Monoclonal Antibody Purification with a High Capacity Protein A Resin

TOYOPEARL
APPLICATION NOTE

TOYOPEARL® AF-rProtein A HC-650F, a high capacity protein A resin for the purification of monoclonal antibodies (mAbs), was recently introduced by Tosoh Corporation. This resin, with dynamic binding capacities (DBC) of 70 g/L at 5 minutes residence time, is the newest member of the TOYOPEARL product line.

TOYOPEARL AF-rProtein A HC-650F resin remains dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL particles do not distort under flow rates that generate up to 0.3 MPa pressure. These resin properties, combined with a narrow particle size distribution, result in superior pressure-flow characteristics for the packed TOYOPEARL bed.

TOYOPEARL AF-rProtein A HC-650F is a hydroxylated methacrylic polymer resin. *Table 1* lists the properties and dynamic binding capacities of this resin. The following experiments detail the purification of a monoclonal antibody (human IgG_1) using TOYOPEARL AF-rProtein A HC-650F.

Table 1. Properties of TOYOPEARL AF-rProtein A HC-650F

	TOYOPEARL AF-rProtein A HC-650F
Particle size	45 µm
Pore diameter	100 nm
DBC (5 min)	70 g/L
DBC (2 min)	50 g/L
Caustic stability	> 200 CIP cycles (0.1 mol/L NaOH)
Max. pressure	0.3 MPa

Introduction

Protein A chromatography, the most widely used type of affinity chromatography, relies on the specific and reversible binding of antibodies to an immobilized ligand; in this case protein A. The protein A ligand can either bind directly to the Fc region of an antibody or to an Fc tag that has been fused to the target of interest.

Protein A chromatography is a very robust purification procedure and is used as a capture step due to its specificity. In protein A chromatography, crude feed stock 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. Low pH elution can have a negative effect on protein stability and it is advised that the eluted protein solution be neutralized to minimize aggregation and denaturation.

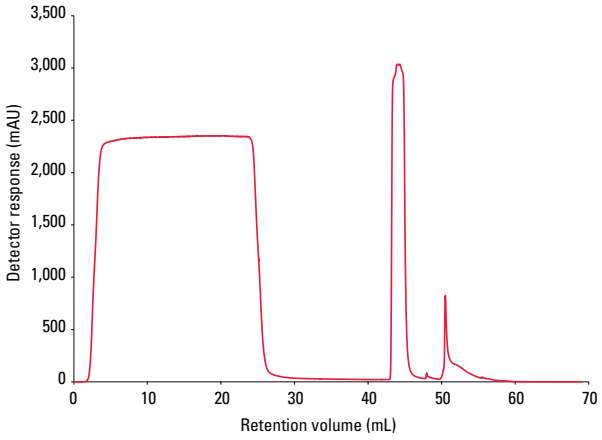
Experimental Conditions/ Results

The data presented here demonstrates the capabilities of TOYOPEARL AF-rProtein A HC-650F to purify a human IgG_1 monoclonal antibody from crude feed stock.

Experiments were carried out on 5.0 mm ID × 9.7 cm column packed with TOYOPEARL AF-rProtein A HC-650F resin. The column was performance tested with a 1% column volume (CV) injection of 3.0 mol/L NaCl with a 350 mmol/L NaCl mobile phase and found to be acceptable for use in these experiments.

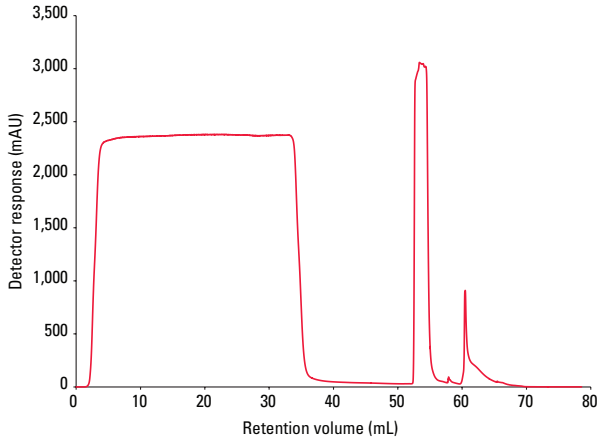
The column was equilibrated with 20 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.39, and loaded with consecutively larger quantities of feedstock so that loads of 35 g/L *(Figure 1)*, 50 g/L *(Figure 2)*, and 65 g/L *(Figure 3)* were achieved. After loading, the column was washed with 5 CV of equilibration buffer to remove any unbound impurities and the column was then eluted with 100 mmol/L Na citrate, pH 3.0.

Figure 1. TOYOPEARL AF-rProtein A HC-650F, 35 g/L



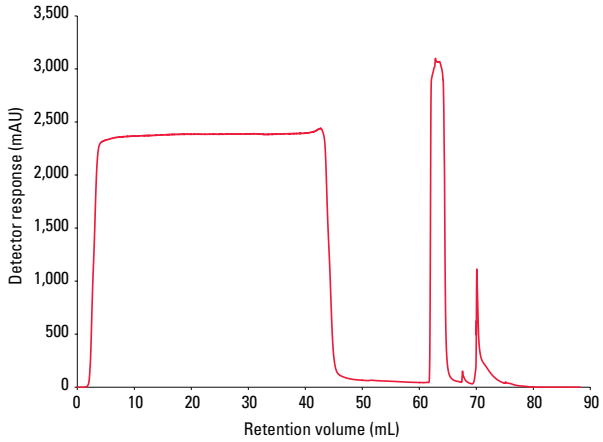
Column size:	5.0 mm ID × 9.7 cm (1.90 mL)
Mobile phase:	A: 20 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.39
	B: 100 mmol/L Na citrate, pH 3.0
	C: 0.1 mol/L NaOH
Flow rate:	116 cm/hr (0.38 mL/min)
Residence time:	5 min
Detection:	UV @ 280 nm
Sample:	crude feedstock (titer = 3.0 g/L)
Sample load:	22.2 mL

