Comprehensive Characterization and Quantitation of Lipid Nanoparticle-Based RNA Formulations

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Abstract

The therapeutic potential of lipid nanoparticles (LNPs) as delivery vehicles has been demonstrated in recent years cumulating in the current emergency use of the mRNA based SARS-CoV-2 vaccines. In order to ensure the safety and efficacy of the LNP-RNA vaccine or therapeutics, various quality attributes of LNP-RNA products need to be measured throughout the product development cycle. In this poster, we will demonstrate the use of a dynamics light scattering (DLS) Plate Reader for fast screening and quality control of the LNP preparations, and multi-angle light scattering (MALS) combined with ultraviolet (UV) and refractive index (dRI) detectors following size exclusion chromatography (SEC) or field flow fractionation (FFF) separation for in-depth characterization. SEC or FFF provides sized based separation, and MALS, UV, and dRI detectors enables biophysical characterization. We will show the data from the online detectors for the quantitation of particle size distribution, particle concentration, molecular weights of RNA and lipids, and sized-based RNA payload distribution of the LNP-RNA samples.

LNP-RNA Physical Attributes and Assays

Attribute	Assay	SEC/FFF-MALS-UV-dRI
mRNA integrity	Gel, qPCR	✓
LNP size	DLS analysis	✓
LNP distribution	DLS analysis	✓
Physical stability	DLS analysis	✓
LNP number	NTA analysis	✓
LNP morphology	TEM, cryo-EM	✓ (R_g/R_h)
LNP charge	Zeta potential	Possibly with EAF4
Encapsulation efficiency	Fluorescence	✓, new
mRNA concentration	Fluorescence	✓, new
Lipid concentration	LC-MS analysis	✓, new

Wyatt Solutions for Quantifying LNP-RNA Attributes

DLS Plate Reader
Size distribution and particle concentration with built-in automation

Mobius
Automated zeta potential measurement with an autosampler

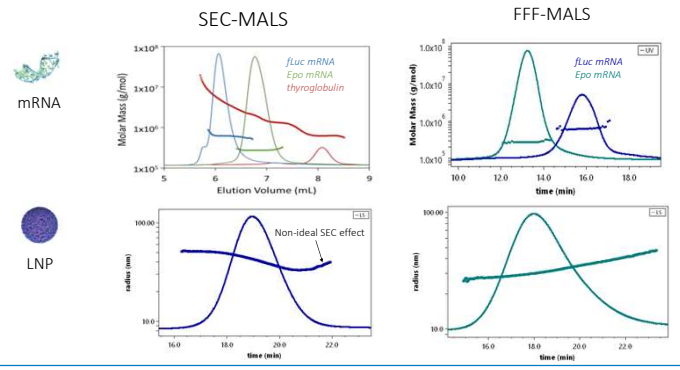
ultraDAWN
Real-time LNP size and concentration for process development and PAT

SEC-MD

FFF-MD

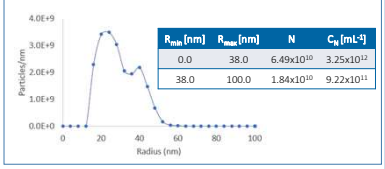
✓ MD (online multiple-detector): DAWN (MALS-DLS), UV (260 nm), Optilab (dRI)
✓ Software packages are 21 CFR Part 11 compliant, IQOQ service available

Size-Based Separation by both SEC and FFF: mRNA and LNP



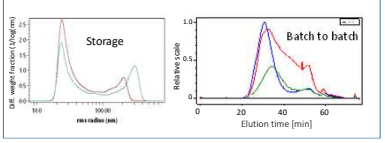
Comprehensive Characterization and Payload Analysis

Size and Concentration

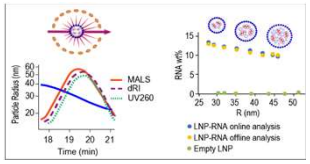


R_{min} [nm]	R_{max} [nm]	N	C_N [mL^{-1}]
0.0	38.0	6.49x10^{10}	3.25x10^{12}
38.0	100.0	1.84x10^{10}	9.22x10^{11}

Physical Stability (FFF)



Size-based RNA payload Analysis

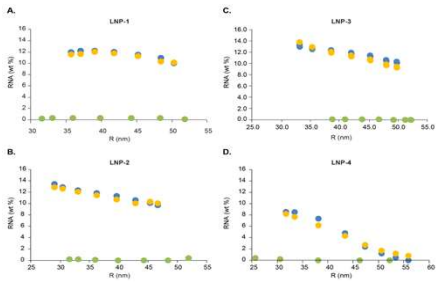


X. Jia, et al, "Enabling online determination of the size-dependent RNA content of lipid nanoparticle-based RNA formulations", submitted.

Offline analysis
Total mass: use dRI signal from SEC
RNA mass:
1) Fractions collected;
2) LNP disrupted by 2% Triton X-100 and diluted;
3) Analyzed by UHP-RPC-UV(260 nm) for RNA quantitation.

Online analysis (Wyatt LNP method)
Use data from ***online multiple-detector*** and ***Wyatt LNP Analysis*** to calculate lipid and RNA concentration of each eluting slice.

Online LNP Payload Analysis: Cross Verification

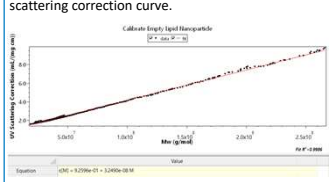


LNP-RNA, offline
LNP-RNA, online
Empty LNP, online

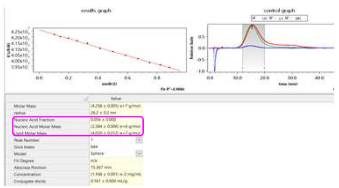
- Online LNP Payload Analysis confirmed that empty LNPs do not contain RNA.
- The agreement of size-dependent payload between the Online LNP Payload Analysis method and the offline approach is outstanding for all four LNP samples, confirming the validity of the online method.

Step-by-Step ASTRA LNP Payload Analysis Procedure

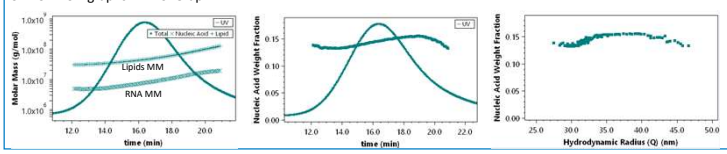
1. Use the method for empty LNP to generate a UV scattering correction curve.



2. Use the method for loaded LNP to get the results.



3. View new graphs in EASI Graph.



Conclusions

- SEC or FFF separates LNP with high resolution.
- Online detectors (MALS, UV at 260 nm, and dRI) provide comprehensive characterization and multi-attribute quantitation.
- The new LNP Analysis method enables size-based nucleic acid payload.
- MD-SEC and MD-FFF are essential tools for measuring LNP size, concentration, payload, and product quality. Software packages are 21 CFR 11 compliant, making both systems suitable in QC environment.
- Both systems are automated, robust, easy to adopt, with minimum hands-on time, less prone to experimental errors.
- MD-SEC and MD-FFF are orthogonal and complementary to the other conventional assays.