Disposable Membrane Chromatography

Performance Analysis and Economic Cost Model

by Jeff Mora, Andrew Sinclair, Noushin Delmdahl, and Uwe Gottschalk

Disposable devices for unit operations in bioprocessing have been commonplace for some time. Additionally, support systems for each unit operation have also become disposable. They include aseptic bags for buffer and product storage and filters to ensure sterility. The most common disposable devices for unit operations are filters used to clarify harvests and protect columns, virus retentive filters, sterilizing-grade filters for fill and finish, and in some cases crossflow filters for product concentration and buffer exchange.

The simplest reason behind this development is that disposables eliminate cleaning validation for stainless steel housings and vessels and streamline development and/or scale-up. FDA cleaning regulations require cleaning, maintenance, and sanitization of equipment and utensils at appropriate intervals to prevent malfunctions or contamination. Furthermore, such cleaning needs to be validated (**1**). Eliminating cleaning validation not only reduces costs, but it also decreases the time it takes to get to a final process. Most validation projects take months or even years to complete, significantly delaying process finalization. Moreover, because disposable devices are modular and available in numerous sizes, scaling up a process is often just a matter of increasing device size by whatever factor is necessary.

The benefits of "going disposable" are well known and generally accepted

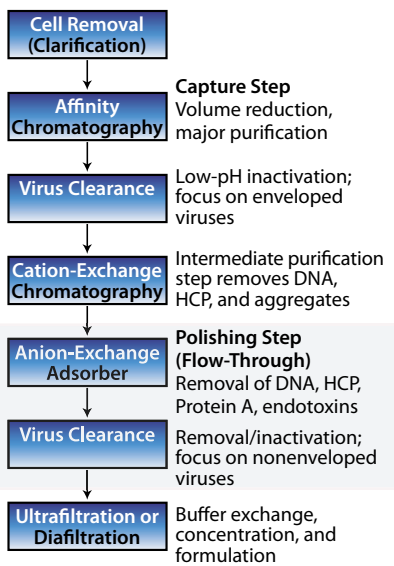
Photo 1: Purification associates are handling a 30-in Q membrane chromatography device. It can be used for virus clearance and trace contaminant removal at large scale.

in the bioprocessing community for certain unit operations. However, the same benefits apply to others that are not as prevalent as filtration and storage steps. Chromatography is one such unit operation, especially when used in the flow-through mode for polishing applications during late-stage purification. This particular application is well suited for disposable membrane chromatography.

The key drivers here are the same as elsewhere: no cleaning validation and ease of scale-up. But other, more compelling reasons are concealed or overlooked until a more rigorous analysis is conducted. This reveals the true benefits of disposable membrane chromatography.

Here we take an in-depth look at such benefits. In addition, recent unprecedented advancements have made disposable chromatography even more reasonable than before. We review them in detail, then incorporate them into an economic cost model. We begin with a cursory review of the flow-through–polishing process step (unit operation) along with membrane chromatography technology itself, with its pragmatic history.

Figure 1: Anion-exchange in the flow-through mode is typically third in the sequence of columns.



Membrane Chromatography

Anion-exchange (AEX) chromatography is a common unit operation for trace-contaminant removal and virus clearance during large-scale biopharmaceutical production (Figure 1). AEX resins are traditionally used in flow-through mode. They are "packed" into columns, a format that enables resin media to function practically and predictably. Conditions in the mobile

phase are adjusted, so product does not bind to positively charged ligands found on the surface and within the pores of the AEX resin (stationary phase): Typically such ligands are either strong anion exchangers such as quaternary ammonium (Q) or weak anion exchangers such as diethylamine (D).

Conditions are optimized to bind trace contaminants as biopharmaceutical product flows through a solid phase or AEX media. Contaminants include but are not limited to host-cell proteins (CHOPs if derived from Chinese hamster ovary cells), recombinant deoxyribonucleic acids (DNA), leached protein A (LProA), and supplements used during fermentation. Contaminant concentration is at the ppm or ppb level.

Most viruses express an acidic isoelectric point and are negatively charged. During scale-down virus clearance studies, they bind to positively charged ligands while product flows through. For this reason, AEX chromatography is a unit operation frequently validated for virus clearance. Typical clearance values for AEX resins are >3 LRV (log reduction values) after 50–100 chromatography cycles (**2**). Load challenge for product flow-through is typically 50–70 g product/L of AEX sorbent (**3**).

