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The isothermal relaxation of the glassy state of 60% w/w ethylene glycol in water: a possible nucleation process below the glass transition[1]

Patrick M. Mehl

American Red Cross, Holland Laboratory for Biomedical R&D, 15601 Crabbs Branch Way, Rockville, MD 20855 (USA)

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Abstract

Calorimetric measurements with or without annealing around the glass transition were performed for the aqueous solution of 60% w/w ethylene glycol. Excess entropies and excess enthalpies were calculated as functions of annealing times and temperatures. The kinetics of the relaxation was analyzed and shows that relaxation does not reach completion over experimental time. Therefore, another phenomenon and the relaxation of the glassy state are overlapping. This phenomenon is assumed to be a nucleation process. The kinetics parameters are, however, determined under isothermal conditions in the range of annealing times and temperatures where the nucleation is still suppressed. Slower warming rates that allow crystal growth show increased crystallization with annealing time which supports the hypothesis of nucleus formation during annealing.

INTRODUCTION

Vitrification is the only possible technique for organ cryopreservation at low temperatures which avoids mechanical damages created by crystallization [1]. Because vitrified organs are planned to be stored for organ and tissue banking, the aging of glassy aqueous solutions at temperatures around the glass transition is a vital factor for organ preservation. Indeed as crystallization must be avoided during the warming subsequent to storage, possible nuclei density increases inside the glassy matrix must be investigated as a function of temperature T and time t of storage. However the storage temperature is also limited by the possible creation of fractures because the brittleness of the glass increases as temperature decreases [2]. A window for storage is defined by a temperature at which the viscosity is high enough to limit nucleation and by a temperature high enough to limit

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fracturing. In the present paper, the first consideration is investigated by DSC using indirect annealing experiments, as previously defined [3].

MATERIALS AND METHODS

Ethylene glycol from Fisher was used as received and diluted to 60% w/w in deionized water. The solution was then passed through a 0.22 micron Millipore filter to avoid heterogeneous nuclei formation during cooling. Experiments were performed in a Perkin-Elmer DSC-4 at different temperatures for different periods *t*. The variations of apparent specific heat from non-annealed and annealed samples were calculated to estimate the excess entropy and excess enthalpy stored during annealing. The cooling and heating rates were 10°C $\min^{-1}$. (A heating rate of 10°C $\min^{-1}$ does not allow formed nuclei to grow.) To reveal the presence of nuclei, similar annealing treatments were pursued by replacing the last warming step with a 2.5°C $\min^{-1}$ warming rate to allow growth of stable nuclei.

RESULTS

Figure 1A shows the variation in specific heat C_p calculated by subtraction of the value for the annealed sample for time *t* from the value

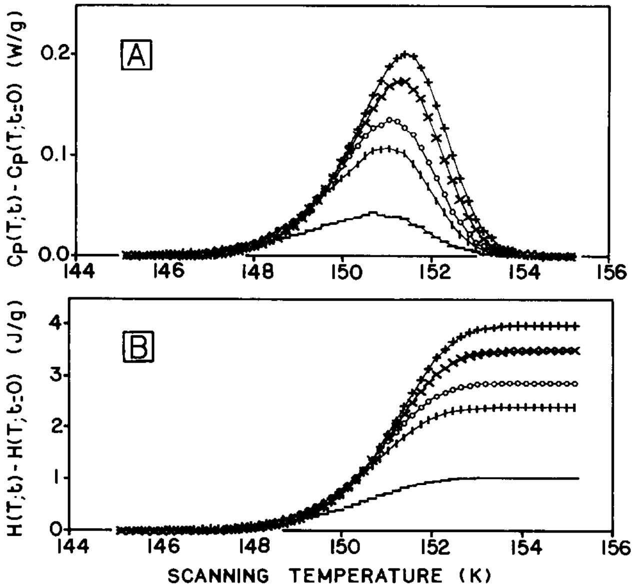

Fig. 1. Difference of warming thermal curves $dQ/dt'(T, t)$ and $dQ/dt'(T, t = 0)$ giving similar values than the difference $C_p(T, t)$ and $C_p(T, t = 0)$ for the same sample of 60% w/w ethylene glycol in water annealed at −133°C for *t* min (A) and the corresponding calculated excess enthalpy (B): —, 5 min; |, 20 min; O, 30 min; ×, 60 min; +, 120 min.

