Advancements in Large Scale Pre-Packed Chromatography Columns

Dana C. Pentia, Matthew J. Heisler, James R. Peyser
Repligen Corporation, Waltham, MA, USA

Summary

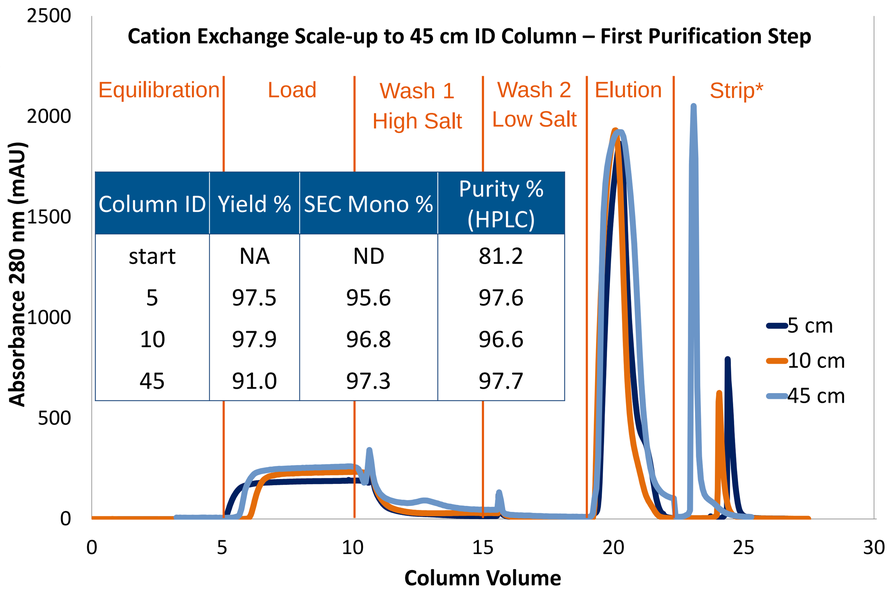
- Pre-packed chromatography columns with internal diameters up to 45 cm are now available for large scale GMP purification of biological products
- Column design permits reliable process scale-up yielding comparable chromatographic output and product quality characteristics– ease of upscaling and downscaling can be achieved with pre-packed disposable chromatography columns
- Efficient cleaning capability makes pre-packed column platform ideal for multi-cycle and campaign use

Scalability of a Recombinant Protein Purification to 45 cm ID Pre-Packed Columns

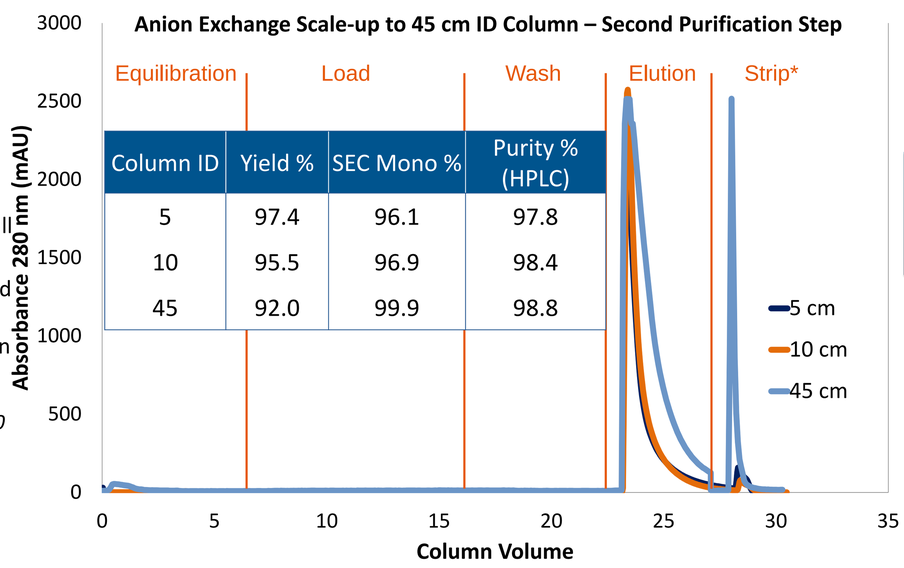
Methods:

- A multi-step purification process for a recombinant protein produced in *E.coli* was scaled-up using OPUS® pre-packed columns with internal diameters of 5, 10 and 45 cm
- The purification process included a cation exchange followed by an anion exchange chromatography step
- Process output: yield, monomeric purity by SEC, product purity by HPLC

Note: Purification process with 45 cm ID columns were performed in a manufacturing setting. Small differences in buffer preparation existed.



