

Research Paper

Comparative Investigation by Two Analytical Approaches of Enthalpy Relaxation for Glassy Glucose, Sucrose, Maltose, and Trehalose

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Purpose. In an effort to understand the stability of glassy sugars such as glucose, sucrose, maltose, and trehalose, the molecular mobility below the glass transition temperature (T_g) was investigated by an enthalpy relaxation measurement with differential scanning calorimetry (DSC).

Methods. The glassy sample was aged over several days at ($T_g - 10$) K to ($T_g - 30$) K, before a DSC heating scan was taken. The relaxed enthalpy (ΔH_{relax}) was estimated from the endothermic peak area. The enthalpy relaxation time was analyzed from the time course of ΔH_{relax} using two different approaches; Kohlrausch–Williams–Watts (KWW) and extended Adam–Gibbs (exAG).

Results. τ^{KWW} , which is defined as the mean average enthalpy relaxation time in a distribution, and τ_0^{eff} and $\tau_{\infty}^{\text{eff}}$, which correspond to the enthalpy relaxation time of the initial minimum and final maximum cooperative rearrangement region, were estimated by KWW and exAG, respectively. And three activation energies for enthalpy relaxation were calculated from the Arrhenius plot.

Conclusions. Although these ΔE s originated from different theoretical backgrounds, almost the same trend was observed for a comparison of the values of the four sugars. The finding that the ΔE s of glassy trehalose were the largest among the four sugars may support the reason that glassy trehalose is an effective stabilizer.

KEY WORDS: enthalpy relaxation; extended Adam–Gibbs; glassy state; KWW; trehalose.

INTRODUCTION

Recently, the development of amorphous pharmaceutical products has been increasing. Such pharmaceutical products, which fall into the glassy state, with glass former excipi-

ents, must be physically and chemically stabilized during processing and storage. The principle of stabilizing has been understood in terms of the frozen molecular mobility of the pharmaceutical system below the glass transition temperature (T_g) (1,2). However, the macroscopic properties such as the volume and the enthalpy of the glassy material are known to relax down toward the equilibrium state over actual long-term scales. These relaxation times have been thought to relate to the molecular mobility at the temperature below the T_g (3,4). In the case of some glassy materials used as pharmaceuticals (such as indomethacin), the slow relaxation processes have been investigated by detecting the recovery of the relaxed enthalpy through isothermal physical aging experiments using differential scanning calorimetry (DSC) (4–12), as well as amorphous polymers (13). Sugars are used as an excipient of pharmaceutical products to in order to maintain the function; therefore, some reports on the properties of sugars have been published (4–9). However, very little systematic relaxation data for glassy sugars is available. Among sugar groups, glassy trehalose has gained considerable attention, because a number of living organisms use the glass transition properties of trehalose for self-preservation under severe circumstances, such as the desert (14,15). In addition, the effective role of glassy trehalose as a lyoprotectant to the unstable proteins has often been reported (16). The superior effects of glassy trehalose have been suggested to be due to a number of physical properties of trehalose including the high T_g (15,17), the ability to form intermolecular hydrogen bonds (18), the hydrated volume (19), and its higher fragility (14,20).

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ABBREVIATIONS: β , nonexponential parameter; ΔC_p , heat capacity change at the T_g [J/(g·K)]; ΔE_0^{eff} , activation energy for enthalpy relaxation obtained by Arrhenius plot of τ_0^{eff} (kJ/mol); $\Delta E_{\infty}^{\text{eff}}$, activation energy for enthalpy relaxation obtained by Arrhenius plot of $\tau_{\infty}^{\text{eff}}$ (kJ/mol); ΔE^{KWW} , activation energy for enthalpy relaxation obtained by Arrhenius plot of τ^{KWW} (kJ/mol); ΔH_{∞} , maximum enthalpy relaxation amount (J/g); ΔH_{relax} , enthalpy relaxation amount (J/g); k_B , Boltzmann constant; $\ln A_0^{\text{eff}}$, pre-exponential parameter obtained by Arrhenius plot of τ_0^{eff} ; $\ln A_{\infty}^{\text{eff}}$, pre-exponential parameter obtained by Arrhenius plot of $\tau_{\infty}^{\text{eff}}$; $\ln A^{\text{KWW}}$, pre-exponential parameter obtained by Arrhenius plot of τ^{KWW} ; R , gas constant; S_c , configurational entropy; S_c^* , configurational entropy of single molecule; T_g , glass transition temperature (K); τ , molecular relaxation time in liquid state; τ_0 , pre-exponential parameter; $\tau_{\infty}^{\text{eff}}$, effective enthalpy relaxation time of the final maximum CRR size at the aging temperature (h); τ_0^{eff} , effective enthalpy relaxation time of the initial minimum CRR size at the aging temperature (h); $\tau^{\text{eff}}(t)$, effective enthalpy relaxation time (h); τ^{KWW} , characteristic enthalpy relaxation time (h); $\Delta \mu$, molar activation free energy; Z , the number of molecules in CRR.

