Would You Like a Carrier With That? – High Resolution Analysis of RNA With and Without a Lipid Nanocarrier System

Callum G. Davidson[a], Panida Punnabhum[a], Yvonne Perrie[a], Zahra Rattray[a]
[a.] University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, United Kingdom.

Introduction

- Ribonucleic acid therapies are currently in the spotlight of nanomedicine field with FDA approval of several therapies, three of which utilize lipid nanoparticle drug delivery carrier systems[1].
- With the growing popularity of a novel therapeutic platform, subsequent analytical methods transferred from nanomedicine analytics have been adopted as routine, gold standard techniques which have not faced similar growth and development.
- LNPs have the potential to revolutionize the drug delivery field, however the complexity of LNPs, and a lack of deep analytical profiling during early development stages, can delay their translation to the clinic.
- Here, we use a model PolyA and cationic lipid nanoparticle system, complexed with Poly(A), and highlight differences in critical quality attributes measured at different manufacture steps, and demonstrate the need for Field-Flow Fractionation high resolution instrumentation [2].

Aim

The aim of this work is to characterize RNA drug without and with a complexed lipid nanocarrier drug delivery platform, through increasing resolution of analytical pipelines.

Methodology

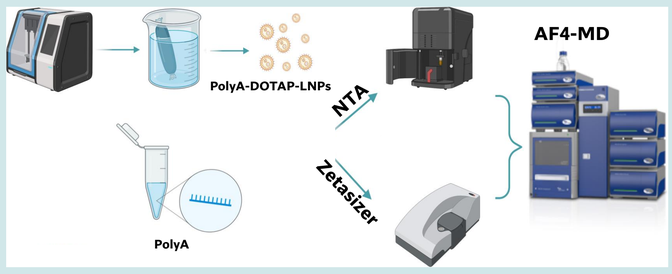


Fig 1. – Flow diagram of PolyA-DOTAP-LNP manufacture, dialysis, PolyA and LNP analysis pipelines. Made with BioRender.

- PolyA-DOTAP-LNPs were manufactured via microfluidics (DOTAP:CHOL:DSPC:DMG-PEG2K) at a molar percentage ratio of 50:38.5:10:1.5. and purified.
- Poly(A) and LNPs were analyzed using dynamic light scattering (DLS), and nanoparticle tracking analysis (NTA).

Table .1 – AF4 Methodology for analysis of PolyA and PolyA-DOTAP-LNPs

AF4 Parameter	PolyA	PolyA-DOTAP-LNPs
Channel	Conventional	Frit-Inlet
Spacer	350 µm	
Membrane	Regenerated Cellulose Amphiphilic (10 kDa)	
Inj. Vol	20 µL	
Eluent	Phosphate Buffered Saline (pH 7.4)	
Cross-Flow (Type)	Varied (Linear)	0.75 mL/min (Exponential, 0.2)
Detector Flow	0.5 mL/min	0.3 mL/min
Hyphenated Detectors	UV	UV-MALS-DLS

Results

PolyA

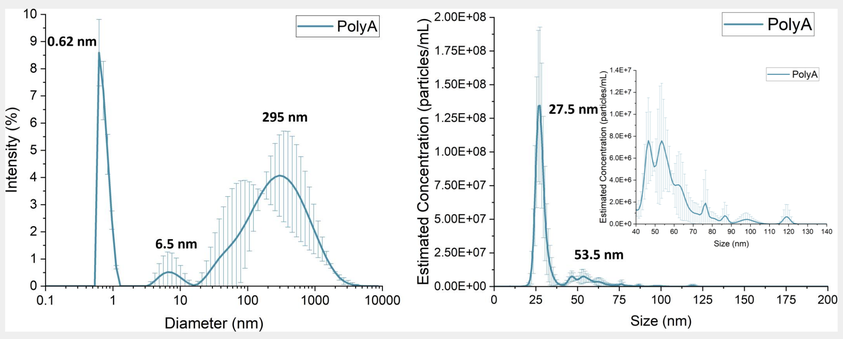


Fig.2 *– Evaluation of PolyA size distribution using,* ***A)*** *Zetasizer DLS (55 µg/mL), and* ***B)*** *NTA (1 mg/mL),* (n=3 ± SD).

- DLS data highlights 3 main PolyA size distributions. (Fig.2A).
- NTA displays one main peak at 27.5 nm and a secondary fractioned peak distribution ~ 53.5 nm. (Fig.2B)
- NTA only suitable for identification of sub-micron particulates, DLS can identify sizes like RNA clusters.

