Effects of Single-Use Pumping Technologies on LNP Quality Attributes during mRNA Manufacturing TFF Operations

Executed By: **bioX**
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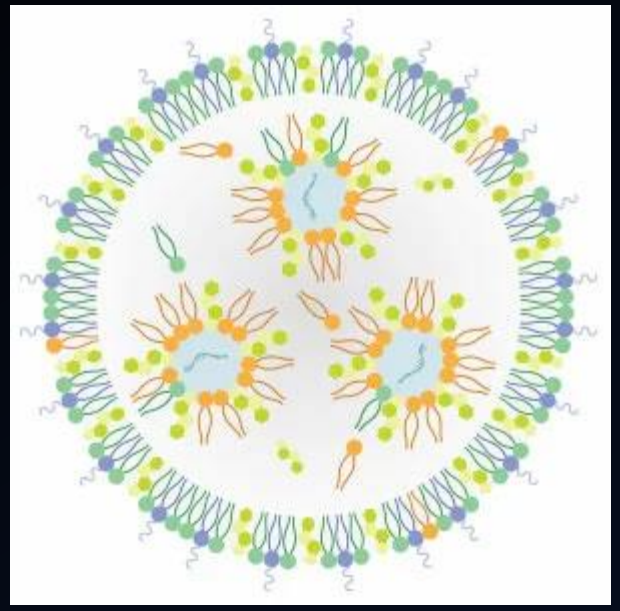
Date: **19Apr23**

Foundation of bioX and what we do

- Completely independent 3rd party applications testing and process development laboratory
- No affiliation with any supplier in the biotech/pharma markets
- Work on behalf of end users to execute comparability and characterization across all modalities and all unit operations
- Full Scale Analytical Capabilities and Process/Pilot Scale to 3K

mRNA MFG Critical Equipment Selection Criteria



- Lipid nanoparticles (LNPs) are the **most developmentally advanced** non-viral gene delivery system that deliver nucleic acids.

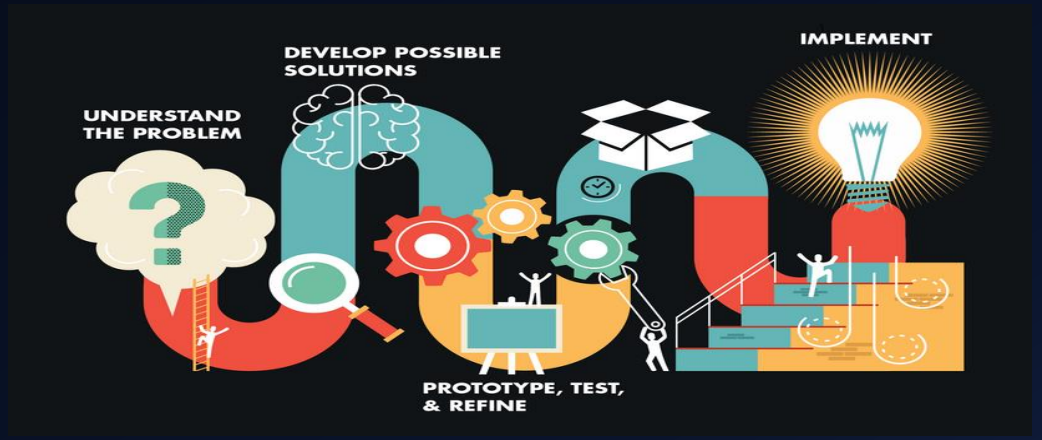
- LNPs have several benefits over previous lipid-based nucleic acid delivery systems through:
  - High nucleic acid encapsulation efficiency
  - Potent transfection capabilities
  - Improved tissue penetration for drug delivery
  - Low cytotoxicity and immunogenicity

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

Equipment Selection and Product Impact

- Which equipment options are available per unit operation?
- What design considerations truly impact the product?
- How well is the process characterized per unit operation?
- For TFF operations, what is the impact of face velocity in the filter membrane?
- Is low shear important to LNPs?

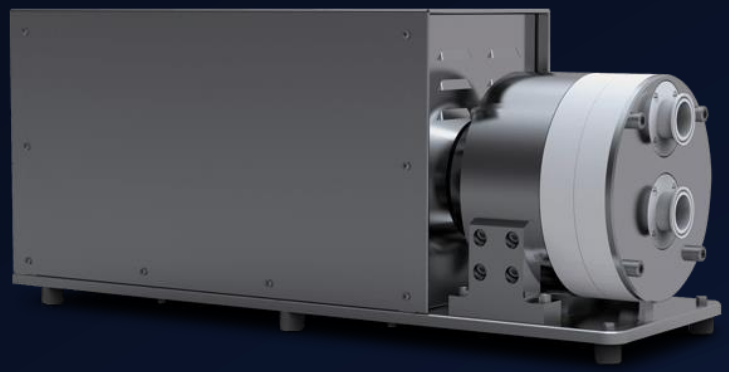
- Hollow Fiber vs Flat Sheet?
- Positive Displacement or Centrifugal Pumps?
- Has an impact assessment of System Valves, DipTubes, Sensors, and Setup been conducted?



SU Centrifugal Pumps and SU Quaternary Pumps

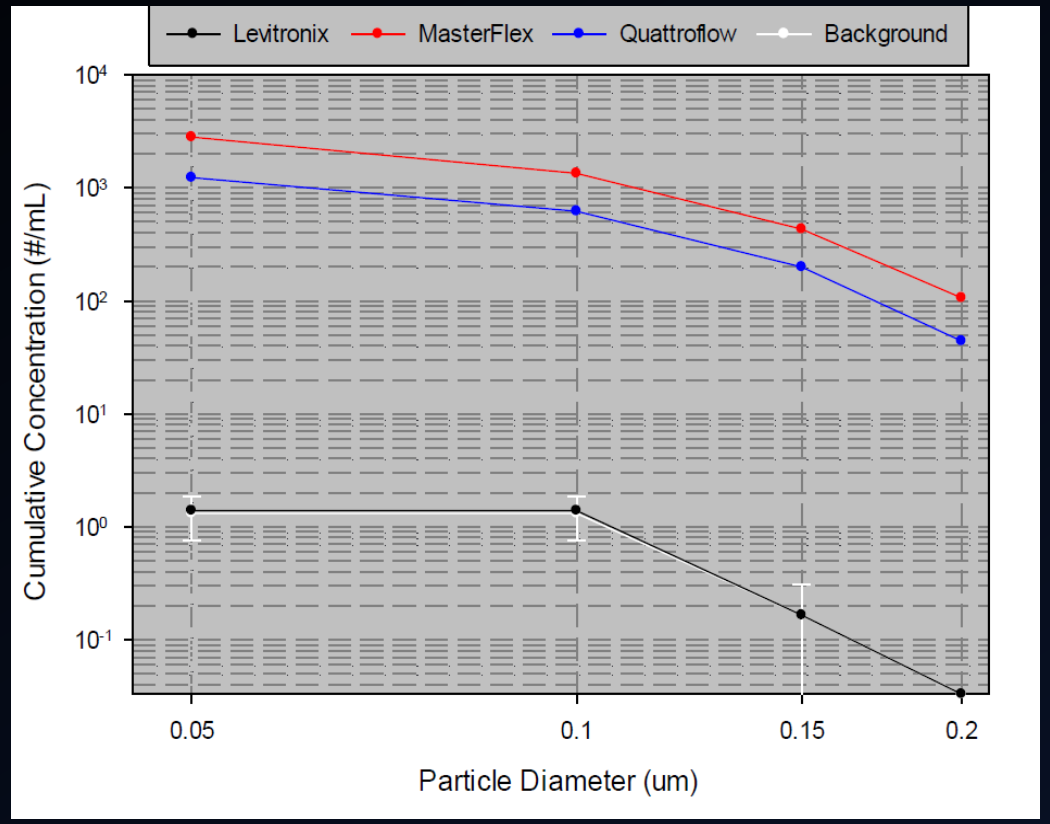
- **SU Centrifugal Pump Benefits**
  - Accurate, stable, and reliable flow without pulsation
  - Significantly smaller footprint than positive displacement pumps
  - No particle shedding or agglomeration
  - High Turndown allows for wide flowrate range

- **SU Quaternary Pump Benefits**
  - Turndown range allows multiple flow duties
  - Self-priming (even when running dry)
  - Low pulsation for high precision
  - No metal-to-metal wear

