Solid–Liquid Phase Transitions and Amorphous States in Ternary Sucrose–Glycine–Water Systems

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The solid–liquid phase behaviour of binary and ternary mixtures containing water, sucrose and glycine has been studied by differential scanning calorimetry. In addition to defining the liquidus and solidus coexistence boundaries, the studies have been extended to the measurement of glass transitions of amorphous binary and ternary mixtures under conditions where phase separation is not spontaneous.

An estimate of the shape of the stable glass surface in the composition–temperature diagram has been made by combining the experimental results with semi-empirical treatments of glass–rubber transitions. The importance of such information for the formulation of mixtures to be freeze-dried is discussed.

During recent years a general realisation has emerged of the importance of water-soluble or water-sensitive amorphous solids in several branches of technology, particularly in the processing of foods[1] and bioproducts, *e.g.* isolated proteins.[2,3] Emphasis is placed on systems of low moisture content (<10% w/w) usually with the aim of achieving shelf-stability in products that have, conventionally, been classified as unstable. In practice, the starting material is often a dilute solution and the removal of >99% of the water present is effected by freezing or evaporation of pure water as ice or vapour, respectively, followed by diffusion and desorption of residual moisture from the interior of the solid residue.[2–4]

The systems under consideration are usually of a multi-component type, with some solutes (*e.g.* salts) able to form eutectic mixtures with ice and with one another,[5,6] but with other solutes (*e.g.* carbohydrates) tending to supersaturation, giving rise to solid solutions which undergo glass transitions. The actual behaviour of such mixtures during concentration by freezing or evaporation is therefore likely to be complex but of great practical importance.

The solid–liquid equilibrium phase behaviour of binary aqueous mixtures subjected to freezing has been investigated in fair detail, and compilations of eutectic data are available for many salt–ice systems. The actual extent of eutectic-phase separation is governed by the rates of nucleation and growth of the crystalline solute in the concentrated solution phase, before the steeply increasing viscosity effectively inhibits crystal growth, especially as the glass transition is approached. Murase and Franks have studied the crystallisation of sodium and potassium phosphates from binary and ternary solutions during freezing.[5] They found that the degree of salt crystallisation depends on the initial solution concentration, that some salts readily crystallise at the eutectic temperature, while others always form supersaturated solutions, and that in ternary phosphate mixtures one salt will inhibit the crystallisation of the other. This has important implications in the use of such salts as pH buffers. More recently, Chan and Randall have classified salts according to their actual phase behaviour during freezing.[6] They distinguish between salts which readily crystallise during freezing (*e.g.* Na₂SO₄), those which partially crystallise (*e.g.* CaCl₂) and those which preferentially give rise to amorphous phases (*e.g.* CH₃COONa).

Solutes that do not crystallise easily from frozen aqueous solutions are commonly employed for this very reason as aids to freeze-drying.[3,4] The thermomechanical behaviour of such solutions during the freezing process has received increasing attention during the past decade.[7] Since eutectic phase separation does not take place spontaneously, the solution becomes supersaturated and thus thermodynamically unstable. Eventually a temperature and concentration is reached at which the mixture undergoes the glass transition. The correct experimental identification of this point on a temperature–composition diagram is still subject to some debate,[4,6,8] but there is general agreement that a range of water soluble solutes, *e.g.* sugars, exhibit typical glass–rubber transitions, in their pure states,[7] as blends[9] or as supersaturated, frozen aqueous solutions.[3,7,8]

