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Techniques of differential scanning calorimetry for quantification of low contents of amorphous phases

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Abstract Differential scanning calorimetry (DSC) is one of the most frequently used techniques for analyzing small concentrations of amorphous phases in a crystalline matrix. In recent years novel enhanced DSC approaches have been intensively looked for to improve parameters such as sensitivity, accuracy, and detection limit of the technique. Low levels of amorphous phases can be quantitatively determined in DSC by measuring the heat capacity change associated with the glass transition. In this short review the potentials provided by the HyperDSC and StepScan DSC techniques are discussed. Examples illustrate the advantages and disadvantages of the techniques and compare their abilities to detect small glass transitions and determine low contents of amorphous phases in samples which are mostly crystalline.

Keywords Thermal analysis · Differential scanning calorimetry · HyperDSC · StepScan DSC · Amorphous content · Glass transition

Introduction

The degree of crystallinity is an important parameter for various food and pharmaceutical systems as it affects their behavior during processing, storage, and consumption. Presence of even a tiny amount of amorphous component(s) in a crystalline product may have considerable impact on the stability, processability, and bioavailability of the material and its performance during product manufacture and use [1–3]. It is, therefore, of great importance to search for means to assess the extent of disorder in a solid quantitatively, down to very low concentration levels.

The amorphous phases in materials such as sugars and pharmaceuticals are typically formed unintentionally, and the challenge arises from the fact that the concentration of disordered material is often high enough to cause changes in product performance but yet too small to be easily detected [4–6]. A number of techniques are available for the detection and quantification of low levels of amorphous components, but the methods are usually sample-specific. There are several studies reported in the literature where different techniques for determination of the content of amorphous phase(s) are compared as analysis methods [5–10]. Each technique has its own advantages and disadvantages. The method of choice depends for example on the concentration level of the amorphous phase and the amount of sample available. In addition, there are differences in the sample preparation procedure and the time required by the measurement. Table 1 summarizes the various techniques employed.

Differential scanning calorimetry (DSC) is one of the most frequently used techniques for the measurement of the degree of amorphicity. Owing to difficulties in detection of very low concentrations of amorphous phases by means of the conventional DSC technique, in recent years novel DSC approaches have been intensively looked for to improve parameters such as sensitivity, accuracy, and detection limit. The high-speed or high-performance differential scanning calorimetry (HyperDSC) technique, in which an increased heating rate is used, significantly improves the sensitivity of DSC as it allows small transitions to be detected more readily [11–26]. Moreover, several versions of another approach have been proposed

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Table 1 Analytical methods employed and their detection limits for quantification of amorphous phases

Method	Detection limit/%	References
X-ray powder diffraction (XRD)	0.4–10	[57–61]
Differential scanning calorimetry (DSC)	1–20	[60–63]
High-speed DSC (HyperDSC)	0.2–1.5	[15–19]
StepScan DSC	0.8	[8, 44]
Temperature-modulated DSC (TMDSC)	0.9	[17, 64, 65]
Gravimetric moisture sorption (GMS)	0.5–1	[66, 67]
Solution calorimetry (SC)	0.5–1.8	[68–70]
Isothermal microcalorimetry (IMC)	0.5–5	[61, 67, 68, 71]
Dynamic mechanical analysis (DMA)	2	[72]
Density measurements	10	[5]
Solid-state nuclear magnetic resonance (SS-NMR)	0.5–3	[57, 63, 71]
Fourier transform infrared spectroscopy (FTIR)	1–2	[9, 73]
Raman spectroscopy	1	[74, 75]
Near infrared spectroscopy (NIR)	0.5–1	[59, 66]
Thermally stimulated current spectrometry	~1	[76]
Inverse phase gas chromatography	1	[77, 78]

including the temperature-modulated DSC (TMDSC) [27–39], TOPEM (advanced temperature-modulated DSC) [40–43], and StepScan DSC [44–56] techniques. These techniques allow for reversible changes to be distinguished from the potentially interfering irreversible kinetic events by way of temperature modulation. In this short review we discuss the abilities of the HyperDSC and StepScan DSC techniques to detect and quantify low contents of amorphous phases in samples which are mostly crystalline. In the earlier reviews dealing with the determination of amorphicity in general, the DSC methods have not been extensively discussed.

