Simplifying Progress

Monolithic Columns Enable Streamlined Downstream Processing and Analytics of Lipid Nanoparticles

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Introduction

Lipid nanoparticles (LNPs) are leading non-viral carriers for therapeutics, offering versatility in encapsulating diverse payloads. Their manufacturing superiority over viral systems allows for modularity, speed, and scalability. However, this modularity poses challenges in purification and characterization due to sample uniqueness. LNPs require downstream processing for in vivo application and adherence to critical quality attributes (CQAs). Analytical methods for those currently predominantly require undesirable particle disassembly beforehand.

Monolithic columns offer ideal chromatography for LNPs due to laminar flow, minimizing shear forces, and surface modification enabling selective options. Here is presented the purification method for LNPs on monolithic columns utilizing the PATfix® analytical chromatographic system, efficiently separating LNPs from free cargo.

An analytical-scale two-dimensional chromatographic tool was developed. It delivers comprehensive characterization of encapsulation efficiency, nucleic acid content, degradation, and separation of co-encapsulated cargos, without any sample pre-treatment. Highly tunable and automatable, this method maximizes efficiency and facilitates precise separation of LNP populations.

1. Experimental setup

- LNP-mRNA Encapsulation

mRNA of 4000 nucleotides (100 µg/mL in 25 mM sodium citrate, pH 3.5) 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 1.5 mol%; 12.5 mM in EtOH) in a flow rate ratio of 3:1, and a total flow rate of 12 mL/min. LNP product (1.05 mL) was immediately diluted 10-fold in PBS.

- LNP-mRNA purification

Utilizing the PATfix chromatographic system and software, the LNP product was loaded onto a CIMac™ OH column and mixed in-line with 2x loading buffer (containing high concentration kosmotropic salt). The purification process was monitored using UV and MALS detectors. Upon loading the sample and washing the column, a step elution was conducted to elution buffer B (low conductivity buffer), which eluted most of the particles. To completely unbind all species, cleaning buffer C was applied after. Fractions were collected and analyzed.

- 2D chromatographic analysis (HIC+IP-RP-HPLC) – LNP Switcher

On PATfix, an analytical amount of LNPs is diluted in loading buffer (containing kosmotropic salt) and loaded onto a CIMac OH column. LNPs bind to the column, while free mRNA flows through towards CIMac™ SDVB column, where it is mixed with a buffer to maintain IP-RP conditions.

Free mRNA is eluted form the SDVB column by increasing acetonitrile composition, facilitating its characterization by UV. Subsequently, LNPs are eluted from the OH column by reducing conductivity and mixed to maintain IP-RP conditions. Passing through a MALS detector enables particle detection and size determination.

LNPs bind to the SDVB column and upon increasing the acetonitrile concentration, the particles dissolve, allowing the mRNA to be eluted and subsequently detected by a UV detector.

Figure 1: PATfix LC based chromatographic system.

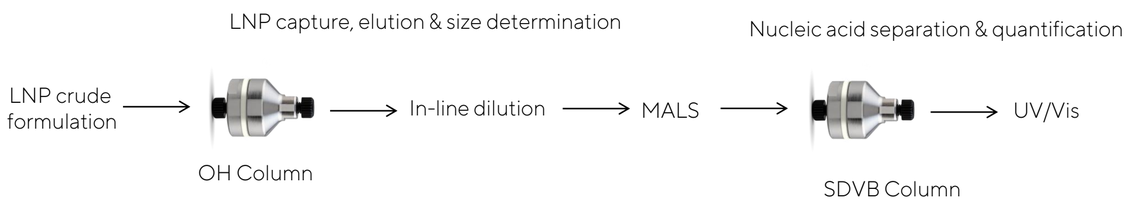

Figure 2: Schematic diagram of the setup for the 2D chromatographic analysis – LNP switcher.

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

2. Results - purification

The below chromatogram was obtained for purification of LNPs on an CIMac OH column, utilizing hydrophobic interaction chromatography (HIC).

