CaptureSMB for the continuous purification of mAbs with high productivity and load

CaptureSMB is a 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 with significant benefits over 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, load of 68 g/L in this study**
- **40-60% reduction in buffer requirements**
- **2- to 3-fold increase in productivity compared to batch chromatography and reduction in equipment footprint, productivity was 73 g/L/h in this study**
- **Least complex and most robust multi-column configuration, allows use of short bed heights (5 cm)**

This application note describes the development of a lab-scale mAb capture purification step employing the CaptureSMB process using the Contichrom CUBE benchtop twin-column system and columns of only 5 cm bed height. Due to the CaptureSMB process principle, high load and high throughput could be achieved simultaneously with this column format.

Introduction

CaptureSMB greatly improves the economics of antibody purification. The simple twin column process configuration of CaptureSMB leads to reduced regulatory effort and operational risk compared to other multi-column configurations that use more columns.

CaptureSMB is a continuous process using two columns of the same type. CaptureSMB operates on all Contichrom CUBE benchtop systems. ChromIQ, the operating software of the Contichrom systems, provides a wizard tool for designing and operating the CaptureSMB process.

Increasing productivity and load at the affinity capture step is the main driver for implementing continuous processing, as large volumes of feed must be processed at the capture step. Both aims can be achieved using CaptureSMB. It is offered with UV-based dynamic process control (AutomAb), which automatically keeps the continuous capture process at an optimum. AutomAb prevents product yield losses due to decline of affinity resin capacity or variations in feed titer. Any CaptureSMB process developed with the Contichrom CUBE system is directly scalable to the Contichrom TWIN GMP system.

CaptureSMB principle

In batch chromatography, an affinity column is loaded up to the point of breakthrough of the product and then loading is stopped to prevent product loss. Usually, a safety margin is applied. As the shape of the breakthrough curve is sigmoidal, a significant portion of the expensive affinity resin is not utilized. With CaptureSMB a second identical column is connected to the outlet of the first column, allowing to continue loading beyond breakthrough of mAb from the first column, thereby fully saturating the first column. Then the fully loaded first column is disconnected from the second column, washed, eluted, cleaned and re-equilibrated 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. Due to the loading beyond breakthrough which can be carried out at low residence time, resin utilization is maximized, and productivity greatly increases compared to a single column batch method. CaptureSMB uses a dual loading flow-rate strategy with different feed flow rates during interconnected and parallel loading phases to further enhance process performance and to match loading and recovery and regeneration protocol durations.

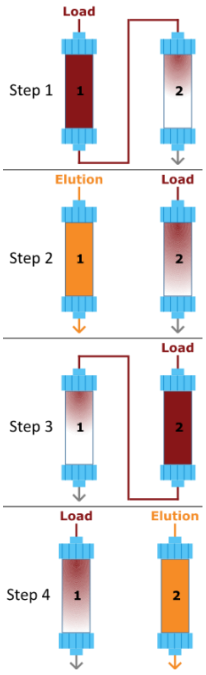

Fig. 1. **Schematic illustration of a single cycle of the twin-column CaptureSMB process. Step 1 & 3: interconnected phases, Step 2 & 4: batch phase.**

Step 1 – In the first interconnected phase, Column 1 is placed upstream of Column 2. Loading of feed proceeds until Column 1 reaches maximum binding capacity while all breakthrough of antibody is captured on Column 2. A wash step is then used to flush any remaining unbound antibody from Column 1 to Column 2.

Step 2 – The process switches to a batch (parallel) phase, where Column 1 is washed, eluted, and cleaned. In parallel, Column 2 is directly loaded with feed until Column 1 is regenerated and ready for Step 3.

Step 3 – In the second interconnected phase, Column 2 is placed upstream of Column 1. Column 2 is loaded to maximum binding capacity while Column 1 captures the breakthrough of antibody in the downstream position. Once loaded, Column 2 is washed in series and Column 1 captures the unbound antibody.

Step 4 – In a second batch (parallel) phase, Column 2 is now washed, eluted, and cleaned. In parallel, Column 1 is directly loaded with feed until Column 2 is regenerated and ready for the next cycle.

