ChromaCon

Dynamic Process Control for 2C-PCC Counter-current Capture Processes (CaptureSMB®)

The Contichrom® CUBE chromatography systems use batch and continuous periodic counter-current (PCC) processes for purification of target proteins by affinity chromatography. PCC optimizes the utilization of the chromatography resin capacity during the capture step, typically providing improvements of 40%-60% compared to traditional batch chromatography. In addition, the throughput can be increased up to two-fold. Faster processing preserves protein integrity during the critical step of feed loading where target proteins are exposed to proteases and glycosidases. The simple twin-column system configuration minimizes the risk of hardware failure. 2C-PCC (CaptureSMB®) is the least complex and most effective PCC process.

This application note shows how AutomAb®, the dynamic process control of the Contichrom® CUBE FPLC system for the CaptureSMB® process, can be used to adjust for variations in binding capacity of the chromatography medium due to resin wear-out or for changes in feed composition when different protein concentrations are used. Dynamic control is in line with FDA's initiative to improve product quality by employing process analytical technology (PAT).

Introduction

Continuous chromatography is provides significant benefits with the advantages of higher throughput and lower cost of goods due to reduced Protein A resin and buffer consumption. As it operates at steady-state it should be also better in providing a consistent product quality. Dynamic process control is needed to keep continuous processes at an optimal set point. The high productivity of PCC processes promises a reduction in the capital costs, as facilities with smaller footprint can provide the same output as much larger batch process facilities.

Basic principle of 2C-PCC (CaptureSMB®)

The 2C-PCC process employs only 2 chromatography columns to create a periodic capture purification step (Fig. 1). It is the least complex and most robust multi-column capture process.

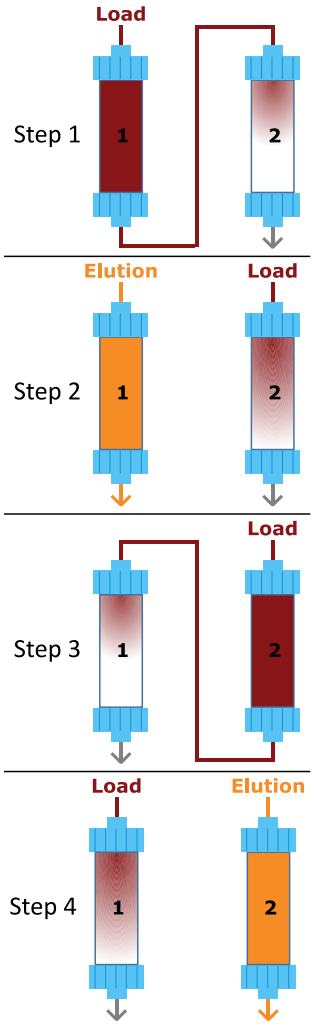


Fig. 1. The principle of 2C-PCC (CaptureSMB®).
Step 1: In the sequential loading phase, columns 1 and 2 are interconnected. Column 1 is fully loaded with sample (red) while its breakthrough is captured on column 2.
Step 2: Column 1 is washed, eluted, cleaned and re-equilibrated while loading continues on column 2.
Step 3: After regeneration of column 1, the columns are inter-connected and column 2 is fully loaded while its breakthrough is captured on column 1.
Step 4: Column 2 is washed, eluted, cleaned and re-equilibrated while loading continues on column 1. This cyclic process is repeated in a continuous way.

It has been scientifically proven to be more effective than other multi-column configurations.* The 2C-PCC process uses several optimized sub-processes for loading, washing, cleaning-in-place and regeneration, where the two columns are appropriately connected or run independently. The loading process is optimized to make full use of the capacity of the expensive affinity resin.

