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Carbohydrate Polymers 74 (2008) 68–78
Carbohydrate Polymers
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Effects of the lyotropic series salts on the gelation of konjac glucomannan in aqueous solutions

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Received 16 September 2007; received in revised form 14 January 2008; accepted 17 January 2008
Available online 26 January 2008

Abstract

Effects of Lyotropic series salts on the gelation behavior of konjac glucomannan (KGM) in aqueous solutions were studied by means of rheometry and electron spin resonance (ESR). Rheological measurements showed that sulfate alone, heretofore never reported, was capable of inducing KGM form a thermally irreversible gel rather than via deacetylation that has been the approbatory origin when the commonly alkaline coagulant of Na_2CO_3 is used. The increase in rotational correlation time that scales with the microviscosity indicated reduced mobilities of KGM chains upon addition of Na_2SO_4, in agreement with the rheological results. In the presence of Na_2CO_3, salting-out salts facilitated the gelation, whereas salting-in salts assisted the gelation at low salt concentrations but suppressed it at high salt concentrations. It was suggested that the Na_2CO_3 be also of salting-out effect in addition to facilitating the deacetylation of KGM chains.

Keywords: Konjac glucomannan; Lyotropic series salts; Gelation; Rheology; Electron spin resonance; Polysaccharide

1. Introduction

The gelation mechanism of polysaccharides has been extensively studied not only for scientific interests but also for the importance of applications in a variety of fields such as food, chemical, biochemical, pharmaceutical, cosmetic, painting and other relevant industries (Stephen, Phillips, & Williams, 2006). As one of the important industrialized polysaccharides, Konjac glucomannan (KGM) has various realized or promising applications as films or membranes in coating, packaging and as controlled release matrices in addition to having utility in food manufacturing (Dave, Sheth, McCarthy, Ratto, & Kaplan, 1998; Gao & Zhang, 2001; Nishinari, Williams, & Phillips, 1992; Wang & He, 2002).

KGM is a neutral polysaccharide derived from the tuber of *Amorphophallus Konjac C.* (Nishinari et al., 1992), composed of β-(1 → 4) linked β-D-mannose and β-D-glucose in a molar ratio of 1.6:1 (Kato & Matsuda, 1969; Katsuraya et al., 2003; Maeda, Shimahara, & Sugiyama, 1980). There may be some branches linked to the backbone though the exact branched position is still in debate (Katsuraya et al., 2003; Nishinari et al., 1992). KGM backbone possesses a low degree of acetyl groups (approximately 1 acetyl group per 19 residues) (Kato & Matsuda, 1969; Maeda et al., 1980; Maekaji, 1974), which is assumed to be an important factor predominating the solubility of KGM in water. The gelation of KGM has been the motif and drawn to attention in the academic studies and industrial applications for quite a long time. It is well-known that KGM generally forms a thermally irreversible gel in the presence of an alkaline coagulant such as Na_2CO_3 upon heating. Conclusion from early studies by Maekaji (1974) was that KGM loses its acetyl groups upon alkaline treatment, and then deacetylated KGM aggregates with one another through linkages such as hydrogen bonds, by which the KGM chains form junction zones, thereby ultimately lead-

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0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.01.016

ing to gel formation. The effect of the addition of alkali is believed to facilitate the deacetylation of the KGM chains. Now it seems to have been widely accepted that it is the deacetylation that induces the gelation of KGM (Huang, Takahashi, Kobayashi, Kawase, & Nishinari, 2002; Jacon, Rao, Cooley, & Walter, 1993; Williams, Foster, Norton, Yoshimura, & Nishinari, 2000; Yoshimura & Nishinari, 1999; Zhang et al., 2001). Alternatively, KGM can also form synergistic gels with other polysaccharides such as κ-carrageenan (Williams, Clegg, Langdon, Nishinari, & Phillips, 1992; Williams, Clegg, Langdon, Nishinari, & Piculell, 1993), xanthan gum (Annable, Williams, & Nishinari, 1994; Brownsey, Cairns, Miles, & Morris, 1988; Goycoolea, Richardson, & Morris, 1995; Paradossi, Chiessi, Barbiroli, & Fessas, 2002), gellan gum (Miyoshi, Takaya, Williams, & Nishinari, 1996), acetan or deacetylated acetan (Ridout, Brownsey, & Morris, 1998; Ridout, Cairns, Brownsey, & Morris, 1998) without involving the above-mentioned deacetylation. The synergistic models of these binary mixtures have been presented on the basis of the intermolecular interaction, likely hydrogen bonding, supported by different characterization methods. In addition to these, Dave et al. (1998) reported a gelation for KGM at a high concentration above 7 wt%, which was attributed to the formation of liquid crystalline phases. Despite the enormous number of articles devoted to the effects of salts on the physicochemical properties of polysaccharides, little is known about the details of the influence of lyotropic series salts on the rheological properties of KGM. By observing gelation kinetics in the presence of lyotropic series salts with alkaline coagulants, Case, Kropp, Hamann, and Schwartz (1992) suggested that hydrophobic interactions play an important role in the gelation process of KGM. The detailed gelation mechanism for KGM under different conditions has been still under clarification. Besides the effects of salts or ions on the gelation are of importance because salts are commonly present in various systems such as food, chemical, pharmaceutical, biological systems and so forth, the consideration of addition of different salts into a polysaccharide system may be used as a reference to develop a better understanding of its gelation mechanism.

Some anions and cations have been noted to be effective in the order of salting-out to salting-in effect: SO_4^{2-}< $H_2PO_4^-$<Cl^-<NO_3^-<ClO_4^-<SCN^- and K^+<Na^+<H^+< Mg^{2+}<Ca^{2+}<Al^{3+}. These series known as lyotropic or Hofmeister series were related to a special physicochemical property of the ion called "lyotropy" (Kunz, Henle, & Ninham, 2004; Omta, Kropman, Woutersen, & Bakker, 2003; Parsegian, 1995). Ions on the left such as strongly hydrated but weakly lyotropic anions of SO_4^{2-} are called kosmotropes, while the weakly hydrated ions, such as lyotropic anions of SCN^- are referred to chaotropes. It has been believed both experimentally and theoretically that the effectiveness is more pronounced for anions than cations, which is classically used to describe the capacity of an ion to enhance or weaken the hydrogen-bond structure of water molecules, or, in other words, to enhance or reduce the solubility of a solute, respectively. The salts that contain lyotropic series ions have been used to study their effects on proteins (von Hipple & Wong, 1964), synthetic polymers (Briscoe, Luckham, & Zhu, 1996; Lii, Tomasik, Hung, & Lai, 2002; Song, Ryoo, & Jhon, 1991; Thiyagarajan, Chaiko, & Hjelm, 1995), methylcellulose (MC) (Xu, Wang, Tam, & Li, 2004). The overall salt effect depends on the nature of salts, surface, substrate, temperature and concentration. Although salt effects are very complex and quite different in different systems, lyotropic salts can be employed to shed light on the presence of molecular interactions in polymer systems (von Hipple & Wong, 1964; Song et al., 1991; Zhang, Furyk, Bergbreiter, & Cremer, 2005).

In this work, the representative salting-out salts, Na_2SO_4 and NaCl, and salting-in salts, $NaNO_3$ and NaSCN, are used to study systematically their effects on the gelation of the non-electrolytical KGM in aqueous solutions by means of rheometry, FT-IR, Circular dichroism (CD) and electron spin resonance (ESR) measurements. The aim of this work was also to identify the roles of the alkaline coagulant commonly used in gelation of KGM to shed further light on the gelation mechanism.

2. Experiment

2.1. Materials

KGM was kindly supplied by Shimizu. Chemical Co., Tokyo, Japan. Purification prior to shipment was achieved by ethanol extraction followed by spray drying. The molecular weight of KGM was approximately 6.9×10^5 and its intrinsic viscosities using cadoxen as a solvent was 3.91 dL/g (Zhang et al., 2001).

