Multi-Cycle Performance Qualification of OPUS® Columns

Introduction

OPUS® (Open Platform User Specified) Pre-packed Disposable Chromatography Columns are designed to deliver the industry required flexibility of multi-use applications in downstream processing. Constructed from a medical grade polypropylene homopolymer, OPUS® Columns are configurable for any industry standard size, chromatography resin, and bioprocessing application. As such, OPUS® Columns must be compatible with standard multi-cycle capture and polish methods to be suitable for use in a typical downstream processing campaign for monoclonal antibodies, recombinant proteins, or vaccines. In this Tech Note, the re-use of OPUS® Columns is qualified through a multi-cycle performance investigation.

Methods and Materials

A 20 x 20 cm OPUS® Column packed with SP Sepharose® was used for the purification of a recombinant protein from *E. coli* culture lysate. The OPUS® 20 cm design is representative of Repligen's pre-packed disposable columns with internal diameters ranging from 10 – 30 cm. All columns in this size range have consistent design parameters, packing procedures, and performance characteristics (visit www.repligen.com/opus for more information).

The purification procedure of the recombinant protein from the *E. coli* clarified lysate is outlined in Table 1 below.

Table 1: Chromatographic Procedure for Purification of a Recombinant Protein

Step	Buffer	Duration
Equilibration	Low conductivity buffer	3 CV
Load	Filtered cell lysate diluted to 10 mg/mL protein in Eq. buffer	20 mg/mL resin
Wash	Equilibration buffer (low conductivity)	3 CV
Elution	High salt buffer (collect 100 mAU – 100 mAU)	3 CV
Sanitization	0.2 N NaOH	2 CV

The flow rate for all purification cycles was 200 cm/h.

Multi-Cycle Performance Case Study

- Multi-Cycle Purification I: The recombinant protein from *E. coli* was purified for 10 cycles on an OPUS® Column packed with SP Sepharose.
- Extensive Buffer Circulation: Following the 10 cycle purification, the column was subjected to extensive re-use testing by circulating high salt buffer for more than 1200 column volumes (roughly equivalent to more than 80 purification cycles with 15 column volumes per cycle).
- Multi-Cycle Purification II: To confirm the integrity of the packed bed after extensive buffer circulation, the column was used for an additional 2 cycles of purification for the *E.coli* derived recombinant protein.

To assess column performance, asymmetry at 10% peak height and theoretical plates per meter were measured before and after the experiment, as well as during intervals throughout the use of the column. A 2% acetone injection of 1% of column volume at a flow rate of 100 cm/h was used for the testing method.

Multi-Cycle Performance Qualification of OPUS® Columns

Case Study Objectives

Process Performance:

- Process Yield: variation of +/- 5% for the multi-cycle purification
- Product Purity: variation of +/- 2%

Column Performance:

- Pressure vs. Flow: variation of +/- 0.2 bar
- Asymmetry:
  - Acceptance criteria for a well packed column is 0.8-1.6
  - For the extensive buffer circulation, consistent chromatographic performance is defined as asymmetry varying within 0.9-1.4 for the purposes of this report
- HETP: greater than 2500 plates/m

Results

Multi-Cycle Purification I

The results for the 10 cycle purification of a recombinant protein from *E. coli* are shown in Figure 1 and Table 2.

