ORIGINAL ARTICLE

Thermal Properties of Freeze-Concentrated Sugar-Phosphate Solutions

**Nathdanai Harnkarnsujarit · Misako Nakajima ·
Kiyoshi Kawai · Manabu Watanabe · Toru Suzuki**

Abstract In order to understand the effect of phosphate salts on the freeze-concentrated glass-like transition temperature (T_g') of aqueous sugar solutions, two types of sugar (glucose and maltose) and five types of phosphate salts (Na_3PO_4, $Na_4P_2O_7$, $Na_5P_3O_{10}$, K_3PO_4, and $K_4P_2O_7$) were employed, and the thermal properties of various sugar-phosphate aqueous systems were investigated using differential scanning calorimetry. The T_g' of glucose increased with increasing sodium phosphates up to a certain phosphate ratio, decreasing thereafter. The maximum T_g' value was slightly higher in the order of $Na_3PO_4 > Na_4P_2O_7 \geq Na_5P_3O_{10}$. Maltose-sodium phosphate also showed a similar trend as glucose-sodium phosphate samples. However, the degree of T_g'-rise of maltose systems was much less than that of glucose. It is thought that the T_g' elevated by the molecular interaction between sugar and phosphate ions will be reduced by hydrated sodium ions. In comparisons between potassium phosphate and sodium phosphate, it was found that sugar-potassium phosphates showed the lower maximum T_g' at a lower phosphate ratio than sugar-sodium phosphates. In addition, the T_g' of potassium phosphates dropped sharply in comparison with sodium phosphates at the high phosphate ratio. These results suggest that potassium phosphates are lower T_g' than sodium phosphates, and that potassium ion plays a better plasticizer than sodium ion. A certain amount of sodium phosphates (Na_3PO_4 and $Na_4P_2O_7$) caused devitrification. Potassium phosphates, however, did not show devitrification which can be explained by the fact that potassium ion can be dynamically restricted by sugar.

Keywords Freeze-concentrated glass-like transition · Devitrification · Phosphate · Sugar · Differential scanning calorimetry (DSC)

Introduction

Ice formation in bio, pharmaceutical, and food materials during cooling process causes phase separation between ice and the freeze-concentrated amorphous matrix. When the viscosity of the freeze-concentrated amorphous matrix increases to $>10^{12}$ Pa·s during further cooling, the freeze-concentrated glass transition occurs in the systems. The temperature of which freeze-concentrated glass transition occurs has been commonly described as T_g' in order to distinguish it from an ordinary glass transition temperature (T_g); the ordinary glass transition means the glass transition of aqueous systems with no ice formation and can be accomplished by a rapid cooling more than the crystallization rate of water molecules [1].

There has been considerable interest in the T_g' of sugars and/or sugar mixtures because their freeze-concentrated glass transition contributes to the cryo- and lyo-preservation of bio [2–5], pharmaceutical [5], and food materials [1, 2, 5]. While the T_g' of sugar systems has been investigated using differential scanning calorimetry (DSC), the DSC thermogram exhibits two endothermic shifts; a small endothermic shift and a subsequent large one are observed. While the origin of the two endothermic shifts has been discussed extensively, there has been no consensus. For example, it is thought that the low-temperature shift corresponds to a maximally freeze-concentrated glass transition and the high-temperature shift is an ante-melting temperature where the viscous flow initiates in the amorphous region occurring a few Kelvin below the

