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Observation of multiple glass transitions in the system water/1,3-butanediol. Effect on ice crystallization [1]

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Abstract

A thermal analysis approach is presented to analyze the interaction between ice nucleation and glass transition. Double glass transitions are observed using calorimetry for the system 1,3-butanediol–water for concentrations less than 30% mole/mole in water as previously shown for 1,2-propanediol. Annealing experiments above the main glass transition temperatures reveal a third endothermic transition identified as a glass transition peak. The thermal range of this third glass transition is − 107 to − 97°C for 15% mole/mole 1,3-butanediol in water. For this concentration, ice crystallization was suppressed during cooling at 10°C min^{1} but still occurred during the subsequent warming. Analysis of the devitrification temperature and of the amount of ice during warming show that annealing conditions do increase the ice nuclei density and do modify the crystal growth kinetics either by dissociating cubic and hexagonal ice nucleus formation or by structurally changing the crystal growth characteristics. The total amount of ice crystallizing during warming remains constant for annealings below − 95°C for several hours. The third glass transition was shown to be independent of the ice nucleus density during annealing. A decrease in the size of both first glass transitions, mostly of the first glass transition, is observed as the magnitude of the third glass transition increases. This relates the existence of the third glass transition to the existence of the first glass transition. The structural relaxation associated with the third glass transition is shown not to affect the ice crystallization kinetics.

Keywords: 1, 3-Butanediol; Enthalpy relaxation; Ice crystallization; Multiple glass transitions; Water

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1. Introduction

Thermal analysis with calorimetry has been shown to be useful for the interpretation of phase transitions in aqueous solutions. The present study applies the calorimetric technique to the interpretation of the observed multiple glass transitions and their interaction with the crystallization of ice. Double glass transitions have been observed for the systems 1,2-propanediol–D_2O [1, 2]and 1,2-propanediol–H_2O [1–3]. Their appearances were reported and investigated as a function of the solute concentration [4]. Their characteristics suggested the possibility of a liquid–liquid phase separation during cooling down below the main glass transition [5] rather than a localization of the structural relaxation within the glassy state [6–8]. Observations of double glass transitions for other polyalcohols, e.g. 1,3-butanediol, were also made previously [9, 10]. However, they were reported without any explanation for these observations.

Multiple glass transitions for polymer glasses have been explained as the consequence of the formation during quenching of non-well-stabilized glasses, in contrast with well-stabilized glasses which present one main glass transition [11]. The associated structural recovery observed through enthalpy recovery for these non-well-stabilized glasses involves the existence of a distribution of retardation times using the KAHR model or the continuous spectrum model with the stretched exponential function [12]. However, the present glass-forming aqueous solutions do not present the same size characteristics as polymers. The present study, therefore, attempts to analyze the data assuming the existence of a liquid–liquid phase separation during cooling.

Another phase separation due to ice nucleation during cooling was observed but the extent of the ice nucleation did not support the hypothesis of this phase separation being responsible for the observation of multiple glass transitions [4, 13, 14]. Ice nucleation below the glass transition has been reported for various aqueous glass-forming solutions [15–25]. However, the mechanism of ice nucleation below the glass transition and its relationship with the structural recovery of the glassy state has not yet been clearly explained. The only supportive explanation for the formation of ice nuclei is the possible diffusion of water molecules within the glassy matrix at temperatures higher than that of the amorphous pure water. Water molecules in a bulk free-water phase might be able to form clusters down to the hypothetical glass transition temperature of pure water for infinitely slow cooling which must be thermally located at lower temperatures than the usual temperature recorded after ultraquenching, i.e., −136°C.

The present hypothesis is, therefore, to assume that the ice nucleation processes below the glass transition are a consequence of the liquid–liquid microphase separation during the initial cooling or during annealing conditions. Understanding the processes of ice nucleation and liquid–liquid phase separation during cooling in aqueous glass-forming solutions is important in the field of cryobiology and for the science of freeze-drying, because the stability of the glassy state is essential and the amount of crystallization needs to be controlled. Polyalcohols are widely used in pharmaceutical industries and in cryobiology as vitrification agents for the preservation of organ or tissue by vitrification or freezing techniques [26, 27]. The observation of multiple glass transitions using 1,3-butanediol is presently tested as a general behavior for vitrification agents such as 1,2-propanediol. A better understanding of the nature of the various phases appearing during cooling is needed to assess the stability of the various products from the available techniques for the preservation of biological materials. The search for other similar polyalcohols similar to 1,2-propanediol is limited to solutes which do not crystallize any hydrate with water. This condition has therefore limited the choice to 1,3-butanediol, glycerol, and 2,4-pentanediol, which have been shown not to crystallize any hydrate with water [28, 29] or which are difficult to crystallize in their pure form [30, 31]. As a first approach, 1,3-butanediol is considered for the specific concentration of 15% mole/mole because the double glass transitions are observed directly after the initial cooling during the subsequent warming [9, 10], and considering the possible effect of glass relaxation on the ice nucleation processes as previously observed for 1,2-propanediol [1–4]. Higher 1,3-butanediol concentrations have also been considered for qualitative experiments to determine the concentration limit for observing the various glass transitions.

2. Materials and methods

Samples were prepared by diluting (dl) -1,3-butanediol (99% +, Aldrich), without further purification, directly in deionized water. The above (dl) -1,3-butanediol was a racemic mixture of the optically active isomers of 1,3-butanediol. Both isomers (*R*)-(−)-1,3-butanediol (98% Aldrich) and (*S*)-(+)-1,3-butanediol (98% aldrich) were also used separately without further purification. The concentration range needed to suppress ice crystallization during the initial cooling was reported previously and checked here [9, 10]. A comparison was also made using 1,2-propanediol (99% Aldrich) and (*S*)-(+)-1,2-propanediol (99% Aldrich) as solutes. 1,3-Butanediol will refer to the racemic mixture.

Calorimetric experiments were performed with a DSC-4 (Perkin-Elmer) adapted for low temperatures down to −160°C. Temperature and energy calibrations of the DSC-4 were done using known melting temperatures and heats of fusion for various pure compounds [24]. This calibration for various scanning rates provided corrections within a standard error of 0.3°C for the temperature and within 3% error for the energies.

The supplemented phase diagram of the present system was determined during warming at 2.5°C min^{-1} after an initial cooling of 2.5°C min^{-1}. The homogeneous ice nucleation temperature T_{hom} was determined as a function of the solute concentration with an emulsion system, allowing separation of the homogeneous nucleation from the heterogeneous nucleation of ice within the sample [32–34]. A 1 : 1 molar ratio mixture of methylcyclohexane and ethylcyclopentane was mixed with a surfactant, SPAN 65 (Fluka), at a concentration of 4% w/v. The various samples were mixed with the emulsifiant solution to form an emulsion by passing the mixture several times through a .2-gage needle. To induce heterogeneous ice nucleation in the samples during cooling, ice nucleating agents were introduced in the deionized water for the chosen samples at a concentration of 1 g l^{-1} of water. In this study, the agent used was *Pseudomonas Syringuae* (Kodak). This technique allows separation of the homogeneous from the heterogeneous ice nucleation [35].

Variation of the glass transition temperature T_g with solute concentration was determined with cooling and warming rates of 1°C min^{-1}. For concentrations below 20% mole/mole, samples were cooled rapidly to temperatures around 100°C above T_g to avoid crystal growth without avoiding ice nucleation. After thermal equilibration, they were cooled at 1°C min^{-1} down to -150°C before recording the subsequent thermal curve during warming. The effect of the cooling and warming rate on the measurement of T_g is known to be non-negligible [36, 37]. However, this effect will not be considered in the present study.

