LNP Manufacturing Small Scale T-Mix Pump Comparison

Final Report

Issued to

Levitronix

Conducted by

bioX

Based on Proposal:

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bioX Execution Signature

bioX Role, Title	Print Name	Signature	Date
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1 INTRODUCTION AND SCOPE

bioX conducted two distinct scopes of work using the Levitronix MagLev centrifugal pump technology. The scopes of work were as follows:

Scope #1: Dual Nucleic Acid Payload T-Mix Performance Testing

Using the following three pump types:

- QF30 from Quattroflow
- MasterFlex L/S Peristaltic Pump
- PuraLev I30SU from Levitronix

bioX assessed the LNP encapsulation performance impact of the three pump technologies used in the T-Mix manufacturing unit operation that uses a dual nucleic acid payload. The targeted LNP formation performance criteria included the following:

- Particle Size
- Polydispersity Index (PDI)
- Encapsulation Efficiency
- Zeta Potential (Z-Average)

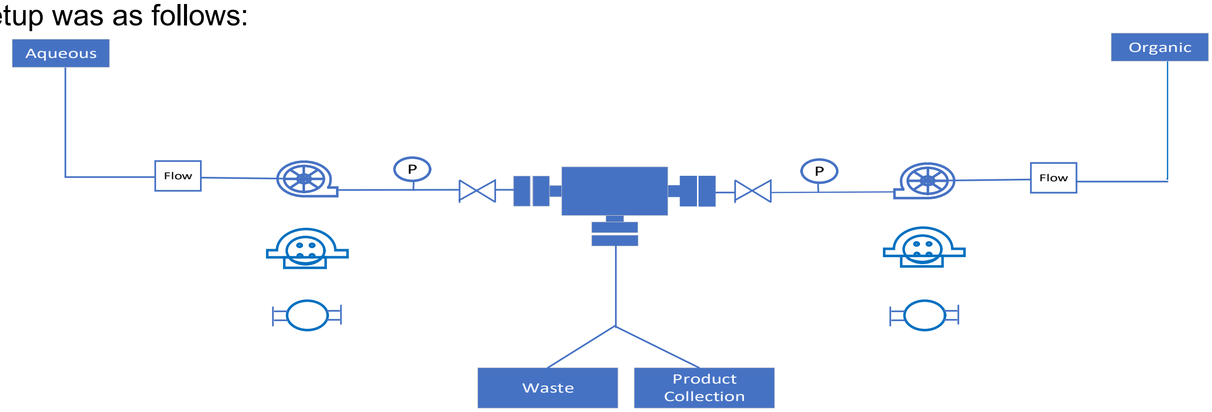
The use of a known LNP manufacturing and encapsulation process was used to execute this testing since it has been well characterized, very repeatable, and thus the impact from changing the pump type was the primary independent variable evaluated.

The following represents the executed testing parameters and performance metrics used in the study:

- Mixing of the Aqueous and Organic liquid stream at a 3:1 Dilution Ratio
- Flow rates of 105 mL/min and 35 ml/min respectively will be tested.
- Target characterization performance targets are as follows post T-Mix:
  - Particle Size: 80nm
  - Polydispersity Index (PDI): <0.20
  - Encapsulation Efficiency: >90%
  - Zeta Potential (Z-Average): -30 to -10

Testing was run in triplicate and thus there were 9 total tests executed. Attachment #1 covers the data generated during the scope of this execution.

T-Mix setup was as follows:



2 TEST EQUIPMENT

All testing will be conducted at the bioX Process Optimization and Development (POD) applications testing lab located in Salem, NH utilized calibrated instrumentation and transmitters and when required, was standardized prior to use. Equipment used was as follows:

- Malvern Zetasizer calibration due date 30Dec24
- Nanodrop UV/Vis Spectrophotometer calibration due date 30Dec24
- Inline Pressure Sensors (Feed, Retentate and Permeate) were zeroed
- Ashcroft Digital Pressure Gauge within calibration due date of 30Oct24

3 PERFORMANCE CRITERIA EXPLAINED

Acceptance criteria for the LNP formulation using the T-mix is based on the following performance characteristics and rationale:

