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Salt-tolerant cation exchange HD-Sb hydrogel membrane: mAb purification performance in flowthrough mode

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mAb Purification Performance in Flowthrough Mode

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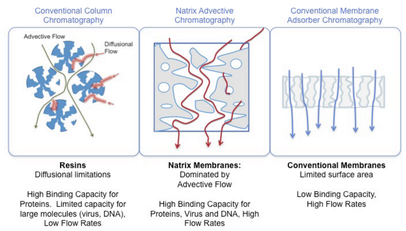
ABSTRACT

Development of Protein A based purification platforms have simplified the downstream processing of monoclonal antibodies (mAb), the largest component of biopharmaceuticals. The ever increasing titer of the cell culture process is putting more pressure on the downstream process to further increase its productivity. The classical Protein A based mAb purification platform consists of two polishing steps, bind and elute cation-exchanger and flowthrough anion-exchanger. Cation-exchange chromatography is very efficient at the separation of HCP, leached Protein A and product-related impurities such as aggregates and fragments in bind and elute mode. However, anion-exchange chromatography is a proven technology to remove DNA, viruses, endotoxins and HCP in flowthrough mode. This study compares bind and elute mAb purification performance with that in flowthrough mode for the Natrix HD-Sb hydrogel membrane.

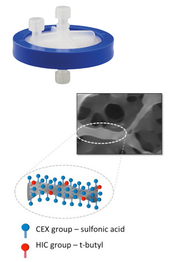
Natrix HD-Sb is a salt-tolerant strong cation-exchange membrane augmented with hydrophobic butyl groups. This study demonstrates the effectiveness of Natrix HD-Sb chemistry in removing aggregates and HCP from challenging feed at high load with significant improvement in productivity and simplicity. The flowthrough separation performance of Natrix HD-Sb around neutral pH will be highlighted to show the potential of having tandem polishing steps (cation-exchange ➞ anion-exchange) without needing any pH or conductivity adjustment. This tandem membrane approach has the potential for streamlining the downstream process for increased productivity & process efficiency.

INTRODUCTION

Natrix HD Hydrogel Advantages over Traditional Media



Natrix HD-Sb Hydrogel Membrane



- Natrix HD-Sb is a salt-tolerant cation exchange (CEX) membrane augmented with hydrophobic (HIC) groups designed to purify biomolecules like mAb in bind & elute or flowthrough mode
- High binding capacity, excellent yield and high aggregate clearance
- High salt tolerance for process robustness

NatriFlo HD-Q Membrane Adsorbers

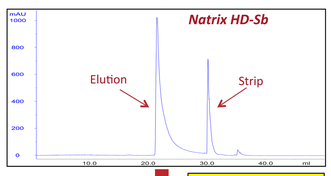
- NatriFlo HD-Q is a high capacity, high throughput strong anion exchange membrane adsorber designed to purify biomolecules such as mAbs in flowthrough mode
- Virus, DNA & HCP clearance with loads up to 10 kg of mAb/L membrane
- Process robustness, salt tolerance, buffer compatibility
- Low operating pressure

For more data, refer to: Y Hou et al (Merck & Co. Inc), "Advective Hydrogel Membrane Chromatography for Monoclonal Antibody Purification in Bioprocessing", Biotechnology Progress, 31 (2015), 974-982

CASE STUDY I: HD-Sb in Bind and Elute vs Flowthrough Mode

Option 1: HD-Sb in Bind and Elute mode

Protein A purified mAb1 → Feed: 1 mg/mL, Aggregate: 8.1%

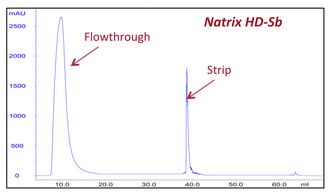


Load: 50 mg/mL
Flow rate: 10 MV/min
Equilibration: 45 mM Na Acetate + NaCl, pH 4.5 & 16 mS/cm
Elution: 20 mM Na phosphate + NaCl, pH 6.3 & 6 mS/cm

Monomer yield: 92%
Aggregate: Not detectable

Option 2: HD-Sb in Flowthrough mode

Protein A purified mAb1 → Feed: 10.4 mg/mL, Aggregate: 10.4%



Load: 300 mg/mL
Flow rate: 10 MV/min
Equilibration: 50 mM Na Acetate + NaCl, pH 5.5 & 10 mS/cm
Elution: 25 mM Tris + 1 M NaCl, pH 8.1

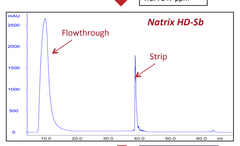
Monomer yield: 93%
Aggregate: 0.5%

Excellent aggregate clearance at high yield in both modes
Much higher load capacity in FT mode

CASE STUDY II (mAb1): High Aggregate Clearance in FT Mode

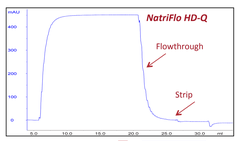
Option 1: Protein A followed by two flowthrough steps
HD-Sb (pH 5.5) --> HD-Q (pH 7.5)

Protein A purified mAb1 → Feed: 10.4 mg/mL, Aggregate: 10.4%, HCP: 247 ppm



Load: 300 mg/mL
Flow rate: 10 MV/min
Equilibration: 50 mM Na Acetate + NaCl, pH 5.5 & 10 mS/cm
Elution: 25 mM Tris + 1 M NaCl, pH 8.1

