Removal of Aggregate from an IgG_4 Product Using CHT™ Ceramic Hydroxyapatite Resin

Tech Note

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Process Separations

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Introduction

The presence of even trace amounts of aggregate in immunoglobulin biopharmaceuticals is of significant concern during all phases of drug development, from process design to quality control. The formation of aggregates can adversely impact process economics by causing decreased product yield, peak broadening (requiring the addition of polishing steps), and loss of activity (Gagnon 1996). Aggregate formation can also adversely affect product safety because it can cause complement activation or even anaphylaxis upon injection (Ritchie and Navolotskaia 1996, WHO Report 1995).

Most chromatographic techniques would be expected to be able to remove aggregated or multimeric species of immunoglobulin from the monomer. However, techniques such as ion exchange and hydrophobic interaction chromatography may also induce the formation of additional aggregate or multimer due to increased protein concentration or changes in salt concentration, pH, or both required for elution. Size exclusion chromatography does not suffer from these disadvantages, but does result in significant dilution of the product. If large quantities of product are required, large size exclusion columns, concentration steps, and support equipment will add considerably to production costs. In some cases, additional product loss due to denaturation may occur during dilution.

CHT Ceramic Hydroxyapatite exhibits electrostatic, repulsive, and coordinate covalent bond formation in interacting with protein species. We have explored its potential in removing aggregate from a purified IgG_4 biopharmaceutical known to contain aggregate, and report here on a simple and effective process to do so.

Results

Size exclusion chromatography analysis of the IgG_4 sample (derived from chromatogram a, Figure 1) indicated that it contained 86.6% monomer of 141.9 kD and 10.1% aggregate, which consisted of nearly equal amounts of 485.3 kD and >1.1 MD species. Possible degradation products with apparent molecular weights below 3,000 were also present (about 1.0% of total protein).

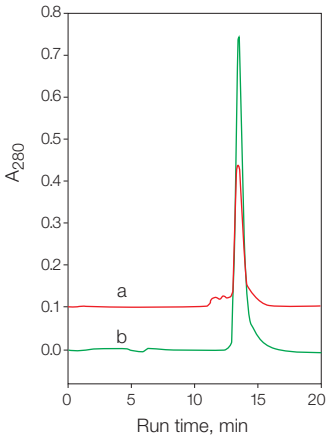

Fig. 1. Size exclusion chromatography analysis of IgG_4 samples. Chromatogram a (—), starting material; chromatogram b (—), main peak (Figure 2, peak a) from the CHT Column (scales are offset for comparison). Column, Bio-Sil® SEC 250-5, 7.8 x 300 mm (catalog #125-0062); buffer, 0.1 M sodium phosphate, pH 6.8, containing 0.15 M NaCl; flow rate, 1.0 ml/min. Molecular weights were calculated by calibration with standards (IgG, ovalbumin, myoglobin, vitamin B_{12}, catalog #151-1901)

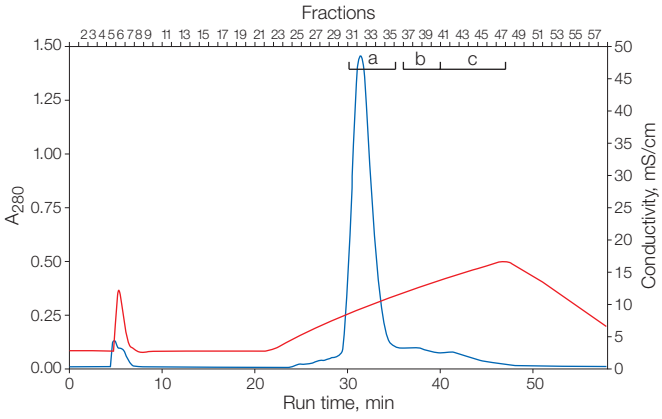

Fig. 2. CHT chromatography of IgG_4 sample containing aggregate. Chromatography was performed on a BioLogic DuoFlow™ 10 System (catalog #760-0037). Sample (12 mg) was loaded onto a 7 x 52 mm Bio-Scale™ MT Column (catalog #751-0081) containing CHT, 20 µm (catalog #158-2000) in 20 mM sodium phosphate, pH 7.0; elution was from 20 to 160 mM sodium phosphate in 20 column volumes. The flow rate was 1.5 ml/min, and the fraction size was 1.5 ml. Peaks were pooled as indicated in the figure: a, fractions 31–35; b, fractions 37–40; c, fractions 41–47. A_{280} (—); conductivity (—).

