T5099

Validation Of Resistive Pulse Sensing (RPS) For Characterizing Nanoparticles In Drug Formulations

Jean-Luc Fraikin, Andrew Cleland, Peter Meinhold, Franklin Monzon and Lew Brown

Spectradyne LLC, Torrance, CA

2017 AAPS ANNUAL MEETING & EXPOSITION

CONTACT INFORMATION: lew.brown@spectradynellc.com

DEVELOPING SCIENCE. IMPACTING HEALTH.

PURPOSE

Resistive pulse sensing (RPS, Figure 1) has emerged as a **much-needed orthogonal method** to optical particle analysis techniques such as dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA), which fail to accurately measure the size distribution of nanoparticles in many real-world formulations. **Spectradyne's nCS1™ instrument** (Figure 2) was used to evaluate the method's accuracy and precision with a **broad set of materials**, including liposomal, polymeric, and protein-based nanoparticles, and to **quantify degradation** in a formulation. The nCS1 was directly compared to DLS in replicate tests of two different lipid nanoparticle formulations produced by a highly controlled microfluidic mixing system.

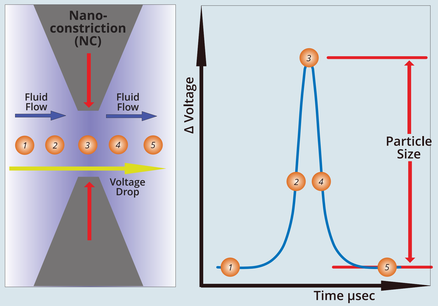


Figure 1: Resistive Pulse Sensing measures particles one by one, by detecting a change in electrical signal as each particle passes through a nano-constriction. The change in signal is directly proportional to the particle *volume*, regardless of particle shape or material.

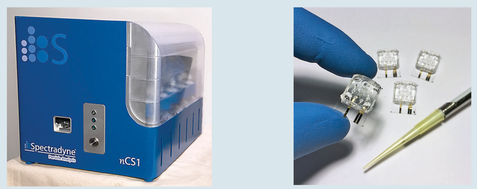


Figure 2: The Spectradyne nCS1 occupies a **small bench top footprint**, approximately 1.5 sq ft (left). **Only 3 µL of a sample is required** for analysis using a disposable microfluidic cartridge (right), which prevents contamination between measurements and eliminates cleaning requirements.

METHODS

Concentration linearity of the ncs1 was demonstrated using a dilution series of polymeric nanoparticles (62 nm diameter). Replicate measurements were performed in different analysis cartridges to quantify repeatability. Sample groups spanning a variety of materials were analyzed on the nCS1 including liposome-, protein- and polymer-based nanoparticle formulations. The particle concentration vs. Size distributions were compared to quantify differences within each group. Polydisperse lipid nanoparticle samples were created and measured on DLS by an independent formulation lab to compare performance versus the nCS1.

RESULTS

1. Concentration Linearity:

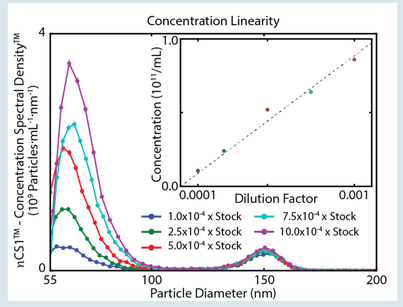
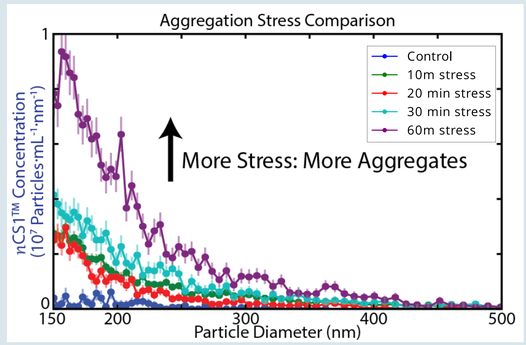


Figure 3: nCS1 demonstrated a highly linear response to concentration for a dilution series of 62 nm diameter polymeric nanoparticles. Particle size distributions and linear fit of concentration versus dilution are shown.

