Increase Monoclonal Antibody Purity With TOYOPEARL® Sulfate-650F Resin: a Strong Cation Exchange Resin for Capture and Removal of mAb Aggregates

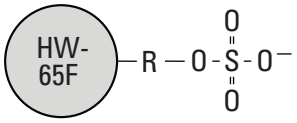
Introduction

Ion exchange chromatography is often used as an intermediate purification step in monoclonal antibody (mAb) purification for the removal of protein aggregates, host cell proteins (HCP), and leached protein A ligand. Industry trends are focusing on the development of continuous downstream processing. Typically scientists in biopharmaceutical settings use cation exchange (CEX) and anion exchange (AEX) chromatography steps in series to further polish a purified mAb after the protein A purification step. In this study, we focus on the development of a CEX step for the removal of aggregates, host cell proteins and leached protein A to improve the purity of the mAb eluate, post-protein A, in a single polishing step.

A strong cation exchange resin, TOYOPEARL Sulfate-650F, is used in this study. It is a novel resin with the following benefits: strong capture of mAb aggregates, high salt-tolerance, wide working pH range, and high dynamic binding capacity. The protocol used with this resin has a minimal pH adjustment of the purified mAb eluate after the protein A step.

Materials and Methods

TOYOPEARL Sulfate-650F resin, 45 µm, 100 nm (see structure) and TOYOPEARL AF-rProtein A HC-650F, 45 µm, 100 nm resins were used in this study. Both resins were obtained from Tosoh Bioscience LLC and were packed separately into two Omnifit® Benchmark columns, 25 mm ID × 25 cm and 6.6 mm ID × 10 cm. A TSKgel® G3000SW$_{XL}$, 5 µm, 25 nm, 7.8 mm ID × 30 cm SEC column was used for analyzing collected IgG_1 fractions after CHO supernatant crude sample containing IgG_1 was passed through and eluted from the TOYOPEARL Sulfate-650F columns.



Purify IgG_1 using TOYOPEARL AF-rProtein A HC-650F Resin

As shown in the steps below, CHO clarified cell supernatant (CCS) containing IgG_1 was passed through a 25 mm ID × 15 cm column packed with TOYOPEARL AF-rProtein A HC-650F resin to purify the IgG_1. The elution peak was collected starting and ending at 100 mAU.

1	Equilibrate (6 CV, 225 cm/hr): 20 mmol/L Tris-acetate, 150 mmol/L NaCl, pH 7.4
2	Load (48 mg/mL-resin, 225 cm/hr): TBL-mAb-01 CCS
3	Wash (10 CV, 225 cm/hr): 20 mmol/L Tris-acetate, 150 mmol/L NaCl, pH 7.4
4	Elute (5 CV, 225 cm/hr): 50 mmol/L acetic acid, pH 3.0
5	Sanitize (4 CV, 225 cm/hr): 0.1 mol/L NaOH
6	Regenerate (4 CV, 225 cm/hr): 20 mmol/L Tris-acetate, 150 mmol/L NaCl, pH 7.4

The IgG_1 eluate from the TOYOPEARL AF-rProtein A HC-650F column was adjusted to pH 5.0 with 1 mol/L Tris base and quantified by UV absorbance at 280 nm. A portion of the protein A eluate was dialyzed by ultrafiltration/diafiltration (7×) into deionized water.

Static Binding Capacity Screening

To obtain static binding capacity (SBC) for the mAb on TOYOPEARL Sulfate-650F, the collected purified IgG_1 eluate was dialyzed and adjusted to various pH and sodium chloride conditions and a final total IgG_1 concentration of 10 mg/mL. The IgG_1 was bound to TOYOPEARL Sulfate-650F resin in Resin Seeker plates (20 µL/well) as described below. Adjusted protein A IgG_1 eluate was bound to the resin by batch adsorption for 1 hour at ambient temperature. Following incubation, resin was removed from each well by vacuum filtration, and 75 µL samples of each well were read for UV absorbance to determine concentration of unbound protein. Static binding capacity was determined. SBC data was analyzed with SAS JMP 12 software.

1	Equilibrate (3 ×200 µL/well): 50 mmol/L Tris-acetate*, various NaCl conc. and pH
2	Bind (200 µL/well, 1 hr, RT) : TBL-mAb-01, 10 g/L, various NaCl conc. and pH

*Note: for pH 4.0 – 5.6, 50 mmol/L acetic acid was titrated with Tris base.
For pH 7.2 – 8.4, 50 mmol/L Tris base was titrated with acetic acid.

