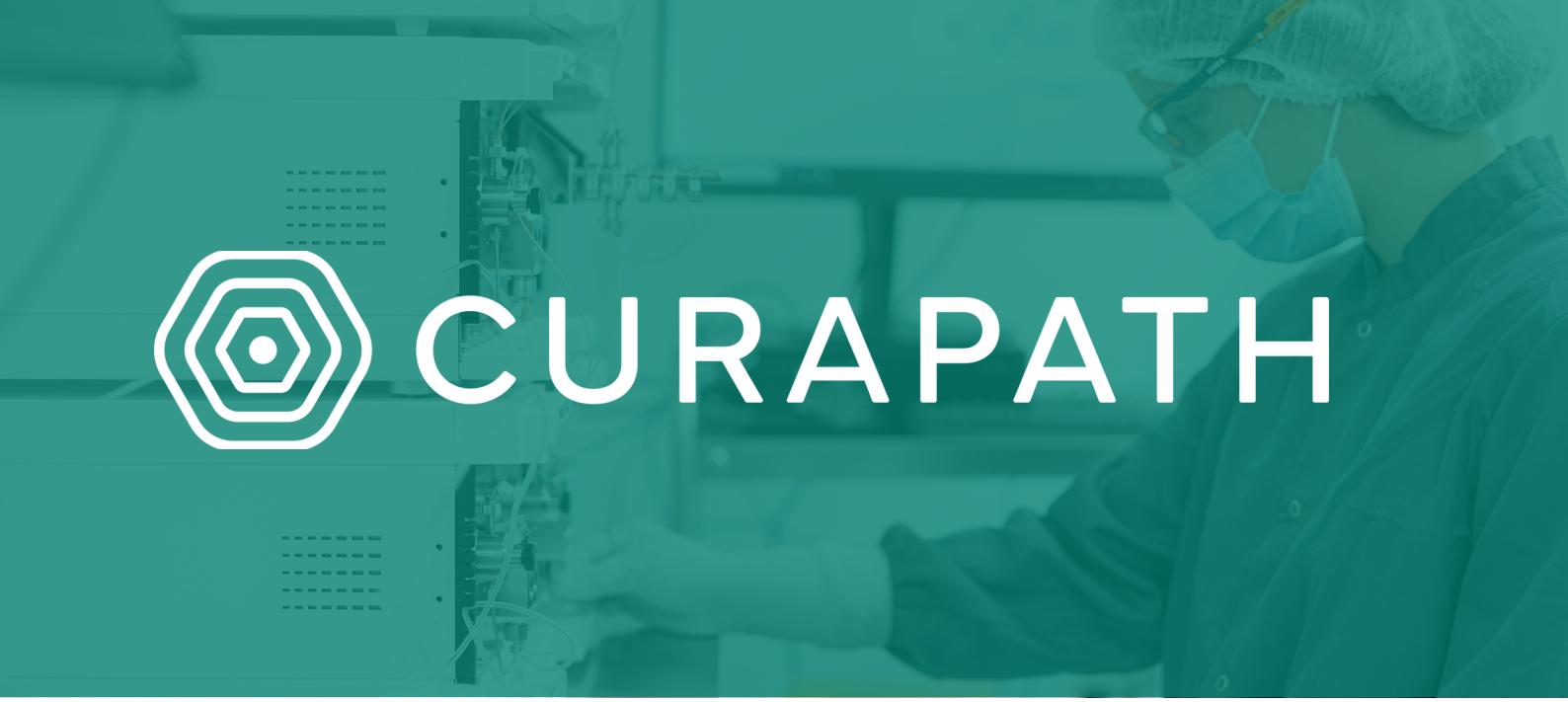

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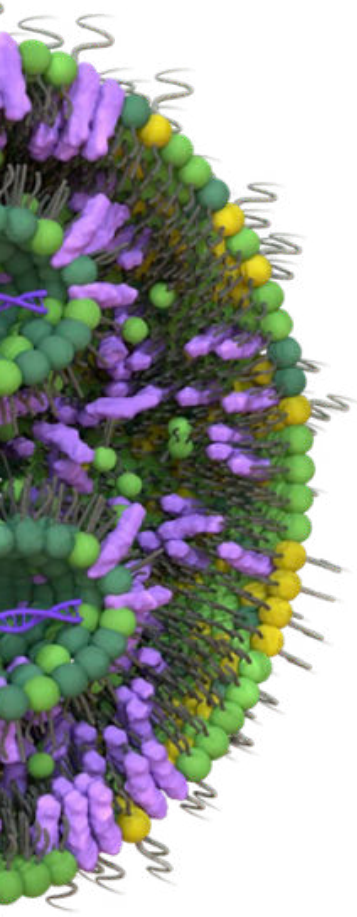