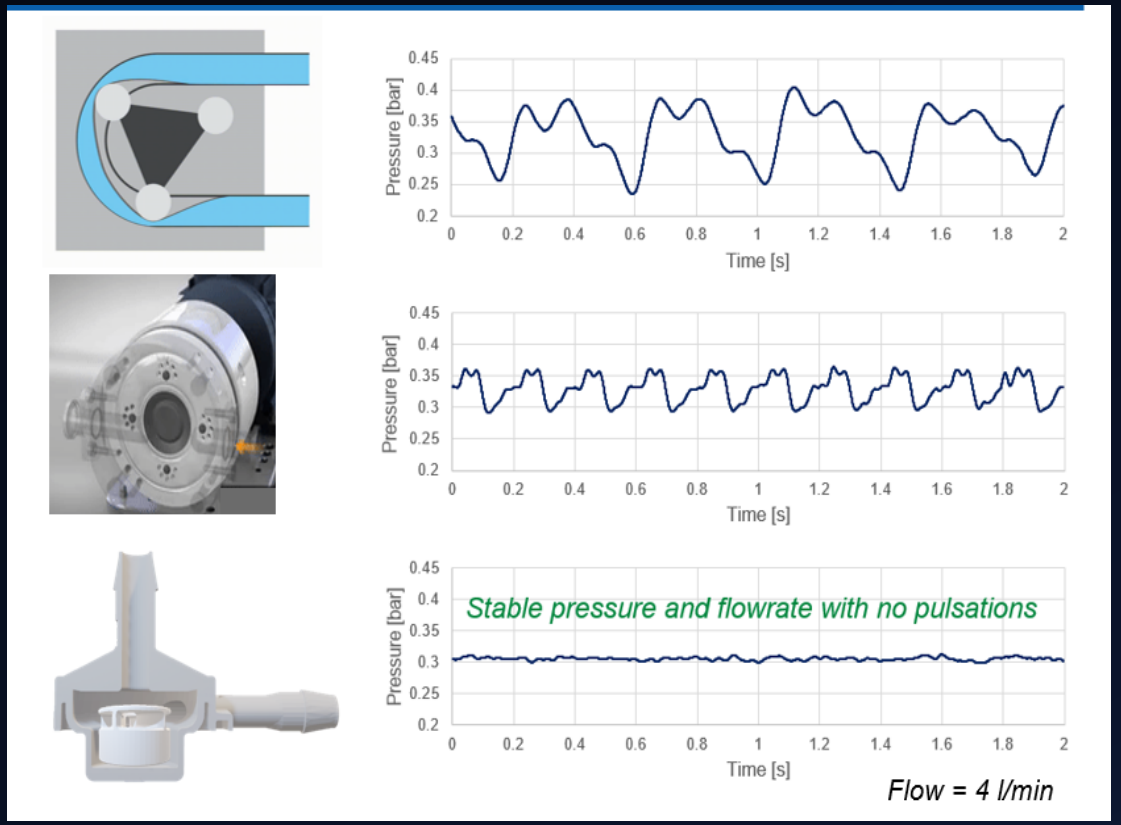


Design Considerations for Pumping Technologies

Particulate Data



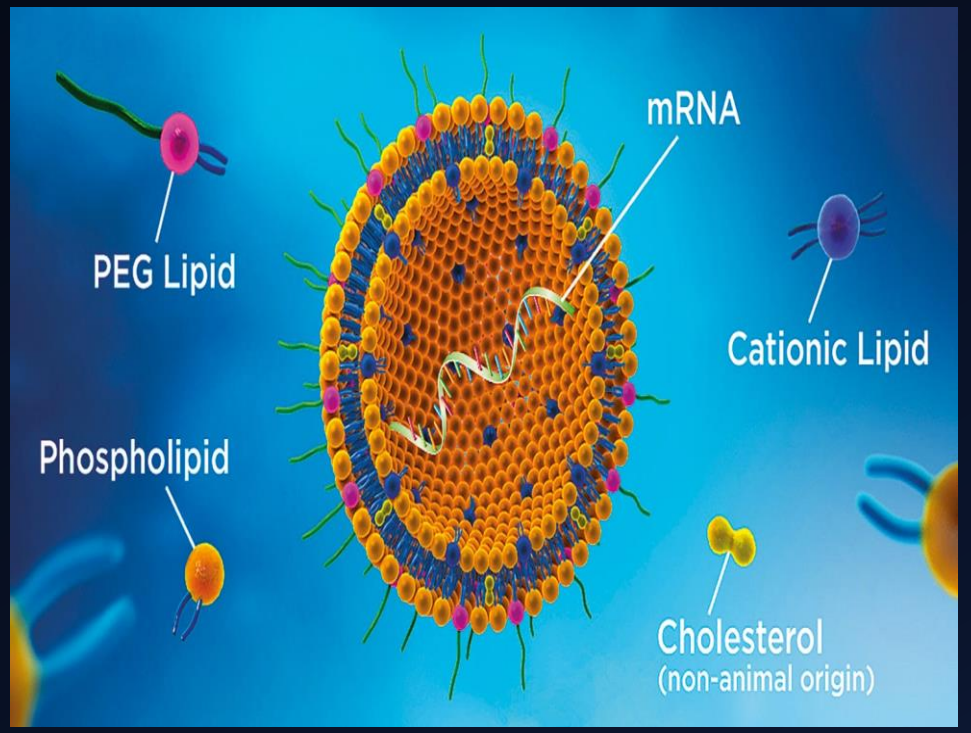
Pulsation Data



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



Test Conditions and Components

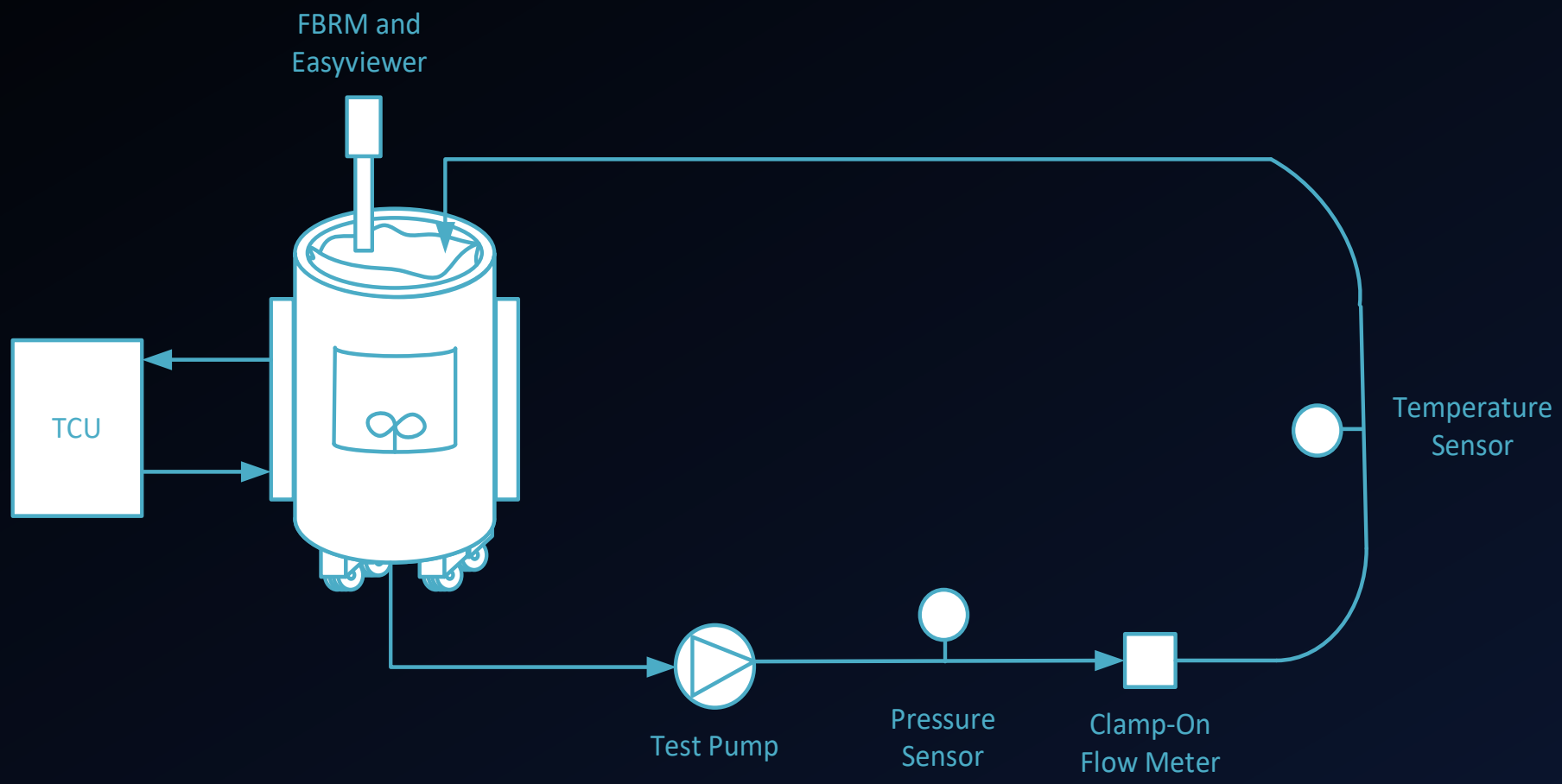
FLOWS AND PRESSURES

- Volume per Test Run: 4L-70L
- Run Time: 12 Hours
- Operating Points:
  - 100ml/min @ 10psi
  - 400ml/min @ 10psi
  - 100ml/min @ 20psi
  - 400ml/min@ 20psi
  - 23 L/min @ 45psi
  - 50 L/min @ 30 psi
  - Fluid Temperature between 18-25^{o}C

PROCESS CONTACT MATERIALS

- Tubing: Pharmed BPT Tubing, AdvantaPure APSH, and Reinforced APSH for pressures > 30psi
- Pressure Sensor: Pendotech (SU) and Ashcroft (Analog)
- Flow Meter: Leviflow LFSC-iX
- In situ Particle Sizing: FBRM and Easy Viewer from MT

Recirculation Loop Setup



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

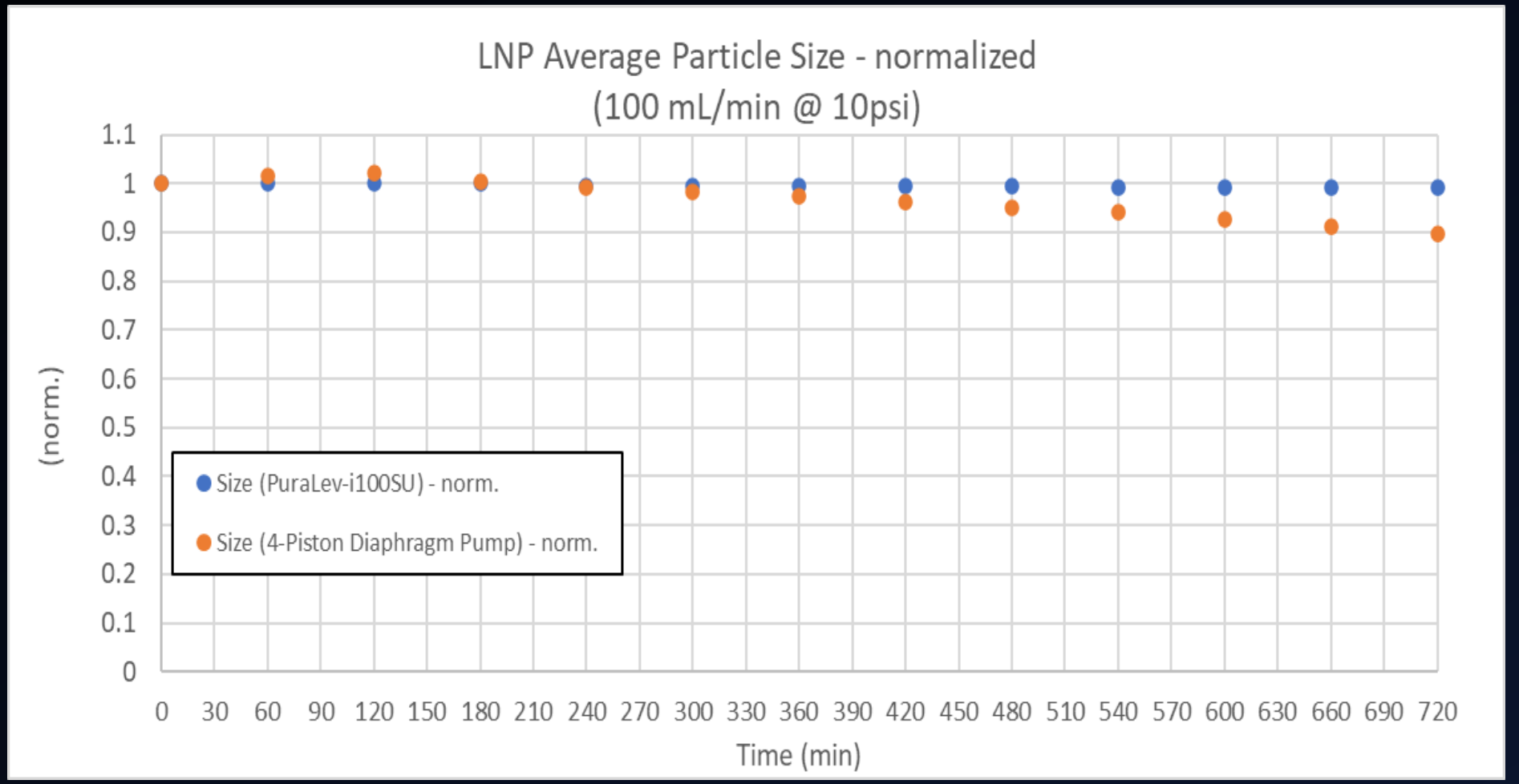
Capillary Gel Electrophoresis and/or DLS

- 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)

Test Results (100ml/min @ 10PSI)

LNP Avg Particle Size

(Lipid Profile 1)



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

Test Results (100ml/min @ 10PSI)

