Using Differential Scanning Calorimetry (DSC) for Optimized Lyophilization Cycle Design

J. Jeff Schwegman, Ph.D. AB BioTechnologies LLC, 1116 Azalea Lane, Bloomington, IN 47401. 812-327-6898

Introduction

Unlike the early days of lyophilization in which the process was considered more of an art, modern freeze-drying involves a much more scientific approach to developing and optimizing lyophilization cycles. Running an optimized process is of great interest to companies that produce freeze-dried products because in a manufacturing process, freeze-drying is the most expensive unit operation. By understanding the physio-thermal characteristics of the formulation and designing the cycle accordingly around these properties, the development scientist can ensure that the cycle will dry the product in the shortest time possible while still maintaining the desired quality and cosmetic attributes. The key pieces of information that are needed to obtain a complete physio-thermal profile include the physical state of the materials in the frozen state (crystalline, amorphous, metastable, mixed), and the critical temperatures associated with those different phases (glass transition temperature, eutectic melting temperature, collapse temperature). Differential Scanning Calorimetry or DSC, is a technique that is well suited to determine both of these key pieces of information, and should be the first step in designing an optimized lyophilization cycle.

DSC Principles

Materials that are exposed to changing temperatures will undergo physical or chemical changes to their structure. An example of this is water which will form crystals at low temperatures, liquid at ambient temperatures, and steam at higher temperatures. As a material goes through these physical or chemical changes as a function of temperature, they will either release a small amount of energy to the surrounding environment (exothermic), or absorb some heat from the surrounding environment (endothermic). This small amount of heat that is either given off or absorbed by the system is the basis for DSC.

The DSC instrument is composed of a sample chamber and reference chamber or sample and reference furnaces. Unlike differential thermal analysis (DTA) which uses a common heating and cooling source, DSC incorporates a separate heating source for both the sample and reference cells. By having separate furnaces for the sample and the reference material, the DSC is capable of calculating the heat flow to the sample material. In simple terms, the instrument wants to keep the sample and reference material at the same temperature during the heating and cooling cycle. It does this by closely monitoring and controlling the temperature of the sample and the reference. When the sample goes through a physical or chemical change in response to a change in temperature, it will give off or absorb a small amount of heat from the environment. The instrument senses this and applies or removes heat to keep the sample at the same temperature as the reference. Since the instrument is measuring heat flow instead of just the temperature difference between the sample and reference, it is now possible to be able to calculate some of the thermodynamic values for the transition. As shown in Figure 1, several simple equations can be used to demonstrate how the heat flow is calculated, and how it can be applied to calculate the heat capacity for the transition. The heat capacity can then be used to calculate other thermodynamic values including the enthalpy and entropy of the transition.

$$\frac{\text{heat}}{\text{time}} = \frac{q}{t} = \text{heat flow}$$

$$\frac{\text{temperature increase}}{\text{time}} = \frac{\Delta T}{t} = \text{heating rate}$$

$$\frac{\frac{q}{t}}{\frac{\Delta T}{t}} = \frac{q}{\Delta T} = C_p = \text{heat capacity}$$

Figure 1

Formulation Considerations and Calibration

Establishing a complete thermal profile for a solution that is to be freeze-dried is crucial for developing an optimized lyophilization cycle that is tailored specifically for that particular product. All of the different components added (active and excipients) will impart different thermal characteristics to that formulation, making it unique in regards to its thermal properties. For this specific reason, it is imperative to characterize each new formulation. A complete thermal profile will include analysis by DSC and freeze-dry microscopy. Freeze-dry microscopy is beyond the scope of this paper, and will be addressed as a separate topic; this paper will focus solely on thermal characterization by DSC.

The first step in a DSC thermal analysis study is to specify the formulation to be freeze-dried. As mentioned in the above paragraph, all of the components in the formulation can contribute to the overall thermal character of the product, so it is important to have the formulation development phase complete and have a final formulation identified. Any changes to the formation either by changing the active or excipients added, or even changing the ratios of these components will require the thermal analysis to be redone.

