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Changes in freezing bound water in water–gellan systems with structure formation

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Thermal behavior of water–gellan systems was investigated by differential scanning calorimetry. Bound water content in water–gellan systems was found to be markedly influenced by structure formation. In the low water content range [W_c = (grams of water)/(gram of dry gellan) = 1–5 g/g], water–gellan systems form a liquid crystalline structure. The amount of freezing bound water was dependent upon the melting and crystallization process of water in the system. In the high water content range (W_c = 20–200 g/g), water–gellan systems form hydrogels, when the freezing bound water content increases with the formation of junction zones. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The phase transition behavior of gellan gels has been intensively studied in recent years. Gel–sol transition was investigated by viscoelastic measurement (Watase & Nishinari, 1993; Nakamura *et al.*, 1993) and differential scanning calorimetry (DSC) (Yuguchi *et al.*, 1993; Watase & Nishinari, 1993). The reported temperatures for the gel–sol transition fell into two ranges; one in the region of 293–320 K and the other between 340 and 378 K. More than two gel–sol transitions, found by DSC, have been reported. It is reported that this temperature difference is a function of the ion type, concentration, measurement method, etc. According to the results obtained by mechanical and thermal measurements (Izumi *et al.*, 1994), two states exist in both the gel and sol states of sodium salt-type gellan hydrogels.

The concentration of gellan in the gels investigated in the above ranged from 0.1 to 5% [(grams of gellan)/(gram of water) × 100, %]. This means that the gels consist of 99.9 to 95% water. Therefore, it is important to investigate the structure of water in the gels in order to understand their physical properties. We have reported that the melting and crystallization behavior of water in gels varies with the structure of the gel (Hatakeyama *et al.*, 1987; Quinn *et al.*, 1993, 1994).

In this study, the phase transition of water in water–gellan systems is investigated in relation to liquid crystal and junction zone formation.

EXPERIMENTAL

Sample preparation

Gellan supplied by San-Ei Chemical Industries, Ltd (Osaka, Japan) was used as a 'common sample' by the research group on gellan gum affiliated to the Society of Polymer Science, Japan. Molecular weight (M_w) = 3.6 × 10^5 (Okamoto *et al.*, 1993), (M_n) = 5.4 × 10^4 (Ogawa, 1993) and the concentrations of metal cations are: Na, 1900 μg/g, K, 10 800 μg/g, Ca, 5120 μg/g and Mg, 1460 μg/g. Details of the sample characteristics are found in a series of papers appearing elsewhere (Food Hydrocolloids, 1993). The sample was used as received without further purification. Aqueous solutions (0.5 to 2 wt%) of gellan were prepared using distilled, deionized water. The solutions were sealed hermetically and pre-heated in an oven at 313 K for 24 h. For DSC measurements, a sample of the solution was hermetically sealed in an aluminium sample vessel.

Samples of low water content were prepared as follows: (1) 1–1.5 mg of gellan powder was weighed in pre-weighed aluminium sample vessels, (2) deionized water was added to the sample using a microsyringe, (3) water in the sample was evaporated until the predetermined weight of water was attained, (4) the DSC sample vessel was sealed using a sealer and weighed, (5) the sample was heated to 365 K and maintained at that temperature for 5 min, cooled to room temperature and kept at room temperature for 24 h, (6) sample vessels were weighed in order to confirm that no weight loss occurred during the above procedure. The samples whose weight decreased during the above heating and

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cooling processes were eliminated, (7) after measurement, the sample vessels were punctured and heated at 380K *in vacuo* (1×10^{-2} mm Hg) and weighed. The water content of samples, W_c, is expressed as follows:

$$W_c = [(\text{grams of water})/(\text{gram of dry gellan gum})], \text{g/g}$$

Measurements

Thermal analysis

A Perkin–Elmer DSC II-C differential scanning calorimeter, equipped with a low temperature cooling apparatus, was used for all thermal analysis experiments. Temperature and energy calibration were achieved using the crystal–crystal and melting transition peaks of spectroscopy grade cyclohexane. Samples were hermetically sealed in aluminium vessels and scanned at 10K/min. The sample weights ranged from 2 to 5mg and no weight loss was observed during the experiments.

Microscopic analysis

A Leitz Orthoplan Pol polarizing light microscope, equipped with a Linkam THMS600 temperature control unit was used to examine the higher order structure of the water–gellan systems. The samples were observed under the crossed nicol condition with a colour sensitive plate (retardation; $R = 530$nm).

