

Slow Molecular Mobility in the Amorphous Solid State of Fructose: Fragility and Aging

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ABSTRACT: The molecular mobility of β -D-fructose was studied by thermally stimulated depolarization currents (TSDC) in the amorphous solid state. The amorphous solid samples were prepared in such a way that the tautomeric mixture was near the equilibrium composition. A broad secondary relaxation was observed, that merges, at high temperatures, with the alpha relaxation. The alpha relaxation temperature provided by the TSDC technique is $T_g = 13\text{ }^\circ\text{C}$ (at 4 K min^{-1}). The fragility index calculated from TSDC data is $m = 34$, significantly lower when compared with the values reported in the literature obtained from Dielectric Relaxation Spectroscopy (DRS). The physical significance of the fragility obtained by the 2 dielectric techniques is discussed. The influence of physical aging on the secondary relaxation in amorphous fructose was analyzed as the glass structurally relaxes. A complex behavior was observed such that the faster components (lower temperature) of the secondary relaxation are negligibly dependent on aging and may be ascribed to intramolecular modes of motion, while the slower motional modes (higher temperature) show a significant dependence on aging and correspond to the genuine Johari–Goldstein β -relaxation.

Keywords: aging, amorphous state, carbohydrate, fragility, glass transition, glassy state, secondary relaxations

Introduction

Carbohydrates are of universal importance playing a key role in the molecular logic of life (Lee and Lee 1995; Dwek and Butters 2002). The use of such compounds in biopreservation (Fox 1995; Crowe 2002), particularly in the cryopreservation of cells, has revived research on this important class of biomolecules.

Their low toxicity (Kuleshova and others 1999) and stabilizer ability confer them a major role in food science (Levine 2002) and pharmaceutical industry (Werz and Seeberger 2005) by assuring processing, storage, and handling stability. Moreover, carbohydrates are recently considered as green raw materials for the chemical industry (Lichtenthaler and Peters 2004).

Among the low molecular weight carbohydrates, fructose is being used as sweetener for replacing glucose since, contrary to glucose, it is found to be innocuous for diabetes patients (Oppenheimer and others 2008). Furthermore, its effectiveness to reduce undesirable effects caused by freezing (for example, dehydration of cells and denaturation of globular proteins) is only exceeded by trehalose and maltose (Green and Angell 1989).

Both crystalline and amorphous states are important in the field of food and pharmaceutical technology. The amorphous form of drugs and excipients is of pharmaceutical interest since it has been reported to improve solubility, accelerate dissolution, and bioavailability and promote therapeutic activity (Yu 2001; Roberts and Debenedetti 2002).

There is a general idea that, as long as the temperature remains below T_g , some undesirable chemical reactions that lead to deteri-

oration may not proceed. However, evidence does exist that even below T_g there are amorphous materials that still exhibit significant molecular mobility (Andronis and Zografis 1997). In the glassy state, the system is in a nonequilibrium state, with respect to both crystalline and “equilibrium” glassy state (Andronis and Zografis 1997). As a consequence, it will evolve at a rate that depends on the temperature and the thermal history of the glass; the evolution that occurs below the glass transition temperature is referred to as structural relaxation or physical aging (Wungtanagorn and Schmidt 2001). Thus, to prevent the molecular processes responsible for destabilization of an amorphous substance over the normal life-time of a pharmaceutical or food product, we need to know the time scales of molecular motion under a variety of conditions (Hancock and others 1995). It is also important to understand the factors that control crystallization, which is often desirable during processing or storage of food (Walstra 2003) or that can occur below T_g , from the solid amorphous state (Andronis and Zografis 1997). Therefore, the purpose of this study was to characterize the time scales for molecular mobility of the monosaccharide fructose in the supercooled and glassy states. While most studies of sugar mobility are carried out in aqueous solutions of these substances (Green and Angell 1989; Magazu and others 1998; Mehl 1998; Feeney and others 2001; Fuchs and Kaatz 2002; Shinyashiki and others 2008), studies of molecular mobility in the pure substances by relaxation techniques are scarce (Tombari and others 2001; Kaminski and others 2006). D-fructose is a monosaccharide with 6 carbon atoms that presents 5 tautomeric structures, 1 linear similar to that of sorbitol and 4 cyclic, that are shown in Figure 1. In the crystalline form, monosaccharides exist only in the pyranose form. The transformation of one sugar isomer to another is a solvent-mediated process and thus cannot occur once the monosaccharide is in the gas phase (Finch 1999). However, in the melt this transition is possible, and the equilibrium liquid is a mixture of different conformers. Crystalline β -D-fructose is an hexose consisting entirely of β -D-fructopyranose, but in its molten state other conformers may form (Tombari and others 2001). Fructose in the liquid state

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appears as a very intricate system (Fan and Angell 1995; Fan and Angell 1996). Part of the complexity is due to the fact that the liquid obtained from the fusion of the crystal is not in an equilibrium state: it contains more β -D-fructopyranose tautomer (which makes up the crystal) than does the equilibrated liquid (with lowest free energy).

