Use of Stepscan DSC for Freeze-Drying Optimization

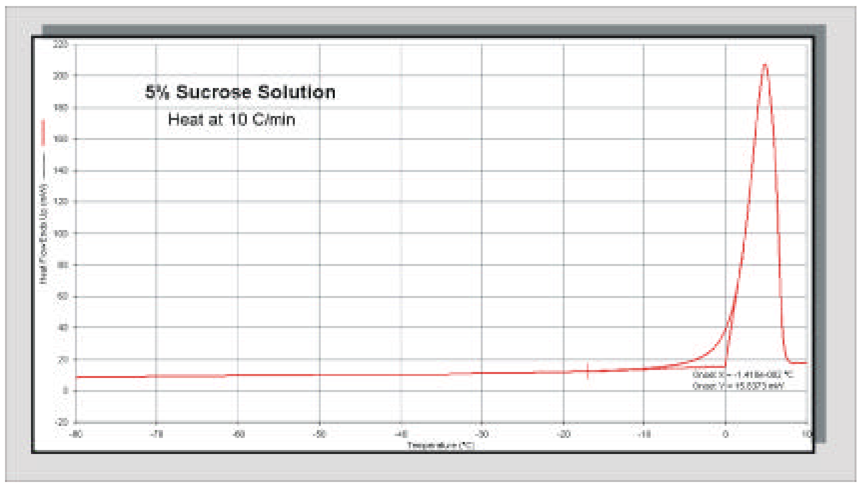
W.J. Sichina, International Marketing Manager

Introduction

The production of many new drugs requires that the active material first be dissolved in water and then freeze-dried or lyophilized in order to generate a compound which is easily ingested and which also has a pleasing or pharmaceutically elegant appearance. The lyophilization process involves cooling an aqueous formulation to subambient temperatures and then pulling a vacuum to drive off the free water. Due to energy consumption considerations, this can be expensive and it is desirable to know the maximum acceptable temperature to which the solution can be cooled before a vacuum is applied. It is also essential to ensure that the temperature is sufficiently low enough to avoid the 'collapse' of the freeze-dried cake, which renders an unsatisfactory or useless product.

One key parameter that has been identified as crucial to understanding the lyophilization process is the subambient glass transition temperature, or Tg, of the given formulation. Generally, the process temperature is set below the Tg of the formulation during primary drying in order to avoid the 'collapse' of the product during lyophilization. In addition to the glass transition temperature, the magnitude of the change in heat capacity (ΔCp) at Tg along with the occurrence of any recrystallization events may have a major effect on the successful avoidance of the collapse of the product during

Figure 1. DSC subambient results on 5% sucrose solution.



processing. Thus, an analytical technique is required which yields accurate, sensitive and reproducible data on Tg(s) and recrystallization transformations in the subambient temperature regions. This information is valuable in the generation of a 'pharmaceutically elegant' freeze-dried product.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) provides a means of addressing the key issues surrounding the production of a successful lyophilized material. In particular, the state-of-the-art PerkinElmer Pyris 1 DSC system is ideally suited for this particular application. The Pyris 1 DSC offers the pharmaceutical scientist the following advantages:

- Very high sensitivity
- Outstanding resolution
- Excellent subambient performance
- Use of platinum resistance thermometers (PRT) for the highest possible accuracy and precision
- Ease of use with Pyris Software for Windows

With the PerkinElmer Pyris 1 DSC system, the scientist can quantitatively examine and test the following characteristics associated with the generation of proper cake formulation:

- cyroprotectors
- bulking agents
- buffer salts
- surfactants

The cryoprotectors are typically simple sugars (generally sucrose or mannitol) that preferentially bind to the

PerkinElmer™ instruments.

Figure 2. Pre-melting DSC results on 5% sucrose solution.

