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Enhanced Anion-Exchange Capture Chromatography of Lentiviral Vectors Using Sartobind® Convec D

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Abstract

Large-scale manufacturing has been a bottleneck in transitioning the use of lentiviral vectors from clinical trials to commercial cell and gene therapy applications.This application note details the development of a robust and scalable method for capturing lentiviral vectors using Sartobind® Convec D membrane chromatography. We evaluated the performance of this membrane adsorber in capturing and purifying lentiviral particles by assessing its ability to retain and recover infective viral particles and remove contaminants under gentle process conditions. Importantly, the membrane is available in a gamma-irradiated version, supporting its use in a bioburden-free environment. The findings highlight the potential of Convec® D membrane chromatography as an effective solution for lentiviral purification, offering significant advantages for large scale production of viral vectors used in gene therapy.

Introduction

Lentiviral vectors (LVs) are increasingly used as a tool for gene and cell therapy. These vectors have become the vector of choice for ex vivo modification of patient cells and are especially popular for chimeric antigen receptor T (CAR-T) cell therapies. However, the purification of these vectors remains challenging due to the low stability and tolerance of these enveloped viruses to process conditions such as pH, salt, temperature, and shear forces. As a result, the ability of viral vector manufacturers to meet clinical demand is still limited by production processes.

Sartobind® membrane chromatography devices are particularly advantageous for the purification of large particles, including viruses. Due to their mainly intrinsic convective flow, membranes offer significantly reduced mass transfer resistance and lower back pressure profiles than resins. In addition, membrane chromatography devices are known to be relatively easy to manufacture, with simple clean-in-place procedures and linear scale-up. The net negative charge of LV particles at a neutral pH makes anion exchange (AEX) chromatography feasible and a popular choice for LV manufacturing processes. However, current technologies are often unsuitable for LVs; besides their labile character, they present a substantial amount of charged species on their surface, resulting in complex adsorption and binding phenomena.

Sartobind® Convec D is a specialized weak AEX chromatography solution designed for the efficient downstream processing of LVs; it has been screened for the most suitable pore size and binding properties for LV purification. The novel membrane family provides an increased surface area due to the optimized pore size, increased homogeneity, and weakened virus-ligand interactions provided by the lower density of ligands.

Maintaining a low bioburden state across chromatographic and non-chromatographic operations is crucial in integrated continuous biomanufacturing. Employing gamma-irradiated disposables offers a significant advantage by ensuring these materials remain bioburden-free. So far, this strategy has not been widely implemented, likely because gamma irradiation is suspected to impact the performance of the chromatographic device. The irradiation process is known to possibly impact parts of the functionalized membrane, e.g., the ligand conformation required for successful binding and capacity for lentiviral vectors. In contrast, other membrane characteristics, e.g., ligand density, might not change.

This study presents an efficient purification solution for LV particles using convective mass transfer and weak AEX chemistry combined with the unique properties of Sartobind® Convec D. The adsorber enabled viral recoveries up to 74% in combination with gentle load and elution conditions while allowing for low contaminant binding profiles. Ultimately, no impact on product recovery nor quality was identified while using the gamma-irradiated products used to support closed and aseptic bioprocessing, reducing contamination and bioburden risks.

Methods

Cell Culture and Clarification
LVs were produced by transient transfection of suspended HEK293 cells using PEIpro® (Sartorius) in a 10 L Univessel® Glass bioreactor controlled by a Biostat® B (Sartorius). An endonuclease step was performed to digest nucleic acids for optimal results during the downstream processing. The harvest clarification was performed using a Sartopure® PP3 20 µm followed by a Sartopure® PP3 0.65 µm and Sartoclean® 2 0.8 µm (Sartorius; all size 9 filters). Harvested LV was stored in aliquots, frozen at –80 °C, and used as feed for all the studies. The titer of the LV material was 1.1×10^8 TU / mL.

Anion-Exchange Chromatography
The chromatographic runs were performed using a Sartobind® Convec D Nano 3 mL 8 mm bed height (Sartorius; Figure 1) with an ÄKTA avant™ 150 (Cytiva). During the chromatographic runs, conductivity and UV absorbance at 280 nm were monitored as well as the multiangle light scattering (MALS) signal with a DAWN detector (Waters) for an online assessment of the viral particles.

