High Frequency Spectroscopic Study of the Bound State of Water in PEG-H₂O System during Heating from Frozen State

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The bound state of water in PEG (polyethylene glycol)-H₂O systems was studied by using HFS and DSC methods. Samples with the mixing ratios EO:H₂O = 1:1 (a) and 1:10 (b), where EO indicates the ethylene oxide units in PEG chain, were heated from frozen state to melt state. Sample (a) gave an HFS peak corresponding to the weakly bound water, while sample (b) gave the peaks of both the bound and free water. DSC measurements gave quite similar patterns to those of HFS measurements, but with additional peaks corresponding to cold crystallization. The difference between the two measurements was reasonably interpreted by considering the origin of the cold crystallization.

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Introduction

Polyethylene glycol (PEG) is a highly hydrophilic and viscous material, which is widely used for rubber molding, lubricants in metal processing, cosmetics, matrices for medical reagents, and other purposes. It is also used as a biocompatible material to suppress the adhesion of biomaterials, such as proteins, by spreading onto the surface of artificial organs.¹⁻⁴ The suppression is considered to arise from the formation of a surface diffusion layer due to the hydration of PEG on the surface of organs. Because of this usefulness, the PEG-H₂O mixed system has been investigated in various fields of research.⁵⁻¹⁰ Results of these studies show that the water involved in PEG can roughly be divided into two kinds of water: free water and bound water. Furthermore, it is reported that the bound water is divided into the non-freezable bound water and the freezable bound water. Besides PEG, poly(2-methoxyethylacrylate) (PMEA), for instance, is known to have the similar effects, where the adsorbed protein is not so easily denatured.¹¹ The hydrated layer on PMEA surface is also effective in suppressing the adhesion of proteins. In a DSC study, a large exothermic peak due to the so-called cold crystallization is observed reproducibly in heating process, within a wide range of water content.¹² The water molecules showing the cold crystallization are considered to be weakly bound to high polymer chains, in the intermediate strength range between non-freezable water and free water. The phenomenon of cold crystallization is characteristic of biocompatible materials such as PEG, polysaccharides and gelatins, and is considered as the key point of achieving biocompatibility. Consequently, the study of the state changes of water at low temperatures is important in developing the biocompatible materials and/or in understanding the mechanisms working in them.

We have proposed a new spectroscopic method, high frequency spectroscopy (HFS), which can detect the change of permittivity

as the shift of resonance frequency of the given sample system.¹³ The resonance frequency of HFS is given as the equation:

$$f_R = \frac{A}{\sqrt{\epsilon_r}}$$

where f_R is the resonance frequency, ϵ_r the permittivity of the sample and A the circuit constant of the measuring system. Therefore, the change of permittivity can be detected as the change of f_R in HFS.

Using HFS, researchers have studied the changes of clustering structure between water and organic molecules in water-alcohol systems, where the characteristic and discontinuous changes of resonance frequencies have been observed.¹⁴ Various changes in solution structure have been reported in the measurements of X-ray diffraction,¹⁵ mass analysis,^{16,17} NMR,¹⁸⁻²⁰ Raman scattering,²¹ and time-domain reflectometry (TDR).²² The results obtained by these measurements are basically in good agreement with those obtained by HFS study. Consequently, HFS is useful for studying the changes of bound condition of water in the polymer-water mixtures. This study has been suggested in a previous study of the state analysis of water in polyethylene polyol, which is known as a starting material for producing polyurethane foam.²³

The permittivity of water is greatly decreased when water comes to frozen state. The permittivity of liquid water is around 80, while that of ice is about 4. This decreasing is detected as a shift to the higher frequency side in HFS measurements. A change of the bound state of water will be detected as a change of permittivity by a series of measurements in freeze-melt process under temperature control.

HFS has an advantage in the sense that it allows one to measure various kinds of sample specimens, provided the sample can be placed between the electrode plates. In addition, it will allow the *in situ* measurement of biological samples, which cannot be conducted by DSC method.

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Fig. 1 High frequency spectra of water at -150°C and at 0°C .

Experimental

Reagents and preparation

PEG reagent having an average molecular weight of 10000 (Wako Pure Chemical Industries, Ltd.) was used without further purification. To about 10 g of PEG, water was added so as to have the molar ratio $\text{EO}:\text{H}_2\text{O} = 1:1 - 1:10$ (mole fraction of water (X_w) is 0.50 to 0.91), where the $\text{EO}:\text{H}_2\text{O}$ ratio means that of one unit of ethylene oxide to a number of H_2O molecules. The water used was the Milli-Q Academic A-10 (Millipore Ltd.) grade with the specific resistance $18.2 \text{ M}\Omega \text{ cm}^{-1}$. Since the PEG in crystalline state is not so hydrophilic and is slow in the hydration process, the PEG ($M_w = 10000$)- H_2O mixed specimen was first kept at 70°C , at somewhat higher temperature than its melting point for more than a week to proceed hydration. After this the specimen was kept at room temperature for more than a week and then was used in experiments.

