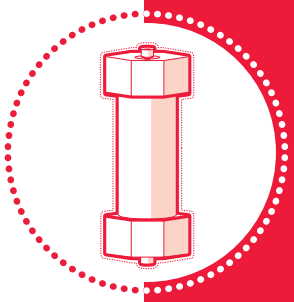
TOSOH



Development of an antibody platform from 1 to 200 mL

Your Challenge

- You deal with an increased demand for mAbs for therapeutic applications.
- You need to reduce the costs of such treatments.

Our Solution

Replacement of traditional methods by a 2-step platform
- Innovative resins packed in SkillPak format

What was done?
- Development on 1 & 5 mL columns; scale up to 200 mL

What was the result?
- Most effective mAb platform with no adjustments to the process parameters from 1 to 5, 50 and 200 mL

TOYOPEARL AF-rProtein A HC-650F and TOYOPEARL NH_2-750F packed in SkillPak columns allow scientists to develop cost-effective purification processes and move from process development to pilot scale quickly, safely, and efficiently.

Your Benefit

Speed up process development and **reduce DSP costs** by using Tosoh's solutions.

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

Purification of monoclonal antibodies

TOSOH BIOSCIENCE

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Application Note

Development and scale-up of an antibody platform using 1 mL to 200 mL SkillPak™ pre-packed columns

The demand for purified monoclonal antibodies (mAb) for therapeutic applications has increased drastically in the clinical pipeline over the last few years. To enable the implementation of such efficient therapies in a larger patient cohort, the biopharmaceutical industry needs to reduce the costs of such treatments. As a partner of the industry, Tosoh Bioscience is constantly working on new ways to improve the productivity of purification processes, reduce the development time, and make such development easier for biopharma professionals.

In this application note, we share how we developed a 2-step platform to purify a specific mAB, Pertuzumab, using 1 mL and 5 mL SkillPak pre-packed columns. The 2-step platform consists of a Protein A capture and a single polishing step on a salt-tolerant anion exchange resin (AEX). Subsequently, we demonstrate how these new SkillPak pre-packed columns enables an easy and direct scale-up from 1 mL to 200 mL.

SkillPak pre-packed columns allow scientists to develop and optimize chromatographic processes on a small scale with low sample and material consumption while enabling robust and safe transfer to larger scales.

Experimental Conditions

All experiments were done using TOYOPEARL® AF-rProtein A HC-650F and TOYOPEARL NH_2-750F in SkillPak pre-packed columns with column volumes of 1 mL (7 mm ID × 2.5 cm L), 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. The feedstock consisted of clarified Pertuzumab.

DBC – TOYOPEARL AF-rProtein A HC-650F

Dynamic binding capacity (DBC) measurement was performed using a SkillPak 1 TOYOPEARL AF-rProtein A HC-650F column (1 mL). After equilibration (5 CV) with 100 mmol/L sodium phosphate buffer pH 7, the column was loaded with Pertuzumab Protein A eluate adjusted to 2 g/L mAb with a residence time of 4 min (39 cm/h). After 10% breakthrough of the maximum UV absorbance, DBC was calculated via sample volume.

Capture – TOYOPEARL AF-rProtein A HC-650F

TOYOPEARL AF-rProtein A HC-650F was equilibrated with 100 mmol/L sodium phosphate pH 7.0 and loaded with 8 CV of clarified cell culture fluid with 4.4 mg/mL Pertuzumab. The first washing step was performed with 100 mmol/L sodium phosphate pH 7.0 for 5 CV, and the second washing step was performed with 100 mmol/L sodium acetate, pH 6.0 for 6 CV. Elution was carried out with 100 mmol/L sodium acetate pH 3.0 (8 CV). The cleaning of the column (CIP) was performed with 200 mmol/L NaOH + 500 mmol/L NaCl for 5 CV. After cleaning, the column was re-equilibrated with 100 mmol/L sodium phosphate pH 7.0. The flow rate in the equilibration, washing, CIP, and re-equilibration steps was 180 cm/h and 150 cm/h during the load.

Polishing – TOYOPEARL NH_2-750F

To find the best process and separation conditions for the flow-through process, a bind-and-elute purification with a 20 mmol/L Tris-HCl pH 8, 8.5, and 9 was carried out on a 5 mL SkillPak column. The column was equilibrated for 3 CV before loading 55 mg of the Protein A eluate sample. Afterward, a washing step with equilibration buffer for 5 CV was performed. The elution was performed with a linear gradient (20 CV) to 20 mmol/L Tris-HCl pH 8, 8.5, and 9 + 1 mol/L NaCl. Afterward, CIP was carried out with 500 mmol/L NaOH. The flow rate during the entire process was 300 cm/h.

