TOSOH

Seamless scale-up of downstream processes

Your Challenge

- You need to quickly scale up downstream processes from lab- to pilot-scale.
- You suffer from poorly packed columns or long lead times.

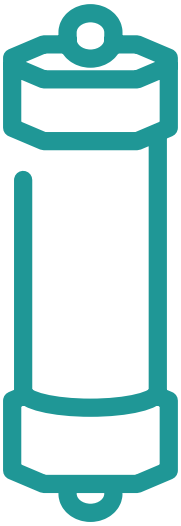
Our Solution

SkillPak pre-packed columns
- Available from 1 mL to 200 mL

What was done?
- We demonstrated the separation performances at various scales using protein standards.

What was the result?
- Reproducible performances at all scales

SkillPak pre-packed column platform enables seamless scale-up. We demonstrated that neither the capacity of the packed resin nor the separation efficiency are affected when increasing column volume.



SkillPak® pre-packed columns

Your Benefit

Quickly scale up your DSP methods from lab- to pilot-scale using SkillPak columns.

https://www.separations.eu.tosohbioscience.com/solutions/process-mediaproducts/process-development/skillpak

TOSOH BIOSCIENCE
SEPARATION & PURIFICATION
CONNECTING MINDS.
TOUCHING LIVES.

TOSOH

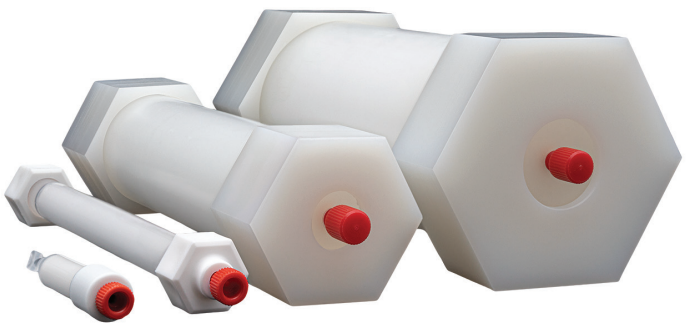
Application Note

Seamless scale-up of downstream processes using SkillPak® pre-packed columns

Today's biopharmaceutical landscape is a rapidly developing and ever-changing environment. For trends like personalized therapeutics, a quick and robust way to scale-up chromatographic processes is more critical than ever. This makes it is possible to shorten process development time, which impacts development cost and duration to get into clinical phases, and is greatly beneficial for the overall biopharmaceutical economics.

The SkillPak pre-packed column platform from Tosoh Bioscience enables seamless scale-up from 5 mL columns to 200 mL columns. With this platform, chromatographic processes can be designed using low amounts of samples and consumables. Once optimal conditions are determined on a small scale, the process can be easily transferred to larger scales.

To demonstrate the excellent scale-up capabilities of SkillPak pre-packed columns, we separated two different protein standards on different column dimensions packed with the anion exchange (AEX) resin TOYOPEARL® NH_2-750F and cation exchange (CEX) resin TOYOPEARL Sulfate-650F. Next, the dynamic binding capacity (DBC) of Bovine Serum Albumin (BSA) on the AEX resin was determined at different scales to demonstrate scale equivalence. Last, the elution profile of BSA in a linear salt gradient was compared at the different scales.



Experimental Conditions

All experiments were done using TOYOPEARL NH_2-750F and TOYOPEARL Sulfate-650F in SkillPak pre-packed columns with column volumes (CV) of 5 mL (8 mm ID × 10 cm L), 50 mL (25 mm ID × 10 cm L), and 200 mL (50 mm ID × 10 cm L) on an ÄKTA® Avant 150 system.

Separation of Protein Standards

Protein standards were separated on these columns to understand the separation capabilities better. Therefore, mixtures of proteins were created. The proteins were selected by their respective isoelectric points (pI), which provides information about their net surface charge at specific pH values. For separation on the AEX resin TOYOPEARL NH_2-750F, mostly proteins with acidic pIs were chosen. The opposite was the case for the protein mixture to be separated on the CEX resin TOYOPEARL Sulfate-650F. *Tables 1 & 2* show the different protein mixtures with concentration and pI for the individual components:

Table 1. Composition of AEX protein standard

Protein	C (g/L)	pI
Papain	0.5	~8.8
Trypsin inhibitor (soy bean)	0.5	~4.1 - 4.5
BSA	1	~4.7
Pepsin	1	~ 2.7

Table 2. Composition of CEX protein standard

Protein	C (g/L)	pI
γ-Globulins (from human blood)	0.5	~ 7.2
Ribonuclease A	0.5	~ 8.5
α-Chymotrypsinogen	0.5	~ 9.6
Lysozyme	0.5	~ 11

The proteins were dissolved in the respective equilibration buffers and then separated using the chromatographic method and buffers shown in *Table 3*. Flow rate was kept constant at 100 cm/h throughout the entire study. UV response was monitored at 280 nm.

