



## DIFFERENTIAL SCANNING CALORIMETRY (DSC) AN ESSENTIAL AND CRITICAL INSTRUMENT IN OPTIMIZING LYOPHILIZATION PROCESS

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### ABSTRACT

At present, lyophilization has become an important in-course process which has been proved to increase the stability of a parenteral product which causes a consequent increase in its bioavailability. A thorough understanding of a formulation provides the basis for developing the freeze-drying cycle parameters, such as freezing temperature, annealing temperature, shelf temperature, vacuum level, and time duration. The steps of lyophilization have three distinctive parts which is to be done with utmost care to restrict the drug products from degrading. Some characterization has to be done to reach a conclusion whether the drug product is as same as was before lyophilization i.e. the drug did not undergo any kind of degradation. Differential scanning calorimetry (DSC) is an experimental technique for measuring the energy necessary to establish a nearly-zero temperature difference between sample and reference. The present review article demonstrates a vivid description of the reason of extreme usefulness of DSC and its modifications for examining the  $T_g$  (Glass temperature) properties of freeze-dried pharmaceutical systems.

**Keywords:** Lyophilization, Differential Scanning calorimetry (DSC),  $T_g$  (Glass Transition Temperature), Collapse Temperature.

### LYOPHILIZATION

The world has entered a modern era where the process of lyophilization has become a lead in the fields of pharmaceutical industry as far as parenteral formulations taken into consideration.<sup>1</sup> The process of freeze drying of food products were first traced in ancient South America, in the regions of Andes to preserve potatoes.<sup>2</sup> In 1890, first reports were submitted with conclusion that biological specimens can be preserved by drying in vacuum condition and temperature below 0°C.<sup>3</sup> Freeze drying or lyophilization is an effective way of drying pharmaceutical products without degradation of its physicochemical and pharmacological properties. The physical phenomenon of sublimation i.e. direct transition between solid state and gaseous state without passing through the liquid state, is used.<sup>4</sup>

Lyophilization is a three-step freeze drying process for removing nearly all the moisture from a target. The steps are “separate and dependent”<sup>5</sup>

*Before discussing the steps in order, for convenience the process can be summarized as below*

- a. A variety of processing steps such as vaccine preparation, extraction, purification and formulation in a suitable medium are required regarding cell or other bioproducts for freeze drying.
- b. First, simple freezing under vacuum is done which causes reduction in denaturation of products, solution component immobilization,

foaming. It induces desired ice crystal structure favorable for drying.

- c. A typical condition in drying chamber is to be maintained during primary drying to sustain water migration from sample ice during drying. Strictly freeze drying interface temperature is to be maintained below eutectic, glass transition, collapse, or melt temperature to minimize sample damage during drying.
- d. Resistant moisture in apparently dry structure gets removed by desorption in secondary drying stage.
- e. Sealing in an inert environment under vacuum to avoid the contamination from reactive, destabilizing, atmospheric gases and dampening of the freeze dried sample.
- f. The samples are then withdrawn from freeze drier, stored and/or distributed for use prior to reconstitution for injection, application or regrowth.<sup>6</sup>

**Now overview of the freeze drying process is described briefly as below**

**a. Freezing stage**

It is the first stage of the freeze drying process which determines the microstructure formed by the solidified solution and also that of the dried product. The performance of the freeze drying process is significantly dependant on this stage. The materials (viz. gel suspension, liquid solution or food stuffs) are cooled down below their specific solidification point. The value of the said temperature should be below eutectic point where the material becomes totally crystalline in nature. Two different behaviors are displayed by materials subjected to freeze drying: (a) liquid phase solidifies (eutectic formation); (b) the liquid phase does not solidify rather it just becomes more and more viscous until it finally takes the form of a very stiff, highly viscous liquid. At the end of freezing step, the water (solvent) and the drug with excipients (solute) are separated as the water solidifies. About 65-90 % of the water is removed and the rest of it is present in the sorbed (unfrozen) state.<sup>4, 7</sup>

