Evaluation of the Critical Quality Attributes of Lipid Nanoparticles Stored Under Different 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. 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.

2. Method

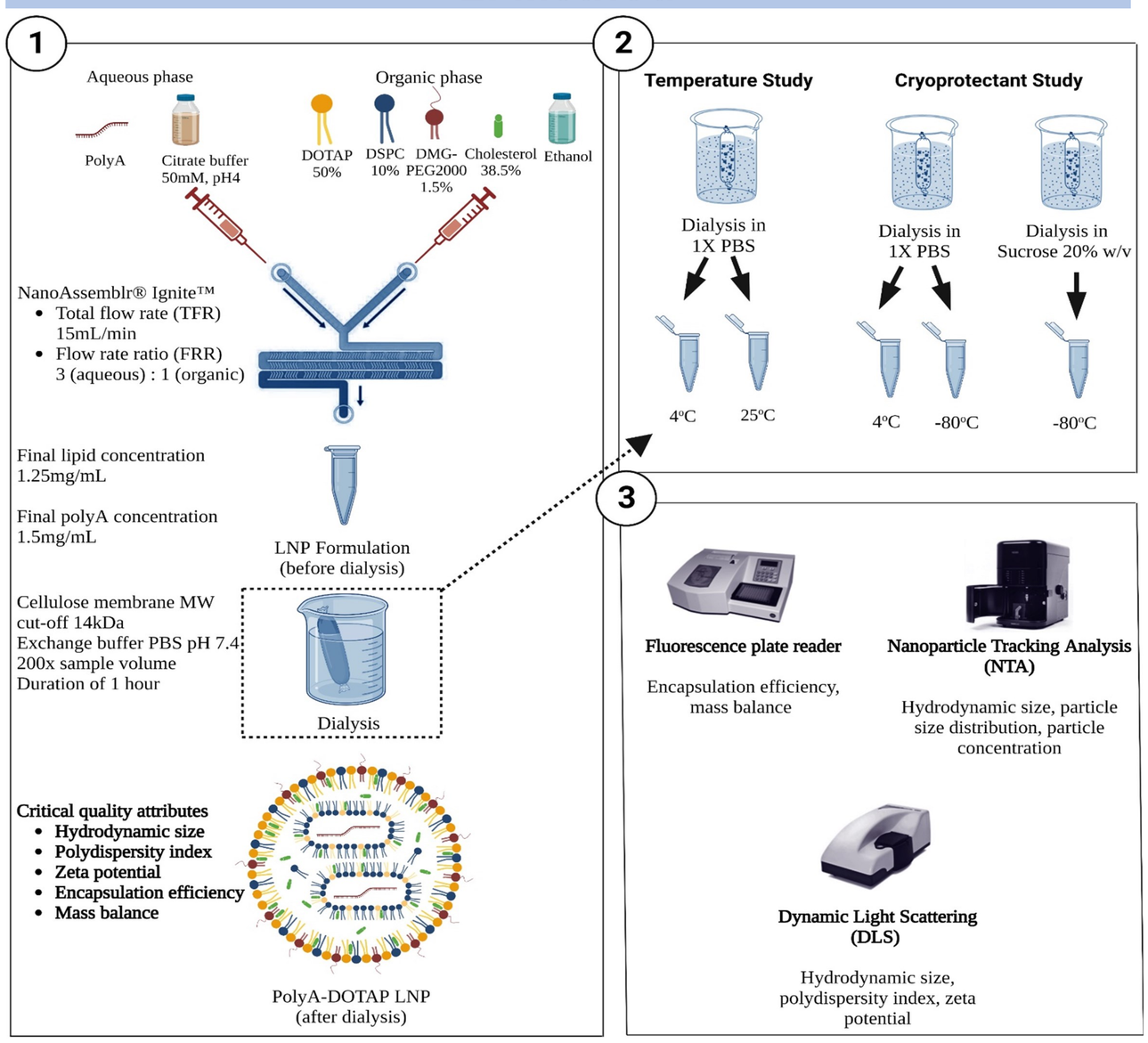


Figure 1: Step-by-step method (1) for LNP manufacture (2) stability study experimental design (3) CQA measurement. Two variables were tested, firstly the formulation storage temperature (4 °C and 25 °C) and secondly the addition of cryoprotectant (i.e., sucrose 20 % w/v) and storage at -80 °C to determine 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

