



# Characterization of a Newly Developed High Capacity, Alkaline Resistant, Recombinant Protein A Resin

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# Introduction

- TOYOPEARL® AF-rProtein A HC-650F media is a *high capacity* affinity resin optimized for the purification of monoclonal antibodies (mAbs).
- TOYOPEARL AF-rProtein A-650F media is an affinity resin optimized for the purification of monoclonal antibodies.
- TOYOPEARL HW-65 resin, the polymeric base bead for this resin, is chemically modified to provide higher capacity. This resin has a pressure rating of 0.3 MPa, and is stable in the pH range 3-13.
- Comparisons were made between the TOYOPEARL AF-rProtein A-650F, TOYOPEARL AF-rProtein A HC-650F, and another commercially available protein A resin for capacity, purity, recovery, and host cell protein removal.



# Table 1: Comparison of Protein A Resins: Manufacturer/Capacity/Residence Time/Stability

Product name	Supplier	Bead diameter	Binding capacity (g/L)		pH stability
			DBC (2 min)	DBC (5 min)	
TOYOPEARL AF-rProtein A HC-650F	Tosoh Bioscience	45 $\mu$ m	50	70	Caustic stable
TOYOPEARL AF-rProtein A-650F	Tosoh Bioscience	45 $\mu$ m	30	40	Caustic stable
MabSelect SuRe™ LX	GE Healthcare	85 $\mu$ m	30	58	Caustic stable

Capacity and stability values for the resins tested in this study were obtained from literature available for each product.



## Table 2: Design Space Parameters

Variables: feedstock titer  
resin load  
HCP spiking



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)



# Experimental Design

- A four factor, central composite, experimental design was executed.
- Experiments were carried out with both citrate and acetate as the elution buffer for a total of 60 experiments per resin.



# Experimental Materials and Methods

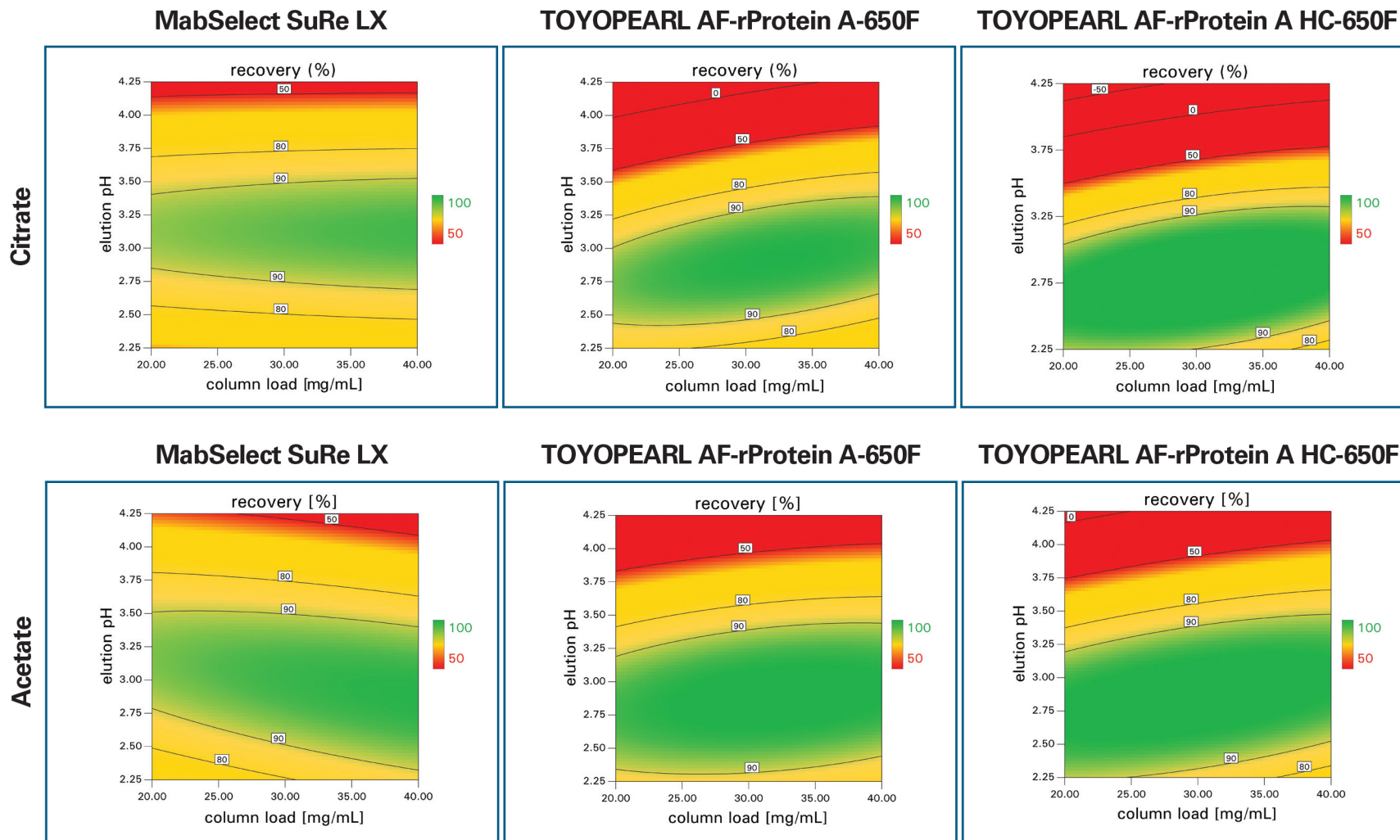
<b>Resins:</b>	TOYOPEARL AF-rProtein A-650F TOYOPEARL AF-rProtein A HC-650F MabSelect SuRe LX
<b>Instrument:</b>	Freedom EVO®
<b>Column Size:</b>	MediaScout® RoboColumn®, 10 mm ID × 5 mm
<b>Mobile Phase:</b>	equilibration/wash: 100 mmol/L phosphate, pH 6.5 elution: 100 mmol/L citrate or acetate, pH as indicated
<b>Gradient:</b>	isocratic
<b>Flow Rates:</b>	equilibration/wash: 150 cm/hr (0.5 mL/min) load: 30 cm/hr (0.1 mL/min) elution: 60 cm/hr (0.2 mL/min)
<b>Temperature:</b>	ambient
<b>Injection vol.:</b>	as indicated
<b>Sample:</b>	monoclonal antibody harvest
<b>Sample Load:</b>	as indicated

The purifications were carried out by the Tecan Freedom EVO robotic liquid handling instrument according to the experimental design protocol generated by the Design-Expert® DOE software.

