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Anomalous ice nucleation behavior in aqueous polyvinyl alcohol solutions

Abstract

The effect of polymers on the ice nucleation temperature (T_f) was studied in a W/O emulsion using ~ 5μm diameter droplets by differential scanning calorimetry (DSC). Four types of polymers were used. Among them, only polyvinyl alcohol (PVA) showed the additional effect of increasing the T_f of the aqueous solutions. This increase was logarithmic with the concentration of PVA and the difference in molecular weight did not have any significant effect on T_f for the same weight concentration. It was shown that the number of the structural unit (CH_2CHOH) was the key parameter for the increasing degree of T_f.

1. Introduction

Polyvinyl alcohol (PVA) is one of general polymers and has been widely used in many fields. The characteristic feature of PVA, which differs from other polymers, is well known to be the formation of physically crosslinked hydrogels under the proper conditions [1-5]. Because of its nontoxic property and good biocompatibility, this hydrogel has been expected to be used in various biomedical and pharmaceutical applications e.g., scaffolds for bioactive molecules and cell delivery, and the immobilization of cells or biopolymers [6-8]. In many cases, PVA physical gels were prepared by freeze/thaw treatments and their performance critically depends on the conditions. Therefore, the mechanism of PVA cryogelation has been largely studied to determine the normalized method for applications until recently [2, 7, 9, 10].

On the other hand, it was also reported that PVA molecules adsorbed on ice crystal surfaces and inhibited the increase in the ice grain size induced by recrystallization, which was not observed in aqueous polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP) solutions [11- 13]. These properties were similar to antifreeze proteins or antifreeze glycoproteins (AF(G)Ps), and therefore, PVA and PVA-derivatives were expected to be used as low cost substitutes for them in the fields of the cryopreservation of blood, tissues, and organs [11-16]. Thus, the cryogenic applications of PVA seem to have become more common, but the study of the ice nucleation behavior of aqueous PVA solutions, which was the primary phenomenon of freezing, has remained less developed than that of the other polymer solutions [17, 18]. According to Wowk et al., PVA seemed to inhibit the heterogeneous nucleation induced by *Pseudomonas syringae* or other organic nucleators, but many small ice grains remained at the same time [16, 19]. In addition, it was reported that the ice nucleation of an aqueous PVA solution often occurred at a temperature higher than that of the control solution [19-21]. There have been no remarks about them and the ice nucleation behavior of aqueous PVA solutions has not yet been clarified. In this study, we investigated their behavior using a W/O (water in oil) emulsion with about 5μm diameter droplets.

2. Experimental

This study tested five PVA samples with different molecular weights and three other types of polymers. These polymers were of commercial origin and used without further purification. Due to the surface-active property of the PVA with a low degree of saponification, which was considered to bring in a fundamental problem for the emulsification preparation, the higher one (>97.9mol%) were used in this study. Their characteristics are listed in Table 1. Aqueous polymer solutions were prepared by dissolving the solutes in distilled water at the bath temperature of 120^{o}C. Concentrations were determined by the weight ratio and mainly expressed by the molality or weight concentration. Low concentrations of the polymer solutions were prepared by adding a calculated amount of water to the highly concentrated ones.

The W/O emulsion were prepared using *n*-heptane (Wako Pure Chemical Industries, Ltd.) as the continuous phase and sorbitan tristearate (Span 65, Wako Pure Chemical Industries, Ltd.) as the emulsifier. The emulsification was carried out by mixing 10ml of an aqueous polymer solution, 20ml of *n*-heptane, and 500mg of Span 65 using a commercial homogenizer (NihonseikiCo., Ltd.) at about 12,000rpm for 6min. The droplets size distribution of the emulsion was determined by observation using a 500-power light microscope BX51 (OLYMPUS Co., Ltd.). When the size distribution was wide after mixing, the emulsion droplets were homogenized by a membrane emulsification technique using a 5μm SPG (Shirasu Porous Glass) membrane (SPG Technology Co., Ltd.). All ice nucleation temperatures (T_f) with the volume of 6μl were measured by a DSC-60 (Shimadzu Corporation) at the rate of -5^{o}C/min and determined as the onset point of the exothermic peak during the cooling process (Fig. 1). The ferroelectric phase transition of $(NH_4)_2SO_4$ at 223.1 K, melting of ice (T= 0^{o}C, 273K), and viscosity of pure water (0.905cP) were used for calibration in this study [22].

