University of Strathclyde Glasgow

Orthogonal Analytical Insights from RNA Drug Substances to Lipid Nanoparticle Drug Products

Callum G. Davidson[a], Eleni Kapsali[a], Yvonne Perrie[a], Zahra Rattray[a]

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

Introduction

- Polyadenylic acid (Poly(A)) has emerged as a popular choice for use as a model RNA drug substance for LNP formulation prototyping.
- In the absence of existing RNA and LNP drug substance/drug product reference materials, Poly(A)-LNPs are widely used as a control formulation to assess the impact of manufacturing process parameters on formulation critical quality attributes (CQAs).
- Since the stability of RNA drug substance (DS) cargo is a major determinant of drug product (DP) performance, both pre-formulation and post-formulation attributes of the RNA must be assessed thus highlighting the need for systematic comparison of DS quality and its effect on DP formulation.
- **This study aims to enhance knowledge of model Poly(A) critical quality attribute impact on LNP formulation attributes, ultimately seeking to produce better standardised controls for LNP bench to bedside translation.**

Methodology

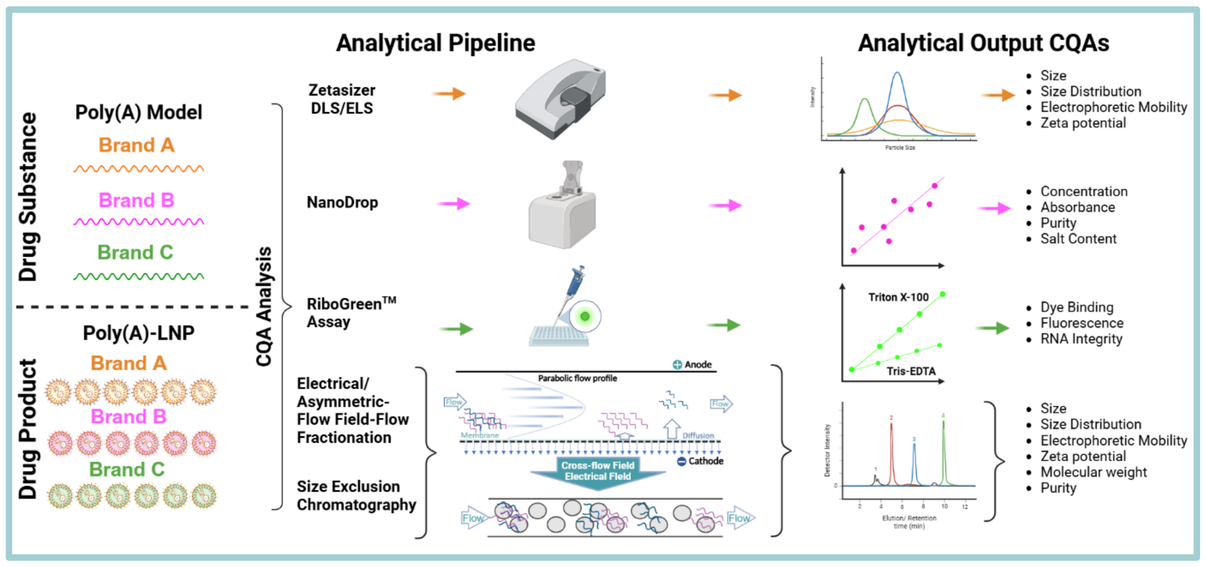

Fig.1 *– An overview of analytical techniques and methodologies employed to evaluate CQAs of Poly(A) drug substances from three commercial vendors and assess their impact within an encapsulated SM102-LNP drug product formulation.*

