Chimeric IgG Monoclonal Antibody Purification: A Comparative Study Using CHT™ Ceramic Hydroxyapatite and CFT™ Ceramic Fluoroapatite Chromatographic Media

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Introduction

Apatite-based media have been shown to be effective chromatographic supports for monoclonal antibody (mAb) purification. CHT ceramic hydroxyapatite and CFT ceramic fluoroapatite media are multimodal chromatographic supports that interact with biomolecules through cation exchange via phosphate groups, through metal affinity via calcium atoms, or both. The two chromatographic media are different from typical polymer-based resins in that they are both the support matrix and the ligand. The protein accessible groups in CHT ceramic hydroxyapatite are Ca^{2+}, PO_4^{3-}, and OH; they are Ca^{2+}, PO_4^{3-}, and F in CFT ceramic fluoroapatite. Substitution is performed by chemical conversion of hydroxyapatite nanocrystals to fluoroapatite with a fluorine reagent.

This tech note describes the purification of a chimeric IgG mAb using a two-step purification platform consisting of UNOsphere SUPrA™ medium capture followed by a CHT ceramic hydroxyapatite or CFT ceramic fluoroapatite media polishing step. The results demonstrate that both CHT and CFT deliver highly purified chimeric IgG by removing leached protein A, host cell protein (HCP), DNA, and mAb aggregates.

Methods

Two-Step Purification of IgG

Chimeric IgG mAb was obtained from Chinese hamster ovary (CHO) cells at a starting titer of 1 mg/ml and purified in two steps. The capture step was performed using UNOsphere SUPrA affinity chromatographic medium, a protein A–based affinity support. The polishing step was performed using either ceramic hydroxyapatite or ceramic fluoroapatite, which are both multimodal apatite-based media.

Capture by UNOsphere SUPrA Affinity Medium

All mAb separations were conducted on a BioLogic DuoFlow™ workstation. Prepacked Bio-Scale™ Mini UNOsphere SUPrA medium cartridges (1 ml) were equilibrated with phosphate-buffered saline (PBS), pH 7.2, at 0.5 ml/min. A total of 40 ml of tissue culture supernatant was loaded evenly over four prepacked 1 ml cartridges and washed with 20 column volumes (CV) of PBS. The captured antibody was eluted with 20 CV of 0.1 M glycine, pH 3.0, at a flow rate of 1 ml/min. Pooled antibody fractions were incubated for 1 hr in elution buffer before neutralization to pH 7.0 with NaOH to simulate a viral inactivation step.

Polishing by CHT Ceramic Hydroxyapatite Chromatography

A 4 ml column (0.5 cm x 20 cm) packed with CHT ceramic hydroxyapatite Type II 40 µm particle size was equilibrated with 10 mM sodium phosphate, pH 7.0 (buffer A). Antibody pooled fractions (5 ml) from UNOsphere SUPrA affinity media chromatography were loaded onto the CHT column. Elution of the bound antibody was performed using a 40 CV NaCl gradient from 0–1 M in buffer A at 1 ml/min (305 cm/hr). The fractions collected were pooled and analyzed by size exclusion chromatography (SEC) on a Bio-Sil® SEC 250-5 column.

Polishing by CFT Ceramic Fluoroapatite Chromatography

A 4 ml column (0.5 cm x 20 cm) packed with CFT ceramic fluoroapatite Type II 40 µm particle size was equilibrated with of buffer A. The pooled fractions (5 ml) from UNOsphere SUPrA affinity media chromatography were loaded onto the column. Elution of the bound antibody was performed using a 40 CV salt gradient from 0–1 M NaCl in buffer A at 1 ml/min (305 cm/hr). The fractions collected were pooled and analyzed by SEC on a Bio-Sil SEC 250-5 column.

Analysis of Protein Fractions

Protein fractions were analyzed for leached protein A levels with a protein A ELISA kit (Cygnus Technologies). A Quant-iT PicoGreen dsDNA quantitation kit (Life Technologies) was used to determine DNA contamination. HCP analysis was performed using an HCP ELISA kit (Cygnus Technologies).

