Diffusion of solutes in supercooled sugar aqueous solutions

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In this study we measured the diffusion coefficients of fluoresceine in aqueous solutions of sucrose and trehalose, cryoprotectant disaccharides, using the fluorescence recovery after photobleaching (FRAP). The results were analyzed on the basis of the classical continuum hydrodynamic theory (Stokes-Einstein relationship). It is shown that below a characteristic temperature the classical friction model fails to describe the diffusion of solutes in supercooled aqueous solutions. The impact of the non-classical diffusion on the prediction of reaction kinetics in these systems is also discussed.

1. Introduction

The addition of certain solutes, such as polyols and saccharides, increase the glass transition temperature of water from 136 K to values close to ambient temperature. For this reason these solutions are commonly used for the stabilization of biomolecules, preventing their degradation by delaying the molecular mobility and, consequently, deterioration reactions.

Since deterioration reactions could be diffusionally controlled, it is very important for the modeling of cryopreservation to measure the diffusion of solutes in supercooled aqueous solutions as a function of temperature and composition.

Solutes diffusion in aqueous systems of low water content and high viscosity presents a complex dynamic [1] near the vitreous transition ($\eta \sim 10^{14}$ Pa·s). The simple continuum hydrodynamic diffusion model expressed by the Stokes-Einstein relationship,

$$D = \frac{kT}{6\pi\eta r} \qquad (1)$$

where D is the translational diffusion coefficient for a solute of radius r in a solvent of viscosity η, is not longer valid because of a decoupling between D and η, which depends on the size relationship between the diffusing molecule and those of the surrounding media [2,3], as well as on the type of interaction [4].

Champion *et al* [1] observed this decoupling for the diffusion of fluorescein in sucrose-water mixtures and a similar results were observed for the decoupling of electrical conductivity and viscosity of simple salts (NaCl, $MgCl_2$) in aqueous solutions of trehalose and sucrose [5].

It is important to determine the diffusion behavior of different solutes in systems where mobility control is a decisive factor for stability, *i.e.* aqueous solutions of trehalose used as models for food conservation.

In this work we studied the translational diffusion of fluorescein, a neutral solute, in trehalose and sucrose aqueous supercooled solutions using the fluorescence recovery after photobleaching (FRAP) technique.

2. Experimental Methods

A few techniques are known to determine D in supercooled fluids. The most important are the *Forced Rayleigh scattering* (FRS) and the *Fluorescence Recovery After Photobleaching* (FRAP), a variant of which is described in this paper. A remarkable issue of the FRAP technique is the possibility of measuring a wide range of diffusion coefficients (10^{-9} m^2/sec to 10^{-17} m^2/sec). This technique may also be used with many fluorophores, but requires a translucent media.

2.1. Experimental Apparatus There are many ways to carry on the photo-bleaching technique. The one developed here consisted in shooting an intense beam in order to generate the photo-bleaching

(*writing* beam) and a second beam with the only purpose of exciting the fluorophore into a radiative state (*reading* beam). At a first stage a gaussian beam of length $\lambda=488$ nm, generated by an argon laser equipment was used. The working power was around *700* mW. The complete experimental set-up is shown in Fig. 1.

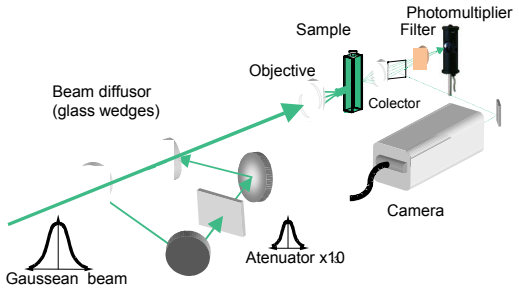

Fig. 1. Experimental set-up for a cylinder-shape photo-bleaching.

As mentioned above, the first stage of the set-up managed with separating the principal beam into the writing and a reading beam. This was done by means of glass wedges (Reflectance ≈ 4%). A neutral Inconel metallic filter (Transmittance = 0,1%) made the necessary attenuation of the reading beam. The bleaching time window was regulated with an automatic stopper. Preliminary tests showed that bleaching periods of 100 ms were suitable for this fluorescent probe.

The sample was placed in the focal plane of a convergent lens (objective) in such a way to obtain a cylinder-shape enlightening of minimum radium $R\cong\omega$. At this point the beam radius was $\omega_f=\lambda f/(\pi\omega_0)$, being ω_0 the gaussian beam radius at the lens and f the focal distance (see Fig. 2).