Dynamic Binding Capacity Optimization

Dynamic binding capacity (DBC) at 10% breakthrough was determined for TOYOPEARL Sulfate-650F. Dialyzed protein A eluate was adjusted to various pH and sodium chloride concentrations and a final total IgG_1 concentration of 5 mg/mL. DBC determination was done as shown below. Chromatograph was primed with protein solution to determine UV absorbance (280 nm) at 100% breakthrough. Protein was bound to column until UV absorbance at 10% breakthrough was reached, and DBC was determined based on the volume of protein solution loaded. DBC data was analyzed with SAS JMP 12 statistical software.

1	Equilibrate (10 CV, 180 cm/hr): 50 mmol/L acetate-Tris, various NaCl conc. and pH
2	Load (45 cm/hr): TBL-mAb-01, 5 g/L to ca. 136 mAU
3	Wash (5 CV, 45 cm/hr): equilibration buffer
4	Elute (5 CV, 45 cm/hr): equilibration buffer + 1 mol/L NaCl
5	Sanitize (5 CV, 60 cm/hr): 0.5 mol/L NaOH
6	Regenerate (5 CV, 60 cm/hr): water

Elution Optimization

Collected IgG_1 eluate was adjusted to pH 5.2 with 1 mol/L acetic acid and/or 1 mol/L Tris base and 12.1 mS/cm conductivity with 4 mol/L NaCl and/or water. Sample was loaded onto a 6.6 mm × 3.0 cm column of TOYOPEARL Sulfate-650F as shown below. Conductivity at elution peak was determined.

1	Equilibrate (10 CV, 180 cm/hr): 50 mmol/L acetate-Tris, 100 mmol/L NaCl, pH 5.2
2	Load (45 cm/hr): TBL-mAb-01, ca. 10 g/L
3	Wash (5 CV, 45 cm/hr): equilibration buffer
4	Elute (20 CV, 45 cm/hr): 50 mmol/L acetate-Tris, 100 – 500 mmol/L NaCl, pH 5.2
5	Sanitize (5 CV, 60 cm/hr): 0.5 mol/L NaOH
6	Regenerate (5 CV, 60 cm/hr): water

Separation was repeated with a 98 mg/mL-resin load, and step gradient elution at 260, 290, or 320 mmol/L NaCl for 10 CV. Throughout step gradient elution, 1-CV fractions were collected and analyzed for protein concentration, aggregate content (SEC chromatography with TSKgel G3000SW$_{XL}$), CHO-HCP (ELISA), and protein A content (ELISA).

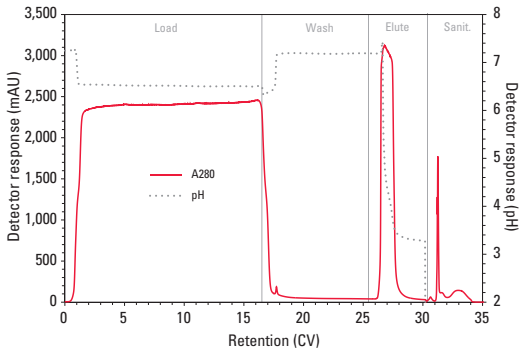
1	Equilibrate (10 CV, 180 cm/hr): 50 mmol/L acetate-Tris, 100 mmol/L NaCl, pH 5.2
2	Load (45 cm/hr): TBL-mAb-01, ca. 10 g/L
3	Wash (5 CV, 45 cm/hr): equilibration buffer
4	Elute (10 CV, 45 cm/hr): Equilibration buffer + 260 – 320 mmol/L NaCl
5	Strip (5 CV, 45 cm/hr): 50 mmol/L acetate-Tris, 1.0 mol/L NaCl, pH 5.2
6	Sanitize (5 CV, 60 cm/hr): 0.5 mol/L NaOH
7	Regenerate: water (5 CV, 60 cm/hr)

Results and Discussions

Purification of IgG$_1$ using TOYOPEARL AF-rProtein A HC-650F Resin

The crude sample containing IgG$_1$ was passed through the protein A column and fractions of IgG$_1$ were collected for further work. *Figure 1* demonstrates that the IgG$_1$ was purified by protein A chromatography. The eluate peak was collected and further analyzed by size exclusion chromatography using a TSKgel G3000SW$_{XL}$ SEC column for monomer and aggregate yield, host cell protein (HCP) content and protein A ligand leaching (see *Table 1*).

