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Application of the Kwei Equation to model the T_g Behavior of Binary Blends of Sugars and Salts

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Abstract

Vitrification of sugar-based solutions plays an important role in cryopreservation, lyophilization, and the emerging field of anhydrous preservation. An understanding of the glass transition characteristics of such formulations is essential for determining an appropriate storage temperature to ensure an extended shelf life of vitrified products. To better understand the effect of salt on the glass transition temperature (T_g) of glass-forming sugars, we investigated several data-fitting models (Fox, Gordon-Taylor and Kwei) for sugar-salt formulations using data from the literature, as well as new data generated on blends of trehalose and choline dihydrogen phosphate (CDHP). CDHP has recently been shown to have promise as a stabilizing agent for proteins and DNA. The Kwei equation, which has a specific parameter characterizing intermolecular interactions, provides good fits to the T_g data for sugar-salt blends, and complements other commonly used models that are frequently used to model T_g data.

Keywords

Glass transition temperature; Kwei; Gordon-Taylor; Fox; Trehalose; Choline dihydrogen phosphate; Anhydrous preservation; Cryoprotectant

Introduction

It has been widely recognized that vitrification confers considerable benefits for food storage, pharmaceutical formulation, and the long-term storage of living cells and tissues [4]. Sugars are commonly used in these vitrification-based applications as stabilizing excipients or cryoprotective agents, and are often used in combination with other components such as inorganic or organic salts. For example, phosphate salts are often used as pH buffers in protein formulations [10]. Despite the fact that sugars are often used in

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combination with salts and polymers in a range of extended shelf-life applications, models that can accurately represent the T_g behavior of multi-component compositions are somewhat limited.

The Fox model, as shown by Eq. (1), describes a weighted-average relationship between T_g and the mass fraction of the component [3].

$$\frac{1}{T_g} = \frac{1-x}{T_{g,1}} + \frac{x}{T_{g,2}} \quad (1)$$

where $T_{g,1}$ and $T_{g,2}$ (in K) are the glass transition temperatures of the pure components with the lower and higher T_g , respectively, and x represents the mass fraction of the higher T_g component. Gordon and Taylor [5] introduced a parameter, k_{G-T} , that can be incorporated into the simple weighted-average relation to better represent the unequal contributions of components in a mixture to the final T_g [1].

$$T_g = \frac{(1-x)T_{g,1} + k_{G-T}xT_{g,2}}{(1-x) + k_{G-T}x} \quad (2)$$

In the G-T equation, $k_{G-T} = (\rho_1/\alpha_1)/(\rho_2/\alpha_2)$ where ρ is the density of each component and α is the increment at T_g of the isobaric expansivity of each component. By the Simha-Boyer rule ($T_g/\alpha = 0.133$), the above relation can be replaced with $k_{G-T} = (\rho_1T_{g,1})/(\rho_2T_{g,2})$ [6]. Couchman and Karasz [2] also proposed that $k_{G-T} = \Delta C_P^2/\Delta C_P^1$, where ΔC_P^i is the change in heat capacity of component i between its liquid-like and glassy states. After wide usage for polymer mixtures, this equation was introduced by Roos [13] in 1993 to predict the T_g of mixtures composed of low molecular weight sugars such as maltose.

Prior studies have employed the Gordon-Taylor model to model the T_g behavior of cryoprotective mixtures such as sucrose/tris or citrate buffer [12]. For most of the mixtures, however, these studies obtained empirical values of k_{G-T} , which were not compared with the theoretical values based on the Couchman-Karasz relation. This is mainly because k_{G-T} has a valid physical meaning only when the assumptions of ideal volume mixing and no interactions between components are valid. Therefore, for non-ideal (real) mixtures with varying degrees of intermolecular interactions the value of k_{G-T} is largely used for data-fitting purposes only [7].

In 1984 Kwei [9] found that several mixtures of resins (e.g., polymethyl methacrylate and phenolic resins) exhibited a T_g behavior which deviated considerably from the mathematical form provided by the G-T equation. This deviation was interpreted as the contribution of the hydrogen bonding (H-bonding) between components [9]. To resolve the deviation, Kwei modified the original G-T equation to include a second parameter, q , as shown by Eq. (3). The parameter k has the same physical meaning as k_{G-T} , while q is used to model the effects of interactions between components, such as hydrogen bonds, on the T_g value.

$$T_g = \frac{(1-x)T_{g,1} + kxT_{g,2}}{(1-x) + kx} + qx(1-x) \quad (3)$$

Very recently we found that the Kwei model, which was proposed originally for polymer science more than two decades ago, may be a promising mixing model for compositions used in the biopreservation field. To date the Kwei model has not been applied to sugar-based mixtures of cryobiological interest. To better model the effects of salt on the properties of glass-forming sugar mixtures, this study applied various models (i.e., Fox, G-T and Kwei) to T_g data over the full compositional range by using data from the literature as well as new data on binary mixtures of trehalose and CDHP first reported in this study. Choline dihydrogen phosphate (CDHP) is a member of a family of biocompatible organic salts that have been investigated recently for their protein stabilizing characteristics.

