Natrix®

NatriFlo™ HD-Q Membrane Adsorbers:

Method Development and Buffer Selection

Introduction

Anion exchange chromatography is a proven technology to remove DNA, viruses, endotoxins and acidic host cell proteins found in process feed streams during the production of biotherapeutics such as monoclonal antibodies (mAb). It is typically used as a polishing step in flow-through mode to remove small amounts of impurities from large amounts of process feed stream during mAb production. The operating process parameters are chosen such that the product of interest is either neutral or positively charged and thereby flows through the media whereas the process impurities are negatively charged and bind tightly to the media.

The amount of product load is dictated by the type and amount of impurities in the process feed, the binding capacity and salt tolerance of the media, as well as the throughput of the chromatographic device or column. Traditional chromatography columns are often oversized due to poor throughput or slow flow requirements for the resin beads. Conventional membrane adsorbers cannot provide sufficient process robustness due to low binding capacity of membrane. These factors impose constraints on the design of purification schemes. However, the NatriFlo HD-Q membrane overcomes these limitations by combining high binding capacity and high flow rates to deliver best-in-class performance. Table 1 compares the NatriFlo HD-Q membrane adsorber with competing technologies.

Table 1: 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**	+	+	–	–

** 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*

1. NatriFlo™ HD-Q Membrane Chromatography

NatriFlo HD-Q products are designed for flow-through (or negative) chromatography with the objective being to capture small amounts of impurities from the process feed stream while the product of interest flows through. **Figure 1 and Figure 2** show the typical steps performed in NatriFlo HD-Q chromatography and a representative chromatogram of a mAb polishing step respectively.

1.1. Feed Sample Preparation

The pH and conductivity of the feed sample should be appropriately adjusted before loading. The conductivity of the sample can be adjusted through dilution in most cases. However, diafiltration may be necessary in some cases to remove specific interfering ions. For example, the presence of citrate ions in the process feed stream reduces the performance of any anion exchange media and thereby necessitates the ultrafiltration/diafiltration step before anion exchange chromatography in the overall purification scheme. Refer to **Section 2** for further guidelines on the selection of buffering ions, pH and conductivity.

