ELSEVIER

PII: S0144-8617(96)00092-6

Carbohydrate Polymers 30 (1996) 193–207
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0144-8617/96/$15.00 + .00

Rheological and DSC studies on the interaction between gellan gum and konjac glucomannan

Katsuyoshi Nishinari,[a]* Emako Miyoshi,[a] TomohisaTakaya[a] & Peter A. Williams[b]

[a]*Department of Food and Nutrition, Faculty of Human Life Science, Osaka City University, Sumiyoshi, 558, Osaka, Japan*
[b]*Faculty of Science, Health and Medical Studies, North East Wales Institute, Connah's Quay, Deeside, CH5 4BR, Clwyd, UK*

Interaction between gellan gum (GELL) and konjac glucomannan (KGM) has been studied by dynamic viscoelastic measurements and differential scanning calorimetry (DSC). Three fractions of konjac glucomannan with different molecular weights (1.17 × 10^6 (ND), 9.5 × 10^5 (LM-1) and 2.51 × 10^5 (LM-2)) were used in this study. Mechanical spectra showed that 1.6% solutions of GELL alone and of LM-2 alone behaved like a dilute polymer solution whilst 1.6% solutions of ND alone and LM-1 alone behaved like a concentrated polymer solution where molecular entanglements play an important role. Thermal scanning rheological measurements showed a step-like change in the loss modulus which is attributed to helix-coil transition, and subsequent aggregation of helices, and an exothermic and endothermic peak appeared in cooling and heating DSC curves at the same temperature of transition. The transition temperature shifted to lower temperatures, and the transition enthalpy decreased with increasing KGM content in the mixture. The elasticity of mixtures originates mainly from aggregated helices of gellan gum molecules for GELL/LM-2 systems whilst the contribution from KGM is more important for GELL/LM-1 and GELL/ND systems. Although the storage modulus of GELL/LM-1 and GELL/LM-2 as a function of mixing ratio showed a maximum, it is concluded that KGM inhibit the formation of the ordered structure of gellan gum. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Many polysaccharides have found widespread applications in the food industry because they form viscous solutions and gels which are extremely useful in the texturing of foodstuffs. The addition of galactomannan to certain polysaccharides (like agarose or κ-carrageenan) is known to improve mechanical properties and reduce brittleness or syneresis of the polysaccharide gel (Morris, 1986). The synergistic effects between galactomannan and these polysaccharides lead to gels with increased elasticity and strength under conditions where the pure components would not gel. Admixtures of galactomannan and xanthan also form gels despite the fact that they neither form gels independently.

The mechanism of gelation of these mixtures has been the subject of much interest since early studies (Dea & Morrison, 1975; Dea, 1979) and is a matter of debate and several models have been proposed (Cairns *et al.*, 1986; 1987; Brownsey *et al.*, 1988; Tako and Nakamura, 1986; Fernandes *et al.*, 1991; Rochas *et al.*, 1990; Williams *et al.*, 1991; Cheetham and Mashimba, 1988; 1991; Shatwell *et al.*, 1991; Turquois *et al.*, 1992; Fernandes *et al.*, 1992; Williams *et al.*, 1993; Kohyama *et al.*, 1993). Three different types of gel structures in these mixed gels have been defined if both polysaccharides contribute to the network; (i) interpenetrating network; (ii) a phase-separated network; (iii) the binding of one polysaccharide to the other to form a coupled network defined by specific junction zones (Morris, 1986). The model for gelation of the mixture postulated interaction between the helical structure of the carrageenan or agarose and the unsubstituted mannan regions, the so-called 'smooth region' of galactomannan chains (Dea & Morrison, 1975; Dea, 1979). X-ray diffraction studies, however, did not provide evidence for a specific interaction or evidence of mannan/κ-carrageenan complexation, and it was suggested that the most likely model for such gels consists of a galactomannan solution contained within a carrageenan network (Cairns *et al.*, 1986, 1987; Brownsey *et al.*, 1988). These workers also suggested, however, that it was possible that other mannan chains adsorbed onto large aggregates of helices and Williams *et al.* (1993) provided further support for this. Recently, Kohyama *et al.* (1993) carried out rheological and DSC

---

*Corresponding author.

studies on the gelation of konjac glucomannan/carrageenan mixtures using three konjac glucomannan fractions with different molecular weights. The elastic modulus at small deformation, the breaking stress and breaking strain of mixed gels increased with increasing molecular weight of konjac glucomannan, however, the gel–sol transition temperatures were not influenced so much by the molecular weight of konjac glucomannan. From these results, another new model was proposed; a main network of the mixture is formed by carrageenan molecules, and konjac glucomannan molecules interact with carrageenan molecules and form weak junction zones which contribute to the elasticity, but not so much to thermal stability.

Gellan gum, an anionic polysaccharide produced by *Pseudomonas elodea* has a complex tetrasaccharide repeat unit (Jansson *et al.*, 1983; O'Neill *et al.*, 1983) and forms a transparent gel in the presence of cations, via a helical intermediate (Grasdalen & Smidsrod, 1987; Crescenzi *et al.*, 1986; 1987; Dentini *et al.*, 1988; Chapman *et al.*, 1990; Milas *et al.*, 1990; Robinson *et al.*, 1991; Manning, 1992; Shi, 1990), in a way analogous to the gelation of carrageenans. Junction zones of gellan gum gels are formed by the aggregation of double helical gellan molecules, and are controlled by the thermodynamic properties of the systems (temperature, cations, solubility of polymer) (Crescenzi *et al.*, 1986; 1987; Dentini *et al.*, 1988; Chapman *et al.*, 1990; Milas *et al.*, 1990; Robinson *et al.*, 1991; Manning, 1992; Shi, 1990). Previous studies (Miyoshi *et al.*, 1994a, b) have shown the changes in rheological and thermal properties of sodium form gellan gum solution caused by the addition of metal ions. We have suggested that salts promote the aggregation of helices of gellan gum molecules at lower temperatures whilst they reduce the coil dimension of gellan gum molecules at higher temperatures. It was also found that the mechanism of gel formation in gellan gum in the presence of divalent cations is markedly different from that in the presence of monovalent cations. In the presence of sufficient divalent cations, these junction zones formed on cooling below the setting temperature became more heat resistant, which were very different from those of thermoreversible gels formed by gellan gum alone or in the presence of monovalent cations.

Konjac glucomannan (KGM) is a glucomannan obtained from the tuber of Amorphophallus konjac K.Koch (Nishinari *et al.*, 1992), and forms a thermally-stable gel on the addition of alkaline coagulant. This gel has been used in Japanese traditional foods (Nishinari, 1988). Recently, the interaction of KGM with other hydrocolloids such as xanthan and κ-carrageenan has been studied (Dea, 1979; Cairns *et al.*, 1986, 1987; Brownsey *et al.*, 1988; Williams *et al.*, 1991, 1993; Kohyama *et al.*, 1993; Annable, 1994a). It was shown that the xanthan/KGM mixtures form thermoreversible gels at neutral pHs although neither KGM alone nor xanthan alone form a gel at neutral pHs. In the present work, the rheological and thermal properties of gellan gum/konjac glucomannan mixtures were studied by dynamic viscoelastic measurement and differential scanning calorimetry in order to elucidate the mechanism of interaction.

MATERIALS AND METHODS

Materials

As described in previous studies (Miyoshi *et al.*, 1994a, b), gellan samples (in powder form) were kindly supplied by Kelco Division of Merck & Co. Inc., California, USA. The contents of the inorganic ions Na^+, K^+, Ca^{2+} and Mg^{2+} were determined by Kelco using an Inductively Coupled Argon Plasma Emission Spectrophotometer (ICP) as Na^+ 3.03%, K^+ 0.19%, Ca^{2+} 0.11% and Mg^{2+} 0.02%, respectively.

The non-degraded native konjac glucomannan (ND) and two fractions of konjac glucomannan with different molecular weights (LM-1 and LM-2), prepared by enzymatic degradation, were kindly supplied by Shimizu Chemical Co., Hiroshima, Japan. The molecular weights of the sample fractions determined by the same method in a previous work (Kohyama *et al.*, 1993) were 1.17×10^6 (ND), 9.5×10^5 (LM-1) and 2.51×10^5 (LM-2). Man/Glc ratio estimated from the peak area detected with a refractive index did not depend so much on the molecular weight of KGM, and was about 2.0.

Solutions of gellan gum/konjac glucomannan at various ratios were prepared by mixing the appropriate amounts of each powder and dispersed by stirring at 40°C overnight. The total polysaccharide concentration was fixed at 1.6% (w/w) and the gellan gum/konjac glucomannan ratio was changed. The solutions of mixtures for viscoelastic and DSC studies were prepared by stirring at 70°C for 2h and then heated at 90°C for 1h to attain complete transparency as described in previous studies (Miyoshi *et al.*, 1994a, b). The solutions of mixtures were then kept at 70°C before each rheological and DSC measurement to prevent gelation.

Rheological measurements

The mechanical spectra measurements were performed with a Dynamic Stress Rheometer DSR from Rheometrics Co. Ltd, NJ, USA at various temperatures. All the measurements were performed within the linear viscoelastic regime. The details of rheological measurements were described previously (Miyoshi *et al.*, 1994a). The frequency dependence of the storage modulus G' and loss modulus G'' at a frequency range from 10^{-2} to 10^1rad/s was observed after attaining the plateau value of both moduli as a function of time at various temperatures from 30 to 0°C. The storage modulus G'

as a function of mixing ratio at various concentrations was determined by values of G′ at 1.0 rad/s in frequency sweep measurements ($10^{-1} \sim 10^{1}$ rad/s) at various temperatures. Temperature dependence of G′ and G″ was observed by cooling the systems from 50 to 5°C and then reheating to 50°C, at the rate of 0.5°C/min, at a constant frequency of 0.1rad/s.

DSC measurements

Differential scanning calorimetry (DSC) measurements were carried out using a Setaram micro DSC-III calorimeter, Caluire, France. The details of DSC measurements were described previously (Miyoshi *et al.*, 1994b). The sample and reference pans were placed inside the calorimeter and heated to 110°C and kept for 10 min to ensure the mixture was in the form of a homogeneous solution. Then the temperature was lowered to 5°C at 0.5°C/min and raised again at the same rate up to 110°C. Finally, the temperature was scanned up and down at a preset rate, and the enthalpy profiles were recorded.

RESULTS AND DISCUSSIONS

Rheological properties

Figure 1 shows the frequency dependence of G′ and G″ for a 1.6% solution of gellan gum alone. At any temperature from 30 to 0°C, the viscoelastic behavior was typical of a dilute polymer solution, i.e. G′ < G″ throughout the accessible frequency range, and both G′ and G″ were strongly frequency dependent (Morris, 1982, Clark & Ross-Murphy, 1987; Te Nijenhuis, 1990).

