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Quantification of low levels of amorphous content in sucrose by hyperDSC

Minna Lappalainen[a,*], Ilkka Pitkänen[a], Päivi Harjunen[b]

[a] *University of Jyväskylä, Department of Chemistry, P.O. Box 35, FIN-40014 University of Jyväskylä, Finland*
[b] *University of Kuopio, Department of Pharmaceutics, P.O. Box 1627, FIN-70211 University of Kuopio, Finland*

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Abstract

A method was developed for the quantification of low levels of amorphous content in sucrose with hyperDSC. The method was based on the fact that the change of specific heat at the glass transition is linearly proportional to the amorphous content. It was found out that as annealing time increased, the glass transition temperature moved to a higher temperature and the change of specific heat increased. ΔC_p for annealed totally amorphous sucrose was $0.761 \pm 0.012\ \mathrm{J\,g^{-1}\,{}^{\circ}C^{-1}}$. Synthetic mixtures with various proportions of crystalline and amorphous sucrose were prepared. The following linear regression between ΔC_p and amorphous content was obtained: $\Delta C_p = 0.0075x - 0.00484$ ($R = 0.999$). The limit of detection (LOD) and the limit of quantification (LOQ) values were 0.062 and 0.207%, respectively. The effect of grinding time on the amorphous content of crystalline sucrose was studied and a correlation between grinding time and amorphous content of sucrose was found. It was also found that the amorphous content could only attain a value of about 80–90% by grinding in the way used in this study.

Keywords: Sucrose; Amorphous content; Glass transition; HyperDSC

1. Introduction

Pharmaceutical processing (milling, lyophilization, granulating, drying) is often responsible for a fully or partially amorphous state of an otherwise highly crystalline material. The presence of amorphous material in crystalline substances can have considerable impact on the physical properties of the sample. In view of the significant effects that the state of disorder in crystalline solids can have on the properties of pharmaceutical materials, it would be important to be able to assess the extent of disorder in a solid quantitatively, down to very low levels. The amorphous state may be generated accidentally and in many ways this can be the most problematic, as the levels of disordered material may be sufficiently large to cause changes in product performance but also too small to be easily detected. A small degree of amorphous character is not often detectable by standard techniques. For that reason, the quantification of amorphous content is a widely studied problem.

There are many techniques for the determination of amorphous content. One of the newest techniques is hyperDSC (high speed or high performance DSC) where the sensitivity is significantly increased by using high heating rates (Robinson, 2002). HyperDSC has been reported to have a high sensitivity using controlled fast heating and cooling rates of 50–500 °C $\mathrm{min^{-1}}$ (Robinson, 2002; Pijpers et al., 2002). This significantly increases the sensitivity because the increased scan rate leads to higher heat flow. The DSC output is measured as a function of heat flow (mW), which can also be rewritten as energy per unit time ($\mathrm{J\,s^{-1}}$). At fast scan rates the same heat flow occurs over a shorter time period and the thermal event becomes larger. Therefore, the use of accelerated heating rates allows extremely low-energy transitions that would be below the limit of detection at the more typical heating rates employed in conventional DSC to be identified and measured with ease and also allows the measurement of much smaller samples (Robinson, 2002; Saunders et al., 2004; McGregor et al., 2004; Gabbott et al., 2003). Whereas amorphous character can be difficult to detect in highly crystalline solids using conventional DSC technique, it is also possible to use higher scanning rates to aid visualization of the T_g. HyperDSC can show glass transitions in less time with much increased sensitivity (Robinson, 2002). HyperDSC is able to provide valuable information rapidly and on small samples, opening a new area for research in amorphous materials (Saunders et al., 2004).

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[*] Corresponding author. Fax: +358 14 2602501.
E-mail address: minna.lappalainen@cc.jyu.fi (M. Lappalainen).

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Sucrose is a very common sugar and the amorphous content in sucrose sample has been determined in many studies. For example, X-ray powder diffraction, density, conventional DSC, water vapour sorption, microcalorimetry and solution calorimetry has been used for quantification of amorphous content in sucrose (Saleki-Gerhardt et al., 1994; Raemy et al., 1993; Gao and Rytting, 1997). In X-ray powder diffraction method, the limit of determination is 10% amorphous content. Density technique is less capable of detecting low amorphous levels than X-ray powder diffraction. With conventional DSC technique there is possibility to determine about 5% amorphous content. Microcalorimetry and water vapour sorption techniques are more sensitive in detecting low amorphous levels and the determination limits are at least 1% (Saleki-Gerhardt et al., 1994; Raemy et al., 1993).

