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Phenomenological theory describing the behaviour of non-freezing water in structure formation process of polysaccharide aqueous solutions

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Abstract

In this study, the experimental results on annealing and subsequent cooling induced gelation obtained by the falling ball method (FBM) and differential scanning calorimetry (DSC) have been summarized. By FBM measurements, it has become apparent that the gel–sol transition temperature, T_{g-s}, increases by the annealing of the solution in the sol state. By DSC measurements, the anomalous behaviour of non-freezing water was observed in the annealing process of the solution in the sol state, i.e. the amount of non-freezing water changed oscillationally in the initial stage of annealing and then approached a constant value. Such an observation suggested that the structure that induces gelation is formed by the homogenization of the system. It is thought that the homogenization of the system is caused by the diffusion of polysaccharide chains removed from polysaccharide molecule assemblies by the dissociation. The phenomenological equation was derived in order to describe the behaviour of non-freezing water taking into account the effect of homogenization of the system assuming that the adsorption and desorption of water molecules on polysaccharide chains takes place. It was found that a large amount of water is adsorbed in junction zones composed of polysaccharide chain assemblies. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Xanthan; Hyaluronic acid; Differential scanning calorimetry; Gelation; Sol–gel transition; Non freezing water

1. Introduction

It is known that various kinds of polysaccharide electrolytes form hydrogels when their aqueous solutions are maintained at a temperature lower than the gel–sol transition temperature (T_{g-s}) (Dickinson, 1991; Nishinari & Doi, 1994). However, it is known that a group of polysaccharide electrolytes, such as xanthan gum (XA) and hyaluronic acid (HA) do not readily form hydrogels by the usual gelation method (Cuveir & Launary, 1986; De Smedt, Deckeyser, Ribitsch, Lauers & Demeester, 1993; Milas, Rinaudo, Knipper & Shuppiser, 1990; Nishinari & Sen-i To Kogyo, 1984; Richardson & Ross-Murphy, 1987; Ross-Murphy, Morris & Morris, 1983; Scott, Cummings, Brass & Chen, 1991; Williams, Clegg, Day, Nishinari & Phillips, 1991; Williams, Day, Nishinari & Phillips, 1991; Williams, Annable, Phillips & Nishinari, 1994; Yanaki & Yamaguchi, 1990). In particular, XA has been considered as a non-gelling polysaccharide in aqueous solution (Cuveir & Launary, 1986; Nishinari & Sen-i To Kogyo, 1984; Richardson & Ross-Murphy, 1987; Ross-Murphy et al., 1983; Milas et al., 1990; Williams et al., 1991; Williams et al., 1991; Williams et al., 1994), and it is usually used as a food additive which increases the viscosity of the foods. XA is a polysaccharide produced by bacteria *Xanthomonaus campestris* (Davodson, 1980). HA is an amino polysaccharide and is a major component of biopolymers found in cartilage, eye vitreous humor and synovial fluid (Atkins, Phelps & Sheehan, 1972). HA retains a large amount of water, and is compatible with biopolymers and hydrophilic polymers (Laurent, 1957; Takigami, Takigami & Phyllips, 1993). On this account, HA is widely used in the medical field and has been investigated under physiological condition (Yanaki & Yamaguchi, 1990). In our previous studies, however it was found that XA and HA form hydrogels when aqueous solutions are annealed at a temperature higher than the characteristic temperature and subsequently cooled (annealing and subsequent cooling induced gelation) (Fujiwara, Takahashi, Hatakeyama & Hatakeyama, 1999; Quinn, Hatakeyama, Takahashi & Hatakeyama, 1994; Yoshida, Takahashi, Hatakeyama & Hatakeyama, 1998; Yoshida, Takahashi, Iwanami, Tanaka, Hatakeyama & Hatakeyama, 1998). By

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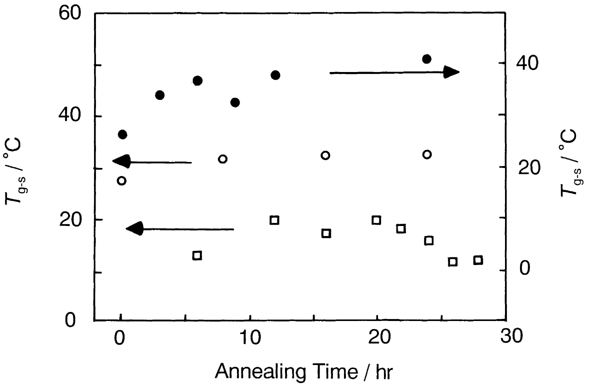

