FlexiPro™ TFF

- **Multi-scale downstream workflows with a single system**
- **Gentle and effective platform for processing LNPs**
- **No detectable degradation of mRNA-loaded LNPs after extended diafiltration**
- **Stable mean volume diameter and polydispersity indices (PDI)**
- **Robust and uniform LNPs using NanoPulse™ technology from Inside Therapeutics**
- **Scaleable and efficent diaflitration using Meissner SepraPor® hollow fiber filter**

Evaluation of shear during diafiltration of NanoPulse™ LNPs with single use FlexiPro™ TFF system and SepraPor® 100kDa filter

Anne Duconseille, VERDOT, Riom, France
Sébastien Lefebvre, VERDOT, Riom, France
Robin Oliveres, Inside Therapeutics, Bordeaux, France
Jessica McRoskey, Meissner, Camarillo, CA USA

Abstract

Lipid nanoparticles (LNPs) were diafiltered using the VERDOT® single use FlexiPro™ TFF system equipped with a Meissner SepraPor® 100 kDa hollow fiber filter (Figure 1). Measurements of mean particle diameter and polydispersity index (PDI) throughout the process showed that both empty and full LNPs maintained particle integrity during buffer exchange from an ethanol/PBS mixture to pure PBS, as well as during extended diafiltration periods that were performed to challenge the system. Therefore, the shear stress applied by the system and filter was sufficiently low to preserve lipid nanoparticles (LNP) structural stability.

These findings demonstrate that the VERDOT FlexiPro TFF system in combination with a Meissner SepraPor® filter provides a gentle and effective platform for purifying and diafiltering fragile nanoparticles.

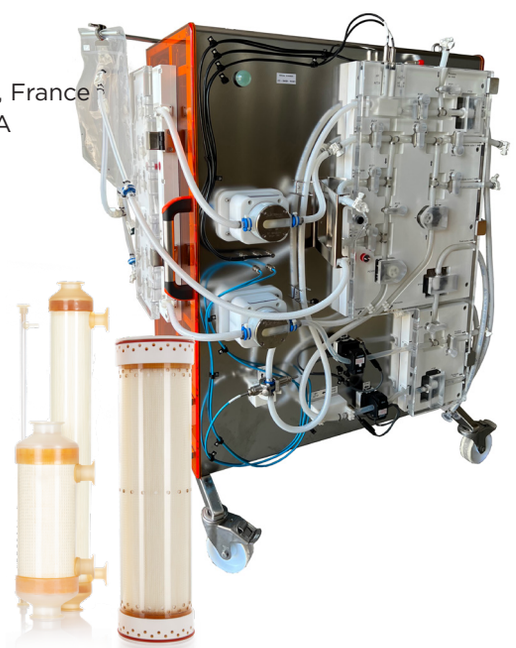

Figure 1. FlexiPro TFF™ offers single-use flexibility with four Flow Kit options and Meissner SepraPor® 100 kDa hollow fiber filter

This study also demonstrates the robustness of the LNPs from Inside Therapeutics' newly patented technology NanoPulse™ with pulsating alternating valve.

