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# Influence of compression on water sorption, glass transition, and enthalpy relaxation behavior of freeze-dried amorphous sugar matrices

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ABSTRACT

An amorphous matrix comprised of sugar molecules are frequently used in the pharmaceutical industry. The compression of the amorphous sugar matrix improves the handling. Herein, the influence of compression on the water sorption of an amorphous sugar matrix was investigated. Amorphous sugar samples were prepared by freeze-drying, using several types of sugars, and compressed at 0–443 MPa. The compressed amorphous sugar samples as well as uncompressed samples were rehumidified at given RHs, and the equilibrium water content and glass transition temperature ($T_g$) were then measured. Compression resulted in a decrease in the equilibrium water content of the matrix, the magnitude of which was more significant for smaller sized sugars. Diffusivity of water vapor in the sample was also decreased to one-hundredth by the compression. The $T_g$ value for a given RH remained unchanged, irrespective of the compression. Accordingly, the decrease in $T_g$ with increasing water content increased as the result of compression. The structural relaxation of the amorphous sugar matrices were also examined and found to be accelerated to the level of a non-porous amorphous sugar matrix as the result of the compression. The findings indicate that pores contained in freeze-dried sugar samples interfere with the propagation of structural relaxation.

## 1. Introduction

When an aqueous solution of a sugar is dehydrated under appropriate conditions, the sugar molecules form a random network in which hydrogen bonds are formed between their hydroxyl groups and consequently are converted into an amorphous solid matrix. Amorphous sugar matrices are frequently used as excipients, bulk-forming agents and stabilizing agents for labile ingredients such as proteins in the pharmaceutical industry (Pikal, 1994; Carpenter et al., 1994; Franks et al., 1994). To date, the physicochemical properties of various amorphous sugar solids have been investigated (Levine. and Slade, 1991; Liu, 2006; Liu et al., 2006; Roos, 2010). In addition, glass transition and water sorption data for a number of commonly used sugars have been well documented (Slade and Levine, 1988; Hancock and Shamblin, 1998).

Amorphous sugar matrices are commonly prepared by freeze- or spray-drying, which results in a product with an extremely low density. The compression of such bulky amorphous sugar materials into a tablets leads to an improved handling and a reduction of the volume of the preparation. It is well known that compression affects the mechanical properties of an amorphous sugar matrix including friability (Inghelbrecht and Remon, 1998), porosity (Sebhatu et al., 1997; Sebhatu and Alderborn, 1999; Berggren et al., 2004), and tensile strength (Ndindayino et al., 2002). The physicochemical properties of an amorphous sugar matrix would also be expected to change, more or less, as the result of compression. In actual fact, the tendency for an amorphous sugar matrix to crystallize was reported to be increased by compression (Kawakami et al., 2006; Imamura et al., 2010) while the glass transition temperature ($T_g$) remained unchanged in the absolutely dried state (Imamura et al., 2010). However, substantially less information is available concerning the impact of compression on some of the physicochemical properties of amorphous sugar matrices.

The impact of compression on the water sorption behavior of an amorphous sugar matrix has not been examined in detail, although water sorption is an important aspect of an amorphous sugar matrix (Hancock and Shamblin, 1998). Since, as is generally accepted, water molecules of hydration serve to plasticize an amorphous sugar matrix (Roos and Karel, 1991a), a change in water sorption behavior as the result of compression is likely to alter the relationship between $T_g$ and the water content of an amorphous sugar matrix. Furthermore, it has been proposed that the structural relaxation of an amorphous sugar matrix is closely related to the water

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sorption behavior (Chung et al., 2002; Tanaka et al., 2005) and thus may also be affected by compression.

In this study, the effect of compression on the water sorption behavior of an amorphous sugar matrix was investigated. Amorphous sugar matrices were prepared by freeze-drying aqueous solutions of sugars, followed by compression of the products under a pressure of 0–443 MPa. The compressed amorphous sugar samples as well as uncompressed samples were then rehumidified at given relative humidities (RHs). The equilibrium water contents as well as the $T_g$ values were compared between the compressed and uncompressed amorphous sugar samples and among different types of sugars. The influence of compression on the relationship between water content and $T_g$ values of amorphous sugar matrices was then examined. Changes in the enthalpy relaxation behavior of amorphous sugar matrices, as the result of compression were also investigated and the findings are discussed with respect to relationships with changes in water sorption behavior.

## 2. Materials and methods

### 2.1. Materials

Sucrose, α-maltose, trehalose, α-lactose, $P_2O_5$, LiCl, $CH_3COOK$, and $MgCl_2$ were purchased from Wako Pure Chemical Industries, Ltd., (Osaka, Japan). Maltotriose and -pentaose were products of Hayashibara Biochemical Laboratories, Inc., (Okayama, Japan). Dextrans (MW ca. 1.5k, by enzymatic synthesis; MW ca. 40k from *Leuconostoc* sp.) were products of Sigma–Aldrich Co., (St. Louis, MO). All other chemicals were of reagent grade.