Fig. 1. Relationship between T_{g-s} and the annealing time. ○: XA 1 wt.% at 40°C, ●: XA 2 wt.% at 40°C, □: HA 3 wt.% at 60°C.

the falling ball method, it was confirmed that this characteristic temperature corresponds with the T_{g-s}. The annealing was also effective in the gelation of gellan gum, which is a representative gel forming polysaccharide electrolyte, i.e. it forms a tough and elastic gel when the gellan aqueous solutions are annealed in the sol state (Quinn, Hatakeyama, Yoshida, Takahashi & Hatakeyama, 1993; Yoshida & Takahashi, 1993). We also reported that structure of non-freezing water surrounding XA and HA changes during the annealing process (Fujiwara et al., 1999; Yoshida et al., 1998; Yoshida et al., 1998).

In this study, the experimental results on the annealing and subsequent cooling induced gelation of XA and HA aqueous solutions are summarized. By theoretical consideration, a phenomenological equation describing the behaviour of non-freezing water in systems is derived.

2. Summary of experimental results

In this section, the experimental results obtained from the falling ball measurements (FBM) for XA and HA gels and DSC measurements in the annealing process of XA and HA solutions are summarized.

In this study, XA powder provided by Mitsubishi Chemical Co. Ltd. and HA powder provided by Kibun Food Chemical Co. Ltd. was used without further purification. The molecular weights of XA and HA were 10^5 and 2.2×10^6, respectively. The limiting viscosity of HA and the content of protein contained in HA were 32 dl/g and 0.06%, respectively. Aqueous solutions of these polysaccharides were prepared using pure water provided by Wako Pure Chemical Industries Ltd. and all glassware was sterilized before use.

2.1. Falling ball method measurements

In the FBM measurements, a desired amount of XA or HA powder and 5 ml of pure water were sealed into a glass tube, which was ca. 35 cm in length and 10 mm in diameter, with a stirrer chip. After maintaining at room temperature for 3 days with occasional stirring, the sample tube was annealed at the desired annealing temperature for a predetermined annealing time. Then, it was immersed in a thermostat at 5°C for 1 day for XA system and 2–3 days for HA system to form gels. The glass tube was opened and a steel ball of ca. 0.5 mg in weight with 0.8 mm diameter was inserted into the gel. The gel in the glass tube was heated from 5°C to a temperature higher than the gel–sol transition temperature at a rate of ca. 0.5°C/min in the thermostat. The height of the steel ball was measured by a cathetometer and recorded. The gel–sol transition temperature was determined as the temperature at which the steel ball started to fall in the gel. The detail of the method to determine gel–sol transition temperature is described in our previous papers (Fujiwara et al., 1999; Yoshida et al., 1998).

In XA systems, solutions were heterogeneous when prepared at room temperature (Yoshida et al., 1998; Yoshida et al., 1998). However, the solutions thus obtained were thermodynamically stable, and no precipitate was observed although the solutions were kept in a refrigerator for more than one week. When the solution was maintained at 40°C for 1 day, it was transformed into a homogeneous solution and by subsequent cooling at 5°C a firm gel was obtained. The gel–sol transition temperature (T_{g-s}) of 1 and 2 wt.% xanthan aqueous solutions was measured by the falling ball method. As shown in Fig. 1, T_{g-s} gradually increased in the initial 10 h with increasing annealing time and then approached a constant value at 33°C within 24 h for 1 wt.%; and 42°C for 2 wt.% system.

In HA systems, the solutions prepared at room temperature were optically homogeneous and clear. The FBM measurements of 1, 2 and 3 wt.% solutions were carried out. Gelation was not observed for 1 and 2 wt.% systems annealed for any time at any temperature. However, 3 wt.% systems formed a gel under the condition of annealing temperature $T = 60$°C and annealing time t longer than 6 h, although 3 wt.% systems annealed at $T = 40$ and 50°C did not form gels (Fujiwara et al., 1999). The gel–sol transition temperatures T_{g-s} measured by FBM are also shown in Fig. 1 as a function of annealing time. The T_{g-s} gradually increased in annealing time region from 6 to 12 h and then decreased after 20 h via the annealing time region. The behaviour of T_{g-s} seems to be different from the behaviour of XA systems. However, HA is a thermolabile polysaccharide, and therefore the decrease of T_{g-s} in the long annealing time region seems to be due to the scission of HA chain because of the annealing at relatively high temperature. Therefore, it can be concluded that the annealing time dependence of T_{g-s} is essentially the same as that of the XA systems.

