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Effect of cryoprotectants on eutectics of $NaCl\cdot 2H_2O$/ice and KCl/ice studied by temperature wave analysis and differential scanning calorimetry

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Abstract

The effect of cryoprotectants (sucrose, glucose, dimethyl sulfoxide (DMSO) and polyvinylpyrrolidone (PVP)) on the eutectics of $NaCl\cdot 2H_2O$/ice and KCl/ice have been studied by temperature wave analysis (TWA) and differential scanning calorimetry (DSC). The eutectic crystallization was inhibited by the cryoprotectant, and a higher inhibitory ability was observed in NaCl systems (water/NaCl/cryoprotectant) than in KCl systems (water/KCl/cryoprotectant) with the same additive concentration. In the above system, sugars showed the higher inhibition ability than DMSO or PVP. The increase of thermal diffusivity with the increasing sugar concentration at a temperature immediately after the eutectic melting in KCl/sugar systems suggested a characteristic hydration of sugar and K^+ ion, this was not observed in NaCl/sugar systems.

Keywords: Cryoprotectant; NaCl aqueous solution; KCl aqueous solution; Eutectic; Temperature wave analysis; Sucrose; Glucose; Hydration; Thermal diffusivity; Freezing

1. Introduction

The living systems are heterogeneous, and the most living organisms contain ions such as sodium and potassium. It is known that the freeze-thaw damage to biological cells is basically attributed to two major mechanisms [1]: one is the osmotic dehydration of cells due to the increased concentration of solute, sodium chloride (NaCl) in particular, in the extra-cellular liquid as ice precipitates, finally eutectic crystallization forms [2,3]. The other is the cell destruction through the formation of intracellular ice [4,5]. To minimize the damage due to the above mechanism, the cryoprotectants have been used to segregate the physiological salt or to inhibit the eutectic crystallization in freezing process. In order to examine such segregation effect, ternary mixture of water, salt and cryoprotectants has been studied by using thermal analysis at low temperature [2,3,6–13], especially in the water–NaCl–cryoprotectant systems (abbreviated as NaCl systems). In the prior papers [14–20], the method of temperature wave analysis (TWA) has been used as an unique technique to study the heat transport property in a continuous temperature scan. This method can provide the thermal diffusivity, which is known to be sensitive to the higher order structure of the materials. In the previous studies of aqueous alkali chloride (NaCl, KCl, RbCl and CsCl) solutions [20,21], thermal diffusivity was determined by TWA during freezing and thawing. A sharp increase of thermal diffusivity during eutectic crystallization was observed with super-cooling phenomena. Thermal diffusivity in the frozen state showed a variation depending on the alkali chloride solute, higher the thermal diffusivity, lower the eutectic melting temperature. A different number of bounding water molecules to each alkali chloride solute was suggested. The melting temperature of

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eutectic is −22 °C for $NaCl{\cdot}2H_2O$/ice [22–24], −10.7 °C for KCl/ice [23]. In contrast to extensive studies devoted to the characterization of the effect of cryoprotective agents on the eutectic in ternary mixture of salt of water–NaCl–cryoprotectant, comparable data concerning the effect of cryoprotectant on the eutectic in ternary mixture of water–KCl–cryoprotectant (abbreviated as KCl system) are not well studied.

The object of this study is to clarify the difference between the eutectic formed in NaCl and KCl systems to which have been added the cryoprotectants such as sucrose, glucose, dimethyl sulfoxide (DMSO) and polyvinylpyrrolidone (PVP). For that purpose, melting behavior ofeutectic in the ternary system was investigated by using differential scanning calorimetry (DSC) and thermal wave analysis (TWA). The concentrations of sodium chloride (NaCl) and potassium chloride (KCl) in this study were kept constant at 0.154 M, corresponding to the physiologicalconcentration.

