

Characterization of Pharmaceutical Materials by Thermal

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1. Introduction

Thermal analysis has been an extremely important analytical tool within the pharmaceutical industry for more than forty (40) years. Although the technique could easily be classified as mature, recent advances in Differential Scanning Calorimetry (DSC) have generated renewed enthusiasm in thermal analysis from pharmaceutical scientists. These new developments include Tzero DSC™ and Modulated DSC (MDSC®) which provide significantly improved performance in critical areas such as sensitivity, resolution and separation of complex transitions.

This paper will illustrate the use of these improved DSC technologies on characterization of a wide variety of pharmaceutical materials including amorphous and crystalline drugs, drug delivery systems such as tablets and biodegradable polymer microspheres, proteins and frozen solutions used for freeze-drying.

2. Recent Advances in DSC Technology

A brief description of the new technologies is provided to help explain how the improved performance is obtained over tradition DSC instrumentation.

2.1. Modulated DSC®

An MDSC® experiment is performed on the same instrument as used for traditional DSC measurements. The difference between the two techniques is in the temperature profile applied to the sample and the deconvolution (separation) of the resulting heat flow signal into several components.

Instead of the simple linear temperature change used by DSC, MDSC® uses two simultaneous heating rates; an average or underlying rate similar to DSC plus a sinusoidal or modulated heating rate. The average rate provides information equivalent to traditional DSC while the modulated heating rate provides unique information about the sample's heat capacity. Figure 1 shows how temperature changes with time in an MDSC® experiment.

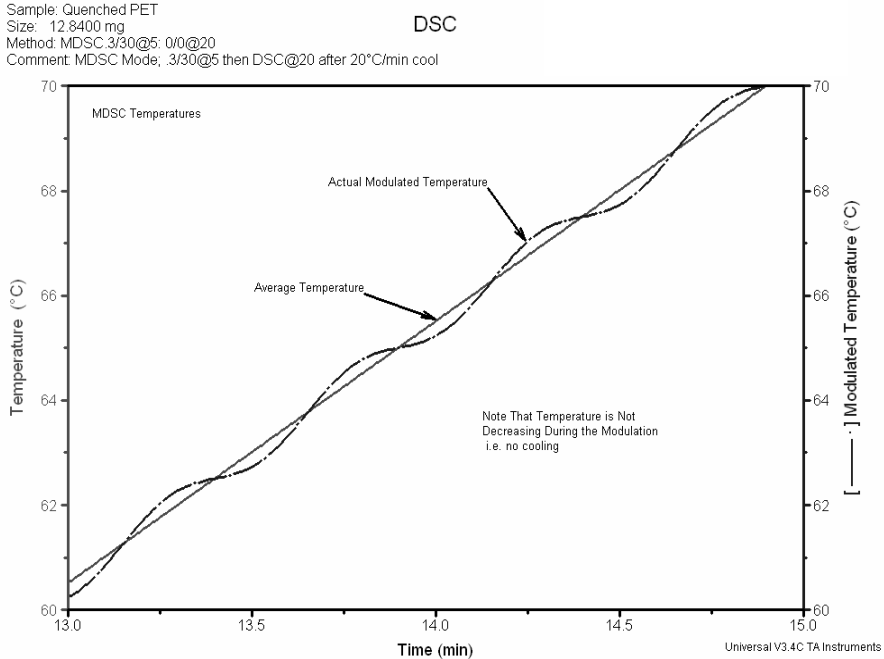


Figure 1.

As stated previously, the reason for applying simultaneous heating rates is to create additional information about the heat capacity or structure of the material. A brief examination of the equation used to describe the heat flow signal from a DSC or MDSC[®] experiment shows the benefit of the dual heating rates.

$$\frac{dH}{dt} = C_p \frac{dT}{dt} + f(T, t)$$

Where:

$\frac{dH}{dt}$ is the Total heat flow due to the underlying or linear heating rate

C_p is the Heat Capacity Component of the Total heat flow and is calculated from just the heat flow that responds to the modulated heating rate

$\frac{dT}{dt}$ is the measured heating rate which has both an average (linear) and amplitude (modulated) component

$f(T, t)$ is the Kinetic Component of the Total heat flow and is calculated from the difference between the Total and Heat Capacity component.

$C_p \frac{dT}{dt}$ is the Reversing Heat Flow Component of the Total Heat Flow

Traditional DSC provides a single signal which is the sum of all thermal events occurring within the temperature range of the experiment. This often makes it difficult to interpret data or detect small transitions. MDSC[®] has a significant advantage over traditional DSC in that it measures both the Heat Capacity Component and the Total, and obtains the Kinetic Component from the difference. Separation of complex transitions into specific components greatly improves interpretation of results. In general, MDSC[®] provides the following advantages over traditional DSC.

- increased sensitivity
- increased resolution
- separation of complex transitions
- more accurate measurement of crystallinity in semicrystalline materials
- direct measurement of heat capacity, either while programming temperature or holding it isothermal

Several of these benefits are illustrated on pharmaceutical materials later in this paper.

2.2. Tzero DSC[™] Technology

Until the recent introduction of this new approach to measuring absolute values of heat flow, DSC technology had not changed in a significant way since its commercialization in the mid-1960s. That technology was based on a single differential measurement and used either a heat-flux or power-compensation approach. The performance limitations of those early technologies could be seen in baselines that were neither flat nor very reproducible, and in peak-widths (resolution) that were much greater than expected from the melting of pure metals. Tzero DSC[™] technology provides very significant improvements in baseline performance, sensitivity and temperature resolution of transitions.

The improved performance of Tzero DSC[™] results from a cell design which produces two simultaneous differential measurements and provides for the ability to calibrate the thermal resistance and heat capacitance of the individual sensors as a function of temperature.

By knowing the actual thermal characteristics of a specific cell, any imbalance in capacitance or resistance of the sensors can be accounted for in the calculation of the absolute heat flow signal. The improved resolution of Tzero DSC[™] technology is seen in Figure 2, a comparison of an indium melt with traditional and Tzero DSC[™] technologies.

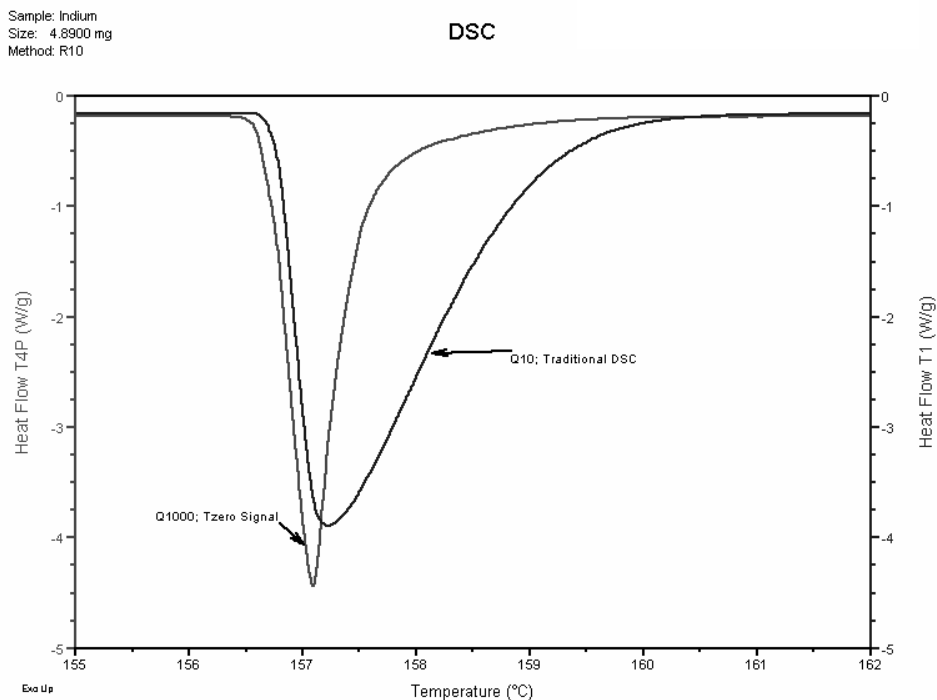


Figure 2.

Tzero DSC™ provides a much more accurate heat flow signal than was previously available. Because of this, heat capacity values can be measured in a single run versus the three runs required for traditional DSC. This kind of performance results directly from the much more complete calibration of the physical components of the system and use of those calibration values in calculating the heat flow due to just the sample. Calibration factors are measured versus temperature and then continuously applied to the four-term heat flow equation used to calculate the sample heat flow.

$$q = -\frac{\Delta T}{R_r} + \Delta T_0 \left(\frac{1}{R_r} - \frac{1}{R_s} \right) + (C_r - C_s) \frac{dT_s}{dt} - C_r \frac{d\Delta T}{dt}$$

Where:

$$q = \text{sample heat flow} = q_s - q_r$$

$$\Delta T = \text{temperature difference between sample and reference}$$

ΔT_0 = temperature difference between sample and Tzero thermocouple located between sample and reference sensors

$-\frac{\Delta T}{R_r}$ = principle heat flow

between $\Delta T_0 \left(\frac{1}{R_r} - \frac{1}{R_s} \right)$ = term to account for any imbalances in thermal Resistance sample and reference sensors

R = thermal Resistance of sample or reference sensors

$(C_r - C_s) \frac{dT_s}{d\tau}$ = term to account for any imbalance in heat Capacitance between the sensors

C = heat Capacitance of sample or reference sensors

$-C_r \frac{d\Delta T}{d\tau}$ = term to account for the difference in heating rates between the sensors (T4) and between the sample pans (T4P) during a transition in the sample

The advantages of Tzero DSC™ technology are illustrated on pharmaceutical materials in the applications section of this paper. In general, benefits fall into five areas:

- flat and reproducible baselines
- higher sensitivity
- higher resolution
- single run measurement of heat capacity
- higher heating rate MDSC®

3. Pharmaceutical Applications

Because all transitions in materials involve the flow of heat (into the sample during endothermic events and from the sample for exothermic events), DSC is the universal detector for measuring a wide variety of transitions in pharmaceutical materials. This paper will focus on some of the most common measurements and illustrate the superior performance of Tzero DSC™ and MDSC® technology.

These applications include measurement of:

- Amorphous Structure
 - Glass transition
 - Detection of amorphous material in semi-crystalline compounds

- Crystallinity
 - Melting and crystallization
 - Purity
 - Polymorphs

- Drug/Excipient Interaction

- Protein Denaturation

- Freeze Drying

- Miscellaneous / Complementary Thermal Analysis Techniques

4. Amorphous Structure

The physical properties of amorphous structure are quite different from crystalline structure. Major differences include dissolution rate (faster bioavailability), storage stability and hygroscopicity, the tendency to absorb moisture or other solvents. It is, therefore, important to know if a drug or drug delivery system has an amorphous component.

The most common DSC measurement of amorphous structure is the measurement of the glass transition. It is important to know both the size of the transition in heat flow or heat capacity units and the temperature (T_g) at which it occurs. The size of the transition provides quantitative information about the amount of amorphous structure in the sample, and the temperature identifies the point where there is a dramatic change in physical properties. Below the glass transition temperature there is limited molecular mobility while above there is high mobility that results in much lower viscosity and potentially much greater chemical interaction between components. Because of this, there is a general desire to store samples at least 40°C below their glass transition temperature.

Since amorphous materials are often hygroscopic and because small amounts of moisture or solvent act to plasticize (lower T_g) the sample, it is important to measure the actual T_g of drug formulations as well as to control their volatile content. Figure 3 shows the glass transitions of an amorphous sucrose sample that was exposed to lab air (approx. 50% RH) for about thirty minutes. The first heat shows the midpoint of the glass transition centered near -28.70°C while the second heat to 100°C shows that it has increased by nearly 40°C to 11.8°C . Even this sample still has several percent moisture since the glass transition of completely dry sucrose is nearly 70°C .

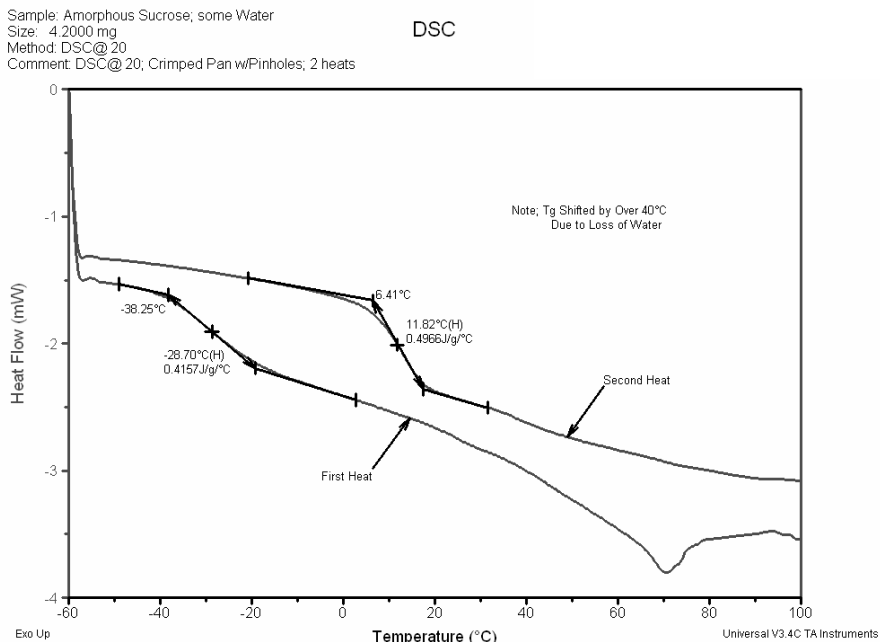


Figure 3.

