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Application Note

August, 2024

Keywords or phrases:
Adeno-Associated Virus (AAV) capture, membrane chromatography, Sartobind® S, cation-exchange chromatography, gene therapy manufacturing

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

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Abstract

Adeno-associated viruses are increasingly used as vectors for gene therapy applications, requiring efficient purification methods to meet clinical and commercial demands. This application note details the development of a robust and scalable method for capturing adeno-associated virus serotype 8 (AAV8) using Sartobind® S membrane chromatography. By employing a design of experiments approach, the study aimed to optimize key process parameters. Thereafter, the reproducibility and scalability of the method was evaluated. The findings highlight the potential of Sartobind® S membrane chromatography as an effective solution for AAV capture, offering significant implications for gene therapy manufacturing.

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Introduction

Adeno-associated viruses (AAV) are the leading platform for delivering gene therapies to treat a variety of diseases. As such, there is an increased need for advanced manufacturing processes to keep up with demand. Efficient and scalable purification of AAV vectors is critical for clinical and commercial success. Sartobind® membrane chromatography consumables are particularly advantageous for the purification of large particles, including AAV vectors. Due to their mainly intrinsic convective flow, membranes offer a significantly reduced mass transfer resistance compared to resin-based chromatography. They can, therefore, be operated at higher flow rates due to a low back pressure profile. In addition, membrane chromatography consumables offer easy handling, simple clean-in-place procedures, and are linearly scalable.

This study focuses on establishing a Sartobind® S-based capture step to purify AAV serotype 8 (AAV8), a serotype widely employed in gene therapy applications. For the development of the capture step, a design of experiments (DoE) approach was employed to optimize process parameters specifically for Sartobind® S. This approach also aided in characterizing the influence of several factors on purification efficiency. Furthermore, the reproducibility and scalability of the established process were demonstrated.

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

Materials and Methods

AAV8 Production, Lysis, Nuclease Treatment and Tangential Flow Filtration

AAV8 was produced by transient transfection of HEK293 cells using FectoVIR®-AAV (Sartorius). Cells were cultivated in a 10 L Univessel® Glass bioreactor controlled by a Biostat® B (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 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 (both Sartorius). Subsequently, tangential flow filtration (TFF) of the AAV8 harvest material (10x ultrafiltration and 5x diafiltration) was conducted using two stacked 100 kDa Hydrosart® Sartocon® Slice cassettes (Sartorius) with 0.14 m² membrane area each (0.28 m² total membrane area) on a Sartoflow® Advanced TFF System (Sartorius). The diafiltration buffer was composed of 20 mM Tris, 200 mM NaCl, 0.1% (w/w) poloxamer 188, 2 mM $MgCl_2$ at pH 7.5. The AAV8 retentate material was aliquoted and stored at –80 °C.

Sample Preparation for Sartobind® S Chromatographic Runs

All the chromatographic runs for process development, including the DoE, were performed using Sartobind® S Nano 1 mL 4 mm bed height consumables (Sartorius; Figure 1) with an ÄKTA avant™ 150 (Cytiva). The loading buffer was composed of 50 mM acetate, 20 mM – 80 mM NaCl (depending on the specific run), 0.01% (w/w) poloxamer 188 and 2 mM $MgCl_2$. The pH of this buffer was set to 3.5, 4.5, or 5.5, depending on the specific run. 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 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 the factors loading and elution buffer pH (3.5 to 5.5) and concentration of NaCl in load buffer (Table 1) using MODDE® software (Sartorius). The factors were systematically varied to identify optimal conditions for maximizing AAV recovery and purity. 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

Results and Discussion

Performance of Sartobind® S Chromatographic Runs

Before the first use of a Sartobind® S membrane consumable, an initial wash with 20 membrane volumes (MV) of 0.5 M NaOH was performed (and kept for 30 mins), followed by 10 MV of load buffer, 10 MV of elution buffer and finally more than 20 MV of load buffer (until stable baseline).