Results were compiled and analyzed for recovery, aggregate content, HCP removal, effects of feedstock titer, and ligand leaching.



# Figure 1: Recovery of mAb



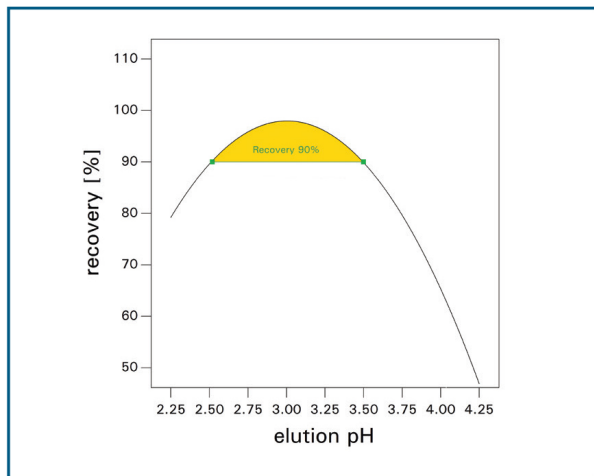


# Figure 2: Recovery of mAb Eluted in Acetate

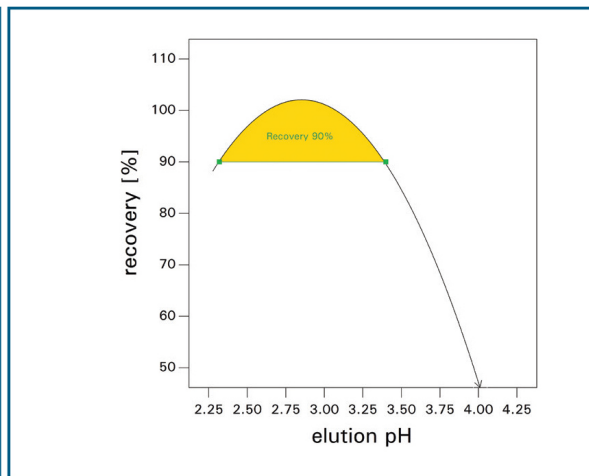
MabSelect SuRe LX

TOYOPEARL AF-rProtein A-650F

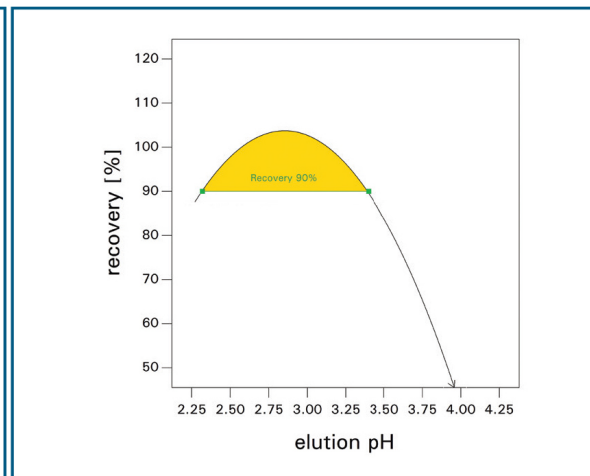
TOYOPEARL AF-rProtein A HC-650F



pH 2.5 – 3.5



pH 2.3 – 3.4



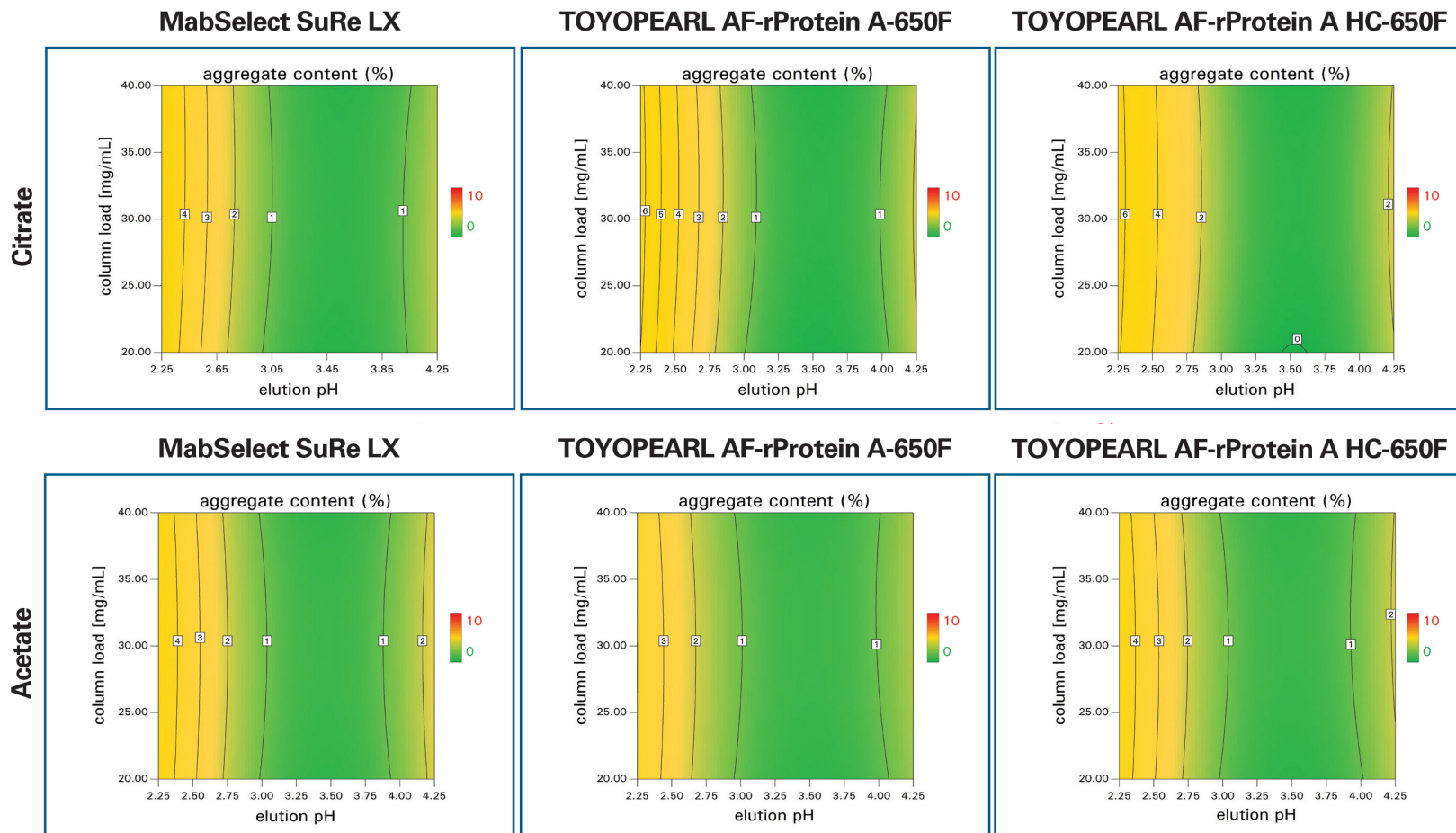
pH 2.3 – 3.4