X-Ray scattering analysis

Synchrotron orbital radiation (SOR) experiments were carried out at the synchrotron facility of the National High Energy Physics Laboratory, Tsukuba, Japan. The details of experimental procedure were described previously (Quinn *et al.*, 1993).

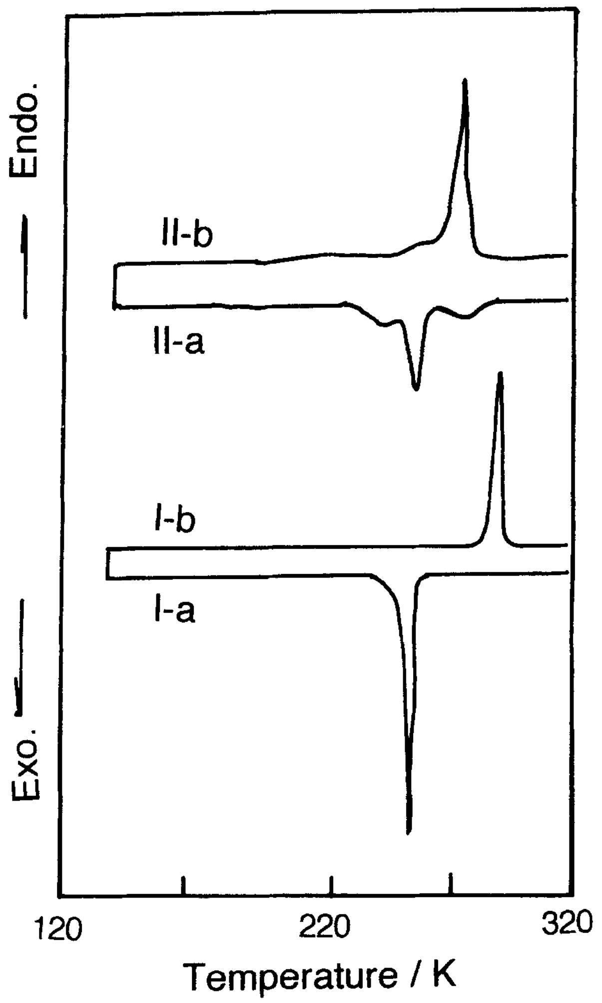

Fig. 1. Representative DSC curves of water–gellan systems. (I) $W_c = 83$g/g, (II) $W_c = 0.97$g/g, (a) cooling, (b) heating, scanning rate = 10K/min.

RESULTS

Figure 1 shows representative DSC curves of water–gellan systems with high ($W_c = 83$g/g, sample I) and low ($W_c = 0.97$g/g, sample II) water contents over the temperature range 130 to 320K at a heating rate of 10K/min. In the cooling curve (I-a) of sample I, crystallization of water is observed and in the heating curve (I-b), melting of water is observed. The gel–sol transition was not detected due to the relatively low sensitivity of the apparatus. In the cooling curve of sample II (curve II-a), the following first order transitions are observed: a small exotherm which was attributed to liquid crystallization at around 271K, crystallization of free water at 253K and that of freezing bound water at 245K. In the heating curve (curve II-b), a slight shift of baseline was detected at around 206K. This baseline shift was attributed to a glass transition. However, this glass transition was not clearly seen in the cooling curve (II-a). In contrast, the peak attributed to liquid crystallization merges into the melting peak of water and is observed as a shoulder at the high temperature side of the main melting peak at 275K.

Figure 2 shows stacked DSC cooling curves of water–gellan systems. All of the cooling curves are from the first cooling scan from 320 to 150K. With increasing W_c, the melting peak approaches a constant value and levels off at around $W_c = 5$g/g. The crystallization peak attributed to freezing bound water and the liquid crystallization peak were observable up to $W_c = 5$g/g.

In the DSC heating curves, it was observed that melting peak temperature increased with increasing W_c and leveled off at $W_c = 5.0$g/g. The liquid crystallization peak merged into the melting peak and was difficult to detect as a separate peak. The heat capacity difference (ΔC_p) at the glass transition was small compared to other polyelectrolyte polysaccharides (Yoshida *et al.*, 1991).

Figure 3 shows polarizing light micrographs of water–gellan systems. W_c was approximately 5g/g. As mentioned above, that transition was not observed as a separate peak in DSC heating curves. Due to this a complete phase transition map could not be established.