Na_2SO_4, NaCl, $NaNO_3$, NaSCN and Na_2CO_3 used in this study were of assay grade reagents (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China), and were used without further purification.

2.2. Preparation of KGM solutions

Powders of KGM were dispersed in double distilled/deionized water with stirring. The solutions were heated to 80 °C and maintained at this temperature for 1 h and cooled to the room temperature.

For samples containing salts, each salt was dissolved beforehand in water at room temperature at certain concentrations, and then, the salt solution was mixed with KGM aqueous solution at a ratio of 1:1, with gently stirring at room temperature. 10 min stirring was needed for diluting before the measurements. The pH values of the solutions were measured by use of Metrohm 744 pH Meter (Bie & Berntsen Co., Herisau, Switzerland) at room temperature.

2.3. Viscoelastic measurements

Tests were carried out in a stress-controlled rheometer, Rheostrees1 (ThermoHaake Ltd., Germany), using a

1.0 mm gap parallel-plate sensor (60 mm in the diameter of the plate). The sample was poured directly onto the lower parallel plate, which was heated to the testing temperature beforehand. The free-surface of all samples was immediately covered with low viscosity silicone oil to prevent the evaporation of water. Experiments were repeated to ensure that the addition of oil did not affect the data. Stress dependence of the storage and loss shear moduli, G' and G'', was examined to determine a linear viscoelastic regime. Oscillatory measurements of G' and G'' were performed at the frequency range from 0.01 to 10 Hz. Steady shear rate sweep measurements were carried out in the range from 0.01 to 100 s^{-1}. For samples with the addition of alkali, 20 μl of 2 M Na_2CO_3 was added to per 1 g KGM solution at the time $t = 0$ and mixed.

2.4. Gelation kinetic tests

When a solution prepared at a non-gelling temperature is kept at a certain gelation temperature, both moduli begin to increase with lapse of time. The evolution of storage shear modulus G', loss shear modulus G'' and tanδ is recorded as a function of time under testing temperature. In order to avoid the destruction of the structure being formed, the testing stress should be as low as possible within the linear regime. In the present work the applied stress was imposed at 0.2 Pa. In this paper, for simplicity, the gelation time, t_0 at 1 rad/s was determined as the intersection of G' and G'' though the first order kinetics equation is commonly used to obtain the kinetic parameters by fitting the rheological data (Huang et al., 2002).

2.5. Circular dichroism

CD spectra were recorded with a Jasco J-715 Spectropolarimeter (Jasco Co., Japan) in the range 190–500 nm with quartz cells with an optical path of 0.1 cm, under a nitrogen atmosphere. Instrument parameters were as follows: time constant 1 sec, scan speed 100 nm/min, slit width 1 nm, step resolution 0.2 nm. Five scans were averaged for each solution and then corrected for background by the subtraction of a buffer blank.

2.6. Fourier Transform-IR spectra measurement

Powders of native KGM were mixed with KBr and cast into a thin film until no reduction in weight was observed. The gels formed by using Na_2SO_4 as the only coagulant, and the spin-labeled KGM as mentioned below were both frozen dried by using LGJ-10 freeze-drying machine (Beijing Four-cycle Scientific Technologic Machine Manufactory, China). Then the lyophilized samples were also mixed with KBr and compressed into a thin film. FT-IR spectra were obtained from these films, using a Paragon 100 Fourier Transform-Infrared Spectrometer (Perkin Elmer Co., USA) in the range from 4000 to 500 cm^{-1}.

2.7. ESR measurement

KGM was spin-labeled with 4-amino Tempo (97%, Sigma Chemical Co., USA) through hydroxyl groups along the polymer chains as described previously (Annable et al., 1994; Williams, Clegg, Day, Phillips, & Nishinari, 1991; Williams et al., 1992, 1993). The spin-labeled KGM solutions were directly used to conduct the ESR measurement. The ESR spectra were recorded at 25 °C on a Bruker EMX-8 spectrometer (Bruker Co., Germany) operating at X-band with 100 kHz magnetic field modulation at 1 K and 9.985 mW microwave power to avoid power saturation. All spectra were determined by using a glass capillary with 0.5 mm diameter to contain the aqueous samples. Before measurements the spin-labeled KGM solutions were mixed with different salt solutions at the weight ratio of 1:1 with stirring for 4 h.

The rotational motion is usually described by rotational correlation time, τ_R, which as an approximation, from the ESR spectra, can be calculated using Kevelson's expression for isotropic rotation in the motional narrowing region by the following equation (Kevelson, 1960):

$$\tau_R = 6.6 \times 10^{-10} W_0 \left[\sqrt{h_0/h_{-1}} + \sqrt{h_0/h_{+1}} - 2 \right] \quad (1)$$

where W_0 represents the peak-to-peak line width of the mid-field line (in gauss); and h_{-1}, h_0 and h_{+1} are the peak-to-peak heights of the low-, mid-, and high-field lines, respectively. The constant 6.6×10^{-10} has been calculated for di-tert-butyl nitroxide (Yoshioka, 1978), but, to a good approximation, it can be used for other nitroxide radicals as well (Day, Phillips, & Williams, 1988; Wang, Lu, & Yan, 1997). The average relative error for the rotational correlation time τ_R is lower than 10%.

3. Results and discussion

From some preliminary tests, suitable salt concentration ranging from 60 mM to 2 M (dependent on the salt solubility in water) and suitable KGM concentration of 0.8 wt% were used. At other concentrations, the obtained solutions were either too viscous to be mixed well with each other or too dilute to show obvious effect within a reasonable testing time. Fig. 1 showed the frequency dependence of G' and G'' for 0.8 wt% KGM in Na_2SO_4 aqueous solutions at different concentrations of Na_2SO_4 and 30 °C. It was found that KGM in Na_2SO_4 solutions showed a typical semi-dilute polymer solution behavior, which can be explained by assuming that the entanglement of molecular chains is transient rather than permanent. At lower frequencies, molecular chains disentangle during a long period of oscillation; while at higher frequencies, KGM molecules can't disentangle during short period of oscillation and form a temporary network structure. Therefore, the solution changed from predominantly liquid-like behavior at low frequencies ($G' < G''$) to solid-like behavior at higher frequencies ($G' > G''$) (Ross-Murphy, 1987). As

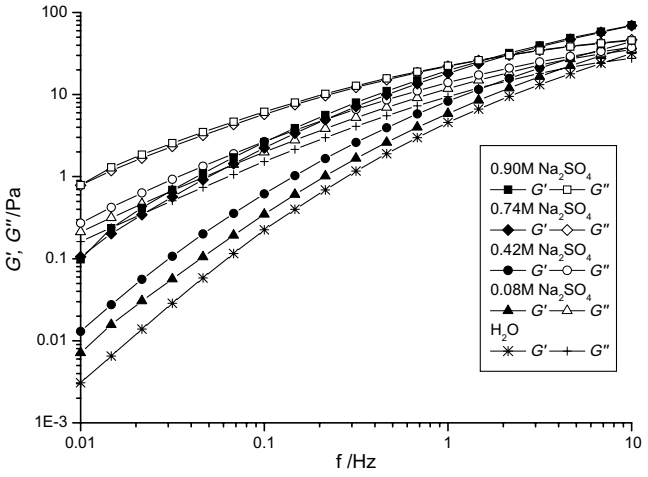

Fig. 1. Frequency dependence of G' and G'' for 0.8 wt% KGM aqueous solutions in the presence of various Na_2SO_4 concentrations at 30 °C.

shown in Fig. 1, the addition of Na_2SO_4 increased both moduli, and shifted the crossover frequency of G' and G'' slightly to a lower value with increasing the concentration of Na_2SO_4, indicating an enhancement of temporary network structures of KGM chains.