- Highlighted areas under the curve (bounded by the indicated pH values) represent recoveries  $\geq 90\%$  for each resin with acetate as the elution buffer.
- The best recovery for all resins tested is in the 2.5 - 3.4 pH range.
- MabSelect SuRe LX recovery range is slightly shifted ( $\Delta$  pH 0.1) to the higher end of the pH spectrum, but the recovery window is smaller.





# Figure 3: Aggregate Content

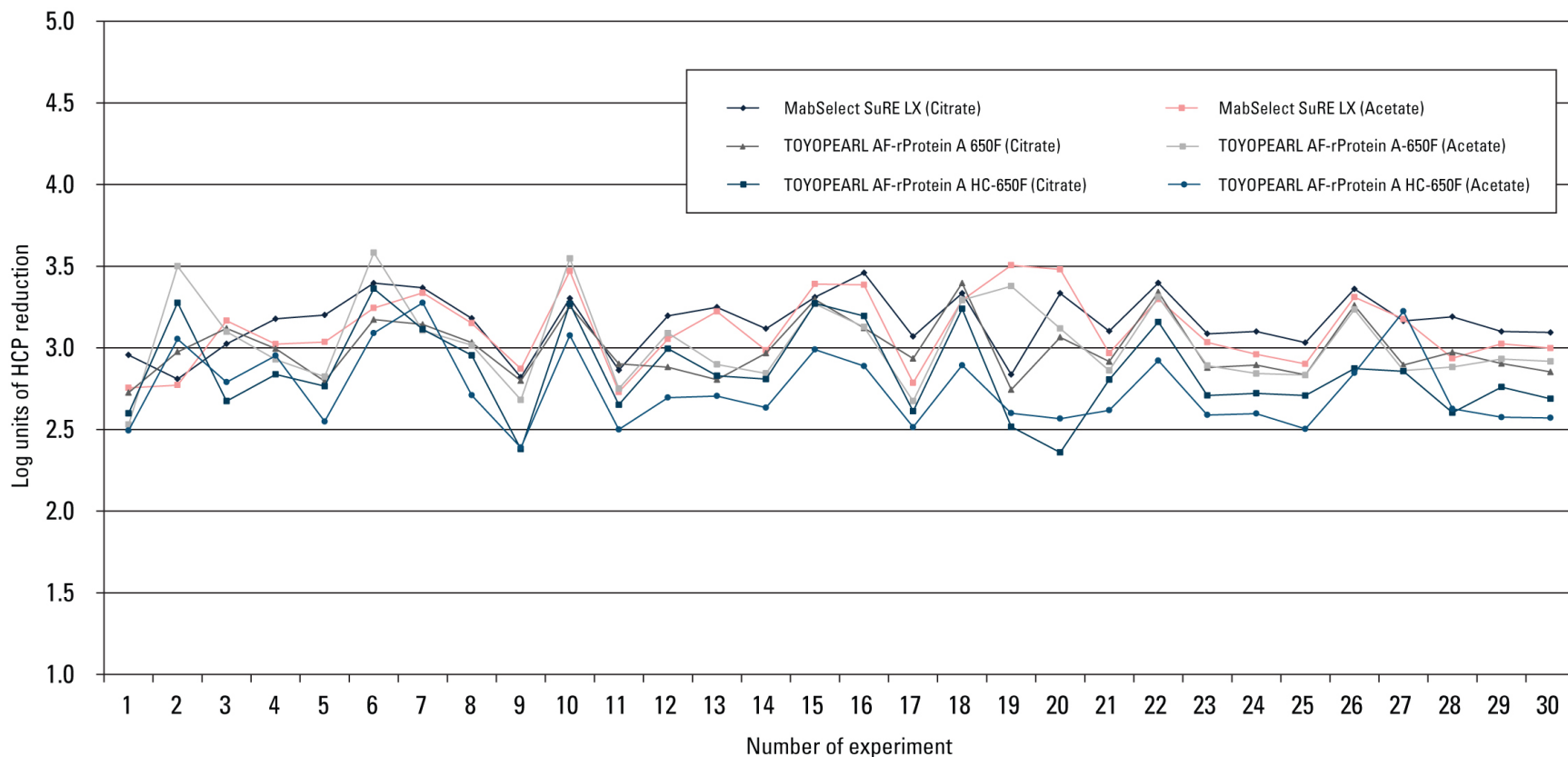


All resins show acceptable aggregate content levels of <4%

Aggregate levels increased with decreasing elution pH. Acetate elution buffer mitigates this effect slightly.



