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Final Concentration and Formulation of Lentiviral Vectors Using Sartocon® Hydrosart® TFF Cassettes

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Abstract

Lentiviral vector (LV) manufacturing requires efficient downstream processing to ensure high titer, purity, and functionality. The final concentration and buffer exchange step is critical for preparing LVs for long-term storage and clinical use, and tangential flow filtration (TFF) offers a scalable solution for this purpose.

This study provides insights into the development of a TFF unit operation using Sartocon® Hydrosart® 300 kDa TFF cassettes to concentrate and formulate LV feeds in a suitable buffer system, performed at the end of a typical downstream workflow in the Sartoflow® Smart TFF benchtop system. Our findings aim to guide and contribute to the design of a robust, scalable, and efficient TFF strategy tailored for LV manufacturing.

Introduction

Downstream processing of lentiviral vectors (LVs) involves a series of critical steps aimed at clarifying, purifying, and concentrating the virus after its production in cell culture systems, typically utilizing HEK293T or similar producer cell lines.[1] The goal is to generate a high-titer, pure, and biologically active vector preparation that is suitable for use in research, preclinical studies, or clinical applications.[2] Among the available strategies, tangential flow filtration (TFF) is widely regarded as the preferred method for large-scale concentration and buffer exchange, owing to its efficiency, robustness, scalability, and alignment with Good Manufacturing Practice (GMP) standards.[3]

In the later stages of a typical downstream process, TFF is frequently employed for the final concentration and formulation of LVs. It plays a crucial role in preparing the vectors for long-term storage and eventual clinical application.[1] To ensure the stability and functionality of LVs throughout this process, a combination of buffer systems and stabilizing agents is used. Commonly employed buffer systems include PIPES, HEPES, histidine-HCl, Tris-HCl, and PBS, all of which serve to maintain the virus in a stable environment during concentration, purification, and storage. Additionally, excipients such as sugars (e.g., sucrose and trehalose) and proteins (e.g., recombinant human albumin and gelatin), as well as stabilizers like magnesium chloride ($MgCl_2$), are commonly used to protect the virus from degradation and to maintain its integrity and therapeutic functionality.[4]

This study aims to develop a concentration and formulation through ultrafiltration and diafiltration (UF | DF) unit operation for LVs using Sartocon® Hydrosart® 300 kDa TFF cassettes and the Sartoflow® Smart system.[5–7] We provide guidance on identifying optimal operational parameters and evaluating functional performance to support the efficient and scalable manufacturing of LVs.

Materials and Methods

LV Production Workflow

The representative feed for the UF | DF (TFF2) trials was produced using a typical workflow for LVs (Figure 1).

Figure 1: *Schematic Overview of the LV Production Process Applied to Establishing the Second TFF Step (TFF2, UF | DF)*

Upstream and Nuclease Digestion
▪ Univessel® Glass controlled by Biostat® B ▪ Transient transfection of HEK293 cells (PEIpro®) ▪ Post-transfection endonuclease treatment
Harvest and Clarification
Sartopore® PP3 8 µm | Sartopore® PP3 0.65 µm (setup commonly followed by sterile filter Sartopore® 2 0.45 µm)
Intermediate Purification (TFF1)
Sartocon® Hydrosart® 300 kDa (10 × UF | 5 × DF)
Intermediate Purification (Capture)
Sartobind Convec® (followed by immediate dilution)
Concentration and Formulation (TFF2)
Sartocon® Hydrosart® 300 kDa (10 × UF | 5 × DF)

UF | DF (TFF2) Experiments

TFF2 experiments were conducted using the Sartoflow® Smart TFF system (Sartorius) under a constant inlet pressure control strategy. A Sartocon® Slice 200 Hydrosart® cassette with a nominal molecular weight cut-off (MWCO) of 300 kDa with an effective membrane area of 180 cm^2 was evaluated. The UF | DF trial was conducted using a loading density of approximately 40 L/m^2 (initial volume of 0.7 L) of processed material after harvest and clarification (filtration) and intermediate purification (TFF1 and capture chromatography), followed by a 10-fold concentration and 5 times diafiltration with a buffer composed 5% sucrose, 20 mM $MgCl_2$, 50 mM HEPES, pH 7.5. After UF | DF, the module was flushed twice with one hold-up volume each by recirculating 50 mL of diafiltration buffer for 5 min through the system. Flush volumes were combined with the retentate.

