Assessment of activation energy of enthalpy relaxation in sucrose-water system: effects of DSC cycle type and sample thermal history

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Abstract

The purpose of this study is to critically analyze different methods of calculation of activation energy of relaxation in sucrose-water system from differential scanning calorimetry data. We consider the use of different thermal cycles for calculations together with Moynihan and Kissinger equations. We study the effect of two methods of glass transition temperature determination (half-step and inflection point) on the activation energy values. Along with experimental DSC data, we use the data simulated using Tool–Narayanaswamy–Moynihan model to validate the use of cooling and heating curves and to check the reproducibility of the activation energy calculations. The obtained results show that the thermal cycle with equal cooling and heating rates provides the most reliable data set and the glass transition temperature definition using inflection point rather than half step can be recommended for calculations. Moreover, due to technical reasons, heating rather than cooling scans provide the most reliable results of activation energy calculations. Furthermore, a simple method based on the width of the glass transition region shows reasonable results for single scan experiments. The activation energies of the glass transition in sucrose-water system with different water contents and different thermal histories were studied. Since it is impossible to apply traditional methods based on Moynihan equation for the activation energy evaluation for freeze-dried samples, we propose using another method based on the properties of the recovery peak. Combining the results obtained by different methods, we present a dependence of activation energy in sucrose-water system on water content. The results show that water decreases the activation energy of relaxation process in sucrose matrix.

Keywords DSC · Glass transition · Activation energy · Sucrose · Freeze-dried samples · Tool–Narayanaswamy–Moynihan model

Introduction

The glassy state has numerous applications in pharmaceutical and food industries. For example, it is used for the improvement of solubility of poorly water-soluble drugs [1–3] and stabilization of proteins and cells by sugars/polymers [4]. According to one of the definitions, “Glass is a non-equilibrium, non-crystalline state of matter that appears solid on a short time scale but continuously relaxes towards the liquid state” [5]. Characterization of the dynamics in such materials is a subject of increasing interest. The main

property of the glassy state is the glass transition temperature. The molecular mobility is relatively slow below the glass transition temperature, and relatively fast above. In the glass transition concept, the term molecular mobility considers as the translational motion of the whole molecule or a segment of the polymer [6]. “Freezing” of molecular motions of protein in glassy sugar matrix is one of the possible explanations of superior protein stability in solid amorphous formulations.

As mentioned above, samples in the glassy state continuously relax towards a state that is characterized by equilibrium values of such thermodynamic parameters as volume and enthalpy. Molecular motions responsible for the relaxation process in the glassy state are complex and can be understood only by combining results obtained by several experimental techniques. The molecular mobility of the glassy state can be assessed by isothermal calorimetry and

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differential scanning calorimetry (DSC) [6–10], or alternatively by such methods as dielectric spectroscopy [11]. Two types of relaxation processes are considered in the literature: α -relaxations and β -relaxations [6, 8]. The α -relaxations (primary relaxations) are responsible for the global mobility and cooperative motions. These primary relaxations are associated with the glass transition temperature in DSC and changes in the viscosity. The β -relaxations or secondary relaxations are associated with groups of motions, rather than the whole molecule mobility, they can be detected by dielectric spectroscopy.

The key parameter needed for understanding structural relaxations in glass-forming systems is the activation energy. In a broader sense, the concept of activation energy is used in many areas of chemistry and physics for the description and modeling of many thermal-induced processes, such as chemical reactions, denaturation, gelatinization, decomposition, etc. In the case of glass-forming systems, knowledge of this parameter is especially important when considering temperature dependences of relaxation and recovery processes in the glassy state. Probably the most widely used and discussed in the literature method of calculation of the activation energy of the relaxation process was proposed by Moynihan [12]. The main assumption of this method is a temperature-independent distribution of relaxation times. In practice, this approach requires a DSC experiment with different cooling or/and heating rates. The accuracy of activation energy depends on the temperature program [13], the algorithm of the T_g evaluation, left and right limits for baselines, and the quality of the DSC signal.

Moynihan equation is very similar to the Kissinger equation, which is also used to determine the activation energy of the glass transition [14–16]. Kissinger equation uses a temperature of the maximum of the recovery endotherm instead of the glass transition temperature (middle of the heat capacity step). The original Kissinger analysis was developed for the study of the dependence of a position of the melting peak on the heating rate in the DSC experiment [14]. Later the Kissinger equation was applied to an evaluation of effective activation energy of relaxation in amorphous materials [15].

The values of activation energy obtained using Moynihan and Kissinger methods are sensitive to experimental conditions [13]. To get meaningful results, one should follow several recommendations. The thermal cycle must include heating and cooling 20–30 degrees above or below the glass transition. If the thermal history of the sample (cooling and heating rates) is unknown, the calculation results are unreliable.

Another method for evaluation of enthalpy relaxation is based on cooperative rearrangement region by Adam and Gibbs [17–19]. This statistical approach includes parameters that describe order/disorder in the system and considers relaxation as the progressive ordering of the matrix.

Alternatively, relaxation times in glassy polymers can be described by KAHR (Kovacs, Aklonis, Hutchinson, and Ramos) equation [20].

For pharmaceutical applications, attention has been focused on the importance of the relaxation process in glassy matrixes during isothermal storage below the glass transition [6, 21, 22]. Global relaxation of amorphous excipients upon aging results in the loss of enthalpy and volume during the evolution of glasses towards the equilibrium supercooled liquid [23, 24]. The volume relaxation might be seen as the shrinking of freeze-dried cake when the storage temperature approaches the glass transition [25]. An increase in enthalpy is seen on DSC heating curves as an endothermic event after the glass transition temperature in amorphous excipients [6, 18, 24]. The intensity of the event increases with storage time indicating greater structural relaxation [24]. A similar effect to the storage time is the storage temperature. The recovery peak increases when the storage temperature approaches the glass transition temperature. The relaxation enthalpy has non-exponential dependence on relaxation time and may be described by the Kohlrausch–Williams–Watts stretched exponential function (KWW equation) [26].