Figure 1. Purification of IgG$_1$ from CHO Supernatant Crude Sample using TOYOPEARL AF-rProtein A HC-650F Resin



Resin:	TOYOPEARL AF-rProtein A HC-650F
Column:	25 mm ID × 15 cm (74 mL)
Mobile phase:	A: 20 mmol/L Tris-acetate, 150 mmol/L NaCl, pH 7.4 B: 50 mmol/L acetic acid C: 0.1 mol/L NaOH
Flow rate:	225 cm/hr (4 min residence time)
Detection:	UV @ 280 nm (mAU), pH
Temperature:	ambient
Injection vol.:	1200 mL (48 mg/mL-resin load ratio)
Sample:	TBL-mAb-01 CSS @ 2.95 g/L
Instrument:	ÄKTA® avant 25

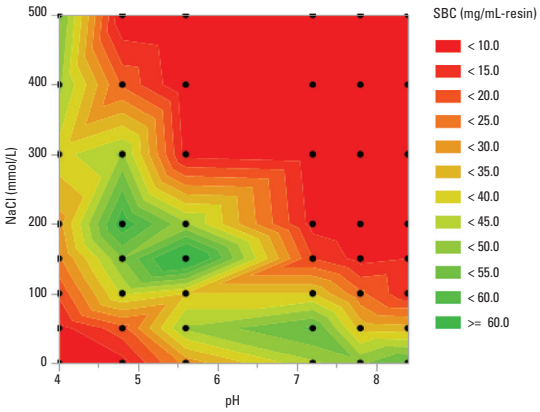
Table 1. Analysis Data for the Collected IgG$_1$ Eluate Peak

Protein A Eluate Analysis	
Yield (total IgG)	99%
Aggregate	4.4% (0.5% HMW, 3.9% dimer)
HCP	1260 ppm
Protein A	1.2 ppm

Static Binding Capacity (SBC) Screening for TOYOPEARL Sulfate-650F Resin

To optimize the binding capacity of TOYOPEARL Sulfate-650F resin, the SBC screening was carried out to find out the maximum amount of protein bound to a chromatography medium at given solvent and protein concentration conditions. *Figure 2* shows a binding map that was created for TOYOPEARL Sulfate-650F resin at pH values from 4.0 – 5.6 or 7.2 – 8.4, and 0 – 500 mmol/L NaCl. Maximum protein binding was noted between pH 4.8 and 5.6, from ca. 100 mmol/L – 200 mmol/L NaCl. At lower pH values, NaCl is necessary for protein binding. At higher pH values, significant binding is noted with little to no NaCl.

Figure 2. SBC Screening for TOYOPEARL Sulfate-650F Resin using the Collected IgG$_1$ Eluate



Dynamic Binding Capacity (DBC) Optimization

DBC was optimized by DoE with a three-level, full-factorial method at pH 4.8 – 5.6 and 100 – 200 mmol/L NaCl (results as shown in *Figure 3* with data points consolidated in *Table 2*). A maximum DBC of >120 mg/mL-resin was noted between pH 4.8, 150 mmol/L NaCl, and pH 5.2, 100 mmol/L NaCl. Conditions of pH 5.2, 12.1 mS/cm were used for the elution optimization experiments for maximum binding.

Figure 3. Dynamic Binding Capacity Optimization for TOYOPEARL Sulfate-650F Resin using the Collected IgG$_1$ Eluate

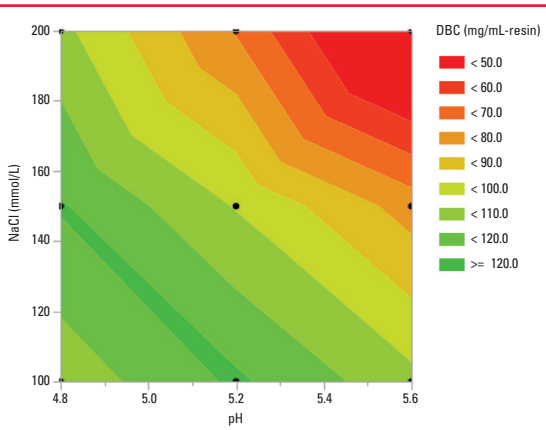


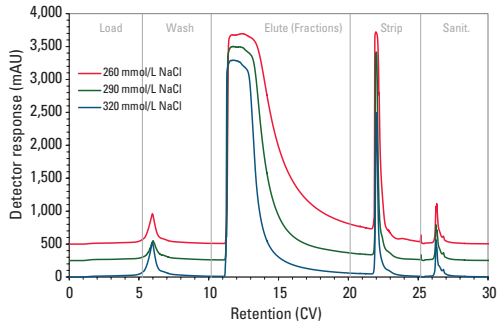
Table 2. Dynamic Binding Capacity Data at Various Conditions (run order of conditions randomized)

