Streamlining Purification

Proof of Concept for a Fully Connected Downstream Process

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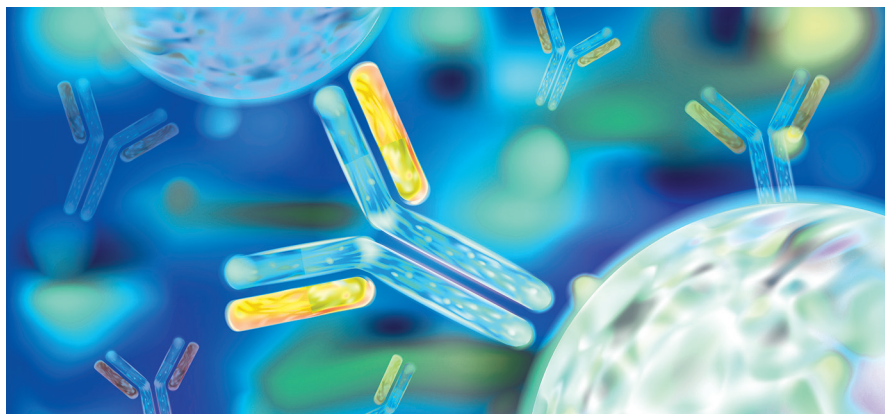
Protein-based therapeutics remain the largest biopharmaceutical market — dominated mainly by monoclonal antibodies (mAbs) and their derivatives (**1**). Through advancing technologies over the past 10 years, companies making such products have experienced a shift in the challenges involved, with mAbs now produced at high expression titers (≥5 g/L), resulting in constraints on the downstream side. Complex engineered constructs such as fusion proteins, bispecifics, and trispecifics usually are produced at lower titers; however, they often are less stable and/or more prone to aggregation than traditional mAb molecules. With large numbers of therapeutic candidates having passed through early clinical trials in the past decade, demand is increasing for production of materials for phase 3 studies and commercial needs.

Those combined phenomena are driving a trend in process intensification and (semi)continuous manufacturing, which have been discussed since the early 2000s but are now finally gaining traction. A number of biomanufacturers are implementing such technologies to help solve challenges brought on by high expression titers, unstable molecules, and growing demands for manufacturing capacity (**2, 3**).

Process intensification encompasses an array of solutions that can make bioprocessing faster, cheaper, and more sustainable than is possible with legacy processes. In downstream processing, such technologies include sequential and batch multicolumn chromatography (S-MCC and B-MCC), rapid-cycling chromatography (RCC), single-pass tangential-flow filtration (SPTFF), and connected processes. Table 1 lists key process intensification technologies and summarizes their expected benefits.



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Among those strategies, RCC and S-MCC especially enable strong productivity increases at the step level. However, the real bottleneck of the downstream process lies in the batch approach itself, which requires that each step begins after the previous one ends. One way to increase the efficiency of biomanufacturing would be the connection of process steps combined with use of membrane chromatography (**4–6**). Below, we showcase a proof-of-concept study of a connected, membrane-based downstream process that increases productivity at the facility level.

Material and Methods

Process Design and Buffer Screening: We defined conditions for each chromatography unit operation through laboratory-scale tests using an Äkta pure 25 purification system (Cytiva). Consumables included Sartobind nano membranes (1.2 mL for Sartobind Rapid A with 4-mm bed height, 1 mL for Sartobind S and STIC with 4-mm bed heights, all from Sartorius).

Proof of Concept for the Connected Membrane Process: For testing purposes, we used a standard immunoglobulin G class 1 (IgG1) antibody at a harvest concentration of 3.0 g/L with 6.3% high–molecular-weight (HMW) content.