Sucrose can be obtained in a highly pure crystalline state and it can also be easily formed into a completely amorphous state by lyophilization (Saleki-Gerhardt and Zografi, 1994) or spray drying (Christensen et al., 2002). Amorphous sucrose has also been formed by melting followed by cooling (Urbani et al., 1997; Vanhal and Blond, 1999; Orford et al., 1990). This produces a good-grade amorphous solid; however, the melting of sucrose is anomalous due to partial thermal decomposition and poorly reproducible amounts of decomposition products are formed (Hurtta et al., 2004; Carstensen and Van Scoik, 1990). For that reason, the amorphous sucrose should be formed some other way. Sucrose can be retained in the amorphous state under dry conditions and room temperature, yet it can be induced to crystallize over short and convenient time periods when the temperature is raised above its glass transition temperature, T_g, or when subjecting the sample to moderate relative humidities and to absorption of water that reduces T_g (Saleki-Gerhardt and Zografi, 1994; Roos and Karel, 1991; Shamblin and Zografi, 1999; Shamblin et al., 1996).

Gloria and Sievert (2001) and Saleki-Gerhardt et al. (1994) used the enthalpy of crystallization peak of amorphous sucrose in quantification of its amorphous content by DSC. In the present work we developed the method of quantification of the amorphous content of sucrose by hyperDSC. This method is based on the fact that the change of specific heat (ΔC_p) in the glass transition is linearly proportional to the amorphous content if the amorphous glasses are in the same state. In our earlier studies (Hurtta and Pitkänen, 2004) this method was tested with maltitol with good results. In addition, because grinding is often used in industry, we studied the effect of grinding time on the amorphous content of crystalline samples.

2. Materials and methods

Crystalline D(+)-sucrose with purity of >99.5% was purchased from Fluka BioChemica. The crystalline sucrose was dried at 50 °C for 2 days and then stored in a desiccator over P_2O_5.

Amorphous sucrose was prepared from a 30% (w/w) sucrose–water solution by spray drying with a Büchi Mini-Spray Drier 190 (Büchi Laboratorium-Technic AG, Switzerland). The spray drying conditions were: air flow rate 15 (dial setting), atomizer air flow rate 500 ml min^{-1}, heating rate 12 (dial setting), inlet temperature 160 °C, outlet temperature 110 °C and feed rate 6 ml min^{-1}. The nozzle diameter was 0.7 mm. Spray dried sucrose was measured with powder diffraction and was found to be totally amorphous because no peaks were found. After spray drying, the samples were stored at room temperature in a desiccator over P_2O_5 before and during the studies.

The particle morphology and shape of spray dried sucrose sample were evaluated with a scanning electron microscope (SEM) (XL 30 ESEM TMP microscope, FEI/Philips, the Czech Republic). Samples were coated with gold under vacuum (Sputter Coater II-E 5100, Polaron Equipment, UK). All micrographs were taken at an acceleration voltage of 15 kV. The spray drying was successful and the spray dried sucrose sample appeared amorphous.

Powder diffraction measurements were carried out using a Huber Imaging Plate Guinier camera G670. The X-ray source was germanium crystal monochromatized Cu $K\alpha_1$ radiation ($\lambda = 1.54056$ Å) and the X-ray tube was operated at 45 kV and 25 mA. The samples were prepared on Mylar film using vaseline. Exposure time was 30 min and the imaging plate was scanned six times. ZDS software was used to process the measurement data.

DSC measurements were carried out on a Perkin-Elmer Pyris DSC Diamond using 50 μl aluminium sample cups with capillary holes and a dynamic nitrogen atmosphere with a flow rate of 50 ml min^{-1}. The sample weights were 4–6 mg. The temperature calibration was done with indium and zinc standards and the heat flow was calibrated by the specific heat of sapphire. The base calibration was done with a heating rate of 10 °C min^{-1}. All measured glass transition temperatures were corrected for a thermal lag of 0.037 min^{-1} (Hurtta and Pitkänen, 2004).

The development of a method for the determination of amorphous content was started by studying the effect of annealing on the change of specific heat at the glass transition. The annealing temperature 60 °C was chosen (Urbani et al., 1997; Shamblin et al., 1999; Shamblin and Zografi, 1998) and the different annealing times of 0.5, 1, 2, 4, 5 and 6 h were used. The temperature program was: (a) heating from room temperature to annealing temperature (from 25 to 60 °C) at a heating rate of 10 °C min^{-1} and (b) annealing in 60 °C for t_a (min), then (c) cooling to the starting temperature (10 °C) at a cooling rate of 10 °C min^{-1} and finally (d) heating from starting temperature over the glass transition region (from 10 to 120 °C) at 100 °C min^{-1}.