Figure 2 shows the frequency dependence of G′ and G″ for mixtures of gellan gum (GELL) and ND (total polysaccharide concentration 1.6%) with various mixing ratios and at various temperatures. For a mixture with GELL/ND = 1.4/0.2 (Fig. 2 (A)), the behavior at 0°C was typical of a concentrated polymer solution, i.e. G′ was smaller at lower frequencies but became larger than G″ at higher frequencies. The frequency dependence of G′ and G″ for concentrated polymer solution has been explained as follows: the entanglement of molecular chains are easy to disentangle during the long period of oscillation at low frequencies, and therefore the solution behaves as a viscous fluid; the molecular chains do not disentangle during the short period of oscillation at high frequencies, and their entanglement plays a role of knots and forms a temporary three-dimensional network, and as a result the solution behavior tends to that of an elastic solid (Morris, 1982; Clark & Ross-Murphy, 1987; Te Nijenhuis, 1990). At 15, 25, and 30°C, it showed a typical dilute polymer solution behavior where G″ predominates G′ at all the frequencies. Mixtures with GELL/ND = 1.0/0.6 (Fig. 2(B)), 0.6/1.0 (Fig. 2(C)), and a 1.6% solution of ND alone (Fig. 2(D)) showed similar tendencies, but the cross-over frequency observed at 0°C shifted to lower frequencies with increasing content of ND. G′ and G″ of the 1.6% solution of ND alone is least frequency dependent and least temperature dependent. The temperature dependence was most conspicuous at lowest frequencies, and the difference between G′ and G″ at 0 and 30°C increased with increasing content of gellan gum, indicating that this is induced by the conformational change of gellan molecules and that the conformation of ND is not changed so much at this temperature range. This will be discussed later in more detail in the light of the thermal scanning rheological measurements.

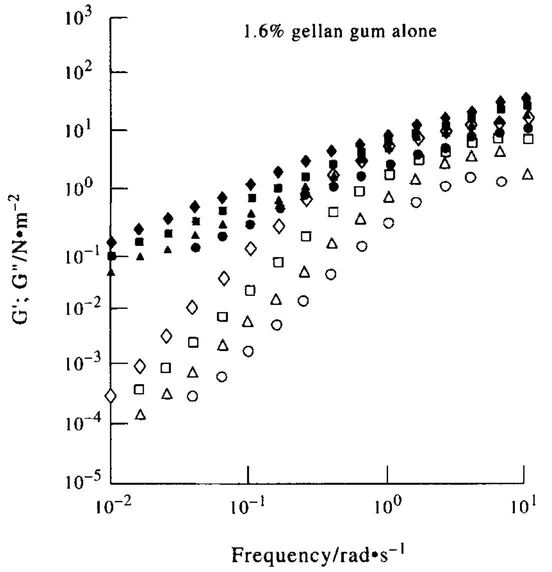

Fig. 1. Frequency dependence of storage modulus G′ and loss modulus G″ for a 1.6% solution of gellan gum alone at various temperatures. (○) G′, (●) G″, 30°C; (△) G′, (▲) G″, 25°C; (□) G′, (■) G″, 15°C; (◇) G′, (◆), G″, 0°C.

Figure 3 shows the frequency dependence of G′ and G″ for mixtures of gellan gum (GELL) and LM-1 (total polysaccharide concentration 1.6%) with various mixing ratios and at various temperatures. For a mixture with GELL/LM-1 = 1.4/0.2 (Fig. 3(A)), the behavior at 30 or 25°C was typical of a dilute solution, however, the behavior at 15°C was typical of a concentrated polymer solution because G′ approached G″ at higher frequencies, and in fact, G′ and G″ actually showed a cross-over at a relatively high frequency. The behavior at 0°C was also typical of a concentrated polymer solution and the cross-over frequency was lower than that at 15°C. The behavior of a mixture with GELL/LM-1 = 1.0/0.6 (Fig. 3(B)) showed a similar tendency to that of a mixture with GELL/LM-1 = 1.4/0.2 (Fig. 3(A)). For a mixture with GELL/LM-1 = 0.6/1.0 (Fig. 3(C)), the behavior even at 30 or 25°C was typical of a concentrated polymer solution, moreover, the behavior at 15 or 0°C tended towards that of a weak

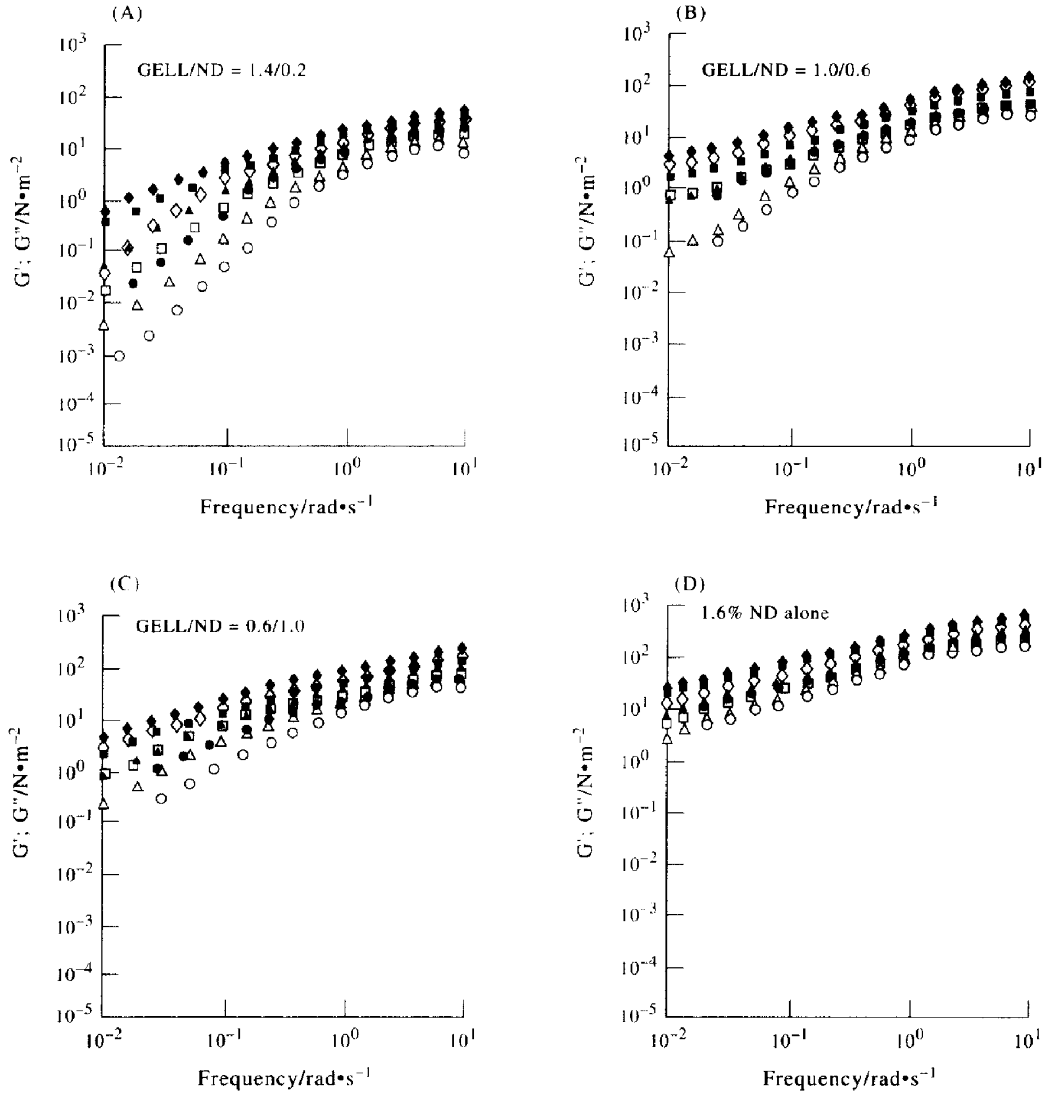

Fig. 2. Frequency dependence of storage modulus G′ and loss modulus G″ for GELL/ND mixtures (total polysaccharide concentration 1.6%) with various mixing ratios at various temperatures. (○) G′, (●) G″, 30°C; (△) G′, (▲) G″, 25°C; (□) G′, (■) G″, 15°C; (◇) G′, (◆), G″, 0°C; mixing ratios of GELL/ND: (A) 1.4:0.2, (B) 1.0:0.6, (C) 0.6:1.0, (D) 0.0:1.6.

gel (Morris, 1982; Clark & Ross-Murphy, 1987; Te Nijenhuis, 1990; Almdal *et al.*, 1993) because G′ was larger than G″ throughout the experimentally-accessible frequency range and both moduli were little dependent on frequency. The weak gel behavior is the intermediate rheological behavior between that of the concentated polymer solution and that of the elastic or true gel. Mechanical spectra of true gels are characterised by the appearance of rubber-like plateau of both moduli and sometimes accompanied with a slight dip of the loss modulus G″ at a certain frequency. True gels are formed by a sufficient number of junction zones in gellan gum solutions in the presence of sufficient salt (Figs 2(f), 5(f) and 6(d) in Miyoshi *et al.*, 1994a). For a 1.6% LM-1 solution (Fig. 3(D)), at any temperature from 30 to 0°C, the behaviour was typical of a concentrated polymer solution and those curves were less dependent on temperature.

Figure 4 shows the frequency dependence of G′ and G″ for mixtures of GELL/LM-2 at various mixing ratios and at various temperatures. G″ is greater than G′ at all the frequencies and at all the temperatures examined for mixtures with GELL/LM-2 = 1.4/0.2 (Fig. 4(A)), 1.0/0.6 (Fig. 4(B)) and G′ was too small to be detected at all the temperatures for a 1.6% solution of LM-2 alone (Fig. 4(D)). The difference in behavior of 1.6% solutions of KGM with different molecular weights (Figs 2(D), 3(D) and 4(D)) is induced by a difference in the entanglement which is often observed for flexible macromolecules such as poly(methyl methacrylate) (Masuda *et al.*, 1970) and polystyrene (Onogi *et al.*, 1970). The cross-over frequency shifted to lower frequencies with increasing molecular weight of KGM (Figs 2(D) and 3(D)) as in poly(methyl methacrylate) or polystyrene molecules, although these macromolecules are slightly more flexible than KGM.

