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Application Note

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A New Way to Perform mAb Capture: Scaling Rapid Cycling Membrane Chromatography From Bench to Commercial Manufacturing

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Abstract

The goal of this study was to demonstrate the successful scale-up of a rapid cycling chromatography (RCC) process from a generic bench chromatography system to the Resolute® RCC MU and Resolute® Flowdrive SU using the Sartobind® Rapid A as the chromatographic matrix of choice. The results demonstrated scalability with no significant loss in product yield or quality. Combining high productivity chromatography methods and SU affinity membranes deliver a viable alternative to the the current packed bed Protein A capture techniques for intensified purification of monoclonal antibodies (mAbs) up to commercial production scale.

Introduction

Traditionally, in the biopharmaceutical industry, monoclonal antibodies (mAbs) are captured with Protein A-based affinity chromatography resins as a first unit operation in downstream processes. Although effective, this methodology has many challenges especially as the process scales into commercial manufacture. Resins occupy a large footprint (including cold storage for re-use) and require specialized resources for column packing activities. Although pre-packed columns overcome some of the inconveniences of self-packing, they are only available in certain sizes and are expensive. Additionally, Protein A resins are often sensitive to caustic chemicals, making cleaning-in-place (CIP) challenging and increasing the risk of contamination.

Membrane-based rapid cycling chromatography (RCC) is a mode of chromatography where a membrane is rapidly cycled to mimic the throughput of larger, less efficient devices. As such, RCC represents an opportunity to replace packed-bed operations with a simple, ready-to-use, intensified solution. RCC eliminates the pain points of capital expenditure on columns, operational challenges (column packing, testing, cleaning, storage, etc.), and resin batch management[1–3], while membrane matrices are also more caustic-stable, simplifying CIP and reducing bioburden contamination risk.

Scalability is also not fully solved by the use of membrane-based matrices, as some solutions available in the market struggle with binding capacity and pressure limitations. Typically, as the size of membrane chromatography devices increases, so does the generated back-pressure, meaning that some portfolios on the market are limited in their operation at larger scales. Sartobind® Rapid A devices overcome these limitations and support affinity capture chromatography unit operations from process development to production scale without a significant increase in pressure.

Sartobind® Rapid A readily supports RCC, enabling up to 14x more productivity during capture chromatography.[2,4,5] Here, we demonstrated the scalability of Sartobind® Rapid A in rapid cycling mode, by comparing cycles, pressure profiles, and critical quality attributes across different devices and scales.

Materials

Hardware

- Resolute® RCC MU chromatography system (Figure 1)
- Resolute® Flowdrive SU chromatography system (Figure 1)
- Sartorius Precision Balance
- Octet® R8 protein Analysis system

Consumables

- Sartobind® Rapid A membranes (Figure 1) (Nano, Mini, 75 mL, 200 mL, Cassette)
- Sartopore® 2 XLG (0.8 | 0.2 µm) filters (inline guard filter for chromatography)
- Sartopore® 2 (0.8 | 0.45 µm) filters (buffer pre-filtration)
- Sartopure® GF+, 0.65 µm Maxicaps® (post-thaw filter for HCCF in cassette trials)
- ⅜ inch and ½ flowkit assembly for Resolute® Flowdrive SU

Table 1: *Resolute® Systems for Rapid Cycling Chromatography*

	Resolute® RCC MU	Resolute® Flowdrive SU
Types	Multi-use cleanable system	Single-use
Flow Rate Ranges	5 – 150 L/hr	8 – 150, 18 – 600, 36 – 900 L/hr*
Pressure Rating	5 bar	4 bar
Recommended Devices	75 mL and 200 mL	75 mL and 200 mL and up to 3 Cassettes

