Simplifying Progress

Re-Imagining Chromatography

Building Efficient, Connected Downstream Processes

November, 2023 | Priyanka Gupta, Katy McLaughlin, Ganesh Kumar, Sanket Jadhav

Keywords or Phrases:
mAbs, Intensified chromatography, Process intensification, Downstream process, Purification

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Introduction

Process intensification strategies represent an excellent opportunity for improving overall efficiency within the biopharmaceutical industry. Significant progress toward process intensification has been made for well-established modalities such as monoclonal antibodies (mAbs), fusion proteins, and bi- | multi-specifics. The industry is also beginning to explore process intensification in newer modalities such as adeno-associated viruses (AAVs).

Most of these advances and process adaptations have been applied upstream, where the industry has many options for adopting intensification, from simple (N-1) perfusion to a steady-state perfusion process in the production (N) bioreactor. However, downstream processes have not kept up with these advances, so while upstream process intensification strategies have greatly improved productivity, the industry faces bottlenecks when purifying the feeds with increased titers.

A recent industry survey reported that the top bottlenecks biomanufacturers encounter during downstream processing are clarification, lower productivity and high costs of Protein A resins for mAb capture, productivity and buffer requirement constraints posed by chromatography resins | columns, and lack of efficient equipment platforms that can be connected to upstream to maintain and improve productivity and footprint to process all the downstream steps.[1] In fact, when asked, "Which new downstream purification (DSP) technologies are you actively considering to address bottlenecks and problems?" the top response (37.7% of respondents) was adopting continuous (or connected) purification systems.[1]

Intensified Downstream Processing Challenges

Clearly, the industry is looking for solutions to connect upstream (whether intensified or fed-batch) and downstream process steps to leverage and maintain increased productivity while reducing cost and facility footprint.

Though there have been efforts made in developing scaled-down models connecting upstream bioreactor with the capture and subsequent chromatography steps, scale-up | GMP-scale adoption has been hindered by the effort, cost, and time required to integrate and connect existing 'islands' of downstream systems to replicate the scale-down models.

Moreover, traditional equipment and process platforms are typically not equipped to handle connected | continuous downstream processes; they are often large, fixed, and inflexible, meaning they cannot accommodate different titers | volumes, products, unit operations, and processing durations.

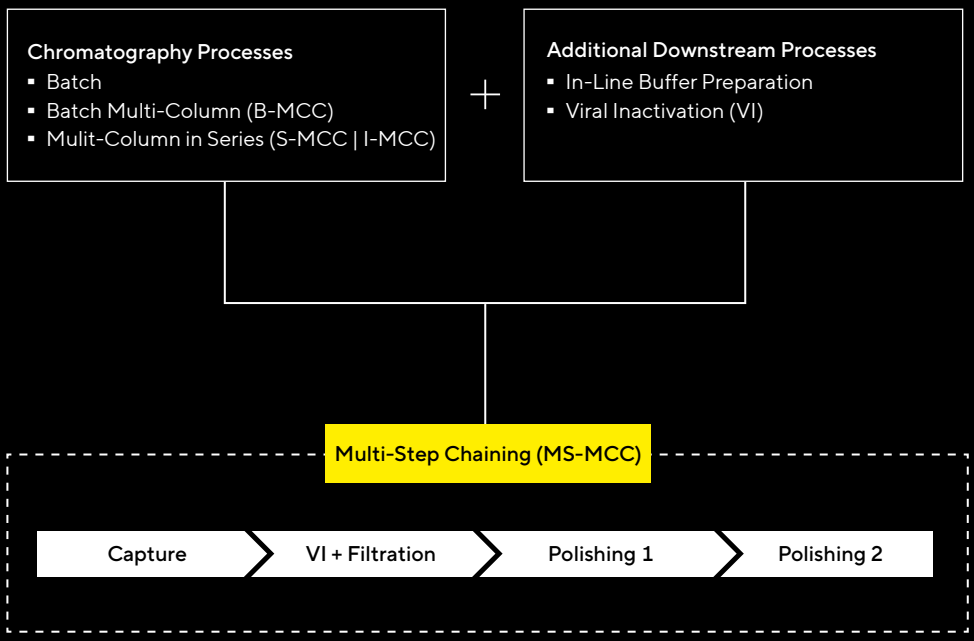
Re-Imagining Chromatography With a Multi-Step Connected Purification System

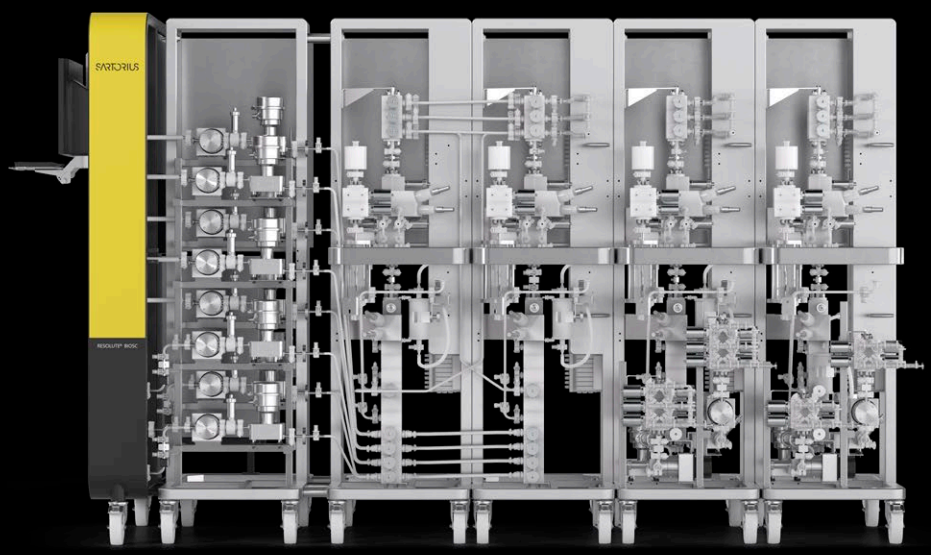
To leverage the benefits of downstream process intensification, a downstream system must:

