Thermal properties of ethylene glycol aqueous solutions

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Abstract

Preventing ice crystallization by transforming liquids into an amorphous state, vitrification can be considered as the most suitable technique allowing complex tissues, and organs cryopreservation. This process requires the use of rapid cooling rates in the presence of cryoprotective solutions highly concentrated in antifreeze compounds, such as polyalcohols. Many of them have already been intensively studied. Their glass forming tendency and the stability of their amorphous state would make vitrification a reality if their biological toxicity did not reduce their usable concentrations often below the concentrations necessary to vitrify organs under achievable thermal conditions. Fortunately, it has been shown that mixtures of cryoprotectants tend to reduce the global toxicity of cryoprotective solutions and various efficient combinations have been proposed containing ethanediol. This work reports on the thermal properties of aqueous solutions with 40, 43, 45, 48, and 50% (w/w) of this compound measured by differential scanning calorimetry. The glass forming tendency and the stability of the amorphous state are evaluated as a function of concentration. They are given by the critical cooling rates \( v_{ccr} \) above which ice crystallization is avoided, and the critical warming rates \( v_{cwr} \) necessary to prevent ice crystallization in the supercooled liquid state during rewarming. Those critical rates are calculated using the same semi-empirical model as previously. This work shows a strong decrease of averaged critical cooling and warming rates when ethanediol concentration increases, \( v_{ccr} = 569 \) and \( v_{cwr} = 1.08 \times 10^{10} \) K/min for 40% (w/w) whereas \( v_{ccr} = 11 \) and \( v_{cwr} = 853 \) K/min for 50% (w/w). Those results are compared with the corresponding properties of other dialcohols obtained by the same method. Ethylene glycol efficiency is between those of 1,2-propanediol and 1,3-propanediol.

Keywords: Ethylene glycol; Ethanediol; Vitrification; Glass transition; DSC; Critical cooling and warming rates

Vitrification might provide a general solution to the problem of organ preservation [32] because this procedure should allow to avoid both the direct and the indirect damaging effects of freezing, related to changes in the extracellular solution toxicity, as well as mechanical damages caused by the ice crystals on the cells [38]. However, it requires generally the use of a rapid cooling, in combination with non-toxic, though high concentrations of cryoprotective agents aiming to form efficient glass-forming mixtures with water. Indeed, one of the cryoprotectants most remarkable properties is that the wholly amorphous states of their aqueous solutions are much more stable than

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that of pure water [9]. Presently, the main challenge in cryobiology is to find the best composition for a cryoprotective solution owing a low biological toxicity while allowing vitrification on cooling. Several compounds have been studied, independently, or in association with other cryoprotectants, but a cryoprotective solution of very low toxicity whose amorphous state has a high stability has proved extremely difficult to overcome.

However, several promising mixtures have been studied recently. Fahy et al. [15] reported the benefit of substituting the weak glass-former ethylene glycol to the strong glass-former 1,2-propanediol on toxicity in their solutions. These new vitrification solutions are substantially less toxic than former VS41A. Interestingly, more recent works from Wowk and Fahy [41] have shown that the addition of simple synthetic ice blocking polymers to concentrated ethylene glycol aqueous solutions, as those used for vitrification, decreases the amount of ice by minimizing nucleation.

Luyet and Rasmussen [20] and MacKenzie [23] studied aqueous solutions of ethylene glycol by differential thermal analysis a long time ago. Boutron and Kaufmann [10] studied the aqueous solution with 45% (w/w) ethylene glycol and ternary solutions with water, glycerol, and ethylene glycol [10], using a differential scanning calorimeter. Later, Hayes and Pegg [18] determined temperature–time–transition curves of ethylene glycol/water solutions. More recently, Wowk et al. [40] published data on 45% (w/w) ethylene glycol and less complete data for 40 and 50% (w/w) ethylene glycol in water. Nevertheless, the glass-forming tendency and stability of this compound was only known for a few concentrations. The work presented in this paper is aiming to fill in this lack of information. Indeed, complete data for the kinetics of ice crystallization in the presence of a given cryoprotectant are essential for ensuring vitrification and for designing optimized technical procedures to cool and to rewarm. The goal of this work was thus to study by DSC other concentrations of ethylene glycol in order to know more precisely the concentration from which aqueous solutions of this compound have an amorphous state sufficiently stable to be obtained experimentally. We limited this work between 40 and 50% (w/w) ethylene glycol in water, in order to compare its efficiency with other polyalcohols whose thermal properties regarding vitrification are already completely characterized. Below 40% (w/w) vitrification is rarely achieved. Above 50% (w/w), the solutions are generally too toxic.

