AutomAb®: UV-based Dynamic Process Control for CaptureSMB

AutomAb is a UV-based dynamic process control method to adjust the CaptureSMB process following decline in binding capacity of the chromatography medium or changes of product compound concentration in the feed.

CaptureSMB is a twin-column continuous chromatography process for the purification of target proteins by affinity chromatography. It 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 (productivity) can be increased 2-3 fold and buffer consumption is typically cut by 40-60%. CaptureSMB offers the least complex multicolumn capture process combined with the benefits of periodic counter-current (PCC) operation.

This application note shows how AutomAb balances a change of 20% in titer and a column capacity decrease of 10%, keeping product yield and process performance of the CaptureSMB process at a maximum. Dynamic process control is in line with FDA's initiative to improve product quality by employing process analytical technology (PAT).

Both CaptureSMB and AutomAb are included with the Contichrom CUBE systems, the versatile benchtop systems for development of continuous twin-column chromatography processes.

Introduction

In affinity chromatography, resin aging due to repeated cleaning is commonly observed. Resin aging leads to a capacity decline of the affinity resin. However, in commercial manufacturing, the intended use time of the resin can be up to 100 or even 200 cycles. Therefore, many affinity capture processes are designed such that future capacity decline is accounted for from the start by including a safety factor in the load set point. For example, affinity columns are loaded up to 90% of the 1% product breakthrough value (1% DBC). In addition, the safety factor accounts for titer variations in the harvest to be processed. Alternatively, to the use of safety factors, robust operation can be achieved by dynamic process control. By monitoring and evaluating the properties of the flow through (or the eluate) in the chromatography process, the control responds to changes in titer and column capacity, keeping the process at optimum load.

Along this rationale, for CaptureSMB, a dynamic process control concept, AutomAb, has been developed.

Basic Principle of CaptureSMB

The CaptureSMB process employs two chromatography columns to create a continuous capture step (Fig. 1). It is the least complex process using the periodic countercurrent principle (PCC).

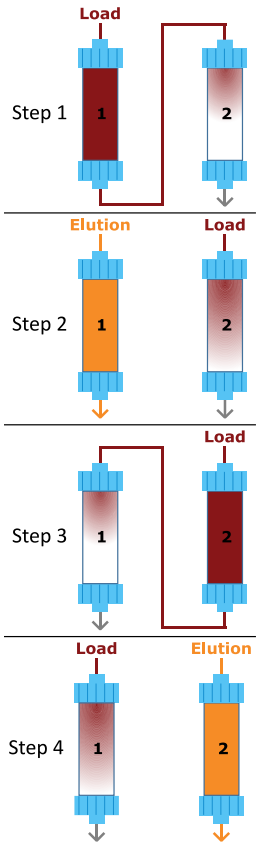

Fig. 1. The principle of CaptureSMB
Step 1: In the interconnected loading phase, columns 1 and 2 are interconnected. Column 1 is fully loaded with sample (red) while its product breakthrough is captured on column 2.
Step 2: Column 1 is washed, eluted, cleaned and re-equilibrated while loading is continued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

The improved throughput and resin capacity utilization of CaptureSMB in comparison to single column batch chromatography is due to the overloading of the first column (beyond breakthrough) while the columns are interconnected as shown in Fig 2.

