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Optimized thermal treatments to obtain reproducible DSC thermograms with sucrose + dextran frozen solutions

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Abstract

The freezing behaviour of concentrated model solutions has been studied by differential scanning calorimetry (DSC) over a large range of sucrose + dextran ratios. When conventional thermal cycles (10°C/min) are used, it is observed that the freezing is dependent on the dextran concentration, i.e. the amount of unfrozen water increases with the dextran concentration.The high viscosity of the concentrated solution limits the ice crystal growth during the cooling step of the DSC analysis. In order to obtain maximally freeze-concentrated samples, a methodology is proposed that corresponds to specific annealing treatments. © 1998 Published by Elsevier Science Ltd. All rights reserved.

1. Introduction

When an aqueous solution is submitted to cooling below its freezing point, ice crystallisation may occur. As the temperature is further reduced, more ice crystallises and the residual liquid phase becomes progressively more concentrated; the viscosity of the unfrozen fraction increases strongly and a liquid–glass transition takes place in the residual cryoconcentrated phase. The temperature at which the glass transition of the maximally freeze-concentrated phase occurs is called T_g' (Levine & Slade, 1988). Each solute in aqueous solution is expected to have a univariant maximal freeze-concentration C_g' and consequently T_g' is expected to have a constant value. This is realised at the condition that the cooling rate allows ice crystallisation and not solute crystallisation.

Measurements carried out using differential scanning calorimetry (DSC) allow us to observe the thermal changes that occur in frozen systems, but DSC experiments show that cooling and heating processes involving liquid↔solid changes for water result in transitions which depend on concentration and thermal history. The addition of solutes appears to modify the water freezing behaviour, but thermodynamic equations alone cannot predict how much water will remain unfrozen. Only a non-equilibrium state due to the ice crystal growth with respect to the cooling rate can explain the variations in the non-frozen water quantity. It is very important to obtain reproducible thermograms, i.e. corresponding to the maximal freeze-concentration state, because the temperatures of the different transitions must be reliable for use in further stability studies.

Numerous papers have discussed DSC studies on frozen sugar solutions; it is impossible to quote all and particularly for sucrose solutions which are of interest here. Some of these papers (Ablett, Izzard, & Lillford, 1992; Roos & Karel, 1991) have described the efficiency of thermal treatments to obtain a maximally freeze-concentrated phase with the aim of improving the accuracy of the T_g' value. The uncertainty about the T_g' value results from the controversial interpretation of the DSC events and from the great dependence of these events on thermal history.

The study of Roos and Karel (1991) showed the DSC thermograms obtained with frozen sucrose solutions on a large concentration range, with thermal treatments depending on the solute concentration. Their experiments led to a constant temperature of the transition onset, but the thermogram shape varied with the

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concentration. Ablett et al. (1992) used isothermal annealings and determined T_g' from the annealing temperature resulting in the highest temperatures for the different glass transition steps. They did not discuss, however, the effects of the glass ageing which may take place due to the duration of the annealing treatments and modifying the shape of the thermograms and thus the transition temperatures.

More, in a recent paper we have shown that T_g' should not be considered as an unique temperature but as a temperature range (Blond, Simatos, Catté, Dussap, & Gros, 1997). The physical changes due to this kinetically controlled transition spread out on a wide range of temperature independently of the heating/cooling rates.

The achievement of maximal freeze-concentration appears to be particularly difficult with solutions containing macromolecules. The development of the ice matrix is the result of two simultaneous kinetic processes, which are strongly interrelated because of the important effect of temperature and viscosity on both of them: nucleation and crystal growth. Generally, the presence of polymers promotes the heterogeneous nucleation in bulk volume, but this effect is limited if they are in the gel state; moreover they always slow down the linear crystallisation rate (Blond, 1985, 1988; Muhr & Blanshard, 1986). In ice creams, the hydrocolloids, although added in very small quantities, have a recognised improving effect on the sensory properties of texture; they have been claimed to have one or more of the following effects: to increase the viscosity of the mix; to directly modify the texture; to restrict the growth of ice crystals during the freezing and storage processes (Cadwell, Goff, & Stanley, 1992; Hagiwara & Hartel, 1996).

