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Application News

No. Q123

Powder Property Analysis

Characterization of Insoluble Subvisible Particles in Biopharmaceuticals by Flow Imaging Method

Because biopharmaceuticals specifically attack pathogens, they have few side effects and have a large effect, but on the other hand, in comparison with small-molecule drugs, they have low resistance to stress and aggregate easily. If aggregation formation of a biopharmaceutical occurs as a result of stress, it has been pointed out that the effect of the biopharmaceutical as a drug may be diminished or lost, and there is also a possibility of severe side effects, including shock induced by an immunologic reaction.

For aggregate characterization of protein preparations, which are one type of biopharmaceutical, the United States Pharmacopeia (USP) and the Japanese Pharmacopoeia (JP) specify evaluation by the light obscuration (LO) method for insoluble subvisible particles with sizes of 10 µm and larger.

For insoluble subvisible particles with sizes of the micrometer-order, examples of evaluation by the flow imaging (FI) method have been reported in recent years [(1), (2)]. In comparison with LO, FI has higher sensitivity for highly transparent particles, and also enables classification of particles from images.

The Shimadzu iSpect™ DIA-10 dynamic particle image analysis system (Fig. 1), which is based on the FI method, can be used in acquiring of particle imaging of particles in liquid samples and analysis of particle size distribution, concentration, and shape. Because the sample size is small (minimum measurement amount 50 µL, dead volume of 50 µL or less) and the optical system features a narrow imaging area that minimizes missed particles (imaging efficiency: 90% or higher), it is particularly suitable for characterization of insoluble subvisible-size particles in biopharmaceuticals. This article introduces an example in which the size and concentration of aggregates in a protein solution were characterized by using the iSpect DIA-10.

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Fig. 1 iSpect™ DIA-10 Dynamic Particle Image Analysis System

Sample and Method

Freeze-dried human immunoglobulin was used in the sample. The sample solution was prepared by dissolving the sample powder in a citric acid-phosphoric acid buffer of pH 5.0 to a concentration of 1 mg/mL and passing the solution through a 100 nm syringe filter. To induce protein aggregate formation, the sample solution was divided into two parts. One part was heated for 3 min with a heat block set at 80 °C (heat stress sample), and the other was stirred for 10 min with a PEEK (polyether ether ketone) resin-made stirring plate (stirring stress sample).

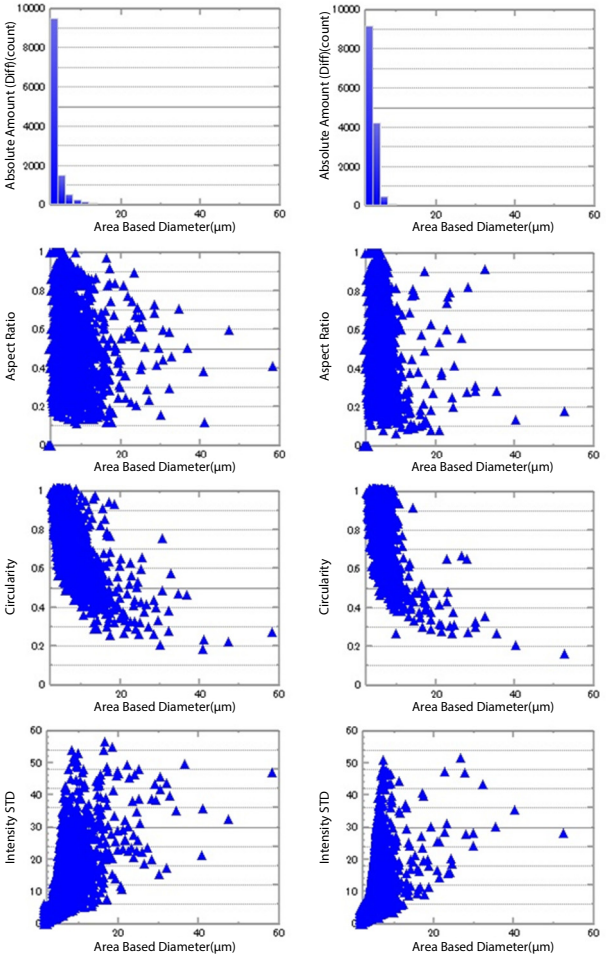
The two types of protein aggregate samples (heat stress, stirring stress samples) prepared as described above were measured under the measurement conditions in the following table.

Table 1 Measurement Conditions

Frame rate	: 8 frame/sec
Efficiency	: 97 %
Sample amount	: 50 µL
Threshold	: 220
Flow rate	: 0.1 mL/min

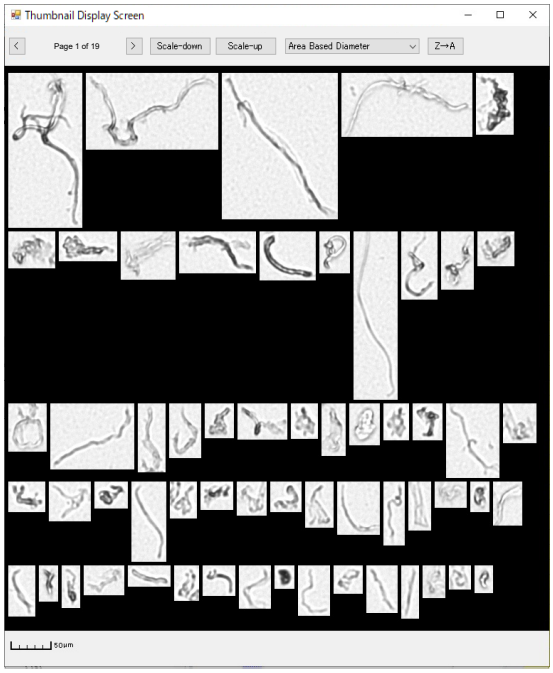
Measurement Results

Fig. 2 shows the particle size distribution and scatter diagrams, and Fig. 3 shows typical particle images. Table 2 and Fig. 4 show the measurement results for the observed particle count and number concentration.