**b. Primary drying**

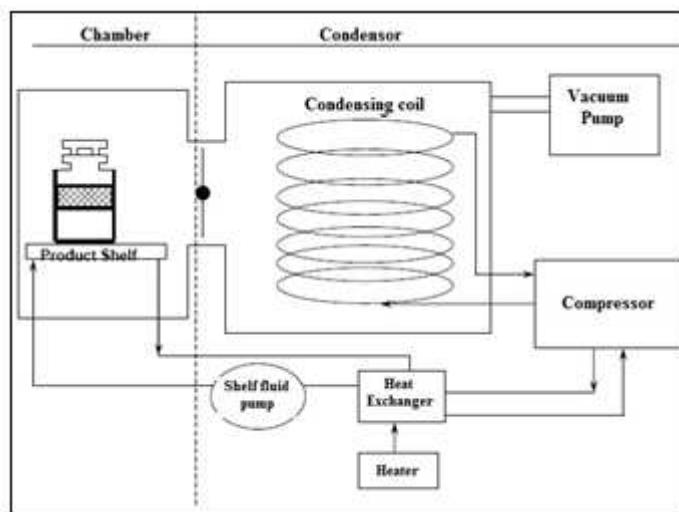
After the process of freezing, the chambers are evacuated and the pressure is reduced to an extent that causes sublimation of solvent to take place in the primary drying stage. Now the water present is sublimed to gaseous state containing latent heat of sublimation (2840KJ/kg ice). Thus, the temperature of the frozen product is again reduced. If heat is not supplied from a respective source, the vapor pressure of the water at the temperature of the product reaches the same value as that of the partial pressure of the water vapor in the drying chamber; therefore, the system reaches equilibrium and no additional water sublimation from the product would occur.<sup>7</sup>

**c. Secondary drying**

This phase of freeze drying involves removal of water (sorbed). Ideally this stage is the continuation of primary drying. It starts as the ice has been removed in the previous stage. The water that is removed in this stage may be due to: - (a) physical adsorption, (b) chemical adsorption, and (c) water of crystallization. This water is removed under vacuum.<sup>7</sup>

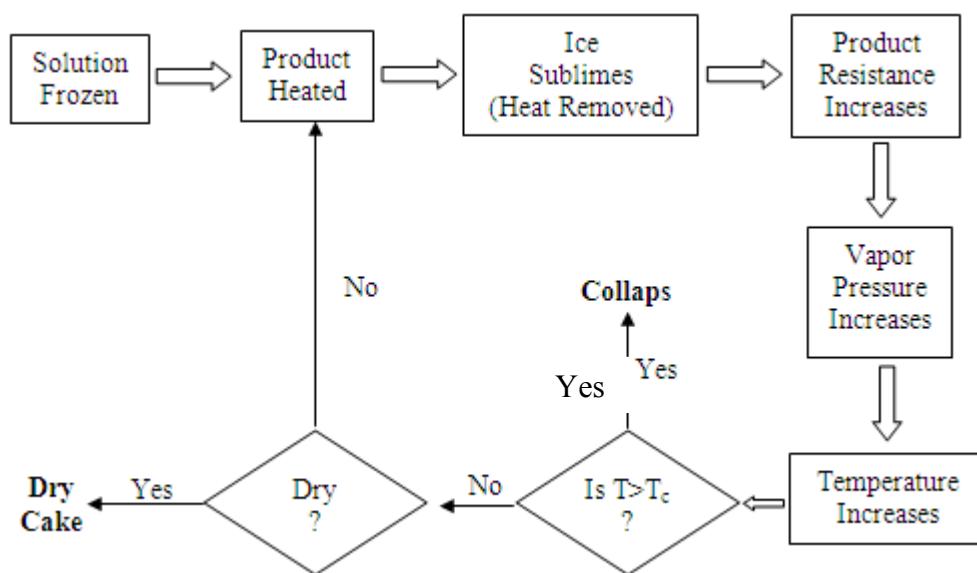
The manner of freezing can have a significant impact on the primary stage of drying characteristics of a formulation. A decrease in the *mass transfer resistance (MTR)* with respect to a particular shelf temperature and chamber pressure will increase the rate of sublimation. Consequently the product temperature will decrease due to enthalpy of ice sublimation. A increase in the primary drying shelf temperature of vertically frozen visible causes an increase in the product temperature for primary drying, which in turn matches that of a standard-frozen material dried at a colder shelf temperature. That higher shelf temperature provides heat transfer, speeding up primary drying. For equivalent product temperatures, the rate of sublimation was by an extent faster for vertically oriented ice than for standard-frozen solutions. Consequently, time required for primary drying decreases which constitutes half of the time spent in the lyophilization process. The duration of the lyophilization cycle should be reduced due such lowering of the primary drying time of the annealed vials.<sup>8</sup>

