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Robust and Scalable Ultrafiltration and Diafiltration for Downstream Adeno-Associated Virus (AAV) Processing Using Sartocon® Hydrosart® Tangential Flow Filtration Cassettes

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Abstract

Effective downstream processing of adeno-associated virus (AAV) vectors is essential to improving patient access to gene therapies. Efficient, consistent, and scalable ultrafiltration and diafiltration are critical aspects of downstream AAV processing, as they significantly impact viral recovery and impurity removal.

This application note provides performance data of Sartocon® Hydrosart® tangential flow filtration (TFF) cassettes for ultrafiltration and diafiltration of AAV8. We evaluated two cassette configurations, ECO and E-Screen, to determine their effectiveness in processing AAV8 from harvested lysate. We identified optimal operating conditions, such as transmembrane pressure and pump rates by performing small-scale trials and compared the performance of the two configurations before testing the reproducibility and scalability of the best-performing cassette.

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Introduction

In the rapidly evolving gene therapy landscape, adeno-associated Virus (AAV) vectors have emerged as indispensable tools for delivering genetic material with unprecedented precision thanks to the existence of diverse AAV serotypes. As the demand for AAV-based therapeutics continues to grow and evolve to treat systemic diseases, developing manufacturing processes that ensure scalability, reproducibility, and cost-effectiveness becomes paramount. A critical aspect of AAV production lies in the downstream purification steps, with ultrafiltration and diafiltration (UF | DF) playing a key role in achieving high product purity and yield while allowing the virus to be formulated into a desired buffer.

Sartorius offers two different Sartocon® cassette geometries for UF | DF operations: the E-Screen and ECO formats. The ECO cassette is designed for concentration and diafiltration of low-viscosity solutions (<3 cp) based on tangential flow filtration (TFF), whereas the E-Screen cassette is suitable for high-viscosity solutions (> 3 cp and protein concentration > 20%).[1] Compared to the E-Screen geometry, the ECO cassette has 26% more surface area per standard cassette width, allowing more area to be installed in the cassette holder and reducing the power requirement of the feed pump. As a result, the need for larger equipment is reduced when scaling up processing with the ECO cassette format. The shear stress applied to the virus particles during processing is also reduced. In addition, Hydrosart® high-performance UF | DF membranes have been optimized for biopharmaceutical process applications with a wide pH and temperature range. These cellulose-based membranes are extremely hydrophilic, making them non-protein binding and virtually non-fouling.[2] As a result, they have extremely high fluxes.

In a previous study with AAV serotype 8 (AAV8), we used Ambr® Crossflow and MODDE® design of experiments (DoE) software to show that 100 kDa TFF cassettes provide superior recovery and removal of protein and DNA.[3] Here, we aimed to establish a robust concentration and diafiltration unit operation of AAV8 using Sartocon® Hydrosart® 100 kDa TFF cassettes. We compared product recovery (viral genome [vg] titer), impurity removal (total protein and dsDNA), and speed (permeate flux) across different cassette configurations, ECO and E-Screen, at a small scale. We also assessed the reproducibility and feasibility of scaling up the established process with the selected cassette configuration.

Figure 1: *The Sartocon® Slice 200 Cassette Represents the Smallest Scale-Down Device in the Sartocon® Cassette Product Family*

Materials and Methods

AAV8 was produced by transient transfection of HEK293 cells using FectoVIR®-AAV (Sartorius). Cells were cultivated in a 10 L Univessel® Glass bioreactor controlled by a Biostat® B (Sartorius). At the time of harvest, Tween was added to the bioreactor to lyse the producing cells to release the AAV particles. An endonuclease step was performed to digest nucleic acids for optimal results during downstream processing. Then, the harvest clarification was done with a Sartoclear® DL75 depth filter followed by a Sartopore® 2 XLG membrane filter (both Sartorius).[4] Harvested AAV8 was stored in aliquots, frozen at –80 °C, and used as feed for all the studies. The titer of the AAV8 material was ~3×10^{10} vg/mL.