Cooling and warming rates of 10°C min^{-1} were used as a standard procedure to investigate the effect of annealing on the appearance of double glass transitions. These rates were shown to be sufficient to suppress ice crystallization during cooling for 15% mole/mole 1,3-butanediol [9, 10]. They were also shown to permit ice crystals to grow during the subsequent warming to check the effect of structural relaxation on the ice crystallization kinetics.

In a first set of experiments, the samples were cooled below the glass transitions down to -150°C and warmed back to various annealing temperatures for various periods of time. They were cooled back to -150°C before recording the subsequent warming, up to temperatures above the end of ice melting. These experimental conditions will be called annealing on warming. A second set was performed by cooling samples directly to annealing temperatures for various time periods, then cooling them down to -150°C before recording the subsequent warming. These experimental conditions will be called annealing on cooling.

The comparison of structural relaxation for glassy states and ice crystallization during warming was analyzed using the correspondence between the ice crystallization characteristics and the devitrification temperature T_d which corresponds to the maximum crystallization rate during heating [10]. The excess enthalpy recovery was defined as the difference between the enthalpy of the annealed sample and that of the annealed sample for 0 min across the glass transition [23, 24]. The previous analysis of ice crystallization with annealing exposure at sub-glass-transition temperatures has shown that structural relaxation interacts with ice nucleation and with ice crystal growth kinetics for the systems ethylene glycol–water [23, 24] and 1,2-propanediol–D_2O [13].

The possibility of crystallizing a hydrate or an unknown compound with an endothermal peak during warming representing its melts, was checked with direct cryomicroscopic observations using a Carl Zeiss Jena cryomicroscope adapted to low temperatures [20–22]. The calibration in temperature for the cryomicroscope was performed as usual as for the DSC-4. Samples were cooled in the coldtage at 10°C min^{-1} down to -130°C and then warmed back to the annealing temperatures with the same thermal treatment as the chosen samples for calorimetry.

3. Results

3.1. Comparison between samples with isomer alone and with racemic mixture. Solute concentration dependence

The supplemented phase diagram of 1,3-butanediol in water is reported in Fig. 1 with the various phase transitions observed during cooling and warming. No hydrate eutectic crystallized during cooling. Only ice crystallized during the initial cooling and during the subsequent warming for concentrations lower than 60% w/w.

The concentration range needed to suppress crystallization of ice during the initial cooling was observed as being when the T_{hom} curve is lower than that of the maximum ice crystallization rate of the T_d or T_{cg} curve (Fig. 1). The kinetics of ice crystallization is dependent on how much ice nucleation and ice crystal growth overlap compared to the cooling and warming rates. However, as T_{hom} is close to the T_g curve, the crystal growth

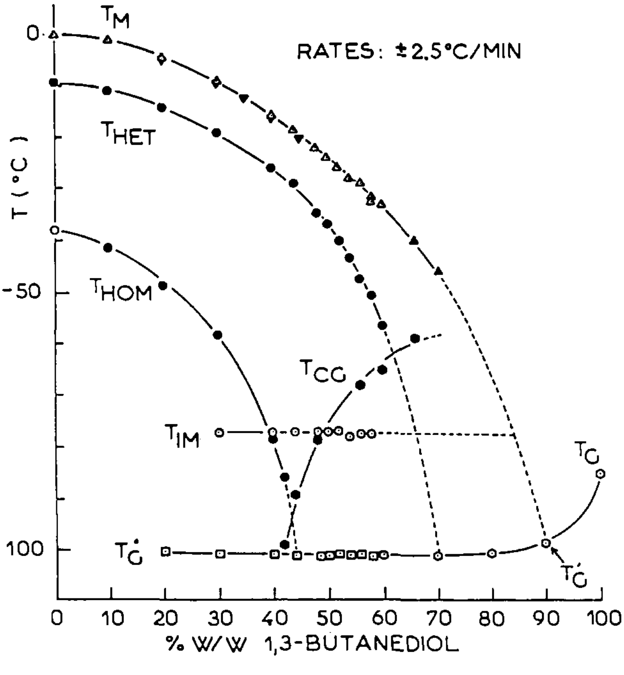

Fig. 1. Phase diagram of the binary system 1,3-butanediol–water: △T_m, end of ice melting temperature; ●, T_{hom}, onset temperature of the homogeneous ice nucleation peak; □, T_g^*, glass transition temperature of the amorphous residue after crystallization of ice; , T_g, glass transition temperature without ice; T_{cg} or T_d in the text, devitrification or ice crystallization temperature during warming (bottom of the DSC crystallization peak). Homogeneous T_{hom} and heterogeneous T_{het} ice nucleation temperatures were determined as described in the method section. The other temperatures were determined in bulk sample during warming at 2.5°C min^{-1} after cooling at "320°C min^{-1}". ▼, data published by Boutron et al. [9]; ▲, T_m recorded for samples cycled several times between -100 and -40°C to include ice crystallization; T_{im} refers to the "incipient melting" temperature as reported by other authors [34].

rates will be too low to permit the ice nuclei to grow into macroscopic ice crystals with the cooling rates presently used. The heat of crystallization as a function of the cooling rate for various 1,3-butanediol concentrations was previously reported [9]. The vitrification range for cooling rates of 10°C min^{-1} was between 40 and 45% w/w [9,10] as checked here.

For a more accurate determination of the T_g curve, T_g was measured during warming after cooling rate of 1°C min^{-1} (Fig. 2). Below the bifurcation point, double glass transitions are observed. The T_g curve is very linear, in contrast with that of the system 1,2-propanediol in water or in deuterium oxide [13, 28] or other glass-forming solutions or compounds in which the glass transition temperature as a function of the solute concentration generally obeys the Couchmann Law [38, 39].

For solute concentrations higher than 15% mole/mole, annealing experiments are necessary to reveal the existence of double glass transitions, as previously noticed for the systems with 1,2-propanediol [1-3]. For concentrations above 30% mole/mole (62.5% w/w), double glass transitions are not observed at present. Annealing experiments below and above the main glass transition do not reveal the presence of any secondary glass transition for these solute concentrations.

However, for lower solute concentrations, double glass transitions are noticeable. Annealing experiments on warming have been done at sub-T_g temperatures for 52.5% w/w 1,3-butanediol, (*R*)-(−)-1,3-butanediol or (*S*)-(+)-1,3-butanediol. Both glass