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 DBC runs a volume corresponding to 1.57×10^{13} viral particles per mL MV (70% of the determined maximum capacity, i.e., when first sample breakthrough occurred) 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% elution buffer was performed over 20 MV, followed by a strip with 100% 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 1) was used on an ÄKTA avant™ 150 (Cytiva). 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.

Figure 1: *Sartobind® S Nano 1 mL (Left) Sartobind® S 75 mL Capsule (Right)*

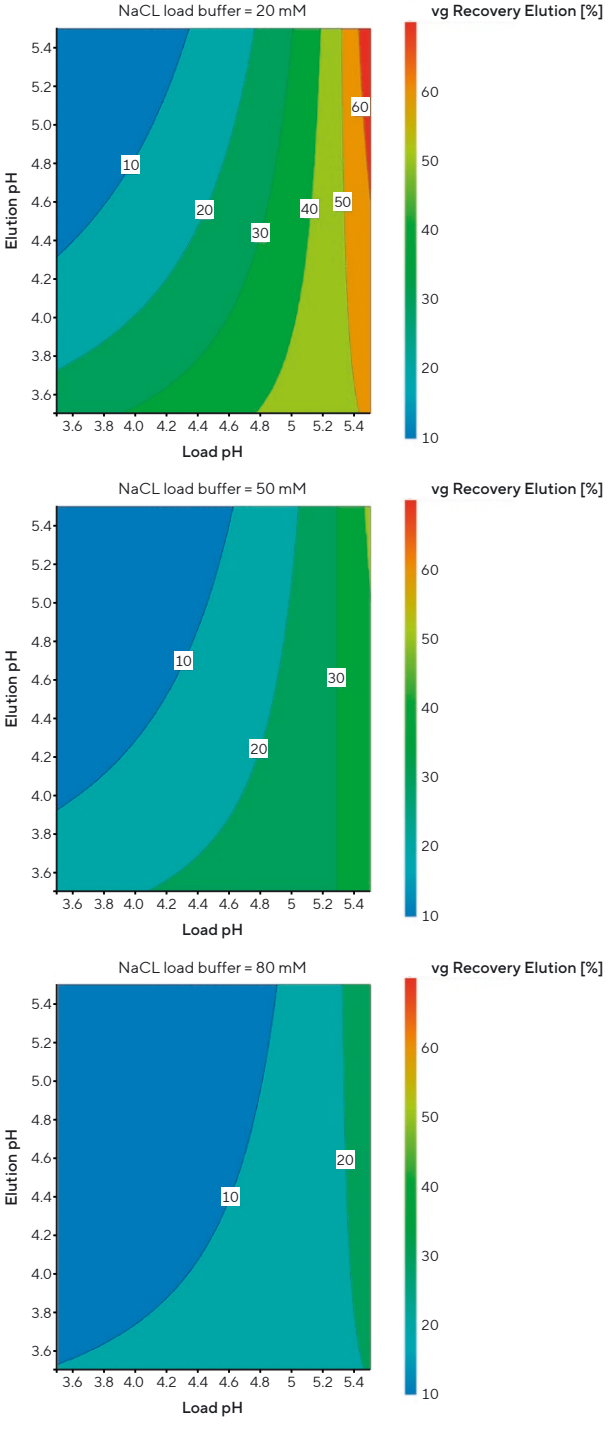
Analytical Methods

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

Development of AAV8 Capture Using Sartobind® S

A DoE approach was employed to establish the AAV8 capture step using Sartobind® S. The experimental design focused on optimizing the binding and elution behavior of AAV8 on the Sartobind® S membrane and identifying the critical factors influencing the effectiveness of the capture step.

Figure 2: *Response Contour Plots of the Viral Genome Recovery as a Function of all Three Factors Evaluated*



The analysis of the results and subsequent modeling of the DoE revealed that all factors significantly affected some of the evaluated responses. Figure 2 presents the response contour plots for viral genome recovery. The highest recovery (80% vg) was achieved at low NaCl concentrations and when the load and elution buffer pH were high.

