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Application Note

Natrix® Q Chromatography Membrane

Comparison of process-related impurity clearance for membrane adsorbers and resin-based chromatography technologies.

Introduction

Strong anion exchange (AEX) chromatography has become an industry standard in polishing steps for monoclonal antibody (MAb) purification. It is a proven technology to remove DNA, viruses, endotoxins and acidic host cell proteins (HCP) from process feed streams in flowthrough mode.

As the industry increasingly pursues high productivity 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.

Traditional, resin-based chromatography columns are often oversized due to throughput limitations and are ill-suited to flexible manufacturing. Conventional membrane adsorbers offer faster throughput but cannot provide sufficient process robustness due to the low binding capacity. These factors impose challenges on the design of purification schemes for manufacturing of biotherapeutics.

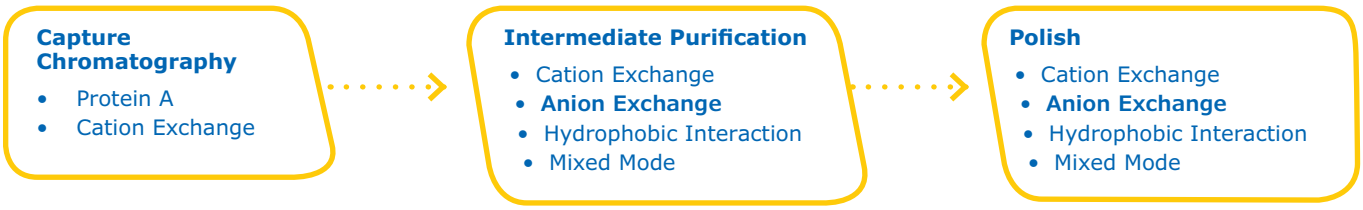
Natrix® Q chromatography membrane overcomes these limitations by combining high binding capacity and high flow rates to deliver best-in-class performance in a single-use, plug-and-play format.

This Application Note examines the performance of Natrix® Q chromatography membrane in comparison to currently available quaternary amine resins, membranes and salt-tolerant primary amine membranes. Comparative data is provided using BSA and DNA as benchmark biomolecules. In addition, the effectiveness and scalability of Natrix® Q chromatography membrane in removing process impurities (HCP, DNA & viruses) from industrial feed streams is also demonstrated.

Overview of study

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 for capture due to its ability to deliver >95% pure product in a single step. After the capture step, mAb is further purified using either one or two additional chromatographic units. Strong anion exchange chromatography is the most popular choice as either the 2nd or 3rd chromatographic step due to its proven performance in large scale mAb purification. Below is the summary of the most popular options for each chromatographic step in a typical mAb production scenario.

Chromatography Steps in a Typical mAb Production Platform



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

- Robust separation performance with high 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 Table 1.

Table 1. Pros and Cons of Existing Technology

Technology	PROS	CONS
Column Chromatography	• Robust separation performance due to high media binding capacity for many impurities • Flexibility in process design	• Oversized column due to throughput limitation • Relatively complex operation requiring column packing, qualification, cleaning or validation; risk of cross-contamination • Not suited to single-use format • Low binding capacity for large molecules (ex. large viruses, DNA) due to small resin bead pore size
Conventional Membrane Adsorber	• High throughput • Good performance when feed is relatively clean • Single-use, plug-and-play format	Limited binding capacity of membrane means: • Lower process robustness and thereby higher risk associated with product quality from one batch to another batch • Limited process design flexibility • Typically needs at least 3 – 5X dilution to achieve high loading in case of challenging feed conditions (ex. high salt concentration)

Competitive evaluation

The dynamic binding capacity under different process conditions for Natrix® Q chromatography membrane were evaluated and compared to a broad range of common chromatography membranes and resins (see Table 2).

Table 2. Chromatography Media used in Competitive Evaluation

Device	Matrix	Chemistry	Media Volume (mL)	Flow Rate (mL/min)
Natrix® Q Recon Mini	Porous Polyacrylamide	Quaternary Amine	0.20	2.0
Membrane 1-Q	Modified hydrophilic polyethersulfone		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
Resin 3-MM	Highly cross-linked agarose	Mixed Mode	1.00	1.0

Membranes were operated at 6 seconds residence time; resins were operated at 1 minute residence time.