The next step in conducting a good thermal analysis study is to ensure that the instrument will report accurate results. This is accomplished through the use of calibration standards. When conducting low temperature DSC studies on frozen samples, the typical temperature range of interest is from approximately -40°C to 0°C. As such, calibration standards should be chosen accordingly to encompass this temperature region. Mercury and gallium have been used successfully as calibration standards for this temperature region, and have melting temperatures of -38.8°C and 29.8°C, respectively[1]. Both of these elements can be obtained cheaply from most chemical supply companies. A simple study using sodium chloride can be conducted to verify the accuracy of the calibration. It is known that sodium chloride forms a eutectic with ice at a concentration of 23%. The melting point of this eutectic occurs with an onset of melt occurring at approximately -21.5°C. A successful calibration should result in the sodium chloride sample having an experimental onset of melt temperature that is within ±1°C of theoretical.

Interpreting a DSC Thermogram

The DSC thermal analysis experiment will give two key pieces of information that will be used for lyophilization cycle development. The first key piece of information gained from these experiments is the physical structure of the solids formed during freezing. Specifically, we are interested in determining if our system is forming an amorphous glassy phase, a crystalline eutectic phase, a metastable glassy phase, or is the system a mixture of these different phases. The second key piece of information gained from these studies is the critical temperature associated with each of these phases. Specifically, what is the temperature where the solid structures formed during the freezing phase go from a rigid solid to a flowing mass? These critical temperatures are important, because they represent the maximum temperature that the product can withstand during primary drying without suffering from collapse or eutectic melting. The critical temperatures of interest determined through DSC thermal analysis are the glass transition temperature (Tg'), the eutectic melting temperature (Te), and the collapse temperature (Tc) of the product, the

latter of which can only be accurately determined using a technique called freeze-dry microscopy briefly mentioned above and discussed in detail in a separate paper.

When interpreting a DSC thermogram, it is important to only consider the warming curve. As the sample cools down prior to freezing, it will generally freeze well below 0°C due to the effects of supercooling and freezing point depression. Additionally, when viewing thermograms, it is important to note which direction endotherms and exotherms are displayed in the graph. This becomes important when trying to identify what each of the different peaks represents in terms of the thermal event that is occurring at that particular temperature. In the United States, it is common practice to display thermograms with the endotherms pointing up. In Europe, it is common to display exotherms pointing up.

Identification of the type of event that is occurring as the sample is warmed (eutectic melt, glass transition) is based upon the characteristic shape of the curve that is generated in the thermogram as the sample goes through that particular thermal event[2]. Figure 2 shows the thermogram from a crystalline species going through a eutectic melt.

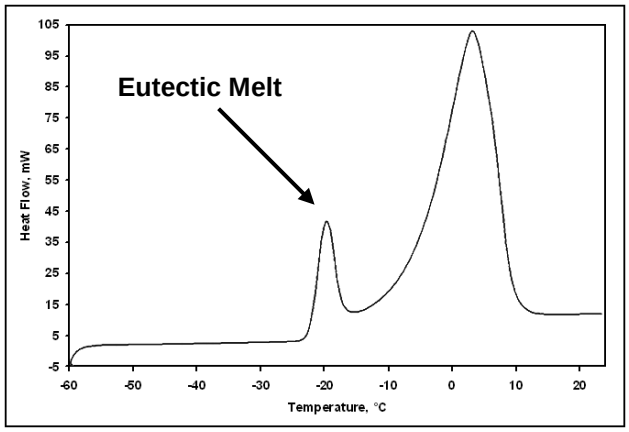

Figure 2

Endothermic peaks that are characteristic of a eutectic melt are large and symmetric as indicated in Figure 2. The additional, large endotherm shown in Figure 2 that is occurring at a warmer temperature is the melting of the ice that is surrounding the interstitial space in the frozen system. The ice melt endotherm is typically a very large, asymmetric peak which goes through its transition near 0°C. This peak is so large and broad, that it is not uncommon for smaller endotherms (characteristic of eutectic melts) to be overlapped and "buried" underneath the large ice melt endotherm. An ice/mannitol eutectic, which has a eutectic melting temperature of -1.0°C, is an example of an endotherm that is routinely not observed when conducting a standard low temperature DSC thermal analysis study because of this issue. The parameters of the DSC method can be changed to resolve these overlapping thermal events which will be discussed in detail below.

