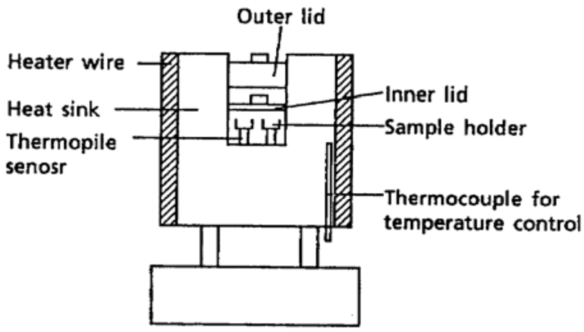
APPLICATIONS OF HIGH SENSITIVITY DSC

The Seiko DSC6100 offers proven high sensitivity performance which is ten to thirty times greater than most DSC instruments on the market. The high sensitivity DSC can detect very weak thermal transitions which cannot be observed by most other DSC's. Applications of the high sensitivity, state-of-the-art DSC6100 include:

- ***Pharmaceuticals,*** such as the detection of the melting and pre-transition events associated with lipids, denaturation of proteins, optimization of lyophilization (freeze-drying) and analysis of low mass samples.
- ***Foods,*** including the detection of multi-component melting points, gelatinization of starches, retrogradation of starches and analysis of low mass samples.
- ***Polymers,*** including the detection of weak heat set middle endothermic peaks for fibers, better characterization of the glass transition event of highly crosslinked or crystalline polymers, analysis of filled highly polymers such as composites, coatings, liquid crystalline polymers and low mass samples.

The high level of sensitivity achieved by the DSC6100 is possible through the following unique hardware design features:

- ***Thermopile sensors***, rather than traditional thermocouples, which minimize baseline noise level since the thermopile sensors provide a large electrical response
- ***Large mass heat sink,*** made of a silver block which functions effectively as a thermal RC-filter
- ***Small thermal distance*** between the sample thermopiles and heater thermocouple 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.



Configuration of the DSC120

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Figure 1

Displayed in Figure 1 is a diagram of the Seiko high sensitivity DSC cell with the specialized design features.

The Seiko DSC6100 provides the following desirable features for the characterization of a wide range of materials:

- ***High sensitivity,*** which is 10 to 30 times greater than most conventional DSC instruments currently on the market
- ***Very stable baseline performance*** necessary for obtaining reproducible results on weak thermal transitions
- ***Excellent subambient performance*** for the accurate and precise measurement of low temperature, weak transitions
- ***Ease of operation*** with DSC design as opposed to more tedious and cumbersome design associated with traditional microcalorimeters
- ***Wide temperature range*** (-150 to 500°C) so that the instrument can be used for many applications including polymers, fibers, elastomers, composites in addition to pharmaceutical and bioscience applications
- ***Fast heating and cooling rates*** (up to 10°C/min) so that DSC6100 can be used for mainstream DSC applications for which the much slower micro-calorimeters cannot be used

The Seiko DSC6100 also provides the following other useful features:

- ***Automated 20 point temperature calibration*** to provide greatest possible accuracy of temperatures across a wide temperature range
- ***Automated 10 point enthalpic calibration*** to provide for the greatest possible accuracy of heats of transition and heat capacities across the entire temperature range
- ***Specialized sample pans*** for the analysis of aqueous solutions, liquids, pastes as well as solids

PHARMACEUTICAL APPLICATIONS

The high sensitivity Seiko DSC is finding increasing usage for pharmaceutical applications including:

- *Lipids*
- *Denaturation of proteins*
- *Optimization of lyophilization*
- *Low mass samples*

