Natrix®

## NatriFlo™ HD-Q Membrane Adsorber:

# *A high capacity, disposable chromatography solution with process flexibility to break the downstream bottleneck for mAb purification*

### INTRODUCTION

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

As the industry pursues an increasing interest in downstream single-use technologies and flexible biomanufacturing due to advancements in cell culture technology and the emergence of cost-sensitive biosimilars, conventional purification technologies present limitations.

Despite their high binding capacity, traditional resin-based chromatography columns are often oversized due to throughput limitation and are not suitable for flexible biomanufacturing. Conventional membrane adsorbers, meanwhile, offer faster throughput, but cannot provide sufficient process robustness due to the low binding capacity typical of most membranes. These factors impose challenges on the design of purification schemes for manufacturing of biotherapeutics.

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.

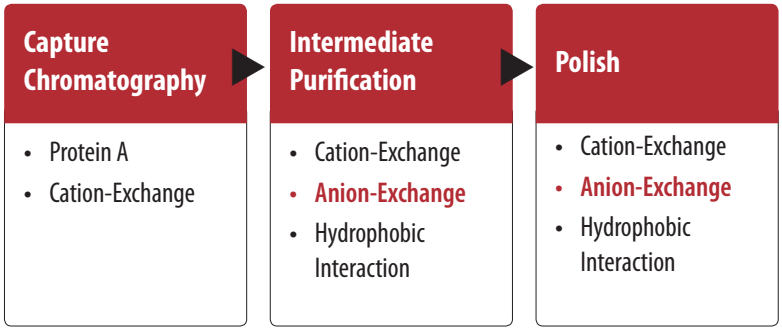
This paper examines the performance of NatriFlo HD-Q adsorbers in comparison to currently available column-based resins, AEX membranes and salt-tolerant primary amine membranes. Comparative data is provided using BSA & DNA as benchmark biomolecules. In addition, data from leading biopharmaceutical firms featuring actual process feed streams is provided to demonstrate NatriFlo HD-Q's performance to clear host cell proteins (HCP), viruses and DNA from mAb solution as well as scalability.

# OVERVIEW OF STUDY

This study compares dynamic BSA binding capacity of NatriFlo HD-Q membrane adsorbers 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.

A typical mAb production platform has three chromatographic steps: 1) Capture chromatography, 2) Intermediate purification and 3) Polish. Currently, Protein A is a popular choice as a capture chromatography due to its ability to deliver >90 - 95% pure product in a single step. After the capture chromatography step, mAb is further purified using two more chromatographic steps, one of which is anion exchange chromatography. Strong anion-exchange chromatography is the most popular choice as either 2nd or 3rd chromatographic step due to its proven performance record in large scale mAb purification. Below is the summary of most popular options for each chromatographic step in a typical mAb production scenario.

**Chromatography Steps in Typical mAb Production Platform**




When designing a modern mAb process with AEX as a purification step, the following objectives are typically desired:

- 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

However, current technologies are limited in their ability to meet all process requirements due to inherent technical limitations, as noted in the tables below.

**PROS/CONS OF EXISTING TECHNOLOGY**

| | PROS | CONS |
|---|---|---|
| COLUMN CHROMATOGRAPHY | • Robust separation performance due to high media binding capacity but often ends up being oversized due to throughput limitation<br>• Flexibility in process design | • Oversized column due to throughput limitation<br>• Cost of GMP facility is very high<br>• Relatively complex operation requiring column packing, qualification, cleaning or validation; risk of cross-contamination<br>• Limited salt tolerance<br>• Not suited to single-use format |
| MEMBRANE CHROMATOGRAPHY | • High throughput<br>• Good performance only when feed is relatively clean | • Limited binding capacity of membrane means:<br>  • Lower process robustness and thereby higher risk associated with product quality from one batch to another batch<br>  • Limited process design flexibility<br>• Typically needs at least 3X – 5X dilution to achieve kg/L loading |

**NatriFlo HD-Q Membrane Adsorber Overview**

NatriFlo HD-Q represent a new format for deploying high capacity, high throughput strong anion exchange chromatography to purify biomolecules such as mAbs in flow-through mode. The NatriFlo™ HD-Q Flow-Through Membrane Adsorber family is the first of several product lines to employ Natrix HD Membrane technology. 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**.

