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Application Note

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Resolute® BioSMB 80/350 System

Scale-Up of Multi-Column Chromatography

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Abstract

The Sartorius Resolute® BioSMB platform is the first flow path, multi-column chromatography solution that is fully scalable from the Process Development (PD) laboratory to GMP manufacturing. The Resolute® BioSMB 80/350 system is specifically designed to allow users to convert an existing PD scale continuous purification into a process scale continuous purification based on feedstreams derived from fed-batch bioreactors of up to 2,000 L. The chromatographic process developed using the Resolute® BioSMB PD system can be readily scaled to the Resolute® BioSMB 80/350 system without changing the resin, buffer system or product quality assays. The Resolute® BioSMB platform provides process consistency across all scales of operation while making it practical to implement in flexible, multi-product facilities.

- Inherent unit operation efficiency of the Resolute® BioSMB platform reduces the cost of chromatographic media by up to 80% without changing either the resin or buffer used in the chromatographic process
- Entire chromatographic process can be conducted in a smaller system using smaller column volumes with associated reductions in buffer than would be required in a standard batch process to produce the same amount of material
- The entire flow path of the Resolute® BioSMB 80/350 system can be replaced within 30 minutes
- The valve system does not require cleaning or cleaning validation
- Ability to use up to 8 columns increases specific productivity and application flexibility

Scale-Up Study

The scale-up of a monoclonal antibody (mAb) capture process was performed using the Resolute® BioSMB platform. A humanized IgG1 was expressed in a CHO-K1 cell line using a 500 L Single-Use Bioreactor (SUB). The harvested cell culture fluid (HCCF) with a titer of 5.8 g/L of expressed mAb was purified using KANEKA KanCapA® Protein A resin. The method development was carried out initially using batch chromatography and following optimization of the chromatographic conditions was transferred to multi-column operation using the Resolute® BioSMB PD system. The multi-column process was then scaled-up 150-fold via the Resolute® BioSMB 350 system. The entire study was accomplished in less than 3 weeks.

Process development via batch chromatography

The dynamic binding capacity of KANEKA KanCapA® resin for the mAb was determined to be ~45 g/L at a residence time of 6 minutes. The non-load stages of the purification (i. e., wash, elution, regeneration, re-equilibration) required 22.5 column volumes (CV) to complete.

Resolute® BioSMB PD system study

The optimal multi-column chromatography process was determined to use a total of five columns:

- 1 primary load column
- 2 secondary load columns in parallel (to accommodate the effluent from Wash 1), and
- 2 columns in the non-load steps

Loading was carried out using 8 CV of HCCF over 10 minutes. A total of 5 × 5 mL KANEKA KanCapA® PRC columns (1.12 cm i. d. × 5 cm) were used for the capture step operating at a flow rate of 4.0 mL/min, giving a residence time of 3 min 2 sec. Under these conditions, the operating binding capacity of KANEKA KanCapA® resin for the mAb was 46.4 g/L (Table 1).

Resolute® BioSMB 350 system study

The 5 column process was scaled up 150-fold using a total of 5 × 0.77 L KANEKA KanCapA OPUS® columns (14 cm i. d. × 5 cm) operating at a flow rate of 37 L/h, giving a residence time of 3 min 02 sec and an operating binding capacity for the mAb using KANEKA KanCapA® sorbent of 46.4 g/L. Under these conditions, 400 L of HCCF containing 2.3 kg of expressed mAb (5.8 g/L) was processed in 13 cycles over 11 hours (Table 1).

	PD Scale	Process Scale
Flow rate	4.0 mL/min	37 L/h
Column dimensions	1.12 cm i. d. × 5 cm	14 cm i. d. × 5 cm
Column volume	5 × 5 mL = 25 mL	5 × 0.77 L = 3.85 L
Residence time	3 min 2 sec	3 min 2 sec
Number of columns	5	5
Operating binding capacity	46.4 g/L	46.4 g/L

Table 1: *Operating Conditions for Scale-Up Study*

Scale-Up Results

Performance data of the Resolute® BioSMB PD and Resolute® BioSMB 350 systems is summarised in Table 2 and compared to the batch benchmark. Product yield and quality was consistent throughout the 150-fold scale-up of the process and comparable to the batch benchmark.

As a consequence of the multi-column loading approach higher protein binding capacity was achieved and the mAb concentration in the eluent from the KANEKA KanCapA® column was increased by 30% compared to the batch procedure.

