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Downstream Processing Workflows for the Intermediate Purification of Lentiviral Vectors

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Abstract

The growing need for high quantities and purities of lentiviral vectors (LVs) has driven the development of cost-effective purification strategies that can scale efficiently to meet these demands. Anion-exchange (AEX) chromatography is a leading unit operation typically used during the intermediate purification phase of large-scale bioprocesses. While a common approach is to apply AEX directly to capture the product after clarification, tangential flow filtration (TFF) can be used prior to chromatography to concentrate the viral product, perform buffer exchange, and remove smaller impurities.

Sartobind® Convec D is a weak AEX chromatography membrane designed for LV capture, optimized to improve virus recoveries while operating under gentle conditions. Hydrosart® high-performance TFF membranes are cellulose-based, optimized for virus purification, and known for their remarkably high fluxes.

This study aims to incorporate these two Sartorius technologies into an intermediate purification workflow for LVs and evaluate the impacts of adding a TFF step prior to AEX chromatography on process performance, assessing the process efficiency of the intermediate LVs purification steps.

Introduction

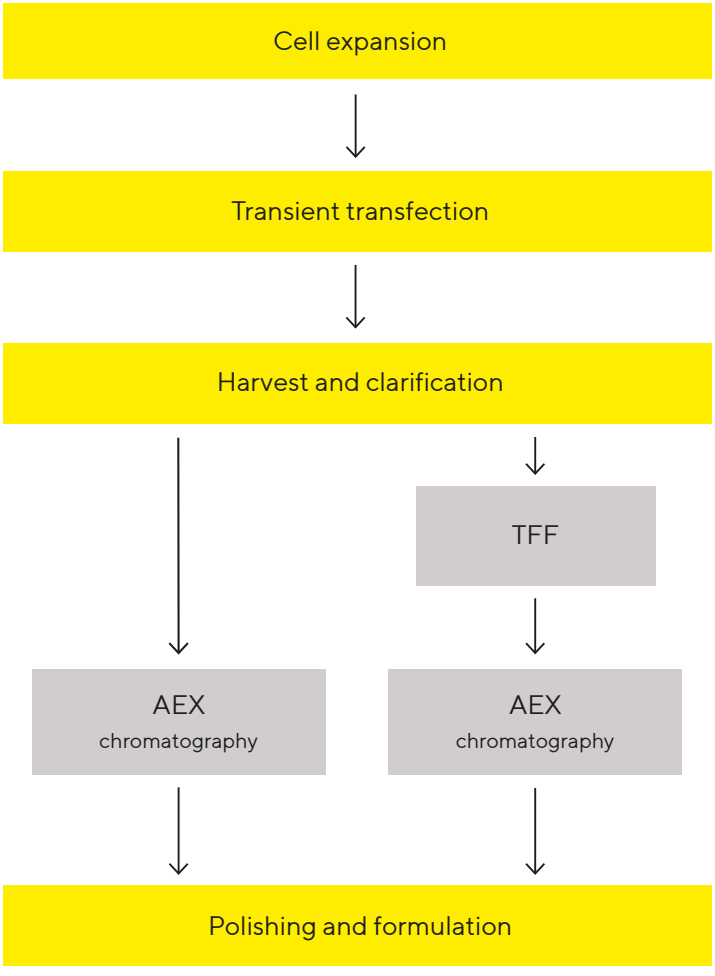
Lentiviral vectors production and purification processes have evolved, but the growing demand for higher quantities and purity is driving the development of more cost-effective strategies. Processing requirements differ based on whether the vectors are used in vivo or ex vivo. In both applications, bioprocess operations should focus on reducing impurities (such as host cell proteins, residual DNA, transfection reagents, and media components) while maintaining high yields and viral efficacy.[1] After clarification, the scalable purification of LVs typically involves chromatography and | or tangential flow filtration (TFF). Resin chromatography offers scalability, high purity, and recovery, but the large hydrodynamic radius of LVs can limit binding capacity and flow rates due to high backpressures. In this context, membrane adsorbers and monoliths, which operate primarily through convective flow, enable faster processing and higher throughput, making them ideal for LV capture. After virus clarification, TFF can be used for concentration, reducing the feed volume for the later steps, whereas small impurities such as proteins and DNA fragments are removed. When dia-filtration is performed, TFF allows for buffer exchange to an appropriate composition or conductivity, which is relevant when a subsequent anion exchange (AEX) chromatographic step is applied.[2]

