Experimental Study of the Hydration Properties of Homologous Disaccharides

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Abstract. To get some insight into the hydration mechanisms of homologous disaccharides, we report measurements on trehalose, maltose, and sucrose aqueous solutions. The interest on these systems is mainly due to the extraordinary properties of disaccharides and especially of trehalose, the most effective bio-protector against freezing and dehydration. To carry out this study we have investigated the volumetric properties of the three disaccharide solutions, by performing density and ultrasonic velocity measurements at different concentration and temperature values. What emerges from these studies is that trehalose shows, in comparison with maltose and sucrose, the greatest structural sensitivity to temperature changes and the smallest values of the partial molar volume in all the investigated temperature range, this circumstance being indicative of a more packed conformational arrangement.

Key words: aqueous solutions, density measurement, disaccharide, ultrasonic technique

1. Introduction

It is well known that among disaccharides trehalose seems to be unique in nature as bioprotector in drying and freezing processes. Trehalose is a naturally occurring, glass-forming disaccharide, exploited by nature for millennia across a range of desiccation-resistant organisms [1,2]. Empirical evidence shows that high concentrations of trehalose in the tissues of certain insects, yeasts, lactobacillus, mushrooms and desert plants (the resurrection plant is a particular example) allow them to survive for decades, and in some cases centuries, in a state of suspended animation under conditions of water deficiency, resuming rapidly their active metabolism when re-hydrated [3–6]. This process is called ‘anhydrobiosis’, to underline that the organisms don’t necessarily freeze-dry but dry at high temperatures [7]. Furthermore, trehalose, is widespread used in nature also as frost protector, being able to preserve living species from the damages caused by freezing [8, 9]. In fact, sugar accumulation, together with undercooling and vitrification, is a useful mechanism that organisms have for dealing with sub-zero temperatures [10]. Up to now many studies have evidenced a remarkably (and sometimes uniquely [6]) effectiveness of trehalose in stabilizing biomolecules, cells and tissues during air-drying and freeze-drying. Although other disaccharides such as maltose and sucrose have shown

similar properties [9–14], it clearly appears that trehalose is, with a great extent, the most effective bioprotector [15].

Green and Angell [8, 9] suggest that the greater trehalose effectiveness in respect to the other disaccharides, is related to its higher glass transition temperature, which implies higher water content at the transition. On the other hand Crowe and co-workers [10] have noted that vitrification is not sufficient for preservation. Vitrification alone doesn't explain, for example, why another carbohydrate, dextran, which has a glass transition temperature significantly higher than that of trehalose, is a much less effective cryoprotectant than trehalose. According to the water replacement hypothesis, first proposed by Crowe [16], as the systems are dried or frozen, the bonding of the trehalose hydrogens with the polar headgroups of the lipids that constitute biomembranes, replace those of hydration water at the membrane-fluid interface. In such a way, in the opinions of these authors, it prevents the phase transition and the accompanying leakage upon rehydration.

The extraordinary properties of trehalose make it attractive for industrial exploitation: it is widely used as excipients in the pharmaceutical industry, as an ingredient for dried and processed food, as a non-toxic cryoprotectant of vaccines and organs for surgical transplants [17], as well as a preservative in medicines, and as a moisture retainer in cosmetics.

From a general point of view, trehalose, maltose and sucrose have the same chemical formula ($C_{12}H_{22}O_{11}$) (molecular weight $M_w = 342.3$), but different structures, which could account for the different effectiveness. More precisely trehalose is a disaccharide of glucose (α -D-glucopyranosil α -D-glucopyranoside) constituted by two pyranose (six-membered) rings in the same α configuration, linked by a glycosidic bond between the chiral carbon atoms C1 of the two rings. Maltose (4-O- α -D-glucopyranosil-D-glucose) is also constituted by two pyranose rings of glucose in the α configuration, but the oxygen bridge links the two carbon atoms C1 and C4 of the two rings. Finally, sucrose (α -D-glucopyranosil β -D-fructofuranoside) is constituted by a glucose ring (pyranose) in the α configuration and a fructose ring (furanose) in the β configuration; the α and β structures of the same monosaccharide differ only in the orientation of the OH groups at some carbon atom in the ring itself (mutarotation equilibria).

Notwithstanding the extraordinary bioprotective and osmoregulative properties of trehalose are well established empirically, the underlying molecular mechanisms remain cryptic. It's out of doubt that to understand the molecular mechanisms determining the effectiveness of trehalose, it is important to characterize the structural and dynamical properties of disaccharide water solutions over a wide temperature range and to compare experimental data to the available simulation results, interpreting them within a single consistent picture. From the microscopic point of view, the interaction of trehalose with water has been principally investigated by three significant molecular dynamics simulation studies [11, 18, 19]. In a recent work reporting on results of statistical analyses on infinitely dilute aqueous solution of trehalose, whose initial configuration was taken from X-Ray data of the dihydrate

crystal, Fornili et al. [19] have found that the total number of molecules within 3.5 Å hydration shell is 35.9, 18.9 of which being hydrogen bonded to trehalose atoms. However using a more strict criterion for the hydrogen bond definition, that is a distance between proton and acceptor less than 2.4 Å and an angle formed by donor, hydrogen and acceptor $>145^\circ$, they have found that the hydration number for the most diluted solutions (6 wt%) at 27 °C decreases to 11.5 according to the findings obtained by Donnamaria et al using the SPC/E water model[10]. More recently, Conrad and De Pablo[18], using a fully-flexible SPC-type water model, have found that the hydration number of trehalose that is the average number of waters hydrogen bonded per trehalose molecule over the course of the simulation, decreases monotonically from 12.8 at 6 wt% to 3.2 at 90 wt% at 85 °C and from 13.4 to 3.4 for the same range at 25 °C. Indeed, besides the good agreement of these data, it is interesting to note that while the conclusions of Donnamaria support the idea that the water dynamics is only slightly altered by the presence of trehalose, Fornili has found that the water self-diffusion coefficient around the whole trehalose and around its oxygen atoms is 25% smaller than in pure water. Such a result is supported by Brady and co-workers in a molecular dynamics (MD) study [20] and by a series of Quasi Elastic Neutron Scattering (QENS) experiments [21, 22] which suggest that trehalose affects significantly the dynamics of water molecules in its neighbourhood consistently with the presence of a series of hydration layers or shells each of which is characterized by a relatively slow diffusion. Concerning the interaction disaccharides-water it has also been found by Raman scattering experiments[23,24] that disaccharides obstruct the crystallization process reducing the amount of freezable water, namely destroying the network of water compatible with that of ice. In addition, it has been evidenced that, among the three disaccharides, trehalose promotes the most substantial *destructuring effect* on the tetrahedral H-bond network of pure water.

In this frame, the present work, whose principal aim is to gain a better understanding of the hydration mechanisms of the three homologous disaccharides, trehalose, maltose and sucrose, with water, can be considered as a useful test for the working hypothesis of current simulation approaches. To carry out this study, we have investigated the volumetric properties of the three disaccharide solutions, by performing density and ultrasonic velocity measurements at different concentration and temperature values. What emerges from these studies is that trehalose shows, in comparison with maltose and sucrose, the smallest values of the partial molar volume in all the investigated temperature range. Far important, it is characterized by the most remarkable partial molar volume increase with temperature, revealing, in respect to the other disaccharides, the greatest structural sensitivity to temperature changes. The higher value of the hydration number together with the lower value of the apparent partial molar volume observed for trehalose, prove the existence of a more packed conformational arrangement, which however can open up itself by increasing temperature.

Table I. Trehalose, maltose and sucrose density values at different concentrations and temperatures

Trehalose							
ρ (gr cm ⁻³)							
m	T = 20	T = 30	T = 40	T = 50	T = 60	T = 70	T = 80
0.206	1.02456	1.02176	1.01816	1.01377	1.00880	1.00321	0.99708
0.556	1.06454	1.06120	1.05731	1.05249	1.04722	1.04151	1.03541
1.374	1.13950	1.13562	1.13151	1.12572	1.11993	1.11391	1.10761
Maltose							
ρ (gr cm ⁻³)							
m	T = 20	T = 30	T = 40	T = 50	T = 60	T = 70	T = 80
0.206	1.02435	1.02175	1.01785	1.01355	1.00860	1.00321	0.99691
0.556	1.06365	1.06055	1.05660	1.05201	1.04685	1.04081	1.03510
1.374	1.13801	1.13340	1.12901	1.12460	1.11860	1.11411	1.10671
Sucrose							
ρ (gr cm ⁻³)							
m	T = 20	T = 30	T = 40	T = 50	T = 60	T = 70	T = 80
0.206	1.02420	1.02130	1.01772	1.01340	1.00850	1.00311	0.99681
0.556	1.06352	1.06030	1.05651	1.05191	1.04680	1.04090	1.03401
1.374	1.13760	1.13360	1.12930	1.12450	1.11810	1.11401	1.10680

2. Experimental Set-Up

The investigated samples were purchased from Aldrich-Chemie. The solutions were prepared by weight using double distilled deionized water. Care was taken in order to obtain stable, clear and dust-free sample; ample time was allowed for equilibration. In addition, the solutions were filtered with 0.45 μm Amicon filters and stored in the dark to minimize biological and photo-chemical degradation.

Density measurements were performed using the standard picnometer technique. The picnometer has capillaries of narrow bore provided with well-fitting glass caps in order to avoid changes in composition because of evaporation. The picnometer was calibrated against densities of water with an accuracy of 0.1%. Density values were reproducible to one part in 10^5 . The temperature range investigated was 20 °C ÷ 80 °C with a temperature control better than 0.1 °C. In Table I the density values of trehalose, maltose and sucrose as a function of temperature at different molality values m , $m = 10^5 \phi / [100 M_d(1-\phi)]$ being M_d and ϕ the disaccharide molecular weight and the weight fraction respectively, are reported.

Figure 1. Apparent partial molar volume of trehalose as a function of concentration at different temperatures. The dashed lines represent the result of a linear fit procedure.

Ultrasonic velocity measurements were performed by using a home-made acoustic interferometer working at the frequency of 3 MHz. The acoustic interferometer was planned and realized in order to optimise the velocity measurements. To this purpose the transducer-reflector distance has been fixed at 12.2 cm. We have checked, by performing measurements at some values of concentration and for frequencies from 3 MHz to 20 MHz, that the sound velocity is almost frequency-independent in this range indicating that we are not in the presence of possible relaxation processes. The electronic equipment consisted of a standard Matec Inc. apparatus; measurements, reproducible within 0.05%, were performed using both the echoes overlapping method and sing-around principle [32] which involves the passing of repeated pulses of ultrasonic radiation through the solution. The ultrasonic cell was calibrated with water as standard, using the data of Del Grosso and Mader [25]. The cell was almost completely immersed in a water bath thermostatically controlled at $25 \pm 0.01^\circ\text{C}$.

3. Results and Discussion

It is well known that the solute partial molar volume, V_ϕ , can be written as the sum of the intrinsic volume of the solute, V_{intr} , and of the volume contribution due to solute-solvent interactions, $V_{solute-solvent}$,

$$V_\phi = V_{intr} + V_{solute-solvent}. \quad (1)$$

Figure 2. Apparent partial molar volume of maltose as a function of concentration at different temperatures. The dashed lines represent the result of a linear fit procedure.

Figure 3. Apparent partial molar volume of sucrose as a function of concentration at different temperatures. The dashed lines represent the result of a linear fit procedure.

Figure 4. Temperature dependence of the partial molar volume of trehalose, maltose and sucrose.

It can be therefore informative of the character of solute-solvent interactions [26]. In particular, from the measured densities it is possible to calculate the apparent partial molar volume, $V_\phi(T)$, by using the equation [27]:

$$V_\phi(T) = \frac{1000(\rho_w - \rho_d)}{m\rho_w\rho_d} + \frac{M_d}{\rho_d}, \quad (2)$$

where $\rho_w(T)$ and $\rho_d(T)$ are the density of water and solution respectively. Figures 1, 2 and 3 show, respectively, the trehalose, maltose, and sucrose apparent partial molar volume as a function of concentration at temperature values of 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C and 80 °C. The dashed lines represent the result of a linear fit procedure. The obtained data are in good agreement with published values where comparison is possible [27]. First of all, for each sample investigated, it is possible to evidence a linear dependence of V_ϕ on concentration, as expected for a non-electrolyte solute. Far important, one finds that there is a slight volume contraction when they dissolve in water, with a rate of change that increases with falling temperature. A noteworthy result is obtained by a comparative analysis of the data obtained for the three disaccharides; in fact, it emerges that, within the investigated concentration and temperature range, trehalose shows the smallest apparent partial molar volume values in respect to the three homologous disaccharides. This finding is confirmed by the evaluation of the partial molar volume obtained by extrapolating at infinite dilution the apparent partial molar volume. Also in this case, the agreement of our data with those reported by other authors, is fairly good [26, 28]. In Figure 4 the temperature dependence of the partial molar

volume of trehalose, maltose and sucrose is reported as a function of temperature. Notwithstanding for all the disaccharides one observes a marked increase of the partial molar volume by increasing the temperature, a peculiar behaviour is evidenced for trehalose. In particular, the most significant features are:

- i) trehalose shows, in comparison with maltose and sucrose, the smallest values of the partial molar volume in all the investigated temperature range, this circumstance being indicative of a more packed conformational arrangement especially at the lowest temperatures;
- ii) trehalose is characterized by the most remarkable partial molar volume increase with temperature, revealing, in respect to the other disaccharides, the greatest structural sensitivity to temperature changes.

These results support the findings obtained by NMR, PCS and MD [29–31] which indicate that the trehalose molecule in the diluted concentration range, is characterized by a high intramolecular flexibility that gives rise to internal motion of the two glucose rings around the glycosidic oxygen.

In order to ascertain whether this volume reduction is due to a folding of the disaccharide and to get information on the solute-solvent interaction strength, ultrasonic velocity measurements were performed on trehalose, maltose and sucrose aqueous solutions as a function of concentration and temperature. In fact, through the evaluation of the adiabatic compressibility coefficient by the Lorentz relationship $\beta = 1/\rho v^2$, we can get information on the disaccharide hydration number, n_H .

We assume, following a molecular model [32], that the volume of the solution V can be partitioned into two contributions: the hydration volume, V_H , where significant interactions between the disaccharide and water occur, and the bulk water volume, V_w . Following this model, the volume of the solution can be written as $V = n_d V_H + (n_w - n_d n_H) V_w$, where n_d and n_w are the mole numbers of disaccharide and of water, respectively. Under the hypothesis of negligible compressibility for hydrated units, we obtain the solute-solvent interaction strength, $n_w/n_d(1 - \beta/\beta_w)$, being β and β_w the adiabatic compressibility of the solution and of water, respectively. From the extrapolation at infinite dilution we can evaluate the hydration number by the formula:

$$n_H = \lim_{n_d \rightarrow 0} \frac{n_w}{n_d} \left(1 - \frac{\beta}{\beta_w}\right). \quad (3)$$

In Figure 5, as an example, the behaviour of the solute-solvent interaction strength as a function of concentration is reported for trehalose, maltose and sucrose at $T = 25^\circ\text{C}$. The continuous lines represent the result of a polynomial fitting procedure.

It clearly emerges that, in respect to the other disaccharides, the trehalose-water system is characterized, in all the investigated concentration range, by both the highest value of the interaction strength and of the hydration number. For example, at $T = 25^\circ\text{C}$, it is $n_H = 15.2$ for trehalose, $n_H = 14.7$ for maltose and $n_H = 14.1$ for sucrose. The higher value of the hydration number together with the lower value

Figure 5. Behaviour of the solute-solvent interaction strength of the three disaccharides as a function of concentration. The continuous lines represent the result of a polynomial fitting procedure.

of the apparent partial molar volume observed for trehalose, definitively prove the existence of a more packed conformation, which however can open up itself by increasing temperature. Furthermore, it is also evident that the conformational differences among the disaccharides tend to diminish by rising temperature.

The main drawback in calculating hydration numbers from ultrasonic measurements is the assumption that the compressibility of trehalose together with its closely associated water molecules, is negligible [32]. Inherent in this model is the further assumption that the compressibility of water molecules near, but not inside the primary hydration shells, is the same as that of pure water. Therefore, in general, the hydration number derived in this way can be different from those derived by means of *local* probes like neutron scattering or EXAFS, also because of differences in the models used with the various approaches and critical assumptions underlying these models. In particular, especially at high concentration values, apart from simple interactions between water and trehalose, other interactions could come into play, and, as a consequence, the compressibility behaviour could be related to the structural arrangement of the whole system rather than to specific entities. However, in our case, in spite of the simplicity of the model, due to the very diluted investigated concentration range which allows to neglect solute-solute interactions, the values obtained for n_H are in good agreement with those calculated by means of MD simulation [11, 18, 19].

The higher interaction strength of trehalose with water supports recent neutron scattering findings[33] obtained by using Mibemol spectrometer (LLB-Saclay)

which have allowed to characterize the different degree of fragility of the trehalose / H₂O and sucrose/H₂O solutions studying their vibrational dynamics. What emerged is that, at a molality $m = 2.92$, trehalose has the the strongest character in comparison with the sucrose mixture. This result agrees, and complements, those obtained by viscosity measurements[34] and Differential Scanning Calorimetry (DSC) [35] data which evidenciate that the trehalose mixture, at the same fixed concentration, shows the weakest glass transition peak ($\Delta C_p = 0.07 \text{ J(g K)}^{-1}$), in comparison with the sucrose mixture ($\Delta C_p = 0.09 \text{ J(g K)}^{-1}$), indicative of a stronger character. All these observations outline a picture in which the trehalose/water system plays the role, in comparison with the sucrose/water mixture, of the strongest system. On a biological point of view, this finding could imply a greater ability of trehalose to encapsulate biomolecules in more rigid and packed structures which could be relevant in avoiding water evaporation.

Concluding Remarks

In this paper we have reported on some experimental findings obtained by density and sound velocity measurements on trehalose, maltose and sucrose aqueous solution. The main point that emerge is that, among the investigated disaccharide solutions, the trehalose system shows, in the 20 °C÷80 °C temperature range, the smallest partial molar volume value, indicative of a more packed conformation, together with a greater partial volume increase with temperature, which indicates a greater structural sensitivity. In addition, ultrasonic data reveal that the trehalose-water system is characterized by the highest values of both the interaction strength parameter and of hydration number, so supporting the hypothesis of a more packed conformation especially at the lowest temperatures. On a biological point of view, this finding could imply a greater ability of trehalose to encapsulate biomolecules in more rigid and packed structures and hence a greater bioprotector effectiveness of trehalose in respect to sucrose.

In order to get more insight into the interaction mechanisms underlying the bioprotective properties of trehalose, further investigations are actually in progress.

References

1. Crowe, J.H., Crowe, L.M., Carpenter, J.F., Prestrelski, S. and Hoekstra, F.A.: Anhydrobiosis: Cellular Adaptation to Extreme Dehydration, In W.H. Dantzler (ed.), *Handbook of Physiology*, Oxford Univ. Press, Oxford, 1997, pp. 1445–1478.
2. Westh, P. and Ramlov, H.: Trehalose Accumulation in the Tardigrade *Adorybiotus Coronifer* during Anhydrobiosis, *J. Exp. Zoology* **258** (1991), 303–311.
3. Grba, S., Oura, E. and Suomalainen, H.: On the Formation of Glycogen and Trehalose in Baker's Yeast, *Eur. J. Appl. Microbiol.* **2** (1975), 29–31.
4. Williams, R.J. and Leopold, A.C.: The Glassy State in Corn Embryos, *Plant Physiol.* **89** (1989), 977–981.
5. Sun, W.Q., Irving, T.C. and Leopold, A.C.: The Role of Sugar, Vitrification and Membrane Phase Transition in Seed Desiccation Tolerance, *Physiol. Plant.* **90** (1994), 621–628.

6. Clegg, J.S.: The Control of Emergence and Metabolism by External Osmotic Pressure and the Role of Free Glycerol in Developing Cysts of *Artemia Salina*, *J. Exp. Biol.* **41** (1964), 879–892.
7. Crowe, J.H., Crowe, L.M., Crowe, Carpenter and Winstrom, C.A.: Stabilization of Dry Phospholipid Bilayers and Proteins by Sugars, *Biochem. J.* **242** (1987), 1–10.
8. Green, J.L. and Angell, C.A.: Phase Relations and Vitrification in Saccharide-Water Solutions and the Trehalose Anomaly, *J. Phys. Chem. B* **93** (1989), 2880–2882.
9. Angell, C.A.: Formation of Glasses from Liquids and Biopolymers, *Science* **267** (1995), 1924–1935.
10. Crowe, J.H., Leslie, S.B. and Crowe, L.M.: Is Vitrification Sufficient to Preserve Liposomes during Freeze-Drying?, *Cryobiology* **31** (1994), 355–366.
11. Donnamaria, M.C., Howard, E.I. and Grigera, J.R.: Interaction of Water with α,α -Trehalose in Solution: Molecular Dynamics Simulation Approach, *J. Chem. Soc. Faraday Trans.* **90** (1994), 2731–2735.
12. Leslie, S.B., Israeli, E., Lighthart, B., Crowe, J.H. and Crowe, L.M.: Trehalose and Sucrose Protect Both Membranes and Proteins in Intact Bacteria During Drying, *Appl. Environ. Microbiol.* **61** (1995), 3592–3597.
13. Fox, K.C.: Putting Protein Under Glass, *Science* **267** (1995), 1922–1923.
14. Chan, R.K., Pathmanathan, K. and Johari, G.P.: Dielectric Relaxations in the Liquid and Glassy States of Glucose and its Water Mixtures, *J. Phys. Chem. B* **90** (1986), 6358–6362.
15. Crowe, J.H., Crowe, L.M. and Jackson S.A.: Preservation of Structural and Functional Activity in Lyophilized Sarcoplasmic Reticulum, *Arch. Biochem. Biophys.* **220** (1983), 477–484.
16. Crowe, J.H., Carpenter, J.F. and Crowe, L.M.: The Role of Vitrification in Anhydrobiosis, *Annu. Rev. Physiol.* **60** (1998), 73–103.
17. Panek, A.D.: Trehalose Metabolism: New Horizons in Technological Applications, *Braz. J. Med. Biol. Res.* **28** (1995), 169–181.
18. Conrad, P.B. and de Pablo, J.: Computer Simulation of the Cryoprotectant Disaccharide α,α -Trehalose in Aqueous Solution, *J. Phys. Chem. A* **103** (1999), 4049–4055.
19. Bonanno, G., Noto, R., Fornili, S.L.: Water Interaction with α,α -Trehalose: Molecular Dynamics Simulation, *J. Chem. Soc., Faraday Trans.* **94** (1998), 2755–2762.
20. Liu, Q. and Brady, J.W.: Anisotropic Solvent Structuring in Aqueous Sugar Solutions, *J. Am. Chem. Soc.* **118** (1977), 12276–12286.
21. Magazù, S., Majolino, D., Middendorf, H.D., Migliardo, P., Musolino, A.M., Sciortino, M.T. and Wanderlingh, U.: α,α -Trehalose-Water Solutions: An Important Bioprotective Liquid System. Diffusive Motions as Probed by Dynamic Light Scattering and IQENS, In S. Cusack (ed.), *Biological Macromolecular Dynamics*, Adenine Press, New York, 1996, pp. 155–159.
22. Magazù, S., Maisano, G., Majolino, D., Migliardo, P., Mugolino, A.M. and Villari, V.: Diffusive Properties of α,α -Trehalose-Water Solutions, *Progr. of Theor. Phys.* **126** (1997), 195–200.
23. Branca, C., Magazù, S., Maisano, G. and Migliardo, P.: α,α -Trehalose-Water Solution. 3. Vibrational Dynamics Studies by Inelastic Light Scattering, *J. Phys. Chem. B* **103** (1999), 1347–1353.
24. Branca, C., Magazù, S., Maisano, G. and Migliardo, P.: Anomalous Cryoprotective Effectiveness of Trehalose: Raman Scattering Evidences, *J. Chem. Phys.* **111** (1999), 281–287.
25. Del Grosso, V.A. and Mader, C.W.: Speed of Sound in Pure Water, *J. Acoustic Soc. Am.* **52** n° 5 (1972), 1442–1446.
26. Galema, S.A. and Høiland, H.: Stereochemical Aspects of Hydration of Carbohydrates in Aqueous Solutions. 3. Density and Ultrasound Measurements, *J. Phys. Chem.* **95** (1991), 5321–5326.
27. Miller, D.P., de Pablo, J.J., Corti, H.: Thermophysical Properties of Trehalose and its Concentrated Aqueous Solutions, *Pharmaceutical Research* **14** (1997), 578–590.
28. Høiland, H. and Holvik, H.: Partial Molal Volumes and Compressibility of Carbohydrates in Water, *J. Solution Chem.* **7** (1978), 587–596.