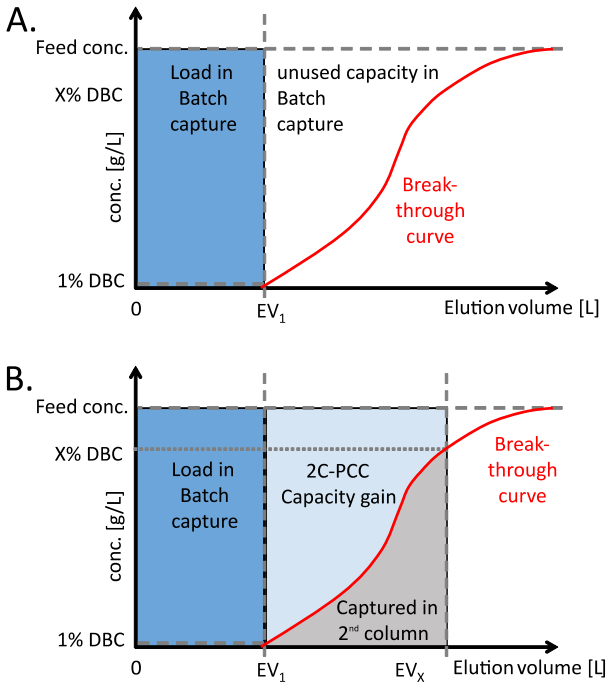


Fig. 2. Capacity utilization for standard vs PCC. A. shows the load in batch capture. In batch capture the load has to be stopped at product breakthrough to avoid product loss. B. In the sequential loading phase of 2C-PCC the first column is loaded beyond breakthrough, e.g. to 70% breakthrough. The breakthrough is captured on the second column. Thereby the capacity utilization of the first column is significantly increased.

2C-PCC using Contichrom® CUBE system

The Contichrom® CUBE systems allow for continuous purification of target molecules. The established twin-column Contichrom® platform is designed to simplify system interaction and operational handling. The dynamic control functionality for capture processes AutomAb® is a key feature of the Contichrom® CUBE. The twin-column process is not only more lean, but also more efficient than batch and other multicolumn processes (Fig. 3).

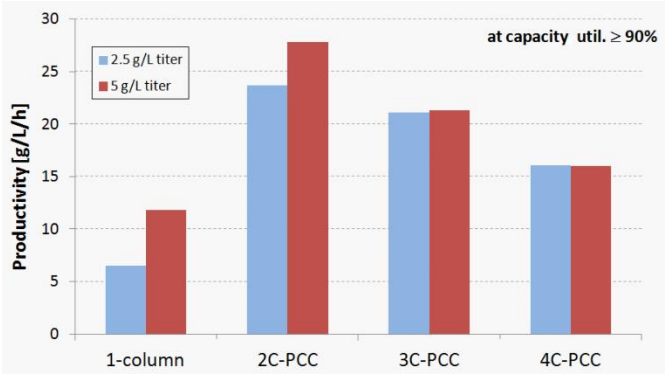


Fig. 3. Productivity of different multicolumn setups for capacity utilization of > 90%. The influence is shown at typical feed concentrations of 2.5 g/L and 5 g/L. The 2C-PCC process is superior to batch and to the more complex three and four column processes.*

The principle of dynamic process control

The Contichrom® CUBE chromatographic system operates with a unique process control algorithm, AutomAb®, that auto-corrects process deviations keeping it at a defined set point. AutomAb® control monitors and controls column saturation levels by automatic adjustments. If process parameters change, for example feed composition or chromatography medium capacity, the loading time is correspondingly adjusted. Feed composition may change in feed quality or concentration of the target protein. Chromatography medium capacity may change due to deterioration of the affinity reagent, for example Protein A for mAbs.

The system can also be operated with static process control, that is, with predefined, fixed column switch times. However any changes introduced into the process are then not absorbed and lead to obtaining varying target product concentrations and quality. Thus the use of dynamic process control is essential when operating continuous processes over many process cycles.

**Reference: Baur D., Angarita M., Müller-Späth T., Steinebach F., Morbidelli M. 2016. Comparison of batch and continuous multi-column protein A capture processes by optimal design. Biotechnology Journal. 11: 1860-7314.*

AutomAb® control concept

AutomAb® is a tool for process control keeping the capture process at an optimum even if process parameters change. When two columns are loaded in series the second column receives the breakthrough (preload) from the first column. AutomAb® keeps the preload area constant by controlling the interconnected loading time, ensuring that always the same amount of product is loaded onto the next column during sequential loading. The preload area can be specified by the user or determined by AutomAb® during the first cycle.

