Why multifaceted physicochemical characterization is key to ensure the stability of your mRNA-LNP drug product

By **Sarah Vandenberghe**
Scientist Formulation

Physicochemical stability is not merely a desirable attribute but an indispensable requirement to ensure the efficacy, safety, and shelf life of mRNA-LNP-based therapeutics. Changes in particle attributes (e.g. size, ultrastructure), mRNA release or decay can all severely impact the functionality of the drug product.

Given the susceptibility of mRNA-LNP formulations to instability, there is a pressing need for characterization assays capable of monitoring their physicochemical and chemical stability over time. Standard methods often lack the sensitivity and specificity required to accurately assess LNP stability. More comprehensive orthogonal techniques capable of generating an in-depth profile of critical quality attributes provide invaluable insights into the structural integrity and colloidal stability of mRNA-LNP complexes. Such characterization profiles can facilitate formulation optimization, storage condition selection, and quality control throughout the development and manufacturing process of LNP products.

This white paper describes a case study which considered identical mRNA-LNPs in two distinct storage buffers (buffer 1 and buffer 2). Four different assays were used to discern the physicochemical characteristics of the mRNA- LNP formulations. Forced degradation of the two formulations was achieved by subjecting them to multiple freeze-thaw (FT) cycles. A comprehensive assessment was conducted, focusing on critical parameters including particle size and distribution, encapsulation efficiency, and LNP morphology (Figure 1).

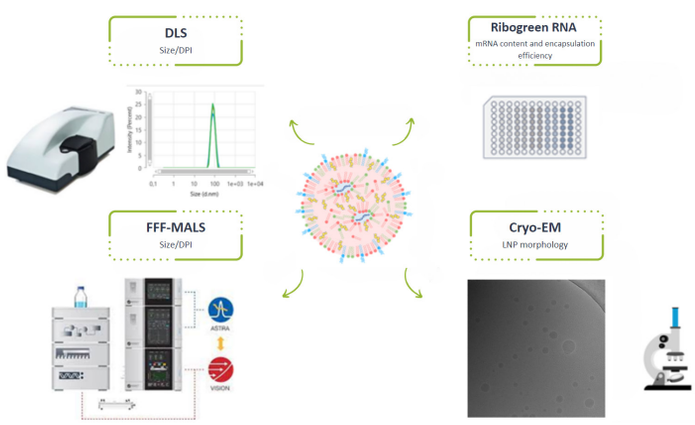

Figure 1

Overview of the characterization assays used to evaluate the stability profile of mRNA-LNPs

Dynamic light scattering (DLS) revealed an increase in particle size and polydispersity index (PDI) for buffer 1, suggesting the occurrence of aggregation. In stark contrast, buffer 2 demonstrated remarkable stability, with no discernible changes in particle size or distribution observed over the course of multiple freeze-thaw (FT) cycles. Even after four FT cycles, the formulation remained homogeneous and monodisperse (Figure 2).

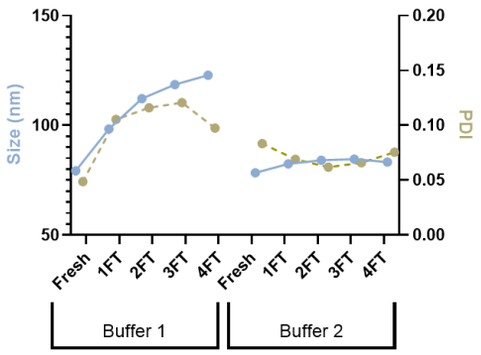

Figure 2

LNP size (Z-average) and PDI for the two storage buffers over multiple FT cycles

This observation was confirmed by analysis of field-flow fractionation coupled with multi-angle light scattering (FFF-MALS). FFF-MALS is a more sensitive technique due to the separation of the sample prior to analysis by different detectors. Complex samples are separated on the FFF-MALS by size, and the different fractions are subsequently characterized by the MALS, DLS, RI and UV/ Vis detectors. The combination of these four detectors provides comprehensive information on various properties such as size, molar mass, particle concentration, conformation and particle payload.

