Go With The Flow – High Resolution Lipid Nanoparticle Metrology

Callum G. Davidson[a], Rand Abdulrahman[a], Panida Punnabhum[a], Yvonne Perrie[a], Zahra Rattray[a]

a. University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, United Kingdom

callum.davidson@strath.ac.uk

Introduction

Asymmetric-Flow Field Flow Fraction (AF4) is a rapidly growing analytical technique for the separation and in-line analysis of RNA-loaded lipid nanoparticles (RNA-LNPs). This high resolution, robust technique will facilitate the clinical translation of new RNA-LNP formulations by evaluating nanoparticle critical quality attributes (CQAs) and enhance the knowledge between RNA-LNP design, manufacture, and associated physical chemical properties [1]. The use of high-resolution analytical techniques can bridge existing knowledge gaps and accelerate the successful clinical translation of RNA-LNP therapies.

The aim of this work was to develop a method for the characterization of RNA-LNPs using Frit Inlet (FI) AF4-Multi angle light scattering (MALS)-UV to determine LNP particle size distribution.

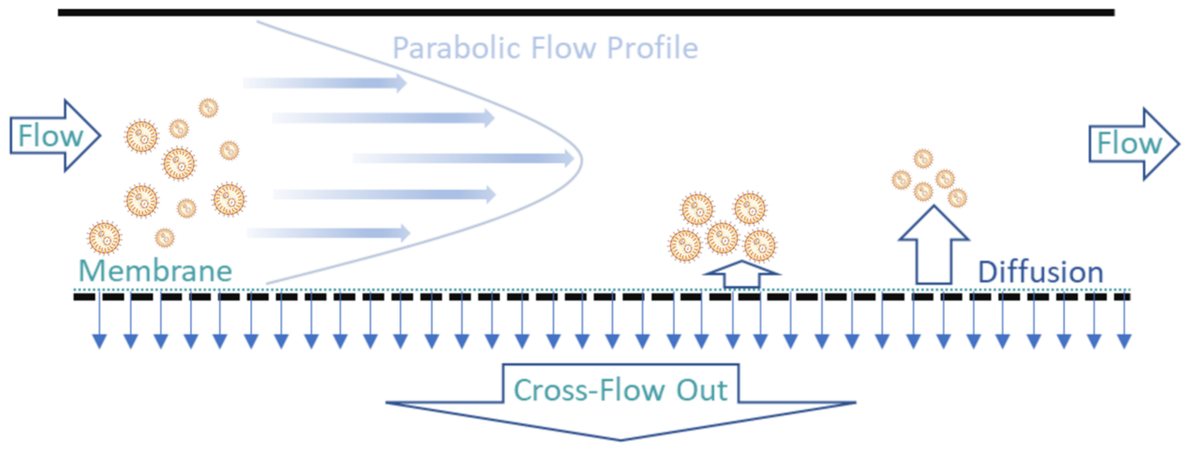

Fig. 1 – AF4 The working principle of FFF. Note the height scale is exaggerated as all particles move only microns away from the membrane covered porous bottom plate. Remade from [1] using BioRender.

Methodology

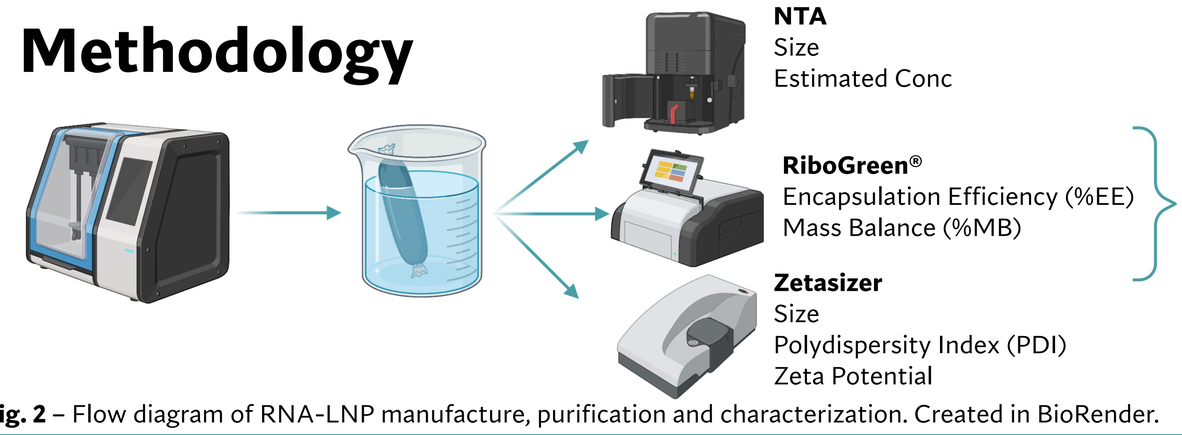

Fig. 2 – Flow diagram of RNA-LNP manufacture, purification and characterization. Created in BioRender.

