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Application News

No.Q115

Powder Property Analysis

Accelerated Testing of Protein Stability Using the Aggregates Sizer TC (With Temperature Control)

Biopharmaceuticals contact a variety of materials during their production, storage, and transport that include metal, plastic and glass. Protein stability differs depending on the materials it comes into contact with. Although investigations must be performed into materials appropriate for contact with biopharmaceuticals, analyzing the many materials a biopharmaceutical contacts during processing increases costs. Furthermore, several months or more are needed to perform a long-term evaluations of storage stability. Accelerated stability testing of contact materials performed in advance would improve the efficiency of investigations into production processes for biopharmaceuticals.
We conducted accelerated stability testing of proteins via the monitoring of aggregate formation during application of physical stress at constant temperature. Three agitator plate materials (PEEK, stainless steel, and glass) attached to the "Aggregates Sizer TC (with temperature control)", Aggregation Analysis System for Biopharmaceuticals was used for testing. The results we obtained indicated the importance of temperature control for stability testing and suggested different materials have different effects on aggregation.

Materials and Methods

A solution of freeze dried bovine-derived γ-globulin adjusted to 1 mg/mL with PBS (pH 7.4) was used as the sample.
Measurements were taken while stirring 5 mL of the sample solution in the batch cell (with temperature control, Fig. 1 (c)) for 40 minutes at 190 strokes/minute. Experiments were performed using three different materials for the stirrer rod: PEEK, stainless steel (SUS316), and glass. During accelerated testing, measurements were taken while maintaining a set temperature of either 23 ˚C, 30 ˚C, or 42 ˚C using a temperature controlled circulator.
Particle size distribution and quantitative measurements were made by the quantitative laser diffraction method (qLD method) using the Aggregates Sizer. A refractive index of 1.46-0.10i and density of 1.37 g/cm^3 were used.

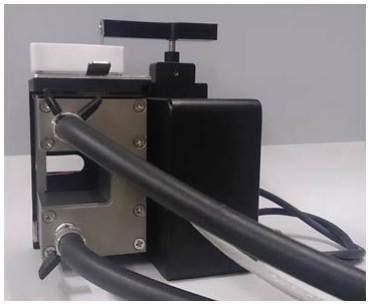
Results and Discussion

The change in particle size distribution over time at 42 ˚C using a PEEK stirrer plate is shown in Fig. 2 as a representative example set of results. Fig. 2 shows that aggregates formed over time. Aggregate formation over 40 minutes at 23 ˚C is shown in Fig. 3 based on the proposed aggregate analysis criteria of aggregate diameter ranges of 0.2 to 2 µm and 2 to 10 µm. Comparing aggregate formation in each size range, particles in the 2 to 10 µm range were most common when using the PEEK stirrer, and particles in the 0.2 to 2 µm range were most common when using the stainless steel stirrer. Results also show the lowest number of aggregates were present in either size range when a glass stirrer plate was used. Aggregated formation at each temperature is compared for PEEK in Fig. 4. This results show that during accelerated testing, aggregate formation increased dependent on temperature. Based on the above findings, temperature control is important for an appropriate analysis of aggregate formation during accelerated testing.

a

b

c



d

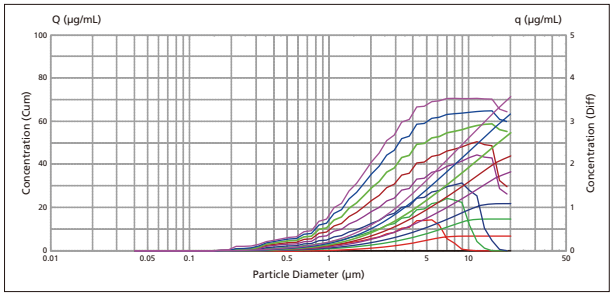


Fig. 1 Aggregation Analysis System for Biopharmaceuticals, "Aggregates Sizer TC (with Temperature Control) "
(a) Main unit, (b) temperature controlled circulator, (c) batch cell (with temperature control), and (d) monitoring screen

