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Enhancing mRNA Capture With Resolute® BioSMB and CIMmultus® Oligo dT

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Abstract

Over the past decade, bioprocessing has undergone a significant transformation toward efficient, continuous manufacturing. Initially applied to established molecules such as recombinant proteins, these methodologies are now being extended to the manufacturing processes of other biological modalities, yielding comparable benefits. In this proof-of-concept study, we demonstrate that the Resolute® BioSMB system can be used to achieve a continuous mRNA capture chromatography application with CIMmultus® Oligo dT monolith devices while maintaining process conditions, performance, and robustness.

Breakthrough curve experiments were first conducted to determine the optimal conditions for continuous bind-and-elute processing of mRNA on the CIMmultus® dT monolith. Using this data, several continuous chromatography scenarios were calculated and tested on the monoliths. These operations demonstrated reproducible elution profiles and high yields (97%).

We demonstrated that continuous loading is possible with monolith devices and that productivity can increase by 57% at a larger scale. As a result, using smaller devices can achieve the same mass throughput as batch mode. Alternatively, if the total volume of the monolithic devices is maintained, the mass throughput will be significantly improved compared to operating in batch mode.

Introduction

Since the COVID-19 pandemic, the critical need for an uninterrupted supply of effective drug substances has become a focal point for regulators and biologics manufacturers.[1] Continuous processing is achieved by running unit operations in a steady or cyclical state for extended periods, avoiding costly stop-start disruptions. This approach has been demonstrated to significantly improve biologics production and overall plant efficiency, without increasing risk to product quality.[2] Consequently, organizations like the U.S. Food and Drug Administration (FDA) have shown increasing interest and investment in continuous manufacturing and multi-column chromatography (MCC).

The main goal of MCC is to achieve a continuous product loading scenario in order to fit within a continuous process or increase the performance of the chromatography step. The general idea of MCC is to cycle more than one column between the loading step and the non-loading steps, enabling continuous product processing. In some cases, multiple columns can be positioned and cycled in series in the loading zone to increase operating binding capacity while minimizing the risk of product breakthrough. This is typically referred to as sequential multi-column chromatography (S-MCC). Such processes can be operated close to the equilibrium or static binding capacity of the chromatographic media, leading to a significant reduction in media costs.

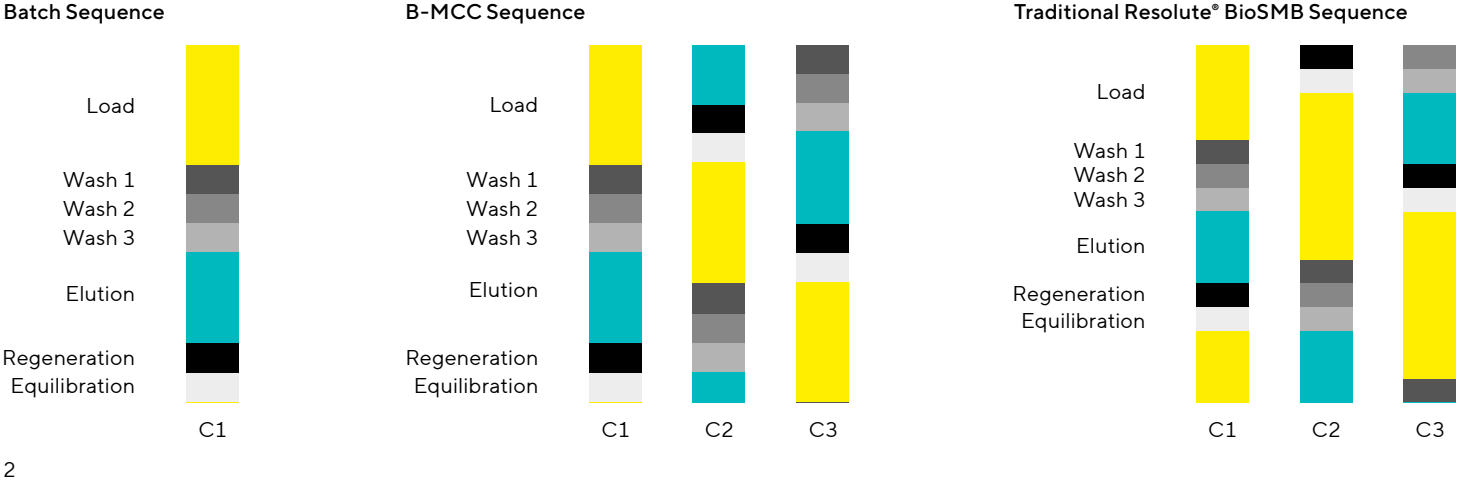
Another approach to enable continuous chromatography is batch multi-column chromatography (B-MCC), otherwise known as parallel batch chromatography. This strategy enables continuous processing while minimizing additional process development. In B-MCC, two or more chromatography columns independently cycle through the loading and non-loading steps of the chromatography process and maintain a continuous product load. The columns in the B-MCC scenario are never connected sequentially or in series, as they would be in S-MCC (Figure 1).