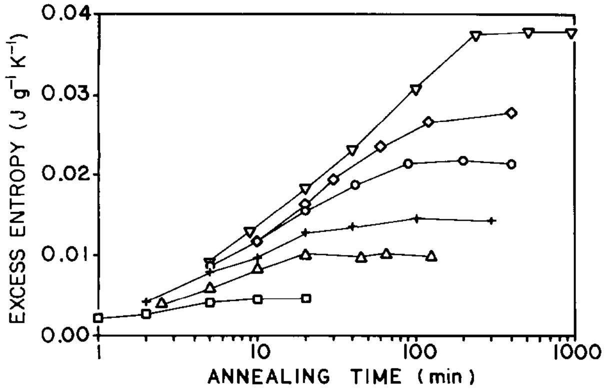

Fig. 2. Calculated excess entropy from the variations in $C_p(T, t) - C_p(T, t = 0)$ for different temperatures and times of annealing for 60% w/w enthylene glycol in water: □, −129°C; △, −130°C; +, −131°C; ○, −132°C; ◇, −133°C; ▽, −134°C.

for the unannealed sample. An assumption is made here by neglecting the transient effect of the initial heating and recooling on the annealing process. From the data, the excess enthalpies are calculated as shown in Fig. 1B. Similarly, the excess entropy can be calculated for the entropy stored after annealing at different temperatures T for different times t (Fig. 2). The enthalpy relaxation function can then be defined as

$$\phi(t, T) = [H(t, T) - H(\infty, T)]/[H(0, T) - H(\infty, T)] \qquad (1)$$

i.e. the ratio of the excess enthalpy at t to the excess enthalpy at $t = \infty$ for annealing at T. The denominator is estimated graphically from the experimental thermal curve of the glass transition during heating with two assumptions: firstly, the specific heat of the liquid over the whole thermal range of the glass transition is considered constant; secondly, the specific

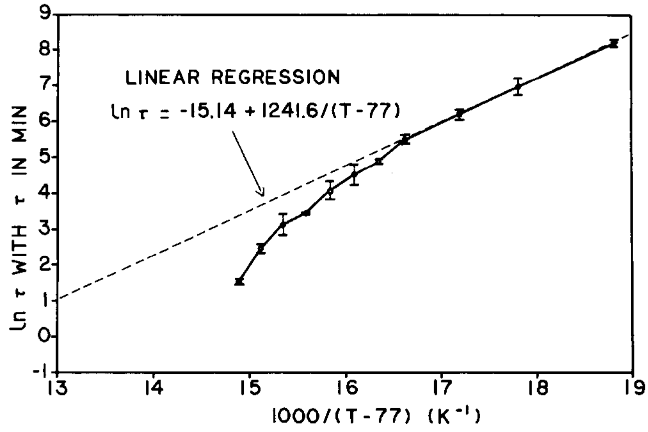

Fig. 3. Ln τ as a function of $1/(T - 77)$ with the relaxation time τ in min and the temperature T in K.

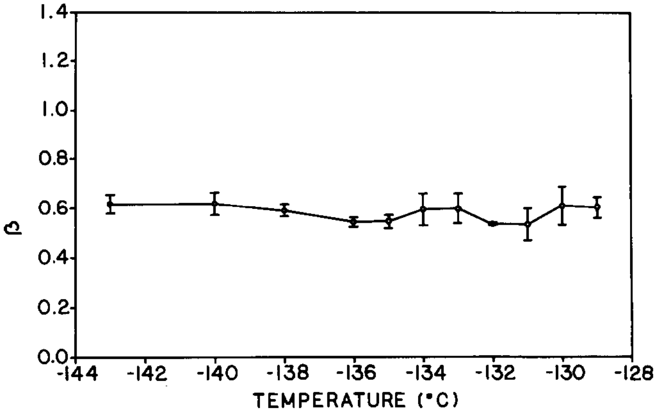

Fig. 4. Exponent β as a function of the annealing temperature T for 60% w/w ethylene glycol in water.

heat of the glassy state below and above the glass transition is considered constant. From integration of the thermal curves, the enthalpy for the liquid is extrapolated linearly to annealing temperatures T to estimate the maximum excess enthalpy between the non-annealed glassy state at T and the liquid. The Kolhrausch–Williams–Watts function gives [4]

$$\phi(t, T) = \exp - [t/\tau(T)]^{\beta(T)} \tag{2}$$

where the parameters $\tau(T)$, the relaxation time, and $\beta(T)$, the non-linearity factor, are calculated as dependent on T. The relaxation, however, does not reach its maximum for the present aqueous solution [3]. Variations in τ and β are reported in Figs. 3 and 4, for only the linear part of $\ln(-\ln(\phi))$ versus $\ln(t)$. The variations in these parameters, especially $\ln(\tau)$ versus the inverse of temperature, are not linear for annealing temperatures in the glass transition [3].

DISCUSSION

The fact that relaxation does not reach its maximum raises two questions concerning the assumptions made to deduce the parameters: (i) the assumption of a stable glass without crystallization; and (ii) the assumption that the relaxation is independent of the initial relaxed state of the glass.

