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APPLICATION NOTE

AN6601: Measuring size, concentration, and zeta potential of LNPs with the DynaPro™ ZetaStar™

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Summary

Microfluidic formulation of RNA-containing lipid nanoparticles (LNP-RNA) is a common means of developing and producing this important non-viral modality of gene delivery. A key challenge during formulation is the selection of the specific lipids and formulation conditions to achieve the desired particle sizes and surface potential. This application note demonstrates the use of the DynaPro™ ZetaStar™ instrument to rapidly characterize size, polydispersity and zeta potential of LNP-RNA suspensions, highlighting the outcomes of two different LNP formulation techniques.

Introduction

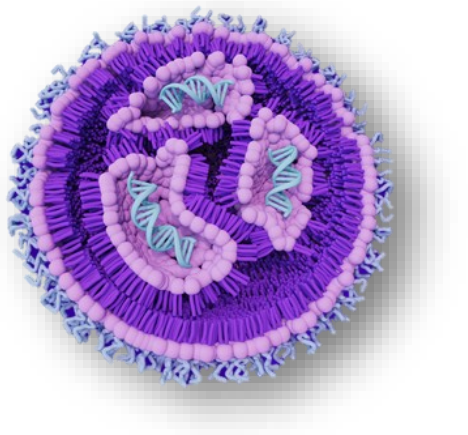
Lipid nanoparticle-based delivery of pharmaceuticals holds much promise for novel gene therapies, oncology, and effective, rapid-response vaccines. COVID messenger RNA (mRNA) vaccines by Pfizer™/BioNTech™ and Moderna™ are two of the latest successful examples.

From formulation to characterization

The main components of LNP-RNA are ionizable lipids, PEGylated lipids, cholesterol and phospholipids, forming the nanoparticle, and the active pharmaceutical ingredient RNA. The LNP surface may be decorated with targeting proteins such as antibodies or fragments thereof, guaranteeing delivery of the payload to the right cell type.

A common means to produce LNPs is mixing RNA, dissolved in aqueous solution, with the lipid mixture, dissolved in an organic solvent, such as ethanol. The addition of aqueous solvent causes the lipids to phase separate, resulting in the formation of lipid nanoparticles that electrostatically encapsulate the RNA. This process is believed to become more efficient and better controlled via rapid microfluidic mixing.

Key biophysical attributes of the intermediate and final formulation products include particle size and polydispersity, zeta potential and particle concentration. The specific lipids used as well as mixing parameters such as flow rate and solution ratio all impact the outcome. Since these attributes affect biodistribution and cellular uptake as well as dosing, they are considered critical for safety and efficacy and must be characterized.



The attributes mentioned above are typically measured by dynamic, electrophoretic and static light scattering (DLS/ELS/SLS). Though several instruments do incorporate all three measurement types, performing a full set on a single LNP-RNA sample tends to be slow and cumbersome. In particular, the zeta potential of LNP-RNA is notoriously difficult to measure accurately and repeatably when sample quantities are restricted, such as in early development.

DynaPro ZetaStar Instrument

Wyatt Technology's DynaPro ZetaStar DLS/ELS/SLS instrument facilitates rapid, streamlined characterization of LNP-RNA. With the microcuvette and dip cell, ELS, DLS,

and SLS are measured for size, polydispersity, zeta potential and particle concentration in a single workflow with just 65 µL of sample. The entire process takes less than a minute. Alternatively, size, polydispersity and zeta potential can be measured automatically for an entire series of formulation conditions by means of an autosampler, requiring about 500 µL per sample.

Figure 1. The DynaPro ZetaStar DLS/ELS/SLS instrument features walk-up operation with an intuitive touch-screen application, DYNAMICS Touch™, that guides users through the measurement process. It can be operated manually, in microcuvette mode, or combined with an autosampler for automated analysis of many samples in DYNAMICS™ software.

Rather than using the same photodetector for each measurement type, the ZetaStar instrument incorporates separate detection channels optimized for each technique. This enables parallel measurements of DLS and SLS (size, polydispersity and particle concentration) or DLS and ELS (size, polydispersity and zeta potential) in the shortest amount of time.