LNP Polydispersity Index

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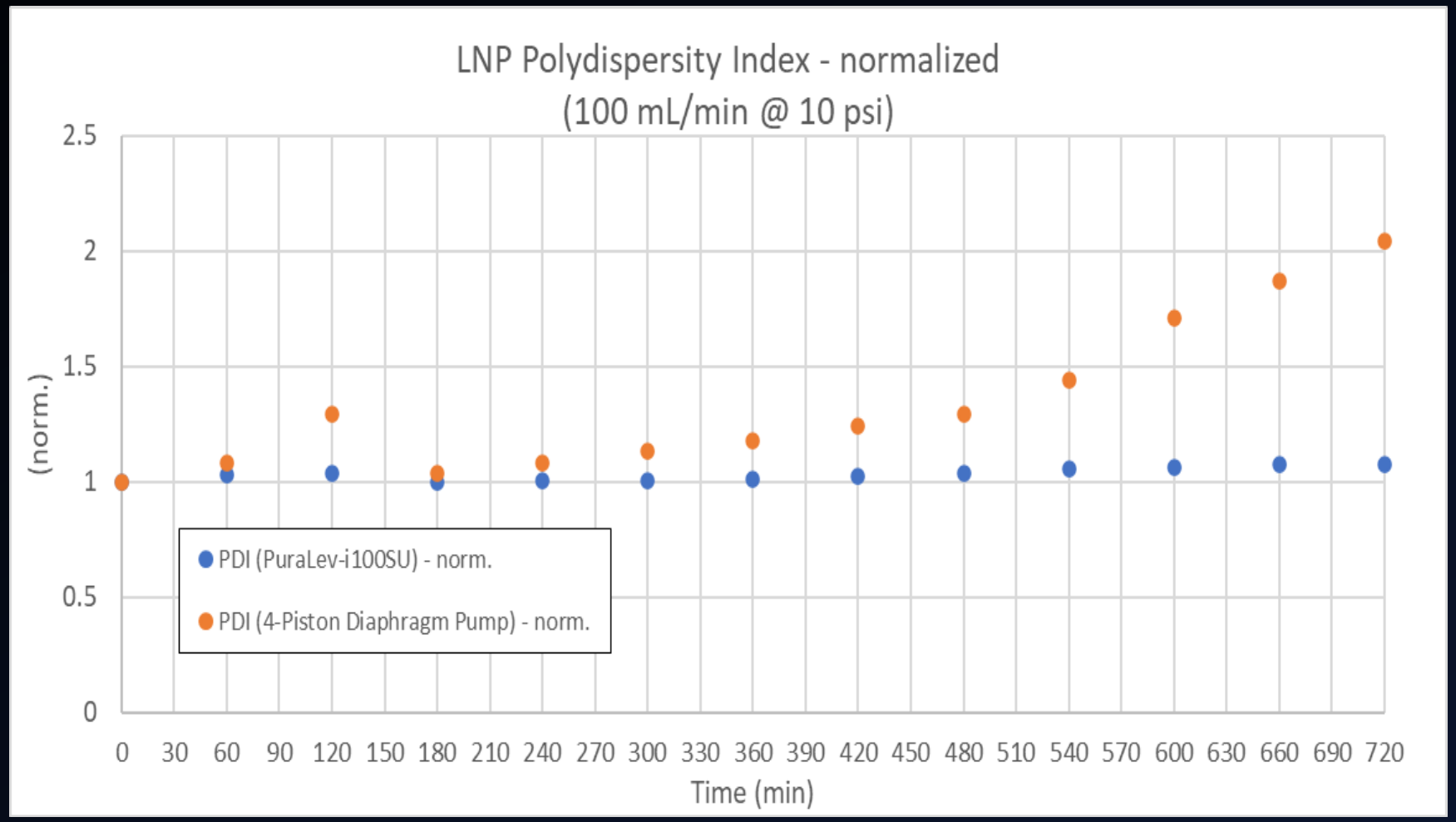

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Test Results (400ml/min @ 10PSI)

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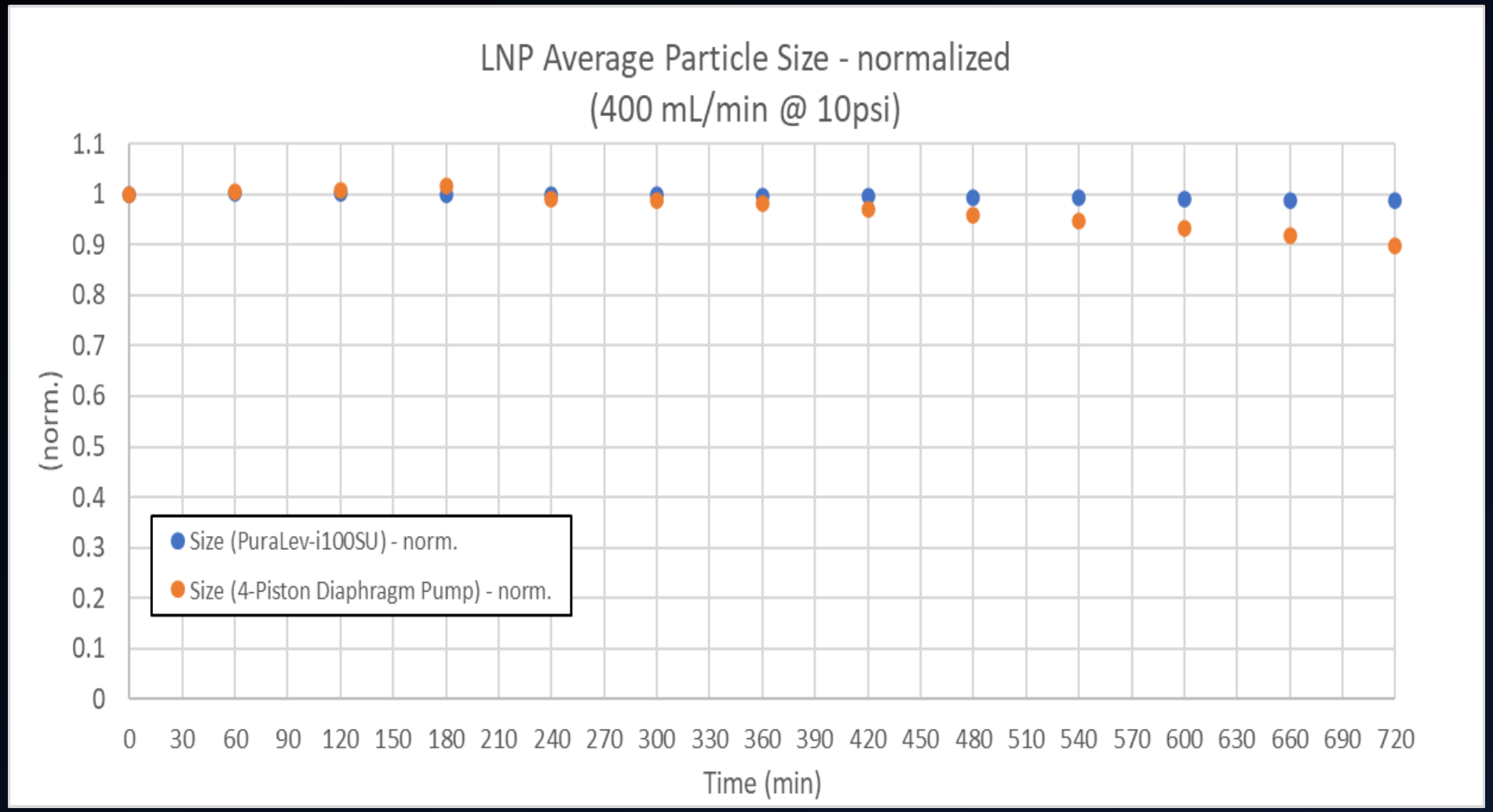

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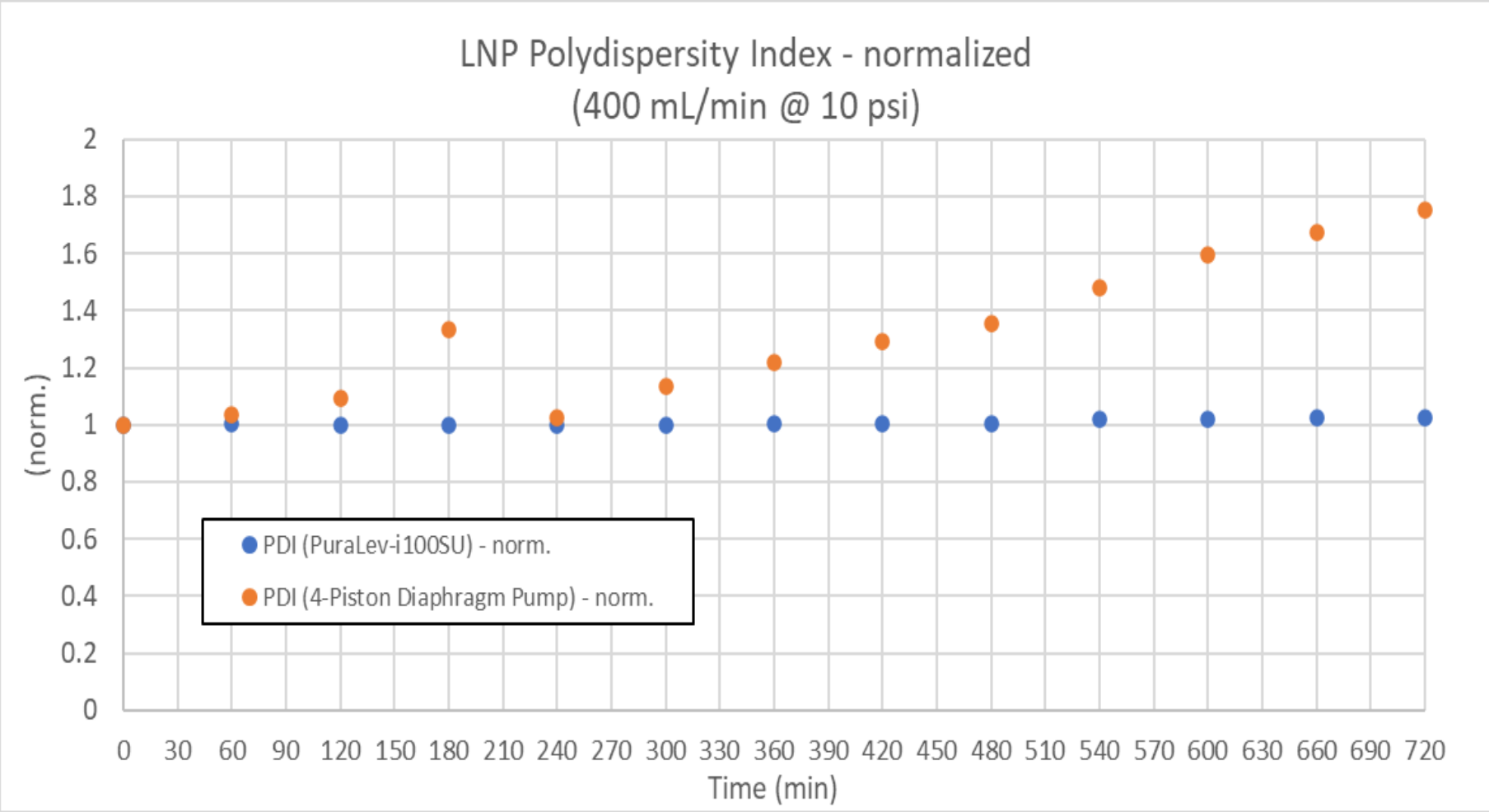

