APPLICATION NOTE

Thermal Analysis

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Pharmaceutical Thermal Analysis: Measurement of Protein Unfolding (T_m)

DSC 8500

The folding and unfolding of a protein in solution has a certain energy associated with it and the ability to measure the temperature and enthalpy of these transitions is an important tool in the modern laboratory. Information on the effect of buffers, buffer concentration, pH, and temperature on the stability of the protein can be obtained as well as kinetic data on the process of unfolding. PerkinElmer's Diamond DSC (Figure 1) has demonstrated the ability to measure these values down to moderately dilute concentrations.

Solutions of lysozyme (Sigma) were prepared in unbuffered, deionized water in the following concentrations: 20 mg/mL, 10 mg/mL, 5 mg/mL, and 2.5 mg/mL. All weights were within 3%. Lower concentration solutions were obtained by successive dilution. Samples were run using high volume 60 µL stainless steel pans. The reference pan contained the solvent but no solvent-solvent baseline was subtracted. Samples were run at 5 °C/min under nitrogen purge using LN2 or an Intercooler 2 for cooling.

Lysozyme is a relatively small protein (MW = 14.6 kD) and is often used as a test case for studying protein behavior. Figures 2 and 3 show DSC results for this series of compounds. Ribonuclease (RNase, MW 13.8 kD) and Bovine Serum Albumin (BSA, MW = 68 kD) were also prepared as above and run in concentrations of 40 mg/mL and 32 mg/mL respectively, and then diluted down. Results for 10 mg/mL BSA and 8 mg/RNase are shown in Figure 3.

The Diamond DSC's power compensation design has the sensitivity to measure protein unfolding in solution at moderate concentrations as well as the temperature range for lyophilization, Tg ,Tg' and excipient purity studies.

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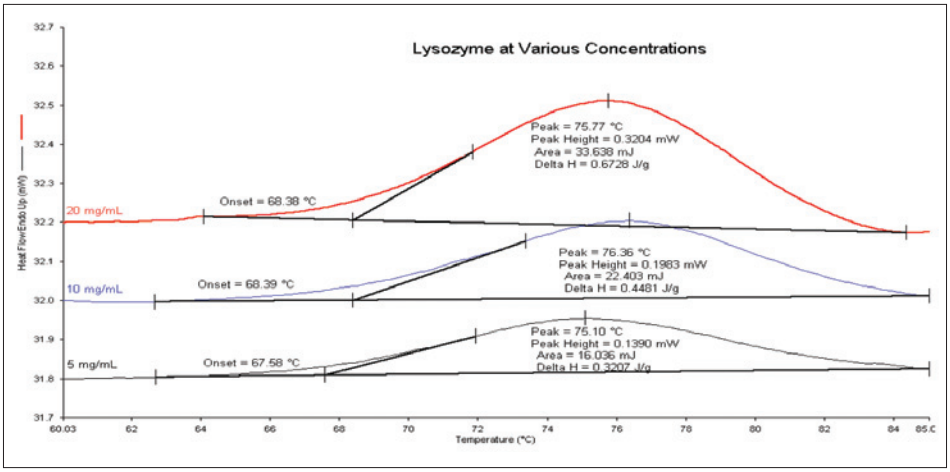

Figure 2. Lysozyme SHC solutions: high concentrations.

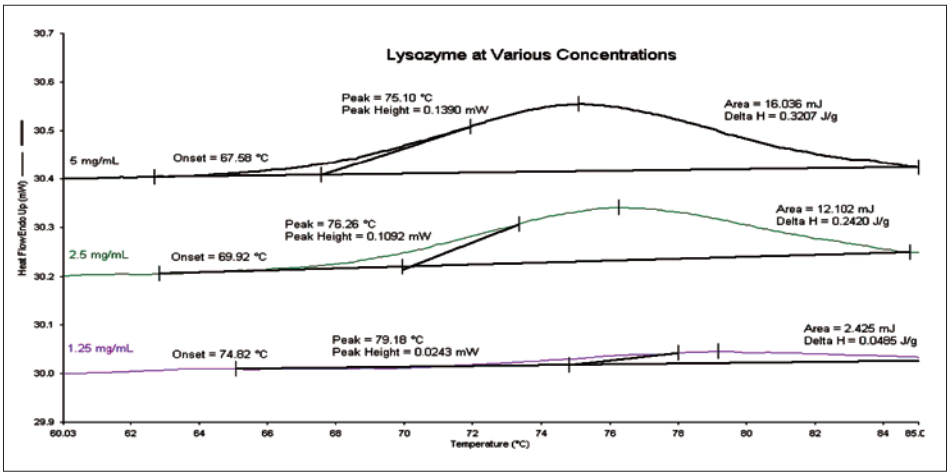

Figure 3. Lysozyme SLC solution: low concentrations

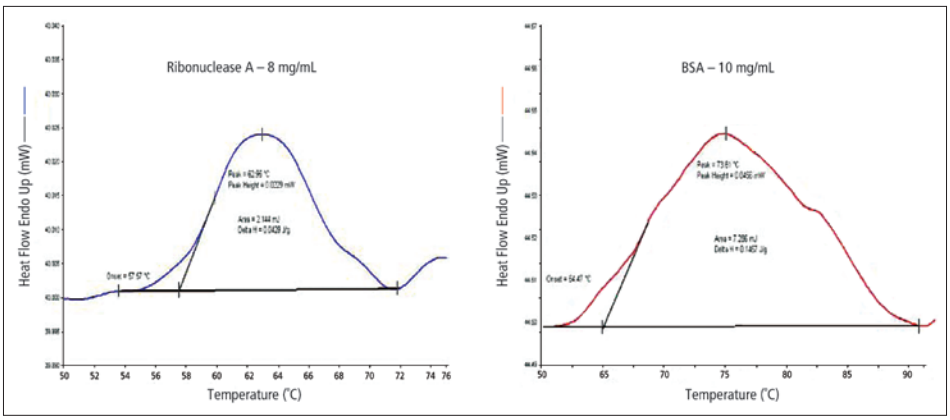

Figure 4. RNase and BSA in deionized water.

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