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
- Electrostatic
- Hydrophobic

Strategies for Nuvia aPrime 4A chromatography

**Equilibration pH 7 ~ 8**

- High pI target protein
- Weak binding
- Electrostatic Repulsion

**Nuvia aPrime 4A**

- Unique selectivity
- Effective impurity clearance

**Low pI target proteins**

- Strong binding
- Electrostatic Interaction

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

Optimization 1: Purification with 10 mM sodium phosphate buffer

- Modest salt tolerance
- Minimum feed stream conditioning
- Good target recovery
- Improved yield and process economics

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 straightforward 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 Ca²⁺ 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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**Nuvia aPrime 4A chromatography in flow-through mode: Rapid condition screening**

- Test protein pI ~ 8.45
- Predicted Target Recovery (%)
- Predicted Binding Capacity (mg/ml)

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

- Test protein pI ~ 6.9
- 10% BT DBC × 50 mg/ml
- Column fractions

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

- 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

**Important parameters**

- Target protein binding
- Flow-through buffer pH
- Flow-through buffer [NaCl]
- Flow-through buffer pH * Flow-through buffer [NaCl]