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Integrated | Connected Downstream Processing of mAbs With Resolute® BioSC for Improved Productivity, Cost, Footprint, and Facility Utilization

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Abstract

Monoclonal antibody (mAb) manufacturing processes have continued to evolve over the last few decades. Improvements in cell line engineering, media, and upstream processing have resulted in higher product titers, creating downstream process limitations. Manufacturers require novel and more efficient platform technologies to meet ever-increasing product demands while being mindful of costs, processing times, and regulatory requirements. Downstream process intensification is one strategy to improve the efficiency of mAb processing. However, a lack of guidance on developing, scaling, and maintaining process and product quality is blocking the more widespread implementation of downstream intensification across the biopharmaceutical industry.

In this application note, we describe how intensifying two chromatography polishing steps by developing unit operations using Sartobind® Q and CMM HyperCel saves buffer volumes and accelerates the downstream process. The downstream platform was then used to develop a connected process by parallel processing multiple unit operations. This resulted in >35% time savings, 2 – 3x footprint reduction, and 16% higher facility capacity than the traditional process (resin-based sequential process). This approach guides mAb developers to apply single unit operation and platform intensification approaches to achieve cost-effectiveness, footprint gains, and high productivity for downstream processes.

ENZENE Biosciences Ltd.

Introduction

Through the successful implementation of process intensification strategies, upstream mAb titers have almost doubled in recent years. Now, the focus has shifted to improvement opportunities in downstream purification processes. Intensified downstream bioprocessing has recently gained significant interest because of various advantages, such as faster operations, smaller equipment and consumable sizes, high volumetric productivity, streamlined process flow, low cycle times, and reduced capital cost.[1]

Downstream intensification approaches started with efforts to adopt high-capacity and | or high-flux consumables. To fully leverage the benefits offered by next-generation purification consumables, the industry requires systems that can:

1. Connect directly with upstream processes.
2. Establish seamless interconnectivity between multiple operations in the process chain under the same automation umbrella.
3. Bring flexibility in operation, easily cater to breakdowns, and enable rapid changeovers.
4. Enable modularity by engaging or disengaging the systems per process and product needs.
5. Reduce facility footprint and production costs.

With the advent of technologies like the Resolute® BioSC Pilot, the possibility of processing multiple operations on a single system has become more realistic. However, a strategic approach is required during process development to leverage the capabilities of such systems, which are associated with benefits like increased throughput, reduced intermediate storage, decreased capital expenditure, lower footprint, and, importantly, improved asset utilization.

All these advantages make the process more productive, and as a result, more cost-effective and sustainable.

To help with the real-life implementation of process intensification as part of continuous process improvement and streamlining industry nomenclature, we have defined four levels of process intensification strategies, from a basic batch process (Level 0) to a continuous process (Level 3), as summarized in Figure 1.[2] In Level 1 intensification, one or multiple standalone operations in the process train are targeted to increase productivity and, as a result, cost efficiency. For a Level 2 intensification process, two or more operations are connected to run in parallel with overarching automation. This substantially reduces processing time and manual intervention while further optimizing asset utilization. Level 3 intensification is achieved if and when a continuous process with a steady state operation for all the process operations is adopted. Applying Level 2 and 3 intensification strategies can reduce the downstream process time significantly, for example, from a 75-hour batch process to a 30-hour connected downstream process.

The work described here leverages an intensified platform (Level 1) recently developed in collaboration with Enzene Biosciences Ltd.[3] The results demonstrate how strategic choice of consumables and a shift to connected processing (Level 2) enable increased cost efficiency, greater productivity, improved facility output, and reduced facility footprint.

Figure 1: *Levels of Process Intensification From Level 0 to Level 3*

L0 – Standard Batch	Stand-alone unit operation.
L1– Intensified, Stand-Alone Unit Operation	Increases the individual step productivity (by, e.g., rapid cycling, multiple columns, in-line buffer generation, operating at higher binding capacity, switching to single-use consumables).
L2 – Connected Process	At least two (standard or intensified) unit operations running simultaneously, including pool tanks with varying fill levels. Software orchestration is beneficial. Also called a clustered or linked process.
L3 – Continuous Process	Fully integrated with steady-state flow, small intermediate tanks, software orchestration, long run times, and closed processing. Also called a semi-continuous or pseudo-continuous process.
L3.1 – Continuous Flow Process	Complete steady-state flow. All bind and elute steps are replaced with flow through mode. The molecule does not stop, and impurities are removed from the stream.

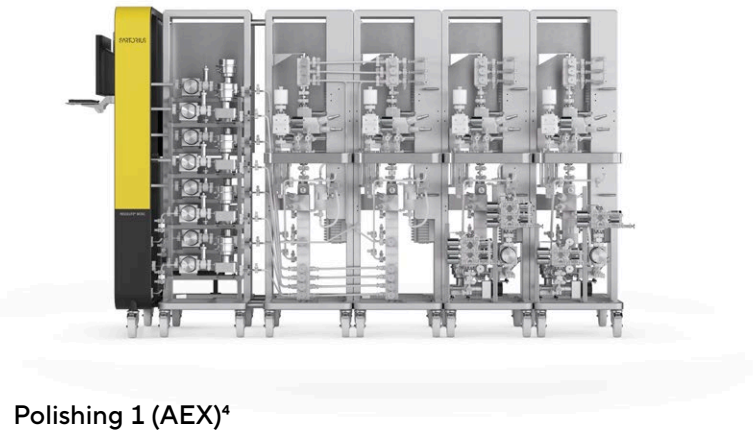
Materials

The model mAb (Adalimumab) harvest was generated from a Cellca™ cell line (Sartorius). The harvest was clarified using Sartoclear® depth filters to remove cell debris, and the filtrate was processed through the chromatography steps.

Automated chromatography systems were used for the execution of all the chromatography experiments at 10 L scale. Glass chromatography columns were used for packing the resins used in the lab-scale process. Post-Protein A and virus inactivation, intermediate filtration was carried out through a dual layer Sartopore® 2 XLG filters (0.8 – 0.2 µm pore size).

Column configurations and buffer details for the three chromatography steps were as follows:

A 200 L scale, Resolute® BioSC system was used to execute the connected process.