- Process the product in <24 – 36 hours (if the upstream is a fed-batch | intensified fed-batch process) or up to 8 weeks (if the upstream is a perfusion process)
- Connect capture chromatography with virus inactivation, aggregate removal | intermediate filtration, polishing chromatography (optionally: virus filtration and ultrafiltration | diafiltration)
- Enable flexibility and rapid change-overs
- Support modularity, multi-functionality, and low footprint
- Facilitate seamless interconnectivity
- Allow in-line dilution (with buffers outside the cleanroom connected through a wall)
- Remain (functionally) closed
- Be capable of operating in a ballroom | dancefloor concept

These requirements can be enabled by the Resolute® BioSC (Figure 1).

Figure 1: *Overview of the Resolute® BioSC Platform for Intensified Downstream Processing*





Enzene Bioscience Case Study

To demonstrate the potential ease of introducing process intensification into your process, we present a simple, gradual approach for connecting downstream unit operations.

We worked in collaboration with Enzene Biosciences to implement a new intensified process platform in a connected mode with the Resolute® BioSC. The aim was to reduce overall downstream processing time, creating further opportunities for even higher productivity at reduced footprint.

The following case study shows the successful application of a gradual approach to downstream mAb process intensification, starting by increasing the productivity of unit operations followed by linking them to create a connected process.

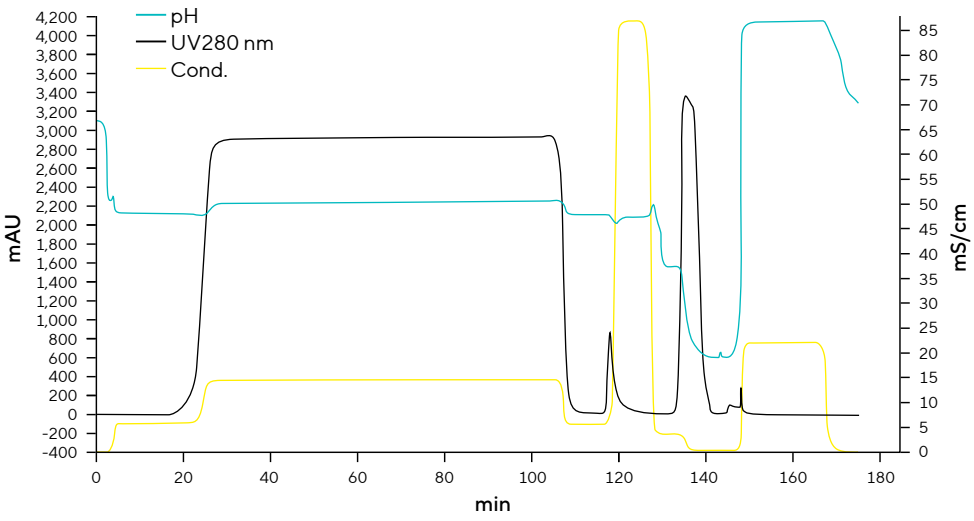
Aims and Approach

When intensifying the downstream process at Enzene, our objectives were to increase productivity, reduce buffer consumption, decrease foot-print, and eliminate intermediate buffer adjustment between the polishing chromatography steps.

We took a three-step approach: (1) optimizing individual unit operations in batch mode, (2) connecting the process steps to generate a proof-of-concept at 10 L scale, and (3) scaling up to 200 L to implement the DSP on the Resolute® BioSC.

Figure 2: *Process Chromatogram of B-MCC (Two Columns) Capture Chromatography Using Protein A Resin*