Sucrose is probably the most used disaccharide in pharmaceutical science for protein stabilization in solid state [4, 27]. As was mentioned above, the molecular motions in glassy sucrose are coupled with the protein motions and keep the protein molecules inflexible in the rigid matrix. The matrix properties can be described in terms of the activation energy of the relaxation process. The activation energies of glass transitions of sucrose and sucrose-water mixtures found in the literature are listed in Table 1. One can see that values differ between different studies, which can be attributed to differences in calculation methods, choice of the glass transition temperature evaluation, and the thermal history of the samples. The large variation in the literature values of activation energy suggests that further studies to determine its accurate value for sucrose and sucrose-water systems are needed.

Table 1 Literature values of sucrose activation energies determined by different methods

System	$E_{act}/\text{kJ mol}^{-1}$	References
Spray-dried sucrose	301 (onset) 260 (midpoint)	[28]
Freeze-dried sucrose	407 (onset) 239 (midpoint) 153 (endset)	[10]
Freeze-dried sucrose	360	[6]
Melt	341 and 320	[18]
Melt	350	[29]
10% sucrose solution in water	230 (midpoint)	[30]

Water has a strong effect on the properties of amorphous materials. It decreases the glass transition temperature of glassy sugars and biopolymers, which can be described by Gordon–Taylor equation [31]. Water acts as a plasticizer; it increases the global mobility of the glassy matrix, which accelerates relaxation processes. In formulations of proteins, the relaxation processes in the sucrose-water glassy matrix can have a big impact on protein molecular motions and as a consequence on protein denaturation and aggregation. While protein degradation is accelerated by water molecules below the T_g [32, 33], the impact of relaxation of the glassy matrix on this process is still unclear.

This work aims to evaluate the activation energy of the relaxation process in the sucrose-water system. For that, we critically examine the procedures of activation energy calculations from different types of DSC thermal cycles and also try to determine activation energies from single scan experiments. Furthermore, we examine the effect of water on the activation energy of the glass transition and the effect of sucrose thermal history, in particular, compare samples obtained by melting and freeze-drying.

Materials and methods

Materials

Crystalline sucrose (CAS 57-50-1) > 99.5% purity, was purchased from Sigma Aldrich. Milli-Q purified water (ELGA, Purelab Flex) was used for all experiments.

Preparation of amorphous sucrose by freeze-drying

Amorphous sucrose was prepared by lyophilization from aqueous solutions using a freeze dryer (Epsilon 2–4 LSC-plus, Martin Christ GmbH, Germany). The samples with solids content of 10 mass% were freeze-dried in 6 mL clear glass vials out in clear tubing glass vials of 6 mL volume and 22 mm of diameter (Schott, Germany) filled in with 2 mL solution. The vials were loaded at room temperature. During freezing, the temperature was lowered to $-45\text{ }^{\circ}\text{C}$ in 3 h held isothermally for 2 h. Then a shelf was heated to $4\text{ }^{\circ}\text{C}$ and the chamber pressure was lower to 0.1 mbar. The primary drying was done at shelf temperature of $4\text{ }^{\circ}\text{C}$ and 0.1 mbar chamber pressure for 16 h. For secondary drying, the temperature was increased to $20\text{ }^{\circ}\text{C}$ in 1 h, the chamber pressure was lower to 0.01 mbar. After the ramp, the shelf was held isothermally in 3 h. At the end of the freeze-drying cycle, the chamber was filled with dry nitrogen, sealed under vacuum, and stored in at $-20\text{ }^{\circ}\text{C}$ until further analysis. No collapse was observed in the vials. The amorphous character of the samples was confirmed by X-ray scattering experiments (data not shown).

Thermal analysis

Samples were analysed using Differential Scanning Calorimeter DSC 1 (Mettler Toledo, Switzerland). Calibration was performed using indium. An empty aluminium crucible was used as a reference. All mass determinations were performed using Mettler AT261 DeltaRange balances. Standard aluminium crucibles ($40\text{ }\mu\text{L}$, Mettler Toledo) were used.

Sucrose-water mixtures with water contents 15–20 mass% were prepared directly in the DSC pans from crystalline sucrose and liquid water. The samples had masses around 25–27 mg. Sucrose was added to the pan and the mass was measured, then the required amount of water was added, the pan was hermetically sealed, and the mass was measured again. These samples were heated at $1\text{ }^{\circ}\text{C min}^{-1}$ to $130\text{ }^{\circ}\text{C}$, above the melting temperature of the binary mixture to get a glassy state. Then samples were subjected to various cooling/heating cycles. The pan's mass was measured after experiments. No significant difference between the sample mass before and after the run was detected, i.e. no water loss occurred during the run. The crucibles were opened after the run and the samples looked like colourless transparent droplets; sucrose caramelization was not observed.

We used equilibration or interrupted equilibration of freeze-dried material at various relative humidities in saturated salt solutions LiCl ($a_w=0.11$), MgCl_2 ($a_w=0.33$), $\text{Mg}(\text{NO}_3)_2$ ($a_w=0.53$), NaCl ($a_w=0.75$) to obtain water content 1–12 mass% in solid samples.

