Recrystallization of freezable bound water in aqueous solutions of medium concentrations*

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For aqueous solutions with freezable bound water, vitrification and recrystallization are mingled, which brings difficulty to application and misleads the interpretation of relevant experiments. Here, we report a quantification scheme for the freezable bound water based on the water-content dependence of glass transition temperature, by which also the concentration range for the solutions that may undergo recrystallization finds a clear definition. Furthermore, we find that depending on the amount of the freezable bound water, different temperature protocols should be devised to achieve a complete recrystallization. Our results may be helpful for understanding the dynamics of supercooled aqueous solutions and for improving their manipulation in various industries.

Keywords: recrystallization, aqueous solution, glass transition, freezable bound water

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1. Introduction

Recrystallization, or cold crystallization, refers to the crystallization process invoked by heating the devitrified liquids, which is a crucial phenomenon that can be observed in cryopreserved foods and living organs,[1–4] polymers,[5–8] metallic glass-forming alloys,[9–12] and aqueous solutions.[13–16] Generally speaking, the recrystallization process can be categorized into two distinct classes according to whether the devitrified liquids totally recrystallize or not. In one case, the devitrified liquid can totally recrystallize; therefore the recrystallized phase has the same composition as the devitrified liquid, as observed in most metallic glasses[10–12,17] and polymers.[6,18–20] In the other case, the devitrified liquid recrystallizes incompletely, and the residual phase maintains the liquid status upon further heating, as widely observed in aqueous solutions[13,21,22] in a particular concentration range depending on the nature of the solute.

The recrystallization process in aqueous solutions has already been investigated by using differential scanning calorimetry (DSC)[13,21–24] and spectroscopic techniques such as x-ray diffraction,[25] nuclear magnetic resonance,[26] and mid-infrared (MIR).[16,27,28] The water molecules within such solutions have been classified into two types. Those water molecules that vitrify upon cooling but can be set free to recrystallize upon reheating have been termed freezing bound water[29] or intermediate water.[28] The other portion of water that can easily vitrify with the solute but cannot recrystallize upon the subsequent reheating process is usually called non-freezing bound water.[29,30] For both scientific and technical purposes, it is essentially important to find a convincing and reliable scheme to exactly quantify these two kinds of water, which we prefer to term them as freezable bound water and non-freezable bound water. In the past, the amount of freezable bound water is generally deduced from the melting enthalpy of precipitated ice, $\Delta H_{\rm ice}$, obtained by integrating the endothermic peak on the DSC heating curve, to which the value of the melting enthalpy for bulk ice (334 $\mathrm{J\cdot g^{-1}}$) is used in conversion. Regretfully, the assumption that $\Delta H_{\rm ice}$ is independent of the solution concentration and/or the temperature may introduce a large error.

The recrystallization of the freezable bound water leaves behind a residual solution which comprises solute and the non-freezable bound water, which is thus referred as the freeze-concentrated phase. Therefore, the amount of non-freezable bound water can be determined so long as the concentration of the freeze-concentrated solution has been fixed. Traditionally, this concentration is viewed as corresponding to the point at which the extrapolated equilibrium liquidus, i.e., $T_{\rm m}$ versus concentration curve, for precipitated ice and the glass–liquid transition temperature, $T_{\rm g}$, versus concentration curve for solute-rich solutions intersect (Fig. 23 in Ref. [31]). This quantification scheme is applicable to solutions of large organic molecules with a narrow gap between the equilibrium liquidus curve and the $T_{\rm g}$ versus concentration curve.[13] Recently, it is suggested that equilibrium liquidus should not be

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exrapolated so far to come across the T_g versus concentration curve due to the broader range of temperature for maximum ice formation (Fig. 1 in Ref. [32]). Rather, at a T'_m slightly higher than the intersection point mentioned above, the freezing process in any water-rich solution would have completely finished; the concentration corresponding to this T'_m is taken to be that of the freeze-concentrated solution. However, up to now there is no consensus on how to determine the value of this T'_m.

