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Scientific and technological aspects of aqueous glasses

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Abstract

The physical nature of a glass, as related to stable liquid and crystalline solid phases was defined by Kauzmann in 1948. Since then, glass research has been almost exclusively confined to inorganic materials. This review aims to demonstrate that many substances, not falling into the category of classical 'materials', can be rendered into amorphous states. In particular, water itself, but also water soluble and water sensitive organic molecules, some of them biomolecules, can be rendered into supersaturated and solid solutions. New ways of studying and applying amorphisation processes have led to major advances in food and pharmaceutical processing aimed mainly at the stabilisation of labile materials. Because of their molecular similarities to water, polyhydroxy compounds are attracting particular interest as potential matrix elements in the preparation of glassy products.

Keywords: Potential; Polyhydroxy; Labile; Pharmaceutical

1. Introduction and history

Glass as a material was probably first produced, accidentally, by the fusion of sand and soda in an open fire, as described by Pliny. The oldest pure fabricated glass is a moulded amulet of deep lapis lazuli colour, dating from approximately 7000 BC The attractive mechanical and optical properties of the material were soon recognised and eventually led to the development of silicate glass manufacturing processes. Over the following centuries glass technology attained a high state of refinement, and to the original mixture of silica and soda ash were added many novel materials. The patent literature bears witness to the continuing importance of glass as a material of construction and decoration. One modern encyclopaedia describes glasses as a '…a wide range of inorganic substances which differ widely in chemical composition and physical properties, but which possess the essential characteristic of having been cooled from a state of fusion to become a solid without crystallisation. Glass at room temperature can be regarded as a liquid, which is of such a high viscosity that it, behaves as a rigid elastic solid.' It is one of the objectives of this article to demonstrate that the above definition is unduly restrictive.

Perusal of the literature demonstrates that the study of glasses has been in the past and is still, at present, almost dominated by materials scientists. Thus, the considerable progress in develop-

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ments of novel materials has been confined mainly to glasses based on metals, silicates, oxides, ceramics and polymers. This is also true as regards fundamental studies into the physical and mechanical properties of the many materials that form the basis of the construction industry. The most significant contribution to an understanding of the physical properties of glasses, and especially to the interpretation of the glass transition, is found in Kauzmann's famous 1948 publication,[1] in which the concept of configurational entropy was first introduced to account for the thermodynamics and kinetics of amorphisation and the behaviour of glasses in relation to the liquid state and the perfect crystal [1].

The realisation that amorphous solids in general, and glasses in particular, also play an interesting and important role in technologies not necessarily related to materials science, came relatively late. Indeed, mention of the word 'glass', used in reference to a system other than a 'real' material, probably dates from the late 1939 [2]. Even then, the significance of substances that could easily be quenched from the melt without crystallising and with eventual vitrification, escaped most of those who might have known better. The idea that the physical properties of sugar candy could be described by the same equations that apply to window glass or to metal alloys was not grasped at the time by materials scientists. The pioneering studies by Luyet and his associates on frozen aqueous solutions demonstrated the fundamental role that glasses play in cryobiology. They developed the technique of cryomicroscopy and coupled it with differential thermal analysis; this enabled them to study the process of freezing, freeze concentration and vitrification under controlled conditions [3]. An important aspect of their work consisted of the determination of glass transition temperatures of several freeze-concentrated aqueous solutions, i.e. of solutes that did not exhibit eutectic phase separations but formed supersaturated solutions. Table 1 summarises a selection of some early results. Yet, even years after intensive study, mysteries still surround several structural and dynamic aspects of glasses and, especially, of the nature of the glass transition. This is illustrated by a recent report entitled 'Why the Glass Transition is still interesting' [4].