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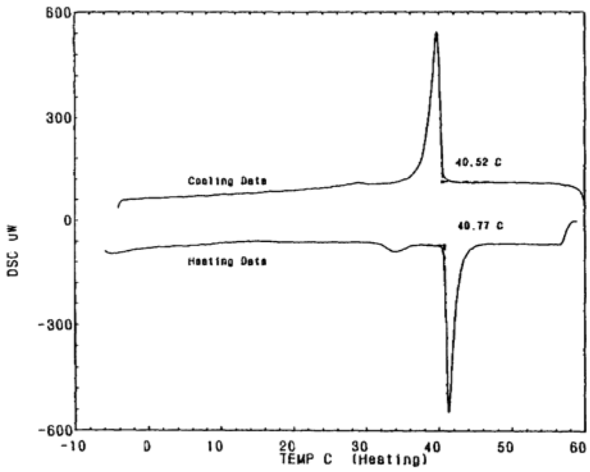
Lipids

One of the more important uses of high sensitivity DSC is in the analysis of lipids. Lipids tend to mimic the properties of tissue and are under investigation as drug delivery system. In solution, lipids form bilayer structures known as liposomes which exhibit melting and a partial disorientation, which produces a weak pre-transition endothermic event. The pre-transition event associated with lipids requires the use of a high sensitivity DSC for its successful detection, especially for dilute concentrations.

The following experimental conditions have been utilized with excellent outcome on the high sensitivity Seiko DSC:

Heating rate:	1 or 2°C/min
Initial temperature:	0°C
Sample container:	70 µL aluminum sealed container
Sample mass:	approx. 55 mg solution
Reference:	70 µL container with 55 mg of pure water



DSC Heating and Cooling Data of DPPC

Figure 2

Displayed in Figure 2 are the results obtained on a DPPC lipid during heating and cooling. The melting transition is observed at 40.8°C

during heating and at 40.5°C during cooling. The weak pre-transition event is observed at 34.4°C during heating and at 29.0°C during the cooling segment.

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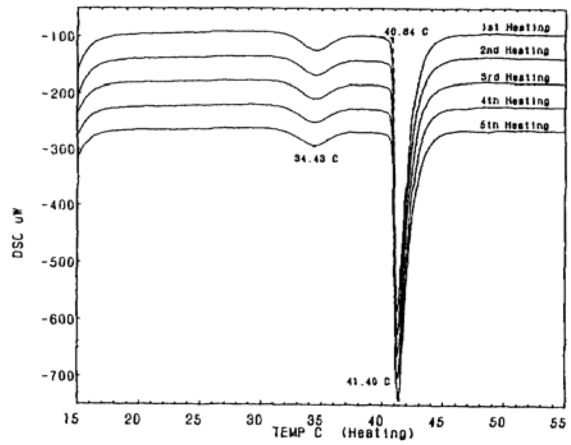
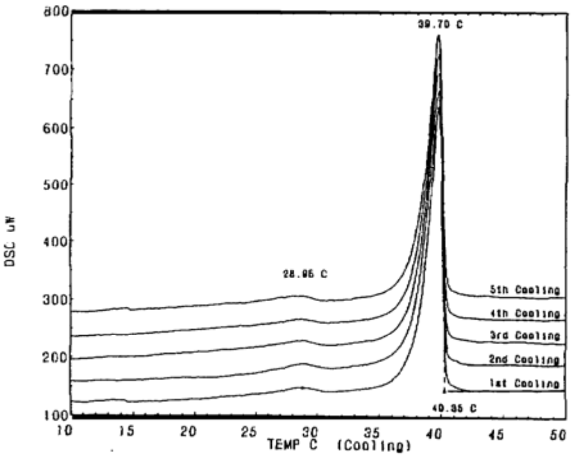


DSC Heating Data of DPPC

Figure 3



DSC Cooling Data of DPPC

Figure 4

The high level of reproducibility associated with the DSC is demonstrated in Figures 3 and 4. Figure 3 shows five consecutive heating experiments performed on the DPPC lipid while Figure 4 displays five cooling experiments on the sample.

