

α,α -Trehalose–Water Solutions. II. Influence of Hydrogen Bond Connectivity on Transport Properties[†]

S. Magazu,^{*,‡} G. Maisano,[‡] H. D. Middendorf,[§] P. Migliardo,[‡] A. M. Musolino,[‡] and V. Villari[‡]

Dipartimento di Fisica and INFN, Università di Messina, P.O. Box 55, Papardo,
98166 S. Agata di Messina, Italy, and Clarendon Laboratory, University of Oxford,
Parks Road, Oxford OX1 3PU, U.K.

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Results of photon correlation spectroscopy and viscosity measurements performed in trehalose aqueous solutions are reported. The light scattering experimental findings reveal a peculiar behavior of the effective diffusion coefficient D_{eff} which, in the dilute region and in a limited temperature range, shows strong changes. This behavior suggests a great sensitivity of the system structure to temperature changes. On the other hand, the temperature dependence of shear viscosity η confirms the noticeable kinetically *fragile* character of these systems.

1. Introduction

In recent years there has been growing interest in polysaccharides, a class of biopolymers of universal importance in living matter.^{1–9} In particular, trehalose, a *disaccharide* of glucose, is synthesized at high concentrations (as much as 20% of dry weight) by many anhydrobiotic organisms. Some of these preserve their structural and functional integrity over several years of dehydration, and resume their metabolic activity coming in contact with water. Some authors^{1,2} have related the survival of these organisms to α -trehalose-imposed glassy state which enables them to avoid degrading reactions. Others^{6,12} suggest that this sugar keeps the organism's internal structure in its native state by binding to polar and charged groups of molecules that are normally hydrated.

As a matter of fact, while the role of trehalose as an effective bioprotector is fairly well-established, the molecular processes involved in its interaction with water remain unclear and are the subject of much current research.

A molecular dynamics (MD)¹⁰ study has attempted to quantify the properties of intra- and intermolecular hydrogen bonds. The former occur when the two hexapyranose rings interact directly because of their rotation around the bridging oxygen. It has been shown that the trehalose molecules tend to create polygonal arrangement (hexagonal) water structures similar to those found in ice structure. A more recent MD simulation¹³ has been used to model the aqueous solvation of trehalose. It was found that trehalose imposes considerable structure on the adjacent water molecules. This structuring results much more extensive than that found in pure liquid water and extends out to at least the third *solvation shell*. The lower value of the water self-diffusion coefficient found for the solution case is consistent with the significant solvent structuring found in the structural analysis. Furthermore, the small differences found in the simulations with other disaccharides could account for their differing effectiveness.

In some previous works,^{14–17} we reported the results of ultrasonic, incoherent quasi elastic neutron scattering (IQENS), photon correlation spectroscopy (PCS), and Raman-scattering experiments on trehalose–water solutions, which evidence structural changes as a function of temperature and concentration. In particular, the adiabatic compressibility,¹⁶ evaluated by ultrasonic technique, furnished information on the strength of trehalose–water interactions and on the temperature dependence of the hydration number. IQENS¹⁴ and preliminary PCS¹⁵ measurements allowed us to separate the self and the collective contribution in the dynamical responses, revealing the presence of two mechanisms assigned to the continuous translational diffusion of the trehalose molecules and to the translational jump diffusion of the bulk water molecules. Moreover, the noticeable *fragile* character of the trehalose–water system was evidenced, in the supercooled regime, by low-frequency depolarized Raman light-scattering experiments.¹⁷

In this paper we present PCS and viscosity data on trehalose aqueous solutions. It will be shown that both the diffusion coefficient D_{eff} and the shear viscosity η display a behavior that strongly depends on concentration and temperature. In particular the initial increase of D_{eff} with concentration, in the dilute regime and at high temperature, indicates the existence of repulsive intramolecular (between the two hexapyranose rings) and intermolecular (among the hydrated trehalose molecules) interactions. In addition the temperature behavior of shear viscosity indicates that the noticeable fragile character of this system increases by increasing the water content.

