Simplifying Progress

Simplifying the Transfer of a Chromatography Process to Multi-Column Chromatography

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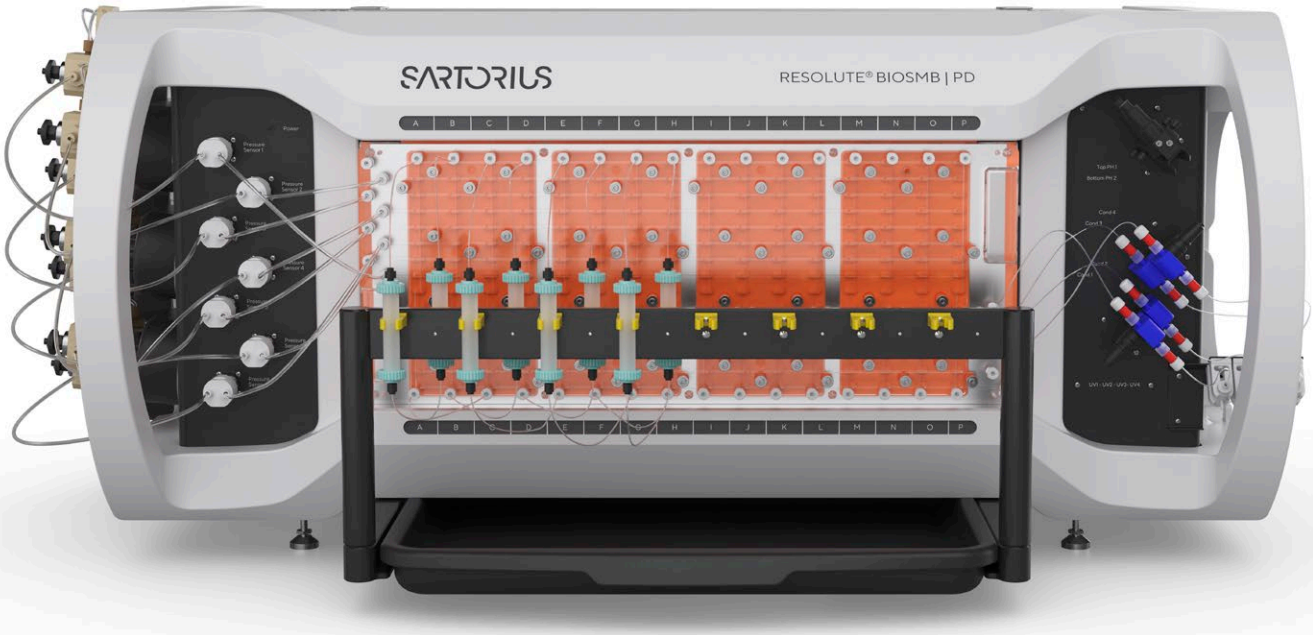
Introduction

For over 30 years, only minor improvements have been introduced to the general operation of chromatographic bioprocessing steps. Despite these improvements, the capture chromatography step remains one of the most costly and time-consuming operations conducted in biopharmaceutical production suites.

Multi-column chromatography (MCC) aims to reduce chromatography's economic and operational burden, especially at the capture step, by maximizing process efficiencies to create value for the manufacturer. The main strategies that MCC employs are continuous column cycling, smaller column sizes, and multiple load zone columns to increase capacity and process yield.

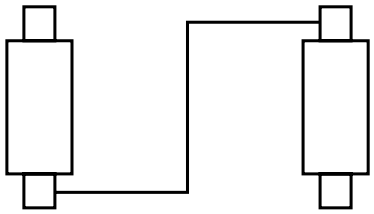
For bioprocess development engineers, the perceived challenges towards transitioning to multi-column from batch chromatography tend to outweigh the benefits. Batch chromatography has many variables that users must control to achieve a successful purification. The added complexity of a process step with multiple columns creates a perceived hurdle causing hesitation towards adopting MCC in conventional processing.

This document serves as a step-by-step beginner's guide to simplify and de-risk the process. This guide includes recommendations for generating supporting experimental data, modeling processes with Sartorius-provided tools, and finally, creating and optimizing a multi-column chromatography process.

