TOSOH

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APPLICATION NOTE

REDUCE DOWNSTREAM PROCESSING COSTS FOR mAbs BY SWITCHING TO TOSOH'S 2-STEP PLATFORM

INTRODUCTION

Downstream processing is responsible for up to 80 percent of the entire production costs of biotherapeutics. Given the current drive to reduce the cost of manufacturing for biological therapeutics, streamlining downstream processing is a necessity for chromatographers and process engineers.

In this study, we showcase the benefits of Tosoh's 2-step process for the purification of monoclonal antibodies (mAb) in comparison to the standard industrial process. Combining high-performance Protein A capturing and a single polishing step on salt-tolerant anion exchange resin, we could reduce the downstream costs by 45 % and increase the production output by 58 %.

MATERIAL AND METHODS

Resins and Pre-packed Columns
TOYOPEARL® AF-rProtein A HC-650F is a high capacity Protein A resin for the purification of mAbs. This resin exhibits dynamic binding capacities (DBC) of 70 g/L at 5 minutes of residence time.

TOYOPEARL® NH_2-750F, a salt-tolerant anion exchange resin, is based on the TOYOPEARL methacrylate backbone and functionalized with primary amine groups. TOYOPEARL NH_2-750F resin is ideal for the intermediate purification of mAbs and other proteins. Impurities, such as DNA, viruses, host cell proteins, and endotoxins, are removed. Furthermore, because of the relatively low pKa-value (between 7 and 9), TOYOPEARL NH_2-750F is also able to remove mAb-aggregates efficiently. With this unique feature, both polishing steps that are usually necessary to remove all impurities are combined in one flow-through polishing step.

All experiments were performed on SkillPak™ 5 mL prepacked columns. The SkillPak columns are designed for fast method development or resin screening. These columns guarantee optimal performance and can be operated with commonly used low or medium pressure liquid chromatography systems. They are reproducibly packed and take into account the varying compressibility of each resin, providing an accurate representation of conditions found in full-scale columns.

PURIFICATION PROTOCOLS

Capturing – TOYOPEARL AF-rProtein A HC-650F
TOYOPEARL AF-rProtein A HC-650F was equilibrated with 100 mM sodium phosphate pH 7.0 and loaded with 20 CV (100 mL) of clarified cell culture fluid with 2 mg/mL Adalimumab. The washing step was performed with 100 mM sodium acetate, pH 7.0 for 10 CV (50 ml). Elution was carried out with 100 mM sodium acetate pH 3.0. The cleaning of the column was performed with 200 mM NaOH. After cleaning, the column was reequilibrated with sodium phosphate buffer. The flow rate in the steps of equilibration, washing, elution, and re-equilibration was 204 cm/h, 150 cm/h for load, and 180 cm/h for CIP.

Virus inactivation
The eluate from the capturing step was held at pH 3.0 for 1 hour before being adjusted back to pH 8.0 and the desired conductivity for the polishing experiments with 1 M Tris. The aggregate content after the incubation at pH 3.0 was 1.05%.

Polishing – TOYOPEARL NH_2-750F
The Protein A purified antibody was diluted to a concentration of 1 mg/ml and adjusted to the conductivity of 20, 22.5 or 25 mS/cm and 20 mM Tris-HCl, pH8. The column was equilibrated with different conductivities (20, 22.5, and 25 mS/cm – adjusted with NaCl) of the equilibration buffer. The sample was loaded for 40 CV (200 ml). Afterward, a washing step with equilibration buffer for 5 CV (25 ml) was performed. The cleaning of the column was carried out with 500 mM NaOH. The flow rate during the entire process was 300 cm/h.

Analytical SEC
The load after 1-hour hold at pH 3.0 and the flow-through of Toyopearl NH_2-750F were analyzed by size exclusion chromatography (SEC) using a TSKgel® UP-SW3000. The method can be found here.

RESULTS AND DISCUSSIONS

Process development

In previous work, we optimized the capturing of the antibody on the TOYOPEARL AF-rProtein A HC-650F. After the capturing step, we introduced a virus inactivation step by holding the eluate for 1 hour at low pH.

The first experiments on the salt-tolerant anion exchanger TOYOPEARL NH_2-750F were performed in bind-and-elute mode with a linear gradient at pH 8.0 to determine the necessary conductivity for the flow-through experiments. (Figure 1).

As the monomer eluted first, the next step consisted in adapting the method to achieve flow-through purification. For the flow-through experiments, we used conductivities between 20 and 25 mS/cm, as the monomer is eluting under these conditions, whereas the aggregates remain bond to the resin. The purities and yields at three different conductivities are listed in Table 1.