Three types of thermal cycles were run for samples with water contents 15–20 mass%. In the constant heating rate (CHR) experiment, the sample at $130\text{ }^{\circ}\text{C}$ was cooled to $-70\text{ }^{\circ}\text{C}$ at a cooling rate of 20 K min^{-1} , equilibrated for 5 min, followed by a heating step with a ramp of 10 K min^{-1} to $30\text{ }^{\circ}\text{C}$, equilibrated for 5 min. Then cooling step performed at varying cooling rates (20, 10, 5, 2.5, and $1, 0.5\text{ K min}^{-1}$) followed by equilibration for 5 min at $-70\text{ }^{\circ}\text{C}$ and uniform heating step (10 K min^{-1}) followed by equilibration for 5 min at $30\text{ }^{\circ}\text{C}$. In the constant cooling rate (CCR) experiment the cooling rate was uniform (5 K min^{-1}), the heating rate was varied (20, 10, 5, 2.5, 1, and 0.5 K min^{-1}). In equal rates (ER) experiments the same rates (20, 10, 5, 2.5, 1, and 0.5 K min^{-1}) were used for both cooling and heating steps. Equilibration for 5 min at $-70\text{ }^{\circ}\text{C}$ and $30\text{ }^{\circ}\text{C}$ was used every time before cooling or heating.

The melted sucrose from crystals was obtained directly in DSC pan by the following procedure: 5 min equilibration at $25\text{ }^{\circ}\text{C}$, heating 20 K min^{-1} to $190\text{ }^{\circ}\text{C}$, cooling 20 K min^{-1} to -70 , isothermal 5 min, heating 10 K min^{-1} to $100\text{ }^{\circ}\text{C}$.

For freeze-dried samples, the next DSC procedure was performed: 5 min equilibration at $25\text{ }^{\circ}\text{C}$ cooling 20, 10 or 1 K min^{-1} to $-70\text{ }^{\circ}\text{C}$, isothermal 5 min, heating 10 K min^{-1} to $100\text{ }^{\circ}\text{C}$. Exact cooling temperature is specified in the text. Annealing DSC program for freeze-dried samples was

following: 5 min equilibration at 25 °C, heating 1 K min⁻¹ to 60 °C, cooling 20 K min⁻¹ to 70°, isothermal 5 min, heating 10 K min⁻¹ to 100 °C. In this program, DSC pans were pierced.

Glass transitions temperature was determined in 2 ways: as half of the step of the glass transition (bisector, according to ISO 11357-2:1999) and as the inflection point of the step (maximum of a derivative of the DSC signal in the glass transition region). Before evaluation of the experimental data, the time correction of the heat flow was applied. A time constant of 3 s was used in all calculations. All data treatment was performed in MATLAB (The MathWorks, Inc.).

Results and discussion

The work presented below is divided into several sections. In the beginning, we study the effect of DSC thermal cycle design and different methodologies of the glass transition temperature evaluation on the activation energy values. Then we use the obtained activation energy values for simulation of cooling and heating data and validation of the methodologies. Afterwards, a simpler method based on the width of the glass transition region was applied to determine the activation energy from single scan experiments. At the end, we analyze possibilities of the activation energy determination in freeze-dried samples.

Effect of thermal cycle design

Several types of thermal cycles were run to see the effect of experimental conditions on the calculation of activation energy of the glass transition. Three types of DSC experiments were performed: constant heating rate (CHR) experiments, where the cooling rate was varied (20–0.5 K min⁻¹)

while the heating range was 10 K min⁻¹; constant cooling rate (CCR) experiments, where the heating rate was varied, while the cooling rate was 5 K min⁻¹; equal rates (ER) experiments where the cooling rate was equal the heating rate. Below we compare the shapes of the cooling and heating curves obtained in the three types of experiments.

Cooling curves

The results of the CHR and ER DSC experiments performed with samples containing about 16 mass% of water are shown in Fig. 1. Reliable DSC curves were not obtained for cooling rates 20 and 10 °C min⁻¹ (data not shown) because the calorimeter could not accurately maintain a high cooling rate at low temperatures. The general shapes of the DSC curves do not strongly depend on the thermal cycle type since in both cases the experiments start from a thermodynamically stable liquid state. However, such details as baseline slope and stability vary between the two types of experiments, which can influence the accuracy of activation energy calculations. All cooling curves presented in Fig. 1 have a step of the heat capacity indicating a glass transition and its temperature depend on the cooling rate: increasing cooling rate leads to higher glass transition temperatures as documented previously [30, 34, 35].

Heating curves

The shape of a DSC heating curve depends on the whole thermal history of the sample including both cooling and heating steps, not only heating but also cooling steps. Therefore, unlike the case of the cooling curves, we present three sets of data: CHR, CCR and ER (Fig. 2). In contrast to the cooling curves, the shapes of the DSC curves obtained in different types of experiments are different, with substantial

Fig. 1 Cooling curves obtained in CHR experiment **a** water content 16.8 mass% and ER experiment **b** water content 16 mass%. Different colours correspond to different cooling rates

Fig. 2 Heating curves obtained in the CHR experiment **a** water content 16.8 mass% (heating rate $10\text{ }^{\circ}\text{C min}^{-1}$), the ER experiment **b** water content 16 mass%, and the CCR **c** water content 16.7 mass%. Different colours correspond to different cooling rates for the CHR and heating rates in the ER and CCR

variations in glass transition temperatures and the intensities of enthalpy recovery peak. The CHR experiments have shown the almost no shift in the T_g but an increase of enthalpy recovery peak upon increasing scan rate (Fig. 2a). The ER experiments have shown smallest enthalpy recovery peaks in agreement with a previous study [36]. The intensity of the recovery peak in the CCR experiment depends on the heating rate (but weaker than the dependence on cooling rate in CHR experiments). The glass transition temperature in the ER and CCR experiments shifts to higher values upon the increase of heating rate.

Calculations of activation energy from scan rates dependences

The dependence of the glass transition temperature on the cooling rate allows calculation of the activation energy E_{act} , using Eq. 1 proposed by Moynihan [35]. However, as it will be discussed below, this method of calculation is often applied on glass transition temperatures obtained in heating experiments and also on data on enthalpy recovery peak position (Eq. 2 and 3, respectively). Moreover, for calculations based on the peak positions, Kissinger equation, which differs by the term T_p^2 is sometimes used. Here we will call Eqs. 1–3 Moynihan equations and Eq. 4 Kissinger equation.