Glass transition seen by differential scanning calorimetry

A typical DSC scan for a totally or partially amorphous sample is shown in Fig. 1; the curve displays a glass transition, a crystallization exotherm, and a melting endotherm [79]. The glass transition is seen as a change in heat capacity (C_p) on heating. The magnitude of ΔC_p at glass transition temperature (T_g) varies from compound to compound, and this variance is the basis for the classification of glass formers to strong and fragile glass formers [80]. Above T_g the sample viscosity gradually decreases with increasing temperature such that at some point (crystallization temperature) the molecules gain enough freedom to spontaneously arrange

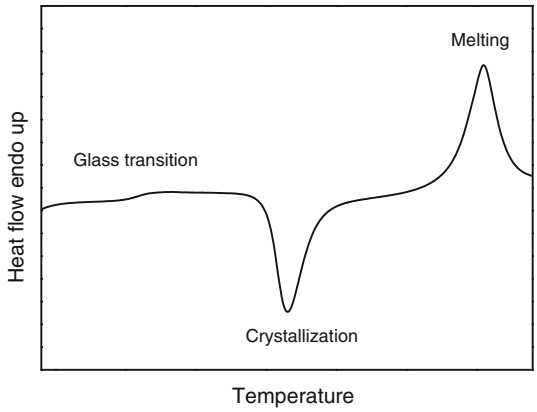

Fig. 1 Schematic DSC curve for a non-annealed amorphous sample upon heating

themselves into a form of crystalline solid. This first-order transition appears as an exothermic peak in the DSC curve. Finally upon further heating the sample melts. This gives an endothermic signal in the DSC data.

A special problem related to DSC studies dealing with the glass transition is caused by the fact that an amorphous system carries a memory of its thermal history. Different cooling rates result in glasses of different degrees of order. Note that the glassy solid is thermodynamically far from equilibrium, and only the liquid or rubber state is at thermodynamic equilibrium. Glasses are known to change their properties when annealed at temperatures below their T_g [81–87]. The structure of a non-annealed glass is close to the structure of the corresponding liquid, and accordingly, ΔC_p at T_g is smaller for a non-annealed than an annealed glass [88].

The glass transition is a second-order transition and it is seen in the DCS data as a step in the baseline in the heat flow versus temperature curve (Figs. 1, 2). The

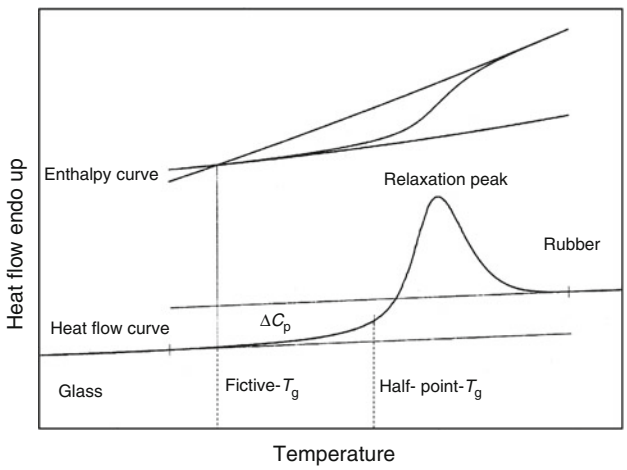

Fig. 2 Glass transition seen in the DSC data, in the heat flow curve, and in the enthalpy curve

endothermic peak often observed on the heat flow curve at the end of the glass transition range (Fig. 2) is known as the enthalpy relaxation peak; it is the recovery of the enthalpy lost during the annealing process below the T_g and it corresponds to the enthalpy difference between an annealed glass and a quenched (non-annealed) glass [79, 84, 85]. The relaxation peak may also arise if the heating and cooling rates are different. When a slowly cooled material is heated with a faster rate over the glass transition than was the case on cooling, rapid heating does not allow sufficient time for molecules to relax at the glass transition and extra energy at a higher temperature is needed for relaxation of molecules to the rubber state [4, 89]. The presence of a relaxation endotherm may complicate the determination of T_g because the baseline shift may sometimes render the identification and quantification of the glass transition extremely difficult.

