Simplifying Progress

Monolith-Based Solutions for Fast and Efficient LNP Purification at High-Throughput and Lab Scales

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Introduction

Lipid nanoparticles (LNPs) are versatile drug delivery systems widely used for encapsulation and delivery of therapeutic molecules, including nucleic acids, proteins, and small molecules. Their biocompatibility, stability, and ability to enhance intracellular delivery have made them central to modern therapeutics, particularly mRNA vaccines. As LNP-based therapeutics continue to advance, rapid screening and optimization of multiple formulations are becoming increasingly important. Small-scale and high-throughput workflows enable efficient evaluation of formulation parameters and critical quality attributes; however, these approaches require purification methods that are efficient, scalable, and easily transferred across development stages.

To address these challenges, we developed a monolithic chromatography workflow, based on Convective Interaction Media (CIM ®), for LNP purification that supports both high-throughput screening and lab-scale process development. In this poster, we demonstrate LNP purification using CIM OH Monolithic 96-Well Plates, alongside lab-scale formats CIM spin OH and CIMmic® OH, followed by a larger scale purification run using a preparative CIMmultus® OH column (Figure 1). The shared CIM technology ensures a consistent and scalable LNP purification from early screening to preparative scale.

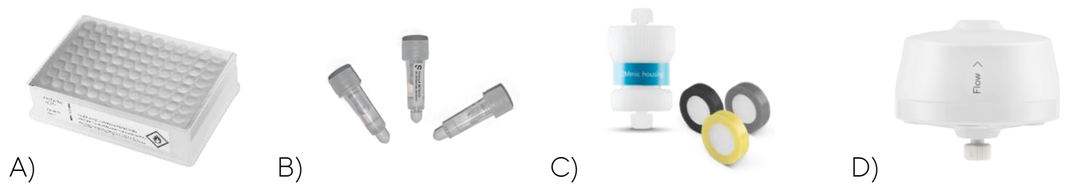

Figure 1: CIM products used in experiments, all with 6 µm channel diameter A) CIM OH 0.05 mL Monolithic 96-Well Plate B) CIM Spin OH 0.1 mL C) CIMmic OH 0.1 mL disc D) CIMmultus OH 1 mL

1. Experimental approach

LNPs were formed with NanoAssemblr™ Ignite (Cytiva) using Moderna-like formulation with SM-102, DSPC, Cholesterol and DMG-PEG-2000 in 25 mM acetate, pH 5. After formulation, LNPs were 5x (on CIM 96-WP, CIM Spin, CIMmic) or 10x (on CIMmultus) diluted with 50 mM Tris, pH 7.4 to neutralize and stabilize the particles. Upon neutralization the sample was further diluted 1:1 with loading buffer either off-line (CIM 96-WP, CIM Spin, CIMmic) or in-line (CIMmultus) to achieve binding conditions of 15 mM Tris, 0.5 M Na-citrate, 200 mM sucrose, pH 7.4. Samples were loaded on the column and eluted with 3 CV (column volumes) of 15 mM Tris, 200 mM sucrose, pH 7.4. Any residuals that remained bound on the column were stripped with 50% Ethanol. 0.2 mg of encapsulated mLuc mRNA per mL of monolith was purified in each workflow (Figure 2). After purification, samples were analyzed using the PATfix® LNP Switcher platform, in vitro cell-based Luciferase activity assay and size measurement with Nanoparticle Tracking Analysis (NTA). Columns were cleaned before and after the runs with 1 M NaOH as stated in the Instructions for Use.

In parallel to the high-throughput and lab scale experiments, a stability study was performed using LNPs purified on the CIMmultus column. Samples were stored at room temperature, 4 °C, –20 °C, and –80 °C, and analyzed after 1 and 6 months using the PATfix LNP Switcher. Biological activity was also evaluated using a cell-based Luciferase activity assay.

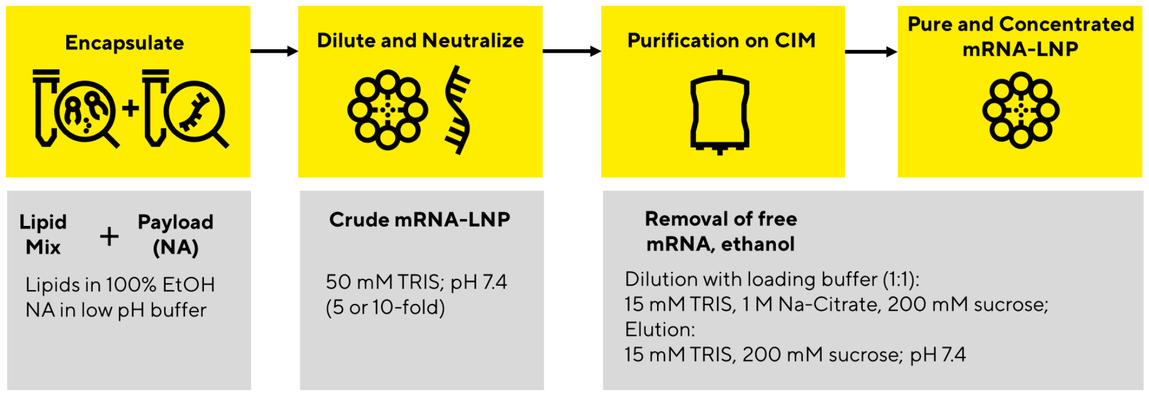

Figure 2: Overview of LNP formulation and purification.

