

Solid aqueous solutions

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Abstract

In most treatments of aqueous "solutions" it is taken for granted that water is present in the liquid state and in excess, with the concentrations of solutes limited by their saturation solubilities. Hence, the thermodynamic reference states are defined as the infinitely dilute solution, a solution of unit molality, etc. In recent years supersaturated aqueous solutions have been receiving increasing attention, mainly because of their importance in the life sciences and in diverse branches of technology. The physical chemistry of structures, dynamics and interactions in aqueous mixtures in which the concentration of water is of the order of 0-5% by weight is largely unexplored. Such mixtures can be produced from unsaturated aqueous solutions either by evaporative drying or freeze-drying. Being thermodynamically unstable, they rely for their (kinetic) stability on their extremely high viscosities. These systems, like other amorphous solids, exhibit the phenomenon of glass transitions with the residual moisture acting as plasticiser, reducing the glass temperature of the solid substrate. The thermomechanical properties of amorphous, water-sensitive solids are important determinants in many technological processes, but also in the acclimation of living organisms to environmental stresses, such as freezing, saline conditions and desiccation.

INTRODUCTION

Modern solution chemistry dates from the times of Raoult, Henry, Ostwald, van't Hoff and Arrhenius. Solution theories were subsequently developed by Bjerrum, Nernst, Brønsted, Debye and, in more recent times, by Eyring, Hildebrand and Friedman and their contemporaries. All such treatments implicitly assume a solution to be a *liquid* mixture, consisting of a solvent and one or more solutes. The concentrations of the solutes range from the limit of infinite dilution to saturation, i.e. within the boundaries of the solid-liquid or liquid-liquid phase coexistence regions in the equilibrium phase diagram. Relaxation processes in such liquid solutions tend to be rapid on the timescale of experimental observations, and the solution is then always in a state of internal equilibrium.

In recent years, mainly resulting from the pioneering work of Slade and Levine (refs. 1, 2), the important role of water, not as the ubiquitous solvent, but as the ubiquitous plasticiser, has received increasing attention by biologists and technologists. The emphasis is then no longer on chemical or physical equilibrium states but on thermodynamically unstable states which achieve an apparent "real time" structural and chemical stability by virtue of their high viscosities. In such systems water is usually a minor, yet very important, component, present in up to 5% by weight. Depending on their chemical compositions and the temperature, the physical states of such homogeneous aqueous "solutions" range from brittle, elastic solids, through viscoelastic, deformable "rubbers" to syrupy, and finally mobile fluids. The thermomechanical and chemical properties of such materials are of considerable importance in several branches of industry, e.g. food processing and storage (ref. 3), pharmaceuticals, biochemicals and other technologies (ref. 4), based on labile, water-sensitive raw materials. Solid or semi-solid solutions of this type cannot be described in terms of dilute solution theories, even where these have been extended by empirical means to make them applicable up to the limit of saturation (ref. 5).

PREPARATION OF AQUEOUS AMORPHOUS SOLIDS

Since we are here concerned with highly supersaturated mixtures, preparative methods must be based on the removal of water from initially unsaturated solutions. This can be achieved either by freeze-drying or by the evaporation of water at ambient or elevated temperatures, possibly under reduced pressure. The conditions must be chosen such that solutes do not precipitate as eutectic mixtures (in the case of freezing) or along the solidus line (in the case of evaporation). An aqueous solution of sucrose will serve as a typical example (ref. 6).

If such a solution is frozen, it becomes increasingly concentrated, but sucrose does not crystallise (precipitate) spontaneously under eutectic conditions (-14°C , 4M). Instead, freezing continues and the solution becomes increasingly supersaturated. As the freezing temperature continues to decrease, so the viscosity rises, increasingly steeply and, eventually, in a non-Arrhenius manner. Freezing becomes progressively slower and eventually ceases, at least within the timescale of the measurement. The supersaturated solution then undergoes a glass transition, with the viscosity increasing by several orders of magnitude over a very narrow temperature interval. The glass transition of the supersaturated, freeze-concentrated solution, T_g' , is not a phase transition. Operationally, it can be defined in terms of viscosity, e.g. the temperature at which the viscosity reaches 10^{13} Pa s. The physical state of the mixture changes from a fluid to a "syrup" and then to a viscoelastic, deformable "rubber" and finally to a brittle, amorphous solid "glass". Thermodynamically such a mixture is a supercooled liquid in which viscous flow takes place at rates of $\mu\text{m}/\text{year}$; in practice the system can be treated as a solid. Other relaxation processes are similarly slow; for instance, isothermal enthalpy relaxation rates can be measured calorimetrically (see below).

The temperature/composition behaviour of the system is shown in Fig. 1, in the form of a so-called *state* diagram (ref. 6). It includes the familiar solid/liquid coexistence boundaries of the equilibrium *phase* diagram, and also the glass transition profile which joins T_g of water (133 K) and sucrose (ca. 343 K). The extrapolated liquidus and the glass transition curve intersect at a point D (241 K, 82% w/w sucrose, i.e. 13.4 mol/kg water); this temperature is often denoted by T_g' in the current literature.

