Physiochemical Characterisation of Poly(A) Lipid Nanoparticles: Effect of Cryoprotectant and Temperature Storage Conditions

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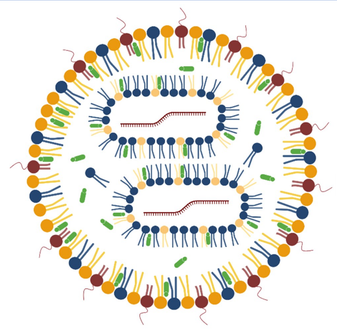
1. Introduction

Lipid nanoparticles (LNPs) are emerging new modalities for mRNA therapeutics which have been in the spotlight for the past decade. Since these are relatively new drug delivery systems compared to conventional medicines, new analytical techniques for the robust characterization of their critical quality attributes (CQAs) are needed [1]. It has been reported that several stimuli can affect the stability of the LNPs such as leakage of the nucleic acid cargo from the nanoparticle and LNP aggregation, resulting in low translation efficiency [2]. Hence, understanding the duration of stability is key during formulation development.

The **aim** of the present study is to evaluate the stability of PolyA-LNPs:

1. Stored at different temperatures (4°C and 25°C)
2. Dialysed in the absence and presence of cryoprotectant sucrose

We measured the impact of the above storage conditions on LNP physicochemical parameters.



Hydrodynamic Size
Polydispersity Index
Zeta potential
Encapsulation Efficiency
Mass Balance

Figure 1. Schematic showing LNP self-assembly and the critical quality attributes (CQAs).

2. Method

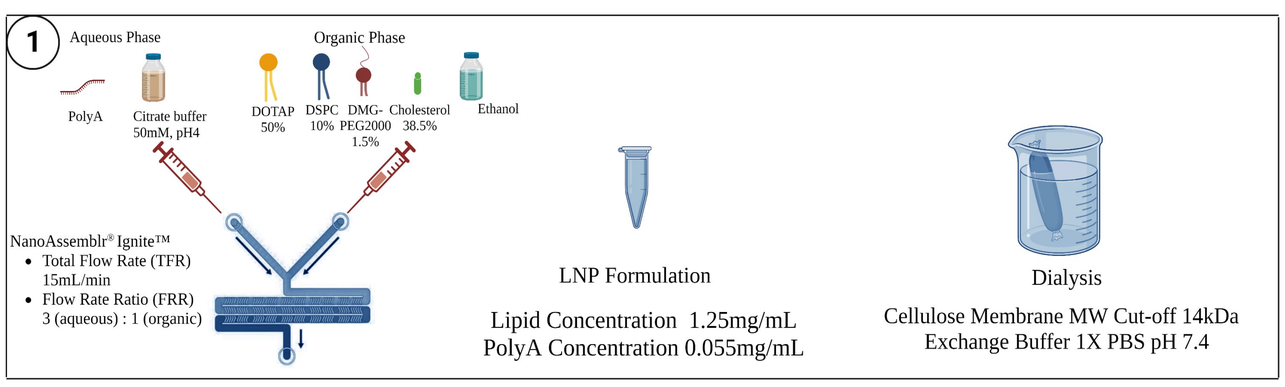
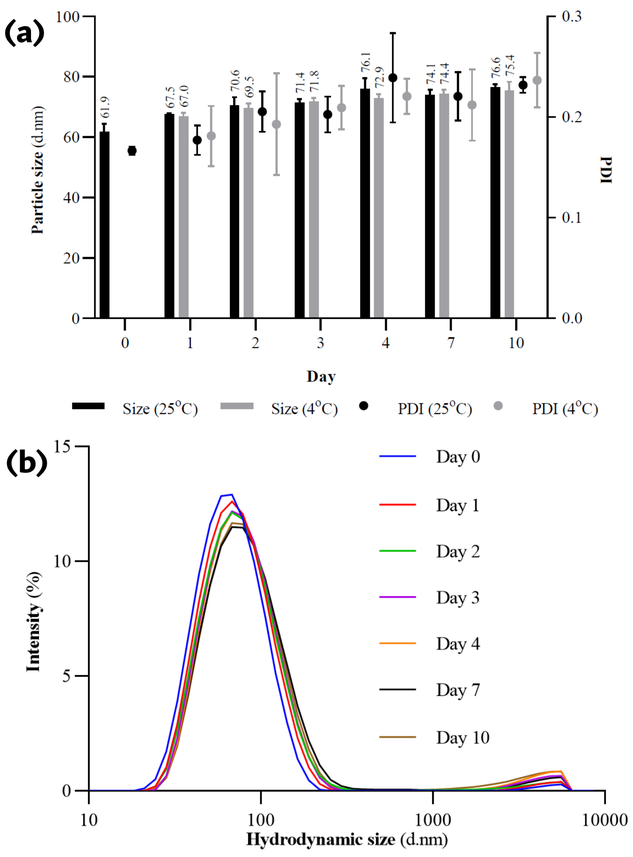