Analytical Methods

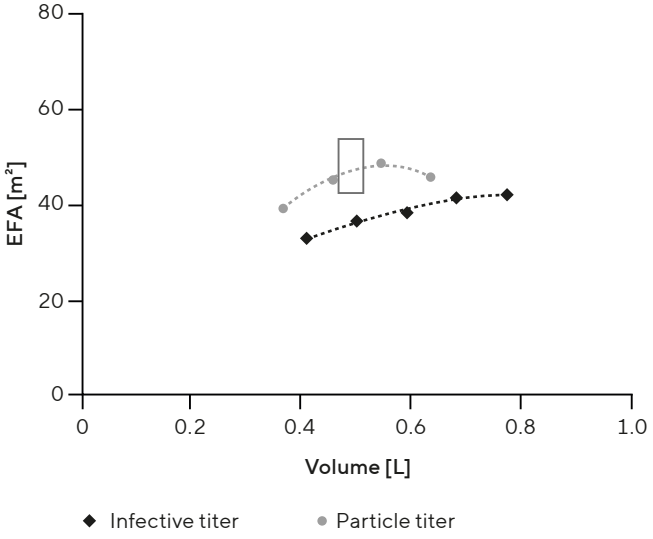
Analytical testing included infectious titer by titer unit (TU) assessment through GFP expression (Incucyte® S3 Live Cell Analysis System) and total particles titer (LV-associated p24 ELISA).

Results

Transmembrane Pressure Optimization

The optimum operating transmembrane pressure (TMP) was identified for two different recirculation rates. The permeate flux was measured at increasing TMP values and two different target Δ pressures (ΔP), starting with the lowest and ending with the highest. Between each target ΔP, a five-minute depolarization was performed, during which the permeate was closed and the feedstock was recirculated (Figure 2).

Figure 2: *Permeate Flux Measured as a Function of TMP at Two Delta Pressure Values (ΔP)*



The optimum TMP range was selected as the range approaching the pressure-independent zone of the process (where further increases of pressure do not increase permeate flux), typically close to where the optimal performance from an ultrafilter is achieved. As no flux advantage was observed for the highest recirculation rate, the selected TMP was 0.5 – 0.6 bar at ΔP 0.8 bar.

TMP scouting generally aims to find a balance between achieving higher flux (to shorten process time) and maintaining gentle operating conditions that help preserve product quality. Using a higher ΔP is only warranted if it produces a noticeably better flux. Additionally, selecting operating conditions that promote enough crossflow and turbulence can further enhance product purity by improving the removal of contaminants. Note that these TMP scouting experiments were performed on the module with depolarization; however, they were conducted without caustic cleaning between each target ΔP.

Since the lower ΔP TMP iterations were run first, we hypothesize that partial membrane fouling occurring during the initial feed flow conditions resulted in lower permeate fluxes at the higher ΔP.

This could potentially be minimized by applying backpressure on the permeate side, which may help redistribute fouling or reduce cake compaction. This outcome emphasizes the importance of controlling parameters like TMP and recirculation rates for a given feed stream, which can be critical for ensuring optimal filtration processing time and yield. In the context of LV production, this exercise helps to maximize the recovery and purity of the viral vectors while minimizing potential damage to the product.

UF | DF

With the selected TMP and ΔP, the flux performance during the concentration and diafiltration of a harvested clarified LVs was evaluated (Table 1).

Table 1: *TFF Parameters From UF | DF*

Sartocon® Slice 200 Hydrosart® 300 kDa	
Δ Pressure (ΔP; Constant) [bar]	0.8
Target Inlet Pressure (P1) [bar]	1.0
TMP [bar]	0.5 – 0.6
Average Permeate Flux (UF | DF) [LMH]	131 | 58
Total Processing Time (UF| DF) [min]	16 | 20

Using a constant pressure-driven approach to control the TFF trial (TMP = 0.5 – 0.6 bar; ΔP = 0.8 bar), average fluxes of 131 and 58 LMH were achieved for UF and DF, respectively, resulting in a total processing time of 36 mins (Table 1).

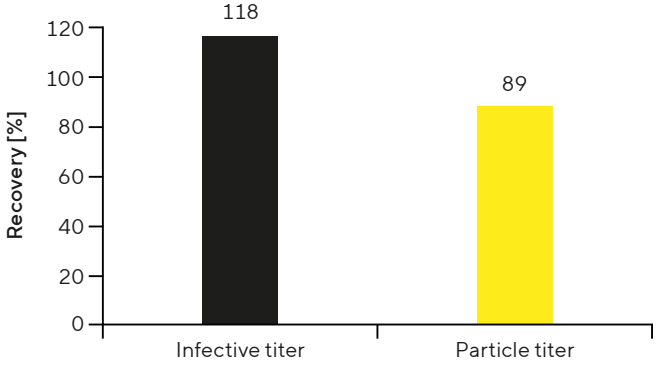
Although the process was intended to function primarily within a membrane resistance–controlled regime, during operation, mass transfer limitations and solute accumulation (such as product impurities) can still occur at the membrane surface. This leads to polarization and fouling effects, which contribute to flux decline from the start of the run. In this regard, the content of contaminants (e.g., trapped proteins and DNA) can significantly influence fouling, leading to increased membrane resistance over filtration time and, in turn, reducing membrane productivity.[8]

While a substantial increase in viscosity could also potentially explain a flux drop at a fixed pressure drop (ΔP), the viscosity was monitored and remained relatively stable (~1.0 – 1.2 cP) throughout the UF | DF run. Therefore, the continuous drop in fluxes is better attributed to fluid dynamics, such as fouling and polarization, during the trial. Although this study did not focus on a detailed analysis of these phenomena, incorporating flux decline and contamination profile modeling in future research would provide valuable insights into how TFF operating conditions impact UF | DF efficiency for a specific LV feed stream.

