INVESTIGATION OF THERMAL DECOMPOSITION AS THE CAUSE OF THE LOSS OF CRYSTALLINE STRUCTURE IN SUCROSE, GLUCOSE, AND FRUCTOSE

BY

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DISSERTATION

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ABSTRACT

Simple sugars (e.g., sucrose, glucose, and fructose) are abundantly used as basic ingredients in the food industry and as excipients in the pharmaceutical industry, due to the quality attributes they contribute to the final products, such as sweet taste, flavor, texture, color, shelf-life (e.g., flavor retention via encapsulation), and carrier function of active pharmaceutical components. These quality attributes directly depend on the processing protocol employed, in particular heating conditions, as well as the type(s) of sugar used. Thus, it is important to accurately and completely characterize the sugars prior to use in specific applications.

Melting has been commonly used for sugar characterization because it is an easy and quick property to measure, yet is usually repeatable and robust. In general, melting is determined by heating a sugar at a specified scanning rate using a thermal analytical technique, such as Differential Scanning Calorimetry. The sugar loses its crystalline structure (commonly termed melting) by adsorbing heat energy, which yields the critical parameters of the resultant melting peak, i.e., the onset melting temperature ($T_{m \text{ onset}}$), the peak melting temperature ($T_{m \text{ peak}}$), and the enthalpy of melting ($\Delta H$). These parameters provide a good deal of information for sugar identification and characterization (e.g., purity, type, size, etc.) purposes.

However, the reported melting temperatures for sugars vary widely. This variation has been attributed to a number of causes, such as differences in melting temperature determination methods, origin, impurity, polymorphs, superheating, liquefaction, and thermal decomposition and/or mutarotation in addition to melting. However, a complete explanation of the substantial variation observed in the sugar melting temperatures as a function of heating rates is not currently found in the literature. Of importance to note is that from a thermodynamic viewpoint, the heating rate dependency of the sugar melting temperatures suggests that the sugars do not
experience thermodynamic melting. Because thermodynamic melting occurs at a single, time independent temperature with a constant enthalpy value ($\Delta H$), where the crystalline solid and corresponding liquid phases are in thermodynamic equilibrium ($\Delta G=0$) at a constant pressure, the melting parameters for the sugars cannot be used for identification purpose.

Therefore, the ultimate objective of this research was to elucidate the fundamental mechanism underlying the loss of crystalline structure (melting) in these sugars. With thermal and chemical analytical approaches, this research found that the kinetic process of thermal decomposition was responsible for the loss of crystalline structure in these sugars, leading to heating rate dependency in their melting parameters. This result distinguishes thermodynamic melting from the loss of crystalline structure caused by thermal decomposition (termed “apparent melting” in this research), which solves the controversy that currently exists in the literature regarding the wide variation in the melting parameters of these sugars. These results prove not only that the loss of crystalline structure in the sugars is caused by thermal decomposition, but also that apparent melting in the sugars is achieved via a time-temperature combination process. This research also attempted to determine the thermodynamic melting temperature of these sugars by suppressing thermal decomposition using fast scanning rates. In the case of fructose, its thermodynamic melting was achieved. In addition, this research explored the effect of different heating conditions on the glass transition parameters for amorphous sucrose prepared by melt-quenching (i.e., melting followed by quick cooling) because melting-quenching is one of the common methods for preparing amorphous materials. The heating conditions employed directly effected the glass transition parameters obtained, where, in general, it was found that the longer time, lower temperature heating conditions resulted in lower glass transition temperature values. Therefore, in practical applications, this research is useful for better understanding the
quality and stability issues associated with heat processed sugar-containing food and pharmaceutical products and for developing new sugar based products.
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CHAPTER 1
INTRODUCTION

1.1. Rationale and significance

Sucrose, glucose, and fructose are abundantly occurring, common simple sugars used as basic ingredients in the food industry and as excipients in the pharmaceutical industry, due to the desirable attributes they contribute to final product quality, such as sweet taste, texture, color, flavor, shelf-life, and carrier function. These quality attributes directly depend on the processing protocol employed, in particular heating conditions, as well as the type(s) of sugar used. Thus, it is important to accurately and completely characterize these crystalline sugars prior to use in specific applications.

Melting is one of the most common properties utilized for material characterization, because melting is a fast and easy property to measure, yet is usually repeatable and robust. In general, melting is determined by heating a material at a specified scanning rate using a thermal analytical technique, such as Differential Scanning Calorimetry (DSC), which yields the critical parameters of the resultant melting peak: the onset melting temperature ($T_{m_{\text{onset}}}$), the peak melting temperature ($T_{m_{\text{peak}}}$), and the enthalpy of melting ($\Delta H$). These parameters, which are unique for each material, provide a good deal of information for material identification and characterization (e.g., purity, type, size, etc.) purposes.

However, the reported melting parameters for sucrose, glucose, and fructose vary widely in the literature. Although a number of explanations have been put forth, including measurement methods (Hurtta and others 2004b), origin (Beckett and others 2006), impurity (Shah and Chakradeo 1936; Hirschmüller 1953; Kamoda 1960; Okuno and others 2003; Beckett and others 2006), polymorphism (Kishihara and others 2001; Lee and Lin 2007; Lee and Chang 2009),
liquefaction (Tombari and others 2007), and thermal decomposition and/or mutarotation in addition to melting (Hurtta and others 2004; Lappalainen and others 2006b), they do not completely explain the large variation observed in the melting parameters of these sugar as a function of heating rates. The heating rate dependency of the sugar melting parameters indicates the occurrence of a time-dependent (kinetic) process during melting (loss of crystalline structure). From a thermodynamic viewpoint, this means that these sugars do not experience thermodynamic melting, since thermodynamic melting occurs at a single, time independent temperature (Wunderlich 1990a). Rather, the observed heating rate dependency suggests that the melting parameters for sucrose, glucose, and fructose cannot be used for identification purposes. Furthermore, this suggests the need for research studies to elucidate the cause(s) underlying this heating rate dependency.

The main theoretical contribution of this research is to ascertain the fundamental mechanism underlying the loss of crystalline structure in sucrose, glucose, and fructose – the loss of crystalline structure in these sugars, and perhaps other compounds that exhibit this heating rate dependency (termed herein “apparent melting”), under typical heating rate conditions. This knowledge will solve the controversy that currently exists in the literature regarding the melting of these sugars. In addition, the main practical contribution of this research is to apply the knowledge gained to improve the quality of current sugar based food and pharmaceutical products and to provide insight for developing new products.

1.2. Statement of research objectives

Thermodynamic melting occurs at a single, time-independent (i.e., heating rate independent) temperature with a constant enthalpy value ($\Delta H$), where the crystalline solid and corresponding
liquid phases are in thermodynamic equilibrium (\(\Delta G=0\)) at a constant pressure (Wunderlich, 1990a). Hence, substantial variation in the melting parameters (\(T_m\) ‚onset, \(T_m\) peak, and \(\Delta H\)) for sucrose, glucose and fructose reported in the literature is not consistent with the strict definition of thermodynamic melting. However, a complete explanation for the observed variation is not currently found in the literature and thus necessitates further investigation.

Therefore, the ultimate objective of this research is to elucidate the fundamental mechanism underlying the loss of crystalline structure (melting) in these sugars. In order to accomplish this purpose, this research was carried out with the following four specific objectives.

**Objective 1:** To investigate the heating rate dependency of the melting parameters for sucrose, glucose, and fructose, which implies the occurrence of a kinetic process during the loss of crystalline structure in these sugars, using a thermal analytical approach, i.e., standard differential scanning calorimetry (SDSC), modulated differential scanning calorimetry (MDSC), and thermogravimetric analysis (TGA). We hypothesize that the loss of crystalline structure in these sugars is caused by a kinetic process, resulting in the heating rate dependency on their melting parameters.

**Objective 2:** To determine if any decomposition components (e.g., glucose, fructose, and 5-HMF) are formed during loss of crystalline structure in sucrose using a chemical analytical approach, i.e., high-performance liquid chromatography (HPLC). Sucrose samples for HPLC analysis will be prepared using two different methods, i.e., a SDSC method (a relatively fast heating method) and a quasi-isothermal MDSC method (a relatively slow heating method). We hypothesize that if the loss of crystalline structure in sucrose is caused by thermal decomposition,
decomposition components, glucose, fructose, and 5-HMF, will be formed and detected by HPLC analysis concomitant with the onset of the loss of crystalline structure.

Objective 3: To measure the thermodynamic melting temperature for sucrose, glucose, and fructose using rapid-scanning differential scanning calorimetry, which uses extremely fast scan rates compared to a conventional DSC. We hypothesize that if the heating rate is fast enough to suppress the kinetic process of thermal decomposition, the thermodynamic melting temperature for these sugars could be measured.

Objective 4: To examine the effect of heating conditions on the glass transition parameters of amorphous sucrose prepared by melt-quenching. Melt-quenching (i.e., melting followed by quick cooling) is one of the common methods for preparing amorphous materials. Depending on the heating conditions employed, the amorphous sucrose prepared by melt-quenching may experience different thermal decomposition histories (i.e., formation of different amounts and types of thermal decomposition components), which in turn may affect their overall resultant quality attributes, of specific interest herein the $T_g$ values. We hypothesize that the heating conditions employed will have a significant influence on the amorphous sucrose $T_g$ values, with slower heating methods resulting in lower $T_g$ values and faster heating methods resulting in higher $T_g$ values.
2.1. Sugars in the food and pharmaceutical industry

Sugars (e.g., sucrose, glucose, and fructose) have been widely used as key ingredients in both the food and pharmaceutical industries because of the quality attributes they contribute to the final product. In addition to contributing sweetness, sugars also play important roles in terms of texture, color, flavor, shelf-life, and carrier function in numerous products, such as bakery goods (e.g., bread, cakes, cookies), fat-phase confectionery (e.g., dark and milk chocolate, couvertures), syrup-phase confectionery (e.g., hard, boiled sweets or candy, caramels, fudges, fondants, soft and hard nougats, toffees), breakfast cereals, dairy products (e.g., sweetened condensed milk, yogurt, ice creams), preserves (e.g., jams, jellies) and pharmaceutical excipients (Jeffery 1993; Mathlouthi 1995; Lindley 1988; Mullarney and others 2003). In food products, for example, sugars act as humectants for preservation and extended shelf-life in bakery products and as binders for increasing crispness, spread and surface porosity in breakfast cereals, in addition to providing desirable flavor, color, and sweetness. In particular, sugar surface coating on breakfast cereals significantly increases their shelf-life. In fat-phase confectioneries, sugar often makes up 40 to 50% of the solids dispersed in the fat phase, thus sugar’s functional properties, such as enhancing mouthfeel and fatty flavor, in the fat phase are critical to product acceptability. In syrup-phase confectioneries, sugars provide different quality attributes, depending on product type; for example, the recrystallization of the syrup phase is required in the case of fondants and fudges, whereas the formation of a stable syrup phase (amorphous form) is required in the case of hard candy and caramels. In pharmaceutical products, sugars are mainly used as excipients, which are inert substances in order to confer a suitable consistency or form to
the active drug component, enabling delivery of medicine in a variety of dosage forms (Smolinske 1992; Moreton 1995). For example, some sugars (e.g., sucrose, lactose, mannose, fructose, trehalose) in crystalline, partially crystalline, or amorphous forms are used as excipients for large-scale tablet and capsule manufacturing and as fillers in chewable tablets (Ritchie and Winfield 1984; Ishikawa and others 2001; Mullarney and others 2003; Joshi and Duriez 2004; Surana and others 2004; Airaksinen and others 2005; Gohel and Jogani 2005; Fu and others 2006).

However, the quality attributes of these products depend on the processing protocol (mostly thermal processing) and the type(s) of sugar used. Therefore, it is vital to accurately and completely determine the characteristics of the sugars prior to their applications, in particular, to high sugar containing products.

2.2. Melting for material characterization

A crystalline material is often characterized by its melting properties (Wundrilich 1980). In general, melting is defined as the transition from the solid crystalline phase (also termed state or form) of a material to the liquid phase (Wunderlich 1990a). The phase transition is complete when the material loses all of its crystalline structure. Melting is commonly measured by heating a material at a specified heating rate using a thermal analytical technique, such as Differential Scanning Calorimetry (DSC) or Differential Thermal Analysis (DTA), which provides the critical parameters of the resultant melting peak; that is, the onset melting temperature (T_m onset), the peak melting temperature (T_m peak), and the enthalpy of melting (ΔH). These melting parameters provide a good deal of information about the characteristics of the material (e.g., purity, type, size, etc.), thus melting has been widely used to identify crystalline materials.
Even though melting is a unique property of a crystalline material, the melting parameters for some crystalline materials reported in the literature vary widely, in spite of using the same instrumentation and the same experimental conditions for determination of the melting parameters. As shown in Table 2.1, the literature reported $T_m$ onset values for crystalline sucrose, glucose, and fructose vary widely, ranging from 160°C to 192°C for sucrose, from 130°C to 171.8°C for glucose, and from 80°C to 125.8°C for fructose. What is the underlying cause(s) of such wide variation in reported melting parameters for these simple sugars? To answer this question, a more detailed thermodynamic based discussion of melting is needed. Because this research was performed using high purity crystalline sucrose, glucose, and fructose, as shown in Appendix A, melting will be discussed for a one-component system.

2.3. Thermodynamic melting and apparent melting

2.3.1. Thermodynamic melting

Melting occurs when, at a given temperature and atmospheric pressure, a crystalline material in the solid phase, which has full long-range order, is converted to an amorphous material in the liquid phase, which does not have order. Melting can thus be thought of as loss of crystalline structure. During the phase transition, the thermal energy required for loss of crystalline structure is called the latent heat of fusion, which is equivalent to the enthalpy of melting ($\Delta H$). Upon completion of the phase transition, molecular order decreases and mobility increases. These changes in the material caused by the phase transition can be thermodynamically described by the following functions of state: enthalpy (H), entropy (S), heat capacity ($C_p$), and Gibbs energy (G).
To define thermodynamic melting, it is necessary to know the relation between these state functions and temperature. Enthalpy (H), which is the amount of energy absorbed in a process in which the material transitions from one phase to another, increases with increasing temperature (Figure 2.1a). The enthalpy of the crystalline phase is less than that of the amorphous phase, because the crystalline phase is more ordered. Entropy (S), which reflects the randomness of the molecules in the material, increases with increasing temperature (Figure 2.1b). As in the case of enthalpy, the crystalline phase has the lowest entropy, because it is the most ordered phase. Heat capacity (C_p), which is the energy required to increase the temperature of a material by a certain temperature interval, increases with increasing temperature (Figure 2.1c). However, Gibbs energy (free enthalpy, G = H-TS), which is related to the overall stability of the material, decreases with increasing temperature (Figure 2.1a).

The schematic illustration of Gibbs energy as a function of temperature, at a constant pressure, for a one-component system as shown in Figure 2.2 is useful for discussing the nature of thermodynamic melting. At low temperatures (below T_m), the material is in the crystalline phase, since the crystalline phase has the lowest Gibbs energy, compared to the liquid phase, and is thus the most stable phase. At T_m, both the crystalline and liquid phases of the material are in equilibrium, since the phases have the same Gibbs energy and ΔG=0. Thermodynamic melting is strictly defined as the intersection of the crystalline and liquid Gibbs energy curves. Therefore, thermodynamic melting occurs at a single, time-independent (i.e., heating rate independent) temperature. At high temperatures (above T_m), the material is in the liquid phase, since the liquid phase has the lowest Gibbs energy, compared to the crystalline phase, making it the most stable phase.
In practice, melting parameters are obtained using thermal analytical techniques, such as the Differential Scanning Calorimeter (DSC) and Differential Thermal Analysis (DTA). DSC, one of the thermal analysis techniques used in this research, measures the heat flow difference between a sample and inert reference (typically an empty pan) as a function of temperature or time, which can be converted to enthalpy (H) and heat capacity (C_p). Integration of the heat flow signal provides enthalpy (H), which is a function of the material’s specific heat (C_p) and energy absorbed or released by the material due to phase transitions. Specific heat or heat capacity is the result of molecular motion, and therefore, changes in heat capacity reflect changes in the molecular mobility of the material. One of the important aspects of thermodynamic melting, which DSC makes visible, is that at any given temperature, there is an absolute difference in enthalpy (∆H, J/g) between the crystalline and amorphous phases. That absolute difference determines the size (J/g) of the endothermic peak when the crystalline material loses its crystalline structure (melts) and the size of the exothermic peak when an amorphous material crystallizes. The amount of heat that must be absorbed to convert the crystalline phase to the amorphous phase is independent of what causes the phase transition. The best way to illustrate the difference in enthalpy between the two phases, as a function of temperature, is with an enthalpy plot. An enthalpy (J/g) plot is created by first taking the absolute integral of a heat flow signal (W/g, where W=J/s) with respect to time or the absolute integral of a heat capacity signal (J/g·°C) with respect to temperature. Enthalpy plots for 100% crystalline and 100% amorphous phases are shown at the top of Figure 2.3. The heat capacity signals used to create the enthalpy plots are shown at the bottom of Figure 2.3.

Two important characteristics can be observed from the enthalpy plot (Figure 2.3, top). First, there is an absolute difference in enthalpy (∆H, J/g) between amorphous and crystalline
phases, and that difference increases with increasing temperature due to differences in the heat
capacity between the two phases. Secondly, the laws of thermodynamics require a crystalline
material to absorb the difference in enthalpy between the two phases in order to become
amorphous. Based on these characteristics, the difference in enthalpy between the crystalline and
amorphous phases at the thermodynamic melting temperature should be a single, constant value,
equal to the area of the endothermic peak (ΔH, J/g) obtained using DSC. Thus, the ΔH value for
thermodynamic melting is also time-independent, i.e., heating rate independent.

Theoretically, as discussed above, thermodynamic melting occurs at a single temperature
with a specific enthalpy. However, both material properties and measurement methods can affect
the experimentally obtained melting parameters. In the case of pure and relatively small
molecules, thermodynamic melting usually occurs over a narrow temperature range. However, in
the case of larger molecules, e.g., polymers, thermodynamic melting occurs over a broad
temperature range due to a number of factors, including molecular weight distribution, crystal
purity, type, size, and crystal perfection during heating. For example, in the case of
macromolecular materials with poor (or imperfect) crystals, crystal perfection during heating can
occur, resulting in an increase in the melting temperature of the original material. Fast heating
rates are sometimes used to avoid crystal perfection during heating; however, using too fast of a
heat rate may lead to superheating (Wunderlich 1990b). Superheating occurs when heat is
supplied to the crystals faster than they can melt. As explained by Wunderlich (1990b, pp. 197),
the result is that "the interior of the crystal heats above the melting temperature and finally melts
with entropy production at a high temperature, when the interface between the crystal and the
melt progresses sufficiently."
In addition to material properties, measurement methods can also affect the experimentally obtained melting parameters, both between methods (e.g., DSC versus a melting point apparatus) and within a method (Hurtta and others 2004b). For example, melting parameters are often determined by heating a sample to a temperature greater than its melting temperature at a specific heating rate using Differential Scanning Calorimetry (DSC). In DSC analysis, the furnace attempts to heat the sample at the user selected heating rate and a thermocouple, or other temperature sensor, is used to measure sample temperature. However, the sample temperature lags behind the thermocouple temperature, because the thermocouple is not in direct contact with the sample (i.e., the heat must flow across a barrier to get to the sample). This difference between the thermocouple and sample temperature, termed thermal lag, affects the determination of the melting temperature. The thermal lag varies with experimental conditions. Fast heating rates, large sample sizes, heavy sample pans, poor thermal conductivity or contact between pan and platform, and high heat capacity materials (e.g., aqueous solutions) all increase thermal lag (Cassel 2008a). However, use of modest heating rates (1 to 25°C/min), sample sizes (1 to 5 mg), and sample pan masses can reduce melting parameter variation caused by thermal lag. For example, in the case of indium (3.8 mg), a common DSC calibration standard, \( T_{\text{m onset}} \), \( T_{\text{m peak}} \), and \( \Delta H \) increased by only 0.75°C, 1.31°C, and 0.38 J/g, respectively, when using heating rates of 1 and 25°C/min with hermetic aluminum DSC pans (~ 50 mg).

2.3.2. Apparent melting

Based on the previous discussion of thermodynamic melting, one might expect that relatively pure and small molecular weight materials, such as sucrose, glucose, and fructose, would exhibit a constant set of melting parameters. However, as shown in Table 2.1, the widely varying melting parameters for sucrose, glucose, and fructose were observed by a number of
studies. Another important finding is that the melting parameters of these sugars were dependent on the heating rate, as specifically reported by Hurtta and others (2004b), which investigated the effect of heating rates (0.5 to 100 °C/min) on sugar melting parameters. In general, the melting parameters (i.e., $T_{m\text{ onset}}$, $T_{m\text{ peak}}$, and $\Delta H$) increase with increasing heating rate. A similar observation was also found in D-xylose and L-xylose (Lappalainen and others 2006b), as shown in Table 2.2.

What is the underlying cause of this heating rate dependency observed in the sugar melting parameters? A complete explanation for this heating rate dependency for all of the sugar melting parameters is not currently available in the literature. However, a number of publications have discussed the large variation and have offered the following possible explanations: a) origin, b) impurity, c) polymorphism (or allotrohism), d) superheating, e) liquefaction, and f) thermal decomposition and/or mutarotation in addition to melting.

a) Origin: The origin of the sugar may be responsible for the wide variation of melting temperatures, since there are different sources (e.g., cane and beet sugar) and manufacturing methods. For example, it is well documented in the literature that sucrose prepared by different manufacturing methods (e.g., reagent grade versus commercial grade) yields different shapes and numbers of DSC endothermic melting peaks (one, two or three), which can give rise to different $T_{m\text{ onset}}$ values (Richards and Shafizadeh 1978; Kelly and Brown 1978/79; Eggleston and others 1996; Kishihara and others 2001; Okuno and others 2002a, 2002b, 2003; Hurtta and others 2004b; Kishihara and others 2004; Beckett and others 2006; Lee and Chang 2009). To date, these findings on the variation in sucrose melting peaks have been mainly related to impurity or polymorphism (discussed below). However, it is important to note the $T_{m\text{ onset}}$ still exhibits a
heating rate dependency for a single type of sucrose (e.g., reagent grade) (Okuno and others 2003; Hurtta and others 2004b; Beckett and others 2006).

b) Impurity: In general, the presence of impurities significantly decreases the melting temperature and broadens the endothermic melting peak (Widmann and Scherrer 1991; Cassel 2008b). However, it has been reported that the melting temperature for sucrose was not always depressed by impurities; but was dependent on the type and amount of impurities, such as organic solvents, mineral salts, water, and thermal decomposition components (Shah and Chakradeo 1936; Hirschmüller 1953; Kamoda 1960; Beckett and others 2006). Okuno and others (2002a, 2002b, 2003) reported a similar observation, however, they concluded that the large variation in the sucrose melting temperature was because the impurities contained in the sucrose solution during crystallization affect the formation of different structure in sucrose crystals, which is termed polymorphism (discussed below).

c) Polymorphism (or allotrophenism): A number of other publications attribute the difference in sucrose melting temperatures to the presence of conformational polymorphs (Kishihara and others 2001; Lee and Lin 2007; Lee and Chang 2009). However, Saska (2008) specifically disagreed with the presence of sucrose polymorphs as reported by Lee and Lin (2007). The idea of sugar polymorphism is not new, but was also mentioned by Shallenberger and Birch (1975), using the term allotropic forms. These researchers suggested that the presence of different allotropic forms can cause problems in the identification of a sugar by its melting temperature, since the sugar crystal can alter its allotropic form during slow heating, such as is employed in the determination of a melting temperature. However, no data was given to support this suggestion.

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1Allotropic forms refers to the way that the molecules in a material are packed into the cell of the crystal lattice, in much the same way that carbon can exist as diamond, graphite, or lamp-black.
**d) Superheating:** Typically, melting is sufficiently fast so that its “rate” is determined by the conduction of the heat of fusion into the crystal; thus, the temperature of the crystal does not rise above the melting temperature until the end of the transition (Wunderlick 2007). However, as mentioned above, superheating occurs when heat is supplied to the crystals faster than they can melt. In superheating, the surface of the crystals still begin to melt at or close to the equilibrium melting temperature, but because melting occurs so slowly that the temperature of the interior of the crystals rises above the equilibrium melting temperature before the end of the transition. Tammann (1910), and later Hellmuth and Wunderlick (1965), ascribed the heating rate dependency they observed in glucose melting curves to superheating. Melting was thought to still begin when the equilibrium melting point was reached, but that the crystals easily superheated because of slow melting due to the H-bond structure of glucose, which is also at the root of the high viscosity of the molten glucose (Wunderlick 2007). In studies performed more recently on glucose, as well as other sugars (Hurtta and others 2004b; Lappalainen and others 2006b), using modern DSC instruments, \( T_{m \text{ onset}} \) has been shown to actually shift to higher starting temperatures with increasing heating rate, proving that superheating is not the underlying cause of the heating rate dependency of the sugar melting parameters. Magoń and Pyda (2009) also reported that sucrose, glucose, and fructose exhibited superheating, but their data clearly shows that \( T_{m \text{ onset}} \) continues to increase gradually with increasing heating rate. Thus, based on the definition of superheating (no change in \( T_{m \text{ onset}} \)) this increase in \( T_{m \text{ onset}} \) strongly suggests that superheating is not the cause of the observed heating rate dependency for these sugars.

**e) Liquefaction:** Spontaneous "liquefaction" is the theory proposed by Tombari and others (2007) to explain the large variation observed in the melting parameters for isomerizable materials, such as fructose, glucose, and galactose (Shallenberger 1978; Horton and Walaszek
Their theory is based on the formation of tautomers via mutarotation during heating. When an isomerizable crystalline material is held isothermally, the energy of the molecules next to a vacancy in the crystal lattice increases, which may be sufficient to isomerize it to a tautomer that no longer fits in the lattice site nor is able to revert to its original state. This gives rise to the formation of a liquid like local region embedding the vacancy. Similar regions may appear elsewhere in the lattice and when the number of such regions becomes high enough they coalesce and the crystal spontaneously liquefies at the isothermal temperature, significantly below the melting temperature of the material. The time required for liquefaction was found to be dependent on the difference between the isothermal temperature and the reported crystalline melting temperature, according to a non-exponential rate kinetics and a temperature dependent rate constant. In addition, they reported that the liquefied materials studied did not crystallize upon cooling, specifically discussing the lack of recrystallization in the case of fructose over a six year time period. Since the authors found that the isomerizable crystalline materials they studied spontaneously liquefy over a period of time (i.e., hours to weeks) at temperatures far below their reported melting temperatures, they concluded that the thermodynamic melting temperature for these crystals could not be determined. A point important to the current study is that Tombari and others (2007) also mentioned that their results could be a consequence of chemical decomposition, however, they did not find evidence that decomposition occurred for fructose within their work or within the literature they cited.

f) Thermal decomposition and/or mutarotation in addition to melting: By comparing the $T_{m\text{onset}}$ measured by DSC, to the initial decomposition temperature ($T_1$), measured by Thermogravimetric Analysis (TGA), Hurtta and others (2004b; sucrose, glucose, and fructose in Table 2.1) and Lappalainen and others (2006b; xylose in Table 2.2) concluded that the observed
heating rate dependency of $T_{m \text{ onset}}$ for these sugars at low heating rates was due to the occurrence of thermal decomposition and mutarotation (except no mutarotation in the case of sucrose), in addition to melting. In the case of sucrose at relatively high heating rates, they reported that melting occurred before thermal decomposition. However, from our observation of their sucrose data, it appears that at relatively high heating rates, $T_{m \text{ onset}}$ continues to increase slightly, in addition to a relatively large increase in enthalpy (no explanation for the large increase in enthalpy was offered). In the case of glucose, fructose, and xylose at relatively high heating rates, the authors concluded that mutarotation was predominantly responsible for the observed increase in $T_{m \text{ onset}}$ with increased heating rate. Regarding the observed increase in enthalpy for all heating rates employed for fructose and glucose, Hurtta and others (2004b) mentioned that endothermic/exothermic changes associated with caramelization/decomposition might have also affected the enthalpy values.

Two aspects of the conclusions drawn by Hurtta and others (2004b) and Lappalainen and others (2006b) that require further discussion are: 1) the use of mutarotation as a possible explanation for the observed heating rate dependency of $T_{m \text{ onset}}$ for glucose, fructose, and xylose and 2) the difference in thermal lag between DSC and TGA measurement methods. Hurtta and others (2004b) and Lappalainen and others (2006b) included mutarotation (in addition to decomposition and melting) as a possible explanation for the observed heating rate dependency of $T_{m \text{ onset}}$ for glucose, fructose, and xylose; however, no specific mechanism of action was suggested. Since mutarotation in crystalline sugars is induced only after melting begins (Wlodarczyk and others 2009), it should only affect the melting parameters associated with the shape of the endothermic melting peak – $T_{m \text{ peak}}$ and $\Delta H$, but not the temperature at which
melting begins – $T_{m\text{ onset}}$. Therefore, we assert that mutarotation is not a possible explanation for the observed heating rate dependency of $T_{m\text{ onset}}$.

The second factor influencing the conclusions drawn by Hurtta and others (2004b) and Lappalainen and others (2006b) is the difference in thermal lag between DSC and TGA measurement methods. Unlike in DSC, the thermocouple in TGA is not in direct contact with the sample pan, so thermal lag in a TGA experiment grows larger as heating rate increases compared to a DSC experiment, resulting in a higher reported initial thermal decomposition temperature ($T_i$) than actually occurs. Hence, it is possible that in these two studies, at relatively high heating rates (up to 100°C/min), $T_i$ is actually much closer to, or even lower than, $T_{m\text{ onset}}$ for the sugars due to the large thermal lag in the TGA measurement method. Thus, caution is needed when comparing $T_i$ to $T_{m\text{ onset}}$ for determining thermal event order especially at high heating rates.

In addition to Hurtta and others (2004b) and Lappalainen and others (2006b), a number of other researchers have concluded that thermal decomposition (but not mutarotation), in addition to melting, are responsible for the observed variation in the sugar melting temperatures (Kamoda 1960; Öris 1973; Roos 1995; Kishihara and others 2004; Sakamoto and others 2006). However, there are some differences in the specific experimental results reported in these studies.

