MELTING BEHAVIOUR AND QUANTIFICATION OF LOW AMORPHOUS LEVELS IN SUGARS AND SUGAR ALCOHOLS WITH DSC TECHNIQUES

Doctoral Dissertation

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Melting behaviour and quantification of low amorphous levels in sugars and sugar alcohols with DSC techniques

Abstract

The present thesis consists of six publications and a summary of the experimental results reviewed together with relevant literature data. The research was divided into three parts: the melting behaviour and the preparation of amorphous samples as well as the development of a method for quantification of low amorphous content.

The melting behaviour of sucrose, glucose, fructose and xylose was investigated. The melting peaks were determined with DSC and the start of decomposition was studied with TG at different rates of heating. In addition, melting points were determined with a melting point apparatus and some StepScan DSC measurements were carried out. There were differences between the different samples of the same sugar and the rate of heating had a remarkable effect on the melting behaviour.

The main objective of this thesis was to develop a method for quantification of low levels of amorphous content in sugars (sucrose) and sugar alcohols (maltitol). The techniques chosen were HyperDSC and StepScan DSC, some of the newest DSC techniques. The amorphous maltitol was prepared from crystalline material by melting followed by rapid cooling and the amorphous sucrose was prepared by spray drying.

In DSC, detection of low concentrations of amorphous components is based on detection of changes in specific heat capacity (ΔC_p) associated with the glass transition. The effects of time and temperature of the low-temperature annealing (below T_g) on the ΔC_p of amorphous samples was studied. The annealing had notable effect on the change in specific heat: as the annealing time was increased, the glass transition temperature moved to a higher temperature and the change of the specific heat increased. The ΔC_p was shown to be a good indicator for the degree of amorphicity in sugar and sugar alcohol samples that are mostly crystalline. With both HyperDSC and StepScan DSC methods experimental conditions were found under which the degree of amorphicity linearly depends on ΔC_p and low limit of detection (LOD) and limit of quantification (LOQ) values were achieved. In addition, the effect of mechanical treatment was tested for sucrose and a correlation between grinding time and amorphous content was found.

Keywords   amorphous content, glass transition, HyperDSC, StepScan DSC, melting behaviour, sugar

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Tiivistelmä

Tämä väitöskirjatutkimus koostuu kuudesta osajulkaisusta ja yhteenveto-osiosta, jossa kokeellisen työn tulokset esitellään tiivistetysti ja referoidaan kirjallisuudesta löytyvä työn kannalta oleellinen teoria. Tutkimus jakautui kolmeen osaan: sulamiskäyttäytyminen, amorfisen aineen valmistus ja menetelmän kehitys vähäisen amorfisuuden määrittämiseksi.

Tässä työssä tutkittiin sakkaroosin, glukoosin, fruktoosin ja ksyloosin sulamiskäyttäytymistä. Sulamispiikit määritettiin DSC:llä eri lämmitysnopeuksilla, ja hajoamista tutkittiin termovaa'alla vastaavilla lämmitysnopeuksilla. Sulamispisteet määritettiin myös sulamispistelaitteella ja lisäksi tehtiin muutamia StepScan DSC mittauksia. Saman sokerin eri näytteiden välillä oli merkittäviä eroja, ja lämmitysnopeudella oli huomattava vaikutus sulamiskäyttäytymiseen.

Työn päätarkoitus oli kehittää menetelmä vähäisen amorfisuuuden määrittämiseksi sokereista (sakkaroosi) ja sokerialkoholeista (maltitoli). Menetelmiksi valittiin uudet DSC tekniikat: HyperDSC ja StepScan DSC. Amorfinen maltitoli valmistettiin kiteisestä maltitolista sulattamalla ja sitä seuraavalla nopealla jäähdyttämisellä, ja amorfinen sakkaroosi valmistettiin sumukuivaamalla.

Pienen amorfisen osuuden määrittäminen DSC:llä perustuu lasitransitioon liittyvän ominaislämpökapasiteetin muutoksen (ΔC_p) havainnointiin. Päästölämpötilan ja päästöajan vaikutusta amorfisten näytteiden ΔCp:hen tutkittiin. Päästöllä oli merkittävä vaikutus ominaislämpökapasiteetin muutokseen: kun päästöaika kasvoi, lasitransitiolämpötila siirtyi korkeampaan lämpötilaan ja ominaislämpökapasiteetin muutos kasvoi. Sokeri- ja sokerialkoholinäytteiden pienen amorfisen osuuden määrityksessä ΔC_p:n määritys osoittautui hyväksi menetelmäksi. Sekä HyperDSC että StepScan DSC menetelmillä löydettiin kokeelliset olosuhteet, jossa ΔC_p oli suoraan verrannollinen näytteessä olevaan amorfiseen osuuteen, ja alhaiset havaitsemis- ja määritysrajat (LOD ja LOQ) saavutettiin. Lisäksi mekaanisen käsittelyn vaikutusta sakkaroosinäytteeseen testattiin, ja jauhamisajan ja amorfisuuden välille löydettiin korrelaatio.

Asiasanat amorfinen osuus, lasitransitio, HyperDSC, StepScan DSC, sulamiskäyttäytyminen, sokeri	

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PREFACE

This thesis summarizes my share of the research on sugars and sugar alcohols carried out in co-operation with Danisco Sweeteners. The experimental work of this thesis was carried out at the Department of Chemistry, University of Jyväskylä during the years 2002-2005. The dissertation was written at the Laboratory of Inorganic Chemistry, the Aalto University School of Science and Technology between September 2009 and April 2010.

I wish to express my deepest gratitude to my instructor, Docent Ilkka Pitkänen, for his endless support and interest in my work during these years. I am also very grateful to my supervisor Prof. Maarit Karppinen for the opportunity to finish off this thesis in Espoo. Her positive attitude and support enabled me to complete my studies. I would like to thank Juha Nurmi and Heikki Heikkilä for good collaboration and Danisco Sweeteners for financial support. Financial support from the Ellen and Artturi Nyyssönen Foundation, the Finnish Chemical Society, the Orion Research Foundation and the city of Kotka is gratefully acknowledged. I also thank the reviewers, Professor Anne Juppo, University of Helsinki and Professor Vesa-Pekka Lehto, University of Eastern Finland, for their valuable comments.

I wish to express my thanks to my co-authors; especially Päivi Harjunen is thanked for kind and expert help with spray drying. Additionally I'm thankful to the whole personnel both in Laboratory of Inorganic Chemistry, Espoo and Department of Chemistry, Jyväskylä. Special thanks go to my closest co-workers from the past and present: Sara, Riika, Pirita, Jenni J., Jenni L., Samuli, Tommi, Outi and Tuomas. I have spent most delightful moments at the work among you.

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Espoo, June 2010

Minna Lappalainen

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their corresponding Roman numerals.

I   M. Hurtta, I. Pitkänen and J. Knuutinen, Melting behaviour of D-sucrose, D-glucose and D-fructose, *Carbohydr. Res.* **339** (2004) 2267-2273.

II   M. Lappalainen, I. Pitkänen, H. Heikkilä and J. Nurmi, Melting behaviour and evolved gas analysis of xylose, *J. Therm. Anal. Cal.* **84** (2006) 367-376.

III   M. Hurtta and I. Pitkänen, Quantification of low levels of amorphous content in maltitol, *Thermochim. Acta* **419** (2004) 19-29.

IV   M. Lappalainen, I. Pitkänen and P. Harjunen, Quantification of low levels of amorphous content in sucrose by hyperDSC, *Int. J. Pharm.* **307** (2006) 150-155.

V   M. Lappalainen and I. Pitkänen, Quantification of amorphous content in maltitol by StepScan DSC, *J. Therm. Anal. Cal.* **84** (2006) 345-353.

VI   M. Lappalainen and M. Karppinen, Techniques of differential scanning calorimetry for quantification of low contents of amorphous phases, *J. Therm. Anal. Cal.* in press (2010).

AUTHOR'S CONTRIBUTION

I   The author defined the research plan together with the co-authors. The author performed all measurements and had a major role in writing the manuscript.

II   The author defined the research plan together with co-authors. The author performed TG and DSC measurements and participated in writing the manuscript.

III   The author defined the research plan together with the co-authors. The author prepared all samples and performed all measurements and participated in writing the manuscript.

IV   The author defined the research plan together with the co-authors. The author performed all measurements and had a major role in writing the manuscript.

V   The author defined the research plan together with the co-authors. The author prepared all samples and performed all measurements and had a major role in writing the manuscript.

VI   The author had a major role in writing the manuscript.

LIST OF ABBREVIATIONS

Methods

DSC	Differential scanning calorimetry
HyperDSC	High-speed DSC
StepScan DSC	Stepwise DSC
TMDSC	Temperature-modulated DSC
TOPEM	Advanced temperature-modulated DSC
DMA	Dynamic mechanical analysis
GMS	Gravimetric moisture sorption
FTIR	Fourier transform infrared spectroscopy
IMC	Isothermal microcalorimetry
NIR	Near infrared spectroscopy
SC	Solution calorimetry
SS-NMR	Solid-state nuclear magnetic resonance spectroscopy
TG	Thermogravimetry
XRD	X-ray powder diffraction

Symbols

C_p	Specific heat at constant pressure
ΔC_p	Change of specific heat at constant pressure
G	Gibbs energy
ΔG	Change of Gibbs energy
H	Enthalpy
ΔH_f	Heat of fusion
Q	Heat flow
S	Entropy
ΔS	Change of entropy
t	Time
T	Temperature
T_g	Glass transition temperature
T_f	Normal freezing point = Normal melting point
T_i	Initial temperature

α and β	The configural symbol of sugar anomer. Two anomers are designated alpha (α) or beta (β), according to the configurational relationship between the anomeric centre (the configuration of hydroxyl substituents) and the anomeric carbon atom (the asymmetric carbon atom created by the ring structure of the sugar): alpha – opposite side, beta – same side. Anomerization is the process of conversion of one anomer to the other.
D and L	Enantiomeric or mirror-image relationships between the sugars are indicated by the use of the small capital letters D and L. A sugar belongs to the D-family of sugars when its highest numbered asymmetric carbon atom has the OH group written to the right in the Fischer projection formula. The sugar belongs to the L-family, when that OH group is written to the left. The configuration is transposed at all asymmetric centers.
l (subscript)	Liquid
a (subscript)	Amorphous
c (subscript)	Crystalline
REV	Reversible
IRREV	Irreversible
LOD	Limit of detection
LOQ	Limit of quantification
R	Correlation coefficient
R^2	Exponential correlation coefficient
a	Intercept of regression line
b	Slope of regression line
s_a	Standard deviation for the intercept of regression line (a)
s_b	Standard deviation for the slope of regression line (b)

1 INTRODUCTION

The degree of crystallinity is an important parameter for various food and pharmaceutical systems as it affects their behaviour 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] Detection and quantification of low levels of amorphous content have became important issues in fields at pharmacy and dairy products.[4] The amorphous phases 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.[5-7] 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.

A number of techniques are available for the detection and quantification of low levels of amorphous components, but the methods are usually sample-specific. In addition, the amount of the amorphous material in the crystalline mass may be small and hence difficult to detect using traditional analytical techniques. There are several studies reported in the literature where different techniques for determination of the content of amorphous phase(s) are compared as analytical methods.[6-11] 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 HyperDSC (high-speed or high-performance differential scanning calorimetry) 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.[12-25] Another possibility is to modulate the heating program such that the reversible changes in specific heat capacity

may be distinguished from potentially interfering irreversible kinetic events such as the enthalpy relaxation. Several versions of this approach have been proposed including the TMDSC (temperature modulated DSC),[26-38] TOPEM (advanced temperature-modulated DSC)[39-42] and StepScan DSC[43-54] techniques.

