# Driving precision in LNP development with the FR-JET® technology

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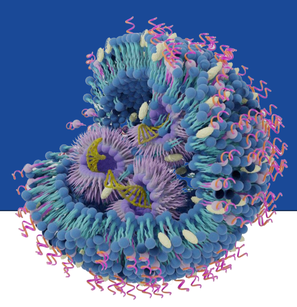
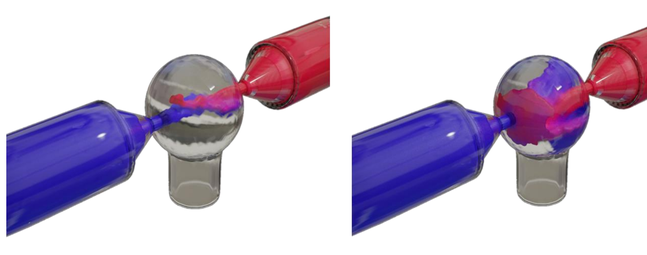


## PURPOSE

Lipid nanoparticle (LNP)-based delivery systems are central to nucleic acid therapeutics due to their efficiency in encapsulating RNA and a range of other payloads, tuneable properties, and biocompatibility. LEON's FR-JET® modular mixer enables scalable and precise LNP formulation through rapid, turbulent mixing of lipid and aqueous phases within a spherical chamber, ensuring uniform nanoparticle size distribution. This study explores the effects of total lipid concentration and total flow rate (TFR) on encapsulation efficiency (EE), payload recovery, and LNP morphology, using three LNP models—SM-102, ALC-0315, and DLin-MC3-DMA—formulated with polyadenosine (polyA) as a model nucleic acid payload.

## METHODS

Each of the three LNP models included an ionizable lipid (SM-102, ALC-0315, or DLin-MC3-DMA), cholesterol, DSPC, and DMG-PEG2000, prepared at an N/P ratio of 6 and a 3:1 aqueous-to-organic flow rate ratio. Formulations were tested at low (15 mg/mL) and high (70 mg/mL) lipid concentrations under TFRs of 30 mL/min and 80mL/min. Particle size, polydispersity index (PDI), and zeta-potential were measured at 25 °C using DLS and ELS. EE and polyA recovery were assessed using the RiboGreen™ assay and fluorescence detection. CryoTEM was used to evaluate LNP morphology and structural features.



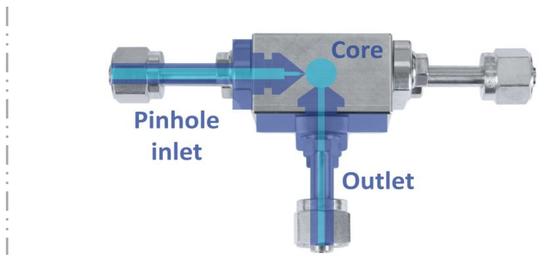



***Figure 1. Left:*** *Representative images of the mixing dynamics within the spherical chamber of the FR-JET® modular mixer.* ***Right:*** *The FR-JET® technology is a modular system with various nozzle and chamber dimensions that enable scalability from lab-bench to commercial scales.*

## RESULTS AND DISCUSSIONS

The FR-JET® modular mixer successfully produced LNPs using SM-102, DLin-MC3-DMA, and ALC-0315 as ionizable lipids at both lower and higher lipid concentrations while maintaining consistent quality. Particle size remained within the desired range of below 100 nm for all LNPs . All formulations showed PDI values below 0.2, indicating uniform particles. Zeta-potential varied by lipid composition and lipid concentration: SM-102 LNPs exhibited an increase in zeta-potential from near neutral to +15 mV at higher lipid concentrations; DLin-MC3-DMA LNPs exhibited zeta-potential values of +3 to +6 mV; and ALC-0315 LNPs exhibited negative zeta-potential values of -6 to -3 mV. EE was 98-100% and 93-95% for LNPs prepared at high and low lipid concentration, respectively. A clear influence of the effect of lipid concentration to encapsulate more payload was observed with SM-102 and DLin-MC3-DMA LNPs, whereas ALC-0315 LNPs were more robust to encapsulate payload even at lower lipid concentration. Payload recovery was above 87% for most formulations, with the exception of two LNPs formulations prepared at low TFR exhibiting a 70-73% recovery of the payload, indicating that formulations processed at higher TFRs improve payload recovery. CryoTEM analysis revealed a higher proportion of solid core structures at elevated concentrations, with over 85% of particles showing spherical morphologies with solid cores. These findings suggest that increased lipid concentrations contribute to the formation of more structurally uniform and stable LNPs suitable for therapeutic delivery. The particle size of LNPs remained stable over 12 weeks of storage at 2-8°C, with a 20 nm, 10 nm, and 2 nm increase in size for SM-102, DLin-MC3-DMA and ALC-0315 LNPs, respectively.