### 2.2. Preparation of amorphous sugar samples

Freeze-drying of aqueous sugar solutions was carried out, as described previously (Imamura et al., 2008). In a typical experiment, 5 mL of an aqueous sugar solution (100 mg/mL) was instantly frozen in liquid nitrogen and then lyophilized at room temperature using a freeze-dryer (UT-80, EYELA TOKYO RIKAKIKAI Co., Tokyo, Japan) connected to a vacuum pump (GLD-100, ULVAC Japan, Ltd., Tokyo, Japan). The freeze-dried samples were thoroughly dehydrated at 37 °C in a vacuum desiccator over $P_2O_5$ for more than 2 days.

### 2.3. Compression and rehumidification of amorphous sugar samples

A 50–200 mg sample of the thoroughly dehydrated amorphous sugar sample was transferred in a 13-mm diameter die and compressed at 0–443 MPa for 5 min using a hand-operated hydraulic compactor (SSP-10A, Shimadzu Rika Co., Tokyo, Japan). The compressed samples as well as uncompressed samples were rehumidified at 25 °C in a vacuum desiccator over saturated solutions of LiCl, $CH_3COOK$, or $MgCl_2$ for 0–40 days. The relative humidities in the desiccators containing saturated LiCl, $CH_3COOK$, and $MgCl_2$ solutions were 11, 23, and 33%, respectively (Greenspan, 1977).

### 2.4. Water content analysis

The water contents of the compressed and rehumidified samples were gravimetrically determined. To accomplish this, the sample weight of a thoroughly dried state (before rehumidification), $m_0$ (g) and the increased mass due to rehumidification, $\Delta m$ (g), were measured, and the water content was determined from the relationship $\Delta m/m_0$. The deviation in measured water content was less than 0.004 g/g-dry matter. Alternatively, a Karl–Fischer titration was also performed in the same manner as described in a previous study (Imamura et al., 2001). The values for the water content obtained from both the gravimetric and titration methods were in good agreement. This confirms that the residual water in the freeze-dried samples was thoroughly removed by desiccation over $P_2O_5$ (before rehumidification) and the gravimetric method can be used to precisely determine the amount of sorbed water.

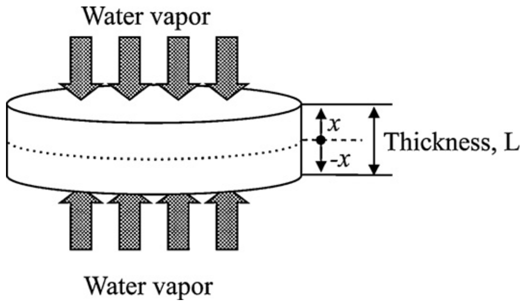


**Fig. 1.** Simplified view of a sample used in simulating water sorption: the thickness of the sample disc was 5 mm for a freeze-dried sugar sample and 0.5 mm for a compressed sample. In the simulation, the permeation of water vapor was assumed to occur from the top and basal, but not via the side face. The position in the thickness direction is represented by the distance from the middle thickness, *x*.

### 2.5. Estimation of the diffusivity of water vapor in an amorphous sugar matrix

In order to verify that the amorphous sugar samples reached sorption equilibrium by the rehumidification procedure used, the diffusion coefficient of water molecules inside the sample were estimated and the water sorption process of the samples were then simulated. In the theoretical investigation, the same procedure and assumptions were used as for the simulation of the water sorption process in polymer films (Shigemoto et al., 2000; Watkins and Anseth, 2005): Freeze-dried sugar samples and the compressed samples were simplified as a disc (Fig. 1), and it was assumed that water vapor permeates only from the top and basal sides of the disc in the direction of the thickness: The permeation from the side face of the sample disc was ignored for model simplification. When the diffusion of water vapor in the thickness direction follows Fick's law, the water content can be correlated with the position in the sample disc and the rehumidification period by the following equation.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{1}$$

where *x* and *t* denote the distance from the thickness center (Fig. 1) and the rehumidification period, respectively, and *C* is the water content as a function of *x* and *t*. *D* represents the diffusion coefficient of water vapor in the sample disc. The boundary conditions were as follows:

$$t = 0, -L > x > L, C = 0; \quad t = 0,\ x = \pm L, C = C_e;$$
$$t = 0, x = 0, \quad \frac{\partial C}{\partial x} = 0 \tag{2}$$

where $C_e$ represents the equilibrium water content. Consequently, Eq. (3) can be obtained by integrating Eq. (1) with the boundary conditions (2) (Watkins and Anseth, 2005).

$$\frac{C}{C_e} = 1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\exp\left\{-D(2n+1)^2\frac{\pi^2 t}{4L^2}\right\}$$
$$\cos\left[\frac{(2n+1)\pi x}{2L}\right] \tag{3}$$