BIND- AND ELUTE CHROMATOGRAM OF A mAb PURIFICATION ON SkillPak TOYOPEARL NH_2-750F 5 mL

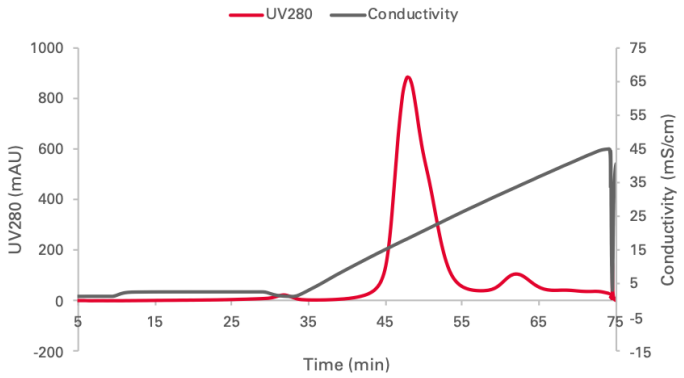

Figure 1

AGGREGATE CONTENT AND YIELD OF THE FLOW-THROUGH PROCESS ON TOYOPEARL NH_2-750F AT DIFFERENT CONDUCTIVITIES

Conductivity	Aggregate	Yield
20 mS/cm	0 %	91.3 %
22.5 mS/cm	0.09 %	93.3 %
25 mS/cm	0.27 %	94.6 %

Table 1

To achieve the desired purity without a subsequent chromatography step, we chose the conductivity of 20 mS/cm for the platform design. The corresponding chromatogram is shown in Figure 2.

Purification platform

Both chromatography steps were now combined in one integrated process, including the intermediate low pH hold. Protein A has a recovery of 98.8 %, while AEX has a recovery of 91.3 % (20 mS/cm), which results in a total recovery of 90.2 %. DNA, HCP, and leached Protein A were removed to the Limit of Detection of the used assays (see Table 2).

FLOW-THROUGH CHROMATOGRAM OF A mAb PURIFICATION ON SkillPak TOYOPEARL NH_2-750F 5 mL

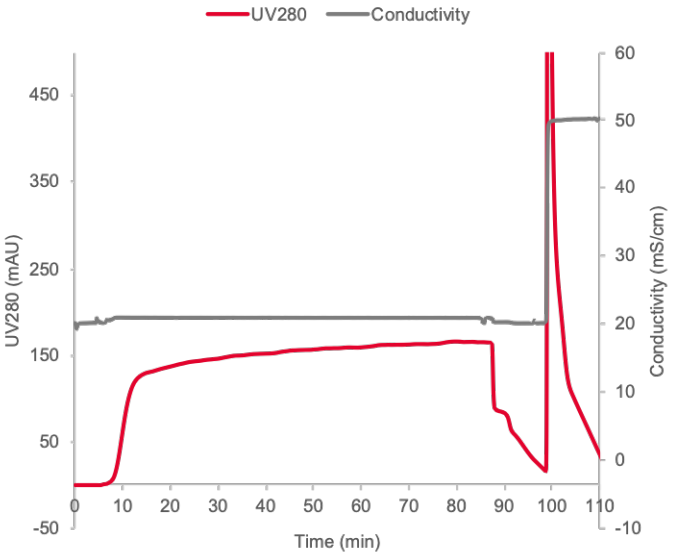

Figure 2

CRITICAL QUALITY ATTRIBUTES OF TOSOH'S 2-STEP PURIFICATION PLATFORM

Critical parameter	Aggregate (%)	Yield (%)	DNA (ppm)	Host Cell Proteins (ppm)	Leached Protein A (ppm)
Feed	0.56		5,500	550,000	< 0.5
After Protein A	1.05	98.8	60	19,000	5.0
After AEX (20 mS/cm)	0.00	90.2	<0.2	< 30	< 0.5

ppm: parts of contaminant per one million parts of product

Table 2

COST ANALYSIS

We used the BioSolve (Biopharm Service Ltd., UK) bioprocess analysis software to compare the downstream costs of the optimized two-step process with the costs of an industry standard process published by BioPhorum Operations Group. As shown in Table 3, the optimized Tosoh process offers 45 % lower costs per gram than the standard industrial process. Besides, process times have been reduced by 58% due to the elimination of one chromatography step and higher flow rates on the Tosoh resins.

CONCLUSION

TOYOPEARL NH_2-750F is an effective anion exchange resin for the removal of dimer and higher-order aggregates from mAb monomer. This is the ideal resin for streamlining purification processes of monoclonal antibodies in combination with high-performance Protein A resins.

Tosoh Bioscience's 2-step antibody purification process presented here using SkillPak pre-packed columns combines high recovery with low process costs and processing time. This platform offers a superior alternative to the industry standard 3-step process. It can easily be scaled up to a pilot plant and eventually to a manufacturing scale for increased productivity and profitability.

COMPARISON OF THE PURIFICATION PROCESSES USING BIOSOLVE SOFTWARE (BIOPHARM SERVICES LTD., UK)

	BioPhorum Process (3 steps)	Tosoh (2 steps)
Batches per year	213	510
Throughput (doses or kg per yr)	717	1,793
Total capital (USD M)	35.8	36.8
Calculated Capacity Utilisation	80%	80%
Cost per gram (USD)	44.49	24.45
Capital Charge	10.38	4.13
Materials	0.91	0.37
Consumables	8.91	4.45
Labour	21.20	14.20
Others	3.09	1.29
Cost Breakdown (%)	100%	100%
Capital Charge	23%	17%
Materials	2%	2%
Consumables	20%	18%
Labour	48%	58%
Others	7%	5%

Table 3