

Using Anion Exchange Membrane Adsorbers to Ensure Effective Virus Clearance of Challenging Parvoviruses

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ABSTRACT

In this article, the authors have detailed the best practices when optimizing and sizing anion exchange (AEX) membrane adsorbers for virus clearance. AEX chromatography is a powerful method to clear all types of virus by separating viruses from the product of interest based on ionic charge. Membrane adsorbers (macroporous membranes with chromatographic ligands attached that function similarly to resins containing identical ligands) offer several advantages over conventional resin chromatography, such as higher binding capacity for large molecules (i.e., viruses), >10-fold faster flow rates, use of up to 75% less buffer, and present a flexible single-use solution that does not require cleaning validation. These qualities make membrane adsorbers a perfect fit for flow through chromatography operations where virus clearance is expected. The authors therefore performed minute virus of mice (MVM) spiking studies with a monoclonal antibody product and small-scale AEX membrane adsorbers to evaluate a range of critical processing conditions, such as conductivity, process pause, product loading, and virus spike purity. The results provide an understanding of the impact of the evaluated process parameters on the clearance of parvovirus.

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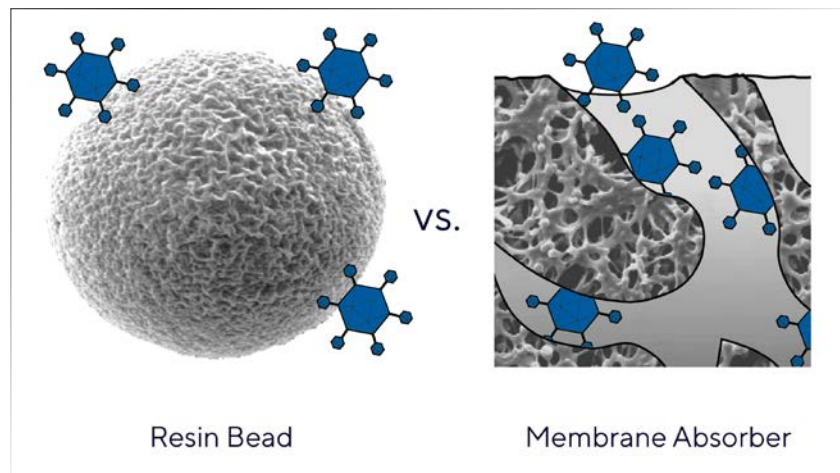
The risk of viral contamination is a feature common to all biopharmaceutical products and vaccines derived from mammalian cell lines and can have serious implications. Contamination can cost millions in investigation, clean-up, corrective action, and manufacturing plant downtime (1). Even if no contaminated biologics reach patients, manufacturing shortfalls can result in lost sales, as well as drug or vaccine shortages, and can cause unnecessary patient suffering.

To ensure the safety of biologics, regulatory agencies require a current good manufacturing practice (CGMP) downstream purification process that is validated to remove and/or inactivate viruses to prevent contamination. The validated process

should conform to an orthogonal approach for viral clearance, which typically includes inactivation by low pH and/or solvent detergent and removal by anion exchange (AEX) chromatography, virus filtration, and other methods (2).

As a virus removal method in the purification of monoclonal antibodies (mAbs), AEX membrane adsorbers show equivalent parvovirus clearance capabilities to AEX resins (3). Using membrane adsorbers instead of resins offers many benefits including higher binding capacity for large molecules, such as viruses. The open pore structure (**Figure 1**) of the membrane adsorber allows large molecules to easily enter the matrix and bind to ligands. Membrane adsorbers also have greater than 10–30-fold faster flow rates

Figure 1. Pore structure of a conventional resin bead compared to a membrane adsorber.



and use up to 75% less buffer, which means AEX membranes can operate at the same level of productivity as resins in a much smaller footprint (4), making them cost-effective for viral clearance.

To ensure that membrane adsorbers provide maximum viral clearance, critical process parameters for virus binding must be established. The authors therefore undertook minute virus of mice (MVM) spiking studies to evaluate the effects of conductivity, process pause, protein loading, and virus preparation purity on retention.