Table 1
Glass transitions temperatures (T_g') of some maximally freeze-concentrated aqueous carbohydrate solutions

	K
Xylose	225
Ribose	226
Glucose	230
Fructose	231
Mannitol	233
Sucrose	241
Trehalose	243
Lactose	245
Raffinose	247
Stachyose	249
Maltohexaose	258
Cyclodextrin	264

2. Vitreous water

The notion that pure liquid water itself might also be quenched into a vitreous state first attracted attention long after it had been shown that an amorphous form of 'ice' resulted from the deposition of water vapour on a cold metal plate [5]. The properties of this amorphous solid water (ASW), especially in relation to liquid water, were reviewed and discussed in detail in 1982 [6]. Problems arose, however, in attempts to determine the behaviour of deeply undercooled water, as a notional glass transition is approached. The homogeneous nucleation temperature of ice from undercooled water lies in the neighbourhood of 233 K, and the crystal growth of ice is extremely rapid. Furthermore, the physical properties of both ice and water, i.e. high specific heat, low density and low thermal conductivity, make for inefficient heat transfer. Very high cooling rates therefore need to be attained in order to prevent freezing in the temperature interval between the nucleation temperature and a (notional) glass transition. Reports of the successful hyperquenching of small volumes of liquid water into the glassy state date from 1980, [7] and the method has since been consid-

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[1] According to the author, the work, which formed the basis of the article, had been completed by 1939, but its publication had to be delayed because he was seconded to the Defence Industry for the duration of World War II.

erably refined and is utilised extensively in the preparation of biological samples for low temperature electron microscopy [8].

More recently a different form of amorphous water was discovered; it is obtained during the pressure melting (100 kPa) of ice-Ih at 77 K. It is also formed by the compression of ice-Ih below 150 K [9]. The two substances differ in density. Thus, the vapour deposited, and the hyperquenched liquid both give rise to the low-density state (LDA), whereas pressure melting or the compression of ice produce a high-density variety (HDA). A first order, reversible transition, with no intermediate states, occurs with a 20% volume change. Although this interpretation is not yet universally accepted, the evidence for and against has been objectively reviewed [10]. Interestingly, the HDA variety is the most abundant form of water in the universe, because it is formed on interstellar dust particles that, eventually, turn into comets [11].

The phenomenon of polyamorphism is not unique to water; it has also been reported for silicon and several binary oxide mixtures, which exhibit tetrahedrally configured crystal structures, akin to those of the ice polymorphs. What gives water its special interest lies in the relationship between the two amorphous states. At present there exists no unified picture of the process liquid water → undercooled water → glass. The situation is made more complex by the reported divergence of the isobaric specific heat, the isothermal compressibility and the coefficient of expansion of undercooled water near 228 K, [12] a temperature that cannot readily be achieved experimentally, because $T_h \approx 233$ K. The question remains whether HDA is a glassy form of compressed water or whether it should be treated as a collapsed crystal. Initial neutron diffraction studies on cooled D_2O were limited to the study of oxygen–oxygen radial distribution functions [13]. They suggested that, at low pressures, the structure changes towards that characteristic of LDA, but at high pressure the structural change is in the direction of HDA. A recent study of the amorphous phases, with the aid of neutron diffraction with isotope substitution, has made possible a full structural analysis [14]. During the transition from HDA through liquid water and LDA to ice-Ih, subtle changes occur in the second neighbour shell. The study has confirmed that both ASW ‘phases’ consist of fully hydrogen-bonded networks, with LDA resembling ice-Ih and HDA resembling liquid water.

An intriguing question arises, whether a liquid/liquid phase separation, of the type frequently encountered in aqueous solutions, might also exist in amorphous solid water. Neutron data provide indirect evidence for a critical point at approximately 230 K and 50 MPa, but this point on the P–T phase diagram lies in the inaccessible ‘no man’s land.’ If such a phase coexistence in the mobile liquid could ever be established, it might take us back to the high and low density ‘mixture’ model for liquid water, hypothesised by Henry Frank in 1945, on the basis of the observed thermodynamic properties, but later discarded as unrealistic [15].

The nature of the problem is illustrated by reference to Fig. 1, which displays the various models that have been proposed for liquid water [10]. Computer simulation has been proposed as the only way of studying the experimentally inaccessible regions of the phase diagram. The Molecular Dynamics (MD) method in particular has been energetically applied to define the ‘structure’ of liquid water. Unfortunately, the inadequacy of available water dimer potential functions must cast serious doubts on the value of currently employed techniques. Thus, one of the shortcomings of even the currently popular TIPS potential functions is their inability to correctly account for the temperature of maximum density of water or its critical temperature.