N. Harnkarnsujarit · M. Nakajima · M. Watanabe · T. Suzuki
Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan

K. Kawai (✉)
Department of Biofunctional Science and Technology, Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan
e-mail: kawai@hiroshima-u.ac.jp

beginning of ice-melting [6, 7] or the temperature of the beginning of ice-melting [1, 8, 9]. On the other hand, there is an interpretation that the low- and the high-temperature shifts correspond to glass transitions of two phases of different solute concentrations, with the former due to the transition in a freeze-concentrated region containing a large amount of un-frozen water and the latter due to the transition in a maximally freeze-concentrated region [10, 11]. In addition, Pyne et al. [11] point out that the high-temperature shift overlaps the onset of ice-melting. Modulated temperature-DSC (MT-DSC), which can separate the reversible part and non-reversible part of the DSC heat flow, is useful for a better understanding of complex glass transition properties. Goff et al. [12] investigated the freeze-concentrated glass transition behavior of sucrose aqueous solution using MT-DSC, and concluded that the low-temperature shift is a maximally freeze-concentrated glass transition, and that the high-temperature shift is simultaneous events of the glass transition of plasticized solute within the ice-crystals and the beginning of ice-melting. As the collapse of freeze-concentrated amorphous matrix and re-crystallization of ice usually occur at temperatures above the high-temperature shift, the high-temperature shift is a more practically important parameter than the low-temperature shift with respect to cryo- and lyo-preservation [13–15]. Thus, this study denotes simply the high-temperature shift as a freeze-concentrated "glass-like" transition (T_g') hereafter.

More recently, the interest in T_g' of oxyanions (e.g., borate and phosphate) mixed with sugars has been stimulated by the synergistic rise in T_g'. For example, Izutsu et al. [16, 17] investigated the effect of sodium tetraborate on the T_g' of sugar and polyol aqueous solutions, and reported the T_g' of mannitol solution increased from −35 °C to −22 °C by the addition of up to 300 mM sodium tetraborate [17]. It was proposed that borate accomplishes this by forming a reversible cross-linked network between hydroxyl compounds. On the other hand, Kawai and Suzuki [18] investigated the effect of sodium tripolyphosphate on the T_g' of sugar aqueous solutions and reported a similar effect to sodium tetraborate on T_g'. Phosphate is of more practical use than borate in pharmaceutical and/or food applications because of toxicity concerns regarding borate. However, very little quantitative data was available on the T_g' of sugar-phosphate aqueous systems. Although mono- and di-phosphates easily crystallizes during cooling (eutectic) or subsequent heating process (devitrification) [14, 19, 20], it was expected that the crystallization of phosphates was prevented by sugar [21]. In addition, there was a possibility that the phosphates can raise the T_g' of sugar.

To elucidate this subject, the T_g' of various sugar-phosphate aqueous systems was systematically investigated using DSC. For this purpose, two types of sugar (glucose and maltose) and four types of phosphate salts (sodium and potassium mono-

and di-phosphate) were employed. Sodium tri-phosphate was also used in order to obtain some additional data of the phosphate number and thermal properties. From the results, the effects of the number of glucose and phosphate units, the type of anion, and the ratio of sugar and phosphate compound on the T_g' value were discussed.

Materials and Methods

Sample Preparation

Analytical grade sodium phosphate (Na_3PO_4) and sodium triphosphate ($Na_5P_3O_{10}$) were purchased from Sigma-Aldrich, Tokyo, Japan. Analytical grade sodium diphosphate ($Na_4P_2O_7$) was purchased from Kanto Chemical Co., Inc., Tokyo, Japan. Analytical grade glucose, maltose, potassium phosphate (K_3PO_4), and potassium diphosphate ($K_4P_2O_7$) were purchased from Wako Pure Chemical Industries, Tokyo, Ltd., Japan.

Sugars (glucose and maltose) and phosphate salts (Na_3PO_4, $Na_4P_2O_7$, $Na_5P_3O_{10}$, K_3PO_4, and $K_4P_2O_7$) were mixed at various phosphate mole fractions (X_p) defined as the mole of a phosphate salt divided by the total mole of all solutes (phosphate salt and sugar). Sugar-phosphate aqueous solutions were prepared at 10 % (w/w) solids. The solute mixtures were dissolved in distilled water at 25 °C for 30 min by magnetic stirring. The samples of up to $X_P=0.86$ were prepared depending on the water solubility of the phosphate salts.