Figure 2: Step-by-step method (1) for LNP manufacture (2) stability study experimental design (3) CQAs measurement. Two variables were tested, firstly the formulation storage temperature (4°C and 25°C) and secondly the addition of cryoprotectant (i.e. sucrose 20% *w/v*) and storage at -80°C to determine stability following freeze-thaw. CQAs such as particle size, Polydispersity Index (PDI) and Zeta Potential (ZP) were measured using Dynamic Light Scattering (DLS), particle size distribution using Nanoparticle Tracking Analysis (NTA), Encapsulation Efficiency (EE) and Mass Balance (MB) analyzed using RiboGreen Assay.

3. Results



No significant changes were observed in size and PDI for LNPs stored at different temperatures and storage durations.

Aggregation of LNPs as well as particle swelling with increase in storage temperature.

A decreased intensity and distribution skewness to the right with longer storage duration in the fridge. This is the opposite case with increased intensity for aggregates with increased storage duration.

This is in good agreement with Figure 3(a) which highlights increased particle hydrodynamic size with time, which is evident from this distribution obtained.

Figure 3: **(a)** Impact of temperature on LNP size and PDI **(b)** Size distribution by intensity measured for LNPs stored in the fridge. LNPs were analysed by DLS providing average mean ± standard deviation (n=3).

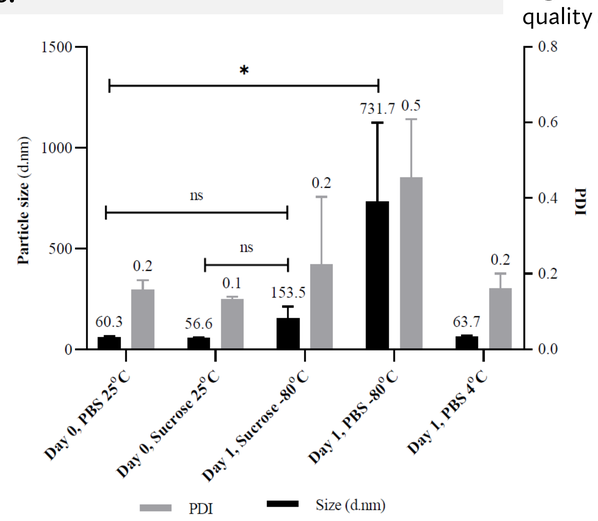
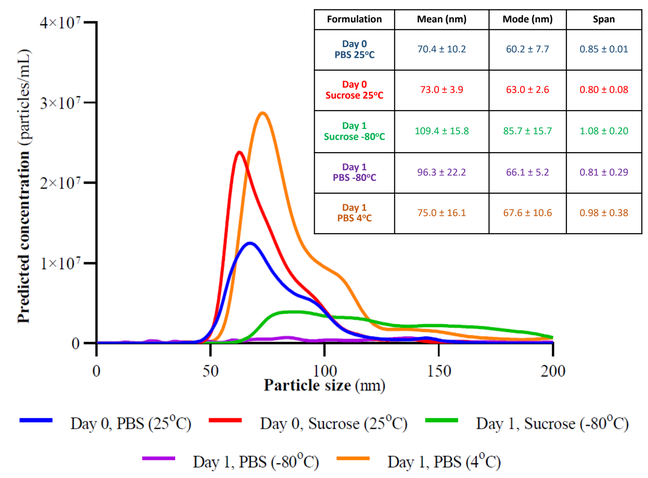


Figure 4: Size and PDI of LNPs following dialysis with 20% *w/v* sucrose vs PBS. LNPs analysed by DLS providing average mean ± standard deviation (n=3). Day 0: day of manufacture and Day 1: 1 freeze-thaw cycle.