Figure 4 is an MDSC[®] experiment that shows how the size of the glass transition increases with increasing amounts of amorphous structure. The sample of Polyethylene Terephthalate (PET) was first quench cooled to produce a 100% amorphous structure then cooled at slower-and-slower rates to produce increasing amounts of crystalline structure. Even with a cooling rate of 0.2°C from above the melting temperature, the material retains a large amorphous component. To quantify the percentage of amorphous phase, the size of the glass transition (0.14 J/g°C) is divided by the size of the glass transition for a 100% amorphous sample (0.35 J/g°C).

$$\% \text{ Amorphous Phase} = \frac{0.14}{0.35} \times 100 = 40\%$$

Although this is a good approximation of the amorphous content of the sample, the actual content is probably slightly higher. Amorphous material that is sometimes trapped within crystalline lattices, often called the rigid amorphous phase, does not contribute to the step change in heat capacity at the glass transition and is therefore undetected.

Sample: PET; AFTER DIFFERENT COOL RATES
 Size: 9.4300 mg
 Method: MDSC_531/40 @ 5°C/MIN
 Comment: MDSC_531/40 @ 5°C/MIN, QUENCH, 20, 10, 5, 2, 1, 0.2 COOL

DSC

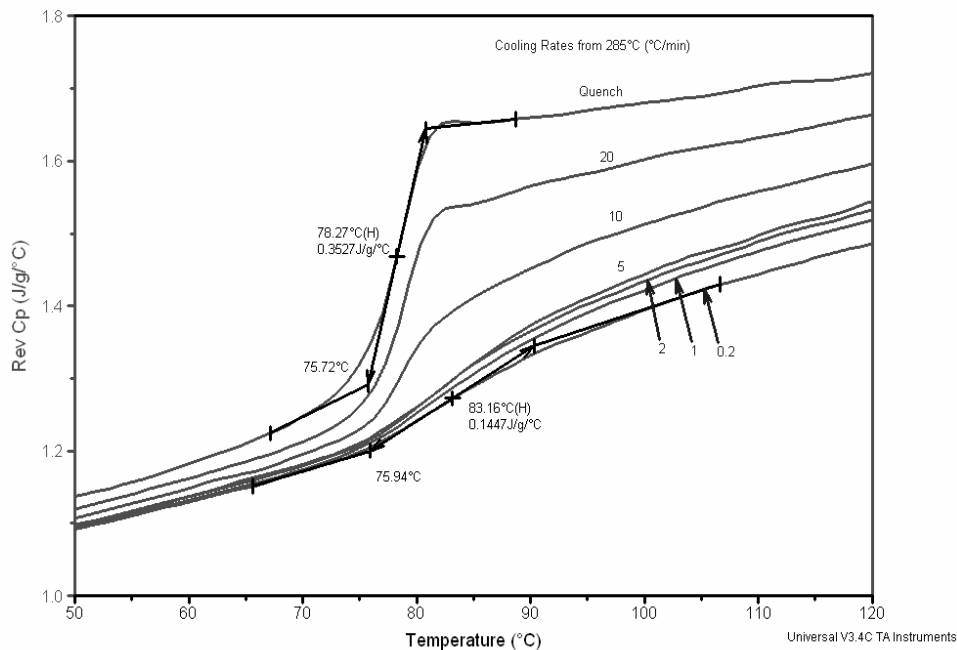


Figure 4.

One of the most difficult measurements for DSC is the detection of small amounts (<5%) of amorphous material in highly crystalline samples. The transition is small and is often hidden by small variations (nonlinear) in the DSC baseline. Figures 5 and 6 show the results of the outstanding baseline obtained with a Tzero™ DSC on a sample of crystalline sucrose that has less than one percent amorphous phase. Figure 5 shows duplicate runs on a very small (180µg) sample of freeze-dried amorphous sucrose. The value of the second heat is to not only check reproducibility but also to verify that the sample is dry. A wet sample with even a few percent moisture would have a lower Tg the first time that it is heated in a crimped (not hermetically sealed) pan. These runs are essentially calibration runs for determining the weight of amorphous material in another sample based on the size of the glass transition.

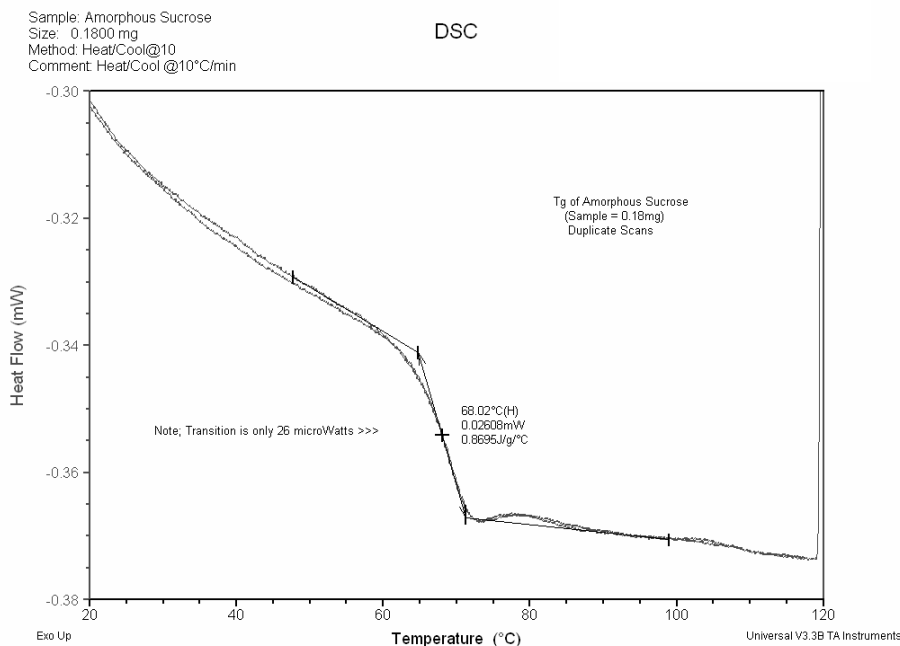


Figure 5.

Figure 6 shows an overlay of the data from Figure 5 along with three other experiments. The first experiment was on a relatively large (15mg) sample of sucrose that was thought to be 100% crystalline. A large sample was used to increase the sensitivity of the measurement in detecting small amounts of amorphous content. At the expected glass transition temperature, a very small step change of $8\mu\text{W}$ is detected. Comparing this change with that of the 100% amorphous sample permits the calculation of the amount of amorphous structure in the crystalline sample.

$$\frac{8.4\mu\text{W}}{X} = \frac{24.6\mu\text{W}}{180\mu\text{g}} \text{ where } X = 61\mu\text{g}$$

$$\% \text{ Amorphous Sucrose} = \frac{61\mu\text{g} \times 100}{15,000\mu\text{g}} = 0.4\%$$

In order to verify that there was a small amount of amorphous material in the crystalline sample, the technique of “standard addition” was applied where a known quantity ($80\mu\text{g}$) of amorphous material was added to a known quantity ($16,000\mu\text{g}$) of the crystalline sample. Based on the amount of amorphous material added, a step change of $10.9\mu\text{W}$ would be expected if there were no amorphous material in the original crystalline sample.

$$\frac{80\mu\text{g}}{180\mu\text{g}} \times 24.6\mu\text{W} = 10.9\mu\text{W}$$

Actual results on duplicate runs shown in the middle of Figure 6 show a step change of $17.3\mu\text{W}$. This equates to a weight of:

$$\frac{24.6\mu\text{W}}{180\mu\text{g}} = \frac{17.3\mu\text{W}}{X} \text{ where } X = 126\mu\text{g}$$

$$\% \text{ Amorphous Sucrose} = \frac{126\mu\text{g} \times 100}{16,080\mu\text{g}} = 0.8\%$$

Since only 0.5% was added, the original crystalline sample must have contained 0.3% which agrees quite well with the 0.4% measured directly.

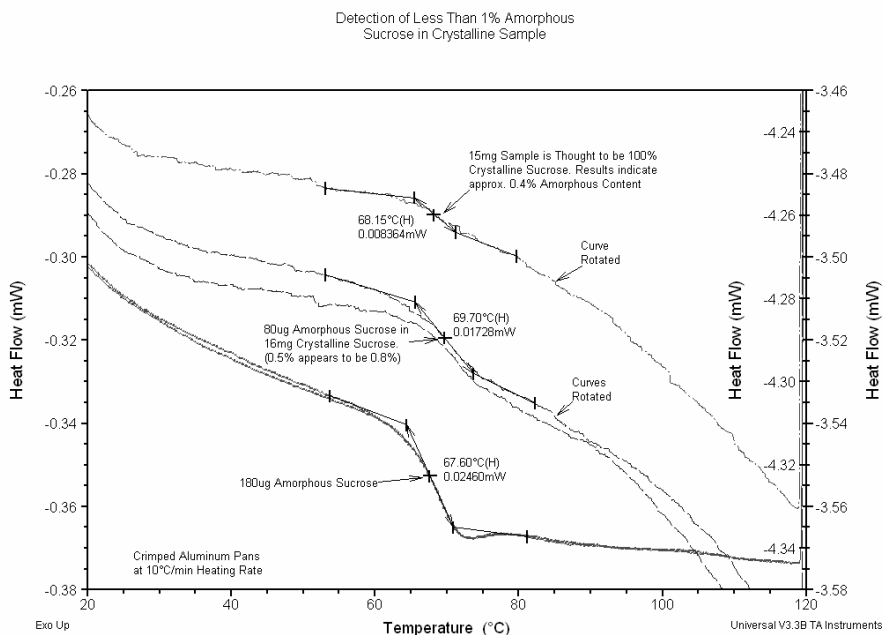


Figure 6.

For many samples, it is often difficult to detect the glass transition by DSC even when the sample has a high amorphous content. This is due to interferences from other transitions that occur over the same temperature range.

The Total signal in Figure 7 (which is equivalent to a standard DSC signal) is almost uninterpretable due to numerous transitions between room temperature and 150°C . Because this was an MDSC[®] experiment, the transitions are separated into the Reversing and Nonreversing signals and can be more easily interpreted as shown. The

Reversing signal, which is just the heat capacity component of the Total signal, is extremely useful for measuring glass transitions in all types of difficult samples.

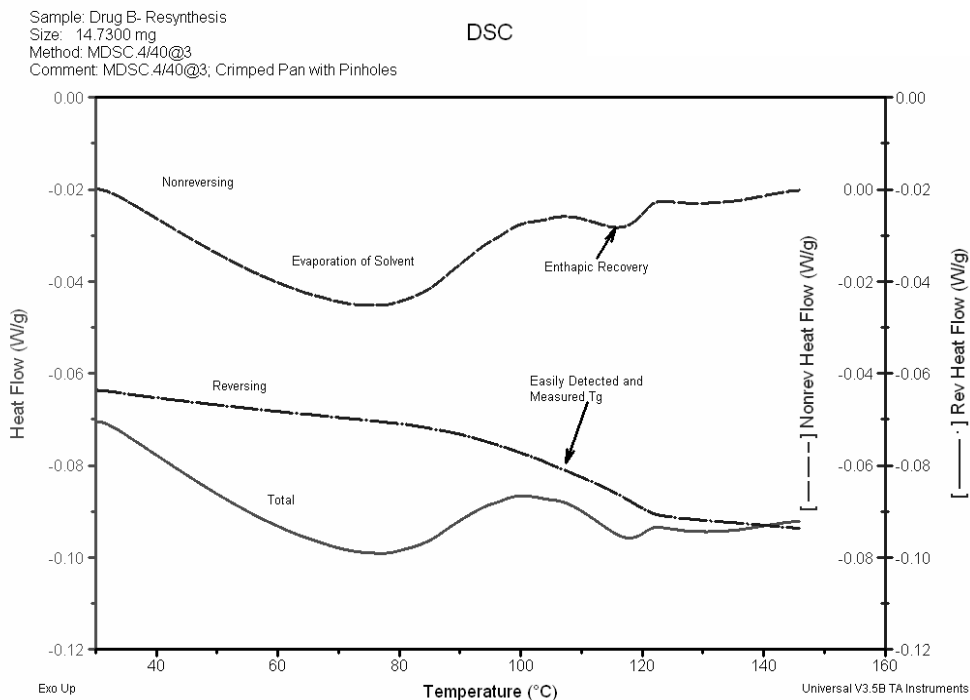


Figure 7.

5. Crystallinity

Unlike glass transitions that are often hard to detect, endotherms associated with material melting are relatively high in energy (J/g) and easily seen. However, this does not mean that it is always easy to measure crystallinity by DSC. The DSC user must constantly be aware of other transitions that appear as endothermic peaks and can be misinterpreted as melting. For example, the endothermic peak between 40 and 100°C in Figure 8 is the result of water evaporation from the pinhole pan as the water molecule is lost from a monohydrate form of a drug. For this material, which is highly (>99%) crystalline according to x-ray diffraction results, the crystal structure is also lost as the water evaporates. The resulting amorphous material crystallizes near 122°C and melts at 174°C.

