Simplifying Progress

Monolithic Columns for a High Recovery Purification Process of RNA-LNP Vaccines and Therapeutics

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Introduction

Lipid nanoparticles (LNPs) provide the most advanced platform for in vivo drug delivery of nucleic acids. However, they are not yet well-characterized biopharmaceuticals. Among others, challenges are of biological, manufacturing, and characterization type. Manufacturing challenges include correctly forming the drug product, maintaining its integrity, achieving sufficient recovery, and obtaining a well-purified and characterized product.

Tangential flow filtration (TFF) has been implemented to scale up downstream processing for the Comirnaty and the Spikevax vaccines. TFF may result in low recovery of the encapsulated RNA, ranging around 50%. Thus, an alternative to the TFF process, a new separation process has been developed using CIM Convective Interaction Media® OH monolithic columns. CIM® monolithic columns are uniquely suitable for LNP purification due to the low shear stress imparted of the purely laminar flow and a wide selection of chemically modified surfaces. LNPs are loaded onto the columns under kosmotropic conditions, directly following the encapsulation process and neutralization.

This process, which is much faster than comparable TFF processes, achieves the desired concentration, ethanol removal, and buffer exchange functions. At the same time, free, non-encapsulated RNA is removed by tuning the buffers and achieving chromatographic separation. Subpopulations of the sample are also collected, with the most active portion of the drug product retained.

Additionally, analytical methods developed using PATfix® LNP Switcher Platform enable the characterization of such formed drug products. They allow the determination of mRNA quantity and purity inside LNPs, mRNA-lipid adduct quantity, and lipid purity.

1. Experimental setup

- LNP-mRNA Encapsulation

mRNA of 2000 nucleotides (mFluc, 100 µg/mL in 50 mM sodium citrate, pH 5) was encapsulated into lipid nanoparticles using NanoAssemblr® Ignite™. The aqueous stream was mixed using microfluidic technology with a stream of lipidic solution in an N/P ratio of 6.1 (SM-102: 46 mol%, Cholesterol 43 mol%, DSPC 9 mol%, DMG-PEG 2K 1.6 mol%; 15 mM in EtOH) in a flow rate ratio of 3:1, and a total flow rate of 12 mL/min. LNP product (1.50 mL) was immediately diluted 10-fold in 15 mM TRIS, pH 7.4.

- LNP-mRNA purification

The LNP product was loaded onto a CIMmultus® OH column and mixed in-line with 2x loading buffer (containing high concentration kosmotropic salt). The purification process was monitored using UV (260 and 280 nm) and MALS (90° angle) detectors. Upon loading the sample and washing the column, a gradient elution was conducted to elution buffer B (low conductivity buffer), which eluted most of the particles. Cleaning buffer C was applied after to wash the residual species. Fractions were collected and analyzed. Herein 3 different processes are presented:

M1 – Kosmotropic salt 1

M2 – Kosmotropic salt 2

M3 – Kosmotropic salt 1 + sucrose

- Ultrafiltration

As a control, an ultrafiltration (UF) was used for sample buffer exchange. Selected samples were buffer exchanged into 15 mM TRIS, pH 7.4 using Amicon® Ultra Centrifugal Filters with 30 kDa molecular weight cut-off and centrifuged at 6.2 rpm for 10 minutes three times.

Above is an example of a representative LNP formulation, this can be extended to preferred formulation of an encapsulated nucleic acid in the LNP consisting of an ionizable cationic lipid, cholesterol, phospholipid, and PEGylated lipid.

2. Results – Analytical and physicochemical

Preparative Chromatography

The below chromatogram was obtained purifying the LNPs using CIMac OH column, utilizing hydrophobic interaction chromatography (HIC) mode.

CIMmultus OH – 1 mL (6 µm)