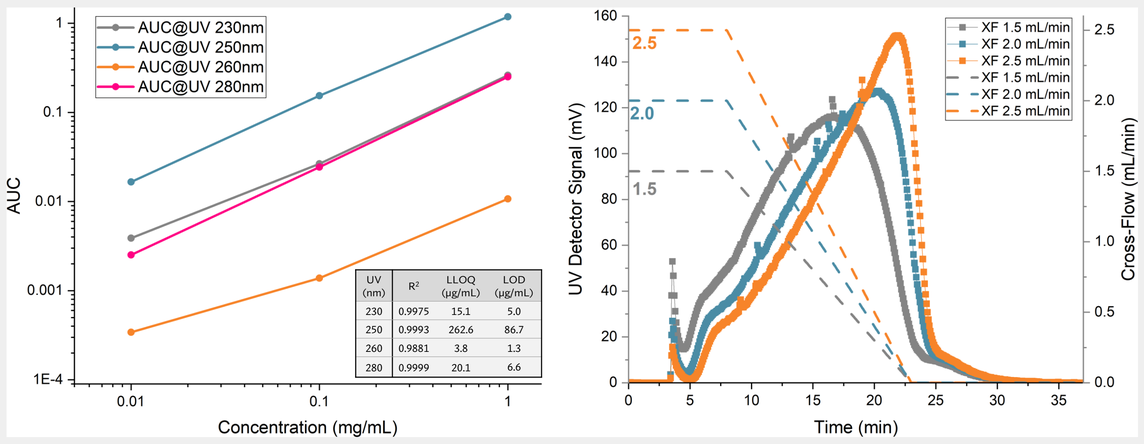


Fig.4 *– AF4 evaluation of PolyA* ***A)*** *calibration curve from direct injection AUC using various UV detector wavelengths.* ***B)*** *PolyA (1 mg/mL) elution profiles at various cross-flow (XF) flow rates, using UV detector 250 nm wavelength.*

- Direct injections (Fig.4A) show 250 nm UV detector wavelength was most suitable for PolyA detection, across all concentrations whilst producing $R^2 > 0.999$.
- Using a UV detector (250 nm), various cross-flow (XF) flow rates were tested to evaluate impact of XF on PolyA detection, producing enhanced signals of upto 150 mV (Fig.4B).

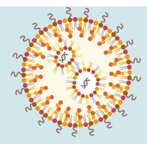
Conclusions

- DLS produced more insightful data at sizes < 20 nm, NTA characterized more sub-populations ~ 50 nm.
- UV detector wavelength 250 nm, most suitable for PolyA quantification, however more optimization required to separate larger molar masses.
- High resolution NTA and AF4 analysis quantify LNP sub-populations, not detectable using DLS.
- AF4 also highlighted a range of morphologies within the LNP population beyond the scope of NTA. Overall showing an unequal loading of PolyA within LNPs.

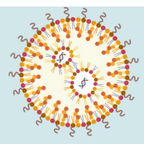
Ongoing & Future Work

- Evaluating RNA loading in/around LNPs on encapsulation within LNPs and exposure to various environmental stressors.
- Develop EAF4 methodology for separation of RNA drug API to determine purity profile.
- Evaluate different formulations of lipids to determine impact of lipid physiochemical properties on overall LNP critical quality attributes.
- Develop lipid quantification methodology to evaluate sub-population fractions within a LNP distribution.

Results



PolyA-DOTAP-LNPs



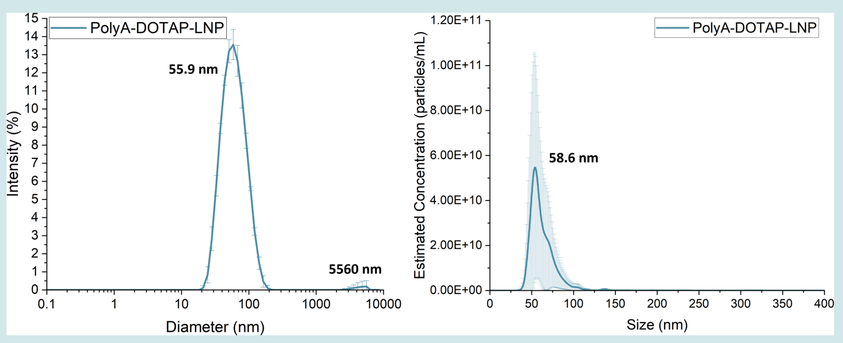


Fig.3 *– PolyA-DOTRAP-LNP size distribution evaluation using,* ***A)*** *Zetasizer DLS, and* ***B)*** *NTA,* (n=3 ± SD).

- DLS data highlights 1 main LNP peak at 56 nm, and larger particulates at 5560 nm. (Fig.3A).
- NTA displays one main peak at 58.6 nm with shouldering peak at 70 nm. (Fig.3B)
- NTA demonstrated the need for high resolution analytics within early development of LNP formulations.

