CaptureSMB for Efficient Affinity Purification of Monoclonal Antibodies

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A novel twin column counter-current chromatography process was used for the capture of monoclonal antibodies (mAbs) from clarified cell culture harvest. The process features improved performance in terms of productivity, buffer consumption, product concentration and capacity utilisation compared to traditional batch chromatography. As presented in this case study, the advantages become more pronounced as loading flow velocities are increased. Due to its cyclic nature, the CaptureSMB process is very well suited for the integration with conventional batch-wise as well as with continuous processing.

Introduction

Affinity chromatography stationary phase costs are one of the largest drivers of consumables costs in the downstream processing of biologics. However, in traditional batch chromatography, the stationary phases are not fully utilised due to the dynamic behaviour of column loading: As the feed is loaded an S-shaped internal product concentration front forms within the column as a result of isotherm and mass transfer effects. This leads to an early breakthrough and losses of the protein of interest before the full resin capacity has been utilised. Typically 30-50% of the maximum capacity, i.e. the static binding capacity, is unused in batch chromatography. After loading, the column is washed, the product is recovered and the column is cleaned. Thereby also the fraction of the resin that has not beed utilised is cleaned. Since cleaning is one the decisive drivers for resin degradation it is obvious that significant cost savings can be obtained by improving the capacity utilisation of the chromatography step.

In the twin-column CaptureSMB process, this is achieved by loading one of the columns until a significant amount of product has broken though and by simultaneously capturing the breakthrough in the interconnected second column *(see Figure 1).*

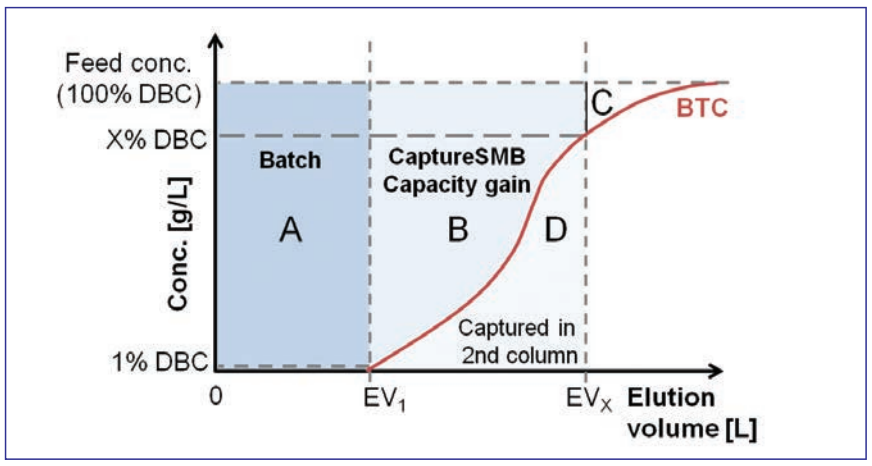

Figure 1. Schematic of a breakthrough curve (BTC) (outlet concentration over elution volume) of column directly loaded with feed,. EV1, EVX: Elution volumes corresponding to 1% Feed concentration (1% DBC), and X% feed concentration, respectively (X% DBC). Area A corresponds to the maximum amount of product that can be loaded in a single column batch process, before product breakthrough. For two interconnected columns, areas A+B and D correspond to the amount of product that is bound on the upstream column and the downstream column, respectively, when the columns are loaded up to EVX. The area A+B+C corresponds to the static capacity.

The process comprises the following steps and is outlined in the schematic in *Figure 2*:

IC – interconnected phase: The two columns are connected in series and loading occurs through the upstream-column: The breakthrough of the first column is captured in the second column. Once the upstream column has been loaded to high capacity utilisation, the loading is stopped and buffer is pumped through the two columns in series in order to flush unbound material from the upstream into the downstream column.

B – batch phase: The columns are disconnected and the previously upstream column is washed, eluted to recover the product, cleaned and re-equilibrated. In parallel, the column that has previously taken up the breakthrough from the upstream column is continued to be loaded at a lower flow rate.

