Available online at www.sciencedirect.com
SCIENCE DIRECT®
Thermochimica Acta 419 (2004) 131–134
thermochimica acta
www.elsevier.com/locate/tca

Comparative study of glass transformation of glycerol–H_2O–NaCl ternary system and glycerol–PBS complex system

Gang Zhao [a,1], Xiaojie Guo [b], Liqun He [a,*], Zhong Liu [b], Dayong Gao [a,c]

[a] *Department of Thermal Science and Energy Engineering, Cryobiomedical Engineering Research Institute, University of Science and Technology of China, Hefbei 230027, PR China*
[b] *Cross Bank and Blood Center of Hefei, Hefei 230022, PR China*
[c] *Department of Mechanical Engineering, University of Kentucky, Lexington, KY 40506-0108, USA*

Received 24 September 2003; received in revised form 17 February 2004; accepted 18 February 2004

Available online 9 April 2004

Abstract

A differential scanning calorimeter was used to study the glass transformation of glycerol–H_2O–NaCl ternary system and glycerol–PBS complex system that are used clinically in order to explore the cryoprotective role of glycerol in preserving frozen biomaterials. These systems are of particular importance in the field of cryobiology, for knowledge of glassy phases may be especially crucial for long-term cryopreservation of biological materials. The melting and glass transition temperatures for the two systems of different concentration (glycerol, mass concentration, from 5 to 100%, interval 5%) were measured, and very simple equations have been derived for predicting the temperatures. A glassy state that is crystallographically amorphous was found for glycerol concentration between 45 and 60%, and a complete glassy state was found at glycerol concentration greater than 60% for both of the systems. These supplemented phase diagram data may help the design of procedures during vitrification preservation process based on glycerol.

Keywords: Glass transition/vitrification; DSC; Glycerol; Phase change

1. Introduction

The challenge to cells during freezing process is not their ability to endure storage at very low temperatures; rather, it is the lethality of an intermediate zone of temperature (from −15 to −60 °C) that a cell must traverse twice—once during cooling and once during warming [1]. Cells must endure the intra- and extracellular ice formation and growth. Vitrification, as an alternative to freezing, has been widely discussed and clinically used especially recently. Vitrification is a process by which cell suspensions are under cooled to the glass transition temperature (T_g) and directly solidified into the amorphous or glassy state [2]. Freezing injury could be maximally avoided by preventing the formation of ice inside and outside the cells during vitrification, and even partial vitrification is now recognized as playing a key role in traditional method of preservation as well [3].

As vitrification is considered to have a great potential for successfully cryopreserving biomaterials, it is necessary to have fundamental knowledge concerning the glass transition temperature (T_g) and the stability of the glassy state of the chosen vitrification medium. Luyet et al. have given the T_g of the binary system glycerol–H_2O of different concentration by using differential thermal analysis [4], in the light of which this paper focuses on the relationship between the glass transition temperature and the concentration of the most widely used, glycerol-based, clinical cryopreservative solutions (glycerol–H_2O–NaCl ternary system and glycerol–PBS complex system). Additionally, the melting temperature (T_m) and the endothermic energies (E_{endo}) are also given as functions of the concentration of these two systems. Finally, the application of these equations

* Corresponding author. Tel.: +86-551-3607146; fax: +86-551-3607346.
E-mail addresses: cooky@mail.ustc.edu.cn (G. Zhao), heliqun@ustc.edu.cn (L. He).
[1] Co-corresponding author.

0040-6031/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2004.02.007

in optimizing the vitrification preservation procedures is discussed.

2. Materials and methods

2.1. Sample preparation

glycerol–NaCl–water ternary solutions were prepared by adding different masses of glycerol to 0.9% (w/v) NaCl aqueous solutions. The 0.9% NaCl aqueous solution was prepared by dissolving 0.9 g of NaCl (Analar grade, Shanghai Chemical Reagent Co. Ltd., Shanghai, PR China) into 100 ml of deionized water produced by KFLOW-R050ACB water clean system (KFLOW water system Co. Ltd., Xiamen, Shanghai, PR China) at room temperature. Then different masses of glycerol (Analar grade, Shanghai Chemical Reagent Company of China Medical Group, Shanghai, PR China) were added into different masses of 0.9% NaCl solutions to make glycerol–NaCl–H_2O water systems with concentratons of 5–100% (interval 5%), the total mass of each sample was 10 g. All the related masses were measured using an electronic semi-micro-analytical and -precision balance (Bejing Sartorius Instrument & System Engineering Co. Ltd., Beijing, PR China).

