CaptureSMB® for the continuous purification of mAbs

CaptureSMB® is an optimized twin column periodic countercurrent (PCC) process used for the continuous purification of monoclonal antibodies (mAbs) and antibody fragments using affinity resins such as Protein A and Protein L. CaptureSMB® has been implemented at lab and GMP production scale offering significant benefits compared to single-column batch chromatography.

Main performance benefits include:
- Automated and continuous capture of mAbs
- 40-60% reduction in affinity resin costs due to the full utilization of resin capacity
- 40-60% reduction in buffer requirements
- 2- to 6-fold increase in productivity compared to batch and concomitant reduction in equipment footprint
- Increased flexibility of production scheduling
- Least complex and most robust multi-column configuration
- Successfully passed virus and process validation studies

This application note describes the development of a lab-scale mAb capture purification step employing the CaptureSMB® process using the Contichrom® CUBE system. A 400 mL batch of CHO cell supernatant was processed with twin Protein A columns (1 mL) during an 8 h period. The results demonstrate the typical CaptureSMB® process performance and productivity gains compared to a batch process.

Introduction

CaptureSMB® is a patented technology allowing antibody process developers to greatly improve the economics of antibody purification without facing additional regulatory hurdles. The simple twin column process configuration allows for reaping the full economic benefits of multi-column configurations, but with reduced regulatory and operational risk compared to other multi-column configurations.

CaptureSMB® is a continuous process which can be operated on all Contichrom® CUBE and Contichrom® CUBE Combined systems. ChromIQ®, the operating software of the Contichrom® systems, provides the wizard for designing and operating the CaptureSMB® processes.

CaptureSMB® differs from a plain 2-column PCC process, as CaptureSMB® allows for an optimized modulation of flow rates leading to an increased productivity compared to a standard 2-column PCC process. Increasing productivity at the mAb capture step is the main driver for implementing continuous processing, as large volumes of feed have to be processed at the capture step. The primary economic benefits of PCC processes are often concomitant with the preservation of product integrity/quality. It has been shown that CaptureSMB® is easier to validate than other multi-column processes, while providing equal or superior process performance. CaptureSMB® is offered with a dynamic process control (AutomAb®), which automatically keeps the continuous capture process at an optimum. AutomAb® prevents product yield losses due to a gradual decrease in Protein A / L capacity or variations in feed titer. CaptureSMB® also offers the benefit that it has been combined with further continuous downstream steps such as virus inactivation and polishing steps, allowing automation solutions for the entire downstream processing train.

Any CaptureSMB® process developed with the Contichrom® system is directly scalable to the EcoPrime® Twin GMP system employing the CaptureSMB® process.

CaptureSMB® principle

In batch chromatography, an affinity column is loaded up to the point of breakthrough of the product and then stopped in order to prevent product losses. As the shape of the breakthrough zone is sigmoid, a significant portion of the expensive affinity matrix is not utilized. With CaptureSMB® a second identical column is connected to the product outlet of the first column, allowing to continue loading beyond the first column breakthrough thereby fully saturating the capacity of the affinity matrix. Then the fully loaded first column is disconnected from the second column, washed / eluted then cleaned and reconditioned for further use (Fig. 1), while in parallel, the second column is loaded with feed. The first cleaned column is now placed behind the second column to again allow the capture of the breakthrough product. This cyclic process is then repeated multiple times until the entire feed material has been processed.
In a CaptureSMB® run, the total number of cycles carried out is determined by the volume of feed to be processed and the capacity of the columns used. Due to cyclic processing over two columns, resin utilization is maximized and productivity greatly increases compared to the batch method.

With advanced process control software, CaptureSMB® automatically applies an optimized dual loading flow-rate strategy to enhance process performance. In the interconnected phases, the columns are operated at maximum possible feed flow rate; in the batch phases, the column that was previously in the downstream position continues loading at the same or lower flow rate, depending on the feed titer. A lower feed flow rate guarantees that no breakthrough of feed from the single column occurs. As a result, this leads to an improved dynamic capacity in the batch feeding steps.

In addition, if the end of a column lifespan is reached during the run then the column can simply be replaced mid-cycle.