To date, continuous manufacturing has mostly been implemented in the production of monoclonal antibodies (mAbs), but its applications are not exclusive to this subcategory of the biologics industry. MCC addresses bottlenecks in the downstream process of all biomolecules by facilitating continuous loading of the molecule of interest.

Here, we demonstrate that the Resolute® BioSMB multi-column chromatography platform can be used to purify firefly luciferase (FLuc) mRNA. We used a monolithic chromatography format (CIMmultus® Oligo dT devices) for capture purification. Due to the predominantly convective nature of monolithic devices, the advantages of placing two devices in series in the load zone (S-MCC) are reduced compared to a traditional resin. Therefore, we placed the monolith columns in a B-MCC configuration on our MCC platform, the Resolute® BioSMB.

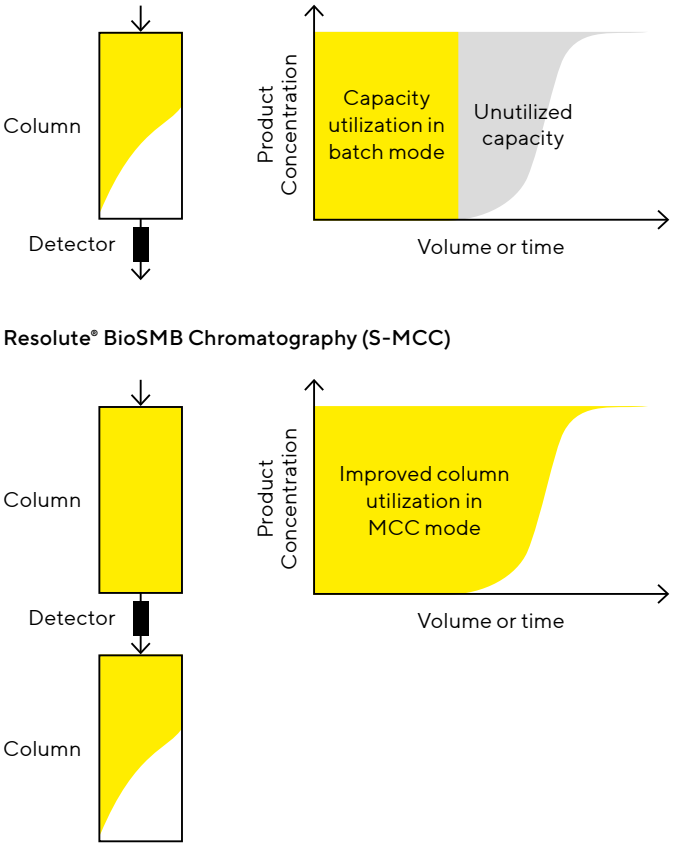
The Resolute® BioSMB platform is fully scalable from process development to commercial manufacturing. The Resolute® BioSMB PD system used in this study is designed for lab-scale process development, facilitating the transition from a batch, single-column process to an intensified multi-column process without altering the chromatographic separation performance, media, buffers, or product quality. In traditional batch chromatographic processing, only 50% to 70% of the chromatographic column's total binding capacity is typically used, leading to significant CAPEX and OPEX for maintenance (Figure 2). In contrast, the Resolute® BioSMB PD system uses a series of substantially smaller, and at times interconnected, columns, optimizing resource utilization and improving overall process efficiency. For manufacturing-scale equipment, two flow range variants are available: Resolute® BioSMB 80 system and Resolute® BioSMB 350 system, providing a scalable platform for MCC.