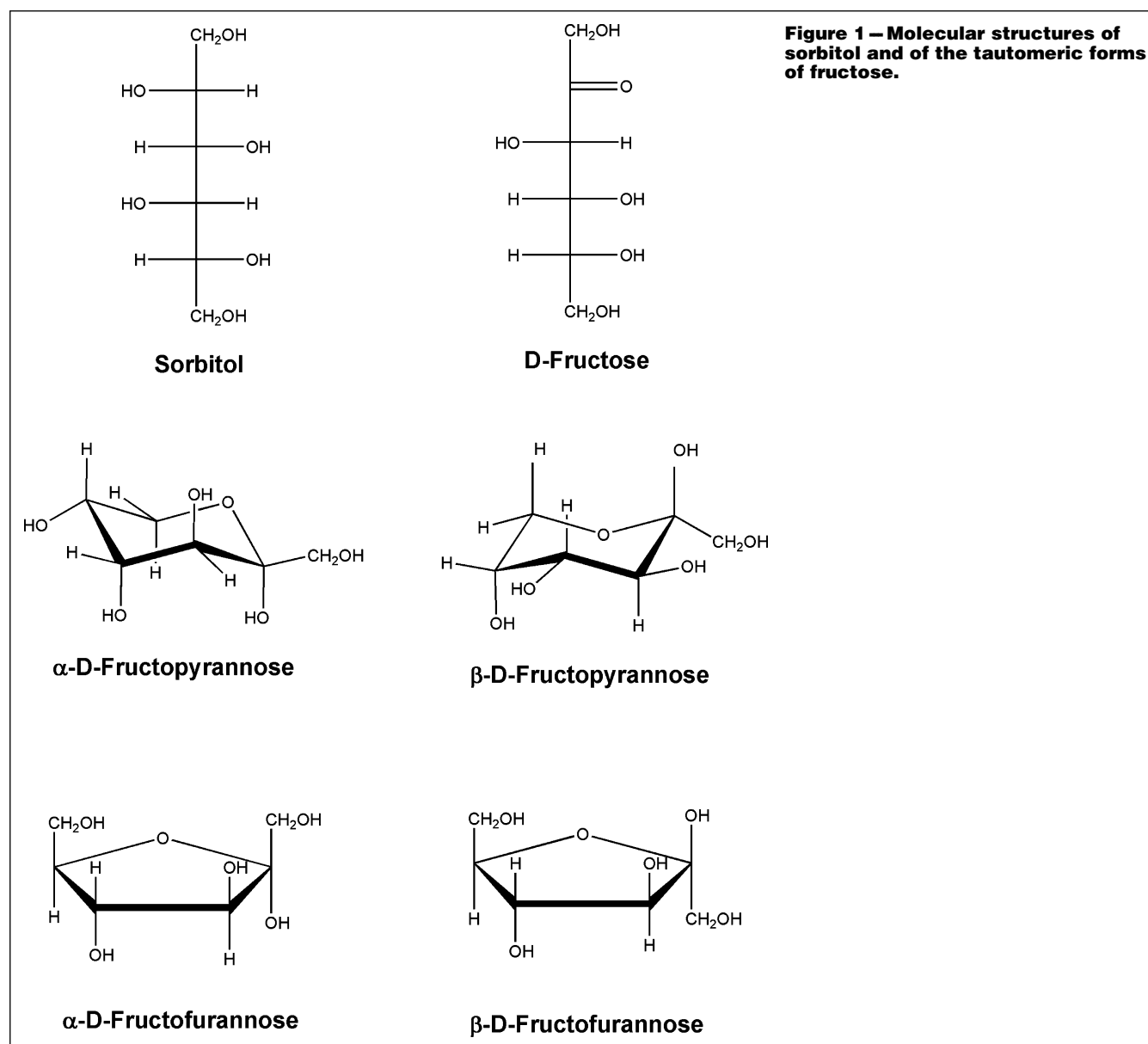
In fact, while the crystal of β -D-fructose is constituted by a single tautomer (β -D-fructopyranose), the equilibrium liquid is a mixture of tautomers where β -D-fructopyranose is dominant, but where the fructofuranose forms are present in appreciable proportions.

Furthermore, the tautomer equilibrium is accomplished on a time scale longer compared with that of the melting process. The equilibrium composition at 98 °C (371 K) is attained after 2.5 to 3 h. Another part of the complexity arises from the decomposition processes that occur around the melting point. However, the time scale of decomposition is fortunately longer than that of equilibration. Finally, the fact that the equilibrium constant is temperature dependent also contributes to the complexity of the system (Fan and Angell 1995). The various conformers (5- and 6-membered rings)

form intermolecular H-bonds meaning that the liquid presents different structures with different H-bonding at different temperatures. Calculations also suggest that although furanosides are capable of stronger (shorter) intramolecular hydrogen bonding, the smaller dihedral angle between adjacent hydroxyl groups cancels some of that stabilizing effect as a consequence of increased steric interactions. Due to steric limitations of the furanose ring, the 5-carbon furanosides are capable only of forming 2 intramolecular hydrogen bonds, whereas the pyranosides can form 3 such bonds. The previously mentioned results suggest that pyranosides are thermodynamically more stable than furanosides (Guler and others 2002). This in itself, rather than solvation effects, could be the main reason for monosaccharides preferring pyranoside structures in solution and in the melted state.

For D-glucose, only pyranose forms are reported in the liquid state, in opposition to D-fructose where a large amount of furanose forms can exist in the melt depending on the thermal history. The presence of substantial proportions of furanose isomers in D-fructose may be at the origin of the different relaxational behavior of this monosaccharide as compared with D-glucose. As stated

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before, the purpose of this study was to characterize the time scales for molecular mobility of the monosaccharide fructose. The dielectric technique of thermostimulated depolarization currents (TSDC) is able to probe slow reorientational motions and, consequently, is suitable to study mobility in solids so that it will be used to this purpose. With these studies, we look at getting relevant information regarding the different modes of motion present in fructose, and to bring a better understanding of the mechanisms leading to changes during storage and handling or even processing of such a substance.