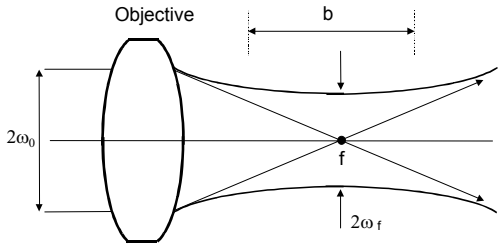

Fig. 2. Detail of the beam after trespassing the objective.

Notice that when the focal distance is reduced, so is ω_f. Also, the confocal distance b (distance between points in which power decays a half) is reduced, because of the known relation $b=2\pi\omega_f^2/\lambda$. In any case, the sample thickness should not exceed b. Particularly, as samples of ~1 mm thickness were used to avoid border effects, a restrictive limit of ω_f >8,81μm arose. The chosen radii were 12 and 16 μm.

The fluorescent emission was collected by a convergent lens and finally detected by a photo-multiplier. A long-pass glass filter was placed in order to prevent the reading beam (488 nm) from reaching the photo-multiplier tube, while allowing the fluorescence detection (~ 517 nm). Before the measurement the beam was deflected toward a camera in order of adjusting the optics and determining the magnitude of the fluorescence signal in the sample.

The sample temperature was controlled by flowing cold nitrogen gas and a proportional regulator actuating on a heating resistance.

Measurements were made on α,α-trehalose aqueous solutions, varying temperature from 5 °C to 35 °C and concentrations from 56% w/w to 89% w/w.

2.2. Methodological analysis The basic idea is to make use of fluorescent molecules as probes in the solution of interest. When these molecules are enlightened, radiative and non radiative transitions are generated depending on the light intensity (I). Very intensive enlightening moves the fluorophore to an irreversible state, which results into full *bleaching* of the enlightened region. Particularly, when a gaussian beam of type

$$I(r)=\frac{2P}{\pi\omega^2}e^{-2r^2/\omega^2}$$

is used, a cylinder-shaped [6] profile is produced after some time. Its radius R is supposed to be similar to ω, the beam radius for an intensity decay of $1/e^2$.

The (molar) concentration of fluorescent molecules is uniform before the photo-bleaching process, which means $c(x,y,z,t)=C$ $(t<0)$. Immediately after the process takes place,

$$\begin{cases} c(x,y,z,t)=0 & \text{for } r<R,\quad t\geq 0 \\ c(x,y,z,t)=C & \text{for } r\geq R,\quad t\geq 0 \end{cases}$$

The concentration of bleached molecules may be

considered as $u(x,y,z,t)=C-c(x,y,z,t)$ ($t \geq 0$). Its total amount remains unchanged, so the following *diffusion equation* may be stated,

$$\frac{\partial u}{\partial t} = D\left[\frac{\partial^2 u}{\partial r^2} + \frac{1}{r}\frac{\partial u}{\partial r}\right]$$

with the border condition $u(R,t)=0$ for $t \geq 0$ and the initial condition $u(r,0)=C$ for $r<R$. The diffusion is considered to be radial.

Solving this equation (variable separation method) [7] leads to the following general solution

$$u(r,t) = \sum_{m=1}^{\infty} a_m J_0\left(\frac{\alpha_m}{R} r\right) e^{-\alpha_m^2 Dt/R^2} \; ; r < R \;,\; t \geq 0$$

being α_m the zeros from the Bessel J_0 first class function. Every exponential term contributes to the solution with a characteristic time,

$$\tau_m = \frac{R^2}{\alpha_m{}^2 D}, \qquad m = 1,2,3..... \qquad (2)$$

Signal detection is generated by enlightening the region of interest with such intensity that collectable radiative transitions are produced. Total emitted fluorescent power is [6]

$$F(t) = \frac{\beta}{A} \int_V I(x,y,z).c(x,y,z,t)dV =$$

$$\frac{\beta Z}{A} \int_S I(r)\left[C - u(r,t)\right] r.dr.d\theta \quad ; \; t \geq 0$$

where β is the product of all quantum efficiencies before reaching the detector, A is an attenuation factor and V is the volume of interest. A signal of type,

$$F(t) = b_0 - b_1 e^{-t/\tau_1} - b_2 e^{-t/\tau_2} - ... - b_m e^{-t/\tau_m} - ...$$

is obtained for our solution $u(r,t)$. The b_0 parameter represents the emitted power after long periods ($t \to \infty$), which as a rule of thumb is 5% less than the power before the photo-bleaching process. This is often thought to be the fraction of steady fluorescent molecules [8].