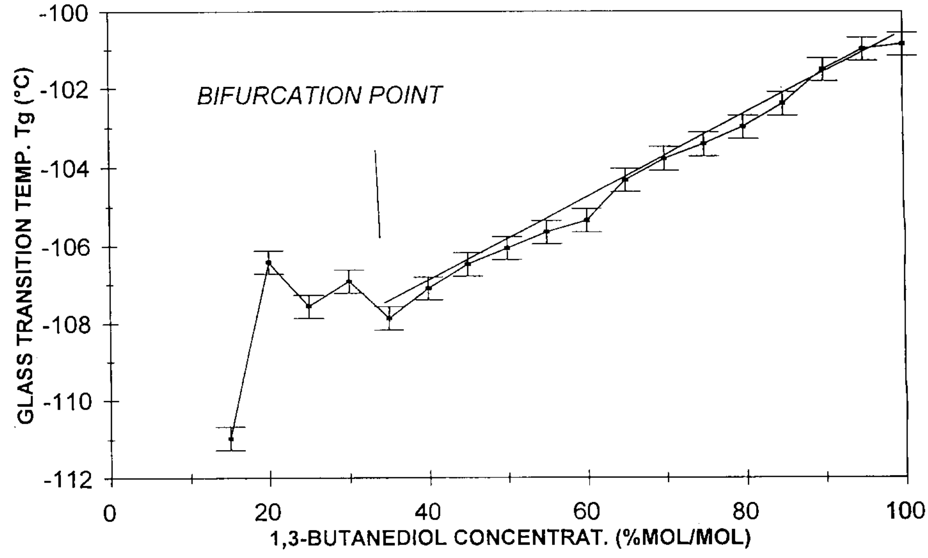

Fig. 2. Temperature T_g (°C) reported as a function of 1,3-butanediol concentration (% mole/mole) in water. The samples were initially cooled at 1°C min^{-1} down to −150°C and the temperatures were measured during the subsequent warming. The T_g values were determined for the first glass transition. The second glass transition temperature was not reported. Direct observation of multiple glass transition was reported for concentrations below the reported bifurcation point.

transitions are observed for these samples. The corresponding thermal curves are reported in Fig. 3 as representative thermal curves. The curves for the various isomer combinations under the same experimental conditions are so similar that they can be superimposed on top of each other. This shows that the existence of these similar thermal events between isomers and racemic mixtures is not the consequence of interaction between isomers. A similar behavior has been observed for the isomers of 1,2-propanediol in water [unpublished results].

3.2. Observation of a third glass transition

Annealing experiments on warming for 2-h periods have been done with a 15% mole/mole sample of (dl)-1,3-butanediol for various annealing temperatures below and above the main glass transition temperature observed directly during the subsequent warming after the initial cooling. The thermal curves recorded after annealing are drawn in Fig. 4 for annealing temperatures below −119°C. For these temperatures, the enthalpy relaxation involves the first two glass transitions. For annealing temperatures above −119°C, the thermal curves representing the glass transitions are reported in Fig. 5, with the appearance of a third endothermic peak at temperatures above those of both first glass transitions.

This peak has an onset temperature that increases with the annealing temperature, and a magnitude that passes through a maximum as the annealing temperature

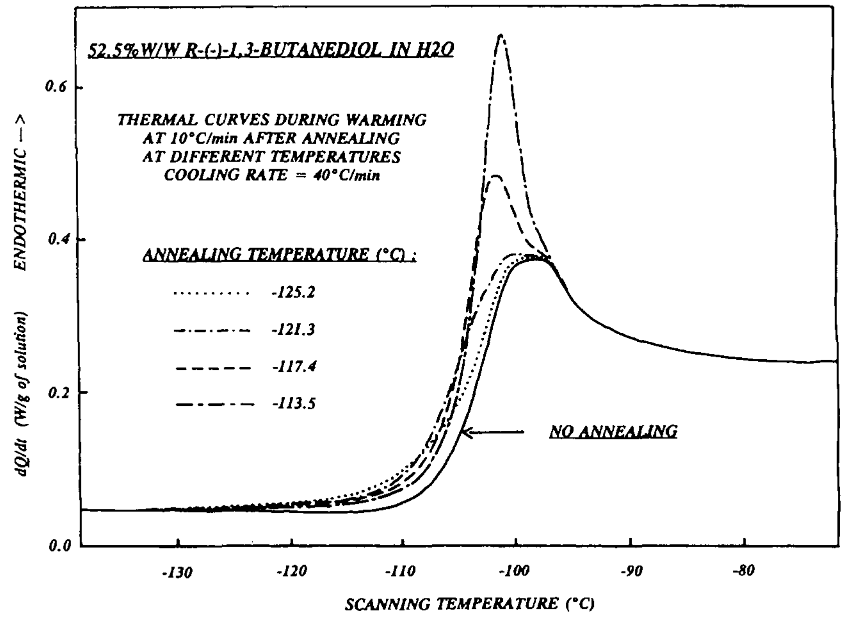

Fig. 3. Thermal curves during warming at 10°C min^{-1} for 52.5% w/w (*R*)-1,3-butanediol in water after various annealing thermal treatments at various temperatures.

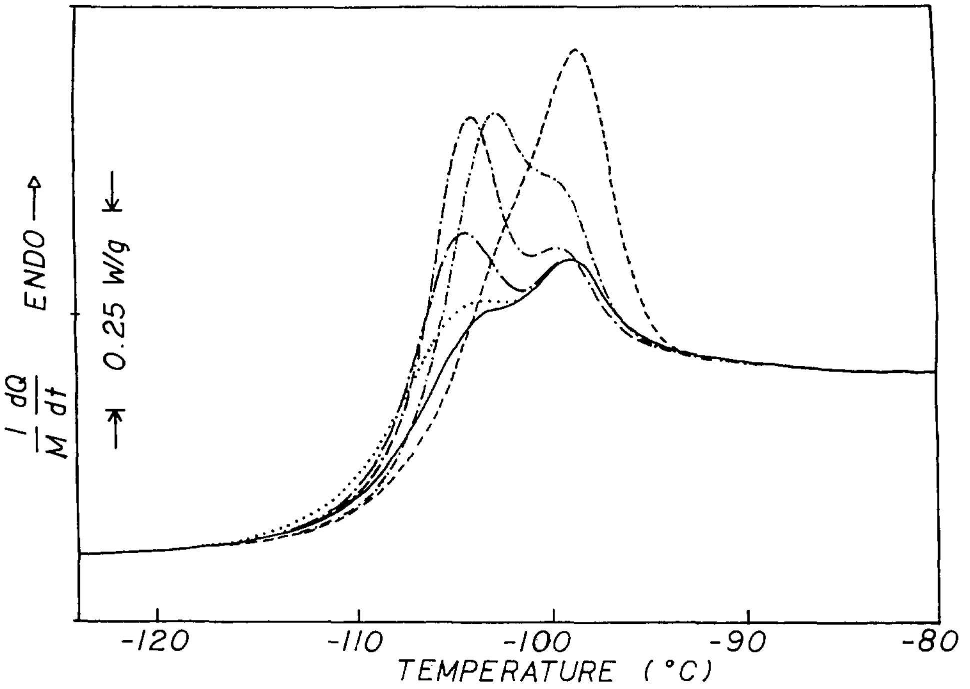

Fig. 4. Thermal curves during warming at 10°C min^{-1} for 15% mole/mole 1,3-butanediol in water after annealing on warming at various temperatures below −111°C for a 2-h annealing period: — — — —, −111°C; · — · —, −115°C; · — — ·, −119°C; ·—·—, −123°C; ·······, −127°C; and —, no annealing.

increases, as for a normal structural recovery for a glass transition [23, 24]. These two characteristics support the hypothesis that this endothermic peak corresponds to a third glass transition. However, its maximum magnitude for the enthalpy recovery for infinite time exposure at each temperature as a function of temperature is not presently observed. The present data do not permit us to deduce the values of the apparent relaxation times associated with this endothermal peak.