Capture Chromatography

Parameter	Details
Column Configuration	
Stationary phase	Protein A resin
Dimensions	See Methods section
Operating mode	Dual column, parallel batch mode
Matrix	Highly cross-linked agarose
Ligand	Alkali-stabilized Protein A derived (*E.coli*)
Buffer Details	
Equilibration	50 mM sodium phosphate, pH 7.0, Cond. 5.85 mS/cm
Wash 2	50 mM sodium phosphate, 1 M sodium chloride, pH 7.0, Cond. 87.67 mS/cm
Wash 3	50 mM sodium acetate, pH 5.5, Cond. 3.55 mS/cm
Elution buffer	200 mM acetic acid, pH 2.7, Cond. 0.271 mS/cm
Regeneration	100 mM sodium hydroxide
Storage	20% v/v ethanol in WFI

Polishing 1 (AEX)[4]

Parameter	Details
Column Configuration	
Stationary phase	Sartobind® Q
Dimensions	See Methods section
Matrix	Stabilized reinforced cellulose
Ligand	Quarternary ammonium
Ligand density	2 – 5 µeq/cm²
Buffer Details	
Equilibration and post-load wash	20 mM sodium phosphate, pH 8.0, Cond. 3.29 mS/cm
Conductivity	20 mM sodium phosphate, 1 M sodium chloride, pH 8.0
High salt strip	20 mM sodium phosphate, 1 M sodium chloride, pH 8.0, Cond. 82.58 mS/cm
Regeneration	1 M sodium hydroxide
Storage	20% v/v ethanol in equilibration buffer

Polishing 2 (Mixed Mode)[5]

Parameter	Details
Column Configuration	
Stationary phase	CMM HyperCel
Dimensions	See Methods section
Matrix	High porosity cross-linked cellulose
Ligand	Aminobenzoic acid
Ligand density	Av. 70 µeq/mL
Buffer Details	
Equilibration	25 mM Tris-HCl, pH 8.0, Cond. 1.4 mS/cm
Wash	25 mM Tris-HCl, pH 7.6, Cond. 1.88 mS/cm
Elution	Tris-HCl, 300 mM NaCl pH 7.4, Cond. 30mS/cm
Regeneration	1 M sodium hydroxide

Methods

Batch Process

A 10 L Univessel® glass bioreactor was run with Sartorius' XtraCHO media for 14 days to reach to a cell density of >30 million cells/mL and consistent viability >98%.
The bioreactor harvest was clarified using Sartoclear® depth filters to generate the feed for the downstream process.
The platform used for downstream processing is shown in Figure 2 (Intensified Platform). Capture chromatography was performed with Protein A resin. Post-capture, the elute was held at pH < 3.5 for 60 minutes (mins) for virus inactivation and then neutralized to pH 8. The neutralization led to an increase in turbidity, which was clarified by intermediate filtration. The filtered and neutralized protein eluate (NPEL) from capture and virus inactivation (VI) was subjected to polishing chromatography after 2-fold dilution to conductivity <4 mS/cm.

The process development for polishing steps is shown in Figure 2 and described in detail in another application note,[3] which details the advantages of the intensified platform (with Sartobind® Q for the first polishing step and CMM HyperCel™ for the second polishing step) over the resin platform. For the batch process, this intensified platform was executed by carrying out the unit operations one after another, as shown in Figure 4A.

Connected Process Strategy

For the connected purification process, clarified cell culture harvest (10 L) was loaded continuously based on the maximum protein that can be loaded on a given affinity capture chromatography column through a batch multi-column chromatography process where the two columns work in parallel (B-MCC - two columns) was implemented for the capture step. Figure 3A shows the phase diagram for the B-MCC (two columns) process, where the black and yellow lines show how a column travels through different phases of the chromatography cycle. Figure 3B shows the difference between how multiple cycles are perfomed for the batch processing and B-MCC (two columns) mode.

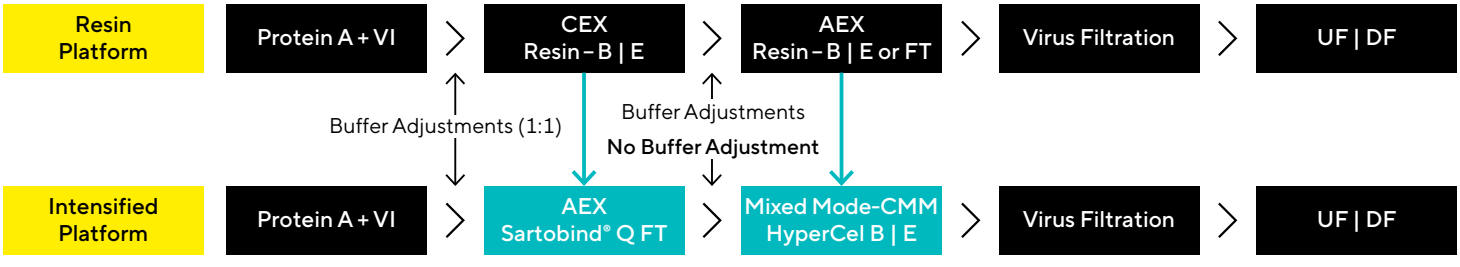
Figure 2: *Process Layout of Basic Resin Platform Against the Intensified Platform (Level 1) Which Is Used Here for Connected Processing*

After one column loading phase is completed, the other column loading phase initiates. The flowthrough of the loaded column is directed to a flowthrough collection instead of loading onto a subsequent column, as in a series mode of operation. In a semi-continuous process setup, columns were switched between the loading and nonloading steps, comprising column wash, elution, cleaning in place (CIP), and equilibration. In the same sequential manner, both capture columns work continuously. In this way, only two columns are necessary, unlike the minimum of three to four required when the process is performed in series mode. The elution phase for capture was fixed at three column volumes (CVs) to avoid volume variability and promote efficient synchronization for the subsequent connected operations.

The capture column 1 (C1) eluate was subjected to viral inactivation and neutralization. It was then stored at 2 – 8 °C until the viral inactivation and neutralization of column 2 (C2) was complete. The elute of two cycles of the capture chromatography step (C1 and C2) were pooled, and later subject to intermediate filtration. The pool was then sent to the first polishing chromatography step (polishing 1) using Sartobind® Q, which offers a high loading capacity of up to 5 kg mAb per liter of membrane. The second polishing chromatography step (polishing 2) was performed using mixed-mode cation exchange resin, CMM HyperCel.
Similarly, the eluates from subsequent cycles from both capture columns (column 1 and column 2) were subject to viral inactivation and neutralization individually, pooled, filtered, and then sent to the polishing 1 and 2 steps sequentially.
This entire procedure was repeated in a connected | semi-continuous operation till the completion of the batch.
The process flow for the connected process (and its comparison to the batch process) is shown in Figure 4B.

While scheduling the operations one after another, it was important to decide the pooling criteria to create an uninterrupted operation and avoid oversizing the columns. The pooling of two cycles from Protein A capture and VI (NPEL pool) onto the Sartobind® Q membrane was done because the flowthrough operation on Sartobind® Q demands higher loading capacities, hence higher load volumes.

Choosing a suitable capture column volume is the key here:



Note. VI = virus inactivation, B | E = bind and elute, FT = flowthrough, CEX = cation exchange, AEX = anion exchange, UF | DF = ultrafiltration | diafiltration

