HELIX BIOTECH

pDNA Lipid Nanoparticle Formulation and Screening using Nova IJM

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Abstract

DNA Lipid Nanoparticles (LNPs) have emerged as a promising platform for gene therapy and gene editing with potential applications in a wide range of diseases and conditions. LNPs protect nucleic acids like RNA and DNA from degradation and facilitate their uptake into cells. While RNA LNPs were proven through the rollout and scaleup of Moderna and Pfizer vaccines for COVID-19, DNA LNP encapsulation is less common, in part due to the challenges posed by DNA's bulkier size and the limited availability of proven manufacturing platforms. To address these limitations, we have developed a new LNP manufacturing platform - the Nova Impinged Jet Mixing (IJM) platform. Our platform offers faster and more efficient production, greater control over the manufacturing configuration, and better scalability than existing platforms. In this article, we will provide a detailed overview of DNA LNP production, including small sample screening and process optimization, using our novel Nova Benchtop (BT) platform. We will also discuss the key advantages of our platform, including its wide range of sample volumes, flow rates, and modularity for variable system design.

Introduction

DNA Lipid Nanoparticles, or DNA LNPs, have emerged as a promising platform for gene therapy and gene editing, with potential applications in a wide range of diseases and conditions. These spherical nanoparticles containing ionizable and structural lipids protect nucleic acids, like RNA and DNA, from degradation in the extracellular environment and inside cells, and facilitate their uptake into cells, allowing for less disruptive and more efficient delivery of genetic material. While RNA LNP encapsulation has been successful in clinical trials, DNA LNP encapsulation is less common, in part due to the challenges posed by DNA's bulkier size and the limited availability of proven manufacturing platforms suitable for DNA LNP synthesis.

LNPs facilitate the uptake of nucleic acid payloads such as RNA or DNA into cells via endosomal envelopment. Successful translation in vivo involves release of nucleic acid from the LNP and endosome. Once RNA is released into the cell, naturally occurring ribosomes, amino acids, and translation machinery translate the RNA to synthesize the coded protein of interest. DNA that is released from LNPs and escapes the endosome may be transcribed to produce functional RNA or RNA that is further translated to produce proteins. As compared to RNA, plasmid DNA delivery can result in extended transgene expression. Although clinical success for encapsulating RNA in LNPs is widely known following the COVID-19 vaccine rollout, DNA is bulkier than RNA and DNA LNP encapsulation is less common/practiced. Further, there are still a limited number of technology platforms that can be used to screen, optimize, and scale-up DNA LNP formulations.

To address limitations in the availability of proven LNP manufacturing techniques, we have developed a new LNP manufacturing platform - the Nova Impinged Jet Mixing (IJM) platform. Our platform offers faster and more efficient production, greater control over the manufacturing configuration and process via modular plug-and-play design, and better scalability than existing platforms. In this article, we will provide a detailed overview of DNA LNP production, including small sample screening and process optimization, using our novel Nova Benchtop (BT) platform.

Nova Benchtop IJM Nanoparticle Manufacturing System

- Reusable IJM
- Easy to Clean
- Fully Automated Production
- Versatile Modular Platform
- Scalable for GMP
- 1-60mL Syringe Volume
- ≤1mL to ≥100mL Sample Volume

Results

PEG Concentration Analysis

The effect of varying PEG content in an ionizable LNP+DNA formulation was evaluated with samples including 0.5, 1.5, 2.5, and 3.5 mol% PEG-DMG. While PEG content is known to be a strong regulator of LNP clearance and repeat dosing effects in vivo, there was no statistically significant difference across the PEG content levels evaluated. All of the LNPs displayed low particle size (<90nm) and PDI (<0.2).

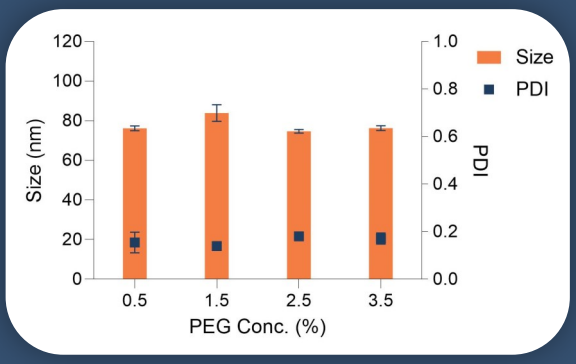


Figure 1. Size and PDI of LNPs made with the Nova BT IJM with varying PEG concentrations.