In common with other glass formers, it might be expected that the two, or possibly even more, distinguishable amorphous water phases should be associated with corresponding glass transitions. Various different T_g values have been reported for LDA and HDA, apparently depending on the experimental technique employed and the thermal history of the sample. On the other hand, some investigators have been unable to detect any glass transitions at all. For many years the value first proposed from extrapolations of calorimetric measurements, 136 K, has been universally used, [16,17] but recent studies on hyperquenched water suggest a revised value, 165 ± 5 K as being more

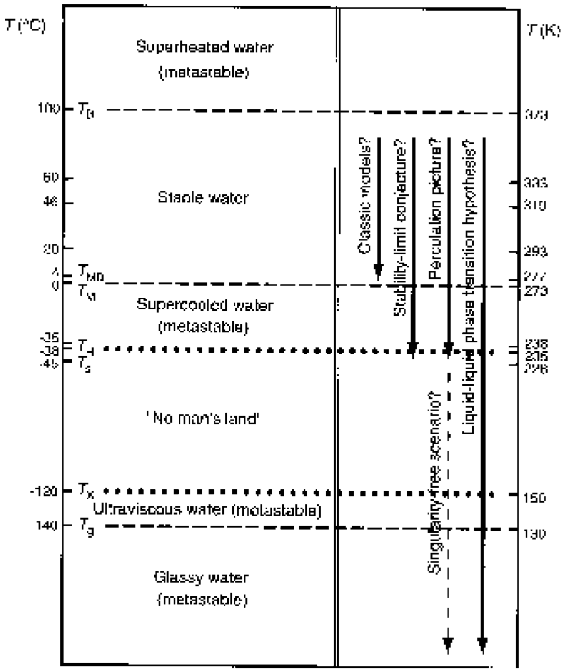

Fig. 1. Speculative phase diagram of 'liquid' water, including metastable superheated and cryoquenched states. The following phase and state transition temperatures at atmospheric pressure are indicated: T_B, boiling point; T_{MD}, maximum density; T_M, melting point; T_H, homogeneous nucleation; T_s, divergence of physical properties, extrapolated from experimental data [12]; T_X, devitrification, T_g, (hypothetical) glass transition. The region between T_H and T_X is inaccessible to experiment. Reproduced, with permission, from ref. [10].

realistic, [18] because it is more in line with the behaviour of other 'good' glass forming systems. It may therefore be that a notional glass transition is 'hidden' by the devitrification, which occurs at a lower temperature.

At the present time, therefore, no consensus exists about the structural nature of the ASW forms and their relation to liquid water and/or ice. It is not even clear whether they can be regarded as metastable 'phases', in the thermodynamic meaning of that term. Indeed, the term metastability appears to have become inappropriate, because a solid 'equilibrium phase' is stable only within a well-defined P–T domain and becomes thermodynamically unstable on passing a P–T coexistence curve. It then becomes a matter of the nucleation and growth rates of the daughter phase within the mother phase. For solid–solid polymorphic or even polyamorphic (ASW) transformations at low temperatures, such kinetic processes are likely to be slow, so that metastable phases can be expected to possess long life times and could be regarded as 'stable' on an appropriate time scale. The dynamic behaviour, e.g. relaxation times and configurational entropies, of amorphous water phases remains to be determined.

3. Aqueous solid solutions

The above-described uncertainties that beset an understanding of the physical nature of ASW in all its manifestations have not prevented considerable progress in the study of supersaturated aqueous solutions. It was recognised some twenty years ago that such systems are basic in a variety of ecologically and technologically important areas, particularly natural frost and drought resistance, [19] food processing [20] and pharmaceutical formulation development [21].

The technological potential of vitreous aqueous solutions was first recognised during thermoanalytical studies of freeze-drying and air drying. In the pharmaceutical industry it had long been known that the stability of drugs, especially those based on proteins, was considerably enhanced by freeze-drying, but that this process required the addition of so-called 'excipients' to the solution to be dried. Empirical approaches had suggested that carbohydrates were particularly effective in providing enhanced drug stability. DSC heating scans of frozen solutions frequently revealed the 'signature' of the glass transition, i.e. an endothermic step in the heat flow (specific heat) at some subzero temperature, which was then followed by the characteristic ice melting endotherm; this is illustrated in Fig. 2. When such a solution was actually freeze-dried (rather than freeze-thawed), a DSC scan of the resulting 'dry' preparation still indicated the presence of a glass transition, although broader and at a much higher temperature, shown in the inset of Fig. 2.