To obtain the melting point (T_m) of the aqueous PVA solutions, a time-temperature curve of cooling process was recorded by measuring the change in temperature of 2ml solutions in glass tubes with stirring at the cooling rate of about -0.7^{o}C/min. The T_m was determined by the Mouline method [23].

The viscosities of the aqueous PVA22 solutions were measured at 25^{o}C by viscometers (Kusano Scientific Instrument MFG Co., Ltd., Sibata Scientific Technology Co., Ltd.). The overlap concentration C* was estimated from the intrinsic viscosity value.

Table 1 Characteristic properties of polymers used in this study

Type of polymer	molecular weights	Saponifications[†]	Manufacturers	Abbr.
PVA	1700	98.7mol%	SIGMA-ALDRICH Japan K.K.	PVA1.7
	13200	98.3mol%	Japan VAM ＆ POVAL Co., Ltd.	PVA13
	17600 – 26400	98.0mol%	Wako Pure Chemical Industries, Ltd.	PVA22
	39600 – 48400	97.9mol%	Wako Pure Chemical Industries, Ltd.	PVA44
	89000 – 98000	99.7mol%	SIGMA-ALDRICH	PVA93
PEG	15000 – 25000	—	Wako Pure Chemical Industries, Ltd.	PEG20
PVP	35000	—	Wako Pure Chemical Industries, Ltd.	PVP35
Dextran	32000 – 45000	—	Wako Pure Chemical Industries, Ltd.	DX38

[†]: Their saponification were determined by ^{1}H-NMR in the ratio of methylene groups (-CH_2-) to acetyl groups (CH_3CO-).

3. Results & Discussion

Emulsification of aqueous solutions was one strategy which could confine both the limited number of contaminant particles and the surface nature of the droplets. This strategy makes the most of the emulsion droplets to avoid the opportunity of specific heterogeneous nucleation induced by them [17, 24]. The uniform size of the droplets was also required in order to measure the T_f. If the size of the droplets increased, then T_f would rise [25]. A linear relationship between the logarithm of the drop volume and the degree of supercooling (T_s = 273 - T) is represented by

$$\ln V = a - bT_s \qquad (1)$$

where V is the droplet volume and both a and b are constants for the specific water used for the sample. Thus, ice nucleation behaves in a volume-dependent manner and 3-10μm droplets size was often used for measuring T_f [17, 26, 27].

Fig. 2 shows the changes in the droplet size of the emulsion and T_f before and after membrane emulsification. As shown in Fig. 2, when the PVA22 concentration increased, the size of droplets without the membrane emulsification became notably bigger than that of the droplets with the membrane emulsification and then the higher T_f (symbols for before) was resulted from Eq. 1. It seems that because the viscosity of the aqueous PVA22 solutions dramatically increased from 1.0mmol/kg (data not shown), it was difficult to prepare uniformed sized droplets of the emulsions using a commercial homogenizer. The droplet size of the emulsion after the membrane emulsification could be very fine with a good homogeneity and T_f decreased with the PVA22 concentration (symbols for after) from that of (symbols for before). Thus the volume effect of the droplets was overcome by the membrane emulsification. Fig. 3 shows the ice nucleation behavior versus the concentration of the four types of aqueous polymer solutions in the W/O emulsion droplets. T_f was depicted as a scale of the logarithmic solute molality. The droplet size used for the measurement was not significantly varied among the four polymers. It was shown that T_f decreased by the addition of PEG20, PVP35 and DX38. The degree of supercooling increased with an increase in their polymer concentration. On the other hand, the ice nucleation behavior of the aqueous PVA22 solutions was different from that of the other (Fig.1, 3). Its T_f logarithmically rose with the concentration of PVA for a concentration less than 1.0mmol/kg, while it was reduced at the higher concentrated solutions. It always existed over the T_f of pure water in the entire concentration region from 0.015 to 11 mmol/kg. That is, it was indicated that the heterogeneous nucleation was always induced by the PVA molecule in aqueous PVA solutions.

Subsequently, the influence of the difference in the PVA molecular weight was examined. When the effect was based on the molality, it revealed that the effectiveness in nucleating ice increased with the increasing molecular weight (Fig.4a). However, once we compared them as a function of the logarithmic weight concentration, the effectiveness was not significantly different among the five types of PVA (Fig.4b). Therefore, it was concluded that the effect on the increasing T_f depends not on the PVA molecular weight, but the number of structural units (CH_2CHOH). An additivity rule could be observed for a much wider molecular weight difference.

