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Gelation Behavior of Native and Acetylated Konjac Glucomannan

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Gelation kinetics of native and acetylated konjac glucomannan (KGM) samples in the presence of alkali (sodium carbonate) was studied by dynamic viscoelastic measurements. Molecular weight and other molecular parameters of KGM were determined by static light scattering and viscosity measurements. It was found that KGM molecules were degraded during acetylation treatment, but the molecular weights of acetylated samples were almost independent of the degree of acetylation (DA) and were about a half of that of a native sample. At a fixed alkaline concentration, increasing concentration of KGM or temperature shortened the gelation time, but increasing DA delayed it. The deacetylation reaction and subsequent aggregation process of acetylated samples needed longer time than that of native sample, and acetylated samples formed finally more elastic gels. It implied that the presence of acetyl groups exerts a strong influence on gelation behavior of KGM. It was suggested that the gelation rate of acetylated KGM and native KGM, which depends on the alkaline concentration and temperature, is an important factor that determines the elastic modulus of gels. This was supported by the experimental finding that the saturated elastic modulus tends to the same value when the ratio of alkali concentration to acetylated groups was kept constant. In slower gelation processes, junction zones are more homogeneously distributed and more numerous, leading to the more elastic gels.

1. Introduction

The gelation mechanism of polysaccharides has been studied by many research groups not only from a scientific interest but also for this mechanism's importance in food, pharmaceutical, biomedical, cosmetic, coating, painting, and related industries.[1−5] Proteins also form a gel, but the minimum concentration required for gel formation is much higher than that for polysaccharides, and therefore, polysaccharides are more frequently used as gelling and thickening agents in food and related industries. Gelation of polysaccharides may be classified into four categories from the viewpoint of the temperature dependence of the elastic modulus:[6] (1) so-called cold set gels like agarose, carrageenans and gellan which form a gel on cooling the solution, (2) so-called heat set gels like some cellulose derivatives such as methyl cellulose (MC), hydroxypropyl methyl cellulose (HPMC), Curdlan and konjac glucomannan (KGM) which form a gel on heating the solution, (3) re-entrant gels like xyloglucan from which some galactose residues are removed (which forms a gel at an intermediate specific temperature range and remains sol state at higher and lower temperatures outside of this specific temperature range), and (4) inverse re-entrant gels like a mixed solution of methyl cellulose and gelatin which forms a gel at higher and lower temperatures and stays in a sol state at an intermediate temperature range. Gelatin may be replaced by some polysaccharides which form a gel on cooling. Gels belonging to the first class are mostly thermoreversible and have been studied extensively although the gelation mechanism has not completely been clarified at the molecular level. The other three classes have not been elucidated so well. Some polysaccharides like MC or HPMC or Curdlan forms a gel on heating by itself; however, KGM forms a gel on heating only in the presence of alkali which removes acetyl groups. Some of these heat-set gels are thermoreversible (MC or HPMC gel, low-set gel of Curdlan), while others (KGM gel and high-set gel of Curdlan) are thermoirreversible.

Konjac glucomannan (KGM) is a neutral polysaccharide derived from the tuber of *Amorphophallus konjac* C. Koch. KGM consists of β-1,4 linked glucose and mannose units.[7] The glucose: mannose ratio has been reported to be between 1:1.6[8] and 1:1.4,[9] and the presence of some branching points at the C-3 position of the mannoses[10] is suggested. The glucomannan backbone possesses 5−10% acetyl-substituted

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residues,[11−13] and it is widely accepted that the presence of this group confers solubility on the glucomannan in aqueous solution. Maekaji[12,13] concluded that the molecules of KGM, which lost their acetyl groups with the aid of alkalis, aggregate in part with one another through a linkage such as the hydrogen bond, by which the molecules come into a network structure. That is, the gel is formed. It was shown recently by Williams et al. based on NMR relaxometry and rheology that the addition of alkali to KGM plays an important solubilizing role in addition to facilitating the deacetylation of the chain.[14] The physicochemical properties have not been fully elucidated mainly because of the difficulty in obtaining easily soluble and well-fractionated KGM samples.