Sartobind® Convec D is a specialized weak AEX chromatography solution designed with suitable 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.[3] Hydrosart® high-performance TFF membranes represent the state-of-the-art technology for virus purification, optimized for biopharmaceutical applications. These cellulose-based membranes are highly hydrophilic, delivering high flux, chemical resistance, and mechanical stability under a wide range of conditions.[4]

This study aims to provide a comprehensive comparison of the two purification workflows to determine the value of including a TFF step before AEX chromatography during downstream LV processing (Figure 1).

By assessing key metrics such as yield, throughput, and impurity removal, the study will offer insights into how best to optimize the intermediate purification of LVs using Sartobind® Convec D to effectively capture the virus particles and Hydrosart® membranes to concentrate the product, reduce buffer conductivity, and remove smaller impurities prior to chromatography.

Figure 1: *Processing Workflow for Intermediate LV Purification Using an AEX-Only Workflow and a Workflow With TFF Followed by AEX*



Materials and Methods

LV Production, Harvest, and Clarification

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

Capture Chromatography (AEX)

Chromatographic runs were performed using a Sartobind® Convec D Nano 3 mL with 8 mm bed height (Sartorius) using an ÄKTA avant™ 150 (Cytiva). During the chromatographic runs, conductivity and UV absorbance at 280 nm were monitored. The multi-angle light scattering (MALS) signal was monitored with a DAWN detector (Wyatt Technology) for an online assessment of the viral particles. A flow rate 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, with 2 M NaCl, 10 mM arginine, and 10 mM $MgCl_2$.

Tangential Flow Filtration (TFF)

TFF was performed using a Sartocon® Hydrosart® Slice 200 TFF cassette with a pore size of 300 kDa (Sartorius) and an effective filter area of 180 cm^2 on the Sartoflow® Smart TFF system (Sartorius). The process was controlled through a constant inlet pressure using the optimal operating delta pressure (ΔP) and transmembrane pressure (TMP).

Analytical Methods

Analytical quantifications included infectious titer by assessing transducing units (TU) via GFP expression (Incucyte® S3 live-cell analysis), total protein by Bradford assay, and residual DNA using a PicoGreen assay.

Result

AEX-Only Workflow

As described in detail in a previous application note[5], the capture step was established using a Sartobind® Convec D Nano 3 mL[3] to capture LVs from a clarified lysate and remove major impurities, such as proteins and DNA.

AEX Chromatography

Dynamic Binding Capacity

The appropriate loading range of LVs per mL of membrane was determined by assessing the dynamic binding capacity (DBC) of the clarified material (Table 1). Prior to loading into the chromatographic membrane, the clarified LVs were diluted to adjust to a final conductivity of 7 – 10 mS/cm.

Table 1: *Loading Volume Corresponding to 10% Breakthrough and Respective $DBC_{10\%}$ (Particles/mL Membrane) of Clarified LV Using Sartobind® Convec D*

Sartobind® Convec D Nano 3 mL	
Volume 10% breakthrough [mL]	120
DBC 10% [particles/mL membrane]	1.5×10^{11}

Note *The DBC is highly dependent on the quality of the clarified harvest material, such as titer and contaminant content; therefore, this value must be estimated for the feed stream intended to be used in the capture step*

Capture in Bind-Elute Mode

The membrane adsorber was saturated until 10% of the breakthrough volume (Figure 2), and the elution strategy was screened by applying a linear gradient (0 – 2 M NaCl) and an isocratic step elution using two different elution buffer conductivities (1 M and 2 M NaCl). The infectious recovery, as well as protein and DNA removal ranges, were evaluated (Table 2).

