119. Supercooling and vitrification of aqueous polyethyleneglycol solutions

4 AUTHORS, INCLUDING:

Hitoshi Kanno
National Defense Academy of Japan
135 PUBLICATIONS **2,043** CITATIONS

[Original Paper]

Supercooling and Vitrification of Aqueous Polyethyleneglycol Solutions

Kazuhito KAJIWARA[1], Yuuma KITADA[1], Kiyoshi TOMIZAWA[2], and Hitoshi KANNO[2]

1. *School of Bioscience and Biotechnology, Tokyo University of Technology, 1404-1 Katakura Hachioji, Tokyo 192-0982, Japan*
2. *Department of Applied Chemistry, National Defense Academy, Yokosuka, Kanagawa 239-8686, Japan*

Supercooling and vitrification were studied for aqueous polyethyleneglycol (PEG) solutions of molecular weights (Ms) = 200, 300, 400, 600, 1000, 1540, 2000, and 3000. It is confirmed that additivity rule holds fairly well in the supercooling of the PEG solutions of M = 1000, 2000, and 3000. Glass formation was tested for the PEG solutions at the PEG concentrations at which they give the supercooling down to -100, -90 or -80 °C. The T_g variations (T_g: glass transition temperature) with M were analyzed and discussed.

INTRODUCTION

Studies of aqueous solutions at low temperatures are important in cryopreservation of living cells and biotechnology, not to say in basic science[1,2]. As there is a wide range of molecular weights in water soluble polyethyleneglycols (PEGs), we can study supercooling and vitrification of their aqueous solutions as a function of the molecular weight in a systematic way. Taking advantage of this merit, Zobrist *et al*[3] have recently measured T_H values (T_H: homogeneous ice nucleation temperature) for aqueous solutions of PEGs of various molecular weights and have shown that the linear relation ($T_H = k\,T_m$) (T_m: melting temperature) holds quite well for all the PEG solutions and that the k value increases with increase in molecular weight of PEG. Kanno *et al*[4] have reported that additivity rule holds in the supercooling of aqueous solutions of alcohols and PEGs.

In this study, we measured the homogeneous ice nucleation temperatures (T_Hs) of aqueous PEG solutions as a function of PEG concentration to test the additivity rule and then we tried to vitrify the PEG solutions at the solute concentrations at which they give T_H of -80, -90 or -100 °C.

MATERIALS AND METHODS

The average molecular weights (Ms) of PEGs used in this study are M = 200, 300, 400, 600, 1000, 1540, 2000, and 3000. The emulsification method developed by Rasmussen and MacKenzie[5] was used to measure T_H of these aqueous solutions. The dispersant phase was a 1:1 volume mixture of methylcyclopentane and methylcyclohexane. The surfactant used in this study was Span 65 (sorbitan tristearate). The emulsions were made by mixing 2 ml sample solution, 4 ml dispersant solution and ~100 mg

Span 65 using a small blender with a rotation rate of 6×10^3 rpm. The mixing time was ~ 3min in most cases. A simple DTA method was used in the T_H and T_g measurements (T_g : glass transition temperature). The cooling rate was about 3 °C/min in the T_H measurements. In the case of the T_g measurements, the bulk solutions were used. The cooling rate was about 1.8×10^3 °C/min in the quenching process and the heating rate was about 4 °C/min in the T_g region.

RESULTS AND DISCUSSION

First we examined the additivity rule of supercooling by using the T_H data for the aqueous PEG solutions of M = 1000, 2000, and 3000. Fig. 1 shows the T_H curves of the M = 1000, 2000 and 3000 solutions and the hypothetical T_H curves for the M = 3000 solutions by increasing the PEG concentration of the M = 3000 solution 1.5(□) or 3(△) times without changing the T_H value.
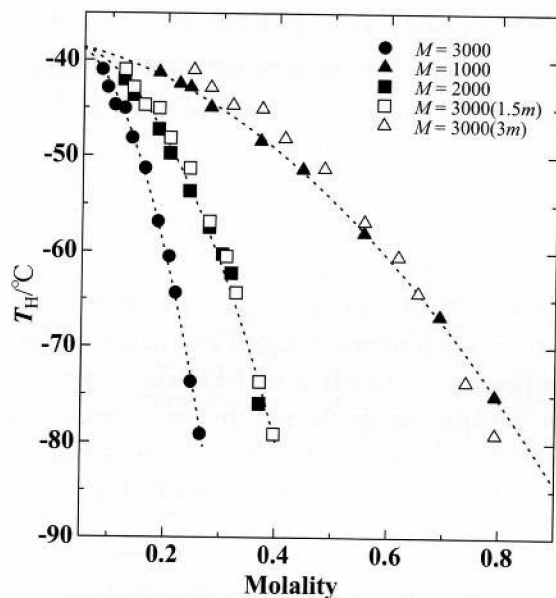