In the present investigation, experiments showed that the DSC thermograms of highly concentrated solutions did not correspond to maximally freeze-concentrated samples with the usual conditions of analysis (cooling/heating rates 5–10°C min^{-1}). A methodology was therefore developed to obtain reproducible thermograms. The model compounds selected for these studies were a sugar, the sucrose, and a very soluble polymer, the dextran. We studied the influence of the dry matter content and of the molecular weight on the freezing process as monitored by DSC analyses, then the effect of the dextran addition on the freezing process of sucrose solutions, particularly by observing the changes in the T_g' values.

2. Materials and methods

2.1. Materials

The model solutions were prepared using sucrose (Merck) and dextran T40 (Pharmacia) MW: 40,000; these materials were used as received. Pure samples were dissolved to a final concentration of 10–50 g solute/100 g solution. For the mixtures, the study was limited to 50% dry matter solutions, in which the ratio of dextran to sucrose was gradually increased. The dissolution was achieved by the addition of the polymer in distilled water; then the preweighed sucrose was gradually added with agitation until a clear solution was obtained. The most viscous solutions were heated to 50°C to improve the dissolution rate.

2.2. Differential scanning calorimetry

DSC was used to determine the glass transition and melting temperatures of the model solutions. The DSC used was a Perkin–Elmer DSC-7 equipped with the liquid nitrogen subambient accessory. Hermetically sealed 20 μl aluminium pans were used for all the measurements; an empty pan was used as reference. The sample weight was around 5 mg. Cooling and heating rates were 10°C min^{-1}.

The glass transition temperature of any amorphous substance is defined as the temperature range where the specific heat (C_p) changes in a stepwise manner. Different temperatures may be chosen to characterise this transition: the onset point (T_{g_o}), the mid-point of the heat capacity change (T_{g_m}) or the end-point (T_{g_e}). Here the glass transition temperatures were expressed as the midpoint of heat capacity increments and determined using the Perkin–Elmer software. This point is accepted as the standard T_g as determined by DSC (ASTM Standard method E 1356-91).

An isothermal annealing treatment involves an isothermal holding time. If the holding temperature is higher than T_g', the treatment should allow either crystal growth or ice melting; if it is lower, only a physical ageing of the glass can be observed. In the present work, thermal cycles were only used to obtain full ice crystallisation; they consisted of a limited first heating, immediately followed by a second cooling. The definition of the maximum temperature to be used for this treatment was one of this study objectives.

3. Results

3.1. Shape of heating thermograms of frozen solutions following a single cooling at 10°C min^{-1}

The heating thermograms of sucrose solutions varying from 10 to 50% dry matter, obtained after a cooling to −70°C, show principally a large endotherm which corresponds to the ice melting [Fig. 1(a) and (b); for clarity only 20 and 50% concentrations are shown]. It is obvious that this endotherm begins at the same temperature independently of concentration. Before this endotherm, one can see the complex feature corresponding to the glass transition of the unfrozen fraction, its temperature range always being the same and its magnitude depending only on the dry matter content. We have already identified this feature as a glass transition associated with an enthalpy relaxation process and characterised it by its onset temperature (T_{g_0}) and by the mid-point temperature of each heat capacity increment (T_{g_1} and T_{g_2}) (Blond, 1989; Blond et al., 1997). The T_g' value determined from the study of a modelled state diagram was shown to be slightly higher than T_{g_1} (Blond & Simatos, 1991; Simatos & Blond, 1993). According to different authors, the value proposed for T_g' can be T_{g_0} (Roos & Karel, 1991), T_{g_1} (Ablett et al., 1992; Shalaev & Franks, 1995) or T_{g_2} (Levine & Slade, 1988).

