Evaluation of shear stress on the FlexiPro™ TFF system

VERDOT

- **No detectable degradation of lipsomes over 5 hour circulation through retentate flowpath**
- **Two pump technologies: PSG Dover Quattroflow® QF150SU and Levitronix® PuraLev® i100-SU**
- **Tested over the full range of flow and Trans Membrane Pressure (TMP)**
- **Evaluation of the liposome integrity with average liposome size and the Poly dispersity Index (PDI)**
- **Two light scattering: instruments: Microtrac MRB NANOTRAC Flex, and Anton Paar's Litesizer™ 500**

Product integrity maintained with 100nm liposomes, representative of AAV, Lentivirus and lipid nanoparticles

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Introduction

In downstream processing for biopharmaceutical manufacturing, Tangential Flow Filtration (TFF) is one of the most used processes for performing concentration and diafiltration. It can also be used for microfiltration. There is also a growing interest in TFF upstream processing for extracting molecules of interest in continuous mode using TFF cartridges.

The application field of TFF covers all kinds of therapies, from small polypeptides to full cells or virus capsids, used in gene therapies. The principle of TFF requires circulation of the target multiple times through the pumps, valves, and TFF filter, which may potentially damage the product over time due to shear stress. For instance, a 500 L product batch concentrated for 5 hours at 40 LPM will average 24 cycles of the product through the equipment.

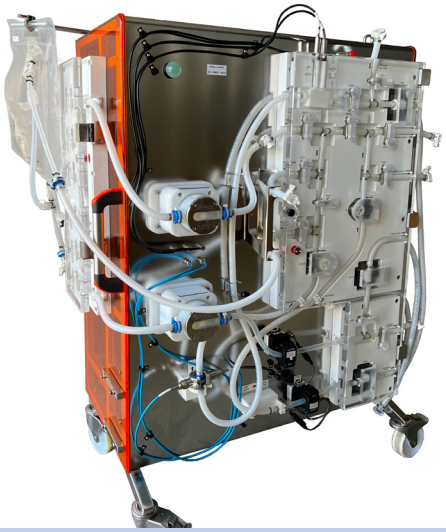


Figure 1. FlexiPro TFF™ offers single-use flexibility with four FlowKit options

Herein, we evaluate the performance of the Verdot FlexiPro™ TFF single-use system (Figure 1) to ensure the integrity of the product during circulation for gene therapy applications, such as an AAV virus, Lentivirus (LV), lipid nanoparticles (LNP) or messenger RNA (mRNA) vaccines.

In this study, the product was simulated with 100nm liposomes. These particles have a similar size to AAVs (80 nm), lentiviruses (100 nm) and LNPs commonly

used, and having a more fragile structure than virus particles, they act as a worst-case analysis. The study consisted in monitoring the evolution of the average liposome size and the Poly dispersity Index (PDI) while being circulated through the FlexiPro TFF over a period of five hours. Several parameters were evaluated, including flow rate (33%, 66% and 100% of the full flow), trans-membrane pressure (TMP) (0.6 bar, 1.0 bar and 1.4 bar) and compared two different types of pump technologies, i.e., the Levitronix® LeviFlow® and PSG Dover Quattroflow® single use pumps.

The study showed no degradation of the liposomes over the 5 hours of circulation with the different configurations tested, although the liposomes have been circulated between 283 and 850 times in average, given the sample volume and the flow rate used, proving that the FlexiPro TFF is a safe system for performing tangential filtration on fragile and non-fragile therapeutic forms.

Materials and Methods

Equipment:
The FlexiPro TFF SN# 22-SKD-1431 equipped with a Low FlowKit (LFK) was used for these experiments. The Low Flow kit has a maximum flow of 170 LPH. The tubing is made of ¼" ID braided silicone.

For the circulation pump, two technologies were compared: the Quattroflow® QF150SU and the Levitronix® PuraLev® i100-SU. The flowrate was regulated in closed loop with a LeviFlow® Clamp-On Sensor LFSC-i10X-001 from Levitronix®.

The pressure control valve used was the patented design of Verdot, where the outer surface of the tube is pinched with a D50mm eccentric roller against a support under a closed loop pressure regulation. PendoTECH's Single Use Pressure Sensors™ were used for the pressure regulation.

The monitoring of the liposomes was performed with two nanoparticle size analyzers based on light scattering: the Microtrac MRB NANOTRAC Flex, and the Anton Paar's Litesizer™ 500. The mean volume diameter was used for the size measurement and size distribution.

Liposomes and mobile phase:
The trials used liposomes with mean particle size: 100 nm (90-120 nm), with phosphatidylcholine and mPEG2000-DSPE lipids (95:5 mol%), from FormuMax Scientific (Article # F10209D, Batch # 06172201). The liposomes were prepared in phosphate buffer saline (PBS), pH 7.4, provided by Th.GEYER Gmbh (Article 8461.0005, Batch 274306) diluted in deionized water ultra-filtrated at 100 kDa for eliminating any particle.

