T1330-01-05

Optimizing lipid nanoparticles (LNPs) processing for siRNA delivery by microfluidics system

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PURPOSE

The success of mRNA vaccines during the COVID-19 pandemic has greatly accelerated the development of nucleic acid delivery. In addition to mRNA therapy, interfering RNA technology could be used to treat many diseases that cannot be targeted by small molecules.[1,2] However, the successful application of RNAi technology in pharmaceutical applications depends on developing efficient and targeted delivery systems. Nanoparticles, particularly lipid nanoparticles (LNPs) fabricated by microfluidics, have emerged as a promising platform for RNAi.[3-5] This study aims to provide a comprehensive technical update on LNPs formulation for siRNA delivery through a microfluidics approach.

OBJECTIVES

In this study, we aimed to explore and optimize the formulation of LNPs for short interfering RNA (siRNA) delivery, leveraging a microfluidics system with a flow-focusing approach.

METHODS

- To prepare siRNA-LNP, a microfluidics device was used. The lipid composition were shown below.

Component	Molar Ratio (%)
DOTAP	45
DPOC	11
Cholesterol	39
DMG-PEG2000	5

- Critical parameters, including total flow rate (TFR), N/P ratio of lipid and RNA, depth of microfluidics channels, and lipid blends concentration were investigated.
- Design of experiments was employed to evaluate the relationship between pH and concentration of PBS and sodium acetate buffers on the physicochemical properties of siRNA-LNPs.