Figure 1: *The Sartobind® Convec D Nano 3 mL (8 mm Bed Height) is Sartorius´ Smallest Commercialized Scalable Device*

A flowrate of 5 MV/min was used for all the chromatographic runs. The equilibration buffer was composed of 20 mM Tris pH 7.0. The elution buffer was composed of 20 mM Tris pH 7.0, 2 M NaCl, 10 mM arginine, and 10 mM $MgCl_2$. Before loading, the conductivity of the LV samples was adjusted to 7 – 10 mS/cm with the equilibration buffer. Upon the first use of a Sartobind® Convec D membrane adsorber, an initial wash with 20 membrane volumes (MV) with 0.5 M NaOH was performed (and held for 30 mins). This was followed by 10 MV of equilibration buffer, 10 MV of elution buffer, and an additional 20 MV of equilibration buffer (or until a stable constant conductivity signal was observed) before sample loading.

To determine the dynamic binding capacity (DBC), flowthrough fractions were analyzed in 25 mL increments during the loading phase for particle DBC10%. The particle density of each fraction was determined from the MALS signal (using the sphere model, ASTRA Software version 8.2). For the bind-elute mode chromatographic runs, the required volume of the equilibrated LV sample was loaded at DBC10%, followed by a wash step with 30 MV of equilibration buffer. Elution was performed over a 40 MV gradient from 0% to 100% elution buffer and in an isocratic mode with 50% or 100% elution buffer for 40 MV. Elution was followed by a strip with 100% elution buffer for 30 MV. The loading sample and elution peaks were collected and immediately diluted 1 : 5 with buffer composed of 5% Sucrose, 20 mM $MgCl_2$, and 50 mM HEPES pH 7.5.

To evaluate the possible impact of gamma irradiation on the functionalized membrane, gamma-irradiated Sartobind® Convec D Nano 3 mL membranes (with an 8 mm bed height) were compared to non-irradiated membrane units of the same material and format. The deviations (%) in total and functional titer recovery, as well as removal of residual DNA and total protein, between the mean values (n = 3) of gamma- and non-irradiated membranes were evaluated as key indicators.

Analytical Methods for Quantifying LVs and Contaminants
Analytical testing included infectious titer by TU assessment through GFP expression (Incucyte® S3 Live-Cell Analysis System), viral particles (vp) titer (LV-associated p24 ELISA), total protein (Bradford), and residual DNA (PicoGreen) assays.

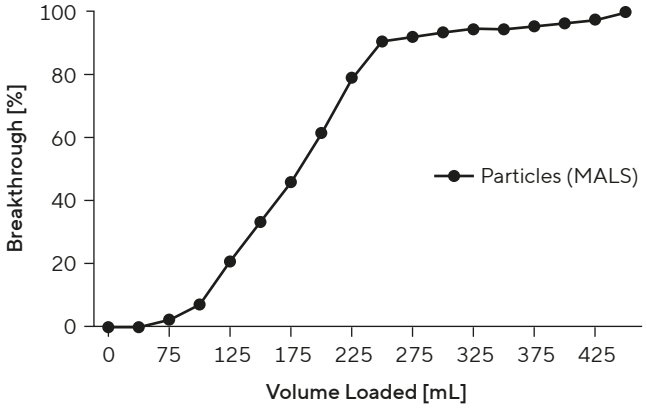
Results and Discussion

The capture step was established using the Sartobind® Convec D Nano (anion-exchanger) to purify LV by removing major impurities, such as DNA and proteins. The purification was performed in a bind-elute mode where the virus particles, which have a negative surface charge at working pH, are adsorbed by the positively charged matrix.

Dynamic Binding Capacity (DBC)

The selection of a suitable loading range of LV amount/mL of membrane was determined based on assessing the dynamic binding capacity (DBC). This value is highly dependent on the quality of the clarified harvest material, such as titer and contaminant content. The DBC of the Sartobind® Convec D was determined via MALS, which was linearly correlated with p24 Elisa assessments for particle load. The flowthrough fractions were analyzed in a 25 mL volume increments to determine the breakthrough of the lentiviral particles (Figure 2).

Figure 2: *Breakthrough Profile During Loading on the Sartobind® Convec D Membrane Adsorber Determined by Multiangle Light Scattering*



The 10% breakthrough of the LV particles was reached after loading approximately 110 – 120 mL of feed material, resulting in DBC values of approximately 1.5×10^{11} total particles/mL of membrane (Table 1).

