HORIBA Scientific

Application Note

Characterization of Sub-Visible Particles in Protein Therapeutic Formulations

AN215

Introduction

The development of stable protein-based formulations with controlled rheological response is an area of increasing interest for the high-growth bio-therapeutic industry. Formulation involves the characterization of a drug's physical, chemical, and mechanical properties in order to choose what other constituents (such as excipients) should be used in the preparation. In dealing with protein formulation, it is important to understand the behavior of a given protein under a variety of stress conditions such as temperature or agitation.

The characterization of sub-visible particle populations in protein formulations is an area of increasing importance. Formulations with high loadings, sub-visible particles are particularly difficult to analyze with traditional light scattering methods. The proteins in solution often have inherent fluorescence properties which generate strong background signals thus impacting the detectability of sub-visible particles.

This note covers the historical significance of optimizing protein formulations. We will demonstrate how sub-visible particles can be effectively visualized and quantified in real-time under stress conditions with use of the ViewSizer® 3000 from MANTA Instruments (Fig. 1).

A case study based on a IgG1 monoclonal antibody at a nominal protein concentration of 100 mg/mL, supplied under MTA from a major pharmaceutical company, subsequently referred to as mAb A, shows how the ViewSizer® 3000 was used to measure increasing trends in sub-visible particle size and concentration as a result of agitation and increasing temperature.

History

The term biologics first emerged in the United States in the early twentieth century in association with the diphtheria antitoxin. These products were initially rushed to market and their use proceeded initially without regulatory safeguards. By 1895, laws regulating biological products had been enacted by governments of France, Germany, Italy, and Russia. The initial reluctance to regulate these new substances in the US changed in 1901, when thirteen children died after being treated with a diphtheria antitoxin. Today biologics are well regulated worldwide.

Fig. 1. The ViewSizer® 3000

Ever-increasing understanding of the cell physiology and stress, as well as the factors involved in gene expressions and protein production contributed to the success of biologics. The introduction of targeted therapies coupled with rising adoption of patient-centric personalized medicine has fueled the demand. The global biologics market size was valued at $276.6 billion in 2015 and is anticipated to reach $399.5 billion by 2025, according to industry report by Grand View Research, Inc. [1].

While biologics are remarkably effective and popular therapeutics, some stability concerns related to protein aggregation are receiving increasing attention in the industry. For example, FDA and Purdue University publications indicate that protein aggregation could be connected to adverse patient experiences [2, 3]. Keynote presentation by J.F. Carpenter of the University of Colorado at Peptalk 2018 also provided interesting insights on real-world triggers of protein aggregation [4].

Theory

Protein aggregation can occur under environmental stresses such as agitation, temperature, pH or oxidative stress. Extreme temperatures can weaken and destabilize the non-covalent interactions between the amino acid residues. Acidity or alkalinity outside of the protein's pH range can change the protonation state of the amino acids, which can increase or decrease the non-covalent interactions. This can also lead to less stable interactions and result in protein unfolding.

Additionally, misfolded/unfolded proteins can aggregate with the same misfolded/unfolded protein or with different proteins. In general, the synthesis of proteins and their amino acid sequence is based on the sequence encoded in DNA. Some mutations in the DNA sequence may affect the sequence of protein amino acids with subsequent abnormal interactions within the protein molecule. This can affect the folding of the protein and lead to exposed regions of the protein, which are responsible for aggregation.

Case study

The mAb A was provided to MANTA Instruments to determine if the ViewSizer® 3000 could detect sub-visible protein aggregates and if those aggregates change under stress conditions.