In the current article, we report a simple scheme for the precise quantification of freezable bound water involving only the water-content dependence of glass transition temperature, T_g. It will make clear the point that the non-freezable bound water is in fact the hydrated water that characterizes the hydration ability of a given solute. Ice recrystallization only occurs in a solution containing more water than specified by the hydration water, but only up to a critical threshold beyond which spontaneous crystallization occurs during the cooling process. Namely, the aqueous solutions that may undergo recrystallization, in a sense, fall within the medium-concentration range. The quantification scheme, and also the temperature protocol to provoke ice recrystallization in solutions of different amounts of freezable bound water, will be discussed on the basis of DSC and Raman spectroscopic data.

2. Experimental

Various aqueous solutions in as large as possible range of concentration, specified by the mass fraction of water therein, X_{aqu}, were prepared with Millipore water and high-purity solutes $ZnCl_2$ (99.99%), $AlCl_3{\cdot}6H_2O$ (99%), glycerol (99.5%), ethylene glycol (99.8%), sorbitol (99.5%), 1,2,4-butanetriol (98%), and polyethylene glycols 300 (PEG 300, Bioultra); all these are Sigma–Aldrich products. The DSC measurements were performed on a calorimeter (PE DSC8000) at a cooling/heating rate of 20 K/min. When cooled down to 123 K, the sample would be held at that temperature for 1 min before the heating procedure started. In this text, following the conventional practice,[33,34] glass transition temperature T_g was extracted from the onset point of the heating curve. During Raman spectra measurements, the temperature was adjusted within ±0.1 K using a cooling unit (Linkam L-600A) equipped with a temperature controller (Linkam TMS 94). Raman spectra were measured on a confocal Raman system (Jobin–Yvon HR800) with the 532-nm diode laser excitation. The laser power of 1 mW was focused onto the sample surface through a fused SiO_2 film of thickness 0.3 mm. The integration time was set to 40 ms per point with a spectral resolution of 1.4 cm^{-1}.

3. Results and discussion

Figure 1 displays the DSC heating curves for the aqueous solutions of $AlCl_3$, $ZnCl_2$, ethylene glycol, glycerol, glycerol+$ZnCl_2$ (molar ratio 3:1), 1,2,4-butanetriol, sorbitol and PEG 300, with the mass fraction of water $X_{aqu} = 0.8$, 0.61, 0.54, 0.53, 0.51, 0.47, 0.44, and 0.49, respectively. The choice of these particular concentration values for the solutions concerned is due to the fact that recrystallization can only be detected in a solution within aparticular concentration range, i.e., zone II of medium concentrations (Cf. Fig. 1 in Ref. [33]), of which the boundaries are defined by X'_{aqu} which refers to the hydration capacity of the solute, and X^{cr}_{aqu} above which the excessive water will spontaneously precipitate prior to the vitrification of the residual freeze-concentrated phase. For the solutes here concerned, the concentration ranges of zone II are as follows: $0.72 < X_{aqu} < 0.81$ for $AlCl_3$, $0.49 < X_{aqu} < 0.65$ for $ZnCl_2$, $0.22 < X_{aqu} < 0.58$ for ethylene glycol, $0.23 < X_{aqu} < 0.53$ for glycerol, $0.23 < X_{aqu} < 0.56$ for glycerol+$ZnCl_2$ (molar ratio 3:1), $0.24 < X_{aqu} < 0.53$ for 1,2,4-butanetriol, $0.19 < X_{aqu} < 0.51$ for sorbitol, and $0.32 < X_{aqu} < 0.51$ for PEG 300, respectively.

