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Monoliths for a Novel, Selective and High-Recovery 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 yet been developed into a well-characterized biopharmaceutical. Manufacturing challenges include the proper formation of the drug product, maintaining its integrity, achieving sufficient recovery, and obtaining a well-separated and characterized product. The focus of characterization should be on determining drug substance purity, excipient purity, and the entire drug product purity.

Filtration methods have been implemented for scale-up of downstream processing for the Comirnaty and the Spikevax vaccines [2]. It is well known in the field that these methods can be challenging to optimize, yielding encapsulated RNA recoveries of 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, the purely laminar flow, and the range of possible chemically modified surfaces. Analytical methods on other monolithic columns enable the characterization of such formed drug products. Key characteristics being obtained include encapsulation efficiency, mRNA quantity and purity within LNPs, mRNA-lipid adduct quantity [3], and lipid purity.

Monolithic columns can be used for efficient purification of LNPs, as an alternative to standard processes (TFF and dialysis). Mobile phases for purification are optimized for high recovery, stability, and functionality. Compared to standard processes, the novel chromatographic method using CIM monoliths demonstrates superior activity and uniformity due to reduced size distribution and enhanced activity. LNPs are loaded onto the columns under kosmotropic conditions, directly following the encapsulation process and neutralization. Elution results in a high recovery of particles. The process is performed up to 16 times faster than comparable processes (e.g. TFF, dialysis). 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.

This process paves the way for a more controlled and advanced purification of LNP-based drug products. Increasing complexity to tackle the most challenging healthcare challenges, such as cancer, immune diseases, and congenital genetic diseases, will require more effective purification and characterization methods. Adding targeting moieties to the surface of the LNP is a complex reaction that is currently not properly controlled, and monolithic columns offer a unique solution for such separations.

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, 314 and 350 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.

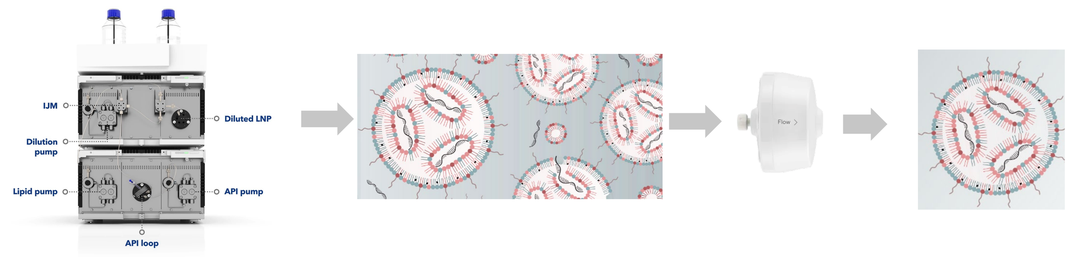

Figure 1: Encapsulation of LNPs followed by purification using CIMmultus OH columns produces a purified LNP particle. [4]

- **Reference TFF Process**

The assembled LNP sample was exposed to a Sartorius Hydrosart 300kDa ECO (50cm²) membrane and diafiltrated for 5 DV into 15 mM TRIS, 150 mM NaCl, pH 7.4 and another 5 DV to 15 mM TRIS, 150 mM NaCl, 240 mM sucrose, pH 7.4. Upon completion of diafiltration, the product was concentrated and collected, including 1 hold-up volume of flush.

2. Results – preparative chromatography

CIMmultus OH – 1 mL (6 µm)

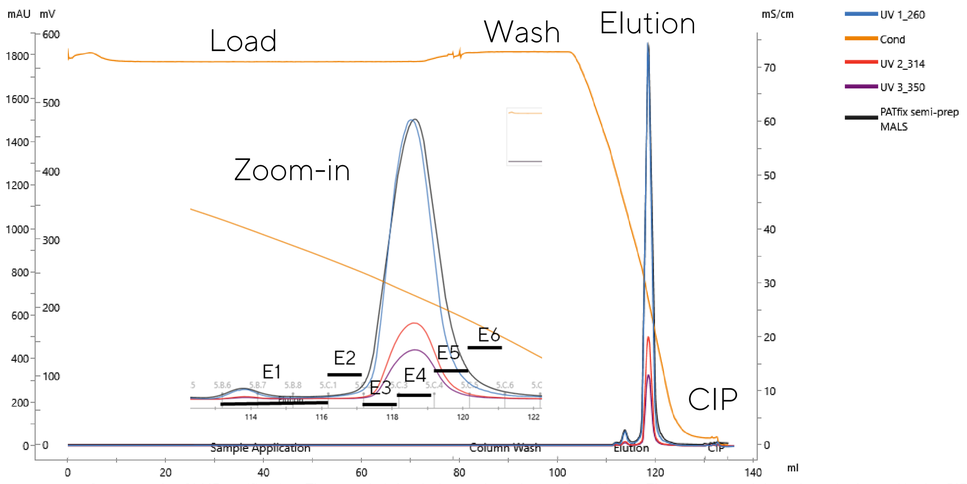

Figure 2: Chromatogram of an example of LNP purification. The sample is loaded onto the column, eluted in the Elution step, and the column is cleaned in the CIP step. The UV signal is used for detection at 260 nm, 314 nm, and 350 nm, and the MALS detector at a 90° angle.