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

Natrix® Q chromatography membrane maintained superior dynamic binding capacity over a wide range of conductivities in comparison to all membrane adsorbers including salt tolerant media (see Figure 1 and Table 2). Resin 1–Q demonstrated similar binding capacity to Natrix® Q chromatography membrane, but suffers from throughput limitations inherent to column chromatography.

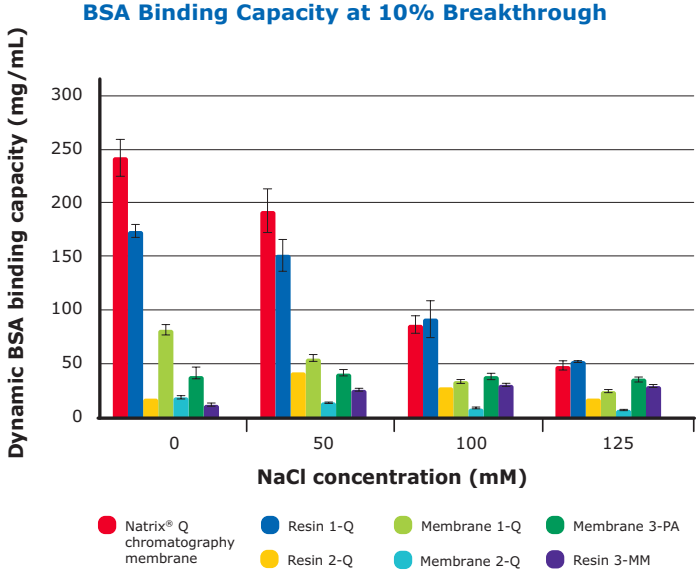
The typical residence time for any column at process scale is at least 2 minutes which puts constraint on the loading capacity of the column. Using this residence time, a typical column would need more than 33 hours to load the feed sample with 10 g/L titer to 10 kg/L capacity. By comparison, Natrix® Q chromatography membrane would need only 1.7 hours to load the same amount of feed at 10 MV/min flow rate (or even less if impurity clearance is maintained at higher flow rate.)

The improved salt tolerance of Natrix® Q chromatography membrane provides greater flexibility during process design. In addition, it is able to achieve high levels of performance without the need for dilution to reduced conductivity.

Table 3. Salt Tolerance Study: Dynamic BSA Binding Capacity at 10% Breakthrough (mg/mL)

Device	NaCl Concentration			
	0 mM	50 mM	100 mM	125 mM
Natrix® Q chromatography membrane	242	192	86	48
Resin 1-Q	174	151	91	52
Resin 2-Q	17	41	27	18
Membrane 1-Q	82	55	33	24
Membrane 2-Q	18	13	8	7
Membrane 3-PA	39	41	38	36
Resin 3-MM	11	25	30	29

Figure 1. Effect of NaCl Concentration on BSA Binding Capacity at 10% Breakthrough



2. Phosphate tolerance study

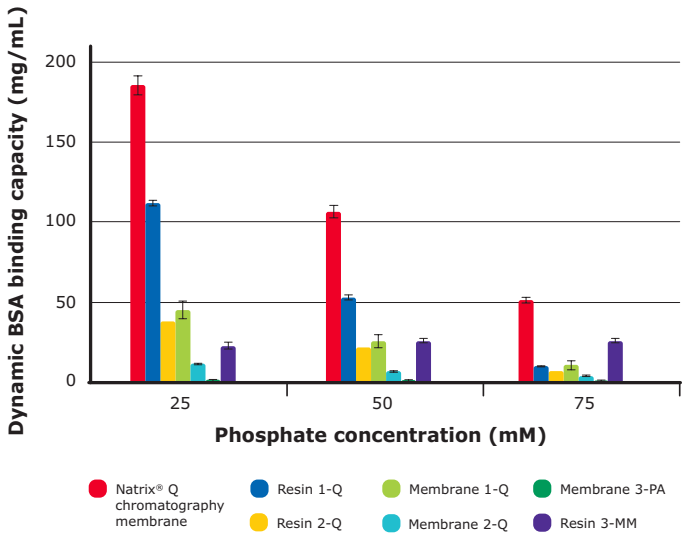
Phosphate is one of the most popular and widely used buffers for the pH range of 5.8 to 8.0. Natrix® Q chromatography membrane can tolerate the presence of phosphate ions unlike other anion exchange media, whose performance is significantly affected in presence of multivalent anionic buffers.

