Measurement of Protein Aggregates in the 150 nm to 1,500 nm Size Range Using Resistive Pulse Sensing

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Spectradyne®
Particle Analysis

Introduction

Particulates in parenteral drug development and production have always been a serious issue. In biologics, the issue is compounded by reported impacts of aggregates and particles on the product's efficacy, safety and immunogenicity. FDA regulations strongly recommend in-depth characterization of the identity and quantity of particles in protein therapeutics.

While techniques for sub-visible (1-100µm) particles are well established, characterization of sub-micron (100-1,000nm) particulates has only become possible recently. Spectradyne's nCS1 offers a new implementation of the resistive pulse sensing method and delivers:

- An alternative to optics-based methods.
- No dependence on particle material type.
- High-resolution size distribution.
- Sizing range: 50nm to 2µm diameter.
- Arbitrary polydispersity.
- Total sample analysis in minutes.

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.

Purpose

The resistive pulse sensing method shows promise in providing a much-needed alternative to light scattering-based methods for characterizing protein aggregates and nanoparticles in pharmaceutical drug development. Spectradyne's nCS1 instrument was used to evaluate the accuracy and precision of the method for use with biologic protein formulations, and to quantify the effect of stress applied to the formulation by measuring the samples' particle size distributions.

Methods

A proprietary protein formulation was supplied to Spectradyne for measurement with the nCS1 instrument. The sample was run "as-is": no dilution or additives were required. The native buffer was an "acetate buffer" with an ionic strength of approximately 20mM. Five samples were provided: One unstressed sample and four samples subjected to increasing levels of stress (10, 20, 30 and 60 minutes of stress). The size and concentration of particles in each sample were measured across a size range spanning 150nm – 1,500nm.

Results

Protein Formulation Samples: The nCS1 clearly distinguished concentration differences in protein aggregates for each of the five samples (control plus four different stress conditions). As expected, increasing stress levels produce higher concentration of protein aggregates.

To capture the complete range desired, 150nm -1,500nm, two disposable cartridges are required with different sized measurement constrictions. The two measurements can then be "stitched together" by the analysis software to show the entire range desired.

Figure 1 shows the results for the control plus four varying stress samples in the range from 150-500nm, as measured in the 1µm constriction cartridge. Clearly, the longer duration stress conditions lead to significantly higher protein aggregate content.

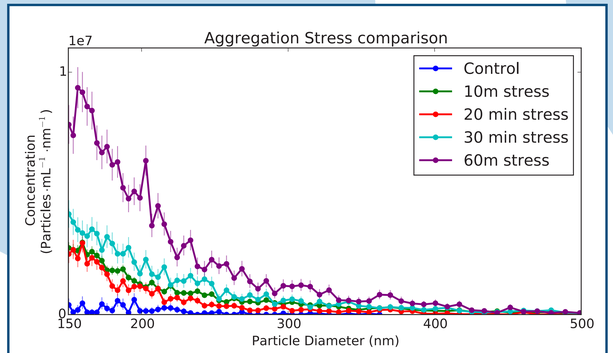

Figure 1: nCS1 measurement results for four protein aggregation samples plus control, showing clear increase in aggregation with increased stress.

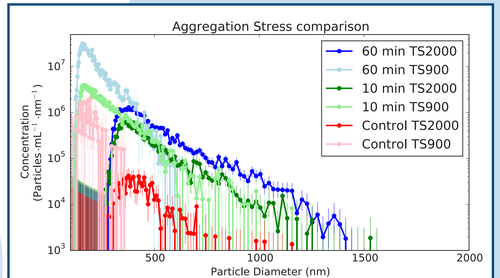

Figure 2: nCS1 measurements from 2µm cartridge (TS-2000) and 1µm cartidge (TS-900) combined to show full range from 150nm to 2µm.

Figure 2 shows the results from both the 1µm constriction and 2µm constriction cartridges plotted together by the software. The log concentration scale hows that the nCS1 can capture concentration measurements over four orders of magnitude. Other measurements have demonstrated high resolution characterization of individual particles over six orders of magnitude in concentration.

Results (continued)

Comparison of different measurement techniques for sub-micron particulates: Nanoparticle suspensions having NIST-certified mean diameters of 52, 94, 122 and 150 nm were mixed together to equal nominal final concentrations ($5x10^9$ particles/mL). One analysis was made on the nCS1, and two aliquots of sample were sent to an independent laboratory for measurement using Dynamic Light Scattering (DLS) and optical tracking (NTA) techniques. Analysis of the mixture by the three different techniques is shown in Figure 3. Spectradyne's nCS1 clearly resolved the four components of the mixture and yielded concentration measurements for each sub-population within ~30% of the estimates of the manufacturer. Neither optical tracking nor DLS could measure the true composition of the sample. Optical tracking reported a total particle concentration nearly 3 times greater.

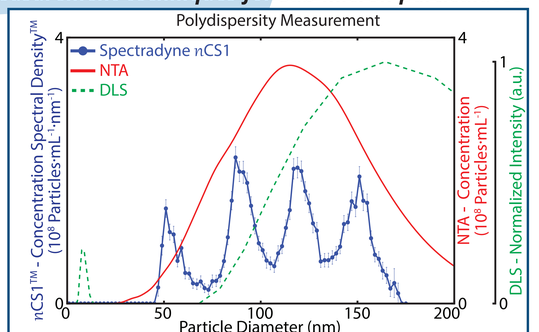

Figure 3: Direct comparison of Spectradyne's nCS1 with single-particle optical tracking and dynamic light scattering. Only the nCS1 reveals the true composition of the polydisperse sample, a mixture of 52, 94, 122 and 150 nm polystyrene particles.

These measurements were made using polystyrene beads in water, where there is a significant difference in refractive index (RI): polystyrene RI = 1.599, water RI = 1.334. This high contrast instance is quite different than is the case for proteins in buffer, where protein solution (100mg/mL) RI = 1.35 to 1.36, water RI = 1.334. Clearly, there is minimal difference in refractive index between the protein aggregates and carrier. This makes it extremely difficult for techniques relying on optical index differences, like DLS and NTA, to properly detect protein aggregates. The nCS1 is not in any way reliant on optical index differences, basing its measurements on electrical detection only. Combined with its high sizing resolution and broad concentration range, this insensitivity to refractive index contrast make the nCS1 uniquely suited to measurement of submicron protein aggregates.

Conclusions

The resistive pulse sensing method as implemented in Spectradyne's nCS1 was able to differentiate particle size distributions for five different samples containing varying amounts of protein aggregates caused by stressing the formulation. This high-resolution discrimination of particle size distributions is not possible using techniques based upon light scattering, such as Dynamic Light Scattering (DLS) or Nanoparticle tracking analysis (NTA).