Scalability and Multi-Cycle Performance of Pre-Packed Chromatography Columns Ranging from Development to Production Scale

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Summary

Disposable manufacturing has been a developing trend in biopharmaceutical manufacturing for the last two decades. In a 2011 Survey of Biopharmaceutical Manufacturing Capacity and Production[1] the authors note "we are on the verge of the first approvals of mainstream biopharmaceutical products manufactured using single use/disposable systems" and "Single use and disposable chromatography units will be the most important trend in the next five years"[2].

Disposable technologies have been adopted by biopharmaceutical industry as a mean for faster product changeover, favorable economics, and improved safety. Until now there has not been a broadly applicable solution for disposable chromatography steps.

Repligen's OPUS™ (Open Platform User Specified) pre-packed columns are disposable and can be packed with almost any bioprocessing resin, at internal diameters ranging from 0.5 cm to 60 cm, and column heights from 5 cm and up, offering a broad, scalable solution for the purification of biological products. These columns provide the flexibility to perform development studies, as well as purification of kilogram batches of biological products within the same chromatography platform. OPUS™ columns make single-use/disposable downstream purification of biologics a reality.

Performance of these columns is demonstrated by few case studies that look at purification of proteins.

Consistency of purification is maintained for various scales of the columns, making them ideal for scalability of a downstream purification process.

The ability of using a single pre-packed column for a multi-cycle purification campaign is addressed. Chromatographic performance is not compromised by repeat cycles of purification and sanitization of the column.

Scalability of OPUS™ Columns

Objective: Demonstrate scalability of OPUS™ columns through consistency of chromatographic performance.

Method:
- Pulse injection of 1% CV of 2% acetone
- Affinity separation of hIgG from BSA on OPUS™ columns with diameters of 1.2, 2.5, 8, 20cm, packed at 20cm bed height with CaptivA™ PriMab (Sepharose® 4FF backbone). Linear flow rate of 100cm/hr

Table 1: Purification procedure for hIgG spiked with BSA on CaptivA™ PriMab resin

Step	Buffer	Duration
Equilibration	Neutral pH buffer	5.0 CV
Load	IgG spiked with 10% (w/w) BSA, loaded at 20mg IgG/mL resin	N/A
Wash 1	Equilibration buffer, neutral pH	2.0 CV
Wash 2	High salt buffer	3.0 CV
Wash 3	Equilibration buffer, neutral pH	2.0 CV
Elution	Low pH buffer (Collect from 100 mAU to 100 mAU)	4.0 CV
Strip	100 mM Phosphoric Acid	2.0 CV

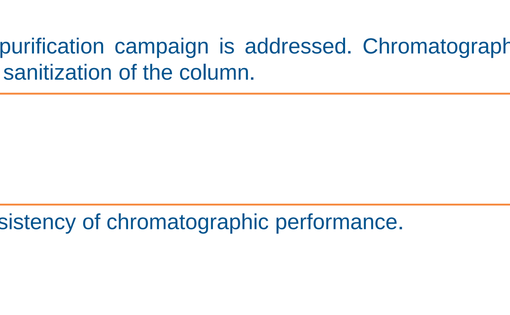
Acceptance Criteria:
- Column quality attributes pass acceptance test: Plates/m: >1500, Asymmetry: 0.8-1.6
- Consistent purification across different scale columns

Results:

Table 2: Column quality attributes for different sizes CaptivA™ PrimMab packed columns

Column ID:	Plates/m Pre-run	Asymmetry Pre-run	Plates/m Post-run	Asymmetry Post-run
1.2 cm	2494	1.1	2485	1.1
2.5 cm	3386	1.1	3355	1.2
8 cm	2490	1.1	3557	1.1
20 cm	2570	1.2	2305	1.3

Figure 1: SDS-PAGE of CaptivA™ PriMab purification scales



Conclusions:
- Demonstrated scalability of OPUS™ platform, quality attributes are maintained for different column diameters
- Chromatographic separation remains unchanged across the OPUS™ platform

