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Glass transition and enthalpy relaxation of ethylene glycol and its aqueous solution

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Abstract

Differential scanning calorimetry (DSC) and cryomicroscopy were employed to investigate the glass transition and enthalpy relaxation behaviors of ethylene glycol (EG) and its aqueous solution (50% EG) with different crystallization percent. Isothermal crystallization method was used in devitrification region to get different crystallinity after samples quenched below glass transition temperature. The DSC thermograms upon warming showed that the pure EG has a single glass transition, while the 50% EG solution has two if the solution crystallized partially. It is believed that the lower temperature transition represents the glass transition of bulk amorphous phase of EG aqueous solution glass state, while the higher one is related to ice inclusions, whose mobility is restricted by ice crystal. Cryomicroscopic observation indicated that the EG crystal has regular shape while the ice crystal in 50% EG aqueous solution glass matrix has no regular surface. Isothermal annealing experiments at temperatures lower than T_g were also conducted on these amorphous samples in DSC, and the results showed that both the two amorphous phases presented in 50% EG experience enthalpy relaxation. The relaxation process of restricted amorphous phase is more sensitive to annealing temperature.

Keywords: Enthalpy relaxation; Glass transition; Ethylene glycol; Differential scanning calorimetry; Cryomicroscopy

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1. Introduction

Ethylene glycol (EG) is a commonly used cryoprotective agent (CPA) for the preservation of simple biological system at low temperatures, it is also an important glass former in the design of vitrification solution for the cryopreservation of organized tissues and organs by vitrification [1,2]. Glass is known to undergo relaxation when stored at temperature below its glass temperature (T_g) and this process will affect preservation quality of biological materials embedded in the glassy matrix [3]. The most available literatures are concerned with the glass forming or nucleation property of polyalcohols/water mixtures [2,4–9], and little attention was given to their relaxation behaviors. Some authors reported the glass transition and (or) enthalpy relaxation of pure compound polyalcohols, such as glycerol [10–14], propylene glycol [11,14], EG [13,14] and their binary mixture systems [14], but there is a lack of information about relaxation of their aqueous solutions below T_g. Furthermore, no report is found up to now about glass transition and relaxation behaviors of polyalcohols and their aqueous solutions with presence of crystal.

In practice, it is very difficult to get completely amorphous solid at accessible cooling rates, even quenched vitrification solution into liquid nitrogen directly. This partially crystallized glass (PCG) has the appearance of an array of usually microscopic or submicroscopic crystal (ice for aqueous solutions) particles embedded in glassy matrix [5]. Thus, it will be more useful to investigated glass transition and enthalpy relaxation behaviors of PCG than purely amorphous glass state.

In the present paper, differential scanning calorimetry (DSC) and cryomicroscopy were used to study 50% EG

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(by mass) with emphasis on the effect of crystallinity on the glass transition and enthalpy relaxation behaviors. Pure compound EG was also studied for compare purpose. Fifty percent EG was chosen only because it has typical thermal behaviors and the DSC results could be easily quantified.

2. Materials and methods

2.1. Reagents

The analytical grade ethylene glycol (EG) was purchased from Sigma Inc. and was used without further purification. Double distilled water for preparation of solutions was passed through a millipore filter to remove any residual particles and was used to prepare solution cyclopentane (99.9%+, m.p. −135.06 °C) was obtained from Perkin-Elmer.

2.2. Differential scanning calorimetry experiments

Calorimetric measurements were made with a Perkin-Elmer Pyris-Diamond differential scanning calorimeter, equipped with nitrogen cooling system (CryoFill^{TM}) and Pyris software 5.0 (both Perkin-Elmer). Standard liquid aluminum pans (Perkin-Elmer) were used in all DSC experiments and all samples were around 2 mg. An empty pan was used as reference. The furnace block of DSC was flushed with dry nitrogen gas to avoid condensation of moisture from the air. Helium gas (99.999%+) was used as sample purge at a rate of about 20 ml/min. The temperature scale of the instrument was calibrated by the melting point of pure ice (0 °C, onset) and crystallization point of cyclopentane (−135.06 °C, onset). And the enthalpy scale was based on the heat of fusion of pure ice (333.88 J/g). All calibrations were performed by using scanning rate of 10 °C/min.