Test methods

The ViewSizer® 3000 characterizes nanoparticles by recording and tracking their Brownian movements; larger, micron-sized particles are analyzed by tracking their settling motion (driven by gravity). The system leverages innovative illumination and detection techniques that enable video recording of scattered light from wide-ranging sizes of individual particles simultaneously. A schematic diagram of ViewSizer® 3000 optical system is shown in Fig. 2:

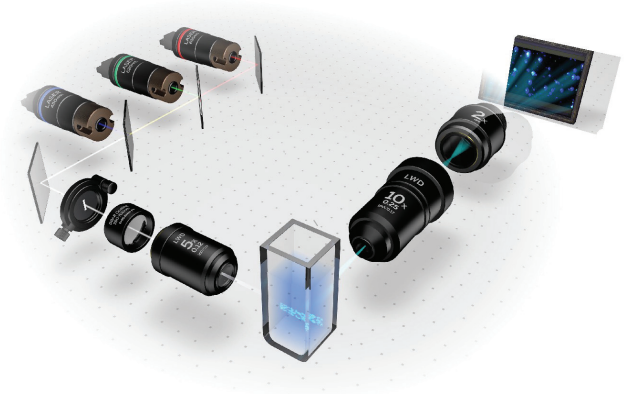

Fig. 2. Schematic diagram of the ViewSizer® 3000 optical system

A key advancement of this system is its ability to address the very large dynamic range of scattered light intensity from differently sized nanoparticles coexisting in polydisperse colloids. Test results from other light scattering techniques typically have significant artifacts and uncertainties that result from this massively disproportionate scattered light intensity. Very intense scattered light from larger particles overwhelms traditional detection systems and obscure the analysis of other particles in the sample.

The ViewSizer® 3000 can also be used to measure viscosity. This is done by adding a small number of polystyrene standards of known, same size to the sample and sizing them with an assumed viscosity of water. The ratio between known particle size and measured particle size is proportional to the ratio of actual viscosity and assumed viscosity.

For typical experiments the instrument records 25 seven-second-long videos of particles Brownian motion. The instrument stirs the sample between each video, which improves sampling statistics by ensuring that a fresh aliquot of sample is used for each video.

The ViewSizer® 3000 software analyzes the videos and provides particle size distribution (PSD) in histogram or cumulative formats. With the ViewSizer® 3000, the PSD data is supplemented by particle visualization whereby images of scattered light from each particle can be viewed in real-time. Such video images provide a desirable visual qualitative validation of the mix of particles in the sample. The videos also provide insights into sample stability.

Test protocols and results

In all tests, the mAb A was measured neat. The samples were transferred to the ViewSizer® 3000 in a cuvette. The only inputs needed for the experiments were temperature, which was controlled at 22°C for the agitation test, and viscosity which was measured with the ViewSizer® 3000.

The protocol for the agitation test was:

Test A - ViewSizer® 3000 video recording settings were adjusted for biologic samples then 25 videos were recorded with minimal stirring between each video to get fresh aliquot for each video. Videos were processed with settings for biologic samples.

Test B - after 30 minutes of vigorous stirring in the instrument repeat measurements were done with the same sample in the cuvette as in Test A.

The agitation test yielded the following results:

