Water–disaccharides interactions in saturated solution and the crystallisation conditions

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Abstract

This paper reports solubility data and measurements of viscosity of the saturated aqueous solutions of sucrose, maltitol, and trehalose. Likewise, the metastable zone width and velocity of nucleation of the three disaccharides are compared. The narrowest metastable zone is observed for maltitol and the largest for trehalose. Such behaviour is due to a higher affinity of trehalose for water. Moreover, the crystallisation of anhydrous disaccharides in aqueous solution necessitates that hydration water be removed and evacuated from crystal integration surface to the bulk of solution to allow the growth of crystals. This step of disassociation and diffusion of hydration water proves to be the controlling step of the crystallisation process. Structural features at the origin of the differences between the three sugars are studied by FTIR spectroscopy. Modifications of frequencies and intensities of the vibrations around the glycosidic bond are interpreted in terms of conformational flexibility. Arguments like H-bond strength or conformational flexibility of the two monomers around the glycosidic oxygen were evoked as possible explanations of the behaviour of disaccharides. Likewise stability of hydration of the disaccharides is derived from the interpretation of FTIR spectra. These structural features help in interpreting the differences in crystallisation conditions and to hypothesize about the cryoprotective ability of the studied molecules.
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1. Introduction

There has been a growing interest in recent years for disaccharides especially as concerns their hydration, glass transition and biopreservation properties. Much less work was devoted to their crystallisation in aqueous medium. Attention was particularly focused on the ability of sugars in general and disaccharides in particular to solidify from solution as glass rather than crystals. The rearrangement of amorphous (freeze-dried) state under varied conditions of temperature and water vapour pressure to yield more or less stable crystalline forms was also studied (Césaro, Magazù, Migliardo, Sussich, & Vadalà,

2004; Sussich, Skopec, Brady, & Cesàro, 2001; Taga, Senma, & Osaki, 1972).

The studied disaccharides, namely, trehalose (α-D-glucopyranosyl(1-1)α-D-glucopyranoside), sucrose (β-D-fructofuranosyl(1-1)α-D-glucopyranoside) and maltitol (α-D-glucopyranosyl(1-4)glucitol) are nonreducing sugars, thermally and chemically stable, frequently used in food and nonfood applications. The ability of trehalose to protect biological material against freezing damage or dehydration deterioration was observed and correlated with its function in "cryptobiotic" and "anhydrobiotic" organisms where it is present at relatively high concentration. Sucrose is also known for its biopreservation efficiency among other numerous properties. Maltitol finds more and more applications as low calorie sweetener and acariogenic additive (Maguire, Rugg-Gunn, & Wright, 2000) in toothpastes, mouthwash and tablets. Arguments used

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to explain the special properties and applications of disaccharides remain unclear despite the different and numerous approaches both experimental (Branca, Magazù, Maisano, & Migliardo, 1999; Crowe, Crowe, & Chapman, 1984; Ediger, Angell, & Nagel, 1996; Green & Angell, 1989; Miller, de Pablo, & Corti, 1999; Miller & de Pablo, 2000; Oku et al., 2003; Orford, Parker, & Ring, 1990; Sussich, Princivalle, & Cesàro, 1999; Talja & Roos, 2001; Taylor & Zografi, 1998; Willart, Danede, De Gusseme, Descamps, & Neves, 2003) and theoretical (Bonanno, Noto, & Fornili, 1998; Conrad & de Pablo, 1999; Ekdawi-Sever, Conrad, & de Pablo, 2001; Ekdawi-Sever, de Pablo, Feick, & von Meerwall, 2003; Engelsen, Monteiro, Herv'e du Penhoat, & Perez, 2001; Liu, Schmidt, Teo, Karplus, & Brady, 1997; Molinero, Çağin, & Goddard, 2003; Naidoo & Kuttel, 2001; Roberts & Debenedetti, 1999; Sakurai, Murata, Inoue, Hino, & Kobayashi, 1997) already published.

Concentration conditions prevailing when a disaccharide is used as a cryoprotectant or when its structure is studied in freeze-dried or concentrated frozen phases are rather high closer to the saturated or supersaturated states than to the dilute solutions usually investigated by molecular modelling. Moreover, saturated solutions, although looking macroscopically as homogeneous liquids are in fact largely heterogeneous at the microscopic level. They contain organised swarms of solute molecules called "protonuclei" preparing the further step of nucleation. Various molecular associations take place in aqueous disaccharides solutions. At least three types of associations (water–water, water–sugar and sugar–sugar) occur together with collisions between the different associates.

Separation and purification of disaccharides generally requires application of the process of crystallisation from solution. As in any mass transfer operation a driving force or state of unbalance is necessary for the process to operate. In crystallisation, this driving force is a concentration difference, which is termed supersaturation. Therefore, it is needed to know the equilibrium state which is given by the solubility curve separating unsaturated region from supersaturated one. In fact, neither growth nor nucleation can take place in unsaturated region. Crystal growth is possible in the whole supersaturated region whereas nucleation can take place in the metastable zone only if seeds are already present and in labile region spontaneously (without seeds). The solubility of water soluble compound (sugars, salts,...) is needed to the determination of metastable zone width (MSZW) as it is one of its limits. The knowledge of the MSZW is necessary for the control of the various stages of crystallisation, mainly by reducing crystallisation process time and to have precise reaction parameters. MSZW was determined for sucrose solutions by use of the saturoscopic method (Bubnik, Kadlec, Pour, & Hinková, 2001). Metastable zone width is in fact the limit of supersaturation corresponding to the driving force needed to generate nucleation. The appearance of nuclei is a key step preceeding crystal growth and resulting from the establishment of sugar–sugar associates as concentration is increased and hydration water removed or it may be due to a decrease in temperature of solution. Nucleation can take place in the metastable zone and nuclei are formed as stable clusters of sugar molecules. This first step involves the gradual formation of sugar swarms in the liquid phase, but for the spontaneous appearance of a solid phase there is a need of very high supersaturation and this generally takes place in the labile zone. The critical size of sucrose nuclei, corresponds to the association of 80–100 molecules to reach a sphere with a radius equal to 20 Å (Van Hook, 1961). According to Kelly and Mak (1975), the minimum swarm of sucrose molecules needed to form a basic unit of crystal is 6. Also, it is possible that these hexamers exist in undersaturated solutions at the range of concentrations from 44% to 67% (w/w) at 20 °C (2/3 to saturation) (Mathlouthi & Genotelle, 1998). Of all problems concerning crystal growth from solutions, structure of solutions may play a crucial role in determining the rate of crystal growth and crystal habit. In an attempt to understand water–disaccharides interactions in aqueous solutions, the dynamic viscosity of these solutions was measured as a function of concentration up to saturation. In particular, viscosity of sugar saturated aqueous solutions is important to know for the interpretation of crystal growth mechanism. Crystal growth of sucrose in a supersaturated solution proceeds in two stages: a stage of diffusion corresponding to the transfer of molecules from bulk solution to the close vicinity of the crystal and a stage of integration of these molecules in the crystal lattice after disassociation of hydration water. Respective rates of these two steps are different and depending on temperature: at high temperature (above 40 °C), diffusion is the limiting step and at low temperature growth is controlled by integration (Van Hook, 1977). The diffusion coefficient is inversely proportional to viscosity. The average crystal growth rate (R_c) can be represented by the following expression (Mathlouthi & Genotelle, 1995):