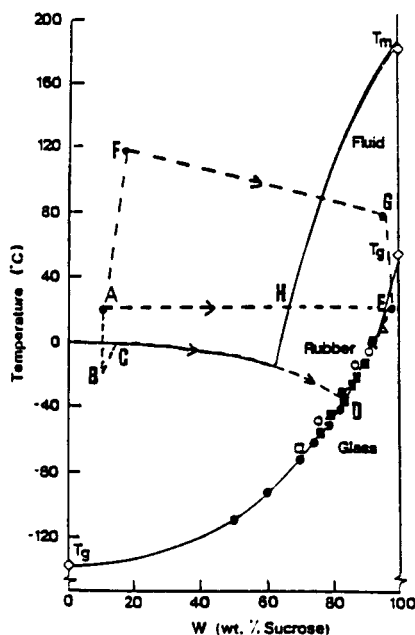


Fig. 1. Solid-liquid state diagram for the binary sucrose-water system; drying pathways are shown by broken lines and arrows, phase and glass transitions are shown by drawn-out lines. The conversion of a dilute solution (A) into a stable glass (E) by the removal of >99% water can be achieved by freeze-drying (ABCDE), ambient temperature evaporation (AHE), or elevated temperature drying processes (AFGE).

Structurally such a supersaturated mixture resulting during the freezing of the sucrose solution, consists of ice crystals dispersed within the continuous supersaturated solution phase (the glass) which typically may consist of only 5% of the total original mass. The ice can be removed by vacuum sublimation, leaving the amorphous solute in the form of a highly porous glass. The remaining moisture (unfrozen at T_g') must then be removed from the solid solution by diffusion and desorption (ref. 8), leaving the anhydrous glass which is mechanically stable up to its T_g . In practice, drying is not generally carried to completion, the final product containing up to 5% residual moisture. Even such low levels of moisture can depress T_g to below ambient temperature. Such a product will therefore not be shelf-stable. Figure 2 illustrates the beginning of viscoelastic collapse in a freeze-dried sucrose glass (ref. 9).

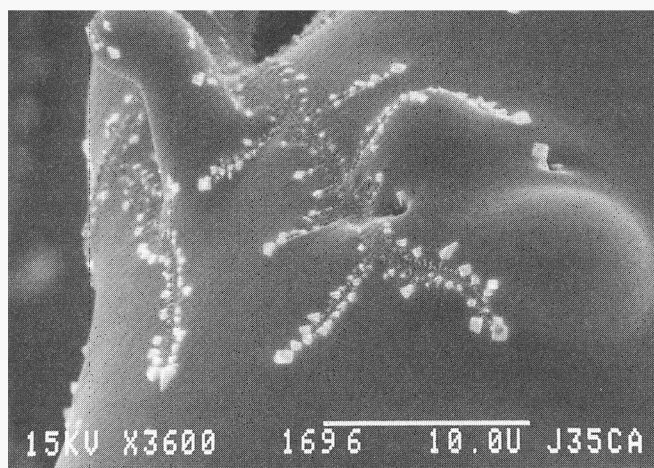


Fig. 2. Scanning electron micrograph of an optimally freeze-dried aqueous solution, containing 10% sucrose and 1% NaCl. Freeze-drying was performed below T_g for several days. The dried specimen was cut, fractured and mounted on a metal stub under dry air. It was then sputter coated with AuPd to give thickness of 17-20 nm. The globules at the end of the fibres indicate the initial stages of collapse, probably because the specimen temperature rose to above T_g for a short period during exposure to the electron beam. Scale bar = 10 μ m.

Instead of freezing, followed by sublimation of ice and diffusion/desorption of the residual unfrozen water (path ABCDE in Fig. 1), water can also be removed isothermally, e.g. by evaporation under reduced pressure at room temperature, along path AHE. Here again, unless sucrose is precipitated when its saturation solubility is exceeded (at point H), the solution will become thermodynamically unstable. It will, however, become kinetically stable once T_g is reached. Under certain circumstances, for instance during a baking process, water is removed at even higher temperatures, schematically shown as path AFGE (ref. 3).

ANALYTICAL TECHNIQUES

Since the glass transition is the manifestation of an almost discontinuous change in thermal and mechanical relaxation properties, the physical techniques best suited to the study of this phenomenon include differential scanning calorimetry (DSC), thermomechanical analysis (TMA), thermogravimetric analysis (TGA) and spectroscopic methods, e.g. NMR and ESR, capable of monitoring relaxation rates in amorphous solids and semisolids. Phenomenologically, glass transitions are accompanied by discontinuities in the second derivatives of the Gibbs free energy with respect to temperature and pressure, i.e. the heat capacity (C) and the coefficients of expansion and compression (ref. 10). This has occasionally led to their description, although incorrectly, as second order (thermodynamic) transitions.

With DSC measurements, the feasibility of detecting T_g depends on the magnitude of ΔC for the material under study. This quantity is by no means constant, and for some glass formers (e.g. silica) is undetectably small. For related substances, e.g. maltose oligomers of increasing degree of polymerisation, ΔC decreases from an initial value of 0.9 J (K⁻¹ g⁻¹) for glucose to a constant value of 0.45 J (K⁻¹ g⁻¹) for maltoheptaose and maltohexaose (ref. 11). The origin of the relationship between the heat capacity change accompanying the glass transition and the degree of polymerisation of the substrate is obscure.

VITRIFICATION OF AQUEOUS SOLUTIONS

In order to vitrify pure water or a dilute aqueous solution, very high cooling rates are required so as to prevent the nucleation and subsequent growth of ice crystals (ref. 12). In practice, the necessary cooling rates can only be achieved with very small volumes of liquid. Vitreous water has been prepared by the rapid immersion in a cryogen of an electron microscope grid containing a film of the liquid (ref. 13). The glass temperature of vitreous water lies in the neighbourhood of -133 K; its physical properties have been described in detail (ref. 14).

