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Structural and Dynamic Properties of Crystalline and Amorphous Phases in Raffinose-Water Mixtures

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Purpose. To obtain an improved characterisation of the raffinose-water solid-solid and solid-liquid state diagram, and to study the thermophysical behaviour of the solid amorphous phase. This information is expected to shed light on the potential of raffinose as a pharmaceutical excipient, for stabilising labile preparations at high temperatures.

Methods. X-ray diffraction, scanning electron microscopy, polarised-light microscopy, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were applied to study raffinose pentahydrate and its behaviour during progressive dehydration.

Results. Isothermal dehydration of raffinose pentahydrate led to its gradual amorphisation, but also to minor changes in the diffractograms, suggesting the probability of lower stable hydrates. Their existence was confirmed by DSC. Anhydrous raffinose was found to be completely amorphous, and this was supported by the gradual disappearance of birefringence during dehydration. In contrast, electron micrographs, taken during the dehydration process, exhibited no changes in the original ultrastructural crystal morphology. The widths of the glass-to-fluid transitions and the absolute specific heats of crystalline and amorphous phases in the vitreous and fluid states were used to estimate some structural and relaxation characteristics of amorphous raffinose-water mixtures.

Conclusions. Raffinose forms the most "fragile" glass of those pharmaceutical excipients for which data are available. In its thermomechanical properties, it is superior to trehalose and should therefore be effective as a long-term stabiliser for dried biopharmaceutical preparations at temperatures up to 65°C.

KEY WORDS: raffinose pentahydrate; vitrification; dehydration; excipient; stabilisation.

INTRODUCTION

Di- and oligosaccharides, as well as other polyhydroxy compounds (PHCs) have found increasing application as lyoprotectant excipients in dried pharmaceutical preparations. The nature of their efficacy is still subject to speculation. An analogy of *in vitro* drying with natural resistance to desiccation by drought or freezing has also been recognised (1). PHCs found in desiccation-resistant organisms include, for example, glucose, trehalose, stachyose, raffinose, glycerol and sorbitol (2–4). The search for a common mechanism of protection has turned to properties, common to PHCs and certain other compounds, which might account for their ability to protect labile molecules and complex supermolecular structures, even whole organisms, against the removal of liquid water.

A property, common to the majority of PHCs, is their propensity to supersaturation during the drying of their solutions, resulting in an eventual vitrification (5). The glass forming potential of carbohydrates was earlier identified as an important chemical and microbiological stabilising factor in low and intermediate moisture food technology (6). Water is now regarded as the universal plasticiser of amorphous, dried natural products, and the thermomechanical properties of natural materials can be correlated with their ability to provide for chemical and microbiological stability.

The same approach has subsequently been employed to investigate the stability and stabilisation of labile therapeutic substances, such as protein-based drugs (1). Here again, it was found that the glass transition temperature (T_g) of a given formulation provided a distinct dividing line between fairly rapid inactivation and an enhanced shelf life. Both in food and in pharmaceutical technology, T_g is now considered as a significant processing parameter in the promotion of physical and chemical stability.

Solid-liquid phase and thermophysical properties of PHC-water mixtures have therefore been subjected to detailed studies. Several unpublished and/or proprietary reports suggest that even T_g cannot, by itself, be taken as a the sole determinant of product stability (7). Studies performed at sub-T_g temperatures, are providing information on slow relaxation processes, characteristic of amorphous materials in general (8). It has also been reported that amorphous PHCs can undergo slow solid phase transitions, which in the presence of water may also involve the partial, or complete, crystallisation of hydrates (9).

The binary system water-raffinose is of major interest, because of its occurrence as a stable pentahydrate (R5W), the highest hydrate known of any oligosaccharide. The amorphisation of R5W during dehydration and the thermophysical changes in the amorphous solid during water vapour sorption have been reported (10). The sorption data and effects of sorption on the recrystallisation of raffinose do, however, pose some unresolved problems.

Similar dehydration-induced amorphisations have been reported for α,α-trehalose dihydrate (11) and β,β-trehalose tetrahydrate (12). It was found impossible to induce crystallisation of the amorphous anhydrous sugars, at least in real time and under the actual experimental conditions employed. Earlier studies on crystalline phase transitions in carbohydrates had, however, produced evidence of an anhydrous, crystalline trehalose, melting at 215°C (13). A more recent report, (14) describes how, under varying conditions of dehydration of trehalose dihydrate, several polymorphs, including the anhydrous sugar, can be obtained.

We earlier identified the possible existence of amorphous and crystalline phases that may coexist and the kinetics of some amorphous-crystalline phase transitions in binary water-raffinose systems (15). Apart from the pentahydrate, the nature of the solidus and liquidus curves suggested the possible existence of lower hydrates. The present report extends our previous

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studies, with emphasis on two aspects: the elucidation of structural changes during the dehydration of R5W and the thermophysical characterisation of the solid amorphous phases.