With the combination of speed and performance enabled by Natrix HD membrane technology, NatriFlo HD-Q membrane adsorbers are able to achieve:

- Best-in-class Viral Clearance, HCP and DNA removal, even with the most challenging feedstreams....with plenty of capacity to spare
- Salt and pH tolerance in an industry-standard strong anion exchange (Q) chemistry (even in phosphate buffer systems)
- Simple, low cost polish operations in a single-use format


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

Natrix is currently finalizing its NatriPur™ Bind-Elute product line for launch in 2014, and product development teams are working on several other chemistries to help solve additional demanding downstream challenges, with the vision of building a complete single-use downstream platform.

The performance advantages of Natrix HD membranes are derived from three primary functional features:

- High functional binding group density
- Readily accessible functional groups
- Multi-point molecular binding

# COMPETITIVE EVALUATION

To evaluate NatriFlo HD-Q membrane adsorbers, data was compared for a broad range of common chromatography devices (see table).

| 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 the recommended flow rate (i.e. membrane adsorbers were run at 10 MV/min whereas 1 mL prepacked columns were run at 1 CV/min).

The feed sample specifications for the various studies were as follows:

- **Salt Tolerance Study:** 1 g/L BSA in 25 mM Tris + NaCl buffer, pH 8.0
- **Phosphate Tolerance Study:** 1 g/L BSA in phosphate buffer, pH 8.0
- **DNA Binding Study:** 0.1 g/L Herring testes DNA in 25 mM Tris buffer, pH 8.0

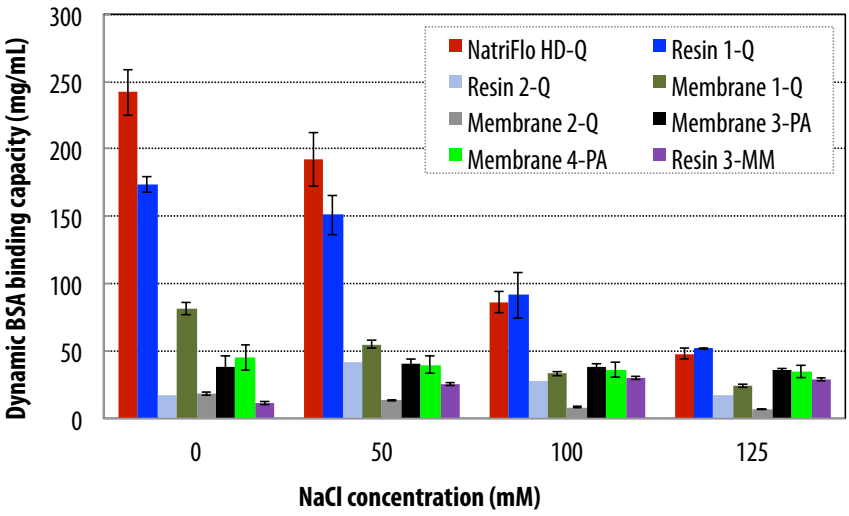
## 1. SALT TOLERANCE STUDY

To evaluate salt tolerance, a feed stream (1 g/L BSA in 25 mM Tris + NaCl buffer, pH 8.0) was processed at a variety of salt concentrations, ranging from 0 to 125 mM NaCl. It is evident from Figure 1 that NatriFlo HD-Q maintained superior dynamic binding capacity over a wide range of conductivities in comparison to all membrane adsorbers including salt-tolerant media. Resin 1–Q demonstrated similar binding capacity as NatriFlo HD-Q but suffers from throughput limitations inherent to column chromatography.

