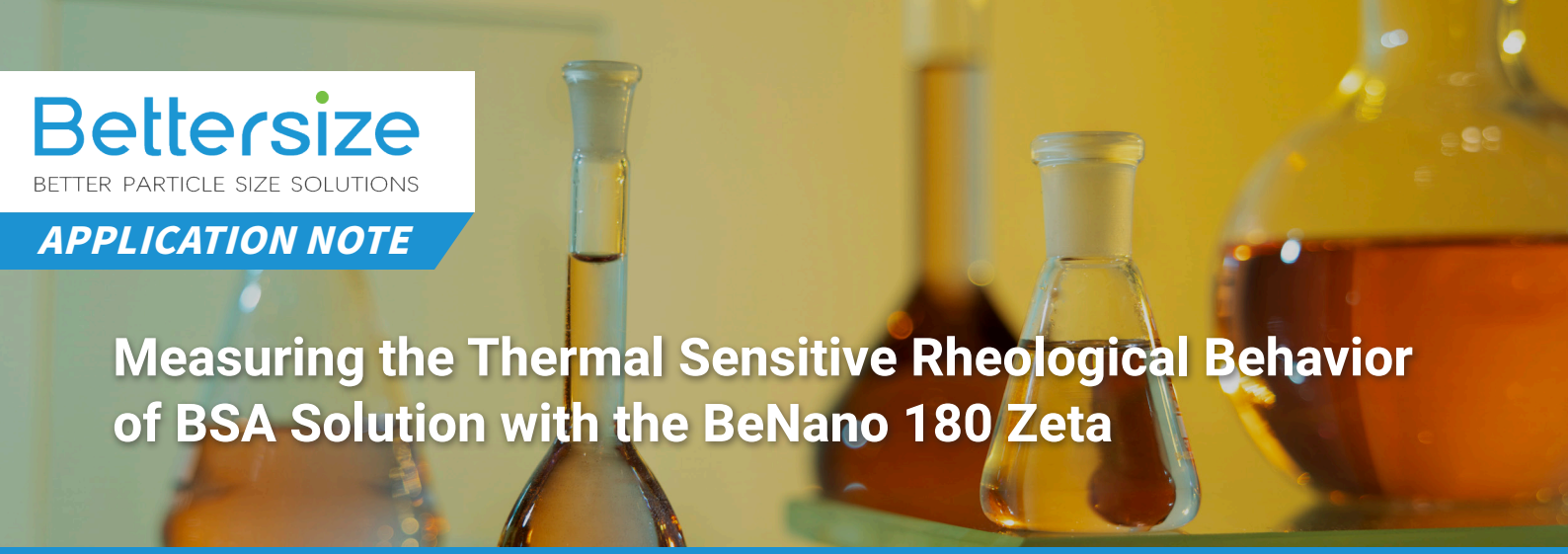

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APPLICATION NOTE

Measuring the Thermal Sensitive Rheological Behavior of BSA Solution with the BeNano 180 Zeta

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Introduction

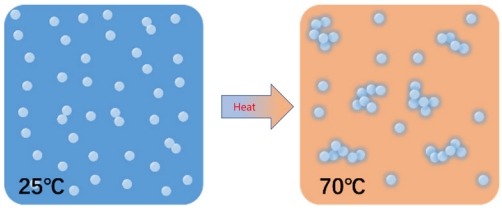

Figure 1. Schematic diagram of the denaturalization of BSA during heating

Dynamic light scattering microrheology (DLS Microrheology) is a technique that uses dynamic light scattering to measure the mean square displacements (MSD) of added inert tracer particles in a solution environment and further obtain the rheological information of solutions. The DLS microrheology can be used to characterize weakly structured polymer and protein solutions, and also gel systems, in which tracer particles can diffuse over significant distances.

Compared with the mechanical rheological technique, DLS microrheology allows for straightforward and fast measurements and data treatment, and can obtain high frequency information in a single measurement.

- The tracer particles are added to the system being studied, such as a protein solution or a dilute polymer solution.
- Particles suspended in a solution system move randomly through thermal energy exchange k_BT.
- The motion of the tracer particles is determined by the rheological properties of the surrounding environment.
- Detecting the motion of tracer particles can provide rheological information.