Exp.	Run	Load pH	Load NaCl (mmol/L)	DBC (mg/mL)
1	5	4.8	100	104
2	1	5.6	100	103
3	2	4.8	200	103
4	4	5.6	200	23
5	8	4.8	150	121
6	9	5.6	150	76
7	7	5.2	100	122
8	6	5.2	200	69
9	3	5.2	150	99
10	10	5.2	150	102

Optimize Conditions for Separation and Elution of Monomer and High Molecular Weight Peaks

To optimize elution conditions, a gradient elution was performed at pH 5.2. A peak conductivity of 30.1 mS/cm was noted (ca. 288 mmol/L NaCl). Experiment was repeated as a step gradient at 260, 290, or 320 mmol/L NaCl (see *Figure 4*). Due to peak tailing during elution, 1-CV fractions were collected throughout elution. Fractions were analyzed for IgG_1 concentration, aggregate, HCP and protein A, and results were analyzed to determine optimum NaCl concentration and peak volume.

Figure 4. Profiling of the Collected IgG_1 Eluate Peak Separated by TOYOPEARL Sulfate-650F Resin at Various Conditions



Resin:	TOYOPEARL Sulfate-650F
Column:	6.6 mm ID × 3.0 cm (1.0 mL)
Mobile phase:	A: 50 mmol/L acetate-Tris, 100 mmol/L NaCl, pH 5.2 B: 50 mmol/L acetate-Tris, pH 5.2, NaCl as indicated C: 50 mmol/L acetate-Tris, 1.0 mol/L NaCl, pH 5.2 D: 0.5 mol/L NaOH
Flow rate:	45 cm/hr (4 min residence time)
Detection:	UV @ 280 nm (mAU)
Temperature:	ambient
Injection vol.:	5.3 mL (97 mg/mL-resin load ratio)
Sample:	TBL-mAb-01, 19.1 mg/mL
Instrument:	ÄKTA avant 25

Peaks were analyzed for recovery, aggregates, HCP and protein A content (*Figure 5*). Data analysis suggests the optimum aggregate and HCP removal are obtained at 260 mmol/L NaCl in elution buffer and maximum (9 CV) elution volume. Protein A ligand content at these conditions (40 ppb) is significantly lower than that found in the load material (1200 ppb). Data is consolidated in *Table 3*.

Figure 5. Analysis Data of the Collected Eluate Peaks from TOYOPEARL Sulfate-650F Resin

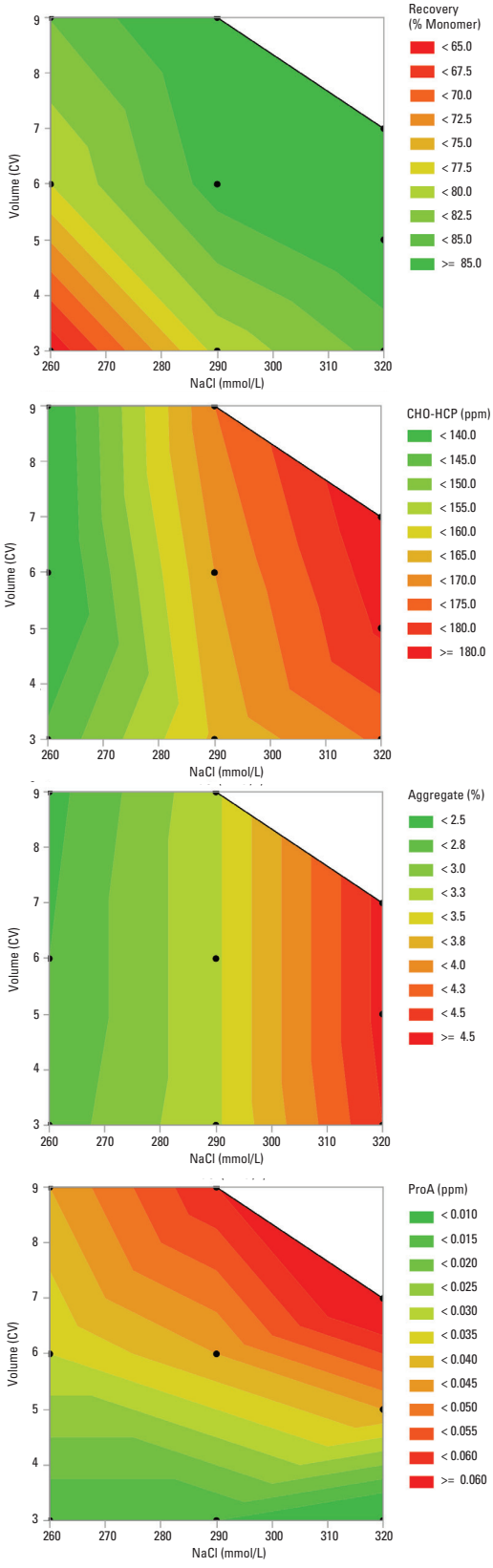
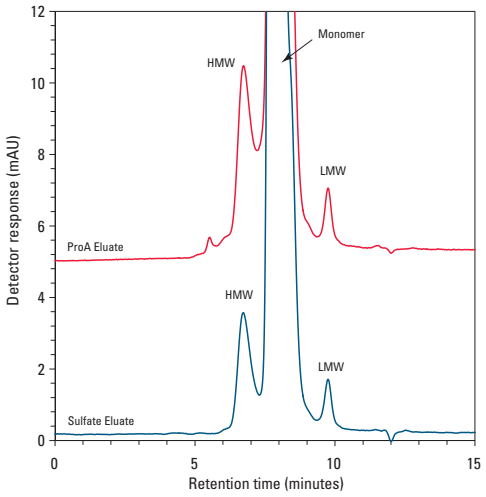