CIMac OH column

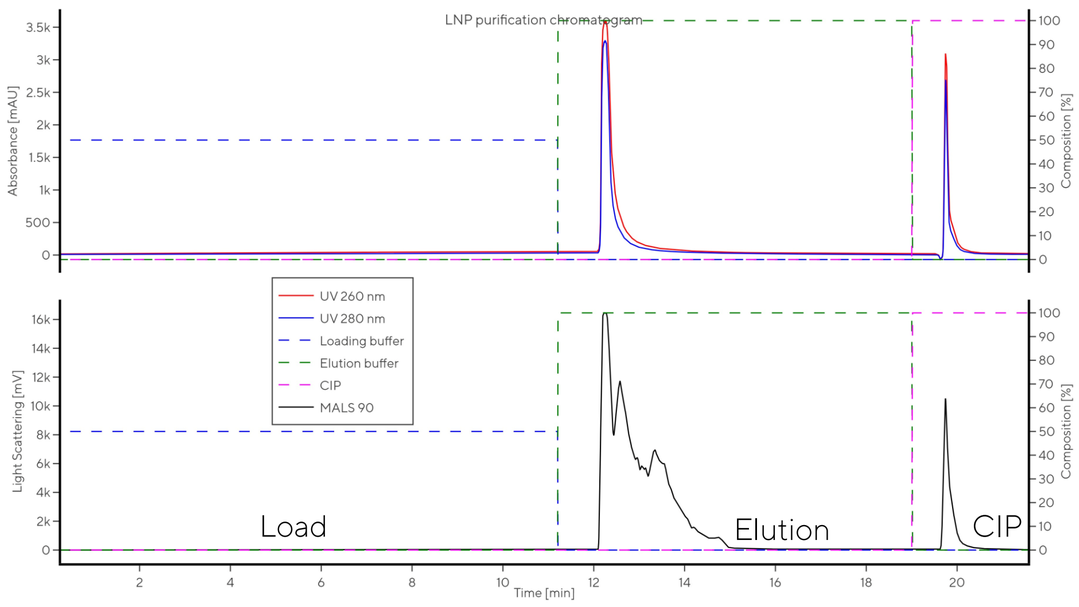

Figure 2: Chromatogram of an example LNP purification. The sample is loaded onto the column in LOAD step, eluted in ELUTION step and the column is cleaned in CIP step. UV signal is used for detection at 260 nm and 280 nm and MALS detector.

3. Results - analytics

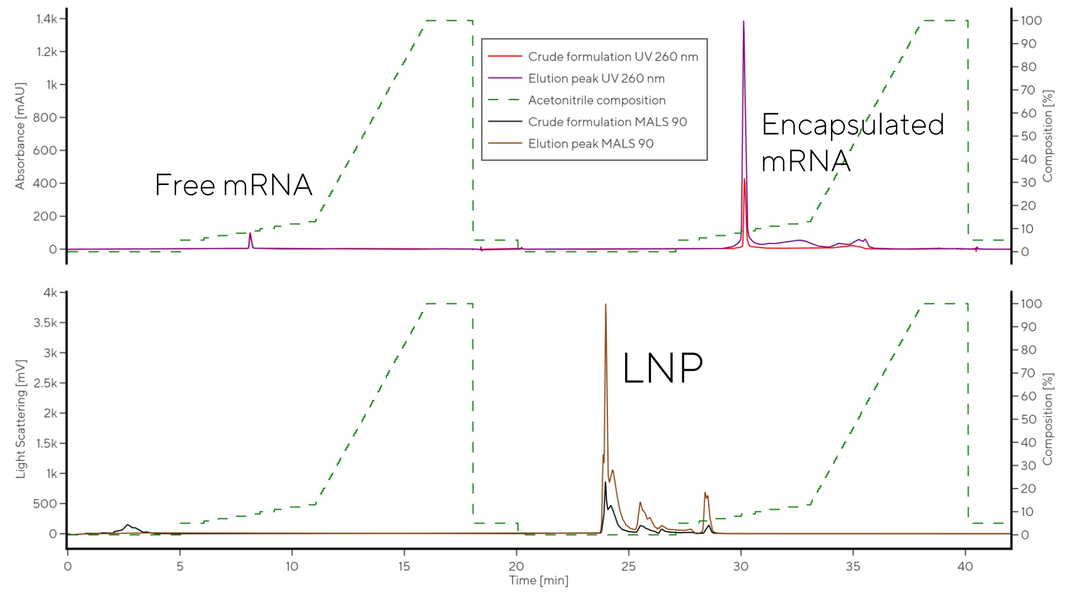

Figure 4: Analytical chromatogram of crude formulation and pure elution peak of LNPs from the above described 2D chromatography. In the UV signal, free and encapsulated mRNA is detected, while in the MALS we can observe the LNPs as particles.

In Figure 4, an overlay of chromatograms is shown of the crude formulation (red UV and black MALS line) and of the collected elution peak (purple UV and brown MALS line) from purification. First part of the chromatogram shows a peak of free mRNA, while the second part of the chromatogram shows encapsulated mRNA.

Purifying LNPs on monolithic column increases encapsulation efficiency.

