Removing the Aggregates of an Acidic Monoclonal Antibody with CHT Ceramic Hydroxyapatite XT Media

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Monoclonal Antibody Purification

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Abstract

CHT Ceramic Hydroxyapatite Media/Resins are known as the industry gold standard for impurity removal during monoclonal antibody (mAb) purification. In the present study, we demonstrate the removal of aggregates from the monomers of mAb S (pI ~6.9) with our newly launched CHT XT Media. mAb S was initially isolated from CHO cell culture using Protein A chromatography. The eluate from this capture step was a mixture of mAb S monomers and aggregates, with the high molecular weight species accounting for ~34% of the total mass of antibodies. Polish purification was then performed on a CHT XT Column, resulting in >96% final monomer content, thereby confirming the robustness of CHT XT in aggregate removal. In addition, excellent clearance of host cell proteins (HCPs), dsDNA, leached recombinant Protein A (rPA) and endotoxins was achieved, making CHT XT a great solution for mAb purification.

Introduction

Monoclonal antibodies (mAbs) are one of the largest and fastest growing areas of research in the pharmaceutical industry thanks to their application in the treatment of a wide range of diseases such as cancer, chronic inflammation, metabolic diseases, and infection. The therapeutic success of any mAb depends heavily on its purity. A major challenge in downstream purification of mAbs is the formation and removal of aggregates (Vazquez-Rey and Lang 2011). The presence of aggregates can hamper the efficacy of therapeutic mAbs due to their different bioactivity, storage stability, immunogenicity, and pharmacokinetic properties relative to the monomeric versions. In many cases, antibody aggregates are the most challenging process/product-related impurities to remove. Extensive process development is necessary to obtain highly pure monomeric mAbs.

Protein A chromatography is often used for capture purification of mAbs. However, the acidic conditions required for eluting many mAbs from a Protein A resin leads to increased aggregate formation (Mazzer et al. 2015). This warrants another chromatographic step for removal of not only the aggregates but also any leached Protein A.

CHT Ceramic Hydroxyapatite Media has been considered to be the gold standard for impurity removal — including aggregates, leached Protein A, dsDNA, HCPs, and endotoxins — during mAb purification (Gagnon 2009, Gagnon et al. 2006).

CHT is a mixed-mode media that can bind to biomolecules by calcium metal affinity, phosphoryl cation exchange interactions, and/or hydrogen bonding. CHT XT is the newest addition to the CHT family. It is manufactured at a high sintering temperature, leading to robustness and a long life cycle for repeated use.

In the present study, we demonstrate the removal of aggregates of an acidic mAb (mAb S, pI ~6.9) in a Protein A eluate, which contained ~34% high molecular weight species or aggregates. Polish purification of this eluate on a CHT XT Column led to significant aggregate reduction and monomer enrichment to >96% final monomer content. In addition, excellent clearance of other impurities, such as HCPs, dsDNA, leached rPA, and endotoxins, was also achieved. These results establish the new CHT XT Media as a great solution for mAb purification.

Materials and Methods

Chromatography Media and Columns

UNOsphere SUPrA (Bio-Rad Laboratories, catalog #1560218) was packed in a 0.7 x 5.5 cm column. CHT XT (Bio-Rad, #12002457) was packed in a 0.5 x 5.1 cm column. The monomeric content in the eluate fractions or pools was determined using ENrich SEC 650 Columns (Bio-Rad, #7801650) with 1x phosphate buffered saline (PBS) as the mobile phase.

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Chromatography System

Chromatographic separations and analysis were performed on an NGC 10 Chromatography System (Bio-Rad, #7880001), which provides simultaneous monitoring of absorbance at 280 nm, conductivity, and pH.