2. Experimental Setup and Data Analysis

The solutions were prepared by weight, using α,α -trehalose dihydrate purchased from Aldrich-Chemie and doubly distilled deionized water. Care was taken in order to obtain stable, clear and dust-free samples; ample time was allowed for equilibration; in addition, the solutions were filtered using 0.8 μm Amicon filters and stored in the dark to minimize biological and photochemical degradation. The concentration range investigated was $\Phi = 0.04$ to $\Phi = 0.54$, $\Phi = (n_{\text{T}}M_{\text{T}}/\rho_{\text{T}})/[(n_{\text{T}}M_{\text{T}} + n_{\text{W}}M_{\text{W}})/\rho_{\text{soluz}}]$ being the trehalose volume fraction. In this expression, the molar numbers and molecular weight for

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[‡] Dipartimento di Fisica and INFN.

[§] Clarendon Laboratory.

trehalose and water are n_T , M_T and n_w , M_w , respectively, and the trehalose and solution densities are denoted by ρ_T and ρ_{soluz} . Lower trehalose concentration values were not taken into account because they do not furnish sufficient optical contrast.

QELS measurements were performed by means of the PCS technique, using a standard scattering apparatus with a photon counting optical system and BROOKHAVEN BI-30 correlator to analyze the scattered light. As exciting source, the 4880 Å vertically polarized line of a unimode Ar⁺ laser (INNOVA model 70) with a power range of 50–400 mW was used. The light scattered, at 90°, was fed by an optical fiber into a photomultiplier tube connected with the correlator. The scattered intensity was automatically normalized for the incoming beam intensity. Samples were sealed in optical cells and then mounted in an optical thermostat designed to minimize unwanted stray-light contributions. The high purity of the sample, as well as the optical purity of the sample holder, enabled us to collect data with good signal to noise ratio and with high reproducibility. The temperature range investigated was 20–85 °C, and the temperature constancy was better than ± 0.02 °C.

For the viscosity measurements we employed standard Ubbelohde viscometers mounted in a suitable constant temperature stable to within ± 0.02 °C in the range $20 \leq T \leq 85$ °C. Long flow time viscosimeters were chosen in order to minimize the kinetic energy correction. Before measuring the viscosity the sample were stabilized for sufficient time at the temperature selected. Experimental data turned out to be reproducible with a relative error of less than 0.001. Auxiliary density measurements, necessary to evaluate the shear viscosity of the solutions from the kinematic one, were performed using a standard picnometer.

3. Results and Discussion

Photon Correlation and Diffusion Coefficient Data. It is well-known that in a QELS experiment¹⁸ the total scattered field E_S can be written as a superposition of fields $E_S^{(n)}$ from N particles. If these are identical and assumed small compared with the wavelength of the incident light, the N particles in the scattering volume give rise to the field:

$$E_S(k,t) = \sum_n E_S^{(n)}(k,t) = A \exp(i\omega_0 t) \sum_{j=1}^N \exp[i\mathbf{k} \cdot \mathbf{r}_j(t)] \quad (1)$$

Here A is the amplitude of the wave scattered by a particle, ω_0 is the angular frequency of the incident light, $\mathbf{r}_j(t)$ denotes the position of the j th particle and \mathbf{k} is the scattering vector. The modulus of \mathbf{k} is related to the scattering angle ϑ by the well-known relation

$$k = |\mathbf{k}| = (4\pi n/\lambda) \sin \vartheta/2 \quad (2)$$

where n denotes the refractive index of the solvent and λ the wavelength of the incident light. For the scattered field a normalized time correlation function, $g_1(k,t)$, can be defined as

$$g_1(k,t) = \frac{\langle E_S(k,0)E_S^*(k,t) \rangle}{\langle |E_S(k)|^2 \rangle} = \frac{\langle E_S(k,0)E_S^*(k,t) \rangle}{\langle I_S(k) \rangle} \quad (3)$$

PCS allows us to measure the normalized autocorrelation function of the total scattered intensity $I_S(k,t)$:

$$g_2(k,t) = \frac{\langle I_S(k,0)I_S(k,t) \rangle}{\langle I_S(k) \rangle^2} \quad (4)$$

In the case of *independence of particle motion*, since E_S is the sum of independent random quantities, it will itself be a *random variable*. With this assumption, the central limit theorem assures that E_S will be a Gaussian distribution, and Siegert's relationship can be applied:

$$g_2(k,t) = 1 + \alpha |g_1(k,t)|^2 \quad (5)$$

where α is a constant which depends on the number of the coherence areas selected (and hence on the experimental setup).