The DSC6100 has the necessary high degree of sensitivity to detect both the

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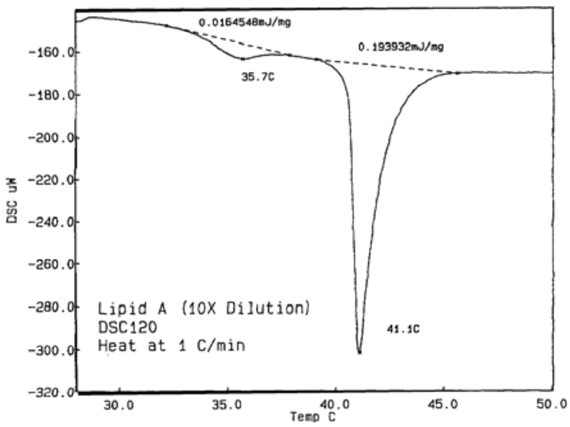

Figure 5

melting and the weak pre-transition events for more dilute concentrations. Shown in Figure 5 are the DSC results obtained on a DPPC lipid which has been diluted by a factor of 10 by the original, standard concentration.

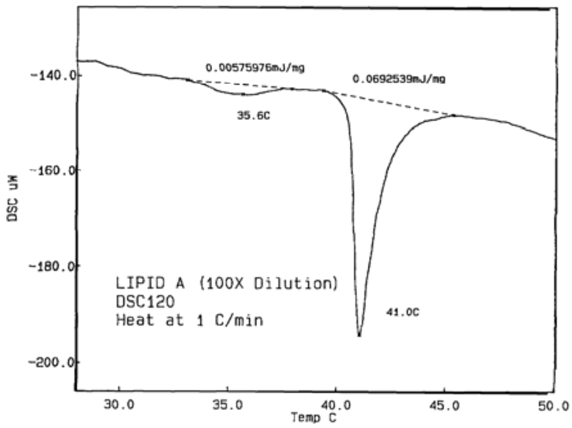

Figure 6

Even at a dilution factor of 100, the DSC6100 can still detect the very weak melting and pre-transition events associated with the DPPC lipid system. This is demonstrated in Figure 6 and both transition are still evident even at under highly dilute concentrations.

A direct overlay of the dilute DPPC lipid results are displayed in Figure 7.

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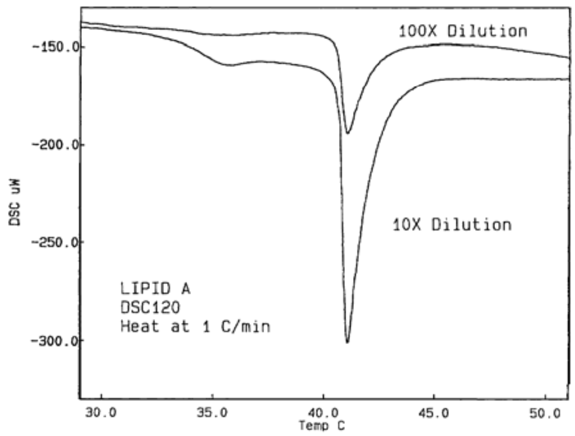

Figure 7

Thermal Denaturation of Proteins

The use of high sensitivity DSC is essential for studying the thermal denaturation of proteins in aqueous solution. When placed into solution, proteins have a specialized three-dimensional shape that allows them to support biological functions. When heat is applied to the protein-water system, this shape breaks down because of molecular thermal motions and thermal denaturation takes place. During this process, the thermal heat adsorption can be detected by DSC; however, a high sensitivity instrument is required.

The kinetics associated with the protein thermal denaturation events necessitates the use of slow heating rates (1 or 2°C/min). The slower rates required for protein denaturation demands a high sensitivity DSC instrument, such as the DSC6100.

Shown in Figure 8 are the DSC6100 results obtained two different protein solutions (myoglobin and lysozyme) at a 1% concentration level. The weak thermal denaturation events associated with these protein systems are well-defined and clearly observed with the high sensitivity DSC.