2. Materials and methods

2.1. Materials

NaCl, KCl and glucose were purchased from Wako Pure Chemical Ind., Ltd. with purity more than 99.5%. Sucrose and dimethyl sulfoxide (DMSO) were purchased from Tokyo Kasei Kogyo Co., Ltd. with the purity of 99.9%. Polyvinylpyrrolidone (PVP, average $M_W = 40\,000$) was from Sigma Chemical Co. with the purity of 99.5%. All chemicals were used without further purification. The pure water with resistivity of at least 15.1 MΩ cm was provided for the solvent.

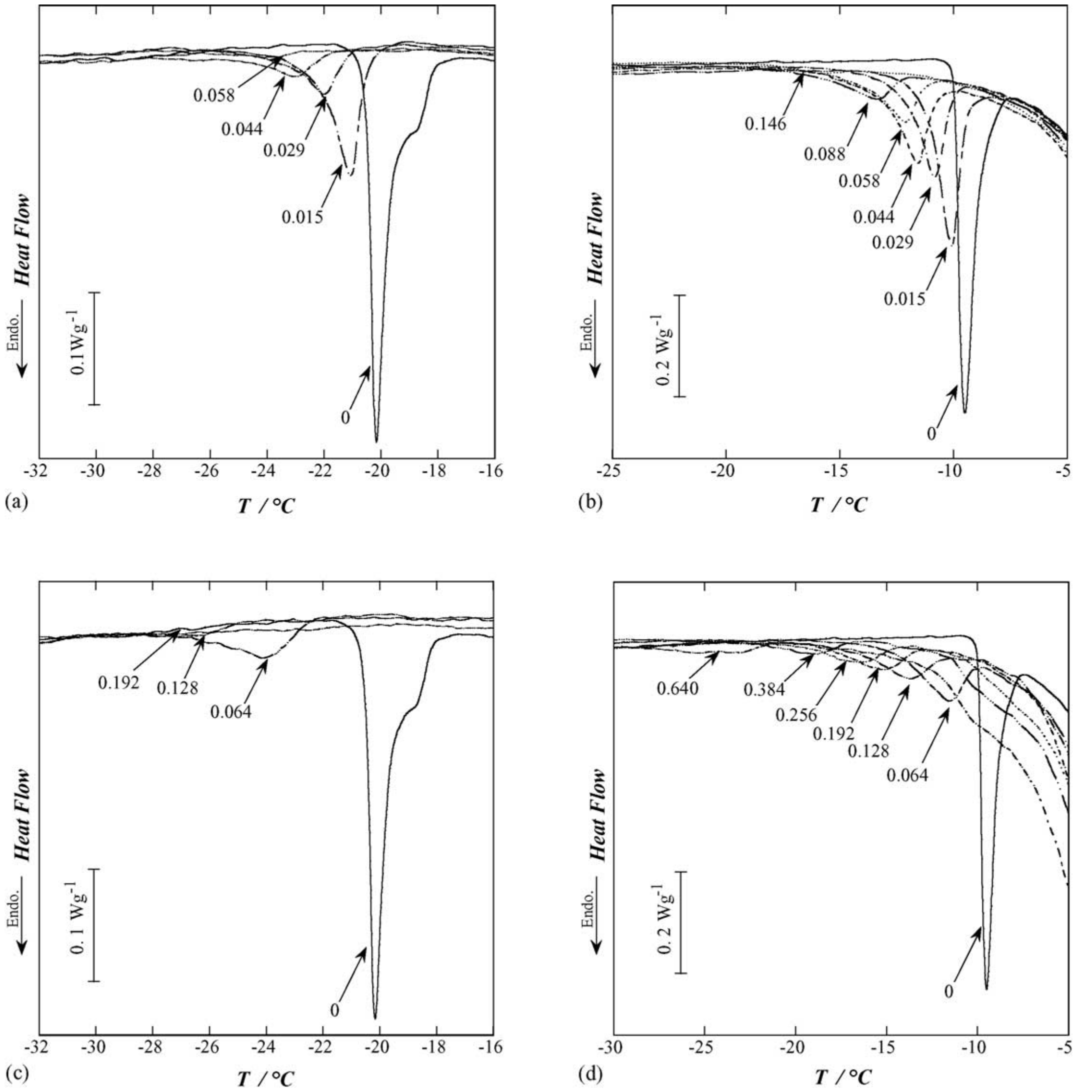

Fig. 1. DSC heating curves for ternary systems of 0.154 M salt and cryoprotectants with various molar concentrations during heating process. The compositions for the ternary systems are: (a) NaCl and sucrose; (b) KCl and sucrose; (c) NaCl and DMSO; (d) KCl and DMSO. Numerals shown in figure are molar concentration of the added cryoprotectants.

2.2. Measurement of DSC

RIGAKU TAS 200 instrument equipped with a cooling accessory was used for the measurement of DSC. Sample of approximately 2 mg was sealed in an aluminum pan, following the procedures described in Ref. [25]. Specimens were pre-cooled to −80 °C at a rate of 5 °C/min from room temperature before the heating scans were recorded at a rate of 2 °C/min.

2.3. Measurement of TWA

