NatriFlo™ HD-Q Membrane Adsorber:

A Study of The Design Space of NatriFlo HD-Q

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ABSTRACT

The current practices in downstream processing of biopharmaceuticals cannot keep pace with upstream production and therefore, more efficient purification platforms are needed. Manufacturers are looking towards continuous processing, and incorporating single-use products to decrease process times and costs.

To meet the modern challenges in purification, Natrix Separations has developed a new chromatographic technology, NatriFlo HD-Q adsorbers, for purifying and polishing protein feeds at all process scales. The Natrix HD-Q membrane is a macroporous hydrogel that provides a high density of binding sites and functions at fast flow rates, in a single-use format. Understanding the evolution of the performance across the design space of NatriFlo HD-Q is crucial for process developers trying to get the most out of this technology, hence this paper discusses the extensive capabilities of NatriFlo HD-Q products in over 500 different process combinations.

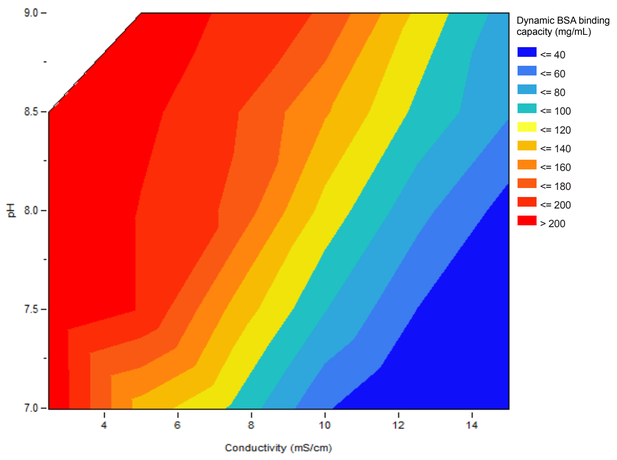
Tested using model BSA over a wide range of conductivity, pH, buffer species, flow rates and at different scales, Natrix HD-Q membrane demonstrated impressive binding capacity, as well as salt and pH tolerance. Some data generated with competing technologies is also discussed. With its extensive range of purification capabilities, NatriFlo HD-Q will add robustness, speed, flexibility and efficiency to the downstream processing of biomaterials.

INTRODUCTION

Monoclonal antibody (mAb) purification is one of the fastest growing areas in the biopharmaceutical industry. Platform approaches for downstream purification are often chosen because they optimize process development time and operational efficiency. However, it can be challenging to fit the molecule and its specific characteristics into the platform. Incorporating chromatography products that perform well in a wide range of operating conditions enables process flexibility and offers solutions at every stage of the downstream process. Moreover, such products offering tolerance to variability provide the advantage of built-in robustness.

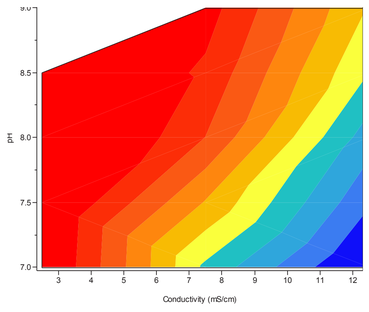
To demonstrate the superior performance and benefits of NatriFlo HD-Q in this context, an extensive study of the design space was conducted varying the critical process parameters traditionally associated with DSP: pH, conductivity, flow rate, buffer species, and product scale. Using BSA as a model protein for impurities clearance, NatriFlo HD-Q was also compared to competing technologies.

EXTENDED OPERATIONAL FLEXIBILITY

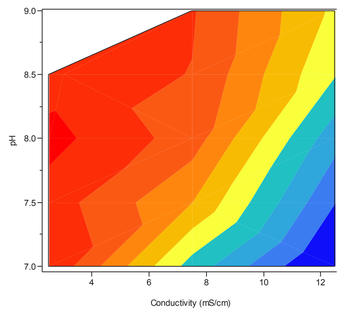


Dynamic binding capacity at 10% breakthrough was evaluated for 2g/L BSA in several process conditions including pH, conductivity, flow rate and buffer species using NatriFlo HD-Q Recon.