DSC isothermal crystallization in devitrification region was performed to obtain different crystallinity fraction in quenched glass matrix. The samples were first cooled at 200 °C/min (nominal cooling rates) from room temperature to −150 °C, which is well below the vitreous transition of the solutions to obtain glass state or partially crystallized glass (PCG) state. Secondly, the samples were heated to some temperature, typically 6–8 K below crystallization peak temperature at a rate of 200 °C/min and allow the crystal to grow isothermally for different time. Then the samples were cooled again at 200 °C/min (nominal cooling rates) to some annealing temperature and kept at this temperature for different time (from 1 to 240 min). At last, the aged samples with different crystallinity degrees were heated at 10 °C/min and the DSC curves were recorded. In order to reduce experimental scatter due to differences in heat transfer between the calorimeter and the sample, we leave the sample undisturbed inside the furnace for a set of experiments. All experiments were repeated three times.

2.3. Cryomicroscopy experiment

Cryomicroscope was locally designed by combining an inverted microscope AE31 (Motic Optical Industrial Group, Ltd., China) with a temperature-controlled accessory. The temperature program was similar to the DSC protocol above, as follows: quenched the samples at about 150 °C/min from room temperature to −150 °C and then heated at 10 °C/min through the devitrification region. Digital images were collected upon warming at 0.5 K intervals throughout the experiments. This experiment was repeated twice. The excess enthalpy released during annealing in glassy state was calculated using the glassy state without annealing as a reference.

3. Results and discussion

A typical dependence of the calorimeter output on time during isothermal crystallization of pure EG after cooling to −150 °C at 200 °C/min (nominal cooling rates) and subsequent warming at the same rate to −88 °C is shown in Fig. 1. It is well known that growth rate of crystal is sensitive to temperature and the different growth rates can be realized by changing the crystallization temperature. In Alves et al. [15] work, different crystallinity degrees of quenched amorphous poly(ethylene terephthalate) were obtained by heating the samples to different temperature in cold crystallization region and then cooled back to a temperature below T_g. In our study, this method was no longer used. Because for PET, the temperature span of cold crystallization is more than 50 K and the crystal grow slowly; while for EG and 50% EG, this span is typically only about 15 K for a normal heating rate such as 10 °C/min. That is to say, the crystallization process of EG solution is too rapidly to obtain expected crystallinity fractions precisely. We found that for EG and 50% EG, the

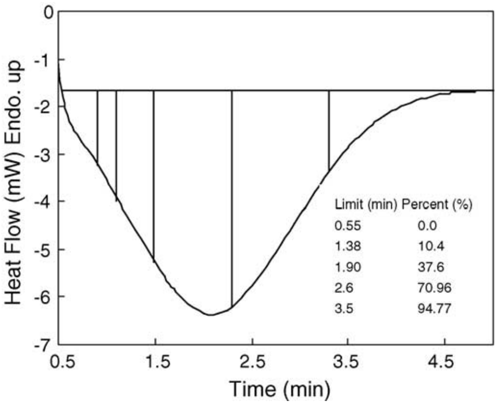

Fig. 1. Dependence of DSC output on time during crystal formation under isothermal conditions and the calculation of the crystal portion in pure EG at −88 °C after cooling from room temperature to −150 °C at a rate of 200 °C/min, and then heating to −88 °C at the same rate. The time 0.55 min corresponds to the beginning of isothermal process.

