J Therm Anal Calorim (2009) 98:203–206
DOI 10.1007/s10973-009-0317-8

The influence of crucible material on the DSC thermal analysis compared to freeze-drying microscopy results

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Received: 18 October 2008 / Accepted: 30 January 2009 / Published online: 7 August 2009

Abstract The aim of this study was to investigate the influence of different crucible materials on the thermal analysis of binary systems. The thermal properties of two distinct solutions were measured both by Differential Scanning Calorimetry (DSC) and freeze-drying microscopy and the results were compared. The glass transition of the maximally freeze-concentrate (T_g') and the eutectic melting temperature (T_{eut}) were not influenced by the crucible material. However the heat of fusion (ΔH) involved during the T_{eut} as well as the ΔC_p involved during the T_g' of the solutions were affected.

Keywords Lyophilization · Eutectic temperature · Glass transition temperature · Differential scanning calorimetry · Freeze-drying microscopy

Introduction

The intent of low-temperature thermal analysis is to identify the critical values for freezing and primary drying as well as the characteristics that contribute to the behavior of a product during the freeze-drying process. Also, in order to decrease the time and energy consumption, it is necessary to study the thermal properties of the product before it is freeze-dried to optimize the drying conditions while maintaining the highest quality of the product. Therefore, it is important to have precise and reliable knowledge of this parameter in order to maintain the product temperature as high as possible during the sublimation period without going beyond the collapse temperature [1]. Cooling a liquid below some temperature usually results in crystallization. However under special conditions, the same liquid undergoes an increase of the viscosity without crystallization, the glass transition that leads to glass formation. Owing to this fact, the glass may be defined as a material of a thermally arrested molecular arrangement of a liquid state. Hence, the generally used glass transition temperature, T_g depends on cooling rate and thermal history [2]. During freezing step if a solute crystallizes from solution, it is well known that the maximum allowable product temperature during primary drying is the eutectic melting temperature (T_{eut}). T_{eut} is the temperature at which a liquid phase begins to appear in the frozen solution during warming. During freeze-drying, the temperature of the product should not reach T_{eut} or a melt-back will occur consequently ruining the product. A more common type of freezing behavior, however, is where the solute remains amorphous during the freezing process, and the freezing-concentrated phase becomes more viscous as freezing proceeds. This continues until the temperature reaches the glass transition of the maximally freeze-concentrated (T_g'), below which the amorphous phase becomes glassy [3]. With primary drying below this transition temperature the product will retain the microstructure, which was established by freezing. At some temperature above this transition, called collapse temperature (T_c), the amorphous phase will undergo viscous flow during the time

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scale of freeze-drying, resulting in a collapsed product and general loss of the desirable properties of a freeze-dried solid [4]. It is very important to point out that T_g' is a second order transition and it represents the temperature at which mobility of amorphous phase in the maximally freeze-concentrate becomes significant in the time scale, while collapse is a dynamic phenomenon taking place in the freeze-drying front where the interstitial water in the frozen matrix becomes significantly mobile. The basis for such definition is that ion conduction in the interstitial region require the presence of mobile water. If mobile water is present, then the interstitial region cannot be completely frozen, in accordance to electrical measurements to determine the collapse temperature [5]. However T_c is also often considered as close to the T_g' and it was shown to take place 1–2 °C above the glass transition temperature [6]. Differential Scanning Calorimetry (DSC) has been used as a mean of assessing physicochemical (endothermic and exothermic) behavior during freezing and warming. Calorimetrically, T_g is usually defined at the midpoint of the ΔC_p (T_g) value. It should be emphasized, there are kinetic and thermodynamic contributions to the C_p value in the glass transition region [7]. It is used to determine phase transitions that correlate the crystallization and melting of a product to various temperatures as well the T_g' of an aqueous formulation [8]. To perform DSC analysis, aqueous formulations are commonly put into an aluminum crucible. Whereas, lyophilized formulations are most often manufactured in a type I glass container. The aim of this study was to investigate the influence of different crucible materials on the results of DSC thermal analysis of two distinct solutions: 5% trehalose solution and 0.9% Sodium Chloride solution. In addition, the T_c and T_{eut} of these solutions were measured by freeze-drying microscopy and systematically compared to the T_g' and T_{eut} measured by DSC in order to confirm (or not) in complex media the similarity of values observed in binary aqueous solutions.

