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THERMAL AND VISCOELASTIC PROPERTIES OF XANTHAN GUM/CHITOSAN COMPLEXES IN AQUEOUS SOLUTIONS

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The viscoelasticity and thermal properties of aqueous solutions of blended xanthan gum (XA) with chitosan were investigated in order to study the electrostatic interaction established between two polysaccharides. Storage modulus G', loss modulus G'' and zero shear rate viscosity η_O attain a maximum at a chitosan concentration C_{max}. The above results indicate that the junction between XA and chitosan is formed in a concentration range lower than C_{max} and the viscoelasticity of systems increases with increasing concentration. In a concentration range higher than C_{max}, junction formation may not occur effectively since the excess amount of chitosan completely screens anions of XA. The chain rigidity of XA decreases by the screening of the repulsive interaction between anions on XA chains. The ineffective junction formation and the decrease of XA chain rigidity may cause the decrease of viscoelasticity of systems with increasing concentration. The value of C_{max} decreases with increasing molecular mass of chitosan. From melting enthalpy of the above system measured by DSC, the amount of non-freezing water (W_{nf}) was evaluated. W_{nf} shows a minimum at the concentration C_{max}. This fact suggests that hydrophobic fields increased by junction structure formation through ion-complexation between XA and chitosan molecules.

Keywords: *chitosan, complex, DSC, viscoelasticity, xanthan*

Introduction

Xanthan gum (XA) (Fig. 1) is a water soluble polysaccharide produced by *Bacterium Xanthomonas Canpestris*. XA takes a double helix conformation with the persistence length 120 nm at room temperature [1–4]. XA is an anionic polymer, since carbonium ions in the side chain are dissociated in aqueous solution. Physical properties of XA aqueous solutions have been widely investigated by many researchers [1–11]. The ability of XA to form hydrogels is very low, and therefore XA was considered to be a non-gelling polysaccharide. We found that aqueous solutions of XA form hydrogels by cooling after annealing at a temperature slightly higher than the gel–sol transition temperature (annealing induced gelation) [12–14]. It is thought that hydrogelation of XA occurs via hydrogen bonding in the annealing induced gelation. The experimental evidence suggested that the XA molecules do not easily form hydrogels in ordinary conditions. On this account, XA is usually used as a viscosity controller in food industries due to its high viscosity in aqueous media. Chemical modification of XA was introduced in order to improve the solubility and flocculation characteristics of XA [15–17].

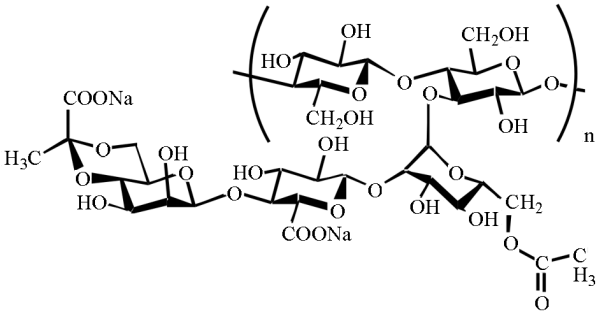

Fig. 1 Chemical structure of XA

Chitosan is a polysaccharide obtained by deacetylation of chitin. Chitin is contained in the shells of various shellfish and also in insects; therefore it is an abundant biomass obtainable from nature. Chitosan is insoluble in water, but soluble in acid solution when molecular mass is high, although oligomeric chitosan is water soluble. Chitosan having amino groups behaves as a cationic polymer as shown in Fig. 2 [18]. Solubility of chitosan in acid solutions depends on pH, ionic strength and the degree of acetylation (DA). Many researchers have investigated the molecular conformation of chitosan chain depending on the factors described above [19–22]. Chemical modification of chitosan was also

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carried out to improve the solubility [23–26]. Chitosan has excellent characteristics, such as antibacterial nature, detoxification, anticancer nature and so on, and has received attention in the medical field, as well as in food, textile, and cosmetics industries and so on.

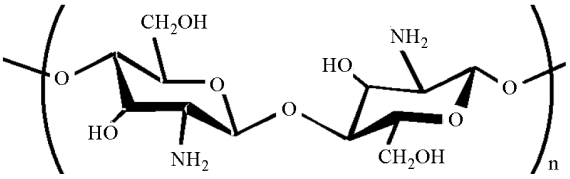

Fig. 2 Chemical structure of chitosan

Biopolymer blends have recently been investigated [27–33], especially in the field of foods science, since various kinds of biopolymers, such as starch, XA and amylose are contained in foods. Biopolymer blends composed of the above polysaccharides have been investigated [29, 31], since they are important in the food industry. In the plastic industry, polyvinyl alcohol blends with agar and hydroxyethylcellulose were investigated in order to improve the mechanical properties of biodegradable films [27].