Since most endothermic transitions that can be confused with melting are kinetic events (evaporation, decomposition, and enthalpic recovery at T_g), it is relatively easy to distinguish between melting and these other transitions. This is done by changing the heating rate over the range of 1 to 20°C/min. The onset of a true melting peak will shift

very little ($<1^{\circ}\text{C}$) with heating rate while evaporation and decomposition peaks will shift by 10°C or more.

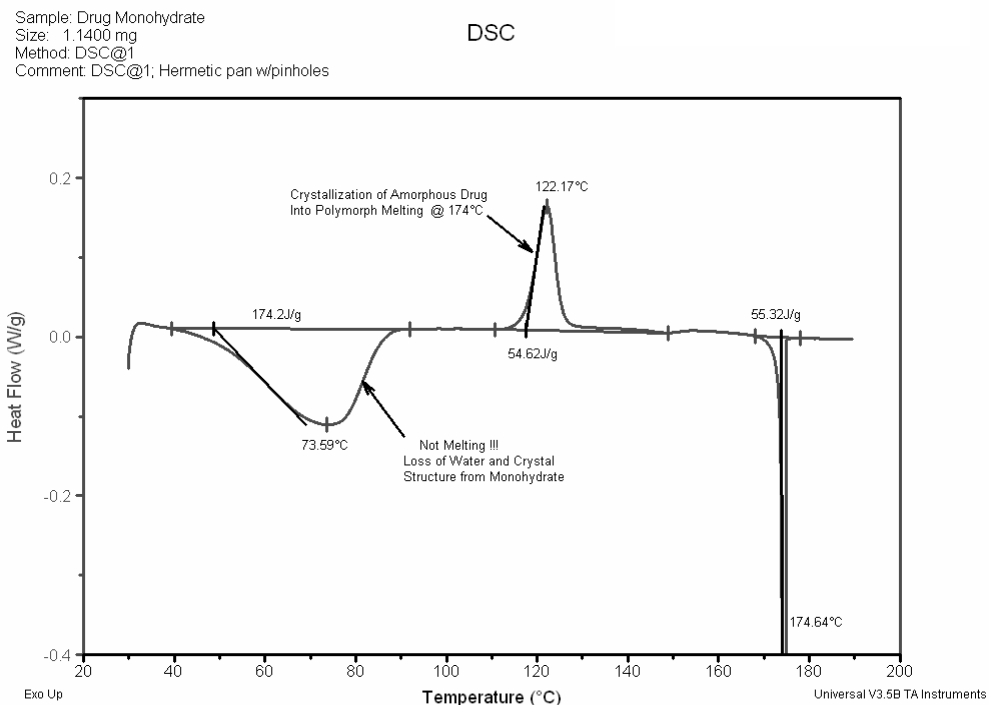


Figure 8.

Figure 9 shows how the melting peak of Phenacetin changes with heating rates of 1, 5 and $20^{\circ}\text{C}/\text{min}$. The shift in the peak onset is only 0.3°C . Peak temperature and width do increase with heating rate but the onset of a true melting transition will change only slightly.

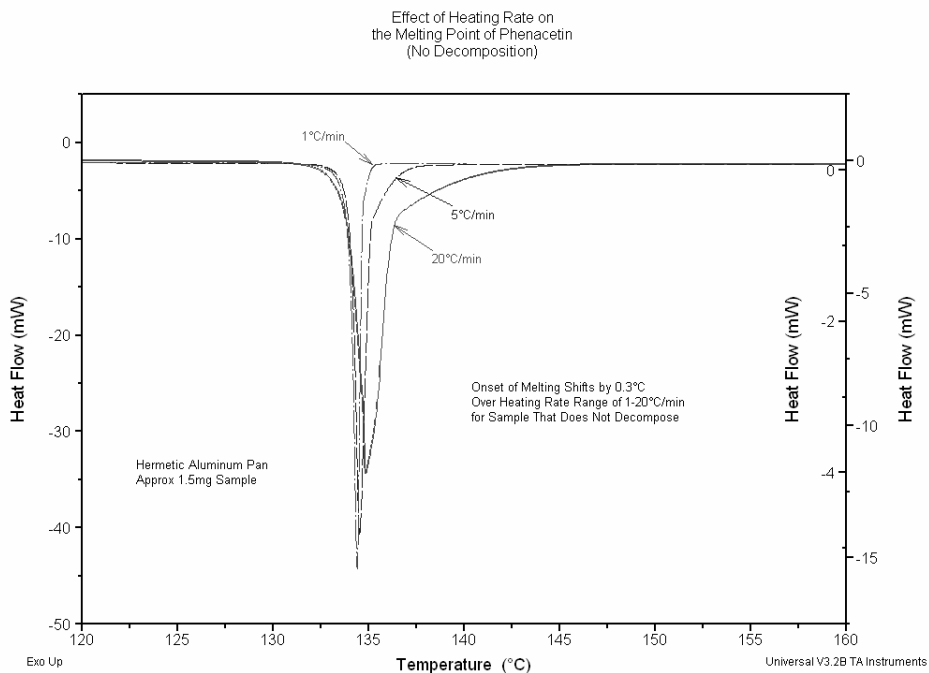


Figure 9.

A very different result is obtained on Ciprofloxacin Hydrochloride at the same three heating rates as seen in Figure 10. The onset of the endotherm shifts by nearly 30°C. This means that the endotherm is really decomposition and not melting. Again, the onset of true melting shifts very little with heating rate when using aluminum sample pans, either crimped or hermetic.

Acetaminophen is an interesting material in that most pharmaceutical grade samples are usually completely crystalline but easily converted to a completely amorphous structure by cooling at rates of 20°C/min or higher from above the melt. In addition, the crystal structure can exist in different forms called polymorphs, which is discussed later in this section. Figure 11 shows the first heat on the as-received sample and the second heat after the sample had been cooled at 20°C/min from 200°C. The first heat shows no glass transition or cold crystallization peak indicating it was highly crystalline. After cooling the sample at 20°C/min from 200°C, the second heat shows a large glass transition and cold crystallization peak indicative of amorphous structure. The melting peak is still very sharp, indicating no decomposition but it has shifted to a lower temperature typical of a less stable polymorph.

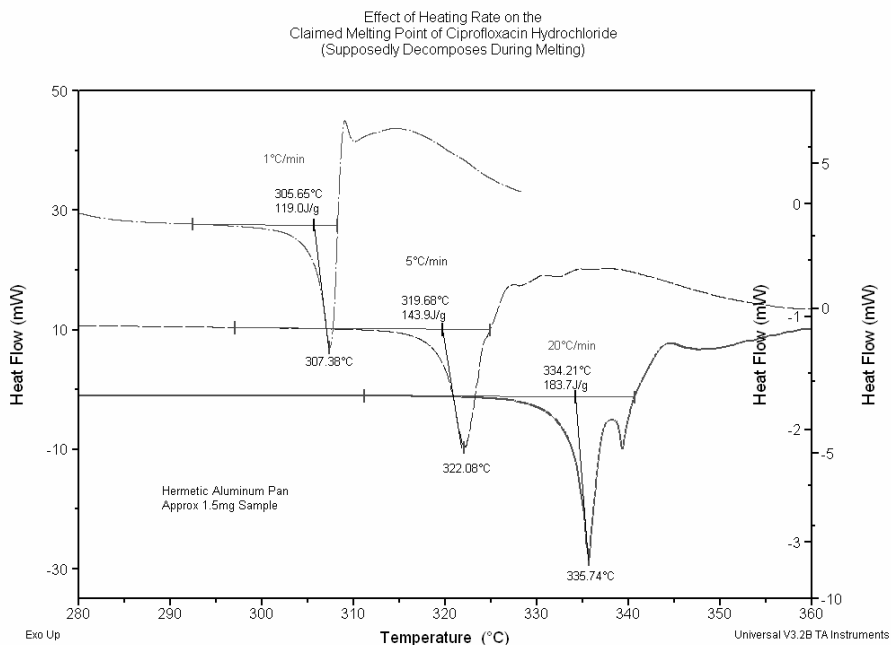


Figure 10.

Melting of Acetaminophen Tablet as Received
and After 20°C/min Cooling from 200°C

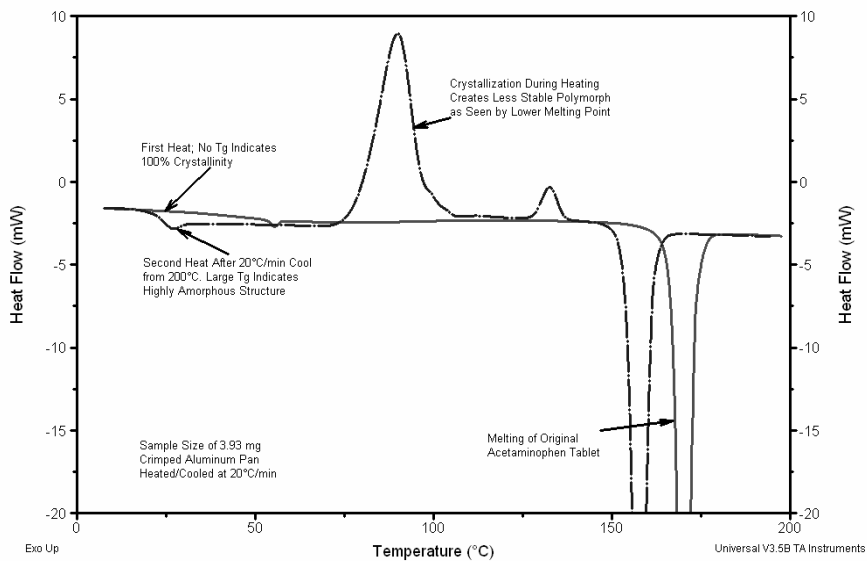


Figure 11.

Most pharmaceutical drugs will not recrystallize in the solid state once they are completely melted. In addition, a high percentage decompose while melting.

- Calorimetric Purity

DSC can be used to measure the absolute purity of some crystalline compounds with very high sensitivity for detecting even small amounts ($\pm 0.01\%$) of impurity. This is due to the melting point depression caused by the impurity which lowers and broadens the temperature range of melting. The effects of 0.7 to 5% mole fraction p-Aminobenzoic Acid on the melting point of Phenacetin are shown in Figure 12. The materials form a eutectic mixture that melts near 113°C and the melting peak of Phenacetin broadens considerably at all concentrations of impurity.

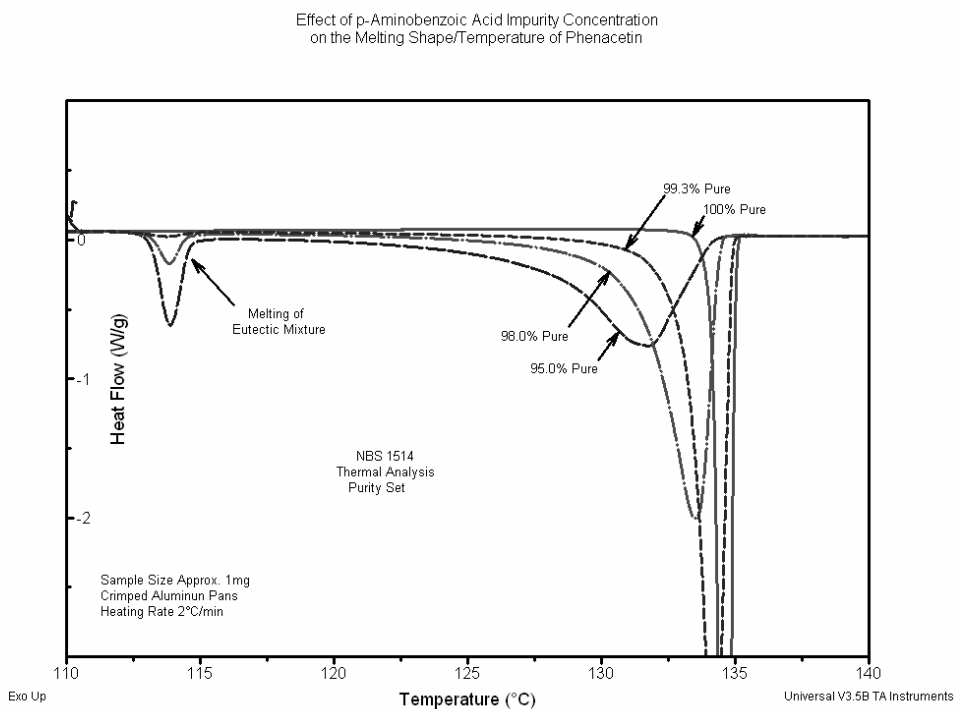


Figure 12.

The calculation of absolute purity is based on the Van't Hoff equation:

$$T_s = T_o - \left(\frac{R X T_o^2}{\Delta H_f} \right) \frac{1}{F}$$

Where: T_s = sample temperature
 T_0 = calculated melting point of 100% pure crystalline sample
 R = gas constant (1.987 cal/mol K)
 X = total mole fraction impurity
 F = fraction melted at T_s

An example of applying the Van't Hoff equation (software program) to a sample of Phenacetin is seen in Figure 13. Based on the Van't Hoff equation, a plot of T_s versus $1/F$ should be a straight line. An iterative process of small corrections is made to linearize the plot and provide the intercept T_0 .

