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Comparing Flow- and Pressure-Driven Tangential Flow Filtration Processes for Adeno-Associated Virus Purification Using Sartocon® Hydrosart® Cassettes

Dr. Sara Cardoso, Dr. Franziska Bollmann
Sartorius Stedim Biotech GmbH, August-Spindler-Straße 11, 37079 Göttingen, Germany

Correspondence
Email: sara.cardoso@sartorius.com | franziska.bollmann@sartorius.com

Abstract

Adeno-associated viruses (AAVs) are becoming increasingly important in the modern clinical landscape, and meeting their manufacturing demand requires the development of efficient purification processes. One common downstream step is concentration and diafiltration by tangential flow filtration (TFF), which plays a critical role in producing AAVs at a high yield and quality.

This application note explores two methodologies to approach TFF process steps—constant flow rate and constant pressure—using Sartocon® Hydrosart® cassettes, which are known for their high-performance filtration capabilities. By evaluating the ECO and E-Screen cassette formats, we aimed to establish the optimal parameters for maximizing AAV recovery and impurity removal and reducing processing time. These insights help inform the development of an efficient TFF strategy, balancing processing time and purification needs, and ultimately enhancing the efficiency and scalability of AAV production processes.

For further information, visit
TFF Cassettes

Introduction

Adeno-associated viruses (AAVs) have become an increasingly popular tool for efficiently transferring therapeutic genes, and their clinical applications are accelerating rapidly. In response to the growing demand for AAV products, significant efforts have been made to develop and optimize methodologies that generate high-quality AAVs with substantially increased production yields.

Tangential flow filtration (TFF) is commonly used for the concentration and diafiltration of AAV during downstream purification. This process step plays a crucial role in achieving high product purity and yields while allowing the viral particles to be formulated in the desired buffer. Hydrosart® is a high-performance, stabilized cellulose-based membrane that has been optimized for biopharmaceutical process applications. It is known for its stability across a broad pH range and is extremely hydrophilic, making it non-protein binding and virtually non-fouling, allowing for exceptionally high fluxes.[1] The performance of Hydrosart® cassettes has already been demonstrated for TFF in AAV serotype 8 (AAV8) processes.[2] Sartorius offers two different TFF cassette geometries for ultrafiltration and diafiltration (UF | DF): the E-Screen and ECO format. Sartocon® ECO cassettes are designed for TFF-based concentration and diafiltration of low-viscosity solutions, whereas E-Screen cassettes are suited for highly viscous solutions.[3] Compared to the E-Screen geometry, the ECO cassette features 26% more surface area per standard cassette width, which allows for a larger filtration surface within the cassette holder, reducing the feed pump power demand. Consequently, the need for larger systems during scaled-up processing is reduced when operating the ECO cassette format. It also decreases the shear stress applied to the virus particles during processing.

A TFF process may be controlled by an interplay of several operating parameters according to specific process or system requirements, usually involving measuring pressure or flow rate at various points in the system. One crucial part of the process control is based on keeping the driving force behind the retentate flow rate, also known as the crossflow rate or recirculation rate, constant. This crossflow action can take the form of maintaining a constant flow rate (i.e., flow per unit area; feed flow rate) or constant pressure (ΔP—feed and retentate pressure, P1 and P2).

In this study, we aimed to develop and establish a concentration and diafiltration unit operation for AAV8 using Sartocon® Hydrosart® 100 kDa TFF cassettes, based on the two methodologies: controlling the process at either a constant flow rate or at a constant pressure. Such a study is crucial for optimizing TFF processes in AAV production, as each method offers distinct advantages and impacts depending on the process conditions. Investigating these approaches enables the identification of optimal parameters, such as pressure and flow rate, to maximize product recovery, improve impurity removal, and achieve faster processing times. A clear understanding of the benefits and trade-offs between constant pressure-driven and constant flow-driven TFF operations can lead to more efficient and scalable viral vector manufacturing processes.

Materials and Methods

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

Subsequently, the TFF experiments were conducted using 100 kDa Hydrosart® Sartocon® Slice 200 ultrafiltration cassettes (Sartorius; Figure 1) with both an "E" channel and "ECO" channel configuration. Small-scale process development was performed on a Sartoflow® Smart TFF System (Sartorius; Figure 2). The consumables were evaluated using two typical ultrafiltration methods: controlling the process through a constant feed flow rate (constant flow rate process method) and through a constant inlet pressure (constant pressure process method).