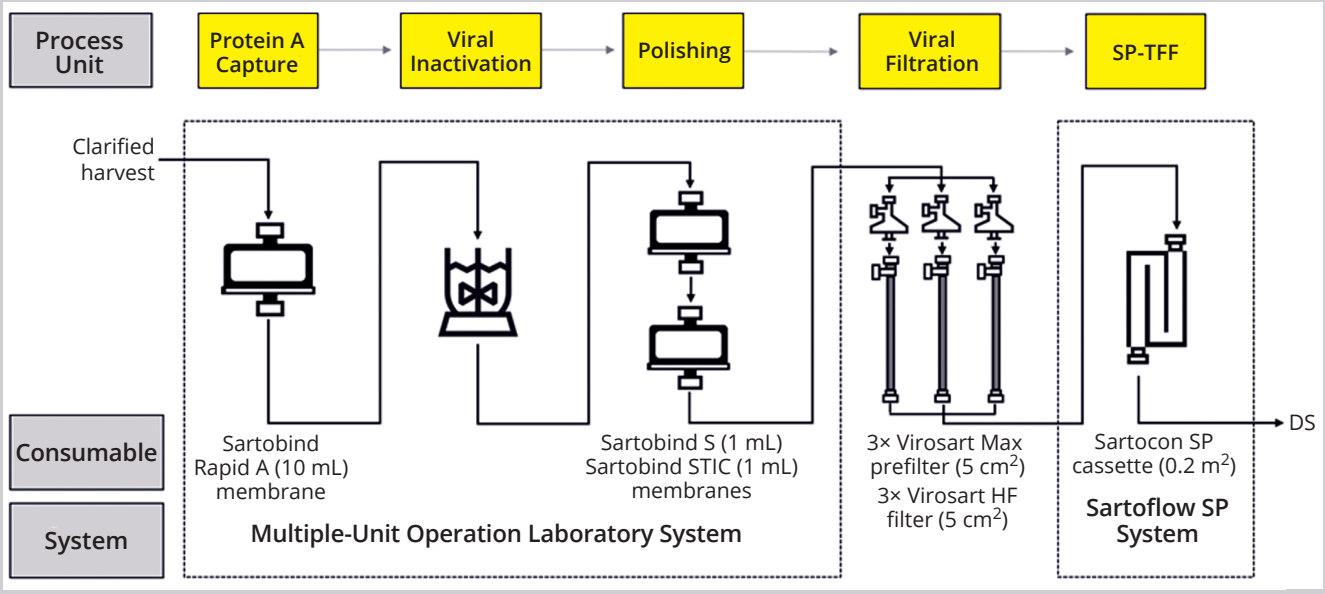
For the connected process experiment, we used a custom Resolute BioSC Lab chromatography system (Sartorius) designed to operate three different steps (three modules), adding a manifold to the system outlet for virus filtration and a Sartoflow SP single-pass tangential-flow filtration (SPTFF) system from Sartorius for final concentration and buffer exchange. Figure 1 shows the system and consumable setup; Figure 2 shows how all steps were orchestrated.

All consumables described herein come from Sartorius. We used a

Table 1: Summary of downstream process-intensification technologies and their benefits

Technology	Principle	Benefits
Batch multicolumn chromatography (B-MCC)	Scaling out: Use two or three smaller columns rather than one large column.	Relative ease of handling; enables continuous loading and processing
Sequential multicolumn chromatography (S-MCC)	Using multiple columns in parallel, including two in series, allows overloading of a column without yield loss.	Enables continuous loading; reduces resin volume (by up to 80%); lowers buffer consumption and potentially processing time
Membrane-based polishing	Use convective sorbents to perform flow-through steps (with or without cycling).	Eliminates packing efforts, column capital expenditure, and maintenance; speeds up both processing and validation; reduces consumables sizing and buffer consumption, facilitates handling, and lowers costs; reduces bioburden risks
Rapid-cycling chromatography (RCC)	Use smaller chromatography matrices with convective mass transfer to make >40 cycles within a batch.	Eliminates packing efforts, column capital expenditure, and maintenance; speeds up both processing and validation; reduces consumable sizing and buffer consumption, facilitates handling and lowers costs; reduces bioburden risks
Single-pass tangential-flow filtration (SPTFF)	Users can change buffer and/or concentrate the product without recirculation in a tank.	Enables buffer exchange and/or product concentration between steps
Connected processing	Orchestrate operations to process material through each step as soon as possible (before the previous one finishes). This can combine a traditional batch approach with RCC or MCC, cutting one batch into small subpools that get processed by fully automated cycling chromatography media.	Uses one central automation platform to reduce the number of systems, column sizes, and intermediate tank sizes, as well as their related footprints and costs; reduces processing time by up to 40% and consequently increases the number of batches that can be processed per year