Binary systems consisting of one crystallising component (water) and one non-crystallising component, usually sucrose, have been studied in detail,[7,8,10,11] but only scant information is available for ternary mixtures containing two crystallisable components (salt, water). Detailed reports are available for aqueous mixtures of NaCl and glycerol or hydroxyethyl starch.[12] They illustrate the complex phase behaviour of such systems. The present investigation of the water–sucrose–glycine system was initiated because of its fundamental role in the design of effective freeze-drying processes, particularly for labile products that are prone to losses of biological activity during such treatments.[2,4] While our work was in progress, a report was published with some details of the solid–liquid equilibrium phase behaviour of this ternary system.[13] In their concluding paragraph, the authors emphasise the need for information on metastable states which, together with phase equilibria '… is needed to develop a scientifically based technology for freeze-drying of biological materials.' It is the aim of our investigation to provide such information, in continuing our earlier studies of glass transitions of carbohydrates.[9]

Experimental

Sucrose and glycine (β-C₂H₅O₂N) were of Sigma analytical grade. The crystalline chemicals were used without further purification. Distilled water was used for all experimental work.

The melting points and latent heats of the pure materials and their molecular weights used for the calculations in this work are listed in Table 1. It was noted that pure glycine decomposes at its melting point.

The Perkin Elmer DSC-2 instrument used in this work was fitted with Auto-scanning Zero and sub-ambient temperature accessories. The DARES data handling system was used for monitoring and processing heat flow–time curves.[14] The

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Table 1 Physical properties of materials

	sucrose	glycine	water
molecular weight	342.3	75.7	18.0
melting temperature/K	458	535	273.2
latent heat of fusion/J mol^{-1}	—	3500[a]	5980
glass transition/K	343	280	134
ΔC_p glass–rubber /J mol^{-1} K^{-1}	107	235[a]	35

[a] Fitted data, see text.

scanning rate for cooling and heating was 5 K min^{-1} for all experiments.

Sample Preparation and Differential Scanning Calorimetry (DSC)

For glycine–water mixtures, two different methods were used to prepare solutions, because of the limited solubility of glycine (20% w/w at 25 °C). To prepare dilute solutions, (<20% w/w glycine), the components were weighed and mixed at room temperature. Each solution was transferred into an aluminium DSC pan, and sealed hermetically. Sample weights were 8–17 mg. The pan was then placed in the DSC oven (preheated to 300 K) and cooled to 240 K. After 3 min, the sample was rewarmed to 300 K. For higher concentrations the solid glycine and water were weighed into a stainless-steel DSC pan. After sealing the pan, it was placed in the DSC. The sample was heated and the heat flow monitored. This scan was defined as a 'first scan'. When the heat flow curve indicated complete dissolution of glycine, the cooling cycle to 240 K was started. After cooling, the rewarming scan was carried out as a 'second scan'.

For the study of sucrose–glycine binary systems, two types of preparation were used: freeze-dried cakes of various compositions and mixtures of powdered crystalline sucrose and glycine. Freeze-dried cakes were prepared from 25–28% w/w solutions of different solute compositions, i.e. different ratios glycine : sucrose. All freeze-drying conditions (freezing, ice sublimation and secondary drying) were carefully controlled.[3] The solutions were frozen in 5 ml vials at a cooling rate of −45 K h^{-1}. The maximally frozen solutions were dried at a pressure of 1.3 N m^{-2} for 2 days at 233 K, followed by 3 days at 313 K. A portion of each cake (8–10 mg) was transferred into a stainless-steel DSC pan, and sealed. All these operations were carried out in an atmosphere of dry nitrogen. The sample was then subjected to cooling and heating in the DSC, as described above. Other samples were prepared by mixing the preweighed crystalline sucrose and glycine in a stainless-steel DSC pan. The samples were sealed under a nitrogen atmosphere, after flushing with dry nitrogen. Sample weights were 25–60 mg. The sequence of DSC scans was the same as for the concentrated samples of glycine–water mixtures, described above.

For ternary systems sucrose–glycine–water, solutions with three different weight ratios of glycine : sucrose were used, though the total solid contents were almost identical (25–28% w/w). The DSC measurements were performed as for the dilute glycine solutions.