* 3 flow rate ranges

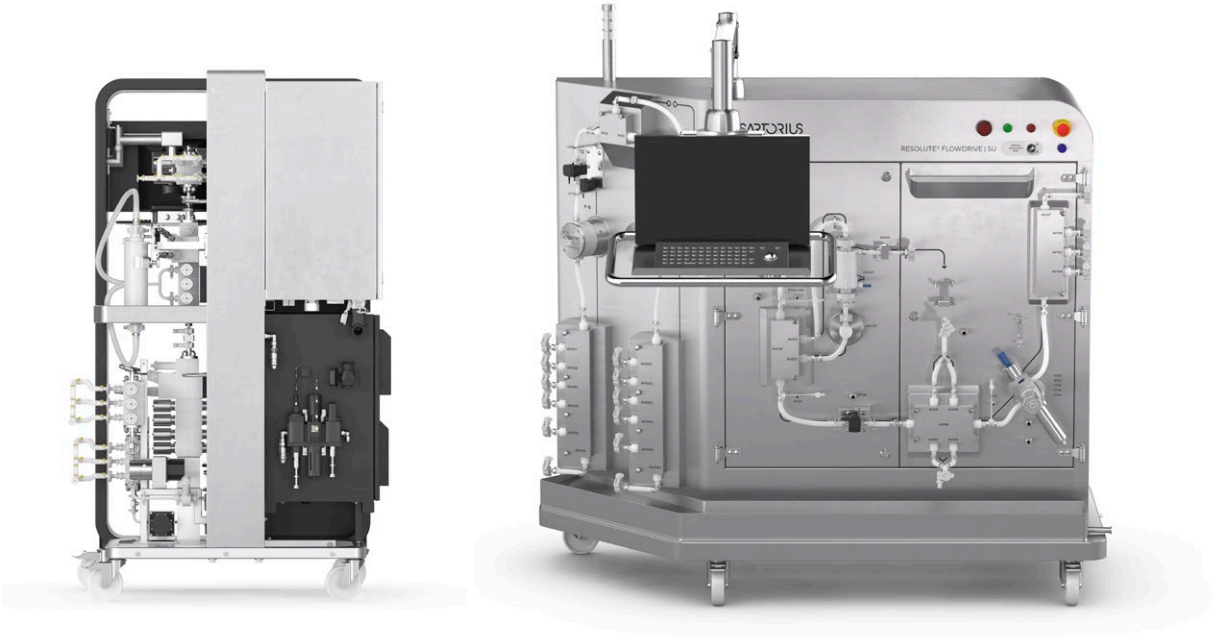
Table 2: *Buffers*

Buffer	Name	Ingredients	pH [–]	Conductivity [mS/cm]
Re-Equilibration | Wash	PBS	137 mM NaCl; 2.7 mM KCl; 10 mM $Na_2HPO_4 \times 2H_2O$; 1.8 mM KH_2PO_4	7.4 ±0.2	16
Elution	AcOH	0.1 M AcOH; 150 mM NaCl	~2.9 ±0.1	~15
CIP	NaOH	0.2 M NaOH	>12.5	>60
Storage	PBS | EtOH	20% (v/v) Ethanol in 1× PBS	N/A	N/A

Feed Material
The same feedstock (1,000 L) was used at all scales to avoid variation. However, volume limitations and capacity restraints meant that all experiments could not be run in parallel. This necessitated intermediate storage, and repeated thaw cycles, which makes the composition of the feedstock more challenging for downstream purification.

Figure 1: *Sartobind® Rapid A Sizes Evaluated and the Chromatography Systems Deployed in This Application Note*

	Nano	Mini	75 mL	200 mL	2 × Cassettes	3 × Cassette
Membrane Volume (MV)	1.2 mL	10 mL	75 mL	200 mL	1.6 L	2.4 L



Methods

The chromatography steps and parameters are shown in Table 3. The Sartobind® Nano and Mini devices were used with classical benchtop batch chromatography systems. The Sartobind® 75 mL device was used with the Resolute® RCC MU system (the 200 mL capsule can also be used on this system), and the 200 mL Cassettes were utilized with the Resolute® Flowdrive SU chromatography system.

