TOYOPEARL GigaCap® Cation Exchange Resins for Aggregate Removal from Monoclonal Antibodies

TOYOPEARL
APPLICATION NOTE

Aggregate removal is a pivotal process step in the purification of monoclonal antibodies (mAbs). The removal of soluble, non-precipitated mAb aggregates is highly challenging due to their close physical and chemical similarity to the drug product itself. Aggregates of host cell proteins (HCP) and DNA, in addition to mAb aggregates, also must be removed during downstream manufacturing, although most of these types of aggregates are sufficiently discrete from the protein of interest and therefore pose less of a challenge in removal.

Introduction

Ion exchange chromatography (IEC) has long been used as an effective means of removing process impurities from biological drug products. Cation exchange chromatography (CEX), in particular, has shown to be effective in separating mAb monomer from dimers and higher order aggregates.

By carefully controlling the pH and conductivity of the mobile phase in a chromatographic separation, it is possible to affect the net electrochemical charge of mAb aggregates such that they have a greater overall charge when compared to the mAb monomer. This charge difference results in preferential binding of the aggregates to the chromatography resin over the monomer.

TOYOPEARL GigaCap S-650 and TOYOPEARL GigaCap CM-650 media are high capacity cation exchange resins optimized for process scale applications, including the purification of monoclonal antibodies. These resins are based on the TOYOPEARL® HW-65 size exclusion resin and have been functionalized with sulfopropyl (TOYOPEARL GigaCap S) or carboxymethyl (TOYOPEARL GigaCap CM) groups. *Table 1* lists the properties of the resins used in this study.

Table 1. Properties of TOYOPEARL GigaCap resins used in this study

	TOYOPEARL GigaCap S-650M	TOYOPEARL GigaCap S-650S	TOYOPEARL GigaCap CM-650M
Particle size (mean)	75 µm	35 µm	75 µm
Pore size (mean)	100 nm	100 nm	100 nm
Ion exchange capacity	0.10 – 0.20 eq/L	0.15 – 0.25 eq/L	0.17 – 0.28 eq/L
Ligand pKa	1.2	1.2	3.6

Since TOYOPEARL GigaCap resins use the same polymethacrylate backbone as all other TOYOPEARL chromatography resins, they exhibit similar pressure-flow and chemical stability characteristics as TOYOPEARL resins of similar particle sizes. TOYOPEARL GigaCap resins are stable at high linear velocities, have excellent pressure-flow characteristics and will withstand back pressures of up to 0.3 MPa.

The smaller particle size of the TOYOPEARL GigaCap S-650S (35 µm vs. 75 µm for M-grade) retains the capacity and mechanical stability of the M-grade variant, though at a reduced linear velocity.

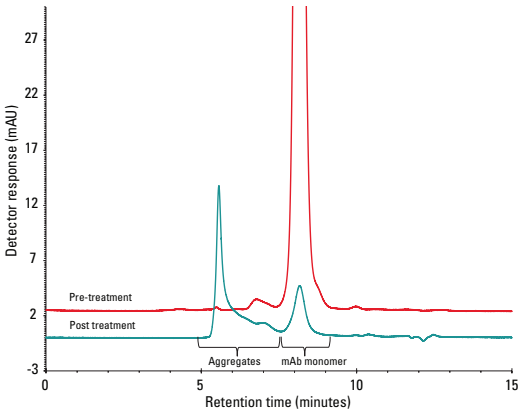
The data presented here demonstrate effectiveness of TOYOPEARL GigaCap S-650M, TOYOPEARL GigaCap S-650S and TOYOPEARL GigaCap CM-650M resins in the elimination of mAb aggregates from protein A purified material.

Experimental Conditions/Results

Monoclonal antibody aggregates were produced by acid treatment of a protein A purified mAb. A 2.85 mL aliquot of protein A purified monoclonal antibody (29.3 g/L) was acidified with 150 µL of 1 mol/L hydrochloric acid (final concentration: 0.05 mol/L HCl) for 1 hour at room temperature.

The 3 mL reaction was quenched by dilution in 81 mL of 0.2 mol/L sodium acetate, pH 5.0 (mobile phase A), to give a final protein concentration of 1.0 g/L. *Figure 1* is an SEC analysis of the pre- and post-acid treatment of the protein A purified mAb.

Figure 1. SEC analysis of acid induced mAb aggregates



Column:	TSKgel® G3000SW$_{XL}$, 7.8 mm ID × 30 cm
Mobile phase:	0.1 mol/L sodium phosphate, 0.1 mol/L sodium sulfate, 0.05% NaN_3, pH 6.7
Gradient:	isocratic
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	pre- and post-aggregated mAb

A 3.1 mL aliquot of unaggregated protein A purified mAb was diluted with 87 mL of 0.2 mol/L sodium acetate, pH 5.0, to a final protein concentration of 1.0 g/L. The diluted unaggregated mAb was spiked with the aggregated mAb to produce a sample with an aggregate content of approximately 22%.

For the initial screening experiments in this study, 6.6 mm ID columns were packed to a bed height of 5 ± 0.5 cm with the TOYOPEARL GigaCap S-650M and TOYOPEARL GigaCap CM-650M resins.

A 20 CV linear gradient from 0-100% mobile phase B (mobile phase A + 1.0 mol/L NaCl) was used to separate the mAb monomer from mAb dimer and aggregates with the TOYOPEARL GigaCap M-grade resins (chromatogram not shown). Fractions were taken throughout the elution at 1 CV intervals.