Figure 1: *Traditional, Single-Column Batch Chromatography Compared to B-MCC and Resolute® BioSMB Sequences*



Materials

Figure 2: *Comparison of Capacity Utilization in Traditional Batch vs. Resolute® BioSMB Chromatography*

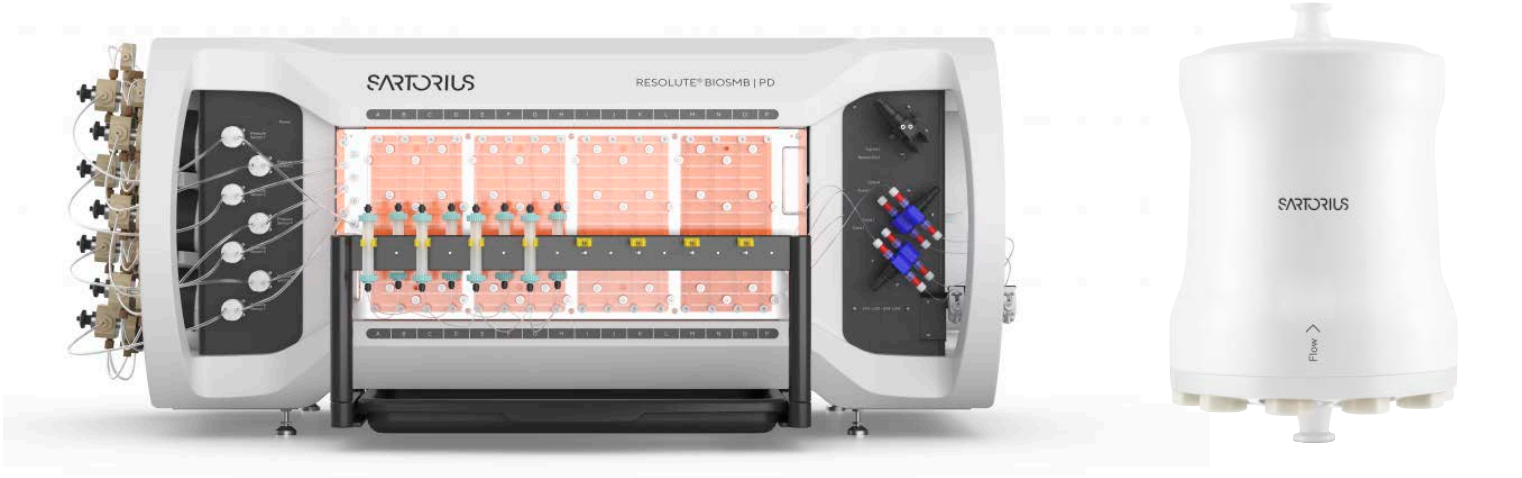


Firefly luciferase (FLuc) mRNA, including a poly (A) sequence, was synthesized by in vitro transcription (IVT). The IVT reaction was subjected to DNAse digestion, followed by purification with tangential flow filtration (TFF). The post-TFF material was diluted 1:2 with 2x Equilibration Buffer (EQ), and 180 mL of this solution was utilized for chromatographic mRNA capture in B-MCC mode with the Resolute® BioSMB.

The CIMmultus® Oligo dT18 (C12 Linker) 1 mL monolithic column (2 µm) from Sartorius was used for mRNA capture. The buffers used for the capture step performed on the Resolute® BioSMB PD (Sartorius) are listed in Table 1.

Table 1: *Buffer Recipe List*

Buffer	pH	Cond [mS/cm]	Notes
100 mM Sodium Phosphate, 400 mM NaCl, 4 mM EDTA	7.0	42.6 ± 4.3	Load (2x EQ buffer) Mixed 1:1 with post-TFF material
50 mM Sodium Phosphate, 200 mM NaCl, 2 mM EDTA	7.0	23.2 ± 2.3	Wash 1
50 mM Sodium Phosphate, 2 mM EDTA	7.0	6.95 ± 3.05	Wash 2
WFI	N/A	N/A	Elution
0.5 M Sodium Hydroxide	13.0 ± 0.25	N/A	Clean-in
50 mM Sodium Phosphate, 200 mM NaCl, 2 mM EDTA	7.0	23.2 ± 2.3	Equilibration