RNA-LNPs were prepared *via* microfluidics by mixing DOTAP:CHOL:DSPC:DMG-PEG2000 at a mol% ratio of 50:38.5:10:1.5. PolyA DOTAP-LNPs were dialysed against PBS pH 7.4.

Critical quality attributes (CQAs) were measured using dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and RNA Quantification RiboGreen® Assay for PolyA encapsulation efficiency (%EE) and mass balance (%MB) prior to AF4 method development.

Results

Table 1 – PolyA DOTAP-LNP Critical Quality Attribute specification and evaluation, (n=3 ± SD).

Sample	DLS Size (nm)	DLS PDI	ZP (mV)	% EE	% MB
Specification	60-80	< 0.2	< + 10	> 95	> 70
PolyA DOTAP-LNP	61.5 ± 2.5	0.16 ± 0.004	+ 6.2 ± 0.5	98.8 ± 0.4	76.6 ± 4.7

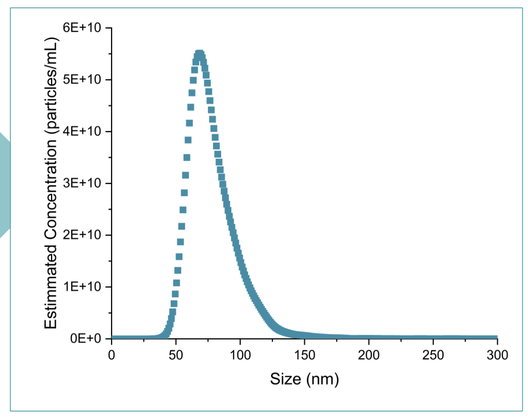

Fig. 3 –PolyA DOTAP-LNPs particle size distribution as measured by NTA (n=3, ± SD).

Fig. 3 denotes a PolyA DOTAP-LNP size distribution using nanoparticle tracking analysis. The averaged LNP size was 76.6 ± 6.3 nm, a mode of 67.0 ± 4.9 nm, D10 distribution of 57.6 ± 5.9 nm, D50 of 72.6 ± 6.3 nm, D90 of 100.1 ± 6.5 nm with a span of 0.59.

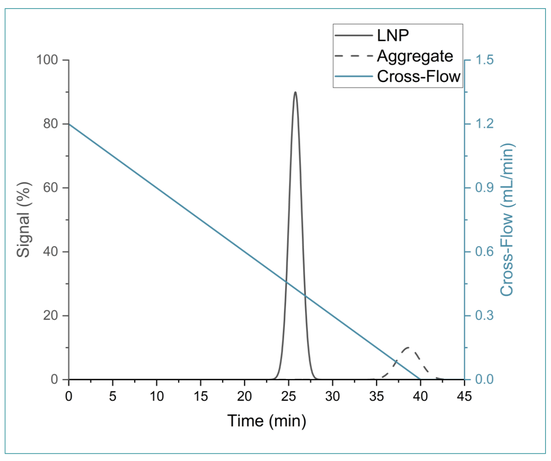

Fig. 4a – Linear cross-flow decay profile (0.2 decay) simulation from 1.2 mL/min to 0 mL/min crossflow for 40 minutes with predicted elution profiles for PolyA DOTAP-LNPs and aggregates.

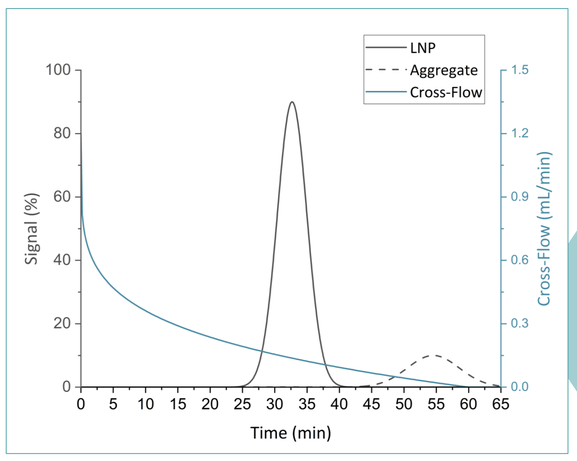

Fig. 4b – Exponential cross-flow decay profile (0.2 decay) modelled from 1.2 mL/min to 0 mL/min crossflow for 60 minutes with predicted elution profiles of PolyA DOTAP-LNPs and aggregates.

