TOSOH

Multi-column scFv Capture Platform

Your Challenge

- You deal with increased demand and throughput concerns for antibody therapies.
- You need to increase the efficiency of traditional capture processes without sacrificing quality.

Our Solution

MCC with Octave systems and SkillPak columns

- Intensification of batch capture processes

What was done?

- A platform Protein L purification process for purifying scFv was transferred from batch to MCC proof of concept.

What was the result?

- A more time, buffer, and resin-efficient process resulting in increased single-mer quantity and comparable recovery and impurity profiles.

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

Your Benefit

Reduce efficiency concerns without compromising on quality.

Intensified purification of antibody therapies

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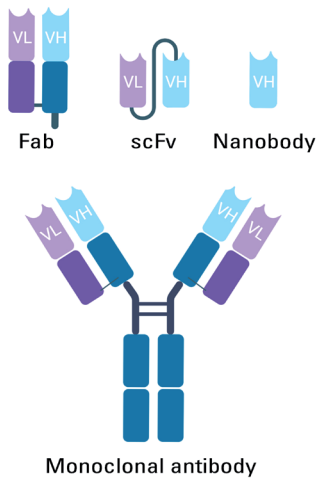
Application Note

Multi-Column Chromatography Platform for Protein L Affinity Capture of scFv Antibody Fragment

Introduction

Antibody fusion proteins and fragments, such as antigen-binding domains (Fabs), single-chain variable fragments (scFv), and heavy chain variable domains (nanobodies) *(Figure 1)* have become increasingly important alternative therapeutics to intact immunoglobulins due to their small size, ease of manufacturing, and ability to effectively penetrate tissues (De Greve and Fioravanti 2024, Gezehagn-Kussia and Tessema 2024, Manoutcharian and Gevorkian 2024). However, for downstream capture, these molecules often lack affinity for the traditional Protein A-based monoclonal antibody (mAb) capture ligand. Instead, Protein L-based purification methods are often used for the capture step due to this ligand's high affinity for various Fab domain structures as well as several types of related antibody fragments including scFv and nanobodies (Bates and Power 2019, Gezehagn-Kussia and Tessema 2024).

Figure 1. Graphic representation of an intact monoclonal antibody, Fab, scFv, and a nanobody antibody fragment.



(created using BioRender.com)

Recent innovations in mAb processing technology, such as the use of multi-column chromatography (MCC), have been shown to significantly reduce operational costs, laboratory footprint, and processing time by increasing process productivity, while maintaining product quality attributes and recovery (e.g. Matte 2022), particularly for affinity process steps like Protein L chromatography. To demonstrate these benefits of MCC, we describe here a comparison between conventional batch chromatography and MCC using the Octave™ BIO instrument *(Figure 2)* for the purification of single chain variable fragments.

Figure 2. Bench-top Octave BIO MCC instrument with SkillPak™ BIO columns.

Experimental Conditions

Sample

scFv-containing CHO cell culture supernatant (titer 2.2 g/Liter) was acquired from ExcellGene (Monthey, Switzerland). The scFv fragment has a molecular weight of 26.7 kDa, length of 250 amino acids, and a theoretical pI of 8.8.

Multi-column chromatography system

The Octave BIO benchtop MCC system consists of six pumps, a manifold block (valve block), and a multi-detector array. The pumps are each designated to one buffer that is required for the purification process. The manifold block, which is hydraulically actuated to regulate flow, enables up to eight columns to be connected to run in series or in parallel. With the multi-detector array, up to four different process streams can be monitored by dual-channel wavelength for UV, conductivity, and pH.

Affinity resin and pre-packed columns

TOYOPEARL® AF-rProtein L-650F affinity chromatography was assessed in batch and multi-column chromatography modes to compare product recovery, resin utilization, process productivity (g of target molecule purified/L resin/hour process time), multimer formation, and host cell protein (HCP) removal. The Protein L liganded resin contains a polymethacrylate backbone with a particle diameter of ~45 µm. The resin has a static binding capacity of 21 mg/mL for the scFv molecule. The resin is stable at pH 2 - 13 and is available in both bulk resin and in pre-packed columns. All batch purification runs were conducted using an ÄKTA Avant™ 25 FPLC system.