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%	55-59
Differential scanning calorimetry (DSC)	1-20%	58-61
High-speed DSC (HyperDSC)	0.2-1.5%	16-18, **III**, **IV**
StepScan DSC	0.8%	9, **V**
Temperature-modulated DSC (TMDSC)	0.9%	18, 62, 63
Gravimetric moisture sorption (GMS)	0.5-1%	64, 65
Solution calorimetry (SC)	0.5-1.8%	66-70
Isothermal microcalorimetry (IMC)	0.5-5%	59, 65, 66, 71-74
Dynamic mechanical analysis (DMA)	2%	75
Density measurements	10%	6
Solid-state nuclear magnetic resonance spectroscopy (SS-NMR)	0.5-3%	55, 61, 71
Fourier transform infrared spectroscopy (FTIR)	1-2%	10, 76
Raman spectroscopy	1%	77-79
Mid infrared spectroscopy (MIR)	1-2%	10
Near infrared spectroscopy (NIR)	0.5-1%	57, 64, 80, 81
Thermally-stimulated current spectrometry	~1%	82
Therahertz pulsed spectroscopy	1-2%	83
Inverse phase gas chromatography	1%	84, 85

For quantification of amorphous content, fully crystalline and fully amorphous reference samples are needed. For that reason, the preparation of an amorphous sample is an essential part of method development. One of the most common methods used for the preparation of an amorphous sample is melting of the crystalline sample followed by rapid cooling. However, when melting temperatures of sugars are reviewed in the literature, it is seen that values for the same sugar may slightly differ from each other.[I,II] The melting of sugars is not an unambiguous event and it has therefore been studied in this theses. It was revealed that the melting of sugars is a multiphase phenomenon and the results are affected by both the determination method and the origin and quality of samples. For that reason, it is important to clarify whether melting can be used for preparation of amorphous sugar.

The main purpose of this thesis was to explore the ability of the HyperDSC and StepScan DSC techniques to detect and quantify low levels of amorphous phases in samples which are mostly crystalline (sugars and sugar alcohols). In this study, new methods for quantification of low concentrations of amorphous components in maltitol and sucrose samples were developed.

2 AMORPHOUS STATE

In the crystalline state the basic constituents (molecules in the present case) have a well-defined regular packing in an ordered lattice structure. Solids that are not crystalline are called amorphous. An amorphous state has no regular crystal organization but instead the molecules exist in a non-uniform array. The three-dimensional long-range order typical of a crystalline material does not exist in the amorphous state, and the position of molecules relative to one another is more random, approaching that in the liquid state. Amorphous solids typically exhibit short-range order over a few molecular dimensions and have physical properties quite different from those of their corresponding crystalline states.[1,5,11,86-90] The structure of the amorphous materials is more difficult to characterise and their properties are more difficult to control than those of crystalline systems.[5] Amorphous states demonstrate greater intermolecular distances and molecular mobilities, as well as higher potential energy levels when compared to crystals.[2] The difference in the structure of amorphous and crystalline materials is illustrated in Fig. 1.
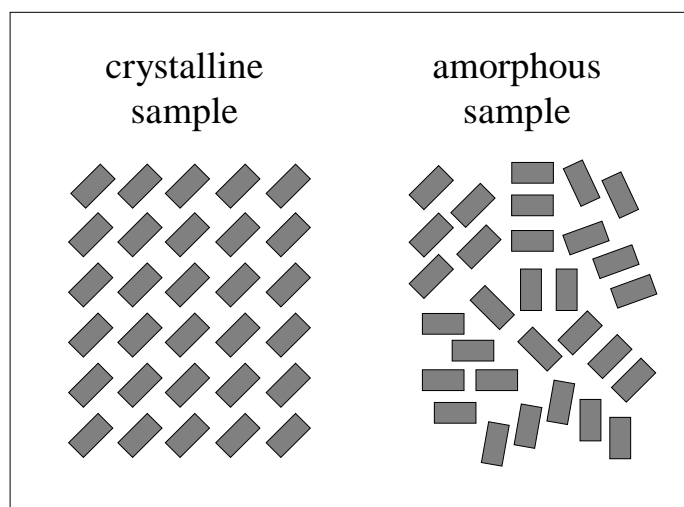
Figure 1. Organization of the basic constituents (atoms, ions or molecules) in amorphous and crystalline samples.

Amorphous material may exist as a viscous (solid) glass or as a more liquid-like rubbery amorphous state. Co-existence of different glassy states is also possible.[91,92] The rubbery amorphous state is considered as an equilibrium supercooled liquid, an amorphous state with the structural characteristics of a liquid, but with a much higher viscosity.[1] Glasses are considered to be "frozen" liquids due to their structural similarity

to liquids. However, in many aspects the glasses behave more like solids, although structurally they are liquid-like.[2] The amorphous state does not define an exact surface nature, consequently amorphous material prepared by different methods could reasonably exhibit a different surface character.[84]

2.1 Glass transition

The transition between the rubbery state and the glassy state is a second-order phase transition, which occurs at the glass transition temperature, T_g.[93-95] At the glass transition temperature there will be a physical change in the amorphous material. The glass transition is a kinetic and relaxation event, depending on the temperature scanning rate and the thermal history of the material.[89] It occurs at a temperature below which some molecular motions become so slow that the liquid takes on the properties of a solid. When a liquid is rapidly cooled so that the molecular motions sharply decrease and crystallization becomes impossible, the viscosity may become so high that all large-amplitude molecular movements are "frozen" and the liquid completely loses its mobility and is quenched into a thermodynamically unstable glassy state.[2,95] The ratio of the glass transition temperature to the melting temperature is typically 0.70.[88] Residual water acts as a plasticizer by increasing the free volume of the material, hence it has a profound effect on the glass transition of amorphous materials leading to a decrease in T_g and increase in mobility.[5,88,96-100]

Many physical properties of glassy amorphous materials are different from those of the corresponding rubbery amorphous materials.[1] The glass transition temperature of an amorphous solid is a critical physical property, and it can influence its chemical and physical stability. Within the transition region many properties change their values such as viscosity, dielectric and especially mechanical properties.[97,101] At the glass transition there is an abrupt change in derivative thermodynamic properties with a change in temperature.[90]

2.2 Relaxation process

The properties of glasses are markedly affected by their thermal history and the temperatures employed in their preparation.[102-105] Thermal history is defined by the way

in which the glass was formed and for how long a time the system was maintained at any given conditions.[106] Differences in molecular coordination of glasses represent different potential energy levels suggesting that in an amorphous system the energy levels of molecules are not uniform. The structural features and energy levels of amorphous phases are process dependent, which means that different amorphous states can be generated.[2,90,103,104] In contrast to equilibrium crystals, there is a multitude of glasses of the same chemical structure, which differ in their cooling or annealing history.[107] Below the glass transition temperature, large-amplitude molecular motions in the amorphous material still occur but on very slow time scales (e.g. hours or days).[2,108]

The non-equilibrium nature of the glassy state leads to structural relaxation, i.e. the spontaneous approach of the material towards a thermodynamically more favourable equilibrium state at a rate that depends on the temperature and the whole thermal history of the glass.[102,109,110] The term structural relaxation refers to changes in atomic/molecular arrangements that occur during the relaxation.[90] The isothermal structural relaxation that occurs below the glass transition temperature is extremely slow and is often referred to as physical ageing or annealing.[89,96,109,110] The annealing of amorphous material allows more of the initial microstructure to convert to the preferred local order.[105] As glass is aged isothermally below the glass transition temperature, both enthalpy and entropy decrease spontaneously with time toward temperature-dependent equilibrium values.[90,111,112] Amorphous states are constantly changing in structure and in energy level, moreover, the changes are not only time dependent but also temperature dependent.[2] Relaxation phenomena are often observed within the glass transition temperature range and enthalpy relaxations may be either endothermic or exothermic, depending on the thermal history of the material and the time scale of observation.[5,96] The theoretical approaches to relaxation in glass-forming liquids and amorphous solids have been broadly reviewed by Angell *et al*.[113] The possible coupling between molecular mobility and the stability of amorphous phases have recently received a lot of attention.[2,106,111,114]

2.3 Preparation of amorphous material

Preparation of amorphous material involves a rapid change of the material from equilibrium state to a non-equilibrium state without allowing the time needed for the

material to adjust to changes occurring in its surroundings and to maintain equilibrium.[96] In order to produce an amorphous system it is necessary to create a highly disordered molecular state and then to stabilise that disordered state so that all molecular motions, which might induce instability, are reduced.[108] For both thermodynamic and kinetic reasons, the preparation of an amorphous solid is straightforward for some materials (good glass formers), but difficult for others (poor glass formers).[89] Figure 2 summarizes the most common ways to prepare amorphous materials.
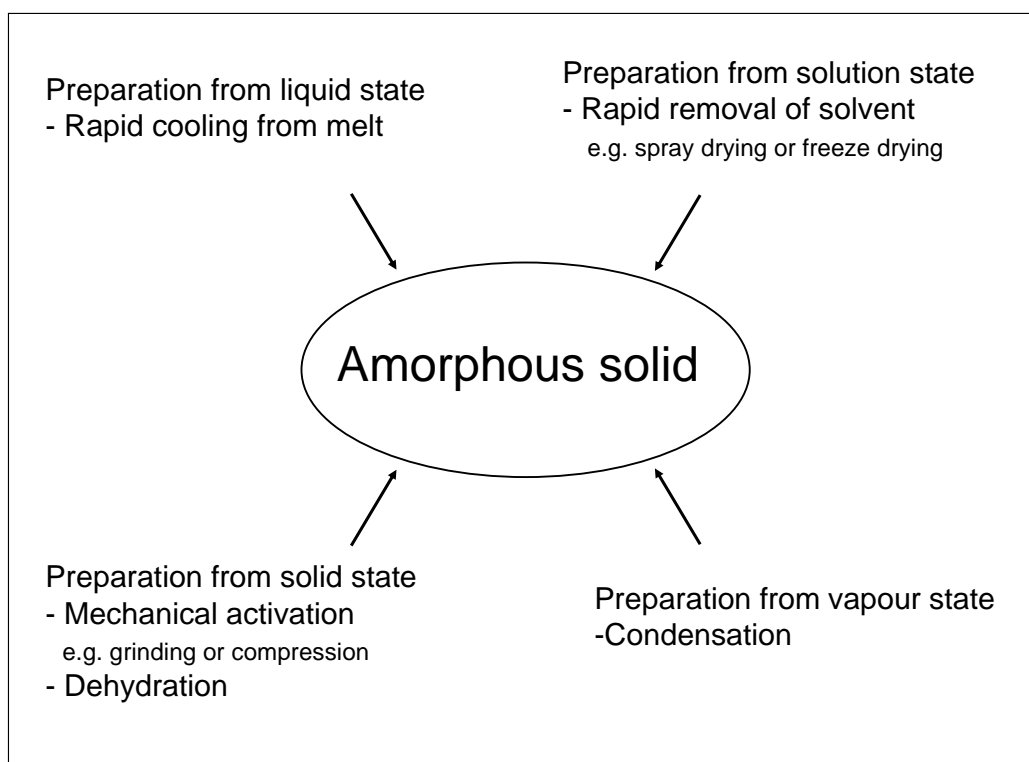
Figure 2. Summary of the most common ways to prepare amorphous materials.

The most common methods for producing amorphous materials are rapid cooling from a melt or rapid removal of the solvent.[5,96] Generally, an amorphous solid is formed if the transition from a liquid state (melt or solution) to a solid state is fast enough to keep the molecules in a random order, i.e. crystallization is kinetically hindered and the molecules are kept in the same coordination as they where in the liquid state.[2] Crystallization requires nucleation, and nucleation tendency is a function of the viscosity of the liquid where it occurs.