As already mentioned above, the glass transition is a dynamic (not thermodynamical) phenomenon. Accordingly, the T_g depends not only on the thermal history of the sample but also on the temperature scanning rate [90–92]. It should also be noted that the glass transition temperatures are extremely sensitive to water. The residual moisture in material acts as a plasticizer by increasing the free volume of the material. Hence, it has a profound influence on the glass transition of amorphous materials leading to a decrease in T_g [4, 89, 93–97].

There are at least three ways to determine the value of T_g from the DSC data. The so-called "half-point-T_g" is the temperature that corresponds to the point on the heat flow curve where ΔC_p reaches 50% of the total change in the specific heat capacity about the transition. At half-point-T_g the heat capacity is midway between those of the glassy and the liquid states. The T_g may also be read at the inflection point of the DSC curve associated with the glass transition. However, in case a large relaxation peak follows the glass transition, the inflection point revealed from the curve is often displaced from the real inflection point of the glass transition [88]. This is why the inflection point is seldom used to define the value of T_g [79]. The third way to determine the value of T_g is to find the intersection of the extrapolated pre-transition and post-transition baselines on the enthalpy curve that is the integral of the specific heat curve [4, 98], see Fig. 2. This point is called "fictive-T_g" (i.e., "thermodynamic" or "enthalpic" glass transition temperature) [79].

Quantitative analysis of amorphous content by differential scanning calorimetry

Traditionally the exothermic crystallization peak which follows—upon heating—the glass transition has been used for quantitative analysis of the content of amorphous phase(s) [5, 59–61, 99]. In some studies (in case no crystallization occurs) even the melting enthalpy has been used for quantifying the degree of amorphicity [57, 62]. However, it is difficult to quantify particularly the small concentration of the amorphous phase with confidence in these techniques. It is hence preferable to use the glass transition for the quantification of the amorphous content. With appropriate calibration, the magnitude of ΔC_p at T_g and thereby the amorphous content may be determined in a highly quantitative way. Note that ΔC_p is linearly proportional to the amorphous content provided that the amorphous glasses are in the same state both in the actual sample studied and in the reference samples used for calibration [38]. Great care is, however, required if the change in the C_p value at T_g is to be measured quantitatively. To obtain reliable results, it is essential to perform a calibration using a standard such as sapphire [4]. Moreover, baseline calibration is needed to ensure as flat baseline as possible.

Quantitative determination of amorphous phases requires 100% pure crystalline and amorphous standards as well as well-defined samples with various degrees of crystallinity for reference. A commonly accepted technique to prepare a standard series of samples with various known degrees of crystallinity is to simply weigh and thoroughly mix fully amorphous and fully crystalline samples at various ratios. A check for homogeneity of such a mixture can be done by observing the standard deviation of several measurements performed with the same mixture [7]. However, preparation of samples with various degrees of crystallinity (in average) by mixing the fully amorphous and fully crystalline samples produces mixtures in which the crystalline and amorphous portions exist separately in different particles [1, 5, 8]. This does not perfectly correspond to the real situation where amorphous and crystalline portions are normally in intimate contact with each other in each individual particle.

A starting point in the development of a method for the determination of amorphous content is to ensure that the ΔC_p value for a 100% amorphous sample can be determined in a reproducible manner. Here the first step is to find the proper annealing temperature and time for the reference sample to eliminate the effect of thermal history of the sample and thereby reach a constant ΔC_p value. After the proper annealing conditions have been found, an appropriate temperature program can be made. This program is then used for the measurements of the fully crystalline and the fully amorphous samples as well as the synthetic mixtures. From the results, the ΔC_p value at the T_g as a function of the degree of amorphicity can be found and a linear regression line can be calculated.

Novel enhanced DSC techniques

There are two new DSC approaches that have already been shown to greatly improve the detection of the glass transition. In the HyperDSC technique a high heating rate is used to allow small transitions to be detected more readily [11–26], whereas in the TMDSC techniques the heating program is modulated such that the reversible changes in specific heat capacity may be distinguished from potentially interfering irreversible kinetic events such as the enthalpy relaxation. In the TMDSC technique, using conventional heat-flux DSC equipment, periodic temperature oscillations are applied to the system. The heating-block temperature is sinusoidally modulated such that the sample temperature is modulated in the same manner about a constant period. The modulated temperature and the resultant modulated heat flow are then typically deconvoluted using a Fourier transform to obtain the reversible and non-reversible components [27–39]. The StepScan DSC method, on the other hand, employs a high-sensitive power-compensated DSC equipment. The sample follows a heat-hold temperature program, and the program allows equilibration of the system after each step in a series of small step-wise increases in temperature. As a result the reversible and non-reversible components are obtained without mathematical operations [44–56]. In the following, the HyperDSC and StepScan DSC techniques are discussed in more detail.