Figure 1: System and consumables setup; DS = drug substance, SPTFF = single-pass tangential-flow filtration



Sartobind Rapid A Mini membrane (10 mL) for mAb capture on the first module. Two Rapid A elutions were pooled and sent to the second module for automatic virus inactivation (VI). In that process, the system automatically lowers the solution pH to a target of 3.5 using 5 M acetic acid (AcOH), performs 30 minutes incubation, and then neutralizes the solution to a target of 7.0 using 2 M tris. Meanwhile, protein A elution continues in a dedicated tank.

Post-VI product was pumped into the third module for a double flow-through combining Sartobind S and STIC Nano membranes (1 mL with a 4-mm bed height) into a single step. Table 2 describes detailed recipes for the chromatography steps based on screening studies that we had performed up front.

For nanofiltration, we directly combined three Virosart Max prefilters (5 cm²) with three Virosart HF filters (5 cm²) using a filtration manifold. Filters were vented and flushed with buffer according to manufacturer recommendations.

Finally, we used a Sartoflow SP filtration system with a Sartocon SP cassette (0.2 m²) for SPTFF. The cassette was equilibrated in diafiltration buffer before product loading. First, product was recirculated on the SPTFF cassette for 10 minutes to build a polarization gel so that we could identify working parameters. Because the cassette is

Figure 2: Recipe scheme for detailed orchestration of all steps

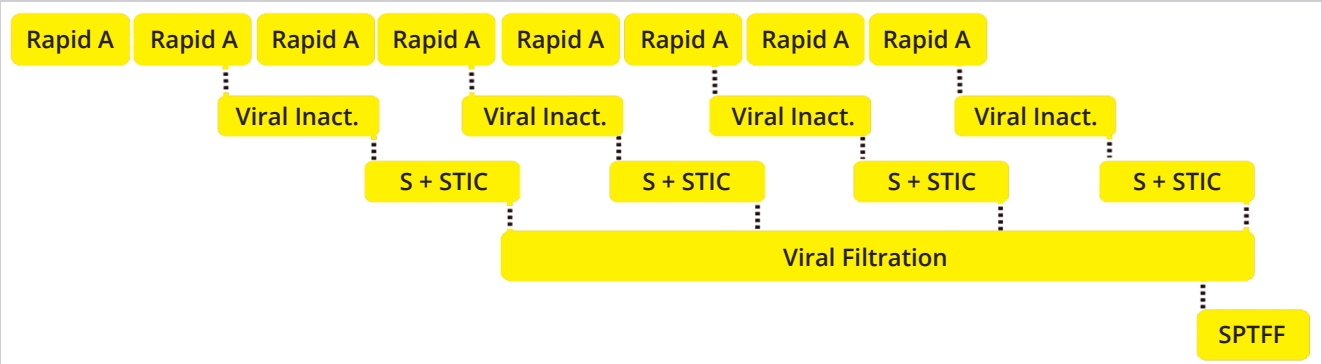


Table 2: Detailed recipe for chromatography steps; flow rate on the Sartobind Rapid A membrane was limited by pump capability of the custom Resolute BioSC Lab control system; mAb = monoclonal antibody, MV = membrane volume, NA = not applicable.