When an amorphous solid is prepared by rapid cooling from a melt (melt quenching) at a given high viscosity (attained below the melting point), the material will have the appearance of an amorphous solid.[86] After melting, among the factors determining the final solid phase are the relative rates of nucleation, crystal growth and cooling.[3] Cooling must take place quickly enough to prohibit nucleation and growth of crystals.[90] Depending on the cooling rate, various glassy states of the same material are formed. The drawback of this approach is its potential for chemical degradation during the melting step.[104] For that reason, melt quenching can only be applied to samples that are thermally stable.[11] Melt quenching is usually used in laboratory scale and the method for large-scale production of amorphous material is melt extrusion.[115]

Random order that exists in solutions can be "frozen" due to a sufficiently rapid removal of the solvent for example by means of spray or freeze drying.[5,96] In spray drying the solution is sprayed into hot air to produce uniform droplets and the solvent is evaporated fast which leads to amorphous particle formation. In freeze drying the solution is rapidly frozen and the water is separated from the solution at low temperature and pressure such that the solute molecules remain in the disordered structure they were frozen in. Extremely low temperatures are used to limit molecular mobility and to prevent nucleation.[3,5,116]

Amorphous material can also be prepared by condensation from the vapour state. If the condensation of the material from the vapour state to the solid state occurs fast enough, the molecules remain disordered.[1,117] Amorphous phase may also be obtained from the solid (crystalline) state under mechanical stress or upon dehydration.[3,118] Mechanical activation of a crystalline material (e.g. during grinding or compression) may render a fully or partially amorphous material. Upon grinding, the crystalline material gradually collapses to the amorphous state with increasing grinding time.[89,105]

The formation of the amorphous state and its relation to equilibrium conditions are illustrated in Fig. 3. Crystals, solution and melt are stable equilibrium states. The amorphous states (glass and rubber) are non-equilibrium states with time-dependent properties. Changes between equilibrium states and the glassy state always occur through the rubbery states.[119] In their review papers, Morris *et al.*[116] have introduced the theoretical approaches to phase transformations whereas Zhang *et al.*[3] have focused on the practical aspect of the subject.
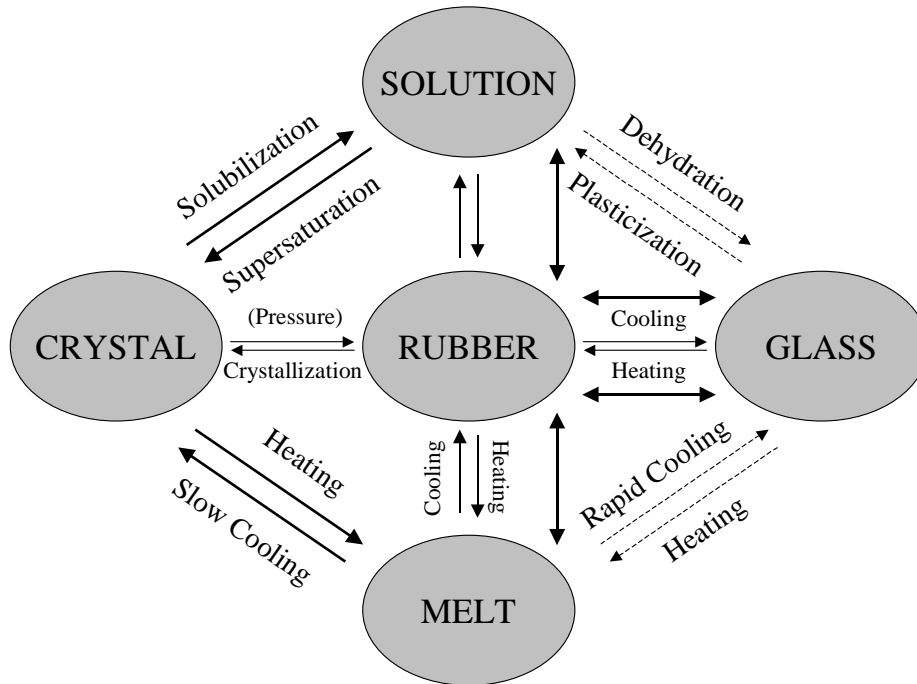
Figure 3. Physical states of material.[119]

2.4 Some thermodynamic aspects

The phase transitions can be classified based on changes in Gibbs energy. A schematic representation of the Gibbs energy (G) and the enthalpy (H) of crystalline, amorphous and liquid phases as a function of temperature at a constant pressure is shown in Fig. 4. The intercept of Gibbs energy lines gives the transition temperatures: the Gibbs energy is the same for the crystalline and liquid states at the melting point, T_f, and for the amorphous and liquid states at the glass transition temperature, T_g.

Amorphous solids have higher energy, entropy, enthalpy and Gibbs energy than the corresponding crystals. The increase in enthalpy with increasing temperature is higher in the supercooled liquid state than in the glass state.[89,96] Enthalpy shows a step change at the T_f. The enthalpy change occurs isothermally and is equal to the latent heat of transition.[96] At the thermodynamic equilibrium for melting the Gibbs energy $\Delta G = \Delta H_f - T\Delta S_f = 0$, which gives $\Delta H_f = T\Delta S_f$. From this equation, the entropy change in melting can be calculated as: $\Delta S_f = \Delta H_f / T$. The entropies can be compared: they follow the

order of $S_l > S_a > S_c$, where the subscripts l, a and c refer to the liquid, amorphous and crystalline states, respectively.
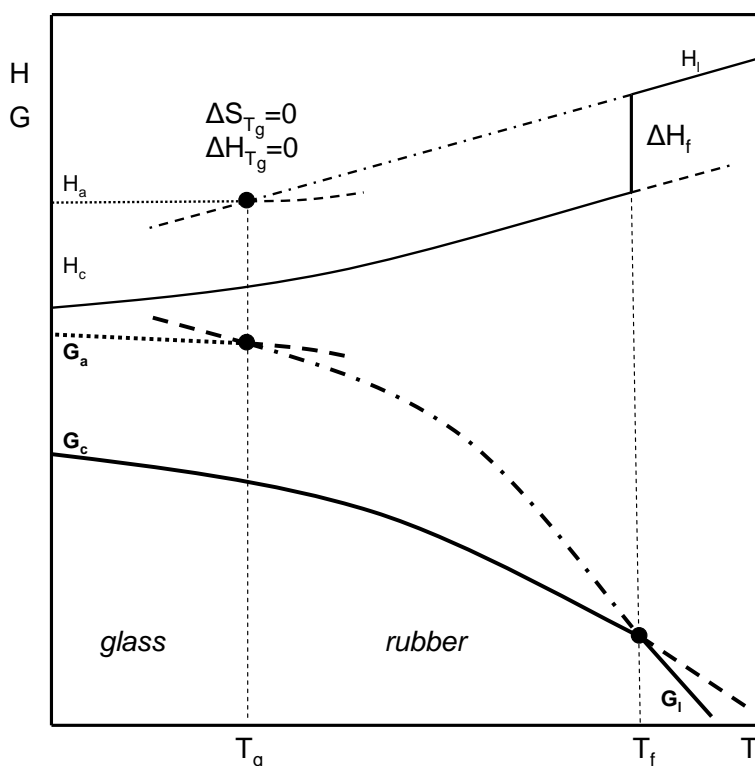
Figure 4. Schematic presentation of the Gibbs energy (G) and enthalpy (H) of crystalline, amorphous and liquid phases as a function of temperature. The subscripts l, a and c refer to the liquid, amorphous and crystalline states, respectively. Stable states are presented as solid lines (_____). Amorphous glass states are presented in short dot lines (……..). The dashed lines (_ _ _ _) describe an extrapolated area and dash dotted lines (_ . _ . _) describe the rubber area.

The glass transition is represented by a change in the derivatives of extensive thermodynamic parameters such as volume, enthalpy and entropy.[1] Because the constant pressure heat capacity C_p is defined as the derivative of the enthalpy versus temperature plot, a step change in C_p occurs at T_g (see Fig. 5). Hence the transition is dependent on molecular mobility with no associated heat of transition for the process.[5,96] If there is no heat of transition, there can also be no entropy of transition.[107] At the glass transition $\Delta S_{T_g} = 0$, which means that $S_l = S_a$.
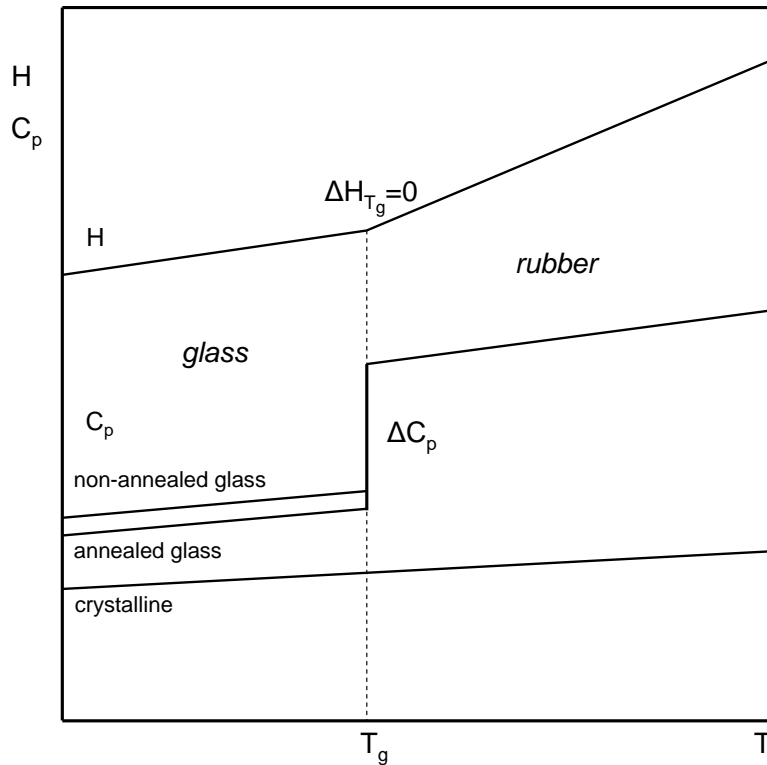
Figure 5. Schematic presentation of the heat capacity (C_p) and enthalpy (H) of crystalline and amorphous phases as a function of temperature about the glass transition region.

