Higher or Lower? – The Resolution of Analytical Pipelines for the Evaluation of Lipid Nanoparticle Critical Quality Attributes

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Introduction

With the approval of Onpattro®, Spikevax® and Comirnaty®[1], research interest into the use of lipid nanoparticles (LNPs) as drug delivery platforms has grown exponentially, with a knock-on effect of growing interest in LNP characterization techniques. Dynamic light scattering (DLS) is the gold standard technique for particle sizing, routinely used to measure the average particle size and polydispersity of an oligo-LNP formulations in a quality control context. However, during early development, more comprehensive knowledge of nanoparticle stability is required to develop optimal formulations. Nanoparticle tracking analysis (NTA) can be used as a high-resolution sizing technique to measure oligo-LNP size distribution. Briefly, NTA measures oligo-LNP size and polydispersity on a particle-by-particle basis through image tracking and correlated movement of oligo-LNPs. Here, we use case studies to compare the orthogonal size, and size distribution analytical techniques.

Aims & Objectives

- **To compare high and low resolution orthogonal analytical techniques in the evaluation of prototype oligo-LNP critical quality attributes.**
- Corresponding objectives are to:
  - Design, microfluidic manufacture of prototype PolyA DOTAP-LNPs and dialyse in selected storage buffer systems.
  - Evaluate associated LNP CQA's over defined stability periods using two independent case studies using both NTA and DLS.

Methodology

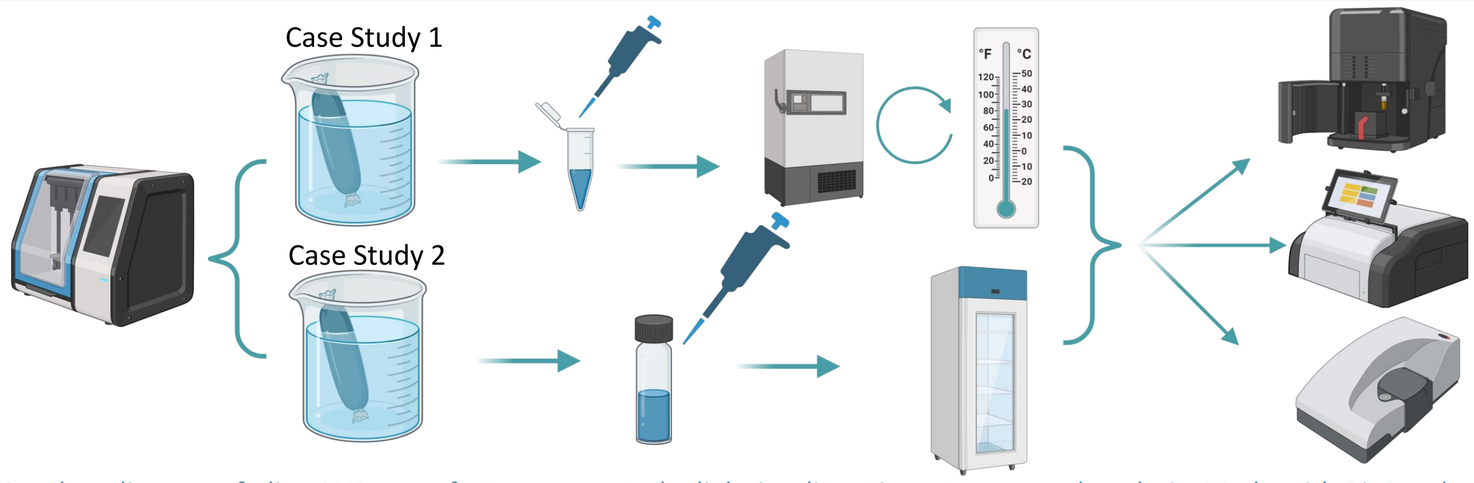

Fig.1 – Flow diagram of oligo-LNP manufacture, case study dialysis, aliquoting, storage, and analysis. Made with BioRender.

LNPs are manufactured by microfluidics dialysed against storage buffers (case study 1: PBS and 20 % sucrose, case study 2: PBS), aliquoted into appropriate containers, stored at defined conditions and analysed. LNP size and span are measured by NTA, LNP size, PDI and zeta potential are measured by the Zetasizer (DLS). Encapsulation efficiency (% EE) is determined using RiboGreen™ assay.

Case Study 1: Evaluating drug-LNP size and size distribution critical quality attributes at frozen storage with the use of 20 % sucrose (w/v) as a cryoprotectant.