All of the above possible explanations do not completely and/or satisfactorily account for the large variation observed in the melting parameters for these sugars. Specifically, variation in $T_{m\text{ onset}}$ is still observed as a function of heating rate, despite using the same sugar and the same measurement method and taking thermal lag into account. However, these studies do support the observation that these sugars do not exhibit thermodynamic melting, but rather appear influenced by a kinetic process. However, it is often impossible to distinguish between thermodynamic melting and the melting caused by a kinetic process if only a single heating rate is used in DSC.
analysis. Because both thermodynamic melting and the melting caused by a kinetic process causes the loss of crystalline structure, their resultant endothermic peaks exhibit similar characteristics, which lead to considerable confusion. Herein, the term “loss of crystalline structure” will be used instead of melting to prevent confusion. In order to distinguish melting that is dependent on heating rate from thermodynamic melting (heating rate independent), we propose implementation of a new term, “apparent melting”, which describes the loss of crystalline structure (crystalline to amorphous phase transition) caused by a kinetic process. Unlike thermodynamic melting, apparent melting may occur at a temperature below the reported thermodynamic melting temperature.

2.4. Kinetic processes causing loss of crystalline structure

Knowing the type of kinetic processes associated with the loss of crystalline structure is of vital importance for elucidating the underlying cause of apparent melting in the case of the sugars. In this section, three possible kinetic processes involved with loss of crystal structure will be discussed; that is, thermal decomposition, dehydration, and chemical interaction (Figure 2.4).

2.4.1. Thermal decomposition

Thermal decomposition (or degradation) is the chemical transformations taking place under the influence of heat at temperatures between 100 and 300°C (Moldoveanu 1998). In other words, thermal decomposition, through the input of heat energy (enthalpy), gives rise to alterations in the chemistry of the molecules (i.e., intermolecular bond breaking, for example, the glycosidic bond between glucose and fructose in the sucrose molecule) that compose the crystalline matrix. Once these intermolecular bonds begin to break, the molecules can no longer maintain their original crystalline structure, resulting in the loss of crystalline structure (matrix is now
amorphous). Herein, in the case of thermal decomposition, the enthalpy includes both the energy of amorphization (the energy difference between the crystalline and amorphous phases, at a specific temperature) and the energy need for bond breaking due to thermal decomposition. Since thermal decomposition is a kinetic (i.e., heating rate dependent) process, this enthalpy value is not constant because it increases as the temperature at which thermal decomposition occurs increases. Therefore, in the present research, thermal decomposition is proposed as the most plausible kinetic process responsible for the loss of crystalline structure in the sugars (i.e., sucrose, glucose, and fructose), but not as an additional process accompanying thermodynamic melting that other studies have concluded.

TGA, which detects weight loss caused by thermal decomposition, is often used with DSC to investigate if thermal decomposition is the cause of the loss of crystalline structure. For example, Figure 2.5 exhibits the $T_{m \text{ onset}}$ and $T_i$ for acetylsalicylic acid measured using simultaneous DSC/TGA at a heating rate of 1°C/min. The thermal decomposition ($T_i$) for acetylsalicylic acid begins at a lower temperature than $T_{m \text{ onset}}$, indicating that the loss of crystalline structure in acetylsalicylic acid is caused by thermal decomposition, not thermodynamic melting.

Although a number of publications have used both DSC (or Differential Thermal Analysis, DTA) and TGA techniques for investigating the effect of thermal decomposition on sugar melting, these studies have reported a wide array of conflicting results. For example, Öris (1973) observed that at a heating rate of 6°C/min, fructose started to decompose around 150°C as measured by TGA, which was much higher than its melting temperature, 120°C, as measured by DTA; whereas, glucose started to decompose just above its melting temperature. Raemy and Schweizer (1983) found that at a heating rate of 1°C/min, D-fructose, D-glucose, and sucrose
began decomposing at 170°C, 200°C, and 190°C, respectively, as measured by a special high pressure-DTA (100 bar), which was much higher than their melting temperatures, 80°C for D-fructose, 130°C for D-glucose, and 160°C for sucrose, measured by conventional heat flow calorimetry. However, Hurtta and others (2004b) and Lappalainen and other (2006b) reported that at relatively low heating rates, such as the 1 and 6°C/min rates used in the above studies, the thermal decomposition measured by TGA for fructose, glucose, sucrose, and xylose took place before their melting temperatures measured by DSC.

The discrepancy in the above results may be due to the difference in the use of instruments and measurement parameters and protocols, sugar origin, and sugar purity or quality. However, as mentioned previously, the larger thermal lag in TGA can lead to an over estimate of Ti. Because both TGA and DSC analyze a material using a specified heating rate, the thermal lag effect cannot be avoided. The important issue is that TGA has a larger thermal lag than DSC because of the non-contact thermocouple position in TGA (discussed in detail in the session on thermal analytical techniques). In addition, the thermal lag in TGA becomes larger with increasing heating rate, compared to DSC. Even though the thermal lag in TGA can be reduced using a large amount of sample and/or a high thermal conductivity gas (e.g. helium), the heating rate which can be used to obtain reliable TGA results is still restricted in the case of materials which undergo apparent melting. Therefore, if the above studies did not carefully consider the thermal lag issue, it would be possible to misinterpret the cause(s) for the differences in temperature between the DSC and TGA data. However, thermal analytical techniques alone may be not sufficient to unambiguously confirm the kinetic process of thermal decomposition as the cause of the loss of crystalline structure in the sugars. Thus, a chemical analytical approach, such
as High Performance Liquid Chromatography (HPLC) would be necessary. HPLC analysis will be discussed in the section on chemical analytical technique.

2.4.2. Dehydration

Melting is generally used to produce an amorphous material. However, the preparation of an amorphous material can be accomplished by a variety of methods, including the unusual method of direct transformation from the crystalline to amorphous phase without the melting of the crystalline material, such as in dehydration, milling (Willart and others 2001), irradiation (Hudson 1992; Baragiola and others 2008), and pressure (Sharma and Sikka 1996). These methods are termed “nonthermal routes of amorphization” (Willart and others 2002). Dehydration is of particular interest, compared to the other nonthermal routes of amorphization, because dehydration can cause the loss of crystalline structure in a crystalline material below its melting temperature when the crystalline material is heated at a specified heating rate using a DSC, which was used in the research herein. For example, Figure 2.6 shows the DSC thermogram of crystalline Drug A monohydrate using sealed and non-sealed (pinhole) pans. In the non-sealed pan, the DSC thermogram showed a broad endothermic peak around 70°C and then a recrystallization peak around 120°C. The recrystallization peak means that the crystalline phase in Drug A was changed to the amorphous phase, because water molecules present in Drug A evaporated through the pinhole, which is indicated by the broad endothermic peak. In the sealed pan, however, only a melting endothermic peak was observed around 109°C, since water molecules could not evaporate from Drug A. The significant piece of information in Figure 2.6 is that dehydration occurred well below the melting temperature of Drug A and was responsible for the loss of crystalline structure.
Actually, a number of publications have pointed out that dehydration prior to melting causes the loss of crystalline structure in the hydrated form of some crystalline sugars. For example, the DSC thermogram for crystalline raffinose pentahydrate ($\text{C}_{18}\text{H}_{32}\text{O}_{16} \cdot 5\text{H}_{2}\text{O}$), which containing 5 mol of water molecule per 1 mol of raffinose, showed several endothermic peaks, indicating the successive removal of the water molecules present in the crystalline raffinose pentahydrate (Frank 2007). The crystalline raffinose pentahydrate in an unsealed pan preheated to 125°C and then isothermally held for 5 min using DSC did not show any endothermic peak in its second DSC scan. Furthermore, during preheating at 125°C, the total weight loss evaluated using TGA was nearly equal to the loss of 5 mol of water from crystalline raffinose pentahydrate (Cheng and Lin 2006). This result indicates that the loss of the crystalline structure in crystalline raffinose pentahydrate is entirely caused by dehydration. Bates and others (2007) also agreed with this result by showing that the crystalline structure in raffinose pentahydrate stored for over 24 hrs in a vacuum oven at 60°C, which is well below its reported melting temperature (79.7°C from Kajiwara and Franks 1997), was changed to the amorphous phase by progressive water evaporation. They also noted that no significant difference was observed between amorphous raffinose formed by dehydration and by freeze-drying in terms of the glass transition temperature ($T_g$) and water sorption profiles.

Figure 2.7 shows that the birefringence (characteristic of crystals) of crystalline raffinose pentahydrate was completely absent after vacuum dehydration at room temperature, which indicates that the crystalline structure of the raffinose pentahydrate became amorphous below its reported melting temperature. Despite the loss of crystalline structure, however, no change in the shape and dimensions of the original crystal was observed (Franks 2007). A similar observation also was found for trehalose dihydrate (Ding and others 1996). Even though the trehalose
dihydrate crystalline structure \((\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot 2\text{H}_{2}\text{O})\) completely disappeared after dehydration was performed well below its reported melting temperature \((T_{m}= 95.7^\circ\text{C}, \text{Ding and others 1996})\) under ambient and vacuum conditions, the original crystal shape was retained.

However, not all crystalline hydrates are converted to only the amorphous phase by dehydration. The structural condition or thermodynamic properties of the material formed by dehydration are substantially influenced by the dehydration rate, which is the kinetics of water loss. Crystalline trehalose dihydrate directly transitioned to anhydrous amorphous trehalose using fast dehydration, whereas it transitioned to anhydrous polymeric crystalline \((\alpha)\) trehalose using slow dehydration \((\text{Willart and others 2002})\). The dehydration rate depends on several factors such as temperature, crystal size, the degree of disorder in the crystals, and the presence of water and its location in the crystals \((\text{Taylor and York 1998})\). In particular, the presence of water in the crystals is necessary for a conversion to take place from the crystalline to the amorphous phase via dehydration. The conversion occurs when water molecules, which support the hydrate crystalline structure, are removed and vacancies are formed. An increase in the number of vacancies (crystal defects) gives rise to an increase in lattice disorder, which subsequently leads to conversion to the amorphous phase \((\text{Bates and others 2007})\).

Similar to raffinose and trehalose, fructose and glucose can be crystallized as hydrate forms. Fructose can exist as crystalline fructose dihydrate \((\text{Young and others 1952}; \text{Ben Gaïda and others 2006})\) and glucose as crystalline \(\alpha\)-D-glucose monohydrate \((\text{Boonyai and Bhandari 2006})\). Due to the presence of water molecules, these hydrate forms have lower melting temperatures, 20.87\(^\circ\text{C}\) for fructose dehydrate \((\text{Ben Gaïda and others 2006})\) and 71.3\(^\circ\text{C}\) for \(\alpha\)-D-glucose monohydrate \((\text{Ben Gaïda and others 2006})\), compared to their anhydrous forms, 127\(^\circ\text{C}\) for fructose and 158\(^\circ\text{C}\) for glucose \((\text{Roos 1995})\). However, the anhydrous crystalline fructose and
glucose used in the research herein were highly pure (greater than 99.5%). Even if they contain their hydrate form, it is unlikely, at such trace amounts, that dehydration (open system) or dissolution (closed system) is responsible for the observed changes in $T_{m\text{ onset}}$ of the anhydrous form as a function of heating rate.

### 2.4.3. Chemical interaction

Loss of crystalline structure caused by chemical interaction (also termed chemical incompatibility), between excipient (carriers) and active ingredient in drugs, has been reported in the pharmaceutical field, negatively impacting the drug effectiveness and shelf-life (Balestrieri and others 1996; Ceschel and others 2003; Oliveira and others 2005; Kiss and others 2006). The partial or complete chemical interaction (incompatibility) between excipient and active ingredient results in a new melting peak, which usually falls between the excipient and active ingredient melting temperatures. For example, the mixture of aspirin (acetylsalicylic acid) and magnesium stearate (an excipient) results in a melting temperature lowering effect for aspirin, caused by their chemical interaction (Figure 2.8, Wissing and others 2000). Melting temperature lowering was ascribed to the intimate contact between the drug and excipient, where the excipient forms a surface film on the active ingredient particles, which may promote the decrease in the active ingredient's melting temperature (Miller and York 1988).

The DSC thermogram for the mixture of an excipient and an active ingredient also shows other endothermic peaks caused by chemical interaction, in addition to their individual melting peaks. For example, Desai and others (2003) observed that the physical mixture of lactose (excipient) and fluconazole (antifungal active ingredient) exhibited additional peaks at 86.1°C and 136.4°C, which were lower than the reported melting temperature of pure fluconazole, 140.2°C (Figure 2.9c, left). By using X-ray diffraction, the authors confirmed that most of the
peaks present in the pure fluconazole (Figure 2.9a, right) were absent in the mixture (Figure 2.9c, right), indicating the loss of most of the crystalline structure in fluconazole due to partial chemical interaction with lactose (Desai and others 2003). A similar observation was found for other drugs, such as acetylcysteine, indomethacin, and glimepiride (Kerč and others 1992; Venkataram and others 1995; Cides and others 2006).

A decrease in melting temperature is possible via chemical interaction when two (or more) incompatible materials are physically mixed together. However, the three sugars used in the present research have a high purity (SigmaUltra, greater than 99.5%), and thus, chemical interaction is not the likely cause of the observed changes in $T_{m\text{ onset}}$ as a function of heating rate.

2.5. Thermal analytical techniques

Most foods undergo moderate to severe heat treatments during processing and/or cooking. The heat treatments give rise to various changes in the physical and chemical properties of the food products, which affect their overall quality attributes, such as taste, flavor, texture, appearance, and shelf life. Thus, it is important for food scientists and food manufacturers to understand the effect of heat on the properties of food products (in particular, high sugar content products which are susceptible to heat) in order to optimize processing conditions for improving food quality, without sacrificing food safety. Several thermal analytical techniques are generally utilized for probing changes in the food properties associated with heat treatments. This section will discuss the principles of the thermal analytical techniques used in the present research, i.e., standard differential scanning calorimetry (SDSC or DSC), modulated differential scanning calorimetry (MDSC), rapid-scanning differential scanning calorimetry (Rapid-scanning DSC), and thermogravimetric analysis (TGA).
2.5.1. Standard differential scanning calorimetry (SDSC)

Standard differential scanning calorimetry (SDSC), which is generally abbreviated as DSC, is a thermal analytical technique in which the difference in the amount of heat inputs into a sample and a reference is measured as a function of temperature, while the sample and the reference are subjected to a controlled temperature program (Note: This definition is that approved by the International Confederation for Thermal Analysis, August 1977). There are two types of DSC instruments; power compensation and heat-flux DSC.

A cross sectional diagram of a power compensation DSC cell is showed in Figure 2.10. A power compensation DSC cell has two separate furnaces; one for the sample pan and the other for the reference pan, which is usually an empty pan. The sample and reference pans are placed in identical environments. The sample and the reference pans are heated at the same rate and maintained at the same temperature. However, while the sample undergoes a thermal event, the sample pan takes more (or less) heat to maintain the same temperature as the reference pan. Hence, the furnace in the sample platform produces more (or less) heat than the furnace in the reference platform. Whether more or less heat must flow to the sample depends on whether the thermal event is exothermic (gives off heat) or endothermic (takes up heat). The difference in heat output of the two furnaces at any given temperature is measured and directly appears as the total heat flow signal plotted as a function of temperature or time, which is a DSC thermogram.

A cross sectional diagram of a heat-flux DSC cell (Q2000 DSC, TA instruments, New Castle, DE) is shown in Figure 2.11. A heat-flux DSC cell includes a sensor with raised sample and reference platforms. The sensor is composed of a thick, flat constantan (a copper-nickel alloy, which has a resistance that is constant over a wide range of temperatures) body and a pair of thin wall tubes. The thin wall section creates the thermal resistance and the two flat end surfaces are
to hold the sample and reference. A thin chromel disk covers the underside of each platform and functions as an area thermocouple junction to reduce sensitivity to variations in contact between the sensor and the pans. A chromel wire is welded to each chromel disk. The base surface of the sensor is brazed to the silver heating block (furnace), which makes the base of the sensor assembly isothermal (Danley, 2003). In typical heat-flux DSC instruments, heat is transferred through the constantan body and up to the sample and reference pans. The differential heat flow to the sample and reference is monitored by chromel-constantan area thermocouples formed by the junction of the constantan body and a chromel disc welded to the underside of each platform. The temperature difference between the sample and reference thermocouple is fed to a variable high gain amplifier, where the signal is amplified, electronically scaled to read directly as heat flow, and then displayed on the DSC thermogram, which is similar to the thermogram in the bottom of Figure 2.10.

Unlike other heat-flux DSC instruments, the Q2000 DSC has another component, a chromel/constantan wire, which is called a chromel/constantan Tzero thermocouple. The wire is located symmetrically between the sample and reference sensor platforms and functions as an independent measurement and furnace control sensor. The incorporation of the new wire results in a dramatic improvement in instrument resolution and baseline stability, which is especially important for heat capacity measurements and for detection and quantification of weak and broad transitions.

Compared to power compensation DSC, heat-flux DSC has a better baseline, because of a much smaller temperature difference between the furnace, which is enclosed in the heat-flux DSC cell, and the sensor. This smaller temperature difference is due to the configuration of the
heat-flux DSC, which has one furnace and one sensor, instead of two furnaces and two sensors as found in compensation DSC.

Both DSC instruments require calibrations in advance of sample analysis, since thermal resistance changes with temperature, which mean that the measured temperature difference is not equal to the difference in temperature between the sample and reference due to the DSC pan as a barrier. Because the Q2000 DSC was used in the present research, basic calibrations for Q2000 DSC are explained in this section. The calibration procedures for the Q2000 DSC are: 1) Tzero calibration (also termed baseline calibration) and 2) Temperature and enthalpy calibrations.

Tzero calibration is performed in two steps. Step one is to run a DSC scan without any pans and measure the temperature difference between the two platforms. Step two is to run a DSC scan with a standard material, such as sapphire (98.3 mg for sample, 99.7 mg for reference), on both the sample and reference platforms at a specific heating rate, usually 20°C/min. The purpose of step two is to obtain the accurate calibration of the sensor thermal capacitance and resistance values. Temperature and enthalpy calibrations are carried out with a known melting point standard, usually indium (melting temperature of 156.6°C, ΔH of 28.71 J/g), by heating through its melting transition at a heating rate of 10°C/min. The calibration tolerance of the indium melting temperature is ± 0.40°C (TA Instruments TGA & DSC Theory and Application Training Courses 2008).

The DSC thermogram shown in the bottom of Figure 2.10 represents a typical thermogram obtained by SDSC. To interpret the DSC thermogram, it is necessary to understand the theoretical basis for the signals. The total heat flow plotted in a DSC thermogram is composed of two components – the heat capacity component, which is a function of the applied heating rate,
and the kinetic component, which is a function of time at an absolute temperature – and is described by the following equation:

\[
\frac{dH}{dt} = C_p \frac{dT}{dt} + f(T, t)
\]  

(Equation 1)

where,

\[
\frac{dH}{dt} = \text{Total heat flow (mW = mJ/sec)}
\]

\[C_p = \text{Sample heat capacity (J/°C) = specific } C_p (J/(g·°C)) \times \text{sample weight (g)}\]

\[\frac{dT}{dt} = \text{Heating rate (°C/min)}\]

\[C_p \left(\frac{dT}{dt}\right) = \text{Heat capacity component of total heat flow (mW)}\]

\[f(T, t) = \text{Kinetic component of total heat flow (mW)}\]

The total heat flow increases linearly with the applied heating rate due to the heat capacity \((C_p)\) of the sample. Any thermal event (e.g., most melting transitions and glass transitions) accompanied by a change in \(C_p\) produces a change in the heat flow signal. For example, as a crystalline material is heated at a specific heating rate over its endothermic peak temperature, the material undergoes a transition from crystalline phase to liquid phase. During the phase transition, the crystalline material adsorbs energy (heat), which breaks intermolecular bonds (loss of crystalline structure) and results in a phase transition to the liquid phase. Because of the energy (\(\Delta H\), enthalpy) absorbed by the material during the phase transition, the thermal event appears as a large endothermic peak on the DSC thermogram. The baseline of the heat flow signal before and after the endothermic peak is different due to a difference in heat capacity (\(\Delta C_p\)) between the crystalline solid phase and the liquid phase, indicating a change in molecular mobility (Figure
The kinetic component is another heat flow that contributes to the total heat flow, due to kinetic processes, such as decomposition, crystallization, and evaporation.

In the SDSC, lack of being able to separate the heat capacity and kinetic components can cause difficulty in DSC data interpretation, especially when the thermal events overlap or take place over the same temperature range. In this case, modulated differential scanning calorimetry (MDSC) is extremely useful, since it measures both the total heat flow and its heat capacity component, obtaining the kinetic component from their difference, using two simultaneous heating rates.

### 2.5.2. Modulated differential scanning calorimetry (MDSC)

Modulated differential scanning calorimetry (MDSC) was developed to overcome the disadvantages of SDCS by applying two simultaneous heating rates to the sample: a linear or average heating rate (same as in SDSC) and a modulated or sinusoidal heating rate. The following discussion regarding MDSC is based on the Modulated DSC Technology manual by Thomas (2006). The modulated heating rate is determined by two parameters (Figure 2.12): the temperature modulation amplitude, which is the sinusoidal temperature change superimposed on the average temperature change, and the temperature modulation period, which is the time, in seconds, to complete one modulation cycle. The heating rate (dT/dt) in Equation 1 for MDSC is a combination of the average heating rate and the modulated heating rate.

The average heating rate is used to provide the same information as SDSC (total heat flow), whereas the modulated heating rate is used to measure the fraction of the total heat flow that responds to a changing heating rate. This fraction is generally caused by heat capacity, changes in heat capacity, and most melting. This fraction is termed the heat capacity component (also termed the reversing heat flow) and is shown as the reversing heat flow signal in a MDSC.
thermogram. The other fraction of the total heat flow does not respond to the changing heating rate, and is calculated by subtracting the reversing heat flow from the total heat flow. This fraction is caused by kinetic processes, such as decomposition, evaporation, chemical reactions, and crystallization. This fraction is termed the kinetic component (also termed the nonreversing heat flow) and is shown as the nonreversing heat flow signal in a MDSC thermogram. Figure 2.13 is a general MDSC thermogram and contains all the three signals. These signals obtained by MDSC and their relationship can be explained using Equation 1. Table 2.3 summarizes the relation between Equation 1 and the thermal events observed in the heat capacity component (reversing heat flow) and the kinetic component (nonreversing heat flow) using MDSC.

Different MDSC methods can be applied to the sample depending on the purpose of the experiment. For example, quasi-isothermal MDSC, where a small temperature modulation (typically <1°C) is applied to a constant average temperature for a period of time, is very useful for measuring heat capacity and a change in heat capacity during thermal events, including kinetic processes. A change in heat capacity indicates a change in sample structure caused by any thermal event. Thus, a change in sample structure caused by kinetic processes can be observed by measuring a change in $C_p$. In quasi-isothermal MDSC, a change in $C_p$ is displayed in the reversing $C_p$ (Rev $C_p$) signal, because Rev $C_p$ is associated with the modulated heating rate, but not the average heating rate, which is zero. To help understand the principles of quasi-isothermal MDSC, the differences between general MDSC and quasi-isothermal MDSC methods are summarized in Table 2.4 and 2.5, in terms of the signals available in both methods.

As shown in Table 2.5, the heat flow amplitude is required to calculate Rev $C_p$. The heat flow amplitude is changed when thermal events cause a change in sample structure during temperature modulation at a constant isothermal temperature. However, as mentioned previously,
kinetic processes do not respond to the modulated heating rate, because they are a function of both time and temperature and MDSC uses very small temperature modulations and relatively short modulation periods. Thus, kinetic processes do not contribute to the modulated heat flow. The only contribution to the Rev $C_p$ signal is the sample’s heat capacity. However, because all changes in sample structure involve a change in heat capacity, any kinetic process that creates a change in sample structure can give rise to a change in the modulated heat flow amplitude signal and subsequently, a change in the Rev $C_p$ signal appeared in quasi-isothermal MDSC.

In the present research, two types of quasi-isothermal MDSC methods were used to investigate the loss of crystalline structure in sugars. In the first type, termed quasi-isothermal MDSC, the modulated heating rate is applied to sugars at a single isothermal temperature until the crystalline structure in the sugars is removed. In the second type, termed stepwise quasi-isothermal MDSC, the modulated heating rate is applied to sugars for a fixed time (e.g., 30 mins) at increasing temperature steps (e.g., 60 to 120°C) across the range of temperatures over which the loss of crystalline structure occurs.

2.5.3. Rapid-scanning differential scanning calorimetry (Rapid-scanning DSC)

Rapid-scanning differential scanning calorimetry (Rapid-scanning DSC) is a new DSC technique which has recently received a great deal of attention due to the advantages of using extremely fast scan rates. Because of the extremely fast scan rates, the new DSC technique is currently referred to by a variety of names in the literature, including high-speed DSC (Pijers and others 2002; McGregor and others 2004; Saunders and others 2004; Lappalainen and others 2006a), high performance DSC (Poel and Mathot 2007), high-sensitivity, high-speed DSC (Gabbott and others 2003), rapid-scanning DSC (Danley and others 2008), and fast scan DSC (Ye and others 2009).
The fast scan rates used in the new rapid-scanning DSC make it possible to overcome a number of material characterization challenges. For example, faster scan rates greatly increase heat flow sensitivity (signal height) for small transitions, since the same amount of energy (the DSC output, mJ/sec) is released (or adsorbed) over a much shorter time, as shown in Figure 2.14 (Gabbott and others 2003). Thus, the detection and quantification of low levels of amorphous content in materials (e.g., lactose, maltitol, and sucrose) could be achieved using this new rapid-scanning DSC technique (Gabbott and others 2003; Saunders and others 2004; Hurtta and Pitkänen 2004a; Lappalainen and others 2006a).

Another example is the application of the rapid-scanning DSC to suppress kinetic processes, which make data interpretation difficult, for accurate material characterization (Pijers and others, 2002). At high enough scan rates, it is possible for materials to not undergo kinetic (time-dependent) processes, such as polymeric conversion (Poel and Mathot 2007; Danley and others 2008; Buanz and Gaisford 2009; Yang and others 2009), recrystallization (McGregor and others 2004; Mathot and others 2006; Poel and Mathot 2007; Abdulkarim and Ghazali 2007; Miltner and others 2008; Van Assche and others 2009), or thermal decomposition (Wurm and others 2009). In the present research, this application is exceptionally important since the rapid-scanning DSC may allow us to reach the thermodynamic melting temperature of the sugars before the kinetic processes can occur.

Currently, the maximum scan rate of commercially available rapid-scanning DSC instruments is 500°C/min (PerklnElmer, Shelton, CT). However, the rapid-scanning DSC, which has been developed under project RHC (Rapid Heating and Cooling) by TA Instruments (New Castle, DE), has a maximum scan rate of 2000°C/min. The extreme fast scan rate is ascribed to the unique features of the rapid-scanning DSC (i.e., use of a small transducer, which is
surrounded by an infrared furnace system instead of a resistance furnace system, and a very small pan and sample size, typically 20-100 μg). The rapid-scanning DSC is compared to a conventional DSC in terms of transducer and pan size in Figure 2.15. The rapid-scanning DSC has a liquid nitrogen cooling system (LNCS), which ranges in temperature from -180°C to 700°C. Dry nitrogen is typically used as the purge gas. The calibration procedures and the operation principle for the rapid-scanning DSC are similar to those used in SDSC.

2.5.4. **Thermogravimetric analysis (TGA)**

TGA is a thermal analytical technique used to evaluate the thermal or oxidative stability of a material by monitoring the amount and rate of weight change in the material, while the material is heated at a constant rate under a specific atmosphere, such as air, nitrogen, oxygen, or helium gas. TGA measures weight loss or gain of a material caused by thermal processes during heating, such as decomposition, evaporation, gas adsorption or desorption. Thus, TGA in the present study is a useful technique to investigate sugar thermal decomposition associated with the loss of crystalline structure in sugars.

In experiments using TGA, weight and temperature are critical parameters to obtain precise results. Hence, both weight calibration and temperature calibration should be done prior to sample analysis. Weight calibration is simply performed by placing a calibration weight on the sample pan (Figure 2.16, top). Temperature calibration is carried out using both a TGA calibration magnet placed under the furnace and Curie point standard composed of well characterized transition metals (e.g., alumel, nickel, cobalt, and alumel/cobalt/nickel alloys). The Curie point standard is a ferromagnetic material, which shows a strong attraction to magnetic fields. When the Curie point standard is heated to over a specific temperature, it loses its magnetic susceptibility (ferromagnetic transition); this temperature is called the Curie point. For
example, when alumel, a typical Curie point standard, is heated over its Curie point (151.48°C), it loses its magnetic susceptibility because of the change in its structural order (a second-order phase transition) caused by the heating, so that it does not exhibit attraction to the TGA calibration magnet position under the furnace. The magnetic transition appears as a weight loss as the temperature increases and a weight gain as the temperature decreases, which recovers the attraction of the Curie point standard to the magnet.

Beside both calibrations, there are other parameters that affect temperature accuracy and balance stability. Unlike DSC, the thermometer in the TGA is not directly in contact with the sample pan, thus it is not possible to directly measure the temperature of the sample (Figure 2.16, top). However, using a more conductive gas (e.g., helium) to improve the contact between the sample and the thermometer can increase temperature accuracy. Balance stability is indicated by baseline stability, which also influences the TGA results; thus, it is important to obtain a stable baseline as a function of time and temperature. Baseline stability can be controlled by the rate of balance and furnace purge gas flow. When purge gas flow rates are higher, undesirable buoyancy effects, which result in an unstable baseline, are decreased.

The weight change of a material in a TGA is associated with the two photodetectors in the balance, which convert light into current or voltage (not shown in Figure 2.16). The balance should be zero in advance of sample analysis. At zero, the same amount of light is emitted into the two photodetectors. When the material is heated by the furnace, according to predetermined experimental conditions, it is subjected to thermal processes that are accompanied by weight loss or gain. When weight loss or gain occurs, an unequal amount of light is emitted onto the two photodetectors, which in turn generates current to return the balance to equilibrium. The amount of additional current is proportional to the material's weight change. This weight change
information is sent to a recorder along with the temperature of the material and elapsed time, producing a TGA thermogram, similar to the one shown in Figure 2.16 (bottom).

A TGA thermogram can provide a variety of information depending on the way in which the data are plotted. Commonly, the derivative weight loss (%/°C) is plotted as a function of temperature, which shows the temperature at which weight loss is most obvious (Figure 2.16, bottom). Another is the derivative temperature (°C/min) plot, which can monitor the change in the material's heating rate during a phase transition. The derivative temperature plot provides critical information in the present study, exploring whether the temperature (T<sub>i</sub>) at which weight loss for the sugar begins due to thermal decomposition corresponds to the temperature (T<sub>m onset</sub>) at which the phase transition begins. During the crystalline to amorphous transition, the heating rate of the sugar should change, because the sugar cannot keep up with the predetermined heating rate due to the energy required for the phase transition. In the absence of the phase transition, all the heat is used to raise the sugar's temperature (sensible heat). During the phase transition, however, the heat is primarily used to change the sugar's phase, not to raise its temperature. Hence, when the sugar undergoes a phase transition, the derivative temperature decreases until all of the crystalline structures are removed and then increases as the temperature of the sugar catches up to the predetermined heating rate. Therefore, the use of TGA derivative temperatures could be useful to explore thermal decomposition as the cause of the loss of crystalline structure in the sugars.