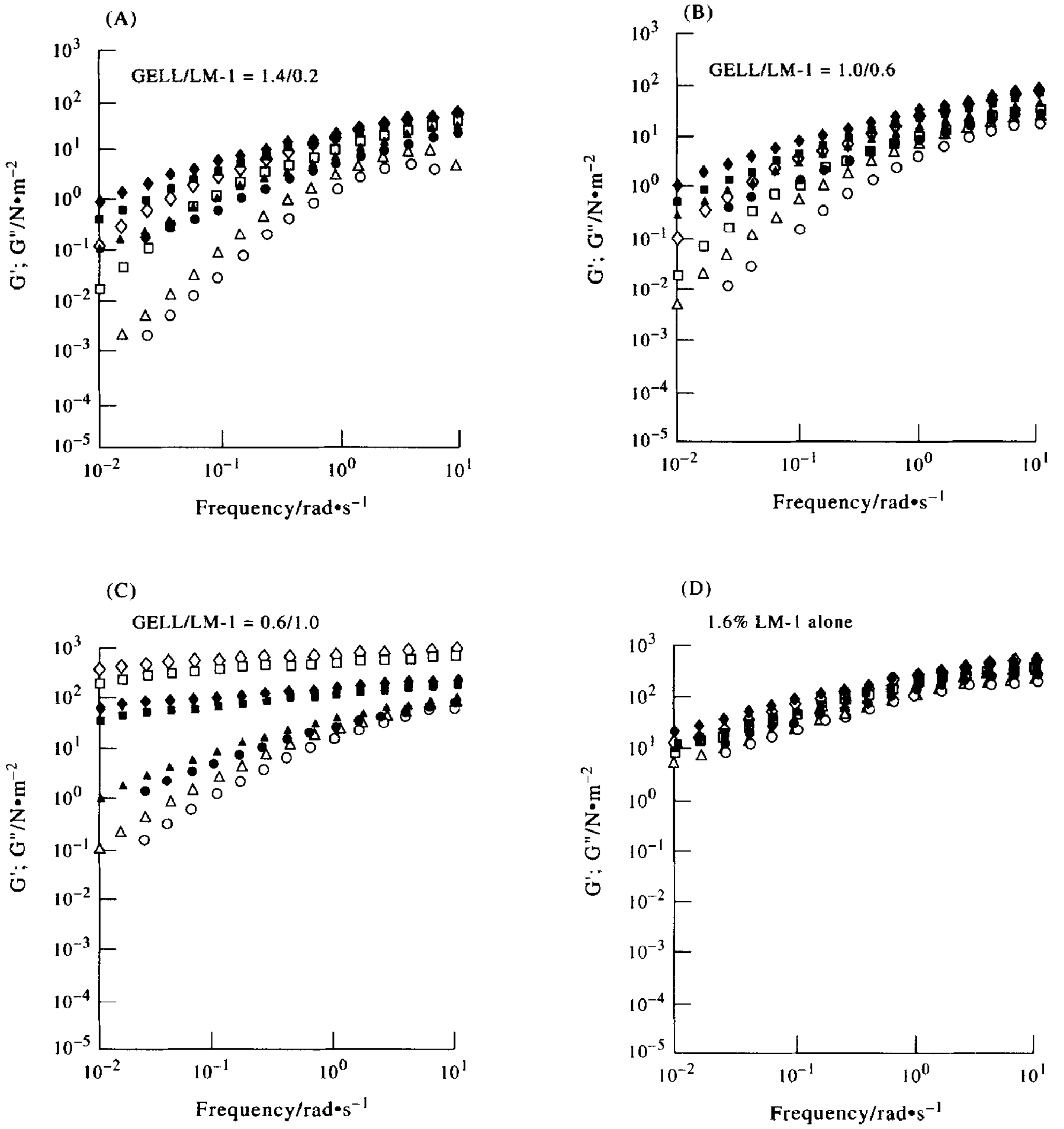

Fig. 3. Frequency dependence of storage modulus G′ and loss modulus G″ for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios at various temperatures. (○) G′, (●) G″, 30°C; (△) G′, (▲) G″, 25°C; (□) G′, (■) G″, 15°C; (◇) G′, (◆), G″, 0°C; mixing ratios of GELL/LM-1: (A) 1.4:0.2, (B) 1.0:0.6, (C) 0.6:1.0, (D) 0.0:1.6.

Figure 5 shows the storage modulus G′ for GELL/ND mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at 1.0 rad/s and at various temperatures. At any temperature from 30 to 0°C, G′ for mixtures increased monotonically with increasing content of ND in the mixtures, though the modulus for a mixture with GELL/ND = 0.8/0.8 showed a shallow dip at any temperature. G′ for a 1.6% solution of ND alone was significantly larger than that for any other mixtures and was little dependent on temperature. Although the highest molecular weight fraction ND seems to inhibit the helix formation of gellan more, as will be discussed later, the storage modulus of GELL/ND mixtures as a function of ND content (Fig. 5) increases with increasing ND content. This is simply because G′ of a solution of ND alone is far larger (more than two decades!) than that of gellan alone. The inhibition seems to be most important at a mixing ratio of GELL/ND = 0.8/0.8. Since ND inhibits most the helix formation of gellan, no synergistic interaction was observed in these mixtures (Fig. 5).

Figure 6 shows the storage modulus G′ for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at 1.0 rad/s and at various temperatures. The behavior of G′ as a function of LM-1 content in gellan gum/LM-1 mixtures depended on temperature. G′ at 30°C increased monotonically with increasing content of LM-1 in mixtures whilst G′ at 25°C was maximum at a mixing ratio of GELL/LM-1 = 0.4/1.2 and G′ at 15 or 0°C was maximum at GELL/LM-1 = 0.6/1.0. These findings suggest that the synergistic interaction occurs only between gellan gum molecules in helix conformation and konjac glucomannan because gellan gum

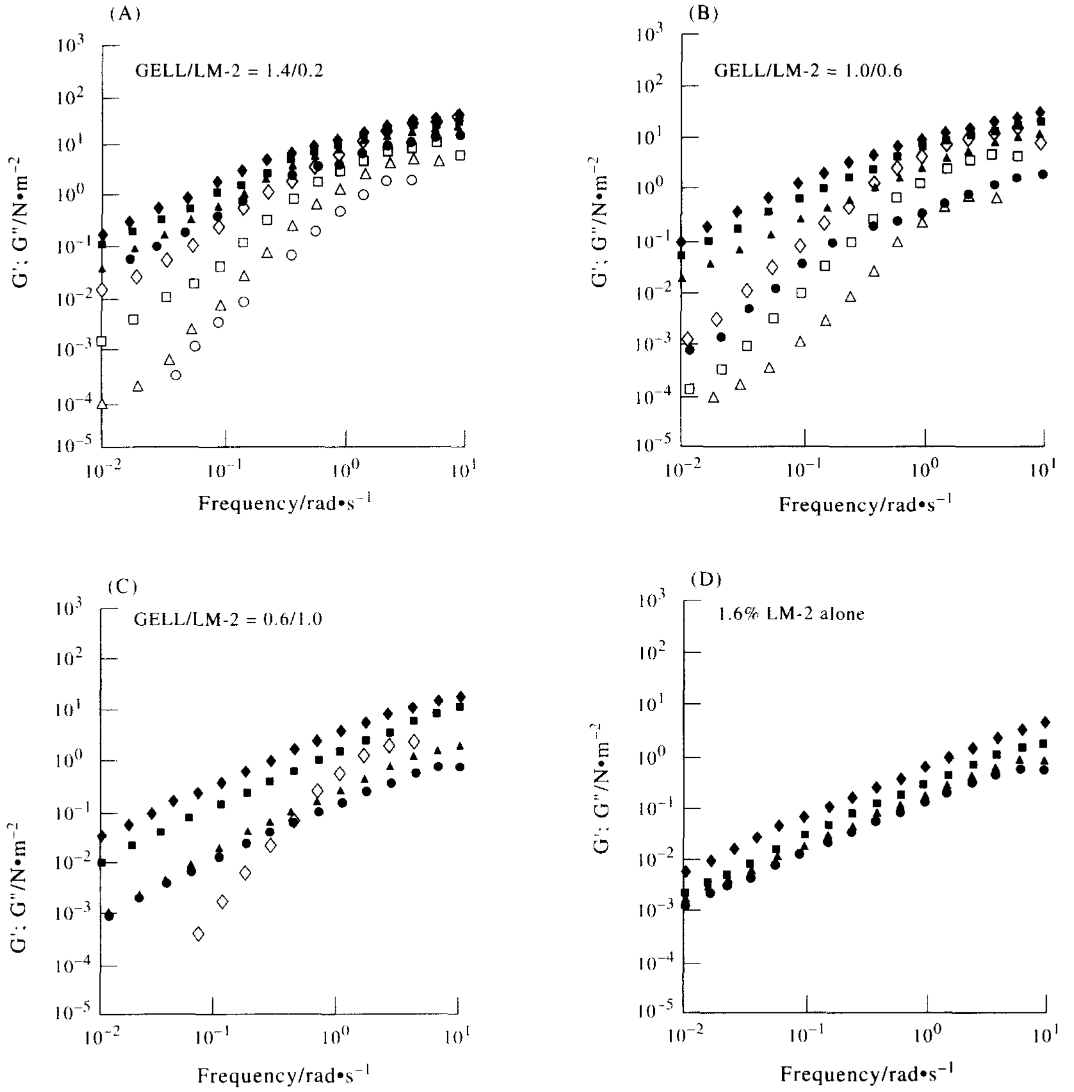

Fig. 4. Frequency dependence of storage modulus G′ and loss modulus G″ for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios at various temperatures. (○) G′, (●) G″, 30°C; (△) G′, (▲) G″, 25°C; (□) G′, (■) G″, 15°C; (◇) G′, (◆), G″, 0°C; mixing ratios of GELL/LM-2: (A) 1.4:0.2, (B) 1.0:0.6, (C) 0.6:1.0, (D) 0.0:1.6.

molecules adopts a helical conformation at lower temperatures.

Figure 7 shows the storage modulus G′ for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at 1.0 rad/s and at various temperatures. The behavior in GELL/LM-2 mixtures was significantly different from that in GELL/LM-1 or GELL/ND mixtures with the same total polymer concentration. G′ as a function of LM-2 content at 0°C slightly increased up to LM-2 content 0.4 and then rapidly decreased with increasing content of LM-2 in the mixtures. At 15, 25 and 30°C, G′ became maximum at LM-2 content 0.2 ~ 0.4. G′ at any temperature for mixtures with more than LM-2 content 1.4 was too small to be detected. Thus, in the case of GELL/LM-2 mixtures, the significant decrease of G′ with increasing concentration of LM-2 in mixtures occurred, so that it seems that G′ for the GELL/LM-2 mixtures is dominated by the modulus of the gellan gum, however, it appeared that synergism occurred with LM-2 only at lower konjac glucomannan content around 0.2. Since the lowest molecular weight fraction LM-2 does not inhibit the helix formation so much with comparison to LM-1 and ND, as will be discussed later, the synergistic interaction may occur over a wider temperature range as shown in Fig. 7.

