



Lipid Nanoparticles (LNP) Pump Characterization Testing

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Experimental Materials and Design

- ❑ Lipid Nanoparticle (LNP) suspension (in a mostly Potassium phosphate and Sodium phosphate buffers, with Sodium Chloride, Dibasic sodium phosphate dihydrate, sucrose, 18%EtOH, etc.) using conventional and self-amplifying mRNA (SAM) comprised of the following 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

- ❑ Volume per Test Run: 50L-70L

- ❑ Run Time: 12 Hours

- ❑ Operating Points:

- 50 L/min @ 30 psi
- 23 L/min @ 45 psi
- Fluid Temperature between 18-25°C

Analytical Methods

❑ RiboGreen Assay

- % mRNA encapsulated in LNP and concentration of mRNA
- Fluorescence based assay
- 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 sample
 - TE buffer, sample, RiboGreen dye, surfactant
- Buffer Blank- adjust samples
 - TE buffer, RiboGreen
- Standard curve Blank- adjust standard curve
 - TE buffer, RiboGreen, surfactant
- **Encapsulation% = (Total mRNA-Free mRNA)/(Total mRNA)**
- **Total mRNA Concentration:** Correlating total mRNA fluorescence with concentration on standard curve
- Decreasing percentage over run indicates damage of LNPs (and increase of free mRNA).

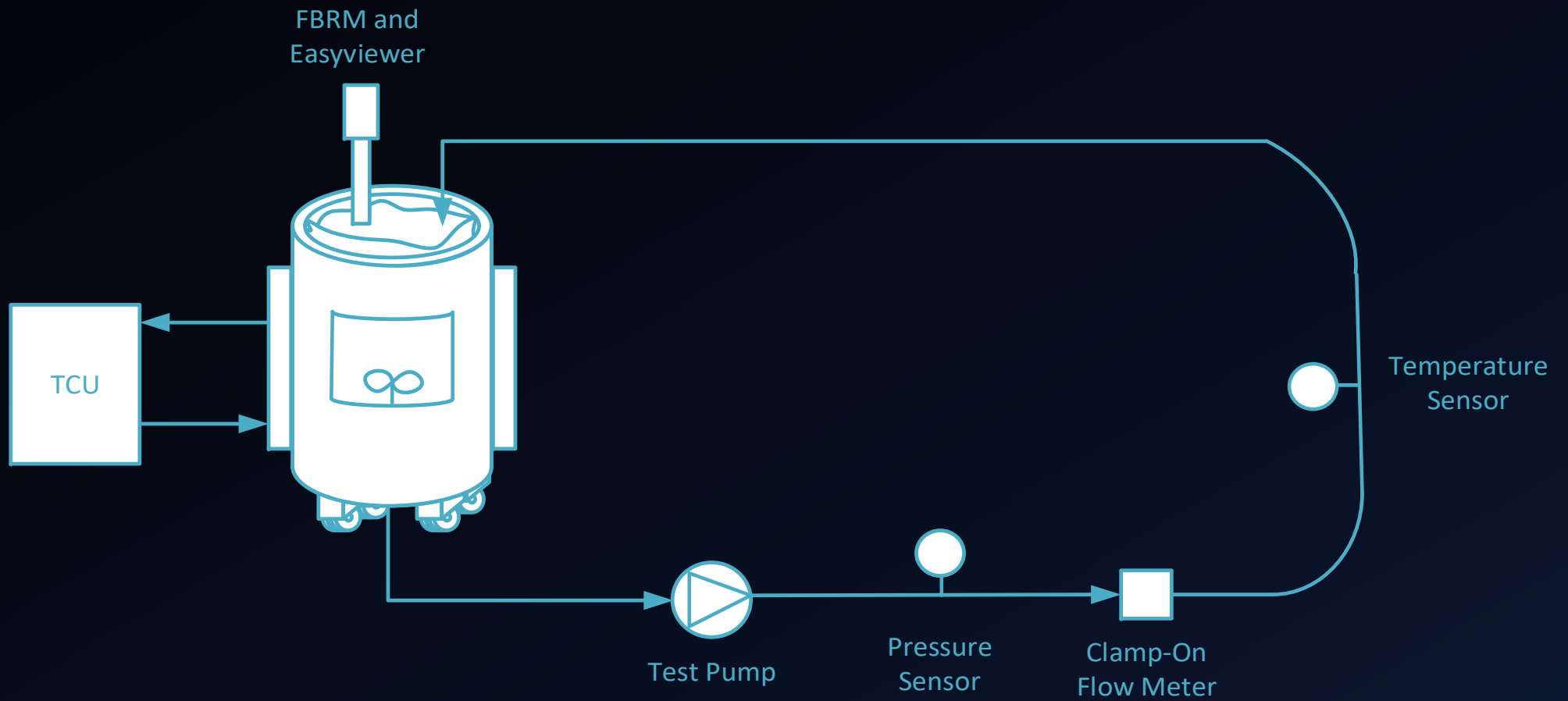
❑ Dynamic Light Scattering (DLS)