- **Particle Size:** Particle size is a critical test attribute in Lipid Nanoparticle (LNP) manufacturing for several important reasons.
    - Drug Delivery Efficiency:
        - Particle size directly affects the cellular uptake and biodistribution of LNPs.
        - Smaller particles (typically 50-200 nm) are generally more efficient at cellular entry and can better evade the reticuloendothelial system.
    - Stability:
        - Particle size influences the physical stability of the LNP formulation.
        - Consistent particle size helps maintain uniform dispersion and prevents aggregation.
    - Encapsulation Efficiency:
        - Particle size can impact the amount of active pharmaceutical ingredient (API) that can be encapsulated within the LNP.
        - Optimal size ensures maximum drug loading while maintaining stability.
    - Pharmacokinetics and Biodistribution:
        - Size affects how LNPs interact with biological systems, influencing their circulation time and tissue distribution.
        - It can determine whether particles can cross certain biological barriers (e.g., blood-brain barrier).
    - Manufacturing Consistency:
        - Particle size is an indicator of manufacturing process control and reproducibility.
        - Consistent size across batches is crucial for ensuring product quality and performance.
    - Regulatory Compliance:
        - Particle size is a key quality attribute that regulatory bodies like the FDA and EMA closely scrutinize.
        - It's often part of the product specifications and release criteria.
    - Safety Profile:
        - Particle size can influence the toxicity profile of LNPs.
        - Extremely small particles might have unintended interactions with biological systems.
    - Drug Release Kinetics:
        - Particle size can affect the rate at which the encapsulated drug is released from the LNP.
        - This impacts the pharmacological effect and duration of action.
    - Sterilization and Filtration:
        - Particle size is crucial for sterile filtration processes, which typically use 0.2 μm filters.

- Consistent and appropriate size ensures successful sterile filtration without product loss.
- Product Performance:
  - For mRNA vaccines or therapeutics, particle size can affect the efficiency of mRNA delivery to target cells.
  - It impacts the overall efficacy of the LNP-based product.

Given these factors, performance target centers around a narrow size distribution (low polydispersity index) and a target size range that balances cellular uptake, stability, and manufacturing feasibility. Particle size analysis is performed using Dynamic Light Scattering (DLS) at bioX using our Malvern Zetasizer.

- **Polydispersity Index**: The polydispersity index (PDI) is a measure used to describe the degree of non-uniformity of a size distribution of particles, polymers, or molecules. The Polydispersity index (PDI) is a dimensionless number and does not have a specific unit of measure.
  - Range:
    - PDI values typically range from 0 to 1. A PDI of 0 would indicate a perfectly uniform sample (monodisperse). Values closer to 1 indicate a broader size distribution (more polydisperse).
  - Calculation:
    - PDI is often calculated as the square of the standard deviation divided by the mean diameter.
    - PDI = $(\sigma/d)^2$, where σ is the standard deviation and d is the mean diameter.
  - Interpretation:
    - PDI < 0.05: Highly monodisperse
    - $0.05 <$ PDI < 0.08: Nearly monodisperse
    - $0.08 <$ PDI < 0.7: Mid-range polydispersity
    - PDI > 0.7: Very polydisperse

- **Encapsulation Efficiency**: Measuring encapsulation efficiency during Lipid Nanoparticle (LNP) manufacturing is crucial for several important reasons.
  - Drug Loading Quantification:
    - Encapsulation efficiency tells you how much of the active pharmaceutical ingredient (API), such as mRNA or small molecules, is successfully entrapped within the LNPs. It's typically expressed as a percentage of the total API that's encapsulated.
  - Process Optimization:
    - It helps in optimizing the manufacturing process by providing feedback on how well the encapsulation step is working. Allows for adjustments in formulation or process parameters to improve efficiency.
  - Batch-to-Batch Consistency:
    - Monitoring encapsulation efficiency helps ensure consistency across different production batches. It's a critical quality attribute for demonstrating reproducibility in manufacturing.
  - Dose Accuracy:
    - Knowing the encapsulation efficiency is essential for accurate dosing of the final product.
    - It ensures that the intended amount of API is delivered to patients.
  - Product Efficacy:
    - The therapeutic effect of the LNP formulation is directly related to how much API is encapsulated and delivered to target cells.
    - Low encapsulation efficiency could result in reduced efficacy.
  - Cost Effectiveness:
    - APIs, especially mRNA, can be expensive.