# High-throughput Screening Application Note

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# Implementation of IJM NanoScaler Pro

## Automated screening system for lipid nanoparticle formulation

Lipid nanoparticles (LNPs) are at the forefront of RNA delivery, as demonstrated by the great success of two mRNA LNP-based COVID-19 vaccines. The potency of an LNP product predominantly depends on the precise tuning of the lipidic components. Nevertheless, the screening of novel LNP compositions is limited by the efficacy of conventional preparation methods.

In response to these challenges, Curapath has tested an automated high-throughput screening (HTS) system developed by Knauer: the NanoScaler Pro. This innovative platform has been engineered to evaluate the scope of LNP formulations, while circumventing the labor-intensive and time-consuming nature of conventional experiments.

By leveraging this automated system, researchers can strategically plan their screenings using a Design of Experiments (DoE) approach for comprehensive screening of LNP compositions, to ultimately identify the optimal LNP formulation with greater efficiency and accuracy.

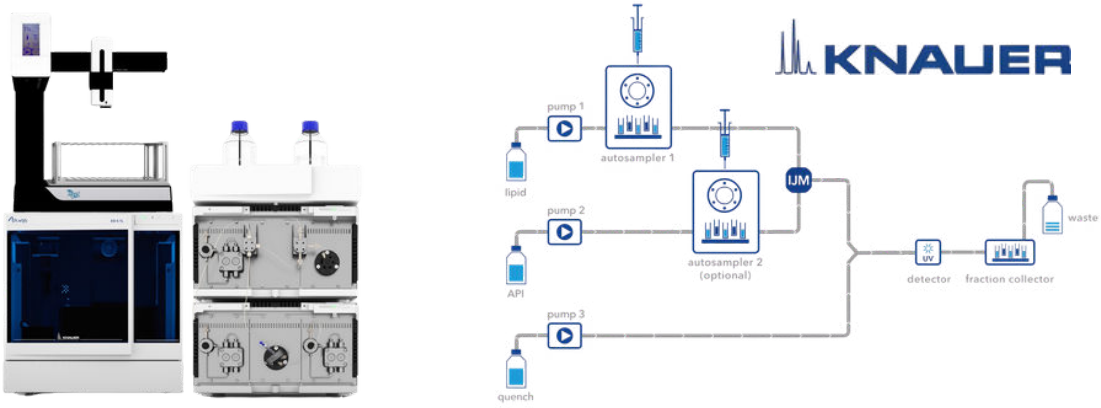



**Figure 1.** IJM Nanoscaler Pro system and flow scheme of the formulation process.

In this application note, we employed the autosampler 1 (Figure 1) for the premixing of the lipid phase. This solution is then injected into a loop, in order to minimize the use of lipids. The payload (mRNA in our case) can be either selected from a second autosampler or injected directly from the pump 2. Next, both solutions are mixed into the IJM and quenched by using the corresponding buffer. Finally, the formulations are collected in the fraction collector in the appropriate time ranges.

Herein, we demonstrate the robustness of the equipment by preparing three replicates of the same formulation. Additionally, we highlight its capacity to automate the screening process of different LNP compositions. All the formulations tested are compared with the ones prepared by the well-established microfluidic system (Knauer Nanoscaler), obtaining a perfect match.

# Automated screening system for LNPs

## Materials & Methods

### NanoScaler

Two different lipid compositions were manually dissolved in ethanol:

**1.** ALC-0315, DSPC, Cholesterol and DMG-PEG at molar ratio of 46.3/9.4/42.7/1.6 (Figure 2).
**2.** C12-200, DOPE, Cholesterol, DMG-PEG at different molar ratios (Figure 3).