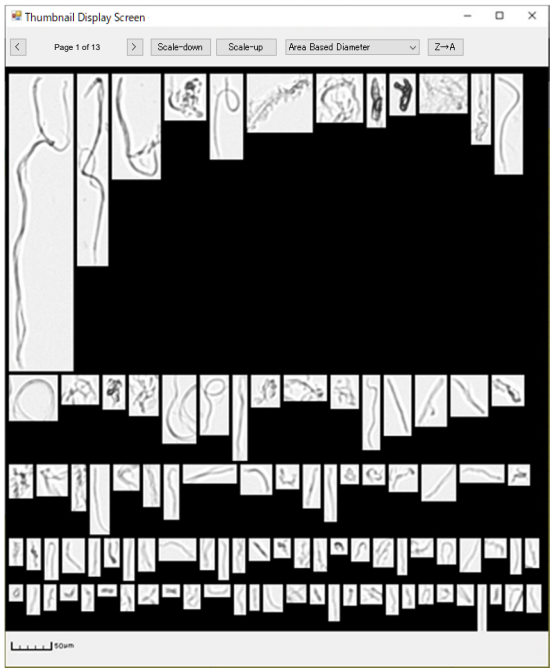


a) Heat stress b) Stirring stress

Fig. 2 Particle Size Distribution and Scatter Diagrams



a) Heat stress



b) Stirring stress

Fig. 3 Typical Particle Images

References

(1) Susumu Uchiyama, "Toward the Proper Biophysical Characterization of Aggregates in Biopharmaceuticals," Yakugaku Zasshi (Journal of the Pharmaceutical Society of Japan), 138, 1503-1507 (2018)
(2) Kiyoshi M et al., "Collaborative study for analysis of subvisible particles using flow imaging and light obscuration: experiences in Japanese biopharmaceutical consortium" Journal of Pharmaceutical Sciences, 108, 832-841 (2019)

Table 2 Observed Particle Count and Number Concentration

		Heat stress	Stirring stress
Observed particle count (particle count)			
(Total)		32246	18813
(By size)	<2 µm	20129	4669
	2 µm - 10 µm	11797	14057
	10 µm - 25 µm	298	78
	≥25 µm	22	9
Particle concentration (count/mL) *1			
(Total)		668102	389784
(By size)	<2 µm	417051	96737
	2 µm - 10 µm	244421	291246
	10 µm - 25 µm	6174	1616
	≥25 µm	456	186

*1 Particle concentration was calculated from the observed particle count, volume of the observed area, and number of recorded frames.

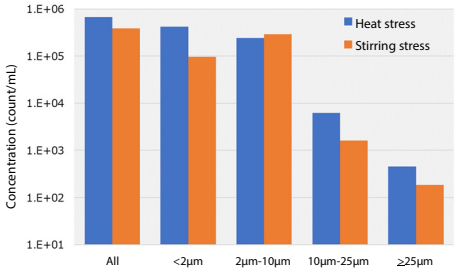


Fig. 4 Number Concentration by Particle Size

From the particle size distribution, scatter diagrams, and particle concentration, it can be understood that the amount and shape parameters of micrometer-order aggregates differ when different stress conditions are applied, even with the same protein concentration. Moreover, string-shaped and lump-shaped particles were observed in the particle images.

Although shape analysis of particles smaller than 5 µm is difficult with the iSpect DIA-10 because one pixel is 0.8625 µm and it is also difficult to obtain adequate contrast with the solvent, detection is possible. As reference, Fig. 5 shows the measurement results for 2 µm polystyrene latex particles.

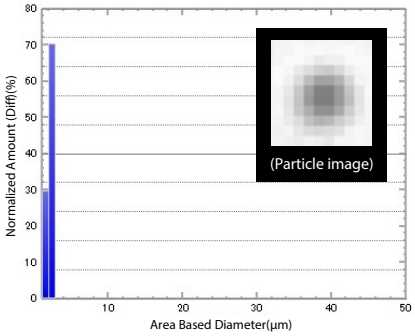


Fig. 5 Measurement Results for Polystyrene Latex Particles (2 µm)

Conclusion

As described above, particle images of micrometer-order insoluble subvisible particles were obtained and the particle concentration was characterized by size by measuring protein solutions prepared under different stress conditions with an iSpect DIA-10. Because even trace samples can be measured with high imaging efficiency, the iSpect DIA-10 is useful for characterization of insoluble subvisible particles in biopharmaceuticals.

First Edition: Apr. 2020

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