Displayed in Figure 9 are the results obtained on a protein system with a higher thermal stability. The protein solutions were prepared

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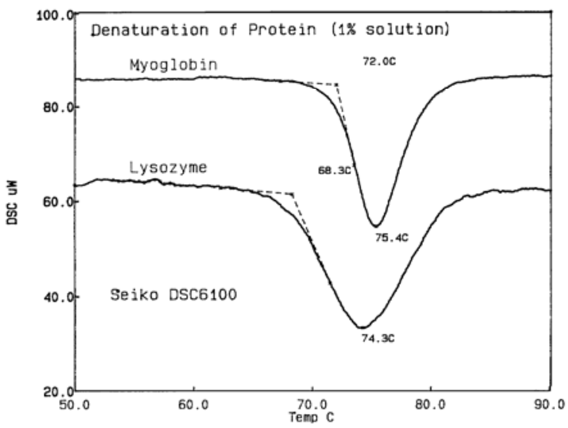

Figure 8

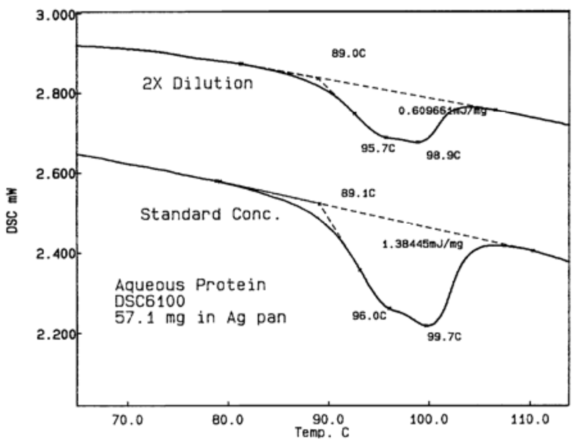

Figure 9

in two different concentrations where one was twice as dilute as the other. As the data demonstrates, the DSC6100 can easily detect the thermal denaturation event associated with the more dilute protein solution.

Lyophilizaton

Another major application area in the pharmaceutical industry is in the optimization of the lyophilization (freeze-drying) process. Lyophilization is necessary for drug formulations which exhibit a lack of liquid stability; and, during the lyophilization process, the aqueous formulation is cooled down to a sufficiently low temperature where

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primary drying can be applied to generate a freeze-dried 'cake'. The selection of the proper lyophilization formulations and conditions required to generate a satisfactory cake mandates a good deal of knowledge about the particular formulation and the effects of time and temperature on its thermal characteristics.

One key parameter that has been identified as crucial to understanding the lyophilization process for a given drug formulation is the glass transition temperature (Tg). Generally, the process temperature is set below the subambient Tg of the formulation during primary drying in order to avoid 'collapse' of the product during lyophilization. In addition to Tg, data indicates that the change in heat capacity, ΔCP, at Tg along with the occurrence of any recrystallization events can have a major effect on the success of producing a successful cake and avoiding collapse. Thus, a sensitive DSC, such as the Seiko DSC6100, is required which yields accurate, sensitive, and reproducible data on Tg and recrystallization events in the subambient temperature region.

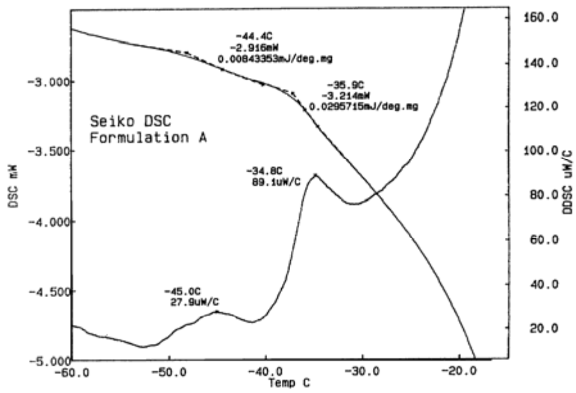

Figure 10

Displayed in Figure 10 are the results obtained from the Seiko DSC for aqueous formulation A. The plot shows the DSC heat flow and derivative as a function of temperature below the main melting of the ice component. Two glass transition events are obtained for this particular formulation at -44 and -36°C. The magnitude of the derivative peak at Tg helps to determine the relative size of the Tg event and its importance to the lyophilization process and of the propensity of the formulation to avoid collapsing during processing. The Seiko DSC has the necessary high degree of sensitivity and stability required to detect the weak Tg's of the formulation even under prolonged subambient operating conditions.

