Simplifying Progress

A Chromatographic Tool to Comprehensively Characterize Complex Novel RNA-LNP Drug Products

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Introduction

Lipid nanoparticles (LNPs) have emerged as the most advanced drug delivery system for gene therapies and vaccines, due to their ability to encapsulate a diverse range of payloads, including nucleic acids and proteins. They provide protection from enzymatic degradation, enhanced cellular uptake, and controlled release.

The complex composition and inherent instability of LNPs pose significant challenges for their characterization, particularly in assessing encapsulation efficiency, payload integrity, and size distribution. To address these challenges, we have developed a novel two-dimensional chromatography (2D) method capable of simultaneously evaluating key parameters of the LNP formulations. The method utilizes a switching chromatography system with two distinct analytical columns, independent pumps, and multiple detectors including an ultraviolet-visible detector (UV-VIS) and a multi-angle light scattering detector (MALS). This configuration enables the direct injection and analysis of the LNPs without prior sample preparation. The 2D technique facilitates the accurate quantification of both encapsulated and non-encapsulated nucleic acids, the assessment of payload integrity, and measurement of the LNP size and size distribution.

The findings indicate that this approach provides a comprehensive and efficient analytical platform, thereby advancing the understanding and development of robust lipid-based drug delivery systems.

1. Chromatographic setup

2D chromatographic analysis – PATfix® LNP Switcher Platform

PATfix LNP Switcher does not require any sample pre-treatment. Samples are only diluted with the loading buffer and applied to the column. In the first step, the LNPs bind to the CIMac OH column, while non-encapsulated RNA flows through towards the CIMac SDVB column, where it is in-line diluted with the binding buffer. Non-encapsulated mRNA is eluted from the SDVB column by increasing acetonitrile concentration, facilitating its quantification by the UV-Vis detector.

Subsequently, LNPs are eluted from the OH column by reducing conductivity, passing the MALS detector to enable particle detection and size determination and after in-line diluted to the binding conditions for the SDVB column. Upon increasing the acetonitrile concentration, the particles adsorbed on the SDVB column dissolve, allowing the mRNA to be eluted and subsequently detected by a UV detector.

This configuration of the 2D chromatographic system enables a simultaneous determination of crucial LNP parameters in an all-in-one analytical assay:

1. RNA quantification
2. Encapsulation efficiency
3. Particle size and size distribution
4. RNA integrity
5. Detection of RNA-lipid adducts

Figure 1: PATfix 2D chromatographic system.

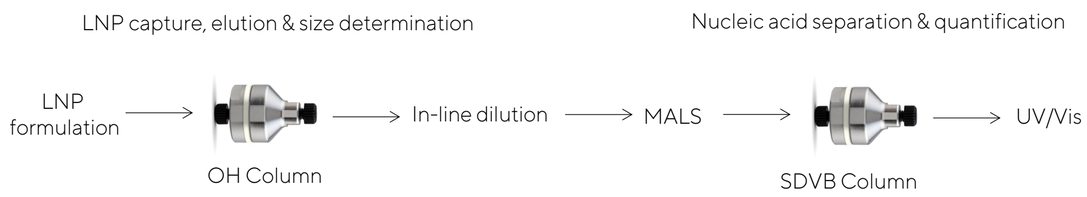

Figure 2: Schematic diagram of the setup for the 2D chromatographic analysis – LNP switcher.