$$R_c = k(\sigma - 1)/\eta \tag{1}$$

where σ is a solution supersaturation and k is a constant.

It is obvious that disaccharides owe some of their functional properties to such structural conformation as glycosidic bonds and hydrogen bonding. In both dilute and concentrated aqueous solutions, folding around the glycosidic linkage and hydrogen bonding plays major roles in the behaviour of disaccharides in solution. It is the case of properties such as solubility, viscosity and molecular arrangements that take place before crystallisation. FTIR spectroscopy only provides information on the vibrations of groups of atoms in a molecule. This can help elucidating structural features like molecular associations or changes in conformation (Kačuráková & Mathlouthi, 1996). To complement these molecular properties, the different physico-chemical properties having a

relation sugar–water or sugar–sugar interactions like solubility, metastable zone width, viscosity and nucleation induction time were determined. Induction time is the time that may elapse prior to the formation of a detectable amount of the new crystalline phase. This time measures the "ability" of the solution to remain out of equilibrium (supersaturated). Moreover, induction time depends largely on the technique used to measure it (Kashchiev & van Rosmalen, 2003). In addition, nucleation of concentrated amorphous solutions was studied. In this state, disaccharides form a fluid, solid-like matrix with a high viscosity and low molecular mobility which makes associations difficult to take place. The phase diagrams of the three studied disaccharide–water binary mixtures were revisited in the light of our metastable zone width and nucleation results with the aim to contribute to the clarification of notions like sugar glass fragility.

2. Materials and methods

2.1. Disaccharide solutions preparation

Trehalose and sucrose were obtained from Sigma and their purity was >99% (HPLC). Maltitol was supplied by Roquette Frères. The solutions, in HPLC grade doubly distilled water, were prepared by weighing, and the concentrations checked with a standardised Abbe refractometer. Concentrated amorphous solutions (CAS) were prepared by a rapid cooling of concentrated disaccharides solutions (85% w/w) obtained by sugar dissolution in HPLC grade doubly distilled water at temperature higher than 85 °C. After that, the three CAS were stored at −18 °C for 24 h.

2.2. Solubility measurements

The solubility of maltitol and trehalose in water was determined using an isothermal method. Three similar and covered double jacket beakers with a controlled temperature at ±0.1 °C by a cryothermostat (Techne TE-8D) were used. Fifty milliliter of distilled water and stir bars were placed in beakers to ensure proper mixing. Sugar powder was added in slight excess of the expected solubility limit. The three solutions were stirred at the same agitation speed for about 6 h, then, stirrers were stopped. After sedimentation of sugar particles suspended in the solution, concentration of the liquid phase was measured with an Abbe refractometer (EUROMEX) previously standardised with maltitol or trehalose known weight concentrations. Solutions were stirred again until the concentration becomes constant. After stabilisation, the concentration given in percentage of disaccharide in the solution was noted. The same experiment was repeated each 10 °C from 10 °C to 90 °C. The solubility values of sucrose in the same range of temperatures are taken from the literature (Bubnik & Kadlec, 1995).

2.3. Metastable zone width (MSZW)

The saturoscopic method (Bubnik et al., 2001) was used for determining the metastable zone width of maltitol and trehalose obtained by cooling at a constant rate (0.2 °C per 30 min) a sugar solution saturated at a given temperature. A saturoscope is a microscope (Hund H 500) equipped with a sample holder constituted of two metallic units including a coil for accurate (±0.1 °C) temperature control by a thermostatic bath (Techne TE-8D). A magnification of 400 was found convenient for observation the beginning of nucleation. A drop of saturated sugar solution is deposited in the saturoscope between a glass strip and a slide to avoid water evaporation. The large mass of metallic units and the small size of sample allow obtaining of stable temperature for the experimented solution sample. The temperature at which the first microscopic speck of a particle appears was noted in each solution. It corresponds to the limit of stability of supersaturated solution (or metastable zone width: MSZW). The same experiment was repeated with maltitol and trehalose solutions saturated in a temperature range from 20 °C to 85 °C. The metastable zone width of sucrose was taken from the literature (Vaccari & Mantovani, 1995).

2.4. Viscosity measurement

For viscosity measurements, a controlled temperature Couette type viscometer (Rheomat RM 225) was used. The viscosity was measured at 20 °C for disaccharide solution at weight fractions ranging from 0% to 58%. Likewise, viscosity of the saturated solutions at different temperatures between 20 °C and 80 °C was measured using the same device. All samples were subjected to an increasing shear rate from 100 to 600 s^{-1}.

2.5. Nucleation rate

To study disaccharides nucleation, saturated solutions at 70 °C were gradually cooled until the appearance of nuclei. The time of induction of nucleation was noted for each sugar. This method was not applicable to concentrated amorphous solutions (CAS) stored at −18 °C. These solutions were subjected to a slow heating of 5 °C per hour. Then, temperature and localisation of nuclei in the bulk of CAS was noted.

