Continuous chromatography beyond affinity capture of monoclonal antibodies

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CY13808-25May20-PT

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Continuous chromatography beyond affinity capture of monoclonal antibodies

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Abstract

The interest in process intensification and process control in the biopharmaceutical industry has heavily increased in recent years, leading to evaluation of continuous and/or semi-continuous processing. The efforts in continuous chromatography so far have mainly been focusing on affinity capture of monoclonal antibodies (mAbs) but the interest in exploring other applications is growing.

In this poster, we demonstrate the use of periodic counter-current chromatography (PCC) in bind-elute, flow-through, and desalting applications using different chromatographic techniques, and a brief example of an integrated process is presented.

Continuous mAb capture and PCC

In the evaluation of 3-column (3C) and 4-column (4C) PCC technology in mAb processes, so far, features that increase process control and support process intensification have shown to be of great value, for example:

- **Dynamic control** enables stable process performance under changing conditions (Fig 1):
  - Sample concentration
  - Column performance
  - Column bed volume
- **Interchangeable UV LED flow cells** to cover a range of titers.
- **Real-time trending** to monitor process performance between cycles:
  - Sample loading volume
  - Peak amount
  - Pressure
- **Resin lifetime studies** confirm that resin performance is consistent when used in a PCC application (Fig 2).

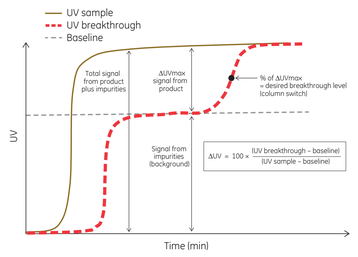


Fig 1. Principle of dynamic control.

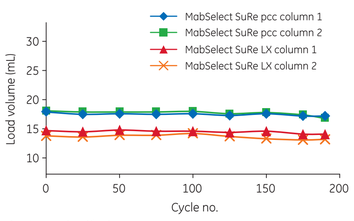


Fig 2. Resin lifetime study.

Dynamic UV control used in mAb purification

Proof of concept presented by Merck, NJ, US shows a robust and efficient process for three different mAbs, when using the ÄKTA™ pcc 75 system (Fig 3) in a broad range of titers:

- Cell culture development not critical for controlling column loading if using robust dynamic control with shorter UV path length (0.4 mm).
- The system can dynamically control continuous column loading up to about 31 g/L feeds independent of cell culture background.
- Significant reduction in resin volume and increase in affinity chromatography productivity using 3C PCC (Table 1).
- Product pool purity consistent across different feeds (Fig 4).

Table 1. Comparison of resin volume and producitivity between a batch and a PCC process

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

Fig 3. ÄKTA™ pcc 75 is controlled by UNICORN™ software.

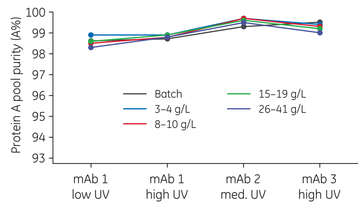


Fig 4. Purity results from runs with continuous loading of varying cell culture feeds.

Acknowledgements

Merck, NJ, US: Darshini Shah, Collette Cutler, Hong Li, Nihal Tugcu, David Roush.

Sanofi Pasteur, Marcy L'Etoile, France: Lucile Vaille, Arthur Leclercq, Eric Calvosa.

GE Healthcare: Christer Eriksson, Erik Östlund, Anders Bjurman, Magnus Nilsson, Lotta Molander.

Application examples

Proof of concept: desalting using ÄKTA pcc 75

Separation of protein and salt peaks in a desalting experiment with a 4C PCC setup (Fig 5). Automated desalting with PCC increases efficiency through parallel sample loadings, elutions, and cleaning sequences.

Columns:
4 × 53 mL HiPrep™ 26/10 Desalting (Sephadex™ G-25 resin).

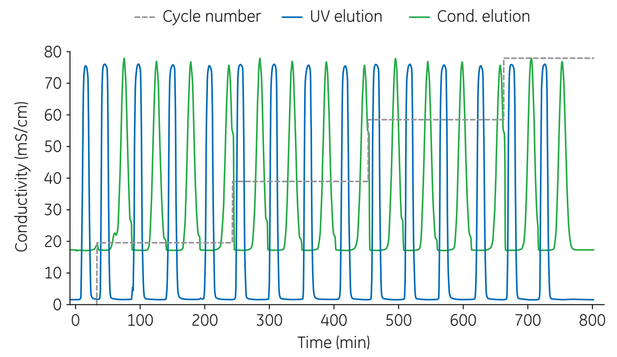
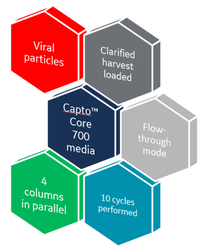


Fig 5. Protein peaks and salt peaks separated by desalting on Sephadex G-25 in 4C PCC mode.