A

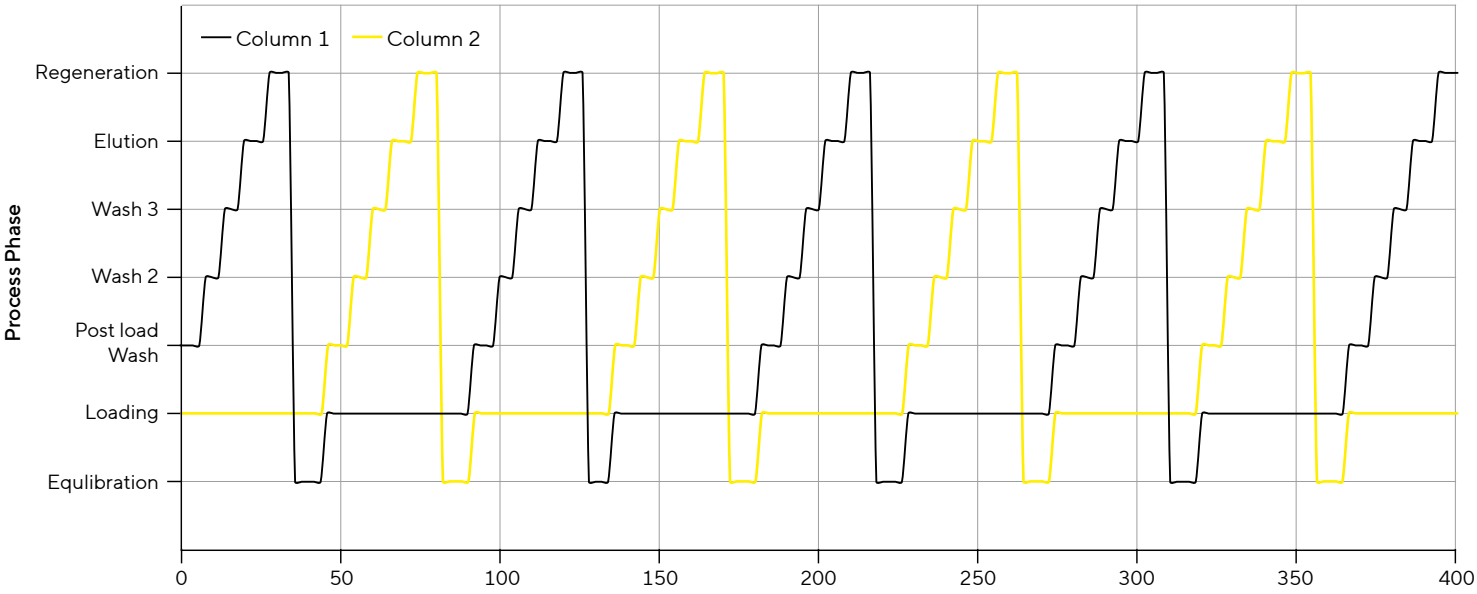


B

Parameter	Details
Column Configuration	
Stationary Phase	Protein A resin
Dimensions (Height | Diameter | Volume)	**10 L:** 10.3 cm | 3.2 cm | 83 mL **200 L:** 10 cm | 20 cm | 3.14 L
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

Note. **(A)** Chromatogram for capture chromatography step. The dark purple line shows UV-280, representing the protein | product absorbance with time. The product can be seen later in the elution peak and is collected using a 50 mAu cut-off on both sides (elution peak from 135 mins to 142 mins). **(B)** Column configuration and buffer conditions (10 L and 200 L are production bioreactor scales)

Figure 3: *Phase Diagram for B-MCC Operations Showing How Two Columns Move Into Different Process Steps Within the Chromatography Cycle*



Explore Additional Resources

More details about the approach, methods, and results are available in two application notes and two webinars.

Intensifying a mAb Polishing Platform – Targeting Time, Cost, and Space Efficiency

Application Note 1

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

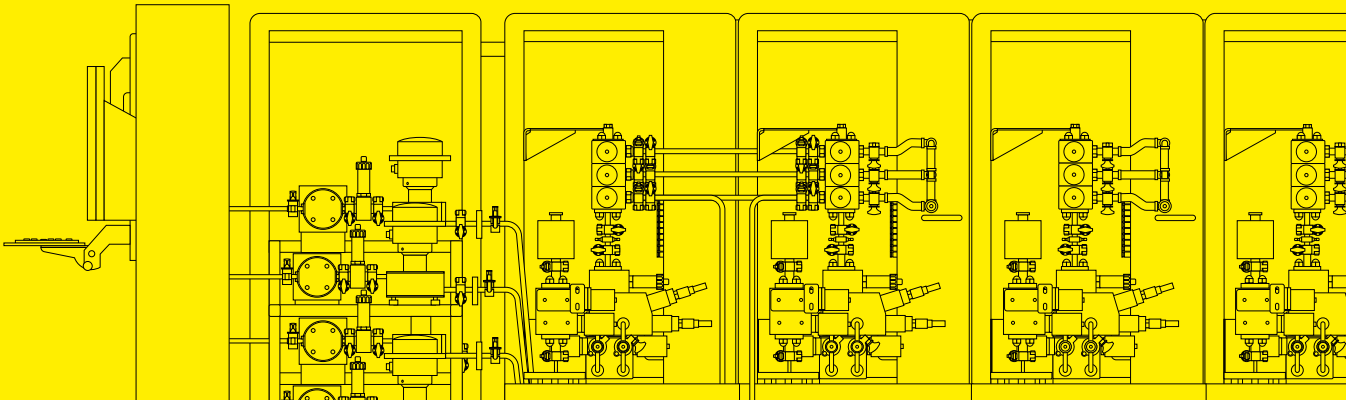
Application Note 2

What Are the Benefits of a Connected Downstream Process Using Resolute® BioSC?

Webinar 1

Intensified Connected Processing of mAbs for Robust Manufacturing with Resolute®BioSC

Webinar 2



Intensified Unit Operations

We first developed the downstream process at a small scale in batch mode to optimize the three chromatography steps:

Capture
Protein A capture in twin column, B-MCC (two columns) mode (Figures 2 and 3).

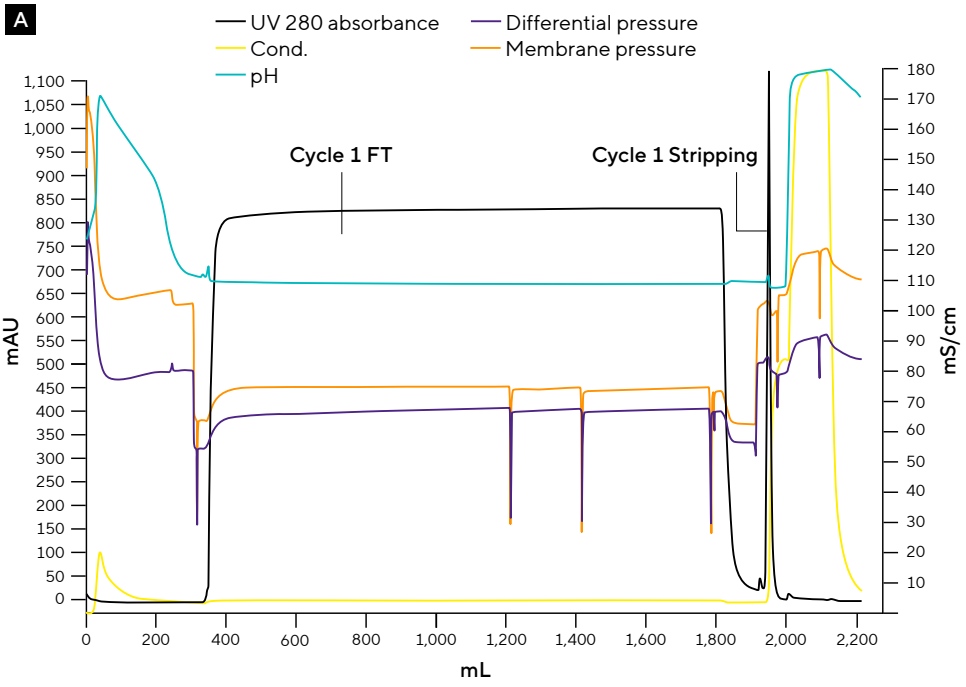
Polishing 1
Sartobind® Q membrane (Figure 4)

