Submicron Protein Aggregation Measurements for Early Assessment of Formulation Instability

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Particle Analysis

Purpose:

Early detection of protein aggregation in drug formulations is critical for the assessment of drug safety and efficacy. Aggregation is a continuous process, with small aggregates forming much sooner than larger ones, and significant time savings can be achieved if particles can be detected earlier. However, the most common techniques for characterizing protein aggregation use light scattering, and are not sufficiently sensitive to reliably detect particles smaller than 100-200nm in diameter. Moreover, instruments based on light scattering give inaccurate concentration measurements when confronted with polydisperse mixtures because the light scattered from larger particles obscures the much weaker scattered light from smaller particles.

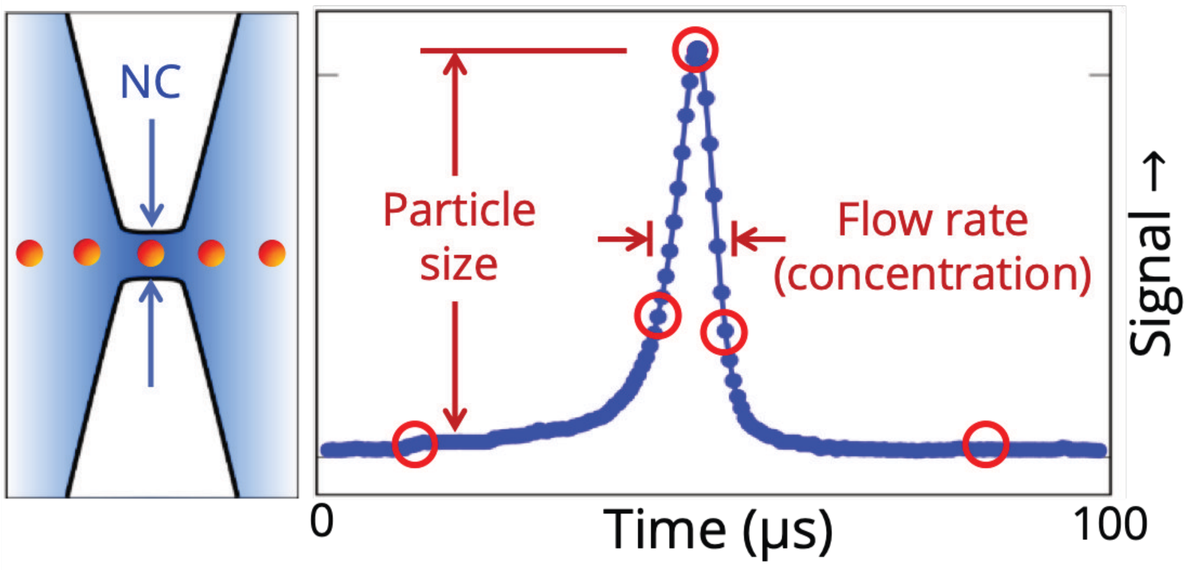
In this report, an electrical method for detection of nanoparticles is used to demonstrate that measurements of nanoscale protein aggregates indeed give an earlier, more sensitive, indication of aggregation than measurements of larger particles.

Methods:

Protein formulations, both stressed and unstressed, were measured using the Spectradyne nCS1™ instrument and associated microfluidic cartridges. Measurements required only 3 microliters sample volume in each case. The principle of operation, Microfluidic Resistive Pulse Sensing (MRPS™), is a non-optical technique in which particles are detected individually as they pass through a nanoconstriction sensing element. Because particles are measured individually, the size and concentration of highly polydisperse mixtures can be measured with high resolution. In addition, the electrical nature of the detection method avoids sensitivity loss associated with the high transparency of biological nanoparticles like protein aggregates and exosomes.