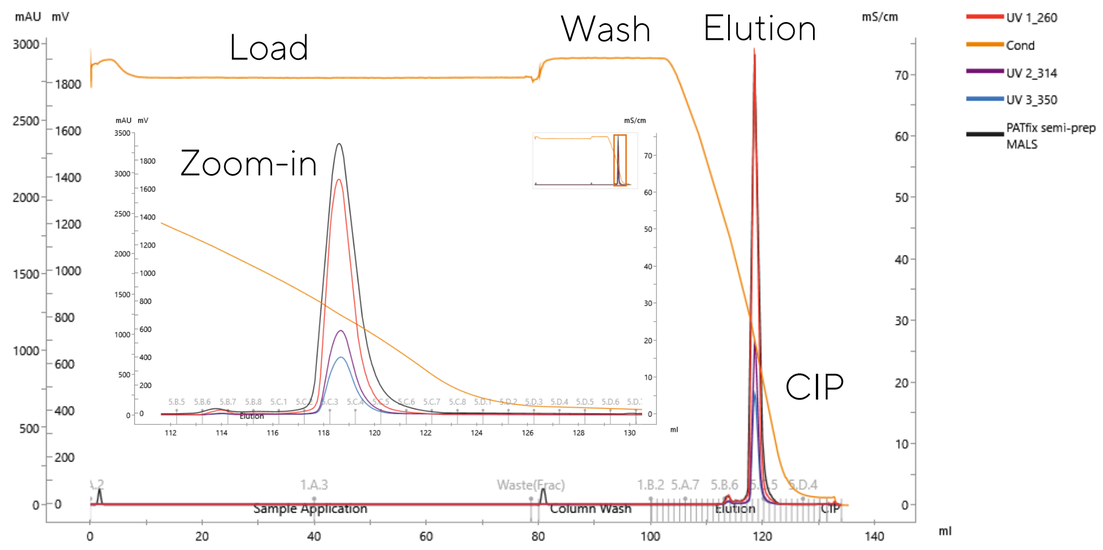

Figure 1: Chromatogram of an example of LNP purification. The sample is loaded onto the column, eluted in Elution step and the column is cleaned in CIP step. UV signal is used for detection at 260 nm, 314 nm and 350 nm and MALS detector at 90°.

Physicochemical characterization of mRNA-LNP formulations

The samples obtained from different processes were analyzed for their encapsulation efficiency using LNP Switcher setup: crude sample, UF and main peak fractions from 3 purification methods using different conditions. The main peak fractions were additionally frozen at -80 °C to determine stability.

	Crude	M1 Elution Peak	UF	M2 Elution Peak	M3 Elution Peak
Z_{avg} (d.nm)	136.2	97.2	94.5	102.4	103.5 nm
PdI	0.24	0.06	0.14	0.22	0.08
EE [%]	86.5	99.1	96.4	98.3	99.7
c [ng/µL]	4.7	42.2	21.6	8.3	36.2

Figure 2: Dynamic Light Scattering (average size and Polydispersity Index (PdI)) and LNP switcher (encapsulation efficiency and concentration) results.

Encapsulation efficiency of LNP samples and mRNA quantity inside LNPs was determined using PATfix® LNP Switcher Platform.

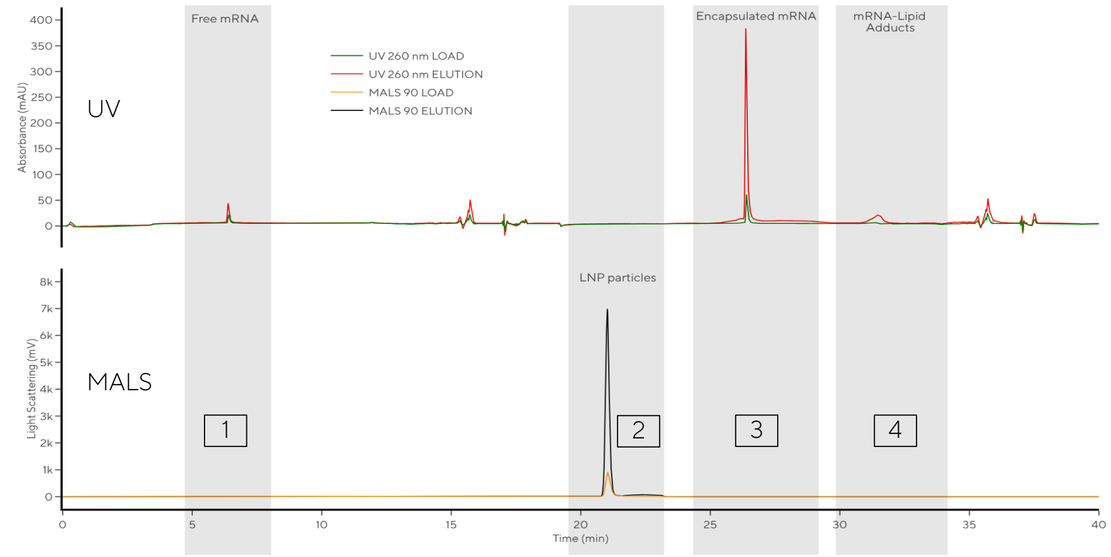

Figure 3: 1 – Free mRNA (21 % in LOAD sample and 5 % in ELUTION sample), 2 – LNP particles, 3 – Encapsulated mRNA, mRNA-Lipid Adducts. Chromatogram of an example LNP switcher run of load sample and elution fraction. UV signal is used for detection at 260 nm and MALS detector at 90° angle.

3. Results – *In Vitro* Protein Expression

In vitro tests were performed on two different cell lines (HEK-293 and Caco-2). Luciferase expression and cell toxicity were assessed.