The next thermal event routinely observed in low temperature DSC thermal analysis work is the glass transition. Figure 3 shows a thermogram from an amorphous, aqueous system.

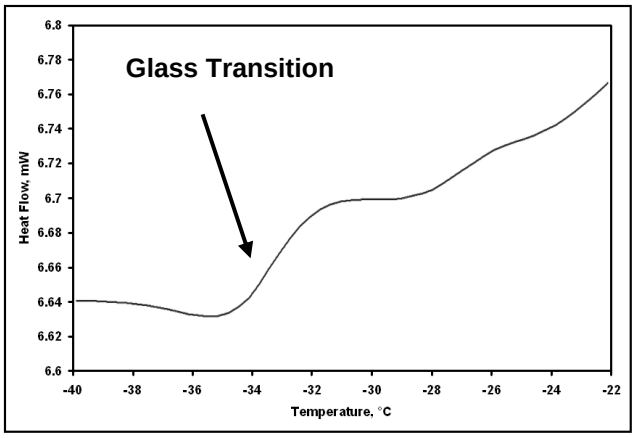

Figure 3

The peak resulting from a glass transition is much different than the peak from a eutectic melt, hence the easy differentiation between the two different phases. The classic endotherm resulting from a glass transition is known as an "S curve". These endothermic events are very low energy events compared to a eutectic melt (note the difference between the scale of the y-axis for the eutectic melt vs. the glass transition. The difference in the energy between the two different phases going

through their respective thermal events as a result of increased temperature is attributed to what is actually going on at the molecular level for each transition. For a eutectic melt, the crystalline lattice of the eutectic structure is being pulled apart as the transition occurs, which in thermodynamics, is a relatively high energy event. On the other hand, when an amorphous, glassy phase goes through a glass transition, only a shift in viscosity occurs, which is a very low energy thermal event as there is no lattice to pull apart or bonds to be broken as the system goes through its transition.

In the case of amorphous or partially amorphous systems, caution must be exercised by the scientist interpreting the DSC thermogram. Because a glass transition is such a low energy thermal event, it can easily be missed by the untrained eye. Figure 4 shows a thermogram that was collected for a frozen, aqueous system containing only an amorphous component.

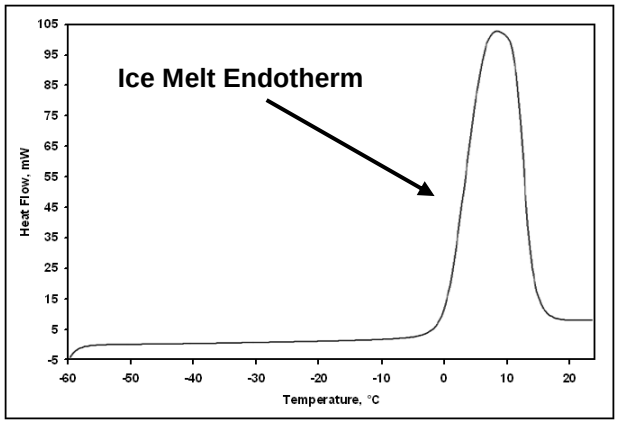

Figure 4

Upon first observation of this thermogram, it appears that only one thermal event is occurring upon warming the system. The large endotherm present in Figure 4 is a result of the melting of the ice surrounding the interstitial space. If this thermogram was taken for face value, and a lyophilization cycle was designed around it, entire batches of product could be lost because of processing them at temperatures that are too high. The proper technique for interpreting thermograms is to expand the y-axis at the lower temperature range to look for low energy glass transitions. The image in Figure 4 was the first thermogram generated for this particular product. Figure 3 represents the low temperature, y-axis expanded region that allowed the identification of a glass transition that was not observed in the initial thermogram. Knowing that an amorphous phase with a low temperature glass transition exists for this formulation, the development scientist can adjust the lyophilization cycle parameters accordingly to account for this.

