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Study on the Unfrozen Water Quantity of Maximally Freeze-Concentrated Solutions for Multicomponent Lyoprotectants

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ABSTRACT

The concentration of maximally freeze-concentrated solutions W'_g and the corresponding glass transition temperature T'_g and ante-melting temperature T'_m of lyoprotectant solutions, are critical parameters for developing lyophilization process. Usually, the lyoprotectant solutions are multicomponent solutions composed of electrolytes, sugars, proteins, polymers, and other chemicals. In this article, the W'_g values of several multicomponent solutions including trehalose/NaCl, bovine serum albumin/NaCl, and hydroxyethyl starch/NaCl with water were determined by differential scanning calorimetry. A linear relationship between the unfrozen water fraction W_{un} and the initial solute concentrations W_i was found: $W_{un} = \sum(a_i \cdot W_i)$, which suggested that in the multicomponent solutions each solute could hydrate a certain amount of water a_i (g water/g solute) that could not be frozen. The hypothesis was compared with more literature data. For the same solute in different solutions, variation in the fitted coefficient a_i is noticed and discussed. If a "universal" value a_i for each solute is adopted, both W'_g and T'_g for a multicomponent solution could be predicted if Couchman-Karasz equation is adopted for calculating glass transition temperature at the same time. The prediction discrepancies for T'_g with experimental data were less than 2°C. The finding is discussed about its molecular basis and applicability.

Introduction

In the lyophilization process, several kinds of cryoprotectants and lyoprotectants including sugars, proteins, and polymers may be added to protect the pharmaceutical or biological materials from freezing and dehydration injuries. Knowledge of thermodynamic properties of these multicomponent solutions are important for the design and optimization of lyophilization procedures, among which the supplemented phase diagrams have been studied extensively (Fig. 1). For amorphous protectants, which are used mostly, the concentration of the maximally freeze-concentrated solution W'_g, the corresponding glass transition temperature T'_g, and the ante-melting temperature T'_m are critical parameters for developing the freezing and drying protocols in the lyophilization process.[1-4]

There have been numerous research studies reported in literature on the phase diagrams of solute/water binary solutions revealed through differential scanning calorimetry (DSC), the solutes include: disaccharides and other low molecular weight carbohydrates,[5-9] polymers and proteins,[10-13] and so on. Several methods were proposed to determine the T'_g or W'_g values: Roos[3] measured T'_g by DSC and fitted glass transition temperature T_g with Gordon-Tayler equation, the W'_g was calculated by extrapolating T'_g to the T_g curve; Miller et al.[14] took the intersection point of extended equilibrium melting curve T_m and glass transition curve T_g as T'_g and W'_g; Levine and Slade[15] calculated W'_g from ice melting endotherm of a single solution; Ablett et al.[16] plotted a line of freezable water content W_{ice} against solution concentration and obtained W'_g by extrapolating the line to.$W_{ice} = 0$. It has been shown that the obtained T'_g or W'_g values of the same solution by various approaches were slightly different.[17]

Up to now there have been few formula, empirical or theoretical, that copes with T'_g or W'_g. Levine et al.[15] found that for low molecular weight carbohydrates, T'_g increased linearly with the molecular weight. Matveev et al.[18,19] applied a water-clustering model to get the T_m curve theoretically, then obtained T'_g and W'_g with

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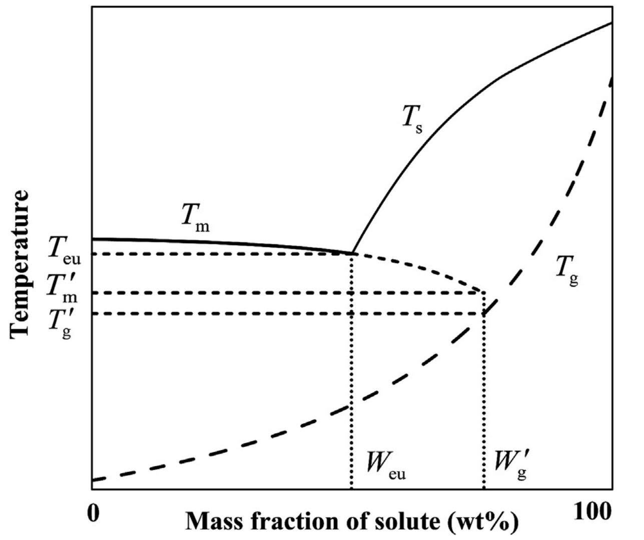

Figure 1. Phase diagram of a model binary solution. (T_m: equilibrium melting curve, T_s: solubility curve, T_{eu} and W_{eu}: eutectic temperature and concentration, for amorphous solute, T_g: glass transition curve, W'_g: maximally freeze-concentrated concentration, T'_g and T'_m: glass transition temperature and ante-melting temperature at maximally freeze-concentrated solution.)