Reusability of Smaller OPUS™ Columns

Objective: To demonstrate reusability of OPUS™ columns in a multi-use experimental setting

Method:
- Purification of hIgG from a CHO culture media using affinity chromatography
- OPUS™ 1.2x20cm columns packed with CaptivA™ PriMab media; linear flow rate of 200 cm/h; 20 cycles

Table 3: Multi-cycle purification procedure

Step	Buffer	Duration
Equilibration	Neutral pH buffer	8.0 CV
Load	hIgG loaded at 30mg IgG/mL resin	N/A
Wash 1	Equilibration buffer, neutral pH	5.0 CV
Wash 2	High salt buffer	5.0 CV
Wash 3	Equilibration buffer, neutral pH	5.0 CV
Elution	Low pH buffer (Collect 100 mAU-100 mAU)	4.0 CV
Strip	100 mM Phosphoric Acid	2.0 CV
Rinse (every 5th cycle)	Equilibration buffer, neutral pH	2.0 CV
Sanitization (every 5th cycle)	0.1 N Sodium hydroxide, 0.5 M Sodium chloride	15 min contact time

Acceptance Criteria:
- No substantial change in run to run consistency

Results:

Figure 2: Overlay of 20 cycles of hIgG purification on 1.2x20 cm OPUS™ column packed with CaptivA™ PriMab resin

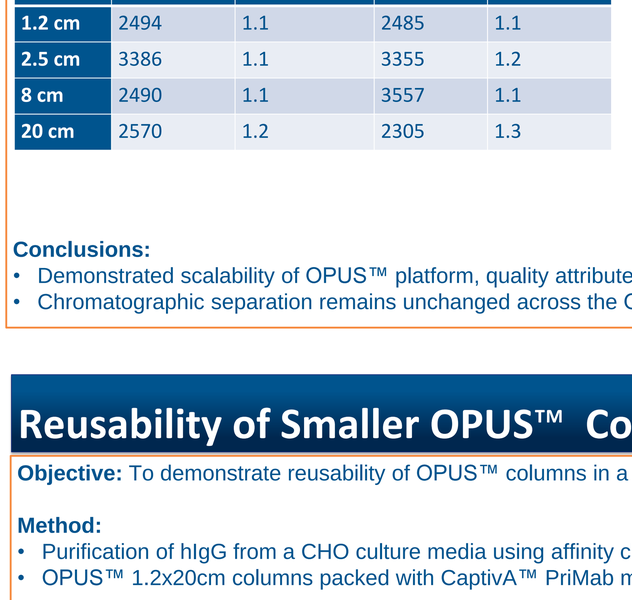


Table 4: Quantitative results of 20 cycles of hIgG purification

Run #	Elution Volume (mL)	Elution Conc. (mg/mL)	% Yield	% HCP decrease
4	26.1	18.4	86	97.5%
7	26.1	18.7	88	97.4%
10	26.3	18.2	86	96.9%
13	27.1	17.8	86	98.7%
16	27.2	17.3	85	98.3%
19	25.7	18.1	84	97.6%
20	25.9	18.4	86	98.0%
Avg	26.3	18.1	86	97.8%
% RSD	2.2%	2.5%	1.4%	0.6%

Conclusion:
- Chromatographic performance is maintained over multiple purification and sanitization runs on the same OPUS™ column
- OPUS™ columns provide robust and dependable performance for process development and characterization work

Multi-cycle Performance of Larger OPUS™ Columns

Objective: To demonstrate that OPUS™ columns maintain chromatographic performance throughout a multi-cycle purification campaign

Method:
- Purification of a recombinant protein from filtered cell lysate on a cation exchange resin
- OPUS™ 20x20cm SP Sepharose® column; linear flow rate of 200 cm/hr
- 10 total cycles with multiple buffer/ load preparations on different days