**Figure 1**  
*Schematic representation of freeze drying process and the instrumentation*



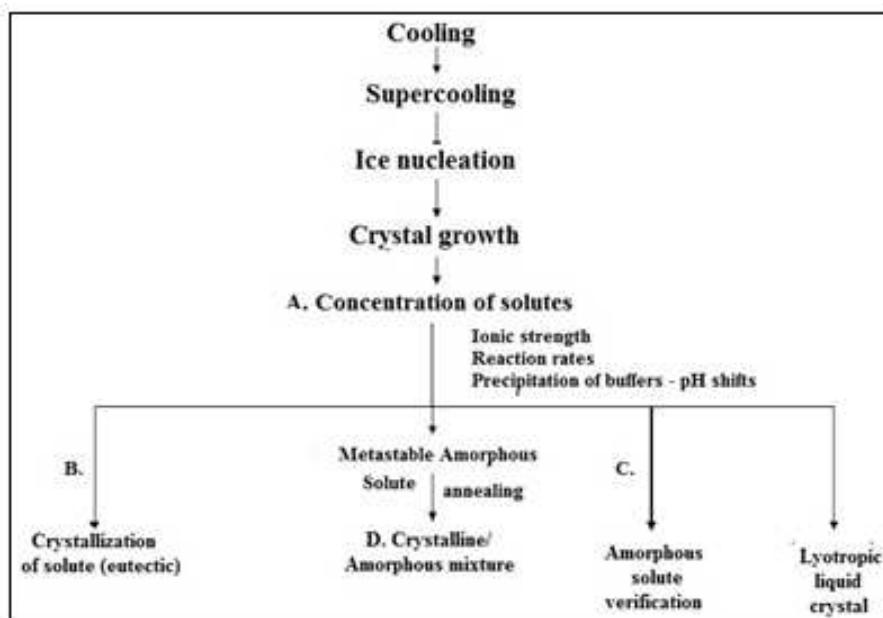
Some products like aqueous sucrose solutions are observed to show some structural changes during the drying process resulting in the phenomenon of *collapse* due to product resistance. Even though, the product is frozen below its eutectic temperature, elevation of temperature during the drying process may affect the structure of the frozen matrix at boundary of drying front.<sup>9</sup>

**Figure 2**  
*Process of collapse of product<sup>10</sup>*



As the process of freeze drying has been explained above thoroughly, a schematic flowchart has been mentioned below as per explanation.