The apparatus used in this study has been published in the prior papers [14–18]. The measuring device of TWA consists of a function synthesizer, a digital lock-in amplifier, a power source of bias current for sensor, a temperature controller, a sample holder on the hot stage and a personal computer. A set of flat plate of Pyrex 7740 sputtered with thin gold layers with an electric resistance of 50 Ω across an area of 1 mm × 4 mm and covered with an electrical insulation coating were used as a measurement cell for thermal diffusivity (one for a heater and the other for a sensor). The aqueous solution of each solute was inserted by capillary action into the cell. The cell was fixed under a load and sealed with a spacer to keep a constant thickness. To avoid the deformation in the thickness direction, the specimen was kept with a periphery free in the cell. The thickness of the specimen was kept 130 μm.

By the passage of ac current on the heater, the temperature wave was generated at the front surface of the specimen and propagated in the thickness direction. The frequency of temperature wave was chosen by considering the thermal diffusion length defined as $\mu = 1/k = (\omega/2\alpha)^{-1/2}$. The temperature variation at the rear surface was detected by the variation of the electrical resistance of the sensor. The relationship between the frequency of the generated temperature wave and the phase delay of the signal on the sensor is

$$\Delta\theta = -\sqrt{\frac{\pi f}{\alpha}}d - \frac{\pi}{4} \qquad (1)$$

where $\Delta\theta$ is a phase delay, α is a thermal diffusivity, f is a frequency of the generated heating wave [$f = \omega/2\pi$] and d is a sample thickness. In this equation, the relationship between

