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Choosing the Optimal MWCO for AAV TFF: 100 kDa vs. 300 kDa

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Abstract

Tangential flow filtration with 100 kDa membranes is widely used for adeno-associated virus (AAV) processing as it enables reliable viral retention. This study evaluates Sartocon® Slice 200 ECO Hydrosart® TFF ultrafiltration cassettes with 100 kDa or 300 kDa molecular weight cut-off (MWCO) membranes for AAV processing. These findings suggest that membranes with a higher MWCO can enhance impurity clearance while maintaining AAV recovery. Overall, both membranes offer effective viral retention, enabling flexible process optimization across development and manufacturing scales.

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Introduction

Adeno-associated virus (AAV) purification processes commonly employ tangential flow filtration (TFF) with 100 kDa membranes due to its reliable viral retention performance.[1,2] However, 300 kDa membranes may offer improved throughput and clearance of impurities while still maintaining effective AAV retention because of the significantly larger size of AAV particles relative to the membrane pores.

Hydrosart® ultrafiltration membranes offer excellent hydrophilicity, virtually non-fouling performance, and scalability across multiple formats, making them ideally suited for viral vector processing applications. The availability of multiple molecular weight cut-off (MWCO) options enables flexible process design to meet specific performance targets across development and manufacturing scales.[3] This application note evaluates the performance of Sartocon® Slice 200 ECO Hydrosart® TFF ultrafiltration cassettes with 100 kDa or 300 kDa MWCO membranes for AAV processing, focusing on viral recovery, filtration efficiency, and removal of impurities.

Materials and Methods

AAV8 was produced in suspension using HEK293 cells via transient transfection in a 10 L bioreactor system. Following harvest, cell lysis was induced to release intracellular AAV, and an endonuclease treatment was applied to reduce nucleic acid contamination. Clarification was performed using Sartoclear® DL75 depth filters; sterile filtration was performed using Sartopore® 2 XLG filters.[4] TFF experiments were conducted using Sartocon® Slice 200 ECO Hydrosart® TFF ultrafiltration cassettes with 100 kDa or 300 kDa MWCO membranes and an ECO-Screen channel configuration.[3,5] A standardized workflow consisting of 10-fold concentration by ultrafiltration (UF) and five diafiltration (DF) volumes was applied using identical feed volumes and operating conditions, with inlet pressure (P1) set to 1 bar and transmembrane pressure (TMP) set to 0.6 bar. Process performance was evaluated using viral genome titration (ddPCR), total protein quantification (Bradford assay), and total dsDNA quantification (PicoGreen™ assay).

Results and Discussion

Flux and process efficiency

Figure 1: Permeate flux profiles for AAV ultrafiltration using Sartocon® Slice 200 ECO Hydrosart® TFF ultrafiltration cassettes with 100 kDa or 300 kDa MWCO membranes.

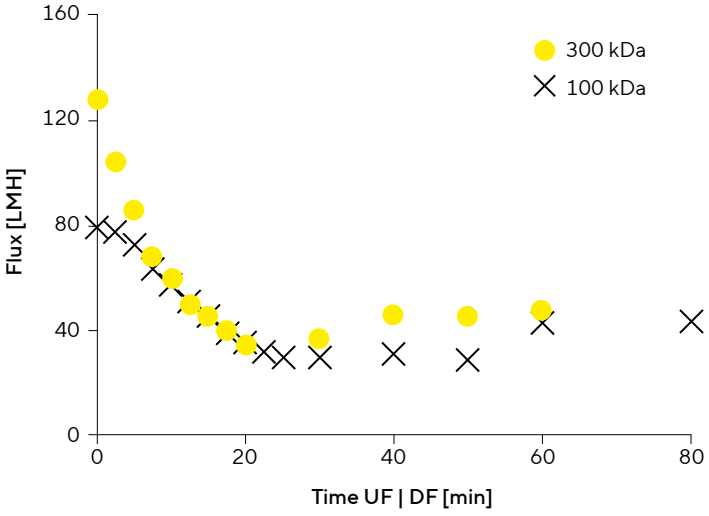
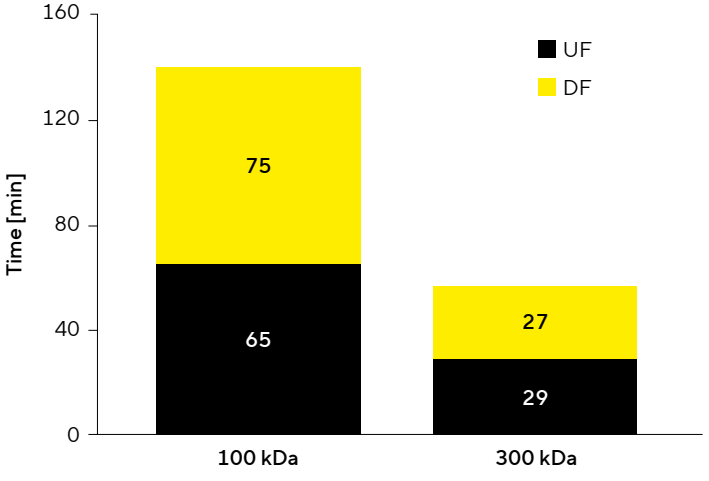