**Figure 3**  
**Flowchart of Freeze drying<sup>10</sup>**



### **Importance of Controlled freezing**

The process of freezing has to be investigated because this process is very difficult to control. It has been observed that normally most of the solutions subjected to the process of freezing get supercooled at approximately about -15°C, which is difficult to overcome by standard processes. Ice may be forced to nucleate at higher temperature close to zero, but then only the bottom surface would undergo such phenomenon and the ice would propagate in a vertical direction resulting in lower MTRs. The crystalline structure of the ice formed in the process of freezing may affect the water vapor flow during primary drying and final product. If the primary drying shelf temperature be increased, a 40 % hike in rate of sublimation in case of vertically frozen material has been recorded with an equivalent product temperature as per studies.<sup>8</sup>

### **Heat transfer in Freeze drying**

The process of freeze drying is totally dependent on heat transfer rate irrespective of the tool use to control the process. The rate of heat transfer vary with deviation in the operating condition, type of the container, equipment and configuration used for the loading the product. The heat transfer coefficient varies with processing condition used for drying. The gas temperature has a minor effect on the coefficient but also, the chamber pressure cannot be neglected. The introduction of a tray for lyoloading reduces the value of heat transfer

coefficient of the entire lot with more variation in case of the vials at edges.<sup>11</sup>

## **DIFFERENTIAL SCANNING CALORIMETRY**

Previously, thermal analysis was accumulation of a group of technique in which a property of a sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere is programmed. Later, the definition was changed to "Analysis of change in the property of sample which is related to an imposed temperature alteration".<sup>12</sup>

### **Thermal analysis comprises the following techniques<sup>13</sup>**

1. Differential Thermal Analysis (DTA)
2. Differential Scanning Calorimetry (DSC)
3. Thermogravimetric Analysis (TGA)
4. Dilatometry (DIL)
5. Evolved Gas Analysis (EGA)
6. Dynamic Mechanical Analysis (DMA)
7. Dielectric Analysis (DEA)

Out of which, Differential Scanning Calorimetry (DSC) has been found to be one of the most commonly used thermal analysis techniques. There are two types of definitions for the said type of thermal analysis. Firstly, it can be defined as the measure of the difference in heat flow rate to

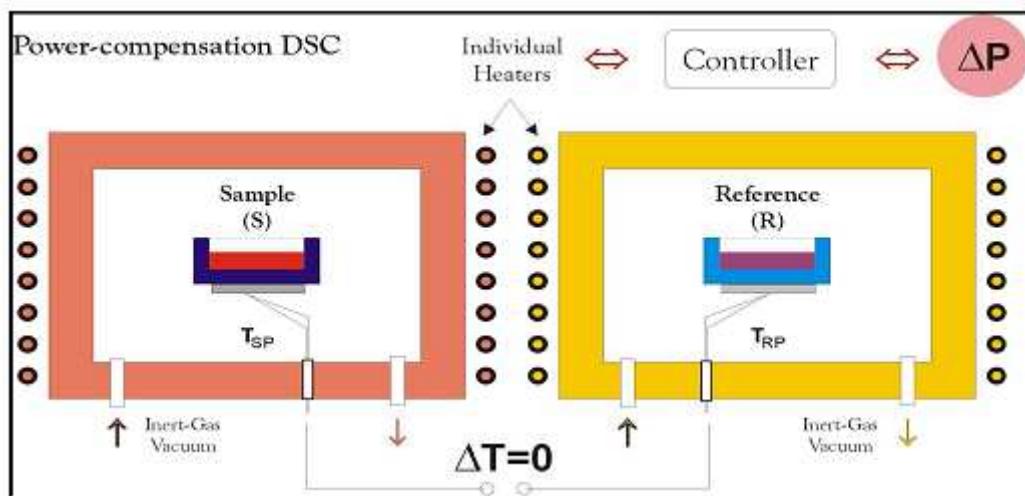
material (sample) and to a reference material while they are subjected to a controlled temperature program.<sup>13</sup> Later the definition was given as an experimental technique for measuring the energy necessary to establish a nearly-zero temperature difference between the test substance (and/or its reaction products) and an inert reference material R, while the two samples are subjected to an identical (heating, cooling or constant) temperature program.<sup>14</sup> Differential scanning calorimetry (DSC) is an experimental technique for measuring the energy necessary to establish a nearly-zero temperature difference between a test substance S (and/or its reaction products) and an inert reference

material R, while the two samples are subjected to an identical (heating, cooling or constant) temperature programme. Two types of DSC systems are commonly in use:

**a. Power-compensation DSC**

The specimen (TS) and reference (TR) temperatures are controlled independently using separate (identical) ovens. The temperature difference between the sample and reference is maintained to zero by varying the power input to the two furnaces. This energy is then a measure of the enthalpy or heat capacity changes in the test specimen S (relative to the reference R).