Capture Purification on UNOsphere SUPrA Column

mAb S was purified from a CHO cell culture harvest by affinity chromatography on a UNOsphere SUPrA Column. Prior to loading, the column was equilibrated with 5 column volumes (CV) of 1x PBS, pH 7.0 at 300 cm/hr. CHO cell culture (10 ml) was applied to the column at 150 cm/hr followed by a post-loading wash with 10 CV of 1x PBS, pH 7.0. mAb S elution was performed with 5 CV of 0.1 M glycine, pH 3. The eluted mAb S solution was adjusted to pH ~8.0 with 1 M Tris base and filtered through a 0.22 µm membrane. The column was stripped with 5 CV of 0.1 M glycine, 1 M NaCl, pH 3 at 300 cm/hr after use and stored in 5 CV of 20% ethanol. The monomer content in the eluted sample was quantified using SEC (Figure 1) as per the protocol described.

Polish Purification on CHT XT Column

The CHT load was prepared by adding 0.3 ml of 0.5 M $NaPO_4$ (monobasic) to 15 ml of neutralized UNOsphere SUPrA eluate. The pH of this sample was adjusted to 7.0 with 1 N NaOH. Polish purification was carried out as described in Figure 2.

Monomer Content Analysis with Size Exclusion Chromatography

The SEC column was equilibrated with 1.5 CV of 1x PBS pH 7.0 at 50 cm/hr. Approximately 0.25 ml of the samples were loaded and eluted with 1 CV of 1x PBS pH 7.0. The column was stored in 1.5 CV of 20% ethanol. The calculation for monomer recovery was carried out as previously described (He 2015).

Analytical Assays

The quantity of dsDNA was measured with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, #P7589). The HCP content was measured with a CHO HCP Assay Kit (Cygnus Technologies, #F550). Leached Protein A was quantitated with a Mix-N-Go Protein A Assay Kit (Cygnus, #F600). Endotoxin contamination was quantitated with a ToxinSensor LAL Endotoxin Assay Kit (GenScript, #L00350) and an EndoLISA Endotoxin Detection Assay (Hyglos GmbH, #609033). Protein concentration was quantified using the Quick Start Bradford 1x Dye Reagent (Bio-Rad, #5000205) and a Quick Start Bovine Gamma-Globulin (BGG) Standard Set (Bio-Rad, #5000209).

Results and Discussion

Aggregate Content of UNOsphere SUPrA Eluate (mAb S)

About 34% mAb S aggregate content was seen in the UNOsphere SUPrA eluate as shown in Figure 1. This result indicates that mAb S is pH sensitive; and the acidic condition needed for its elution from the Protein A column triggered structural changes as well as oligomerization. The harvested eluate was subjected to further purification on CHT XT.

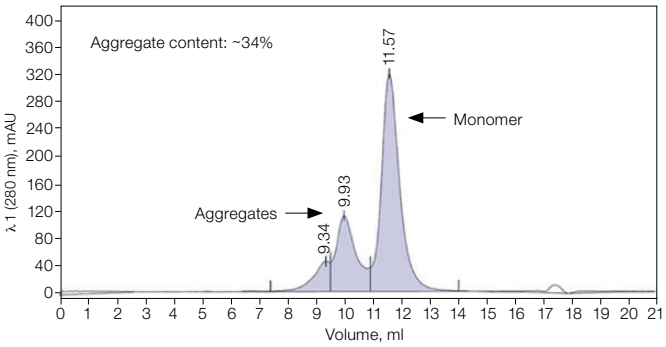

Fig. 1. SEC analysis of mAb S eluted from UNOsphere SUPrA. SEC reveals the level of mAb S aggregates in the eluate from UNOsphere SUPrA.

Separation of mAb S Monomers from Aggregates on CHT XT

The UNOsphere SUPrA eluate was preconditioned, as described in Materials and Methods, before loading on the CHT XT Column. Polish purification on CHT XT was carried out as detailed in Figure 2. The mAb S monomers were eluted as a sharp peak between fractions 10 and 14, which were pooled together for monomer content analysis by SEC. The high molecular weight fractions were eluted later and seen as multiple peaks in the chromatogram.