Polishing 2
CMM HyperCel Mixed Mode Resin (Figure 5)

Taking a unit operation-based intensification approach using the above consumables, we aimed to increase productivity, reduce buffer consumption, and eliminate intermediate buffer adjustment between the polishing chromatography steps. Cost modeling showed that the application of such an intensified polishing platform on a commercial scale could already reduce the cost of goods by approximately 13%.[2]

Figure 4: *Process Chromatogram of Polishing 1 Using Sartobind® Q*

A



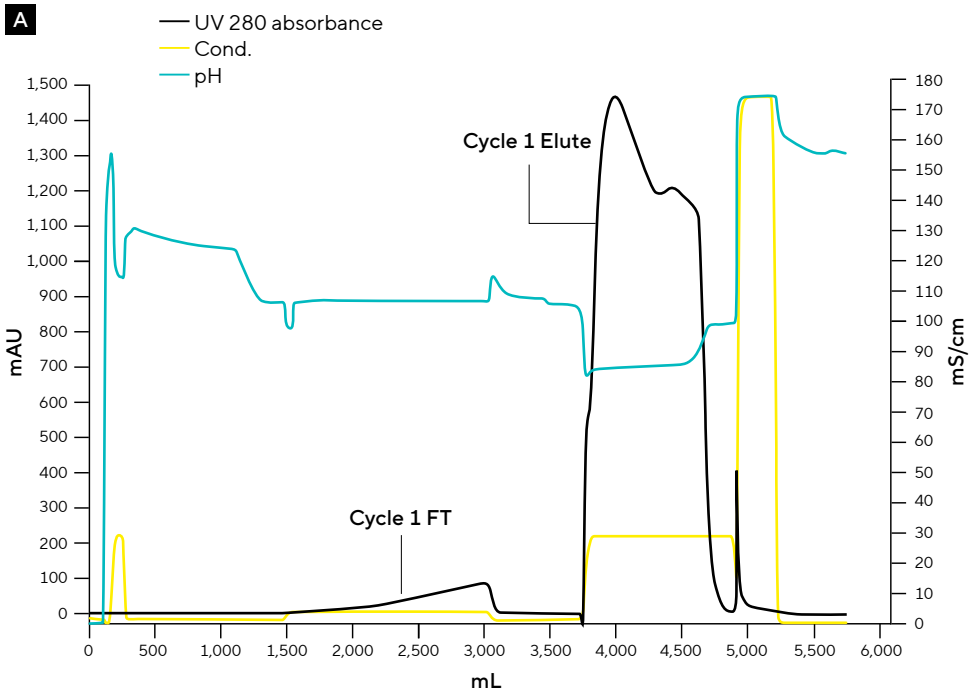
B

Parameter	Details
Membrane Configuration	
Stationary phase	Sartobind® Q
Dimensions (Height | Diameter | Volume)	**10 L:** 4 mm | 3.3 cm| 2 × 1 mL **200 L:** 4 mm | 7.7 cm| 75 mL
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

Note. **(A)** Chromatogram of the first polishing step. The black line shows UV-280, representing the protein | product absorbance with time. The product is released in the flowthrough (FT peak) and collected using a 50 mAU cut-off on both sides (FT peak from 370 to 1,920 mL). **(B)** Membrane configuration and buffer conditions (10 L and 200 L are production bioreactor scales)

Figure 5: *Process Chromatogram of Polishing 2 Using CMM HyperCel*

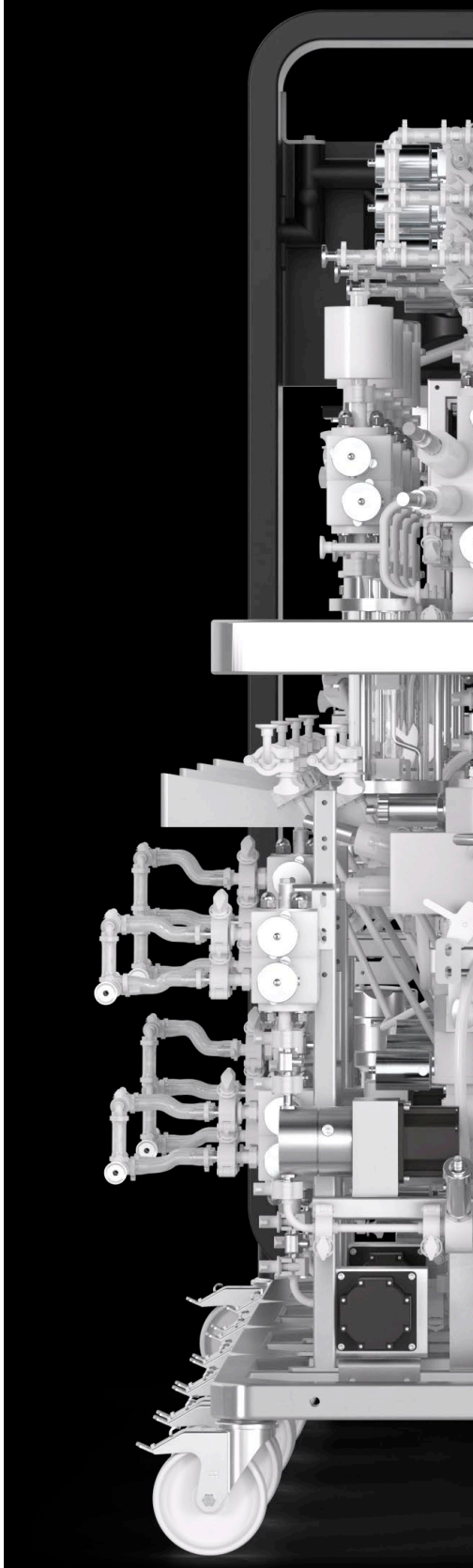
A



B

Parameter	Details
Column Configuration	
Stationary phase	CMM HyperCel
Dimensions (Height | Diameter | Volume)	**10 L:** 9.3 cm | 4.4 cm| 140 mL **200 L:** 16.9 cm | 20 cm| 5 L
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