After an ideal pH value was determined, flow-through experiments were carried out at different conductivities to find the best separation conditions.

Scale-up of the 2-step process

The Protein A purification and flow-through polishing methods were transferred from 5 mL to 50 mL and 200 mL columns, keeping loading (mg/mL), linear flow rate (cm/h), and column volumes per step constant.

Analytical SEC

The feed and the flow-through of TOYOPEARL NH_2-750F were analyzed by size exclusion chromatography (SEC) using a TSKgel® UP-SW3000 to determine the content of monomers and aggregates of mAb.

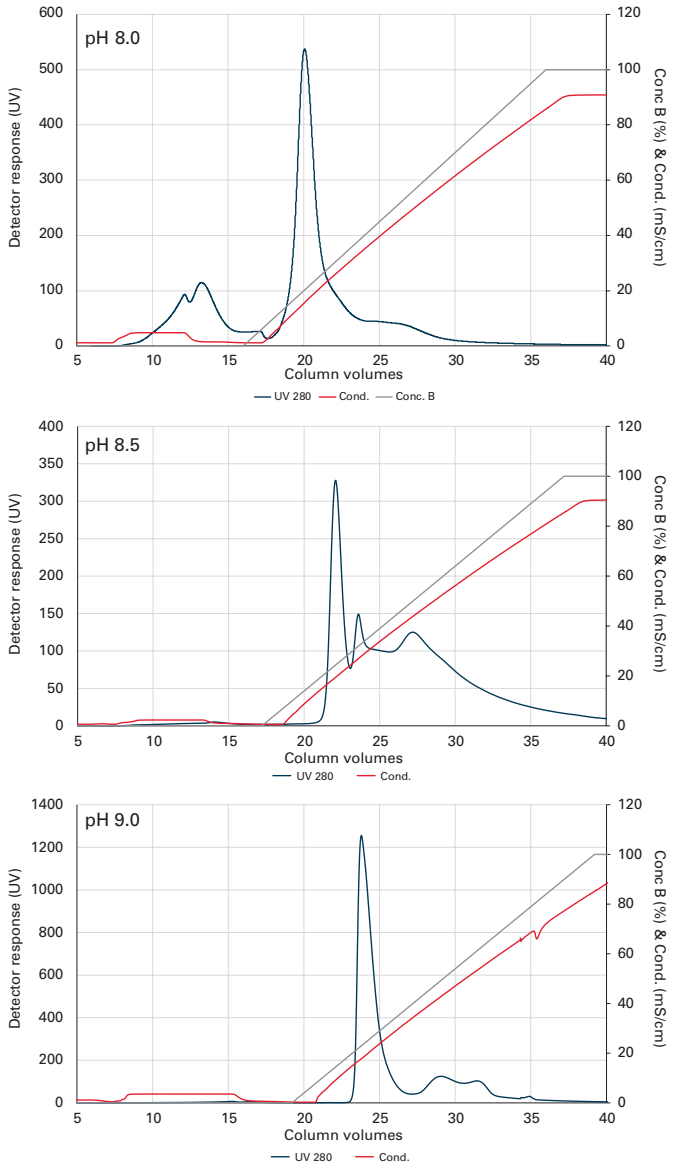
Results and Discussion

Process development

The first experiments on AEX were performed in bind-and-elute mode with a linear salt gradient at pH 8.0, 8.5, and 9.0 to determine the best pH value for the separation of Pertuzumab. Due to the lower net charge, the monomer eluted before the aggregates, which allowed for the development of a flow-through process when setting the conductivity between the monomer and aggregate elution range.

The pH had an impact on the binding behavior based on the isoelectric point (pI) of mAb. The best separation conditions of the mAb were found at pH 9 with two separated sample peaks *(Figure 1)*.

Figure 1. Bind/elute chromatograms of TOYOPEARL NH_2-750F at different pH values.



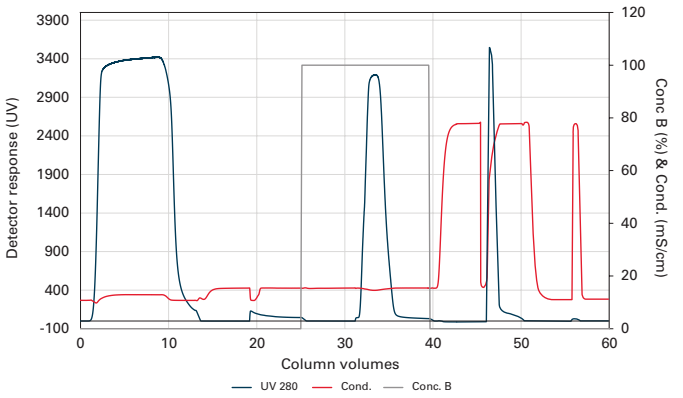
From the experiment with AEX in bind-and-elute mode at pH 9, we determined which conductivity range allows the elution of monomer from aggregate. Based on this range, we carried out flow-through tests at three different conductivities (17 mS/cm, 20 mS/cm, and 25 mS/cm). The purities and yields at those conductivities are listed in *Table 1*.

