Simplifying Progress

Use of High-Throughput and DoE Tools for Robust Development of DSP Technologies for AAV

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Introduction

Adeno-associated viral vectors (AAV) are commonly used as a delivery tool for gene therapy. However, producing them is still posing a challenge to manufacturers as platform production processes are challenging to develop and downstream processing is somewhat inefficient. With the potential diversification of adeno-associated virus (AAV) capsid serotypes for gene therapy applications, a downstream processing platform, that can purify a variety of serotypes with similar recovery and purity is of particular interest to reduce process development timelines and manufacturing complexity. In our study, we are focusing on the development of the three major downstream processing steps to purify AAV particles: harvest clarification, tangential flow filtration (TFF), and capture chromatography. To do so, we are screening several different materials and process parameters using design of experiments (DoE) methodology and screening devices.

1. Experimental Approach

Adeno-associated virus was produced in suspension by transient transfection of HEK293 cells. At the time of harvest, tween was added to the bioreactor to lyse the producing cells in order to release the AAV particles. Figure 1 shows the applied AAV downstream processing workflow, starting with harvest clarification of the bioreactor material by normal flow filtration, followed by a TFF step to concentrate the feed material and remove larger impurities like host-cell DNA and proteins. For optimal results during the capture chromatography step, an endonuclease step, using Kryptonase™, is performed to digest nucleic acids, followed by an optional second TFF step. Purification of AAV particles is done by cation-exchange chromatography using monolithic columns. Further downstream processing steps, especially the separation of full and empty AAV particles, are currently under development with promising preliminary results.

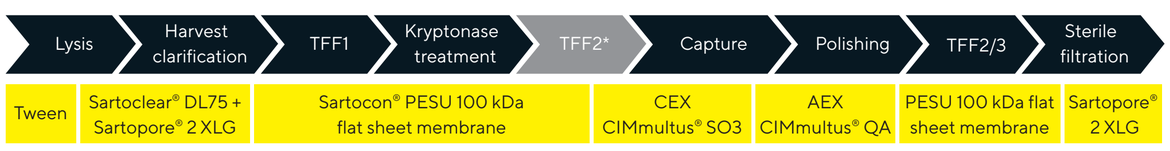
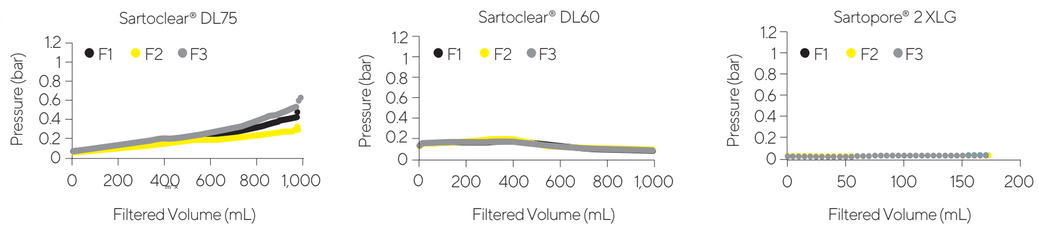


Figure 1: *Schematic Diagram of the Developed and Optimized AAV Production Process (*Note: TFF2 Step Is Optional), Including Positioning of Consumables That Showed Best Performance.*

2. Results – Harvest Clarification

When developing an AAV downstream process, firstly, AAV particles must be efficiently separated from as many process and product-related impurities as possible, which can be challenging due to obligatory lysis of the cells. We have performed a study to benchmark marketed Sartorius filters, prototype filters, and competitor filters and to optimize process parameters, e.g., flow rate. Overall, five Sartorius filter and five competitor filter combinations were assessed, followed by a Sartopore® 2 XLG (0.8 | 0.2 µm) membrane filter for each combination. Filters were assessed at the optimal flow rate (200 L/m² × h, identified in a separate experiment set) by filtering a fixed volume.