Dextran solutions at the same concentration levels show a different behaviour [Fig. 1(c) and (d)]; the feature in front of the ice melting endotherm appears as a distinct peak (**p**), particularly for high dextran concentrations. The comparison of DSC thermograms for different dextran-sucrose ratios after freezing with the same cooling rate shows that the feature in front of the ice melting endotherm also changes with the dextran concentration (Fig. 2). Its appearance is between the ones of pure sucrose and pure dextran solutions. Apparently, the transitions are shifted towards higher temperatures when the dextran concentration is increased and are becoming distinctive peaks.

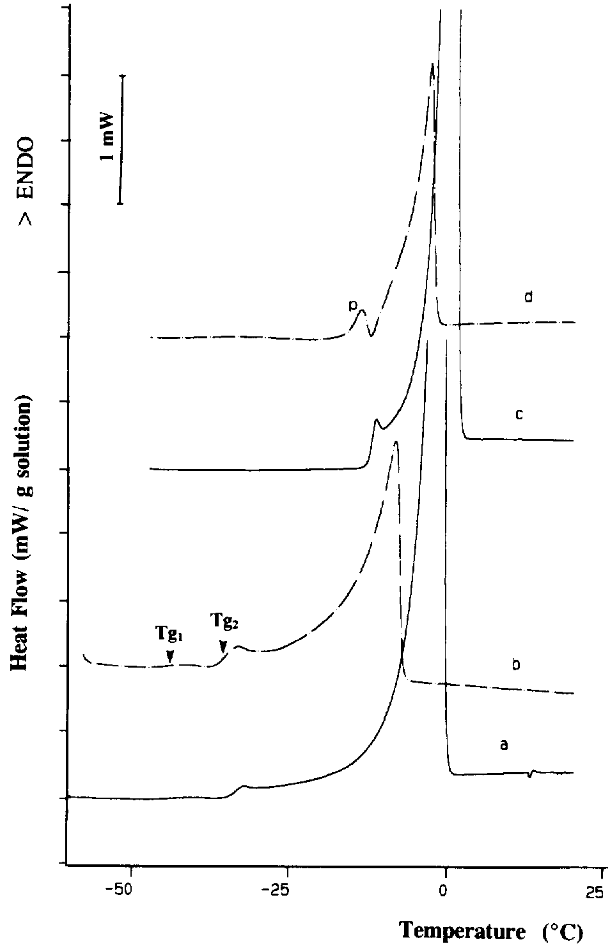

Fig. 1. DSC heating traces obtained after a single cooling for sucrose and dextran solutions. (a) 20%sucrose; (b) 50% sucrose; (c) 20% dextran; (d) 50% dextran.

An advanced analysis of thermograms obtained after a single cooling (Fig. 2) is without interest; the shape of the thermal features observed before the ice melting transition is too dependent on experimental conditions. These features can be modified with changes in sample size and/or cooling and heating conditions. In thermal analysis the samples have a very small size, and their cooling/heating rates can be considered as identical to the programmed rates, these ones being often too fast to allow a full crystallisation. A large undercooling can be observed [Fig. 3(1)]; the ice nucleation temperature is greatly dependent upon the sample size and its purity, the presence of particles increasing the number of nucleation sites.

These experiments show that the amount of ice formed during the first cooling and heating treatment varies depending on the solute concentration and on the solute molecular size; with the same concentration