The typical residence time for any column at process scale is at least 2 minutes which puts constraint on the loading capacity of the column. For example, a typical column would need more than 66 hours just to load the feed sample having 5 g/L titer to 10 kg/L capacity at 2 minutes of residence time. By comparison, 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 for the same impurities clearance performance).

NatriFlo HD-Q offers superior dynamic binding capacity over a wide range of conductivity without throughput constraints. The improved salt tolerance of Natrix HD-Q membranes provides greater flexibility during process design. In addition, NatriFlo HD-Q is able to achieve high levels of performance without the need to dilute salt concentrations to reduced conductivity.

**Figure 1: 10% BT BSA Binding Capacity: Salt Tolerance**




**Data Table: Salt Tolerance Study**

Dynamic BSA Binding Capacity (mg/mL)

| | | NaCl (mM) | | | |
|---|---|---|---|---|---|
| **Device** | **Flow Rate** (MV/Min or CV/Min) | 0 mM | 50 mM | 100 mM | 125 mM |
| NatriFlo HD-Q | 10 | 242 | 192 | 86 | 48 |
| Resin 1-Q | 1 | 174 | 151 | 91 | 52 |
| Resin 2-Q | 1 | 17 | 41 | 27 | 18 |
| Membrane 1-Q | 10 | 82 | 55 | 33 | 24 |
| Membrane 2-Q | 10 | 18 | 13 | 8 | 7 |
| Membrane 3-PA | 10 | 39 | 41 | 38 | 36 |
| Membrane 4-PA | 10 | 45 | 40 | 36 | 35 |
| Resin 3-MM | 1 | 11 | 25 | 30 | 29 |

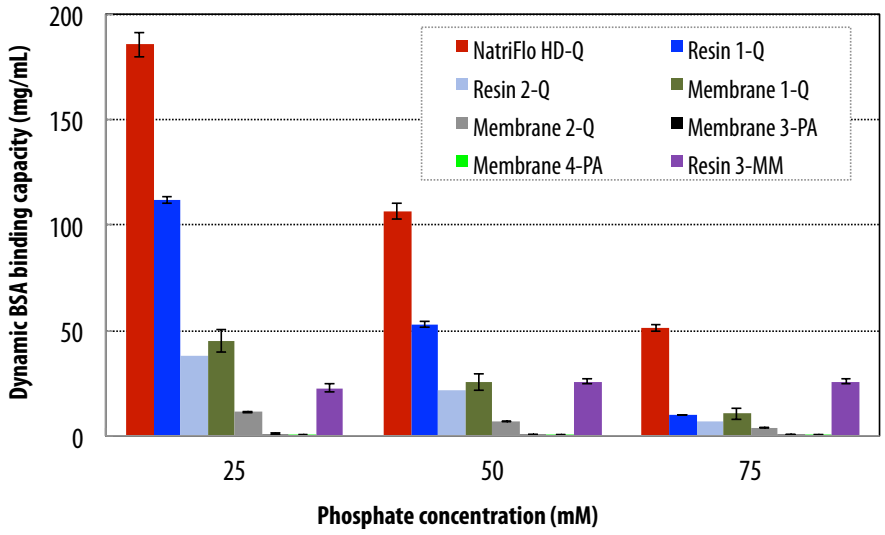
## 2. PHOSPHATE TOLERANCE STUDY

Phosphate is one of the most popular and widely used buffers in the pH range of 5.8 to 8.0. Natrix HD-Q membrane can tolerate the presence of phosphate ions unlike other salt tolerant anion exchange membrane adsorbers, which can be used only with monovalent buffers. Figure 2 compares phosphate tolerance of the NatriFlo HD-Q membrane with competing technologies. All the competing devices were run as per the instructions provided by the vendors. All the membrane adsorbers were run at 10 MV/min flow rate whereas 1 mL prepacked columns were run at 1 CV/min as recommended by the supplier. The feed sample (1 g/L BSA, pH 8.0) was prepared in phosphate buffers having concentration from 25 to 75 mM.