When an unsaturated solution of a salt is concentrated, either by the freezing or evaporation of water, then, according to the phase rule, the salt is expected to precipitate when its eutectic concentration is reached, or the appropriate concentration along the solidus curve, in the case of evaporation. In practice, however, the kinetics of precipitation depend on the nature of the salt, its initial concentration, its crystal structure and other factors. Complete precipitation is observed only for initial concentrations in excess of 0.5M (ref. 15).

Many salts readily form supersaturated solutions during cooling and eventually the freeze-concentrated solutions vitrify; upon warming they exhibit glass transitions, followed by eutectic crystallisation. Some salts will not crystallise at all in real time, even when their frozen solutions are warmed to temperatures above T_g' and annealed. Some examples, and their respective T_g' and T_e values are collected in Table 1 (ref. 16).

TABLE 1. Glass formation and eutectic phase separation in frozen salt solutions. Temperatures are shown in °C, for reasons of familiarity.

Salt	T_g' (°C)	T_e (°C)
NaCl.2H ₂ O	< -60	-23
NaHCO ₃	-52	-4
Tris base	-55	-4
Glycine	-70 (estim.)	-4
Tris-HCl	-65	-13
β -Alanine	-65	-14
CaCl ₂	-95	-52
Histidine	-33	-
Na citrate	-41	-
K citrate	-62	-
Na acetate	-64	-
K acetate	-76	-

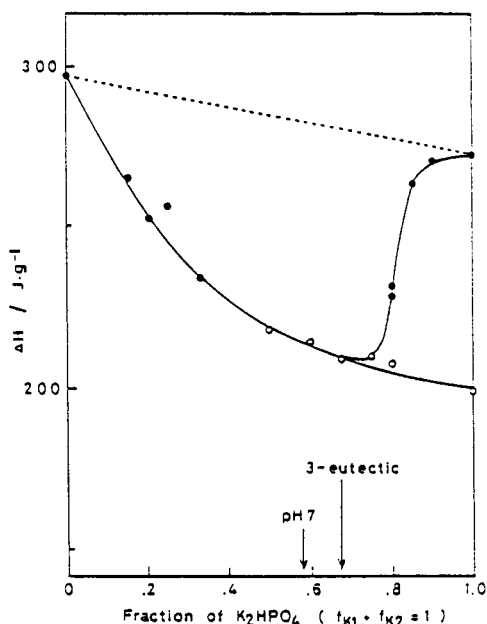


Fig. 3. The enthalpy of autectic melting, ΔH , of frozen potassium phosphate buffer mixtures as a function of the mol ratio $\text{KH}_2\text{PO}_4:\text{K}_2\text{HPO}_4$. The broken line corresponds to complete eutectic phase separation (ideal). The experimental points for annealed (\bullet) and non-annealed mixtures (\circ) indicate the occurrence of partial vitrification. Reproduced, with permission, from ref. 15.

While most binary solutions of salts generally tend to exhibit eutectic phase separation to some degree, this is not necessarily the case for ternary salt mixtures (two salts + water). Figure 3 illustrates the mutual inhibition of precipitation of K_2HPO_4 and KH_2PO_4 , as measured by the heat of eutectic melting (ref. 15). The maximum inhibition is observed in the region of the ternary eutectic mol ratio 0.48 which, in the case of these two salts, happens to lie close to the mol ratio 0.72 corresponding to a pH 7 buffer mixture. For the sodium phosphates the ternary eutectic mol ratio is 57. Partial eutectic formation would then be accompanied by large pH changes in the freeze concentrate.

Other classes of solutes, in particular carbohydrates, are frequently employed for the very purpose of inhibiting precipitation in supersaturated mixtures. With the notable exception of mannitol, sugars do not crystallise spontaneously from freezing solutions. For this reason sugars frequently find use as "stabilisers" or "excipients" in freeze-drying operations (ref. 8). The T_g' values of di- and trisaccharides lie well above those for salts, usually in the region of -30 to -20°C (refs. 2,17). In freeze-drying operations the choice of a stabiliser with as high a T_g' as practicable will lead to considerable improvements in the process economics, since, for obvious reasons, the ice sublimation must be performed at or below T_g' (ref. 6).

STATIC PHYSICAL PROPERTIES OF GLASSY AQUEOUS MIXTURES

Although glass transitions and amorphous states of metals, synthetic polymers and ceramics have long been subjects of extensive study (ref. 10), a realisation of the importance of amorphous aqueous solids, especially those based on naturally occurring materials, dates only from the 1980s. Even now, the available physico-chemical literature is almost exclusively confined to properties of carbohydrate/water mixtures. The general principles governing the physical behaviour of these systems do not differ from those of inorganic materials. The properties discussed in this section have been termed "static", rather than "equilibrium". Although of a kinetic nature, they are characterised by very low rates.

While there is still some debate about the correct molecular interpretation of the glass transition (ref. 10), its measurement is quite straightforward. Figure 4 shows a DSC heating scan of a previously frozen aqueous solution of Ficoll (a condensation polymer of sucrose and ethylene chlorhydrin). Two thermal events are indicated: T_g' at 255 K and the endotherm corresponding to the melting of ice into the freeze-concentrated solution (ref. 18). The latter is frequently employed to estimate the amount of "unfrozen water" in the freeze-concentrate. However, the endotherm profile also contains contributions from the heat of dilution and the heat capacity of fusion which make the exact determination of unfrozen water an uncertain affair (refs. 19, 20).