Materials and Methods

Fructose, $C_6H_{12}O_6$, molecular weight $MW = 180.1572 \text{ g}\cdot\text{mol}^{-1}$, was from Sigma-Aldrich (Steinheim, Germany), catalog n° 23,970-4, degree of purity 99%+, and was used without further purification.

The DSC thermogram of our crystalline fructose, obtained with a heating rate of $10 \text{ K}/\text{min}$, showed a melting peak with an onset at $T_{on} = 114.7 \text{ }^\circ\text{C}$ (387.7 K) and a maximum intensity at $T_m = 131.4 \text{ }^\circ\text{C}$ (404.5 K). The large difference between the temperatures of the onset and of the maximum of the melting peak (17 K) is to be underlined. Care must be taken to minimize the temperature excursion above T_{fus} to avoid decomposition. According to ^{13}C NMR, a 6-h isotherm at 374 K leads to small scale development of new peaks (Fan and Angell 1995). Decomposition is prevented if the samples are left at 378 K for few minutes.

The differential scanning calorimetry (DSC) experiments were performed with a 2920 MDSC system from TA Instruments Inc. Dry high purity He gas with a flow rate of $30 \text{ cm}^3/\text{min}$ was purged through the sample. Cooling was accomplished with the liquid nitrogen cooling accessory (LNCA), which provides automatic and continuous programmed sample cooling down to $-150 \text{ }^\circ\text{C}$ (123 K). The baseline was calibrated scanning the temperature domain of the experiments with an empty pan. Details of the calibration procedure are given elsewhere (Moura Ramos and others 2004).

Thermally stimulated depolarization current (TSDC) experiments were carried out with a TSC/RMA 9000 spectrometer (Thermold Partners, Stamford, Conn., U.S.A.) covering the temperature range between -170 and $+400 \text{ }^\circ\text{C}$. The melting point of indium was used to calibrate the instrument at different linear rates, from $-20 \text{ K}\cdot\text{min}^{-1}$ to $+20 \text{ K}\cdot\text{min}^{-1}$. The software of the instrument provides a calibration method that produces a specific data file. When the calibration file is loaded into the “analysis” software application, the software recognizes this file, and allows the calibration of the instrument. The configuration of the cell is a parallel plate capacitor with an effective area of approximately 38 mm^2 . The sample, in the pellet form and placed between the 2 electrodes, is immersed in an atmosphere of high purity helium (1.1 bar). Our samples were studied in the temperature interval from $-160 \text{ }^\circ\text{C}$ up to $+40 \text{ }^\circ\text{C}$, and several baselines were performed (experiments obtained by heating the depolarized sample) to verify that no singular electric event (space charge effects for example) was detected in the same temperature range. A constant and very weak current intensity was obtained in all those experiments (flat baseline). Fructose in the solid amorphous phase was prepared inside the apparatus by melting a pellet of the crystal (thickness of approximately 0.5 mm) and cooling the isotropic liquid. The substance displayed excellent glass forming ability, with a wide supercooled liquid temperature region and high thermal stability against crystallization. On the other hand, the preparation conditions previously indicated (helium atmosphere and melting above $100 \text{ }^\circ\text{C}$) ensure a very low water content of the sample. A total of 3 inde-

pendent fructose pellets were studied, and the results showed full reproducibility.

Several references explaining the physical background of TSDC are available (van Turnhout 1975; Chen and Kirsch 1981; Teyssedre and others 1997) and can be useful for the reader not familiar with this experimental technique. Moreover, several studies (Teyssedre and Lacabanne 1995; Alvarez and others 2000; Correia and others 2001a, 2001b) can be helpful to explain the experimental procedures used in TSDC, and the physical meaning of the data provided by this technique.

Results and Discussion

DSC and TSDC signatures of the tautomer equilibrium

Considering the importance of the time-temperature histories in the properties of liquid fructose, we decided to prepare the samples for study in different ways, as explained in the following. We will call “fresh melt” (Fan and Angell 1995) a sample that was completely melted and immediately cooled down to more than $30 \text{ }^\circ\text{C}$ below the melting point. Our fresh melt samples were prepared using 2 different procedures: (1) heating at $T = 115 \text{ }^\circ\text{C}$ (388.2 K) and annealing 7 min at this temperature, followed by fast cooling to $-20 \text{ }^\circ\text{C}$; (2) heating at $T = 155 \text{ }^\circ\text{C}$ (428.2 K) immediately followed by fast cooling to $-20 \text{ }^\circ\text{C}$. In the 1st procedure the lower heating temperature is supposed to prevent decomposition, while the annealing period is designed to ensure complete melting; in the 2nd procedure, inversely, the higher heating temperature ensures complete melting, while the absence of annealing minimizes decomposition. Let us note that both ways of preparing the fresh melt lead to the same results. At the opposite of the fresh melt, we will call “relaxed melt” a sample where the tautomer mixture has the equilibrium composition, that is, where the equilibration was allowed to occur. To allow the equilibration of the fresh melt, we annealed it at temperatures above T_g , in the supercooled liquid state, specifically at $T_a = 30 \text{ }^\circ\text{C}$. Figure 2 shows the calorimetric signature of