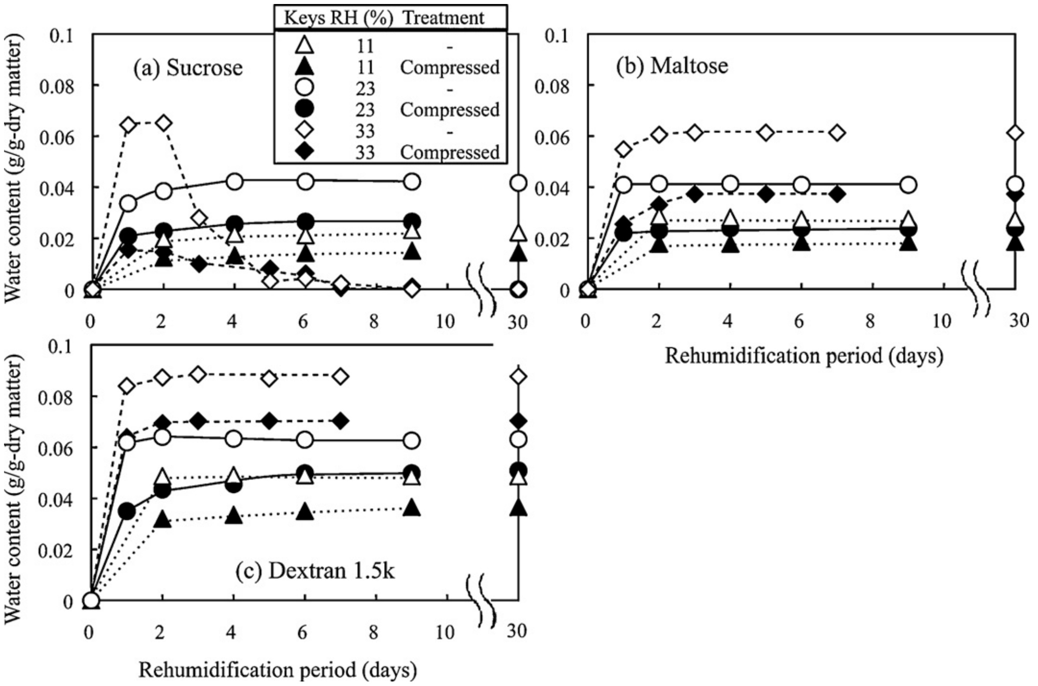


**Fig. 2.** Courses for the increase in water content for compressed and uncompressed freeze-dried amorphous sucrose (a), maltose (b), and dextran 1.5k (c) at different RHs. The compression was carried out at 443 MPa for 5 min. The deviation in water content was 0.004 g-water/g-dry matter at most.

Using Eq. (3), the water content profiles in a sample disc at given periods of rehumidification were simulated for different diffusion coefficients and the overall water contents of the sample at different rehumidification periods were then calculated by averaging the water content profile. The one-dimensional diffusion model, employed herein, has been reported to successfully simulate the desorption process of freeze-dried carbohydrates under a vacuum (Spieles et al., 1995).

### 2.6. *Differential scanning calorimetry*

The $T_g$ values for the compressed and uncompressed amorphous sugar samples were determined using a Perkin–Elmer Pyris differential scanning calorimeter by the same procedure as used in our previous study (Imamura et al., 2010). In an typical experiment, 3–10 mg of a sample that had been hermetically sealed in 20 μL aluminum pans was scanned at a rate of 10 °C/min from a temperature at least 50 °C lower than the $T_g$ to 180 °C, using an empty aluminum pan as a reference. The $T_g$ value was determined as the onset temperature in the shift in apparent specific heat due to the glass-to-rubber transition using data obtained from the DSC thermogram. Herein, the prewarming of the sample for eliminating the overestimation of $T_g$ due to an enthalpy relaxation was omitted because it could have diminished the effect of the compression.

The extent of structural relaxation of the compressed and uncompressed samples were evaluated by means of DSC, following the procedure outlined by Hancock et al. (1995). The compressed and uncompressed samples (sucrose) were scanned using a DSC in the same manner as described above. The heat quantity of the endothermic peak, located at the end of the glass transition region, was determined as the extent of structural relaxation of the sample by calculating the area between the DSC curve and the extrapolated base line for the temperature range above $T_g$ (Hancock et al., 1995; Shamblin et al., 2000). The structural relaxation behavior of a non-porous amorphous sucrose sample was also measured. The non-porous amorphous sucrose sample was prepared by melting sucrose crystals and quenching.

All the analyses were performed in triplicate, at a minimum for each sample.