2.6. Fourier transform infrared spectroscopy (FTIR)

FTIR–ATR spectra were recorded with a Nicolet Impact 410 spectrometer interfaced with a OMNIC data processor (Happ-Genzel algorithm). An average of 200 scans was recorded at 2 cm^{-1} resolution. FTIR spectra of disaccharides aqueous solutions were obtained using a thunderdome horizontal ATR liquid cell with a ZnSe crystal (incident angle = 90°). For the three studied disaccharides, the wavenumber range was 3600–800 cm^{-1}.

3. Results and discussion

3.1. Macroscopic properties of disaccharides solutions

3.1.1. Solubility

Fig. 1 shows the solubility of maltitol, trehalose and sucrose in water as a function of temperature. As can be observed, disaccharide solubility values are relatively high, very likely because of the large number of equatorial hydroxyl groups in the disaccharide molecules. Such hydroxyl groups are more hydrated than axial hydroxyl groups. Vicinal hydroxyl groups in the equatorial configuration possess an O–O spacing similar to that of water molecules and therefore fit easily into the existing water structure. In Fig. 1 it may be observed that, for temperatures below 60 °C, sucrose is more soluble than maltitol and trehalose and above 60 °C, the situation is reversed. The establishment of water–maltitol bonds becomes, then, much easier than that of water with trehalose or sucrose. Such differences might originate from differences in the molecular structures of the three disaccharides (Fig. 2). Moreover, hydration number of sucrose is known to correspond approximately to five water molecules per sucrose
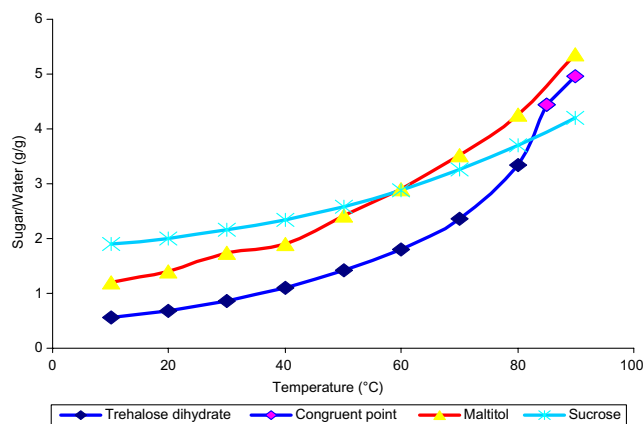
molecule (Starzak, Peacock, & Mathlouthi, 2000), whereas for maltitol the hydration corresponds on average to seven water molecules per maltitol molecule in diluted solution (Mathlouthi, Hutteau, & Angiboust, 1996). Rather than having a quasi-spherical shape like the sucrose molecule folded up with its two intramolecular bonds, the molecule of maltitol is unfolded; its acyclic half (D-glucitol) is flexible, and in aqueous solution a rotation around C(3′)–C(4′) and C(2′)–C(3′) bonds can take place. This leads to modifications of conformation of the maltitol molecule as concentration is varied which may help explaining solubility and crystallisation differences between the studied sugars. As for trehalose dihydrate, solubility curve is characterised by a remarkable increase at high temperatures and a congruent point is observed at 85 °C. This point can be considered as the intersection of solubility curves of the monohydrate on the one hand of the dihydrate on the other.

3.1.2. Metastable zone width

If a saturated sugar syrup drop is slowly cooled between blade and plate in the saturoscope, we quickly obtain a supersaturated solution. This state, although thermodynamically unstable (Mullin, 1972), can be maintained until the appearance of a solid phase. The maltitol showed a specific behaviour in supersaturated solutions characterised by a narrow supersaturation zone which slightly increases as temperature is raised showing a narrower metastable zone at low temperature (Fig. 3a). The obtained metastable zone width varies from 1.05 at 20 °C to 1.09 at 85 °C (Fig. 3a). This narrow metastable zone may originate from the motion of the D-glucitol moiety of maltitol; this flexibility leads to an easier establishment and rupture of hydrogen bonds between maltitol molecules. However, sucrose in supersaturated solutions adopts a molecular conformation with two intramolecular hydrogen bonds (Mathlouthi, 1981; Mathlouthi, Cholli, & Koenig, 1986) and the similar to that found in the crystal. Therefore, sucrose remains stable in solution at high supersaturation with a metastable limit situated at a supersaturation of 1.35 (Fig. 3b). Among
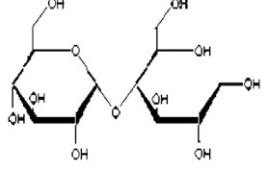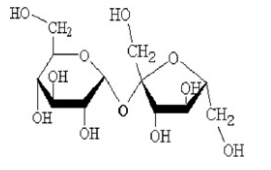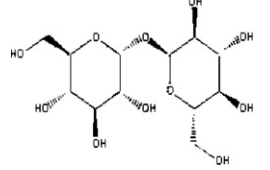

Fig. 1. Solubility of disaccharides in water.

Maltitol	Sucrose	Trehalose
α-D-Glcp(1-4) glucitol	α-D-Glcp (1-2) -D-Fruf	α-D-Glcp(1-1) -D- Glcp
Anhydrous crystal (m.p. 146°C)	Anhydrous crystal (m.p. 186°C)	Dihydrate T_h (m.p. 100°C) Anhydrous T_β (m.p. 215°C) Unstable polymorphs T_α; T_γ; T_ε; T_κ

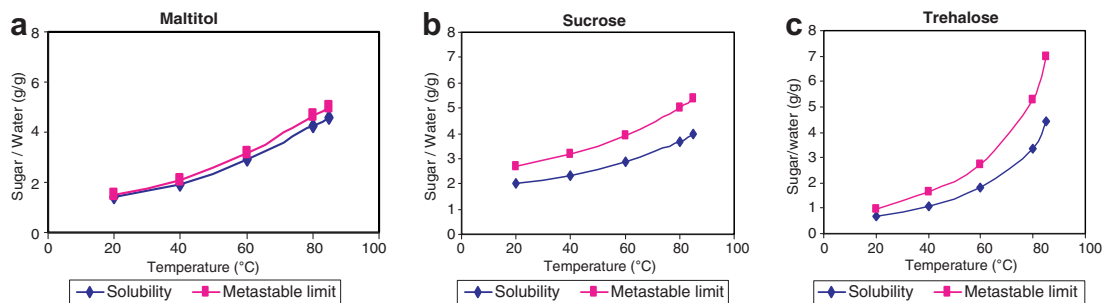Fig. 2. Structures and crystal characteristics of the studied disaccharides.