MATERIALS AND METHODS

Model biological

A Cellca Chinese hamster ovary (CHO) cell line expressing a mAb, known as Cellca2, was used as the model biological for these studies. This mAb was chosen because it is a commonly manufactured commercial biologic and is representative of the type of protein produced in biopharmaceutical manufacturing.

Viral model

MVM was selected for this study taking into consideration the concepts of specific and nonspecific model viruses as described in International Conference on Harmonisation of Technical Requirements for

Registration of Pharmaceuticals for Human Use Q5A (5). The virus is closely related to known and/or potential contaminants of biologically-derived material and has physicochemical characteristics that test the potential viral removal capacity of AEX. MVM is one of the most challenging viruses to remove by AEX chromatography because small viruses, such as parvovirus, have fewer binding sites that can interact with the chromatography ligand when compared to large viruses (such as retroviruses). In addition, the isoelectric point (pI) of MVM is 6.2, which is the highest pI of all the viruses typically tested in mAb–virus validation studies (6,7).

MVM for spiking studies

The MVM for the spiking studies was cultured in mouse cells with a proprietary media. The virus was purified by cell clarification, concentration, and chromatography to remove impurities. The MVM titer was measured using a standard tissue culture infectious dose that causes 50% cell death ($TCID_{50}$) assay and was used at approximately 10^8 $TCID_{50}$ /mL for each spiking study.

AEX membrane adsorbers

Membrane adsorbers (Sartobind, Sartorius GmbH) that were flexible and single-use were used because they did

not require column packing or cleaning validation. The membrane adsorbers also came in a range of device sizes, which means that a viral clearance process can be validated at small-scale and transferred quickly and easily to manufacturing scale.

AEX chromatography is often used in flow through mode to remove viruses from biologics. The anion exchange media will separate viruses from product by utilizing differences in ionic charge. Under optimal conditions, the virus will be negatively charged and bind to the adsorber, while the product will be positively charged, and will not bind.

Experimental approach

To determine the optimum process conditions, the authors assessed the following critical process parameters: conductivity, flow stop/pressure pause, protein (mAb) loading, and virus spike purity.

In all studies, the membrane adsorbers were wetted and flushed with buffer (according to Sartorius' user manual) (8). Following these directions is critical to ensure the membrane is fully wetted and all air is removed from the device to prevent low and/or variable virus removal (especially with hydrophobic proteins). The wetting procedure is also needed to remove storage solution from the device. In addition, a prefilter was placed in-line before the small-scale membrane (Sartobind). The prefilter was used to remove any large particles that may cause fouling on the membrane adsorber as well as trap any air bubbles originating from the system that may try to enter the membrane adsorber. After wetting and flushing, the membrane adsorbers were sanitized with sodium hydroxide and equilibrated with buffer.

For all studies, 1% (v/v) of MVM at a concentration of approximately 10^8 $TCID_{50}$ /mL was added to the samples. Fractions were collected and analyzed to determine any virus breakthrough. Virus infectivity was determined via a $TCID_{50}$ assay. A logarithmic reduction

Figure 2. Effect of conductivity on minute virus of mice (MVM) retention. ↑ denotes no virus particles detected. LRV is logarithmic reduction value.