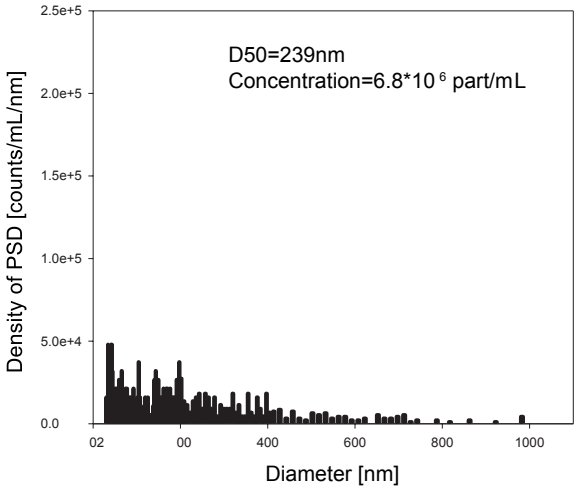

Fig. 3. Particle size distribution before agitation

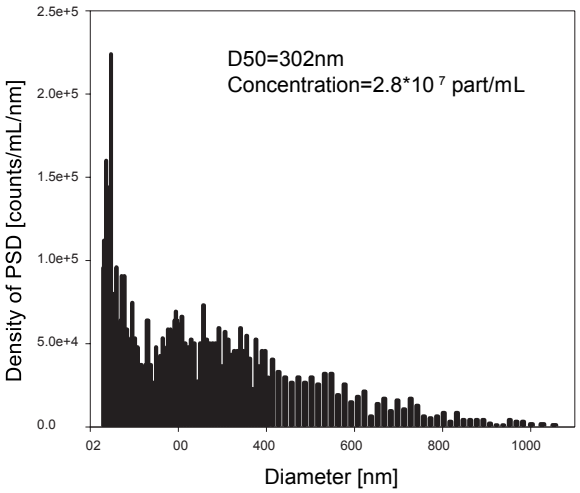

Fig. 4. Particle size distribution after agitation

In summary, these results show that stirring for 30 minutes caused a 27% increase in D50 size and a 4-fold increase in particle concentration.

These increases can also be visualized by examining recorded videos:

Fig. 5. Video frame of sample before agitation

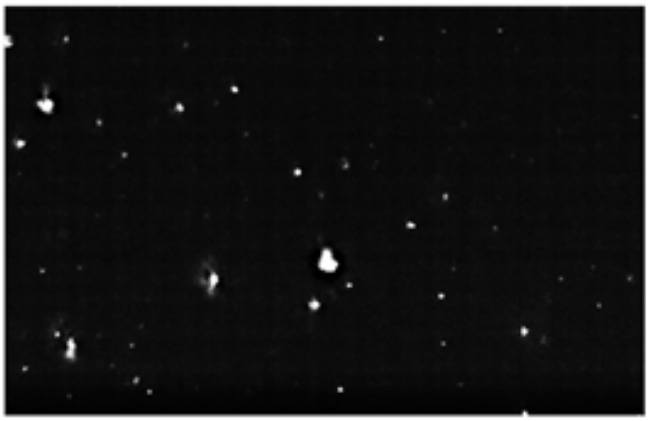

Fig. 6. Video frame of sample after agitation

The second half of the study was focused on gauging effects of temperature on protein aggregation. The protocol for temperature test was as follows:

Prior to testing of PSDs at different temperatures, the viscosity was measured and found to be 2.7 cP at 20°C and 1.6 cP at 40°C, respectively.

PSD Test 1 – ViewSizer® 3000 settings were adjusted for biologics. Temperature was stabilized at 20°C. Ten videos were recorded with no stirring between each video.

PSD Test 2 through Test 6 - Temperature was increased in increments of 4°C, up to 40°C. Once temperature stabilized, sample was soaked at that temperature for 3 minutes. Then ten videos were recorded with no stirring between each video.

The temperature test results are summarized in Figures 7 through 10 below, which clearly show increasing trends in particle size and particle concentration as the sample temperature increases:

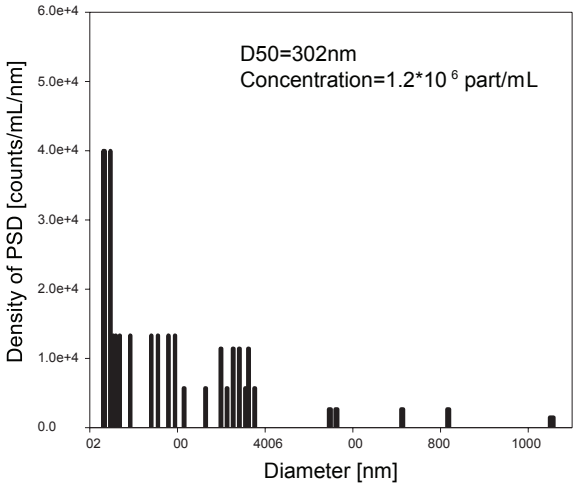

Fig. 7. PSD after Test 1 (20°C)