Results and Discussion

Capture of Chimeric IgG by UNOsphere SUPrA Affinity Medium

The analytical SEC chromatogram of the starting tissue culture supernatant is shown in Figure 1. The peak at 8.8 min was identified as IgG by SDS-PAGE analysis. A significant amount of material with smaller and larger molecular weights can be seen. Evaluation of the UNOsphere SUPrA medium affinity purification pool in Figure 2 indicates a high degree of IgG purity, with approximately 0.8% dimers and 0.4% aggregates.

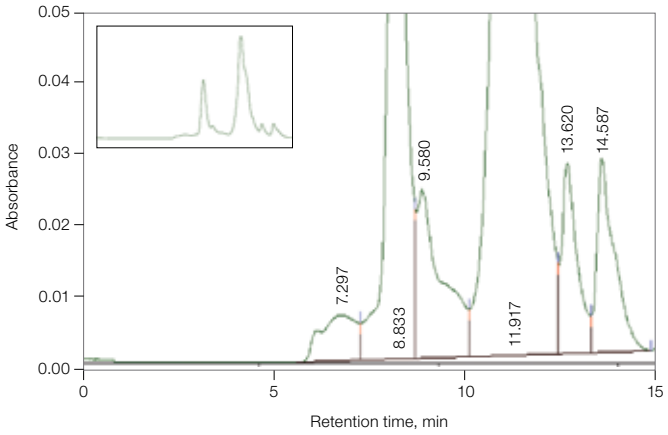

Fig. 1. Evaluation of starting tissue culture supernatant. A sample of chimeric IgG mAb supernatant derived from CHO cells was analyzed by SEC-HPLC to evaluate initial levels of contamination. The peak at 8.8 min represents the chimeric IgG. Inset shows zoomed out view of chromatogram.

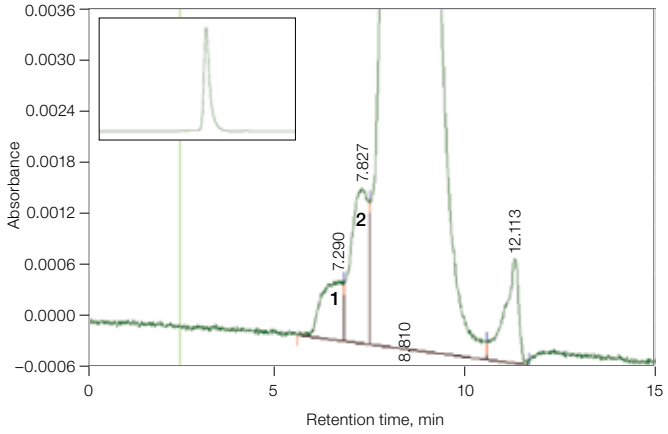

Fig. 2. Capture of chimeric IgG by UNOsphere SUPrA affinity medium. Elution fractions from the chromatographic capture step were analyzed by SEC-HPLC to evaluate levels of contamination. Peak 1 and peak 2 correspond to dimer contaminants and aggregate contaminants, respectively. Inset shows zoomed out view of chromatogram.

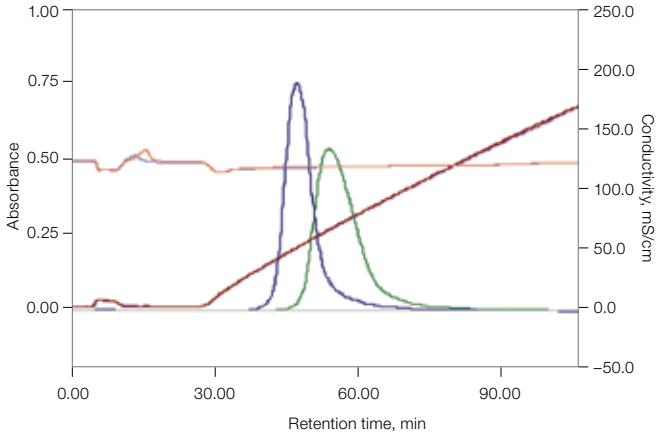

Fig. 3. Comparison of CFT and CHT media in the final polishing of chimeric IgG. The blue and green traces correspond to chimeric IgG undergoing final polishing by CFT media chromatography and by CHT media chromatography, respectively. The salt gradient profiles of the two chromatographic purifications are superimposed.

