Simplifying Progress

Monoliths for a Novel Selective and High Recovering Purification Process of LNP-based Biopharmaceuticals

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Introduction

Lipid nanoparticles (LNPs) provide the most advanced platform for in vivo drug delivery of nucleic acids. [1] However, they have not been yet developed into a well-characterized biopharmaceutical. The challenges are both of biological and CMC nature.

Filtration methods have been implemented for scale-up of downstream processing for the Comirnaty and the Spikevax vaccines [2]. It is known in the field that these methods can be difficult to optimize and can give encapsulated RNA recoveries around 50%. Thus, we implemented CIM Convective Interaction Media® monolithic columns as they are uniquely suitable for LNP separations due to the lack of shear stress imparted by them. Mobile phases for purification are optimized for high recovery, stability, and functionality. LNPs are loaded onto the columns under kosmotropic conditions, directly following the encapsulation process and neutralization. Elution produces a high recovery collection of particles. The process is performed up to 16-times faster than comparable processes. This process achieves the desired concentration, ethanol removal and buffer exchange functions. In addition to those, free, non-encapsulated, RNA is removed by this process by tuning the buffers and achieving chromatographic separation.

Compared to standard processes, this novel chromatographic method demonstrates superior activity and uniformity due to reduced size distribution and enhanced activity. Additionally, analytical methods on other monolithic columns enable the characterization of such formed drug products. Key characteristics being obtained are encapsulation efficiency, mRNA quantity and purity inside LNPs, mRNA-lipid adduct quantity [3] and lipid purity.

1. Experimental setup

- LNP Assembly

mRNA of 2000 nucleotides (mFluc, 100 µg/mL in 25 mM sodium acetate, pH 5.0) was encapsulated into lipid nanoparticles using Knauer IJM Nanoscaler. The aqueous stream was mixed using microfluidic technology with a stream of lipidic solution in an N/P ratio of 6.0 (SM-102: 50 mol%, Cholesterol 38.5 mol%, DSPC 10.0 mol%, DMG-PEG 2K 1.5 mol%; 15 mM in EtOH) in a flow rate ratio of 3:1, and a total flow rate of 12 mL/min. LNP product was immediately diluted 10-fold in 50 mM TRIS, pH 7.4.

- Chromatographic Purification of LNP Drug product using CIMmultus® OH 6 µm

The LNP product was loaded onto a CIMmultus OH column and mixed in-line with 2x loading buffer (containing 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 analysed.

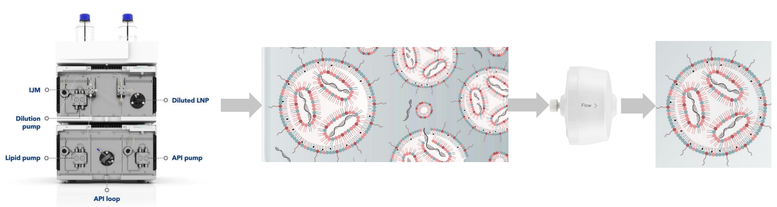

Figure 1: Encapsulation of LNPs followed by purification using CIMmultus OH columns produces a purified LNP particle. [4]

- Reference TFF Process

The LNP product was loaded onto a Sartorius Hydrosart 300kDa ECO (50cm2) membrane and diafiltrated for 5 DV into 15 mM TRIS, 150 mM NaCl and another 5 DV volumes to 15 mM TRIS, 150 mM NaCl, 240 mM sucrose, pH 7.4. Upon completion, the product was diafiltrated. The TMP during processing was 3.7-4.7 psi at a flow of 15 mL/min. Product was collected and analysed.

2. Results – preparative chromatography

A purification was successfully performed to collect the most active fractions of the LNP drug product. The recovery of the purification in regard to total RNA was 92%. The E1 and E6 fractions are less active in terms of luciferase production. This can mean either more empty particles or particles that are either too small or too big, respectively, to properly transfect the cells. There is no response in CIP, showcasing a good recovery of the process.