Proof of concept: continuous chromatography for vaccine process purification

Initial trial results from Sanofi Pasteur, France demonstrate:



- Consistent 10 chromatography cycles using ÄKTA pcc 75 (Fig 6).
- Baseline detection and dynamic control efficiency.
- Purity and yield in accordance with expectations.
- Dynamic control functionality useful for process adaptation.
- Trend curves for real time process monitoring to ensure a constant product quality.
- Purity and yield similar to batch mode, however, the productivity increase is moderate due to Capto™ Core 700 specificity that offers high load rate already in batch mode.
- Quick operations with known and robust hardware and software.
- System targeting bind-elute mode: limitations in software to support flow-through applications*.
- Low number of columns required is compatible with large-scale process, where equipment already is available.

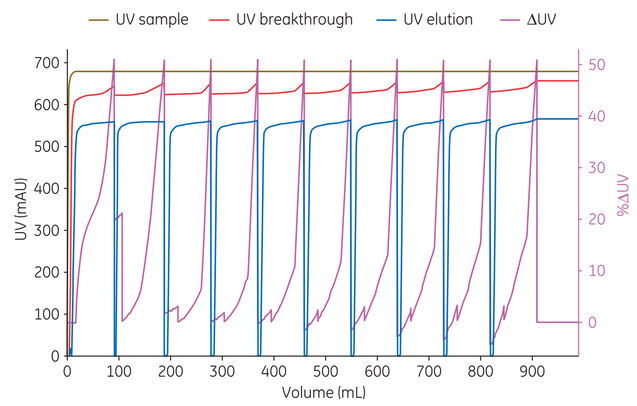


Fig 6. Chromatogram from viral purification on Capto Core 700 in 4C PCC mode on ÄKTA pcc 75.

*GE will release software package with improved support for flow-through applications.

PCC used in polishing and concentration steps in a plasma protein purification process

Example results from purification of human IgG, using the process outlined in Figures 7 and 8, are shown in Figures 9 to 11.

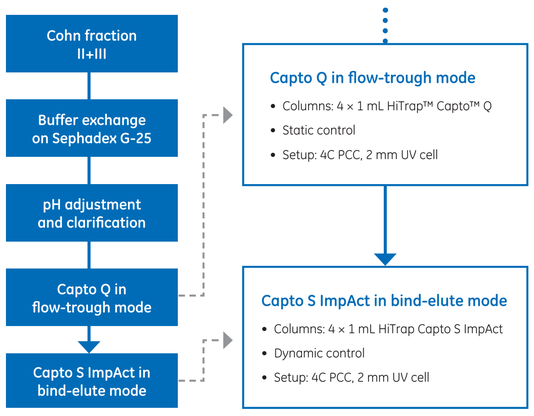


Fig 7. Traditional plasma protein purification process.

Fig 8. Process steps run in 4C PCC mode in ÄKTA pcc 75.

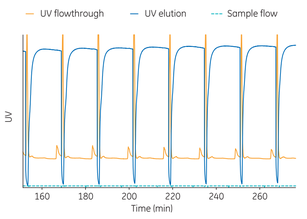


Fig 9. Protein peaks from anion exchange in flow-through mode using 4C PCC.

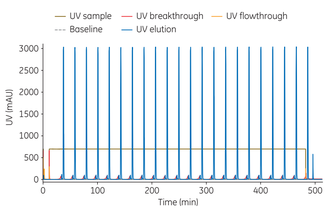


Fig 10. Protein peaks from cation exchange in bind-elute mode using 4C PCC.

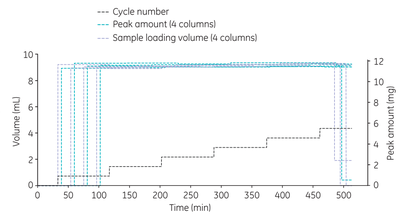


Fig 11. Real-time trending of sample loading volume and elution peak amount from cation exchange in bind-elute mode using 4C PCC show consistency in operation.

Test bed for integrated processes

Overarching test software (Fig 12) controlling unit operations to:

- define critical user needs for integrated process development.
- find solutions to support integrated bioprocessing, enabling real-time release testing, and use of process analytical technologies to control processes.

Fig 12. Overarching test software to monitor and control an integrated process.

Conclusions

The proof of concepts presented show that the PCC technology can be adapted to most of the common chromatographic techniques in both binding and flow-through mode as well as in desalting. This makes PCC suitable for a variety of application areas, such as purification of recombinant proteins, native proteins, and vaccines. Continuous chromatography might not be the most optimal solution for all processes/companies/facilities, but it should be considered as one of the tools in the toolbox to be evaluated when developing or optimizing different types of processes. The emerging focus on integrated processes with process control functions and intelligent automation is an interesting area for the industry to explore further, learn more about, and develop for efficient processing of biomolecules in the future.

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29284412 AA 09/2017

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