EXPERIMENTAL

Raffinose pentahydrate, α-D-galactopyranosyl-(1 → 6)-O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside (ex Sigma, >99% pure),[6] was used without further purification. Distilled water was used for all experimental work. Samples were usually prepared as follows: powdered R5W was dried at temperatures ranging from ambient to 60°C, under reduced pressure (50–100 mbar). Residual water contents were determined by mass loss during evaporation and by a coulometric Karl Fischer method. In contrast to earlier reported studies (10,15), R5W samples were carefully dehydrated so as to arrive at stoichiometries corresponding to R4W, R3W, R2W, R1W and AR (anhydrous raffinose). Some samples for DSC were prepared by holding R5W powder in a desiccator at 25 or 35°C over saturated NaOH solution. Samples were periodically weighed, until the water content had been reduced to the desired value. Samples were then hermetically sealed in DSC pans.

X-Ray Diffraction

Crystalline phases in the partially and completely vacuum-dried samples were identified by XRD on a Philips PW BASED diffractometer, using a 45 kV × 40 mA wavelength of CuKα = 1.54 Å, at room temperature and ambient relative humidity. Samples were loaded into a sample holder of 2 mm thickness and scanned at a rate of 5° (2θ min^{-1}). In order to ensure adequate spherical averaging, the samples were rotated in the beam. A sample of completely dried raffinose (AR) was remeasured after 30 min exposure to ambient conditions. No difference in the diffraction patterns could be observed, indicating that water uptake from the atmosphere was negligible in the short term.

Scanning Electron Microscopy

Small amounts of R5W and AR were sprinkled onto the surfaces of two separate cleaned and polished aluminium specimen stubs. Each sample was loaded via an air lock onto the cold stage of an Oxford Instruments CM1500 Cryopreparation Module which had been precooled to −150°C. Samples were allowed to equilibrate for 10 min before being transferred, via a second air lock, onto an Oxford Instruments Low Temperature Module, attached to the stage of a Philips Instruments XL30 Hot FEG scanning electron microscope and precooled to −150°C. The microscope is fitted with turbomolecular and ion pumps in order to maintain a high and clean vacuum. The specimen stub and sample were allowed to equilibrate for a further 10 min before examination. To minimise surface contamination, a fenestrated liquid nitrogen cold trap was located about 5 mm above the sample surface.

Samples were examined uncoated over a wide range of temperatures at 2 kV and a beam current of 65 pA and photographed at 25 deg intervals from −150 to +125°C. After each photograph was recorded the temperature of the stage was increased by 25 deg, and the samples were allowed to equilibrate for 10 min before re-examination. The same region of the sample was examined throughout the experiment. During the three hours needed to complete each experimental run the pressure inside the microscope chamber increased from 980 μPa to 1.4 mPa for the R5W sample, and from 300 μPa to 450 μPa for the AR sample.

Polarised-Light Microscopy

R5W and AR were observed by crossed nicol polarised-light microscopy on a Nikon OPTIPHOT2-POL microscope. A small amount of the solid powder was sprinkled onto to the surfaces of glass slides. Monobromonaphthalene was used as immersion oil and cover slips were placed over the preparations prior to examination.

Differential Scanning Calorimetry

Measurements were performed with a Perkin Elmer Model 2 DSC, equipped with an Intracooler-2 unit (or with a Shimadzu DSC-50). The instrument had been modified to significantly enhance its sensitivity and provide for a data processing facility (16). Temperature calibration was performed at a scanning rate of 5 deg min^{-1} to within 1°C, with water, cyclohexane and indium. Experimental heating and cooling rates were also 5 deg min^{-1}. Usually solid samples were heated from 25 to 125°C, except where noted. Melting points, heats of fusion and/or glass transitions were measured during this first heating scan. Samples were then cooled from 125°C to −28°C and reheated back to 125°C. Glass transitions were again recorded. They were taken as the onset temperatures of heat flow discontinuities. Widths of the glass transitions, ΔT_g, i.e., the temperature difference between the extrapolated onset and completion of the specific heat discontinuities, were also recorded.

Absolute heat capacities of crystal and glass phases and of the fluid phase at the completion of the glass transition were also measured; an experimental procedure previously described for this purpose was employed (17,18).

Enthalpic relaxation rates of anhydrous raffinose glasses were determined at four temperatures below T_g by the standard method of integrating relaxation endotherms, corresponding to increasing annealing times (19). The energy of activation, ΔH^* was obtained from an Arrhenius plot.