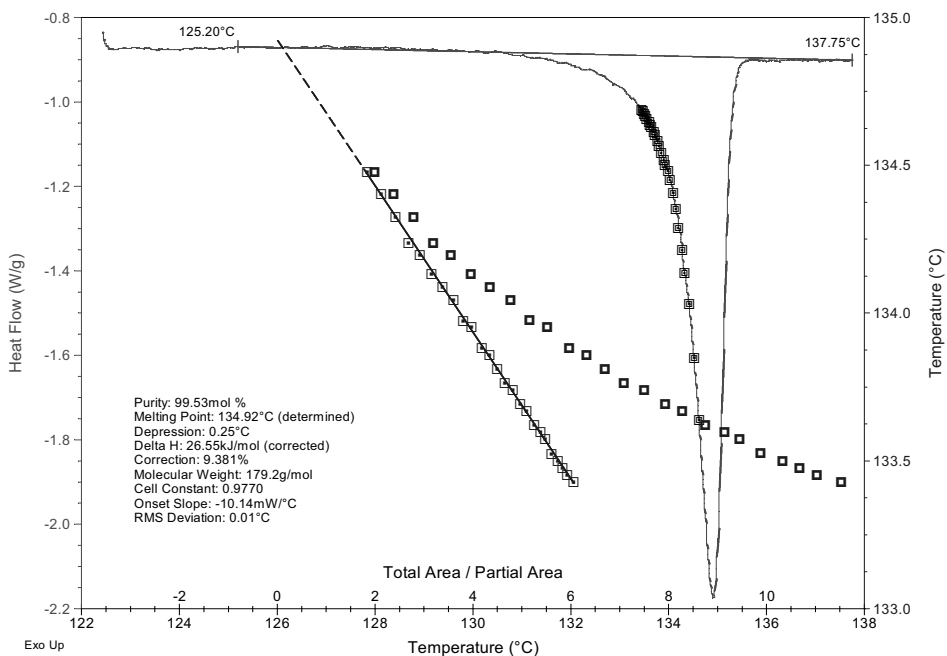


Figure 13.

The DSC purity technique has several advantages including:

- Fast: less than 30 minutes
- Uses small samples: typically 1 mg
- Does not require a 100% pure sample of the material to be analyzed

However, there are limitations that must be considered as well, including:

- Purity should be greater than 98%
 - Sample cannot decompose during melting
 - Impurity cannot form a solid-solid solution; must be insoluble in solid and soluble in melt
 - Does not provide the identity of the impurity
- Polymorphs

Some materials can exist in multiple crystal forms called polymorphs. They have the same chemical structure but a different crystalline structure which can result in significant differences in physical properties such as solubility, bioavailability and storage stability. The most stable form typically has the lowest dissolution rate and may not be the ideal form for a particular application. For all of these reasons, plus others associated with the development and manufacture of efficient and effective drug delivery systems, it is important to know if a specific compound can and does exist in different polymorphic forms.

DSC is the most widely used analytical technique for measuring crystallinity and crystalline polymorphs. However, the results are often misinterpreted by the novice user who fails to realize that the sample may be changing as it is heated. The fact that it can change means that kinetic processes are involved. These include crystallization of amorphous material (as seen during the second heat of Acetaminophen in Figure 11) and conversion of less stable polymorphic forms into more stable forms that melt at a higher temperature. Two techniques that can be used to better understand what is happening to the sample as it is heated are Modulated DSC[®] and multiple heating rate DSC. The benefit of MDSC[®] can be seen in a comparison of Figures 14 and 15 for a polymer microsphere with approximately 30% drug. The standard DSC data in Figure 14 was run at a relatively low heating rate of 5°C/min to optimize resolution of multiple transitions. It is nearly impossible to interpret the data. Figure 15 is MDSC[®] data on the same material. The Reversing Heat Flow signal shows a very clear glass transition at about 30°C and two melting peaks between 125 and 175°C. Since melting happens after the cold crystallization exotherm in the Total signal, the sample was primarily amorphous. Other transitions that complicate the Total signal of DSC include enthalpic recovery at the end of T_g, evaporation of about 2% volatiles (from TGA) and crystallization of the amorphous drug just above 100°C.

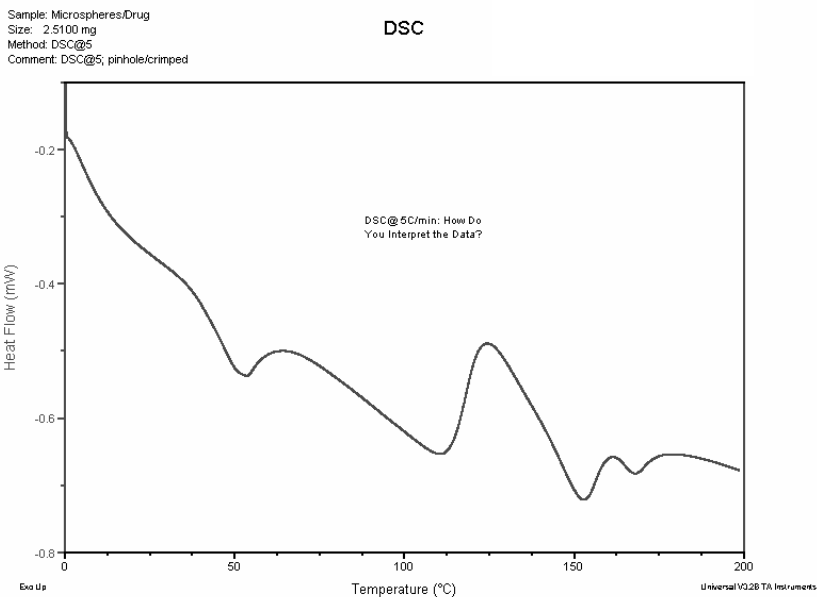


Figure 14.

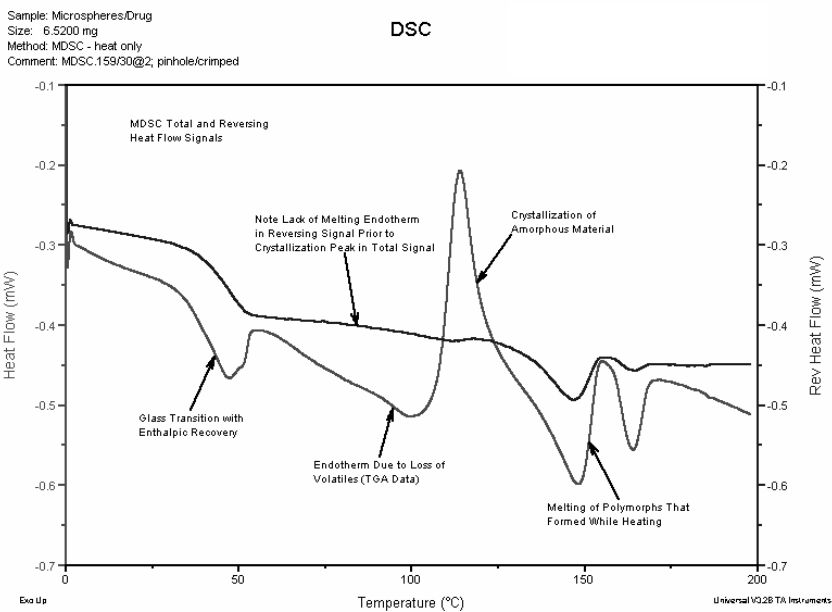


Figure 15.

The benefit of multiple heating rates will be illustrated on several drugs. The first is sulfanilimide which is reported to have three polymorphic forms. These are easily detected as seen in Figure 16 which is a comparison of data created at 1 and 10°C/min. Note that heating rate has very little effect on the number and shape of the melting peaks except for the slight broadening of the large peak at 165°C. This means that each polymorphic form is relatively stable and does not transform from one form to another during the experiment. It is very easy to characterize the relative amount of each form in this kind of sample.

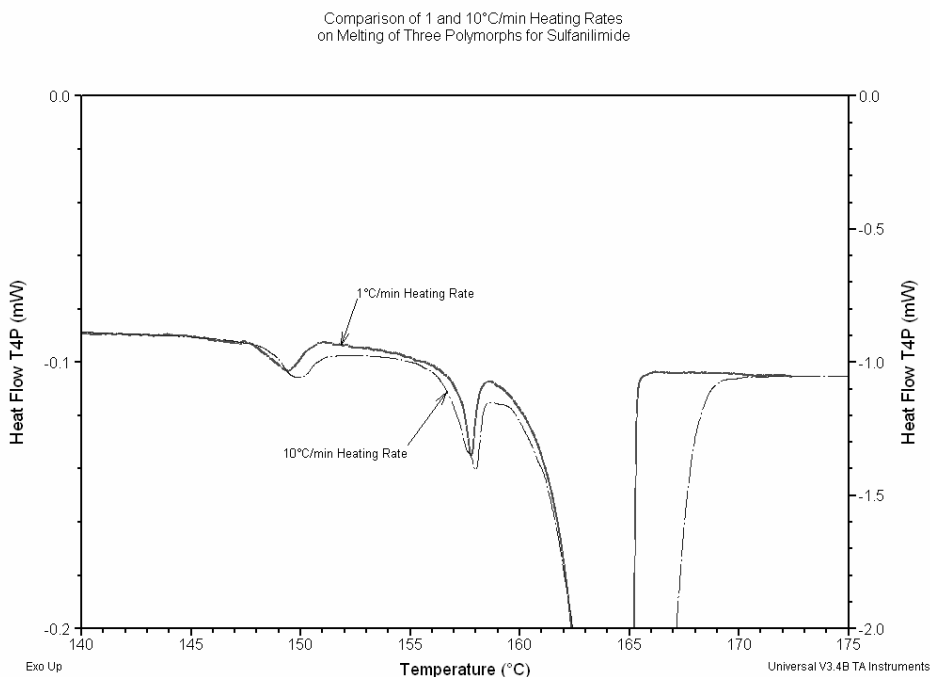


Figure 16.

The second sample to be analyzed with multiple heating rates is a drug monohydrate. The data in Figures 17 and 18 show data at 10 and 1°C/min respectively. Note that both experiments were performed with hermetic (sealed) pans to prevent evaporation of the water (5% by weight from TGA) from the hydrate. The importance of this will be illustrated a little later.

Figure 17 shows the data from the 10°C/min experiment at two sensitivities in order to illustrate some of the finer points. The slight step at the leading edge of the melt is not a glass transition. This was verified by MDSC[®] data which was heated and cooled over this temperature range. In addition, the glass transition of the amorphous

drug is near 50°C. The baseline shift from the beginning to the end of the melt is caused by the higher heat capacity of the liquid phase as compared to the solid phase. Because of this step, the most accurate way to integrate the peak is with a sigmoidal baseline. The most important information from this scan is that there is only a single melting peak (one polymorph).

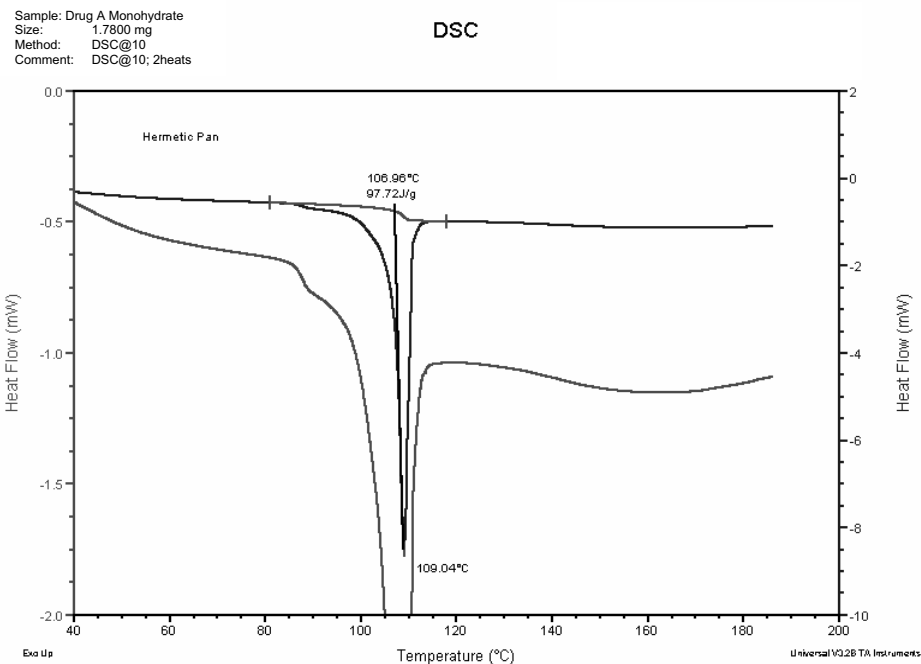


Figure 17.

Figure 18 is the same material run at 1°C/min. The shape of the end of the melt is slightly different, plus there is an additional melting peak near 160°C. At the slower heating rate, a small amount of the material has time to change into another polymorphic form which melts at a higher temperature.

Data shown in Figure 19 appears to be from a totally different sample than the data from Figures 17 and 18; however, it is the same material. The huge difference in the results is simply the result of using an unsealed versus sealed pan. Whereas Figures 17 and 18 were created with sealed hermetic pans, Figure 19 used a hermetic pan with a pinhole in the top. This pinhole allowed water (from the hydrate) to escape from the pan which also caused the conversion of the crystalline material into an amorphous form. The amorphous form crystallized near 120°C and then melted near 174°C. In a sealed pan with water (5%) present, the sample showed very little tendency to crystallize after it had melted.