Note. **(A)** Chromatogram of the second polishing step. The dark blue line shows UV-280, representing the protein | product absorbance with time. The elution was collected using a 50 mAu cut-off on both sides (elution peak from 3,750 to 4,750 mL). **(B)** Column configuration and buffer conditions (10 L and 200 L are production bioreactor scales)



Connected Process and Scale-Up

Once the above steps were developed and optimized in a batch mode with fed-batch upstream as a proof of concept (Cellca 2 CHO Cell Line, Model mAb: Adalimumab, upstream titer: 4 g/L, viable cell density: 30 million cells/mL) and connected downstream process run was executed successfully at a 10 L scale, the process was scaled up to 200 L (using the Biostat® STR 200).

In the 200 L process, the capture step (with Protein A resin in dual column B-MCC mode), virus inactivation, intermediate filtration, polishing 1, and polishing 2, were run in a semi-continuous | connected mode, using a unique recipe with Resolute® BioSC Pilot four module system (Figure 6).

The setup enabled the pre-virus filtration steps to be processed in less than 27 hours—significantly faster than the 41-hour timeline for processing in conventional | staggered batch mode. This increased productivity by up to 54% and reduced the required downstream process time by up to 35% (Figure 7).

Importantly, all process and product parameters (including bioburden and endotoxin) were found to be consistent between the 200 L and the 10 L connected downstream process, demonstrating consistency, scalability, and reproducibility (Refer to Application Note 2 for the data and more details on how to develop and successfully scale a connected downstream process for mAbs).[3]

Figure 6: *Process Flow Diagram for 200 L Connected Run with Resolute® BioSC Pilot*

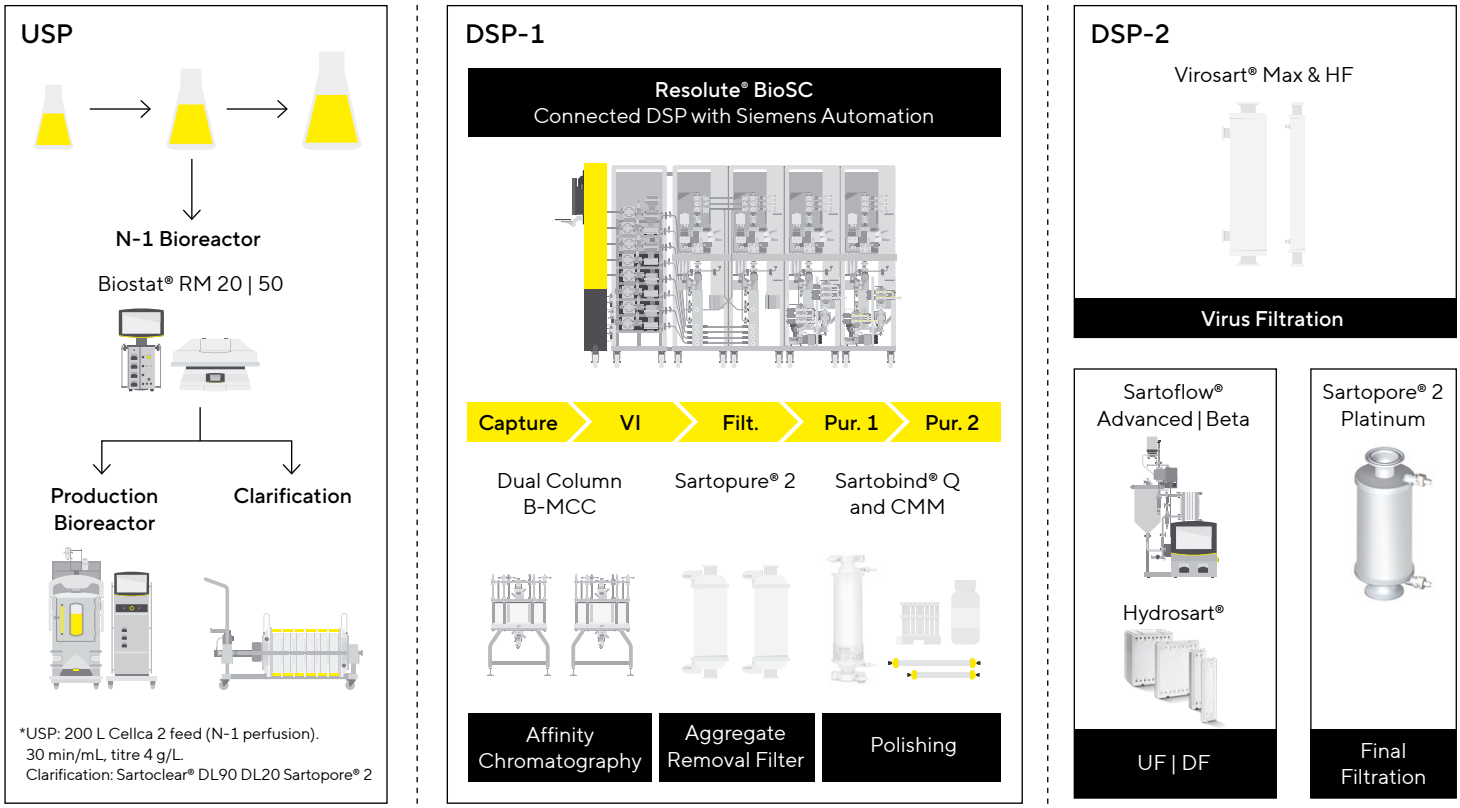
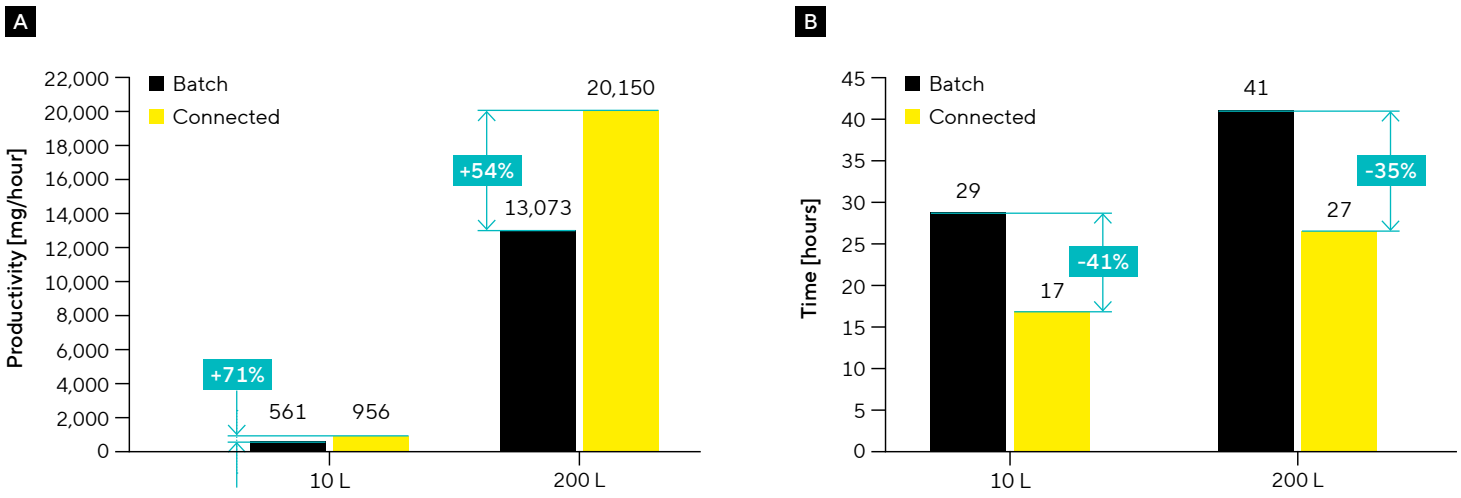