Moynihan equations:

$$\frac{d \ln |q^-|}{d(1/T_g)} = -\frac{E_{\text{act}}}{R} \quad (1)$$

$$\frac{d \ln |q^+|}{d(1/T_g)} = -\frac{E_{\text{act}}}{R} \quad (2)$$

$$\frac{d \ln |q^+|}{d(1/T_p)} = -\frac{E_{\text{act}}}{R} \quad (3)$$

Kissinger equation:

$$\frac{d \ln \left(\frac{q^+}{T_p^2} \right)}{d(1/T_p)} = -\frac{E_{\text{act}}}{R} \quad (4)$$

In the equations above, T_g is the glass transition temperature (either midpoint of inflection point) in K, T_p is the maximum of the recovery peak in K, $|q^-|$ is the absolute cooling rate in K s^{-1} , $|q^+|$ is the absolute heating rate in K s^{-1} , R is universal gas constant in $\text{J mol}^{-1} \text{K}^{-1}$, E_{act} is the activation energy of the glass transition in J mol^{-1} .

The Moynihan equation (Eq. 1) was originally developed for cooling experiments [35] and is commonly used for calculations of the activation energy. However, in many

cases, it is problematic to collect reliable cooling DSC curves. The temperature program is not so precisely controlled during cooling as during heating scans. Moreover, due to supercooling [37], the temperature calibration is less reliable in cooling scans. In consequence, in most research papers the glass transitions are evaluated from heating DSC experiments and then the activation energies are calculated using Eq. 2. One more problem is a meaningful determination of the glass transition temperature. There is no golden standard of the glass transition determination, there are several methods used by different researchers, the differences in the obtained T_g values can be several degrees. We consider two ways of the glass transition determination in this work: a half-step of the heat capacity change and the inflection point. The half step method defines the glass transition temperature as an intersection point of the bisector of the left and right baselines and the DSC curve. The inflection method defines the glass transition temperature as an inflection point of the step at higher temperatures [38]. As a consequence, there is no universally accepted procedure for calculation of activation energy either. The method based on cooling DSC data seems to have the most clear theoretical basis. On the other hand, due to technical problems mentioned above, it would not necessarily give the most accurate results. For example, it was shown in [13], that using the peak maximum (Eq. 3) instead of the glass transition step gives more accurate results in calculation of activation energy. Since Kissinger equation (Eq. 4) differs from Eq. 3 only by the T_p^2 term that is not strongly dependent on heating rate, the accuracies of the calculations using Eqs. 3 and 4 are expected to be similar.

We used Eqs. 1–4 for the evaluation of activation energy in several sucrose-water samples in the range of water contents of 16–19 mass% (Table 2). Activation energies obtained from cooling experiments show values substantially higher than those obtained on heating. A similar trend was observed before for sorbitol and fructose [34]

and polyvinylpyrrolidone [10]. The exact reason for that is unclear, but the following factors may play a role. The scan rate range for cooling experiments is shorter (0.5, 1, 2.5, and 5) than in heating experiments, where two more fast scan rates (10 and 20 K min⁻¹) are present. The dependence of $\ln |q|$ on $1/T_g$ has a negative second derivative (see Figure S1), which implies a steeper slope of the dependence at lower scan rates and thus higher activation energy. However, the existence of the second derivative in the data can also indicate possible problems with temperature control upon cooling, which are insignificant for most types of DSC experiments, but substantial enough to influence calculations of activation energy. Moreover, the standard deviations of E_{act} obtained in cooling scans (see Table 2) are much higher than for heating scans, which supports the interpretation described above.

In order to further investigate if the disagreement between the values presented in Table 2 is a result of technical imperfections of the calorimetric method or a result of inapplicability of Eqs. 1–4 to the data obtained by certain types of thermal cycles, we tested these equations on computer-generated heat capacity data. The data were generated using Tool-Narayanaswamy-Moynihan (TNM) model defined by two equations [12, 39, 40]:

$$\Phi(t) = \exp \left[- \left(\int_0^t \frac{dt}{\tau(T, T_f)} \right)^\beta \right] \quad (5)$$

and

$$\tau(T, T_f) = A \cdot \exp \left[x \frac{\Delta E_{act}}{RT} + (1-x) \frac{\Delta E_{act}}{RT_f} \right] \quad (6)$$

where $\Phi(t)$ is the relaxation function of the given material property, t is the time, τ is the relaxation time, β is the non-exponentiality parameter, A is the pre-exponential factor, x is the non-linearity parameter, T_f is the fictive temperature. The fictive temperature was calculated numerically [41] using a temperature step ΔT of 1 K:

Table 2 Activation energy of glass transition, samples (16–19 mass% water), scan rate range 0.5–20 K min⁻¹, kJ mol⁻¹

Type of thermal cycle	Cooling		Heating			
	Moynihan, Eq. 1, $T_g, 1/2$ (0.5–5 K min ⁻¹)	Moynihan T_g , Eq. 1, inflection (0.5–5 K min ⁻¹)	Moynihan, Eq. 2, $T_g, 1/2$	Moynihan Eq. 2, T_g inflection	Moynihan, Eq. 3 peak	Kissinger Eq. 4 peak
CHR	426 ± 42	411 ± 87	–	–	–	–
CCR	–	–	308 ± 15	256 ± 18	174 ± 7	170 ± 7
ER	381 ± 27	520 ± 115	254 ± 6	213 ± 19	193 ± 6	189 ± 6

The presented values are the average values for 3 different samples with slightly varied water concentrations

$$T_{f,n} = T_0 + \sum_{j=1}^n \Delta T_j \left\{ 1 - \exp \left[- \left(\sum_{k=j}^n \Delta T_k / q_k \tau_k \right)^\beta \right] \right\} \quad (7)$$

The activation energy value of 220 kJ mol^{-1} (similar to typical values shown in Table 2) was used for generating the data. The values of β and x were taken as 0.5 and 0.7, respectively. Cooling and heating curves corresponding to CHR, CCR and ER thermal cycles were simulated; examples of simulated data for ER thermal cycles are presented in Fig. 3. Then the activation energy was calculated from simulated data according to Eqs. 1–4. Obtained values of the activation energy are shown in Table 3.

