SARTORIUS

Application Note

December, 2024

Keywords or phrases:
AAV2, Sartobind® Convec SC capture, membrane chromatography

Adeno-Associated Virus 2 (AAV2) Capture with Sartobind® Convec SC Membrane Chromatography

Daniel Last[1], Mathias Kahl[1], Marius Lechner[2], Swapnil Chaudhari[2]*, David Dauer[2], André Krause[2], Fabien Rousset[2]
[1]IDT Biologika GmbH
[2]Sartorius Stedim Biotech GmbH

Correspondence
*Email: swapnil.chaudhari@sartorius.com

Abstract

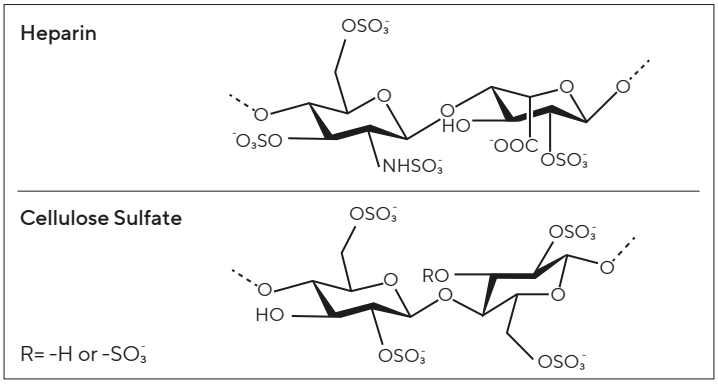
This study evaluates Sartobind® Convec SC for AAV2 pseudo-affinity capture. Sartobind® Convec SC employs sulfated cellulose ligands to mimic heparin, enabling efficient purification under mild elution conditions. With a dynamic binding capacity more than 0.8×10^{13} capsid particle (cp)/mL Sartobind® Convec SC achieves over 96% capsid recovery and 95% HCP reduction at neutral pH elution. The rapid 18-second contact time and pre-sterilized format reduce processing time and costs, making Sartobind® Convec SC a high-productivity, cost-reducing alternative to conventional resins for capturing various AAV serotypes and engineered capsids.

Introduction

Many immunological adeno-associated viral vector (AAV) serotypes are currently used in the biotechnology industry. Of these, AAV serotype 2 (AAV2)—the first fully characterized serotype—has been most extensively evaluated in pre-clinical studies[1]. The AAV2 capsid is composed of three structural proteins (VP1, VP2, and VP3) that are derived from the same gene but differ in their lengths and functionalities[2-4].

A heparin-binding motif has been previously identified in AAV2[5]. This has created a new opportunity to use chromatography media with sulfated cellulose ligands for pseudo-affinity capture of AAV serotypes. As shown in Figure 1, sulfated cellulose mimics heparin, a heavily sulfated glycosaminoglycan. The negatively charged sulfated cellulose enables ionic and pseudo-affinity interactions with proteins with heparin-binding motifs from viruses such as influenza, vaccinia, rabies, herpes, or microbial antigens.

Figure 1: *Comparison of Heparin Structure (Top) and Sulfated Cellulose Structure (Bottom)*

