TOSOH

Continuous multi-column Protein L purification

Your Challenge

- You deal with increased demand and throughput concerns for antibody therapies.
- You need to reduce the costs of producing these therapies.

Our Solution

MCC with Octave systems and SkillPak columns

- Replacement of traditional chromatography methods

What was done?

- Protein L purification method transferred from batch to MCC proof of concept at a bench scale.

What was the result?

- Increased process efficiency and reduced buffer consumption, while maintaining high recovery.

Together, Octave BIO and SkillPak BIO platforms provide an powerful and straight-forward solution to transfer batch processes and unlock the benefits of multi-column chromatography.

Your Benefit

Reduce DSP costs and efficiency concerns with Tosoh's integrated MCC solutions.

Intensified purification of antibody therapies

TOSOH BIOSCIENCE

SEPARATION & PURIFICATION

CONNECTING MINDS.
TOUCHING LIVES.

https://www.separations.eu.tosohbioscience.com/solutions/multi-column-chromatography/octave-bio-system

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Application Note

Protein L Chromatography Process Transfer for the Intensified Purification of Fab-Fragments

Antibody fragments, such as antigen-binding domains (Fab), single chain variable fragments (ScFv) and heavy chain variable domains (nanobodies) have emerged as increasingly important therapeutic and diagnostic alternatives to full-length mAbs for a multitude of diseases.

Protein L affinity chromatography is typically used for the capture of antibody fragments containing Fab part since they generally lack affinity to protein A ligand. Here we compare capture steps performed in batch mode and multi-column chromatography (MCC) mode, along with two different Protein L resins, using Tosoh's Octave BIO bench-top MCC system.

We demonstrated that transitioning from conventional batch chromatography to MCC significantly increases productivity and leads to resin and buffer volume reduction and reduced costs. The bench-top Protein L MCC process presented here could be scaled to pilot or manufacturing scale.

Experimental Conditions

Columns and resin

Two Protein L resins, TOYOPEARL® AF r-Protein L-650F and Cytiva's Capto™ L, were compared in both batch and MCC mode regarding product recovery, resin utilization, process productivity and buffer consumption.

TOYOPEARL AF-rProtein L-650F is based on polymethacrylate resin particles with a mean particle diameter of 45 µm, to which the recombinant Protein L ligands are attached. TOYOPEARL AF-rProtein L-650F offers a static binding capacity of 54 mg/mL for Fab fragments. It is stable at pH 2-12 and can be purchased in bulk or different scales of pre-packed columns. For most experiments shown here, TOYOPEARL AF-rProtein L-650F was used in the SkillPak™1 BIO (1 mL) pre-packed column format (see *Figure 1*).

Figure 1. SkillPak 1 BIO (1 mL)

Fragment used

Since large quantities of Fab fragment was required for these experiments, fragmented material was produced by proteolytic digestion of a biosimilar of the mAb Adalimumab. The enzyme papain was used to cleave the mAb after His-228 in a hinge region and to separate both Fab fragments individually from the Fc portion. Protein A-purified mAb was mixed with activated papain, and the reaction was stopped after 16 hours with 30 mmol/L iodoacetamide. The reaction mixture was spiked with cell culture supernatant from CHO cells to simulate impurities typically present before the capture step. The final Fab concentration was 2.23 mg/mL.