2.6. Chemical analytical technique

2.6.1. High-performance liquid chromatography (HPLC)
High-performance liquid chromatography (HPLC) is known as a successful technique for separating, identifying, and quantifying sugars and their thermal decomposition components (Bonn 1985; Gomis and others 1991; Porretta 1992; Lo Coco and others 1994; Yuan and others 1996; Rounds and Gregory 1998; Yuan and Chen 1999). In the present study, HPLC was used as a chemical analytical technique to investigate thermal decomposition as the kinetic process causing the loss of crystalline structure in the sugars, as we hypothesize. For HPLC analysis, sucrose was chosen because it does not form tautomers via mutarotation and crystalline solid phase hydrates, which would complicate the HPLC analysis, and it is also the most commonly used sugar in the food and pharmaceutical industries.

Sucrose thermal decomposition is a set of chemical reactions achieved through a myriad of complex mechanisms, which are not fully understood, in spite of extensive studies. In the present study, however, it is important to detect only the initial decomposition components formed, which we hypothesize are formed concomitantly with the onset of the loss of crystalline structure in sucrose, if thermal decomposition is the cause of the loss of crystalline structure. Therefore, our discussion of sucrose thermal decomposition will concentrate on its primary reaction and associated thermal decomposition components.

Most studies for sucrose thermal decomposition (or caramelization) have been performed in dilute or concentrated aqueous solution (Gardiner 1966; Mauch 1971; Richards and Shafizadeh 1978; Richards 1986; Kroh 1994; and Quintas and others 2007). According to these studies, the first reaction step in the thermal decomposition of sucrose takes place via protonation of the oxygen of the glycosidic bond between the glucose and fructose moieties, resulting in the splitting of the glycosidic linkage, termed sucrose hydrolysis. The hydrogen ion (H+) required for this step is provided by water. Because water is more highly dissociated at higher temperatures,
it can provide a medium which is more conducive for catalysis ($pK = 14.00$ at $24^\circ$C and $12.23$ at $100^\circ$C), accelerating sucrose hydrolysis (Kelly and Brown, 1978/79).

In the absence (anhydrous conditions) of an aqueous solution, sucrose thermal decomposition seems to be somewhat different, in terms of the mechanism and decomposition components. Quintas and others (2007) observed that the global model to predict sucrose thermal degradation failed to express the behavior of thermal decomposition for sucrose with extremely low water content ($3.58\%, w/w$), which suggests the presence of different reaction mechanisms and, thus, different thermal decomposition components. However, there is lack of information about sucrose thermal decomposition in the absence of an aqueous solution. Since no water is present to provide the $H^+$ for sucrose hydrolysis in the absence of an aqueous solution, it is unlikely that sucrose hydrolysis would be the first reaction step of sucrose thermal decomposition in the absence of an aqueous solution. However, it was mentioned by Quintas and others (2007), based on the work of Richards (1986) and Lowary and Richards (1988), that the $H^+$ required for sucrose hydrolysis could be derived from the dissociation of the sucrose molecule itself at high temperatures. In addition, we hypothesize that another possible source of the $H^+$ is surface water on the sucrose crystals. Kelly and Brown (1978/79) reported that $pK_a$ values of sucrose decreased to $11.07$ at $90^\circ$C from $12.43$ at $20^\circ$C, which indicates that sucrose has acidic properties. This indicates that sucrose hydrolysis can occur in the absence of an aqueous solution. Actually, Šimkovic and others (2003) observed, using GC/MS, that splitting of the glycosidic bond was the most prominent primary reaction of sucrose thermal decomposition in the absence of an aqueous solution. Although in the absence of a solvent, the initial thermal degradation of pure sucrose would be extremely slow, trace amounts of the thermal degradation components (probably acids such as acetic, formic, laevulinic acids) formed via this step may
themselves undergo more rapid degradation reactions, which was termed an "acid-autocatalyzed process" by Richards (1986).

In conclusion, regardless of the presence or absence of an aqueous solution, sucrose thermal decomposition primarily occurs via sucrose hydrolysis as shown in Figure 2.17. Once sucrose is broken down into glucose [1] and fructose carbocation [1] via sucrose hydrolysis, glucose forms acidic and other decomposition components through further degradation reactions (not shown in Figure 2.17). Fructose carbocation, due to its instability, immediately participates in subsequent, more complex reactions, resulting in the formation of various decomposition components, including anhydrofructose [2a] by cyclization; a wide range of products, such as 5-(hydroxymethyl)furfural (5-HMF) [2b], by non-specific degradation (e.g., condensation); oligosaccharides [2c] by combining with the hydroxyl oxygen of another saccharide (mostly sucrose); and fructose [2d] by accepting a hydroxyl ion (OH⁻) from water. However, the production of fructose [2d] may not occur in the absence of an aqueous solution because there is no water to react with fructose carbocation until a condensation product, such as 5-HMF, is produced, or unless surface water is available. These intermediate products are produced through similar mechanisms in the presence and absence of an aqueous solution. However, in the absence of an aqueous solution, minor products such as anhydrous sucrose [3] and sucrose isomers [4] are also produced through minor reaction mechanisms.

As mentioned above, though sucrose thermal decomposition continues through numerous reaction mechanisms, in the present study, it is important to measure only the initial decomposition components formed in the early stages of sucrose thermal decomposition (i.e., glucose, fructose, and 5-HMF), because this study aims to elucidate whether the loss of crystalline structure in sucrose is caused by thermal decomposition. Therefore, in the present
study, glucose, fructose, and 5-HMF were selected as thermal decomposition indicator components for HPLC analysis.

In general, the basic components of a HPLC system include a solvent reservoir (also termed as the mobile phase), pump, injector, columns (guard and analytical columns), detector(s), recorder/integrator/data system, and waste reservoir, as shown in Figure 2.18. A HPLC system begins with the mobile phase which carries the sample of interest through the system. The pump delivers the mobile phase into the system. The injector allows the sample to be placed into the flowing mobile phase for introduction onto the guard column. Prior to injection, the sample is generally filtered using a sample filter because particulate matter included in the sample often causes injector and column damage. The guard column is often located just before the analytical column to chemically remove components of the sample that may foul the analytical column. In the analytical column following the guard column, the sample separation occurs primarily based on the differential attraction of the sample components for the mobile phase and the packing material within the column. The components separated by the analytical column pass through a detector, such as a UV-visible absorption detector, fluorescence detector, refractive index detector, electrochemical detector, to name a few detector types. The separated components prompt an electrical response from the detector, which is digitized and sent to a recorder. While the recorder serves to record the basic results of a chromatographic separation, the electronic integrators and computer-controlled data system interpret the data.

Depending on the components targeted for separation, different separation modes are utilized in HPLC analysis. A separation mode used commonly for sugars and sugar derivatives is cation-exchange HPLC, which is based on the difference in affinity between solute ions and charged sites bound to the column packing material (stationary phase). The stationary phase of
the cation-exchange HPLC contains fixed functional groups that are positively changed. For example, crosslinked styrene-divinylbenzen copolymer (polystyrene) used as the column packing material in the present study is modified to produce cation exchange resins with Ca\(^{2+}\). The mobile phase of the cation-exchange HPLC is usually an aqueous buffer and its constituents compete with solutes for binding to sites on the stationary phase. Solute retention time, which indicates component separation, is controlled by changing the mobile phase ionic strength and/or pH. For example, as the ionic strength of the mobile phase increases, the mobile phase constituents compete more effectively with solutes for binding to sites on the stationary phase, resulting in a decrease in solute retention time. Solute retention time is also affected by the degree of crosslinking in the stationary phase and the type of molecules being analyzed. Components of the sample with different retention times are monitored by the detector(s). In the present study, two detectors were used: Refractive index (RI) detector for sucrose, glucose, and fructose and a photodiode array (PDA) detector for 5-HMF.
2.7. Figures and Tables

Figure 2.1 Temperature (T) dependence of enthalpy (H), Gibbs energy (G), entropy (S), and heat capacity (C_p) of crystalline and glass solid phases (adapted from Wunderlich, 1990a).
Figure 2.2 Schematic illustration of Gibbs energy as a function of temperature, at a constant pressure, for a one-component system. Thermodynamic melting ($T_m$) occurs at the intersection of the crystalline and liquid phases, where the phases have the same Gibbs energy, $\Delta G=0$ (adapted from Wunderlich 1990a).
Figure 2.3 Temperature dependence of the latent heat of fusion (also termed enthalpy, $H$ in J/g) and heat capacity ($C_p$ in J/g°C) of a material in the crystalline and amorphous phases. $T_g$, glass transition temperature; $T_c$, cold crystallization temperature; and $T_m$, melting temperature.
Figure 2.4 Kinetic processes causing the loss of crystalline structure, thermal decomposition, dehydration, and chemical interaction. The possible locations of the kinetic processes are indicated on the diagram; however, the energy associated with each process is not shown. $T_g$, glass transition temperature; $T_c$, cold crystallization temperature; and $T_m$, melting temperature.
Figure 2.5 Simultaneous DSC/TGA thermogram for acetylsalicylic acid determined at a heating rate of 1°C/min.
Figure 2.6 The DSC thermogram of crystalline Drug A monohydrate using sealed and unsealed (pinhole) pans.
Figure 2.7 Micrographs of a raffinose crystal (left), showing pronounced birefringence and the same crystal (right) after dehydration by vacuum treatment at room temperature. The ultrastructure resembles that of the crystal, but the absence of birefringence is evidence of the amorphous phase (Frank 2007).
Figure 2.8 DSC thermogram for aspirin (acetylsalicylic acid) and magnesium stearate alone and as a 50 w/w mixture (Wissing and others 2000).
Figure 2.9 DSC thermogram (left) and X-ray diffractogram (right) of a. fluconazole, b. lactose, and c. 1:1 fluconazole/lactose mixture (Desai and others 2003).
Figure 2.10 Schematic diagram of a power compensation DSC (top) and a typical DSC thermogram of an endothermic thermal event (bottom). $T_{m \text{ onset}}$ is the onset temperature of endothermic peak in °C, $T_{m \text{ peak}}$ is the peak temperature of endothermic peak in °C, $\Delta H$ is the enthalpy in J/g, and $\Delta C_p$ is the heat capacity in J/(g·°C).
Figure 2.11 Schematic diagram of a heat-flux DSC (adapted from Danley 2003).
Figure 2.12 Plots showing the temperature modulation amplitude and period, average heating rate, and modulated heating rate used in MDSC.
Figure 2.13 Total heat flow, reversing heat flow, and nonreversing heat flow shown in the MDSC thermogram for fructose candy containing both amorphous and crystalline phases.
Figure 2.14 Higher sensitivity (signal height) at fast scan rates in thermoplastic polymer (Goth and Clénet 2008).
Figure 2.15 Comparison of the rapid-scanning DSC to a conventional DSC in terms of transducer and pan size (adapted from Aubuchon and others 2008).
Figure 2.16 Schematic diagram of a TGA (top) and a typical TGA thermogram (bottom).
Figure 2.17 The predominant mechanism of sucrose thermal decomposition in the presence of solvent (dash arrow) and in the absence of solvent (solid arrow) (synthesized from Mauch 1971; Richard 1986; Šimkovic and others 2003; Quintas and others 2007).
Figure 2.18 A schematic illustration of a general high-performance liquid chromatography (HPLC) system.
<table>
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<th>Sugar type</th>
<th>Heating rate ({°C/min})</th>
<th>Melting temp. (°C)</th>
<th>Enthalpy (ΔH, J/g)</th>
<th>Analytical technique</th>
<th>Initial decomposition temp. (T_i, °C)**</th>
<th>Reference</th>
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<td>54.8/119.8</td>
<td>DSC</td>
<td>159.6/167.0</td>
<td>Hurtta and others 2004b</td>
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<td>173.7/184.5</td>
<td>72.1/126.6</td>
<td>DSC</td>
<td>161.1/171.3</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
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<td>120</td>
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<td></td>
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<td>111.4/128</td>
<td>DSC</td>
<td>169.6/178.8</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>173</td>
<td>190</td>
<td>DSC</td>
<td></td>
<td>Roos 1995; Bonelli and others 1997</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>183.5</td>
<td>118</td>
<td>DSC</td>
<td></td>
<td>Roos and Karel 1990, 1991a</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>186</td>
<td></td>
<td>DSC</td>
<td></td>
<td>Weitz and Wunderlich 1974</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>176</td>
<td>135</td>
<td>DSC</td>
<td></td>
<td>Gloria and Sievert 2001</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>188</td>
<td></td>
<td>DSC</td>
<td></td>
<td>Saleki-Gerhardt and Zografi 1994</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>190</td>
<td></td>
<td>DSC</td>
<td></td>
<td>Vanhal and Blond 1999</td>
</tr>
<tr>
<td>Sucrose A/B</td>
<td>10</td>
<td>185.9/188.9</td>
<td>126.4/134.4</td>
<td>DSC</td>
<td>179.7/189.2</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Sucrose A/B</td>
<td>20</td>
<td>187.5/189.6</td>
<td>130.8/135.4</td>
<td>DSC</td>
<td>192.2/200.7</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Sucrose A/B</td>
<td>50</td>
<td>188.3/191.1</td>
<td>136.9/138.8</td>
<td>DSC</td>
<td>207.5/214.9</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Sucrose A/B</td>
<td>100</td>
<td>189.0/190.8</td>
<td>143.2/145.4</td>
<td>DSC</td>
<td>235.3/228.4</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Glucose A/B*</td>
<td>0.5</td>
<td>147.5/145.1</td>
<td>182.7/180.1</td>
<td>DSC</td>
<td>146.4/147.0</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>α-D-glucose</td>
<td>1</td>
<td>135</td>
<td>180</td>
<td>HP-DTA</td>
<td></td>
<td>Raemy 1983</td>
</tr>
<tr>
<td>β-D-glucose</td>
<td>1</td>
<td>130</td>
<td>150</td>
<td>HP-DTA</td>
<td></td>
<td>Raemy 1983</td>
</tr>
<tr>
<td>Glucose A/B</td>
<td>1</td>
<td>149.8/146.5</td>
<td>189.1/185.4</td>
<td>DSC</td>
<td>149.8/152.0</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Glucose A/B</td>
<td>2</td>
<td>152.8/148.9</td>
<td>189.0/187.1</td>
<td>DSC</td>
<td>151.4/159.1</td>
<td>Hurtta and others 2004b</td>
</tr>
</tbody>
</table>
Table 2.1 (cont.)

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Temperature 1</th>
<th>Temperature 2</th>
<th>Temperature 3</th>
<th>Method</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose</td>
<td>5</td>
<td>143</td>
<td>158</td>
<td>DSC</td>
<td>Roos 1995</td>
</tr>
<tr>
<td>Glucose</td>
<td>6</td>
<td>165</td>
<td></td>
<td>DTA</td>
<td>Őrsi 1973</td>
</tr>
<tr>
<td>D-(+)-glucose</td>
<td>10</td>
<td>158.24</td>
<td>163.92</td>
<td>DSC</td>
<td>Wungtanagorn and Schmidt 2001ab</td>
</tr>
<tr>
<td>Glucose A/B</td>
<td>10</td>
<td>160.4/155.2</td>
<td>163.1/159.4</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Glucose A/B</td>
<td>20</td>
<td>164.8/158.3</td>
<td>167.4/163.8</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Glucose A/B</td>
<td>50</td>
<td>169.4/164.4</td>
<td>172.6/168.9</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Glucose A/B</td>
<td>100</td>
<td>171.8/166.7</td>
<td>176.1/173.8</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Fructose A/B</td>
<td>0.5</td>
<td>108.2/110.0</td>
<td>114.3/113.0</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>D-fructose</td>
<td>1</td>
<td>113.6/112.7</td>
<td>118.4/116.7</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Fructose A/B</td>
<td>2</td>
<td>112/116.2</td>
<td>123.2/121.0</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>D-fructose</td>
<td>5</td>
<td>108</td>
<td>127</td>
<td>DSC</td>
<td>Roos 1995</td>
</tr>
<tr>
<td>Fructose</td>
<td>6</td>
<td>120</td>
<td></td>
<td>DTA</td>
<td>Őrsi 1973</td>
</tr>
<tr>
<td>Fructose A/B</td>
<td>10</td>
<td>125.8/125.7</td>
<td>131.4/131.7</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>D-(-)-fructose</td>
<td>10</td>
<td>113.58</td>
<td>132.03</td>
<td>DSC</td>
<td>Wungtanagorn and Schmidt 2001ab</td>
</tr>
<tr>
<td>Fructose</td>
<td>10</td>
<td>133</td>
<td></td>
<td>DSC</td>
<td>Truong and others 2002, 2004</td>
</tr>
<tr>
<td>Fructose A/B</td>
<td>20</td>
<td>131.3/130.0</td>
<td>137.8/136.0</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Fructose A/B</td>
<td>50</td>
<td>135.7/134.9</td>
<td>140.6/139.8</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
<tr>
<td>Fructose A/B</td>
<td>100</td>
<td>137/136.8</td>
<td>142.6/142.0</td>
<td>DSC</td>
<td>Hurtta and others 2004b</td>
</tr>
</tbody>
</table>

*A/B indicates two different sugar samples. Sucrose A and fructose A were bulk materials, the others were fine chemicals made for laboratory use.

**Initial decomposition temperature was determined using TGA. ***HP-DTA: High pressure DTA apparatus.
Table 2.2 Melting parameters ($T_{m \text{ onset}}$, $T_{m \text{ peak}}$, and $\Delta H$) measured using DSC and initial decomposition temperature ($T_{i}$) measured using TGA for D-xylose and L-xylose as a function of heating rate.

<table>
<thead>
<tr>
<th>Heating rate ($^\circ\text{C/min}$)</th>
<th>Melting temp. ($^\circ\text{C}$)</th>
<th>Enthalpy ($\Delta H$, J/g)</th>
<th>Initial decomposition temp. ($T_{i}$, $^\circ\text{C}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{m \text{ onset}}$</td>
<td>$T_{m \text{ peak}}$</td>
<td></td>
</tr>
<tr>
<td>L-xylose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>143.9</td>
<td>147.3</td>
<td>208</td>
</tr>
<tr>
<td>1</td>
<td>147.1</td>
<td>150.3</td>
<td>213</td>
</tr>
<tr>
<td>2</td>
<td>151.4</td>
<td>155.3</td>
<td>222</td>
</tr>
<tr>
<td>10</td>
<td>155.8</td>
<td>160.8</td>
<td>237</td>
</tr>
<tr>
<td>20</td>
<td>159.2</td>
<td>164.8</td>
<td>240</td>
</tr>
<tr>
<td>40</td>
<td>162.2</td>
<td>168.4</td>
<td>251</td>
</tr>
<tr>
<td>D-xylose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>136.8</td>
<td>142.4</td>
<td>207</td>
</tr>
<tr>
<td>1</td>
<td>139.7</td>
<td>145.8</td>
<td>216</td>
</tr>
<tr>
<td>2</td>
<td>143.7</td>
<td>150.1</td>
<td>222</td>
</tr>
<tr>
<td>10</td>
<td>152.8</td>
<td>157.7</td>
<td>228</td>
</tr>
<tr>
<td>20</td>
<td>156.3</td>
<td>162.7</td>
<td>236</td>
</tr>
<tr>
<td>40</td>
<td>158.3</td>
<td>166.3</td>
<td>239</td>
</tr>
</tbody>
</table>
Table 2.3 Thermal events observed in the heat capacity component (reversing heat flow) and the kinetic component (nonreversing heat flow) using MDSC (adapted from Thomas 2006).

\[
\frac{dH}{dt} = \frac{C_p}{dt} dT + f(T,t) \quad \text{(Equation 1)}
\]

\( \frac{dH}{dt} = \text{Total heat flow (mW = mJ/sec)} \)

: It is due to the average (linear) heating rate and is equivalent to standard DSC at the same average heating rate.

\( C_p \) = Sample heat capacity (J/°C) = specific \( C_p \) (J/(g·°C)) x sample weight (g)

\( \frac{dT}{dt} = \text{Heating rate (°C/min)} \)

: It is the measured heating rate, which has both a linear and modulated component.

\( C_p \frac{dT}{dt} = \text{Heat flow component = Reversing heat flow} \)

: It is heat capacity component of the total heat flow. It is calculated from the heat flow that responds to the modulated heating rate.

\( f(T, t) = \text{Kinetic component = Nonreversing heat flow} \)

: Heat flow that is a function of time at an absolute temperature. It is calculated from the difference between the total heat flow and the reversing heat flow component. It does not respond to the modulated heating rate.

<table>
<thead>
<tr>
<th>Total heat flow</th>
<th>Heat capacity component (Reversing heat flow)</th>
<th>Kinetic component (Nonreversing heat flow)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal processes</td>
<td>Heat capacity</td>
<td>Decomposition</td>
</tr>
<tr>
<td></td>
<td>Glass transition (( T_g ))</td>
<td>Evaporation</td>
</tr>
<tr>
<td></td>
<td>Most melting</td>
<td>Crystallization</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enthalpy recovery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thermoset cure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Starch gelatinization</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protein denaturation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Some melting</td>
</tr>
</tbody>
</table>
### Table 2.4 Signals available in general MDSC and equations used to calculate the signals.

<table>
<thead>
<tr>
<th>Signals</th>
<th>Is the signal available?</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total heat flow</td>
<td>Yes (Measured signal)</td>
<td>= The average value of the modulated heat flow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= Heat flow signal at the average heating rate</td>
</tr>
<tr>
<td>Rev heat flow</td>
<td>Yes (Calculated signal)</td>
<td>= Rev $C_p$ x average heating rate</td>
</tr>
<tr>
<td>Non-rev heat flow</td>
<td>Yes (Calculated signal)</td>
<td>= Total heat flow - Rev heat flow</td>
</tr>
<tr>
<td>Total $C_p$</td>
<td>Yes (Calculated signal)</td>
<td>= Total heat flow x $K_{C_p}\text{ Total} / \text{average heating rate}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>($K_{C_p}\text{ Total}: \text{Calibration constant for Total }C_p\text{ which is obtained by heat capacity calibration}$)</td>
</tr>
<tr>
<td>Rev $C_p$</td>
<td>Yes (Calculated signal)</td>
<td>= Heat flow amplitude x $K_{C_p}\text{ Rev}/ \text{heating rate amplitude}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>($K_{C_p}\text{ Rev}: \text{Calibration constant for Reversing }C_p\text{ which is obtained by heat capacity calibration}$)</td>
</tr>
<tr>
<td>Non-rev $C_p$</td>
<td>Yes (Calculated signal)</td>
<td>= Total $C_p$ - Rev $C_p$</td>
</tr>
</tbody>
</table>

### Table 2.5 Signals available in quasi-isothermal MDSC.

<table>
<thead>
<tr>
<th>Signals</th>
<th>Is the signal available?</th>
<th>Why is the signal not available?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total heat flow</td>
<td>Yes (Measured signal)</td>
<td>= The average value of the modulated heat flow</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= The heat flow signal at the average heating rate</td>
</tr>
<tr>
<td>Rev heat flow</td>
<td>No</td>
<td>Average heating rate = 0</td>
</tr>
<tr>
<td>Non-rev heat flow</td>
<td>No</td>
<td>Rev heat flow cannot be calculated due to average heating rate = 0</td>
</tr>
<tr>
<td>Total $C_p$</td>
<td>No</td>
<td>Average heating rate = 0</td>
</tr>
<tr>
<td>Rev $C_p$</td>
<td>Yes</td>
<td>The heat flow amplitude and heating rate amplitude required to calculate Rev $C_p$ depend on modulated heating rate, which results from a modulated temperature amplitude and a modulated temperature period.</td>
</tr>
<tr>
<td>Non-rev $C_p$</td>
<td>No</td>
<td>Total $C_p$ cannot be calculated because average heating rate = 0</td>
</tr>
</tbody>
</table>
2.8. References


TA Instruments TGA & DSC Theory and Application Training Courses; 2008 July 29-30; Rolling Meadows, IL.


CHAPTER 3

INVESTIGATION OF THE HEATING RATE DEPENDENCY ASSOCIATED WITH THE LOSS OF CRYSTALLINE STRUCTURE IN SUCROSE, GLUCOSE, AND FRUCTOSE USING A THERMAL ANALYTICAL APPROACH

3.1. Abstract

According to thermodynamic principles, a crystalline material melts at a single, time-independent temperature with a constant enthalpy value. However, substantial variation in the melting parameters ($T_{m\text{ onset}}$, $T_{m\text{ peak}}$, and $\Delta H$) for sucrose, glucose and fructose (as well as similar materials) has been a longstanding, troublesome, and controversial issue in the literature. Even though a number of explanations have been put forth, including origin, impurity, polymorphism, superheating, liquefaction, and thermal decomposition and/or mutarotation in addition to melting, they do not completely and/or satisfactorily account for the observed variation. Thus, the ultimate objective of this research was to elucidate the fundamental mechanism underlying the loss of crystalline structure in these sugars using both thermal (Standard DSC, Modulated DSC, and TGA on sucrose, glucose, and fructose) and chemical (HPLC on sucrose) analytical approaches. The melting parameters for all three sugars exhibited a strong heating rate dependency, which implies the occurrence of a kinetic process during the loss of crystalline structure in these sugars. The difference in the stepwise quasi-isothermal MDSC heat capacity and modulated heat flow amplitude data for the three sugars compared to indium and mannitol (thermodynamic melting comparison materials) strongly suggests that thermal decomposition is the kinetic process responsible for the loss of crystalline structure. The critical difference between our conclusion regarding the role of thermal decomposition in sugar melting and others is that we hypothesize that thermal decomposition is the kinetic process that is responsible for the loss of crystalline structure, not that thermal decomposition occurs in addition to
thermodynamic melting. We propose the term “apparent melting” to distinguish the loss of crystalline structure due to a kinetic process, such as thermal decomposition, from thermodynamic melting. Thermal decomposition as the kinetic process causing apparent melting for these sugars is further investigated via a chemical analysis approach in Chapter 4.

3.2. Introduction

Melting (sometimes termed fusion) is a first-order phase transition from the crystalline solid phase to the liquid phase (Roos 1995; Wunderlich 1990a), with no change in chemical composition. The parameters associated with the melting process (the onset melting temperature, $T_{m\text{ onset}}$; the peak melting temperature, $T_{m\text{ peak}}$; and the enthalpy of melting, $\Delta H$) are usually measured by heating a crystalline material at a specified rate to a temperature where the melting endothermic peak is complete, using a thermal analytical technique, such as Differential Scanning Calorimetry (DSC) or Differential Thermal Analysis (DTA). The melting parameters provide a good deal of information about the characteristics of the crystalline material (e.g., purity, type, size, etc.), thus, melting parameters have been used as unique material properties for identification and characterization of crystalline materials.

However, for some crystalline sugars (e.g., sucrose, glucose, and fructose) a wide range of melting parameters has been reported in the literature. For example, literature reported $T_{m\text{ onset}}$ values range from approximately 160°C to 192°C for sucrose, from 130°C to 172°C for glucose, and from 80°C to 126°C for fructose (Table 3.1). An important observation contrary to the definition of thermodynamic melting based on the data in Table 3.1 is that, within each sugar, the melting parameters strongly tend to increase with increasing heating rate. Thermodynamic melting occurs at a single, time-independent (i.e., heating rate independent) temperature (most
often reported as $T_{m,\text{onset}}$, where the crystalline solid and corresponding liquid phases are in thermodynamic equilibrium at a constant pressure; that is, the temperature at which the Gibbs energy ($G$; also called free enthalpy) of the crystalline solid phase is equal to that of the corresponding liquid phase, $\Delta G=0$ (Figure 3.1) (Wunderlich, 1990a). Thus, from a thermodynamic viewpoint, the widely varying sugar melting parameters are not consistent with the strict definition of thermodynamic melting and thus necessitate further investigation.

DSC, one of the thermal analysis techniques used in this research, measures the heat flow difference between a sample and inert reference (typically an empty pan) as a function of temperature and time. Integration of the heat flow signal provides enthalpy ($H$), which is a function of the material’s specific heat ($C_p$) and energy absorbed or released by the material due to phase transitions. Specific heat or heat capacity is the result of molecular motion, and therefore, changes in heat capacity reflect changes in the molecular mobility of the material. One of the important aspects of thermodynamic melting, which DSC makes visible, is that at any given temperature, there is an absolute difference in enthalpy ($H$, J/g) between the crystalline and amorphous phases. That absolute difference determines the size (J/g) of the endothermic peak when the crystalline material melts and the size of the exothermic peak when an amorphous material crystallizes. The amount of heat that must be absorbed to convert the crystalline phase to the amorphous phase is essentially independent of what causes the phase transition (if a kinetic process causes loss of crystalline structure, the size of the endothermic peak can change slightly due to the energy of the process). The best way to illustrate the difference in enthalpy between the crystalline and amorphous phases, as a function of temperature, is with an enthalpy plot. An enthalpy (J/g) plot is created by taking the absolute integral of a heat flow signal (W/g, where $W = J/s$) with respect to time or the absolute integral of a heat capacity signal (J/g°C) with
respect to temperature. Figure 3.2 (top) is an example of enthalpy plots for 100% amorphous and 100% crystalline materials. The heat capacity signals used to create the enthalpy plots are shown at the bottom of Figure 3.2.

Two important characteristics associated with the loss of crystalline structure (i.e., crystalline to amorphous phase transition) can be observed from the enthalpy plot (Figure 3.2, top). First, there is an absolute difference in enthalpy (J/g) between the amorphous and crystalline phases (i.e., the enthalpy difference of amorphization\(^2\)), and that difference increases with increasing temperature due to differences in the heat capacity between the two phases. Second, the laws of thermodynamics require a crystalline material to absorb the difference in enthalpy between the two phases in order to become amorphous. Based on these characteristics, the difference in enthalpy between the crystalline and amorphous phases at the thermodynamic melting temperature should be a single, constant value, equal to the area of the endothermic peak (\(\Delta H, J/g\)) obtained using DSC. Thus, the \(\Delta H\) value for thermodynamic melting is also independent of time (i.e., heating rate independent).