During the past two decades it became increasingly clear that investigations of food and pharmaceutical processing technologies could greatly

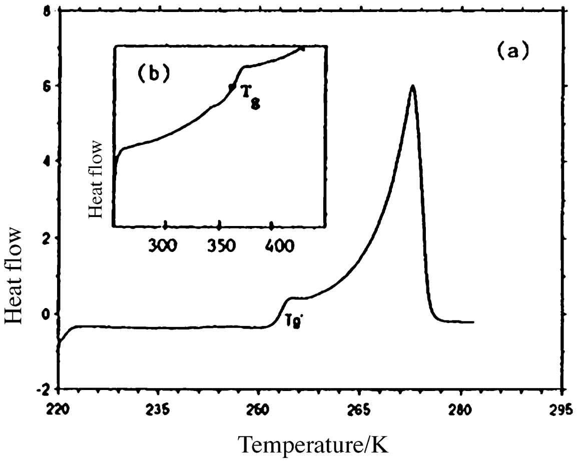

Fig. 2. (a) DSC heating scan of a frozen aqueous solution of Ficoll® (a copolymer of sucrose and ethylene chlorhydrin). The endothermic step in the heat flow is symptomatic of a glass transition; it is followed by the melting endotherm, which is a superposition of the latent heat of fusion, the heat of dilution and a heat capacity of fusion term. (b) Scan of the same solution, after freeze-drying to a low residual water content ($<$ 2%).

benefit from a materials (or polymer) science oriented approach [22]. It also became clear that product shelf lives appeared to be closely correlated with glass transitions. This led to the construction of so-called state diagrams, combining conventional solid–liquid and solid–solid phase coexistence data with (kinetic) temperature-composition nucleation and glass transition profiles [23]. Fig. 3 shows a popular state diagram, for the system sucrose–water. In accordance with phase rule, and in the absence of one or several sucrose hydrates, the cooling of an unsaturated solution should lead to a eutectic point, T_e, at which liquidus and solidus curves meet and where anhydrous sucrose precipitates (crystallises). This temperature is shown as 255 K in Fig. 3. The phase diagram as shown is, however, misleading, because at ordinary cooling rates, the probability of sucrose nucleation at T_e or below is extremely low, effectively zero, so that continued cooling will produce a supersaturated solution which eventually undergoes vitrification at T_g' (241 K). At this point the glass consists of an aqueous 80% w/w sucrose solution and is usually referred to as 'maximally freeze-concentrated'. It is in the form of a two-phase system of ice crystals embedded in the residual solid solution. In a typical freeze-drying process the crystalline ice is then removed by sublimation, leaving behind the supersaturated solid solution in the form of a three-dimensional 'spider web', as shown in Fig. 4. The residual, unfrozen water has to be removed by diffusion, eventually yielding a dried product with a T_g value lying on the glass profile and depending on the residual water content. For the case of sucrose, shown in Fig. 3, the maximum T_g that can be achieved is approximately 345 K.[2]

The earlier observation that acceptable product shelf lives depended on the presence of suitable excipients in the initial solution, coupled with the

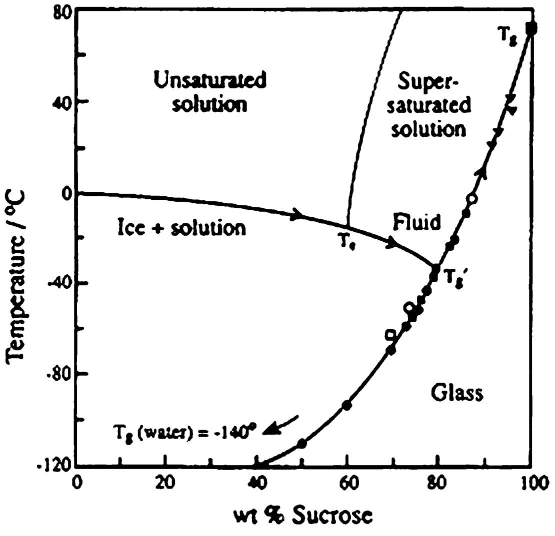

Fig. 3. 'State diagram' for the water–sucrose system, combining equilibrium phase coexistence curves with metastable, supersaturated states and the glass transition profile. T_e is the (notional) eutectic point and T_g' is the glass transition of the maximally freeze-concentrated mixture. Redrawn from ref. [23].

