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The primary ice phase field in the H_2O-NaCl-dimethyl sulphoxide ternary system

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The determination and application of H_2O–NaCl–cryoprotective agent phase diagram information appears to be a fruitful area for co-operative research between materials scientists and biologists. Dimethyl sulphoxide (DMSO) is a widely used cryoprotective agent, and in this present work the primary ice phase field in the H_2O–NaCl–DMSO system has been determined including the extension into this system of the lines of two-fold saturation from the ice–$NaCl \cdot 2H_2O$ and the ice–$DMSO \cdot 3H_2O$ binary eutectics. Liquidus surface data are presented for isoplethal sections taken through the ternary system along composition lines defined by weight ratios of DMSO to NaCl of 1/5, 1/2 and 9. Liquidus surface data obtained from an additional thirteen isoplethal sections are used to construct the complete isothermal projections of the primary ice phase field along 10° C contours. The distribution and occurrence of glassy phases in this ternary system are also discussed.

1. Introduction

Damage to biological materials during freezing and thawing has no single clearly defined cause. Two mechanisms of damage believed to be of primary importance are: (a) osmotic dehydration of cells due to increasing concentration of solutes, particularly NaCl, in the extracellular liquid as ice precipitates [1, 2]; and (b) cell rupture through the formation of intracellular ice [3, 4]. With respect to the first damage mechanism, Lovelock [1] found that a concentration of 4.4 wt% NaCl was toxic for red blood cells. Extracellular fluids, having a composition of approximately 0.85 wt% NaCl initially, reach this composition due to crystallization of ice when cooled to only −3° C.

Beginning with the work of Polge *et al.* [5] in 1949, the addition of cryoprotective compounds (CPC) to physiologic materials has been shown to reduce the damaging effects of freezing and thawing. The most commonly used cryoprotective compounds are dimethyl sulphoxide (DMSO) and glycerol. Such compounds reduce salt concentration during freezing by dilution of the residual liquid since ice has no solubility for either NaCl or the cryoprotective compounds. In order to predict optimal concentration of cryoprotective agents, it is essential that the ternary phase diagrams of H_2O–NaCl–CPC be known, and the determination and application of low temperature phase diagram information would seem to be a unique area for the co-operation of materials scientists and biologists.

There are at least two other instances where knowledge of such ternary phase diagrams is crucial for reducing freeze–thaw damage of biological materials. Silvares *et al.* [6] have extended the dehydration dynamics for biological cells as a function of cooling rate to account for changes in cell membrane permeability as a function of temperature and osmolarity of the extracellular solution. These calculations rely on information obtainable from phase diagrams of relevant systems. The formation and subsequent devitrification of glassy phases has been observed in many systems of interest to cryobiologists [7–10]. The study of such glassy phases is also dependent on phase diagram information and the degree of metastability of these glasses is a function of residual liquid composition. The metastability of these glasses may be high; that is, they may devitrify at relatively high temperatures. Devitrification is sometimes absent, however, for glasses formed in CPC-rich solutions, while those glasses formed in NaCl-rich solutions are generally unstable. The presence of a stable glass in the extracellular region is believed to enhance cryoprotection in two ways. The formation of the glass reduces the maximum salt concentration reached during freezing by reducing the total amount of ice formed. In addition, the amount of dehydration experienced by the biological material during freezing is also reduced.

Limited investigations of the H_2O–NaCl–DMSO ternary system have been made by Cocks and Brower [7] and by Rasmussen *et al.* [8]. The effects of physiological support media on this system have also been studied [9]. The present work extends these studies to include the entire primary ice phase field. A previous paper has described similar work on the ternary system H_2O–NaCl–glycerol [10].

2. Experimental

Equilibrium and non-equilibrium phase transformations in the H_2O–NaCl–DMSO ternary system were studied by differential thermal analysis (DTA) techniques [7, 11]. Using these methods, liquidus, eutectic, glass transition and devitrification (crystallization) temperature were determined.

Differential thermal analysis is a technique that measures temperature differences between a sample and a thermally inert reference material as the temperature is changed at a linear rate. Investigations were made using a Dupont 900 Thermal Analyzer with rapid cool attachment. Ternary solutions were made from distilled water, certified ACS NaCl, and certified ACS DMSO. Solutions were prepared with constant weight ratios of DMSO to NaCl and varying water contents. Constituents were weighed to one part in ten thousand and compositions calculated to the nearest tenth of 1 wt %. The DTA samples consisted of 0.02 ml of solution dispersed over thermally inert glass beads in a 4 mm diameter glass tube.

