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Quantitative Laser Diffraction Method for the Assessment of Subvisible Protein Particles

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Robert Buco[1], Shinichiro Totoki[2]
[1]Shimadzu Corporation, Marlborough, MA 01752;
[2]Shimadzu Corporation, Kyoto, Japan

Introduction

Risks associated with aggregated protein particles

Aggregated protein particles in the size range of 0.2-20 µm have a strong potential to be immunogenic, and in the case of therapeutic protein products, these aggregates can lead to the patient developing resistance to the therapy. Analysis of the size distribution and quantity of aggregates in these therapeutics natively and in response to mechanical stresses can be useful in determining efficacy and safety as well as informing labelling decisions for storage conditions and shelf life.

Figure 1 Aggregation of antibodies

Measurement methods for protein particles

It is relatively easy to determine a high amount of aggregation in protein solutions by visual inspection. As is shown in Figure 3, solutions with a high amount of aggregates appear cloudy, however, solutions with low concentrations of aggregates can appear to be nearly transparent. There are many analytical techniques that can be used to evaluate the size distribution of particles; some of the more common ones are shown in Figure 2. None of the existing methods adequately cover the entire size range of concern for aggregates in protein therapeutics, requiring laboratories that perform this testing to employ multiple techniques with little overlap to cover the entire range. Quantitative Laser Diffraction (qLD) is potentially applicable over the entire range from 0.2-20 µm and is able to measure both the particle size distribution and concentration of low abundance aggregates in solution.

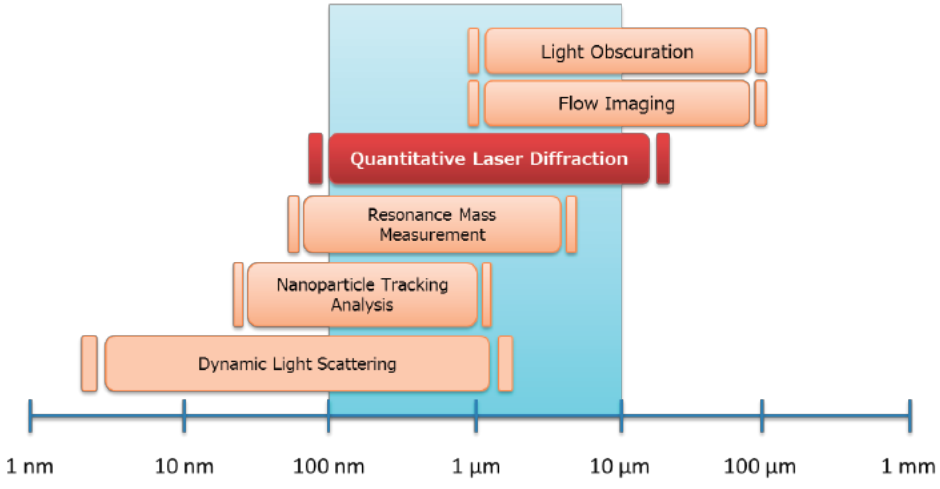
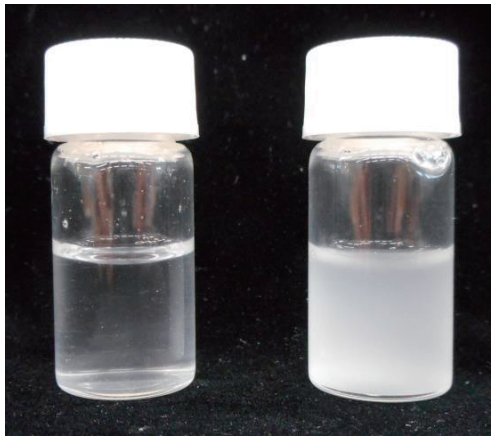


Figure 2 Particle size measurement methods



Non-stressed Stressed (heat)

Figure 3 Aggregated protein solutions

Principle of Quantitative Laser Diffraction (qLD)

qLD estimates the size and quantity of particles by measuring the intensity and angle of scattered diffractions of incident LASER light combined with known physical properties, such as the refractive index of the particle and solvent.