As illustrated in Figure 2 and Table 4, Natrix® Q chromatography membrane achieved more than 50% greater dynamic binding capacity than the closest competitior. This advantage was most pronounced at higher phosphate concentration.

Table 4. Phosphate Tolerance Study: Dynamic BSA Binding Capacity at 10% Breakthrough (mg/mL)

Device	Phosphate Concentration		
	25 mM	50 mM	75 mM
Natrix® Q chromatography membrane	186	107	51
Resin 1-Q	112	53	10
Resin 2-Q	38	22	7
Membrane 1-Q	45	26	10
Membrane 2-Q	11	7	4
Membrane 3-PA	1	1	1
Resin 3-MM	23	26	26

Figure 2. Effect of Phosphate Concentration on BSA Binding Capacity at 10% Breakthrough



3. DNA binding capacity study

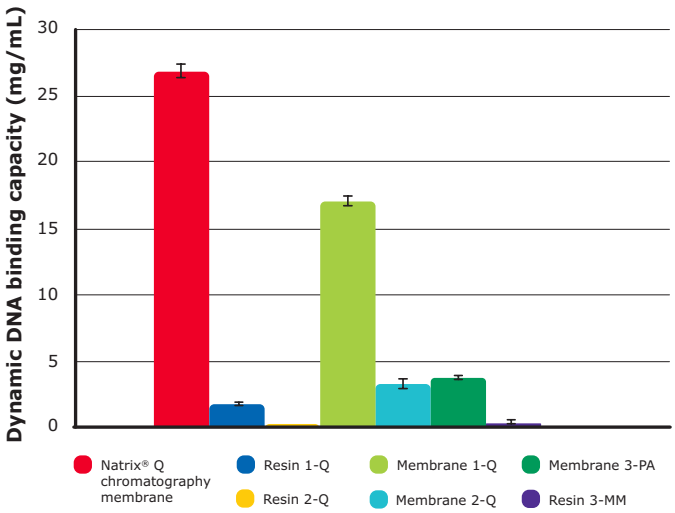
Natrix® Q chromatography membrane achieved an average DNA dynamic binding capacity of 27 mg/mL, 59% higher than the next closest competitor (Membrane 1-Q at 17 mg/mL). None of the other AEX media exceeded 5 mg/mL (see Table 5 and Figure 3).

Only the Natrix® Q chromatography membrane achieved excellent binding capacity for both protein and DNA without throughput limitation. This is due to the easily accessible, high functional group density of the Natrix® chromatography membranes.

Table 5. Dynamic DNA Binding Capacity (mg/mL)

Device	DBC at 10% breakthrough (mg/mL)
Natrix® Q chromatography membrane	26.9
Resin 1-Q	1.8
Resin 2-Q	0.1
Membrane 1-Q	17.1
Membrane 2-Q	3.4
Membrane 3-PA	3.6
Resin 3-MM	0.1

Figure 3. DNA Binding Capacity at 10% Breakthough



Summary

- The superior dynamic binding capacity of Natrix® Q chromatography membrane over a wide range of operating conditions combined with impressive throughput enable simple and robust polishing operations in a single-use format.
- Resin 1-Q achieved good dynamic BSA binding capacity but very low DNA dynamic binding capacity at 1 minute of residence time. In most cases, 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.

Purification Performance in MAb Polishing Application - Collaborator Data

Several major biopharmaceutical firms have evaluated the performance of Natrix® Q chromatography membrane in their existing mAb purification platform. The impurity (virus, HCP & DNA) clearance and process scalability data provided in this Application Note represent the achieved performance based on their actual proprietary antibody feed streams.