Table 1: *Load Density and DBC10% of the Sartobind® Convec D Membrane Determined via Multiangle Light Scattering (MALS) and p24 ELISA*

	Load Density [Particles/mL]	DBC10%/mL Membrane
MALS	3.93×10^9	~1.50×10^{11}
p24 ELISA	3.99×10^9	~1.53×10^{11}

Isocratic vs. Gradient Elution on Sartobind® Convec D Membranes

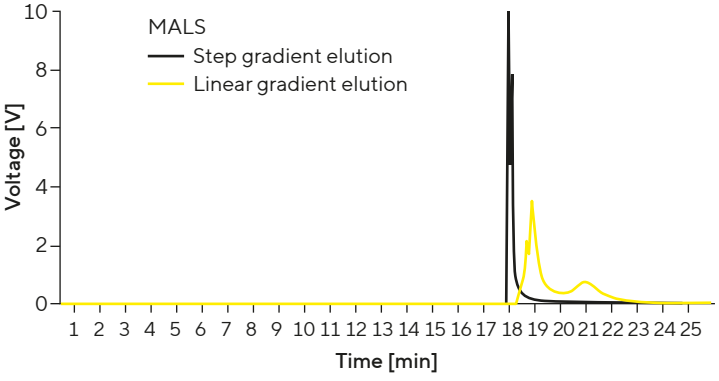
After defining the LV loading amount, the bind-elute runs were performed by saturating the membrane adsorbers until ~10% breakthrough by loading 120 mL of LV feed sample (corresponding to 40 MV). The elution was performed using linear gradient elution (0% to 100% elution buffer) and in isocratic mode by applying a single-step elution (50% or 100% elution buffer) during 40 MV (Table 2).

Table 2: *Chromatographic Elution Strategies Applied to the Sartobind® Convec D to Recover LVs*

Elution	Load [MV] [7 – 10 mS/cm]	NaCl [M]	Conductivity MPB [mS/cm]
Linear gradient	40	0 – 2	92 – 158
Isocratic	40	1	92
Isocratic	40	2	15

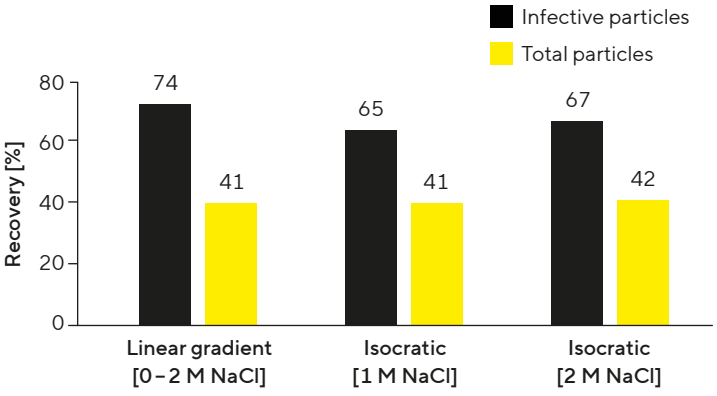
In Figure 3, the LV particles are represented by the MALS (V) signal during the entire chromatographic run, using a linear gradient and isocratic elution.

Figure 3: *MALS (V) Signal for the Two Strategies of LV Elution on the Sartobind® Convec D Membrane Adsorber (Step-Gradient Elution and Linear Gradient Elution)*



A summary of the recovery of infective and total LV particles, determined by the cell-based infectivity assay and p24 ELISA, is shown in Figure 4.

Figure 4: *Recovery of Infective and Total Particles After Purification of LV Feedstreams Using Sartobind® Convec D Nano 3 mL*



A linear gradient elution (0% to 100% elution buffer) was used as an initial approach. However, a step elution is typically preferred due to the lower elution volumes, supporting operational efficiency in large-scale manufacturing. During gradient elution, the elution peak tends to lengthen due to the gradual increase in salt concentration, resulting in a broader peak. When a step elution is applied, the peaks tend to sharpen.

Examining the chromatogram from the gradient elution of Sartobind® Convec D, the MALS signal reveals a broad 'two-peak' profile (Figure 3). Separate LV populations with inherent structural differences are described in the literature. Those differences, which show different binding species on their envelope, have been reported as potential causes of the individual peak elution.[1] However, the same phenomenon is not observed when an isocratic elution is applied (with 50% or 100% elution buffer), where a single sharp peak is observed.

Traditional AEX chromatographic stationary phases are usually operated at high salt concentrations (up to 1.5–2 M) to recover the LV particles. Still, the reported recoveries are often relatively poor (10–30%).[2] With Sartobind® Convec D, both gradient and isocratic elution approaches showed good compatibility with the enveloped infectious virus particles, yielding recoveries ranging from 65–74% (Figure 4). The slight improvement observed in the recovery of infectious particles from elution with a linear gradient (74%) can potentially be explained by the lower salt content. The elution of the LV particles was achieved with less than 750 mM NaCl, compared to isocratic elution with 1 M or 2 M NaCl, which resulted in 65% and 67% recovery, respectively. It is important to note that these differences fall within the assay variation of the infectivity technique. In addition, no improvement was observed when applying an isocratic elution with 2 M NaCl, compared with 1 M NaCl. LVs are known to be sensitive to high salt concentrations, which can increase virus inactivation levels. Therefore, the ability to elute the LVs at lower salt concentrations is a key process advantage.