Figure 1: Overlay of 10 Purification Runs of Recombinant Protein Purification

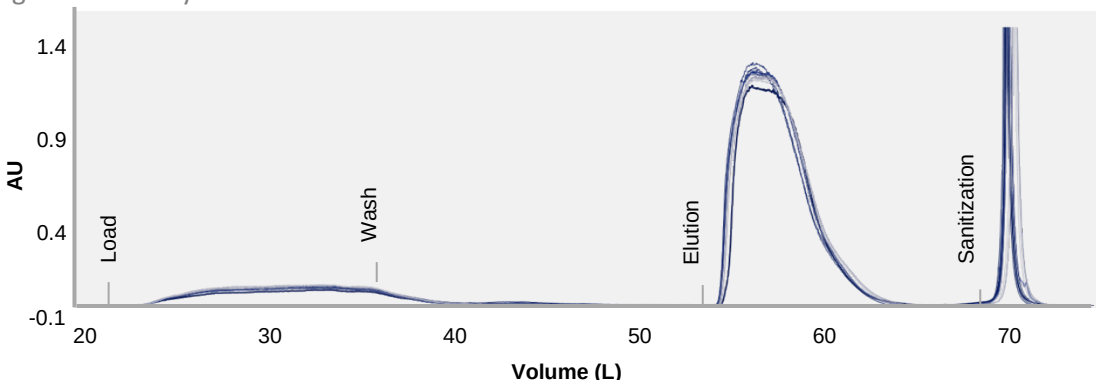

Table 2: Results for the 10 Purification Runs of a Recombinant Protein

Run #	Elution Volume (L)	Elution Conc. (mg/mL)	% Yield	% Purity (by HPLC)	Flow ΔP 200 cm/h (bar)
1	7.0	16.2	92.9	75.9	0.91
2	6.6	16.9	90.4	76.0	0.86
3	6.7	16.9	92.5	76.2	0.85
4	6.8	16.9	93.8	76.2	0.88
5	6.8	17.0	94.2	75.9	0.88
6	6.8	17.1	94.5	76.1	0.88
7	6.8	16.3	90.9	76.5	0.88
8	6.8	16.6	92.6	76.2	0.88
9	6.7	16.7	91.9	75.9	0.88
10	6.8	16.4	91.8	76.2	0.86
Avg	6.8	16.7	92.6	76.1	0.88
% RSD	1.5%	1.9%	1.5%	0.3%	1.9%

The 10 purification cycles show similar chromatographic profiles and eluate quality, in addition to low percent residual standard deviations. More importantly, the pressure drop (flow delta P) over the column is maintained, thus demonstrating the packed bed is not altered.

Table 3: Performance Characteristics Summary

	HETP (plates/m)	Asymmetry
Pre-run	2815	1.3
Post-run	3512	1.2

Column characteristics of HETP and asymmetry did not decline (Table 3). The theoretical plates per meter and asymmetry results are within the acceptance criteria for both the pre and post-multi-cycle runs.

Extensive Buffer Circulation Study Simulating Multi-Campaign Use

An extensive re-use study was performed to investigate the performance of OPUS® columns beyond 10 cycles. For this study, buffer was circulated through the column for more than 1200 column volumes at a flow rate of 200 cm/h. The column performance characteristics were measured regularly, and summarized in Table 4.

Table 4: Performance Characteristics of the 20 x 20 cm OPUS® Column

CV buffer	Plate count (Plates/m)	Asymmetry
0	2820	1.1
95	2890	1.0
355	2885	1.2
470	3535	1.1
985	3113	1.3
1225	2840	1.3

As shown by the data in Table 4, the column performance characteristics are well maintained throughout the extensive buffer circulation study.

Multi-Cycle Purification II

After the extensive buffer circulation, the column was tested again by using the same purification procedure outline in Table 1.

Figure 2: Overlaid Chromatograms of Two Additional Purification Cycles

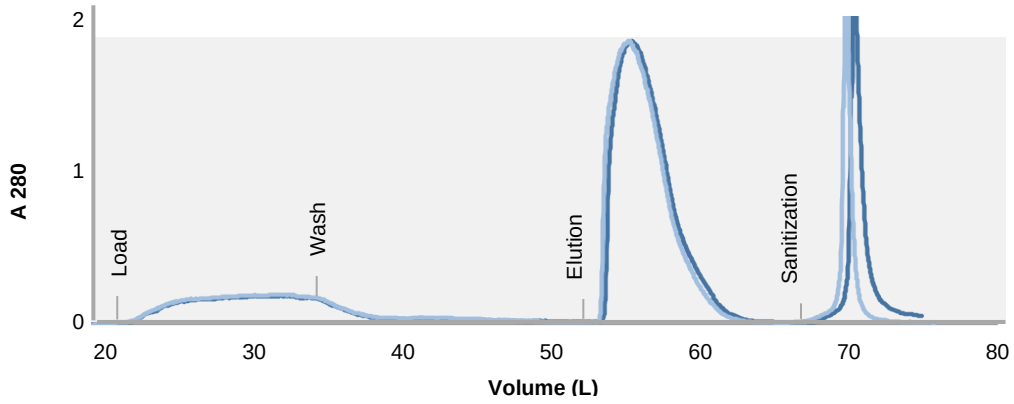

Table 5: Eluate Results from Two Additional Purification Cycles

Run #	Volume (L)	Conc. (mg/mL)	Yield %	% Purity (by HPLC)	Flow DP @ 200 cm/h (bar)
1	8.4*	15.9	92.5	77.5	0.89
2	8.3*	16.3	93.4	77.5	0.89

The additional two purification cycles show the same chromatographic profile, and eluate quality. The pressure drop over the column is maintained, and is similar to the pressure drop observed during the first 10 cycle runs. This demonstrates the stability of the packed bed and the reliability of OPUS® columns when used over multiple campaigns.

Conclusions

The Multi-cycle performance case study shows the well-engineered design of OPUS® columns are compatible with multi-use. The results confirm there is no substantial difference in run-run consistency and chromatographic performance is maintained over the course of many cycles. Therefore, OPUS® columns are suitable for replacing traditional columns in multi-cycle or multi-campaign use applications.