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

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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 rPA and endotoxins was achieved, making CHT XT a great solution for mAb purification.

Introduction

A major challenge in downstream purification of monoclonal antibodies (mAbs) is the formation and removal of aggregates (Rey V.M. et al., 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. Protein A chromatography, often used for capture purification of mAbs, leads to increased aggregate formation (Bracewell et al., 2015) due to the acidic conditions required for elution.

CHT Ceramic Hydroxyapatite Media has been shown 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 from an acidic mAb (mAb S, pI ~6.9) Protein A eluate, which contained ~34% high-molecular-weight species or aggregates. Polish purification of this eluate on a CHT XT Column lead to aggregate removal 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

SEC chromatography method: (Enrich SEC650 column: 10 x 300 mm, Bio-Rad, Cat#780-1650)
Equilibration: 1.5 CV of SEC running buffer - 1xPBS, pH 7, 50 cm/h
Load: 0.25 ml of sample, Elution: 1 CV of SEC running buffer, Storage: 1.5 CV of 20% EtOH
SuPrA chromatography method: (SuPrA, Bio-Rad Cat # 1560218 : 0.7 x 5.5 cm, CV = 2 ml)
Equilibration: 5 CV of equilibration buffer - 1xPBS, pH 7, 300 cm/h
Load: 10 ml of MAb S cell culture harvest, 150 cm/h, Wash: 10 CV of equilibration buffer, Elution: 5 CV of elution buffer - 0.1 M Glycine, pH 3, Strip: 5 CV of strip buffer - 0.1 M Glycine, 1 M NaCl, pH 3, 300 cm/h, Storage: 5 CV of 20% EtOH
CHT XT chromatography method: (CHT XT, Bio-Rad Cat # 12002457 : 0.5 x 5.1 cm, CV = 1 ml)
Equilibration: 10 CV of equilibration buffer - 10 mM $NaPO_4$, pH 7, 300 cm/h
Load: 5 ml of CHT load, Wash: 3 CV of equilibration buffer
Elution: Linear gradient of 0 to 100% elution buffer 10 mM $NaPO_4$, 1 M NaCl, pH 7, over 20 CV, collected 1-ml fractions
Strip: 4 CV of regeneration buffer - 400 mM $NaPO_4$, pH 7, Sanitization: 3 CV of 1 N NaOH
Analytical assay reagents and kits:

- Syringe filter: PALL, 32 mm, 0.2 um Supor membrane, Ref: 4652
- Quant-iT PicoGreen dsDNA assay kit: Invitrogen, Ref.#P7589
- CHO HCP assay kit: Cygnus Tech., Cat#F550
- Mix-N-Go Protein A assay kit: Cygnus Tech., Cat#F600
- ToxinSensor LAL endotoxin assay kit: GenScript, Cat#L00350
- EndoLISA assay kit: Hyglos GmbH, Ref#609033
- QuickStart Bradford 1x Dye reagent: Bio-Rad, Cat#500-0205
- QuickStart Bovine Gamma-Globulin (BGG) Standard: Bio-Rad, Cat#500-0209

Results

Aggregate Content of UNOsphere SuprA Eluate (mAb S) Using SEC-HPLC: About 34% mAb S aggregate content was seen in the UNOsphere SuprA eluate as shown in Figure 1. This was expected, since Protein A chromatography is known to trigger structural changes and promote

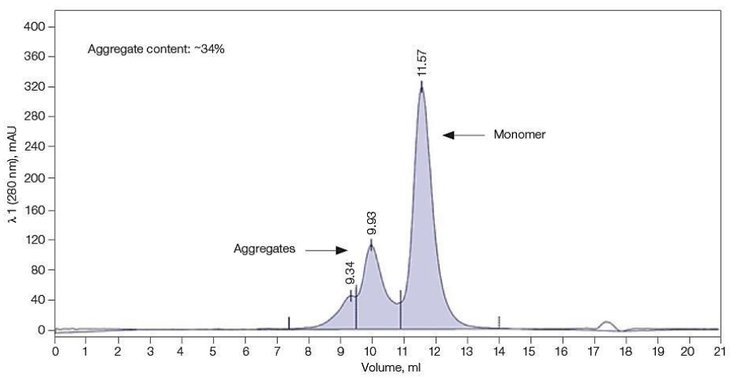

Figure 1. SEC analysis of mAb S eluted from UNOsphere SuprA. SEC showing the level of mAb S aggregates in the eluate from UNOsphere SUPrA. This will be the load on CHT XT. OD 280 (-)

Separation of mAb S monomer from Aggregates on CHT XT

The CHT load was prepared by adding 0.3 ml of 0.5 M NaPO4 (monobasic) to 15 ml of neutralized UNOsphere SuprA eluate. The pH of this sample was adjusted to 7.0 with 1 N NaOH. The polish purification on CHT XT was carried out as detailed in the methods section. The mAb S monomers were eluted as a sharp peak between fractions 10 to 14, which were pooled together for monomer content analysis by SEC. The HMW fractions eluted later and were seen as multiple peaks in the chromatogram.