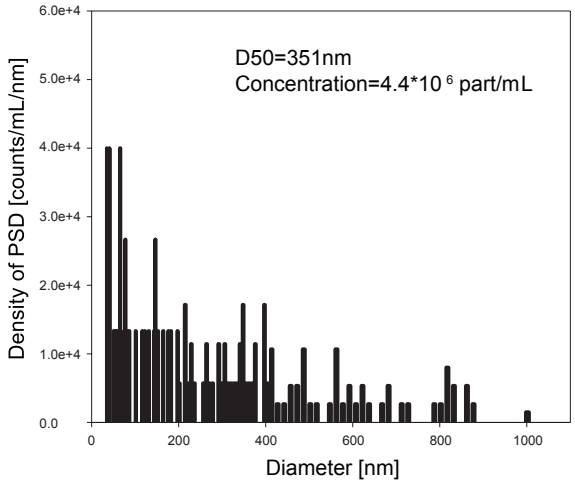

Fig. 8. PSD after Test 6 (40°C)

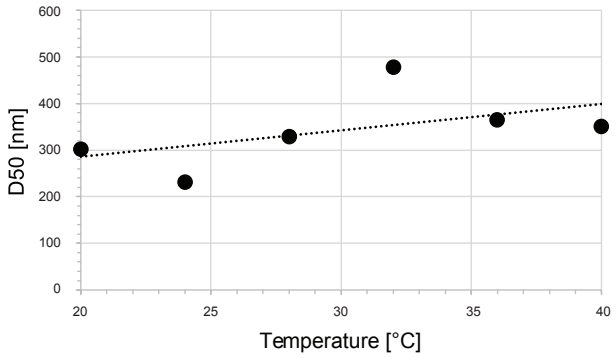

Fig. 9. D50 as a function of temperature

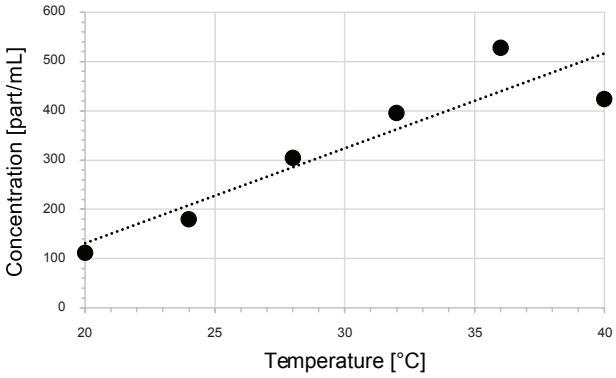

Fig. 10. Concentration as a function of temperature

Summary

Biologic formulations are complex and under certain circumstances can experience protein aggregation or poor stability. Understanding triggers of these phenomena and their complex effects are important for the development and optimization of protein formulations. The ViewSizer ® 3000 enables scientists to visualize and quantify the kinetics of protein aggregation in real-time for biologic therapeutics under a variety of stress conditions including agitation, temperature and the addition of contaminants. The case study featured here validates these capabilities for agitation and temperature stress conditions with the 100 mg/mL mAb A. These insights support the development of stable, effective and safe biologic products.

References

[1] *Biologics Market Size Worth $399.5 Billion By 2025*, Grand View Research, March 2018;

[2] Kotarek J. et al., *Subvisible Particle Content, Formulation, and Dose of an Erythropoietin Peptide Mimetic Product Are Associated with Severe Adverse Postmarketing Events*, Journal of Pharmaceutical Sciences, vol.105 (3), 1023-1027;

[3] Moussa E.M. et al., *Immunogenicity of Therapeutic Protein Aggregates*, Journal of Pharmaceutical Sciences, vol. 105 (2), 417-430;

[4] Carpenter J.F., *Mishandling of Therapeutic Protein Products by End Users: Particle Formation and Potential Roles in Adverse Immunogenicity*, Peptalk at Protein Science Week, San Diego, CA, January 12, 2018.

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