Figure 2 clearly shows superior dynamic binding capacity of NatriFlo HD-Q over competitive technologies in phosphate buffer. The salt-tolerant membrane with primary amine based chemistry did not bind BSA from phosphate buffer whereas Natrix HD-Q membrane achieved dynamic BSA binding capacity as high as 186 mg/mL at 10% breakthrough in the presence of 25 mM phosphate at pH 8.

Overall, NatriFlo HD-Q achieved more than 50% higher dynamic binding capacity compared to the next best alternative, column chromatography. This advantage was most pronounced at higher phosphate concentration.

**Figure 2: 10% BT BSA Binding Capacity: Phosphate Tolerance**




**Data Table: Phosphate Tolerance Study**

Dynamic BSA Binding Capacity (mg/mL)

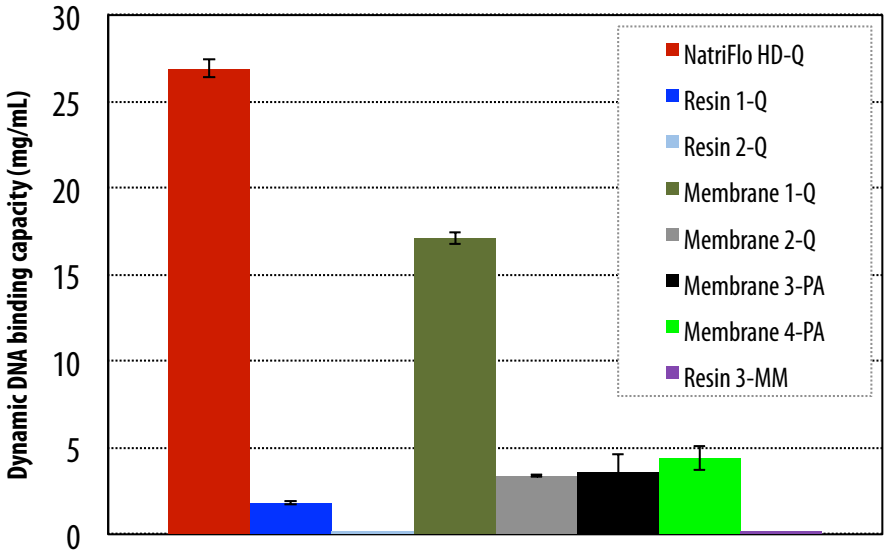
| | | Phosphate (mM) | | |
|---|---|---|---|---|
| **Device** | **Flow Rate** (MV/Min or CV/Min) | 25 mM | 50 mM | 75 mM |
| NatriFlo HD-Q | 10 | 186 | 107 | 51 |
| Resin 1-Q | 1 | 112 | 53 | 10 |
| Resin 2-Q | 1 | 38 | 22 | 7 |
| Membrane 1-Q | 10 | 45 | 26 | 10 |
| Membrane 2-Q | 10 | 11 | 7 | 4 |
| Membrane 3-PA | 10 | 1 | 1 | 1 |
| Membrane 4-PA | 10 | 0 | 0 | 0 |
| Resin 3-MM | 1 | 23 | 26 | 26 |

## 3. DNA BINDING CAPACITY STUDY

Dynamic DNA binding capacity was tested by processing a feed stream (0.1 g/L Herring testes DNA in 25 mM Tris buffer, pH 8.0) through the various devices. NatriFlo HD-Q achieved an average DNA dynamic binding capacity of 27 mg/mL, 59% higher than the next closest competitor (Membrane 1-Q at 17 mg/mL). None of the other devices exceeded 5 mg/mL.

