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Application News

No. Q118

Powder Property Analysis

Evaluation of Dispersibility and Stability of Sensitized Latex

- Utilizing the qLD Method in the Field of Drugs for In Vitro Diagnostics (Clinical Reagents) -

Among the drugs used for in vitro diagnostics (clinical reagents), there are drugs that consist of latex with a protein, such as an antigen or antibody, adsorbed to the surface (sensitized latex). Since tests using sensitized latex can be performed easily and quickly compared to other techniques, it is adopted widely in tests such as those for illnesses and pregnancy. Among the techniques that use sensitized latex, some perform testing by utilizing the agglutination that occurs due to the antigen-antibody reaction between the sensitized latex and the target substance. For such techniques, agglutination with the target substance must be accelerated in order to increase sensitivity. However, self-agglutination and non-specific agglutination with substances other than the target substance need to be suppressed because they can lead to a reduced usable period and incorrect test results. This means that controlling agglutination characteristics is an important issue. Since multiple factors including sensitized latex concentration and solution composition (pH, salt concentration, etc.) affect agglutination characteristics, drug development requires investigation of various conditions and evaluation of the relationship between dispersibility and stability.

One system that is effective in increasing efficiency of such investigations on various conditions is the Aggregates Sizer TC, Aggregatetion Analysis System for Biopharmaceuticals (hereafter "Aggregates Sizer TC"), which completes a single quantitative measurement of particle size distribution in a few seconds. Also, accelerated stability testing is possible by agitation testing using a batch cell. This research evaluates the dispersibility and stability of latex sensitized with Protein A or Protein G using the Aggregates Sizer TC. In regard to dispersibility, after causing agglutination by storing Protein A-sensitized latex past its usable period, dispersion treatment was performed on both the stock solution and the diluted solution and the effect of concentration on dispersibility was evaluated. To check stability, the effect of solution composition on stability was evaluated by applying agitation stress to Protein G-sensitized latex in solutions containing different pH levels and salt concentrations to accelerate agglutination. This article introduces the results, which confirm differences in dispersibility and stability for each set of conditions.

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Materials and Methods

Dispersibility was evaluated using commercially-available Protein A-sensitized latex (particle size: 1 µm, stock solution concentration: 25 mg/mL). First, agglutination was promoted by keeping samples in chilled storage until they had exceeded their usable periods. Next, dispersion treatment was performed on sample stock solution and sample solution diluted by a factor of 10 using an ultrasonic bath (100 W) for one minute. Each solution was further diluted to a factor of 5000 and subjected to particle size distribution measurement.

For stability evaluation, commercially-available Protein G-sensitized latex (particle size: 1 µm, stock solution concentration: 14 mg/mL) was diluted by a factor of 2800 with each of phosphate-buffered saline (pH 7.4), phosphate-buffered saline + 1 M sodium chloride, and citrate-phosphate buffer solution pH 5.0. Then while agitating these three solutions at 25 °C and 180 strokes/min using a stainless steel stirring bar, particle size distribution was continuously measured for one hour.

Particle size distributions and quantitative values were measured according to the quantitative laser diffraction method (qLD method) using Aggregate Sizer TC. A batch cell was used. The refractive index of 1.62-0.00i and density of 1.05 g/cm^3 were used for the calculation parameters.

Results and Discussion

The results of dispersibility evaluation are shown in Fig. 1 by the particle size distribution before and after dispersion treatment. We see that without dispersion treatment, agglutination has occurred because the usable period is exceeded.

Looking at the results of dispersion treatment on the two concentration types, aggregates were dispersed to primary particles for the solution diluted by a factor of 10, but for the stock solution, dispersion hardly progressed compared to that without dispersion treatment. This shows that concentration affects dispersibility.

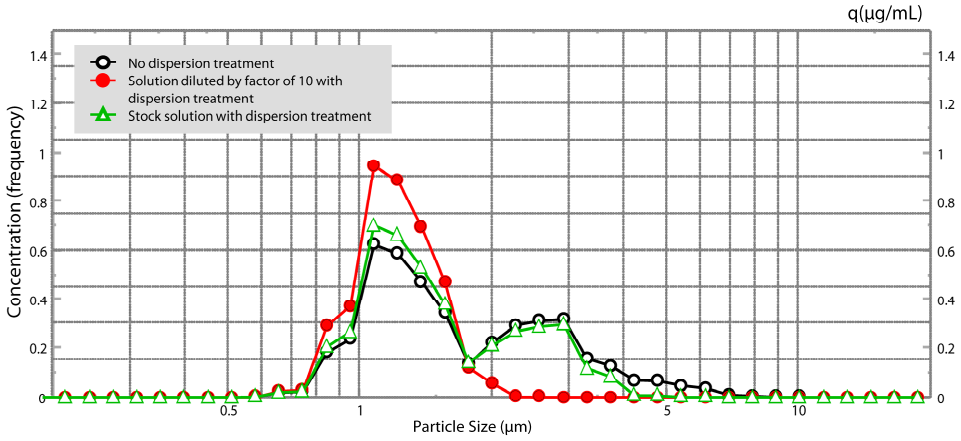

Fig. 1 Particle Size Distribution of Protein A-Sensitized Latex Before and After Dispersion Treatment