Thermogravimetric Analysis

TGA measurements were performed with a Perkin-Elmer TGA7. About 10 mg of R5W powder was loaded into a sample container and heated from 25 to 125°C at rates of 1.0, 0.5 and 0.1 deg min^{-1}, respectively. Like other temperature scanning techniques, the method has limitations when even the lowest scan rate (0.1 deg min^{-1}) becomes "fast" relative to the process being measured.

RESULTS

X-Ray Diffraction

X-ray powder diffractograms corresponding to R5W and partly dehydrated forms, corresponding to stoichiometries R4W

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[6] In our earlier publication (11) and also in ref (12) an incorrect formula for raffinose was printed.

and R3W, are shown in Fig. 1a–c. At the resolution employed, the diffractogram for R5W agrees in every respect with that reported in ref. 10.

X-ray measurements here reported are suggestive of the existence of more than one crystalline phase but are not of a high enough resolution to identify unambiguously small changes in atomic positions, such as might be expected to occur during solid-solid phase changes involving R5W, R4W and/or R3W. Diffraction measurements of a higher resolution are currently in progress. Even so, the diffractograms, shown in reduced form in Figs. 1a–c, reveal a number of line shifts, especially in the region $10° < 2\Theta < 25°$. Partial amorphisation becomes detectable in Fig. 1c, i.e., at dehydration levels beyond R4W, but the present data do not permit a detailed analysis of the residual crystalline part of the diffractograms for lines that are not characteristic of R5W.

Polarised-Light Microscopy

Polarised-light micrographs of a R5W crystal in its fully hydrated and its dehydrated form (AR) showed that the pronounced birefringence exhibited by R5W almost disappears during dehydration, but some residual birefringence is still detectable, even though residual water could no longer be detected by Karl Fischer analysis. Despite amorphisation, the original crystal ultrastructure is maintained during dehydration.

Birefringence alone is not necessarily an indicator of crystallinity. For instance a residual birefringence can be caused by stresses frozen into the material during its formation. The sharpness of contrast of the dehydrated samples obtained did not have the appearance of smooth, curved contours which would be expected for birefringence contours in a stressed glass.

Scanning Electron Microscopy

Four micrographs are shown in Fig. 2, taken before and after the dehydration of R5W at −150°C and 100°C, respectively. The period of heating was 3 h in both cases. No detectable changes in the crystal ultrastructure of either sample can be observed as a result of heating. It is unlikely that that under conditions of high vacuum (ca. 1 mPa) in the microscope chamber, water of crystallisation could be lost from R5W at −150°C. The saturation vapour pressure of water at −150°C is 668 mPa. While the potential for water sublimation at temperatures above −100°C cannot be altogether discounted, the rate of such water removal would be very low during the observation period. The morphological stability towards dehydration and towards temperature may be considered to be at odds with the diffraction data which unambiguously indicate progressive amorphisation. The dehydration in the electron microscope chamber was, however, carried out under conditions that would leave the resulting amorphous products well below their respective glass transition temperatures and, therefore, subject to no observable viscous flow or structural collapse on the time scale of the experiments.

Differential Scanning Calorimetry (DSC)

From earlier DSC results (15), the melting point and heat of fusion of the putative tetrahydrate are 357.3 ± 0.3 K and 72.7 ± 2.2 kJ mol^{-1}, respectively. The widths of glass transitions, ΔT_g are shown in Fig. 3 as a function of the raffinose mol fraction, x_r, for the range R5W → AR. Data points refer