Figure 1: Steps in NatriFlo HD-Q chromatography

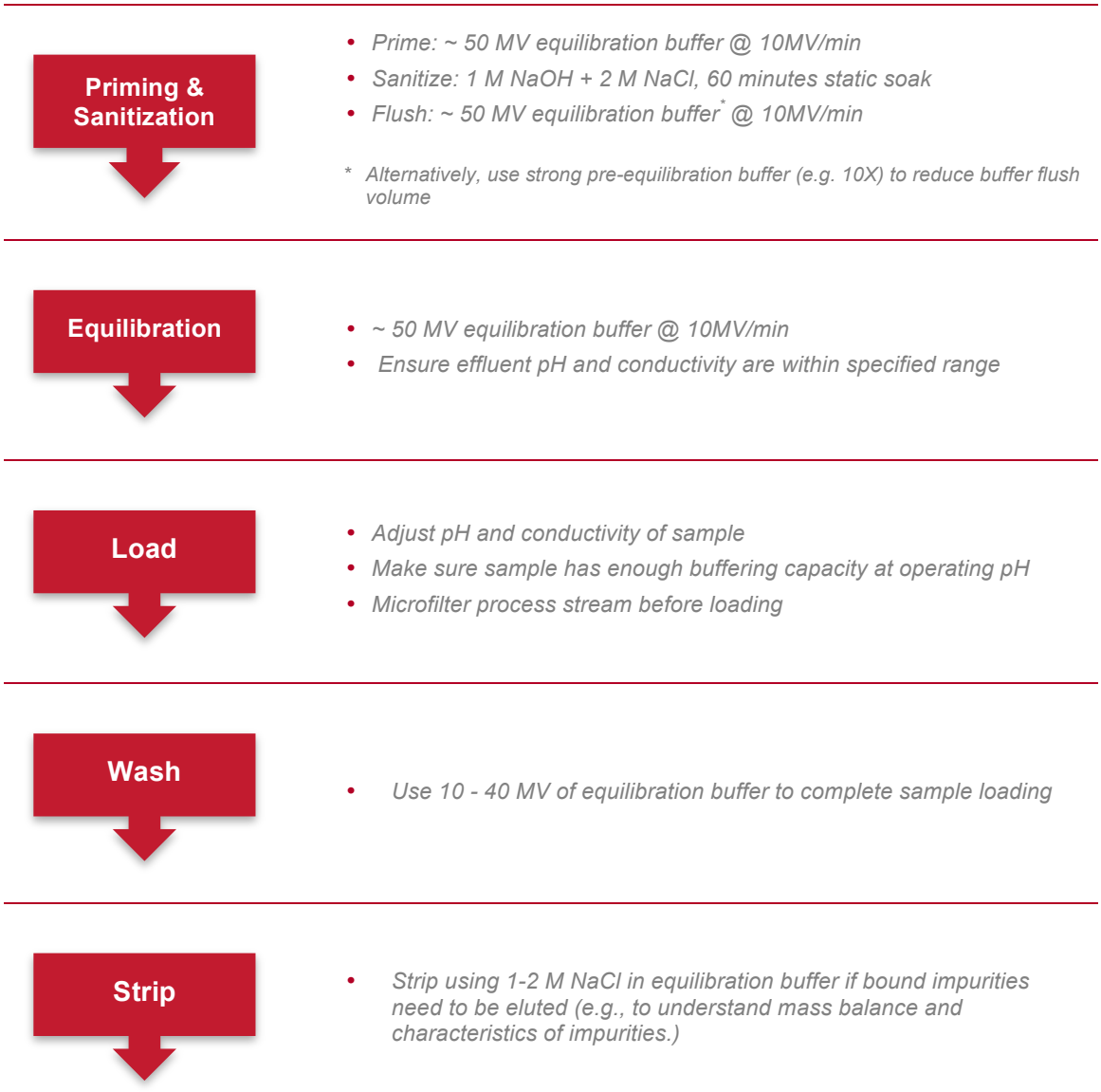
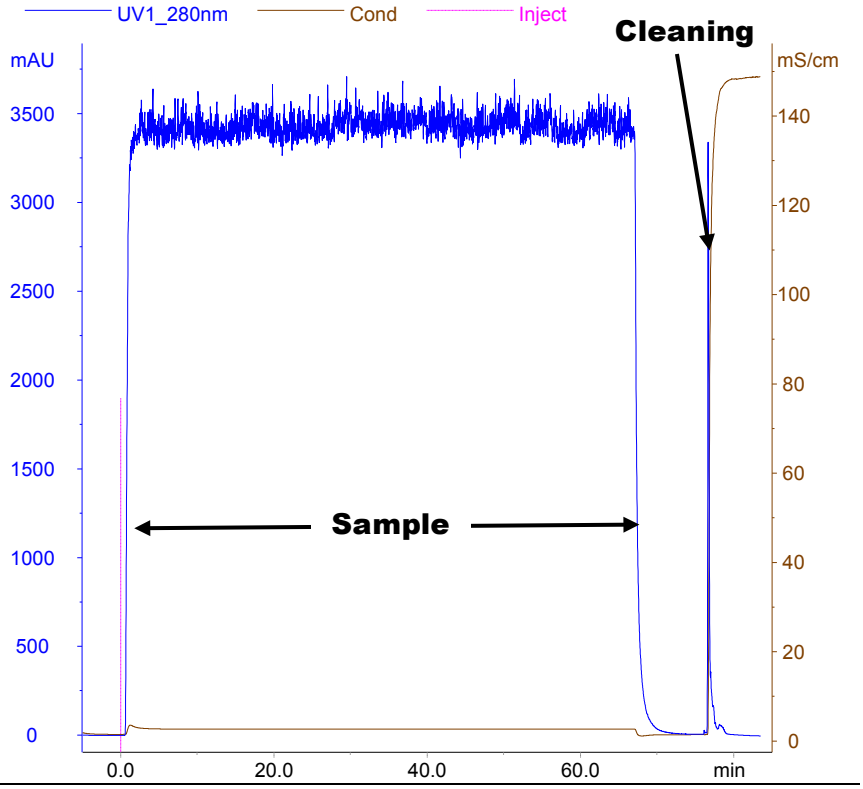


Figure 2: Representative chromatogram of NatriFlo HD-Q chromatography for mAb polishing.