RESULTS

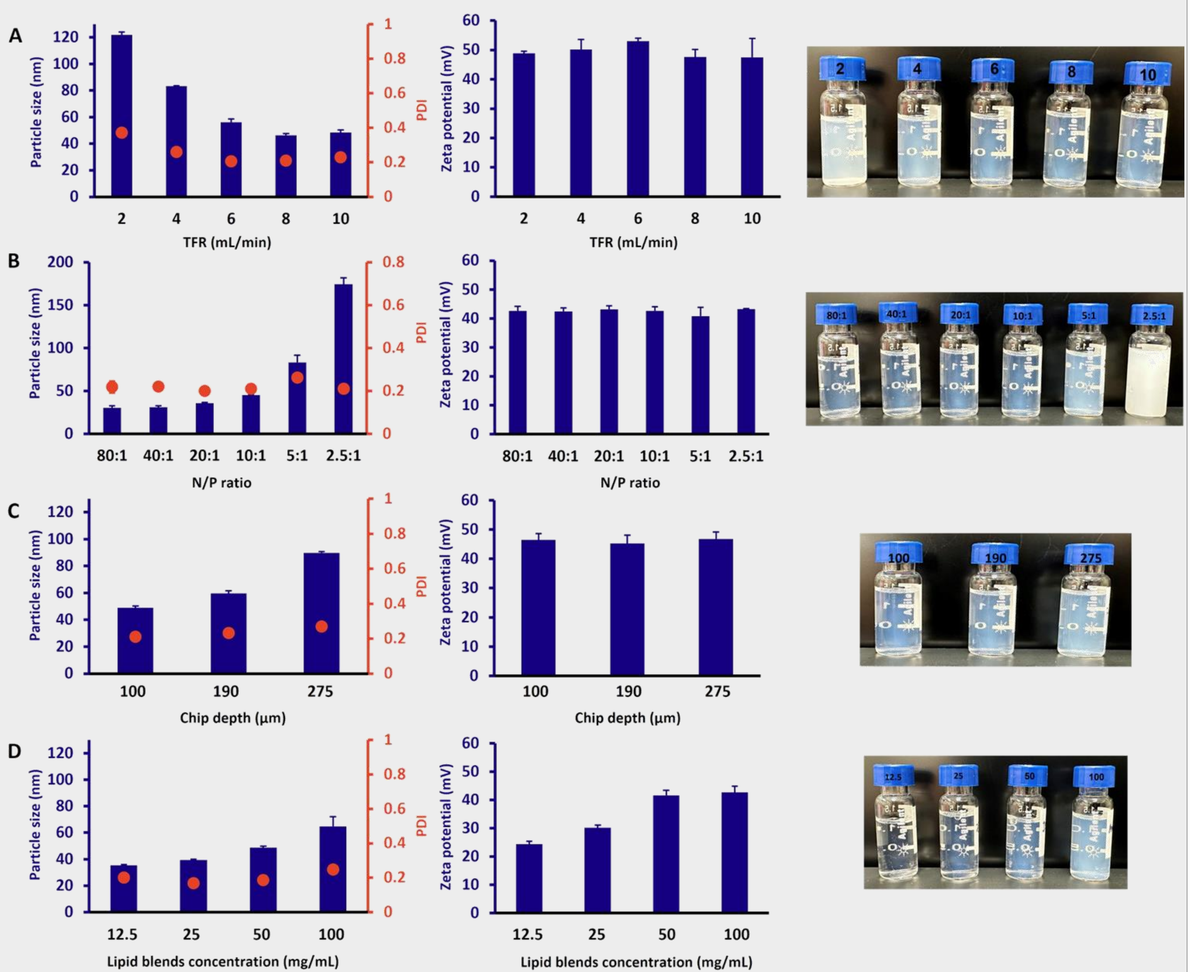

Figure 1. Effect of TFR (A), N/P ratio (B), chip depth (C), and lipid component concentration (D) on particle size, PDI, zeta potential, and appearance of siRNA-LNPs.

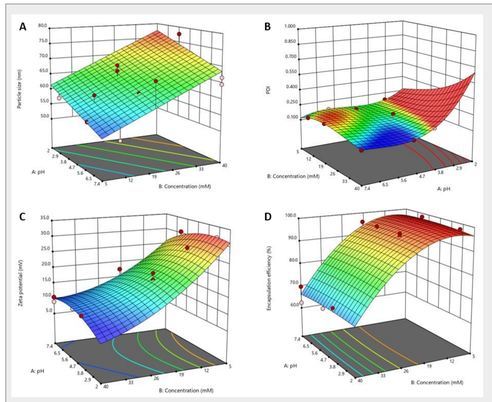

Figure 2. Surface response plots showing the effect of pH and conc. of PBS buffer on particle size (A), PDI (B), zeta potential (C), and encapsulation efficiency (EE) (D) of siRNA-LNPs

- The concentration of PBS buffer significantly affect zeta potential and EE. With decreasing PBS buffer concentration, both zeta potential and EE significantly increased.
- The result also indicated that zeta potential can impact the loading capacity of LNP.

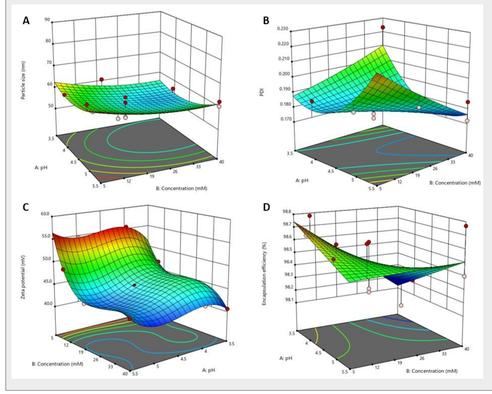

Figure 3. Surface response plots showing the effect of pH and conc. of sodium acetate buffer on particle size (A), PDI (B), zeta potential (C), and EE (D) of siRNA-LNPs

- The response-fitting results indicated that, the pH in range of 3.5-5.5 and concentration in range of 5-40 mM were not significant factors affecting the physicochemical properties of siRNA-LNPs.

CONCLUSIONS

- By establishing these optimized parameters, including **8 mL/min TFR, 10:1 N/P ratio, 100 μm chip depth size, and 25 mg/mL lipid concentration,** we have provided an understanding of developing effective nucleic acid delivery systems.
- For **PBS buffer** with decreasing concentration, both zeta potential and EE significantly increased.

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