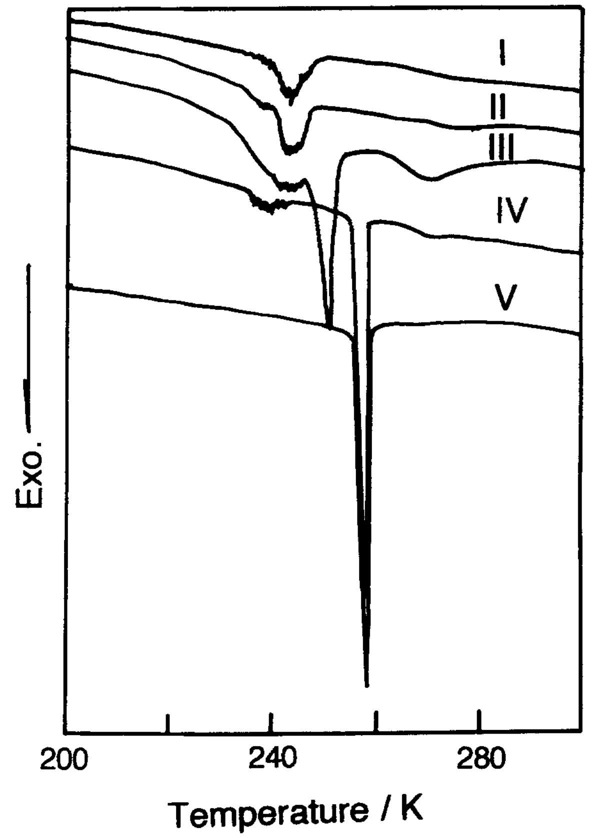

Fig. 2. Stacked DSC cooling curves of water–gellan systems: W_c (I) 0.76g/g, (II) 0.91, (III) 1.07, (IV) 9.92, (V) 83.3; scanning rate = 10K/min.

Moreover, thermal behavior of the liquid crystalline state varied according to a number of factors such as, repeated scans and annealing at room temperature. This suggests that the original state of the sample is difficult to maintain once an ordered state of the system is established.

Figure 4 shows the relationship between amounts of free water (W_f), freezing bound water (W_{fb}), non-freezing water (W_{nf}) and total water content (W_c). W_f was calculated from enthalpy of the high temperature side of crystallization and W_{fb} from the low temperature side crystallization peak. In the high water content range, no first order phase transition was observed in a fraction of the water. W_{nf} was calculated by subtracting the summation of W_f and W_{fb} from W_c. W_{nf} content of the water–gellan systems was 0.7g/g. This means that when the W_c is less than 0.7g/g, freezing water is no longer present. The W_{nf} value increased slightly with increasing W_c. W_{nf} values of many polysaccharides are constant over the entire W_c range. The W_{nf} value of 0.7g/g for the water–gellan system is comparatively large when compared to the amount of non-freezing water in the water–carboxymethylcellulose system, 0.7g/g (Hatakeyama *et al.*, 1990), water–sodium cellulose sulfate, 0.4g/g (Hatakeyama *et al.*, 1987), and water–alginic acid, 0.5g/g (Nakamura *et al.*, 1991).

The thermal behavior of the water–gellan system was affected by the thermal history of the samples. The influence of thermal history of gellans was more marked compared with that of the other water–polysaccharide system. Figure 5 shows representative DSC cooling curves of the water–gellan system with $W_c = 0.76$g/g. (Curve 1 in this figure corresponds to the enlarged curve I in Fig. 2) Sharp peaks due to the size and irregular distribution of ice in the system were observed. On repeated crystallization and melting of water in the system, crystallization peaks gradually shifted to the low temperature side. At the same time, the area of the low temperature peaks increased. This suggests that the amount of irregular ice increased with repeated melting and crystallization. The W_c of the sample shown in Fig. 5 is very high, and it may be appropriate to consider that water molecules are tightly associated by gellan molecules. The number of binding sites and the space available to water molecules varies according to the melting and crystallization conditions.