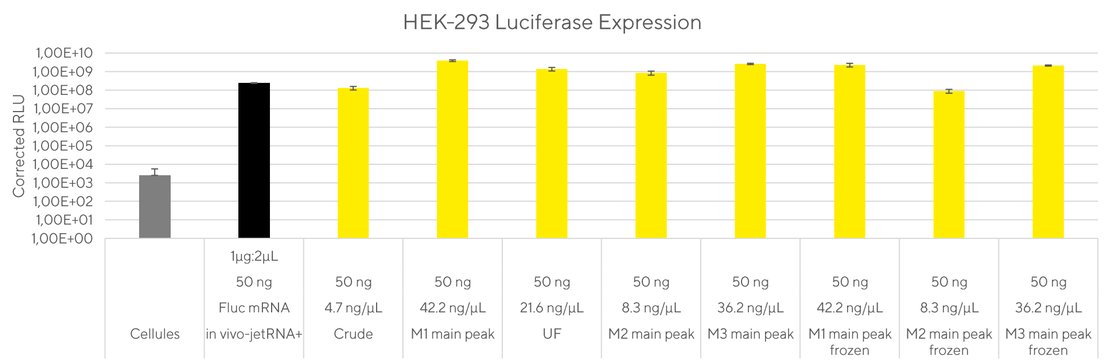

Figure 4: Luciferase expression of different LNP samples on HEK-293 cell line.

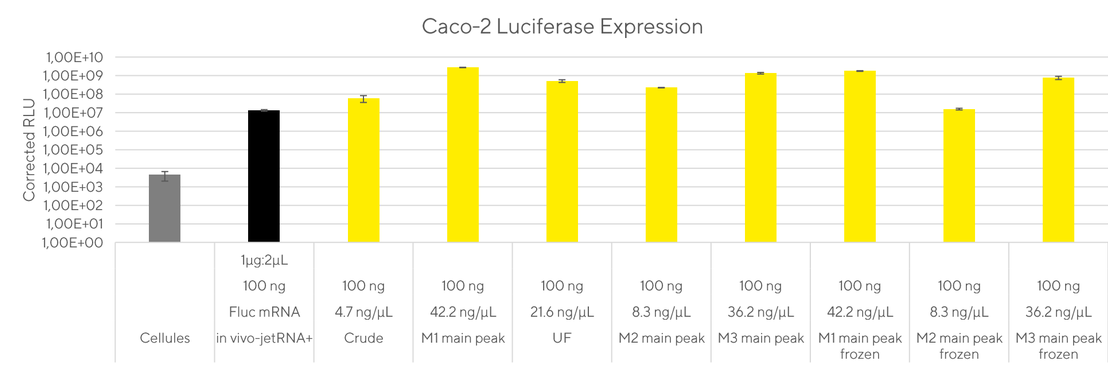

Figure 5: Luciferase expression of different LNP samples on Caco-2 cell line.

	Cellules	mFluc	Crude	M1 main peak	UF	M2 main peak	M3 main peak	M1 main peak frozen	M2 main peak frozen	M3 main peak frozen
HEK-293	100.0 %	93.2 %	88.5 %	89.3 %	95.3 %	88.5 %	98.4 %	91.3 %	94.0 %	98.8 %
Caco-2	100.0 %	100.2 %	96.1 %	97.1 %	101.5 %	92.0 %	103.6 %	98.0 %	97.4 %	99.2 %

Figure 6: Relative protein expression in % of LNP samples on HEK-293 and Caco-2 cell lines. Lower percent means higher cell toxicity. Samples show slightly higher toxicity on HEK-293 cell line,

Evident from *in vitro* protein expression, the best performing process is the M1 process, followed by the M3. This process gives particles that are relatively more active than the control ultrafiltration samples, even 3x more active in HEK-293 cell line and 5x more active in the Caco-2 cell line. This result is robust across cell lines. Even freezing the samples maintains the activity, especially in the process of the M3 where sucrose is added to chromatography.

Toxicity observed was not significant and was particularly non-toxic for the process M3, where sucrose is added to buffers.

4. Results – mRNA Recovery

The total mRNA recovery obtained through the purification process using CIMmultus OH column is 93% for the M1 process, while the UF process yields 73%. The lower mRNA recovery from the UF process is likely due to shear stress during centrifugation and the adhesion of the LNP particles to membranes and plastic vials. The unique characteristics of monoliths facilitate achieving significantly higher mRNA recovery compared to UF.

5. Conclusion

- Elution from the CIMmultus OH with reducing conductivity resulted in high recovery of the LNP particles. A robust recovery (based on RNA quantification) of >90 % is achieved. Alternative elution conditions with cryopreserving saccharose enabled freezing and long-term stable particles.
- Particles collected from the CIMmultus OH column show a higher encapsulation efficiency, smaller average size and lower PdI when compared with the control filtration process.
- Particles fractionated using the CIMmultus HIC column immediately after formulation shows up to three times higher *in vitro* protein expression and comparable particle toxicity comparing to control filtration and was robust across experiments and cell lines tested (HEK-293 and Caco-2).