

Validation of Resistive Pulse Sensing for Characterizing Nanoparticles in Drug Formulations

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Introduction

Despite widespread use, dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) fail to accurately measure the size distribution of nanoparticles in many real-world formulations. All formulations are polydisperse to some degree, confounding DLS, and low index contrast materials such as proteins, viruses, and liposomes evade clear detection by any light scattering technique. Resistive pulse sensing (RPS) has emerged as a much-needed orthogonal method. Spectradyne's implementation (the nCS1 instrument) was used to evaluate the method's accuracy and precision with a broad set of materials, including liposomal, polymeric, protein-based, and natural nanoparticles, and to quantify degradation in a formulation. The nCS1 was directly compared to DLS and NTA in a head-to-head test.



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 samples were measured by an independent testing lab to compare performance of the nCS1 to DLS and NTA.

Results

1.) Concentration Linearity:

A sample of biocomposite nanoparticles (62 nm in diameter) was used to create a dilution series. The nCS1 demonstrated a highly linear concentration response over the entire dilution range of 10^{10} to 10^{11} particles/ml ($R^2 = 0.98$), as shown in Figure 1. The measured absolute concentration of particles in the undiluted formulation was $(9.6 \pm 0.9) \times 10^{13}$ 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 %).

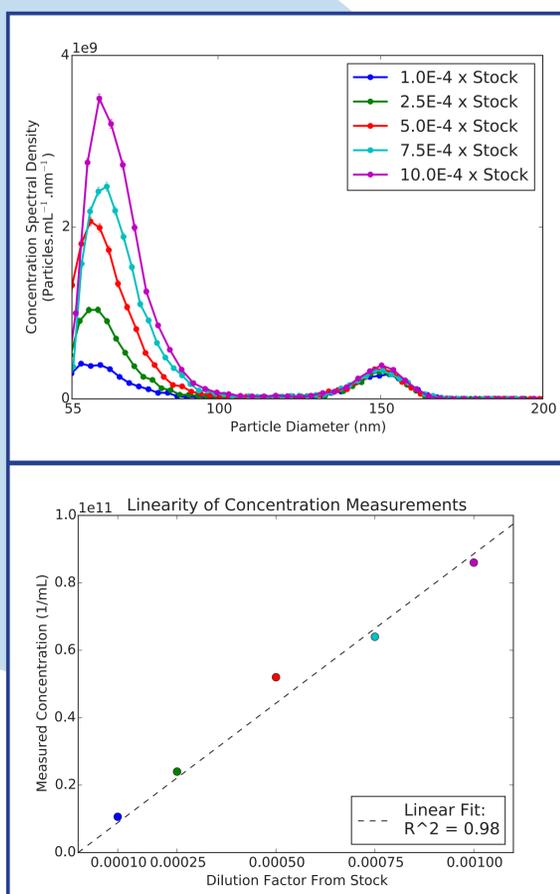


Figure 1: nCS1 measurement results for dilution series with 62 nm diameter nanoparticles, showing particle size distributions (top) and linear fit of concentration versus dilution (bottom).

Results (continued)

2.) Measurement of Nanoparticle Formulations of Different Materials:

Nanoparticle drug formulations made from both liposomal and polymeric materials were successfully measured with the nCS1 as shown in the following figures:

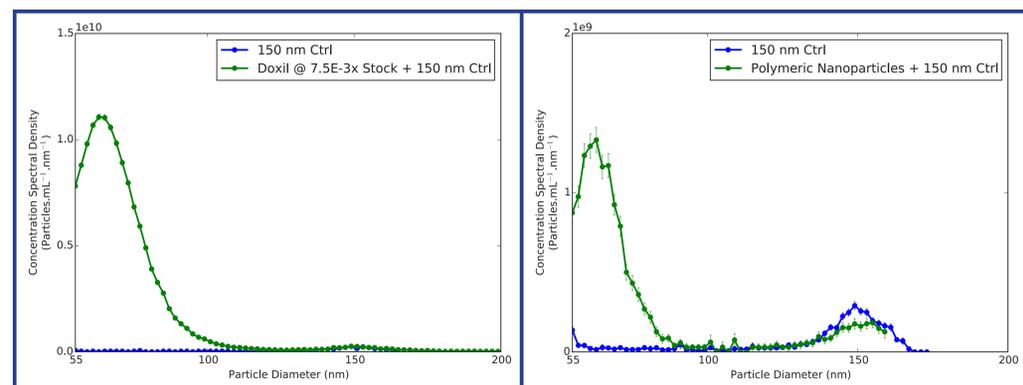


Figure 2: Nanoparticle formulations measured with the nCS1. Left graph shows Doxil, a liposomal formulation, while the right graph shows another formulation made from polymeric nanoparticles.