	Sartobind Rapid A Mini Membrane (10 mL, 2.8 MV/min)	Sartobind S and STIC Membranes (1 mL, 5 MV/min for both types)
Equilibration	2 MV of 50 mM tris and 20 mM NaCl at pH 7.5	70 MV of 50 mM tris at pH 7.0
Load	10 MV of mAb solution at 3.0 g/L	110 MV from previous step
Wash	2 MV of 50 mM tris and 20 mM NaCl at pH 7.5 + 4 MV of 50 mM tris and 1 M NaCl at pH 7.5 + 10 MV of 50 mM tris and 20 mM NaCl at pH 7.5	50 MV of 50 mM tris at pH 7.0
Elution (with peak cut)	12 MV of 25 mM NaOAc at pH 3.7	NA
Strip	3 MV of 0.5 M AcOH	35 MV of 50 mM tris and 1 M NaCl at pH 7.5
Equilibration	18 MV of 50mM tris and 20mM NaCl at pH 7.5	35 MV of 50 mM tris at pH 7.0
Clean in place	Only the day before the test using 0.2 M NaOH with a 10-minute contact time	35 MV of 0.2 M NaOH

relatively large (no smaller model was available), we initiated SPTFF only toward the end of the connected process sequence when a sufficient pool of Sartobind eluate was gathered in the feed vessel for the Sartocon SP cassette. That was a design limitation of the small-scale skids available. At larger scales, the goal would be to start SPTFF once enough product has gone through the virus-retentive filters (ideally, soon after product exits the filter).

Analytics: Only the input material (clarified bulk harvest) and process-output material (post SPTFF) were tested by our quality control (QC) group. No intermediate samples were taken between process steps, with one exception: Product exiting the polishing train was tested for mAb concentration to confirm a concentration factor for the SPTFF step. We tested mAb concentration using size-exclusion high-performance liquid chromatography (SEC-HPLC) and determined total host-cell protein (HCP) content using a disc-based immunoassay with the Gyrolab CHO-HCP E3G kit on a Gyrolab xP workstation (all from Gyros Protein Technologies). HMW content was assessed using SEC-HPLC.

Results and Discussion

Connected Process Execution: The test was successful. The custom Resolute BioSC Lab system carried out all chromatography steps and tank-based VI. Including all subcycles and steps (Figure 2), four cycles were completed within five hours.

We did encounter a minor issue: Flow-through material exiting the double polishing step at 10 mL/min ran through the virus-retentive filters with a rising pressure — up to 3.5 bar in the first cycle — which caused leaks from the single-use tubing. With the Virosart Max and HF filters positioned on the polishing outlet, they were loaded directly by the chromatography system pumps, so the pressure on the Sartobind S + STIC membranes went higher than 4 bar, which is the maximum pressure allowed for those capsules.

Consequently, after the first cycle's completion, we decided to change the nanofiltration installation by incorporating an independent pump to prevent backpressure on the polishing operation. That pump was unable to deliver flow rates <10 mL/min, however, so we could not carry on using the filter train as set up. We believe that using a smaller independent pump would solve this problem easily in the future. Our experiment highlighted the benefits of using a constant flow instead of connecting a virus-retentive filter directly at the polishing outlet. Therefore, we do not consider this event to be an indication that the connected process would not work, only that a minor adjustment would be required.

Once a sufficient pool of polished product was collected, the SPTFF started. After testing various settings, we targeted a concentration factor of 5.5. The transmembrane pressure (TMP) remained stable at 0.2 bar.

Simultaneously, the Sartocon SP cassette also provided in-line diafiltration of the feed with three diavolumes. The system automatically

We have shown that a **FULLY CONNECTED** membrane-based process can produce purified product for clinical trials.