Results and Discussion

Glycine–Water Binary Systems

Fig. 1 shows some representative DSC heating scans of previously frozen, dilute glycine solutions. Where a eutectic mixture of water and glycine exists, the power–time curve
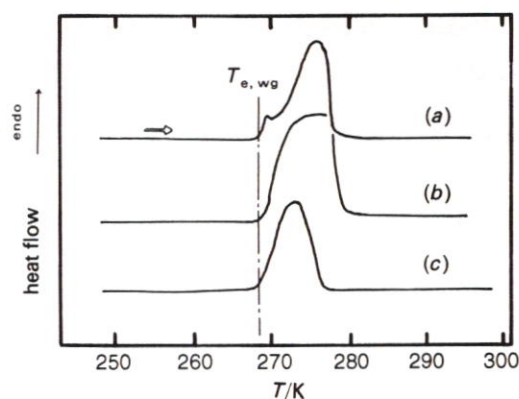

Fig. 1 DSC heating power–time curves of frozen glycine solutions (a) 0.9%, (b) 4.4% and (c) 20.0%. Cooling and heating rate 5.0 K min^{-1}. Sample weight 8–17 mg. The $T_{e,wg}$ line shows the estimated eutectic temperature.

should exhibit two endothermic peaks, unless the composition corresponds to the eutectic. Since two separable endotherms could be identified in the 0.9% w/w power–time curve, it was concluded that this system forms a eutectic mixture. The small peak corresponds to the eutectic melt and the large peak to the melting of ice. On the other hand, the 4.4% and 20% solutions are close enough to the eutectic composition that the ice-melting peaks disguise the eutectic melt. This is also supported by the fact that the extrapolated onset temperature $T_{e,wg}$ of the small peak for the 0.9% w/w solution coincides with those of peaks for the other solutions, since the eutectic temperature is independent of the solution concentration.

The eutectic temperature of the glycine–water system was therefore determined as 268.5 K, from the extrapolated onset temperature $T_{e,wg}$. Further, it was noted that none of the DSC curves for dilute solutions showed evidence of a glass transition, at least not within the measured temperature range.

Fig. 2 shows some 'second' heating scans for concentrated glycine–water mixtures. All 'first' heating runs (not shown) displayed only one broad endotherm at high temperatures, corresponding to the dissolution of glycine. Again, no glass transitions were observed.

All 'second' heating scans show the same complex pattern. Since glycine is known to exhibit polymorphism,[15] this pattern can be interpreted as follows: The eutectic mixture
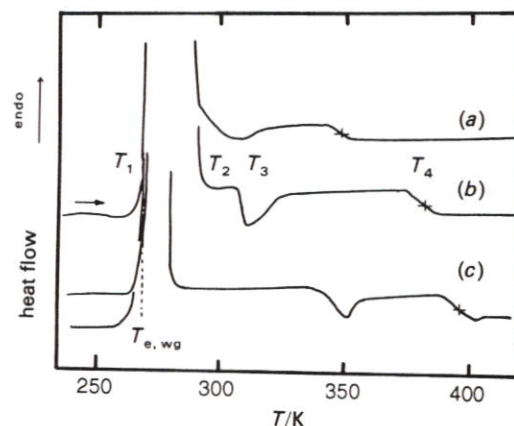

Fig. 2 'Second' DSC heating power–time curves of concentrated glycine solutions (a) 27%, (b) 37% and (c) 45%. Cooling and heating rate 5.0 K min^{-1}. Sample weight 45–80 mg. T_1 eutectic temperature ice–β-glycine, T_2 = dissolution of β-glycine, T_3 = crystallisation of α-glycine. T_4 = dissolution of α-glycine.

melts at the eutectic temperature T_1, and the β-glycine crystals which separate from frozen solutions, dissolve at T_2. After that, α-glycine crystals (the stable form) recrystallise continuously at T_3, and finally at T_4 α-glycine dissolves. Although it was difficult to determine the dissolution temperatures exactly, we could take the temperatures, marked by crosses in Fig. 2, as the dissolution temperature T_4 of α-glycine. As expected, T_4 increases as the glycine concentration increases.

