Scalable continuous chromatography process for enhanced efficiency in biomanufacturing

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Scalable continuous chromatography process for enhanced efficiency in biomanufacturing

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Abstract

The cost pressure on biopharmaceuticals drives the industry towards exploring process intensification options that will maintain or improve quality, stability, and manufacturability of the product while increasing productivity. One such option explored is continuous, or connected, biomanufacturing. For continuous chromatography, the interest in techniques such as periodic counter-current chromatography (PCC) is increasing.

In this poster, based on data from a collaboration between Merck and GE Healthcare Life Sciences, we use two case studies to demonstrate that PCC is a robust and scalable technology: a 50-fold scale-up of a three-column PCC (3C PCC) mAb capture step within the lab-scale ÄKTA™ pcc system and a 294-fold scale-up directly from lab to manufacturing scale.

Introduction

Continuous processing—one tool in the tool box

The key question when designing a process is "what problem needs to be solved?". Together with gains and constraints, this will be the basis for selecting the most suitable technology and manufacturing strategy, for example, straight-through, continuous, hybrid, or traditional processing (Fig 1).

Gains and constraints	**Research, PD, manufacturing**	**Volumes**	**Purification protocols**	**Molecule characteristics**	**Running frequency**
Process flexibility	**Interest and support**	**Available equipment/ facilities**	**Funding**	**Willing to take risk**	**Personnel and knowledge**

Fig 1. Examples of contributing factors when deciding on technology and manufacturing strategy.

Continuous chromatography rationale

The drivers for using continuous chromatography technologies, such as PCC, can be divided in two categories:

- **Process benefits:** Operational flexibility by reduced equipment footprint and manpower; cost benefits for protein A mAb capture step; lower buffer consumption; enhanced productivity.
- **Product benefits:** Consistent product quality; faster purification for unstable molecules; applicable for many protein modalities.

Increased efficiency with PCC

Proof of concept presented by Merck & Co., Inc., Kenilworth, NJ, USA, shows a significant reduction in resin volume and increased productivity (Table 1) with maintained product quality (Table 2) when using PCC compared to conventional batch in process development scale.

Table 1. Comparison of resin volume and productivity in the affinity capture step between a batch and a PCC process

Results (normalized to batch)	Batch process	PCC process
Resin volume	100%	40%
Productivity	100%	280%

Table 2. Quality data in anion exchange chromatography (AIEX) pool connected to perfusion bioreactor, affinity capture in PCC mode and virus inactivation

Results	Batch process AIEX pool	PCC process AIEX pool
Yield (%)	85	90
Residual HCP (ppm)	18	11
Residual DNA (ppb)	LOQ	LOQ
Residual protein A (ppm)	LOQ	0.5

LOQ = limit of quantification

Material and methods

Case study 1: 50-fold scale-up within a lab-scale PCC system

In the first case study, performed by GE, a 3C PCC set-up for a mAb capture step was run on ÄKTA pcc (Fig 2A). The step was first run with three 2 × 1 mL HiTrap™ columns connected in series, and then scaled up 50 fold and run under the same conditions on three 94 mL AxiChrom™ 50 columns. The mAb was purified on columns packed with a new protein A resin, MabSelect™ PrismA, and sample titer was 1.1 g/L mAb in cell culture supernatant, loaded to 50% breakthrough. A 1 M NaOH cleaning-in-place (CIP) step was included in the cyclic chromatographic process.

Case study 2: 294-fold scale-up directly from lab to manufacturing scale

In the second case study, performed by Merck, a mAb capture step was scaled from a lab-scale ÄKTA pcc to a manufacturing-scale BioProcess™ pcc system (Fig 2).

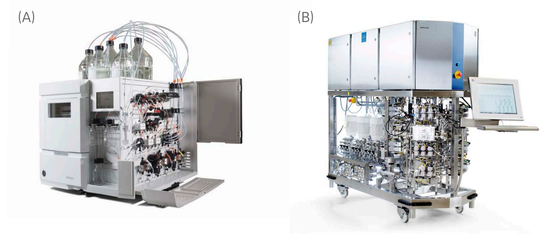

Fig 2. (A) Lab-scale ÄKTA pcc, with UNICORN™ control software, used in case study 1 and 2; (B) BioProcess pcc, with UNICORN software and DeltaV™ distribution control system, used in case study 2.