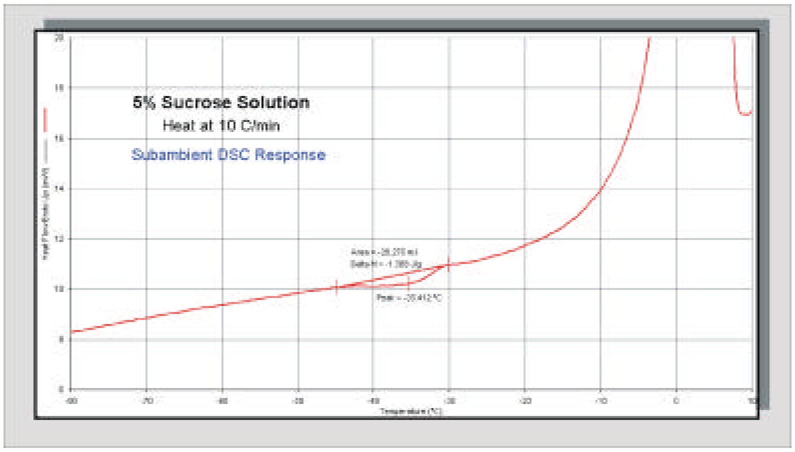
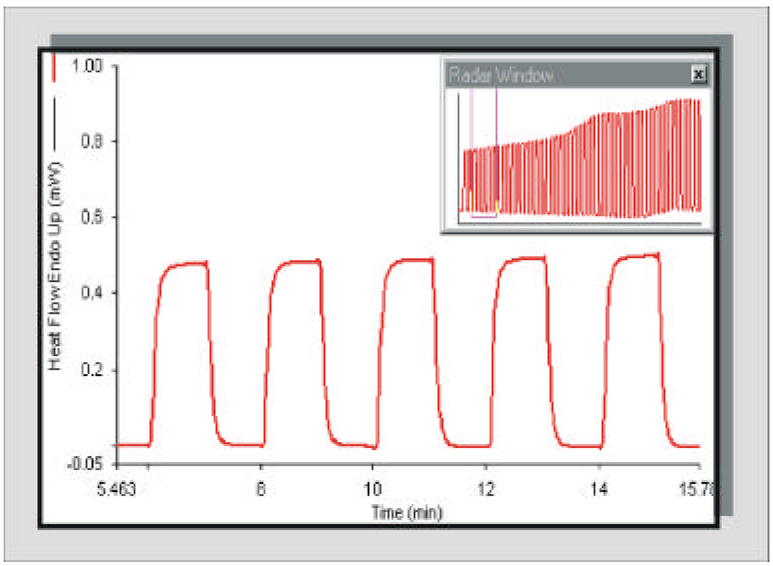


Figure 3. StepScan DSC results showing repetitive heat-isothermal hold segments.



active drug or protein and protect it against denaturation during freezing of the bulk solution. The effects of the different additives on the thermal properties of the formulations can be determined using the Pyris 1 DSC.

Aqueous lyophilization formulations are best analyzed in an open aluminum pan since the properties of interest are below 0 C. Between 10 to 20 mg of solution is injected into an open pan and then analyzed with the DSC. The sample is quickly cooled to a temperature of approximately -80 C, held for 2 minutes, and then heated at a rate of 5 or 10 C/min back to room temperature.

Displayed in Figure 1 are the DSC results obtained on a 5%, by weight, solution of sucrose cryo-protector dissolved in water.

The sample was heated at a rate of 10 C/min between –80 and 20 C and the melting of the bulk ice phase at 0 C dominates the DSC response of the formulation. However, an enlarged or magnified view of the subambient DSC response of the sucrose solution does reveal that other thermal events occur below the main ice melting peak, as is shown in Figure 2.

The 5% sucrose formulation does yield what appears to be an exothermic transition at approximately –35 C well below the main ice melting peak. The interpretation of this thermal event is not straightforward, as it is known that the formulation does undergo a glass transition in this region. What is needed is a means of enhancing the characterization of the subambient response of the 5% sucrose formulation in order to clarify the interpretation of the results. This can be accomplished with the new StepScan DSC software from PerkinElmer which is used in conjunction with the Pyris 1 DSC.

