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iSpect DIA-10 Dynamic Particle Image Analysis System

Application News

Characterization of Subvisible Particulate Matter in Biopharmaceuticals by Flow Imaging Method

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User Benefits

- It is possible to evaluate using the flow imaging method, which is included as reference information in USP and JP.
- The sample volume for measurement using iSpect DIA-10 is small.
  (minimum measurement amount: 50 µL, dead volume: 50 µL or less)
- The microcell minimizes missed particles (imaging efficiency: 90% or higher).

■ Introduction

Biopharmaceuticals possess high specificity and efficacy; however, they are more susceptible to stress and prone to aggregation compared to small molecule drugs. The occurrence of aggregation can not only lead to reduced or lost efficacy but may also cause side effects due to immune responses.

For the evaluation of particulate matter, such as aggregates in biopharmaceuticals, the light obscuration method (LO) is widely used, as it is established as a testing method in the United States Pharmacopeia (USP) and the Japanese Pharmacopoeia (JP). In recent years, there have been reports of evaluations using flow imaging method (FI), which has higher sensitivity to highly transparent particles and allows for particle classification from images, compared to light obscuration method. This method is also included as reference information in both USP and JP[1), 2), 3), 4)].

iSpect DIA-10 Dynamic Particle Image Analysis System is an analysis device based on the FI method, which requires a small sample volume and has an optical system that minimizes the chances of missing particles, making it suitable for the characterization of subvisible particulate matter in biopharmaceuticals. This article introduces an example in which the size and concentration of subvisible particulate matter in a protein solution were characterized by using the iSpect DIA-10.

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).

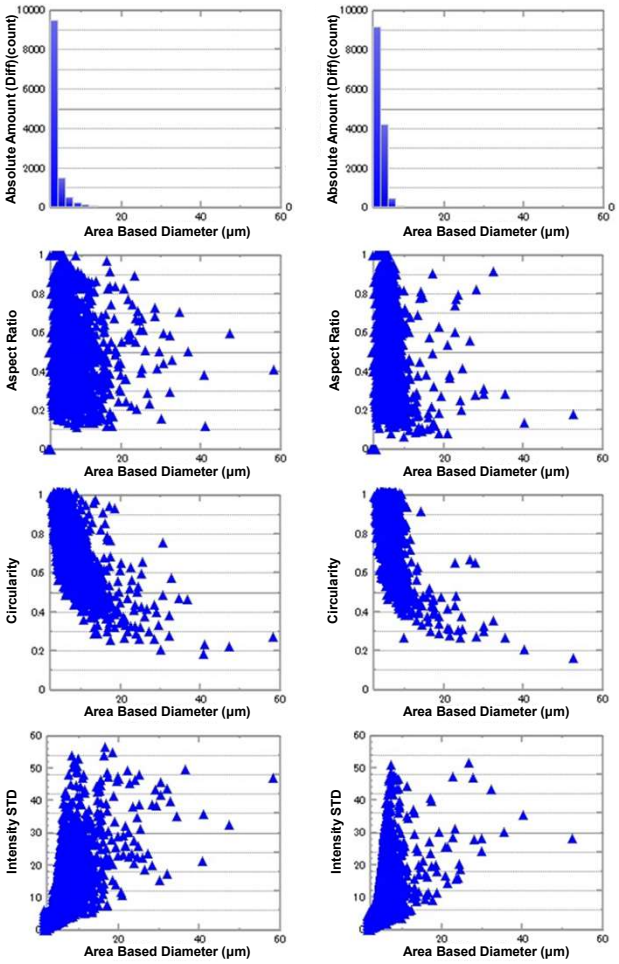
The two types of protein aggregate samples (heat stress, stirring stress samples) prepared as described above were measured under the measurement conditions in the following table.