Only Natrix HD-Q membrane achieved excellent binding capacity for both protein and DNA without throughput limitation. This is due to the easily accessible high ligand density in Natrix HD membrane.

**Figure 3:** ***10% BT DNA*** **Binding Capacity**




**Data Table**: **Dynamic DNA Binding Capacity Study**

Dynamic DNA Binding Capacity (mg/mL)

| **Device** | **Flow Rate** (MV/Min or CV/Min) | **Avg. DBC** (mg/mL) |
|---|---|---|
| NatriFlo HD-Q | 10 | 26.9 |
| Resin 1-Q | 1 | 1.8 |
| Resin 2-Q | 1 | 0.1 |
| Membrane 1-Q | 10 | 17.1 |
| Membrane 2-Q | 10 | 3.4 |
| Membrane 3-PA | 10 | 3.6 |
| Membrane 4-PA | 10 | 4.4 |

## SUMMARY FINDINGS

- 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 at process scale due to throughput limitation even though resins offer good protein binding capacity.
- Conventional membrane adsorbers lack the process robustness & design flexibility due to poor binding capacity of media.

# COLLABORATOR DATA

Competitive data provided above clearly demonstrate the competitive edge of NatriFlo HD-Q membrane adsorbers for the purification of biomolecules. Several major biopharmaceutical firms (including CMOs) have evaluated the performance of NatriFlo HD-Q membrane adsorbers in their existing mAb purification platform. The impurity (virus, HCP & DNA) clearance and process scalability data provided below are the representative performance data based on their actual proprietary antibody feed streams.

## Collaborator 1: Viral and DNA Clearance

Collaborator 1 was interested in single-use anion exchange chromatography which can reduce DNA levels down to <10 ppb in their mAb feedstream while providing > 4 LRV clearance of two model viruses, xenotropic murine leukemia virus (xMuLV, retrovirus, enveloped, ssRNA, 80-120 nm) and murine minute virus (MMV, parvovirus, non-enveloped, ssDNA, 18-26 nm). The study was conducted at viral testing facility (Lancaster Laboratories, Lancaster, PA).

Quite often, the loading capacity of anion exchange step is dictated by virus breakthrough. In order to understand the design space for virus clearance, the study was conducted over a wide range of conductivity (5 - 15 mS/cm) and load (up to 10 kg of mAb/L of membrane). The feed sample was diafiltered in 25 mM Tris + NaCl buffer at pH 7.5 and has mAb titer 15 g/L with 1.3% aggregates, 84 ppm HCP and 83 ppb DNA. The feed sample was appropriately spiked with virus or DNA (CHO genome DNA) just before the experiment to understand virus and DNA clearance.

**SUMMARY**

The Natrix HD-Q membrane achieved excellent clearance (≥ 4.8 LRV) of xMuLV virus from partially purified mAb feed over a wide conductivity (5 – 15 mS/cm) and flow rate (10 – 25 MV/min) at 10 kg/L load.

- No xMuLV breakthrough was detected in the flow through product for load capacity as high as 10 kg/L and flow rate being either 10 or 25 MV/min. The processing time for feed with 15 g/L titer at 25 MV/min is less than an hour with load capacity being 10 kg mAb/L membrane
- Flow independent clearance of xMuLV (size: 80 – 120 nm) demonstrates a convection dominant mass transport phenomenon in Natrix hydrogel technology

The Natrix HD-Q membrane achieved excellent clearance (> 4 LRV) of MMV virus from partially purified mAb feed with conductivity as high as 10 mS/cm at 10 kg mAb/L membrane load and 10 MV/min (residence time ~ 6 seconds) flow rate

- At 5 mS/cm conductivity, MMV clearance remains constant at 4.8 LRV between load values of 3 to 10 kg mAb/L membrane
- At 3 kg/L load, MMV clearance remains the same (4.8 LRV) from 5 to 10 mS/cm

The data reveal excellent clearance of both viruses at very high mAb loads over a broad range of conductivities with residence time in the order of a few seconds. The high binding capacity of the membrane combined with excellent flow properties and improved salt tolerance provides greater process economy without sacrificing the process robustness or design flexibility.