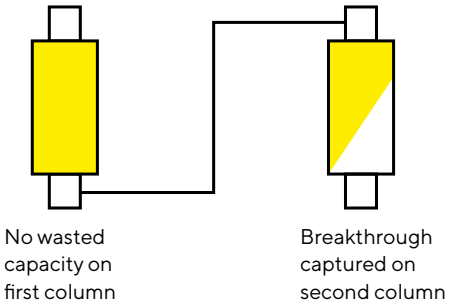


Fundamentals of Moving to BioSMB – Capture Chromatography

- Add a column to the load zone and **increase operating flow rate**



- **Increase binding capacity** and capture breakthrough on second column



- Add columns to operate non-load and load steps **concurrently**

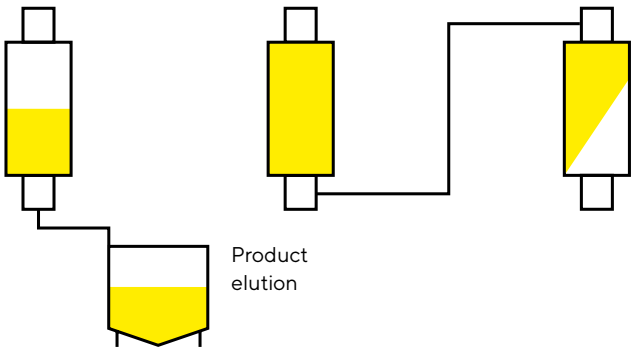


Figure 1: *How Multi-Column Capture Chromatography Increases Speed and Throughput While Reducing Resin Requirements.*

How Does MCC Work?

In standard batch chromatography, a single large column is employed to capture a product. Excess resin is required to prevent product loss via breakthrough, resulting in an overall underutilization of resin. Multi-column chromatography uses a series of smaller columns operated in parallel. This arrangement allows users to run multiple process steps simultaneously, creating an efficient continuous process. The smaller columns also improve resin utilization increasing productivity and saving costs.

Benefits of MCC

As a mode of capture chromatography, MCC brings users the benefits of better resin utilization and higher throughput. When the process is tuned for total resin reduction, users can achieve up to an 80% lower resin requirement compared to their batch process. The process can also be adjusted for higher throughput, allowing users to complete their chromatography step in a fraction of the time required by their batch chromatography process. These benefits are achieved by two main principles illustrated in Figure 1: the controlled overloading of columns by linking multiple columns in series and the sequential and parallel processing of multiple smaller columns. These two levers can be manipulated to enable further optimization into a continuous process, whereby a controlled flow rate may drive the operating conditions.

Transfer from Batch to MCC – Where to Start?

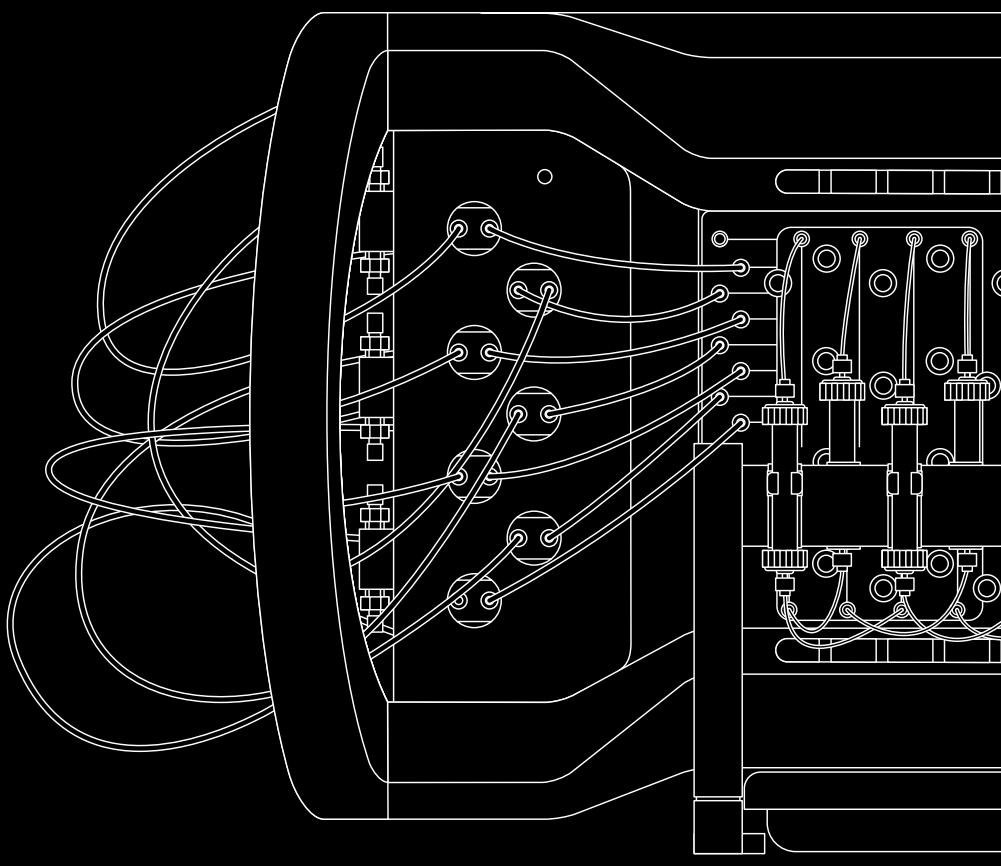
Before considering MCC, it is important to understand that MCC is a production method that drives higher efficiency into a chromatography manufacturing step. Therefore, process development strategies are required to determine optimal separation conditions before moving the operations to MCC. The transition to MCC usually happens after establishing resins, buffers, solutions, and process step volumes but before scaling up the chromatography protocol for tech transfer and production.