BSA solution is relatively stable under low-temperature conditions, but it will be thermally denatured and form protein agglomerates during heating, therefore significantly changing the solution properties. The viscoelastic information of BSA solutions at different temperatures can be characterized by the DLS microrheology.

Theory

In the DLS microrheology, colloidal particles in the size range of 0.3 - 2.0 μm with known size information are added as tracer particles. The rheological properties of the environment are reflected in the motion of the tracer particles. For a purely viscous fluid sample (Newtonian fluid), the tracer particles diffuse freely throughout the sample environment, and the MSD $\langle \Delta r^2(\tau) \rangle$ of the particle increases linearly with time:

$$\langle \Delta r^2(\tau) \rangle = 6D\tau$$

where D is the diffusion coefficient of the particle. According to the standard Stokes-Einstein equation:

$$D = \frac{k_BT}{6\pi\eta R}$$

where R is the radius of the tracer particle, the relation between MSD and viscosity can be obtained as follows:

$$\langle \Delta r^2(\tau) \rangle = \frac{k_B T}{\pi \eta R} \tau$$

The viscosity η of the Newtonian fluid can be obtained by fitting the curve of $\langle \Delta r^2(\tau) \rangle$ with time. However, for non-Newtonian fluids which contain elastic components, we can use the generalized Stokes-Einstein equation:

$$G^*(\omega) = \frac{k_B T}{\pi R i \langle \Delta r^2(i\omega) \rangle} = G'(\omega) + iG''(\omega)$$

by which the elastic (storage) modulus G' and the viscous (loss) modulus G'' are derived by $\langle \Delta r^2(\tau) \rangle$ as a function of frequency. And the complex viscosity $\eta^*(\omega)$ and creep compliance J(t) can be obtained as follows, respectively:

$$\eta^*(\omega) = \frac{G^*(\omega)}{\omega}$$

$$J(t) = \frac{\pi \alpha}{k_B T} \langle \Delta r^2(t) \rangle$$

Instrumentation

The BeNano 180 Zeta nanoparticle size and zeta potential analyzer (Bettersize Instruments Ltd.) is used in this application note. The instrument is equipped with a laser beam with a wavelength of 671 nm and a power of 50 mW as a light source, and avalanche photodiode detectors (APD) are set up to collect scattered light signals at 173°.

Sample Preparation and Measurement Condition

The measurement temperatures were controlled from 25 °C to 70 °C by the built-in temperature control system of the BeNano, with at least 300 seconds of temperature equilibration for each measurement. 10 μL of negatively charged 400 nm polystyrene spheres were added into 1 mL of the 10 mg/mL BSA solution as tracer particles.

Results and Discussion

First, in the same environment, the zeta potentials of the BSA solutions and the tracer particle suspension were measured respectively. The zeta potential of the BSA solution was -14.35 mV, and the zeta potential of the 400 nm tracer particle suspension was -51 mV. Both BSA and tracer particles are negatively charged, which can prevent agglomeration caused by the interaction of positive and negative charges.

The correlation functions of the samples were obtained by calculating the fluctuation of the scattered signals:

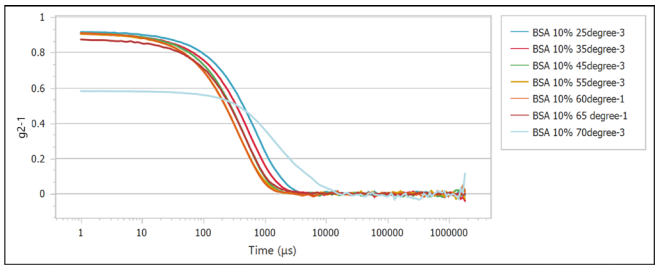

Figure 2. Correlation functions of BSA solutions at different temperatures

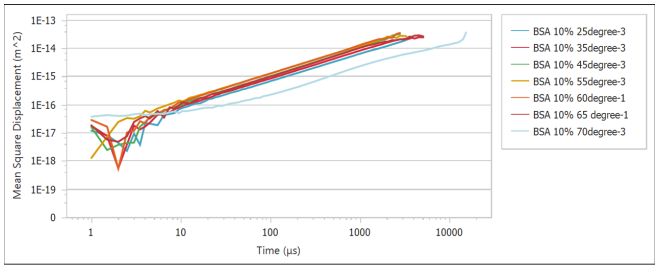

Figure 3. MSD curves of BSA solutions at different temperatures

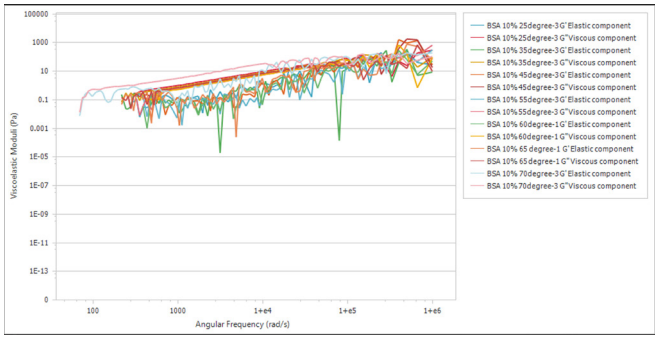

Figure 4. Viscoelastic modulus curves of BSA solutions at different temperatures

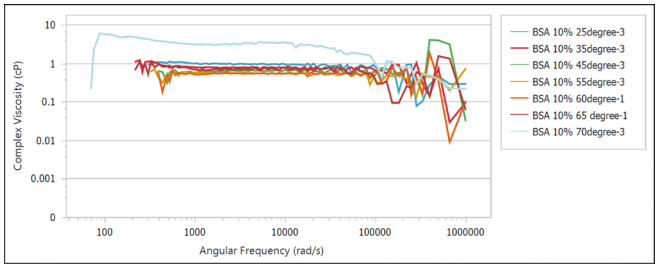

Figure 5. Complex viscosity curves of BSA solutions at different temperatures

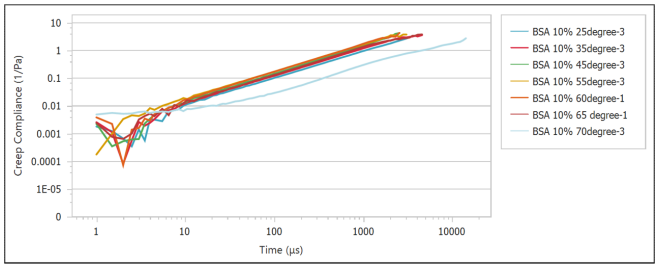

Figure 6. Creep compliance curves of BSA solutions at different temperatures

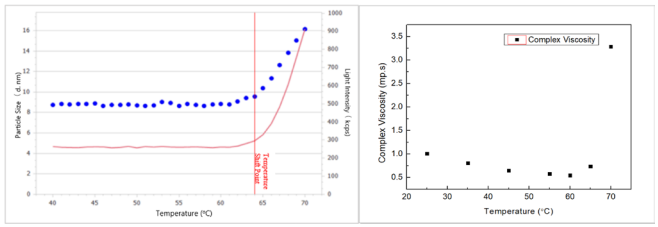

Figure 7. Curves of BSA particle size and intensity versus temperature (left) and complex viscosity versus temperature @ 2096 rad/s (right)

Figures 1-6 show that the correlation functions decayed faster as the temperature increased between 25°C and 60°C. This suggests that the velocity of tracer particle motion increases with temperature due to the decrease in solution viscosity, while the viscoelastic moduli of the solutions decrease throughout this temperature range. Furthermore, MSD curves show that for this temperature range, higher temperatures are associated with higher MSD values and faster tracer particle velocities.

However, the correlation functions decayed slower with increasing temperature in the range of 60 °C to 70 °C. This indicates that the motion of the tracer particles decreases with the increasing temperature, which is due to the formation of aggregates by the thermal denaturation of BSA in this temperature range, and the formation of aggregates dramatically increases the solution viscoelasticity.

As shown in Figure 7, the complex viscosity obtained by the DLS microrheolgy increased rapidly at about 65 °C, by which the temperature dependence of particle size and complex viscosity was cross-verified.

Conclusion

The measurement results show the microrheology detection capability of the BeNano for a protein sample. The thermal-sensitive microrheological information can sensitively and accurately reflect the denaturing process of a protein solution with temperature. By the microrheology measurements, the rheological parameters of the sample, such as mean square displacement, complex viscosity, viscoelastic moduli, and creep compliance, can be obtained quickly in the high frequency range, which provides a powerful tool for characterizing the rheological properties of liquids.

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