StepScan DSC

StepScan DSC is new software for the enhanced characterization of the thermal properties of materials. The method is straightforward and utilizes the traditional approach for measuring the heat capacity, Cp, for the highest possible reliability of results without interfering experimental problems. The StepScan DSC approach is only possible with the design of the power compensated Pyris 1 DSC, with its very low mass sample and reference furnaces and rapid response time.

PerkinElmer™ instruments.

Visit our website at **www.perkinelmer.com.**

PerkinElmer Instruments
761 Main Avenue
Norwalk, CT 06859-0010 USA
Tel: 80O-762-4000 or
(1+) 203-762-4000
Fax: (1+) 203-762-4228

ISO 9001

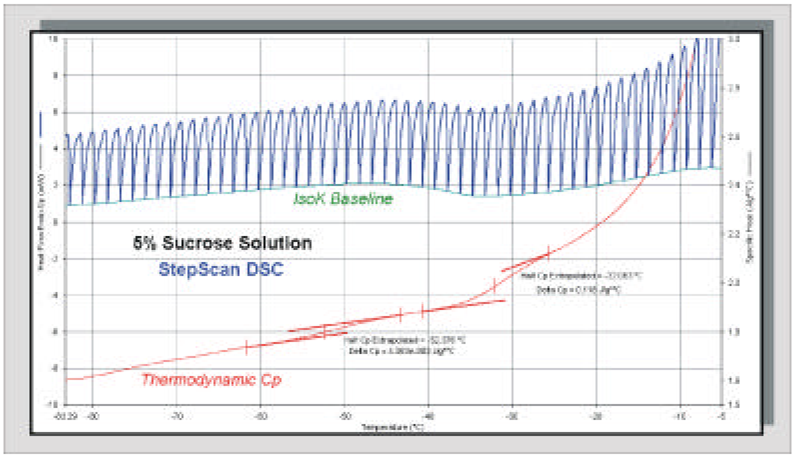
Figure 3 shows the StepScan DSC approach with the application of a repetitive sequence of short heating – isothermal hold segments.

Visit our website at **www.perkinelmer.com**.

PerkinElmer instruments.

PerkinElmer Instruments
761 Main Avenue
Norwalk, CT 06859-0010 USA
Tel: 80O-762-4000 or
(1+) 203-762-4000
Fax: (1+) 203-762-4228

Figure 4. StepScan DSC subambient results on 5% sucrose formulation.



With the application of heating (10°C/min) over small temperature increments (1.5 or 2°C), and by holding for a short time interval (e.g., 30 seconds), the heat capacity that is yielded reflects the reversible aspects of the sample. Kinetic or irreversible effects (on the time scale of the experiment) are eliminated in the ***Thermodynamic Cp*** data set, which reflects 'fast' phenomenon, such as the sample's heat capacity (molecular vibrations) or Tg (molecular rotations). For example, if a sample has a glass transition, Tg, which has an overlapping enthalpic relaxation, moisture loss or crystallization event, the Thermodynamic Cp signal will show the classic, stepwise change in the heat capacity, which makes it simple and straightforward to analyze and interpret. The StepScan DSC approach also provides the kinetic or ***IsoK Baseline*** data set, which is reflective of the irreversible or 'slow' processes taking place during the experiment. The enthalpic relaxation event, which can occur on physically aged samples at Tg, will show up in the IsoK Baseline data set.

Because the StepScan DSC approach requires rapid DSC response times, the technique is only feasible with the power compensated DSC, which allows for fast heating and thermal equilibration. The application of the StepScan approach to a large mass furnace, heat flux DSC instrument would be difficult or technically unfeasible due to the inability to rapidly respond and equilibrate.