Purification on CIMmultus column can be monitored in real time using a range of in-line detectors, such as UV, light scattering and conductivity. A representative chromatogram of LNP purification (Figure 3A) shows negligible UV signal during loading, followed by a pronounced elution peak, indicating efficient binding on OH column and high recovery of LNPs.

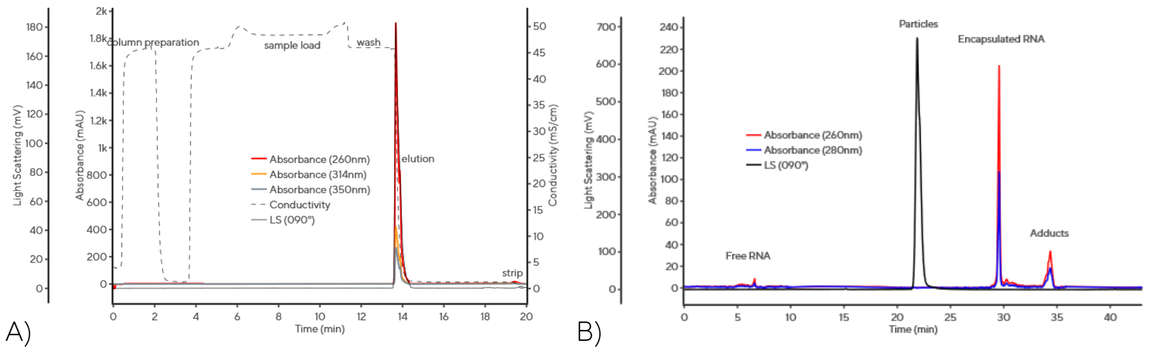

Figure 3: A) Representative chromatogram of LNP purification using 1 mL CIMmultus OH column. B) PATfix LNP Switcher analytical chromatogram

2. Results – PATfix LNP Switcher

To evaluate consistency across CIM purification formats, LNP samples were first characterized using PATfix LNP Switcher, which enables single-run, multiparameter analysis without sample pretreatment. The platform simultaneously measures key quality attributes, including encapsulation efficiency (EE), nucleic acid integrity, nucleic acid–lipid adduct formation, and particle heterogeneity, with represetative analytical chromatogram shown in Figure 3B. Additionally, RNA concentration can be determined by applying a calibration curve to the PATfix LNP Switcher data, from which overall recovery can be calculated.

As shown in Figure 4A, high recovery was obtained with both the CIM Spin column and the corresponding preparative CIMmultus column, demonstrating strong and consistent performance across formats. Lower recovery was observed with the CIMmic disc, primarily due to sample retention within the syringe during elution, an effect that becomes more pronounced at low LNP quantities. Recovery may be further improved through an additional elution buffer wash step.

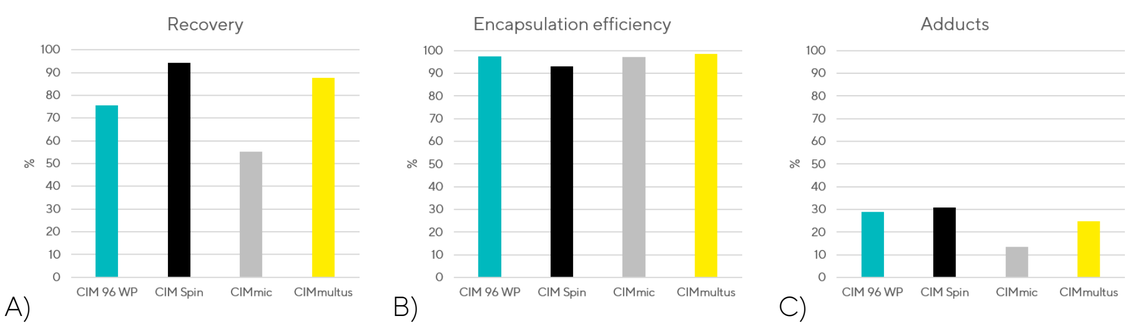

Figure 4: results obtained with PATfix LNP Switcher A) Recovery calculated from concentration measurement B) Encapsulation efficiency in elution fractions C) Adduct presence in elution fractions

EE is a critical quality attribute of LNP formulations, reflecting successful incorporation of nucleic acid into the lipid matrix. As shown in Figure 4B, high EE values were consistent across all tested formats, indicating preserved LNP integrity following purification. These findings suggest that the improved purification conditions, including vacuum and centrifugal forces, were sufficiently mild to maintain particle structure.

Adducts can form during formulation, purification, or storage and may negatively impact biological activity, as adduct-bound RNA is unavailable for protein expression. Adduct levels ranged from 13–30% across all purification approaches (Figure 4C), demonstrating comparable impurity profiles.