Once optimal conditions for the chromatography separation have been established in batch mode, or if a batch chromatography process already exists, then the process can easily be intensified to MCC with a high degree of precision, resulting in minimal or no qualification runs.

Transferring a batch chromatography process to MCC results in two fundamental changes:

1. The loading zone of the process is adjusted to have multiple columns in series, resulting in higher utilized capacity

2. The process is executed at shorter contact times due to reduced column height (contact time is used to describe the combined contact time of the product over all columns in series)



Factors Affecting MCC Performance

When transferring a process to MCC, three factors will impact the performance of the operation:

Binding Capacity of the Resin for the Product Molecule

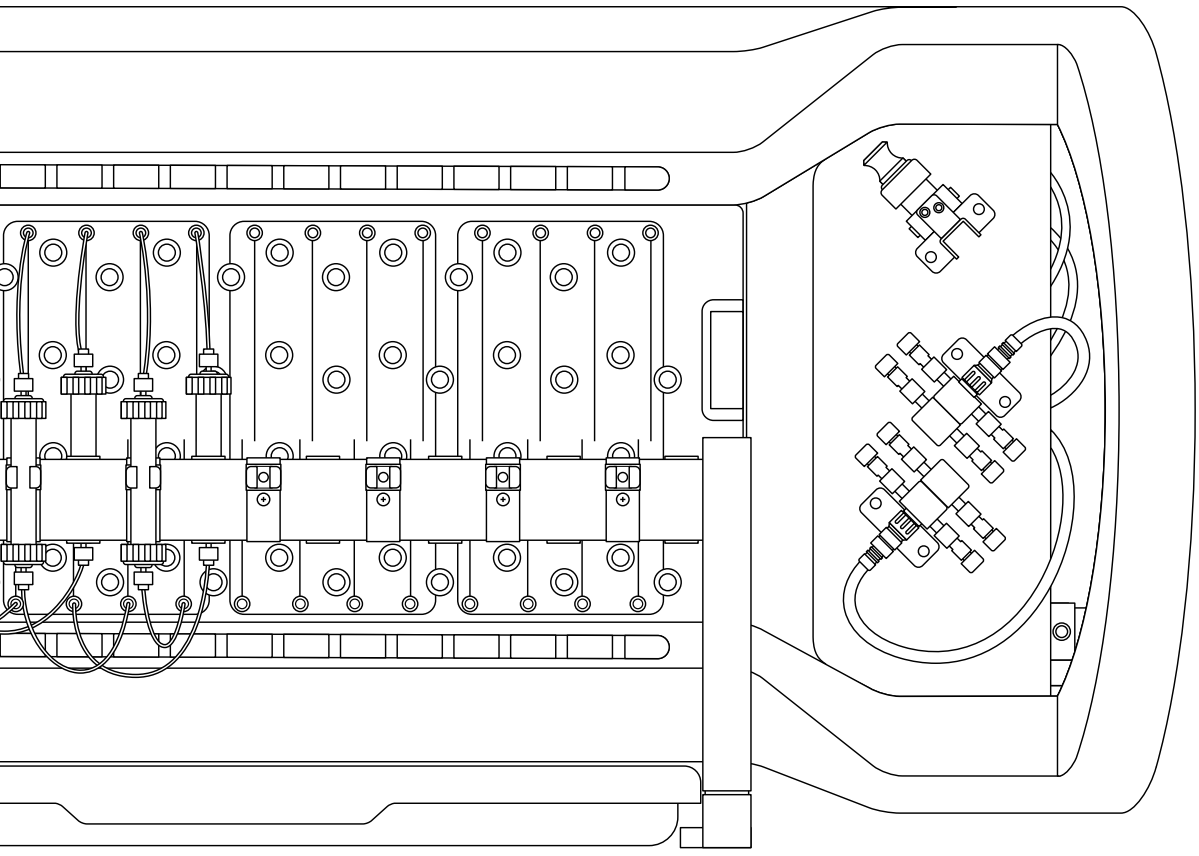
The standard operating binding capacity (OBC) used for batch chromatography (e.g., 80% of 10% breakthrough) is not applicable for MCC. Instead, a complete product breakthrough curve is used to identify an operating binding capacity that utilizes column capacity close to the static binding capacity (100% breakthrough).

