

Robust and Scalable Ultrafiltration and Diafiltration for Downstream Adeno-Associated Virus (AAV) Processing

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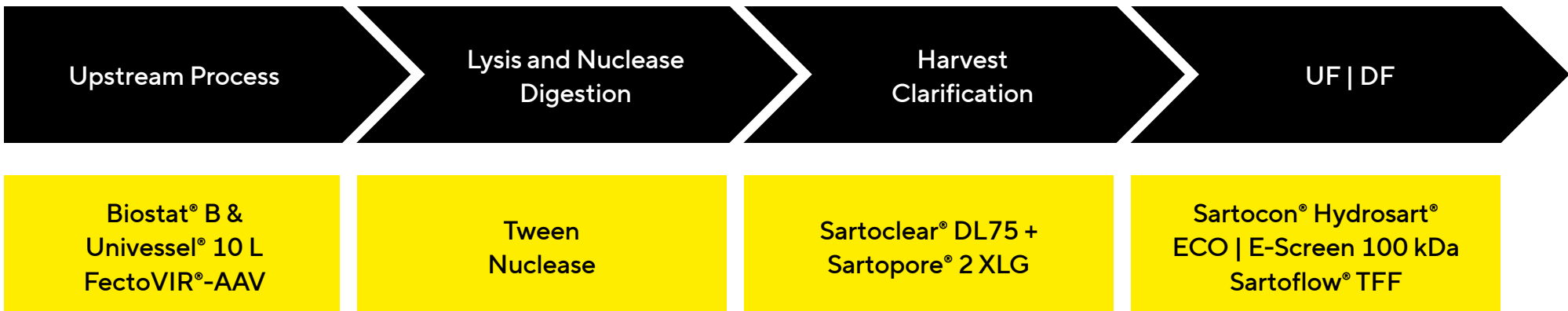
1. Introduction

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 Sartoclon[®] 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.

2. Materials & Methods

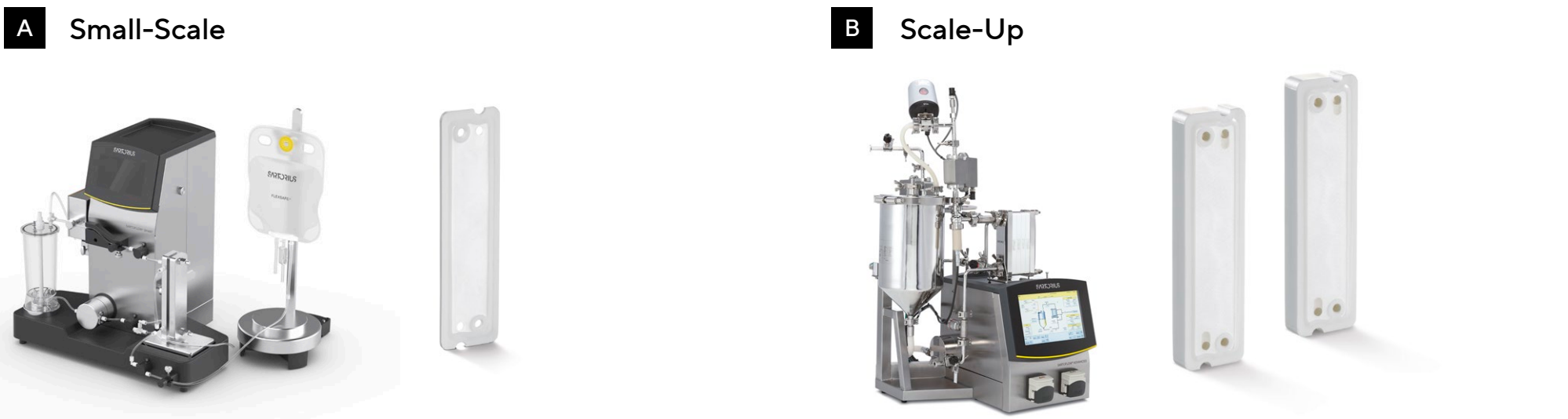
AAV Production Process Overview

Figure 1: Schematic of the Applied AAV Production Process for Establishing the UF | DF Protocol



TFF Experiments Using Sartoclon[®] Hydrosart[®] Cassettes

Figure 2: (A) Sartoflow[®] Smart TFF System in the Configuration Used for the Study; The Sartoclon[®] Slice 200 Cassette Represents the Smallest Scale-Down Device in the Sartoclon[®] Cassette Product Family (180 cm² membrane area). (B) Sartoflow[®] Advanced TFF System; Sartoclon[®] Slice Cassette (2 × 0.14 m² membrane area each)



All experiments were conducted using the same initial total loading volume of 1 L (Sartoclon[®] Slice 200) or 10 L (Sartoclon[®] Slice) harvested AAV8 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 MgCl₂ at pH 7.5.