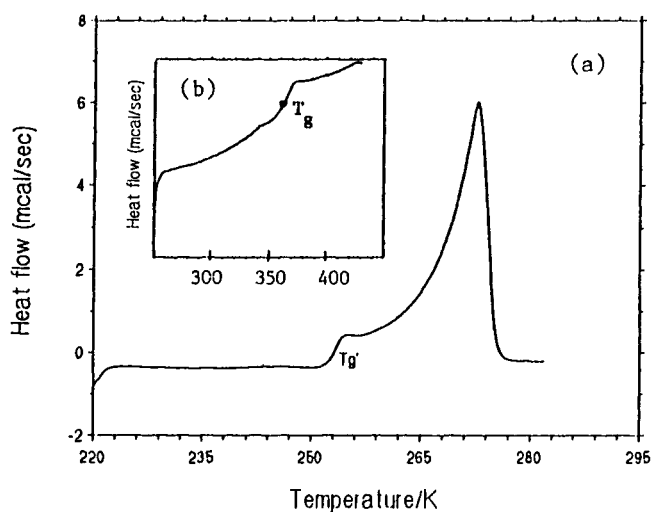


Fig. 4. DSC power-time trace corresponding to the reheating of (a) a frozen 20% Ficoll solution and (b) the same product after drying. The glass transition is indicated by the discontinuity in the heat capacity.

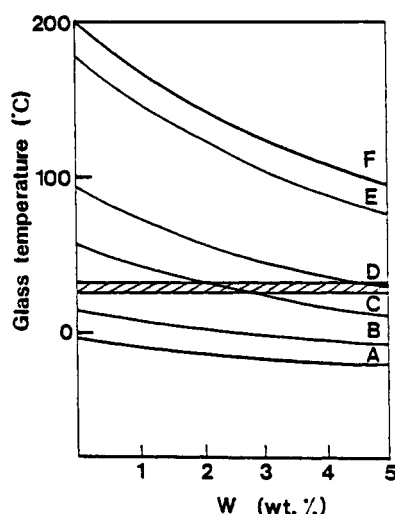


Fig. 5. The effect of residual moisture (w) on the glass transition temperatures of A: sorbitol, B: fructose, C: sucrose, D: maltose, E: maltohexaose and F: lignin. The $T_g(w)$ profiles define the residual moisture conditions for ambient temperature stability (shaded area). Modified and redrawn from ref. 4.

After the sublimation of ice, followed by further drying to remove the residual moisture, the scan shown in the inset was obtained. The glass transition is now observed in the neighbourhood of 370 K, an indication of the plasticising power of water. Figure 5 compares the T_g -depressant effects of low water concentrations (w) on a range of water soluble materials of differing molecular weights and structures. For amorphous polymers, it has been shown that, as a first approximation, T_g values of the anhydrous substances show a dependence on the molecular weight. This behaviour has been also established for glucose and its oligomers, as shown in Fig. 6 (ref. 21). The molecular weight dependence levels off at the so-called entanglement limit.

Apart from the molecular weight dependence of T_g for a series of oligomers, within each group of substances of almost identical molecular weights, marked differences in T_g occur; compare for instance glucose (304 K), fructose (293 K) and sorbitol (270 K); the reasons are not obvious. The observed behaviour is likely to be related to the packing of the molecules and their flexibility in the amorphous state, and the resulting distribution of the free volume in the system.

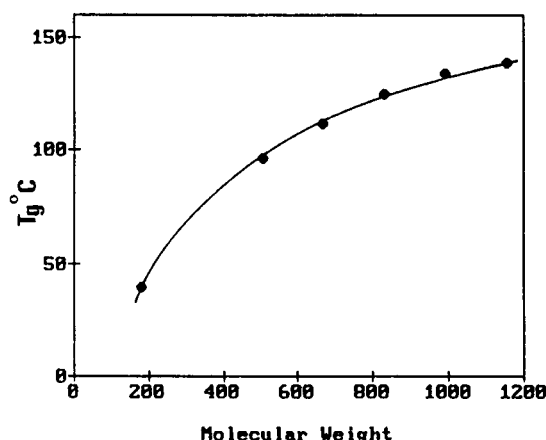


Fig. 6. The glass transition temperatures of a series of malto-oligosaccharides as a function of molecular weight. Reproduced, with permission, from ref. 3.

The prediction of glass transition temperatures of mixtures 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. Nevertheless, it is useful to estimate T_g of binary or ternary systems by an equation, even if of an empirical nature. Equation (1) or some simplified derivative is commonly used for different types of binary systems, in particular polymer blends and polymer-diluent systems (refs. 11, 22, 23):

$$T_g = \left[\sum_{i=1}^n x_i \Delta C_{pi} T_{gi} \right] / \left[\sum_{i=1}^n x_i \Delta C_{pi} \right] \quad \dots (1)$$

where x_i is the mol fraction, T_{gi} 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 T_g is the glass transition temperature of the mixture. This equation was derived from a consideration of the entropy of mixing 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. (1) is that DSC measurements are able to yield all the quantities in this equation.