Interrupted heating experiments were used to verify the presence of metastable phases in samples that exhibited glass transitions. This technique involves quenching the sample from a temperature between the devitrification and liquidus temperatures, followed by a second heating [7]. The absence of both the glass transition and the devitrification peak during the second heating verifies the metastable nature of such phases.

Ice, the first solid phase to crystallize from physiologic solutions, has been shown to have little or no solid solubility for solute materials, presumably because of its hydrogen-bonding characteristics [12]. Precipitation of ice continues from the onset of freezing until the composition of the residual liquid phase reaches a line of two-fold saturation. Thus, the crystallization paths of solutions whose overall compositions lie in the primary ice phase field, will extend from pure H_2O into the ternary system along a line of constant ratio of the other two components. Such ratio lines, R, can be defined by the weight ratio of DMSO to NaCl; thus ternary compositions can be specified by the notation $R = A + B\%\ H_2O$. For example, the ternary composition, 10% DMSO–5% NaCl–85% H_2O can be expressed as $R = 2 + 85\%$ H_2O with $R\,(=2)$ equal to the ratio of the weight percentage of DMSO to that of NaCl.

The composition of extracellular physiological solutions is normally taken to be 99 wt % H_2O, 0.87 wt % NaCl, and 0.13 wt % proteins and other biological materials. Hence, the portion of the H_2O–NaCl–DMSO phase diagram of primary

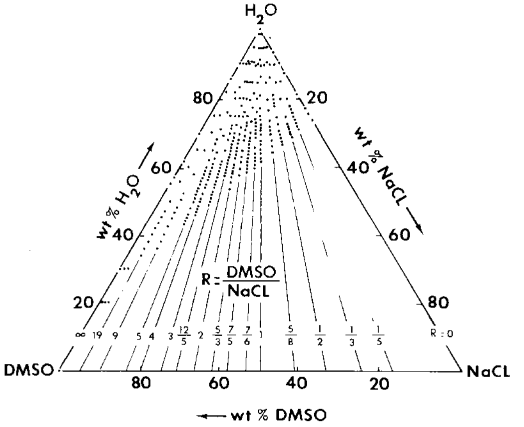

Figure 1 Compositions investigated in the H_2O–NaCl–DMSO ternary system. Compositions were made for specific ratios, R, of DMSO to NaCl.

interest to the cryobiologist is the water-rich corner of the ternary system. Fig. 1 shows the compositions investigated to establish both constant ratio isoplethal sections, and ultimately the liquidus surface for the water rich corner of the H_2O–NaCl–DMSO ternary phase diagram.

3. Results and discussion

Water-rich solutions in this system exhibit both exothermic and endothermic peaks when subjected to differential thermal analysis. Figs. 2 to 4 show composites of the thermograms obtained for solutions of various water content lying on the $R = 1/5$, $1/2$, and 9 ratio lines, respectively. Examples of all of the various thermal transformations observed are contained in these composites. The thermal features observed during freezing and thawing such solutions are labelled in Figs. 2 and 3. Equilibrium melting at the liquidus surface is shown by an endothermic peak, T, while melting associated with a line of two-fold saturation, i.e. that line of solution compositions and temperature along which a liquid is in equilibrium with two

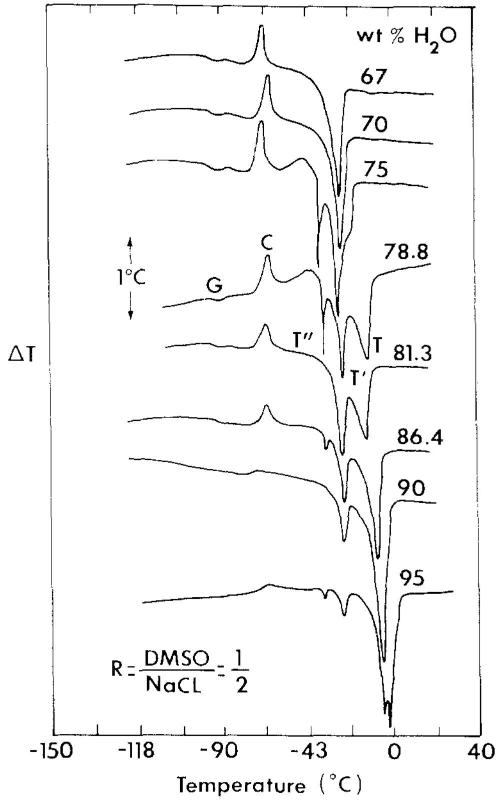

Figure 3 Composite thermograms for the DMSO/NaCl ratio, $R = 1/2$.