KGM is regarded as a noncalorie food, and one of the primary benefits of traditional Japanese foods made with konjac flour is the content of indigestible dietary fiber, the role of which has been demonstrated in weight reduction, modification of carbohydrate metabolism in diabetics, and cholesterol reduction.[5] KGM is effective for improving the vitamin B-6 nutritional state,[15] modifying the intestinal microbial metabolism,[16] or lowering plasma cholesterol in rats.[17] The introduction of KGM fiber in a diet may improve metabolic control in human beings.[18] Additionally, KGM can be extruded into films[19] or forms blend membranes[20,21] for coating and packaging applications, and KGM gels have promising applications for a controlled release matrix.[22] Recently the interaction of KGM with other hydrocolloids was studied, for example, KGM with κ-carrageenan,[23−28] xanthan,[29−31] gellan gum,[32,33] corn starch,[34,35] and acetan[36,37] to develop further utilization.

In our previous study,[38] the critical gelation time t_{cr} for KGM aqueous dispersions in the presence of Na_2CO_3 was determined by using Winter−Chambon's method.[39] They have proposed a method to determine the gelation point from mechanical spectra.[39] According to their proposition, the gelation point corresponding to the specific instant t_{cr} is defined as a point at which relations $G'(\omega) \sim G''(\omega) \sim \omega^n$ ($0 < n < 1$), and $\tan \delta = G''/G' = \tan(n\pi/2)$ hold simultaneously, where n is the relaxation exponent. In the previous study,[38] we observed that the values of t_{cr} were independent of frequency, and both of the storage and loss shear moduli, G' and G'' showed a power law dependence in the double logarithmic plot against frequency at t_{cr}. The gelation time t_0 corresponding to the intersection of G' and G'' was also suggested to mark the instant of gelation point,[40] but the time of intersection was found to be a function of frequency for KGM.[39] However, for experimental simplicity, in our studies which follow, t_0 at 1 rad s^{-1} was used instead of t_{cr} to determine the apparent onset time of gelation.

Although the deacetylation by alkali is believed to be a key reaction which leads to the gelation, the role of acetyl groups is not fully understood despite recent experimental efforts.[14] In the present study, the native KGM was treated with acetic anhydride, and five acetylated fractions with different degrees of acetylation (DA) were obtained. The rheological behavior during gelation of KGM with different degrees of acetylation on addition of an alkali Na_2CO_3 was investigated by dynamic viscoelasticity to gain further insight into the gelation mechanism.

Table 1. Molecular Characteristics of KGM Samples

sample	Rs	Ac20	Ac21	Ac26	Ac27	Ac32
DA (%)	1.6	2.2	2.6	2.9	4.0	5.3
DS	0.06	0.09	0.10	0.11	0.16	0.21
$10^{-2}[\eta]$ ($cm^3\ g^{-1}$)[a]	2.83	2.00	2.12	2.07	2.08	2.11
k' [a]	0.50	0.45	0.40	0.38	0.42	0.41
$10^{-3}M_w$ [b]	608	327	317	341	346	295
$R_{G,z}$ (nm)[b]	48.5	44.4	42.8	44.9	44.5	42.9
10^4A_2 (mol $cm^3\ g^{-2}$)[b]	0.97	1.11	0.67	1.02	0.94	0.70

[a] Measurement by Ubbelohde type viscometer in cadoxen at 25 °C. [b] Measurement by static light scattering in cadoxen at 25 °C.

2. Materials and Methods

2.1. Materials. Six fractions of KGM with different degrees of acetylation were prepared in the Central Research Institute of the Mitsukan Group Co. Ltd. (Aichi, Japan). The native KGM, denoted as Rs, was purchased from Shimizu Chemical Co. (Hiroshima, Japan), which was treated with acetic anhydride at different reaction temperatures in the presence of different quantities of catalyst (zinc chloride) as described below, and then Ac20, Ac21, Ac26, Ac27, and Ac32 fractions with different acetylation levels were obtained. DA (%) and the degree of substitution (DS) of Rs, Ac20, Ac21, Ac26, Ac27, and Ac32 fractions were determined by a method of alkaline titration and are listed in Table 1. DA is defined as the weight percent of acetyl-substituted residues in KGM backbone. DS was calculated from DA as the (moles of acetyl-substituted residues)/(moles of monosaccharide residues).

Measurement was performed by static light scattering in cadoxen at 25 °C.

Pretreatment: 20 mL of 50vol % acetic acid was added into 10 g of Rs (native KGM) and mixed sufficiently and then dried at 60 °C for 30 min.