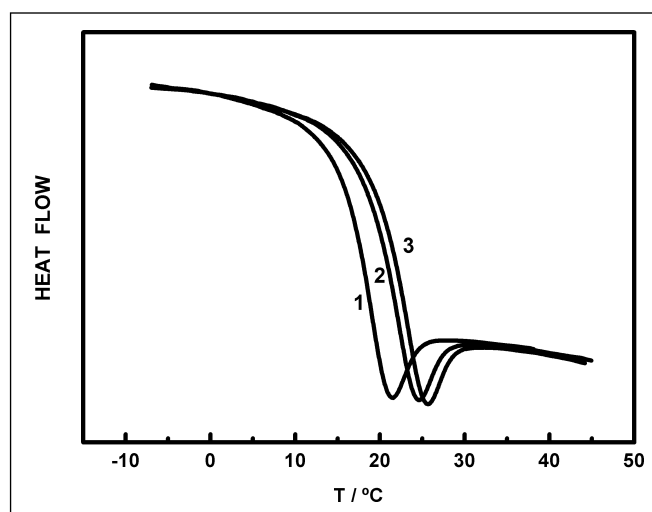


Figure 2—DSC glass transition signature of D-fructose in different states of tautomer equilibration. Curve 1 corresponds to the so-called fresh melt (see text). Curve 2 was obtained after annealing the fresh melt for 3 h at $T_a = 30 \text{ }^\circ\text{C}$. The sample corresponding to curve 2 left at room temperature ($20 \text{ }^\circ\text{C}$) for 14 h gave rise to the signal shown in curve 3, which nearly corresponds to the so-called relaxed melt. The heating rate of the experiments was $10 \text{ K}\cdot\text{min}^{-1}$, and the sample was always kept under helium atmosphere.

the glass transition of D-fructose in 3 different degrees of anomeric equilibration.

We conclude from Figure 2 that the signal is displaced to higher temperatures as the equilibrium evolves from the fresh melt to the relaxed melt, in agreement with some researchers (Truong and others 2002), but in apparent contradiction with others (Fan and Angell 1995).

Let us stress that this evolution from the fresh to the relaxed melt, induced by annealing above T_g , corresponds to a slow chemical equilibration process that cannot be confused with physical aging (Truong and others 2002).

The onset temperature of the DSC glass transition signal we obtained for the glass made by quenching the fresh melt was $T_{on} = 14.5$ °C and by quenching the equilibrated melt was $T_{on} = 19.2$ °C (at 10 K/min on heating), which can be compared with the values reported by Fan and Angell (1996): 13 and 7 °C, respectively. Other values reported in the literature, also obtained by DSC, are: 1 °C (Wungtanagorn and Schmidt 2001), 5 °C (Roos 1993), and 11 °C (Noel and others 2000). One of the reasons for the dispersion of these later literature values probably lies in the fact that the measured samples were in different states of the anomeric composition; another reason can be the presence of adsorbed water. The heat capacity jump in the glass transformation range was found to be $\Delta C_p = (0.80 \pm 0.01) \text{ J g}^{-1} \text{ K}^{-1} = (144 \pm 2) \text{ J mol}^{-1} \text{ K}^{-1}$ (average over 33 experiments, the uncertainty indicated is the standard deviation of the mean), in reasonable agreement with the values $\Delta C_p = 0.77 \pm 0.01 \text{ J K}^{-1} \text{ g}^{-1}$ (Wungtanagorn and Schmidt 2001), $\Delta C_p = 0.74 \text{ J K}^{-1} \text{ g}^{-1}$ (Fan and Angell 1995), and $\Delta C_p = 0.75 \text{ J K}^{-1} \text{ g}^{-1}$ (Roos 1993) previously reported. It should also be pointed out that liquid β -D-fructose does not readily crystallize on cooling remaining in the supercooled liquid state for indefinitely long periods.

The influence in the TSDC thermogram of D-fructose of the degree of anomeric equilibration was also analyzed, and the results are shown in Figure 3. Figure 3(a) depicts the effect of the equilibration degree in the secondary relaxation of amorphous solid fructose, while Figure 3(b) refers to the main relaxation. A general conclusion that stands out from Figure 3 is that the dielectric strength of both α and β -relaxations decreases as the anomeric mixture goes on from the fresh melt to the relaxed melt. Given their localized nature, the amplitude of the reorientational motions associated with the secondary relaxations is not expected to be influenced by equilibration. The observed decrease in the polarization in both relaxations is probably accounted by a decrease in the polarity of the anomeric mixture upon equilibration, suggesting that the β -D-fructopyranose anomer, that is more abundant in the fresh melt compared with the relaxed melt, is more polar than the other anomers. Another conclusion that follows from Figure 3(b) is that the temperature of maximum intensity of the α -peaks increases as the equilibrium goes on, indicating a corresponding increase of the glass transition temperature. This result is in full agreement with the DSC results reported previously. The shift and renormalization of the peaks in Figure 3(b) lead to a good superposition of the peaks, indicating that the change of the anomeric composition of the amorphous solid does not correspond to any appreciable modification of the distribution of relaxation times.

Let us finally note that it was reported (Truong and others 2004) (and confirmed in the present study) that this effect on T_g of the evolution of the anomeric equilibrium can be erased by heating up to the vicinity of the melting temperature.

The reported results show that we can isolate β -D-fructose in distinct amorphous solid states. However, we are not in the presence of a true polyamorphism, whose definition is the existence, usually coexistence, in a 1-component system of 2 condensed

amorphous phases separated by a clear phase transition (Hancock and others 2002; Shalaev and Zografi 2002; Tarjus and others 2003). The number of instances of true polyamorphism reported in the literature appears likely to be small. Nevertheless, amorphous forms of the same substance with distinct chemical and/or physical properties can be isolated, which are not true polyamorphs according to the strict definition provided previously. These are referred to as pseudo-polyamorphs. In the case of β -D-fructose, we have the possibility of isolating conformational pseudo-polyamorphs, which do not represent distinct thermodynamic phases, but rather a continuum of conformer mixtures that are primarily differentiated by the extent of their departure from the conformational equilibrium composition.