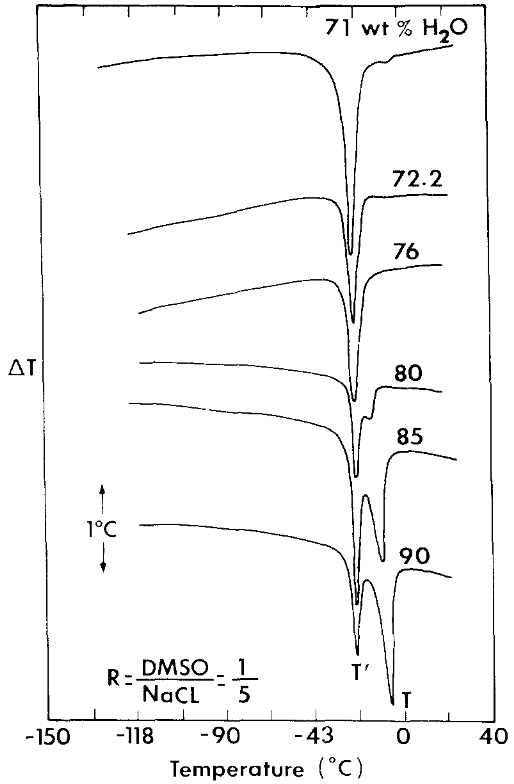

Figure 2 Composite thermograms for the DMSO/NaCl ratio, $R = 1/5$.

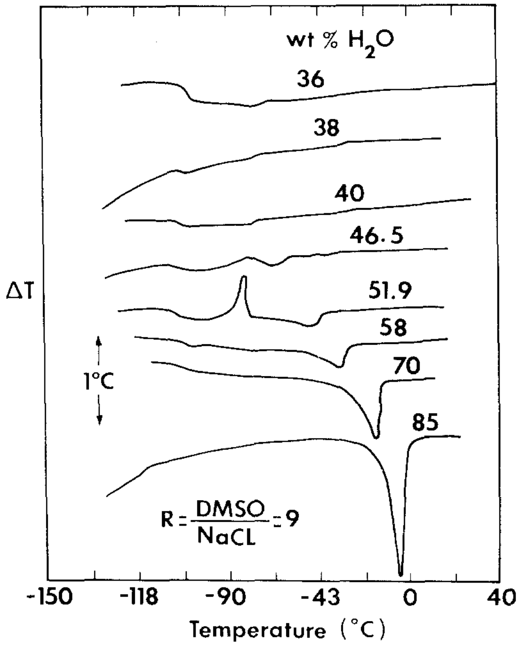

Figure 4 Composite thermograms for the DMSO/NaCl ratio, $R = 9$.

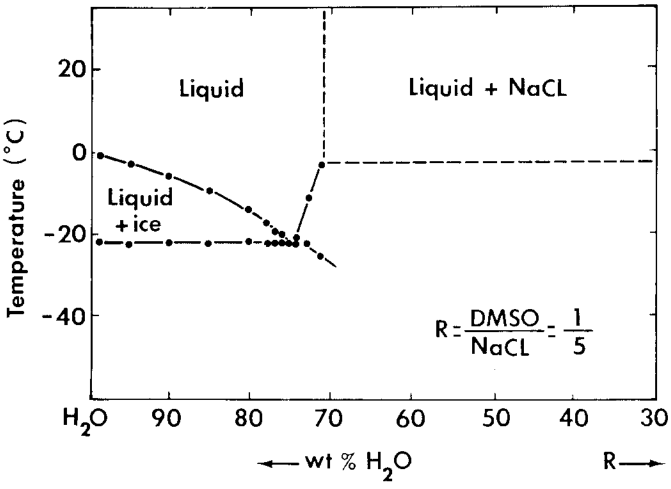

Figure 5 $R = 1/5$ isoplethal section. ●, equilibrium melting.

solids, is labelled T′. Some thermograms exhibited a third endothermic peak, T″, believed to be associated with a ternary eutectic reaction. The transition temperature for metastable glasses formed in many of these solutions is identified as G, and the temperature at which these glasses devitrify, i.e., crystallize with the concomitant formation of ice on heating, is marked C.