Another measurement has been made to test the nature of this endothermic peak by using annealing experiments on cooling. The sample was cooled from room temperature down to −105°C and annealed for 2 h before being cooled down to −150°C. The subsequent warming was recorded for the observation of the various thermal events and reported in Fig. 6 for comparison with the same sample directly cooled to −150°C, then annealed on warming at the same temperature for the same time period of 2 h. The two curves are almost identical as demonstrated by the curve obtained by subtraction of one curve from the other. A slight difference is observed for the third endothermic peak and no difference is noticeable for the double glass transitions. A higher difference is observed for the devitrification due to the difference in ice nucleus density because the sample crosses the main thermal range of ice nucleation four times for the annealing on warming conditions, compared to only twice for the annealing conditions on cooling.

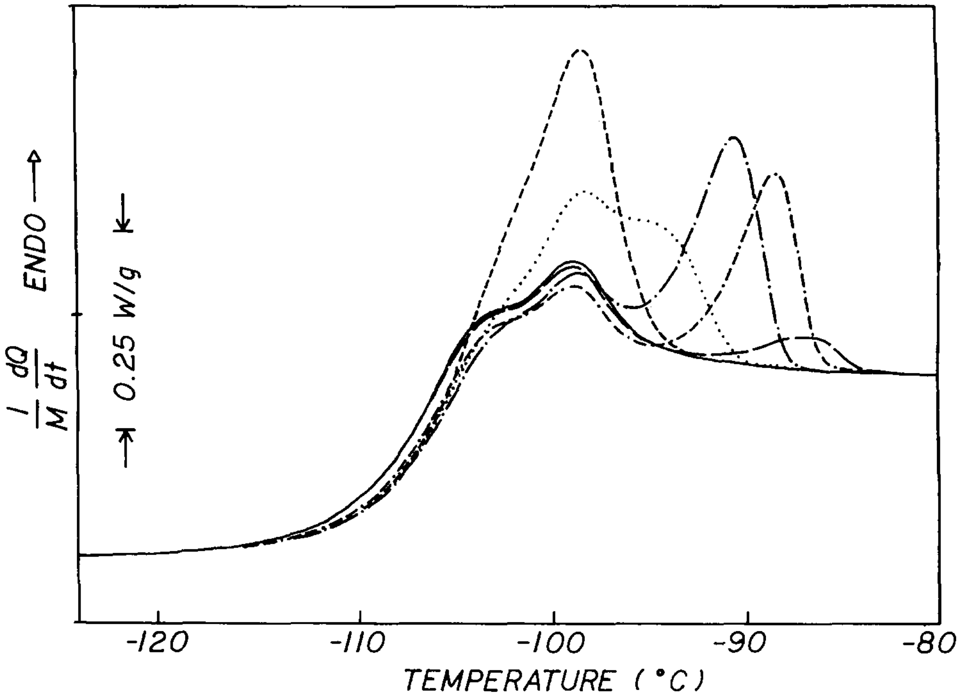

Fig. 5. Thermal curves during warming at 10°C min^{-1} for 15% mole/mole 1,3-butanediol in water after annealing on warming at various temperatures above −111°C for a 2-h annealing period: – – – –, −111°C; ····, −107°C; ·—·, −105°C; · – – ·, −103°C; ——, −101°C; —, −99°C. It should be noted that the glass transition after annealing at −99°C and that of the unannealed sample are similar.

This last experimental condition also excludes the possibility of fracture formation and healing during warming as a reason for the observation of the third endothermal peak. This hypothesis is supported by cryomicroscopic conditions where no fractures were observed.

Similar tests have been performed with one isomer alone. The thermal curves are also reported in Fig. 6. These thermal curves are similar to those of the racemic mixture in the presence of the third endothermic peak. However, a predicted lower devitrification temperature is observed for the isomers compared to that for the racemic mixture. Indeed, observation of a lowering of the melting temperature when isomers are mixed in comparison to when they are used alone is expected, as previously observed for the isomers of 2,3-butanediol [40] and also observed in Fig. 6. This will induce a lower suppression of ice crystallization for the isomers than for the racemic mixture (Fig. 6) [40].

Cryomicroscopic experiments with similar experimental conditions as for calorimetry were performed for 15% mole/mole 1,3-butanediol in water. Several annealing experiments on warming at −106 °C in the cold stage of the cryomicroscope for 1 h failed to produce any observable crystallization followed by melting at around −99 to −90°C. This is not a proof that a microscopic solid phase has not formed

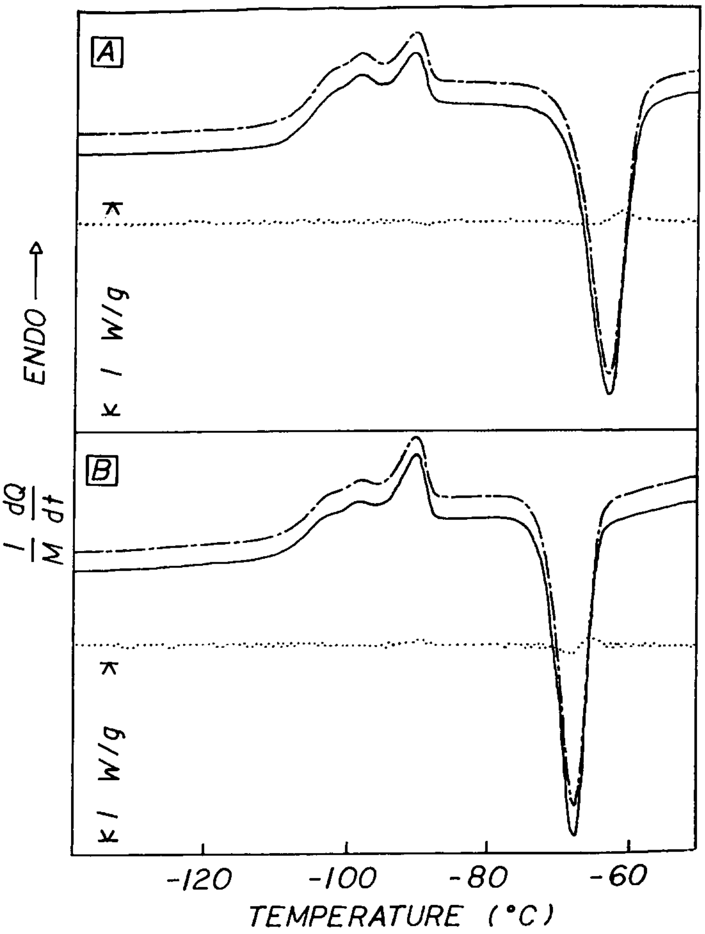

Fig. 6. Comparison of the thermal curves during warming at 10°C min^{-1} for 15% mole/mole (A) 1,3-butanediol and (B) (*R*)-(−)-1,3-butanediol in water after annealing: - - -, on warming at −105°C for a 2-h exposure; — on cooling at −105°C for a 2-h exposure. The subtraction of the second curve from the first thermal curve is reported below ·····.

during annealing. It does, however, support the nature of the third endothermal peak being a glass transition.