Figure 8 shows the storage modulus G′ for GELL/ND, GELL/LM-1 and GELL/LM-2 (total polysaccharide concentration 1.6%) as a function of mixing ratio at 1.0rad/s and at 30°C (Fig. 8(A)) and at 0°C (Fig. 8(B)). Closed diamond represents G′ for gellan alone. At all the mixing ratios and at both temperatures, G′ for gellan alone is smaller than that for mixtures. The reason for the increase of G′ by addition of KGM may be ascribed to one of the following two possibilites: (1) an increase of effective concentration of gellan because

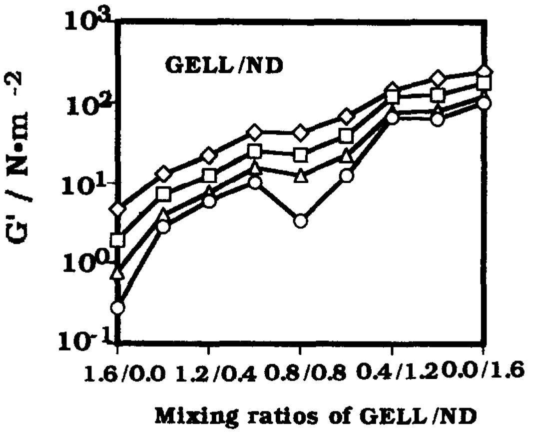

Fig. 5. Storage modulus G′ for GELL/ND mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at various temperatures: (○) 30°C, (△) 25°C, (□) 15°C, (◇) 0°C; freq. ω = 1.0 rad/s.

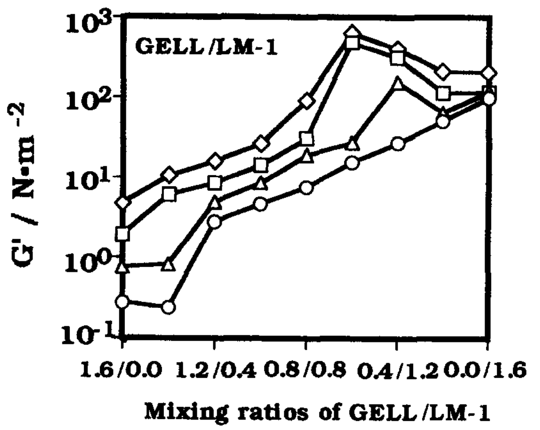

Fig. 6. Storage modulus G′ for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at various temperatures: (○) 30°C, (△) 25°C, (□) 15°C, (◇) 0°C; freq. ω = 1.0 rad/s.

KGM immobilizes water, or in other words, the volume which gellan can occupy is excluded by the presence of KGM; (2) a synergistic interaction between KGM and gellan occurs so that the topological entanglement may play a role of a temporary crosslinking point. The synergistic interaction occurs only at lower temperatures around GELL/LM-1 ratio 0.6/1.0 ~ 0.4/1.2, and at wider temperature range for GELL/LM-2 around the mixing ratio 1.4/0.2 ~ 1.2/0.4 as shown in Figs 6 and 7. As will be shown later (Fig. 13(A) and (B)), however, the synergism between gellan gum and konjac glucomannan does not seem to contribute so much to the increase of G′, and therefore, the first possibility is more plausible. The crosses represent G′ for KGM alone, ✧: ND and +: LM-1. At the mixing ratios with higher KGM contents, values of G′ for mixtures were larger than those for KGM alone. The synergistic interaction seems to be important at lower temperatures.

In order to see whether molecular association between gellan and konjac glucomannan occurs or not, the following experiment was carried out: the storage shear modulus G′ of GELL/KGM mixtures with constant GELL concentration 1% and various LM-2 concentrations was observed. Figure 9 shows G′ as a function of LM-2 concentration, G′ increased with increasing concentration of LM-2, however, it decreased above the concentration of LM-2 ~ 0.3%. Although the total polymer concentration increased, G′ began to decrease at a certain concentration of LM-2, indicating the occurrence of the phase separation. Incompatibility between polymers is a common phenomenon (Dea & Morrison, 1975; Watase & Nishinari, 1980; Clark *et al.*, 1982; 1983; Clark & Ross-Murphy, 1985; Clark & Lee-Tuffnell, 1986; Leloup *et al.*, 1990; Annable *et al.*, 1994b) and frequently leads to a phase separation.

Figure 10 shows the temperature dependence of G′ and G″ during a cooling or a heating process for a 1.6% solution of gellan gum alone at 0.1 rad/s at a cooling or heating rate of 0.5°C/min. During the cooling process, G′ for a 1.6% solution of gellan gum alone began to be detected around 26°C, corresponding to the setting temperature T_s, and increased gradually with decreasing temperature, while G″ could be detected from 50°C and began to increase rapidly at ~ 35°C and then increased gradually. During the heating process, G′ for a 1.6% solution of gellan gum alone decreased gradually with increasing temperature and then became too small to be detected, corresponding to the melting temperature T_m, although G″ began to decrease rapidly at ~30°C and then decreased gradually. The temperature at which G″ drastically changes should be a kind of transition temperature. The midpoint temperature of the transition T_M is defined as the temperature at which G″ changes most steeply, and the relaxational strength ΔG is defined as the difference between G″ at the lower and higher temperaure sides at T_M. The relaxational strength ΔG is usually defined using the storage modulus (Wada, 1971). However, G″ could be detected over a

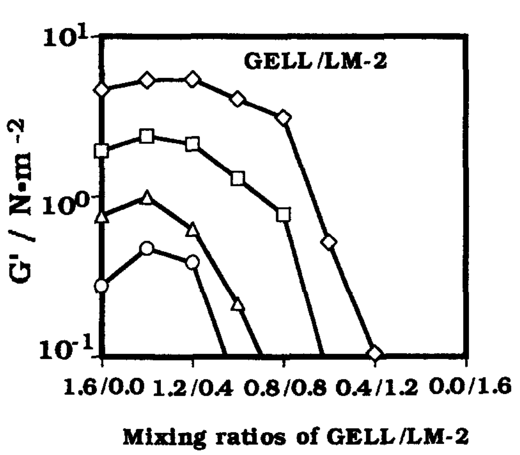

Fig. 7. Storage modulus G′ for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio at various temperatures. (○) 30°C, (△) 25°C, (□) 15°C, (◇) 0°C; freq. ω = 1.0 rad/s.

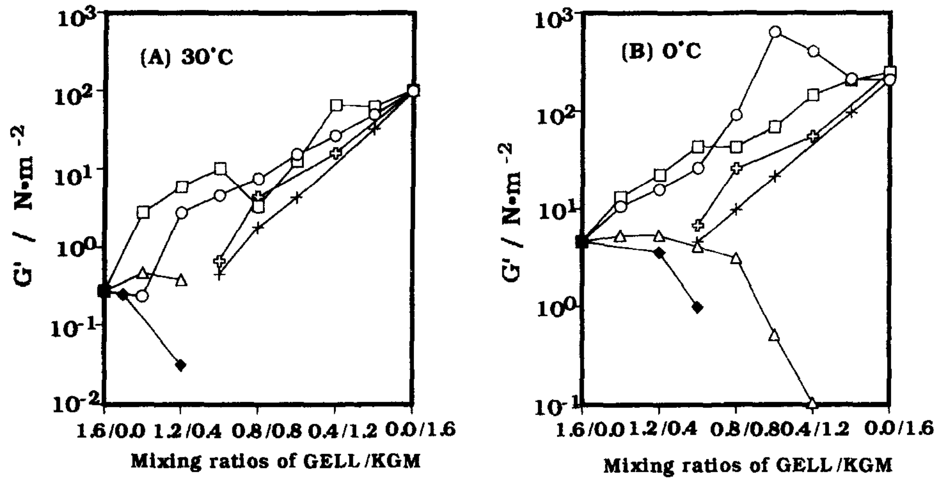

Fig. 8. Storage shear modulus G′ of GELL/KGM mixtures (total polysaccharide concentration 1.6%), gellan gum alone and konjac glucomannan alone as a function of mixing ratio at 30°C (A) and at 0°C (B). (◆) Gellan gum alone, (△) GELL/LM-2 mixtures, (○) GELL/LM-1 mixtures, (□) GELL/ND mixtures, (+) LM-1 alone, (⌖) ND alone.

wider temperature range than G′ in the present study, and the change in G″ at the transition was used as the relaxational strength.

Figure 11 shows the temperature dependence of G′ and G″ during cooling or heating process for GELL/ND mixtures (total polysaccharide concentration 1.6%) with various mixing ratios and at 0.1 rad/s and at 0.5°C/min. The thermal behavior of G′ and G″ for a mixture with GELL/ND = 1.4/0.2 (Fig. 11(A)) was similar to that for a gellan gum alone (Fig. 10), although the temperature at which G′ begins to be detected in the cooling process shifted to higher temperatures. There was little thermal hysteresis for a mixture with GELL/ND = 1.0/0.6 (Fig. 11(B)) or 0.6/1.0 (Fig. 11(C)), and G′ and G″ became less temperature dependent with increasing content of ND. For a 1.6% solution of ND alone (Fig. 11(D)), both moduli were significantly larger throughout the accessible temperature range than those of any other mixtures (Fig. 11(A) ~ (C)) and became less temperature dependent. The midpoint temperature T_M of the transition shifted to lower temperatures and the relaxational strength ΔG at T_M decreased monotonically with increasing content of KGM. As was described above, the behavior of the mixtures of 1.6% total polysaccharide concentration are characteristic of dilute or concentrated solutions. Therefore, the transition observed from the temperature dependence of G′ and G″ should not be attributed to sol–gel transition. This may be induced by the coil-helix transition. At low concentrations of gellan gum molecules, the helix formation and its aggregation to form a certain ordered

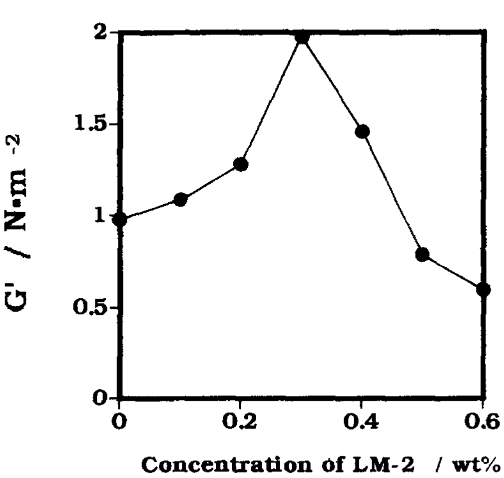

Fig. 9. Storage shear modulus G′ of GELL/KGM mixtures with a constant concentration of GELL(1%) and various concentration of LM-2 as a function of LM-2 concentration at 0°C. freq. = 1.0 rad/s.