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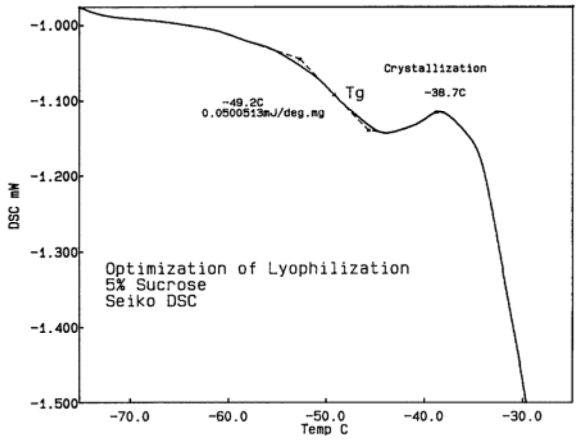

Figure 11

Displayed in Figure 11 are the DSC results obtained from an aqueous formulation containing 5% sucrose cryoprotector. The Tg of this solution is obtained at -49°C. With further heating, this formulation undergoes recrystallization at -39°C. There is evidence that formulations which exhibit a recrystallization event during heating will lyophilize successfully regardless of the product temperature and the magnitude and temperature.

FOODS

Food samples oftentimes require the use of high sensitivity DSC. The high sensitivity performance is necessary when working with foods containing a number of components or additives, low mass samples or samples with weak thermal transitions.

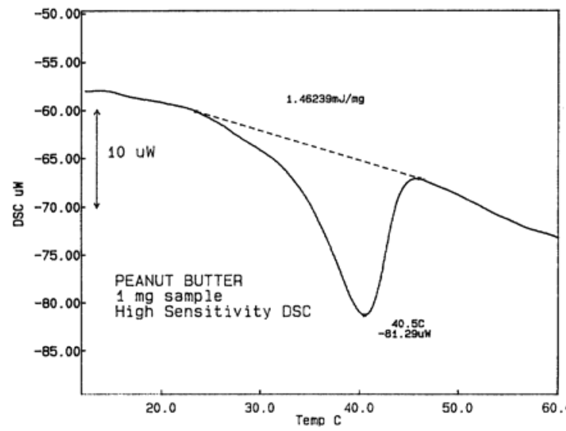

Figure 12

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Displayed in Figure 12 is a sample of a 1.00 mg sample of a commercial peanut butter. An endothermic transition is obtained at 40.5°C and this represents the melting of the stabilizer component found in the peanut butter. The heat of melting is only 1.38 mJ/mg. The high sensitivity capability of the DSC6100 permits this transition to be easily observed even at a relatively low mass.

The textural properties of food products containing starch, such as rice, wheat or potatoes, can be affected by physical transformations known as gelatinization and retrogradation. Gelatinization refers to the process by which a starch/water system undergoes an order-disorder transition during heating. It is believed that the starch loses its crystallinity during gelatinization. Retrogradation refers to the process by which a cooked or heated aqueous starch slurry reverts back to some sort of ordered or crystalline form over time. The retrogradation process is analogous to the material becoming stale during storage.

The retrogradation process of starch and water slurries is a low energy transformation and thus requires the use of a high sensitivity DSC, such as the Seiko DSC6100.

Displayed in Figure 13 are the DSC6100 results obtained on a rice starch/water slurry that had been stored in a refrigerator at a temperature of 5°C and then sampled after 7 days. The rice slurry was heated at a rate of 1°C/mm in a sealed silver DSC container. The

