

CHARACTERIZATION OF WATER IN POLYSACCHARIDE HYDROGELS BY DSC

H. Yoshida^{1}, T. Hatakeyama² and H. Hatakeyama³*

¹DEPARTMENT OF INDUSTRIAL CHEMISTRY, TOKYO METROPOLITAN UNIVERSITY,
MINAMI-OHSAWA, HACHIOJI, TOKYO 192-03, JAPAN

²RESEARCH INSTITUTE FOR POLYMERS AND TEXTILES, TSUKUBA 305, JAPAN

³INDUSTRIAL PRODUCTS RESEARCH INSTITUTE, TSUKUBA 305, JAPAN

Water molecules in hydrogels were classified into three categories according to phase transition behavior; non-freezing, freezing bound and free water. Melting, crystallization, and glass transition of water in hydrogels reflected the state of the water interacting with polysaccharides. Freezing bound water formed metastable ice by slow cooling and formed amorphous ice by quenching. From the isothermal crystallization measurement, nucleation rate and crystal growth rate were obtained. The crystal growth rate of freezing bound water was about ten times slower than that of free water. The DSC characterization of water in hydrogels was summarized.

Keywords: characterization of water, DSC, polysaccharide hydrogels

Introduction

Polysaccharide hydrogels have received considerable interest as intelligent materials, such as artificial tissues and drug delivery systems [1]. The functional properties and higher-order structure of hydrogels are influenced by the hydration state of polysaccharides [2]. The characterization of water is important in clarifying the role of water in hydrogels from the aspects of functionality and structure. The state of water in various polymers, including polymers in aqueous solutions, have been studied using thermal, dielectrical and spectroscopic analysis [3-7].

We have reported the nature and the phase transition behavior of water in water-hydrophilic polymers [8, 9], water-polyelectrolytes [10] and water-polysaccharide systems [11-13] by differential scanning calorimetry (DSC) and a

* To whom correspondence and proofs should be sent.

nuclear magnetic resonance spectroscopy (NMR). Water in the above systems is classified into three categories; such as non-freezing, freezing bound and free water from the view point of phase transition and molecular mobility [10–12]. The molecular mobility and molecular coagulation of the above systems are influenced by both non-freezing water and freezing bound water which are restrained by polymers [13, 14]. In the case of the water-hydrophilic polymer systems, the amount of freezing bound water is calculated by the heat of crystallization observed in the cooling process, since the crystallization exothermic peak of freezing bound water is observed separately from that of free water. For the water-polyelectrolytes and the water-polysaccharide systems, however, it is difficult to determine the amount of freezing bound water in the cooling process as freezing bound water and free water crystallize continuously. We have reported that the crystal growth rate of freezing bound water was slower than that of free water [15, 16].

In this paper, the DSC characterization of water in polysaccharide hydrogels was summarized based on the difference in crystallization behaviour between free and freezing bound water.

Experimental

Sample

Polysaccharide samples used in this experiment were sodium salts of xanthan, supplied from Kelco Co. Ltd., U.S.A., and of hyaluronic acid, supplied from Kibun Food Chemicals Co. Ltd., Japan. The sample purification and preparation were carried out as previously reported [12, 13]. Samples weighing 0.5–2 mg were dissolved in water in aluminum DSC pans used for liquid samples. After obtaining a homogeneous solution, excess water was allowed to evaporate slowly at room temperature in order to control the water content. Water content (W_c) was defined as follows.

$$W_c = (\text{grams of water})/(\text{grams of dry sample}) \text{ (g/g)}$$

Dry sample weight was determined by weighing after heating to 450 K, at which temperature observation by thermogravimetry/differential thermal analysis (TG/DTA) showed that the water had evaporated completely.

Apparatus

Transition temperatures and heat of transition were obtained using a Seiko DSC200 differential scanning calorimeter equipped with a cooling apparatus controlled by a Seiko SSC5000 Thermal Analysis System. Pure water and tin were used as standard materials for temperature and enthalpy calibration. Samples

were cooled from 303 K to 153 K at $10 \text{ deg}\cdot\text{min}^{-1}$ and then heated to 313 K at $10 \text{ deg}\cdot\text{min}^{-1}$. The melting peak temperature (T_{mp}) and the starting temperature of melting (T_{mi}) were used as melting temperature [17]. The onset temperature of glass transition was determined from DSC heating curves [18].