- Average particle size of sample
- Measures intensity of light scattering over time

❑ Capillary Gel Electrophoresis and/or DLS

- Polydispersity Index (PDI) = $\frac{Std\ dev^2}{mean\ particle\ diameter}$
- Variation of particle size of sample
(Higher PDI: could be aggregation or fragments present in sample)

Recirculation Loop Setup

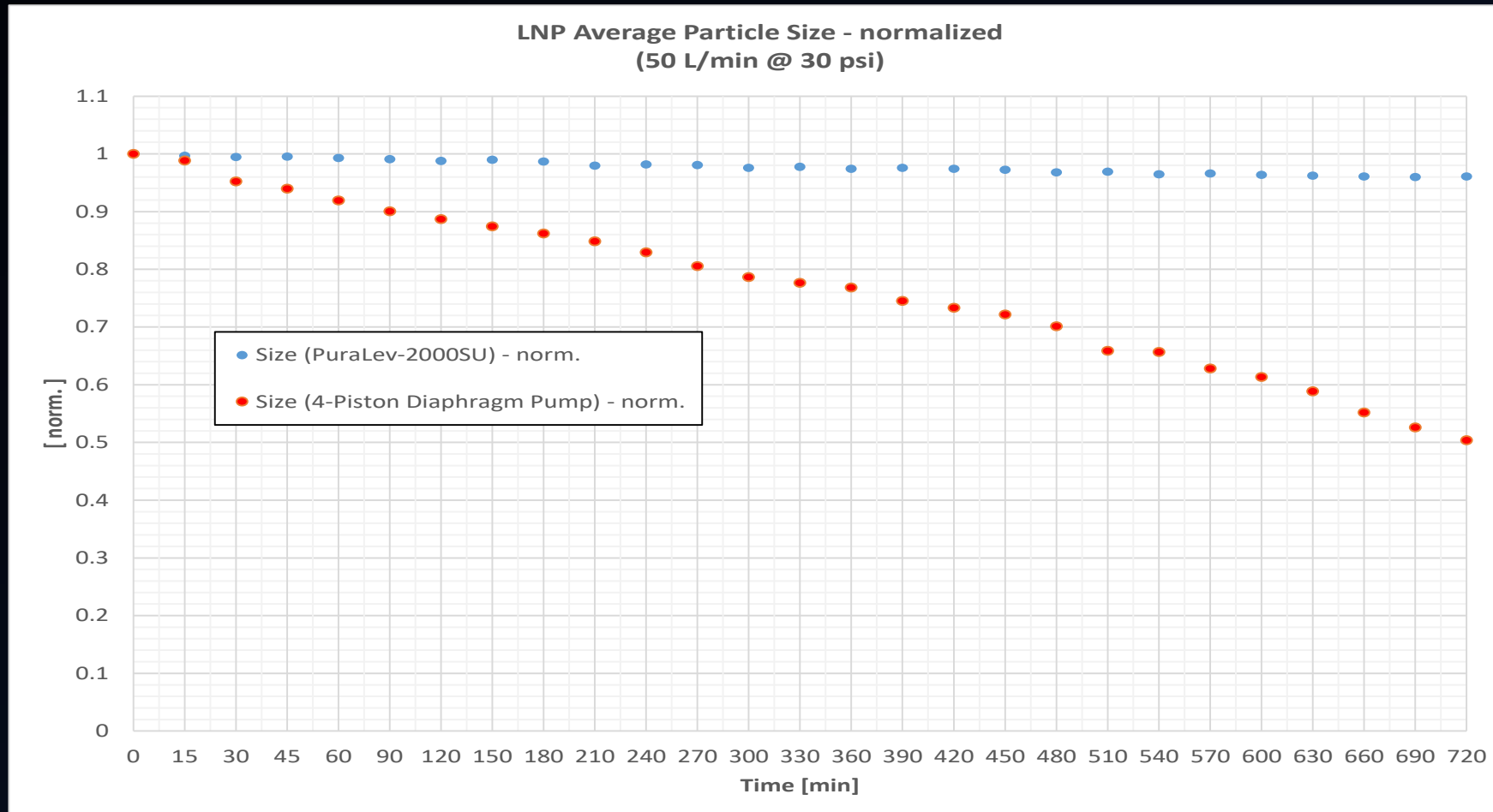


Test Results (50LPM @ 30PSI)



LNP Avg Particle Size

(Lipid Profile 1)



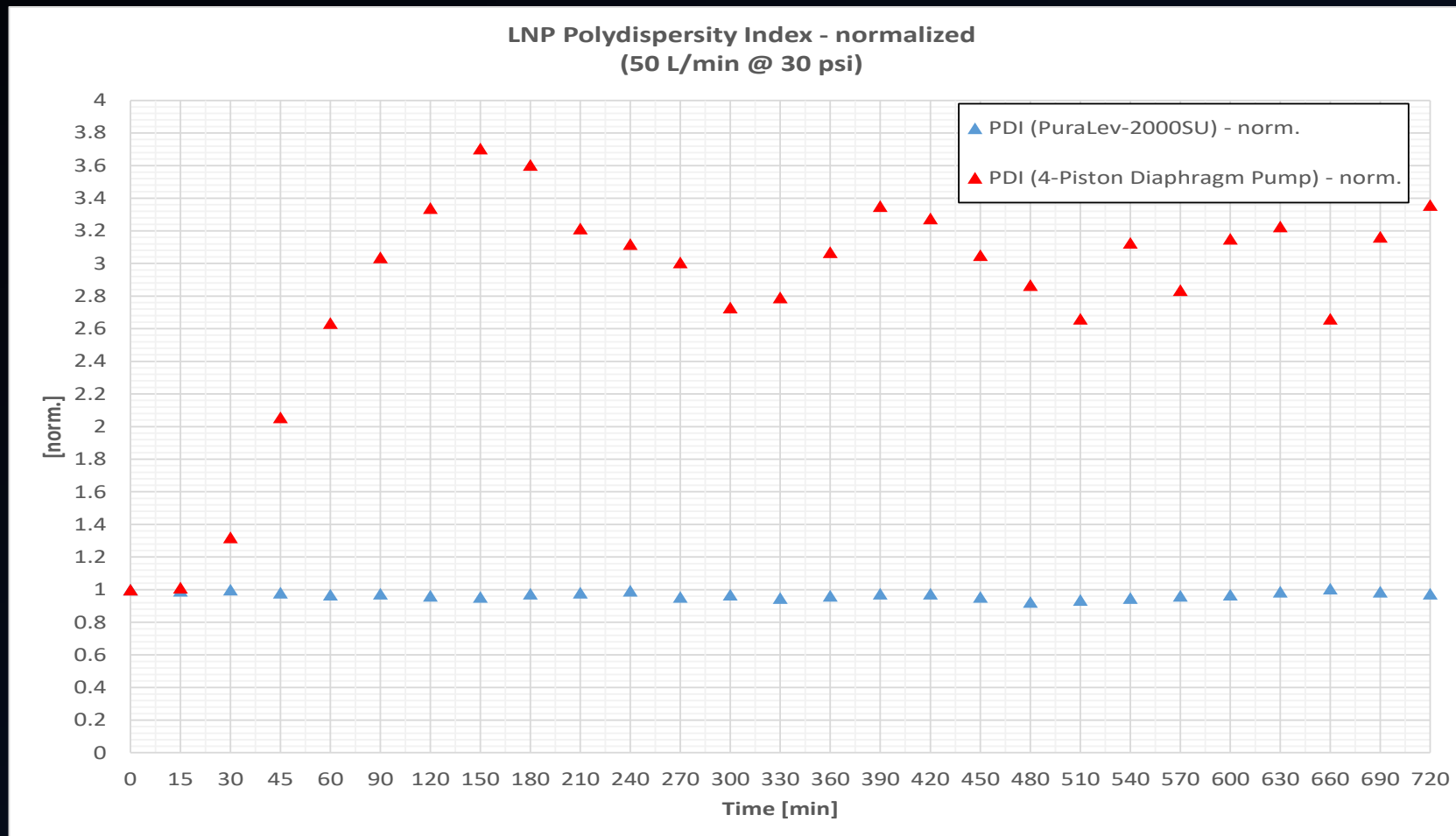
Average particle size of sample, measured with intensity of light scattering over time.