Formulation Parameters	
PEG conc.	Varied, tested
Lipid Type	DLin-MC3-DMA
N/P Ratio	6
Lipid Mix Concentrations	6.25 mM
Flow Rate Ratio	3
Total Flow Rate	15 mL/min
Formulation Buffer Content	100mM Sodium Acetate

Lipid Type Analysis

The effect of varying ionizable or cationic lipid type in a typical 4-component LNP+DNA formulation was evaluated with samples including DOTAP or DOTMA (cationic), or DODAP, DODMA, ALC-0315, or SM-102 (ionizable).

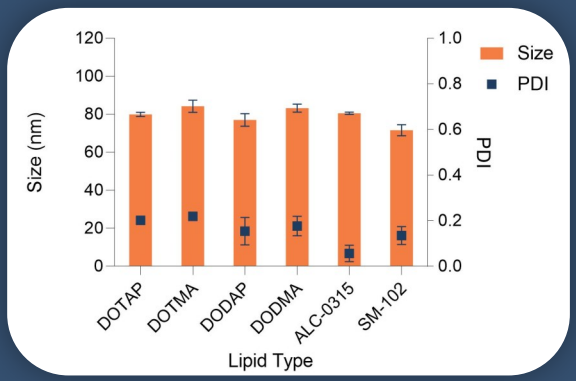


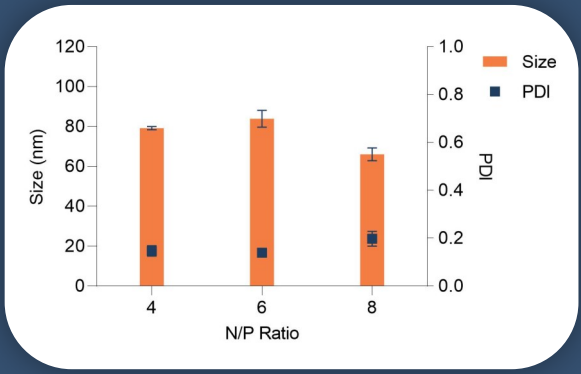
Figure 2. Size and PDI of LNPs with varying lipid types.

Formulation Parameters	
PEG conc.	1.5%
Lipid Type	Varied, tested
N/P Ratio	6
Lipid Mix Concentrations	6.25 mM
Flow Rate Ratio	3
Total Flow Rate	15 mL/min
Formulation Buffer Content	100mM Sodium Acetate

Results

N/P Analysis

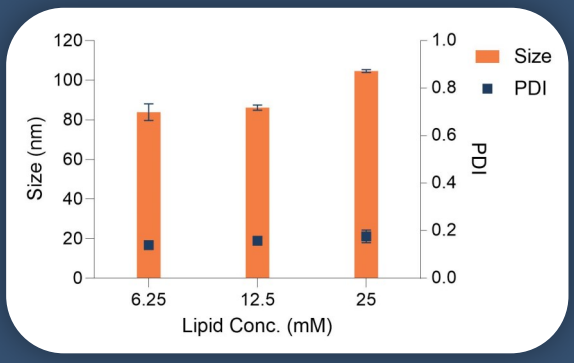
The effect of varying N/P ratio in an ionizable LNP+DNA formulation was evaluated with samples formulated at N/P ratios of 4, 6 , and 8.



Formulation Parameters	
PEG conc.	1.5%
Lipid Type	DLin-MC3-DMA
N/P Ratio	Varied, Tested
Lipid Mix Concentrations	6.25 mM
Flow Rate Ratio	3
Total Flow Rate	15 mL/min
Formulation Buffer Content	100mM Sodium Acetate

Figure 3. Size and PDI of LNPs made with the Nova BT IJM with varying N/P Ratios.

Lipid Conc. Analysis



Formulation Parameters	
PEG conc.	1.5%
Lipid Type	DLin-MC3-DMA
N/P Ratio	6
Lipid Mix Concentrations	Varied, Tested
Flow Rate Ratio	3
Total Flow Rate	15 mL/min
Formulation Buffer Content	100mM Sodium Acetate

Figure 4. Size and PDI of LNPs made with varying lipid concentrations.

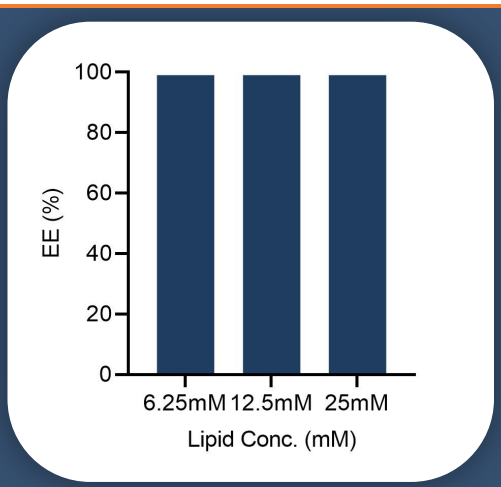


Figure 5. Encapsulation Efficiency (EE%) of LNPs with varying lipid concentrations.