Equation (1) has been tested for oligomers of glucose and water (ref. 11). The agreement between measured and calculated T_g values increases with increasing molecular weight, i.e. for maltose the deviation reaches 60%, whereas for maltohexaose the deviation does not exceed 35%. The uncertainty about the correct value of the glass temperature of water may be partly responsible for the lack of agreement. On the other hand, specific (hydrogen bonding) interactions between the components may also contribute to the deviations. For anhydrous binary sugar mixtures, the agreement with eqn. (1) has been found to be much better (ref.24). In that case the sugar with the lower T_g value (e.g. fructose) acts as diluent for the other component (e.g. glucose or sucrose).

DYNAMIC PROPERTIES OF GLASSY AQUEOUS SOLUTIONS

Rates of physical and chemical processes in liquid phases can be adequately accounted for by the Arrhenius relationship. As water is removed and the solution becomes supersaturated, deviations from the classical behaviour are observed and become increasingly marked as the glass transition is approached. Several empirical equations have been proposed to account for the observed behaviour, e.g. the Vogel-Tammann-Fulcher (VTF) and Williams-Landel-Ferry (WLF) equations relate the logarithm of a rate process to $(T - T_g)$. In other words, T_g is then the reference temperature, instead of zero Kelvin, as is the case for the Arrhenius relationship. In most cases, the empirical rate equations apply over $(T - T_g)$ ranges of approx. 100 degrees; beyond that, kinetics are adequately described by the Arrhenius equation. Slade and Levine have discussed the relevance of empirical rate equations to low moisture food materials (e.g. cereals) in the neighbourhood of T_g (ref. 3).

By definition, chemical reaction rates in glasses, and relaxation rates of glassy matrices are low, especially when measured at temperatures well below T_g . For instance, viscous flow in ordinary window glass at ambient temperatures is expressed in terms of mm/century. By careful adjustment of the experimental conditions, relaxation processes in the glass become amenable to measurement.

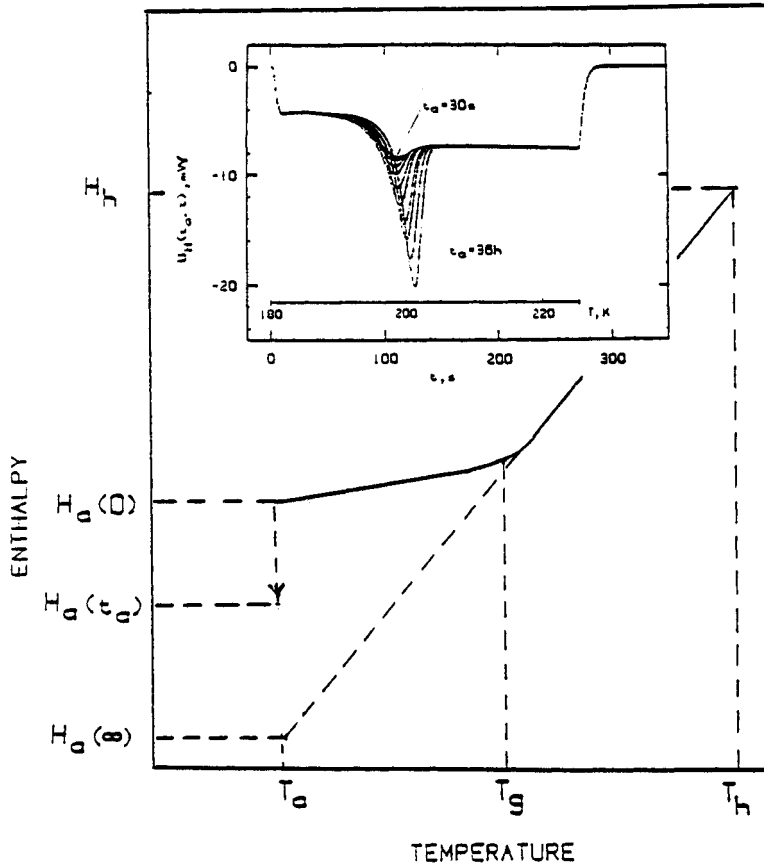


Fig. 7. Diagrammatic representation of enthalpy as a function of temperature. Solid curve: cooling path during glass formation. For details, see text. Inset: DSC power-time signal recorded during the heating of a glycerol glass subjected to different annealing times at an annealing temperature of 180K; heating rate 0.667 K s^{-1} . Redrawn, with changes, from ref. 25.

Thus, DSC can be employed to measure isothermal enthalpy relaxation rates in glasses; the calorimeter then functions as a spectrometer! The principle is illustrated in Fig. 7. The sample is held in the undercooled liquid state (T_h) where relaxation is instantaneous. It is then further cooled at a constant rate through the glass transition to an annealing temperature T_a , where it is held for a time t_a . The sample is then returned to T_h . By repeating this cycle for different values of t_a and integrating the calorimeter power signal U_H , the enthalpy is obtained as a function of time. This is illustrated in the inset of Fig. 7. $H_a(t_a)$ is then given by:

$$H_a(t_a) = \text{constant} - k \int U_H(t_a, t) dt \quad \dots (2)$$

A normalized isothermal relaxation function can be written as:

$$\begin{aligned} \phi(ta) &= [H_a(t_a) - H_a(\infty)] / [H_a(0) - H_a(\infty)] \\ &= \exp[-(t_a/\tau_0)^\beta] \end{aligned} \quad \dots (3)$$

where τ_0 is the relaxation time and β a distribution parameter.

The technique has been employed to measure enthalpy relaxation in glycerol glasses ($T_g = 195 \text{ K}$) (ref. 25). At the highest annealing temperature used, 185 K, relaxation is fast, 36h. At 170 K, however, the relaxation process was estimated to take 4 months. No comparative data are available for other carbohydrates or mixtures.

