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Robust and Scalable Membrane Chromatography for Adeno-Associated Virus Capture Using Sartobind® S

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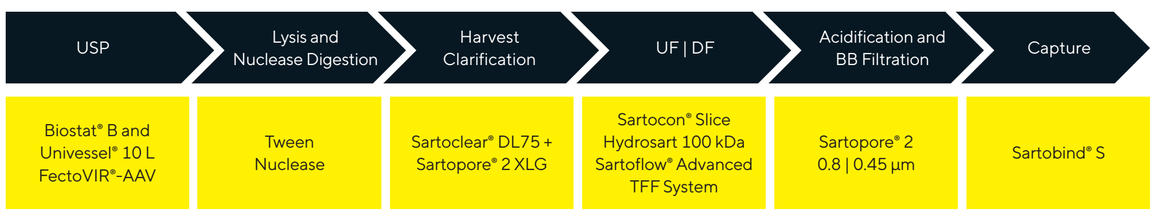
Introduction

Adeno-associated viruses (AAV) are widely used in gene delivery for treating various diseases, driving demand for improved manufacturing processes. Efficient AAV purification is essential for clinical and commercial success, and Sartobind® membrane chromatography devices excel in purifying large biomolecules like viruses. These membranes enable low mass transfer resistance, allowing high flow rates and easy scalability. In this study, we developed a Sartobind® S-based capture step to purify AAV8 using a design of experiments (DoE) approach to optimize parameters. The process demonstrated reproducibility and scalability, making it a robust option for AAV8 purification.

Experimental Approach

AAV8 Production Process

Figure 1: *Schematic Diagram of the AAV Production Process for Establishing the Capture Step Protocol*



Sample Preparation for Sartobind® S Chromatographic Runs

All the chromatographic runs for process development were performed using Sartobind® S Nano 1 mL 4 mm bed height devices (Sartorius; Figure 2A). The loading buffer was composed of 50 mM acetate, 0.01% (w/w) poloxamer 188 and 2 mM $MgCl_2$. The pH and NaCl concentration of this buffer were varied depending on the specific (DoE) run (Table 1). The elution buffer had the same composition, except for NaCl being added at 2 M. Prior to loading, AAV8 samples were diluted 10 times with the load buffer, adjusted to the target pH and NaCl concentration, and filtered with a Sartopore® 2 0.8 | 0.45 µm bioburden reduction filter (Sartorius).

Sartobind® S DoE Design

A three-factor, two-level full-factorial DoE with two center-point replicates was designed with load and elution buffer pH and concentration of NaCl in load buffer as factors (Table 1) using MODDE® software (Sartorius). Overall, 12 runs were performed for the DoE study.

Table 1: *Factors and Ranges of the DoE*

DoE Factor	Factor Range
Concentration of NaCl in load buffer (mM)	20 – 80
Load buffer pH	3.5 – 5.5
Elution buffer pH	3.5 – 5.5

Performance of Sartobind® S Chromatographic Runs

Dynamic binding capacity (DBC) was initially assessed to estimate the target AAV8 loading amount for the planned purification runs. Based on the results from the BC runs, a volume corresponding to 1.57×10^{13} viral particles per mL MV of the equilibrated AAV8 sample was loaded, followed by a wash step with 30 MV of load buffer. A gradient elution from 0 to 100% of elution buffer was performed over 20 MV, followed by a strip with 100% of elution buffer for 20 MV. The flow rate was 5 MV/min for all steps. Elution peak fractions were collected, and immediately neutralized with 10% of 1 M Tris, pH 9.0.

For the scale up run, a Sartobind® S Capsule 75 mL (Figure 2B) was used. The amount of sample loaded was adjusted to the larger membrane volume. Otherwise, the operating procedure remained the same as in the 1 mL small scale runs.