Table 3. Chromatographic method for separation of protein standards

Step	CV	Buffer AEX	Buffer CEX
Equilibration	5	20 mmol/L TRIS, pH 8.0	100 mmol/L NaAc, pH 5.0
Sample application	1	AEX - Protein Standard (see Table 1)	CEX - Protein Standard (see Table 2)
Wash	5	20 mmol/L TRIS, pH 8.0	100 mmol/L NaAc, pH 5.0
Gradient Elution	10	20 mmol/L TRIS + 1 mol/L NaCl, pH 8.0	100 mmol/L NaAc, pH 5.0 + 1 mol/L NaCl
High salt wash	5	20 mmol/L TRIS + 1 mol/L NaCl, pH 8.0	100 mmol/L NaAc, pH 5.0 + 1 mol/L NaCl
CIP	5	0.5 mol/L NaOH	0.5 mol/L NaOH
Re-equilibration	5	20 mmol/L TRIS, pH 8.0	100 mmol/L NaAc, pH 5.0

Table 4. Chromatographic method for comparison of BSA elution profiles on TOYOPEARL NH_2-750F in different column scales

Step	CV	Buffer CEX
Equilibration	4	20 mmol/L MES, 20 mmol/L HEPES, 20 mmol/L Acetic acid, 20 mmol/L NaCl, pH 7.3
Sample application	variable	3.5 g/L BSA in Equilibration Buffer
Wash	2	Equilibration Buffer
Elution	8.8 Gradient + 2 100 %	Equilibration Buffer + 1 mol/L NaCl
CIP	2 + 1	0.5 mol/L NaOH (18 min hold after 2 CV flush)
Flush	2	Equilibration Buffer + 1 mol/L NaCl
Re-equilibration	2	Equilibration Buffer

DBC and elution profiles of BSA on TOYOPEARL NH_2-750F

As a further evaluation, the DBC of BSA on TOYOPEARL NH_2-750F was measured in the three SkillPak pre-packed columns of different CVs to show independence of binding capacity from column scale. During loading, residence time was kept constant at four minutes.

Additionally, the three SkillPak pre-packed columns containing TOYOPEARL NH_2-750F were loaded with BSA until 85% of the DBC was reached. Afterward, BSA was eluted in a linear salt gradient according to the method shown in *Table 4* to compare elution profiles.

Flow rates were kept constant at 100 cm/h during the entire process.

Results and Discussion

Separation of Protein Standards on AEX and CEX

Figures 1 & 2 show the comparison of UV signals for the separation of the protein standards on TOYOPEARL NH_2-750F and TOYOPEARL Sulfate-650F, respectively, in different scales using SkillPak pre-packed columns.

The separation of the protein standards on AEX and CEX leads to similar elution profiles on the different column scales. The proteins elute with increasing net surface charge during the linear salt gradient. In both cases, a shift to the right in retention volume can be observed with decreasing column volume. This can be attributed to the different influences of the system dead volume, leading to a higher retention volume when referenced as CV.

Figure 1. Separation of a protein standard on TOYOPEARL NH_2-750F in different scales

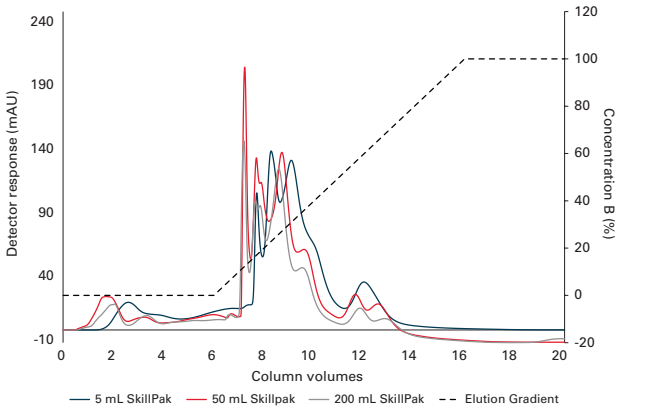
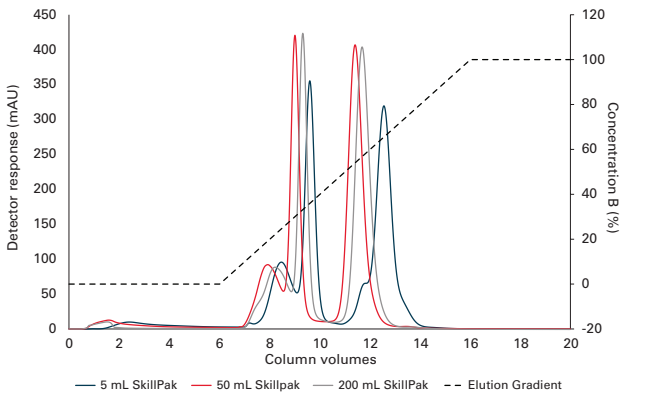