Figure 2: Comparison of the durations of UF (10-fold concentration) and DF (5 volumes) for Sartocon® Slice 200 ECO Hydrosart® TFF ultrafiltration cassettes with 100 kDa or 300 kDa MWCO membranes



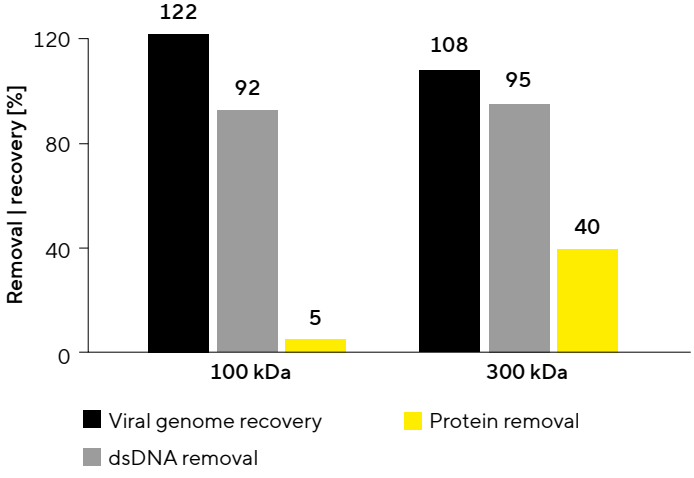
Flux profiles demonstrated consistently higher initial flux values for the 300 kDa membrane with stable flux over time, while the 100 kDa membrane exhibited a lower initial flux (Figure 1). These results suggest that the larger pore structure of the 300 kDa membrane can potentially reduce flow resistance or fouling effects and thereby support higher throughput.

In addition, a clear improvement in processing time was observed when using the 300 kDa membrane, as the UF and DF durations were significantly reduced compared with the 100 kDa reference process (Figure 2). This corresponds to an approximately 2 – 3-fold reduction in processing time, highlighting a substantial opportunity for process intensification.

Viral recovery and impurity clearance

Both the 100 kDa and 300 kDa MWCO membranes demonstrated high AAV recovery during TFF processing (Figure 3). Viral genome recovery was slightly higher for the 100 kDa membrane compared with the 300 kDa membrane; this difference is consistent with minor viral passage through the larger 300 kDa pores, as confirmed by detection of a low percentage (approximately 1%) of viral DNA in the permeate sample. However, accounting for analytical variability, the estimated viral loss associated with the 300 kDa membrane is approximately 1 – 5%, which is generally considered acceptable in downstream processing workflows. The 300 kDa membrane demonstrated superior impurity removal compared with the 100 kDa membrane. In particular, the significantly higher host cell protein removal highlights the advantage of increased membrane permeability for removal of impurities.

Figure 3: Comparison of viral genome recovery, dsDNA removal, and protein removal for Sartocon® Slice 200 ECO Hydrosart® TFF ultrafiltration cassettes with 100 kDa or 300 kDa MWCO membranes.



Conclusion

This study demonstrates that Sartocon® Slice 200 ECO Hydrosart® TFF ultrafiltration cassettes with 300 kDa MWCO membranes provide a compelling alternative to conventional 100 kDa membranes for AAV downstream processing. Compared with 100 kDa membranes, the 300 kDa configuration enables:

- Substantially shorter processing times
- Increased permeate flux and throughput
- Reduced fouling propensity
- Improved host cell protein clearance

Importantly, these benefits are achieved while maintaining comparable AAV retention, with only minor and operationally acceptable viral losses. Overall, our results support the use of higher MWCO membranes as an effective strategy for process intensification and performance optimization in AAV TFF operations.

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