Collaborator 1: Viral and DNA Clearance

The process targets in this study were to use single-use anion exchange chromatography to reduce DNA levels down to <10 ppb in the mAb feed stream while providing > 4 LRV clearance of two model viruses, xenotropic murine leukemia virus (xMuLV, retrovirus, enveloped, ssRNA, 80-120 nm) and minute virus of mice (MVM, parvovirus, non-enveloped, ssDNA, 18-26 nm). The study was conducted at an external testing facility.

The loading capacity of an anion exchange step is often 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 loading capacity (up to 10 kg of mAb/L of membrane). The partially purified mAb feed was diafiltered in 25 mM Tris + NaCl buffer at pH 7.5. The final sample has a 15 g/L titer 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® Q chromatography membrane achieved excellent xMuLV clearance (≥ 4.8 LRV) over a wide conductivity (5 – 15 mS/cm) and flow rate (10 – 25 MV/min) range at 10 kg/L load (see Figure 4).

- No xMuLV breakthrough was detected in the flow through for all evaluated scenarios. The processing time at 25 MV/min was less than an hour.
- Flow independent clearance of xMuLV demonstrates a convection dominant mass transport phenomenon in Natrix® membrane technology.

Natrix® Q achieved > 4 LRV MVM clearance with conductivity up to 10 mS/cm at 10 kg load and 10 MV/min flow rate (residence time = 6 seconds) (see Figure 5).

- At 5 mS/cm conductivity, MVM clearance remains constant at 4.8 LRV for up to 10 kg/L load.
- At 3 kg/L load, MVM 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 loading capacity combined with excellent flow properties and improved salt tolerance provides great process economy without sacrificing the process robustness or design flexibility.

In addition, with the same feed material Natrix® Q chromatography membrane demonstrated > 2.9 LRV DNA clearance (from 612 ppb to < 0.7 ppb, as measured by qPCR assay) at 10 kg/L load.

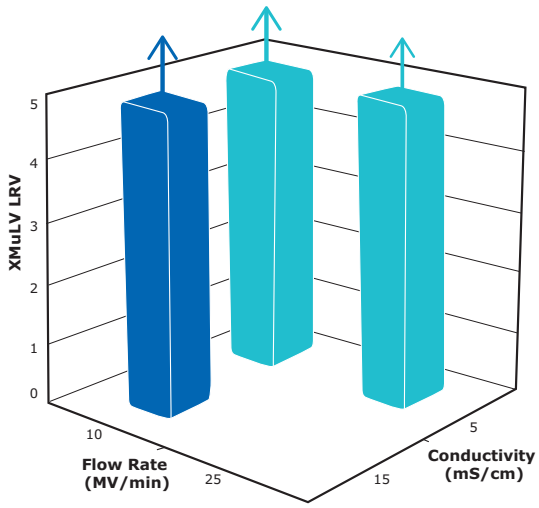


Figure 4. xMuLV clearance at 10 kg/L load

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

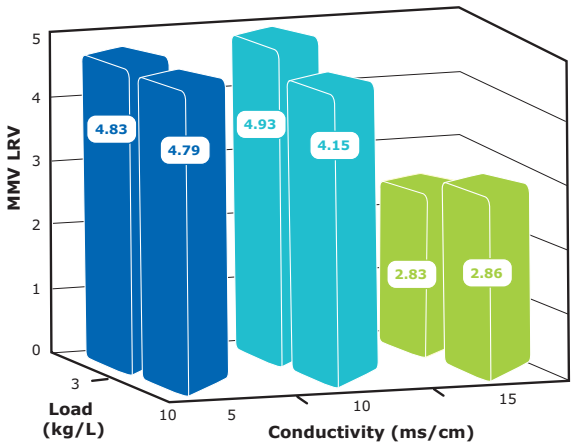


Figure 5. MVM clearance at 10 MV/min flow rate

Collaborator 2 - HCP Clearance and Scalability

The objective of this study was to evaluate Natrix® Q chromatography membrane for HCP removal at high load (up to 10 kg/L) and scalability to pilot and process scale devices.