Measurements

About a 5-mL volume of PEG- H_2O mixture was put in a sample holder, into which the measuring electrode was inserted. Then liquid nitrogen was poured around the specimen to cool it quickly down to -150°C , to a frozen state. Then the HFS measurements were made in the heating process with the rate $0.8^{\circ}\text{C}/\text{min}$ average. During the measurement, the temperature was monitored by a fluorescence type thermometer (Anritsu Meter Co. Ltd., FL-2000), which uses the change of the fluorescence lifetime due to the change of temperature. The sensor part of it is made of glass fiber. It was confirmed that the setting of the sensor close to the measuring electrode gave no harmful effect to the measuring of HFS spectra. It was also confirmed that this thermometer is free from any harmful electric interaction. DSC measurements were made with a Thermoplus 2 (Rigaku, DSC 8240D) with the attachment of a cooling unit. The range of measuring temperature was $-100 - 50^{\circ}\text{C}$, with a heating rate of $1^{\circ}\text{C}/\text{min}$.

Results and Discussion

The HFS spectra of pure water in a frozen state (-150°C) and in liquid state (0°C) are shown in Fig. 1. The spectrum at -150°C

Fig. 2 Resonance frequency vs. temperature for pure water sample.

Fig. 3 Plots of $df(T)/dT$ against temperature for water and 10 wt% NaCl aqueous solution.

shows a broad peak at around 1800 MHz, while that at 0°C shows a sharp peak at 460 MHz. Since the water molecules in frozen state do not easily reorient with the change of applied electric field, that the permittivity of water is greatly reduced to near 4. On the other hand, water molecules in liquid state can easily reorient in accord to the change of electric field, and they bring about the rapid increase of permittivity.

Figure 2 shows the $f(T)$ curve, which shows the change of resonance frequency as a function of temperature T . As seen in the figure, the frequency remains constant from -150 to near -1°C , while it shows sudden decrease of frequency as much as about 1300 MHz between $-1^{\circ}\text{C} < T < 1^{\circ}\text{C}$.

Figure 3 shows the derivative curve $df(T)/dT$ of the function $f(T)$. For reference, the similar derivative curve for NaCl solution is shown. In the curve for the water sample, a sharp peak near 0°C is observed, while in that of the NaCl solution a peak is observed around at -22°C , which agrees with the eutectic point known in the H_2O -NaCl system.

The HFS spectra of PEG- H_2O mixed system ($X_w = 0.50$) measured at -150°C and at 0°C are shown in Fig. 4. A broad resonance peak is observed around at 1600 MHz for the -150°C

Fig. 4 High frequency spectra for the PEG-H₂O mixture having molar ratios EO:H₂O = 1:1 ($X_w = 0.5$).

Fig. 5 Plots of frequency vs. temperature for PEG-H₂O mixture having mole fractions (X_w) 0.50 and 0.91.

curve. This shows that the permittivity of the sample specimen is decreased down to around $\epsilon_r = 6 - 7$, which is somewhat higher than that of PEG ($\epsilon_r = 2 - 3$) or ice ($\epsilon_r \approx 4$). As seen in Fig. 1, the completely frozen water gives the peak at around 1800 MHz, which corresponds to the permittivity of around 4. These suggest that a part of the water molecules in the sample are not completely frozen, but exist as non-freezable water molecules and/or amorphous ice. These show good agreement with the study of Bogdanov *et al.*, who reported that this system tends to supercool and become partially or completely glassy state by cooling.²⁴ On the contrary, by the melting of the frozen water the resonance peak shifts to around 1050 MHz showing a sudden increase of permittivity. The frequency shift Δf in this change is about 550 MHz.

The frequency change Δf as a function of temperature T for the PEG-H₂O mixed system is shown in Fig. 5. Two curves (a) and (b) correspond to the samples with $X_w = 0.50$ and $X_w = 0.91$, respectively. The curve (a) does not show any frequency change up to -40°C , while a shift to lower frequencies are observed with the increase of temperature between -40 and -10°C . At higher temperatures no big change of frequency is

Fig. 6 The $df(T)/dT$ curves of the PEG-H₂O mixed samples. The curves (a) and (b) show the results of $X_w = 0.50$ and $X_w = 0.91$, respectively.

observed. These observations suggest that the change of permittivity occurs in one step due to the melt of bound water in the sample. On the contrary, the curve (b) shows two step changes in temperature ranges $-20^\circ\text{C} < T < -10^\circ\text{C}$ and $-10^\circ\text{C} < T < 0^\circ\text{C}$. The change observed in lower temperature region is considered as coming from the melting of bound water as well as for the sample with $X_w = 0.50$. On the contrary, the change observed in higher temperature region is continuously observed up to the melting point of ice, suggesting that it is due to the melting of free water.