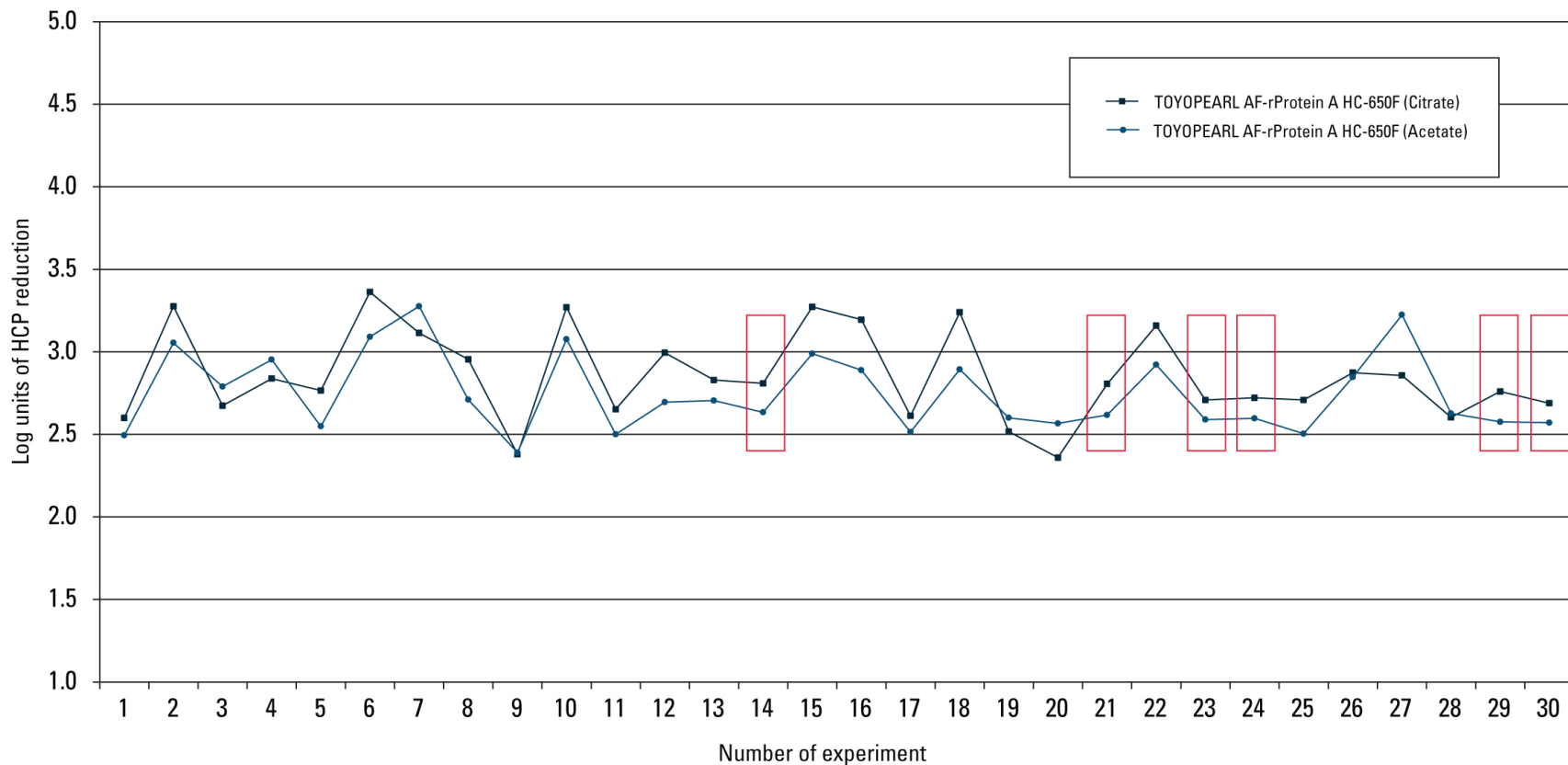
# Figure 4: Host Cell Protein (HCP) Removal for all Resins Tested



All resins show HCP removal values between 2.4 log and 3.6 log for citrate and acetate elution buffers.

