

In Situ pH-Decrease-Induced Gelation of Sodium Alginate/Carboxymethylated Konjac Glucomannan

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Received 19 June 2007; accepted 16 October 2007

DOI 10.1002/app.27591

Published online 25 February 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The gelation kinetics and rheological properties of a mixture system containing two anionic polysaccharides, sodium alginate and carboxymethylated konjac glucomannan, in the presence of glucono- δ -lactone were investigated with dynamic viscoelastic measurements. The slow release of protons induced by glucono- δ -lactone gave birth to partly uncharged polymer chains and partly shielded electrostatic repulsion, resulting in an interpolymer association. This segregative interaction brought about a significant improvement in the elastic modulus of the resultant gel. The strongest synergism occurred at a carboxymethyl-

lated konjac glucomannan concentration of 29.9 wt %, exhibiting the highest gel strength. Increasing the content or the degree of substitution of carboxymethylated konjac glucomannan resulted in slower gelation kinetics. The fractal dimension of the incipient gel, which was not greatly affected by the mixing ratio or the degree of substitution of carboxymethylated konjac glucomannan, reflected a rather compact gel structure network. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 108: 2825–2832, 2008

Key words: biopolymers; gels; rheology

INTRODUCTION

A few binary mixtures of polysaccharides exhibit synergistic interactions that lead to or enhance gelation, resulting in controlled properties. In particular, most of the known synergisms involve mixtures of an anionic helix-forming polysaccharide such as xanthan, acetan, or kappa carrageenan with certain nongelling plant galactomannans (e.g., guar and locust bean gums) or glucomannans [e.g., konjac glucomannan (KGM)].^{1–11} There is academic interest in identifying the molecular mechanisms of gelation of the complexes, and there is industrial interest due to their important applications in food engineering, drug delivery, and oil/gas recovery.

KGM is a neutral polysaccharide isolated from the tubers of *Amorphophallus konjac* C. Koch. It consists of β -1,4-linked glucose and mannose units, and the glucose/mannose ratio has been reported to be around 1 : 1.6.¹² There are some branches linked to the backbone, but the exact branched position is still

under debate.¹³ The glucomannan backbone of KGM possesses 5–10% acetyl substituted residues,^{15,16} and it is widely accepted that the presence of this group confers water solubility on the glucomannan. Deacetylation, generated by the addition of a mild alkali, results in the association of KGM molecular chains into a gel network supported by intermolecular hydrogen bonding.^{16,17} The stable gel made by the heating of konjac flour in the presence of alkali has long been used as a noncalorie health-care food classified as indigestible dietary fiber in Japan.¹⁸ Chemically crosslinked KGM gel beads have been used as fillers of chromatography columns to fractionate water-soluble biomacromolecules.¹⁹ As a dietary fiber with one of the highest viscosities among food biopolymers, KGM fiber shows a certain degree of physiological function in reducing coronary heart disease risk factors (hyperglycemia, hyperlipidemia, and hypertension),²⁰ improving glycemic control and lipid profile,²¹ modifying the intestinal microbial metabolism,²² and lowering plasma cholesterol.²³ KGM is known to form thermoreversible gels with xanthan^{4–6} and acetan or deacetylated acetan^{7–9} under conditions in which the individual components do not gel. Such synergistic behavior is normally attributed to intermolecular binding between the two polysaccharides. The addition of KGM to a gelling polysaccharide such as κ -carrageenan or agarose can enhance gelation or lead to gelation at lower concentrations.^{10,11,24}

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Contract grant sponsor: Japan Society for the Promotion of Science.

Contract grant sponsor: SRF for ROCS (SEM).

Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 50703031.

Journal of Applied Polymer Science, Vol. 108, 2825–2832 (2008)
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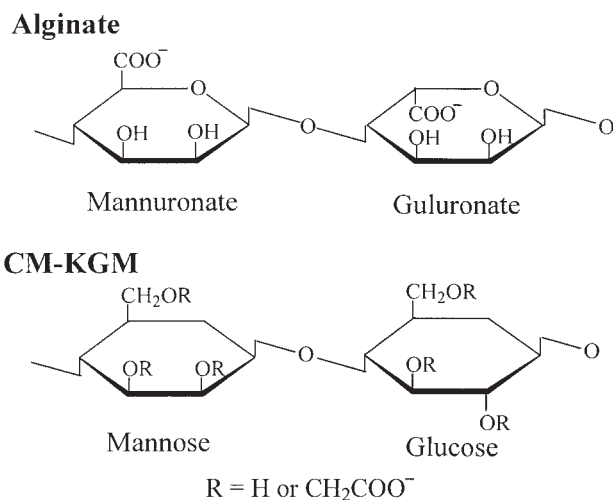


Figure 1 Schematic illustration of the principal structures of alginate and CM-KGM.