Test results (50LPM @ 30PSI)



LNP Polydispersity Index

(Lipid Profile 1)



$$\text{Polydispersity Index (PDI)} = \frac{\text{Std dev}^2}{\text{mean particle diameter}}$$

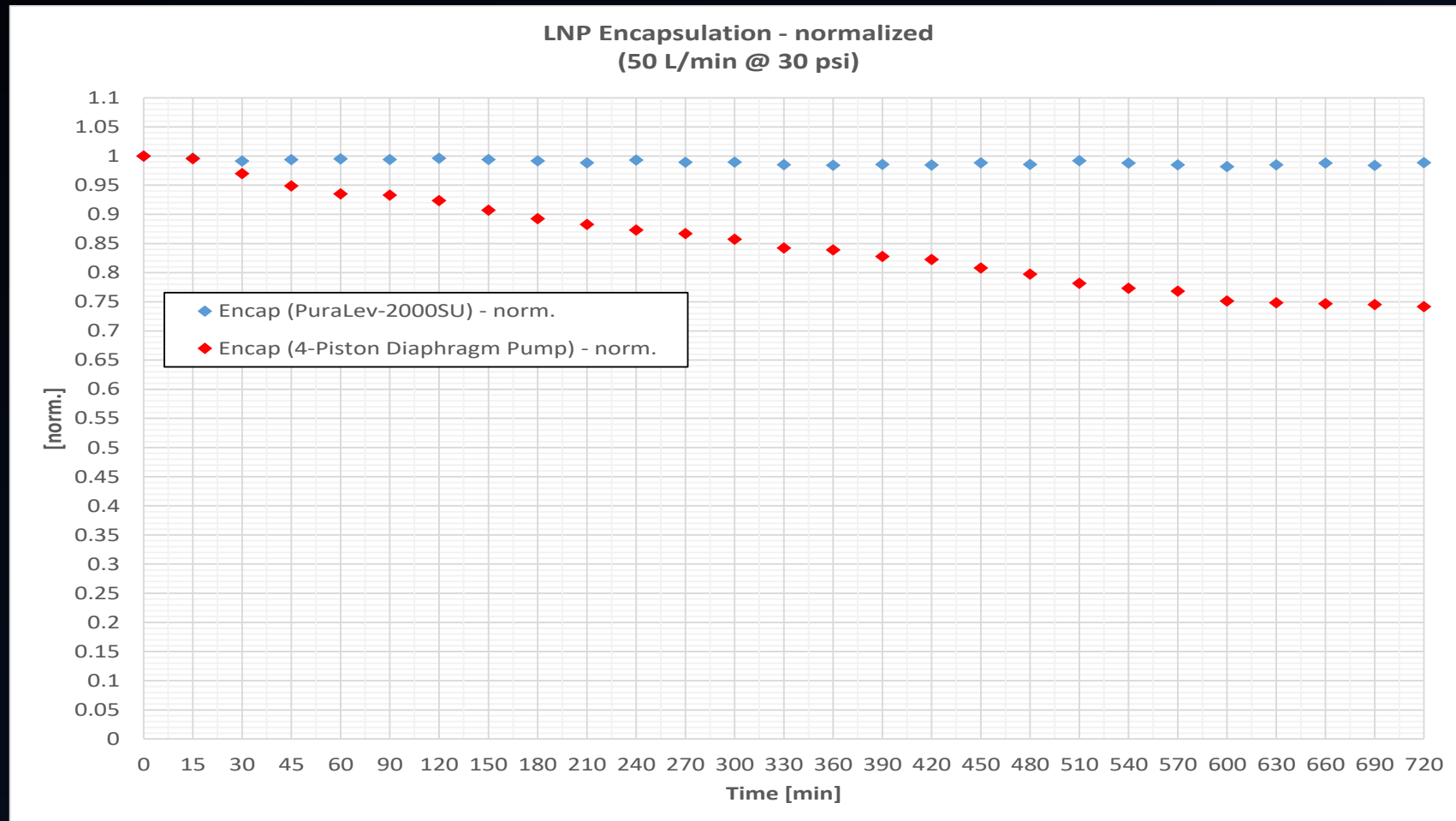
represents variation of particle size of sample
(Higher PDI: could be aggregation or fragments present in sample)