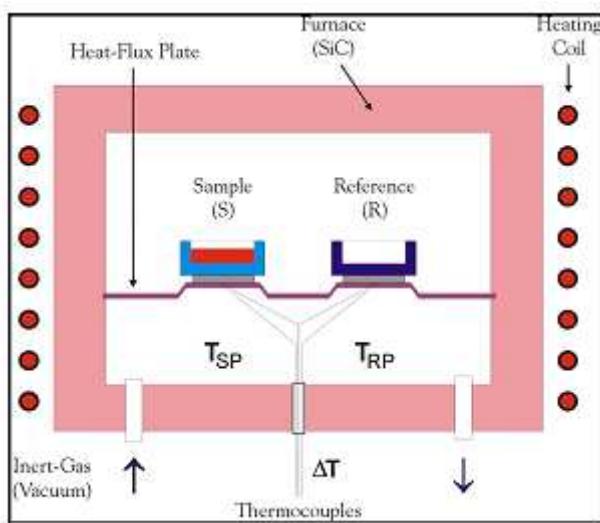
**Figure 4**  
*Schematic Representation of Power-compensation DSC<sup>11</sup>*



**b. Heat-flux DSC**

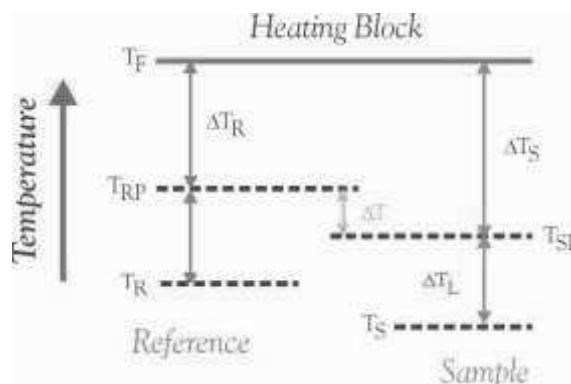
The test specimen S and reference material R (usually an empty sample pan + lid) are enclosed in the same furnace together with a metallic block with high thermal conductivity that ensures a good heat-flow path between S and R. The enthalpy or heat capacity changes in the specimen S lead to temperature differences relative to R. This results in a certain heat-flow between S and R, however small compared to those in DTA, because of the good thermal contact between S and R. The temperature difference DT between S and R is recorded and further related to the enthalpy change in the specimen using calibration experiments. The heat-flux DSC system is thus a slightly-modified DTA system: the only important difference is the good heat-flow path between the specimen and reference crucibles. The DSC instrument used in the present experiment is a heat-flux type NETZSCH 404C Pegasus calorimeter. Therefore special attention will be given to this type of instruments.

**Figure 5**  
**Schematic Representation of Heat-flux DSC<sup>12</sup>**



The assembly of thermal resistances of the heat-flux DSC system changes with temperature. Nevertheless, DSC instruments may be used in the so-called 'calibrated' mode: the amplification of the DSC signal is then also automatically varied with temperature to maintain a constant calorimetric sensitivity with temperature. Temperature shifts may occur between the samples (S and R) and thermocouples, since these are not in direct physical contact with the samples. The measured temperature difference  $\Delta T_{\text{exp}}$  is not equal to  $(T_S - T_R)$  where  $T_S$  and  $T_R$  are the sample and reference temperatures, respectively. The temperature difference of interest  $(T_S - T_R)$  may however be deduced by considering the heat-flow paths in the DSC system.<sup>14</sup>

**Figure 6**  
**Thermal-resistance diagram for a typical heat-flux DSC system<sup>14</sup>**



Where,

$T_{SP}$ ,  $T_{RP}$  = temperature of the sample (SP) and reference (RP) platforms as measured by the thermocouples.  $T_{SP}$  is plotted as the abscissa of the measured DSC curve.