Slow molecular mobility in D-fructose studied by TSDC

The molecular mobility in fructose was studied in the amorphous solid state with equilibrium anomeric composition, using the experimental procedure of partial polarization (PP). The PP method, where the polarizing field is applied in a narrow

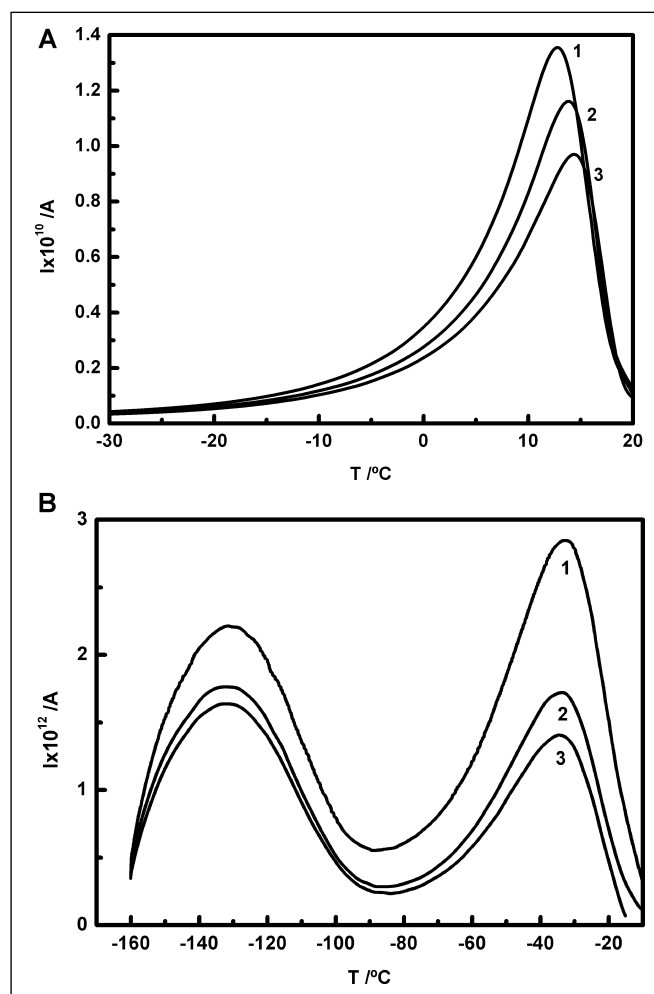


Figure 3—Effect of the degree of equilibration of D-fructose on the molecular mobility in the amorphous solid state. Curves 1, 2, and 3 correspond to samples prepared as described in the caption of Figure 2. The experimental protocols were: (A) polarization temperature, $T_p = -35$ °C, freezing temperature, $T_0 = -160$ °C, strength of the polarizing electric field, $E = 450 \text{ V mm}^{-1}$, heating rate, $r = 8 \text{ K min}^{-1}$; (B) polarization temperature, $T_p = 10$ °C, freezing temperature, $T_0 = -80$ °C, strength of the polarizing electric field, $E = 450 \text{ V mm}^{-1}$, heating rate, $r = 8 \text{ K min}^{-1}$.

temperature interval, enables to resolve a complex relaxation peak into its individual relaxation modes. In the limit of a very narrow polarization temperature window, the retained polarization (and, of course, the current peak that is the result of a partial polarization [PP] experiment) would correspond to a single, individual dipolar motion (Teyssedre and Lacabanne 1995; Teyssedre and others 1996).

The temperature dependent relaxation time of each motional mode was obtained from the experimentally determined partial polarization peaks using the usual Bucci method (Bucci and Fieschi 1964; Bucci and others 1966; McCrum 1982). The temperature dependence of the relaxation time of a single reorientational motion can often be described by the Eyring equation:

$$\tau(T) = \frac{h}{kT} \exp\left(-\frac{\Delta S^\ddagger}{R}\right) \exp\left(\frac{\Delta H^\ddagger}{RT}\right) \quad (1)$$

where h , k , and R are, respectively, the Planck, the Boltzmann, and the gas constants, and ΔH^\ddagger and ΔS^\ddagger are, respectively, the molar enthalpy and the molar entropy of activation of the molecular motion. If the motional process occurs without cooperativity, that is, without involving the neighboring molecules, we have $\Delta S^\ddagger = 0$, so that the Arrhenius prefactor, τ_0 , is close to the quantity $\tau_D = \frac{h}{kT}$, the so-called Debye time. This quantity, whose value is 1.76×10^{-13} s at room temperature ($T = 273.15$ K), represents the characteristic time of a process with no activation Gibbs energy (preexponential factor of the Eyring equation). The deviation of the Arrhenius prefactor, τ_0 , from τ_D correlates to the activation entropy associated with reorientation in the relaxation process (Sauer and others 1990). High values of the activation entropy, or smaller value of τ_0 relative to τ_D , can be interpreted to arise from cooperativity of the orientational motion: the smaller the τ_0 value the higher the cooperativity of the motion. The so-called Starkweather plot (Starkweather 1981, 1988, 1991), shown in Figure 4, displays the general features of the slow molecular mobility of D-fructose and is a useful way of presenting TSDC data to highlight the degree of cooperativity of the different motional processes.