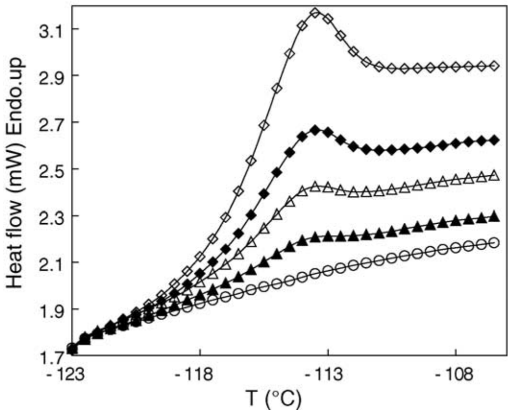

Fig. 2. Temperature dependence of heat flow measured in heating DSC scans on samples of amorphous EG with 21% original crystallinity crystallized at −88 °C isothermally for different time. Thermograms measured after crystallized at −88 °C for: (◇) 0 s, (◆) 50 s, (△) 60 s, (▲) 90 s and (○) 250 s.

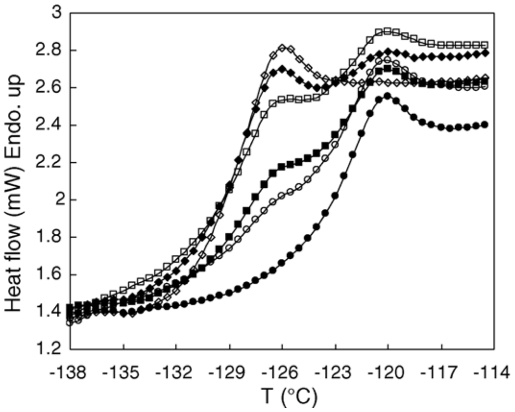

Fig. 3. Temperature dependence of heat flow measured in heating DSC scans on samples of amorphous 50% EG crystallized at −88 °C isothermally for different time. Thermograms measured after crystallized at −88 °C for: (◇) 0 s, (◆) 30 s, (□) 60 s, (■, ▲) 90 s, (○) 120 s and (●) 250 s.

devitrification peak temperature were about −80 and −78 °C respectively. Using isothermal crystallization method as described above, the devitrification processes could be finished in 5 min. It should be mentioned that for pure compound EG, it is difficult to obtain complete amorphous glassy state but PCG (21% crystallinity degree) when cooling at 200 °C/min (nominal cooling rates) in our study. Although this result differs greatly from other reports [13,14], which showed that pure EG could not be vitrified by the quenching at accessible rate in DSC, we owned it to the difference between calorimeters. It should be pointed out that for 50% EG, no EG crystal but ice crystal was formed during either cooling or subsequent heating process.

The glass transition temperature of the glass state samples was identified by analyzing the stepwise changes of heat flow or heat capacity (ΔC_p) during heating in DSC experiments. Heat flow changes of glass matrix of pure EG with 21% original crystallinity degree in glass transition region were show in Fig. 2. Only one glass transition was found with different crystal fractions; and the glass transition temperatures kept almost constant with the change of crystal fractions. Fig. 2 also indicated that ΔC_p, the difference between the heat capacity in equilibrium liquid state and that of the glassy state decreased with the crystallinity increasing, since the baselines of heat flow shift downward after the glass transition processes. When all the liquid phase EG change into crystal, no ΔC_p signal was detect since no amorphous state presented in the samples.

With the different amount of ice, two glass-like transitions were distinguished in glassy matrix of 50% EG solution except for the purely amorphous and the freezable water completely crystallized case, as shown in Fig. 3. The fraction of freezable water in aqueous solutions can be estimated by the equation [16,17]:

$$\alpha = 1 - \frac{\Delta H(T_\mathrm{f})_\mathrm{DSC}}{\Delta H(T_\mathrm{f})}(1-x)^{-1}$$

with

$$\Delta H(T_\mathrm{f}) = -334 - 2.05(T_\mathrm{f} - 273)$$

where T_f is freezing temperature and $\Delta H(T_\mathrm{f})_\mathrm{DSC}$ is freezing enthalpy measured by DSC during cooling process. x is the solute fraction. We found the amount of ice is rather small (less than 8% by mass) compared to the glassy matrix even if all of the freezable water frozen in 50% EG solution. But it is interesting that the small amount of ice crystal change the glass transition behaviors notably.