The exact estimation of the eutectic point from the power–time curves was also difficult. The broken line which indicates the eutectic temperature $T_{e, wg}$ obtained from Fig. 1 is shown in Fig. 2 as a reference.

The relationship between T_4, T_1 and glycine concentration is plotted in Fig. 3. In order to establish the complete phase diagram, we applied the usual equations for solubility, derived from a balance of chemical potentials between the pure solid and the same component in solution.

For a simple binary system, if the melting heats of the pure materials and the activity coefficients are known, the melting point depressions of each component, i.e. the mutual solubilities, can be estimated by the following relationships:

For a depression of the freezing point of water:

$$-\ln(1 - X_g)Y_w = (\Delta h_w^\circ / R)(1/T_w - 1/T_{m, w}^\circ) \qquad (1)$$

where X_g is the mol fraction of glycine, Y_w the activity coefficient of water, Δh_w^0 the latent heat of fusion of ice, 5980 J mol^{-1}, $T_{m, w}^\circ$ the melting temperature of ice, 273.15 K, and T_w is the actual melting temperature of the solution.

Similarly, for glycine:

$$-\ln X_g Y_g = (\Delta h_g^\circ / R)(1/T_g - 1/T_{m, g}^\circ) \qquad (2)$$

where, Y_g is the activity coefficient of glycine, Δh_g° the melting heat of pure α-glycine, $T_{m, g}^\circ$ its melting temperature, 535 K, and T_g is the actual melting temperature, depressed by water.

Activity data for glycine and water in aqueous solutions are available only over a very limited concentration range (up to 3 mol kg^{-1} at 25 °C), and no information exists about Δh_g° for α-glycine, because of its instability at the melting point. We therefore set Y_w and Y_g constant, equal to unity, and search for the best fit to the experimental data, treating Δh_g° as an adjustable parameter. With $\Delta h_g^\circ = 3600$ J mol^{-1}, the best fit was attained. The result is shown as a solid line in Fig. 3. It seems to be a good approximation for the whole solidus curve. The point at which eqn. (1) and (2) intersect is the eutectic point. Its composition and temperature were 11.4% w/w and 235 K, respectively. Recently Shalaev et al. have reported eutectic data for the glycine–water system as 11.34% w/w and $T_{e, wg} = 269.6$ K.[13] The composition agrees

very closely with our calculated result; however, their reported temperature differs substantially from our calculated value. We have the direct result from the DSC curve in Fig. 1, which indicates a eutectic temperature of 268.5 K. Judging from the available data, the eutectic temperature seems to be ca. 269 K. The large discrepancy of our calculated result is likely to be due to setting the activity coefficients as equal to unity. As regards the correct value for the (hypothetical) melting heat of glycine, Δh_g°, the decomposition of glycine makes its determination problematical.

Eqn. (1) and (2), as written, imply that the latent heats are independent of temperature. To include a correction for heat capacity effects would require temperature measurements of a higher degree of accuracy than could be achieved by DSC. Additionally, no information is available regarding heats and heat capacities of dilution even for the aqueous solutions, let alone sucrose–glycine mixtures.