3.3. Comparison of structural relaxation and ice crystallization

The effect of the structural recovery of glassy states on the kinetics of ice crystallization has been studied by comparing the enthalpy recovery with the devitrification temperatures T_d for various annealing conditions. The values of T_d are reported in Fig. 7 for annealing experiments at various temperatures T_a with exposure times of

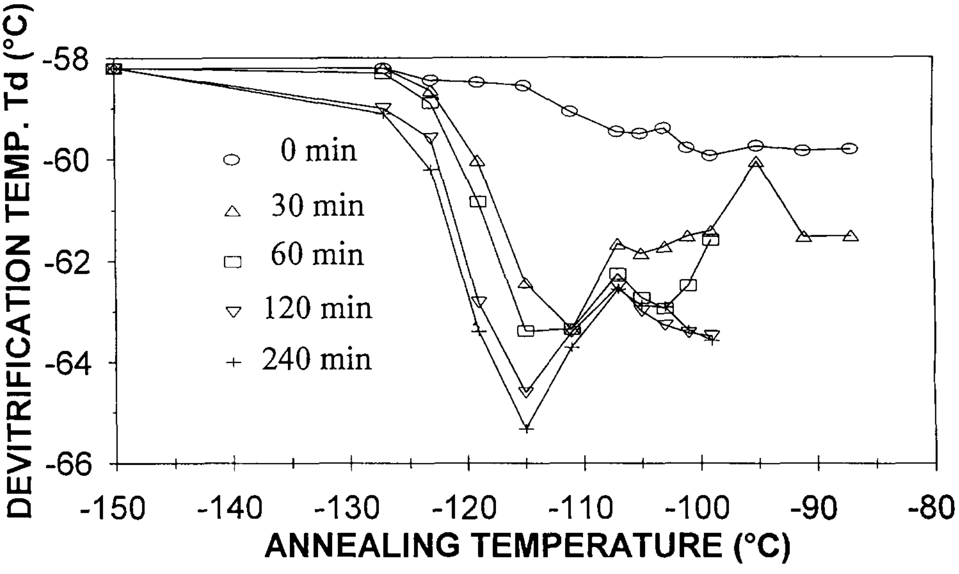

Fig. 7. Effect of the annealing temperature T_a for 15% mole/mole 1,3-butanediol in water on the devitrification temperature T_d (°C). The annealing time periods are reported in the figure.

0 min, 30 min, 1 h, 2 h and 4 h. This figure shows two thermal domains which activate ice crystallization during subsequent warming. The first thermal domain is below −107°C and the second thermal domain is between −107 and −95°C. This second thermal domain may be due to the beginning of the ice crystal growth thermal range, because for 30 and 60 min exposure at this thermal range, the devitrification temperature T_d decreases for the highest temperatures studied. For the highest annealing temperatures above −91°C, Fig. 7 also reveals that the devitrification temperature decreases again for a period of annealing of 30 min. Comparison of the devitrification peak shape shows that the peak is wider. The heat of crystallization also decreases from 13.3 to 12.3 cal g^{-1} after 30 min or 4 annealing at respectively −87 or −95°C. For lower temperatures, as for the experiments reported in Fig. 4, the heat of crystallization during subsequent warmings remains the same within 1% variation. These points can be considered as located below the TTT-curve for isothermal crystallization as defined by other authors [18]. This shows that annealing experiments can locate the thermal gap between the ice nucleation thermal domain and that of ice crystal growth , as previously described for other systems [22]. The beginning of noticeable ice crystal growth can be located at temperatures close to −95°C as the thermal range for ice nucleation is located below −99°C. Therefore, the existence of the previous two apparent thermal ranges for the ice nucleation is real and cannot be explained by a possible overlap with the ice crystal growth thermal range. This also shows that there is a complex process relating the structural relaxation and the ice of nucleation, and that the second ice nucleation thermal domain may be related to the structural change of the glassy states within the sample. These changes may delay the ice crystal growth

during the subsequent warming, as observed previously for 1,2-propanediol in D_2O [1, 2, 4, 13]. The first ice nucleation thermal range is located around $-107°C$ and can be associated with the homogeneous ice nucleation. An explanation is that the annealing process with structural relaxation of the glassy states within the sample interacts selectively with the ice nucleation, without ruling out the possibility of a slowing down of the ice crystal growth during warming as previously observed in the system 1,2-propanediol–D_2O [2, 4].

An alternative interpretation is a change in the nature of ice nucleation at low temperatures. Cubic ice has been found to be the initial step for the formation of ice nuclei [41]. This possibility of a dissociation of the nucleation of cubic ice instead of hexagonal ice has been previously presented, with the assumption that the higher temperature peak was associated with cubic ice nucleation [21]. These cubic ice nuclei are, however, more stable at low temperatures. Above a critical size which is temperature dependent, they must be transformed into hexagonal ice to initialiate crystal growth [41]. Therefore, the lowest thermal range must be associated with the formation of cubic ice nuclei. The highest thermal range is associated with the direct formation of hexagonal ice nuclei which will induce faster crystal growth rates than the cubic ice nuclei which need to go through the cubic-to-hexagonal ice transformation before allowing the ice crystal to grow. Previous studies have reported the stabilization of cubic ice by various solutes at high temperatures up to the devitrification peaks using X-ray diffraction patterns [9, 25, 42]. However, X-ray diffraction powder patterns for evanescent ice crystals have also been observed by MacFarlane and Forsyth to be similar to those of cubic ice which might also include errors in the interpretation of the ice crystal structure (unpublished results, Hey, 1995, personal communication). On the basis of these contradictory observations, the hypothesis of dissociation between the cubic and hexagonal ice nucleation below T_g is weakly supported by the present data, however, with the higher temperature peak corresponding to the hexagonal ice nucleation and the lower peak with the cubic ice nucleation, contrary to the hypothesis of Bronshteyn and Steponkus [21]. This interpretation must be checked using other thermal analysis approaches.

To analyze the connection between the third glass transition and the ice nucleation, the contribution of the enthalpy recovery corresponding to this transition during annealing at various temperatures is reported in Fig. 8 as a function of the annealing time period. From these values of enthalpy recovery, a relationship can be looked for using the representation of these contributions of enthalpy recovery during annealing at various temperatures for various times as a function of the recorded devitrification temperature T_d. The increase in the enthalpy recovery corresponding to the third glass transition does not change the devitrification temperature T_d. Therefore, either ice nucleus formation occurs earlier or does not continue as the structural relaxation proceeds with the third glass transition.

The effect of the structural relaxation from the two main glass transitions is now considered more precisely. The effect of annealing at various annealing temperatures for various time periods on the measured values of T_d is shown in Fig. 9. The curves are complex, as expected. The various contributions for each annealing temperature result in several crossing points. The devitrification temperature generally decreases as

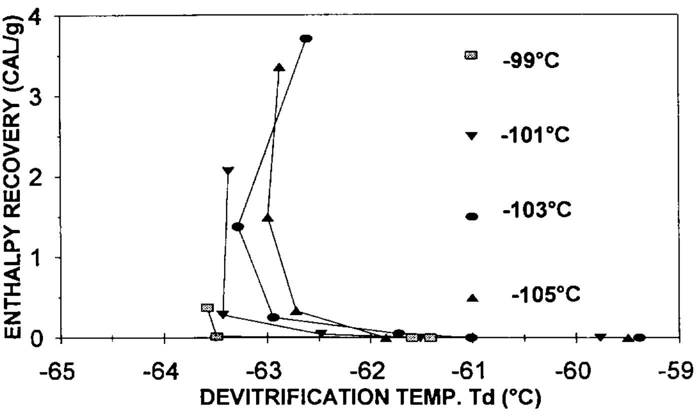

Fig. 8. Contribution of the enthalpy recovery during annealing from the third glass transition for 15% mole/mole 1,3-butanediol in water as a function of the devitrification temperature T_d for various annealing temperatures T_a reported within the figure.