Table 1 Measurement Conditions

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

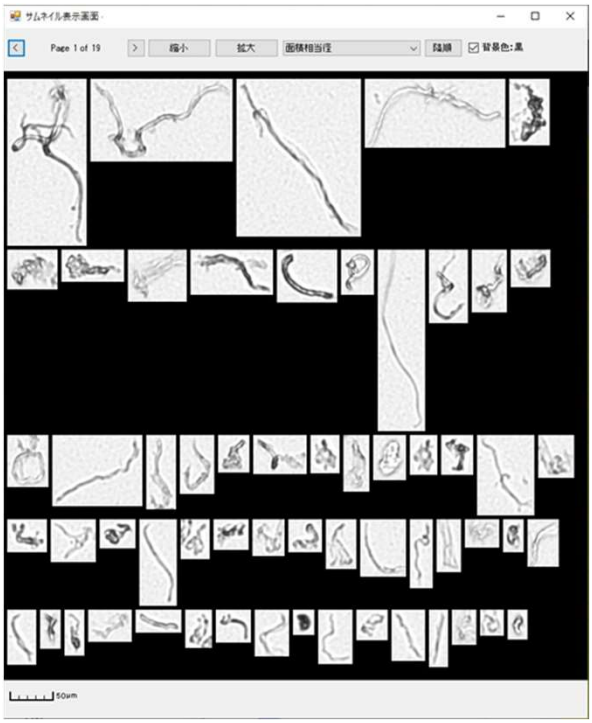
■ Measurement Results

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.

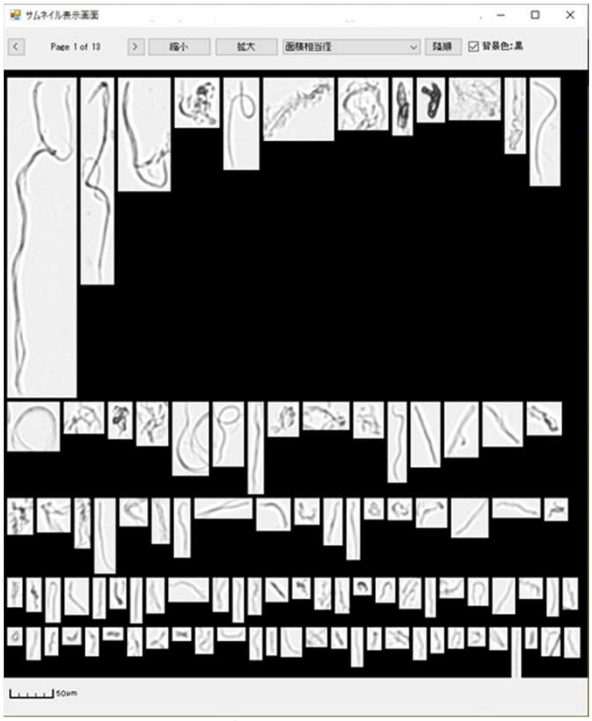


a) Heat stress b) Stirring stress

Fig. 2 Particle Size Distribution and Scatter Diagrams



a) Heat stress



b) Stirring stress

Fig. 3 Typical Particle Images

		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.

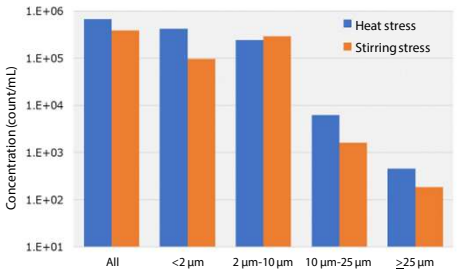


Fig. 4 Number Concentration by Particle Size

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.

As shown in Figure 5, it has been confirmed that by adjusting threshold, it is also possible to detect 2 µm polystyrene latex particles.

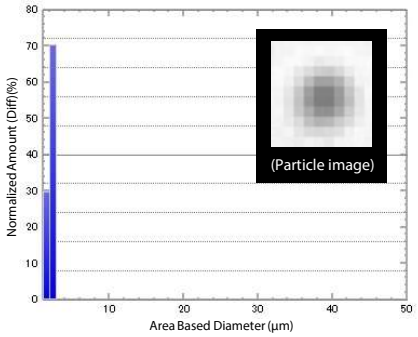


Fig. 5 Measurement Results for Polystyrene Latex Particles (2 µm)

■ Conclusion

As described above, particle images of subvisible particulate matter 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 in subvisible particulate matter in biopharmaceuticals.

<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)
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