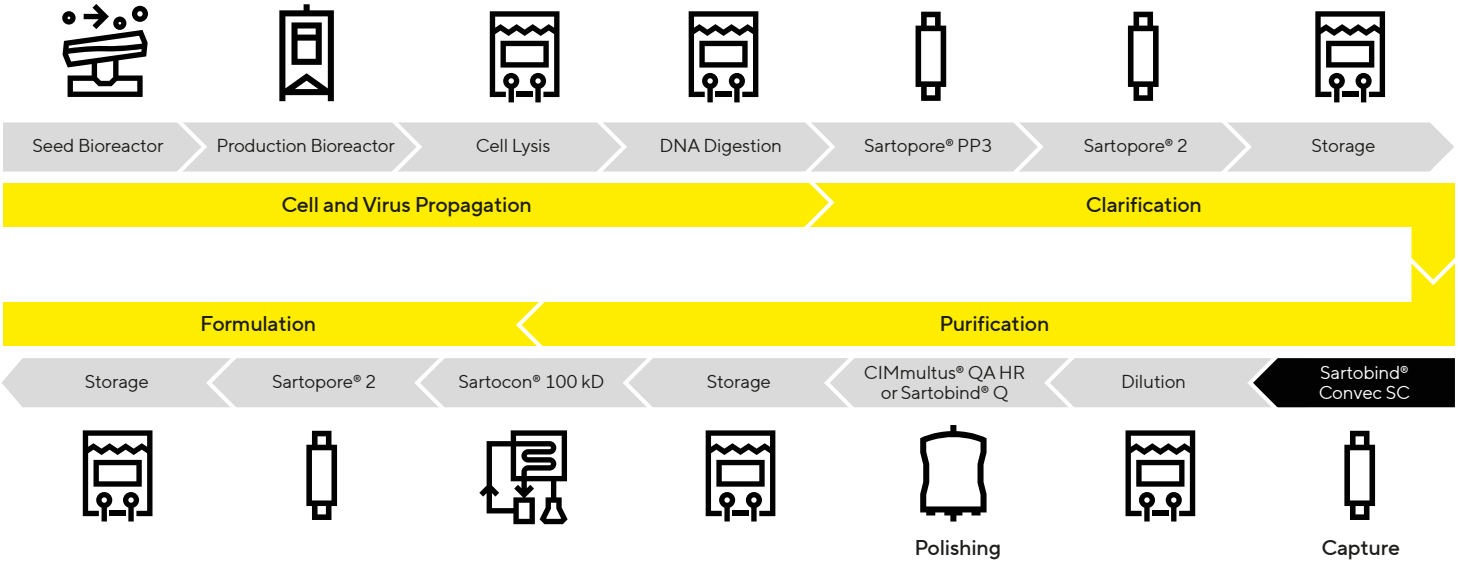


The use of membrane chromatography techniques for purifying AAVs is well-documented in the literature[1, 6, 7]. Membrane chromatography offers several advantages over traditional resin-based methods, including faster mass transfer due to convective transport and low-pressure drops. Additionally, its single-use format eliminates the need for column packing, cleaning, regeneration, and validation, significantly reducing labor and costs[8, 9, 11, 12, 13].

A newly developed Sartobind® Convec SC matrix allows proper convective mass transport of large biomolecules through the membrane structure thanks to its optimized porosity. Its channel size of ~1.1 µm makes it optimal for large viral particle capture. Compared to classic ion exchangers, the ligand density is optimized to improve ligand interaction for large biomolecules. It avoids excessive interaction on the surface of the chromatography matrix, which often leads to the deformation or degradation of viral particles. Sartobind® Convec SC helps to concentrate and purify viral capsids while removing most impurities at the same time[11]. A typical workflow of an AAV process using Sartobind® Convec SC for viral capture is shown in Figure 2.

This application note describes the use of Sartobind® Convec SC for AAV2 capture, performed by IDT Biologika. IDT is a global contract development and manufacturing organization for vaccine, gene, and immune therapeutics, as well as aseptic fill-finish of biologics and sterile injectables with sites in Germany and the US.

Figure 2: *Example of an AAV Manufacturing Process Using Sartobind® Convec SC as a Capture Medium*



Materials

Consumables

The consumables used in this study and their specifications are listed in Table 2.

Table 1: *Consumables Used for AAV2 Pseudo-Affinity Capture*

Sartobind® Convec SC Nano	Ligand	-OSO_3 (sulfated cellulose)
	Membrane volume (area)	3 mL (114 cm^2)
	Bed height	8 mm
	Maximum pressure bar at 20 °C	4 bar (0.4 MPa, 58 psig)
	Capsule material	Polypropylene
Sartopore® 2	Pore sizes	0.45 | 0.2 µm
	Filter area	0.015 m^2

Buffer, Reagents, and AAV-2 Feed

All buffers used are listed in Table 2.