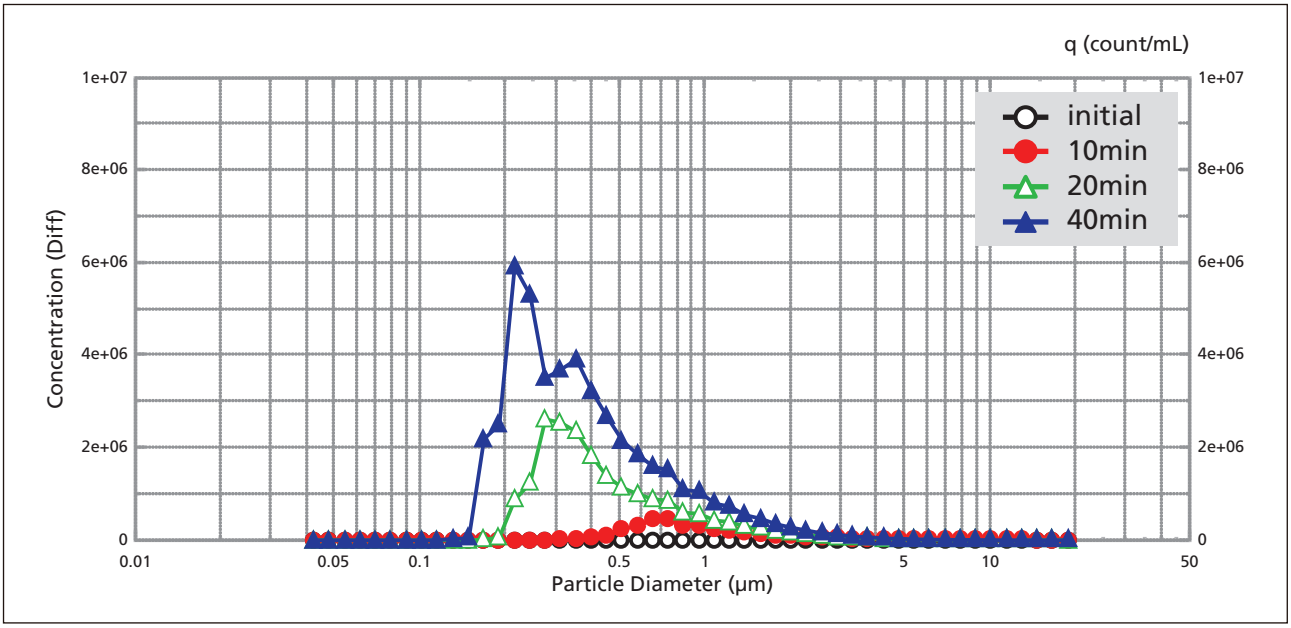


Fig. 2 Aggregate Formation over Time During Accelerated Testing (PEEK at 42 ˚C)

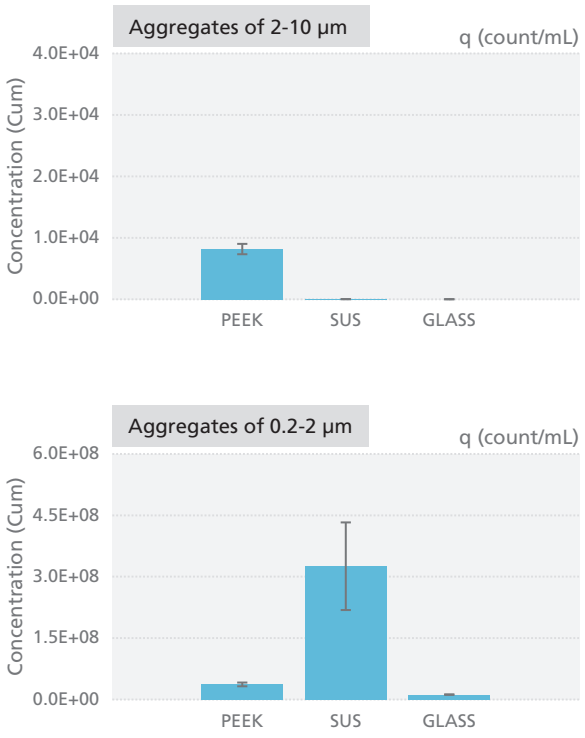


Fig. 3 Comparison of Aggregate Formation in Each Size Range for Each Stirrer Material (After 40 Minutes at 23 ˚C)

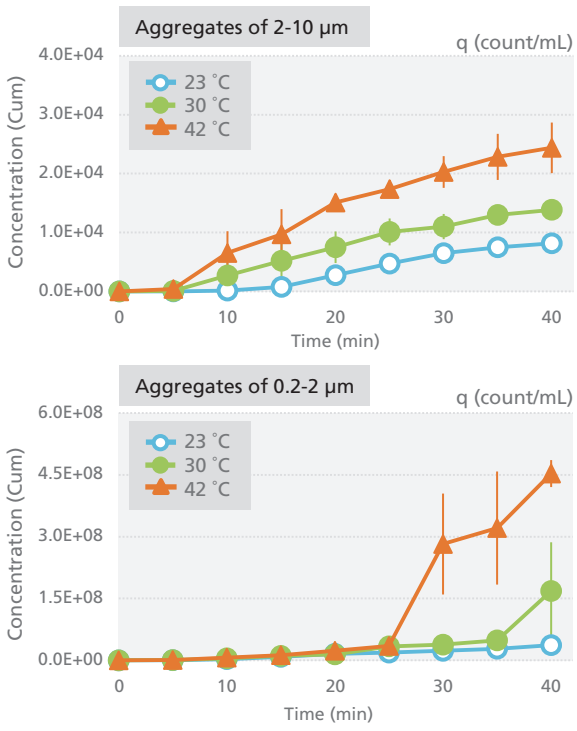


Fig. 4 Aggregate Formation over Time at Each Temperature (PEEK Stirrer)

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