However, such explanations have merely been based on the physical properties in the liquid state, supercooled liquid state, or at the T_g . And very few investigations of the properties of the glassy state have been reported.

The purpose of this evaluation is to provide information on the molecular mobility of amorphous trehalose and other sugars such as glucose, sucrose, and maltose by elucidating the temperature dependence of the enthalpy relaxation time, obtained from an isothermal aging experiment using DSC. In the obtained relaxed enthalpy-time course data analysis, however, the following serious problems remain: the enthalpy relaxation process (relaxed enthalpy-time course) in most glassy materials never obeys a simple exponential decay, therefore the physical interpretation of the relaxation process remains unclear (21). A number of approaches have been attempted in order to analyze and accurately predict the enthalpy relaxation process (22). However, among them two different types of approaches have successfully been applied for the most part to aging data lower than T_g . The approaches, which originate from different physical models, are seemingly capable of predicting the nonexponential decay of the enthalpy relaxation. The Kohlrausch-Williams-Watts (KWW) formula approach (4-13) expresses the stretched exponential decay by assuming a special type of distribution of the relaxation time. The second approach is an extended Adam-Gibbs approach, based on decreasing the configurational entropy (S_c) with aging (3,23-26). Other approaches such as the Tool-Narayananaswamy-Moynihan equation (22) seem to have some difficulty in the actual application; however, the theory is worth considering.

In this research, the two approaches described above were used in order to attempt to analyze the enthalpy relaxation time for four types of sugars. The temperature dependence of the enthalpy relaxation time was compared for the four sugars.

MATERIALS AND METHODS

Maltose monohydrate, sucrose, and glucose were purchased from KOKUSAN Chem. Co. Ltd. (Tokyo, Japan), WAKO Pure Chem. Ind. Ltd. (Osaka, Japan), and Sigma-Aldrich Co. (St. Louis, MO, USA), respectively. Trehalose dihydrate was provided by Hayashibara Co. (Okayama, Japan). In order to prepare samples that do not contain water as much as possible, each reagent was melted in an open aluminum DSC pan placed on a heater that was controlled to the melting temperature of each sugar in the anhydrous state reported in literature (27). And the molten sugar in DSC pan was sealed immediately after being quenched to room temperature. After the quenching, the weights of the samples ranged from 5 to 7 mg.

DSC measurement was performed on a Shimadzu DSC-50 (heat flux type), with the temperature and heat capacity calibrated with indium (WAKO Pure Chem. Ind. Ltd., Osaka, Japan) and pure water. All cooling or heating scans conducted in this study were performed at 5 K/min. The temperature sequence for measuring the enthalpy relaxation is shown in Fig. 1. Prior to the mean measurement, the glassy samples were heated and cooled in order to erase the thermal history (process 1). The T_g of each sample was determined from the onset of the endothermic shift upon reheating (process 2). The samples were subsequently cooled-down to

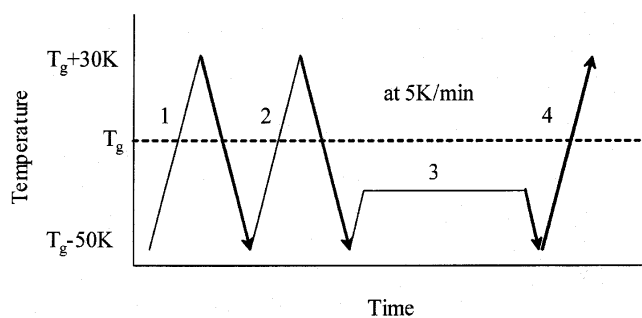


Fig. 1. Temperature sequence in ΔH_{relax} measurements.