Glycine–Sucrose Binary Systems

Fig. 4 shows representative DSC heating traces for a mixture of solid glycine and sucrose and for freeze-dried cakes. In particular, corresponding 'first' and 'second' heating runs are shown for the crystalline mixture. 'First' scans for every composition clearly showed the melting peak above 450 K, and also indicated a minor eutectic peak at ca. 417 K. During the cooling run, following the 'first' heating, no exotherm due to crystallisation could be detected, indicating that the homogeneous mixture had been quenched from the liquid to the glass state. Glass transitions, shown in Fig. 4, were detected in all 'second' heating curves. As expected, the glass-transition temperature, θ, depends on the composition, decreasing with increasing ratio of glycine : sucrose. On the other hand, freeze-dried samples gave typical DSC scans showing glass, recrystallisation and melting transitions. The θ values of freeze-dried samples also depended on the glycine : sucrose ratios, but the dependence of the melting points of the devitrified samples on the composition was not clear because of the difficulty encountered in the determination of the exact melting points from the broad peaks. The relationships between θ and the composition of fused mixtures and freeze-dried samples are shown in Fig. 5. It was found that θ values of freeze-dried samples are consistently lower than those of previously fused mixtures.
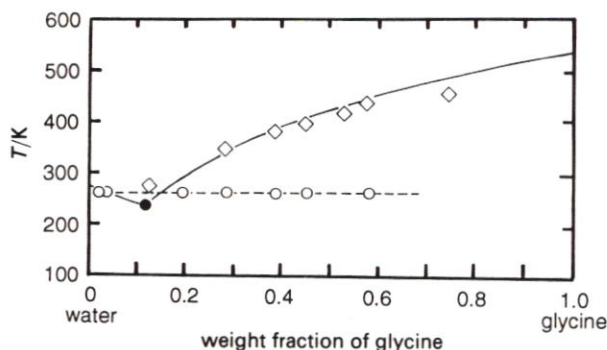

Fig. 3 Phase diagram of the glycine–water binary system. ($\diamond$) and ($\bigcirc$) are the experimental dissolution temperatures of glycine and the eutectic temperatures at various concentrations, respectively. The solid line shows the calculated temperatures, using eqn. (1) and (2), ($\bullet$) the calculated eutectic point.
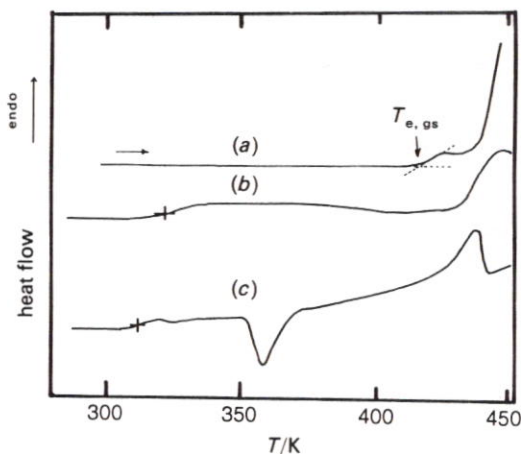

Fig. 4 Typical DSC heating power–time curves of the binary sucrose–glycine blends. (a) 'First', (b) 'second' heating scan of the fused crystal mixture (glycine 3.4% w/w), and (c) 'first' scan of the freeze-dried cake from a sucrose–glycine solution (glycine 1.6% w/w of total solid weight). Heating rate 5.0 K min^{-1}. (+) θ, the glass transition; $T_{e, gs}$ is the eutectic point of the sucrose–glycine system.
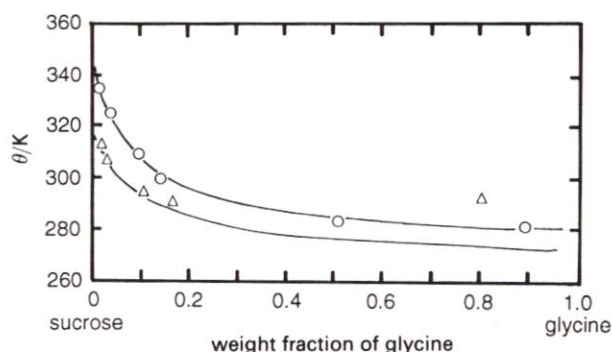
Fig. 5 Glass-transition temperature, θ, vs. glycine weight fraction in sucrose–glycine blends. ($\bigcirc$) Mixtures of glycine and sucrose crystals, ($\triangle$) freeze-dried cakes from aqueous solutions. Solid lines show the calculated data, using eqn. (3).