Extracting Critical Temperature Data

So far, it has been shown that the shape of the peak can be used to identify the different phases of material within a formulated product when they are frozen. In addition to phase identification, critical temperature data can also be obtained from these thermograms.

After a eutectic phase has been identified, as shown and discussed in Figure 2, the eutectic melting temperature (Te) can be determined. When discussing the melting temperature of a eutectic, it is customary to talk about the "Onset of Melt" for that eutectic. This is the temperature when the eutectic first begins to go through its transition. The onset of melt is determined by drawing a tangent to the baseline leading up to the peak, and a tangent to the leading edge of the peak. The classic eutectic melting temperature is the temperature where these tangent lines intersect. Modern DSC computer software packages have programs that can do this task automatically with a little help from the analyst.

After an amorphous phase has been identified, as shown and discussed in Figure 3, the glass transition temperature (Tg') can be determined. When discussing the transition of a glassy phase, it is customary to talk about the temperature where the glassy phase is halfway through its shift in viscosity. The glass transition temperature is determined by drawing a tangent to the baseline leading up to the S curve, and a tangent to the baseline leading away from the S curve. The midpoint

on the S curve between these tangents is the classic glass transition temperature. As with eutectic melting temperatures, modern DSC computer software packages have programs that can do this task automatically with a little help from the analyst.

These critical temperature values (Tg', Te) are used for designing an optimized lyophilization cycle, and represent the maximum allowable product temperature that can be achieved during primary drying without loss of the product.

Improving Resolution and Sensitivity

Occasionally, sensitivity and or resolution enhancement of a thermogram will be needed. For example some glass transitions occur with such low energy, that they cannot be detected using a typical DSC heating protocol. Additionally, sometimes transitions will overlap because they are so close in temperature. This was the case discussed above with the eutectic melting of a Mannitol eutectic and the ice melt endotherm.

In these particular cases, resolution and sensitivity of the instrument can be improved by altering the warming rate through the different transitions[1]. As a rule of thumb, increasing the heating rate through the transitions increases the sensitivity, and decreasing the heating rate through the transitions, increases the resolution. A standard protocol for a DSC thermal analysis study would involve cooling the sample from ambient to -60°C at 5 to 10°C/minute. Once the temperature stabilized, the sample would then be warmed back to ambient at approximately 20°C/minute. To increase the sensitivity of the instrument, a heating rate as high as 60°C/minute might be employed. To increase the resolution of the instrument a heating rate as low at 0.1°C/'minute might be employed.

Metastable Glassy Systems

Systems that contain components that have the potential to form a metastable glass (a phase that should have crystallized during freezing but instead formed a glass) pose an interesting challenge to the analyst conducting the thermal analysis study. First of all, the system must be identified as a metastable glassy system, and second of all, an annealing protocol must be developed to convert the metastable glassy phase back to the more stable crystalline form (eutectic). A thermogram from a typical system that forms a metastable glass is shown in Figure 5.

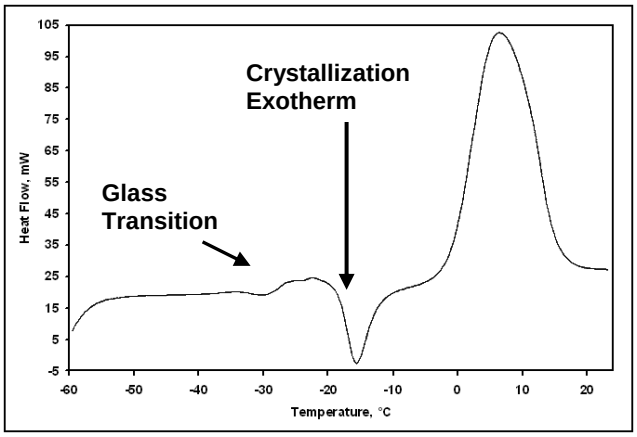

Figure 5

As this system is warmed, the metastable glass goes through the glass transition at approximately -28°C as indicated. As the system continues to warm, an exothermic crystallization event occurs at approximately -18°C, which is the crystallization of the metastable glass into the more stable eutectic mixture.