A)



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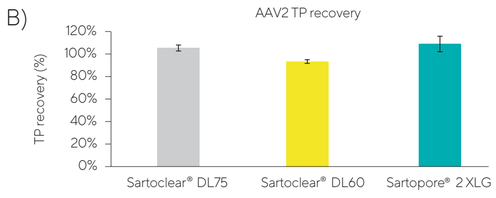


C)

Filter	Turbidity (NTU)
Start	167.4
Sartoclear® DL75	3.4 ±0.12
Sartoclear® DL60	2.2 ±0.09
Sartopore® 2 XLG	1.7 ±0.12

D)

Figure 2: *A) Diagrams of the Filtration Runs of Each Filter. B) AAV2 Particle Recovery After Each Filtration. C) Turbidity of Starting Material and Filtrates. D) Image of the Used Device Sartoclear® Caps. N=3*

Figure 2 shows the results of the best-performing filter combination from Sartorius. The double-layer cellulose-based depth filter Sartoclear® DL75 (10 | 2 µm) was able to efficiently and reproducibly clarify AAV2 from lysed cells (final turbidity 3.4 NTU) while retaining an excellent viral particle recovery (~100%). With Sartoclear® DL75, capacities of more than 400 L/m² were achieved, without observing filter blockage. We have identified, that a second cellulose-based depth filter, like in this case Sartoclear® DL60 is not necessary to be included in the filter train as the performance of the Sartoclear® DL75 filter is sufficient. Subsequent sterile filtration with Sartopore® 2 XLG could be performed without blocking, i.e., pressure increase, until a capacity of at least 400 L/m² and without particle loss (~ 100% vp recovery).

3. Results – TFF

Next, we have been optimizing the TFF step. The early selection of high-performing filters as a base for developing tangential flow filtration can represent a challenge when the feed material is scarce and the development timeline is short. A comparison of multiple flat sheet cassettes performances as well as the optimization of process parameters for an AAV8 concentration and diafiltration TFF process was performed.

A)

MWCO (kDa)	Membrane Material	Feed Flow Rate (mL/min)	TMP (mbar)
100	Hydrosart®	50–75	300–600
100	PES	50–75	300–600
30	Hydrosart®	50–75	600–900
30	PES	50–75	600–900

B)

Figure 3: *A) DoE Design of the Flat Sheet Membrane Cassette Screening Experiment Performed With Ambr® CF (TMP = Transmembrane Pressure, V = 70 mL, 10× Concentration, 5× Diafiltration, 10 cm² Cassettes). B) Image of an Ambr® CF High-Throughput TFF Screening System.*

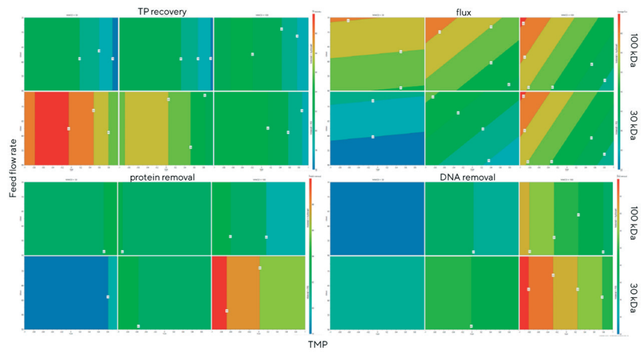


Figure 4: *Contour Plot of the Results of the DoE Performed, Comparing Several Flat Sheet Membrane Cassettes for TFF of AAV8.*

Screening for different TFF conditions and consumables could be easily performed by running the DoE shown in figure 3 with the high-throughput TFF development system Ambr® CF. The resulting contour plot, which was created with MODDE® software, is shown in figure 4. In general, AAV particle recovery was similar for all tested conditions. However, contaminant removal and permeate flux were better with cassettes with a 100 kDa cut-off. According to the DoE model, the 100 kDa PES cassette run at a high feed flow rate (75 mL/min) and a low TMP (300 mbar) was performing best. Next, a scale-up of the process to the Sartoflow® Smart TFF system was performed (triplicate).

A)

	TP recovery (%)	Permeate flux (LMH)	Protein removal (%)	DNA removal (%)
Average (SF Smart)	91	37	79	36
CV (%)	2	3	1	18
DoE Ambr® CF	90	63	89	73

B)

Figure 5: *A) Results of the Triplicate Runs Performed With the Sartoflow® Smart Including Results of the Comparable DoE Run Performed With Ambr® CF (100 kDa PES, Slice 200, 180 cm², TMP = 600 mbar, Feed Flow Rate = 260 mL/min, V = 1 L, 10× Concentration, 5× Diafiltration). B) Image of a Sartoflow® Smart TFF System.*

According to the results shown in figure 5, the scale-up of the TFF step from Ambr® CF to the Sartoflow® Smart was directly feasible with comparable results and overall good reproducibility. With the optimized process, AAV8 particle recoveries of >90% can be obtained, with good impurity removal levels.