In a previous study by Kanno et al., it was reported that a higher supercooling was obtained as the number of OH groups in a solute molecule increased [28]. It seems opposite to the effect induced by the addition of PVA. However, there is a basal difference between the two behaviors. Although the degree of increasing supercooling by the addition of methanol, ethylene glycol, glycerol, and sugars, was linear with the solute molality, the degree of reducing the supercooling by PVA logarithmically increased. However, a similar relation to Fig.4a was then observed in the case of an ice nucleating agent(INA) from *Pseudomonas syringae* [29]. It was reported that T_f of the aqueous INA monomers of *Pseudomonas syringae* solution logarithmically increased with the molecular weight similar to Fig. 4.

In the simple theory represented by Fletcher, the nucleation frequency J was given by [30],

$$J=K \exp(-\Delta G^*/kT) \qquad (2)$$

where K is a constant, ΔG^* is the energy barrier required for the formation of critical embryos, k is Boltzmann's constant and T is the absolute temperature. If the ice embryo with volume V_S and surface area A was formed, their free energy of formation could be represented as follows:

$$\Delta G^*=V_S\Delta G_V+\sigma_{SL}A_{SL}+(\sigma_{SP}-\sigma_{PL})A_{SP} \qquad (3)$$

where σ is the interfacial tension and the abbreviations of the subscript are as follows: P= ice nucleating particle, S= solid and L= liquid. A_{sp} denotes the total surface area of the ice embryo in contact with the ice nucleating agent. Because of this third term, the heterogeneous nucleation becomes more energetically-favored than the homogeneous nucleation. In other words, the relation of both nucleation frequencies was given as $J_{het} > J_{hom}$. J in equation (2) shows the nucleation frequency per unit volume. Here, the nucleation frequency J_{het} would be regarded as, the nucleation frequency per unit surface area, J_{sur}, and then it represented,

$$J_{het} = S\, J_{sur} \tag{4}$$

where S is the total surface area of ice nucleating agents. In this study, if the PVA coils worked as the ice nucleating agent and the surface area of their coil per one molecule was uniform, the S would be proportional to its concentration. The nucleation temperature would then logarithmically rise with the PVA concentration. The additivity rule for the increasing of T_f supports the idea that the PVA coils consisted of a structural unit (CH_2CHOH) that provides the nucleation site.

We estimated the overlap concentration C* by the reciprocal of the intrinsic viscosity [31, 32]. C* was considered as the boundary concentration at which an individual polymer coil starts to make contact with the surrounding ones. Below C*, it was considered that the PVA coils did not interact with each other, therefore, the number of PVA free coils proportionally increased with the increase in the PVA concentration. C* of the aqueous PVA22 solution was determined to be 1.0 mmol/kg in this work. If the PVA coils worked as the ice nucleating agent as mentioned above, the number of the ice nucleating agents would continue to increase until the PVA concentration reached C*, 1.0 mmol/kg, with enhancement of the ice nucleation probability due to the increase in J. Consequently T_f would rise. Above C*, T_f would not linearly rise with the logarithmic increase in their concentration because the activities of the ice nucleating agents would be reduced due to the start of the interchain interaction between the PVA coils for the rate of the heterogeneous nucleation depends on the S of the ice nucleating agent as seen in equation (4).

The reason why T_f stopped rising and subsequently started decreasing with the PVA concentration would be due to another factor. It is well known that the depth of the supercooling closely correlates with the T_m. The empirical relationship between T_f and T_m is represented by

$$\Delta T_f = \lambda \Delta T_m \tag{5}$$

where $\Delta T_f = T_{f,\ H2O} - T_f$, $\Delta T_m = T_{m,\ H2O} - T_m$ and λ is a constant. $T_{f,\ H2O}$ and T_f are the nucleation temperatures of pure water and aqueous solution, and $T_{m,\ H2O}$ and T_m are the melting points of pure water and the aqueous solution, respectively. Numerous researchers have reported that this equation is able to be used for polymers, low molecular alcohols, sugars, electrolytes and heterogeneous nucleation systems [17, 18, 24, 26-29, 33-35].