Differential Scanning Calorimetry (DSC)

Thermal properties of the samples were investigated using a DSC (DSC-50; Shimadzu Co., Tokyo, Japan). The DSC was calibrated using indium and distilled water. The samples (approximately 7 mg) were hermetically sealed in an aluminum pan, and cooled to −80 °C at 6 °C/min, then heated to 25 °C at 2 °C/min. DSC thermograms were analyzed using a TA-60WS (Shimadzu Co., Tokyo, Japan) interfaced with the DSC. The values of T_g' and devitrification temperature (T_d) were evaluated from the onset point of endothermic shift and the onset point of exothermic peak of heat flow, respectively. All measurements were performed in triplicate and the results were averaged.

Results and Discussion

DSC Thermograms

Typical DSC thermograms for maltose-Na_3PO_4 are shown in Fig. 1. One or two endothermic shifts, exothermic peak (devitrification), and endothermic peak (ice-melting) were

Fig. 1 Typical DSC thermograms for maltose-Na_3PO_4 system. The X_P indicates the mole of Na_3PO_4 divided by the total mole of all solutes (maltose and Na_3PO_4). From the onset of the high-temperature shift, freeze-concentrated glass-like transition (T_g') was evaluated. In addition, devitrification temperature (T_d) was evaluated from the onset of exothermic peak
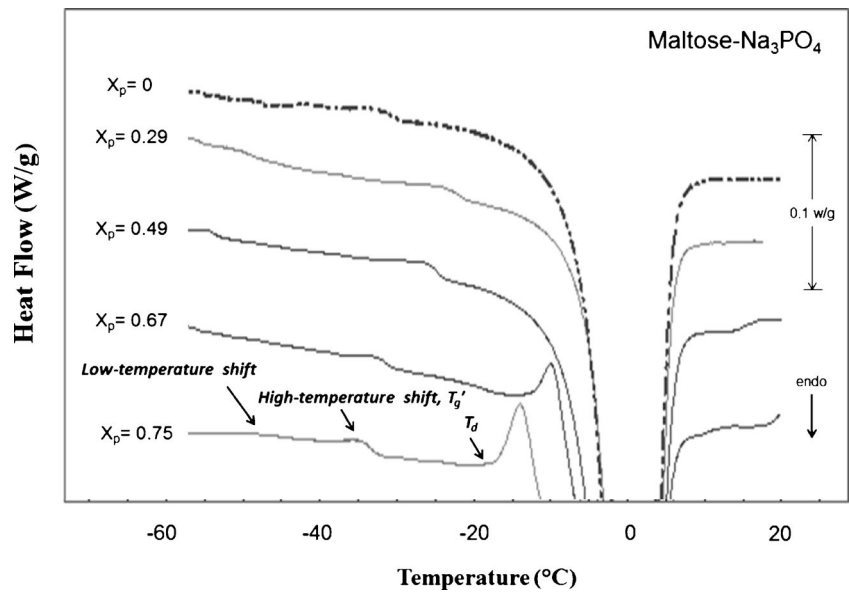
observed in the DSC thermograms, depending on the sample. As mentioned above, two endothermic shifts have been commonly observed in sugar solutions, but their origin has not been completely understood. The low-temperature shift was a minor thermal response and unclear for some samples. The high-temperature shift, on the other hand, was observed clearly in all the samples. This study focused on the high-temperature shift for the practical reason of which the collapse of freeze-concentrated amorphous matrix and recrystallization of ice usually occur at temperatures above the high-temperature shift [13–15], and the T_g' was determined from the onset point of the shift. The T_g' of maltose shown at $X_P=0$ (−32.5 °C) was in good agreement with a previous result (−32 °C) [1]. The other samples also showed a similar glass transition behavior to Fig. 1, and the T_g' was evaluated reasonably. On the other hand, a certain amount of Na_3PO_4 caused devitrification during heat-scanning. This was also confirmed in glucose-Na_3PO_4, glucose-$Na_4P_2O_7$, maltose-Na_3PO_4 and maltose-$Na_4P_2O_7$ samples. The T_d of the samples was evaluated from the onset point of the peak.