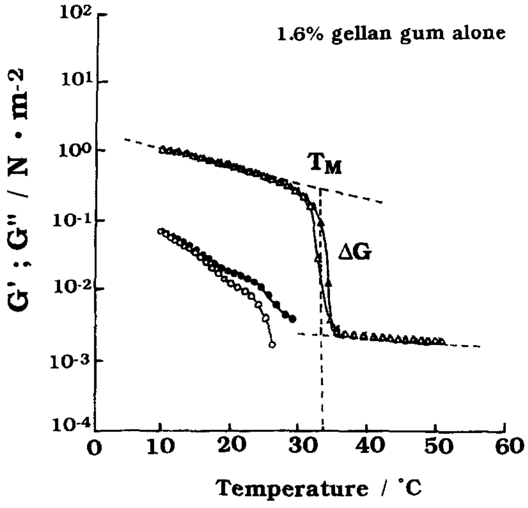

Fig. 10. Temperature dependence of storage modulus G′ and loss modulus G″ during cooling or heating process for a 1.6% solution of gellan gum alone. (○) G′, (△) G″, cooling; (●) G′, (▲) G″, heating; cooling and heating rate: 0.5°C/min; freq. ω = 0.1 rad/s; ΔG represents the relaxational strength, and T_M stands for the midpoint temperature of the transition.

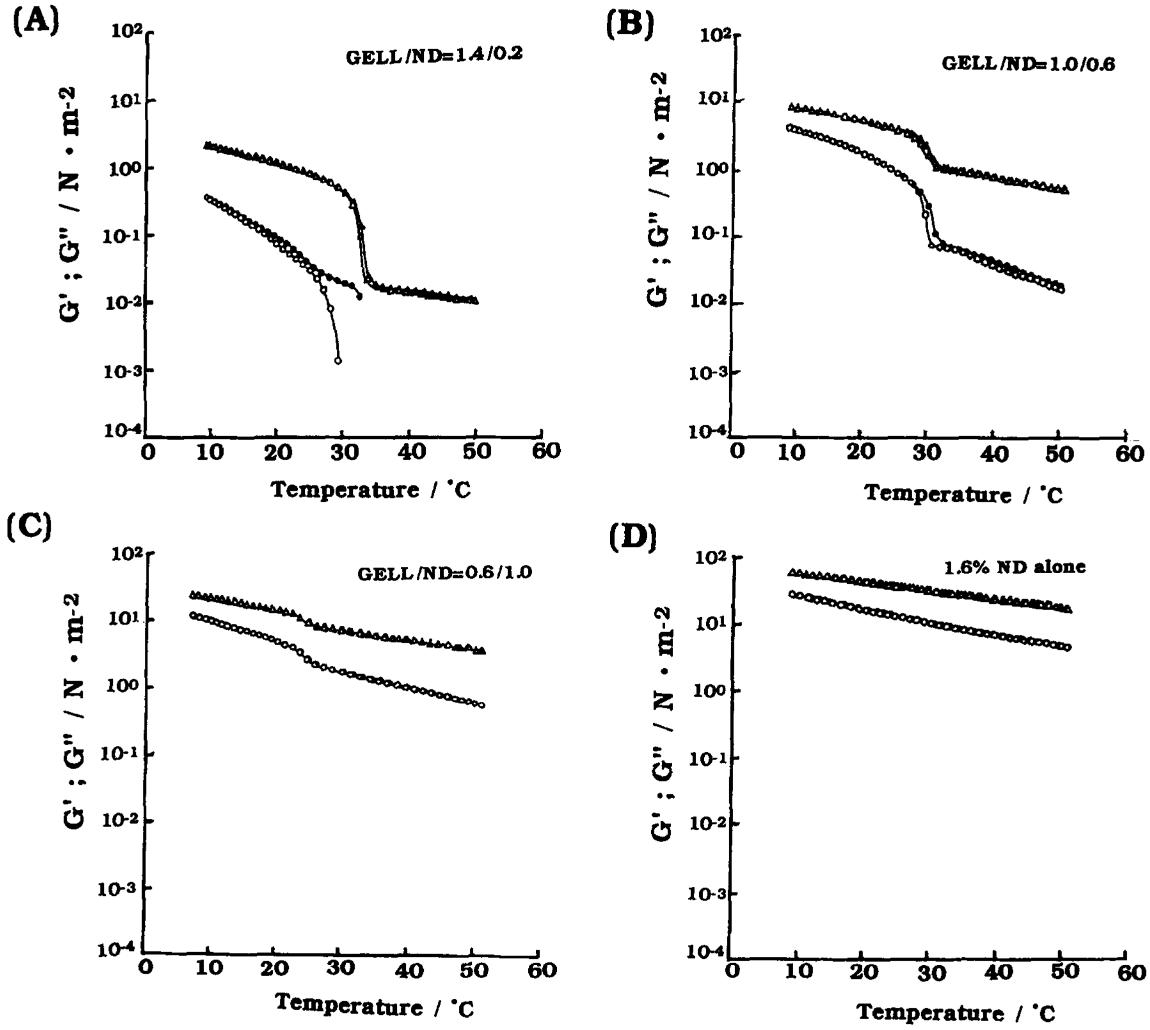

Fig. 11. Temperature dependence of storage modulus G′ and loss modulus G″ during cooling or heating process for GELL/ND mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. (○) G′, (△) G″, cooling; (●) G′,(▲) G″, heating; cooling and heating rate: 0.5°C/min; freq. ω = 0.1 rad/s; mixing ratios of GELL/ND: (A) 1.4:0.2, (B) 1.0:0.6, (C) 0.6:1.0, (D) 0.0:1.6.

structure does not lead to gel formation because the number of helical aggregates is not sufficient to give a continuous network throughout the whole solution. Therefore, the apparent plateau at lower temperatures should not be identified as the rubbery plateau, but it is similar to the second plateau for suspensions discussed by Onogi *et al.* (1970) and Masuda *et al.* (1970).

Figure 12 shows the temperature dependence of G′ and G″ during the cooling or heating process for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios at 0.1 rad/s and at a cooling or heating rate of 0.5°C/min. The thermal behavior of G′ and G″ for a mixture with GELL/LM-1 = 1.4/0.2 (Fig. 12(A)) was similar to that for a gellan gum alone. However, the temperature at which G′ begins to be detected in the cooling process shifted to higher temperatures and G′ and G″ increased with increasing content of LM-1. G′ for a mixture with GELL/LM-1 = 1.0/0.6 (Fig. 12(B)) could be also detected even at 50°C in both cooling and heating process, and G′ and G″ became less temperature dependent. Moreover, the curves of both moduli in the cooling process almost coincided with those in the heating process, so that little thermal hysteresis was exhibited. G′ and G″ for a 1.6% solution of LM-1 alone (Fig. 12(D)) were significantly larger throughout the accessible temperature range than those of any other mixtures (Figs 12(A)–(C)) and became less temperature dependent.

Figure 13 shows the temperature dependence of G′ and G″ during the cooling or heating process for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. The thermal behavior of G′ and G″ for GELL/LM-2 mixtures was significantly different from that for GELL/ND mixtures (Fig. 11) or GELL/LM-1 mixtures (Fig. 12). Mixtures with GELL/LM-2 = 1.4/0.2 (Fig. 13(A)) and 1.0/0.6 (Fig. 13(B)) exhibited thermal hysteresis, however, the temperature at which G′ begins to be detected in the

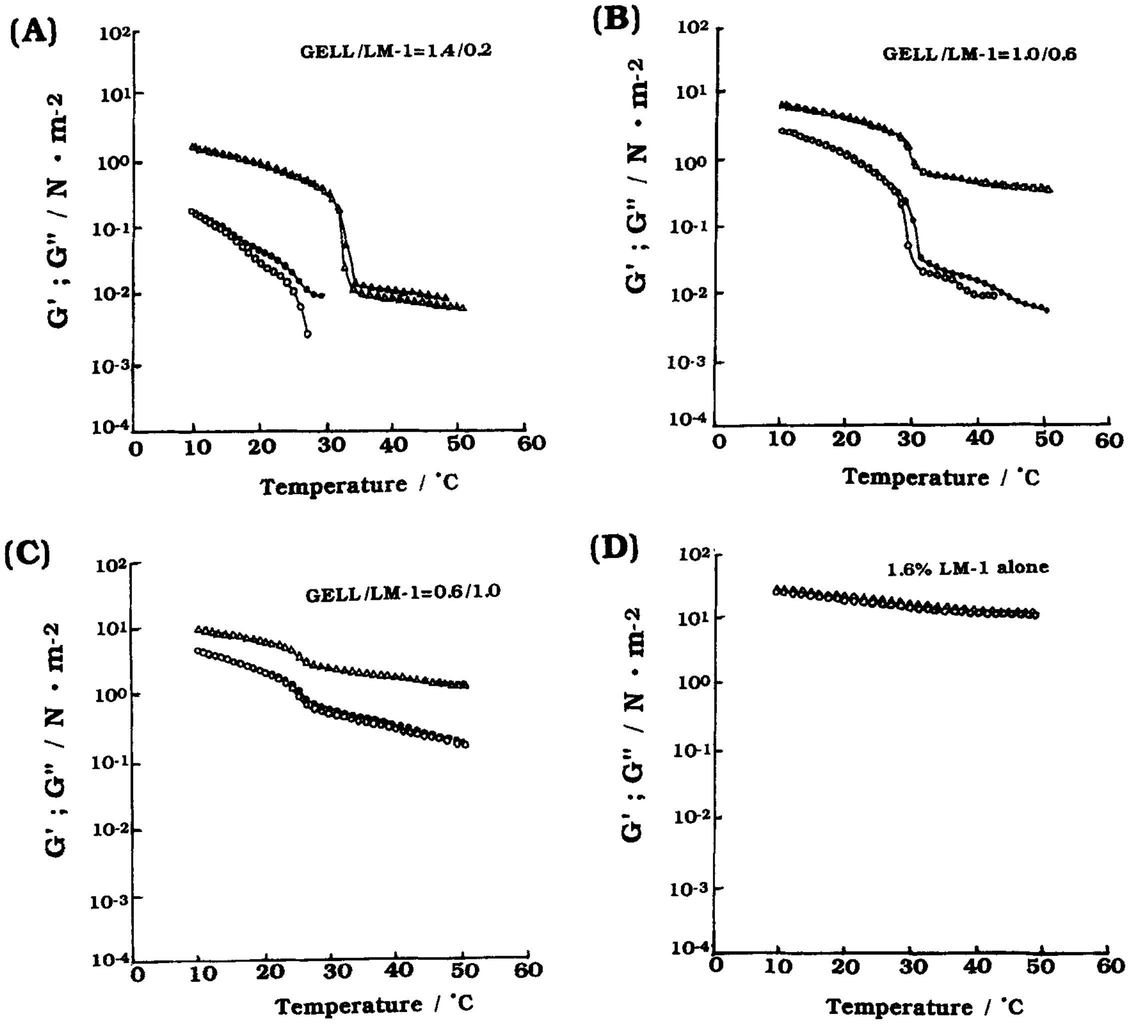

Fig. 12. Temperature dependence of storage modulus G′ and loss modulus G″ during cooling or heating process for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. (○) G′, (△) G″, cooling; (●) G′, (▲) G″, heating; cooling and heating rate: 0.5°C/min; freq. ω = 0.1 rad/s; mixing ratios of GELL/LM-1: (A) 1.4:0.2, (B) 1.0:0.6, (C) 0.6:1.0, (D) 0.0:1.6.

cooling process and the temperature at which G′ becomes too small to be detected in the heating process shifted to lower temperatures with increasing content of LM-2. Moreover, the values of G′ and G″ for these mixtures with GELL/LM-2 became smaller with increasing content of LM-2. G′ for a mixture with LM-2 content 1.0% (Fig. 13(C)) was too small to be detected, and the temperature at which G″ increased steeply shifted to a lower temperature and G″ became less temperature dependent with increasing content of LM-2.