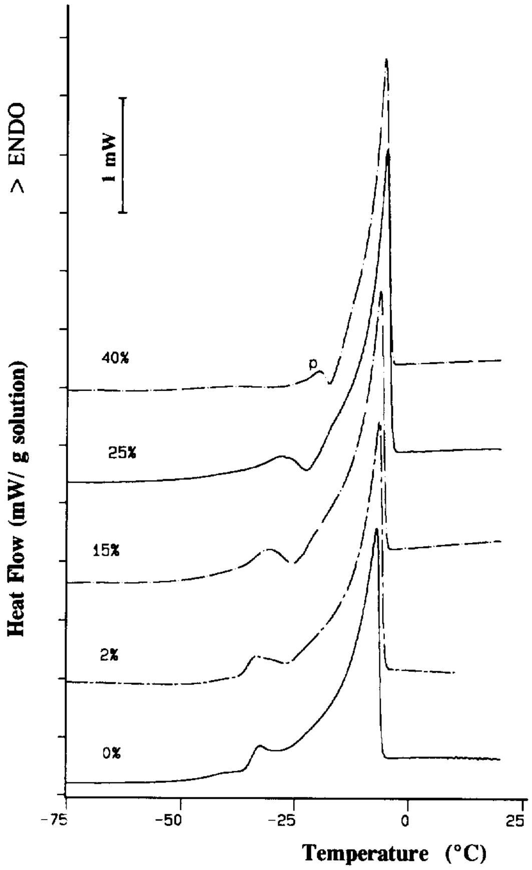

Fig. 2. Influence of dextran content on the setting up of the freezing as obtained in DSC scans with a cooling rate of 10°C min^{-1}. The dry matter of all solutions was 50%.

range, the freezing is more complete in a sugar solution than in a polymer solution. The increasing dextran concentration slows down the setting up of ice crystals; after the cooling step, the sample is not fully stabilised since a crystallisation exotherm can be visible during rewarming, it corresponds to the decreasing part of the **p** peak (Fig. 3). The difference of enthalpy measured from the cooling and rewarming thermograms was also shown by Quinn, Kampff, Smyth, and McBrierty (1988) with hydrogels. The high viscosity of partly freeze-concentrated solutions increases when the temperature decreases, thus limiting the water diffusion towards ice crystals and a complete freezing could be expected during rewarming only.

3.2. Annealing treatment

Different thermal cycles, by changing only the maximum temperature of the first heating, were tested in order to be sure that maximally frozen samples were obtained; the working hypothesis was that the objective was reached when thermograms obtained further were always reproducible, even if the maximum temperature was raised 2 or 3°C. Experimental results showed that the samples had to be heated just above the temperature of the end of the first small peak (**p**) [Fig. 1(d)].

Fig. 3(4) shows the thermogram for the 50% dextran solution obtained after an annealing treatment to the maximum temperature of −11°C; we can observe that the characteristic reproducible glass transition feature has replaced the peak (**p**). Fig. 4 allows us to ascertain the inadequacy of a lower temperature treatment for obtaining a stabilized sample: curves 3 and 4 obtained after annealing at −13 and −11°C, respectively, show an important decrease of the exothermic effect; annealing at −11°C results in a further increase of the temperature T_{g_2}.

Similar annealing treatments were carried out on the sucrose + dextran solutions. The resulting thermograms are presented in Fig. 5; the glass transition shows a two-step pattern as with the sucrose solution only for the low dextran concentrations; it is shifted to higher temperatures with increasing dextran concentration. The T_{g_2} step appears visible for all the dextran concentrations; its magnitude is higher than the one which can be expected for the glass transition of the freeze-concentrated phase (Blond & Simatos, 1991). Fig. 6 shows the maximum heating temperatures which must be used for the optimized annealing treatment. They are compared with the melting (T_m) and T_{g_2} temperatures of the tested mixtures. Increasing dextran content shifts the glass transition towards higher temperatures and consequently the maximum temperature of the annealing treatment. McCurdly, Goff, Standley, & Stone (1994) gave higher temperatures for T_g' (T_{g_2}) of frozen solutions with similar sucrose–dextran ratios; the difference could be due to the dextran used, which had a very high molecular weight.