The pH of both the load and elution buffers, as well as the conductivity | NaCl concentration of the load material, were identified as significant factors influencing vg recovery through the DoE analysis (Table 2). Although elution pH negatively correlates with vg recovery, high pH levels in both load and elution buffers lead to high viral genome recovery. This is due to a positive interaction between the two factors, which correlates positively with viral genome recovery. A similar trend was observed for capsid recovery in terms of the load and elution buffer pH as factors. However, a negative interaction between load buffer pH and NaCl concentration was detected in this case. Overall, we found that a low NaCl concentration in the load buffer enhances virus recovery and total protein removal.

For impurity removal, we observed a lower reduction under conditions that yielded the highest viral genome recovery. To increase protein removal, both the load pH and NaCl concentration should be low, which contrasts with their effects on viral genome recovery. Therefore, optimal parameter set-points for virus recovery must be balanced with some trade-offs in protein removal efficiency. DNA removal was highest at the setpoints that supported high virus recovery, further supported by its positive correlation with elution pH.

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

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

Note. Blank = non-significant term, ↑ = positive correlation, ↓ negative correlation, vp = viral particle (capsids), vg = viral genome

In conclusion, the optimal setpoints identified were a pH of 5.5 for both the load and elution buffers and a NaCl concentration of 20 mM in the load buffer.

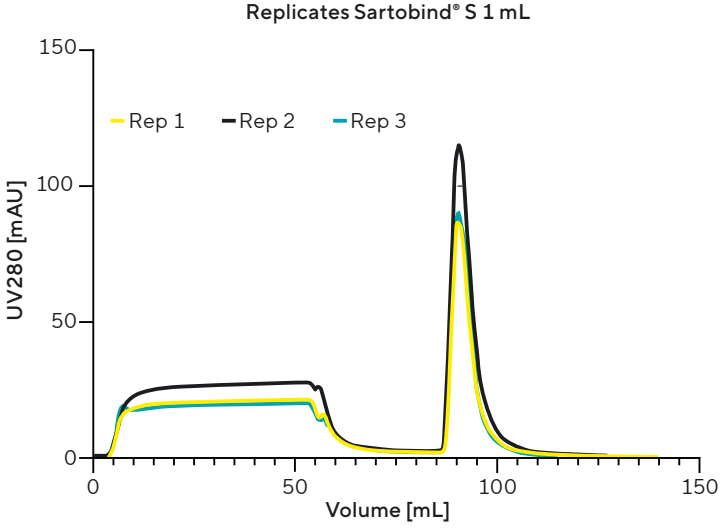
When analyzing the response contour plot for vg recovery, we observed the potential for even lower NaCl concentrations in the load buffer to enhance vg recoveries. To explore this effect further, we conducted a separate chromatographic purification experiment.

We performed two runs in parallel: one with 10 mM NaCl and the other with 20 mM NaCl in the load buffer, both at pH 5.5. Based on the functional readout results, reducing the NaCl concentration in the load buffer did not provide any further benefit but yielded recoveries similar to the runs with 20 mM NaCl in the load buffer. However, this could be a factor worth evaluating for the development of purification protocols for other serotypes.

Reproducibility Analysis of the Developed AAV8 Capture Protocol Using Sartobind® S

Three independent AAV8 capture chromatography runs were performed to assess the reproducibility of the established process. A Sartobind® S Nano 1 mL consumable was employed with the best parameters set points identified from the DoE study (Figure 3, Table 3).

Figure 3: *Overlay Of Chromatograms Of The Three Replicates Of AAV8 Capture Chromatography Performed With Sartobind® S Nano 1 mL Consumable*



Note. Runs were performed using the optimal conditions identified through the DoE study.

Table 3: *Capsids, Viral Genomes, dsDNA, and Protein Recovery of Three Replicates of AAV8 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. Mean recovery and coefficients of variation (CV) between the three replicates are shown. Runs were performed at pH 5.5 and 20 mM NaCl in the load buffer.