Contaminant Removal with CHT Support Followed by Polishing with CFT Support

Figure 3 shows the chromatograms of chimeric IgG purification on CHT and CFT columns. The antibody peak on the CHT column eluted later (~69 ms/cm) than did the antibody peak on the CFT column (~54 ms/cm), which indicates that this mAb has a higher affinity for CHT than for CFT.

Analysis of the CHT and CFT pools by SEC-HPLC (Figure 4A, 4B) shows no evidence (<0.03%) of dimers or aggregates (Figure 4). This represents higher purity than that of the supplied chimeric IgG standard (Table 1).

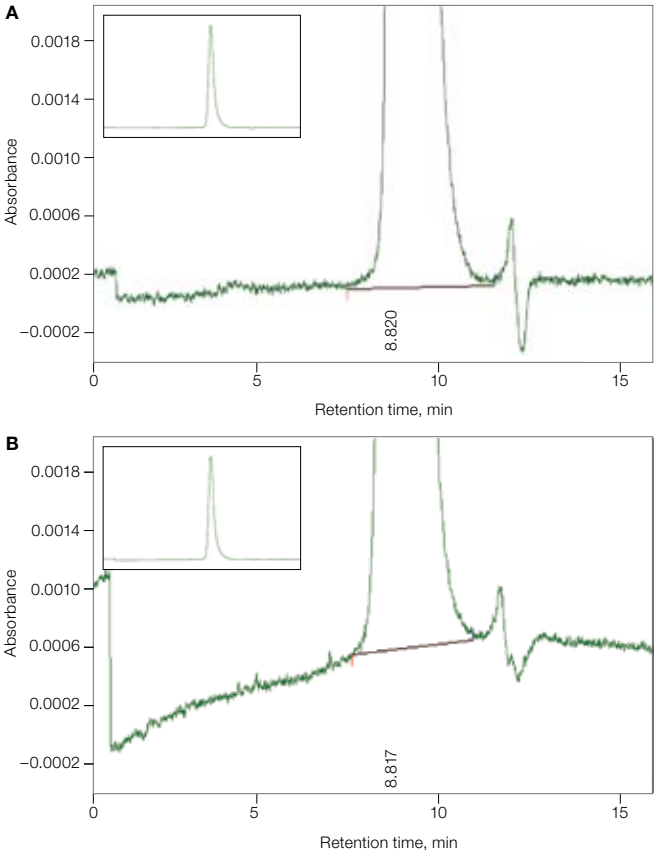

Fig. 4. Removal of dimer and aggregate impurities by CHT and CFT media chromatography. Elution fractions from the final polishing step using CHT (**A**) or CFT (**B**) were analyzed by SEC-HPLC to evaluate levels of contamination. Dimer and aggregate levels of less than 0.03% were detected. Insets show zoomed out view of chromatograms.

A summary of the purification levels of IgG across the complete two-step procedure is presented in Table 1. Both CHT and CFT chromatographic steps reduced protein A, HCP, and dimer/aggregate levels to below the limits of detection.

Table 1. Chimeric IgG impurity clearance data.

	IgG, mg/ml	Protein A, ppm	HCP, ng/ml	HCP reduction	DNA, ppm	DNA reduction	Dimer/ Aggregate, %
Tissue culture	1	NA	5.94×10^4	—	7.58×10^3	—	2.48
UNOsphere SUPrA medium pool	2.03	3.17×10^1	2.40×10^1	>3 log	9.57	>2 log	1.24
CHT pool	0.52	<0.96	<1.3	>4 log	5.90×10^1	>4 log	<0.03
CFT pool	0.66	<0.75	<1.1	>4 log	1.18	>3 log	<0.03
Chimeric IgG standard	10	10.98	139.3	—	0.87	—	2.47

Conclusion

This work demonstrates that both CHT ceramic hydroxyapatite and CFT ceramic fluoroapatite media are suitable as a second polishing step in a chimeric IgG monoclonal antibody purification process. Leached protein A and HCP were reduced to below the limits of detection with both matrices. The study also demonstrates that mAb purified on CHT and CFT media is of higher purity than that of commercial standard chimeric IgG.

Information in this tech note was current as of the date of the writing (2009) and not necessarily the date this version (Rev A, 2009) was published.

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