Fig.5 shows the relationship between T_f and T_m in aqueous PVA22 solutions. It was observed that the decrease in ΔT_f was about 4.3 times greater than that of ΔT_m above 3.0 mmol/kg. How many of the ice nucleating agent increase above 3.0 mmol/kg with the concentration increase could not be confirmed due to their interchain interaction, while the good agreement with curves T_f and T_m at λ = 4.3 indicated that the reduction of T_f would be mostly dependent on the decreasing T_m followed by equation (5).

Finally, we will discuss the reason why T_f increased in only the aqueous PVA solution. It was considered that an effective ice nucleating agent which could produce the heterogeneous nucleation was needed to be at least a 10nm particle [36]. As mentioned in the introduction, only PVA was known to form a physical crosslinked gel among polymers which were investigated in this study. The physical crosslinked gel was considered to be formed by the interchain or intrachain interaction with the hydrogen bonding [37]. Although a macroscopic gel could not be formed below the C*, the intrachain interaction was always present, resulting in the formation of a microgel with a hydrodynamic radius over 10nm at room temperature [38]. Such a minute gel could not be filtered from the aqueous solution by passing through a 0.20μm filter. Actually, it was confirmed that T_f of aqueous the PVA solutions did not significantly change with or without the filtration. Thus, it was considered that the microgel of PVA formed an apparent transparent solution and the heterogeneous nucleation would be induced by them.

In conclusion, we discovered the anomalous ice nucleation behavior of an aqueous PVA solution in which T_f logarithmically rises with the increase in the PVA concentration. We also found that the structural unit (CH_2CHOH) was a key for the extent of the ice nucleation temperature elevation. It is assumed that the anomalous behavior would be induced by the microgels formed by the PVA molecule which works as a nucleating agent. The PVA property as an ice nucleating agent would affect various cryogenic applications such as cryogelation technology for biomedicine and pharmacy, the cryopreservation of blood, tissues, and organs, and frozen foods.

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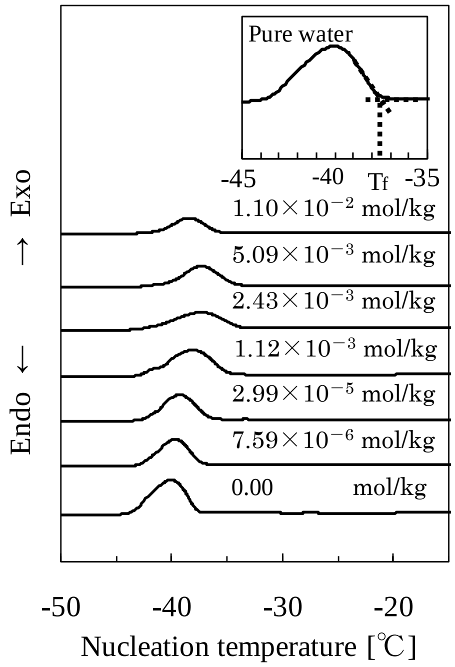

Fig.1 DSC curves for PVA emulsion. The exothermic peaks result from the ice nucleation in aqueous PVA solutions. The inset shows that the onset point of the exothermic peak is defined as the ice nucleation temperature T_f.

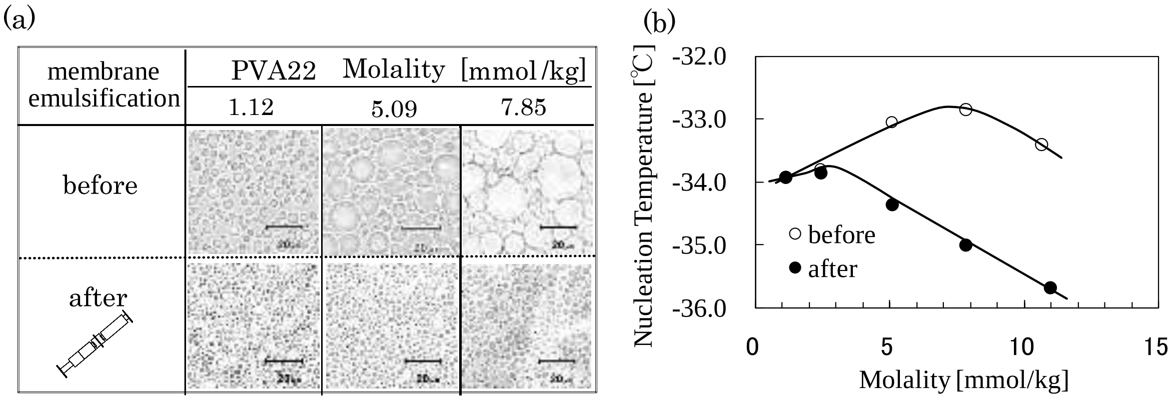

Fig.2 The effect of membrane emulsification on the droplet size in the PVA emulsion and their size on T_f of the aqueous PVA solutions. Symbols of before and after show before and after the membrane emulsification respectively. (a) Optical microscopic observation. (b) T_f vs. Molality of aqueous PVA22 solutions.