In addition, NatriFlo HD-Q demonstrated excellent DNA clearance (> 2.96 LRV) at 10 kg/L load from protein A purified mAb feed at 10 mS/cm conductivity. The DNA reduction was from 612 ppb to <0.7 ppb at 10 kg/L load (as measured by qPCR assay).

## Virus/DNA Clearance & Process Robustness

**Figure 4: xMuLV clearance at 10 kg/L load**

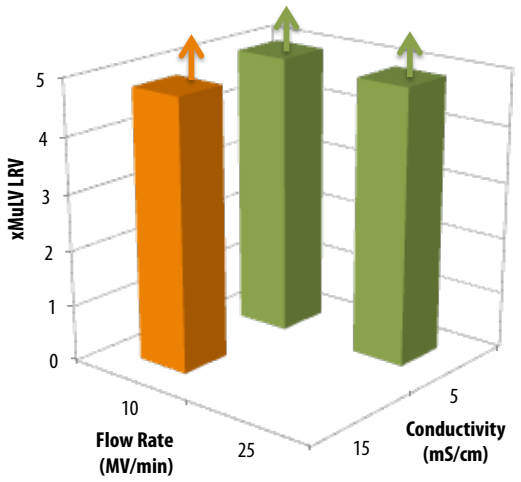
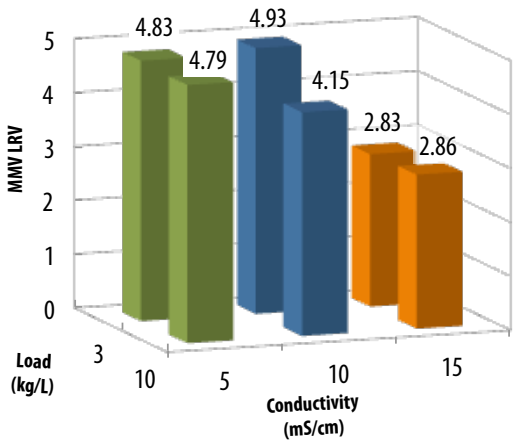



**Figure 5: MMV clearance at 10 MV/min flow rate**




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

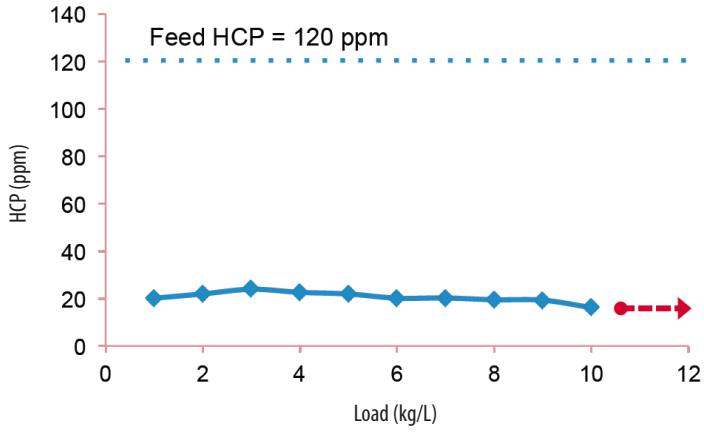
### Collaborator 2 - Gallus BioPharmaceuticals: HCP Clearance & Scalability

Working in collaboration with Natrix, Gallus BioPharmaceuticals (St. Louis, MO) evaluated lab-scale NatriFlo HD-Q membrane adsorbers for their ability to remove acidic host cell protein (HCP) at protein loads of up to 10 kg/L.