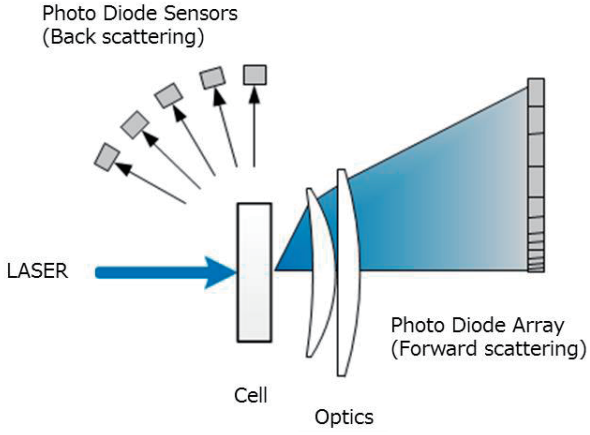


Figure 4 The optical system of Quantitative Laser Diffraction method

Figure 5 Aggregates Sizer

Materials and methods

Silica particles were purchased from micromod Partikeltechnologie GmbH (Rostock, Germany) and Polysciences, Inc. (PA, USA). Polyclonal antibody (Ab) were purchased from Nihon Pharmaceutical co.,ltd. (Tokyo, Japan.). Measurement and analysis of aggregated protein particles in the size range of 0.2-20 µm was performed by qLD using the Aggregates Sizer instrument (Shimadzu Corp., Kyoto, Japan) equipped with WingSALD Bio software Ver3.1.5 (Shimadzu Corp., Kyoto, Japan). The refractive index and density values used in this analysis were 1.43-0.00i and 2.00 g/cm^3 for Silica; 1.46-0.10i and 1.37 g/cm^3 for Ab.

Results

Measurement of silica particles

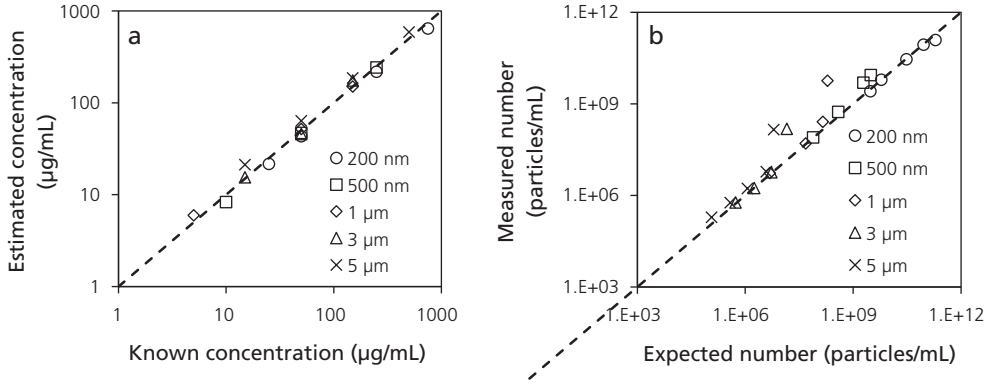


Figure 7 Correlation of known concentration and expected particle size distribution of silica particles with experimental measurements; a. concentration, b. particle size distribution

Measurement of heat stressed Ab solution

Ab solutions were prepared at a concentration of 5 mg/mL and heated for 5, 7 and 9 minutes at 70 °C in a heater. Particles were detected between 0.1-0.2 µm.