How MRPS works:

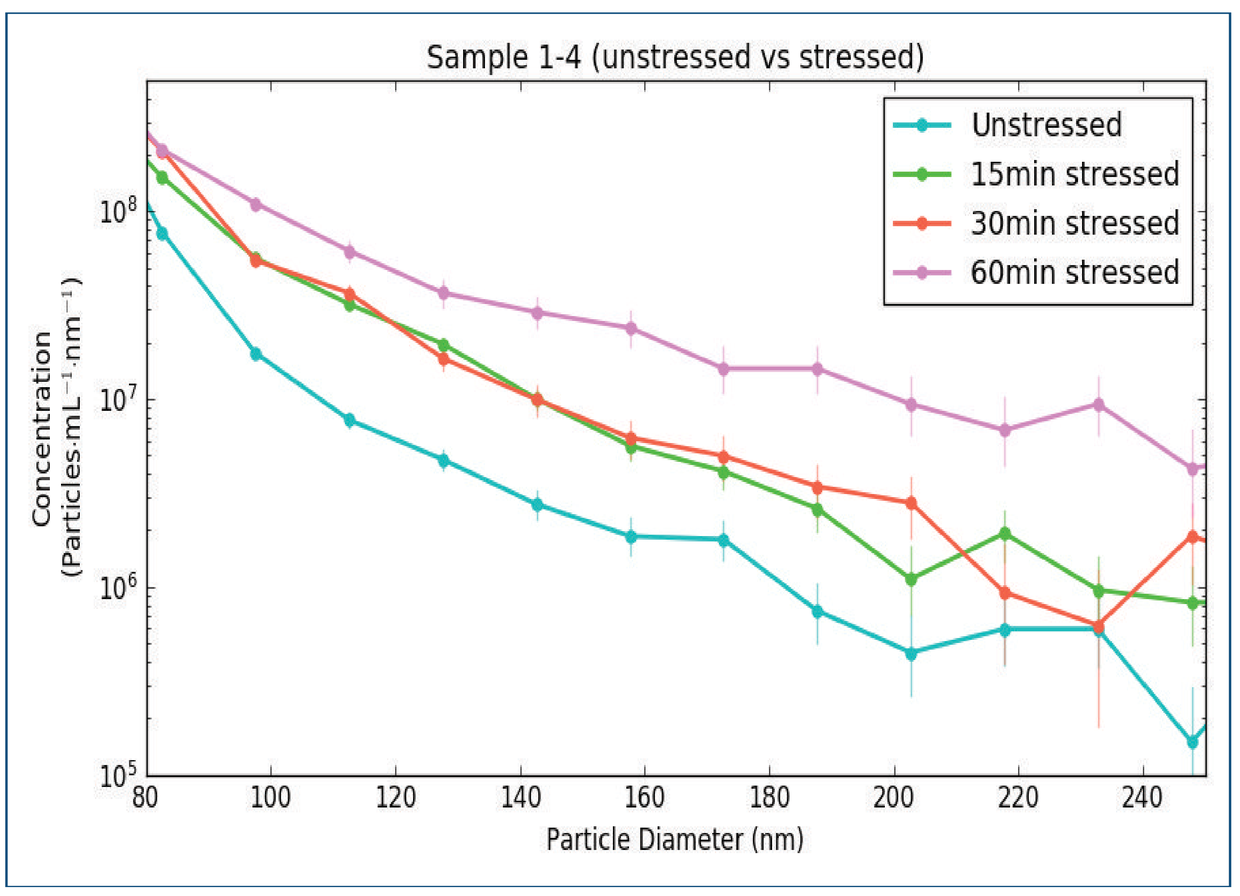
MRPS is a state-of-the-art microfluidic implementation of Resistive Pulse Sensing, aka the Coulter Principle. In RPS, the electrical resistance of a conducting fluid is monitored as particles flow through a constriction (see illustration below), thereby blocking the flow of ions and temporarily increasing the resistance. Maximum resistance modulation is obtained when the particle is midway through the constriction. The size of the resistance spike is directly proportional to the particle volume, regardless of particle material, and the transit time gives the fluid flow rate, which enables concentration measurements.