Materials and methods

Materials

Ethylene glycol (EG) is an organic dialcohol, liquid at room temperature, non-volatile, and colorless. It is completely miscible with water and has a sweet taste. It can be obtained by oxidizing olefin in an aqueous solution of potassium permanganate. This compound is largely used in plastics manufacturing, as well as an hydraulic braking liquid and an antifreeze in cars. In cryobiology, EG is classified as a permeable cryoprotectant. Mixed with other compounds, it has been used in different studies concerning cryopreservation of different kinds of tissues, and organs. EG has been used successfully for the freeze storage of many microorganisms [19]. Unfortunately, this compound is extremely toxic to some protozoans, due to a general problem of diols which act as solvents for some microbial polysaccharides, leading to toxicity. EG has been shown to be a successful cryoprotectant for mammalian zygotes and embryos, as well as Drosophilae embryos [18]. It appears to be well tolerated by embryos [37] as it penetrates more easily than propanediol or glycerol, diminishing osmotic stresses and facilitating mass transfers. Other studies conducted on rat islets of Langerhans [35,36] indicated that it may be less toxic to pancreatic islet than dimethyl sulfoxide. However, EG has substantial toxic effects on both the smooth muscle and the vascular endothelium of the rabbit common carotid artery [43], which makes this compound a non suitable cryoprotectant for vascular tissue, and its toxicity has been observed also on rat kidneys [17].

To determine the thermal properties of EG aqueous solutions, we conducted a series of experiments using a differential scanning calorimeter DSC2 from Perkin–Elmer. DSC measurements
allow the observation of phase transitions under dynamic conditions. This leads to the evaluation of the critical cooling rate above which a given solution vitrifies entirely, and the critical warming rate necessary to avoid devitrification on warming, which results from the instability of the amorphous state produced by rapid cooling. We have studied aqueous solutions with 40, 43, 45, 48, and 50% (w/w) EG. Cryoprotective solutions were prepared in weight per weight with EG from Sigma Chemical (Lot 71K0190, 99+%%) without further purification and deionized water. The calibration of the DSC2 for the temperatures and for the heat flow was made from the melting of ice from deionized water (\(T = 0\,^\circ C\) and \(\Delta H = 333.8\) J/g) and the crystallographic transition of cyclohexane in its solid state (\(T = -87.1\,^\circ C\)). Temperature values were found to be reproducible within ±0.5°C. All cryoprotective solutions were studied during cooling and warming at a constant rate which varied from one experiment to another from 2.5 to 320°C/min, between −153°C (which is below the vitreous transition of the solutions) and 12°C. Heats of solidification were measured at selected programmed cooling rates of 2.5, 5, 10, 20, 40, 80, 160, and 320°C/min. According to our previous studies on a DSC2, we know that the actual cooling rates are identical to the indicated rates up to 80°C/min [1]. The temperatures of the transitions on warming were determined at warming rates of 2.5, 5, 10, 20, 40, and 80°C/min, after quenching (programmed cooling rate of 320°C/min).

For DSC2 measurements, samples weight is critical, as they do have to be heavy enough to allow a good thermal contact between the oven and the filled pan, but they must not induce a saturation of the DSC2 recorder leading to a loss of information on the thermograms. Recent measurements undertaken on this DSC2 were done successfully with weights contained between 3 and 5 mg [1,2]. In the present study, we worked with weights of 3.41 ± 0.11 mg. The experiments were conducted using standard hermetically sealed aluminum pans (Perkin–Elmer, 0219-0062) designed for volatile samples. To ensure the sealed pans insulation, their weights were measured at the end of the experiments and compared with the weight obtained before the DSC2 measurements. It must be notified that at the end of 1995, the aspect of the sample holders surfaces changed. As a consequence, some differences have been observed in the kinetics of ice crystallization [2] leading to less reproducible results. For this reason, each composition has been tested at least three times. Between measurements, the solutions were preserved in properly insulated flasks because of their high hygroscopy. Finally, to ensure a good reproducibility of our measurements, the droplets of liquid were deposited on the side of the pan using a syringe. The goal was to have the largest contact area between the solution and the aluminum since this area acts upon the width and the accuracy of the peaks [30].

