Direct visualization, sizing and concentration measurement of protein aggregates using NTA

NANO**SIGHT RANGE**

Visualize and measure nanoparticle size and concentration

PARTICLE CONCENTRATION

PARTICLE SIZE

Introduction

Protein aggregation can occur at all steps in the manufacturing process (cell culture, purification and formulation), storage, distribution and handling of products. It results from various kinds of stress such as agitation and exposure to extremes of pH, temperature, ionic strength, or various interfaces (e.g., air–liquid interface). High protein concentrations (as in the case of some biotherapeutic formulations) can further increase the likelihood of aggregation.

A wide variety of aggregates are encountered in biopharmaceutical samples, with a range of sizes and characteristics (e.g. soluble or insoluble, covalent or non-covalent, reversible or irreversible). Protein aggregates span a broad size range, from small oligomers (nanometers) to insoluble micron-sized aggregates that can contain millions of monomer units.

Aggregation needs to be carefully characterized and controlled during development, manufacture, and subsequent storage of a formulated product. Similarly, by monitoring the state of aggregation, modification or optimization of the production process can be achieved.

Nanoparticle Tracking Analysis (NTA)

Nanoparticle Tracking Analysis (NTA) is a method of visualizing and analyzing particles in liquids that relates the rate of Brownian motion to particle size (Figure 1). The rate of movement is related only to the viscosity of the liquid, the temperature, and the size of the particles, and generates a high resolution particle size distribution by sizing each particle individually along with giving the concentration of particles present in the sample (Figure 2). Due to the low refractive index of protein, the lower limit of detection in NTA measurement is approximately 30 nm diameter. This means that protein monomer units, which are typically in the range 3 nm - 10 nm are not measurable by NTA, but aggregates comprised of just a few 10s of monomers to many thousands of units can be sized and counted. As it is typically not necessary to dilute the sample to obtain the particle size distribution, the aggregation profile is not changed due to sample processing.

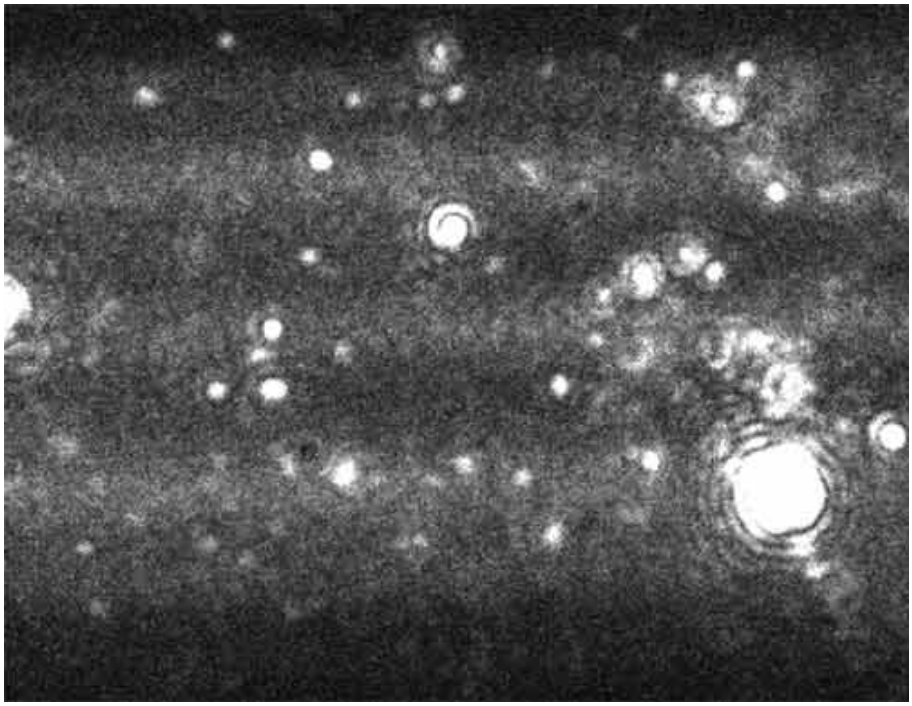

Figure 1. A typical image produced by the NTA technique. The image allows the users to instantly recognize certain features about their sample and the presence of aggregates.