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[2] Actually, the phase diagram of the sucrose-water system is more complex through the existence of several hydrates [24], two of which have actually been structurally characterised by X-ray diffraction; the others are inferred from various older literature data. Since none of these hydrates or even anhydrous sucrose will crystallise during the 'real time' cooling of a dilute solution, they have been omitted from the simplified representation in Fig. 3.

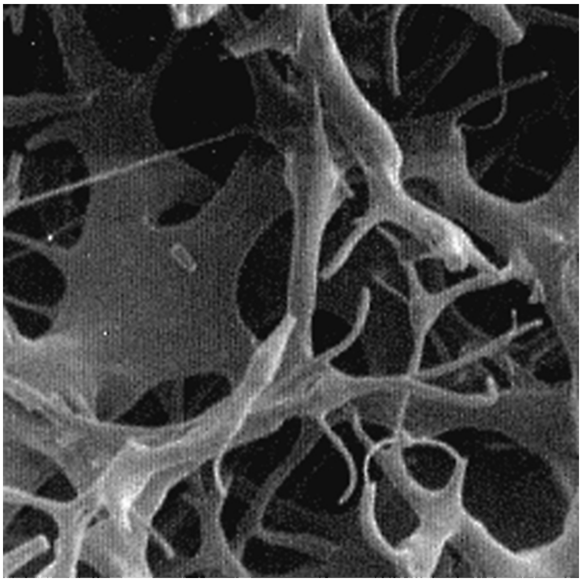

Fig. 4. Scanning electron micrograph of a maximally freeze-concentrated aqueous sucrose solution after the sublimation of ice. The cavities denote spaces previously occupied by ice crystals. The filaments represent the glassy sucrose, containing dissolved residual water.

realisation of the significance of T_g' led to a rationalisation of the efficacy of carbohydrates as protectants during and after the drying of labile biomaterials. Polyhydroxy compounds (PHCs) as a group possess some common properties, one of them being their reluctance to crystallise from aqueous solution. This reluctance derives from their marked similarities with water, in particular the nature of their interactions with water and with one another [25]. Like ice and liquid water, they form three-dimensional hydrogen bonded networks in the crystal and probably also in the glassy state [26]. Because of their stereochemical and topological complexities, however, crystal nucleation rates are low, especially in aqueous solution, where sugar–sugar –OH interactions closely resemble those with water. It is the resulting tendency to supersaturation that produces the vitrification of pharmaceutical preparations, and helps to confer an improved degree of stability on such products [27].

It is still unclear what molecular attributes of individual PHCs determine their observed T_g values. A selection of 'best value' T_g data are summarised in Table 2. Adopting the PHC generic formula $(CH_2O)_n$, then as a first order approximation, T_g increases with increasing n for the two series triose, tetraose, pentose, hexose, and mono-, di-, trisaccharide, etc. This molecular weight dependence does not, however, account for distinct T_g differences between PHCs with identical molecular weights, e.g. glucose, mannose, fructose, sorbitol and mannitol. In fact, mannitol is the only common PHC, which readily crystallises from a freezing aqueous solution and exhibits a clean eutectic phase separation. Its closely related stereoisomer, sorbitol, has a glass temperature of 200 K and cannot be made to crystallise from a cold aqueous solution. It is therefore not clear which features of pyranoses, furanoses and alicyclic PHCs influence the thermophysical properties of their respective glasses. In those disaccharides for which reliable glass transition data are available, it is observed that a furanose ring has a lower

Table 2
Glass transition temperatures of dried (anhydrous) amorphous polyhydroxy compounds; 'best values' selected from recent publications

	T_g/K
Trioses	
Glycerol	180
Pentoses	
Ribose	263
Xylose	263
Arabinose	271
Hexoses	
Fructose	286
Glucose	312
Mannitol	303
Sorbitol	270
Disaccharides	
Sucrose	343
Trehalose	379
Maltose·H_2O	343
Trisaccharides	
Raffinose	382
Maltotriose	368
Tetrasaccharides	
Stachyose	405

propensity for vitrification than does a pyranose ring.

