Effects of Buffer Composition on Protein Purification with a Hydrophobic Anion Exchange Resin, Nuvia aPrime 4A

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Abstract

Nuvia aPrime 4A is a newly launched hydrophobic anion exchange resin for the purification of therapeutic proteins. In the present study, we show that buffer components have significant effects on the purity and recovery of the target protein during Nuvia aPrime 4A chromatography. The use of sodium phosphate buffer led to high recovery of a test protein (pI ~9.2), while a Bis-Tris propane buffer (Good's buffer) offered high impurity clearance.

Introduction

The elimination of product- and process-related impurities is essential to the safety and efficacy of biopharmaceuticals. To achieve this goal, multiple separation steps are often required at the expense of target yield. This results in low process productivity and economy. We have recently developed a mixed-mode chromatography resin, Nuvia aPrime 4A, that has a positively charged hydrophobic functional ligand. The ligand density and hydrophobicity has been optimized for simultaneous maximal purity and recovery/yield. With Nuvia aPrime 4A, a broad range of impurities can be removed in a single chromatography step. This mixed-mode resin can tolerate the modest concentration of salts typically present in feedstocks, making it an effective tool for capturing target protein molecules from crude extracts as well as for polish purification.

Nuvia aPrime 4A can be operated in flow-through and bind-and-elute modes under gentle conditions (bulletin 7193). Here we discuss the possible orthogonal interactions between the Nuvia aPrime 4A ligand and incoming biomolecules and the rational design of chromatography conditions to be used with this resin. We also demonstrate the impact of buffer components on target recovery and impurity removal during Nuvia aPrime 4A chromatography. Results from the present study can be used as guidance for selecting appropriate buffer systems to achieve desired column chromatography performance.

Materials and Methods

Chromatography System

Chromatographic separations and analyses were performed on an NGC 10 Chromatography System (Bio-Rad Laboratories), which provides simultaneous monitoring of absorbance at 280 nm, conductivity, and pH and peak area quantitation for the determination of target protein aggregate content. ENrich SEC Columns (Bio-Rad) providing high capacity and reproducibility were used with the NGC Chromatography System.

Purification Condition Screening

A design of experiments (DOE) study was performed to obtain purification conditions for a test protein (isoelectric point (pI) ~9.2) using Nuvia aPrime 4A. The effects of sodium phosphate, Bis-Tris propane (Good's buffer), sodium chloride concentration, loading levels, buffer pH, and chromatography residence time on purification of the target were investigated. A two-level full factorial screening design suggested by JMP Software (SAS Institute) was employed (Table 1).

Mini Bio-Spin Columns, each containing 100 µl of Nuvia aPrime 4A Resin, were pre-equilibrated with the respective resin equilibration buffers. The partially purified test protein was exchanged into its respective test resin equilibration buffer using Econo-Pac 10DG Desalting Columns (Bio-Rad). Feedstock (1 ml, containing ~3 mg target protein) was loaded to each pre-equilibrated spin column and mixed with resin at room temperature for 3–7 min with agitation according to Table 1. Unbound materials were removed at the end of the incubation by spinning at 1,600 x g for 30 sec. The protein concentration of a sample was quantified by its absorbance reading (280 nm) with a NanoDrop Spectrophotometer (Thermo Fisher Scientific), using a coefficient of 1.4 OD at 1 mg/ml. The clearance of

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host cell proteins (HCPs) and double-stranded DNA (dsDNA) was determined using the CHO HCP ELISA Kit (Cygnus Technologies) and high-sensitivity Quant-iT dsDNA Assay Kit (Life Technologies Corporation), respectively. Endotoxin contamination in protein samples was quantitated using the EndoLISA Endotoxin Detection Assay (Hyglos GmbH). Analytical grade or higher chemicals were purchased from commercial vendors for preparation of the buffers. A standard least squares regression was employed to obtain the critical parameters for target purification using JMP Software.

Table 1. DOE setup for flow-through mode.

Experiment	Pattern	Resin Equilibration Buffer, pH	Resin Equilibration Buffer [NaCl], mM	Residence Time, min	Protein Load Level, mg/ml
1	−+−+	6	150	3	70
2	+++−	8	150	7	30
3	−+++	6	150	7	70
4	−−+−	6	0	7	30
5	+−++	8	0	7	70
6	+−−−	8	0	3	30
7	0	7	75	5	50
8	−+−−	6	150	3	30
9	−−−−	6	0	3	30
10	+−−+	8	0	3	70
11	++++	8	150	7	70
12	++−+	8	150	3	70
13	−−−+	6	0	3	70
14	++−−	8	150	3	30
15	−−++	6	0	7	70
16	+−+−	8	0	7	30
17	0	7	75	5	50
18	−++−	6	150	7	30

Note: Experiments 7 and 17 are the center points.