The prediction of glass-transition temperatures from the properties of the pure components and the mixture composition is uncertain, because the very nature of the glass transition itself is not yet fully understood.[16] Nevertheless, it is useful to describe θ of binary or ternary systems by an equation, even if of an empirical nature. Eqn. (3) is commonly used for different types of binary systems, such as polymer blends and polymer-diluent systems.[17–19]

$$\theta = \left[\sum_{i=1}^{n} X_i \Delta C_{pi} \theta_i \right] \bigg/ \left[\sum_{i=1}^{n} X_i \Delta C_{pi} \right] \qquad (3)$$

where X_i is the mol fraction, θ_i the glass-transition temperature, and ΔC_{pi} is the incremental change in isobaric heat capacity of the pure component i at its respective transition, and θ is the glass transition temperature of the mixture. This equation was derived from a consideration of the entropy of mixing,[17,18] and is based on a classical thermodynamic approach. It is argued that a glass transition resembles a second-order transition, despite its underlying kinetic origin. A useful feature of eqn. (3) is that DSC measurements are able to yield all the quantities in this equation.

In order to apply eqn. (3) to the sucrose–glycine binary system, the values of both θ_i and ΔC_{pi} for sucrose and glycine are required. Literature data for pure sucrose are included in Table 1. We obtained the values, $\theta_s = 343$ K and $\Delta C_{ps} = 167$ J mol^{-1} K^{-1} by an extrapolation, to 100% w/w sucrose, of our own experimental data for the binary mixtures. ΔC_p is actually the heat capacity increment at θ for a mixture containing 1.6% w/w glycine. We regard this as almost equal to the heat capacity increment of pure sucrose. Of the published ΔC_p values we regard 107 J mol^{-1} K^{-1} as the most reliable, because it is based on absolute $C_p(T)$ determinations for the rubber and glass states.[9] On the other hand, although θ of pure glycine was determined as 280 K in a similar manner, there are no reported data for ΔC_p for comparison. It was too difficult to determine the value from experimental data, since the measured heat capacity increment decreased rapidly, as the composition approached pure glycine. We therefore fitted eqn. (3), using the numerical values in Table 1, to the experimental data for the mixtures by treating ΔC_p of glycine as an adjustable parameter. The best fit was obtained by putting $\Delta C_p = 235$ J mol^{-1} K^{-1}, as shown in Fig. 5. The reliability of this value is, however, uncertain. It seems large compared to that of sucrose. Nevertheless, if the result is accepted as valid, then the discrepancy between glass-transition temperatures of the fused mixtures and the freeze-dried samples probably arises from residual moisture in the freeze-dried products. In practice, freeze-drying can rarely be performed to completion, so that the 'dry' preparation contains a few

per cent of residual moisture which would depress θ of the 'anhydrous' preparation.[19] There is also a possibility of sorption of water by the freeze-dried samples during the transfer to the DSC pan, even though the operation was performed under a nitrogen atmosphere. On the basis of this assumption, we treated freeze-dried samples as ternary systems of sucrose, glycine and water. If water was contained in the ternary system, then eqn. (3) can be employed to calculate its mol fraction.

θ_w and ΔC_p of pure water are known to be 134 K and 34.9 J mol^{-1} K^{-1} respectively,[20] based on measurements on vapour-deposited water (amorphous ice) at liquid nitrogen temperatures. Using the experimentally determined glass-transition temperatures and heat capacity increments of the other two components, it was possible to fit eqn. (3) to the θ data for freeze-dried samples, by regarding the moisture content X_3 as an adjustable parameter. The solid line in Fig. 5, corresponding to $X_3 = 0.4$, provided the best fit. This value converts to 4–8.5% w/w, depending on the solute composition, i.e. the ratio of glycine : sucrose. This range is considered reasonable, judging from our practical freeze-drying experience.

