Enhancing mAb platform process using Membrane chromatography (Affinity/Protein-A, using Sartobind Rapid A) for FIH molecules with focus on Optimizing cost and Accelerating timelines

Indraneel Sanyal[$], Nuresh Manukonda[$], Ankitha Hirematha[$] and Mukesh Dakua[$], Buwadas R Raut[1], Madhav Naik[1]

Aragen Biologics Private Limited, Bangalore, India. **$. Presenting Author and authors for correspondence, 1. Sartorius Stedim India Pvt.Ltd.**
Sr. Director, Process Development, Aragen Biologics Private Limited, Email: indraneel.sanyal@aragen.com.

Abstract

Membrane chromatography offers significant advantages over traditional column chromatography, including higher throughput, reduced processing times, and enhanced scalability, making it an interesting tool in biologics production. This study investigates the use of Protein A membrane chromatography for monoclonal antibody (mAb) purification in a Contract Development and Manufacturing Organization (CDMO) setting involved in biologics development and manufacturing of First-In-Human (FIH) molecules. We adapted our mAb platform from resin-based to membrane Protein A chromatography, evaluating productivity, material cost, and plant occupancy at 50L, 500L, and 2000L scales. Results indicate this technology's potential benefits for clinical development and improved operational flexibility

Background

Challenges with Affinity Capture Step using Traditional Columns

- Mass transfer limitations and operating pressure constraints.
- Oversized and underutilized due to process time limitations.
- Result in disposal of underused resin or costly storage, cleaning, and revalidation.

Membrane Technology in Downstream Processing

- Labor and Cleaning: Reduces labour-intensive cleaning validation and column packing.
- Changeover: Decreases product changeover times in multi-product facilities.
- Contamination: Minimizes cross-contamination risks and lowers bioburden.
- Reduces the upfront cost of purchasing large volumes of resin that remain unused for most of their useful life after early-phase clinical material production is completed.

Advantages of Membrane-Based Protein A Chromatography

- Ready for use Capability: Significantly higher productivity (g/L/h)..
- Scalability: Easily scalable from laboratory to manufacturing scale.
- CDMO Operations: Enhances quick purification of mAbs and reduces turnaround times in multi-product facilities.

Methodology

- Resin to membrane adaptation of the Platform process
- Establishing the DBC and adjusting the washing & Elution conditions.
- Membrane cycling studies for useful life-span.
- Evaluated Productivity, Buffer usage, Plant occupancy, and mAb cost/gram at two different Expression titres and three different scales using Sartorius ExCIT tool (Expert Chromatography Intensifier tool).
- The data derived assumes 3 GMP batches for the early-phase clinical campaign.

Sartobind® Rapid A Membrane volume (1.2 mL)						
Step	**Buffer**	**MV**	**Volume (mL)**	**Flow rate (mL /min)**	**Residence time (min)**	**Time (min)**
Equilibration	1xPBS	20	24	12	0.1	2
sample load	Sample	10.0	12.0	6	0.2	2
Sample wash	1x PBS	20	24	12	0.1	2
Elution	30mM Sodium acetate	20	24	6	0.2	4
Regeneration	0.1M NaOH	10	12	6	0.2	2
Wash	Type-1 water	10	12	12	0.1	1
Total		**90.0**	**108.0**			**13.00**

Observations:

- Membrane Protein A chromatography (Sartobind® Rapid A) can reduce the cost per gram of mAb by approximately threefold in the affinity capture step.
- Productivity (g/L/h) with Rapid A is about 20 times higher than with resin.
- Productivity (g/k€/h) with Rapid A is about 7 times higher than with resin.
- The cost-effectiveness of resin improves with an increasing number of usage cycles.
- Rapid A uses at least twice the buffer volume compared to resin, which can impact manufacturing costs.

Conclusions

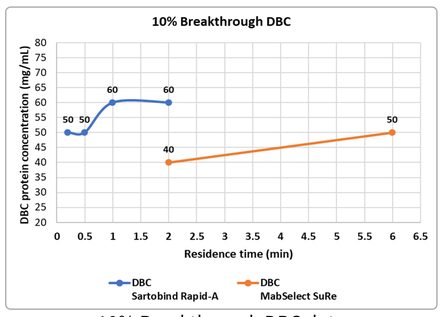
Membrane-based Protein A chromatography is highly productive, scalable, and suitable for GMP production of antibody therapeutics , enhancing efficiency in multi-product manufacturing. In First In Human (FIH) applications, it offers reduced upfront costs, quick product changeovers, and lower facility occupancy. Despite lower buffer consumption and being cost-effective at scale, has higher TOC (Total Ownership Costs) for FIH that includes high upfront costs, labor-intensive processes, and storage challenges. In CDMO setups, membrane chromatography benefits FIH manufacturing by reducing both material upfront costs and plant occupancy.

