Performing Production Scale Chromatographic Separations in Pre-Packed Disposable Columns

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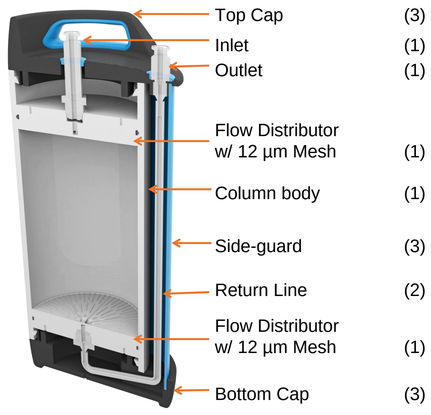
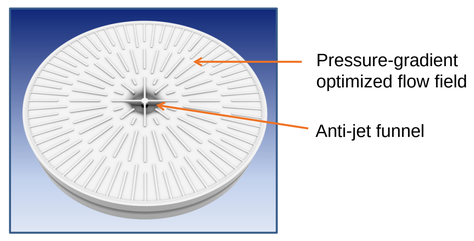
REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING



Legend:

Product Contact Material:
1 = Polypropylene
2 = Platinum Cured Silicone

Non-product Contact Materials:
3 = Acrylonitrile Butadiene Styrene (ABS) copolymer



Summary

- Disposable and single-use technologies have become standard in many of the world's leading biopharmaceutical companies. Faster product changeover, favorable economics, and improved safety have driven this paradigm shift. Until now, however, there has not been a broadly applicable solution for disposable column chromatography.
- Repligen's OPUS® (Open Platform User Specified) Pre-Packed Disposable Columns with internal diameters from 1.2 to 30 cm, and column heights from 5 cm and up, offer a scalable solution for the purification of biological products.
- Design of the flow distributor permits uniform flow distribution for all column sizes. Consistency of purification is maintained for various scales of the columns, making OPUS columns ideal for upscaling and downscaling purification processes. Column characteristics are not compromised during transportation.
- The ability to use a single pre-packed column for a multi-cycle campaign is addressed by extensive re-usability tests that include purification of a biological molecule followed by column sanitization. Cleaning of small molecules, bioburden and endotoxins is investigated quantitatively.

Packing Performance

Method:

- Column packing quality testing for different diameters OPUS columns pre-packed with Sepharose® 6 FF resin
- Separation resolution of molecular weight markers on different sizes of OPUS columns

Results:

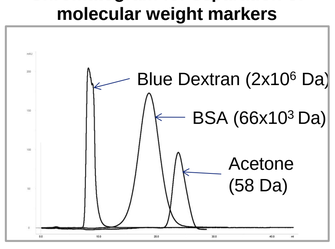
Quality of OPUS columns pre-packed with Sepharose® 6FF

Column ID	Plates/m @ 100cm/h	Asymmetry @ 100 cm/h
1.2 cm	3010	1.1
2.5 cm	3465	1.2
8 cm	2959	1.2
20 cm	3295	1.2

Separation resolutions for various sizes of OPUS columns

Column ID	Resolution Dextran/BSA	Resolution Blue Dextran/Acetone	Resolution BSA/Acetone
1.2 cm	2.2	4.6	0.9
2.5 cm	2.6	6.1	1.1
8 cm	2.3	6.1	1.2
20 cm	2.8	7.1	1.2

Chromatogram for separation of molecular weight markers



Conclusions:

- Consistency of resolution factors and column properties show optimum flow distribution
- Chromatographic performance is demonstrated and maintained over a range of OPUS column diameters

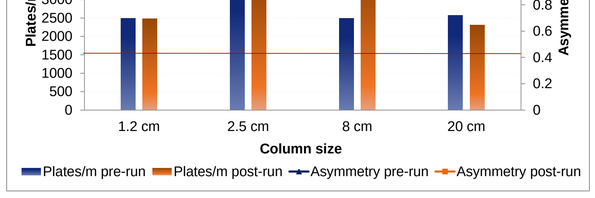
Separation Performance

Method:

- Quality testing of different diameters OPUS columns packed with an affinity media
- Affinity separation of hIgG from BSA on OPUS columns with diameters of 1.2, 2.5, 8, 20 cm, packed at 20 cm bed height with CaptivA™ PriMab™ (Sepharose® 4FF backbone)

Results:

Column properties for different sizes CaptivA PriMab OPUS columns



SDS-PAGE of CaptivA PriMab purification

Conclusions:

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

Transportation Qualification

Method:

- A 20 x 20 cm OPUS column packed with SP Sepharose® was shipped 6000 miles by truck and air.