2.5 Consequences of amorphous components

The behaviour of powders will change depending upon their processing history.[1-3,72] Most crystalline solids undergoing processing are likely to take on a certain degree of amorphous character, i.e. they become partially or wholly disordered. Such processing includes freeze drying, spray drying, conventional drying, wet granulation, aqueous film coating, milling, grinding, compression and compaction.[5,6] The presence of additives or processing conditions might stabilize or favour the amorphous state.[1] As a result, the storage and processing conditions have to be controlled carefully.[3] In typical pharmaceutical and food systems two types of situations should be considered. In the first case, the material exists intrinsically in the amorphous state or it is purposefully rendered amorphous. In the second case, crystalline material has been inadvertently converted to amorphous during processing. The latter type of amorphous character usually exists predominantly on surfaces at levels not easily detected; it has the potential to produce unwanted changes in the physical and chemical properties of the sample.[1]

The formation of disorder in a solid generally produces thermodynamically unstable regions that are in a higher energy state when compared to the crystalline form. The amorphous regions are reactive "hot spots" in which physical changes and/or chemical degradation can be initiated.[6,7,100] Amorphous materials, although perhaps occupying only a very small proportion of the bulk, may contribute greatly to the behaviour of the material as it resides primarily on the particle surfaces.[1,72] Amorphous material is thermodynamically unstable and therefore liable, under certain circumstances such as elevated temperature or relative humidity, to undergo changes in its amorphous structure, or susceptible to crystallization.[1,3,11,88,90,120,121]

The presence of amorphous material in crystalline pharmaceutical and food substances can have considerable impact on the bioavailability, processability and physical/chemical stability of the system and the performance of the material during product manufacture and use.[2,120,122] Typical amorphous solids have physical properties (heat capacity, vapour pressure, adsorption behaviour e.g. enhanced water sorption, mechanical and rheological properties, solubility) quite different from those of their corresponding crystalline forms.[120] Amorphous forms, due their low packing efficiency and lack of long-range order, present higher internal energy, specific volume and molecular mobility than their crystalline counterparts, which may lead to enhanced dissolution behaviour and bioavailability.[1,2,5,6,88,89,120] In some instances, these properties may be advantageous and it is desirable to produce and maintain the amorphous state,[77] however, the increased molecular mobility of the amorphous state above the glass transition temperature may decrease the chemical stability.[120] Amorphous forms often exhibit stronger chemical reactivity and a faster chemical degradation rate. Potential conversion to a thermodynamically more stable crystalline form may occur over time. In addition, most amorphous solids are increasingly hygroscopic resulting in spontaneous absorption of water vapour.[2,88,97] Moisture also acts as anti-solvent enhancing the driving force for crystallization.[3]

2.6 Amorphous sugars

Sugars may exist as crystalline, semicrystalline, partially crystalline or totally amorphous. Amorphous sugar may be present as a thin layer on the surface of crystalline sugar.[123] Amorphous sugar exists in a thermodynamically metastable, nonequilibrium state that tends to crystallize under the influence of a number of factors, mainly temperature and moisture. Molecules of amorphous solids in the glassy state are not able to change their spatial arrangement but as the temperature is increased above T_g the molecular mobility increases and crystallization becomes possible at temperatures higher than the glass transition temperature but below the melting temperature.[96,97,121,124-126] Amorphous sugar is very hygroscopic and capable of absorbing water from the environment during processing. Most of the physical and structural properties of amorphous sugar are moisture dependent (for example the stickiness, caking and recrystallization).[4,123]

The glass transition temperature of sugars generally depends on the molecular weight and several studies[93,102,127] have reported the T_g values for common sugars and sugar alcohols. The glass transition temperature of amorphous sugar is extremely sensitive to water and it decreases with increasing water content.[97-99] Roos[93] reported an equation to predict the T_g values of carbohydrates at various water contents, for example, the glass transition temperature may change as much as some 10°C for mixtures containing only 1% water.[110] The differences between the reported T_g values for common sugar glasses are probably due to residual water in the samples, differences in sample handling techniques, and differences in techniques used to determine the value of T_g. Variations in the T_g values may also be due to the different thermal histories of the samples.[93,95,96,108] The glass transition temperature seems to be a good indicator of the transitions that may occur in an amorphous sugar, like collapse, stickiness and caking during storage.[123]

As the amorphous sugar glass is annealed at a constant annealing temperature below its T_g, its enthalpy relaxes toward equilibrium, i.e. the enthalpy decreases as ageing time increases. As the glass is reheated, the enthalpy increases and crosses the equilibrium liquid line. Amorphous sugar in products containing sugar can undergo physical ageing during storage below their glass transition temperatures. Physical ageing in food materials may produce unfavourable changes in some physical properties (density,

hardness and brittleness), which in turn affect the quality, stability and shelf life of those products.[108-110,128-133]

The amorphous state of sugars is widely known. It is typically formed by dry-milling, quenching of melt, rapid drying of solutions (spray-drying), freeze-concentration of aqueous solutions and their freeze-drying, as well as boiling or extrusion of highly concentrated solutions followed by cooling.[110,123] Most studies describe amorphous materials formed by rapid cooling from a melt.[5,77,82,95,99,108-110,127-130,134-136]

3 QUANTITATIVE ANALYSIS OF AMORPHOUS CONTENT BY DSC

Differential scanning calorimetry (DSC) is one of the most frequently used techniques for the measurement of the degree of amorphicity. A typical DSC scan for a totally or partially amorphous sample is shown in Fig. 6; the curve displays a glass transition, a crystallization exotherm, and a melting endotherm.[101] The glass transition is seen as a change in heat capacity on heating. The magnitude of ΔC_p at T_g varies from compound to compound, and this variance is the basis for the classification of glass formers from strong to fragile glass formers.[90]
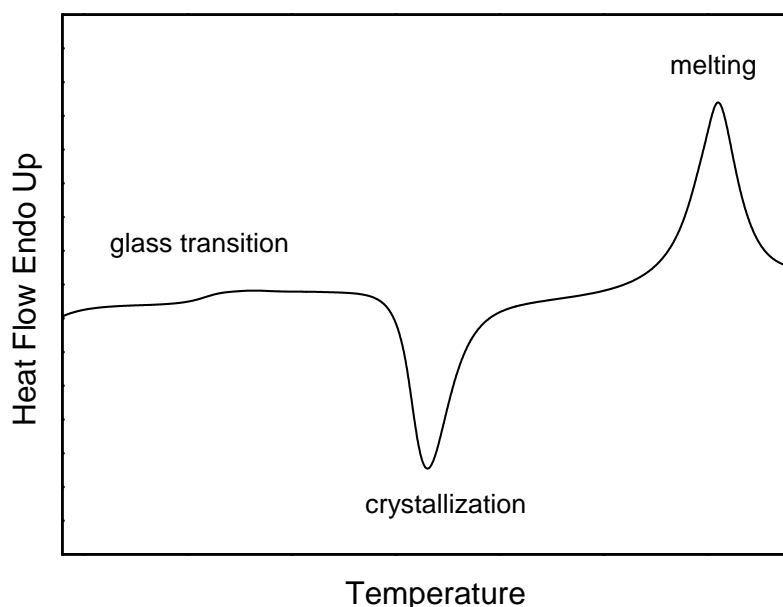
Figure 6. Schematic DSC curve for a non-annealed amorphous sample upon heating.

Traditionally the exothermic crystallization peak which follows - upon heating - the glass transition has been used for the quantitative analysis of the content of amorphous phase(s).[6,57-59,137] In some studies (in case no crystallization occurs) even the melting enthalpy has been used for quantifying the degree of amorphicity.[55,60] However, it is difficult to quantify particularly the small concentration of the amorphous phase with confidence in these techniques, therefore it is 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.[37] 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[5] and baseline calibration is also needed to ensure as flat baseline as possible.

3.1 Glass transition seen by DSC

The glass transition is a second-order transition and it is seen in the DSC data as a step in the baseline in the heat flow versus temperature curve (Fig. 7). The endothermic peak often observed on the heat flow curve at the end of the glass transition range is known as the enthalpy relaxation peak; it is the recovery of the enthalpy lost during the annealing process below the glass transition temperature and it corresponds to the enthalpy difference between an annealed glass and a quenched (non-annealed) glass.[101,129,130] 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 the relaxation of molecules to the rubber state.[5,96] 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.

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 glass transition temperature may also be read at the inflection point of the DSC curve associated with the glass transition. However, in the case where a large relaxation peak follows the glass transition, the inflection point determined from the curve is often displaced from the real inflection point of the glass transition,[107] hence this is why the inflection point is seldom used to define the value of T_g.[101] 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 (see Fig. 7).[5,138] This point is called "fictive-T_g" (*i.e.* "thermodynamic" or "enthalpic" glass transition temperature).[101]
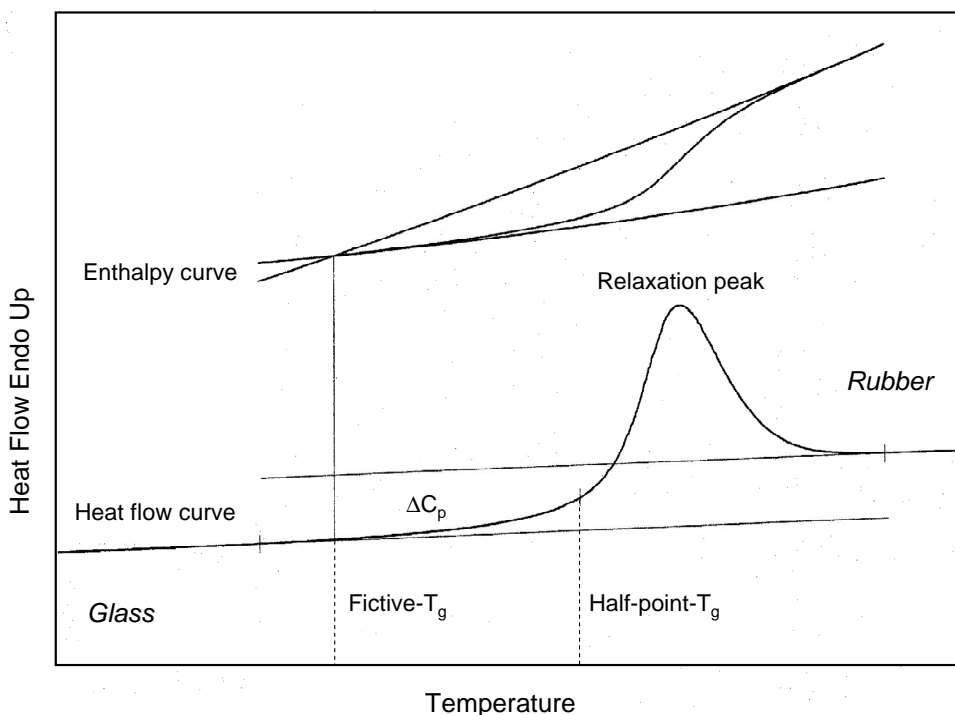
Figure 7. Glass transition seen in the DSC data, in the heat flow curve, and in the enthalpy curve.

A particular 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. Glasses are known to change their properties when annealed at temperatures below their glass transition temperature.[108,110,128-132] 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.[107]

The glass transition is a dynamic (not thermodynamical) phenomenon. Accordingly, the glass transition temperature depends not only on the thermal history of the sample but also on the temperature scanning rate.[89,102,134] It should also be noted that the glass transition temperatures are extremely sensitive to water, as the residual moisture in the material acts as a plasticizer by increasing the free volume of the material. Hence, it has

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a profound influence on the glass transition of amorphous materials leading to a decrease in T_g.[5,88,96-100]

3.2 Basic principles of method development

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.[8] However, preparation of samples with various degrees of crystallinity (on average) by mixing the fully amorphous and fully crystalline samples produces mixtures in which the crystalline and amorphous portions exist separately in different particles.[1,6,9] 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 glass transition temperature as a function of the degree of amorphicity can be found and a linear regression line can be calculated.

3.3 Novel enhanced DSC techniques

Because of 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 to improve parameters such as sensitivity, accuracy and

detection limit. There are two new DSC approaches, the HyperDSC and the StepScan DSC, that have already been shown to greatly improve the detection of the glass transition. In the following, these techniques are discussed in more detail.

3.3.1 HyperDSC

The high-speed 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°Cmin[-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.[12-15] The high heating rate significantly increases the sensitivity because an increased scan rate leads to a higher heat flow. The DSC output is measured in 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[12,13,16-20] 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.[13-25]

Higher scanning rates also aid the visualisation of the T_g in the samples with only a small amount of the amorphous phase embedded in crystalline matrix[5,17,18,20] and this is illustrated in Fig. 8. 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,[12,18] and is able to provide us with valuable information rapidly and on small samples, opening a new area for research on amorphous materials.[16]
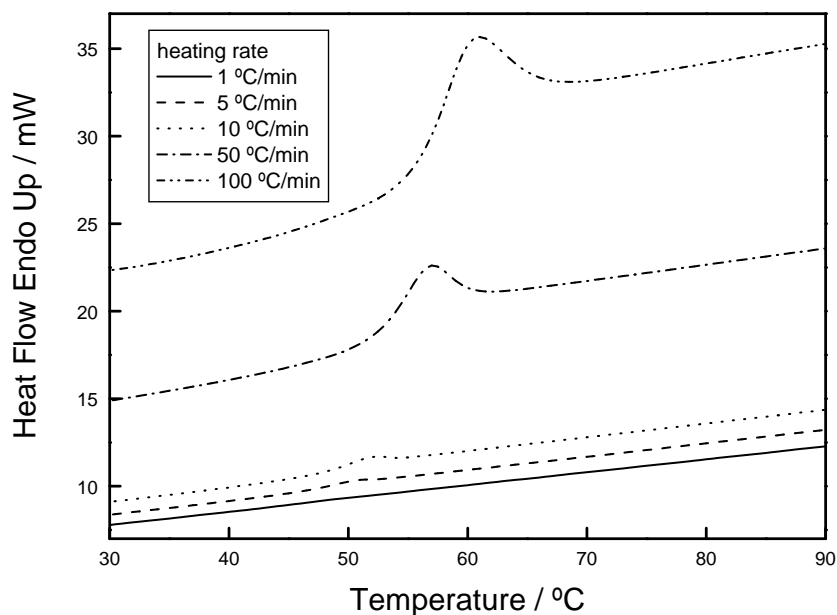
Figure 8. The effect of heating rate on the sensitivity of glass transition measurements.