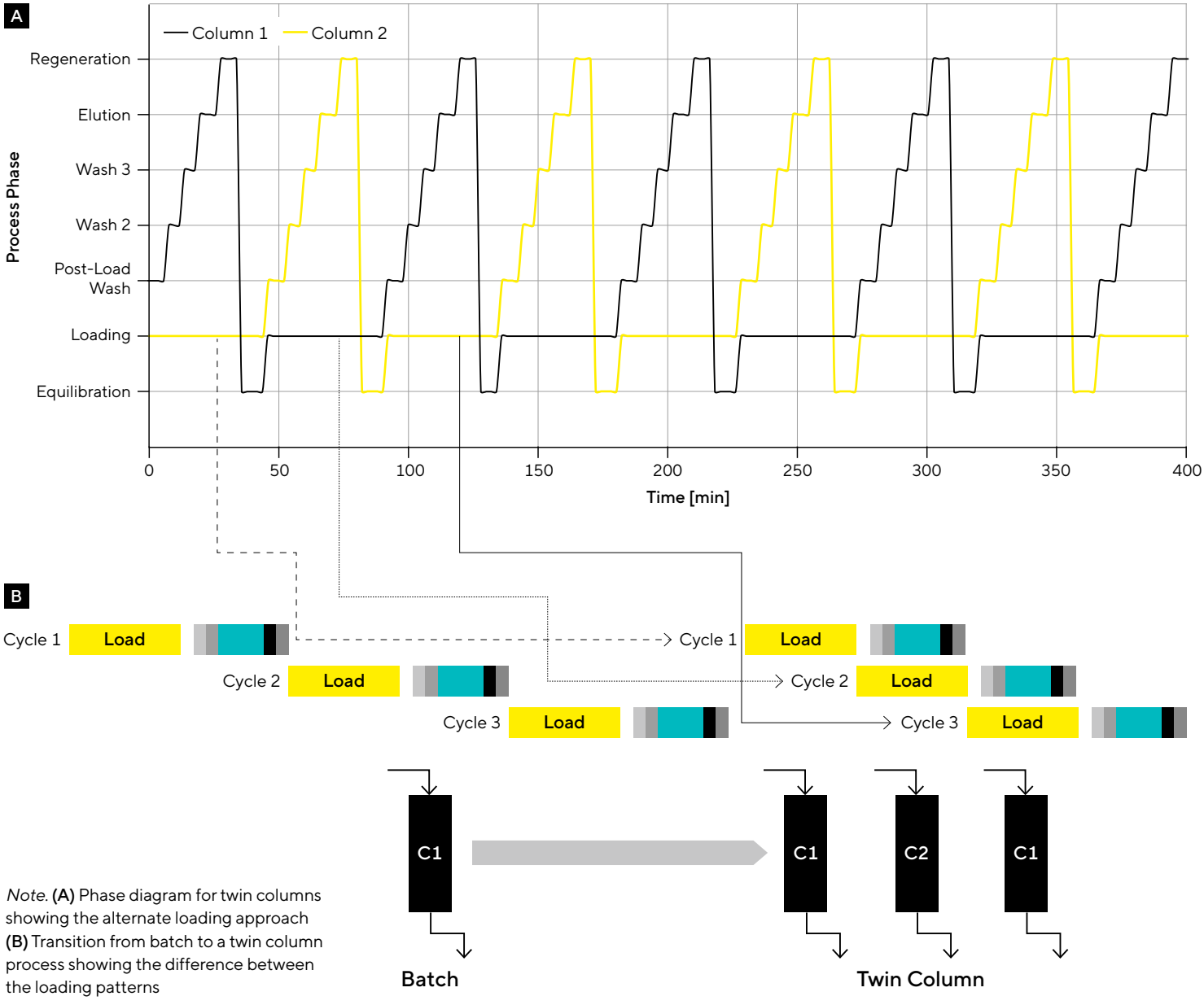
columns can be sized to have one cycle per Sartobind® Q cycle. The loading capacity used here was 2.5 kg/L membrane instead of 5 kg/L to reduce the column sizing for the remainder of the steps. We opted to pool 2 cycles of capture for 1 Sartobind® Q load to further reduce the column size. Column sizing can be reduced even further, but this would require pooling >2 cycles which would create longer waiting times in the process.

If Sartobind® Q capacity was considered as 5 kg/L membrane, even larger columns would be required for capture and polishing 2, or four cycles would have had to be pooled from the capture step with the same column sizing.

The current setup is the most optimized with respect to column sizing and loading capacity on Sartobind® Q. It is recommended to perform process-specific optimization to balance column sizing and waiting times.

As CMM HyperCel was chosen to enable direct loading from Sartobind® Q without any intermediate dilution | buffer adjustment, the sizing of the CMM HyperCel column was selected to cater to one complete Sartobind® Q cycle elute. Briefly, for a single 10 L clarified harvest processed with the connected process, eight capture cycles were carried out in B-MCC mode (two columns), which led to four cycles of polishing 1 using the Sartobind® Q membrane module and four cycles of polishing 2 with CMM HyperCel column.

Figure 3: *The Capture Step Was Carried Out in a Semi-Continuous B-MCC Mode (Two Columns)*



Note. **(A)** Phase diagram for twin columns showing the alternate loading approach **(B)** Transition from batch to a twin column process showing the difference between the loading patterns

This helped assess the intra-batch usability of Sartobind® Q, as multiple cycles were run one after another on the membrane (Figure 4B). All the experiments up to polishing 2 at small scale were performed on individual standard chromatography systems, and the connected process was run through pseudo connections by scheduling the operations in parallel. Post-polishing 2, the product from all four cycles was pooled to carry out virus filtration and ultrafiltration | diafiltration (UF | DF) in a batch mode.

Most importantly, the residence time for loading on capture was calculated based on the loading capacity and time required for polishing 1 and 2 system readiness (to load the subsequent cycle input material) so that the entire batch could be run in connected mode. Hence, the residence time of 4 min used for the loading phase during process development runs[1,4] was changed to 11 mins to accommodate other cycle times at polishing 1 and polishing 2, enabling connected operation. The process layout for the 10 L scale connected process is given in the following tables.

Column and Run Information – Capture Resin: Protein A

Parameter	Value
Binding capacity [g/L resin]	~30.0
Diameter [cm]	3.2
Bed height [cm]	Column 1: 10.3 Column 2: 10.5
Cross sectional area [cm²]	8.04
Column volume [mL]	Column 1: 83.4 Column 2: 84.0
Loading capacity | cycle [mg on each column]	2,520.0
Volumetric flowrate (non-loading) [mL/min]	21.1
Volumetric flowrate (loading) [mL/min]	7.67
Residence time (non-loading) [min]	4.0
Residence time (loading)* [min]	11.0
Column cycles required [cycles per column]	4
Number of columns	2
Total number of cycles run during 10 L batch [cycles per batch]	8
Asymmetry range (based on manufacturer's range of 0.8 – 1.8)	Observed asymmetry ▪ Column 1: 1.54 ▪ Column 2: 1.11

*Residence time was changed from 4 min to 11 min to accommodate the flow requirements of polishing 2