Theoretically, as discussed above, thermodynamic melting occurs at a single temperature with a constant enthalpy value. However, both material properties and measurement methods can affect experimentally obtained melting parameters. In the case of pure and relatively small molecules, thermodynamic melting usually occurs over a narrow temperature range. However, in the case of larger molecules (e.g., polymers), thermodynamic melting occurs over a broad temperature range due to a number of factors, including molecular weight distribution, crystal purity, type, size, and crystal perfection during heating. For example, in the case of macromolecular materials with poor (or imperfect) crystals, crystal perfection during heating can

\(^2\)Conversion from a crystalline to an amorphous structure.
occur, resulting in an increase in the melting temperature of the original material. Fast heating rates are sometimes used to avoid crystal perfection during heating; however, using too fast of a heating rate may lead to superheating (Wunderlich 1990b). Superheating occurs when heat is supplied to the crystals faster than they can melt. As explained by Wunderlich (1990b, pp. 197), the result is that "the interior of the crystal heats above the melting temperature and finally melts with entropy production at a higher temperature, when the interface between the crystal and the melt progress sufficiently."

Additionally, measurement methods can influence the experimentally obtained melting parameters, both between methods (e.g., DSC versus a melting point apparatus) and within a method (Hurtta and others 2004). An example of within a method is thermal lag in DSC measurements. In DSC analysis, the furnace attempts to heat the sample at the user selected heating rate and a thermocouple, or other temperature sensor, is used to measure sample temperature. However the thermocouple is not in direct contact with the sample, and there is a thermal lag that varies with experimental conditions. This difference between the thermocouple and sample temperatures affects the determination of the melting temperature. Fast heating rates, large sample sizes, heavy sample pans, poor thermal conductivity or contact between pan and platform, and high heat capacity materials (e.g., aqueous solutions) all increase thermal lag (Cassel 2008a). However, use of modest heating rates (1 to 25°C/min), sample sizes (1 to 5 mg), and sample pan mass can reduce melting parameter variation caused by thermal lag. For example, in the case of indium (3.8 mg), a common DSC calibration standard, $T_{\text{m onset}}$, $T_{\text{m peak}}$, and $\Delta H$ increased by only 0.75°C, 1.31°C and 0.38 J/g, respectively, when using heating rates of 1 and 25°C/min with hermetic aluminum DSC pans (~ 50 mg).
However, the small variations above cannot explain the heating rate dependency and large differences observed for the sugar melting parameters shown in Table 3.1. Then what is the underlying cause of the heating rate dependency observed in these melting parameters? Even though a complete explanation was not found in the literature, a number of publications have discussed the large variation and have offered the following possible explanations: origin, impurity, polymorphism (or allotrophism), superheating, liquefaction, and thermal decomposition and/or mutarotation in addition to melting.

The origin of the sugar may be responsible for the wide variation of melting points, since there are different sources (e.g., cane and beet sugar) and manufacturing methods. For example, it is well documented in the literature that sucrose prepared by different manufacturing methods (e.g., reagent grade versus commercial grade) yields different shapes and numbers of DSC endothermic melting peaks (one, two or three), which can give rise to different $T_{m \text{ onset}}$ values (Richards and Shafizadeh 1978; Kelly and Brown 1978/79; Eggleston and others 1996; Kishihara and others 2001; Okuno and others 2002a, 2002b, 2003; Hurtta and others 2004; Kishihara and others 2004; Beckett and others 2006; Lee and Chang 2009). To date, these findings on the variation in sucrose melting peaks have mainly been related to impurity or polymorphism (discussed below). However, it is important to note the $T_{m \text{ onset}}$ still exhibits a heating rate dependency for a single type of sucrose (e.g., reagent grade) (Okuno and others 2003; Hurtta and others 2004; Beckett and others 2006).

In general, the presence of impurities significantly decreases the melting temperature and broadens the endothermic melting peak (Widmann and Scherrer 1991; Cassel 2008b). However, it has been reported that the melting temperature for sucrose was not always depressed by impurities; but was dependent on the type and amount of impurities, such as organic solvents,
mineral salts, water, and thermal decomposition components (Shah and Chakradeo 1936; Hirschmuler 1953; Kamoda 1960; Beckett and others 2006). Okuno and others (2002a, 2002b, 2003) reported a similar observation, however, they concluded that the large variation in the sucrose melting temperature was because during crystallization the impurities contained in the sucrose solution cause formation of an additional crystal structure (polymorphism). A number of other publications also attribute the difference in sucrose melting temperatures to the presence of conformational polymorphs (Kishihara and others 2001; Lee and Lin 2007; Lee and Chang 2009). However, Saska (2008) specifically disagreed with the presence of sucrose polymorphs as reported by Lee and Lin (2007). The idea of sugar polymorphism is not new, but was mentioned by Shallenberger and Birch (1975), using the term allotropic forms. These researchers suggested that the presence of different allotropic forms can cause problems in the identification of a sugar by its melting temperature, because the sugar crystal can alter its allotropic form during slow heating, such as is employed in the determination of a melting temperature.

Superheating, as mentioned above, occurs when heat is supply to the crystals faster than they can melt. In superheating, the surface of the crystals still begin to melt at or close to the equilibrium melting temperature, but because melting occurs so slowly the temperature of the interior of the crystals rises above the equilibrium melting temperature before the end of the transition. Tammann (1910), and later Hellmuth and Wunderlick (1965), ascribed the heating rate dependency they observed in glucose melting curves to superheating. Melting was thought to still begin when the equilibrium melting point was reached, but that the glucose crystals easily

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3 Allotrophic forms refers to the way that the molecules in a material are packed into the cell of the crystal lattice, in much the same way that carbon can exist as diamond, graphite, or lamp-black. 4 Typically, melting is sufficiently fast so that its “rate” is determined by the conduction of the heat of fusion into the crystal; thus, the temperature of the crystal does not rise above the melting temperature until the end of the transition (Wunderlick 2007).
superheated because of slow melting due to the H-bond structure of glucose, which is also at the root of the high viscosity of the molten glucose (Wunderlick 2007). In studies performed more recently on glucose, as well as other sugars (e.g., Hurtta and others 2004; Lappalainen and others 2006; Magoń and Pyda 2009⁵), using modern DSC instruments, Tₘ onset has been shown to actually shift to higher starting temperatures with increasing heating rate, proving that superheating is not the underlying cause of the heating rate dependency of the sugar melting parameters.

Tombari and others (2007) proposed the theory of spontaneous "liquefaction" to explain why isomerizable crystals, such as fructose, glucose, and galactose, exhibit a large variation in their melting temperatures. Their theory is based on the formation of tautomers via mutarotation during heating. When an isomerizable crystalline material is held isothermally, the energy of the molecules next to a vacancy in the crystal lattice increases, which may be sufficient to isomerize it to a tautomer that no longer fits in the lattice site nor is able to revert to its original state. This gives rise to the formation of a liquid like local region embedding the vacancy. Similar regions may appear elsewhere in the lattice and when the number of such regions becomes high enough they coalesce and the crystal spontaneously liquefies at the isothermal temperature, significantly below the melting temperature of the material. The time required for liquefaction was dependent on the difference between the isothermal temperature and the reported crystalline melting temperature, according to a non-exponential rate kinetics and a temperature dependent rate

⁵ Magoń and Pyda (2009) reported that sucrose, glucose, and fructose exhibited superheating, but their data clearly shows that Tₘ onset continues to increase gradually with increasing heating rate. It appears that they incorrectly attempted to quantify the amount of superheating by subtracting the Tₘ onset at the lowest heating rate from the Tₘ onset at the highest heating rate. However, based on the definition of superheating there should be no change in Tₘ onset, rather just a change in Tₘ peak.
constant. In addition, they reported that the liquefied materials studied did not crystallize upon cooling, specifically discussing the lack of recrystallization in the case of fructose over a six year time period. Since the authors found that the isomerizable crystalline materials they studied spontaneously liquefy over a period of time (i.e., hours to weeks) at temperatures far below their reported melting temperatures, they concluded that the thermodynamic melting temperature for these crystals could not be determined. A point important to the current study is that Tombari and others (2007) also mentioned that their results could be a consequence of chemical decomposition, however, they did not find evidence that decomposition occurred for fructose within their work or within the literature they cited.

By comparing the $T_{m\text{ onstart}}$, measured by DSC, to the initial decomposition temperature ($T_i$), measured by Thermogravimetric Analysis (TGA), Hurtta and others (2004; sucrose, glucose, and fructose) and Lappalainen and others (2006; xylose) concluded that the observed heating rate dependency of $T_{m\text{ onstart}}$ for sucrose, glucose, fructose, and xylose at low heating rates was due to the occurrence of thermal decomposition and mutarotation (except no mutarotation in the case of sucrose), in addition to melting. In the case of sucrose at relatively high heating rates, they reported that melting occurred before thermal decomposition. However, from our observation of their sucrose data, it appears that at relatively high heating rates, $T_{m\text{ onstart}}$ continues to increase slightly, in addition to a relatively large increase in enthalpy (no explanation for the large increase in enthalpy was offered). In the case of glucose, fructose, and xylose at relatively high heating rates, the authors concluded that mutarotation was predominantly responsible for the observed increase in $T_{m\text{ onstart}}$ with increased heating rate. Regarding the observed increase in enthalpy for all heating rates employed for fructose and glucose, Hurtta and others (2004)
mentioned that endothermic/exothermic changes associated with caramelization/decomposition might have also affected the enthalpy values.

Two aspects of the conclusions drawn by Hurtta and others (2004) and Lappalainen and others (2006) that require further discussion are: 1) the use of mutarotation as a possible explanation for the observed heating rate dependency of \( T_{m\text{ onset}} \) for glucose, fructose, and xylose and 2) the difference in thermal lag between DSC and TGA measurement methods. Hurtta and others (2004) and Lappalainen and others (2006) included mutarotation (in addition to decomposition and melting) as a possible explanation for the observed heating rate dependency of \( T_{m\text{ onset}} \) for glucose, fructose, and xylose; however, no specific mechanism of action was suggested. Since mutarotation in crystalline sugars is induced only after melting begins (Wlodarczyk and others 2009), it should only affect the melting parameters associated with the shape of the endothermic melting peak – \( T_{m\text{ peak}} \) and \( \Delta H \), but not the temperature at which melting begins – \( T_{m\text{ onset}} \). Therefore, we assert that mutarotation is not a possible explanation for the observed heating rate dependency of \( T_{m\text{ onset}} \).

The second factor influencing the conclusions drawn by Hurtta and others (2004) and Lappalainen and others (2006) is the difference in thermal lag between DSC and TGA measurement methods. Unlike in DSC, the thermocouple in TGA is not in direct contact with the sample pan, so thermal lag in a TGA experiment grows larger as heating rate increases compared to a DSC experiment, resulting in a higher reported initial thermal decomposition temperature (\( T_i \)) than actually occurs. Therefore, it is possible that in these two studies, at relatively high heating rates (up to 100°C/min), \( T_i \) is actually much closer to, or even lower than, \( T_{m\text{ onset}} \) for the sugars due to the large thermal lag in the TGA measurement method. Thus, caution is needed when comparing \( T_i \) to \( T_{m\text{ onset}} \) for determining thermal event order especially at high heating rates.
In addition to Hurtta and others (2004) and Lappalainen and others (2006), a number of other researchers have concluded that thermal decomposition (but not mutarotation), in addition to melting, are responsible for the observed variation in the sugar melting temperatures (Kamoda 1960; Öris 1973; Roos 1995; Kishihara and others 2004; Sakamoto and others 2006; Magoń and Pyda 2009). However, there are some differences in the specific experimental results reported in these studies.

The above explanations do not completely and/or satisfactorily account for the large variation observed in the melting parameters for these sugars. Specifically, variation in $T_{m \text{ onset}}$ is still observed as a function of heating rate, despite using the same sugar and the same measurement method and taking thermal lag into account. However, these studies do support the observation that these sugars do not exhibit thermodynamic melting, but rather appear influenced by a kinetic process. We hypothesize that the kinetic process responsible for the “melting” of sucrose, glucose, and fructose (as well as similar materials) is thermal decomposition, not thermodynamic melting, and that the “melting” of materials that exhibit heat rate dependency, such as the sugars studied in this research, should be distinguished from materials that exhibit thermodynamic melting. Herein, to prevent confusion, the term “loss of crystalline structure” will be used instead of melting. Furthermore, since thermal decomposition is a time-temperature combination process, the loss of crystal structure occurs not as a single, time independent temperature, but as function of time and temperature. Thus, loss of crystalline structure in these sugars can occur at temperatures well below their literature reported “melting” temperatures, if held for a long enough time. Therefore, the ultimate objective of this study is to elucidate the fundamental mechanism underlying the loss of crystalline structure in these sugars, addressing a major controversy that currently exists in the literature. The results of this study are presented in
two companion papers – the first focuses on thermal analysis for all three sugars and the second on chemical analysis for sucrose. The specific objectives of this part of the study are: 1) to explore the heating rate dependency associated with the loss of crystalline structure in sucrose, glucose, and fructose using Standard DSC (SDSC) and 2) to investigate the underlying kinetic process responsible for the loss of crystalline structure in sucrose, glucose, and fructose using Modulated DSC (MDSC) and SDSC/TGA.

3.3. Materials and Methods

3.3.1. Materials

Crystalline sucrose (S0389, ≥ 99.5%), D-(−)-fructose (F2543, ≥ 99.5%), D-(+)-glucose (G7528, 99.5%), and mannitol (M9546, ≥ 99.9%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Material information for the three sugars and mannitol was given in Appendix A and B, respectively. These analytical reagent grade materials were used without further purification and stored under room conditions in their original containers, wrapped tightly with parafilm after opening. The water contents of the three sugars and mannitol were measured by coulometric Karl Fischer Titration with Hydranal Coulomat AG as a solvent and were 0.004 % wb for sucrose, 0.048% wb for glucose, 0.033% wb for fructose, and 0.060% wb for mannitol.

3.3.2. Methods

Standard DSC (SDSC) and stepwise quasi-isothermal MDSC experiments were carried out using a DSC Q2000 (TA instruments, New Castle, DE), equipped with a Refrigerated Cooling System (RCS 90). The TGA experiments were carried out using a TGA Q500 (TA instruments, New Castle, DE).
3.3.2.1. Standard DSC (SDSC) experiments

The DSC Q2000 was calibrated for enthalpy (cell constant) and temperature prior to sample measurements. Temperature calibration was performed to correct the difference between the known melting temperature of a standard (indium, \( T_{\text{m onset}} \) of 156.6\(^\circ\)C, \( \Delta H \) of 28.71 J/g, Lot# A10R020, TA instruments, New Castle, DE) and its measured melting temperature. Hermetic aluminum Tzero pans (PN 901683.90, TA instruments, New Castle, DE) and lids (PN309684.901, TA instruments, New Castle, DE) were used for all calibration and sample measurements, including an empty pan as the reference. Dry nitrogen, at a flow rate of 50 mL/min, was used as the purge gas.

Hermetically sealed sucrose, glucose, and fructose (approximately 2.75 mg) samples were equilibrated at 25\(^\circ\)C and then heated at heating rates of 2, 5, and 10\(^\circ\)C/min over the temperature range where an entire endothermic peak was obtained. Using a similar method, mannitol (approximately 2 mg) and indium (3.8 mg) were analyzed at heating rates of 1, 5, and 25\(^\circ\)C/min. All samples were measured in triplicate at each heating rate. Universal Analysis (UA) software (TA instruments, New Castle, DE, version 4.4A) was used to obtain the melting parameters (\( T_{\text{m onset}} \), \( T_{\text{m peak}} \), and \( \Delta H \)) and to plot the average heat flow signal of triplicate measurements at each heating rate against temperature.

3.3.2.2. Stepwise-isothermal MDSC experiments

Stepwise-isothermal MDSC experiments were performed using the modulated mode in the DSC Q2000. In advance of sample measurements, DSC heat capacity calibration was done using a 22.93 mg sapphire disk (PN 970370.901, TA instrument, New Castle, DE) hermetically sealed in a pan for accurate determination of changes in heat capacity (Rev \( C_p \)). With the same sapphire disk, MDSC heat capacity calibration was conducted using the temperature modulation
conditions used for the sample measurements; that is, a modulation amplitude of ± 1.0°C and a period of 100 sec for sucrose, glucose, and fructose, and a modulation amplitude of ± 0.1°C and a period of 60 sec period for indium and mannitol. Dry nitrogen purge gas was set at a flow rate of 50 mL/min.

For sample measurements, sucrose (approximately 2 mg) was heated from 80°C to 160°C using a modulation amplitude of ± 1.0°C, a period of 100 sec, a stepwise temperature-increment of 1°C, and an isothermal time of 25 min with a data off period of 5 min for initial equilibration. With the same modulation condition as used for sucrose, glucose and fructose were heated from 70°C to 160°C and from 60°C to 120°C, respectively. Indium (4.7 mg) was heated from 155°C to 165°C using a modulation amplitude of ± 0.1°C, a period of 60 sec, a stepwise temperature-increment of 0.1°C, and an isothermal time of 25 min with a data off period of 5 min for initial equilibration. Mannitol (approximately 2 mg) was heated from 149°C to 159°C using the same modulation condition as in indium. All sample measurements were done in duplicate. Results were displayed as plots of Rev C_p, modulated heat flow, and temperature against isothermal time using the UA software.

3.3.2.3. TGA experiments

The TGA Q500 was calibrated for weight and temperature in advance of sample measurements. Weight calibration was done by placing a calibration weight on the sample pan. Unlike in DSC, the thermocouple in TGA is not directly in contact with the sample pan, which causes a larger thermal lag than in DSC. Thus, in order to reduce the effect of this large thermal lag three things were done. First, the TGA thermal couple was positioned as close as possible to the sample pan. Second, since thermal lag in TGA becomes larger with increasing heating rate, the maximum heating rate of 10°C/min (identified via preliminary experiments) was used and
the TGA was calibrated at each heating rate (2, 5, and 10°C/min) using indium (67.05 mg). Indium was used for TGA temperature calibration so as to make the experimental condition for both TGA and DSC as identical as possible. Third, helium at a flow rate of 50 ml/min was used as the furnace purge gas to improve the contact between the sample and the thermocouple. Dry nitrogen at a flow rate of 10 ml/min was used for the balance purge gas. The DSC Q2000 was also calibrated using the same methods as described in the SDSC experiments.

For TGA experiments, sucrose, glucose, and fructose (approximately 84 mg) were heated at heating rates of 2, 5, and 10°C/min over the same temperature range as in the SDSC experiments. Mannitol (approximately 25 mg) was heated at a heating rate of 10°C/min from 25 to 230°C. All TGA measurements were done in triplicate. In order to facilitate comparison, the average TGA thermal data were overlaid with the average SDSC thermal data using the UA software. The TGA thermal data was displayed as %weight loss and TGA derivative temperature signals against temperature, while the SDSC thermal data was displayed as heat flow and SDSC derivative temperature signals against temperature.

3.4. Results and Discussion

3.4.1. Standard DSC (SDSC) experiments

SDSC experiments were performed in order to explore the heating rate dependency of the melting parameters ($T_{\text{m onset}}$, $T_{\text{m peak}}$, and $\Delta H$) for sucrose, glucose, and fructose by comparing them to a thermodynamic melting material, such as indium. Although indium is a well-established thermodynamic melting material, it is not an organic material like the sugars. It would be desirable to employ a material more similar to the sugars than indium, as a comparison thermodynamic melting material. Sugar alcohols, such as mannitol and sorbitol, are frequently
used to prevent food products from browning during heat processing, since they are very stable to heat and are thought to melt without thermal decomposition (Gombás and others 2003). Even though no literature was found regarding the use of sugar alcohols as comparison thermodynamic melting materials, a recent report by Lappalainen and others (2006) used xylitol instead of indium to measure thermal lag in their experiments on L- and D-xyloses, noting that xylitol’s melting behavior is normal, that is, it appears to be a thermodynamic melting material. Therefore, mannitol, the alcohol form of fructose, and sorbitol, the alcohol form of glucose, were selected as possible comparison thermodynamic melting materials, in addition to indium. In preliminary SDSC experiments (data for sorbitol given in Appendix C), mannitol was selected as the comparison material, because it more closely resembled the thermal behavior of indium.

Figure 3.3 shows the melting parameters ($T_{m\text{ onsets}}$, $T_{m\text{ peaks}}$, and $\Delta H$) for indium and mannitol at heating rates of 1, 5, and 25°C/min. For both indium and mannitol these parameters did not exhibit significant heating rate dependency. Over the heating rate range of 1 to 25°C/min, $T_{m\text{ onset}}$ and $T_{m\text{ peak}}$ for indium increased by 0.75°C and 1.31°C, respectively, and $\Delta H$ changed by 0.38 J/g. Similar to indium, mannitol showed only a slight increase in $T_{m\text{ onset}}$ and $T_{m\text{ peak}}$, 0.30°C and 0.73°C, respectively, and $\Delta H$ changed by 1.54 J/g. Although the differences in $T_{m\text{ onset}}$ and $T_{m\text{ peak}}$ for indium and mannitol were statistically significant ($p = 0.05$) as a function of heating rate (Table 3.2), the difference is not meaningful, rather the result of a very small standard deviation. In addition to similar $T_m$ parameters, the endothermic peak shape of mannitol was very similar to that of indium, however mannitol exhibited a larger $\Delta H$ standard deviation. These results suggest that the use of mannitol as a thermodynamic melting comparison material is justified.

Unlike indium and mannitol, the melting parameters of sucrose, glucose, and fructose exhibited strong heating rate dependency as shown in Figure 3.4. Over the heating rate range of 2
to 10°C/min, $T_{m\text{onset}}$, $T_{m\text{peak}}$, and $\Delta H$ increased by 12.95°C, 10.71°C, and 12.03 J/g for sucrose (large endothermic peak), by 7.51°C, 8.21°C, and 23.60 J/g for glucose, and by 6.34°C, 9.40°C, and 15.66 J/g for fructose, respectively. This strong heating rate dependency suggests that there is a kinetic process associated with the loss of crystalline structure occurring in these sugars.

Similar increases in heating rate dependency were reported by Hurtta and others (2004), also studying sucrose, glucose, and fructose, though the magnitude of the increases varied somewhat between the present study and Hurtta and others (2004). If the loss of crystalline structure in these sugars is due to thermal decomposition, the discrepancy in the magnitude of values between the present study and Hurtta and others (2004) can be attributed to the non-reproducible (non-uniform) nature of kinetic processes. An additional observation is that in Hurtta and others (2004) the two sucrose samples used (bulk [A] and fine chemical [B]) showed different numbers of endothermic melting peaks, with sucrose A exhibiting two peaks and sucrose B one peak. However, in the present study, the analytical reagent grade sucrose exhibited two endothermic melting peaks. The presence of two endothermic melting peaks for analytical reagent grade sucrose has been previously reported (Richards and Shafizadeh 1978; Eggleston and others 1996; Beckett and others 2006; and Lee and Lin 2007). The difference in the number of peaks between different sucrose sources is currently under study in our laboratory, however, an important point in regards to this study is that both sucrose samples used by Hurtta and others (2004) and the sucrose sample used in this study, as well as other studies (Okuno and others 2003; Hurtta and others 2004; Beckett and others 2006), all exhibited heating rate dependency (Figure 3.4).

3.4.2. Stepwise-isothermal MDSC experiments

Stepwise quasi-isothermal MDSC experiments were conducted to further investigate the presence of a kinetic process occurring during the loss of crystalline structure in sucrose, glucose,
and fructose, as suggested by the heating rate dependency experiments presented above. Stepwise quasi-isothermal MDSC is a very useful technique for measuring heat capacity ($C_p$) and heat capacity changes ($\Delta C_p$) caused by structural transformations, such as phase transitions, in a material during heating. In the case of thermodynamic melting, the phase transition (structural change) of a material from the crystalline solid phase to the corresponding liquid phase results in an increase in molecular mobility, such as vibrational, rotational, and translational motions, and is reflected in a corresponding increase in $C_p$. Once the phase transition is completed, $C_p$ is larger for the liquid phase than it was for the crystalline solid phase. However, for a material where the loss of crystalline structure involves a kinetic process, the change in $C_p$ associated with the phase transition may be different than for a thermodynamic material melting, because the kinetic process may chemically alter the material. For example, in the case of thermal decomposition as the kinetic process, the material’s molecules are, at least initially, broken down into smaller components, which result in an increase in molecular mobility and thus a continuous increase in $C_p$ with temperature.

It is important here to mention that the $C_p$ of a thermodynamic melting material is also dependent on temperature (and to a lesser degree, other factors, such as atmospheric pressure) before and after a phase transition, but increases predictably (able to be modeled) with temperature and at a much slower rate than the changes in $C_p$ with temperature associated with a material that looses its crystalline structure via a kinetic process. All changes in $C_p$ involving a structural change in a material are displayed in the Rev $C_p$ signal in stepwise-isothermal MDSC. The Rev $C_p$ signal is calculated from the amplitude of the modulated heat flow (Thomas 2006), which reflects the response of the material to the modulated heating rate used in stepwise quasi-isothermal MDSC. Therefore, if a kinetic process is occurring during the loss of crystalline
structure in the three sugars, it should be able to be observed by comparing the changes in the Rev $C_p$ and modulated heat flow of the three sugars to those of indium and mannitol.

Figure 3.5 shows the stepwise quasi-isothermal MDSC thermograms for indium and mannitol. For indium, a thermodynamic melting material, the solid to liquid phase transition in the Rev $C_p$ signal occurred over a very narrow temperature range (~0.3°C) and then the Rev $C_p$ leveled off and regained its predictable and small temperature dependency (as mentioned above) after the phase transition, as it had before the phase transition. The amplitude of the modulated heat flow dramatically changed during the phase transition and also leveled off after the phase transition. Similar observations were found for mannitol, used herein as a thermodynamic melting comparison material, where the change in the Rev $C_p$ took place over ~4.09°C.

Another important finding, which should be noted in Figure 3.5 for both indium and mannitol, is the collections of exothermic and endothermic peaks that appear in the modulated heat flow during the phase transition. These collections of peaks are the result of the sinusoidal heat/cool temperature profile used in the stepwise quasi-isothermal MDSC experiment. During the phase transition, when the modulation temperature increases above the average temperature, some of the material melts and when the modulation temperature drops below the average temperature, some of the material crystallizes. Thus, while the modulation temperature is applied to the material at an isothermal temperature, the modulated heat flow amplitude remains almost constant because the material melts and recrystallizes continuously. However, when the isothermal temperature is increased or decreased even a small amount, the modulated heat flow changes a great deal, since a different amount of material melts and recrystallizes at each different isothermal temperature. For indium and mannitol, therefore, the presence of the exothermic and endothermic collections of peaks in Figure 3.5 shows that both materials were
able to melt and recrystallize successively, which suggests that from a structural perspective the molecules can easily respond to the applied modulated temperature and, in turn, from a chemical perspective that the molecules were not altered during the phase transition, that is, the chemical composition of the solid phase was the same as the liquid phase.

The stepwise quasi-isothermal MDSC thermograms for sucrose, glucose, and fructose showed completely different thermal behaviors compared to indium and mannitol in terms of the temperature range over which the phase transition (i.e., the loss of crystalline structure) occurs, the $\text{Rev } C_p$ value after the phase transition, and the absence of the collections of exothermic and endothermic peaks (Figure 3.6). However, the thermal behavior of the three sugars was similar to each other. During the phase transition, the change in the $\text{Rev } C_p$ occurred over a relatively broad temperature range (~23°C for sucrose, ~9°C for glucose, and 9°C for fructose). Once the $\text{Rev } C_p$ began to increase (~120°C for sucrose, ~130°C for glucose, and ~92°C for fructose), unlike indium and mannitol, it never decreased, but rather kept increasing, though more gradually, after the complete loss of crystalline structure (~143°C for sucrose, ~139°C for glucose, and ~101°C for fructose). As discussed above, this result can be interpreted in the light of the relation between the $\text{Rev } C_p$ and molecular mobility. The sugars are broken down into smaller components by a kinetic process, specifically thermal decomposition. The formation of smaller components leads to an increase in molecular mobility and thus $\text{Rev } C_p$. This continuous increase in the $\text{Rev } C_p$ indicates that thermal decomposition continues after all crystalline structure was removed in the sugars. As can be observed from Figure 3.6, the increase in the $\text{Rev } C_p$ after the phase change is different for each sugar and is much larger than in the case of indium and mannitol.
Unlike indium and mannitol, the three sugars exhibited no exothermic and endothermic collections of peaks in the modulated heat flow. In particular, the absence of the collection of exothermic peaks (i.e., absence of recrystallization) further supports that thermal decomposition is the kinetic process causing the loss of crystalline structure in the sugars. We propose that the absence of recrystallization is due to chemical alteration of the sugar molecules via thermal decomposition, that is, the chemical composition of the solid phase was not the same as the liquid phase. The chemically altered molecules in the material can no longer support the original crystalline structure, leading to the loss of crystalline structure. The presence of thermal decomposition products would make recrystallization difficult, especially over the short modulation period used in the quasi-isothermal MDSC experiment.

It is important to mention that another possible explanation for the absence of the collection of exothermic peaks is mutarotation. Once the loss of crystalline structure is initiated, mutarotation can occur and the tautomer(s) formed could interfere with the recrystallization process (especially over the short MDSC experimental time frame as mentioned above). Two observations make it very unlikely that mutarotation is solely responsible for the absence of the collection of exothermic peaks observed in Figure 3.6. First, mutarotation is not possible in the case of sucrose, so the absence of the collection of exothermic peaks for sucrose cannot be explained by mutarotation only, leaving thermal decomposition as the likely cause. Second, even if mutarotation were responsible for the absence of the collection of exothermic peaks, it would not be the cause of the heating rate dependency of $T_{m\text{onset}}$, since mutarotation affects the peak

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6To clarify, mutarotation may occur in addition to thermal decomposition in the case of glucose and fructose, but for the reasons given in the text, it is not solely responsible for the absence of the collection of exothermic peaks observed in Figure 3.6.
shape parameters ($T_{m \text{ peak}}$ and $\Delta H$), but not the beginning of the loss of crystal structure ($T_{m \text{ onset}}$, as previously discussed in the introduction).

It is important here to clarify the role of mutarotation in sugar melting hypothesized by Tombari and others (2007). As discussed in the introduction, Tombari and others (2007) proposed that mutarotation was the cause of the loss of crystalline structure in isomerizable sugars, e.g., glucose, fructose, and galactose. However, we found that sucrose, a non-isomerizable sugar, exhibited the same behavior as the isomerizable sugars (glucose, fructose, and galactose studied by Tombari and others (2007) and glucose and fructose studied herein), which leads us back to our hypothesis that thermal decomposition is responsible for the loss crystalline structure in the three sugars.