HyperDSC

The HyperDSC technique is based on a relatively new technology but has already attracted considerable interest as a method of high sensitivity. The technique employs very fast heating and cooling rates of up to 500 °C min^{-1}, and requires an instrument with an extremely fast response time with respect to the chosen temperature program together with a very high resolution. This can only be achieved using a power-compensated DSC apparatus in which the low furnace mass and small dimensions ensure much faster heat transfer compared to the situation with the heat-flux DSCs [11–14]. The high heating rate significantly increases the sensitivity because an increased scan rate leads to a higher heat flow. The DSC output is measured as a function of heat flow (mW), which can also be expressed as energy per unit time (J/s). At fast scan rates, the same amount of heat flows over a shorter time period. Therefore, the use of increased heating rates allows extremely small transitions that would be below the limit of detection at the heating rates employed in conventional DSC to be measured. The HyperDSC technique also allows the measurement of much smaller samples [11, 12, 15–17, 20, 21], and has over the last few years found a variety of applications in the fields of pharmaceuticals and polymers ranging from studies of polymorphism and glass transitions to those of the kinetics of macromolecular and pharmaceutical systems [12–26].

Higher scanning rates also aid the visualization of the T_g in samples with only a small amount of the amorphous phase embedded in crystalline matrix [4, 16, 17, 21]. Hence HyperDSC should be considered as a higly plausible technique for studying glass transitions. The technique can offer a huge improvement in sensitivity and speed over the conventional and modulated DSC techniques [11, 17], and is able to provide us with valuable information rapidly and on small samples, opening a new area for research on amorphous materials [15].

There are, however, some limitations to the technique. Resolution is one of the concerns to be addressed [17]. At high heating rates, thermal gradients within the sample may have an impact, widening the signals detected from the glass transition and other thermal events and superimposing these phenomena inside the material. One way to reduce the thermal gradient is to use samples of very small masses. On the other hand, a certain minimum mass is needed to ensure an acceptable signal-to-noise ratio and a measurable signal. Hence, a compromise has to be found [12]. To achieve optimal thermal contact between the sample and the sample container, aluminum foil or a thin-foil sample pan may be used [12, 14, 25]. Corrections concerning thermal lag are often found to be necessary and have been used and discussed [25, 26].

Both Saunders et al. [15] and Gabbott et al. [16] have evaluated the potential of the HyperDSC technique to characterize low amorphous contents in lactose samples. Different heating rates were tested, and the linear control of the heating rate for all the high scan rates used (50–500 °C min^{-1}) was demonstrated. The size of the DSC response increased substantially as the scan rate was increased, and it became easier to detect the glass transition even for samples with $\sim$1% amorphicity when using very low sample masses. Mixtures of crystalline and amorphous (spray dried) lactose were prepared, and the heating rate of 500 °C min^{-1} was chosen for the measurements because this rate was found to show the largest glass transition on the spray-dried lactose. The step height change of the glass transition was measured from the onset to the maximum height for each sample, and a linear relationship between this value and amorphicity was found [15, 16]. An independent sample that had been found to contain 1% amorphicity by a solution calorimetry method was measured under the same conditions, a clear glass transition was seen which well verified the sensitivity of the HyperDSC technique [16].

The sensitivity of HyperDSC has also been tested for various maltitol samples. Even for a sample with 1%