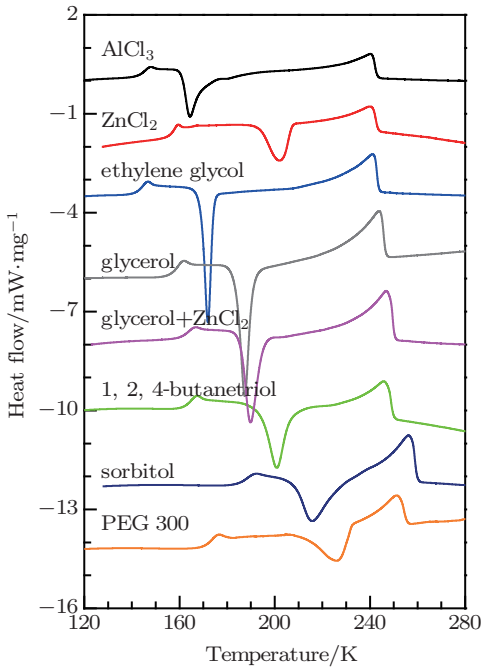


Fig. 1. (color online) DSC heating curves for aqueous solutions of $AlCl_3$, $ZnCl_2$, ethylene glycol, glycerol, glycerol+$ZnCl_2$ (molar ratio 3:1), 1,2,4-butanetriol, sorbitol, and PEG 300, with a mass fraction of water of 0.8, 0.61, 0.54, 0.53, 0.51, 0.47, 0.44, and 0.49, respectively. The curves are shifted vertically for clarity.

The salient feature of the curves in Fig. 1 is the large exothermic peak following a minor endothermic peak arising from the devitrification process. This is to say that it is heating that induces this icing process, i.e., recrystallization, in the devitrified solution. For a given solution within zone II, the

recrystallization temperature rises with decreasing water content, behaving just as the glass transition of the solution with a water content below X'_{aqu}. Moreover, a very slow heating rate or even an annealing treatment performed at temperature above T_g is needed for achieving recrystallization (for details, see the Supplementary Information in Ref. [33]). One point needs to be clarified: this large exothermic peak may arise from the crystallization of free water and/or the hydrated solute. In the following section, we will verify the chemical nature of this recrystallized phase and quantify its content more accurately.

First, two different protocols for the cooling/heating process in DSC measurements were applied to prepare the solution in different status, and Raman spectra were recorded to reveal the chemical nature of the recrystallized phase induced by annealing treatment. Taking the aqueous $ZnCl_2$ solution with $X_{aqu} = 0.61$ as an example, this solution totally vitrifies at $T_g = 155.1$ K (see Fig. 2(a)). Unlike the conventional cooling/heating process (black line), protocol 2 (red line) was slightly complicated so as to ensure the completeness of recrystallization induced in the first round of heating and allow the detection of glass–liquid transition of the residual freeze-concentrated phase. Toward this end, the sample, when heated from 123 K up to 208 K passing the exothermic peak due to recrystallization, was maintained for 1 min at 208 K, which was followed by cooling down to 123 K once again and subsequently by reheating to room temperature. In the second round of heating, a new glass–liquid transition was observed at a transition temperature $T_g = 164$ K, which is higher than that of the original solution as revealed in the conventional cooling/heating process (black line in Fig. 2(a)) but is very close to the T_g of $ZnCl_2$ solution with $X_{aqu} = 0.49$ (blue line in Fig. 2(a)). On the DSC curve for the second-round heating process, only an endothermic peak which corresponds to the melting process of the recrystallized phase can be observed; the exothermic peak corresponding to the recrystallization process is now unavailable. The roughly equal area of two endothermic peaks of these two protocols indicates that recrystallization in protocol 2 has completed during heating the sample from 123 K to 208 K prior to the second cooling/heating cycle. A higher T_g is attributed to a higher concentration of the solution, so it is reasonable to speculate that the solution having undergone the exothermic process comprises pure ice dispersed in a solution phase of larger concentration. This speculation can be justified by the following Raman spectroscopic measurements. As shown in Fig. 2(b), the black dashed line represents the Raman spectrum of the aqueous $ZnCl_2$ solution measured at 298 K, where the broad main peak centered at about 3473 cm^{-1} refers to the OH stretching mode of water. The difference spectrum between those obtained at 183 K on the solution before and after the recrystallization, respectively, is plotted in a red solid line. The negative peak centered at around 3469 cm^{-1} and the accompanying positive peak centered at about 3114 cm^{-1} are both caused by the recrystallization event. The peaks at about 3114 cm^{-1}, 3232 cm^{-1}, and 3338 cm^{-1} in the difference spectrum are the feature of bulk ice, which can be confirmed by comparing with the Raman spectrum of pure ice measured also at 183 K (blue dotted line). Accordingly, it is reasonable to conclude that the recrystallized phase is pure ice.