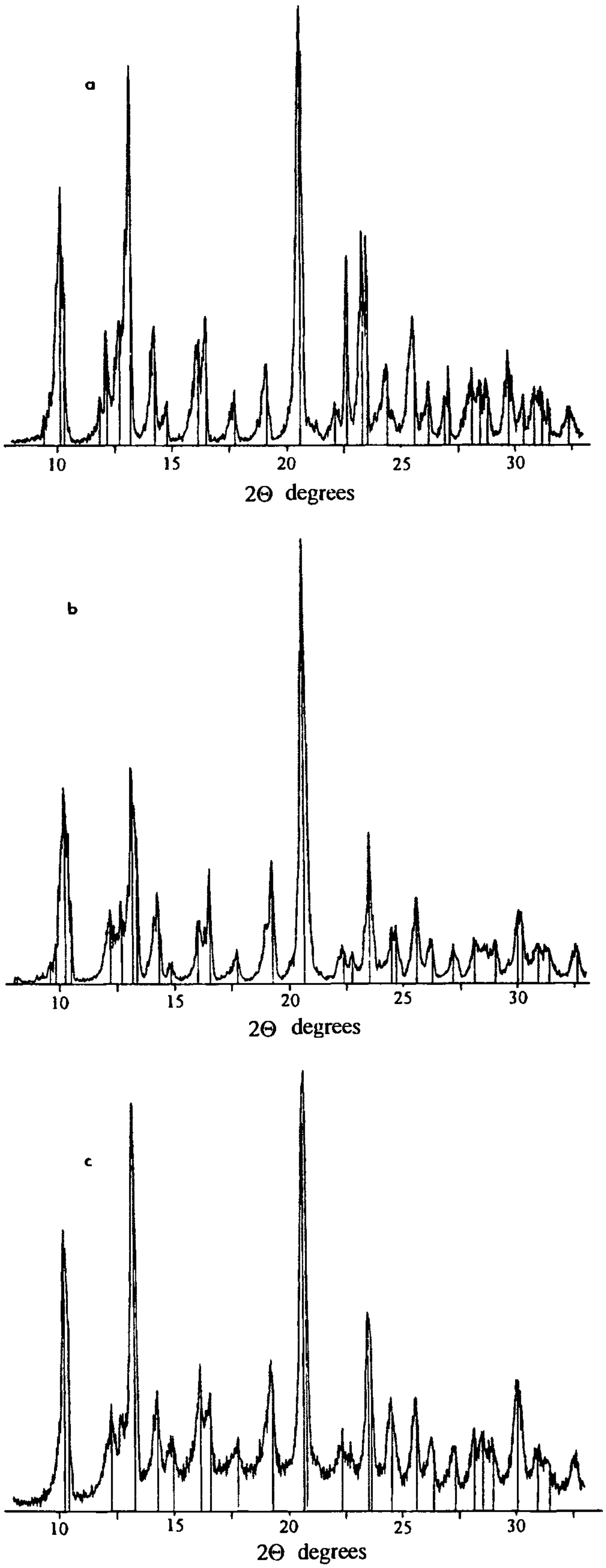


Fig. 1. X-ray powder diffractograms of R5W (a) and partially dehydrated forms, corresponding to stoichiometries R4W (b) and R3W (c).