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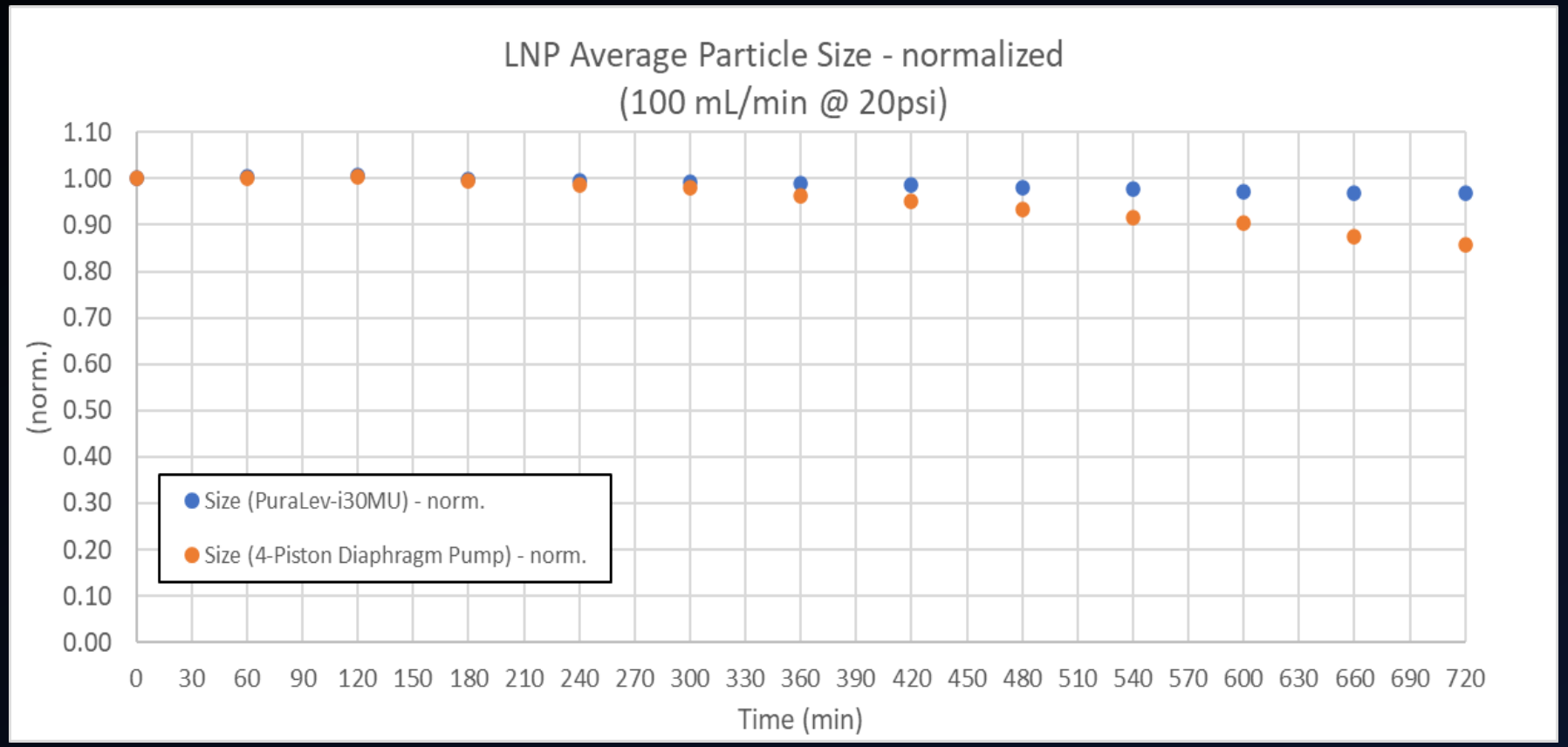

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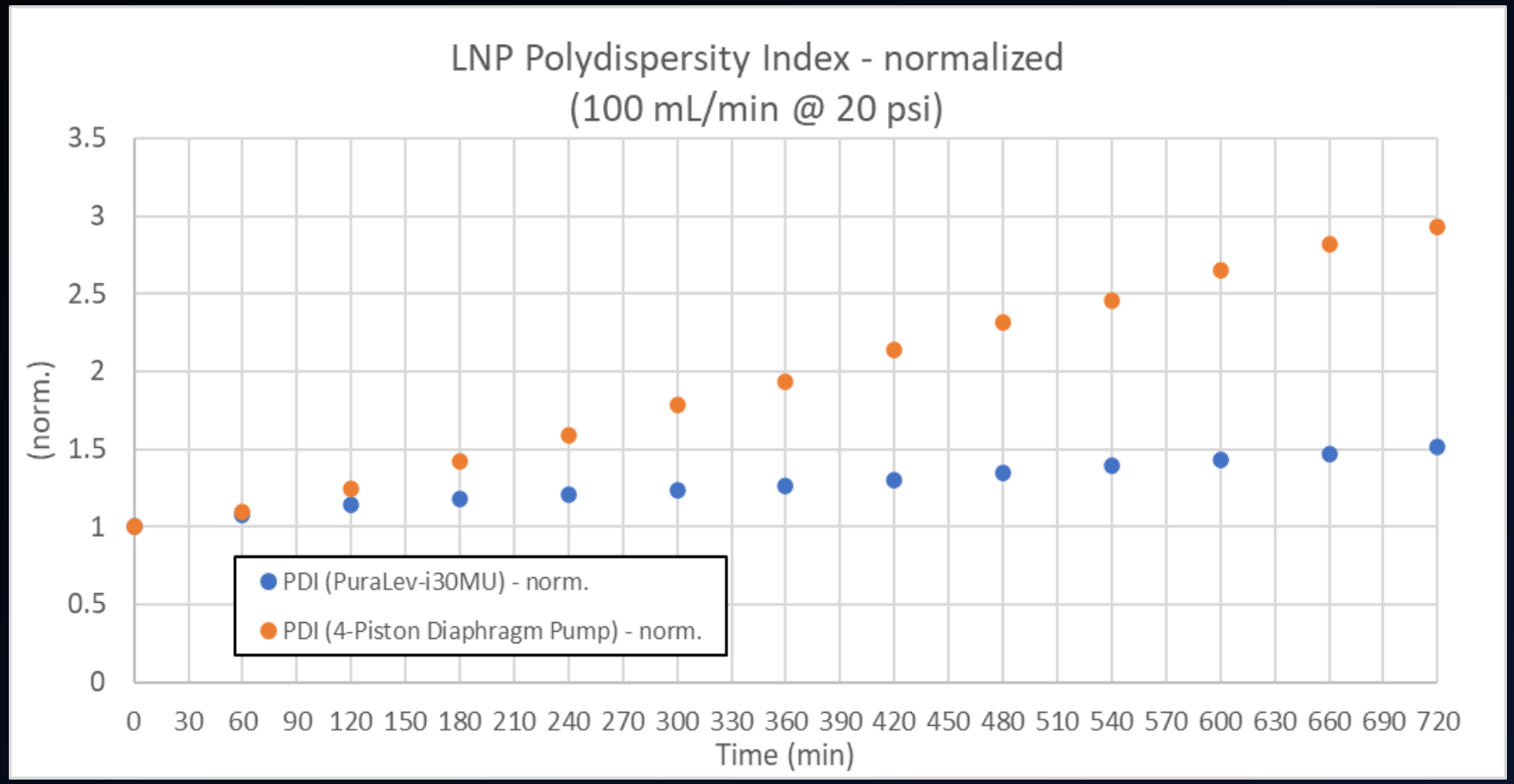

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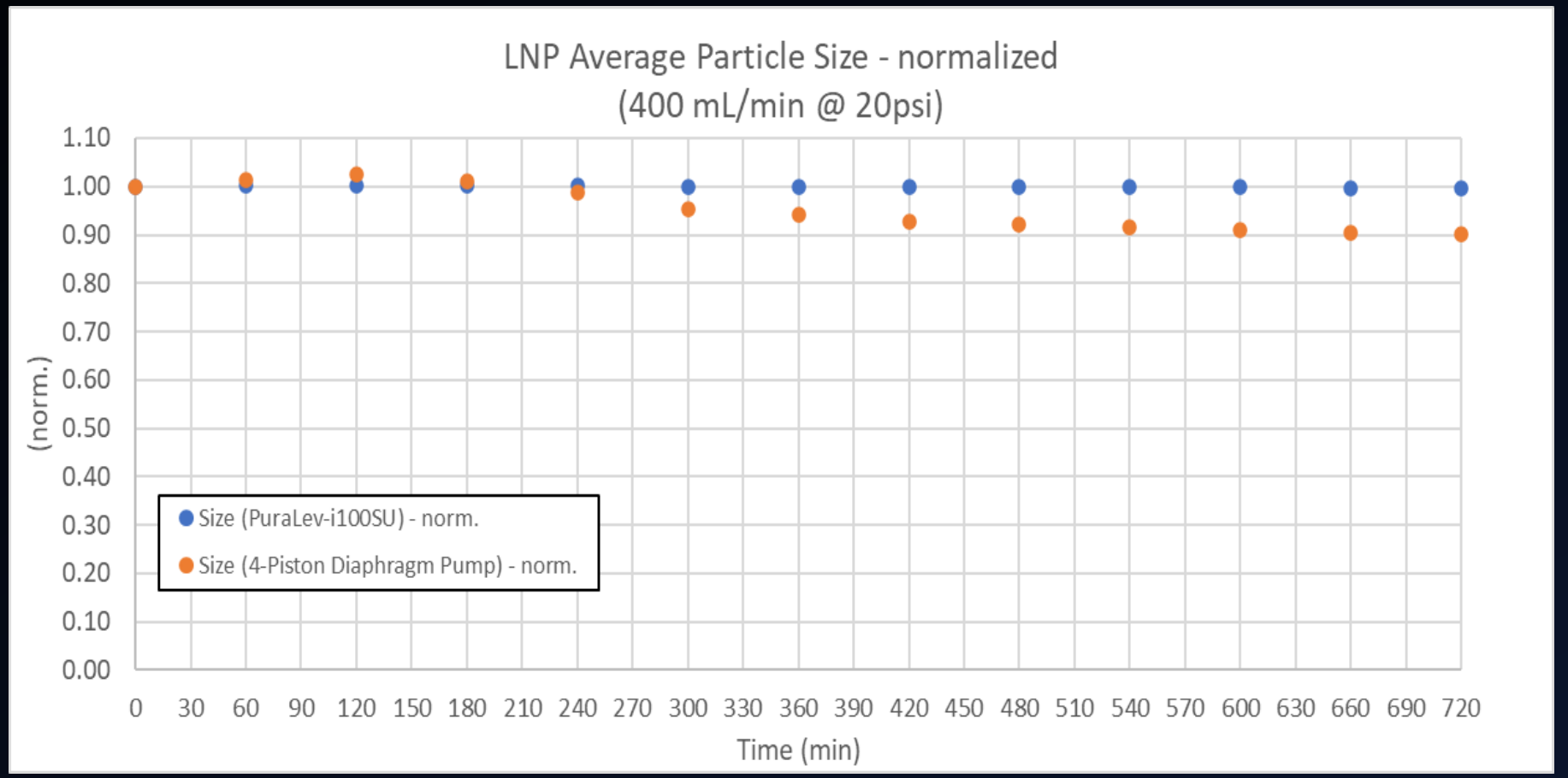