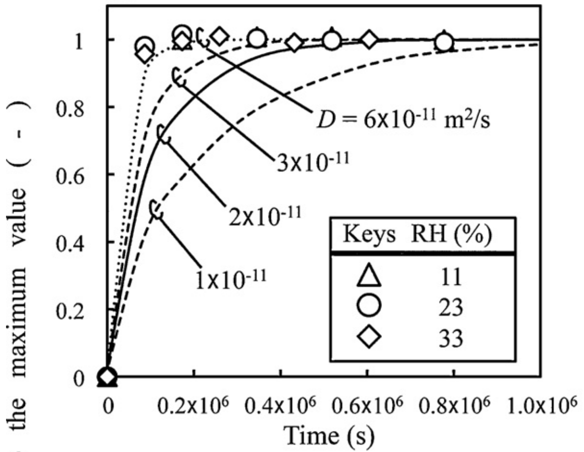
## 3. Results and discussion

### 3.1. *Water sorption of compressed amorphous sugar matrix*

Fig. 2 shows representative time courses for the increase in water content for compressed and uncompressed amorphous sugar samples during rehumidification at selected RHs. The water contents for freeze-dried (uncompressed) sugar samples reached a maximum within one or two days, which is typical for the rehumidification of a freeze-dried sugar sample under reduced pressure (Roos and Karel, 1991a). In the case for the compressed samples, approximately 7 days were required to reach the maximum water content. This indicates that compression decreases the diffusivity of water vapor in a freeze-dried sugar matrix to a substantial extent.

The time courses for the relative water content to the maximum were simulated for different diffusion coefficients and the obtained data were compared with the experimental data in Fig. 3. The simulated time course for freeze-dried (uncompressed) sample was in good agreement with the experimental data, when the diffusion coefficient is in the range above $6 \times 10^{-11}$ m$^2$/s (Fig. 3(a)). On the other hand, Fig. 3(b) suggests that the water vapor diffusion coefficient for the compressed samples is between $2 \times 10^{-13}$ and $6 \times 10^{-13}$ m$^2$/s. The predicted range of the diffusion coefficient is less than one-hundredth that for the uncompressed samples but is approximately 10–100 times greater than that reported for a sugar paste ($10^{-14}$–$10^{-15}$ m$^2$/s (Alavi et al., 2003)). This may be closely related to pores in the samples. Namely, a freeze-dried sugar sample contains a large number of cylindrical pores that had been occupied by ice crystals and the pores serve as channels for the water vapor permeation. Compression substantially decreases the number of pores present in a sample (Imamura et al., 2010) and thus lowers the water diffusivity relative to that for an uncompressed sample. However, the remaining pores in the compressed

(a) Freeze-dried trehalose



(b) Compressed trehalose

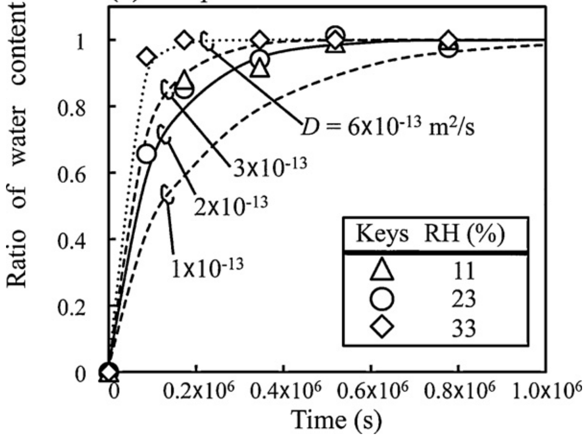


**Fig. 3.** Simulated water sorption processes (increase in water content) of freeze-dried (a) and compressed samples (b) during rehumidification (curves) and experimental values (plots). The compression was conducted for 5 min at 443 MPa. The vertical axis denotes the relative water content to the maximum. The calculated curves were determined using different diffusion coefficients.

sample results in the water diffusivity being higher than that for a nonporous sugar paste.

The water content profiles for compressed samples at different rehumidification periods were simulated with the diffusion coefficient, $D = 2 \times 10^{-13}$ m$^2$/s (Fig. 4). Fig. 4 indicates that the water content distribution in the compressed sample becomes flat within $6 \times 10^5$ s (∼7 days). Therefore the maximum water contents for the compressed samples as well as the uncompressed samples can be assumed to be equilibrium values.

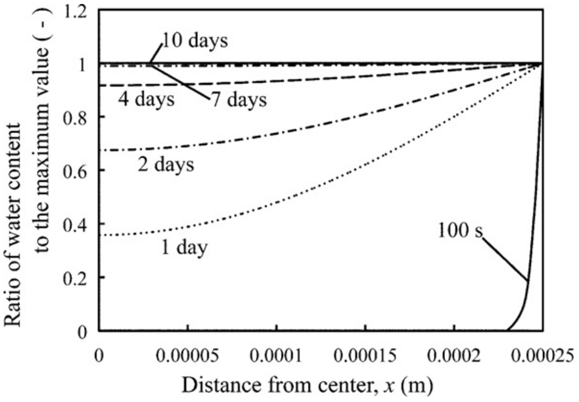


**Fig. 4.** Simulated water content distribution in a compressed sugar sample at different stages of rehumidification. The diffusion coefficient was assumed to be $2 \times 10^{-13}$ m$^2$/s, based on the simulation results (Fig. 3).