Resin Maximum Linear Velocity (Or Minimum Contact Time)

MCC enables faster processing by allowing an increased loading flow rate. Nevertheless, understanding the physical resin limitations to prevent high operational pressures and column performance degradation is critical to success. These limits are commonly supplied by the resin manufacturer.

Column Bed Height for Contact Time Calculations

The load zone in an MCC process typically involves more than one column; contact time calculations should consider this adjustment. MCC users will typically aim to minimize bed height to manage the back pressure impact of having two columns operating in series.



Making the Switch – What Does it Involve?

The fastest and easiest method to transfer a process to MCC would be to increase the operating binding capacity of the batch process by 50% and to reduce the bed height by 50%. The bed height is reduced to mimic the total bed height of the original batch process and to avoid back pressure and linear velocity limitations. The engineer should be aware that this method is based on general assumptions of capacity and resin performance; further optimization may be required during MCC experimentation.

For a more optimized approach to an MCC transfer, the process engineer will first need to establish the MCC load operating capacity and contact time by analyzing breakthrough curves. Once the load conditions are determined, they should determine the load zone column configuration, considering the recirculation of the unbound material in the wash step and the recirculation of this material into the second pass column(s). Finally, they should configure the remaining non-load steps into the MCC process (using the same volumes from batch processing) to minimize changes in linear velocity and calculate the total number of columns required for the process.

The following sections explain the rationale for the above changes, along with procedural guidance on implementing these changes to a chromatography process. These changes can generally be applied to all bind and elute chromatography operations.

Step 1 – Perform Breakthrough Curve Experiments

The relationship between flow rate and dynamic binding capacity must be fully understood to prevent the MCC process from negatively impacting yield. This knowledge can be gathered using breakthrough curves and the resulting binding capacity limits observed at complete breakthrough.

Although flow rates are agnostic to process parameters extracted from break-through performance, these experiments should be conducted at three different flow rates and the column loaded as close as possible to the static binding capacity, allowing the extrapolation of characteristic mass transfer coefficients (process limits) from the breakthrough curve data while accounting for any irregularities in raw data. The resulting analysis provides the user with a flow rate-dependent set of bounds, allowing the MCC process to be set to optimal capture efficiency depending on the chosen flow rate, resin utilization, or short operation time.

Tips for Experimental Setup

- System - Breakthrough curve experiments can be conducted on the same system used in process development to optimize the purification process. Note: a fraction collector will likely be necessary to analyze the product breakthrough curves.

- Process – Aside from the changing volumetric flow rates, the chromatography process characteristics should remain unchanged. The buffers, solutions, and resin should be equivalent to those established from process development to achieve optimal separation. For all subsequent experiments, ensure that the column is fully washed, cleaned, and equilibrated.