Alginate is a family of unbranched binary copolymers of (1→4)-linked β-D-mannuronic acid and α-L-guluronic acid residues of widely varying compositions and sequential structures (Fig. 1). The most attractive property of alginate is the gel formation induced by the addition of various divalent cations, and this gelling ability renders its application in numerous food and pharmaceutical applications.²⁵ A synergistic gel from alginate and pectin has been extensively studied. This mixed gel can be formed at low pH values under conditions in which neither of the pure samples would gel, that is, in the absence of divalent cations and with high water activity.²⁶ The pH value is crucial for the gel formation, and a pH value above 4 hinders the gel formation. The fact that the pK_a values of the uronic acid residues in the alginate and pectin chains are between 3.4 and 3.7 indicates that the molecular chains must be partly protonated before the appearance of interpolymer interactions to reduce electrostatic repulsion between the two anionic polymers.²⁷ The gelation mechanism of the alginate/pectin mixed system is believed to be ascribable to an incompatibility or exclusion effect at the expense of a heterogeneous association of the poly(guluronic acid) blocks of alginate and the methyl ester regions of pectin with a low charge.²⁸

In this study, we report the sol-gel transition in aqueous mixtures of sodium alginate (SA) and carboxymethylated konjac glucomannan (CM-KGM; a charged derivative from KGM synthesized with 6 wt % NaOH/5 wt % urea as a reaction medium; see Fig. 1) induced by an *in situ* pH decrease in the presence of glucono- δ -lactone (GDL), a slow acidifier that is widely used in the processing of tofu (a traditional gel-like food) from soybean protein.²⁹ The gel formation kinetics and the gel characteristics were studied with dynamic small-deformation oscillation

measurements. The effects of the mixing ratios and degrees of substitution (DSs) of CM-KGM on the gelation kinetics and gel strength were investigated.

EXPERIMENTAL

Materials

The raw KGM sample was a gift from Shimizu Chemical Co. (Hiroshima, Japan) and was further purified by being mixed with three times its weight of 50 and 80 wt % ethanol for 2 h and with waterless ethanol for 4 h and then vacuum-dried at 60°C for 4 h. SA and all other chemicals (reagent pure grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and used as received. The molar ratio of mannuronate residue (M) to guluronate residue (G) and the molar fractions of the GG, MM, and GM (MG) doublet sequences of the SA sample, determined by ¹H-NMR measurements (Unity Plus 500, Varian, San Francisco, CA) according to Grasdalen et al.³⁰ with a phosphate-D₂O (pD = 6.96) solution of SA (14 g/L) at 80°C, were 0.89, 0.30, 0.24, and 0.23, respectively. The intrinsic viscosity measurements of the SA solutions in 0.1M NaCl were carried out at 25 ± 0.02°C with an Ubbelohde-type viscometer (Kaburagi Scientific Instruments Co., Ltd., Tokyo, Japan). Extrapolation to infinite dilution was performed with both Huggins and Kraemer plots, from which the intrinsic viscosity was calculated to be 910 cm³/g.

Determination of the solubility of KGM in NaOH/urea

To determine the optimum composition for the NaOH/urea solution, we checked the degree of solubility (S_a) of KGM in the NaOH/urea system by fixing the NaOH concentration at 6 wt % and varying the concentration of urea between 1 and 9 wt %. Dry KGM flour (5 g) was dispersed in 495 g of the NaOH/urea solution by mechanical agitation (a spiral propeller with a diameter of 80 mm) at 600 rpm for 18 h at room temperature. The dissolved and insoluble parts of KGM were isolated by centrifugation at 9000g and 15°C for 45 min. The insoluble precipitate was suspended in methanol/water (6 : 4 w/w) and neutralized with 6M HCl and then was filtered off with a G3 glass filter and repeatedly washed with acetone. This part of insoluble KGM was vacuum-dried at 80°C to a constant weight. The NaOH/urea solution containing the dissolved KGM was first neutralized with 6M HCl and then mixed with 600 g of acetone. The resultant precipitate was processed with the same method for the insoluble part. S_a of KGM was calculated as follows:

TABLE I
Effects of the Amounts of ClCH₂COOH and KI, Reaction Temperature, and Reaction Time on the Extent of Carboxymethylation

Sample	CM-KGM1	CM-KGM2	CM-KGM3	CM-KGM4
ClCH ₂ COOH (g)	20	20	30	35
KI (g)	0.4	0.4	0.6	0.7
Temperature (°C)	25	40	40	45
Time (h)	16	22	24	32
DS	0.26	0.75	0.94	1.40
Intrinsic viscosity (cm ³ /g)	680	655	662	614

$$S_a = [W_{\text{sol}} / (W_{\text{sol}} + W_{\text{in}})] \times 100 \%$$

where W_{sol} and W_{in} are the weights of the dissolved and insoluble parts of KGM, respectively. The solubility tests were repeated three times, and the average value was considered S_a .

