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Simplifying Progress

Non-Sterile and Sterile Membrane Chromatography Cassettes for Bind & Elute Applications of Viruses and Large Proteins

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1. Introduction

For flow-through polishing applications membrane adsorbers have become a well-established technology. However, there is an increasing demand for bind and elute purifications for larger targets as adeno- and lentiviruses, virus like particles (VLP) and influenza.[1] The reason is the higher binding capacity of macroporous membranes compared to conventional resins having much smaller pores and excluding them by size.

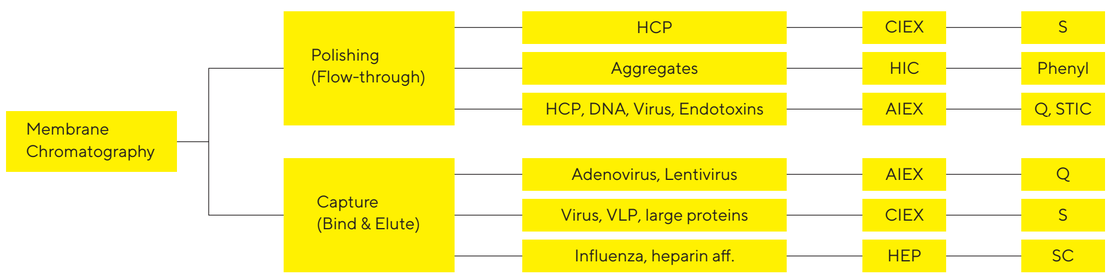


Figure 1: Typical modes of operations and applications for membrane adsorbers in the biopharmaceutical industry. Ligands: S = sulfonic acid for cation exchange (CIEX), Phenyl as hydrobhobic interaction chromatography ligand (HIC), Q = quaternary ammonium, STIC = STIC PA primary amine (salt tolerant) for anion exchange (AIEX), SC = sulfated cellulose

But capture applications with such devices suffered from the current size limitation of 5 liters e.g. in the Sartobind® Q or S Jumbo 5 L. (Fig. 2a). Here we describe a modular cassette system which has been tested for scale-up and flow performance in comparison with void volume optimized capsules. The goals were to create a system up to 20 L membrane volume which can be optionally expanded to ~100 liter and, be able adapt exactly to the size needed (modular), using the same 4 and 8 mm bed height as the capsules and membranes for single- or intra batch re-use. The system should also allow an option for sterile chromatography as some viruses are too large to finally sterile filter.

2. The Cassette Design: 2 Membrane Stacks 4 | 8 mm

In capsules the membrane is rolled up. To achieve the same flow pattern in the cassette, a cut through a capsule suggests two stacks of membrane with a central inlet flow channel. The fluid enters on top between the stacks and travels through these to the outside (down- stream) channels and then to the outlet (Fig. 2b). By this design approach the principal fluid path is maintained.

Figure 2a: Sartobind® Jumbo 5 L as largest adsorber size

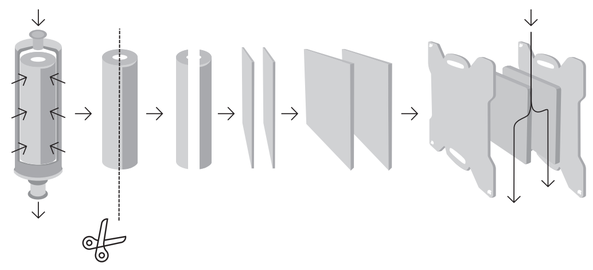


Figure 2b: Design for the cassette is derived from the capsule by cutting and creating two membrane stacks.

Figure 3 shows the realized cassette with a size of 634 × 387 × 47 mm and a dry weight of 4.9 kg (6.0 kg wet). The cassette can be assembled in a pilot filter holder which accommodates up to 13 cassettes (20.8 L, Fig. 4c) and are run with one manifold feeding the liquid in the upstream distribution channels and one manifold of the downstream channel (Fig. 4a). A process holder has been designed for up to ~100 L membrane (Fig 4b).