($T_g - 50$) K, and reheated up to each aging temperature ranging from ($T_g - 10$) K to ($T_g - 30$) K. Most of the samples were kept for isothermal aging from 2 to 960 h at the aging temperature set in the DSC equipment. The remaining samples were kept in an outer oven (process 3). After the aging period, the samples were cooled once to ($T_g - 50$) K, and again reheated to ($T_g + 30$) K (process 4). The enthalpy relaxation ΔH_{relax} was calculated from the endothermic peak area around T_g , which appeared on the heating curve as shown in Fig. 2.

RESULTS

Determination of Enthalpy Relaxation

DSC heating curves for an amorphous trehalose sample aged under several different conditions are shown in Fig. 2 as an example of a typical result for the materials investigated. Fresh samples showed a simple baseline shift to the endothermic level, representing a typical glass transition feature. Thus, T_g and its heat capacity change (ΔC_p) could be extrapolated

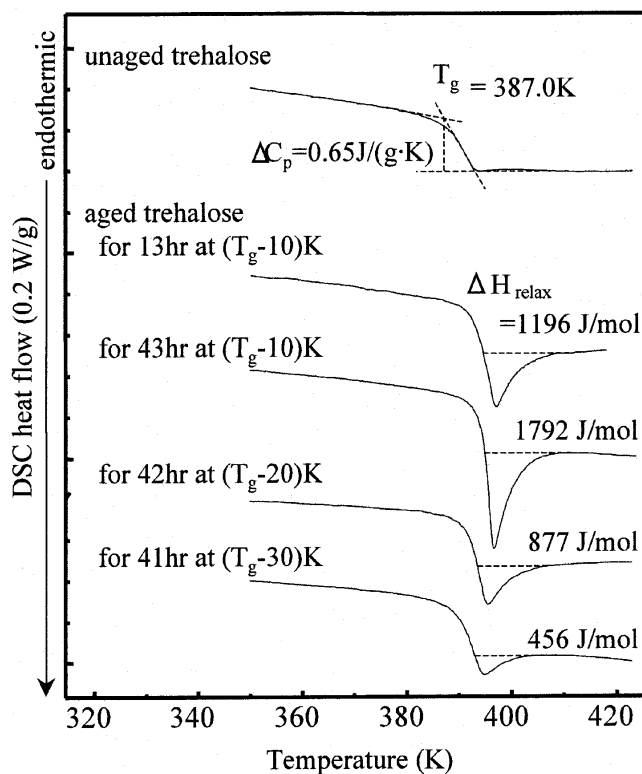


Fig. 2. DSC thermograms for amorphous trehalose with and without various aging treatments.

from the DSC heating curve. Although a T_g value decreases sensitively by the residual moisture, the obtained $T_g = 387.0$ K agreed well with that (i.e., 388.2 K) of an anhydrous amorphous trehalose reported by literature (15). On the other hand, the sharp endothermic peak around T_g , which corresponds to the recovery of enthalpy relaxation, could be detected in the results of samples aged at any temperature. The relaxed enthalpic recovery peak was confirmed to increase with aging time. In addition, the rate of formation of the peak was observed to decrease as the aging temperature dropped. The other samples also showed the same trend as that shown in Fig. 2 (not shown). The endothermic peak area was estimated from the enthalpic relaxation amount (ΔH_{relax}).