Determination of zeta potential of LNP-RNA in a solution that contains appreciable ionic strength (more than about 10 mM NaCl) has been a vexing issue for most ELS instruments. ELS relies on applying an electric field, under which the charged analyte particles move at a velocity that is relative to their zeta potential. High applied voltage enables fast measurements but can degrade the particles, making the results unreliable. Low applied voltage results in a weak response, necessitating long measurement times. The ZetaStar instrument implements an advanced electro-optical technique that enables highly sensitive detection even at low applied electric fields. This technique, dubbed FIDELIS for 'Fiber Interferometric Doppler Electrophoretic Light Scattering', provides rapid, reliable zeta potential determination, simultaneously with measurements of size and polydispersity in the DLS channel, as shown below.

The ZetaStar instrument can be operated in walk-up mode via the intuitive DYNAMICS Touch onboard app, or with full 21CFR Part 11 compliance via comprehensive DYNAMICS software on a host PC. Quantifying multiple quality attributes on a single instrument, the ZetaStar Instrument contributes to pharmaceutical studies of dosing, formulation, stability and lot-to-lot variability across product and process development cycles as well as quality control in the manufacture of a marketed product.

Materials and Methods

LNPs were kindly provided by the University of Pennsylvania. LNPs were prepared either via microfluidic-mixing or hand-mixing. Prior to measurement, the neat LNPs were then diluted 100-fold in 0.02 µm filtered 20 mM tris buffer (pH 7.4). The samples were as follows:

- S1: Empty LNP prepared via microfluidic mixing.
- S2: LNP loaded with mRNA, prepared via microfluidic mixing.
- S3: LNP loaded with mRNA, prepared via hand mixing.

Batch DLS, ELS and SLS measurements were performed in a dip cell with the ZetaStar instrument. Data acquisition and analysis were performed with DYNAMICS software. Adaptive collection mode was enabled for optimization of the applied current and measurement time, which resulted in 60 seconds on average for each sample.

Results and Discussion

Size and size distribution

DLS measurements revealed large differences in the size distribution for the LNPs prepared by microfluidic mixing compared to the LNPs prepared by hand mixing. Figure 2 shows size distribution results for all three LNPs. The empty LNP prepared using microfluidic mixing (S1) appears to be the smallest among all three LNPs with a mean radius ~30 nm with a small amount of aggregate

around couple hundred nanometers. Using the same technique to create mRNA-loaded LNP produces particles with an average radius of ~40 nm and single broad size distribution (S2). These distributions agree with the expectation and are within the desired range for optimal circulation and cellular uptake.

Not only does the presence of nucleic acid affect the size of LNPs, the processing parameters used for generating LNPs or even the process itself can also significantly influence the size of final production particles. As shown in Figure 2, hand-mixing process (S3) produces much larger LNP with comparable polydispersity. Their average radius is nearly two times as big as the LNPs created using microfluidic mixing approach. This larger size may be undesirable for bioavailability and functionality.

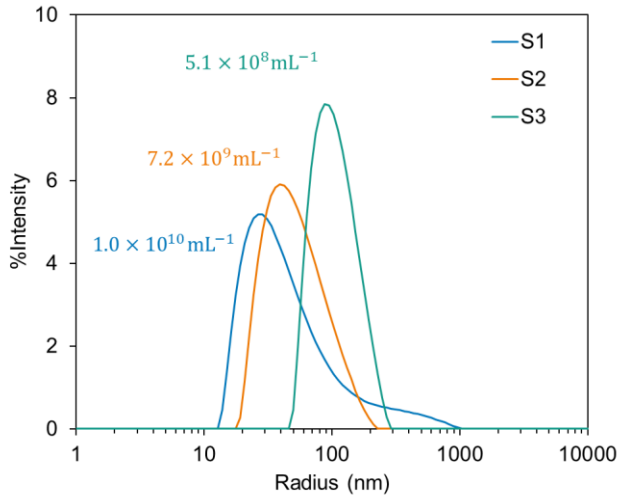


Figure 2. Size, size distribution, and concentration of empty LNP prepared via microfluidic-mixing (S1), LNP with mRNA prepared via microfluidic-mixing (S2), LNP with mRNA prepared via hand-mixing (S3).