Figure 2. Separation of a protein standard on TOYOPEARL Sulfate-650F in different scales



DBC and elution profile of BSA on different scales of TOYOPEARL NH_2-750F

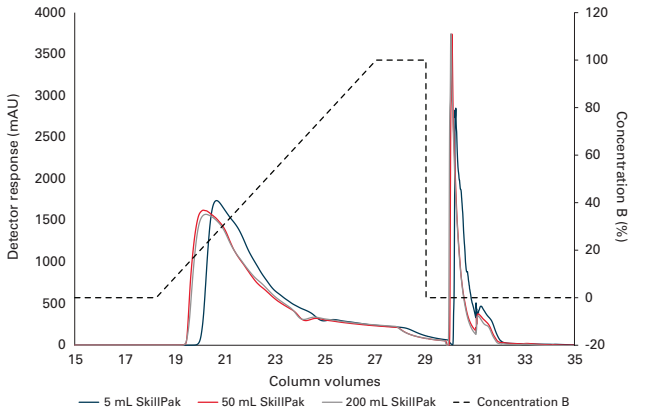
The values for DBC of BSA on the different scales of TOYOPEARL NH_2-750F are very close, with a maximum deviation of 4% *(see Table 5)*. This shows the independence of binding capacity from column scale, which can be very important with regard to loading mass in scaled-up processes. With the given independence, binding capacity can be determined on a small scale to conserve both product and buffers. Later the determined capacity and thus maximum loading masses can be confidently assumed for larger-scale columns.

Table 5. DBC of BSA on different scales of TOYOPEARL NH_2-750F

CV	DBC 10%
5	67.10
50	64.89
200	64.58

Figure 3 shows the elution profiles of BSA on different scales of TOYOPEARL NH_2-750F.

Figure 3. Elution profile of BSA on different scales of TOYOPEARL NH_2-750F



Once again, the scale does not influence the elution profile. The chromatograms overlay very well. The only difference, as previously explained, is an offset in retention volume for the smallest column volume, which is again explained by the larger influence of the system dead volume.

Conclusion

The SkillPak pre-packed column platform enables seamless scale-up from 5 mL to 200 mL in column volume. We demonstrated that neither the capacity of the packed resin nor the separation efficiency are affected when increasing column volume. This can facilitate process development by decreasing working volumes in early stages without worrying about the processes' performance on larger pre-packed scales in later stages. The results presented here were generated using two different ion exchange resins. Other methods using Hydrophobic Interaction Chromatography (HIC) or Affinity Chromatography (AFC) could be scaled-up similarly. Entire processes employing orthogonal separation principles can easily be scaled-up or down using SkillPak pre-packed columns.

Tosoh Bioscience, TSKgel and TOYOPEARL are registered trademarks of Tosoh Corporation. SkillPak is a registered trademark of Tosoh Bioscience LLC in the USA, EU, and India. ÄKTA is a registered trademark of Cytiva.

Ordering Information

Part #	Description	Resin volume	Column dimensions
45245	SkillPak 5 TOYOPEARL NH_2-750F	5 mL	0.8 cm ID × 10 cm
45310	SkillPak 50 TOYOPEARL NH_2-750F	50 mL	2.5 cm ID × 10 cm
45311	SkillPak 200 TOYOPEARL NH_2-750F	200 mL	5.0 cm ID × 10 cm
45241	SkillPak 5 TOYOPEARL Sulfate-650F	5 mL	0.8 cm ID × 10 cm
45318	SkillPak 50 TOYOPEARL Sulfate-650F	50 mL	2.5 cm ID × 10 cm
45319	SkillPak 200 TOYOPEARL Sulfate-650F	200 mL	5.0 cm ID × 10 cm