Estimation of Characteristic Enthalpy Relaxation Time by KWW

The time course data for the ΔH_{relax} of trehalose are shown in Fig. 3. It is clear that the increase in ΔH_{relax} becomes larger as the aging temperature nears T_g . The solid lines in Fig. 3 represent the results of best fitting to the experimental data using the KWW equation, expressed by the following,

$$\Delta H_{\text{relax}} = \Delta H_{\infty} \{1 - \exp[-(t/\tau^{\text{KWW}})^{\beta}]\} \quad (1)$$

where t , τ^{KWW} , and β are the aging time, characteristic enthalpy relaxation time, and the nonexponential parameter, respectively. The nonexponential parameter β has been generally considered to be related to the distribution of enthalpy relaxation time (4–13), which changes between 0 and 1. In the KWW equation, both τ^{KWW} and β are adjustable parameters that can be determined from fitting. ΔH_{∞} is the maximum enthalpy recovery at any given temperature and is calculated from the following equation,

$$\Delta H_{\infty} = \Delta C_p(T_g - T) \quad (2)$$

where ΔC_p is a heat capacity change at T_g , and T is the aging temperature. However, in the fitting procedure used in this investigation, there was a possibility a significant error may exist for τ^{KWW} , depending on the deviation of β , as described

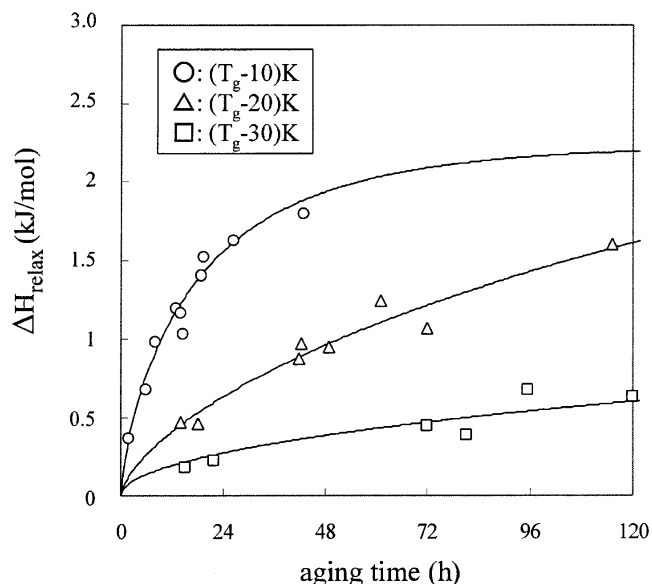


Fig. 3. Time course of ΔH_{relax} for glassy trehalose.

by Hancock *et al.* (28). Despite a remaining similar problem involved in the determination of β , Dudde *et al.* adopted a β value determined using alternative methods, such as dielectric relaxation measurements (5). In addition, Duncan *et al.* used a fixed value for β , $\beta = 1$, although there was no theoretical means for this value (6). In this investigation, a method in which the initial values were $\beta = 0.5$, $\tau^{\text{KWW}} = 100$ in the fitting calculation was adopted in order to get good agreement with values used in other reports (4,9).

The best fitting for trehalose samples was obtained within the standard error of 0.65 to 0.98. The estimated β values varied between 0.46 and 0.75, depending on the aging temperature, and the corresponding τ^{KWW} s were evaluated. Although the figures for other sugars are not shown here, the aging effects and the time course of ΔH_{relax} showed the same trend as those of trehalose.

An Arrhenius plot was obtained in order to elucidate the temperature dependence of the enthalpy relaxation time, as shown in Fig. 4. The plots were determined to have fairly good linearity for every sugar investigated in the temperature range of $(T_g - 10)$ K to $(T_g - 30)$ K. Shamblin *et al.* suggested that the temperature dependence of τ^{KWW} would show little deviation from the Arrhenius formula when it was estimated up a temperature below the T_g (29). Apart from the detailed discussion of whether the application of the Arrhenius formula for τ^{KWW} is reasonable or not, the activation energy (ΔE^{KWW}) and the pre-exponential parameters ($\ln A^{\text{KWW}}$) could be estimated from the slope and intercept, respectively, in Fig. 4. The following results are summarized in Table I; T_g , ΔC_p , the range of β change, ΔE^{KWW} , and $\ln A^{\text{KWW}}$.