Freeze-Concentrated Glass-Like Transition Temperature

The effect of sodium phosphates (Na_3PO_4, $Na_4P_2O_7$, and $Na_5P_3O_{10}$) on the T_g' of glucose is shown in Fig. 2. The lines were obtained by fitting a cubic equation to the T_g' values ($R^2>0.97$), and given as a guide to the eye. The guide lines were also given for the other systems ($R^2>0.87$) as shown in afterwards. The T_g' of glucose increased with increasing sodium phosphate in the range of up to $X_P=0.37\sim0.45$, decreasing thereafter. The maximum T_g' value evaluated from the guide line was slightly higher in the order of Na_3PO_4 (−29.2 °C) > $Na_4P_2O_7$ (−31.6 °C) ≥ $Na_5P_3O_{10}$ (−32.1 °C). It is known that a certain amount of phosphate raises the glass transition

temperature of dehydrated sugar as a result of intermolecular interaction. For example, Ohtake et al. [22, 23] demonstrated that dehydrated mixtures of monophosphate (KH_2PO_4 and K_2HPO_4) and disaccharide (trehalose and sucrose) showed higher glass transition temperatures than that of trehalose and sucrose alone. However, in the case of the freeze-concentrated state, the contribution of water molecules to the glass transition temperature should also be considered. As mentioned above, phosphate ions and glucose interact in the freeze-concentrated state [18]. Sodium ions, on the other hand, hydrate, and thus hydrate water molecules exist as "unfrozen water" in the freeze-concentrated state. Since water plays a role of plasticizer, the unfrozen water in the freeze-concentrated system reduce the T_g' [10, 24–26]. For example, Inoue and Ishikawa [25] investigated the effect of NaCl
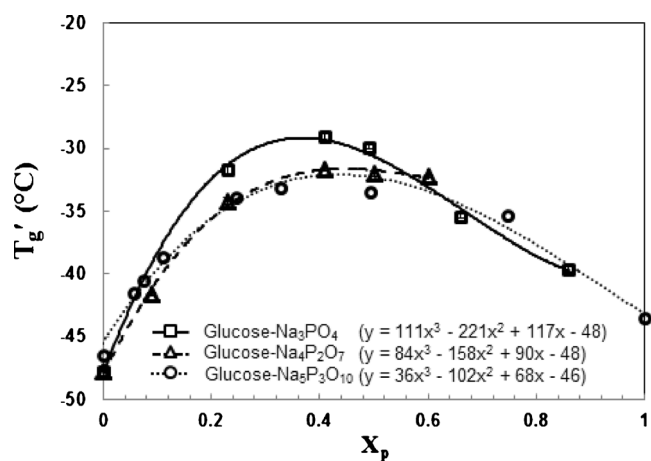

Fig. 2 Effect of sodium phosphates (Na_3PO_4, $Na_4P_2O_7$, and $Na_5P_3O_{10}$) on the T_g' of glucose. Values are expressed as mean ± SD ($n=3$). The lines were obtained by fitting a cubic equation to the T_g' values ($R^2>0.97$), and given as a guide to the eye. The T_g' values for glucose-$Na_5P_3O_{10}$ sample were taken from our previous results [18]

addition on the T_g' of bovine serum albumin aqueous solution, and demonstrated that unfrozen water increased and T_g' decreased with increased NaCl. Taking this into account, the T_g' elevated by the molecular interaction between sugar and phosphate ion will be reduced by hydrated sodium ion; the number of sodium ions ($Na_3PO_4 < Na_4P_2O_7 < Na_5P_3O_{10}$) explains the slight difference in maximum T_g' ($Na_3PO_4 > Na_4P_2O_7 \geq Na_5P_3O_{10}$). There was a minor difference of T_g' between $Na_4P_2O_7$ and $Na_5P_3O_{10}$ in spite of the different number of sodium ions. This was caused by a limitation of the plasticizing effect of unfrozen water on the freeze-concentrated glass phase [10].

