Comparison of Maglev Centrifugal Pump and Quaternary Diaphragm Pump Effects on mRNA encapsulated LNPs

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1. MOTIVATION

Lipid nanoparticles (LNPs) are the most developmentally advanced non-viral gene delivery system that delivers nucleic acids. LNPs have several benefits over previous lipid-based nucleic acid delivery systems through:

- High nucleic acid encapsulation efficiency
- Potent transfection capabilities
- Improved tissue penetration for drug delivery
- Low cytotoxicity and immunogenicity

The MFG of LNPs in a process fluid stream is critical, and there are some equipment options for consideration that many have ignored.

2. INTRODUCTION

Messenger ribonucleic acid (mRNA) encapsulated in lipid nanoparticles (LNP) is a vaccine modality that has found recent effectiveness and prominence within the industry. Both the mRNA and LNP are susceptible to physical degradation and may present as fragmentation, aggregation, precipitation, fusion, or leakage of mRNA from the LNP.[1]

One proposed mechanism of physical degradation is processing vaccine solutions using recirculated pumping. Some prolonged recirculated pumping involves tangential flow filtration (TFF), typically used throughout the industry to concentrate and often diafilter a product pool. This study evaluates and compares maglev single-use centrifugal pump, and single-use quaternary diaphragm pump and their effects on mRNA encapsulated LNPs at target flow rates, pressures, and durations.

3. MATERIALS AND METHODS

3.1. mRNA and LNP Formulation

An LNP suspension (in mainly Potassium phosphate and Sodium phosphate buffers, with Sodium Chloride, Dibasic sodium phosphate dihydrate, sucrose, 18%EtOH, etc.) was formulated using conventional and self-amplifying mRNA (SAM) comprised of the following lipid profiles captured in **Table 1**:

Table 1: Lipid Profiles

Profile 1	ALC-0315 = (4-hydroxybutyl) azanediyl)bis (hexane-6,1-diyl)bis(2-hexyldecanoate)
	ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N ditetradecylacetamide
	1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC)
	Cholesterol
Profile 2	DLin-MC3-DMA: (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate
	1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC)
	PEG2000-DMG = Alpha-(3'-{[1,2-di(myristyloxy)propanoxy] carbonylamino}propyl)-ω-methoxy, polyoxyethylene
	Cholesterol

Parameters and Detection Methods

The parameters monitored in this testing have been:

- The average particle size of the LNPs
- The Polydispersity Index (PDI) represents the variation in sample particle size. Higher PDI is represented by aggregation or fragments present in a sample
- % mRNA encapsulated in LNP relative to total mRNA (free plus encapsulated). A decreasing percentage of encapsulation over the run indicates damage to LNPs (and an increase of free mRNA).

Capillary Gel Electrophoresis and/or Dynamic Light Scattering (DLS) have been shown to relate log normal particle size distribution for small spherical particles[2]. Yet another use for DLS is to determine the Polydispersity Index (PDI) of particles. PDI indicates a variation in the particle size of the sample per Equation 1 below. An increase in PDI may be indicative of aggregation or fragments present in the sample.

$$\text{PDI} = \frac{Std\ dev^2}{mean\ particle\ diameter} \qquad \text{Equation 1}$$

LNP's used in this experiment were analyzed using Focused Beam Reflectance Measurement (FBRM) technology and determined to exhibit the appropriate sphericity for DLS as a detection method.