Fig. 3. Disaccharide metastable zone widths.

the three studied disaccharides, trehalose can be considered as the most stable in the supersaturated state. In fact, nucleation begins at supersaturation limit of 1.40 at 20 °C and reaches 1.58 at 85 °C (Fig. 3c). Such strong affinity with water, together with its chemical stability and glass-forming ability are at the origin of trehalose's success as a protectant of organisms which suffer dehydration stress. In addition, molecular modelling performed by Paul, Conrad, and de Pablo (1999) shows the possibility to that trehalose molecules possess an internal hydrogen bond at high concentration which reinforces its stability.

3.1.3. Viscosity

Fig. 4 shows that the rheological behaviour of aqueous solutions of maltitol and trehalose is very similar to that reported for sucrose (Mathlouthi & Genotelle, 1995; Bubnik, Kadlec, Urban, & Bruhns, 1995). All three disaccharides show a gradual increase in viscosity when mass concentration is varied from 10% to 58%. In fact, viscosity-concentration data obtained at 20 °C show that maltitol has the highest viscosity values in all studied concentration range and trehalose the lowest ones. This behaviour seems associated with molecular structure of maltitol which involves a free lateral chain responsible of increased maltitol–maltitol interactions. Oppositely, trehalose–water interactions are preponderant which leads to lower viscosity at the same concentration.
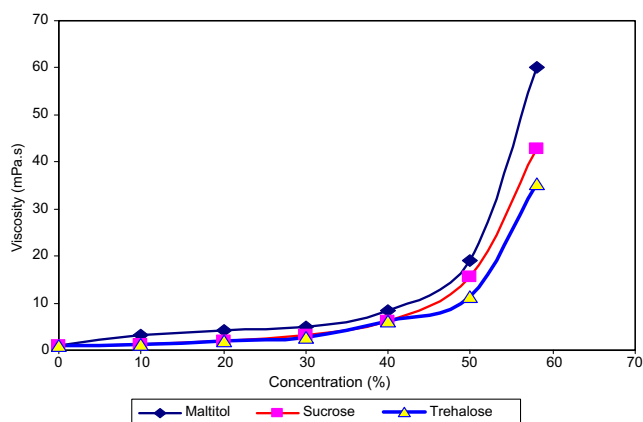
Dynamic viscosity of saturated disaccharide aqueous solutions is reported as a function of temperature (Fig. 5). It can be observed that viscosity of maltitol and trehalose saturated solutions increase as temperature is raised. This augmentation of viscosity was usually attributed to the formation of pre-critical molecular clusters in solution (Zhu et al., 2003). For that, in the maltitol and trehalose crystallisation processes, high temperatures must be avoided if not agitation must be improved. Moreover, the variation of dynamic viscosity of a saturated solution with increasing temperature is influenced by two opposite effect factors:

– concentration at saturation, which while increasing, tends to increase viscosity at constant temperature;
– temperature, which while increasing, tends to decrease viscosity at constant concentration.

Observation of Fig. 5 shows that maltitol and trehalose are more influenced by the "concentration effect" whereas sucrose is more influenced by the "temperature effect". The decrease in saturation concentration of sucrose as temperature is increased is predominant while the saturation concentration of trehalose and maltitol seems to rapidly augment and so is the case for viscosity.

3.1.4. Nucleation frequency

It is well known that nucleation depends largely on supersaturation and its induction time is the shorter the narrower the metastable zone width (Table 1). Therefore,

Fig. 4. Viscosity of disaccharide aqueous solution versus mass concentration.
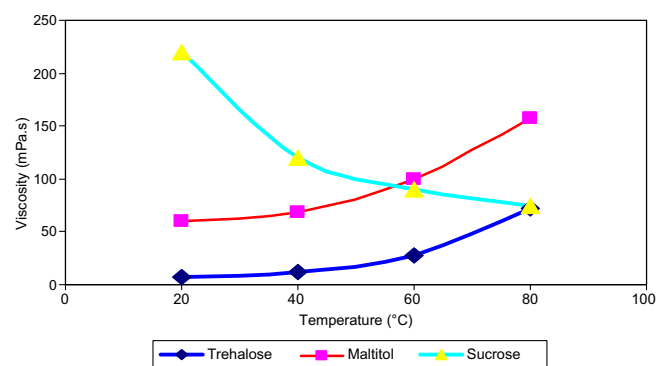

Fig. 5. Viscosity of disaccharide saturated solutions at different temperatures.

Table 1
Induction time and supersaturation of nucleation obtained by cooling solutions previously saturated with disaccharides at 70 °C

Disaccharides	Maltitol	Sucrose	Trehalose
Nucleation time (min)	22	48	85
Supersaturation	1.10	1.35	1.50

maltitol has the highest nucleation rate whereas trehalose has the lowest. The difference in nucleation frequency can be explained according to the physico-chemical properties of disaccharide saturated solutions. Indeed, at 70 °C maltitol is the most soluble sugar of the three studied ones (Fig. 1) and it has the narrowest metastable zone (Fig. 6). However, it seems that nucleation rate has not been affected by viscosity because at this temperature maltitol saturated solution has the highest viscosity value (Fig. 5). Nucleation rate is usually calculated according to theoretical thermodynamic models (Schoen, 1961). From experimental results, an empirical formula giving nucleation rate (V_N) as a function of the previous studied parameters can be proposed independently from viscosity:

$$V_N = K_R TS / \sigma_L \qquad (2)$$

where T is the temperature in K, S the ratio sugar/water given by solubility curves, σ_L the supersaturation coefficient at the limit of the metastable zone and K_R is a constant for the reaction between sugar molecules. K_R depends largely on sugar chemical structure and molecular mobility due essentially to the number of unbound hydroxyl groups. Trehalose solution is difficult to nucleate because of the strong trehalose–water interactions. This character has been studied using neutron diffraction and authors qualified it as a "noticeable kosmotrope" character (Césaro et al., 2004). In addition, trehalose–water systems are considered more homogeneous than sucrose and maltose ones and results of molecular dynamic simulations have shown that trehalose molecules possess similar capabilities to interact with both sugar and water molecules (Lerbret, Bordat, Affouard, Descamps, & Migliardo, 2005).