**CaptureSMB® for mAb purification**

CaptureSMB® is often used for the isolation of monoclonal antibodies. Three steps are required to execute a successful run:

**Step 1: Defining batch run parameters**

CaptureSMB® uses many of the same process parameters as batch purification and the majority of protocol optimization should be carried out in batch mode to save feed material. Improvements made to the washing, equilibration, elution or cleaning-in-place (CIP) steps in batch mode will translate directly to the CaptureSMB® method. A generic purification protocol for mAbs used in this application note works as a good starting point. The feed material has to be pre-filtered.

The method outlined in Tables 1 & 2 was carried out using the Contichrom® CUBE Combined using the ChromIQ® batch purification wizard.

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In addition, if the end of a column lifespan is reached during the run then the column can simply be replaced mid-cycle.
Step 2: Generation of breakthrough curves

Before initiating a CaptureSMB® method, an experimentally generated breakthrough curve is required to calculate an optimal time when the switch from the “interconnected” to “batch” phases should occur. This prevents breakthrough of antibody from the downstream column during loading, while at the same time ensuring the highest possible capacity utilization.

The characteristics of the breakthrough curve depend upon a combination of factors including the Protein A resin source, column dimensions, feed composition and loading flow rate. In order to obtain the necessary information for the CaptureSMB® setup, it is necessary that a breakthrough curve is experimentally generated each time column dimensions or loading flow rates are changed.

A single column breakthrough curve was generated at a flow rate of 600 cm/h (Fig. 2). The concentration of mAb in the flow-through was determined at intervals during the loading of a single column. Feed and breakthrough concentrations were determined using offline analytics (data not shown).

![Breakthrough profile of a mAb loaded on a single Protein A batch column at a flow rate of 600 cm/hr. The UV A280nm signal is shown. Breakthrough occurs on the column after 10 min. The arrows indicate the five fractions of flow-through that were analyzed offline for mAb content. The mAb concentration and the corresponding load volume obtained from the five fractions were then computed to obtain a dynamic breakthrough curve shown in Fig. 3.](image)

Antibody breakthrough concentrations were entered into the Loading Tab of the ChromIQ® wizard and used to fit a breakthrough curve from which the method was automatically optimized (Fig. 3). A breakthrough value of 70% of the maximum of the dynamic breakthrough curve (DBC) was selected for process design, meaning that during the interconnected loading phase the first column is loaded to 70% breakthrough with respect to the mAb concentration in the feed. Choosing a value of 65-85% DBC typically ensures a Protein A resin capacity utilization of > 90%. Going to higher DBC values would lead to only marginal improvements of resin capacity utilization at the cost of productivity.
Fig. 3. Dynamic Breakthrough Curve (DBC) automatically fitted using the experimentally generated antibody breakthrough concentrations. The values of the five fractions from the breakthrough curve indicated in Figure 2 were inserted into the table on the left, automatically generating the breakthrough curve shown on the right. The 70% breakthrough value is shown as a red vertical line.

In order to ensure that at high flow rates (>300 cm/h using columns < 10 cm bed height) no breakthrough happens with a second column connected in series, another breakthrough curve using two columns connected in series is needed. The breakthrough profile for two connected Protein A columns loaded with 70 mg/ml mAb at a flow rate of 600 cm/h is shown in Fig. 4. The chromatograms confirm that breakthrough from Column 2 does not occur prior to the 70% DBC value, i.e. no product losses are expected during the interconnected loading step of the CaptureSMB® process. This second breakthrough curve is done for verification purposes only and does not need to be done for low flowrates or columns with at least 10 cm bed height.

Fig. 4. Breakthrough profile for two Protein A columns connected in series with a load of 70 mg/mL mAb at a flow rate of 600 cm/h. The UV A280nm signal is shown for Column 1 (red line) & Column 2 (blue line). Breakthrough occurs on the first column after having loaded 10 mL of starting material. The arrow indicates when breakthrough occurs from Column 2 (at 45 mL). The red area under the curve indicates the breakthrough of product from the first column to the second column and the green area indicates the breakthrough form the second column.
Step 3: Design and execution of the CaptureSMB® run

Loading Tab:
Information about column dimensions and flow rate used in the breakthrough experiments, as well as the feed titer was entered into the Loading Tab:

![CaptureSMB Design Wizard](image)