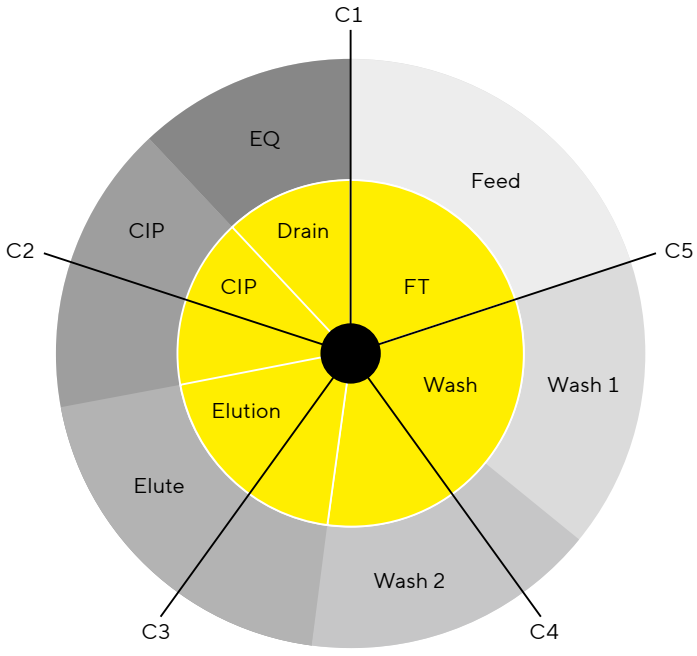
Methods

The FLuc mRNA breakthrough profile with the monolithic devices was evaluated with a preparative batch chromatography system. These breakthrough curves dictated the loading conditions of the monolithic devices, analogous to the operation of a batch chromatography column. The dynamic binding capacity (DBC) of the device was calculated at 10% breakthrough. Then, the operating binding capacity (OBC) was set at 80% DBC for the B-MCC evaluation on the Resolute® BioSMB PD system. The batch parameters, including the OBC, were used to create a phase within the Resolute® BioSMB Phase Editor software, which required five monolithic devices for this capture step (Figure 3 and Table 2). To enable continuous loading and elution, the duration of these two steps was equal (one switch time), while all other non-loading steps remained unchanged.

Table 2: *Executed Phase Conditions*

Inlet Name	Volume	Flow Rate	Time
Feed	10.67 CV	1.2 mL/min	10.67 min
Wash 1	5 CV	0.7 mL/min	0.80 switch times
Wash 2	5 CV	0.7 mL/min	0.80 switch times
Elute	10 CV	1.1 mL/min	1.00 switch times
CIP	5 CV	0.7 mL/min	0.80 switch times
EQ	5 CV	0.9 mL/min	0.60 switch times

Figure 3: *Chronogram Associated With the Executed Phase*



Note. Five monolithic devices (C1-C5) were used to perform the capture step with 1 mL CIMmultus® Oligo dT18 monoliths.

Results

DBC Assessment

Breakthrough of the mRNA molecule was evaluated at residence times of 0.5 min and 1 min. The 0.5-min breakthrough curve used to determine DBC is shown in Figure 4. The results of the 0.5-min and 1-min breakthrough curves are summarized in Table 3. The Resolute® BioSMB loading reflects the calculated capacity from the 1-min residence time breakthrough curve.

Figure 4: *Breakthrough Curve for 0.5-Minute Residence Time*

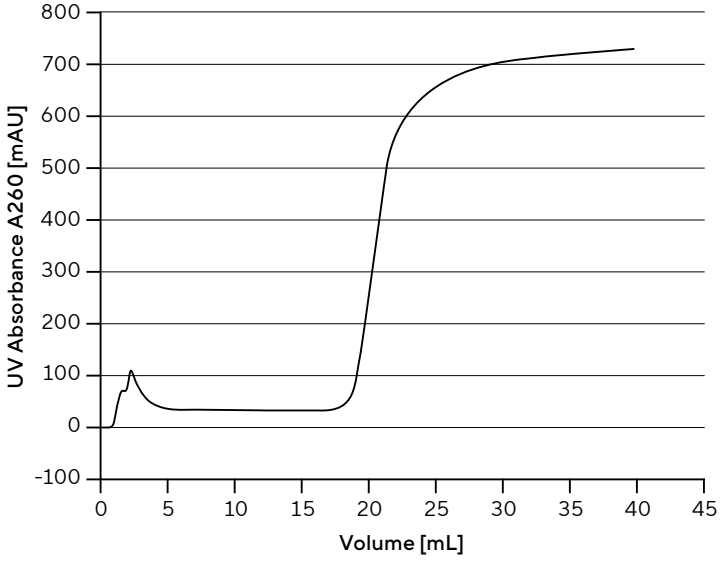


Table 3: *DBC Breakthrough Curve Results*

Residence Time [min]	Load Concentration [mg/mL]	Volume at 10% BT [mL]	UV at 10% BT [mAU]	Load Mass at 10% BT [mg]	80% of 10% BT Load [mg mRNA/mL device]	Load CV
0.5	0.25	19.11	70.79	3.98	2.65	10.6
1	0.25	16.26	100.39	3.39	2.26	9.04