Bulletin DP-MKT-381-00
December 2025

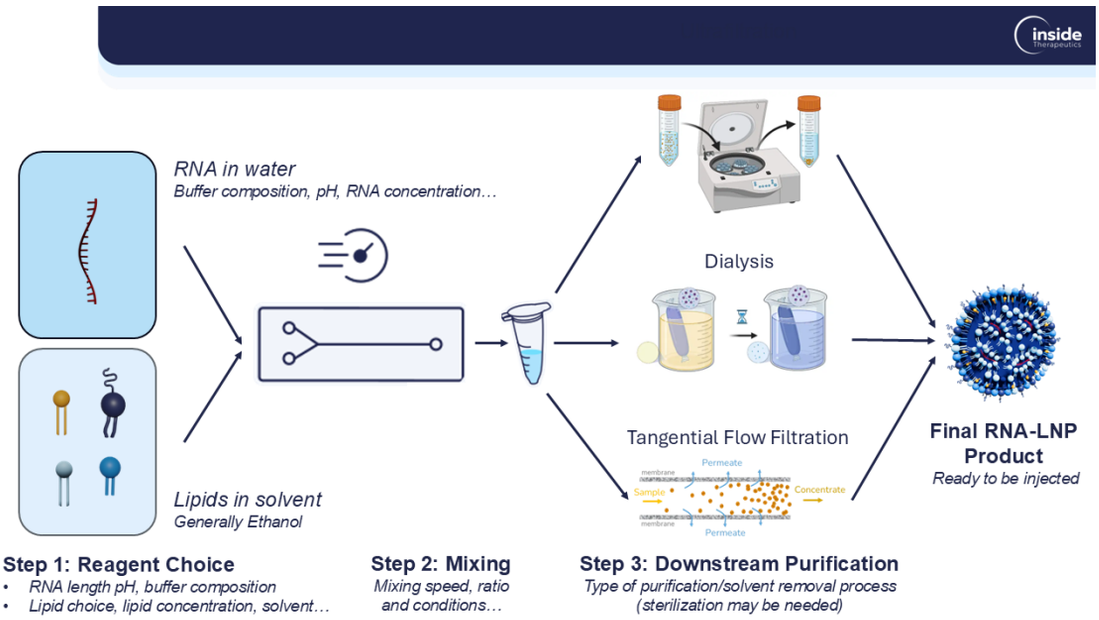

Figure 2. LNP manufacturing process

Introduction

LNP encapsulation of mRNA is often depicted as perfectly spherical liposomes that fully enclose the mRNA molecule. In practice, however, microscopy and biophysical analyses reveal a far more complex reality — LNPs vary significantly in size, internal structure, and the degree of mRNA encapsulation and protection. Their morphology is influenced by multiple parameters, including the mRNA length, lipid composition, and the manufacturing process employed.

The LNP structural and functional properties are directly connected to the formulation conditions and purification methods. Moreover, the LNP production process has historically been difficult to scale up. Recently patented technology from Inside Therapeutics uses high-frequency pulsating valves to maintain high encapsulation quality at any production scale (Figure 2). One of the objectives of this study was to confirm the integrity of the liposomes during downstream processing such as diafiltration following mRNA encapsulation.

The LNP manufacturing process generally involves two main steps:

1. Formulation:
This initial step consists of combining the mRNA in an aqueous buffer with the four lipid components (i.e., ionizable lipid, helper lipid, cholesterol, and PEG-lipid), dissolved in an organic solvent such as ethanol. The two phases are rapidly mixed to enable spontaneous nanoparticle formation. Over the years, various mixing strategies have been developed — ranging from simple T-mixers to microfluidic systems with more advanced platforms such as NanoPulse™ from Inside Therapeutics — each offering different levels of control over mixing time, particle size, reproducibility, and scalability.

2. Purification:
Following formulation, a purification step is required to remove excess solvent and exchange the buffer to improve particle stability and biocompatibility. At the laboratory scale, this is often achieved using dialysis, while tangential flow filtration (TFF) is preferred at larger scales for efficiency and scalability.

Tangential flow filtration is a widely used technique for purifying and concentrating LNPs. However, the shear forces that are generated by fluid flow and membrane interactions can lead to aggregation or deformation of the particles, which can compromise their performance. Maintaining structural stability of the particles throughout the production process is essential to ensure their efficacy and safety as carriers for active molecules in pharmaceutical and cosmetic applications. Therefore, it is crucial to optimize TFF conditions to minimize shear stress and preserve LNP integrity.

Empty and full liposomes from Inside Therapeutics were diafiltered using the VERDOT FlexiPro TFF system equipped with a Meissner SepraPor® hollow fiber filter using standard conditions as well as extended processing times. The objective was to determine whether the structural integrity of LNPs was maintained throughout the process, thereby confirming the performance of the system and filter for sensitive nanoparticles.

Materials

Lipid nanoparticles (LNPs):
Empty and full LNPs (mRNA) were stored at 4°C in 20% ethanol for approximately one month. The mixture was diluted 1:1 with phosphate-buffered saline (PBS) prior to processing. (*Inside Therapeutics - Bordeaux, France*).

Buffers:
The diafiltration buffer was PBS from a 10X stock solution diluted with deionized water, pH 7.4 (*Dutscher - Bernolsheim, France*).

Equipment:
Dynamic light scattering (DLS) measurements were performed using a NANOTRAC FLEX 1.5 m system equipped with a FlowGuard for measurements in moving liquid (*Microtrac - Toulouse, France*).