Before the UF | DF trials, a permeate flow rate characterization study was carried out for all cassettes to determine the optimal operating recirculation rate and TMP conditions. The constant feed flow method studies were carried out by adjusting the pump rate (%) for several incremental values. The constant pressure method studies were carried out by adjusting the inlet (P1) and retentate pressures (P2) for several incremental delta (Δ) pressure values (ΔP = P1 – P2). In both cases, for each module and recirculation rate, the TMP was ramped up until a decrease in the corresponding permeate flow rate was observed. For both cases, 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 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 50 mL of diafiltration buffer for 5 min through the system. The flushes were then combined with the retentates.

Analytical testing included viral particle/capsids (vp) titer (ELISA), viral genome (vg) titer (ddPCR), total protein (BCA), and total dsDNA (PicoGreen) assays.

Figure 1: *The Sartocon® Slice 200 Cassette is the Smallest Device in the Sartocon® Cassette Product Family*

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

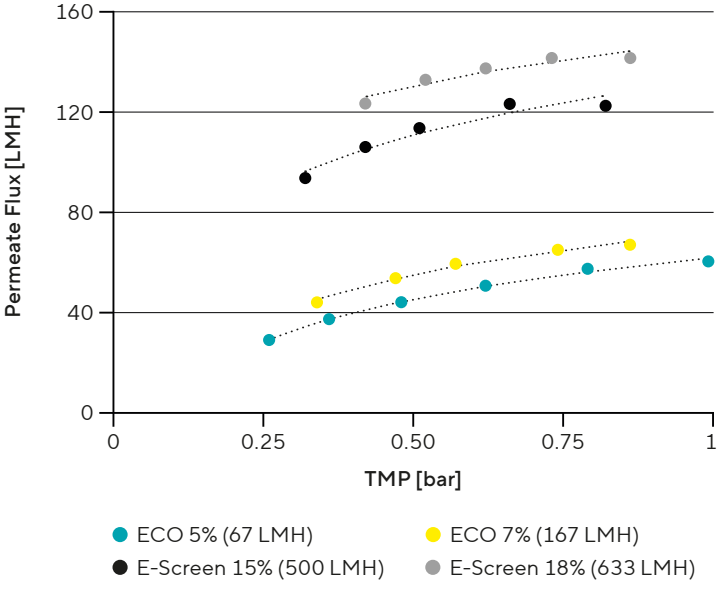
Results and Discussion

TMP Optimization

Constant Flow Rate

Initially, the optimal operating TMP for each cassette was identified at a small scale using the Sartocon® Slice 200 cassette and the Sartoflow® Smart TFF system. The permeate flux was measured at various increasing TMP values and two different target pump rates for each cassette type (Figure 3).

Figure 3: *Permeate Flux Measured as a Function of TMP at Two Different Pump Rates for Sartocon® Slice 200 Hydrosart® 100 kDa E-Screen and ECO Cassettes Using the Constant Flow Rate Method*

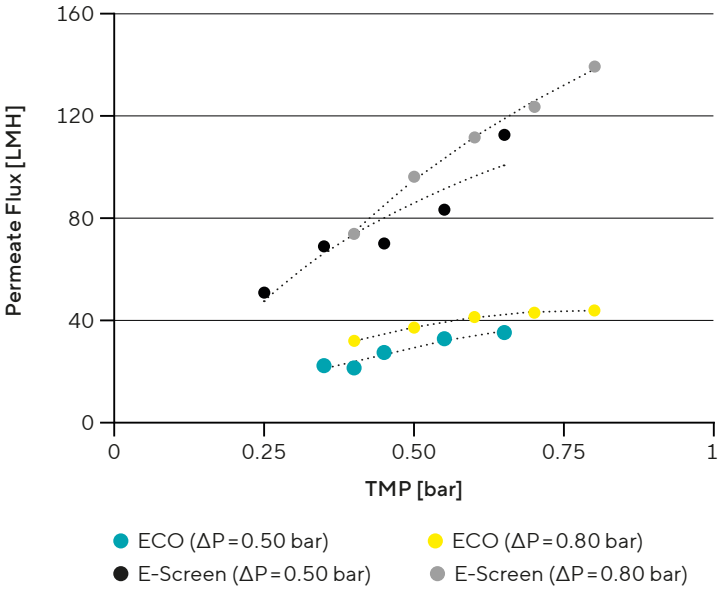


The optimum TMP range was selected as the range approaching the pressure-independent zone of the process (further increases of pressure do not increase permeate flux), typically close to where one achieves optimal performance from an ultrafilter. The selected TMP ranges were 0.65–0.70 bar (at 7% pump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

Constant Pressure (ΔP)

Similar to the flux characterization study carried out using the constant flow rate method, the optimum operating TMP for each cassette was also identified for the constant pressure method. The permeate flux was measured at various increasing TMP values and two different target Δ pressure (ΔP) values for each cassette type (Figure 4).