The difference in the crystallisation mechanisms of sucrose, trehalose and maltitol is certainly due to their interactions with water. During sucrose crystal growth,

incorporation of the sucrose molecules into the crystal lattice requires that hydration water molecules are disassociated. Because it seems to constitute the highest energy barrier to crystal growth, this step of dehydration has been considered as the rate-limiting step. Oppositely, maltitol molecules easily get rid of their hydration water and show a very high nucleation rate. This high nucleation rate could be the cause of lower crystal growth rates of this disaccharide (Longinotti, Mazzobre, Buera, & Corti, 2002). As for trehalose, the strong network of hydration water and its relatively rigid molecular conformation in aqueous solution provokes a resistance to crystallisation and to the removal of hydration water.

3.2. FTIR spectra

The 1200–800 cm^{-1} region of the Fourier transform infrared (FTIR) spectra, commonly called "finger print" region was explored for the three disaccharides in aqueous solution at concentrations ranging from 10% to 60%. This frequency region was especially used in this study to differentiate sucrose, trehalose and maltitol by their molecular vibrations sensitive to flexibility around the glycosidic bond. These vibrations are mainly due to C–O stretching in ring and inter-rings C–O–C as well as to C–O–H and C–C–H bending and also to the symmetrical deformations of CH$_2$ groups. The second studied FTIR spectra region is that situated between 1800 and 1600 cm^{-1} usually attributed to the H–O–H bending of water molecules. In fact this band gives important information about the effect of disaccharides on the hydrogen bonded network of water. FTIR spectra obtained were analysed by comparison with the literature on the one hand and using fractal dimensions of the normalised spectra on the other.

3.2.1. Analysis of the observed frequencies in the 1200–900 cm^{-1} range

FTIR spectra of aqueous solutions of disaccharides are reported in Fig. 7 and the observed frequencies listed in Table 2. Analysis of frequencies observed in the finger print region (1200–800 cm^{-1}) show some differences although the general aspects of spectra are comparable. Band similarities are more evident at concentrations below 40%. However, in concentrated and saturated solutions, bands become well-resolved as in the crystal spectra. The two major bands are centred at 1149 and 1049 cm^{-1} for trehalose, whereas for sucrose and maltitol slight frequency shifts are observed. These shifts of the C–O stretching and C–O–H bending could originate from a modification of the level of hydration of the C–O–H groups. Indeed, dihydrate trehalose contains two bonded water molecules that generate a more stable hydration shell of this disaccharide. One other band was observed for trehalose and maltitol but not for sucrose. This band is centred at 1033 cm^{-1} for trehalose and 1025 cm^{-1} for maltitol. It may be assigned to a ν(C–C), ν(C–O) stretching and δ(C–O–H) symmetrical bending originating from the C-4–O groups.
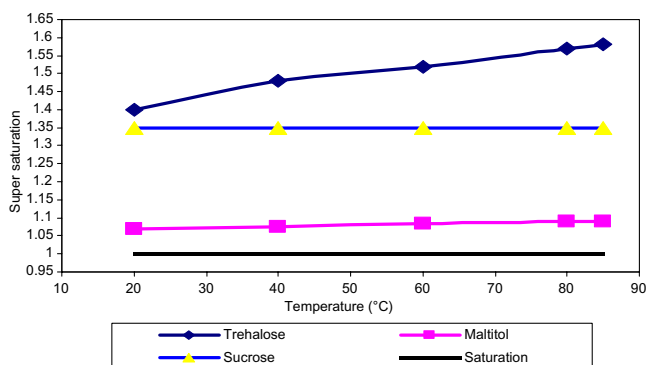