Test results (50LPM @ 30PSI)



LNP Encapsulation

(Lipid Profile 1)



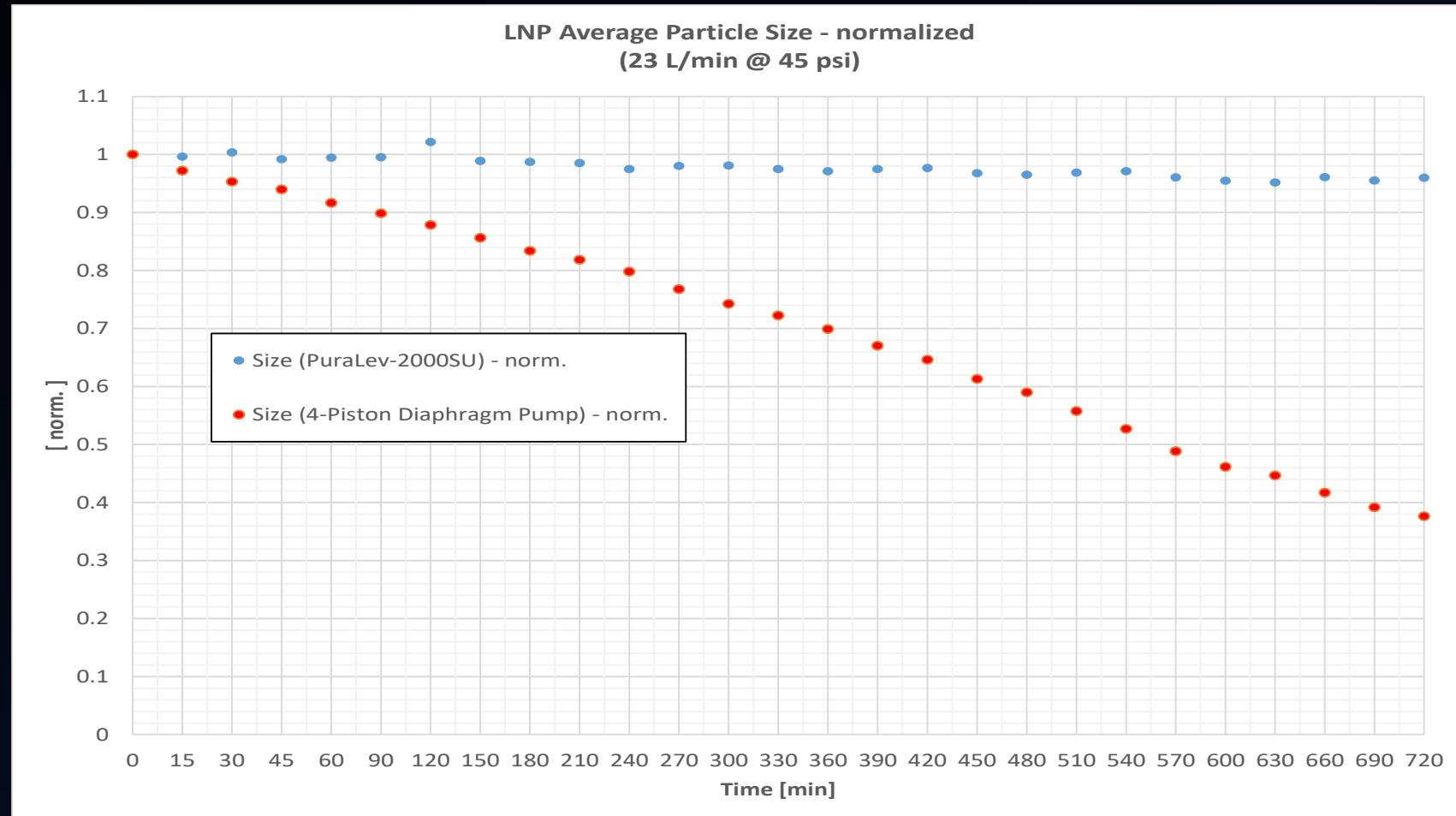
% mRNA encapsulated in LNP in relation to total mRNA (free plus encapsulated). Decreasing percentage over run indicates damage of LNPs (and increase of free mRNA).