Corresponding to the dynamic viscoelastic measurements, the steady shear viscosity measurements were shown in Fig. 2 denoting the viscosity as a function of shear rate for the same samples at the same temperature of 30 °C. It was found that the steady shear viscosity showed a Newtonian plateau at low shear rates, and then decreased with increasing shear rate at high shear rates. It is known that intermolecular entanglements disrupted by the imposed deformation resulted in new interactions at low shear rates, and so there was no overall change in the extent of entanglement, which corresponds to the Newtonian region in the flow curves. When the rate of disruption becomes greater than the rate of formation of new entanglements with increasing shear rate, the onset of shear thinning occurs. The extent of re-entanglement decreases with increasing shear rate, and the viscosity of the solution thus gradually decreases. As shown in Fig. 2, the effect of adding Na_2SO_4 is, primarily, to increase the steady shear viscosity above that of the pure KGM aqueous solution, and the higher the concentration of Na_2SO_4, the stronger the effect. This is also a result of enhancement of frictions or interactions between solvents and solutes, consistent with the above dynamical measurements. The difference between η^* and η was also shown in Fig. 3 for 0.8 wt% KGM aqueous solutions with various Na_2SO_4 concentrations at 30 °C. A remarkable deviation from the Cox-Merz rule was observed, the complex viscosity higher than the apparent viscosity, i.e., $\eta*(\omega) > \eta(\dot{\gamma})|_{\omega=\dot{\gamma}}$, especially in the cases of higher Na_2SO_4 concentrations. Such a deviation is clearly related to the effect of Na_2SO_4 in the system.

The increase in both the moduli and the viscosity upon addition of NaCl, $NaNO_3$ and NaSCN into KGM solu-

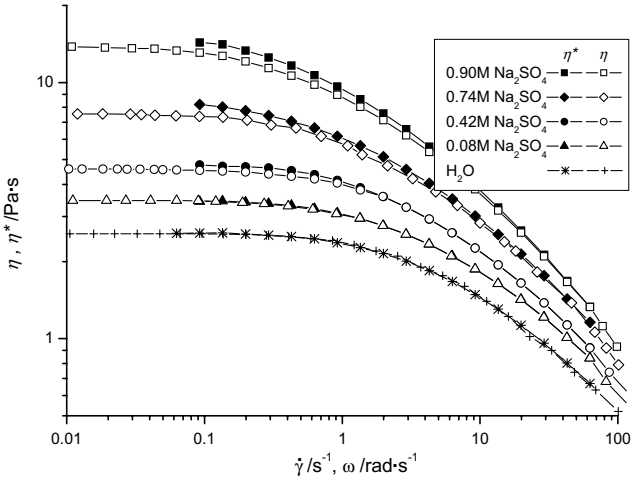

Fig. 3. Cox-Merz plot of 0.8 wt% KGM aqueous solutions with various Na_2SO_4 concentrations at 30 °C.

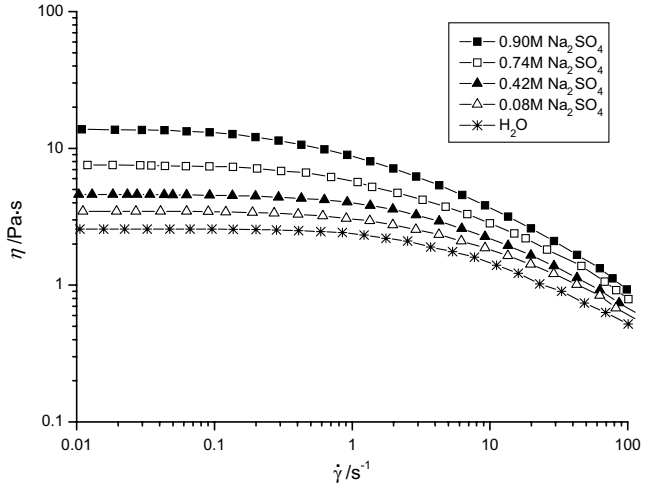

Fig. 2. Shear rate dependence of viscosity for 0.8 wt% KGM aqueous solutions in the presence of various Na_2SO_4 concentrations at 30 °C.

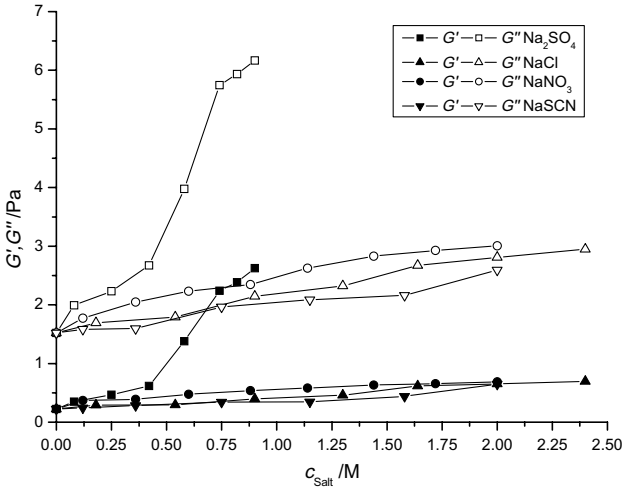

Fig. 4. G' and G'' ($\omega = 0.1$ Hz) as a function of added salts concentration, c_{salt}, for 0.8 wt% KGM in aqueous solutions, at 30 °C.

tions were also observed and found to be similar to that of adding Na_2SO_4, but their influences on KGM solutions were not so significant as that of Na_2SO_4, as seen in Figs. 4 and 5 which compared the effects of adding different salts on both moduli, G' and G'', at a frequency of 0.1 Hz, and the zero-shear viscosity, η_o, for 0.8 wt% KGM aqueous solutions at 30 °C, respectively. The explanation of the effects for all the salts is as follows.

Taking into account that Na_2SO_4 is one of the strongest salting-out salts and NaCl is relatively weak salting-out salt while NaSCN is a potent salting-in salt and $NaNO_3$ is relatively weak salting-in salt, the increase in both moduli and viscosity upon addition of salting-out salts can be considered to be the enhancement of the salt-induced water structure perturbations or the strong interactions between ions and specific sites on the polymers. The stronger the salting-out, the greater the effectiveness just as shown in Figs. 1–5 in the case of Na_2SO_4. As for NaSCN, its strong salting-in effect may increase the solubility of KGM. Because of the low hydrophilicity of KGM molecules, the increased viscosity of KGM aqueous solutions is a result of increasing solubility of KGM. Thus, the addition of NaSCN would make KGM chains more extended in water, consequently increasing the moduli or the viscosity of the solution. As for NaCl and $NaNO_3$, their effects could be understood according to Hofmeister series order.