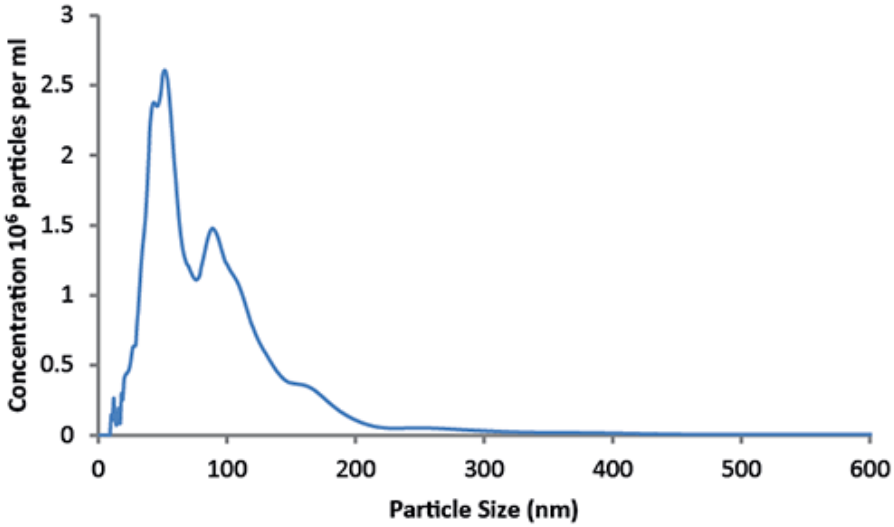

Figure 2. Particle size distribution (number distribution) produced from the sample shown in Figure 1.

Example 1 - Shear Stress

In the following example a virus was correctly measured by NTA at 45 nm diameter (Figure 3a). However, following agitation of the same sample by simple shaking for a few seconds, shear stress was seen to have induced aggregation in the virus sample (Figure 3b).

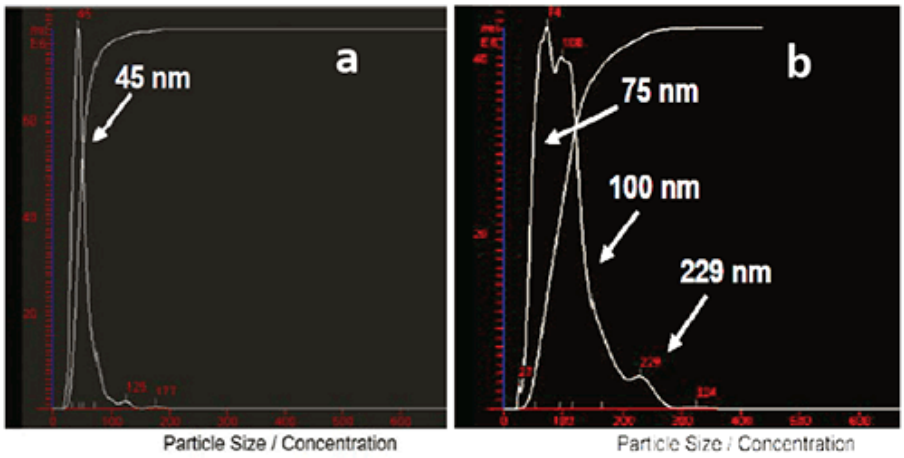

Figure 3. Particle size distribution profile of a virus sample a) before and b) after shear stress induced aggregation. Note the change in scale of the normalized vertical axis shows a drop in the concentration of particles on aggregation (from approximately $80x10^7$ particles/mL to approximately $50x10^7$ particles/mL).

From; Moser M., (2008) Emerging analytical techniques to characterize vaccines, Proc. Intl. Conf. Vaccines Europe, Brussels, December 2008.