In this case, FFF-MALS analysis revealed that LNPs formulated in Buffer 1 showed higher elution times, indicating larger sized particles. With every additional freeze-thaw cycle, this shift became more pronounced. Conversely, a perfect overlay of the FFF-MALS profiles was observed for the second storage buffer, representing a uniform particle size distribution and absence of aggregation events throughout the different FT cycles (Figure 3).

Ribogreen results showed that the mRNA content remained constant over the different freeze-thaw cycles for both formulations. When assessing the encapsulation efficiency (EE), a subtle yet noteworthy decrease in EE was seen for buffer 1 which suggests the presence of additional free mRNA. No change in EE was detected for buffer 2, which reaffirms its superior stability and payload retention capabilities (Figure 4).

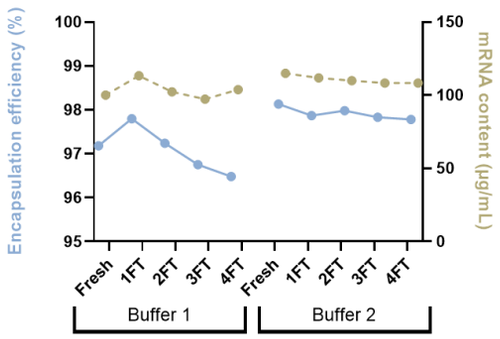

Figure 4

Encapsulation efficiency and mRNA content for the two storage buffers over multiple FT cycles

Assessment of particle homogeneity by cryogenic electron microscopy (cryo-EM) provides insights in the ultrastructure of lipid nanoparticles. As described by Brader et al.[1], physically stressed LNPs can form bleb structures, which may lead to further degradation and loss of mRNA encapsulation by bursting of the LNP upon freeze thaw. Analyses of both formulations after one FT cycle showed that storage buffer 2 counted a slightly lower percentage of biphasic dense particles whilst having a higher percentage of solid core particles (Figure 5).

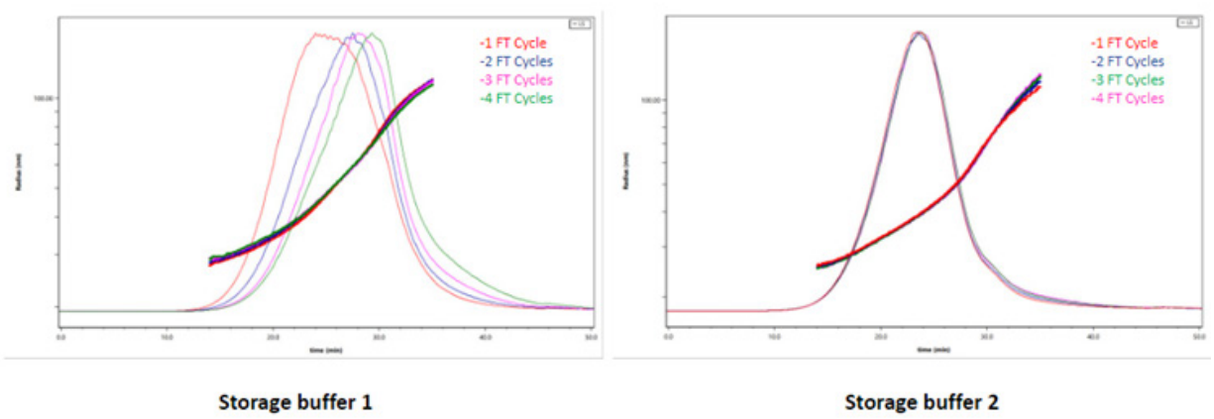

Figure 3

FFF-MALS profiles of mRNA-LNPs in two different storage buffers after subjecting them to freeze-thaw cycles