Analytical Methods

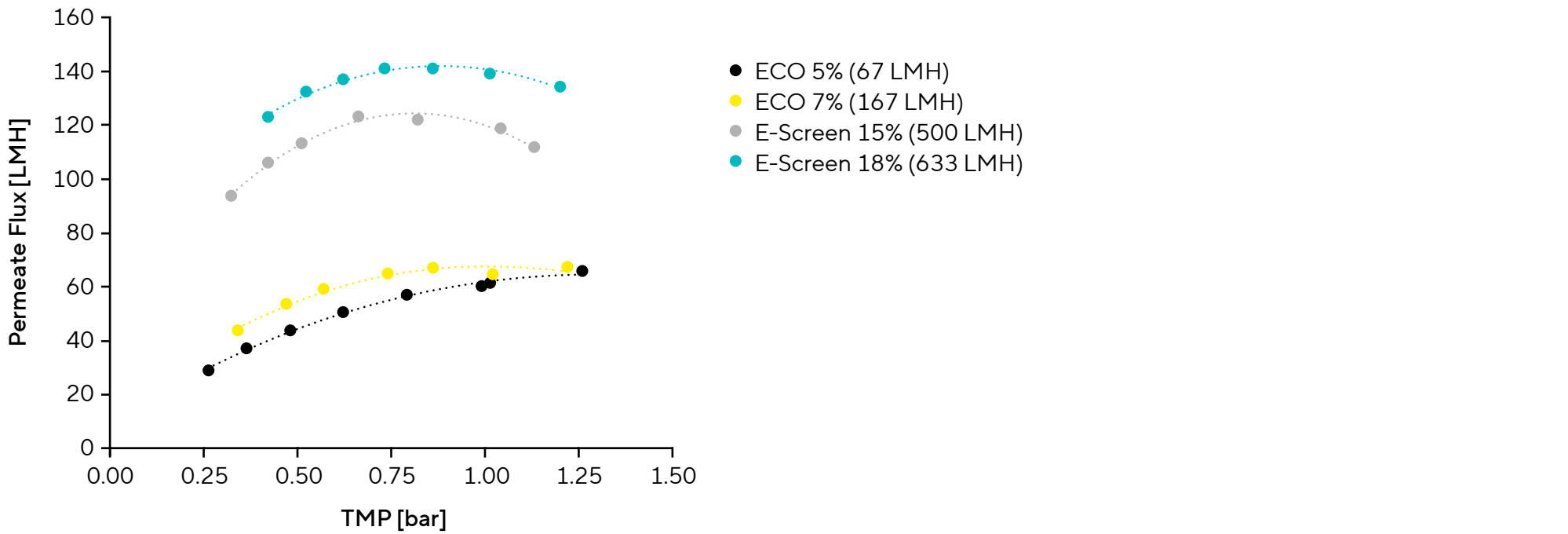
Analytical testing included vg titer by ddPCR, total protein by BCA, and total dsDNA by PicoGreen[™] assays.

3. Results

TMP Optimization

Operating TMP was determined by measuring the permeate flux at increasing TMP values and two different target pump rates for each cassette type.

Figure 3: Permeate Flux Measured as a Function of TMP at Two Different Pump Rates for Both Sartoclon[®] Slice 200 Hydrosart[®] 100 kDa ECO and E-Screen Cassettes



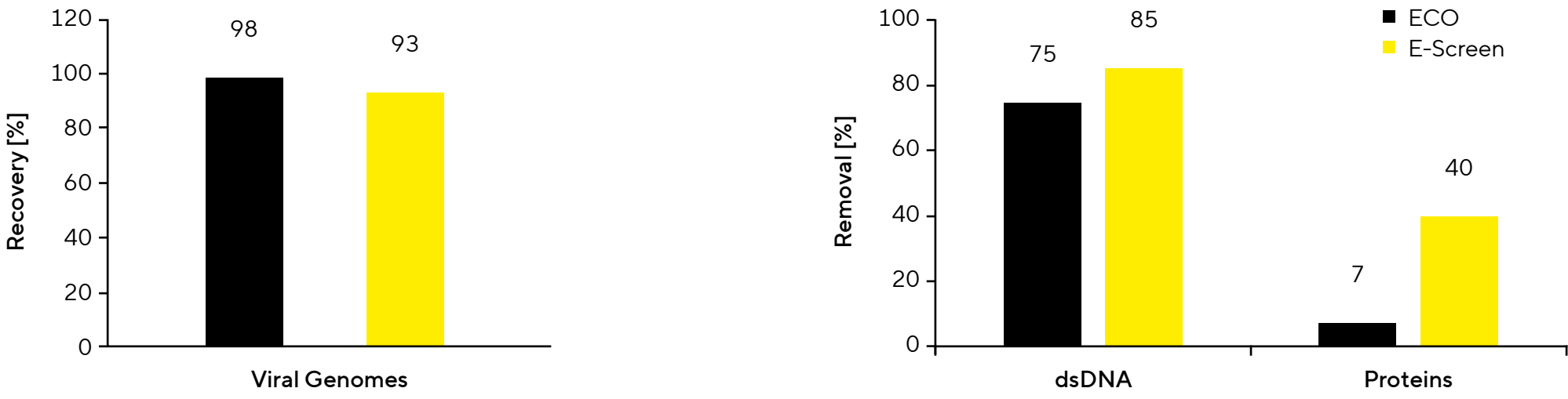
The optimum TMP range was selected as the range approaching the pressure-independent zone of the process (further increases of pressure do not increase permeate flux), typically close to where one achieves optimal performance from an ultrafilter.