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The IgG_4 sample was applied to a CHT Column and eluted with a linear phosphate gradient. A single major peak, flanked by two or three broader minor peaks, was obtained (Figure 2).

The major peak from the CHT Column (peak a) was analyzed on a size exclusion column and was found to consist of only monomeric IgG_4 (Figure 1, chromatogram b). Spectrophotometric analysis at 280 nm indicated that it represented about 72.4% of the protein applied to the CHT Column. The major CHT column pools were also analyzed by nonreducing 5% SDS-PAGE (Figure 3).

A major band comigrating with the 150 kD standard was presumed to be IgG_4 monomer. Some nine or ten components with molecular weights greater than that of the IgG_4 monomer were apparent in the CHT column load (Figure 3, lane 2) and were presumed to be aggregates. The major CHT column peak (lane 3) appeared to be free of these aggregates and had a trace of a single lower molecular weight species, which may be the $F(ab')_2$ fragment. Lanes 4 and 5, which contained the later eluting minor peaks (b and c, respectively) from the CHT Column, contained an array of aggregates and some lower molecular weight species, which may be IgG_4 degradation products. A major band, presumed to be the IgG_4 light chain, ran slightly ahead of the 75 kD standard under these conditions. Some of the components observed in the IgG_4 fractions were also found in a commercially available human IgG preparation (lane 6).

A reducing 4–20% SDS gel was also run (Figure 4), which shows that some aggregated species were present under these conditions as well and may have arisen from covalent crosslinking. These components were also removed by CHT chromatography. The heavy and light chains migrated as expected in this gel.

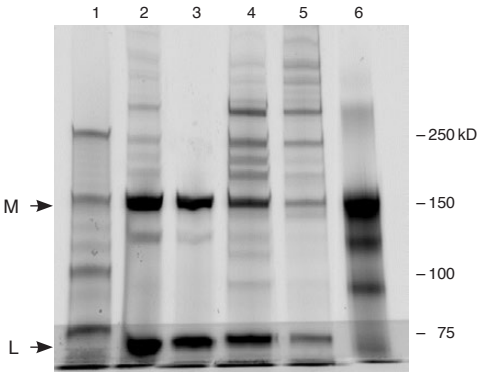

Fig. 3. Nonreducing SDS-PAGE analysis of CHT column pools. Lane 1, MW standards; lane 2, IgG_4 starting material; lane 3, main CHT column peak (peak a); lane 4, CHT peak b; lane 5, CHT peak c; lane 6, human IgG (Sigma). The gel was a 5% Ready Gel® Precast Gel (catalog #161-1213), and the stain was an experimental stain about 50-fold more sensitive than Coomassie Blue. Positions of the IgG_4 monomer (M) and light chain (L) are indicated by arrows.

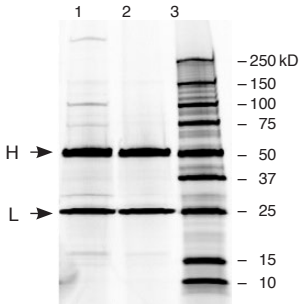

Fig. 4. Reducing SDS-PAGE analysis of CHT column pools. Lane 1, IgG_4 starting material; lane 2, main CHT column pool (peak a); lane 3, MW standards. The gel was a 4–20% Ready Gel Precast Gel (catalog #161-1159); the stain was the same as in Figure 3. Positions of the IgG_4 heavy (H) and light (L) chains are indicated by arrows.

Discussion

The results reported here indicate that CHT column chromatography is capable of removing essentially all of the aggregation and degradation products found in a human IgG_4 biopharmaceutical in a single step.

The aggregates found in this preparation did not appear to be simple multimers of the 141.9 kD IgG_4 monomer. The absence of simple multimers may have been due to the formation of various complexes with the monomer, $F(ab')_2$ fragment, and other degradation products. Both disulfide and nonreducible crosslinks (possibly enzyme-mediated) appeared to exist in this preparation.

The CHT Column performed well at a relatively high sample load (6 mg/ml) and with a relatively short bed height (52 mm). No reaggregation was evident in these experiments. The procedure is simple and should be easily scaled up. This suggests CHT chromatography may be useful for immunoglobulin polishing in numerous circumstances.

References

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