As mentioned above, the melting and crystallization peaks of water in the systems, even in a very low W_c range, varied according to the thermal pre-history of the sample. As previously reported (Hatakeyama *et al.*, 1987; Quinn *et al.*, 1993), the crystallization peak of water in the gellan gels with W_c ranging from 20 to 200g/g was separated into two peaks by annealing in the temperature range from 340 to 365K for 5–40min. As schematically shown in the upper frame of Fig. 6, a low temperature peak (Exo_l) appeared by annealing, and the relative area of the exothermic peaks varied as a function of annealing time. In this figure, the total area ($Exo_l + Exo_h$) is taken as 1.0. The low temperature peak attributed to the freezing bound water increased with increasing annealing time. Similar behavior was also found in xanthan gels (Quinn *et al.*, 1994) over a similar W_c range.

Figure 7 shows the relationship between the relative amount of freezing bound water and the size of the junction zones, calculated from small angle X-ray scattering (SAXS). It is clearly seen that the amount of freezing bound water in gellan gels is related to the structural dimensional change in the junction zone.

DISCUSSION

Over a wide water content range, water–gellan systems form glasses. The glass transition temperature (T_g) was observed at around 206K for a water content of 1.0g/g, although the heat capacity difference (ΔC_p) at T_g was rather difficult to detect, especially after repeated scans (Fig. 1). A similar tendency was also observed for liquid crystallization. As shown in Figs 1 and 2, a liquid crystallization peak was observed at W_c's ranging from 0.7 to 5.0g/g in the first cooling curve. However, after repeated scans, the liquid crystallization peak was diffi-

Fig. 3. Polarizing light micrographs of water–gellan systems. Scale bar is 200μm.

cult to detect. This indicates that once the liquid crystalline state is established, disruption is difficult even at a temperature corresponding to the sol state. These observations suggest that the stable liquid crystalline structure is maintained by freezing and that the amorphous regions of the system are comparatively limited in size order over this temperature range. This results in the small ΔC_p value at T_g. Because of this a full phase diagram is not easy to establish.

Even at high water contents, the amount of freezing bound water increased with increasing annealing time at 365K (Fig. 6). According to Yuguchi *et al.* (1993), the main gel–sol transition was observed at around 293K and residual small transition peaks terminated at around 360K. From these results, it is considered that the annealing temperature at 365K corresponds to the sol state. As reported in our previous paper (Quinn *et al.*, 1993), the sol state of the water–gellan system is thermodynamically a non-equilibrium state.

Although the total amount of bound water ($W_{fb} + W_{nf}$) of the gellan is not particularly high (Fig. 4) compared to that of other polyelectrolyte polysaccharides, the phase transition temperature of water is strongly affected by the pre-history of the sample. This phenomenon is attributable to the fact that molecular aggregation proceeds in the sol, gel and liquid crystalline states. Nakajima *et al.* (1994a) directly observed the aggregated structure of gellan by scanning tunneling microscopy and reported that gellan molecules aggregate into a 3.4nm crosslinked domain of 30–40nm length strands depending on the species of cations present in the system.

As shown in Fig. 7, the size of the junction zone calculated from the peak observed in the SAXS pattern,

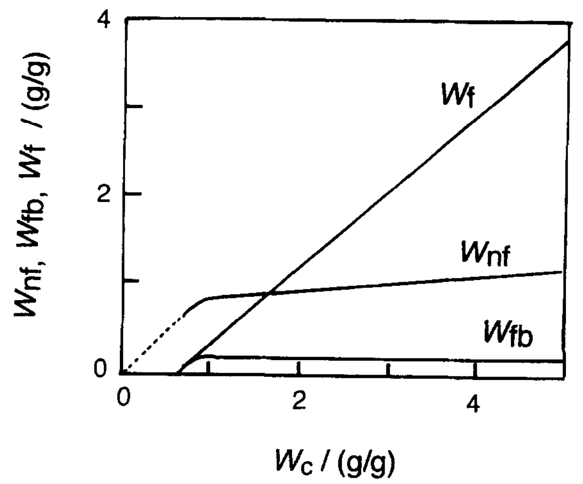

Fig. 4. Relationships between amounts of free water (W_f), freezing bound water (W_{fb}), non-freezable water (W_{nf}) and W_c of water–gellan systems.

was 16–20nm, and the amount of freezing bound water increased with increasing junction zone size (Fig. 7). Judging from the above results, it is appropriate to consider that free water in molecular aggregates is separated into small portions and that the amount of free water decreases. The phase transition behavior of water in the water–gellan system reflects directly the molecular rearrangements. Thermal data suggests that gellan molecules stabilize gradually as a function of factors such as, temperature, water content and time, although the equilibrium state could not be attained in the time scale of these observations.

