TOYOPEARL® AF-rProtein A HC-650F rProtein A Ligand Leakage

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 *(see AN61)*, 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 amount of leached protein A ligand that is present in the purified mAb post-capture when using 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 10.0 mm ID × 5 mm MediaScout® RoboColumns® 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 product recovery, aggregates, leached protein A ligand, and 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 eluted mAb was analyzed for leached protein A ligand using ELISA kits specific to each protein A resin. TOYOPEARL AF-rProtein A-650F ELISA (item 22815) and TOYOPEARL AF-rProtein A HC-650F ELISA (item 23433) were used for the Tosoh resins. A MabSelect SuRe ELISA (Repligen item 9333-1) was used for the GE Healthcare resin. *Figure 2* shows the results of the DOE experiments for ligand leakage (ng/mL) for all three resins using both citrate and acetate as an elution buffer. Acceptable levels of ligand leakage were seen for all resins tested; however, the TOYOPEARL AF-rProtein A HC-650F showed levels of leakage an order of magnitude lower than that seen with the MabSelect SuRe LX.

Figure 2. Results of DOE ligand leaching tests

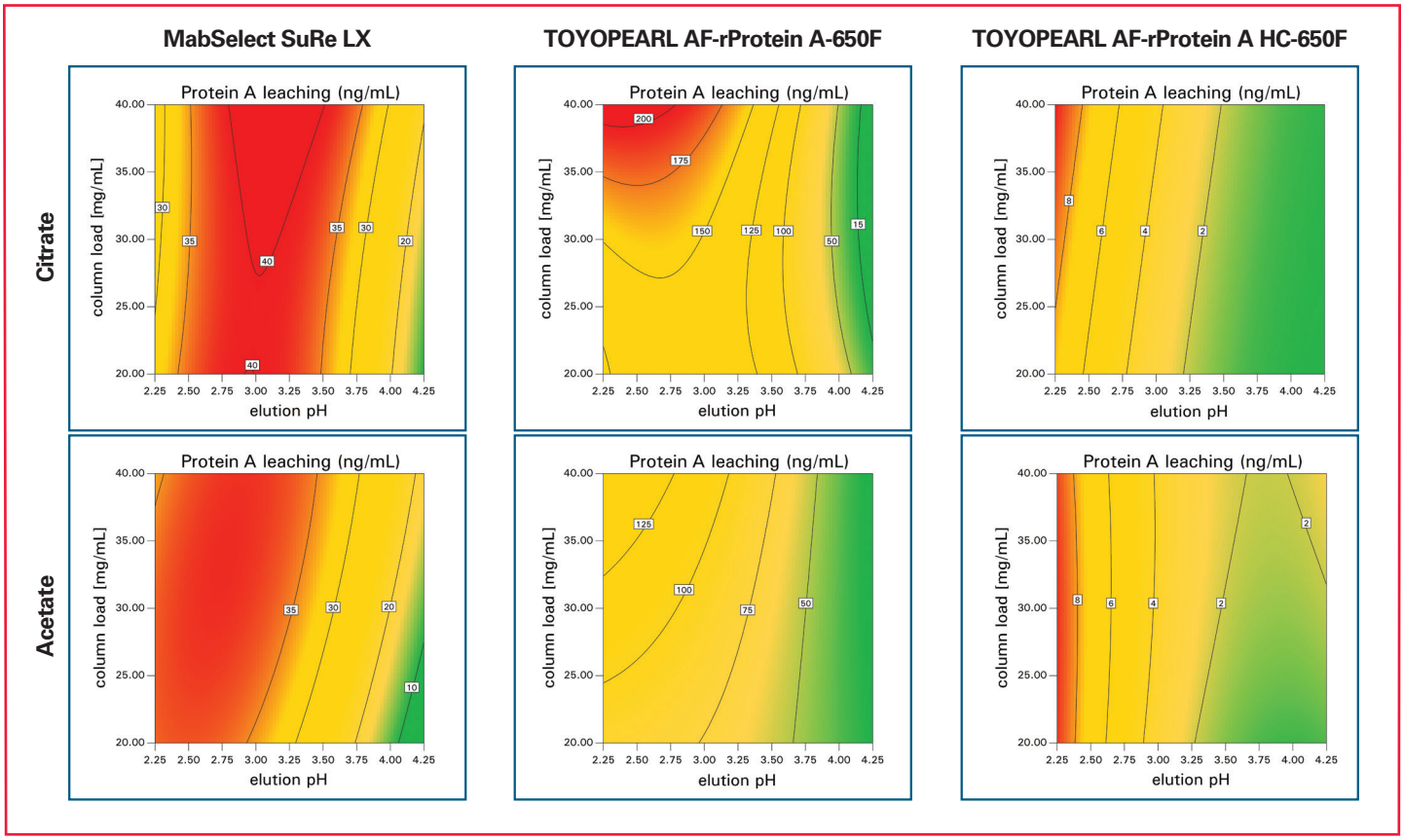


Figure 3. TOYOPEARL AF-rProtein A HC-650F ligand stability, 0.2 mol/L NaOH CIP

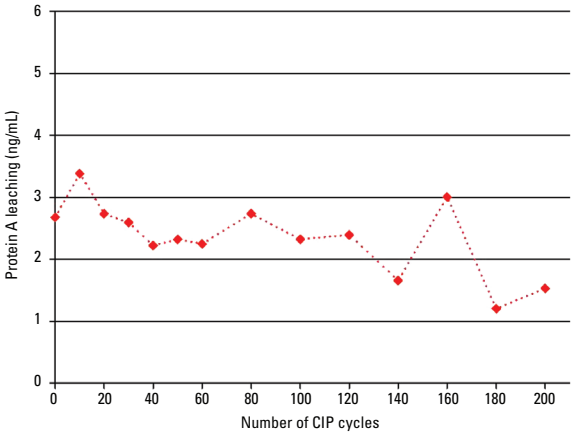
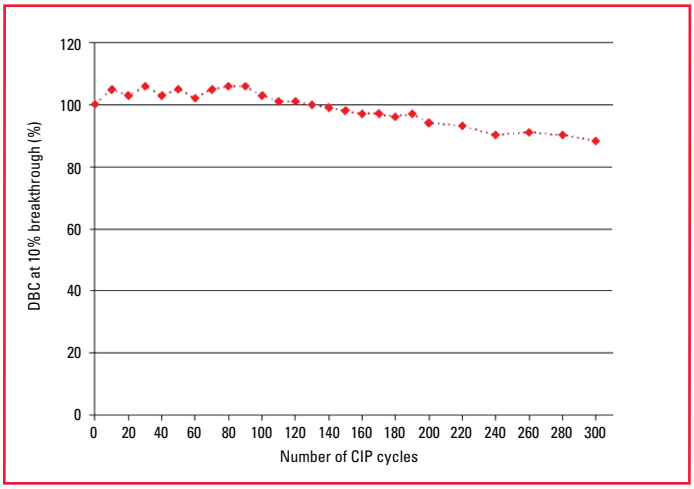


Figure 3 shows the amount of ligand eluted from the TOYOPEARL AF-rProtein A HC-650F resin over 200 cycles using 0.2 mol/L NaOH to clean in place (CIP) between each cycle. As the number of CIP cycles increased, the amount of ligand present in the eluted product decreased. This indicates that the TOYOPEARL AF-rProtein A HC-650F resin has a very stable ligand attachment and meets the performance expectations required in the biopharmaceutical industry for ligand leaching.