Diafiltration was performed using the following equipment:

- SepraPor® XFC100C024-7711 hollow fiber filter; 60 cm length, 100 kDa molecular weight cut-off, 1 mm internal diameter *(Meissner - Camarillo, California, USA).*
- FlexiPro TFF single use system equipped with a Very Low Flow Kit (VLFK). The VLFK uses 1/8" tubing and allows flow rates up to 30 L/hr *(VERDOT – Riom, France).*

Methods and Results:

1. Preliminary Filter Characterization:
To characterize the filter performance, a series of experiments was conducted to evaluate the permeate flux as a function of operating conditions. The data was then used to determine the optimal transmembrane pressure (TMP) for processing the LNPs.

First, ultra-pure water was circulated through the filtration module, keeping the feed rate constant at 20 L/hr while varying the TMP. Table 1 shows the permeate flow rate and feed pressure obtained from this experiment.

Feed Flow Rate (L/hr)	TMP (Bar)	Permeate Flow Rate (L/hr)	Feed Pressure (Bar)
20	0.35	1.48	0.5
20	0.6	2.44	0.76
20	0.8	3.27	1.05
20	1.0	3.97	1.25
20	1.2	4.6	1.45
20	1.4	5.1	1.59

Table 1. Permeate Flux vs. Transmembrane Pressure for Ultra-pure Water

A suspension of empty LNPs was then circulated through the filtration module, repeating the constant feed flow rate and varying TMP conditions from the ultra-pure water run. Table 2 shows the resulting permeate flow rate and feed pressure.

Feed Flow Rate (L/hr)	TMP (Bar)	Permeate Flow Rate (L/hr)	Feed Pressure (Bar)
20	0.4	1.58	0.6
20	0.6	2	0.8
20	0.8	2.35	1.0
20	1	2.16	1.3
20	1.2	2.03	1.3
20	1.4	2.03	1.5

Table 2. Permeate Flux vs. Transmembrane Pressure for Empty LNP Suspension

The results of these experiments were used to calculate the additional resistance induced by the filter cake on the membrane surface which is caused by LNP particle accumulation. The data is summarized in Figure 3, which shows the normalized flux vs. TMP. The degradation of the normalized flux with increasing TMP suggests membrane polarization and increased cake thickness. The further regeneration of the membrane confirmed fouling was not involved. To mitigate these effects, a maximum TMP of 0.5 bar was used for further LNP processing.

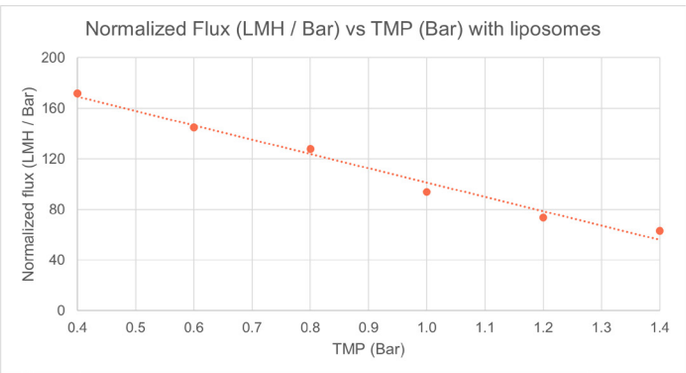

Figure 3. Normalized Flux (LMH/Bar) vs. TMP (Bar) with empty liposomes

2. Diafiltration of Empty LNPs

Initial diafiltration to exchange buffer (Ethanol/PBS to PBS)

A solution of 200 mL empty LNP suspension in 20% ethanol was diluted 1:1 with phosphate-buffered saline (PBS) to a total volume of 400 mL. The solution was then diafiltered to exchange the buffer from the ethanol/PBS solution to pure PBS.

The VERDOT FlexiPro TFF system precisely maintained the initial volume of 400 mL using automatic regulation based on retentate weight. Process parameters such as feed flow rate, TMP, permeate flow rate, and conductivity were monitored to ensure controlled conditions and complete buffer exchange to PBS.

Diafiltration volumes (DV) are expressed as multiples of the retentate volume. Because the retentate volume and the permeate flow are kept constant, the diafiltration volume can be calculated by dividing the total permeate flow volume by the retentate volume.