Yet one further stereochemical property affects the T_g values. The amorphisation of trehalose has been intensively studied over the past decade, because at one time it was claimed to possess a unique ability to stabilise labile molecules [28]. With one exception, experimental studies on trehalose are always performed with α,α-anomer ($T_g = 382$ K), although this is hardly ever mentioned in published reports. β,β-Trehalose differs from the more commonly available anomer in two respects: it occurs as a tetrahydrate, compared to the dihydrate of α,α-trehalose, and in its anhydrous state it exhibits a glass transition, higher that that of α,α-trehalose [29].

Nevertheless, despite the above-mentioned uncertainties, the observed correlation of the glassy state with stability led to the development of improved, and more rational pharmaceutical formulation strategies. Pilot studies on the drying of highly labile proteins from aqueous solution demonstrated that by observing the constraints set by the glass transition profile, shelf stable products could be obtained where it had not previously been possible [30]. Empirical stability tests on a variety of products suggested that a dried preparation with an acceptable shelf life of several months could generally be obtained by storage at temperatures in the neighbourhood of (T_g −20) degree. Since T_g is sensitively dependent on the residual water content, a desired storage temperature can partly be controlled by the degree of drying. Figs. 5 and 6 illustrate the superior ambient temperature stabilities of two vacuum-dried preparations containing compatible PHC excipients with high glass transition temperatures. Neither of these substances, human growth hormone (unpublished results) and the restriction enzyme *Pst*I [31] could previously have been dried or stored in the liquid state, without the addition of glycerol and the employment of very low subzero temperatures.

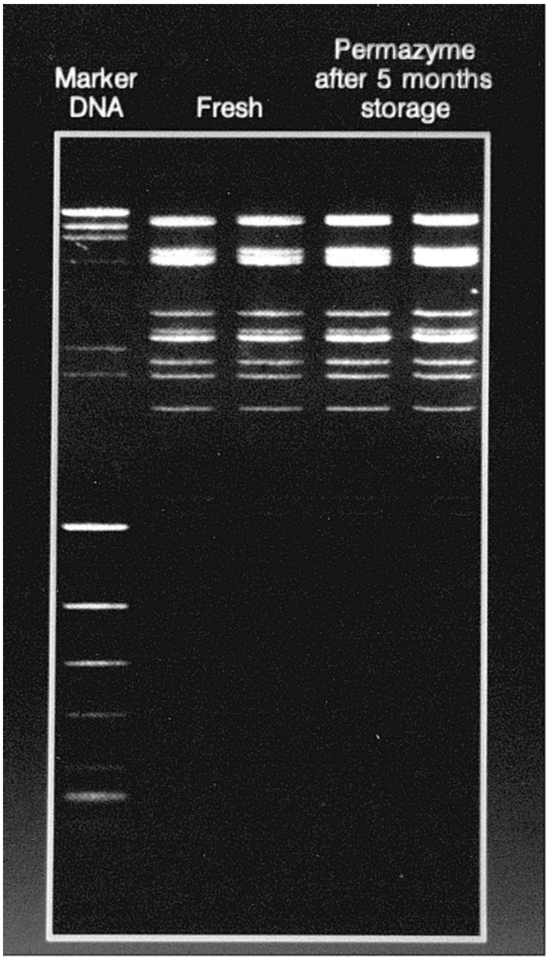

Fig. 5. Restriction digest of λ phage DNA by *Pst*I. Lane 1, marker DNA; Lanes 2 and 3, DNA digested with fresh *Pst*I; Lanes 4 and 5, DNA digested with *Pst*I enzyme that had been stabilised by vitrification and stored at room temperature for 5 months. For details, see ref. [31].