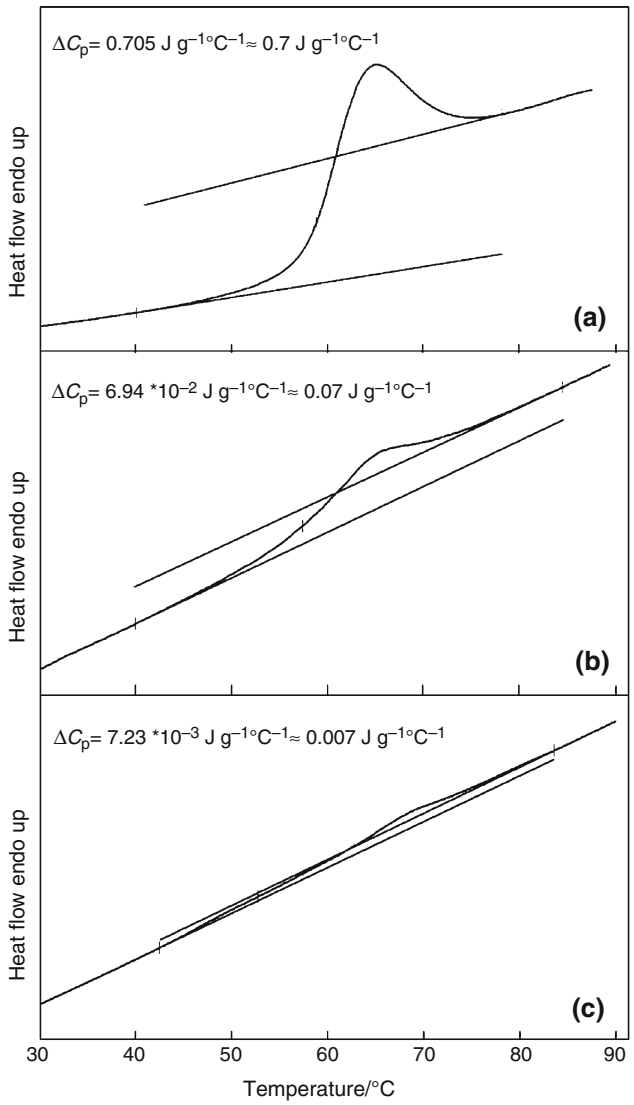

Fig. 3 HyperDSC glass transitions for samples containing **a** 100%, **b** 10%, and **c** 1% amorphous maltitol. Heating rate was 100 °C min^{-1} and sample mass ca. 5 mg [18]

amorphous content the glass transition was easily seen such that the ΔC_p value could be calculated (Fig. 3).

StepScan DSC

The new stepwise DSC technique, StepScan DSC, is software for the characterization of thermal properties of materials [44–56]. The technique permits the separation of DSC data into thermodynamic (reversible) and kinetic (irreversible) components for better interpretation. StepScan produces a temperature program that consists of a series of short heat-hold steps [45–47]. After each step the heat flow is equilibrated until a given criterion is satisfied, and then the next step is started. The StepScan DSC approach is only possible with the design of the power-compensated DSC, with its very low-mass sample and reference furnaces and rapid response time [47–49]. An empty-pan baseline should be run if an accurate C_p is needed [50].

The "Thermodynamic-C_p" data set reflects reversible (or fast) events, such as the sample's heat capacity or glass transition. The kinetic or so-called "IsoK Baseline" data set reflects irreversible (or slow) processes taking place during the experiment, such as relaxation or crystallization. The temperature program used in StepScan DSC consists of alternate steps of heating with constant rate and isothermal holding. The reversible component is only observed in the heating part of the cycle and the irreversible one only in the isothermal part [46, 48, 51]. The duration of the isothermal part varies and the variation is controlled by the software which allows the sample to achieve a state close to the thermal equilibrium at each temperature step. No special mathematical operations, like Fourier transformation, are needed to obtain the results by the StepScan DSC technique [52]. The basic equation describing the heat flow response in a StepScan DSC experiment is given as: $dQ/dt = C_p(dT/dt) + K(T,t)$. In this equation, dQ/dt is the DSC heat flow, C_p is the sample's specific heat capacity, dT/dt is the heating rate employed, and $K(T,t)$ is the kinetic component [48, 51].

There are many measurement parameters in the StepScan DSC method. The three most important parameters are the length of the isothermal segment, the temperature jump between two subsequent isothermal segments, and the linear heating rate in the dynamic segments [46, 51]. There is also a criteria-parameter (= criteria to shorten the isotherm) which determines the length of the isotherms [45, 50]. A fine criterion requires a longer time but it allows higher accuracy in the measurement. The sensitivity of the measurement can be improved by using a high heating rate or a large sample. For calibration a relatively slow heating rate (e.g., 2 °C min^{-1}) is recommended because, in spite of a high partial heating rate in the dynamic segment, the overall heating rate is usually quite slow in StepScan measurements. Slow scan speeds in order to maintain steady state through the required number of oscillations result in longer experimental running times. StepScan DSC requires two separate scans, a blank scan and a sample scan, for any given set of experimental parameters, which results in a very time-consuming procedure.