The collected fractions were analyzed by size exclusion chromatography using a TSKgel G3000SW$_{XL}$ column and fractions were pooled to maximize purity and recovery. Analysis of the pooled fractions in *Table 2* indicated that further optimization was required.

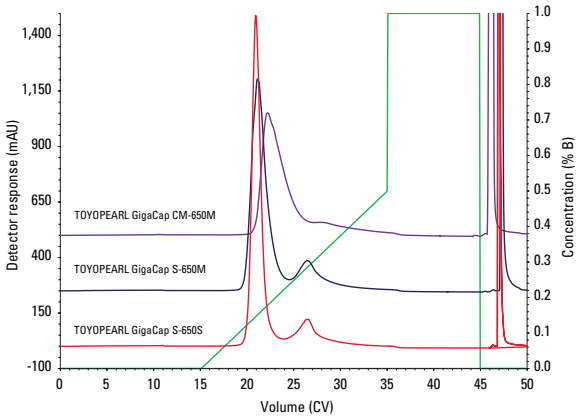
Table 2. Analysis of aggregate removal from screening runs (N = 3)

	Aggregate concentration (%)	Δ Aggregate concentration (%)	Aggregate removed
Protein A purified mAb (load)	22.6 ± 0.4	n/a	n/a
TOYOPEARL GigaCap S-650M (mAb pool)	5.5 ± 0.6	17.0 ± 0.7	75.0 ± 3.0
TOYOPEARL GigaCap CM-650M (mAb pool)	3.0 ± 1.0	20.0 ± 1.0	87.0 ± 5.0

The optimized experiments in this study were carried out on 6.6 mm ID columns, packed to a bed height of 20.5 ± 0.5 cm with the TOYOPEARL GigaCap S-650M, TOYOPEARL GigaCap S-650S, and TOYOPEARL GigaCap CM-650M resins. In addition, the elution gradient was changed to 0-50% mobile phase B over 20 CV.

The optimized separations can be seen in *Figure 2*. As was done in the screening experiments, fractions were taken throughout the elution at 1 CV intervals. After elution, the column was sanitized with 0.5 mol/L NaOH.

Figure 2. Removal of aggregates with TOYOPEARL GigaCap resins at optimized conditions



Resins:	TOYOPEARL GigaCap CM-650M TOYOPEARL GigaCap S-650M TOYOPEARL GigaCap S-650S
Column size:	6.6 mm ID × 20.5 cm (7.0 mL)
Mobile phase:	A: 0.2 mol/L sodium acetate, pH 5.0 B: mobile phase A + 1.0 mol/L NaCl
Gradient:	0 – 50% mobile phase B (20 CV)
Flow rate:	M-grades: 610 cm/hr (3.49 mL/min) S-grade: 251 cm/hr (1.43 ml/min)
Detection:	UV @ 280 nm
Sample:	protein A purified mAb
Sample load:	10 g/L resin

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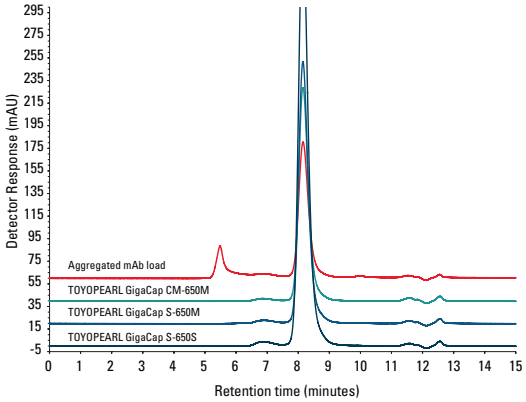
The collected fractions were analyzed by size exclusion chromatography using a TSKgel G3000SW$_{XL}$ column and fractions were pooled to maximize purity and recovery. Analysis of the pooled mAb fractions in *Figure 3* and *Table 3* demonstrate that TOYOPEARL GigaCap cation exchange resins are capable of removing > 92% of process aggregates from a protein A purified mAb.

Table 3. Analysis of aggregate removal from optimized runs (N = 1)

	Aggregate concentration (%)	Δ Aggregate concentration (%)	Aggregate removed
Protein A purified mAb (load)	21.4	n/a	n/a
TOYOPEARL GigaCap S-650M (mAb pool)	1.7	19.7	92.1
TOYOPEARL GigaCap S-650S (mAb pool)	1.3	20.1	93.9
TOYOPEARL GigaCap CM-650M (mAb pool)	1.7	19.7	92.1

Figure 3. SEC analysis of optimized TOYOPEARL GigaCap purified mAb



Column:	TSKgel G3000SW$_{XL}$, 7.8 mm ID × 30 cm
Mobile phase:	0.1 mol/L sodium phosphate, 0.1 mol/L sodium sulfate, 0.05% NaN_3, pH 6.7
Gradient:	isocratic
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Sample:	pooled fractions from purification

Conclusions

TOYOPEARL GigaCap S-650M, TOYOPEARL GigaCap S-650S and TOYOPEARL GigaCap CM-650M cation exchange resins are an effective means for the removal of dimer and higher order aggregates from mAb monomer in a protein A purified antibody.

Though some aggregate was present in the purified mAb pool, further method development with a step gradient may further reduce the amount of aggregate carryover to less than 1% and increase product yield in the monomer peak.

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