There are, however, some limitations to the technique. Resolution is one of the concerns that needs to be addressed.[18] 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.[13] To achieve optimal thermal contact between the sample and the sample container, aluminum foil or a thin-foil sample pan may be used.[13,15,24] Corrections concerning thermal lag are often found to be necessary and have been used and discussed.[24,25]

Both Saunders *et al*.[16] and Gabbott *et al*.[17] 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°Cmin^{-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 ~1% amorphicity when using very low sample masses. Mixtures of crystalline and amorphous (spray dried) lactose were prepared, and

the heating rate of 500°Cmin[-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.[16,17] 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 clearly verified the sensitivity of the HyperDSC technique.[17]

Saklatvala *et al*.[18] compared the HyperDSC and TMDSC techniques using polyvinylpyrrolidone 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 a more straightforward detection of the glass transitions, however, detecting small glass transitions of amorphous components was a challenge.

3.3.2 StepScan DSC

StepScan DSC is the stepwise DSC technique for the characterization of thermal properties of materials.[43-54] 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.[43-45] 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.[45-47] An empty-pan baseline should be run if an accurate C_p is needed.[48]

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.[44,46,49] 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.[50] 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.[46,49]

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.[44,49] There is also a criteria-parameter (= criteria to shorten the isotherm) which determines the length of the isotherms.[43,48] 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. $2°Cmin^{-1}$) 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[45,47,48,51] and is shown in Fig. 9.
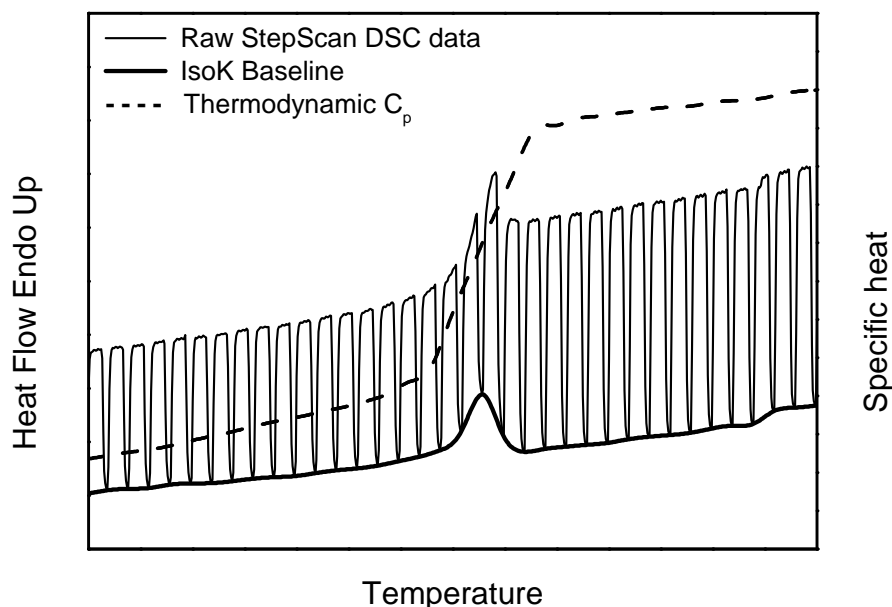
Figure 9. Typical StepScan DSC data for a glass transition: from the raw StepScan DSC data the IsoK Baseline curve and the Thermodynamic C_p curve are calculated.

Lehto *et al.*[9] 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.[9]

Černošek *et al.*[50] evaluated the capability of the StepScan DSC technique to measure the glass transition of a model glass (arsenic trisulfide). 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.

4 AIMS OF THE STUDY

The research was divided into three parts: the melting behaviour of sugars and the preparation of amorphous samples as well as the development of a method for quantification of low amorphous contents.

The specific aims of this study were:

- to explore the ability of the HyperDSC and StepScan DSC techniques to detect and quantify low levels of amorphous phases in samples which are mostly crystalline (sugars and sugar alcohols)

- to clarify whether melt quenching can be used for preparation of amorphous sugar and sugar alcohol samples

- to study the melting behaviour of sugars

- to study the effect of mechanical treatment on the amorphous content by grinding crystalline sucrose samples and to test the HyperDSC method for quantification of amorphous contents of ground samples

5 MATERIALS AND METHODS

5.1 Reagents and samples

The materials examined in melting studies of the present thesis were sucrose, glucose, fructose and xylose. Before melting measurements sucrose, glucose and fructose samples were dried at 50°C for two days and then stored in a desiccator over P_2O_5. Xylose samples were dried over P_2O_5.[I,II]

The samples for the quantification of low levels of amorphous content were maltitol and sucrose. Crystalline maltitol was dried and stored over P_2O_5. Amorphous maltitol was prepared from crystalline maltitol by melting it in an oven at 165°C (mp 150°C) and keeping it at that temperature for 15 minutes. The degradation temperature of maltitol was checked with TG before the preparation of amorphous maltitol. The bright and colourless liquid was poured on to a cooled metal plate and the plate was put in a desiccator. The desiccator was refrigerated at 5°C for at least 1 hour and the final product (glass) consisted of glassy "pearls". After cooling, amorphous maltitol was crushed in a porcelain mortar and the powder was stored in a desiccator over P_2O_5 at 5°C. Experiments showed that the powder started to crystallize if it was stored outside the desiccator at room temperature; the change started already within a couple of hours.[III,V]

Crystalline sucrose was dried at 50°C for two 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. After spray drying, the samples were stored at room temperature in a desiccator over P_2O_5.[IV]

Mixtures of both maltitol and sucrose samples were prepared by weighing known quantities of amorphous and crystalline material at various ratios and by mixing them thoroughly in a porcelain mortar. In addition, 100 and 0% amorphous samples were used. The mixtures were prepared one at a time and measured immediately. Two to five parallel measurements were taken at each test point.[III-V]

Table 2 summarizes the samples employed in this study. Maltitol was chosen because it offers a practical advantage for the investigation of quantification of amorphous level as no interfering re-crystallization effect is observed. The glucose, fructose and sucrose

were chosen for model sugars because they are the most common sugars in industrial use. Xylose was investigated because of the interest of the sponsor.

Table 2. Samples employed in this study.

Sample	Manufacturer
Melting studies	
Sucrose (2820 Sucrose Pharma 51115) [sucrose A]	Danisco Sweeteners, Finland
D(+)-sucrose >99.5% [sucrose B]	Fluka BioChemica
D(+)-glucose AnalR [glucose A]	BDH
D(+)-glucose anhydrous >99.5% [glucose B]	Fluka BioChemica
Fructose (A125 Fructofin C 10098) [fructose A]	Danisco Sweeteners, Finland
D(-)-fructose >99.5% [fructose B]	Fluka BioChemica
Crystalline L-xylose (Xylose lot 1 22.8.2001)	Danisco Sweeteners, Finland
D-xylose (Lot Z121296, >99.8%)	Danisco Sweeteners, Finland
L(-)-xylose (minimum 99%,)	Sigma
D(+)-xylose (Sigmaultra 99%)	Sigma-Aldrich
Amorphicity studies	
Crystalline maltitol (Lot M010803) 99.8%	Danisco Sweeteners, Finland
Crystalline maltitol >98%	Sigma
Crystalline D(+)-sucrose >99.5%	Fluka BioChemica

5.2 Analysis techniques

The aim of the X-ray powder diffraction measurements was to identify the phases of the samples[I,II] or to ensure the amorphicity of the samples[III-V].

The decomposition of samples was investigated using TG analysis. Conventional DSC was employed to study the melting behaviour of samples. Several heating rates were used in both the decomposition and the melting studies of the samples.[I,II] In addition, some StepScan DSC measurements with different measurement parameters were performed.[II] Also a melting point apparatus was used for melting point measurements.[I]

Many different series of DSC measurements were carried out in order to develop a method for quantification of low levels of amorphous content in maltitol and sucrose. Both HyperDSC and StepScan DSC techniques were used. The experimental conditions of the DSC measurements are described in Publications III-V.

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 for 2.5 to 60 minutes and 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 hour and then in a desiccator over silica gel for at least two hours every time before grinding. The ground samples were measured immediately after grinding with DSC.[IV]

6 STUDIES ON SUGARS AND SUGAR ALCOHOLS

6.1 Melting of sugars

Melting refers to the transformation of an organized solid crystalline structure to a disorganized liquid state.[139] In melting, the solid and liquid are in thermodynamic equilibrium at constant temperature and pressure.[107] Usually melting is determined by raising the temperature at a certain heating rate over the range of melting point, this causes a temperature gradient in the sample and an apparent rise of the observed melting temperature. For that reason, the melting proceeds over an apparent temperature range as a result of the lag in the heat transfer and is larger the faster the heating rate is.[139] The melting can be said to be anomalous if the form recrystallized from the melt is different from the original form or if the conformation of molecules changes during melting. Also a decomposition taking place in the melting temperature range causes the melting to be anomalous. The anomalous melting temperature is strongly dependent on the heating rate.

Heating of crystalline sugars above melting point followed by rapid cooling often results in the formation of a solid, transparent, glassy material.[96] This technique is often used to prepare amorphous sugars and sugar alcohols.[93,95,99,109,110,127,129,130,135,136] Crystalline sugar melts when it is heated to or above its melting point. Sugars do not have sharp melting points and their melting proceeds over a temperature range and as a result the melting endotherms are fairly broad. The melting points of sugars are sensitive to water, impurities and crystallinity. Some sugars may caramelize and become brown concomitantly with the melting process. Sugars may also decompose before their melting begins.[124,140,141] The melting points may differ between sugar anomers.[96] α- and β-anomers of the same sugar are in equilibrium in water, but in the crystalline state one anomer is usually the dominating form.[140,142,143] Also the conformation of the sugar may change in the melting process.[99,144-149]

The melting of sugar is not a well-defined process because the liquid formed immediately on melting is not an equilibrium state of the system. A sugar in the molten state, above its melting point, is composed of more than one conformer (epimers, tautomers and anomers) and the conformer equilibrium is established on a longer time scale than that of the melting process. Hence, observing a true equilibrium fusion

process, e.g. by placing a crystal into an equilibrated melt, is probably not possible in all cases.[129,131,150] Part of the complexity of the molten state of sugar is due to an initial non-equilibrium state produced on fusion and another part results from the decomposition processes which set in around the melting point. In addition, the equilibrium state of liquid itself is temperature-dependent, with widely different time scales for equilibration with respect to its different degrees of freedom.[131]

When melting points of sugars are reviewed in the literature, it is found that values for the same sugar may slightly differ from each other (see Table 3). The articles are mainly general and the melting temperatures of various sugars are just summarized into one table. The melting behaviour of each individual sugar is usually not studied. However, the melting point is one of the parameters commonly used to identify and characterize the material and the amorphous sugar is often formed by heating of crystalline sugar above its melting temperature followed by rapid cooling. The melting of sugar is not an unambiguous event, for that reason, it should be studied in order to determine if melting can be used for preparation of amorphous sugar.