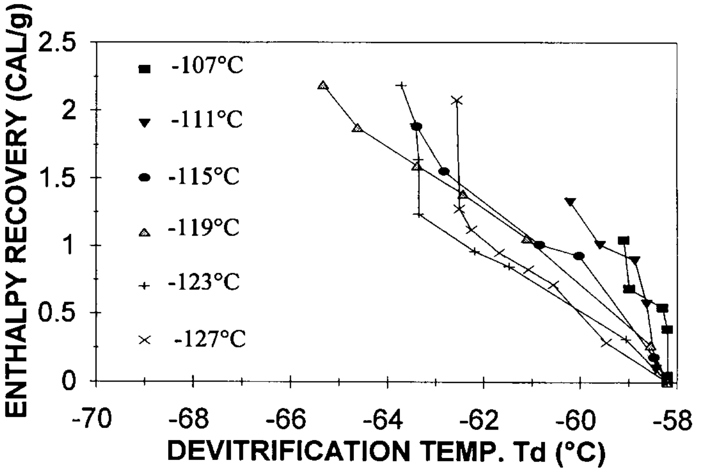

Fig. 9. Contribution to the enthalpy recovery during annealing from the three glass transitions for 15% mole/mole 1,3-butanediol in water as a function of the corresponding devitrification temperature T_d (°C) recorded during the subsequent warming after various annealing exposures at various temperatures reported within the figure.

previously observed and reported by various investigators [13, 14, 16, 17, 19, 20]. It also stabilizes at similar temperatures and even increases slightly as the time of exposure increases (Fig. 10A and B). This has previously been reported for the system 1,2-propanediol–D_2O with the argument of the existence of interaction between the structural relaxation and the ice crystal growth during subsequent warming [1, 2, 4, 13]. For the present system, the enthalpy relaxation is still progressing as the ice nucleation process becomes saturated, see Figs. 10 and 11.

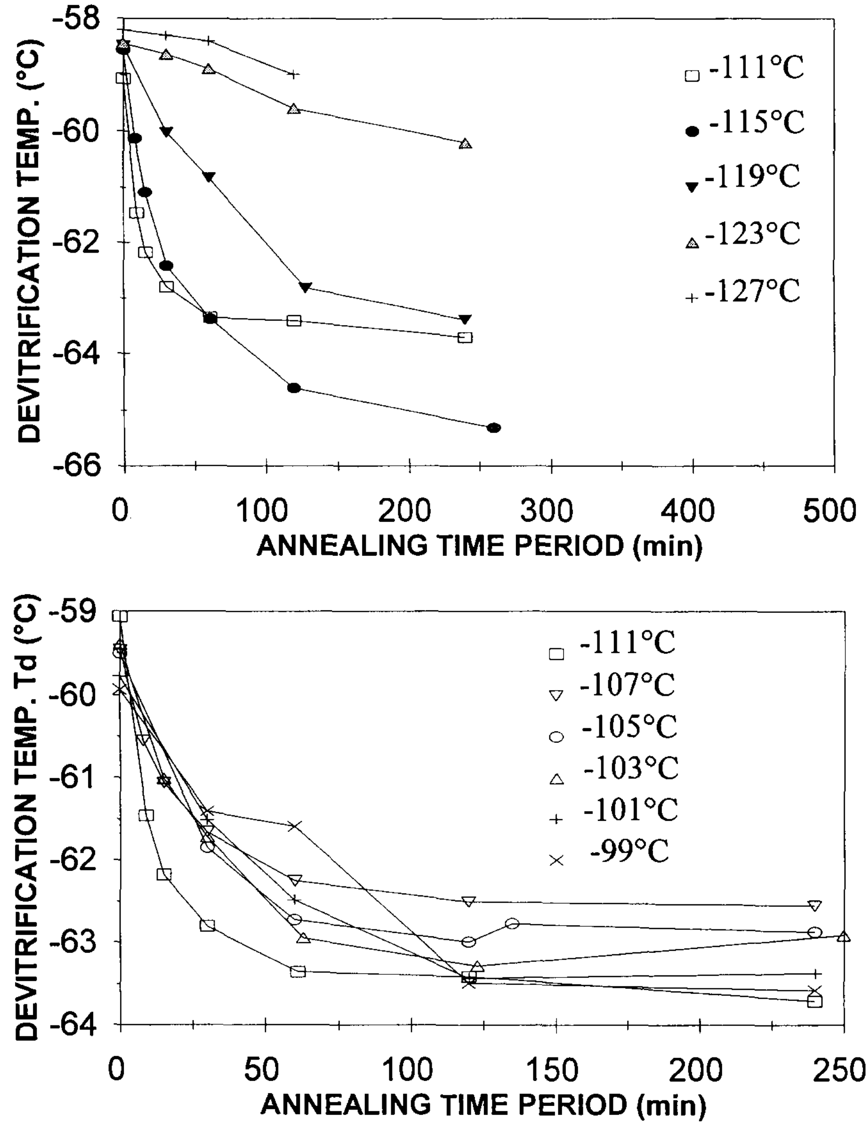

Fig. 10. Variation of the devitrification temperature T_d (°C) for 15% mole/mole 1,3-butanediol in water as a function of the annealing time period (min) for various annealing temperatures T_a as reported in the figures: A, for T_a below −111°C; and B, for T_a above −111°C.

For the lowest annealing temperature, the effect of the structural relaxation is not sensitive to variation in the devitrification temperature. As the overlap of the structural relaxation between the first and second glass transitions increases with the annealing temperature and exposure, the devitrification temperature decreases. This shows that

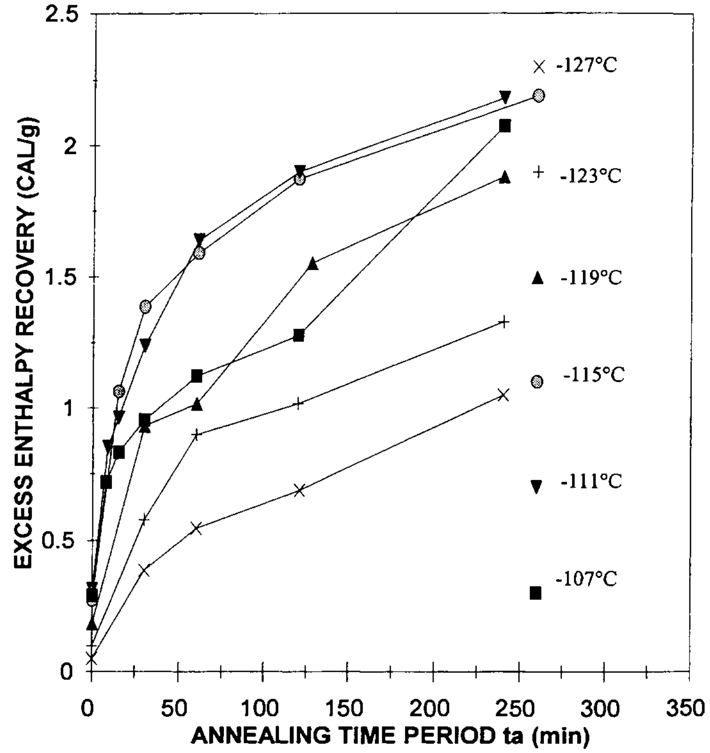

Fig. 11. Excess enthalpy recovery (cal g^{-1}) as a function of the annealing time for various annealing temperatures for the glass transitions of 15% mole/mole 1,3-butanediol in water.

the structural relaxation of the second or main glass transition peak in the main contributor to the variation in the devitrification temperature.