A significant increase in particle size (p<0.05) was observed following one freeze-thaw cycle with PBS vs sucrose formulations.

DOTAP LNPs here demonstrated limited freeze-thaw stability, requiring further optimization through the inclusion of cryoprotectants.



Formulation	Mean (nm)	Mode (nm)	Span
Day 0 PBS 25°C	70.4 ± 10.2	60.2 ± 7.7	0.85 ± 0.01
Day 0 Sucrose 25°C	73.0 ± 3.9	63.0 ± 2.6	0.80 ± 0.08
Day 1 Sucrose -80°C	109.4 ± 15.8	85.7 ± 15.7	1.08 ± 0.20
Day 1 PBS -80°C	96.3 ± 22.2	66.1 ± 5.2	0.81 ± 0.29
Day 1 PBS 4°C	75.0 ± 16.1	67.6 ± 10.6	0.98 ± 0.38

Figure 5: Particle size distribution obtained from NTA for LNP formulation dialysed in PBS and 20% *w/v* sucrose (n=3).

Sucrose -80°C had the peak with the highest span of 1.08 ± 0.20 signifying a wider size distribution compared to the other storage conditions. Same storage condition of sucrose -80°C has also given the highest mode size of 85.7 ± 15.7 nm.

NTA is a separation technique which could provide data on particle concentration and mode which cannot be obtained from DLS analysis.

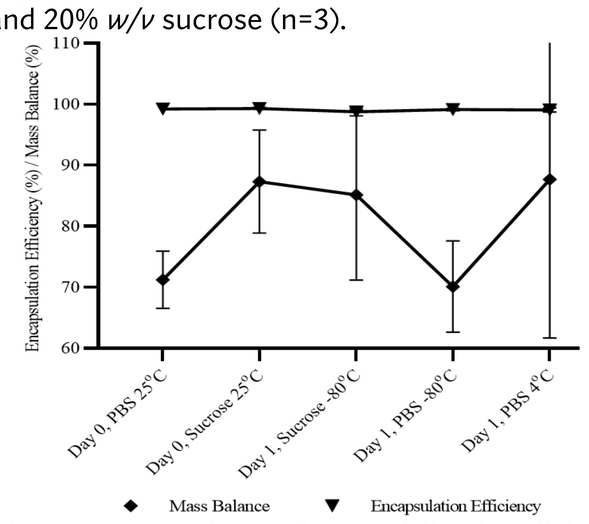


Figure 6: EE and MB of LNPs following dialysis with 20% *w/v* sucrose vs PBS measured by RiboGreen Assay.

For all formulations dialysed with sucrose and PBS, EE ranged from 99% to 100% whereas MB 60% to 100%.

The addition of sucrose as well as undergoing a single freeze-thaw cycle does not significantly change the %EE and %MB.

4. Conclusion & Future Directions

DLS and NTA are characterisation methods which both give hydrodynamic size, however NTA provides higher resolution information on particle concentration and size parameters (Figure 3 and Figure 5).

Sucrose has effectively retained the LNP structure minimizing particle aggregation (Figure 4). Hence, LNPs can be cryopreserved at -80°C for the duration of formulation development. Storage temperature and the use of cryoprotectant does not cause mRNA leakage from the LNP for the conditions examined (Figure 6).

The results of CQAs measured above can change with different LNP prototypes. In future studies, the stability of additional LNP prototypes constructed from MC3 and SM-102 lipids and different oligonucleotide sequences will be examined. Ongoing methods are being developed for the application of Asymmetric Flow Field-Flow Fractionation (AF4) hyphenated with multiple detectors (e.g. DLS and multiangle light scattering) for the high resolution separation and analysis.

References

[1] Parot, J., et al. (2020) *Journal of Controlled Release*, 320.

[2] Kamiya, M., et al. (2022), *Pharmaceutics*, 14.

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MMS Multiscale Metrology Suite for Next-Generation Health Nanotechnologies

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