Materials and methods

Chemicals

The model solutions were prepared as followed: 5% trehalose solution (m/m) of d(+)-Trehalose dihydrate (Sigma-Aldrich-USA) and a Sodium Chloride solution 0.9% (m/m) (Aster produtos médicos Ltda—Brazil).

Crucible materials (DSC pans)

Three different DSC pans were used during the analysis: 40 μL (microliter) aluminum standard crucible, 40 μL quartz crucible and 40 μL type I glass crucible, each with different thermal conductivity values (Table 1) [9]. The quartz and type I glass DSC pans were constructed following the same dimensions of the aluminum pan to prevent the influence of mass on the crucible material heat capacity during the analysis. The crucibles were made by Wiesberg—glass materials, RJ, Brazil.

Table 1 Thermal conductivity of materials

Crucible material	Thermal conductivity/w m^{-1} $°C^{-1}$
Aluminum	237
Type I Glass (borosilicate)	1.14
Quartz	1.4

Differential scanning calorimetry

DSC was used to determine the T_g' and T_{eut} of these binary systems. The DSC used was a Mettler-822 equipped with cooler sub-ambient accessory. Temperature calibration was done using distillated water (melting point at 0 °C and $\Delta H_{fusion} = 335$ J g^{-1}). For each different crucible material an empty pan of the same material was used as reference. DSC experiments were conducted under atmosphere of 50 mL min^{-1} nitrogen flow rate. To eliminate the influence of the fill volume on the crystallization temperature, exactly 40 μL of each solution were poured into each open crucible, and then cooled from 25 °C to −50 °C at 5 °C min^{-1} cooling rate followed by a 5 min isotherm to ensure temperature stability and sample equilibration. After cooling step, the samples were heated to 25 °C using a heating rate of 2 °C min^{-1}. All T_g' were recorded as midpoint temperatures and the relative variation of the heat capacity across the glass transition (ΔC_p, in J g^{-1} $°C^{-1}$) were calculated.

Freeze-drying microscopy

Solution collapse temperatures were determined by a freeze-drying microscopy (FDCS 196, Linkam Scientific Instruments, Surrey, U.K.) equipped with a liquid nitrogen cooling system and a programmable temperature controller (TMS94, Linkam). The stage was calibrated for temperature measurement with an aqueous solution of NaCl 0.9% (m/m) (T_{eut} of approximately −21.1 °C). The samples were frozen at −50 °C (5 °C min^{-1}) followed by a 5 min isotherm to ensure temperature stability and sample equilibration. During the drying step the system was evacuated to a pressure of 100 mTorr (Edwards vacuum pump E2M1.5) maintained by a Pirani pressure monitor gauge. The frozen sample was heated using a 2 °C min^{-1} heating rate. Direct

observation of freezing and freeze-drying was done by a Nikon Elipse E600 (Nikon, Japan) polarized microscope using a 10× magnification.

Results

Differential scanning calorimetry

Figure 1 compares the influence of different crucible materials in the T_{eut} of NaCl solution. The NaCl solution readily crystallizes during cooling of the frozen solution and is known to form a eutectic mixture from binary solutions. It was observed that NaCl solution T_{eut} peak in glass (−19.96 °C) and quartz crucibles (−20.13 °C) decreased by 0.87 and 0.7 °C, respectively compared to the aluminum experiments (−20.83 °C). However, there was an almost 3-fold increase of enthalpic energy involved during the eutectic melting (ΔH_{eut}) between the glass ($\Delta H_{eut} = -10.81$ J g^{-1}) and quartz crucibles ($\Delta H_{eut} = -9.84$ J g^{-1}) compared to the aluminum crucible ($\Delta H_{eut} = -3.65$ J g^{-1}). This behavior could be explained due to the specific thermal conductivity differences between each crucible material (Table 1). Higher thermal conductivity (aluminum crucible) dissipated energy in shorter time did not allowing enough time to the sample response, it was confirmed by the difference of integrated area under each peak. On the other hand the crucibles (quartz and glass) that had presented lower thermal conductivity had enough time to transfer the energy to the sample increasing the integrated area under each peak (higher ΔH_{fusion}). The same differences were observed during freezing crystallization on each sample and crucibles (data not shown). According to Willemer et al., differences on $\Delta H_{crystallization}$ were observed studying the influence of different glass containers used during freezing in freeze-drying 1 and 5% mannitol and 10% sucrose solution. He stated that during freezing, the different forces between the walls and liquid influence the structure of the freezing product and it subcooling. The freezing speed in the quartz vials was up to 16% greater than standard vials (glass) but in the resin vials the freezing speed was 14% lower [10].