CaptureSMB for mAb purification

Three steps, that are described in the following, are required to execute a successful CaptureSMB run:

Step 1: Defining batch run parameters

CaptureSMB uses many of the same process parameters as batch purification and most of the 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 method outlined in Tables 1 & 2 was carried out using the Contichrom CUBE system. The feed material was pre-filtered.

Table 1. - Batch method & materials

Buffer A – Equilibration / Wash	20 mM Na phos, 150 mM NaCl, pH 7
Buffer B – High Salt Wash	20mM Na phos, 1 M NaCl, pH 7.0
Buffer C – Elution/Desorption	0.1 M Sodium Citrate, pH 3.0
Buffer D - CIP	0.1 M Sodium Hydroxide
Feed	clarified harvest 4.8 g/L mAb
Column dimensions (x2)	Volume: 1 mL (Diameter: 0.5 cm, Bed height: 5 cm)
Resin	Purolite Praesto Jetted A50

Table 2. - Batch method parameters

Step	Column Volumes [CV]	Flow rate [cm/h]	Buffer
Equilibration	5	600	Buffer A
Load	5.6	300	Feed
Wash 1	3	600	Buffer A
Wash 2	5	600	Buffer B
Wash 3	5	600	Buffer A
Elute	4	600	Buffer C
CIP	5 (2 for 1 min CIP)	100 (600 for 1 min CIP)	Buffer D
Re-equilibration 1	3	600	Buffer B
Re-equilibration 2	5	600	Buffer A

Step 2: Generating a breakthrough curve

Before initiating a CaptureSMB method, a single experimentally generated breakthrough curve is required to determine CaptureSMB operating parameters such as the load volume of feed material during the interconnected and batch process phases. The characteristics of the breakthrough curve depend upon a combination of factors including the Protein A resin, feed composition and loading flow rate.

Single column breakthrough curves were generated at flow rates of 300 cm/h (residence time RT = 1 min) and 150 cm/h (residence time RT = 2 min). In each case, the flow-through of the column was fractionated and the mAb concentrations in the fractions and the feed were determined by offline analytics (see Fig. 2). For CaptureSMB design, only the 1 min RT curve

was used. The 2 min RT curve was used to determine the optimal load for a single column reference run (See Table 3).

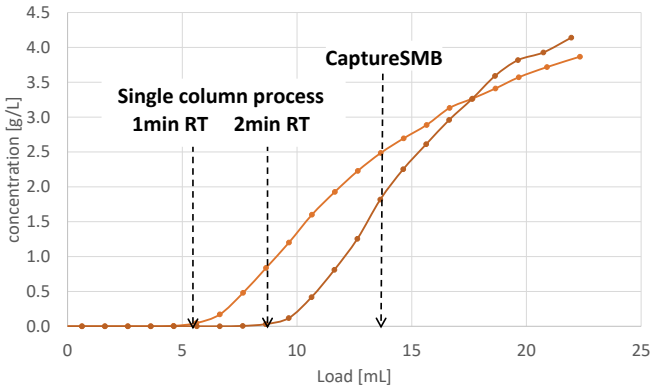

Fig. 2 **Single column breakthrough curves (at 1 and 2 min Residence times. The vertical arrows indicate the degree of loading of the single column processes and CaptureSMB as reported in Table 3**

Step 3: Designing and running CaptureSMB

The CaptureSMB methods to be run on the Contichrom CUBE system can be easily generated using the CaptureSMB wizard, a software tool embedded in the ChromIQ operating software. For process design, the breakthrough curve data from a single column, column dimensions, feed concentration and the recovery and regeneration protocol are required. From these inputs, the CaptureSMB wizard determines the CaptureSMB operating parameters, including the feed flow rates and the loading time. Thereby the load volume of CaptureSMB exceeds the load volume of single column chromatography (see Fig. 2). Next, the UV-based dynamic process control AutomAb® is enabled and the number of CaptureSMB cycles is entered by the user depending on the amount of starting material to be processed. Finally, the methods are generated in the CaptureSMB wizard and the process is run on the Contichrom system. The process design of CaptureSMB was carried out using the 1 min RT breakthrough curve data.