Estimation of Enthalpy Relaxation Time Using exAG

An alternative approach to describe the enthalpy relaxation process based on the cooperative rearrangement region (CRR) model proposed by Adams and Gibbs exists (23,24). The original form is expressed by Eq. (3),

$$\tau = \tau_0 \exp(Z\Delta\mu / RT) = \tau_0 \exp(S_c^* \Delta\mu / k_B T S_C) \quad (3)$$

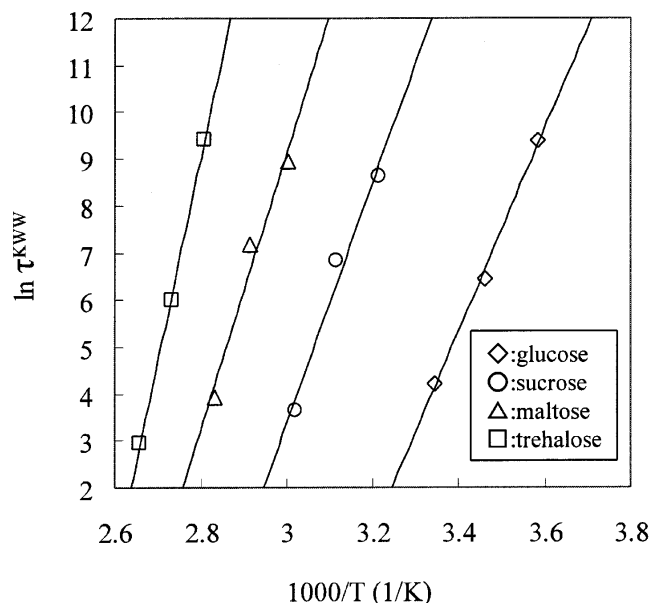


Fig. 4. Arrhenius plot of τ^{KWW} (h).

Table I. The Result of KWW Fitting and Arrhenius Plot of τ^{KWW}

Compound	T_g (K)	ΔC_p [J/(g · K)]	β	$\ln A^{\text{KWW}}$	ΔE^{KWW} (kJ/mol)
Glucose	309	0.79	0.46–0.53	–68.1	179.5
Sucrose	341	0.57	0.53–0.62	–73.2	212.2
Maltose	363	0.65	0.47–0.55	–79.4	245.4
Trehalose	386	0.65	0.51–0.75	–112.5	360.8

where τ , τ_0 , Z , $\Delta\mu$, R , S_c^* , and k_B are the molecular relaxation time, the pre-exponential parameter, the number of molecules in CRR, the molar activation free energy, the gas constant, the configurational entropy of a single molecule, and the Boltzmann constant, respectively. Because $\Delta\mu/R$ or $S_c^*\Delta\mu/k_B$ is a constant, the logarithm of τ becomes proportional to Z/T or $1/TS_c$. Equation (3) has often been used in order to describe the temperature dependence of the molecular relaxation time in the supercooled liquid state. However, it has also been extended to describe the enthalpy relaxation process in the glassy state (23,24). In this extended model, when enthalpy relaxation proceeds during isothermal aging, it is assumed that the molecular numbers involved in the CRR [Z in Eq. (3)] would increase, leading to a decrease in the S_c . Therefore, the τ increases with enthalpy relaxation during isothermal aging. From these considerations, the enthalpy relaxation data for these glassy materials can be expressed by Eq. (4).

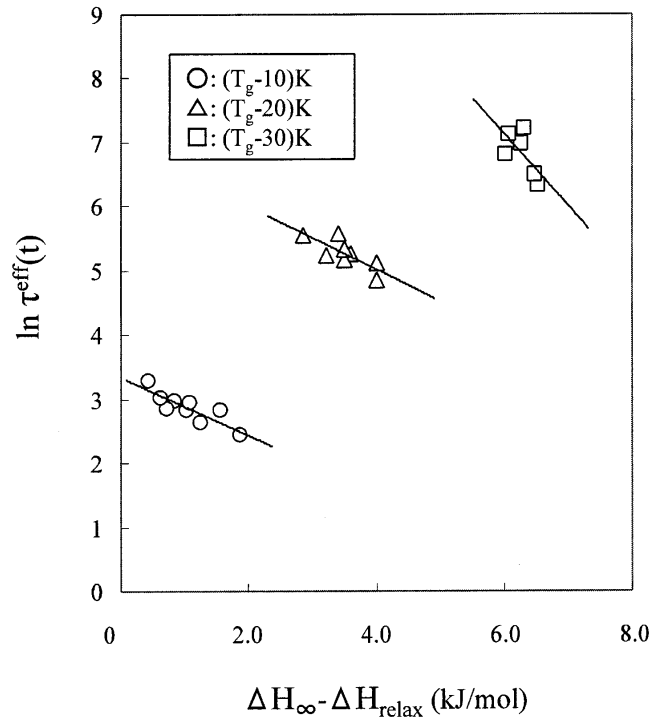
$$\frac{d[\Delta H_\infty - \Delta H_{\text{relax}}]}{dt} = -\frac{1}{\tau^{\text{eff}}(t)} [\Delta H_\infty - \Delta H_{\text{relax}}] \quad (4)$$