Particle concentration

Particle concentration reveals yield information and informs dosing. Thanks to the dedicated SLS detector, particle concentration measurements can be made in under a minute and without reagents or particle concentration standards. The ZetaStar instrument enables simultaneous size and particle concentration measurement in as little as 2 µL with a quartz cuvette. The particle concentration results from three LNPs can be found in Figure 2 and summarized in Table 1. These data suggest that the hand-mixing procedure creates fewer larger particles while microfluidic-mixing produces more smaller particles.

Zeta potential

The ZetaStar instrument performs simultaneous dynamic and electrophoretic light scattering (DLS/ELS) to determine size and zeta potential. The zeta potential analysis (Figure 3) reveals that all three LNPs exhibit a consistent negative charge, regardless of their preparation method or whether they are loaded or empty.

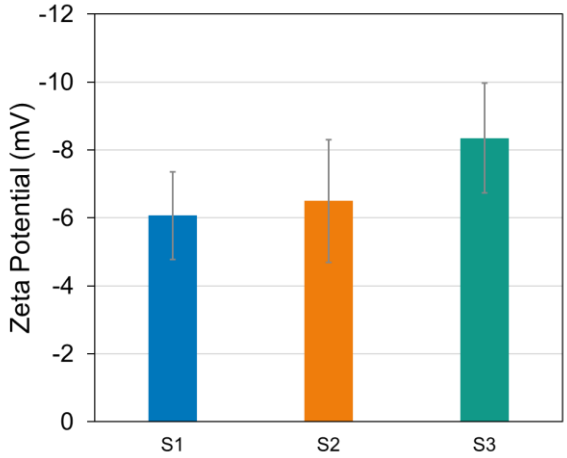


Figure 3. Zeta potential of empty LNP prepared via microfluidic-mixing (S1), LNP with mRNA prepared via microfluidic-mixing (S2), LNP with mRNA prepared via hand-mixing (S3).

The results are also summarized in Table 1. It is important to note that the zeta potential is a measure of the surface charge of the nanoparticles and does not include the charge of buried mRNA. In this case, the presence of the negatively charged mRNA inside the LNP did not produce a significant change in the net surface charge of the particle.

Table 1. Average hydrodynamic radius, zeta potential, and particle concentration of LNP samples

LNP condition	Radius (nm)	Zeta potential (mV)	Particle concentration (mL^{-1})
S1: Empty, microfluidic mixing	38 ± 2	-6.1 ± 1.3	(1.0 ± 0.3) x 10^{10}
S2: Loaded, microfluidic mixing	44 ± 1	-6.5 ± 1.8	(7.2 ± 1.1) x 10^{9}
S3: Loaded, hand mixing	98 ± 2	-8.4 ± 1.6	(5.1 ± 0.5) x 10^{8}

All values are average and standard deviation of 10 measurements.

Conclusions

Measuring LNP-RNA size, size distribution, zeta potential and particle concentration with a DynaPro ZetaStar instrument provided a streamlined approach to rapid screening of LNP formulation and/or process conditions. The ZetaStar instrument is a simple to use, powerful and versatile tool that, in addition to size, polydispersity, zeta potential and particle concentration for nanoparticles, can further determine molar mass, turbidity, interactions and thermal behavior for macromolecules such as polymers, proteins or nucleic acids.

While a ZetaStar instrument is ideal for quick screening, LNP-RNA analysis can be further enhanced with detailed characterization of particle size distributions, concentration, encapsulation efficiency and size-based payload (the amount of RNA encapsulated in each LNP particle size). The more detailed studies require separation-based techniques of SEC-MALS and/or FFF-MALS, wherein all of these attributes can be determined in a single run using Wyatt's online DAWN™ light scattering and Optilab™ refractive index detectors, Waters™ chromatographic modules, Eclipse™ field-flow fractionation instrument and ASTRA™ software.

Acknowledgements

We thank the University of Pennsylvania for kindly providing LNPs.

To learn more about the Wyatt's solutions for lipid nanoparticle characterization, please click here.

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