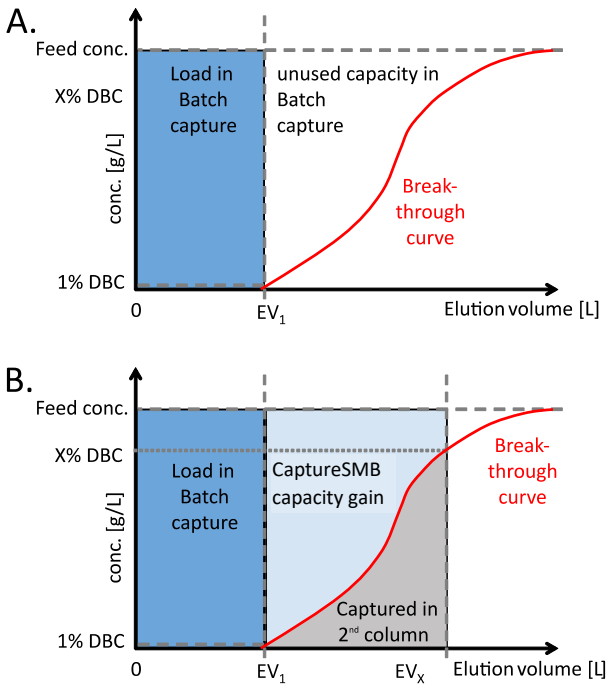

Fig. 2. Capacity utilization of Batch chromatography (A.) vs. CaptureSMB (B). In batch capture (A.) the load has to be stopped at product breakthrough to avoid product loss. B. In the interconnected loading phase of CaptureSMB (B.) the first column is loaded beyond breakthrough, e.g. to 70% breakthrough. The breakthrough is captured on the second column. Thereby the capacity utilization of the first column is significantly increased.

AutomAb control concept

AutomAb monitors the breakthrough curve of product during the interconnected loading stage (Steps 1 and 3 in Fig. 1) using a UV detector that is located between the two columns (position "UV 1" in Fig. 3). The product breakthrough curve (see red line in Fig. 2) is typically visible as rise of the UV signal above the impurity baseline, as shown in (Fig. 3). Using the impurity baseline, AutomAb continuously integrates the rising UV signal while the two columns are loaded in interconnected mode. The area under the rising breakthrough curve is directly proportional to the mass of product that is transferred from the first to the second column. This area is denoted as "preload area" in Fig. 3 and represents the control parameter of AutomAb. 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 interconnected loading. The preload area can be specified by the user or automatically determined by AutomAb during the first cycle of CaptureSMB operation. The impurity baseline values for integration are determined separately by each detector while in position "UV 2" (Fig. 3). Thus, the area measurement of each of the two detectors used in the twin-column CaptureSMB process relies on its own baseline.

This is an advantage as the detectors do not need to be calibrated against each other or an external reference. AutomAb thus eliminates the risk of faulty adjustments due to relative de-calibration of UV detectors.

AutomAb benefits from the flexibility that the CaptureSMB process provides: Due to the two-column setup, the duration of the interconnected loading stage (Steps 1 and 3 in Fig. 1) can be fully controlled by AutomAb as there are no parallel process steps which would require waiting times.

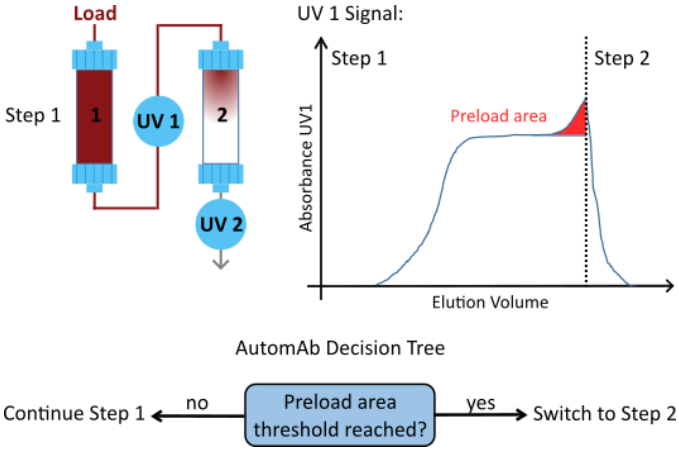

Fig. 3. In the CaptureSMB process each column has its own UV detector located behind the column. When the columns are interconnected, the UV signal of the outflow of the first column is at the same time the UV signal of the inflow of the second column (see above UV1). AutomAb monitors the UV signal of the outflow of the first column in interconnected mode and keeps the mAb load onto the second column constant by controlling the preload area.