A number of publications have implicated that thermal decomposition accompanies (along with or right after) sugar melting in sucrose, glucose, and fructose (Kamoda 1960; Ōris 1973; Mauch and Asseily, 1975; Roos 1995; Hurtta and others 2004; Kishihara and others 2004; Sakamoto and others 2006; Liu and others 2006; Magoń and Pyda 2009). The critical difference between our conclusion regarding the role of thermal decomposition in sugar melting and others is that we propose that thermal decomposition is the kinetic process that is responsible for the loss of crystalline structure in sucrose, glucose, and fructose; not that thermal decomposition occurs in addition to thermodynamic melting. Thermal decomposition, through the input of heat energy (enthalpy), gives rise to alterations in the chemistry of the molecules (i.e., intermolecular bond breaking, for example, the glycosidic bond between glucose and fructose in the sucrose molecule) that compose the crystalline matrix. Once these intermolecular bonds begin to break, the molecules can no longer maintain their original crystalline structure, resulting in the loss of crystalline structure (matrix is now amorphous) and a commensurate increase in enthalpy equal
to the absolute difference in enthalpy (ΔH, J/g) between the crystalline and amorphous phases (as previously discussed in association with Figure 3.2). However, in the case of thermal decomposition as the kinetic process this enthalpy value is not a constant, as it is in thermodynamic melting (i.e., heating rate independent), but rather increases as the temperature at which thermal decomposition occurs increases (i.e., heating rate dependent). Therefore, the enthalpy of the endothermic “melting” peaks for sucrose, glucose, and fructose in Figure 3.4 includes both the enthalpy required for bond breaking due to thermal decomposition and the enthalpy (ΔH, J/g) required for amorphization (loss of crystalline structure) at the temperature at which thermal decomposition occurs.

Because of the fundamental differences between the loss of crystalline structure caused by a kinetic process, such as thermal decomposition proposed herein, and the loss of crystalline structure caused by thermodynamic melting, we propose implementation of a new term, “apparent melting”, to describe the loss of crystalline structure caused by a kinetic process. Apparent melting is the amorphization of a material at temperatures below the thermodynamic melting temperature caused by a kinetic process. Kinetic processes that can cause apparent melting include decomposition (studied herein), dehydration (Ding and others 1996; Kajiwara and Franks 1997; Cheng and Lin 2006; Bates and others 2007; Franks 2007), and chemical interactions/reactions (Miller and York 1988; Kerč and others 1992; Venkataram and others 1995; Wissing and others 2000; Desai and others 2003; Cides and others 2006). Table 3.4 summarizes the similarities and differences between thermodynamic melting and apparent melting specifically caused via thermal decomposition. It important to note that, in general, the enthalpy associated with apparent melting may be larger or smaller than that associated with thermodynamic melting, since although amorphization is always an endothermic process
(increase in entropy), the kinetic process may be endothermic (making $\Delta H$ larger) or exothermic (making $\Delta H$ smaller).

### 3.4.3. TGA experiments

TGA experiments were performed and compared to the SDSC experiments (previously discussed) in an attempt to further investigate our hypothesis that thermal decomposition is the kinetic process causing the loss of crystalline structure in the three sugars, regardless of the heating rate employed. TGA is generally utilized for investigating kinetic processes (e.g., thermal decomposition, evaporation, and gas desorption) by monitoring weight change in a material during heating, at a constant rate under a specified atmosphere. Although, TGA experiments alone cannot identify the specific type of kinetic process.

A comparison was made between the SDSC measured temperature ($T_{m \text{onset}}$), at which the phase transition (the loss of crystalline structure) occurs, and the TGA measured temperature ($T_i$), at which weight loss occurs, hypothesized to be due to thermal decomposition, for the three sugars at the three different heating rates (2, 5, and 10 °C/min). The SDSC and TGA thermal data for the sugars also was compared to that for mannitol, as a comparison thermodynamic melting material. In addition to comparing $T_{m \text{onset}}$ and $T_i$, SDSC and TGA derivative temperature signals (in units of °C/min) were used to monitor changes in the heating rate of the sugars during the phase transition in order to more accurately compare the SDSC and TGA thermal data.

It is difficult to ensure the accurate comparison of SDSC and TGA thermal data. Thus, it would be helpful to have an internal indicator (i.e., within the TGA experiment) of the temperature at which the sample undergoes its crystalline to amorphous phase transition (provided also by the external SDSC experiment). The derivative temperature signal provides this internal indicator, since when a sample undergoes a crystalline to amorphous phase
transition the heating rate of the sample exhibits an abrupt decrease. This abrupt decrease in the derivative temperature signal results from the fact that during the phase transition the sample cannot keep up with the predetermined heating rate, due to the large amount of energy required for the phase transition. Thus, the abrupt decrease in the derivative temperature signal indicates the temperature at which the loss of crystalline structure in the sugars begins within the TGA experiment. This same phase transition temperature is also obtained via the SDSC experiment (both as the heat flow signal and the SDSC derivative temperature signal), but is an external indicator (is from a separate instrument) used for comparison with the TGA experimental data.

Figure 3.7 shows the SDSC and TGA thermograms for indium at a heating rate of 10°C/min. An abrupt decrease in the TGA derivative temperature signal was observed at the same temperature (as measured using the extrapolated onset point) as the thermodynamic melting temperature of indium (156.6°C) and, as expected, no weight loss in the TGA weight loss signal was observed, since, based on literature findings, indium does not experience any weight loss events until its boiling point at 2080°C (Mehdipour and others 2005).

Figure 3.8 shows the SDSC and TGA thermograms for mannitol at a heating rate of 10°C/min. Originally, no weight loss was expected for mannitol, because it exhibited similar thermal behavior to indium in the SDCS and stepwise-quasi isothermal MDSC experiments discussed above. However, as can be seen in Figure 3.8, a large weight loss is observed for mannitol beginning at its melting temperature ($T_{\text{m onset}} = 165°C$). This large weight loss is attributed to evaporation of liquid mannitol. Unlike in SDSC, the TGA sample pan is open, allowing evaporation of volatile materials. All the mannitol in the sample pan completely evaporated, without a trace of decomposition (sample pan was empty and clean), during the TGA experiment.

For additional information on the vapor pressure and evaporation of sugar alcohols the interested reader is referred to Barone and others (1990) and Yan and Suppes (2008).
experiments (TGA experimental end temperature was 290°C). A number of other researchers also observed weight loss after melting for mannitol using a TGA, however the temperature at which appreciable weight loss began varied among these studies, ranging from approximately 190 to 250°C (240°C Schwarz and others 1972; 230°C Landín and others 2005; 250°C Tomassetti and others 2005; 190°C Hulse and others 2009). Two additional studies (Naini and others 1998; Bruni and others 2009) reported no appreciable weight loss for mannitol after melting using TGA, however the end temperatures used in these studies were much lower (200 and 180°C, respectively) than in the studies that reported appreciable weight loss.

Also worthy of mentioning is the small, but gradual, weight loss observed for mannitol beginning around 120°C. This weight loss is attributed to the slow sublimation of mannitol (Chickos and Acree 2002; Barone and others 1990). Walter-Levy (1968) reported sublimation of D-mannitol at 130°C, however, the sublimation of mannitol was not observed by Schwarz and others (1972). The difference between these results (observation of sublimation or not) may be because the very small amount of weight loss due to sublimation was overlooked compared to the large weight loss due to evaporation. In addition, a similar TGA weight loss observation (sublimation and evaporation) was found in the case of acetaminophen and phenacetin, which did not exhibit strong heating rate dependency on their T_m parameters over the heating rate range of 2 to 10°C/min using SDSC (Appendix D and E).

Figure 3.9 shows the SDSC and TGA thermograms for sucrose analyzed at heating rates of 2, 5, and 10°C/min, respectively. For all three heating rates, the SDSC heat flow signal simultaneously decreased with the TGA weight loss signal, indicating that the T_i was fairly close to the T_m onset. When sucrose began to lose its crystalline structure, an abrupt decrease was observed in both SDSC and TGA derivative temperature signals because of the energy required
for the loss of crystalline structure. After complete removal of the crystalline structure, the
derivative temperature signal of the sucrose jumped to a higher value as the temperature of the
sucrose caught up to that of the reference. A similar observation was found for glucose (Figure
3.10) and fructose (Figure 3.11). In the case of glucose, however, over the heating rate range of 2
to \(10^\circ\)C/min, an additional, but very small, weight loss (less than 0.05%) was observed in the
temperature range from 53°C to 60°C (data shown in Appendix F). We hypothesize that this
weight loss was due to water loss associated with the presence of a small amount of \(\alpha\)-D-glucose
monohydrate in the anhydrous glucose sample. Hence, the weight (%) in Figure 3.10 was lower
than 100% even before the loss of crystalline structure in glucose occurred.

In summary, mannitol was being used in this research as a thermodynamic melting
comparison material, since it is chemically more similar to the sugars than indium. However,
since mannitol exhibited appreciable weight loss immediately after melting due to evaporation in
the TGA experiments, the TGA thermogram for mannitol is not distinguishable from that for the
simple sugars (weight loss hypothesized to be due to thermal decomposition) in terms of the
cause of weight loss. Therefore, SDSC and TGA experiments alone are not sufficient to identify
the specific kinetic process responsible for weight loss. Thus, to determine the cause of weight
loss in the case of the sugars, chemical analysis (specifically High Performance Liquid
Chromatography, HPLC) was proposed and the results reported in Chapter 4. HPLC was
selected since it is known to be a successful technique for separating, identifying, and
quantifying sugars and their thermal decomposition components (Bonn 1985; Gomis and others
1991; Porretta 1992; Lo Coco and others 1994; Yuan and others 1996; Yuan and Chen 1999).
Since the presence of tautomers in the case of glucose and fructose would complicate the HPLC
analysis, sucrose was chosen for the further study because it does not form tautomers and it is also the most commonly used sugar in the food and pharmaceutical industries.

3.5. Conclusions

This study, using a thermal analytical approach, was conducted to elucidate the fundamental mechanism underlying the loss of crystalline structure in sucrose, glucose, and fructose. Based on the SDSC experiments, all three melting parameters ($T_{m\text{ onset}}$, $T_{m\text{ peak}}$, and $\Delta H$) exhibited a strong heating rate dependency, compared to indium and mannitol, the thermodynamic melting comparison materials. These SDSC results suggested that a kinetic process was responsible for the loss of crystalline structure in the sugars. The stepwise quasi-isothermal MDSC thermograms for sucrose, glucose, and fructose showed completely different thermal behaviors compared to indium and mannitol. The temperature range over which the loss of crystalline structure occurred was much broader for the sugars, the $C_p$ value after the phase transition was much larger and unique for each sugar, and there were no collections of exothermic and endothermic peaks for the sugars, suggesting that thermal decomposition was the kinetic process responsible for the loss of crystalline structure in the sugars. Because of the fundamental differences between the loss of crystalline structure caused by a kinetic process, such as thermal decomposition, and the loss of crystalline structure caused by thermodynamic melting, we propose the implementation of a new term, “apparent melting”, to describe the loss of crystalline structure caused by kinetic processes. In the SDSC and TGA comparison experiments, the temperature ($T_i$) at which weight loss for these sugars began due to thermal decomposition corresponded to the temperature ($T_{m\text{ onset}}$) at which the phase transition began regardless of heating rate. These results support our hypothesis that thermal decomposition is the kinetic process responsible for the loss of crystalline structure
in the three sugars. However, based on the results for mannitol as a thermodynamic melting comparison material, SDSC and TGA experiments alone are not sufficient to identify the specific kinetic process responsible for weight loss. Thus, to identify the specific cause of weight loss in the case of the sugars, chemical analysis, i.e., HPLC, was proposed and carried out on sucrose in Chapter 4.

This research is significant because melting is not only a common property used in sugar characterization, but also a general method used to prepare amorphous sugars, which are widely used as ingredients in the food industry and as excipients in the pharmaceutical industry. Since, as proposed herein, thermal decomposition chemically alters the sugar molecules during heating, the subsequent amorphous matrix (sugar molecules plus decomposition components) produced by the melt-quenching method may affect the physico-chemical properties of the final product, such as glass transition temperature, textural attributes, flavor profile and ability to protect active pharmaceutical components, as well as the product’s shelf-life.
3.6. Figures and Tables

Figure 3.1 Schematic illustration of Gibbs energy as a function of temperature, at a constant pressure, for a one-component system. Thermodynamic melting ($T_m$) occurs at the intersection of the crystalline and liquid phases, where the phases have the same Gibbs energy, $\Delta G=0$ (adapted from Wunderlich 1990a).
Figure 3.2 Temperature dependence of the latent heat of fusion (also termed enthalpy, H in J/g) and heat capacity (C<sub>p</sub> in J/g·°C) of a material in the crystalline and amorphous phases. T<sub>g</sub>, glass transition temperature; T<sub>c</sub>, cold crystallization temperature; and T<sub>m</sub>, melting temperature.
Figure 3.3 SDSC scans and melting parameters ($T_{m \text{onset}}$, $T_{m \text{peak}}$, and $\Delta H$) associated with the loss of crystalline structure in indium and mannitol over the heating rate (HR) range of 1 to 25°C/min.
Figure 3.4 SDSC scans and melting parameters ($T_{m\text{onset}}$, $T_{m\text{peak}}$, and $\Delta H$) associated with the loss of crystalline structure in sucrose, glucose, and fructose at heating rates (HR) of 1, 5, and $10^\circ\text{C/min}$.
Figure 3.5 Changes in heat capacity (Rev $C_p$) and modulated heat flow amplitude during the loss of crystalline structure in indium and mannitol, measured using stepwise quasi-isothermal MDSC.
Figure 3.6 Changes in heat capacity (Rev C<sub>p</sub>) and modulated heat flow amplitude during the loss of crystalline structure in sucrose, glucose, and fructose, measured using stepwise quasi-isothermal MDSC.
Figure 3.7 Weight loss, TGA derivative temperature, heat flow, and SDSC derivative temperature for indium heated at a heating rate of 10°C/min.
Figure 3.8 Weight loss, TGA derivative temperature, heat flow, and SDSC derivative temperature for crystalline mannitol heated at a heating rate of 10°C/min.
Figure 3.9 Weight loss, TGA derivative temperature, heat flow, and SDSC derivative temperature for crystalline sucrose heated at heating rates of 2, 5, and 10°C/min.
Figure 3.10 Weight loss, TGA derivative temperature, heat flow, and SDSC derivative temperature for crystalline glucose heated at heating rates of 2, 5, and 10°C/min.
Figure 3.11 Weight loss, TGA derivative temperature, heat flow, and SDSC derivative temperature for crystalline fructose heated at heating rates of 2, 5, and 10°C/min.
Table 3.1 Melting parameters ($T_{m\text{ onset}}$, $T_{m\text{ peak}}$, and $\Delta H$) and initial decomposition temperature ($T_i$) reported in the literatures for sucrose, glucose, and fructose as a function of heating rate.

<table>
<thead>
<tr>
<th>Sugar type</th>
<th>Heating rate (${^\circ}\text{C/min}$)</th>
<th>Melting temp. (${^\circ}\text{C}$)</th>
<th>Enthalpy ($\Delta H, J/g$)</th>
<th>Analytical technique</th>
<th>Initial decomposition temp. ($T_i, {^\circ}\text{C}$)**</th>
<th>Reference</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>$T_{m\text{ onset}}$</td>
<td>$T_{m\text{ peak}}$</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sucrose A/B*</td>
<td>0.5</td>
<td>167.9/181.4</td>
<td>169.9/182.7</td>
<td>54.8/119.8</td>
<td>DSC</td>
<td>159.6/167.0</td>
</tr>
<tr>
<td>Sucrose A/B</td>
<td>1</td>
<td>173.7/184.5</td>
<td>176.6/186.6</td>
<td>72.1/126.6</td>
<td>DSC</td>
<td>161.1/171.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1</td>
<td>160</td>
<td>185</td>
<td>120</td>
<td>HP-DTA</td>
<td>159.6/167.0</td>
</tr>
<tr>
<td>Sucrose A/B</td>
<td>2</td>
<td>178.2/187.1</td>
<td>181.4/189.3</td>
<td>111.4/128</td>
<td>DSC</td>
<td>169.6/178.8</td>
</tr>
<tr>
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<td>173</td>
<td>190</td>
<td>118</td>
<td>DSC</td>
<td>159.6/167.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>183.5</td>
<td></td>
<td>DSC</td>
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<td>146.4/147.0</td>
</tr>
<tr>
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<td>DSC</td>
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<tr>
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<td></td>
<td>DSC</td>
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<tr>
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<td>DSC</td>
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<tr>
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<td>135.7</td>
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<td>235.3/228.4</td>
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<td>149.1/147.5</td>
<td>182.7/180.1</td>
<td>DSC</td>
<td>146.4/147.0</td>
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<tr>
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<td>135</td>
<td>150</td>
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<td>150</td>
<td>180</td>
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<td>154.8/151.9</td>
<td>189.0/187.1</td>
<td>DSC</td>
<td>151.4/159.1</td>
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**References:**
- Hurtta et al, 2004
- Weitz and Wunderlich, 1974
- Gloria and Sievert, 2001
- Vanhal and Blond, 1999
- Smidova and others, 2003
Table 3.1 (cont.)

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<td>157 (peak 215) Órsi, 1973</td>
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<td>Wungtanagorn and Schmidt, 2001ab</td>
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<tr>
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<td>DSC</td>
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<td>122.8/119.0 Hurtta et al, 2004</td>
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<td>108</td>
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<td>140.6/139.8</td>
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<td>142.6/142.0</td>
<td>212.8/203.7</td>
<td>DSC</td>
<td>166.1/165.4 Hurtta et al, 2004</td>
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</table>

*A/B indicates two different sugar samples. Sucrose A and fructose A were bulk materials, the others were fine chemicals made for laboratory use. **Initial decomposition temperature was determined using TGA. ***HP-DTA: High pressure DTA apparatus.
Table 3.2 Melting parameters ($T_{m\,\text{onset}}$, $T_{m\,\text{peak}}$, $\Delta H$) for indium and mannitol as a function of heating rate (N=3, n=3; where N = replication number and n = data analysis number). A linear baseline* for indium and a sigmoidal tangent baseline* for mannitol were used for determining their melting parameters using the UA software.

<table>
<thead>
<tr>
<th>Heating rate (°C/min)</th>
<th>Thermodynamic melting materials</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indium</td>
<td>Mannitol</td>
</tr>
<tr>
<td></td>
<td>$T_{m,\text{onset}}$ (°C)</td>
<td>$T_{m,\text{onset}}$ (°C)</td>
</tr>
<tr>
<td>1</td>
<td>156.45 ± 0.01$^a$</td>
<td>156.67 ± 0.01$^a$</td>
</tr>
<tr>
<td>5</td>
<td>156.60 ± 0.00$^b$</td>
<td>157.02 ± 0.01$^b$</td>
</tr>
<tr>
<td>25</td>
<td>157.20 ± 0.00$^c$</td>
<td>157.98 ± 0.00$^c$</td>
</tr>
</tbody>
</table>

* A linear baseline was used in the case of indium because the difference in the $C_p$ between the crystalline and liquid phases was very small, whereas a sigmoidal tangent baseline was used for mannitol because the difference in the $C_p$ between the crystalline and liquid phases was large (see Figure 3.5).

** Means with the same letter within a column (difference among different heating temperatures) are not significantly different (p=0.05).
Table 3.3 Melting parameters ($T_{m\text{ onset}}$, $T_{m\text{ peak}}$, $\Delta H$) for crystalline sucrose, glucose, and fructose as a function of heating rate (N=3, n=3; where N = replication number and n = data analysis number). A sigmoidal tangent baseline and perpendicular drop function in the UA software was used for determining the melting parameters for sucrose, since sucrose showed two overlapped endothermic peak. A sigmoidal tangent baseline* was used for determining the melting parameters for glucose and fructose using the UA software.

<table>
<thead>
<tr>
<th>Heating rate (°C/min)</th>
<th>Apparent melting materials</th>
<th>Apparent melting materials</th>
<th>Apparent melting materials</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Sucrose (small endothermic peak)</td>
<td>Sucrose (large endothermic peak)</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_{m\text{ onset}}$ (°C)</td>
<td>$T_{m\text{ peak}}$ (°C)</td>
<td>Enthalpy ($\Delta H$, J/g)</td>
<td>$T_{m\text{ onset}}$ (°C)</td>
</tr>
<tr>
<td>2</td>
<td>138.01 ± 0.12$^a$</td>
<td>146.00 ± 0.68$^a$</td>
<td>9.77 ± 0.80$^b$</td>
<td>173.24 ± 2.26$^a$</td>
</tr>
<tr>
<td>5</td>
<td>145.15 ± 0.49$^b$</td>
<td>154.48 ± 0.51$^b$</td>
<td>6.59 ± 0.60$^a$</td>
<td>179.64 ± 0.67$^b$</td>
</tr>
<tr>
<td>10</td>
<td>150.97 ± 0.51$^c$</td>
<td>156.64 ± 0.92$^c$</td>
<td>10.03 ± 0.66$^b$</td>
<td>186.19 ± 0.27$^c$</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td>Fructose</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_{m\text{ onset}}$ (°C)</td>
<td>$T_{m\text{ peak}}$ (°C)</td>
<td>Enthalpy ($\Delta H$, J/g)</td>
<td>$T_{m\text{ onset}}$ (°C)</td>
</tr>
<tr>
<td>2</td>
<td>150.89 ± 0.08$^a$</td>
<td>154.06 ± 0.08$^a$</td>
<td>185.11 ± 5.91$^a$</td>
<td>107.25 ± 1.05$^a$</td>
</tr>
<tr>
<td>5</td>
<td>155.11 ± 0.10$^b$</td>
<td>158.58 ± 0.13$^b$</td>
<td>199.21 ± 5.52$^b$</td>
<td>110.45 ± 0.30$^a$</td>
</tr>
<tr>
<td>10</td>
<td>158.40 ± 0.14$^c$</td>
<td>162.27 ± 0.10$^c$</td>
<td>208.71 ± 5.58$^c$</td>
<td>113.59 ± 1.51$^c$</td>
</tr>
</tbody>
</table>

* A sigmoidal tangent baseline was used for the sugars because the difference in the $C_p$ between their crystalline and liquid phases was large (see Figure 3.6).
** Means with the same letter within a column for each sugar (difference among different heating temperatures) are not significantly different (p=0.05).
Table 3.4 Similarities and differences between thermodynamic melting and apparent melting.

<table>
<thead>
<tr>
<th>Similarities</th>
<th>Thermodynamic melting</th>
<th>Apparent melting (caused by thermal decomposition)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Similarities</strong></td>
<td>– Loss of crystalline structure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Produces an endothermic peak</td>
<td></td>
</tr>
<tr>
<td><strong>Differences</strong></td>
<td>– A single temperature</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Time-independent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Constant ΔH (amorphization enthalpy at constant T)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– No chemical alteration in material’s molecules</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(pure crystalline material = pure liquid material)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Cause: Phases have equal Gibbs energy (ΔG=0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Temperature depending on heating rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Time-dependent (kinetics)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Variable ΔH (amorphization enthalpy dependent on heating rate plus decomposition)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Chemical alteration in material’s molecules</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(pure crystalline material ≠ chemically altered liquid)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– Cause: kinetic process, e.g., thermal decomposition</td>
<td></td>
</tr>
</tbody>
</table>
3.7. References


CHAPTER 4

INVESTIGATION OF THERMAL DECOMPOSITION AS THE KINETIC PROCESS THAT CAUSES THE LOSS OF CRYSTALLINE STRUCTURE IN SUCROSE USING A CHEMICAL ANALYTICAL APPROACH

4.1. Abstract

High Performance Liquid Chromatography (HPLC) was used to investigate thermal decomposition as the cause of the loss of crystalline structure in sucrose. The loss of crystalline structure in sucrose was accomplished using two different methods: a SDSC method, which is a relatively fast heating method (10°C/min), and a quasi-isothermal MDSC method, which is a relatively slow heating method (120°C for 3100 min). In the fast heating method, initial decomposition components, glucose (0.365%) and 5-HMF (0.003%), were found in the sucrose sample coincident with the onset temperature of the 1st endothermic peak. In the slow heating method, the same initial decomposition components, glucose (0.411%) and 5-HMF (0.003%), were found in the sucrose sample coincident with the time (50 min) that the reversing heat capacity (Rev C_p) began to increase. In both fast and slow heating methods, even before all crystalline sucrose structure was completely removed, unidentified thermal decomposition components were formed. The extent of formation of these unidentified components significantly increased as the heating temperature approached the reported melting temperature (fast method) and as the holding time was longer (slow method). These results prove not only that the loss of crystalline structure in sucrose is caused by thermal decomposition, but also that it is achieved via a time-temperature combination process. This knowledge is important for quality assurance purposes and for developing new sugar based food and pharmaceutical products. Specifically, this research provides new insights into the caramalization process, showing that in addition to
occurring at high temperatures, caramalization can occur under low temperature (significantly below the literature reported melting temperature), albeit longer time, conditions.

4.2. Introduction

Melting of a crystalline material occurs at a single, heating rate independent temperature, where the crystalline solid and corresponding liquid phases are in thermodynamic equilibrium, \( \Delta \text{Gibbs energy} = 0 \) at a constant pressure (Wunderlich 1990), with no change in chemical composition. However, as detailed in Table 3.1 in Chapter 3, a number of investigators have reported a large variation in the melting parameters (the onset melting temperature, \( T_{m \text{ onset}} \); the peak melting temperature, \( T_{m \text{ peak}} \); and the enthalpy of melting, \( \Delta H \)) for sucrose, glucose, and fructose. A critical observation regarding these literature reported melting parameters, which was confirmed using standard Differential Scanning Calorimetry (SDSC) in Chapter 3, is that they all increase with increasing heating rate. This heating rate dependency led to the hypothesis that a kinetic process was responsible for the loss of crystalline structure (as in Chapter 3, to minimize confusion, the term “loss of crystalline structure” is used instead of melting) in sucrose, glucose, and fructose. In addition, stepwise quasi-isotherm modulated DSC (MDSC) and Thermogravimetric Analysis (TGA) results suggested that the most plausible kinetic process was thermal decomposition\(^8\), but not as an additional process accompanying thermodynamic melting, but as the sole kinetic process responsible for the loss of crystalline structure.

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\(^8\) A number of publications have suggested that thermal decomposition accompanies (along with or right after) sugar melting in sucrose, glucose, and fructose (Kamoda 1960; Öris 1973; Mauch and Asseily 1975; Roos 1995; Hurtta and others 2004; Kishihara and others 2004; Sakamoto and others 2006; Liu and others 2006). The critical difference between our conclusion regarding the role of thermal decomposition in sugar melting and others is that we propose that thermal decomposition is the kinetic process that is responsible for the loss of crystalline structure in
However, these thermal analysis experiments alone were not sufficient to unambiguously confirm the specific type of kinetic process. Thus, to identify the specific type of kinetic process responsible for the loss of crystalline structure a chemical analysis approach, specifically High Performance Liquid Chromatography (HPLC), was proposed. HPLC was selected since it is known to be a successful technique for separating, identifying, and quantifying sugars and their thermal decomposition components (Bonn 1985; Gomis and others 1991; Porretta 1992; Lo Coco and others 1994; Yuan and others 1996; Yuan and Chen 1999). Since the presence of tautomers in the case of glucose and fructose would complicate the HPLC analysis, sucrose was chosen for the further study because it does not form tautomers and it is also the most commonly used sugar in the food and pharmaceutical industries.

We hypothesize that if the loss of crystalline structure in sucrose is caused by thermal decomposition, initial decomposition components, specifically glucose, fructose, and 5-HMF, will be formed and detected by HPLC analysis, at the same temperature (in SDCS experiments) or time (in quasi-isothermal MDSC experiments) at which apparent melting begins. The term “apparent melting” was proposed in Chapter 3 to distinguish the loss of crystalline structure due to a kinetic process, such as thermal decomposition, from thermodynamic melting.

4.2.1. Sucrose Thermal Decomposition

Numerous publications have investigated the thermal decomposition of sucrose both in the presence (dilute and concentrated) and absence (anhydrous conditions) of an aqueous solution (Hirschmüller 1953; Gardiner 1966; Mauch 1971; Kelly and Brown 1978/79; Richards and Shafizadeh 1978; Richards 1986; Lowary and Richards 1988; Tomasik 1989; Kroh 1994; Eggleston and others 1996; Šimkovic and others 2003; Quintas and others 2007). A schematic sucrose, glucose, and fructose; not that thermal decomposition occurs in addition to thermodynamic melting. For additional details the interested reader is referred to Chapter 3.
overview of the thermal decomposition of sucrose under both conditions is shown in Figure 4.1. In the case of dilute or concentrated aqueous solutions the first step in sucrose thermal decomposition is the splitting of the glycosidic linkage between the glucose and fructose moieties (i.e., sucrose hydrolysis), via protonation of the oxygen atom of the glycosidic linkage. The hydrogen ion (H⁺) required for this step is provided by water. In the absence of an aqueous solution, the mechanism and decomposition components of sucrose thermal decomposition, as illustrated in Figure 4.1, are somewhat different. However, Šimkovic and others (2003), studying anhydrous sucrose using GC/MS, showed that the splitting of the glycosidic linkage was still the most prominent primary reaction of sucrose thermal decomposition. It was mentioned by Quintas and others (2007), based on the work of Richards (1986) and Lowary and Richards (1988), that the H⁺ required for sucrose hydrolysis could be derived from the dissociation of the sucrose molecule itself at high temperatures. In addition, we hypothesize that another possible source of the H⁺ is surface water on the sucrose crystals. Thus, regardless of the presence or absence of an aqueous solution, sucrose thermal decomposition primarily occurs via sucrose hydrolysis as shown in Figure 4.1.

Once sucrose is broken down into glucose [1] and fructose carbocation [1] via sucrose hydrolysis, glucose forms acidic and other decomposition components through further reactions (not shown in Figure 4.1). Fructose carbocation, due to its instability, immediately participates in subsequent, more complex reactions, resulting in the formation of various decomposition components, including anhydrofructose [2a] by cyclization; a wide range of products, such as 5-(hydroxymethyl)furfural (5-HMF) [2b], by non-specific degradation (e.g., condensation); oligosaccharides [2c] by combining with the hydroxyl oxygen of another saccharide (mostly sucrose); and fructose [2d] by accepting a hydroxyl ion (OH⁻) from water. These intermediate
products are produced through similar mechanisms in the presence and absence of an aqueous solution. However, in the absence of an aqueous solution, minor products such as anhydrous sucrose [3] and sucrose isomers [4] are also produced through minor reaction pathways.