Samples were collected every 15 minutes throughout the run and analyzed by DLS to determine the mean volume diameter and the PDI. These two parameters are an indication of shear stress on LNP integrity.

Figure 4 shows that the retentate conductivity achieved 99% of the PBS diafiltration buffer conductivity after 159 minutes. The steady-state permeate flow rate was 0.95±0.05 L/hr which corresponds to a total permeate flow volume of 2.5 L, indicating that 6.3 DV is sufficient to replace buffer from the initial storage solution (400 mL). The diafiltration process continued until 6 DV.

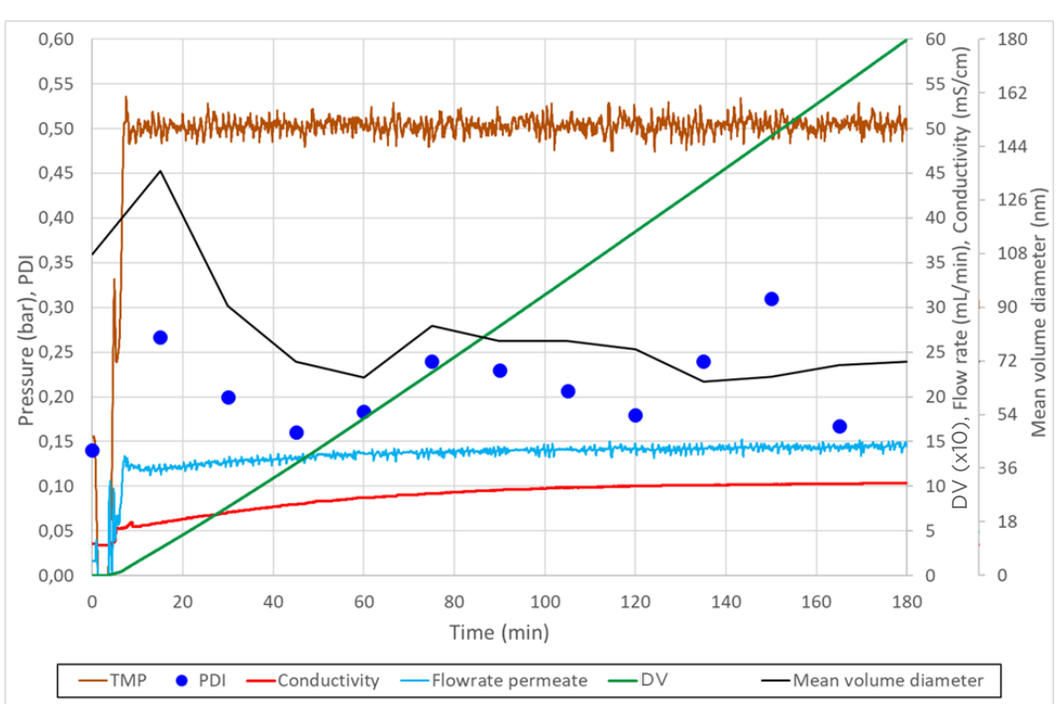

Figure 4. Run data for initial diafiltration of empty LNPs

During the first 120 min of the buffer exchange, where the conductivity increases from 3.5 mS/cm to approximately 10 mS/cm, the mean volume diameter of the LNPs decreased rapidly from 135 nm to 75.9 nm. The mean volume diameter then remained stable at 70.0±4.3 nm for the remainder of the experiment where the retentate conductivity was maintained at 10.3 mS/cm, up to 180 minutes (Table 3).

In a similar pattern, the PDI increased from the initial value of 0.13 to 0.18 during the buffer exchange but then remained stable (0.22±0.07) during the extended diafiltration period (from 120 to 180 minutes), indicating that the LNPs remained structurally intact and did not fracture. This is further elucidated in the Discussion section.

	Initial Value (0 min)	After initial buffer exchange (at 120 min)	During extended diafiltration
Mean volume diameter (nm)	135	75.9	70.0±4.3
PDI value	0.13	0.18	0.22±0.07

Table 3. Results from Diafiltration of Empty LNPs

Secondary extended diafiltration (only PBS)

In order to challenge the system without any impact of buffer exchange on the LNP structural integrity, a secondary diafiltration process was performed for a period of 3 hours (an additional 6 DV). This second diafiltration was performed in an isocratic manner (i.e., from PBS to fresh PBS) by constantly injecting new PBS buffer with the diafiltration pump to maintain the same volume of LNP suspension. The automated retentate weight regulation was programmed on the FlexiPro TFF as in the previous experiment.