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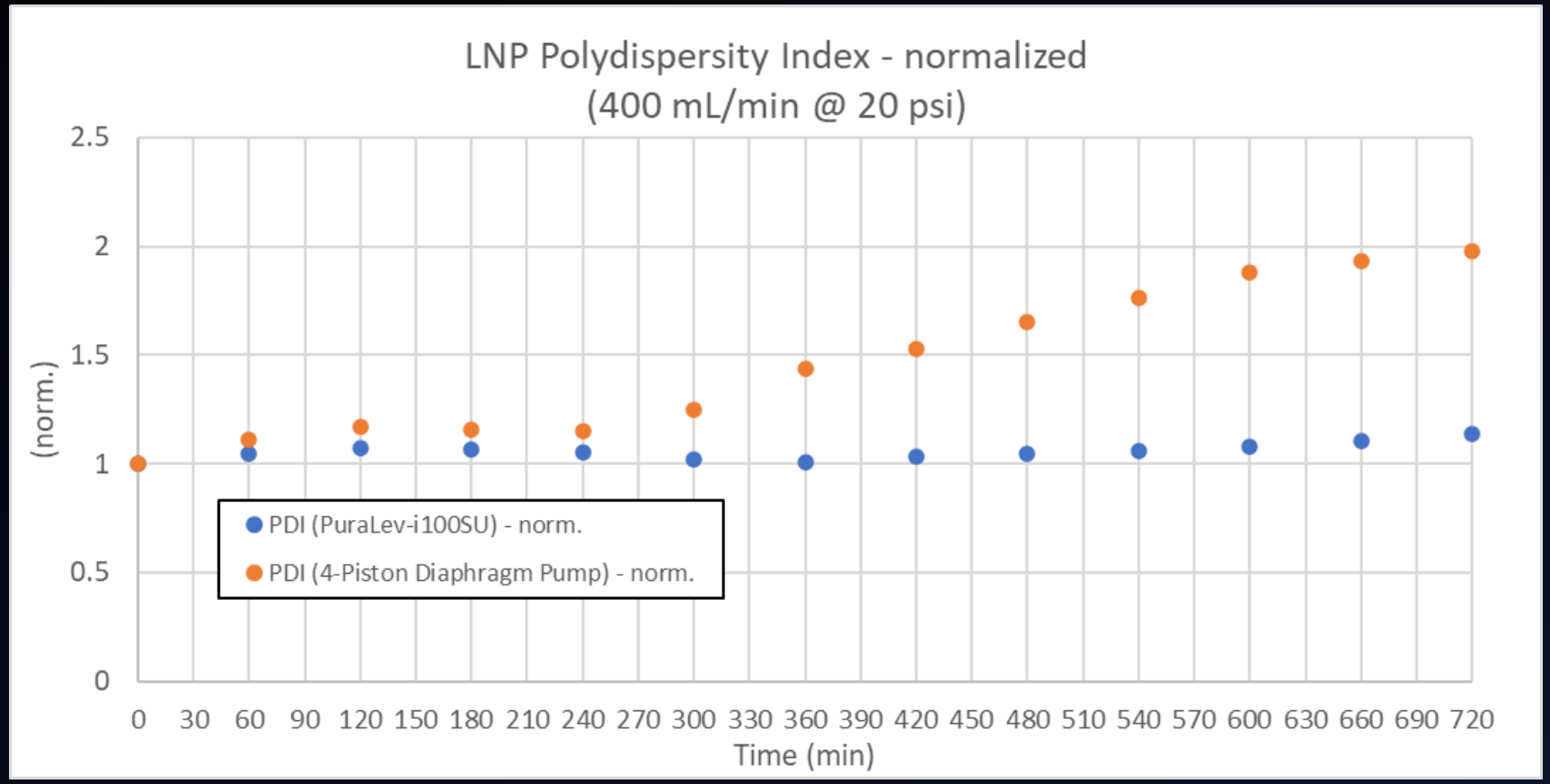

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Test Results (50LPM @ 30PSI)

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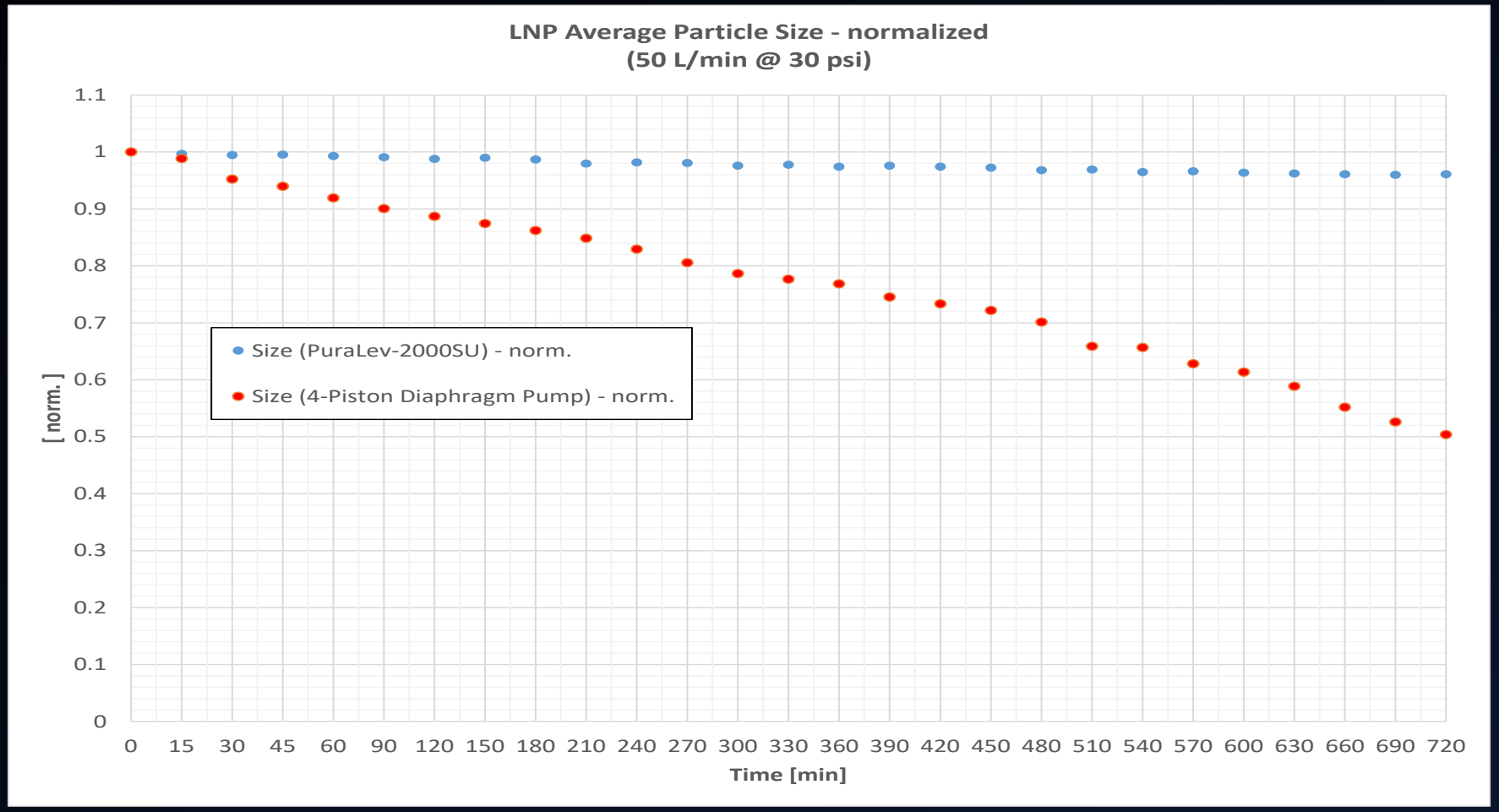

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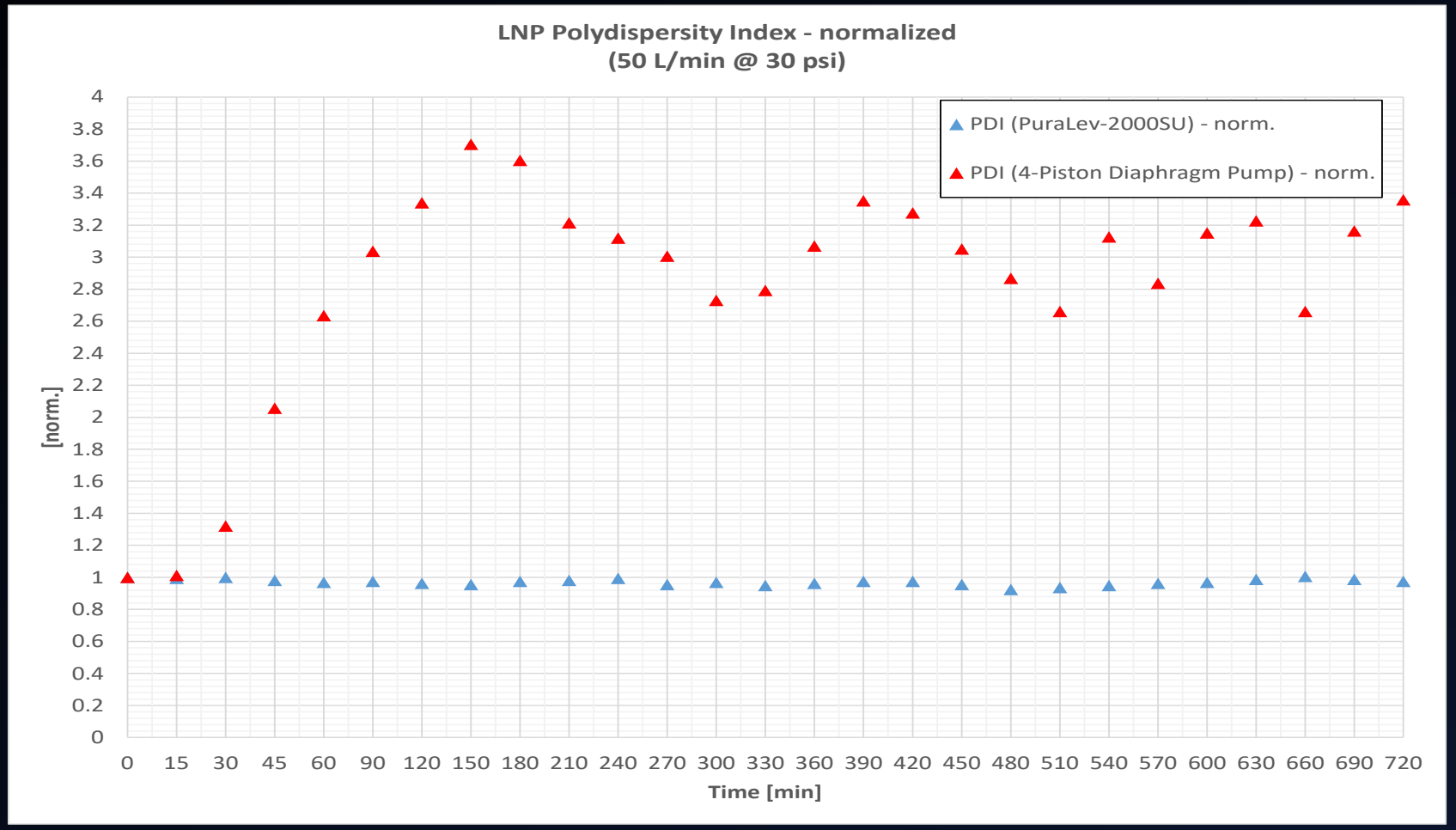