**Methods**

To estimate the critical cooling rates, we used the semi-empirical model developed from the classical theory of crystallization by Boutron [7]. Its basic hypothesis states that the ice crystals are spherical and have equal size, which has been verified for some polyalcohols by cryomicroscopic measurements [25]. The rate of growth is considered as a function of temperature and the resulting equation includes a term that takes into consideration the reduction in growth rate due to crystal impingement [7]. By measuring on the thermograms the area of the exothermic peak of crystallization, we can determine the heat of ice solidification \(q\) for each tested cooling rate. These values being plotted as a function of the cooling rate, the model is used to fit a theoretical curve to the experimental points and estimate the critical cooling rate, which is defined as the rate above which less than 0.2% of the solution will crystallize. The theoretical curves correspond as usual to the fourth model [7] given by

\[
A_1(x) = -\ln(1 - x^{1/3}) + \frac{1}{2} \ln(1 + x^{1/3} + x^{2/3}) + \sqrt{3} \arctan \left( \frac{\sqrt{3}x^{1/3}}{2 + x^{1/3}} \right) = k_4 |v|,
\]

where \(x\) is the ratio of the total quantity of ice crystallized on cooling to the maximum crystallizable ice (0 ≤ \(x\) ≤ 1), \(v\) is the cooling rate, and \(k_4\) is...
a constant. The theoretical value of \( q \), considered as before [1–4,7,8,13,33] to be proportional to \( x \), is chosen such that its maximum value \( q_{\text{max}} \) fits the experimental values. Since \( x = 1 \) corresponds to \( q = q_{\text{max}} \), \( x = q/q_{\text{max}} \). In our measurements with EG solutions, after cooling at 2.5°C/min, no devitrification peak occurred on subsequent warming, meaning that \( q = q_{\text{max}} \) at this slow cooling rate, except for 50% (w/w) EG. In this case, a rough estimation of \( q_{\text{max}} \) has been calculated from the area of the melting peak of ice at 2.5°C/min, neglecting the variation of the latent heat of melting with the temperature due to the difference between the specific heats of water and ice. This method gives an over-evaluation of \( q \) between the specific heats of water and ice. This neglecting the variation of the latent heat of melting with the temperature due to the difference between the specific heats of water and ice. This method gives an over-evaluation of \( q \) between the specific heats of water and ice.

\[
q = \frac{v_{\text{ccr}}}{C_{176}} \quad \text{and is calculated from the values of } k_4 \text{ and } q_{\text{max}}.
\]

The constant \( k_4 \) can be considered as characteristic of the glass-forming tendency since the smaller \( k_4 \), the larger is the glass-forming tendency [7].