PolyA DOTAP-LNP elution profile was modelled using NovaAnalysis software using the AF4-FI channel with the following parameters: amphiphilic regenerated cellulose 10 kDa membrane, 350 µm spacer, 1 x PBS (pH 7.4) eluent, detector flow 0.5 mL/min, injection flow 0.2 mL/min, 2 min delay time, linear (Fig.4a) and power 0.2, (Fig. 4b) crossflow decay profiles, and 0.05 mL/min pump rise for 5 minutes.

PolyA DOTAP-LNP sample was separated based on the method in fig. 5b as a power decay method proved to enhance sample recovery from the membrane compared with linear decay methods. Our sample was injected at 0.25 mg/mL lipid final concentration. Fig. 5b denotes the elution profile of our PolyA DOTAP-LNP sample.

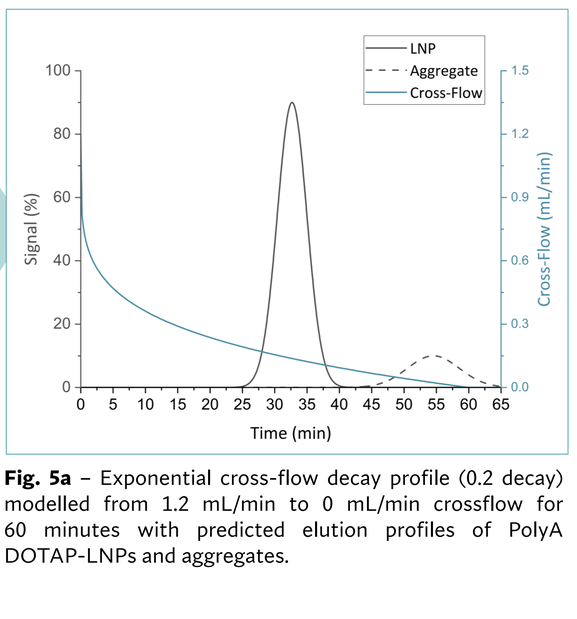

Fig. 5a – Exponential cross-flow decay profile (0.2 decay) modelled from 1.2 mL/min to 0 mL/min crossflow for 60 minutes with predicted elution profiles of PolyA DOTAP-LNPs and aggregates.

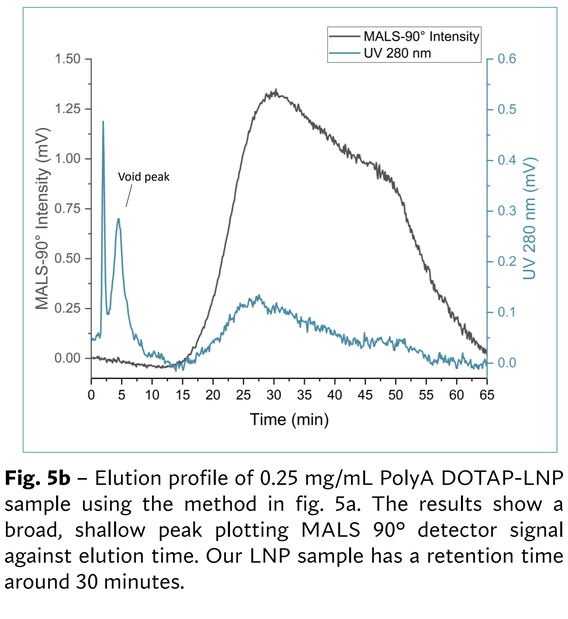

Fig. 5b – Elution profile of 0.25 mg/mL PolyA DOTAP-LNP sample using the method in fig. 5a. The results show a broad, shallow peak plotting MALS 90° detector signal against elution time. Our LNP sample has a retention time around 30 minutes.

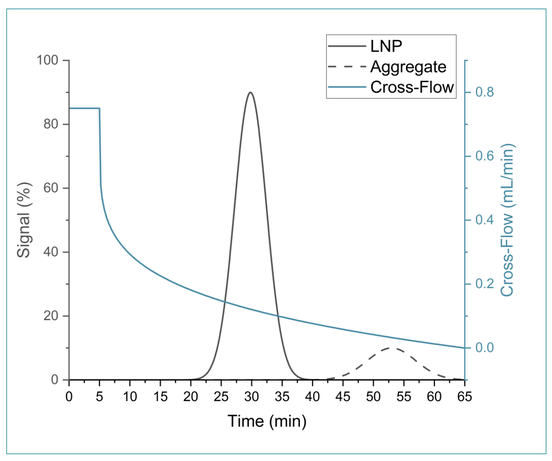

Fig. 6a – Exponential cross-flow decay profile (0.2 decay) modelled from 0.75 mL/min to 0 mL/min crossflow for 60 minutes with predicted elution profiles of PolyA DOTAP-LNPs and aggregates.