For solutions composed of solutes with different molecular weights, the shift of T_{g_2} towards higher

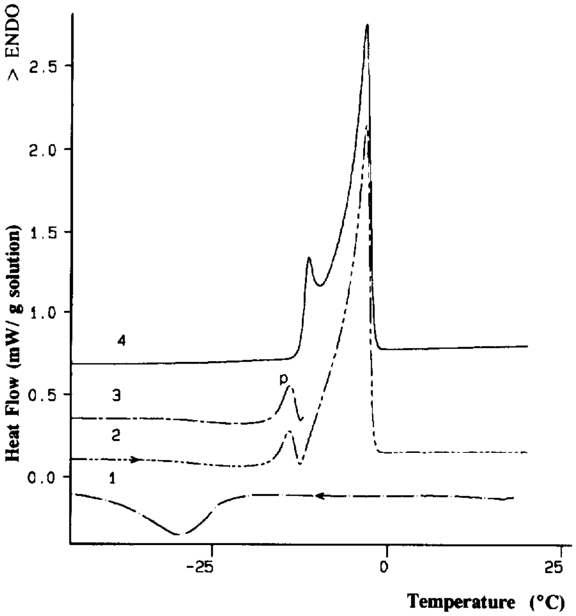

Fig. 3. Annealing treatment used to obtain a maximally freeze-concentrated system from a 50% dextran solution. (1) cooling to −70°C (10°C min^{-1}); (2) rewarming without annealing treatment (10°C min^{-1}); (3) annealing treatment, i. e. rewarming to −11°C (10°C min^{-1}); (4) rewarming (10°C min^{-1}). For better clarity, the cooling curves before step 3 and between steps 3 and 4 are not presented.

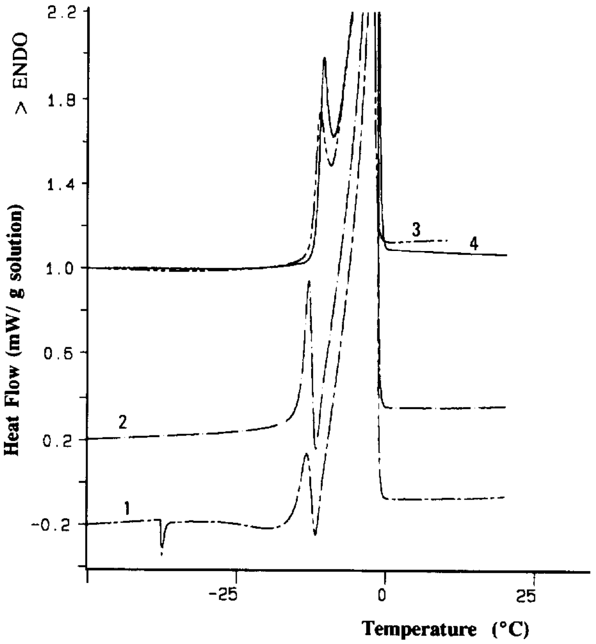

Fig. 4. Influence of the annealing temperature on the shape of the glass transition features as observed by DSC: curve 1, without thermal treatment; curve 2, −15°C; curve 3, −13°C; curve 4, −11°C. (Curves 3 and 4 have been superimposed to better see their difference.) 50% dextran solution.