Results

Poly(A) – Drug Substance

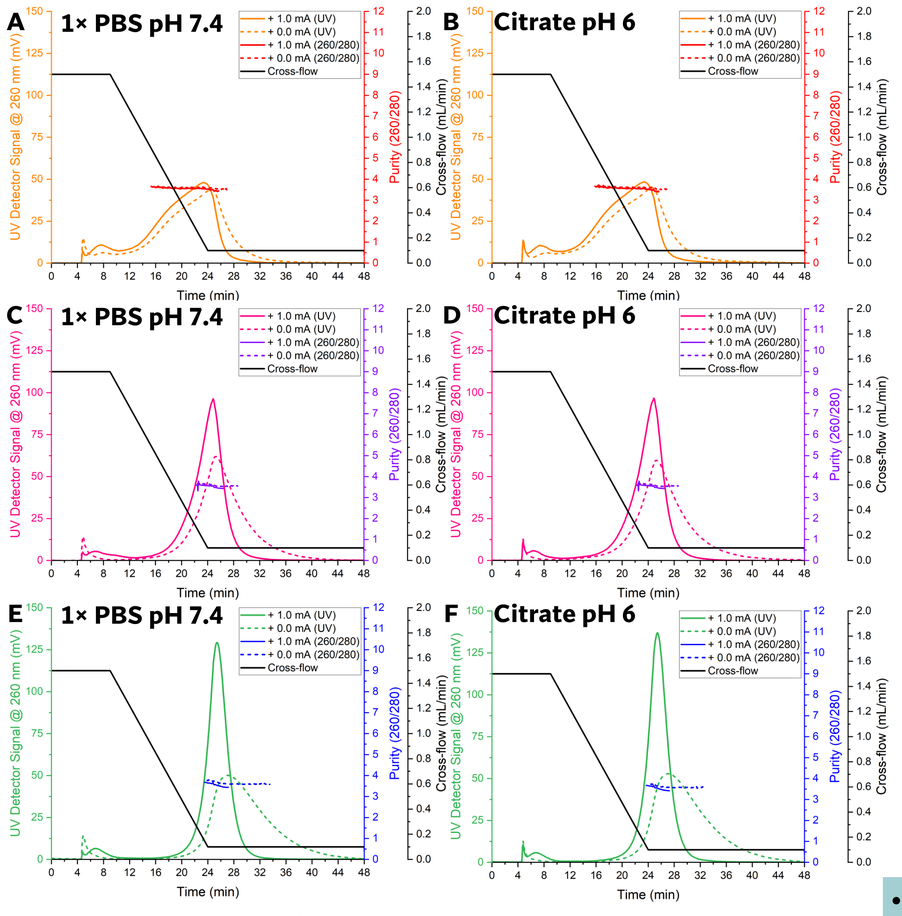

Fig.2 *– EAF4-UV traces using 0.0 mA and +1.0 mA currents to separate Brand A Poly(A) in A) PBS, B) Citrate pH 6. Brand B C) PBS, and D) Citrate pH 6. Brand C in E) PBS, F) Citrate pH 6. 5 µg Poly(A). UV profiles n=3, purity ratio (260/280) n=2.*

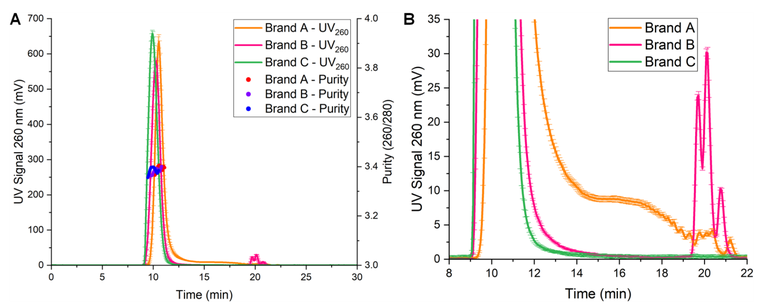

Fig.3 *– SEC-UV analysis of Branded Poly(A) drug substances A) 260 nm absorbance and purity (260/280 FWHM), B) focussed UV detector separation profiles, n=2 ± SD).*

Table.1 *– EAF4 enhanced Direct Injection estimated chain lengths of Poly(A) brands diluted in 1× PBS pH 7.4, n=3.*

Brand	Est. Chain Length (MW/0.343)	Est. Chain Length D10 (D10/0.343)	Est. Chain Length D50 (D50/0.343)	Est. Chain Length D90 (D90/0.343)
Brand A	876	620	889	1073
Brand B	921	605	1000	1219
Brand C	1482	871	1540	1891