Test results (23LPM @ 45PSI)



LNP Average Particle Size

(Lipid Profile 2)



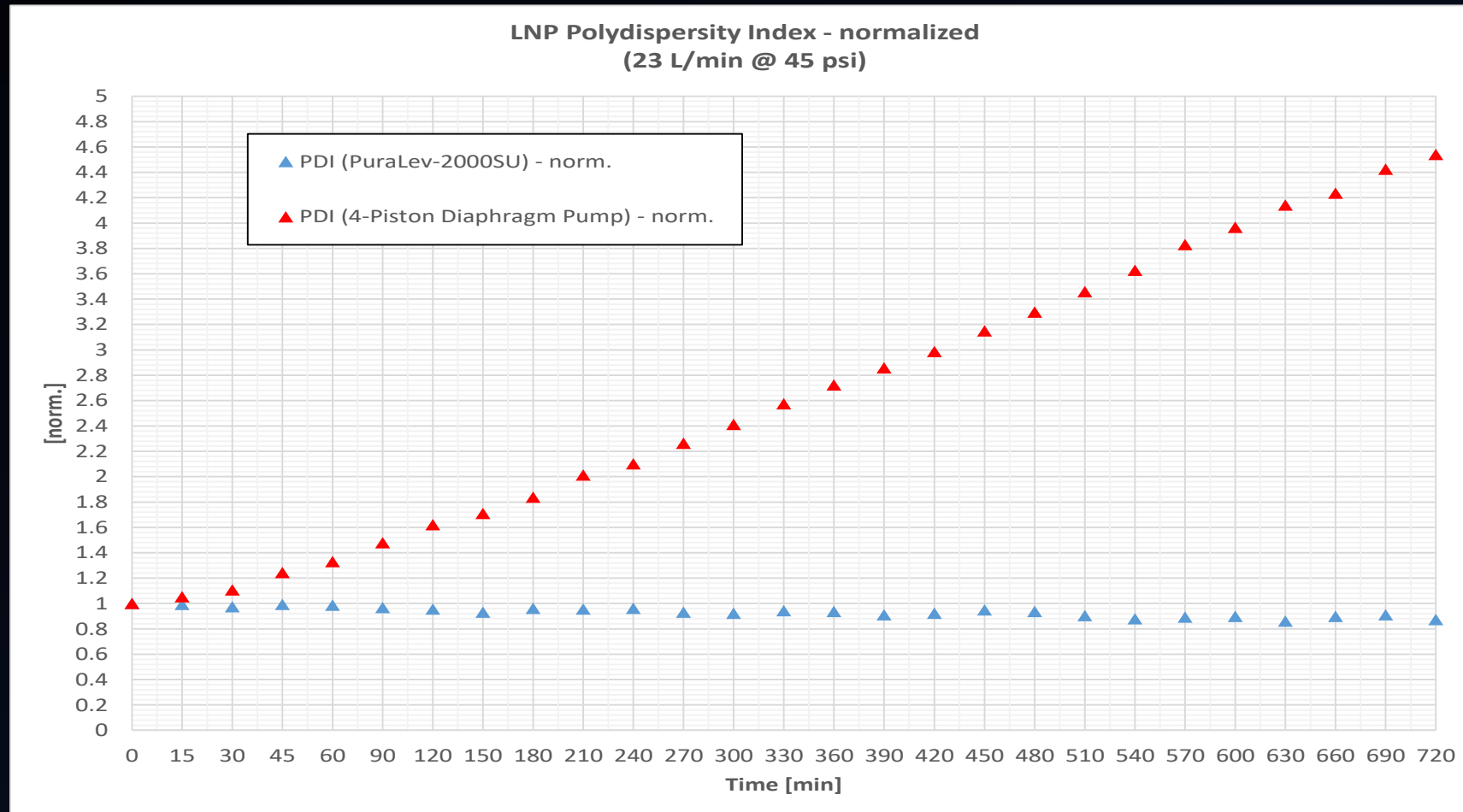
Average particle size of sample, measured with intensity of light scattering over time.

Test results (23LPM @ 45PSI)



LNP Polydispersity Index

(Lipid Profile 2)