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Figure 2: *MALS (V) Signal During Linear Gradient Elution of Clarified LVs Using Sartobind® Convec D*

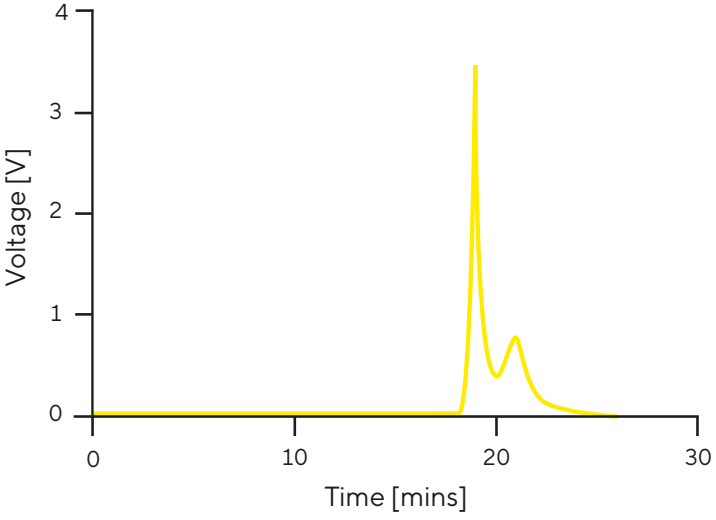


Table 2: *Infectious Particle Recovery [%], Protein Removal [%], and DNA Removal [%] for UF | DF of Clarified LV Using Sartobind® Convec D*

Sartobind® Convec D Nano 3 mL	Elution Sample
Infectious particle recovery [%]	65 - 74
Protein removal [%]	85 - 90
DNA removal [%]	30 - 60

Both gradient and isocratic elution strategies yielded recoveries ranging from 65 – 74%. Regarding contaminants, 85 – 90% protein removal was observed during loading. To a lesser extent, approximately 30 – 60% residual DNA removal was achieved, resulting in partial co-elution of the DNA with the LVs.

TFF-AEX Workflow

TFF

As described in detail in an earlier application note,[6] the UF | DF step was optimized and established using Sartocon® Hydrosart® Slice 200 TFF cassettes[4] to concentrate and diafiltrate LVs from a clarified lysate, while removing small impurities such as proteins and DNA fragments.

The optimal operating TMP was determined based on permeate flux and suitable delta pressure (ΔP) recirculation rate (Figure 3).

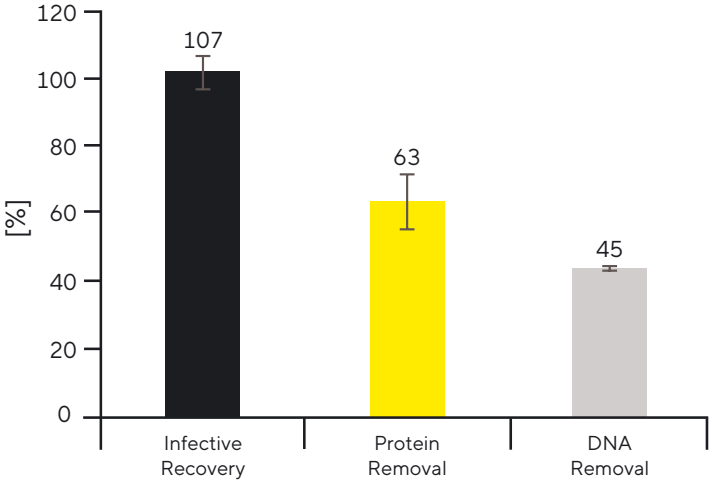
Table 3: *Parameters and Total Processing Time for UF | DF of Clarified LVs Using a Sartocon® Hydrosart® Slice 200 TFF Cassette*

Sartocon® Hydrosart® Slice 200 300 kDa	
Inlet pressure [fixed bar]	0.95
Δ pressure [ΔP; bar]	0.80
TMP [bar]	0.55
Target UF | DF [fold change]*	10 x | 5 x
Processing time [UF | DF; min]	23 – 30

*Two flushes (one hold-up volume each) were added to the retentate sample

The TFF trial was further evaluated with regard to the recovery of infectious particles as well as protein and DNA removal ranges (Table 4).