Example 2 - Heat Stress

In this example using heat (50^oC), 1 mg/mL IgG has aggregated over time, with the particles scattering light increasing in number and intensity when observed in the NS500 fitted with a 642 nm laser. At each time point, NTA and and also dynamic light scattering (DLS) measurements were taken and size data for both the monomer and the aggregates could be observed (Figure 4). After 20 minutes of thermally induced aggregation, the monomer peak described with DLS showed a sphere equivalent hydrodynamic diameter of approximately 10 nm, with NTA measuring aggregate particles starting at approximately 30 nm, with peaks observed at 50 nm and 85 nm and the largest aggregates at approximately 300 nm. Since NTA also gives particle concentration, the increase in particle number during the time course of the thermal aggregation could also be tracked (Figure 5). This data suggests that for the first thirty minutes there are minimal aggregates above 30 nm in size. From 30 to 100 minutes, the number of aggregates measuring 30 nm or larger remains stable and after 100 minutes the number of aggregates appears to increase in a more exponential manner. Using NTA and DLS size data and NTA concentration data from the same sample gives an information-rich solution when studying the complex area of protein aggregation.

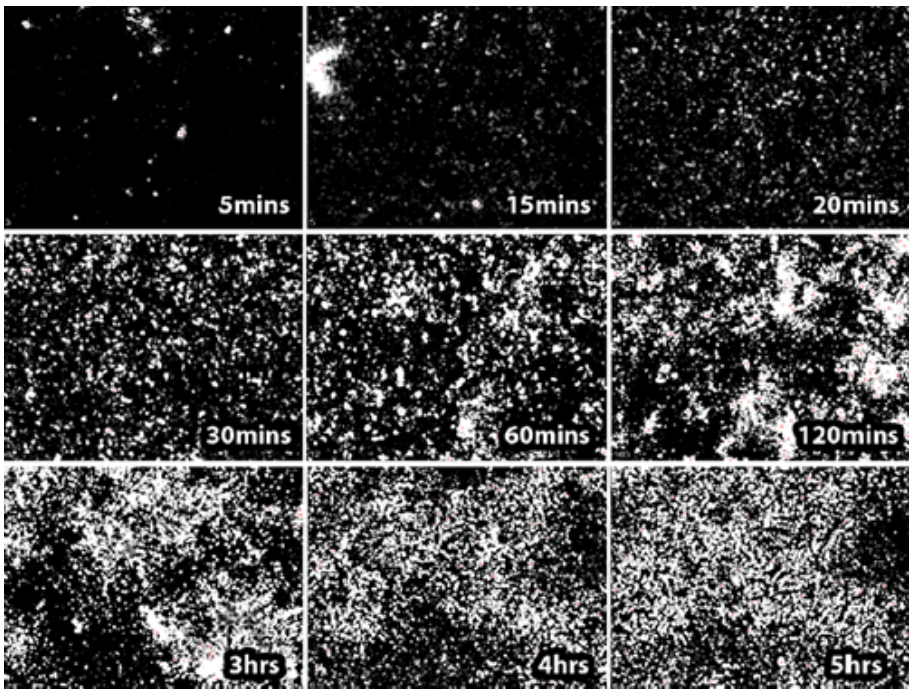

Figure 4. Aggregation of IgG over time

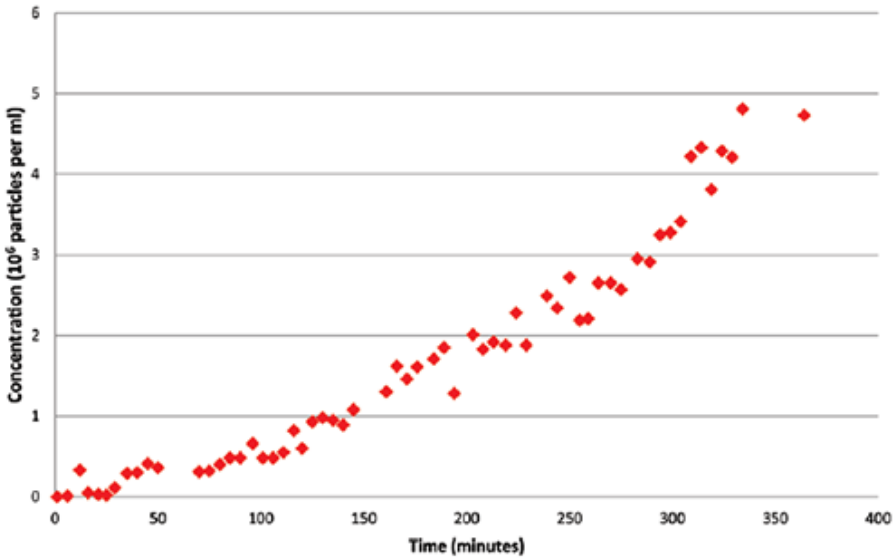

Figure 5. IgG aggregation over time as measured with NTA.