As is seen from Figs 11–13, the temperature dependence of moduli for GELL/KGM mixtures is strongly dependent on molecular weight. In all three cases, the midpoint transition temperature T_M shifted to lower temperatures, and the relaxation strength ΔG decreased with increasing content of KGM (Fig. 14(A) and (B)). T_M and ΔG for gellan alone is also shown in this Fig. 14 (A) and (B) for the comparison with those for mixtures. At all the mixing ratios and for all the KGM fractions, ΔG for gellan alone is larger than that for mixtures. This is in good agreement with DSC results as will be shown later. Since the main contribution to the magnitude of ΔG is by the formation and the subsequent aggregation of helices of gellan molecules, this suggests that the process is inhibited by the presence of KGM. The relaxation strength ΔG decreased with increasing molecular weight of KGM, therefore, the inhibition becomes more important with increasing molecular weight of KGM. However, as is shown in Figs 6 and 7, some synergistic effects were observed at a certain mixing ratio where G′ for a mixture is larger than G′ for a pure component at lower temperatures.

DSC

Figure 15 shows cooling and heating DSC curves for gellan gum/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios of

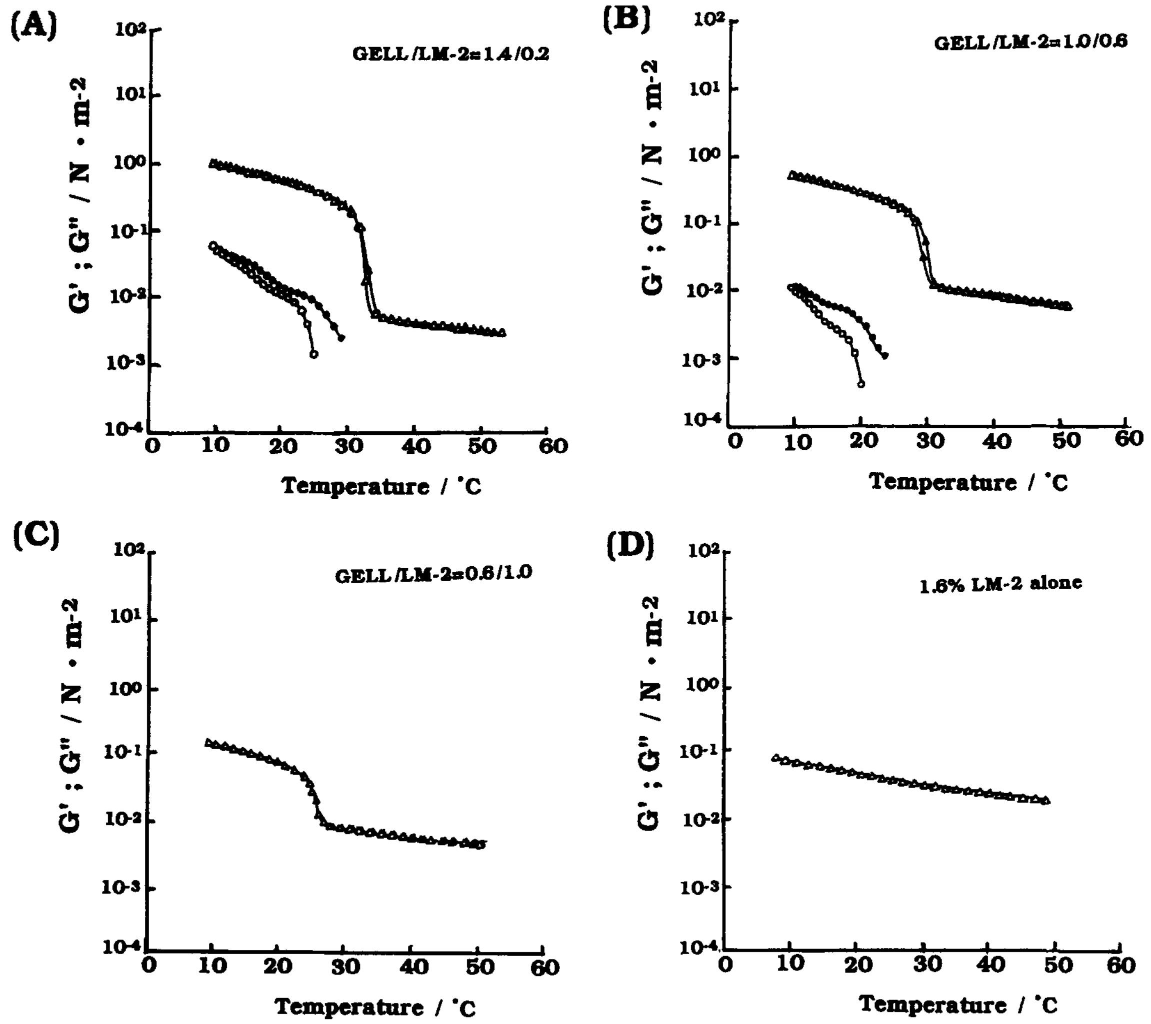

Fig. 13.. Temperature dependence of storage modulus G′ and loss modulus G″ during cooling or heating process for GELL/LM-2 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. (○) G′, (△) G″, cooling; (●) G′, (▲) G″, heating; cooling and heating rate: 0.5°C/min; freq. ω = 0.1 rad/s; mixing ratios of GELL/LM-2: (A) 1.4:0.2, (B) 1.0:0.6, (C) 0.6:1.0, (D) 0.0:1.6.

GELL/LM-1. The cooling curve for a 1.6% solution of gellan gum alone (Fig. 15(A)(a)) showed a main exothermic peak at 32.3°C and another small peak at 95.1°C whilst the heating curve (Fig. 15(B)(a)) showed only a main endothermic peak at 33.7°C. The experimental result that the endothermic peak temperature T_m in a heating DSC curve is higher than the exothermic peak temperature T_s in a cooling DSC curve is commomly observed for many thermoreversible systems and could be understood by a zipper model approach (Nishinari *et al.*, 1990). As reported previously, however, the extrapolation of scan rate to zero will make this difference between T_m and T_s negligible for gellan gum (Manning, 1992; Miyoshi *et al.*, 1994b). Both these exothermic and endothermic peaks should not be attributed to gel–sol transition because in all these cases, the frequency dependence described above shows that these mixed systems are not in a gel state, but in sol state. The cooling or heating curves for all mixed gellan gum/LM-1 solutions also showed a main exothermic or a main endothermic peak at a temperature range from 15 to 35°C, however, both exothermic peak temperature T_s and endothermic peak temperature T_m shifted to lower temperatures with increasing content of LM-1 in mixtures. The midpoint temperature of transition T_M determined from rheological measurements seems to be inbetween T_s and T_m determined by DSC measurements. The exothermic and endothermic enthalpies for mixtures decreased with increasing content of LM-1 in mixtures, moreover, for mixtures where LM-1 is in excess, both exothermic peak and endothermic peak became very broad. Eventually, the cooling or heating DSC curve for a 1.6% solution of konjac mannan (LM-1) alone (Fig. 15(A) curve(i) and (B) curve(i)) did not show any exothermic or endothermic peak, suggesting that konjac glucomannan alone does not gel or does not show any conformational change such as helix-coil transition, and is consistent

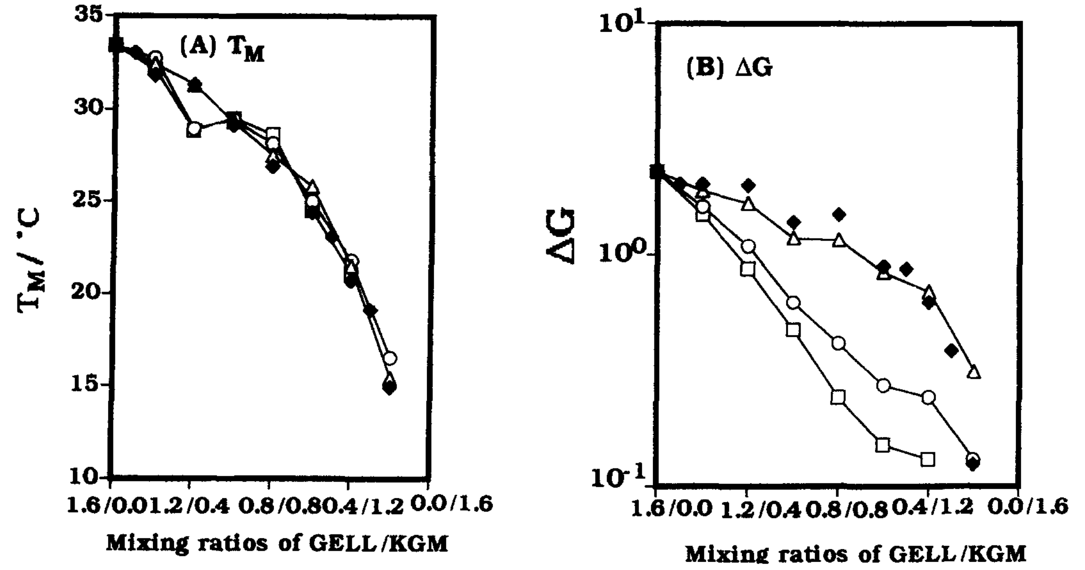

Fig. 14. Midpoint temperature T_M (A) and the relaxation strength ΔG (B) for GELL/KGM mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio. (◆) Gellan gum alone, (□) GELL/ND mixtures, (○) GELL/LM-1 mixtures, (△) GELL/LM-2 mixtures.