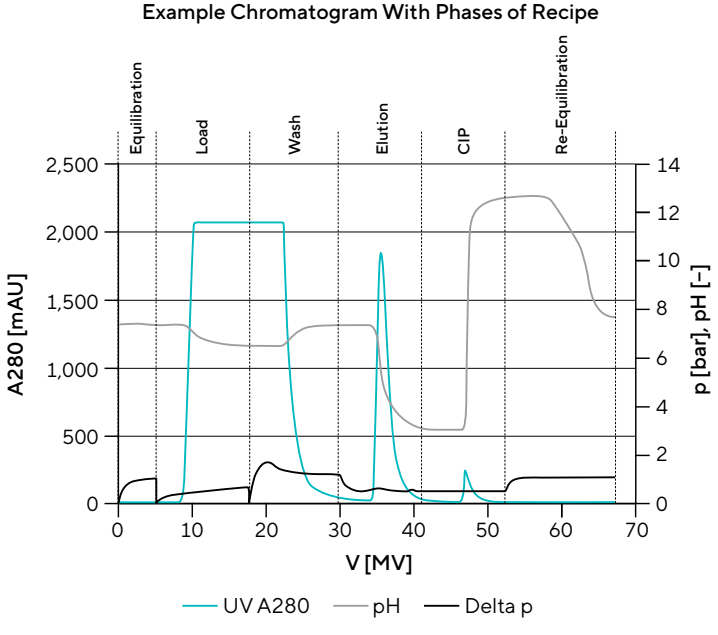
The number of cycles for each device (and respective feed volume used) are shown in Table 4. An example of a typical chromatogram for membrane-based RCC is shown in Figure 2.

Table 3: *Chromatography Recipe*

Phase	Buffer	Flow Rate [MV/min]	Volume [MV] Load [g/L]
Equilibration	1×PBS	10	Hold until pH ≤ 7.6 or max. 5 MV
Load	Adalimumab HCCF*	5	32.5 g/L
Wash	1×PBS	10	12
Elution (0.1–0.1 AU)	AcOH	5	12
CIP	NaOH	5	Hold until pH > 12.3 (max. 10 MV) then 3 MV
Re-equilibration	1×PBS	10	Hold until pH ≤ 7.6 or max. 15 MV

*HCCF = harvest cell culture fluid

Figure 2: *Typical RCC Chromatogram*



The following critical quality attributes were assessed for all process scales:

- Protein concentration
- Monomers, high molecular weight species (HMWS) and low molecular weight species (LMWS)
- Host cell proteins (HCP)
- Host cell DNA (hcDNA)
- Leached Protein A

Table 4: *Harvested Cell Culture Fluid (HCCF) Volume and Targeted Number of Cycles for Each Device*

Sartobind® Rapid A Device	Number of Cycles Performed	HCCF Volume	Max Lifetime Cycles
Nano	20	~1 L	
Mini	45	~6 L	300*
75 mL	20	~30 L	100*
200 mL	60	~160 L	200*
Cassette	2 cassettes: 19 3 cassettes: 5	2 cassettes: ~500 L 3 cassettes: ~200 L	200** 200**

Note. Cycles were stopped when material was depleted feed volume and targeted number of cycles for each device

* Experimentally proven
** Extrapolated

Results

Process Performance

To test the scalability of the device portfolio from a process perspective, we compared chromatograms and differential pressures across the capture chromatography operation for each device. Figure 3 shows similar chromatograms for RCC across five devices | scales.

Peak shapes are highly comparable across scales for each phase of the chromatography recipe, indicating excellent consistency across device sizes in the portfolio. Different peak intensities are a result of the different UV detectors and sensitivities of the chromatography systems.

We then tested the differential pressure across the different devices (Figure 4). The operating pressure was 1.2 – 2 bar at 10 MV/min, depending on device size. The differential pressure profile (< 2 bar) was comparable across all Sartobind® Rapid A devices.

Figure 4 shows that the observed pressures are well within the limits of existing SU chromatography skids (0 – 4 bar), and the flow rate does not need to be slowed down to accommodate pressure fluctuations.