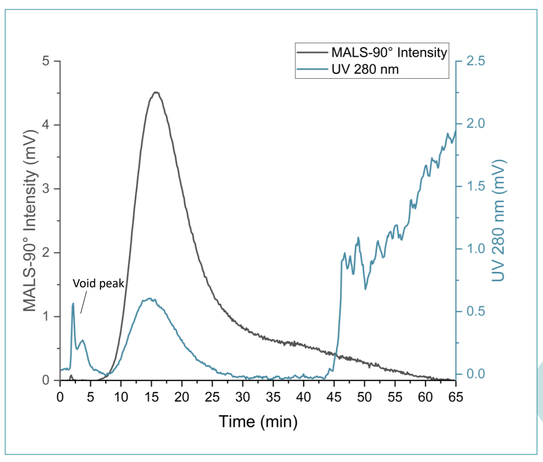

Fig. 6b – Elution profile of 0.25 mg/mL PolyA DOTAP-LNP sample using the method in fig. 6a. The results show narrower, intense peak plotting MALS 90° detector signal against elution time. The corresponding retention time is ~17 minutes.

From introducing a longer sample hold and decreasing the initial cross-flow decay to 0.75 mL/min, a narrower, more intense elution profile was noted with a PolyA DOTAP-LNP retention around 17 minutes. With a 3.5x higher signal intensity.

PolyA DOTAP-LNP concentration was increased to 0.5 mg/mL and injected using the same method from fig. 6a. Sample signal intensity increased 3-fold with corresponding elution profiles observed in fig. 7a. Mean radius distribution for injections 5, 6 and 7 plotted in fig. 7b. The elution profiles are like Mildner *et al.[1]* study. Our results produced a mean radius of gyration around 20 nm.

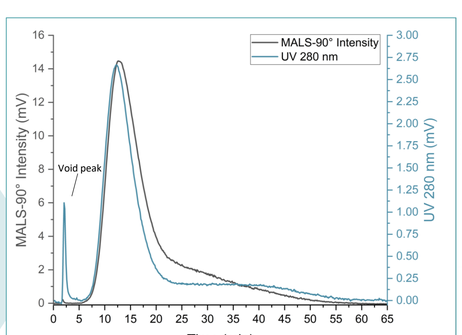

Fig. 7a – Elution profile of 0.50 mg/mL PolyA DOTAP-LNP sample using the method in fig. 6a. The results show narrower, intense peak plotting MALS 90° detector signal against elution time. LNP samples have a retention time around 13 minutes.

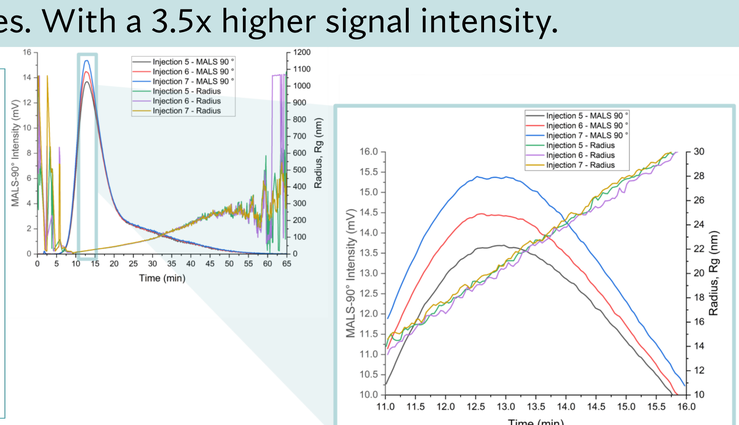

Fig. 7b – **i)** Processed results of MALS-90° intensity and radius of gyration (Rg) of injections 5-7, plotted against elution time. **ii)** Data shows MALS-90° peak maxima at 13-minute retention time corresponding to an approximately 20 nm particle radius of gyration.

Conclusions & Ongoing Work

- Initial AF4 method develop for PolyA DOTAP-LNPs.
- Additional method optimization required to understand critical parameters for FFF analysis and its relevance to other RNA-LNP formulations (SM102, MC3).
- Data processing pipeline to probe particle geometry (MALS/DLS) and colloidal stability.

References:

1. Mildner, R., et al., Eur J Pharm Biopharm, 2021. **163**: p. 252-265.