In frozen concentrated solutions of sucrose [18] and trehalose [19], the authors also found two glass-like transitions, whose physical significance is still not completely understood now. In 1,3-butanediol aqueous solutions, three glass-like transition were found by the author [20]. But it is difficult to identify the nature of the two or three glass transition by calorimetry. For double glass-like transitions in frozen concentrated aqueous solutions, different authors provided different explanations. Luyet and Rusmussen [21] regarded the lower temperature transition to be the true glass transition, and the higher temperature transition to be due to an interfacial interaction between ice crystals and the amorphous phase. Many authors suggested that the higher temperature transition was due to the onset of the ice melting [22–24]. The thermal history of samples in our study was different from those of frozen concentrated disaccharide aqueous solutions, and the temperature difference between two transitions (less than 6 K) was also not the same (about 12 K) as that in sucrose and trehalose aqueous solutions. The devitrification (cold crystallization) temperature for 50% EG is about −80 °C and the “hot” crystallization temperature is about −65 °C (cooling at a relative slow rate, such as 2 °C/min) in our study. While the second glass-like transition temperature is about −121 °C. So assumed the second transition to be the onset of the ice melting seemed to be unreasonable since the melting temperature is much lower than both the “hot” and cold crystallization temperature.

It is well known that double peaks can also appear during glass transition when certain glasses experience certain enthalpy relaxation process. But only some polymers can exhibit extremely slow relaxation process above their glass transition temperature. For small molecular hydrogen-bond liquids, the thermal treatment on supercooling liquids seems to be no impact on the enthalpy recovery process during glass transition. Although no similar report about double transitions phenomenon in partially crystallized polyalcohols solutions is available now, we consider both transitions may be the glass transitions rather than other thermal events such as onset of the ice melting based on the assumption that the mobility of some amorphous matrix is restricted by microscopic or submicroscopic crystals. In Ref. [7], two nucleation events were found in 50% EG solution by the authors. One was observed over the range of −80 to −100 °C with the maximal nucleation rate at about −87 °C and the other over the range of −120 to −130 °C with the maximal nucleation rate at about −124 °C. The heating rate in our study was 200 °C/min and the small ice crystals formed in lower temperature nucleation had no time to grow. So, the increase of crystallinity fractions at −88 °C can be owned to ripening process of both old and new ice nuclei. In cryomicroscope experiments, many newly formed nuclei were observed and the growth process could be identified clearly in our study. We could speculate intuitively that the amount of amorphous phase restricted by the ice crystals increased as the isothermal crystallization time increased. As show in Fig. 3, the step shape of the glass transition changes with different amount of ice crystals. The first transition processes decaying while the second enhancing with the growth of the ice crystals. Since the stepwise signal is proportional to the fraction of amorphous phase in partially crystallized glass (Fig. 2), it is easy to speculate that the amount of bulk amorphous state reduced while restricted amorphous state increased as the ice crystals growing.

Crystal morphology of both pure EG and 50% EG detected by cryomicroscope are shown in Fig. 4. The image of crystals in pure EG illustrates that the crystals have detectable interfacial. While in 50% EG, the nucleation during isothermal crystallization results in a cloud of crystals too small to be detectable (as seen by the darkening in Fig. 4(b)). Although no quantitative method is available to estimate the total surface area of crystals, it can be concluded from the images that the specific surface area of ice crystal in 50% EG/w/w is much larger than that in the EG, and the interaction between ice crystal and water/EG mixture amorphous phase was stronger than that between EG crystal and its amorphous state. These pictures are also helpful to explain why partially crystallized EG has only one glass transition process.

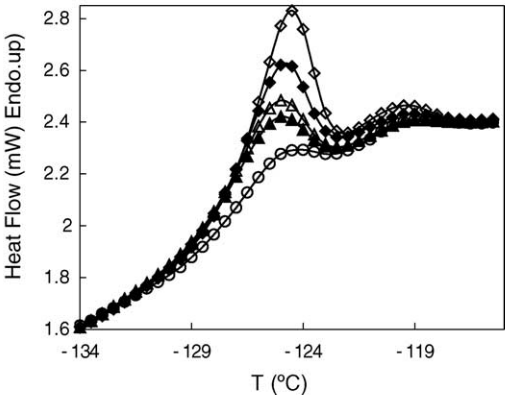

Fig. 5. Temperature dependence of heat flow measured in heating DSC scan on samples of amorphous 50% EG with 55% freezable water frozen after annealing at −136 °C for different time: (◇) 60 min, (◆) 30 min, (△) 10 min, (▲) 5 min, (○) 0 min.