Figure 3: *Process Performance Is Consistent Across Scales*

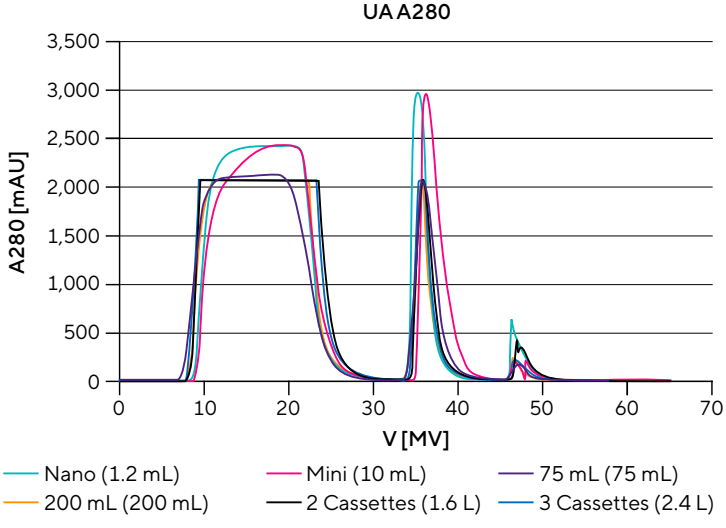
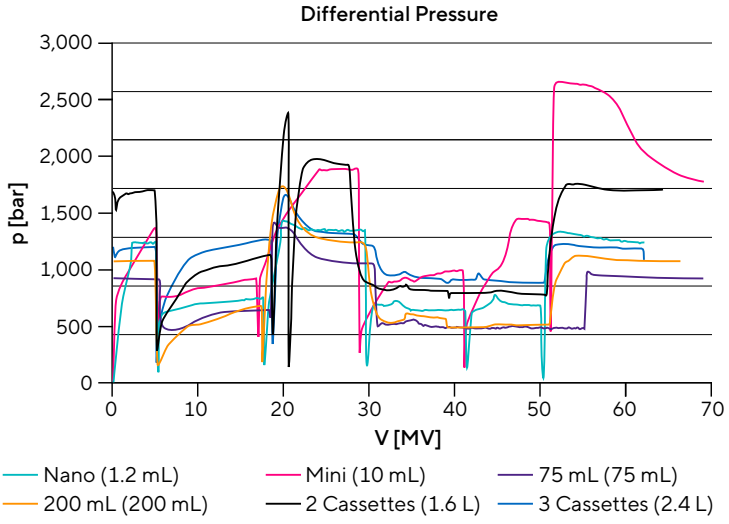


Figure 4: *Differential Pressure Profiles of Each Device*



Critical Quality Attributes

We then analyzed yield, HMWS, LMWS, HCP, hcDNA and leached Protein A were investigated to ensure these critical quality attributes remained consistent across scales.

The average elution volume, concentration, and yield are shown in Figure 5. Overall productivity of the capture step is shown in Table 5. While there were some differences, variation was within a narrow range, and yield and productivity were largely consistent across process scales.

Figure 5: *Average Elution Volume | Concentration And Resulting Yield*

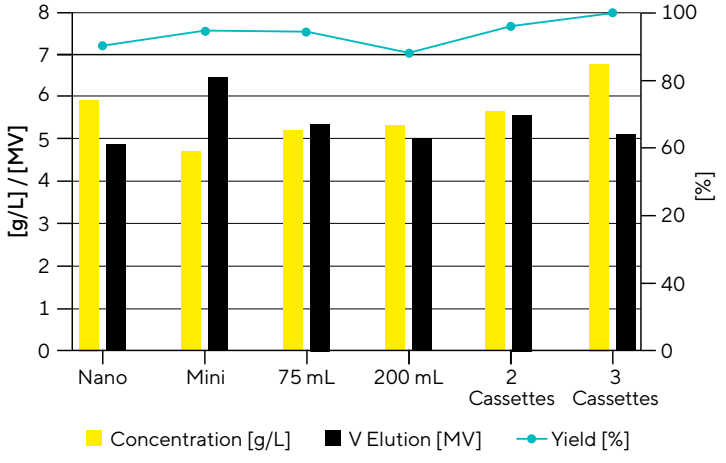
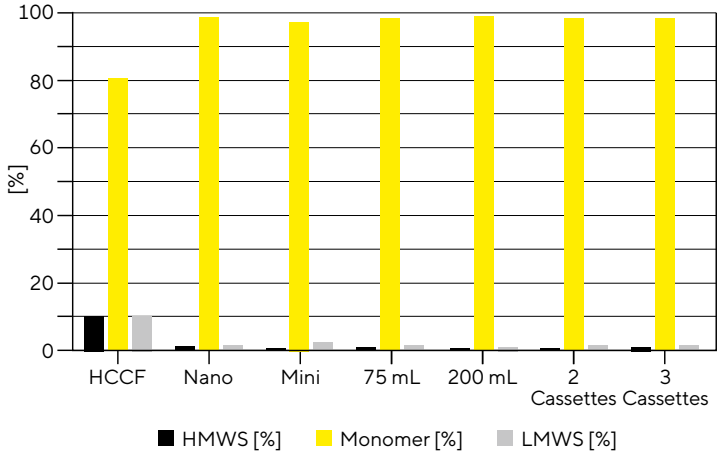