Though sucrose thermal decomposition continues through a myriad of reaction pathways, in the present study it is important to measure only the initial decomposition components, such as glucose, fructose, and 5-HMF, formed in the early stages of sucrose thermal decomposition, because this study aims to elucidate whether the loss of crystalline structure in sucrose is caused by thermal decomposition, not to detect and quantify all possible sucrose decomposition components. Thus, the specific objective of the present study is to determine if glucose, fructose, and 5-HMF, the three selected thermal decomposition indicator components, are formed at the onset of the loss of crystalline structure in sucrose using HPLC analysis. Sucrose samples for HPLC analysis were prepared using both SDSC (fast heating method) and quasi-isothermal MDSC (slow heating method).

4.3. Materials and Methods

4.3.1. Materials

Crystalline sucrose (S0389, ≥ 99.5%), D-(-)-fructose (F2543, ≥ 99.5%), D-(+)-glucose (G7528, 99.5%), mannitol (M9546, ≥ 99.9%), and 5-(hydroxymethyl)furfural (5-HMF, W501808, ≥ 99%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and were used without further purification. HPLC grade water (Ricca Chemical Company, Arlington, TX, USA) was used for the preparation of standard and sample solutions. The water contents of the three sugars and mannitol were measured by coulometric Karl Fischer Titration with Hydranal
Coulomat AG as a solvent and were 0.004 % wb for sucrose, 0.048% wb for glucose, 0.033% wb for fructose, and 0.060% wb for mannitol.

4.3.2. Sample preparation

Sucrose, as an apparent melting material, and mannitol, as a thermodynamic melting comparison material, were individually heated using two methods; a standard DSC (SDSC) method, which is a fast heating method, and a quasi-isothermal MDSC method, which is a slow heating method. A DSC Q2000 (TA instruments, New Castle, DE), equipped with a Refrigerated Cooling System (RCS 90), was utilized for both methods.

4.3.2.1. SDSC: Fast heating method

Prior to sample preparation, enthalpy (cell constant) and temperature calibrations were done using indium (T_m onset of 156.6°C, ΔH of 28.71 J/g, Lot# A10R020, TA instruments, New Castle, DE) hermetically sealed in a Tzero aluminum DSC pan (PN 901683.90, TA instruments, New Castle, DE) and lid (PN309684.901, TA instruments, New Castle, DE). The same type of pans and lids were used for the calibrations and sample preparations. An empty pan was used as the reference. Dry nitrogen, at a flow rate of 50 mL/min, was used as the purge gas.

Sucrose (approximately 9.75 mg) was hermetically sealed in a pan. Three sample pans of sucrose were loaded in the DSC cell; one pan was placed on the sample platform and the others were on the bottom of the DSC cell. These three pans were heated at a heating rate of 10°C/min to six different final heating temperatures (termed target temperatures), corresponding to the following SDSC heat flow signal attributes shown in Figure 4.2: 1) before the appearance of any endothermic peaks (target temperature 100.0°C; sample temperature 98.7 ± 0.1°C), 2) the onset of the first endothermic peak (target temperature 151°C; sample temperature 149.6 ± 0.1°C), 3) at the end of the first endothermic peak (target temperature 160.0°C; sample temperature 158.6 ±
0.1°C), 4) the onset of the second endothermic peak (target temperature 186.0°C; sample temperature 184.1 ± 0.1°C), 5) the end of the second endothermic peak (target temperature 195.0°C; sample temperature 192.7 ± 0.2°C), and 6) 15°C above the completion of the second endothermic peak (target temperature 210.0°C; sample temperature 208.4 ± 0.2°C) and then immediately cooled at a heating rate of 50°C/min to 25°C. Because the sample temperature lags behind the furnace temperature when heating sample using DSC, the sample temperatures above are always slightly lower (approximately 1.6°C) than the target temperatures. In addition, a sample that was not heated at all was prepared and termed the “as is” sample. Images of the “as-is” and SDSC sucrose samples in DSC pans are shown in Table 4.2. Using the same procedure, three more sample pans of sucrose were prepared at each final heating temperature to make one batch of sucrose samples for HPLC analysis.

For each batch (a total of six sample pans), after heating in the DSC, the DSC lids were removed and each sample plus pan was weighed and then transferred into a small plastic bottle using 5 ml HPLC water. After the samples were completely dissolved in the HPLC water, they were filtered through a 0.22 μm Millex syringe filter (Millipore Corp., Temecula, CA) and kept in amber colored HPLC vials (National Scientific Company, Rockwood, TN) in a -70°C freezer until HPLC analysis. The DSC pans were removed from the plastic bottle, dried off, and reweighed (in case the pan weight had changed due to lid removal). The difference in weight between the dried pan and the sample plus pan before dissolving was used to calculate the sucrose concentration.

Mannitol samples (approximately 9.50 mg per pan) for HPLC analysis were prepared using the same procedures as for sucrose (10°C/min), except different final heating temperatures were used. Five different final heating temperatures (termed target temperatures), corresponding to the
following SDSC heat flow signal attributes shown in Figure 4.3, were used: 1) before the appearance of an endothermic peak (target temperature 158.0°C; sample temperature 156.7 ± 0.0°C), 2) the onset of the endothermic peak (target temperature 165.0°C; sample temperature 163.5 ± 0.0°C), 3) right after the peak temperature (target temperature 174.0°C; sample temperature 172.6 ± 0.0°C), 4) at the end of the endothermic peak (target temperature 182.0°C; sample temperature 180.6 ± 0.0°C), and 5) 8°C above the completion of the endothermic peak (target temperature 190.0°C; sample temperature 188.6 ± 0.0°C) and then immediately cooled at a heating rate of 50°C/min to 25°C. In addition, an “as is” mannitol sample with no heat treatment was prepared. Regardless of the preparation methods, e.g., melt-quenching and freeze-drying, partially or completely melted mannitol crystallized upon cooling to room temperature (Kim and others 1998; and Yu and others 1998); thus, no mannitol sample images were taken. The SDSC mannitol samples for HPLC analysis were prepared using the same procedure as detailed for SDSC sucrose.

Average T_m parameters for sucrose and mannitol were obtained from the complete SDSC thermogram, heated to 210.0°C for sucrose and 190.0°C for mannitol (Table 4.1). These T_m parameters are very similar to those reported in Chapter 3 (Table 3.3), for a smaller sample size (2.75 mg for sucrose and 2.00 mg for mannitol) compared to the larger small sizes (9.75 mg for sucrose and 9.5 mg for mannitol) used for HPLC sample preparation used herein.

**4.3.2.2. Quasi-isothermal MDSC: Slow heating method**

Prior to sample preparation, DSC and MDSC heat capacity calibrations were done using a 22.93 mg sapphire disk (PN 970370.901, TA instrument, New Castle, DE) hermetically sealed in a pan. MDSC heat capacity calibration was carried out using a modulation amplitude of ± 1.0°C and a period of 100 sec, which were the same values used for sucrose sample preparation. An
empty pan was used as the reference. Dry nitrogen, at a flow rate of 50 mL/min, was used as the purge gas.

As described in the SDSC method section, three sample pans of sucrose (approximately 9.75 mg per pan) were loaded in the DSC cell and isothermally held at 120.0°C for different times (i.e., 0, 10, 50, 100, 200, 250, 300, 350, 400, 450, 500, 1200, 2000, and 3100 min) with a modulation amplitude of ± 1.0°C and a period of 100 sec. The 120°C isothermal temperature was chosen based on the result of stepwise-quasi isothermal MDSC experiments for sucrose performed in Chapter 3, since at 120.0°C the Cp began to gradually increase (within the 30 min time step used in the experiment), indicating the onset of the loss of crystalline structure in sucrose. The isothermal time required to remove all crystalline structure in sucrose at 120.0°C, 3100 min, was determined by adding two standard deviations to the average isothermal time of triplicate measurements obtained via preliminary experiments (data not shown). The sucrose samples held at 120.0°C at the different times were immediately cooled to 25°C at a heating rate of 50°C/min in the DSC. Images of the “as-is” and quasi-isothermal MDSC sucrose samples in DSC pans are shown in Table 4.2. The MDSC sucrose samples for HPLC analysis were prepared using the same procedure as detailed for SDSC sucrose.

Since mannitol is a thermodynamic melting material, it melts over a very narrow temperature range and thus different quasi-isothermal MDSC experimental conditions were required for mannitol sample preparation compared to sucrose: a modulation amplitude of ± 0.5°C and a period of 120 sec, a gas flow rate of 1 ml/min, and use of an internal lid (PN 901671.901, TA instrument, New Castle, DE) in addition to a Tzero hermetic pans and lids. Thus, all calibrations for enthalpy, temperature, and DSC and MDSC heat capacity were carried out using these experimental conditions prior to mannitol sample preparation. An empty pan with an
internal lid was used as the reference for all calibration and mannitol sample preparation. Dry nitrogen, at a flow rate of 1 mL/min, was used as the purge gas. Enthalpy and temperature calibrations were done using indium and DSC and MDSC (modulation amplitude of ± 0.5°C and a period of 120 sec) heat capacity calibrations were done using a sapphire disk. All calibration standards were hermetically sealed in a DSC pan with an internal lid.

Mannitol (approximately 9.50 mg per pan) was hermetically sealed in a pan with an internal lid. Three sample pans of mannitol were loaded in the DSC cell as described above for sucrose. These pans were isothermally held at 159.9°C for 5555 min, using a modulation amplitude of ± 0.5°C and a period of 120 sec, and were immediately cooled to 25°C at a heating rate of 50°C/min in the DSC. The isothermal temperature, 159.9°C, was also selected based on the result of stepwise-quasi isothermal MDSC experiments for mannitol performed in Chapter 3. The isothermal time required to remove all crystalline structure in mannitol at 159.9°C, 5555 min, was determined by adding two standard deviations to the average isothermal time of triplicate measurements obtained through preliminary experiments (data not shown). The MDSC mannitol samples for HPLC analysis were prepared using the same procedure as detailed for SDSC sucrose.

4.3.3. HPLC analysis

The chromatographic analysis was conducted using a Waters 2695 Alliance High-Performance Liquid Chromatography (HPLC) system (Waters, Milford, MA), equipped with a Hewlett-Packard interface 35900E A/A converter. The analytical column was an Aminex HPX-87C calcium form cation exchange resin-based column (300 x 7.8 mm) packed with sulfonated divinyl benzene-styrene copolymer with a particle size of 9 μm (Bio-Rad Lab., Richmond, CA, USA). The guard column was a Carbo-C Refill cartridge (30 x 4.6 mm) (Bio-Rad Lab.,
Richmond, CA). HPLC grade water (Ricca Chemical Company, Arlington, TX) was used for the mobile phase. The analytical column temperature was maintained at 85°C and the guard column at 30°C. The flow rate was set to 0.6 mL/min. All samples were injected into the HPLC system using a 20 μl loop injector.

A Waters 410 refractive index (RI) detector (Waters, Milford, MA) was connected to a Hewlett Packard Series 1050 photodiode array (PDA) detector (Hewlett Packard, Palo Alto, CA) for the sucrose samples. While sucrose, glucose, and fructose were determined using the RI detector, 5-HMF was simultaneously measured using the PDA detector at a wavelength of 284 nm. A Waters 410 refractive index (RI) detector was used for the mannitol samples. Chromatographic peaks were identified by comparing retention times and spectra to those of known standard solutions. A mixed standard solution, containing sucrose, glucose, fructose, and 5-HMF, was used for HPLC analysis of all sucrose samples. A mannitol standard solution was used for the HPLC analysis of all mannitol samples. All computations were performed using an Agilent ChemStation (ChemStation for LC 3D Rev A. 08. 03, Agilent Technologies, Inc., Santa Clara, CA). HPLC analysis was done in duplicate for each batch of sucrose and mannitol samples.

Sucrose and mannitol results were displayed as the average % ratio of sucrose remaining (g/L) to the total sample concentration (g/L). Glucose, fructose, and 5-HMF results were displayed as the average % ratio of the decomposition component concentration (g/L) formed during the loss of crystalline structure to the total sample concentration (g/L). The limit of quantification (LOQ) of the HPLC analysis was 0.044 g/L for glucose, 0.010 g/L for fructose, and 0.001 g/L for 5-HMF.
4.4. Results and Discussion

4.4.1. SDSC: Fast heating method

Figure 4.2 shows the SDSC heat flow signal for sucrose, the percent glucose, fructose, and 5-HMF (the three selected thermal decomposition indicator components) formed in the sample, and the percent sucrose remaining in the sample during the loss of crystalline structure when heating sucrose at 10°C/min. As observed in the heat flow signal of Figure 4.2, sucrose exhibited two endothermic peaks\(^9\) during the loss of crystalline structure. Table 4.1 contains the corresponding sucrose melting parameters for both the small and large endothermic peaks. In addition, images of the “as is” and heated sucrose samples in DSC pans are shown in Table 4.2.

As can be observed in Figure 4.2, no thermal decomposition indicator compounds were detected via HPLC analysis for either the “as is” or the 98.7°C sample (target temperature 100°C). However, concomitant with the \(T_{\text{m onset}}\) of the 1\(^{\text{st}}\) endothermic peak (sample temperature 149.6°C ± 0.1°C; target temperature 151.0°C), a small amount of glucose (0.365%) and 5-HMF (0.003%) were detected in the sample via HPLC analysis (Figure 4.2 and Appendix G). However, no obvious changes in crystal appearance were noted until the 158.6°C sample temperature (target temperature 160.0°C), where very slight yellowing was observed (Table 4.2). The amount of glucose and 5-HMF increased gradually, until the \(T_{\text{m onset}}\) of the 2\(^{\text{nd}}\) endothermic peak (sample temperature 184.1 ± 0.1°C; target temperature 186.0°C), where these indicator compounds began to increase more substantially and the change in crystal appearance and color became more obvious (Table 4.2).

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\(^9\) The number of endothermic peaks obtained for sucrose varies in the literature from one to three, with one peak often being obtained when analyzing commercial sources (e.g., Domino, C&H, and United Sugar), two peaks for analytical grade sucrose (e.g., Sigma and Fischer), and three peaks for laboratory-recrystallized sucrose (e.g., using methanol or ethanol). The difference in the number of peaks between different sucrose sources is currently under study in our laboratory.
Also at \( T_{m, \text{onset}} \) of the 2\textsuperscript{nd} endothermic peak (sample temperature 184.1 ± 0.1°C; target temperature 186.0°C), fructose (0.218%) was first detected (Figure 4.2 and Appendix G). The later detection of fructose compared to glucose and 5-HMF was discussed by Mauch (1971) and attributed to the lack of free water\textsuperscript{10}, which is required to form fructose from fructose carbocation, until the occurrence of condensation reactions. In addition to the formation of these indicator components, a number of unidentified HPLC peaks began to be observed in the sucrose samples at a sample temperature of 158.6°C (target temperature 160.0°C). These unidentified peaks indicate the formation of a number of diverse decomposition components, most likely produced from fructose carbocation via further reactions, as shown in Figure 4.1. At a sample temperature of 184.1°C (target temperature 186.0°C) and beyond, the amount and diversity of these additional decomposition components increased rapidly as evidenced by the large number and size of unidentified HPLC peaks and the corresponding large decrease in %sucrose. It is interesting to note that despite the considerable decrease in sucrose content at sample temperatures of 192.7°C (target temperature 195.0°C) (61.87%) and 208.4°C (target temperature 210.0°C) (27.82%), the samples were only light and medium yellow in color, respectively (Table 4.2 and Appendix G).

These results indicate that thermal decomposition causes the loss of crystalline structure in sucrose, and the observed endothermic peak measured by SDSC is due to the energy of amorphization (the energy difference between the crystalline and amorphous phases, at a specific temperature) plus the energy associated with thermal decomposition. As proposed in Chapter 3,

\textsuperscript{10} The minute quantity of surface water may also be a source of free water for fructose formation, but, more than likely, it is used primarily for the initial sucrose hydrolysis reaction. In addition, if surface water were available for fructose formation, the fructose would probably have been detected earlier.
the loss of crystalline structure caused by a kinetic process (thermal decomposition herein) is termed apparent melting in order to distinguish it from thermodynamic melting.

Unlike sucrose, no decomposition components were observed during the loss of crystalline structure in mannitol by heating at 10°C/min using SDSC method (Figure 4.3). Although the mannitol used was of very high purity (99.9%), an unknown peak in the HPLC chromatographs was observed at a retention time of 22 min (data not shown), which was identified as sorbitol. The percent sorbitol peak area, based on the total area, was the same for all samples (0.95 ± 0.01), including the “as is” mannitol sample (Appendix H). Hence, sorbitol was found to be a minor impurity in the mannitol sample, but in the case of SDSC, was not a thermal decomposition component.

Even at a sample temperature of 188.6°C (target temperature 190.0°C), which is much higher than the temperature at which crystalline structure is completely removed in mannitol, no decomposition components were detected. This result shows that the loss of crystalline structure in mannitol is accomplished via thermodynamic melting. Furthermore, it confirms that, as suggested in Chapter 3, the very small weight loss for mannitol observed in the proximity of its melting temperature is due to sublimation, not thermal decomposition.

4.4.2. Quasi-isothermal MDSC: Slow heating method

Figure 4.4 shows the $C_p$ signal for sucrose, the percent glucose, fructose, and 5-HMF (the three selected thermal decomposition indicator components) formed in the sample, and the percent sucrose remaining in the sample during the loss of crystalline structure when holding the sucrose at 120.0°C using the quasi-isothermal MDSC method. A holding time of 3100 min was required to completely remove the crystalline structure in sucrose at 120.0°C.
As can be observed in Figure 4.4B, no thermal decomposition indicator compounds were detected via HPLC analysis for either the “as is” or the 10 min sample. However, at a holding time of 50 min, where the $C_p$ began to increase (indicating the onset of the loss of crystalline structure), small amounts of glucose (0.411%) and 5-HMF (0.003%) were detected. This result confirms that the loss of crystalline structures in sucrose is due to thermal decomposition. Since thermal decomposition is a kinetic process, the loss of crystalline structure in sucrose can take place even at 120.0°C, which is far below the reported melting temperature for sucrose (approximately 169 to 192°C as shown in Table 3.1 in Chapter 3), if the holding time is sufficiently long.

Others have mentioned the possibility of sucrose decomposition at temperatures below its melting temperature. Hirschmüller (1953) stated, “At temperatures below the melting point, the decomposition of sucrose is slow.” Sakamoto and others (2006) reported, using HPLC that a commercial granulated sucrose turned to a light brown color along with the formation of glucose and fructose after holding for 48 hrs at 100°C. However, no complete theoretical framework was available to explain how this low temperature decomposition was possible, until the studies herein (Chapters 3 and 4).

Similar to the SDSC results discussed above, fructose was not detected until a holding time of 200 min, which was later than the detection of glucose and 5-HMF (Figure 4.4). Peaks corresponding to unidentified decomposition components began to appear at a holding time of 200 min. Up to the holding time of 1200 min, the $C_p$ increased relatively slowly. Sucrose samples at holding times of 500 and 1200 mins, were visually intact (i.e., retained their outer crystalline shape), but had slightly yellowed (Table 4.2). After a holding time of 1200 min, a considerable increase in $C_p$ was observed and glucose, fructose, and 5-HMF production
increased. At a holding time of 2000 min, sucrose crystals had turned a deep yellow color and had partially adhered to each other (Table 4.2). At a holding time of 3100 min, the time at which all crystalline structure was removed, the sucrose sample turned a dark brown color and only 24.043% sucrose content remained in the sample (Appendix G).

Unlike mannitol samples prepared by the fast heating (SDSC) method, mannitol samples prepared by the slow heating (quasi-isothermal MDSC) method (Figure 4.5) exhibited a small, but measurable decrease in % mannitol content, a 2.47% decrease after 5555 min at a holding temperature of 159.9°C (Appendix H). In addition, after 5555 min at 159.9°C, trace amounts of two unidentified decomposition components were also observed in mannitol samples. The amount of these two unidentified decomposition components was only 1.49%, calculated based on the % ratio of unidentified components peak area to total peak area. This small value of unidentified decomposition components in the case of mannitol is negligible and as such supports that mannitol is a thermodynamic melting material. The formation of unidentified decomposition components in the mannitol samples is not surprising since it was held for 5555 min at 159.9°C, which is only 5.5°C lower than its $T_m$ onset of 165.4°C (Table 4.1). In comparison, in the case of the slow heating method for sucrose only 24.043% sucrose remained after being held for 3100 min at 120.0°C, which was 30.5°C lower than its $T_m$ onset for the 1st endothermic peak of 150.5°C (Table 4.1). Therefore, based on this comparison, it is very unlikely that the loss of crystalline structure in mannitol is caused by thermal decomposition.

### 4.4.3. Comparison of fast (SDSC) and slow (quasi-isothermal MDSC) heating methods

Compared to the fast heating (SDSC) method, complete loss of crystalline structure occurred more slowly in the slow heating (quasi-isothermal MDSC) method. Since thermal decomposition is a kinetic (time-dependent) process, we hypothesize that the loss of crystalline structure in
sucrose is a time-temperature combination process. That is, the loss of crystalline structure in sucrose occurs more quickly at higher temperatures (shorter times) compared to lower temperatures (longer times); however, the degree of thermal decomposition (measured as the amount of sucrose remaining as well as the amount of thermal decomposition components produced) is more extensive at lower temperatures. As observed in the present study, upon complete loss of crystalline structure in the fast heating method (SDSC; sample temperature of 192.7°C), the sucrose content decreased to 61.871% and the glucose, fructose, and 5-HMF formed were 14.088%, 0.882%, and 0.053%, respectively (Appendix G). Whereas, in the slow heating method (quasi-isothermal MDSC, 120.0°C for 3100 mins), the sucrose content decreased to 24.043% and the glucose, fructose, and 5-HMF formed were 30.322%, 6.499%, and 1.043%, respectively (Appendix G). In addition, the amount and the diversity of unidentified decomposition components were greater in the slow heating method, compared to the fast heating method.

An interesting observation is the dramatic decrease in sucrose content between the sample heated to 192.7°C (the temperature for the complete loss of crystalline structure) and the sample heated to 208.4°C using the fast heating (SDSC) method (Figure 4.2 and Table 4.2). During this change in temperature, which took only 1.6 min, the sucrose content decreased from 61.871% to 27.819%, nearly equal to the sucrose content (24.043%) in the slow heating (quasi-isothermal MDSC) method (3100 min, the holding time for the complete loss of crystalline structure at 120.0°C). In addition, the amount of indicator components in the sample heated to 208.4°C (33.167% glucose, 6.082% fructose, and 0.627% 5-HMF) also became similar to the slow heating (quasi-isothermal MDSC) method (values given above). Despite these component
content similarities, the 208.4°C sample was only medium yellow in color, while the 120.0°C for 3100 min sample was deep brown in color (Table 4.2).

This dissimilarity in color was attributed to the difference in the diversity of thermal decomposition components between the two heating conditions. Based on the HPLC data, more unidentified peaks with larger peak areas, which indicate the formation of a large amount and wide variety of decomposition components, were observed in the 120.0°C for 3100 min sample than in the 208.4°C sample. The more diverse thermal decomposition components formed in the 120.0°C for 3100 min sample were themselves subjected to further, more advanced stages of the thermal decomposition reaction scheme, yielding complex color producing components. Whereas, the 208.4°C sample, even though it contained a similar amount of indicator components (detailed above), had fewer and smaller unidentified peaks compared to the 120.0°C for 3100 min sample, indicating that less advanced thermal decomposition reactions had occurred. These results further support that the slow heating method (longer heating time at a lower temperature) causes more severe thermal decomposition (measured as the amount of thermal decomposition components produced, not as the amount of sucrose remaining) than the fast heating method (faster heating rate to a higher temperature).

These results provide new insights into the caramelization process. Caramelization has generally been thought of as a complex series of reactions occurring under higher temperature conditions and involving degradation of sugar molecules and polymerization of the reaction intermediates and reactants (Coultate 1996; Jiang and others 2008). However, based on the research reported herein, since the loss of crystalline structure in sucrose occurs via a time-temperature combination process, caramelization does not have to be carried out under higher temperature conditions, but can also be carried out under lower temperature, albeit longer time,
conditions. Thus, the desired degree of caramelization required for producing a specific final product can be controlled by selecting appropriate time-temperature conditions. Therefore, from a practical viewpoint caramelization of sucrose can simply be defined as browning of sucrose (or other apparent melting sugar) by applying heat for a length of time. In turn, the conversion of crystalline sucrose to amorphous sucrose by applying heat for a length of time (i.e., apparent melting) can be thought of as “controlled caramelization.”

These results further suggest that, since the applied heating conditions determine the amounts and types of thermal decomposition components, apparent melting of sucrose influences several important final product characters, such as sweet taste, flavor, color, texture, and shelf-life. For example, in regards to flavor, the applied heating conditions could be optimized so as to maximize the key caramel flavor compounds. Another example, in regards to shelf-life, is the stability of amorphous sucrose prepared by melt-quenching\textsuperscript{11} (heating followed by quick cooling), where the decomposition components formed during heating will have a significant impact on the resultant glass transition temperature, a critical factor determining the physicochemical stability of amorphous materials. Therefore, the elucidation of the cause of the loss of crystalline structure in sucrose is very important from both a theoretical and practical viewpoint.

4.5. Conclusions

Regardless of the heating method employed, the formation of initial decomposition components was concomitant with the loss of crystalline structure in sucrose. In sucrose samples

\textsuperscript{11} It is important to point out that in the case of preparing amorphous sucrose by melt-quenching it is probably not necessary to quench cool the sample to keep the sucrose from crystallizing, since the formation of thermal decomposition components formed during melting serve to retard crystallizing.
prepared using the fast heating (SDSC) method, glucose and 5-HMF were observed immediately after the $T_{m\text{onset}}$ of the 1st endothermic peak, at a sample temperature 149.6 ± 0.1°C (target temperature 151.0°C). In sucrose samples prepared using the slow heating (quasi-isothermal MDSC) method, glucose and 5-HMF were detected at the holding time of 50 min (at 120.0°C), where the $C_p$ began to increase, signaling the onset of the loss of crystalline structure. In addition to glucose and 5-HMF, a variety of unidentified thermal decomposition components were continuously formed even before all crystalline structure was removed in sucrose. These results confirm our hypothesis that the loss of crystalline structure in sucrose is caused by thermal decomposition; thus, sucrose does not go through thermodynamic melting, but rather apparent melting.

In addition, a higher amount and more diverse thermal decomposition components were observed in the slow heating (quasi-isothermal MDSC) method, compared to the fast heating (SDSC) method. This result suggests that the loss of crystalline structure in sucrose is a time-temperature combination process. That is, longer times are required for the loss of crystalline structure in sucrose at lower temperatures, whereas, shorter times are required at higher temperatures. This knowledge that the loss of crystalline structure in sucrose is caused by the time-temperature thermal decomposition process is significance because melting is not only a common property used in sugar characterization, but also a general method used to prepare amorphous sugar, which is widely used as a key ingredient in the food and pharmaceutical industries. Consequently, heating conditions employed can affect the final properties of sugar-based products, such as the product’s flavor and glass transition temperature.

In addition, this research provides new insights into the caramelization process. Caramelization has generally been thought of as a complex series of reactions occurring under
higher temperature conditions. However, based on the research reported herein, caramalization can occur under lower temperature, albeit longer time, conditions. Therefore, the caramalization reaction can be controlled via selection of appropriate heating conditions from a wide range of time-temperature combinations.

Lastly, besides sucrose, the melting parameters of other materials, both organic (e.g., glucose and fructose [Chapter 3, Figure 3.4] and acetylsalicylic acid) and inorganic (e.g., silicon), have been shown to exhibit a heating rate dependency. Thus, additional fundamental studies are needed to investigate the possibility of apparent melting in these materials, as well as the resultant consequences to the properties of the finish products.
4.6. Figures and Tables

Figure 4.1 The predominant mechanism of sucrose thermal decomposition in the presence of solvent (dash arrow) and in the absence of solvent (solid arrow) (synthesized from Mauch 1971; Richard 1986; Šimkovic and others 2003; Quintas and others 2007).
Figure 4.2 SDSC heat flow scan (at 10°C/min) and HPLC results for sucrose and indicator thermal decomposition components: (A) Full temperature scale, (B) High temperature region for sucrose and glucose, and (C) High temperature region for fructose and 5-HMF.
Figure 4.3 SDSC heat flow scan (at 10°C/min) and HPLC results for mannitol: (A) Full temperature scale and (B) High temperature region.
Figure 4.4. $C_p$ obtained via quasi-isothermal MDSC (at 120.0°C for 3100 mins) and HPLC analysis for sucrose and indicator thermal decomposition components: (A) Full time scale, (B) Short time region for sucrose and glucose, and (C) Short time region for fructose and 5-HMF.
Figure 4.5 $C_p$ obtained via quasi-isothermal MDSC (at 159.9°C for 5555 mins) and HPLC results for mannitol.
Table 4.1 $T_m$ parameters ($T_{m\text{ onset}}$, $T_{m\text{ peak}}$, and $\Delta H$) for sucrose and mannitol scanned at a heating rate of 10°C/min up to a final temperature of 210.0°C for sucrose and 190.0°C for mannitol using a SDSC (**N = 6 and n = 3***).