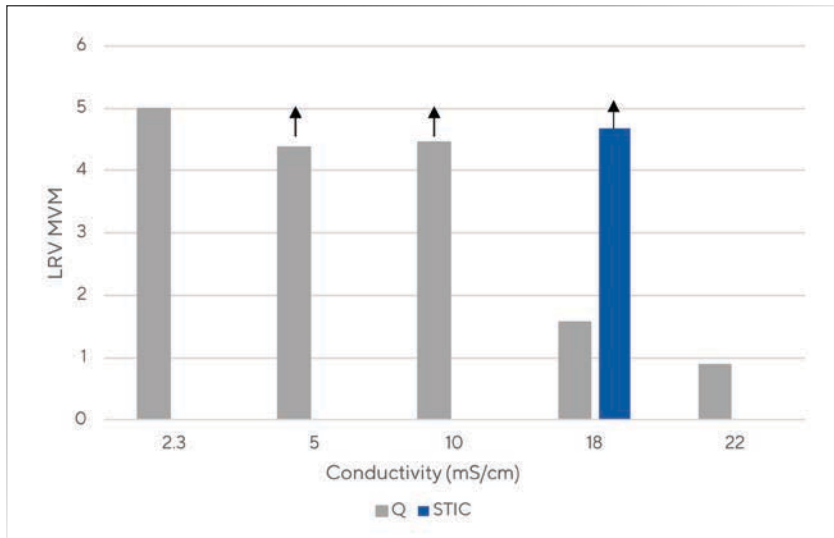
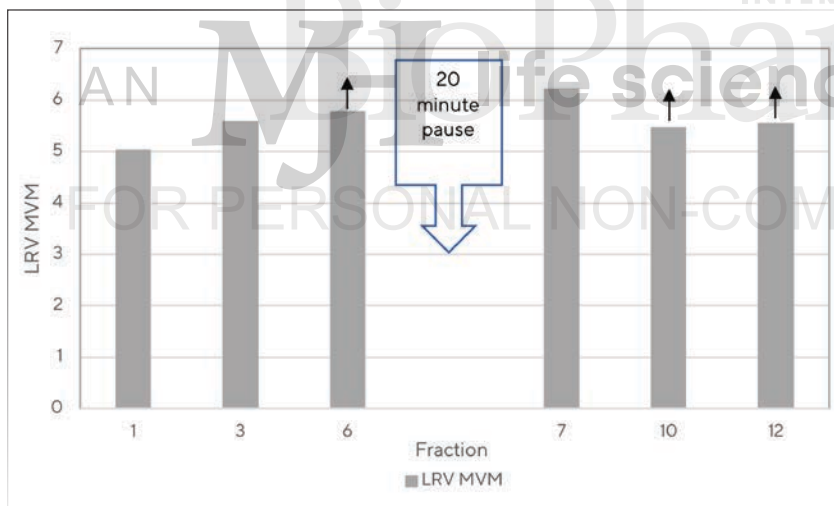


Figure 3. Effect of process flow pause on minute virus of mice (MVM) retention (Sartobind Q pico). LRV is logarithmic reduction value.



value (LRV) of the virus was calculated by comparing the quantity of virus recovered in the mAb filtrate to the quantity of virus loaded onto the AEX membrane adsorber.

Assessing conductivity

Cellca2 mAb stock solution (20 g/L) was diluted to 5 g/L with 20 mM Tris, pH 7.5 buffer. The conductivity was adjusted to six different values ranging from 2–20 mS/cm by adding the appropriate amount of sodium chlo-

ride (NaCl). The diluted solutions were then spiked with 1% (v/v) MVM (10^8 TCID₅₀/mL) and loaded onto the Q membrane adsorber (Sartobind). For the (Sartobind) STIC study, Cellca2 mAb stock solution was diluted into 20 mM Tris, 150 mM NaCl, pH 7.5 buffer with a conductivity of 18 mS/cm, spiked with MVM, and loaded onto the membrane. Both membranes were loaded to 1 kg/L at a flow rate of 10 membrane volume (MV)/min. Filtrate fractions were collected and analyzed for any virus break-

through using a TCID₅₀ assay. **Figure 2** shows the LRV results of the pooled fractions under the different conductivities.

Process pause/flow stop

Process pauses due to disconnecting and reconnecting single-use bags to and from an AEX membrane adsorber or unexpected power loss can occur in biopharmaceutical manufacturing environments. There is evidence that this may affect MVM retention on virus clearance filters (9). To assess whether there is a similar impact on membrane adsorbers, a 20-min pressure pause/flow stop was initiated half-way through the loading. Cellca2 mAb was diluted with 20 mM Tris, 25 mM NaCl, pH 7.2 buffer to a concentration of 5g/L, and 1% (v/v) MVM (10^8 TCID₅₀/mL) was added. The solution was loaded onto the Q pico (0.08 mL) membrane adsorber (Sartobind) to a capacity of 12.5 kg/L at a flow rate of 10 MV/min. **Figure 3** shows the LRV results for the 12 fractions that were collected and analyzed for any virus breakthrough using TCID₅₀ assay.