Glycerol–PBS complex solutions were prepared using the same method except that phosphate buffered saline (PBS) was used instead of the 0.9% NaCl aqueous solutions. The PBS was prepared by dissolving all the salts (NaCl: 8 g, KCl: 0.2 g, Na_2HPO_4: 1.44 g, KH_2PO_4: 0.24 g) in 800 ml double-distilled water (SZ-97 automatic tripartite pure water distillatory, Shanghai Yarong Biochemical Instrument Factory, Shanghai, PR China) at room temperature. Then the pH value was adjusted to 7.4 using HCl aqueous solution (1 M) and the total volume was fixed at 1 litre.

Glycerol-based solutions of different concentration were pipeted (10–12 μl, about 12 mg) into pre-weighed DSC aluminum pans, and the pans were hermetically sealed. The weight of each solution sample was obtained by subtracting the weight of the empty pan from the total weight of the sample.

2.2. Thermal analysis

2.2.1. Calibration of DSC

All the measurements were carried out on a Pyris-1 (Perkin-Elmer Corporation, Norwalk, CT) DSC. Before measurement, the temperature scale of the DSC was calibrated by the melting point of *n*-decane ($C_{10}H_{22}$, 243.45 K or −29.7 °C for 99.9% purity) and indium (156.7 °C for 99.9% purity). The transition enthalpies were based on the heat of fusion of pure ice (333.9 J/g). The instrument was calibrated two–three times. To minimize the thermal lag, a low and evenly distributed sample mass (9–15 mg) was used.

2.2.2. Thermal history and analysis of T_g, T_m, and E_{endo}

The sample initially equilibrated at 25 °C was cooled at 100 °C/min to −150 °C/min, and then it was equilibrated for 2–3 min, after that heated from −150 to 25 °C at 10 °C/min. The thermograms were analyzed using Pyris software for windows (version 3.81, Perkin-Elmer). The T_g was determinated as the onset point of the change in the specific heat capacity. Two tangents were drawn on the thermogram to determine the T_g, the one below the transition was aligned to the heat flow and the other one above the transition was set at the highest point of the transition and aligned to a parallel fit to the first one, as described in ref. [5]. The latent endothermic energy during the heating process from the pre-frozen sample was also calculated using the Pyris-1 DSC software, the linear baseline was drawn between the start and the end temperature of the phase change (if the phase change can be detected obviously). The onset point of the phase change process was regarded as the melting point (T_m).

3. Results and discussion

3.1. Heating DSC thermograms

Heating DSC curves for glycerol–PBS and glycerol–NaCl–H_2O are shown in Figs. 1 and 2. Both of the figures indicate that the melting temperature decreases with the

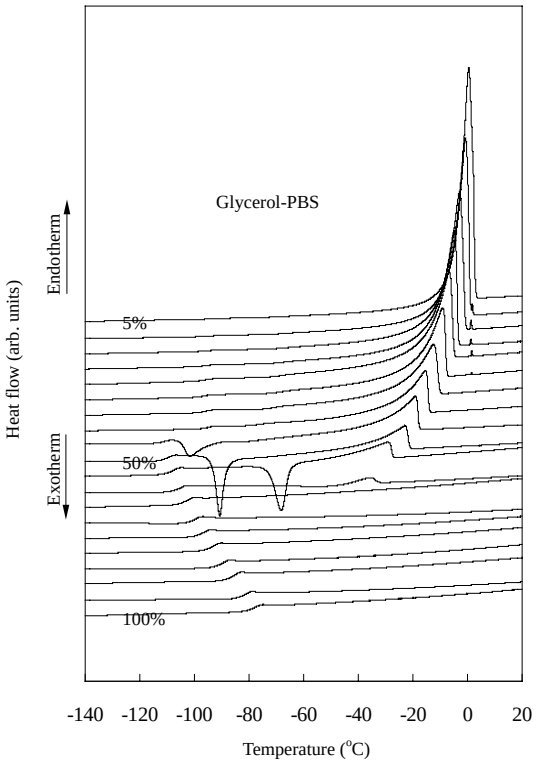

Fig. 1. Heating DSC curves for glycerol–PBS (from the top line to the bottom line, the concentration of glycerol increases from 5 to 100%, interval 5%).