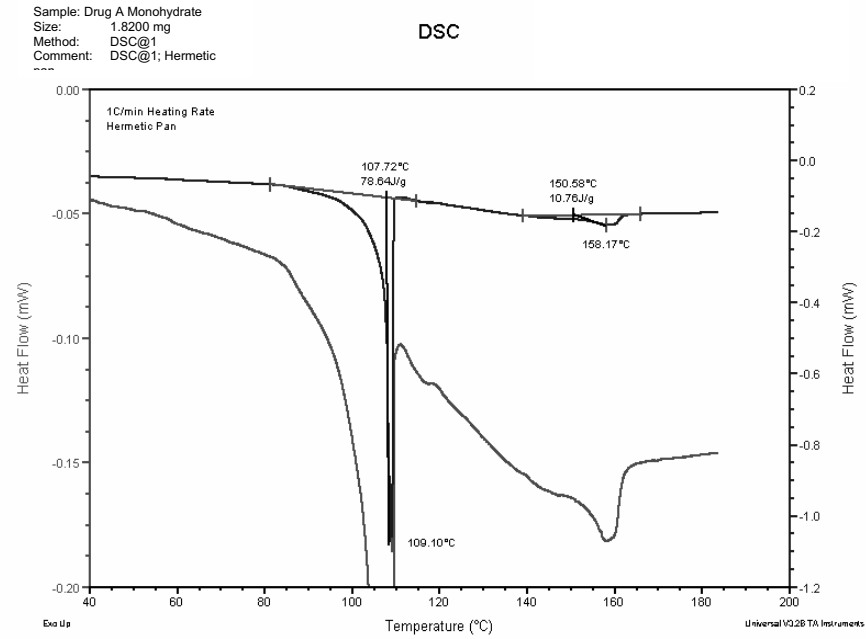


Figure 18.

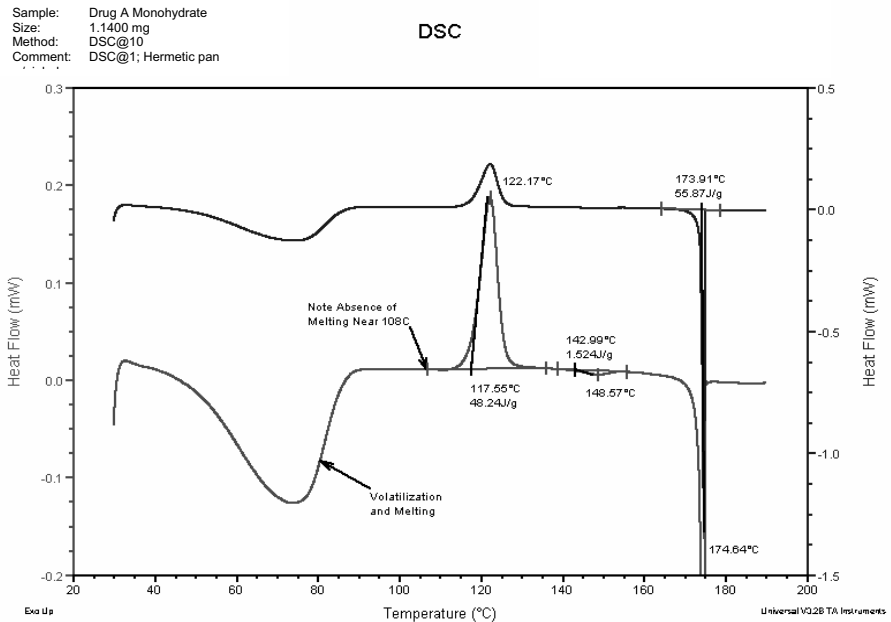


Figure 19.

Figure 20 is a comparison of Figures 19 and 20 which were both run at 1°C/min with the only experimental difference being the sealed versus unsealed pans.

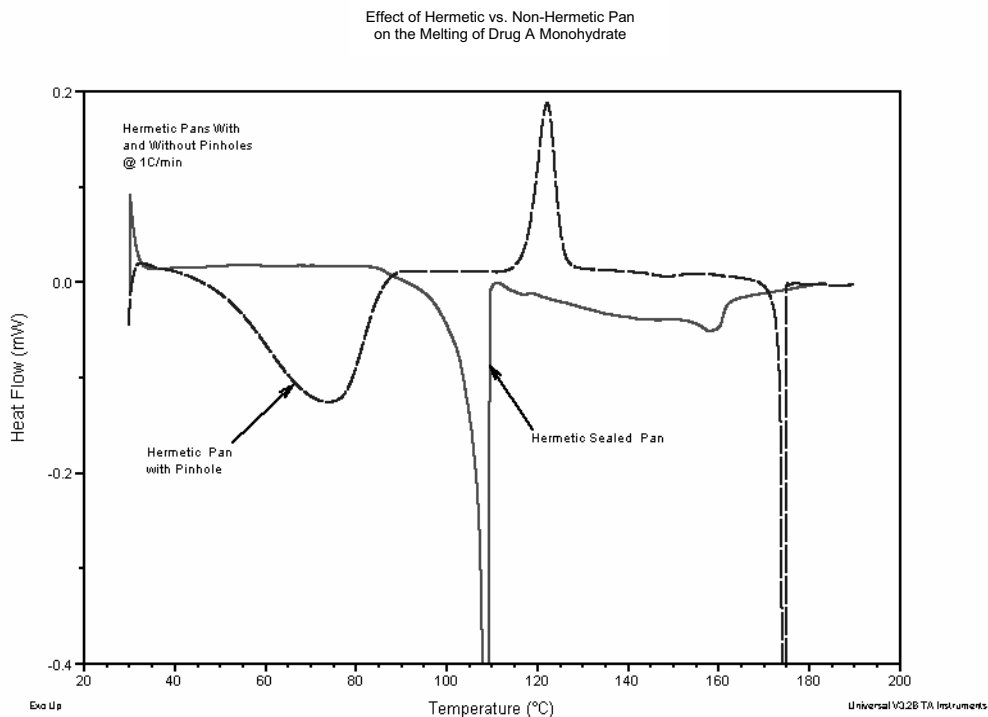


Figure 20.

There is an important point to be learned from this data on the drug monohydrate. The presence of moisture or solvents can have a significant effect on DSC results. Therefore, always run thermogravimetric experiments on new samples to determine the temperature and amount of weight loss. When it exceeds about 0.5%, always compare the effect of sealed versus unsealed pans on the results. Use the type of pan that provides the most meaningful information on the properties of the material.

The third and last polymorphic drug to be characterized at multiple heating rates is anhydrous and contains less than 0.05% volatiles (from TGA). Therefore, it was run in standard crimped aluminum pans which are not sealed. This example best illustrates the value of using multiple heating rates to characterize the ability of the drug to convert from one polymorphic form to another. In general, start with a heating rate of 10°C/min. If only a single melting peak is detected, then there is probably no need to use other conditions. If multiple peaks or shoulders on the major peak are seen, then a lower heating rate experiment should be performed to see if overlapping peaks can be separated or additional peaks form.

Figure 21 shows the data for the anhydrous drug at 10°C/min. There are clearly two peaks and the data might be integrated with a perpendicular drop from the baseline to try to quantify the amount of each polymorph.

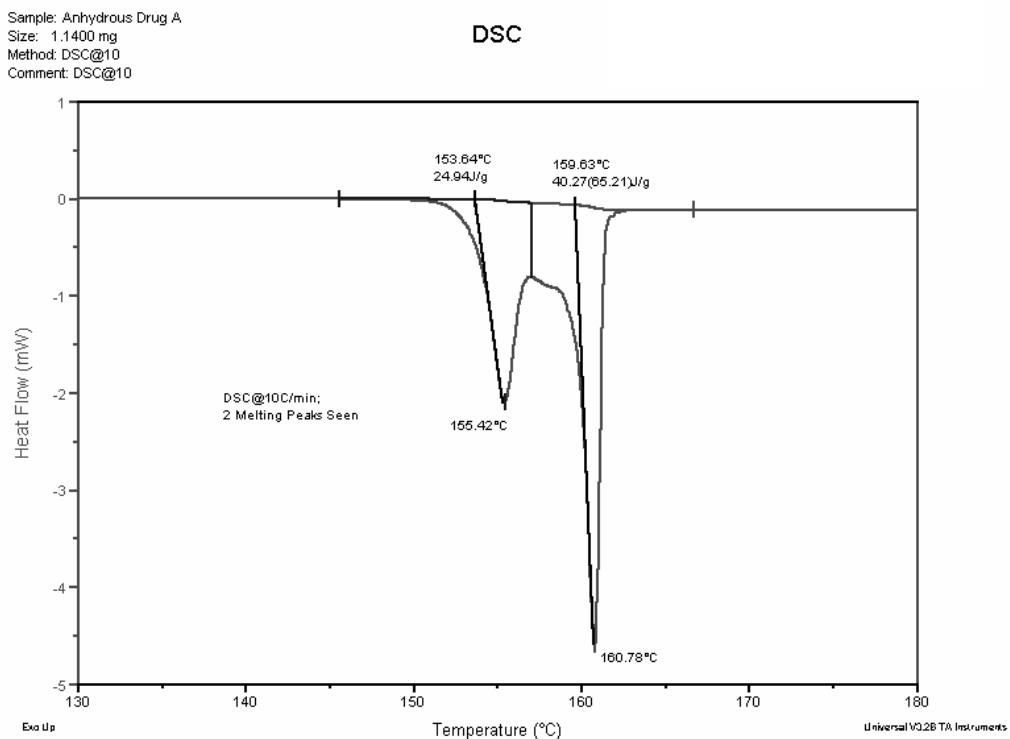


Figure 21.

However, the results would be totally wrong as seen from the data in Figure 22 which is the same material but heated at only 1°C/min. In this case, there is an additional peak at 175°C and a very different ratio of peak sizes for the peaks near 155 and 161°C. In both experiments, the total energy of melting is the same (65 J/g), but it is distributed very differently among the various polymorphic crystal forms. Since this sample changes significantly with lower heating rates, it is necessary to use higher heating rates (50 - 100°C/min) to minimize the time and opportunity of one polymorphic form to convert to another as will be illustrated.

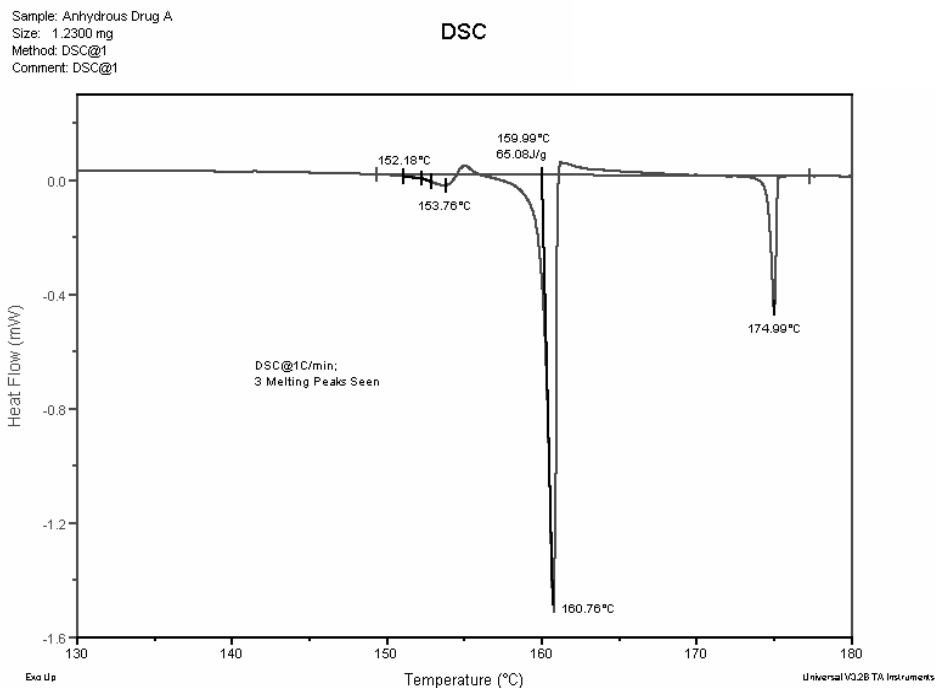


Figure 22.

Figure 23 shows data at 50°C/min heating rate with the results from the 1 and 10°C/min experiments overlaid for comparison. At the high heating rate only a single melting peak is seen which means that there is only one polymorphic form in the original sample. Since it begins to melt near 153°C, as also seen in the 1 and 10°C/min data, the crystal form in the original sample is the lowest temperature (least stable) polymorph.

One concern at higher heating rates is the loss of resolution. A major benefit of the new Tzero DSC™ technology is the higher resolution provided by the T4 and T4P heat flow signals which account for thermal lags that occur due to the sensors and pans. The higher resolution results often make the difference between seeing or not seeing a small amount of one polymorph in a mixture of other polymorphs. Figure 24 is a comparison of the T4P signal of a Tzero DSC™ with the traditional one-term (T₁) signal of conventional DSC. The polymorph detected at 170°C was verified to be real by hot-stage microscopy results. The lower resolution signal of conventional DSC was not able to detect it at the high heating rate of 50°C/min while lower heating rates could not be used due to the sample changing during the experiment.