The characteristic times τ_m decrease with m, while α take the values $\alpha_1 \cong 2{,}4048$, $\alpha_2 \cong 5{,}5201$, $\alpha_3 \cong 8{,}6537$, etc.. Though $F(t)$ will be dominated by τ_1, taking into account only the first exponential term generates a biased estimation. Two outputs were considered, one of type $F(t) \cong b_0 - b_1 exp(-t/\tau_1)$ and another which took τ_1, τ_2, τ_3 and τ_4 into account.

The first approach had a 20% bias compared with the latter one. τ_1 was estimated by a non linear fit using the Gauss-Newton method. The fitting model included the first four exponential terms of $F(t)$ to keep the error auto-correlation under control. The calculation of D is immediate at this point because of the equation (2).

3. Results and Discussion

3.1. Diffusion coefficients

The diffusion coefficients of fluorescein in sucrose aqueous solutions were determined over a limited range of temperature and concentration to compare with the values reported by Champion *et al* [1]. The measurements of the diffusion coefficient of fluorescein in trehalose aqueous solutions extend on a wider temperature range, as shown in Fig. 3.

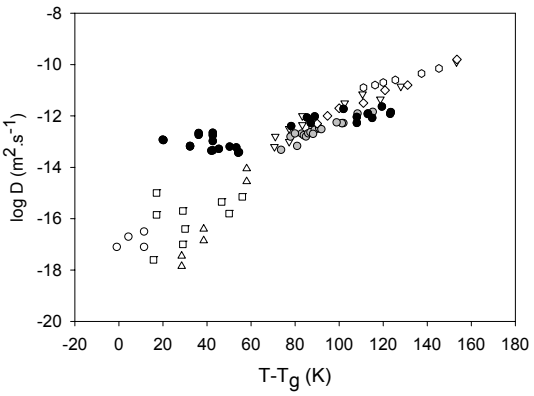

Fig.3. Diffusion coefficient of fluorescein in trehalose (●) and sucrose (○), this work. Open symbols: results for sucrose from ref. [1].

It can be seen that for both sugars the diffusion coefficient of fluorescein decreases exponentially with temperature, the diffusion coefficient in trehalose changing weaker than in sucrose.

3.2. Viscosity calculation The analysis of the continuum hydrodynamic diffusion model requires to know the dynamic viscosity of the sugar solutions.

The calculation of η in sucrose and trehalose were performed by using interpolation equations for the experimental results reported for aqueous solutions of both sugars [1,9].

The viscosity equations used for the interpolation were the Williams-Landel-Ferry (WLF) [10],

$$\log\left(\frac{\eta(T)}{\eta(T_g)}\right) = \frac{C_{1g}(T-T_g)}{C_{2g}+(T-T_g)} \quad ; \quad T > T_g$$

and the power-law equation [11],

$$\eta(T) = A\left(T-T_C\right)^{-\gamma} \quad ; \quad T > T_C \approx 1.2T_g$$

WLF should be used for temperatures above T_g, while for $T > T_c$ the power-law gives a better representation of the temperature dependence [12]. Both equations seem to need three parameters to be estimated for any known T_g. This is excessive for the limited amount of data available (related to trehalose). The reasonable choice was to settle $\eta(T_g) \cong 10^{14}$ Pa·s (WLF) and $T_c \cong 1.2\ T_g$ (mode coupling theory).

For sucrose solutions we used the WLF equation with the parameters reported by Champion *et al* [1].

For trehalose solutions the data from Miller *et al* [9] were fitted to the WLF equation for $(T\text{-}T_g) < 45$ K and to the power-law equation above that range. The T_g values for trehalose solutions were estimated from the experimental values by Miller *et al* [9] (see Table 1).

Table 1. Estimated values of T_g for trehalose aqueous solution from data in ref. [9].

% w/w trehalose	T_g (°C)
60.0	-80
65.0	-68
68.5	-60
72.4	-51
80.0	-27
83.0	-15

The fitting results represented in Fig. 4 were obtained with the WLF parameters C_{1g} = -13.91, C_{2g} = 5.92 and the power-law parameters A = 454.37 , γ = 1.597.

3.3. The validity of the hydrodynamic model With the measured values of *D(T)* and $\eta(T)$ the validity of Stokes-Einstein relationship can be verified. In Fig. 5 we plotted $T/D\eta$ as a function of T_g/T for the mobility of fluorescein in trehalose and sucrose solutions.