Results

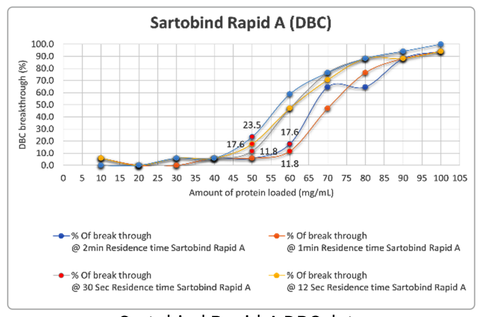
Our studies with Sartobind Rapid A revealed that it binds up to 50 mg/mL at both 12 seconds and 30 seconds residence times. When the residence time was increased to 1 minute, the binding improved to 60 mg/mL, but further increasing the residence time to 2 minutes did not enhance the DBC any further.

Based on these findings, we recommend operating the membrane at a 30-second residence time for sample loading. This approach offers optimal binding without significant improvements at higher residence times, increases productivity by completing cycles quickly, and mitigates backpressure during scale-up.

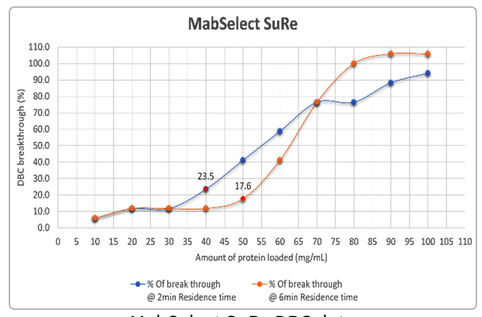
For resin, we tested the DBC at residence times of 2 minutes and 6 minutes. We suggest operating the resin at a 4-minute residence time to reduce process time and enhance productivity (g/L/h).



