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Application News

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Particle Property Analysis

Aggregates Sizer Enables Evaluation of Biopharmaceutical Additives to Inhibit Protein Aggregation

When performing formulation studies for biopharmaceuticals, investigation into the conditions for inhibiting aggregate formation is required because it is known that aggregate formation in biopharmaceuticals can cause serious side effects in the human body such as anaphylaxis. Solution composition is one such condition, yet it in itself comprises a wide range of conditions which require investigation because protein stability depends on combinations of pH, additive types, and concentrations. Therefore, evaluation that can be performed in a short time is desirable when evaluating stability.

The Aggregates Sizer Aggregation Analysis System for Biopharmaceuticals (referred to as Aggregates Sizer hereafter) is useful for improving efficiency of formulation studies because a large number of analytes can be processed in the time taken to perform a single measurement, which is only a few seconds. We here introduce a study in which we confirmed the differences in the ability of each additive to inhibit protein aggregation, by using Aggregates Sizer to measure the concentrations of aggregate formation in protein solutions containing different additives that have repeatedly been frozen and thawed.

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Samples and Measurement Method

Freeze-dried human gamma globulin was used. A total of seven conditions were examined. Solutions of phosphate-buffered saline (PBS; pH 7.4) and PBS with pH changed to 5.8, 6.8, and 7.8 comprised four conditions. Solutions of PBS containing an additive of either 0.1% of polysorbate 20, 100 mM of L-arginine, or 100 mM of D-sorbitol comprised the remaining three conditions. Each of these seven solutions was used to prepare sample solutions with protein concentrations of 1 mg/ml.

In order to induce aggregate formation, sample solutions were frozen in a freezer at -80 °C and thawed in a water bath at room temperature (this process is referred to as the FT cycle hereafter) repeatedly 16 times. Concentrations of aggregate formation were measured after FT cycle 0 (prior to initial freezing), 1, 2, 4, 8, and 16.

Measurements of the particle size distributions and quantitative values were performed by the quantitative laser diffraction method (qLD method) using Aggregate Sizer. Micro cells, as shown in Fig. 1, were used. The refractive index of 1.46-0.10i and density of 1.37 g/cm^3 were used for the calculation parameters.

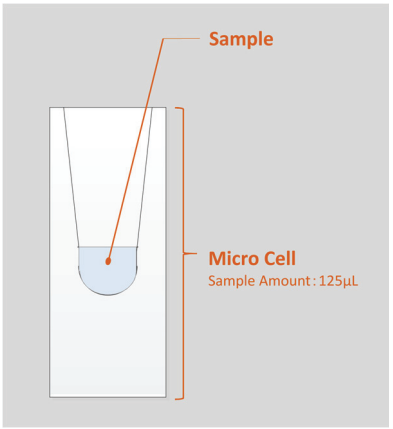

Fig. 1 Micro Cell

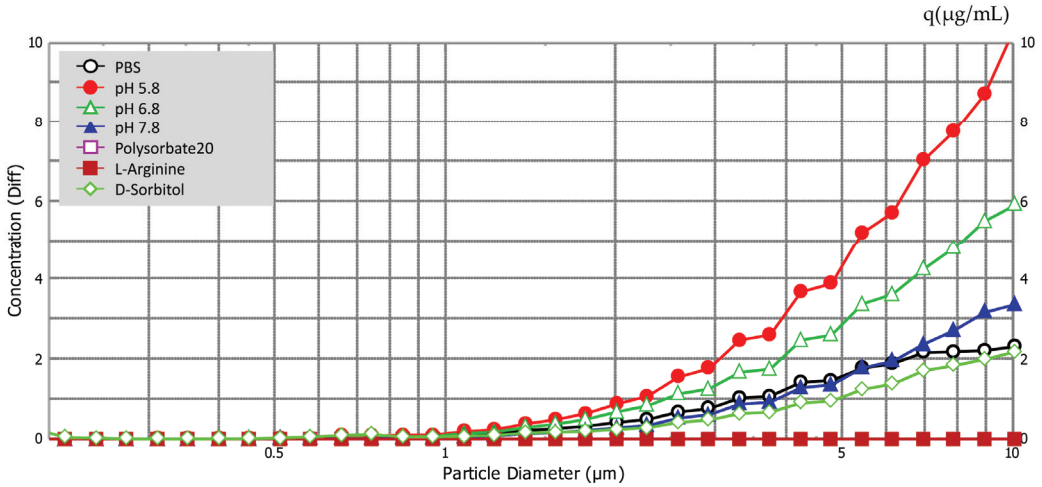

Fig. 2 Particle Size Distribution After the 16th FT Cycle

Results and Observations

Fig. 2 shows the particle size distribution of aggregates for each condition after the 16th FT cycle. We can see that aggregate formation is occurring in the 0.2 µm to 10 µm range.

Fig. 3 shows the concentration of aggregate formation in the 0.2 µm to 2 µm range and the 2 µm to 10 µm range for each condition in Table 1. Regarding pH, we can see that lower values correspond to higher concentrations of aggregates, indicating that changing the PBS to a higher acidity increases aggregate concentration. Regarding additives, for polysorbate 20 and L-arginine additives the intensity of scattered light originating from aggregates was less than the detection sensitivity and almost no aggregates were formed. For D-sorbitol, while aggregate formation was observed, it was inhibited to approximately half compared to PBS only.