Figure 4: *Permeate Flux Measured as a Function of TMP at Two Different Delta Pressure Values (ΔP) for Sartocon® Slice 200 Hydrosart® 100 kDa E-Screen and ECO Cassettes Using the Constant Pressure (ΔP) Method*



The selected TMP range was 0.60–0.65 bar (at ΔP = 0.80) for the ECO and for the E-Screen cassette.

AAV8 Concentration and Diafiltration Performance

Constant Flow Rate

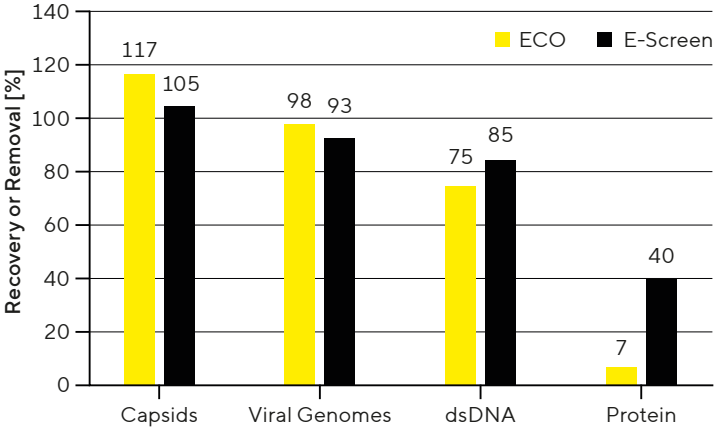
With the identified optimal range for TMP and pump rate | flow rate for each ultrafiltration cassette type, we evaluated their performance in the concentration and diafiltration of a harvested AAV8 lysate (Table 1 and Figure 5).

Table 1: *Parameters and Results of Small-Scale AAV8 UF | DF Runs Performed With Sartocon® Slice 200 Hydrosart® ECO and E-Screen Using the Constant Flow Rate Method*

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
Average permeate flux (UF) [LMH]	63	80
Average permeate flux (DF) [LMH]	42	47
Run time (UF) [min]	47	34
Run time (DF) [min]	38	35

We observed that, to achieve a similar TMP (0.6 – 0.7 bar), the pump rate is 2 – 3 times lower for the ECO (7 – 8%) compared to the E-Screen (18 – 20%) cassette format. Lower processing times were achieved for the E-Screen (69 min) compared to the ECO cassette (84 min, Table 1).

Figure 5: *Recovery of AAV8 Capsids and Viral Genomes and Removal of dsDNA and Protein Following UF | DF Performed With Sartocon® Slice 200 Hydrosart® 100 kDa ECO and E-Screen TFF Cassettes Using the Constant Flow Rate Method*



While capsid recoveries were very similar for both geometries (117 and 105%), genome recovery was slightly higher with the ECO (98%) compared to the E-Screen (93%) cassette. Both cassettes demonstrated a generally high dsDNA removal efficiency (75 and 85%), but protein removal was much higher with the E-Screen cassette (40%) than with the ECO cassette format (7%; Figure 5).

Constant Pressure (ΔP)

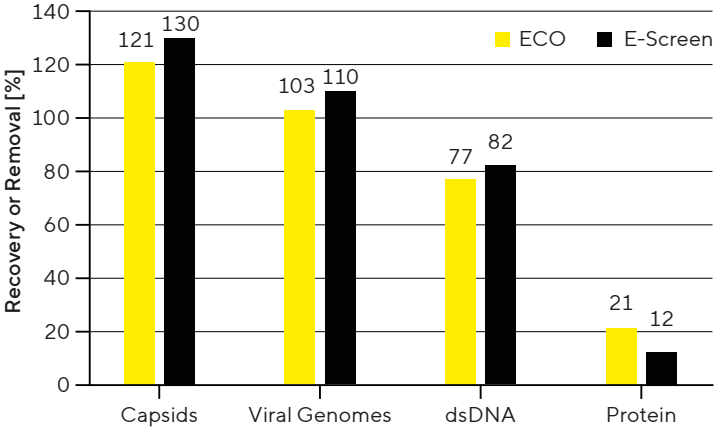
With the identified optimum setpoint for TMP and ΔP for each ultrafiltration cassette type, we evaluated their performance in the concentration and diafiltration of a harvested AAV8 lysate (Table 2).