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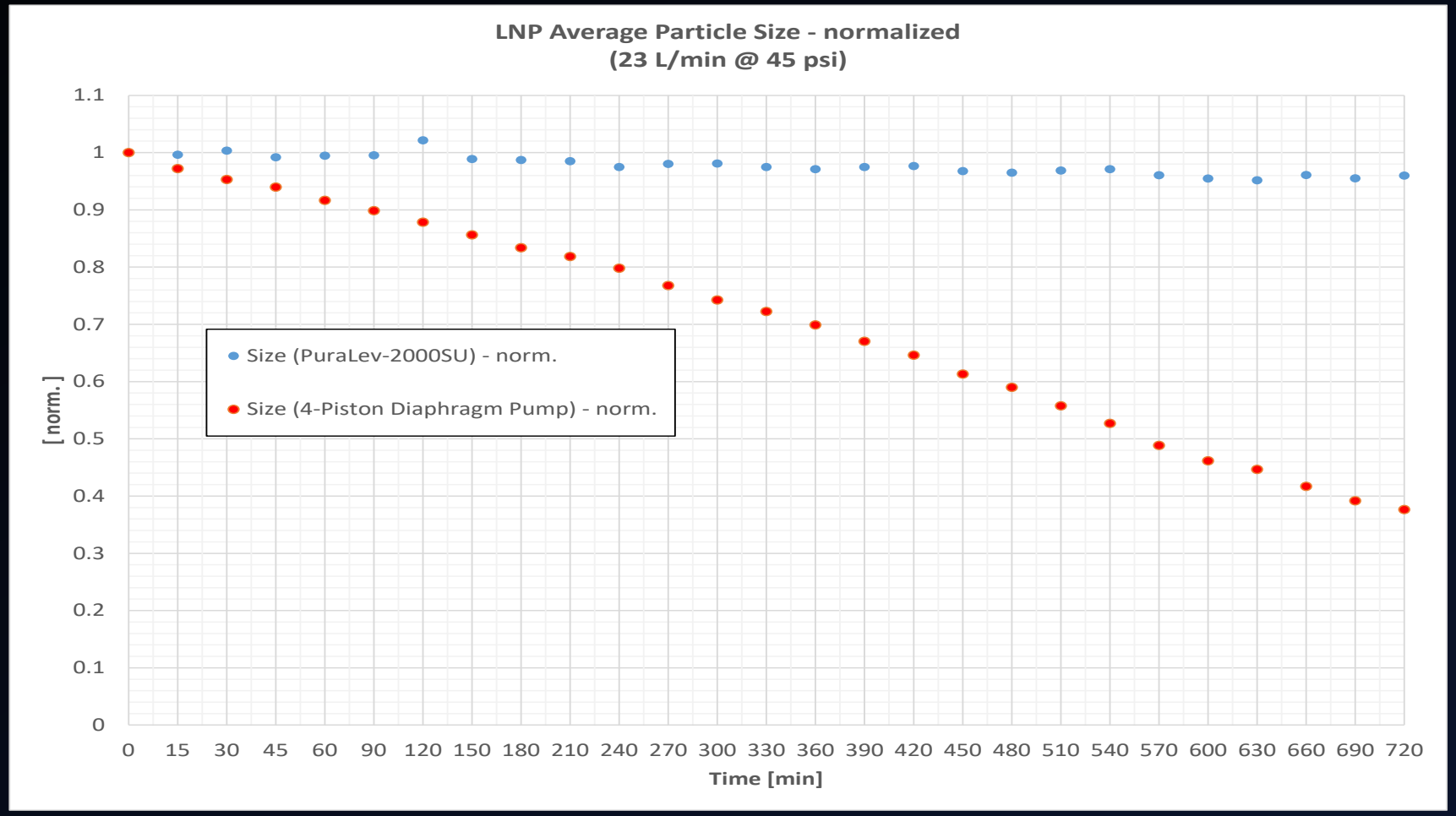
Test results (23LPM @ 45PSI)

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

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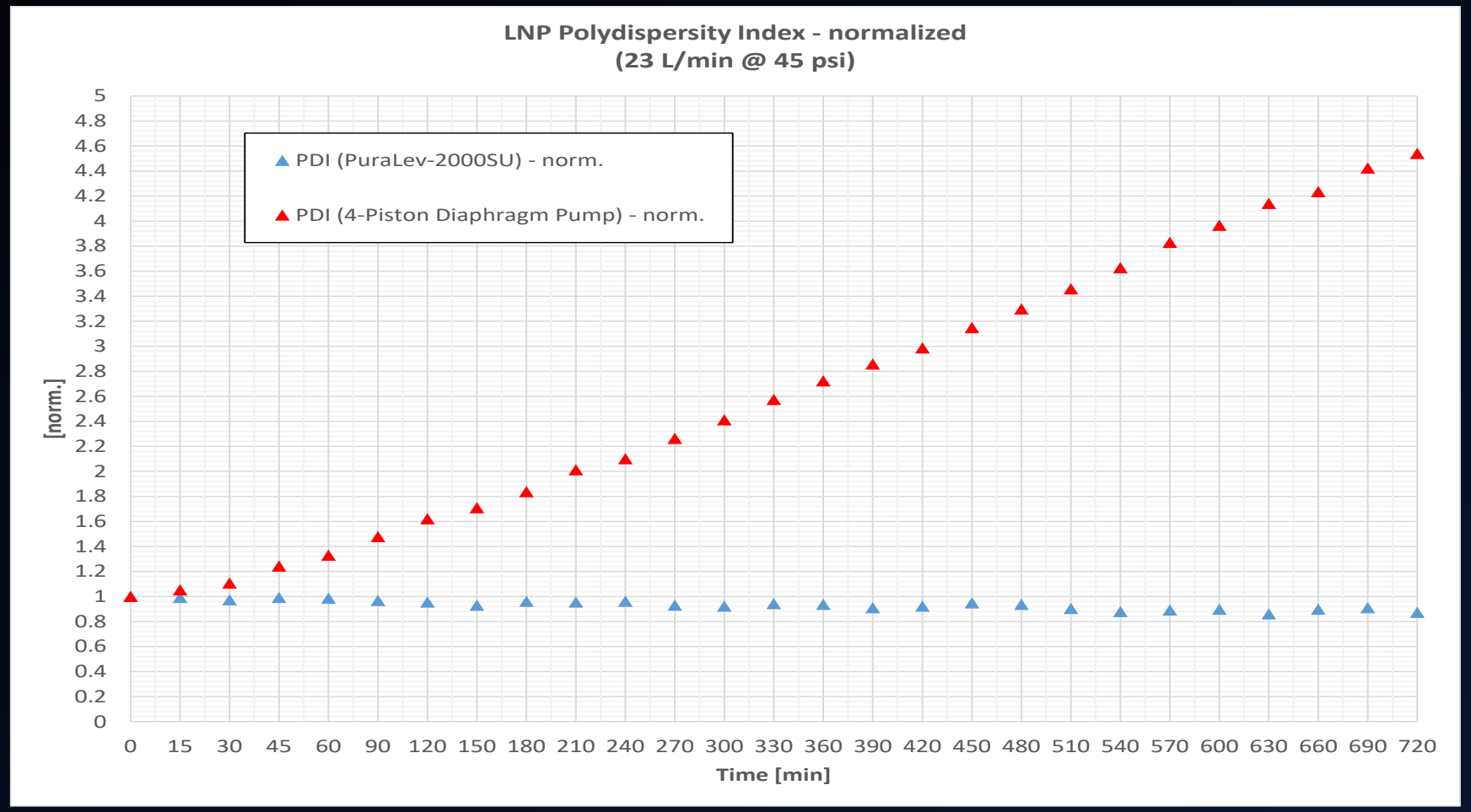

Polydispersity Index (PDI)=

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

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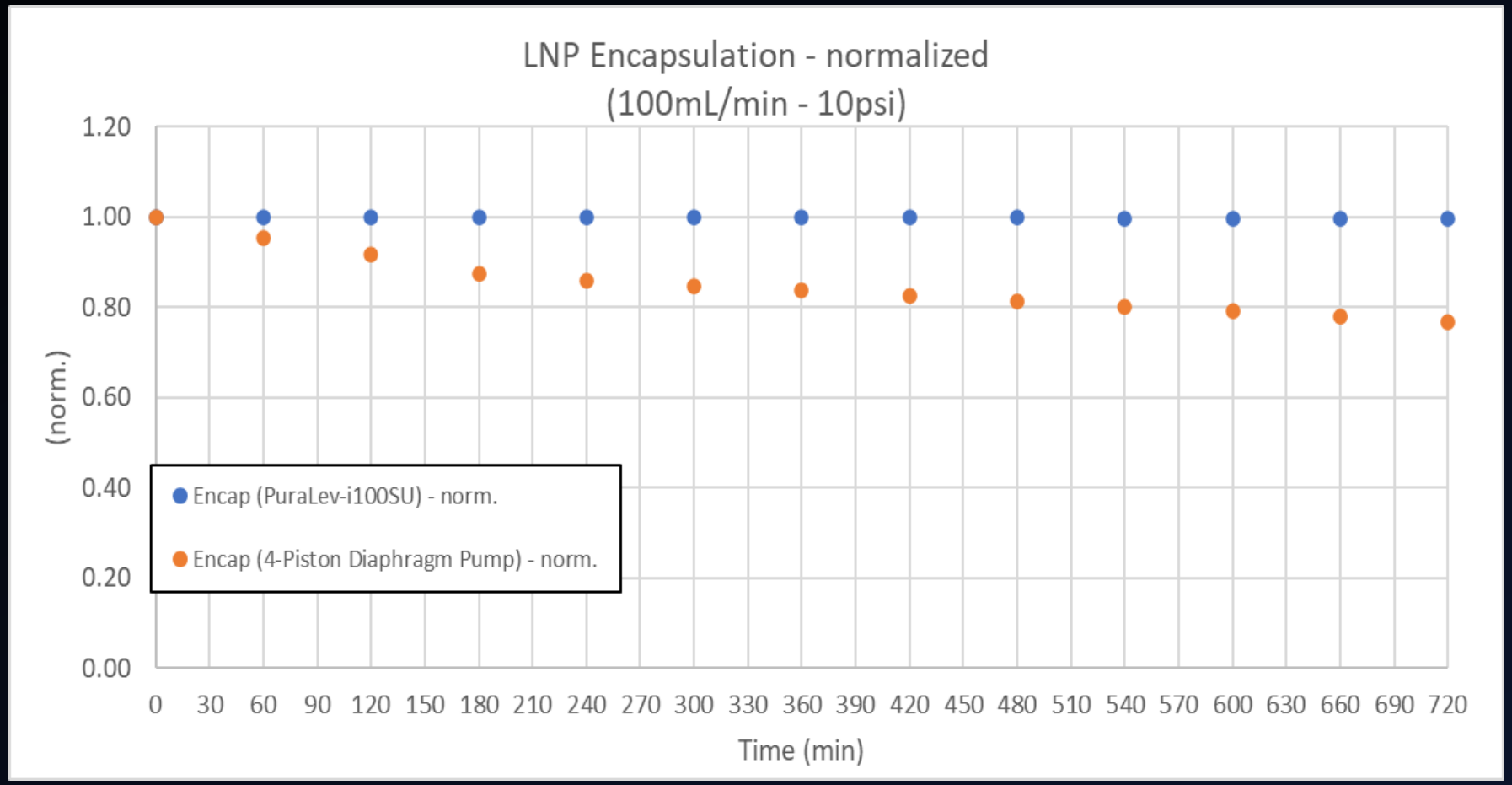
Test results (100ml/min @ 10PSI)

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 (400ml/min @ 10PSI)