The FBM measurements revealed that T_{g-s} gradually increased with increasing annealing time and approached a constant value. These experimental facts are interpreted to be as a result of a structural change, which enables the systems to form gels, during annealing of these polysaccharide aqueous solutions.

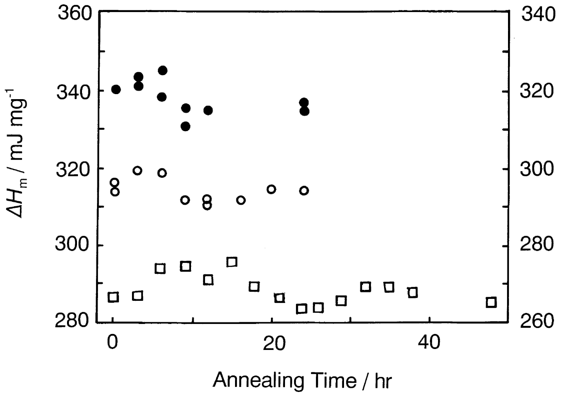

Fig. 2. Changes of ΔH_m as a function of annealing time. ○: XA 1 wt.% at 40°C, ●: XA 2 wt.% at 40°C, □: HA 3 wt.% at 60°C.

2.2. Differential scanning calorimetry

Water molecules in polysaccharide electrolyte/water systems are categorized into three kinds of water, i.e. non-freezing water, freezing bound water and free water. Non-freezing water is tightly bound to the polysaccharide electrolyte molecules and therefore cannot crystallize. Freezing bound water is weakly bound to polysaccharide electrolyte molecules. Consequently, it can crystallize, but the melting temperature is lower than that of bulk water. Free water is not influenced by polysaccharide electrolyte molecules, and hence the physical properties of free water are the same as those of bulk water. It is known that bound water, i.e. non-freezing water and freezing bound water, plays an important role in forming the junction zones in gel forming polysaccharides (Hatakeyama, Nakamura & Hatakeyama, 1979; Nakamura, Hatakeyama & Hatakeyama, 1981; Nakamura, Hatakeyama & Hatakeyama, 1982; Nakamura, Hatakeyama & Hatakeyama, 1985). In 1 and 2 wt.% XA and 3 wt.% HA systems, freezing bound water did not exist. Consequently, it is thought that the structural change of XA and HA aqueous solutions during the annealing process can be revealed through the change of non-freezing water content. The amount of non-freezing water restrained in polysaccharide hydrogels can be calculated based on the enthalpy of melting or crystallization of water ΔH_m. On this account, the time dependence of the amount of non-freezing water in XA and HA aqueous solutions during the annealing process were measured by DSC. The experimental conditions of the DSC measurements were described in detail elsewhere (Yoshida et al., 1998; Fujiwara et al., 1999).

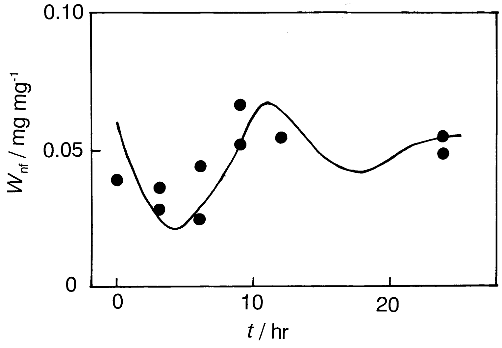

Fig. 4. Comparison between experimental data and calculated values using Eq. (9) of XA (2 wt.%). The parameter values are shown in the text.

Annealing time dependence of ΔH_m of 1 and 2 wt% XA aqueous solutions at 40°C and 3 wt.% HA aqueous solution at 60°C are shown in Fig. 2. The ΔH_m measured by DSC changed oscillationally during annealing via maximum at annealing time $t \sim 6$ h and minimum at $t \sim 10$ h in XA system: for HA system the maximum is at 10 h and the minimum is at 25 h, and then it approached a constant value. The final value of ΔH_m seemed to be slightly smaller than the initial value. The annealing time dependence of ΔH_m shows that the amount of non-freezing water decreased in the initial 6 h and then increased and approached a constant value via a maximum at t around 10 hs in the XA system and 10 and 25 h for the HA system, respectively. Annealing time dependence of the amount of non-freezing water Wnf (mg/mg) per 1 mg of water in the systems was calculated from the data of ΔH_m in Fig. 2 by assuming that the relation $\Delta H_m = 333$ mJ/mg for bulk water is applicable to the free water in the systems studied here. The obtained results are shown in Figs. 3–5. From the results of the DSC measurements, the structural change in the annealing process can be considered as described below.