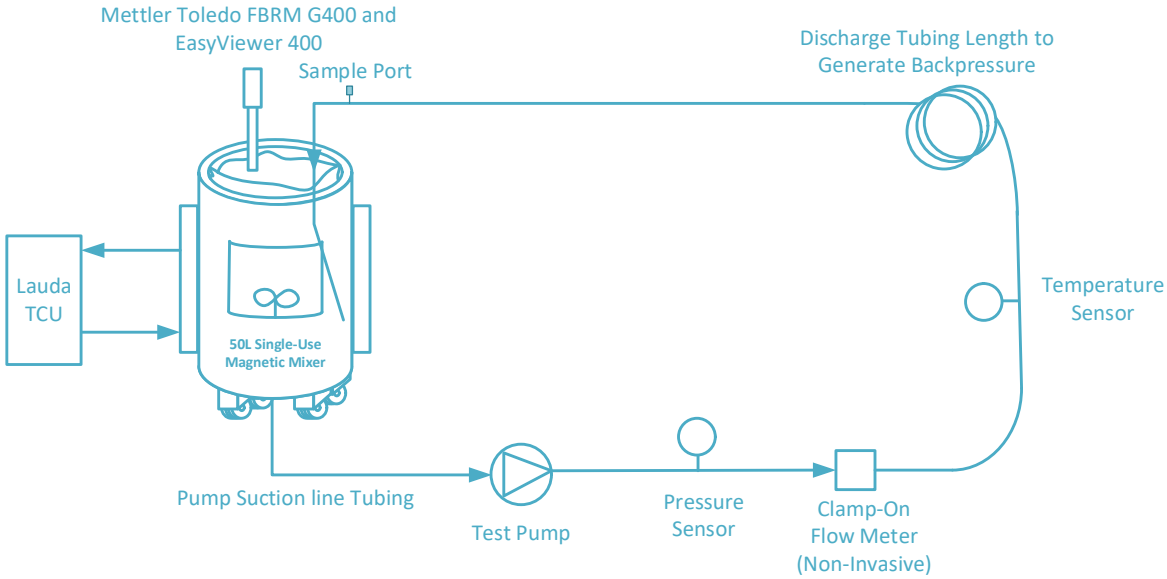
- RiboGreen Assay is a fluorescence based assay that has published detection down to 1.0 ng/mL RNA and linearity with fluorescence changes verses RNA concentration[3]. This assay was used to determine % encapsulation of mRNA.

$$\text{Encapsulation}\% = \frac{Total\ mRNA - Free\ mRNA}{Total\ mRNA} \qquad \text{Equation 2}$$

- Free mRNA: RiboGreen binds to mRNA outside the LNP
    - TE buffer, sample, RiboGreen dye
- Total mRNA: surfactant breaks open LNP, and RiboGreen binds to all mRNA in a sample
    - TE buffer, sample, RiboGreen dye, surfactant
- Buffer Blank- adjust samples
    - TE buffer, RiboGreen
- Standard curve Blank- adjusts the standard curve
    - TE buffer, RiboGreen, surfactant
- **Encapsulation%** = (Total mRNA-Free mRNA)/(Total mRNA)
- **Total mRNA Concentration:** Correlating total mRNA fluorescence with a concentration on the standard curve

3.2. Experimental Setup

Figure 1: Experimental Equipment Setup



The physical testing system consisted of a 50L single-use magnetic mixing system (Sartorius). A Focused Beam Reflectance Measurement (FBRM) device and an EasyViewer imaging tool were affixed/secured through a sanitary fitting at the top of the mixing bag. A recirculation loop of disposable tubing was attached to the bottom outlet of the bag and pumped to the top inlet on the bag through a sub-surface polypropylene dip tube. The recirculation loop included the pumping technology being tested and a pressure sensor, temperature sensor, sample port, and a (non-invasive) clamp-on flow meter. It was ensured that all transitions between the different inline components with variable inner diameters/bore sizes have smooth transitions. The Single-Use Mixer (SUM) was jacketed and cooled via a temperature control unit to counter the temperature increase effects of speed, pressure, and duration of the recirculated pumping. The

primary goal of this testing was to assess the impact of different pump technologies on mRNA-encapsulated LNPs; therefore – by purpose – all other devices which cause or can cause shear have been eliminated in the test loop.

