Reducing residual DNA with no impact on critical quality attributes: A case study using modified Ceramic Hydroxyapatite (CHT) conditions to selectively remove host cell DNA contaminants

Payam Farahani, Misa Shaw, Jeanette Doerr, Michael Wang, Connie Chang

ABSTRACT

Food and Drug Administration (FDA) specifications for the highest degree of purity for biopharmaceuticals require final products to be essentially free of residual host cell DNA contaminants. Host cell DNA are an important potential impurities that must be monitored during development and production of biotherapeutic proteins. DNA contaminants that remain in products administered to patients could result in adverse immunological reactions during administration and continued treatment. Avid Bioservices successfully developed a Ceramic Hydroxyapatite (CHT) purification step that was able to selectively remove residual host cell DNA levels while not effecting critical quality attributes. This poster discusses how the high affinity of CHT for DNA at optimized pH and phosphate concentrations resulted in a single purification step therefore reducing the amount of residual DNA to acceptable levels for clinical development.

PROCEDURE/Results

Procedure

To test the DNA removal capability of ceramic hydroxyapatite (CHT), 20 mL sample of Clarified Harvest was adjusted to the desired phosphate and NaCl concentration. The sample was then pH adjusted and spiked with 1400 ng/mL of genomic DNA. The sample was then loaded on a pre-equilibrated CHT type II (40 µm) column. The flow through was collected and the column was chased with the equilibration buffer. The column was stripped with 500mM $NaPO^4$, pH 7.0. The samples (Harvest, spiked harvest, Flow through and strip) were submitted for residual DNA analysis, SDS-PAGE and BCA.

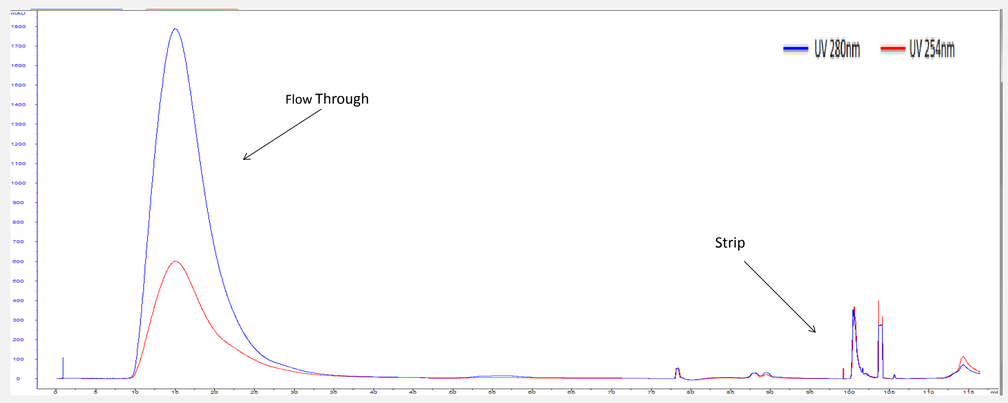


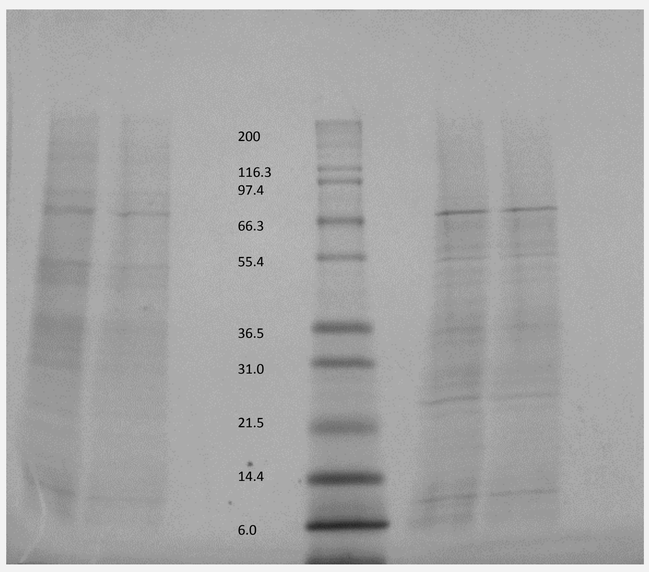
Figure 1. CHT chromatogram. Arrows indicate the location of flow through and strip peaks

Sample	A280	A260	Ratio (A_{280}:A_{260})
CHT Start	1.65	1.38	1.19
CHT Flow Through	0.406	0.32	1.3
CHT Strip	0.013	0.021	0.62

Table 1. Absorbance readings (A_{280} and A_{260}) and Ratio for each of the CHT samples.

Sample ID	Concentration	Total Volume	Total Protein	% Recovery
	(µg/mL protein)	(mL)	(mg)	
CHT Start	885.8	20	17.76	100%
CHT Flow Through	254.8	69	17.58	99%
CHT Strip	BDL	17	NA	NA

Table 2. Micro BCA results.



1. Starting Material
2. CHT Flow Through
3. CHT Strip
4. MW
5. Starting Material - R
6. CHT Flow Through - R
7. CHT Strip - R

Figure 2. SDS-PAGE analysis of CHT samples. Lanes 1, 2 and 3 are non-reduced. Lanes 6, 7 and 8 are reduced.

Sample ID	Concentration	Total Residual DNA Content	Fold Reduction	Results
	(mg/mL protein)	(pg/mL)	(vs. the previous step)	(ng DNA/mg protein)
CHT Start	0.885	1,400,610.00	NA	1,187.00
CHT Flow Through	0.26	20.8	67337.02	<LOQ
CHT Strip	0.01	133,579.20	NA	13,357.90

Table 3. Residual DNA results.

CONCLUSION

- Results from the A_{280} : A_{260} ratio suggested very good separation of the proteins from DNA in the flow-through mode
- The A_{280} : A_{260} ratio decreases to 1:1 in the strip, suggesting separation of DNA and Protein
- Micro-BCA analysis indicate a 99% recovery of proteins
- SDS-PAGE analysis shows no impact on the product profile
- Residual DNA analysis indicate a ~67,000 fold reduction in residual DNA contaminants in the flow through with the majority of the DNA being removed during the strip

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