The activation energies calculated from the cooling scans are in good agreement with the value used for the generation of the heat capacity curves. Likewise, the activation energies calculated from heating runs of ER thermal cycles are in good agreement with the expected value. In contrast, the values obtained from CCR cycles are not close to the initial value of activation energy in simulated heating curves. This suggests that CCR cycles should not be used for the evaluation of activation energy with Eqs. 1–4.

Comparison of Tables 2 and 3 reveals that while E_{act} calculations from generated data show a good agreement between ER heating data and cooling data, this agreement

is not observed in calculations based on real DSC scans. Since the cooling scans are stronger affected by technical problems (as mentioned above), we concluded that calculations based on heating data of ER cycles are most reliable. In particular, since the Moynihan (ER, inflection) and Kissinger methods give the most consistent results working with both simulated and DSC data, for further calculations their mean value of 201 kJ mol^{-1} was taken as the activation energy for this concentration range (16–19 mass% water). The difference between the input value (220 kJ mol^{-1}) and obtained results (Table 3) for the ER cycle is about 2.5%, which is similar or lower than the systematic error of activation energy determination observed in previous works [42].

Using the fictive temperature T_f for determining the activation energy from cooling scans is more theoretically justified than using T_g because T_f has a clearer interpretation in the frame of TNM theory. For comparison, we evaluated the activation energy from computer-generated data using the fictive temperature values, see Table 3 and Figure S6 in SI. Calculations based on T_f give similar results as those based on T_g (cooling and heating curves) and T_p . On the other hand, T_f determination is usually not provided in commercial software of calorimeters and hence requires additional programming for DSC data evaluation.

Fig. 3 Glass transitions in C_p data generated using TNM model for ER scans. $E_a = 220 \text{ kJ mol}^{-1}$, $\beta = 0.5$, $x = 0.7$. **a** cooling, E_a calculated from Eq. 1: $224.8 \text{ kJ mol}^{-1}$ (half-step) $226.0 \text{ kJ mol}^{-1}$ (inflection point). **b** heating, E_a calculated from Eq. 1: $223.8 \text{ kJ mol}^{-1}$ (half-step) $223.9 \text{ kJ mol}^{-1}$ (inflection point). Different colors correspond with different scan rates

Table 3 Activation energies calculated from computer-generated TNM data using Eqs. 1–4

Type of thermal cycle	Cooling			Heating			
	Moynihan, Eq. 1, T_g , $\frac{1}{2}$ (0.01–20 K min^{-1})	Moynihan T_g , Eq. 1, inflection (0.01–20 K min^{-1})	Moynihan T_f , Eq. 1, (0.01–20 K min^{-1})	Moynihan, Eq. 2, T_g , $\frac{1}{2}$	Moynihan Eq. 2, T_g inflection	Moynihan, Eq. 3 peak	Kissinger Eq. 4 peak
CHR	225	226	224	–	–	–	–
CCR	–	–	–	365	264	312	307
ER	225	226	224	224	224	221	216

Parameters of the model: $E_a = 220 \text{ kJ mol}^{-1}$, $\beta = 0.5$, $x = 0.7$

Calculation of activation energy from the width of the glass transition region

Dependence of the glass transition region width on thermal cycle design

A simpler method of the activation energy determination that does not require cycles with varying scan rates is described in the literature [10, 43, 44]. It is based on the correlation between the width of the glass transition region (ΔT_g) measured during heating and the activation energy:

$$\frac{C}{\Delta T_g} = \frac{E_{\text{act}}}{RT_g^2} \quad (8)$$

where C is a constant. Since the Eq. 8 does not require a set of cooling/heating scans, it is possible to determine the activation energy from single scan experiments. Below we will check the dependence of the width of the glass transition region on the scan rate and applicability of the Eq. 8 for the evaluation of the activation energy.

An example of widths of the glass transition region at various experimental conditions is presented in Fig. 4. One can observe changes in ΔT_g with scan rate, type of experiment (ER or CCR), and methodology of evaluation of the glass transition temperature. The transition width increases with increasing scan rate, in agreement with the trend reported in the literature [10, 43]. The transition width is greater for the ER scans than for the CCR. The evaluation of glass transition as 1/2 step consistently gives larger glass transition region widths. Plots analogous to Fig. 4 were obtained

Fig. 4 The width of the glass transition region under different experimental conditions, water content 18.3 mass% ($T_g = -39.84$ °C, scan rate 5 K min⁻¹) in ER experiment; water content 18.1 mass% ($T_g = -37.585$ °C, scan rate 5 K min⁻¹) in CCR

using data for several water contents in the range of 16–20 mass%. The ER experiments give bigger widths than the CCR. Besides, there is the discrepancy in width at scan rate 5 °C min⁻¹, where both methods are supposed to give similar values when cooling rate is equal to heating rate since the starting state and thermal histories are equal. One reason might be spontaneous crystallization of sucrose from the sucrose-water melt, but the comparison of the heat capacity steps does not provide a support to this idea. Another reason can be the presence of water concentration gradient in the samples that can be different in different thermal cycles.