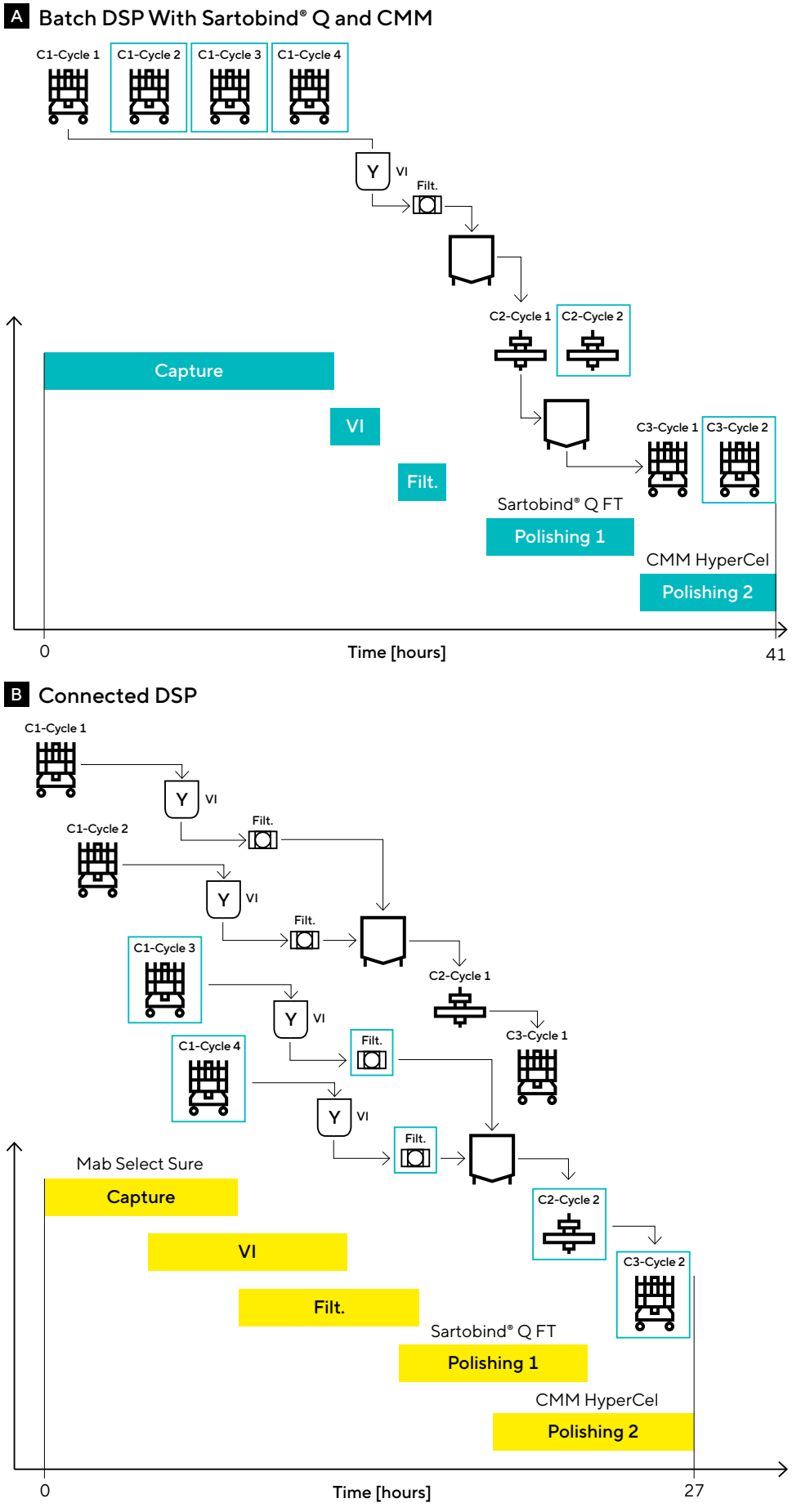
Column and Run Information – Polishing 1 Membrane: Sartobind® Q

Parameter	Value
Loading capacity [g/mL membrane]	2.5
Membrane diameter [cm]	3.3
Membrane height [mm]	4.0
Membrane area [cm²]	36.4
Membrane volume [mL]	2*1.0
Loading capacity | cycle [g | 2 mL]	5
Volumetric flowrate (non-loading) [mL/min]	20
Volumetric flowrate (loading) [mL/min]	10
Residence time (non-loading) [min]	0.10
Residence time (loading) [min]	0.20
Number of cycles	4
Loading density (observed) [g/mL]	~2.2
Pre-filter | in-line used*	Sartopore® 2 XLG; 0.021 m² area

Column and Run Information – Polishing 2 Resin: CMM HyperCel

Parameter	Value
Binding capacity [g/L resin]	~34
Diameter [cm]	4.4
Bed height [cm]	9.3
Cross sectional area [cm²]	15.20
Column volume [mL]	140.58
Loading capacity | cycle [mg]	4779.65
Volumetric flowrate (all phases) [mL/min]	35
Residence time (all phases) [mins]	4
Volumetric flowrate [mL/min]	35
Number of Cycles	4
Asymmetry range (based on manufacturer's range of 0.8 – 1.8)	Observed asymmetry: 1.00

Figure 4: *Comparison of (A) Batch Process Flow vs (B) Connected Process Flow*



Note. Blue boxes indicate where the same consumables were used.

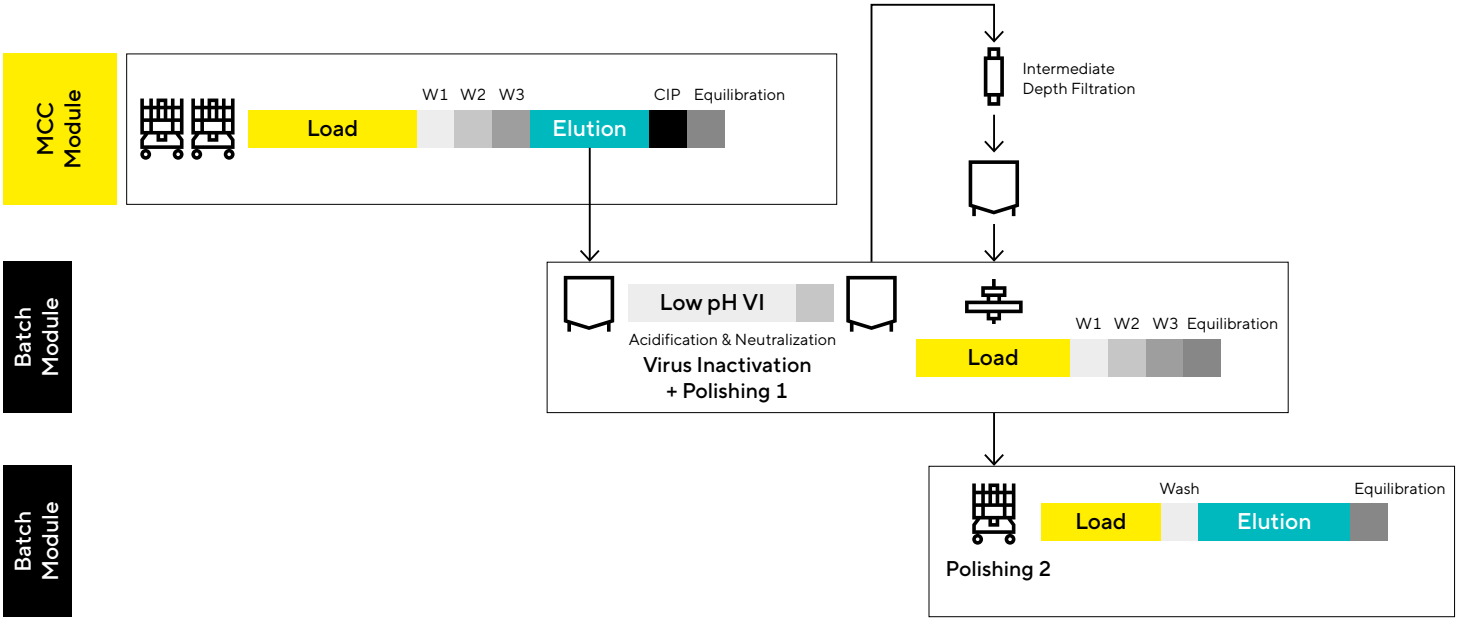
Connected Process – Proof of Concept at 200 L Scale
After the successful execution of the 10 L connected process, the operation was scaled up to 200 L. Cells were cultured in a Biostat® STR 200 Generation 3 bioreactor, and the harvest was clarified through a series of Sartoclear® depth filters (DL90 and DL20) to achieve a titer of 2.8 g/L in 265 L clarified harvest for loading onto the capture column.