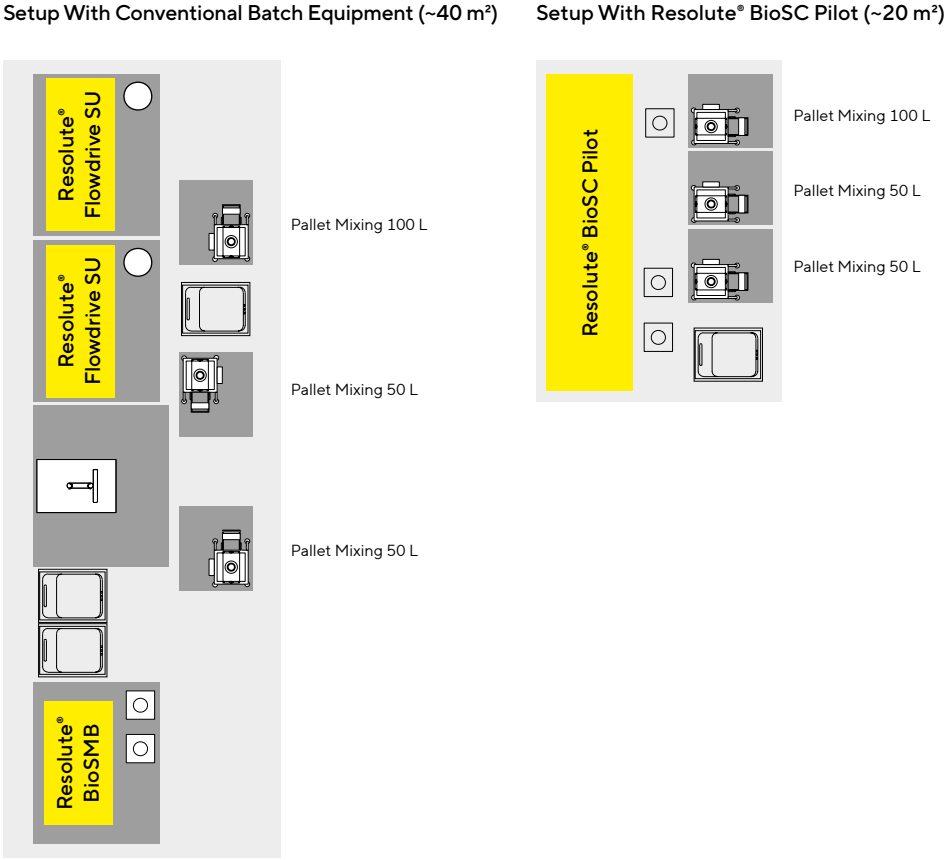
Figure 7: *Comparison of **(A)** Productivity and **(B)** Time for Batch and Connected Processes at 10 L and 200 L Scales*



Analyzing the Benefits of a Multi-Step System

To assess the footprint savings achieved by employing the Resolute® BioSC—which performs multiple steps on a single system—we carried out a footprint analysis at a 200 L scale. We compared a connected downstream process carried out with conventional single-use (SU) equipment (1 × Resolute® BioSMB Process 80, 2 × Resolute® Flowdrive SU for chromatography steps, and 1 × FlexAct Modular for virus inactivation) to the same process with all steps executed with the Resolute® BioSC Pilot. Figure 8 shows that using the Resolute® BioSC Pilot for a connected downstream process reduced footprint by >2-fold.