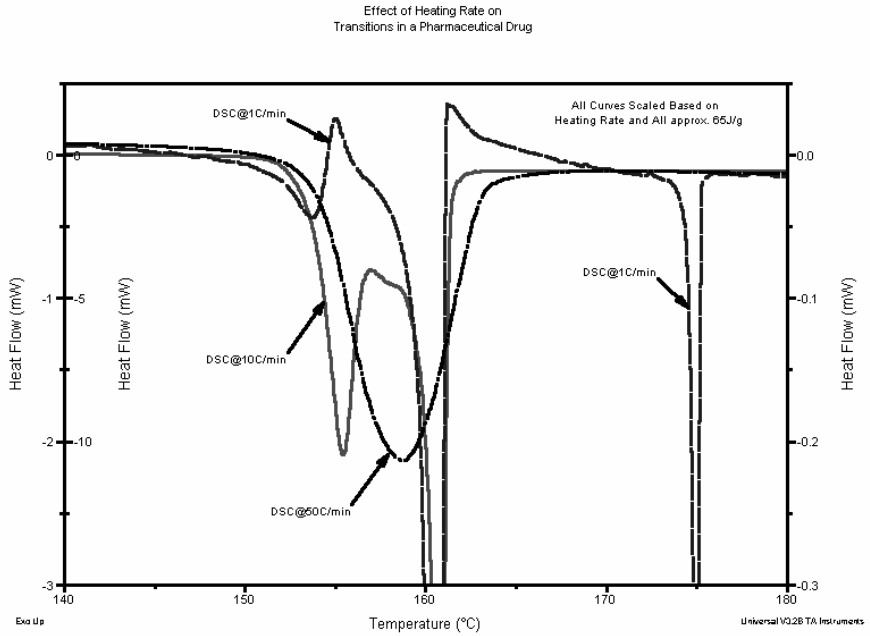


Figure 23.

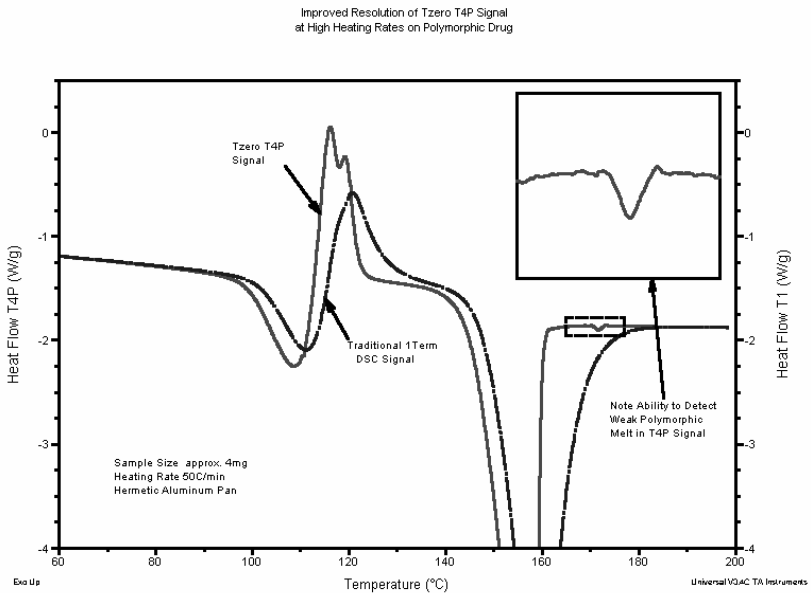


Figure 24.

6. Drug Delivery System Using Polymer Microspheres

Until now, we have focused on DSC analysis of individual amorphous and/or crystalline drugs. We will now apply what we have learned from those experiments to a much more complex sample consisting of amorphous and hydrated crystalline drug dispersed in biodegradable polymer microspheres of 50 – 200 microns.

TGA results (Figure 25) show the sample loses just over 2% weight by 150°C. Therefore, it is necessary to use hermetic pans for the DSC experiments. The next step in determining the crystalline content of the drug in the microspheres is to run at different heating rates to see if the sample undergoes polymorphic transformation at low heating rates.

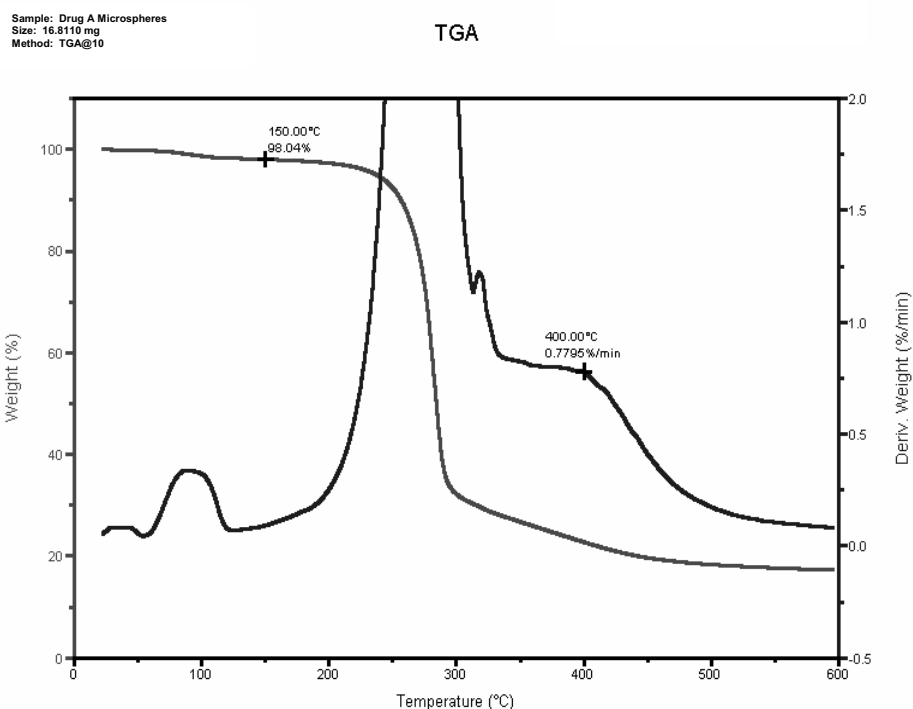


Figure 25.

Figure 26 is a comparison of the Total Heat Capacity signals from experiments run at 1, 10 and 50°C/min. Results show that only at 50°C/min is a single melting peak obtained. This means that the sample must be run at 50°C/min in order to measure the original crystalline form of the drug instead of the other polymorphs that form at slower heating rates.

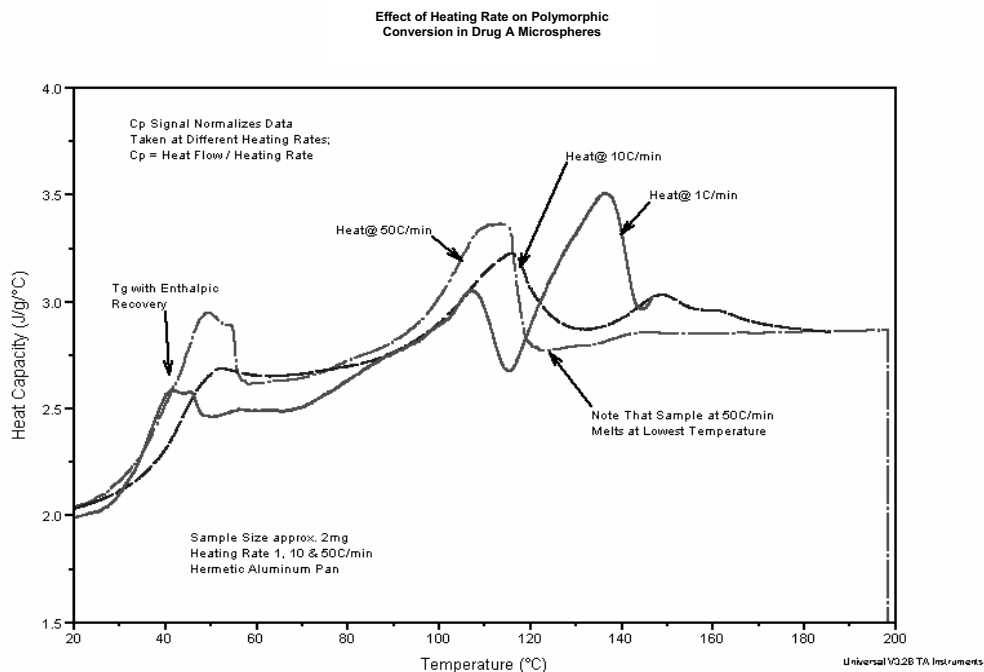


Figure 26.

At 50°C/min in Figure 27, the first heat shows a total heat of fusion of 12.58 J/g with 97% of that coming from the peak near 115°C. Since the pure drug has a heat of fusion of about 98 J/g, this means that the microspheres contain about 13% (12.58/98) crystalline drug. To confirm reproducibility of the measurement at 50°C/min heating rate, the sample was run in triplicate with excellent results shown in Figure 28. In addition to crystalline drug, the microspheres contain an even higher concentration of amorphous drug. However, all of the data shown in Figures 27 and 28 show only a single glass transition near 35°C. This means that the amorphous drug and the amorphous polymer of the microspheres are completely miscible and it is not possible to measure the amount of amorphous drug in the sample by DSC; another approach such as TGA is needed.

Comparison of Data From Three Experiments on Drug A Monohydrate Microspheres

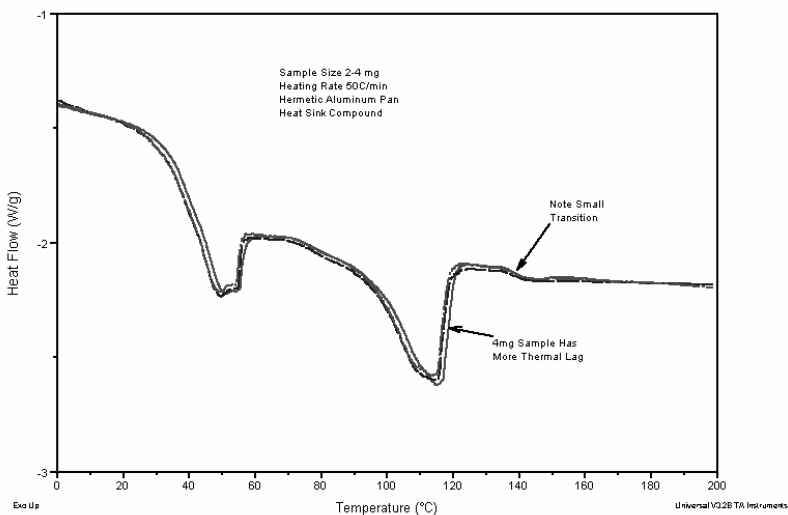


Figure 27.

Sample: Drug A Monohydrate Microspheres
 Size: 4.3900 mg
 Method: DSC@50

DSC

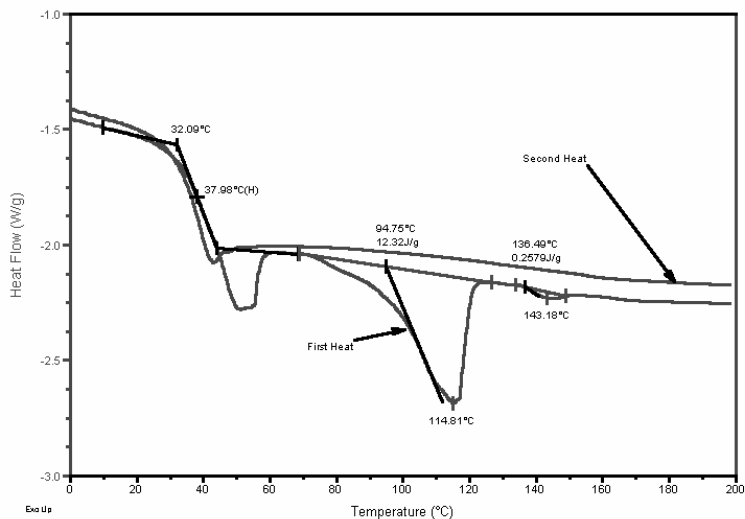


Figure 28.

TGA data of placebo microspheres is shown in Figure 29. It shows that the polymer microspheres (no drug) are essentially fully decomposed by 400°C where the rate of weight loss is 0.04%/min. This is in contrast to the microspheres with the drug (Figure 25) which are still losing weight at a relatively high rate (0.78%/min) due to the ongoing decomposition of the drug. Since all of the drug is amorphous by 400°C, it is possible to calculate the total drug loading from a ratio of the rates of weight loss if the rate of weight loss for a 100% drug sample is known. Although not shown, a pure drug sample showed a rate of weight loss of 2.24%/min at 400°C. Therefore:

$$\% \text{ Total Drug} = \frac{0.78 - 0.04}{2.24} = 30\%$$

The target-loading was 32% and so there is good agreement. Since the sample was known to have 13% crystalline drug from the DSC data, it must have had 17% amorphous drug as well. Since the purpose of the microspheres is to provide a controlled rate of drug release into the body and since amorphous and crystalline drugs have different dissolution rates, it is not surprising that the microspheres were formulated with both amorphous and crystalline drug.