where $(\Delta H_\infty - \Delta H_{\text{relax}})$ is the enthalpy deviation from the equilibrium, and $\tau^{\text{eff}}(t)$ is defined namely as the effective enthalpy relaxation time (3,23–26). The logarithm of $\tau^{\text{eff}}(t)$ in addition to the τ of supercooled liquid becomes proportional to $1/TS_c$ for a glassy system (23,24). Likewise, increasing $\tau^{\text{eff}}(t)$ by enthalpy relaxation has also been experimentally presented by decreasing the excess enthalpy $(\Delta H_\infty - \Delta H_{\text{relax}})$ (3,25,26) as follows,

$$\ln \tau^{\text{eff}}(t) = \ln \tau_\infty^{\text{eff}} + B(\Delta H_\infty - \Delta H_{\text{relax}}) \quad (5)$$

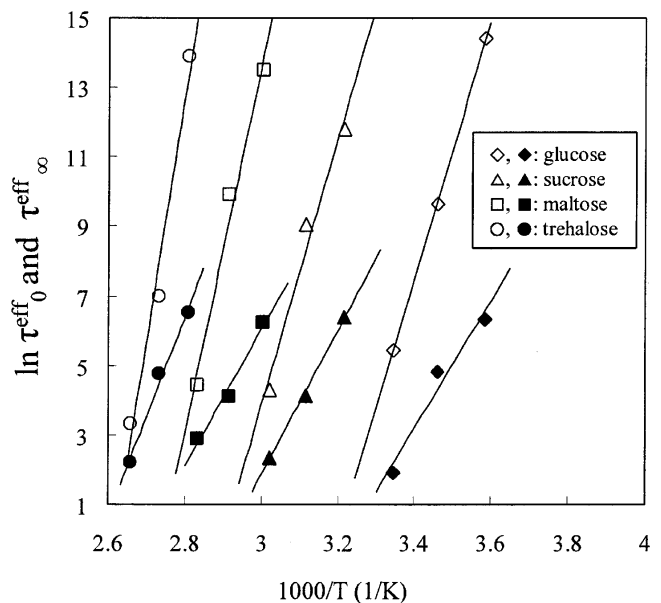
where B and τ_∞^{eff} are constants that can be estimated from the slope and intercept of the $\ln \tau^{\text{eff}}(t)$ vs. $(\Delta H_\infty - \Delta H_{\text{relax}})$ plot, respectively. Here, τ_∞^{eff} corresponds to the $\tau^{\text{eff}}(t)$ at which the enthalpy has relaxed up to the equilibrium state [$\Delta H_{\text{relax}} = \Delta H_\infty$ in Eq. (5)]. All studies of the temperature dependence of $\tau^{\text{eff}}(t)$ have only considered the value of τ_∞^{eff} (3,25,26), although $\tau^{\text{eff}}(t)$ itself changes during aging. According to Eq. 5, the $\tau^{\text{eff}}(t)$ at which the enthalpy has not yet relaxed [$\Delta H_{\text{relax}} = 0$ in Eq. (5)] can be also calculated. In the current investigation, the effective relaxation time is referred to as τ_0^{eff} and is used in addition to τ_∞^{eff} . In the CRR model proposed by Adam and Gibbs, τ_0^{eff} and τ_∞^{eff} correspond to the relaxation time of the minimum and maximum CRR size, respectively, during isothermal aging at a given aging temperature.