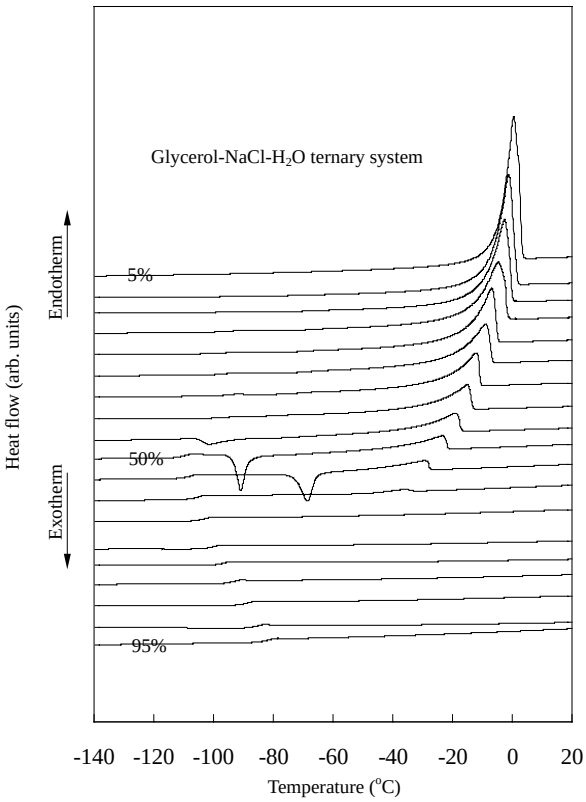

Fig. 2. Heating DSC curves for glycerol–NaCl–H_2O ternary system (from the top line to the bottom line, the concentration of glycerol increases from 5 to 95%, interval 5%).

increasing glycerol concentration from 5 to 60%, and the endothermic peaks broadened with increasing glycerol concentration. No detectable melting peak exits for solutions containing more than 60% glycerol. The thermal offset corresponding to the glass transition of the solution is evident at glycerol concentrations of 45–100%. For glycerol concentration of 45–55%, exothermic peaks appeared, indicating that re-crystallization or de-vitrification occurred during the heating process. It can be seen that these glycerol solutions were in a partial glassy state, that is crystallographically amorphous, during the cooling process [6]. From the figures, the vitrification of solution with glycerol concentration of 45–55% is unstable.

3.2. The glass transition temperature (T_g)

The relationship between glycerol concentration and the glass transion temperature (T_g) are shown in Fig. 3. From Fig. 3, it can be seen that both the glycerol–PBS complex system and glycerol–NaCl–H_2O ternary system have very similar trend of T_g versus T, and they have almost the same fitting curve. The glass transition temperature (T_g) for the glycerol–PBS complex system and the glycerol–NaCl–H_2O ternary system can be described separately as follows:

$$y = 0.005x^2 - 0.1154x - 119.40 \quad (45 \le x \le 100)$$
$$y = 0.005x^2 - 0.0902x - 120.71 \quad (45 \le x \le 100)$$

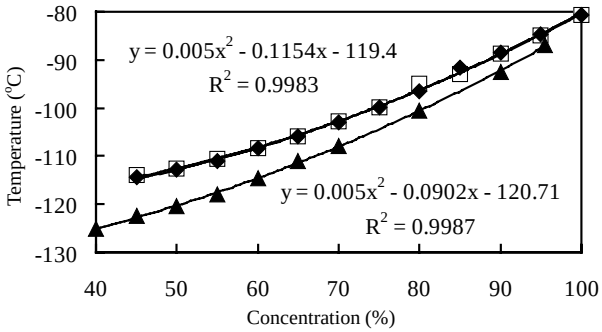

Fig. 3. Glass transition temperature (T_g) vs. glycerol concentration ((□) points to glycerol–PBS complex system, and the equation in the top left corner is the fitting formula for them; (◆) points to glycerol–NaCl–H_2O ternary system, and the equation in the bottom right corner is the fitting formula for them; (▲) points to glycerol–H_2O binary system, from Ref. [2]).

where x is the glycerol concentration (%) for the systems and y the glass transition temperature T_g (°C) for the systems.

From Fig. 3, it can also be seen that compared to the glycerol–H_2O binary system of, both the studied systems (glycerol–PBS and glycerol–NaCl–H_2O) have a higher T_g, perhaps because more ions are imported into these systems.

3.3. The melting temperature (T_m) and the endothermic energy (E_{endo})

The melting temperatures (T_m) for these two systems are plotted in Fig. 4, from which it can be seen the T_m and the concentration of glycerol have a quadratic relationship. The melting temperature decreases with increasing glycerol concentration, until the concentration reaches 60%. For glycerol-based systems, if the concentration is larger than 60%, there are no detectable melting endothermic peaks, indicating that these systems have a completely vitrified state.