In case the sample exhibits a glass transition with overlapping enthalpic relaxation, the Thermodynamic-C_p signal would show the classic, stepwise change in the heat capacity, which simplifies the interpretation and makes the calculation of ΔC_p much easier. The enthalpic relaxation event would then show up in the IsoK Baseline data set [47, 49, 50, 53]. This is illustrated in Fig. 4.

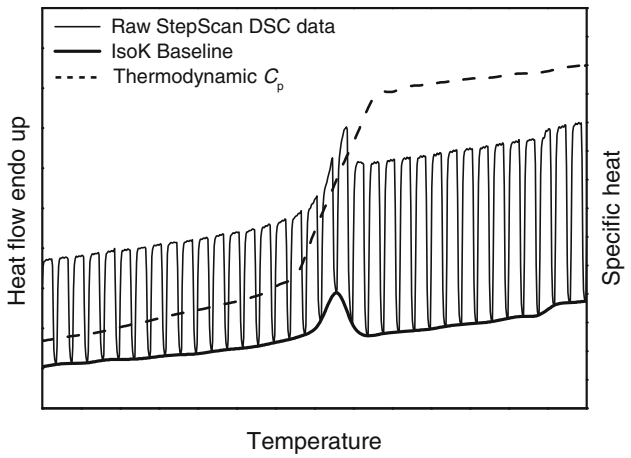

Fig. 4 Typical StepScan DSC data for a glass transition: from the raw StepScan DSC data the IsoK Baseline curve and the thermodynamic-C_p curve are calculated

Lehto et al. [8] determined the degree of amorphicity of lactose samples using StepScan DSC and compared the method to other methods that are widely used to quantify the degree of amorphicity (XRD, conventional DSC, IMC, SC, Raman spectrometry and GMS). The work revealed that the determination of ΔC_p may be hampered in StepScan DSC by the fact that the baseline of the heat capacity curve is not always a straight line. It was found that the interpretation of the glass transition was very difficult when the change in specific heat is small (as in the case with a sample with 2% amorphicity). However, good correlation was found between the degree of amorphicity and ΔC_p. Moreover, the StepScan DSC results were in good agreement with the results obtained with IMC, SC, Raman and GMS for the same samples [8].

Černošek et al. [52] evaluated the capability of the StepScan DSC technique to measure the glass transition of a model glass (As_2S_3). The reversing (thermodynamic) part of the StepScan data about the glass transition region was found to remain completely unaffected by the choice of the experimental parameters such that both the ΔC_p and T_g could be determined without influence of the thermal history experienced by the sample or the experimental conditions employed.

Comparison of HyperDSC and StepScan DSC techniques

The sensitivity of the technique depends on the relative magnitude of the property change being measured. In DSC, detection of low concentrations of amorphous components is based on detection of changes in specific heat capacity associated with the glass transition. HyperDSC increases the sensitivity of DSC using a high heating rate. However, this approach also results in broadening and shifting of the glass transition, and does not always yield the desired sensitivity. Nevertheless, it should be emphasized that even though the value of T_g and also the heat capacity C_p will shift up with increasing heating rate, the magnitude of ΔC_p does not change [52]. StepScan DSC, on the other hand, eliminates all baseline curvature and drift from the heat capacity signal and provides higher sensitivity for straightforward detection of weak glass transitions. However, in samples with extremely low concentrations of amorphous components, detecting the glass transitions may be a challenge and the calculation of the heat capacity change is impossible. Hence the suitability, selectivity, and limit of detection of each technique are sample-specific.

The capabilities of the two methods, HyperDSC and StepScan DSC, have been compared in quantification of low concentrations of amorphous components in sugar and sugar alcohol samples (maltitol and sucrose) [18, 19, 44]. In the StepScan measurements, two different heating rates were compared. For calibrations done at a heating rate of 2 °C min^{-1} the temperature program was: temperature increment 2 °C, heating rate 2 °C min^{-1}, isothermal period 1 min and criteria 0.01 mW (= method 1). For calibrations done at a heating rate of 10 °C min^{-1} the temperature program was: temperature increment 2 °C, heating rate 10 °C min^{-1}, isothermal period 1 min and criteria 0.0001 mW (= method 2). In the HyperDSC studies, the measurements were performed at a heating rate of 100 °C min^{-1}. The ΔC_p values obtained for synthetic mixtures were used to calculate the average and standard deviation values and at least three parallel results were used for every lot. Moreover, the ΔC_p values were calculated for both the fictive and the half-point glass transition temperatures for the sake of comparison, but no significant differences in the results were observed. The mean values of ΔC_p about the glass transition region were plotted against the degree of amorphicity. The linear regression between ΔC_p and the degree of amorphicity was obtained for both the techniques, as illustrated in Fig. 5.