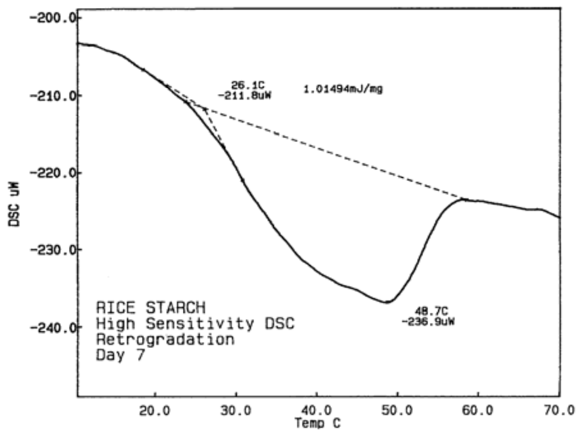

Figure 13

transition observed between 30 an 50°C represents the retrogradation of the aged starch/water slurry. The total heat of transition is only 1.02 mJ/mg. This low energy, coupled with the required slow heating rate, necessitates the use of a high sensitivity DSC.

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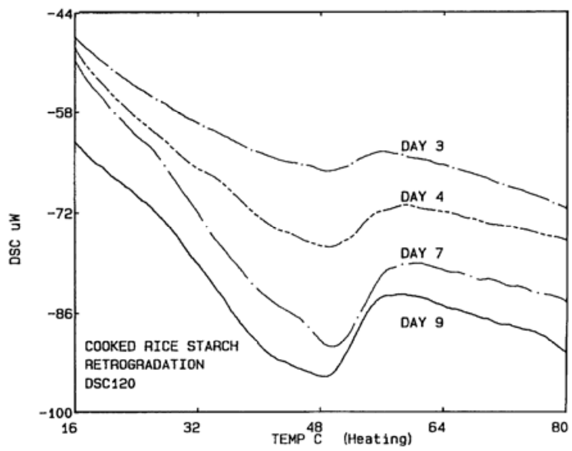

Figure 14

Displayed in Figure 14 is an overlay of the starch/water retrogradation results at various aging time intervals. The data shows that as the slurry is aged for longer times, the retrogradation endotherm increases in magnitude reflecting the increasing 'staleness' of the starch/water slurry.

POLYMERS

One potential major area for applications of high sensitivity DSC would be for the characterization

of polymeric materials, including:

- Better measurement of weak Tg's
- Coatings
- Highly filled polymer systems, such as composites
- Better understanding of semi-crystalline polymers, such as PET or nylon
- Characterization of highly cross-linked thermoset materials
- Liquid crystalline polymers
- Heat set histories of fibers

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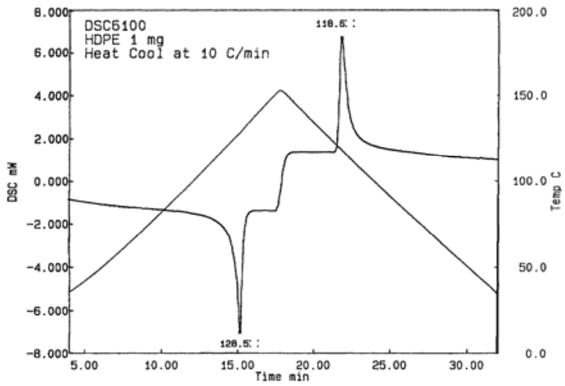

Figure 15

The DSC6100 can be used to study typical polymeric materials and its improved, faster heating and cooling rate capability (up to 10°C/min) permits the DSC to be utilized for such mainstream applications. Displayed in Figure 15 are the results obtained from the DSC6100 for a 1.00 mg sample of high density polyethylene when heated and cooled at a rate of 10°C/min. The DSC6100 provides linear heating and cooling ramps.

The real high sensitivity performance of the DSC6100 for polymeric applications is demonstrated with the results shown in Figure 16 which are those for a sample of low density polyethylene with a mass of only 30 μg (0.030 mg). The DSC6100 provides high quality, low noise data even at very low sample masses.