Figure 6 shows the derivative curve $df(T)/dT$ for the PEG-H₂O mixed sample. The curves (a) and (b) correspond to those of Fig. 5. For the sample with $X_w = 0.50$, a peak is observed at -19°C . This peak shows that the melting of water occurs as a single process in this sample. Further, the temperature of this process corresponds to the melting of the bound water, bound strongly to PEG. On the other hand, for the sample with $X_w = 0.91$, two sharp peaks are observed: at -14°C and at -2°C . The peak at -14°C is considered to come from the same type of origin as the peak in the curve (a). This is the melting of bound water which is strongly bound to PEG. The peak at -2°C corresponds to free water and melted water, which are not bound to PEG.

In Fig. 7 the DSC curves for the same samples as in Fig. 6 are shown. The curves (a) and (b) correspond to the samples of $X_w = 0.50$ and of $X_w = 0.91$, respectively. For the sample of $X_w = 0.50$, an endothermic peak is observed at around -14°C , which is assigned to the melting peak of bound water. For the sample $X_w = 0.91$, two peaks are observed at around -14°C and at 0°C . These peaks can be assigned to the bound water and free water, respectively. Both of the samples show exothermic peaks around -60°C with similar intensity. These are assigned to the cold crystallization* of water. Both the $df(T)/dT$ curve

* If the bound water molecules in a high polymer are cooled down rapidly, they are solidified in amorphous state and the thermal motion of water stops completely. With the increase of temperature above T_g , the transition from amorphous state to crystalline state begins, and a release of heat energy with the amount of crystallization energy is observed. This phenomenon is called cold crystallization, and it can be observed as an endothermic peak in DSC measurements.

Fig. 7 DSC curves of the sample of mole fractions (X_w) 0.50 and 0.91.

(Fig. 6) and DSC curves are considered in good agreement except for the peaks of the cold crystallization and melting peak of $X_w = 0.50$. The reason why the cold crystallization is not observed in HFS measurement can be explained as follows: the water molecules which are frozen in amorphous state do not easily reorient with the change of electric field, giving a small value of permittivity like that of crystalline state. Consequently, the HFS peaks shifted to high frequency side. When the amorphous state of water changes into crystalline state with the increase of temperature, a small amount of energy is released and thus a DSC peak is observed. On the contrary, the HFS peaks are not explicitly observed due to the negligible change of water mobility. The reason why the temperatures of melting peaks differ in HFS and DSC measurements would be explained as follows: 1) The measuring method of temperature in DSC is not exactly the same as in HFS, in which the temperature of the specimen is directly measured; 2) temperature distribution can occur in the specimen because a larger amount of sample is required in HFS than in DSC.

In Fig. 8, schematic images of the state change of water in PEG-H₂O mixed system due to temperature changes are shown. At sample temperatures less than glass transition temperature T_g (a), the free waters are crystallized in ice state. The water molecules bound to PEG are in glassy state and their thermal motion is frozen. The results of DSC²⁴ and dielectric measurements^{25,26} suggest that the PEG-H₂O mixed system has a tendency of supercooling to form a partial and/or complete amorphous phase. In the temperature range of (b), $T_g < T < T_{ml}$, where T_{ml} is melting temperature of bound water, the PEG chain is allowed to move to some extent, and consequently the water molecules which are bound to the PEG chain are also allowed to move. Further, the water molecules which are frozen in amorphous state are induced to crystallize. This is considered as cold crystallization. Hatakeyama *et al.* reported from the standpoint of enthalpy balance of freezing and melting processes that cold crystallization is attributable to the molecular rearrangement of PEG molecules associated with amorphous ice.²⁷ This is in good agreement with our results. In the range of (c), $T_{ml} < T < T_{mh}$, where T_{mh} is melting temperature of free water, the bound water in frozen state begins to melt. In this range, the PEG chain and H₂O molecules begin to move freely so that the reorientation of water molecules is active, resulting in the rapid increase of permittivity. In the temperature range of

Fig. 8 Images of the state change of PEG-H₂O mixed system caused by the temperature.

$T_{mh} < T$, which is not given in Fig. 8, the free water molecules in frozen state turn into molten state, and show a big increase of permittivity.

The so far presented experimental results of HFS method show the possibility of detecting the state changes of water molecules in macromolecule-H₂O mixed system. Further, the $d\epsilon(T)/dT$ curve derived from HFS measurement agrees well with the DSC curve, except for the signal arising from the cold crystallization. In the research of the cross-linked dextrin gel, it has been reported that a cold crystallization is observed when the gel has suitable pore size with H₂O under a special environment.^{28,29} A similar mechanism would be applicable to PEG-H₂O systems. This is informative in the point of biocompatibility.

The HFS method, compared with DSC method, has the advantages of 1) not needing a reference sample; 2) allowing the measurement without sample holder, provided the electrode can be inserted in the sample solution. This method could be expanded to *in situ*, *in vitro*, and *in vivo* measurements in the field of preservation of organs and in the medical recovery.

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