The AutomAb controller can be constrained to allow control parameter changes only within the design space. AutomAb does require visibility of the product breakthrough and therefore a sufficiently high product titer in the starting material. In purification of mAbs, typically a titer of 0.5-1.0 g/L is sufficient, depending on the impurity level.

AutomAb response to change in titer

Two CaptureSMB runs were designed using the CaptureSMB Wizard that is embedded in the ChromIQ operating software of the Contichrom CUBE system. The load material was clarified cell culture harvest containing mAb. The runs were operated on using 2 Protein A affinity columns of 0.8 cm i.D. and 10 cm bed height. Each run was operated for 5 cycles. For the first run (Run A), the original harvest with a mAb titer of 6.0 g/L was used. For the second run (Run B), the harvest, diluted to 5.0 g/L using de-ionized water, was used. AutomAb was activated for both runs using the same preload area setpoint value. The resulting chromatograms are shown in Fig. 4. Both chromatograms show the repetitive pattern of loading and elutions from both columns that are characteristic of CaptureSMB.

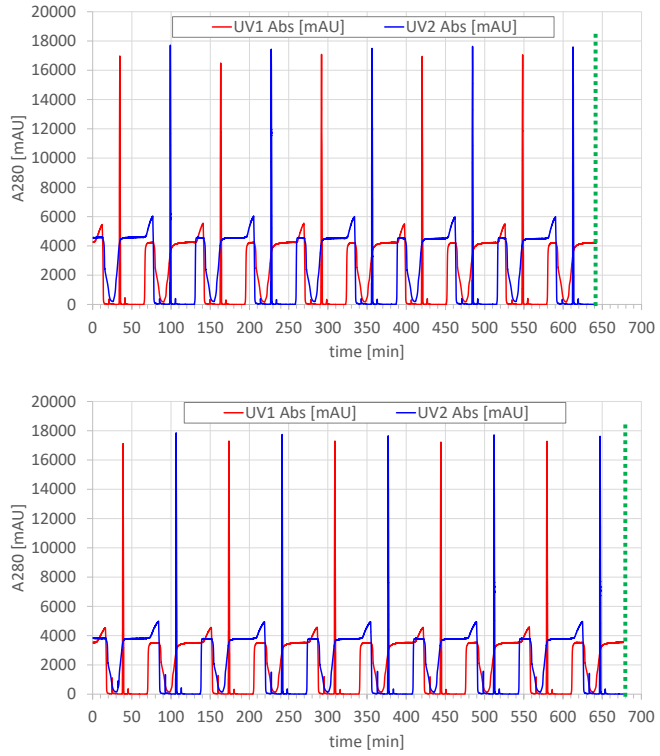

Fig. 4. Chromatogram of CaptureSMB Run A (top), 6 g/L titer, and Run B (bottom), 5 g/L titer. Red and Blue signals correspond to detectors UV1 and UV2, respectively. The end of the runs is marked by a green dashed vertical line.

However, a difference in run duration becomes obvious. While the duration of run A is 640 min, the duration of run B is 680 min. The difference is due to AutomAb, that has prolonged the interconnected loading times in response to the lower feed titer in Run B. An evaluation of the feed material consumption showed that, in Run A, 418.1 mL of harvest had been loaded and in Run B, 505.3 mL of harvest was loaded. Thus, in Run B, almost exactly 20% more feed material was loaded, which corresponds to the titer difference of the starting material (5.0 g/L vs. 6.0 g/L) as expected. A closer look at the pre-load area values and the product pool concentrations reveals that, despite the 20% in feed titer, the values were very similar between Run A and Run B.

Table 1: Preload areas of UV1 [mAUs] and product pool concentrations [g/L] of Runs A and B listed by cycle. The last row shows the average values.