Table 3: Analytical results; HCP = host-cell protein, HMW = high–molecular-weight species, mAb = monoclonal antibody, SPTFF = single-pass tangential-flow filtration

Fraction	mAb Titer	HMW	HCP	Yield
Load Rapid A (clarified bulk harvest)	3.0 g/L	6.3%	64,315 ppm	—
SPTFF pool (including buffer flushes)	9.8 g/L	4.0%	35 ppm	67%

Table 4: Scale-up projections for steps of a 200-L clinical batch; SPTFF = single-pass tangential-flow filtration

		Time	
Step Consumables	Cycles	Step Starts	Step Ends
Capture: Sartobind Rapid A membrane (200 mL)	90	0 hours	14.5 hours
Virus Inactivation: Flexsafe bag in Pro Mixer system (20 L)	15	1.5 hours	15.6 hours
Polishing: Sartobind S membrane (75 mL) + Sartobind STIC membrane (75 mL)	15	2.1 hours	15.8 hours
Nanofiltration: Virosart Max filter (0.2 m²) + Virosart HF filter (0.2 m²)	1	2.3 hours	16.0 hours
SPTFF: Sartocon SP filter (1.7 m²)	1	2.5 hours	16.2 hours

measured conductivities on the feed, buffer, and retentate lines. Input conductivity of the feed was 10.0 mS/cm, and it was diafiltered against phosphate-buffered saline (PBS) with a conductivity of 16.5 mS/cm. The retentate conductivity value stabilized at 15.2 mS/cm, giving an estimated diafiltration efficiency of 80%. Without further optimization, that value is admittedly below the expected performance of a final TFF step used for formulation of drug substance. However, it is an interesting performance for a continuous TFF step that could run for long periods as part of a connected process. The final buffer adjustment for formulation could be improved either by optimizing the method further (by using two SPTFF cassettes in series) or by adding a final standard TFF to concentrate product further at the end of the process. Sizing of that extra step would be greatly reduced by the preconcentration and buffer exchange already performed by the SPTFF.

In total, the system operated in single-pass mode for 31 minutes. The Sartocon SP cassette processed an incoming feed titrated at 2.3 g/L. After pooling with a buffer flush, the system recovered 176 mL of mAb concentrated at 9.0 g/L from the retentate of the cassette. Therefore, the observed in-line concentration factor was 3.9. So this entire connected process allowed us to produce 1.6 g of drug substance, starting from the clarified harvest, within 5 hours and 20 minutes. That translates to an overall process performance of 25 g/h/L membrane, including VI, virus filtration, and SPTFF. Note that flow rates were nonoptimal, especially for the Rapid A membrane, due to system limitations, so this value could be increased further. The setup outperforms the standard resin-based process for this mAb, which culminates at 2.5 g/h/L resin.

Analytical Results: Table 3 gives our analytical results. Note that those data match these targets for a phase 1 process: <5% of HMW species in pool SPTFF and HCP levels of <100 ppm. Also, the HMW concentration in the load was high only because the product had been stored for some time; the purity would be better in real process conditions. Adding a depth filter after VI could provide further purity regarding HMWs and HCPs.

Considering the results above, a sample taken during the third cycle after the polishing steps showed a total yield of 78% for all chromatography steps and VI combined. That result is comparable to the batch process that this continuous setup would replace. However, a yield gap manifests with the SPTFF, which reduced that total to 67%. We needed a high buffer flush to recover the mAb, given the large size of the SPTFF cassette. As a consequence, it is likely that some product remained present on the membrane. Further time spent on process optimization using more adequately sized SPTFF consumables would increase both the concentration and yield further. Thus, we consider our unoptimized test results to be very promising.

Large-Scale Implementation: Based on the small-scale process in this study, we calculated process projections for a clinical batch of 200 L at 3 g/L. The entire batch could be processed within two work shifts using a Resolute BioSC Pilot system with a 200-mL Rapid A capsule together with Sartobind S and STIC capsules of 75 mL connected. Rapid A elutions would be pooled for VI, and each post-VI product would correspond to a polishing cycle.

The virus filter size would require confirmation through a sizing study. Based on standard performance and available filter sizes, however, the Virosart HF filter (0.2 m²) would be a good fit with enough of a safety margin. As mentioned above, that filter should be operated by an independent pump. Finally, the SPTFF

Ultimately, this connected process strategy should generate **DRUG PRODUCT** from clarified harvest within a day, using only very small and relatively inexpensive consumables.

could be carried out with a 1.7-m² cassette running continuously. Certainly, further optimization would be required; in our experiment, we were limited by low flow of product exiting the polishing train relative to the cassette that we used for SPTFF.