3.) Monitoring of Stressed Nanoparticle Formulations:

The following figures show degradation found in drug formulations during stability testing:

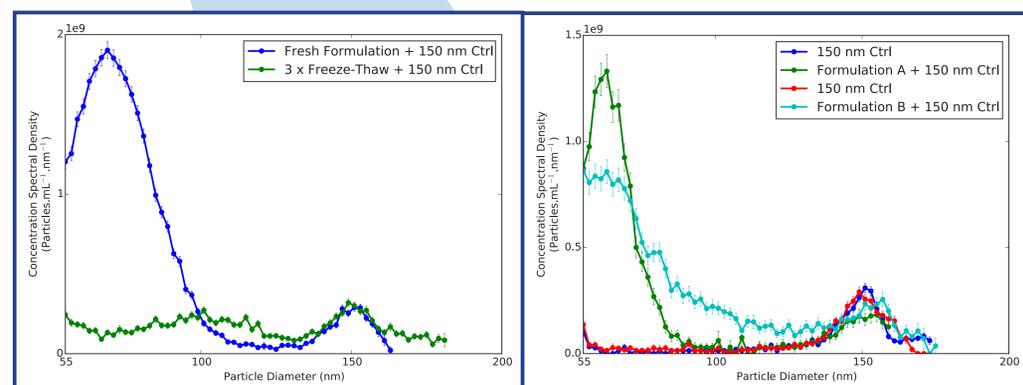
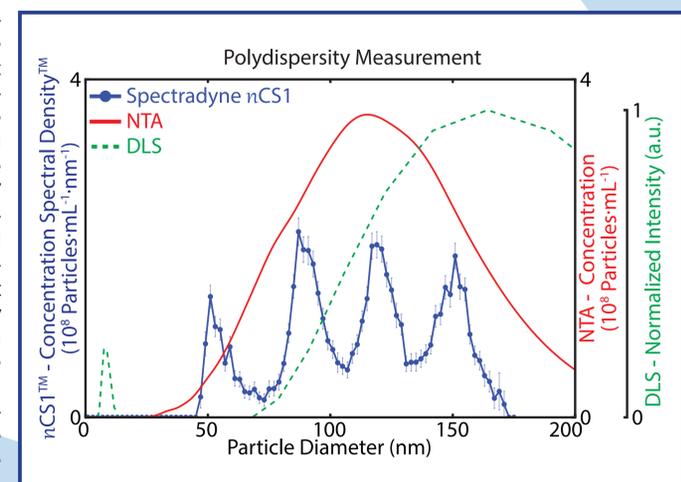


Figure 3: Stability testing of nanoparticle drug formulations measured with the nCS1. The left graph shows a dramatic change in particle size distribution for a formulation after undergoing a 3X freeze/thaw cycle. On the right graph, we see the more subtle effect of shelf-life on a single drug product with two different formulations: Formulation A in green holds up better over time than its alternate Formulation B.

4.) Comparison of Performance Versus DLS and NTA:

Figure 4 (right): Comparison of results obtained with the same sample as measured by the nCS1, Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA). The sample consisted of 52, 94, 122 and 150nm NIST-traceable polystyrene spheres at roughly 5×10^9 particles/mL. The DLS and NTA measurements were performed at a 3rd party independent testing laboratory. The results clearly show that only the nCS1 is able to accurately characterize the sample. DLS is an ensemble measurement, therefore not useful for polydisperse samples. While NTA does measure each particle individually, it does not have the precision necessary to resolve the four components.



Conclusions

The resistive pulse sensing method as implemented in Spectradyne's nCS1 was validated as a high-precision technique for sizing and quantifying nanoparticle-containing drug formulations. The nCS1 demonstrated a highly linear concentration response, repeatability < 3%, and accurate characterization of a variety of material types, outperforming both DLS and NTA in a direct head-to-head comparison. Resistive pulse sensing was validated as an accurate technique for quantifying nanoparticles that is orthogonal to light scattering methods such as DLS and optical tracking.