As with the 10 L scale process and based on the binding capacity of Protein A resin, the load was divided into eight cycles for loading. For 200 L scale, columns used for capture were scaled up to 2×3.14 L instead of 2×83 mL, 75 mL instead of 2 mL Sartobind® Q membrane was used, and a 5.3 L CMM HyperCel column was used instead of a 140 mL column. The overall scale-up factor for stationary phase consumables was 38x higher than for 10 L scale. The connected process was set up as described for the 10 L, but the large-scale process required additional adjustments:

- A four-module Resolute® BioSC Pilot system with a control module was used, a two column module (MCC module), and two batch modules to run the process from capture to polishing 2. The MCC module was used to run the capture step B-MCC mode with two columns and the two batch modules were used for VI and two polishing steps. A representative recipe diagram is shown in Figure 5.
- Detailed individual recipes were created on the Resolute® BioSC Pilot modules for the designated operations. Handshakes between these recipes from individual modules were incorporated to coordinate the processes for better time synchronization.
- The residence time for the capture step was extended to 14 mins to accommodate the polishing 1 and 2 steps and the intermediate filtration time between the VI and polishing 1 step.
- The extended residence time also provides the additional time required to wash the Resolute® BioSC Pilot batch module flow paths which are used for multiple operations.

The in-process samples were collected at the single-use surge tanks at all steps for every cycle and sent for analysis to assess purity and recovery.

Figure 5: *Representative Recipe Diagram Showing Respective Modules Operating Different Process Operations on the Resolute® BioSC*



Results

We initially performed the connected process at 10 L to assess the performance at lab scale in comparison to the batch processing results from the first application note.[3]

Protein A — Capture
A total of eight cycles were run in parallel batch mode (four on each column) to generate semi-continuous output. The average monomer recovery for the capture step was similar for both connected (94.7%) and batch (91%) modes. The purity obtained was >97%, comparable to the batch process data.

Overall, the following highlights were observed in the B-MCC (two columns) process:

- Protein A resin achieved a binding capacity of 30 mg/mL.
- Elution length was kept to 3 CV, enabling the pH to 3.5 ± 0.2 for VI and fixed volume elution for time scheduling.
- Impurity removal
  - Host cell protein (HCP) reduction: 172,085 to 300 ppm (3-log)
  - Host cell DNA (hcDNA) reduction: 2.07 ng/L to below the limit of quantification (LOQ)

Sartobind® Q — Polishing 1
Sartobind® Q removes traces of process impurities (residual HCP and hcDNA) and also acts as an orthogonal virus removal step.[6] The average monomer recovery and purity obtained for four cycles of this step were found to be 98.57 % and >97%, consistent with the batch process results.

The process highlights were as follows:

- During the connected run, up to 2.5 kg/L of membrane loading was considered based on the batch process run time, productivity, and throughput calculations. This also enabled us to run more cycles on the same Sartobind® Q membrane to assess intra-batch usage.
- Reduced loading on Sartobind® Q enabled smaller Protein A and CMM HyperCel column volumes than if the loading was done at 5 kg/L.
- A constant volume was generated for each cycle due to fixed elution volumes on the capture step.
- The observed output values for flowthrough volume collected, pH and conductivity of flowthrough, and protein concentration in flowthrough were consistent for all cycles.
- Sartobind® Q membrane removed the HCP content from the load sample to less than 20 ppm (1-log reduction) and was consistent for all four cycles. The hcDNA and residual leached Protein A content was below LOQ.

The same Sartobind® Q membranes were used for all four cycles without exceeding pressure limits, highlighting the reusability of the Sartobind® Q membranes for up to four cycles. The pressure for the fourth cycle reached 2.5 bar without a drop in the flowrate (max pressure allowed is 3 bar). However, there was no impact on the quality or recovery. To enhance usability for multiple cycles within a batch, it is advisable to incorporate a 0.45 or 0.2 µm pre-filter before proceeding to the Sartobind® Q step. The data here does not reveal any information on inter-batch usage, as the membrane was not used over multiple batches.

CMM HyperCel — Polishing 2
Each cycle of Sartobind® Q flowthrough was loaded onto the CMM HyperCel column without any buffer adjustment. This approach eliminates additional sample preparation and holding tank steps, reducing processing time. The elution volumes were consistent with the elution volumes of the batch process.

A summary of the connected platform results is given in Figure 6.

Figure 6: *Summary of Connected 10 L Run*

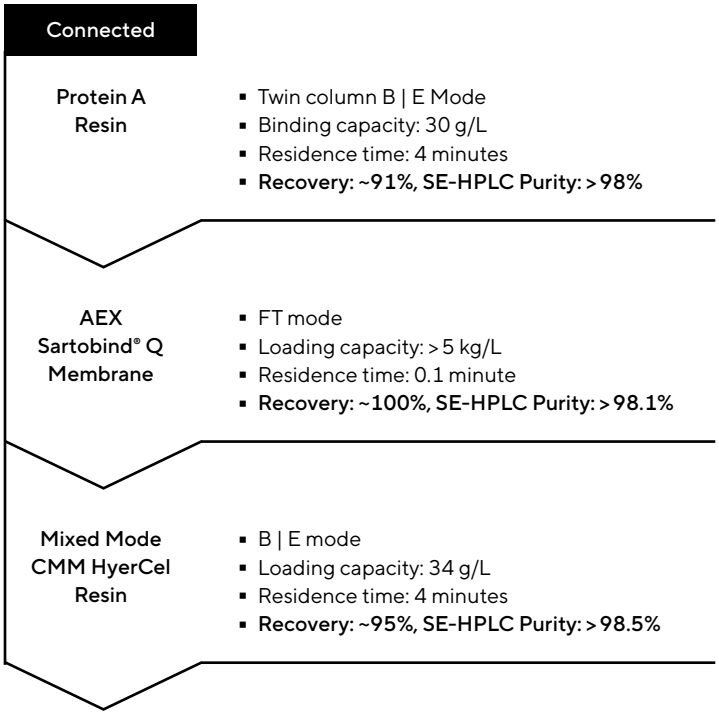


Table 1: *Connected Process Purity Results at 10 L Scale*

Sample Details	% HMW	% Monomer	% LMW
Standard mAb 1 mg/mL	0.3	99.2	0.4
Cellca model mAb	0.8	99.2	ND*