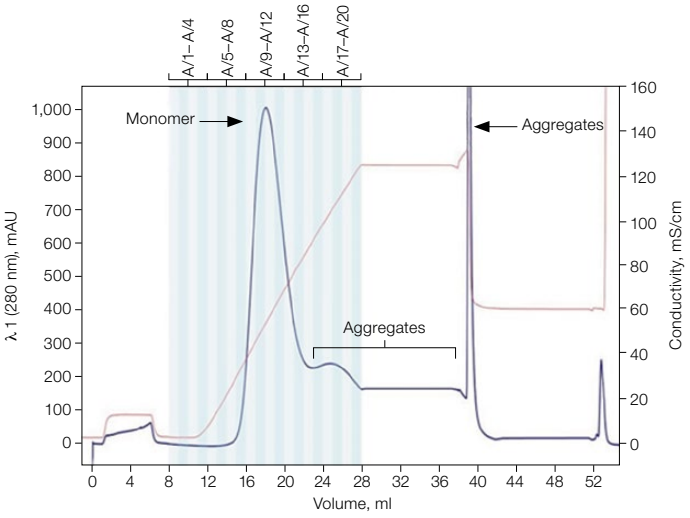

Fig. 2. Separation of mAb S monomers from aggregates on a CHT XT Column. The blue vertical bands represent collected column fractions. Fractions 10 to 14 were pooled. OD 280 (—); conductivity (—).

Equilibration:	10 CV of 10 mM $NaPO_4$, pH 7, 300 cm/hr
Load:	5 ml of CHT load (containing 8.5 mg mAb S at a concentration of 1.7 mg/ml)
Wash:	3 CV of 10 mM $NaPO_4$, pH 7
Elution:	20 CV of linear gradient: 0 to 100% of 10 mM $NaPO_4$, 1 M NaCl, pH 7
Strip:	4 CV of 400 mM $NaPO_4$, pH 7
Sanitization:	3 CV of 1 N NaOH

Aggregate Content of CHT XT Eluate (mAb S)

Significant reduction of the mAb S aggregate was achieved after purification of the Protein A eluate on CHT XT as shown in Figure 3. The final aggregate content was quantitated to be only ~3% as opposed to the initial ~34% seen in the UNOsphere SUPrA eluate. Overall purification performance of CHT XT chromatography is summarized in Table 1.

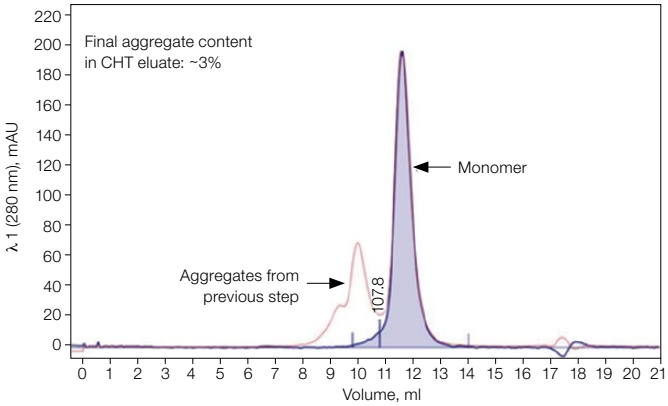

Fig. 3. SEC analysis of mAb S eluted from CHT XT. SEC showing the level of mAb S aggregates in the eluate from CHT XT. Load (—); eluate (■).

Table 1. Reduction of mAb S aggregate contamination by CHT XT.

Sample	Monomer content, %
CHT XT load	66.0
CHT XT eluate	96.9

Clearance of HCPs, DNA, Leached Protein A, and Endotoxins by CHT XT

To further assess the levels of other impurities in the CHT XT eluate, various analytical assays (as mentioned in Materials and Methods) were performed. The results are shown in Table 2. CHT XT showed multiple fold–removal of all four impurities.

Table 2. Impurity clearance by CHT XT.

Impurity	Load	Pool
HCP, ppm	2,635	229
dsDNA, ppm	112	3
Leached rPA, ppm	11	1
Endotoxin (LAL Endotoxin Assay), EU/mg	11	1
Endotoxin (EndoLISA Assay), EU/mg	422	16

Conclusions

This study shows that CHT XT is capable of minimizing the aggregate content during mAb purification. It can also reduce other product- and process-related impurities to significantly low levels. These data demonstrate that CHT XT is an exceptional tool for polish purification of monoclonal antibodies.

References

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