Validation of the Resistive Pulse Sensing Method for Characterizing Nanoparticle Formulations for Drug Delivery
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Introduction
Precise characterization of nanomaterials in drug formulations is critical at all stages of their development and production. However, instrumentation for the accurate and rapid analysis of nanoparticle size and concentration has until now been unavailable. Spectradyne’s nCS1 offers a new implementation of the resistive pulse sensing method and delivers:

- An alternative to optics-based methods.
- High resolution size distributions.
- Absolute concentration measurements.
- All particle materials.
- Arbitrary polydispersity.
- Total scan time in minutes.

Purpose
The resistive pulse sensing method shows promise in providing a much-needed alternative to light scattering-based methods for characterizing nanoparticles in pharmaceutical drug development. Spectradyne’s nCS1 instrument was used to evaluate the accuracy and precision of the method for use with formulated biocomposite nanoparticles, and to distinguish normal from degraded formulations by measuring the samples’ particle size distributions. Finally, a head-to-head test comparing resistive pulse sensing to single-particle optical tracking and dynamic light scattering was performed, and clearly demonstrates the power of the high-resolution measurements delivered by the nCS1 instrument.

Methods
A formulation containing 62 nm (mean diameter) nanoparticles for delivery of a commercial cancer therapy was diluted over an order of magnitude in concentration. The nCS1 was used to measure the concentration of particles at each dilution, and the repeatability of measurement results between cartridges from the same mold lot was analyzed. In a subsequent experiment, high-resolution particle size and concentration distributions of two nanoparticle-based drug formulations were obtained with the nCS1 and used to identify degradation in one of the samples. Finally, a polydisperse mixture of polystyrene particles was analyzed by an independent contractor using two conventional particle analysis methods: Dynamic Light Scattering (DLS) and single-particle optical tracking. The results were compared to the analysis of the same samples using the nCS1.

Effective Formulation Stability Analysis.
Subsequent measurements of two different nanoparticle-based drug formulations clearly identified a difference in particle size distribution between the samples. A clear peak in particle concentration was observed in the normal sample (mean 62 nm, width 15%), while the degraded sample displayed a broad distribution of sizes and no identifiable peak. Other methods such as dynamic light scattering would be unable to provide such high resolution size distribution information.

Superior Accuracy and Resolution.
Nanoparticle suspensions having NIST-certified mean diameter 52, 94, 122 150 nm were mixed together to equal nominal final concentrations (1x10^9 particles/mL). Analysis of the mixture by the three different techniques is shown at right. Spectradyne’s nCS1 clearly resolved the four components of the mixture and yielded concentration measurements for each sub-population within ~20% of the estimates of the manufacturer. Neither optical tracking nor DLS were able to measure the true composition of the sample. Optical tracking reported a total particle concentration nearly 3 times greater.

Impacts for Drug Development and Delivery
1. Formulation Analysis. The resistive pulse sensing method as implemented in Spectadynne’s nCS1 delivers two fundamentally new metrics that can be used to characterize a nanoparticle formulation more precisely and effectively. First, the instrument reports absolute particle concentration, a parameter which relates to the bioavailability of a drug and directly informs the production process. Second, the instrument generates high resolution, statistically significant measurements of particle size distributions, enabling the accurate sizing of a particle population and the detection of spurious particles in nearby size ranges.  

2. Stability Testing. The nCS1’s ability to provide high resolution size and concentration measurements makes the instrument ideally suited for establishing the stability of formulations under different conditions. The technology has been used to directly measure the effects of freeze-thaw cycling on the particles in a commercial lipid emulsion-based drug product, and to detect the degradation over time of biocomposite particles in a cancer therapeutic formulation. In each case, clear signatures were observed in the Concentration Spectral Density that readily distinguished the formulations, and that would have been undetectable using other means.

3. Process Characterization. Different manufacturing methods and processing parameters have significant effects on the physical characteristics of a nanoparticle product. A complete understanding of the impact of these variables on the quality of the particles is critical at all stages of development and production. Spectradyne’s implementation of the resistive pulse sensing method has been used to characterize the detailed difference in particle size distributions between liposomal formulations extruded with different pore diameters (Figure 4).

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 response to varying particle concentration, and yielded a precise, repeatable characterization of the distribution of mean 63 nm diameter nanoparticles in a commercial nanoparticle formulation. The nCS1 clearly distinguished normal and degraded formulations by providing high-resolution particle size distributions, data unobtainable with other techniques. Finally, in a direct comparison with other leading particle analysis techniques, the nCS1 clearly demonstrated its superiority in measuring polydisperse samples.