In the amorphous solid state, 2 different kinds of mobility are present: (1) a lower temperature relaxation whose motional modes

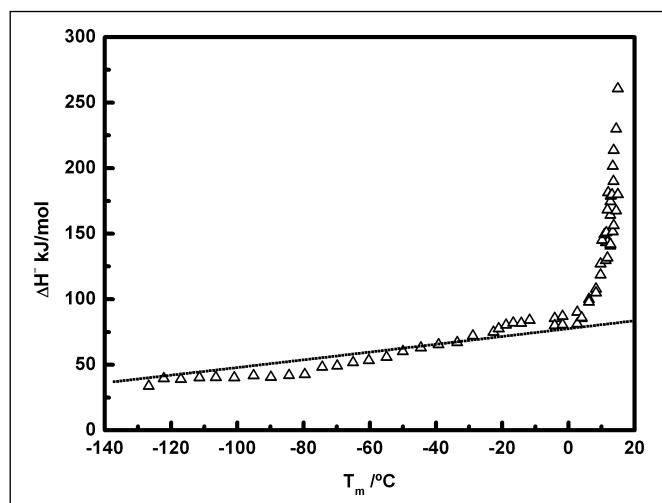


Figure 4—Activation enthalpy, ΔH^\ddagger , of the partial polarization components of the relaxations in D-fructose as a function of the peak's location, T_m . The uncertainty in the enthalpy values is less than 2%. The line describes the zero entropy behavior. The points with $T_m < 0$ °C correspond to the secondary relaxations, while the other ($0 < T_m < 15$ °C) correspond to motional modes of the α -relaxation.

are activated below 0 °C and nearly obey to the zero entropy line (the secondary relaxations); (2) a higher temperature relaxation whose motional modes are activated between 0 and 15 °C and that show a dramatic deviation from the zero entropy line (the main or α -relaxation). Figure 5(a) shows some partial polarization peaks that have been obtained in the sub- T_g region (open triangles with $T_m < 0$ °C in Figure 4), while Figure 5(b) displays partial polarization peaks in the glass transition region (open triangles with $0 < T_m < 15$ °C in Figure 4).

Aging effect on the secondary relaxations. It can be seen in Figure 5(a) (as well as in Figure 3a) that the secondary relaxation of fructose is broad and presents some kind of structure, with 2 local maxima separated by a saddle. This structure of the secondary relaxation is also visible in Figure 6 where the results of 3 wide polarization window experiments are displayed (see experimental conditions in the caption of the figure).

Furthermore, the results displayed in Figure 6 show that the higher temperature part of the secondary relaxation is influenced

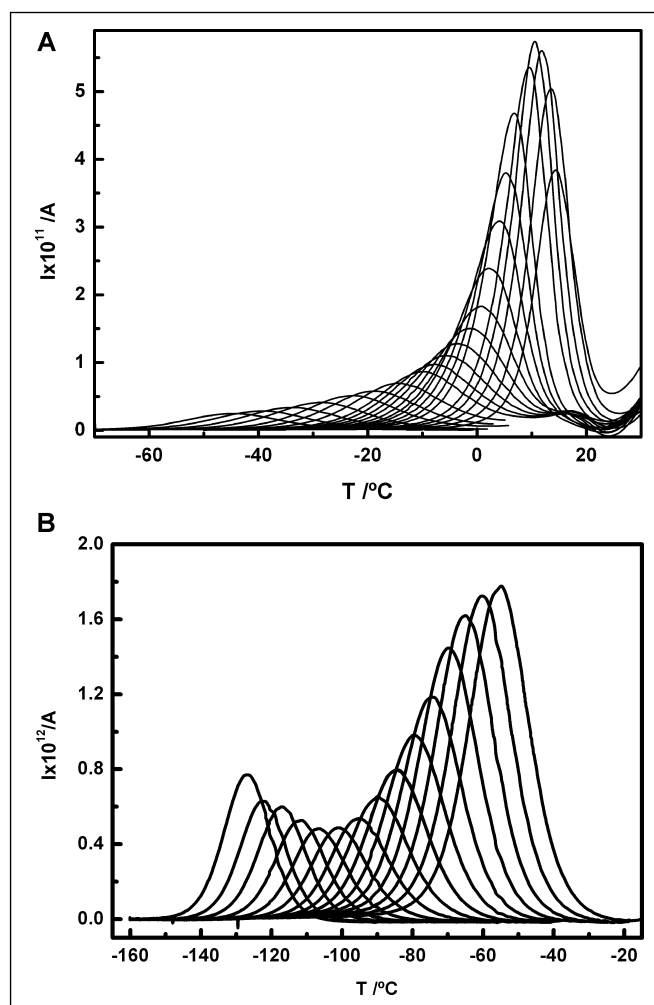


Figure 5—(A) Partial polarization components of the sub- T_g molecular mobility of amorphous fructose. The polarization temperatures, T_p , were from -130 to -60 °C, with intervals of 5 °C. (B) Partial polarization peaks of fructose in the temperature region of the glass transition. The polarization temperatures were $T_p = -50, -45, -40, -35, -30, -26, -22$ °C and from -18 to $+12$ °C with intervals of 2 °C. The other relevant experimental conditions, common to (A) and (B), were: strength of the polarizing electric field, $E = 400$ V mm $^{-1}$; polarization time, $t_p = 5$ min; width of the polarization window, $\Delta T = 2$ °C; heating rate, $r = 4$ K min $^{-1}$.