BT = breakthrough

Resolute® BioSMB Evaluation

We performed a parallel batch operation on the Resolute® BioSMB PD as described in the introduction. The Resolute® BioSMB recipe consisted of five monolithic devices, and three cycles were executed, with one washdown cycle included to recover the product once loading was completed. Only elutions containing the product were collected. This approach enabled continuous loading and elution on the Resolute® BioSMB, with the load and elution both operating for one switch time.

The yield was calculated by dividing the total mass loaded by the total mass in the elution. mRNA concentration was measured using the A260 reading on a NanoDrop spectrophotometer, while mRNA purity was assessed using the A260/280 ratio. The results are displayed in Table 4. The yield for the experiment was calculated to be approximately 97%, with around 97% of the product detected in the eluate and approximately 4.8% of the product in the flowthrough, which was below the instrument's detection limit.

Table 4: *Analytics Results — mRNA Concentration (A260) and Purity (A260/A280)*

Sample Name	Concentration [ng/µL]	A260/A280
Post-TFF Load Post-Thaw	1668	1.87
Post-TFF Load Post-Dilution	247	1.94
Resolute® BioSMB Flowthrough	12	1.76
Resolute® BioSMB Elution	255	1.92
Resolute® BioSMB Post-TFF Load Post-Dilution	247	1.94

Using the data and the recipe from this evaluation, we created a variety of theoretical scenarios, as shown in Table 5. In these scenarios, the productivity and processing time options of the Resolute® BioSMB were compared to the current batch process conditions using a 400 mL monolithic device. By deploying the Resolute® BioSMB system, every scenario was more productive than the current batch process. In addition, depending on the desired processing time and device scale, the desired mass throughput could be adjusted to meet the process needs.

When considering these scenarios, a 400 mL monolith with a 1 min residence time (row 1, Table 5) is used as a base case. Each of the subsequent comparisons is made based on this starting point ("Productivity Increase Compared to Base-Case Batch"). Each of the MCC rows assumes a B-MCC approach, such as those used in the previously described experiments. In these scenarios, five devices were not required due to the ratio of the duration of the load versus the non-load steps. The OBC for the base case was supplied to Sartorius while the other OBC values were determined experimentally.

Table 5: *Productivity Scenarios*

Device Volume [mL]	Batch (B) or Multicolumn Chromatography (MCC)	Number of Devices	Operating Binding Capacity [mg/mL]	Productivity [mg product/mL sorbent/hr]	Mass Throughput [mg product/hr]
400	B	1	2.65	5.3	2120
1.2	MCC	4	2.65	7.53	36
40	MCC	4	2.65	7.53	1205
80	MCC	4	2.65	7.53	2410

Note. Continuous loading and elution are the assumptions for MCC.

*Assumption all steps performed with a 1-min residence time.

**Assumption of capacity equal to the 0.5-min RT breakthrough curve and all steps operated with a residence time equal to 0.5 min.

Discussion

The data presented here show that the Resolute® BioSMB system demonstrated higher productivity in every scenario compared to the current batch process. In addition, depending on the desired processing time and device scale, the desired mass throughput could be adjusted to meet the process needs. This is particularly important for manufacturing metrics like facility flexibility, clean room suite sizing, and meeting drug product demand with available capital equipment.

These findings suggest that the B-MCC configuration on the Resolute® BioSMB PD system maintains mRNA product quality while enabling continuous loading and elution of the target molecule.

Conclusion

Employing MCC in a bioprocess enables productivity improvements across various modalities. By implementing a B-MCC or parallel batch approach, smaller devices can be used for the same process. Additionally, transitioning to a B-MCC approach requires no additional process development, streamlining scale-up activities. Continuous loading is also enabled, removing bottlenecks that would otherwise persist downstream.

The efficiency improvements enabled by MCC have been widely demonstrated in the literature when referencing mAbs. The data presented here clearly demonstrate that the same approaches also work for other biologics, such as mRNA.

References

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