2. Typical 2D chromatogram

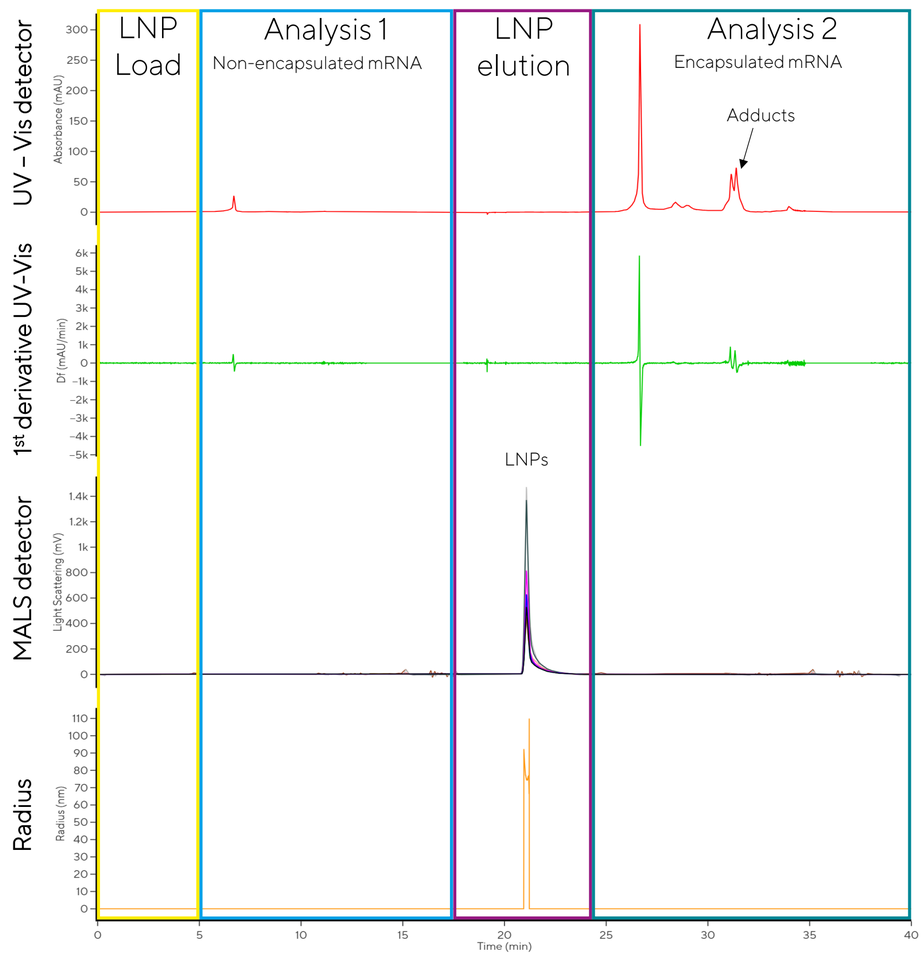

Figure 3: Typical chromatogram of LNPs encapsulating mRNA (4000 nt).