TFF experiments were conducted using 100 kDa Hydrosart® Sartocon® Slice 200 ultrafiltration cassettes (Sartorius; Figure 1) with both an E-Screen and ECO channel configuration. Small-scale process development was performed on a Sartoflow® Smart TFF System using a Sartocon® Slice 200 with 180 cm² membrane area (Sartorius; Figure 2). The scaled-up experiment was performed using a Sartoflow® Advanced TFF System using 2 × Sartocon® Slice with 0.14 m² membrane area (Sartorius; Figure 3).

Figure 2: *Sartoflow® Smart TFF System in the Configuration Used for the Study*

Figure 3: *Sartoflow® Advanced TFF System*

The cassettes tested were evaluated under a constant feed flow rate. During the trials, a permeate flow rate characterization study was carried out for all cassettes to determine the optimal operating pump rate and transmembrane pressure (TMP) condition for UF | DF operation. For each module, the pump rate and TMP were ramped up until a decrease in the corresponding permeate flow rate was observed. The optimal TMP was selected as the inflection point of the permeate flow rate.

All experiments were conducted using the same initial total loading volume of 1 L (Sartocon® Slice 200) or 10 L (Sartocon® Slice) followed by a 10-fold concentration and 5 times diafiltration with a buffer composed of 20 mM Tris, 200 mM NaCl, 0.1% (w/w) poloxamer 188, 2 mM $MgCl_2$, at pH 7.5. After UF | DF, the system and cassettes were flushed twice with one hold-up volume each by recirculating diafiltration buffer (50 mL for the Sartocon® Slice 200 and 200 mL for the Sartocon® Slice) for 5 min through the system. The flushes were then combined with the retentates.

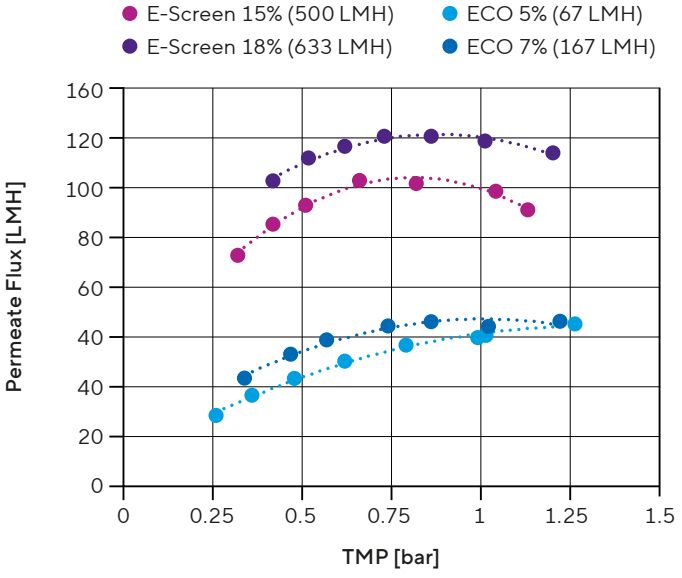
Analytical testing included vg titer by ddPCR, total protein by BCA, and total dsDNA by PicoGreen™ assays.

Results

TMP Optimization

We first determined the optimal operating TMP for each cassette at a small scale using the Sartocon® Slice 200 cassette and the Sartoflow® Smart TFF system. To this end, the permeate flux was measured at increasing TMP values and two different target pump rates for each cassette type (Figure 4).

Figure 4: *Permeate Flux Measured as a Function of TMP at Two Different Pump Rates for Both Sartocon® Slice 200 Hydrosart® 100 kDa ECO and E-Screen Cassettes*



The optimum TMP range was selected as the range approaching the pressure-independent zone of the process (further increases of pressure do not increase permeate flux), typically close to where one achieves optimal performance from an ultrafilter. The selected TMP ranges were 0.65 – 0.70 bar (at 7% pump rate) for the ECO and 0.60 – 0.65 bar (at 18% pump rate) for the E-Screen cassette.