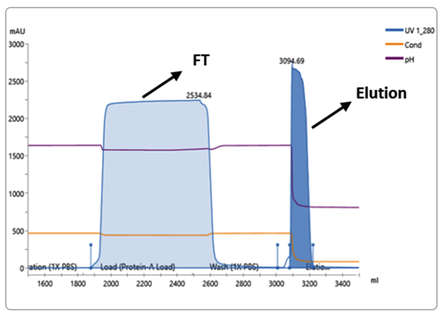
10% Breakthrough DBC data



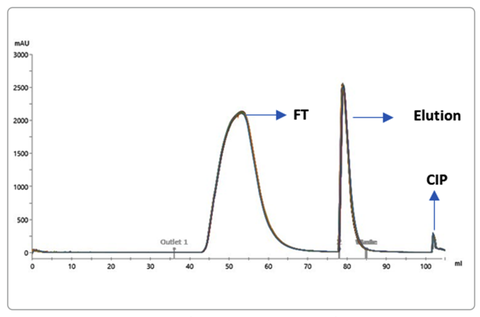
Sartobind Rapid-A DBC data



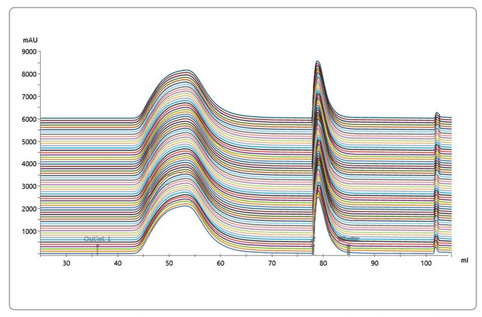
MabSelect SuRe DBC data



Protein A resin chromatogram

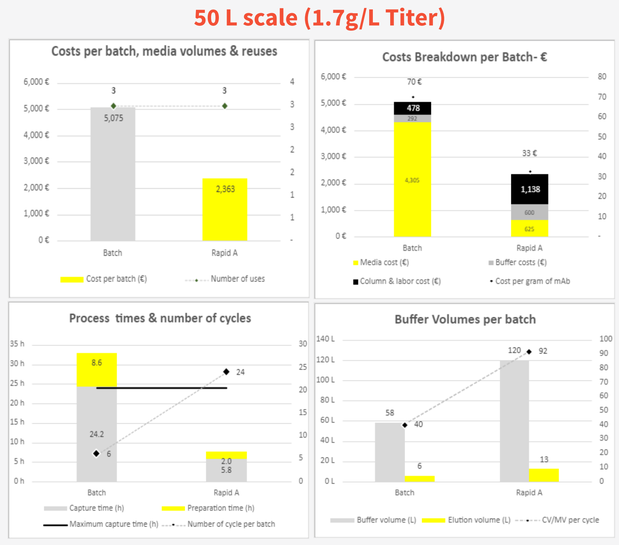


Resin to Membrane Process Adaptation

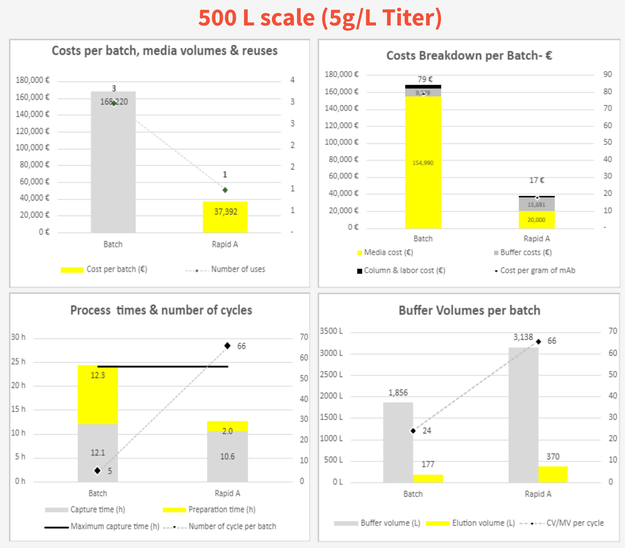


60 runs overlay at 4% Y axis on Sartobind Rapid A

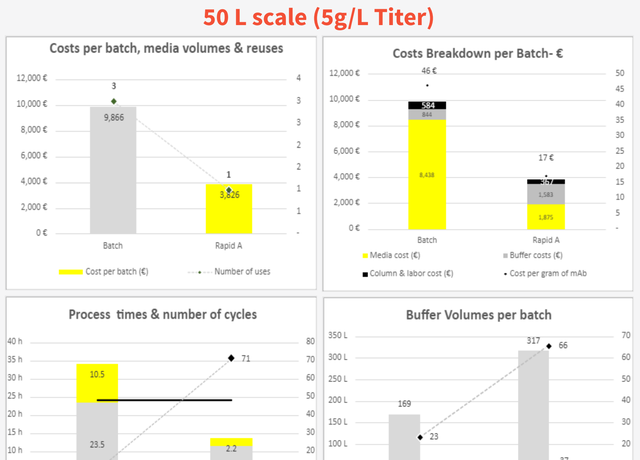
50 L scale (1.7g/L Titer)



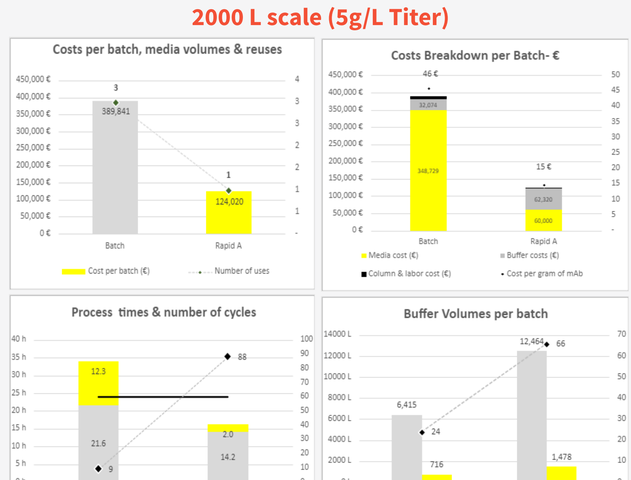
500 L scale (5g/L Titer)



50 L scale (5g/L Titer)



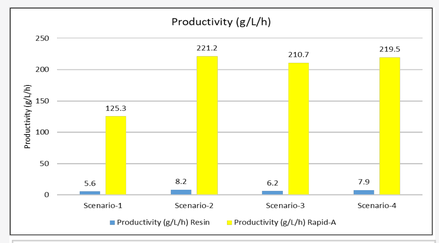
2000 L scale (5g/L Titer)

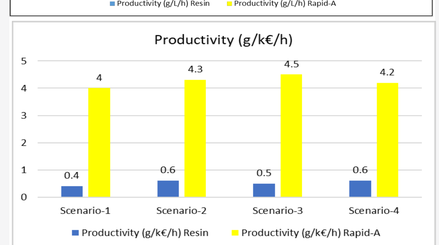


Comparative data of four scenarios

Pictorial representation of columns vs membrane chromatography formats available								
Attributes (Estimated values for protein-A step)	**50L Scale**		**50L Scale**		**500L Scale**		**2kL Scale**	
	Resin	**Sartobind Rapid-A**	**Resin**	**Sartobind Rapid-A**	**Resin**	**Sartobind Rapid-A**	**Resin**	**Sartobind Rapid-A**
Resin or media volume (L)	0.5	0.075	1	0.075	18	0.8	40	2.4
Number of runs per batch	6	24	10	71	5	66	9	88
Upfront cost (€)	5,075	2,363	9,866	3,826	1,68,220	37,392	3,89,841	1,24,020
Plant occupancy (hrs)	32.8	7.8	34	13.6	24.4	12.6	33.9	16.2
Buffer volume (L)	58	120	169	317	1856	3138	6,415	12,464
mAb Per gram cost (€)	70	33	46	17	79	17	46	15
Productivity (g/L/h)	5.6	125.3	8.2	221.2	6.2	210.7	7.9	219.5
Productivity (g/k€/h)	0.4	4	0.6	4.3	0.5	4.5	0.6	4.2

Productivity comparison





Acknowledgment: Sartorius Separation Technologies team: Jean-Marc Cappia Head of Market Development, Intensified Chromatography; and Ana Raquel Fortuna, Product Manager Sartobind® Rapid Platform