Table 2: *Buffers Used for AAV2 Pseudo-Affinity Capture on Sartobind® Convec SC*

Phase	Ingredients	pH
Equilibration	10 mM Tris-HCl, 50 mM NaCl	7.4
Feed	Clarified cell culture lysate containing 2.5×10^{13} AAV-2 capsid particles	7.6
Wash	10 mM Tris-HCl, 50 mM NaCl	7.4
Elution 1	10 mM Tris-HCl, 500 mM NaCl	7.4
Elution 2	10 mM Tris-HCl, 1,000 mM NaCl	7.4
Elution 3	10 mM Tris-HCl, 2,000 mM NaCl	7.4
Regeneration	1 M NaOH, 2 M NaCl	14.0
Re-equilibration	10 mM Tris-HCl, 50 mM NaCl	7.4

Methods

HEK cells producing AAV2 were lysed using high salt treatment. Following clarification and enzymatic DNA digestion, the lysate was diluted 1:10 in 10 mM Tris-HCl buffer, pH 7.4, resulting in a final sample conductivity of 6.7 mS/cm and a pH of 7.6. Prior to capture on Sartobind® Convec SC, the diluted sample was filtered through a 0.45 | 0.2 µm Sartopore® 2 sterile filter. The capture process was then carried out according to the protocol described in Table 3.

Table 3: *AAV2 Pseudo-Affinity Capture Recipe on Sartobind® Convec SC*

Phase	Volume [MV]	Flowrate [MV/min]
Equilibration	15	3.33
Feed	333	3.33
Wash	15	3.33
Elution 1	10	3.33
Elution 2	10	3.33
Elution 3	10	3.33
Regeneration	15	0.25
Re-Equilibration	15	3.33

Results and Discussion

High Binding Efficiency and Protein Removal With Mild Elution Conditions

In this proof-of-principle study, 2.5×10^{13} AAV2 capsid particles (cp) were loaded on a 3 mL Sartobind® Convec SC Nano Capsule, demonstrating its potential for pseudo-affinity capture. The chromatogram on the right (Figure 3) shows elution at 0.5, 1, and 2 M NaCl.

Remarkably, only about 3% of AAV2 capsid and viral genome were detected in the flowthrough (Figure 4), indicating a dynamic binding capacity (DBC) exceeding 0.8×10^{13} cp/mL and a binding efficiency of 97%. This is consistent with the measured total AAV capsid recovery of more than 96% across all three elution fractions.

Notably, over 95% of the AAV2 capsids were eluted in the first elution fraction at 500 mM NaCl, pH 7.4, showcasing the mild elution conditions (Figure 4). The 14% difference in recovery between AAV capsid and viral genome falls within the expected assay fluctuation range, with the capsid titer being a more reliable measurement. A 95% reduction of host cell proteins (HCP) was also achieved.

High Productivity Due to Short Contact Times

In contrast to conventional AAV affinity chromatography media, which require a contact time of one minute or more, in this study, Sartobind® Convec SC bound AAV2 in just 18 seconds. This demonstrates its potential as a high-productivity alternative to traditional resin-based chromatography while leaving room for further improvement of flow rates, productivity, and binding capacity

Figure 3: ***(A)*** *Chromatogram of AAV2 Pseudo-Affinity Capture on Sartobind® Convec SC,* ***(B)*** *A Zoomed-in View Showing Flowthrough (FT) Elution at 0.5, 1, and 2 M NaCl*