by aging but that the lower temperature one is not. In fact, the experimental protocol of the 3 experiments whose results are displayed in Figure 6 is identical, but in each experiment the sample is allowed to stay at temperatures in the range of -15 to -5 °C where physical aging readily occurs (the calorimetric glass transition temperature of fructose in the range 0 to 15 °C as reported before in section “DSC and TSDC signatures of the tautomer equilibrium”). The decrease in intensity of the higher temperature part of the secondary relaxation global peak is thus a consequence of aging, allowing the conclusion that we are in the presence of 2 different mechanisms of local mobility (Moura Ramos and others 2007, 2008; Diogo and Moura-Ramos 2008). The faster motional modes, at lower temperatures, not affected by aging, have probably an intramolecular origin. These are local motions that consist of internal rotations of one part of a molecule relative to the other part or conformational modifications of a cyclic unit, which occur without significant interference of the neighboring molecules. On the other hand, the slower motional modes, at higher temperatures, that are affected by aging, have probably an intermolecular origin and correspond to the genuine β -relaxation or Johari–Goldstein relaxation, which is considered as a universal feature of the amorphous solid state. Note that this secondary relaxation peak is a feature of the amorphous state given that it is absent in the studied crystalline form of fructose. Furthermore, those manifestations of the physical aging on the secondary relaxations are reversible as the aging effects can be erased by heating above T_g to the equilibrium metastable liquid state.

The alpha relaxation temperature provided by TSDC. Among the partial polarization TSDC peaks of the glass transition region shown in Figure 5(b), that with higher intensity is important for the characterization of the glass transition. Above T_M , the depolarization becomes very fast and the retention of the polarization becomes increasingly difficult. That is why the intensity of the maximum of the PP peaks above T_M in the glass transition region decreases sharply as the polarization temperature, T_p , increases (dotted peaks in Figure 5(b)). This is also the reason why the PP peaks in the glass transition region present a shape such that there

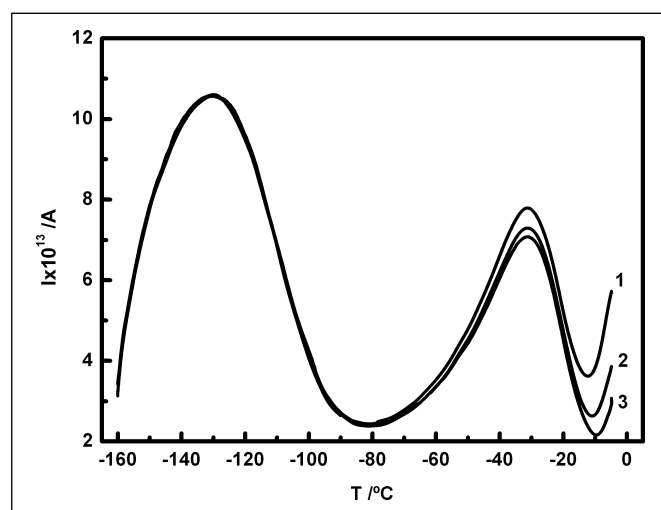


Figure 6 – TSDC thermogram showing the secondary relaxations of amorphous fructose with different degrees of aging. The numbers indicate the order of the successive identical experiments. The relevant experimental parameters for the 3 experiments were: strength of the polarizing electric field, $E = 450 \text{ V mm}^{-1}$, polarization time, $t_p = 5 \text{ min}$, polarization temperature, $T_p = -35$ °C, freezing temperature, $T_o = -160$ °C, final temperature, $T_f = -5$ °C, heating rate, $r = 8 \text{ K min}^{-1}$.

is a sharp decrease of the current intensity (a fast depolarization) above the temperature of maximum intensity. Above T_M the “freezing-in” of the polarization is prevented, indicating the transformation to the equilibrium (ergodic) metastable supercooled liquid state. The temperature T_M of the PP peak with maximum intensity in the glass transition region thus represents the lower limit (the onset) of the transition range between the nonequilibrium glass and the phase into which it transforms by heating (metastable supercooled liquid, orientationally disordered crystal, liquid crystal, and so on). It can thus be considered as the glass transition temperature provided by the TSDC technique, at the heating rate of the experiment. In the case of fructose this temperature is $T_M = 13$ °C (at 4 K min^{-1}) for the glass with equilibrium anomeric composition, value comparable with the values of the calorimetric glass transition reported before.

It was claimed (Saiter and others 2003) that the dielectric TSDC manifestation of the glass transition, T_M , corresponds to a temperature lower than that of the onset of the calorimetric glass transition signal, T_g , and it was argued that the difference between those temperatures was a consequence of the different nature of the experimental probes and of the different time response of these probes. However, our TSDC results obtained on a wide diversity of glass-forming systems (Moura Ramos 1997; Moura Ramos and others 2006a, 2006b; Diogo and others 2007; Diogo and Moura-Ramos 2008) do not support that claim and show that there is a very reasonable agreement between T_M and T_g , without any systematic trend.

The main relaxation and fragility. A 2nd reason that makes the partial polarization peak with higher intensity in the glass transition region important for the characterization of the glass transition is that it is used as a reference peak to calculate the fragility or steepness index of the glass forming system (Alvarez and others 2000; Correia and others 2000a, 2000b). From the analysis of the experimental data relative to this peak, it can be concluded that, in the case of fructose, the relaxation time at the glass transition temperature is $\tau(T_M) = 58 \text{ s}$, and that the activation energy at the same temperature is $E_a(T_M) = 188 \text{ kJ mol}^{-1}$. The fragility index from the TSDC data is thus given by

$$m = \left[\frac{d \log_{10} \tau(T)}{d(T_M/T)} \right]_{T=T_M} = \frac{E_a(T_M)}{2.303 RT_M} = 34 \quad (2)$$