It is known that various interactions, such as electrostatic interaction, hydrogen bonding, hydrophobic interaction, are established between biopolymers. In order to clarify the association of biopolymers through structure formation, it is important to study molecular interactions of different kinds of biopolymers. It is thought that the structural formation of biopolymer blends is an important subject from both industrial and scientific view points.

The aim of this study is to investigate XA and chitosan blends by thermal analysis and rheological measurement in order to clarify electrostatic interaction between the above biopolymers. Maurstad *et al*. investigated XA/chitosan blends prepared in acid solutions [28, 30, 32]. In this investigation, thermal and viscoelastic properties were measured in pure water by removing acetic acid in solutions utilizing dialysis technique.

Experimental

Sample preparation

XA from Mitsubishi Rayon Co. Ltd. and two kinds of chitosans, chitosan 10 and chitosan 100, from Wako Pure Chemical Industries, Ltd. were used. Numerals attached to chitosans indicated the values of viscosity at 20°C of solution with concentration 5 g L^{-1}. Aqueous solutions of XA/chitosan were prepared by the process described below. XA aqueous solution and 0.3 mol L^{-1} acetic acid solution of chitosan were prepared by stirring for 3 days at room temperature. By mixing two solutions 1.5 mol L^{-1} acetic acid solution of XA/chitosan was obtained. The solution immediately after mixing was not homogeneous and contained suspended particles composed of XA and chitosan. Further stirring dissolved the suspending particles and homogenized the solution. From the solution thus obtained, acetic acid was removed by dialysis for about 1 day. The concentration of solutions was controlled by vaporization of water in a vacuum oven at 40°C. Concentration of XA in solutions used for rheological measurements was 1 mass%. The concentration of chitosan was calculated based on the mass of XA.

Viscoelastic measurements

Viscoelastic properties were measured using a cone-plate type rheometer (NRM2000 Nihon Rheology KIKI Inc.). The diameter and slope of the cone was 3.1 cm and 3.0°, respectively. Measured frequency- and shear rate ranges were 10^{-2}–10 Hz and 10^{-2}–100 s^{-1}. Temperature of the sample was detected by a thermocouple inserted in the shaft of the cone and controlled within ±0.1°C by a box type controler utilizing a Peltier device. The controler surrounds the cone and plate and stabilizes the air in it. In order to avoid the temperature inhomogeneity of the sample, measurements were carried out for 10 min after which the temperature indicated on the controler was stabilized.

Theramal analysis

Thermal analysis was carried out using two kinds of differential scanning calorimeters DSC200 and DSC6200 (SII Nano Technology Inc.) for samples with various water contents and chitosan concentrations. 3~5 mg of the sample was placed in an Al open pan. The sample pan mounted on the DSC was cooled to –150°C and maintained at that temperature for 10 min. DSC curves were obtained in the subsequent heating process from –150 to 90°C. The heating and cooling rate in the forgoing processes is 10°C min^{-1}.

After the measurement was carried out, the sample mass was determined by weighing the sample pan. Then, a small pinhole was made on the cover of the sample pan. The sample in the pierced pan was dried in a vacuum oven at 120°C. Water content (W_c) of the sample was defined by

$$W_c = \frac{\text{mass of water contained in sample}}{\text{mass of dry sample}} \quad (1)$$

Results and discussion

Figure 3 shows frequency dependency of storage modulus *G*' and loss modulus *G*"of aqueous solutions of XA and XA/chitosan 10 with chitosan concentration 0.3 mass%. It is clearly seen that both *G*' and *G*" increases with increasing frequency. *G*' is larger than loss modulus *G*" in the whole frequency range.