Example 3 - High Concentration Samples

Protein production techniques are increasingly able to generate higher concentration stocks at 150 mg/mL or more. BSA samples of 50 mg/mL, 100 mg/mL and 150 mg/mL were loaded into the sample chamber. As the concentration increases, the scatter observed due to non-resolvable monomers and smaller aggregates increases, but NTA is still able to identify and track aggregates from approximately 40 nm diameter (Figure 6).

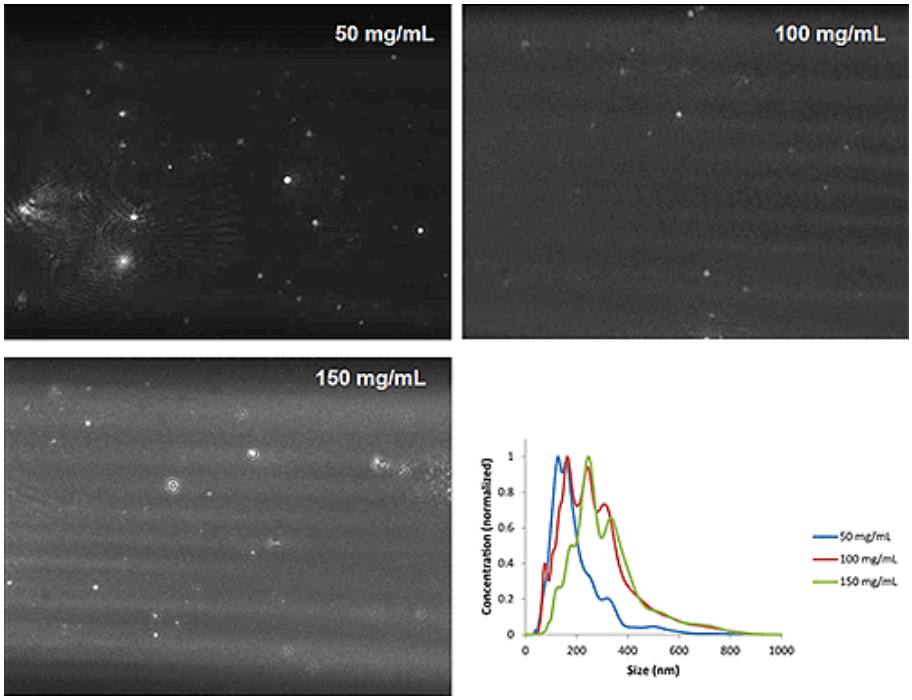

Figure 6. NTA video frames and size distribution profiles for BSA samples of 50 mg/mL, 100 mg/mL and 150 mg/mL.

Recent Literature Examples

NTA has been used to investigate the influence of percentage ammonium sulphate used in three-phase partitioning on the size and size distribution of α-chymotrypsin (Rather et al., PLoS ONE, 2012. 7(12): e49241. doi:10.1371/journal. pone.00492410).

Torosantucci et al. investigated the effect of different antioxidants on copper/ascorbic acid induced aggregation of insulin using NTA to generate size distribution profiles compared to the non-aggregated state (Torosantucci et al., Eur J Pharm Biopharm. 2013 Aug;84(3):464-71. doi: 10.1016/j.ejpb.2013.01.011. Epub 2013 Feb 9.).

Conclusion

To prevent the presence of large aggregates rendering a protein therapeutic unsuitable for patients, scientists need to have an understanding of where in the process of synthesis, purification, packaging, transport, storage and use the protein's monomer units begin to aggregate together. By taking size distribution measurements with NTA at different points in the process, scientists are able to identify the point at which aggregation begins. This point/step can then be reviewed and potentially modified to prevent or slow the formation of protein aggregates.

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