Simplifying Progress

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

M. Leuthold[1], S. Weisshaar[1], F. Taft[1], M. Hirai[1]

[1]Sartorius Stedim Biotech GmbH, August-Spindler-Str. 11, D-37079 Goettingen, Germany
Contact: Polishing-Technologies@Sartorius.com

1. Introduction

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

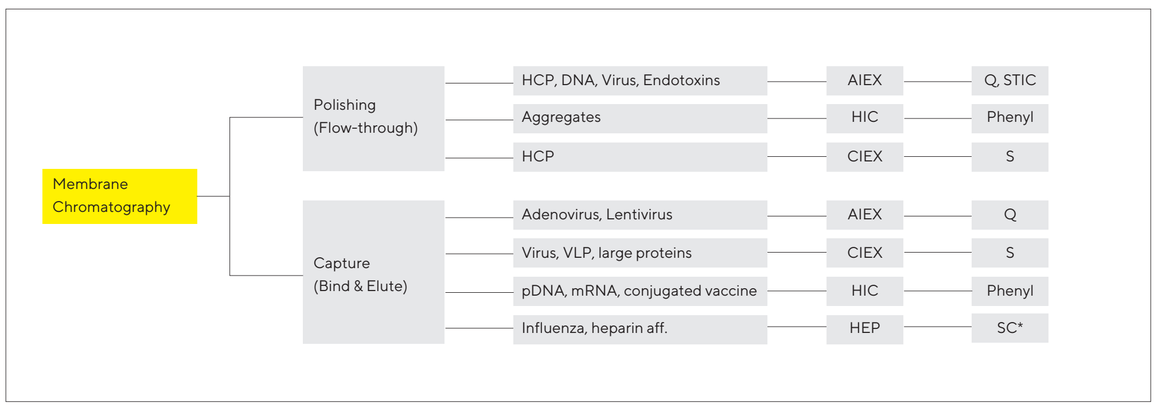


Figure 1: Typical modes of operations and applications for membrane adsorbers in the biopharmaceutical industry. Ligands: S = sulfonic acid for cation exchange (CIEX), Phenyl as hydrobhobic interaction chromatography ligand (HIC), Q = quaternary ammonium, STIC = STIC PA primary amine (salt tolerant)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

Capture applications very often require a larger amount of chromatographic matrix and our current device portfolio was limited to 5 liter of membrane in the largest capsule 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 goal was to develop 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 capsule format.

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 (downstream) channels and then to the outlet (Fig. 2b). By this design approach the principal fluid path is maintained.

Figure 2a: Sartobind® Jumbo 5 L is the largest capsule size.

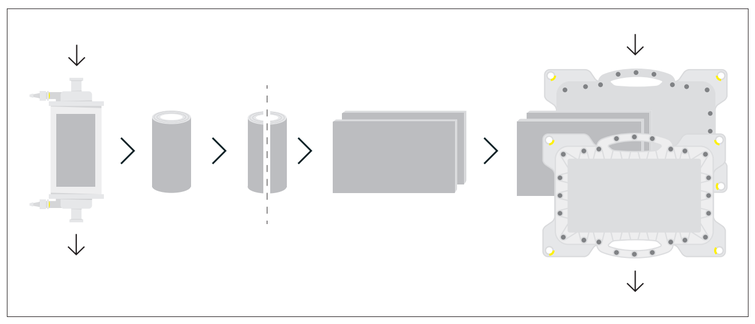


Figure 2b: Design for the cassette is derived from the capsule by cutting and creating two membrane stacks.

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 of membrane, 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).