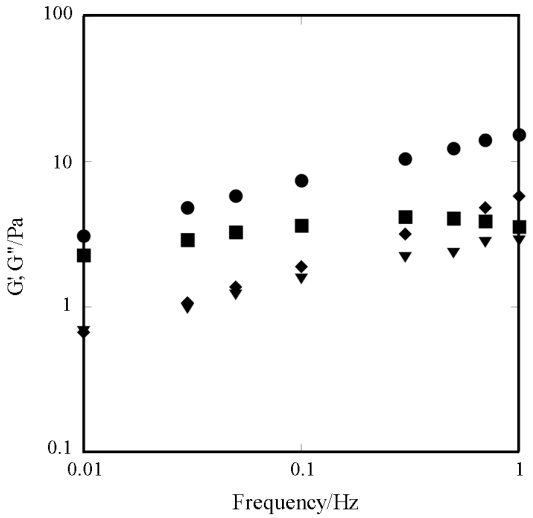

Fig. 3 Frequency dependence of storage modulus *G'* and loss modulus *G''* for XA aqueous solution (◆ – *G'*, ▼ – *G''*) and 0.3 mass% XA/chitosan 10 aqueous solution (● – *G'*, ■ – *G''*)

Figure 4 shows shear rate dependences of viscosity η of XA and XA/chitosan 10 with chitosan concentration 0.3 mass%. Newtonian behaviour is observable in a very narrow shear rate range, $\dot{\gamma} \leq 0.05 s^{-1}$. At a shear rate range larger than 0.05 s^{-1}, η linearly decreases with increasing $\dot{\gamma}$. Since the slopes of *G*' and *G*" *vs*. frequency are positive, the systems measured here are considered to be elastic liquids showing gel-like characteristics.

Figure 5 shows the relationship between *G*' and chitosan concentration measured at frequency 0.5 Hz. With the increase of chitosan concentration, *G*' increased initially and then decreased after reaching a maximum value. The concentration where *G*' maximum was observed was designated as C_{max}.

Figure 6 shows relationships between *G*" and concentration measured at frequency 0.5 Hz, and Fig. 7 shows relationships between zero shear rate viscosity η_0 and concentration. Both *G*" and η_0 show a maximum at the same concentration C_{max} where *G*' maximum was observed. The above experimental results indicate that the junction between XA and chitosan is formed in a concentration range lower than C_{max}. The fact that viscoelasticity of systems increases suggests that link formation is enhanced with increasing concentration. In a concentration range higher than C_{max}, an excess amount of chitosan completely screens anions of XA, and therefore junction formation may not occur effectively. Further, it is considered that chain rigidity of XA decreased since the repulsive interaction between anions attached to XA chains decreased by the screening. The ineffective junction formation and the decrease of XA chain rigidity may cause a decrease in the viscoelasticity of systems with increasing concentration. Among various kinds of molecular interaction to stabilize the biopolymers including DNA and enzymes, the elec-

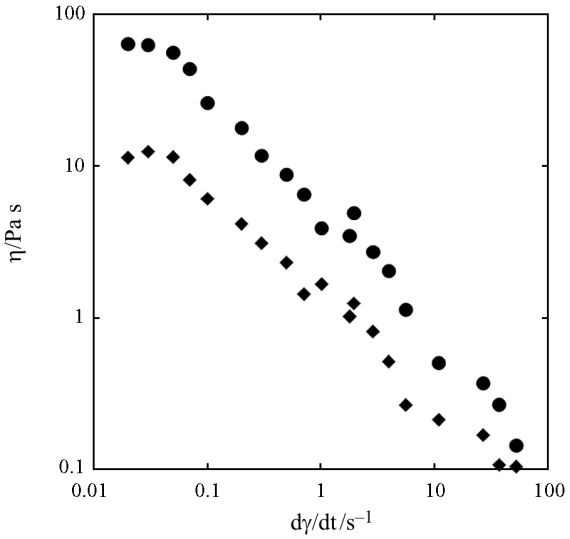

Fig. 4 Shear rate dependence of viscosity η for ◆ – XA aqueous solution and ● – 0.3 mass% XA/chitosan 10 aqueous solution

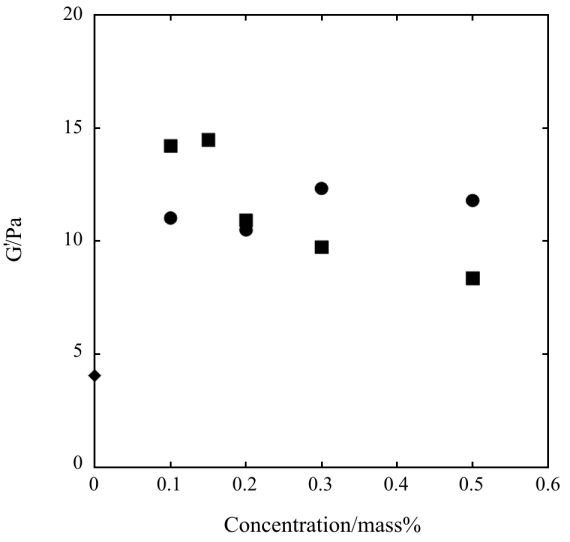

Fig. 5 Chitosan concentration dependence of *G*' for ◆ – XA aqueous solution and 0.3 mass% XA/chitosan aqueous solutions including ● – chitosan 10 and ■ – chitosan 100