Table 1. Aggregate content and recovery after polishing on TOYOPEARL NH_2-750F at different conductivities during flow-through process development.

Conductivity (mS/cm)	Aggregates (%)	Recovery (%)
17	0.08	87.76
20	0.27	94.17
25	1.47	98.41

A 17 mS/cm conductivity was chosen for the platform design to achieve the desired purity without a subsequent chromatography step. The corresponding chromatogram is shown in *Figure 2*.

Figure 2. Flow-through chromatogram of the Protein A eluate on TOYOPEARL NH_2-750F at pH 9 and 17 mS/cm.



Scale-up

The process generated on the 5 mL scale was then transferred to SkillPak columns with volumes of 50 mL (2.5 cm ID × 10 cm L) and 200 mL (5.0 cm ID × 10 cmL) packed with TOYOPEARL AF-rProtein A HC-650F and TOYOPEARL NH_2-750F resins.

The results are summarized in *Table 2*.

Table 2. Summary of 2-step scale-up from 5 mL to 50 mL to 200 mL using TOYOPEARL AF-rProtein A HC-650F and TOYOPEARL NH_2-750F in SkillPak pre-packed columns

CV (mL)	Loaded mAb (mg)	Recovery Pro A (%)	Recovery NH_2 (FT) (%)	Overall recovery (%)	Monomer purity (%)
5	176	98.14	87.76	86.13	99.92
50	1932	98.58	94.97	93.62	99.69
200	7728	97.58	92.63	90.38	99.75

Table 2 shows the individual recoveries for TOYOPEARL AF-rProtein A-650F and TOYOPEARL NH_2-750F steps as well as the combined overall recovery of the process at the different scales. In the last column, we shared the monomer purity at the end of the process. Overall recoveries of around 90 % and a high monomer purity of over 99.9 % were achieved. When comparing the performance at different scales, some deviations become apparent, especially regarding the recovery of the flowthrough AEX step. The explanation for these deviations is that the fractionation volume was not adapted to the changes in column volume. In other words, the last collected fraction contained varying amounts of high molecular weight impurities, decreasing the purity, and increasing the recovery. For another run, where the cutoff might have been earlier due to how the different factions spaced out, the purity was higher while the recovery was lower.

These results are sufficient as a scale-up experiment. The next step before moving into pilot purification would be the optimization of the fraction collection to ensure optimal recoveries and purities while maintaining maximum efficiency and avoiding implementing a third chromatography step.

Conclusion

In this application note, we highlighted the efficient development of a 2-step antibody purification platform with TOYOPEARL AF-rProtein A HC-650F and TOYOPEARL NH_2-750F using 1 mL and 5 mL SkillPak pre-packed columns. Development at these small scales allows for low resin, feedstock, and buffer consumption. Using 50 mL and 200 mL SkillPak columns, this process was effortlessly scaled-up without any adjustments to the process parameters determined in the previous small-scale experiments. This seamless scale-up using SkillPak pre-packed columns allows scientists to move from process development to pilot scale quickly, safely, and efficiently.

Ordering Information

Part #	Description	Resin volume	Column dimensions
0045209	SkillPak 1 TOYOPEARL NH_2-750F	1 mL	0.7 cm ID × 2.5 cm
0045245	SkillPak 5 TOYOPEARL NH_2-750F	5 mL	0.8 cm ID × 10 cm
0045310	SkillPak 50 TOYOPEARL NH_2-750F	50 mL	2.5 cm ID × 10 cm
0045311	SkillPak 200 TOYOPEARL NH_2-750F	200 mL	5.0 cm ID × 10 cm
0045201	SkillPak 1 TOYOPEARL AF-rProtein A HC-650F	1 mL	0.7 cm ID × 2.5 cm
0045258	SkillPak 5 TOYOPEARL AF-rProtein A HC-650F	5 mL	0.8 cm ID × 10 cm
0045338	SkillPak 50 TOYOPEARL AF-rProtein A HC-650F	50 mL	2.5 cm ID × 10 cm
0045339	SkillPak 200 TOYOPEARL AF-rProtein A HC-650F	200 mL	5.0 cm ID × 10 cm