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# SOLUBILITY AND GLASS TRANSITION IN THE SYSTEM α-*D*-TREHALOSE/WATER

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## Abstract

Precipitation of trehalose dihydrate in water is observed at room temperature for trehalose concentrations higher than 47.5%*w/w*. Direct observations of crystal melting in water and measures of the solution density determine the thermal variations of trehalose saturation $S(T)$ (*mM*) in water: $\ln(S(T))=\ln(0.1223)-(1330/T)$ with $R^2=0.9982$. The glass transition ($T_g$) curve measured by DSC is lower at low concentrations and higher at high concentrations than previously reported. $T_g$ is also measured as a function of the cooling/warming rates. Analysis of specific heat changes at $T_g$ and associated activation energy leads to identify a most stable glassy state around the second eutectic concentration.

**Keywords:** glass transition, phase diagram, solubility, trehalose, water

## Introduction

Trehalose was found to be a good agent for the protection of biological cells during either freezing or drying stresses [1] and was nicknamed the drought preserver sugar in 1920s. Trehalose is actually tested for storage of human blood cells by freeze-thaw or freeze-drying [2]. This protection was interpreted by Green and Angell as due to anomalously high glass transition temperatures $T_g$ for trehalose compared to other sugars [3]. Franks argued however that trehalose is clustering water molecules around itself as forming a dihydrate and protecting cells by removing the available water molecules [4]. To analyze these hypothesis, the system α-*D*-trehalose/water is presently studied by DSC for its phase diagram, with the thermal variation of the saturation concentration of trehalose in water and the $T_g$ curve and its dependence with cooling/warming rates [5–7] to estimate the stability and fragility of formed glasses.

## Materials and methods

Samples are prepared by dissolving α-*D*-trehalose dihydrate (Sigma, reduced or non-reduced metal content) in deionized water at high temperature in glass vials. Growing crystals around 300 K are observed for concentrations as low as 47.5%*w/w* as samples are cooled back to room temperatures. Their growth is

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slower for a reduced metal content as metals act as nucleation sites. Using the dry sample weight, they are identified as trehalose dihydrate crystals and used for preparing samples. Solutions are dried in the DSC pans at various temperatures and times to achieve high concentrations. Sample transparency is checked for the absence of white anhydrous trehalose crystals. Final concentrations are checked using the sample dry weight after removing of water by heat. Both Sigma dihydrate and precipitate have been tested by DSC using open pans from 163.15 to 503.15 K. They both melt at 372.2±3 K, then water evaporates enabling the anhydrous trehalose to crystallize before melting at 488.2 K. The present temperatures are within less than 2 K from those reported [8].

### *Solubility*

No complete trehalose saturation curve has previously been published. Two sets of samples with various concentrations in scintillation vials are exposed, while shaken, to step by step increasing temperatures in a water bath 181 from Precision Scientific Inc., to measure the temperature of complete dissolution of trehalose dihydrate crystals. Warming rates are less than 0.5 K $\min^{-1}$. Temperature reading errors are less than 1 to 3 K for higher temperatures due to the slow crystal dissolution.

### *Density*

The solution density is measured using a Mettler/Paar DMA40 (Anton/Paar, Graz, Austria) calibrated using deionized water, deuterium water (Sigma 100%) and glycerol (Sigma) with a temperature controlled by a Mettler Exacal EX-100 bath and measured with a Bat-12 thermocouple within 0.1 K. The density error is less than 0.1%.

### *DSC*

Melting $T_m$ and glass transition $T_g$ temperatures are recorded with a DSC-4 Perkin-Elmer calibrated as usual with various organics [5] and indium providing a temperature error less than 0.4 K. Cooling/warming rates are from 0.5 to 80 K $\min^{-1}$. $T_g$ is here defined as the transition onset temperature. The fictive temperature would have been a better choice to take into account the width of the transition [9] but is not presently determined. The dependence of $T_g$ with the scanning rate $V$ is analyzed using the method of Moynihan [6]:

$$\ln(V) = A + (Eg/RT_g) \tag{1}$$

or using a more recent dynamics approach reported by Shi [7]:

$$\ln(V) = \ln(T_g/B) + [Eg'/T_g - T_s)] \tag{2}$$

with $A$, $B$ are constant, $Eg$ and $Eg'$ are apparent activation energies.