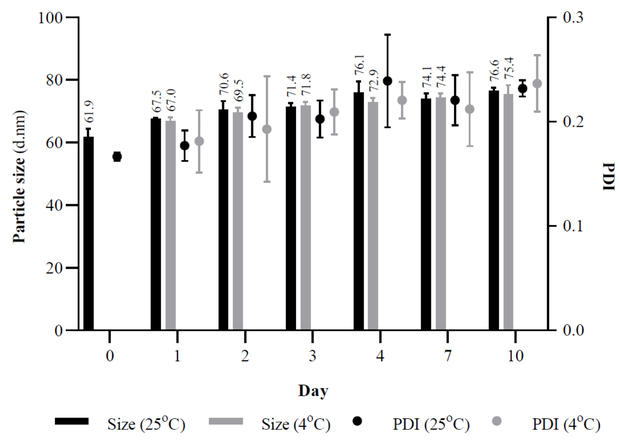


Figure 2: The impact of temperature on LNP size and polydispersity index (PDI). LNPs were analysed by DLS providing average mean ± standard deviation (n=3).

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.

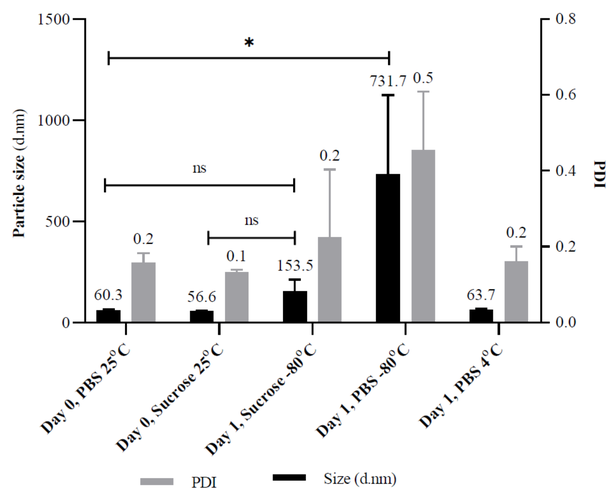
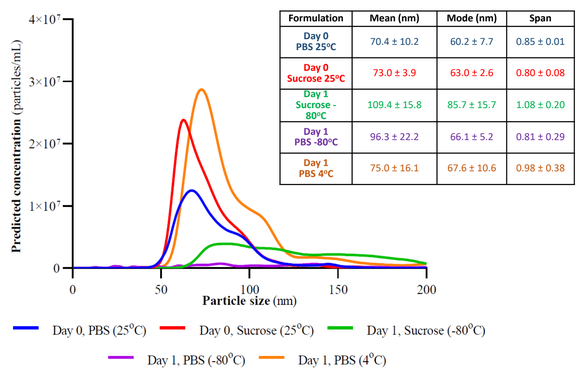


Figure 3: Size and PDI of LNPs following dialysis with 20% *w/v* sucrose vs PBS. LNPs analysed by DLS providing average mean ± standard deviation (n=3). Day 0: day of manufacture and Day 1: 1 freeze-thaw cycle.

A significant increase in particle size (p<0.05) was observed following one freeze-thaw cycle with PBS versus 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 4: Particle size distribution obtained from NTA for LNP formulation dialyzed 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.

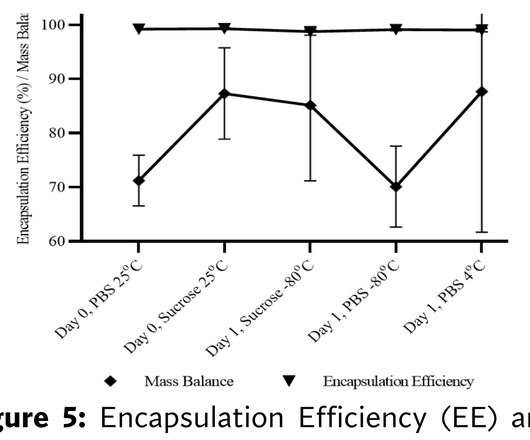


Figure 5: Encapsulation Efficiency (EE) and Mass Balance (MB) measured by Ribogreen Assay.

For all formulations, 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

- We used DLS and NTA characterization techniques to measure the stability of DOTAP LNPs encapsulating poly(A) as a model manufactured by microfluidics. These two methods both give hydrodynamic size however NTA provides higher resolution information on particle concentration and size parameters (Figure 2 and Figure 4).
- Mean particle diameter and PDI increased during the 10 days storage at 4°C and 25°C, suggesting aggregation of LNPs and particle swelling with an increase in storage temperature (Figure 2).
- Storing LNPs at -80°C in the absence of sugar increased particle size, with the distribution skewed to a higher particle size for aggregates. In the presence of the cryoprotectant, sucrose has effectively retained the LNP structure minimizing particle aggregation (Figure 3). 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 5).
- 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: pp. 495-510.
[2] Kamiya, M., et al. (2022), *Pharmaceutics*, 14(11).

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MMS Multiscale Metrology Suite for Next-Generation Health Nanotechnologies

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