3. Analyzing Complex LNP Formulations

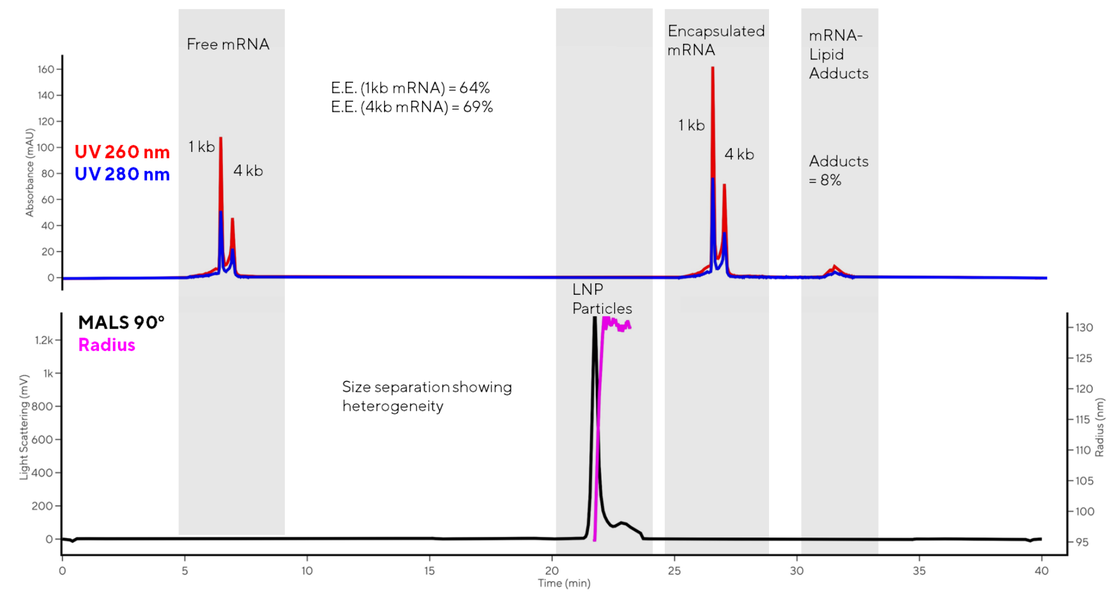

Figure 4: An LNP Switcher chromatogram showing analysis of an LNP with co-encapsulated cargo of 2 different mRNAs.

Encapsulating multiple cargos into one formulation introduces additional complexity into LNP drug products. Using two mRNAs is common for novel CAR-T applications, requiring chromatographic separation to achieve accurate determination. This chromatogram shows the separation and accurate quantification of a 1 kb and 4 kb mRNA. Additionally, separation on the OH column shows heterogeneity of particles, with multiple subpopulations within such samples.

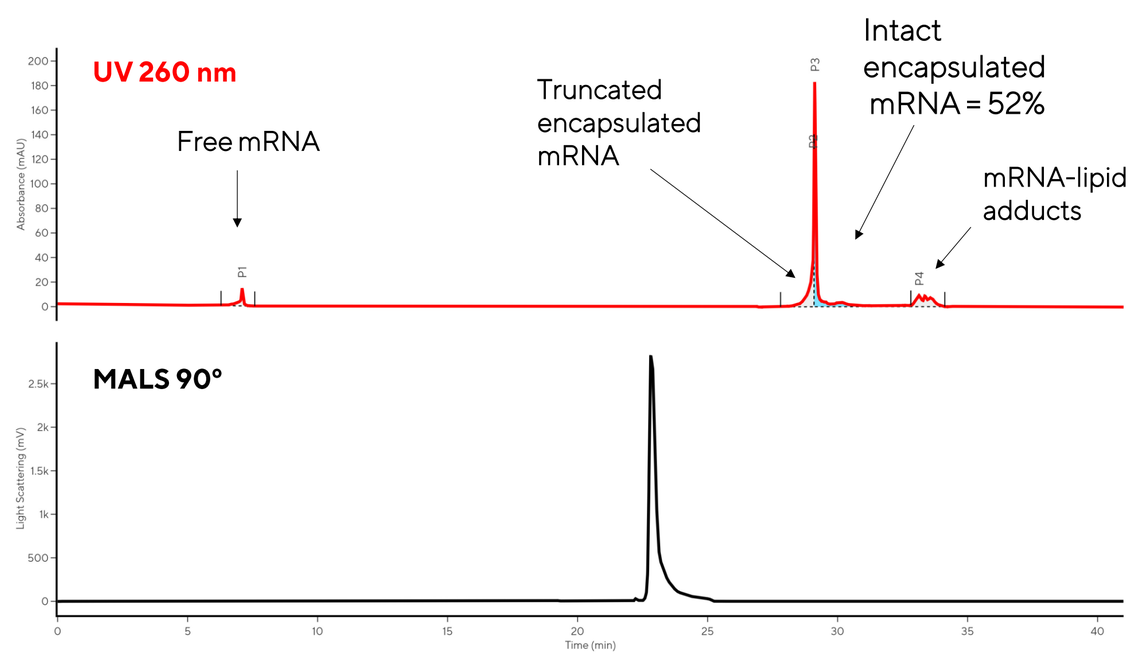

Figure 5: An LNP Switcher chromatogram showing the actual effective mRNA represents only 52% of the total mRNA, even though encapsulation efficiency is 94%.

