Extending Ceramic Hydroxyapatite (CHT) Column Life at Manufacturing Scale through Small-Scale Optimization

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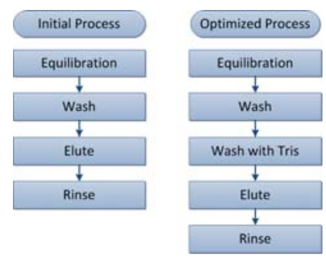
ABSTRACT

Ceramic Hydroxyapatite (CHT) chromatography is frequently utilized as the final polishing step in purifying monoclonal IgG antibodies to remove residual DNA, endotoxin, host cell proteins, protein A, viruses, and aggregates. Avid Bioservices, Inc. significantly increased productivity of a proprietary cell line which necessitated optimizing the downstream process. CHT resin damage was observed in initial scale up purification runs as a result of increased back pressure and Ca^{2+} depletion. Through our investigation and collaboration with the vendor, we observed Ca^{2+} depletion occurred as contaminants were removed once the number of cycles was increased to accommodate the increased antibody titer; once depleted, the resin lost the ability to remove contaminants. To extend the CHT column lifetime at manufacturing scale, a Tris wash step was implemented to maintain the pH from dropping prior to elution, as a decrease in pH can lead to CHT resin damage, hence increased back pressure. In addition, compensating for Ca^{2+} depletion after every cycle was also implemented. As a result of these minor modifications, the CHT column integrity was maintained and the CHT column life extended from 3-4 cycles to a minimum of 8 cycles.

SUMMARY

A scaled down model of the manufacturing process was utilized and determined that the original CHT chromatography process, developed for a less concentrated material, was unable to handle the increased product titer resulting from the greater productivity of the optimized upstream process.

We found that two simple modifications to the original CHT process would accommodate increased cycling of material over the column. A decrease in pH prior to elution and depletion of Ca^{2+} have been addressed by adding a second wash step and supplementing Ca^{2+} throughout the process. These two modifications were successfully implemented at manufacturing scaled and were found to increase column life while improving yield.



OPTIMIZATION 1: ADDRESS pH FLUCTUATION

Initial scale down studies were conducted with CHT chromatography conditions determined for the original, less concentrated material. This process involved one loading buffer wash prior to elution (Figure 1, gray arrow). When the more concentrated material from the optimized upstream process was applied to the CHT column, a decrease in pH was seen (Figure 1, red arrow). This led to an increase in back pressure, which would subsequently lead to CHT resin damage over time when material is cycled over the column.

A second wash step with Tris was included prior to elution (Figure 2, green arrow); the addition of this wash step minimized the decrease in pH (Figure 2, red arrow) and did not affect the elution of the material.

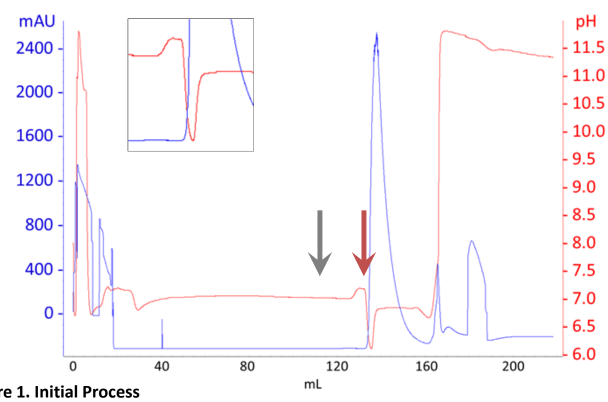


Figure 1. Initial Process
Gray arrow, loading buffer wash. Red arrow and inset image, pH drop.

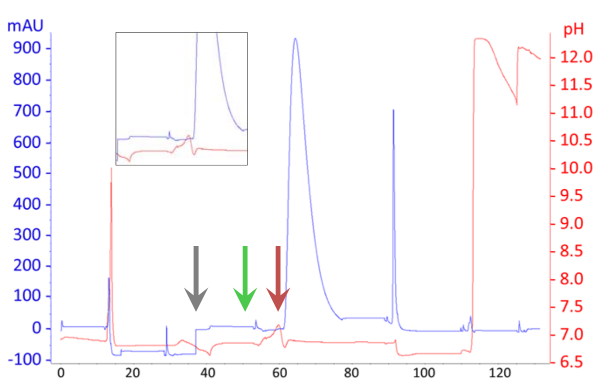


Figure 2. Wash Step Optimization
Gray arrow, loading buffer wash. Green arrow, Tris wash. Red arrow and inset image, minimized pH drop.

OPTIMIZATION 2: ADDRESS CA^{2+} DEPLETION

Samples from each step of the manufacturing CHT chromatography process were assayed for Ca^{2+} content using a colorimetric assay. Ca^{2+} was detected in all samples and thus found to be leaching from the resin throughout the process (Table 1).

Each sample was supplemented with Ca^{2+} before proceeding to the next step in a scaled down model and Ca^{2+} content determined. All samples had 0 ppm Ca^{2+}, indicating that supplementing prevented Ca^{2+} depletion.

Table 1. Ca^{2+} Content (ppm)

CHT Process Sample	Without Ca^{2+} Supplemented	Ca^{2+} Supplemented
Equilibration	16	0
Wash	10	0
Wash + Tris	6	0
Elute	9	0
Rinse	3	0
Sanitization	15	0

RESULTS AND RECOMMENDATIONS

The inclusion of a second wash step with Tris and Ca^{2+} supplementation has been tested in the scale down model numerous times to determine robustness.

As a result of these two minor modifications, the column life at the manufacturing scale increased from 3-4 cycles to at least 8 cycles. The benefits of this optimized CHT process include minimizing material costs along with decreasing the column preparation and handling time.

Although these modifications have been successfully implemented at the manufacturing scale, additional CHT optimization may be undertaken to further increase the column life by working to control and decrease back pressure through modulating flow rate..

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