Fig. 4 shows the transition in the concentration of aggregate formation for each FT cycle with respect to the pH 5.8 condition. We can confirm that the concentration of aggregate formation increases with the number of FT cycles.

The above results show that Aggregates Sizer is effective for solution composition studies.

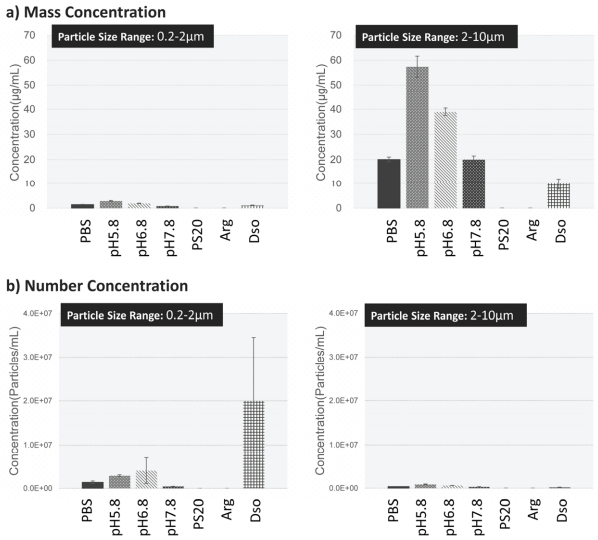

Fig. 3 Concentration of Aggregate Formation After the 16th FT Cycle for Each Condition

Table 1 Concentration of Aggregate Formation After the 16th FT Cycle for Each Condition

	Concentration of aggregate formation (µg/mL)			
	0.2-2 µm		2-10 µm	
	Avg.	SE	Avg.	SE
PBS	1.44	0.04	20.10	0.95
pH5.8	2.85	0.20	57.40	4.25
pH6.8	1.89	0.10	38.99	1.49
pH7.8	0.82	0.01	20.07	1.37
PS20	-	-	-	-
Arg	-	-	-	-
Dso	1.07	0.12	9.93	2.26

** Measured with n = 3

** Abbreviations in the table have the following meanings:
PBS: Phosphate-buffered saline (pH 7.4)
pH 5.8 /pH 6.8 /pH 7.8: PBS with pH changed to the indicated values
PS20: Polysorbate 20
Arg: L-arginine
Dso: D-sorbitol

** Particles were not detected for PS20 and Arg because the scattered light intensity was less than the quantitative lower limit.

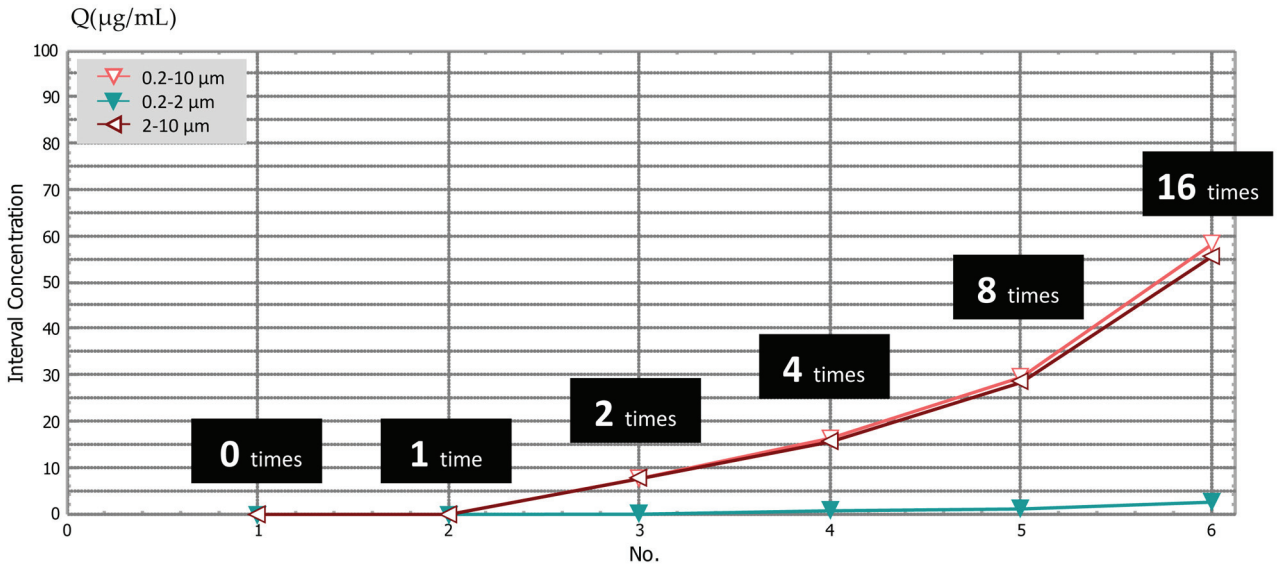

Fig. 4 Transition in the Concentration of Aggregate Formation for the Number of FT Cycles with Respect to the pH 5.8 Condition

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