Results

Nuvia aPrime 4A is a mixed-mode anion exchange resin with a positively charged hydrophobic ligand (Figure 1). Theoretically, it would electrostatically repel basic biomolecules (pI >7) and attract (bind to) acidic biomolecules (pI <7). Our main goal was to study the effects of buffer species, sodium phosphate vs. Bis-Tris propane, for the polish purification of a basic protein with a predicted pI of ~9.2. The intact target protein molecules should be positively charged at near neutral pH (6–8) and would, therefore, appear in the flow-through fractions of Nuvia aPrime 4A Spin Columns in the DOE study. However, the aggregates of the target protein may be more hydrophobic due to partial denaturation. Other impurities in the feedstock included the negatively charged dsDNA, various proteins from the expression host cells, and the negatively charged but somewhat hydrophobic contaminant endotoxins. Their interactions with Nuvia aPrime 4A Resin are dependent on the conductivity, or sodium chloride concentration, and pH of the buffers. Two important considerations in process economics, residence time and protein load level, were also included as

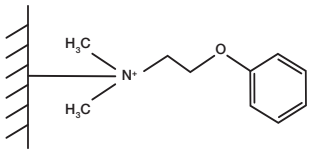

Fig. 1. Mixed-mode ligand for Nuvia aPrime 4A Resin.

parameters in our DOE investigation for their effects on target protein recovery and impurity clearance.

Purification with 10 mM Sodium Phosphate Buffer

A prediction profile was created for five responses — total protein recovery, monomer content, and contamination by endotoxins, dsDNA, and HCP — at different buffer pH, sodium chloride concentrations, residence times, and protein load levels. As shown in Figure 2, the buffer pH has significant influence on the final product purity and recovery; higher target protein monomer content and better dsDNA clearance can be achieved at higher pH, at the expense of target recovery in the flow-through fractions. At lower pH, endotoxin molecules are probably more hydrophobic due to the protonation of their phosphoryl groups and are therefore removed by the Nuvia aPrime 4A Columns more efficiently. The HCP content is independent of the buffer pH (Figure 2A).

The protein load level is another important parameter for flow-through purification of the test protein with Nuvia aPrime 4A. With increasing protein load, the recovery of target protein in flow-through fractions was improved but higher contamination by HCP was also observed, suggesting the saturation of protein binding sites on the resin. On the other hand, the relative contamination level of endotoxin, defined as EU/mg of

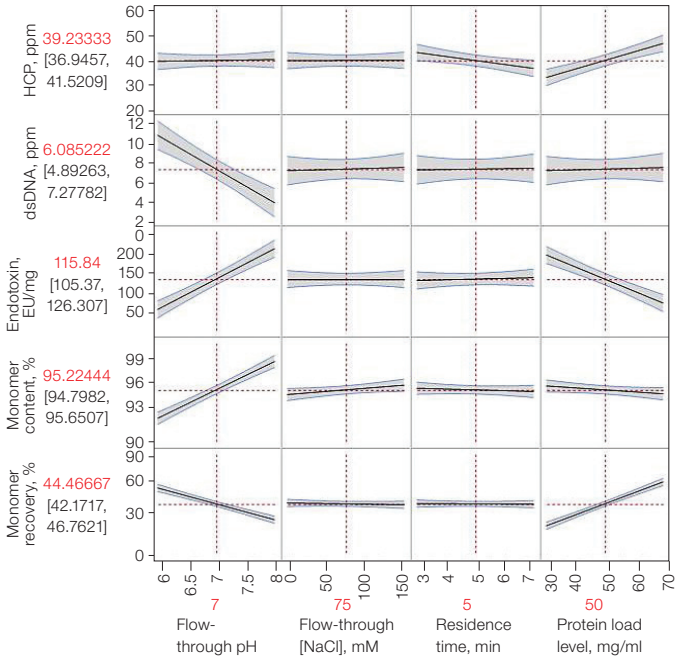

Fig. 2A. Prediction profile with the sodium phosphate buffer.