The effect of sodium phosphates (Na_3PO_4, $Na_4P_2O_7$, and $Na_5P_3O_{10}$) on the T_g' of maltose is shown in Fig. 3. A similar trend to glucose-sodium phosphate systems was observed; the maximum T_g' value observed at around $X_P=0.3$ was slightly higher in the order of Na_3PO_4 (−23.7 °C) > $Na_4P_2O_7$ (−26.1 °C) ≥ $Na_5P_3O_{10}$ (−27.6 °C). The degree of T_g'-rise, on the other hand, was much less than glucose-sodium phosphate systems. This result was in good agreement with previous results for sugar-sodium tripolyphosphate [18] and sugar-sodium tetraborate [16] systems. Since low-T_g' sugar aqueous systems often crystallize or collapse during freeze-drying, it is of practical importance to synergistically raise their T_g'.

Effects of potassium phosphates (K_3PO_4 and $K_4P_2O_7$) on the T_g' of glucose and maltose are shown in Figs. 4 and 5, respectively. For comparison, the guide lines for Na_3PO_4 and $Na_4P_2O_7$ were taken from Figs. 2 and 3. There was little difference in effect between K_3PO_4 and $K_4P_2O_7$ on the T_g' of glucose (Fig. 4) and maltose (Fig. 5). On the other hand, a major difference was found in the comparison between potassium phosphates and sodium phosphates. The maximum T_g' of potassium phosphate systems (−37.2~−37.8°C for glucose and−27.3~−29.6°C for maltose) was much lower than sodium
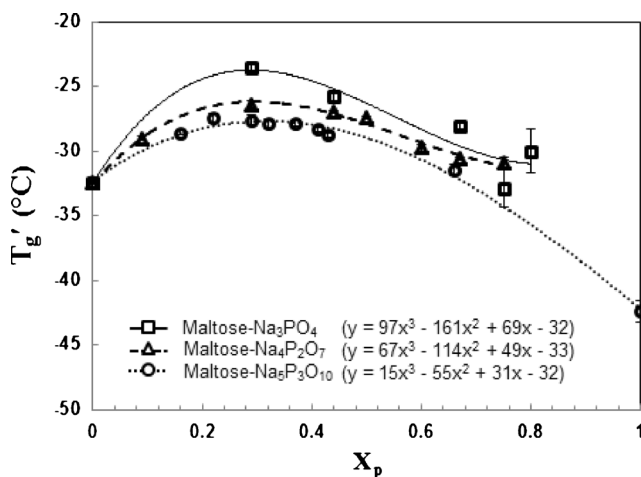

Fig. 4 Effect of potassium phosphates (K_3PO_4, $K_4P_2O_7$) on the T_g' of glucose. Values are expressed as mean ± SD (n=3). The lines were obtained by fitting a cubic equation to the T_g' values ($R^2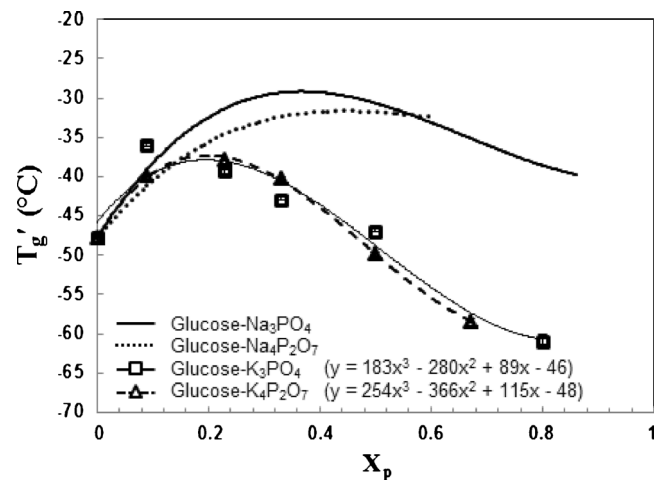$>0.91), and given as a guide to the eye. For comparisons, the guide lines of glucose-Na_3PO_4 and glucose-$Na_4P_2O_7$ samples were also taken from Fig. 2