Monomer yield: 93%
Aggregate: 0.5%
HCP: 47 ppm

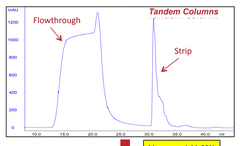


Load: 500 mg/mL
Flow rate: 10 MV/min
Equilibration: 25 mM Tris + NaCl, pH 7.5 & 5 mS/cm
Elution: 25 mM Tris + 1 M NaCl, pH 8.1

Yield: 95%
Aggregate: 0.5%
HCP: 5 ppm

Option 2: Protein A followed by one flowthrough step (tandem process)
[HD-Sb + HD-Q] (pH 7.5)

Protein A purified mAb1 → Feed: 3.5 mg/mL, Aggregate: 9.9%, HCP: 239 ppm



Load: 300 mg/mL
Flow rate: 10 MV/min
Equilibration: 20 mM Na phosphate, pH 7.5 & 2 mS/cm
Elution: 25 mM Tris + 1 M NaCl, pH 8.1

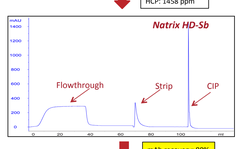
Monomer yield: 90%
Aggregate: 0.6%
HCP: 26 ppm

Tandem columns:
Two devices stacked in series
Top (HD-Sb); Bottom (HD-Q)

CASE STUDY III (mAb2): High HCP Clearance in FT Mode

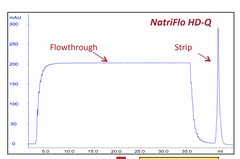
Option 1: Protein A followed by two flowthrough steps
HD-Sb (pH 5.5) --> HD-Q (pH 7.5)

Protein A purified mAb2 → Feed: 0.8 mg/mL, Aggregate: 1.7%, HCP: 1458 ppm



Load: 300 mg/mL
Flow rate: 10 MV/min
Equilibration: 50 mM Na Acetate + NaCl, pH 5.5 & 8.5 mS/cm
Elution: 50 mM Na Acetate + NaCl, pH 5.5 & 10.5 mS/cm

mAb recovery: 80%
Aggregate: 0.5%
HCP: 107 ppm

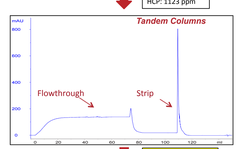


Load: 500 mg/mL
Flow rate: 10 MV/min
Equilibration: 25 mM Tris + NaCl, pH 7.5 & 8.5 mS/cm
Elution: 25 mM Tris + 1 M NaCl, pH 8.1

Monomer yield: 95%
Aggregate: 0.5%
HCP: 43 ppm

Option 2: Protein A followed by one flowthrough step (tandem process)
[HD-Sb + HD-Q] (pH 7.5)

Protein A purified mAb2 → Feed: 0.42 mg/mL, Aggregate: 1.9%, HCP: 1123 ppm



Load: 300 mg/mL
Flow rate: 10 MV/min
Equilibration: 20 mM Na phosphate, pH 7.5 & 4 mS/cm
Elution: 25 mM Tris + 1 M NaCl, pH 8.1

Monomer yield: 89%
Aggregate: 0.7%
HCP: 75 ppm

Tandem columns:
Two devices stacked in series
Top (HD-Sb); Bottom (HD-Q)

CONCLUSIONS

This work demonstrates that the Natrix HD-Sb cation-exchanger could be used efficiently in flowthrough mode with similar performance to bind and elute mode for HCP clearance and aggregates removal. This negative chromatography mode of operation, binding impurities instead of mAb, promotes increased loading capacity and enhances the utilization of the media. Moreover, HD-Sb used in FT does not require an elution step since impurities can be eliminated in the strip / regeneration step. When combined with the short residence time of Natrix hydrogel membranes (6 seconds or less), this mode achieves very high productivity for process development and production due to the reduced cycle time.

The success of the two-step flowthrough polishing process (HD-Sb followed by HD-Q) demonstrated here inspired the tandem approach. This novel approach enables continuous loading of a Protein A purified feed on the cation-exchanger and the anion-exchanger, with no intermediate pH or conductivity adjustment. Though further testing with increased loading capacity and additional mAbs with different properties are still required to determine broad applicability, this approach has the potential to contribute to realizing continuous processing for downstream mAb purification, which will improve productivity and process efficiency significantly.

ABOUT NATRIX SEPARATIONS

Natrix Separations is the developer and manufacturer of Natrix HD membrane technology, an advanced chromatography material that enables significant speed and capacity improvements for the capture and purification of biologics. Natrix products utilize established industry-standard chemistries in a single-use format to provide a low cost manufacturing advantage for drug developers. The Natrix team is comprised of industry leaders in downstream processing, as well as engineering, design, quality and manufacturing. Natrix is privately-held and based in Burlington, Ontario, Canada.

About Natrix Technology

Natrix HD Membranes offer a breakthrough in membrane architecture that will change downstream purification. With a three-dimensional macroporous hydrogel structure that provides a High Density of binding sites and rapid mass transfer, Natrix HD Membranes deliver binding capacity that exceeds resin-based columns with the fast flow rates typical of membrane adsorbers. Additionally, Natrix HD Membrane technology is highly versatile, and can be deployed in flow-through or bind-elute mode, with nearly any ion exchange, affinity or customized chemistry.

Natrix wishes to thank its industrial collaborators for testing NatriPur and NatriFlo products

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