The B phase is followed by another IC phase whereby the previously loaded column is placed in the upstream position and the equilibrated column is placed in the downstream position and the columns are loaded again in series. The IC phase is then followed by another B phase that is run as described above, just with the columns in opposite order. This completes one cycle and the CaptureSMB process can be carried out for as many cycles as desired.

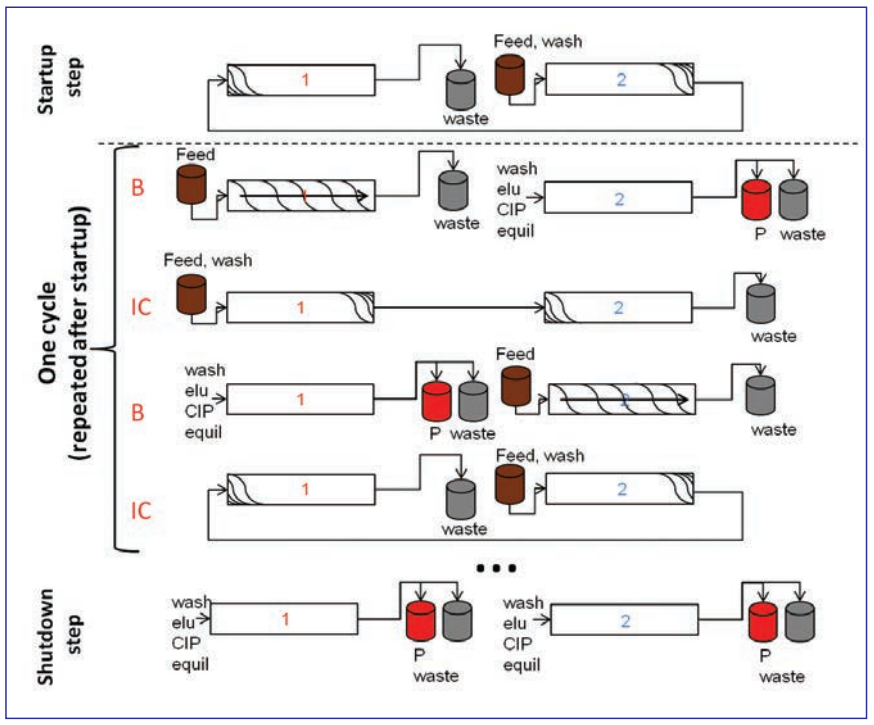

Figure 2. Schematic illustration of the twin-column CaptureSMB process.

Performance Parameter Definition

CaptureSMB and batch processes are compared based on a number of performance parameters that are defined below.

The purity P is defined by the area ratio of the product peak and the total peak area of the analytical chromatograms and is given in percent [%]. This purity definition is typically applied, when calculating the purity with respect to product-related impurities such as aggregates or fragments. The areas are extracted from the analytical chromatograms of product fractions:

$$P = \frac{A_{pool}}{A_{total}}$$

With respect to other impurities like Host Cell Proteins (HCP) or DNA, the purity is expressed in terms of amount impurity per amount of product, e.g. [ng HCP/ mg mAb] or [ppm HCP] and is typically determined by ELISA or other fluorescent assays.

The process yield Y is defined as the ratio of the product mass recovered in the product pool mpool and the product mass supplied through the feed mfeed, within one cycle. In CaptureSMB chromatography, the yield is measured after the startup cycle, i.e. when the process has started up and the UV profiles do not change anymore from cycle to cycle (after the startup cycle):

$$Y = \frac{m_{pool}}{m_{feed}}$$

The mass balance closure mb is defined as the ratio of the product mass leaving the system mout including all outlet streams and the product mass entering the system via the feed within the same time period (mfeed). In batch and CaptureSMB chromatography (after the startup cycle) the mass balance closure is typically referred to one cycle:

$$mb = \frac{m_{out}}{m_{feed}}$$

The load L is defined as the ratio between product mass in the feed and the total bed volume Vcol (in CaptureSMB including all columns), measured within the same time period (typically one cycle):

$$L = \frac{m_{feed}}{V_{col}}$$

The productivity Prod is defined as the ratio of the product mass contained in the product pool, mpool, of one cycle, the cycle duration tcycle and the total stationary phase volume Vcol:

$$Prod = \frac{m_{pool}}{t_{cycle} \cdot V_{col}} = \frac{L}{t_{cycle}} \cdot Y$$