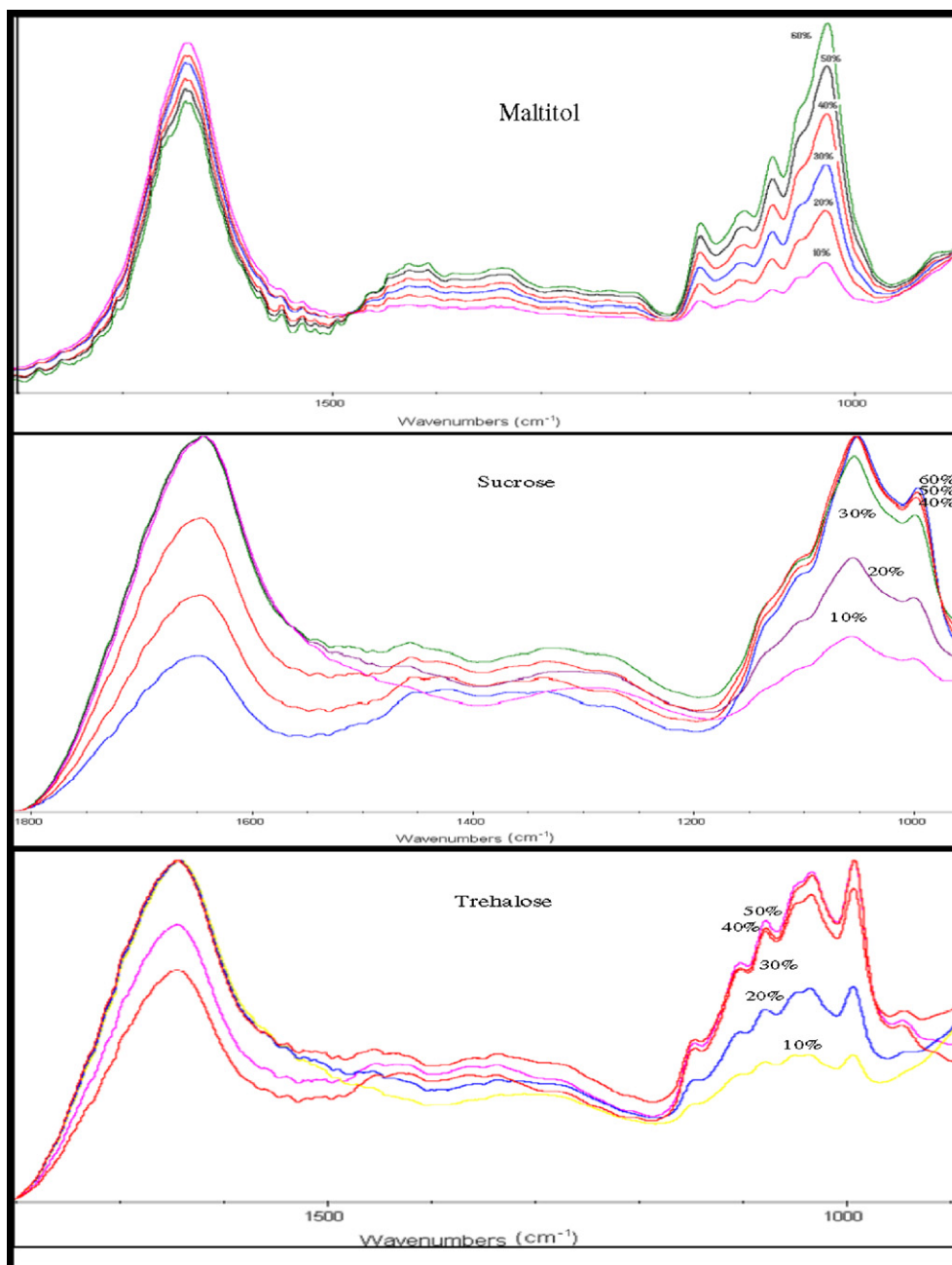Fig. 6. Comparison of three disaccharides metastable zone widths.

Fig. 7. General profiles of FTIR spectra of aqueous solution of disaccharides at different concentrations.

Because of maltitol molecular structure (stability of C-1–O-1–C-4 bridge), there are no observed peaks in the 950–900 cm^{-1} which is characteristic of the glycosidic bond. Moreover, the bands observed at 993 cm^{-1} for trehalose and 1000 cm^{-1} for concentrated sucrose solutions are very likely due to the anti-symmetric and symmetric stretching modes of C–O in the glycosidic linkage.

3.2.2. Fractal analysis of FTIR spectra in the 1200–900 cm^{-1} region

The fractal analysis of FTIR spectra was used to quantify differences between spectra shapes. Fractal dimensions of the recorded spectra were calculated as a function of

sugar concentration for the three disaccharides using a method previously detailed (Rogé, Gilli, & Mathlouthi, 2006). When mass concentration increases from 10% to 85%, fractal dimensions of the FTIR spectra of the three disaccharides present different trends (Fig. 8). As a general rule fractal dimension is sensitive to the fine structure of molecules (clustering, pre-nucleation,...). Therefore, observation of Fig. 8 shows that maltitol clustering seems independent from concentration which can be explained by its much more flexible D-glucitol moiety. However, the organisation of trehalose molecules increases gradually with concentration. In fact, trehalose molecules could not involve intramolecular hydrogen bonds like sucrose which was

Table 2
Observed frequencies (cm^{-1}) in the FTIR spectra of disaccharide aqueous solution at concentrations between 10% and 60%

| Suc (%w/w)						Tre (%w/w)					Mal (%w/w)					
10	20	30	40	50	60	10	20	30	40	50	10	20	30	40	50	60
–	–	–	–	–	–	1683	1682	1683	1683	1683	–	–	–	–	–	
1673	1674	1673	1673	1673	1673	–	–	–	–	–	–	–	–	–	–	–
1640	1640	1640	1641	1641	1640	1641	1642	1642	1642	1641	1641	1641	1641	1641	1640	1641
1145	1142	1141	1140	1137	1136	1149	1149	1149	1149	1149	1148	1148	1147	1148	1147	1147
–	–	1106	1105	1104	1100	1107	1105	1105	1105	1103	–	–	–	1107	1108	1108
						1081	1079	1079	1079	1078	1077	1078	1077	1078	1078	1078
1059	1056	1055	1054	1052	1050	1053	1050	1049	1049	1048	1055	1055	1056	1052	1051	1051
						1035	1033	1033	1032	1032						
–	–	–	–	–	–	–	–	–	–	–	1025	1025	1025	1026	1025	1025
–	–	–	–	–	–	–	–	1018	1017	1017	–	–	–	–	–	–
–	–	1001	1000	999	997	993	993	993	992	992	–	–	–	–	–	–
–	–	–	–	–	–	–	947	947	947	945	–	–	–	–	–	–
–	–	–	927	927	927	–	–	–	–	–	–	–	–	–	–	–
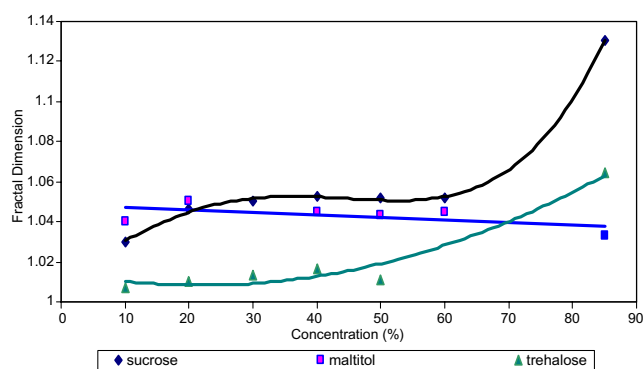
Fig. 8. Fractal dimensions of disaccharide FTIR spectra as a function of concentration.

Fig. 9. Integrated intensity ratio $\delta(C-O-H)/\delta(H-C-H)$ of disaccharide FTIR spectra.

found to have a molecular organisation changing around 20% and 60% because of the establishment of a first and then a second intramolecular H-bond (Mathlouthi, 1981; Mathlouthi et al., 1986).