Disposable AEX membrane chromatography operates in the same way as resin-based chromatography. Product flows through the media, to which contaminants bind. However, there is one critical exception: Membrane chromatography does not require long residence times because its ligands are 100% exposed to the mobile medium. The ligands are attached to an open porous membrane, not to a bead with convolutions that encrypt those ligands on an inner pore surface (Figure 2). Long residence times with resins or beads require greater column bed heights or slower linear flow rates (or both). Those two characteristics are manipulated to increase residence time. This explains why most columns for polishing or removing trace contaminants are oversized in relation to contaminant loads.

For flow-through and polishing applications, columns typically run at 100–150 cm/h. For that reason, they have large diameters (100–160 cm). Large diameters yield higher frontal surface areas, enabling greater flow rates (20–50 Lpm) at low linear-flow rates. However, such diameters drive column volumes up to 150–225 L (frontal surface area × column height 10–20 cm). That forces resin capacity to low levels (50–70 g product/L of AEX sorbent).

Conversely, polishing or trace contaminant removal can be conducted with an AEX membrane

Photo 2: 10, 20, and 30-in. membrane chromatography capsules can be run single and in parallel.

Figure 2: Key differences in base matrix; convective flow brings molecules in the mobile medium to the bead, but pore diffusion must take place to optimize binding; convective flow brings molecules in the mobile medium directly to the ligands on the membrane, requiring no pore diffusion.

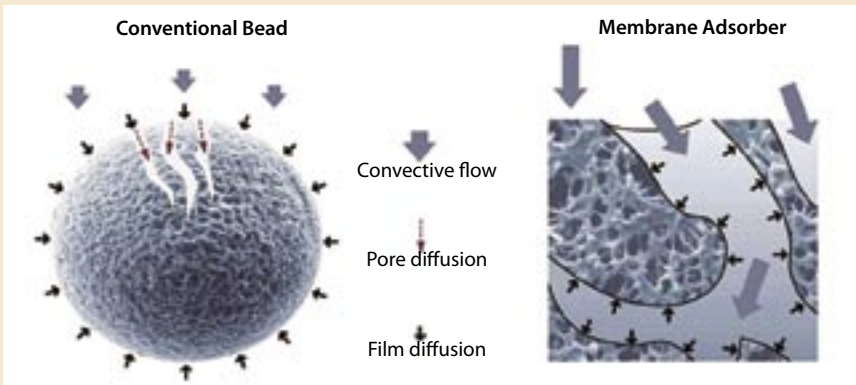


Table 1: Virus clearance and trace contaminant clearance values for the Sartobind Q unit operation in the CAMPATH 1-H process

Viruses	Size (nm)	Enveloped	Clearance by Sartobind Q Factor (log_{10})
SV-40: Simian Virus-40	16–25	no	1.34 ± 0.43
Reo-3: Respiratory Enteric Orphan III	75–80	no	3.62 ± 0.42
MuLV: Murine Leukemia Virus	80–110	yes	4.40 ± 0.56
PRV: Pseudorabies Virus	150–250	yes	3.88 ± 0.38
DNA			3
Host Cell Proteins			1
Endotoxin			Decreased

Table 2: Removal of very low levels of host cell protein by Q-Sepharose FF and a Sartobind Q 15 membrane adsorber (**5**)

	Host Cell Protein (ng/mg antibody)	Antibody Loaded (g antibody/L sorbent)	Flow Rate (cm/h)	Flow Rate (mL/min)	Flow Rate (CV/min)
Load	10.6	—	—	—	—
Q Sepharose FF	<2	50	76	0.43	0.06
Sartobind Q 15	<2	15,000	620	52	123

[a] A 1930.66-cm Q-Sepharose FF column was loaded to 50 g antibody/L resin at 76 cm/h (0.43 mL/min), and a Q15 membrane was loaded to 15,000g antibody/L membrane at 620 cm/h (37.6 mL/min). The effluent was collected in fractions across the load. No host-cell protein was detected in any of the fractions. Load material was type I, further purified by cation-exchange chromatography, then adjusted to pH 8, conductivity 7 mS/cm, with 1.5M Tris base and purified water to a final concentration of 2.5 g/L. Chromatography consisted of a 45-min exposure to 0.5M NaOH, 10–15 mL 250 mM Tris, 0.5 M NaCl, pH 8 (buffer B), 30–35 mL 25-mM Tris, 50-mM NaCl, pH 8 (buffer A), load, 20-mL buffer A, 10–15 mL buffer B, 45-min exposure to 0.5M NaOH, and 20mL 0.1 M NaOH.