Acetylation: 50 mL of acetic anhydride was added into the pretreated materials at different temperatures (Ac20 and Ac21, 50 °C; Ac26 and Ac27, 70 °C; Ac32, 90 °C)and then mixed for 30 min. Zinc chloride as catalyst in different quantities (Ac20, Ac26, and Ac32, 0.1 g; Ac21 and Ac27, 0.2 g) was added, and then the recation was mixed for 2 h. Then, 50 mL of deionized water was added and mixed for 10 min. The precipitate was obtained by adding 100 mL of ethanol and then filtered off by using a G3 glass filter. One hundred milliliters of 60% ethanol was added and then mixed for 30 min and filtered, and this process was repeated twice. One hundred milliliters 100% ethanol was added and then filtered. After drying in a draft, the material was evacuated at 60 °C for 4 h.

2.2. Preparation of Aqueous Dispersion and Cadoxen Solution of KGM. Powders of KGM fractions (Rs, Ac20, Ac21, Ac26, Ac27, and Ac32) were dispersed in distilled water at room temperature for 1 h and were heated to 80 °C and then maintained at 80 °C for 1 h and cooled to room temperature. The aqueous dispersions of KGM with a concentration range 0.5−3.0 wt % were used in the dynamic viscoelastic measurements to study the mechanism of gelation.

The solvent cadoxen was freshly prepared before each measurement. About 28 wt % aqueous solution of ethylenediamine was saturated with cadmium oxide (CdO) at 0 °C under vigorous stirring and kept for 10 h below 5 °C. The solution was centrifuged at 9000*g* for 30 min, and then the supernatant was filtered through a G4 glass filter. The cadoxen prepared in this way, which was transparent and stable at 25 °C, was kept in a refrigerator below 5 °C until use. Cadmium content in cadoxen was 4.5 wt %. The

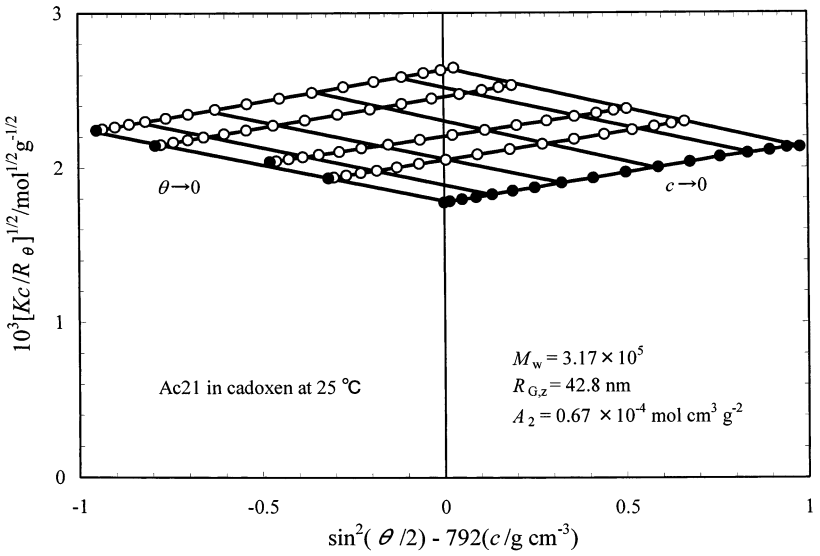

Figure 1. Zimm plots for Ac21 in cadoxen at 25 °C.

refractive index n_0 of cadoxen was taken to be 1.388 at 633 nm at 25 °C.[41] The cadoxen solutions of KGM were used in intrinsic viscosity and static light scattering measurements to determine the molecular weight and other molecular parameters of KGM.

Our cadoxen differed from that of Henley[42] in regard to the NaOH content; Henley's cadoxen contained about 0.5 M NaOH, while ours is free of NaOH. The reason is that the addition of NaOH possibly changes DA in KGM samples. Our preliminary tests also showed that addition of NaOH barely enhanced the solubility of KGM in cadoxen. This kind of cadoxen without NaOH was also used by Sato et al.[43] in the study of xanthan and by Zhang et al.[41] in the study of β-D-glucan.

2.3. Measurements of Intrinsic Viscosity. The intrinsic viscosities of KGM cadoxen solutions were measured at 25 ± 0.02 °C by using an Ubbelohde type viscometer (Kaburagi Scientific Instruments Co. Ltd., Tokyo, Japan). Extrapolation to infinite dilution was made using both Huggins and Kraemer plots from which the intrinsic viscosity $[\eta]$ and Huggins constants k' were calculated. The flow time for the cadoxen solvent was 504 s.