$T_F$  = temperature of the heating block (furnace).

$\Delta T_R$  = temperature difference of the reference platform RP relative to the furnace.

$\Delta T_S$  = temperature difference of the sample platform SP relative to the furnace.

$\Delta T_L$  = temperature difference of the sample S relative to the sample thermocouple.

Lyophilization or freeze-drying involves freezing product followed by reducing water through sublimation and desorption so as to limit chemical and biological activity. The lyophilization is used in pharmaceutical industry for preserving

pharmaceutical dosage which is prone to physical or chemical instability when packed in solution form. The drying step has to be carried out below a critical temperature i.e. collapse temperature. Collapse temperature is the point at which softening

progresses leading to structural collapse of the freezed cake. The amorphous nature of the material temperature is determined by glass transition temperature ( $T_g$ ), thus identification of  $T_g$  for lyophilized product serves as a key parameter for the process development, formulation development, stability and storage condition for lyophilized product. This experimental note demonstrates the change in  $T_g$  of Lyophilized Pharmaceutical Dosage accompanied by small change in heat capacity measured using DSC. The graph is plotted as heat flow endo-up at y-axis with temperature at x-axis.<sup>15</sup> A thermal analytical scientist working for a pharmaceutical R&D center needs to accurately determine the glass transition temperature ( $T_g$ ) of aqueous solutions containing an active drug, cryoprotectant, and other agents to optimize the lyophilization (freeze-drying) process. Lyophilization is necessary for drug formulations which exhibit low liquid stability; and during the lyophilization process, the aqueous formulation is cooled down to a sufficiently low temperature where primary drying is applied to generate a freeze-dried 'cake'. However, a good deal of knowledge about the particular formulation and the effects of temperature and time on its thermal characteristics is required to select a lyophilization formulations and conditions for generating a satisfactory cake mandates. The first most criteria in understanding the lyophilization process is the glass transition temperature, or  $T_g$ , of the given formulation. Generally, the process temperature is set below the subambient  $T_g$  of the formulation during primary drying to avoid 'collapse' of the product during lyophilization. Along with the glass transition temperature, data indicates that the magnitude of the change in heat capacity ( $C_p$ ) at  $T_g$  along with the occurrence of any recrystallization events can have a major effect on the success of avoiding collapse of the product. Thus, an analytical technique is required that produces accurate yields, sensitivity, reproducibility and recrystallization transformations in the subambient temperature regions.

### **Solution**

Differential scanning calorimetry (DSC) meets all the criteria that have to be kept in mind for production of successful cake during lyophilization. The DSC should offer the pharmaceutical scientist the following advantages:

- High sensitivity (the highest sensitivity of any commercial DSC instrument)
- Excellent sub ambient performance
- ease of use
- Highly stable baseline characteristics

With an apparatus of such caliber, the scientists can quantitatively examine and test the following characteristics associated with the generation of proper cake formulation: cryoprotectors

- Buffer salts
- Surfactants

The cryoprotectors are typically sugars (e.g., sucrose or mannitol) that preferentially bind to the 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 a good DSC system, which is increasingly becoming a demand in a well-equipped pharmaceutical R&D center. 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.<sup>16</sup>