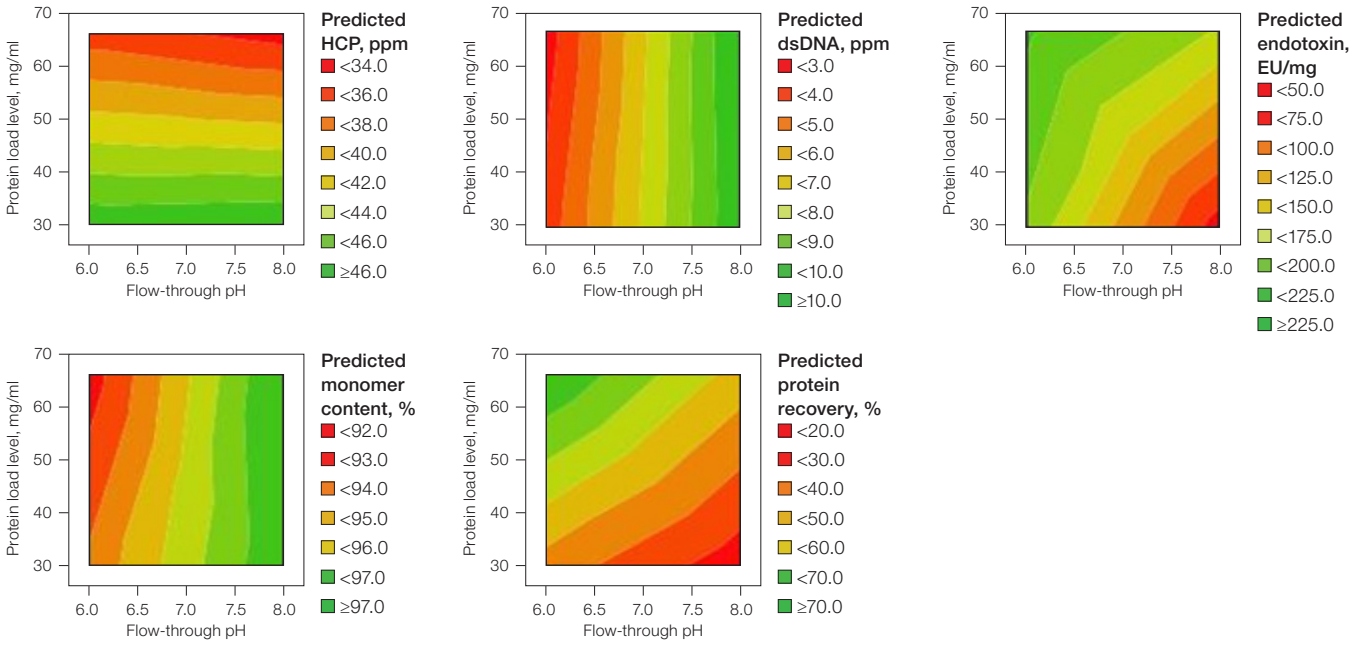

Fig. 2B. Predicted profile against pH variables in a phosphate buffer.

target protein in the flow-through fractions, was lower at higher pH when the phosphoryl groups on endotoxin molecules are fully ionized. The endotoxin molecules predominantly interact with the Nuvia aPrime 4A ligand via electrostatic interactions at higher pH. We observed no effect of sodium chloride concentration in the buffer. Results from the DOE screening are also illustrated as contour plots (Figure 2B).

Purification with 10 mM Bis-Tris Propane Buffer

When Good's buffer, Bis-Tris propane, was used as the purification buffer, the product purification and recovery profiles (Figure 3A) were similar to those observed for sodium phosphate–based buffers, except that the concentration of sodium chloride in the buffer showed an impact on the clearance of dsDNA, endotoxin, and target protein aggregates. Compared with sodium phosphate, the Bis-Tris propane buffer has a lower overall conductivity and is more hydrophobic, which probably reduces the solubility of dsDNAs in the buffer and promotes their absorption by Nuvia aPrime 4A. On the other hand, the solubility of endotoxins in the buffer appeared to be higher in the Bis-Tris propane buffer, resulting in a higher contamination level in the flow-through fractions. The aggregates of target protein, which is more hydrophobic than the monomers, also bound better in the presence of higher sodium chloride concentration. Sodium chloride had no effect when a sodium phosphate buffer was used. Results from the DOE screening are also illustrated as contour plots (Figure 3B).