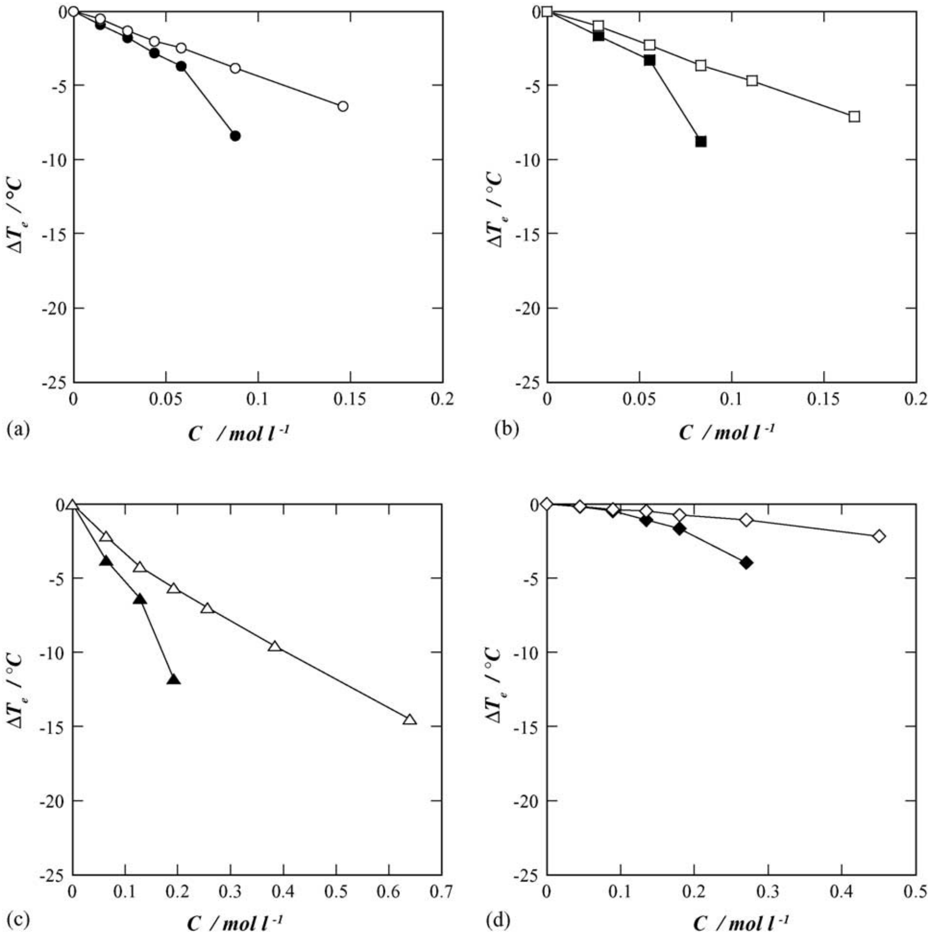

Fig. 2. Depression of the melting peak temperature of eutectic (ΔT_e) with various molar concentrations of cryoprotectants. The compositions for the ternary systems are: (a) –●– NaCl and sucrose, –○– KCl and sucrose; (b) –■– NaCl and glucose, –□– KCl and glucose; (c) –▲– NaCl and DMSO, –△– KCl and DMSO; (d) –◆– NaCl and PVP, –◇– KCl and PVP.

the square root of f and $\Delta\theta$ is verified to be a linear. Therefore, when the thickness of the specimen is known, the thermal diffusivity α can be obtained from the slope of $\sqrt{f}$ versus $\Delta\theta$. Eq. (1) can also be written as follows:

$$\alpha = \frac{\pi f d^2}{(\Delta\theta + \pi/4)^2} \quad (2)$$

From to Eq. (2), α can be determined by $\Delta\theta$ under a constant frequency. When the temperature is scanned at a constant heating or cooling rate, thermal diffusivity can be obtained as a function of temperature.

The specimen was first cooled to −70 °C from room temperature with a cooling rate of 0.5 °C/min, and then a phase delay at a fixed frequency of 17.6 Hz was measured in a heating scan from −70 to 25 °C at a heating rate of 0.2 °C/min.

3. Results and discussions

Heat flow rate measured by DSC at the melting region of eutectic exist in ternary systems composed of 0.154 M salts (NaCl or KCl) and cryoprotectants (sucrose or DMSO) are shown in Fig. 1. Molar concentration for sucrose is from 0 to 0.146 M, for DMSO is from 0 to 0.640 M. Numerals shown in figure are molar concentrations of each cryoprotectants. In Fig. 1(a) and (c), for NaCl binary system, endothermic peak occurs at around −20.2 °C is ascribed to the eutectic melting of $NaCl{\cdot}2H_2O$/ice. Concerning the eutectic melting temperature, the peak temperature is used in the following discussion. The endothermic peak becomes smaller and shifts to the lower temperature as the increasing additive concentration, and it disappears in the addition of 0.146 M of sucrose and 0.256 M of DMSO. In Fig. 1(b) and (d), for the KCl binary system, the endothermic peak occurs at around −9.6 °C is ascribed as the eutectic melting of KCl/ice. The endothermic peak becomes smaller and shifts to the lower temperature with the increasing cryoprotectant concentration, and it can still be observed even under 0.146 M of sucrose and 0.640 M of DMSO in KCl systems.