LV Recovery

In terms of functional performance parameters, we evaluated particle and infectious titer and LV yield (Figure 3).

Figure 3: *Infectious Titer and Total Particle Recovery [%]*



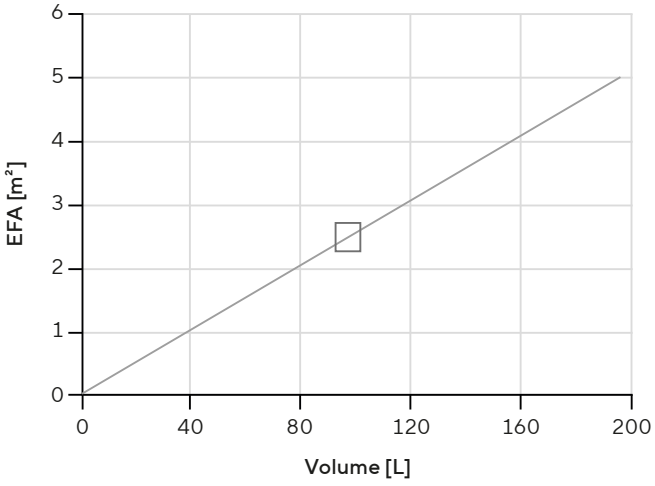
The UF | DF trial demonstrated effective retention of infectious viral particles, with yields of ~ 118% and 89% recovery of total particles at the final concentration and formulation step. No viral particles were detected in the permeate, with measured values consistently falling below the assay's limit of detection for permeate samples.

While impurity removal is not the main target of the TFF2 step—and was therefore not included in this analysis—total protein levels were very low or inexistent (below the limit of detection of the assay) at this stage in the process. However, concentrated residual DNA was still identified (~ 200 ng/mL) in this case. The incomplete removal of high-molecular-weight DNA species, including histones, could be addressed by introducing a DNase treatment step, which breaks down remaining large DNA fragments, simplifying LV polishing after TFF2.

Filtration Area: Example Calculation

Lastly, we applied the results to an example of concentration and formulation through TFF at the end of a downstream LV process workflow. The effective filtration area (EFA) required for 10 × UF and 5 × DF of a given feed volume using the average permeate flux was plotted (Figure 4).

Figure 4: *Effective Filtration Area Necessary for a 36-Min 10 × UF and 5 × DF of a Given Feed Volume*



Membrane area may be an important selection criterion, especially at the final formulation step. Depending on the feed characteristics and the selected design for the downstream workflow, the volume to be processed at the end of the process can be highly variable. Using the scalable Sartoflow® Smart and the Sartocon® Hydrosart® Slice 200 cassette format, which represents the smallest scale-down device in the Sartocon® product family, enables process development before further scaling. The ability to select a TFF cassette configuration and optimize process parameters at small scale offers a major advantage, as it avoids the need for large feed volumes during process development before scaling to bigger systems with larger effective filtration areas.

A previous study tested different TFF cassette configurations (varying format and pore size) in the Sartoflow® Smart, followed by a scale-up to the Sartoflow® Advanced. The results demonstrated strong process robustness and linear scalability using LV feed streams.[9,10] Using our calculation, for a 100 L final formulation volume, approximately 2.5 m² of membrane area would be required for a 10 × UF 5 × DF in 36 mins (Figure 4, black box).

Conclusion

In conclusion, this study identified optimal parameters for UF | DF of LVs using Sartocon® Hydrosart® 300 kDa cassettes, with conditions supporting stable operation and efficient virus retention. This included:

- High lentiviral vector recovery—The process achieved very high recovery (~ 100%) of infectious LVs, with no detectable viral particles in the permeate, confirming effective virus retention and minimal product loss.
- Short processing time—The process yielded a total UF | DF runtime of 36 minutes, with high permeate fluxes, indicating efficient performance for final concentration and buffer exchange.
- Process scalability—The use of Sartocon® Slice 200 cassettes in the Sartoflow® Smart system providesd a scalable development platform. The results are also transferable to larger-scale systems (e.g., Sartoflow® Advance), supporting linear scalability.
- Design flexibility for variable volumes The EFA can be calculated based on permeate flux to support rapid and predictable scale-up.

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