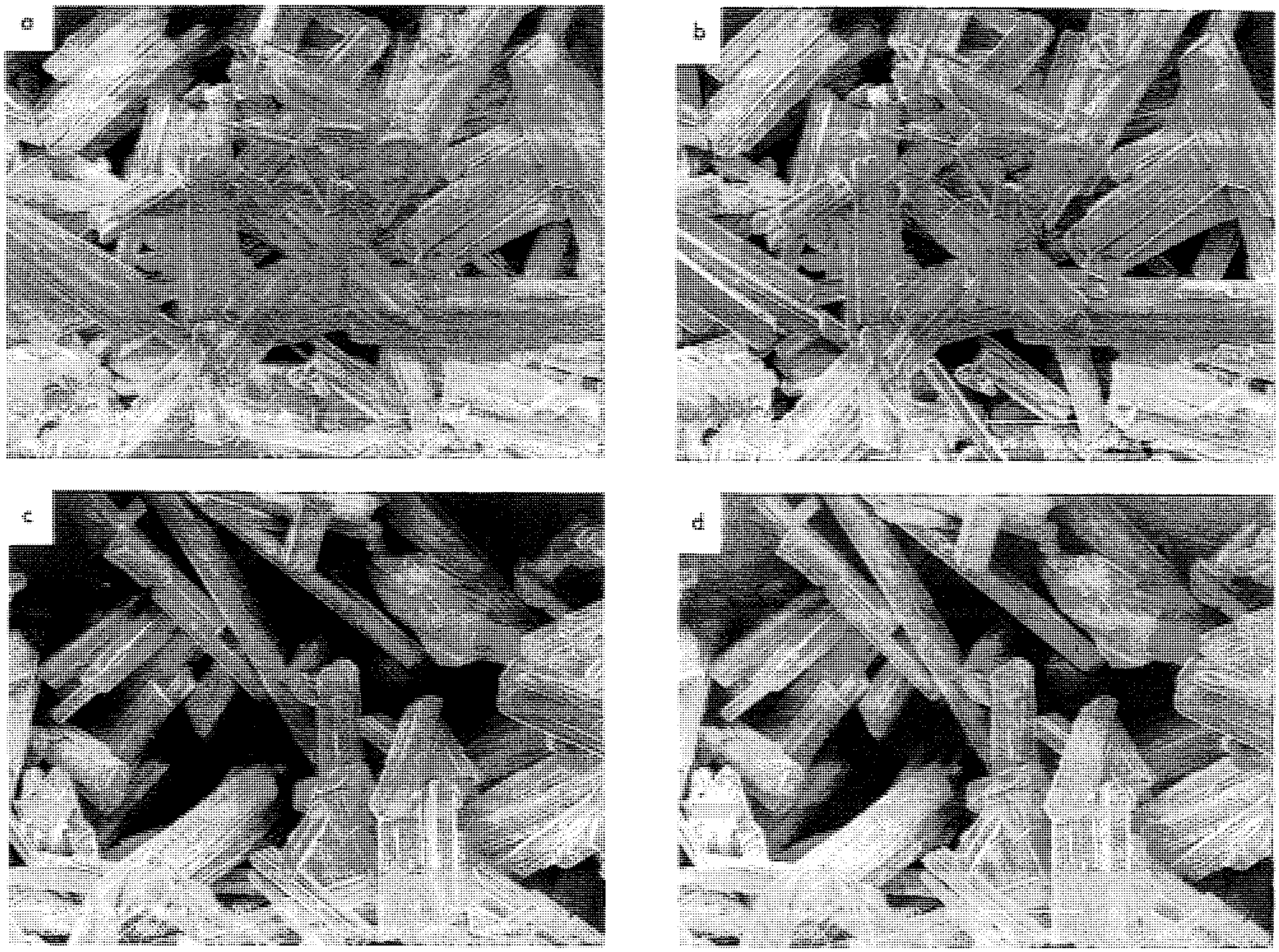


Fig. 2. Scanning electron micrographs of crystalline R5W at (a) −150 and (b) + 100°C and of amorphous AR, after dehydration at (c) −150 and (d) + 100°C. Scale bars correspond to 100 μm. For details, see text.

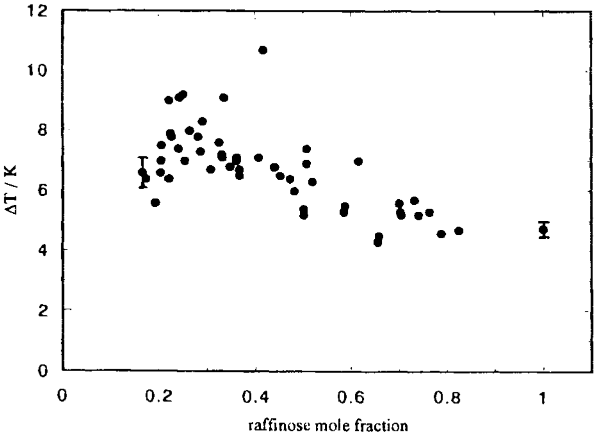


Fig. 3. Widths of the glass transition ΔT_g of raffinose-water mixtures as a function of the raffinose mol fraction. The scatter in the data points most probably due to the fact that they were obtained from different samples over a period of several months. The two points, shown with error bars were obtained under controlled conditions, on six or eight independent samples, scanned in sequence over a short period of time, at identical cooling and heating rates.

to single measurements on different samples, except for the two circles with error bars which correspond to the mean of seven or eight measurements on different samples of the same composition. Measurements were conducted over a period covering several months, which might account for the scatter of the data. Despite the scatter, however, a non-monotonic trend in the composition dependence of ΔT_g is noticeable.

Molar heat capacities of R5W and AR in respective crystalline and amorphous states (glass and fluid) are collected in Table 1; respective standard deviations are also indicated. For purposes of comparison, earlier data for glucose, fructose and sucrose are included in Table 1 (17). It should be noted that in some earlier work, glass transitions were measured as the midpoints of the heat flow discontinuities. This should, however, not affect the measured heat capacity changes.

The activation energy of structural relaxation, ΔH^*, determined from DSC measurements, following annealing at sub-T_g temperatures, was estimated as 1,205 kJ mol^{-1}. It falls within the range of structural relaxation activation energies of a wide variety of inorganic glasses (9).

Thermogravimetry (TGA)

Figure 4 shows a TGA scan for a R5W sample, performed at a heating rate of 0.1 deg min^{-1}. No weight loss can be

Table 1. Glass Temperatures and Molar Heat Capacities of Saccharides ($J\ K^{-1}\ mol^{-1}$)

		$C_p/(J\ mol^{-1}K^{-1})$			
		Crystal	Glass	Fluid	
	T_g/K	at 300 K	at T_g	at 300 K	at T_g
R5W	262	875 ± 21	966 ± 16	1460 ± 28	1289 ± 29
AR	376	—	660 ± 35[a] 890 ± 65		1192 ± 81
glucose	311	221	282		382
fructose	289	230	157		239
sucrose	343	430	658		765

[a] Temperatures in the tables are given in Kelvin, because they are used in the calculations of thermodynamic and relaxational properties at 300 K.

detected below ca. 36°C. Thereafter, weight loss is rapid until a concentration of 97% w/w is attained, corresponding to the R4W stoichiometry. The slope of the scan changes at this composition. A further change in the slope is observed at 73°C and a concentration which would correspond to a crystalline $R\frac{1}{2}W$. The X-ray data for this composition do, however, indicate an almost complete amorphisation. Similar results were also obtained at higher scanning rates. At advanced stages of dehydration the results become scan rate dependent, suggesting that the water loss is then substantially slower than even the slowest scan speed; results thus become unreliable.