As shown above, the diffusion coefficient of water vapor is assumed to be constant in the above-described theoretical investigations of the water diffusion coefficient and the water sorption process. However, it has been reported that the diffusion coefficient of water vapor varies, depending on the water content (Bruin et al., 1980; Yamamoto, 2001; Alavi et al., 2003) (e.g. The diffusion coefficients of water vapor in an amorphous sugar paste are estimated to be $0.8 \times 10^{-15}$, $1.4 \times 10^{-14}$, and $8.5 \times 10^{-14}$ m$^2$/s for water contents of 0.02, 0.04, and 0.06 g/g-dry matter, respectively, using the reported correlation equation (Alavi et al., 2003)). However, since most of the rehumidification period, except for first day, shows more than 80% of the equilibrium water content, the assumptions for constant water diffusivity can be considered to be reasonable.

As shown in Fig. 2, the compressed samples have a markedly lower maximum (equilibrium) water content than the uncompressed samples. Since the hydration states of the amorphous sugar matrices appear to reach equilibrium within several days, as indicated above, the lower maximum water content of the compressed sample indicates that compression results in a decrease in the number of hydration sites in an amorphous sugar matrix.

Our previous study demonstrated that the compression at hundreds of MPa markedly decreased the surface area of freeze-dried sugar samples (Imamura et al., 2010). Taking into account the fact that water sorption involves "adsorption" on the surface as well as "absorption" into the bulk, the decrease in water content as the result of the compression (Fig. 2) can be considered to be due to the decrease in surface area. However, the amount of water adsorbed on the matrix surface was estimated to be less than 0.001 g-water/g-dry sugar; Reported specific surface areas of freeze-dried carbohydrates are at most 2 m$^2$/g (Abdul-Fattah et al., 2007), and the area occupied by a single water molecule is approximately $6 \times 10^{-20}$ m$^2$. Therefore, the maximum amount of water molecules that could adsorb on the sample surface is estimated to be 0.00075 g-water/g-dry sample $(= [1.5\ \mathrm{m^2/g}]/[6 \times 10^{-20}\ \mathrm{m^2}]/[6.02 \times 10^{23}\ \text{molecules}] \times [18\ \mathrm{g/mol}])$. The estimated maximum amount of "adsorbed" water is less than one-eighth of the total decrease in the water content due to the compression. Consequently, the decrease in the water content as the result of compression cannot be explained simply by the decrease in surface area at present stage. Clearly, further investigations are needed to clarify this issue.

In the case of sucrose (Fig. 2(a)), both the compressed and uncompressed samples began to release water of hydration at approximately the second day of the rehumidification at RH 33%, because of the crystallization of sucrose (Makower and Dye, 1956). It should be noted that the water content of the compressed sucrose sample decreases to almost zero by rehumidification at RH 23% for more than 20 days while that of the uncompressed sample remains nearly constant after 4 days. A powder X-ray diffractometric analysis revealed that the compressed sucrose sample that had been rehumidified at RH 23%, had completely crystallized after 30 days (data not shown). This demonstrates that compression facilitates the crystallization of an amorphous sucrose matrix. It has been reported that compression increases the tendency of an amorphous sucrose matrix to crystallize, when the sample is in a thoroughly dehydrated state (Kawakami et al., 2006; Imamura et al., 2010), and such an impact of compression on this tendency appears to also hold for the case of a humidified system.

The rehumidification of sucrose samples at RH 33% caused a marked collapse due to the glass-to-rubber transition. It should also be noted that, at RH 33%, the water content for the compressed sucrose sample was markedly lower than that for uncompressed

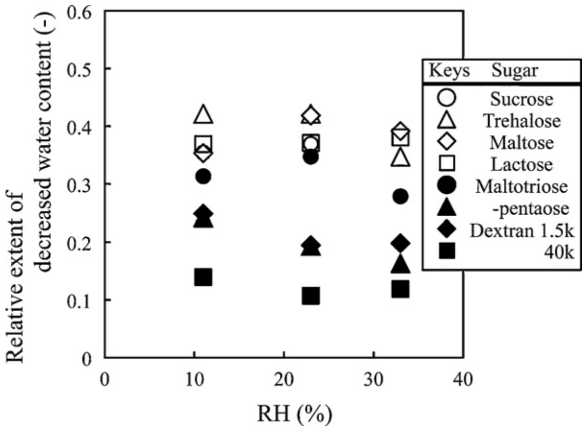


**Fig. 5.** Decrease in water content of freeze-dried sugars due to compression at 443 MPa for 5 min. The extents of decrease in water content are shown relative to the water content for uncompressed sample.