Fig. 1 Test of the additivity rule on the T_H data for the PEG solutions of M = 1000 (●), 2000 (■) and 3000 (▲) with hypothetical T_H data (□, △).
Note: m = molality

Complete and fairly well overlaps between the observed T_H and the hypothetical T_H values clearly indicate that the additivity rule holds fairly well for the PEG solutions of M = 1000, 2000, and 3000. Close examination, however, reveals that overlap between the observed T_H values and the hypothetical T_H values is better

between M = 1000 and M = 2000 than between M = 1000 and M = 3000. Therefore, it is clear that the additivity rule holds quite well as long as the difference of the PEG molecular weight is not large.

Oguni and Angell[6] examined the supercooling behavior for aqueous solutions of methanol, ethanol and 1-propanol and showed that supercooling is governed by the sum of the fractional supercooling contributions arising from the groups of a solute compound (additivity rule). Subsequently, Kanno et al[4] measured T_H curves for many aqueous solutions of alcohols and sugars and established that additivity rule holds fairly well for the supercooling of aqueous solutions.

Glass transition of water is considered to be − 135 °C although Angell[7] has recently argued that it should be around −108 °C. Actually vitrification of neat liquid water is extremely difficult and is only feasible when fine aerosol is cooled at a extremely fast cooling rate (> 10^5 °C). On the other hand, aqueous solutions of hygroscopic salts and glass-forming solutes are usually glass forming in certain solute concentration range at easily accessible cooling rate (~ 10^3 °C/min)[8]. When an aqueous solution is glass-forming, its T_g value approaches to −135 °C with decreasing solute concentration. Thus it is interesting to test whether or not an aqueous solution, which shows supercooling down to ~ −100 °C, can be vitrified with a cooling rate of ~ 2×10^3 °C/min.

Next we examined the glass forming behavior of the PEG solutions which give T_H value of -100 °C. Fig. 2 shows the T_g and T_c values of the PEG

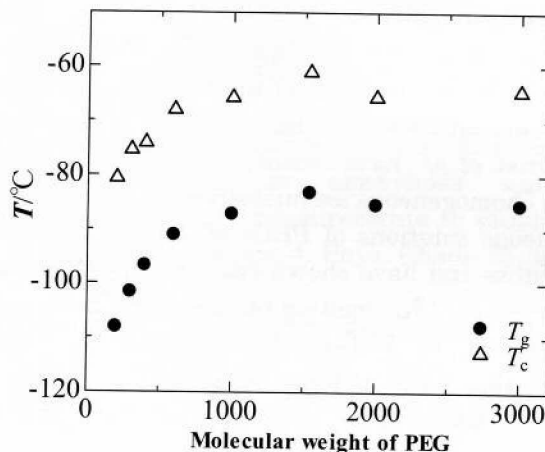**Fig. 2** Variation of T_g with molecular weight of PEG for the PEG solutions giving the T_H values of −100°C

solutions (T_c: crystallization temperature).

Vitrification of the PEG solutions was made by cooling them with a cooling rate of 1.8×10^3 °C/min. An interesting point is that both T_g and T_c rise with increase in PEG molecular weight up to $M = 1000$ and then give an plateau value till $M = 3000$. Apparently the T_g (or T_c) value for the $M = 1540$ solution is a little higher than the value expected from the smoothed line between the T_g (or T_c) values for the $M = 1000$ solution and the M $= 2000$ (or 3000) solution. At the moment we can not give a clear-cut explanation. As the PEG solutions of $T_H = -100$ °C are glass-forming, we next tried the vitrification of the PEG solutions of $T_H = -90$ °C.