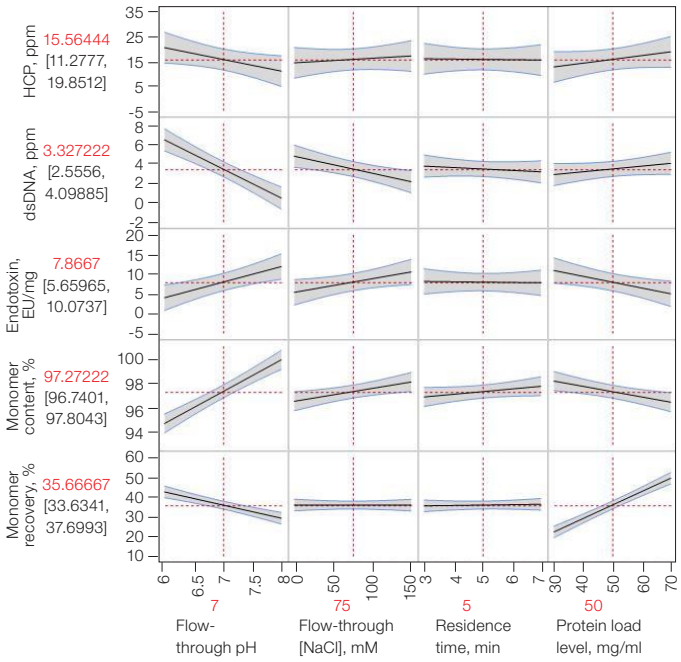

Fig. 3A. Prediction profile with the Bis-Tris propane buffer.

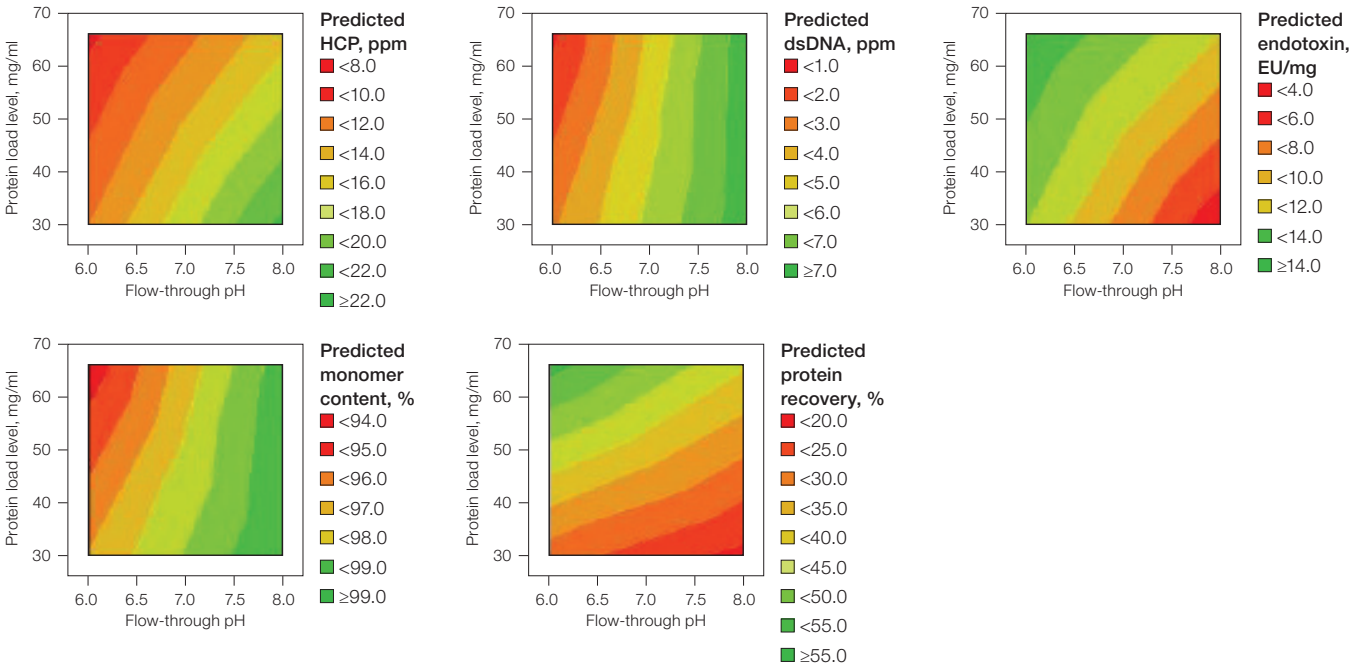

Fig. 3B. Predicted profile against Bis-Tris propane buffer and pH variables.

In general, purification performed with sodium phosphate buffer provided better target protein recovery, while Bis-Tris propane buffer offered better impurity clearance and higher product purity. However, sample residence time had no effect on purification performance in either case, confirming the notion that Nuvia aPrime 4A offers efficient mass transfer of biomolecules (bulletin 7193). As a result, increasing processing flow rate will not compromise its purification performance, making Nuvia aPrime 4A suitable for large-scale bioprocessing.

Conclusions

In summary, we have shown that buffer composition has a significant effect on target purity and recovery. A user should select an appropriate buffer based on the specific goals for a particular purification step, such as the feedstock contamination level, desired target recovery, and overall process economics. In addition, residence time has no effect on any of the parameters tested, indicating that Nuvia aPrime 4A Resin can be used efficiently at the fast flow rates required by today's high-throughput biologics manufacturing industry.

Visit bio-rad.com/Nuvia4ABuffer for more information.

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