CaPure-HA Resin for Aggregate Removal from Monoclonal Antibodies

Chromatography Resin
APPLICATION NOTE

The past five years or so have brought improvements in cell culture making ever increasing upstream product titer a reality. While increased upstream titer has many advantages, including reduced production costs; one of the drawbacks is a marked increase in the presence of dimer and other higher order aggregates. Scientists in the field of process chromatography methods development are constantly on the lookout for better and more selective ways to remove aggregates and other process related impurities from monoclonal antibody 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.

Introduction

CaPure-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, CaPure-HA is both the ligand and the base bead. Hydroxyapatite has unique separation properties for biomolecules and CaPure-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.

CaPure-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 CaPure-HA.

Table 1. Properties of CaPure-HA

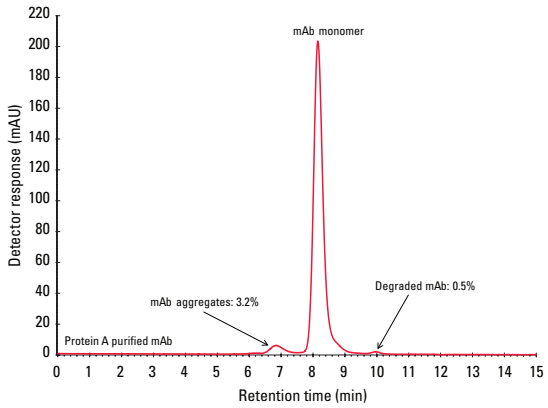
Particle size (mean):	39 µm
Pressure rating:	10 MPa
Shipped as:	dry powder
pH stability:	6.5 – 14
Shelf life (estimated):	10 years

The data presented here demonstrate the capabilities of CaPure-HA to remove degradation products, dimer, and higher order aggregates from the monomer of a protein A purified IgG_1 monoclonal antibody.

Experimental Conditions/Results

Figure 1 is a size exclusion chromatography analysis of an aliquot of a protein A purified IgG_1 mAb on a TSKgel® G3000SWXL column. This sample is representative of the material that will be purified using a column packed with CaPure-HA. In this figure, the presence of degradation products, monomer, dimer, and higher order aggregates can be seen.

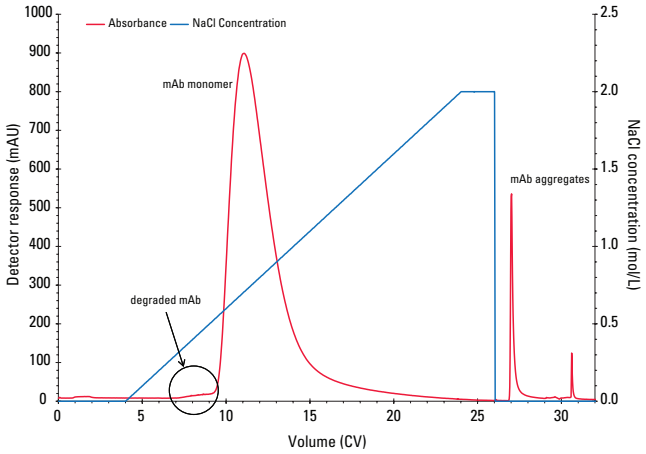
Figure 1. SEC analysis of mAb prior to aggregate removal by CaPure-HA



Column:	TSKgel G3000SWXL, 7.8 mm ID × 30 cm
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.8
Gradient:	isocratic
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Injection vol:	20 µL
Sample:	protein A purified mAb IgG_1

For the aggregate removal experiment *(Figure 2)*, a 1.0 cm ID × 10 cm column was packed with CaPure-HA resin. The column was equilibrated with 5 mmol/L sodium phosphate, pH 6.8 (mobile phase A) for 5 CV. A 0.5 g/L sample of protein A purified mAb (IgG_1) was then loaded onto the column, and the column was washed with mobile phase A.

Figure 2. Purification of mAb on CaPure-HA column

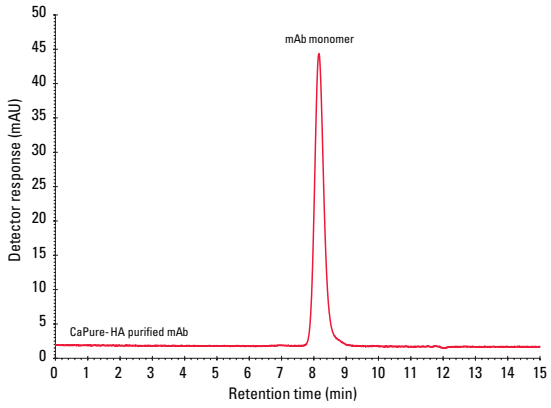


Resin:	CaPure-HA
Column size:	1.0 cm ID × 10 cm (7.85 mL)
Mobile phase:	A: 5 mmol/L sodium phosphate, pH 6.8 B: mobile phase A + 2.0 mol/L NaCl
Gradient:	0-100% mobile phase B (20 CV)
Flow rate:	3.93 mL/min (300 cm/hr)
Detection:	UV @ 280 nm
Sample:	protein A purified mAb, 10.0 g/L
Sample load:	10.0 g/L-resin

A 20 CV linear gradient from 0-100% mobile phase B (mobile phase A + 2.0 mol/L NaCl) was used to separate the mAb monomer from mAb degradation products, dimer, and higher order aggregates. After elution, the column was sanitized using 0.5 mol/L NaOH.

The purified mAb monomer peak was analyzed by size exclusion chromatography using a TSKgel G3000SW$_{XL}$ column to verify the removal of degradation products, dimer, and larger aggregates from the mAb monomer *(Figure 3)*. The presence of mAb degradation products, dimer or higher order aggregates is not detectable by analytical size exclusion HPLC.

Figure 3. SEC analysis of purified mAb monomer from CaPure-HA



Column:	TSKgel G3000SW$_{XL}$, 7.8 mm ID × 30 cm
Mobile phase:	0.2 mol/L phosphate buffer, pH 6.8
Gradient:	isocratic
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Injection vol:	20 µL
Sample:	CaPure-HA purified mAb IgG$_1$

Conclusions

CaPure-HA is an effective resin for the removal of degradation products, dimer, and higher order aggregates from mAb monomer in a protein A purified antibody. The highly selective and robust nature of CaPure-HA gives chromatographers the flexibility to use this resin for aggregate removal at almost any stage in the manufacturing process.

TOSOH BIOSCIENCE

TOSOH BIOSCIENCE LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Tel: 800-366-4875
email: info.tbl@tosoh.com
www.tosohbioscience.com

AN72
1014