For the MCC experiments, Protein L-650F was used in the SkillPak 1 BIO (0.8 cm inner diameter (ID) × 2.0 cm bed height (BH), 1 mL) pre-packed column format designed for MCC systems *(Figure 3A)*. For the batch purification experiments, Protein L-650F was used in the SkillPak 5 (0.8 cm ID × 10 cm BH, 5 mL) pre-packed column format *(Figure 3B)*.

Figure 3. Pre-packed columns for MCC and batch chromatography

A Tosoh TSKgel® UP-SW3000 column (2 µm particles, 4.6 mm ID × 30 cm L connected to Thermo Scientific Ultimate™ 3000 U/HPLC instrument was used for analytical size exclusion chromatography (SEC) to compare scFv purity after batch and MCC purification runs. The ELISA assay kit for host cell protein (HCP) measurement was from Cygnus® Technologies. Protein concentration was determined via UV_{280} spectroscopy.

Batch purification

To develop baseline chromatographic performance of the Protein L affinity capture steps, a batch-scale purification process was performed with a 5 mL Protein L pre-packed column *(Table 1)*.

Table 1. Process method summary for batch purification of scFv using Protein L.

Process step	CV	Buffer	Flow rate (cm/h)
Equilibration	5	100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4	120
Load	Variable	Feedstock containing scFv	150
Wash 1	5	100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4	120
Wash 2	10	100 mmol/L sodium acetate, pH 4.5	120
Elution	10	100 mmol/L acetic acid, pH 3.2	120
Strip	5	100 mmol/L acetic acid, pH 2.8	120
CIP	5	100 mmol/L NaOH	120
Re-equilibration	10	100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4	120

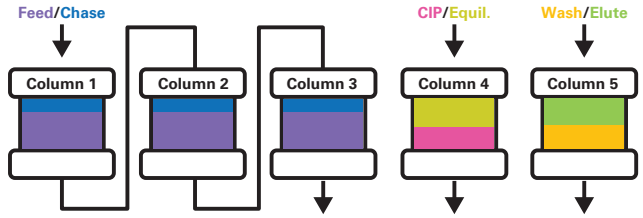
The column was loaded with 45% saturation (9.6 mg scFv/mL resin) of the previously determined static binding capacity (SBC) to prevent loss of any product during loading. The elution peak was collected for determination of the protein concentration, impurity profile, and efficiency of HCP removal.

MCC process

The batch chromatography process was converted to an MCC process with the goal of improving process productivity while maintaining all key quality attributes. To achieve this, the Octave BIO, a benchtop MCC system, was equipped with five 1 mL Protein L SkillPak BIO pre-packed affinity columns.

In this method adjusted to five-columns, three columns in series were in the loading phase *(Figure 4)*. Once the first column was completely saturated, it was moved to the washing phase, and the second column that was capturing the breakthrough moves into the primary capture position. Subsequently, the columns were washed, and the scFv-containing fraction was eluted through a separate outlet. The columns were then cleaned and re-equilibrated before reentering the loading zone.

Figure 4. MCC Protein L capture method.



Since product breakthrough and loss is of little concern during the loading phase in MCC processes due to secondary and tertiary load columns, the columns were loaded to 90% saturation (18.9 mg scFv/mL resin) of the static binding capacity. The residence time during loading was 0.5 minutes. Method parameters are listed in *Table 2*.

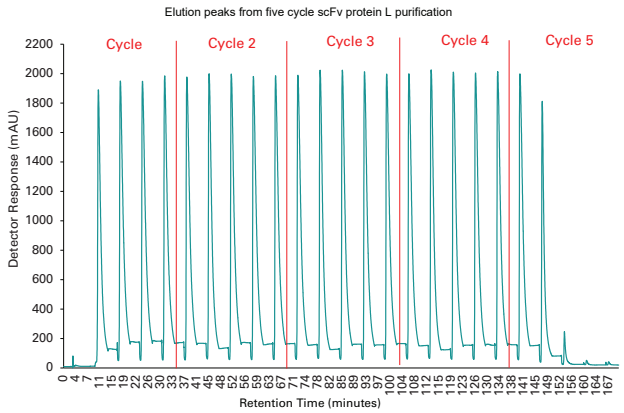
Table 2. Process method summary for MCC purification of scFv using Protein L.