Carboxymethylation of KGM

Native KGM (10 g) was added to 667 g of a 6 wt % NaOH/5 wt % urea aqueous solution in a three-necked flask, and then the mixture was mixed under mechanical stirring at 600 rpm/min for 18 h to obtain an alkaline KGM solution with a KGM concentration of around 1.5 wt %. The predetermined amounts of chloroacetic acid (ClCH₂COOH) and potassium iodide (KI) were added. The carboxymethylation reaction was carried out under the desired conditions, including the amounts of the chemicals, temperature, and time, as listed in Table I. The reaction was stopped by the addition of a 800-g mixture of acetone/methanol (4 : 1 w/w) to obtain a white, fiberlike precipitate. The resultant mixture was centrifuged at 9000g and 15°C for 30 min. The separated precipitate was washed with 200 g of 50, 80, and 95 wt % ethanol. Then, the crude production was dissolved in 600 g of distilled water by mechanical stirring for 24 h. The solution was neutralized with 6M HCl to a pH value of 10 ± 0.2 , and then 800 g of methanol was added to the solution under vigorous stirring. The resultant precipitate was filtered off with a G3 glass filter and then repeatedly washed with 80 wt % methanol four times and finally with pure methanol four times. The final product was vacuum-dried at 60°C for 48 h. The DS values of the carboxymethylated samples, determined by the titration method according to a previous report,³¹ are listed in Table I. The intrinsic viscosities of the carboxymethylated samples measured with the same method for SA are listed in Table I.

Preparation of SA/CM-KGM mixtures

The polysaccharides were blended at different mixing ratios and dissolved in deionized water under magnetic agitation at room temperature for 8 h. In

the Results and Discussion section, the mixing ratios are denoted by the content of CM-KGM (W_{CM}) in the mixture, which is defined as $W_{\text{CM}} = m_{\text{CM-KGM}} / (m_{\text{CM-KGM}} + m_{\text{SA}})$, where $m_{\text{CM-KGM}}$ and m_{SA} denote the masses of CM-KGM and SA, respectively. A constant polysaccharide content of 1.5 wt % was used in the whole investigation. SA/CM-KGM mixtures with W_{CM} values of 5.5, 13.3, 29.9, 43.5, 64.9, and 88.6 wt % were used in this study. The pH value of the 1.5 wt % SA aqueous solution was 6.92. The pH values of the 1.5 wt % aqueous solutions of CM-KGM samples with different DS values were 7.0 ± 0.1 .

Rheological measurements

Aqueous solutions with a polysaccharide concentration of 1.5 wt % and with different W_{CM} values were equilibrated in a thermostated water bath at 20°C for 2 h before dynamic viscoelastic measurements.

Dynamic viscoelastic measurements were carried out with an RFS II fluids spectrometer (Rheometrics Co., Ltd., New Castle, DE) with a cone-plate geometry (25 mm in diameter, 0.1° cone angle, and 0.05-mm gap). The strain in all measurements of this work was set at 0.5%, which was prechecked to be within a linear viscoelastic regime. One gram of a KGM aqueous dispersion was mixed with the required amount of GDL, and the mixture then was poured onto the plate of the instrument, which was kept at the measurement temperature (20°C in all investigations unless otherwise specified). A constant GDL concentration of 1.5 wt % was used in this study. The gelation kinetics were recorded as the time course of the rheological parameters [storage shear modulus (G'), loss shear modulus (G''), and loss tangent ($\tan \delta$)] at different frequencies programmed by a multiwave temperature ramp test. The starting time, $t = 0$, corresponded to the time when the SA/CM-KGM mixture was mixed with the GDL and then transferred onto the sample plate.

RESULTS

Carboxymethylation

In contrast to the widespread application of KGM as a gelling agent or textural modifier in the food

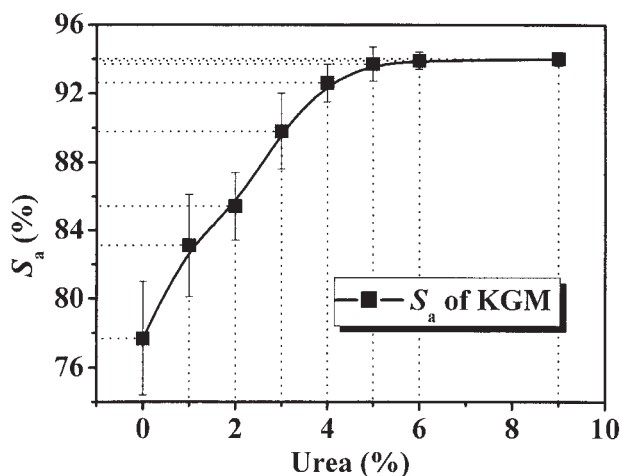


Figure 2 Dependence of the solubility of KGM (S_a) on the concentration of urea in a NaOH/urea solution. The concentration of NaOH was 6 wt %.