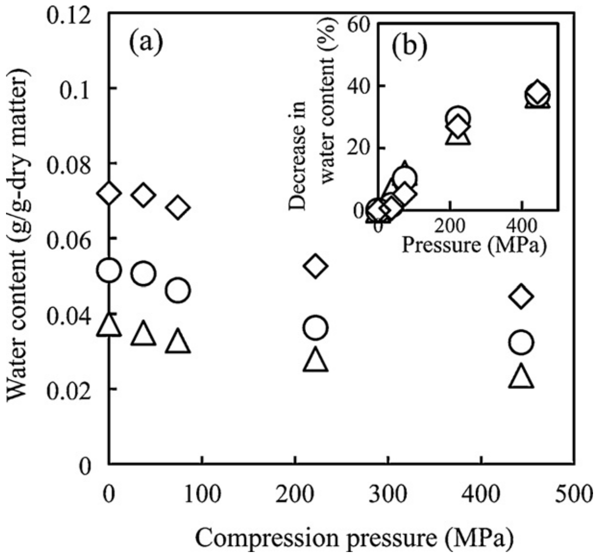


**Fig. 6.** (a) Water contents of freeze-dried amorphous sugar (α-lactose) compressed under different pressures and rehumidified at RH 11% (triangles), 23% (circles), and 33% (diamonds) at 25 °C. The compression was carried out for 5 min. (b) Magnitudes of the decrease in water content due to compression for different pressures. The vertical axis indicates the value relative to the water contents for the uncompressed samples. The deviation in water content was 0.004 g/g-dry matter at most, and the values at 0 MPa of compression pressure means those for the uncompressed samples.

samples of amorphous sucrose, which were largely in the crystalline state (~4 days), as shown in Fig. 2. This suggests that the impact of compression on the water sorption of an amorphous sugar matrix is maintained in spite of a glass-to-rubber transition.

The maximum water contents for the compressed and uncompressed amorphous sugar samples during rehumidification (Fig. 2) were plotted against the RHs (Fig. S1 in Supplemental Information) and, from the obtained water sorption isotherms, the extent of decrease in the equilibrium water content due to compression were calculated and these data are shown in Fig. 5. As shown in Fig. 5, the RH for rehumidification does not make any significant influence on the relative decrease in water content due to compression, suggesting that the water sorption isotherms for the compressed and uncompressed samples are similar in shape although different in value. The relative decreases in the equilibrium water content for all disaccharides are approximately 40%, and as the molecular size of sugar increases, the decrease in water content due to compression tends to be smaller. This suggests that an amorphous matrix of a larger oligomer would be more resistant to micro-structural deformation that occurs as the result of compression.

Fig. 6 shows representative data for the dependence of the equilibrium water content of an amorphous sample on the compression pressure used. The water content decreases with increasing compression pressure, which becomes less significant at a compression pressure equal to and above 222 MPa, as shown in Fig. 6.

The differences between the water contents of the compressed and uncompressed samples was calculated for different compression pressures and RHs and these data are shown as the ratios to the water contents of the uncompressed samples in Fig. 6(b). The results for different RHs are roughly consistent with each other. It would be expected that, among the hydration sites in an amorphous sugar matrix, that there would be some variation in the ability to bind water molecules: the water sorption isotherm therefore, represents the distribution of water binding abilities of the various hydration sites. Therefore, based on the results for different RHs shown in Fig. 6(b), it can be concluded that the hydration sites, with various differences in water binding ability, are similarly decreased by the compression.

### 3.2. Glass transition temperature

Fig. 7 shows DSC thermograms for uncompressed and compressed sucrose samples that were rehumidified for different periods. As shown in Fig. 7, the uncompressed samples exhibit a distinct exothermic peak due to the crystallization of sucrose, regardless of whether the length of the rehumidification period was more than a single day (data not shown). Furthermore, the $T_g$ values for the uncompressed sugar samples are consistent with reported values (Roos and Karel, 1991a,b; Imamura et al., 2002). These facts demonstrate that the water sorption of the uncompressed samples reaches an almost maximum value within one day, as indicated by Figs. 2. In contrast, the DSC thermogram for the compressed and one-day-rehumidified sucrose sample (Fig. 7) shows multiple exothermic peaks due to crystallization, indicating the spatial non-uniformity of the water content in the sample as the result of insufficient rehumidification. On the other hand, the compressed

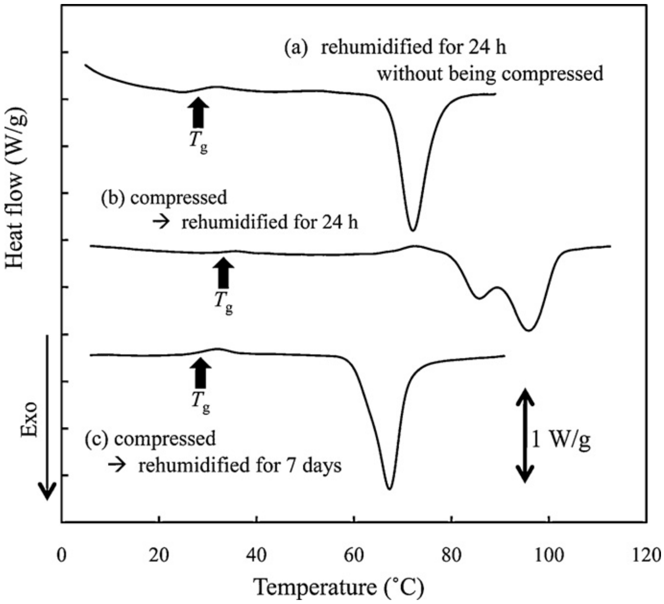


**Fig. 7.** DSC thermograms of freeze-dried sucrose samples, (a) rehumidified for 24 h without being compressed, (b) compressed and then rehumidified for 24 h, and (c) compressed and then rehumidified for 7 days. Compression was conducted at 443 MPa for 5 min, and RH for rehumidification was 23% at 25 °C.

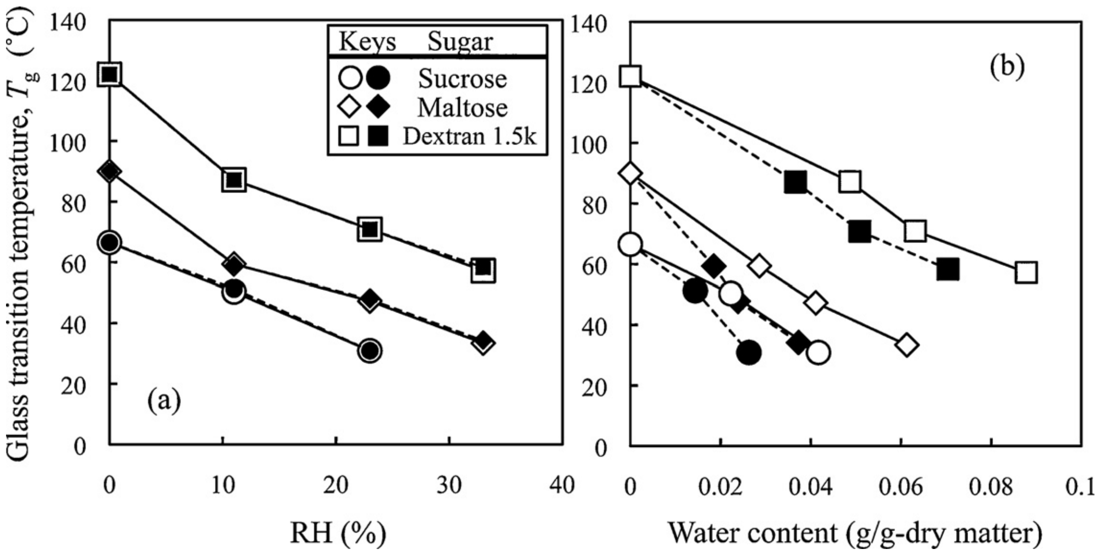


**Fig. 8.** Dependences of glass transition temperatures for compressed (closed keys) and uncompressed freeze-dried amorphous sugars (open keys) on (a) RH in the rehumidification and (b) water content of the samples. The compression was carried out at 443 MPa for 5 min. The deviation in glass transition temperature was within ±2 °C.

samples, rehumidified for more than 7 days, show a single event corresponding to crystallization, similar to the uncompressed sample. The $T_g$ and the crystallization temperature were also constant when samples were rehumidified for more than 7 days. These findings strongly support the above conclusion that the water content of the compressed sample reaches equilibrium within 7 days.

The glass transition temperatures, $T_g$, for the compressed and uncompressed amorphous sugar samples are plotted against RH and water content, respectively, in Fig. 8(a) and (B). Fig. 8(a) indicates that compression does not lead to any change in the $T_g$ values. However, as shown above (Figs. 2, 5 and 6), the equilibrium water content of an amorphous sugar matrix is markedly decreased by compression. It thus follows that compression alters the dependence of $T_g$ on the water content: As is well known, the $T_g$ value of an amorphous sugar matrix decreases with increasing water content of the matrix, the degree of which is increased by compression, as shown in Fig. 8(b).

The slope of the relationship between $T_g$ and water content (Fig. 8(b)) indicates the extent to which the increase in water content lowers the $T_g$ value of an amorphous sugar matrix, that is, the sensitivity of $T_g$ to the water content. Hence, the values of the slopes for all of the tested compressed and uncompressed samples were determined and the values are listed in Table 1. The increase in the slope of the plot of $T_g$ against water content by compression is significant for disaccharides and less so for a larger oligomer. This is because the decrease in water content due to compression is greater for a smaller sized sugar, as described above.

**Table 1**
Changes in $T_g$ of compressed and uncompressed freeze-dried amorphous sugars due to increase in water content by 0.01 g-water/g-dry matter, $\Delta T_g/\Delta w_{0.01}$.

| Sugar | $\Delta T_g/\Delta w_{0.01}$ (°C/[0.01 g-water/g-dry matter]) | |
|---|---|---|
| | Uncompressed | Compressed |
| Sucrose | 7.0 | 10.9 |
| Maltose | 10.7 | 16.5 |
| Trehalose | 12.0 | 20.8 |
| Lactose | 9.0 | 14.3 |
| Maltotriose | 7.9 | 11.5 |
| Maltopentaose | 9.6 | 12.7 |
| Dextran 1.5k | 7.1 | 9.5 |
| Dextran 40k | 7.1 | 8.2 |

It is generally accepted that the $T_g$ value of an amorphous sugar matrix varies markedly, depending on the water content (Roos and Karel, 1991a). Accordingly, the agreement of the $T_g$ values for the compressed and uncompressed samples for given RHs (Fig. 8(a)) appears to be in conflict with the general assumptions concerning the $T_g$-water content relationship: the $T_g$ value of the compressed sample should have been higher than that of the uncompressed sample by the decreased amount of water content due to compression.