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<th>Small endothermic peak (1st)</th>
<th>Large endothermic peak (2nd)</th>
<th>Total</th>
</tr>
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<tr>
<td>$T_{m\text{ onset}}$ (°C)</td>
<td>$T_{m\text{ onset}}$ (°C)</td>
<td>$\Delta H$ (°C)</td>
</tr>
<tr>
<td>150.54 ± 0.34</td>
<td>157.34 ± 0.42</td>
<td>7.03 ± 1.23</td>
</tr>
<tr>
<td>Mannitol</td>
<td></td>
<td></td>
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</table>

* Sigmoidal tangent baseline was used for crystalline sucrose and mannitol using the UA software.
** The average values above were obtained by measuring samples in six replicate and by analyzing the $T_m$ parameters for each sample measurement in triplicate.
<table>
<thead>
<tr>
<th>Sample temp. (°C)</th>
<th>Target temp. (°C)</th>
<th>Images</th>
<th>Holding time (min)</th>
<th>Target temp. (°C)</th>
<th>Images</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>“as is”</td>
<td><img src="image2.png" alt="Image" /></td>
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<td>100.0</td>
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<td>120.0</td>
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</tr>
<tr>
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<td>151.0</td>
<td><img src="image5.png" alt="Image" /></td>
<td>1200</td>
<td>120.0</td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
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<td>2000</td>
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<td>120.0</td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
<tr>
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<td>210.0</td>
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</table>

Table 4.2 Images of “as is” and thermal treated sucrose samples.
4.7. References


5.1. Abstract

The loss of crystalline structure in sucrose, glucose, and fructose has been shown to be due to the kinetic process of thermal decomposition (termed apparent melting), rather than thermodynamic melting (Chapters 3 and 4). Thus, the purpose of this research was to investigate whether or not it is possible to scan fast enough to suppress the kinetic process of thermal decomposition and reach the thermodynamic melting temperature of these sugars using a new rapid-scanning DSC. Indium, a thermodynamic melting material, and sucrose, glucose, and fructose were analyzed at three heating rates from 1 to 25°C/min using Standard DSC and at seven heating rates from 50 to 2000°C/min using rapid-scanning DSC. Thermodynamic melting was achieved when the onset temperature ($T_{m\text{ onset}}$) of the endothermic peak leveled off to a constant value independent of heating rate. The $T_{m\text{ onset}}$ for indium was constant ($156.74 \pm 0.42°C$) at all heating rates. Whereas, the $T_{m\text{ onset}}$ for fructose increased considerably until a heating rate of approximately 698°C/min, after which the average $T_{m\text{ onset}}$ for the remaining three heating rates was constant at $135.83 \pm 1.14°C$. Thus, we propose $135.83°C$ to be the thermodynamic melting temperature ($T_{m\text{ onset}}$) of fructose. However, for sucrose and glucose, thermodynamic melting temperatures were not able to be obtained, since the upper limit heating rate used was not fast enough to inhibit thermal decomposition and achieve thermodynamic melting, perhaps due to the higher apparent $T_{m\text{ onset}}$ of sucrose and glucose compared to fructose. This research suggests the possibility of using the new fast-scanning DSC for measuring thermal properties of materials that exhibit similar thermal behavior to the sugars studied herein, without interference from kinetic processes.
5.2. Introduction

Differential scanning calorimetry (DSC) has been extensively used for material characterization in a wide variety of research areas, including food science, pharmaceutics, material science, (bio)chemistry, and physics due to a number of significant advantages, such as ease of sample preparation, applicability to solid and liquid samples, fast analysis time, and a broad temperature range (Verdonck and others 1999). Because of its many advantages, DSC is the most frequently used thermal analysis technique, accounting for probably 70% of all thermal analysis measurements (Thomas and Schmidt 2010). DSC provides both quantitative and qualitative thermal/physical material property information\(^\text{12}\) (e.g., phase transition, glass transition, cold crystallization, polymorphism, and purity) as a function of time and temperature.

Because of a number of material characterization challenges, a new DSC technique, which performs at much faster heating and cooling rates than attainable with a conventional DSC, has recently received a great deal of attention (Danley and others 2008). This new faster heating and cooling DSC technique is currently referred to by a variety of names in the literature, including high-speed DSC (Pijers and others 2002; McGregor and others 2004; Saunders and others 2004; Lappalainen and others 2006), high performance DSC (Poel and Mathot 2007), high-sensitivity, high-speed DSC (Gabbott and others 2003), rapid-scanning DSC (Danley and others 2008), and fast scan DSC (Ye and others 2009).

As demonstrated in the literature, this new rapid-scanning DSC technique has a number of exciting applications. Faster scanning rates greatly increase heat flow sensitivity (signal height) for small transitions, since the same amount of energy (the DSC output, mJ/sec) is released (or adsorbed) over a much shorter time, although this benefit is somewhat tempered by the

\(^{12}\) Since every change in structure (transition) either absorbs or releases heat, DSC is the universal detector for measuring structural changes.
requirement of using small sample sizes to attain the fast scanning rates. For example, a number of publications have shown the possibility of detecting and quantifying low levels of amorphous content in lactose (Gabbott and others 2003; Saunders and others 2004), maltitol (Hurtta and Pitkänen 2004), and sucrose (Lappalainen and others 2006), using rapid-scanning DSC.

An application of importance to the present research is the use of rapid-scanning DSC for studies that require suppression of time-dependent (kinetic) transitions (e.g., polymeric conversion [Poel and Mathot 2007; Danley and others 2008; Buanz and Gaisford 2009; Yang and others 2009], recrystallization [McGregor and others 2004; Mathot and others 2006; Poel and Mathot 2007; Abdulkarim and Ghazali 2007; Miltner and others 2008; Van Assche and others 2009], or thermal decomposition [Wurm and others 2009]) for accurate material characterization. For example, Wurm and others (2009) reported reaching the melting temperature of fibrous silk protein without interference from thermal decomposition using rapid-scan DSC (300K/min scanning rate), whereas using conventional DSC (10K/min) resulted in thermal decomposition before melting.

In the previous chapters (Chapter 3 and 4), it has been shown that the loss of crystalline structure in sucrose, glucose, and fructose is due to the kinetic process of thermal decomposition (termed apparent melting), rather than thermodynamic melting. Thus, the purpose of this research was to investigate whether or not it is possible to scan fast enough to suppress the kinetic process of thermal decomposition and reach the thermodynamic melting temperature of these sugars using rapid-scanning DSC.

5.3. Materials and Methods

5.3.1. Materials
Crystalline sucrose (S0389, ≥ 99.5%), D-(+)-glucose (G7528, 99.5%), and D-(−)-fructose (F2543, ≥ 99.5%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). These analytical reagent grade samples were used without further purification and stored under room conditions in their original containers, wrapped tightly with parafilm after opening.

5.3.2. Standard DSC (SDSC) measurements

Sample measurements with relative slow heating rates (1 to 25°C/min) were performed using a DSC Q2000 (TA instruments, New Castle, DE), equipped with a Refrigerated Cooling System (RCS 90). Prior to sample measurements, the calibration for enthalpy (cell constant) and temperature was completed using indium (T(onset) of 156.6°C and ΔH of 28.71 J/g). Hermetic aluminum Tzero pans (PN 901683.90, TA instruments, New Castle, DE) and lids (PN309684.901, TA instruments, New Castle, DE) were used for calibration and all sample measurements. An empty pan was used as the reference and dry nitrogen, at a flow rate of 50 mL/min, was used as the purge gas. Sucrose, glucose, and fructose (approximately 2.75 mg) sealed in hermetic pans were equilibrated at 25°C and then heated at heating rates of 2, 5, and 10°C/min to temperatures after completion of the endothermic peak (220°C for sucrose, 195°C glucose, and 180°C fructose). Measurements for each heating rate were done in duplicate. Indium also was heated at heating rates of 1, 5, and 25°C/min in order to compare with the T(onset) for the sugars as a function of heating rate.

5.3.3. Rapid-scanning DSC measurements

The rapid-scanning DSC used in the present study was developed by TA instruments (New Castle, DE), known as project RHC (Rapid Heating and Cooling). The rapid-scanning DSC has a liquid nitrogen cooling system (LNCS), which ranges in temperature from -180°C to 700°C. The unique features of the rapid-scanning DSC (i.e., use of a small transducer, which is surrounded
by an infrared furnace system instead of a resistance furnace system, and a very small pan and sample size, typically 20-100 μg) make it possible to achieve extremely fast scan rates (up to 2000°C/min).

Prior to sample measurements, initial temperature calibration was carried out using indium at a heating rate of 500°C/min. In order to insure the reliability of the rapid-scanning DSC temperature data, indium was heated at the same heating rates to be used in subsequent sample measurements. The heating rates employed were 50, 100, 250, 500, 1000, 1500, and 2000°C/min. Overall the heating rates, the standard deviation in T_{m, onset} for indium was ± 0.42°C. For temperature calibration and all sample measurements, rapid-scanning DSC aluminum pans with crimped lids were used and an empty pan as the reference. Sample measurements were begun at room temperature and heated at the rates specified below to just after peak temperatures appeared. Dry nitrogen, at a flow rate of 25 mL/min, was used as the purge gas. In addition to measuring indium alone, indium was added to the fructose sample as an internal temperature standard to insure the reliability of the rapid-scanning DSC temperature data. A 1:2 weight ratio (w/w) of indium to crystalline fructose was analyzed at the same heating rates as indium alone. For fructose (approximately 100 μg), the rapid-scanning DSC measurements were done in duplicate over the heating rate range of 50 to 2000°C/min, except for the heating rates of 50, 100, and 2000°C/min, which were done only once, due to limited use of the new rapid-scanning DCS. For sucrose and glucose (approximately 100 μg), the rapid-scanning DSC measurements were performed in duplicate at the heating rates of 250, 500, 1000, and 1500°C/min. Experiments at the highest heating rate (2000°C/min) were attempted for sucrose and glucose, however, the rapid-scanning DSC was not able to maintain the 2000°C/min at the higher temperatures required for loss of crystalline structure in sucrose and glucose compared to fructose, due to the fact that
at higher temperatures there is more heat loss to the environment around the furnace. TA Instruments is currently working to increase the robustness of high heating rates in the high temperature range.

5.3.4. Determination of thermodynamic melting temperatures

The Universal Analysis (UA) software (TA instruments, New Castle, DE) was used to analyze the \( T_{m \text{ onset}} \) values for all sample measurements obtained using standard DSC and rapid-scanning DSC. The \( T_{m \text{ onset}} \) values were plotted against heating rate. Thermodynamic melting temperature (\( T_{m \text{ onset}} \)) was determined as the temperature where \( T_{m \text{ onset}} \) leveled off to a constant value regardless of heating rate. The heating rate at the \( T_{m \text{ onset}} \) was decided as the minimum heating rate required for reaching thermodynamic melting.

Statistical analyses were carried out using SAS software (SAS Institute Inc., Cary, NC). For the three sugars and the mixture of fructose and indium, the general linear model (GLM) procedure was utilized for the analysis of variance. The Tukey’s studentized range test was used to determine any significant difference between means at a \( p=0.05 \). For indium, single-sample t test at a \( p=0.05 \) was used to compare the \( T_{m \text{ onset}} \) for indium to the population mean (156.60\(^\circ\)C).

5.4. Results and Discussion

\( T_{m \text{ onset}} \) for sucrose, glucose, fructose, mixture of fructose and indium, and indium as a function of heating rate are given in Table 5.1 and plotted in Figure 5.1 (except for the mixture since these data would directly overlap with the individual indium and fructose data). As expected, since indium is a thermodynamic melting material, the \( T_{m \text{ onset}} \) values for indium remained relatively constant (156.74 ± 0.42\(^\circ\)C) over the entire heating rate range employed and were not significantly different from the population mean (156.60\(^\circ\)C) at a \( p=0.05 \). In contrast, the
sugars exhibited an increase in T_{m\:onset} as a function of heating rate. For sucrose and glucose, this heating rate dependency continued over the entire heating rate range, whereas for fructose the T_{m\:onset} significantly increased with heating rate until 1000^\circ C/min, after which T_{m\:onset} remained relatively constant at 135.83 \pm 1.14^\circ C for the remaining three heating rates. Thus, we propose 135.83^\circ C to be the thermodynamic melting temperature (T_{m\:onset}) of fructose. By extrapolation, the minimum heating rate required to reach the thermodynamic melting (heating rate independent T_{m\:onset}) of fructose was 698^\circ C/min. The calculated fructose T_{m\:onset} at 698^\circ C/min was 135^\circ C. The mixture of fructose and indium exhibited very similar T_{m\:onset} values as those for fructose and indium alone, respectively (Table 5.1).

Unlike fructose, the thermodynamic melting temperatures for both sucrose and glucose were not attained. Possibly, the 1500^\circ C/min upper limit heating rate was not fast enough to suppress the kinetic process, i.e., thermal decomposition, and achieve thermodynamic melting, because of the higher apparent T_{m\:onset} of sucrose and glucose compared to fructose.

5.5. Conclusions

This study demonstrates the ability of the rapid-scanning DSC to determine the thermodynamic melting temperature for fructose by eliminating the kinetic-based interference of thermal decomposition. Accurate determination and interpretation of material properties are critical to process and product development as well as quality improvement. Therefore, the new rapid-scanning DSC will be of great benefit to the food, pharmaceutical, and material industries for investigating materials that exhibit complicated thermal behaviors, such as the sugars studies herein.
5.6. Figures and Tables

Figure 5.1 $T_{m \text{ onset}}$ values and standard deviation error bars for sucrose, glucose, fructose, and indium as a function of heating rate. Empty symbols indicate apparent melting and solid symbols indicate thermodynamic melting.

![Diagram](image-url)
Table 5.1 $T_{m\text{ onset}}$ values and standard deviations for sucrose, glucose, fructose, mixture of fructose and indium, and indium as a function of heating rate using rapid-scanning DSC.

<table>
<thead>
<tr>
<th>Heating rate (°C/min)</th>
<th>$T_{m\text{ onset}}$ (°C)</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Fructose in the mixture of fructose and indium*</th>
<th>Indium***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>156.45</td>
</tr>
<tr>
<td>2</td>
<td>138.01 ± 0.12f</td>
<td>150.89 ± 0.08f</td>
<td>107.25 ± 1.05f</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>145.15 ± 0.49f</td>
<td>155.11 ± 0.10f</td>
<td>110.45 ± 0.30e</td>
<td>-</td>
<td>-</td>
<td>156.60</td>
</tr>
<tr>
<td>10</td>
<td>150.97 ± 0.51c</td>
<td>158.40 ± 0.14e</td>
<td>113.59 ± 1.51d</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>157.20</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>114.30 ± 0.13e</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>116.77 ± 0.08cd*</td>
<td>117.44 ± 0.10d</td>
<td>155.70</td>
</tr>
<tr>
<td>250</td>
<td>186.09 ± 0.56d</td>
<td>172.84 ± 0.27d</td>
<td>118.56 ± 0.04e</td>
<td>118.38 ± 0.15c</td>
<td>156.82</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>193.01 ± 1.29c</td>
<td>175.23 ± 0.12c</td>
<td>127.91 ± 3.97b</td>
<td>128.04 ± 0.05b</td>
<td>156.68</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>196.70 ± 0.20b</td>
<td>180.66 ± 1.06b</td>
<td>135.02 ± 1.10a</td>
<td>135.86 ± 0.05a</td>
<td>157.01</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>200.62 ± 0.09a</td>
<td>183.57 ± 0.07a</td>
<td>136.30 ± 1.04a</td>
<td>135.92 ± 0.09a</td>
<td>157.23</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>136.54 ± 0.07a</td>
<td>136.37 ± 0.12a</td>
<td>157.30</td>
</tr>
</tbody>
</table>

* Individual measurement. For each measurement, data analysis was done in triplicate.

** Means with the same letter within a column (difference among different heating rates) are not significantly different (p=0.05).

*** Using a single-sample t test, the $T_{m\text{ onset}}$ values for indium were not significantly different from the population mean (156.60°C) at a p=0.05.
5.7. Reference


CHAPTER 6
EFFECTS OF HEATING CONDITIONS ON THE GLASS TRANSITION PARAMETERS OF AMORPHOUS SUCROSE PRODUCED BY MELT-QUENCHING

6.1. Abstract

Sucrose, a key ingredient in many food and pharmaceutical products, is often exposed to various heating conditions during manufacturing. As shown in Chapters 3 and 4, the loss of crystalline structure (melting) in sucrose is caused by the kinetic process of thermal decomposition. Therefore, the purpose of this study was to elucidate the effects of different heating conditions on the T_g parameters of amorphous sucrose produced by melt-quenching, prepared by heating at three different heating rates (1, 10, and 25°C/min) using a standard DSC (SDSC) method and by holding at three different isothermal temperatures (120, 132, and 138°C) using a quasi-isothermal MDSC. In general, the quasi-isothermal MDSC method (lower temperatures for longer times) exhibited lower T_g values, larger ΔC_p values, and broader glass transition ranges (i.e., T_g end minus T_g onset) than the SDSC method (at higher temperatures for shorter times), except at a heating rate of 1°C/min, which exhibited the lowest T_g values, the highest ΔC_p, and the broadest glass transition range for all heating conditions. This research showed that, depending on the heating conditions used, a different amount and variety of sucrose thermal decomposition components may be formed, giving rise to wide variation in the amorphous sucrose T_g values obtained. Thus, the variation observed in the literature T_g values for amorphous sucrose produced by thermal methods, such as melt-quenching, are, in part, due to difference in heating conditions. This research is important for the food and pharmaceutical industries for better understanding the effects of heat processing conditions and associated stability relations (e.g., T_g) for enhancing the quality and stability of sugar-based products.
6.2. Introduction

Amorphous sugars are essential ingredients in many food and pharmaceutical products, due to their useful properties, such as encapsulation ability, high dissolution rate, and high solubility (Mathlouthi 1995; Yu 2001). Examples of food ingredients and products containing amorphous sugars are sprayed dried flavors, boiled sweets, milk powder, coffee powder, infant formula, and fondants (Vanhal and Blond 1999; Van Scoik 1987) and examples of pharmaceutical ingredients and products are excipients for large-scale tablet and capsule manufacturing (Ritchie and Winfield 1984; Ishikawa and others 2001; Shimizu and others 2003; Joshi and Duriez 2004; Surana and others 2004; Airaksinen and others 2005; Fu and others 2006) and fillers in chewable tablets (Mullarney and others 2003; Gohel and Jogani 2005).

However, amorphous sugars are known to undergo structural changes induced by heat and/or moisture uptake, leading to undesirable events, such as stickiness, caking, and collapse or recrystallization (Mathlouthi 1995; Gerggren and Alderborn 2003; Airaksine and others 2005). These undesirable events are detrimental to product quality attributes, such as texture, taste, aroma retention, shelf-life, and delivery and stability characteristics of drug products, which are critical to final product acceptance. These stability concerns associated with amorphous sugar based products are of practical and economical importance, thus, they has been the subject of much research over the past several years.

A parameter of critical importance to the stability of amorphous materials is the glass transition temperature ($T_g$), which is the temperature where a reversible transition occurs between the solid amorphous (glassy) state and the supercooled liquid (rubbery) state. Since the glassy to rubbery transition takes place over a range of temperatures, rather than at a single temperature, it is important to report the onset, midpoint, and endpoint of the transition and to
specify the method and parameters used to obtain the \( T_g \) values. Upon inspection of literature reported \( T_g \) values for the same amorphous material, differences are often observed. For example, the \( T_g \) onset for amorphous sucrose reported by Roos (1995) was 62°C, whereas Slade and Levine (1991) reported a value of 52°C. The dissimilarity between reported \( T_g \) values for the same sugar has been ascribed to differences in: 1) residual water content, 2) samples handling techniques (e.g., preparation methods), 3) \( T_g \) measurement techniques, and 4) \( T_g \) analysis conditions and methods (Roos 1995, 2010; Abiad and others 2009).

In the case of sucrose, the factor influencing \( T_g \) of interest in this study is the heating conditions used to produce amorphous sucrose. The heating conditions used are important because the loss of crystalline structure (melting) in sucrose is due to the kinetic process of thermal decomposition (termed apparent melting), rather than thermodynamic melting, as shown in Chapters 3 and 4. Sucrose melting is a time-temperature combination process, that is, thermal decomposition is more extensive under lower temperature-longer time conditions, than under higher temperature-shorter time conditions. Thus, depending on the heating condition employed, different amounts and types of thermal decomposition components are formed in the amorphous sucrose, and consequently give rise to different \( T_g \) values.

Both Vanhal and Blond (1999), studying sucrose, and Jiang and others (2008) studying sucrose, glucose, and fructose, applied different heating conditions to these sugars in order to explore the relationship between thermal decomposition and \( T_g \) values. Vanhal and Blond (1999) investigated the \( T_g \) values for amorphous sucrose prepared by various heating conditions (i.e., final heating temperature, the residence time at the final temperature, and heating rate). The \( T_g \) values for amorphous sucrose decreased in the final heating temperature range of 190 to 210°C and then increased in the final heating temperature range of 215 to 225°C. When sucrose was
held for various lengths of time at its melting peak temperature, 190°C, the \( T_g \) values initially decreased and then increased with increasing residence time. Vanhal and Blond (1999) attributed the trend of decreasing and then increasing \( T_g \) values for amorphous sucrose in these experiments to the formation of different types and amounts of thermal decomposition components. That is, the decrease in \( T_g \) values was due to the formation of small molecular weight components via bond breaking (under relatively mild heating conditions) and the increase in \( T_g \) values was due to formation of various high molecular weight components via polymerization (under relatively severe heating conditions). Jiang and others (2008) found a similar trend of heating condition dependent changes in \( T_g \) values for fructose and glucose, in addition to sucrose. Additionally, Jiang and others (2008) suggested that water formed via dehydration, which occurs during sugar decomposition, as another possible reason for decreasing \( T_g \) values.

The residence time (or holding time) at the final temperature experiments by Vanhal and Blond (1999) and Jiang and others (2008) were carried out at and above the melting peak temperature of the sugars. Since loss of crystalline structure (melting) in sucrose is due to the kinetic process of thermal decomposition (Chapters 3 and 4) what still needs to be assessed is the effect on \( T_g \) of amorphous sucrose produced under low temperature long time conditions.

Vanhal and Blond (1999) also investigated the effect of heating rates on the \( T_g \) values of amorphous sucrose. These researchers found that \( T_g \) mid decreased from approximately 61 to 71°C as a function of heating rate, from 5 to 40°C/min, respectively. These researchers concluded that at slower heating rates the \( T_g \) value was lower because at slower heating rates the sucrose remains for a longer time at each temperature, which results in formation of more thermal decomposition components and, consequently, lower \( T_g \) values. However, the effect of

\[ ^{13} \text{The type of heating conditions employed by Jiang and others (2008) were final heating temperature and holding time (equivalent to the residence time) at the final temperature.} \]
the heating rate on the amorphous sucrose \( T_g \) values could be confounded with their selection of a constant final heating temperature of 200\(^\circ\)C, which was applied to all heating rates (5, 10, 20, 30, and 40\(^\circ\)C/min). In DSC analysis, the temperature at which the endothermic melting peak for sucrose, glucose, and fructose is completed depends on the heating rate. In other words, as the heating rate increases, the temperature at which the endothermic melting curve is completed increases. As discussed previously, this is because the loss of crystalline structure (melting) of these sugars is due to the kinetic process of thermal decomposition (termed apparent melting), rather than thermodynamic melting. As shown in Figure 6.1, the final heating temperature required for obtaining the entire apparent melting curve for sucrose was approximately 184\(^\circ\)C at a heating rate of 1\(^\circ\)C/min, whereas it was approximately 206\(^\circ\)C at a heating rate of 25\(^\circ\)C/min. Thus, if a constant final heating temperature (e.g., 206\(^\circ\)C) is applied to both heating rates, the amorphous sucrose heated at a heating rate of 1\(^\circ\)C/min would still be exposed to heat until the final heating temperature of 206\(^\circ\)C was reached. It remains ambiguous whether a change in the \( T_g \) for the amorphous sucrose was caused only by the heating rate effect or also by the additional heat received to reach the final heating temperature.

Therefore, the objective of this research was to determine: 1) the effect of heating rate only on the \( T_g \) for amorphous sucrose (final heating temperature determined by heating rate) using SDSC and 2) the effect on \( T_g \) of producing amorphous sucrose under low temperature long time conditions using quasi-isothermal MDSC.

6.3. Materials and Methods

6.3.1. Materials
High purity crystalline sucrose (S0389, ≥ 99.5) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and used without further purification. The water content of sucrose measured by coulometric Karl Fischer Titration (Hydranal Coulomat AG solvent) was 0.004 % wb.

6.3.2. Methods

Amorphous sucrose samples were prepared by heating at different heating rates (1, 10, and 25°C/min) using SDSC and by holding at different isothermal temperatures (120°C, 132°C, and 138°C) using quasi-isothermal MDSC. A DSC Q2000 (TA instruments, New Castle, DE), equipped with a Refrigerated Cooling System (RCS 90), was utilized for both SDSC and quasi-isothermal MDSC. Prior to the preparation and measurement of amorphous sucrose, the calibration for enthalpy (cell constant) and temperature was performed with indium (T_{m\, \text{onset}} of 156.6°C, ΔH of 28.71 J/g, Lot# A10R020, TA instruments, New Castle, DE). In advance of using the quasi-isothermal MDSC, DSC and MDSC heat capacity calibrations (a modulation amplitude of ± 1°C and a period of 100 sec) were individually conducted using a 22.93 mg sapphire disk (PN 970370.901, TA instrument, New Castle, DE) hermetically sealed in a pan. Hermetic aluminum Tzero pans (PN 901683.90, TA instruments, New Castle, DE) and lids (PN309684.901, TA instruments, New Castle, DE) were used for all calibrations, sample preparations, and sample measurements. An empty pan was used as the reference. Dry nitrogen, at a flow rate of 50 mL/min, was used as the purge gas.

6.3.2.1. Sample preparation using different heating rates by SDSC

As previously mentioned in the introduction, the desired final heating temperature required for obtaining an entire apparent melting peak for sucrose depends on the heating rate employed. The final heating temperature for each heating rate was determined as follows. First, the apparent melting end temperature was determined by linear extrapolation for triplicate measurements at
each heating rate. Then, the final heating temperature for each heating rate was calculated as the average end temperature plus two standard deviations. The two standard deviations were added to account for run-to-run variation, ensuring complete loss of crystalline structure for each measurement. The final heating temperatures for each heating rate were: 184°C at 1°C/min, 196°C at 10°C/min, and 206°C at 25°C/min.

Crystalline sucrose (approximately 2.75 mg) hermetically sealed in a pan was heated from 25 °C to the final heating temperature calculated for each heating rate. The total heating time at each heating rate was: 159 min at 1°C/min, 78.4 min 10°C/min, and 7.24 min at 25°C/min. Immediately after the DSC heat flow signal reached the final heating temperature at each heating rate, the melted sucrose was cooled at a cooling rate of 50°C/min to -50°C, equilibrated at -50°C, and then reheated at a heating rate of 10°C/min to 220°C. In the 2nd DSC scan to measure T_g parameters, no measurable endothermic melting peaks were found in all SDSC amorphous sucrose samples, which indicate complete removal of crystalline structure. All amorphous sucrose samples prepared at each heating rate were measured in triplicate. Universal Analysis (UA) software (TA instruments, New Castle, DE, version 4.4A) was used to determine the T_g parameters (T_g onset, T_g inflection, T_g end, ΔC_p) for the amorphous sucrose samples as follows: T_g onset – the temperature at the intersection of the regression line from the starting point of the specified limit (the first tangent) and the inflection tangent of the step change (the second tangent); T_g inflection (by inflection) – the temperature at the steepest slope on the DSC curve between the first tangent and the regression line after the transition (the third tangent); T_g end – the temperature at the intersection of the inflection tangent and the third tangent; and ΔC_p – the difference between the linear extrapolated glass and the liquid heat capacity curves of material at T_g inflection (Wungtanagorn and Schmidt 2001).
6.3.2.2. Sample preparation using different isothermal temperatures by quasi-isothermal MDSC

In order to prepare amorphous sucrose samples using quasi-isothermal MDSC, three isothermal temperatures (120°C, 132°C, and 138°C) were chosen based on the results of the stepwise quasi-isothermal MDSC experiment for sucrose conducted in Chapter 3 (Figures 3.6 and Figure 6.2). In the stepwise quasi-isothermal MDSC experiment, the heat capacity (Rev C_p) signal began to gradually increase at 120°C, indicating the onset of the loss of crystalline structure in sucrose, and then slightly leveled off at approximately 143°C, indicating the removal of all crystalline structure in sucrose. Hence, 120°C, the onset temperature of the loss of crystalline structure in sucrose and 132°C and 138°C, the temperatures at which the loss of crystalline structure occurs relatively quickly, were selected as the isothermal temperatures.

Since the loss of crystalline structure in sucrose is caused by the kinetic process of thermal decomposition, as shown in Chapter 3 and 4, the holding time required for removal of all crystalline structure in sucrose depends on the isothermal temperature employed. Thus, to determine the final holding time for each isothermal temperature, crystalline sucrose (approximately 2.75 mg), hermetically sealed in a pan, was isothermally held at 120°C, 132°C, and 138°C using quasi-isothermal MDSC (a modulation amplitude of ± 1°C and a period of 100 sec) well over the time at which the Rev C_p signal slightly leveled off. The point at which the Rev C_p signal slightly leveled off was regarded as the time required for removal of all crystalline structure in sucrose at each isothermal temperature. The end holding time was obtained by linear extrapolation for triplicate measurements at each isothermal temperature. Then, the final holding time for each isothermal temperature was calculated as the average end holding time plus two standard deviations. As in the case of SDSC, the two standard deviations were added to account
for run-to-run variation, ensuring complete loss of crystalline structure for each measurement. The final holding times for each isothermal temperature were: 3014 min at 120°C, 883 min at 132°C, and 510 min at 138°C (Figure 6.3).

Crystalline sucrose (approximately 2.75 mg) hermetically sealed in a pan was isothermally held at each isothermal temperature for the corresponding time using quasi-isothermal MDSC (a modulation amplitude of ± 1°C and a period of 100 sec). After that, the melted sucrose was immediately cooled at a cooling rate of 50°C/min to -50°C, equilibrated at -50°C, and then reheated at a heating rate of 10°C/min to 220°C. In the 2nd DSC scan to measure T_g parameters, no measurable endothermic melting peaks were found in all the MDSC amorphous sucrose samples. All amorphous sucrose samples prepared at each isothermal temperature were measured in triplicate. The T_g parameters (T_g onset, T_g mid, T_g end, ΔC_p) for the amorphous sucrose samples were analyzed using UA software as described in 6.3.2.1.

6.3.3. Statistical analysis

Statistical analyses were carried out using SAS software (SAS 9.2 TS Level, SAS Institute Inc., Cary, NC). The general linear model (GLM) procedure was utilized for the analysis of variance (ANOVA). The Tukey’s studentized range test was used to determine any significant differences between means at a p = 0.05.

6.4. Results and Discussion

6.4.1. Effect of heating rate on the T_g parameters for amorphous sucrose

The T_g parameters and DSC scans for amorphous sucrose samples prepared by heating at three different heating rates of 1, 10, and 25°C/min using the SDSC are given in Table 6.1 and Figure 6.3. At 1°C/min, the amorphous sucrose exhibited the lowest T_g values and the highest
ΔC_p. At 10 and 25°C/min, the amorphous sucrose showed much higher T_g values and lower ΔC_p values, compared to 1°C/min. However, unexpectedly, the T_g parameters for amorphous sucrose were slightly higher at 10°C/min than at 25°C/min; however, their T_g parameters were very close to each other, compared to those at 1°C/min. It was expected that the slow heating rate would have lower T_g values, since as discussed more below a slower heating rate is thought to result in more thermal decomposition. But, perhaps, the difference in thermal decomposition that occurred at the 10°C/min heating rate was not different enough compared to the 25°C/min heating rate to produce a distinguishable difference in the resultant T_g values. The T_g_mid value (69.83°C) for amorphous sucrose at 10°C/min obtained in the current study was similar to literature reported T_g_mid values (67.5°C Vanhal and Blond 1999; 67°C te Booy and others 1992) for amorphous sucrose prepared and analyzed using similar conditions, except for use of a higher final heating temperature (200°C for both studies). In addition, as shown in Table 6.1, the T_g range increased with decreasing heating rates. According to Vanhal and Blond (1999), as the degree of sucrose thermal decomposition increased, the T_g range (i.e., T_g_end minus T_g_onset) became broader, indicating an increase in the complexity of thermal decomposition components.