Sample: Placebo Microspheres
Size: 25.1520 mg
Method: R10

TGA

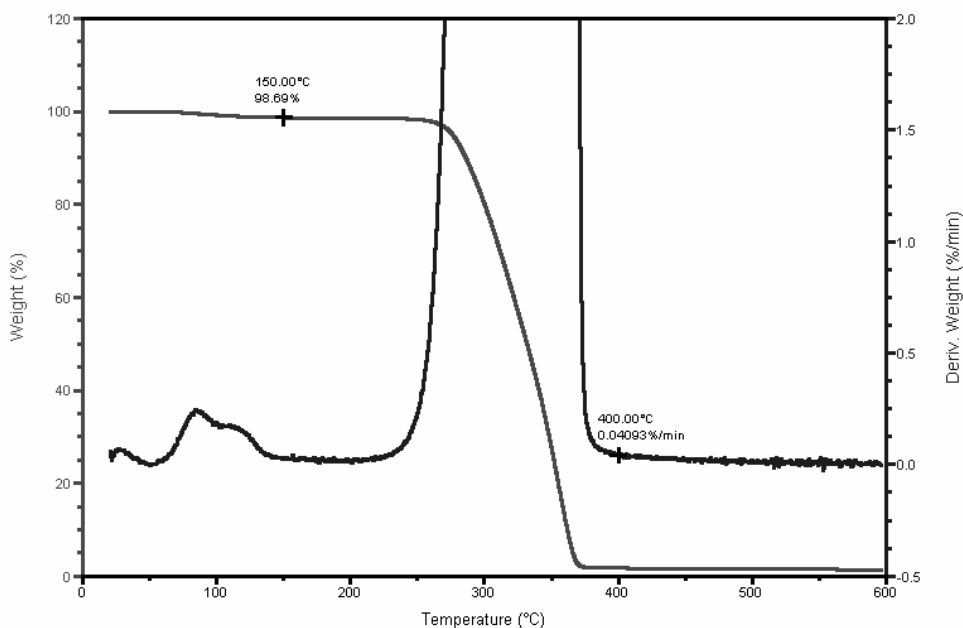


Figure 29.

7. Drug-Excipient Interaction

Actual drug dosage forms are seldom just the pure drug or protein. Instead, they are usually composed of multiple ingredients that aid in the manufacture, storage or delivery of the active ingredient. Because the dosage form must be stored over a period of time at some temperature and relative humidity, there is a need to confirm that the efficacy of a drug formulation will not change with time. Two ways used by pharmaceutical companies to improve storage stability are to keep the sample below the glass transition temperature of any ingredient (minimizes molecular mobility and, therefore, possible chemical interaction) and to use crystalline drugs that are more thermally stable than amorphous drugs.

It is usually the responsibility of the drug formulations group to determine if the drug will interact with any of the other ingredients (excipients) in the final formulation. This is often a very tedious task where multiple samples must be stored under different conditions for long periods of time and tested regularly with a variety of analytical techniques.

DSC has proven to be an excellent tool for detecting drug-excipient interaction. A “finger print” of the fresh formulation is made and then compared to aged samples to look for differences in the transitions of any ingredient especially the active compound. For crystalline drugs that melt without decomposition, this is a relatively easy measurement because the peak area for the melt is a quantitative measure of the crystalline drug. For amorphous materials that are often miscible with other amorphous excipients or for crystalline drugs that decompose instead of melt, the measurement of drug-excipient interaction by DSC is often much more difficult.

Just as with many of the previous examples, always start the analysis of new materials with TGA. This will save a lot of time in the end and help provide the correct interpretation of many transitions. Figure 30 shows TGA results on a Cold/Allergy tablet that used a crystalline drug as the active ingredient. Analysis of the weight and derivative curves show the sample contains more than 1% volatiles and starts to slowly decompose above 100°C. This means that the DSC experiment needs to be performed in hermetic pans in order to avoid a large endothermic evaporation peak that could hide other weak transitions.

Sample: Cold/Allergy Tablet
 Size: 18.4890 mg
 Method: Heat@10
 Comment: N2 Purge=40/60mL/min.: TGA@10

TGA

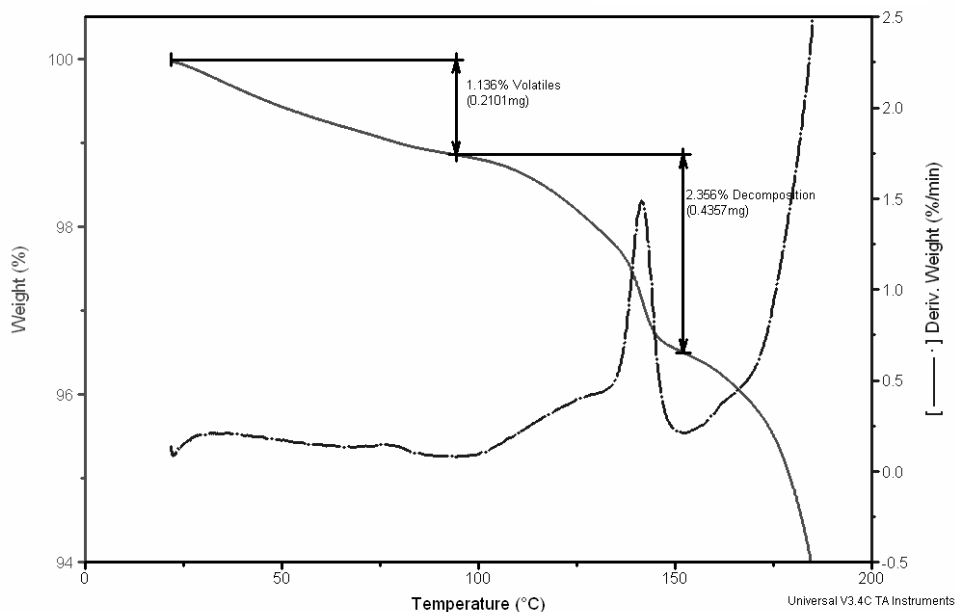


Figure 30.

The DSC data for this material is shown in Figure 31 which is a comparison of three separate heating experiments at 10°C/min on the same sample. The first heat in the sealed hermetic pan does not show any transitions until just below 100°C where the sample is known to decompose from the TGA data of Figure 30. The endotherm between 100 and 150°C could easily be misinterpreted as a melt if it were not for the TGA data. After the first heat to 175°C, the sample was cooled and heated a second time in the sealed hermetic pan (volatiles not lost). This run shows only a glass transition just above 0°C. The drug was crystalline to begin with (no T_g) but has converted to an amorphous form as the result of the decomposition that occurred starting at 100°C on the first heat. When evaluating storage stability of this formulation, the researcher should look for the formation of a glass transition over time. One question that still needs to be answered is the actual temperature of the glass transition.

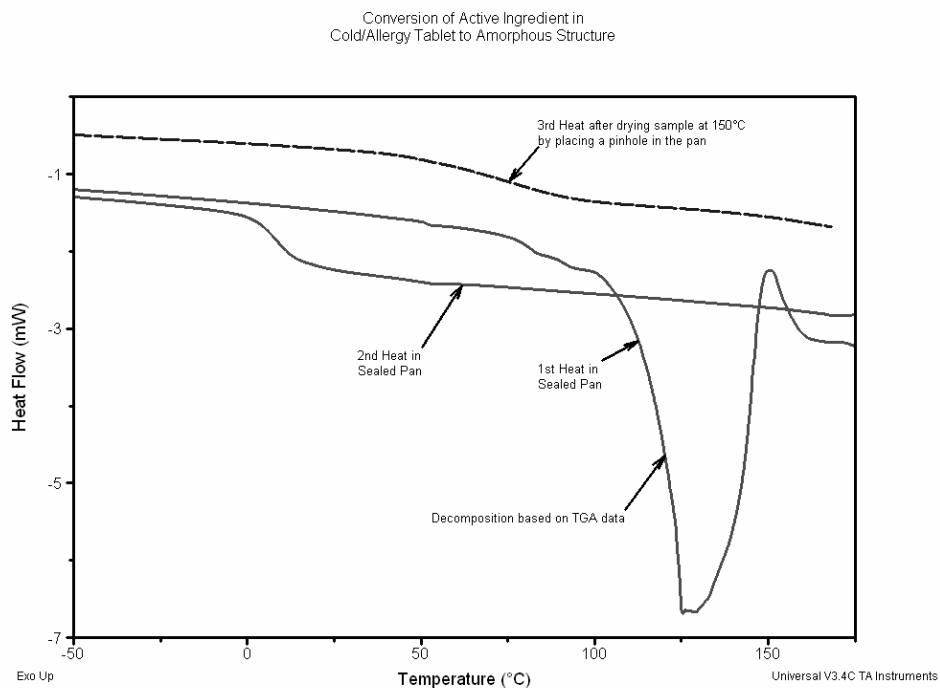


Figure 31.

As discussed earlier, the temperature of a glass transition decreases with increasing amounts of moisture or solvents. The TGA results show that the sample probably contains slightly more than one percent water. To determine the maximum glass transition of a dry sample, a pinhole was placed in the lid of the hermetic pan at the end of the second run and the sample dried at 150°C in the DSC cell for 30 minutes. After drying, the sample was heated a third time and the glass transition is seen starting at 50°C and ending near 100°C. Therefore, the analytical chemist that is evaluating the storage stability of the formulation would look for the development of a glass transition between 0 and 100°C as the sample ages. The wide temperature range is due to possible variations in moisture from batch to batch.

8. Protein Denaturation

Protein denaturation is a general term used to describe a change in structure of a protein. This change usually results in a nonreversible unfolding of the protein that affects its shape and, therefore, its biological activity. There are a variety of techniques used to measure protein denaturation including changes in physical properties (solubility) and changes in reactivity such as with enzymatic proteins.

With DSC, protein denaturation is the measurement of the thermal stability of a protein in solution. A low-energy endothermic peak is observed over a temperature range. The temperature of the peak provides information about thermal stability and the area of the peak is a quantitative measure of the energy absorbed by the protein in order to change structure. Experimental conditions such as heating rate, pH, ionic strength (salt concentration) and even protein concentration can affect results.

In order to minimize the opportunity for aggregation of the protein, most measurements are performed at relatively low concentration (1% or 10mg/ml of solution). DSC data for the denaturation of albumin from chicken eggs is shown in Figure 32 which is a comparison of 1 and 10% concentrations. At 1% concentration, a peak of about $40\mu\text{W}$ in height and 0.21 J/g in area is easily detected. The sharpness of the minor detail associated with peak is, however, less clear because of low signal strength. At 10% concentration, the peak is about $400\mu\text{W}$ in size and there is excellent detail showing minor shoulders on both the low and high temperature sides of the peak. The fact that peak temperatures differ by only 0.02°C provides confidence that minimal aggregation occurs at 10% concentration.

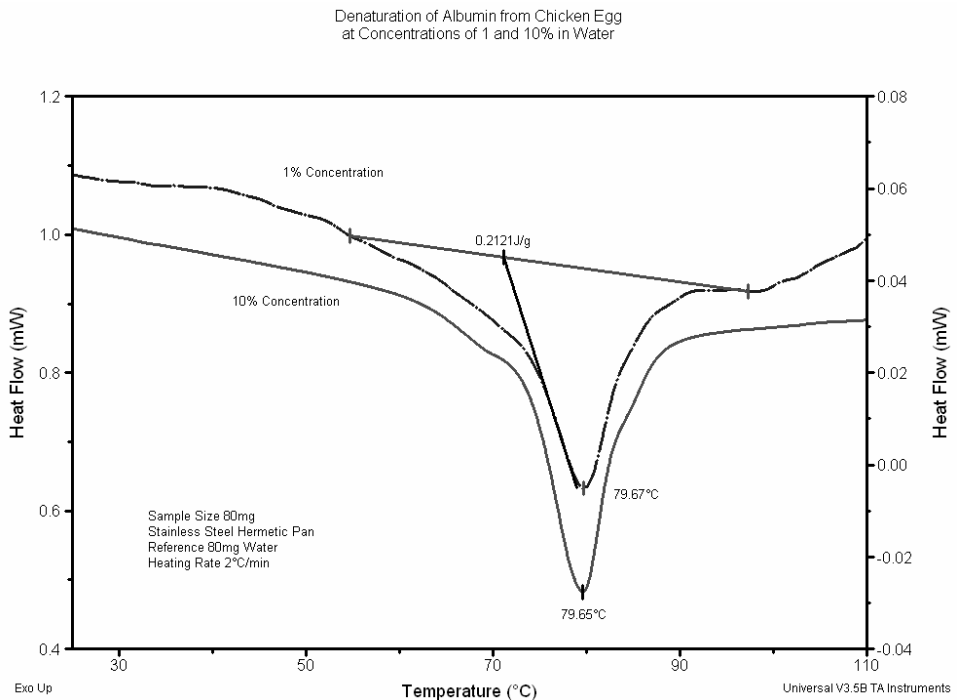


Figure 32.

There is great flexibility in analyzing data. In Figure 33, the 10% concentration data is shown with a sigmoidal baseline and the percent denaturation is plotted as a function of temperature. A plot of time during the experiment shows that the entire

experiment took only 60 minutes which is much faster than typically obtained with microcalorimeters or solution calorimeters.