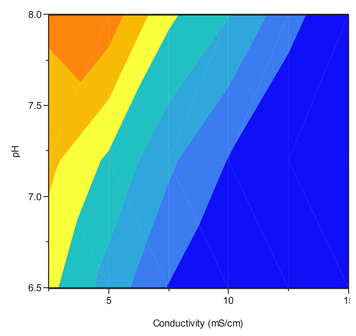
- 25 mM Tris buffer & 10MV/min: NatriFlo HD-Q has binding capacity greater than 50 across a wide design space of pH and conductivity.
  - Most products on the market don't have a binding capacity above 50 mg/mL, even in the most favorable conditions.



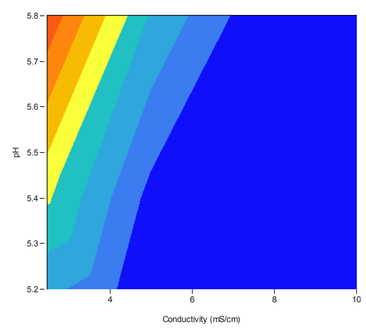
- 25 mM Tris buffer & 5 MV/min.



- 25 mM Tris buffer & 25 MV/min.
- NatriFlo HD-Q provides freedom to operate at very high flow rate (25MV/min) with no loss of performance when in challenging conditions (low pH & high conductivity), or to maximize the binding capacity when reducing the flow rate (5MV/min).



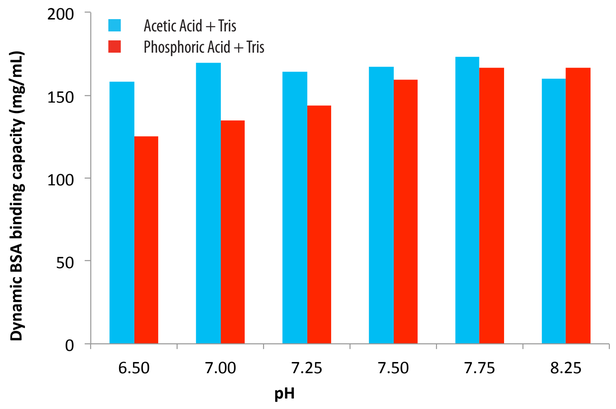
- 20 mM Phosphate buffer & 10 MV/min.



- 25 mM Acetate buffer & 10 MV/min.

Note: the pI of BSA is 4.7 and explains low performance at pH lower than 5.5

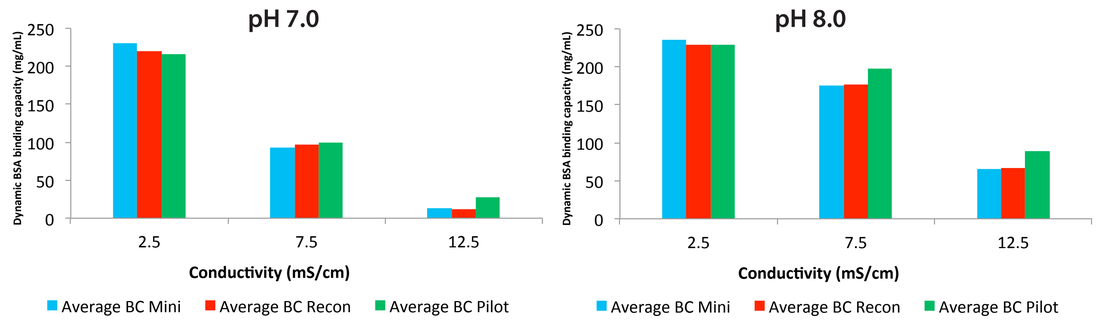
- Even in challenging conditions like anionic buffers at concentrations well known to dramatically reduce the binding capacity of other anion exchangers, NatriFlo HD-Q still performs very well, providing additional options for process developers.



- 2g/L BSA BSA using Protein A elution conditions: 50 mM acetic acid or 25 mM phosphoric acid, pH adjusted with 1M Tris.
- Protein A elution pool can be directly loaded on NatriFlo HD-Q membrane adsorber after pH adjustment due to its high binding capacity.
- This feature has the potential to enable continuous processing and major costs savings.

NatriFlo HD-Q maintains superior binding capacity over a wide range of operating conditions. This extended design space provides tolerance to process variability, creating robustness and reducing risks in GMP manufacturing.

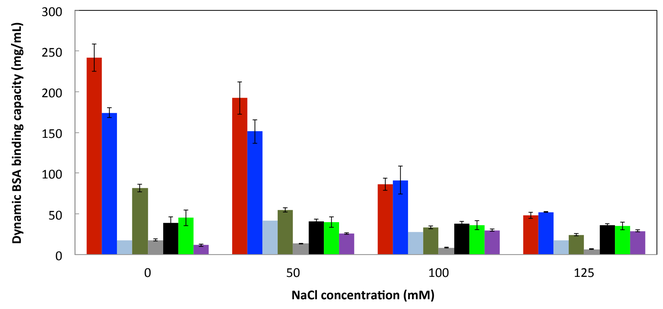
SCALABLE PERFORMANCE



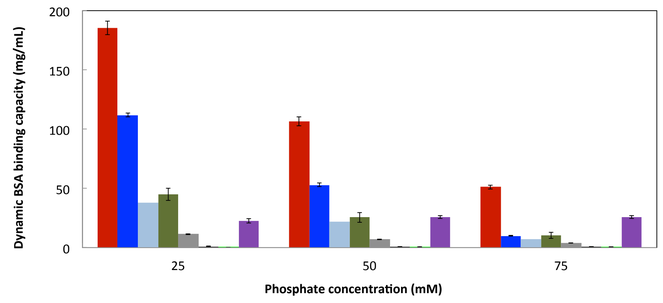
- Dynamic binding capacity at 10% breakthrough was evaluated for 2g/L BSA in several process conditions including pH, conductivity, flow rate and buffer species using NatriFlo HD-Q Recon mini (MV= 0.2mL), Recon (MV= 0.8mL) and Pilot (MV= 15mL).
- The binding capacity of Natrix HD-Q membrane remains constant at all scales in each process condition.

NatriFlo HD-Q adsorbers demonstrated consistent performance from lab to GMP scale for each condition evaluated.

COMPETITIVE ASSESSMENT



- NatriFlo HD-Q outperforms resins in terms of both flow rate and binding capacity, over wide salt conditions.
- NatriFlo HD-Q demonstrates better performance even compared to salt tolerant primary amine-based products



- NatriFlo HD-Q has significantly better phosphate tolerance than any other product evaluated.
- NatriFlo HD-Q membrane adsorber bound >50 mg/mL BSA at 10% breakthrough even from 75 mM phosphate, pH 8.0

- Feed sample:
  - 1 g/L BSA in 25 mM Tris + NaCl buffer, pH 8.0 (salt tolerance study)
  - 1 g/L BSA in phosphate buffer, pH 8.0 (phosphate tolerance study)
- **Device:** HD-Q Recon Mini & scale-down model of commercially available media
- Flow rates:
  - Resins: 1 CV/min
  - Membranes: 10 MV/min

LEGEND FOR BINDING CAPACITY CHARTS

- NatriFlo HD-Q
- Resin 1-Q
- Resin 2-Q
- Membrane 1-Q
- Membrane 2-Q
- Membrane 3-PA
- Membrane 4-PA
- Resin 3-MM

Compared to conventional membrane and resin products, NatriFlo HD-Q demonstrated remarkably better purification performance and throughput at high salt and phosphate conditions.

CONCLUSIONS

Together with single-use technologies, platform approaches for the purification process are keys to solving the DSP bottleneck in mAb production. With excellent salt and phosphate tolerance that outperforms competitors' products, NatriFlo HD-Q achieves outstanding purification performance at all scales despite changes in buffer conditions and flow rates.

NatriFlo HD-Q products provide superior flexibility for process development and built-in robustness for manufacturing. Incorporating NatriFlo HD-Q into purification platforms will eliminate additional investment in capital and operational resources for feed adjustment, leading to improved process economics without trade-offs in product quality.

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 forever change downstream purification. With a revolutionary 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 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.

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