Brief Communication

Viscosity of supercooled aqueous glycerol solutions, validity of the Stokes–Einstein relationship, and implications for cryopreservation

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ABSTRACT

The viscosity of supercooled glycerol aqueous solutions, with glycerol mass fractions between 0.70 and 0.90, have been determined to confirm that the Avramov–Milchev equation describes very well the temperature dependence of the viscosity of the binary mixtures including the supercooled regime. On the contrary, it is shown that the free volume model of viscosity, with the parameters proposed in a recent work (He, Fowler, Toner, J. Appl. Phys. 100 (2006) 074702), overestimates the viscosity of the glycerol-rich mixtures at low temperatures by several orders of magnitude. Moreover, the free volume model for the water diffusion leads to predictions of the Stokes–Einstein product, which are incompatible with the experimental findings. We conclude that the use of these free volume models, with parameters obtained by fitting experimental data far from the supercooled and glassy regions, lead to incorrect predictions of the deterioration rates of biomolecules, overestimating their life times in these cryopreservation media.

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Glass forming systems, such as aqueous glycerol solutions, are commonly used to prevent damage by ice crystallization during cryopreservation by vitrification of biomolecules, cells, and tissues. Properties such as the homogeneous nucleation temperature and the glass transition temperature of glycerol aqueous solutions as a function of composition are extremely important to assess their cryoprotective efficiency, and they have been addressed previously in this journal [7,9]. Considering that deterioration reactions in these media are controlled by the diffusion of the reactants [8] that, depending on the temperature region, is proportional to η^{-1} (Stokes–Einstein equation) or $\eta^{-\alpha}$, with $\alpha < 1$ [5], knowledge of the viscosity temperature dependence is fundamental for the analysis of diffusion-controlled deterioration processes in these media.

Thus, Morris et al. [11] measured the viscosity of residual unfrozen glycerol solutions during freezing and, assuming that the water diffusion coefficient is given by the Stokes–Einstein equation, estimated the distance of water displacement over time at different cooling rates. The viscosities of solutions at temperatures and compositions on the freezing curve of the glycerol–water phase diagram were determined, and the results were used to explain the resultant composition of the unfrozen solutions under different cooling rates and the protecting effect of these media on the stor-

age of living cells. He et al. [6] have predicted maximum storage temperature for long-term degradation of a biological sample by resorting to free volume models of viscosity and diffusion coefficient. The parameters of the model were adjusted by resorting using viscosity and diffusion coefficients in pure water and glycerol solutions. Viscosity data of the glycerol solutions down to 273 K were used in the modeling, in spite of the fact that viscosities were reported above the freezing and solubility curves of the glycerol–water phase diagram [15]. The model results for the glycerol–water system were extrapolated down to 139 K in the case of viscosity and down to 163 K for water self- diffusion. Using these estimations the authors determined the ideal working conditions for different cryoprotective media, including glycerol.

These two examples clearly indicate the need of: (i) measuring the viscosity of the glycerol–water system in the supercooled region, below the solubility and freezing curves, in order to validate the predictions of the free volume models, and (ii) analyzing the validity of the Stokes–Einstein equation or the free volume model to calculate diffusion coefficients in the supercooled region, including the zone close to the glass transition, of interest in cryopreservation.

Viscosity data for pure water and pure glycerol are available in the stable and supercooled regimes for both components. Water viscosity determinations [12] extend down to temperatures close to the homogeneous nucleation (T_h = 235 K), well above the glass transition ($T_{gw} \approx 136$ K), while in pure glycerol the measurements extend down to its glass transition temperature ($T_{gg} \approx 193$ K) [4].

Regarding glycerol–water mixtures, viscosity determinations are abundant in the stable liquid regime, but in the supercooled region only few data for concentrated glycerol solutions (glycerol mass fractions, w, above 0.80) were reported down to 20 K below the solubility equilibrium curve [15]. Considering this scenario we extended viscosity measurements of aqueous solutions with w between 0.70 and 1.00, to the supercooled region. Our measurements were performed using the falling ball method, with supercooled liquid samples contained in a cylindrical acrylic tube 22.2 mm in diameter (D) and 450 mm length (L), surrounded by a concentric tube used for circulating a silicone thermostatic fluid. Four windows having light emitting diodes (LEDs), located at 85, 100, 200 and 400 mm from the top of the tube, allow the visualization of the falling ball at these points. Viscosity measurements were performed at fixed temperatures between 273.0 and 219.7 K, controlled within ±0.1 K. Teflon, stainless steel and glass balls with diameters (d) between 1.57 and 5.55 mm were used, depending on the viscosity of the test solution. Ball diameters were measured with a caliper and their densities were determined by weight.