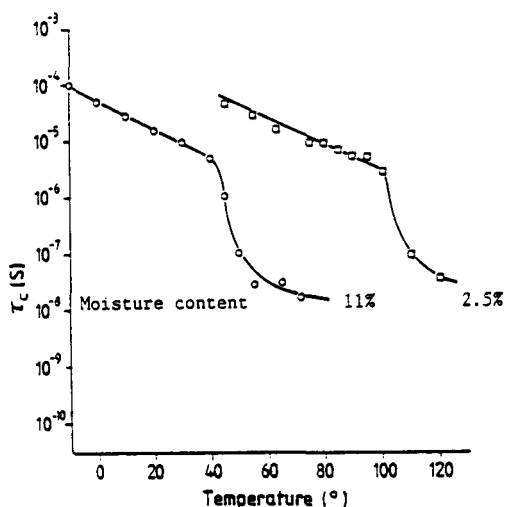


Fig. 8. Rotational diffusion times of an ESR spin probe in maltoheptaose equilibrated at 33% RH (\circ) and oven-dried at 102°C (\square) as a function of temperature. Reproduced, with permission, from ref. 26.

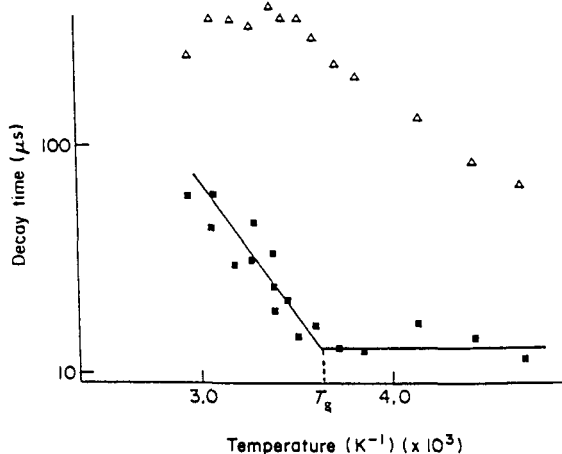


Fig. 9. Proton magnetic spin-spin relaxation times of amylopectin (\blacksquare) and water (Δ) in a low-moisture product in the neighbourhood of the glass transition. Reproduced, with permission, from ref. 30.

The rotational dynamics of probes (actually free radicals) in carbohydrate/water systems have been investigated by electron paramagnetic resonance spectroscopy (ref. 26). Figure 8 shows the temperature dependence of the rotational reorientation time τ_c of the TEMPO spin probe in maltoheptaose, containing 11.5% (left) and 3.5% (right) water, respectively. The glass transitions are clearly indicated by the abrupt change in the temperature dependence of τ_c . It appears that, apart from the shift in T_g with changing water content, the shape of $\tau_c(T)$, both below and above T_g , is unaffected by the moisture content.

Although in dilute aqueous solutions of low viscosity, the rotational and translational diffusion coefficients are related, this does not appear to be the case in supersaturated mixtures in the neighbourhood of T_g , where translational migration is limited by the small free volume in the system. The diffusion of small organic molecules in glassy polymer films has been measured by a variety of techniques, but the interpretation of experimental data is problematical (ref. 27). Sharp boundaries at the polymer/diluent interface have been observed which, only in some cases, move with a constant velocity. As mentioned earlier, the glassy matrix is subject to slow relaxation processes that might modify diffusion and these processes are themselves modified by the diluent concentration.

The migration of water within, and its removal from amorphous matrices containing hydrogen bonding groups is of particular scientific interest and technological importance. It is likely to affect the nature and rates of chemical reactions within aqueous glasses. Experimental information is scarce; diffusion studies on polymer-solvent systems are limited to organic systems, where it has been shown that the processes are complex and concentration dependent (ref. 28).

Based on TGA drying rate measurements of carbohydrate polymers (Ficoll) with similar initial moisture contents, no major changes in the kinetics could be observed at, or above T_g (ref. 29). The Arrhenius relationship appears to hold over the temperature range $T_g \pm 30^{\circ}$, with an activation energy of 29 kJ mol^{-1} , which is of the same order of magnitude as the activation energy of self-diffusion of water in liquid water at ambient temperatures. The water migration rates in the polymer matrix are fitted adequately by a diffusion-limited model.

Recent results from measurements of proton nuclear magnetic spin-spin relaxation times (T_2) on polysaccharide/water systems are shown in Fig. 9 (ref. 30): with decreasing temperature, the polymer segmental motions abruptly change to $\approx 10 \mu\text{s}$ at T_g , whereas the rate of motion of water molecules continues to decline, even below T_g , and that there is no apparent change in the temperature dependence at the glass transition. A linear extrapolation of the water data suggests that the diffusion rates of water

molecules and polymer segments become equal at approximately 165 K. In dilute aqueous solutions, water protons typically exhibit $T_2 \approx 1 \mu\text{s}$ and, since T_2 is inversely proportional to the so-called diffusion time, it can be seen that in the glass the water molecule motions are severely retarded, but hardly to the extent of complete inhibition.

Altogether, therefore, the available evidence suggests that the water molecules constituting the "residual moisture" in a solid solution are able to diffuse rapidly, compared to the motions of the molecules (or polymer segments) making up the glassy phase. The mobility of water within glassy matrices may contribute to the observed chemical reactions that can occur at measurable rates, even below the measured (DSC) T_g .