Initial mRNA concentration was adjusted to 67ng/µL in acetate buffer and the N/P ratio was established between 6 and 8. The total flow rate (TFR) was set to 7.5 mL/min (including the in-line dilution with PBS buffer) and the ratio between aqueous phase and organic phase to 3:1. The aqueous phase was injected via a 1 mL loop and the lipid phase with a 0.5 mL loop, both designed to minimize the dead volume. We selected IJM1 for all the formulations tested. After formulation, LNPs were purified with the Vivaspin® 20, 100kDa MWCO, PES (Sartorius) to remove ethanol and exchange the buffer to PBS. All experiments have been performed at least in duplicate.

### NanoScaler Pro

In this case, the 4 different lipid stock solutions in ethanol were prepared and placed into the autosampler. The parameters when employing the autosampler module in IJM Nanoscaler Pro were the same as previously described, but the autosampler 1 prepared the lipid solution automatically. Once collected by the fraction collector, the LNPs were purified following the same procedure as in NanoScaler formulations.

## Results

In the first experiment, our objective was to evaluate the reproducibility of the experimental setup conditions. The methodology involved formulating three replicates of a standardized lipid composition (lipid composition 1). Following each formulation, a 10 minutes wash step was incorporated. The operating parameters and purification steps remained identical across both systems.

# Automated screening system for LNPs

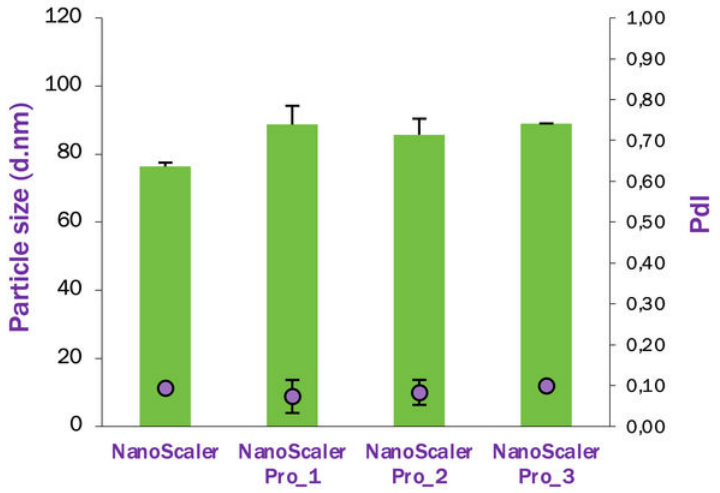



**Figure 2.** Comparison of size and PdI of mRNALNPs formulated with NanoScaler and a sequence of 3 formulations with NanoScaler Pro. Lipid composition 1. (n=3)

In Figure 2, we observed no significant difference in size, PdI and encapsulation efficiency (>90%, data not shown) when comparing both conditions with and without the autosampler module. Therefore, we demonstrated that the NanoScaler Pro is able to formulate automatically replicates of the same LNPs formulation obtaining outstanding results in terms of size, polydispersities (PdI) and Encapsulation Efficiency EE%.

The integration of high-throughput screening (HTS) methodologies is of pivotal importance as it facilitates the exploration of optimal ranges of LNP molar ratios, such as those designed within DoEs. Subsequently, to proof the utility of Nanoscaler Pro in combination with DoEs we performed the following experiment varying lipid molar ratios. In Table 1, the molar ratios and parameters are defined for each formulation:

| FORMULATION | TFR (ML/MIN) | PAYLOAD | IONIZABLE LIPID | HELPER LIPID | CHOLESTEROL | SHIELDING LIPID | N/P |
|---|---|---|---|---|---|---|---|
| 1 | 7.5 | mRNA | 45% | 10% | 41.5% | 3.5% | 8.7 |
| 2 | 7.5 | mRNA | 25% | 22% | 50.5% | 2.5% | 8.7 |
| 3 | 7.5 | mRNA | 25% | 22% | 49.5% | 3.5% | 8.7 |
| 4 | 7.5 | mRNA | 35% | 10% | 53.5% | 1.5% | 8.7 |
| 5 | 7.5 | mRNA | 35% | 19% | 46.5% | 2.5% | 8.7 |

**Table 1.** Composition of different LNP formulations performed with IJM Nanoscaler Pro. Lipid composition 2: C12-200, DOPE, Cholesterol, DMG-PEG.

