Purification of Clinical Grade mAb using Hydrophobic Cation Exchange Chromatography

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Abstract

A three-step non-rProtein A capture process shows how the selectivity afforded by Nuvia™ cPrime™ can be used to clear a light chain contaminant that has a homologous sequence and similar pI to the target mAb monomer. The unique balance between the hydrophobic and charged character of Nuvia cPrime allows for a compact process with reduced buffer consumption and feed conditioning.

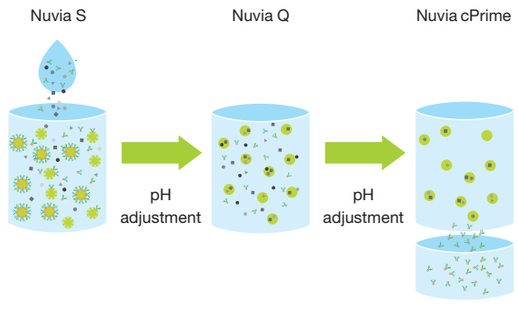
Introduction

Increases in fermentation volume and the protein mass produced have made the timely processing of harvested material extremely challenging. This is further compounded by the elevated levels of process- and/or product-related impurities resulting from prolonged fermentation and substantially higher cell density in the expression culture. Chromatographic resin with high capacity and improved chromatographic and operational performance offer the latest productivity tools to address downstream process challenges. We have employed two next-generation ultra-high capacity ion exchangers, Nuvia™ S and Nuvia™ Q Resin, and Nuvia cPrime Hydrophobic Cation Exchange Resin, to effectively purify a monoclonal antibody from Chinese hamster ovary (CHO) cell culture harvest. Our results demonstrate that this three-step nonaffinity workflow can effectively deliver highly purified monoclonal antibodies, by eliminating closely related fragment impurities, while maintaining a high level of recovery.

Materials and Methods

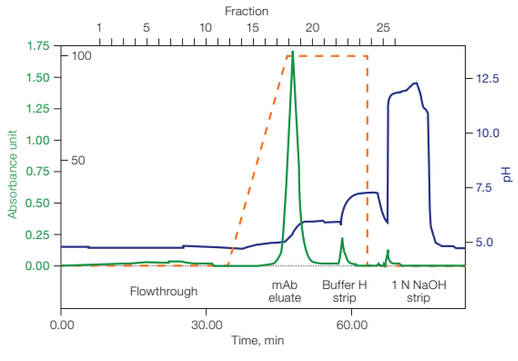
Monoclonal antibody mAb1 was produced in CHO cell culture. Protein fractions were analyzed by SDS-PAGE using linear gradient 4–20% Criterion™ Tris-HCl Gels (Bio-Rad Laboratories, Inc.) stained with Bio-Safe™ Coomassie Stain (Bio-Rad Laboratories, Inc.). Mass spectrometry analysis of gel-purified proteins was performed on an autoflex II MALDI-TOF Mass Spectrometer (Bruker Daltonics). Size exclusion (SEC) high performance liquid chromatography (HPLC) was carried out with a Bio-Sil® SEC 250 Column (Bio-Rad Laboratories, Inc.), and the content of high molecular weight antibody aggregates was quantified by integration using EZLogic™ Integration Software (Bio-Rad Laboratories, Inc.). The clearance of host cell proteins (HCP) and double-stranded DNA (dsDNA) was determined by a CHO-CM HCP ELISA Kit (Cygnus Technologies) and Quant-iT dsDNA High-Sensitivity Assay Kit (Life Technologies Corporation), respectively. mAb1 concentration was determined by UV absorption at 280 nm using a coefficient of 1.4 OD at 1 mg/ml.

Non-rProtein A three-step mAb purification process

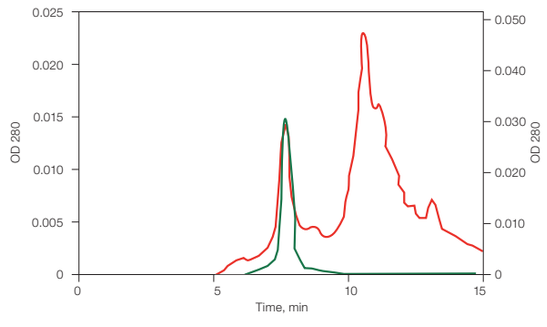


Scan to see more comparative data on our website.

A. Final polish in a three-step non-rProtein A process



B. SEC-HPLC comparison of cell culture supernatant (—) and purified mAb (—)



Conclusion

With mild acidic pH and modest concentration of NaCl, only the desired full-length antibody was bound by the column while the light chain fragment was eliminated in the flowthrough. We have also demonstrated that the proposed three-step purification process can effectively remove product and process impurities, thus yielding clinical grade mAb.

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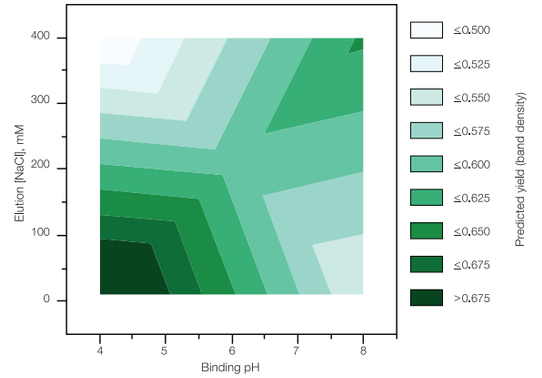
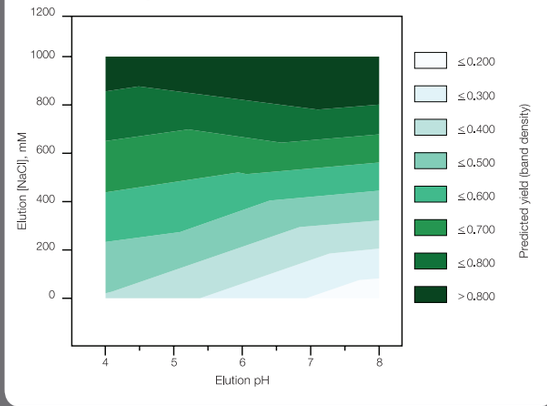
Conducting a simple DOE while elucidating protein-ligand interaction

A two-parameter, two-level study at pH 4–8 was conducted. Eleven experiments were carried out with [NaCl] of 10–400 mM binding or 10–1,000 mM elution.

Binding DOE study.



Elution DOE study.



DOE Results

Lysozyme from *Escherichia coli* lysate, with a pI of 9.5, is positively charged in the working pH range and interacts with Nuvia cPrime via electrostatic charge interaction. Elution can be achieved by an increase in salt concentration across the entire pH range, which also efficiently removes DNA and HCP.

Binding Conditions	DBC, 10%	Recovery	Purity
20 mM NaOAc 150 mM NaOAc, pH 4.0	59 mg/ml	94%	91%
20 mM NaOAc 150 mM NaOAc, pH 4.5	67 mg/ml	100%	92%