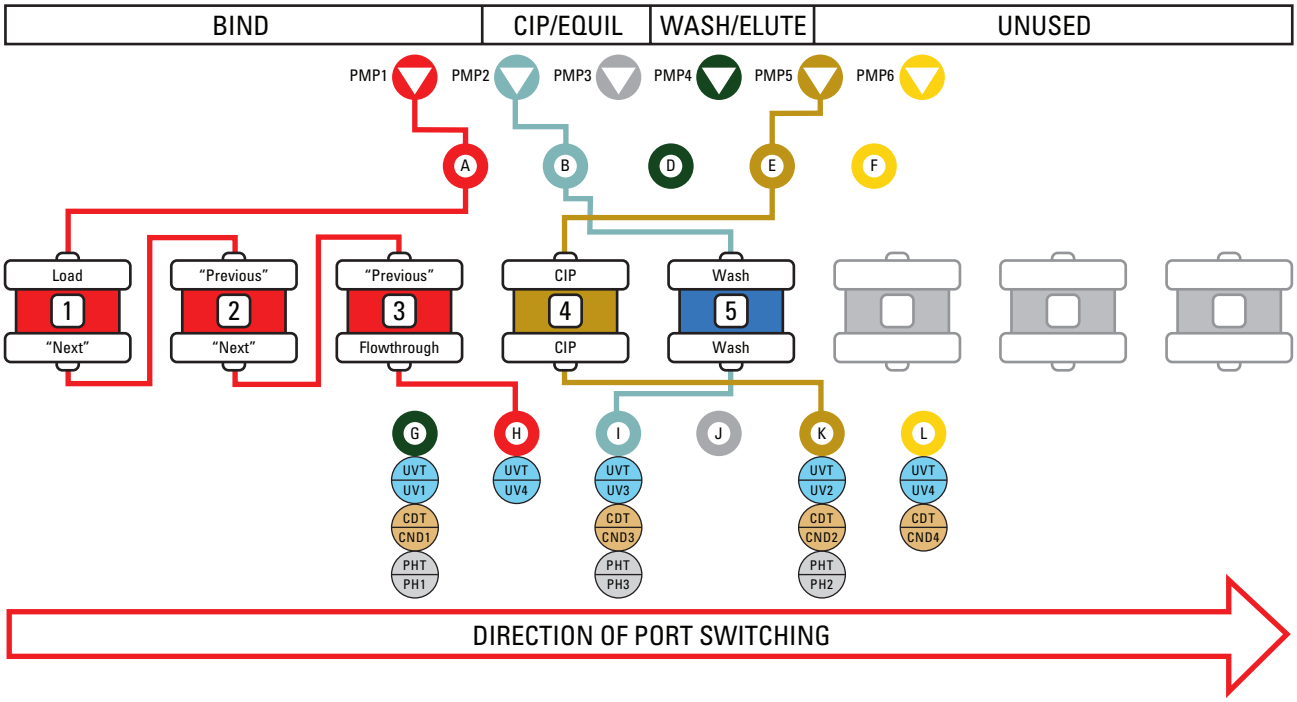
Batch purification runs

Batch purification runs were performed to provide comparison to MCC mode. The two different Protein L resins were packed into columns with an inner diameter of 0.66 mm and a length of 7 cm, resulting in a column volume of 2.4 mL. Afterwards the following method was applied to both of the columns (see *Table 1*).

Table 1. Process summary for batch purification runs

Process step	CV	Buffer	Flow rate (cm/h)
Equilibration	5	100 mmol/L sodium phosphate, pH 7.0	175
Load	Variable	Feed containing Fab fragment	150
Wash 1	10	100 mmol/L sodium phosphate, pH 7.0	175
Wash 2	5	100 mmol/L sodium acetate, pH 5.5	175
Elution	5	100 mmol/L glycine/ HCl, pH 2.5	175
CIP	5	50 mmol/L sodium hydroxide	175
Re-equilibration	5	100 mmol/L sodium phosphate, pH 7.0	175

The columns were loaded with 85% of the previously determined DBC (dynamic binding capacity), as otherwise too much of the target molecule would be lost in the flow-through fraction during the loading phase. This resulted in a loading volume of 15 CV (33.5 mg/mL resin) for TOYOPEARL AF r-Protein L-650F and 6 CV (13.4 mg/mL resin) for Capto L. All runs were conducted using a ÄKTA™ Avant 150 FPLC system.



The collected elution peak was analyzed using size exclusion-UHPLC for impurity profile analysis and analytical Protein L chromatography for Fab concentration determination and respective recovery calculation.

Multi-column chromatography purification runs

The purification process was translated to a sequential multi-column chromatography (MCC) process with the goal of improving process productivity while decreasing buffer consumption. To achieve this, the Octave™ BIO (see *Figure 2*), a bench-top MCC system was equipped with five 1 mL SkillPak BIO pre-packed columns containing the respective Protein L resin.

Figure 2. Octave BIO System

The Octave consists of six pumps, eight column positions, a detector array (four conductivity sensors, four pH probes, and four dual-wavelength UV detectors), and a valve block. The valve block is a low hold-up volume replaceable manifold, which is pneumatically actuated to direct flow from any given system inlet to any given column, and from any given column to any other column or outlet.