For (i), nuclei formation is invisible by DSC measurements. This formation is visible only during their growth. Therefore, annealing treatments at two temperatures were performed; the final warming rate used allows the growth of the nuclei and therefore an estimate of the increase in nuclei is possible as reported in Fig. 5. The thermal treatment is reported in the figure caption. As the annealing time increases, the amount of crystallization observed during the last warming increases. This suggests that storage at these temperatures allows either an increase in the nucleus density below the glass transition or a stabilization of the glass towards the

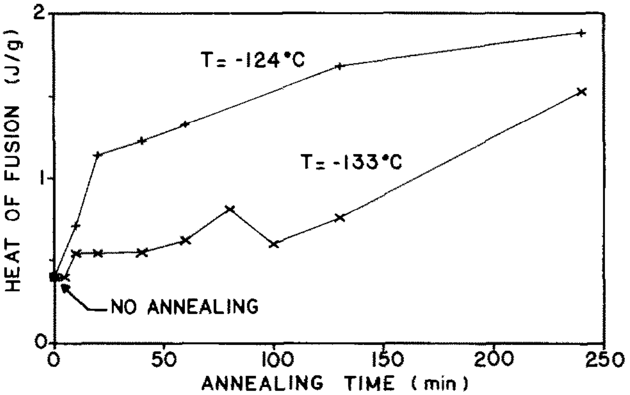

Fig. 5. Heat of fusion (J/g of solution) measured during the final warming of the thermal treatment (described below) versus the annealing time t (min) at −124°C and −133°C. The samples were cooled at 10°C min^{-1} down to −160°C, heated at 10°C min^{-1} up to −124°C or −133°C for annealing and then cooled at 10°C min^{-1} down to −160°C. The samples were finally heated at 2.5°C min^{-1} up to the melting temperature to record the heat of fusion.

formation of a larger number of nuclei during the rewarming. It can be noticed that the increase in the heat of fusion observed in Fig. 5 and the slowing down of the function $\phi(t, -133°\mathrm{C})$ in Fig. 6 correspond to a similar time of annealing.

For assumption (ii), because the relaxation function used is not really that which is defined as being due to a transient relaxation, we have instead

$$\phi_{\mathrm{meas}}(t, T) = [H(t, T) - H(\infty, T)]/[H(t_0, T) - H(\infty, T)]$$

related to the Adams–Gibbs expression of $\phi(t, T)$

$$\phi_{\mathrm{meas}}(t, T) = [\phi(t, T)/\phi(t_0, T)]$$

Therefore, for an identical reference curve as taken for an annealing time

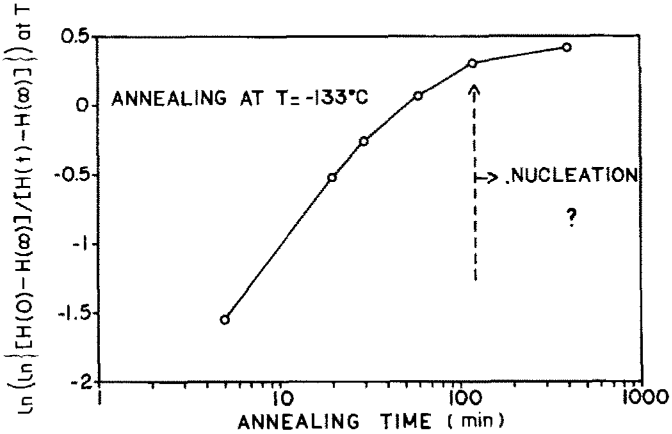

Fig. 6. Ln[−ln($\phi(t, T)$)] as a function of the annealing time t (min) in a logarithmic scale at $T = -133°\mathrm{C}$. The relaxation does not reach completion.

of t_0 for the same annealing temperature set, $\phi_{\mathrm{meas}}(t, T)$ is proportional to $\phi(t, T)$, which verifies the hypothesis.

CONCLUSIONS

The indirect method for isothermal annealing of the glassy state of 60% w/w ethylene glycol in water allows access to the relaxation time τ and to the non-linear exponent β in the relaxation function ϕ [3] within specific assumptions for the physical behavior of the liquid and the glassy states close to the glass transition.

Moreover, this system shows that the decrease in the relaxation time is more rapid when the annealing temperature is close to that of the end of the glass transition on heating. Two possible mechanisms can explain this behavior. The first represents a possible behavior of the liquid and the glassy state around the glass transition which does not verify the assumptions of the constancy of the specific heat of the liquid and of the glassy state around the glass transition. A slow decrease of the difference in their specific heat will lead to experimental observations similar to those presented here. However, it is not possible to check this hypothesis on the basis of the present data. Another possibility is the superposition of nucleation and relaxation. The present data support the hypothesis of an increase in nucleation resulting from the annealing time.

This last hypothesis is important and must be checked for cryobiological purposes: nucleation is a factor leading to crystallization during warming and, therefore, to damage of vitrified organs [1]. An increase in nucleation during storage below the glass transition might be critical if the time of storage is long enough to favor phase separation at low temperatures which will not induce crack formation. However, other thermal analysis techniques are necessary to confirm a nucleation increase during storage, such as neutron or X-ray diffraction experiments, to prove that phase separation occurs.

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REFERENCES

1 G.M. Fahy, D.R. MacFarlane, C.A. Angell and H.T. Meryman, Cryobiology, 21 (1984) 407.
2 P.M. Mehl, Thermochim. Acta, 155 (1989) 187.
3 P.M. Mehl, Thermochim. Acta, 213 (1993) 177–197.
4 K.L. Ngai and G.B. Wright, Relaxations in complex systems, National Technical Information Service, US Department of Commerce, Arlington, VA, 1988.