industry, there have been only a few reports about its chemical functionalization. However, heterogeneous methods have been the primary choices for chemical modifications such as acetylation,^{32,33} nitration,³⁴ palmitoylation,³⁵ and carboxymethylation³⁶ because no proper solvent system for KGM has been proposed so far other than water. There are two disadvantages for the utilization of water as a reaction medium: one is the low water solubility of refined KGM,³⁷ and the other is the highest viscosity of KGM dispersions (even with a very low concentration) among the common hydrocolloids.³⁸ Recently, NaOH/urea³⁹ and NaOH/thiourea^{40,41} aqueous mixtures have been proposed to be two effective solvent systems for the dissolution of cellulose and preparation of cellulose membranes or cellulose/KGM blend membranes. It has been proposed that the addition of urea or thiourea to an aqueous NaOH solution with a certain concentration can significantly break the intermolecular association by hydrogen bonding. Indeed, urea and thiourea have been proved to be active reagents with peptizing power to dissolve KGM gels once formed.⁴²

From the dependence of the values of S_a for KGM on the urea concentration in NaOH/urea solutions, as shown in Figure 2, it can be clearly seen that KGM exhibited higher solubility in NaOH/urea solutions than in water. The value of S_a gradually increased from 78 to 94% with the concentration of urea increasing from 0 to 5 wt % and then leveled off when the concentration of urea still increased to 9 wt %. Therefore, a 6 wt % NaOH/5 wt % urea solution was used as a reaction medium for the carboxymethylation of KGM. According to Figure 3, which shows the shear viscosities of a 1.0 wt % KGM dispersion in water and a 6 wt % NaOH/5 wt % urea solution, the values of the shear viscosity in

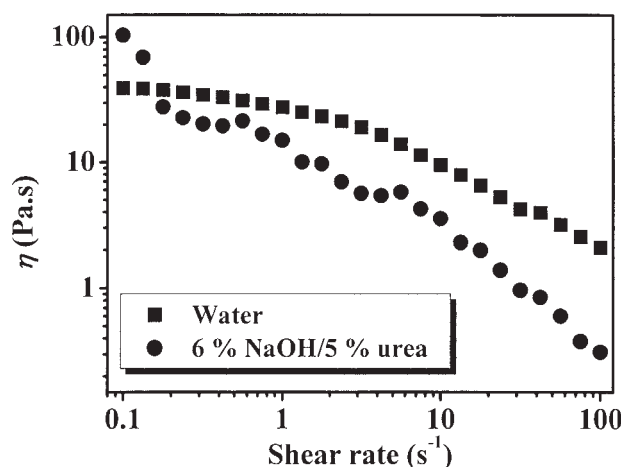


Figure 3 Shear viscosity (η) of a 1.0 wt % KGM dispersion in water and a 6 wt % NaOH/5 wt % urea solution.

the 6 wt % NaOH/5 wt % urea solution were obviously lower than those in water at the same shear rate when the shear rate exceeded $0.2 s^{-1}$. Table I shows the effects of the reaction conditions on the degree of carboxymethylation of KGM. Through changes in the amounts of $ClCH_2COOH$ and KI (the weight ratio of KI to $ClCH_2COOH$ was fixed at 0.02), the reaction temperature, or the reaction time, four carboxymethylated fractions with different DS values were obtained.

Effect of W_{CM} on the gelation of SA/CM-KGM2

Figure 4 shows the typical gelation process of 1.5 wt % SA/CM-KGM2 ($W_{CM} = 43.5\%$) and pure 1.5 wt % SA solutions in the presence of 1.5 wt % GDL at 1 rad/s. For the pure SA solution, both G' and G'' rapidly increased with increasing time and attained pla-

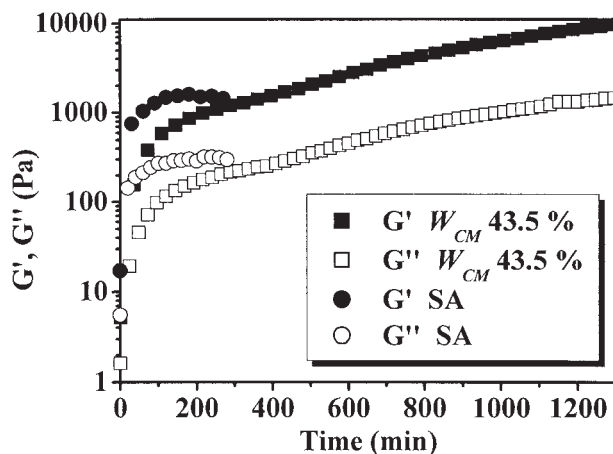


Figure 4 Typical time dependence of G' and G'' of a 1.5 wt % SA/CM-KGM2 mixture system ($W_{CM} = 43.5\%$) and a 1.5 wt % SA solution at the frequency of 1 rad/s and at $20^\circ C$ in the presence of 1.5 wt % GDL.