# Automated screening system for LNPs

In this evaluation, we conducted a comparative analysis of the mean sizes of the five LNPs obtained with IJM Nanoscaler Pro and its non-automated counterpart, NanoScaler. We observed comparable sizes and PdI across all batches examined. In addition, LNPs showed no free mRNA detected by gel electrophoresis (data not shown).

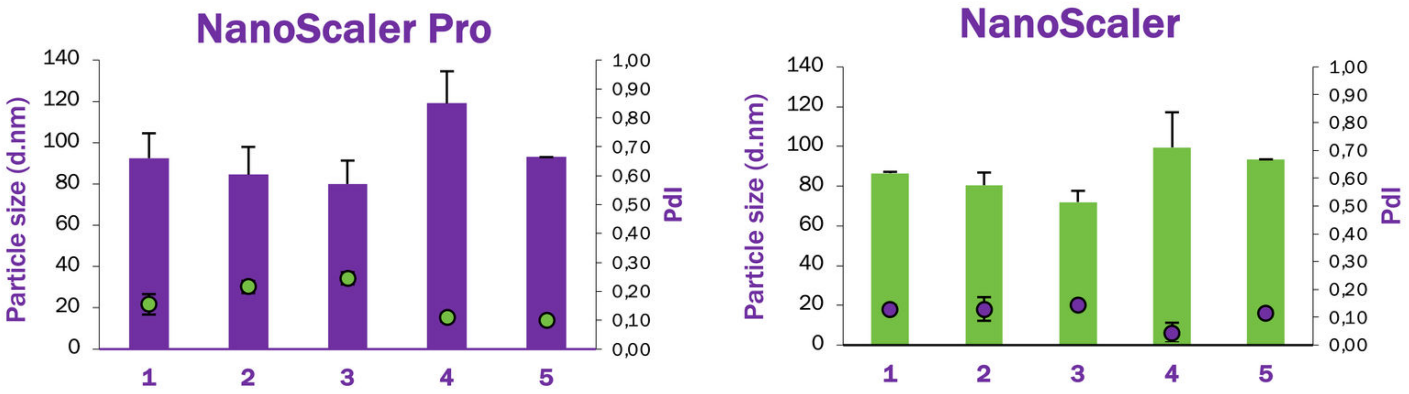


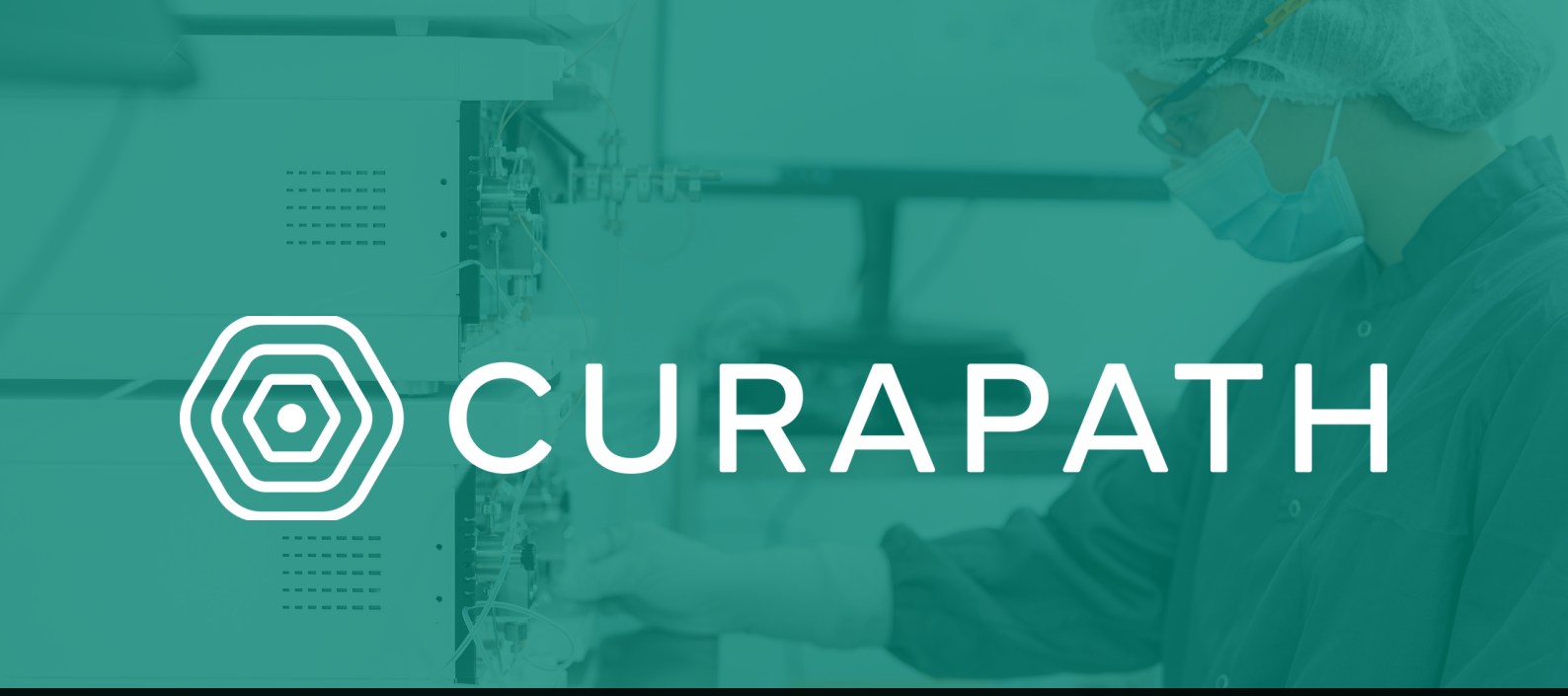

**Figure 3.** Comparison of size and PdI of mRNA-LNPs formulated with NanoScaler and a sequence of 5 formulations with NanoScaler Pro varying the LNPs lipid composition (in Table 1). (n=2)

Finally, we can conclude that our evaluation confirms the robustness of the automated process as evidenced by the reproducibility of LNP batches of the same composition as well as the appropriateness to perform large screenings with different compositions as demonstrated using five different formulations herein.