As shown in Chapters 3 and 4, sucrose melting is caused by the kinetic process of thermal decomposition. Hence, a slower heating rate allows sucrose to remain for a longer time at each temperature, resulting in the formation of more and different thermal decomposition components. In the early stages of sucrose thermal decomposition, small molecular weight decomposition components (e.g., glucose, fructose, 5-HMF, water, and acids) are produced through a variety of reaction pathways. As heating condition becomes severe, high molecular weight decomposition components are formed through polymerization (Hirschmüller 1953; Gardiner 1966; Mauch 1971; Kelly and Brown 1978/79; Richards and Shafizadeh 1978; Richards 1986; Lowary and
Richards 1988; Tomasik 1989; Kroh 1994; Eggleston and others 1996; Šimkovic and others 2003; Quintas and others 2007). Consequently, in the present study, a decrease in $T_g$ values and an increase in $\Delta C_p$ as heating rate decreases is accounted for by the plasticizing effect of the small molecular weight decomposition components. The plasticizing effect of small molecular weight components, in particular water, on $T_g$ is a well-known property (Fox and Flory 1950; Levine and Slade 1987; and Roos and Karel 1991a, b; Brent and others 1997). Both Fang and Angell (1995), studying fructose and galactose, and Okuno and others (2003), studying sucrose, reported that the depression of $T_g$ values for these amorphous sugars prepared by melt-quenching may be due to the small molecular weight fragmentation products formed by the decomposition process in the vicinity of their melting temperatures. As shown in Table 6.1, the $\Delta C_p$ at 1°C/min is higher than the $\Delta C_p$ at 10 and 25°C/min. The higher $\Delta C_p$ at 1°C/min indicates an increase in molecular mobility, which, we hypothesize, is due to the formation of a larger amount of small molecular weight decomposition components. The presence of smaller molecules increases the space between the original molecules by blocking their attractive forces, giving rise to greater free volume and molecular mobility (Abida and others 2009; Roos 2010).

In addition, it is important to note the use of different final heating temperatures for each heating rate. Different final heating temperatures at each heating rate were used to avoid the effect of the additional heat received to reach a common final heating temperature on the $T_g$ parameters for amorphous sucrose (Figure 6.1). However, the amorphous sucrose prepared by heating at 1°C/min to 184°C exhibited the lowest $T_g$ values and the highest $\Delta C_p$. Thus, it seems that more extensive thermal decomposition occurs at slower heating rates, where the sucrose sample remained for a longer amount of time at each temperature, compared to faster heating rates to higher final heating temperatures.
6.4.2. Effect of isothermal temperature on the T\textsubscript{g} parameters for amorphous sucrose

The T\textsubscript{g} parameters and DSC scans for amorphous sucrose prepared by holding at three isothermal temperatures, 120, 132, and 138°C, using quasi-isothermal MDSC are given in Table 6.1 and Figure 6.3. As can be seen in Table 6.1, the T\textsubscript{g} values for amorphous sucrose were lower at 120°C than at 132°C and 138°C. However, the T\textsubscript{g} values were slightly higher at 132°C than at 138°C, though only statistically different for T\textsubscript{g end}. In general, it was expected that lower temperature, longer time heat treatments would lead to lower T\textsubscript{g} values (that is, until extensive polymerization\textsuperscript{14} would occur and then the T\textsubscript{g} values are postulated to begin to increase), since the thermal decomposition process could take place over a longer period of time. This was the case for the 120°C isothermal temperature, but not for 132°C and 138°C. In terms of ΔC\textsubscript{p}, the amorphous sucrose at 138°C had a slightly higher value than those at 120 and 132°C, but the ΔC\textsubscript{p} at 132°C was not statistically different from the value at 120 °C.

Of importance to note is the variability in the data both within each isothermal temperature and between isothermal temperatures. The variation within each isothermal temperature is illustrated by the variation in the triplicate MDSC Rev C\textsubscript{p} signals obtained at each isothermal temperature (Figure 6.4) and the relatively large standard deviations associated with each isothermal temperature (Table 6.1). The variation between isothermal temperatures is illustrated, as discussed above, by the lack of the expected trend in the T\textsubscript{g} values for 132°C and 138°C holding temperatures.

\textsuperscript{14} Both the SDSC and quasi-isothermal MDSC methods used in the present study were the minimum heating condition required for the complete removal of sucrose crystalline structure. Even though the quasi-isothermal MDSC method is generally less gentle than the SDSC method in terms of the degree of thermal decomposition, it seems that both methods were not severe enough to cause extensive polymerization (i.e., production of a significant amount of higher molecular weight compounds), where an increase in T\textsubscript{g} would be expected, as postulated by both Vanhal and Blond (1999) and Jiang and others (2008).
Both sources of variability are attributed to the complex, non-uniform (i.e., non-reproducible) nature of the thermal decomposition reaction. There are a number of factors that can affect both the thermal decomposition reaction pathway and rate, such as aqueous concentration (i.e., water), time, temperature, and presence and concentration of catalysts, such as natural salts (e.g., chlorides, nitrates), alkaline or acidic substances, reducing sugars (e.g., glucose, fructose), and buffering compounds (Mauch 1971; Kelly and Brown 1978/79; Richards 1986). For example, a number of publications have reported the accelerative effect of water on sucrose hydrolysis (Mauch 1971; Kelly and Brown 1978/79; Richards 1986). Kelly and Brown (1978/79) specifically mentioned that the presence of moisture on the surface of sugar crystals seemed to be necessary for thermal decomposition, since the hydrogen ion (H+) from water is required for sucrose hydrolysis. Thus, because thermal decomposition progresses through a myriad of complex reaction pathway involving a variety of chemical transformations, even a small change in the above factors could lead to different decomposition results, and in turn increase the variability in the $T_g$ parameters.

6.4.3. Comparison of $T_g$ parameters obtained by heating rate versus isothermal temperature

Compared to the SDSC method, the quasi-isothermal MDSC method results in greater sucrose thermal decomposition (Chapter 4), producing a greater amount and more diverse decomposition components. Thus, as expected, the quasi-isothermal MDSC amorphous sucrose showed much lower $T_g$ values and higher $\Delta C_p$ values than the SDSC amorphous sucrose, except for the amorphous sucrose at a heating rate of 1°C/min, which only took a total of 159 min, but resulted in the lowest $T_g$ values and the highest $\Delta C_p$ value and the broadest glass transition range ($T_{g_{end}} - T_{g_{onset}}$). Perhaps, the relatively slow 1°C/min heating rate provided a similar initial thermal decomposition scenario as in the MDSC isothermal temperature experiments (low
temperature, long time conditions), but then the higher temperatures (due to scanning up to 184\(^\circ\)C) served to accelerate the further stages of the thermal decomposition process. Additional research is needed to fully elucidate the observed dramatic decrease in \(T_g\) values for the 1\(^\circ\)C/min heating rate compared to 10 and 25\(^\circ\)C/min heating rates.

It is important to mention that an attempted was made to compare the \(T_g\) parameters obtained herein to the \(T_g\) parameters for an amorphous sucrose sample prepared by the non-thermal process of freeze-drying and analyzed using a SDSC heating rate of 10\(^\circ\)C/min. It was hypothesized that the freeze-dried sample would exhibit the highest \(T_g\) values, since it would have experienced no thermal decomposition, thus no lowering of the \(T_g\) values by formation of small molecular weight components. However, unexpectedly, the freeze-dried sample \(T_g\) values \((T_g\,\text{onset} = 53.52\,\text{°C}, \, T_g\,\text{mid} = 60.68\,\text{°C}, \, T_g\,\text{end} = 62.37)\) were lower than the SDSC samples at heating rates of 10 and 25\(^\circ\)C/min and higher than all the quasi-isothermal MDSC samples and the SDSC sample at 1\(^\circ\)C/min. The freeze-dried sample \(\Delta C_p\) value (0.8984 J/g\(_\circ\)C) was slightly higher than the SDSC sample at 1\(^\circ\)C/min, which was the sample with the highest thermal decomposition. This result is ascribed to the relatively high moisture content of the freeze-dried sample. Even after 14 days in a dessicator over \(\text{P}_2\text{O}_5\), the moisture content of the freeze-dried sample was 2.3\% (wb), which lowered the \(T_g\) values and raised the \(\Delta C_p\) value compared to the 10 and 25\(^\circ\)C/min SDSC heating rate samples. Further investigation is needed to obtain \(T_g\) parameters for amorphous sucrose with a much lower moisture content.

**6.5. Conclusions**

This research was performed to investigate the effect of heating conditions on the \(T_g\) parameters for amorphous sucrose prepared by melt-quenching. Overall, it was found that the
quasi-isothermal MDSC method (lower temperatures for longer times) exhibited lower \( T_g \) values, larger \( \Delta C_p \) values, and broader glass transition ranges (i.e., \( T_{g \text{ end}} \) minus \( T_{g \text{ onset}} \)) than the SDSC method (at higher temperatures for shorter times), except at a heating rate of 1°C/min, which exhibited the lowest \( T_g \) values, the highest \( \Delta C_p \), and the broadest glass transition range for all heating conditions. Because the kinetic process of thermal decomposition is responsible for the loss of crystalline structure (melting) in sucrose, the observed decrease in \( T_g \) values was ascribed to the plasticizing effect of small molecular weight decomposition components. It was hypothesized that perhaps, the relatively slow 1°C/min heating rate provided a similar initial thermal decomposition scenario as in the MDSC isothermal temperature experiments (low temperature, long time conditions), but then the higher temperatures (due to scanning up to 184°C) served to accelerate the further stages of the thermal decomposition process. Additional research is needed to fully elucidate the observed dramatic decrease in \( T_g \) values for the 1°C/min heating rate compared to the 10 and 25°C heating rates. In addition, this research showed that the heating conditions employed to produce the amorphous sucrose are an additional important contributing factor to explaining the wide variation of \( T_g \) values observed in the literature. Since glucose and fructose have also been shown to lose their crystalline structure via thermal decomposition (Chapter 3), the effect of heating conditions on their \( T_g \) parameters is hypothesized to be similar to sucrose studied herein. This research is useful for better understanding the quality and stability issues associated with heat processed sugar-containing food and pharmaceutical products.
6.6. Figures and Tables

Figure 6.1 The temperature at which the apparent melting for sucrose is completed depends on the heating rate employed in DSC analysis. Dotted vertical lines are the final temperatures (end temperature plus two standard deviations) employed at each heating rate. Arrows illustrate the additional heating that the sucrose samples would experience if the final heating temperature of 206°C were used for all heating rates.
Figure 6.2 The stepwise quasi-isothermal MDSC thermogram for sucrose (a modulation amplitude of ± 1.0°C, a period of 100 sec, a stepwise temperature-increment of 1°C, and an isothermal time of 25 min with a data off period of 5 min for initial equilibration) used to determine the three isothermal temperatures (120, 132, and 138°C) for preparing amorphous sucrose samples using quasi-isothermal MDSC.
Figure 6.3 Change in $T_g$ mid (by inflection) values for amorphous sucrose samples prepared by heating at three heating rates (HR) of 1, 10, and 25°C/min using SDSC and by holding at three isothermal temperatures (IT) of 120, 132, and 138°C using quasi-isothermal MDSC.
Figure 6.4 Variations in the Rev \( C_p \) change during the loss of crystalline structure in sucrose by holding at isothermal temperatures (IT) of 120\(^\circ\)C for 3014 min, 132\(^\circ\)C for 883 min, and 138\(^\circ\)C for 510 min using quasi-isothermal MDSC.
Table 6.1 $T_g$ parameters ($T_g$ onset, $T_g$ mid, $T_g$ end, and $\Delta C_p$) for amorphous sucrose samples produced by heating at three different heating rates of 1, 10, and 25$^\circ$C/min using SDSC and by holding at three different isothermal temperatures of 120, 132, and 138$^\circ$C using quasi-isothermal MDSC, respectively (**N=3, n=3).

<table>
<thead>
<tr>
<th>Heating condition for preparing amorphous sucrose samples</th>
<th>$T_g$ onset ($^\circ$C)</th>
<th>$T_g$ mid ($^\circ$C)</th>
<th>$T_g$ end ($^\circ$C)</th>
<th>$\Delta C_p$ (J/(g$^\circ$C))</th>
<th>$T_g$ end - $T_g$ onset ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SDSC method</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heating at 1$^\circ$C/min to 184$^\circ$C</td>
<td>25.67 ± 2.08$^{b4*}$</td>
<td>37.45 ± 0.51$^{c4}$</td>
<td>44.84 ± 0.68$^{c8}$</td>
<td>0.8684 ± 0.0227$^{a1}$</td>
<td>19.17 ± 2.61$^{a1}$</td>
</tr>
<tr>
<td>Heating at 10$^\circ$C/min to 196$^\circ$C</td>
<td>64.05 ± 1.38$^{a1}$</td>
<td>69.83 ± 0.51$^{a1}$</td>
<td>75.46 ± 1.72$^{a1}$</td>
<td>0.7230 ± 0.0207$^{b34}$</td>
<td>11.41 ± 3.01$^{b34}$</td>
</tr>
<tr>
<td>Heating at 25$^\circ$C/min to 206$^\circ$C</td>
<td>63.83 ± 0.65$^{a1}$</td>
<td>68.35 ± 0.43$^{b1}$</td>
<td>72.94 ± 0.81$^{b2}$</td>
<td>0.7003 ± 0.0223$^{b4}$</td>
<td>9.11 ± 1.05$^{b4}$</td>
</tr>
<tr>
<td><strong>Quasi-isothermal MDSC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holding at 120$^\circ$C for 3014 min</td>
<td>30.92 ± 1.88$^{b3}$</td>
<td>39.59 ± 1.57$^{b3}$</td>
<td>45.36 ± 1.40$^{c5}$</td>
<td>0.7474 ± 0.0164$^{b3}$</td>
<td>14.44 ± 1.08$^{ab2}$</td>
</tr>
<tr>
<td>Holding at 132$^\circ$C for 883 min</td>
<td>35.26 ± 3.00$^{a2}$</td>
<td>44.27 ± 2.86$^{a2}$</td>
<td>50.67 ± 2.57$^{a3}$</td>
<td>0.7445 ± 0.0088$^{b3}$</td>
<td>15.41 ± 1.09$^{a2}$</td>
</tr>
<tr>
<td>Holding at 138$^\circ$C for 510 min</td>
<td>34.79 ± 0.59$^{a2}$</td>
<td>42.21 ± 0.40$^{a2}$</td>
<td>48.38 ± 0.64$^{b4}$</td>
<td>0.7844 ± 0.0318$^{a2}$</td>
<td>13.59 ± 1.20$^{b23}$</td>
</tr>
</tbody>
</table>

*Means with the same letter (difference among SDSC method or difference among quasi-isothermal MDSC method) are not significantly different (p=0.05).
Means with the same number (difference among both SDSC method and quasi-isothermal MDSC methods) are not significantly different (p=0.05).

**N= a number of samples replication; n= a number of data analysis.
6.7. References

Abiad MG, Carvajal MT, Campanella OH. 2009. A review on methods and theories to describe the glass transition phenomenon: applications in food and pharmaceutical products. Food Engineering Reviews 1:105-132.


Series) and low substituted-hydroxypropylcellulose or spherical sugar granules by direct compression method. Chemical & Pharmaceutical Bulletin 49(2):134-139.


APPENDIX A

Material information data sheet for sucrose, glucose, and fructose from Sigma-Aldrich Co.

<table>
<thead>
<tr>
<th></th>
<th>Sucrose (cane sugar)</th>
<th>Glucose (D- (+)-glucose)</th>
<th>Fructose (D- (-)-fructose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalog no.</td>
<td>S0398 Sigma</td>
<td>G7528 Sigma</td>
<td>F2543 Sigma</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Sucrose Structure" /></td>
<td><img src="image" alt="Glucose Structure" /></td>
<td><img src="image" alt="Fructose Structure" /></td>
</tr>
<tr>
<td>Synonyms</td>
<td>α-D-Glc-(1→2)-β-D-Fru, D(+)-Saccharose, Sugar, β-D-Fructofuranosyl-α-D-glucopyranoside, α-D-Glucopyranosyl β-D-fructofuranoside</td>
<td>Dextrose</td>
<td>D-Levulose, fruit sugar</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C₁₂H₂₂O₁₁</td>
<td>C₆H₁₂O₆</td>
<td>C₆H₁₂O₆</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>342.3</td>
<td>180.16</td>
<td>180.16</td>
</tr>
<tr>
<td>Properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>For molecular biology</td>
<td>SigmaUltra</td>
<td>SigmaUltra</td>
</tr>
<tr>
<td>Assay</td>
<td>≥ 99.5% (GC)</td>
<td>99.5% (GC)</td>
<td></td>
</tr>
<tr>
<td>Melting point</td>
<td>185-187°C (lit.)</td>
<td>150-152°C (lit.)</td>
<td>119-122°C (dec.) (lit.)</td>
</tr>
<tr>
<td>Absorbance</td>
<td>A50%/260 &lt; 0.15</td>
<td>A1M/260, H₂O &lt; 0.02</td>
<td>A1M/280, H₂O &lt; 0.02</td>
</tr>
<tr>
<td></td>
<td>A50%/280 &lt; 0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubility</td>
<td>H₂O: 1M at 20°C, clear, colorless</td>
<td>H₂O: 1M at 20°C, clear, colorless</td>
<td></td>
</tr>
<tr>
<td>Optical activity</td>
<td>[α]20/546 +62±2°, 3hr, c = 10 in H₂O (lit.)</td>
<td>[α]20/D +53±2°, 3hr, c = 10 in H₂O (lit.)</td>
<td></td>
</tr>
<tr>
<td>Total impurities</td>
<td>Free glucose</td>
<td>Insoluble matter, passes filter test</td>
<td>Content</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------</td>
<td>--------------------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>Ignition residue</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.0005% Phosphorus (P)</td>
<td></td>
</tr>
<tr>
<td>Loss</td>
<td>&lt; 0.1%</td>
<td>&lt; 0.05% Glucose (enzymatic)</td>
<td></td>
</tr>
<tr>
<td>Anion traces</td>
<td></td>
<td>&lt; 0.1% Insoluble matter</td>
<td></td>
</tr>
<tr>
<td>Cation traces</td>
<td>Heavy metals (as Pb): &lt; 5 ppm</td>
<td>&lt; 0.1% loss on drying</td>
<td></td>
</tr>
<tr>
<td>Chloride (Cl(^-))</td>
<td>&lt; 0.005%</td>
<td>Chloride (Cl(^-)): &lt; 0.05%</td>
<td></td>
</tr>
<tr>
<td>Sulfate (SO(_4^{2-}))</td>
<td>&lt; 0.005%</td>
<td>Sulfate (SO(_4^{2-})): &lt; 0.05%</td>
<td></td>
</tr>
<tr>
<td>Al: &lt; 0.0005%</td>
<td>Al: &lt; 0.0005%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As: &lt; 0.0001%</td>
<td>Ca: &lt; 0.0005%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ba: &lt; 0.0005%</td>
<td>Cu: &lt; 0.0005%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bi: &lt; 0.0005%</td>
<td>Fe: &lt; 0.0005%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca: &lt; 0.001%</td>
<td>K: &lt; 0.005%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd: &lt; 0.0005%</td>
<td>Mg: &lt; 0.0005%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co: &lt; 0.0005%</td>
<td>NH(^4+): &lt; 0.05%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr: &lt; 0.0005%</td>
<td>Na: &lt; 0.005%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu: &lt; 0.0005%</td>
<td>Pb: &lt; 0.001%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe: &lt; 0.0005%</td>
<td>Zn: &lt; 0.0005%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**APPENDIX B**

Material information data sheet for mannitol from Sigma-Aldrich Co.

**D-Mannitol**

<table>
<thead>
<tr>
<th>Properties</th>
<th>SigmaUltra</th>
<th>Assay</th>
<th>Chloride (Cl): &lt; 0.05%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>SigmaUltra</td>
<td>≥ 99.9%</td>
<td></td>
</tr>
<tr>
<td>Melting point</td>
<td>167-170°C (lit.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubility</td>
<td>H₂O: 1M at 20°C, clear, colorless</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total impurities</td>
<td>&lt; 0.0005%</td>
<td>Phosphorus (P)</td>
<td>Mg: &lt; 0.0005%</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.01%</td>
<td>Insoluble matter</td>
<td>NH₄⁺: &lt; 0.0005%</td>
</tr>
<tr>
<td>Ignition residue</td>
<td>&lt; 0.01%</td>
<td></td>
<td>Na: &lt; 0.005%</td>
</tr>
</tbody>
</table>

**Empirical formula** C₆H₁₄O₆

**Molecular weight** 182.17

**Synonyms** Mannite

**Molecular weight** M9546 Sigma-Aldrich

**Empirical formula** C₆H₁₄O₆

**Molecular weight** 182.17

**Properties**

- **Grade**: SigmaUltra
- **Assay**: ≥ 99.9%
- **Melting point**: 167-170°C (lit.)
- **Solubility**: H₂O: 1M at 20°C, clear, colorless
- **Total impurities**: < 0.0005%
- **Insoluble matter**: < 0.01%
- **Phosphorus (P)**: < 0.0005%
- **Insoluble matter**: < 0.01%
- **Ignition residue**: < 0.01%
- **Cation traces**
  - Al: < 0.0005%
  - Ca: < 0.0005%
  - Cu: < 0.0005%
  - Fe: < 0.0005%
  - K: < 0.005%
  - Mg: < 0.0005%
  - NH₄⁺: < 0.0005%
  - Na: < 0.005%
  - Pb: < 0.001%
  - Zn: < 0.0005%
- **Anion traces**
  - Chloride (Cl): < 0.05%
  - Sulfate (SO₄²⁻): < 0.05%
APPENDIX C

SDSC scans and T_m parameters (T_m onset, T_m peak, and ΔH) for the endothermic peaks associated with loss of crystalline structure in sorbitol at heating rates (HR) of 1, 5, and 25°C/min (N=3, n=3; where N = replication number and n = data analysis number).

<table>
<thead>
<tr>
<th>Heating rate (°C/min)</th>
<th>Sorbitol (T_m parameters)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T_m onset (°C)</td>
</tr>
<tr>
<td>1</td>
<td>93.96 ± 0.08^a</td>
</tr>
<tr>
<td>5</td>
<td>93.99 ± 0.24^a</td>
</tr>
<tr>
<td>25</td>
<td>94.53 ± 0.07^b</td>
</tr>
</tbody>
</table>

* A sigmoidal tangent baseline was used for sorbitol because the difference in the C_p between the crystalline and liquid phases was large.
** Means with the same letter within a column (difference among different heating temperatures) are not significantly different (p=0.05).
APPENDIX D

SDSC thermogram and $T_m$ parameters ($T_m$ onset, $T_m$ peak, and $\Delta H$) for acetaminophen analyzed at heating rates (HR) of 2, 5, and 10°C/min (top) and SDSC/TGA thermogram for acetaminophen analyzed at a heating rate of 10°C/min (bottom).
APPENDIX E

SDSC thermogram and $T_m$ parameters ($T_m$ onset, $T_m$ peak, and $\Delta H$) for phenacetin analyzed at heating rates (HR) of 2, 5, and 10°C/min (top) and SDSC/TGA thermogram for phenacetin analyzed at a heating rate of 10°C/min (bottom).
APPENDIX F

Over the heating rate range of 2 to 10°C/min, an additional, very small, weight loss (less than 0.05%) in crystalline glucose was observed in the temperature range from 53°C to 60°C.
APPENDIX G

Results of HPLC analysis for sucrose samples prepared by both SDSC and quasi-isothermal MDSC methods (refer to Figures 4.2 and 4.4).

<table>
<thead>
<tr>
<th>Final heating temperature (°C)</th>
<th>Sucrose (%)</th>
<th>Glucose (%)</th>
<th>Fructose (%)</th>
<th>5-HMF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDSC method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.0 (as-is)</td>
<td>99.8404 ± 0.8786</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>98.7 ± 0.1</td>
<td>100.7852 ± 1.7107</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>149.6 ± 0.1</td>
<td>101.1415 ± 1.2961</td>
<td>0.3645 ± 0.0593</td>
<td>-</td>
<td>0.0030 ± 0.0003</td>
</tr>
<tr>
<td>158.7 ± 0.1</td>
<td>98.4255 ± 0.5874</td>
<td>1.2668 ± 0.0906</td>
<td>-</td>
<td>0.0056 ± 0.0014</td>
</tr>
<tr>
<td>184.1 ± 0.1</td>
<td>90.2205 ± 1.3538</td>
<td>3.8069 ± 0.1853</td>
<td>0.2518 ± 0.0402</td>
<td>0.0104 ± 0.0024</td>
</tr>
<tr>
<td>192.7 ± 0.2</td>
<td>61.8711 ± 1.3333</td>
<td>14.0878 ± 1.0464</td>
<td>0.8817 ± 0.1985</td>
<td>0.0526 ± 0.0034</td>
</tr>
<tr>
<td>208.4 ± 0.2</td>
<td>27.8192 ± 1.5139</td>
<td>33.1673 ± 0.5488</td>
<td>6.0816 ± 0.1020</td>
<td>0.6269 ± 0.0278</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Holding time at 120°C (min)</th>
<th>Sucrose (%)</th>
<th>Glucose (%)</th>
<th>Fructose (%)</th>
<th>5-HMF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quasi-isothermal MDSC method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (as-is)</td>
<td>99.8404 ± 0.8686</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>98.3217 ± 1.1138</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>98.5637 ± 0.4959</td>
<td>0.4114 ± 0.0430</td>
<td>-</td>
<td>0.0029 ± 0.0001</td>
</tr>
<tr>
<td>100</td>
<td>95.9399 ± 2.9622</td>
<td>1.0876 ± 0.0618</td>
<td>-</td>
<td>0.0067 ± 0.0007</td>
</tr>
<tr>
<td>200</td>
<td>95.1588 ± 0.7662</td>
<td>1.7321 ± 0.0302</td>
<td>0.2411 ± 0.0304</td>
<td>0.0130 ± 0.0009</td>
</tr>
<tr>
<td>250</td>
<td>94.2627 ± 0.8164</td>
<td>2.0535 ± 0.1218</td>
<td>0.2799 ± 0.0307</td>
<td>0.0156 ± 0.0014</td>
</tr>
<tr>
<td>300</td>
<td>93.2208 ± 2.1095</td>
<td>2.2298 ± 0.0655</td>
<td>0.2725 ± 0.0371</td>
<td>0.0168 ± 0.0007</td>
</tr>
<tr>
<td>350</td>
<td>93.5356 ± 0.4518</td>
<td>2.4587 ± 0.0489</td>
<td>0.2878 ± 0.0346</td>
<td>0.0187 ± 0.0004</td>
</tr>
<tr>
<td>400</td>
<td>92.7795 ± 1.5919</td>
<td>2.6727 ± 0.1012</td>
<td>0.3529 ± 0.0206</td>
<td>0.0217 ± 0.0010</td>
</tr>
<tr>
<td>450</td>
<td>91.8528 ± 5.0484</td>
<td>2.9099 ± 0.0819</td>
<td>0.3755 ± 0.0579</td>
<td>0.0240 ± 0.0014</td>
</tr>
<tr>
<td>500</td>
<td>89.3103 ± 4.8850</td>
<td>3.0835 ± 0.0570</td>
<td>0.3885 ± 0.0295</td>
<td>0.0262 ± 0.0004</td>
</tr>
<tr>
<td>1200</td>
<td>73.8966 ± 8.2639</td>
<td>7.6331 ± 0.7757</td>
<td>1.1517 ± 0.2700</td>
<td>0.0856 ± 0.0050</td>
</tr>
<tr>
<td>2000</td>
<td>48.3538 ± 3.4060</td>
<td>24.0177 ± 1.4945</td>
<td>4.2586 ± 0.5612</td>
<td>0.4218 ± 0.0370</td>
</tr>
<tr>
<td>3100</td>
<td>24.0427 ± 0.8495</td>
<td>30.3222 ± 0.4809</td>
<td>6.4987 ± 0.3347</td>
<td>1.0425 ± 0.0554</td>
</tr>
</tbody>
</table>
Results of HPLC analysis for sucrose samples prepared by both SDSC and quasi-isothermal MDSC methods (refer to Figures 4.3 and 4.5).

<table>
<thead>
<tr>
<th>Final heating temperature (°C)</th>
<th>Mannitol (%)</th>
<th>Sorbitol area/total area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDSC method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.00 (as-is)</td>
<td>99.1879 ± 0.1404</td>
<td>0.9315 ± 0.0177</td>
</tr>
<tr>
<td>156.7 ± 0.03</td>
<td>99.5815 ± 0.5827</td>
<td>0.9560 ± 0.0234</td>
</tr>
<tr>
<td>163.5 ± 0.02</td>
<td>99.0318 ± 0.6808</td>
<td>0.9532 ± 0.0246</td>
</tr>
<tr>
<td>172.6 ± 0.03</td>
<td>99.7164 ± 0.7354</td>
<td>0.9571 ± 0.0331</td>
</tr>
<tr>
<td>180.6 ± 0.02</td>
<td>99.9452 ± 0.7409</td>
<td>0.9403 ± 0.0334</td>
</tr>
<tr>
<td>188.6 ± 0.03</td>
<td>99.9484 ± 1.1325</td>
<td>0.9419 ± 0.0121</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Holding time at 159.9°C (min)</th>
<th>Mannitol (%)</th>
<th>Sorbitol area/total area (%)</th>
<th>Unknown components area/total area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quasi-isothermal MDSC method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (as-is)</td>
<td>98.5520 ± 0.8949</td>
<td>0.9543 ± 0.0167</td>
<td></td>
</tr>
<tr>
<td>5555</td>
<td>96.0816 ± 0.4547</td>
<td>0.7753 ± 0.0387</td>
<td>1.4887 ± 0.2191</td>
</tr>
</tbody>
</table>
AUTHOR’S BIOGRAPHY

Joo Won Lee graduated from Korea University in 1999 with a Bachelor of Science degree in Food Science and Technology and in 2001 with a Master of Science degree in Life Science and Biotechnology. After graduation, Joo Won worked for two years as a researcher for Post-harvest and Food Packaging Research Team in Korean Food Research Institute in South Korea before relocating to Champaign, Illinois, to pursue graduate study in Food Science. Upon the completion of her Ph.D., Joo Won will begin her career with Dr. Schmidt’s lab as a postdoc.