teau values after 100 min. For the SA/CM-KGM2 mixture solution, both G' and G'' increased at a relatively low rate, but they exceeded those of the pure SA solution after 500 min. Even at the start time of the experiment, the elastic response was dominating ($G' > G''$), and this trend was strengthened as time passed. The marked increase of the dynamic moduli with time indicated the evolution of the gel. One can clearly see from Figure 4 that the values of the pseudo-equilibrium moduli (G'_{sat} and G''_{sat}) of the SA/CM-KGM2 gel were higher than those of the pure SA solution.

To quantify the critical gelation time (t_{cr}) for the mixture of SA and CM-KGM2, a method proposed by Winter and Chambon⁴³ can be used (Fig. 5). According to this method, t_{cr} can be determined by the observation of a frequency-independent value of $\tan \delta$ [$\tan \delta = G''/G' = \tan(n\pi/2)$] obtained from a multifrequency plot of $\tan \delta$ versus time. The application of this gelation criterion was proposed originally for chemically crosslinked gels because it is important to access low-frequency data to be confident that the interactions being observed are permanent and not physical interactions with some infinite timescale.⁴⁴ However, it has been proved that this criterion can be applied to some synthetically or naturally physical gels including KGM,³³ poly(vinyl chloride),⁴⁵ and schizophyllan.⁴⁶ A typical illustration of the evolution of $\tan \delta$ as a function of time for a 1.5 wt % SA/CM-KGM2 ($W_{\text{CM}} = 43.5$ wt %) solution in the presence of 1.5 wt % GDL at different frequencies is shown in Figure 5. $\tan \delta$ was frequency-dependent and decreased with time during gel formation, indicating that the SA/CM-KGM2 complex became more and more elastic. t_{cr} for the case of a 1.5 wt % SA/CM-KGM2 solution was identified to be 24.1 min, and the corresponding relaxation exponent (n) was calculated to be 0.22. An alter-

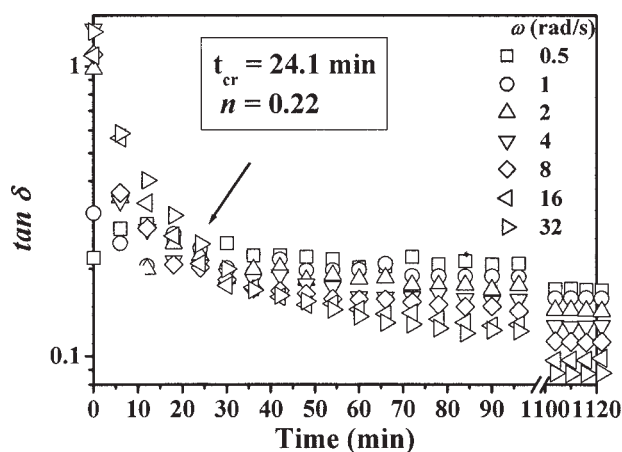


Figure 5 $\tan \delta$ as a function of time for a 1.5 wt % SA/CM-KGM2 mixture system ($W_{\text{CM}} = 43.5$ wt %) in the presence of 1.5 wt % GDL at different frequencies.

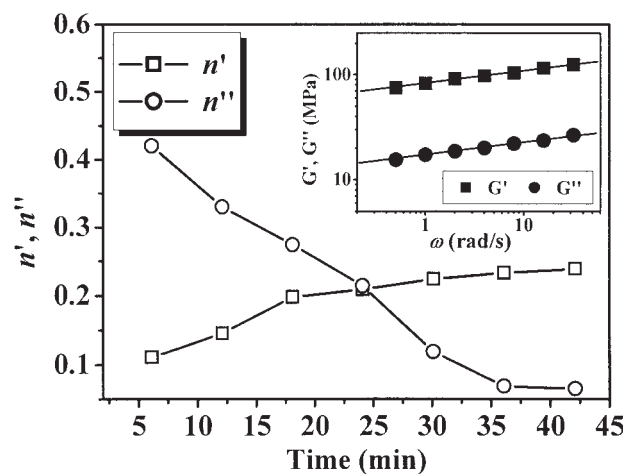


Figure 6 n' for G' and n'' for G'' during the gelation process of a 1.5 wt % SA/CM-KGM2 mixture system ($W_{\text{CM}} = 43.5$ wt %) in the presence of 1.5 wt % GDL. The inset shows the power-law dependence of G' and G'' on the frequency at t_{cr} .