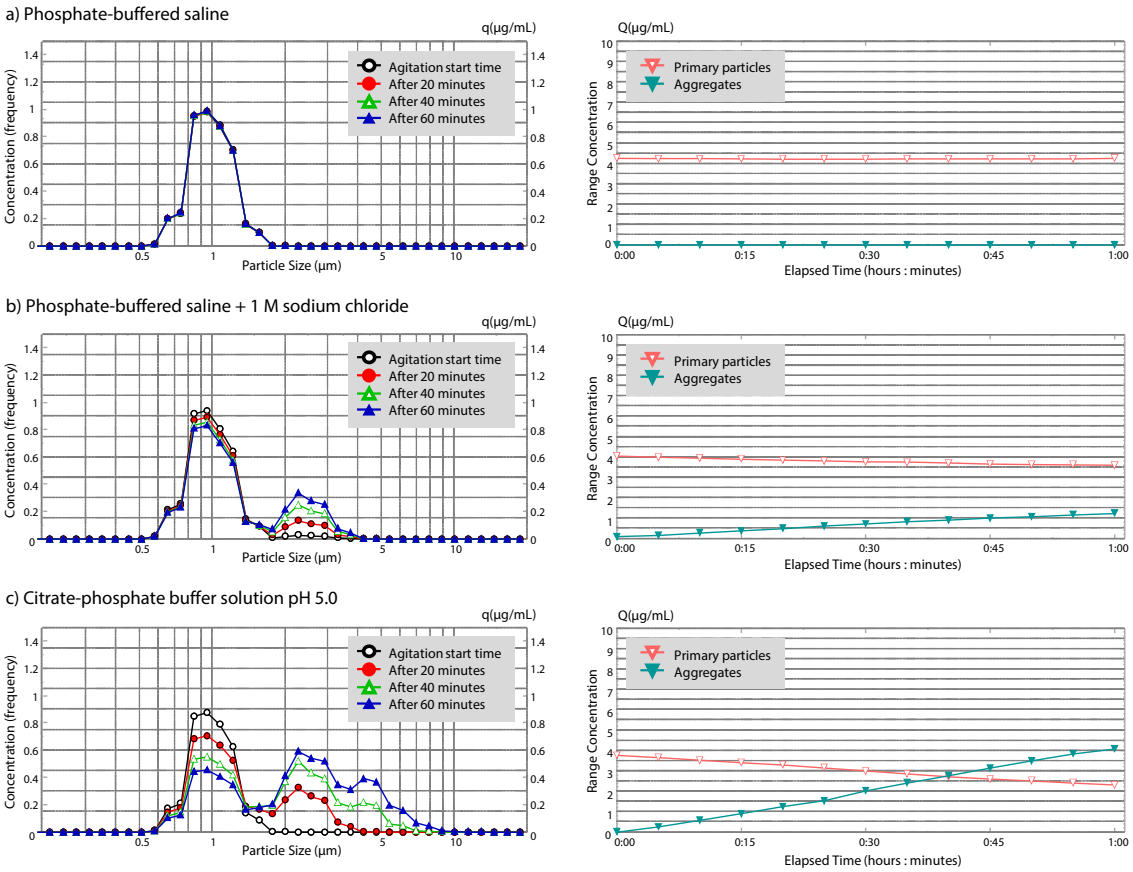

Fig. 2 Particle Size Distribution of Protein G-Sensitized Latex and Time Series Variation of Particle Mass for Each Solution Composition
Left: Particle Size Distribution Right: Time Series Variation of Particle Mass
*** The primary particles and aggregates in the time series variation graph are calculated from the particle masses of the 0.50 to 1.74 μm and 1.74 to 20 μm ranges respectively.**

Fig. 2 and Fig. 3 show the results of stability evaluation. From the particle size distribution of each solution composition in Fig. 2, we see that no aggregates occurred in the phosphate-buffered saline, aggregates with a peak in the 2 to 3 μm range occurred in the phosphate-buffered saline + 1 M sodium chloride, and aggregates distributed broadly over the 2 to 7 μm range occurred in the citrate-phosphate buffer solution pH 5.0. From the time series variation graph of the particle mass of primary particles and aggregates, we see no change in the particle mass for the phosphate-buffered saline meaning that no aggregates occurred. For the phosphate-buffered saline + 1 M sodium chloride and the citrate-phosphate buffer solution pH 5.0, a decrease in primary particles and increase in aggregates was observed and the gradients were steeper for citrate-phosphate buffer solution pH 5.0 (in this research, primary particle mass is calculated as the particle mass of the 0.50 to 1.74 μm range and the aggregate mass is calculated as the particle mass of the 1.74 to 20 μm range).

Fig. 3 shows the time series variation of the ratio of primary particles occupying the total particle mass. After 60 minutes of agitation, we see that the ratio of primary particles is about 3/4 in the phosphate-buffered saline + 1 M sodium chloride and about 1/2 in the citrate-phosphate buffer solution pH 5.0. We can therefore consider that stability decreases in the order of phosphate-buffered saline, phosphate-buffered saline + 1 M sodium chloride, and citrate-phosphate buffer solution pH 5.0.

The above results show that the Aggregates Sizer TC is effective for evaluating the dispersibility and stability of sensitized latex.

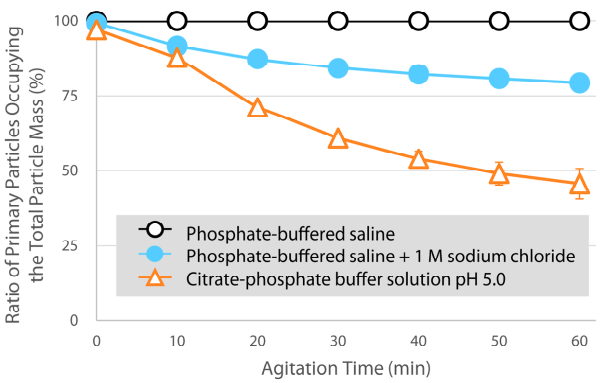

Fig. 3 Time Series Variation Graph of the Ratio of Primary Particles Occupying the Total Particle Mass of Each Solution Composition in Protein G-Sensitized Latex
*** The error bar indicates the standard error at n = 3.**

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