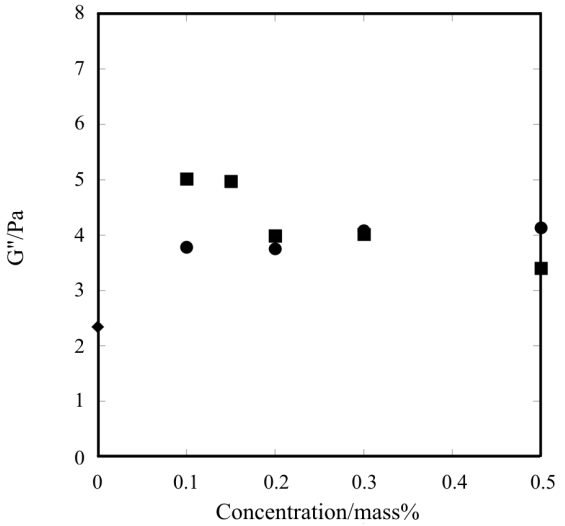

Fig. 6 Chitosan concentration dependence of *G*'' for ◆ – XA aqueous solution and 0.3 mass% XA/chitosan aqueous solutions including ● – chitosan 10 and ■ – chitosan 100

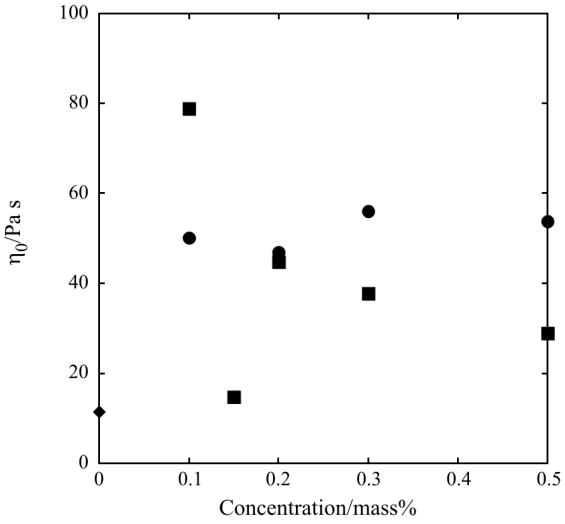

Fig. 7 Chitosan concentration dependence of zero shear rate viscosity η_0 for ◆ – XA aqueous solution and 0.3 mass% XA/chitosan aqueous solutions including ● – chitosan 10 and ■ – chitosan 100

trostatic interaction is the strongest and is reported to relate closely with chromosomal packing of DNA. Maurstad *et al*. observed a similar compaction for XA/chitosan systems [28, 30, 32].

The value of C_{max} for lower molecular mass chitosan 10 was higher than that of chitosan 100. The values of C_{max} are observed at 0.3 mass% for chitosan 10 systems and at 0.15 mass% for chitosan 100 systems, respectively. The above results indicate that one bond is electrostatically formed per 125 repeating units of XA in XA/chitosan 10 systems and one bond is formed 250 repeating of XA in XA/chitosan 100 systems. In fact, the number of repeating units between cross-linking points for XA chains is considerably larger. When the molecular chains of chitosan are long, the probability that a chitosan molecule bridges different XA chains increases. In contrast, chitosan chains with low molecular mass form molecular assembly with the same XA chain without bridging other XA chains. This fact indicates that the size of chitosan affects the screening of anions. It was found that anions in XA/Chitosan 100 systems were screened at a concentration lower than in XA/chitosan 10 systems.

Figure 8 shows representative DSC curves measured in heating process. The endothermic peak at around 0°C is due to the melting of water in the system. The melting enthalpy, ΔH_m, of water per 1 g of water contained in the sample is calculated from the peak area divided by the mass of water contained in the sample. The amount of non-freezing water (W_{nf}) contained in 1 g of the sample was calculated by the following equation.

$$W_{nf} = \frac{334 - \Delta H_m}{334} \times$$

(mass of water contained in 1 g of sample)

Figure 9 shows the relationship between chitosan concentration and the amount of non-freezing water W_{nf} contained in XA/chitosan systems with water content $W_c = 4$. W_{nf} shows the minimum at the concentration C_{max}.