The sample feed (8 g/L Protein A purified mAb in 20mM phosphate + 100 mM NaCl, pH 7) containing 120 ppm HCP was loaded to 10 kg/L of membrane at 10 MV/min (residence time = 6 seconds). Figure 6 illustrates that Natrix® Q chromatography membrane achieved excellent HCP clearance with no sign of increasing breakthrough. Testing was not continued beyond 10 kg/L because of limited supplies of the Protein A purified mAb feed.

The HCP removal with Pilot (membrane volume = 15mL) and Process 150 (membrane volume = 115mL) devices were also evaluated. While the lab scale device was tested up to 10 kg/L (see Figure 6), the Pilot and Process 150 devices were tested only up to 3 kg/L load due to limited supply of Protein A purified mAb. As seen in Figure 7, excellent and consistent HCP clearance from lab to process scale was achieved.

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

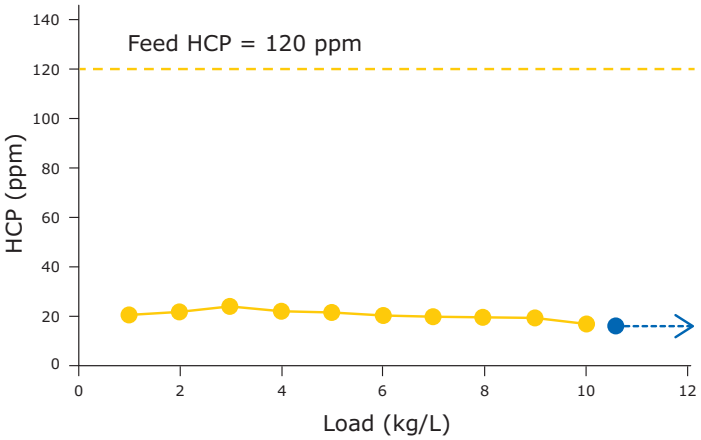
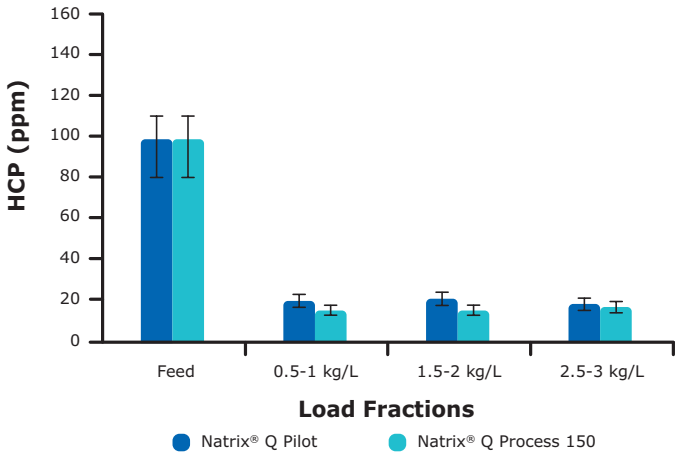


Figure 7. Scalable HCP Removal Across Different Device Sizes



Conclusion

Natrix® Q chromatography membrane delivers improved economy and greater process design flexibility

As described in the preceding data and discussion, Natrix® Q chromatography membrane represents a new class of chromatography tool that delivers enhanced process flexibility and robustness (see Table 6), with cost and risk reduction benefits associated with single-use technology.

1. **Improved salt tolerance and wider buffer compatibility**
   - Superior binding capacity even in the presence of high salt or phosphate concentrations
2. **High loading capacity without compromising process robustness**
   - Excellent virus (xMuLV & MVM) and DNA clearance 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 and consistent HCP clearance from lab scale to production
4. **Truly single-use, plug-and-play format**
   - No column packing, qualification, cleaning, storage, and associated validation
   - No risk of cross-contamination

Table 6. Summary Comparison of Performance Characteristics: Natrix® Q Chromatography Membrane vs. Other Available Chromatography Technologies

Device	Natrix® Q Chromatography Membrane	Resin	Conventional Membrane Adsorbers	Salt Tolerant Membrane Adsorbers
High-Throughput Media	+	–	+	+
High Media Binding Capacity	+	+	–	–
Process Robustness*	+	+	–	–
Compact Footprint	+	–	+	+
Single-Use, Plug-and-Play 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

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