Below the glass transition temperature, amorphous materials are in non-equilibrium and usually undergo relaxation process since their enthalpy, entropy and free volume are relatively higher. If the two processes do indeed represent double glass transitions, then two relaxation peaks were expected after the samples annealing at a given temperature (T_a) below T_g of lower temperature glass transition and the results were given in Fig. 5 ($T_a = -136\,°C$) and Fig. 6 ($T_a = -132\,°C$). The rates of these processes greatly depend

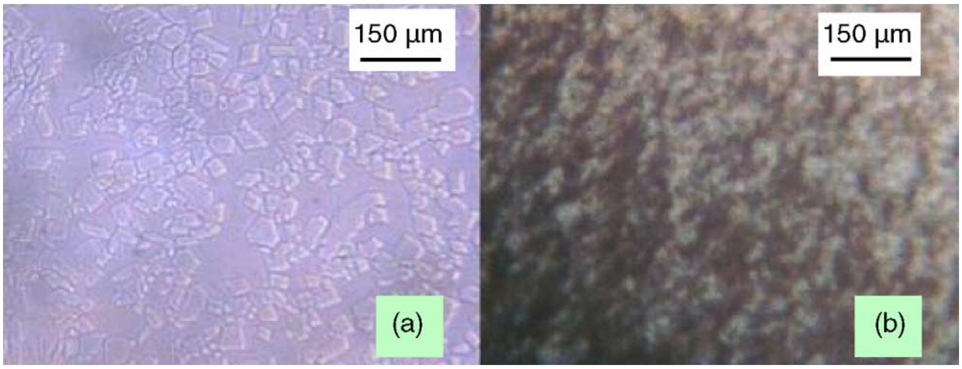

Fig. 4. Image of pure EG and 50% EG solution during isothermal crystallization processes, following the same temperature regime as DSC experiments. The sample were cooling from room temperature to −150 °C at a rate of 200 °C/min and then heating to −88 °C at the same rate: (a) crystal in pure EG; (b) ice crystal in 50% EG solution.

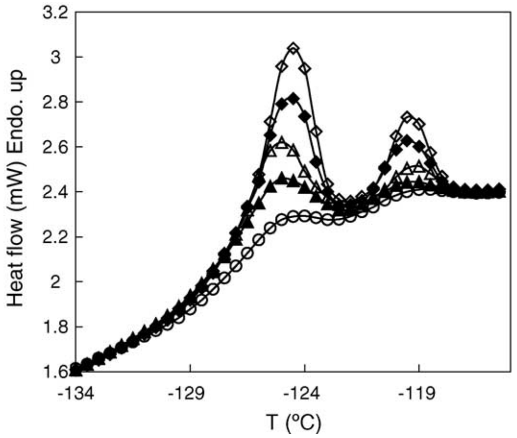

Fig. 6. Temperature dependence of heat flow measured in heating DSC scan on samples of amorphous 50% EG with 55% freezable water frozen after annealing at −132 °C for different time: (◇) 60 min, (◆) 30 min, (△) 10 min, (▲) 5 min, (◯) 0 min.

on the difference between T_a and the glass transition temperature. Thus at $T_a = -136$ °C (Fig. 5), the enthalpy recovery peaks of low-temperature transition are more obvious than those of high-temperature transition. At $T_a = -132$ °C, recovery peaks of high-temperature transition became more identified than those at −136 °C on given experimental timescales (Fig. 6). One possible explanation is that the relaxation process of amorphous state included in submicroscopic crystal is more sensitive to the annealing temperature. But more precise methods are needed to clarify it.