Table 5: *Elution Volumes, Yield, and Productivity Across Scales*

	MV* [mL]	Step Yield [%]	Productivity [$gL^{-1}h^{-1}$]
Nano	1.2	90.4	168
Mini	10	94.7	144
75 mL	75	94.2	173
200 mL	200	88.2	154
2 Cassettes	1,600	94.9	176
3 Cassettes	2,400	98.2	167

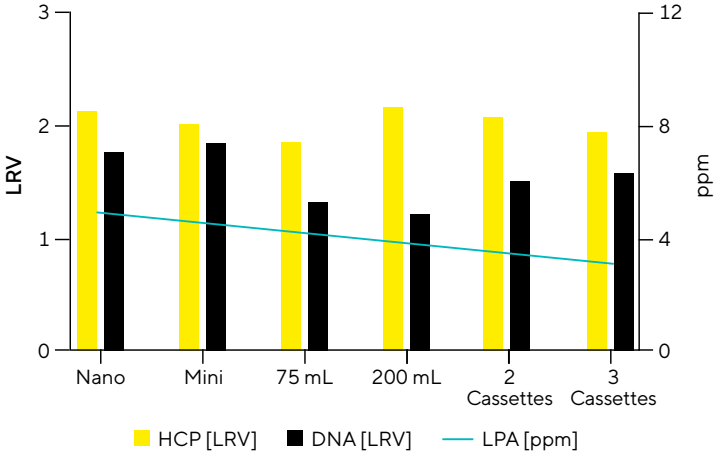
The percentage of monomers, HMWS, and LMWS across scales (compared to load material) are shown in Figure 6. The monomer content in elution fraction on average was 98%, with an average aggregate reduction of > 95%, and an average fragment reduction of > 86%.

Figure 6: *Average Percentage Distribution of Monomer | HMWS | LMWS Relative to Input Material (HCCF)*



Comparable HCP and hcDNA reduction were achieved with the device portfolio (Figure 7), with more than 3 log reduction possible (depending on mAb | load material constitution). Finally, we found that leached Protein A was comparable between the devices and to a resin-purified sample (Figure 7). Robust maintenance of CQAs have also been independently demonstrated.[5]

Figure 7: *Average Impurity Removal Across Sartobind® Rapid A Devices*



Note. HCP: Host Cell Protein, LPA: Leached Protein A

Discussion

The data presented here demonstrates the robust scalability of the Sartobind® Rapid A membrane device portfolio when used in rapid cycling mode, both in terms of critical quality attributes consistency as well as process performance.

Minor deviations between device sizes can be explained by the specifics of different chromatography systems (PID control and void volumes) and consumable design. These differences can influence elution volumes | concentrations, buffer consumption, cycle times, and—as a result—performance indicators such as productivity and removal of host cell impurities. In these experiments, impurity removal was satisfactory and relatively consistent across scales, but clearance was not as high as previously reported,[3,5,6] likely due to the challenging feedstock conditions.

Concerning durability, previous trials have shown that a high number of consecutive cycles is possible without impairment of performance (300 cycles of IgG1 capture with the Sartobind® Protein A Nano, 200 cycles bispecific mAb capture with the 75 mL device; data not shown).

Conclusion

The results summarized above demonstrate that Sartobind® Rapid A is not only suitable as a chromatographic matrix for RCC, but that it builds on a foundation of scalability and consistent performance across sizes. Further advantages, only mentioned tangentially, like the reduction of hands-on time, footprint, cost, and risk, support the choice of Sartobind® Rapid A for the main capture step in intensified purification processes for mAbs.

Learn more about Resolute® Flowdrive | intesified batch chromatography
www.sartorius.com/sartobind-rapid-a

Learn more about the Sartobind® Rapid A portfolio
www.sartorius.com/intensified-batch-chromatography

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