Results:

- Column and packaging found undamaged and intact

20 x 20 cm OPUS column quality testing pre and post transportation

	2815	1.3
	2820	1.1
Post-ship, up-flow @ 100 cm/h	2850	1.2

Conclusions:

- Packaging withstood the rigors of a commercial shipping environment
- Chromatographic performance maintained after shipping

Note: An International Safe Transit Association's (ISTA) test was also performed; OPUS column packaging passed the test, and the column quality was not compromised (data not shown)

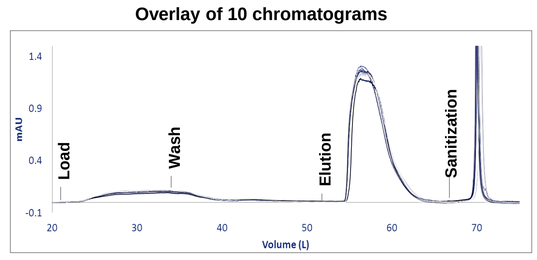
Multi-cycle Performance of a 20 cm ID OPUS Column

Method:

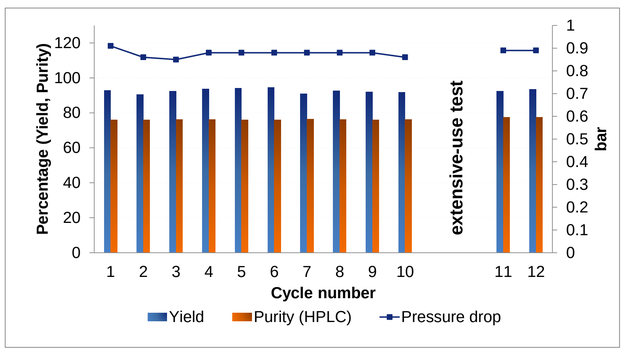
- Purification of a recombinant protein from filtered cell lysate on an OPUS 20 x 20 cm column packed with SP Sepharose®; for 10 cycles, plus 2 additional cycles after extensive re-use test
- Extensive use test: re-circulate the same OPUS column with high salt buffer for 2 weeks

Results:

Overlay of 10 chromatograms



Quantitative results for 10 cycles of purification followed by additional 2 cycles after the extensive re-use test



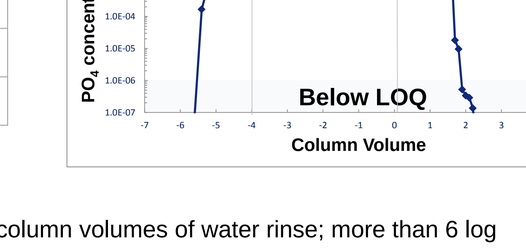
Column quality testing pre and post-run

Conditions	Plate Count plates/m	Asymmetry
Pre-run	2815	1.3
Post-run	3512	1.2

Column quality during extensive use test

Column Volumes of 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

Overlay of 2 additional cycles of purification



Conclusions:

- Column packing quality attributes and consistency of purification are maintained throughout the study
- Performance characteristics are maintained for simulated equivalent flow of >100 process cycle

Cleaning of Small Molecules, Bioburden, and Endotoxin

Method:

- Colorimetric measurement of phosphate reduction on a 20 x 20 cm OPUS column packed with Sepharose® 6FF washed with water
- Sanitization with 1M sodium hydroxide of a 20 x 20 cm OPUS column packed with Sepharose® 6FF, loaded with *E. coli*, followed by bioburden and endotoxin quantitative measurement

Results:

Bioburden and endotoxin test results before and after sanitization of contaminated OPUS column

Sample	CFU/mL @ 2 days	CFU/mL @ 4 days	Endotoxin (EU/mL)
Pre-inoculation water rinse	0	0	< 0.25
Flow-through after *E. coli* incubation	$9x10^6$	$9x10^6$	> 0.25
Post-sanitization water rinse	0	0	< 0.25

Phosphate detection in the column effluent

Conclusions:

- Phosphate is reduced to undetectable levels after two column volumes of water rinse; more than 6 log reduction
- Endotoxin and bioburden can be effectively removed from OPUS columns with the appropriate sanitization protocol

Conclusions

- Pre-packed disposable columns of the OPUS platform are **ideal for the purification of biological molecules** due to the well-engineered design which delivers consistent chromatographic performance, robust packed bed stability for commercial shipping, and industry standard product contact materials
- Consistency in chromatographic performance at different column sizes makes OPUS column platform ideal for **development and scale-up** of purification processes
- Reliable cleaning and sanitization, along with demonstrated reusability make pre-packed disposable columns suitable for **production scale manufacturing** purifications in single-use or multi-cycle processes

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