Other dynamic process controllers base their adjustments on relative measurements of two UV signals before and after the column that is being loaded, requiring an additional detector. AutomAb® on the other hand requires only a single UV measurement between the two columns (Fig. 4). AutomAb® thus eliminates the risk of faulty adjustments due to relative de-calibration of UV detectors.

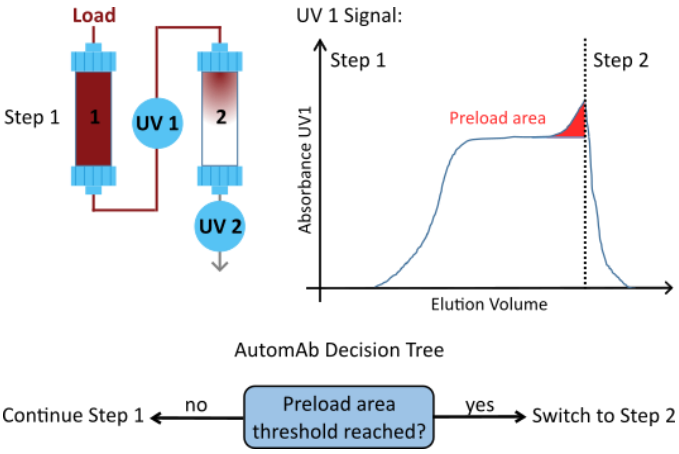


Fig. 4. In the 2C-PCC process each column has its own UV detector located behind the column. When the columns are interconnected, the UV signal of the outflow of the first column is at the same time the UV signal of the inflow of the second column (see above UV1). AutomAb® monitors the UV signal of the outflow of the first column in interconnected mode and keeps the mAb load onto the second column constant.

AutomAb® control in operation

When 2C-PCC is operated over multiple cycles, as in single column capture, the column capacity may decrease, for example due to the depletion of active protein A density on the resin. This effect is commonly observed after repetitive cleaning/washing and caustic treatment. As a result, the preload area value will be reached earlier. Likewise, the protein titer may increase or decrease over time (e.g. in perfusion cell culture), leading to the preload being reached earlier or later. No matter if the preload area is reached earlier or later than in previous cycles, AutomAb® will automatically adjust the loading time to keep the preload area constant. Thus, resin utilization and antibody recovery rates remain at optimal levels. The controller also balances differences in column quality leading to different breakthrough curve shapes (Fig. 5).

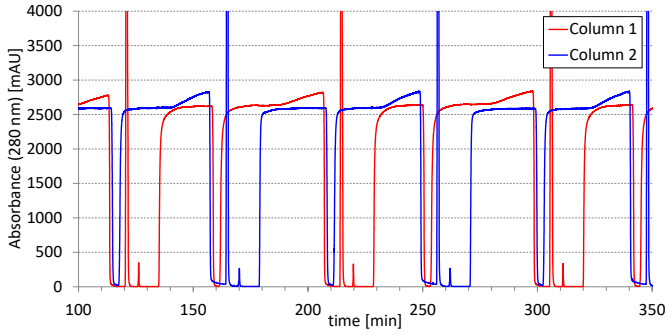
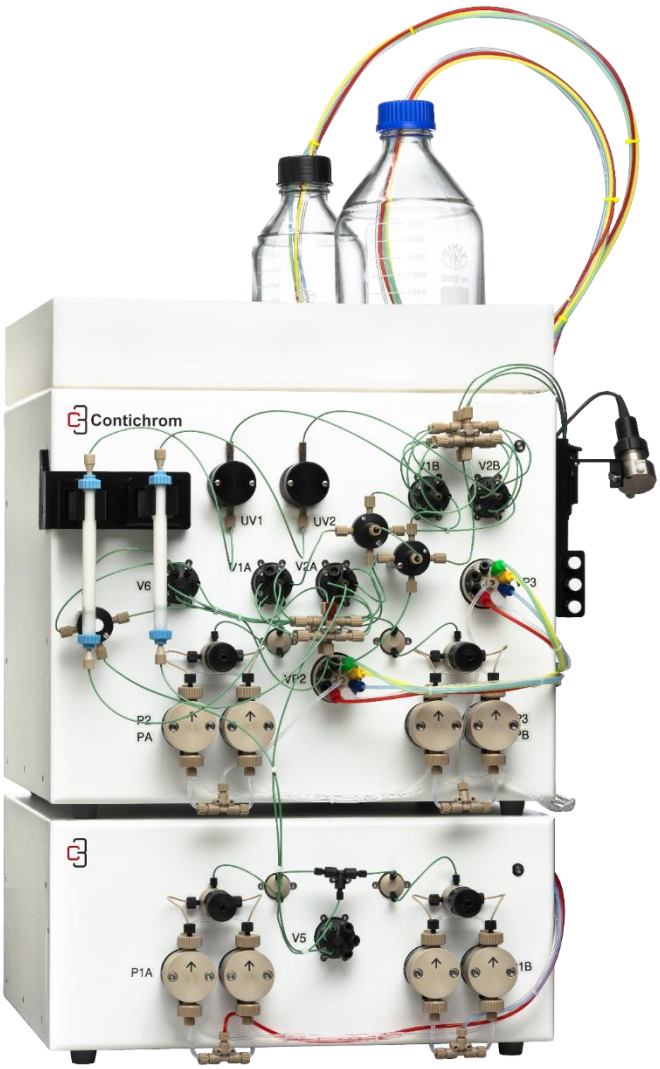