The XA and HA molecules in the pre-annealed samples

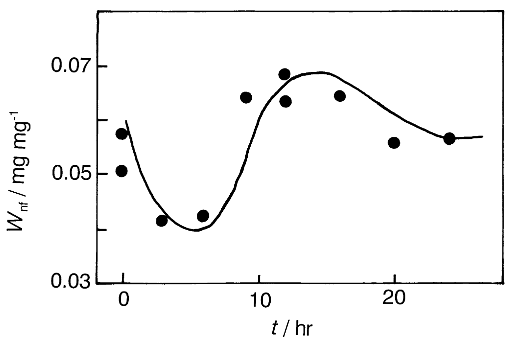

Fig. 3. Comparison between experimental data and calculated values using Eq. (9) of XA (1 wt.%). The parameter values are shown in the text.

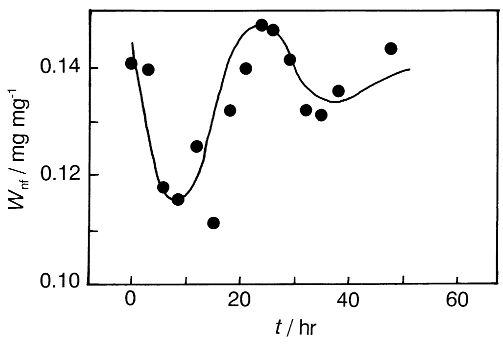

Fig. 5. Comparison between experimental data and calculated values using Eq. (9) of of HA (3 wt.%). The parameter values are shown in the text.

form molecular assemblies. In the initial stage of the annealing, the assemblies of polysaccharide molecules dissociate by desorbing water molecules. The decrease of non-freezing water observed in the initial stage of the annealing can be explained to be as a result of such dissociation of the assemblies of polysaccharide molecules. Subsequently, the solution homogenizes by the diffusion of polysaccharide molecules, which are removed from the assemblies. After the homogenization of the system was attained, the XA and HA molecules form a junction structure which enables the system to form gels by adsorbing the non-freezing water.

3. Phenomenological theory

In order to explain the behaviour of non-freezing water in the systems, we attempted to derive the phenomenological expression by the model described below. In our previous paper, the behaviour of water was considered to be phenomenological and was due to the competition between the homogenization of solution by the diffusion of polysaccharide molecules and the association of polysaccharide molecules in which adsorption and desorption of water molecules take place. Assuming that the total number of repeating units of polysaccharide molecules is N, N can be divided into three groups as shown in Eq. (1). The number of repeating units which adsorbed water molecules N_o, those not adsorbing water molecules N_u and those being incapable of adsorbing water molecules N_s are expressed as follows:

$$N = N_o + N_u + N_s. \tag{1}$$

A repeating unit belonging to $N_o + N_u \quad (= N - N_s)$ constitutes an adsorption site of water molecules and is considered to exist in the region where the density of repeating units is high. However, a repeating unit belonging to N_s is considered to exist in the region where the density of repeating units is low and can be regarded as a portion of a dangling and freely moving chain of a polyelectrolyte. If s-monomers are needed to adsorb one water molecule, the number of water molecules n adsorbed in the polysaccharide molecular assemblies and the number of adsorption sites n_u unoccupied by water molecules in polysaccharide molecular assemblies are given by

$$N_o = sn, \qquad N_u = sn_u. \tag{2}$$

The number of water molecules n is expected to increase when n_u is sufficiently large and/or n is sufficiently small. Therefore, the time evolution of n is expected to obey

$$\frac{dn}{dt} = k_1 n_u - k_2 n \tag{3}$$

here k_1 (> 0) and k_2 (> 0) represent the rate constants for adsorption and desorption of water molecules. Eq. (3) has the simple solution $n_{eq} = k_1 nu/k_2$ at the steady state ($dn/dt = 0$). However, n changes with time t according to Eq. (3) if n deviates from the equilibrium value n_{eq}, i.e. the system is in non-equilibrium state. Eqs. (1)–(3) give

$$\frac{dn}{dt} + (k_1 + k_2)n = \frac{k_1}{s}(N - N_s). \tag{4}$$

The right-hand side of Eq. (4) is expected to change with annealing time t by the removal of polysaccharide molecules from assemblies and the subsequent homogenization of the system. We assume the simple relation to describe the time evolution of N–N_s

$$\frac{d(N - N_s)}{dt} = -\alpha(n - n_{eq}(T)). \tag{5}$$

Here, α (> 0) is the phenomenological constant, and n_{eq} (T) the equilibrium value of n at the annealing temperature T. From Eqs. (4) and (5), the differential equation

$$\frac{d^2n}{dt^2} + (k_1 + k_2)\frac{dn}{dt} + \frac{k_1 \alpha n}{s} = \frac{k_1 \alpha n_{eq}}{s} \tag{6}$$

is obtained. Here, the dependency of n_{eq} on T was not explicitly expressed for the sake of simplification. Here, we redefine new parameters γ and ω and new variables n by the relations

$$\gamma = \frac{(k_1 + k_2)}{2}, \qquad \frac{k_1 \alpha}{s} = \omega_0^2, \qquad n = n - n_{eq}. \tag{7}$$