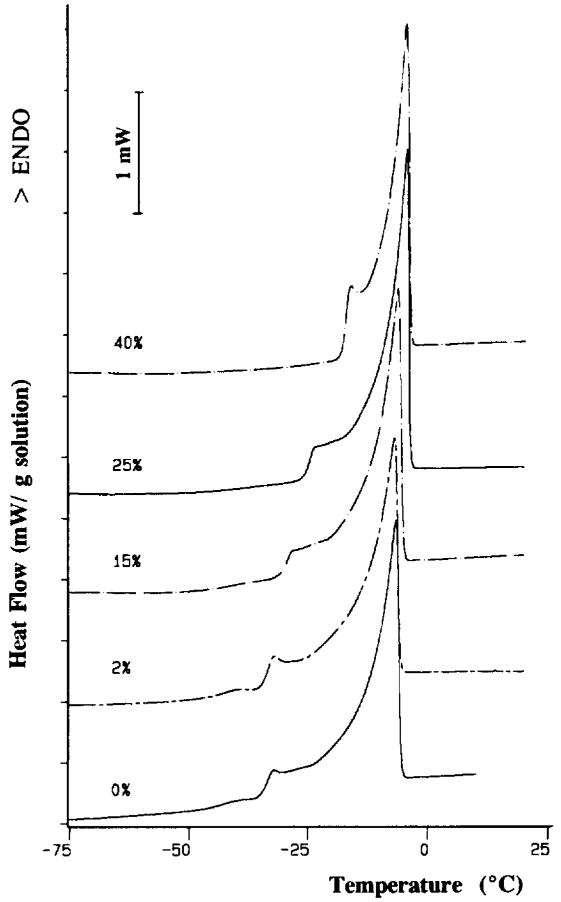

Fig. 5. Resulting heating thermograms following the optimised annealing treatment for sucrose+dextran solutions (dextran concentration 0–40%; total dry matter 50% w/w).

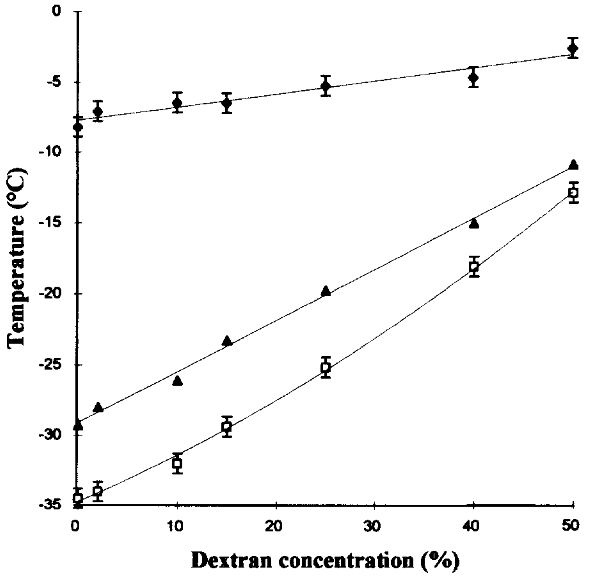

Fig. 6. Comparison of the different transition temperatures (T_m and T_{g_2}) with the maximum temperature determined to obtain the efficient annealing treatment. ◆, T_m; ▲, $T_{annealing}$; □, T_{g_2}.

temperatures appears as the broadening of the glass transition. The temperature onset that should correspond to the onset of the molecular mobility remains constant (Simatos, Blond, & Martin, 1995); the heterogeneous composition could increase the dispersion of the relaxation times (Senapati & Angell, 1991).

4. Discussion

The increasing viscosity of the solutions does not allow a complete freezing during the conventional DSC cycle [Fig. 3(1)]. In these conditions the cooling thermogram for the 50% dextran solution only shows a small exothermic peak. This indicates that the freezing is incomplete during the cooling step, the cooling rate being too high compared with the crystallisation kinetic. Some water crystallisation occurs during the rewarming, an exothermic peak can then be observed just before the ice melting endotherm [Fig. 3(2) and (3)]. Similar results have been described for the PVP–water system (Mackensie & Rasmussen, 1972) and hydrogels (Murase, 1993).

If, during the cooling step, the glass transition temperature of the unfrozen fraction is reached before the maximum freeze-concentration, the glassy state kinetically prevents a complete crystallisation of the freezable water. To further ice crystallisation, the product temperature must be raised above the glass transition temperature of its unfrozen fraction.

During the heating step, the apparent multiple character of the 'melting' peak in DSC scans is then due to the occurrence of this devitrification process, the first small peak (**p**) actually corresponds in its first part to the glass transition of the unfrozen fraction, then to the exothermic devitrification of a part of the still liquid water. On the thermogram obtained after the thermal treatment [Figs. 3(4) and 4(3)] the heat capacity increase revealing the glass transition appears at a higher temperature. In incompletely frozen materials, the glass transition appears at a lower temperature than in a maximally freeze-concentrated system, owing to the lower concentration of the amorphous fraction.