There are two possible explanations for this. One is that compression distorts the conformation of sugar molecules in the matrix (Imamura et al., 2010), which may lead to an enhancement in the plasticizing effect of water of hydration. The other explanation is based on the assumption that sorbed water molecules exist in multiple hydration states; water molecules in some hydration states may have no plasticizing effect and could be preferentially eliminated by the compression, while other water of hydration molecules could cause a lower $T_g$ value. We plan to investigate these possible explanations in the near future.

### 3.3. Enthalpy relaxation

Fig. 9 shows the time courses for the increase in enthalpy due to the structural relaxation of different forms of amorphous sucrose samples at 37 °C. As shown in Fig. 9, the structural relaxation of the freeze-dried (uncompressed) sucrose sample proceeds much more slowly than that of a non-porous amorphous sucrose sample. On the other hand, the compressed amorphous sucrose sample shows almost the same relaxation process as the non-porous sample, indicating that the structural relaxation rate of an amorphous sugar matrix is significantly accelerated by compression.

An amorphous sugar matrix prepared by freeze-drying contains numerous pores that had formerly been occupied by ice crystals (Imamura et al., 2010; van Scoik and Carstensen, 1990). The pores in the freeze-dried sugar matrix were found to be markedly diminished as the result of compression at 443 MPa for 5 min in our previous study (Imamura et al., 2010). Considering these findings, the pores in the freeze-dried matrix may serve to interfere with the propagation of the structural relaxation of an amorphous sugar matrix. Namely, allocational and conformational changes of sugar molecules, which are responsible for the structural relaxation, may

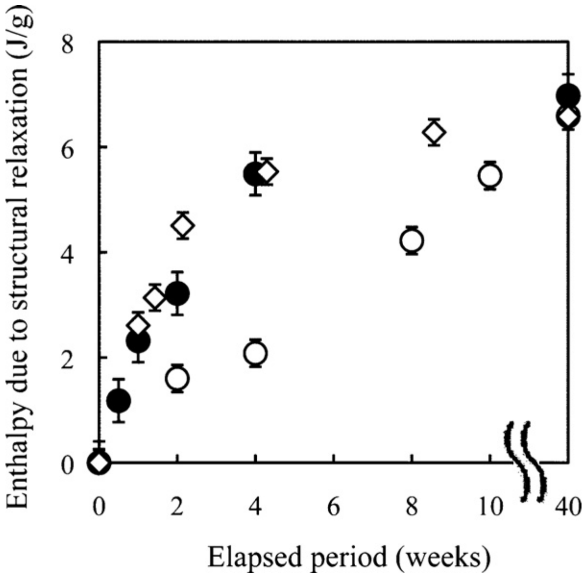


**Fig. 9.** Courses for the relaxation of structural enthalpy of compressed (closed circle) and uncompressed freeze-dried amorphous sucrose (open circles) at 37 °C and RH 0%. The compression was carried out at 443 MPa for 5 min. Results for a sucrose paste, prepared by heating freeze-dried sucrose above the $T_g$ are also shown (open diamonds).

propagate through the matrix, and the propagation would be terminated when pore surfaces are reached. Therefore, the diminution in the number of pores due to compression could increase the propagation rate of the structural relaxation to the level that for a non-porous sample, as shown in Fig. 9.

As shown above, the compression of an amorphous sugar matrix tends to decrease the equilibrium water content at a given RH (Figs. 2, 5 and 6) and accelerate the extent of structural relaxation (Fig. 9). On the other hand, Kim et al. (2001a) reported that the ball-milling of an amorphous food carbohydrate (starch) accelerated structural relaxation and simultaneously decreased the hygroscopicity of the sample (Kim et al., 2001b). Considering that ball-milling involves the creation of partial high pressure regions, these findings clearly demonstrate the existence of a close relationship between the compression of an amorphous sugar matrix, water sorption behavior, and structural relaxation. As discussed above, the decreased number of pores inside a freeze-dried sugar matrix due to compression appears to largely contribute to the acceleration in structural relaxation. Therefore, the decrease in hygroscopicity as the result of compression may also be closely related to the deformation to a nonporous structure although further investigations will be required to verify the mechanism of this process.

## 4. Conclusion

The effects of compression on water sorption, the glass-to-rubber transition, and structural relaxation behaviors of amorphous sugar matrices prepared by freeze-drying were investigated. The compression of an amorphous sugar matrix lowers the equilibrium water content at a given RH, the extent of which is more significant as the size of the sugar molecule decreases and as the compression pressure increases. The $T_g$ value for amorphous sugar matrices that had been rehumidified at a given RH, remained unchanged as the result of compression. However, the decrease in $T_g$ with increasing water content was increased by the compression. The findings indicate that compression accelerates the structural relaxation of the matrix. This may be closely related to the fact that the porous structure of a freeze-dried amorphous sugar is converted into a non-porous structure as the result of compression.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.01.052.

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