3.4. The third glass transition

The observation of the third glass transition is limited to annealing experiments below −99°C. However, its observation might be possible with a longer annealing exposure, e.g. 4 h. at −99°C. To illustrate the observation of the third glass transition during warming, Fig. 12 reports the contribution of the third glass transition to the enthalpy recovery during annealing at various temperatures as a function of the time of exposure at those temperatures. This figure suggests that the observation of this transition is subject to kinetics limitation.

Observations of higher glass transition temperatures than the main glass transition are generally related to a modification of the structure of the glassy state during annealing, such as an increase in the solute concentration, or to the presence of a new phase such as another glassy state. For polymer solutions, this observation has been related to the possible localization of structural relaxation and to the redistribution of

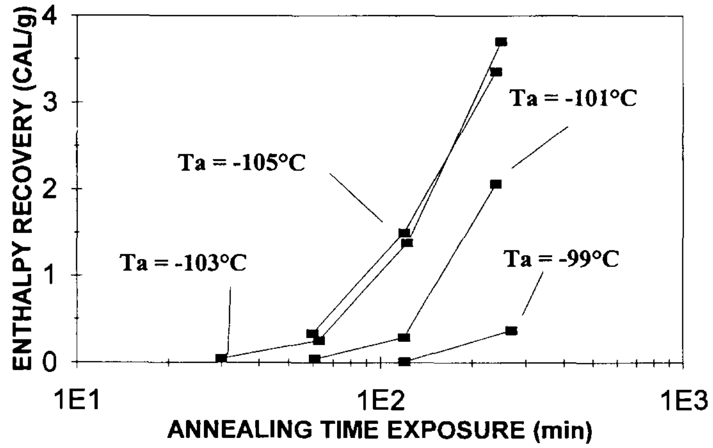

Fig. 12. Excess enthalpy recovery of the third glass transition during annealing at various temperatures as a function of the annealing time period t.

the retardation times during structural recovery [11, 12]. The present transformation during annealing is shown to be related to a depletion of the first glass transition and, partially, of the second glass transition, without apparently modifying the amount of ice crystallization. The magnitude of the third glass transition is related to the transformation ratio from the other glass transitions to this third glassy state and also to the corresponding maximum excess enthalpy between this third glassy transition and its corresponding liquid state at the annealing temperature. However, its temperature above the fictive temperature of the main glass transition clearly suggests that the notion of a non-well-stabilized glassy state may not be applicable to this third glass transition.

The limit glass transition temperature for pure 1,3-butanediol is -100.5°C from Fig. 2 for samples cooled and warmed at 1°C min^{-1}. Assuming some variations of a few degrees higher for the onset temperature of the glass transition corresponding to the higher scanning rate of the DSC-4, this strongly suggests that the amorphous state of pure 1,3-butanediol or a high solute concentration solution was extracted from the sample during annealing at sufficiently high temperatures as the only possible observation of such a high temperature glass transition. Assuming this hypothesis, the observation of the third glass transition after annealing on cooling suggests that this form with 1,3- butanediol will liquid–liquid phase-separate during the initial cooling and exposure at various annealing temperatures up to -97°C. Then the glass transition of this form with 1,3-butanediol must appear during subsequent warming at the corresponding temperature.

3.5. Comparison with 1,2-propanediol as solute

The systems of 1,2-propanediol in water and in deuterium oxide [1–5] exhibit double glass transitions and have initiated the present study. For 1,3 butanediol, the existence of these double glass transitions is independent of the presence of the various optical isomers. Double glass transitions are also observed for the optical isomer of 1,2-propanediol for 42.5% w/w (*S*)-(+)-1,2-propanediol in water (unpublished results). To complete this comparison between 1,2-propanediol and 1,3-butanediol, the search of the third glass transition has been made for various concentrations of 1,2-propanediol in water and in deuterium oxide. The difficulty arises with the thermal range of the third glass transition which overlaps with the main glass transition. The observation of the third glass transition is reported for 37.4% w/w 1,2-propanediol in deuterium oxide in Fig. 13 for annealing experiments at various temperatures for 1 h exposure. Similar experiments for 40% 1,2-propanediol in water are reported in Fig. 14. For both

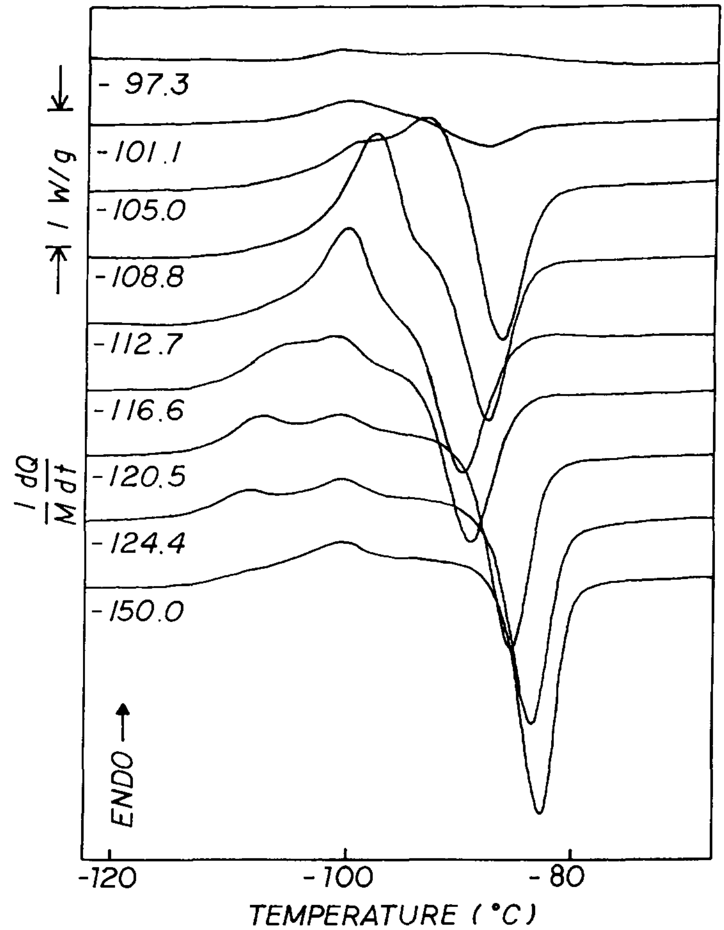

Fig. 13. Thermal curves during warming at 10°C min^{-1} after annealing for 37.4% w/w 1,2-propanediol in deuterium oxide cooled at 320°C min^{-1}. Annealing exposure for 1 h were performed at various temperatures as indicated. A sample without annealing was also tested.