Disposable bags of 50L, ref. BioHub® 2D 50L from Bio-Link, were used for the storage of the deionized and ultrafiltrated water. Disposable bags of 2L, BioHub® 2D 2L from Bio-Link, were used for the storage and as retentate vessel for the liposome suspension. The sampling for analysis online was made with a syringe with needle ID 0.8 mm placed through the low piping of the bag, on the pump load side.

For each configuration tested, a sample of 1 L of liposomes was used. Given that the void volume of the circulation loop is 200 mL, initially primed with PBS, the liposomes were diluted in a 800 mL volume to enclose the volume of the circulation loop.

Each vial of 5 mL lipid was diluted for making 9 samples. Given that the lipid concentration is 50 mM, each 1 L sample comprises:

(0.050 mol/L) x (0.005 L) x (6.022×10^{23}) / 9 = (1.673×10^{19}) lipid chains.

$$N_{tot} = \frac{\left[4\pi\left(\frac{d}{2}\right)^2 + 4\pi\left(\frac{d}{2} - h\right)^2\right]}{a}$$

d = liposome diameter (nm)
h = lipid double layer thickness (5 nm for phosphatidylcholine and DOTAP)
a = area of hydrophilic group (0.71 nm^2 for phosphatidylcholine)
N = 80 088

The number of liposomes per sample of 1 L was 1.673×10^{19} / 80 088 = 2.1×10^{14} liposomes per liter, hence 2.1×10^{11} liposomes /mL which correspond to a titer of product before concentration obtained with performant AAV process.

Methods:
Determination of the PCV setpoints to obtain target TMP
The amount of liposomes in each sample being small, it was not possible to directly recirculate the suspension through the Hollow fiber cartridge as the layering of the liposomes against the filter surface reduced the concentration of the liposomes in the supernatant too much for a reliable measurement by light scattering.

An initial test was performed with a hollow fiber filter to determine the pressure added by the PCV for obtaining the target TMP values for each flow setpoint. As a wide range of filters may be used in the range of flow of the Low FlowKit (5-170LPH), an oversized cartridge was deliberately chosen as "worst case" to minimize the back pressure of the filter and hence maximize the back pressure added by the PCV for obtaining the target TMP,

thus providing the “worst case” shear given by the PCV.

For this purpose, a cartridge with 100kDa cut-off, with ID0.9mm x 300mm hollow fibers for a total surface of 0.46m^2 was chosen.

Rinsing
Before each test with liposome the circulation loop of the FlexiPro TFF was rinsed with 5 L deionized and ultrafiltered water. The water return was analysed in triplicate with the light scattering instruments to ensure no residual presence of particles or residual liposomes from former tests.

Liposomes tests
Given the three flow rates (33%, 66%, 100%), the three TMP (0.6 bar, 1.0 bar, 1.4 bar) and the two pumps technologies (PSG Dover Quattroflow®, Levitronix® PuraLev®), a total of 18 configurations were identified (Tables 1 and 2). The Quattroflow® pump was tested first with the most stressful configurations (i.e., with highest flow and highest TMP). Given that no degradation of liposomes was identified (Figures 2-4), configuration #7 was not tested, as configurations #8 and #9 were more stressful.

With the Levitronix pump, with the base of the former tests, only the most stressful configurations were tested (Figure 5). The sampling and analysis was performed every 15 minutes for the first 2 hours, then every 20 minutes for the remainder of the test.

Table 1. Quattroflow® QF150SU setup

	TMP		
Flow Rate	**0.6 bar**	**1.0 bar**	**1.4 bar**
33% flow	Config #1	Config #2	Config #3
66% flow	Config #4	Config #5	Config #6
100% flow	Config #7	Config #8	Config #9

Table 2. Levitronix® Puralev® i100SU setup

	TMP		
Flow Rate	**0.6 bar**	**1.0 bar**	**1.4 bar**
33% flow	Config #10	Config #11	Config #12
66% flow	Config #13	Config #14	Config #15
100% flow	Config #16	Config #17	Config #18

Each test was started with a new suspension of 0.8 L liposome in PBS, in a 2 L bag connected to the circulation loop.