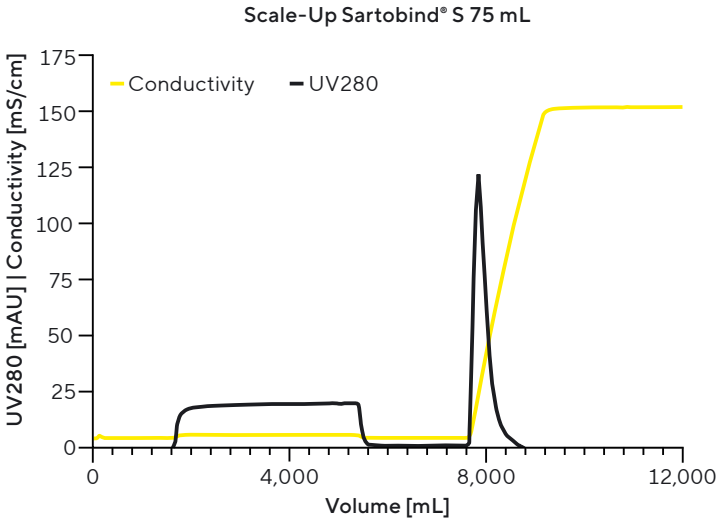
The results obtained for virus recovery and impurity removal of the replicate runs corresponded to the results obtained during the initial experiment from the DoE study. Overall, good reproducibility between the three runs was observed not only when evaluating the chromatographic run profiles (Figure 3) but also in terms of functional readouts with an average of 54% viral protein and 72% viral genome recovery and depletion of contaminants on average of >87% and >93% for proteins and DNA, respectively (Table 3). Generally, low coefficients of variation (CV) were observed, except for the DNA removal, which can be attributed to general assay variation.

Based on the results obtained, we conclude that the established capture chromatographic process for AAV8 using the Sartobind® S membrane consumable is very efficient and robust, yielding highly reproducible results.

Scale-Up of AAV8 Capture Using Sartobind® S

Next, we assessed the ability of the established process to scale linearly by performing a chromatographic run using a 75-fold larger AAV8 sample volume and a larger consumable: the Sartobind® S Capsule 75 mL (Figure 4).

Figure 4: *Chromatogram of AAV8 Capture Chromatography on Sartobind® S 75 mL Capsule*



Note. Run was performed using the optimal conditions identified through the DoE study.

We observed a higher capsid recovery with a similar viral genome recovery for the scaled-up run compared to the small-scale runs (84% vs. 54% capsids and 73% vs. 72% viral genomes, Table 4).

Similar contaminant removals were achieved following scale-up of the established AAV8 capture process (17% vs. 13% protein recovery, <LLOD vs. 7% dsDNA recovery, Table 4). Deviations are in the range of assay variability.

Table 4: *Comparison of Capsid, Viral Genome, dsDNA, and Protein Recovery From AAV8 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. Sartobind® S Nano 1 mL recoveries are the mean from three replicates.

Overall, the virus recoveries and contaminant removal levels obtained at the small scale aligned with the scale-up run results, suggesting linear and predictable scaling from the Sartobind S® Nano 1 mL consumable to the Sartobind® S 75 mL Capsule.

Conclusion

This study evaluated the Sartobind® S membrane chromatography consumable for its effectiveness in purifying AAV8 using cation exchange chromatography. A DoE approach was employed to identify optimal conditions for efficiently binding and eluting AAV8 capsids while significantly reducing DNA and protein impurities. This strategy also facilitated the characterization of key factors influencing purification efficiency.

The optimized conditions for AAV8 capture chromatography with the Sartobind® S membrane achieved:

- High viral genome recovery: 72%, with an 87% reduction in total protein and a 93% reduction in total DNA
- Robust reproducibility: Triplicate runs under optimized conditions produced consistent results
- Exceptional scalability: A 75-fold scale-up preserved high virus recovery and impurity removal rates

These results highlight the suitability and competitive performance of Sartobind® S membrane chromatography for AAV8 capture during downstream purification.
The streamlined and efficient purification protocol developed in this study offers a promising solution for AAV8 and potentially other AAV serotypes. By ensuring the availability of high-quality AAV vectors, our findings contribute to advancing gene therapy applications.

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Status: 02 | 2025