Figure 8: *Footprint Comparison of Connected Downstream Processes Executed With Conventional SU Equipment vs Resolute® BioSC Pilot*



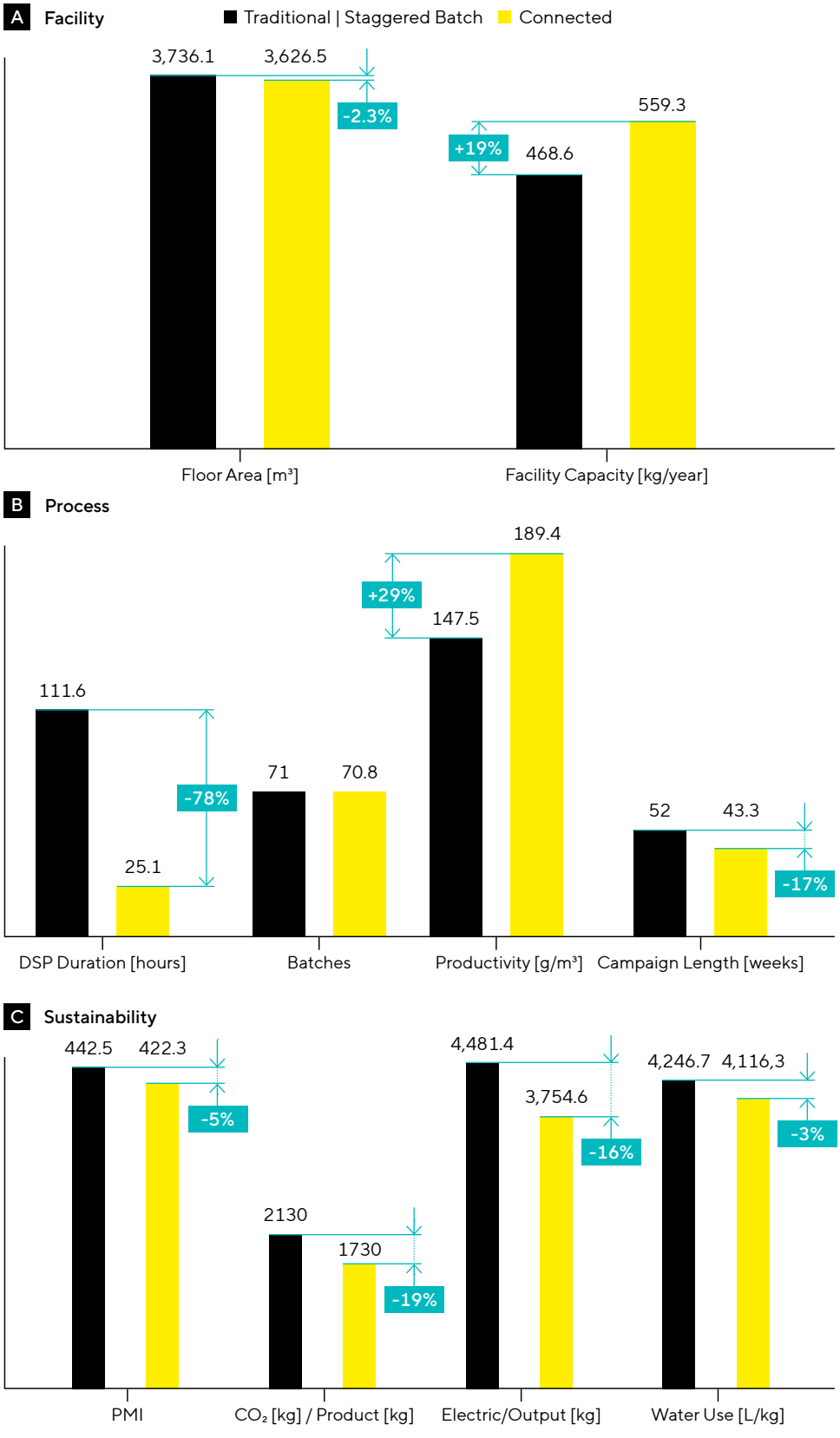
Commercial Process Simulations

The output of the 200 L Resolute® BioSC Pilot scale run and the footprint analysis was subsequently used as an input to simulate (with Biosolve® Process) a 4 × 2,000 L facility intended to produce 500 kg/year of mAb with a high-titer fed-batch upstream process, comparing a batch and connected downstream process. Figure 9 shows the results for a variety of modeled facility factors (floor area and facility capacity), process factors (process duration, batches, productivity, and campaign length), and sustainability factors (process mass intensity, CO_2 emissions, electricity consumption, and water use).

The simulation results demonstrate that employing a connected downstream process resulted in a high throughput facility by raising productivity (~1.3x), increasing facility capacity (1.2x), and reducing campaign length (~17%) compared to a traditional batch downstream process set up. Connected processing also supported improved sustainability by reducing process mass intensity (~5%), CO_2 emissions (~19%), and electrical consumption (~16%) (Figure 9).

In summary, while most of the focus of downstream process intensification is on improving footprint and productivity, our modeling data shows that connected processing can also increase the overall facility output and limit environmental impact. This helps manufacturers build more sustainable processes and meet increasingly ambitious sustainability targets.