### **SOME EVENTS TO PROVE THE NEED OF DSC IN LYOPHILIZATION**

Many pharmaceutical products are prepared in a freeze-dried (lyophilized) format. However, these materials can be difficult to characterize particularly as far as stability with respect to storage temperature and the effect of water is concerned. DSC and its modifications are extremely useful tools for examining the  $T_g$  properties of freeze-dried pharmaceutical systems. These analysis methods when combined with TGA (Thermogravimetric analysis) provide a powerful means for investigating the effect of temperature and water content on the stability of this inherently unstable systems.<sup>17</sup> DSC analysis of diclofenac sodium performed under dynamic flow of either synthetic air or helium or nitrogen did not produce any sharp endothermic peak characteristic of melting peak of a pure substance. Both the rate of scanning of the sample and the environmental atmospheric condition significantly affected the thermographic profile of diclofenac sodium. An exothermic peak prior to an endothermic peak corresponding to melting of the substance appeared when heated under dynamic flow of synthetic air suggesting oxidation (decomposition) of diclofenac sodium

before reaching its melting point. In fact, at a scanning rate of 1 °C/min only the exothermic peak appeared in the thermogram, suggesting complete decomposition prior to melting under the dynamic flow of synthetic air. At 126°C the drug were found to produce a sharp endothermic peak characterizing its melting point.<sup>18</sup> The difficulty of formulating cephalosporins as freeze-dried products lies in the freezing characteristics of their aqueous solutions. The thermal characteristics of aqueous solutions of several cephalosporins were examined by Differential Scanning Calorimetry (DSC) during freezing and warming cycles. It was found that DSC studies of cephalosporin solutions show that they typically do not crystallize during freezing and do not form eutectics. The ones examined also do not go into metastable states during freezing. In addition, the physical state of the “frozen” matrix (in terms of presence or absence of a liquid phase) cannot be clearly ascertained by DSC because the compounds do not form eutectics with water. Therefore, in the determination of parameters to be employed in the design of an optimum lyophilization cycle for compounds like these, DSC may be of limited use. The compound did not crystallize from such solutions and do not form eutectic mixtures with water. Also, freezing did not produce any detectable metastable states of the solutes. Therefore, in the determination of parameters to be used in designing an optimum freeze-drying cycle for compounds such as these, DSC may be of limited use.<sup>19</sup> It is very important to characterize the freeze-dried matrix and to investigate the conservation of the nanoparticle properties. Furthermore such characterization may validate the applied conditions of the process and the optimized formulation. During storage, freeze-

dried nanoparticles included within a vitrified matrix of lyoprotectant must be stored at a temperature below the temperature of glass transition ( $T_g$ ) of the dried formulation to prevent any shrinkage of the freeze-dried cake or any destabilization of included nanoparticles as a result of lyoprotectant crystallization. The temperature of glass transition may be determined by differential scanning calorimetry. Furthermore, this technique is very useful to study the interaction between the lyoprotectant and the nanoparticles. For example, in the case of solid lipid nanocapsules freeze-dried with trehalose, DSC study points out a complexation between lecithin (forming the shell of nanocapsules) and trehalose, reinforcing the stabilizing properties of lecithin.<sup>20</sup> Studies have suggested that modulated temperature differential scanning calorimetry (MTDSC) has been used for the detection of the glass transition temperature ( $T_g$ ) in freeze-dried cakes of lyoprotected liposomes and analysis of frozen carbohydrate solutions. The glass transition appeared in the reversing heat flow, whereas the bilayer melting endotherm was observed in the nonreversing heat flow. This enabled the detection of  $T_g$  even in samples where the glass and bilayer transition overlapped. Along with this, relaxation processes occurring in nonannealed freeze-dried carbohydrate–liposome mixtures, which hinder the determination of  $T_g$  with conventional DSC, were also separated from the heat capacity related heat flow. Analysis of frozen carbohydrate solutions with MTDSC facilitated the identification of the glass transition, devitrification peak, and “softening” transition, which could help to further optimize freeze-drying conditions by rationale.<sup>21</sup>

## CONCLUSION

Lyophilization being an critical process in pharmaceutical manufacturing, it has been found to be very difficult to optimize the detection of glass transition events in frozen formulations, presenting a challenge to the formulation scientists due to the inherently weak nature of these transitions. Thus DSC presenting a necessary high degree of sensitivity, resolution and stable sub-ambient performance necessary to observe the weak glass transition and

recrystallization events associated with lyophilized formulations. It has been found to be more efficient in providing information on thermal properties of formulations, and aid in the knowledge regarding the propensity of formulations to avoid collapse during processing by taking care of certain key parameters v.i.z glass transition temperature, magnitude of glass transition and occurrence of recrystallization. Thus DSC presents one of the best way for generation of pharmaceutically elegant lyophilized formulations.