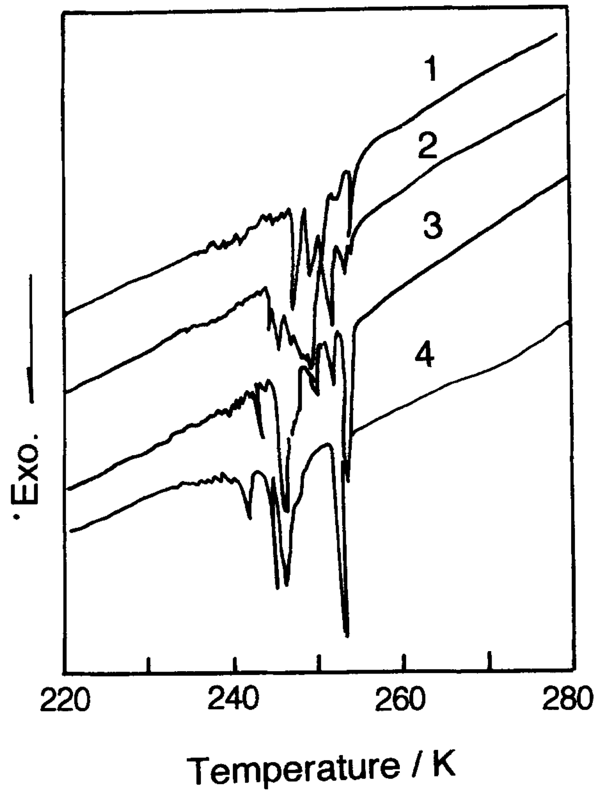

Fig. 5. Stacked DSC cooling curves of water–gellan system with $W_c = 0.76$g/g. Numbers show repeated number of scans.

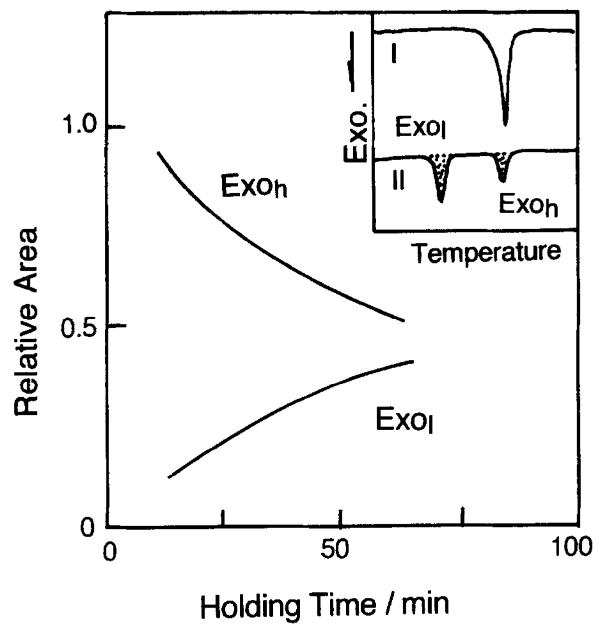

Fig. 6. Relationships between relative height of crystallization peaks of water in gellan gels and time of annealing. $\mathrm{Exo_h}$; high temperature peak (free water). $\mathrm{Exo_l}$; low temperature peak (freezing bound water).

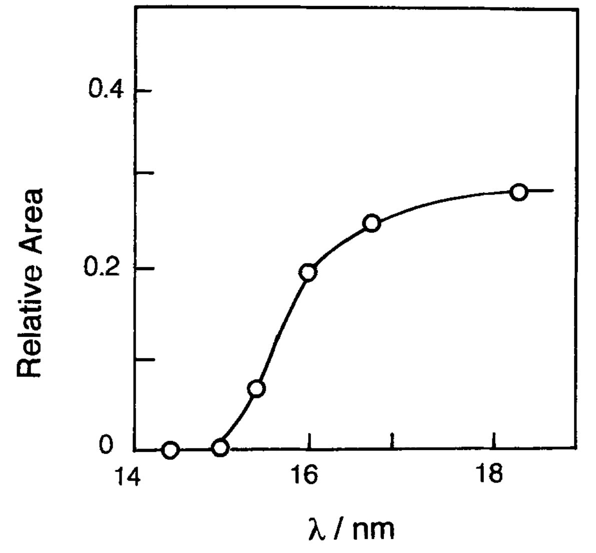

Fig. 7. Relationship between relative freezing bound water content and size of junction zone (λ).

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