Interestingly, during the sample preparation, it was expected despite that at room temperature, when a small amount of KGM aqueous solution was dropped into a large amount of Na_2SO_4 solutions at high concentrations, e.g., mixing a drop of 0.8 wt% KGM solution with a large amount of 2 M Na_2SO_4 solution under stirring, KGM quickly formed a small agglomerated gel. When the granule was placed again into a large amount of distilled water for sufficient time at the room temperature, it melted into a homogeneous solution. At room temperature, this phenomenon could not be observed in solutions of NaCl or of the other salts. The strong salting-out effect of Na_2SO_4 on KGM was revelatory. Accordingly it was indeed observed that Na_2SO_4 was capable of making KGM form an opaque thermally irreversible gel upon heating, its effect apparently similar to Na_2CO_3. High concentration of Na_2SO_4 and elevated temperatures were obviously favorable for such a gel formation, which was shown in Figs. 6 and 7 as the time evolution of G' and G'' for 0.8 wt% KGM in Na_2SO_4 solutions at different concentrations and temperatures, respectively. In fact even though the concentration of Na_2SO_4 was as low as 0.25 M, KGM also formed a gel after a long time. The resulting gel did not dissolve when immerged in a large amount of water with a sufficient time (at least two weeks) and also did not melt

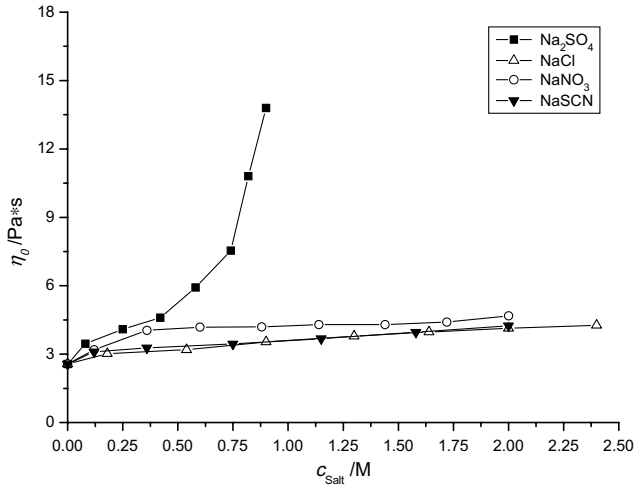

Fig. 5. Zero-shear viscosity as a function of added salt concentration, c_{salt}, for 0.8 wt% KGM in different salts aqueous solutions, at 30 °C.

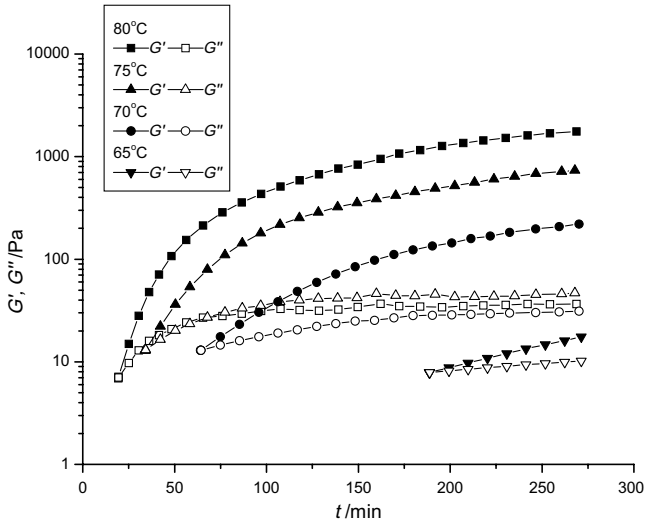

Fig. 6. Time dependence of G' and G'' for 0.8 wt% KGM in 1 M Na_2SO_4 solutions at different heating temperatures.

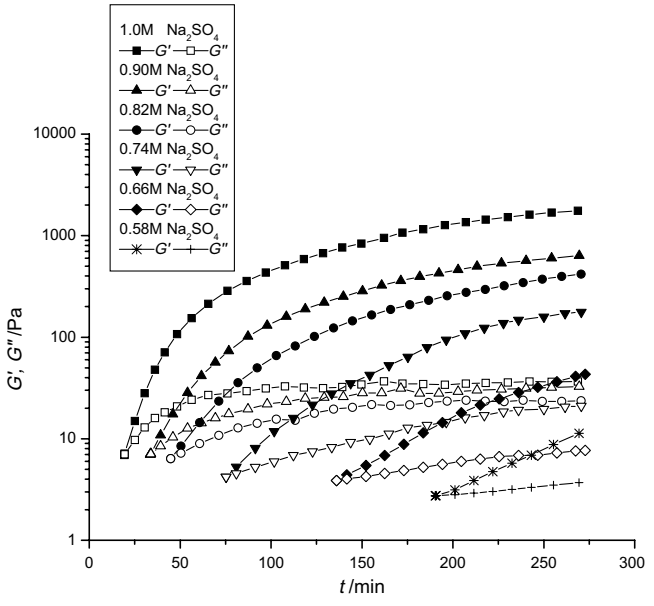

Fig. 7. Time dependence of G' and G'' for 0.8 wt% KGM aqueous solutions in the presence of various Na_2SO_4 concentrations at 80 °C.

on subsequent cooling. This phenomenon was first noted that without any alkaline coagulants, the KGM/Na_2SO_4 mixed systems could form elastic gels with a plateau value of tanδ even less than ca. 0.1. The formation of sulfate-induced KGM gels may interestingly provide a novel clue different from traditional ways, to prepare absolutely natural KGM gels because the chemical structures of KGM chains are unmodified.

It is a well-known phenomenon for a KGM aqueous solution (without any salts) as for many other hydrophilic polymer solutions that rheologically both the moduli and viscosity will drop with increasing temperature. This is commonly due to that the interactions such as hydrogen bonds between water and polymers become weaker as temperature increases. The explanation of the fact in Figs. 6 and 7 in which the moduli increase with concentration and temperature is believed to be the salting-out contribution of Na_2SO_4. Because SO_4^{2-} is a strong salting-out ion, it facilitates forming a complex tightly bonded by water molecules, which leads to a poorer solvent environment for KGM chains. In addition to this, as the concentration of SO_4^{2-} increases, more and more SO_4^{2-} ions compete with KGM chains for the water molecules, attracting more water molecules surrounding them and resulting in less and less water molecules available to hydrate KGM chains, simultaneously resulting in the aggregation of KGM chains. The result of the addition of SO_4^{2-} is thus to make the aggregated KGM chains interact less favorably with the modified aqueous environment, consequently resulting in the formation of the gel. The ion effects on water structure are caused by a competition between ion-water interactions and water-water interactions. Generally, for a heat-setting gelling system the gelation proceeds faster at higher temperatures while it proceeds faster at lower temperatures for a cold-setting gelling system (Nishinari, 1997; Nishinari & Zhang, 2004). Higher temperature is of more benefit to ion hydration and breakdown of hydrogen bonds between water molecules. In other words, elevated temperature favoring the step of gelation may be thus ascribed to higher temperatures increasing the salting-out power of a concentrated salt solution. Increasing the concentration of Na_2SO_4 has an analogous effect on the gelation of KGM accessibly as increasing the temperature. The higher the temperature or the concentration of SO_4^{2-}, the stronger the salting-out effect. This explanation complies with the observed increase in moduli as seen in Figs. 6 and 7 as either temperature or concentration of SO_4^{2-} increases.

Though Na_2SO_4 is a neutral salt, in order to exclude the likelihood of the occurrence of deacetylation of KGM in the presence of Na_2SO_4 and at high temperature, FT-IR spectrum of KGM dried gel formed from Na_2SO_4 solutions was recorded (data not shown). It was found that the spectrum was identical to that of pure KGM, the characteristic band of acetyl group appearing at 1730 cm^{-1} (Maekaji, 1974), indicating that no deacetylation occurred (Huang et al., 2002; Jacon et al., 1993; Williams et al., 2000; Yoshimura & Nishinari, 1999; Zhang et al., 2001). In addition, since the addition of salts may make conformational changes for proteins (von Hipple et al., 1964) and some polysaccharides (Morris, 1994), taking into account the presence of the acetyl groups in the KGM backbones, optically active in the range 200–240 nm (Paradossi et al., 2002), we carried out CD measurements (data not shown) to investigate the likelihood of the conformation change of KGM, and found that upon adding all the salts, no directly obvious evidence for conformational changes were observed, suggesting that the remarkable rheological properties of KGM in the presence of Na_2SO_4 be due to the enhancement of intermolecular association rather than to a chain conformation change.