The constant C was calculated for the ER and CCR experiments according to Eq. 8, using the activation energy value of 201 kJ mol⁻¹. The value 201 kJ mol⁻¹ is taken as the average of values obtained in ER experiments for the inflection point, peak and the Kissinger equation (Table 2, last 3 columns). The C values expectedly follow the behavior of the width of the glass transition (Fig. 5). The values are found in the range of 3 to 6, the average values for 10 °C min⁻¹ are 5.5 ± 0.2 (1/2 step) and 4.1 ± 0.1 (inflection point) are close to 5.0 ± 0.5 reported in [43].

It is of interest to check if the dependencies observed in DSC experiments are valid in the case of heat capacity data simulated using TNM model. Here we present the width of the glass transition region in simulated data for the ER experiment (Fig. 6.a) and the CCR experiment (Fig. 6.b). To obtain ΔT_g , the TNM simulated curves were evaluated in the same way as the experimental data. One can see similar trends for the width for simulated data (Fig. 6) and experimental data (Fig. 4): ΔT_g increases when the scan rate increases. The glass transition width determined using the

Fig. 5 Dependence of constant C (defined in Eq. 8) on scan rate in heating experiments, water content 18.3 mass% ($T_g = -39.84$ °C, scan rate 5 K min⁻¹) in ER experiment; water content 18.1 mass% ($T_g = -37.585$ °C, scan rate 5 K min⁻¹) in CCR. $E_{\text{act}} = 201$ kJ mol⁻¹

Fig. 6 Simulated data for heating scans in ER (a) and CCR (b, cooling rate 5 K min^{-1}) experiments. $E_a = 220 \text{ kJ mol}^{-1}$, $\beta = 0.5$, $x = 0.7$

inflection point has a weaker dependence on the scan rate (compared to the half-step case) in simulated data (Fig. 6). For the inflection point the variation of ΔT_g is about 10% or less in the studied range of scan rates for both ER and CCR experiments. Hence the use of the inflection point seems to be more accurate for the activation energy calculations using this method.

Effects of non-linearity and non-exponentiality parameters on the width of the glass transition region

Further, we check the effect of β and x parameters of TNM equation on the width of the glass transition region in simulated data. For that, heat capacity data were simulated using the same activation energy of 220 kJ mol^{-1} and varying values of β and x . The effect of non-exponentiality parameter β is illustrated in Fig. 7a.

Clearly, the glass transition width is strongly dependent on the value of β parameter, the results obtained by both methods (half-step and inflection point) follow the same trend. The data are shown in Fig. 7. a indicates that ΔT_g is inversely proportional to β , in line with the idea by Pikal et al. who suggested that Eq. 8 allows calculation of βE_{act} rather than E_{act} [43].

In case of the dependence of ΔT_g on the nonlinearity parameter x (Fig. 7.b), the half-step and the inflection

point methods of the glass transition width determination provide substantially different results. While ΔT_g values determined using the half-step method differ by a factor of 2 in the selected range of x , the T_g width determined using the inflection point has a variation of about 15%. This confirms that the inflection point method is preferable to use for the activation energy calculation.

The results shown above indicate that ΔT_g values determined using the inflection point method are expected to be relatively stable to variations of scan rate and nonlinearity parameter x . On the other hand, ΔT_g is strongly dependent on the non-exponentiality parameter β . Hence, Eq. 8 can be used for the approximate calculation of activation energy provided that coefficient C is determined from data on a system that has a β value similar to that of the system under study. The method based on the width of the glass transition region is approximate, but nevertheless, it can give a useful estimate of the activation energy keeping in mind a very large variation of the activation energy values obtained by other methods, see Table 1. Below we will use Eq. 8 and constant C obtained in thermal cycle experiments to calculate the energy barrier of the relaxation process from single scan experiments.

Fig. 7 The dependences of the width of the glass transition region on β (a) and x (b) calculated from simulated TNM model data for $E_a = 220 \text{ kJ mol}^{-1}$, $q = 5 \text{ K min}^{-1}$

Evaluation of activation energy from single scan experiments

Performing thermal cycles with different cooling and heating rates with one sample might be labor-intensive and even not possible for some types of samples. For instance, crystallization or chemical degradation of the material can occur during an experiment. As described above, we calibrated constant C from the data obtained in thermal cycles with different heating rates, the next step is applying Eq. 8 for the single scan rate runs. The single scan experiments were performed at the most used in scanning calorimetry heating rate 10 K min^{-1} . Two types of sucrose-water mixtures were studied: quench-cooled melts and freeze-dried samples. The widths of the glass transition for samples with different water contents and thermal histories were evaluated and presented in Fig. 8.

In samples obtained from the melt, when thermal history of the sample is known, the ΔT_g values are scattered from 7.7 to 10.5 with a small decreasing trend. The freeze-dried materials exhibit much smaller ΔT_g values and show a decreasing trend upon the increase of T_g (Fig. 8, blue squares). To understand this pronounced difference in the behavior between melted and freeze-dried samples, it is instructive to compare the DSC signals obtained from the two types of samples.

The shapes of DSC curves of quench-cooled sucrose and freeze-dried sucrose are substantially different (Fig. 9). The melted material has a classical shape of glass transition

Fig. 8 The width of the glass transition region in single scan rate experiments. Samples obtained by melting of the sample are marked as black cycles, freeze-dried samples are marked as blue squares. The three points around -40° to 30° C were taken from the thermal cycle ER experiments. The points between 0° C and 70° C were taken for cooling rates from 1 to 20 K min^{-1} and the heating rate 10 K min^{-1}

Fig. 9 DSC scans heating scan (10 K min^{-1}) of glassy dry sucrose with different thermal histories. The black curve is the second scan of crystalline sucrose (water content 0 mass%), the blue curve is the first scan of freeze-dried (FD) material with annealing (water content 0 mass%), the red curve is the first scan of freeze-dried sucrose without annealing (water content 2 mass%)

region—a step of heat capacity with a small peak afterwards, related to enthalpy recovery. In the freeze-dried annealed material (the blue curve) it is easy to notice two different regimes before the glass transition step. Below 20° C the signal can be described as a typical baseline of the glassy material, but at about 20° C its slope changes. This endothermic deviation from the baseline in DSC heating scans of glassy sucrose was reported previously [45] and attributed to the sub- T_g phenomenon.