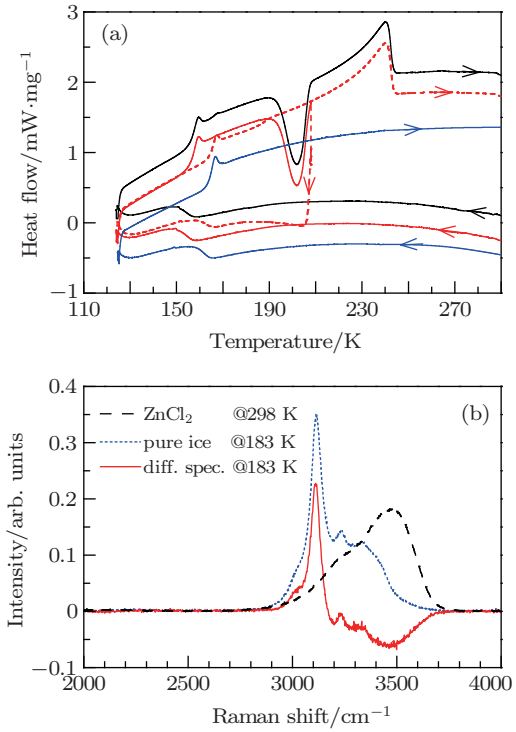


Fig. 2. (color online) (a) DSC thermograms for two distinct temperature protocols as demonstrated on the aqueous $ZnCl_2$ solution with $X_{aqu} = 0.61$ and the solution with $X_{aqu} = 0.49$ for comparison. Protocol 1 (black line and blue line): conventional cooling/heating process; protocol 2 (red line): the heating process is interrupted at 208 K. After being maintained there for 1 min to complete recrystallization, the sample was again cooled down to 123 K and then heated to room temperature (emphasized with a dashed line). The curves are defaulted each by 0.3 mW/mg for clarity. (b) Raman spectra for the aqueous $ZnCl_2$ solution with $X_{aqu} = 0.61$ at 298 K (black dashed line), and for pure ice at 183 K (blue dotted line). The difference spectrum (red solid line) refers to difference in measurements at 183 K on the solution before and after recrystallization.

Next, to further reveal the chemical nature of the recrystallized phase, we check the water-content, X_{aqu}, dependence of T_g data for the aqueous $ZnCl_2$ solution in the whole concentration range available (Fig. 3(a)). With the given X_{aqu} dependence of T_g, the recrystallization behavior can be better elucidated and the possible underlying mechanism can be deciphered. For concentrated solutions with an X_{aqu} below the critical value X^{cr}_{aqu}, they can easily totally vitrify in the cooling process, and the corresponding T_g decreases monotonically with increasing X_{aqu} due to the plastic effect of the added water. In contrast, for the water-rich solutions with $X_{aqu} > X^{cr}_{aqu}$,

the glass-transition temperature T_g suddenly jumps to a higher level T_g' (so does the temperature for the solution to devitrify in the heating process), and accordingly the remarkable feature is an exothermic peak prior to the glass transition in the cooling process, indicating the occurrence of precipitation in the solution. As roughly the same T_g is measured in these water-rich solutions with $X_{aqu} > X_{aqu}^{cr}$, it is reasonable to suggest that the freeze-concentrated phases arising in different water-rich solutions have one and the same composition, which can be directly read from the point $T_g = T_g'$ on the monotonic part of the T_g versus X_{aqu} curve for concentrated solutions. From Fig. 3(a), it can be determined that for an aqueous solution of $ZnCl_2$, $T_g' = 163.85$, $X_{aqu}^{cr} = 0.65$, and $X_{aqu}' = 0.49$ corresponding roughly to $ZnCl_2{\cdot}7H_2O$. The above-mentioned proposition is supported by the linear relationship between X_{aqu} and the heat flow change at T_g per unit mass of solution, denoted here as δ_{T_g}. As shown in Fig. 3(b), δ_{T_g} versus X_{aqu} for water-rich solutions can be well fitted by $\delta_{T_g} = \delta'_{T_g}(1 - X_{aqu})/(1 - X'_{aqu})$, where $(1 - X_{aqu})/(1 - X'_{aqu})$ is the amount of the vitrified freeze-concentrated phase with a water content of X'_{aqu}, and δ'_{T_g} is the heat flow change at T_g'. With the concentration of the freeze-concentrated solution, the amount of bound water that is in strong association with solutes can be accurately determined. Furthermore, as shown in Fig. 2(a), the freeze-concentrated phase in the solution of $ZnCl_2$ with $X_{aqu} = 0.61$ devitrifies at a higher temperature (triangle in Fig. 3(a)), which is very close to T_g'. At the same time, the corresponding δ_{T_g} (triangle in Fig. 3(b)) is also very close to the values extrapolated from the linear relation of δ_{T_g} versus X_{aqu} for water-rich solutions. Therefore, it can be confirmed that the concentration of the freeze-concentrated phase in the exemplar solution with $X_{aqu} = 0.61$ is specified by X'_{aqu}. In fact, for all the solutions of medium concentration with a mass fraction of water falling in the range $X'_{aqu} < X_{aqu} < X_{aqu}^{cr}$, recrystallization process can always be provoked upon annealing the devitrified solution. In a solution within this concentration range, all the water molecules can vitrify together with the solute on the cooling process (for a cooling rate above 1 K·min^{-1} confirmed in this work). These vitrified water molecules are inclusively denoted as bound water. Upon reheating over the devitrification temperature, a portion of the bound water molecules will be set free again and recrystallize at a slightly higher temperature, thus they can be specified as freezable bound water. Accordingly, the water molecules that do not run into recrystallization remain bound to the solute even after a long-time annealing treatment, and they are thus referred to as non-freezable bound water. The amount of this part of water corresponds to X'_{aqu}, which in fact characterizes the hydration capacity of the solute. The amount of the water involved in recrystallization is given by $X_{aqu} - X'_{aqu}$.

