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Application News

No.Q114

Powder Property Analysis

Evaluation of Protein Aggregation Under Various Stress Conditions Using the Aggregates Sizer

Biopharmaceuticals have recently gained attention for their specificity in attacking pathogens, relative lack of side effects, and potent effect. However, compared with low molecular weight pharmaceuticals, biopharmaceuticals are more susceptible to stress, and more likely to aggregate. When a biopharmaceutical aggregates due to stress, this results in a decrease or disappearance of its pharmacological effect, along with the potential for causing serious side effects such as shock symptoms from an immunological reaction. Consequently, a framework is being established that evaluates the stability of biopharmaceuticals in terms of their susceptibility to likely stresses (heat and physical stresses during transport, storage, and at use).
Protein preparations are a type of biopharmaceutical and aggregate to form sub visible particles (SVP) in the size range of 0.2 to 10 µm. Problems with conventional methods of evaluating protein aggregates have been the inability to analyze the SVP size range in a single measurement, inability to take measurements while stress is applied, inability to recover samples after measurement, and inability to perform quantitative measurements. The Aggregates Sizer biopharmaceutical aggregation analysis system (Fig. 1) was developed to overcome these problems.
This article describes how we applied heat and physical stress to intravenous immunoglobulin (IVIG), then evaluated aggregate formation using the Aggregates Sizer. We show how different aggregate formation processes and speeds occur based on stress type and stirrer bar material by quantifying aggregates in the SVP size range.

Materials and Methods

IVIG was used as the sample. A freeze-dried sample was dialyzed with pH 7.4 phosphate buffer saline (PBS) as the external solution, and used as the stock solution (stored at 4 °C). The above stock solution was diluted with PBS (pH 7.4) to 0.87 mg/mL and used as the solution for measurement.
Heat stress was applied by placing 1 mL of IVIG solution (0.87 mg/mL) in a 1.5 mL tube, incubating at 70 °C for 5, 7 and 9 minutes, then taking measurements with a small volume cell (0.4 mL). Stirring stress was evaluated using three different stirrer bars, made of glass, stainless steel and PEEK. The batch cell (Fig. 2) provided with the Aggregates Sizer was filled with 5 mL of IVIG solution (0.87 mg/mL), and measurements were taken while stirring for 8 hours at 190 strokes/min at room temperature.
Particle size distribution and quantitative measurements in the SVP size range were performed by quantitative laser diffraction (qLD) method using the Aggregates Sizer. The Aggregates Sizer is a particle size analyzer that uses a 405 nm semiconductor laser and laser diffraction/scattering to detect the intensity of light scattered by particles between 0.04° and 160°. According to the Mie theory of light scattering, spherical particles of a specific diameter and concentration give rise to a certain scattering pattern and intensity. The particle size distribution can be obtained for the absolute concentration by applying this theory to the scattering pattern of a sample. Quantitative laser diffraction method using the Mie theory requires the refractive index and density of the sample substance. In this experiment, the refractive index was experimentally determined at 1.46-0.10i using a sucrose concentration gradient, and the density used was 1.37 g/cm^3.

Fig. 1 Aggregates Sizer Aggregation Analysis System for Biopharmaceuticals

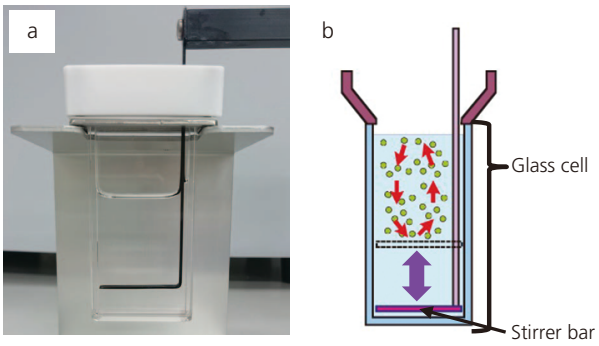

Fig. 2 Batch Cell Structure
(a) Actual batch cell (b) Diagram
The stirrer bar applies physical stress by shaking in a vertical direction.

Results and Discussion

As a representative example, the particle size distribution and amounts of the IVIG aggregates arising from heat stress and stirring stress caused by a glass bar are shown in Fig. 3. Exposure to heat stress only caused an increase in aggregates of around 0.2 µm in size, and no aggregates of 1 µm or larger were formed. Exposure to stirring stress resulted in an increase in aggregates in the region of 0.2 to 10 µm in size over time. The FDA suggests that particles in the SVP size range be evaluated by dividing into two size ranges of 0.2 to 2 µm and 2 to 10 µm, and the Aggregates Sizer is capable of measuring changes in aggregate amounts in both these size ranges simultaneously in a single measurement.