- High encapsulation efficiency means less waste of these costly materials. It impacts the overall economics of the manufacturing process.
    - Stability Indication:
        - Changes in encapsulation efficiency over time can be an indicator of product instability.
        - It's often monitored as part of stability studies.
    - Regulatory Compliance:
        - Encapsulation efficiency is typically a key specification that needs to be reported to regulatory agencies. It's often a part of the product's critical quality attributes.
    - Formulation Development:
        - During early-stage development, it helps in selecting the best lipid composition and ratios for optimal encapsulation.
    - Release Testing:
        - It's often part of the release criteria for batch approval before the product can be distributed.
    - Process Troubleshooting:
        - If encapsulation efficiency drops unexpectedly, it can signal issues in the manufacturing process that need to be addressed.
    - Prediction of In Vivo Performance:
        - Encapsulation efficiency can give insights into how the product might perform in biological systems.

bioX deploys the following measurement techniques for encapsulation efficiency:

- Separating free API from encapsulated API (e.g., through centrifugation or gel filtration)
- Quantifying the encapsulated API (e.g., through spectrophotometry, fluorescence, or HPLC)
- For nucleic acids, techniques like RiboGreen assay or qPCR might be used

The target encapsulation efficiency can vary based on the specific LNP formulation and application, but generally, higher efficiencies (often >80-90%) are desirable for most pharmaceutical applications.

- **Zeta Potential** Zeta potential is a crucial parameter to measure during Lipid Nanoparticle (LNP) manufacturing for several important reasons.
    - Colloidal Stability:
        - Zeta potential is a key indicator of the stability of colloidal systems like LNPs.
        - Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are considered stable.
    - Aggregation Prediction:
        - It helps predict the likelihood of particle aggregation over time.
        - LNPs with zeta potentials close to neutral (between -10 mV and +10 mV) are more prone to aggregation.
    - Shelf-Life Estimation:
        - Zeta potential measurements can help in predicting the shelf life of the LNP formulation.
        - Stable zeta potential often correlates with longer shelf lives.
    - Surface Charge Characterization:
        - It provides information about the surface charge of the LNPs.
        - This is crucial for understanding how LNPs will interact with biological systems.
    - Batch-to-Batch Consistency:
        - Zeta potential is used as a quality control parameter to ensure consistency between different manufacturing batches.

- Formulation Optimization:
  - During development, zeta potential measurements help in optimizing the LNP formulation.
  - It can guide adjustments in lipid composition or ratios.
- Cellular Uptake Prediction:
  - The surface charge of LNPs can influence their cellular uptake.
  - Generally, slightly negative charges are preferred for most applications.
- Drug Release Kinetics:
  - Zeta potential can affect how the encapsulated drug is released from the LNPs.
  - It may influence the interaction with target tissues and cells.
- Stealth Properties:
  - For LNPs designed to evade the immune system, zeta potential is crucial.
  - Near-neutral charges often contribute to "stealth" properties.
- Process Control:
  - Changes in zeta potential during manufacturing can indicate issues in the production process.
  - It's a sensitive indicator of changes in particle surface properties.
- Regulatory Compliance:
  - Zeta potential is often part of the critical quality attributes required by regulatory agencies.
  - It's typically included in batch release specifications.
- Prediction of In Vivo Behavior:
  - Zeta potential can give insights into how LNPs might behave in biological fluids.
  - It can affect protein corona formation and biodistribution.
- Storage Condition Optimization:
  - Zeta potential measurements can help in determining optimal storage conditions for the LNP formulation.
- Functionalization Verification:
  - If LNPs are functionalized (e.g., with targeting ligands), zeta potential can confirm successful modification.

Measurement of zeta potential is typically done using electrophoretic light scattering techniques. At bioX we utilize our Malvern Zetasizer. The specific value considered optimal can vary depending on the LNP application, but generally, a moderate negative charge (e.g., -10 to -30 mV) is often desirable for many pharmaceutical applications.

It's worth noting that zeta potential can be affected by factors like pH, ionic strength of the medium, and the presence of any adsorbed molecules on the LNP surface.