The depressions of the eutectic melting peak temperature (ΔT_e) in ternary systems of 0.154 M salts added with various kind and concentrations of cryoprotectant (sucrose, glucose, DMSO and PVP) are shown in Fig. 2. The larger eutectic temperature depression ΔT_e is observed in the order of sucrose/glucose > DMSO > PVP for both NaCl and KCl ternary systems. NaCl ternary system shows a larger ΔT_e than KCl ternary system under the same cryoprotectant con-

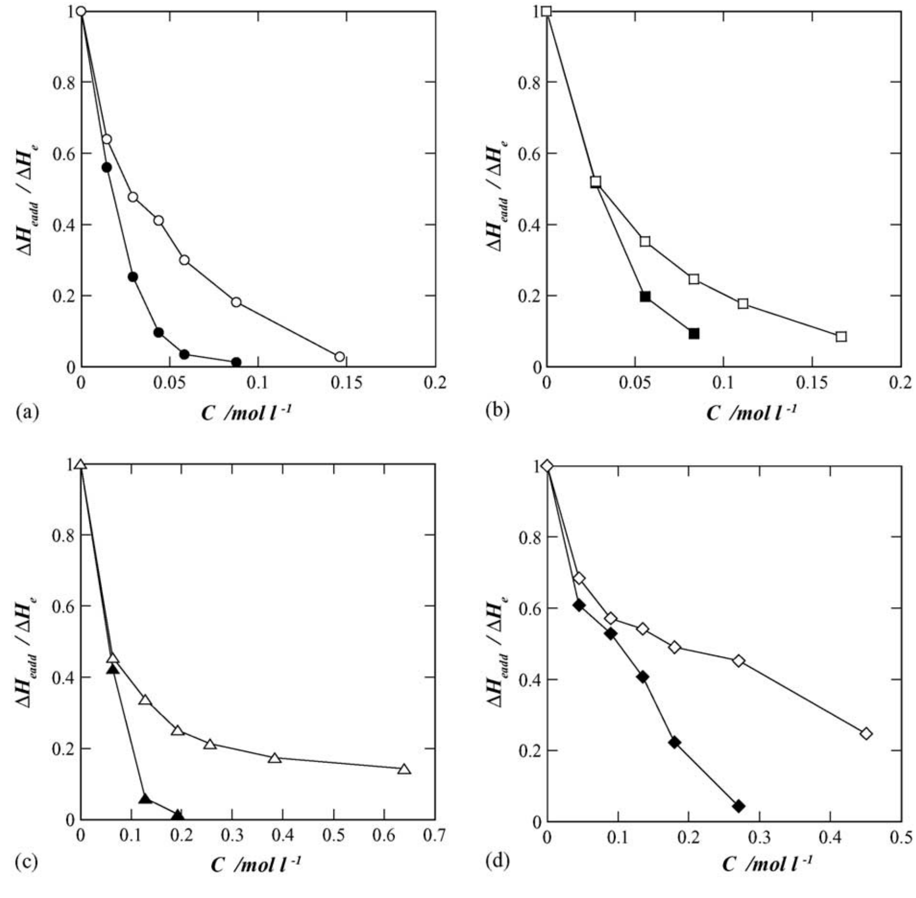

Fig. 3. Ratio of melting enthalpy of eutectic formed in ternary systems with various molar concentrations of cryoprotectants (ΔH_{eadd}) against that formed in corresponding binary salt system (ΔH_e). The compositions for the ternary systems are: (a) –●– NaCl and sucrose, –○– KCl and sucrose; (b) –■– NaCl and glucose, –□– KCl and glucose; (c) –▲– NaCl and DMSO, –△– KCl and DMSO; (d) –◆– NaCl and PVP, –◇– KCl and PVP.

centration. While the melting temperature depression of ice is much smaller in these dilute systems (data not shown).