The actual amount of effective mRNA within LNP samples can be misleading if not using appropriate orthogonal analytics. Taking into account the free mRNA, the fragmented shorter mRNAs and the mRNA-lipid adducts, the actual effective mRNA normally represents <50% of the sample.

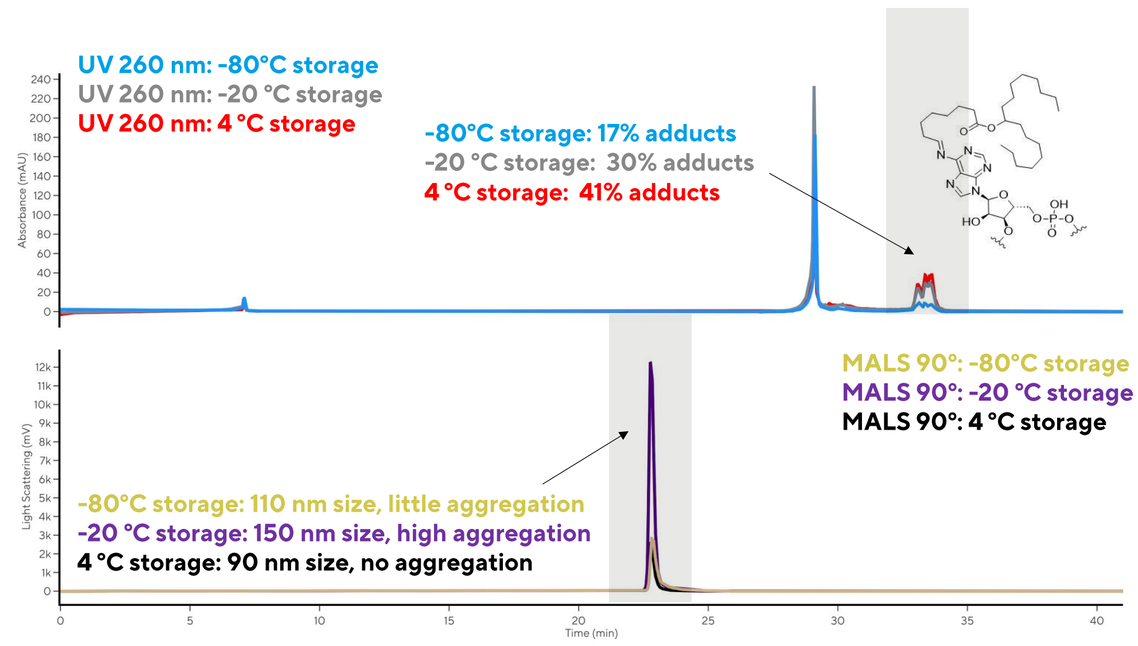

Figure 6: An LNP Switcher chromatogram showing the effect of storage temperature on the percentage of RNA-lipid adduct formation and particle size.

RNA-lipid adduct formation can be monitored effectively using chromatography on the SDVB and shows that it is highly temperature dependent, with increasing ionizable lipid degradation and adduct formation at higher temperatures. Additionally, MALS detector can be useful for observing aggregation, such as here at non-optimal 20 °C.

4. Conclusion

- A 2D chromatographic tool, the PATfix LNP Switcher Platform, has been developed and provides information of encapsulation efficiency, mRNA quantification, size determination, mRNA fragmentation and adduct detection in a single injection without sample pre-treatment.
- Using this chromatographic tool, we show how LNP formulations can be comprehensively characterized by all-in-one orthogonal analytics that monitor crucial parameters which can be then better correlated with *in vitro* behaviour.
- This gets exceedingly more important with complex formulations like multiple cargo encapsulation.
- This 2D chromatographic tool demonstrates great potential for use in new LNP formulation development, LNP formulation quality control and LNP formulation stability studies.