The time required for the ball falling fixed distances was determined by eye visualization and measured with a chronometer with a precision of 0.15 s. Thus, the error in time measurements was ±0.75% for the shortest time, and 0.02% for the longest one. All viscosity measurements, with exception of the sample with the highest viscosity, were performed at least by duplicate, either by measuring the time required for the ball to fall different distances or by using different balls.

The Stokes theory, which allows expressing the viscosity according to the stationary velocity of the falling ball,

$$\eta = \frac{2}{9 v_\infty}(\rho_b - \rho_l)gr^2 \tag{1}$$

where ρ_b is the density of the ball, ρ_l the density of the liquid, g the gravity acceleration, r the ball radius and v_∞ the ball stationary velocity, applies only if the ball falls in an infinite media without inertial effects; that is in the limit of Reynolds number, Re, equal to zero. A correction factor due to wall effects, which depends on the ratio between the diameter of the falling ball (d) and the tube diameter (D), was applied to the velocity of the ball [3]. For the experiments performed in this work the ratio d/D was varied between 0.14 and 0.45 and Re was always smaller than 0.008. Therefore, corrections due to inertial effects were not considered. The ratio between the ball diameter and the distance between the last measuring point and the end of the tube was always bigger than seven. Consequently, end effect corrections were not needed in this work.

Fig. 1 shows the temperature dependence of the viscosity of glycerol–water mixtures measured in this work along with previous published results (see Ref. [15] for a complete list of references on viscosity data). Viscosities of pure glycerol at several temperatures measured in this work are in good agreement with the set of data reported in the literature, within the range of temperature studied, validating the experimental method.

As it was shown in our previous study [15], the three-parameters Avramov–Milchev model (AM model) [1,2] describes quite well the viscosity of the glycerol–water solutions on the whole range of temperature where data are available. The AM equation for viscosity-temperature correlation is,

$$\eta = \eta_0 \cdot \exp\left[(\ln \eta_g - \ln \eta_0) \cdot \left(\frac{T_g}{T} \right)^\alpha \right] \tag{2}$$

where T_g is the solution glass transition temperature, used as an adjustable parameter while $\eta_g = 10^{12.5}$ Pa.s is the viscosity at T_g, and η_0 and α are the additional adjustable parameters. In our
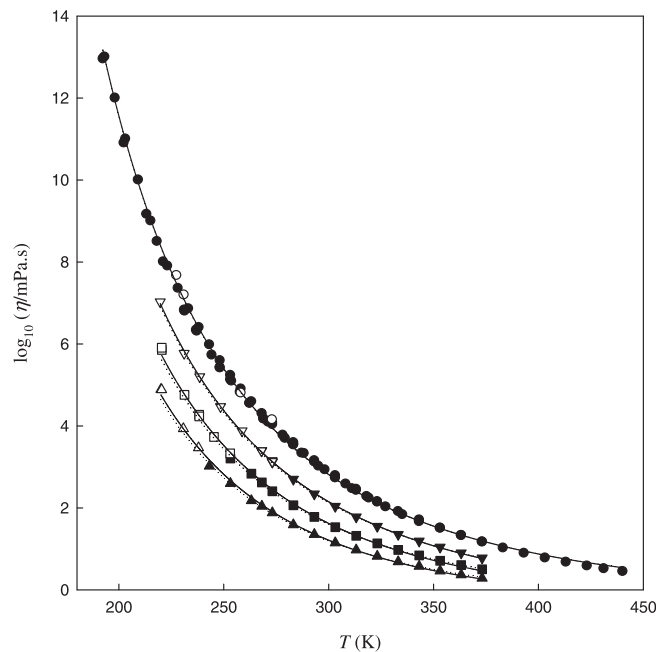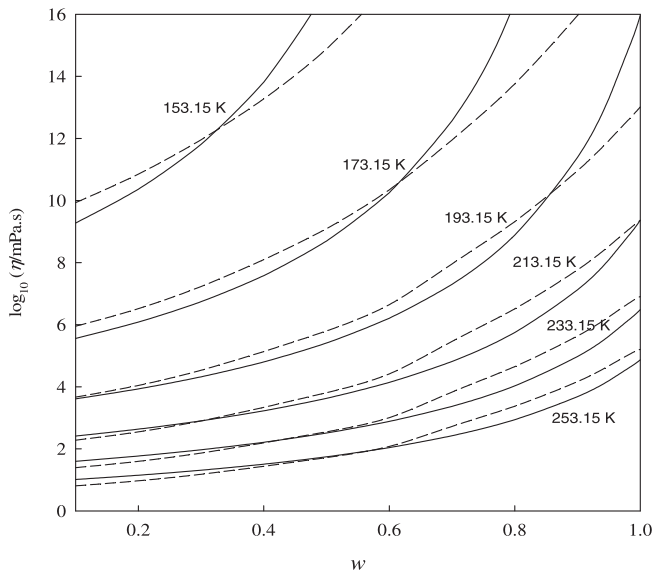