LNP Encapsulation

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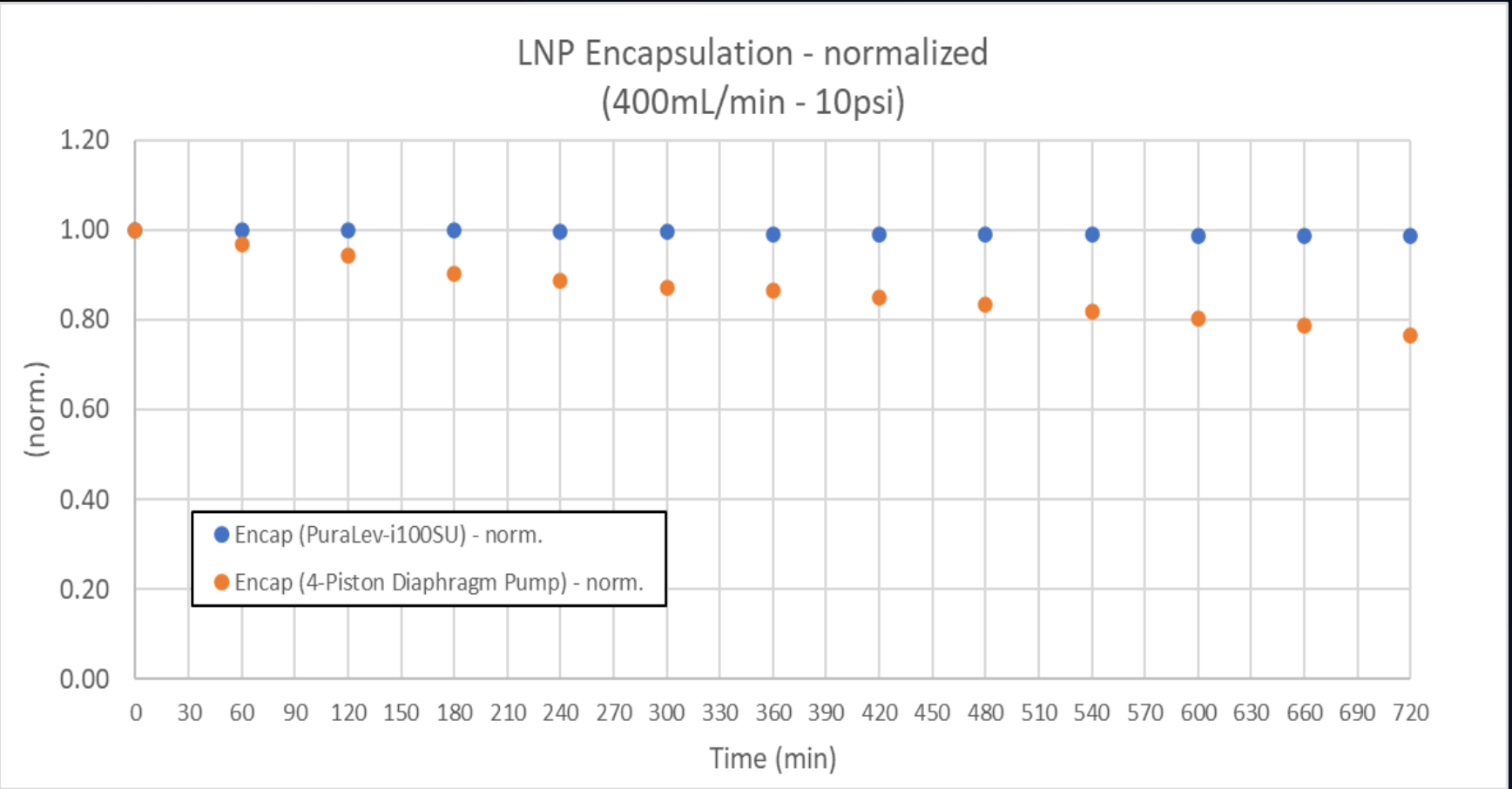

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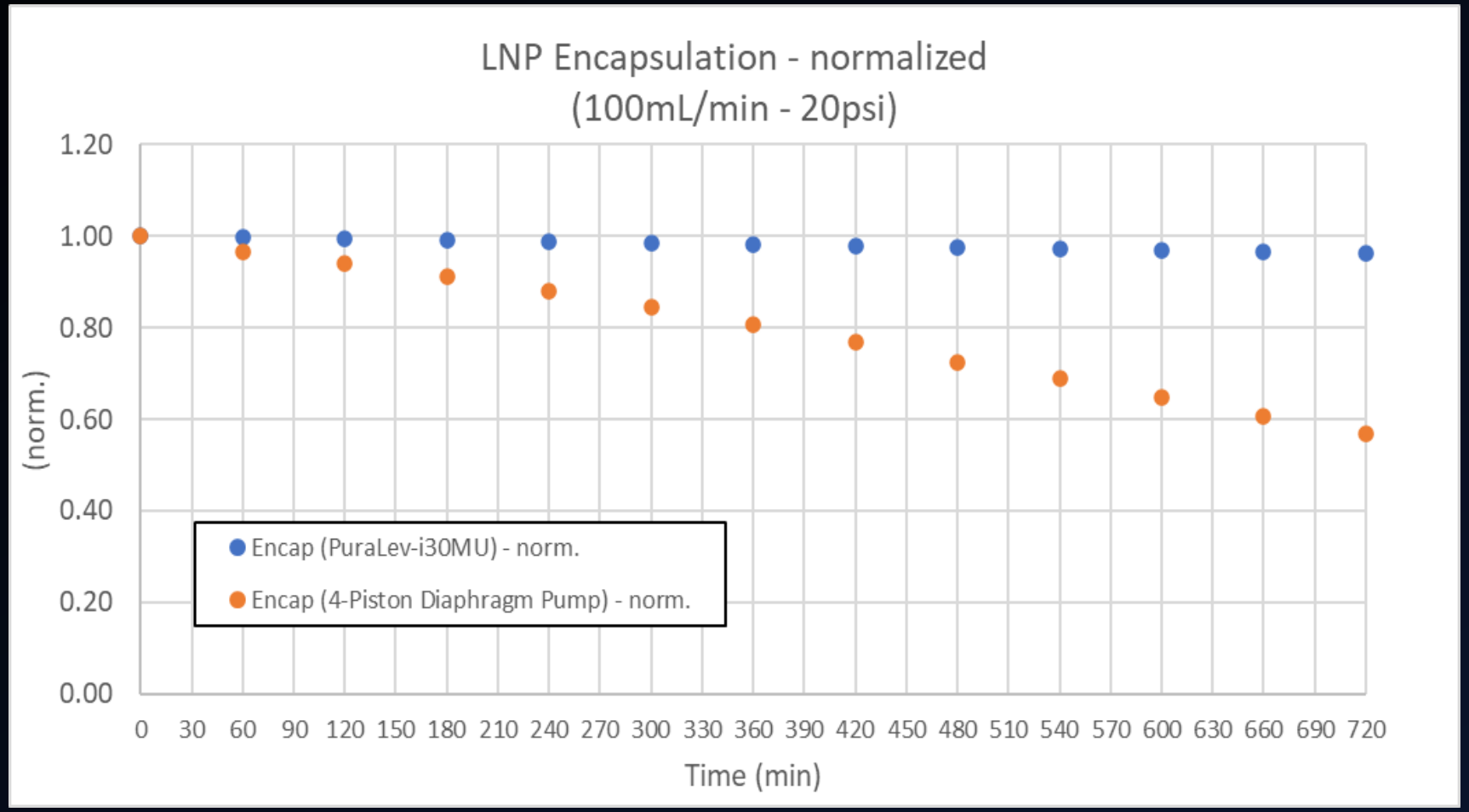

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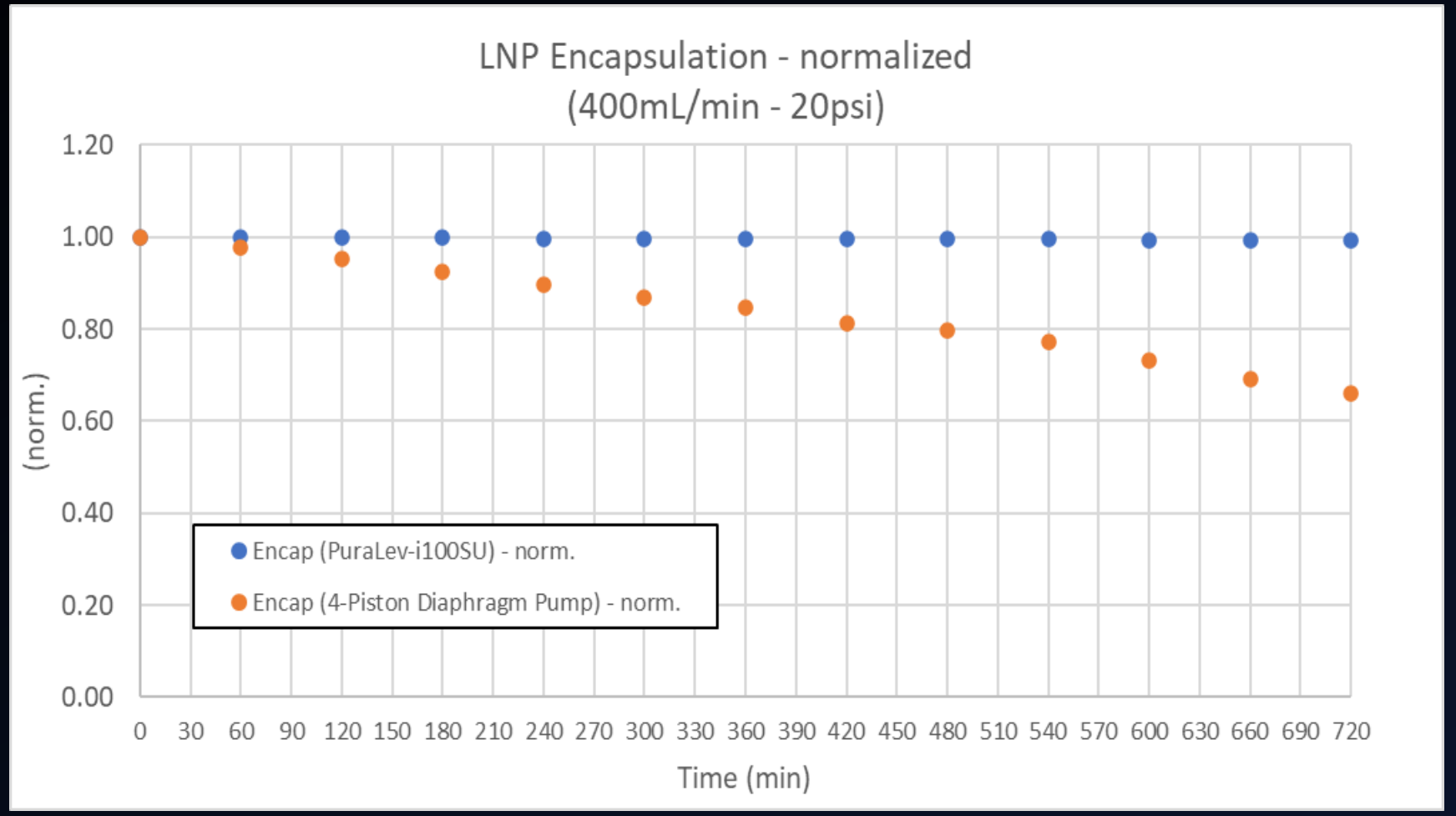