In contrast with the addition of sulfate salts, such a gelation of KGM was not observed in the presence of either NaCl, or $NaNO_3$ or NaSCN, even at a salt concentration of 4 M or at elevated temperatures. Comparing with SO_4^{2-}, an opposite effect on KGM chains should be expected for SCN^-, a strong salting-in ion, capable of inducing a better solvent for KGM by forming a complex with water that drive an increase in the solubility of KGM in this solvent, thus to impede the aggregation of KGM chains. Considering that Cl^- or NO_3^- is a relatively weak salting-out or salting-in ion, it is reasonable to observe the fact that the different effects of added salts follow the Hofmeister series. The gel promoting effect by sulfate salts is more remarkable than that by NaCl, which is attributed to the different salting-out power that may originate from the different charge densities between sulfate ions and chloride ions while their radii are not so different (bivalence, 2.30 Å for SO_4^{2-} (Zhang, Guan, Ji, & Yao, 2006) and univalence, 1.81 Å for Cl^- (Dean, 1985), respectively).

Since the effect of SO_4^{2-} on the gelation of KGM is so strong, the role of CO_3^{2-} in the gelation of KGM should be reconsidered in detail because this commonly used alkaline coagulant can also be classified as a salting-out salt (Briscoe et al., 1996; Thiyagarajan et al., 1995; von Hipple & Schleich, 1969; Zafarani-Moattar & Sadeghi, 2005; Zhang et al., 2005), and the effectiveness of CO_3^{2-} may be as strong as that of SO_4^{2-} at least in the Hofmeister series as was revealed in many polymer aqueous systems (Zafarani-Moattar & Sadeghi, 2005; Zhang et al., 2005). Although we can not draw definite conclusions from these rheological results, it is possible to make hypothetical prediction in the present work that the addition of the alkaline coagulants might play another non-negligible role, i.e., salting-out effect, in addition to the approbatory role in facilitating the deacetylation of the KGM chains (Huang et al., 2002; Williams et al., 2000; Yoshimura & Nishinari, 1999; Zhang et al., 2001). Deacetylation may not be the necessary prerequisite in the gelation though undoubtedly deacetylated KGM is of less water solubility than native KGM and the occurrence of deacetylation can thus significantly promote the gelation process of KGM. Further detailed studies regarding the salting-out effect of CO_3^{2-} are necessary to clarify this.

Many previous papers have claimed that the rate of gelation process of KGM is a function of concentration of Na_2CO_3 as well as the temperature (Huang et al., 2002; Nishinari & Zhang, 2004; Yoshimura & Nishinari, 1999; Zhang et al., 2001). Too strongly alkaline environment may damage the KGM chains, but within a suitable concentration range the higher the concentration of Na_2CO_3, the faster the gelation process. This previously realized observations maybe have already strongly implied that deacetylation along with salting-out effect of Na_2CO_3 play important roles in gelation. High temperature and concentration of Na_2CO_3 assist the deacetylation, but they may also facilitate the salting-out effect of Na_2CO_3 as was shown in Figs. 6 and 7 for Na_2SO_4. The concurrence of the deacetylation and salting-out effect of Na_2CO_3 on the gelation is difficult to distinguish at high temperatures because they contribute simultaneously (Williams et al., 2000). To further identify the effects of Na_2CO_3 on the gelation and also in comparison with the aforementioned rheological results that were obtained in the absence of Na_2CO_3, we first prepared three kinds of KGM solutions with 1 M Na_2SO_4 (sample I), 40 mM Na_2CO_3 (sample II) and combined 1 M Na_2SO_4 and 40 mM Na_2CO_3 (sample III), respectively, and stored them at 4 °C in a refrigerator since at this temperature the deacetylation caused by Na_2CO_3 and sulfate-induced gelation cannot occur within quite a long time. It was found that in about 25 days there were no apparent changes for samples I and II, but aggregates of KGM were observed for sample III (no deacetylation occurred for the three samples, confirmed by FT-IR). However, once upon heating, all the three samples formed a gel immediately. These phenomena clearly show that the addition of combination of Na_2SO_4 and Na_2CO_3 exhibits salting-out effect, strongly implying that Na_2CO_3 possesses a salting-out effect on KGM chain that has never been mentioned before.

And second, the effects of adding the above four salts on the gelation of KGM in the presence of Na_2CO_3 were further investigated. If Na_2SO_4 or NaCl is added along with Na_2CO_3, a combinatively salting-out effect would be expected. In the same way if $NaNO_3$ or NaSCN is added along with Na_2CO_3, a competition between salting-in and salting-out effects would be expected. Accordingly the likely salting-out effect of Na_2CO_3 might be revealed. Figs. 8–11 showed the time dependence of G' for 0.8 wt% KGM at different concentrations of Na_2SO_4, NaCl, $NaNO_3$ and NaSCN solutions, respectively, upon addition of 40 mM Na_2CO_3 at 70 °C. The concentration of Na_2SO_4 was chosen at which KGM did not form a gel in the presence of Na_2SO_4 alone at 70 °C within the test time. The pH values of KGM/salts mixed solutions in the presence of Na_2CO_3 remained ca. 11.4. In Figs. 8–11 without the addition of salts, the growth in moduli in the presence of alkali was attributed to the evolution of deactylation with time (Williams et al., 2000). It is evident that the addition of Na_2SO_4 and NaCl greatly promoted the gelation process, i.e., the gelation time became shorter, and the gelation process

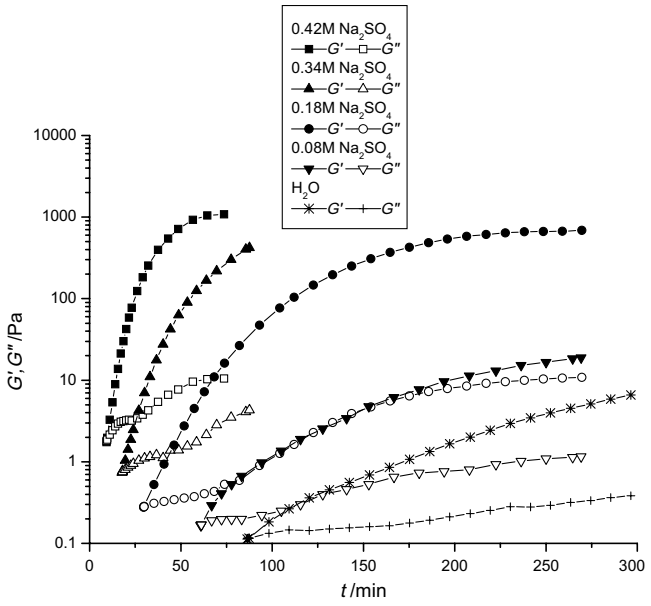

Fig. 8. Time dependence of G' and G'' for 0.8 wt% KGM aqueous solutions in the presence of various Na_2SO_4 concentrations and 40 mM Na_2CO_3 at 70 °C.

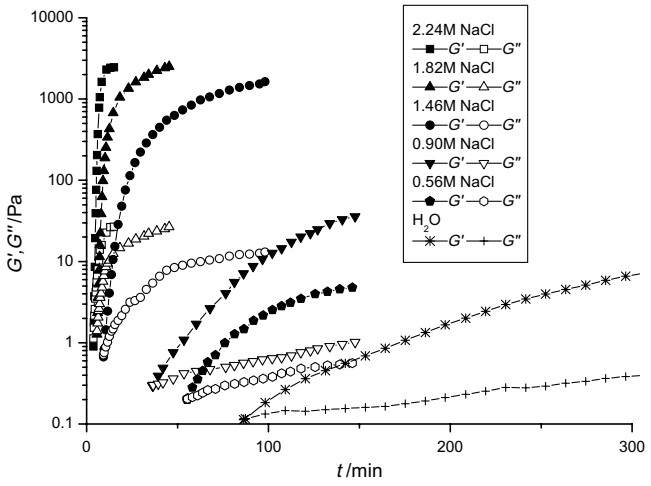

Fig. 9. Time dependence of G' and G'' for 0.8 wt% KGM aqueous solutions in the presence of various NaCl concentrations and 40 mM Na_2CO_3 at 70 °C.

was expedited with increasing concentration of added salts. However, the effects of $NaNO_3$ and NaSCN shown in Figs. 10 and 11 respectively are significantly different from those of Na_2SO_4 and NaCl. With increasing concentration of salts, the gelation time became shorter firstly, but then became longer while their gelation evolutions were not so different. Particularly, it was found that when the concentration of NaSCN is up to 1 M, KGM could not form a gel even in the presence of alkali. The different effects on the gelation of KGM with different sodium salts were summarized as shown in Fig. 12, characteristically denoting by the gelation time as a function of the salt concentration. In the cases of Na_2SO_4 and NaCl, the monotonous drop in gelation time indicated their salting-out nature, and that

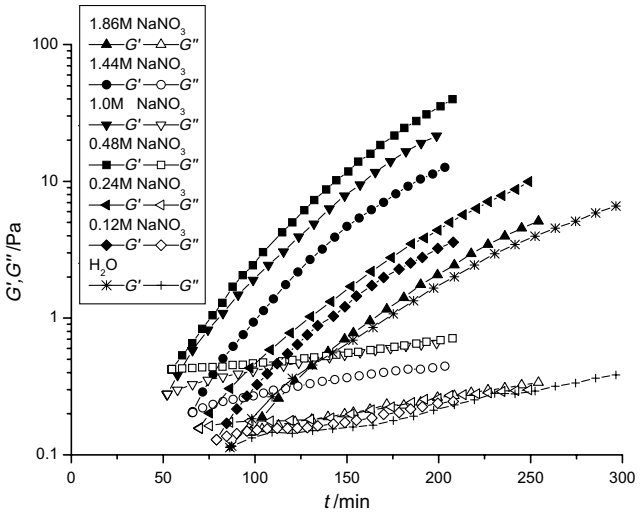

Fig. 10. Time dependence of G' and G'' for 0.8 wt% KGM aqueous solutions in the presence of various $NaNO_3$ concentrations and 40 mM Na_2CO_3 at 70 °C.

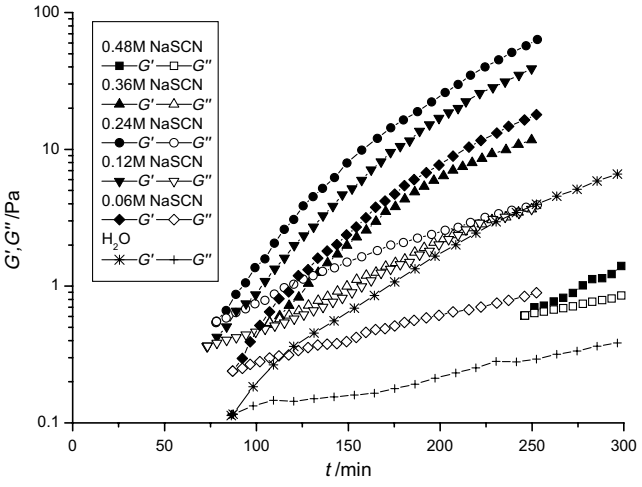

Fig. 11. Time dependence of G' and G'' for 0.8 wt% KGM aqueous solutions in the presence of various NaSCN concentrations and 40 mM Na_2CO_3 at 70 °C.

the salting-out power increased with increase of the salt concentration. In the presence of alkali, both of the deacetylation by alkali and dehydration caused by slating-out ions play roles, thus, the gelation was promoted greatly due to the combinational effects of Na_2CO_3 and Na_2SO_4 or NaCl. As for $NaNO_3$ and NaSCN, the increase in gelation time at higher salt concentrations indicated their salting-in nature while the slight decrease in gelation time in the lower salt concentrations range was due to their relatively weaker salting-in effects. When the concentrations of salting-in salts were lower, their salting-in effects were enshrouded by the strong salting-out effect of Na_2CO_3. The salting-in effect can only emerge significantly when the salt concentration is high enough. Obviously, the salting-in effect of NaSCN is remarkable while that of $NaNO_3$ is relatively flat in agreement with Hofmeister series.

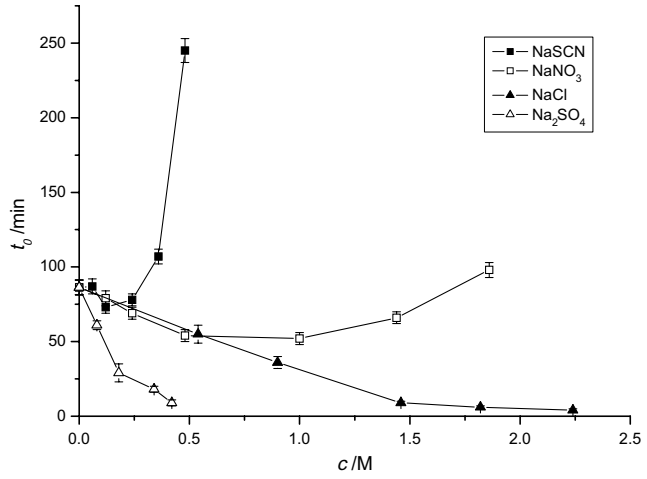

Fig. 12. The gelation time (t_0) as a function of the concentration of various added sodium salts in the presence of 40 mM Na_2CO_3 at 70 °C.

The remarkable feature in Fig. 12 indicated that the gelation likely including deacetylation could be accelerated or delayed (1) by the small amount of salts and (2) dependent on the salt type. This fact also strongly suggested that in addition to facilitating the deacetylation as traditionally considered, CO_3^{2-} itself also has a strong salting-out effect as revealed previously (Briscoe et al., 1996; Thiyagarajan et al., 1995; Zafarani-Moattar & Sadeghi, 2005; Zhang et al., 2005). CO_3^{2-} decreases the water solubilities of both deacetylated and native (non-deacetylated) KGM chains and it seems that CO_3^{2-} is much more sensitive than SO_4^{2-} or Cl^-.

To further elucidate the effect lyotropic series salts, ESR measurements were carried out. The spin-label or spin-probe technique has been used to study a wide variety of polymers, both in bulk and in solution; and KGM has been also studied by the ESR method (Annable et al., 1994; Williams et al., 1991, 1992, 1993). The shape and width of the ESR spectrum of a spin-label or probe is sensitive to the mode and rate of rotation of the radical (Cameron & Davidson, 1996). The information about the influence of the lyotropic series salts on the bulk water and the polymer mobilities can be obtained by ESR spectroscopy, denoting as the change in rotation correlation time τ_R of the bulk and the polymer solutions. τ_R is regarded as the time needed for a molecule to rotate for an angle of π, which can be correlated with the microviscosity of the probe by the Debye equation (Wang et al., 1997):

$$\tau_R = 4\pi\eta\alpha^3/3kT \quad (2)$$

where α is the hydrodynamic radius of the probe, η is the microviscosity, and k and T represent the Boltzmann constant and the temperature, respectively.

