Purification of a Monoclonal Antibody with TOYOPEARL® AF-rProtein A HC-650F at Various Bed Heights

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 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. 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.

Introduction

TOYOPEARL AF-rProtein A HC-650F is a high capacity protein A resin for the purification of monoclonal antibodies (mAbs). This resin exhibits dynamic binding capacities (DBC) of 70 g/L at 5 minutes residence time.

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 excellent 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 data presented here demonstrates the capabilities of TOYOPEARL AF-rProtein A HC-650F to purify a human IgG_1 monoclonal antibody from crude feedstock with a fixed column volume at multiple bed heights and constant residence times.

Table 1. Properties of 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

Experimental Conditions/Results

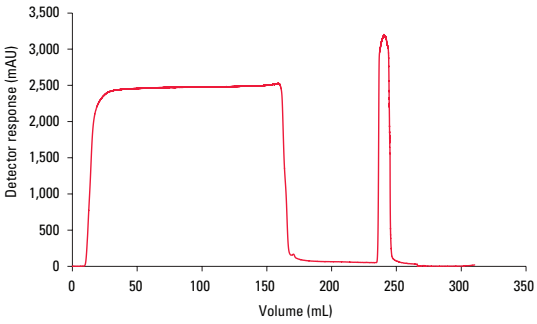
Experiments were carried out on 6.6 mm ID × 21.5 cm, 1.0 cm × 9.3 cm, and 1.5 cm × 4.2 cm columns packed with TOYOPEARL AF-rProtein A HC-650F resin. The columns were performance tested and found to be acceptable for use in these experiments.

The columns were equilibrated with 10 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.40, and loaded with clarified feedstock at approximately 90% of the resin DBC (61 g/L-resin) at 5 minutes residence time. After loading, the column was washed with 5 CV of equilibration buffer to remove any unbound impurities and then eluted with 100 mmol/L Na citrate, pH 3.0.

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).

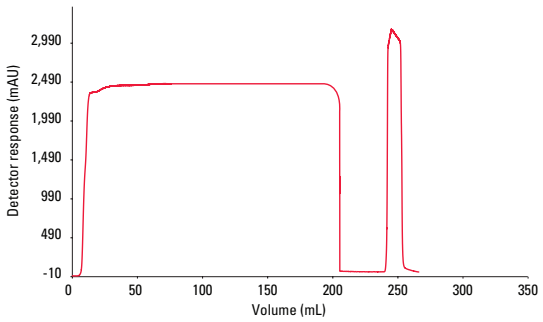
As can be seen in *Figure 1* (6.6 mm ID × 21.5 cm column size), *Figure 2* (1.0 cm × 9.3 cm column size) and *Figure 3* (1.5 cm × 4.2 cm column size), the elution peaks are sharply defined and exhibit minimal tailing. *Table 2* shows the load, yield and purity for each of the purifications performed.

Figure 1. 6.6 mm ID × 21.5 cm column



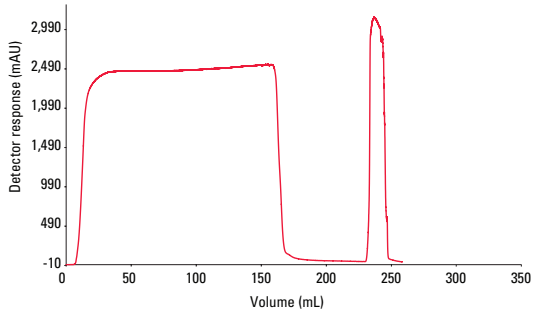
Column size:	6.6 mm ID × 21.5 cm (7.35 mL)
Mobile phase:	A: 10 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.40 B: 100 mmol/L Na citrate, pH 3.0 C: 0.1 mol/L NaOH
Flow rate:	258 cm/hr (1.47 mL/min)
Residence time:	5 min
Detection:	UV @ 280 nm
Sample:	crude feedstock (titer = 3.0 g/L)
Sample load:	150 mL

Figure 2. 1.0 cm ID × 9.3 cm column



Column size:	1.0 cm ID x 9.3 cm (7.30 mL)
Mobile phase:	A: 10 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.40 B: 100 mmol/L Na citrate, pH 3.0 C: 0.1 mol/L NaOH
Flow rate:	112 cm/hr (1.47 mL/min)
Residence time:	5 min
Detection:	UV @ 280 nm
Sample:	crude feedstock (titer = 3.0 g/L)
Sample load:	150 mL

Figure 3. 1.5 cm ID × 4.2 cm column

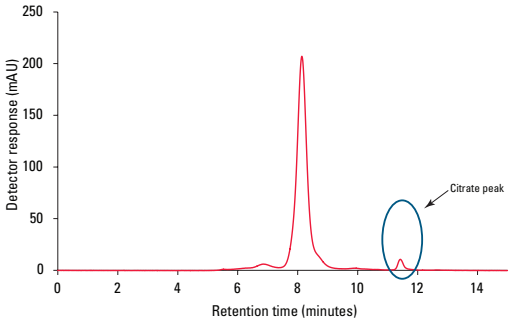


Column size:	1.5 cm ID x 4.2 cm (7.40 mL)
Mobile phase:	A: 10 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH 7.40 B: 100 mmol/L Na citrate, pH 3.0 C: 0.1 mol/L NaOH
Flow rate:	50.4 cm/hr (1.47 mL/min)
Residence time:	5 min
Detection:	UV @ 280 nm
Sample:	crude feedstock (titer = 3.0 g/L)
Sample load:	150 mL

A representative HPLC analysis of the elution pool by SEC *(Figure 4)* indicates that column bed height has no effect on the amount of aggregates present in the purified product.

Recovery was determined by comparing the amount of mAb present in the crude sample loaded onto the column to the amount of mAb present in the elution pool.

Figure 4. Representative SEC HPLC analysis of eluted mAb



Column:	TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm
Mobile phase:	0.1 mol/L phosphate, 0.1mol/L sodium sulfate, 0.05% azide, pH 6.7
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Temperature:	ambient
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 in columns with bed heights as short as 4.2 cm without any increase in aggregate levels or loss of product yield. This level of performance will allow chromatographers to make use of shorter bed heights with the same residence times used in taller bed heights without the loss of product yield or purity.

Table 2. mAb purity and yield

Column dimensions (cm ID × cm)	Sample loaded (mg)	mAb Concentration 1:20 dilution (g/L)	mAb Concentration 1:50 dilution (g/L)	Mean concentration (g/L)	Eluate volume (mL)	mAb recovered (mg)	Yield (%)	Purity (%)
0.66 × 21.5	449.7	12.02	12.33	12.18	36	438.30	97.46	96.0
1.0 × 9.3	449.7	11.11	11.73	11.42	39	445.38	99.04	96.1
1.5 × 4.2	449.7	11.98	12.02	12.00	36	432.00	96.06	94.9

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