Table 1 – Corresponding PolyA DOTAP-LNP CQAs from day of manufacture (DoM) and 1 x freeze/thawed (F/T) samples, (mean ± standard deviation) n=3.

Sample	PDI (DLS)	Span (NTA)	Zeta Potential (mV)	% EE
PBS (DoM)	0.16 ± 0.02	0.56 ± 0.05	+ 7.0 ± 1.0	99.1 ± 0.3
PBS (F/T)	0.45 ± 0.18	1.38 ± 0.48	+ 7.8 ± 1.0	99.0 ± 0.2
20 % Sucrose (DoM)	0.13 ± 0.02	0.53 ± 0.03	+ 5.2 ± 0.8	99.3 ± 0.1
20 % Sucrose (F/T)	0.22 ± 0.15	1.01 ± 0.36	+ 6.5 ± 0.6	99.2 ± 0.2

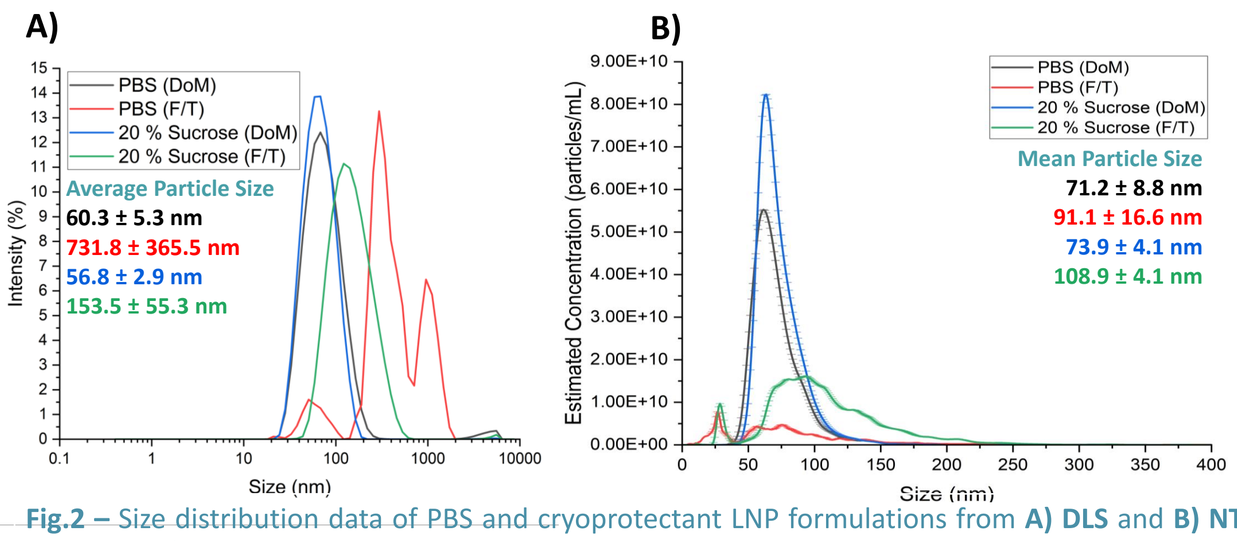

Fig.2 – Size distribution data of PBS and cryoprotectant LNP formulations from **A) DLS** and **B) NTA** after 1 x F/T. DLS data corresponds to mean (n=3), NTA data corresponds to mean ± standard deviation (n=3).

- DLS results were monodisperse for both DoM and sucrose F/T samples **(Fig.2A-B)**. Three peaks were observed for PBS F/T DLS intensity-based size distributions **(Fig.2A)**.
- Corresponding NTA particle size distribution data **(Fig.2B)** were monodisperse for both DoM samples, where multiple subpopulations for both F/T PolyA DOTAP-LNP samples was observed.
- Mean particle size across both DoM samples increased with NTA compared to DLS- whereas overall measured particle size was lower for NTA.
- **NTA detected PolyA DOTAP-LNP sample subpopulations after F/T, whereas DLS data lacked such resolution.**

Case Study 2: Evaluating drug-LNP size and size distribution critical quality attributes over a 28-day stability period under refrigerated conditions.

Table 2 – Corresponding PolyA DOTAP-LNP CQA's from day of manufacture (day 0) days 7, 14, 21, and 28 timepoints, (mean ± standard deviation), n=2 batches.