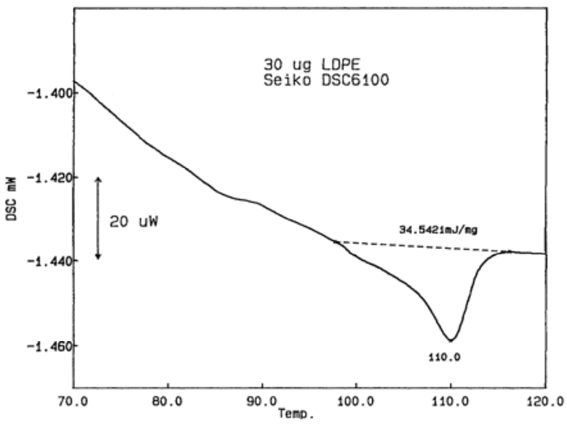

Figure 16

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As another example, DSC can be use to detect the processing conditions utilized to generate synthetic fibers or yarns. Fibers are generally heat set which involves running the spun fibers over a hot surface or a heated tunnel an this procedure stabilizes the physical and thermal properties exhibited by the fibers. The passage of the fibers over the heated surface results in small changes to the amorphous/crystalline structure of the polymer and these changes can be detected by DSC as small middle endothermic events between the glass transition event and the melt peak. The detection of the heat set, middle endothermic peak is critical for fibers since it is related to important end use properties such as dye uptake or streaking problems. For some fibers, such as nylon 6 which have been heat set using a pressurized steam treatment, the heat set endotherm can be very difficult or impossible to detect using conventional DSC instruments simply because the endotherm is so small. The direct detection of the subtle differences due to processing associated with nylon 6 and other fibers requires the use of a high sensitivity DSC, such as the DSC6100.

Displayed in Figure 17 are the results obtained from the DSC6100 on a sample of nylon 6 yarn subjected to the steam heat set treatment. The melting of the crystalline component is observed as a large endotherm at 220°C.

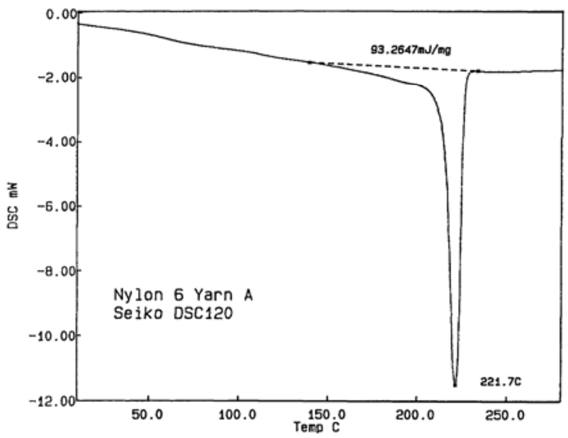

Figure 17

The value of the heat of melting can be used to estimate the percent crystallinity of the yarn.

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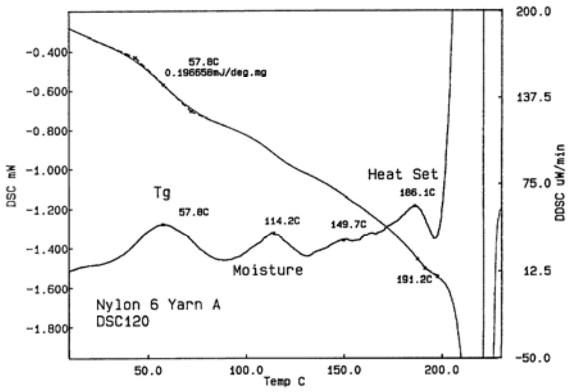

Figure 18

The heat set properties associated with the generation of the nylon 6 fibers can be observed in a magnified view of the DSC data obtained below the melting transition and this is displayed in Figure 18 for the sample. The derivative data, displayed in this figure, helps to more clearly identify the subtle heat set conditions associated with the nylon 6 fibers. For this sample, the Tg is observed as a small, step wise change in the heat flow at 57.8°C with an associated peak in the derivative at 57.8°C. Another peak in the derivative trace is obtained at 114°C and this most likely reflects the evolution of a small amount of remaining moisture from the hydrophilic nylon 6 fibers. The derivative peak detected at 186.1°C is the

main heat setting peak and the temperature and magnitude of this particular peak reflects the particular heat setting conditions (dwell time and steam temperature/pressure in the tunnel). The DSC6100 has the necessary high degree of sensitivity to directly detect the heat set treatment.

