TOSOH

Application Note

Effective removal of mAb aggregate using Ca++Pure-HA® media with potassium salts

Introduction

Downstream process chromatography scientists are constantly on the lookout for better and more selective ways to remove aggregates and other process related impurities from a monoclonal antibody (mAb) monomer. Making use of chromatography resins with better selectivity, resolution and capacity is one approach to solving the problem of aggregate removal in monoclonal antibody production.

Ca++Pure-HA (hydroxyapatite: $Ca_{10}(PO_4)_6(OH)_2$) is a form of calcium phosphate used in the chromatographic separation of biomolecules. Unlike other resins available from Tosoh Bioscience, Ca++Pure-HA is both the ligand and the base bead. Hydroxyapatite has unique separation properties for biomolecules and Ca++Pure-HA offers unparalleled selectivity and resolution for process scale operations. Its highly selective nature often separates proteins otherwise shown to be homogeneous by electrophoresis and other chromatographic techniques.

Ca++Pure-HA resin is a spherical, macroporous form of the hexagonal crystalline structure of hydroxyapatite. It has been sintered at high temperatures for increased mechanical and chemical stability, allowing it to withstand the rigors of industrial-scale applications. *Table 1* lists the properties of Ca++Pure-HA.

Table 1. Properties of Ca++Pure-HA

	Ca++Pure-HA
Particle size (mean):	39 µm
Pressure rating:	10 MPa
Shipped as:	dry powder
pH stability:	6.5 – 14
Shelf life (estimated):	10 years

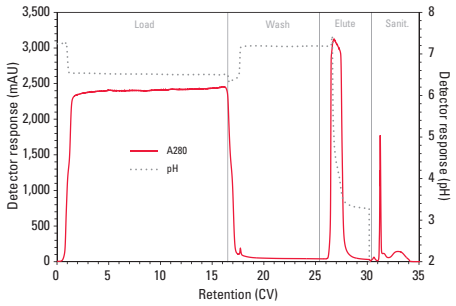
The data presented here demonstrates the capabilities of Ca++Pure-HA media operated with potassium salts such as potassium phosphate and potassium chloride, to remove dimer and higher order aggregates from the monomer of a protein A purified IgG_1 monoclonal antibody.

Experimental Conditions/Results

Purification of IgG_1 using TOYOPEARL AF-rProtein A HC-650F Resin

A crude sample containing IgG_1 was passed through a 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® G3000SWXL SEC column for monomer and aggregate yield, host cell protein (HCP) content and protein A ligand leaching (see data in *Table 2*).

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 2. 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

Removal of mAb aggregates using Ca++Pure-HA media

To remove mAb aggregates from a post-protein A purified sample, Ca++Pure-HA media was used in a polishing chromatography step. The below protocol was used.

Column:	5 mm ID × 5 cm (1.0 mL)
Mobile phase:	A: 50 mmol/L HEPES, 5 mmol/L KPO_4, pH as indicated B: mobile phase A + 2.0 mol/L potassium chloride, pH as indicated C: 500 mmol/L KPO_4, pH as indicated D: 1.0 mol/L KOH
Gradient:	69.4% B (chloride), 10 CV gradient delay, 5 CV
Flow rate:	300 cm/hr (1 min residence time)
Detection:	UV @ 280 nm (mAU), Conductivity (mS/cm), pH
Temperature:	ambient
Injection vol.:	5 µL
Sample:	2.0 mg/mL-media partially-purified mAb-01 (0.2 mL injection)
Instrument:	ÄKTA avant 25
Method:	Pre-equilibrate, mobile phase C, 3 CV Equilibrate, mobile phase A, 10 CV Load Wash, mobile phase A, 5 CV Elution, gradient as indicated, 25 CV Strip, mobile phase C, 5 CV Sanitize, mobile phase D, 5 CV

Data from *Figure 2* shows a high resolution separation between the monomer peak and the aggregate peak across three different pH conditions. The elution of the monomer peak at pH 6.5 was delayed and broader (see *Figure 2*).

Figure 2. Removal of mAb aggregates from the post-protein A purification sample using Ca++Pure-HA media

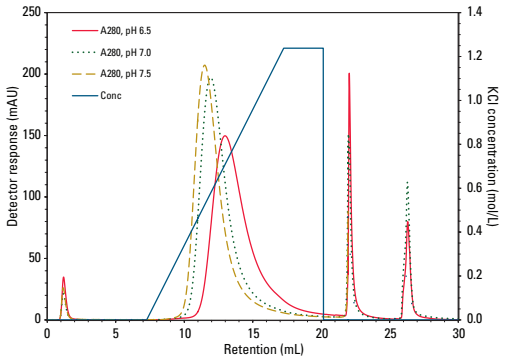
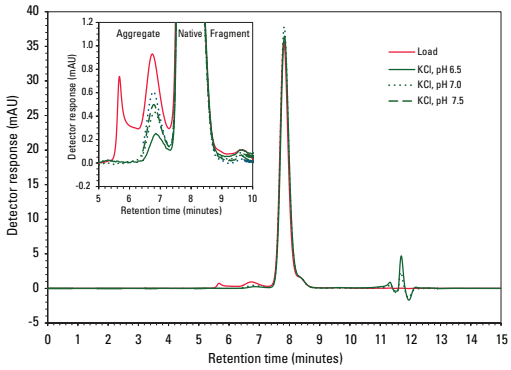


Figure 3 shows a size exclusion chromatography analysis of pooled monomer samples from *Figure 2* for the analysis of aggregate content. These samples were injected onto a TSKgel G3000SWXL column. Data analysis show that after the sample passed through Ca++Pure HA media under potassium phosphate buffer and potassium chloride operating conditions, mAb aggregates were reduced significantly. In fact, at pH 6.5 operating conditions, the aggregate amount was reduced from 6.6% to as low as 1.3% *(Table 3)*.

Figure 3. Aggregate analysis of pooled mAb monomer peaks eluted from different pH buffers using size exclusion chromatography



Column:	TSKgel G3000SWXL, 7.8 mm ID × 30 cm
Mobile phase:	0.1 mol/L phosphate, 0.1 mol/L Na_2SO_4, 0.3% sodium azide, pH 6.7
Flow rate:	1.0 mL/min
Gradient:	isocratic
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection:	10 µg native
Instrument :	HPLC (400 bar pressure)

Table 3. Aggregate analysis data of pooled mAb monomer peak eluted from different pH conditions

Salt	pH	Peak molarity (mmol/L)	Recovery (% native)	Aggregate (%)	Fragment (%)
Load				**6.6**	**0.6**
KCl	6.5	814	72.9	1.3	0.5
	7.0	615	80.0	1.8	0.3
	7.5	509	81.0	2.2	0.3

Conclusions

Ca++Pure-HA media is effective for the removal of dimer and higher order aggregates from a purified mAb sample, post-protein A purification step. In fact, when Ca++Pure-HA media was operated using potassium phosphate buffer as a loading buffer and potassium chloride as an elution buffer, the aggregate content was reduced from 6.6% to as low as 1.3%.