2.4. Light-Scattering Measurements. Static light scattering measurements were made at approximately 25 °C using a multiangle light scattering detector DAWN DSP (Wyatt Technology Inc.). A vertically polarized He−Ne laser operated at the wavelength λ_0 of 632.8 nm was used as the incident beam. The photometer was calibrated with pure toluene and aqueous solution of dextran with low molecular weight. Optical clarification of cadoxen solution of KGM was achieved by filtration through hydrophilic PTFE filters (0.45 μm pore-size). The specific refractive index increments of KGM in cadoxen plus water mixed solvents, $(\partial n/\partial c)_{\mu,\text{mix}}$, at 25 °C were measured using a double-beam differential refractometer of DRM-1021 (Otsuka Electronics Co. Ltd., Osaka, Japan), and $(\partial n/\partial c)_\mu$ in 100% cadoxen were estimated according to the method of Sato−Norisuye−Fujita.[43] Here, the subscript μ denotes the chemical potentials of the diffusible components in the cadoxen solution. The $(\partial n/\partial c)_\mu$ value of 0.136 cm^3 g^{-1} was almost independent of DA.

A Zimm plot in the square-root form was employed to obtain the weight-average molecular weight, M_w, the second virial coefficient, A_2, and the z-average radius of gyration, $R_{G,z}$, expressed as

$$(Kc/R_\theta)^{1/2} = M_w^{-1/2}[1 + (^1/_6)R_{G,z}{}^2k^2 + A_2M_wc]$$

where K, c, and R_θ are the optical constant, the polymer mass concentration, and the reduced scattering intensity at scattering angle θ, respectively. k is the momentum transfer vector defined as $k = (4\pi n_0/\lambda_0)\sin^2(\theta/2)$, with n_0 being the refractive index of the medium (cadoxen solution).

2.5. Dynamic Viscoelasticity. Dynamic viscoelastic measurements were carried out using a Fluids spectrometer RFS II (Rheometrics Co. Ltd.) with a parallel plate geometry (25 mm in diameter, 1.5 mm gap). The strain was set as 0.5% which is within a linear viscoelastic regime. One milliliter of the KGM aqueous dispersion was poured directly onto the plate of the instrument which had been kept at each measurement temperature. The gelation kinetics was studied at constant temperatures from 45 to 80 °C. A solution of Na_2CO_3 (20 μL) with various concentrations was added at time $t = 0$ to the KGM dispersion and mixed, and then the storage shear modulus G' and the loss shear modulus G'' were measured as a function of time at a constant frequency 1 rad s^{-1}. The alkaline concentration was represented by the concentration ratio of Na_2CO_3 to KGM in the investigation of the effects of KGM concentration on gelation. The alkaline concentration was represented by wt % for all the other measurements. The pH values of aqueous dispersions, aqueous dispersions immediately after the addition of Na_2CO_3 solution, and after gelation were measured for some KGM samples at 5 °C.

3. Results and Discussion

3.1. Molecular Weight and Other Molecular Parameters of KGM. The intrinsic viscosities $[\eta]$ and Huggins' coefficient k' of KGM samples in cadoxen at 25 °C are summarized in Table 1. We selected cadoxen solution to dissolve KGM samples since this solvent has been known to dissolve KGM,[26,27] β-D-glucan,[41] cellulose,[42] and xanthan[43] and to make a clear, colorless, and stable solution. As shown in Table 1, the values (0.38−0.50) of Huggins' coefficient were obtained. Intrinsic viscosities of acetylated KGM samples are (2.00−2.12) × 10^2 cm^3 g^{-1} without a marked dependence on DA, and that of native Rs is far larger than those of acetylated samples.