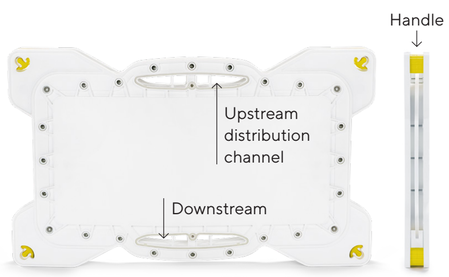


Figure 3: Design of Sartobind® Cassette

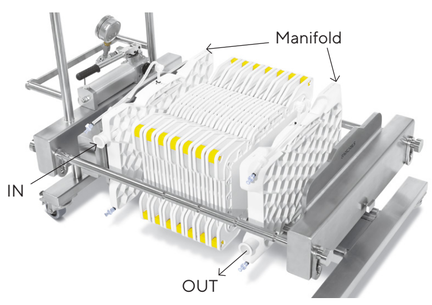


Figure 4a: Symmetrical manifolds at both ends of cassette pack are needed to connect the setup to a chromatography skid

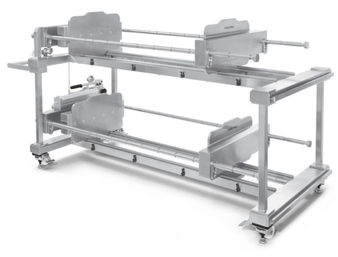


Figure 4b: 100 L Process Holder

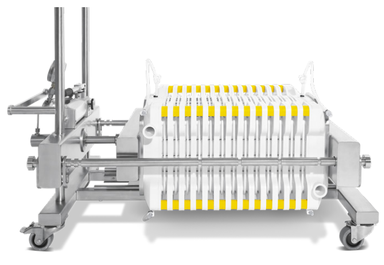


Figure 4c: 13 Cassettes (20.8 L) in the Pilot Filter Holder

Bed height	Nano mL	5" mL	10" mL	20" mL	30" mL	Jumbo mL	Cassette mL
4 mm	1	75	200	400	600	2500	800*
8 mm	3	150	400	800	1200	5000	1600*

Table 1: Existing Sartobind® membrane chromatography capsule portfolio with 4 mm and 8 mm bed height and Cassette membrane volumes; *sterile validated option

3. Sterile Chromatography on Q Cassettes 4 mm and 8 mm

Figure 5 shows the size of some viruses and the sterile filtration barrier at 200 nm. Viruses above that barrier typically cannot be sterile filtered at the final downstream purification step. To overcome this bottleneck, cassettes have been developed in a sterile validated version. Bacteria or viruses cannot replicate after implanting into cassettes and irradiating with 7 – 8 kiloGrays. As commercial cassettes are irradiated at ≥ 25 kGy they can be stated as sterile and be free of bacteria | viruses. Standard and gamma irradiated membranes showed no difference when loaded with standard BSA and virus loads. Extractables stayed within limits of USP88.

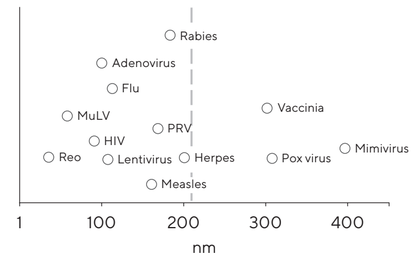


Figure 5: Sterile filtration barrier of about 200 nm avoids sterile filtration of larger viruses.

4. Scaleability

The scale-up from existing adsorber capsules to cassettes is essential.

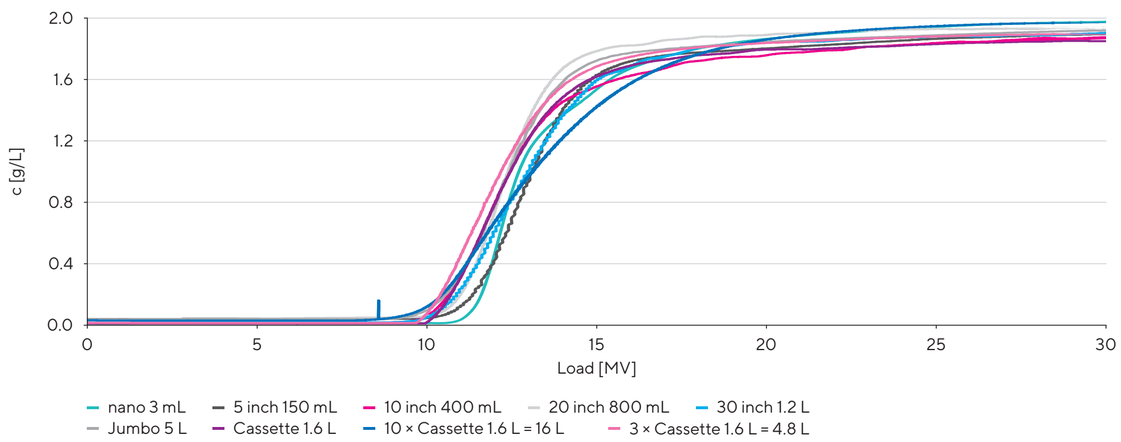
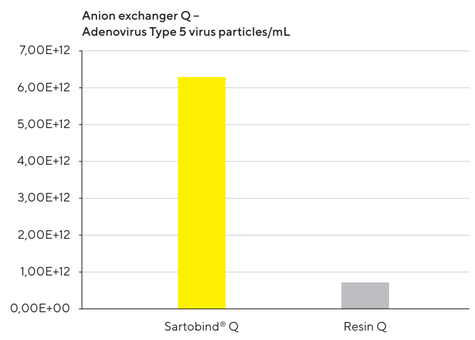