Figure 2. TOYOPEARL AF-rProtein A HC-650F, 50 g/L



Column size:	5.0 mm ID × 9.7 cm (1.90 mL)
Mobile phase:	A: 20 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.39
	B: 100 mmol/L Na citrate, pH 3.0
	C: 0.1 mol/L NaOH
Flow rate:	116 cm/hr (0.38 mL/min)
Residence time:	5 min
Detection:	UV @ 280 nm
Sample:	crude feedstock (titer = 3.0 g/L)
Sample load:	31.7 mL

Figure 3. TOYOPEARL AF-rProtein A HC-650F, 65 g/L



Column size:	5.0 mm ID × 9.7 cm (1.90 mL)
Mobile phase:	A: 20 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.39
	B: 100 mmol/L Na citrate, pH 3.0
	C: 0.1 mol/L NaOH
Flow rate:	116 cm/hr (0.38 mL/min)
Residence time:	5 min
Detection:	UV @ 280 nm
Sample:	crude feedstock (titer = 3.0 g/L)
Sample load:	41.2 mL

Post elution, the column was washed with 3 CV of equilibration buffer and sanitized with 3 CV of 0.1 mol/L NaOH (15 minutes contact time).

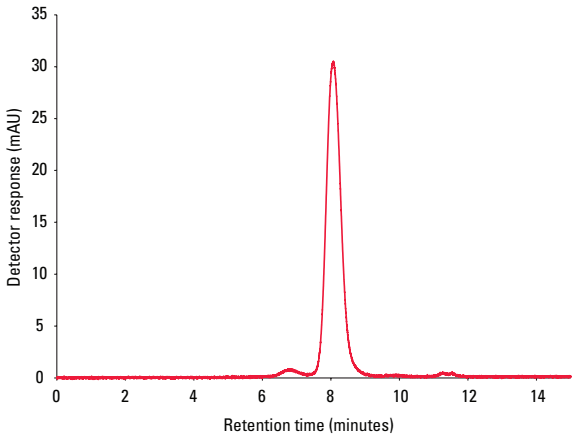
As can be seen from these chromatograms, the elution peaks are sharply defined and exhibit minimal tailing, even at the 65 g/L load. *Table 2* shows the load, yield and purity for each of the purifications performed.

Table 2. mAb purity and yield

Load	% Monomer	% Recovery
35 g/L	96.1	87.2
50 g/L	96.8	86.5
65 g/L	96.1	89.5

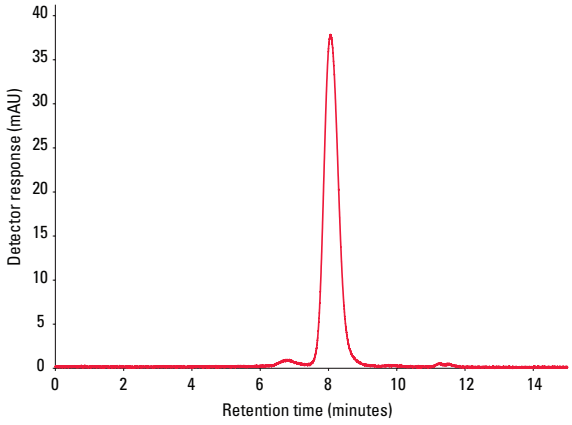
HPLC analysis of the elution pool by SEC *(Figures 4-6)* indicates that increased loading concentrations have no effect on the amount of aggregate present in the purified product.

Figure 4. SEC Analysis of 35 g/L elution



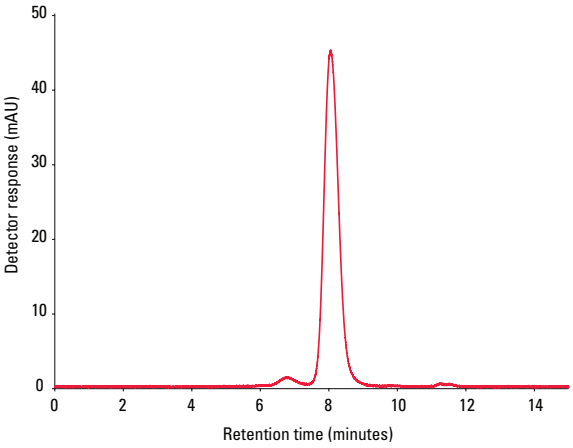
Column:	TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm
Mobile phase:	0.1 mol/L phosphate, 0.1mol/L NaCl,
	0.05% azide, pH 6.7
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Injection vol:	1.0 µL
Sample:	protein A purified mAb

Figure 5. SEC Analysis of 50 g/L elution



Column:	TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm
Mobile phase:	0.1 mol/L phosphate, 0.1mol/L NaCl, 0.05% azide, pH 6.7
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Injection vol:	1.0 µL
Sample:	protein A purified mAb

Figure 6. SEC Analysis of 65 g/L elution



Column:	TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm
Mobile phase:	0.1 mol/L phosphate, 0.1mol/L NaCl, 0.05% azide, pH 6.7
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Injection vol:	1.0 µL
Sample:	protein A purified mAb

Conclusions

TOYOPEARL AF-rProtein A HC-650F is capable of delivering high purity monoclonal antibodies with excellent recovery at loading levels approaching the resin capacity without any increase in aggregate levels. This level of performance will allow chromatographers to fully utilize the increased capacity of this resin without compromising quality while at the same time increasing the productivity of the antibody capture step.

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