The exothermic peak after the glass transition shows that the freeze-dried material crystallizes upon heating while the melted material does not. It was shown that the melting of sucrose crystal can involve the partial decomposition of the substance [46]. One can speculate that the melted sample has some products from the partial chemical decomposition of sucrose after melting. These compounds can prevent crystallization. The freeze-dried powder was not exposed to high temperatures, there are no impurities that can hamper the packing of molecules into the crystalline lattice.

The shapes of the recovery peaks of the two materials are different: the freeze-dried samples exhibit sharper peaks. Therefore, the derivative in the glass transition region of freeze-dried material is higher and the formal T_g width decreases. The decrease of the ΔT_g for the freeze-dried samples leads to unrealistic results of calculation of the activation energy for the dry sucrose of about $900\text{--}1000 \text{ kJ mol}^{-1}$. Therefore, we do not use the ΔT_g data obtained for freeze-dried samples for calculations of activation energy.

The width of the glass transition region (for melted samples) was used for the calculation of the activation energy of

the relaxation process according to Eq. 8. The coefficient C was chosen from the thermal cycle experiments at the same conditions (cooling/heating rate) as in the single scan experiment. The activation energy of the glass transition increases with the increase of sucrose concentration (Fig. 10) due to the increase of the glass transition temperature of the mixture (see Eq. 8). Water acts as a plasticizer and decreases the activation barrier. Our data are in good agreement with data obtained in mechanical spectroscopy experiments and viscosity tests.

TNM fitting

Besides the methods considered above, the activation energy can be obtained from the direct fitting of DSC data by TNM (Tool-Narayanaswamy-Moynihan) model. In addition, the values of non-exponentiality and non-linearity parameters can be obtained in this procedure. On the other hand, this method requires high-quality DSC data and a strictly controlled thermal history of the sample. The later requirement should however be met for all reasonable methods of accurate calculations of activation energy (a possible exception from this rule is discussed in the next section). In the procedure used in this work, the non-linear least-square fitting was performed in MATLAB. In this fitting procedure, a heat capacity curve for the whole cooling-heating cycle is generated using Eqs. 5–7, then only a part of the generated curve corresponding to the glass transition observed upon heating is used for optimization of the fitting parameters.

Fig. 10 The activation energy of the glass transition in the sucrose-water system. . Blue symbols show data obtained in this work using: a method based on the width of the glass transition region (circles), the minimal model for freeze-dried samples (square) and TNM fitting (star). Blue dashed line is a linear approximation of all data points from this work. Literature data obtained by mechanical spectroscopy in [50] are marked as a downward-pointing black triangle, viscosity data [51] are presented as black stars

An example of the fitting results for sucrose-water system is shown in Fig. 11.

As one can easily see, the obtained TNM fit almost perfectly reproduces the experimental data, which suggests that the fitting parameters are accurate. The obtained value of activation energy (179 kJ mol^{-1}) is in reasonable agreement with the data obtained from heating cycles (Eqs. 2–4). The value of the non-exponentiality parameter β is close to 0.5 and the non-linearity parameter x is close to 0.8.

The non-exponentiality parameter β is reflecting the distribution of relaxation times [40]. If β is equal to 1, there is one relaxation process, while lower values correspond to a broader spectrum of relaxation times. The obtained value for sucrose-water system of about 0.5 can be compared with the values for spray-dried sucrose of $\beta = 0.61$ (below the glass transition) and 0.69 (above) measured by dielectric spectroscopy [28]. The value of $\beta = 0.58$ was reported for aspartame-sucrose formulations from TNM fitting [47].

The non-linearity parameter x is reflecting relative contributions to the activation energy governing the glassy and structural changes [40]. Interestingly, the values of β and x parameters are positively correlated [23]. The value for sucrose-water system obtained in this study is about 0.8. While to the best of our knowledge the data of nonlinearity parameters for sucrose-water system were not presented in literature before, one can mention the value of $x = 0.43$ for aspartame-sucrose formulation obtained from TNM fitting [47].

For the fitting procedure used here, the full thermal history of the sample needs to be known. Hence it can be used for assessment of the activation energy in amorphous samples obtained via melting if the data obtained upon cooling

Fig. 11 The experimental DSC data on sucrose-water system with water content 16 mass% (black curve) and the result of the fitting of the data with TNM model. The cooling and heating rates are 10 K min^{-1}

from the liquid state is available. For amorphous samples obtained by freeze-drying, this condition cannot be met. Still, as we will show below, fitting using TNM model can be used as an additional tool for characterization of freeze-dried samples.

Freeze-dried samples

As we showed above, the use of Eq. 8 for freeze-dried sucrose samples gives unrealistically high values of activation energy due to specific features of the peak shapes. Therefore, another approach should be used for this type of sample. The shapes of DSC curves of freeze-dried samples are strongly affected by the pronounced enthalpy recovery peaks, see Figs. 9 and 12.a. These peaks occur at the end of the glass transition and hence characterize the transition to the equilibrium liquid. At these conditions, one can expect that non-linearity [48] and non-exponentiality parameters characterizing the glassy state have a lower influence on the system behavior. This opens up an opportunity to use simpler models of glass transition for the description of the enthalpy recovery peaks. Recently, a so-called minimal model of glass transition was developed in our group [49] for the description of glass transition in systems not exhibiting substantial non-linearity and non-exponentiality. Even though this condition is typically not met, this model can still be useful in certain cases because it provides analytical solutions to several glass transition-related problems. According to this approach, the activation energy can be obtained from DSC heating scan using the following equation:

$$\frac{E_{\text{act}}^*}{RT} - \ln \left(\frac{1}{q\tau_0} \right) = \ln \left(\int_{T_e}^T (\Delta C_p - C_p(x)) dx \right) - \ln C_p(T) \quad (9)$$

where ΔC_p is the heat capacity step and T_e is the temperature where the normalized heat capacity crosses the baseline of the glassy state after possible local minimum.