Table 3: Virus clearance values for Sartobind Q when load challenge is >10.7 kg MAb/L membrane

Viruses	Size (nm)	Enveloped	Clearance by Sartobind Q Factor (log_{10}) Run 1	Clearance by Sartobind Q Factor (log_{10}) Run 2
MMV: Minute Mouse Virus	16–25	no	≥6.03	≥6.03
Reo-3: Respiratory Enteric Orphan III	75–80	no	≥7.00	≥6.94
MuLV: Murine Leukemia Virus	80–110	yes	≥5.35	≥5.52
PRV: Pseudorabies virus	150–250	yes	≥5.58	≥5.58

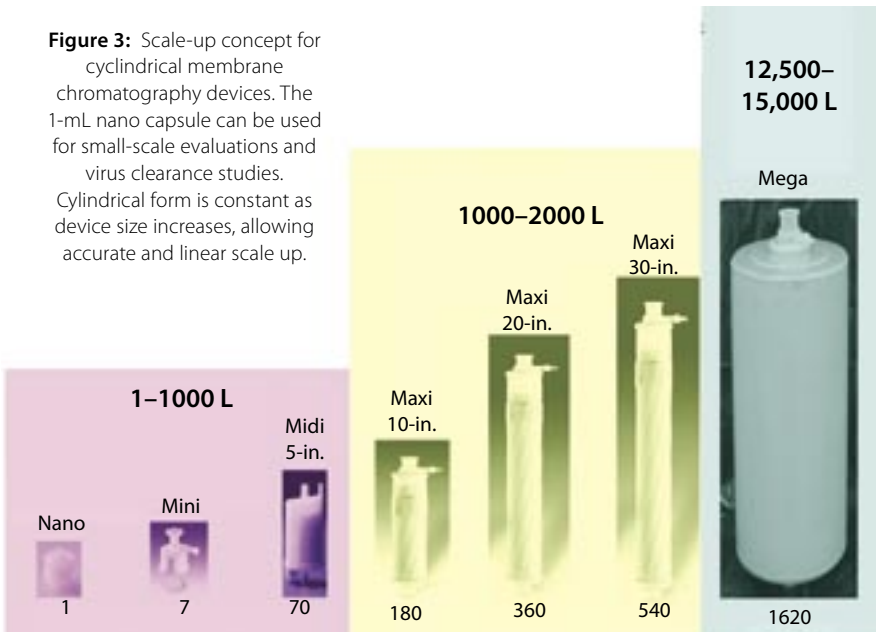


Figure 3: Scale-up concept for cyclindrical membrane chromatography devices. The 1-mL nano capsule can be used for small-scale evaluations and virus clearance studies. Cylindrical form is constant as device size increases, allowing accurate and linear scale up.

chromatography device that has a bed height of 4 mm at flow rates >450 cm/h. Higher linear flow rates reduce frontal surface area requirements. The result is a small-volume disposable membrane chromatography device that handles >50 Lpm. Such smaller devices (Photo 2) are still oversized with regard to trace contaminants and sized for increased flow rates. Disposable membrane chromatography devices also can be validated for virus clearance. We explore this concept in more detail below.

History of Use: The first disposable membrane chromatography step was implemented in a validated process for an FDA-approved product in 2001 (**4**). The unit operation was a product flow-through–polishing step. Its primary objective was to remove trace amounts of DNA. The process step was also validated as a virus clearance step for murine leukemia virus (MuLV), pseudorabies virus (PRV), respiratory enteric orphan III (Reo-III), and simian virus-40 (SV-40). Overall product flow-through capacity was ~1.6 kg MAb/L Q membrane at 5.2 Lpm or 240 cm/h.

Table 1 shows that virus and contaminant clearance values for this unit operation were significant enough to contribute to overall process clearance.

During the same year, a load capacity of 15 kg MAb/L at 620 cm/h was reported for a Q membrane (**5**). This was also a flow-through application for a polishing unit operation. Host-cell protein clearance for the Q membrane was comparable with QSFF (Q Sepharose fast flow) loaded at 50 g/L at 76 cm/h and was removed to below detection (Table 2).

At this point, it became apparent that membrane chromatography devices could be challenged with nearly 300× the amount of product than a column and still clear process-derived contaminants below the detection limit. However, the same publication reported a virus clearance value of 2.3 LRV for MuLV when the Q membrane was challenged to 2 kg-MAb/L at 620 cm/h. That value was hardly competitive with Q resins, but it must be noted that resins are typically challenged to 50–70 g MAb/L.