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4 TEST EXECUTION DATA

4.1 Dual Nucleic Acid Payload T-Mix Performance Testing

Phase 1 Testing: Dual Nucleic Acid Payload T-Mix Performance Testing

Levitronix

Levitronix i30SU Centrifugal Pump		
Process Condition	**Value**	**UOM**
T-Mix Dimension	0.03	Inch
Reynolds Number	3909	Re
Liinear Velocity	16.8	ft/sec
Dilution Ratio	3:1	N/A
Organic Volume	70.0	ml
Organic Flow Rate	35.0	ml/min
Aqueous Volume	315.0	ml
Aqueous Flow Rate	105	ml/min
Particle Size Range	80-90	nm
PDI Target	<0.20	N/A
Encapsulation Efficiency	>80	%
Zeta Potential Range	-10 to -30	mV

Levitronix Test #1 Output		
Test Attribute	**Value**	**UOM**
Avg Particle Size	82	nm
PDI	0.14	N/A
Encapsulation Efficiency	91	%
Zeta Potential	-26	mV

Levitronix Test #2 Output		
Test Attribute	**Value**	**UOM**
Avg Particle Size	85	nm
PDI	0.13	N/A
Encapsulation Efficiency	92	%
Zeta Potential	-18	mV

Levitronix Test #3 Output		
Test Attribute	**Value**	**UOM**
Avg Particle Size	82	nm
PDI	0.13	N/A
Encapsulation Efficiency	89	%
Zeta Potential	-22	mV

Quattroflow

Quattroflow QF30 Quaternary Diaphragm Pump		
Description	**Value**	**UOM**
T-Mix Dimension	0.03	Inch
Reynolds Number	3909	Re
Liinear Velocity	16.8	ft/sec
Dilution Ratio	3:1	N/A
Organic Volume	70.0	ml
Organic Flow Rate	35.0	ml/min
Aqueous Volume	315.0	ml
Aqueous Flow Rate	105	ml/min
Particle Size Range	80-90	nm
PDI Target	<0.20	N/A
Encapsulation Efficiency	>80	%
Zeta Potential Range	-10 to -30	mV

Quattroflow Test #1 Output		
Test Attribute	**Value**	**UOM**
Avg Particle Size	91	nm
PDI	0.21	N/A
Encapsulation Efficiency	84	%
Zeta Potential	-10	mV

Quattroflow Test #2 Output		
Test Attribute	**Value**	**UOM**
Avg Particle Size	88	nm
PDI	0.23	N/A
Encapsulation Efficiency	80	%
Zeta Potential	-12	mV

Quattroflow Test #3 Output		
Test Attribute	**Concentration (g/L)**	**Particle Size (kDa)**
Avg Particle Size	94	nm
PDI	0.22	N/A
Encapsulation Efficiency	82	%
Zeta Potential	-16	mV

Masterflex L/S Peristaltic Pump

Masterflex L/S Peristaltic Pump		
Description	**Value**	**UOM**
T-Mix Dimension	0.03	Inch
Reynolds Number	3909	Re
Liinear Velocity	16.8	ft/sec
Dilution Ratio	3:1	N/A
Organic Volume	70.0	ml
Organic Flow Rate	35.0	ml/min
Aqueous Volume	315.0	ml
Aqueous Flow Rate	105	ml/min
Particle Size Range	80-90	nm
PDI Target	<0.20	N/A
Encapsulation Efficiency	>80	%
Zeta Potential Range	-10 to -30	mV

Masterflex L/S Peristaltic Pump Test #1 Output		
Test Attribute	**Value**	**UOM**
Avg Particle Size	102	nm
PDI	0.26	N/A
Encapsulation Efficiency	72	%
Zeta Potential	4	mV

Masterflex L/S Peristaltic Pump Test #2 Output		
Test Attribute	**Value**	**UOM**
Avg Particle Size	97	nm
PDI	0.32	N/A
Encapsulation Efficiency	72	%
Zeta Potential	6	mV

Masterflex L/S Peristaltic Pump Test #3 Output		
Test Attribute	**Concentration (g/L)**	**Particle Size (kDa)**
Avg Particle Size	96	nm
PDI	0.28	N/A
Encapsulation Efficiency	74	%
Zeta Potential	-5	mV

6 CONCLUSIONS

The results of the testing demonstrated a statistically significant, and measurable performance benefit when using the Levitronix pumps to provide the motive force for the T-mix unit operation used in LNP manufacturing when compared to MasterFlex L/S Peristaltic Pump and the Quattroflow QF30 quaternary diaphragm pumps.

The benefit of using the Levitronix i30SU in the configuration and formulation tested by bioX was demonstrated through the stability of the particle size, encapsulation efficiency and the average particle size. The Levitronix i30SU pump performance, when compared to the other pump options tested, demonstrated superior stability during the T-mix unit operation.