Optimizing LNP-RNA Formulations: Key Insights into Size, Zeta Potential, and Stability

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Abstract

The microfluidic formulation of RNA-containing lipid nanoparticles (LNP-RNA) is a widely utilized approach for developing and producing non-viral gene delivery systems. A critical challenge in this process is optimizing lipid selection and formulation conditions to achieve targeted particle sizes and surface potential.

This study highlights the application of the DLS/SLS/ELS instrument for rapid characterization of LNP-RNA therapeutics, focusing on size, polydispersity, and zeta potential.

Results demonstrate the impact of production methods on LNP formulations, colloidal stability across various buffer conditions, and structural changes following freeze-thaw cycles.

These findings underscore the importance of precise characterization for optimizing LNP-RNA formulations and ensuring their stability and efficacy.



The DynaPro™ ZetaStar ™ instrument combines dynamic, static and electrophoretic light scattering (DLS/SLS/ELS) all in one platform.

Particle Concentration Charge and Isoelectric Point Size and Polydispersity **Thermal Denaturation** k_n and A₂ **Molar Mass Viscosity Turbidity**

Features



Designed for Casual and Expert users

DYNAMICS Touch™ software intuitively guides with virtually no training.

Measure particle concentration, size, and zeta potential in less than 2 minutes with 65 µL.



Hands-free automation

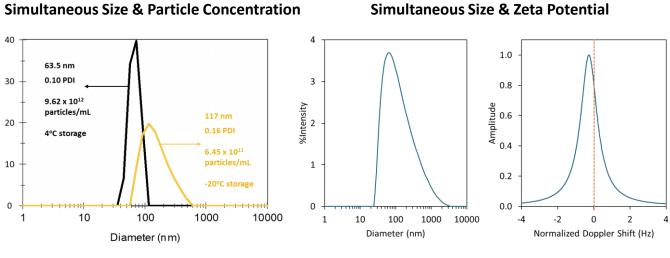
Combine the DynaPro ZetaStar instrument with an autosampler for unattended measurements of dozens of **samples**; probe stability with automated temperature



ELS in native buffer.

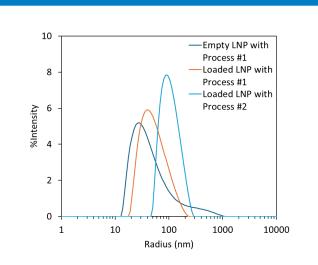
The ZetaStar instrument was designed for biopharma applications and with the addition of the ATLAS™ pressurization accessory, excels in high salt buffers. Obtain size and zeta potential in formulation buffer without dilution.

Rapid quality check



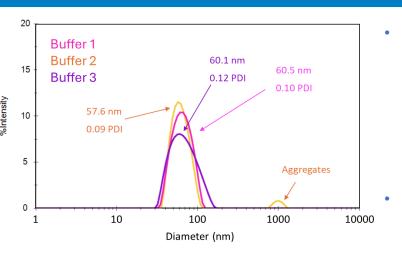
- LNP size, polydispersity, and zeta potential are important metrics for both effectiveness and stability.
- The DynaPro ZetaStar instrument's dedicated detectors allow simultaneous DLS-SLS or DLS-ELS measurements.
- Save overall time and hands-on time: DLS-SLS measurement ~15 seconds,
 - DLS-ELS measurement ~30 seconds
- Collecting the two data streams simultaneously also allows us to probe for potential changes that may occur during the measurement

Mixing Process Repeatability Assessment



- Empty LNPs exhibit a noticeable broad distribution and smaller size.
- Prep 2 produces larger particles than Prep 1.
- ELS shows that all samples are negatively charged under the test conditions; however, Process 1 and Process 2 conditions seem to have little influence on the LNP surface charge.
- The LNP mixing method, total flow rate, and organic-aqueous ratio make a significant difference in the particles produced and the surface charge of the lipids.
- The DynaPro ZetaStar simultaneously measures size and zeta potential and can be integrated with an autosampler, saving time and effort.

Zeta Potential Measurement in Buffer Formulation



■ LNP 1 Buffer 1 ■ LNP 1 Buffer 2 ■ LNP 1 Buffer 3

Three LNP buffers of increasing ionic strength were tested: Buffer 1 (lowest strength) to Buffer 3 (highest).

The ZetaStar instrument, designed for

salt buffers with the ATLAS

buffers without dilution.

biopharma applications, excels in high-

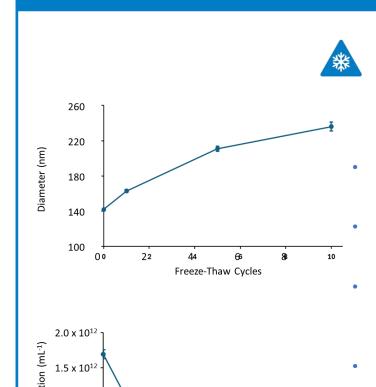
pressurization accessory or Arc™ pump

& autosampler, enabling size and zeta

potential measurements in formulation

- Ionic strength had no noticeable effect on particle size but influenced zeta potential.
- Buffer 1 showed a positive zeta potential, while Buffers 2 and 3 were negative.
- In all cases, zeta potential remained below ±30 mV, indicating a higher risk of particle aggregation.

Freeze Thaw Stability



Freeze-Thaw Cycles

 1.0×10^{12}

0.5 x 10¹²

concentration

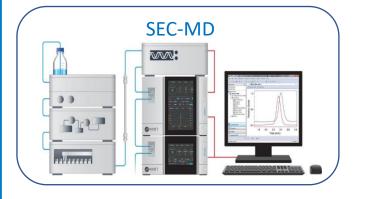
- Freezing and thawing can introduce concentration gradients and mechanical stress. Zeta potential is sensitive to ionic strength changes. LNP size, polydispersity, and particle concentration across freeze-thaw cycles (before
- freezing, and after 1 to 10 cycles). Average size increases after the first cycle and continues to grow, while polydispersity remains stable, suggesting uniform size growth without large aggregates.
- Aggregation is evident from decreasing particle concentration, indicating smaller LNPs form larger particles over time.
- This study highlights the importance of understanding freezing effects on LNP properties and demonstrates the DynaPro ZetaStar instrument's capability to monitor these changes during freeze-thaw experiments.

Wyatt Solutions for LNP analysis











Conclusion

-1.91

Zeta Potential (mV)

- The DynaPro ZetaStar instrument provides a streamlined approach for rapid screening of LNP formulation and process conditions. It measures LNP-RNA size, size distribution, zeta potential, and particle concentration.
- The DynaPro ZetaStar instrument is a simple, powerful, and versatile tool that can also determine molar mass, turbidity, interactions, and thermal behavior for macromolecules like polymers, proteins, or nucleic acids.



Conductivity (mS/cm)

SEC-MALS and/or FFF-MALS are recommended.



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