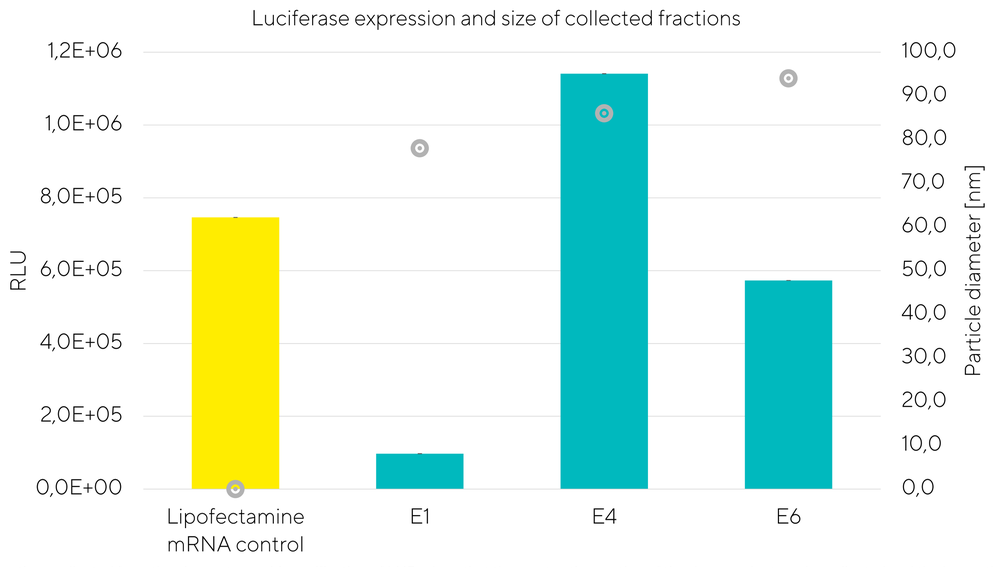

Figure 3: Fractions collected from the chromatographic purification of LNPs show that the most active portion of the drug product can be collected and the less active portions removed. The size increases across the peak, showcasing the size separation properties of the CIM OH column,

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.

3. Comparison of Chromatographic Purification and TFF

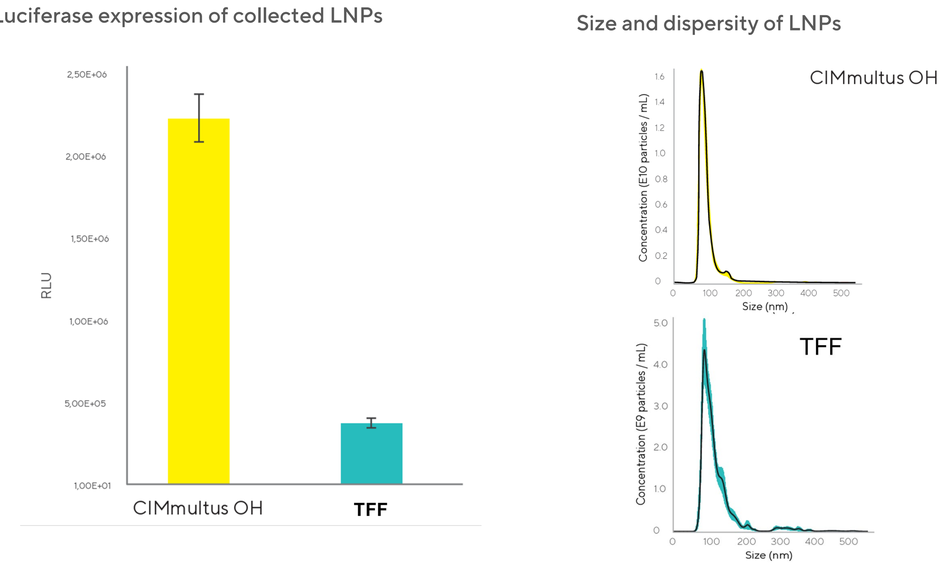

Figure 4: Comparison of LNP Drug product purification with TFF and OH column.

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%

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 be diluted down with the appropriate dilution buffer to form the final formulation. Using the monolith also gives you an option to operate in sterile conditions and avoid sterile filtration.