The incompleteness of the freezing process is important with highly concentrated polymer systems compared with small molecule solutions, because of the viscosity and also of the mechanic hindrance due to the entanglement of the large molecules. The freeze-concentration allows the solution to reach the critical concentration of the polymer at which the viscosity abruptly increases (Morris, 1989).

We can deduce from our experiments that the sample must be heated up to a few degrees above the temperature of the minimum between the 'two' peaks, i.e. just above the temperature at which the crystallisation can be considered as complete; then the sample is cooled until 20–30°C below the expected T_g' range. The second heating scan obtained is considered as corresponding to that of the maximally freeze-concentrated sample; in these conditions, the glass transition yields the well-identified shape with an enthalpy relaxation peak and at a temperature independent of the initial concentration. For unknown samples, a first essay is necessary.

Isothermal annealing treatments were proposed (Ablett et al., 1992; Roos & Karel, 1991), i.e. the holding of the sample for some time at a given temperature. But the choice of this temperature is difficult: at a too high temperature a fraction of the ice melts, diluting the unfrozen medium; if it is too low, i.e. below T_g', a holding time is inefficient because the amorphous unfrozen phase is a glass, and neither nucleation nor crystal growth are possible within the experimental time. In the latter case, an annealing treatment will only be useful to reach a temperature equilibrium; however, if long enough, it may work as physical ageing of the glass, modifying the glass transition shape as a result of increasing enthalpy relaxation (Blond, 1989).

The difficulty in choosing the optimum annealing temperature originates in the complex kinetic feature of the glass transition of the freeze-concentrated fraction, as observed by DSC, as compared with the glass transition of the same material without ice; it spreads over several degrees and, as indicated by the magnitude of its apparent ΔC_p which is much larger than expected (Blond & Simatos, 1991), the mobility due to the glass–rubber change cannot be separated from the large mobility due to the ice melting. We think that no work has yet allowed the accurate determination of the limit between the end of the glass transition of the unfrozen fraction and the beginning of the ice melting.

5. Conclusion

In conclusion, it is useful to make a few comments based on commonly accepted facts and also some prospective views.

T_g' is defined as the glass transition temperature of the maximally freeze-concentrated solution remaining outside the ice crystals. With highly concentrated and/or viscous systems, the ice crystallisation is slowed down; the presence of unfrozen water, which had been interpreted as an indication of bound water, is now widely recognised as the result of the non-equilibrium state. We have shown that annealing treatments are essential, particularly with highly concentrated solutions, when the objective is to obtain a maximally freeze-concentrated product to do comparative and accurate experiments.

Optimum annealing conditions should be obtained when the samples are held just above T_g', but this suggestion is only theoretical because it is difficult to differentiate the end of the glass transition from the beginning of the ice melting and then to determine the true value of T_g'.

The nucleation and crystal growth rates are limited by viscosity; according to Angell et al. (1995), the crystallisation would only be possible when the viscosity is below 10^4 Pas. It could then be envisaged to predict the optimum annealing temperature from the evolution of viscosity at temperatures above the glass transition. If crystallisation curves however are drawn in a state diagram (Chang & Baust, 1991; Hayes & Pegg, 1996; Luyet & Rasmussen, 1968), they are not parallel to the T_g curve and so the irruptive crystallisation does not appear as an isoviscosity feature if we assume the glass transition is.

The viscosity decrease, just above the glass transition, is dependent on the system properties and particularly on its fragility. The liquid fragility can be interpreted in terms of the resistance of the short-range order of the initially glassy structure to thermal disruption above T_g (Angell et al., 1995). An evaluation of the fragility of the concentrated solutions could perhaps give some information on the crystallisation ability during the annealing treatments.

Concentrations above 50% have not been studied here and certainly the choice of time and/or temperature for the thermal treatments should be even more critical.

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