Performance of Sartocon® ECO and E-Screen Cassette Formats in AAV8 Concentration and Diafiltration

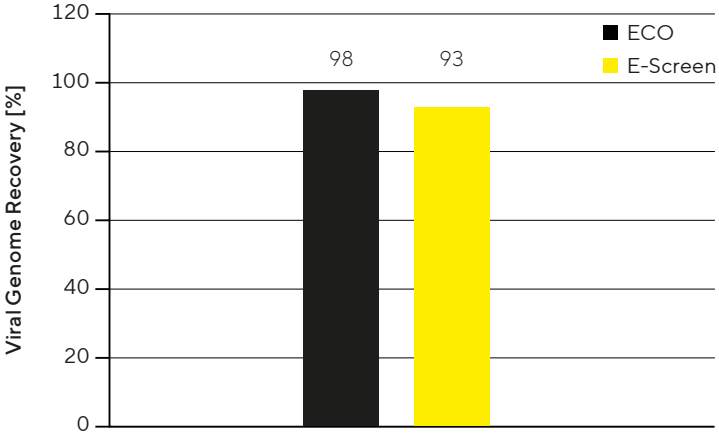
With the optimal range for the TMP and pump rate identified for each ultrafiltration cassette type, we evaluated the performance of each cassette type in the concentration and diafiltration of harvested AAV8 lysate (Table 1, Figure 5, and Figure 6).

Table 1: *Parameters and Results of Small-Scale AAV8 UF | DF Runs Performed with Sartocon® Slice 200 Hydrosart® ECO and E-Screen*

Hydrosart® 100 kDa	ECO	E-Screen
Pump rate [%] (constant)	7 – 8%	18 – 20%
Target feed flow-rate [LMH]	167	633
TMP [bar]	0.65 – 0.70	0.60 – 0.65
Run time [min]	85	70

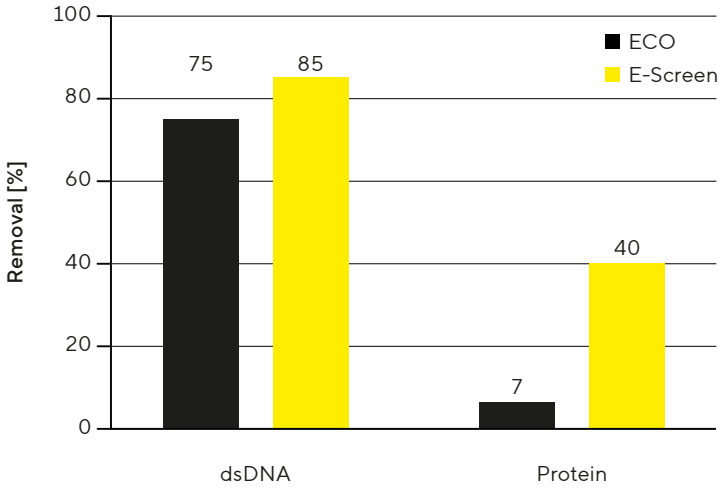
We observed that the pump rate is 2 – 3 times lower for the ECO (7 – 8%) compared to the E-Screen (18 – 20%) cassette format (Table 1) to achieve a similar TMP (0.6 – 0.7 bar). Processing time was also around 17% lower for the E-Screen compared to the ECO cassette format.

Figure 5: *AAV8 Viral Genome Recovery After UF | DF Performed With Sartocon® Slice 200 Hydrosart® 100 kDa ECO and E-Screen TFF Cassettes*



For functional performance parameters, we observed that genome recovery was slightly higher with the ECO (98%) compared to the E-Screen (93%) cassette (Figure 5). Both cassettes demonstrated a generally high dsDNA removal efficiency (75 and 85%). However, protein removal was considerably higher with the E-Screen cassette (40%) than with the ECO cassette format (7%) (Figure 6).