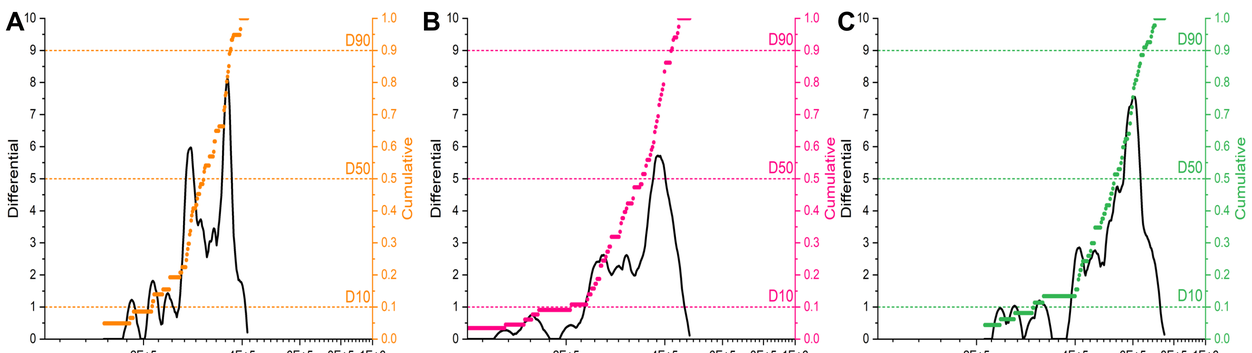

Fig.4 *– Direct Injection (12.5 µg) Poly(A) molar mass evaluation of A) Brand A B) Brand B, C) Brand C in 1× PBS pH 7.4. Molar mass distributions integrated at FWHM MALS-90° detector signal. n=3*

- **EAF4 separation highlights a 3-fold UV detection enhancement when applying a positive current external field.**
- **Orthogonal aSEC-UV profiles confirm vendor-specific chain lengths (A<B<C), and polydispersity (A>B>C).**
- **MALS detector direct injection circumvents low separation detector signals for batch-based size profiling.**

Poly(A)-LNP – Drug Product

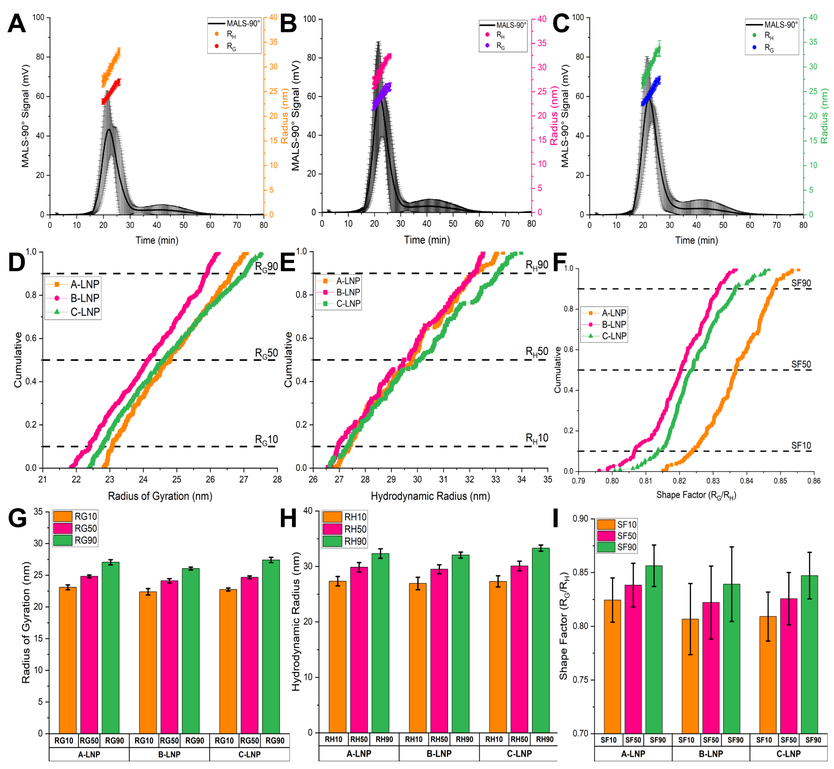

Fig.5 *– FI-AF4-MD of Poly(A) LNP formulations. MALS-90° elution profiles for A) A-LNPs, B) B-LNPs, and C) C-LNPs. (n=3 ± SD). Cumulative distributions for D) radius of gyration (Rg), E) hydrodynamic radius (Rh), and F) shape factor (SF) (n=3), with respective distributions for G) Rg, H) Rh, and I) SF (n=3 ± SD).*