The other advantage of the StepScan DSC approach is that it provides a direct heat capacity measurement using the traditional and time-proven means without the need for deconvolution or the extraction of sine wave amplitudes. Other techniques, such as TMDSC (temperature modulated DSC), as applied to a large mass, heat flux furnace, oftentimes have problems due to distorted sine waves and phase lag. Because of the direct nature of the StepScan DSC experimental approach for assessing the thermodynamic heat capacities, the results are not plagued with these experimental difficulties.

StepScan DSC Results on Sucrose Formulations

Displayed in Figure 4 are the StepScan DSC generated on the 5% sucrose solution in the subambient region below the main ice melting transition.

The blue or uppermost curve is the StepScan DSC 'raw' data and shows the individual heat-hold segments. The IsoK Baseline, green or center curve, reflects the slow or irreversible thermal events. The exothermic transition, observed in the standard DSC results, appears in the IsoK Baseline data set. The Thermodynamic Cp data appears in red as the lowermost curve and this reflects the 'fast' changes taking place in the sample. The Thermodynamic Cp data is particularly beneficial for sample characterization purposes as it substantially 'cleans up' the detection and identification of the glass transition event(s).

For the 5% sucrose formulation, the Thermodynamic Cp data set reveals that two Tg's occur in the subambient regions, at approximately –52 and –32 C. This is very useful information for freeze-drying purposes as it helps to identify the key transitions for lyophilization optimization. Certainly, the formulation must be cooled below –33 C as this is where the primary Tg is

PerkinElmer™ instruments.

Visit our website at **www.perkinelmer.com**.

PerkinElmer Instruments
761 Main Avenue
Norwalk, CT 06859-0010 USA
Tel: 80O-762-4000 or
(1+) 203-762-4000
Fax: (1+) 203-762-4228

ISO 9001

Figure 5. Thermodynamic and IsoK Baseline data sets for the 5% sucrose solution demonstrating the ability of StepScan DSC to separate out 'fast' and 'slow' events.

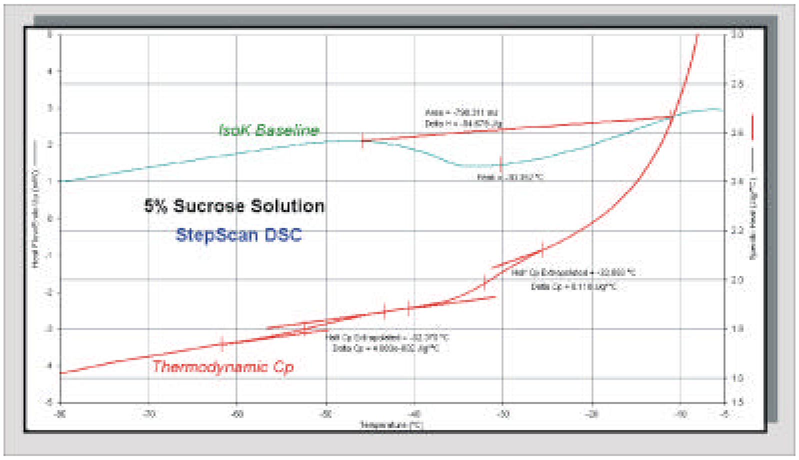
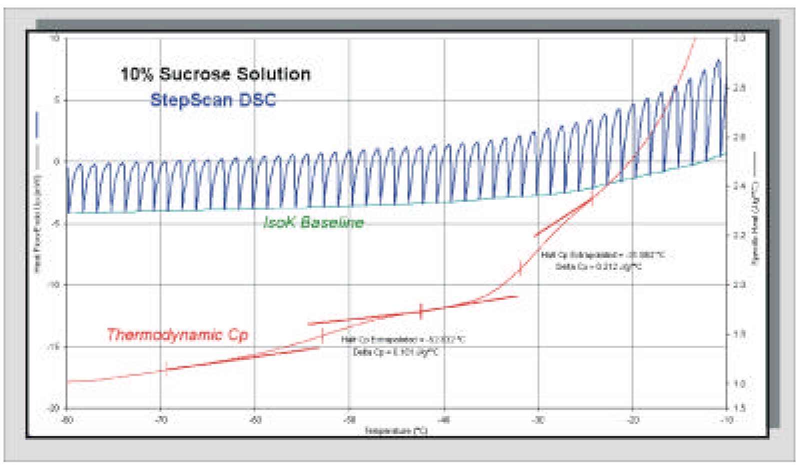