4. Pharmaceutical glasses at sub-T_g temperatures

Following the initial successes of the improved pharmaceutical formulation developments, attempts were made to obtain quantitative relationships between T_g values and storage stability. It soon became clear that a simple relationship probably does not exist, and that chemical deterioration processes of dried proteins and other labile molecules continue, albeit at reduced rates, well below the glass temperature. An example is shown in Fig. 7 for the unimolecular dissociation of 2-(4-nitrophenoxy) tetrahydropyran, dried to a constant residual water content in the presence of several

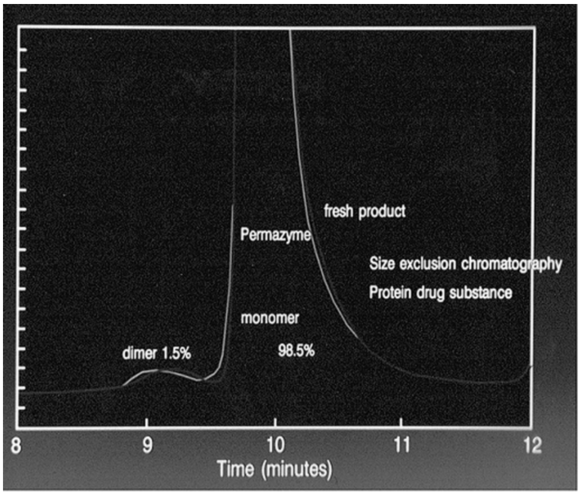

Fig. 6. Size exclusion chromatograms of two human growth hormone preparations, showing the pure, monomer peptide and the 1.5% contamination by dimer. The faint outline is the chrmoatogram of a fresh product and the bold, superimposed outline section is the corresponding profile for a product, vacuum dried from solution of a carbohydrate stabiliser (Permazyme™ process) and stored in the glassy state at room temperature for 9 months (F. Franks, unpublished results).

excipients with different T_g values [32]. The results show two striking features: (1) unexpectedly, excipients with low T_g values are the best stabilisers, and (2) the drop in the rate constant for the dissociation in the neighbourhood of T_g is most pronounced for excipients with high T_g values.

Attention has now turned to a characterisation of sub-T_g molecular mobility in multicomponent pharmaceutical preparations. Experimental studies in this temperature domain are difficult because of the long relaxation times for structural and chemical changes, and also because thermal history (cooling/heating/drying rates) affect the observed results. As a starting point the so-called Kauzmann temperature (T_K) is estimated from the extrapolation of experimental thermophysical data. Measurements on various therapeutic protein preparations have indicated that their chemical stabilities correlate well with their T_K values, while no such correlation is found with T_g values [33].

Standard experimental techniques to probe sub-T_g behaviour include measurements of relaxation enthalpies, heat capacities, thermomechanical properties and dielectric and nuclear magnetic relaxation. Relaxation times (τ) are calculated with the aid of the 'stretched exponential' equation

$$\Phi_t = \exp(-t/\tau)^\beta$$

where Φ_t is the extent of relaxation at time t and β is a distribution parameter, with $0<\beta<1$. In order to compare the relaxation behaviour of different materials, the well-known VTF equation (Vogel–Tammann–Fulcher) is used:

$$\tau = A \exp[B/(T-C)]$$

The significance of the constants A, B and C and the relation of the VTF equation to the Arrhenius and WLF (Williams–Landel–Ferry) equations has been discussed ever since the importance of glasses in food and pharmaceutical processing became to be realised [22,34,35]. It is now generally realised that chemical stability of amorphous pharmaceutical preparations correlates well with structural relaxation rates. For practical shelf lives, in the order of 1–2 years, a storage temperature of at least (T_g–50) degrees should be aimed at during formulation development. That means that for a product, including residual water, des-

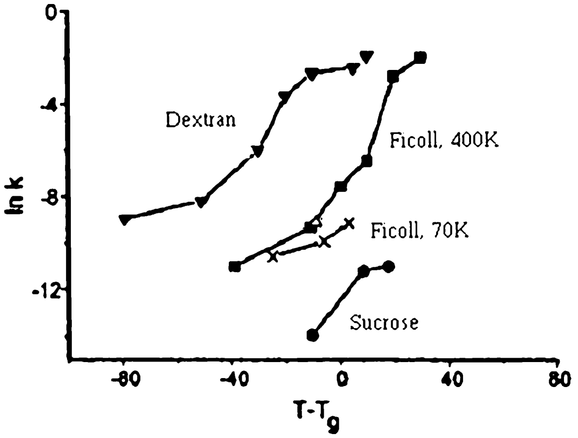

Fig. 7. Chemical reactivity in glasses at sub-T_g temperatures. Rate constants of the unimolecular dissociation of 2-(4-nitrophenoxy) tetrahydropyran in freeze-dried glassy matrices of sucrose, dextran and Ficoll® at identical residual water contents. Modified from ref. [32].