Process step	CV	Buffer	Flow rate (cm/h)
Equilibration	9.1	100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4	240
Load	Variable	Feed containing scFv	240
Chase	5	100 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4	240
Wash	5.5	100 mmol/L sodium acetate, pH 4.5	240
Elution	8.1	100 mmol/L acetic acid, pH 3.2	240
CIP	4.5	100 mmol/L NaOH	240

Results and Discussion

Batch and MCC purification runs were compared for several key parameters: recovery, utilized resin capacity, productivity, aggregate %, and HCP removal. Utilized resin capacity shows how much product can be recovered from a run with respect to the column bed height. By utilizing three columns in the load zone during MCC, high levels of recovery could be achieved while increasing resin capacity compared to batch processes. Chromatograms from the five-cycle MCC purification run are presented in *Figure 5*.

Figure 5. MCC protein L elution chromatograph for scFv



AU280 profile for the elution of scFv during run is in teal. Cycle changes are indicated by red lines. Cycle 1 represents the startup cycle, in which the first elution at ~5 min lacks protein. Cycle 5 is the shutdown cycle, with the last elution steps from columns with no loaded protein.

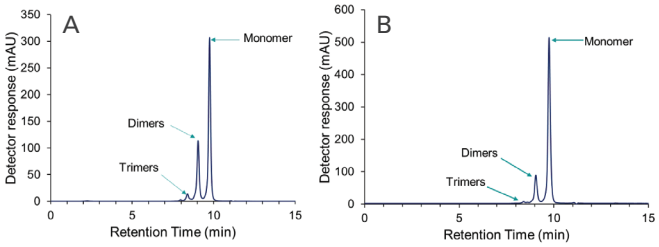
Comparison of the results for scFv purification using batch chromatography vs. MCC are presented in *Table 3*. The percentage recoveries between the batch and MCC process for scFv were highly comparable. However, by maximizing the resin's capacity through loading multiple columns in series and closer to full saturation, the process productivity for MCC substantially increased compared to the batch process. The batch column productivity was also lower than MCC due to loading only ~40% of the DBC to avoid product loss during the loading step. Further MCC improvement was achieved from lower processing time since the residence time in loading was decreased from 4.0 min for batch to 0.5 min for MCC. The faster residence time was possible due to the MCC columns being connected in-series as well as the unique pressure/flow characteristics of the SkillPak 1 BIO columns. The resulting MCC method was able to process almost 8-fold more scFv compared to batch (378 mg vs. 48 mg), with the same total resin volume and in a slightly shorter overall process time.

Table 3. Comparison of purification parameters for batch chromatography vs. MCC modes.

Chromatographic mode	Batch	MCC
Residence Time (min)	4.0	0.5
Recovery (%)	94.8	94.3
Utilized resin capacity (g scFv/L resin)	9.6	18.9
Productivity (g scFv/L resin/h)	4.1	33.4
Multimer (%)	33.3	17.8
HCP clearance (log)	2.98	2.94
Total process time (h)	3.2	2.8
Total scFv processed (mg)	48	378

Another important attribute of batch vs. MCC processes was the improved scFv purity using MCC. Product and process related impurity levels from the MCC process were lower or comparable to batch, while HCP clearance was comparable between the two processes *(Table 3)*. Analytical SEC shows that the MCC purified product had a slightly lower amount of higher molecular weight protein impurities than conventional batch chromatography *(Figure 6)*. Antibody fragments are often prone to multimerization and aggregation which diminishes the proportion of the desired monomer product (Bates and Power 2019). We confirmed the identity of the higher molecular weight impurities seen in *Figure 6* as scFv dimers and trimers using SEC-MALS analysis (data not shown).

Figure 6. Analytical SEC with UV detection at 280 nm of (A) the batch and (B) MCC processes.



Conclusions

Comparison between conventional batch purification and multi-column chromatography platform highlights the multiple advantages of a continuous multi-column purification process. The advantages of MCC over batch chromatography included (1) higher process purification productivity, (2) lower resin consumption, (3) reduced protein multimerization, and (4) ~8-fold increase in the amount of protein processed while slightly reducing the overall process time. Comparison of MCC and batch purification modes here strongly supports the use of MCC processes for optimized biologics purification. The bench-top MCC purification scheme presented here can easily be scaled to a pilot plant or manufacturing process using an Octave PRO skid, which operates with the same capabilities on a larger scale.

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