## Conclusions

Curapath, by employing the IJM Nanoscaler Pro provided by KNAUER, has developed a method to automatically generate LNPs by programming a sequence of LNP formulations, which could vary lipid composition as well as molar ratios. Our findings underscore the suitability of this method in conducting feasibility studies for emerging technologies through HTS-DoE screenings. In addition, the use of the IJM NanoScaler Pro, not only streamlines the screening process but also facilitates the scalability, as it is based on the same technology as KNAUER's GMP production systems.

Consequently, the use of NanoScaler Pro paves the way to accelerate technology transfer from the small-scale feasibility studies to the large-scale production, thus propelling advancements in the field of lipid nanoparticle formulation.



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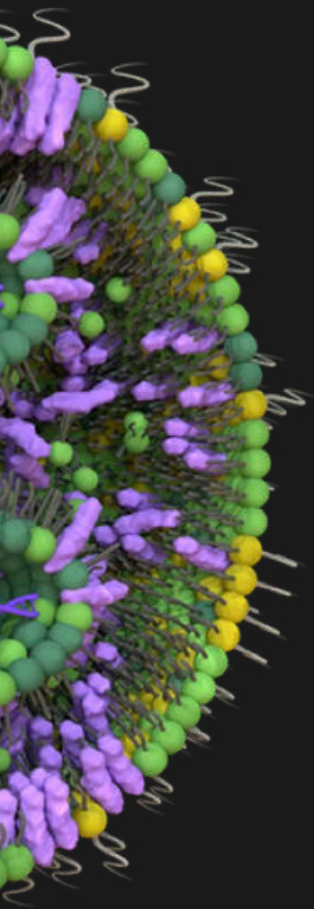

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