The average relaxation rate for the diffusion, $\langle \Gamma \rangle$, is the relevant parameter to be determined by PCS measurement. It is given by the first cumulant of the normalized dynamic structure function $S(k,t)$:

$$\langle \Gamma \rangle = - \left. \frac{d \ln g_1(k,t)}{dt} \right|_{t=0} = - \left. \frac{d \ln S(k,t)}{dt} \right|_{t=0} \quad (6)$$

A Brownian relaxation time may be defined by $\tau_B \equiv m/\xi$, where m and ξ are the mass and the friction coefficient, respectively. For times greater than τ_B , but smaller than the configurational relaxation time of the system, the dynamics obeys the Smoluchowski equation and $S(k,t)$ is known to decay exponentially according to $S(k,t) = S(k)e^{-\Gamma(k)t}$, $S(k)$ being the structure factor and where it results:

$$\Gamma(k) = k^2 D_{\text{eff}}(k) = k^2 D_0 \frac{H(k)}{S(k)} \quad (7)$$

Here $D_{\text{eff}}(k)$ is an *effective* k -dependent diffusion coefficient and D_0 is the free diffusion coefficient. The hydrodynamic function $H(k)$ depends on the particle equilibrium distribution as well as on the form of the interparticle potential. If k_m denotes the position of the main peak of the static structure factor, we have $k_m \approx 2\pi/d$, where d is the mean interparticle spacing. It is easy to see that, for $k \gg k_m$, since $S(k) \rightarrow 1$, $D_{\text{eff}}(k)$ tends to the self-diffusion coefficient D_S according $D_{\text{eff}}(k) = D_S = D_0 H(k)$.

In the opposite limit $k \ll k_m$, the effective diffusion coefficient is identical to the collective diffusion coefficient D_0 defined by the generalized Stokes–Einstein relation $D_c = (\partial \Pi / \partial C)_T / \xi$, $(\partial C / \partial \Pi)_T$ being the osmotic compressibility and ξ a frictional coefficient. D_c is connected with $H(k)$ and $S(k)$ in the $k \rightarrow 0$ limit through the relationship

$$D_{\text{eff}}(k \rightarrow 0) = D_c = \frac{H(0)}{S(0)} \quad (9)$$

It should be noted that, in principle, since $S(k \rightarrow 0)$ can become very small compared to 1, the collective diffusion coefficient D_c can be much larger than the free diffusion coefficient at infinite dilution. In order to evaluate the $S(k)$ function for our systems, we have also performed measurements of the scattered intensity as a function of the scattering angle. The obtained scattered intensity profiles do not reveal, in the investigated k range, any dependence of $S(k)$ on the exchanged wave vector. In addition, the effective diffusion coefficient $D_{\text{eff}} = \Gamma(k)/k^2$ evaluated from the scattered intensity correlation function at different scattering angles, does not depend on the exchanged wave vector. The evolution of D_{eff} vs volume fraction Φ at different temperatures is shown in Figure 1. The most important feature is the drastic change of D_{eff} , in the dilute regime, when temperature rises. The initial increase of D_{eff} at low Φ can be

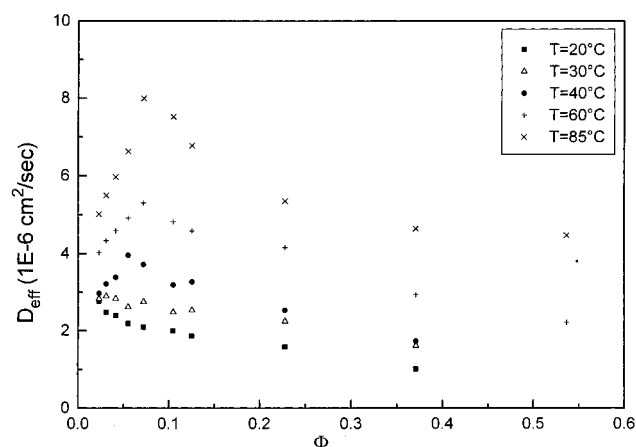


Figure 1. Translational diffusion coefficient as a function of volume fraction Φ at five temperatures.