Table 2: Equipment | Material List of Test Setup

Equipment | Material	Type	Manufacturer
Tank/Vessel	50L single-use magnetic mixing system	Sartorius
Test Pump A	PuraLev-2000SU with DCP-2000.2	Levitronix
Test Pump B	Quattroflow QF-4400	Quattroflow
Suction Line Tubing	1" x 1 3/8" STHT-R x 38" in Length	Saint-Gobain
Discharge Line Tubing	1" x 1 3/8" STHT-R x 16" in Length	Saint-Gobain
Backpressure Tubing Loop for 30psi @ 50 L/min	¾" (ID) x 1" (OD) x 182' Length Clear Braided Vinyl Tubing	HydroMaxx
Backpressure Tubing Loop for 45psi @ 23 L/min	¼" (ID) x ½" (OD) X 78' Length PharMed BPT	Saint-Gobain
Single-Use Pressure Sensor	PREPS-N-1-1	Pendotech
Clamp-On Flow Sensor	LeviFlow LFSC-i25x	Levitronix
Temperature Sensor	TEMPS-N-025	Pendotech
Temperature Control Unit (TCU)	Variocool VC3000	Lauda

Table 3: Measurement Equipment

Measurement Equipment	Type	Manufacturer
Focused Beam Reflectance Measurement (FBRM) device	G400	Mettler-Toledo
EasyViewer	400	Mettler-Toledo
Particle Sizing (Off-Line)	Zetasizer Pro	Malvern
RNA Quantitation	Quant-It	ThermoFisher Scientific

3.3. Experimental Method

The following measures were taken during the experiment to minimize variation:

- A consistent volume of LNPs at a target concentration was filled into the 50L single-use mixing system for each test.
- The single-use mixing system was actively cooled to maintain and control ambient liquid temperatures (18-25^{o}C) throughout all tests.
- All instrumentation used was within the appropriate calibration and standardization periods.
- Compendial grade materials were used throughout all testing to ensure quality and consistency.
- All testing was performed continuously at the same flow rates across pumps for each pump condition to eliminate flow rate as a test variable. Pumps were swapped in the system layout, with no other changes made to the flow path.

- The tubing inner diameter and lengths were consistent across all testing per-flow rate and pressure.
- TFF recirculation pumping conditions varied based on processing needs hence a 12-hour duration for each test condition was used to observe any physical degradation effects.

The single-use maglev centrifugal pump and single-use quaternary diaphragm (4-piston) pump were both tested under the following two conditions:

Table 4: Testing Conditions

Test Condition	Flow Rate (LPM)	Pressure (psig)	Volume per Test Run [L]	Run Time (hours]	Fluid Temperature [C]
Lipid Profile 1	50	30	50-70	12	18-25
Lipid Profile 2	23	45	50-70	12	18-25

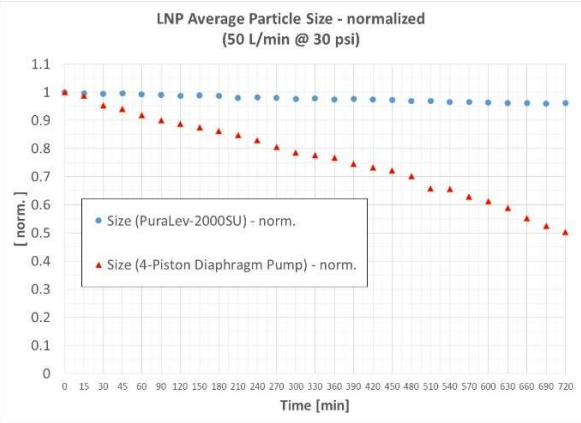
4. RESULTS

Particle Size (see Figure 2)