Results

Flow Rate Ratio Analysis

The effect of varying FRR on an ionizable LNP+DNA formulation was evaluated with samples formulated at flow rate ratios of 3, 4, and 5.

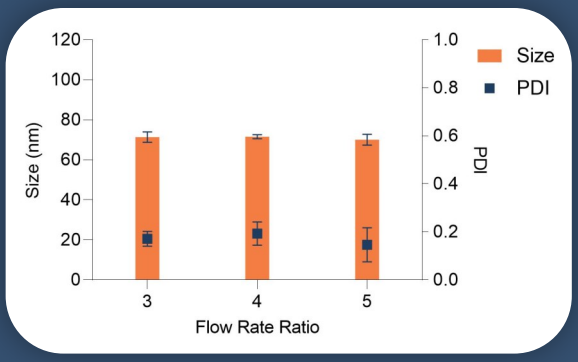


Figure 6. Size and PDI of LNPs made with the Nova BT IJM with varying Flow Rate Ratios.

Formulation Parameters	
PEG conc.	1.5%
Lipid Type	DLin-MC3-DMA
N/P Ratio	6
Lipid Mix Concentrations	6.25 mM
Flow Rate Ratio	Varied, Tested
Total Flow Rate	15 mL/min
Formulation Buffer Content	100mM Sodium Acetate

Total Flow Rate Analysis

The effect of varying TFR on an ionizable LNP+DNA formulation was evaluated with samples formulated at total flow rates of 5, 10, 15, and 20 mL/min.

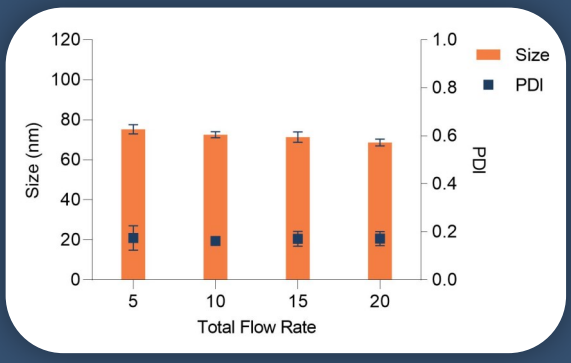
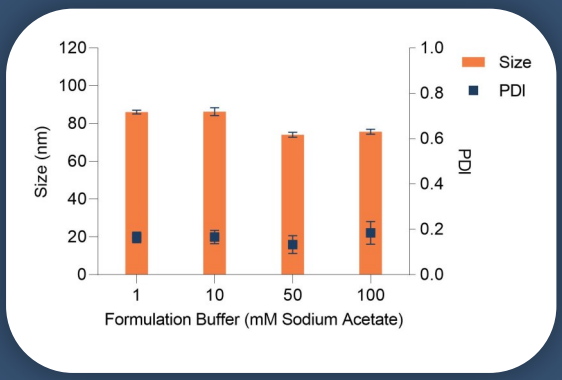


Figure 7. Size and PDI of LNPs made with varying Total Flow Rates.

Formulation Parameters	
PEG conc.	1.5%
Lipid Type	DLin-MC3-DMA
N/P Ratio	6
Lipid Mix Concentrations	6.25 mM
Flow Rate Ratio	3
Total Flow Rate	Varied, Tested
Formulation Buffer Content	100mM Sodium Acetate

Formulation Buffer Analysis

The effect of formulation buffer concentration on ionizable LNP+DNA formulation was evaluated with samples formulated using 1, 10, 50, and 100mM sodium acetate (pH4).



Formulation Parameters	
PEG conc.	1.5%
Lipid Type	DLin-MC3-DMA
N/P Ratio	6
Lipid Mix Concentrations	6.25 mM
Flow Rate Ratio	3
Total Flow Rate	15 mL/min
Formulation Buffer Content	Varied, Tested

Figure 8. Size and PDI of LNPs made with the Nova BT IJM with varying Formulation Buffer concentrations.

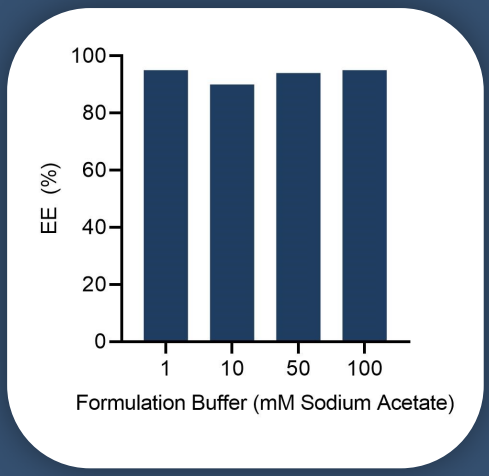


Figure 9. Encapsulation Efficiency (EE%) of LNPs with varying Formulation Buffer concentrations.

Discussion

In this study, we evaluated the effects of formulation and process parameters on the characteristics of DNA+LNP nanoparticles using our novel Nova Benchtop platform. We found that the type of ionizable lipid, PEG content, N/P ratio, and lipid stock concentration all had some impact on nanoparticle size, polydispersity, and encapsulation efficiency. Notably, lipid stock concentration positively correlated with nanoparticle size, which may be due to increased lipid aggregation at higher concentrations. It is worth noting that while our study focused on small-scale screening and optimization, the successful translation of DNA+LNP formulations to in vivo applications involves many additional factors beyond the scope of this study, such as stability, toxicity, biodistribution, and target specificity. Future studies would typically be needed to address these factors in order to fully evaluate the potential of DNA+LNP as a gene therapy platform.

The Nova BT platform represents a significant step forward in the development of DNA+LNP formulations for gene therapy and gene editing. Overall, we observed low particle size (~80nm), low PDI (~0.2) and high encapsulation efficiency (~95%) across all samples with few statistically significant differences. This suggests that our Nova BT platform is a robust and reliable tool for small-scale screening and optimization of DNA+LNP formulations. In addition, our platform offers several advantages over existing manufacturing platforms, including greater control over the manufacturing configuration and process via modular plug-and-play design, and better scalability. Each dual pump module accommodate two syringes with volumes from 1-60mL, providing a total sample production capacity of <1mL to over 100mL. Pump flow rates range from 0.1-100mL/minute, and reproducibility of the process allow production of over 1L via multiple runs. The modular platform design also allows for easy implementation of additional pumps and mixers for in-line dilution or nanoparticle modification, or increased throughput. The ability to rapidly and efficiently screen, optimize, and scale LNP formulations using our platform will enable researchers to more effectively develop and refine their formulations, potentially leading to more successful clinical outcomes.

Conclusion

In conclusion, DNA+LNP nanoparticles have emerged as a promising platform for gene therapy and gene editing with potential applications in a wide range of diseases and conditions. Our study demonstrates the effectiveness of our novel Nova BT platform in producing high-quality DNA+LNP nanoparticles, with the ability to optimize formulation and process parameters to achieve desired nanoparticle properties. Our platform offers faster and more efficient production, greater control over the manufacturing process, and better scalability than existing platforms. We believe that our study can contribute to the advancement of gene therapy and gene editing applications using DNA+LNP nanoparticles, and we hope that our findings will inspire further research in this field.

Materials and methods

Stock Solutions: 100mM Sodium Acetate pH 4 was used for all samples (apart from the testing scenario that examined Formulation Buffer Concentration, where Sodium Acetate concentration ranged from 1-100 mM.) The pDNA that was used for all testing scenarios, gWiz GFP, was sourced from Aldevron. The lipids used were DOTAP, DOTMA, DODAP, DODMA, ALC– 0315, SM-102, DLIN-MC3-DMA. DLIN -MC3-DMA was used as the main ionizable lipid for all of the main parameter screening studies, using a 50/10/38.5/1.5 mol% ratio of MC3/DSPC/Chol/DMG-PEG2k.

LNP and Post-Processing: The default parameters for LNP formulation for each of the samples were 1.5 % PEG concentration, DLin-MC3-DMA lipid, an N/P ratio of 6, 6.25 mM lipid mix concentration, flow rate ratio of 3, total flow rate of 15 mL/min, and 100 mM sodium Acetate Buffer. All samples were diluted 4x in PBS post processing.

LNP Characterization and Analysis: Particle Size and PDI were measured with an Anton Paar Litesizer 500 Particle Analyzer. The samples were diluted with PBS for measurements. Free and Total DNA content was found via Ribogreen Analysis. The Encapsulation Efficiency was found by subtracting the Free DNA divided by the Total DNA from 1. DNA concentrations were found by averaging the measurements of Total DNA samples.

References

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Zhu, Y., Shen, R., Vuong, I. *et al.* Multi-step screening of DNA/lipid nanoparticles and co-delivery with siRNA to enhance and prolong gene expression. *Nat Commun* **13**, 4282 (2022). https://doi.org/10.1038/s41467-022-31993-y

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