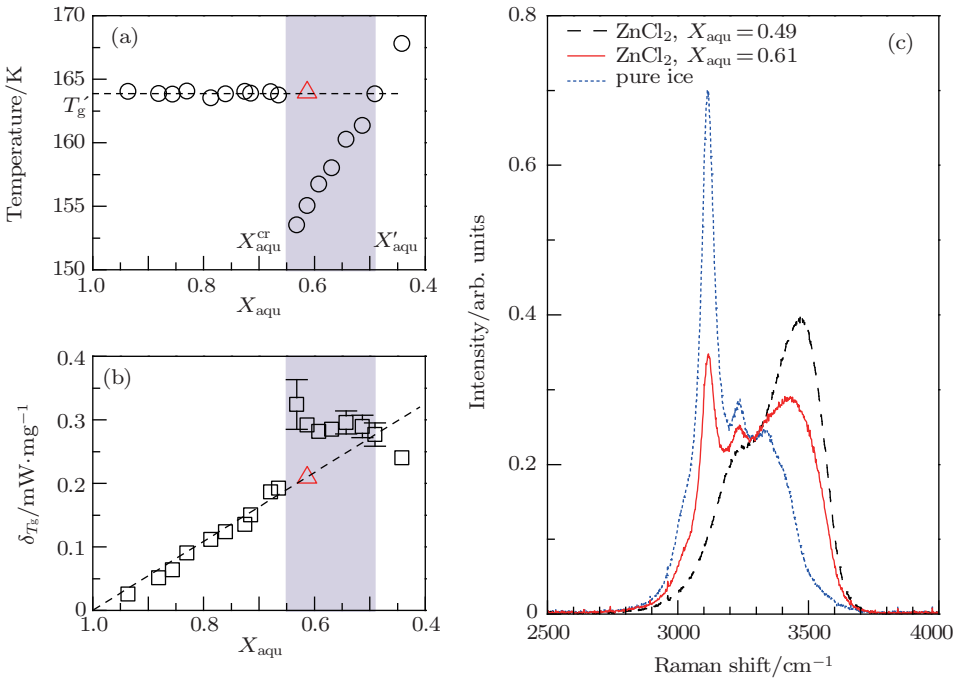


Fig. 3. (color online) (a) Glass transition temperature, T_g, against the mass fraction of water, X_{aqu}, for aqueous $ZnCl_2$ solutions obtained under conventional cooling/heating process (circle). In those solutions with an $X_{aqu} > X_{aqu}^{cr}$, ice precipitation occurs first in the cooling process. The freeze-concentrated phases then vitrify at a constant T_g' and the corresponding X'_{aqu} can be directly read from the monotonous part of the T_g versus X_{aqu} curve at the point where $T_g = T_g'$. (b) Mass-normalized heat flow change at glass transition, δ_{T_g}, as a function of X_{aqu} for aqueous $ZnCl_2$ solutions. The red up triangle in panels (a) and (b) indicates the data point measured in the second round of protocol 2 for the sample with $X_{aqu} = 0.61$. (c) Raman spectra measured at 183 K for pure ice (blue dotted line), the solution with $X_{aqu} = 0.49$ (black dashed line), and the solution with $X_{aqu} = 0.61$ after recrystallization of water (red solid line). The experimental error, approximately 0.5% for T_g and approximately 10% for δ_{T_g}, arises mainly from in calibrating furnace temperature for the former and from extrapolating the heat flow curve at two sides of T_g for the letter. Error bars are omitted when they are smaller than the symbol size.

After recrystallization, the solutions of medium concentration at this stage comprise a mixture of pure ice and the freeze-concentrated solution with $X_{\rm aqu} = X'_{\rm aqu}$. This observation is also confirmed by fitting the Raman spectrum for the solution with $X_{\rm aqu} = 0.61$ (red solid line in Fig. 3(c)) with the supposition of the spectrum for the solution with $X_{\rm aqu} = X'_{\rm aqu} = 0.49$ (black dashed line) and that for pure bulk ice (blue dotted line) measured at the same temperature, namely, $R_{\rm fit} = \alpha R_{X_{\rm aqu}=0.49} + (1-\alpha)R_{\rm ice}$, where R represents the area-normalized Raman spectrum, and $\alpha = 0.6$. It is consistent with a molar ratio of 0.63 for solute against water referred to as the aqueous $ZnCl_2$ solution with $X_{\rm aqu} = 0.61$.

Based on the aforementioned results, some insights over the recrystallization of freezable bound water can be drawn. Freezable bound water is a concept in between the bound water contributing to hydration and free water which can easily crystallize spontaneously during the cooling process. It can vitrify together with the hydrated solute ions while recrystallize in the devitrified solution upon the continuously heating process or subjected to annealing treatment at temperatures between $T_{\rm g}$ and the