Table.2 *– Branded Poly(A)-LNP drug CQAs, z-average/PDI (DLS), zeta potential (ELS), and RiboGreen™ Assay encapsulation efficiency (%EE) (n=3 ± SD).*

LNP	Z-avg. (nm)	PDI	ZP (mV)	% EE
A	65.7 ± 7.1	0.12 ± 0.05	-3.1 ± 1.9	99.4 ± 0.8
B	65.9 ± 6.9	0.12 ± 0.05	-3.8 ± 2.9	99.1 ± 1.0
C	68.5 ± 6.6	0.13 ± 0.07	-3.4 ± 2.3	98.9 ± 1.3

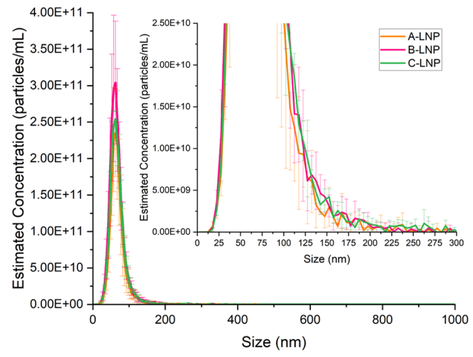

Fig.6 *– Nanoparticle Tracking Analysis particle size distribution profiles of Branded Poly(A)-LNP s using raw data exports, (n=3, ± SD).*

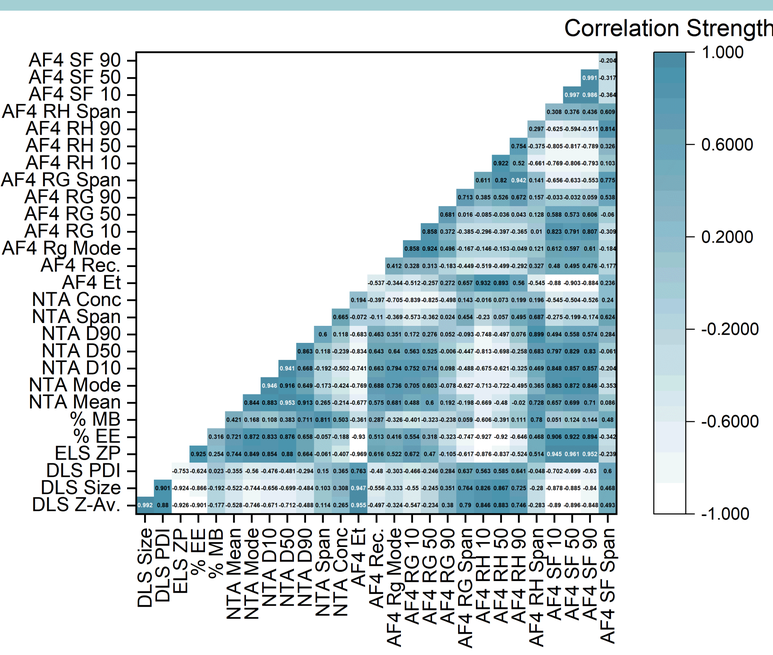

Fig.7 – *Pearson Correlation heatmap of Poly(A)-LNP drug product correlation strength combining all measurable LNP CQAs for deeper insights parameter relationship.*

- **No significant differences were noted between vendor-specific LNP drug products across low-high resolution orthogonal techniques. AF4 highlights enhanced analytical insights into vendor specific LNP formulations.**
- **Correlated CQAs demonstrate formulation parameter relationships which can be built into LNP design for enhanced formulation optimisation within early therapeutic discovery and development.**

Conclusion & Future Work

- **Evaluate different lipid formulations to assess impact on overall LNP CQAs.**
- **Encapsulate mRNA Fluc, GFP, OVA and Cas9 in LNPs assessing secondary, tertiary and quaternary higher order structures.**

Acknowledgements

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

Contact

callum.davidson@strath.ac.uk

mms-facility@strath.ac.uk