4. Column capacity and scalability

Capacity = 3.7 mg of encapsulated nucleic acid per 1 mL of column volume.

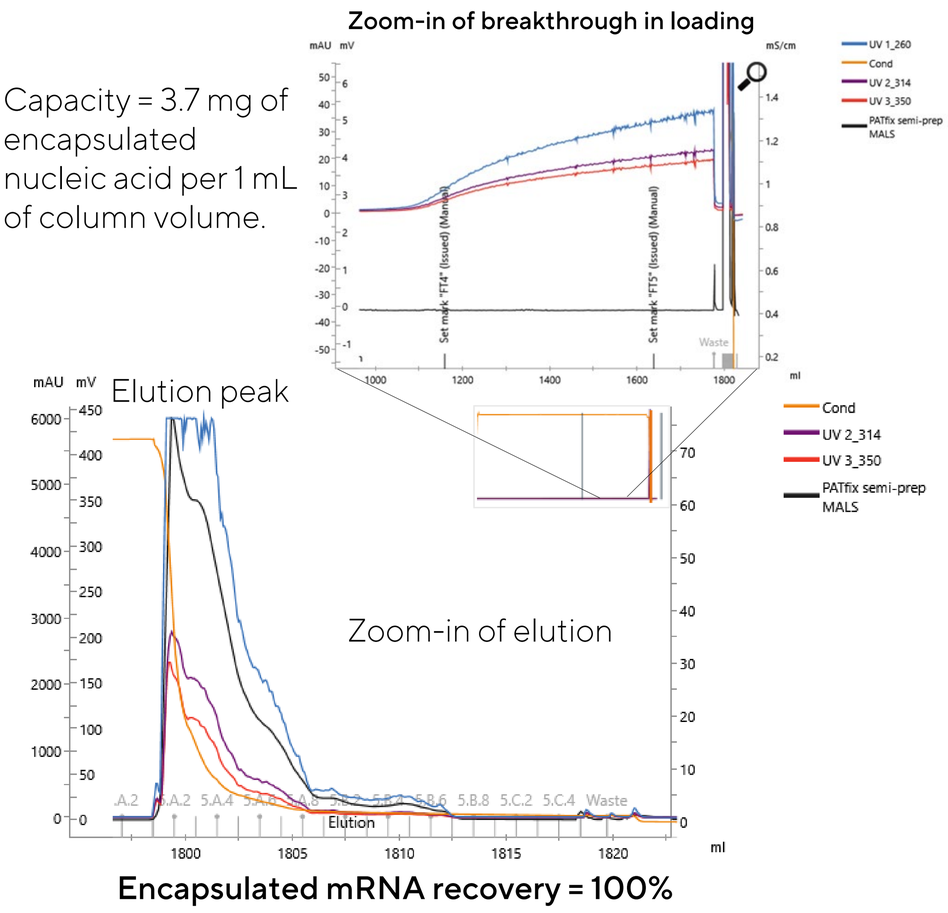

Figure 5: Chromatogram of preparative chromatography for purification of LNPs, showing the breakthrough in flowthrough at capacity and the elution peak of the particles.

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.

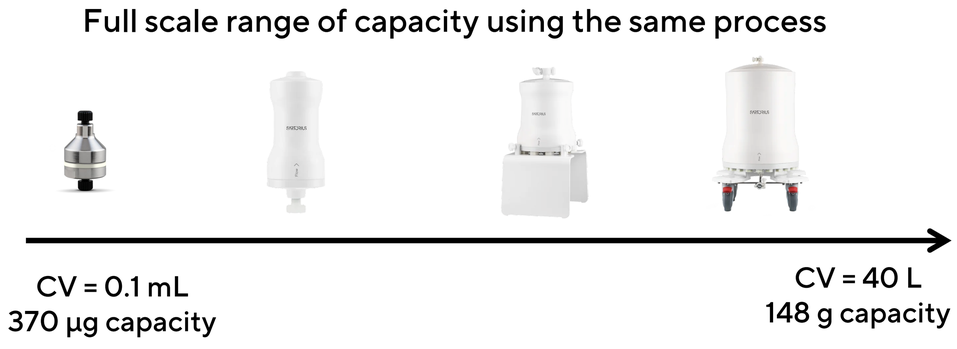

Figure 6: Capacity for LNP purification on CIM OH columns and the ranges of purification scales possible using the columns in BIA portfolio.

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 show up to three times higher *in vitro* protein expression and no particle cytotoxicity compared to TFF. They were robust across experiments and cell lines tested.
- The capacity of the column is 3.7 mg of encapsulated mRNA per 1 mL of column volume, offering a wide range of scales from micrograms to hundreds of grams, utilising the inherent scalability of monolithic columns.