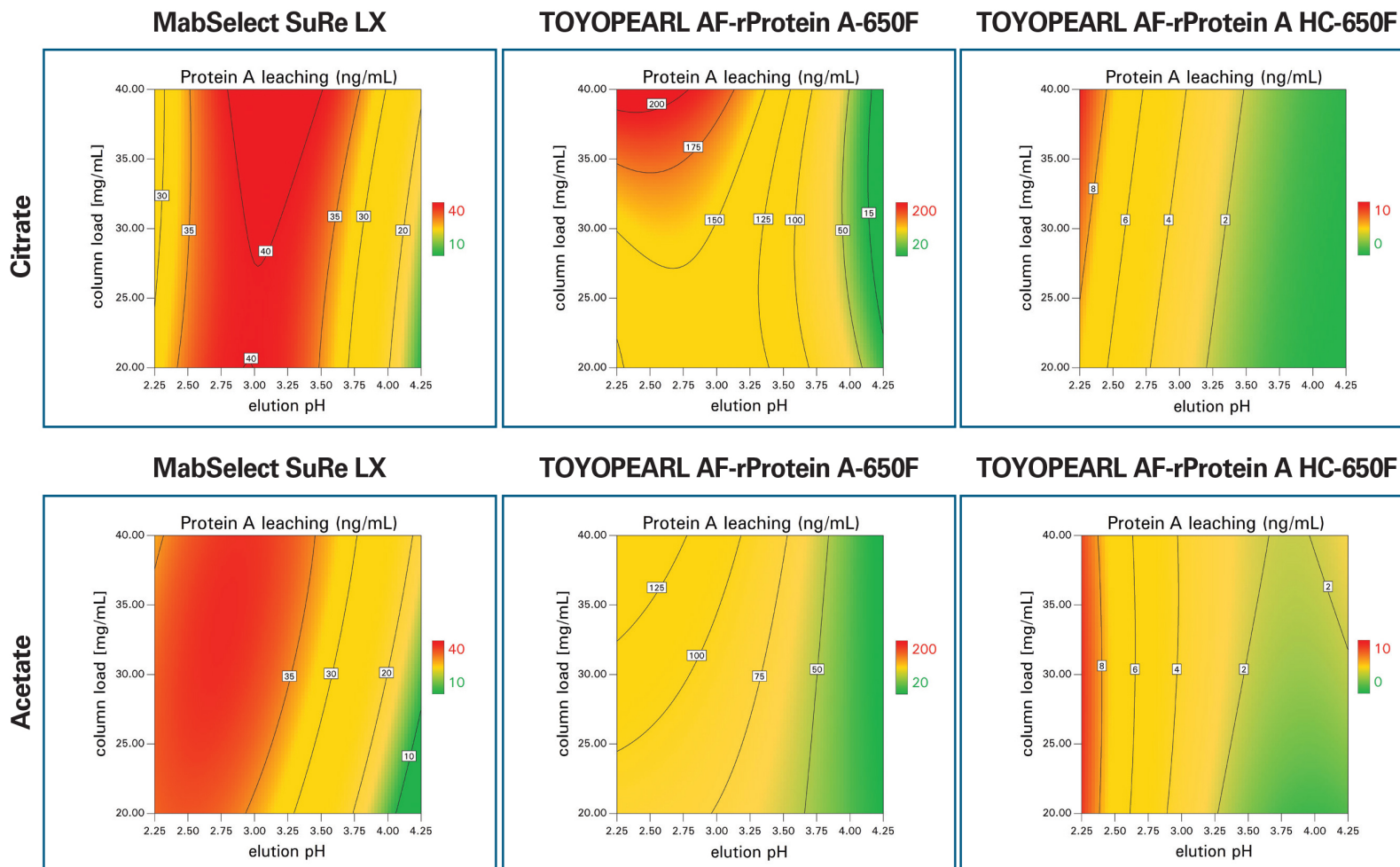


# Figure 5: Host Cell Protein (HCP) Removal for TOYOPEARL AF-rProtein A HC-650F



TOYOPEARL AF-rProtein A HC-650F shows HCP removal values of 2.7 log and 2.6 log for citrate and acetate elution buffers, respectively, for the experiments carried out at the center point values.

# Figure 6: Protein A Ligand Leakage



TOYOPEARL AF-rProtein A HC-650F shows superior levels of ligand leakage (<15 ng/mL) in process relevant pH ranges for both citrate and acetate elution buffers.



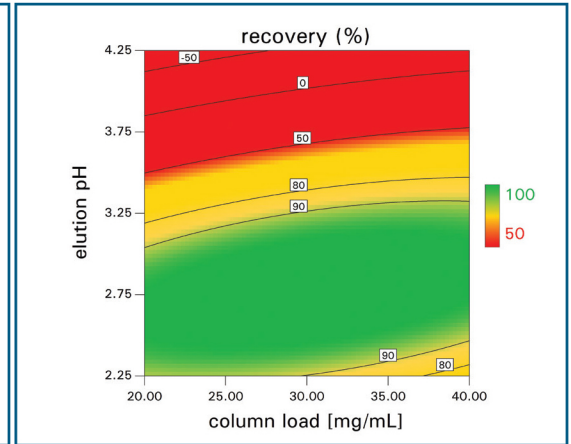
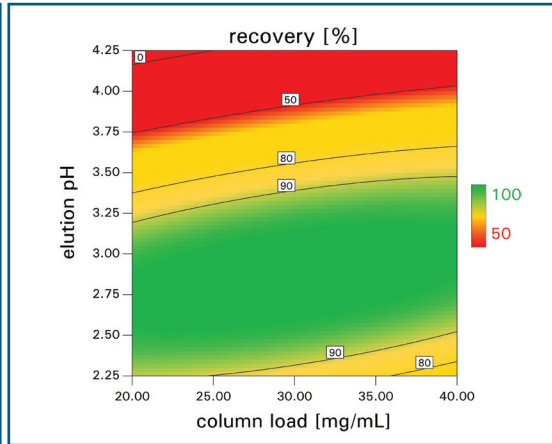
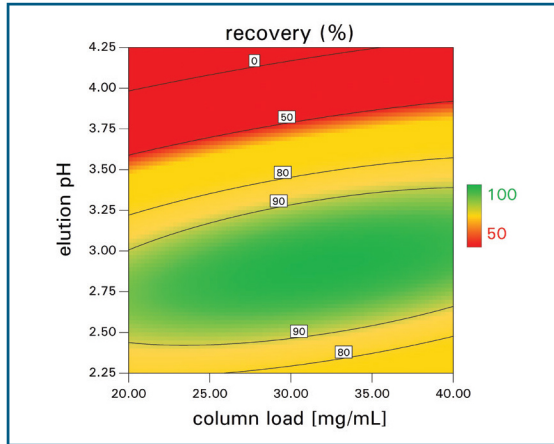
# Figure 7: Effects of Feedstock Titer on Process Critical Values for TOYOPEARL AF-rProtein A HC-650F