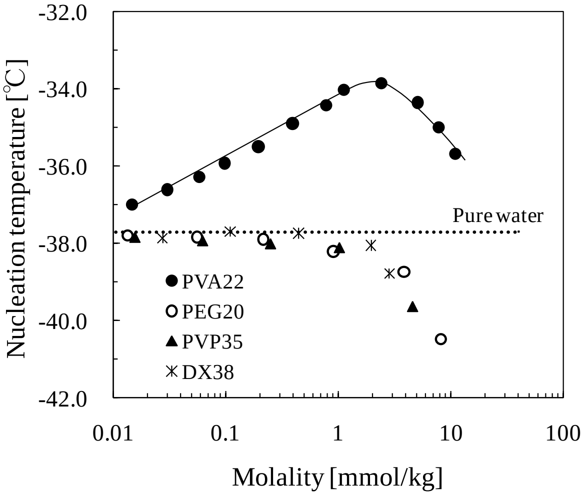

Fig.3 The difference of T_f among the four types of polymers. The dotted line shows T_f of pure water.

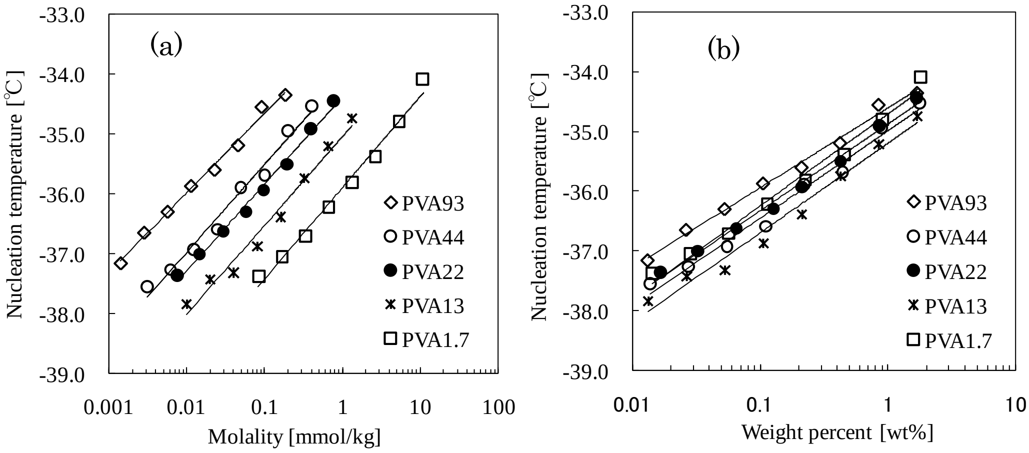

Fig.4 The effect of molecular weight of PVA on T_f. (a) T_f vs. logarithmic molality of aqueous PVA solutions. (b) T_f vs. weight% of aqueous PVA solutions.

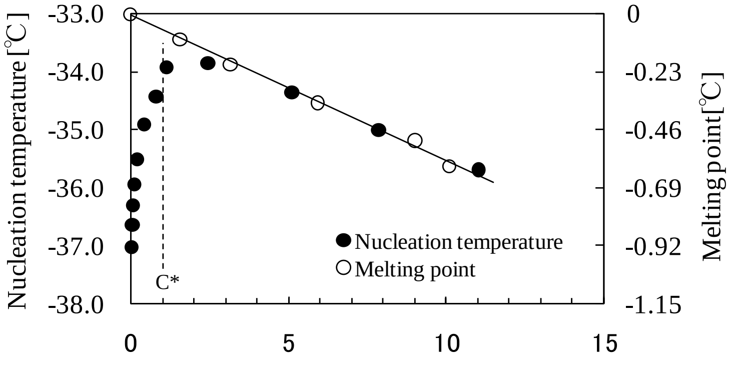

Fig.5 The relationship between T_f and T_m on aqueous PVA22 solutions. The dotted line shows the concentration of C*.