Column ID	Yield %	SEC Mono %	Purity % (HPLC)
start	NA	ND	81.2
5	97.5	95.6	97.6
10	97.9	96.8	96.6
45	91.0	97.3	97.7



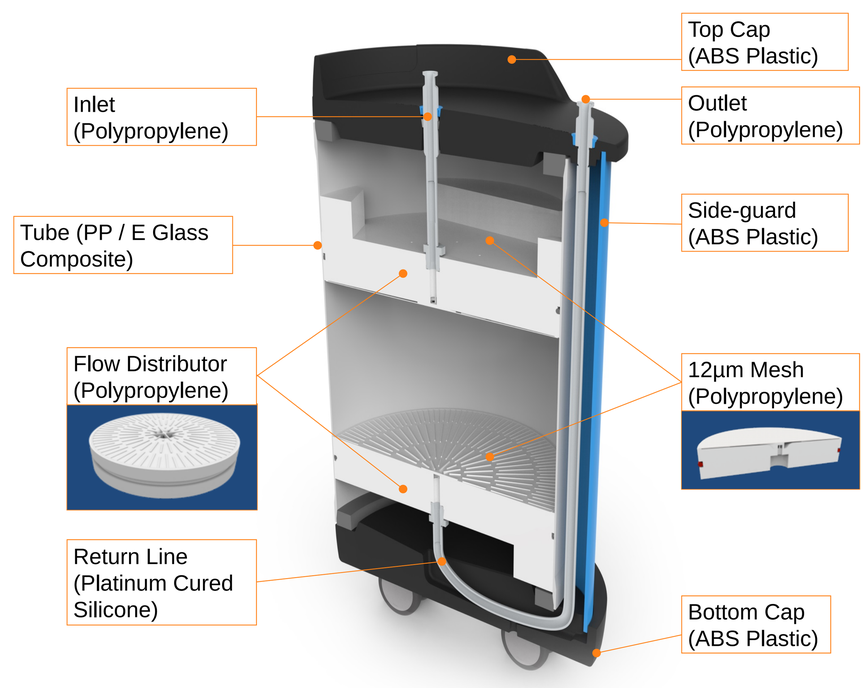
Column ID	Yield %	SEC Mono %	Purity % (HPLC)
5	97.4	96.1	97.8
10	95.5	96.9	98.4
45	92.0	99.9	98.8

Conclusions:

- Chromatographic profiles for all column sizes were similar
- Column step yields were greater than 90% for all scales of the process
- Purified protein had similar characteristics in terms of monomeric and product purity at all scales of the process
- This purification process was scaled to a 45cm ID pre-packed column platform for commercial production

** The strip step on the 45 cm ID columns was run in up-flow, while the 5 cm and 10 cm ID were run in down-flow*

Design Schematic of a 45 cm ID Pre-Packed Disposable Column



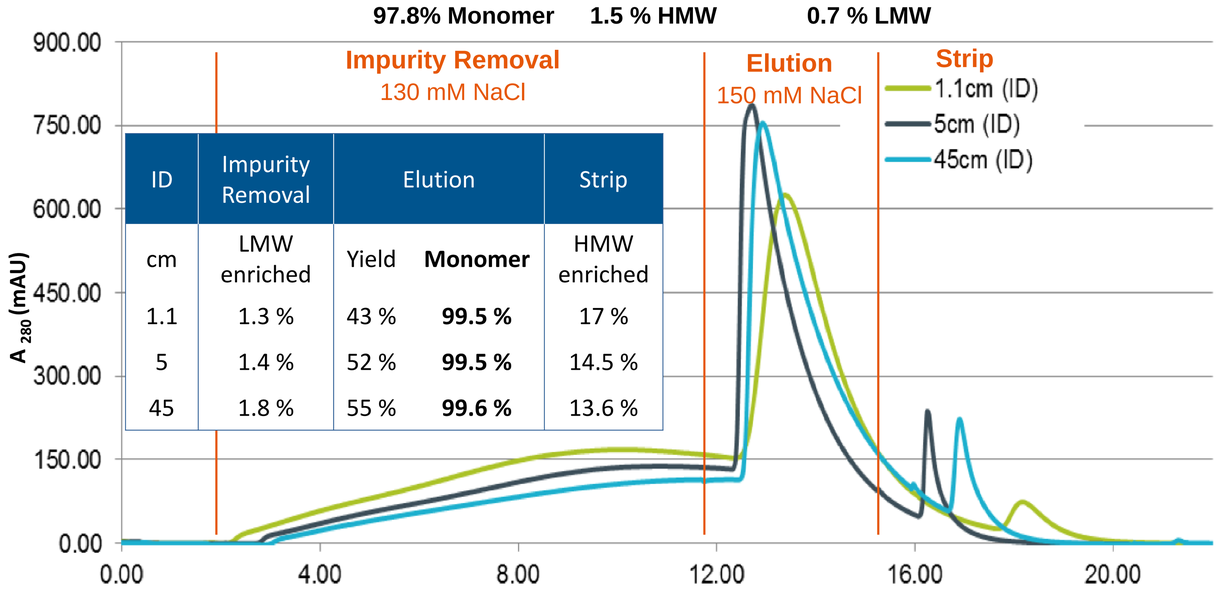
Scale-up of a Challenging Separation of Antibody Species to a 45 cm ID Column