- Column Sizing - The breakthrough curve experiments can be executed with small process development columns in the range of 1 mL to 5 mL. For MCC processes, columns generally have shorter bed heights and wider inner diameters, which helps to reduce backpressure at higher contact times. When scaling up, columns typically maintain a similar bed height while increasing the inner diameter (rather than increasing the bed height).

- Flow Rates (Contact Times) – This is the most critical factor for transferring the process to MCC. Since the second column in-series in the loading zone prevents product loss, the goal of MCC is to operate at the highest flow rates acceptable to the physical limitations of the column and resin. As such, generating breakthrough curve data at higher flow rates (compared to batch) is most beneficial. The overall goal of flow rate determination is to produce three breakthrough curves at different contact times in the likely range of MCC operation. For example, for a standard agarose-based resin, Sartorius advises performing breakthrough experiments at 0.75-, 1.5-, and 2.25-minute contact times. Note: before experimentation, it is crucial to understand the linear velocity limits of the chromatography resin.

- Non-Load Steps – All steps in the phase should be executed at the three contact times, including the non-load steps. These experiments will highlight whether any pressure or performance concerns might arise due to the high flow rates.

Step 2 - Breakthrough Curve Analysis

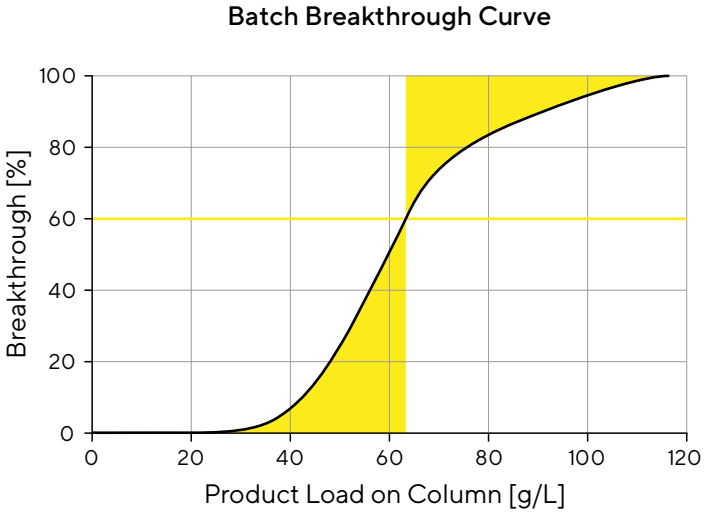
Determination of the operational binding capacity and contact time is critical to balancing process efficiency and product recovery across a loading zone consisting of multiple columns. Therefore, there are two general ways to transfer the loading step to MCC:

Option 1 – Simple Estimates and Experimental Load Zone Determination
An experimental method to estimate operating binding capacity based on evaluating breakthrough curves is useful for many applications and utilized by Sartorius when quick transfer to MCC is required.

Experimental load zone determination does not apply to all chromatography scenarios, but it can allow a simple indication of the column capacity and assist with determining optimal contact times. An experimental approach can quickly show the benefits of the technology, even if multiple breakthrough curves are not available for analysis.

In many cases, the static binding capacity is nearly equivalent to the product load at 60% breakthrough of the feed product concentration C_o. In Figure 2, the static binding capacity can be estimated to be 62 g/L (using the 60% breakthrough). The operating binding capacity for the first MCC experiment can be set at 90% of the static binding capacity, in this case 56 g/L.