Analytical Methods

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

Figure 2: *A) Sartobind® S Nano 1 mL, B) Sartobind® S 75 mL Capsule*

Results

Development of AAV Capture Using Sartobind® S

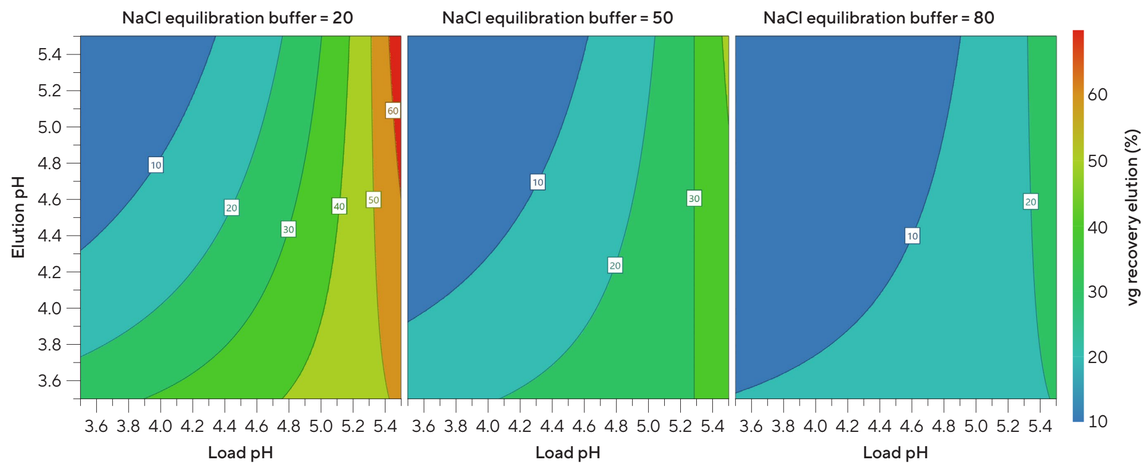
A DoE approach was used to optimize AAV8 capture using Sartobind® S. It identified key factors affecting viral genome recovery, with the highest recovery (80%) at low NaCl and high pH in both load and elution buffers (Table 2, Figure 3). Despite a negative correlation between elution pH and vg recovery, high pH in both buffers led to high vg recovery due to their positive interaction. Low NaCl concentration improved virus recovery and protein removal, though trade-offs were observed between virus recovery and impurity removal due to interactions between the factors that negatively influence protein removal. The same factor dependencies for vg recovery were observed for virus capsid recovery. DNA removal was highest at the setpoints that also led to high virus recovery, further supported by its positive correlation with elution pH. Optimal conditions were a pH of 5.5 for both buffers and 20 mM NaCl in the load buffer.

Table 2: *Overview of Significant Factors Influencing the Respective Responses and Their Correlation*

Factor	vp Recovery	vg Recovery	Protein Recovery	DNA Recovery
Load pH	↑	↑	↑	
Elution pH	↓	↓		↓
Load conc. NaCl	↓	↓	↑	
Interactions	Negative interaction between load pH and salt conc.	Positive interaction between load and elution pH	Positive interaction between elution pH and salt conc. and load and elution pH	

blank = non-significant term, ↑ = positive correlation, ↓ negative correlation

Figure 3: *Response Contour Plots of the Viral Genome Recovery as a Function of All Three Factors Evaluated*



Note. x-axis = load pH, y-axis = elution pH. NaCl concentration from left to right plot: 20 – 50 – 80 mM.

Reproducibility Analysis of Developed AAV Capture Protocol Using Sartobind® S

Three independent AAV8 capture chromatography runs were conducted using the Sartobind® S Nano 1 mL device, applying the optimal parameters from the DoE study (Figure 4, Table 3). Virus recovery and impurity removal closely matched the results from the initial DoE experiment, demonstrating good reproducibility. On average, the runs achieved 54% viral particle recovery, 72% viral genome recovery, and contaminant removal of over 87% and 93% for proteins and DNA, respectively. Low coefficients of variation (CV) were observed, except for dsDNA removal. Overall, the process showed consistent chromatographic profiles across runs. These results confirm the efficiency and robustness of the established AAV8 capture process using Sartobind® S, delivering highly reproducible outcomes.