Time point (Day)	PDI (DLS)	Span (NTA)	Zeta Potential (mV)	EE (%)
0	0.17 ± 0.05	0.68 ± 0.07	+ 7.37 ± 0.11	98.6 ± 0.1
7	0.20 ± 0.02	0.68 ± 0.01	+ 6.16 ± 1.24	98.7 ± 0.1
14	0.23 ± 0.05	0.64 ± 0.09	+ 7.25 ± 0.02	98.6 ± 0.1
21	0.20 ± 0.01	0.70 ± 0.01	+ 6.34 ± 0.64	98.6 ± 0.2
28	0.21 ± 0.02	0.64 ± 0.04	+ 6.14 ± 0.04	98.8 ± 0.1

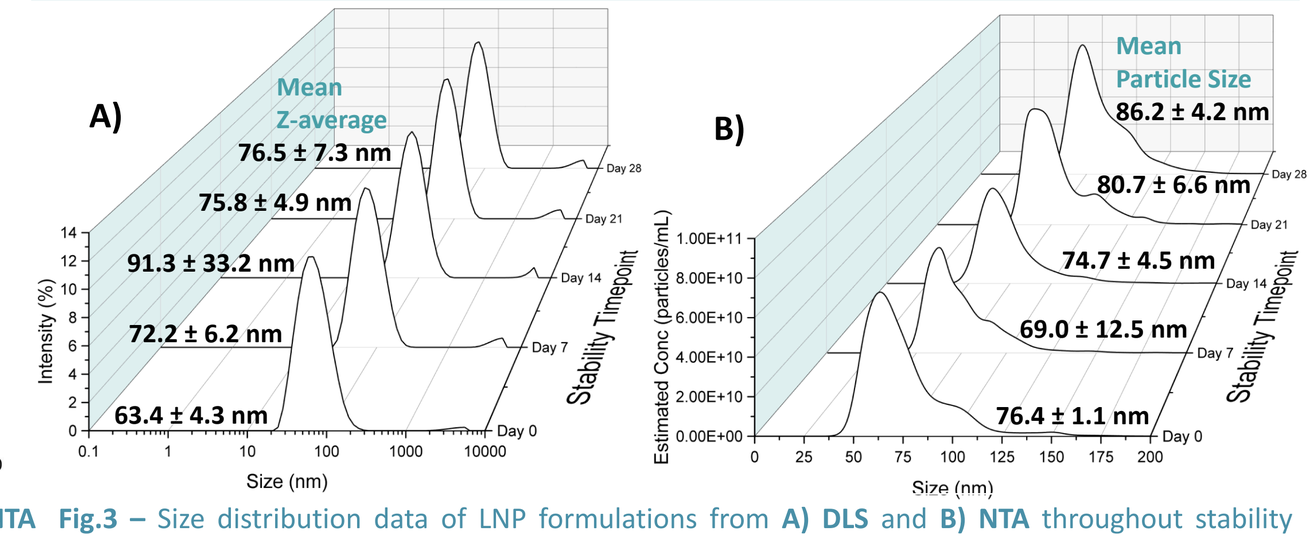

Fig.3 – Size distribution data of LNP formulations from **A) DLS** and **B) NTA** throughout stability study. Reported parameters correspond to mean, n=2 (no standard deviation plotted due to sample variance).

- DLS size data were monodisperse for all sample stability timepoints **(Fig.3A)**.
- NTA size distributions were not monodisperse for all sample stability timepoints, as subpopulation were detected producing fraction peak maxima **(Fig.3B)** for PolyA DOTAP-LNPs.
- Mean particle size across both techniques varied as the stability study evaluation increased in duration- with both techniques trending to a higher particle size on days 21 and 28 **(Fig.3A-B).**
- **NTA detected PolyA DOTAP-LNP sample subpopulations throughout stability study, whereas quantification was unsuccessful using DLS.**

Conclusions & Ongoing Work

- We have demonstrated the need for high resolution analytical techniques to measure LNP CQAs during early-stage development.
- We evaluated different prototype drug-LNP formulations using biorelevant lipid nanoparticle compositions.
- We are developing Flow Field Flow Fractionation methods to evaluated CQAs beyond the scope of DLS and NTA for LNP refrigerated and frozen storage stability.

Acknowledgments

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References

1. Suzuki, Y., Ishihara, H., 2021. Difference in the lipid nanoparticle technology employed in three approved siRNA (Patisiran) and mRNA (COVID-19 vaccine) drugs. Drug Metab Pharmacokinet 41, 100424.