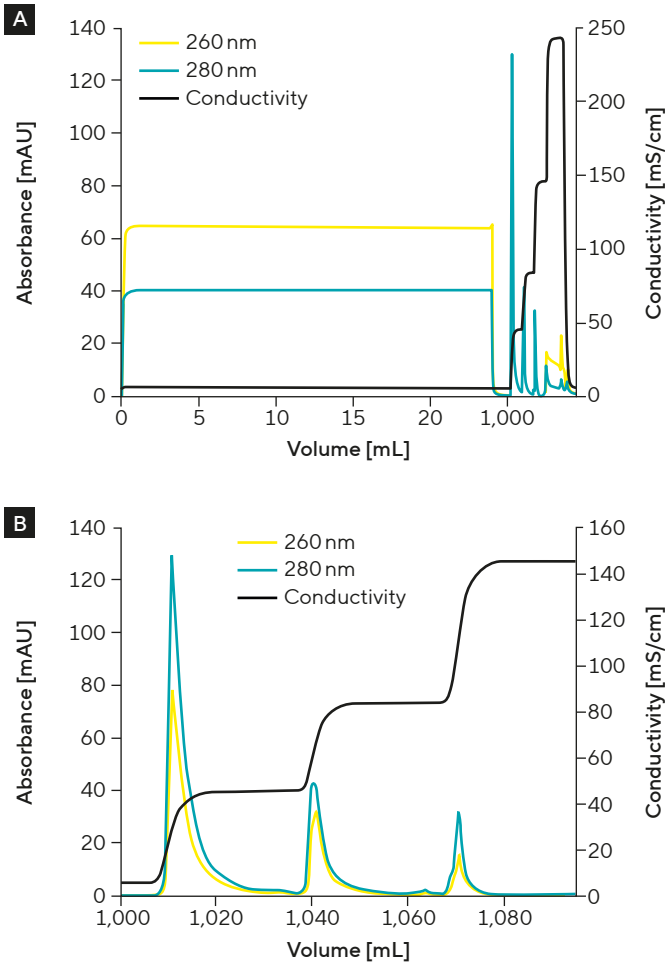
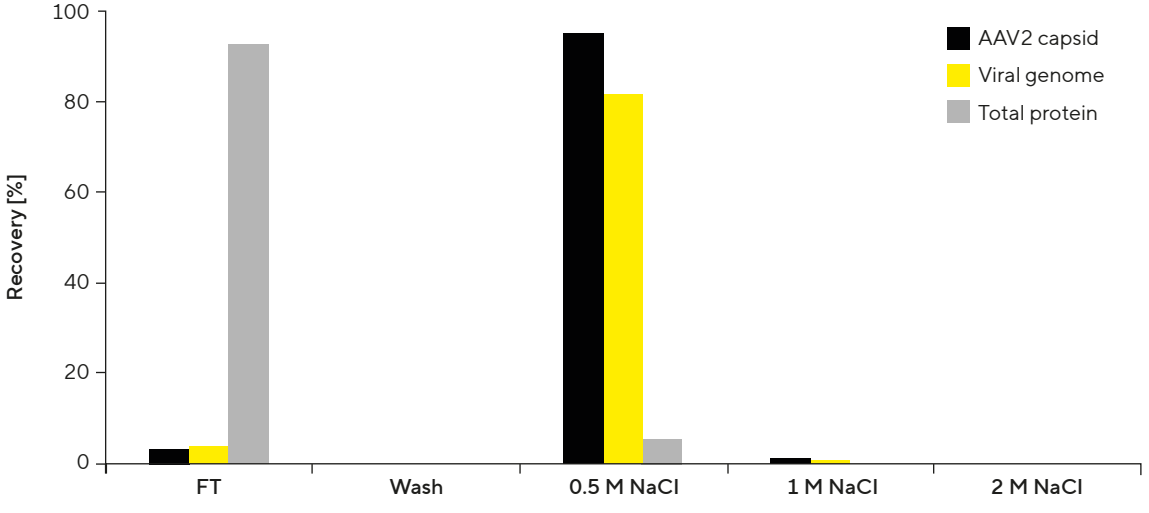


Figure 4: *AAV2 Capsid, Viral Genome, and Host Cell Protein (HCP; Total Protein) Recovery in the Flowthrough (FT), Wash, and Elution Fractions at 0.5, 1, and 2 M NaCl After Purification with Sartobind® Convec SC*



Conclusion

This study highlights the potential of Sartobind® Convec SC as a high-productivity alternative to conventional AAV affinity resins. Its high flow rates and short residence times facilitate rapid cycling within a single batch, significantly reducing the required consumable volume. The gamma-irradiated, ready-to-use formats Sartobind® Convec SC eliminates the need for packing, cleaning, and validation, minimizing processing time and costs. Additionally, the mild elution conditions at physiological pH enhance capsid stability, obviating the need for neutralization steps, and further streamlining the process while increasing total yield.

With the DBC for AAV2 expected to exceed 10^{13} cp/mL, Sartobind® Convec SC is also anticipated to effectively bind AAV serotypes 3, 6, 13 and engineered AAV capsids with heparin-binding motifs[10], making it an efficient and versatile and choice for AAV capture.

Table 4 lists the available options for Sartobind® Convec SC in the Nano product format. Sartorius will launch the entire product portfolio, ranging from Mini (20 mL MV) to 30" capsule (1,200 mL MV), in early 2025 (Figure 5).