The Aggregates Sizer can also be used to calculate aggregate amounts based on a numerical conversion as well as a mass conversion. In light of these results, we consider quantitative laser diffraction method to be an effective means of evaluating the effect of heat and stirring on proteins in manufacturing and purification operations.

Reference

This article was summarized in part in "Quantitative Laser Diffraction Method for the Assessment of Protein Subvisible Particles" (Totoki et al, 2014, J. Pharm. Sci. 104 (2): 618-626), an article published based on joint research with Susumu Uchiyama et al. of Osaka University. Please refer to the original article for details.

http://onlinelibrary.wiley.com/doi/10.1002/jps.24288/references (open access)

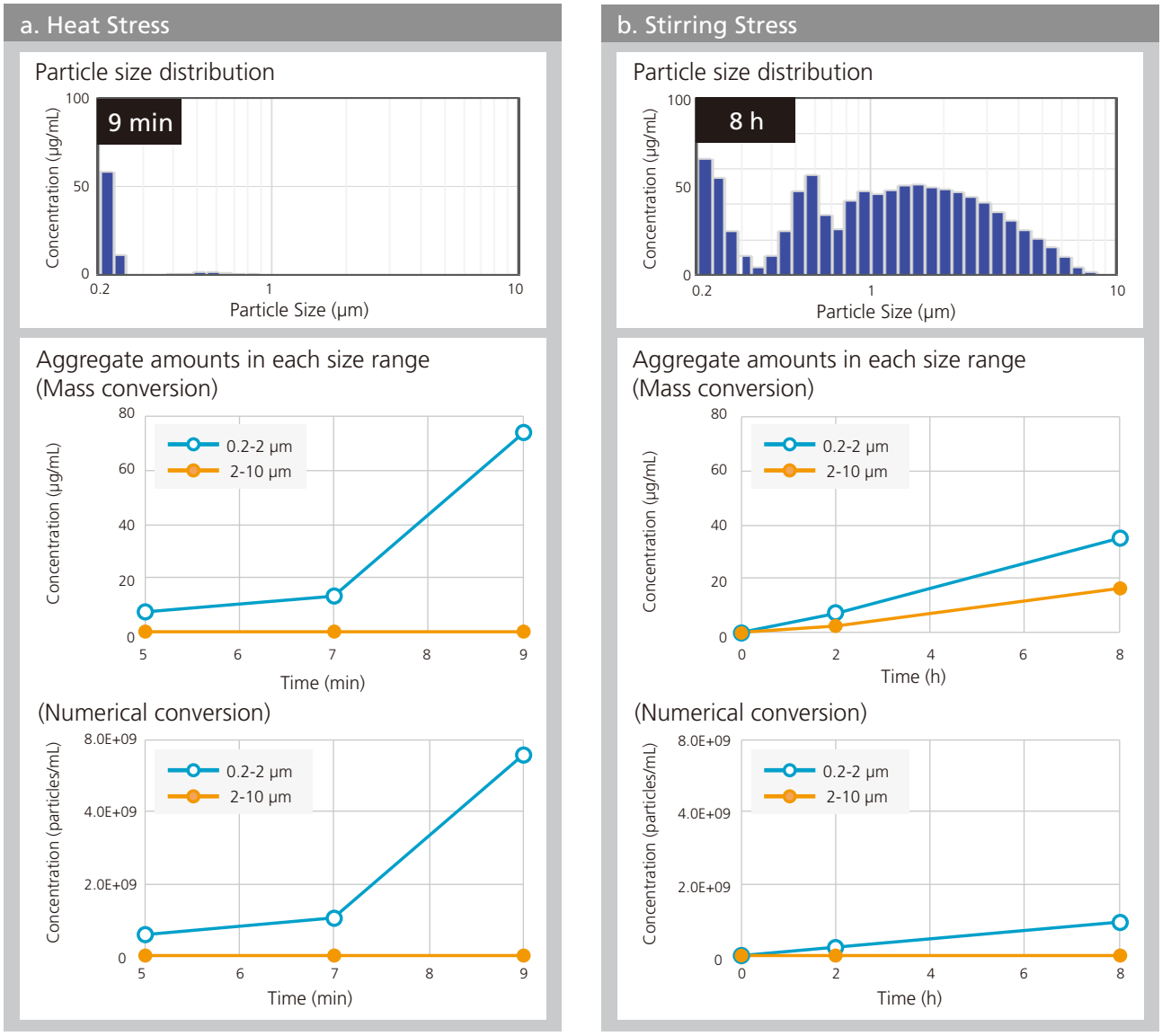

Fig. 3 Particle Size Distributions and Amounts of IVIG Aggregates Formed Under Heat or Stirring Stress

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