29. Magazù, S., Maisano, G., Migliardo, P., Tettamanti, E. and Villari, V.: Transport Phenomena and Snomalous Glass-Forming Behaviour in α,α -Trehalose Aqueous Solutions, *Molecular Physics* **96** (1999), 381.
30. Magazù, S., Maisano, G., Middendorf, H.D., Migliardo, P. and Villari, V.: Hydration and Transport Properties of Aqueous Solutions of α,α -Trehalose, *J. Chem. Phys.* **109** (1998), 1170–1174.
31. Magazù, S., Maisano, G., Middendorf, H.D., Migliardo, P., Musolino, A.M. and Villari, V.: α,α -Trehalose-Water Solution. 2. Influence of Hydrogen Bond Connectivity on Transport Properties, *J. Phys. Chem. B* **102** (1998), 2060–2063.
32. Stuher, J. and Yeager, E.: *Physical Acoustic*, P.W. Mason (ed.), Academic Press, New York, 1965, p. 351.
33. Branca, C., Faraone, A., Magazù, S., Maisano, G., Migliardo, P., Migliardo, F. and Romeo, G.: Comparison of Disaccharide Solutions across Glass Transition, *Physica B*, to be published.
34. Branca, C., Magazù, S., Maisano, G., Migliardo, P. and Villari, V.: The Fragile Character and Structure-Breaker Role of α,α -Trehalose: Viscosity and Raman Scattering Findings, *J. Phys.: Condensed Matter* **11** (1999), 3823–3832.
35. Wang, G.M. and Haymet A.D.J.: Trehalose and Other Sugar Solutions at Low Temperature: Modulated Differential Scanning Calorimetry (MDSC), *J. Phys. Chem.B* **102** (1998), 5341–5347.