IJM NanoScaler Pro: From high throughput screening to commercial manufacturing of lipid nanoparticles for the delivery of RNA vaccines and therapeutics

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INTRODUCTION

KNAUER's NanoProducer system played a crucial role in manufacturing a COVID-19 vaccine. Since then, research on nucleic-acid-based lipid nanoparticles (LNPs) has expanded. These LNPs, composed of 4-5 lipids, vary in ratios and types, affecting their delivery and performance. Buffer composition has also been shown to significantly influence the properties, performance, and stability of LNPs. For developing new LNPs high throughput screening is essential, as well as scalability from lab to production is key. KNAUER's new NanoScalerPro supports this screening, using the same Impingement Jets Mixing (IJM) technology as the NanoProducer.

IJM NanoScaler Pro

Optimized for high throughput screening

- Low volumes
- Minimal residual carry over
- Reproducibility
- Scalability for clinical trials

RESULTS AND DISCUSSION

The NanoScaler Pro, recently released by KNAUER, meets the needs of high throughput screening of LNPs. It can produce between 0.5–50 mL in high throughput mode and can be converted to continuous manufacturing for mid-scale batches. The number of formulations produced depends on the formulation size and flow rates. The NanoScaler Pro comes with 5 different sized Impingement Jets Mixers (IJMs) to allow for reasonable scale-up to mid-scale. Minimal residual carryover, reproducibility, and scalability to clinical manufacturing are several key factors that a high throughput screening system should have.

Minimal Residual Carryover

In Figure 1, LNPs were initially prepared under poor mixing conditions, characterized by low mixing flow rates that produced larger particles. This was immediately followed by a formulation using known mixing conditions to achieve the desired particle size and PdI. This process was repeated multiple times. The data in Figure 1 indicates no carry-over effects from the poorly mixed formulation, as the subsequent properly mixed formulation achieved the expected particle size and PdI.

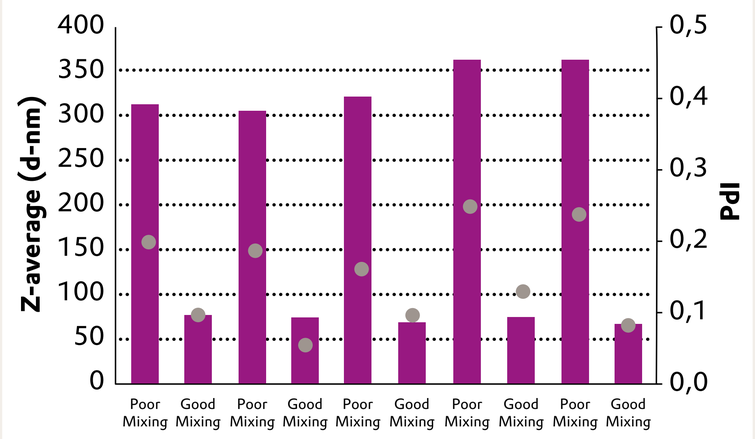


Figure 1: Post-mixing particle size of formulations prepared under poor mixing and good mixing conditions to demonstrate that no residual carryover from the poor mixing conditions is evident in the subsequent good mixing condition.

Reproducibility

The reproducibility of the KNAUER's NanoScaler Pro is shown in Figure 2 as post-mixing particle size analysis of formulations prepared at 0.75 mL and 3 mL sample volumes. The data in Figure 2 shows that the difference between particle size and PdI of the LNP formulations is insignificant and that the system has good reproducibility.

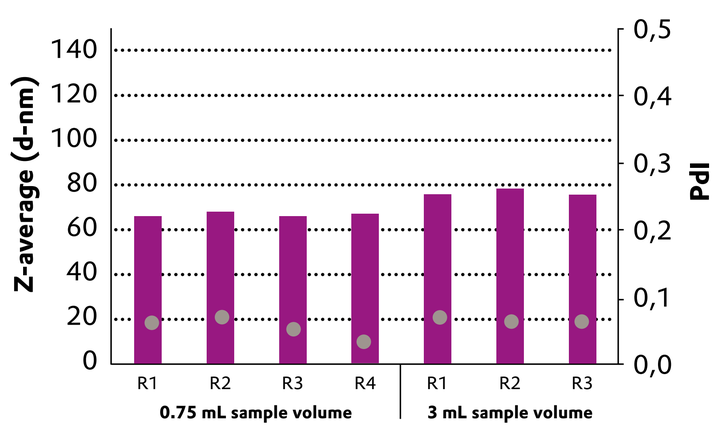


Figure 2: Consecutive formulations were prepared at both 0.75 mL and 3 mL formulation sizes to demonstrate good reproducibility between identical formulations.

Scalability

KNAUER's NanoScaler Pro utilizes the same IJM technology as the NanoProducer systems used for the Pfizer/BioNTech COVID-19 vaccine. The aim was to determine optimal scaled-down flow rates and assess if the NanoScaler Pro could produce particles comparable in size to those from the NanoProducer. Figure 3 shows the particle size and PdI of LNPs generated across various flow rates. The results showed that the optimal scaled-down flow rate produced the smallest homogeneous particles, while deviations from these conditions negatively affected size and PdI.