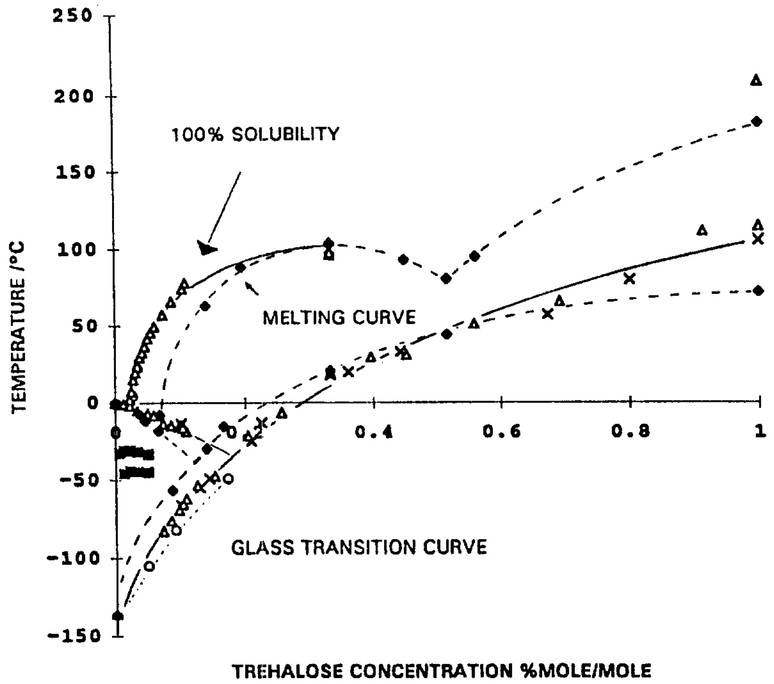

**Fig. 1** Phase diagram reporting the temperature of 100% dissolution of trehalose dihydrate in water, the glass transition temperature $T_g$ and the melting temperature $T_m$ of ice during warming for the present study with the Sigma trehalose dihydrate (Δ) or the precipitate crystals (×). The points (■) are glass transition temperatures of the remaining solution after all ice has crystallized during the initial cooling. Published data from [3] (♦) and [10] (o) have been reported for comparison

## Discussion

The curve for complete crystal dissolution (100% solubility in Fig. 1) must be similar to the $T_m$ curve but is different from the published data [3]. Williams reported a few similar points [10]. Using corresponding densities, saturation concentrations of trehalose in water are calculated and reported in Fig. 2 *vs.*$1/T$. $T_g$ values from this study and from literature [3] are reported in Fig. 1. Both Sigma product and precipitate give similar $T_g$ values. Various $T_g$ values have been reported for anhydrous trehalose with 80°C onset [3], 114°C [2] and 116°C at mid-glass transition [11]. Green and Angell reported $T_m$ for anhydrous trehalose 33 K lower than our data and previous data [2, 11]. A residual moisture is possibly responsible for the these variations [11] and might explain the present discrepancies with their results [3]. The $T_m$ curve for ice intersects the $T_g$ curve at a temperature $T_g'$ that is important to design the primary drying than the complete $T_g$ curve to design the secondary drying for freeze-drying processus [12]. Indeed, collapse and crystallization must be avoided during the secondary drying towards the storage temperature [12].

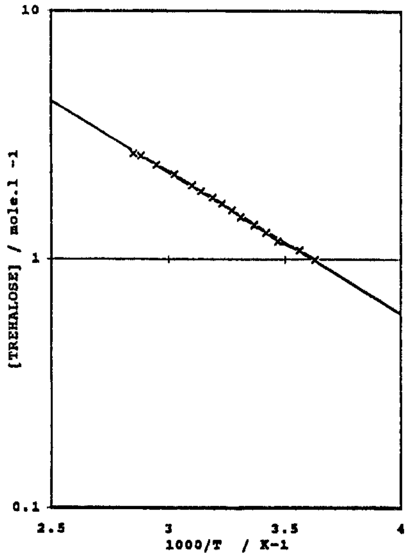

**Fig. 2** Arrhenius representation of the saturation concentrations (mol l$^{-1}$) of trehalose in water

The relaxation kinetics below $T_g$ is critical to estimate for understanding the glassy state stability during storage and the possible molecular motions leading to nucleation/crystallization or liquid/liquid phase separation through relaxation processes. Crowe and Crowe have reported the possibility not to form the anhydrous form during freeze-drying [2]. However, the dihydrate can form upon warming during the second drying from $T_g'$ concentration if time allowed, supporting Franks hypothesis of protection by hydrate formation [4] without excluding a high $T_g$ effect.