Fig. 3 shows the T_g and T_c results for the PEG solutions which give the T_H value of -90 °C. As easily noticed, overall trend of the variations of T_g and T_c values with M is very similar to that for the solutions which give the T_H value of -100 °C.
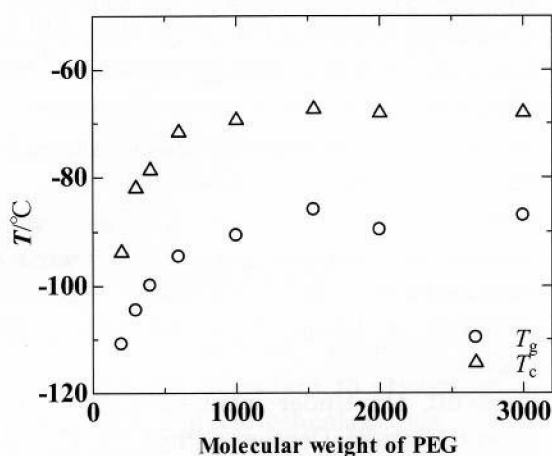

Fig. 3 Variation of T_g with molecular weight of PEG for the PEG solutions giving the T_H values of -90°C

Both T_g and T_c rise with M up to $M = 1000$ and subsequently become a plateau value to $M = 3000$. On the other hand, they are lower in temperature than those for the corresponding solution which gives the T_H value of -100 °C. This is only natural since the PEG concentration of the solutions which give the T_H value of -90 °C is lower than that of the corresponding solution which gives the T_H value of -100 °C. It is well known that the extrapolation of T_g values of an aqueous solution to zero solute concentration give the T_g value of ~ -135 °C. Therefore, T_g of a dilute aqueous solution is lower than that of a concentrated

aqueous solution. It must be noted that similar results are obtained for the PEG solutions giving the T_H values of -80 °C. From the results, we know that viscosity of the PEG solution increases with M and becomes a plateau value above $M = 1000$ although we do not have any experimental datum for the PEG solution of $M > 3000$.

The T_c / T_g ratio gives a measure of thermal stability of supercooled liquid just above T_g. In the cases of the PEG solutions of $T_H = -100$ °C, the T_c / T_g ratio ranges from 1.17 for the $M = 200$ solution to 1.10 for the $M = 3000$ solution. In addition, the ratio varies from 1.11 for the $M = 200$ solution to 1.10 for the $M = 3000$ solution in the cases of the PEG solutions of $T_H = -90$ °C. Therefore, there is a weak trend that aqueous PEG solutions of $T_H = -100$ °C is thermally more stable than that of the corresponding PEG solution of $T_H = -90$ °C. There is a clear trend that the T_c/T_g ratio is larger for the solution of smaller molecular weight than that of larger moleculer weight when we compare the data of the solutions of the same T_H value. This feature may be the indication that liquid sate of higher molecular weight is less disordered than that of smaller molecular weight.

It is important to test whether neat liquid PEG can be vitrified with the cooling rate of 1.8×10^3 °C/min, which we used for the vitrification of aqueous PEG solutions. Fig. 4 shows the results.
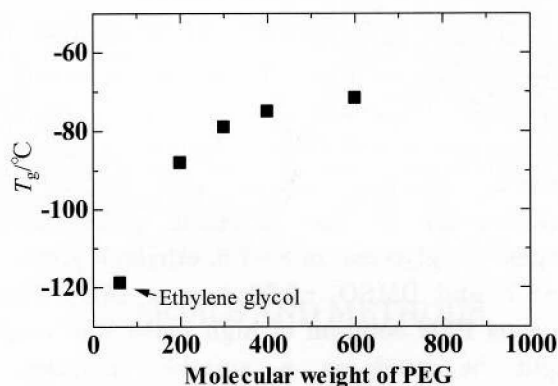

Fig. 4 Variation of T_g with molecular weight of PEG for the neat PEG liquids.

The significant point is that the PEG liquids of $M > 600$ are not glass-forming. It seems that the liquid state of neat PEG of $M > 600$ is not so disordered since high viscous liquid is usually considered to be glass-forming[9]. In this relation, Kimizuka et al[10] reported that viscosity, T_H, T_m, and k values show a trend change around $M = 600$

when we see them as a function of M. They ascribed the changes to the conformational changes of PEG molecules in water and we agree to the interpretation.