Polydispersity Index (PDI)=

$$\frac{Std\ dev^2}{mean\ particle\ diameter}$$

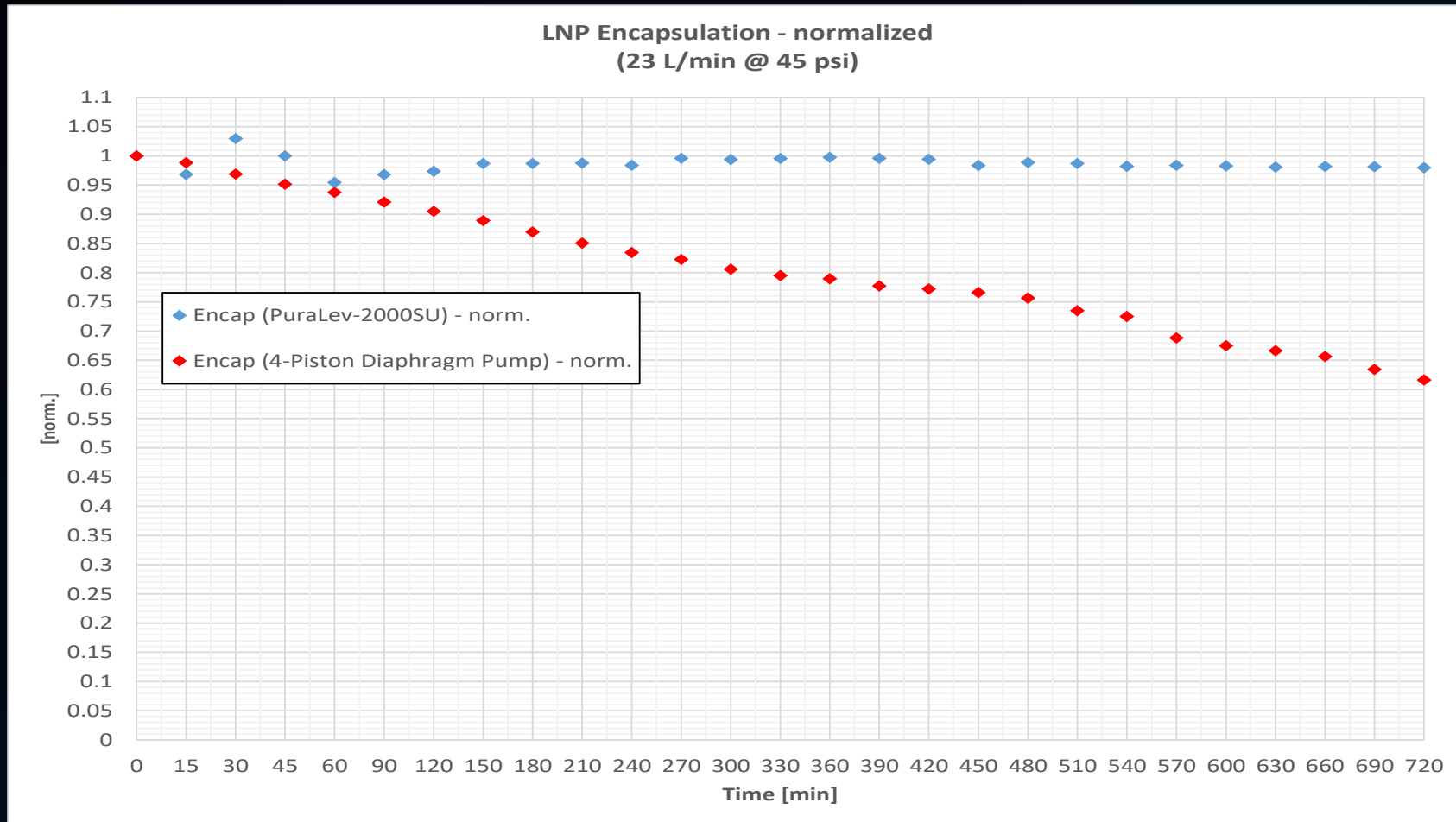
Represents variation of particle size of sample (Higher PDI: could be aggregation or fragments present in sample)

Test results (23LPM @ 45PSI)



LNP Encapsulation

(Lipid Profile 2)



% mRNA encapsulated in LNP in relation to total mRNA (free plus encapsulated). Decreasing percentage over run indicates damage of LNPs (and increase of free mRNA).

Test Results



- ❑ Side by side comparison testing using two pump technologies (Levitronix Maglev Pump PuraLev-2000SU and 4-Piston Diaphragm Pump) were evaluated. The fluid stream used for test execution contained Lipid Nanoparticles (LNP's) similar to those used in mRNA Vaccine manufacturing unit operations (for example, TFF). The fluid stream was comprised primarily of Phosphate Buffer or Citrate Buffer and EtOH at 18%.
- ❑ Pump technology impact to the process stream utilized a fluid stream characterization based on Particle Size, Polydispersity Index (PDI) and mRNA Encapsulation using the following processing parameters:
 - Test Run "A": Flow Rate: 50 L/min | Pressure: 30 psi (2.07 bar)
 - Test Run "B": Flow Rate: 23 L/min | Pressure: 45 psi (3.10 bar)
 - Duration: 12 Hours
 - Fluid Temperature: 18°C- 25°C
- ❑ **The Levitronix MagLev Pump technology showed no statistically relevant impact on the LNP fluid stream during the 12 hour test execution. The 4-Piston Diaphragm pump showed a measured and statistically relevant impact to the measured LNP parameters.**

Final Report Status



Final Report Status

- bioX is in the process of completing final reporting for publication.
- Peer review of the data will occur along with the final report
- Industry references are being collected and will be assessed against the test execution to ensure objective analysis
- bioX intends on providing the final report prior to 01Sep21

Questions?