Materials and methods

Choline hydroxide (20 wt% in water) and phosphoric acid (85 wt% in water) were procured from Sigma Aldrich while high-purity trehalose dihydrate was purchased from Ferro Pfanstiehl Laboratories. The synthesis of CDHP was performed according to methods previously reported in the literature [11]. Briefly, it was synthesized by a slow addition of an aqueous solution (85 wt%) of phosphoric acid (5.7 g) to 20 wt% aqueous choline hydroxide solution (30.1 g) in an ice bath with stirring for 2 hours at room temperature. The filtrate was evaporated to obtain a pure white solid (9.8 g) in 98% yield. Characterization was performed by electrospray mass spectroscopy (cone: ± 35 V), m/z (relative intensity, %): ES^+ , 103.7 ($\text{C}_5\text{H}_{14}\text{NO}^+$, 100); ES^- , 96.8 (H_2PO_4^- , 100).

Different mass ratios of trehalose dihydrate and CDHP were mixed in 5 g of water to generate various weight percentages of trehalose (i.e., 29.8, 45.9, 54.8, 58.7, 63.0, 66.2, 71.9, 77.3, 83.6, 89.1 and 100 wt%, calculated on the dry basis of binary blends of anhydrous trehalose and CDHP). These ternary aqueous solutions were initially freeze dried for 3 days and then kept in a vacuum desiccator at room temperature for 2 days prior to thermal analysis, thus yielding binary blends of trehalose and CDHP.

T_g of neat anhydrous trehalose and its mixtures with CDHP was determined using a Q-100 differential scanning calorimeter (DSC) model (TA Instrument) with heating and cooling rates of $10^\circ\text{C}/\text{min}$. The T_g was defined as the midpoint of the endothermic step-change observed during the controlled rate heating scan. To ensure that no water remained in the dried sample, the sample was further annealed in an open pan at 130°C in the DSC instrument under flowing nitrogen and then heated above 210°C . The specific instrument protocol is given below:

1. Anneal at 130°C for 1 h, isothermal mode;
2. Cool to -30°C and hold for 2 min;
3. Heat to 210°C at $10^\circ\text{C}/\text{min}$ and hold for 2 min;
4. Cool to -30°C and hold for 2 min;

5. Heat to 150 °C at 10 °C/min and hold for 2 min.

Results and discussion

Fig. 1 illustrates the composition dependence of the T_g of four sugar-salt blends (i.e., trehalose-CDHP, sucrose-tris buffer, trehalose-tris buffer and sucrose-citrate buffer [12]). Table 1 also gives the values of parameters in the G-T and Kwei equation. Note that the data point for neat CDHP was not measured in this study but instead given by reference [11]. Here we consider the anhydroustris or citrate buffer as a single component in the aforementioned equations as in the original study [12]. The DSC results of the T_g of binary blends of trehalose and CDHP show a clear “fall-and-rise” trend within the range from 70 to 90 wt% and they follow an S-shape curve within the entire concentration range. A similar pattern is observed for sucrose-tris blends. By contrast the trehalose-tris buffer and citrate-sucrose blends exhibit U-shaped behavior over the full compositional range.

As mentioned earlier, the parameter k_{G-T} in the G-T equation and k in the Kwei equation are equivalent in that each represents the unequal contribution of each component in the mixture to the final T_g . The G-T description for trehalose-CDHP blends yields a k_{G-T} value of 2.47, suggesting that trehalose is dominant in its contribution to the mixture T_g . While the G-T model appears to provide a good fit to the data when the CDHP component is dominant, it does not model the high trehalose compositional range very well. The Kwei equation provides a good fit of the T_g data in this case with $k=0.20$ and $q=338.26$, yielding a higher goodness of fit (0.9664) compared to the G-T equation (0.9157). This higher R^2 value is to be expected based on the extra fitting parameter. However, in contrast to the G-T model, a value of $k=0.20$ in the Kwei model suggests that trehalose contributes a proportion that is smaller than its mass fraction to the final T_g . As a result, the curve depicted by the term

$\frac{(1-x)T_{g,1}+0.2xT_{g,2}}{(1-x)+0.2x}$ obtains a negative deviation from the simple weight-averaged description (i.e. CDHP is dominating in the T_g behavior of the mixture). However, because $q>0$ (in this case, 338.26), the quadratic term $qx(1-x)$ in the Kwei equation results in an increase in the final T_g over and above the weight-averaged term. These two effects dominate in different compositional ranges, leading to the S-curve behavior. The parameter q , as discussed earlier, corresponds to the strength of specific interactions like H-bonding, reflecting the balance between the breaking of self-associations and the forming of inter-associations between the two components [8]. Both self-associations and H-bonds between components will increase the T_g of the mixture [9], due to the formation of a rigid network. In other words, fewer self-associations may decrease the mixture T_g but, at the same time, the formation of inter-component H-bonds will increase the final T_g . A positive q implies that the increase of T_g due to forming inter-associations (H-bonds between trehalose and CDHP in this case) overcomes the decrease associated with breaking self-associations such as H-bonds between trehalose molecules. The larger the absolute value of q , the more pronounced the net increase or decrease of T_g will be. Therefore, one can speculate that the H-bonding network between trehalose and CDHP should be significantly stronger or more rigid than the self-associations of trehalose or choline and dihydrogen phosphate ions. Furthermore, the term $qx(1-x)$ reasonably indicates that such a balance can reach its maximum when the mass fractions of each component are the same, in which case the