Our rheological results showing the salt effects on the gelation of KGM were found to be able to further support by the corresponding ESR measurements. Fig. 13 illustrated the ESR spectra for 10^{-4} M 4-amino Tempo in water in the presence of various salts. In pure water Tempo

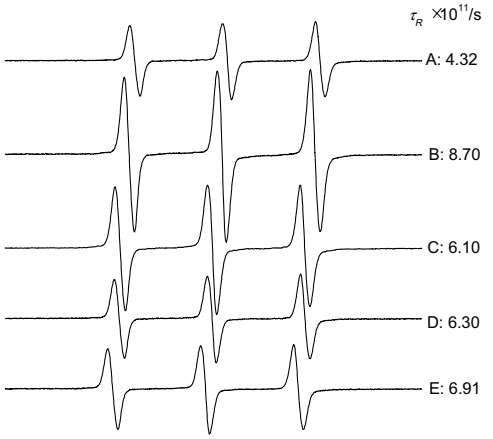

Fig. 13. ESR spectra for 10^{-4} M 4-amino Tempo (spin-probe) in: (A) H_2O; (B) 2 M Na_2SO_4; (C) 4 M NaCl; (D) 4 M $NaNO_3$; (E) 4 M NaSCN solution ($T = 25$ °C).

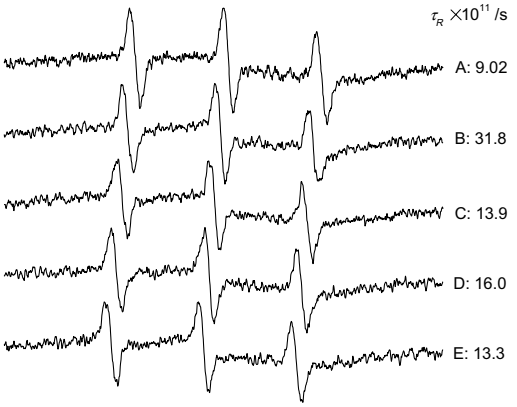

Fig. 14. ESR spectra for 0.1wt% KGM (spin-labeled) in: (A) H_2O; (B) 1 M Na_2SO_4; (C) 2 M NaCl; (D) 2 M $NaNO_3$; (E) 2 M NaSCN solution ($T = 25$ °C).

itself has a motionally narrowed three-lined ESR spectrum due to rapid isotropic tumbling ($\tau_R = 43$ ps). However, it has a weaker mobility (longer rotational correlation time) in the salts solutions than in water. Especially, the value of τ_R in Na_2SO_4 is the largest, 87 ps, about twice as that in water, while τ_R in NaCl (61 ps), $NaNO_3$ (63 ps) and NaSCN (69 ps) are almost the same, longer than in water but much shorter than in Na_2SO_4, though here the anion concentrations (4 M) are twice as that of Na_2SO_4 (2 M). According to Eq. (2), taking into account that τ_R is proportional to the microviscosity, η, this increase in τ_R is a result of reduced water mobilities. The ESR results were also in agreement with the reported sulfate ions tending to increase the order of the water structure and increase its viscosity (Omta et al., 2003). In other words, the increase in τ_R indicates the enhancement of the hydrogen-bond structure of water molecules.

Fig. 14 illustrated the ESR spectrum for 0.1 wt% spin-labeled KGM in various salt solutions. We have also confirmed by FT-IR spectrum that the spin-labeled KGM is identical to native KGM in chemical structure. Especially, the acetyl groups appearing at 1730 cm^{-1} are not removed after the spin-labeled reaction. Thus the behavior of spin-labeled KGM could represent that of the native KGM. In contrast with Fig. 13, it is not surprising to see in Fig. 14 that the value of τ_R for 0.1 wt% spin-labeled KGM in aqueous solutions (90 ps) is much larger than that of Tempo in pure water (43 ps), indicative of slower molecular tumbling of the radicals, because in such a case the Tempo has been covalently labeled on the chain of KGM, predominantly reflecting the segmental motion of the KGM. A large value of radius of the probe is thus expected, which leads to a longer τ_R according to Eq. (2). Here again, as shown in both Figs. 13 and 14, consistent with the aforementioned rheological results, among all the salts the effectiveness of Na_2SO_4 either with or without the KGM is the biggest. In Fig. 14 the addition of Na_2SO_4 significantly increased the τ_R (318 ps, referring to as "weakly immobilized" (Day et al., 1988)) of the probe in KGM solutions, and the line width of ESR became broader. Conversely, the addition of NaCl, $NaNO_3$ and NaSCN at a concentration even twice (2 M) as much as that of Na_2SO_4 (1 M), only resulted in a half value in τ_R (139, 160 and 133 ps, respectively), indicating that the probe experienced low rotational friction just as in water. Compared with Fig. 13, the considerable increase in τ_R in Fig. 14 (note in these cases the concentrations of added salts are only half of those in Fig. 13) is a result of reduced KGM molecular mobilities, equivalent to enhancement of network structures of KGM chains. The reduced polymer mobility may be attributed to the reduced mobilities of not only the single KGM chains, but the aggregates of KGM chains as well. The microviscosities represent the mobilities of solutes in water. It is well-known that polymer solutions are characterized by different macro- and microviscosities since the mobilities of small solutes and polymer chains differ significantly. Thus microscopically, the evidence of ESR for the decrease in mobilities of KGM chains is agreement with the rheological results of the increase in macro-module and macroviscosity.

4. Conclusion

The gelation behavior of KGM was studied as a function of lyotropic salt type, salt concentration and temperature. The influence of salts on the gelation of KGM was quite diversiform. Even in the absence of alkali, the salting-out sodium sulfate was found to be capable of making KGM form a thermally irreversible gel at neutral conditions upon heating while sodium chloride, a weak salting-out salt, sodium nitrate and sodium thiocyanate, salting-in salts, had no such a role on the gelation. Upon addition of sodium sulfate the behavior of KGM in water changed from a viscoelastic fluid at room temperature to an increasing stiff gel at elevated temperatures. The formation of KGM gel in the presence of sodium sulfate alone may imply a different gelation mechanism from that shown in the presence of sodium carbonate via deacetylation. ESR

measurements showed that the addition of sodium sulfate into either water or KGM aqueous solution leaded to a significant increase in rotational correlation time of the radical, consistent with the increase in viscosity and moduli in rheological experiments. In the presence of sodium carbonate, the influence of the lyotropic salts on the gelation of KGM is dependent on the essential salting-out or salting-in nature of the salt. In addition, a revised look at the effect of the commonly adopted sodium carbonate was proposed and its salting-out effect regarding the gelation of KGM was emphasized.

References

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

Briscoe, B., Luckham, P., & Zhu, S. (1996). Rheological study of poly(ethylene oxide) in aqueous salt solutions at high temperature and pressure. *Macromolecules, 29*, 6208–6211.

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

Cameron, G. G., & Davidson, I. G. (1996). Chapter 9: Spin-label studies of heterogeneous polymer systems. In A. H. Fawcett (Ed.), *Polymer spectroscopy* (pp. 231–252). Eastbourne, UK: John Wiley & Sons.

Case, S. E., Kropp, J. A., Hamann, D. D., & Schwartz, S. J. (1992). Characterisation of gelation of konjac mannan using lyotropic salts and rheological measurements. In G. O. Phillips, D. J. Wedlock, & P. A. Williams (Eds.). *Gums and stabilizers for the food industry* (Vol. 6, pp. 489–500). Oxford, UK: IRL.

Dave, V., Sheth, M., McCarthy, S. P., Ratto, J. A., & Kaplan, D. L. (1998). Liquid crystalline, rheological and thermal properties of konjac glucomannan. *Polymer, 39*, 1139–1148.

Day, D. H., Phillips, G. O., & Williams, P. A. (1988). Gelation of kappa carrageenan as studied by electron spin resonance spectroscopy. *Food Hydrocolloids, 2*, 19–30.

Dean, J. A. (1985). *Lange's handbook of chemistry* (13th ed.). New York, USA: McGraw-Hill.

Gao, S., & Zhang, L. (2001). Molecular weight effects on properties of polyurethane/nitrokonjac glucomannan semiinterpenetrating polymer networks. *Macromolecules, 34*, 2202–2207.