incorporation of Couchman-Karasz (C-K) equation, the predicted values were in good agreement with experimental results for some binary solutions. The formula developed by Levine et al. or Matveev et al. are applicable for single solute solutions and difficult to be extended for describing the behavior of practical lyophilization solutions which usually consist of at least 2 solutes. There were also many experiments to measure the T'_g or W'_g values for ternary or quaternary solutions, some qualitative conclusions had been drawn, for example, the addition of electrolytes decreases T'_g and W'_g; increasing the mass contents of protein, starch, or other macromolecules decreases the T'_g of disaccharide solutions.[10,20-24] However, a quantitative relationship for multicomponent solutions is still urgently needed if it exists. Fonseca et al.[25] made such an attempt; they proposed an empirical equation to calculate the T'_g of a culture medium composed of lactose, galactose, lactic acid, and glucose based on experimental data, but they did not show the applicability of the equation for other solutions.

In this article, an equation was put forward to estimate W'_g for multicomponent solutions based on the concept of water hydration. Experimental W'_g data for ternary solutions of trehalose/NaCl/water, bovine serum albumin (BSA)/NaCl/water, hydroxyethyl starch (HES) (200/0.5)/NaCl/water at different mass ratios were measured and used to check the equation. Because T'_g can be further predicted along with C-K equation, more literature data involving W'_g and T'_g values for multicomponent solutions were also used to verify this relationship. Similar water hydration capabilities for the same solute in different systems were noted, so "universal" values for frequently used solutes were adopted, the prediction of W'_g and T'_g thus becomes possible. A comparison was made between our proposed method and that of Fonseca et al.[25]

Theory and Experiment

Theory for Estimation of W'_g for Multicomponent Solutions

It is well known that W'_g for a binary aqueous solutions is independent of initial solution concentration, this implicates that the unfrozen water content hydrated by or associated with a certain amorphous solute may be constant. For multicomponent solutions composed of several solutes, assuming that the unfrozen water content hydrated by each solute is not affected by the interaction forces between solute molecules or ions, the unfrozen water fraction W_{un} can be expressed as the sum of hydrated water:

$$W_{un} = \sum (a_i \cdot W_i) \tag{1}$$

where parameter a_i (g water/g solute) indicates the hydration ratio of solute i, W_i is the initial weight fraction of solute i. The concentration of maximally freeze-concentrated solution, W'_g, can be calculated as follows:

$$W'_g = \sum W_i \Big/ \sum [(1 + a_i) \cdot W_i] \times 100\% \tag{2}$$

Prediction of T'_g for Multicomponent Solutions

For a multicomponent solution without electrolytes, its glass transition temperature may be calculated by "modified" C-K equation,[26] treating all the solutes as one component, the glass transition temperature is:

$$T_{gmix} = \sum (\Delta C_{pi} \cdot W_i \cdot T_{gi}) \Big/ \sum (\Delta C_{pi} \cdot W_i) \tag{3}$$

$$\Delta C_{pmix} = \sum (\Delta C_{pi} \cdot W_i) \Big/ \sum W_i \tag{4}$$

where, ΔC_{pmix} is the heat increment for solute mixture, ΔC_{pi}, W_i, and T_{gi} are heat increment, weight fraction, and glass transition temperature for solute i, respectively. Thus, the glass transition temperature for the solute mixture and water binary solution is:

$$T_g = \frac{\Delta C_{pmix} \cdot T_{gmix} \cdot \sum W_i + \Delta C_{pw} \cdot T_{gw} \cdot (1 - \sum W_i)}{\Delta C_{pmix} \cdot \sum W_i + \Delta C_{pw} \cdot (1 - \sum W_i)} \tag{5}$$

where ΔC_{pw} and T_{gw} are heat increment and glass transition temperature for water. With the definition of C-K coefficient: $k_i = \Delta C_{pw} / \Delta C_{pi}$, Equation 5 can be re-expressed as:

$$T_g = \frac{\sum (W_i \cdot T_{gi} / k_i) + T_{gw} \cdot (1 - \sum W_i)}{\sum (W_i / k_i) + (1 - \sum W_i)} \tag{6}$$

For a multicomponent solution containing electrolytes, there has been no equation proposed to calculate its glass transition temperature so far. Some experiments showed that the addition of NaCl or $MgCl_2$ had no effect on T_g value of sugar/water binary systems.[20,22] If this conclusion holds true for other solutions containing electrolytes, Equation 6 can be modified slightly to calculate their T_g values:

$$T_g = \frac{\sum (W_i \cdot T_{gi} / k_i) \cdot (\sum W_e / \sum W_i + 1) + T_{gw} \cdot (1 - \sum W_i - \sum W_e)}{\sum (W_i / k_i) \cdot (\sum W_e / \sum W_i + 1) + (1 - \sum W_i - \sum W_e)} \tag{7}$$

where W_e is the initial weight fraction of electrolytes.

Combining Equation 7 with Equation 2, the T'_g for a multicomponent solution can be deduced: