TOYOPEARL® AF-rProtein A HC-650F Host Cell Protein Removal

TOYOPEARL® AF-rProtein A HC-650F, produced by Tosoh Corporation, is a high capacity Protein A resin for the purification of monoclonal antibodies (mAbs). This resin, with dynamic binding capacities (DBC) of 70 g/L at 5 minutes residence time, represents the latest in high capacity affinity resin technology being used in the biopharmaceutical industry.

Introduction

Protein A chromatography is a critical step in the purification of monoclonal antibody products. It is a very robust purification procedure and is used as a capture step to remove impurities, such as host cell proteins, due to its specificity. Choosing a protein A resin that is capable of adequately removing such process impurities across a wide range of operating parameters is essential in developing a robust manufacturing process.

In protein A chromatography, crude feedstock is passed through a column under conditions that promote binding. After loading is complete, the column is washed under conditions that do not interrupt the specific interaction between the target and ligand, but that will disrupt any nonspecific interactions between process impurities (host cell proteins, etc.) and the stationary phase.

The bound protein is then eluted with mobile phase conditions that disrupt the target/ligand interactions. Elution of the target molecule from protein A resin is most commonly accomplished by lowering the pH of the mobile phase, creating an environment whereby the structure of the target molecule is altered in such a way as to inhibit binding.

The following experiment compares the host cell protein (HCP) removal capabilities of TOYOPEARL AF-rProtein A HC-650F, TOYOPEARL AF-rProtein A-650F, and another commercially available high capacity protein A resin. *Table 1* lists the properties and dynamic binding capacities of the resins used in this experiment.

Table 1. Properties of protein A resins

			Binding capacity (g/L)	
Product name	**Supplier**	**Bead diameter**	**DBC (2 min)**	**DBC (5 min)**
TOYOPEARL AF-rProtein A HC-650F	Tosoh Bioscience	45 µm	50	70
TOYOPEARL AF-rProtein A-650F	Tosoh Bioscience	45 µm	30	40
MabSelect SuRe™ LX	GE Healthcare	85 µm	30	58

Experimental Conditions/Results

TOYOPEARL AF-rProtein A-650F, TOYOPEARL AF-rProtein A HC-650F and MabSelect SuRe LX resins were packed into multiple MediaScout® RoboColumn® 10.0 mm ID × 5 mm columns for a packed bed volume of 200 µL per column.

A four factor, central composite, experimental design was developed to compare the performance of these resins in terms of host cell protein removal. Factors included in the experimental design are elution pH, resin load, feedstock titer, and initial HCP concentration. *Figure 1* shows the design space parameters for the experiments carried out with the protein A resins.

Figure 1. Design space parameters

Variables: feedstock titer
resin load
HCP spiking
pH

Center point values

Four factor, central composite experimental design						
Factor	**Variable**	**Min. value**	**Max. value**	**-1 Actual**	**+1 Actual**	**Mean value**
A	Elution pH	2.25	4.25	2.75	3.75	3.25
B	Resin load (g/L)	10.0	50.0	20.0	40.0	30.0
C	Feedstock titer (g/L)	0.25	9.25	2.5	7.00	4.75
D	HCP Spike %	5.0	25.0	10.0	20.0	15.0
	HCP concentration (µg/mL)	100	500	200	400	300

Variable: elution buffer (citrate or acetate)

Purifications were carried out using the Tecan Freedom EVO® robotic liquid handling instrument according to the experimental design protocol generated by the Design-Expert® DOE software. Experiments were carried out with both citrate and acetate as the elution buffer for a total of 60 experiments performed per resin.

The feedstock material and eluted mAb was analyzed for host cell protein content using a Cygnus Technologies third generation CHO HCP ELISA kit. *Figure 2* shows the host cell protein removal for each experiment conveyed in terms of log reduction of HCP from the feedstock material while *Figure 3* shows the effects of feedstock titer on the amount of HCP eluted from the TOYOPEARL AF-rProtein A HC-650F resin.

Figure 2 shows the log reduction of HCP for all resins evaluated in these experiments. Acceptable log reduction values are typically greater than 2.5 for protein A purification. All of the resins evaluated show HCP reduction values between 2.4 and 3.6 log for both citrate and acetate elution buffers.

Figure 2. HCP removal for all resins evaluated

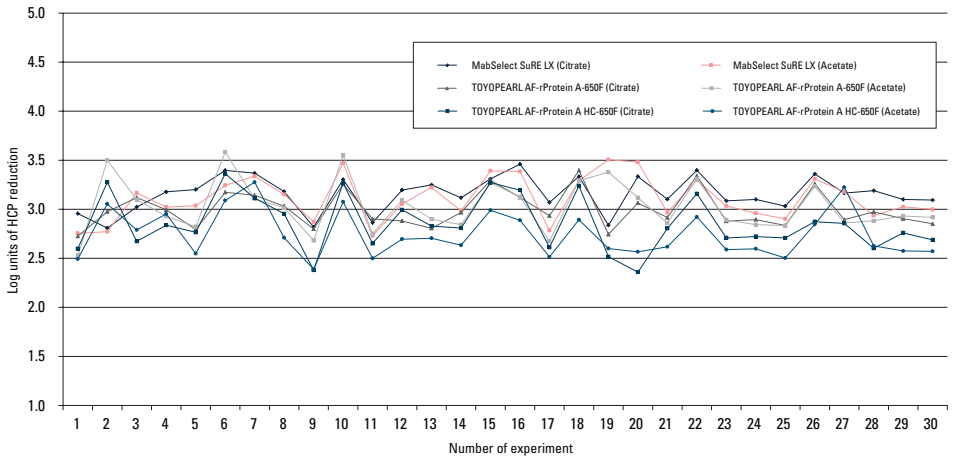
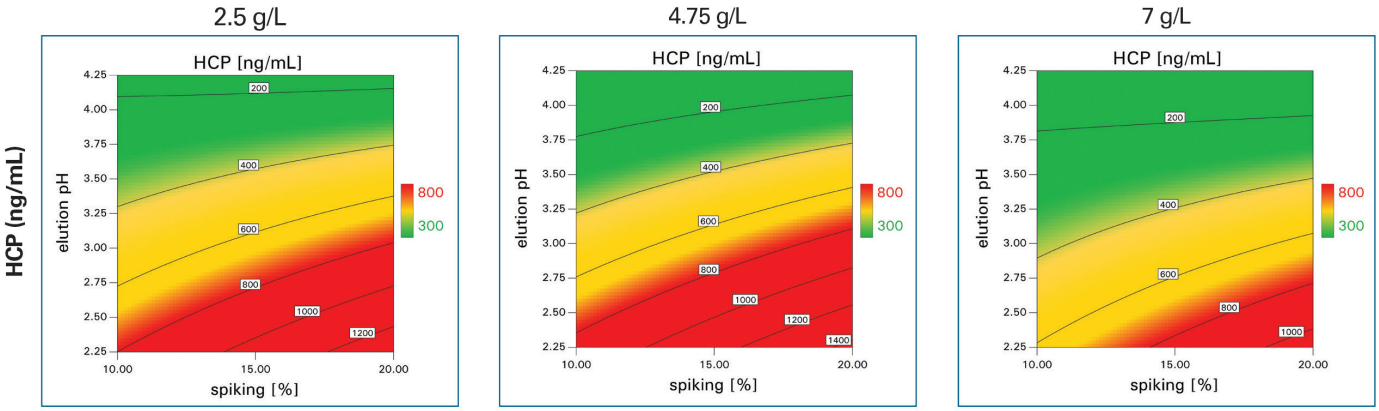


Figure 3. Effect of feedstock titer on HCP concentration in column elution



The effects of mAb titer in the crude feedstock on the concentration of HCP present in the eluted mAb product for TOYOPEARL AF-rProtein A HC-650F is shown in *Figure 3*. As the mAb titer in the crude feedstock increased, the concentration of HCP present in the eluted product decreased. This indicates that for even high feedstock titers, the TOYOPEARL AF-rProtein A HC-650F resin meets the performance expectations required in the biopharmaceutical industry for the removal of host cell proteins.

Conclusions

Protein A chromatography is a critical step in the purification of monoclonal antibody products. Given its ability to remove upwards of 95% of cell culture related impurities in a single step, a chromatographer's choice of protein A resin can ease the burden required of subsequent downstream steps to remove such process impurities as host cell proteins. A protein A resin that is capable of adequately removing such process impurities across a wide range of operating parameters is essential in developing a robust manufacturing process.

TOYOPEARL AF-rProtein A-650F, TOYOPEARL AF-rProtein A HC-650F and MabSelect SuRe LX all show very acceptable log reduction values for HCP removal. For the TOYOPEARL AF-rProtein A HC-650F, as the mAb titer in the crude feedstock increased, the concentration of HCP present in the eluted product decreased. This demonstrates the successful use of TOYOPEARL AF-rProtein A HC-650F for host cell protein removal.

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