Between the two diafiltration experiments, the empty LNP solution was stored at 4°C overnight. The results of the second diafiltration step are illustrated in Figure 5 below.

As shown in Table 4, the mean volume diameter of empty LNPs was initially 71.7 nm. After the second diafiltration, the mean volume diameter was measured at 78.2 nm. The overall mean value for all measurements during the process was 77.5±5.4 nm. Considering the standard deviation, these results indicate no significant change in particle size. Additionally, the PDI decreased from 0.34 to 0.22, with a mean value of 0.22±0.06, which is consistent with the values observed during the first diafiltration.

	Initial Value (0 min)	Final Value (180 min)	Mean Value (0-180 min)
Mean volume diameter (nm)	71.7	78.2	77.5±5.4
PDI value	0.34	0.22	0.22±0.06

Table 4. Results for Extended Diafiltration of Empty LNPs

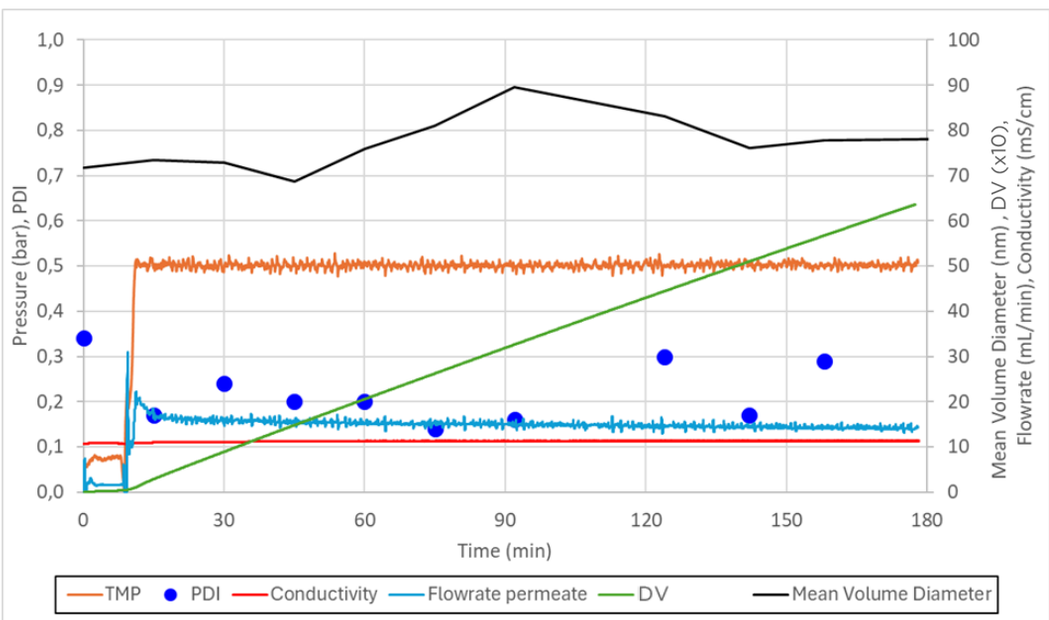

Figure 5. Run data for extended diafiltration of empty LNPs

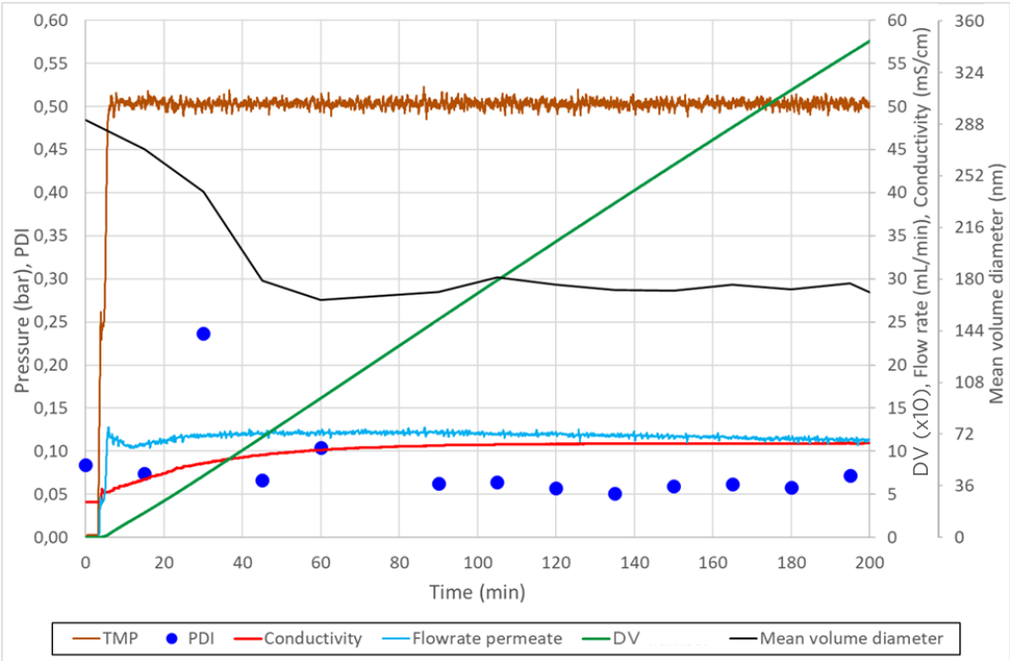

Figure 6. Run data for initial diafiltration of mRNA-loaded LNPs

Both the mean volume diameter and PDI values remained stable throughout the extended diafiltration of the empty LNPs, suggesting that the colloidal stability of the LNPs was preserved. These results demonstrate that the shear stress generated by the filtration system and membrane did not alter the structural integrity of the lipid nanoparticles.

3. Diafiltration of mRNA-loaded LNPs

Following the success of the previous experiments, the system was then tested with LNPs that were loaded with mRNA. With the previous experiment using empty LNPs, a series of two diafiltration steps were performed, the first consisting of a buffer exchange from ethanol/PBS to pure PBS and the second to further challenge the system's impact on LNP structural integrity without buffer exchange. For the diafiltration of the mRNA-loaded LNPs, the buffer exchange and prolonged diafiltration periods were combined into a single process step to more accurately represent normal processing conditions.