Key considerations

Please consider the following key points while using NatriFlo HD-Q membrane adsorbers:

- The equilibration buffer and sample should have the same pH.
- The conductivity of the equilibration buffer should be less than or equal to that of the sample.
- The sample and equilibration buffer should have sufficient buffering capacity at the operating pH.
- Before loading the sample, make sure that the Natrix HD-Q media is adequately equilibrated with buffer. Ensure effluent pH & conductivity are within desired range at the end of equilibration step.
- Prime the sample line with equilibration buffer before connecting it to the sample container.
- If the sample contains interfering anions such as citrate, consider a diafiltration step to remove them. Citrate ions can reduce the binding capacity of any anion exchange media.
- A wash step is essential to ensure the sample is completely pushed through to achieve good yields.
- Consider eluting bound impurities using a strip solution to confirm the mass balance and understand their characteristics.

2. Considerations for Method Development

2.1. Chromatography System Considerations

The combination of higher flow rates with superior binding capacity requires special considerations when designing and performing chromatographic separations. The following two important points should be considered when transitioning from inherently slow column chromatography to fast NatriFlo HD-Q chromatography:

- **System back pressure**: Some modifications in the flow path of the chromatographic system may be needed to accommodate the high flow capability of NatriFlo HD-Q membrane adsorbers. This may include, for example, modification or removal of the flow restrictor after the UV detector, bypassing unnecessary control valves after the device, increasing the tubing size, etc.

- **System hold-up volume**: For NatriFlo HD-Q, the system hold-up volume (fluid volume from sample inlet to product outlet through the membrane adsorber) is generally much higher than the membrane volume at any process scale. Therefore, estimation of system hold-up volume is suggested to accurately calculate the amount of wash buffer needed to complete the sample loading. System hold-up volume can be estimated by a step response method using saline solution.

2.2. Effect of Process Parameters

Optimization of process parameters such as pH, conductivity and flow rate is important to maximize the performance of any anion exchange chromatography media. The effect of these parameters on the performance such as loading, impurity clearance and yield should be studied through screening experiments.

2.2.1.pH

Below the pI, protein carries a net positive charge and has negligible affinity for anion exchangers whereas above the pI, it will bind. The pH of the feed sample should be screened over a wide range to determine the balance between impurity clearance, yield and loading. Normally, higher pH gives better impurity clearance but beyond optimum pH, yield and loading may decrease due to product binding.

2.2.2.Conductivity

The conductivity (or salt concentration) of the process feed stream affects the protein binding capacity of Q chemistry media. Typically, lower conductivity (in the range of 2–5 mS/cm) provides the best performance. **Figure 3** shows the effect of conductivity (or NaCl addition) on the dynamic BSA binding capacity of Natrix HD-Q membrane versus HiTrap Q FF from GE Healthcare Life Sciences at 10% breakthrough along with the published binding capacity data for Sartobind Q and STIC membranes. **Figure 3** shows that at conductivity of 12 mS/cm, the Natrix HD-Q membrane binds well in excess of 100 mg/mL, which is significantly higher than the competitive alternatives. The improved salt tolerance of Natrix HD-Q membranes provides greater flexibility during the process design.

Figure 3: Effect of conductivity (due to addition of NaCl) on BSA dynamic binding capacity at 10% breakthrough

NatriFlo HD-Q Recon versus HiTrap Q FF.

Both the devices were run at manufacturer's recommended flow rate using: 25 mM Tris + NaCl, pH 8.2 buffer.