Comparison of 10 L and 200 L Connected Runs

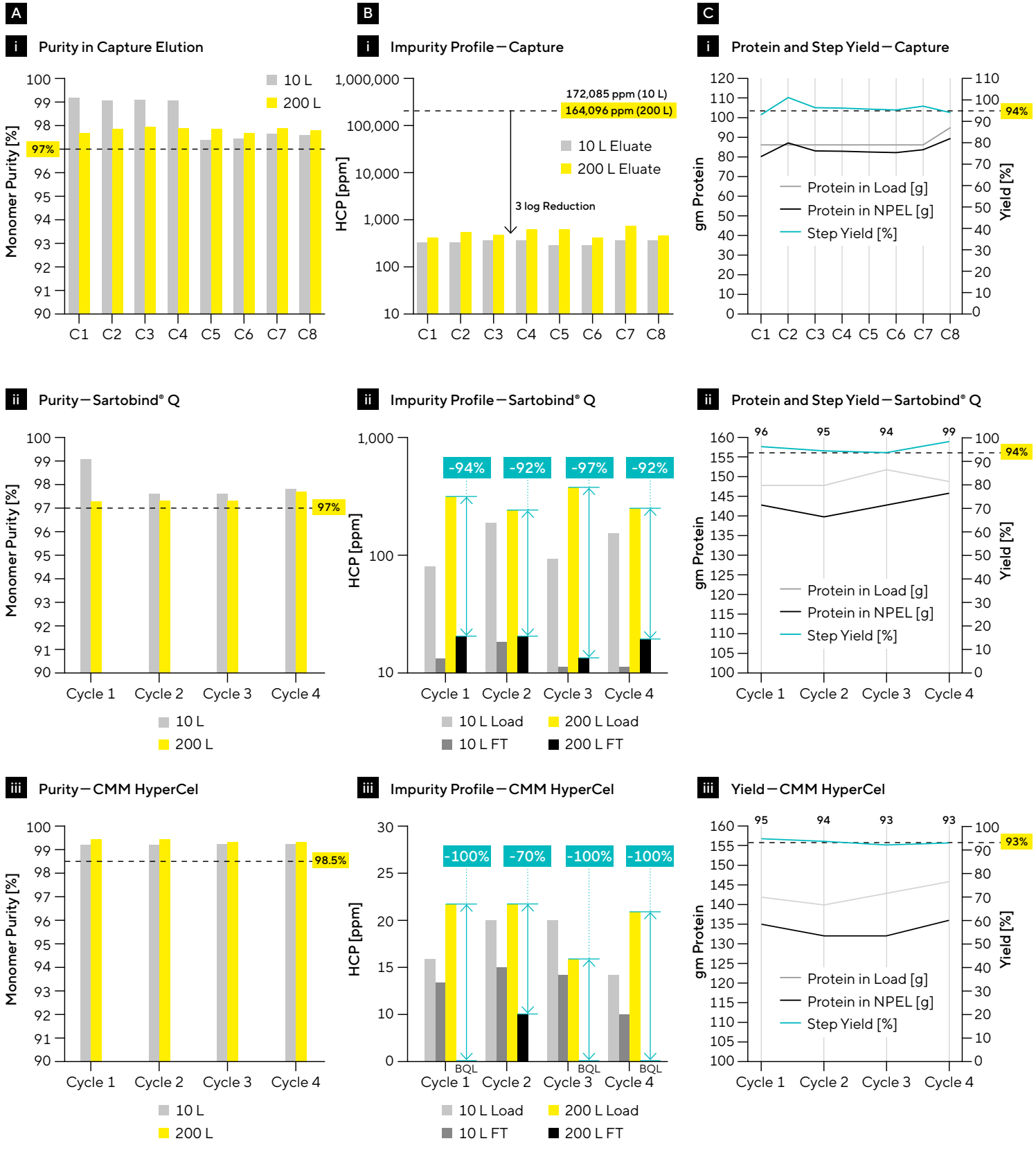
Ensuring proper adaptation of the 10 L connected process and gauging variations resulting from scale up and process adjustments, the process was run at 200 L on the Resolute® BioSC. Figure 7A shows that the achieved purities (% monomer by SE-HPLC) for the three chromatography steps were comparable between the 200 L and 10 L scales. Figure 7B shows the comparison of HCP levels across the three chromatography steps. It should be noted that the intermediate filtration step post virus inactivation and neutralization helped remove HCP (average 50% removal for 4 pools of consecutive cycles) and prepared a cleaner feed for Sartobind Q. Tables 1 and 2 show the values of high molecular weight (% HMW), low molecular weight (% LMW), and % monomer for 10 L scale and 200 L scale respectively. All these figures indicate that the two runs at different scales were comparable to each other in performance when it comes to purity of product and impurity removal. In addition, yield at 200 L was found to meet the 10 L benchmark (Figure 7C). Some additional observations made during the scale-up of the connected run are mentioned below:

- The change in capture residence times from 11 mins to 14 mins did not cause any drop or variance in the performance as higher residence times increase the opportunity for the molecule to bind and should not lead to any losses. Consistent performance was demonstrated in the results obtained on the Resolute® BioSC B-MCC (two columns) process: >97% purity and >95% yield were observed.
- For the VI, the recipe was controlled by the Resolute® BioSC system, and intermediate filtration was carried out manually in coordination with the recipe. Figure 8 shows that the process and quality indicators achieved through an automated recipe on Resolute® BioSC at 200 L scale matched the manual control at 10 L scale, verifying the accuracy of execution by the system. The sizing of the filter and precisely timing the filtration operation were key to saving as much time as possible. No impact on quality was observed due to increased turbidity during neutralization.
- During polishing 1 cycles, the maximum pressure on the pre-filter was < 1 bar, and for the Sartobind® Q filter was <0.7 bar, indicating that the area of the pre-filter and Sartobind® Q sizing for chromatography was well designed for four cycles. As the maximum pressure can go up to 2 bar, these devices can be used for more than four cycles (estimated 8-10 cycles intra-batch usage).
- Polishing 2 elution volumes observed for all cycles were 9-10 CVs, in contrast to 10 L batches, which showed 6-7 CVs. There was no unusual observation during polishing 2, and all the process parameters were well within the acceptance criteria (pH and conductivity). Hence, the increase in CVs may be attributed to a change in scale-related parameters and | or lot-to-lot variability of the resin. The higher number of CVs increased elution volume by 30%, creating a proportionately lower concentration for virus filtration. As the virus filter was already oversized for the volumes estimated, the volume increase did not cause any loading issues on Virosart® Max and HF filters.
- Virus filtration and UF | DF were scaled up and run smoothly. The final product obtained was at 102.1 g/L concentration. These unit operations are not the scope of this application note, so they are not discussed in detail here.
- Process samples were also taken for endotoxin and bioburden assessment, as it was important to demonstrate the safety attributes of the process setups. All the samples were found to comply with the endotoxin limit of <5 EU/mg, and the bioburden was found to be <1 CFU/10 mL.

The summary of results obtained at 200 L scale is shown in Tables 2 and 3.