Trehalose, a non-reducing disaccharide that is retained amorphous in frozen aqueous solutions, was chosen as the model amorphous compound. There has been considerable attention to trehalose in the recent past owing to its ability to serve as a lyoprotectant. During cooling it tends to form a supersaturated freeze-concentrated solution due to incomplete crystallization of water. In this case, the lyophilization characteristics of the formulation are determined by the glass transition temperature of the maximally freeze-concentrate solute (T_g') [11].

Figure 2 shows the influence of different crucibles on the T_g' of a 5% trehalose solution. It is characterized by a discontinuity on the DSC baseline due to the change in heat capacity that occurs in the glass transition. Trehalose T_g' value (−30 °C) is in accordance with published works [12–14]. The T_g' was not influenced by the crucible material, however, a considerable difference in the specific heat capacity (ΔC_p) between the crucible materials was observed during the experiments. A 4-fold increase on the ΔC_p was observed for the quartz ($\Delta C_p = 0.242$ J g^{-1} °C^{-1}) and glass ($\Delta C_p = 0.275$ J g^{-1} °C^{-1}) compared to aluminum crucible ($\Delta C_p = 0.069$ J g^{-1} °C^{-1}). This difference could be explained by the thermal conductivity and heat capacity specific for each crucible material.

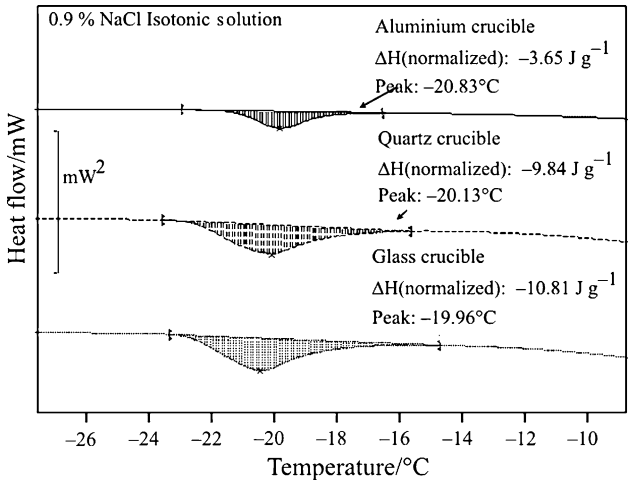

Fig. 1 Eutectic melting (T_{im}) temperature of NaCl using crucible of different materials. The sample was cooled to −50 °C at 5 °C min^{-1} followed by a heating at 5 °C min^{-1} until 25 °C

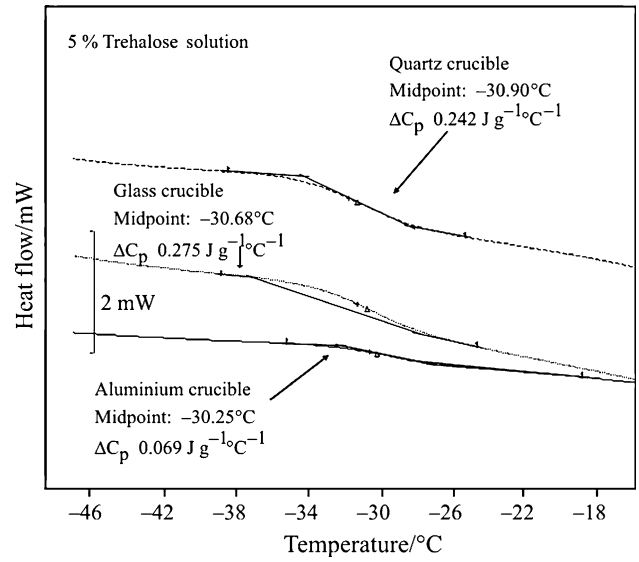

Fig. 2 DSC curves showing the T_g' of a 5% trehalose solution using different crucible materials. The sample was cooled to −50 °C at 5 °C min^{-1} followed by a heating rate at 5 °C min^{-1}

