NatriFlo™ HD-Q Membrane Adsorber:

A new single-use high-capacity polishing tool with excellent salt tolerance and process robustness for mAb purification

NATRIX SEPARATIONS (Burlington, ON, Canada): Dharmesh Kanani, Renaud Jacquemart, Navneet Sidhu, Annabel Shang, Jim Stout • *For more information, please contact Dharmesh Kanani, Natrix Separations, kanani@natrixseparations.com*

ABSTRACT

Strong anion exchange (Q) chromatography has become an industry standard in mAb production. It is a proven technology to remove DNA, viruses, endotoxins and acidic host cell proteins from process feed streams in flowthrough mode.

Recent trends show an increasing interest in downstream single-use technologies and flexible biomanufacturing due to advancement in cell culture technology and emergence of biosimilars. Traditional chromatography columns are slow, often oversized and not suitable for flexible biomanufacturing. Conventional membrane adsorbers cannot provide sufficient process robustness due to low binding capacity of membranes. These factors impose challenges on the design of purification schemes for manufacturing of biotherapeutics. However, the NatriFlo HD-Q membrane adsorbers overcome these limitations by combining high binding capacity and high flow rates to deliver best-in-class performance in single-use plug & flow format.

OBJECTIVES

This study compares dynamic BSA binding capacity of NatriFlo HD-Q with competing technologies to demonstrate performance in challenging process conditions (salt & phosphate background). It also demonstrates the effectiveness of NatriFlo HD-Q in removing process impurities (HCP, DNA & viruses) from industrial feed streams.

INTRODUCTION

Typical mAb Production Platform

Capture Chromatography	Intermediate Purification	Polish
• Protein A • Cation-Exchange	• Cation-Exchange • **Anion-Exchange** • Hydrophobic Interaction	• Cation-Exchange • **Anion-Exchange** • Hydrophobic Interaction

Anion Exchange (AEX): Process Requirements

- Better & robust separation performance with increased throughput and capacity in flowthrough mode
- Flexibility in process design (such as positioning of AEX step in the platform, salt tolerance and buffer compatibility)
- Risk-free, scalable & easy operation in single-use format

AEX: Pros/Cons of Existing Technologies

Column chromatography:

- Robust separation performance due to high media binding capacity but often ends up being oversized due to throughput limitation
- Relatively complex operation & not amicable to single-use format

Membrane chromatography:

- High throughput & good performance only when feed is relatively clean due to limited media binding capacity
- Lower process robustness and less design flexibility

NatriFlo HD-Q Membrane Adsorber Overview

NatriFlo HD-Q Family offers scalable polishing from R&D to cGMP manufacturing (Recon mini, Recon, Pilot & Process devices shown)

- NatriFlo HD-Q is a new high capacity, high throughput strong anion exchange membrane adsorber designed to purify biomolecules such as mAbs in flow-through mode
- Features advanced membrane material with a 3D macroporous hydrogel structure that provides High Density of binding sites and rapid mass transfer