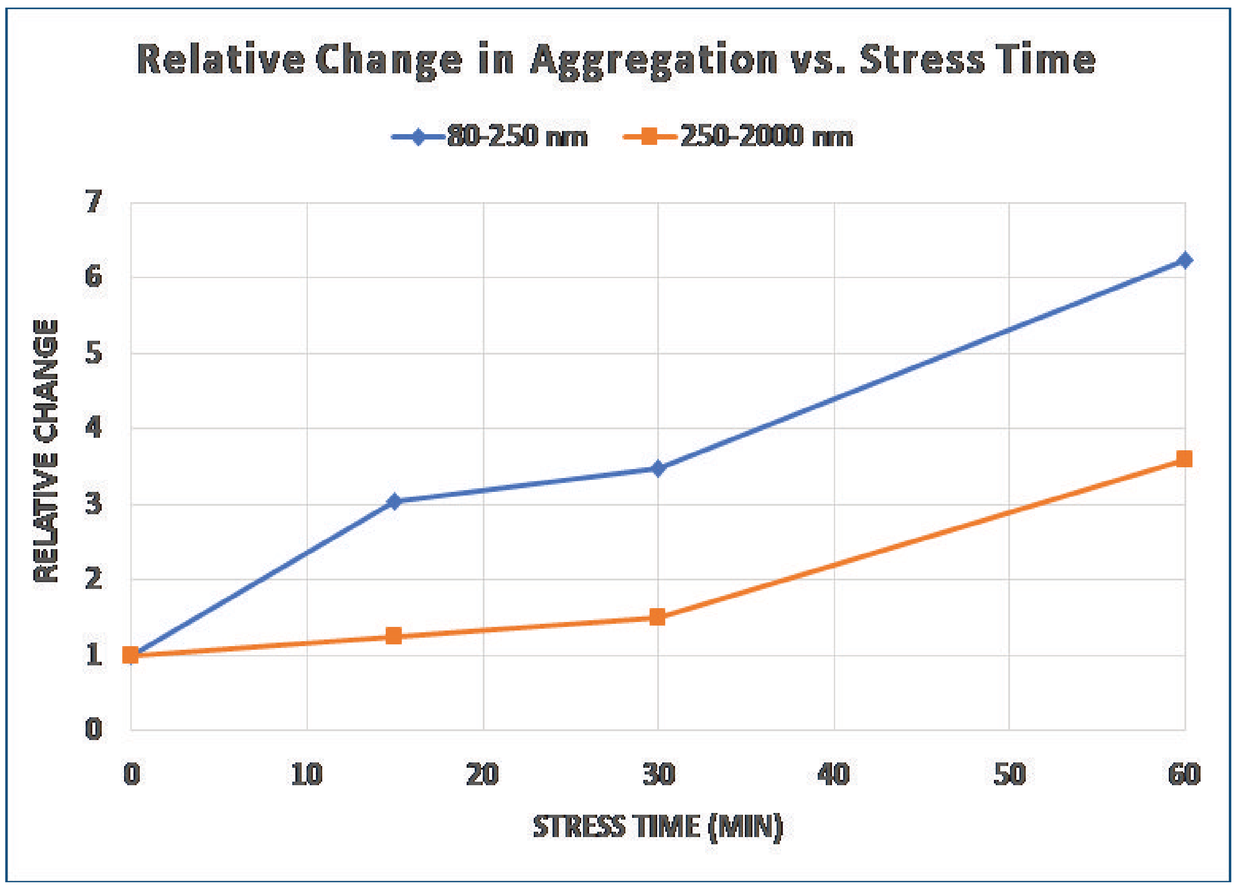
There is no dependance on the index of refraction of the particle and, because particles are detected and sized individually, high resolution measurements are obtained. Spectradyne's proprietary MRPS technology utilizes disposable cartridges to greatly improve ease-of-use and reduce measurement time compared to other nanoparticle implementations of RPS.

Results:

Protein aggregates were detected over a size range from less than 100 nm to 10 µm on samples with varying amounts of stress. Increased aggregation was easily measured, in the smallest size ranges, after only 15 minutes of storage at 40°C. And increasing amounts of aggregation were detected as stress time increased (Figure 1).



Generally, a clear trend of increasing particle concentration with increasing stress time was observed over the full range of measured diameters. The relative change for small diameters, however, increased significantly more than for large diameters, as shown in Figure 2.



Conclusion:

This report demonstrates that the detection of smaller protein aggregates enables earlier detection of aggregation processes. While this demonstration is not surprising, it is a result that is not widely utilized in the industry, despite the importance of minimizing aggregation phenomena. Partly this is because methods that can measure both size and concentration robustly in the nanoscale range have, historically, been unavailable. The MRPS method addresses this metrology deficiency and gives formulation scientists a tool for identifying failing formulations earlier in the drug development process. This in turn allows resources to be redirected to successful formulations sooner, saving significant time and cost.