with rheological results mentioned above, which agreed with previous results reported by many other workers. The fact that a cooling DSC curve shows two exothermic peaks, whilst a heating DSC curve shows only one endothermic peak, has often been observed for many thermoreversible gel systems (Williams *et al.*, 1993). The higher temperature exothermic peak in cooling DSC curves for GELL/LM-1 mixtures in Fig. 13(A) may be attributed to the formation of ordered structure by the presence of small amount of calcium ions. As was

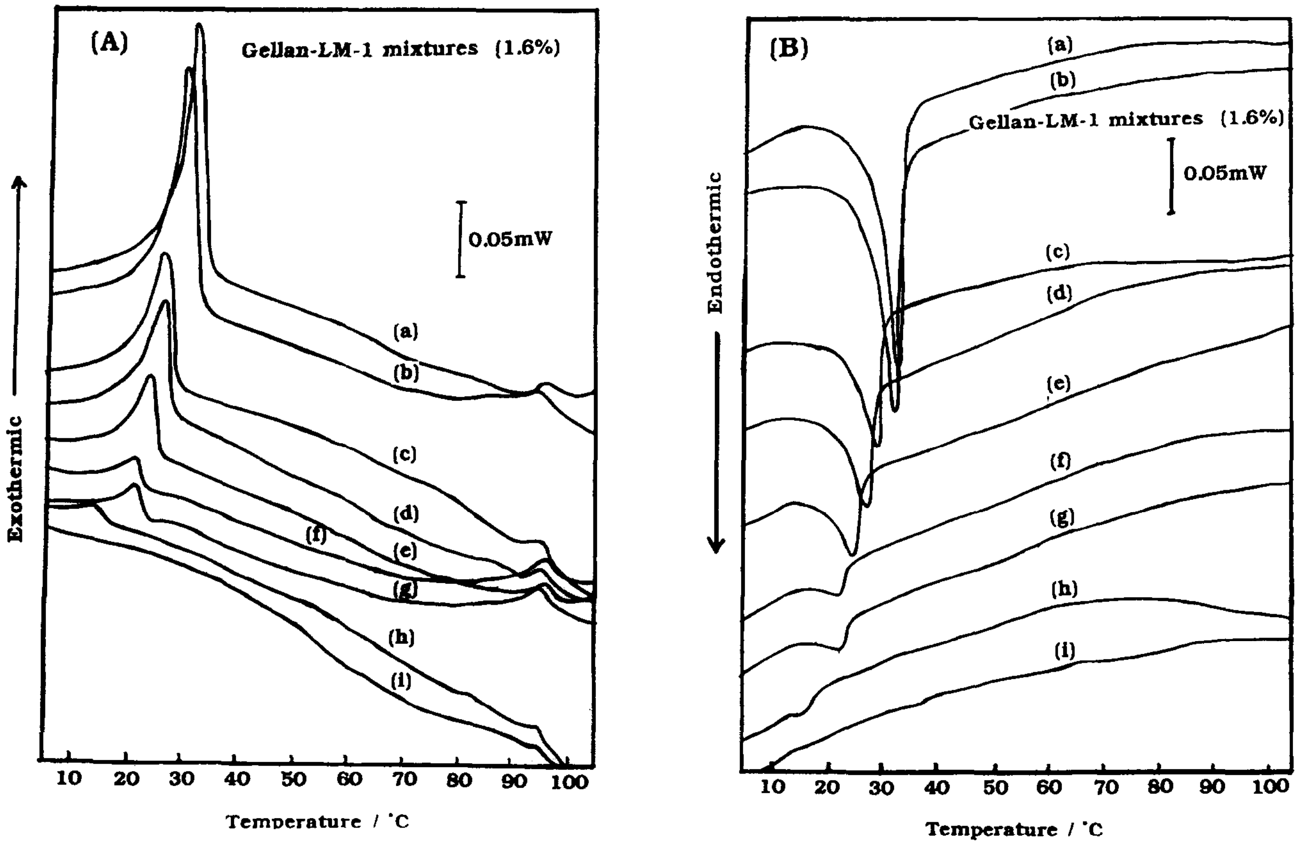

Fig. 15. Cooling (A) and heating (B) DSC curves for GELL/LM-1 mixtures (total polysaccharide concentration 1.6%) with various mixing ratios. Cooling and heating rate: 0.5°C/min; mixing ratios of GELL/LM-1: (a) 1.6/0.0, (b) 1.4/0.2, (c) 1.2/0.4, (d) 1.0/0.6, (e) 0.8/0.8, (f) 0.6/1.0, (g) 0.4/1.2, (h) 0.2/1.4, (i) 0.0/1.6.

shown in a previous study (Miyoshi *et al.*, 1994b) an exothermic peak in cooling DSC curves splits into multiple peaks in the presence of excessive cations. It is improbable that the helix-coil transition of gellan molecules shifted to such a higher temperature on addition of konjac glucomannan.

In the case of ND or LM-2, cooling and heating DSC curves for mixtures showed a similar tendency to those of gellan gum/LM-1 mixtures (data not shown), i.e. the exothermic peak temperature T_s in cooling DSC curves as well as the endothermic peak temperature T_m in heating DSC curves shifted to lower temperatures with increasing content of KGM.

The experimental findings that both T_s and T_m for the mixtures with 1.6% total polysaccharide concentration shifted to lower temperatures with decreasing gellan gum content may be ascribed to the decrease of gellan content itself and/or to the inhibition of helix formation of gellan by konjac glucomannan. Figure 16 shows the dependence of T_s(A) or T_m(B) for the gellan gum/various konjac mannan mixtures as a function of mixing ratio, compared to those for solutions of gellan gum alone at various concentrations.

It is clearly demonstrated that the T_s or T_m of gellan gum/konjac glucomannan mixed systems in the DSC measurement was shifted to lower temperatures with increasing content of konjac glucomannan whatever its molecular weight. This may suggest that the ordered structure was little dependent on the molecular weight of konjac glucomannan. However, as stated above, it was shown that the temperature and frequency dependence of moduli for gellan gum/konjac glucomannan mixtures showed a strong dependence on the molecular weight of konjac glucomannan. Therefore, the effects of molecular weight of konjac glucomannan would be more significant for rheological properties than for thermal properties as in the case of κ-carrageenan/konjac glucomannan mixed systems reported previously (Kohyama *et al.*, 1993). In all cases, at lower concentrations of gellan gum (hence higher content of konjac mannan), both T_s and T_m for mixed systems were slightly higher than those for gellan gum itself. When the concentration of gellan gum in the mixture reached 0.8% (the mixing ratio of GELL/KGM reached 1:1), both T_s and T_m for the mixed system were slightly lower than those for gellan gum alone. At higher concentrations of gellan gum (hence lower content of konjac mannan), both T_s and T_m for the mixed systems were slightly lower than those for gellan gum alone although both T_s and T_m for the mixture with GELL/LM-1 = 1.4/0.2 were slightly higher than those for gellan gum alone. Therefore, the shift of T_s and T_m to lower temperatures for the mixtures in which KGM content is higher than 50% is attributed to the decrease of gellan content and it is noteworthy that the excess KGM promotes the coil-helix transition of gellan. It is possible to expect some synergistic interaction at higher KGM content as also shown in the rheological results (Fig. 6). On the other hand, the shift of T_s and T_m to lower temperatures for the mixtures in which KGM content is lower than 50% is attributed to the inhibition of gellan helix formation by KGM. In all cases, T_s and T_m for the mixed systems decreased in the order of ND, LM-1, LM-2.

Recently, it was suggested that the interaction between konjac glucomannan and κ-carrageenan is weaker than that between κ-carrageenan and κ-carrageenan, but strong enough to produce another elastically-active chains (Kohyama *et al.*, 1993). In gellan/konjac glucomannan mixtures, the main ordered structure may be formed by gellan molecules, and konjac

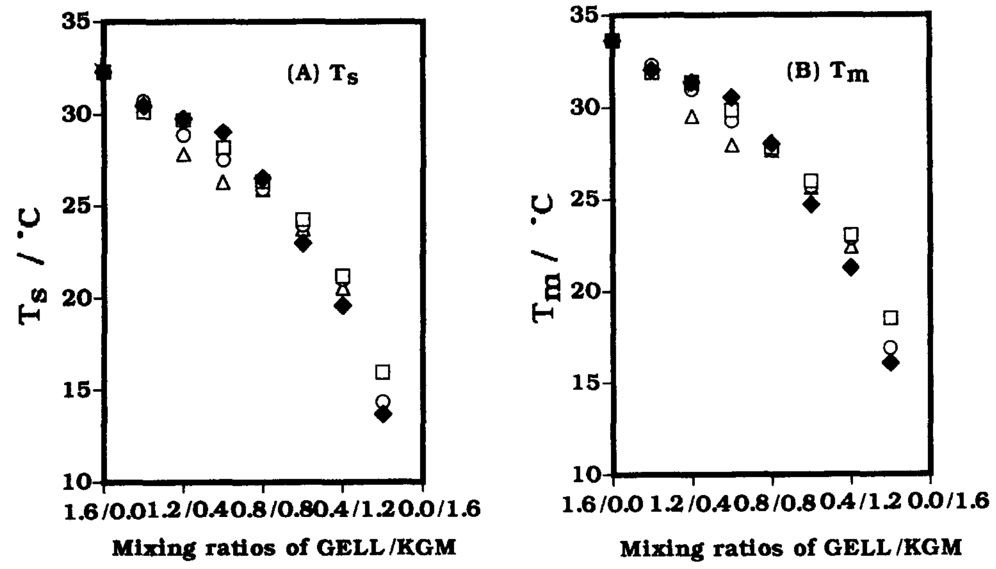

Fig. 16. Exothermic peak temperature T_s(A) and endothermic peak temperature T_m(B) for a solutions of gellan gum alone and GELL/KGM mixtures (total polysaccharide concentration 1.6%) as a function of mixing ratio. (◆) Gellan gum alone, (□) GELL/ND mixtures, (○) GELL/LM-1 mixtures, (△) GELL/LM-2 mixtures.

glucomannan molecules inhibit gellan molecules to form the ordered structure. However, the present data were obtained only from rheological and thermal measurements, and, investigation by other methods is required.

REFERENCES

Almdal, K., Dyre, J., Hvidt, S. & Kramer, O. (1993). Towards a phenomenological definition of the term 'Gel'. *Polymer Gels and Networks*, **1**, 5–17.

Annable, P., Williams, P.A. & Nishinari, K. (1994a). Interaction in xanthan–glucomannan mixtures and the influence of electrolyte. *Macromolecules*, **27**, 4204–4211.