Saklatvala et al. [17] compared the HyperDSC and TMDSC techniques using polyvinylpyrrolidone (PVP) samples. As a result of the higher scanning rate, the step change about the glass transition was much larger in the case of HyperDSC confirming that the faster scanning rate leads to an improvement in the sensitivity. At the same time, however, the transition was broader in HyperDSC. Modulated DSC enabled the separation of the enthalpic relaxation from the heat capacity change allowing for more straightforward detection of the glass transitions. However, detecting small glass transitions of amorphous components was a challenge.

The limit of detection (LOD) is defined as the analyte concentration giving a signal equal to the blank response

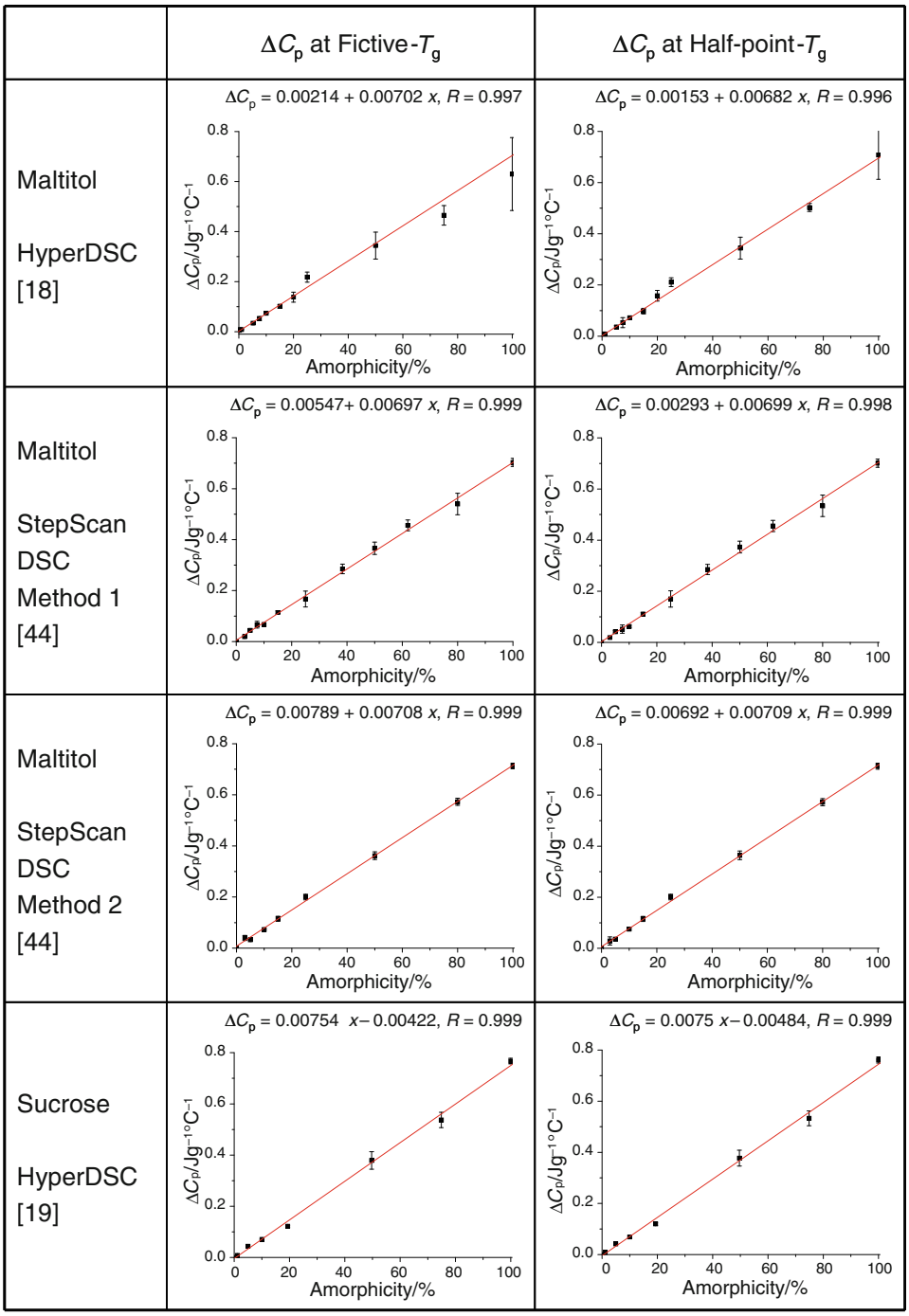

Fig. 5 The average and standard deviation values for the specific heat change at glass transition temperature as function of amorphous content measured with HyperDSC and StepScan DSC for maltitol and sucrose samples [18, 19, 44]. The linear regression lines between ΔC_p and amorphicity (x) with R-values (R = correlation coefficient) are attached

plus three standard deviations of this value, whereas the limit of quantification (LOQ) is the analyte concentration giving a signal equal to the blank response plus 10 standard deviations of this value. From the regression lines the numerical information for constants b, a, s_a, and s_b can be obtained using the well-known equation of $a = bx + y$ (s_a and s_b are standard deviations of a and b). The LOD and LOQ values can be calculated using the equations $X_L = 3s_a/b$ for LOD and $X_L = 10s_a/b$ for LOQ [100]. Table 2 summarizes the LOD and LOQ values calculated from data published for various lactose, maltitol, and sucrose samples. The values are appreciably low for both the HyperDSC and StepScan DSC techniques.

There are some characteristic differences in the HyperDSC and StepScan DSC methods when employed for the quantification of low amorphous contents. In HyperDSC the sensitivity is higher owing to the high heating rates used. The limit of quantification is determined by the measurement noise. Owing to the lower noise level in HyperDSC, lower LOD and LOQ values can be reached,

Table 2 Calculated LOD and LOQ values from different references