Results and Discussion

Figure 2. Quattroflow® QF150SU, TMP = 0.6 bar

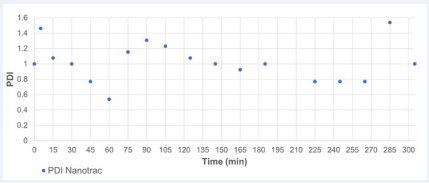
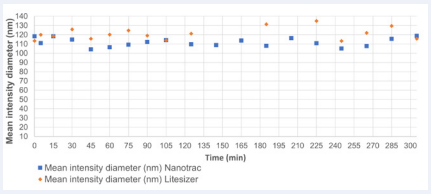
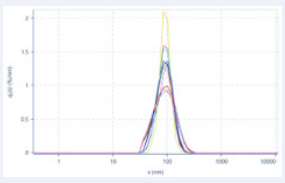
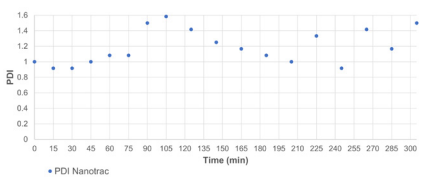
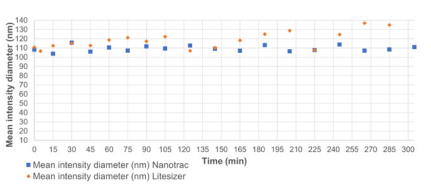
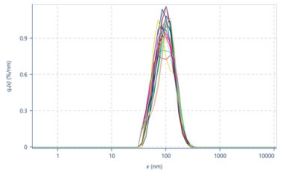
Flow Rate	PDI Normalized	Mean Particle Size	Size Distribution
33% Flow Configuration 1			
66% Flow Configuration 4			

Figure 3. Quattroflow® QF150SU, TMP = 1.0 bar

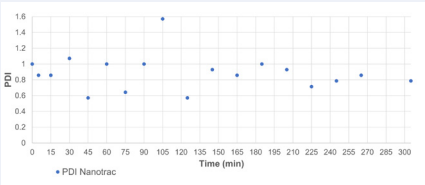
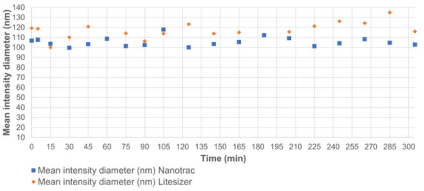
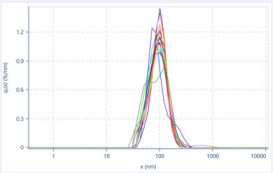
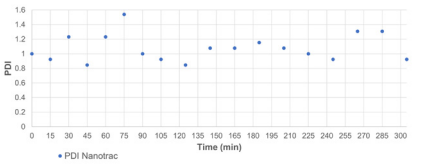
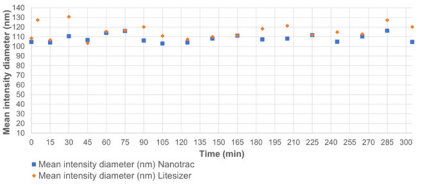
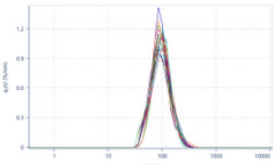
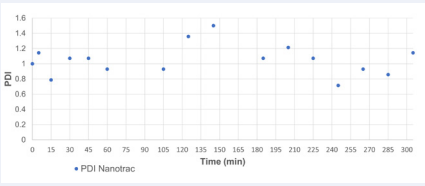
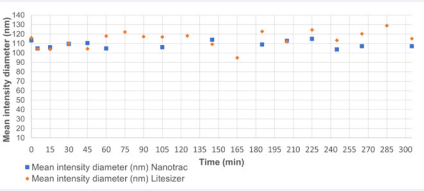
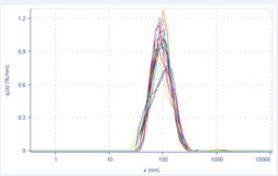
Flow Rate	PDI Normalized	Mean Particle Size	Size Distribution
33% Flow Configuration 2			
66% Flow Configuration 5			
33% Flow Configuration 8			