The plot of $\ln \tau^{\text{eff}}(t)$ vs. $(\Delta H_\infty - \Delta H_{\text{relax}})$ for trehalose is shown in Fig. 5 as a typical result. Figure 5 clearly shows that $\ln \tau^{\text{eff}}(t)$ increased linearly with enthalpy relaxation at any aging temperature in the range of $(T_g - 10)$ to $(T_g - 30)$ K. This confirmed that the τ_0^{eff} and τ_∞^{eff} could be well estimated from


Fig. 5. $\ln \tau^{\text{eff}}(t)$ (h) vs. $\Delta H_\infty - \Delta H_{\text{relax}}$ for trehalose.

Eq. (5). Other samples also showed almost the same trend as trehalose, though not shown here.

Furthermore, Arrhenius plots of the τ_0^{eff} and τ_∞^{eff} in Fig. 6 were analyzed in order to attempt to obtain the activation energy for enthalpy relaxation, similarly to that attempted in other reports (3,25,26). This approach was used because it has often been successfully applied to various glassy materials in numerous previous studies (3,25,26). However, these previous studies provided only the $\Delta E_{\text{relax}}^{\text{eff}}$, which could be estimated from the Arrhenius plot of τ_∞^{eff} as the activation energy for enthalpy relaxation. The τ_0^{eff} was also obtained in


Fig. 6. Arrhenius plot of τ_∞^{eff} (h) (open) and τ_0^{eff} (h) (solid).

this study, therefore, we calculated not only $\Delta E_{\infty}^{\text{eff}}$, but also ΔE_0^{eff} as estimated from the Arrhenius plot of τ_0^{eff} . The obtained ΔE_0^{eff} and $\Delta E_{\infty}^{\text{eff}}$ represent the activation energy for the enthalpy relaxation in the minimum and maximum CRR state, respectively. Both values are listed in Table II.

DISCUSSION

The temperature dependence of the enthalpy relaxation time was estimated as the activation energy by analyzing the enthalpy relaxation time by both KWW and exAG. In the exAG model, the nonexponential enthalpy relaxation process was assumed to be a single enthalpy relaxation process. Moreover, the enthalpy relaxation rate itself was expected to increase by the development of CRR with the enthalpy relaxation. Thus, the τ_0^{eff} and $\tau_{\infty}^{\text{eff}}$ were thought to correspond to the enthalpy relaxation time of the initial minimum and final maximum CRR size at the aging temperature, respectively. Consequently, the ΔE_0^{eff} and $\Delta E_{\infty}^{\text{eff}}$ corresponded to the activation energies of the enthalpy relaxation of the minimum and maximum CRR. Contrastingly, KWW explains that the origin of the nonexponential enthalpy decay is the distribution of the enthalpy relaxation process. Thus, τ^{KWW} is defined as a mean average enthalpy relaxation time in the distribution. Therefore, the activation energy, ΔE^{KWW} , obtained from the temperature dependence of τ^{KWW} , is expected to be between ΔE_0^{eff} and $\Delta E_{\infty}^{\text{eff}}$. In order to compare the ΔE s for trehalose, the three obtained ΔE s were shown in the Arrhenius plot normalized to T_g in Fig. 7. As expected, each enthalpy relaxation time was increased in the order $\tau_{\infty}^{\text{eff}}$, τ^{KWW} , and τ_0^{eff} in the measured temperature range. Thus, the ΔE obtained by each enthalpy relaxation time was also increased in the order $\Delta E_{\infty}^{\text{eff}}$, ΔE^{KWW} , and ΔE_0^{eff} , as shown in Fig. 7. The other samples also showed the same trend as that shown in Fig. 7 (not shown). These results suggest the validity of the above-mentioned interpretation of KWW and exAG.