When a cryoprotective solution is warmed from a wholly amorphous state (no crystallization peak observed on previous cooling), and when warming is not fast enough to avoid ice formation, one observes first the glass transition where the solution becomes a supercooled liquid and where the molecules, which previously were capable only of vibratory motion, acquire rotational, and translational motion. At a higher temperature, they acquire enough mobility to pass from the random arrangement of the amorphous state to the ordered structure of the crystal and there is the devitrification peak corresponding to ice formation on warming. At a still higher temperature, molecular motility is improved. The smaller of the crystals lose their stability, their surface-to-volume ratio being too high. They melt and their molecules are transferred to the large crystals which grow larger. This phenomenon is designated as recrystallization. Afterwards, the crystalline material passes from the solid to the liquid state, inducing a non-isothermal peak corresponding to ice melting. The vitreous transition temperature \( T_g \) is determined at the inflexion point of the rapid increase of specific heat corresponding to the glass transition. The top of the devitrification peak is called \( T_\text{d} \) as usual [1–4]. The temperature \( T_\text{m} \) of the end of ice melting can be defined as the temperature at the top of the melting peak. Indeed, the corrections using the leading edge method [8,11] are negligible at 2.5°C/min for the concentrations of EG presently studied. The temperature corresponding to the peak of devitrification, \( T_\text{d} \), rises as the warming rate increases. The critical warming rate is reached when \( T_\text{d} \) approaches the melting temperature, \( T_\text{m} \), and ice crystallization is avoided. Several authors have observed that \( 1/T_\text{d} \) decreases linearly with the logarithm of the warming rate [12]. Hence, to estimate the critical warming rate \( v_{\text{cwr}} \), above which there is not enough time for crystallization on warming the wholly amorphous solution, \( T_\text{m} \) is divided by \( T_\text{d} \) and the experimental values of \( T_\text{m}/T_\text{d} \) are plotted versus the logarithm of warming rate. \( v_{\text{cwr}} \) is evaluated by extrapolating the experimental values of \( T_\text{m}/T_\text{d} \) to 1.05, which corresponds to about 0.5% crystallization [3,12].


Another definition of the critical warming rate has been proposed [26], depending on the nuclei density which would be low enough that damages in organized tissues would not affect the survival of the vitrified organs. However, this technique to access the non-damaging critical warming rate is less direct than the one we use as it implies the determination of kinetics parameters from the general Johnson–Mehl–Avrami method.

Results

On Fig. 1 are presented the results that we obtained on cooling. The mean experimental values of \( q \) versus the cooling rate have been plotted together with the corresponding standard error bars and the theoretical curves. The experimental points are in good agreement with the theoretical curves for all the concentrations tested. At
40% (w/w) points are missing in the zone of decrease of $q$, due to the capabilities of the DSC2. The parameters of the theoretical curves ($q_{\text{max}}$ and $k_4$) are given in Table 1, with the corresponding values of $v_{\text{ccr}}$.

To study on warming the stability of the amorphous state, thermograms have been obtained after quenching the cryoprotective solutions. Fig. 2 shows the evolution of the mean values of $T_m/T_d$ as a function of the logarithm of the warming rate for all the tested concentrations. The straight lines were drawn using the least squares methods. We observed a satisfactory reproducibility of our measurements within $\pm 0.5 ^\circ\text{C}$, and as one can see on Fig. 2, the experimental points fit well with the linear theoretical prediction. The calculated values of the corresponding critical warming rates are presented on Table 1. As for the previously studied compounds, the critical warming rates are particularly high, often well

### Table 1

Values of $T_m$, of the parameters ($q_{\text{max}}$, $k_4$) necessary to evaluate the critical cooling rate $v_{\text{ccr}}$ ($T_m$ and $q_{\text{max}}$ are given as mean value $\pm$ SE) and calculated values of $v_{\text{ccr}}$ and $v_{\text{cwr}}$ for all the concentrations of ethylene glycol tested.

<table>
<thead>
<tr>
<th>Ethylene glycol % (w/w)</th>
<th>$T_m$ (°C)</th>
<th>$q_{\text{max}}$ (%)</th>
<th>$k_4$ (°C/min)</th>
<th>$v_{\text{ccr}}$ (°C/min)</th>
<th>$v_{\text{cwr}}$ (°C/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>-22.35 ± 0.66 ($n = 3$)</td>
<td>21.3 ($n = 1$)</td>
<td>360</td>
<td>569</td>
<td>1.08 × 10^10</td>
</tr>
<tr>
<td>43</td>
<td>-26.43 ± 0.48 ($n = 3$)</td>
<td>20.02 ± 2.02 ($n = 3$)</td>
<td>67</td>
<td>105</td>
<td>2.85 × 10^7</td>
</tr>
<tr>
<td>45</td>
<td>-28.47 ± 0.42 ($n = 3$)</td>
<td>15.78 ± 1.98 ($n = 3$)</td>
<td>45</td>
<td>63</td>
<td>1.04 × 10^4</td>
</tr>
<tr>
<td>48</td>
<td>-32.62 ± 0.02 ($n = 3$)</td>
<td>11.45 ± 0.23 ($n = 3$)</td>
<td>14</td>
<td>18</td>
<td>1.08 × 10^4</td>
</tr>
<tr>
<td>50</td>
<td>-36.17 ± 0.46 ($n = 6$)</td>
<td>12.81 ± 0.15 ($n = 6$)</td>
<td>8</td>
<td>11</td>
<td>853</td>
</tr>
</tbody>
</table>
beyond the capabilities of the DSC 2. As a result, the determination of the warming rate is somewhat less reliable than that of the critical cooling rate.