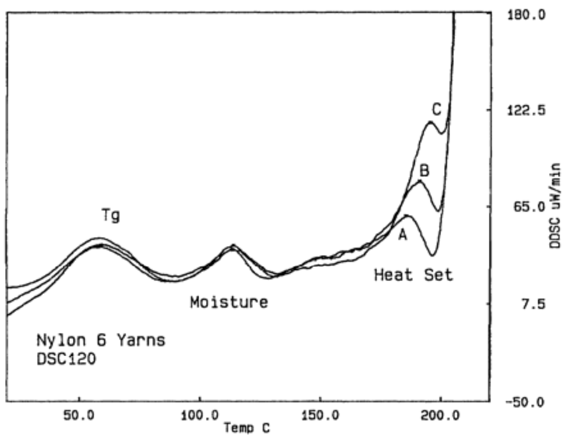

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Figure 19

Displayed in Figure 19 is an overlay of the DSC derivative profiles of three nylon 6 yarns (A, B, and C) which have been exposed to increasing rigorous processing conditions. As the tunnel temperature increases, both the temperature and magnitude of the DSC derivative peak increases. Thus, the Seiko DSC6100 provides a very useful characterization parameter to ascertain the given heat set conditions utilized to generate nylon 6 fibers or yarns.

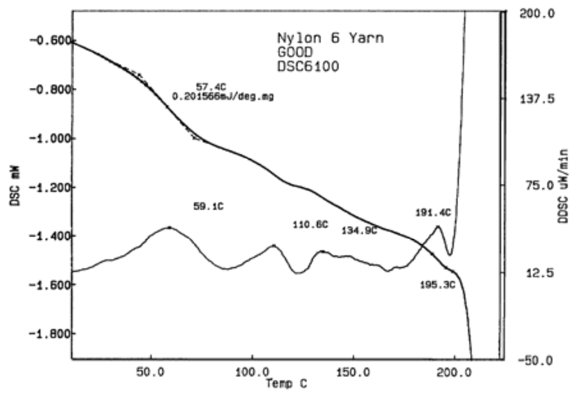

Figure 20

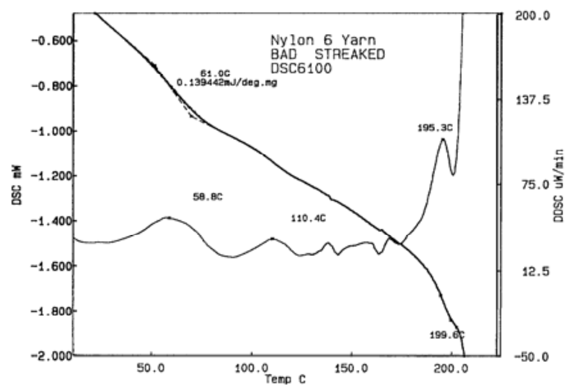

Figure 21

Displayed in Figures 20 and 21 are the DSC6100 results obtained on two nylon 6 fibers, one of which had good dye uptake characteristics and the other which was bad since it produced streaking problems. The bad yarn yielded a significantly higher heat set peak at 195°C as compared to the good yarn whose heat set peak was at 191°C. The

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DSC6100 data indicates that the bad yarn had been exposed to overly severe heat set conditions which resulted in streaking problems. The DSC6100 has the high sensitivity needed to detect the differences in the heat set conditions of the good and bad yarns.

Summary

The Seiko DSC6100 possesses proven high sensitivity which is a factor of 10 to 30 times better than most DSC instruments currently on the market. This is made possible by a number of unique hardware design features. The DSC61OO offers the user high sensitivity, wide temperature range, ability to heat and cool at relatively fast rates, and excellent baseline stability. The DSC6100 is ideally suited for applications in the pharmaceutical, bioscience, foods and polymer fields where high sensitivity and ease of use are required.

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