*ND = not detectable

Figure 7: *Purities, Impurity Profiles, and Yields*



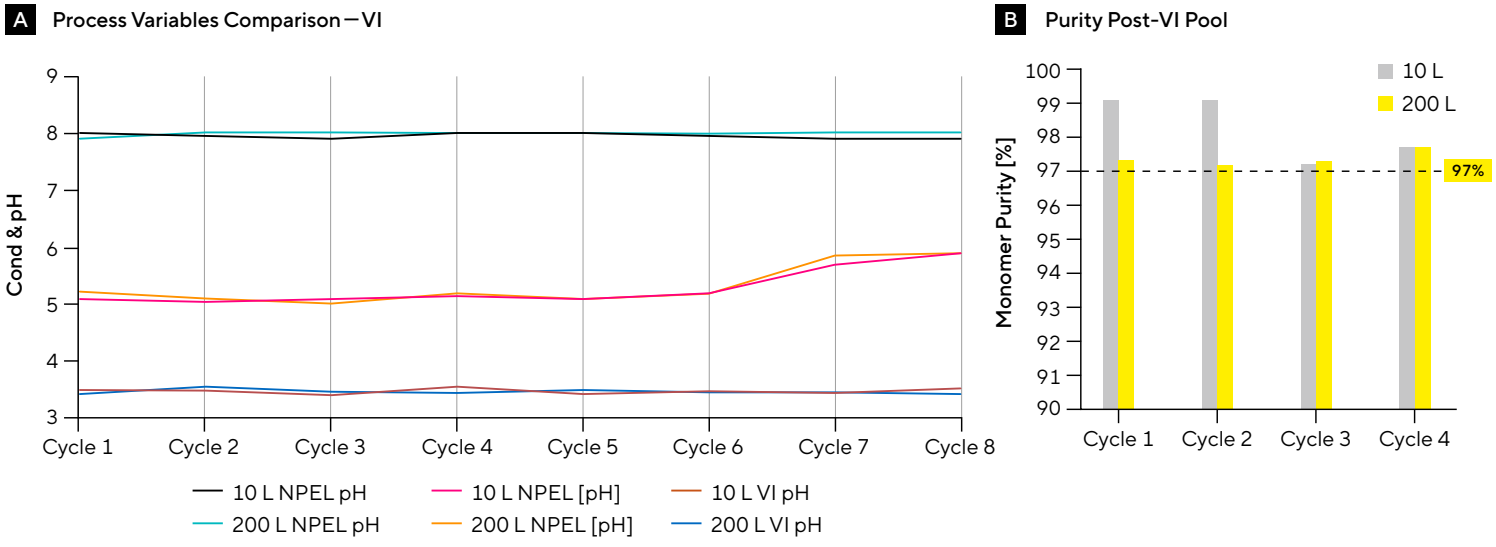
Note. **(A)** Comparison of product purity between 200 and 10 L scale following (i) capture, (ii) polishing 1, and (iii) polishing 2, **(B)** Comparison of impurity profiles between 200 and 10 L scale following (i) capture, (ii) polishing 1, and (iii) polishing 2. (HCP is shown in graphs, hcDNA was 10-20 ng/mL for 200 L and below the LOQ for 10 L, residual Protein A (rProtA) was 5-7 ppm for 200 L and below LOQ for 10 L scale following capture). **(C)** Product yield at 200 L scale following (i) capture, (ii) polishing 1, (iii) polishing 2 for all three chrom steps.

Table 2: *Connected Process Purity Results at 200 L Scale*

Sample Details	% HMW	% Monomer	% LMW
Standard mAb 1 mg/mL	0.5	99.4	0.1
Cycle 1	0.6	99.4	ND*
Cycle 2	0.7	99.3	ND*
Cycle 3	0.7	99.3	ND*
Cycle 4	0.7	99.3	ND*

Note. Compare to Table 1 for 10 L scale

Figure 8: *Process Variables and Purity During Virus Inactivation at 10 L and 200 L Scale*



Note. **(A)** Comparison of the automated regulation of process parameters (pH and conductivity) before and after VI on Resolute® BioSC to manual operation at 10 L scale, **(B)** Comparison of post-VI purity between 10 L and 200 L scales

Table 3: *Analysis of Recovery, Purity, Bioburden, and Endotoxins Following Each Step of the 200 L Run*

Process Step	Recovery [%]	Purity [%]	Bioburden [CFU/10 mL]**	Endotoxin [EU/mg]**
Capture	95	>97.5	<1	<5
VI + filtration	94	>97.2	<1	<5
Polishing 1 (Sartobind® Q)	96	>97.3	<1	<5
Polishing 2 (CMM HyperCel)	94	>99.3	<1	<5
Virus filtration (Virosart® Max and Virosart® HF (20 nm))	92	>99.3	<1	<5
UF | DF (Hydrosart® 30 kDa)	100	>97.5*	<1	<5
Sterile filtration (Sartopore® Platinum)	100	>98.8	<1	<5

*Increase in HMW due to high concentration >140 mg/mL
**CFU-colony forming unit, EU-Endotoxin unit