Table 3. Literature values of melting points of sucrose, glucose, fructose and xylose (the values in parentheses are onset temperatures).

Melting point / °C				References
Sucrose	Glucose	Fructose	D-Xylose	
160-186	146 (α) 148-150 (β)	102-104		151
	146			146
(173) 190	(143) 158	(108) 127	(143) 157	93
(160) 185	(135) 150	(80) 115	(135) 150	152
192	158	124		153
	(158) 164	(114) 132		129
		105		131
188				121
	165	120		141
188				126
190				95
			155	144, 145
185-186	146 (α) 150 (β)	103-105	145	154
180	156	121		155

6.1.1 Melting studies of sucrose, glucose, fructose and xylose

The differences between the literature values for the same sugars indicate that further work is needed in this area. In this study the apparent changes in the melting temperature were investigated and the reasons for the differences were examined. The samples studied were sucrose, glucose, fructose and xylose. Their melting behaviour was studied with conventional DSC (all samples) and also StepScan DSC method (xylose samples), and the decomposition of the same sugars was observed with TG analysis. Different heating rates were used in both types of measurements. Melting points were also determined using a melting point apparatus (sucrose, glucose and fructose samples).

The results of thermal analysis measurements (TG and DSC) are collected in Tables 4-7. Onset and peak temperatures of melting and heat of fusion (ΔH_f) are from DSC. T_i is the initial temperature of decomposition (from TG) (see Fig. 10). Increasing heating rates increased both the onset and the peak temperatures in all samples studied. The enthalpy of melting increased as the heating rate increased and in addition, the initial temperatures of TG measurements were moved higher as the heating rates increased.
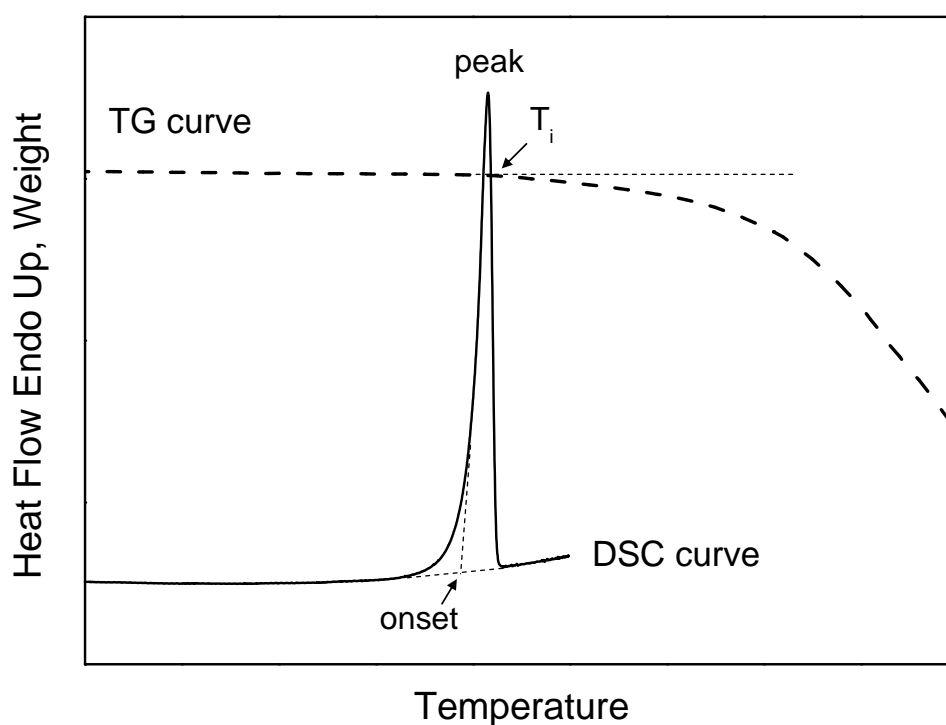

Figure 10. Melting peak (DSC curve) and the start of decomposition (TG curve).

From the x-ray powder diffraction measurements, both sucroses studied were identified as D-sucrose, both glucoses as α-D-glucopyranose and both fructoses as β-D-fructopyranose. Two enantiomeric forms of xylose were identified as α-D-xylopyranose and α-L-xylopyranose. All identifications were unambiguous.

In the case of sucrose samples (see Table 4), the peak and onset temperatures clearly increased as the heating rate was increased to $10°Cmin^{-1}$ but onset was nearly constant at higher heating rates. The peaks also became slightly broader as the heating rate was increased. When TG and DSC results were compared, it could be noticed that the decomposition started before melting at slower heating rates, especially in case of sucrose A. The onset temperature was seen to change because of the decomposition.

Table 4. Results of DSC and TG measurements and the calculated melting entropy values for sucrose.

	Heating rate /$°Cmin^{-1}$	Onset temperature /$°C$	Peak temperature /$°C$	T_i /$°C$	ΔH_f /Jg^{-1}	ΔS_f /$Jmol^{-1}°C^{-1}$
SUCROSE A	0.5	167.9	169.9	159.6	55	112
	1	173.7	176.6	161.1	72	142
	2	178.2	181.4	169.6	111	214
	10	185.9	190.5	179.7	126	233
	20	187.5	191.9	192.2	131	239
	50	188.3	193.7	207.5	137	249
	100	189.0	196.1	235.3	143	259
SUCROSE B	0.5	181.4	182.7	167.0	120	226
	1	184.5	186.6	171.3	127	235
	2	187.1	189.3	178.8	128	234
	10	188.9	191.5	189.2	134	244
	20	189.6	192.9	200.7	135	244
	50	191.1	196.1	214.9	139	249
	100	190.8	196.5	228.4	145	261

The two sucrose samples measured behaved in different ways at slow heating rates and melting occurred at a lower temperature for sucrose A than sucrose B. Distinct changes could also be seen in the shape of the melting peak and the enthalpy of melting. The low enthalpy can be explained by the enthalpy of decomposition being lower than the enthalpy of melting. On the other hand, at a heating rate of $10°Cmin^{-1}$ and above the

behaviour of both sucrose samples was quite similar. Onset temperatures were nearly constant and the peak temperatures changed only slightly. The caramellization could be seen before the melting peak of sucrose A but not in the melting peak of sucrose B. At high heating rates, the melting was normal and occurred clearly before the decomposition started. The melting of sucrose was found to be anomalous, especially at slow heating rates.

In the glucose samples studied (see Table 5), the melting peaks moved to a higher temperature as the heating rate was increased and the shift was almost constant throughout the heating rate range. The peaks were narrow and their shapes were competent but at faster heating rates the peak broadened towards higher temperature. The significant differences between glucose samples were in temperatures: glucose A melted at a higher temperature than glucose B, but its decomposition started at a lower temperature, though their enthalpies of melting were similar. In contrast to sucrose, the increase of the onset temperature continued also at high heating rates in glucose.

The decomposition started before melting was stopped only at slow heating rates and so there has to be another explanation for the shift of onset temperatures at high heating rates. Broido *et al.*[146] studied the mutarotation of α- and β-D-glucose near their melting point. The process of mutarotation was found to become very fast as soon as the bulk material melted and the equilibrium α/β ratio was found to be 44% / 56% in melt. As a consequence the shift of onset temperature can be explained by partial decomposition but also by mutarotation.[146] The melting of glucose was anomalous: besides the melting, also thermal decomposition and mutarotation occurred at slow heating rates, whereas at high heating rates the dominant process was mutarotation.

Table 5. Results of DSC and TG measurements and the calculated melting entropy values for glucose.

	heating rate	onset temperature	peak temperature	T_i	ΔH_f	ΔS_f
	/°Cmin⁻¹	/°C	/°C	/°C	/Jg⁻¹	/Jmol⁻¹°C⁻¹
GLUCOSE A	0.5	147.5	149.1	146.4	183	223
	1	149.8	151.7	149.8	189	227
	2	152.8	154.8	151.4	189	223
	10	160.4	163.1	166.4	196	220
	20	164.8	167.4	176.5	200	219
	50	169.4	172.6	191.6	208	221
	100	171.8	176.1	194.4	221	231
GLUCOSE B	0.5	145.1	147.5	147.0	180	224
	1	146.5	149.3	152.0	185	228
	2	148.9	151.9	159.1	187	226
	10	155.2	159.4	170.3	194	226
	20	158.3	163.8	183.5	199	227
	50	163.3	168.9	201.1	207	228
	100	166.7	173.8	204.3	219	237

The melting peaks of the fructose samples studied were shifted most of all (Table 6). The difference between the results at the slowest and fastest heating rate was as much as 30°C and the shift of the melting peak was regular over the whole heating rate range. Fructose samples behaved in the same way in the tests with the only visible difference the shape of the melting peaks. The peaks were broad already at low heating rates in both fructose samples, but overall a little broader in fructose A. At the slow heating rates the decomposition of fructose samples started before melting but at the faster heating rates the decomposition started only after the melting. In fructose, the onset temperature increased at all heating rates in the same way as in the case of glucose, however, the change was larger at slow heating rates than at fast rates.

Farhoudi and Mauch[147,148] studied mutarotation of D-fructose, and they noticed that during melting β-D-fructopyranose was converted into α- and β-D-fructofuranose, but D-fructofuranose in the melted state was reconverted into D-fructopyranose. The effect of mutarotation on the melting temperature was significant at all heating rates used. The melting of fructose was anomalous because the melting temperature was lowered by both thermal decomposition and mutarotation at low heating rates. On the grounds of

values given by TG, the mutarotation was the predominant factor in the change of the melting point, especially at high heating rates.

Table 6. Results of DSC and TG measurements and the calculated melting entropy values for fructose.

	Heating rate /°Cmin^{-1}	Onset temperature /°C	Peak temperature /°C	T_i /°C	ΔH_f /Jg^{-1}	ΔS_f /Jmol^{-1}°C^{-1}
FRUCTOSE A	0.5	108.2	114.3	110.7	148	246
	1	113.6	118.4	116.3	156	247
	2	112.0	123.2	122.8	162	260
	10	125.8	134.1	138.7	175	250
	20	131.3	137.8	145.0	186	255
	50	135.7	140.6	161.6	198	263
	100	137.0	142.6	166.1	213	280
FRUCTOSE B	0.5	110.0	113.0	110.4	152	248
	1	112.7	116.7	113.9	154	246
	2	116.2	121.0	119.0	164	254
	10	125.7	131.7	136.8	177	253
	20	130.0	136.0	147.1	186	257
	50	134.9	139.8	157.0	199	266
	100	136.8	142.0	165.4	204	268

In the xylose samples (see Table 7), increasing the heating rate caused the onset and peak temperatures to move higher and the peaks to become slightly broader. The enthalpy of melting increased as well. The temperatures were overall higher for the L-form than for the D-form. In both samples studied, the shape of peaks was normal but the peaks of D-form were in general broader than those of L-form. The enthalpy of melting of both samples was nearly equal at slow heating rates but was a little higher for L-form than D-form when higher heating rates were used. The decomposition of L-xylose started at slightly higher temperatures than that of D-xylose. The thermal behaviour of different xylose forms differed especially at low heating rates and there were also differences in melting temperatures among the different samples of the same sugar. It was obvious that decomposition of both enantiomeric forms of xylose began at slow heating rates before the melting ended, whereas at high heating rates the melting clearly occurred before the decomposition started. At slow heating rates the change of onset temperature was larger than at high heating rates (see Table 7).

Table 7. Results of DSC and TG measurements and the calculated melting entropy values for L-xylose and D-xylose.