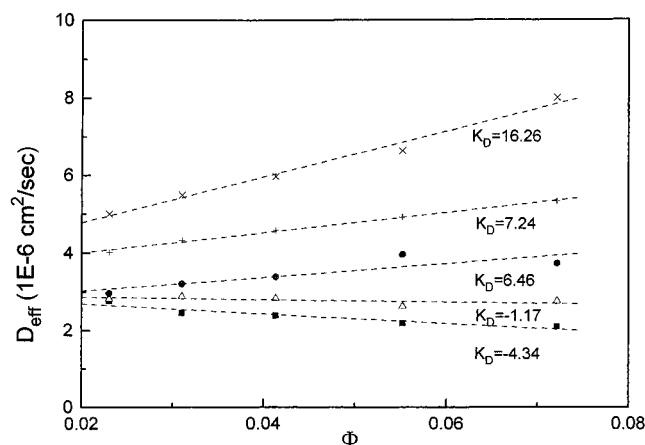


Figure 2. D_{eff} as a function of Φ at low concentration values. The dashed lines represent linear fit according to $D_{\text{eff}} = D_0(1 + K_D\Phi)$.

explained by the existence of repulsive interactions among the hydrated trehalose molecules and among the two rings of the disaccharide. However, as Φ is raised further, D_{eff} goes through a maximum and thereafter decreases. The transition from a collective to a cooperative regime could be attributed to the increase of hydrodynamic interactions or to the presence of aggregative processes. This trend is in good qualitative agreement with the general trend of D_{eff} in colloidal suspensions.¹⁸ In this case the dynamical properties are fairly well-understood in two limiting cases. One consists of a suspension of charged particles, where repulsive interactions produce significant correlations already at low volume fractions so that hydrodynamic interactions can be ignored. The other case refers to a hard-spheres suspension, where hydrodynamic interactions play the main role. Both types of interaction are expected to contribute at moderate volume fractions.

The initial slope of D_{eff} as a function of Φ can give useful information about the nature of interactions among the trehalose molecules. This behavior can be represented by the relation $D_{\text{eff}} = D_0(1 + K_D\Phi)$ where K_D can be positive or negative depending on the repulsive or attractive character of the interactions. As it can be seen from an inspection of Figure 2, K_D shows a large variation passing from negative values $K_D = -4.34$ at $T = 20^\circ\text{C}$, up to $K_D = 16.26$ at $T = 85^\circ\text{C}$. This variation reveals once more the great sensitivity of the system to the temperature changes and can be interpreted on the basis of an enhancing, by increasing temperature, of the intermolecular repulsive interactions among the hydrated trehalose molecules. It should be also stressed that a trehalose molecule can be

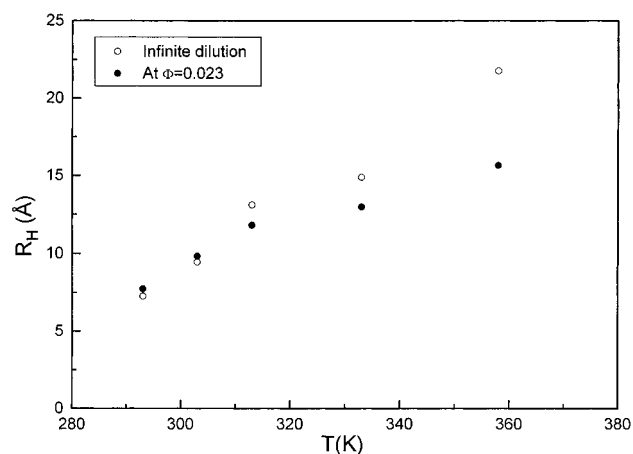


Figure 3. Temperature dependence of the hydrodynamic radius R_H for the most dilute solution ($\Phi = 0.023$) and at infinite dilution.