Figure 6: BSA breakthrough curves of void volume optimized capsules and single, 3 + 1.6 L cassette and 10 × 1.6 L cassette, 8 mm bed height versions.

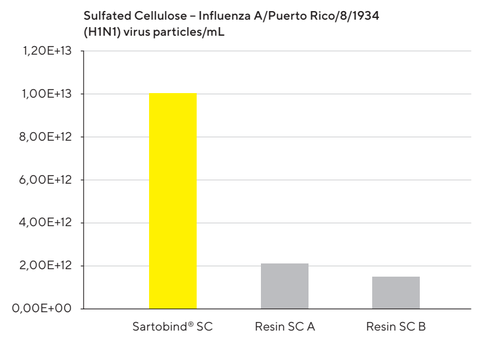
To compare breakthrough performance, devices were loaded with a 2 g/L bovine serum albumin (BSA) solution in 20 mM Tris/HCl pH 7.2. The equilibration was performed using 5 MV of equilibration buffer. The flow rate was 5 MV/min. 1.6 L Q Cassette setups displayed the same shape of breakthrough as the optimized capsule product line (Fig. 6).

5. Virus Capture on AIEX (Q) and Sulfated Cellulose (SC) Adsorbers vs. Resins



Column volumes: 1.0 mL (Sartobind® Q), 20 mL (Resin Q)
Flow rate: 15 CV/min (Sartobind® Q), 0.15 CV/min (Resin Q)
Feed: 8.3 × 10^{12} VP/mL in 50 mM HEPES, 200 mM NaCl, pH 7.5

Comparison of Sartobind® Q binding capacity for Ad 5 displays about 9 times more binding capacity and ~100 times higher flow rate compared to a conventional resin.[2]



Column volumes: 0.08 mL (SC), 0.18 mL (Resin A, Resin B)
Flow rate: 3.75 CV/min (SC), 0.67 CV/min (Resin A), 1 CV/min (Resin B)
Feed: 6 × 10^{07} VP/mL, 30 kHAU/mL in 10 mM TRIS, pH 7.4

Evaluation of the recently developed SC displays ~5 to 7 × higher binding capacity of virus particles (VP)/mL, HAU/mL and ~6 × higher flow rate than conventional resin columns.[3] Comparison with influenza B/Phuket/3073/2013 showed even up to 23-fold higher binding capacity compared to resins.[4]

6. Summary

The Sartobind® Cassette system is a prerequisite for large scale bind & elute membrane chromatography. Combined with anion exchangers and newly developed virus capture membranes such as sulfated cellulose adsorbers it intensifies manufacturing of virus and VLP. The gamma irradiated versions remove a bottleneck in purification of viruses with a size above > 200 nm, as they cannot be sterile filtered. To keep the product sterile also the chromatography step must be run in a sterile validated manner.

7. References

(1) Opitz L.: Sulfated membrane adsorbers for economic pseudo-affinity capture of influenza virus particles. Biotechnol Bioeng 2009 103(6), 1144-1154.

(2) Application Note Sartorius Stedim Biotech: Optimizing Adenovirus Purification Processes, April 2017

(3) Taft, F., Köhler, R. van Teeffelen, S., Fortuna, A. R., Wolff, M., Reichl, U., Villain, L.: Influenza virus capture using membrane chromatography: Improving selectivity by matrix design and pseudo-affinity ligand interactions, PREP Int. Symposium, Preparative and Process Chromatography, Philadelphia, USA, July 19-20, 2016, poster

(4) Fortuna AR, et al., Sulfated cellulose membrane adsorbers as a platform technology for purification of cell-culture derived influenza vaccines, 11th Int Congress on Membranes and Membrane Processes, 2017 July 29 – August 4, San Francisco, USA