NanoPULSE: The First Micromixing Method Delivering Consistent LNP Formulations Seamlessly Across All Scales

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ABSTRACT

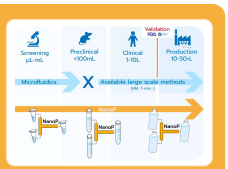
The field of nanomedicine has been attracting experts from various disciplines, aiming to develop nanocarriers capable of encapsulating potent drugs and nucleic acids to treat or prevent critical diseases, exemplified by RNA-LNP therapies. This progress created a demand for devices that enable the precise **synthesis of monodisperse nanoparticles**. However, the field currently lacks a reliable method to **scale production up** from laboratory research (µLs) to clinical production (>10L), hindering the translation from drug development stages to clinical applications. Here, we introduce **NanoPULSE**, the first micromixer capable of leveraging **Taylor-Aris Dispersion** to deliver **consistent LNP characteristics across all production scales**, closing the scale-up gap and meeting the needs of the pharmaceutical industry.

LIMITATIONS

1. **Small volume limitation:** Large-scale synthesis methods (e.g., IJM, T-mixer) cannot be used for lab-scale production (< 1 mL).

2. **Quality issues:** Microfluidic techniques can only be used for limited production times due to agglomeration issues which lead to higher polydispersity levels and make scale-up difficult.

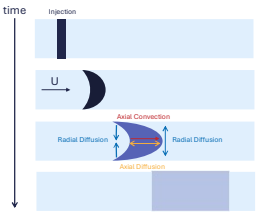
3. **Scalability bottleneck:** Traditional micromixing techniques for LNP production rely on chaotic mixing, resulting in flow-rate- dependent RNA-LNP characteristics.



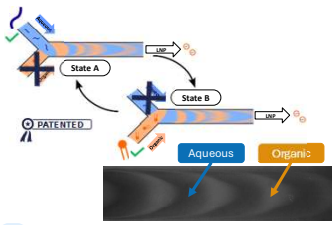
Staggered Herringbone Mixer, after 30 mins of continuous LNP synthesis

1. NanoPULSE Technology:

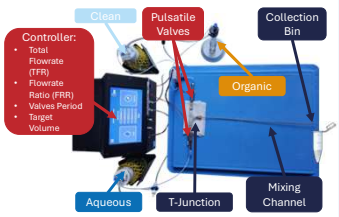
NanoPULSE is a micromixing technology inspired by microfluidic systems, allowing a highly controlled LNP synthesis from low volumes (<1mL) for screening purposes to multiple liters of production for preclinical development. This **highly scalable micromixer** is based on the **Taylor-Aris Dispersion (A)**. The organic phase containing the lipids is sequentially injected with the aqueous phase containing the cargo (e.g., DNA, mRNA, siRNA), to formulate lipids nanoparticles **(B)**, the fringes can be observed via a microscope using a fluorescein dye to highlight the organic phase. Input parameters are entered in the controller, which sets the flowrate (TFR), the flowrate ratio (FRR), and the valve opening times **(C)**.



A Taylor-Aris Dispersion



B NanoPULSE Technology



C NanoPULSE Prototype

2. Simulations:

Live simulation

Lagrangian | Eulerian

SWIPE FOR THE NEXT VIDEO

Simulations properties:
- Maximum velocity: 4.244 m/s
- TFR: 25 mL/min = 1.5 L/h
- FRR: 3:1
- Period: 4 ms
- Channel Diameter: 500 µm
- Total simulation time: 0.3 s

The **Lagrangian** approach permits to **follow the dispersion of two succeeding fringes** among a sea of others as they flow in the mixing channel.

The **Eulerian** approach illustrates the **complete mixing process in the entire mixing channel**.

3. Experimental Results:

Results In rolling sphere format

Formulation of **liposomes and RNA-LNPs with yeast-RNA** has been performed using NanoPULSE {Figure 1}. TAMARA was used as a control in these experiments.

Thanks to dynamic light scattering (DLS) measurements, we were able to confirm that NanoPULSE can **function continuously for several hours without causing any changes** in nanoparticles' size (Z-average) or dispersity (PDI) {Figure 2}.