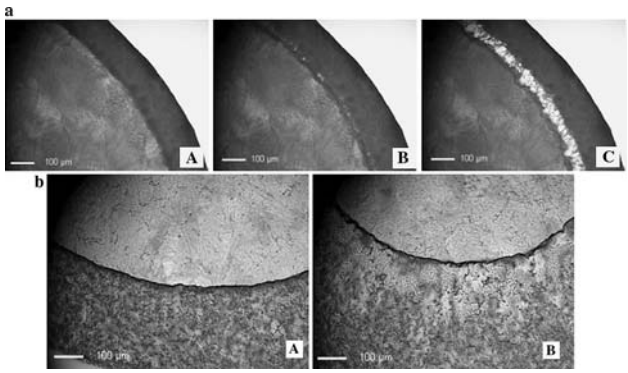

Fig. 3 **a** Microscopic observations of freeze-drying 5% trehalose solution: (A) structure retention in the dried region ("not collapsed") at –28 °C; (B) local loss of structure ("microcollapse") at –25.2 °C; (C) overall loss of structure ("collapse") at −23.9 °C. **b** Microscopic observations of freeze-drying 0.9% NaCl solution: (A) structure retention in the dried region ("not collapsed") at –30 °C; (B) overall loss of structure ("collapse") at –21 °C

Freeze-drying microscopy

The notion that T_c is often considered as close to the T_g' (1–2 °C above the glass transition temperature) should be carefully considered. We observed that trehalose freeze-drying front collapsed around −25 °C, a 5 °C above the T_g' by DSC analysis (Fig. 3a). There are specific experiments using freeze-drying microscopy and DSC where differences of more than 4 °C between T_c and T_g' were previous observed.

The loss of structure for a system composed of a glassy state occurs at the freeze-drying front, and the remainder of the ice matrix retains its original structure. However when an eutectic is present in the interstitial region, the entire matrix undergoes a structural change when the sample temperature reaches the eutectic temperature. This was the case for the NaCl solution (Fig. 3b), where the $T_{eut} = -21$ was closely related to the T_{eut} determined by DSC (Fig. 2).

Discussion

DSC is a very useful tool to determine the critical temperatures (T_g' and T_{eut}) during the lyophilization process. However DSC is performed on microliter volumes, with samples sealed in aluminum crucibles, whereas the solutions to be lyophilized are likely to be dosed in milliliter quantities in open glass vials. In this study, the thermal characterization of two distinct formulations was performed by DSC using three different crucible materials: aluminum, type I glass and quartz and the results were compared to thermal characterization trough freeze-drying microscopy. The results showed that T_g' and T_{eut} were not influenced by the crucible material type. However, the ΔH involved in the eutectic melting and the ΔC_p involved during the T_g' of the formulations were affected. This behavior could be explained by the differences in the specific heat capacity and thermal conductivity of each material used in this work. The aluminum crucible dissipated the energy involved in each thermal event faster than type I glass and quartz. During solution crystallization, it could influence the ice crystal size distribution and morphology. The aluminum crucible normally used in DSC thermal analysis does not represent the real values involved during the eutectic melting and the glass transition temperature occurred during the lyophilization of a formulation in glass vials.

According to the results obtained, both techniques, freeze-drying microscopy and DSC, showed good agreement between them, however more care should be taken regarding the notion that T_c is often considered 1–2 °C above the T_g'.

Acknowledgements The authors are grateful to FAPESP (The State of Sao Paulo Research Foundation) and to CNPq (National Council for Scientific and Technological Development) for the financial support.

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