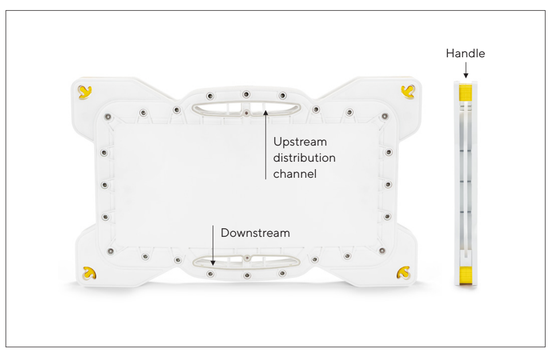


Figure 3: Design of Sartobind® cassette

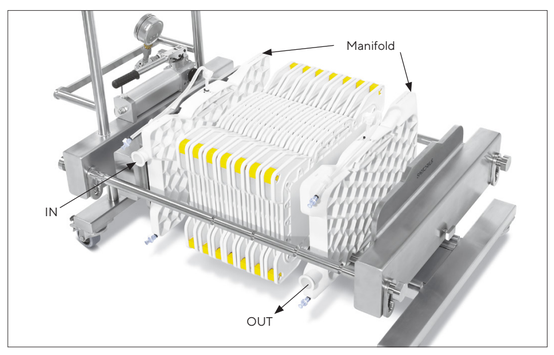


Figure 4a: Symmetrical manifolds at both ends of cassette pack are needed to connect the setup to a chromatography skid

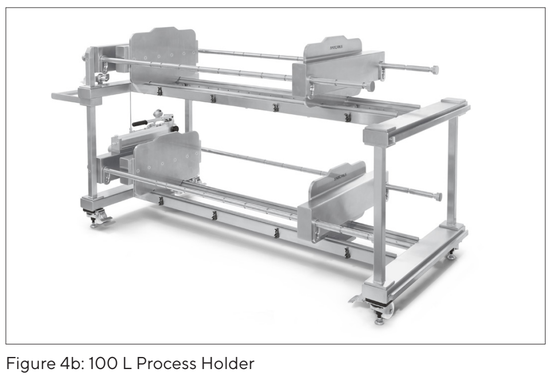


Figure 4b: 100 L Process Holder

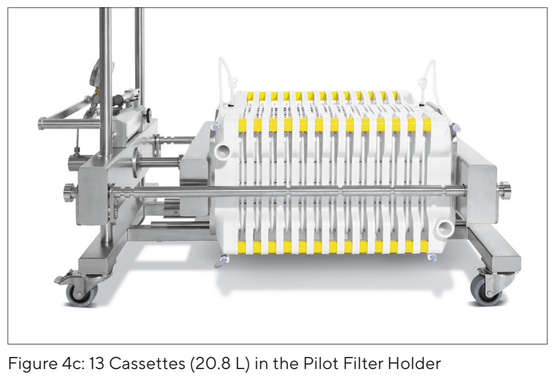


Figure 4c: 13 Cassettes (20.8 L) in the Pilot Filter Holder

Table 1: Existing Sartobind® membrane chromatography capsule portfolio with 4 mm and 8 mm bed height and cassette membrane volumes

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

3. Comparison of capsule and cassette fluid paths

Figure 5 shows the construction of the standard membrane adsorber devices with 4 and 8 mm bed height. In the cassette (Fig. 6) the same flow principle is applied as in the existing capsules as feed flows perpendicular through the membrane and through fluid channels.

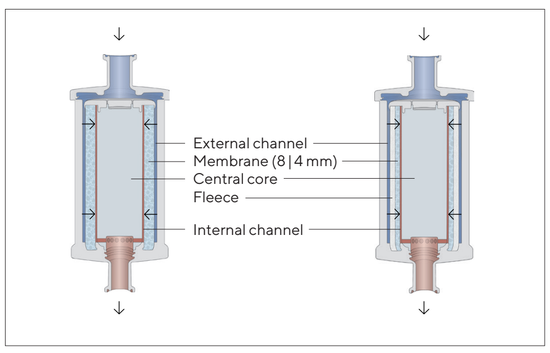


Figure 5: Design and flow path of membrane adsorber capsules with 8 mm (left) and 4 mm (right) bed height.

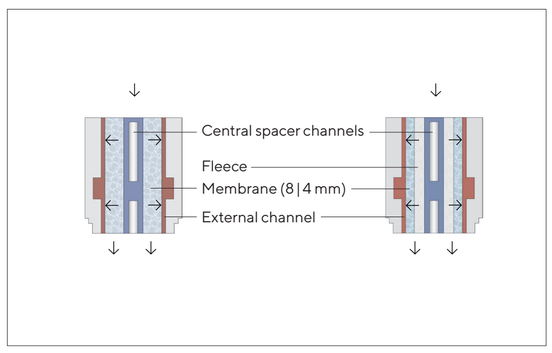


Figure 6: Design and flow path of the scalable cassette format with 8 mm (left) and 4 mm (right) bed height.