In Fig. 3, the ratio of the melting enthalpy of eutectic formed in ternary system against that formed in corresponding binary salt system ($\Delta H_{\mathrm{eadd}}/\Delta H_{\mathrm{e}}$) is plotted as a function of the additive concentration. A larger decrease in $\Delta H_{\mathrm{eadd}}/\Delta H_{\mathrm{e}}$ is found in the order of sucrose > glucose > DMSO > PVP in each system. And $\Delta H_{\mathrm{eadd}}/\Delta H_{\mathrm{e}}$ shows a larger decrease in NaCl system than that in KCl system under the same cryoprotectant concentration.

From the eutectic temperature depression and the enthalpy decrease showed in the above two figures, a larger additive effect is found in NaCl system than in KCl system.

Temperature dependence of thermal diffusivity for ternary systems composed of 0.154 M salts (NaCl or KCl) and cryoprotectants (sucrose or DMSO) in the heating scan are shown in Fig. 4. Numerals shown in figure are molar concentrations of each cryoprotectants. In Fig. 4(a) and (c), for 0.154 M NaCl binary system, thermal diffusivity decreases gradually in the heating scan from −60 °C, a first steep descent occurs at around −22 °C corresponding to the eutectic melting. Then it decreases gradually and the second steep descent occurs which is ascribed to the melting of ice. Finally in the liquid phase, thermal diffusivity slightly increases with increasing the temperature. In the ternary system, with increasing the additive concentration thermal diffusivity is lowered in the frozen state and the steep descent during the eutectic melting becomes more and more unclear. The decreasing of thermal diffusivity which is occurred as a flexion at about −47 °C in the ternary system composed of 0.146 M sucrose corresponds to the glass transition. It accords well to the formation of amorphous phase containing NaCl, sucrose and unfrozen water among ice crystals studied by NMR [6]. Heat flow rate by DSC for 0.154 M NaCl solution added with 0.146 M sucrose in the heating process was shown in the inset of Fig. 4(a). It is found that the changing of the heat flow rate around glass transition cannot be observed clearly. Maybe the concentration of the solution is very dilute, which causing the change cannot be found remarkably. In Fig. 4(b) and (d), comparing at the same additive concentration, a larger decrease of thermal diffusivity is observed in the frozen state of KCl system. The decreasing of thermal diffusivity also oc-