The buffer consumption BC is defined as the ratio of the buffer volume consumed Vbuff and the mass obtained in the product pool mpool, measured within the same time period (typically one cycle):

$$BC = \frac{V_{buff}}{m_{pool}}$$

The capacity utilisation CU is defined as the ratio of the load L and the static capacity Qsat, which corresponds to the maximum binding capacity.

$$CU = \frac{L}{Q_{sat}}$$

Monoclonal Antibody Capture Case Study

Monoclonal antibody (IgG1 with 1.2 mg/mL titre) was captured from clarified cell culture harvest using the CaptureSMB process [1]. The process was run on Contichrom® Lab-10 equipment from ChromaCon, using the ChromIQ® operating software.

Two columns of 0.5 cm inner diameter and 10 cm length were packed with Amsphere™ JWT-203 protein A (JSR Life Sciences), 1 column volume (CV) was 2.0 mL. One UV detector was mounted at the outlet of each column.

The recovery and regeneration protocol had been determined previously through single column batch capture runs and is reported in *Table 1*. Buffer A was 20 mM Phos, 150 mM NaCl, pH 7.5; buffer B was 20 mM Phos, 1M NaCl, pH 7.5; buffer C was 50 mM Na-Cit, pH 3.2; and buffer D was 0.1 M NaOH. The flow rate was 1 mL/min (300 cm/h) except for the CIP step were it was 0.33 mL/min (100 cm/h).

Table 1. Recovery and regeneration protocol of batch chromatography

Step	Buffer	Number of column volumes [CV]
Wash 1	A	2
Wash 2	B	5
Wash 3	A	5
Elution	C	5
CIP	D	7.5 (100 cm/h)
Equilibrate 1	C	2
Equilibrate 2	A	3

The product pool concentration was determined using analytical Protein A chromatography (Poros A20 column, 2.1 mm x 30 mm, Life technologies), aggregate content was measured by size exclusion chromatography (TSK-Gel G3000SWXL, 7.8 mm x 300 mm, Tosoh), and HCP values were determined using a CHO-HCP ELISA kit (# F550, Cygnustechnologies), and DNA content was quantified by fluorescence (Quant-iTTM PicoGreen® dsDNA kit, Life technologies).

The CaptureSMB operating parameters were determined from a breakthrough curve of clarified harvest with a single column (10 cm length, feed flow rate of 300 cm/h). The ChromIQ® operating software of the Contichrom® equipment platform allows for fully automated determination of CaptureSMB operating parameters.

Essentially, the calculations include the determination of the amount of mAb that is contained in the upstream and downstream columns, respectively, in dependence of the amount loaded on the two interconnected columns. In the presented case, the columns were loaded until 70% of the feed concentration was reached at the column outlet of the upstream column (determined by offline fraction analysis of the breakthrough curve). Since the time required for B is fixed by the recovery and regeneration protocol, and the feed flow rate of IC is known (maximum desired feed flow rate should be chosen, in this case 300 cm/h), the remaining parameters QB (feed flow rate of batch phase) and tIC (duration of interconnected phase) can be determined. QB was multiplied with a safety factor of 80% to account for resin degradation over time.