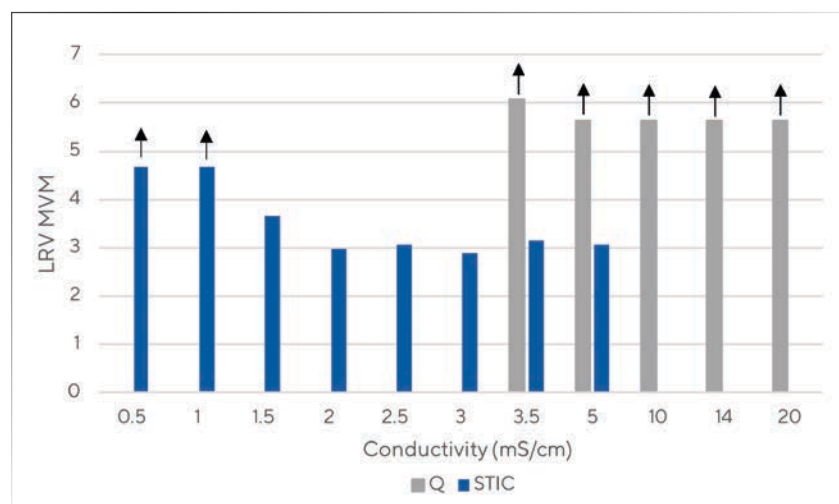
Determining maximum loading capacity

Determining an accurate loading capacity can be critical to successfully utilizing AEX membrane adsorbers. Underloading the membrane will result in excessive costs for large-scale commercial manufacturing operations, while overloading the membrane will result in lower-than-expected virus and impurity clearance. When using membrane adsorbers primarily for virus clearance, as would be the case when the membrane adsorber is used as a third chromatography step, the expected loading capacity would be between 2 kg/L to > 20 kg/L. However, when using the membrane adsorber to also remove impurities, such as when the membrane adsorber is positioned as a second chromatography step, the expected loading capacity is between 0.1 kg/L to 3 kg/L.

A loading study using the Q membrane (Sartobind) was performed to determine the maximum amount of mAb that can be loaded onto the membrane adsorber (in the flow through mode) before the virus removal capacity is reduced. This study was performed using Cellca2 mAb diluted to 5 g/L in 20 mM Tris, 25 mM NaCl, pH 7.2 buffer. In addition, 1% (v/v) MVM (10^8 TCID₅₀/mL) was added to the mAb. The mAb was then loaded onto the Q pico (0.08 mL) (Sartobind) device at a flow rate of 20 MV/min to a final capacity of 20 kg/L. As shown in **Figure 4**, a total of five filtrate fractions were taken and analyzed for the presence of virus.

In addition, a loading study using the STIC membrane (Sartobind) was performed to determine the maximum amount of mAb that can be loaded before virus removal capacity is reduced. This study was performed using Cellca2 mAb diluted to 5 g/L in 20 mM Tris, 150 mM NaCl, pH 7.5, which had a conductivity of 18 mS/cm, and 1% (v/v) MVM (10^8 TCID₅₀/mL) was added to the mAb. STIC membranes are designed to be used at high conductivities (e.g., >10 mS/cm) because, at lower conductivities, the product may possibly bind to the membrane, which would limit virus removal. However, the authors did not perform any analyses at low conductivity in these studies. The STIC pico (0.08 mL) (Sartobind) device was run at a 20-MV/min flow rate and loaded up to 5 kg/L. In these loading studies, a higher flow rate was used to shorten the loading time needed when loading the membranes to high capacity. The typical flow rate for membrane adsorbers is between 10–30 MV/min (Sartorius has [unpublished] data from internal Sartorius studies showing flow rate does not impact protein binding). Also, higher flow rate and higher loading will represent the worst case as if there is an effect; the higher flow rate will have lower binding capacity. As