swollen or collapsed due to intramolecular repulsive or attractive forces between its two rings.^{10,19} At high temperature, the interaction between the two trehalose's rings, mediated by hydrogen-bond interactions, is dominated by repulsive interactions, whereas at lower temperatures the attractive contribution dominates with a *net attraction* of the two hexapyranose rings. Therefore the increase of D_{eff} at low concentration can be attributed also to the straightening of trehalose molecules at high temperature which, reducing the interparticle spacings, further augments the increase in D_{eff} . The temperature dependence of the translational diffusion coefficient D_{eff} can be traced back to both the increased thermal contribution and to the loss of water molecules not tightly bounded to the diffusing trehalose molecules, as evidenced by ultrasonic measurements.¹⁶ Furthermore, Arrhenius plots of D_{eff} according to $D_{\text{eff}} = D \exp(E_D/k_B T)$, give straight lines which furnish activation energy values ranging from ~ 2.5 Kcal/mol at the lowest concentration to ~ 6 Kcal/mol at the highest concentration. These values are typical H-bond energies and suggest that the kinetic process driving the translational diffusive motion is mainly due to the breaking and reforming of the intermolecular H-bonds.

The knowledge of D_{eff} allow us an evolution of the hydrodynamic radius R_H . It is well-known,^{18,19} in fact, from theories of hydration, that the diffusing particle and its primary hydrated sphere may be regarded as forming a kinetic entity with respect to translation, although the water molecules in the hydration shell exchange from time to time with those from the bulk solvent. In the case of spherically shaped particles with sizes large compared with those of the solvent molecules, assuming stick boundary conditions at the interface, the frictional resistance of a particle according to the Stokes' law is given by $1/6\pi\eta_0 R_H$. On the basis of this relation it easy to calculate the radius of the diffusing species from the limiting value at infinite dilution. However, since in our case the size of the diffusing entities (i.e., disaccharide + primary hydration) is not much larger than that of a water molecule, it is not entirely justified to use macroscopic viscosities and the frictional coefficient can differ appreciably. In addition, since our solutions cannot be considered highly diluted, Stokes-Einstein law, $R_H = k_B T / 6\pi\eta_0 D_{\text{eff}}$, can only give a semiquantitative estimate of the trend in mobility with the change in radius. In Figure 3 we report the temperature dependence of the hydrodynamic radius, evaluated from the diffusion coefficient relative to the most dilute solution ($\Phi = 0.023$) and from the diffusion coefficient extrapolated to infinite dilution. Also, this parameter is seen to strongly depend on temperature. It ranges from ~ 7.5 Å at $T = 20^\circ\text{C}$ to ~ 16 Å at $T = 85^\circ\text{C}$, when valuated from the

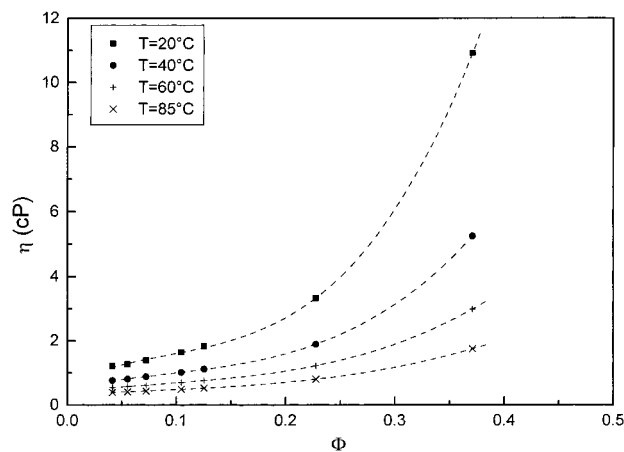


Figure 4. Viscosity η as a function of volume fraction Φ at four temperatures. The dashed lines are guides for the eye.