3. Results – Cell Assay, Nanoparticle Tracking Analysis (NTA)

In addition to PATfix LNP Switcher, other analytical methods were used to confirm suitability of tested products for LNP purification. Functional activity, assessed by luciferase expression in HEK293 cells (Figure 5A), was comparable across all purification strategies, with only minor differences in relative light units (RLU). This demonstrates that none of the approaches adversely affected the biological performance of the LNPs and that the process can be translated from smaller to preparative scale without loss of activity.

NTA (Figure 5B) revealed largely consistent LNP size distributions between purification approaches, with only slight variations in mode diameter. An example NTA profile of the final eluate (Figure 5C) shows a stable and uniform particle population.

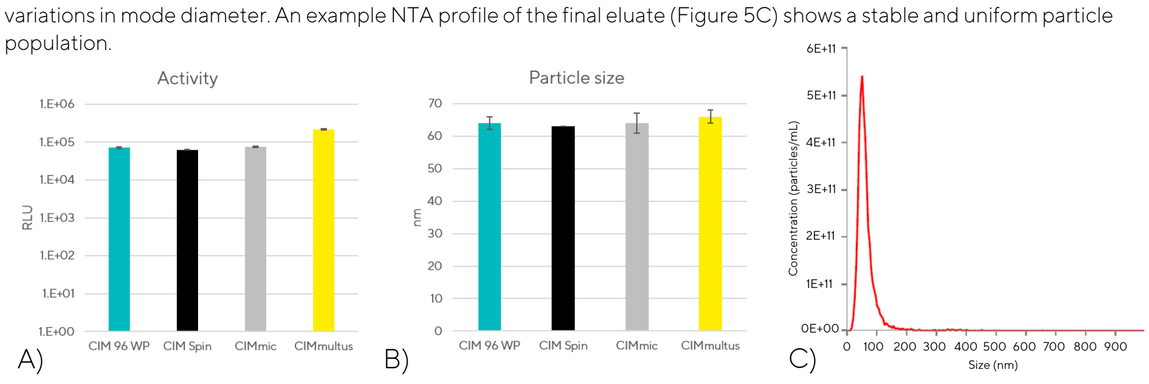

Figure 5: A) Luciferase expression B) Particle size measured with NTA C) Representative NTA measurement profile

4. Results – Stability

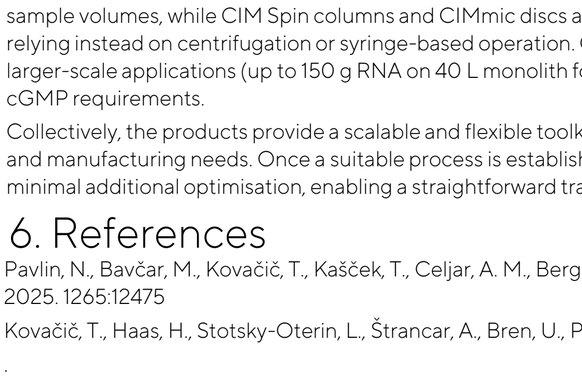

Figure 6: Stability study A) Encapsulation efficiency B) Luciferaze activity

PATfix LNP Switcher results demonstrate that LNPs purified using the CIMmultus column exhibit good long-term stability. EE remained consistent across all measured time points in the stability study, indicating that the formulation integrity was preserved during extended storage (Figure 6A).
In contrast, functional activity after 6 months of storage was strongly dependent on temperature. LNPs stored at –80 °C retained high biological activity, whereas storage at higher temperatures led to a progressive decrease in activity, highlighting the importance of low-temperature conditions for maintaining functional performance (Figure 6B).

5. Conclusion

Together, these results summarized in Table 1 demonstrate that HTS and lab-scale formats enable efficient purification of small LNP quantities with high recovery, without altering impurity profiles, resulting in highly active and stable formulations. The small-scale results are comparable to those obtained at large scale, supporting their suitability for robust and scalable process development.

Table 1: Consistent resuts of LNP purification from small to large scale usim CIM monolithic columns

Product	Recovery	Encapsulation efficiency	Adducts	Size
CIM 96-Well Plate	76 %	97 %	29 %	64 nm
CIM Spin	94 %	93 %	31 %	63 nm
CIMmic disc	55 %	97 %	13 %	64 nm
CIMmultus	88 %	99 %	25 %	66 nm

Each format serves a distinct purpose: the 96-well plates enable rapid screening and high-throughput purification of small sample volumes, while CIM Spin columns and CIMmic discs allow LNP purification without a chromatographic system, relying instead on centrifugation or syringe-based operation. CIMmultus columns, on the other hand, are suitable for larger-scale applications (up to 150 g RNA on 40 L monolith format) and can be operated in workflows compatible with cGMP requirements.

Collectively, the products provide a scalable and flexible toolkit that supports different stages of process development and manufacturing needs. Once a suitable process is established at small scale, it can be transferred to larger formats with minimal additional optimisation, enabling a straightforward transition from screening to production scale.

6. References

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