4. Scalability

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

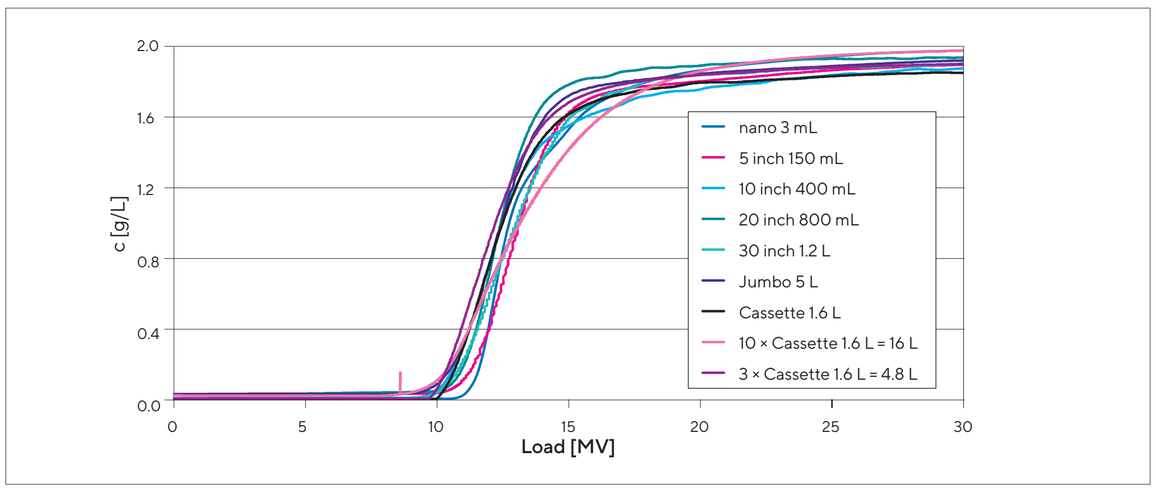
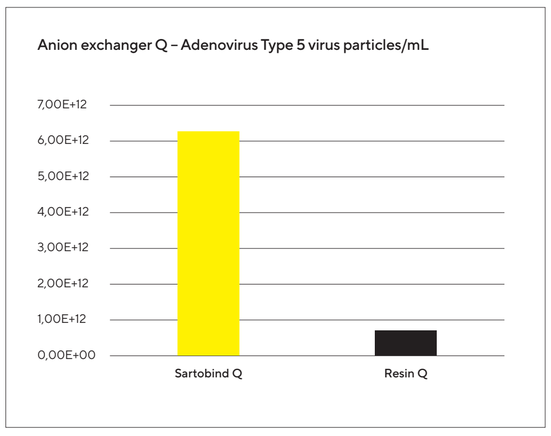


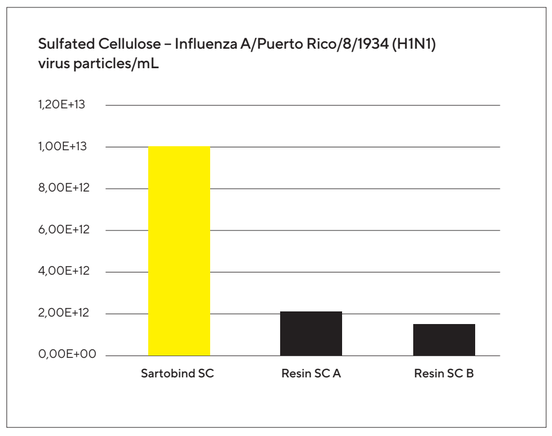
Figure 7: BSA breakthrough curves of capsules and single, 3 × 1.6 L cassette and 10 × 1.6 L cassette, 8 mm bed height versions.

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 Sartobind® Q cassette setups displayed the same shape of breakthrough as the capsule product line (Fig. 7).

5. Virus capture on AIEX (Q) and sulfated cellulose (SC) adsorbers vs. resins



Comparison of Sartobind Q binding capacity for Ad 5 results in 9 fold higher binding capacity and ~100 times higher flow rate compared to a conventional resin.[2]



Evaluation of the recently developed SC membrane shows ~5 to 7 fold higher binding capacity of virus particles (VP)/mL, HAU/mL and ~6 fold higher flow rate than conventional resin columns.[3]

6. Summary

The modular and scalable Sartobind® cassette format overcomes the size limitations 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.

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