The above results suggest that θ of a ternary system can generally be predicted with a reasonable degree of accuracy from its composition with the aid of eqn. (3), especially for the limiting case where the concentration of one component (usually water) approaches zero.[19]

Sucrose–Glycine–Water Ternary Systems

When a sucrose solution is maximally freeze-concentrated, its glass transition θ' and the onset of ice melting occur at a temperature of 241 K which is independent of the initial solute concentration. Experimentally, θ' is detected as the discontinuity in the heat capacity observed in the DSC power–time curve of the frozen solution. In the present study the effect on θ' of the addition of glycine was investigated. Fig. 6 shows DSC heating scans for frozen solutions at three glycine : sucrose ratios. Definite glass transitions, corresponding to θ', were detected in every case. It was noted that θ' decreased with increasing ratio glycine : sucrose. This trend agrees qualitatively with that of θ in anhydrous sucrose–glycine binary systems.

By combining the known state diagram of the sucrose–water system[10,11] with the results obtained in this study, i.e. the liquid–solid equilibrium data for glycine–water systems, and the glass transition and equilibrium eutectic data of sucrose–glycine systems, we attempt to construct the state diagram of sucrose–glycine–water ternary systems, as shown in Fig. 7. Despite a lack of information for some parts of the
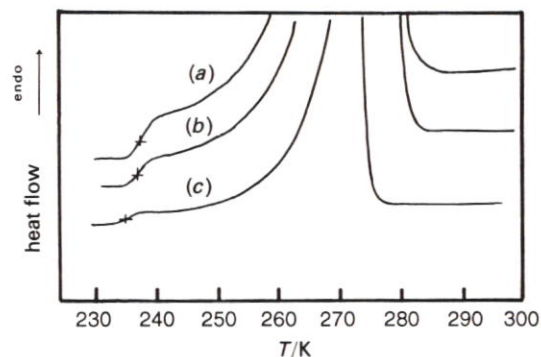

Fig. 6 DSC rewarming curves of frozen sucrose–glycine solutions. Solute concentrations for all curves are 25–28% w/w; the weight ratios glycine : sucrose are (a) 1 : 30, (b) 1 : 9 and (c) 1 : 5. Cooling and heating rate 5.0 K min^{-1}. (+) θ'.
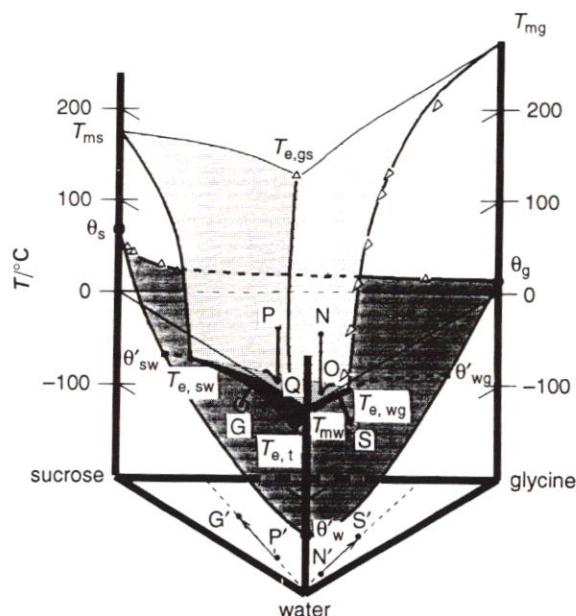
Fig. 7 State diagram of the sucrose–glycine–water ternary system. (△) data obtained in this study. $T_{m,s}$, $T_{m,w}$ and $T_{m,g}$ are the melting temperatures of pure sucrose, water and glycine. $T_{e,sw}$, $T_{e,wg}$ and $T_{e,gs}$ are the respective eutectic points of the binary systems, $T_{e,t}$ is the ternary eutectic point. $T_{e,wg}$ and $T_{e,gs}$ were reliably established in this study, but other eutectic points are uncertain. θ_s, θ_w and θ_g indicate the glass-transition temperatures of the pure materials. The data in ref. 11 and 20 were used for θ_s and θ_w. θ_g is the extrapolated value from our own data. A point θ'_{wg} is the intersection between the extrapolated liquidus curve and the sucrose–water glass-transition curve. Similarly, a point θ'_{wg} refers to the glycine–water system. The θ' values denote the limits of freeze concentration of the respective supersaturated mixtures; for details, see text and ref. 4, 7 and 8.