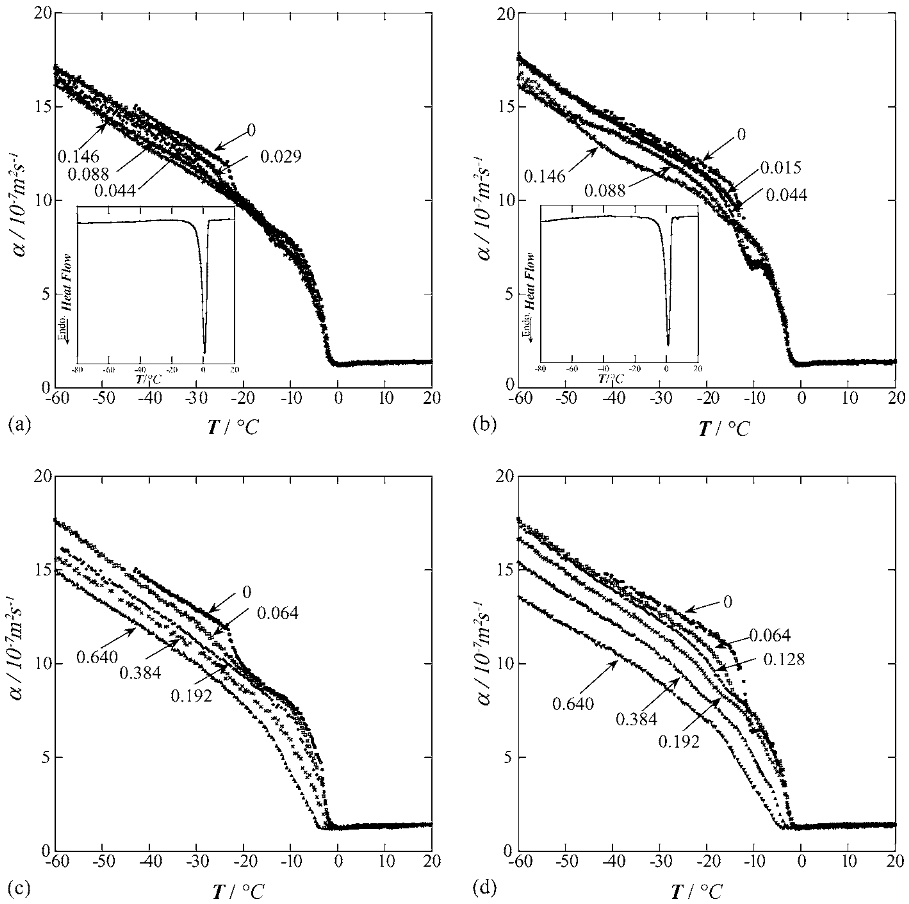

Fig. 4. Thermal diffusivities for ternary systems of 0.154 M salt and cryoprotectants with various molar concentration during heating process. Frequency of temperature wave is 17.6 Hz, temperature scan rate is 0.2 °C/min of heating, the sample thickness is 130 μm. The compositions for the ternary systems are: (a) NaCl and sucrose; (b) KCl and sucrose; (c) NaCl and DMSO; (d) KCl and DMSO. Numerals shown in figure are molar concentration of the added cryoprotectants. Insets in (a) and (b) are the heat flow by DSC under the heating scan of 2 °C/min for ternary systems of 0.154 M NaCl/0.146 M sucrose and 0.154 M KCl/0.146 M sucrose, respectively.

curred as a flexion at about −47 °C due to the glass transition and the range of which is more wider than that exist in corresponding NaCl systems. On the other hand, the heat flow rate by DSC for 0.154 M KCl solution with 0.146 M sucrose, which was shown in the inset of Fig. 4(b), does not show an obviously changing at around the corresponding temperature range. Furthermore, at the temperature region where the eutectic completes melting, thermal diffusivity in KCl system shows characteristic behavior, it increases with increasing additive concentration, whereas this phenomenon is not observed in NaCl system. For NaCl system in the corresponding temperature region at around −20 °C where eutectic completes melting, thermal diffusivity decreases with increasing additive concentration. It has been reported that thermal diffusivity of the frozen solution is mainly due to the ice fraction, because thermal diffusivity of ice is 10 times larger than that of solutions [26–28]. The cryoprotectant hydrates with water molecules when dissolves into aqueous solution, and this will cause the formation of an amorphous cryoprotectant/water phase among ice crystals in the frozen state [6]. Normally the amount of water turned into ice would decrease with increasing concentration of cryoprotectant, and cause the decreasing of thermal diffusivity of the system. While for KCl system in the corresponding temperature region at around −10 °C, it is obviously seen that thermal diffusivity value increases with increasing concentration of additives.