For the MCC process, three of the five columns are connected in series via the valve block, enabling increased loading amounts and higher resin utilization, since product break through is caught on subsequent columns. While these three columns are in the loading zone, the remaining two columns go through the process steps of Wash and Elute, and CIP and Equilibration (see *Figure 3*). Once the first column in the loading zone is fully loaded, the column ports are switched one position to the right in the process scheme (*Figure 3*), simulating column movement to the left into the next processing zone.

Each MCC experiment consisted of five cycles, in which columns are rotated though the different process steps.

Since the serial column configuration in MCC minimizes target molecule breakthough, the columns were able to be loaded to 85% of the previously determined static binding capacity (SBC), resulting in loading masses of 45.2 mg/mL resin for TOYOPEARL AF-rProtein L-650F and 21.8 mg/ mL resin for Capto L. For both resins two runs were performed, varying the residence time during the loading phase from 0.5 minutes to 0.25 minutes.

Results and Discussion

the following six runs (2 batch, 4 MCC) were compared based on several key parameters: recovery, utilized capacity, productivity, and buffer consumption.

Utilized capacity states how much product can be recovered per run with respect to the resin bed volume. Employing three load columns on the Octave system proved effective in maintaining high recovery, while increasing the capacity utilization of the resin when compared to batch processes. While comparing MCC runs, the results show a minor decrease in recovery when reducing the residence time during the loading phase from 0.5 min to 0.25 min, which is to be expected and can be attributed to flowthrough losses. However, recovery is still acceptable.

Table 2. Comparison of evaluation parameters for the different Fab purification runs

Chromatographic process	Resin	Residence time (min)	Recovery (%)	Utilized capacity (mg prod/mL resin)	Buffer consumption (mL buffer/mg prod)
Batch	TOYOPEARL	2.8	95.3	31.9	0.9
MCC	TOYOPEARL	0.5	97.0	45.3	0.8
MCC	TOYOPEARL	0.2	93.8	43.8	0.7
Batch	Capto L	2.8	92.7	12.4	2.4
MCC	Capto L	0.5	95.2	20.6	1.6
MCC	Capto L	0.2	94.3	20.4	1.4

Maximizing the utilized capacity positively influences process economy, since per liter of purchased resin, a higher mass of protein can be purified. Since in MCC the first columns in the loading phase columns can be loaded with more protein due to the subsequent columns catching product breaking through, the utilized capacity can be improved by 42% for TOYOPEARL AF-rProtein L-650F and 66% for Capto L. Comparison of the two resins studied revealed that approximately 2.2 times more protein mass can be loaded onto TOYOPEARL AF-rProtein L-650F than onto Capto L, which can be attributed to the different static binding capacities.

Comparing the buffer consumption (the volume of buffer required to purify a given mass of protein) of the two chromatographic methods, it is clear that MCC reduces chemical consumption and waste generation due to better resin utilization. This improvement in process economy can be further enhanced by the use of a resin with higher binding, as shown by the comparison of Toyopearl and Capto L results *(Table 3)*.

Table 3. Comparison of productivity for the different Fab purification runs

Chromatographic process	Resin	Residence time (min)	Productivity [mg Fab/(mL resin*h]
Batch	TOYOPEARL	2.81	16.80
MCC	TOYOPEARL	0.50	45.09
MCC	TOYOPEARL	0.25	84.72
Batch	Capto L	2.81	8.40
MCC	Capto L	0.50	41.26
MCC	Capto L	0.25	79.64

Productivity is a measure of the efficiency of a process, measured in terms of mass of product processed per unit volume of resin and time.

Productivity is mostly dependent on the process duration. When reducing the residence time during the loading phase, productivity is increased by almost the same factor. Compared to the batch runs, productivity can be increased by roughly 170% by reducing the load residence time to 0.5 min., and increased by another 235% by reducing the residence time to 0.25 min.

Conclusions

This comparison highlights the benefit of single column batch capture step to an MCC process. The higher utilized capacity, increased productivity and reduced buffer consumption that could be shown here all positively affect process economics, making it more feasible to purify large amounts of mAbs or mAb fragments in a shorter time. The Octave product line is a solution for those wishing to decrease resin volume and protein purification costs.