BSA binding capacity data for Sartobind® Q & STIC were extracted from Sartorious's application note in Nature Methods, 2010 (Reference: Sartobind STIC® salt-tolerant membrane chromatography, Fischer-Frühholz et al., Application Note in Nature Methods, 7 December 2010)

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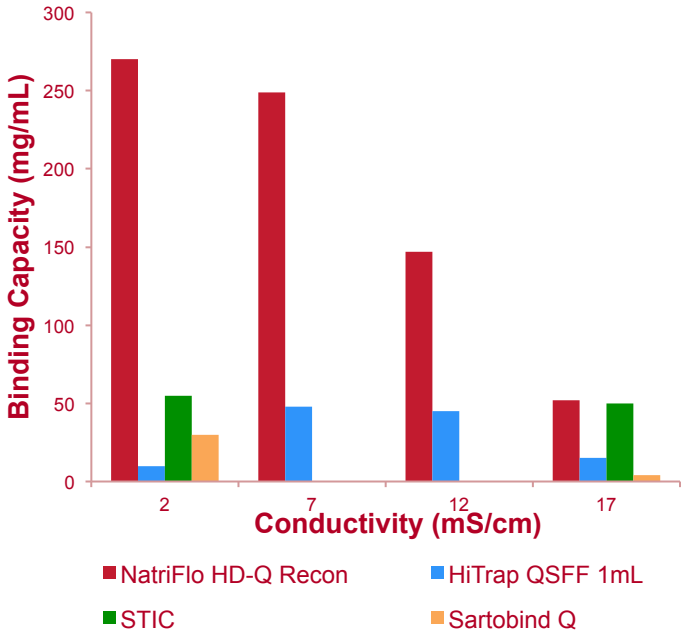


2.2.3. Buffers and salts

Whenever possible, buffer ions should be cationic or zwitterionic and the buffers and process feed streams should have sufficient buffering capacity to control the pH variation at operating conditions. **Table 2** lists the common buffer systems for anion exchange chromatography.

Table 2: Common buffers for anion-exchange chromatography

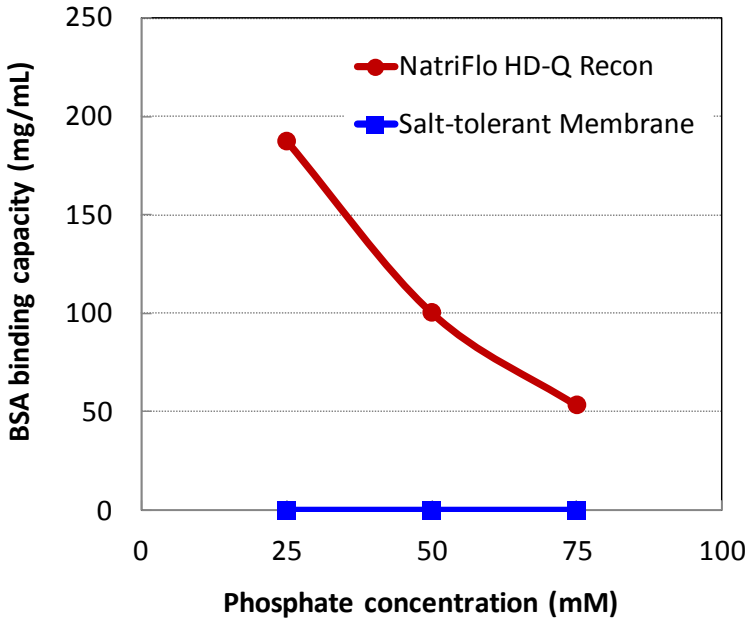
pH range	pKa	Buffer ion	Counter-ion
3.6 – 5.6	4.76	Acetate	Na^+
5.5 – 6.7	6.10	MES	Na^+
5.5 – 7.4	6.04	L-histidine	Cl^-
5.8 – 7.2	6.46	Bis-Tris	Cl^-
6.4 – 7.3	6.80	Bis-Tris propane	Cl^-
6.2 – 7.8	6.95	Imidazole	Cl^-
6.5 – 7.9	7.14	MOPS	Na^+
5.8 – 8.0	7.20	Phosphate (pK2)	Na^+
6.8 – 8.2	7.48	HEPES	Na^+
7.0 – 8.3	7.76	Triethanolamine	Cl^-
7.5 – 9.0	8.06	Tris	Cl^- / CH_3COO^-

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 media, which can be used with monovalent buffers only. Figure 4 compares phosphate tolerance of NatriFlo HD-Q membrane with a commercially available salt-tolerant membrane. The salt-tolerant membrane did not bind BSA from phosphate buffer whereas Natrix HD-Q membrane achieved dynamic BSA binding capacity as high as 180 mg/mL at 10% breakthrough in the presence of 25 mM phosphate at pH 8.