	Preload area UV1		Product conc [g/L]	
Cycle	Run A	Run B	Run A	Run B
1	6211	6542	31.8	30.8
2	6739	6374	32.9	32.5
3	6691	6446	33.2	32.2
4	6505	6472	33.1	32.5
5	6480	6478	33.2	32.5
average	**6525**	**6462**	**32.8**	**32.1**

With static process control, that is, with predefined, fixed interconnected loading times, the durations of Run A and Run B would have been identical as the same volume of feed material would have been loaded. Consequently, one would have obtained a 20% lower product concentration in Run B due to the lower feed titer.

AutomAb response to change in column capacity

A CaptureSMB run (Run C) was operated on the Contichrom CUBE system using two different sets of Protein A affinity columns of 0.8 cm and 10 cm bed height. Initially, the process was operated for 7 cycles with a set of fresh columns. Then, after elution of column 2, this column was replaced by an aged affinity column with 10% lower static capacity and the process was continued for 4 more cycles (see Fig 5). The product pool concentrations from each cycle were determined using analytical Protein A chromatography and load and yield were calculated for each cycle. During the first seven cycles of the run these values were constant with an average yield of 94% and a load of 48.8 g/L, indicating that the process had reached a cyclic steady state. During the last 4 cycles, the average yield was still at 92% while the load had dropped to 45.7 g/L. The observations can be explained through AutomAb: After column exchange, the column with the lower capacity exhibited an earlier breakthrough of product. This was recognized by AutomAb and the interconnected loading time was abbreviated as soon as the target preload area was reached, leading to a smaller load per cycle. Through the decrease in load, the yield was kept high. Assuming a CaptureSMB process without

AutomAb and fixed process control, one would have observed a drop in yield to 85-89% as consequence of the overloading of the column with the decreased capacity. These results show that AutomAb can be used to operate CaptureSMB at optimal load while maintaining a high yield of > 90%.

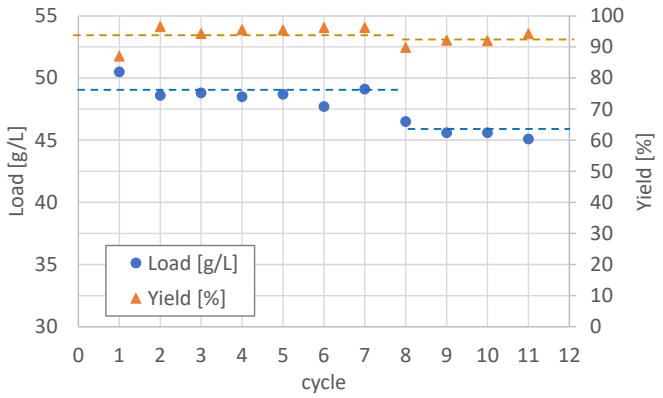

Fig. 5. Load and Yield of CaptureSMB Run C (top), showing the first 7 cycles with original columns, and the next 4 cycles operated with a column set with lower capacity. The dashed lines represent the average values of Load and yield for the 7 first and the last 4 cycles, respectively.

Fig. 6 Contichrom CUBE benchtop chromatography system

Conclusions

The AutomAb dynamic control function supports the optimal and robust operation of the CaptureSMB in response to titer variations and column capacity decline. With AutomAb keeping the process demonstrably under control, the use of safety factors can be minimized. AutomAb is available for Contichom CUBE benchtop and Contichrom TWIN pilot/production systems.

Fig. 7 Contichrom TWIN process scale chromatography system

Contichrom® CUBE 30/100 System Specifications	
Flow rate range	0.1 – 36 / 0.1 – 100 mL/min
Pressure rating	100 bar
Number of columns	2
Number of buffers	Up to 18
Fraction collector	Foxy R1/R2 with multiple rack options
UV Detectors	2x external 4-Channel detectors. Abs 200-600nm. + detector behind each column
Conductivity/pH detectors	2/1 included

Ordering information

Product	*Order #*
Contichrom® CUBE 30	CC220029
Contichrom® CUBE 100	CC220030

For inquiries regarding the Contichrom systems, please visit www.chromacon.com or contact sales@chromacon.com

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