For many amorphous pharmaceuticals, enthalpy relaxation processes is negligible at about 50 K below the T_g [25]. For EG partially crystallized glass, our study showed that the relaxation enthalpy decreased dramatically when annealing temperatures were 30 K below the T_g (Fig. 7), though still slight increase was found with the increasing of annealing time. For amorphous phases in semi-crystalline 50% EG glass, no obvious relaxation was observed when the value of $(T_g - T_a)$ is about 18 K (bulk amorphous state, Fig. 8) and 15 K (restricted amorphous state, Fig. 9). It can be concluded that the enthalpy relaxation of EG aqueous glass is more sensitive to annealing temperature than that of EG glass. For similar cryopreservation system, the authors [10] found that the annealing below T_g will decrease the devitrification temperature, which means higher heating rate is needed to avoid the crystallization. Our study indicated that for EG cryopreservation systems, the enthalpy relaxation at store temperature (about −196 °C) is negligible, since the value of $T_g - T_a$ is about 70 K.

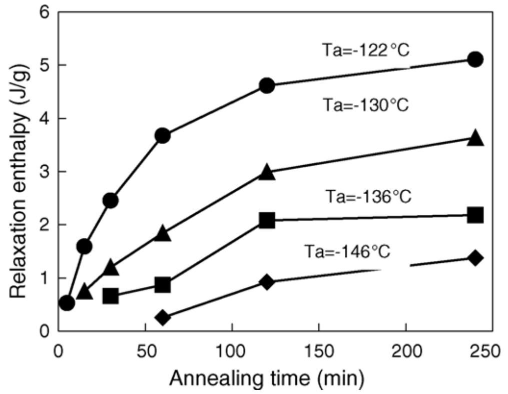

Fig. 7. Relaxation enthalpy of EG partially crystallized glass (21% crystallinity).

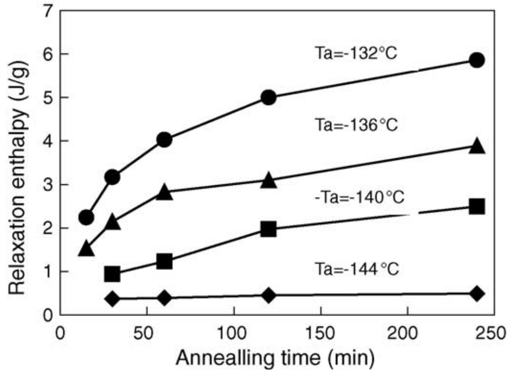

Fig. 8. Relaxation enthalpy of amorphous phase I in 50% EG aqueous glass (55% freezable water frozen).

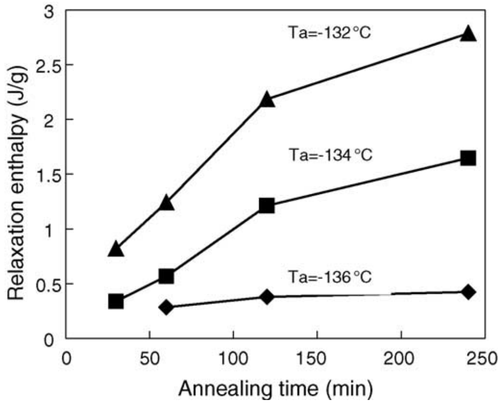

Fig. 9. Relaxation enthalpy of amorphous phase II in 50% EG aqueous glass (55% freezable water frozen).

4. Summary

Both differential scanning calorimetry and cryomicroscopy were used to study the glass transition and the enthalpy relaxation behaviors of EG and its aqueous solution. Only one glass transition process is found in partially crystallized glassy matrix of pure EG. With the presence of ice crystal, 50% EG had two glass-like transitions and the amount of ice crystals can change the peak shapes of the calorimetric glass transition obviously. It is believed that the transition at lower temperature represents the glass transition of bulk amorphous phase of EG aqueous solution glass state, while the higher temperature transition is related to ice inclusions, whose mobility is restricted by ice crystal. Annealing experiments showed that both amorphous phases in 50% EG experienced enthalpy relaxation processes at temperatures below T_g, which can be seen as another proof of the two glass transition assumption. But the relaxation process of constrained phase seemed to be more sensitive to the annealing temperature.

Acknowledgements

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