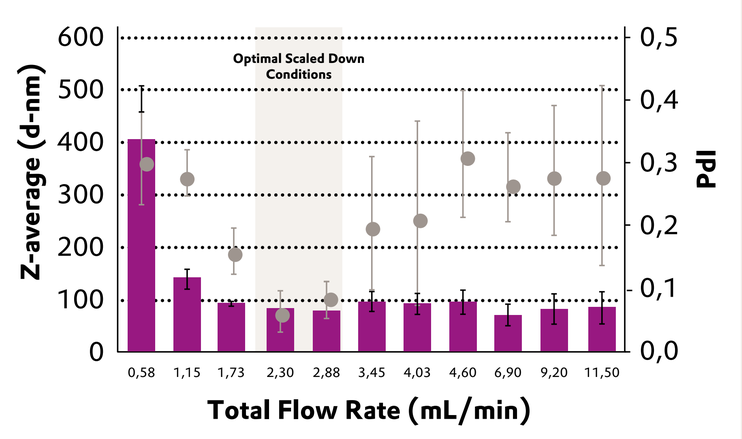
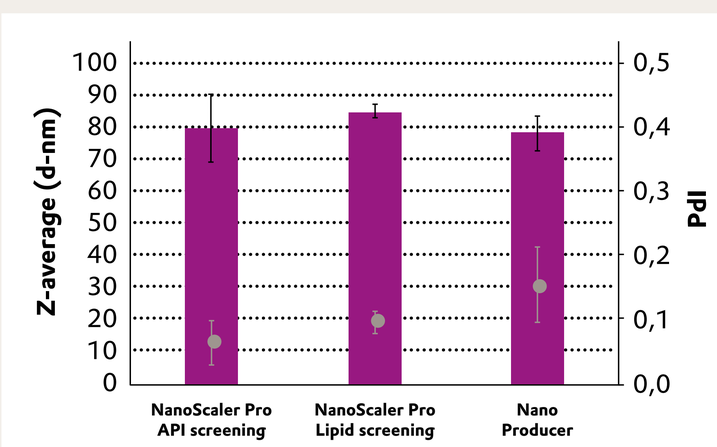


Figure 3: Particle size analysis of particles prepared using the IJM1 mixer over a range of total flow rates. Particles generated at flow rates within the optimal scaled down condition had the smallest particle size and best PdI.

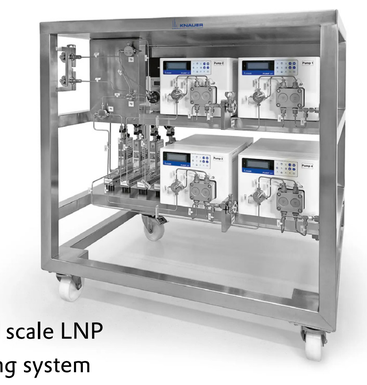
Bridging the Gap: Assessment of Scaled-Up Equipment Versus R&D Systems

The NanoScaler Pro was assessed in two key configurations: the API screening configuration, where the API is injected using the autosampler, which is particularly useful for testing multiple nucleic acids, and the lipid screening configuration, designed for high-throughput testing of various lipids. The LNPs produced under these conditions were compared to those generated by the NanoProducer system. Figure 4 illustrates that the particle size and PdI of LNPs generated by both the NanoProducer and NanoScaler Pro, when appropriately scaled, fall within the expected range of deviation.

Figure 4: Comparison of particles generated with the NanoScaler Pro at conditions scaled down from the NanoProducer.



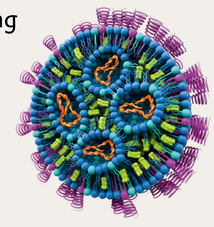
IJM SingleCore NanoProducer



Pilot/clinical scale LNP manufacturing system

Results

KNAUER's NanoScaler Pro was evaluated by Evonik. The collected data suggests that the NanoScaler Pro is highly versatile, which differentiates it from other systems on the market. Further it meets the key factors when selecting a system for high throughput screening of LNP formulations. KNAUER's high-end versatile LNP systems are able to fulfil the needs of partners in industry and research. Based on flexibility and durability our systems are a reliable partner from small-scale development to large-scale LNP production.



Material and methods

Lipid Nanoparticle Formation

Lipid Nanoparticles loaded with either Poly(A) or Fluc-mRNA were prepared by mixing a lipid solvent solution containing an ionizable lipid (D-lin-MC_3), DSPC, cholesterol, and DMG-PEG2000 with nucleic acid dissolved in 50 mM citrate buffer. The solutions were mixed at a ratio of 3 parts aqueous to 1 part solvent at various total flow rates. If required, post-mixed samples were dialyzed using a Slide-A-Lyzer G2 dialysis cassette against 150mM sucrose, 75mM NaCl, 10mM Phosphate, pH 7.4 buffer. Samples were diluted with 20% Phosphate buffered saline, and the particle size and polydispersity index (PdI) were determined by dynamic light scattering using a Malvern Zetasizer Nano-ZS90.

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