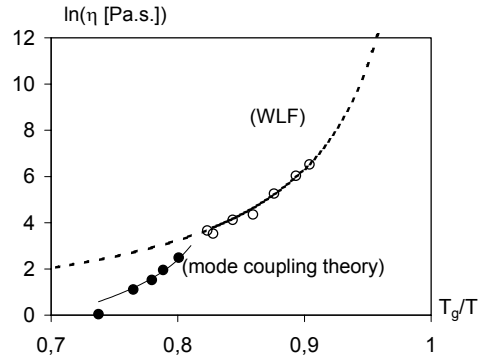

Fig. 4. Dynamic viscosity of aqueous trehalose as a function of temperature: (○) 72.5% w/w; (●) 65.0 % w/w. The dashed line is the WLF fitting curve, the full line is the mode coupling fitting.

For comparison we also plotted the values in sucrose solutions reported by Champion *et al* [1].

It is clear that at temperatures above a critical value the Stokes-Einstein relationship holds. A mean hydrodynamic radius of 0.61 nm was obtained for fluorescein, in reasonable agreement with a previous reported value (~ 0.50 nm) [5].

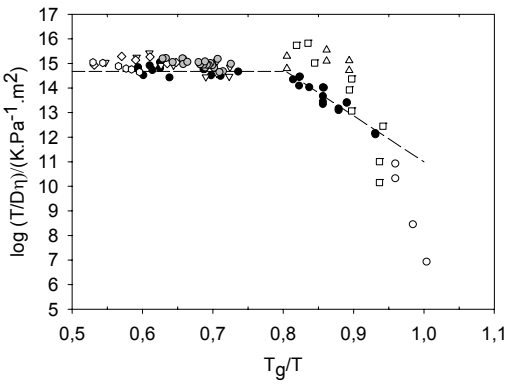

Fig. 5. Stokes-Einstein plot for the diffusion of fluorescein in trehalose (●) and sucrose (○), this work. Open symbols: results for sucrose from ref. [1]. Lines correspond to fitting of trehalose data.

The decoupling of diffusion and viscosity occurs at $T_g/T \approx 0.80$ for trehalose and at $T_g/T \approx 0.86$ [1] for sucrose.

4. Conclusions

The decoupling of diffusion and viscosity of a neutral solute was observed in aqueous supercooled solutions of trehalose. The results are similar to those previously reported for sucrose by other authors, except that the decoupling in trehalose takes place at a higher T/T_g ratio.

This behavior is similar to that found for ionic solutes and it is related to the presence of structural heterogeneities due to the presence of water rich regions. Small solutes can diffuse more easily through these regions having local viscosities lower than the bulk viscosity.

The diffusion of small solutes in trehalose and sucrose at temperatures below the decoupling is much higher than predicted using the Stokes-Einstein relationship. Thus, for $T \approx 0.9T_g$ the diffusion coefficient of fluorescein is a factor 100 larger than that obtained by extrapolating the high temperature value using the hydrodynamic model. Accordingly, the rate of diffusionally controlled deterioration reactions in aqueous systems close to the glass transition temperature could be several orders higher than predictions based on equation (1).

Acknowledgements

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References and Notes

[1] D. Champion, H. Hevert, G. Blond, M. Le Meste and D. Simatos, *J.Phys.Chem. B*, **101**, 10674 (1997)

[2] I. Chang, F. Fujara, B. Geil, G. Heuberger, T. Mangel and H. Silescu, *J. Non-Cryst. Solids*, **248**, 172, (1994).

[3] M. T. Cicerone, F.R.Blackburn, and M.D.Ediger, *J.Chem.Phys.*, **102**, 471 (1995).

[4] M. Le Meste, and A. J Voilley, *J.Phys.Chem.*, **92**, 1612 (1988).

[5] M. P. Longinotti, M. F. Mazzobre, M. P. Buera and H. R. Corti, *Phys.Chem.Chem.Phys*. **4**, 533 (2002).

[6] D. Axelrod, D. E. Koppel, J. Schlessinger, E. Elson, and W. W. Webb, *Biophys.J.*, **16**, 1055 (1976).

[7] Churchil, Ruel Vance, *Fourier series and boundary value problems,* Mc Graw-Hill, New York (1941).

[8] D. Champion, H. Hevert, G. Blond, and D. Simatos, *J. Agric. Food Chem*, **43**, 2887 (1995).

[9] D. P. Miller, J .J. de Pablo, and H. R. Corti, *Pharm. Res.*, **14**, 578 (1997).

[10] M. Williams, R. Landel and J. Ferry, *J. Am. Chem. Soc.*, **77**, 3700 (1955).

[11] D. P. Miller, J. J. de Pablo and H.R.Corti, *J.Phys. Chem.B*, **102**, 10213 (1999).

[12] E. Rössler, *Phys Rev.Lett.*, **65**, 1595 (1990).