native method for determining t_{cr} is to plot against time the viscoelastic relaxation exponents n' and n'' ($G' \sim \omega^{n'}$, $G'' \sim \omega^{n''}$) calculated from the dependence of G' and G'' at each time of measurement and to track a crossover where $n' = n'' = n$ (Fig. 6). Both methods give the same t_{cr} value (ca. 24.1 min) for the system of the 1.5 wt % SA/CM-KGM2 solution ($W_{\text{CM}} = 43.5$ wt %). At t_{cr} , $G'(\omega)$ and $G''(\omega)$ profiles became parallel, and both showed the power-law dependence of the frequency (see the inset of Fig. 6). In this study, the Winter–Chambon criterion was found to be applicable for the determination of t_{cr} for all mixture solutions of SA/CM-KGM with different mixing ratios. For the pure SA solution, however, this criterion lost its validity because no frequency-independent point for $\tan \delta$ was observed (data not shown).

Muthukumar⁴⁷ proposed a theoretical model to rationalize values of viscoelastic relaxation exponent n in the whole physically accessible range ($0 < n < 1$) on the basis of the hypothesis that variations in the strand length between crosslinking points of a gel network give rise to changes in the excluded volume interactions. This model establishes a relationship between n and the fractal morphology of the incipient gel network:

$$n = [d(d + 2 - 2d_f)]/[2(d + 2 - d_f)] \quad (1)$$

where d (which is 3) is the spatial dimension and d_f is the fractal dimension, which is related to the mass of a molecular cluster (M) by its radius of gyration (R^{d_f}) as $R^{d_f} \sim M$. From this equation, all values of the relaxation exponent ($0 < n < 1$) are possible for a fractal in the physically realizable domain ($1 \leq d_f \leq 3$). The value of d_f obtained for the SA/CM-KGM

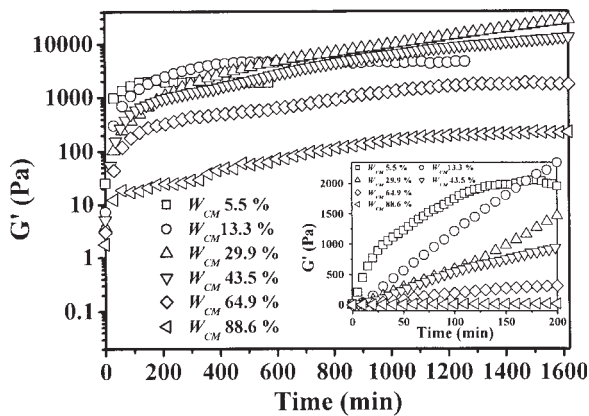


Figure 7 Time dependence of G' for 1.5 wt % SA/CM-KGM2 mixture systems with W_{CM} values of 5.5, 13.3, 29.9, 43.5, 64.9, and 88.6 wt % in the presence of 1.5 wt % GDL.

gel shown in Figure 6 is 2.3, which suggests a rather compact homogeneous network structure.⁴⁸ Values of d_f around 2.5 have been reported for guar gum aggregates in deuterium oxide⁴⁹ and pectin/chitosan mixed gels.⁵⁰

Figure 7 shows the time dependence of G' for SA/CM-KGM2 solutions with different contents of CM-KGM2 ($W_{CM} = 5.5, 13.3, 29.9, 43.5, 64.9, \text{ or } 88.6$ wt %) at 1 rad/s in the presence of 1.5 wt % GDL. It is obvious that G'_{sat} is strongly dependent on W_{CM} . In the initial gelation course, G' linearly increased with time (see the inset of Fig. 7). According to the initial $\Delta G'/\Delta t$ slope of the G' curve, the gelation rate gradually decreased with the weight ratio of CM-KGM2 increasing in the mixture solution. The t_{cr} and G'_{sat} values of the SA/CM-KGM2 mixtures obtained from Figure 7 were plotted against W_{CM} , as shown in Figure 8. t_{cr} monotonically increased with increasing W_{CM} , and this suggested that gelation of the system became slower and slower when the content of CM-KGM2 was gradually increased. Another observation from Figure 8 is that the gel strength was strongly

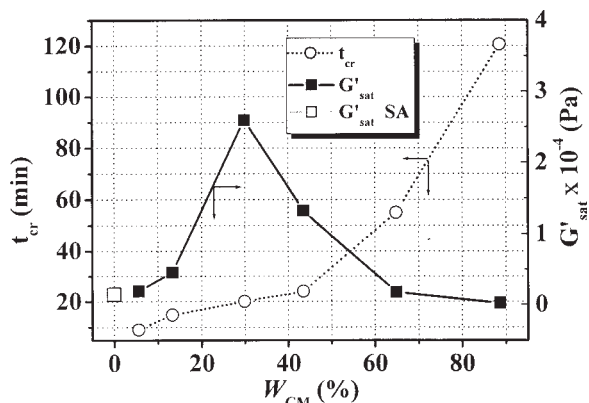


Figure 8 t_{cr} and G'_{sat} of SA/CM-KGM2 solutions (Fig. 7) as a function of W_{CM} .