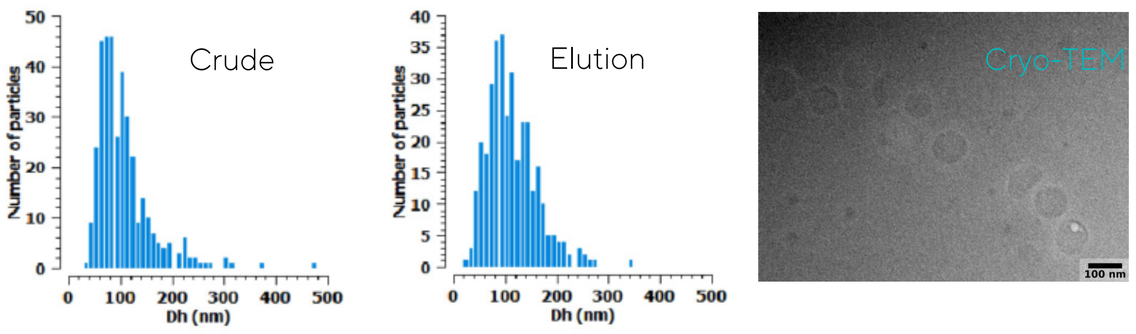

Figure 5: Size distribution of: (a) crude sample and (b) collected elution peak, by Videodrop measurement. (c) A Cryo-TEM image of the collected elution peak.

In Figure 5, it can be observed that offline size determination with Videodrop shows size is maintained and particles are concentrated. Online size determination with MALS correlates well with offline techniques.

Cryo-TEM images of elution peak show well formed, electron-dense particles.

Sample	Encapsulation efficiency	Mean size-Videodrop	MALS size	Concentration	mRNA mass
Crude formulation	85%	107 nm	110 nm	3,06 E+10	117 µg
Elution peak	97%	114 nm	114 nm	6,03 E+10	111 µg

Table 1: Offline analysis of collected LNP fraction during purification.

4. Results – selectivity difference of columns

Diverse physicochemical properties can be explored to analyze and purify LNPs on monolith, such as anion exchange chromatography (AEX) on CIMac™ QA column.

CIMac QA column

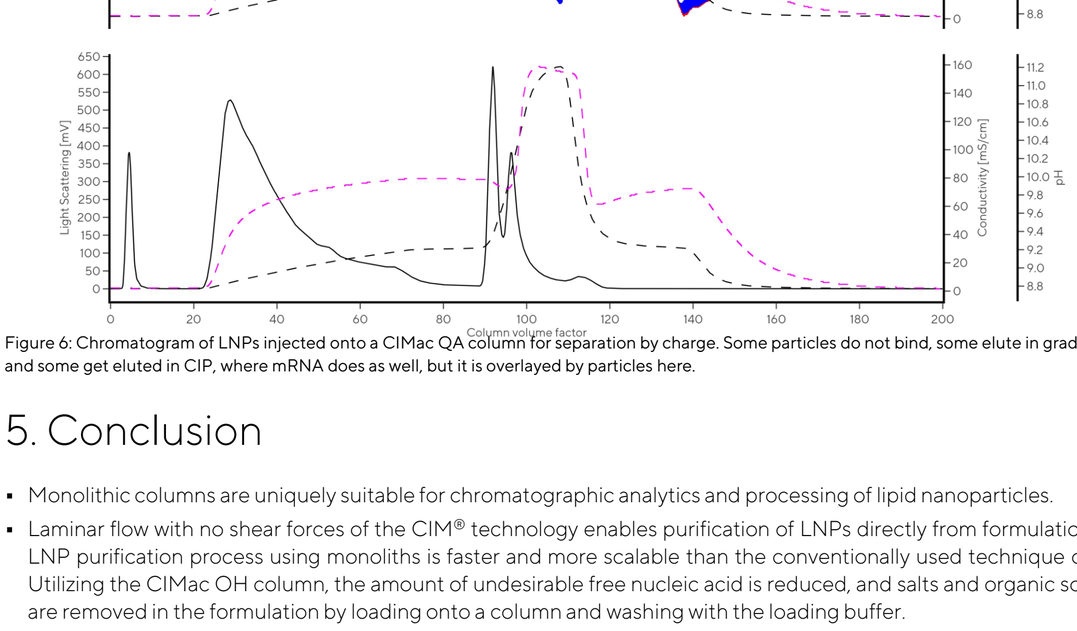

Figure 6: Chromatogram of LNPs injected onto a CIMac QA column for separation by charge. Some particles do not bind, some elute in gradient, and some get eluted in CIP, where mRNA does as well, but it is overlayed by particles here.

5. Conclusion

- Monolithic columns are uniquely suitable for chromatographic analytics and processing of lipid nanoparticles.
- Laminar flow with no shear forces of the CIM® technology enables purification of LNPs directly from formulation. The LNP purification process using monoliths is faster and more scalable than the conventionally used technique of TFF. Utilizing the CIMac OH column, the amount of undesirable free nucleic acid is reduced, and salts and organic solvents are removed in the formulation by loading onto a column and washing with the loading buffer.
- A 2D chromatographic tool LNP switcher was developed that provides encapsulation efficiency, mRNA quantification and size determination in a single injection without any sample pretreatment.
- Different column chemistries can be utilized for the desired selectivity, as shown by the example of HIC and AEX chromatography.