Figure 3: *Infectious Particle Recovery [%], Protein Removal [%], and DNA Removal [%] for UF | DF of Clarified LVs Using a Sartocon® Hydrosart® Slice 200 TFF Cassette*



The optimized process parameters resulted in infectious LVs recoveries exceeding 100%, while achieving a significant removal of proteins (63%) and DNA (45%).

AEX Chromatography

DBC

Similar to the AEX-only workflow, the selection of a suitable loading range of LVs per mL of membrane was determined based on the DBC for the ultrafiltered and diafiltered LVs from the TFF step (Table 4). In contrast to the AEX-only approach, the retentate sample from the TFF was directly loaded into the chromatographic membrane with no further buffer conductivity adjustments.

Table 4: *Loading Volume Corresponding to 10% Breakthrough and Respective $DBC_{10\%}$ (Particles/mL Membrane) of Ultrafiltered and Diafiltered LVs Using Sartobind® Convec D*

Sartobind® Convec D Nano 3 mL	
Volume 10% breakthrough [mL]	25
DBC 10% [particles/mL membrane]	6.2×10^{11}

Capture in Bind-Elute Mode

The membrane adsorber was saturated until 10% of breakthrough volume (Figure 4), and a linear gradient was used to elute the LVs (0 – 2 M NaCl). The recovery of infectious particles, as well as protein and DNA removal ranges achieved, were evaluated (Table 5).

Figure 4: *MALS [V] Signal Using a Linear Gradient Elution of Ultrafiltered and Diafiltered LVs Using Sartobind® Convec D*

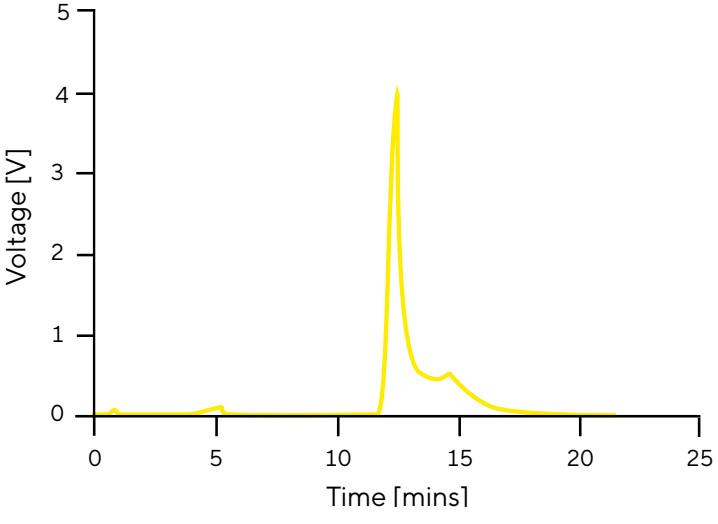


Table 5: *Infectious Recovery [%], Protein Removal, and DNA Removal [%] of Ultrafiltered and Diafiltered LV Using Sartobind® Convec D*

Sartobind® Convec D Nano 3 mL	Elution Sample
Infectious particle recovery [%]	60
Protein removal [%]*	Undetectable
DNA removal [%]	49

*Below the lower limit of quantification of the assay

The capture step of the ultrafiltered and diafiltered LVs achieved a recovery of 60% for infectious virus particles. Regarding contaminants, no proteins were detected with the selected analytical assay, and around 49% of residual DNA removal was achieved after this step.