Table 3. Consolidated Data of the Collected Eluate Peaks from TOYOPEARL Sulfate-650F Resin at Various Conditions (Run Order of Conditions Randomized)

Exp.	Run	Elution NaCl (mmol/L)	Pool volume (CV)	Recovery (% Monomer)	Aggregate (% Dimer/HMW)	HCP (ppm)	Protein A (ppm)
Load					*3.9 / 0.54*	*1260*	*1.2*
1	2	260	3	63.1	2.6 / 0.09	141	0.009
		260	6	77.5	2.4 / 0.07	133	0.033
		260	9	82.6	2.4 / 0.07	134	0.040
2	1	290	3	78.3	3.0 / 0.13	161	0.007
		290	6	86.3	3.0 / 0.12	165	0.042
		290	9	88.7	3.1 / 0.12	170	0.060
3	3	320	3	83.4	4.3 / 0.20	171	0.003
		320	5	87.7	4.4 / 0.19	181	0.039
		320	7	90.2	4.5 / 0.19	185	0.067

Figure 6 shows data from the SEC analysis of the eluate pool at 260 mmol/L NaCl, 9 CV volume. Data shows there is a reduction in aggregate content (in particular HMW impurities), relative to the collected IgG_1 eluate peak material from the protein A resin eluate peak.

Figure 6. Collected Monomer Peaks Analyzed by SEC Column



Resin:	TSKgel G3000SW$_{XL}$
Column:	7.8 mm ID × 30.0 cm
Mobile phase:	0.1 mol/L phosphate, 0.1 mol/L sodium sulfate, 0.1% sodium azide, pH 6.7
Gradient:	isocratic
Flow rate:	1.00 mL/min
Detection:	UV @ 280 nm (mAU)
Temperature:	25° C
Injection vol.:	10 µL (100 µg total IgG)
Sample:	ProA or Sulfate eluate as indicated
Instrument:	Agilent 1100

The peaks from the SEC column were analyzed for high molecular weight, HCP and protein A ligand content. *Table 4* shows that after passing through the TOYOPEARL Sulfate-650F resin, the collected IgG_1 peak has significantly reduced amounts of HMW, HCP and protein A ligand. This suggests that TOYOPEARL Sulfate-650F resin can effectively remove and reduce the impurities of IgG_1.

Table 4. Integrated Peak Data from SEC Column

Impurity	ProA eluate	Sulfate eluate
Dimer (%)	3.9	2.4
HMW (%)	0.54	0.07
HCP (ppm)	1260	134
ProA (ppm)	1.2	0.040

Conclusions

The TOYOPEARL Sulfate-650F resin offers a high dynamic binding capacity (>120 mg/mL-resin) with DBC maxima at pH 4.8, 150 mmol/L NaCl and pH 5.2, 100 mmol/L NaCl. With elution at pH 5.2, recovery and impurity removal (aggregate, HCP, leached protein A) is optimal. In fact, analyzed data of the collected IgG_1 monomer peak from the TOYOPEARL Sulfate-650F resin column showed that its purity was significantly improved with an acceptable amount of HMW proteins and HCP while nearly no protein A ligand was detected in the collected IgG_1 peak. By selecting this strong cation exchange resin as a step after mAb post-protein A purification, only a minimal adjustment to pH or salt concentration to the sample is needed.

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