Figure 4. Effect of increasing protein loading on minute virus of mice (MVM) retention with Q and STIC membrane adsorbers (Sartobind). LRV is logarithmic reduction value. MVM is minute virus of mice.



shown in **Figure 4**, a total of five filtrate fractions were taken and analyzed for the presence of virus

Scalability and reproducibility

Robust virus clearance and scalability of the two small-scale Q devices (1-mL nano and 0.08-mL pico) (Sartobind) were accessed by performing six runs on the pico and six runs on the nano device. Four runs were performed on each device by adding MVM to buffer (runs 1–4) and two runs were performed on each device by adding MVM to Cellca2 mAb (runs 5–6). For runs 1–4, 20mM Tris, 25 mM NaCl, pH 7.2 buffer (loaded at 0.5 L/mL membrane) was spiked with 1% (v/v) of MVM (10^8 TCID₅₀/mL). For runs 5–6, Cellca2 mAb was diluted to 5 g/L with 20 mM Tris, 25 mM NaCl, pH 7.2 and loaded onto the membrane to a capacity of 5 kg/L. All runs were performed at a flow rate of 10 MV/min. **Figure 5** shows the LRV results from the pooled fractions of each run.

Effect of virus purity

Crude virus preparations are commonly used to validate chromatography resins. However, since membrane adsorbers are sized based on capacity, crude virus preparations containing impurities, such

as DNA and host cell proteins (HCPs), may bind to the membrane adsorber, limiting the virus' binding capacity.

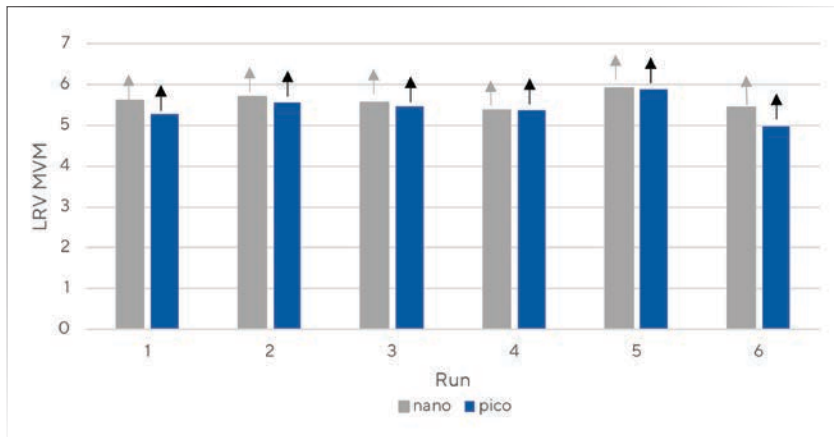
To determine the effects that using crude virus preparation and purified virus preparation has on the loading capacity, crude MVM (10^9 TCID₅₀/mL) clarified by crossflow filtration and purified MVM (10^8 TCID₅₀/mL) purified by chromatography, were added to Cellca2 mAb (10 g/L) and loaded onto Q pico adsorbers (0.08 mL) (Sartobind) at a flow rate of 20 MV/min. The buffer was 20 mM Tris, 25 mM NaCl, pH 7.2, and mAb was loaded to 10 kg/L for the crude virus preparation and 20 kg/L for the purified virus preparation. Five filtrate fractions were taken and analyzed for the presence of virus.

RESULTS AND DISCUSSION

Buffer conditions for optimal binding

The results from this study (**Figure 2**) show the effect of buffer conductivity on the retention of MVM spiked into Cellca2 mAb solution. The conductivity range tested was 2 mS/cm up to 20 mS/cm. As expected, the Q membrane shows good virus retention (>4 LRV) up to 10 mS/cm, after which the virus retention declines at con-

Figure 5. Performance comparison of two scales of Q membrane adsorbers (Sartobind). LRV is logarithmic reduction value. MVM is minute virus of mice.



ductivities of 18 and 20 mS/cm. The STIC membrane (Sartobind) chemistry is a primary amine and is designed to bind impurities with higher conductivity buffers. This membrane shows good virus retention (>4 LRV) at 18 mS/cm conductivity, indicating that the STIC membrane is the best choice if there is a need to process under high conductivity conditions.