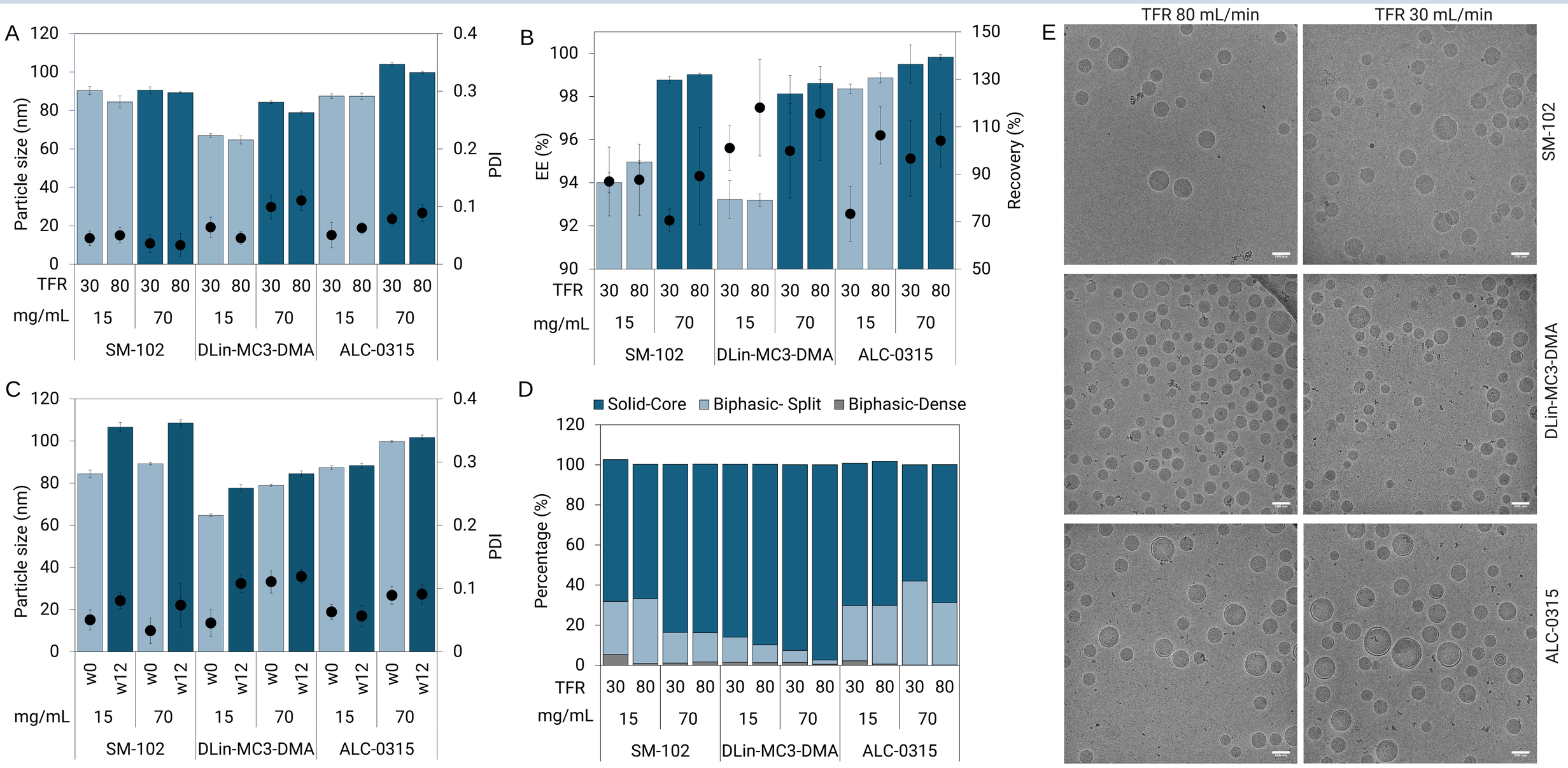



***Figure 2. (A)*** *Particle size & PDI,* ***(B)*** *EE & polyA recovery, were evaluated at lipid concentrations of 15 and 70 mg/mL.* ***(C)*** *Storage stability was evaluated for LNPs stored in PBS at 2–8°C, with particle size and PDI measured by DLS at Weeks 0 and 12 at TFR 80 mL/min. Error bars represent standard deviation (n = 3).* ***(D)*** *Morphological distribution of LNPs (solid cores, biphasic-split and biphasic-dense structures) under different formulation conditions.* ***(E)*** *CryoTEM images show LNP morphology at 70 mg/mL and TFR of 30 and 80 mL/min (scale bar: 100 nm).*

## CONCLUSIONS

The FR-JET® modular mixer enabled production of LNPs at both lower and higher lipid concentrations without compromising core LNP quality attributes. All tested formulations showed consistent particle sizes with low PDI values, high encapsulation efficiency, and good physical storage stability at 2-8 °C for up to 12 weeks. Notably, higher concentrations led to a greater proportion of solid core structures, suggesting improved structural uniformity of the LNPs. These results highlight the suitability of the FR-JET® technology for scalable LNP manufacturing, establishing it as a valuable platform for developing mRNA therapeutics utilizing nanoparticle delivery systems. Formulating LNPs at higher lipid concentrations enables process intensification, improving manufacturing efficiency and optimizing product throughput.