Fig. 3 presents the “non-equilibrium phase diagram” published by Luyet and Rasmussen [20] on warming at 5 °C/min in which the melting temperature $T_m$, the glass transition temperature $T_g$, the devitrification temperature $T_d$, and the recrystallization temperature $T_r$ are plotted against the solute concentration. Our experimental points on warming at 2.5 °C/min after quenching have been added together with the DSC 2 measurements of Hayes and Pegg [18] in similar conditions and DSC 7 measurements by Wowk et al. [40]. Apart from $T_d$, the agreement between our values, and the other published results is satisfactory. The differences observed for $T_d$ could be due to the definition of $T_d$ itself (this temperature is for us the temperature at the top of the devitrification peak) as well as to the method of detection, as this value depends on the shape of the peak. Considering only DSC measurements, the differences between Wowk et al. [40] results and ours are among others due to a dynamic effect since Wowk et al. [40] measurements were conducted at 5 °C/min whereas our measurements were conducted at 2.5 °C/min. Concerning $T_r$, it must be said that on our thermograms, $T_r$ was defined at the minimum of the small exothermic peak observed on the re-warming before the melting. Figs. 3 and 4 of [3] illustrate the type of information given by the thermograms obtained on warming EG solutions, the small feature at $T_r$ being indicated by a circle. In our measurements, we observed recrystallization peaks at 2.5 °C/min only for the highest concentrated solutions of 48 and 50% (w/w) EG. The fact that no recrystallization peak has been detected at that rate at lower concentrations does not mean that it doesn’t exist, since we observed it sometimes at higher warming rates. In fact, this transition was not detectable by DTA [20] and Luyet and Rasmussen [20] characterized recrystallization by a change in transparency of their solution. Forsyth and MacFarlane [16] confirmed
that recrystallization is generally not detectable thermally. However, they observed an exception in the case of EG–water solutions since they found a small exothermic transition by DSC experiments at the opacity/c213s completion temperature of the sample that they give at 80°C/176°C/min [16]. The difference observed on Fig. 3 with Luyet and Rasmussen for \( T_r \) values is then probably due to the difference in the recrystallization detection's methods and their own accuracy, and possibly to differences in the recrystallization kinetics itself in different apparatus.

The phase diagram of EG in water has been intensively studied in the past [34]. Ott et al. [31] reported a 1:1 compound (corresponding to a molar fraction of 0.5) in the phase diagram using melting-point determination. Later, Murthy [29] confirmed by DSC and dielectric measurements the evidence of this hydrate formation. Using differential scanning calorimetric measurements, the equilibrium phase diagram of EG in water was determined and several authors found the eutectic point on the water-rich side (EG molar fraction less than 0.5) around 60% (w/w) EG at a temperature of −48.6°C [29,45]. In practice, this eutectic is difficult to observe. We only noticed it once at 2.5°C/min for 43% (w/w) EG. Its temperature of −49.4°C was very close to Murthy [29] and Zinchenko [45] results. The scarceness of the eutectic observation in our measurements indicates that EG offers a particular resistance to eutectic freezing and confirms previous results obtained by Boutron with other compounds of the polyalcohol family. That is the reason why several authors have only published incomplete phase diagrams [20,39].