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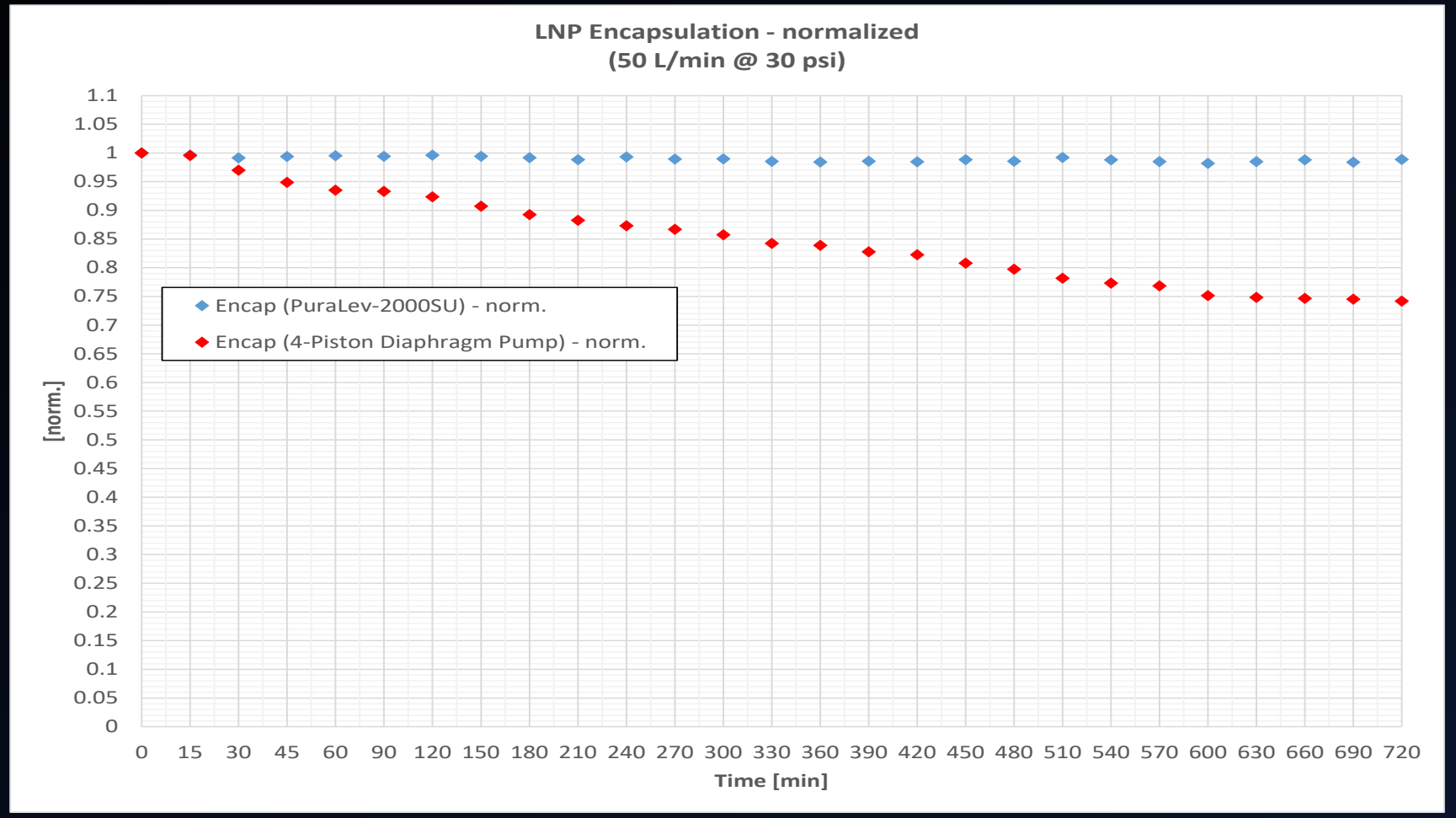

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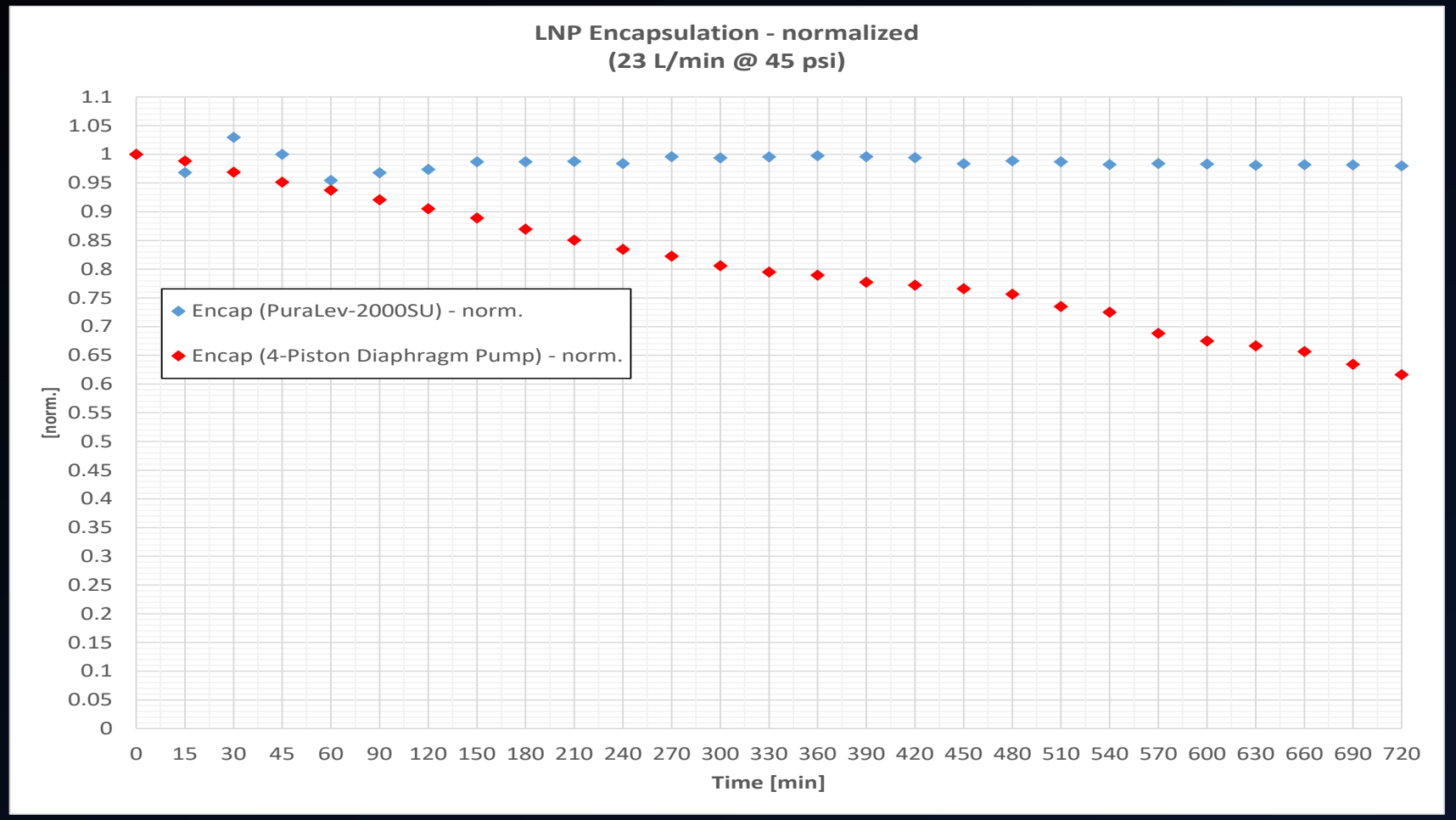

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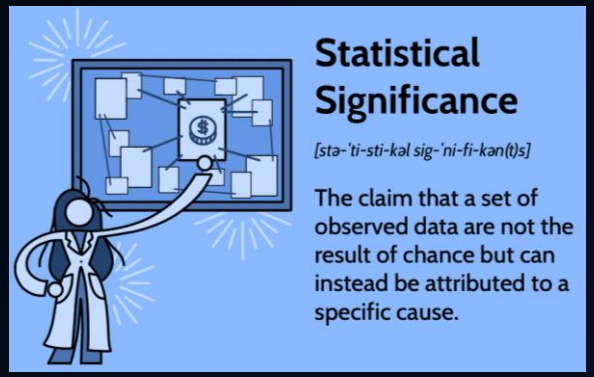
Test Results

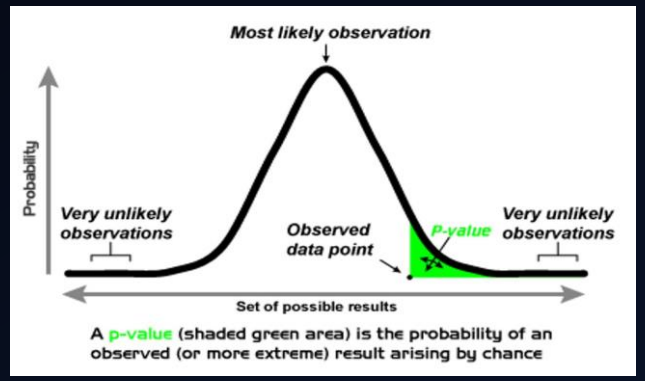
- Side by side comparison testing using two pump technologies (Levitronix Maglev Pump PuraLev-2000SU and 4-Piston Diaphragm Pump) were evaluated. The fluid stream used for test execution contained Lipid Nanoparticles (LNP's) similar to those used in mRNA Vaccine manufacturing unit operations (for example, TFF). The fluid stream was comprised primarily of Phosphate Buffer or Citrate Buffer and EtOH at 18%.

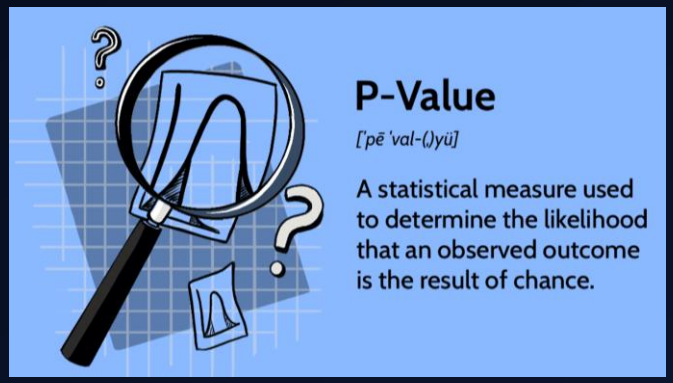
- Pump technology impact to the process stream utilized a fluid stream characterization based on Particle Size, Polydispersity Index (PDI) and mRNA Encapsulation using the following processing parameters:
  - Test Run "A": Flow Rate: 100 ml/min | Pressure: 10 psi (0.69 bar)
  - Test Run "B": Flow Rate: 400ml/min | Pressure: 10 psi (0.69 bar)
  - Test Run "C": Flow Rate: 100ml/min | Pressure: 20 psi (1.38 bar)
  - Test Run "D": Flow Rate: 400ml/min | Pressure: 20 psi (1.38 bar)
  - Test Run "E": Flow Rate: 50 L/min | Pressure: 30 psi (2.07 bar)
  - Test Run "F": Flow Rate: 23 L/min | Pressure: 45 psi (3.10 bar)
  - Duration: 12 Hours
  - Fluid Temperature: 18°C- 25°C

Test Conclusions

- Each of the Levitronix MagLev Pump technologies showed no statistically relevant impact on the LNP fluid streams across all flow rates and pressures during the entirety of the 12-hour test execution. The 4-Piston Diaphragm pumps tested showed a measured and statistically relevant impact to the LNP fluid stream across all of the measured LNP performance parameters.







Questions?