The Q membrane challenged to 2 kg MAb/L was only 10 layers thick rather than 15 layers, as are used for process-scale membrane chromatography devices. The disparity in layers may have been the reason why the virus clearance value was so low. With five fewer layers, a membrane device will have a shortened bed height, thus providing decreased residence time for particles in the mobile phase to bind to it. The same paper supports this concept using binding studies with one layer up to 60 layers. Also, subsequent virus clearance values with 15-layer devices demonstrate good virus clearance. For instance, Zhang et al. demonstrated virus clearance values >5 LRV for MuLV, PRV, Reo-III, and minute virus of mice (MVM) at 1.8 kg MAb/L Q membrane at 240 cm/h (**6**).

Recent Advancements

Recent advancements in membrane chromatography have increased flow-through capacities to >10.7 kg MAb/L of membrane and even greater: 35 kg MAb/mL of membrane has been achieved (**7**). Loading is at 450 cm/h, with process flow rates between 30 and 50 Lpm. Even at high load challenges, the membrane chromatography device provides >5 LRV virus clearance values for MuLV, PRV, Reo-III, and MVM (Table 3) (**8**). As with most polishing applications, DNA and CHOP are at relatively low levels. Thus, the load material was spiked with DNA, CHOP, and leached protein A (LProA) to demonstrate that removal is taking place. Results show very

good DNA removal, acceptable CHOP, and acceptable LProA during spiking experiments (**9**).

The development did not alter the base membrane or ligand density. But the downscale device was shaped into a cylinder, differing from previous scale-down devices in disc format. Discs show signs of membrane fouling not experienced with large-scale membrane chromatography devices. Hence, they show high pressure at low product–flow-through challenges. In return, disc format membranes validated were only for low product throughputs (1–2 kg/L). The new development enables aggressive product loading in the flow through-mode and accurate scale-up for large-scale manufacturing (Figure 3).

AEX resin columns and disposable membrane chromatography devices are both capable of trace contaminant removal and virus clearance. The difference between the two formats is load capacity at flow rates acceptable for large-scale manufacturing and disposability. Capacity and disposability are critical factors to consider when calculating unit operation costs for new products and processes.

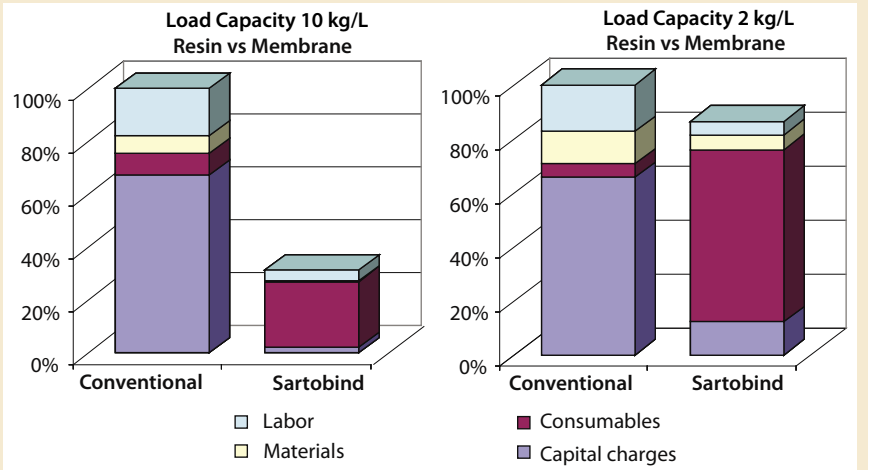
Process lifetime can be up to 10 years: generally the lifetime of a product before generics may compete and erode its price. That 10-year process is assumed to encompass 400 batch production runs and column cycling up to 100 times (four columns, total). Consider that each batch production run stems from a 15,000-L bioreactor. MAb yield at cell harvest is 1 g/L CHO. Thus, each batch produces 15 kg MAb — assuming a 100% process yield. The column size will be 215–225 L, and the membrane chromatography device will be 1.6 L.

Cost Models: Joe Zhou and Tim Tressel from Amgen have developed a disposable cost model for a 10-year process (**9**). The model compares resin and disposable membrane-based chromatography. Input data are from the aforementioned load capacity. Results demonstrate where cost benefits are and are not encountered. This economic model yields a 23% cost reduction when disposable membrane chromatography is used in place of resin-based chromatography. Overall operating costs for the FT AEX step are reduced in spite of increased consumable costs, although there is an economy of scale that was not considered in the evaluation. Media costs increase because a new membrane chromatography capsule is used for each chromatography cycle. Conversely, a column is reusable and can be cycled up to 100 times.