Table 4: *Sartobid Convec® SC Nano Product Formats*

Order Number	Description	Quantity	Bed Height [mm]	Recommended Flow Rate [mL/min]	Maximum Pressure [MPa (bar | psig)]
97SC-04E-C11	Sartobind® Convec SC Nano 3 mL, 8 mm luer female connectors, 2 PEEK adapters luer male to UNF 10-32 female, manual, certificate	1	8	10	0.4 (4 | 58)
97SC-04E-C11-A	Sartobind® Convec SC Nano 3 mL, 8 mm luer female connectors, 2 PEEK adapters luer male to UNF 10-32 female, manual, certificate	4	8	10	0.4 (4 | 58)

Gamma irradiated version is also available

Figure 5: *Sartobind Convec® SC Product Portfolio*

Capsule Size	Nano	Mini	5" Capsule	10" Capsule	20" Capsule	30" Capsule
Membrane volume	3 mL	20 mL	150 mL	400 mL	800 mL	1,200 mL

References

1. Rieser, R., Koch, J., Faccioli, G., Richter, K., Menzen, T., Biel, M., Winter, G., & Michalakis, S. (2021). Comparison of different liquid chromatography-based purification strategies for adeno-associated virus vectors. Pharmaceutics, 13(5), 748. https://doi.org/10.3390/pharmaceutics13050748

2. Becerra, S. P., Koczot, F., Fabisch, P., & Rose, J. A. (1988). Synthesis of adeno-associated virus structural proteins requires both alternative mRNA splicing and alternative initiations from a single transcript. Journal of Virology, 62(8), 2745-2754. https://doi.org/10.1128/jvi.62.8.2745-2754.1988

3. Trempe, J. P., & Carter, B. J. (1988). Alternate mRNA splicing is required for synthesis of adeno-associated virus VP1 capsid protein. Journal of Virology, 62(9), 3356-3363. https://doi.org/10.1128/JVI.62.9.3356-3363.1988

4. Srivastava, A., Lusby, E. W., & Berns, K. I. (1983). Nucleotide sequence and organization of the adeno-associated virus 2 genome. Journal of Virology, 45(2), 555-564. https://doi.org/10.1128/JVI.45.2.555-564.1983

5. Kern, A., Schmidt, K., Leder, C., Müller, O. J., Wobus, C. E., Bettinger, K., Von der Lieth, C. W., King, J. A., & Kleinschmidt, J. A. (2003). Identification of a heparin-binding motif on adeno-associated virus type 2 capsids. Journal of Virology, 77(20), 11072-11081. https://doi.org/10.1128/jvi.77.20.11072-11081.2003

6. Crowley, J. J. L. (2023). Purifying viral vectors: A review of chromatography solutions. In Bioprocess and analytics development for virus-based advanced therapeutics and medicinal products (ATMPs). Springer, Cham.

7. Okada, T., Nonaka-Sarukawa, M., Uchibori, R., Kinoshita, K., Hayashita-Kinoh, H., Nitahara-Kasahara, Y., Takeda, S., & Ozawa, K. (2009). Scalable purification of adeno-associated virus serotype 1 (AAV1) and AAV8 vectors, using dual ion-exchange adsorptive membranes. Human Gene Therapy, 20(9), 1013-1021. https://doi.org/10.1089/hum.2009.006

8. Zydney, A. L. (2021). New developments in membranes for bioprocessing – A review. Journal of Membrane Science, 620, 118804. https://doi.org/10.1016/j.memsci.2020.118804

9. Orr, V., Zhong, L., Moo-Young, M., & Chou, C. P. (2013). Recent advances in bioprocessing application of membrane chromatography. Biotechnology Advances, 31(4), 450-465. https://doi.org/10.1016/j.biotechadv.2013.01.007

10. Van Lieshout, L. P., Stegelmeier, A. A., Rindler, T. N., Lawder, J. J., Sorensen, D. L., Frost, K. L., Booth, S. A., Bridges, J. P., & Wootton, S. K. (2023). Engineered AAV8 capsid acquires heparin and AVB sepharose binding capacity but has altered in vivo transduction efficiency. Gene Therapy, 30(3-4), 236-244. https://doi.org/10.1038/s41434-020-00198-7

11. Fortuna, A. R., Taft, F., Villain, L., Wolff, M. W., & Reichl, U. (2017). Optimization of cell culture-derived influenza A virus particles purification using sulfated cellulose membrane adsorbers. Engineering in Life Sciences, 18(1), 29-39. https://doi.org/10.1002/elsc.201700108

12. Liu, J. R. S. (2013). USA Patent No. US8357376 B2.

13. Villain, L. H. H. (2015). WO Patent No. WO 2015/055269 A1.

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

Status: 03 | 2025