SII NanoTechnology Inc.

DSC AS PROBLEM-SOLVING TOOL: MEASUREMENT OF THE DENATURATION OF PROTEINS

Problem

A scientist, working at a pharmaceutical R&D center, has a pressing need to characterize the thermal denaturation of proteins in solution, which requires a high sensitivity calorimeter. Since technicians will be performing the analyses of the proteins, the scientist would like to use a DSC, for its ease of use, rather than a microcalorimeter, but the applications requires high sensitivity which DSC instruments cannot generally provide.

Solution

In aqueous solution, proteins have a specialized three-dimensional shape that allows them to support biological functions. When heat is applied to the water-protein system, this shape breaks down because of molecular thermal motion and thermal denaturation takes place. During this process, the thermal heat adsorption can be detected by DSC; however, a high sensitivity instrument is required since the endothermic transition is small.

The Seiko DSC6100 provides the following features for the characterization of pharmaceutical samples, such as denaturation of proteins or the analysis of lipids:

- high sensitivity which is 10 to 30 times greater than most conventional DSC instruments on the market
- very stable baseline performance necessary for detecting weak transition
- excellent subambient response for the accurate and precise measurement of low temperature transitions
- DSC design makes instrument easy to operate as opposed to the traditional microcalorimeter
- wide temperature range (-150 to 500°C) so that instrument can be used for many applications including polymers, fibers, elastomers, composites in addition to pharmaceutical and bioscience purposes
- ability to operate at fast heating and cooling rates (10°C/min) so that DSC6100 can be used for mainstream applications which the much slower micro-calorimeters cannot

The high level of sensitivity is made possible through the unique hardware featured with the Seiko DSC6100:

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- thermopile sensors rather than thermocouples which minimize baseline noise levels since the thermopile sensors provide a large electrical response
- large mass heat sink made of a silver block which functions effectively as thermal RC-filter
- small thermal distance between the heater and sample thermocouples for better temperature control
- small inner diameter of the heat sink which provides for a more uniform temperature profile at the bottom of the heat sink

The following experimental procedure gives the best results on the characterization of the thermal denaturation of proteins:

- heating rate of 1 or 2°C/min
- starting temperature of 25°C
- use of 70 ☐L silver sealed containers with approximately 50 mg of solution
- use of equivalent mass of pure water in reference container

The use of slow heating rates is necessary given the kinetics of the denaturation event associated with proteins in solution. The use of slow heating rates demands a high sensitivity DSC instrument, such as the DSC6100.

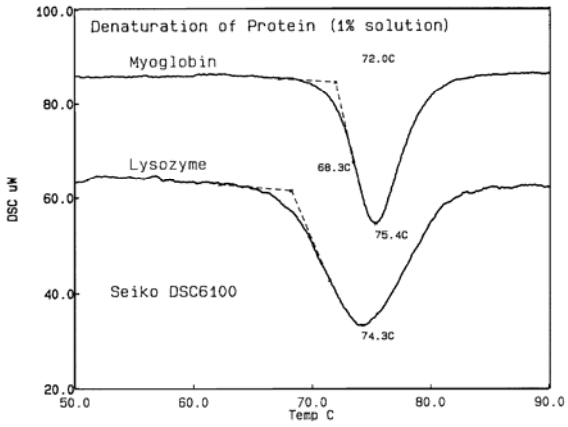

Figure 1

Displayed in Figure 1 are the results obtained from the Seiko high sensitivity DSC for 1% aqueous solutions of lysozyme and myoglobin proteins. The results demonstrate that the DSC6100 has the high degree of sensitivity to detect the weak denaturation thermal event at 74°C. The detection of the denaturation event aids in the evaluation of the thermal stability of proteins for pharmaceutical or bioscience applications.

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Shown in Figure 2 are the DSC6100 results obtained on a protein with a higher thermal

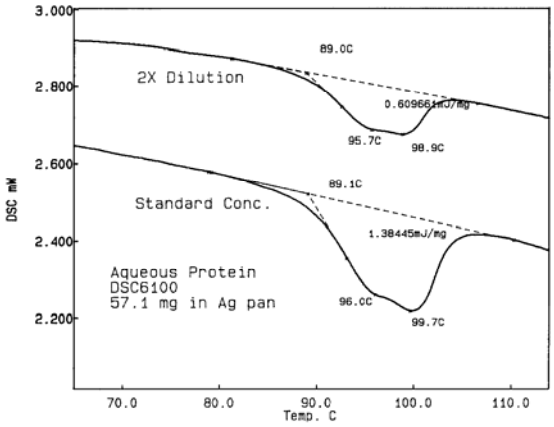

Figure 2

stability. The protein solutions were prepared in two different concentrations where one was two times more dilute than the other. As the data in Figure 2 demonstrates, the DSC6100 can easily detect the thermal denaturation event associated with the more dilute protein solution.

Summary

The Seiko DSC6100 provides the necessary high degree of sensitivity required to detect the weak thermal denaturation endotherm associated with aqueous protein solutions. The denaturation event can be easily detected down to 1% levels.

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