Here, γ represents the averaged rate constant for adsorption and desorption of water molecules and ω_0 the constant representing the effect of the change of the number of adsorption sites by the removal of polysaccharide molecules and subsequent homogenization of the system on the time evolution of n. It seems to be very strange that ω_0 does not depend on k_2 explicitly. However, α represents the rate constant for time evolution of the number of adsorption sites (Eq. (5)) by the removal of the polysaccharide molecules from assemblies and the subsequent homogenization of the system. The removal of polysaccharide molecules resulted from the dissociation of assemblies of polysaccharide molecules accompanied by desorption of water molecules. Therefore, α should be considered to depend on k_2, i.e. ω_0 depends on k_2 implicitly. By the replacement of Eq. (7), Eq. (6) is transformed to

$$\frac{d^2n}{dt^2} + \frac{2\gamma dn}{dt} + \omega_0^2 n = 0. \tag{8}$$

In the case of $\gamma < \omega_0$, Eq. (8) has the oscillational solution

$$n = C \exp(-\gamma t) \cos(\omega t + \delta) \tag{9}$$

where $\omega = (\omega_0^2 - \gamma^2)^{1/2}$. In Figs. 3–5, the theoretical curve calculated by Eq. (9) is drawn by the solid line along with the experimental data of W_{nf} which is evaluated from the data of ΔH_m in Fig. 2. Here, parameters n_{eq}, C, γ, ω and δ were selected as $n_{eq} = 0.06000$, $C = 0.03080$, $\gamma = 0.08660$, $\omega = 0.3142$ and $\delta = 1.5708$ for the 1 wt.% XA system, $n_{eq} = 0.05100$, $C = 0.04297$, $\gamma = 0.08980$, $\omega = 0.4488$ and $\delta = 1.3464$ for the 2 wt.% XA system and $n_{eq} =$

0.1375, $C = -0.03643$, $\gamma = 0.05598$, $\omega = 0.2094$ and $\delta = -1.885$ for the HA systems, respectively. The experimental data agrees semi-quantitatively well with the calculated value. Consequently, Eq. (9) qualitatively describes the behaviour of water in annealing process of solution.

In our phenomenological theory, k_1 and k_2 represent the rate constant for adsorption and desorption of water molecules on polysaccharide chains. Therefore, k_1 and k_2 are expected to have the same order of magnitude, i.e. $k_1 = k_2 = k$. In this case, the condition $\gamma < \omega_0$ can be written as

$$sk < \alpha. \tag{10}$$

As already described above, the constant α represents the rate constant for time evolution of the number of adsorption sites (Eq. (5)) by the removal of polysaccharide molecules from assemblies and the subsequent homogenization of the system. The homogenization of the system is based on the diffusion of the polysaccharide chain. Since the relaxation time of the diffusion of polysaccharide chain is expected to be largely longer than that of adsorption and desorption of water molecules, it cannot be concluded that α is larger than k. Therefore, s must be a small value less than one so as to hold the condition $\gamma < \omega_0$. This means that a large amount of water molecules was adsorbed in polysaccharide molecular assemblies. In fact, 0.06 mg of water was adsorbed in 0.01 mg of XA chains in 1 wt.% XA system, and 0.05 mg of water for 0.02 mg of XA in 2 wt.% XA system and 0.14 mg of water for 0.03 mg of HA in 3 wt.% HA system.

4. Conclusion

The following conclusions are obtained in this study: (1) gelation of the polysaccharide is markedly affected by the structure of the solution in sol state; (2) annealing the solution in sol state is effective to obtain firm gels; (3) a structural change of the solution accompanied by the complex behaviour of non-freezing water takes place in the annealing process of the solution in the sol state; (4) the complex behaviour of non-freezing water is explained by the competition process between the change of adsorption site, which resulted from the removal of polysaccharide chains from assemblies and subsequent diffusion of polysaccharide chains, and the association of polysaccharide molecules in which adsorption and desorption of water molecules take place.

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