Process pause/flow stop

Flow stop or process pause can affect MVM removal on some types of virus retentive filters but is dependent on the biologic being purified (9) as well as the membrane type. Results of this study (Figure 3) show that a 20-minute flow stop during processing with the Cellca2 mAb does not significantly affect MVM retention with AEX membrane adsorbers, indicating that routine connection and reconnection tasks to single-use bags, for example, should not affect viral clearance.

Loading capacity and economic analysis

Membrane adsorbers have high flow rates that allow for the membrane capacity to be fully utilized. Correct sizing of the membrane will assure the membrane is not overloaded, which may cause virus or impurity breakthrough. In addition, correct sizing will prevent

underloading the membrane, which can result in excessive costs for large-scale commercial operations.

The typical capacity for membrane adsorbers is >20 kg/L when used with products that have low impurity levels, for example, when the membrane adsorber is used as the third chromatography step. When impurity removal is also desired, the typical capacity can range from 0.1 kg/L–3 kg/L, depending on the level of impurities. Impurity clearance can be measured and the capacity of the membrane to remove these impurities can be determined during development. However, to accurately determine the loading capacity for the highest virus clearance, a virus clearance study must be performed. The maximum loading capacity is evaluated by collecting filtrate fractions during the virus spiking study and analyzing the fractions for the presence of virus. The membrane should be loaded until virus breakthrough occurs, at which point the membrane has reached the maximum loading capacity.

Figure 4 shows the results of a typical loading study. In the Q membrane (Sartobind) study, all fractions showed complete virus retention, and the resulting pool was able to remove 5.77 LRV at a loading capacity of 20 kg/L.

The STIC membrane (Sartobind) study shows complete virus retention

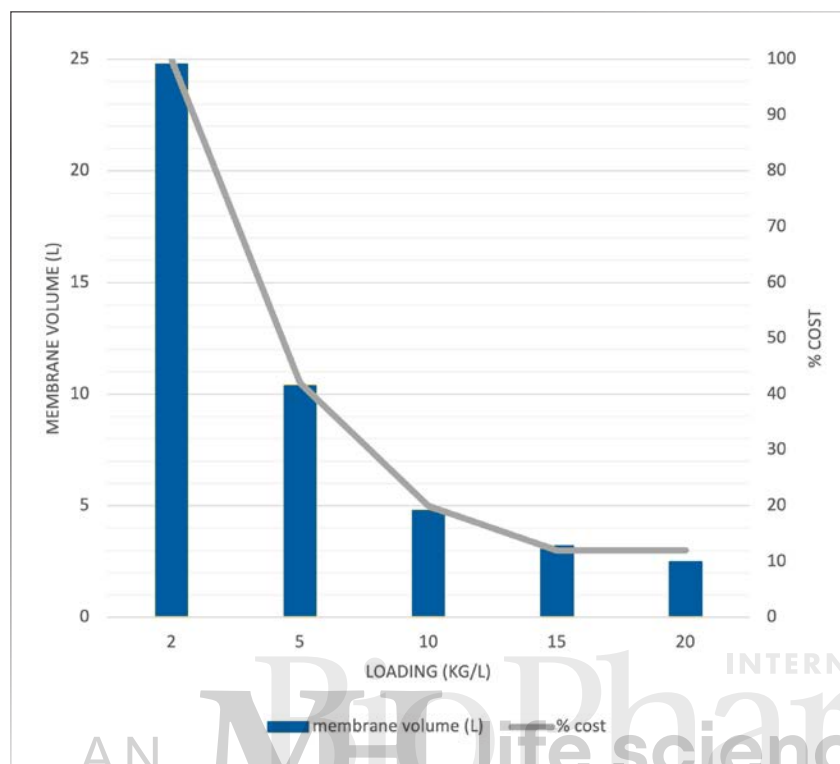
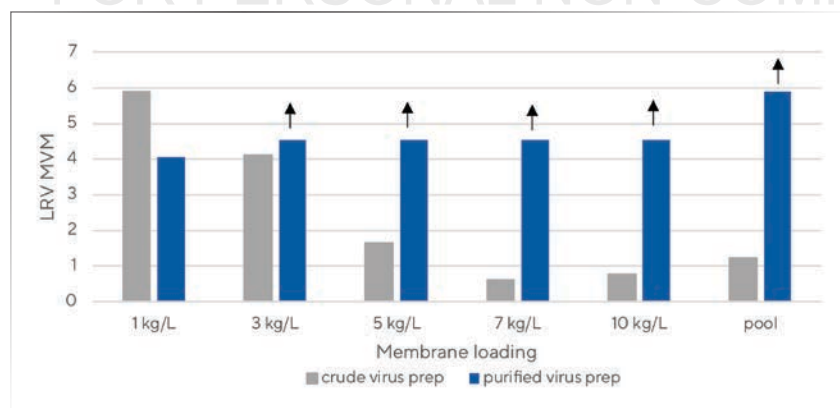
for the first two fractions, followed by a loss in retention with increased loading past the loading capacity of 1 kg/L. For this example, pooling the appropriate fractions to achieve the desired LRV is critical. If all fractions were pooled together, the resulting retention would be 3.15 LRV at the loading capacity of 5 kg/L. In order to reach a target of >4 LRV, pooling the first two fractions (or using a loading capacity of 1 kg/L), would result in retention of >4.67 LRV. Therefore, for this molecule, the recommended loading capacity is 1 kg/L.