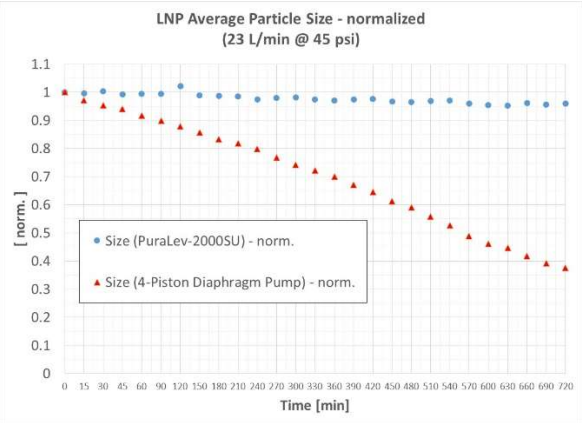
The maglev centrifugal pump exhibited a consistent particle-size profile with minimal variation over the 12-hour testing period with both Lipid profiles and flow rate/pressure conditions. The diaphragm (4-piston) pump consistently exhibited a significant particle size profile shift downwards.

Figure 2: Particle Size Test Results

Lipid Profile 1



Lipid Profile 2

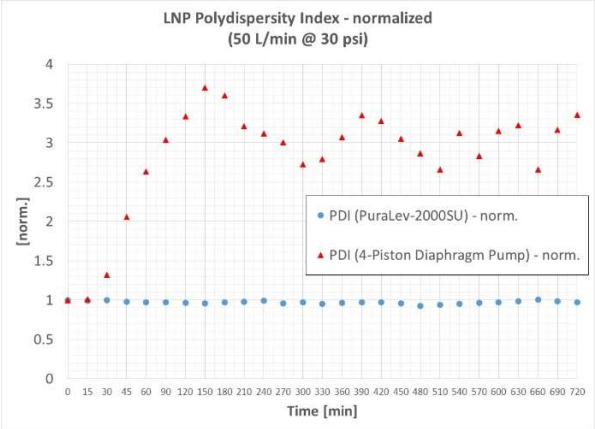


Polydispersity Index (PDI) (see Figure 3)

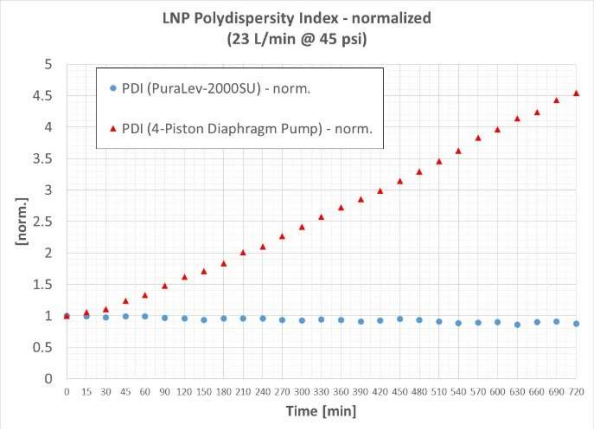
The maglev centrifugal pump exhibited a consistent PDI profile with minimal variation over the 12-hour testing period with both Lipid profiles and flow rate/pressure conditions. However, the diaphragm (4-piston) pump exhibited a significant PDI profile shift upwards.

Figure 3: PDI Test Results for Lipid Profile 1

Lipid Profile 1



Lipid Profile 2

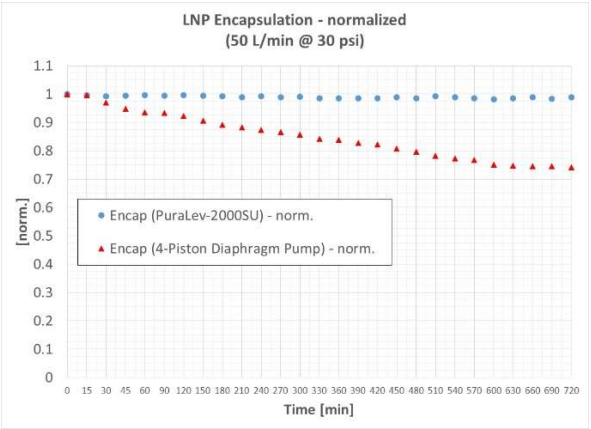


% Encapsulation (see Figure 4)

The maglev centrifugal pump exhibited a consistent % encapsulation profile with minimal variation over the 12-hour testing period with both Lipid profiles and flow rate/pressure conditions. Of note, the first 1.5 hours of data exhibited slight variation but within +/-5%.
The diaphragm (4-piston) pump exhibited a significant % encapsulation profile shift downwards.