Spectradyne's nCS1 demonstrated a highly linear concentration response (Figure 3) over the entire dilution range of 10^{10} -10^{11} particles/mL (R^2 = 0.99). The measured absolute concentration of particles in the undiluted formulation was (9.6 +/- 0.9) x 10^{13} particles/mL, consistent with the manufacturer's estimates from material input to the synthesis process. Measurements in 5 different analysis cartridges yielded mean particle diameter 63 +/- 2 nm, demonstrating low variability (3 %).

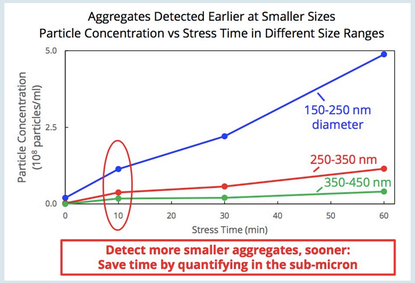
2. Stress-Induced Protein Aggregation:



The nCS1 accurately quantified stress-induced aggregation in a protein therapeutic (Figure 4), and demonstrated the value that measuring smaller particles can bring to formulation development: Aggregation can be detected earlier by measuring smaller particles (Figure 5).

Figure 4 (left): nCS1 measurement results for stressed protein therapeutic showing clear increase in aggregates with stress time.

Figure 5 (right): nCS1 measurements clearly show that increased aggregation is detected much earlier in the smaller (150nm-250nm) size range. Detecting aggregation sooner will save time and money in formulation development. Aggregation activity can be detected and acted upon long before sub-visible methods (flow imaging, light obscuration) might indicate a production issue.



RESULTS (CONTINUED)

3. Comparison to DLS on Two Lipid Formulations:

The nCS1 yielded accurate, high-resolution size and concentration distributions of replicate lipid nanoparticle formulations, while DLS measurements gave incorrect and misleading results (Figure 6).

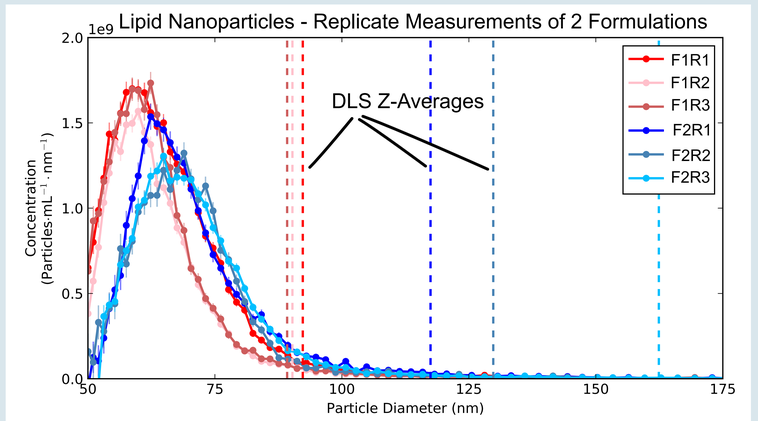


Figure 6: nCS1 measurements of two different lipid nanoparticle formulations (F1 and F2), manufactured in three different replicates each. The three red distributions show the nCS1 measurements for the three different Formulation 1 samples, while the three blue distributions show the nCS1 measurements for the three different Formulation 2 samples. The vertical dotted lines show the measured DLS Z-Averages for each sample as measured by the formulation manufacturer.

The DLS measurements not only misrepresent the mean sizes of each sample, they also show very poor repeatability on the Formulation 2 measurements, due to the longer tail in the distributions and light-scattering's tendency to emphasize larger particles.

CONCLUSIONS

Spectradyne's nCS1 was validated as a method for quantifying nanoparticles that is **truly orthogonal to light scattering techniques** such as DLS and NTA, and delivered insights that could not otherwise be obtained. The nCS1 demonstrated a **highly linear** concentration response, **robust measurements**, and **accurate characterization** of a variety of material types, **outperforming DLS** in a direct head-to-head comparison.

Spectradyne® Particle Analysis