Figure 4 shows the extended stability of the TOYOPEARL AF-rProtein A HC-650F resin when subjected to 300 purification cycles. CIP was done with 0.2 mol/L NaOH following each cycle, with a contact time of 15 minutes. As can be seen from the figure, the capacity, normalized to 100% prior to the first CIP cycle, remains virtually unchanged for approximately 200 cycles. This data, in conjunction with the information presented in figure 3, indicates that the TOYOPEARL AF-rProtein A HC-650F resin is exceptionally stable when cleaned with NaOH. This stability helps to ensure a very long operational lifetime for this resin.

Figure 4. TOYOPEARL AF-rProtein A HC-650F long term stability with 0.2 mol/L NaOH CIP



Conclusions

Protein A chromatography is a critical step in the purification of monoclonal antibody (mAb) products. Given its superior capacity and extremely stable ligand attachment, the choice of TOYOPEARL AF-rProtein A HC-650F as a capture resin can ease the burden required of subsequent downstream process steps to remove protein A ligand. In addition, a more stable ligand attachment and very low levels of ligand leaching allow a greatly expanded operational lifetime and reduced resin costs for mAb capture steps in downstream processing.

TOYOPEARL AF-rProtein A-650F, TOYOPEARL AF-rProtein A HC-650F and MabSelect SuRe LX all show very acceptable levels of ligand leaching. For the TOYOPEARL AF-rProtein A HC-650F, the level of ligand leaching was at least an order of magnitude lower than the other resins tested. This demonstrates the potential for long term use of TOYOPEARL AF-rProtein A HC-650F resin before its effective operational life is complete.

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