Synthetic mixtures were prepared by weighing known quantities of amorphous and crystalline sucrose at various ratios (1, 5, 10, 20, 50 and 75 w/w) amorphous content and by mixing them thoroughly but gentle (without grinding) in a porcelain mortar. Mixtures were prepared one at the time and the measurements were done immediately afterwards the mixture was ready. In addition, amorphous and crystalline samples were used. Three parallel measurements were taken at each test point. Synthetic mixtures were measured with the above mentioned temperature program, where t_a (annealing time) (step (b)) was 300 min (5 h) and the heating scan (step (d)) was carried out from 10 to 140 °C.

The effect of mechanical treatment was studied by grinding crystalline sucrose for different times. The grinding was done at room temperature in a ball mill (FRITSCH pulverizette, type 07.302, Germany) for 2.5–60 min at a speed 8 (scale 1–10). The amount of sucrose was about 1 g. The volume of grinding vessel was 25.0 cm^3 (±1.0 cm^3) and the diameters of the three metal balls used were 15.00 mm (±0.10 mm) for each. The crystalline sucrose was dried in a desiccator over P_2O_5 before grinding. In addition, the grinding vessel and the metal balls were dried in an oven at about 115 °C for 1 h and then in a desiccator over silica gel for at least 2 h every time before grinding. The ground samples were measured immediately after grinding with DSC using the same temperature program that was used for the synthetic mixtures.

Table 1
The effect of annealing time on the T_g and ΔC_p results. The heating rate used was 100 °C min^{-1}

Annealing time (h)	Fictive temperature		Half point temperature	
	T_g (°C)	ΔC_p (J g^{-1} °C^{-1})	T_g (°C)	ΔC_p (J g^{-1} °C^{-1})
0	72.9	0.47	75.5	0.45
0.5	74.3	0.59	83.5	0.59
1	74.8	0.62	84.7	0.62
2	74.8	0.65	85.6	0.65
3	74.6	0.62	86.9	0.66
4	78.7	0.72	87.2	0.72
5	78.0	0.76	86.5	0.75
6	78.5	0.77	87.6	0.76

3. Results and discussion

3.1. Calculation of glass transition and ΔC_p

There are many ways to determine the glass transition temperature. Standard T_g is the temperature at which the heat capacity is midway between those of the liquid and glassy states, or the temperature corresponding to the point of the heat flow curve where the specific heat change is 50% of the change in the complete transition (Wunderlich, 1990). In our earlier study (Hurtta and Pitkänen, 2004) we also used the fictive temperature, which refers to the point on the enthalpy curve where the change of slope occurs (Perkin-Elmer). However, we noticed that there were no significant differences between the results of ΔC_p calculated from the half point temperature and the fictive temperature, and the calculations of synthetic mixtures in this study were performed using only the half point method (Hurtta and Pitkänen, 2004).

The calculation of glass transition was problematic in synthetic mixtures because the crystallization of amorphous sucrose started soon after the relaxation peak and for that reason, the baseline after the glass transition was not a straight line. Therefore, the glass transition was calculated adjusting first the tangent before glass transition and then adjusting the tangent after glass transition being parallel to it (Hurtta and Pitkänen, 2004).

For ground samples, the crystallization started just after glass transition and the crystallization temperature was lower than in the synthetic mixtures. This might be due to the small particle size of ground samples. Also Font et al. (1997) noted that the amorphous sucrose obtained by grinding is less stable than that prepared by other means. The appearance of crystallization confirms this fact. The presence of crystalline phase significantly reduces the induction period, presumably by acting as seeds in the sample and increasing the rate of crystal growth (Saleki-Gerhardt and Zografi, 1994). In these measurements, the final temperature needed for the calculation of glass transition was found by using the baseline after the crystallization peak.

