Nuvia aPrime 4A: A hydrophobic anion exchange resin for refined selectivity and recovery

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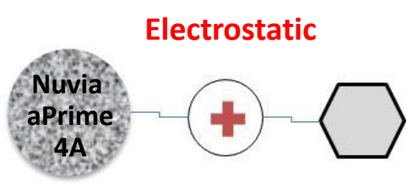
Introduction

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. We have recently developed a new mixed-mode chromatography media, Nuvia aPrime 4A, with a positively charged hydrophobic functional ligand. The ligand density and hydrophobicity has been optimized to facilitate the selective and reversible binding of target molecules for maximal purity and recovery, while a broad range of impurities can be removed in a single chromatography step. In this presentation, we will illustrate the possible orthogonal interactions between the Nuvia aPrime 4A ligand and the incoming biomolecules, and the rational design of chromatography conditions. Nuvia aPrime 4A can be operated in flow-through as well as bind-and-elute modes under gentle conditions. We will demonstrate case study examples using protein molecules with acidic or basic isoelectric point (pI) to showcase the advantages of using this new chromatography media to address protein purification challenges. Nuvia aPrime 4A can tolerate modest salt concentration typically present in feedstocks, making it an effective tool for capturing target protein molecules from crude extracts. Our data indicate that Nuvia aPrime 4A is more efficient in clearing a wide variety of contaminants from expression host cells than the traditional ion exchange chromatography resins.

Characteristics of Nuvia aPrime 4A

A hydrophobic anion exchange resin



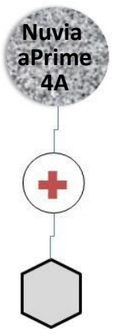
- **Modest salt tolerance**
  - **Minimum feed stream conditioning**
- **Good target recovery**
  - **Improved yield and process economics**
- **Unique selectivity**
  - **Effective impurity clearance**

Strategies for Nuvia aPrime 4A chromatography

Equilibration pH 7 ~ 8

High p*I* target protein
Weak binding

Electrostatic Repulsion



Electrostatic Interaction

Low p*I* target proteins
Strong binding

Flow-through mode

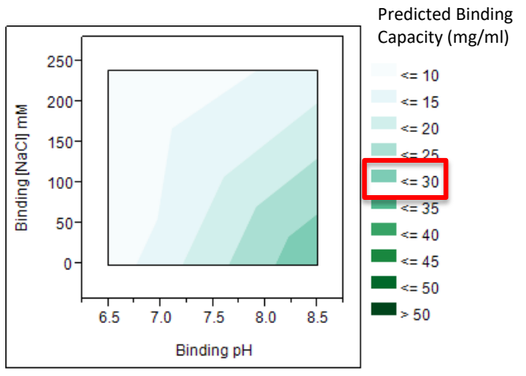
- Recover target in flow-through fraction
- Retain impurities on column
- Balance target recovery & impurity clearance

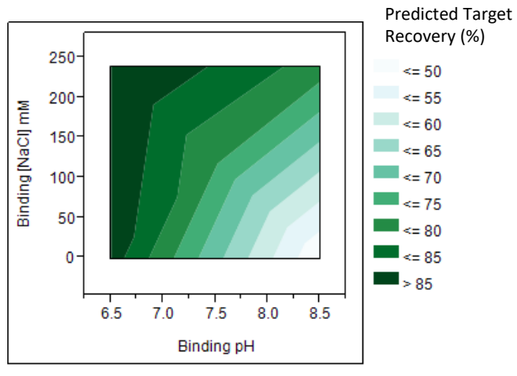
Bind-and-elute mode

- Maximize target binding
- Resolve target from impurities on column
- Protect target integrity with gentle purification condition