Fig. 1. Viscosity of aqueous glycerol solutions as a function of temperature. Bold symbols correspond to published data and empty ones to viscosity measurements performed in this work for: $w = 1.00$ (●), $w = 0.90$ (▼), $w = 0.80$ (■), $w = 0.70$ (▲). The lines correspond to fits of Eq. (2) using previous published data (dotted line), and including the new data (full line).

previous work [15] we analyzed the viscosity of pure glycerol by fixing the value of T_g and interpolated the value of η_g to that temperature, obtaining a value equal to $10^{12.5}$ mPa.s. These parameters values gave the best results and convert the AM Eq. (2) in a two-parameters equation. We followed the same procedure for aqueous solutions, by fitting T_g of aqueous glycerol solutions reported in the literature as a function of composition, using the Gordon–Taylor equation with $T_{gg} = 195.4$ K, $T_{gw} = 141.8$ K, and $k_{GT} = 1.95$ [15]. With the obtained T_g, and fixing $\eta_g = 10^{12.5}$ mPa.s, we adjusted the viscosity of aqueous solutions, for w between 0.7 and 0.9, as a function of temperature. The results of the fits using the AM Eq. (2) are shown in Fig. 1, where it can be observed that the viscosity data of aqueous glycerol solutions ($w = 0.7, 0.8,$ and 0.9) in the supercooled region (220–273 K) agree quite well with the extrapolations of our previous fits performed with Eq. (2) including only viscosity data in the stable liquid region. Thus, the best fitting values of η_0 and α, differ slightly from those reported in our previous work [15], where the values of these parameters for $w = 0.1$–0.6 can be also found. This conclusion is relevant for the predictions of viscosities of supercooled water–glycerol mixtures in the water-rich region, where the crystallization of water prevent us from performing viscosity determinations.

In Fig. 2 we compare the predictions of the AM equation with those of the equation of Williams, Landel and Ferry (WLF) with the parameters reported by He et al. [6]. The AM equation fits very precisely the experimental data of water-rich solutions ($w < 0.6$) at temperatures between 293.15 and 213.15 K [15]. The WLF equation also fits quite precisely the viscosity in this temperature range for $w < 0.6$, while it overestimates the viscosity of the mixtures at temperatures below 193.15 K. At very low temperatures, the region of interest in cryo-preservation, the WLF equation overestimates the mixture viscosity by three orders of magnitude for $w = 1.0$ at 193.15 K, 2.5 orders of magnitude for $w = 0.8$ at 173.15 K, and 1.5 orders of magnitude at 153.15 K for $w = 0.5$, as compared to the AM equation, and the difference between both equations increases with decreasing temperature.