The melting temperature (T_m) for the glycerol–PBS complex system and the glycerol–NaCl–H_2O ternary system can be described separately as follows:

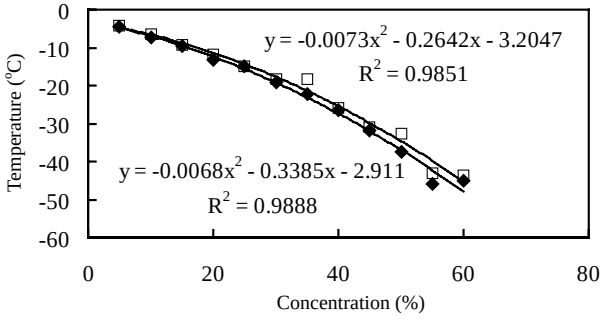

Fig. 4. Melting temperature (T_m) as a function of glycerol concentration ((□) points to glycerol–PBS complex system, and the equation in the top right corner is the fitting formula for them; (◆) points to glycerol–NaCl–H_2O ternary system, and the equation in the bottom left corner is the fitting formula for them).

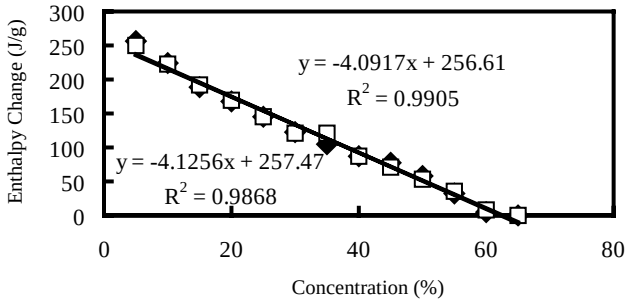

Fig. 5. Endothermic energies (E_{endo}) as functions of glycerol concentration ((□) points to glycerol–PBS complex system, and the equation in the top right corner is the fitting formula for them; (◆) points to glycerol–NaCl–H_2O ternary system, and the equation in the bottom left corner is the fitting formula for them).

$$y = -0.0073x^2 - 0.2642x - 3.2047 \quad 5 \le x \le 60$$
$$y = -0.0068x^2 - 0.3385x - 2.911 \quad 5 \le x \le 60$$

Either of the equations can be used for both the systems due to close arrangement of the two curves, or the average one of the two equations could be used.

The enthalpy change for melting of the two systems are plotted in Fig. 5. With increasing glycerol concentration, the enthalpy change decreases. Both the systems have fine linear relationship between the enthalpy change and the concentration of glycerol (Fig. 5). The heat absorbed during the heating process is caused by the melting of the ice crystal produced during the rapid cooling process. Because the water occupies a relative less mass with the increasing concentration of glycerol, this result is reasonable.

The relationship between the enthalpy change and the concentration of the glycerol for the glycerol–PBS complex system and the glycerol–Nacl–H_2O ternary system can be written as follows:

$$y = \begin{cases} -4.0917x + 256.61, & 5 \le x \le 60 \\ 0, & 60 \le x \le 100 \end{cases}$$

$$y = \begin{cases} -4.1256x + 257.47, & 5 \le x \le 60 \\ 0, & 60 \le x \le 100 \end{cases}$$

These equations can be used to optimize the cryopreservation procedure based on glycerol.

The damaging re-crystallization/de-vitrification phenomena in cryopreservation of cell suspension may be eliminated in certain concentration range. From the DSC curves in this paper, if the re-crystallization/de-vitrification peak and the melting peak can be moved to overlap each other, then the re-crystallization/de-vitrification will be eliminated.

References

[1] P. Mazur, Freezing of living cells: mechanisms and implications, Am. J. Physiol. 143 (1984) C125–C142.
[2] H.S. Ren, Y. Wei, T.C. Hua, J. Zhang, Theoretical prediction of vtrification and de-vitrification tendencies for cryoprotective solutions, Cryobiology 31 (1994) 47–56.
[3] G.M. Fahy, Vitrification, in: J.J. McGrath, K.R. Diller (Eds.), Low Temperature Biotechnology, ASME, New York, 1990.
[4] B. Luyet, D. Rasmussen, Study by differential thermal analysis of the temperatures of instability of rapidly cooled solutions of glycerol, ethylene glycol, sucrose and glucose, Biodynamica 10 (1968) 167–191.
[5] Jens Liesebach, Thomas Rades, Miang Lim, A new method for the determination of the unfrozen matrix concentration and the maximal freeze-concentration, Thermochim. Acta 401 (2003) 159–168.
[6] Tetsuo Iijima, Thermal analysis of cryoprotective solutions for red blood cells, Cryobiology 36 (1998) 165–173.