



Protein L Chromatography Process Transfer for the Continuous 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. With proteolytically produced Fab fragment as an example, here we compare two different protein L chromatography resins for the capture step in a batch mode.

We also demonstrate how the transfer from a conventional batch mode to a multi-column continuous chromatography (MCC) process significantly increases the productivity of the Fab fragment capture. This leads to resin savings while simultaneously decreasing buffer consumption and consequently lower process operation costs. The complete bench-top protein L-based chromatography MCC platform presented here can feasibly be expanded to a pilot or manufacturing scale.

Experimental Conditions

Columns and resin

Two Protein L resins, TOYOPEARL® AF r-Protein L-650F and Capto™ L, were compared in both batch and continuous 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

For facilitated access to larger quantities of Fab fragment 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

To identify baseline chromatographic performance of a Protein L step, a batch process was considered. 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 |
| Reequilibration | 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 elution peak was collected and analyzed using size exclusion-UHPLC for determination of the impurity profile and analytical Protein L chromatography for concentration determination. From the results obtained in this analysis, product recovery was calculated.

Continuous 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 system (see Figure 2), a benchtop MCC system was equipped with five 1 mL SkillPak BIO pre-packed columns containing the respective Protein L resin.

Figure 2. Octave BIO System



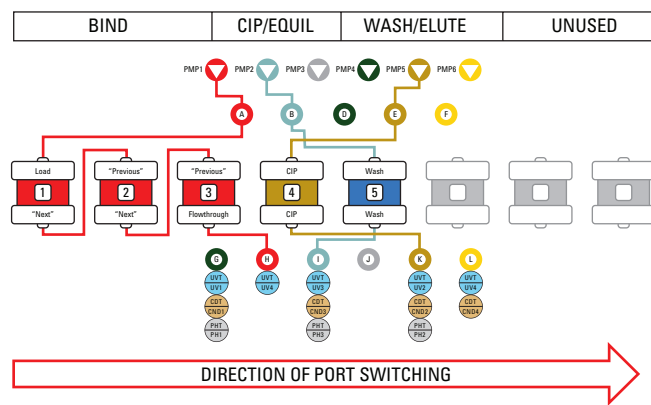
The system consists of 6 pumps, a switching valve block and a detector array. The pumps are all designated to one of the buffers required in the process. Via the switching valve block up to 8 connected columns can either be addressed by different pumps in parallel or be connected to each other in series. The detector array allows for precise process control. Up to four different process streams can be observed with regards to UV-absorption, conductivity, and pH.

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 phase, the remaining two columns go through the phases Wash and Elute, and CIP and Equilibration (see Figure 3). Once the first column in the loading series is fully loaded, the column ports are switched on position against the flow current.

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 | 2.81 | 95.32 | 31.87 | 0.94 |
| MCC | TOYOPEARL | 0.50 | 97.04 | 45.30 | 0.78 |
| MCC | TOYOPEARL | 0.25 | 93.83 | 43.81 | 0.67 |
| Batch | Capto L | 2.81 | 92.70 | 12.40 | 2.42 |
| MCC | Capto L | 0.50 | 95.205 | 20.58 | 1.63 |
| MCC | Capto L | 0.25 | 94.39 | 20.41 | 1.45 |

Figure 3. Process scheme for a 5 column MCC process



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

Since in MCC product breakthrough during the loading phase is less of a risk due to the secondary load columns, the columns were loaded with 85% of the previously determined SBC (static binding capacity), 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 six runs (2 batch, 4 MCC) were compared based on several key parameters: recovery, utilized capacity, productivity, and buffer consumption.

Table 2 (below) shows the tabulated results for the six Fab purification runs.

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, although recovery is still within acceptable levels.

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 contributes significantly to reducing chemical consumption or 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 P/(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.

This reduction in process duration manifests itself in the operating costs, as productivity and process efficiency are proportional to resin savings and time savings.

Conclusions

This comparison highlights the benefit of converting the capture step to a continuous 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 BIO system facilitates the transition from batch to continuous processing by its intuitive handling and by simplifying the method transfer.

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