Furthermore, by operating at a lower residence time compared to batch, the multi-column approach generated a 3.5-fold productivity improvement yielding 56 g mAb/L KANEKA KanCapA® resin/h versus 16 g/L/h for the comparable batch process.

This study represents ~10% of the maximum operational throughput of the Resolute® BioSMB 350 system.

	Batch Benchmark	Resolute® BioSMB PD System	Resolute® BioSMB 350 System
Eluted mAb concentration	9.54 g/L	13.84 g/L	13.85 g/L
MAb yield	98%	97%	97%
Aggregate	0.45%	0.72%	0.65%
LRV* DNA	n. a.	4.16	5.02
LRV HCP	2.4	2.6	2.5
Specific productivity**	16 g/L/h	56 g/L/h	56 g/L/h

* log reduction value
** g mAb/L KANEKA KanCapA® sorbent/h

Table 2: *Performance Data of the Resolute® BioSMB PD and Resolute® BioSMB 350 System*

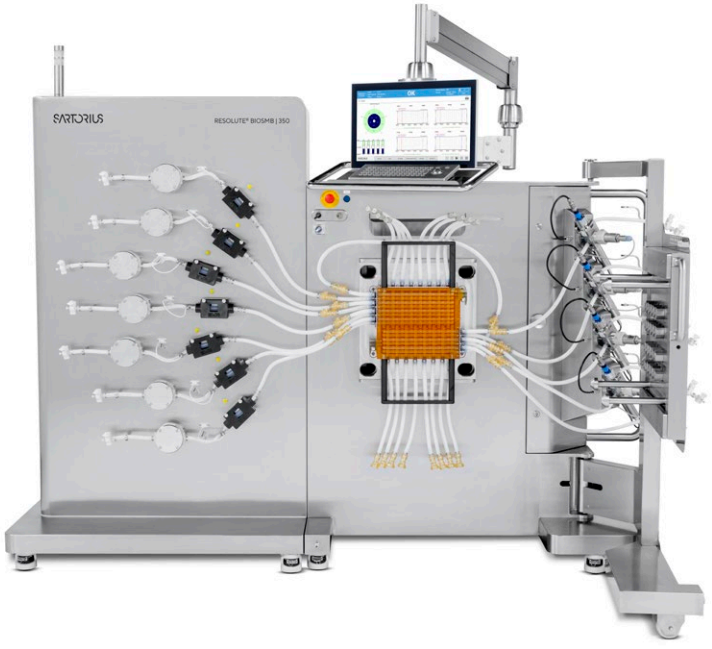

Figure 1: *Resolute® BioSMB 350 System*

Process Comparison

The batch process can be modelled in order to compare how it would perform compared to the scale-up study using the Resolute® BioSMB 350 system. The data are summarised in Table 3. In order to purify mAb from 400 L of HCCF using a batch process under similar processing times, one option is to run a 20 L Protein A chromatography column over 3 cycles, compared to the multi-column process where the optimal process requires just 3.85 L of Protein A resin packed into 5 columns each of 0.77 L volume, and operated over 13 cycles.

This demonstrates a potential overall saving of 16.15 L Protein A resin using the Resolute® BioSMB 350 system compared to a conventional batch technique.

	Batch Process	Resolute® BioSMB Process
Number of cycles	3	13
Column diameter	40 cm	14 cm
Column height	16 cm	5 cm
Number of columns	1	5
Volume of KANEKA KanCapA® sorbent	20 L	3.85 L

Table 3: *Comparative Design of a Batch Versus Multi-Column Process*

Summary

- The Resolute® BioSMB platform offers a scalable path to clinical manufacturing
- A total of 2.3 kg mAb was purified in 11 hours (13 process cycles) using the Resolute® BioSMB 350 system
- Product yield and quality was consistent with a batch process
- Only 3.85 L KANEKA KanCapA® resin was required
- A batch process would require ~20 L KANEKA KanCapA® resin
- 80% reduction in Protein A volume and associated storage, handling, processing and disposal costs
- 5 columns was the optimal configuration
- The batch process was converted to multi-column and scaled up all within 3 weeks
- The Resolute® BioSMB PD process was successfully scaled up >150-fold
- Chromatographic performance was comparable using the Resolute® BioSMB PD and Resolute® BioSMB 350 systems
- Product quality attributes were similar throughout scale-up

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Figure 2: *Resolute® BioSMB 350 System With Optional Lower Flow Pump Module Resolute® BioSMB 80*

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