	Heating rate /°Cmin^{-1}	Onset temperature /°C	Peak temperature /°C	T_i /°C	ΔH_f /Jg^{-1}	ΔS_f /Jmol^{-1}°C^{-1}
L-XYLOSE	0.5	143.9	147.3	145.1	208	217
	1	147.1	150.5	146.9	213	217
	2	151.4	155.3	149.2	222	220
	10	155.8	160.8	164.6	237	228
	20	159.2	164.8	187.5	240	226
	40	162.2	168.4	198.0	251	232
D-XYLOSE	0.5	136.8	142.4	138.5	207	227
	1	139.7	145.8	147.0	216	232
	2	143.7	150.1	147.1	222	232
	10	152.8	157.7	160.7	228	224
	20	156.3	162.7	174.0	236	227
	40	158.3	166.3	184.4	239	227

Two StepScan DSC measurements with different measurement parameters were performed for both L- and D-xylose samples. At the melting point, the change was seen in both thermodynamic and kinetic curves. There were differences between D- and L-xylose and also between different measurements of the same sample. The peaks of kinetic IsoK baseline curves indicated that melting was not only a thermodynamic equilibrium process. The melting enthalpies calculated from the kinetic curves were only slightly smaller than the enthalpies obtained by conventional DSC that showed how considerable the kinetic part of melting was.

According to Shafizadeh *et al.*[144,145] α-D-xylopyranose changes to β-D-xylopyranose upon heating. The crystalline α-D-anomer contains about 10% of the β-D-anomer up to about 125°C where the endotherm begins. It then continues to equilibrate until the melting is completed and the ratio of anomers becomes constant. At a temperature of 154°C, the ratio of α-D-xylopyranose and β-D-xylopyranose is 48%:52%, respectively.[144,145] The melting of xylose was anomalous at slow heating rates because, besides the melting, also partial thermal decomposition and mutarotation occurred. At higher heating rates, judged from the TG data, the mutarotation was the predominant factor in the change of the melting point.

In thermodynamic melting, the onset temperature should not change although the heating rate changes, except for the amount of thermal lag. Thermal lag depends on numerous factors, such as the capability of the instrument to change energy during heating, the response time of the instrument, the heat conduction from the heater to the pan and further to the sample, and the heat capacity and enthalpy of melting of the sample. The crystalline state and melt state are two different thermodynamic systems. An ideal melting process takes place at a constant temperature and is accompanied by an increase in the entropy of system, ΔS_{REV} or the melting entropy. In practice, non-reversible processes cause the observed absorption of heat to differ from the ideal enthalpy of melting, and the calculated entropy change, ΔS_{IRREV}, will differ from ΔS_{REV}. The entropy changes in melting at the different heating rates used in this study were calculated and the results are shown in Tables 4-7. As the results show, the melting entropy changes of sucrose, glucose, fructose and xylose were quite similar. The change of entropy increased with increasing heating rate; especially for sucrose there were notable differences in the melting entropies between low and high heating rates. For comparison, the melting entropy of maltitol was also calculated. The melting of maltitol is known to be normal[130,135] and the melting entropy is evidently higher (376 Jmol^{-1}°C^{-1}) than that of sucrose, glucose, fructose or xylose. The melting of maltitol is reversible whereas the melting of sucrose, glucose and fructose is irreversible because of simultaneous decomposition and/or mutarotation. The lower entropy values are due to the irreversibility of melting, especially at low heating rates.

The DSC and TG measurements in the present study were performed by raising the temperature from room temperature over the melting point at different heating rates. In official melting point determination methods,[156] the temperature of the bath should be raised to about 10°C below the presumed melting point and the sample should then be inserted into the apparatus only 5-10°C below the presumed melting point, so that thermally sensitive samples do not decompose during the measurement. For thermally sensitive samples, for example for different sugars, the melting point results should therefore always be validated.[156,157] In this study, melting points were determined also using a melting point apparatus. As the results of melting point apparatus were compared with melting temperatures determined with DSC, the general conclusion was that the melting point apparatus gave lower melting points than DSC. The values

obtained by the melting point apparatus were comparable with the slowest heating rates (0.5 – 1°Cmin^{-1}) of DSC.[I]

Based on all the results, the absolute melting can be seen in DSC best at a high heating rate because the other processes are slower. The one standardized melting point analysis method is the pharmacopeian method,[156] if this is taken into account, the heating rate of DSC should be 1°Cmin^{-1} and measurements should be performed in an inert atmosphere. The melting point determined in this way is anomalous and the differences between samples are clearly seen at this heating rate.

This work shows that the melting of sugars is a multiphase phenomenon and the results are affected by both the determination method and the origin and quality of samples. The results show that there can be differences between different samples of the same sugar although the measurements are performed identically. In addition the melting point alone cannot be used for identification of sugar samples in all cases.

6.2 DSC method development for quantification of amorphous content

The sensitivity of the technique for quantification of amorphicity 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 upwards with increasing heating rate, the magnitude of ΔC_p does not change.[50] 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 impossible. Hence the suitability, selectivity, and limit of detection of each technique are sample-specific.

In this study, the methods of quantification of low concentrations of amorphous components in sugar and sugar alcohol samples by HyperDSC and StepScan DSC were developed.[III-V] The samples were maltitol (both HyperDSC and StepScan DSC) and sucrose (HyperDSC). Amorphous maltitol was prepared from crystalline by melting. Maltitol occurs in one form only (no polymorphism was observed) and additionally no recrystallization was detected in the glass transition range during the measurements.[130] Once melted, maltitol can be undercooled without further cold crystallization.[135] The amorphous form of sucrose could not be prepared in the same way due to chemical degradation during melting, therefore, the amorphous sucrose was prepared by spray drying. Spray drying is widely used in pharmaceutical industries and it is known to produce predominantly amorphous material due to rapid solidification.[64,137,158]

The method for quantification of amorphicity was developed by first finding out the annealing time that resulted in a constant ΔC_p for totally amorphous samples. The purpose of annealing was to eliminate the effect of the thermal history of the sample. The change of annealing time affected the glass transition temperature and the change of specific heat: as annealing time increased, T_g became higher and ΔC_p became larger.

The outcome of annealing measurements was a measuring program which produced a constant ΔC_p at T_g for totally amorphous sample.

In the HyperDSC experiments of maltitol, the annealing temperature was 40°C[110] and annealing times 35 and 60 min were tested. The following temperature program was used to run the experiments: (a) heating from room temperature to annealing temperature (from 30°C to 40°C) at a heating rate of 10°Cmin[-1] and (b) annealing at 40°C for 60 minutes, then (c) cooling to the starting temperature (- 10°C) at a cooling rate of 10°Cmin[-1] and finally (d) heating from starting temperature over the glass transition region (from - 10°C to 100°C) at 100°Cmin[-1].**III**

In the sucrose experiments, an annealing temperature 60°C was chosen[110,132,133] and the different annealing times of 0.5, 1, 2, 4, 5 and 6 hours were tested. The temperature program chosen to final measurements was: (a) heating from room temperature to annealing temperature (from 25°C to 60°C) at a heating rate of 10°Cmin[-1] and (b) annealing at 60°C for 5 hours, then (c) cooling to the starting temperature (10°C) at a cooling rate of 10°Cmin[-1] and finally (d) heating from starting temperature over the glass transition region (from 10°C to 120°C) at 100°Cmin[-1].**IV**

At the beginning the StepScan DSC measurements of maltitol, the influence of the measurement parameters of the StepScan DSC method on the shape of the glass transition and the size of ΔC_p was first evaluated. This was done by changing one parameter at a time while the others were kept constant. Also two different calibration heating rates were tested. It was found that the heating rate used in the calibration had a significant effect on the shape of the specific heat curve at different StepScan parameters. On the basis of the shapes of the curves and the values of ΔC_p two heating rates were used in the calibration and for that reason two measurement methods were carried out and compared during the following measurements. For calibrations done at a heating rate of 2°Cmin[-1] the temperature program was: a heating rate of 2°Cmin[-1], a temperature increment of 2°C, a isothermal segment of 1 min, an equilibrium criteria of 0.01 mW (= Method 1). For calibrations done at a heating rate of 10°Cmin[-1] the temperature program was: a heating rate of 10°Cmin[-1], a temperature increment of 2°C, an isothermal segment of 1 min, an equilibrium criteria of 0.0001 mW (= Method 2).**V**

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 ΔC_p values of the P_2O_5 dried, totally amorphous samples are illustrated in Table 8. The results of maltitol are comparable with values of different authors[130,135,136,III,V] and the result for sucrose is in line with literature values.[93,108,127,132,IV]

Table 8. The ΔC_p values of parallel measurements of P_2O_5 dried totally amorphous samples of maltitol (n=6) and sucrose (n=4) measured with different methods.

Sample	Method	Calculated from	ΔC_p / $Jg^{-1}°C^{-1}$
Maltitol	HyperDSC	Fictive-T_g	0.73 ± 0.03
		Half-point-T_g	0.71 ± 0.04
Maltitol	StepScan DSC	Method 1	
		Fictive-T_g	0.71 ± 0.01
		Half-point-T_g	0.71 ± 0.01
	StepScan DSC	Method 2	
		Fictive-T_g	0.72 ± 0.01
		Half-point-T_g	0.72 ± 0.01
Sucrose	HyperDSC	Fictive-T_g	0.77 ± 0.01
		Half-point-T_g	0.76 ± 0.01

The mixtures with varying proportions of 100% amorphous and 100% crystalline sample were measured with a sample-specific measuring program. The ΔC_p values of synthetic mixtures were used to calculate average and standard deviation values and at least 3 parallel results were used for every point. The mean values of specific heat change at 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. 11.

	ΔC_p at Fictive-T_g	ΔC_p at Half-point-T_g
maltitol\n\nHyperDSC	$\Delta C_p = 0.00214 + 0.00702\ x,\ R = 0.997$	$\Delta C_p = 0.00153 + 0.00682\ x,\ R = 0.996$
maltitol\n\nStepScan DSC Method1	$\Delta C_p = 0.00547 + 0.00697\ x,\ R = 0.999$	$\Delta C_p = 0.00293 + 0.00699\ x,\ R = 0.998$
maltitol\n\nStepScan DSC Method2	$\Delta C_p = 0.00789 + 0.00708\ x,\ R = 0.999$	$\Delta C_p = 0.00692 + 0.00709\ x,\ R = 0.999$
sucrose\n\nHyperDSC	$\Delta C_p = 0.00754\ x - 0.00422,\ R = 0.999$	$\Delta C_p = 0.0075\ x - 0.00484,\ R = 0.999$
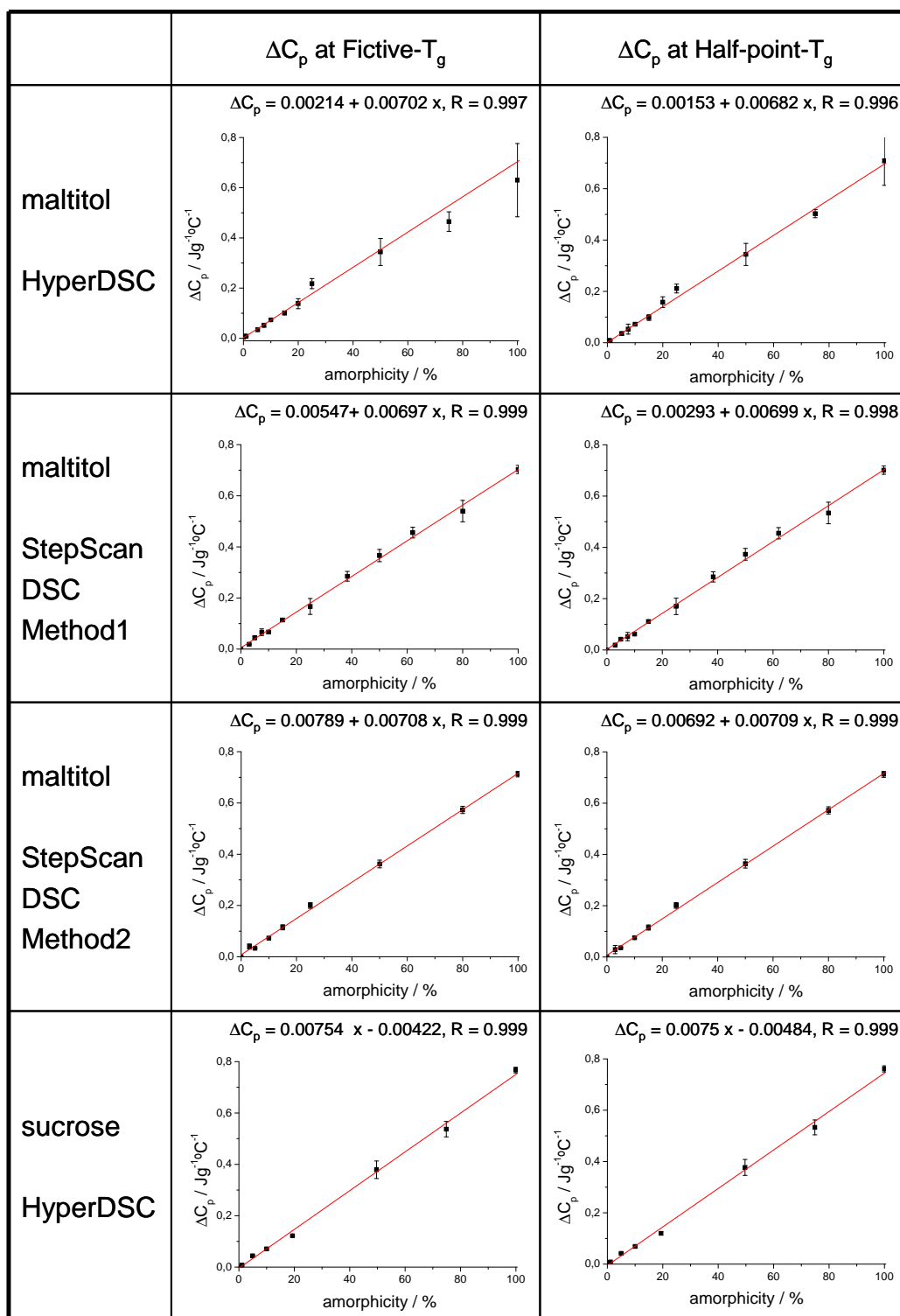
Figure 11. The average and standard deviation values for the specific heat change at glass transition temperature as function of amorphous content measured with HyperDSC and StepScan DSC for maltitol and sucrose samples.[III-V]