Effects of underloading

If the membrane capacity is not utilized, there will be a substantial cost increase, especially when producing large volumes of product. Figure 6 illustrates the amount of membrane area required to process a 10,000-L bioreactor (with an output titer of 5 g/L) at loading capacities ranging from 2 kg/L up to 20 kg/L. Increasing the loading capacity from 5 kg/L to 20 kg/L results in 75% less membrane surface area needed. This decrease in membrane area will provide a 30% cost savings for membrane alone, per bioreactor run. Proper sizing does not only provide savings with consumables, but perhaps, more importantly, buffer usage will be cut by 75%. The buffer needed for each bioreactor run can be reduced from 3600 L to 900 L, which results in a savings of 2700 L less buffer per run. Additionally, the footprint will be much smaller, hence the processing skid and pump size can be reduced.

Scalability and reproducibility

Figure 5 shows robust (>5 LRV) and reproducible virus retention for two different small-scale Q membrane adsorber (Sartobind) devices. The small pico device (0.08 mL) is useful when loading the membrane to high capacities because less product is needed. The larger nano device (1 mL) may be used when lower capacities are validated or

Figure 6. Financial impact of under-loading in late-phase manufacturing.**Figure 7.** Comparison of the effects of loading crude versus purified virus has on virus retention with Q membrane adsorbers (Sartobind). LRV is logarithmic reduction value. MVM is minute virus of mice.

for small-scale process development work. Complete virus removal is shown for all studies.

Virus spike purity

Figure 7 shows that using crude virus preparations, which contain impurities, may interfere with virus binding and cause early virus break-

through. As shown with the crude preparation, the ability of the membrane to retain virus dramatically decreases during increasing loading, which results in a pool of only 1.23 LRV clearance. However, when using the purified preparation, there is complete retention of virus and the resulting pool LRV is 5.77. Therefore, it is

important to use purified virus preparations, such as those used to validate virus removal filters, to not overload the membrane with contaminants from the crude virus preparation. In addition, the process material used for loading capacity studies should also be representative of production material in terms of impurities, including CHO HCPs and DNA, since the amount of impurities present in the product may limit the virus clearance capability of the membrane.

CONCLUSION

Robust, high levels of virus removal (>4–6 LRV) can be achieved using membrane adsorbers (Sartobind). As discussed, choosing optimal buffer conditions, using scalable small-scale devices, and evaluating accurate loading capacity are critical to achieving high LRVs. Determining an accurate loading capacity is crucial to eliminate overloading issues and ensures an economical process at manufacturing scale. Finally, performing validation studies using purified virus preparations and representative process material along with properly wetting small-scale devices will also ensure optimum performance.

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