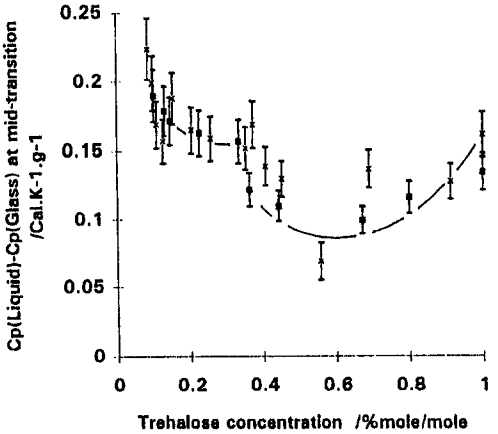

**Fig. 3** Thermal variation of the difference of specific heat at midglass transition between the extrapolated supercooled liquid and the glassy state formed during cooling for the Sigma dihydrate (×) and for the precipitate crystals (■). The full line is placed for support

For estimating the relaxation kinetics, the specific heat difference $\Delta C_p$ at mid-glass transition is first reported in Fig. 3. $\Delta C_p$ can be used to calculate the excess stored enthalpy within the glassy state depending on cooling rates while knowing $T_g$ and the ideal glass temperature defined either by the isentropic temperature of Kauzmann $T_K$ or by a dynamical temperature $T_s$ from Eq. (2). Activation energies deduced from Eqs. (1) and (2), are respectively reported in Figs 4A and 4B.

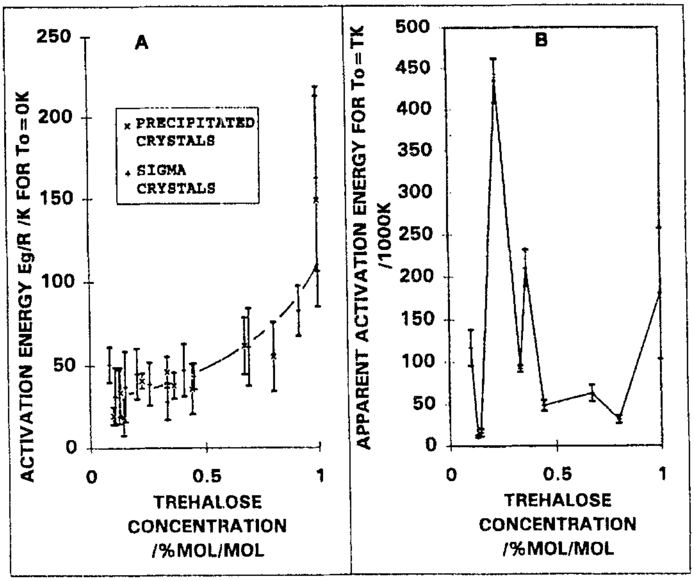

Fig. 4 A - Activation energy calculated using Eq. (1) applied to the variations of $T_g$ with the cooling/warming rates. The full line is placed for support
B - Activation energy calculated using Eq. (2) applied to the variations of $T_g$ with the cooling/warming rates

The combination of Figs 3, 4A and 4B shows the possible existence of a strongest and most stable glassy state between 94 and 100%*w/w* around the second eutectic (Fig. 1). One notes also a relative minimum for $\Delta C_p$ at $T_g$ around the first eutectic concentration.

## Conclusions

This study reports:

1 – the saturation concentration curve of trehalose in water is found different from previously reported data,

2 – $T_g$ values are lower at low concentrations and higher at high concentrations than previously reported data,

3 – the existence of a stronger glassy state between 94 and 100%*w/w* trehalose in water corresponding to the second eutectic of the phase diagram.

Naval Medical Research and Development Command Work Unit # 1642. The opinions and assertions contained herein are the private ones of the writer and are not construed as official at large.

## References

1 A. S. Rudolph and J. H. Crowe, Cryobiology, 22 (1983) 367.
2 L. M. Crowe and J. H. Crowe, Proc. Conference on Frozen Platelets and Platelet Substitutes in Transfusion Medicine, USUHS, Bethesda MD, 1996.
3 J. L. Green and C. A. Angell J. Phys. Chem., 93 (1989) 2880.
4 B. J . Aldous, A. D. Auffret and F. Franks, Proc.of the 1996 Colorado Protein Stability Conference, Breckenridge, Co, 7/10-13/1996.
5 P. M. Mehl, Thermochim. Acta,272 (1996) 201.
6 C. T. Moynihan, in "Assignment of the glass transition" R. J. Seyler, Ed., 5TP1249, ASTM, Baltimore 1995, p. 32.
7 F. G. Shi, J. Materials Res., (1996) in press.
8 F. Shafizadeh and R. A. Susott, J. Org. Chem. 38 (1973) 3710.
9 O. S. Narayanaswami, J. Am. Ceram. Soc., 54 (1971) 491.
10 R. J. Williams, Cryobiology, 22 (1985) 632 (Abst.).
11 R. H. M. Hartley, B. Roser, J. Kampinga and J. Maki, Proc. of the 1996 Colorado Protein Stability Conference, Breckenridge, Co, 7/10-13,1996.
12 E. Y. Shalaev and F. Franks, Cryobiology, 33 (1996) 14.