Redefining The Characterization Paradigm of RNA Lipid Nanoparticles

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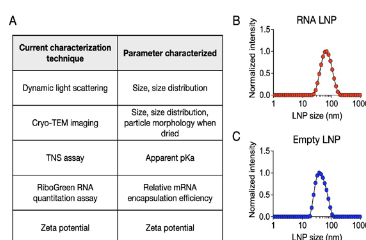
Lipid nanoparticles (LNPs) are potent delivery vehicles that have accelerated the translation of RNA therapeutics and led to the FDA-approval of mRNA COVID-19 vaccines. However, one major barrier that has prevented researchers from unlocking the full potential of LNP-based therapies is the low sensitivity of current characterization techniques, which rely on bulk analysis of LNPs. These techniques cannot evaluate essential parameters, such as RNA loading distribution, particle morphology in ambient conditions, and the percentage of unloaded LNPs, which are critical features to monitor for pharmaceutical development. In this study, we combine standard characterization techniques with a multitude of advanced characterization techniques, including multi-wavelength analytical ultracentrifugation (MWL- AUC), size exclusion chromatography-small angle X-ray scattering (SEC-SAXS), and size exclusion chromatography-multi-angle light scattering (SEC-MALS), to: (1) compare the resolution of each technique and (2) elucidate the advantages and disadvantages for each technique for LNP analysis. As a demonstration of the enhanced resolution of these advanced characterization techniques, we will compare clinically relevant LNP formulations when prepared by either microfluidic rapid mixing or bulk mixing, which are two common methods for small-scale LNP production. With current characterization techniques, it is difficult to assess the quality of new formulation methods; however, these advanced characterization techniques were able to provide insight into how these preparation methods affects LNP structure and RNA loading. Additionally, by combining these techniques together, we demonstrate the effect of mRNA size on RNA loading distribution. The characterization techniques employed here can enhance our understanding of LNP structure-property-function relationships and enable researchers to precisely define their RNA LNP products, which can improve LNP quality and potentially accelerate pharmaceutical development.



Current characterization technique	Parameter characterized
Dynamic light scattering	Size, size distribution
Cryo-TEM imaging	Size, size distribution, particle morphology when dried
TNS assay	Apparent pKa
RiboGreen RNA quantitation assay	Relative mRNA encapsulation efficiency
Zeta potential	Zeta potential

Figure 1. Current characterization techniques for LNPs. (A) Table of current techniques and the parameters quantified. (B-C) Representative intensity-based dynamic light scattering plots showing the polydispersity of RNA LNPs (B) or empty (unloaded) LNPs (C).

Figure 1

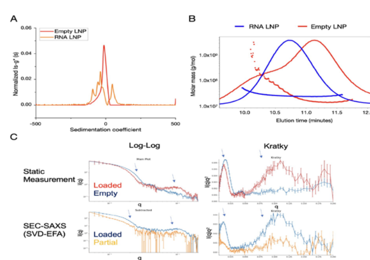


Figure 2. Differences in LNP physical properties detected by advanced characterization techniques between RNA LNPs and empty (unloaded) LNPs. (A) Multi-wavelength sedimentation velocity analytical ultracentrifugation analysis of empty LNPs (red) and RNA LNPs (orange); from this graph, approximately 70% of LNPs present in the RNA LNP samples do not contain RNA. (B) Size exclusion chromatography-multi-angle light scattering (SEC-MALS) analysis of RNA LNPs (blue) and empty LNPs (red). (C) Size exclusion chromatography-small angle X-ray scattering (SEC-SAXS) was used to analyze RNA LNP and empty LNP samples. There is evidence of partially loaded species in RNA LNPs.

Figure 2