Fig. 5. 2C-PCC run controlled by AutomAb®. Differences in column quality affecting the breakthrough curve shape (red line, column 1, versus blue line, column 2) do not affect the preload.

Increased control flexibility

In contrast to other multicolumn PCC processes the sequential loading time is completely independent of recovery and regeneration tasks being performed in parallel in additional columns. This avoids rate-limiting steps (e.g. long CIP time) going on in parallel to the sequential loading. Therefore AutomAb® has full control of the sequential loading time. This ensures that even under conditions of varying feed titer and column degradation the sequential loading step can be run at maximum flow rate.



2C-PCC design and AutomAb®

Using the CaptureSMB® wizard, methods for 2C-PCC runs are created based on data from a single breakthrough curve (see Fig 6.). These methods reflect optimal 2C-PCC operating points and the wizard predicts the expected run performance in terms of productivity, capacity utilization, buffer and feed consumption, product concentration and run time. AutomAb® control can be selected in the wizard and is activated when the run is started. AutomAb® then maintains the optimal operating point.

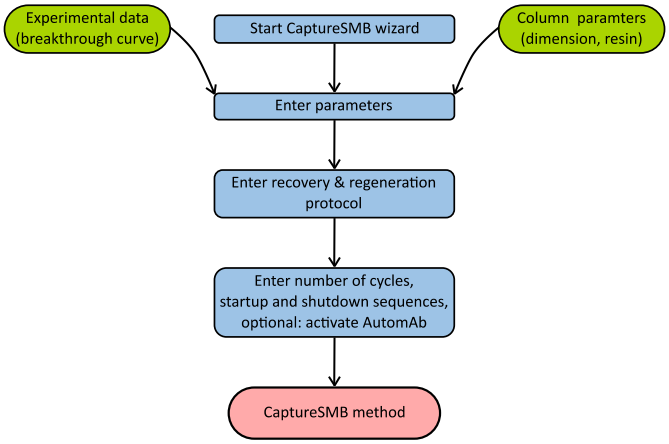


Fig. 6. Flow diagram for creating methods with the CaptureSMB wizard. AutomAb® control can be selected as option.

Conclusions

Undoubtedly, continuous chromatography can tremendously increase the productivity of downstream processing. However, without dynamic process control, additional safety margins have to be set to avoid variations in the eluted product that could lead to a lack in compliance and/or loss of product.

The AutomAb® dynamic control function enables the optimal operation of the 2C-PCC process. Varying process conditions, for example changes in feed concentration or chromatography medium capacity, are compensated. AutomAb® thus enables the use of continuous chromatography for processing perfusion cell culture feeds, where changes in the feed composition may occur.

ChromaCon AG
Technoparkstrasse 1
CH-8005 Zurich
Switzerland
www.chromacon.ch