Figure 2: *Product Unbound on the Column at the 60% Breakthrough Point.*

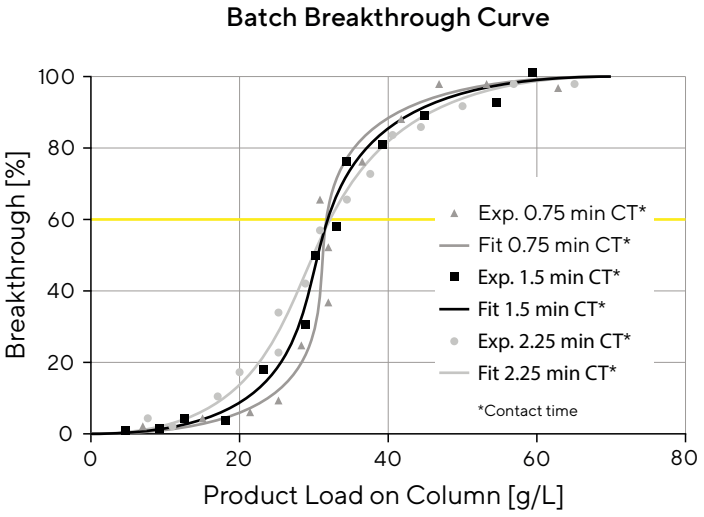


Note. In many standard bind and elute chromatography applications, the amount of product unbound on the column at the 60% breakthrough point (a) is nearly equivalent to the amount of product bound on the column after the 60% breakthrough point (b). In this example, the static binding capacity can be estimated at 62 g/L, assessed from the amount of product loaded on the column when 60% breakthrough is achieved.

Option 2 - Curve Fitting and Capacity | Contact Time Analysis

Analyzing the breakthrough curves can give the process engineer a better understanding of the MCC process's operational capacity and contact time. This method aims to optimize the MCC process through modeling, reducing the number of subsequent experiments. For most chromatographic processes with a breakthrough curve that exhibits lower-order polynomial characteristics (as shown in Figure 3), an analysis that estimates static binding capacity and the mass transfer coefficient is likely all that is needed. Sartorius has developed a simple tool that allows users to input breakthrough curve data through Microsoft Excel® and quickly estimate the static binding capacity and mass transfer coefficient.

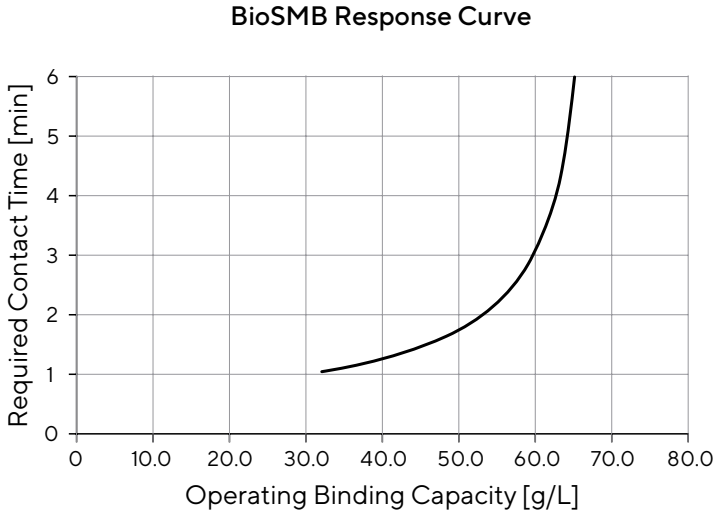
Figure 3: *Example of Curve Fitting to Three Breakthrough Curves.*



Note. Curve fitting is used to estimate resin static binding capacity and mass transfer coefficients.

The output of this analysis will be an understanding of the MCC load step operating space (Figure 4). This knowledge allows the engineer to optimize the process loading step for different factors, including capacity or throughput, without jeopardizing the capture efficiency. Figure 4 shows the limit of the MCC load step. The curve represents a 99% capture efficiency, as predicted by the modeling tool, which enables the end user to identify loading conditions based on contact time, meaning the process can be tailored to a specific loading flow rate or contact time as desired. Load step conditions to the left of this line are likely to result in capture efficiencies higher than the desired inputted capture efficiency, underutilizing column capacity but also enabling the end user to tailor the load flow rate or contact time. Load step conditions to the right of the curve are likely to result in capture efficiencies lower than the desired inputted capture efficiency, resulting in potential product loss in breakthrough.

Figure 4: *MCC Load Zone Contact Time and Operational Binding Capacity Plot to Achieve a Set Capture Efficiency.*



Note. Resolute® BioSMB response curve representing the model approximation of the inputted capture efficiency, usually 99% capture efficiency (<1% product loss through breakthrough), indicating acceptable operation limits for an MCC process given the user-defined acceptable limit of product recovery. Operation below or on the right side of the curve results in product loss.

Step 3 - Completing the MCC Process

Once the load zone conditions are determined, the process engineer can establish the remaining MCC process. In an MCC process, the columns will go through the steps in the following order: 1. Second pass 2. Load (direct feed) 3. Wash 4. Other non-load steps, in the same sequence defined by the process. Columns first enter the second pass portion of the loading phase before the direct feed load step to capture all breakthrough from the column in the direct load position.