In order to describe the phenomena more clearly, for each solution, thermal diffusivity at −10 °C in KCl system or at −20 °C in NaCl system (at the temperature immediately after the eutectic melting) is plotted against the added cryoprotectant concentration, as shown in Fig. 5(a) and (b), respectively. Thermal diffusivity of NaCl systems decreases with increasing additive concentration, which is ascribed to the decreasing amount of ice. On the other hand, in KCl systems (except for the PVP added system), thermal diffusivity shows an increase with increasing additive concentration first, reaches the maximum at 0.1–0.15 M concentration and then decreases. Kajiwara et al. [13] suggested that in KCl system glucose molecules can be substituted for water molecules in the hydration sphere of K^+ ions and glucose molecules come into direct contact with K^+ ions. While in the NaCl system, glucose molecules cannot substitute the water molecules in the hydration structures of Na^+. In this study, the sucrose will resolve into two kinds of monosaccharide units of glucose and fructose when dissolved into water. It is considered that the dissolved monosaccharide units substitute the water molecules coming into direct contact with K^+ ions, and the displaced water molecules form ice during the cooling process, then the increased amount of ice causes the increase of thermal diffusivity. In contrast in the NaCl system, the monosaccharide units cannot substitute the water molecules in the hydration sphere of Na^+ ions and cannot come into direct contact with Na^+ ions, water would remain intact in the hydrated Na^+ sphere, and accordingly no displaced water exists. As shown in Fig. 5, thermal diffusivities for the corresponding NaCl solution do not have the increasing tendency with the increasing sucrose concentrations. The results obtained by the TWA study support Kajiwara's suggestion.

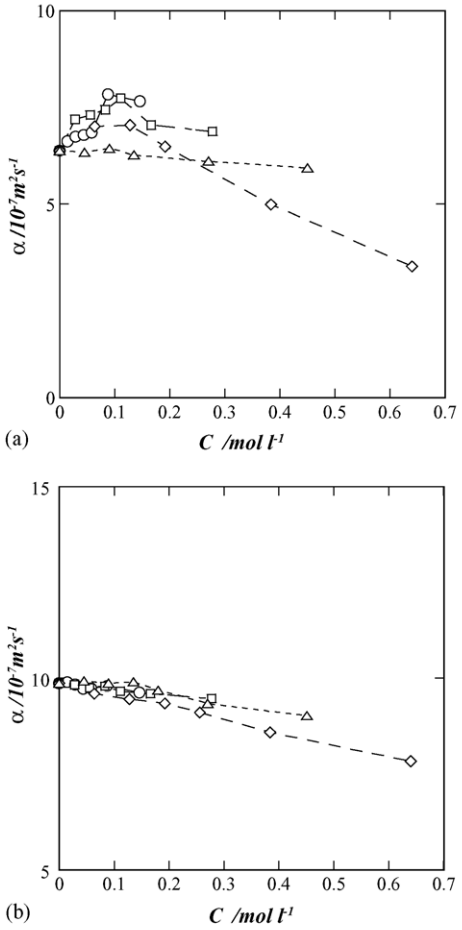

Fig. 5. Thermal diffusivity value for ternary KCl systems: (a) at −10 °C and ternary NaCl systems; (b) at −20 °C plotted against the various cryoprotectant molar concentrations. The added cryoprotectants are sucrose (◯); glucose (☐); DMSO (◊) and PVP (△).

4. Conclusion

The effects of cryoprotectants on the eutectics formed in NaCl systems and KCl systems have been studied by using temperature wave analysis (TWA) and differential scanning calorimetry (DSC). A higher inhibitory ability on eutectic crystallization was observed in NaCl systems than in KCl systems under the same additive concentration. Sugars showed a higher inhibitory ability than DMSO and PVP.

The characteristic increase of thermal diffusivity of KCl systems with the increasing additive concentration (less than 0.15 M) at the temperature range immediately after the eutectic melting suggested that the additive molecules of cryoprotectants substituted the water molecules in the hydration sphere of K^+ ions and come into direct contact with K^+ ions, causing further forming of ice during the cooling process.

While for the corresponding NaCl system, such phenomenon was not found because of the un-substitutable property of the cryoprotectant molecules for the water molecules in the hydration sphere of Na^+ ions.

By temperature modulation technique, it was shown that thermal diffusivity is highly sensitive to observe the feature of the subtle structural change of eutectic and the co-existing state of eutectic melting and ice in alkali chloride/cryoprotectant/water ternary systems, not affected by the latent heat by selecting the higher frequency.

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