Figure 4. Quattroflow® QF150SU, TMP = 1.4 bar

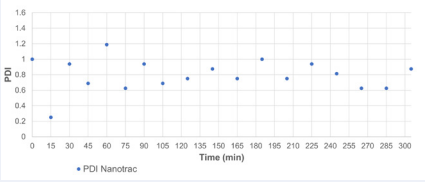
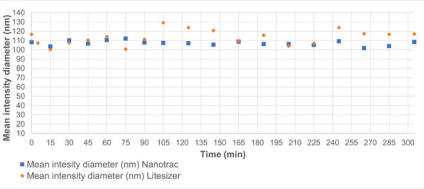
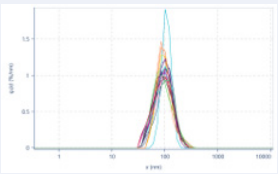
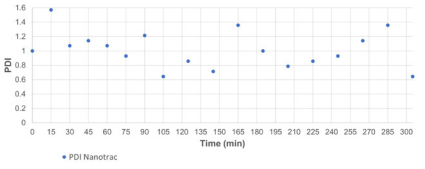
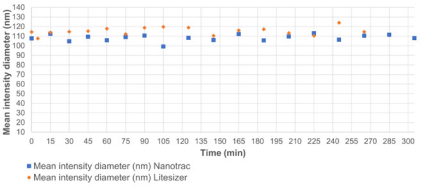
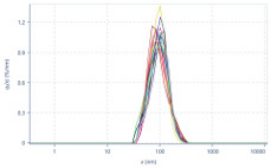
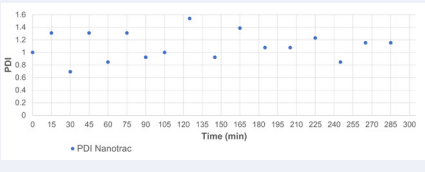
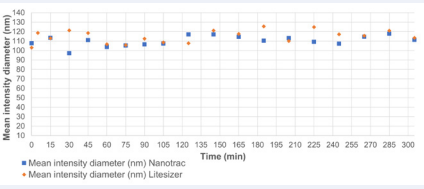
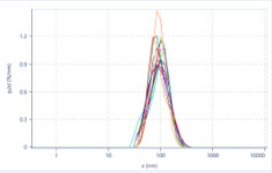
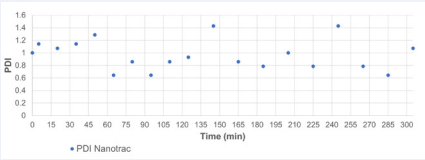
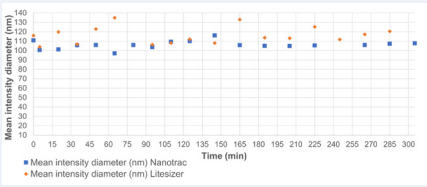
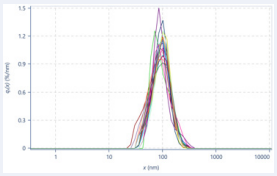
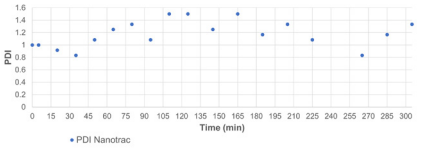
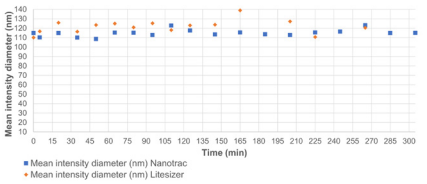
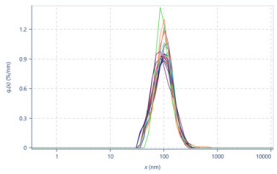
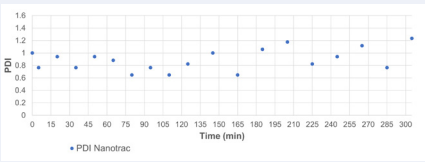
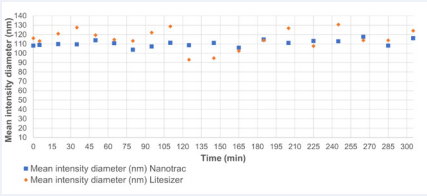
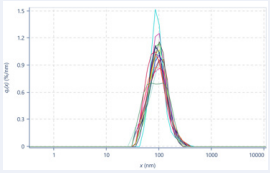
Flow Rate	PDI Normalized	Mean Particle Size	Size Distribution
33% Flow Configuration 3			
66% Flow Configuration 6			
33% Flow Configuration 9			

Figure 5. Levitronix® Puralev® i100SU, TMP = 1.4 bar

Flow Rate	PDI Normalized	Mean Particle Size	Size Distribution
33% Flow Configuration 12			
66% Flow Configuration 15			
33% Flow Configuration 18			

Conclusion

The data demonstrate that even in the worst conditions, with high TMP and high flow, the PDI doesn't increase over time, and that the average particle size does not decrease over time, indicating degradation of the liposomes. It can be therefore concluded that no degradation is noticeable over the 5 hours period. At the highest flow, the 1 L liposome suspension was circulated at 170 LPH (100% flow), thus the liposomes would have been circulated through the system 850 times, which is far more than usual process conditions. Thus, it can be concluded that the FlexiPro™ TFFsystem has a low shear stress on fragile cells or products such as the liposomes and can be safely used for gene therapy process applications.

The two nano-particles size instruments, the Microtrac MRB NANOTRAC Flex, and the Anton Paar's Litesizer™ 500, were also performant and consistent in measurements in the range of concentration currently obtained after AAV upstream manufacturing. The mean intensity diameter was used for the particle size measurement showed in the present document. However, the mean volume diameter, area diameter and number diameter showed the same stability of particle size over the 5 hours duration of each test.