Fig. 2. Viscosities of aqueous glycerol solutions as a function of composition calculated with the AM equation (dashed line), with the parameters reported in this work for $w = 0.7$–1.0 and in Ref. [7] for $w = 0.1$–0.6, and predictions with the WLF equation (full line) with the parameters given by He et al. [6]. Temperatures are indicated on the corresponding curves.
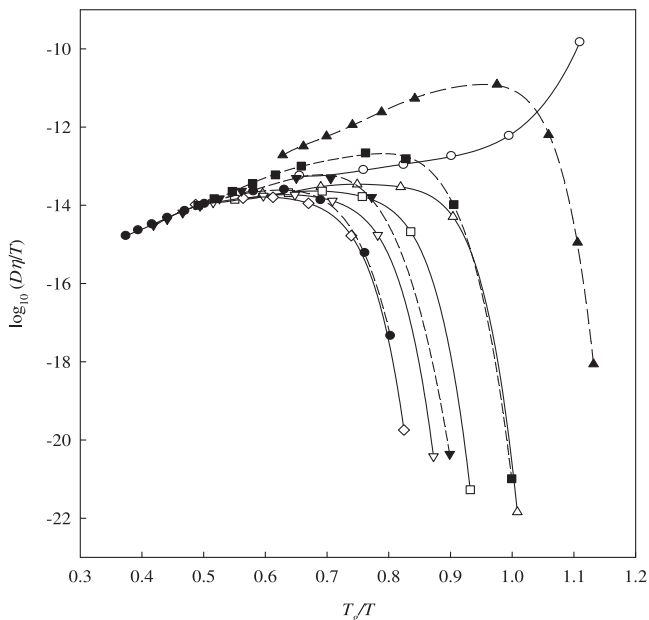
Fig. 3. Stokes–Einstein product for the diffusion of water in glycerol (open symbols) and trehalose (closed symbols) aqueous solutions, calculated with the free volume models [6]. Polyol mass fraction: $w = 1.00$ (○), $w = 0.80$ (△), $w = 0.60$ (□), $w = 0.70$ (▽), $w = 0.20$ (◇).

Low temperature viscosity of cryoprotectants aqueous solutions is one of the parameters to consider in their ability to preserve biological samples, but the knowledge of the diffusion coefficients of the stored molecules in these media are of extreme importance in order to predict the rate of deterioration processes. Therefore, it is important to evaluate the predictions of the free volume model for the diffusion in aqueous solutions of cryoprotectants.

Fig. 3 shows the Stokes–Einstein (SE) product ($D \cdot \eta / T$) for the diffusion of water in glycerol and trehalose water mixtures of different compositions as a function of a reduced inverse temperature (T_g/T), for D and η calculated using the free volume model parameters reported by He et al. [6]. It can be observed, that the

SE product for the diffusion of water in glycerol aqueous solutions exhibits the expected behavior [14] for pure glycerol ($w = 1$), that is, the SE product is much higher than predicted by the SE equation using a reasonable hydrodynamic radius for water, particularly in the glassy state ($T_g/T > 1$). However, for $w < 1$, the SE equation is obeyed up to values of T_g/T between 0.7 and 0.9, depending of the glycerol concentration, but the predicted water diffusion coefficient is dramatically lower than that expected by the SE equation when approaching the glass transition temperature. This behavior is contrary to the experimental observation that the SE product for water and solutes in supercooled polyols and electrolytes aqueous solutions is either constant or increases close to T_g, depending on the specie analyzed [10,14]. The behavior of the SE product is rather similar for water diffusion in trehalose aqueous solutions: although at high temperatures the SE product increases with increasing T_g/T, an unexpected dramatic drop of the SE product is predicted when T_g is approached. Considering that the WLF equation, with the parameters reported by He et al. [6], overestimates the viscosity of glycerol water mixtures at low temperatures (high T_g/T values), it is clear that the free volume model used by these authors largely underestimates the water diffusion coefficients at low temperatures.

These systematic biases in the estimation of the viscosity and the diffusion coefficient of water in binary glycerol–water solutions could lead to misinterpretations on the rate of processes occurring in these media or the life time of stored bio molecules. For instance, if the diffusion coefficient of water calculated with the free volume model were used to predict the rate of water crystallization, the kinetics of this process would be underestimated. On the other hand, if the diffusion coefficient of a given molecule in the supercooled glycerol aqueous solution is calculated using the viscosity calculated with the free volume model [6], assuming that the SE equation holds, the molecule mobility would be underestimated as well as the deterioration rates. Consequently, the life time of biomolecules cryopreserved in these media would be overestimated [13].

In summary, we have extended viscosity measurements of glycerol aqueous solutions to the supercooled regime for aqueous solutions with glycerol mass fractions between 0.70 and 0.90, confirming that the AM equation describes very well the temperature dependence of the viscosity of glycerol aqueous solutions including the supercooled regime. Moreover, we demonstrated that the free volume models for viscosity and diffusion coefficients with parameters obtained by fitting experimental data in the region far from the supercooled and glassy regions, lead to predictions for the SE product which are incompatible with the experimental findings and incorrectly predict the deterioration rates of biomolecules yielding to overestimations of their life times in these cryopreservation media.

Acknowledgments

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