Isothermal crystallization of water was measured using a Perkin Elmer DSC II equipped with cooling apparatus. Samples were maintained at 307 K for 5 mins and quenched to the predetermined crystallization temperatures (T_c). After measuring isothermal crystallization of water, the sample was cooled to 10 K lower than T_c and heated to 307 K at $10 \text{ deg}\cdot\text{min}^{-1}$.

Results and discussion

Quantitative analysis

DSC cooling and heating curves of hyaluronic acid hydrogels with different W_c were shown in Fig. 1. Hard gels with W_c lower than 0.5 (A) shows only glass

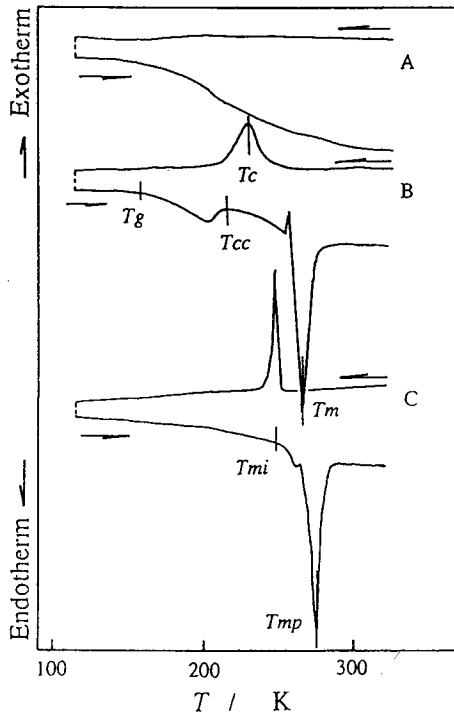


Fig. 1 DSC cooling and heating curves of hyaluronic acid hydrogels with $W_c = 0.4$ (A), 1.2 (B) and 14 (C)

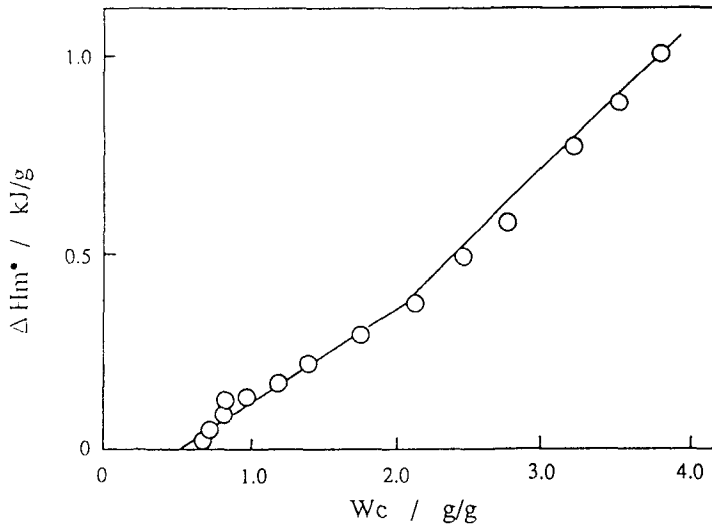


Fig. 2 Relationship between observed heat of fusion normalized by dry sample weight and W_c for hyaluronic acid hydrogels

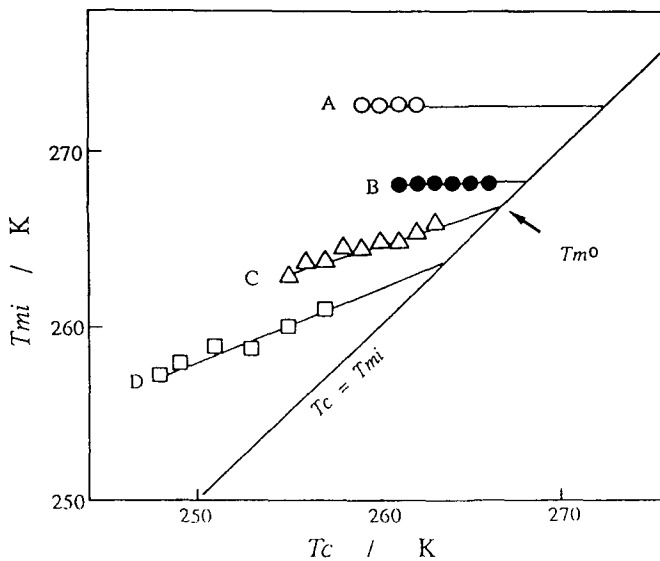


Fig. 3 Relationship between T_{mi} and T_c for pure water (A) and hyaluronic acid hydrogels with $W_c = 14$ (B), 2.1 (C) and 1.2 (D)

transition on DSC cooling and heating curves. Water molecules are present in a form of non-freezing water in this system. Hyaluronic acid hydrogel with W_c about 1 (B) shows a broad crystallization exothermic peak on DSC cooling curve, and glass transition, cold crystallization of water, and melting of ice on DSC heating curve. The hyaluronic acid hydrogel with W_c ranging from 0.5 to 1.8 contains non-freezing bound water and freezing bound water. Soft gels with W_c above 2 (C) shows a melting peak of ice on DSC heating curve, although the system contains non-freezing, freezing bound and free water.