Table 5: Purification procedure for a recombinant protein on SP Sepharose®

Step	Buffer	Duration
Equilibration	Low conductivity buffer	3 CV
Load	Filtered cell lysate containing 10mg/mL protein diluted in Eq. buffer	N/A
Wash	Equilibration buffer (low conductivity)	3 CV
Elution	High salt buffer	3 CV
Sanitization	0.2 N Sodium hydroxide	2 CV

Acceptance Criteria:
- Column quality attributes pre and post run pass specifications: Plates/m: >1500, Asymmetry: 0.8-1.6
- No substantial change in run to run consistency

Results:

Figure3: Overlay of 10 cycles of purification

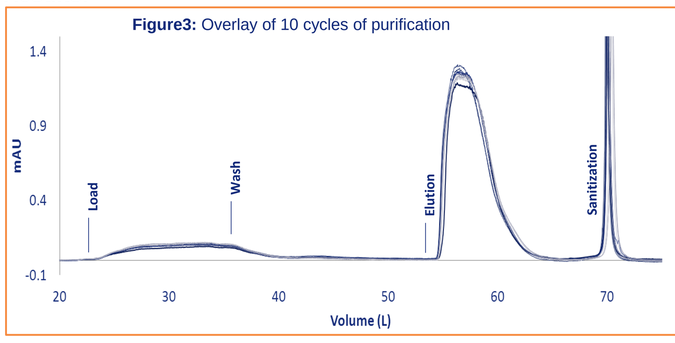


Figure 4: Column quality testing pre and post-run

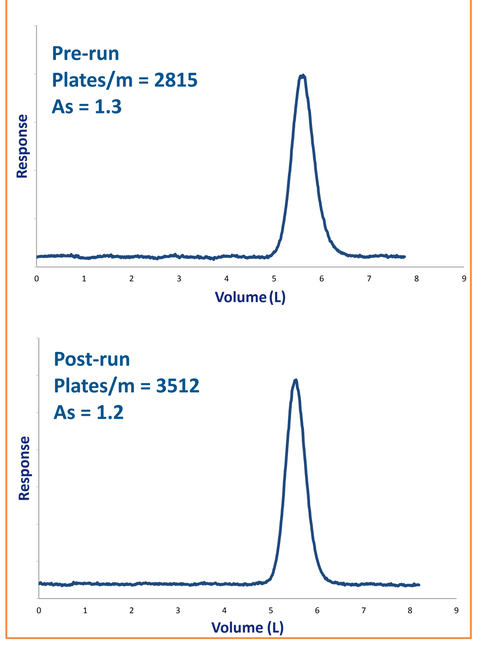


Table 7: Quantitative results of 10 cycles of purification

Run #	Elution Volume (L)	Elution Conc. (mg/mL)	% Yield	% Purity Increase (by HPLC)	Flow ΔP 200cm/hr (bar)
1	7.0	16.2	92.9	23%	0.91
2	6.6	16.9	90.4	21%	0.86
3	6.7	16.9	92.5	20%	0.85
4	6.8	16.9	93.8	21%	0.88
5	6.8	17.0	94.2	22%	0.88
6	6.8	17.1	94.5	21%	0.88
7	6.8	16.3	90.9	23%	0.88
8	6.8	16.6	92.6	22%	0.88
9	6.7	16.7	91.9	22%	0.88
10	6.8	16.4	91.8	24%	0.86
% RSD	1.5%	1.9%	1.5%	5.5%	1.9%

Conclusions:
- Column quality attributes and consistency of purification are maintained over multi-cycle use

Conclusions

- Reproducible purification process performance and the preservation of critical column quality characteristics demonstrates consistency across a range of column diameters (1.2 cm to 20 cm).
- Smaller OPUS™ columns can be reused, making them ideal for process development studies
- Larger OPUS™ columns are robust, reproducible and rugged, permitting a multi-cycle purification campaign
- OPUS™ chromatography columns can be used from development scale to production scale manufacturing, providing a pre-packed disposable chromatography solution

References:
1. 8th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production
2. Dr. Stefan Schmidt, CSO, ERA Biotech

Company Info

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