Figure 10 shows the relationship between *G*' and W_{nf}. *G*' decreases with increasing W_{nf}. This fact shows that the junction structure formed through

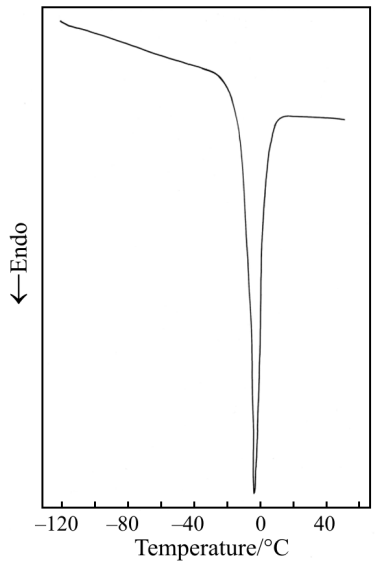

Fig. 8 Representative DSC heating curve measured for 0.3 mass% XA/chitosan system with W_c= 3

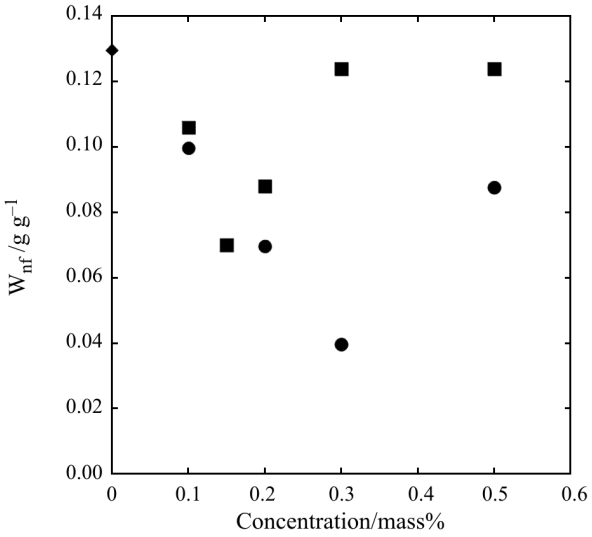

Fig. 9 Chitosan concentration dependence of the amount of non-frezing water W_{nf} contained in 1 g of the sample with water content $W_c = 4$ ◆ – XA ● – XA/chitosan 10 and ■ – XA/chitosan 100

ion-complexation between XA and chitosan molecules forms hydrophobic fields. In a concentration range lower than $C_{max.}$ The amount of non-freezing water bound to XA chains decreases according to the increase of junction points with increasing chitosan concentration. In a concentration range higher than C_{max} the amount of non-freezing water bound to chitosan chains increases with the increase of chitosan concentration.

The above results obtained by viscoelastic measurements and differential scanning calorimetry, indicate that XA-chitosan systems form inter-molecular linking via electrostatic interaction. The concentration required to form linking is limited to a narrow range, however, the molecular properties are markedly influenced by loose loop formed between XA and chitosan.

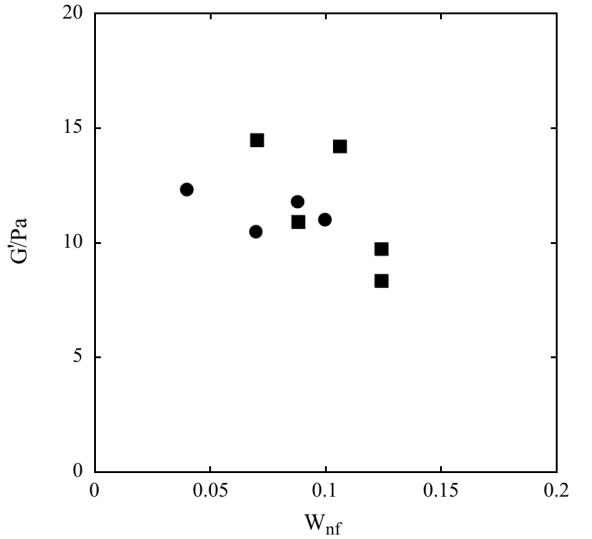

Fig. 10 Relationship between G' and W_{nf} ● – chitosan 10 and ■ – chitosan 100

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