In accordance with the lever rule, it should be noted that the fraction of liquid present, whose composition is indicated by the line of two-fold solution, increases with decreasing water content for solutions whose compositions lie in the primary ice phase field. This is shown by an increasing area enclosed by the endothermic line of two-fold saturation peaks, T′, with decreasing water content. Correspondingly, the areas enclosed by both devitrification and liquidus melting peaks are proportional to the fraction of material transformed [9]. The fraction of primary ice decreases with decreasing water content while the amount of glass undergoing devitrification increases.

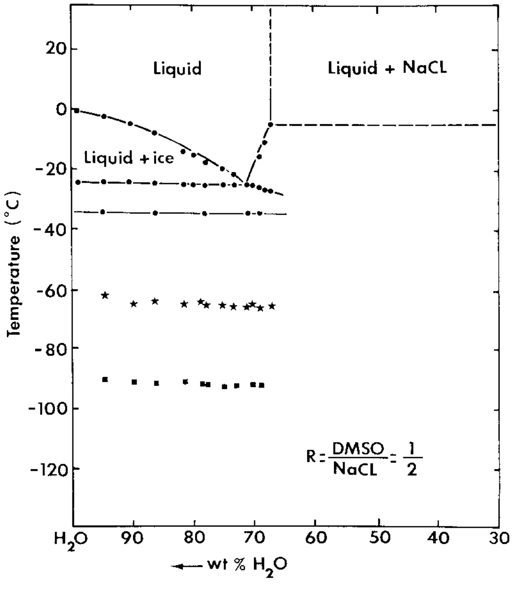

Figure 6 $R = 1/2$ isoplethal section. ●, Equilibrium melting; ■, glass transition temperatures; ★, recrystallization temperatures.

An isoplethal section of a ternary phase diagram is a vertical section for which the ratio of two of the components is constant through the composition–temperature triangular prism. Such sections are useful for illustrating the phase relationships indicated by the thermal features on DTA thermograms.

Isoplethal sections for the ratio lines 1/5, 1/2, and 9 are shown in Figs. 5 to 7, respectively. The solidification behaviour of solutions whose weight ratio of DMSO to NaCl is 1/5 is similar to H_2O–NaCl binary behaviour. The eutectic-like line of two-fold saturation bounding the primary ice phase field is intersected by the isoplethal section at –22° C and a water content of 75 wt%. By comparison, the corresponding point in the H_2O–NaCl binary system occurs at –21.2° C and 78 wt% H_2O. Between 72 and 75 wt% H_2O, primary solidification of $NaCl \cdot 2H_2O$ occurs while, as may be seen, NaCl is the first solid phase to form for initial water contents less than 72 wt%.

Increasing the ratio of DMSO/NaCl from 1/5 to 1/2 significantly changes the solidification behaviour. The intersection of the liquidus curve with the line of two-fold saturation has shifted only slightly, but a third endothermic reaction, indicated by T″ in Fig. 3, is present at several composi-

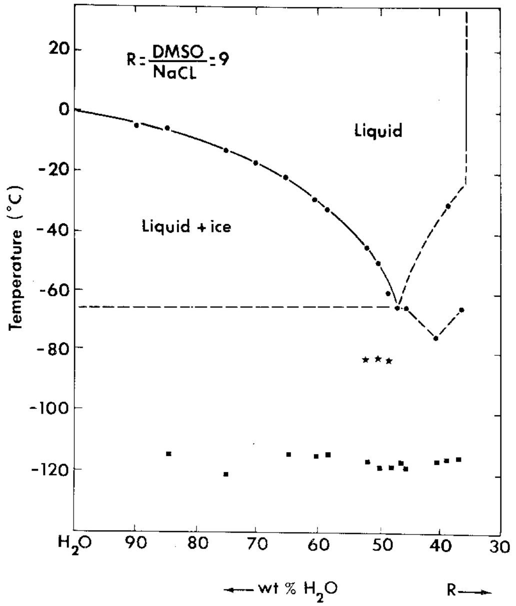

Figure 7 $R = 9$ isoplethal section. ● Equilibrium melting; ▪, glass transition temperatures; ★, recrystallization temperatures.

tions. This thermal feature is believed to indicate ternary eutectic solidification because of its existence in other isoplethal sections, 1/3 and 5/7, at the same temperature, −34.5° C. The absence of T'' behaviour in some solutions is related to degree of metastability of glassy phases formed in those solutions.

Beginning with a DMSO/NaCl ratio of 1/3 and continuing to $R = \infty$, most of the ternary solutions lying in the primary ice phase field formed non-equilibrium glassy phases during initial solidification. The glass transition and devitrification temperatures of the glasses in the $R = 1/2$ isoplethal section were found to be independent of the original water content (Fig. 6). This suggests that these glasses are all similar in nature, probably forming from the two-fold or three-fold saturated solutions residual to primary ice precipitation. A comparison of Figs. 3 and 6 shows that the absence of an endothermic peak at T'' is generally correlated with either a diminished devitrification peak or the absence of such a peak.