diagram, such as the liquidus curve of sucrose–glycine mixtures and the glass-transition curve of glycine–water mixtures, it is possible to construct a comprehensive diagram in Fig. 7. Our experimental data are shown by triangles.

It is demonstrated that a glass-transition surface, supported by the three glass-transition points of the pure components, i.e. θ_s, θ_w and θ_g, is formed, the shape of which is expressed by eqn. (3). When a solution of the composition and the temperature corresponding to the point P is cooled, it first reaches a point Q on the solid–liquid equilibrium surface, from where freeze-concentration progresses along the surface, maintaining a constant glycine : sucrose ratio. Freeze-concentration proceeds, with the state of the system passing through a $T_{e,t}$–$T_{e,sw}$ line, in a similar manner to the freezing process of a binary aqueous sucrose solution, and finally the glass-transition surface is reached at a point G which defines the maximum freeze concentration and the ternary glass-transition temperature θ', it being assumed that glycine remains in the amorphous phase. This process can also be traced along the isopleth of the solute composition on the projection. Thus, during the freeze-concentration of a ternary solution, the initial water content has no effect on the glass-transition point. On the other hand, the ratio of glycine : sucrose has an important effect. If the initial glycine : sucrose ratio is greater than that of ternary eutectic composition, the operational line on the projection becomes N'S'. Under these conditions it was difficult to maintain supersaturation all the way to the glass-transition surface, because of the tendency of glycine to crystallise.

It is admitted that Fig. 7 hardly lends itself to the evaluation of quantitative information on the phase behaviour of the ternary system. Such information could, in principle, be

derived from plots of the total solute concentration against transition temperatures,[9] from projections to isoplethal sections, or arithmetically, from the coefficients of the fitting equations. This latter device was employed by Pegg to evaluate melting and eutectic temperatures for water–glycerol–NaCl mixtures.[21] In the present investigation, however, the solid–liquid coexistence lines for the sucrose–glycine mixtures could not be measured, even though the binary eutectic temperature could be estimated from Fig. 4. Quantitative information can be extracted by solving the simultaneous eqn. (1) and (2) and/or eqn. (3) and using the parameters in Table 1.

The above findings have practical implications for the stabilisation of labile substances by freeze-drying. Both sucrose and glycine find application as stabilisers for isolated proteins. They can only fulfil such a role when present in the amorphous state, i.e. forming a homogeneous mixture (solid solution) with the substance to be stabilised. Furthermore, to render a product stable for long periods of time, the amorphous phase must have a composition which ensures the glassy state at the temperature of storage. Fig. 7 provides a reasonable estimate of the composition–temperature boundaries which set the limits to stability. It also provides an indication of changes in the physical state and chemical composition of such a system if one of the components undergoes inadvertent crystallisation from the amorphous, rubbery state. Such changes can have a profound influence on long-term stability and biochemical activity of a labile substance which is one of the components of the amorphous phase.[22] The phenomenon of spontaneous NaCl crystallisation from its solid solution in sucrose has recently been described as an example of such inadvertent phase separation.[23]

Conclusion

The proposed state diagram for the sucrose–glycine–water ternary system is not complete. It does, however, help with an understanding of the behaviour during freezing or drying of multi-component systems with tendencies to supersaturation and vitrification. For a ternary system, we propose a glass-transition surface as a novel concept. Furthermore, it is suggested that the use of semi-empirical treatments, based on eqn. (3) allow the prediction of multi-component glass transitions.

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