Nuvia aPrime 4A chromatography in flow-through mode: Rapid condition screening

Test protein p*I* ~ 8.45

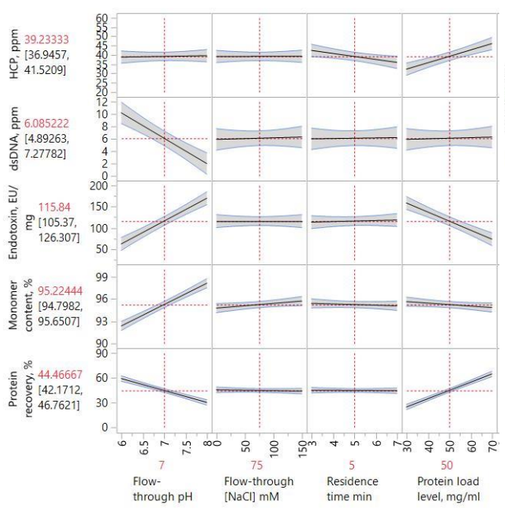


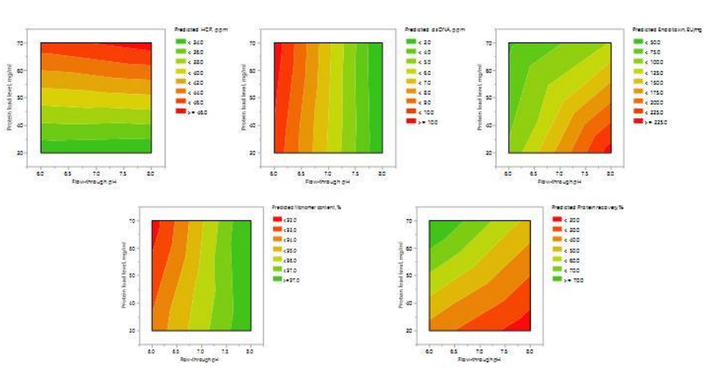


- Binding capacity is low under tested conditions
- Purification should Proceed with flow-through mode

- Target recovery is higher at low pH
- Recovery is improved by modest [NaCl]

Optimization 1: Purification with 10 mM sodium phosphate buffer

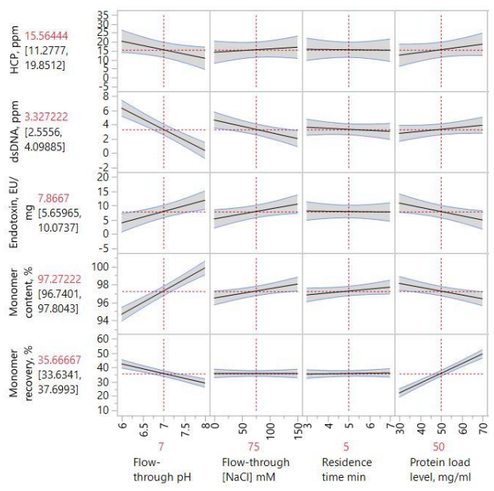


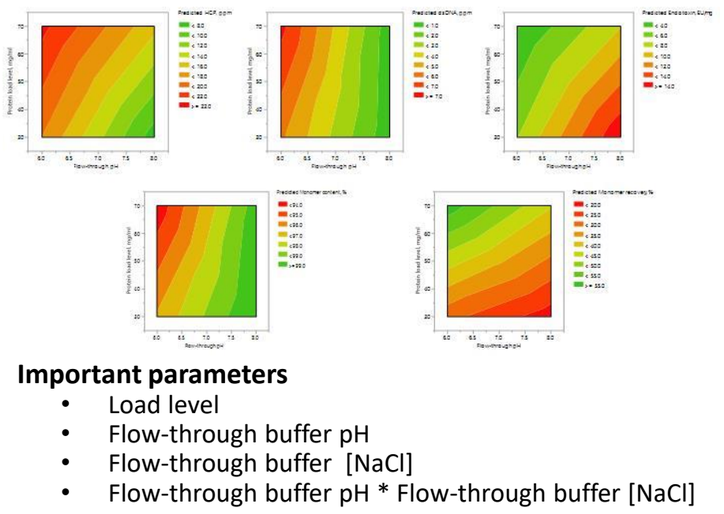


Important parameters

- Load level
- Flow-through buffer pH
- Flow-through buffer pH * Load level
- Flow-through buffer pH * Flow-through buffer [NaCl]