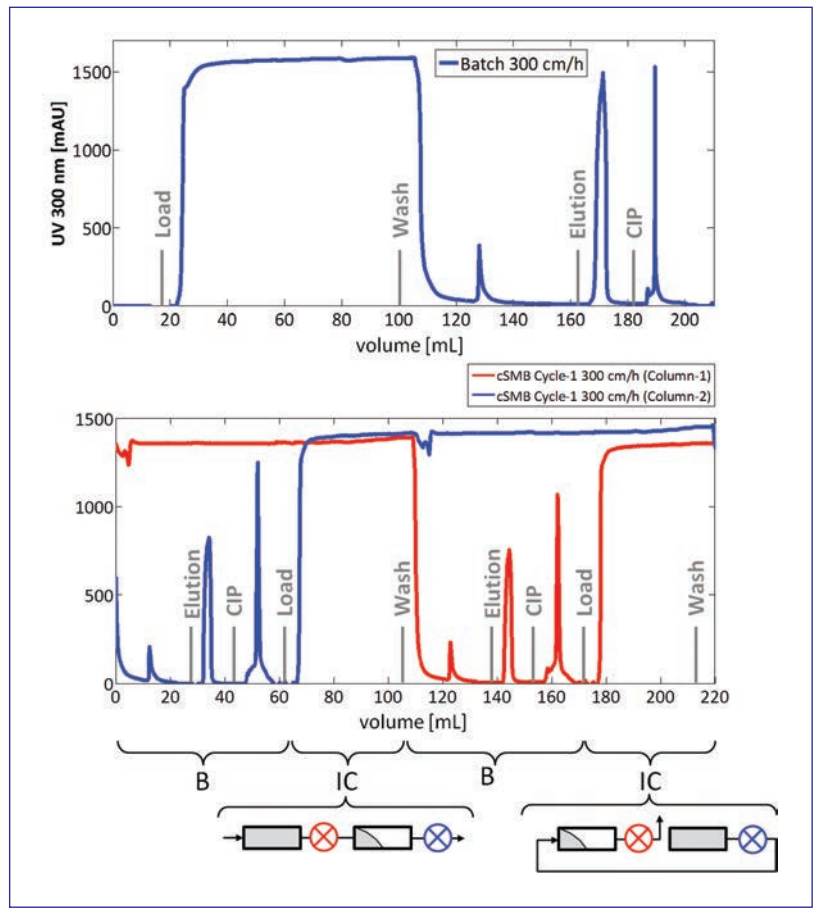

Figure 3. Chromatograms of a batch (top) and CaptureSMB cycle (bottom). The markers indicate the beginning of load, wash, elution and CIP phases, respectively. In the first interconnected (IC) phase of the process column 1 (red UV profile) is upstream of column 2 (blue UV profile) and in the second IC phase vice versa.

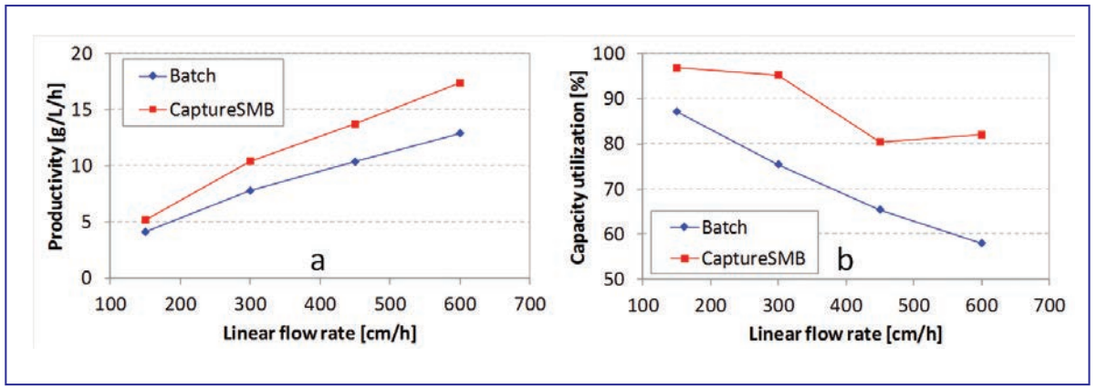

Figure 4. Productivity (left) and capacity utilisation (right) as a function of the linear flow rate for batch (blue symbols) and CaptureSMB (red symbols) processes.

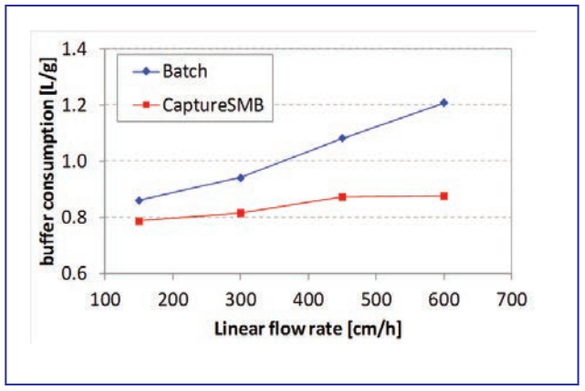

Figure 5. Buffer consumption as a function of the linear flow rate for batch (blue symbols) and CaptureSMB (red symbols) processes.