Taking up PEG of M = 1000 as a solute, we determined the glass-forming composition region for aqueous PEG (M = 1000) solution. The T_g results are shown in Fig. 5. The PEG (M = 1000) solution is shown to be glass-forming from m = ~0.74 to m = ~3.7 with the cooling rate of 1.8×10^3 °C/min.
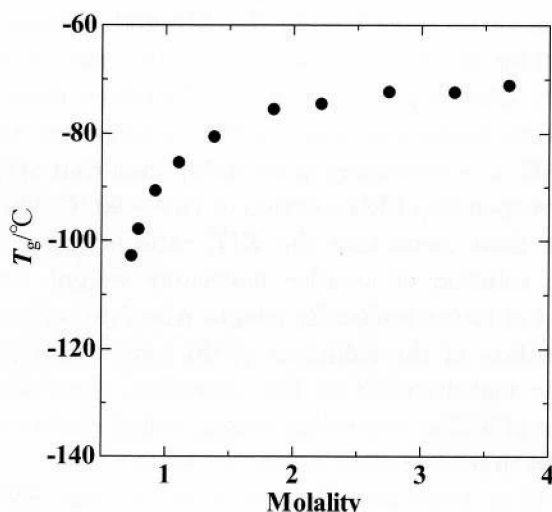

Fig. 5 Glass-forming composition region for aqueous PEG solution of M = 1000.

At this stage, it would be useful to compare the glass-forming abilities of aqueous solutions of biologically important solutes such as glycerol, ethylene glycol, and DMSO. Aqueous solutions of glycerol, ethylene glycol, and DMSO are glass-forming in the following concentration ranges[11,12]; glycerol: m > ~7.6, ethylene glycol: m > ~9.7, and DMSO: ~8.53 < m < ~51.2. Thus, aqueous PEG solution of high molecular weight might be used as a suitable additive to cryo-protective solution for glass formation.

REFERENCES

1) Franks, F., Biophysics and Biochemistry at Low Temperatures, Cambridge University, Cambridge (1985).

2) Angell, C. A. : Supercooled water, Ann. Rev. Phys. Chem. 34, 593 - 630 (1983).

3) Zobrist, B., Weers, U, and Koop, T.: Ice nucleation in aqueous solutions of poly(ethylene glycol) with different molar mass, J. Chem. Phys. 118, 10254 - 10261 (2003).

4) Kanno, H., Miyata, K., Tomizawa, K., and Tanaka, H.: Additivity rule holds in supercooling of aqueous solutions, J. Phys. Chem. B, 108, 6079 - 6082 (2004).

5) Rasmussen, D. H. and MacKenzie, A. P.: Effect of solute on ice-solution interfacial free energy; calculation from measured homogeneous nucleation temperatures. In "Water Structure at the Water Polymer Interface", Edited by Jellinek, H. H. G., Plenum Press, N. Y. p.126 - 145 (1972).

6) Oguni, M.and Angell, C. A.: Hyrophobic and hydrophilic solute effects on the homogeneous nucleation temperature of ice from aqueous solutions, J. Phys. Chem., 87, 1848 - 1851 (1983).

7) Angell, C. A.: Amorphous water, Annu. Rev. Phys. Chem., 55, 559 - 583 (2004).

8) Angell, C. A. and Sare, E. J.: Glass-forming composition regions and glass transition temperatures for aqueous electrolyte solutions, J. Chem. Phys. 52, 1058 - 1068 (1970).

9) Turnbull, D.: Under what conditions can a glass be formed, Contemp. Phys. 10, 473 - 482 (1969).

10) Kimizuka, N., Chotika, V. and Suzuki, T.: Ice nucleation and supercooling behavior of polymer aqueous solutions, Cryobiology, 56, 80 - 87(2008).

11) Rasmussen, D. H. and MacKenzie, A. P.: The glass transition in amorphous water; application of the measurements to problem arising in cryobiology, J. Phys. Chem. 75, 967 - 973 (1971).

12) Rasmussen, D. H. and MacKenzie, A. P.: Phase diagram for the system water-dimethylsulphoxide, Nature, 220, 1315 - 1317 (1968).

ポリエチレングリコール水溶液の過冷却とガラス化：梶原一人 [1]、北田祐馬 [1]、冨澤　清 [2]、菅野　等 [2] ([1] 東京工科大学応用生物学部、[2] 防衛大学校応用化学科) [キーワード：過冷却、ポリエチレングリコール、ガラス化、加成則、ガラス生成]