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

Callum G. Davidson[a], Rand Abdulrahman[a], Michael Cairns[a], Yvonne Perrie[a], Zahra Rattray[a]*

[a] Strathclyde Institute of Pharmacy and Biomedical Sciences, 161 Cathedral Street, G4 0RE, United Kingdom

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*Corresponding author.
E-mail:
zahra.rattray@strath.ac.uk

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SUMMARY

Dynamic light scattering (DLS) is a routinely used analytical technique to measure the size distribution of a colloidal sample. Nanoparticle tracking analysis (NTA) has more recently emerged as an orthogonal technique to determine particle size and estimated concentration, although widespread use of NTA for lipid nanoparticle (LNP) analysis is not reported. Here, we use DLS and NTA to screen the stability of oligonucleotide drug loaded lipid nanoparticles (oligo-LNPs) at ultra-low storage temperatures (-80 °C) in the presence and absence of a cryoprotectant (20 % sucrose, w/v) as a case study to highlight differences in technique resolution. NTA was able to detect additional LNP subpopulations samples for all measured samples, whereas DLS was unable to detect these subpopulations. Our study conveys that the use of orthogonal sizing techniques can support early-stage product development, where a more in-depth analysis of formulation and process parameters on LNP stability would support comprehensive understanding of variable defining product stability.

INTRODUCTION

The design and manufacture of oligo-LNPs contribute to their associated particle size, polydispersity, surface charge, oligo loading and their physical stability. These oligo-LNP parameters are defined as critical quality attributes (CQAs), which impact therapeutic action and biological performance of oligo-LNP candidates.

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. DLS is widely known as a low-resolution technique as it produces ensemble readout. DLS also biases to larger particle sizes in terms of light scattering which can skew particle size results.

Nanoparticle tracking analysis (NTA) can be used as a high-resolution orthogonal sizing technique to measure oligo-LNP size and size distribution CQAs. 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 a case study evaluating oligo-LNP size and size distribution critical quality attributes at frozen storage with the use of 20 % sucrose (w/v) as a cryoprotectant, comparing low-resolution DLS and high-resolution NTA measurements.

MATERIALS AND METHODS

PolyA (oligo) was encapsulated in LNPs composed of DOTAP:CHOL:DSPC:DMG-PEG2000. PolyA DOTAP-

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LNPs were formulated at a 50:38.5:10:1.5 molar percentage ratio using microfluidics, and PolyA DOTAP-LNPs were purified via dialysis against 1 x PBS (pH7.4) with and without the inclusion of the cryoprotectant (20 % sucrose w/v). Resultant formulations were stored at at – 80 °C.

Corresponding LNP hydrodynamic size (Z-average), and polydispersity index (PDI), were measured using a Zetasizer Nano ZS (Malvern, Worcestershire, UK). Particle size and particle concentration of LNPs were measured using a NanoSight NS300 (Malvern, Worcestershire, UK), equipped with a low volume flow cell, a 488 nm laser, and a sCMOS camera.

RESULTS AND DISCUSSION

Size and size distribution data highlight the fundamental impact of freeze/thaw cycling PolyA DOTAP-LNP formulations with and without the use of a cryoprotectant using DLS and NTA sizing techniques. Size distributions were visualised in figures 1 and 2.

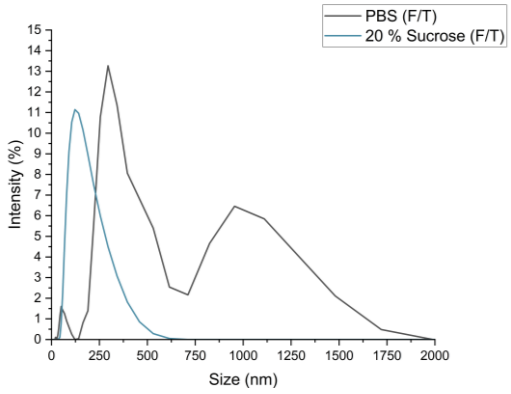

Fig.1. *Corresponding nanoparticle size and Intensity measurements using DLS for PolyA DOTAP LNPs stored in PBS and 20 % Sucrose after freeze/thawing. PolyA DOTAP LNPs were diluted ten-fold in PBS and analysed at 25 °C. Reported parameters correspond to mean (no standard deviation shown due to large variation between samples), n=3.*

Comparing size distribution profiles between techniques demonstrated that PBS LNP subpopulations were quantified using NTA as > 50 % fraction maxima were observed within the NTA size profile (fig.2) than in DLS size profile(fig.1). A similar trend was noted for LNPs containing the cryoprotectant, where one intense peak around 150 nm was produced for LNPs using DLS(fig.1). Whereas NTA size distribution data produced two distinguished peaks varying from 25-275 nm in size, with the second peak including multiple sample fraction maxima indicating subpopulation formation.

Subpopulations within LNPs were detected on NTA instrumentation however undetectable using standardised DLS technology.

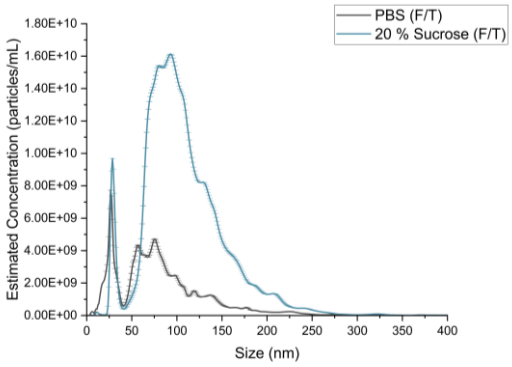

Fig.2. *Nanoparticle tracking analysis (NTA) corresponding size (diameter, nm) and size distribution for PolyA DOTAP LNPs in PBS and PolyA DOTAP LNPs with the use of a cryoprotectant (20 % Sucrose, w/v). PolyA DOTAP-LNP formulations were diluted 4000-fold in PBS and analysed at 25 °C in triplicate reported parameters correspond to mean, ± standard deviation n=3.*

CONCLUSIONS

Our study has demonstrated the need for high resolution analytical techniques to evaluate LNP formulation size and size distribution CQAs. DLS is a robust technique which is extremely useful in QC/QA settings. However, NTA provided more in-depth analysis of LNP CQAs after freeze/thaw allowing determination of LNP subpopulation formation. Future work utilising LNP developed Field Flow Fractionation analytical methods will investigate subpopulation formation beyond NTA capabilities to support comprehensive understanding of variable defining product stability.