**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)

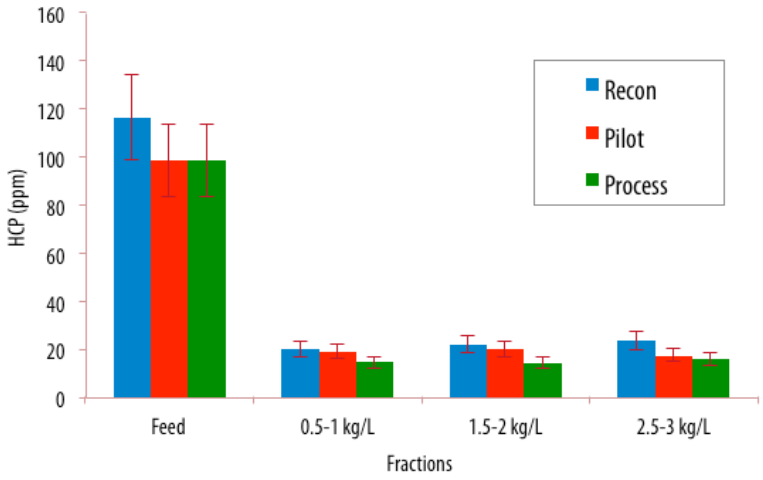
The sample feed contained 120 ppm HCP. Figure 6 illustrates that NatriFlo HD-Q achieved excellent HCP clearance with no sign of increasing breakthrough, even at 10 kg/L load. Testing was not continued beyond 10kg/L because of limited supplies of the protein A purified mAb feed. *(Data provided courtesy of Gallus BioPharmaceuticals.)*

**Figure 6: HCP Reduction Performance at Lab Scale for 10 kg/L Load**



Gallus also evaluated NatriFlo HD-Q for HCP removal across a range of process scales. Gallus included the lab scale Recon membrane adsorber, as well as Pilot and Process scale units. As seen in Figure 7, excellent & consistent HCP clearance from lab to process scale was achieved, even in phosphate buffer. While the Recon (lab scale) device was tested up to 10 kg/L (see Figure 6), the Pilot & Process devices were tested only up to 3 kg/L load due to limited supply of protein A purified mAb.

**Figure 7: Linear Scalability**




**Membrane Volume for NatriFlo HD-Q Membrane Adsorbers**

| | Nominal Membrane Volume (ml of media) |
|---|---|
| **Recon** | 0.8 mL |
| **Pilot** | 15 mL |
| **Process** | 115 mL |

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

## **Conclusions:** *NatriFlo HD-Q delivers improved economy & greater process design flexibility while reducing Risk*

As described in the preceding data and discussion, NatriFlo HD-Q Membrane Adsorbers represent a new class of chromatography tool that delivers enhanced process flexibility and robustness, while reducing risks and costs associated with mAb purification.

1. **Improved salt tolerance and wider buffer compatibility**

   Superior dynamic BSA binding capacity in the presence of salt or phosphate background

2. **High loading capacity without compromising process robustness**

   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

   Excellent HCP clearance with no sign of increasing breakthrough even at 10 kg/L load

3. **Simple scale-up**

   Excellent & consistent HCP clearance from lab to process scale

4. **Truly single-use plug & play format**

   No column packing, qualification, cleaning or validation

   No risk of cross-contamination

### Summary Comparison of Performance Characteristics: NatriFlo HD-Q vs. Other Available Technologies

| | 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** | + | + | – | – |

* Process robustness is defined as ability of the process to tolerate variability in operating parameters such as pH, conductivity, buffer type & concentration, impurities in load

** Process design flexibility is defined as ability of the process to tolerate broad range of conditions and flexibility to change the order of unit operations in the purification process

## 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.

FOR ADDITIONAL INFORMATION

| | | | |
|---|---|---|---|
| SALES INQUIRIES: | Contact JSR Life Sciences | +1 408-543-8976 | bio@jsrmicro.com |
| TECHNICAL INQUIRIES: | Contact Natrix Separations | +1 905-319-2682 | info@natrixseparations.com |