The experimental data shown in Fig. 9 (red curve, water content 2 mass%) was used for evaluation. For fitting that

was performed in MATLAB, the right-hand side of the Eq. 9 was plotted as a function of reverse temperature, see Fig. 12b. The linear part of this plot (corresponding to the ascending part of the peak in Fig. 12a) was used for the fitting. The obtained value is $E_a = 405.2 \text{ kJ mol}^{-1}$ and $\tau_0 = 2.26 \times 10^{-64} \text{ s}$. This activation energy value is in a good agreement with results calculated from Eq. 8 using the melt data (Fig. 10). The main advantage of Eq. 9 over Eq. 1–4 is that variation of scan rate is not needed for the calculation. However, the minimal model does not take into account the non-exponentiality parameter β . Since the fitting value 406 kJ mol^{-1} is in agreement with the previous results, it suggests that at chosen conditions (moving towards equilibrium liquid) parameter β is close to 1.

In order to prove it and get an independent estimate of the value of β parameter, we performed TNM fitting of the same DSC data. As we mentioned above, for fitting using TNM model one needs to know the thermal history of the sample, including the cooling rate used during glass formation. For freeze-dried samples it is undefined, on the overhand the thermodynamic state of the glass can be approximated as obtained from a liquid using a certain cooling rate. To implement this idea, we used several nominal cooling rates together with the heating rate of the experiment (10 K min^{-1}) for fitting the data. The results of the calculation of activation energy in this procedure turned out to be sensitive to the used value of the cooling rate. On the other hand, the non-exponentiality parameter β had values close to 1 in a broad range of tested nominal cooling rates. For the illustration of fitting results, in Fig. 13 we show the fit obtained with a nominal cooling rate of 0.005 K min^{-1} , which gives the activation energy value close to that obtained from Eq. 9. The obtained values of the fitting parameters are: $E_a = 405.1 \text{ kJ mol}^{-1}$, $A = 3.9 \times 10^{-65} \text{ s}$, $\beta = 1$, $x = 0.92$. The obtained value of beta is exactly 1.0, which is due to the limitation imposed on the value of this parameter in the fitting procedure (from 0.0 to 1.0). If no limitation is imposed, it obtains a value of slightly higher than 1. This result confirms that the minimal model approach can be used for the

Fig. 12 The data on freeze-fried sucrose-water sample (water content 2 mass%). Heating rate 10 K min^{-1} . **a** Experimental DSC data. **b** Same data plotted in coordinates of Eq. 9. The fitting range is 320–323 K

Fig. 13 The experimental DSC data on freeze-dried sucrose-water sample with water content 2 mass % (black curve) and the result of the fitting of the data with TNM model. The heating rate is 10 K/min. The cooling rate is chosen as 0.005 K min⁻¹

characterization of activation energy in freeze-dried and annealed samples.

The TNM fitting of the data is in reasonable agreement with the experiment (Fig. 13), however, this agreement is not as good as in the case of melts (compare with Fig. 11). The main deviation in the fitting occurs before the recovery peak (310–315 K), which can be a result of the endothermic event similar to observed for the annealed sample (blue curve in Fig. 9). This sub- T_g endotherm was not possible to fit or describe with any of discussed models (TNM and minimal model). Hence, this phenomenon presents an additional complication for the calculation of activation energy in sucrose-water mixtures, which can partly explain the diversity of the E_{act} values shown in Table 1.

Conclusions

Three thermal cycles were used for the activation energy determination of the glass transition by several methodologies. Despite the fact that the use of cooling curves is better justified theoretically, the evaluations based on a shift of T_g and T_p on heating curves give the most accurate values in simulated data and the most reproducible results in experimental data. Technical problems as inaccurate thermal flow measurements on cooling and high-temperature gradients cause data distortions. Out of three tested thermal cycles, data obtained from ER thermal cycles is the most consistent and accurate. A big impact on data has the way of the glass transition temperature determination. Our simulated data shows that the inflection point is less affected by

non-exponentiality and non-linearity parameters β and x as well as by scan rate.

The activation energy calculation method based on the width of the glass transition region gives a reasonable estimation without a set of scan rates. However, this method has a limitation: if β of the studied sample strongly differs from that of the sample used for the coefficient C calculation, the results can be inaccurate. In other words, the T_g width method should be applied for the samples with similar nature of the relaxation process.

It is not possible to use well-established methods of activation energy assessment for freeze-dried samples. The shape of the glass transition curve differs between freeze-dried and melted materials: the DSC curves of freeze-dried samples have a sharp enthalpy recovery peak, which leads to distortion of results. We propose to apply the minimal model of glass transition evaluation for freeze-dried samples. The TNM fitting confirms that the enthalpy recovery peak in freeze-dried samples can be described without considering non-exponentiality.

In order to obtain meaningful results in the activation energy calculation one should follow recommendations: use freshly prepared samples with known thermal history, samples that do not have a pronounced recovery peak on the heating scan, use ER thermal cycle, and use Moynihan equation for heating scans to evaluate the scan rate dependence of peak maximum or inflection point temperature.

The activation energy of the relaxation process in sucrose–water system decreases with increasing water content. Water molecules act as lubricants, facilitating translational motions of sugar molecules.

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