BioPharm Services provides a cost model that allows end users to input their own data to a software program. This program demonstrates that membrane chromatography costs break even to columns when membranes are loaded to 2 kg MAb/mL and 80% less than columns if loaded to 10 kg MAb/mL. The cost model considers all aspects of a Q FT AEX unit operation, including user interface, utilities, production, labor, consumables, materials, and capital equipment (**10**).

Dramatic reductions in buffer consumption, labor, and overhead are a direct result of smaller membrane chromatography devices. Column volumes — or in this case membrane volumes — are significantly reduced because a membrane chromatography device is 1.6 L, rather than 220 L. Smaller membrane volumes translate into less buffer needed for equilibration and wash steps while eliminating cleaning steps. In fact, buffer consumption is reduced >95%.

Figure 4: Graphic results from the Biopharm Services cost model. Component costs are added up to 100% percent for the column. Results show that membrane chromatography is 80% more affordable than columns if load capacity is 10 kg MAb/L and that membrane chromatography breaks even if loaded to 2 kg MAb/L of membrane.



Disposability eliminates significant upfront costs such as cleaning validation, a column lifetime study, assay development, packing studies, hardware, and columns. Those costs are not required with a disposable chromatography device. The cost benefits provided by reduced buffer consumption, processing time, and upfront costs overcompensate for the increased cost of membrane chromatography media cost (Figure 4).

Of Proven Benefit

Disposable membrane chromatography has proven itself to be beneficial for downstream and late-stage purification in the flow-through mode. Q membranes can offer the same or better virus clearance and trace contaminant removal as columns at equal or greater flow rates. However, a Q membrane will most likely be 200× smaller. A smaller device consumes less buffer and demands less processing time. Its disposable implementation eliminates considerable up-front validation and qualification costs. Disposable membrane chromatography provides a solution to a community that is continuously looking for viable disposable alternatives — not only as negative capture, but for protection of capture columns and for binding and elution applications.

References

1 Equipment Cleaning and Maintenance. *Code of Federal Regulations* Part 211.67, Title 21, Rev. 25 May 2004; www.access.gpo.gov/nara/cfr/waisidx_03/21cfr211_03.html.

2 O'Leary RM, et al. Determining the Useful Lifetime of Chromatography Resins: Prospective Small-Scale Studies. *BioPharm* 14(9) 2001: 10–18.

3 Zhou J, Tressel T. Membrane Chromatography as a Robust Purification System for Large Scale Antibody Production. *BioProcess International* 3(9, supplement) 2005: 32–37.

4 Galliher P, Fowler E. Validation of Impurity Removal by the CAMPATH 1-H Biomanufacturing Process. IBC's Biomanufacturing Week. San Diego, CA USA. November 2001.

5 Knudsen H, et al. Membrane Ion-Exchange Chromatography for Process-Scale Antibody Purification. *J. Chromatography A* 907(1–2) 2001: 145–154.

6 Zhang R, et al. Viral Clearance Feasibility Study with Sartobind Q Membrane for Human Antibody Purification. IBC's 3rd European Event, BioProduction 2004. Antibody Production and Downstream Processing. Munich, Germany. 26-27 October 2004.

7 Li F, et al. Current Therapeutic Antibody Production and Process Optimization. *Bioprocessing J.* September–October 2005: 28–30.

8 Zhou J, Tressel T. Concepts in Q Membrane Chromatography for Large-Scale Antibody Production. *Biotechnol. Prog.* 22(2) 2006: 341-349.

9 Zhou J, et al. Disposable Anionic Membrane Chromatography. IBC Technology Transfer for Biopharmaceuticals. Carlsbad CA USA March 2006.

10 Sinclair A, Monge M. Quantitative Economic Evaluation of Single Use Disposables in Bioprocessing. *Pharmaceut. Eng.* 22(3) 2002: 20–34.

*Corresponding author **Jeff Mora** is membrane chromatography product manager for Sartorius Purification Technologies, 131 Heartland Boulevard, Edgewood, NY 11717, 1-510-432-1887, fax 1-925-803-8984, jeff.mora@sartorius.com, www. sartorius.com. **Andrew Sinclair** is manager director of Biopharm Services, Lancer House East Street, Chesham HP5 1DG, United Kingdom. **Noushin Delmdahl** is product manager and **Uwe Gottschalk** is vice president of purification technologies at Sartorius AG, Weender Landstrasse 94-108, 37075 Germany.*