Table 2: *Parameters and Results of Small-Scale AAV8 UF | DF Runs Performed With Sartocon® Slice 200 Hydrosart® ECO and E-Screen Cassettes Using the Constant Pressure (ΔP) Method*

Hydrosart® 100 kDa	ECO	E-Screen
Inlet pressure [bar] (constant)	1	1
Pump rate [%] (variable | automatic)	6 – 8	25 – 29
Δ pressure (ΔP) [bar] (constant)	0.80	0.80
TMP [bar]	0.65 – 0.70	0.60 – 0.65
Average permeate flux (UF) [LMH]	55	108
Average permeate flux (DF) [LMH]	33	67
Run time (UF) [min]	55	25
Run time (DF) [min]	50	25

Using the constant pressure-driven method, to achieve a similar TMP (0.65 – 0.65 bar) and ΔP (0.8 bar), the pump rate (automatically adjusted by the system) was 3 – 5 times lower for the ECO (6 – 8%) compared to the E-Screen (25 – 29%) cassette format (Table 2). Lower total process times were achieved for the E-Screen (50 min) compared to the ECO cassette (105 min; Table 2).

Figure 6: *Recovery of AAV8 Capsids and Viral Genomes and Removal of dsDNA and Protein Following UF | DF Performed With Sartocon® Slice 200 Hydrosart® 100 kDa ECO and E-Screen TFF Cassettes Using the Constant Pressure (ΔP) Method*



Capsid and genome recoveries were very similar for the ECO and E-Screen configurations (121 and 130%, respectively). In addition, both cassettes exhibited generally high dsDNA removal efficiencies (77 and 82%) and lower protein removal efficiencies (21 and 12%; Figure 6).

Discussion and Conclusion

This study evaluated the performance of flow- and pressure-driven TFF operations for AAV8 processes using Sartocon® Hydrosart® cassettes. The findings, summarized in Table 3, highlight the efficiency and effectiveness of these methodologies under various conditions.

The methodologies of constant ΔP (pressure) and constant feed-flow-rate each present distinct advantages and disadvantages. Constant ΔP offers a simple, fast, and reliable measurement with reduced risk of clogging and consistent TMP after scale-up. It is compatible with all Sartorius systems, making it particularly beneficial for small-scale development. However, potential pressure drops in the middle points of the holder when scaling up with stacked cassettes can increase the gel layer due to lower shear in the middle region.

Conversely, the constant feed flow rate method provides better control over shear throughout the process duration. However, it requires feed pump calibration before experiments, and flow rate measurement can be inaccurate, especially at small scales. Additionally, the inlet pressure rises after scale-up, necessitating a reduction in P2 (retentate natural backpressure), and there is a higher risk of clogging.

In summary, the choice between flow-rate and pressure-driven methods, should be based on specific process requirements.

Recommendations

- Use pressure-driven TFF approaches when simplicity, reliability, and reduced risk of clogging are priorities, especially in small-scale developments where consistent TMP is beneficial.
- Opt for flow-driven TFF methods when precise control over shear is required throughout the process, particularly in scenarios where maintaining viral genome integrity is crucial.
- Consider the balance between processing time and purification needs when choosing between flow rate and pressure-driven methods. Both operating methods yield comparable virus recovery and impurity removal, providing flexibility in TFF operations.

Key Findings

- The ECO cassette is preferable for processes prioritizing lower shear stress, while E-Screen supports faster processing times.
- Both Hydrosart® ECO and E-Screen cassettes showed comparable performance in terms of virus recovery and impurity removal.

Table 3: *Summary of AAV8 UF | DF Performance of Sartocon® Slice 200 Hydrosart® 100 kDa ECO and E-Screen TFF Cassettes Using Constant Flow Rate and Pressure (ΔP) Methods*

		Recovery [%]		Removal [%]		Total Processing Time (UF | DF) [min]
		Capsids	Viral Genomes	dsDNA	Protein	
ECO	Constant flow rate	117	98	75	7	84
	Constant pressure (ΔP)	121	103	77	21	105
E-Screen	Constant flow rate	105	93	85	40	69
	Constant pressure (ΔP)	130	110	82	12	50

Note. Capsid and viral genome recovery and dsDNA and protein removal, as well as processing time are shown.

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Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
sartorius.com

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