The majority of particles in typical LV feed streams are not infective[3] because they are damaged, lack RNA | capsid, or are otherwise incomplete. As such, a key advantage of a chromatography solution would be its ability to retain the infective particles while removing as many non-infective particles as possible. This work also demonstrated that the ratio of infectious particles to total particles could be increased using all elution strategies (up to ~62% enrichment).

It is also known that the binding mechanism for LV particles during sample loading can significantly impact the number of recovered particles during the elution. As such, it is common practice to apply high salt concentrations to increase the conductivity during the loading phase, reducing the strength of the interaction between the particles and the stationary phase. The lower density of AEX ligands inherent to the Sartobind® Convec D offers a huge process advantage compared with traditional AEX chromatographic stationary phases by negating the need to add salt to the feed streams.

Since binding is driven by electrostatic interactions, AEX matrices are usually not selective for LVs: negatively charged nucleic acids and proteins also bind to the chromatographic media. Sartobind® Convec D was very efficient in depleting proteins during the loading phase. High total protein removal was observed in the flowthrough samples, with 85–90% removal during loading, while particles were retained, leading to a higher number of free binding spaces for virus particles.

DNA was removed to a lesser extent (30 – 60% residual DNA removal), resulting in co-elution of the DNA with the LVs. The presence of chromatin is described as a possible explanation for this behavior in AEX adsorbers. At this operating pH range, the complexes of DNA, which are wrapped around histones, carry charges that bind to the positive ligands of the AEX adsorber and are only eluted when higher salt concentrations are applied.[4]

To assess the possible impact of gamma irradiation on the functionalized membrane, we compared the deviation of the performance of gamma-irradiated (n = 3) and non-irradiated Sartobind® Convec D Nano devices (n = 3). We observed ≤ 8% and 12% deviation in the level of infective and total titer recovery, respectively, between mean values of gamma- and non-irradiated membranes. Comparable total protein and residual DNA clearance profiles were also observed, with a deviation of ≤ 5% in residual DNA and total protein removal between the mean values of gamma- and non-irradiated membranes. Taking into consideration the known and expected variance of the assays, no impact on the membrane functionalization by the gamma-irradiation process was identified.

Conclusion

In this study, we evaluated the performance of the Sartobind® Convec D membrane in capturing and purifying LV particles through AEX chromatography. This new cellulose-structured membrane — tailored for LV application — offered high recoveries while enabling lower salt concentrations for LV recovery. This represents a pivotal process advantage due to the sensitivity of these viral particles to harsh process conditions. In addition, the Sartobind® Convec D exhibits minimal protein binding during the loading of the viral feed, freeing up more binding sites to retain the viral particles, leading to relatively high DBCs. Lastly, this study shows that gamma-irradiation did not impact the performance of the functionalized membranes for the LV application, offering a valuable process alternative to maintain sterility during integrated continuous biomanufacturing. Overall, the results of this study highlight the potential of Sartobind® Convec D membrane adsorbers to establish an efficient and robust LV manufacturing process.

References

1. Pamenter, G., Davies, L., Lamont, C., Rahim, D., Knevelman, C., Miskin, J., Mitrophanous, K., Dikicioglu, D., & Bracewell, D. G. (2024b). Lentiviral vector determinants of anion-exchange chromatography elution heterogeneity. Biotechnology and Bioengineering, 121(9), 29362951.https://doi.org/10.1002/bit.28766

2. Moreira, A. S., Cavaco, D. G., Faria, T. Q., Alves, P. M., Carrondo, M. J. T., & Peixoto, C. (2021). Advances in lentivirus purification. Biotechnology Journal, 16(1), e2000019. https://doi.org/10.1002/biot.202000019

3. Soldi, M., Sergi, L. S., Unali, G., Kerzel, T., Cuccovillo, I., Capasso, P., Annoni, A., Biffi, M., Rancoita, P. M. V., Cantore, A., Lombardo, A., Naldini, L., Squadrito, M. L., & Kajaste-Rudnitski, A. (2020). Laboratory-scale lentiviral vector production and purification for enhanced ex vivo and in vivo genetic engineering. Molecular Therapy — Methods & Clinical Development, 19, 411–425. https://doi.org/10.1016/j.omtm.2020.10.009

4. Nian, R., & Gagnon, P. (2016). Advance chromatin extraction enhances performance and productivity of cation exchange chromatography-based capture of immunoglobulin G monoclonal antibodies. Journal of Chromatography A, 1453, 54–61. https://doi.org/10.1016/j.chroma.2016.05.029

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