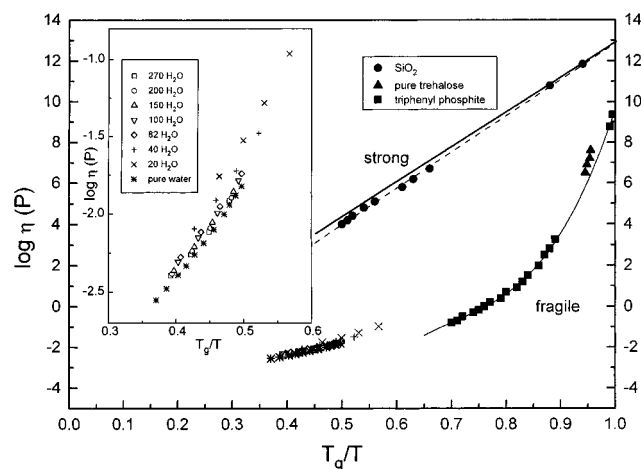


Figure 5. Strong–fragile pattern of viscosity–temperature relations for T_g -based normalization of trehalose aqueous solutions.

diffusion coefficient relative to $\Phi = 0.023$, and from ~ 7.5 Å at $T = 20$ °C to ~ 22 Å at $T = 85$ °C, when valuated from the diffusion coefficient extrapolated to infinite dilution.

It is likely that this dependence is due to an increase of the trehalose molecular volume because of the repulsive interaction between the two hexapyranose rings (i.e., a tendency toward a more opened configuration), which increases with temperature. This contribution largely compensates the radius decrease connected with the decrease in the hydration number.

Shear Viscosity Data. As far as the shear viscosity behavior is concerned, Figure 4 shows the evolution of η as a function of concentration at four temperatures. As it can be seen, the most salient feature is the rapid rise of this macroscopic parameter when concentration is increased and temperature lowered. The shear viscosity data, when represented in the *strong–fragile* liquid classification scheme proposed by Angell, see Figure 5, show strongly curved profiles with respect to those observed in conventionally referred *strong* systems such as glass-forming SiO_2 and GeO_2 . It is well-known²⁰ that this diagram, which has been accorded a lot of attention, is based on the choice of an invariant viscosity at the scaling temperature T_g (10^{13} poise at the glass transition). The departure from the Arrhenius law is taken as a *signature* of the *fragility* degree of the system. The more rapid the temperature dependence of shear viscosity (or relaxation time) is in supercooled range, the more fragile is the system. Our data suggest that trehalose aqueous solutions, similarly to other systems characterized by simple nondirectional Coulomb attractions or by van der Waals

interactions, are extremely *fragile* in character. In particular the flexibility of the two hexapyranose rings around the glycosidic link gives rise to a high density of configurational states, namely a large degeneracy, which leads to a rapid smearing out of the structure with increasing temperature.

The insert of Figure 5 shows in a magnified scale the behaviour of $\log \eta$ vs T_g/T for the investigated solutions. As it can be seen the noticeable *fragile* character of the system increases by decreasing concentration. This relevant result indicates that at high dilution the system can reorganize its structures over a wider variety of different conformations.

Concluding Remarks

In this paper we have analyzed the behavior of the transport properties D_{eff} and η of aqueous trehalose solutions. These parameters, together with other derived quantities, reveal a noticeable temperature and concentration dependence linked to the making and breaking H-bond dynamics. What emerges from a retrospective glance to these findings is that the protective action of trehalose on biological structures could be attributed to the noticeable fragile character, namely, to its great structural sensitivity to temperature and concentration changes. In particular the flexibility around the glycoside oxygen among two rings, especially in the dilute region where the system reveals to be more fragile, could make trehalose able to conform the irregular surface of proteins and to replace a certain number of water molecules that are hydrogen-bonded to the protein surface in a similar way to water itself. Such facts, together with the highest value of the glass transition temperature in comparison with the other disaccharides, which implies a high water content at the glass transition, could explain the exceptional features of trehalose in living cells.

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