The sensitivity of HyperDSC is illustrated in Fig. 12 where DSC curves for various maltitol samples are shown. Even for a sample with 1% amorphous content the glass transition was easily seen such that the ΔC_p value could be calculated.

Figure 12. HyperDSC glass transitions for samples containing (a) 100%, (b) 10% and (c) 1% amorphous maltitol. Heating rate was 100°Cmin^{-1} and sample mass ca. 5 mg.[III]

6.2.1 Detection limits

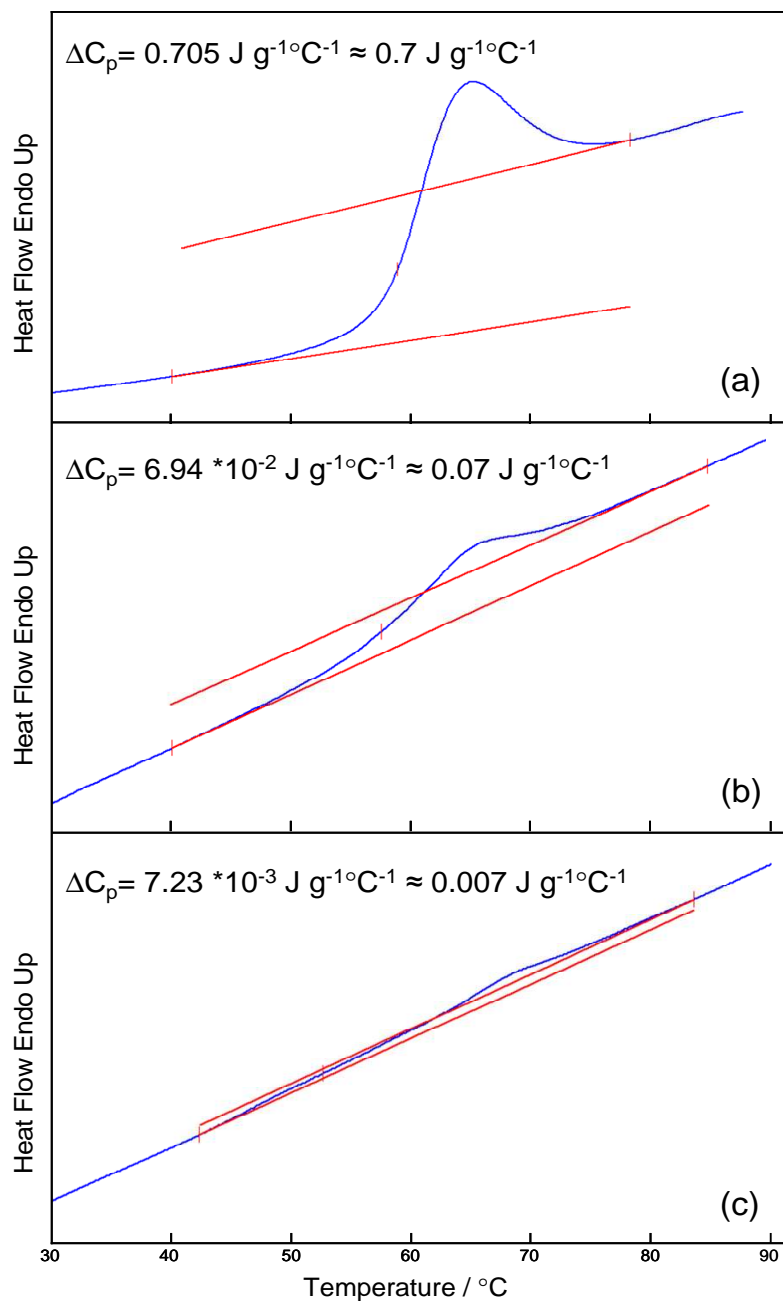The limit of detection (LOD) is defined as the analyte concentration giving a signal equal to the blank response plus three standard deviations of this value, whereas the limit of quantification (LOQ) is the analyte concentration giving a signal equal to the blank response plus ten standard deviations of this value. From the regression lines the numerical information for constants b, a, s_a and s_b can be obtained for the equation of a=bx+y. 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.[159] Table 9 summarizes the calculated LOD and LOQ values. The values are appreciably low for both the HyperDSC and StepScan DSC techniques.

Table 9. Calculated LOD and LOQ values.[III-V]

Sample	Method	Calculated from	LOD / %	LOQ / %
Maltitol	HyperDSC	Fictive-T_g	0.31	1.04
		Half-point-T_g	0.11	0.36
Sucrose	HyperDSC	Half-point-T_g	0.06	0.21
Maltitol	StepScan DSC	Method 1		
		Fictive-T_g	0.24	0.81
		Half-point-T_g	0.27	0.92
Maltitol	StepScan DSC	Method 2		
		Fictive-T_g	0.18	0.61
		Half-point-T_g	0.16	0.52

In addition, instrumental sensitivity and detection limit were determined. The calculated LOD value for a heating rate of 100°Cmin^{-1} and a 10 mg sample was 0.001%. Comparing the value of detection limit at a heating rate of 100°Cmin^{-1} with the experimental LOD value (Publication III: Table 8), it is possible to see that the experimental LOD is much higher than the instrumental limit of detection. This means that the preparation of the mixtures of samples was the weakest point in the determination of low amorphous levels.

6.2.2 Sources of error

There are a number of practical considerations associated with quantification of amorphicity using DSC. It is essential that baseline calibration is performed to ensure as flat baseline as possible. This is extremely important especially in the case of small glass transitions. The presence of relaxation endotherm can present a problem with the measurement of T_g because the baseline shift may sometimes make the identification and quantification of glass transition extremely difficult.

The largest source of error appears to be the inhomogeneity of mixing during the preparation of samples. Sample preparation and storage conditions must be carefully controlled because sugars and sugar alcohols are very hygroscopic and capable of absorbing water. The level of residual moisture has a profound influence on the measured glass transition.

The reliability of results could be improved if some other measurement techniques were used in parallel.

6.2.3 Comparison of 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 levels. In HyperDSC the sensitivity is higher owing to the high heating rates used. The limit of quantification is determined by the measurement noise and due to the lower noise level in HyperDSC, lower LOD and LOQ values can be reached 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 to be 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. In addition the calibration method also 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.

6.2.4 Quantification of amorphicity in ground sucrose samples

Most crystalline solids undergo processing that might produce partially or wholly disordered materials, and one of these, grinding, is often used in industry. In this study the effect of grinding time on the amorphous content of crystalline sucrose was investigated.[IV] Crystalline samples were ground and measured with HyperDSC using the same temperature program that was used for the measurements of test mixtures. The ΔC_p values of ground samples were plotted against grinding time and an exponential correlation was found ($R^2 = 0.998$) (Publication IV: Fig. 4). ΔC_p increased with grinding time. As illustrated earlier, ΔC_p is linearly proportional to amorphous content. By combining some equations also a correlation between grinding time and amorphous content in sucrose were found (Publication IV: Fig 5). The amorphous content increased rapidly at the start of grinding, but it stabilized as grinding time became longer (about two hours). Only an amorphous content of 80-90% could be attained by the grinding method used in this study.

Font *et al.*[160] ground crystalline sucrose in a ball mill for 1 to 60 hours and observed that amorphization degree increases with increasing grinding time. Crystalline sucrose were ground with a vibrating mill for 2 to 16 hours at room temperature by Tsukushi *et al.*[161,162] and Yamamuro *et al.*[163] They reported that only partly amorphized sucrose could be attained with this technique.[161-163]

The aim of the grinding studies was also to test the HyperDSC method for amorphous content determination. It was found that the determination of the change of the specific heat at the glass transition by HyperDSC is suitable for determining amorphous content in both synthetic mixtures and ground samples.

7 CONCLUSIONS

The purpose of this thesis was to explore the ability of the HyperDSC and StepScan DSC techniques to quantify low contents of amorphous phases in food and pharmaceutical samples which are mostly crystalline (sugar and sugar alcohol). For quantification of amorphous content, crystalline and amorphous reference samples are needed. As an amorphous material is prepared by melting of a crystalline material, it must be ascertained that no other events are taking place at the same time.

The melting of sugars is a multiphase phenomenon and the results are affected by both the determination method and the origin and quality of the sample. There can be differences between different samples of the same sugar. The differences in the literature values can be explained by different origins of materials and the different determination methods. The melting point alone cannot be used for identification of sugar samples in all cases.

The preparation of test samples for the determination of small amorphous levels is difficult because amorphous sugars and sugar alcohols readily absorb moisture. In addition, dried crystalline samples may easily absorb small amounts of moisture if stored under normal humidity at room temperature, however, the results of experimental samples stored over P_2O_5 were satisfactory.

In this study, new methods for quantification of low concentrations of amorphous components in sugar and sugar alcohol samples (maltitol and sucrose) were developed. The change in the specific heat (ΔC_p) at the glass transition was shown to be a good indicator for the degree of amorphicity in samples that are mostly crystalline. It was found that the annealing time and temperature must be carefully chosen. The two relatively new DSC techniques, HyperDSC and StepScan DSC, provide us with obvious benefits for the accurate ΔC_p measurements. 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. The HyperDSC method was tested by grinding the crystalline sucrose for different time periods and good correlation between the grinding time and the amorphous content of the sucrose was found. This study has established that the two new DSC approaches should both be seriously considered as potential techniques for quantifying low concentrations of amorphous phases in various crystalline matrices.

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