An isoplethal section for $R = 9$ constructed from the thermal data given in Fig. 4 is shown in Fig. 7. The most notable difference between the thermal behaviour of these DMSO-rich solutions and those previously discussed is the absence of any solidification at a line of two-fold saturation. This behaviour is probably related to the high degree of metastability of the glasses formed in these solutions. Since the thermograms shown in Fig.4 were determined during heating, the absence of both a devitrification peak and a eutectic melting peak suggest that the relatively stable glasses form from the liquid which remains after primary ice precipitation.

A projection of the H_2O–NaCl–DMSO liquidus surface obtained from DTA measurements of all solutions shown in Fig. 1 onto a ternary composition triangle for water rich solutions is shown in Fig. 8. As may be seen, isothermal contour lines at10° C intervals down to −60° C are shown. The isothermal contours at −10° C and −20° C show anomalous behaviour in the vicinity of a ratio of DMSO/NaCl of one. This anomalous behaviour is also present in the line of two-fold saturation. The DTA data suggest that the point of 58 wt% H_2O, 13 wt% NaCl, and 28 wt% DMSO represents a ternary invariant reaction at −34.5° C. The difficulty in determining the exact nature of this reaction lies with the high propensity for glass formations in this region of the system. The addition of DMSO to brine solutions retards both two-phase and three-phase crystallization, and the glassy phases which form exhibit a variation even in their ability to devitrify on heating (cf. Fig. 3). Other investigators [8–13] have conjectured that a low temperature ternary reaction occurs for high values of R and low water contents. As in the present work, they also were unable to determine the exact nature of the reaction due to the formation of glassy phases.

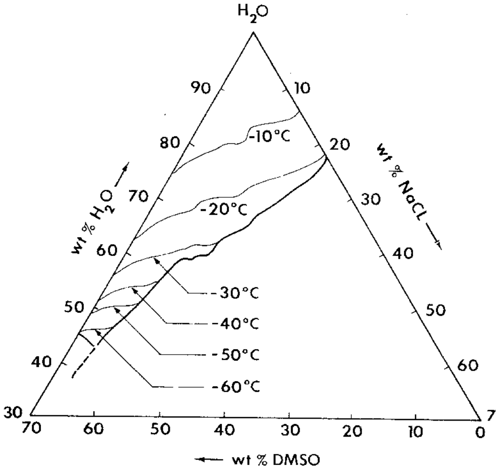

Figure 8 Projection onto the composition plane of the liquidus surface for the primary ice phase field in the ternary system H_2O–DMSO–NaCl.

Recent studies have shown that the formation of stable glassy phases in ternary solutions based on an aqueous medium and containing both NaCl and cryoprotective compound may enhance the viability of cells frozen in those solutions [4]. The presence of the glass will reduce the concentration level reached by NaCl in the extracellular space due to the absence of eutectic ice precipitation. If the salt concentration has not already reached a lethal level when glass formation occurs, the cells will be suspended in a mixture of primary ice and an innocuous glass. Based on these studies, it appears that at least some of the known benefits of DMSO as a cryoprotective compound may be to a large extent be related to its high propensity to form glasses.

4. Conclusions.

Addition of the cryoprotective compound DMSO to binary solutions of H_2O–NaCl depresses the temperature at which primary ice first appears during solidification. Depression of the binary H_2O–$NaCl \cdot 2H_2O$ eutectic point occurs smoothly along a line of two-fold saturation until a region containing approximately 58 wt% H_2O and 28 wt % DMSO is reached. In this region anomalous behaviour believed to be associated with the presence of a ternary invariant reaction exists. The exact nature of this reaction could not be determined because of complications arising out of a stable glass formation. Beyond this region the line of two-fold saturation bounding the primary ice phase field continues to fall until it intersects a line emanating from the ice + $DMSO \cdot 3H_2O$ binary eutectic point. This intersection lies at approximately 43 wt% H_2O, 54 wt% DMSO, and 3 wt% NaCl and at a temperature of approximately –70° C. Frequently solutions having a DMSO/NaCl weight ratio greater than 1/3 generally results in the formation of glassy phases, which may devitrify on warming. The availability of this ternary phase diagram information is expected to be of use in the planning and the interpretation of cryobiological experiments in which DMSO is used as a cryoprotective agent.

Acknowledgement

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