3.2. The effect of annealing time on ΔC_p

It is known that amorphous glasses can contain a varying degree of order depending on the thermal history of the sample. The non-equilibrium nature of the glassy state leads to structural relaxation, i.e. the spontaneous approach of the material towards equilibrium at a rate that depends on the temperature and the complete thermal history of the glass. The isothermal structural relaxation that occurs below the glass transition temperature is extremely slow and is often referred to as structural relaxation, physical aging or annealing (Urbani et al., 1997; Wungtanagorn and Schmidt, 2001; Kerč and Srčič, 1995; Roos, 1995; Yu, 2001). Glasses are known to change their properties when annealed at below their glass transition temperature (Urbani et al., 1997; Shamblin et al., 1999; Shamblin and Zografi, 1998; Claudy et al., 2002; Hancock et al., 1995). The release of the relaxation enthalpy that follows the glass transition corresponds to the enthalpy difference between the annealed and the quenched (non-annealed) glass (Claudy et al., 2002). Different cooling rates produce glasses of different degrees of order. The structure of non-annealed glass is close to the structure of liquid state and the change of specific heat is smaller for non-annealed than annealed glass (Wunderlich, 1990). In addition, the glass transition temperatures of amorphous sugars and sugar alcohols are extremely sensitive to water (Roos, 1995; Hancock et al., 1995). Annealing affects the change of specific heat so that ΔC_p is larger for annealed amorphous glass than for quenched amorphous glass. Also annealing time is known to affect the ΔC_p (Hurtta and Pitkänen, 2004; Wunderlich, 1990).

The development of a method for quantification of amorphous content of sucrose was started by finding out the annealing time that resulted in a constant ΔC_p. The purpose of annealing was to eliminate the effect of the thermal history of the sample. The results of annealing measurements are shown in Table 1. The change of annealing time affected on the glass transition temperature and the change of specific heat: as annealing time increased, the glass transition temperature became higher and the change of specific heat became larger. The annealing time chosen in measurements was 5 h, because the ΔC_p was almost the same as that for 6 h and the measurement time was a little shorter.

The glass transition of sucrose has been extensively studied and there are many published values, which are collected in Table 2. The values differ slightly from each other; the reason might be differences in the preparation of amorphous samples and different measurement techniques. A comparison indicates that our values of fictive glass transition temperatures are very close to temperatures reported in the literature. Also the half

Table 2
The literature values of T_g and ΔC_p of sucrose

T_g (°C)	ΔC_p ($J\,g^{-1}\,{}^\circ C^{-1}$)	Reference
48		Zeng et al. (2001)
52		Slade and Levine (1991)
57		Gloria and Sievert (2001)
66	0.55	Christensen et al. (2002)
67		Urbani et al. (1997)
67	0.60	Roos (1993)
70	0.77	Orford et al. (1990)
72		Vanhal and Blond (1999)
74		Saleki-Gerhardt and Zografi (1994)
74		Shamblin et al. (1996)
77	0.54	Hancock et al. (1995)
78	0.64	Shamblin and Zografi (1998)
78	0.56	Shamblin et al. (1999)

point glass transition temperature of non-annealed amorphous sucrose is close to literature values but the values of annealed samples are about 10 °C higher because of the high heating rate of 100 °C min^{-1} used. The values of ΔC_p of sucrose vary between different references. However, they are comparable with our results. Some values of ΔC_p are equal to our results of non-annealed samples (Christensen et al., 2002; Hancock et al., 1995; Shamblin et al., 1999). On the other hand, some values are close to our results for annealed samples (Roos, 1993; Orford et al., 1990; Shamblin and Zografi, 1998).

3.3. Synthetic mixtures

The ΔC_p values of synthetic mixtures were used to calculate average and standard deviation values. The mean values of specific heat change at the glass transition region were plotted against amorphous content and a linear regression line with R (correlation coefficient) values was calculated (Fig. 1). The equation is (x is amorphous content%):

$$\Delta C_p = 0.0075x - 0.00484, \quad R = 0.999.$$

Fig. 1 demonstrates the clear correlation between the ΔC_p and the amorphous content ($R = 0.999$). Limit of detection (LOD) and limit of quantification (LOQ) values were calculated as described earlier (Hurtta and Pitkänen, 2004). The LOD and LOQ values were 0.062% (amorphous content) and 0.207%. The corresponding values for maltitol were 0.107% (LOD) and 0.358% (LOQ) (Hurtta and Pitkänen, 2004). These values show that very low determination limits for the quantification of amorphous content can be attained with the hyperDSC method.

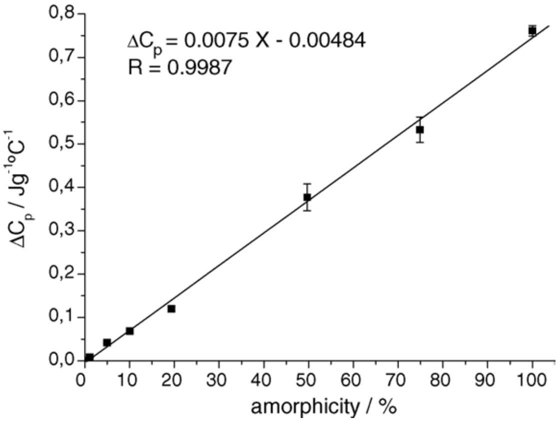

Fig. 1. The results of hyperDSC measurements. Average and standard deviation values of the change of specific heat at glass transition temperature as function of amorphous content ($n = 3$).