It is important to note that the ΔE s in the current study were significantly larger than those of secondary relaxation process (β -relaxation), which has been often reported for relaxation of the glassy system below T_g (30). The β -relaxation, which is readily detected from the dielectric relaxation measurement, has been thought to reflect the local mobility, such as the rotational or vibrational movement of the side chain or group. The ΔE for β -relaxation is generally of the order 10 kJ/mol. Noel *et al.* reported that the ΔE for the β -relaxation of glassy glucose and maltose were 42 and 45 kJ/mol, respectively, as measured by dielectric relaxation (30). On the other hand, the three ΔE s obtained from the current enthalpy relaxation measurements of both sugars below the T_g were of the order 100. The ΔE s for enthalpy relaxation are thought to correspond to the macroscopic mobility of molecules in the

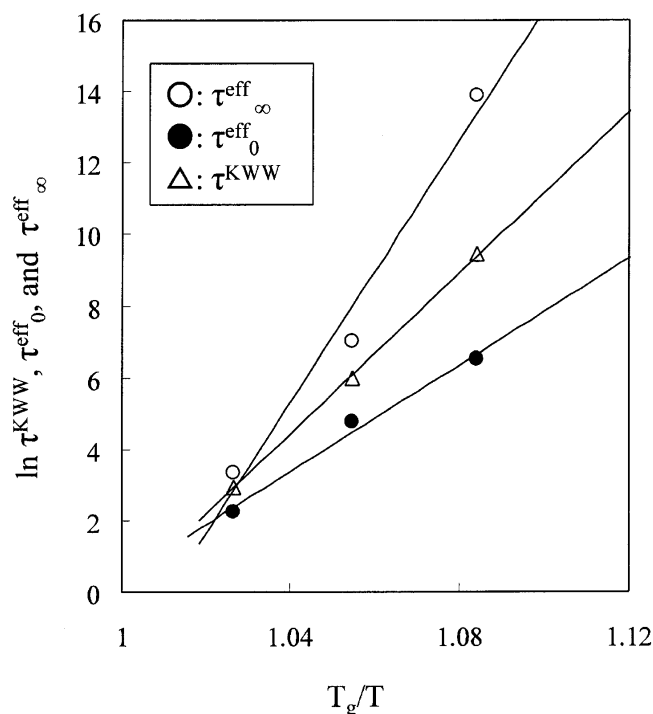


Fig. 7. Three enthalpy relaxation times (h) for trehalose vs. the T_g/T .

glassy state (translational diffusion) in order to directly affect the chemical or physical stability of the glassy product.

Both ΔE^{KWW} and $\Delta E_{\infty}^{\text{eff}}$ increased in the order trehalose, maltose, sucrose, and glucose. Although each ΔE had a different theoretical background, the trends were consistent. On the other hand, ΔE_0^{eff} , an original concept proposed in the current investigation, was increased in the order trehalose, sucrose, maltose, and glucose. These results indicated that all types of ΔE for glassy trehalose were the largest among the four sugars. This finding confirmed that trehalose was the most stable glass below the T_g among the four sugars. Trehalose is known to be one of the most effective glass turnable excipients of the pharmaceutical system. Results of the current study may facilitate an understanding of the mechanism of effective stabilization of pharmaceuticals by trehalose.

CONCLUSIONS

The molecular mobility below the T_g for the amorphous pharmaceutical materials has been a primary focus of current research interests. However, most researches have applied only the KWW approach to the analysis of the enthalpy relaxation time. Herein, the enthalpy relaxation time was analyzed by two approaches, KWW and exAG, in an effort to elucidate the activation energy ΔE . Although the theoretical background of each analysis differed, similar trends were observed for the obtained ΔE s. The finding that the ΔE s for glassy trehalose were the largest among four sugars analyzed suggests that trehalose is the most stable glass below the T_g .

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Table II. The Result of Arrhenius Plot of τ_0^{eff} and $\tau_{\infty}^{\text{eff}}$

Compound	$\ln A_{\infty}^{\text{eff}}$	$\Delta E_{\infty}^{\text{eff}}$ (kJ/mol)	$\ln A_0^{\text{eff}}$	ΔE_0^{eff} (kJ/mol)
Glucose	-119.6	310.9	-59.3	152.8
Maltose	-145.6	441.4	-53.3	164.6
Sucrose	-108.9	320.0	-60.5	172.9
Trehalose	-185.6	589.1	-74.0	238.8

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