LNP Self-Assembly Kinetics with Simultaneous Multi-Angle DLS

Mohit Agarwal (Swabian Instruments GmbH). In collaboration with XNApharma GmbH, Gera, Germany

INTRODUCTION

Lipid nanoparticles (LNPs) have become a key platform technology for the delivery of nucleic acids such as mRNA, siRNA, and DNA in vaccines and gene-based therapeutics. Their physicochemical properties, particularly particle size, size distribution, and structural stability, are established within seconds of formation, where rapid mixing of lipid and aqueous phases initiates nucleation, growth, and structural rearrangement. Therefore, to understand particle evolution, formulation effects, and colloidal stability, it is essential to capture these early self-assembly dynamics [1].

Conventional characterization methods rely on static, endpoint measurements that miss transient processes critical to understanding particle evolution [2]. Time-resolved DLS addresses this gap by continuously monitoring hydrodynamic radius and polydispersity from the moment of mixing. Swabian Instruments' DLScat is purpose-built for this application, combining simultaneous multi-angle detection with automated, long-duration data acquisition. Applied to LNPs prepared in water and sodium acetate buffer (25 mM, pH 4.5), the DLScat resolved clear formulation-dependent differences in self-assembly kinetics over 10 minutes, demonstrating its capability as a powerful tool for real-time nanoparticle characterization.

INSTRUMENTATION

The DLScat records a continuous photon arrival stream simultaneously across multiple scattering angles, commencing within seconds of sample preparation. This raw photon stream from the 600s experiment is partitioned into short, consecutive time slices, each yielding an independent intensity autocorrelation function. Hydrodynamic radius (R_h) and polydispersity index (PDI) are extracted from each slice via cumulant analysis, producing a high-temporal-resolution sequence of size measurements throughout the experiment without interruption or remeasurement.

Key instrument features:

- **Simultaneous multi-angle detection** at up to five angles, improving measurement accuracy and reliability over single-angle approaches
- **Post-processing** via time-sliced photon stream analysis
- **Automated long-duration measurements** from minutes to hours with no user intervention required
- **Optical density (OD) calibration** before each run to optimize detector performance
- **Integrated time window** for flexible extraction of defined measurement intervals
- **Immediate data acquisition** commencing within seconds of sample preparation

EXPERIMENTAL

LNPs were formulated by hand-mixing an ethanolic lipid phase with an aqueous phase directly inside the measurement cuvette at room temperature. Two aqueous media were compared: ultrapure water and 25 mM sodium acetate buffer (pH 4.5). Measurements were initiated within 5s of mixing. Scattering data were acquired simultaneously at four angles (69°, 90°, 111°, and 157°) over 600s. R_h and PDI values, calculated using the cumulant algorithm, represent the mean of three independent measurements evaluated across all detection angles.

RESULTS AND DISCUSSION

Time-resolved DLS measurements provided direct insight into the self-assembly kinetics and temporal evolution of LNPs under both buffer conditions (Figure 1).

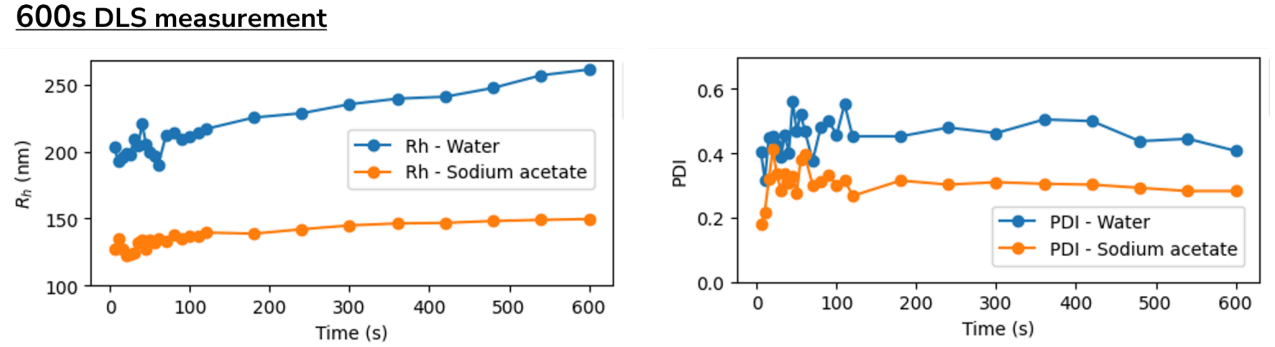

Figure 1: Time-resolved R_h and PDI vs. time for Water and Sodium acetate formulations

Water-based formulations exhibited a continuous increase in R_h over the full 600s measurement window, accompanied by persistently elevated PDI values. This behavior indicates ongoing post-assembly particle growth and structural rearrangement, consistent with the absence of ionic stabilization.

Sodium acetate buffer formulations produced particles with smaller initial sizes and significantly reduced growth dynamics, reaching near-stable Rh values within the first 100s. PDI values were consistently lower throughout, reflecting improved colloidal uniformity. This stabilization is attributed to buffer-mediated modulation of electrostatic interactions, ion screening effects, and lipid packing behavior, resulting in more controlled particle organization.

These results demonstrate that the DLScat clearly resolves formulation-dependent differences in self-assembly kinetics that would be entirely invisible to conventional endpoint measurements.

CONCLUSION

The DLScat enables real-time, multi-angle characterization of nanoparticle self-assembly under realistic preparation conditions. By continuously recording and time-slicing the photon arrival stream across simultaneous detection angles, it resolves transient structural changes with a temporal granularity that no single-angle or sequential approach can match. Applied to LNP formation, the instrument revealed clear, quantitative differences between buffer systems within the first minutes of assembly.

The DLScat is ideally suited for:

- **Formulation development:** identifying optimal buffer and excipient conditions
- **Stability screening:** tracking colloidal evolution over extended timeframes
- **Kinetic self-assembly studies:** resolving nucleation, growth, and maturation phases
- **Process development and quality control:** real-time monitoring without workflow interruption

REFERENCES

[1] Calibration for a count rate-dependent time correlation function and a random noise reduction in pulsed dynamic light scattering. ANAL. SCI. 2022, 38, 607–611. https://doi.org/10.1007/s44211-022-00071-0

[2] Toward time resolved dynamic light scattering microscopy: Retrieving particle size distributions at high temporal resolutions, Rev. Sci. Instrum. 2023, 94, 083101; https://doi.org/10.1063/5.0160156

For more information about the DLScat, visit www.swabianinstruments.com

www.swabianinstruments.com | info@swabianinstruments.com | +49 711 40044790
Swabian Instruments GmbH | Stammheimer Str. 41 | 70435 Stuttgart | Germany