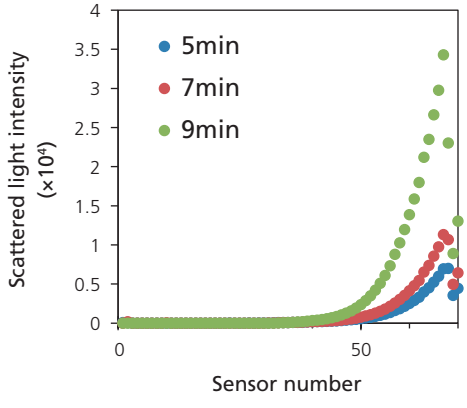


Figure 8 Scattered light intensity from heat stressed protein aggregates

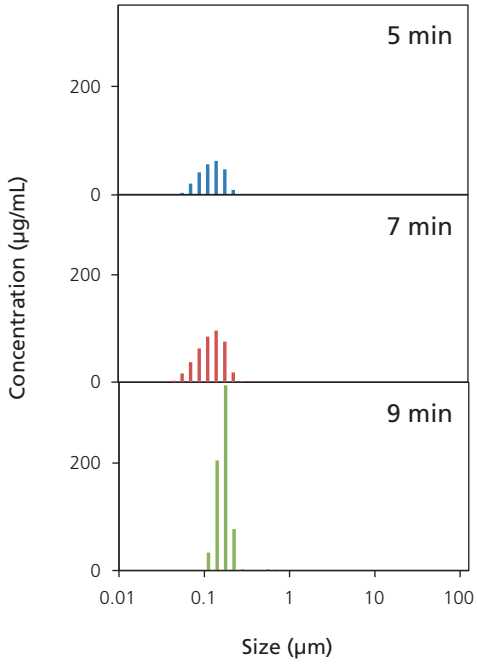


Figure 9 Particle size distribution of heat stressed protein aggregates

Measurement of stir stressed Ab solution

Ab solution (1 mg/mL) was stirred by a blade for 8 hours at 190 rpm at room temperature. Particles were detected between 0.1 -10 µm.

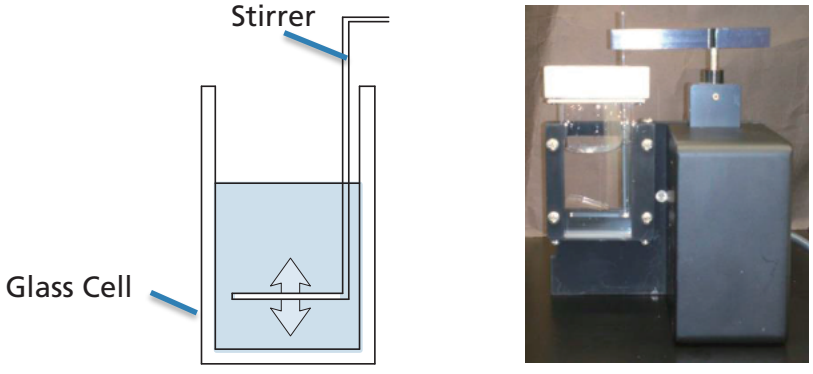


Figure 10 Glass cell with stirrer

Quantitative Laser Diffraction Method for the Assessment of Subvisible Protein Particles