recrystallization temperature. Of course, freezable bound water can also crystallize together with free water in the water-rich solutions upon cooling. Depending on the amount available, the characteristic nature of the freezable bound water can be more prone to behave as either the bound water or the free water, thus recrystallization of the freezable bound water in the medium-concentration solutions can only be observed with properly chosen cooling/heating protocols.

Lastly, we want to emphasize that the method for quantifying the freezable and non-freezable bound water in the current work can be applied not only to systems illustrated in Fig. 1 and alike, but also to the solutions of some large organic molecules such as polysaccharides. For solutions of polysaccharides, the concentration of the freeze-concentrated phase which comprises a mixture of non-freezable bound water and the solute is usually taken as the intersection point of the extrapolated $T_{\rm m}$ versus concentration curve for the precipitated ice with the $T_{\rm g}$ versus concentration curve obtained on concentrated solutions. The error introduced in this procedure by extrapolating the $T_{\rm m}$ curve can be neglected due to the narrow gap between the $T_{\rm m}$ curve and the $T_{\rm g}$ curve. However, such an error cannot be neglected anymore for solutions of electrolytes and some small organic molecules due to the obviously low-lying $T_{\rm g}$ values. This problem can be well resolved by the method here presented in which only the $T_{\rm g}$ values are on account.

4. Conclusion

In summary, DSC measurement and Raman spectroscopy were employed to record the general features of recrystallization in aqueous solutions of several electrolytes, organic molecules, and their mixtures. Our results clearly revealed that ice recrystallization is a phenomenon to be anticipated in the aqueous solutions in a particular concentration range, and it involves only the freezable bound water. For a solution of medium concentration with a water content slightly larger than that characterized by the hydration formula, a deliberately designed temperature protocol is needed to provoke the ice recrystallization. A quantification scheme to determine the amount of the freezable bound water is provided based on the water-content dependence of glass-transition temperature, which can be exactly established by experiment. Our results have gained some insights into the dynamics of supercooled aqueous solutions; they may be helpful for improving the manipulation of supercooled solutions in various industries.

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