## REFERENCES

1. Kasper JC. Formulation of nucleic acid nanoparticles. Formulation development, stabilization mechanism and process control. Dissertation zur Erlangung des Doktorgrades, der Fakultat für Chemie und Pharmazie, der Ludwig-Maximilians-Universitat Munchen, aus Günzburg, 2012.
2. Carpenter JF, Pikal MJ, Chang B.S, Randolph TW. Rational Design of stable lyophilized protein formulations: Some practical advice. *Prarm. Res.* 1997; 14; 969-975.
3. Wang W. Lyophilization and development of solid protein pharmaceuticals. *Int. J. Pharm.* 2000; 203; 60.
4. Christ M. Smart freeze drying. Basic principles, optimum procedures and applications. Crirst, Postfach. 1713.
5. Goliath website. Biopharm. International. 2007.
6. Day JG, Stacey GN. Cryopreservation and freeze-drying protocols. *Methods in molecular biology* 2007; 368(2).
7. Liapis AI, Bruttini R. *Freeze drying*. Taylor and Francis Group, LLC. 2006.
8. Patapoff TW, Overcashier DE. The importance of freezing on lyophilization cycle development. *BioPharm.* 2002; Info #16; 16-22.
9. A guide to freeze drying for the laboratory. Labconco Corporation, U.S.A. 2004.
10. Milton N. *Pharmaceutical freeze drying: the lyophilization process*. Product Development. Eli Lilly and Co.
11. Pisano R, Fissore D, Barresi AA. Heat transfer in freeze-drying apparatus. Chapter No. 6, Development of heat transfer. (Cited 2013 Aug28). Available from [www.intechopen.com](http://www.intechopen.com), 91-115.
12. Brown ME. *Introduction to thermal analysis: techniques and application*. Kluwer Academic Publishers. 2001.
13. Klancnik G, Medved J, Mrvar P. Differential thermal analysis (DTA) and differential scanning calorimetry (DCS) as a method material investigation. *RMZ-Materials and Geoenvironment.* 2010; 57(1); 127-142.
14. Nicula R. *Introduction to differential scanning calorimetry*. Physics department. Rostock University.
15. Satpute, Y. *Thermal DCS 8500. Experimental Notes*. Thermal Pharma.
16. DSC as problem-solving tool: optimization of the lyophilization process. *Thermal analysis and surface solutions GmbH*.
17. Kett, V. *Thermal analysis – the use of DSC and MTDSC in analysing freeze-dried formulations and products*. *Am. Pharm. Rev.* 46-49.
18. Tudia P, Khan ZI, Mestrovic E, Horvat M, Golja P. Thermal behavior of Diclofenac Sodium: decomposition and melting characteristics. *Chem. Pharm. Bull.* 2001; 49(10); 1245-1250.
19. Williams NA, Polli GP. Differential scanning colorimetric studies on frozen cephalosporin solutions. *Int. J. Pharmaceutics.* 1988; 44; 205-212.
20. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: formulation, process and storage considerations. *Adv. Drug Deliv. Rev.* 2006; 58; 1688-1713.
21. Winden ECAV, Talsma H, Crommelin DJA. Thermal analysis of freeze-dried liposome-carbohydrate mixtures with modulated temperature differential scanning calorimetry. *Int. J. Pharm. Sc.* 1998; 87(2); 231-237.