CaptureSMB Results

The CaptureSMB process was run over 6 cycles. Fig. 3 and Fig. 4 show the 280nm signal for Column 1 and Column 2 recorded during three cycles of the CaptureSMB run. Fig. 3 gives an overall visualization of the CaptureSMB run whereas Fig. 4 is an overlay for cycle comparison. Having a UV detector placed at the outlet of each column, the profiles for both columns result in an alternating pattern of loading and elution phases. The interconnected loading phases clearly show breakthrough of mAb from the upstream columns with only minimal rise of the UV signals of the downstream columns above the impurity baselines, i.e., minimal product loss from the outlet of the downstream column of the two interconnected columns. An overlay of the UV signals of 3 CaptureSMB cycles shows that there are negligible deviations in the UV signals from cycle to cycle. This indicates that the process has reached a cyclic steady state, i.e., that product concentration and quality is expected to be very similar from cycle-to-cycle.

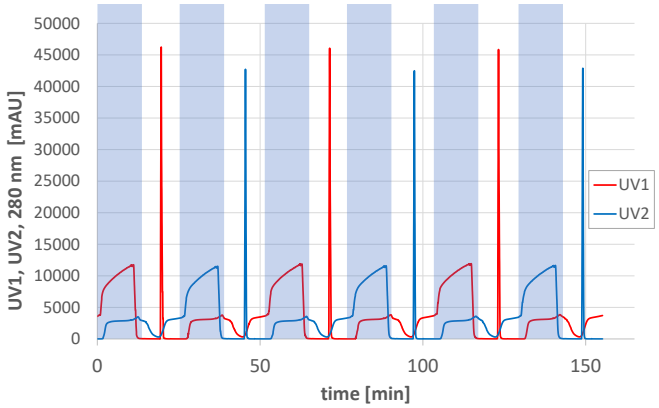

Fig. 3 **UV A280nm signals from 3 cycles of the CaptureSMB run. The interconnected loading phases are indicated by the shaded areas.**

Table 3 shows a summary of the CaptureSMB steady state process performance data compared to single column reference processes with 2 min and 1 min residence times in the loading step and the same recovery and regeneration protocol. The data clearly show a trade-off between Load and Productivity for the single-column process.

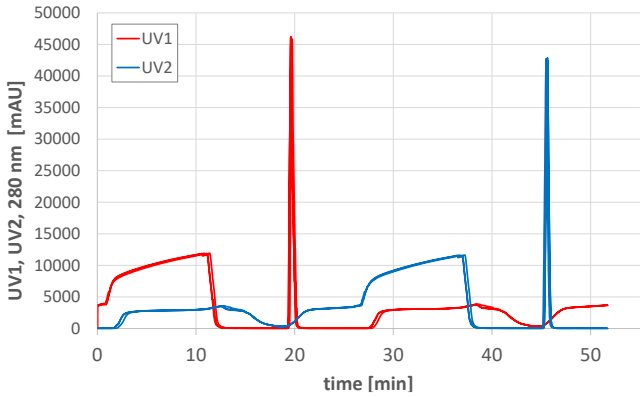

Fig. 4 **Overlay of the UV signals of 3 consecutive CaptureSMB cycles.**

Table 3. Data Summary

		CaptureSMB	Batch (2min)	Batch (1min)
Feed volume processed/cycle	[mL]	27.8	8.8	5.6
Feed concentration	[g/L]	4.8	4.8	4.8
Product pool conc.	[g/L]	15.8	10.1	6.4
Yield	[%]	97.6	95.3	95.5
Productivity/resin volume	[g/L/h]	73.2	47.3	63.9
Buffer consumption	[L/g]	0.43	0.77	1.25
Load	[g/L]	68.0	42.2	26.9
Capacity utilization	[%]	93%	58%	37%
Cycle time	[min]	22.6	41.1	24.1

The results demonstrate the superiority of CaptureSMB over the batch chromatography processes, including up to 50% improvement in productivity (compared to 2 min batch), or a 2.5-fold improvement in resin capacity utilization, a 2.9-fold reduction in buffer consumption and a 2.5-fold higher product concentration (compared to 1 min batch). The results show clearly that CaptureSMB enables the use of short bed heights (5 cm), allowing to reach high productivity and high resin utilization at the same time. For batch chromatography, despite a high productivity value at small residence times (1 min), the resin utilization is extremely low (< 40%).