In analogy to the abovementioned procedure, operating parameters were determined also for maximum feed flow rates of 150 cm/h, 450 cm/h and 600 cm/h.

The UV signals recorded at the outlet of each column are shown in *Figure 3* (bottom) for a run with a maximum feed flow rate of 300 cm/h. The disconnected phases (61 mL length) and interconnected phases (41 mL length) are clearly distinguishable. During column loading, a rapid increase and the reaching of a plateau value of the UV signal can be observed. The plateau UV signal corresponds to the unbound impurities that are flowing through. During the interconnected phase the breakthrough of mAb from the upstream column is visible as rise from the impurity plateau level. At the same time, a rise of the UV profile is not visible at the UV of the downstream column, which indicates that the entire mAb that breaks through is adsorbed in the downstream column.

Process control based on the breakthrough UV-signals [2] is straightforward to implement and have been validated for twin-column CaptureSMB.

As reference processes, batch chromatography runs with equal resin volume (0.5 i.D. x 20 cm L column) were carried out with a load corresponding to 90% of the 1% breakthrough value [3], loading flow velocities of 150 cm/h, 300 cm/h, 450 cm/h and 600 cm/h, respectively, and the same recovery and regeneration protocol *(Table 1)*.

The results for the maximum feed flow rates of 150 cm/h to 600 cm/h showed comparable purities of batch chromatography and CaptureSMB. (Batch runs: 2.0-3.0% aggregates, 7000-12'000 ng HCP/ mg mAb, 1.0-5.0 ng DNA / mg mAb. CaptureSMB runs: 1.5-2.5% aggregates, 5000-12000 ng HCP/ mg mAb, 2.0-4.0 ng DNA / mg mAb).

Figure 4a shows the productivity as a function of the flow rate for the CaptureSMB and batch processes. The productivity of twin-column Capture SMB is larger than the productivity of batch single column capture for any feed flow rate. This is due to two reasons: Firstly, the load of the CaptureSMB process is larger (the columns are loaded far beyond 1% DBC). Secondly, the recovery and regeneration protocol is carried out twice as fast, since the bed height during recovery and regeneration is only half the bed height (10 cm) of the batch reference run (20 cm). It can be further observed that the productivity difference with respect to batch chromatography increases with increasing flow rate *(Figure 4a)*.

This can be attributed to the fact that a broadening of the breakthrough curves as a consequence of increasing flow rate has less effect on CaptureSMB than on batch chromatography since in CaptureSMB material that breaks through earlier due to increased feed flow rate can be likewise captured in the second column.

Therefore the load reduction required to obtain an acceptable yield (preferably > 90%) is much smaller for CaptureSMB compared to batch chromatography leading to a far less dramatic decrease in capacity utilisation *(Figure 4b)*.

Since the load decrease (and capacity utilisation decrease) with increasing feed flow rates is less relevant for CaptureSMB, the buffer consumption remains almost constant for CaptureSMB while it increases for batch chromatography *(Figure 5)*.

Conclusions

The CaptureSMB process offers significant performance advantages in comparison to batch chromatography. The productivity of CaptureSMB in the investigated mAb capture case was on average 35% larger while the capacity utilisation increase was 25%, which translates into 25% resin costs reduction. The buffer consumption was reduced by 20% on average. The advantages of CaptureSMB with respect to batch chromatography are resin-dependent. Preliminary studies indicate that, in the case of resins with broader breakthrough curves (i.e. larger particles) and larger static capacities, the advantages of CaptureSMB can exceed 50% increase in productivity, 40% resin cost reduction and 40% buffer savings at high feed flow rates (600 cm/h).

Due its cyclic nature CaptureSMB is very well suited for combination with continuous upstream production such as perfusion fermentation. By continuous manufacturing the equipment size (pumps, columns) can be typically reduced by at least one order of magnitude.

References

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