*ND = not detectable

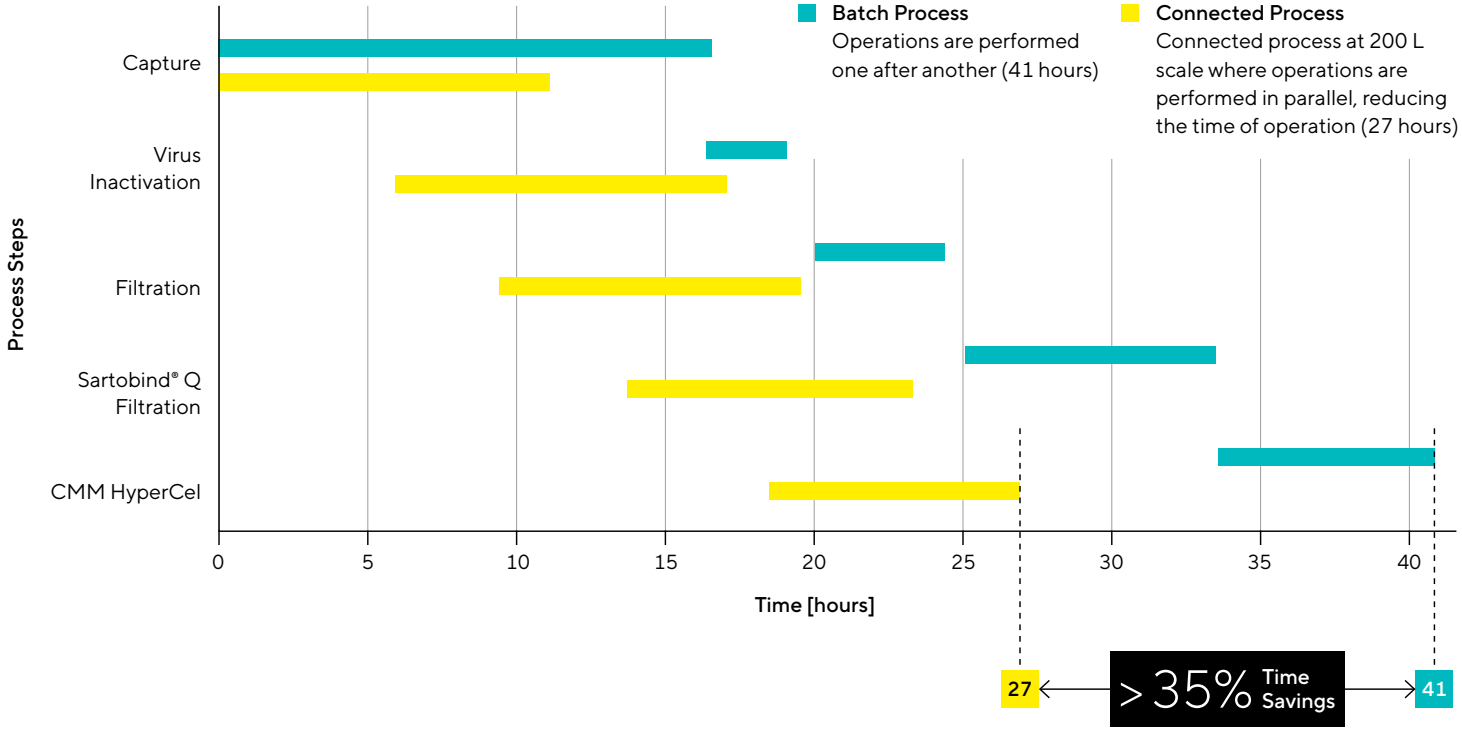
Discussion

Process Outlook

The downstream process run in connected mode at 10 L scale showed a consistent performance to a batch mode over eight cycles of capture followed by four cycles of polishing 1 on Sartobind® Q and polishing 2 on CMM HyperCel. The 200 L scale results were consistent with the 10 L scale, with an overall downstream process recovery of 72%, demonstrating successful scale-up. As shown in Table 3, endotoxin and bioburden were found within acceptable limits at 200 L scale, showing the cleaning efficiency of the Resolute® BioSC system.

Although connected processes bring massive advantages, an interruption or unplanned hold in one of the steps during the process run would result in the collapse of the process flow and synchronization. Hence, an adjustment was made in the loading time duration of the capture step to enable synchronization of overall process steps. Instead of including a hold in the process, our recommendation is to look critically at the step times and identify the rate-limiting step in the whole process. Once found, the residence times of the rest of the processes can be adjusted to give enough time for the slowest step to finish and enable continuos operation.

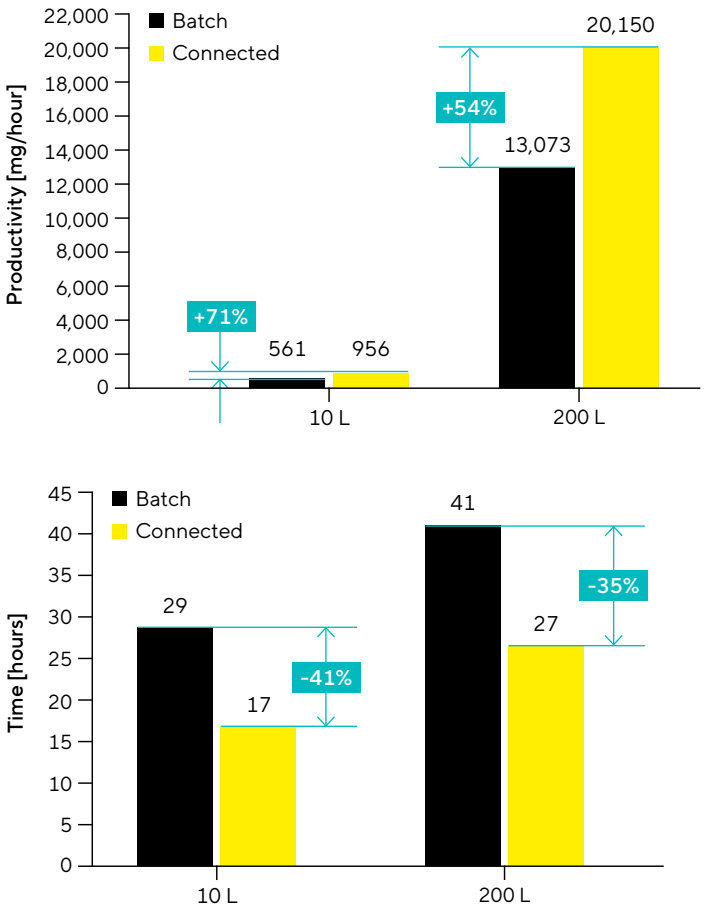
Figure 9: *Comparison of Batch and Connected Process Layouts and Process Time*



Figures 9 and 10 compare the time of processing for batch vs connected mode and show that running the process in connected mode saves significant time (40% and ~35% at 10 L and 200 L pilot scales, respectively). These time savings directly improved the productivity of the connected process by 71% at lab scale and 54% at 200 L scale. As such, any campaign with a connected process can be finished faster than the batch mode operation, improving efficiency and facility capacity.

Even if the process is well organized, marrying different systems with handshakes in between to implement a connected process in an automated way is a cumbersome task. At 10 L scale, we ran the process manually as multiple systems were used and centralised automation was not possible for all these steps. For 200 L scale, the connected process was efficiently carried out on the Resolute® BioSC without any inconsistencies in the output. Recipes for different modules on Resolute® BioSC and the handshake between them allowed seamless synchronization between individual unit operations, resulting in robust process control throughout the process.

Figure 10: *Productivity and Time Comparison at 10 L and 200 L Pilot Scales for Batch and Connected Operations*



Another advantage that the Resolute® BioSC system brings is footprint savings. It is common to have dedicated liquid handling systems for different unit operations in the manufacturing plants. For a mAb process, the footprint is generally occupied by three chromatography systems for the capture, polishing 1 and polishing 2, and the pumping skid for VI. The Resolute® BioSC carries out four different unit operations on a single system, leading to a <2x footprint reduction. From a facility design perspective, this saves significant costs due to reduced area and HVAC requirements.

In summary, the takeaway messages from this work are as follows:

- Process intensification at the unit operation level and further conversion into connected process can bring multiple advantages to the manufacturers.
- Successful recipe creation, handshakes, centralized automation, and automated monitoring of process variables between the modules of Resolute® BioSC ensured the accurate execution of the process without a single process synchronization issue.
- Downstream processing time per batch was reduced by >35% for 200 L scale, leading to 54% higher cumulative productivity than a pilot-scale batch process. Reduced downstream process time can also increase facility capacity by 16% due to faster campaign times.
- Process modifications are needed while scaling up the processes from lab to production scale in a connected mode; the focus should be on solving time management challenges to synchronize the operations while meticulously accounting for flexibility and possible failures without losing productivity.
- A connected process can also be achieved by having large columns and removing intermediate pooling. The process scheme designed here has smaller columns and more cycles for pooling in preparation for the polishing steps. This reduces the resin consumption per batch and drives footprint savings due to smaller column volumes.
- The use of the Resolute® BioSC reduced process footprint by >2x when compared to a traditional connected downstream setup. This reduces both capital and operational expenditure.
- Closed loop operation on the Resolute® BioSC for four operations by a centralized recipe (as opposed to handling them on four different systems) reduced overall manual intervention. This helps lower bioburden and reduces the risk of contamination due to handling issues.

Conclusion

The application note shows that integrating intensification principles into existing processes is a promising approach for improving productivity and efficiency in bioprocesses. Even though the choice of consumables can help connect the processes efficiently, as previously shown,[3] the real secret for success lies in the process integration by adjustments in the process at lab scale followed by transfer to a larger scale. At a large scale, it is difficult for multiple systems to work together to execute the process in connected mode. However, this challenge can be successfully solved by Resolute® BioSC, which enables efficient integration with capable hardware and automation.

In conclusion, intensifying processes by connected processing is a powerful tool for achieving higher efficiency. The Resolute® BioSC delivers flexible and efficient ways to make this possible.

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