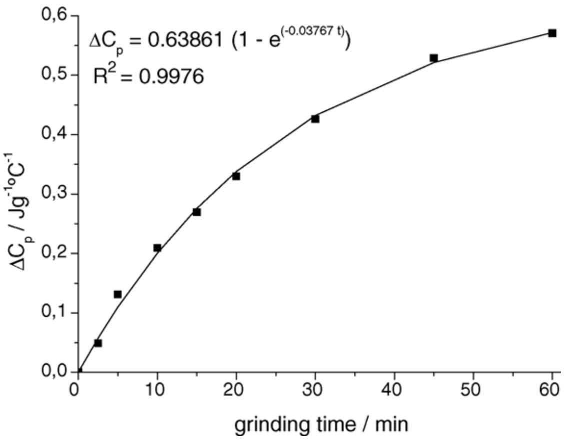

Fig. 2. The change of specific heat at glass transition temperature as a function of grinding time.

3.4. The effect of grinding on amorphous content

The effect of mechanical treatment was studied and a correlation was found between the grinding time and amorphous content. The ΔC_p values of ground samples were plotted against grinding time and the results are shown in Fig. 2.

ΔC_p increases with time (Fig. 2). An exponential fit for data points between ΔC_p values and grinding time with R^2 values was calculated and the experimental equation is:

$$\Delta C_p = 0.63861 - 0.63861\,e^{(-0.03767t)}, \quad R^2 = 0.998$$

where t is grinding time (min). As discussed earlier, ΔC_p is linearly proportional to amorphous content. At the start of grinding the sample was in a crystalline form and the measured ΔC_p was zero. As the grinding time was short, the ΔC_p increased fast because there was a large amount of crystalline material that became amorphous because of grinding. As the grinding time was increased, the amount of crystalline material remaining was reduced. During a long period of grinding the ΔC_p would approach constant value.

As the equation of ΔC_p and grinding time are connected to earlier equations between ΔC_p and amorphous content, the correlation between the grinding time and amorphous content can be calculated. The equation is

$$x = 85.7933 - 85.148\,e^{(-0.03767t)}$$

where x is amorphous content% and t is grinding time in minutes. This is illustrated in Fig. 3.

The amount of amorphous material generated during grinding is a function of many factors including the particle size distribution of the un-milled sample, the solid state properties of the

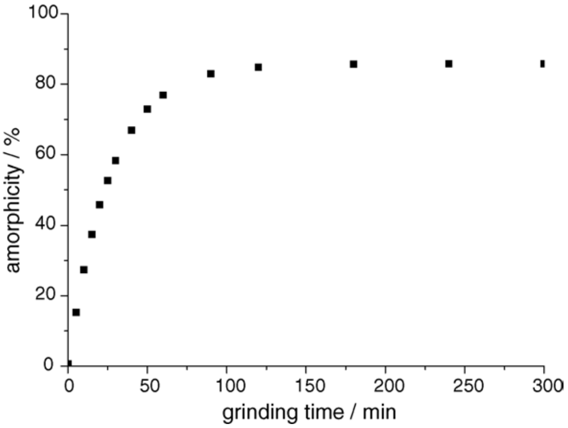

Fig. 3. Amorphicity as a function of grinding time. Calculated values.

sample, the type of milling technology and the milling conditions used. In the case of sucrose, the amorphous content increased rapidly in the first 60 min but it stabilized already in 2 h (120 min) (Fig. 3). According to this study, an amorphous content of only 80–90% can be attained by grinding in the way used in this study. Apparently recrystallization takes place during grinding, and for that reason totally amorphous sample is not able to form. This result is in line with earlier reports (Tsukushi et al., 1995, 1997; Font et al., 1997; Yamamuro et al., 1996).

4. Conclusions

In this study we established that the amorphous content of sucrose can be determined by measuring the change of its specific heat at the glass transition with hyperDSC.

We found the change of the specific heat at the glass transition (ΔC_p) to be a good indicator for the amorphous content on sucrose. As annealing and moisture have a substantial effect on the ΔC_p, samples had to be stored over P_2O_5 and measured immediately. Low LOD and LOQ values were attained due to rapid heating rate used in hyperDSC; thus, the method is suitable for determination of low levels of amorphous material. The method was used to find a correlation between grinding time and amorphous content of sucrose.

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