Another important aspect of the dynamics in solid aqueous solutions concerns their long-term physical stability. Referring once again to Fig. 1, the area to the right of the solidus curve denotes thermodynamic instability (supersaturation), but in the area to the right of the glass transition profile a system will be quasi-stable for long periods. The onset of instability within a measurable time period becomes increasingly more probable as increases in the temperature and/or moisture content take the system across the glass transition boundary and towards the solidus, resulting in a marked reduction in its viscosity. The consequences of allowing this to occur are graphically demonstrated by the DSC traces in Fig. 10 (ref. 6). The first DSC scan was obtained by warming a previously dried amorphous product, containing 93% lactose, 5% of a biologically active substance (to be stabilised) and 2% residual moisture. The glass temperature is clearly indicated at 315 K. It can therefore be assumed that the product is stable at ambient temperature. On heating to temperatures above T_g , amorphous lactose becomes vulnerable to crystallisation, as indicated by the steep exotherm. Thereafter, the amorphous phase consists of the biologically active substance (70%) and water (30%). The mixture was cooled and reheated, when the DSC trace indicated an endotherm, associated with the melting of a small quantity of ice ($T_m = 255 \text{ K}$). Under such conditions (40% "residual" moisture), most biological product would be inactivated at ambient temperatures. To maintain their activity, they would need to be stored below T_g' .

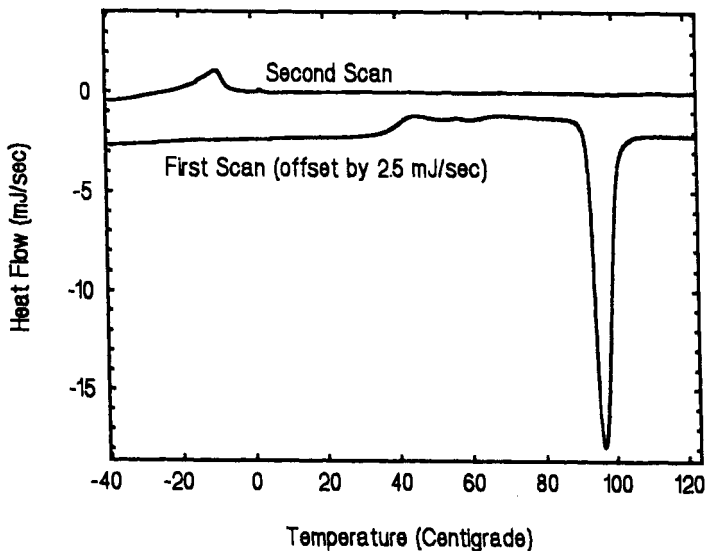


Fig. 10. Two DSC heating scans of a freeze-dried amorphous product containing 93% lactose, 5% protein and 2% water. First scan: heating to/above T_g leads to the crystallisation of lactose. Second scan (after cooling): the amorphous product is now composed of $\approx 70\%$ protein and $\approx 30\%$ water. Some water, frozen during cooling and the ice melting endotherm, is clearly indicated by the ice melting endotherm. From ref. 6.

The *actual*, as distinct from the the predicted equilibrium behaviour of multicomponent systems are of current interest, especially those in which, depending on the composition, crystalline or amorphous phases are produced during drying. Thus, the solid/liquid state diagram of the water/sucrose/glycine system has been mapped (corresponding to the two-dimensional analogue in Fig. 1) and those domains identified where, during freeze-drying, amorphous solids are likely to be produced (Ref. 31).

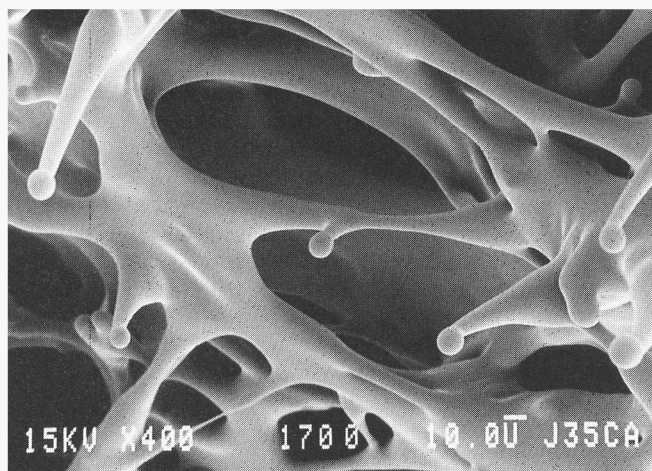


Fig. 11. Scanning electron micrograph of a freeze-dried solution containing 10% of sucrose + NaCl with a mass ratio 5:1. For method of e.m. preparation, see caption to Fig. 2. After exposure to a temperature above T_g , NaCl·2H₂O crystals grow in the amorphous substrate (solvent). Scale bar = 10 μ m.

A more dramatic example of the instability of a ternary solid aqueous solution is the crystallisation of NaCl from amorphous sucrose containing a few percent of residual moisture, shown in Fig. 11 (Ref. 9). The amorphous mixture was prepared by freeze-drying a dilute solution of sucrose and NaCl and exposing the resulting solid to a temperature slightly in excess of T_g . Well defined cubic crystals of NaCl·2H₂O of micrometer dimensions are seen to grow in the amorphous substrate, especially at the edges of the stress cracks in the amorphous solid; their rate of growth may be controlled by careful adjustment of the temperature.