Antibody titer 2.5 g/L

4.75 g/L

7 g/L

Recovery (%) Little effect

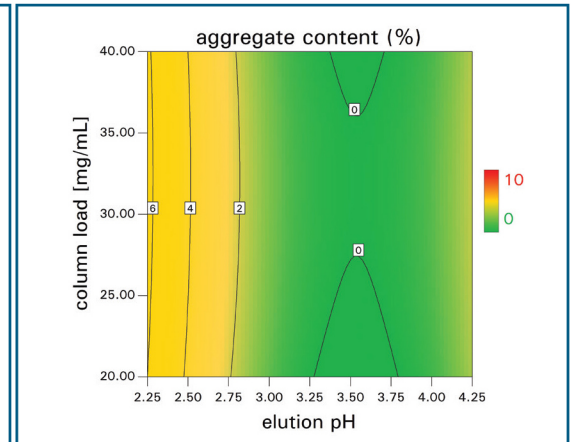
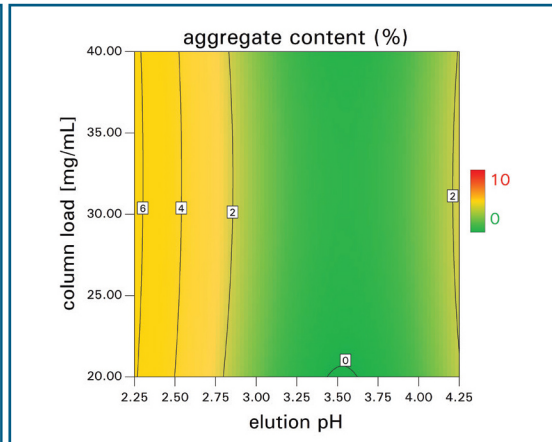
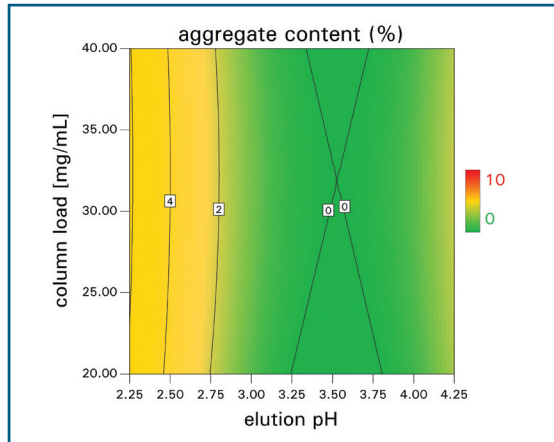


Antibody titer 2.5 g/L

4.75 g/L

7 g/L

Aggregate content (%) No effect

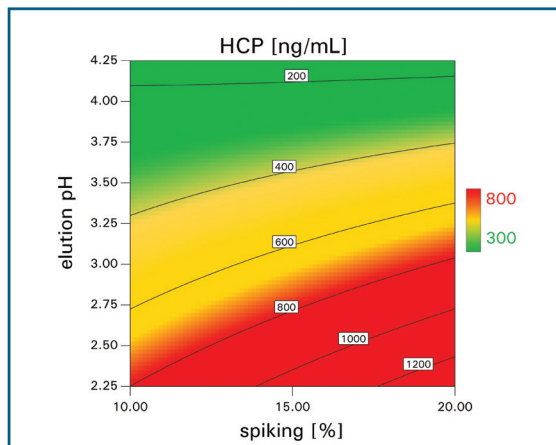




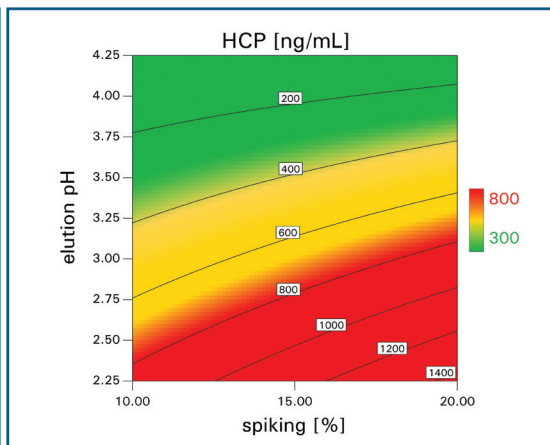
# Figure 7: Effects of Feedstock Titer on Process Critical Values for TOYOPEARL AF-rProtein A HC-650F, Continued