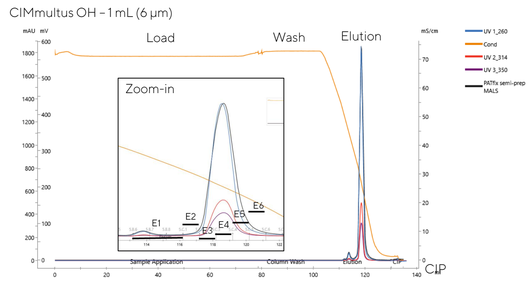

Figure 2: Chromatogram of an example of LNP purification. The sample is loaded onto the column, eluted in Elution step and the column is cleaned in CIP step. UV signal is used for detection at 260 nm, 314 nm and 350 nm and MALS detector at 90°.

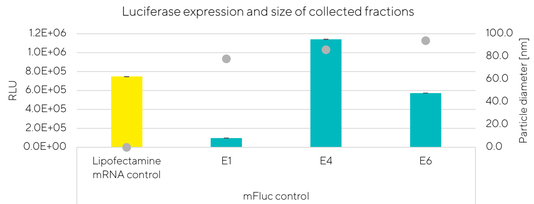

Figure 3: Fractions collected from the chromatographic purification of LNPs show that the most active portion of the drug product can be collected and the less active portions removed. The size increases across the peak, showcasing the size separation properties of the CIM OH column.

3. Comparison of Chromatographic Purification and TFF

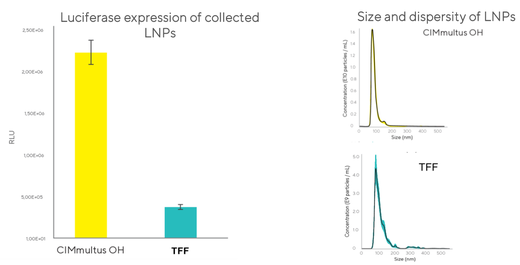

Figure 4: Comparison of LNP Drug product purification with TFF and OH column.

An LNP sample was produced and purified 2 different ways. Utilising the chromatographic purification method, the recovery was nearly quantitative, and the particle and its size distribution were small. The TFF purification with flat sheets gave particles of a broader size distribution that were also less active in terms of luciferase production. The concentration factor was also much higher using a step gradient for collecting the particles and getting a concentration of nearly 2 mg/mL. Ideally, these particles would dilute down with the appropriate dilution buffer to form the final formulation. Using the monolith also gives you an option to operate and sterile conditions and avoid sterile filtration.

Table 1: Comparison of LNP Drug product purification with TFF and OH column.

	CIMmultus OH	TFF
Diameter [nm]	84	91
Size distribution	Narrow	Some larger fractions
EE [%]	99.0	98.0
c [ng/µL]	1700	117
Total mRNA recovery	>95%	87%

4. Column capacity and scalability

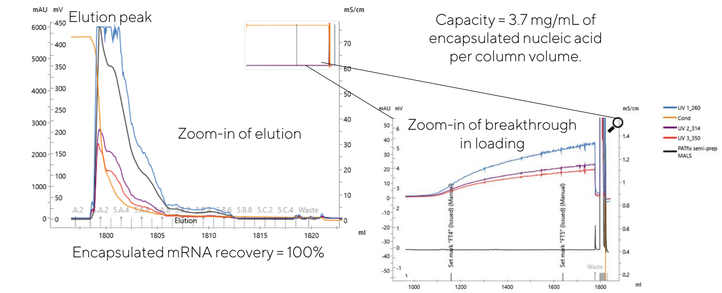

Figure 5: Study of capacity of the OH column; a chromatogram of elution and zoom-in of the breakthrough curve.

A CIMmultus OH 1 mL column with 6 µm pores was loaded with mRNA-LNP particles until breakthrough occurred. The dynamic binding capacity (DBC) for this process was determined to be 3.7 mg/mL of encapsulated nucleic acid or 90 mg/mL with respect to total mass of LNP.

Figure 6: Full scale range of capacity using the same process.

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 >95 % is achieved.
- 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 TFF and was robust across experiments and cell lines tested (HEK-293 and Caco-2).
- The capacity of the column is 3.7 mg/mL of encapsulated mRNA, offering a wide range of scales from micrograms to hundreds of grams utilising the inherent scalability of monolithic columns.

References

[1] Sharma et al, Adv Drug Deliv Rev, 2024
[2] Geng et al, JCR, 2023
[3] Packer et al, Nat Commun, 2021
[4] https://www.knauer.net/lnp-nanoscaler

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