Load Zone Column Configuration

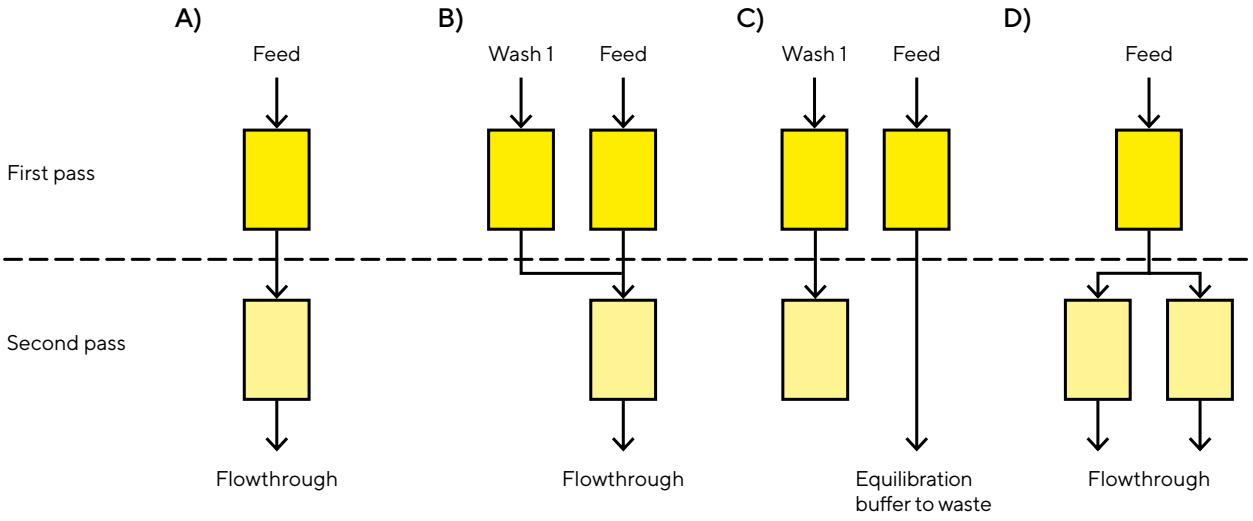
The first step in building the column configuration will be establishing the number of columns in the loading zone. Sartorius recommends two different scenarios: two columns in series (Figure 5a) or one column connected in series to two columns in parallel second pass operation (Figure 5d). The main driver of this decision will be the flow rates of both the load and the first wash steps.

Once a column is switched out of the load zone and begins the wash phase, it is usually important that unbound material is retained and not sent to waste with the rest of the wash step output. Therefore, the first column volume of the wash step should be redirected to the second pass load column to retain the unbound product in the mobile phase within the column. If a ´two columns in series´ (Figure 5a) is employed, then the second pass column will see higher flow rates (Figure 5b). A different column configuration can be used if the linear flow rate is higher than the recommended operating condition.

One option the engineer may utilize is to direct the wash column output to the second pass column, avoiding the second pass for the outlet of the feed column (Figure 5c). This is achievable because the first column volumes on the feed column do not typically result in product breakthrough.

The second option is to utilize a 1–2 load zone (Figure 5d). In the 1–2 load zone column configuration, the output of the first load zone column is distributed to two second pass columns. When unbound material from the first wash is cycled into the second pass, the resulting column configuration will be 2–2, and high linear flow rates can be managed.

Figure 5: *Four Recommended Load Zone Column Configurations.*



Note. (a) Two columns connected in series. (b) Two columns connected in series with the primary wash step redirected to the second loading column, resulting in temporal higher flow rates in the second pass column. (c) Two columns connected in series, the first CV of primary wash is recirculated to the second pass column while the first CV of the primary load column is sent to waste. During the first CV of loading, the primary load column does not have the risk of breakthrough. Therefore, this approach maintains the same linear velocity across the loading process and does not induce product breakthrough. (d) Two columns connected in series with two columns in parallel in the second pass. This reduces the linear velocity of the second pass columns by 50% and enables the primary wash step to be recirculated without an increase in overall linear velocity.