DISCUSSION

Crystalline Phases

The only significant data available on R5W are its crystal structure (20) and its amorphisation/recrystallisation behaviour as water is withdrawn and readmitted. It has been stated that no lower crystal hydrate structures exist. That is, however, incompatible with the water sorption data. The results in Fig. 1 and the birefringence data suggest that some form of crystallinity is completely maintained during dehydration down to R4W, and that only thereafter does partial amorphisation occur. If true, this would be in accord with our earlier DSC studies of the solid-liquid phase diagram which provide evidence of lower hydrates, in particular R4W and/or R3W.

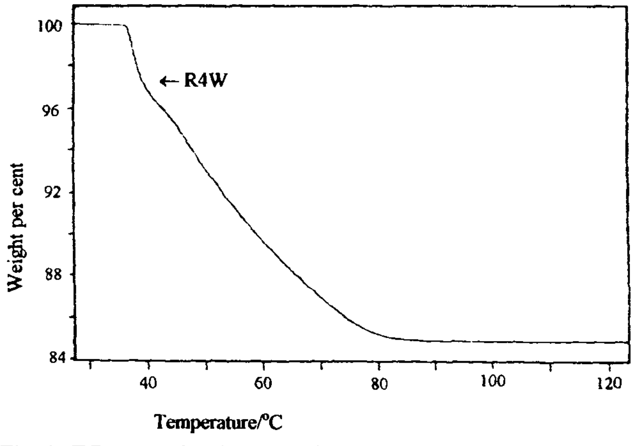


Fig. 4. TGA scan showing the weight (water) loss of crystalline R5W as a function of temperature. Scan rate: 0.1 K/min.

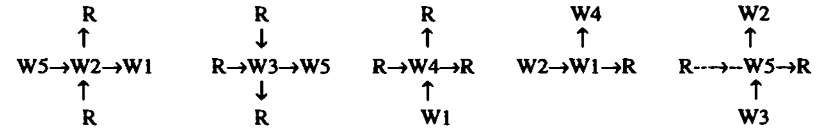
The structure analysis of R5W demonstrates that all oxygen atoms in raffinose and water molecules participate in hydrogen bonding (20). The structure superficially resembles those of other sugars, consisting of an infinite, three-dimensional network of sugar molecule chains. The difference from most other PHC structures is that the chains intersect only at the locations of water molecules, with all water molecules involved in at least three hydrogen bonds. Four of the five water molecules form two acceptor and two donor hydrogen bonds, with only W1 acting as double donor and single acceptor. A simplified water hydrogen bonding scheme at the chain intersection points is shown in Scheme 1, showing that water molecules at chain intersections form hydrogen bonds with sugar residues but also with other water molecules.

By analogy with many systems which exhibit multiple stable hydrates it might be inferred that progressive drying of R5W should give rise to a succession of lower hydrates, eventually leading to the anhydrous crystalline sugar. This is found for monohydrate sugars, but the situation for sugars which form higher hydrates appears to be more complex. If the rate of desiccation exceeds the rate required for the necessary hydrogen bonding rearrangements to occur in the increasingly viscous, supersaturated sugar-water mixture, then it is probable that bond mismatches will occur and become frozen into the network, giving rise to amorphisation. As an example of this type of behaviour attention is drawn to the well-known residual entropy of ice which results from the "freezing-in" of hydrogen atoms in random positions along the O-O axes.

Conceptually, the removal of water molecules from the crystal might proceed in two distinct ways, probably depending on the differential hydrogen bonding energies. Either groups of specific water molecules (e.g., all those designated as W1 in Scheme 1) are removed sequentially, or water molecules are removed from the network randomly. The progressive removal of water molecules from the lattice must in any case lead to a perturbation of the hydrogen bond topology. This might be accompanied by one of several possible "repair" mechanisms: either new sugar-sugar bonds will be formed to replace the sugar-water bonds, giving rise to a new crystal structure, or a new network will form, but in a random manner, thus producing amorphous domains within the remaining R5W crystal structure. The latter mechanism is more likely to occur if water had previously been removed from the crystal in a random manner. The vapour sorption data (10), our DSC studies (15) and the TGA results here reported all suggest that controlled (i.e., slow) desiccation, either isothermally or by heating, leads to the sequential loss of specific water molecules from the lattice, at least down to R3W. Further desiccation, as performed experimentally, may then become too "fast" for new, ordered lattices to become established, with the practical result of progressive amorphisation. Recent evidence of a "cold crystallisation" of anhydrous trehalose at high temperatures raises the question whether prolonged heating of fluid AR might also result in such a "cold crystallisation." This possibility remains to be studied.