Figure 6: *Impurity (dsDNA and Protein) Removal After UF | DF Performed With Sartocon® Slice 200 Hydrosart® 100 kDa ECO and E-Screen TFF Cassettes*



Based on the lower pump rate requirement and higher product recovery, we continued with the ECO cassette format to further evaluate the AAV8 UF | DF process.

Reproducibility of the Established AAV8 UF | DF Process

Three independent UF | DF runs of the harvested AAV8 lysate were performed using the Sartocon® Slice 200 Hydrosart® 100kDa ECO cassettes and the Sartoflow® Smart TFF system to assess the reproducibility of the established process. The parameters were applied as shown in Table 1 (ECO cassette).

In general, the virus recovery and impurity removal results aligned with those obtained during the initial experiments (Table 2). Overall, good reproducibility was observed between the three runs, with a standard deviation of 18% for viral genome recovery. Similar reproducibility was seen for dsDNA and protein removal with only 3 and 6% standard deviation, respectively.

Table 2: *AAV8 Recovery (Viral Genome) and Impurity (dsDNA and Protein) Removal After UF | DF Performed With Sartocon® Slice 200 Hydrosart® 100kDa ECO*

Hydrosart® 100 kDa ECO		
	Recovery [%] ± Stdev	Removal [%] ± Stdev
Viral genome	121 ± 18	
dsDNA		79 ± 3
Protein		13 ± 6

Note. Values shown are % mean ± standard deviation (n = 3).

Based on the results obtained, we conclude that the established UF | DF process for AAV8 using the Hydrosart® 100 kDa ECO TFF cassette is very efficient and robust, yielding highly reproducible results.

Scale-Up of AAV8 UF | DF Using Sartocon® Cassettes

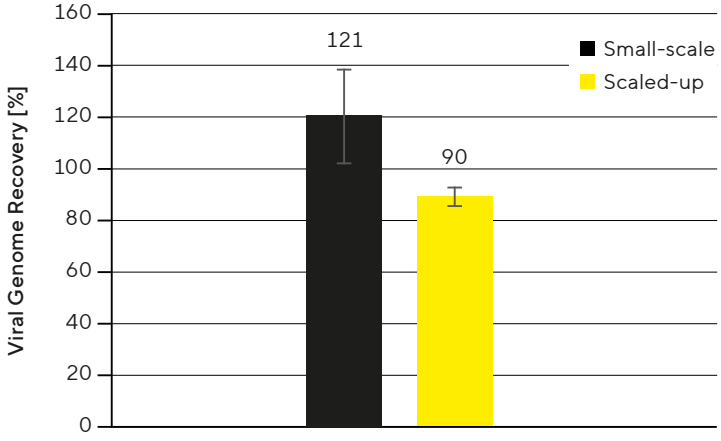
We next assessed the ability of the established process to scale linearly. We performed a UF | DF run with a 10-fold larger volume of harvested AAV8 lysate and scaled the process parameters accordingly. We used two Sartocon® Slice cassettes to provide the membrane area required for a 10-fold increase of lysate volume from the Sartocon® Slice 200 cassette format. Process parameters, such as TMP and pump rate, were scaled to achieve the same feed flux as for the small-scale Sartocon® Slice 200 cassette runs (Table 3).

Table 3: *Parameters of Small-Scale and Scaled-Up AAV8 UF | DF Runs Performed With Either Sartocon® Slice 200 Hydrosart® ECO (Small-Scale) or 2× Sartocon® Slice Hydrosart® 100kDa ECO (Scaled-Up)*

Hydrosart® 100 kDa ECO	Small-Scale	Scaled-Up
Pump rate [%] (constant)	8	11
Target feed flow-rate [LMH]	167	167
TMP [bar]	0.65 – 0.70	0.63 – 0.65
Run time [min]	89	58