The fragility index obtained by TSDC using procedures based on the influence of the heating rate on the features of the higher intensity PP peak (Moura Ramos and others 2002; Diogo and Moura Ramos 2006), namely on the temperature location, T_M , and on the intensity of the maximum, $I(T_M)$, are very close to 34. Note again that the value of the fragility we calculate refer to the equilibrated melt. However, it is worth recalling that the previous equilibration of the melt does not give rise to a significant modification of the distribution of relaxation times of the α -relaxation, so that we expect the fragility index to be independent of the anomeric composition. The value $m = 34$ obtained for fructose is lower than that previously obtained by TSDC for glucose ($m = 43$ [Diogo and Moura-Ramos 2008]), confirming that glucose is a more fragile glass forming liquid compared with fructose (Gangasharan and Murthy 1995; Wungtanagorn and Schmidt 2001). There is some discrepancy between the fragility values obtained for fructose by Dielectric Relaxation Spectroscopy (DRS): $m = 92$ (Kaminski and others 2006) and $m = 53$ (Noel and others 2000). Different values of the fragility index of fructose have also been obtained using procedures based on DSC (Simatos and others 1996; Mehl 1998; Wungtanagorn and Schmidt 2001). This discrepancy in the m values is often underlined

(Wungtanagorn and Schmidt 2001; Diogo and Moura Ramos 2006; Moura Ramos and others 2006a; Wang and others 2006) and seems to become more pronounced for high-fragility liquids (Moura Ramos and others 2006a; Wang and others 2006). It may arise from the difference in the type of relaxation used (viscosity, dielectric, mechanical, enthalpy relaxation, and different procedures of obtaining data and of calculation), that is, from the different nature of the experimental probes.

Comparison between the fragility index obtained by TSDC and by DRS. It is interesting to compare the fragility values provided by the 2 dielectric related techniques, and to try to understand their differences. It is well known that the values obtained by TSDC, $m(tsd)$, are in general lower than those obtained by DRS, $m(drs)$ (Davies and others 1973; Saiter and others 2003; Dargent and others 2005). Some researchers (Davies and others 1973) attributed these differences to the nonexponentiality of the main relaxation, which is not accounted for in TSDC data treatment. In this context it was suggested (Davies and others 1973) that $m(tsd)/m(drs) = \beta$, where $0 < \beta \leq 1$ is the shape factor of the KWW stretched exponential. Other authors (Saiter and others 2003; Dargent and others 2005) consider that the observed differences are related to the fact that TSDC looks at the glass transition from the lower temperature side (from the glassy state), while DRS analyses the glass transition using data obtained at temperatures higher than T_g (supercooled liquid). In fact, it is expected that the slope of the Arrhenius plot at $T = T_g$ will change from a higher value at higher temperatures (equilibrium melt) to a lower value at lower temperatures (constant structure glass), the so-called "return to Arrhenius." In this context, the lower value of the slope is related to the activation energy of the motions in the frozen, nonannealed glass, which can be identified with $E_a(T_M)$, the activation energy determined by TSDC (Hutchinson 1998; Saiter and others 2003; Dargent and others 2005). The change of the slope of the $\tau(T)$ line at T_g is interpreted by some researchers on the basis of the Tool–Narayanawamy–Moynihan (TNM) model (Moynihan and others 1976; Hodge 1994; Hutchinson 1995; Borde and others 2002) in such a way that the ratio of the activation energies (or of the fragility indexes) is identified with the so-called nonlinearity parameter, x ($0 \leq x \leq 1$). Accordingly we have $m(tsd)/m(drs) = x$. Taking $m(drs) = 72$ (the mean of the 2 values published in the literature (Noel and others 2000; Kaminski and others 2006), we obtain $m(tsd)/m(drs) = 0.47$, which is in good agreement with the shape factor of the KWW exponential $\beta = 0.43 - 0.46$ reported for fructose (Tombari and others 2001), but somewhat different from the published nonlinearity parameter $x = 0.755$ (Wungtanagorn and Schmidt 2001). Other agreements were found for glucose and for poly(ethylene terephthalate) (Diogo and Moura-Ramos 2008). Since the parameters β and x often present values of the same order of magnitude (not far from 0.5), it is not easy to determine if the difference between $m(tsd)$ and $m(drs)$ arises from the nonexponentiality of the glass transition relaxation or if, contrarily, it reflects a different significance of the activation energy obtained by the 2 dielectric techniques.

Conclusions

The evolution to equilibrium of the tautomeric composition, which occurs in the supercooled liquid fructose immediately after melting, was monitored by DSC and by TSDC, and it was found that annealing the supercooled liquid for 2 to 3 d leads the system to a situation near the equilibrium. On one hand, evidence from both techniques indicates that the glass transition temperature increases as equilibrium approaches. On the other hand, TSDC results showed that the dielectric strength of both secondary and

main relaxations decreases upon equilibration. The study of the re-orientational mobility in the amorphous state was carried out on samples that were vitrified after annealing the metastable liquid, to warrant that the tautomeric equilibrium was attained (the so-called relaxed melt). The analysis of the motional components of the secondary relaxations showed that they are distributed in energy but not in the prefactor, indicating that they correspond to a local, non-cooperative mobility. The study of the influence of aging showed, on the other hand, that the secondary relaxation of fructose is composed by 2 different types of mobility. At lower temperature, the mobility modes are not affected by aging and are attributed to intramolecular motions, while at higher temperature, the motional modes of the secondary relaxation are influenced by aging and are supposed to correspond to the Johari–Goldstein relaxation.

The fragility index of fructose was calculated from TSDC data. The difference between this value and that obtained by DRS was discussed.

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