Figure 4: NatriFlo HD-Q Recon vs. Salt-tolerant Membrane: BSA binding capacity as a function of phosphate concentration

Dynamic BSA binding capacity were measured at 10% breakthrough at pH 8 in phosphate buffer.

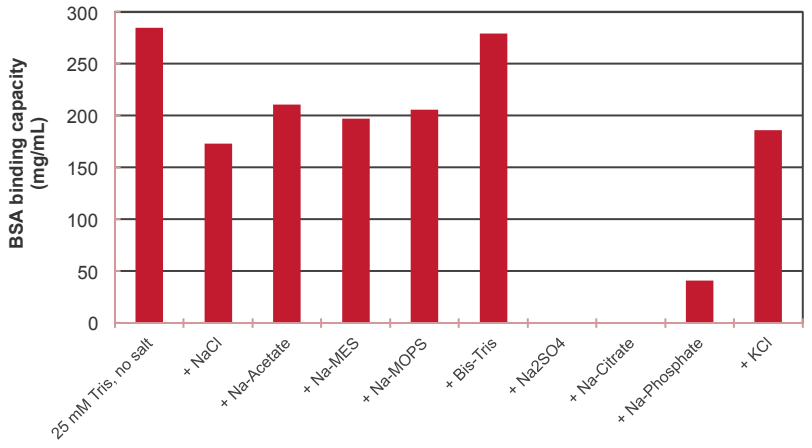
Flow rate: 10 MV/min



In order to understand the impact of common anions in the process feed stream on protein binding capacity of the anion exchange media, dynamic BSA binding capacity of Natrix HD-Q membrane adsorber was measured in the presence of different salts. **Figure 5** shows the effect of different salts at 75 mM concentration level on BSA binding capacity of Natrix HD-Q membrane at 10% breakthrough. BSA solution in the control experiment was prepared using 25 mM Tris, pH 8.1 buffer whereas other experiments were run using BSA solution made in 25 mM Tris + 75 mM various salts, pH adjusted to 8.1. **Figure 5** shows that Natrix HD-Q membrane maintains good BSA binding capacity in the presence of sodium chloride, sodium acetate, Bis-Tris and the sodium salts of MES & MOPS as well as potassium chloride. However, BSA binding capacity is impacted by the presence of 75 mM of sodium sulfate, sodium citrate and sodium phosphate.

Figure 5: Effect of various added salt species (at 75 mM) on Natrix HD-Q BSA binding capacity

Dynamic BSA binding capacity of Natrix HD-Q membrane was measured at 10% breakthrough using BSA solution prepared in 25 mM Tris buffer at pH 8.1 having various salts (at 75 mM). Flow rate – 10 MV/min