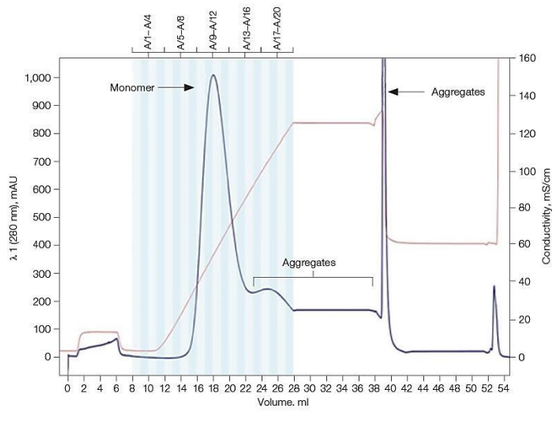

Figure 2. Elution of mAb S monomers from a CHT XT column. The blue vertical bands represent where samples were collected. Fractions 10 to 14 were pooled. OD 280 (purple) %B (red).

Aggregate content of CHT XT eluate (mAb S) using SEC-HPLC

Significant reduction of the mAb S aggregate was seen after purification of the 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.

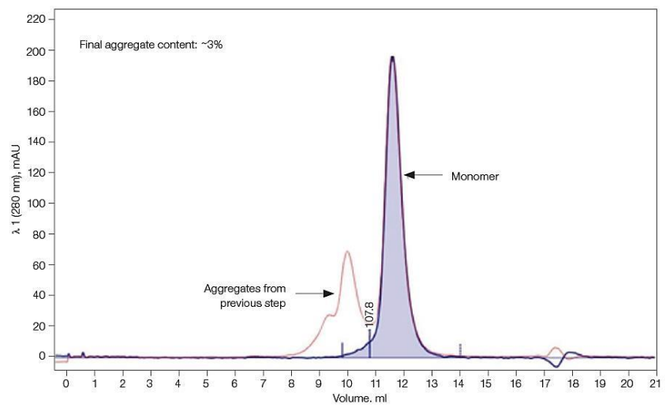

Figure 3. SEC analysis of mAb S eluted from CHT XT. SEC showing the level of mAb S aggregates in the eluate from CHT XT. OD 280 (-)

Sample	Monomer content (%)	Monomer recovery (%)
CHT XT load	66.0	---
CHT XT eluate	96.9	57.0

Table 1: Removal of mAb S aggregates by CHT XT

Clearance of host cell proteins, DNA, leached Protein A and endotoxins by CHT XT

To further assess the levels of other impurities after purification, various analytical assays as mentioned above were performed. The results are shown in Table 2. CHT XT showed removal of all four impurities by multiple folds.

Impurity	Load	Pool	% Reduction
HCP (ppm)	2635	229	91
dsDNA (ppm)	112	3	97
Leached rPA (ppm)	11	1	91
Endotoxin (LAL endotoxin assay) (EU/mg)	11	1	98
Endotoxin (EndoLISA assay) (EU/mg)	422	16	96

Table 2. Impurity clearance by CHT XT

Conclusion

This study shows that CHT XT is capable of minimizing the aggregate levels during mAb purification and can also remove other product and process related impurities to significantly low levels. This presents CHT XT as an exceptional tool for polish purification of monoclonal antibodies.

References

- Rey VM et al., Aggregates in monoclonal antibody manufacturing processes. Biotechnol Bioeng. 2011 Jul;108(7):1494-508.
- Bracewell et al., Protein A chromatography increases monoclonal antibody aggregation rate during subsequent low pH virus inactivation hold. J Chromatogr A. 2015 Oct 9; 1415: 83–90.
- Gagnon, P., Monoclonal antibody purification with hydroxyapatite. N Biotechnol. 2009 Jun;25(5):287-93.
- Gagnon, P et. al., A Ceramic Hydroxyapatite–Based Purification Platform. Simultaneous Removal of Leached Protein A, Aggregates, DNA, and Endotoxins from Mabs, 2006
- Xuemei He, 2015, Mixed-Mode Chromatography for mAb S Aggregate Removal. Comparison of CHT™ Ceramic Hydroxyapatite, Capto adhere and Capto adhere ImpRes.