Antibody titer 2.5 g/L

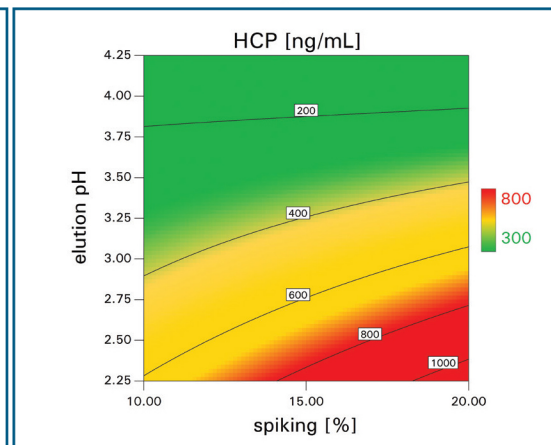
HCP (ng/mL) Moderate effect



4.75 g/L

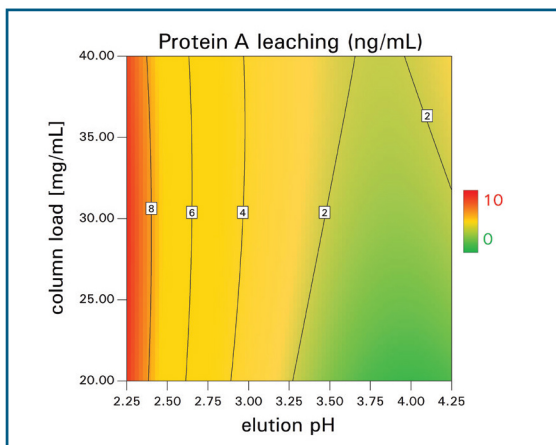


7 g/L

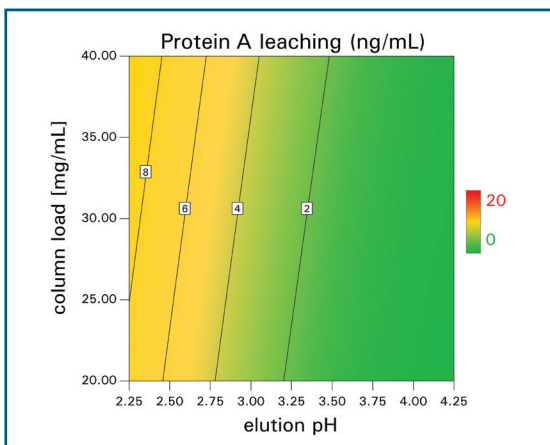


Antibody titer 2.5 g/L

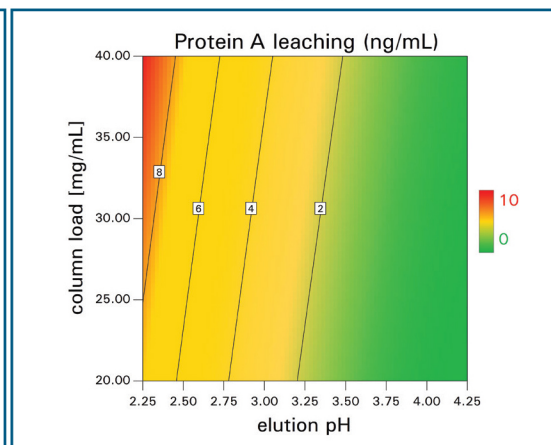
Protein A (ng/mL) No effect



4.75 g/L

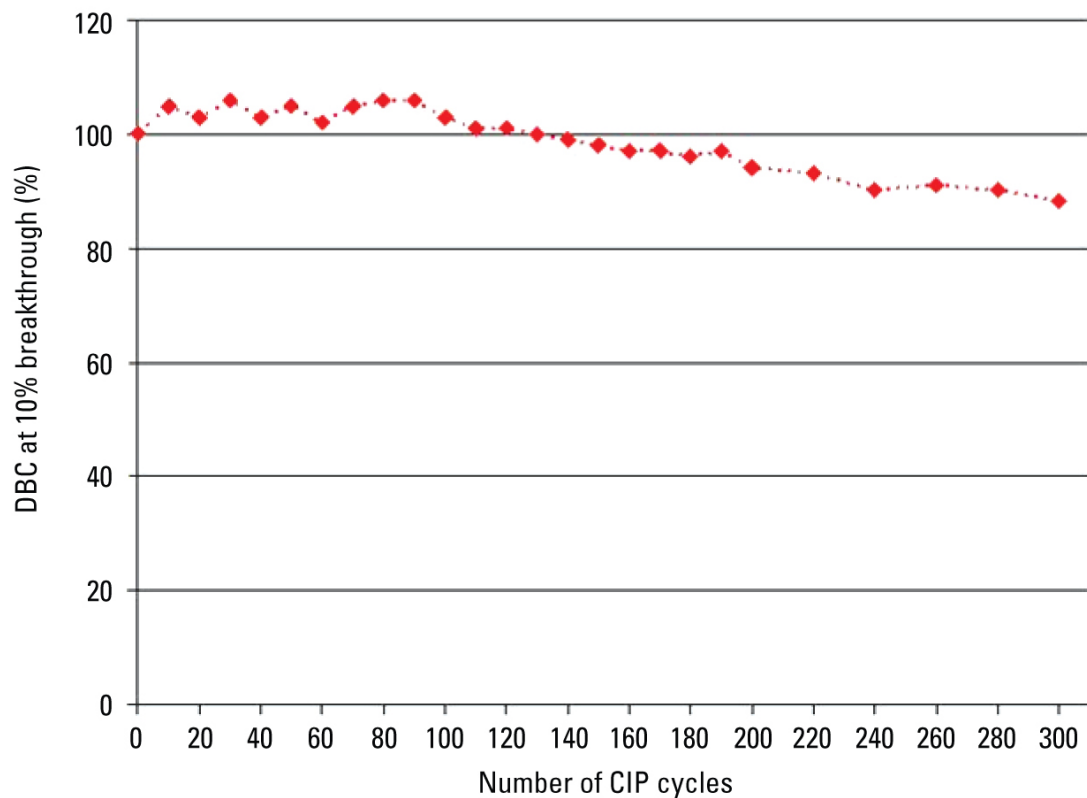


7 g/L





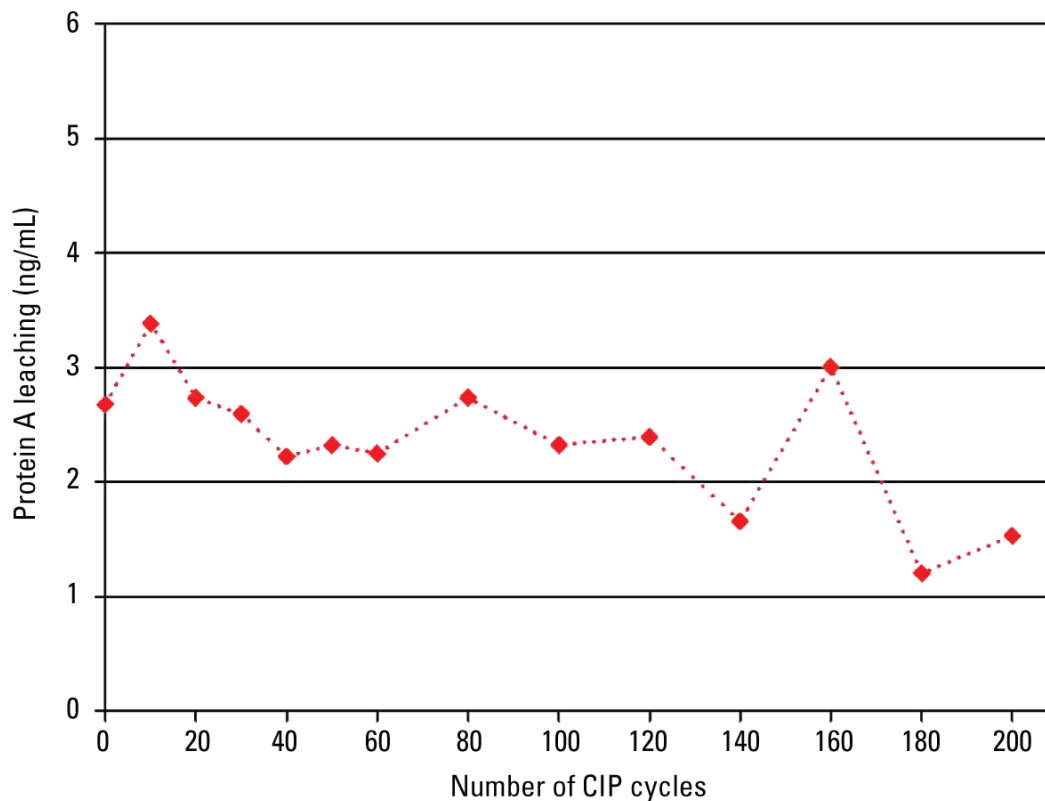
## Figure 8: Alkaline Stability over TOYOPEARL AF-rProtein A HC-650F Resin Lifetime