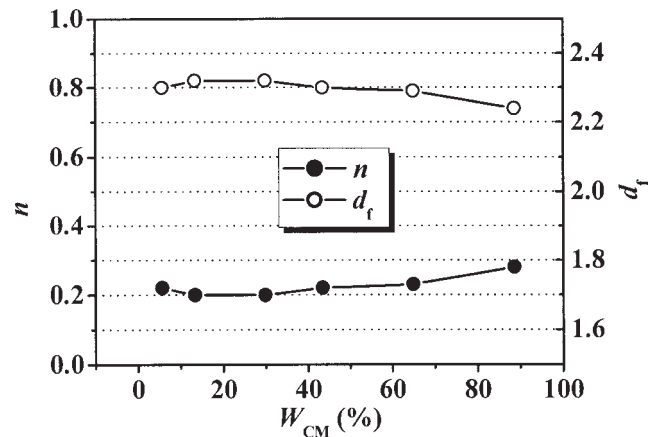


Figure 9 n and d_f as a function of W_{CM} .

dependent on the value of W_{CM} , and the maximum of G'_{sat} (ca. 25,000 Pa) appeared at $W_{CM} = 29.9\%$. The G'_{sat} value of the mixed gels with W_{CM} values of 5.5, 13.3, 29.9, and 43.5% was higher than that of the pure SA gel (ca. 1420 Pa; see the open square point in Fig. 8). However, the opposite result was observed for the mixed gels with W_{CM} values of 64.9 and 88.6%, which were formed at a lower gelation rate.

Figure 9 shows n and d_f as a function of mixing ratio W_{CM} . It is obvious that the value of d_f for the incipient gels is around 2.3 and is basically independent of the mixing ratio; this suggests that there is a rather compact and homogeneous gel network structure and that the fractal morphology of the gel remains unchanged while the composition of the mixture system varies.

Effect of DS of CM-KGM samples on the gelation kinetics

To clarify the effect of DS of CM-KGM samples on the gelation kinetics, the time course of G' for 1.5 wt % SA/CM-KGM mixture systems with different DS values of CM-KGM was examined (Fig. 10). Because of the observation of the maximum of G'_{sat} at $W_{CM} = 29.9\%$ (Fig. 8), a constant of $W_{CM} = 29.9\%$ was used. For the mixture system involving the CM-KGM1 with the lowest DS (0.26), the value of G' was maintained around 35 Pa, which is close to that of a pure SA solution in the absence of GDL (data not shown) and significantly lower than that of a pure SA solution in the presence of GDL. Therefore, we think that no obvious gelation can be observed for this mixture system. For the SA/CM-KGM4 (DS = 1.40) system, a clear lag phase before the start of the development of a gel network (corresponding to the rapid increase of G') was observed, which was

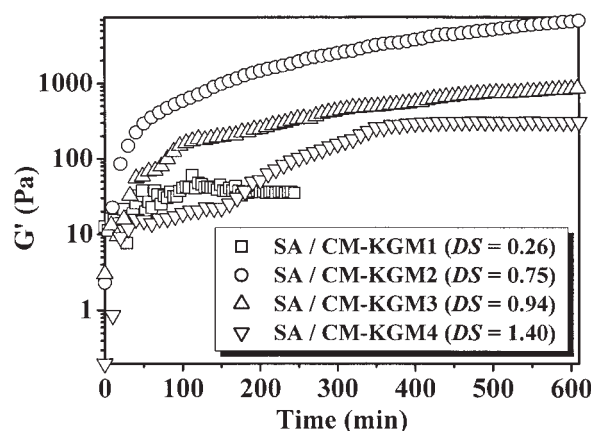


Figure 10 Time dependence of G' for 1.5 wt % SA/CM-KGM mixture systems ($W_{\text{CM}} = 29.9\%$) with different DS values of CM-KGM.

absent from the gelation processes of all the mixture systems involving CM-KGM2 with different mixing ratios (Fig. 7) and the system involving CM-KGM3 (Fig. 10). This lag phase is rationally attributed to the longer time related to the protonation of CM-KGM molecular chains. The fact that G'_{sat} became gradually lower with increasing DS of the CM-KGM samples can also be observed in Figure 10.