As an example, Table 1 builds an MCC process using load conditions determined through breakthrough curve analysis. The load zone column configuration was subsequently optimized, and the scenario chosen was two columns connected in series with two columns in parallel in the second pass. (Figure 5d). In this scenario, the first CV of the primary wash is redirected to the second pass loading column, but since two columns are placed in parallel in the second pass, the linear velocity never exceeds that of the primary load column, alleviating any concern of shortened contact time and product breakthrough. The remainder of the non-loading steps are described in the next section.

Step	Volume [CV]	Time [min]	Columns in Parallel
Second Pass	10	10	2
Load	10	10	1
Wash 1	3 (first CV to second pass)	3	

Table 1: *Load Conditions and Column Configurations for an Mcc Process.*

Non-Load Zone Process Steps

Once the load zone column configuration is established, the remaining steps are to build the rest of the process using the non-load steps (subsequent washes, elution, clean | regeneration, equilibration, etc.). To do this, we recommend that the operational volumes established during the optimization of the purification process are not adjusted. The flow rate and contact time of the non-load steps should be designed to operate as closely as possible to the linear velocity of the load step to prevent column compression and expansion throughout the process.

Step	Volume [CV]	Time [min]	Linear Velocity (cm/hr)
Second Pass (2 columns)	10	10	150 – 300
Load	10	10	300
Wash 1	3 (first CV to second pass)	3	300
Wash 2	5	5	300
Wash 3	3	3	300
Elution	5	5	300
Clean | Regeneration	3	4.5	200
Equilibration	5	5	300

Note. The operational flow rates are set to minimize differences between load and non-load linear velocities.

Table 2: *The Remaining Non-load Steps Are Built Into the MCC Process With the Same Volume Determined During Purification Optimization.*

The number of columns required for the process can be calculated using the following formula:

$$N_c = \text{RoundUp}\left(\frac{\Sigma T_{nl}}{T_l}\right) + N_{cl}$$

Note. N_c is the number of columns, ΣT_{nl} is the sum of the time of all the non-loading and non-second pass steps, T_l is the load step time, and N_{cl} is the number of columns in the load and second pass steps

In this case, a roundup function is required to calculate the number of columns to ensure that the number of columns is an integer number. An MCC process cannot be executed with a partial column, but an idle column for a moment is easily possible. In the scenario shown in Table 2, the sum of the time for the non-load steps is 25.5 minutes, and the load time is 10 minutes. The number of columns in the load zone is equal to 3 (with 2 columns in the second pass), so the total number of columns required for this process is 6.

Author's Recommendations

- It is best to closely monitor the wash and elution steps to determine if step volumes need to be adjusted to account for the increased column loading.
- If there is product in the flow-through pool or the elution pool mass is not equivalent to the amount of product loaded onto the column, then the MCC load step may be too aggressive, and the operating binding capacity should be reduced or the contact time increased.
- Product loss might also occur during column wash steps. Monitor these fractions to adjust washing or loading conditions.

General Terms
Second Pass: The column(s) that are connected last in a series of two columns.
First Pass (or load, or direct feed): The column(s) that are receiving direct product feed and not connected to the outlet of a column.
Contact time: Equivalent to residence time. Calculated by dividing the volume of a column by the flow rate of the fluid going through the column.

Conclusion

Making the switch from batch to continuous or multi-column chromatography can significantly reduce resin utilization, decrease costs and eliminate production bottlenecks without impacting product quality attributes.

The process of transitioning from one system to another can appear challenging; however, transferring a chromatographic process to an MCC process is straightforward.

It can be completed within 1 – 2 days of experimental work, excluding subsequent MCC optimization and verification experiments.

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Jason Forte is a Product Specialist at Sartorius, focusing on intensified processing solutions. He has several years of experience with the Resolute® BioSMB technology devoted to customer implementation of intensified and continuous technologies and the development of process control strategies.

Prior to joining Sartorius in 2020, Jason served as a continuous processing engineer at Pall Biotech. Jason has an additional three years of experience in the industry focused on early-stage drug and process development.
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Karl Rogler earned his bachelor's degree in Chemical Engineering from Rensselaer Polytechnic Institute, where he specialized in chromatography applications for protein purification. He has been developing chromatography applications on the Resolute® BioSMB system since 2013.

In 2015, he became project manager for several Resolute® BioSMB projects and serves today as the Resolute® BioSMB Systems Product Manager.

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