Figure 4: *Overlay of Chromatograms of the Three Replicates of AAV8 Capture Chromatography Performed With Sartobind® S Nano 1 mL Device Using the Optimal Conditions Identified Through the DOE Study*

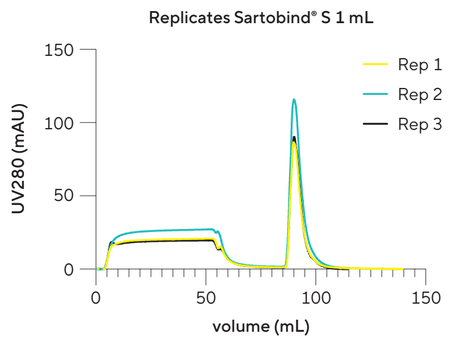


Table 3: *Recovery Following Capture Chromatography With Sartobind® S Nano 1 mL*

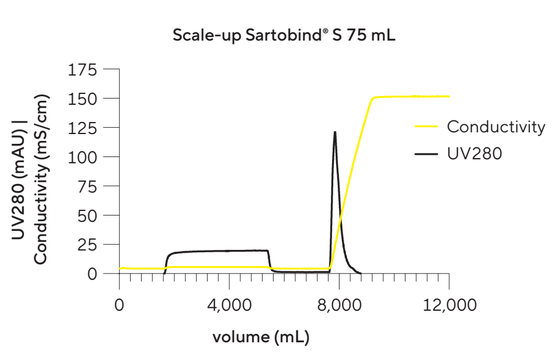
Recovery (%)	vp (Capsids)	vg	Protein	DNA
Rep 1 (DoE)	50	80	12	4
Rep 2	51	75	13	8
Rep 3	60	62	11	8
Mean	**54**	**72**	**13**	**7**
CV	8	10	7	28

Note. Capsids, viral genomes, dsDNA, and protein recoveries (mean and CV) from three replicates of AAV8 capture chromatography are shown. Loading buffer was pH 5.5 and 20 mM NaCl.

Scale-up of AAV Capture Using Sartobind® S

Next, we assessed the linear scalability of the established process by performing a chromatographic run with a 75-fold larger AAV8 sample volume using the Sartobind® S Capsule 75 mL (Figure 5). The scale-up run showed higher capsid recovery (84% vs. 54%) and similar viral genome recovery (73% vs. 72%) compared to small-scale runs (Table 4). Contaminant removal results were also consistent between the two scales, with only minor deviations that are within the range of assay variability. Protein recovery was 17% vs. 13%, and dsDNA recovery was below the limit of detection vs. 7%. Overall, virus recovery and contaminant removal were highly consistent, demonstrating the process' linear and predictable scalability from Sartobind® S Nano 1 mL to Sartobind® S 75 mL.

Figure 5: *Chromatogram of Scaled-Up AAV8 Capture*



Note. Run performed using Sartobind® S 75 mL capsule using the optimal conditions identified through DoE.

Table 4: *Comparison of Recoveries Following Capture Chromatography With Sartobind® S Nano 1 mL and Sartobind® S Capsule 75 mL*

Recovery (%)	vp (Capsids)	vg	Protein	DNA
Nano 1 mL	54	72	13	7
Capsule 75 mL	84	73	17	< LLOD

Note. The mean recoveries for capsids, viral genomes, dsDNA, and protein from three replicates of AAV8 capture chromatography are shown.

Conclusion

In this study we evaluated the performance of the Sartobind S® membrane chromatography device for purifying AAV8 through cation exchange chromatography. DoE facilitated the screening of optimal conditions to efficiently bind and elute AAV8 capsids, while significantly reducing DNA and protein content. This approach also aided in characterizing the influence of several factors on purification efficiency.

The cation exchange-based capture step was successfully established, resulting in a viral genome recovery of approximately 72%, and high protein and DNA clearance (> 87%). Similar results were obtained from a 75-fold scale-up run, demonstrating a scalable and robust alternative to existing AAV purification methods.

The results of our study provide valuable insights into the use of Sartobind® membrane chromatography devices for gene therapy manufacturing. The optimized protocol provides a streamlined and effective solution for the capture of AAV8, with potential implications for other AAV serotypes.