The t_{cr} values of 1.5 wt % SA/CM-KGM mixture systems ($W_{\text{CM}} = 29.9\%$) with different DS values of CM-KGM were determined by the Winter–Chambon criterion⁴³ (Fig. 11). A tendency was observed in which the value of t_{cr} was markedly prolonged with increasing DS of CM-KGM, suggesting that the gelation kinetics were greatly influenced by the amount of carboxymethyl groups in CM-KGM. Viscoelastic relaxation exponent n of the incipient gel on the threshold of gelation was 0.20, 0.24, and 0.26 for the mixture systems involving CM-KGM2 (DS = 0.75), CM-KGM3 (DS = 0.94), and CM-KGM4 (DS = 1.40), respectively. Accordingly, d_f was calculated according to eq. (1) to be approximately 2.3 for all three mixture systems, suggesting that the fractal morphology was unaffected by the DS values of the CM-KGM sample used in this study.

DISCUSSION AND CONCLUSIONS

It is well known that an abrupt decrease in pH causes a precipitation of alginic acid because of the low solubility of alginic acid in an acidic environment, whereas a slow and controlled release of protons may result in the formation of an alginic acid gel.^{51,52} It was recently reported⁵³ that scleroglucan gel was prepared by neutralization of an aqueous alkaline solution of scleroglucan by the *in situ* release of acid with methanoic acid or ethyl acetate. In this study, no precipitate was observed in the measure-

ments, and this implied that the formed gels from the SA/CM-KGM systems induced by a pH decrease were homogeneous.

When another anionic polysaccharide is added to an alginate solution (also an anionic polymer), an electrostatic repulsion will occur. The molecular chains of SA and CM-KGM became partly uncharged with the gradual release of protons induced by GDL, and hence the gelation was realized when the degree of segregative association between the two molecular chains was enough for the formation of a network structure (Fig. 4). The presence of CM-KGM indeed enhanced the gelation of SA, in that a significant improvement of the gel strength was observed for the mixture system (Figs. 4, 7, and 8). Although no other investigations about this mixture system could be used for reference so far, a segregative interaction between these two anionic polysaccharides might be responsible for this phenomenon of gelling enhancement. A similar segregative interaction is widely accepted for the rationalization of the gelation mechanism of an alginate/pectin mixture system.^{26–28,54}

The dependence of the gelation kinetics of the SA/CM-KGM complex on W_{CM} (Figs. 7 and 8) reflects the fact that the buffering capacities of the mixture systems are dependent on their component compositions. For an alginate/pectin system, it has been concluded that the buffering action of alginate and pectin delays the GDL-induced decrease in the pH value more than in a polymer-free solution.⁵⁵ It has also been suggested that the buffering action of the polymers results in a reduction in the charge on

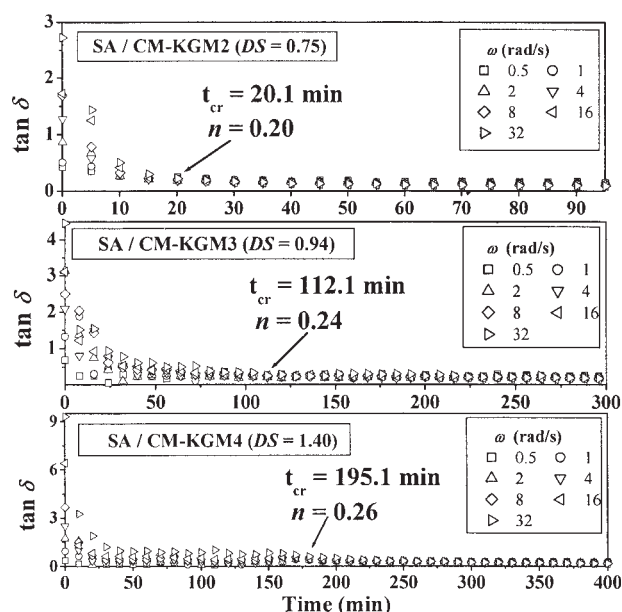


Figure 11 Determination of t_{cr} for 1.5 wt % SA/CM-KGM mixture systems ($W_{\text{CM}} = 29.9\%$) with different DS values of CM-KGM.

the polymer chain, which makes such chains less soluble and hence promotes interpolymer association.⁵⁵ The experimental findings shown in Figures 7 and 8 also indicate that the prerequisite for the interpolymer segregative interaction and the following gel formation is that the molecular chains of CM-KGM must be partly uncharged.

The applicability of the Winter–Chambon criterion⁴³ to all the mixture systems (Figs. 5, 6, and 11) in this study also supports the conclusion that homogeneously complex gels can be obtained at the expense of a pH decrease induced by GDL and in a wide range of mixing ratios. The fractal morphology of the mixed gels was compact and unaffected by the component compositions and DS values of the CM-KGM samples.

The authors acknowledge Takemasa (Graduate School of Human Life Science, Osaka City University) for his helpful discussion about NMR measurement.

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