A solution of 200 mL of full LNP suspension in 20% ethanol was diluted 1:1 with PBS to a total volume of 400 mL. As in the previous experiment, the VERDOT FlexiPro TFF system precisely maintained the initial volume of 400 mL using automatic regulation based on retentate weight. The conductivity of the retentate was monitored to ensure complete buffer exchange to PBS. Once the retentate conductivity was stable, the buffer exchange was considered complete. The solution was then diafiltered for an additional 100 minutes to challenge the system's impact on LNP structural integrity.

Samples were collected throughout the process and analyzed by DLS to monitor mean volume diameter and PDI.

Figure 6 shows that the retentate conductivity achieved 99% of the PBS diafiltration buffer conductivity after 113 minutes. The steady-state permeate flow rate was 0.72±0.03 L/hr which corresponds to a total permeate flow volume of 1.29 L, indicating that 3.2 DV is sufficient to replace buffer from the initial storage solution (400 mL). The diafiltration process continued until 6 DV.

As seen with the diafiltration of the empty LNPs, an initial decrease in mean volume diameter was observed during the buffer exchange from ethanol/PBS solution to PBS, followed by stable values for the remainder of the diafiltration with only PBS (Table 5). Furthermore, the PDI did not change during the extended processing period, indicating stability of LNP integrity.

	Initial Value (0 min)	After buffer exchange (at 120 min)	After extended diafiltration (at 200 min)
Mean volume diameter (nm)	290.7	173±7.0	176.9±7.0
PDI value	0.13	0.07±0.02	0.07

Table 5. Results for Diafiltration of Full LNPs

Discussion

Complete buffer exchange is generally measured by the conductivity of the retentate relative to the conductivity of the diafiltration buffer. When the conductivity of the retentate is 99% of the value of the diafiltration buffer, the diafiltration is considered complete. The rationale for this criterion is based on the ICH Q3C Class 3 guideline, which limits ethanol content in injectables to less than 0.5%. Since the initial ethanol concentration after LNP preparation is typically 30% or less, a 1:100 reduction results in less than 0.3% residual ethanol.