gallus Uniquely Flexible CMO™

Methods:

- Cation exchange separation of low and high molecular weight species from a monoclonal antibody product – scaled–up to 45 cm ID column
- The 45 cm column was OPUS® pre-packed, all other columns were traditional self-packed

Results:



ID	Impurity Removal	Elution		Strip
cm	LMW enriched	Yield	**Monomer**	HMW enriched
1.1	1.3 %	43 %	**99.5 %**	17 %
5	1.4 %	52 %	**99.5 %**	14.5 %
45	1.8 %	55 %	**99.6 %**	13.6 %

Conclusions:

- 45 cm ID columns deliver expected scalability outcome for a difficult separation process
- Product purified on 45 cm ID column had similar quality attributes as that purified on smaller columns IDs

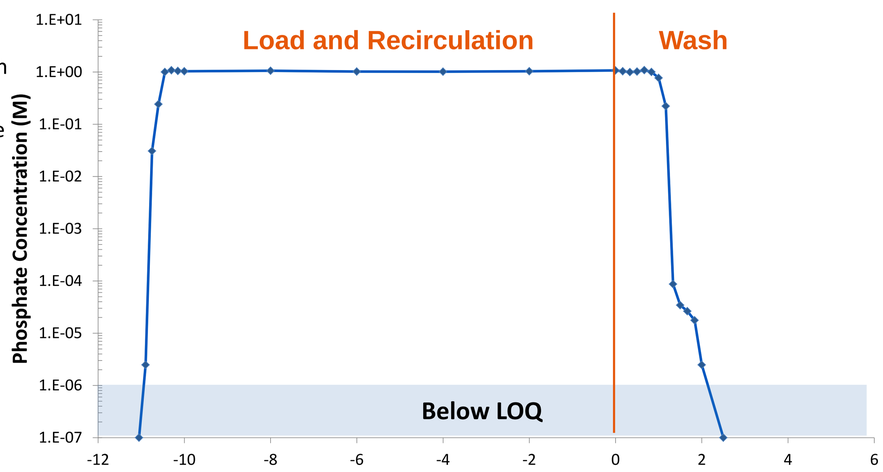
Cleanability of the 45 cm ID OPUS® Column

Methods:

- 45 x 20 cm OPUS® pre-packed with Sepharose® 6FF
- Saturation and wash of phosphate – colorimetric determination of PO_4 traces
- Sanitization of a *E.coli saturated* column – removal of bioburden and endotoxin

Conclusions:

- 45 cm ID pre-packed columns allow for cleaning of small molecules in less than 2 CVs
- Efficient reduction of bioburden, and endotoxin
- Columns are suitable for multi-cycle campaigns that require cleaning, sanitization and/or storage in between runs



Sample	CFU @ 2 days	CFU @ 7 days	Endotoxin (EU/mL)
After overnight incubation with E. coli	TNTC	TNTC	> 1
After cleaning	0	0	< 1

Conclusions

- Large scale pre-packed chromatography columns **fill the gap** in adoption of single-use disposable biologics manufacturing platforms
- **Purification of biological molecules** produced in bioreactors of 500 & 1000 L scale can be achieved with OPUS® platform of pre-packed columns
- Complex purification processes can be **scaled easily** from laboratory to manufacturing scale
- Reliable **cleaning and sanitization** design make pre-packed columns suitable for GMP production scale manufacturing purifications in single-use or multi-cycle processes

Acknowledgments:
Repligen team: Alan Chan, Travis Ward, William Wilde, Downstream Manufacturing Purification Team, Michael Podolski, Fletcher Malcom
Gallus team: Kristina Pleitt, Kelly Wei, Paul Jorjorian, Suite 5 Downstream Purification

Company information:
Repligen Bioprocessing
41 Seyon Street
Waltham, MA 02453
www.repligen.com/bioprocessing
+1-781-215-0111