CONCLUSIONS

The introduction of materials science concepts is leading to new and more rational ways of interpreting the physical and chemical behaviour, and even some physiological implication of solid aqueous solutions in which water is a minor component. In the food industry, quality, processability and safety can now be expressed in terms of the thermomechanical parameters of the solid substrates, with the glass transition playing an important role. The properties of thermodynamically unstable states thus assume a great importance, and "stability" is seen to depend, not on equilibrium phase coexistence, but on kinetic properties of homogeneous phases.

Amorphous glassy states assume a special role in the long-term stabilisation of biological products (e.g. isolated enzymes), which, in liquid solution, have very limited shelf lives.

Finally, all the indications are that at least some ectotherms employ the device of *in vivo* vitrification during the so-called acclimation period to render themselves resistant to extremes of osmotic dehydration by freezing, drought or salinity. They can achieve this by the conversion of their glycogen reserves into high concentrations of sugars which, upon dehydration, eventually cause the cytoplasm to undergo the glass transition at the temperature of their environment.

In view, therefore, of the undoubted technological and ecological importance of highly supersaturated aqueous mixtures and their past neglect by physical scientists, it is hoped that their structures and the processes occurring within them will now receive the attention they deserve.

Acknowledgements

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REFERENCES

1. H. Levine and L. Slade, *Water Sci. Rev.* **3**, 79-185 (1988).
2. L. Slade and H. Levine, *Pure Appl. Chem.* **60**, 1841-1864 (1988).
3. L. Slade and H. Levine, *Crit. Rev. Food Sci. Nutrition* **30**, 115-360 (1991).
4. F. Franks, R.H.M. Hatley and S.F. Mathias, *BioPharm.* **4**, 38-42 (1991).
5. K. Pitzer and J.J. Kim, *J. Amer. Chem. Soc.* **96**, 5701-5710 (1974).
6. F. Franks, *Jap. J. Freezing Drying* **38**, 5-16 (1992).
7. A.P. MacKenzie, *Phil. Trans. Roy. Soc. B* **278**, 167-188 (1977).
8. M.J. Pikal, *BioPharm.* **4**, 18-27 (1991).
9. F. Franks and C. van den Berg, in D.J.A. Crommelin and K.K. Midha (eds.) *Topics in Pharmaceutical Sciences*, p.233, Medpharm Scientific Publishers, Stuttgart, (1991).
10. S.R. Elliott, *Physics of Amorphous Materials*, Longman Scientific & Technical, Harlow (1990).
11. P.D. Orford, R. Parker, S.G. Ring and A.C. Smith, *Int. J. Biol. Macromol.* **11**, 91-96 (1989).
12. F. Franks in F. Franks (ed.) *Water - A Comprehensive Treatise*, Vol.7, p.215, Plenum, New York (1982).
13. E. Mayer, *Cryo-Lett.* **9**, 66-77 (1988).
14. M.G. Sceats and S.A. Rice, in ref. 12, p.83.
15. N. Murase and F. Franks, *Biophys. Chem.* **34**, 293-300 (1989).
16. B.S. Chang and C.S. Randall, *Cryobiology* **29**, 632-656 (1992).
17. Y. Roos, *Carbohydrate Res.* **238**, 39-48 (1993).
18. R.H.M. Hatley, *Dev. Biol. Standard.* **74**, 105-119 (1992).
19. R.H.M. Hatley, C. van den Berg and F. Franks, *Cryo-Lett.* **12**, 113-124 (1991).
20. S. Ablett, M.J. Izzard and P.J. Lillford, *J. Chem. Soc. Faraday Trans.* **88**, 789-794 (1992).
21. H. Levine and L. Slade, in T.M. Hardman (ed.) *"Water and Food Quality"*, p.71, Elsevier, London (1989).
22. P.R. Couchman, *Macromolecules* **11**, 1157-1161 (1978).
23. P.R. Couchman, *Polymer Eng. Sci.* **27**, 618-621 (1987).
24. L. Finegold, F. Franks and R.H.M. Hatley, *J. Chem. Soc. Faraday Trans. I* **85**, 2945-2951 (1989).
25. A. Fransson and G. Bäckström, *Int. J. Thermophysics* **8**, 351-362 (1987).
26. M.J.G.W. Roozen, M.A. Hemminga and P. Walstra, *Carbohydrate Res.* **215**, 229-237 (1991).
27. N.E. Schlotter, *J. Phys. Chem.* **94**, 1692-1699 (1990).
28. J.M. Zielinski and J.L. Duda, *J. Polymer Sci.* **30**, 1081-1088 (1992).
29. B.J. Aldous and A.L. Greer, *unpublished results*.
30. M.T. Kalichevsky, E.M. Jaroszkiewicz and J.M.V. Blanshard, *Polymer* **34**, 346-358 (1993).
31. T. Suzuki and F. Franks, *J. Chem. Soc. Faraday Trans.*, in the press (1993).

NOTE ADDED BEFORE PUBLICATION

Before this volume went to press, the author received a manuscript entitled: *Liquid Fragility and The Glass Transition in Water and Aqueous Solutions* by C. A. Angell, due for publication in *J. Food. Sci.* The properties of hydrophilic glass-forming materials are discussed in terms of the classification of fluids as "strong" or "fragile" which is based on a corresponding states representation of viscosity, using T_g scaling in the Arrhenius equation.

In the opinion of the author, the discussion by Angell provides the most penetrating insight into the problems posed by water-compatible amorphous solids since the pioneering studies of Slade and Levine during the early 1980s.