- DBC at 10% breakthrough is stable for over 300 CIP cycles using 0.2 mol/L NaOH with 15 minutes contact time per cycle.
- The binding capacity begins to decline slightly after 200 cycles.



## Figure 9: Ligand Leakage over TOYOPEARL AF-rProtein A HC-650F Resin Lifetime

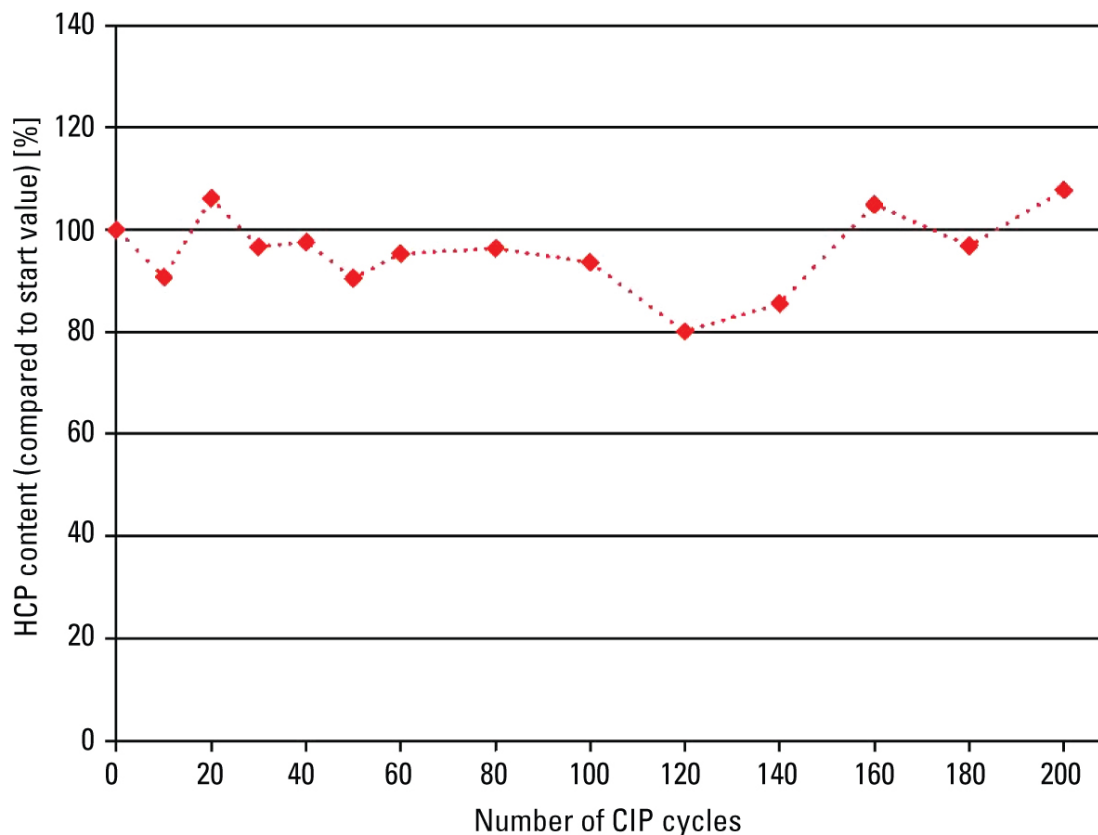


Ligand leakage is stable for over 200 CIP cycles using 0.2 mol/L NaOH with 15 minutes contact time per cycle.





## Figure 10: mAb Purity over TOYOPEARL AF-rProtein A HC-650F Resin Lifetime



Product purity is stable for over 200 CIP cycles using 0.2 mol/L NaOH with 15 minutes contact time per cycle.



# Conclusions

- Product recovery is greater and the recovery window is larger for both TOYOPEARL protein A resins compared to MabSelect SuRe LX.
- Optimal elution pH is slightly higher for MabSelect SuRe LX.
- Aggregate content is less than 4% for all resins tested and aggregate levels increase with decreasing pH, though the use of acetate instead of citrate mitigates this effect slightly.
- HCP log reduction for all resins is very acceptable with the absolute values for TOYOPEARL AF-rProtein A HC-650F being approximately 500 - 600 ng HCP per mL.
- Protein purity increased for all three resins with increasing mAb titer in the feedstock.
- Ligand leakage for the TOYOPEARL AF-rProtein A HC-650F was far superior to the other resins tested.
- Product purity and ligand leakage remains stable over the operating lifetime of the TOYOPEARL AF-rProtein A HC-650F resin.