Optimization 2: Purification with 10 mM Bis-Tris propane buffer



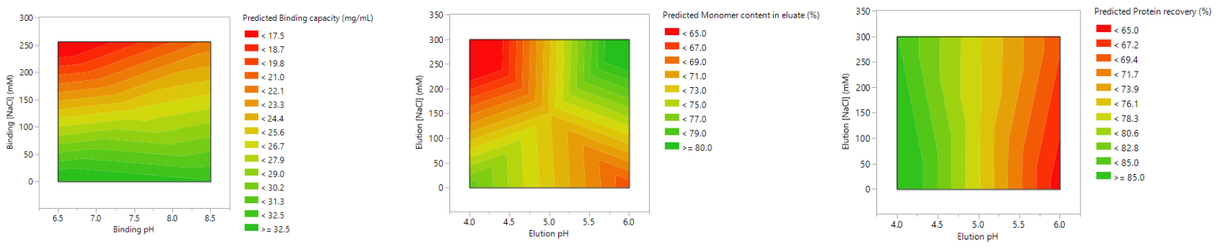


Important parameters

- Load level
- Flow-through buffer pH
- Flow-through buffer [NaCl]
- Flow-through buffer pH * Flow-through buffer [NaCl]

Chromatography method development for purification of an acidic protein on Nuvia aPrime 4A

Test protein p*I* ~ 6.9

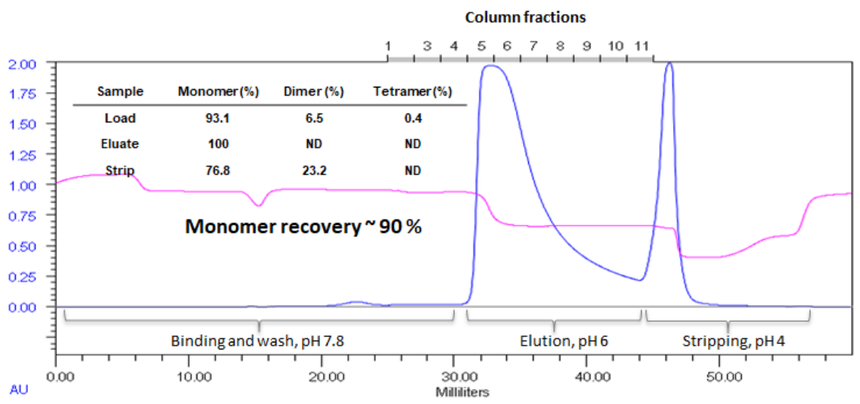


- Target protein is bound efficiently in the presence of modest concentration of NaCl
- Target protein recovery can be improved by more stringent elution at lower pH
- Target protein aggregate in eluate can be minimized by using buffer at pH >= 6

Nuvia aPrime 4 A Chromatography in bind-and-elute mode: Removal of product aggregates

Test protein p*I* ~ 6.9

10% BT DBC
> 50 mg/ml



Conclusions / Nuvia aPrime 4A Summary

- Ligands and biomolecules are engaged through charge and/or hydrophobic interactions
- Chromatography separation can be performed in flow-through or bind-and-elute mode
- Chromatography method development is straight forward with DoE
  - Buffer composition may have significant effects on target purity and recovery
    - For present test case ---
      - Sodium phosphate buffer provides better target recovery
      - Bis-Tris propane buffer offers better impurity clearance
      - The addition of Ca^{2+} may enhance the clearance of endotoxin at acidic pH
- Operation at fast flow rate is feasible as residence time has no effect on product purity or yield
- Resin is ready for large scale process manufacturing of biologics

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