AEX-Only vs. TFF-AEX Workflow

Table 6: *Loading Volume Corresponding to 10% Breakthrough and Respective $DBC_{10\%}$ [Particles/mL Membrane], Overall Infectious Particle Recovery [%], Protein Removal [%], and DNA Removal [%] for AEX-Only and TFF-AEX Workflows*

Sartobind® Convec D Nano 3 mL	AEX-Only	TFF-AEX
Loaded volume (mL)	120	25
Loaded particles [particles]	4.8×10^{11}	1.9×10^{12}
Loading time (min)	24	5
DBC10% [particles/mL membrane]	1.5×10^{11}	6.2×10^{11}
After the Intermediate Workflow	**AEX-Only**	**TFF-AEX**
Infectious recovery [%]	65 - 74	60
Protein removal [%]	85 - 90	Undetectable
DNA removal [%]	30 - 60	81

Placing chromatography earlier in the process (i.e., the AEX-only workflow) requires processing larger volumes of viral feed streams (Table 6). In this example, it was possible to reduce the loading time almost 5-fold (from 24 to 5 minutes) to load 3 – 4-fold more particles while keeping the same membrane adsorber volume. At manufacturing scale, processing larger volumes will translate to larger volumes of chromatography matrices.

In this context, moving from bead-based to convective-based matrices, such as membrane adsorbers, enables very large flow rates, making this type of matrix especially convenient to process larger volumes.

For the TFF-AEX workflow, Hydrosart® TFF cassettes are an excellent choice for reducing the volume of viral feed streams to process in the AEX step (Table 6). Apart from the simplicity inherent to TFF, provided an appropriate balance of cassette type, pore size, and process parameters is achieved, generally high infectious yields can be achieved (Figure 3). Additionally, applying a TFF diafiltration step enables the removal of significant amounts of salts typically found in clarified harvests, preparing the process solution for AEX chromatography, which is sensitive to salt. Thus, TFF is especially useful when lower conductivities need to be reached by avoiding the need for dilution of the loading sample prior to chromatography. In both intermediate workflows, Sartobind® Convec D achieved high infectious recoveries ≥ 60%.

Furthermore, in an AEX-only workflow, there is the added challenge of handling a highly impure solution by chromatography, despite it being a strategy pursued often in LV manufacturing processes. Again, TFF can play a pivotal role by removing smaller contaminants such as proteins and small DNA fragments prior to chromatography and after the overall intermediate workflow (Table 6). Even though Sartobind® Convec D was very efficient in depleting proteins during the loading phase directly to the flow through, this factor could potentially be involved in the higher DBCs observed in the TFF-AEX workflow. Additionally, the fact that adsorption dynamics might differ between two samples with different viral particle concentrations, impacting the binding behavior, should not be ignored.

One drawback of the pre-chromatography TFF is its inability to remove the larger molecular weight DNA species, like histones (Figure 3). Therefore, an important part of the intermediate step is the prior use of DNase. This enzymatic digestion fragments DNA molecules and renders the subsequent purification of LVs, especially AEX-based, much easier to accomplish. AEX chromatography and | or TFF in the intermediate clarification stage should be combined with a more apt strategy to clear impurities like DNA.

Conclusion

The study's primary goal was to compare the effectiveness of two purification workflows for intermediate LV purification, focusing on AEX chromatography and TFF unit operations.

AEX-Only Workflow:
AEX chromatography was applied directly after clarification without the intermediate TFF step. Convective-based matrices like Sartobind® Convec D membrane adsorbers are highly beneficial in handling large flow rates, making them suitable for early processing stages.

TFF-AEX Workflow:
TFF was applied before AEX chromatography to concentrate the viral product, reduce buffer conductivity, and remove smaller impurities. TFF plays a role in reducing viral feed stream volumes for AEX chromatography and removing smaller contaminants, which enhances DBCs. Employing TFF prior to AEX chromatography was also useful in achieving lower conductivities without sample dilution before chromatography. This resulted in 5-fold reduced loading times, enabling the loading of a 3 – 4-fold higher particle number using the same membrane adsorber volume. Overall, excellent recovery was achieved during the UF | DF step using Sartocon® Hydrosart® Slice 200 TFF cassette, when the right pore size and process parameters are selected.

Both unit operations struggle with removing larger DNA molecules; therefore, combining AEX chromatography and | or TFF with strategies to clear DNA impurities, like DNase digestion, is necessary in LV purification processes. Incorporating a TFF step prior to AEX chromatography results in incremental contaminant reduction without significant product loss when operated under optimal conditions. This translates to higher DBCs for virus particles using the same membrane adsorber volume, significantly downsizing the necessary adsorber in a manufacturing process scale.

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