The Amorphous State

The most significant attributes of a pharmaceutical lyoprotectant are its behaviour during the drying of a dilute solution which is likely to contain at least the drug substance and buffer



Schematic 1. Water-centred intermolecular hydrogen bonding patterns in crystalline raffinose pentahydrate. Arrows indicate the direction of proton transfer from donor to acceptor. A broken line indicates a long (weak) hydrogen bond.

salts, its ability to promote the eventual vitrification of the supersaturated solution and to maintain the physical and chemical stability of the dried product under specified conditions of storage. Polarised-light microscopy demonstrates the almost complete vitrification of AR.

The electron micrographs in Fig. 3 illustrate the uncoupling of diffusional modes in supersaturated aqueous solutions. Thus, water is readily removed from the crystalline hydrates by reduced pressure, and this process is accompanied by progressive amorphisation of the residual sugar matrix. On the other hand, this amorphous phase does not exhibit collapse on any practical time scale. The macroscopic ultrastructure of the original crystalline phase is thus maintained, despite the removal of long-range order within the phase, produced by the diffusive removal of water.

The glass temperature T_g has long been recognised as an essential parameter in setting the border between fairly rapid deterioration and an improved stability. Early assumption that a glassy state is sufficient to achieve a long-term stability of pharmaceutical products have, however, had to be revised in the light of more recent fundamental research into the thermomechanical properties of water-soluble glasses. As the "glass transition" stability hypothesis has been put to practical tests, it has become apparent that the correlation between T_g and stability is, at best, an oversimplification. PHC glasses undergo slow relaxation processes, even well below T_g, so that additional physical properties of amorphous solids need to be considered.

A significant advance classified amorphous fluids (e.g., glasses) according to their "fragility" (21), a parameter which describes the effects of temperature on the internal degrees of freedom and hence the relaxation rate of the disordered, thermodynamically unstable molecular ensemble towards its global equilibrium state. According to this concept, "strong" fluids display an Arrhenius-type response when heated at temperatures above T_g, whereas the transport and relaxation rates of "fragile" fluids change more rapidly with temperature than would be predicted by the Arrhenius relationship. In temperature scanning analytical techniques the fragility is characterised by the width of the glass transition, with strong fluids displaying broad transitions, usually in excess of 20 deg, with small changes in the heat capacity, whereas fragile fluids give rise to narrow transitions, typically < 10 deg, accompanied by pronounced heat capacity changes.

A knowledge of the width of the glass transition provides an additional method to calculate the enthalpy of activation of structural relaxation processes, ΔH^*, which presumably involve hydrogen bond rearrangements. Knowledge of ΔH^* allows the fragility parameter δ to be estimated which, in turn, is related to the so-called "temperature of zero mobility," T_0 (22). This is the notional temperature at which the free volume, and therefore also the configurational entropy, approach zero and which is therefore considered to be the limit of "infinite" physical stability of a thermodynamically unstable system. For details of the relationships between T_g, δ, T_0, and free volume, the reader is referred to refs. 9, 21, 22 and 23.

ΔH^* can be obtained independently by direct DSC and by mechanical thermomechanical measurements on samples that have been stored for various periods well below T_g. Such measurements led Hancock *et al.* to the conclusion that sucrose needs to be stored at temperatures ca. 50 degrees below T_g, before molecular motions (as detectable by DSC) become negligible over practical shelf lives (24).

Although these various relationships have been extensively tested on "conventional" inorganic glasses of different chemical types, it is only recently that they have been applied to studies of PHCs (25,26). With the aid of the results in Fig. 3, we have calculated the salient parameters for R5W and AR; they are summarised and compared with available data for several other PHCs in Table 2.

There is currently an ongoing debate whether treatments developed for "real" materials (23) can be readily applied to PHCs and other pharmaceutical excipients. We believe that the fragility concept does appear to hold for such materials. Furthermore, the methods here used for the estimation of T_0 from ΔT_g and δ have been found to be valid for pure solids and solid solutions of quite different chemical types, e.g., metals, salts, silicates, oxides, etc. There appears to be no *a priori* reason why they should not apply to PHCs. Even if some of the numerical constants used in the derivation of T_0 might require modification, the ranking of PHCs, according to their T_0 values would be unaffected. It is encouraging to note that the T_0 values for sucrose and raffinose, estimated from ΔT_g, agree reasonably closely with those derived from direct enthalpic relaxation measurements (24).