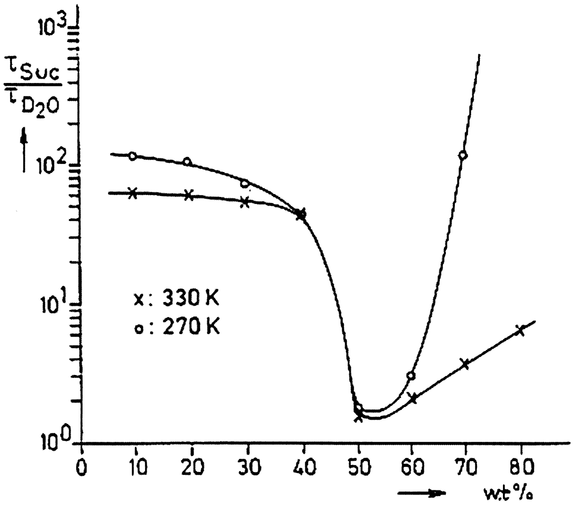

Fig. 8. Rotational diffusion time ratios $\tau_{sucrose}/\tau_{water}$ of sucrose and water at two temperatures, as a function of composition, in unsaturated and supersaturated solutions. Adapted and redrawn from ref. [36].

tined to be stored at room temperature, the glass temperature should exceed 373 K, no mean feat for the product developer!

5. Aspects of multicomponent aqueous glasses

In closing this review of recent progress, it is useful to touch upon some important aspects requiring further study. Glasses are characterised mainly in terms of their bulk properties, although they may contain molecules differing widely in size and shape. Thus, reference to 'molecular mobility' and configurational entropy may well account for the thermomechanical behaviour of the glassy matrix but may disguise motions, even molecular translation, of small molecules, e.g. water molecules within the matrix. Such motions can play an important role in chemical reactions that may occur with labile molecules, proteins, even at low sub-T_g temperatures.

Fig. 3 displays the monotonic dependence of T_g on composition. The glass transition profile does not, however, reveal the complex dynamic behaviour of such mixtures, as revealed by nuclear magnetic relaxation studies [36]. Fig. 8 illustrates the ratio of the rotational diffusion times of sucrose and water $\tau_{sucrose}/\tau_{water}$ in their mixtures. As expected, in dilute solutions the rotations are decoupled, but at higher sucrose concentration, corresponding to supersaturation conditions, the motions undergo a gradual coupling process, symptomatic of network formation, involving both sucrose and water. At even higher concentrations, and approaching T_g, the coupling is reversed, implying that water molecules regain a high degree of mobility by being excluded from the hydrogen-bonded network. The chemical consequences, if any, of 'high mobility water' in a PHC glass remain to be investigated.

Another process involving network rearrangement is observed in the gradual dehydration of crystalline PHC hydrates. The process of controlled dehydration of raffinose pentahydrate involves complex recrystallisation and amorphisation steps, which can be reversed, although slowly, by the addition of low amounts of water or storage of the anhydrous sugar in a humid atmosphere [37,38].

Finally, a frequently cited 'rule' in pharmaceutical chemistry states that crystalline drug substances are more stable towards chemical degradation than their amorphous counterparts. A thorough test of this 'rule' has been performed with insulin, showing that the glassy form exhibits a superior stability over the crystalline form [39]. The reasons are obscure, but the finding serves to illustrate that, despite advances in an understanding of complex amorphous preparations, unresolved problems remain to be addressed in the physics and chemistry of highly supersaturated aqueous mixtures.

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publication, [1] opened our eyes to the basic foundation that underpins the edifice, which the rest of us have constructed during the past 50 years.

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