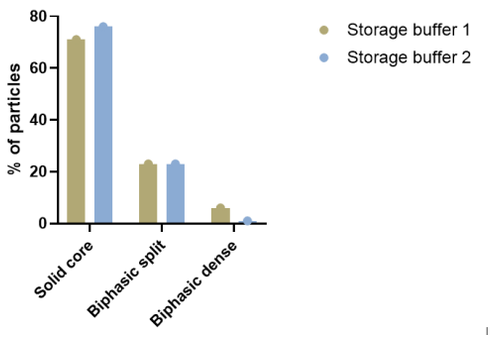

Figure 5

LNP morphology class distribution after 1 FT cycle

In biphasic dense particles the mRNA is densely accumulated in one phase while the second phase consists of morphologically more homogenous solid core LNP material. Both formulations have an equal population of biphasic split particles who consist of separated compartments of solid core LNP material and buffer without any mRNA. These two types of biphasic particles are identified as blebs. Several bleb-like structures are shown in Figure 6.

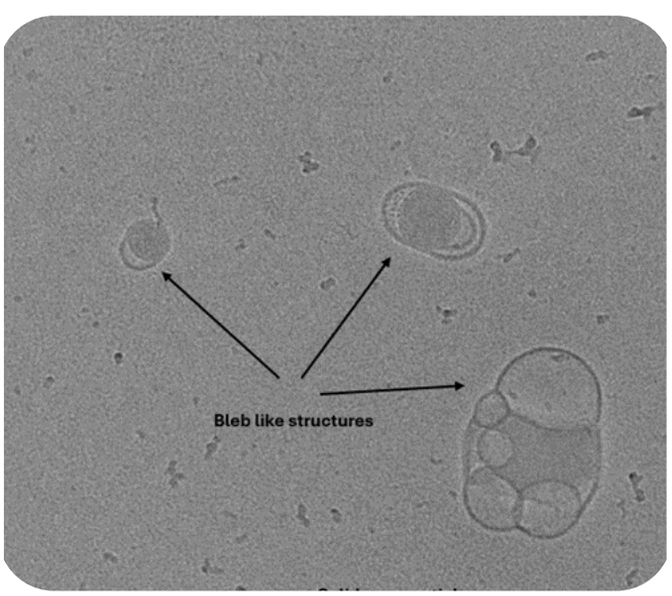

Figure 6

Cryo-EM image of mRNA-LNPs formulated in storage buffer 1 after 1 FT cycle

The reported results underscore the importance of an appropriate storage buffer in preserving stable LNPs formulations and illustrate the need for orthogonal analytical tools. Alterations in encapsulation efficiency, particle size and particle distribution are not desired as they are a sign of instability. It is reasonable to speculate that variations in size could potentially affect the biodistribution of the formulation. It has been described by Di et al. [2] that smaller particles are more prone to distribute to the liver while bigger particles tend to stay more locally, at the site of injection. Next to the impact on biodistribution, literature also highlights the influence of LNP size on the potency and immunogenicity of the mRNA-LNP product.[3,4]

Conclusion

In conclusion, by employing a multifaceted approach that encompasses a range of different techniques, a solid foundation for understanding the formulation's stability profile is provided.

The use of characterization assays is of unprecedented importance during the different phases of drug development. This case study showcases the capabilities of etherna in assessing LNP stability during formulation and process development.

References

1. Brader, M., et al., Encapsulation state of messenger RNA inside lipid nanoparticles. Biophysical Journal, 2021. 120: p. 2766-2770.
2. Di, J., et al., Biodistribution and non-linear gene expression of mRNA LNPs affected by delivery route and particle size. Pharm Res, 2022. 39(1): p. 105-114.
3. Lam, K., et al., Optimizing lipid nanoparticles for delivery in primates. Advanced materials, 2023. 35: 2211420.
4. Hassett K., et al., Impact of lipid nanoparticle size on mRNA vaccine immunogenicity. Journal of controlled release, 2021. 335: p. 237-246.

etherna

+32 3 369 17 40
info@etherna.be

Galileilaan 19
2845 Niel (Belgium)