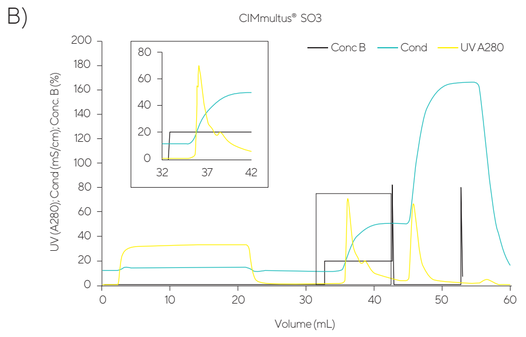
4. Results – Capture Chromatography

To efficiently purify a variety of AAV serotypes and capsid configurations, an alternative to affinity-based capture chromatography was developed using monolith technology. Strong cation exchange chromatography can become a universal capture platform where binding, wash and elution conditions can be quickly optimized for each new AAV serotype instead of relying on the development of a new affinity ligand. We developed a capture chromatography method based on the CIMmultus® SO3 monolith 1 mL screening device by optimizing multiple parameters with DoE.

A)

Factor	Range | Setpoints
Starting material	TFF1, TFF2
pH	3.5 – 4.5
% B in elution (Step)	20 – 60

B)



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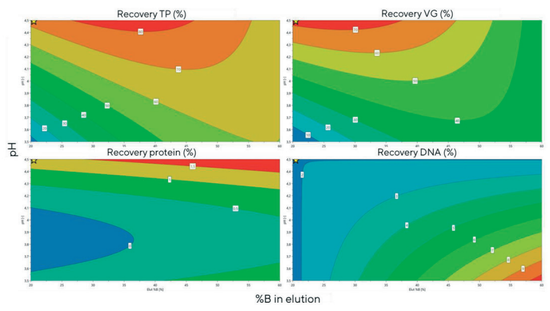

D)

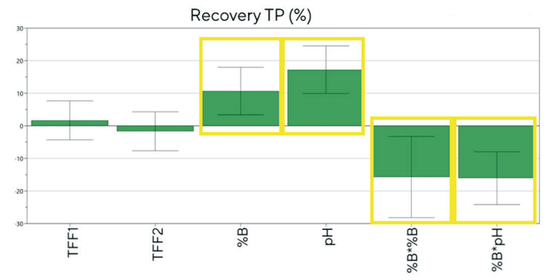

Figure 6: *A) Layout of the DoE Performed to Develop the Capture Chromatography Step for AAV8 With CIMmultus® SO3. B) Chromatogram of the Optimal Condition. C) Contour Plot of the DoE Model (TFF2 Results Shown) Created With MODDE® Software. D) Coefficients Plot of the DoE Model*

According to the results obtained and the analysis of the DoE model shown in figure 6 D, the percentage of buffer B and the pH of the buffer solutions had a significant impact on the AAV particle recovery, whereas the choice of the starting material (TFF1 or 2) didn't have an effect. High pH (4.5) and low %B (20) were the most optimal conditions to achieve high AAV recovery and impurity removal. Triplicate runs performed under these conditions yielded ~70% vp and ~75% vg recovery, respectively. Over 90% of contaminating protein and DNA (close to LLOD) could be removed.

5. Summary | Conclusion

The data presented demonstrate the potential of screening tools combined with data analytics software to rapidly and efficiently develop an AAV downstream process independently of the serotype used. We could show the potential of the Sartoclear® DL 75, a double-layer cellulosic depth filter, for effective AAV harvesting from lysed cells with excellent and reproducible particle recovery and filter capacity. Furthermore, the unique capabilities of the high-throughput TFF development system Ambr® CF allowed for the rapid identification of CPPs and optimal TFF consumables. These results could be easily translated through a scale-up to the Sartoflow® Smart TFF system as we obtained excellent recoveries, that were highly reproducible. Lastly, we developed a capture chromatography step based on a cation-exchange monolithic column that showed to be a promising alternative to affinity-based purification of AAV, which can be rapidly developed.