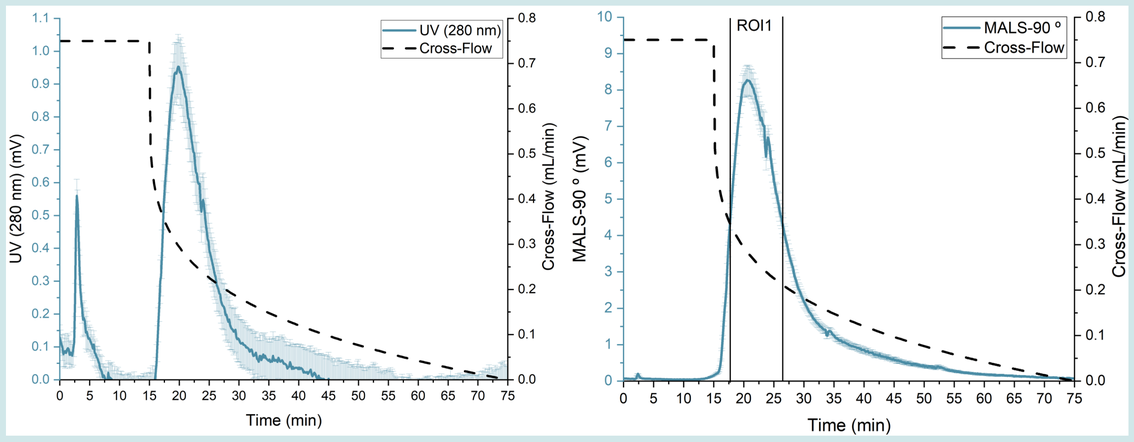


Fig.5 *– PolyA-DOTAP-LNP* ***A)****UV Detector signal and elution method and* ***B)*** *MALS-90° detector signal and elution profile annotated with region of interest (ROI)1. (n=3* ± SD).

- UV-MALS detectors produced fractioned elution profiles, highlighting sub-populations within the general size distribution of LNPs.
- MALS detector produced higher signal than UV due higher light scattering potential of LNPs than light-absorbing components within LNP system.

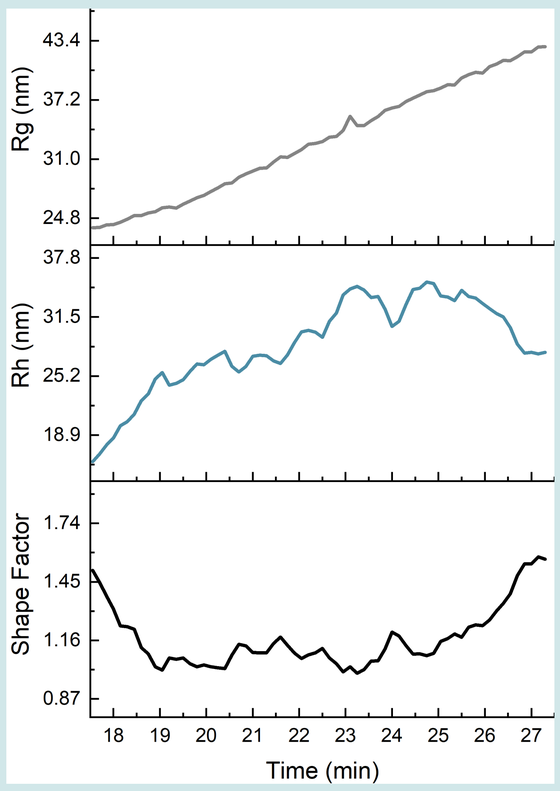


Fig.6*– PolyA-DOTAP-LNP ROI1 FWHM, radius of gyration (Rg), hydrodynamic radius (Rh) and shape factor ratio (Rg/Rh).*

Table.2 *– PolyA-DOTAP-LNP ROI from MALS-90° detector associated retention time (Rt), processing model fit, radius of gyration (Rg), hydrodynamic radius (Rh) and shape factor ratio (Rg/Rh).*

	FI-AF4-MD				
ROI	R_t (min)	Model Fit	Rg (nm)	Rh (nm)	Shape Factor
1	21.0	Sphere	29.7	27.3	1.1

- ROI1 data processed using sphere model fit, shoulder peak (R_t 25 min) also processed using sphere model.
- MALS radius of gyration data produced higher average radii than DLS hydrodynamic radii, producing a shape factor ratio ~ 1.
- Shape factor indicates PolyA-DOTAP-LNPs have an irregular range of shapes within the formulation, as we would expected spherical particles.
- Shape factor also highlights a heterogenous distribution of PolyA within the LNP population.

Acknowledgements

We acknowledge funding from EPRSC (EP/V028960/01) Multiscale Metrology Suite for next-generation health nanotechnologies.

References

1. Zhu, Y., et al., Cell Death & Disease, 2022. **13**(7): p. 644.
2. Mildner, R., et al.,. Eur J Pharm Biopharm, 2021. **163**: p. 252-265.