**Discussion**

**Correction of ice maximum quantity deduced from the thermograms**

As shown in Fig. 1 and Table 1, the total amount of heat of ice crystallization \( q_{max} \) tends to decrease when the EG concentration in water increases. Taking into account the heat of mixture and the evolution of the crystallization latent heat of water with temperature, Boutron calculated the real quantity of ice crystallized for the glycerol–
water system and compared it with the quantity of ice in equilibrium with the eutectic. He observed a good agreement, with a residual error less than ±5% [6]. We made the same corrections from \( q_{\text{max}} \) values of Table 1 for the EG–water system [5,6,44] and we obtained close values of the real quantity of ice crystallized with Thom’s results measured on freezing using an expansion method [39] (the mean relative error is of 11% for comparable cooling rates between 40 and 50% (w/w) EG). Considering Murthy’s result for the eutectic composition, we plotted on Fig. 4 the corrected maximum quantity of ice crystallized as a function of EG concentration together with \( q_{\text{max}} \) values of Table 1. We notice that the real quantities of ice crystallized are in good agreement with the thermodynamic equilibrium in the range of tested concentrations, with a mean residual error of ±5.5%, just above what would be the eutectic melting temperature at thermodynamic equilibrium: the composition of the residual solution is that of the eutectic within this uncertainty, as already observed for the glycerol–water system [6]. However, in our experiments, the eutectic crystallization is extremely scarce. Below the eutectic melting temperature the whole solution is no more in equilibrium when no eutectic freezing takes place.

**Analysis of the shape of the thermograms on cooling**

On the thermograms on cooling, an unusual and systematic effect has been observed. For 40% (w/w) EG, there were two separated bumps in the crystallization peaks at high cooling rates (\( \geq 40^\circ\text{C}/\text{min} \)) the second one decreasing when the cooling rate decreases to the advantage of the first one as shown on Fig. 5. Contrarily, for higher EG concentrations, only one peak was observed whatever the cooling rate when ice crystallized. Some authors conducted detailed investigations in EG concentrated solutions of 48 and 53% (w/w) to study the nucleation and growth of ice crystals. Differential scanning calorimetric measurements [14] supported by cryomicroscopy were analyzed with Johnson–Mehl–Avrami model and showed a two-stage ice nucleation process. The mentioned work concluded that on cooling, a secondary ice nucleation occurs approximately 20°C lower than that at which the initial nucleation event was completed. Although we observed a systematic difference of almost 30°C between the two peak minimums at −80°C/min, this two-stage ice nucleation process could explain the two bumps observed for 40% (w/w) EG, the dynamic effect usually stretching the peaks. Such a phenomenon was also observed in solutions of 45% glycerol plus 1% PEG, and was suggested to result from heterogeneous and homogeneous nucleation process [42]. Because of the limited DCS2 sensitivity, this phenomenon is logically more visible at high cooling rates. Nevertheless, this two-stage nucleation process could also be more visible at high cooling rates because rapid coolings prevent the completion of ice crystallization after the first nucleation event, allowing therefore the second nucleation process to take place. On the contrary, slow cooling rates favor ice crystals growth from the first nucleation step, leaving less and less freezable water to nucleate at lower temperature. As a consequence, for a given cooling rate, the observation of this phenomenon depends on the cryoprotectant concentration since it depends on the amount of crystallizable ice. It was not observed for 45, 48, and 50% (w/w) EG since the corresponding critical cooling rates are smaller than 80°C/min. For 43% (w/w), the amount of ice crystallized was about 0.8% at 80 and 5% at 40°C/min. Only a special program of deconvolution would have allowed to properly examine the peak.
in order to distinguish if there was only one or two separated crystallization peaks. Moreover, it is possible that the double nucleation observed at 40% (w/w) EG might be invisible for the higher concentrations because one of the nucleation mechanism is suppressed. Indeed, ice crystallization is more sluggish in these solutions when EG concentration increases.

**Analysis of the shape of the thermograms on warming**

On the thermograms on warming, we observed two different effects which, though often observed, seem not yet completely understood. On the first hand, a kind of endothermic peak in heat capacity is observed at the end of the glass transition temperature for 43% (w/w) EG or more. This endothermic effect is represented on Fig. 6. It is considered as the consequence of a delayed response of the glass to the supercooled liquid state transition process as the temperature rises. Such a heat capacity overshoot can often arise for entropic reasons [21] or because of differing transient relaxation rates and is not uncommon in aqueous solutions [21, 27], depending on the heating rate [24]. In practice, these distortions made more difficult the determination of \( T_g \). On the second hand, for the highest concentrated solutions (at 48 and 50% (w/w) EG) we observed a small exothermic effect between \( T_d \) and \( T_m \). It cannot correspond to the transformation of metastable cubic ice into hexagonal ice which has been observed by X-ray diffraction on warming initially amorphous aqueous solutions [28] since this transformation is not energetic enough to be detected by differential scanning calorimetry. According to [39], we attributed it to the recrystallization following devitrification during the rewarming. Indeed, pictures have been obtained by cryomicroscopy in comparable concentrated solutions of EG, showing recrystallization during the rewarming.