Figure 6. StepScan DSC results on subambient properties of 10% sucrose aqueous formulation.



observed. A secondary and weaker Tg is also obtained at –53 C for the sucrose solution. This lower amorphous phase transition will no doubt have a bearing on the subsequent freeze-drying properties of the formulation and may have an impact on the propensity of the formulation to undergo (or avoid) 'collapse' during processing.

StepScan DSC proves very useful and effective in separating out the irreversible and reversible transitions associated with the sucrose formulation, which makes subsequent data interpretation much easier. This is shown in Figure 5, which displays the Thermodynamic Cp and IsoK Baseline data sets for the 5% sucrose formulation.

In the case of the 5% sucrose formulation, it would appear that the solution undergoes two well-resolved Tg's at –52 and –32 C (due to two different amorphous phases) with a simultaneous recrystallization transition occurring at about –30 C. StepScan DSC makes this interpretation possible with its ability to separate out 'fast' and 'slow' thermal events.

The subambient characteristics of a more concentrated (10%, by weight) sucrose aqueous solution were measured using StepScan DSC and these results are displayed in Figure 6.

The more concentrated sucrose solution exhibits the two subambient glass transition events at approximately –52 and –32 C and the intensities of the Tg's are greater than observed for the 5% solution (Figure 4). The greater intensity of the glass transition events is consistent with the fact that the 10% solution contains twice as much solute as the 5% formulation. In addition, the recrystallization transition in the IsoK Baseline data set is no longer observed. This indicates that the solution chemistry has been significantly altered between the 5 and 10% concentrations and this would no doubt have an impact on the freeze-drying characteristics of the two different formulations.

Summary

Detection of glass transition events in frozen formulations can be challenging and difficult due to the inherently weak nature of these transitions. The state-of-the-art PerkinElmer Pyris 1 DSC system provides the necessary high degree of sensitivity, resolution and stable subambient performance necessary to observe the weak glass transition(s) and

Visit our website at **www.perkinelmer.com.**

PerkinElmer Instruments
761 Main Avenue
Norwalk, CT 06859-0010 USA
Tel: 80O-762-4000 or
(1+) 203-762-4000
Fax: (1+) 203-762-4228

recrystallization events associated with formulations undergoing lyophilization. The StepScan DSC approach further aids in the interpretation and characterization of the formulations through the separation of ‘fast’ and ‘slow’ events, such as Tg (fast) and recrystallization (slow). The Thermodynamic Cp data set, obtained from StepScan DSC, showed that two resolved subambient Tg’s are observed for the sucrose formulations. One of the primary benefits of StepScan DSC is its ability to greatly enhance the detection and analysis of glass transition events.

The Pyris 1 DSC and StepScan DSC can provide valuable information on the thermal properties of formulations and aid in the knowledge regarding the propensity of the solution to avoid collapse during processing by measuring the following key parameters:

- temperature of Tg(s)
- magnitude of Tg(s) or ΔCp
- occurrence of recrystallization

The StepScan DSC data is valuable in the efficient generation of a ‘pharmaceutically elegant’ freeze-dried product.

Visit our website at **www.perkinelmer.com**.

PerkinElmer Instruments
761 Main Avenue
Norwalk, CT 06859-0010 USA
Tel: 80O-762-4000 or
(1+) 203-762-4000
Fax: (1+) 203-762-4228