probability of inter-component interactions will be the highest. In total, the negative

deviation attributed to $\frac{(1-x)T_{g,1}+0.2xT_{g,2}}{(1-x)+0.2x}$ and the positive deviation caused by $qx(1-x)$ result in an S-shape T_g -composition relationship as seen in Fig. 1 (a). Further investigations at the molecular level are needed in order to compare the H-bonding characteristics of the mixture in terms of binding energy and H-bonding quantity in order to verify the validity of these proposed mixture characteristics.

In the case of the sucrose-tris buffer blends shown in Fig. 1 (b), both the G-T equation and the Kwei equation suggest that sucrose is dominating in its contribution to the T_g of the mixture, as evidenced by the high k values for both fits. Both models provide good fits to the data in the composition range where the tris concentration is high. In the intermediate composition range, significant deviations are observed, which is captured with a negative q parameter ($q=-44.27$) in the Kwei equation, and a corresponding significant increase in the goodness of fit over the G-T equation. Consequently, an S-shape composition dependence of T_g is produced for the blends of sucrose and tris buffer. These fitting parameters suggest that that the breaking of H-bonds between sucrose molecules due to interference caused by the tris buffer can decrease the final T_g more than the formation of inter-component (tris-sucrose) H-bonds can increase it.

As shown in Figs. 1 (c) and (d), the T_g data of trehalose-tris and sucrose-citrate blends both exhibit U-shaped curves. Both the G-T and Kwei equations yield values of k that exhibit a negative deviation from the simple weight-averaged curve, demonstrating the smaller contribution given by the higher T_g component (trehalose in the blend of trehalose-tris buffer and citrate buffer in the blend of sucrose-citrate buffer). Additionally, the negative q determined for both blends means that the breaking of the self-associations decreases the T_g more than the forming of H-bonds between the salt and sugar can increase it. By comparing CDHP with tris or citrate buffer, one can observe that the interactions between CDHP and trehalose are significantly beneficial in terms of increasing T_g while the disturbances caused by tris or citrate to the initial self-associations of sugars result in a net decrease in the final T_g compared to the weight-averaged results.

Conclusions

The widely-used Gordon-Taylor model assumes ideal volume mixing. While it can account for unequally weighted component contributions to the mixture T_g it does not account for the interactions between components in a mixture, and thus cannot accurately model the T_g behavior of cryobiologically relevant sugar-based mixtures that have strong interactions between components. Results from DSC showed that the T_g data for trehalose-CDHP blends exhibited strong deviations from the Gordon-Taylor prediction. We have shown that the Kwei equation, which includes a parameter that accounts for the magnitude in intermolecular interactions, can model the T_g over the full compositional range, not only for trehalose-CDHP blends, but also for other blends of interest in preservation science. It is hypothesized that the deviations from the G-T curve originate from the strong interactions between two components in a mixture as well as their differences in size and shape. With k characterizing the unequal contributions of components to T_g , and q capturing the inter-

component interaction, we have shown that the Kwei model can complement the Fox and G-T models that are routinely used to predict the T_g of mixtures, especially for compositions that have strong inter-molecular interactions. Furthermore, the Kwei model can provide insights into molecular behavior that can be further evaluated by other methods of investigation.

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Fig. 1. The composition dependence of the T_g of sugar-salt blends including (a) trehalose-CDHP, (b) sucrose-tris buffer, (c) trehalose-tris buffer, (d) sucrose-citrate buffer

Table 1

The values of k , q and k_{G-T} and the goodness of fit (R^2) for binary blends of cryobiological interest^a

Mixtures ($T_{g,2}/T_{g,1}$)	$T_{g,2}$	$T_{g,1}$	k	q	R^2	k_{G-T}	R^2
Trehalose/CDHP	394.15	251.15	0.20	338.26	0.9664	2.47	0.9157
Sucrose/Tris	333.15	301.71	36.81	-44.27	0.9715	19.36	0.6978
Trehalose/Tris	388.15	301.71	0.99	-140.61	0.9877	0.26	0.9458
Citrate/Sucrose	406.55	333.15	0.07	-56.30	0.9427	0.04	0.9092

^aThe values of T_g for neat trehalose and sucrose are those listed in the original references, therefore they may not be identical in different mixtures.