**Fig. 5.** Loading Tab of CaptureSMB Wizard in ChromIQ. The column and feed parameters are entered in top part. The lower part of the tab shows the output data of the breakthrough curve fitting.
Recovery & Regeneration Tab:
Wash, elution and cleaning steps of the batch process were inserted in the Recovery & Regeneration Tab:

![CaptureSMB Design Wizard for ChromIQ](image)

Fig. 6. Recovery & Regeneration Tab of CaptureSMB Wizard in ChromIQ. The wash, elution and cleaning protocol values of the batch run were inserted into the lines on the left side.
Method Settings & Performance Tab:

**Fig. 7.** Method Settings and Performance Tab of CaptureSMB Wizard in ChromIQ. The number of Capture SMB cycles was entered to match the feed volume available for the run (left side). The “Performance Computations” window on the right side shows the full set of performance parameters as an output.

After completing data entry into the three tabs, the CaptureSMB® method is generated automatically. The method was then executed.
CaptureSMB® Results:

Fig. 8 and 9 show the A280nm signal for Column 1 & Column 2 recorded during the CaptureSMB® run. Fig. 8 gives an overall visualization of the CaptureSMB® run whereas Fig. 9 is a comparison of 10 overlaid cycles. Cycles are highly comparable and no loss of product from breakthrough is seen.

![Fig. 8. UV A280nm signal form 10 cycles of the CaptureSMB® run.](image)

![Fig. 9. An overlay of all the UV signals of the individual CaptureSMB® cycles from one run shows that there is minimal deviation in performance between each cycle and a predictable elution profile due to the dynamic process control algorithm, AutomAb®.](image)

Table 3 shows a summary of the CaptureSMB® process performance data compared to the same task completed in batch mode.

<table>
<thead>
<tr>
<th></th>
<th>CaptureSMB®</th>
<th>Batch</th>
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<tbody>
<tr>
<td>Feed volume processed/cycle</td>
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<td>38</td>
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<tr>
<td>Feed concentration [g/L]</td>
<td>2.7</td>
<td>2.7</td>
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<tr>
<td>Product pool conc. [g/L]</td>
<td>23.7</td>
<td>2</td>
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<tr>
<td>Yield [%]</td>
<td>98.8%</td>
<td>98.0%</td>
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<tr>
<td>Productivity/resin volume [g/L/h]</td>
<td>65</td>
<td>33</td>
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<tr>
<td>Buffer consumption [L/g]</td>
<td>0.5</td>
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<tr>
<td>Capacity utilization [%]</td>
<td>98%</td>
<td>16%</td>
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<tr>
<td>Cycle time [min]</td>
<td>48</td>
<td>14.5</td>
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</table>

The results show the superiority of CaptureSMB® over the batch chromatography process, including a 2-fold improvement in productivity, a 6-fold improvement in resin capacity utilization, a 7-fold reduction in buffer consumption and a 10-fold higher product concentration.

Dynamic Process Control

AutomAb® dynamic process control can be operated together with CaptureSMB® allowing for optimized performance. Fluctuations in feed quality, target protein concentration or gradual decrease in the capacity of the affinity matrix are automatically compensated for and process performance remains stable over time. 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. The principles of AutomAb® are explained in detail in a separate Application Note.

Summary

Using the Contichrom® CUBE system with the CaptureSMB® wizard, a batch capture method could be quickly converted into a continuous CaptureSMB® process. The results clearly demonstrate the superiority of CaptureSMB® over batch chromatography processes. The results showed a

- 2-fold improvement in productivity
- 6-fold improvement in resin utilization
- 7-fold reduction in buffer consumption
- 10-fold higher product concentration
Contichrom® CUBE Combined 30/100 System Specifications

<table>
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<th>Specification</th>
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<tr>
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<td>Pressure rating</td>
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<tr>
<td>Number of columns</td>
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<tr>
<td>Number of buffers</td>
<td>Up to 18</td>
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<tr>
<td>Fractionation</td>
<td>3 fractions (valve), optional fraction collector</td>
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<tr>
<td>UV Detectors</td>
<td>Fixed wavelengths A280, A254, detection behind each column</td>
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<td>Conductivity/pH detectors</td>
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Ordering information

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For enquiries regarding the Contichrom® systems, please visit [www.chromacon.com](http://www.chromacon.com) or contact [sales@chromacon.com](mailto:sales@chromacon.com).
References