Dynamic Process Control

AutomAb dynamic process control can be enabled for CaptureSMB in the wizard. Fluctuations in feed quality, target protein concentration (as for example in perfusion cell culture) or gradual decrease in the capacity of the affinity matrix are automatically compensated for and process performance remains stable over time. AutomAb monitors and controls column saturation levels by automatic adjustments to the interconnected loading time based on UV detection.

Scale-up scenarios

Scenario 1: Fixed processing time

Under the constraint of a processing time of 24 hours, in this case study, with a productivity of 73 g/L/h and a load of 68 g/L, CaptureSMB can process a harvest volume of 2000L (at 4.8 g/L titer) with just 5.5 Liters of Protein A column volume (2x 25 cm i.D) and a buffer demand of approx. 4130 Liters. Conventional batch chromatography would require 8.5 Liters of Protein A column volume (30 cm i.D. column) and a buffer demand of almost 7400 Liters (see Table 4). Thus, in this scenario, CaptureSMB can save 3 Liters (35%) of Protein A resin per 2000L harvest, which is especially interesting in clinical trial manufacturing where the columns are used only for one harvest. Moreover, the smaller columns size in CaptureSMB makes it more attractive to use pre-packed columns.

Table 4. Scale-up scenario (fixed processing time)

		CaptureSMB	Batch (2min)
Harvest volume	[L]	2000	2000
Feed concentration	[g/L]	4.8	4.8
Processing time	[hrs]	24	24
Column i.D.	[cm]	25	30
Bed height	[cm]	5.6	12
Total resin vol	[L]	5.5	8.5
Buffer consumption	[L]	4130	7390

Scenario 2: Fixed column volume

Under the constraint of a fixed resin volume of 8.5 Liters, with a productivity of 73 g/L/h and a load of 68 g/L, CaptureSMB can process a harvest volume of 2000L (at 4.8 g/L titer) with (2x 30 cm i.D and 6 cm bed height) in 16 hrs while conventional batch chromatography would require 24 hrs processing time (30 cm i.D. column and 12 cm bed height), as shown in Table 5. The buffer demand would be the same as described in scenario 1. Thus, in this scenario, CaptureSMB can save 50% of the processing time, reducing it from a 24 hours operation to a 16 hours operation, freeing up time for the next project. The scenario would also allow the change from a three-shift operation to a two-shift operation, while maintaining the same output per day.

Table 5. Scale-up scenario (fixed processing time)

		CaptureSMB	Batch (2min)
Harvest volume	[L]	2000	2000
Feed concentration	[g/L]	4.8	4.8
Processing time	[hrs]	16	24
Column i.D.	[cm]	30	30
Bed height	[cm]	6	12
Total resin vol	[L]	8.5	8.5
Buffer consumption	[L]	4130	7390

Summary

Using the Contichrom CUBE system (Fig. **5**) 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:

- Productivity of 73 g/L/h (up to 50% improved)
- Resin capacity utilization of 93% (up from 58%)
- Buffer consumption of 0.43 L/g (down from 0.77 L/g)
- Product concentration of 15.8 g/L (up from 10.1 g/L)
- Simultaneous achievement of high productivity and load (capacity utilization) using 5 cm columns.

CaptureSMB is a scalable process. Several users in the biopharmaceutical industry are operating scale-up equipment, the Contichrom TWIN series, in a GMP environment (Fig. 6). The Contichrom TWIN is available in four different standard sizes with flow rates up to 20 L/min.

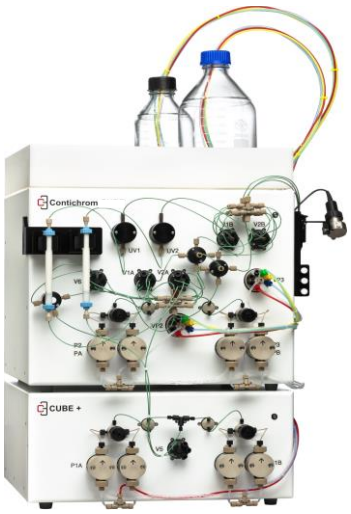

Fig. 5 **Contichrom CUBE benchtop chromatography system**

Fig. 6 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	1-2
Number of buffers	Up to 18
Fractionation	3 fractions (valve), optional fraction collector
UV Detectors	Fixed wavelengths A280, A300, detection 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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