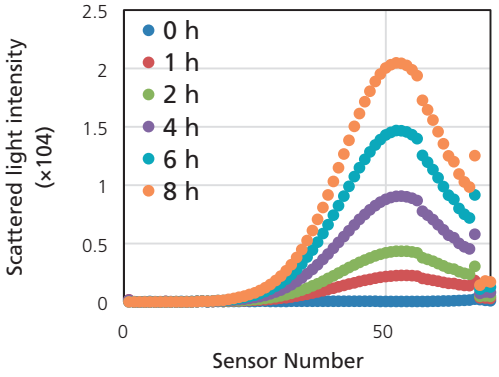


Figure 11 Scattered light intensity from stir stressed protein aggregates

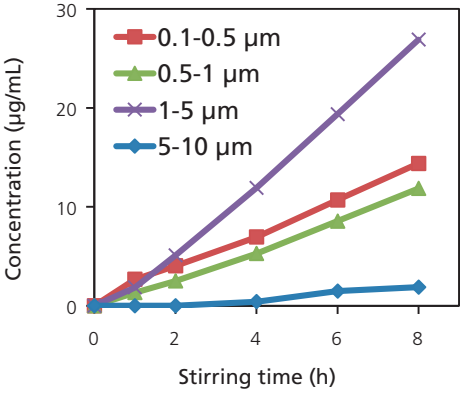


Figure 12 Weight concentration of protein aggregates induced by stir stress

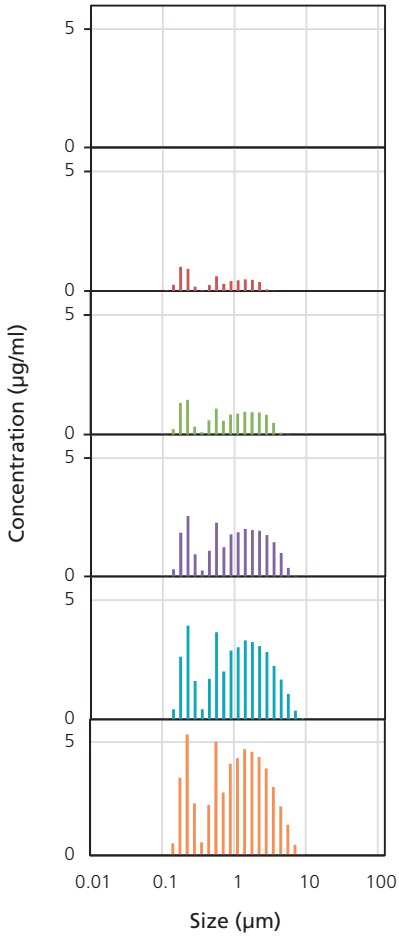


Figure 13 Particle Size distribution of protein aggregates induced by stir stress

Comparison with Flow Microscopy method

Ab solution (5 mg/mL) were heated for 15 min at 70 °C, and diluted in 20 times with PBS (pH7.4). The number concentration estimated by qLD method (Aggregates Sizer) and Flow Microscopy method had a good agreement between 1 - 5 µm.

Quantitative Laser Diffraction Method for the Assessment of Subvisible Protein Particles

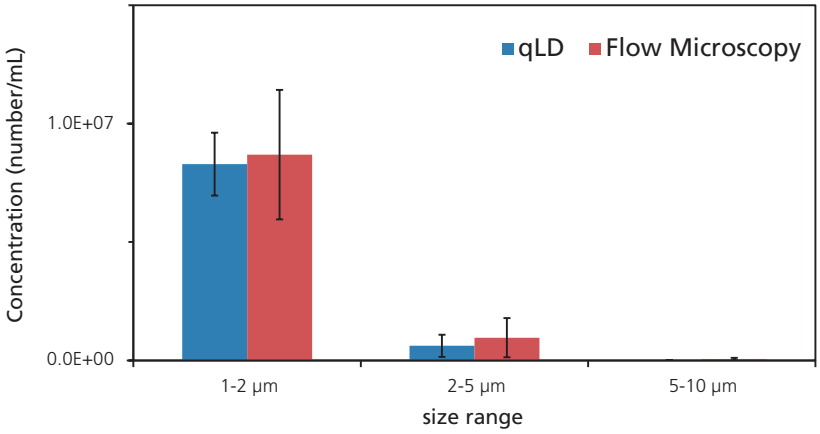


Figure 14 Number concentration of heat stressed protein aggregates measured by qLD (Aggregates Sizer) and Flow Microscopy.

Conclusion

- Aggregated protein particles produced by heat and stir stress could be estimated by Quantitative Laser Diffraction (qLD) method.
- Particle size distributions were different between heat stressed and stir stressed Ab solutions.
- The number estimated by qLD method showed a good agreement with that by Flow Microscopy method.

References

Totoki. S, Yamamoto. G, Tsumoto. K, Uchiyama. S, Fukui. K. 2014. Quantitative Laser Diffraction Method for the Assessment of Protein Subvisible Particles. J Pharma Sci 104(2); 618-626

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