phosphate systems (−29.2~−31.6 °C for glucose and −23.7~−26.1 °C for maltose). In addition, the maximum T_g' of potassium phosphate systems was observed at a lower X_P (0.17~0.25) than sodium phosphate systems (0.30~0.45). Furthermore, the T_g' of potassium phosphate samples dropped sharply in comparison with sodium phosphate samples at a high phosphate ratio. These results suggest that potassium ion plays a better plasticizer than sodium ion, and that potassium phosphates are lower T_g' than sodium phosphates. As mentioned above, sugar and phosphate ion can interact in the freeze-concentrated state, thus the T_g' of their mixture can be elevated synergistically. In contrast, hydrated sodium ion and/ or potassium ion play a role of plasticizer, and thus the T_g' of
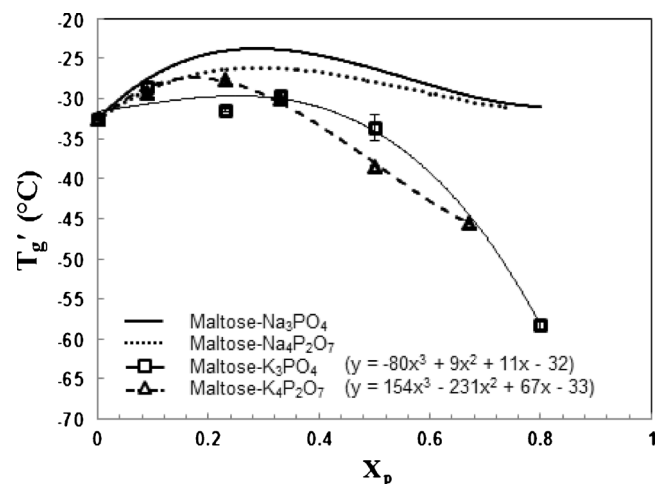

Fig. 3 Effect of sodium phosphates (Na_3PO_4, $Na_4P_2O_7$, and $Na_5P_3O_{10}$) on the T_g' of maltose. Values are expressed as mean ± SD (n=3). The lines were obtained by fitting a cubic equation to the T_g' values (R^2>0.87), and given as a guide to the eye

Fig. 5 Effect of potassium phosphates (K_3PO_4, $K_4P_2O_7$) on the T_g' of malose. Values are expressed as mean ± SD (n=3). The lines were obtained by fitting a cubic equation to the T_g' values (R^2>0.98), and given as a guide to the eye. For comparisons, the guide lines of maltose-Na_3PO_4 and maltose-$Na_4P_2O_7$ samples were also taken from Fig. 3

sugar-phosphate mixture is reduced more or less. If potassium ion is a better plasticizer than sodium ion, potassium ion will prevent the synergistic rise in T_g' greater than sodium ion. As the result, sugar-potassium phosphates had a lower maximum T_g' at a lower X_P and larger T_g'-drop than sugar-sodium phosphates.