3.2.3. Analysis of IR bands sensitive to hydration

In order to have a better insight of the differences between the studied disaccharides in their sensitivity to hydration, the ratio of integrated intensities (area) of IR bands characteristic of sugar on the one hand and sensitive to water on the other were calculated. The surface area of bands in the range of frequencies 1150–950 cm^{-1} assigned to C–O–H bending vibrations was compared to that attributed to CH$_2$ bending (1470–1200 cm^{-1}) unaffected by water interactions and the ratio of integrated intensity $\delta(C-O-H)/\delta(H-C-H)$ reported in Fig. 9. This ratio is the lowest for maltitol in the whole range of concentrations (20–85%), which indicates that this sugar is the less sensitive to hydration. This evoked property of maltitol can be at the origin of the highest nucleation rate and the easiest crystallisation behaviour of this disaccharide. The second interesting information which can be drawn from Fig. 9 is that the limit separating dilute from concentrated state is located at approxi-
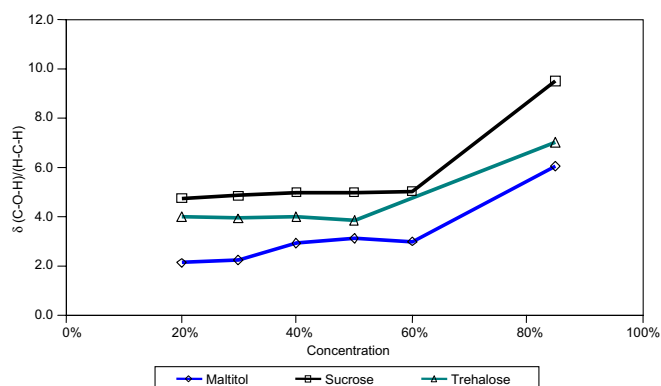
mately 60% for the three disaccharides. The change in slope of the integrated intensities ratio in function of concentration occurs around 60%, probably because of folding around glycosidic bond and sugar–sugar interactions which lead to a decrease in $\delta(CH_2)$ vibration intensity.

3.2.4. Analysis of the 1800–1600 cm^{-1} region

In the 1800–1600 cm^{-1} FTIR region, observed bands were assigned to the H–O–H bending mode. In this frequency region characteristic of water bending, it is possible to distinguish hydration from bulk water. Composite broad bands (Fig. 7) are observed at 1640 and 1674 cm^{-1} for sucrose and 1642 and 1680 cm^{-1} for trehalose. For maltitol, only one nearly symmetrical band was observed at 1640 cm^{-1} for all concentrations studied. Sucrose is known to have a relatively small hydration number and compact shape which explains its high mobility in solution (Ekdawi-Sever et al., 2003). On the other hand trehalose hydration seems not to depend on concentration. Molecular modelling allowed showing that trehalose hydration pattern in dilute solution is surprisingly close to that of the solid state dihydrate structure (Engelsen & Pérez, 2000).

3.2.5. FTIR spectra of concentrated amorphous solutions

FTIR spectra of concentrated amorphous solutions of the three analysed disaccharides are reported in Fig. 10 in the 1200–900 cm^{-1} region. This range of frequencies includes the highly coupled v(C–O), v(C–C) and δ(C–O–H) vibrational modes. Two sub-regions can be separately discussed:

At 960–900 cm^{-1} is the region characterising the C–O–C glycosidic bond folding that is typical for carbohydrates. The most intense absorbance in this region was observed for sucrose whose conformation is the more flexible even at high concentration (85% (w/w)). The low flexibility of trehalose molecule manifested by the lower intensity in this region can be explained by the symmetrical (1–1) bond as well as by the hydration which lead to a relatively stable conformation also responsible of the difficulties of nucleation. As for maltitol, it does not show any intense peak in this region because of the motion of the glucitol moiety.

At 1200–960 cm^{-1} is the frequency range affected by sugar–sugar interactions. Maltitol shows an arranged structure of molecules in the very concentrated solution characterised by a peak centred at 1020 cm^{-1}. Analysis of the general shape of spectra in this region allows classification of studied disaccharides in function of sugar–sugar interaction strength. Accordingly, trehalose concentrated amorphous solution shows the weakest interactions (less intense peak) and maltitol the strongest. Sucrose, has an intermediate position in agreement with metastable zone width and viscosity results. In these very concentrated solutions, the incremental formation of sugar–sugar hydrogen bonds gives solutions their characteristic "polymeric" properties (Molinero et al., 2003).

3.3. Phase diagrams revisited

Phase diagrams of the binary maltitol–water, sucrose–water and trehalose–water systems are reported in Fig. 11. Data from five literature sources are plotted as temperature versus disaccharide mass fraction. The phase diagrams help predicting of glass transition temperature for a given binary mixture. It provides also equilibrium conditions (freezing of water; solubility of sugar). To this information, we have added metastable zone limit and concentrated amorphous
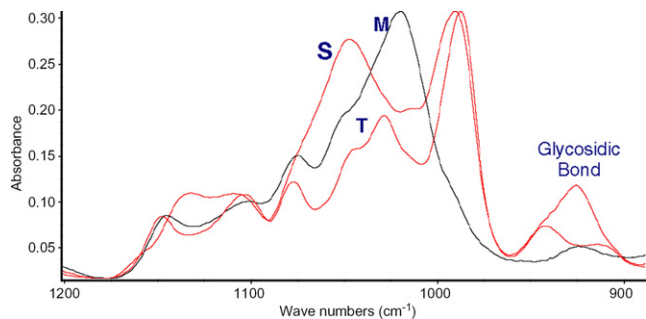