Performance in the concentration and diafiltration of harvested AAV8 lysate was assessed using the optimal range for the TMP and pump rate identified for each ultrafiltration cassette type.

Table 1: 100 kDa ECO and E-Screen TFF Cassettes

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 (UF DF) [min]	85	70

Figure 4: AAV8 Viral Genome Recovery (Left) and Impurity (dsDNA and Protein) Removal (Right) After UF | DF Performed With Sartoclon[®] Slice 200 Hydrosart[®] 100 kDa ECO and E-Screen TFF Cassettes



- Pump rate was 2–3 times lower for the ECO compared to the E-Screen cassette format with a similar TMP, resulting in a lower processing time for the E-Screen compared to the ECO cassette format.
- Both cassettes demonstrated a generally high viral genome recovery and dsDNA removal efficiency. However, protein removal was considerably higher with the E-Screen cassette than with the ECO cassette format.

Reproducibility of the Established AAV8 UF | DF Process

- Based on the lower pump rate, we continued with the ECO cassette format to further evaluate the AAV8 UF | DF process.
- Three independent UF | DF runs of the harvested AAV8 lysate were performed using the Sartoclon[®] Slice 200 Hydrosart[®] 100 kDa ECO cassette type.

Table 2: AAV8 Recovery (Viral Genome) and Impurity (dsDNA and Protein) Removal After UF | DF Performed With Sartoclon[®] Slice 200 Hydrosart[®] 100 kDa 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).

Virus recovery and impurity removal results aligned with those obtained during the initial experiments and good reproducibility was observed between the three runs.

Scale-Up of AAV8 UF | DF Using Sartoclon[®] Cassettes

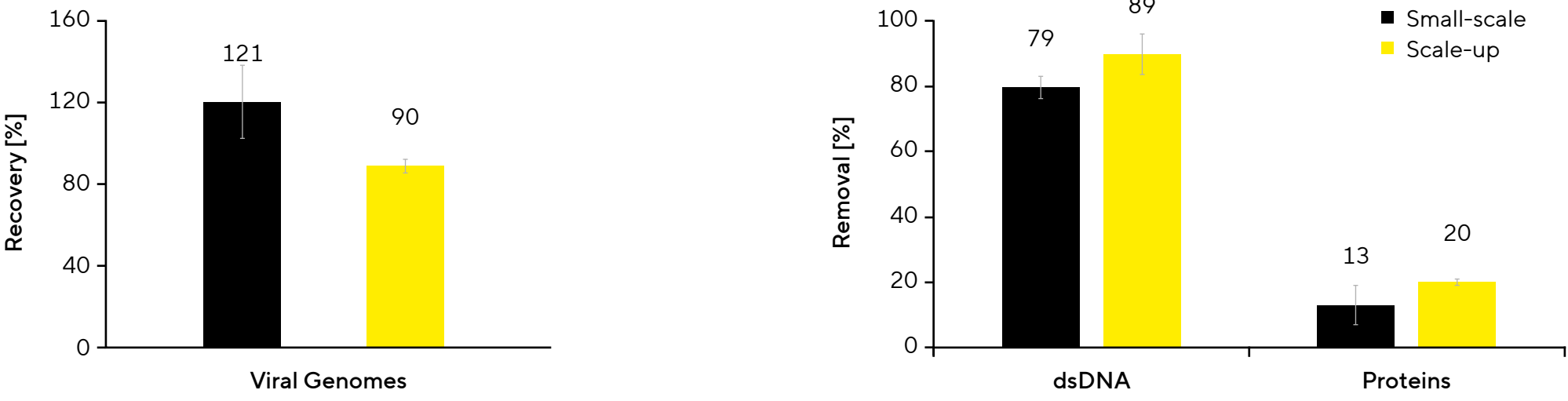
To evaluate scalability of the established UF | DF process, a TFF run with a 10-fold larger volume of harvested AAV8 lysate was performed, with the process parameters being scaled accordingly.

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

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

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

Figure 5: AAV8 Viral Genome Recovery (Left) and Impurity (dsDNA and Protein) Removal (Right) After UF | DF Performed With Hydrosart[®] 100 kDa ECO Cassettes on the Sartoclon[®] Slice 200 (Small-Scale) and 2 × Sartoclon[®] Slice (Scaled-Up)



The virus recovery 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 Sartoclon[®] Slice 200 cassette format to the Sartoclon[®] Slice format.

4. Conclusion

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

- Exceptional AAV8 recovery
 - 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 m²
- Both 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)