Annable, P., Fitton, M.G., Harris, B., Phillips, G.O. & Williams, P.A. (1994b). Phase behaviour and rheology of mixed polymer systems containing starch. *Food Hydrocoll.*, **8**, 351–359.

Brownsey, G.J., Cairns, P., Miles, M.J. & Morris, V.J. (1988). Evidence for intermolecular binding between xanthan and the glucomannan konjac mannan. *Carbohydr. Res.*, **176**, 329–334.

Cairns, P., Morris, V.J., Miles, M.J. & Brownsey, G.J. (1986). Effect of the molecular fine structure of galactomannans on their interaction properties. *Food Hydrocoll.*, **1**, 89–93.

Cairns, P., Miles, M.J., Morris, V.J. & Brownsey, G.J. (1987). X-ray fibre diffraction studies of synergistic, binary polysaccharide gel. *Carbohydr. Res.*, **160**, 411–423.

Chapman, H.D., Chilvers, G.R., Miles, M.J. & Morris, V.J. (1990). Studies on the gelation of the microbial polysaccharides XM6 and gellan gum. In *Gums and Stabilisers for the Food Industry*, Vol.5, eds. G.O. Phillips, D.J. Wedlock & P.A. Williams, Pergamon Press, Oxford and New York, pp. 147–155.

Cheetham, N.W.H. & Mashimba, E.N.M. (1988). Comformational aspects of xanthan–galactomannan. *Carbohydr. Polym.*, **9**, 195–212.

Cheetham, N.W.H. & Mashimba, E.N.M. (1991). Comformational aspects of xanthan–galactomannan gelation. Further evidence from optical–rotation studies. *Carbohydr. Polym.*, **14**, 17–21.

Clark, A.H. & Lee–Tuffnell, C.D. (1986). Gelation of globular proteins. In *Functional Properties of Food Macromolecules*, 1st edn, eds J.R. Mitchell & D.A. Ledward, Elsevier Applied Science, London and New York. pp. 203–272.

Clark, A.H., Richardson, R.K., Ross–Murphy, S.B. & Weaver, A.C. (1982). Structure and mechanical properties of agar/BAS co-gels. In *Gum and Stabilisers for Food Industry*, Vol. 1, eds G.O. Phillips, D.J. Wedlock & P.A. Williams, Pergamon Press, Oxford and New York, pp. 149–160.

Clark, A.H., Richardson, R.K., Ross–Murphy, S.B. & Stubbs, J.M. (1983). Structural and mechanical properties of agar/gelatin co-gels. Small-deformation studies, *Macromolecules*, **16**, 1367–1374.

Clark, A.H. & Ross-Murphy, S.B. (1985). The concentration dependence of biopolymer gel modulus. *Brit. Polym. J.*, **17**, 164–168.

Clark, A.H. & Ross-Murphy, S.B. (1987). Structural and mechanical properties of biopolymers gels. *Adv. Polym. Sci.*, **83**, 57–192.

Crescenzi, V., Dentini, M., Coviello, T. & Rizzo, R. (1986). Comparative analysis of the behaviour of gellan gum (S-60) and welan gum (S-130) in dilute aqueous solution. *Carbohydr. Res.*, **149**, 425–432.

Crescenzi,V., Dentini, M. & Dea, I.C.M. (1987). The influence of side-chains on the dilute-solution properties of three structurally related, bacterial anionic polysaccharides. *Carbohydr. Res.*, 283–302.

Dea, I.C.M. & Morrison, A. (1975). Chemistry and interactions seed galactomannans. *Adv. Cabohydr. Chem. Biochem.*, **31**, 241–312.

Dea, I.C.M. (1979). Interactions of ordered polysaccharide structures-synergism and freeze-thaw phenomena. In *Polysaccharides in Food*, eds J.M.V. Blanshard & J.R. Mitchell, Butterworths, London, pp. 229–247.

Dentini, M., Coviello, T., Burchard, W., Crescenzi, V. (1988). Solution properties of exocellular microbial polysaccharides. 3. Light scattering from gellan and from the exocellular polysaccharide of *Rhizobium trifolii* (strain TA-1) in the ordered state. *Macromolecules*, **21**, 3312–3320.

Fernandes, P.B., Goncalves, M.P. & Doublier, J.L. (1991). A rheological characterization of kappa-carrageenan/galactomannan mixed gels: a comparison of locust bean gum samples. *Carbohydr. Polym.*, **16**, 253–274.

Fernandes, P.B., Goncalves, M.P., Doublier, J.L. (1992). Effect of galactomannan. Addition on the Thermal Behaviour of κ-Carrageenan Gels. *Carbohydr. Polym.*, **19**, 261–269.

Grasdalen, H. & Smidsrod, O. (1987). Gelation of gellan gum. *Carbohydr. Polym.*, **7**, 371–393.

Graessley, W.W. (1974). The Entanglement Concept in Polymer Rheology. *Adv. in Polym. Sci.*, **16**, 1–179.

Jansson, P., Lindberg, B. & Sandford, P.A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*. *Carbohydr. Res.*, **124**, 135–139.

Kohyama, K., Iida, H. & Nishinari, K. (1993). A mixed system composed of different molecular weights konjac glucomannan and kappa-carrageenan: Large deformation and dynamic viscoelastic study. *Food Hydrocoll.*, **7**, 213–226.

Leloup, V.M., Colonna, P. & Buleon, A. (1991). Influence of amylose–amylopectin ratio on gel properties. *J. Cereal Sci.*, **13**, 1–13.

Manning, C.E. (1992). Formation and melting of gellan polysaccharide gels, Thesis, Cranfield Institute of Technology, Silsoe College, Silsoe.

Masuda, T., Kitagawa, K. & Onogi, S. (1970). Viscoelastic properties of poly(methacrylates) prepared by anionic polymerization. *S. Polymer J.*, **1**, 418–424.

Milas, M., Shi, X. & Rinaudo, M. (1990). On the physicochemical properties of gellan gum. *Biopolymers*, **30**, 451–464.

Miyoshi, E., Takaya, T. & Nishinari, K. (1994a). Gel–sol transition in gellan gum solutions I. Rheological studies on the effects of salts. *Food Hydrocoll.*, **8**, in press.

Miyoshi, E., Takaya, T. & Nishinari, K. (1994b) Gel–sol transition in gellan gum solutions II. DSC studies on the effects of salts. *Food Hydrocoll.*, **8**, in press.

Morris, V.J. (1986). In *Gums and Stabilisers for the Food Industry*, Vol. 3, eds G.O. Phillips, D.J. Wedlock & P.A. Williams, Elsevier Applied Science Publishers, London and New York, pp. 87–99.

Morris, E.R. (1990). Mixed polymer gels. In Food Gels, Elsevier Applied Science, ed. P. Harris, London, pp. 291–359.

Morris, E.R. (1982). Rheology of hydrocolloids. In *Gums and Stabilisers for the Food Industry*, Vol. 2, eds G.O. Phillips, D.J. Wedlock & P.A. Williams, Pergamon Press, Oxford and New York, pp. 57–77.

Nishinari, K., Williams, P.A. & Phillips, O.G. (1992). Review of the physico–chemical characteristics and properties of konjac mannan. *Food Hydrocoll.*, **6**, 199–222.

Nishinari, K., Koide, S., Williams, P.A. & Phillips, G.O. (1990). A zipper model approach to the thermoreversible gel–sol transition. *J. Phys. France*, **51**, 1759–1768.

Nishinari, K. (1988). Food hydrocolloids in Japan. In *Gums and Stabilisers for the Food Industry*, Vol. 4, eds G.O. Phillips, D.J. Wedlock & P.A. Williams, IRL Press, Oxford, pp. 373–390.

O'Neill, M.A., Selvendran, R.R. & Morris, V.J. (1983). Structure of the acidic extracellular gelling polysaccharide produced by *Pseudomonas elodea*. *Carbohydr. Res.*, **124**, 123–133.

Onogi, S., Masuda, T. & Kitagawa, K. (1970). Rheological properties of anionic polystyrenes. I. Dynamic viscoelasticity of narrow-distribution polystyrenes. *Macromolecules*, **3**, 109–116.

Robinson, G., Manning, C.E. & Morris, E.R. (1991). Conformation and physical properties of the bacterial polysaccharides gellan, welan, and rhamsan. In '*Food Polymers, Gels, and Colloids*', ed. E. Dickinson, *Roy. Soc. Chem.*, UK, pp. 22–33.

Rochas, C., Taravel, F.R. & Turquois, T. (1990). N.m.r. studies of synergstic kappa-carrageenan/carob galactomannan gels. *Int. J .Biol. Macromolecules*, **12**, 353–358.

Shatwell, K.P., Sutherland, I.W., Ross-Murphy, S.B. & Dea, I.C.M. (1991). Influence of the acetyl substituent on the interaction of xanthan with plant polysaccharides-III. Xanthan–konjac mannan systems *Carbohydr. Polym.*, **14**, 29–51, 115–130, 131–147.

Shi, X. (1990). Relation entre la conformation et les proprietes d'un polysaccharide bacterien, le gellane, Thesis, Centre de Recherches sur les Macromolecules Vegetales-CNRS, l'Universite Joseph-Fourier.

Tako, M. & Nakamura, S. (1986). Synergistic interaction between kappa-carrageenan and locust-bean gum in aqueous media. *Agric. Biol. Chem.*, **50**, 2817–2822.

Te Nijenhuis, K. (1990). Viscoelastic properties of thermoreversible gels. In *Physical Networks: Polymers and Gels*, eds W. Burchard & S.B. Ross-Murphy, Elsevier Applied Science, London, pp. 15–33.

Turquois, T., Rochas, C. & Taravel, F.R. (1992). Rheological studies of synergistic kappa-carrageenan-carob galactomannan gels. *Cabohydr. Polym.*, **17**, 263–268.

Wada, Y. (1971). In Solid State Physics of Polymers (Kobunshi no kotai-bussei), Baifukan, Tokyo, pp. 260–312, 402–412.

Watase, M. & Nishinari, K. (1980). Rheological properties of agarose–gelatin gels. *Rheol. Acta*, **19**, 220–225.

Williams, P.A., Day, D.A., Langdon, M.J., Phillips, O.G. & Nishinari, K. (1991). Synergistic interaction of xanthan gum with glucomannans and galactomannans. *Food Hydrocoll.*, **6**, 489–493.

Williams, P.A., Clegg, S.M., Langdon, M.J., Nishinari, K. & Piculell, L. (1993). Investigation of the gelation mechanism in κ-carrageenan/konjac mannan mixtures using differential scanning calorimetry and electron spin resonance spectroscopy. *Macromolecules*, **26**, 5441–5446.