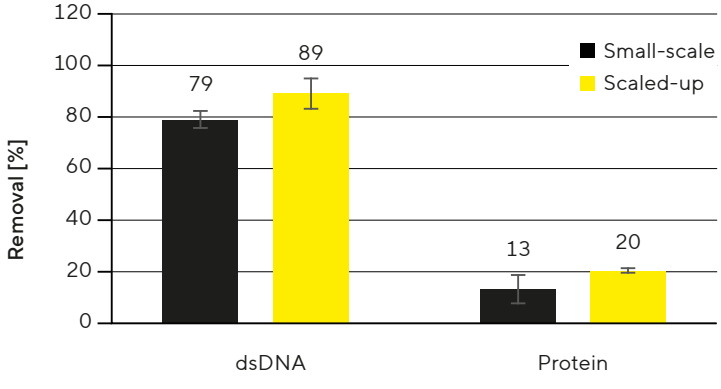
A slightly lower virus recovery was obtained (90 vs. 121% vg; Figure 7). However, contaminant removal results were largely unchanged when scaling up the established AAV8 ultrafiltration and diafiltration process (89 vs. 79% dsDNA and 20 vs. 13% protein; Figure 8).

Figure 7: *AAV8 Recovery Following UF | DF With Hydrosart® 100 kDa ECO Cassettes on the Sartocon® Slice 200 (Small-Scale) and 2x Sartocon® Slice (Scaled-Up)*



Note. Small-scale results are the mean from n = 3, and scaled-up results are the mean from n = 2.

Figure 8: *Impurity (dsDNA and Protein) Removal After UF | DF Performed With Hydrosart® 100 kDa ECO Cassettes on the Sartocon® Slice 200 (Small-Scale) and 2x Sartocon® Slice (Scaled-Up)*



Note. Small-scale results are the mean from n = 3, and scaled-up results are the mean from n = 2.

Overall, the virus recoveries and contaminant removal levels obtained at small scale were very much aligned with the results from the scaled-up run, suggesting linear and predictable scaling from the Sartocon® Slice 200 cassette format to the Sartocon® Slice format.

Conclusion

In this study, we evaluated the performance of two Hydrosart® 100 kDa TFF cassette configurations, ECO and E-Screen, during the UF | DF steps of downstream AAV8 processing. The UF | DF process was conducted at a constant feed flow rate.

Our optimized process parameters resulted in impressive AAV8 genome recoveries exceeding 93%, while achieving significant removal of dsDNA (> 75%) and proteins (7–40%), irrespective of the cassette type used. Both the ECO and E-Screen cassettes demonstrated comparable performance with only minor distinctions:

- The ECO cassettes provided slightly higher viral genome recovery
- The E-Screen cassettes generally exhibited superior impurity removal

Although the E-Screen cassettes required 2 – 3 times more pump power than the ECO cassettes, they achieved higher permeate flux and reduced processing times. Despite this, the ECO cassettes facilitated efficient AAV concentration within a reasonable timeframe and under milder processing conditions, making them advantageous for larger-scale processes where reduced pump capacity might be necessary.

Both different cassette configurations are viable options, depending on specific application and process requirements. Key considerations when selecting the appropriate cassette include:

- Pump power availability at larger scales (E-Screen cassettes demand higher pump rates)
- Shear stress concerns, which can be addressed by selecting consumables that allow for milder flow rates or designing faster runs to reduce exposure times
- Process duration (E-Screen offers higher permeate flux and consequently shorter processing times)

This study underscores the suitability and competitiveness of Sartocon® Hydrosart® TFF cassettes for UF | DF steps in AAV processes. Both the ECO and E-Screen configurations consistently demonstrate:

- Exceptional AAV8 recovery (93%)
- High reproducibility across different runs
- Scalability from small-scale (1 L feed stream) to larger-scale applications (10 L), with membrane surface areas ranging from 0.018 to 0.28 m^2

In conclusion, Sartocon® Hydrosart® TFF cassettes are an outstanding choice for UF | DF in AAV production processes, offering the potential for similar performance across various AAV serotypes.

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