**Discussion about the critical rates**

One sees on Table 1 that the critical cooling rates obtained for 48% (w/w) and more EG in water seem technically achievable for freezing small organs. The equilibrium phase diagram of EG with water containing a hydrate, strong interactions between this component and water molecules must lead to the compound formation. This can be one of the reasons why EG appears to be a good glass-former around the eutectic concentrations. In addition, the critical warming rate decreases with the concentration of the cryoprotectant more rapidly than the critical cooling rate, as usually observed [1–4, 8, 13, 33]. Nevertheless, the critical warming rates for 48% (w/w) and more of EG in water are still too high for simple rewarming of small organs involving only the cryoprotective solution thermal diffusivity and require the use of an electromagnetic rewarming. In Table 2, the critical cooling and warming rates of other polyalcohols are given for comparison with 40, 45, and 50% (w/w) EG. One sees that for 40 and 50% (w/w), the order concerning the efficiency of the polyalcohols is the same as was previously observed with 45% (w/w) EG [12]. It must be noticed that our critical rates are generally close but slightly higher than Wowk et al. [40] values for comparable concentration, both on cooling and on warming. The method of determination being the same, this might be due to a difference in purity of EG, our one being only 99+%.

Using the critical rates to compare the dialcohols efficiency regarding vitrification, it is possible to study the effect of the molecular structure on the cryoprotectant ability to prevent ice crystallization. As shown by the comparison of EG, 1,2-propanediol and 2,3-butanediol critical rates, the length of the carbon chain can be of a great importance. When the chain is longer, the molecule is more flexible. That reduces steric congestion and
facilitates interactions between water and cryoprotectant molecules via hydrogen bonds. They reduce water molecules mobility and disturb ice crystallization. As a consequence, for linear dialcohols, the critical rates become lower when the length of the carbon chain becomes longer. Interestingly, Table 2 gives as well the opportunity to observe the effect of the hydroxyl group position demonstrated by MacFarlane and Forsyth [22] from NMR measurements. It has been underscored that, when a methyl function is attached to the same carbon atom as a hydroxyl group, the electron density is shifted from the methyl group toward the hydroxyl oxygen, making the oxygen a stronger base [22]. As a consequence, the hydrogen bond in which it engages is enhanced and water molecules become less free. Comparisons between critical rates of 1,2-propanediol and 1,3-propanediol on one side, and 1,3-butanediol and 2,3-butanediol on the other side, confirm this effect, both on cooling and on warming.

**Conclusion**

The evolution of cryobiology, based on observations and experiments, can be helped by a systematic thermal characterization of cryoprotectants. The object of this work was to study the thermal properties of ethylene glycol in the presence of water regarding vitrification. Our results for transition temperatures are in agreement with other authors published values. Concerning ice crystallization, DSC measurements on cooling showed that the quantity of ice formed is depending on the cryoprotectant concentration, as usually observed, and that the more the cryoprotectant, the less the critical rates. However, the biological toxicity of a cryoprotectant being a function of its concentration too, this is why this compound is now mixed with other cryoprotectants in the newly developed cryoprotective solutions [15,41]. Concerning DSC measurements on warming from the complete amorphous state, we still have some doubts about our interpretation of the small exothermic peak we observed between $T_d$ and $T_m$ and we attributed for the moment to recrystallization. Globally, this study confirms the effectiveness of this cryoprotec-
tant for the glass forming tendency and the stability of the amorphous state but shows that on a thermal point of view, it is less efficient than other dialcohols with 3 or 4 carbons. Its main interest is thus the low toxicity of the mixtures in which ethylene glycol is used.

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