Enhanced alkaline stability of protein A resin

The new protein A resin, MabSelect PrismA has enhanced capacity and alkaline stability compared to its predecessor MabSelect SuRe™ LX resin. Data from conventional chromatography show that it exhibits up to 40% higher binding capacity at 2.4 min residence time (data not shown) and 90% relative remaining binding capacity after 150 cycles with a CIP solution of 1 M NaOH, compared with 50% relative remaining binding capacity of its predecessor when subjected to the same treatment (Fig 3).

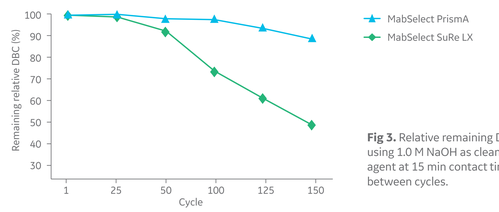

Fig 3. Relative remaining DBC using 1.0 M NaOH as cleaning agent at 15 min contact time between cycles.

Results

Case study 1: 50-fold scale-up within a lab-scale PCC system

- Consistency and quality data was comparable between scales. For example, recovery was 94% for the small scale and 93% for larger scale.
- Dynamic control functionality enabled consistent sample application even when sample varied (Fig 4).
- The new protein A resin, MabSelect PrismA, did not show any decrease in capacity over time, indicating that tougher CIP conditions can be used for long continuous processes. Figure 4 shows the stable real-time trend curves.

Case study 2: 294-fold scale-up directly from lab to manufacturing scale

Yield and quality parameters were comparable between scales and when comparing conventional batch with the 3C PCC set up at 2000 L scale (Table 3). The study also showed that the BioProcess pcc system enabled:

- 65% decrease in protein A resin (31.4 to 11.1 L)
- 40% decrease in buffer volume (3500 to 2200 L)
- 33% decrease in manpower
- successful cleaning and sanitization of the system

Table 3. Comparison of quality data in the affinity capture step between a traditional batch process and a 3C PCC process at 2000 L scale

Results (normalized to batch process run 1)	Batch process run 1	Batch process run 2	PCC process
Yield	100%	98%	96%
Residual HCP	100%	166%	79%
Residual DNA	LOQ	LOQ	LOQ
Residual protein A	100%	88%	165%
HMW	100%	111%	133%
Monomer	100%	100%	100%
Total acidic	100%	111%	112%
Total main	100%	96%	97%
Total basics	100%	108%	101%
Potency	100%	101%	105%

Note: all batches were within acceptable impurities ranges for the final drug substance.

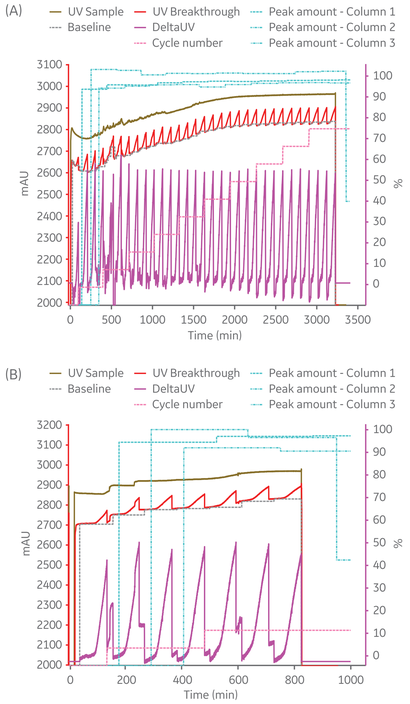

Fig 4. Chromatograms showing delta UV dynamic control and peak amount trend curves from 3C PCC runs for (A) 10 cycles in three 2 mL (2 × 1 mL) HiTrap columns; (B) two cycles in three 94 mL AxiChrom 50 columns.

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Conclusions

The case studies presented show that the PCC processes can be scaled both within the lab-scale system and directly from lab- to manufacturing-scale PCC systems with remained product quality. Additional benefits shown were significant decreases in buffer and resin consumption as well as reduced manpower required compared to traditional batch processing in 2000 L bioreactor scale. Moreover, data show that MabSelect PrismA resin withstands a 1 M NaOH CIP in a cyclic chromatographic process. This result indicates that more efficient cleaning of the resin can be done over many cycles in longer continuous processes. The PCC technology and resin with higher alkaline stability and capacity have the potential to increase productivity in clinical and commercial production and reduce the manufacturing cost per gram of therapeutic protein purified.

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