The T_g' of mono- and di-phosphate solutions ($X_P=1.0$) was not investigated in this study, because they easily crystallizes during cooling process (eutectic) or subsequent heating process (devitrification) [14, 19, 20]. Even if there was no crystallization of the phosphates, their freeze-concentrated glass-like transition will not have been detected because of a lower T_g' than the scanned temperature. However, the suggestion that potassium phosphates are lower T_g' than sodium phosphates is supported by many previous results: ganciclovir sodium ($T_g'=-34.9$ °C) and potassium (-46.2 °C) [27], indomethacin sodium (-35.4 °C) and potassium (-38.9 °C) [28], sodium citrate (-41 °C) and potassium (-62 °C) [14], and sodium acetate (-64 °C) and potassium (-76 °C) [14]. Differences in effect between sodium and potassium ions on glucose solutions have also been demonstrated. For example, Soga et al. [29] investigated the effect of NaCl and KCl on the viscosity of glucose aqueous solutions. Although the viscosity of glucose aqueous solutions increased with increasing NaCl content, a certain amount of KCl reduced the viscosity of glucose aqueous solutions. This indicates that the potassium ion acts as a plasticizer in hydrogen bonding aqueous systems.

Devitrification Temperature

Most phosphate solutions are eutectic during freezing [19, 20]. Although sugar can prevent the crystallization of phosphate in dehydrated systems [22], a certain amount of sodium phosphate (Na_3PO_4 and $Na_4P_2O_7$) in solutions caused devitrification during heat-scanning. Potassium phosphate samples, on the other hand, did not show devitrification. This can be explained by the fact that potassium ion can be dynamically restricted by sugar. For example, NaCl has a salting-out effect for glucose, and thus two hydration structures of which NaCl and glucose are incompatible to each other are formed in NaCl-glucose aqueous solution. On the other hand, KCl has a salting-in effect for glucose, and thus the hydration structure of KCl is compatible with that of glucose [30]. Similar effects are also found in the comparison between $NaNO_3$ and KNO_3 [30]. In addition, potassium ion and glucose interact directly, and thus potassium ion plays a role of cross-linker for dextran gel [31].

The relationship between T_d and X_P is shown in Fig. 6. The T_d decreased with increasing X_P. This was because the T_g' decreased with increasing phosphate ratio at above $X_P=0.30$ $\sim$0.45, since devitrification occurs at a temperature above T_g'. In a comparison of Na_3PO_4 and $Na_4P_2O_7$ samples, it was noted that the T_d of $Na_4P_2O_7$ samples was higher than that of Na_3PO_4 samples. This indicates that $Na_4P_2O_7$ is more resistant to crystallization than Na_3PO_4. As the $Na_5P_3O_{10}$ solution is not eutectic [18], it is thought that the large number of phosphates prevents its crystallization during freeze-concentration.

Conclusions

The T_g' of sugar and phosphate mixture systems was investigated systematically, and the effects of the number of glucose and phosphate units, the type of cation, and the ratio of sugar and phosphate compound on T_g' and T_d values were demonstrated. The synergistic increase of T_g' is expected to improve the stability of frozen and freeze-dried foods and pharmaceutical products.

Fig. 6 Devitrification temperature (T_d) of glucose and maltose mixed with sodium phosphates (Na_3PO_4 and $Na_4P_2O_7$) systems. Values are expressed as mean ± SD ($n=3$). The lines were given as a guide to the eye

References

1. Y.H. Roos, *Phase Transitions in Foods* (Academic, California, 1995), pp. 19–48. 73–107
2. H.D. Goff, M.E. Sahagian, Thermochim. Acta **280**, 449 (1996)
3. P.R. Harrigan, T.D. Madden, P.R. Cullis, Chem. Phys. Lipids **52**, 139 (1990)
4. Z. Hubalek, Cryobiology **46**, 205 (2003)
5. A. Patist, H. Zoerb, Colloids Surf. B **40**, 107 (2005)
6. A.P. MacKenzie, Phil. Trans. R. Soc. B **278**, 167 (1977)
7. E.Y. Shalaev, A.N. Kanev, Cryobiology **31**, 374 (1994)
8. S. Ablett, M.J. Izzard, P.J. Lillford, J. Chem Soc. Faraday Trans. **88**, 789 (1992)