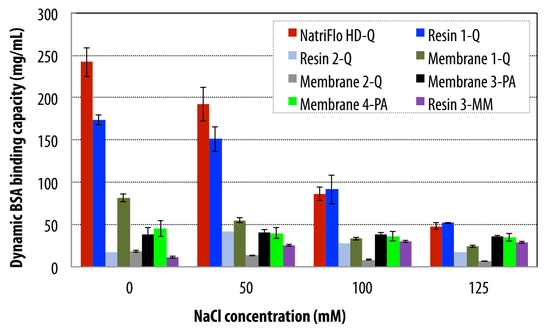
COMPETITIVE EVALUATION

Experimental

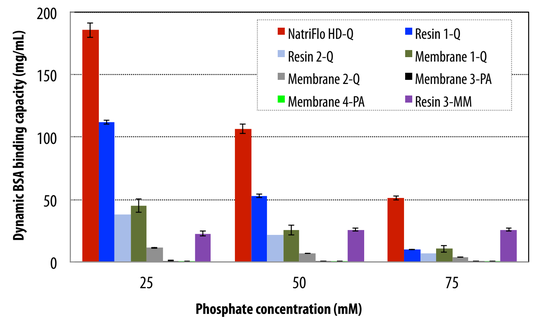
DEVICE	MATRIX	CHEMISTRY	MEDIA VOLUME (mL)	FLOW RATE (mL/min)
NatriFlo HD-Q Recon Mini	Porous Polyacrylamide	Q	0.20	2.0
Membrane 1-Q	Modified hydrophilic polyethersulfone	Q	0.18	1.8
Membrane 2-Q	Stabilized reinforced cellulose		0.08	0.8
Resin 1-Q	Highly cross-linked agarose		1.00	1.0
Resin 2-Q	6% Highly cross-linked agarose		1.00	1.0
Membrane 3-PA	Stabilized reinforced cellulose	Primary Amine	0.08	0.8
Membrane 4-PA	Ultra high MW polyethylene		0.08	0.8
Resin 3-MM	Highly cross-linked agarose	Mixed Mode	1.00	1.0

- All the devices were run as per the instructions provided by the vendors at recommended flow rate (i.e. membrane adsorbers were run at 10 MV/min whereas 1 mL prepacked columns were run at 1 CV/min)
- 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)
  - 0.1 g/L Herring testes DNA in 25 mM tris buffer, pH 8.0 (DNA binding study)

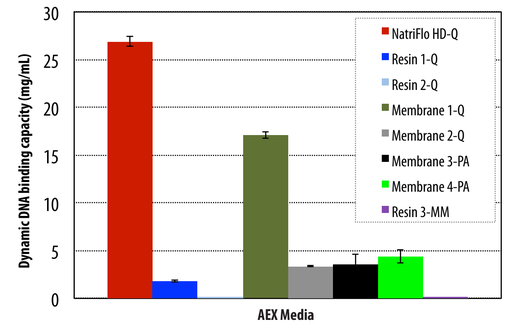
10% BT BSA Binding Capacity: *Salt Tolerance*



10% BT BSA Binding Capacity: *Phosphate Tolerance*



10% BT DNA Binding Capacity

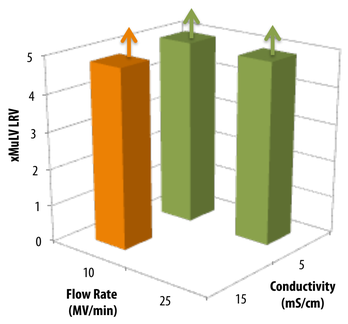


- The **superior dynamic binding capacity of NatriFlo HD-Q membrane adsorber over a wide range of operating conditions combined with impressive throughput** enable simple, robust, low cost polish operations in a single-use format.
- Resin 1-Q achieved good dynamic BSA binding capacity **but very low dynamic DNA binding capacity** at 1 minute of residence time .
- Most of the time, column chromatography **ends up being oversized due to throughput limitation**. For example, *a typical column would need > 66 hrs* just to load the feed sample having 5 g/L titer to 10 kg/L capacity at 2 minutes of residence time. On the other hand, *NatriFlo HD-Q would need only 3.3 hours to load the same feed* at 10 MV/min flow rate (or 1.3 hours at 25 MV/min flow rate).
- C**onventional membrane adsorbers lack the process robustness & design flexibility** due to poor binding capacity of media.

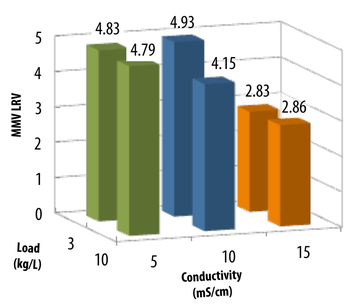
COLLABORATOR'S DATA

Virus/DNA Clearance & Process Robustness

xMuLV clearance at 10 kg/L load



MMV clearance at 10 MV/min flow rate



- **xMuLV:** Excellent clearance (≥ 4.8 LRV) from feed sample having 15 mS/cm conductivity at 10 kg/L load
- **MMV:** Excellent clearance (> 4 LRV) from feed sample having 10 mS/cm conductivity at 10 kg/L load
- **DNA:** Excellent clearance (> 2.96 LRV) from feed sample having 10 mS/cm conductivity at 10 kg/L load
  - 612 ppb to <0.7 ppb (as measured by qPCR assay)

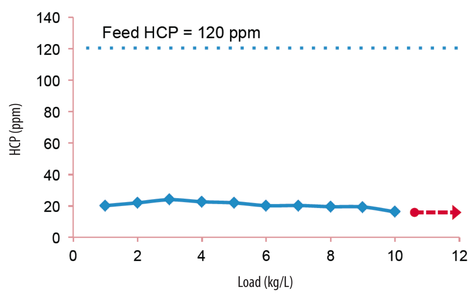
Acknowledgement*: Virus clearance study was conducted using client's material at Eurofins Lancaster Laboratories (Lancaster, PA)*

Trial at Collaborator 1:

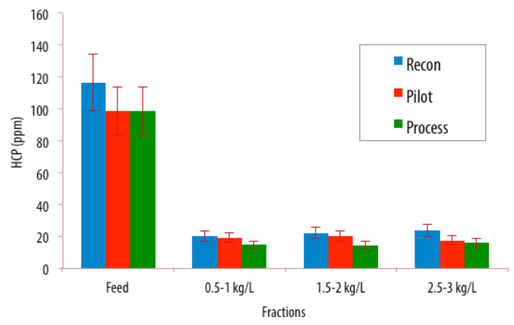
Sample: 15 g/L mAb (partially purified) in 25 mM Tris, pH 7.5 + NaCl

Note: *The up arrow (↑) indicates limit of detection; Combination of 25 MV/min and 15 mS/cm was not tested*

HCP Reduction Performance at Lab Scale for 10 kg/L Load



Linear Scalability



Trial at collaborator (Courtesy of Gallus BioPharmaceuticals)

- Excellent HCP clearance with no sign of increasing breakthrough even at 10 kg/L load (lab scale)
- Excellent & consistent HCP clearance from lab to process scale, even in phosphate buffer (Pilot & Process devices were tested only up to 3 kg/L load due to limited supply of protein A purified mAb)
- **Sample:** 8 g/L protein A purified mAb in **20 mM phosphate + 100 mM NaCl, pH 7**
- **Flow rate:** 10 MV/min (residence time – 6 seconds)

NATRIFLO HD-Q ADVANTAGES

NatriFlo HD-Q membrane adsorber versus column chromatography and other membrane adsorbers

	NatriFlo HD-Q Membrane Adsorbers	Columns	Membrane Adsorbers	Salt Tolerant Membrane Adsorbers
High-Throughput Media	+	–	+	+
High Media Binding Capacity	+	+	–	–
Process Robustness	+	+	–	–
Compact Footprint	+	–	+	+
Single-use Plug & Flow Format	+	–	+	+
Salt tolerance	+	+	–	+
Process Design Flexibility	+	+	–	–

CONCLUSIONS

NatriFlo HD-Q Benefits: *Reduced Risk, Greater Flexibility & Improved Economy*

1. **Improved salt tolerance and wider buffer compatibility**
   a. Superior dynamic BSA binding capacity in the presence of salt or phosphate background
2. **High loading capacity without compromising process robustness**
   a. Excellent virus (xMuLV & MMV) and DNA clearance from partially purified mAb sample for load as high as 10 kg/L over a broad design space
   b. Excellent HCP clearance with no sign of increasing breakthrough even at 10 kg/L load
3. **Simple scale-up**
   a. Excellent & consistent HCP clearance from lab to process scale
4. **Truly single-use plug & play format**
   a. No column packing, qualification, cleaning or validation
   b. No risk of cross-contamination

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.

Natrix wishes to thank Gallus BioPharmaceuticals and its other industrial collaborators for testing and evaluating NatriFlo products.

Natrix Separations, Inc. 5295 John Lucas Drive - Unit 6, Burlington, ON L7L 6A8 Canada 1-866-990-6972, info@natrixseparations.com

www.natrixseparations.com