Fig. 10. FTIR spectra of disaccharide concentrated amorphous solutions (**M**: maltitol, **S**: sucrose, **T**: trehalose).
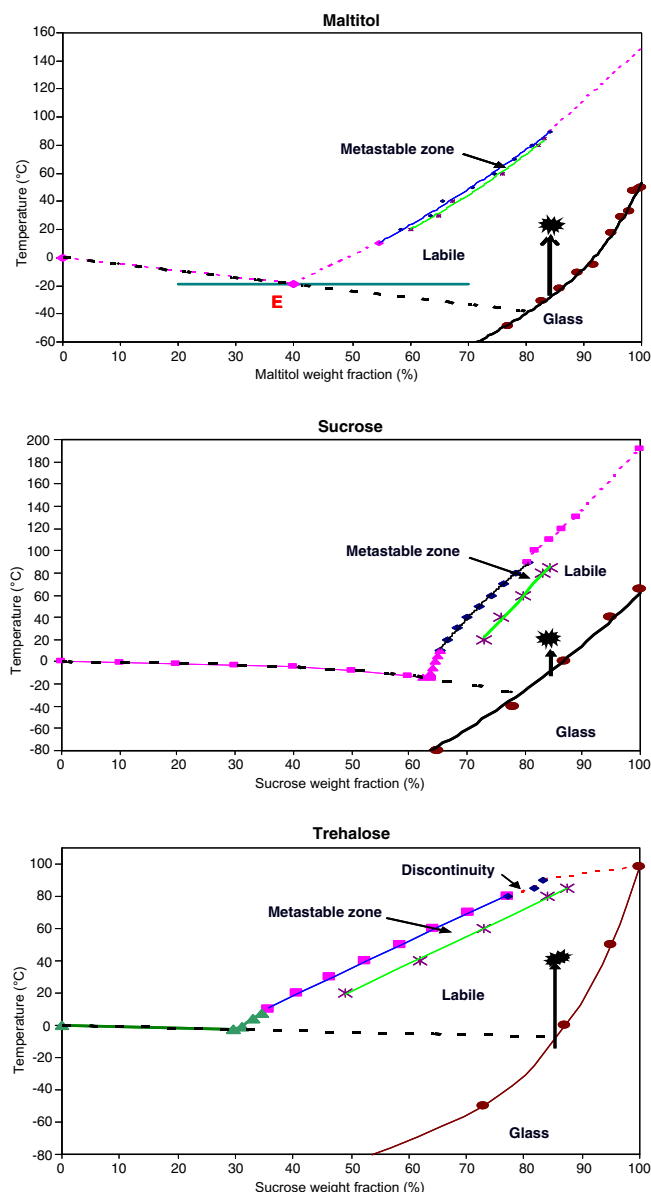

Fig. 11. Disaccharide phase diagrams and CAS nucleation (*: CAS nucleation temperature). [References: Maltitol T_g: Siniti et al. (1999). Sucrose undercooling: Bubnik and Kadlec (1995). Sucrose T_g: Roos and Karel (1991). Trehalose undercooling: Miller et al. (1997). Trehalose T_g: Chen et al. (2000)].

solution nucleation conditions for each disaccharide. This nucleation, assimilated to the recrystallisation of amorphous state (CAS) is highly dependent on water content and mobility (McGarvey, Kett, & Craig, 2003). As observed in Fig. 11, it takes place in the labile zone (supersaturation above metastable zone limit). The difference between glass transition and CAS nucleation temperature is proposed as an indicator of system fragility. Results summarised in Table 3 show that trehalose CAS is the most fragile of the three studied disaccharides. It is the most prompt to crystallise even 70 °C below T_g, which may be expected as trehalose viscosity is the lowest among all studied disaccharides. As noticed by Angell (2002), "fragility" is a

Table 3
Correlation between CAS nucleation and disaccharide glass fragility

Disaccharide	Nucleation temperature (T_N) (°C)	$T_g - T_N$ (glass fragility) (°C)	Crystal growth characteristics
Trehalose	45	70	Rapid (middle of sample)
Sucrose	15	45	Slow (dispersed in all the sample)
Maltitol	25	29	Very slow (surface of solution)

notion difficult to explain satisfactorily. It seems to have both kinetics and thermodynamic origins. So is the nucleation in concentrated amorphous system. Controversy about the definition of fragility remains. For example, Magazù, Migliardo, Mondelli, and Vadalà (2005) state that the higher effectiveness of trehalose's 'cryptobiotic' action can be warranted by the lower fragility of the trehalose–H_2O mixture. Differences in stability of disaccharide concentrated amorphous solutions and the ease of nucleation can be attributed to the differences in water–sugar and sugar–sugar interactions as well as to T_g values. As a general rule, high T_g sugars exhibit a greater degree of freedom to rearrange hydrogen bonds during changes in temperature than low T_g sugars (Wolkers, Oliver, Tablin, & Crowe, 2004). We have also described empirical observation of growth after nucleation in each of CAS samples. Again, a rapid evolution of trehalose–water system as compared to sucrose and maltitol is observed. Trehalose dihydrate predominates in the solution preventing water crystallisation, which very likely is the clue to "cryoprotection".

4. Conclusion

Solution properties and crystallisation conditions of three disaccharides (maltitol, sucrose and trehalose) were determined. Of particular interest are viscosity of saturated solution, metastable zone width and nucleation frequency. The differences between the three disaccharides seem to be linked to their different molecular interactions in the aqueous medium and to the stability of glycosidic bond. From analysis of the FTIR spectra of the aqueous solutions of disaccharides, it appears that sucrose remains to have the most flexible conformation around glycosidic bond whereas maltitol shows lateral motion of its glucitol moiety allowing easier maltitol–maltitol interactions, hence a high nucleation rate. Analysis of FTIR spectra also informs on the sensitivity of peripheral groups of atoms in disaccharides to hydration water. As expected, trehalose and sucrose are more sensitive to hydration than maltitol. Moreover, evidence is made for the presence of hydration and free water in sucrose and trehalose solutions while only one type of water (free) is found in presence of concentrated maltitol. Investigation of concentrated amorphous solutions (85%) stored at freezing temperature (−18 °C) allowed revealing that trehalose

has the highest glass fragility estimated from the difference between glass transition and nucleation temperature. The ease of nucleation due to a lower viscosity and a "water structure breaker effect" seems to be a piece of information to add to the current effort led by different workers to elucidate the cryoprotection mechanism. Moreover, phase diagrams can be usefully complemented by the representation of MSZW and spontaneous nucleation of concentrated amorphous solution.

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