Inspection of the results for R5W and AR indicates the plasticising potency of water, as reflected in the δ values and T_0. The water-plasticised glass is thus seen to be less fragile than its anhydrous counterpart. If the trend in ΔT_g with composition,

Table 2. Characteristic Thermophysical Properties of Amorphous Sugars

Sugar	T_g/K	ΔT_g/K	$\Delta H^*/RT_g$	δ	T_0/K
R5W	261.7	6.5	198	9.7	210
AR	376.4	4.7	389	4.4	338
Trehalose	389[a]	7.0	258	7.0	317
Sucrose	337[a] 343[b]	7.5	258[a] 224[b]	8.6[a] 8.3[b]	276[a] 283[b]
Fructose	283[b]	6.0	232	7.9	235

Note: For Details, see text and refs. 9, 21, 22, 24 and 26.

[a] Data from ref. 26; sugars contained low amounts (< 1%) of residual water.

[b] Data from ref. 18; fused and quenched anhydrous sugars contained no water.

shown in Fig. 3, is taken at face value, then it suggests a corresponding trend in the fragility of raffinose-water mixtures.

The comparison of anhydrous raffinose and trehalose illustrates the greater fragility of raffinose, producing a substantially higher T_0 value than that reported for trehalose (26). If, therefore, the T_0 value is also to be taken as a measure of the long-term stabilising capacity of a pharmaceutical excipient (25), then raffinose would clearly be the preferred choice. According to the figures here reported, AR should be able to provide extended shelf lives at temperatures up to 65°C!

Raffinose and trehalose have already been shown to undergo crystallisation to their respective hydrates, in the presence of water (9,15). Such an *in situ* drying by crystallisation of a hydrate will maintain T_0 of the residual amorphous phase at its original high level, even when low amounts of water might inadvertently have entered a dry product during prolonged storage.

Heat Capacities

We defer a detailed discussion of the results in Table 1 until full results of the synchrotron data are available. A missing quantity is the heat capacity of a (hypothetical) crystalline AR. If such a substance could be produced, perhaps by "cold crystallisation", in an analogous manner to anhydrous trehalose (14), it would greatly assist the interpretation of the available C_p data. To complete the picture, heat capacity data for the lower raffinose hydrates would also assist such an analysis.

The relationships between heat capacities of crystalline and vitreous sugars in terms of the Debye theta function have been discussed earlier (17). For extensively hydrogen-bonded crystals (and glasses?), the simple relationship between the Debye theta and the specific heat cannot be expected to hold. This conclusion is supported by the neutron scattering data for glucose in its fused and vitrified states, which provide the only currently available experimental information about differences and similarities between the intermolecular hydrogen bonding patterns in the crystalline and amorphous states (27). A study of the various intermolecular atom pair distribution functions reveals that the number of hydrogen bonds decreases by ca. 20% upon fusion and in the undercooled state at 80°C but returns to near the crystal value during cooling into the vitreous state. The main change then observed is a distortion of hydrogen bonds in the glass. A fused or vitrified sugar can thus hardly be treated as a classical liquid in terms of its thermophysical properties, and a detailed analysis of the comparative heat capacity data must await a fundamental theoretical analysis of vitrified networks linked by weak, orientation-specific bonds.

SUMMARY AND CONCLUSIONS

Further evidence of the complexities encountered during the drying of PHC solutions is presented, with special reference to raffinose. Evidence for lower hydrates, in addition to the well-known pentahydrate, has been strengthened, but complete confirmation must await a full structural analysis of high resolution X-ray data.

With the aid of relationships developed primarily for conventional inorganic glasses, the salient thermophysical parameters have been calculated for R5W and AR and compared to similar data for other sugars. Methods which have proven useful for the characterisation of inorganic glasses also yield realistic results for anhydrous or water-plasticised PHC glasses.

To apply PHCs as physical and/or chemical stabilisers of pharmaceutical products, it is desirable to develop a good understanding of slow physical and chemical changes which they can undergo during drying and thereafter, and the possible influence of such changes on bioactive materials incorporated in their glassy matrices.

The advantages of anhydrous amorphous sugars which are able to crystallise as hydrates have been placed on a firmer basis; such sugars can act as *in situ* desiccants. Coupled with this attribute is a requirement that any such crystallisation should be slow and must not occur during the drying stage, whether it be by lyophilisation or some other process.

Its superior thermophysical properties, and also its unique ability among oligosaccharides to crystallise as a pentahydrate, render raffinose suitable as a stabiliser, especially where formulated products might suffer temperature abuse during distribution, or moisture pickup during prolonged storage.

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