Figure 4: LNP Encapsulation Test Results

Lipid Profile 1



Lipid Profile 2

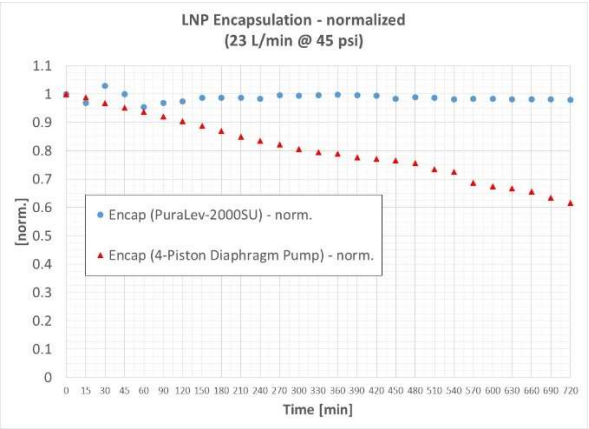
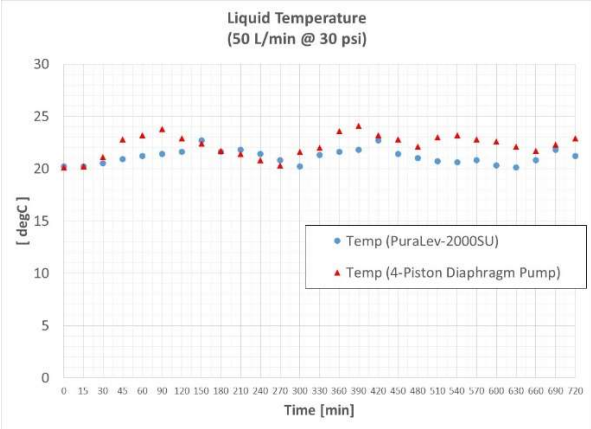
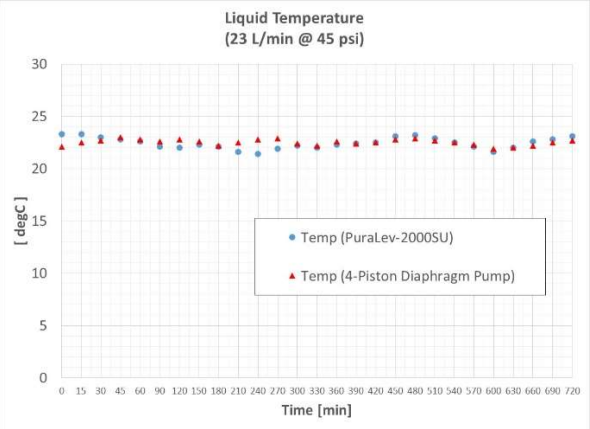


Figure 5: Liquid Temperature

Lipid Profile 1



Lipid Profile 2



The temperature fluctuations have been in a similar range across all test conditions.

5. CONCLUSIONS

The maglev centrifugal pump technology exhibited consistent results and did not impact the LNP fluid stream during the 12-hour test execution under the conditions investigated.

The diaphragm (4-Piston) pump technology under the same conditions yielded a statistically relevant impact on each of the LNP parameters measured. The particle size shift (smaller particles), PDI upward shift, and % encapsulation downward shift are all consistent with physical degradation. The PDI shift indicates that either the mean particle size is shifting, the standard deviation is varying, or a combination of both is occurring. The experimental results are evidence of fragmentation and leakage of mRNA from the LNP under the conditions investigated.

In conclusion, pump choice and operating conditions need further attention to avoid the failure mechanism of physical degradation of mRNA encapsulated LNPs.

6. REFERENCES

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