Sample	Method	Calculated from	LOD/ %	LOQ/ %	References
Maltitol	HyperDSC	Fictive-T_g	0.31	1.04	[18]
		Half-point-T_g	0.11	0.36	[18]
Sucrose	HyperDSC	Half-point-T_g	0.06	0.21	[19]
Lactose	HyperDSC	Half-point-T_g	0.57	1.89	[15]
Lactose	HyperDSC	Half-point-T_g		<1.5	[16]
Maltitol	StepScan DSC	Method 1			
		Fictive-T_g	0.24	0.81	[44]
		Half-point-T_g	0.27	0.92	[44]
Maltitol	StepScan DSC	Method 2			
		Fictive-T_g	0.18	0.61	[44]
		Half-point-T_g	0.16	0.52	[44]

and accordingly, smaller glass transitions can be detected. HyperDSC is also clearly faster than the StepScan DSC method. In some cases, however, distinguishing the glass transition from other thermal events (such as recrystallization and relaxation) has been found difficult in HyperDSC but can be readily achieved in StepScan DSC. The StepScan DSC measurements are more complicated to perform than the HyperDSC measurements. In the StepScan method, the proper choice of the various measurement parameters is important because they have a substantial effect on the results. Also the calibration method influences the results. However, the main advantage of the StepScan DSC method for quantification of an amorphous content is that the glass transition and relaxation peaks are separated to different curves such that the calculation of ΔC_p becomes much easier.

Conclusions

The change in the specific heat (ΔC_p) at the glass transition has been shown to be a good indicator for the degree of amorphicity in samples that are mostly crystalline. The two relatively new DSC techniques, HyperDSC and StepScan DSC, provide us with obvious benefits for the accurate ΔC_p measurements. The former techniques improve in particular the sensitivity of the measurement whereas the main advantage of the StepScan DSC method is the easier data interpretation as it allows better distinguishment of the glass transition from other thermal events. With both the methods, experimental conditions can be found under which the degree of amorphicity linearly depends on ΔC_p and low enough LOD and LOQ values are achieved. Hence, the two new DSC approaches should both be considered as highly potential techniques for quantifying low concentrations of amorphous phases in various crystalline matrices.

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