Goycoolea, F. M., Richardson, R. K., & Morris, E. R. (1995). Stoichiometry and conformation of xanthan in synergistic gelation with Locust bean gum or konjac glucomannan: Evidence for Heterotypic Bind. *Macromolecules, 28*, 8308–8320.

Huang, L., Takahashi, R., Kobayashi, S., Kawase, T., & Nishinari, K. (2002). Gelation behavior of native and acetylated konjac glucomannan. *Biomacromolecules, 3*, 1296–1303.

Jacon, S. A., Rao, M. A., Cooley, H. J., & Walter, R. H. (1993). The isolation and characterization of a water extract of konjac flour gum. *Carbohydrate Polymers, 20*, 35–41.

Kato, K., & Matsuda, K. (1969). Studies on the chemical structure of konjac mannan Part I. Isolation and characterization of oligosaccharides from the partial acid hydrolyzate of the mannan. *Agricultural and Biological Chemistry, 33*, 1446–1453.

Katsuraya, K., Okuyama, K., Hatanaka, K., Oshima, R., Sato, T., & Matsuzaki, K. (2003). Constitution of konjac glucomannan: Chemical analysis and ^{13}C NMR spectroscopy. *Carbohydrate Polymers, 53*, 183–189.

Kevelson, D. (1960). Theory of ESR linewidths of free radicals. *Journal of Chemical Physics, 33*, 1094–1106.

Kunz, W., Henle, J., & Ninham, B. W. (2004). 'Zur Lehre von der Wirkung der Salze' (about the science of the effect of salts): Franz Hofmeister's historical papers. *Current Opinion in Colloid and Interface Science, 9*, 19–37.

Lii, C., Tomasik, P., Hung, W., & Lai, V. M. F. (2002). Revised look at the interaction of starch with electrolyte: Effect of salts from the first non-transition group. *Food Hydrocolloids, 16*, 35–45.

Maeda, M., Shimahara, H., & Sugiyama, N. (1980). Detailed examination of the branched structure of konjac glucomannan. *Agricultural and Biological Chemistry, 44*, 245–252.

Maekaji, K. (1974). The mechanism of gelation of konjac mannan. *Agricultural and Biological Chemistry, 38*, 315–321.

Miyoshi, E., Takaya, T., Williams, P. A., & Nishinari, K. (1996). Effects of sodium chloride and calcium chloride on the interaction between gellan gum and konjac glucomannan. *Journal of Agricultural and Food Chemistry, 44*, 2486–2495.

Morris, E. R. (1994). Chapter 2: Dichroism. In S. B. Ross-Murphy (Ed.), *Physical techniques for the study of food biopolymers* (pp. 15–65). London, UK: Chapman & Hall.

Nishinari, K. (1997). Rheological and DSC study of sol-gel transition in aqueous dispersions of industrially important polymers and colloids. *Colloid and Polymer Science, 275*, 1093–1107.

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

Nishinari, K., & Zhang, H. (2004). Recent advances in the understanding of heat set gelling polysaccharides. *Trends in Food Science and Technology, 15*, 305–312.

Omta, A. W., Kropman, M. F., Woutersen, S., & Bakker, H. J. (2003). Negligible effect of ions on the hydrogen-bond structure in liquid water. *Science, 301*, 347–349.

Paradossi, G., Chiessi, E., Barbiroli, A., & Fessas, D. (2002). Xanthan and glucomannan mixtures: Synergistic interactions and gelation. *Biomacromolecules, 3*, 498–504.

Parsegian, V. A. (1995). Hopes for Hofmeister. *Nature, 378*, 335–336.

Ridout, M. J., Brownsey, G. J., & Morris, V. J. (1998). Synergistic interactions of acetan with carob or konjac mannan. *Macromolecules, 31*, 2539–2544.

Ridout, M. J., Cairns, P., Brownsey, G. J., & Morris, V. J. (1998). Evidence for intermolecular binding between deacetylated acetan and the glucomannan konjac mannan. *Carbohydrate Research, 309*, 375–379.

Ross-Murphy, S. B. (1987). Physical gelation of biopolymers. *Food Hydrocolloids, 1*, 485–495.

Song, J. D., Ryoo, R., & Jhon, M. S. (1991). Anion binding properties of poly(vinylpryrrolidone) in aqueous solution studied by halide NMR spectroscopy. *Macromolecules, 24*, 1727–1730.

Stephen, A. M., Phillips, G. O., & Williams, P. A. (2006). *Food polysaccharides and their applications* (2nd ed.). New York, USA: CRC.

Thiyagarajan, P., Chaiko, D. J., & Hjelm, R. P. (1995). A neutron scattering study of poly(ethylene glycol) in electrolyte solutions. *Macromolecules, 28*, 7730–7736.

von Hipple, P. H., & Schleich, T. (1969). Ion effects on the solution structure of biological macromolecules. *Accounts of Chemical Research, 2*, 257–265.

von Hipple, P. H., & Wong, K. Y. (1964). Neutral salts: The generality of their effects on the stability of macromolecular conformations. *Science, 145*, 577–580.

Wang, K., & He, Z. (2002). Alginate-konjac glucomannan-chitosan beads as controlled release matrix. *International Journal of Pharmaceutics, 244*, 117–126.

Wang, Y., Lu, D., & Yan, H. (1997). An ESR spin probe study of the interaction between poly(ethylene oxide) and dodecyl sulfate surfactants with different monovalent metal counter ions. *Journal of Physical Chemistry B, 101*, 3953–3956.

Williams, P. A., Clegg, S. M., Day, D. H., Phillips, G. O., & Nishinari, K. (1991). Mixed gels formed with konjac mannan and xanthan gum. In E. Dickinson (Ed.). *Food polymers, gels and colloids* (pp. 339–348). Herb, UK: Royal Society of Chemistry.

Williams, P. A., Clegg, S. M., Langdon, M. J., Nishinari, K., & Phillips, G. O. (1992). Studies on the synergistic interaction of konjac mannan

and locust bean gum with kappa carrageenan. In G. O. Phillips, D. J. Wedlock, & P. A. Williams (Eds.). *Gums and stabilizers for the food industry* (Vol. 6, pp. 209–216). Oxford, UK: IRL.

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

Williams, M. A., Foster, T. J., Norton, I. T., Yoshimura, M., & Nishinari, K. (2000). A molecular description of the gelation mechanism of konjac mannan. *Biomacromolecules, 1*, 440–450.

Xu, Y., Wang, C., Tam, K. C., & Li, L. (2004). Salt-assisted and salt-suppressed sol-gel transitions of methylcellulose in water. *Langmuir, 20*, 646–652.

Yoshimura, M., & Nishinari, K. (1999). Dynamic viscoelastic study on the gelation of konjac glumannan with different molecular weights. *Food Hydrocolloids, 13*, 227–233.

Yoshioka, H. (1978). Spin probe study of concentrated aqueous solutions of nonionic surfactant. *Journal of Colloid and Interface Science, 63*, 378–382.

Zafarani-Moattar, M. T., & Sadeghi, R. (2005). Phase diagram data for several PPG + salt aqueous biphasic systems at 25 °C. *Journal of Chemical Engineering Data, 50*, 947–950.

Zhang, Y., Furyk, S., Bergbreiter, D. E., & Cremer, P. S. (2005). Specific ion effects on the water solubility of macromolecules: PNIPAM and the Hofmeister series. *Journal of American Chemical Society, 127*, 14505–14510.

Zhang, J., Guan, Y., Ji, Z., & Yao, S. J. (2006). Effects on membrane properties of NaCS-PDMDAAC capsules by adding inorganic salts. *Journal of Membrane Science, 277*, 270–276.

Zhang, H., Yoshimura, M., Nishinari, K., Williams, M. A. K., Foster, T. J., & Norton, I. T. (2001). Gelation behavior of konjac glucomannan with different molecular weights. *Biopolymers, 59*, 38–50.