Theoretically, as the system is warmed, the metastable glass will go through its glass transition, and the highly viscous phase will soften and begin to flow. The molecules within this flowing state now have time to come together and form a stable crystalline lattice and crystallize. Since crystallization is a high energy event, a large, symmetric, downward peak is easily observed as indicated in Figure 5.

The development scientist now has several key pieces of information regarding their formulation. First of all, the characteristic

thermogram indicates that this formulation has the potential to form a metastable glass during freezing and will need to be annealed. Second of all, the temperate that the product will need to be taken to in order to successfully anneal the product is also available. To adequately anneal a system, the temperature needs to be taken above both the glass transition and the crystallization exotherm. Care must be taken not to warm the sample too far above the crystallization exotherm as there may be other thermal events that will occur at higher temperatures that should not be disturbed. In the example shown in Figure 5, the higher temperature thermal event would be the ice melt endotherm. Based on the example shown in Figure 5, a good annealing temperature for this particular product would be approximately -10°C. At this temperature, the product has gone through the glass transition and the crystallization; however, it has not warmed into the leading edge of the ice melt endotherm.

The protocol for determining the effectiveness of the annealing protocol is as follows: The initial, standard thermal analysis is conducted, and the thermogram in Figure 5 is the result. An annealing temperature of -10°C is chosen, and the DSC experiment is repeated, only this time after the initial cool to -60°C, the sample is warmed to -10°C and held for a certain period of time (typically 10 minutes for the first experiment). The sample is then re-cooled back to the original freezing temperature and then warmed through to ambient temperature like a normal thermal analysis study. If the annealing process was successful, a thermogram like the one shown in Figure 6 will be observed.

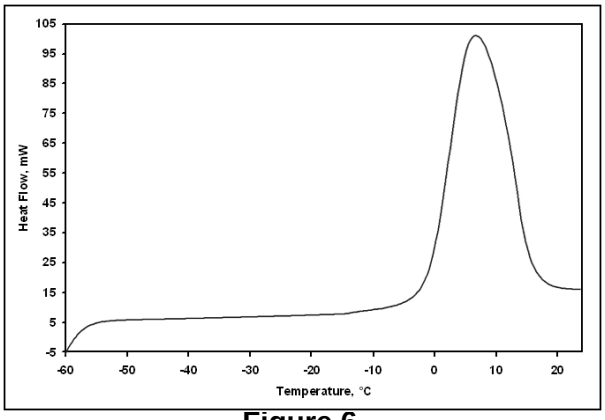

Figure 6

The key elements that this thermogram is missing are the glass transition and the crystallization exotherm which is to be expected if the annealing process was successful. All that should be left is a eutectic melt and the ice melt endotherm. In this particular case, the eutectic that formed was a mannitol eutectic which is co-melting under the ice melt endotherm and is not observed.

Based on some simple thermal analysis experiments, the development scientist now has a wealth of information regarding their product that can be used in pilot studies for designing an optimized lyophilization cycle.

References

1. SL Nail, JJ Schwegman, V Kamp, “Analytical Tools for Characterization of Frozen Systems in the Development of Freeze-Dried Pharmaceuticals”, American Pharmaceutical Review, 3(4): 2000, p. 17-25.
2. SL Nail and LA Gatlin, “Freeze Drying: Principles and Practices”, in Pharmaceutical Dosage Forms: ParenteralMedications, Vol 2, Avis, et al, eds., Marcel Dekker, 1992, p. 171, 174

About the Author: J. Jeff Schwegman, Ph.D. is a formulation and process development scientist in the pharmaceutical industry, where he has over 17 years of experience in developing freeze-dried products consisting of both small and large molecules. He is currently the founder and CEO of AB BioTechnologies which offers teaching and consulting services in pre-formulation, formulation, and lyophilization cycle development for freeze-dried products. He is the author of several articles in peer reviewed journals, he holds several patents in formulation development and equipment design, and he routinely lectures around the world on the development and manufacture of freeze-dried products. Please feel free to contact him at 812-327-6898 or at jjschwegman@gmail.com for consulting or teaching services in lyophilization.