The total amount of freezing water in hydrogels was calculated from the area of endothermic melting peak and the standard heat of melting of water. In order to determine the standard heat of melting of water, DSC heating curves were obtained for the systems with various W_c s. The heat of melting of water obtained from DSC heating curves, which were normalized by the weight of dry polysaccharide, was denoted as δH_m^* . The obtained δH_m^* of hyaluronic acid hydrogels is plotted against W_c in Fig. 2. The line in Fig. 2, shows the inflection point at $W_c = 2.0$. The slope of the line below 2.0 was 278.4 J/g, while the slope above $W_c = 1.0$ was 312.2 J/g. The above facts suggested that the heat of melting of water in the hyaluronic acid system depends on W_c . In the case of xanthan which is a rigid polysaccharide molecule, the value of 333.1 J/g is obtained as the standard heat of melting of water in all W_c regions [19]. The difference between hyaluronic acid and xanthan suggested that molecular flexibility of polysaccharide chain affects the structural change of water.

The freezing water content (W_f) and the non-freezing water content (W_{nf}) are defined as follows:

$$W_{nf} = W_c - W_f$$

The changes of W_f and W_{nf} with W_c for hyaluronic acid (A) and xanthan were reported [12, 14]. Freezing water appeared in samples with W_c above 0.5 for both hyaluronic acid and xanthan. In the case of hyaluronic acid, the change of W_{nf} showed a maximum at $W_c = 0.5$ due to the overlapping of hydration shell [20].

Transition temperatures

The changes of phase transition temperatures with W_c for hyaluronic acid and xanthan are reported elsewhere [12, 14]. T_{mp} of ice restrained in hydrogels is lower than that of pure water. T_{mp} of both samples approached that of pure water with increasing W_c . The value of W_c at which T_{mp} shows a constant value depended on the type of polysaccharide. Wide angle X-ray diffraction analysis of ice in hydrogels showed that freezing bound water formed cubic ice and free water formed hexagonal ice [21]. Similar ice was observed in poly(2-hydroxyethylmethacrylate)hydrogel [22]. Hydrogels contained freezing bound water and free water formed mixed crystal of hexagonal and cubic ice [21].

The relationship between T_{mi} and T_c for hyaluronic acid hydrogels with various W_c 's is shown in Fig. 3. The values of T_{mi} for free water and pure water

are constant regardless of T_c . However, T_{mi} of freezing bound water depends on T_c . The linear relationship between T_{mi} and T_c for freezing bound water was observed as shown in Fig. 3. The slope of the linear relationship between T_{mi} and T_c ($k = dT_{mi}/dT_c$) changes from 0 to 1 depending on crystal size and dimension of crystal growth [23]. For xanthan hydrogel, the k value decreased with increasing W_c from 0.6 to 0 [19]. These facts suggest that cubic ice from freezing bound water is metastable. The temperature at which the extrapolation of the linear relationship between T_{mi} and T_c intersects the line showing $T_c = T_m$ is defined as the equilibrium melting temperature (T_m°). (T_m°) is the thermodynamic value which is independent of the thermal history of sample.

The glass transition behavior of hydrogels is separated into four regions [13]. In the first region, in which the system contains only non-freezing bound water, T_g of the system decreases marked as the result of plasticization of water. The decrease of T_g of hydrogel is observed even if freezing bound water exists in the system in the second region. Freezing bound water formed amorphous ice by cooling at $10 \text{ deg}\cdot\text{min}^{-1}$ in the second region. The main chain motion of the polysaccharide molecules absorbed non-freezing water was plasticized by the amorphous ice in this region. T_g increases with increasing W_c after passing the minimum value because of the presence of regular ice which formed when the system in the third region was cooled. The glass transition of the system appeared as the result of the cooperative motion of polysaccharide, non-freezing water and amorphous ice in the second and third regions. In the fourth region where free water exists, T_g of the system approaches a constant value irrespective of the type of polysaccharide.