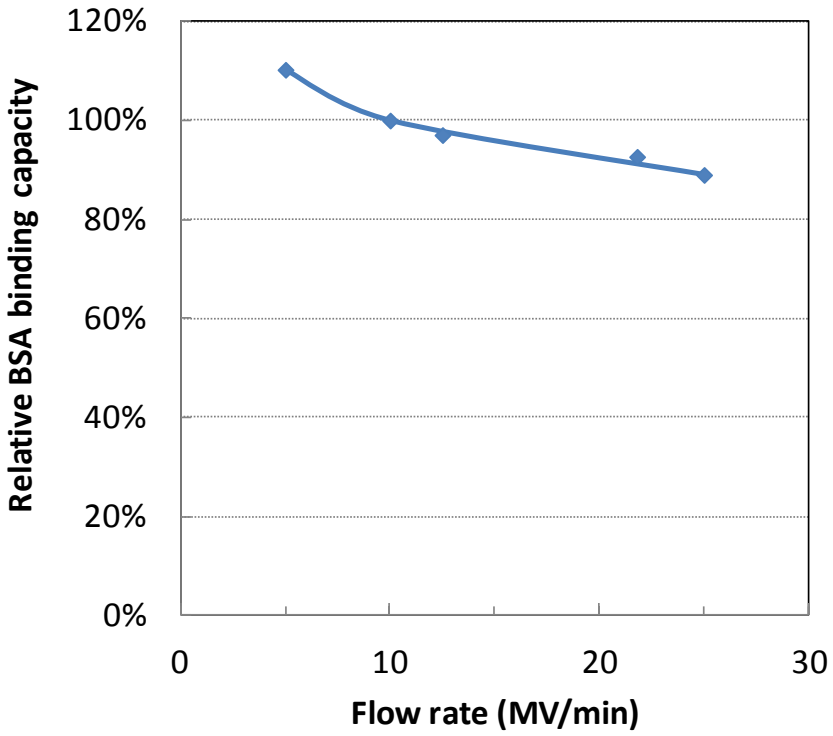
2.2.4. Flow Rate

As shown in **Figure 6**, Natrix HD-Q membrane adsorbers maintain very good binding capacity over a wide flow rate range and can be operated as high as 25 MV/min without significant loss in binding capacity. The high flow rate capability provides flexibility in choosing the operating flow rate based on feed titer and load amount (see section 3.2 to calculate the load time). A typical flow rate is 10 MV/min which results in a residence time of 6 seconds.

Figure 6: Effect of flow rate on 10% breakthrough BSA binding capacity of NatriFlo HD-Q membrane adsorber

The dynamic BSA binding capacity of NatriFlo HD-Q membrane adsorber was measured using BSA solution prepared in 25 mM Tris, pH 8.1 buffer.

The data are normalized with respect to 10 MV/min flow rate (100% = 10 MV/min)



3. NatriFlo HD-Q Membrane Chromatography as a Flow-through Polishing Step in mAb Production

Typical mAb molecules have an isoelectric point (pI) in the range of 7–9 and therefore the process is operated in the pH range of 6–8.5 (generally, higher pH and lower conductivity provide better performance). At these conditions, the mAb is either neutral or positively charged and flows through the media whereas the acidic contaminant molecules bind. The pI of the product and its stability must be carefully considered when defining the design space for pH and conductivity.

3.1. Method Development/Process Optimization

The “plug-and-flow” format of single use NatriFlo HD-Q adsorbers eliminates costly, time-consuming column cleaning and packing validation resulting in simple process development. In general, only the feed stream pH and conductivity (or dilution) need to be optimized to maximize the loading amount with sufficient process robustness for the desired/specified impurity clearance. Below are some general guidelines for developing the method for NatriFlo HD-Q flow-through chromatography:

- First, understand the effect of pH and conductivity (or dilution) of the process feed stream on the clearance of target impurity (most frequently, HCP). This can be achieved by running a small number of experiments at different feed sample pH and conductivities, and analyzing product flow-through and impurity breakthrough in each. Since the Natrix HD-Q membrane offers very high binding capacity and impurity clearance is measured through offline assay,

these initial breakthrough experiments should be run with at least 5 – 10 kg of mAb/L of membrane load to understand the effect of pH and conductivity on the clearance of small amounts of impurities. These experiments may be run at the recommended flow rate, i.e. 10 MV/min flow rate or higher depending on the process throughput requirement.

- If the mAb purity specifications allow, you may want to test higher loading capability of NatriFlo HD-Q membrane adsorbers at optimized pH and conductivity at different flow rates. Clearance of other impurities such as DNA, viruses and endotoxin at optimized conditions should also be assessed. The mAb yield is typically >95% for anion exchange flow-through chromatography with several kg/L loading.

3.2. Process Calculations

The cycle (processing) time of NatriFlo HD-Q chromatography mainly consists of the load time with minor contributions (approximately 15 – 20 minutes) from the set-up, equilibration, wash and strip steps. The loading time depends on the media load capacity, feed titer and flow rate. Load time can be calculated using the following formula:

$$Load\ time\ (minute) = \frac{Load\ (\frac{mg\ of\ mAb}{mL\ of\ membrane})}{Feed\ titer\ \left(\frac{mg}{mL}\right)\ x\ Flow\ rate\ (\frac{MV}{min})}$$

The throughput or processing speed of membrane chromatography can be compared with that of column chromatography using “residence time” as a recognizable term:

$$Residence\ time\ (minute) = \frac{Media\ volume\ (mL)}{Flow\ rate\ (\frac{mL}{min})}$$

Natrix Support

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