For LNP solutions, complete buffer exchange is generally achieved in 5 to 10 DV (Liu *et al*. 2021). For example in the current study, a 400 mL LNP suspension would need 2 to 4 liters of buffer for complete diafiltration. It is important to note that while these volumes are common, the optimal diafiltration volume can vary based on the specific characteristics of the LNPs and the goals of the purification process.

In the present study, the DV was 5.2 with empty LNPs and 3.2 for mRNA-loaded LNPs, indicating that the experimental conditions enabled rapid and complete diafiltration while maintaining particle integrity. In both experiments, additional diafiltration periods (up to 12 DV for empty LNP and 6 DV for full LNP) were performed for the purpose of challenging the system and LNP integrity.

For both empty and mRNA-loaded LNPs, the initial buffer exchange from ethanol/PBS solution to PBS resulted in a reduction of the LNP mean volume diameter, while the PDI remained relatively unchanged. As a polar solvent, ethanol can disrupt the ordered structure of lipid bilayers, leading to increased membrane permeability and water influx. This disruption can cause the bilayer to adopt non-lamellar phases, such as hexagonal or cubic structures which are more prone to swelling. Additionally, ethanol can alter the packing density of lipids by intercalating between lipid molecules, reducing the bilayer's rigidity and allowing for greater water absorption (Zhang *et al*. 2005; Maeki *et al*. 2024). These processes are reversible when the ethanol is removed during buffer exchange, explaining the observed decrease in LNP mean volume diameter at that step. The use of PBS as a diafiltration buffer effectively reduced particle diameters even after prolonged storage in 20% ethanol, while maintaining particle homogeneity.

Since the particle size did not continue to decrease during the prolonged diafiltration steps, the shear stress generated by the system and the filter can be considered insignificant and thus sufficiently low to preserve LNP integrity.

Conclusion

This study demonstrates that the VERDOT single use FlexiPro TFF system, in combination with a Meissner SepraPor® 100 kDa filter, can perform extended diafiltration of lipid nanoparticles (LNPs) without compromising their structural integrity. Both empty and full LNPs maintained stable mean volume diameters and polydispersity indices (PDI) during the extended filtration periods.

The FlexiPro TFF system accurately monitored diafiltration progress via permeate conductivity. The diafiltration was successfully completed within standard processing conditions and LNP integrity was preserved even under intentionally extended durations that rigorously challenged the system.

Overall, these results confirm that the VERDOT FlexiPro TFF system and Meissner SepraPor® filter provide an effective and gentle solution for the purification and buffer exchange of fragile nanoparticles such as LNPs, supporting their safe and effective use in pharmaceutical and cosmetic applications.

References

Liu HW, Hu Y, Ren Y, Nam H, Santos JL, Ng S, Gong L, Brummet M, Carrington CA, Ullman CG, Pomper MG, Minn I, Mao HQ. Scalable Purification of Plasmid DNA Nanoparticles by Tangential Flow Filtration for Systemic Delivery. ACS Appl Mater Interfaces. 2021 Jul 7;13(26):30326-30336. doi: 10.1021/acsami.1c05750. Epub 2021 Jun 23. PMID: 34162211; PMCID: PMC9701136.

Maeki M, Kimura N, Okada Y, Shimizu K, Shibata K, Miyazaki Y, Ishida A, Yonezawa K, Shimizu N, Shinoda W, Tokeshi M. Understanding the effects of ethanol on the liposome bilayer structure using microfluidic-based time-resolved small-angle X-ray scattering and molecular dynamics simulations. Nanoscale Adv. 2024 Mar 25;6(8):2166-2176. doi: 10.1039/d3na01073b. PMID: 38633055; PMCID: PMC11019499.

Zhang, Jianbing and Cao, Honghua and Jing, Bingwen and Regen, Steven L. Ethanol-Induced Reorganization of the Liquid-Ordered Phase: Enhancement of Cholesterol–Phospholipid Association. Journal of the American Chemical Society. 2006 128;1;265-269. doi: 10.1021/ja056918d.PMID: 16390156. https://doi.org/10.1021/ja056918d