Ultimately, this connected process strategy should generate drug product from clarified harvest within a day, using only very small and relatively inexpensive consumables. Indeed, the specific overall productivity for the entire chromatography train would be 72 g/h/L at a cost of 35 €/g (about US$37/g) of mAb produced (considering only the chromatography membranes for a single-use process). This process outperforms not only the current resin-based batch process, but even its connected version. Indeed, connecting the three current resins using the same system would result in costs of €65/g ($68/g) to produce three clinical batches. The productivity of this connected, resin-based process would be 2.5 g/L/h, as mentioned above (29-fold lower than with membranes).

Conclusion

This first proof of concept for a fully connected downstream process — including VI, virus-retentive filtration, and SPTFF — has been a success. For follow-on studies, minor design adjustments would be required to enable full inclusion of the virus-retentive filtration step and improve the SPTFF performance. However, we have shown that a fully connected membrane-based process can produce purified product for clinical trials. Membranes enable purification of large amounts of product with small consumables. They are a perfect fit with connected skids such as the Resolute BioSC system for leveraging a rapid cycling approach, not only for capture, but also for the polishing — which substantially reduces overall costs.

The footprint of the entire process is reduced compared with that of a batch operation. First, only one system (rather than two or three) can be used to control all the chromatography steps. Second, smaller chromatography consumables can be applied, with an entire 200-L harvest processed using only 0.35 L of membrane. Finally, the intermediate tanks between steps and for VI are also reduced in size. In this case, just four mixing tanks of 10–20 L would be required for our 200-L process. This connected, membrane-based downstream process therefore offers major advantages first for production of clinical materials and later for commercial manufacturing.

References

1 Grilo AL, Mantalaris A. The Increasingly Human and Profitable Monoclonal Antibody Market. *Trends Biotechnol.* 37, 2019: 9–16; https://doi.org/10.1016/j.tibtech.2018.05.014.

2 Warikoo V, et al. Integrated Continuous Production of Recombinant Therapeutic Proteins. *Biotechnol. Bioeng.* 109, 2012: 3018–3029; https://doi.org/10.1002/bit.24584.

3 Coolbaugh MJ, et al. Pilot-Scale Demonstration of an End-to-End Integrated and Continuous Biomanufacturing Process. *Biotechnol. Bioeng.* 118(9) 2021: 3287–3301; https://doi.org/10.1002/bit.27670.

4 Schmitz F, et al. Integrated Double Flow-Through Purification of Monoclonal Antibodies Using Membrane Adsorbers and Single-Pass Tangential Flow Filtration. *Biochem. Eng. J.* 195, March 2023; http://dx.doi.org/10.1016/j.bej.2023.108913.

5 Mothes B, et al. Accelerated, Seamless Antibody Purification Process Intensification with Continuous Disposable Technology. *BioProcess Int.* 14(5) 2016: 54–58; https://www.bioprocessintl.com/single-use/accelerated-seamless-antibody-purification-process-intensification-with-continuous-disposable-technology.

6 Pasquier V, et al. Assessment of Membrane-Based Downstream Purification Processes As a Replacement to Traditional Resin Bead for Monoclonal Antibody Purification. *Biotechnol. Progr.* 3508, September 2024; https://doi.org/10.1002/btpr.3508.

Further Reading

Re-Imagine Chromatography: A Stepwise Approach to Downstream Process Intensification (white paper). *BioProcess Int*. 16 April 2024; https://www.bioprocessintl.com/sponsored-content/re-imagine-chromatography-a-stepwise-approach-to-downstream-process-intensification.

McLaughlin K, Nestola P, Rousset F. Re-Imagining Chromatography: Navigating the Path to Downstream Intensification. *BioProcess Int.* 21(10) 2023: 44–46; https://www.bioprocessintl.com/chromatography/re-imagining-chromatography-navigating-the-path-to-downstream-intensification.

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