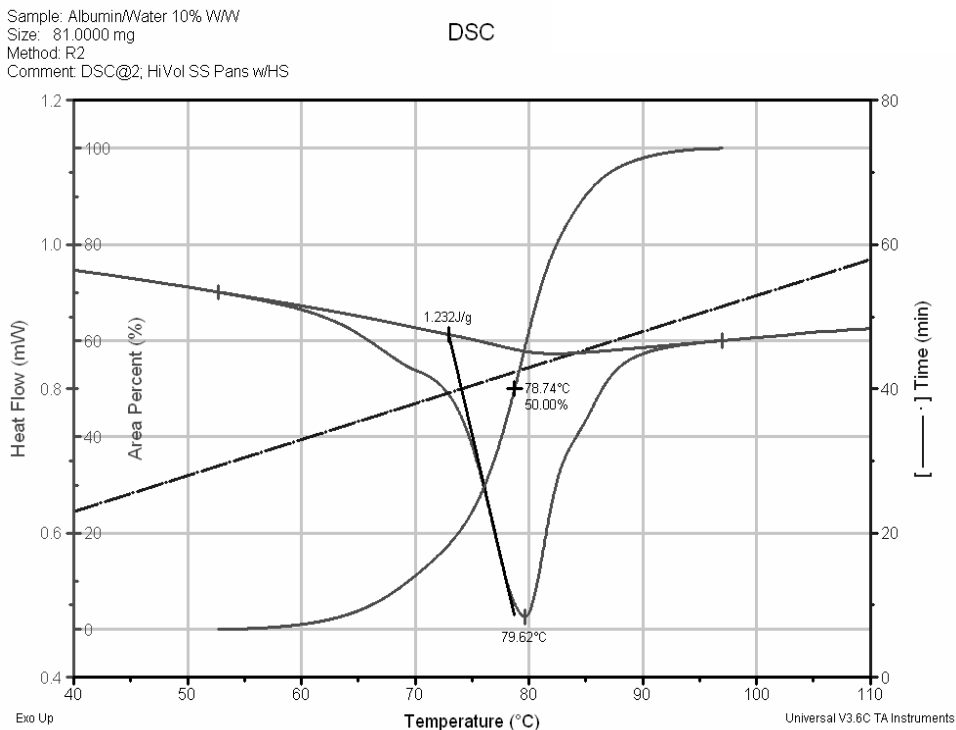


Figure 33.

9. Freeze-Drying

Freeze-drying, or lyophilization, has become a standard process in the pharmaceutical industry for the manufacture of biologically active substances. However, it is not without limitations due to its high cost in capital and energy, long processing time and difficulty in selecting manufacturing conditions of time, temperature, vacuum and component concentration. All of these parameters must be optimized in order to achieve a final product with the desirable characteristics of:

- Full activity of the protein or drug
- Easy reconstruction
- Acceptable appearance of freeze-dried cake
- Good storage stability

The process of freeze-drying relies on the vapor pressure of ice. Even at temperatures as low as -50° , ice sublimates and leaves a very porous, low density cake containing the stabilized drug. Since the sublimation rate (drying rate) is very temperature dependent, use of the highest possible temperature during primary drying provides maximum drying efficiency and lowest process cost.

In order to select the optimum drying temperature, it is necessary to understand the physical characteristics of the components used in the formulation that is to be freeze-dried. In decreasing order of mass, these are typically water, bulking agents, buffers or stabilizers and finally the drug itself. The bulking agent, which can be either crystalline or amorphous, and its interaction with frozen and unfrozen water in the frozen solution, define the physical structure which is essential to successful freeze-drying. This structure manifests itself in the form of transitions that occur at specific temperatures. Physical properties of the bulking agent, such as modulus or viscosity, can change by orders of magnitude depending on whether the process temperature is a few degrees above or below the transition temperature. Therefore, knowledge of this structure and how it changes with time and temperature is required for successful drying.

DSC has been used with only modest success in the characterization of frozen solutions used for freeze-drying. The reason is that there are numerous components and transitions happening within a narrow temperature range and DSC can only measure the sum of these. In addition, DSC must use relatively high heating rates ($10 - 20^{\circ}\text{C}$) in order to optimize sensitivity while the best resolution is obtained at low heating rates of 0.5 to $1^{\circ}\text{C}/\text{min}$.

In the introduction section on new DSC technologies, it was explained how Modulated DSC[®] has both an average and modulated heating rate. The combination of two heating rates allows the operator to select a slow average heating rate in order to obtain good resolution and a higher modulated heating rate to obtain increased sensitivity during the same experiment. In addition, the resulting modulated heat flow can be separated into the heat capacity and kinetic components of the total heat flow in order to improve ease of data interpretation.

Figure 34 shows the raw modulated heating rate and modulated heat flow signals from an MDSC[®] experiment performed at an average heating rate of $0.5^{\circ}\text{C}/\text{min}$ on a frozen solution of 40% sucrose in water. The change in heating rate (modulated) is approximately $3^{\circ}\text{C}/\text{min}$ which causes the modulation in the heat flow signal.

Sample: 40% Sucros/Water WW
 Size: 15.8700 mg
 Method: MDSC 5/120@0.5
 Comment: MDSC 5/120@0.5°C/min; Quenched in LN2; Al Hermetic Pans

DSC

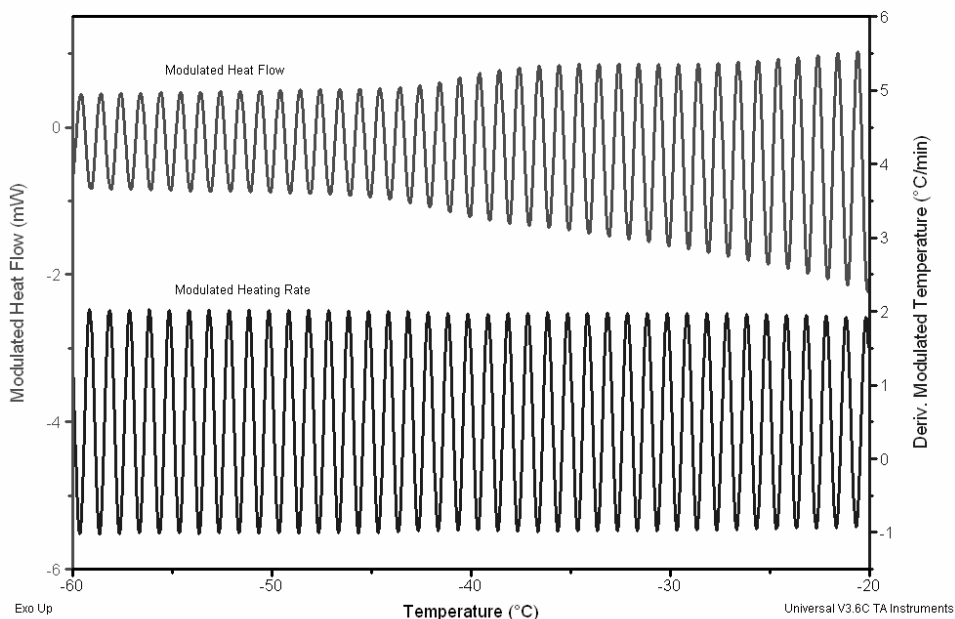


Figure 34.

Figure 35 shows the calculated signals from this MDSC[®] experiment. The Total signal is very difficult to interpret because it is equivalent to standard DSC at the same heating rate and contains two different transitions. The first of these is the important glass transition seen in the Reversing signal between -43.6 and -39.4°C . The second transition is an exotherm in the Nonreversing signal caused by crystallization of free water that could not crystallize during the quench cooling of the sample. This peak shows a maximum at about -36°C and a heat of crystallization of 5.7 J/g . It is not surprising that unfrozen water would begin to crystallize near -42°C since this is near the onset of the glass transition where a significant increase in molecular mobility and diffusion can occur.

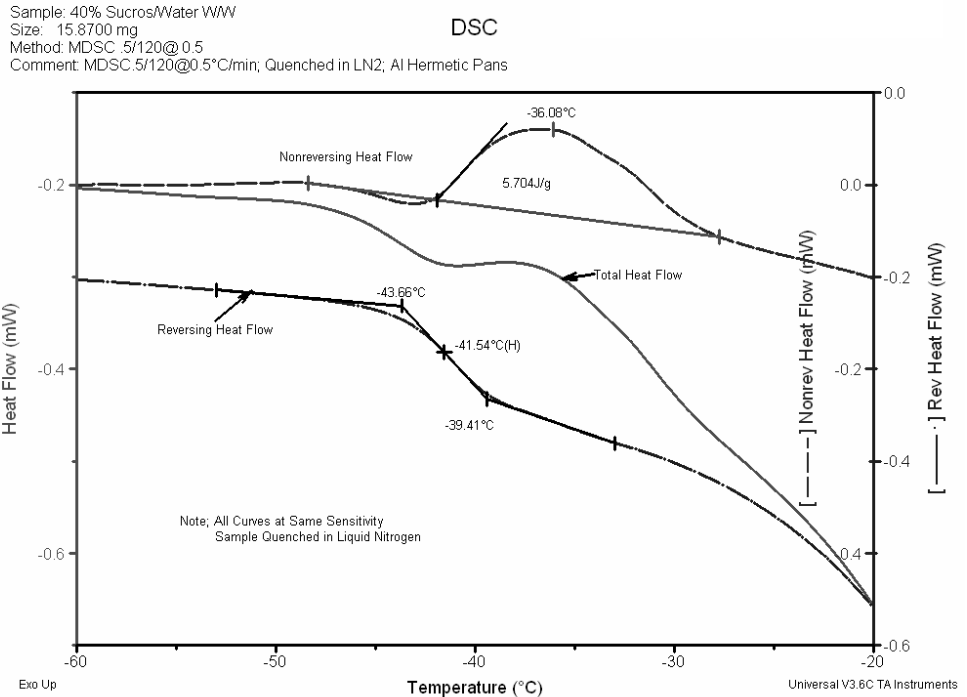


Figure 35.

Figure 36 shows MDSC[®] data obtained on the same sample as above except that it was cooled at 0.5°C/min as compared to the quench cooling that was used in the previous run. The cooling and heating data are plotted in heat capacity units so that they can be visually compared. Notice that the slow cooling produces a more complex structure which has two step changes in heat capacity. The derivative signal more clearly shows the double transition and shows that there are very minor temperature lags between heating and cooling with the Tzero DSC[™] technology. Even at the slow average heating rate of 0.5°C/min, MDSC[®] provides extremely high sensitivity for characterizing the complex structure of frozen solutions used for freeze-drying.

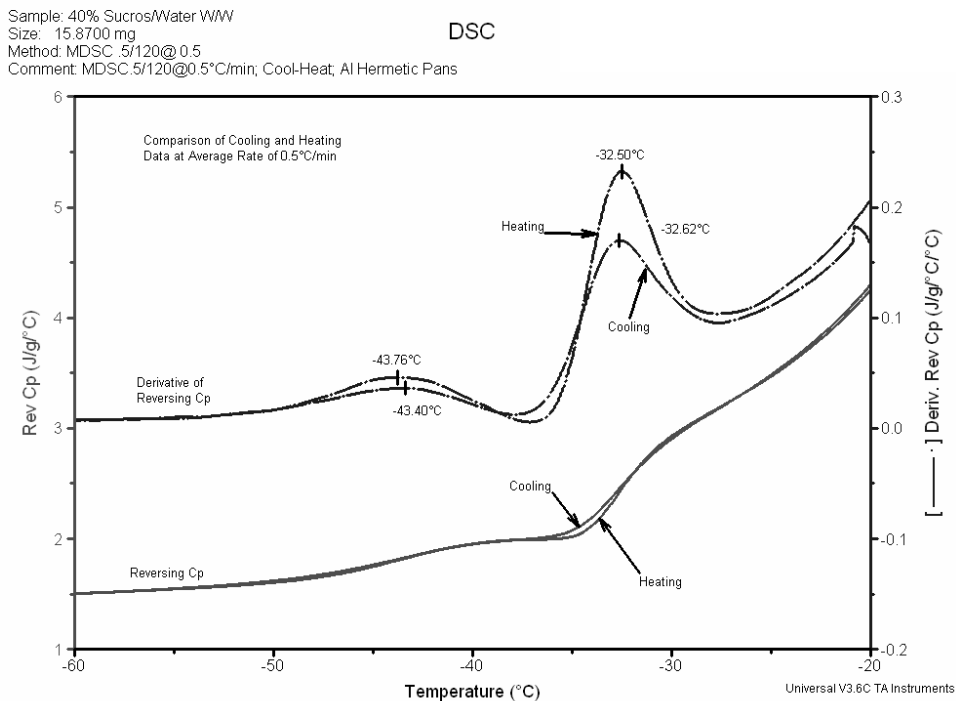


Figure 50.

10. Miscellaneous

This paper has focused primarily on use of DSC and MDSC[®] for characterizing pharmaceutical materials. However, no analytical laboratory would be complete without several other thermal analysis instruments that provide complementary information to DSC and MDSC[®]. These include:

Thermogravimetric Analysis (TGA): Weight Changes

- Moisture content
- Solvate/hydrate content
- Decomposition analysis
 - Can be combined with FTIR and GCMS

Thermomechanical Analysis (TMA): Dimensional Changes

- Coefficient of thermal expansion
- Dimensional stability of fibers and films

Dynamic Mechanical Analysis (DMA): Viscoelastic Properties of Solids

- Modulus of coatings and packaging materials
- Branching of polymers

Rheology: Viscoelastic Properties of Fluids

- Application of topical ointments
- Stability of suspensions and dispersions
- Viscosity of fluids