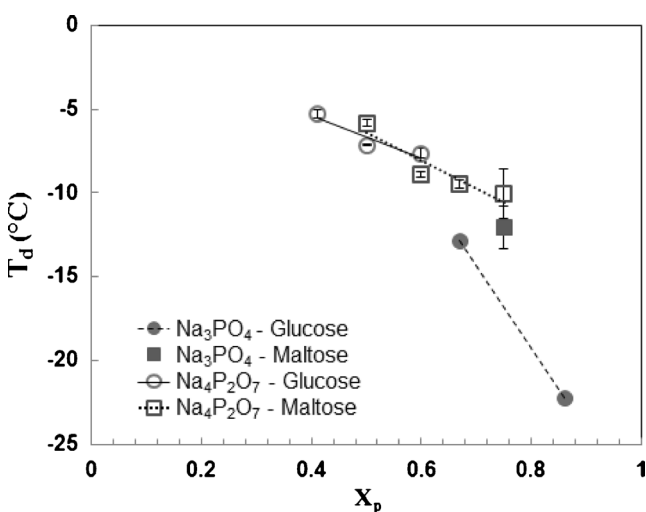9. E.Y. Shalaev, F. Franks, J. Chem Soc. Faraday Trans. **91**, 1551 (1995)
10. H. Levine, L. Slade, Cryo-Lett. **9**, 21 (1988)
11. A. Pyne, R. Surana, R. Suryanarayanan, Thermochim. Acta **405**, 225 (2003)
12. H.D. Goff, E. Verespej, D. Jermann, Thermochim. Acta **399**, 43–55 (2003)
13. Y.H. Roos, J. Therm. Anal. **48**, 535 (1997)
14. B.S. Chang, C.S. Randall, Cryobiology **29**, 632 (1992)
15. W. Wang, Int. J. Pharm. **203**, 1 (2000)
16. K. Izutsu, A. Rimand, N. Aoyagi, S. Kojima, Chem. Pharm. Bull. **51**, 663 (2003)
17. K. Izutsu, A. Rimand, N. Aoyagi, S. Kojima, Int. J. Pharm. **273**, 85 (2004)
18. K. Kawai, T. Suzuki, Cryo-Lett. **27**, 107 (2006)
19. N. Murase, F. Franks, Biophys. Chem. **34**, 293 (1989)
20. N. Murase, P. Echlin, F. Franks, Cryobiology **28**, 364 (1991)
21. K.A. Pikal-Cleland, J.L. Cleland, T.J. Anchordoquy, J.F. Carpenter, J. Pharm. Sci. **91**, 1969 (2002)
22. S. Ohtake, C. Schebor, S.P. Palecek, J.J. Pablode, Cryobiology **48**, 81 (2004)
23. S. Ohtake, C. Schebor, S.P. Palecek, J.J. de Pablo, Pharm. Res. **21**, 1615 (2004)
24. G. Blond, D. Simatos, Food Hydrocoll. **12**, 133 (1998)
25. C. Inoue, M. Ishikawa, J. Food Sci. **65**, 1187 (2000)
26. M.F. Mazzobre, M.P. Longinotti, H.R. Corti, M.P. Buera, Cryobiology **43**, 199 (2001)
27. L. Kumar, A. Baheti, A.K. Bansal, Mol. Pharm. **8**, 309 (2010)
28. L. Kumar, A. Baheti, A. Mokashi, A.K. Bansal, Eur. J. Pharm. Sci. **44**, 136 (2011)
29. M. Soga, K. Kurosaki, K. Kajiwara, in *Water Properties in Food, Health, Pharmaceutical and Biological Systems: ISOPOW10*, ed. by D.S. Reid, T. Sajjaanantakul, P.J. Lillford, S. Charoenrein (Wiley, Singapore, 2010), pp. 473–475
30. K. Kajiwara, A. Motegi, N. Murase, Cryo-Lett. **22**, 311 (2001)
31. T. Watanabe, A. Ohtsuka, N. Murase, P. Barth, K. Gersonde, Magn. Reson. Med. **35**, 697 (1996)