Liposomes and RNA-LNPs were stored in the fridge at 4°C, and their size was measured regularly. **No significant changes** were observed in particle size or PDI for **at least one month** for liposomes, and **after 10 days** for RNA-LNPs {Figure 3}.

Other user-adjustable parameters on the NanoPULSE system, such as TFR, FFR, and pulse period, have been evaluated. For the period, a first study suggests that it **directly impacts the size of the particles obtained**, which is in line with NanoPULSE theory.

A shorter period, corresponding to a higher frequency, results in **smaller nanoparticles**. We project that by increasing the frequency sufficiently, particle sizes similar to those obtained with TAMARA can be achieved {Figure 4}.

The mixing channel measures currently 75 cm, but the impact of its length on particle properties has been evaluated both theoretically and experimentally. A threshold was observed for particle size and PDI, corresponding to the decay of the calculated mixing rate (MR). Preliminary results show that nanoparticles' size and PDI remain unchanged for lengths above 40 cm {Figure 5}.

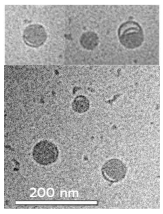


Figure 1

Cryo-TEM LNP, TFR: 36mL/min, Period: 40ms, FRR: 3:1, [lipids]: 5mg/mL N/P: 6:1 DSPC:Chloresterol:SM-102:DMG-PEG200 (10:38.5:50:1.5)

Figure 2

Size and PDI of a 4L formulation of liposomes S100, TFR: 36mL/min, Period: 60ms, FRR: 3:1, [lipids]: 2mg/mL SPC:Chloresterol:DMG-PEG2000 (59:40:1)

Figure 3

Stability of 250mL of ALC LNPs, TFR: 36mL/min, Period: 40ms, FRR: 3:1, [lipids]: 2mg/mL DSPC:Chloresterol:ALC-0315:DMG-PEG2000 (10:38.5:50:1.5)

Figure 4

Impact of the period on the size and PDI of liposomes, TFR: 36mL/min, FRR: 3:1, [lipids]: 2mg/mL, V: 3mL DSPC:Chloresterol:ALC-0315:DMG-PEG2000 (10:38.5:50:1.5)

Figure 5

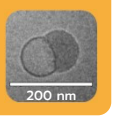
Influence of the mixing channel length on the mixing rate (theoretical) and the liposome size (experimental) SPC:Chloresterol:DMG-PEG2000 (59:40:1)

HIGHLIGHTS

NanoPULSE has shown to be:
- ✓ **highly repeatable**;
- ✓ **100% agglomeration-free** in its mixing channel, even after long production times.

NanoPULSE was proven capable of producing:
- ✓ liposomes and RNA-LNPs from **1mL to 4L in a single run**;
- ✓ **stable** liposomes and RNA-LNPs for at least **10 days**.

Numerical simulations highlight the **mixing performance** of NanoPULSE; hence, **predicting the LNP characteristics**



CONCLUSION

NanoPULSE presents the first micromixing technology that ensures a **seamless scale-up of LNP synthesis** from low-volume research applications all the way to high-volume continuous production using a **single device**. The technology permits a **repeatable, agglomeration-free and volume-independent synthesis** of LNPs via the sequential injection of the organic and aqueous phases in the mixing channel, promoting an enhanced mixing following the Taylor-Aris Dispersion principle. The NanoPULSE prototype permits the user to tune the TFR, FRR, and valves opening times, which allows for a **precise control over the nanoparticle's size**. Future steps include the validation of the preliminary results using a larger dataset, correlation of the simulation results with experimental data, and confirmation of the consistency of the LNPs' critical quality attributes such as zeta potential and encapsulation efficiency. We also aim to validate the continuous, agglomeration-free production of LNPs at larger scales. These findings would confirm NanoPULSE's capability of supporting **all drug development stages**, scaling-up the production from sub-milliliters to liters of synthesis volume while **keeping the LNPs' characteristics intact**.

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