Figure 9: *Biosolve Process Simulations for an End-to-End Process From USP (High Titer) to DSP Using Traditional Batch vs Connected DSP.* ***(A)*** *Facility,* ***(B)****, Process,* ***(C)*** *Sustainability*



Note. 4 × 2,000 L facility producing 500 kg/year of mAb PMI = Process mass intensity

Conclusion

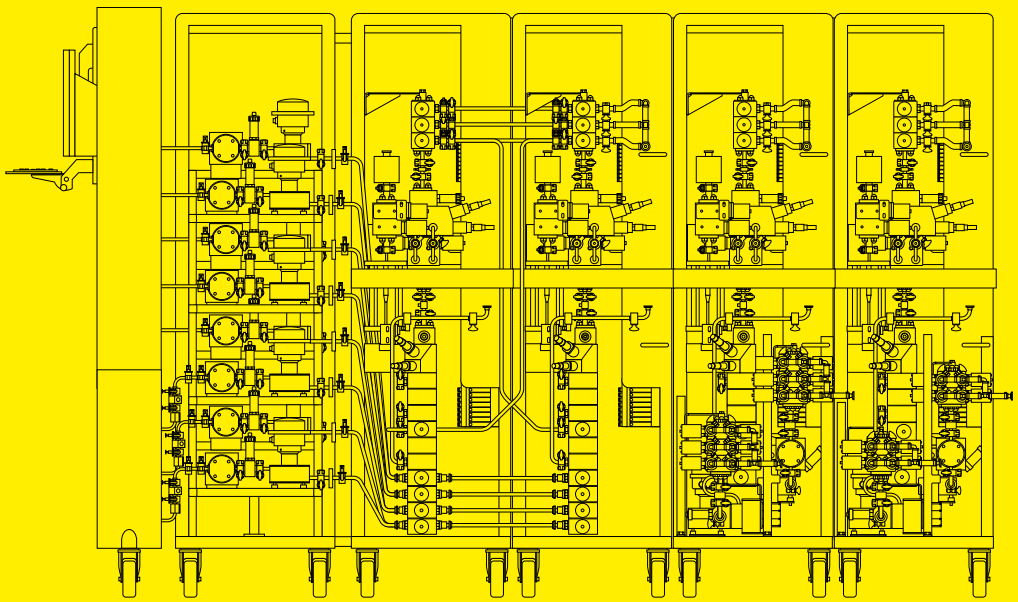
Performing multiple downstream steps in parallel and in a connected process is a powerful approach to intensify existing and new platforms irrespective of the intensification status | readiness of the upstream process.

The case study summarized here shows a stepwise approach for integrating process intensification principles into an existing process to increase productivity, reduce costs, and improve process sustainability. Compared to a traditional batch downstream process, applying connected downstream process intensification with Resolute® BioSC for fed-batch processes supports:

- **Up to 54% increased productivity and 35% reduced processing times**
- **Reduced facility footprint by >2x, enabling higher volumetric output (increased product mass with a smaller equipment footprint.)**
- **Improved process sustainability through a reduction in process mass intensity (5%), CO_2 emissions (~19%), and electrical consumption (~16%).**

In conclusion, the insights derived from this case study offer a tangible example of the application of process intensification principles in a downstream process. The results highlight the Resolute® BioSC as an optimal platform for executing connected, multi-step purification within a singular system, making it easier to reap the benefits of process intensification.

Explore Our Process Chromatography Solutions
https://www.sartorius.com/en/products/process-chromatography



Author Bio

Priyanka Gupta
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Priyanka Gupta heads the External Collaboration Group for Separation Technologies Marketing. In this role, she works closely with external customers to generate valuable data for Sartorius downstream technologies for various modalities. Priyanka joined Sartorius in 2007 following several years at Amgen.

Before her current position, Priyanka led the Market Entry Strategy Group for protein-based therapeutics. Here, she built the global segment market entry strategy and led the team to promote offerings that reflect the distinct needs of target customer groups.

She has a master's degree in Chemical Engineering and a bachelor's and master's degree in Biomedical Engineering and Biotechnology.

Katy McLaughlin
PhD, Scientific Content Writer, Sartorius

Katy is part of the Marketing Communications team at Sartorius, where she supports the creation of a variety of written pieces, from published articles to web content.

Before joining Sartorius in 2021, Katy was employed as a Post-Doctoral Research Associate at the University of Edinburgh, where she also completed her doctoral studies. Here, she carried out research in genetics and cellular biology and began taking on writing projects, eventually entering into a career as a freelance writer for various biotech companies and agencies.

Ganesh Kumar
Head of BPS Sales, Eastern Europe, Middle East & Africa (EEMEA), Sartorius

Ganesh has over a decade of experience in the biopharmaceutical industry with CDMO and solution providers. He started his career at Lonza, Singapore, where he was actively involved in the tech transfer, validation, and large-scale commercial manufacturing of blockbuster mAbs.

Ganesh joined Sartorius in 2016 and through various roles, has led programs on building end-to-end process platforms, conceptual design services, market strategy, go-to-market, acquisitions, establishing strategic customer collaborations for process intensification and in market development | sales for traditional and newer modalities. He is based in Göttingen, Germany.

Sanket Jadhav
PhD, External Collaborations Manager, Sartorius

Sanket is an External Collaborations Manager at Sartorius, leading various customer collaborations as a project technical lead and project manager.

Sanket started his career as a process engineer at Biocon in Bangalore, India, where he led projects on process design, production, and facility design for insulin. Sanket earned his PhD in Chemical Engineering in Australia and then moved to the Netherlands, where he led consulting projects in designing tools for SMB systems, optimizing processes and facilities, establishing USP perfusion processes, and troubleshooting end-to-end processes for customers in Europe and the USA.

With >10 years of biopharmaceutical research and industrial experience, Sanket has a penchant for developing cost-effective, sustainable, and intensified platform technologies for modalities like proteins, mRNA, and other gene therapy applications.

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Status: 12 | 2024