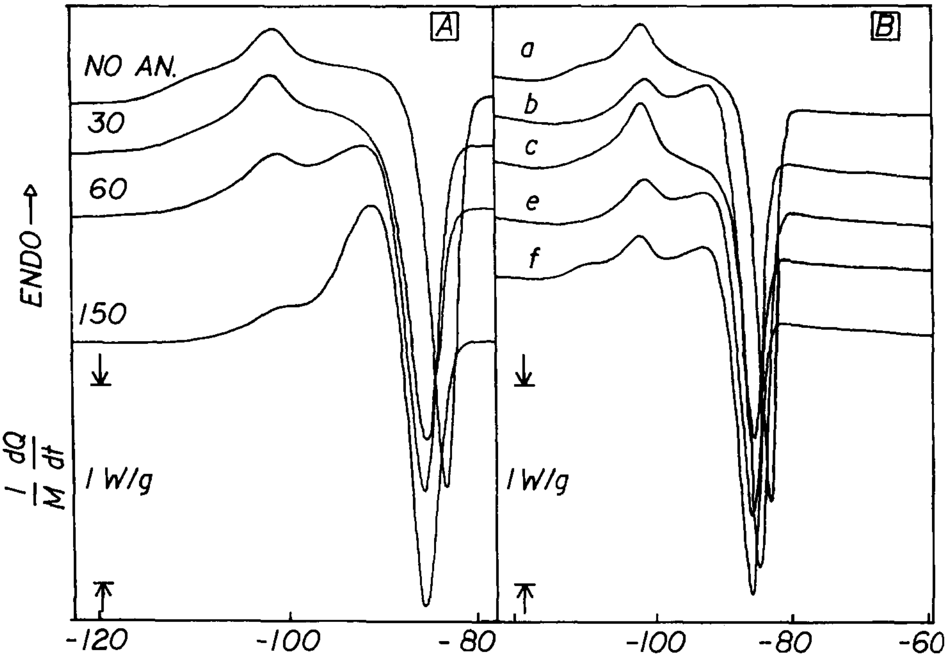

Fig. 14. Thermal curves during warming at 10°C min^{-1} after annealing for 40% w/w 1,2-propanediol in water cooled at 320°C min^{-1}: A, samples cooled then warmed for annealing at −108°C for various periods of time; B samples subjected to various thermal treatments as represented by the temperature of annealing then followed by the time exposure: a, no annealing; b, −105°C, 1 h; c, −124.5°C, 30 min, −105°C, 1min; e, −124.5°C, 30 min, −105°C, 1 h; and f, −105°C, 1h, −124.5°C, 30 min.

systems, the so-called third glass transition peak is present as the first glass transition either decreases strongly in magnitude or disappears completely. The third glass transition, however, still exists at higher temperatures than those of the main glass transitions. This third glass transition temperature appears at higher temperature than the fictive temperature corresponding to the main glass transition, as previously observed for 1,3-butanediol in water.

Observations of these third glass transitions are limited to the thermal range of the main glass transition, which will overlap the overshoot of the third glass transition and will then be invisible or difficult to dissociate from the main glass transition. As the solute concentration increases, the main glass transition temperature will also increase. This is the reason why this third glass transition is observed for solute concentrations at the limit of vitrification during cooling. However, as the sample is annealed at higher temperatures than the temperature of the main glass transition, the third glass transition will arise as the first glass transition decreases or disappears from the thermal curves, as observed for the system with 1,2-propanediol (Figs. 13 and 14). Therefore, this suggests that the origin of the third glass transition is linked to the presence of the first glass transition.

4. Discussion

The plasticizing effect observed by addition of water molecules to glassy materials is known. The calorimetric glass transition for vitrified aqueous solutions is generally associated with the translational mobility of solute molecules. An increase in the water molecule content will lower the glass transition temperature but also destabilize the solution towards ice formation. Observations of double glass transitions for various aqueous glass-forming solutions have previously been reported by various authors [1–3, 9–10]. These observations can be interpreted as the consequence of a liquid–liquid phase separation during cooling [4, 5] or also as the consequence of a localized structural relaxation [6–8]. The dependence of the magnitude of the lowest temperature glass transition on the water content of the sample suggests that this glass transition peak is strongly related to the water phase or to the low solute phase in the sample. Assuming a localized structural relaxation and using the previous observations also lead to the conclusion of a partial liquid–liquid phase separation taking place during cooling. This phase separation has already been discussed for the systems 1,2-propanediol in D_2O and in H_2O [2, 4]. The fact that the double glass transitions are not observed for 1,3-butanediol concentrations higher than 30% mole/mole shows that both 1,3-butanediol and 1,2-propanediol present similar properties. Indeed, for 1,2-propanediol, the double glass transitions were not observed for concentrations higher than 60% w/w, corresponding to 26% mole/mole of 1,2-propanediol in H_2O or in D_2O [2, 4]. Moreover, the existence of a higher and lower concentration range association between water and 1,2-propanediol, at respectively 50 and 2% mole/mole for the system [2, 4, 13, 43, 44], supports the possibility of a liquid–liquid phase separation during cooling, explaining the observation of the multiple glass transitions.

The existence of a third glass transition for such simple binary systems is peculiar as it was found at higher temperatures than the main glass transitions observed during warming. This third glass transition is not reversible because annealing experimental conditions are necessary to reveal its presence. The existence of the third glass transition for the systems 1,2-propanediol in H_2O or in D_2O shows a similarity between both 1,2-propanediol and 1,3-butanediol interacting with the solvents. The separation of high- and low-solute concentration liquids during cooling is a possibility that should be tested using other thermal analysis techniques.

The present observations support the relationship between the first and third glass transitions. As observed for 1,3-butanediol or 1,2-propanediol as solutes, the first glass transition is strongly suppressed as the third glass transition appears. A comparison of the respective structural relaxation contribution for the various glass transitions shows that the second glass transition is mainly responsible for the formation of ice nuclei within the samples. The two other glass transitions are weakly related to the ice nucleation.

The comparison between the ice crystallization and the structural relaxation of the glassy states also shows two thermal ranges for the ice nucleation. These two thermal ranges may be the consequence of a dissociation between cubic ice nucleation and hexagonal ice nucleation or of the effect of the structural recovery below T_g on the crystal growth rates. Both interpretations must be checked using other thermal analysis approaches.

5. Conclusion

Calorimetry has been shown to be capable of revealing the importance of thermal history for vitrified samples such as aqueous glass-forming solutions. However, it is also apparent that calorimetry is not sensitive enough to conclude definitively on the nature of multiple glass transitions.

The existence of multiple glass transitions for low solute concentrations of glass-forming aqueous solutions shows that the vitrification of aqueous solutions remains a complex process which needs further investigations. This is indeed the first report of the observation of triple glass transitions in binary systems for aqueous glass-forming solutions. Observations of this third glass transition in the systems of 1,2-propanediol in water or deuterium oxide is indicative of a more general behavior of these low temperatures. This third glass transition may be the result of a phase separation or of a localized structural relaxation within the water molecule network, such as an interfacial water network. However, more precise methods, such as spectroscopic techniques, are needed to distinguish between the causes.

Understanding of the mechanism of interaction between ice nucleation and structural relaxation of the glassy states for glass-forming aqueous solutions is still limited to qualitative appraoches. Ice nucleation is a consequence of the structural relaxation of the associated glassy state phase. It also affects ice crystal growth, to a wider extent, with the same glassy state or with the non-related glassy state phase.

The hypothesis of a liquid–liquid microphase separation during the initial cooling is supported by the present data by the dependence of the double peaks on the solvent concentration. The similarity between the present system and that of 1,2-propanediol in water or in deuterium oxide suggests a general behavior for aqueous glass-forming solutions [1–4]. This work presents a new attempt to observe these multiple glass transitions in aqueous glass-forming solutions. They may have an effect on scientific and industrial applications where the quality control of products is related to the stability of aqueous glassy states. These applications can be related to the vitrification technique applied for the preservation of organs and biological tissues at low temperatures [24]. As the phase separation occurs during storage above the glass transition, application of the present knowledge can improve the methods of freeze-drying for the preservation of biological materials, e.g., proteins, enzymes, etc., or other preservation techniques at low temperatures.

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