From the viewpoint of DSC characterization of water in hydrogels, the crystallization process of freezing bound water differed from that of free water. As the rate of crystallization of freezing bound water is about ten times slower than that of free water [16], the freezing bound water in hydrogels forms amorphous ice by quenching and forms metastable ice by slow cooling whereas free water in hydrogels forms the stable hexagonal ice.

References

- 1 J. D. Andrade, 'Hydrogels for Medical and Related Applications', ACS Symp. Ser. 31, Washington DC: American Chemical Society, 1976.
- 2 H. Yoshida and Y. Miura, *J. Memb. Sci.*, 68 (1992) 1.
- 3 K. Nishinari, *J. Soc. Rheol. Jpn*, 17 (1989) 100.
- 4 K. A. Mauritz and R. M. Fu, *Macromolecules*, 21 (1988) 1324.
- 5 W. B. Wise and P. E. Pfeffer, *Macromolecules*, 20 (1987) 1550.
- 6 F. X. Quinn, E. Kampff, G. Smith and M. McBrierty, *Macromolecules*, 21 (1988) 3192.
- 7 H. Uedaira, *Hyomen*, 13 (1975) 297.
- 8 K. Nakamura, T. Hatakeyama and H. Hatakeyama, *Polymer*, 22 (1981) 473.
- 9 K. Nakamura, T. Hatakeyama and H. Hatakeyama, *Tex. Res. J.*, 51 (1981) 607.
- 10 T. Hatakeyama, K. Nakamura, H. Yoshida and H. Hatakeyama, *Thermochim Acta*, 88 (1985) 223.

- 11 T. Hatakeyama, H. Hatakeyama and H. Hatakeyama, *Polymer*, 28 (1987) 1282.
- 12 H. Yoshida, T. Hatakeyama and H. Hatakeyama, *Polymer*, 31 (1990) 693.
- 13 H. Yoshida, T. Hatakeyama and H. Hatakeyama, *ACS Symposium Series* 489 (1992) 217.
- 14 H. Yoshida, T. Hatakeyama and H. Hatakeyama, *Koubunshi Ronbunshu*, 46 (1989) 597.
- 15 T. Hatakeyama, H. Yoshida and H. Hatakeyama, 'Physics and Chemistry of Ice' Ed. by N. Maeno, Hokkaidou Univ. Press, Sapporo 1992, p. 262.
- 16 H. Yoshida, T. Hatakeyama and H. Hatakeyama, 'Physics and Chemistry of Ice' Ed. by N. Maeno, Hokkaidou Univ. Press, Sapporo 1992, p. 284.
- 17 S. Nakamura, M. Todoki, K. Nakamura and H. Kanetsuna, *Thermochim Acta*, 136 (1988) 363.
- 18 T. Hatakeyama and H. Kanetsuna, *Thermochim Acta*, 138 (1989) 327.
- 19 H. Yoshida, T. Hatakeyama and H. Hatakeyama, *J. Intelligent Mat. Sci.*, to be published.
- 20 R. Kjellander and E. Florin, *J. Chem. Soc., Faraday Trans.*, 77 (1981) 1.
- 21 H. Yoshida, T. Hatakeyama and H. Hatakeyama, *Polymer Preprints Japan*, 41 (1992).
- 22 L. Bosio, G. P. Johari, M. Oumezzine and J. Teixeira, *Chem. Phys. Lett.*, 188 (1992) 113.
- 23 N. Okui, *Polymer Bulletin*, 23 (1990) 111.

Zusammenfassung — In Übereinstimmung mit dem Phasenumwandlungsverhalten werden Wassermoleküle in Hydrogelen in drei Kategorien eingestuft; nicht gefrierendes, gefrierendes gebundenes und freies Wasser. Das Schmelzen, die Kristallisation und die Glasumwandlung von Wasser in Hydrogelen widerspiegeln den Zustand von Wasser, welches in Wechselwirkung mit Polysacchariden tritt. Gefrierendes gebundenes Wasser bildet metastabiles Eis durch langsames Abkühlen und amorphes Eis durch Abschrecken. Anhand isothermer Kristallisationsmessungen wurden Keimbildungsgeschwindigkeit und Kristallwachstumsgeschwindigkeit erhalten. Die Kristallwachstumsgeschwindigkeit von gefrierendem gebundenem Wasser liegt zehnmal niedriger als die von freiem Wasser.