The typical result of scattered light intensity measurements is shown in Figure 1 for sample Ac21 in cadoxen at 25 °C. From the intercept with the ordinate and the slopes against

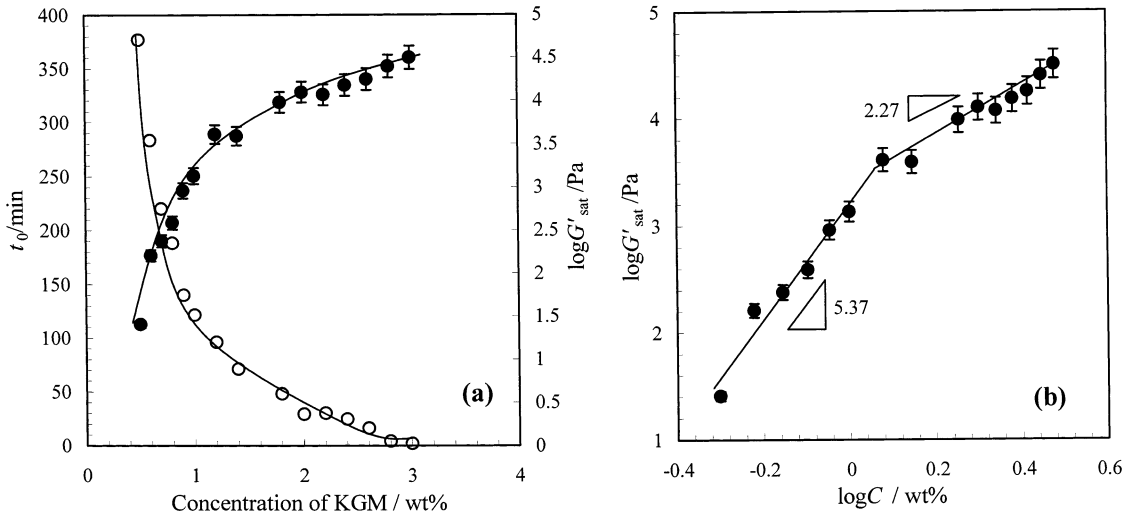

Figure 2. Gelation time t_0 (○) and G'_{sat} (●) as a function of concentration for Rs aqueous dispersions in the presence of Na_2CO_3 at 45 °C. The concentration ratio of Na_2CO_3 to KGM was fixed to 0.1.

the concentration and scattering angle, weight-averaged molecular weight, M_w, second virial coefficient, A_2, and z-average radius of gyration, $R_{G,z}$ were evaluated, respectively. The numerical data from light scattering measurements are summarized in Table 1. According to Sugiyama et al.,[44] the magnitude of $R_{G,z}$ is strongly dependent on strain and place of cultivation. In the present work, the molecular weights of acetylated samples were found to be almost independent of DA, and were about a half of that of a native sample, Rs. In comparison with the molecular weights of native KGM reported by Kohyama et al.[26,27] and Sugiyama et al.,[44] the lower values of molecular weight and radius of gyration obtained for nitro-KGM by Torigata[45] and those for acetylated samples in the present work might be attributed to the degradation of KGM molecules during nitration or acetylation. However, in the present work, the various acetylation reaction conditions, such as different acetylation temperature and the amount of catalyst, did not lead to a remarkable difference in molecular weight. Therefore, in the following discussions, a slight difference in molecular weight between the acetylated samples was not taken into account, and attention was paid mainly to the influence of the degree of acetylation (DA). Acetylation apparently breaks a set of sensitive backbone bonds very rapidly, leading to an immediate drop in molecular weight, but then no subsequent degradation seems to occur. The detailed mechanism of the main chain scission should be explored in the future to get better or ideal samples with different DA without changing the molecular weight.

3.2. Effects of KGM Concentration on Gelation. Both storage and loss moduli G' and G'' of Rs aqueous dispersions in the presence of Na_2CO_3 at a fixed concentration ratio of Na_2CO_3 to KGM (0.1) were found to increase monotonically and attained plateau values, the saturated storage modulus G'_{sat} and loss modulus G''_{sat}, respectively, after a certain time (data not shown) as was observed previously.[38,46] The gelation time[40] t_0 defined as the time of the crossover of G' and G'' and G'_{sat} of Rs aqueous dispersions in the presence of Na_2CO_3 at 45 °C as a function of KGM concentrations are shown in Figure 2, parts a and b. t_0 increased sharply with decreasing concentration and G'_{sat} increased with increasing concentration. Since molecular chains are close each other at higher concentrations, the probability of the formation of a junction zone is higher than that at lower concentrations. Gelation would begin even before the complete loss of acetyl groups at higher concentrations; therefore, t_0 became shorter with increasing concentration of KGM as expected.

The concentration dependence of the elastic modulus of gels has been explained by Clark and Ross-Murphy[47] using a cascade treatment and by Oakenfull[48] using a modified theory of rubber elasticity. Both theories predict a steep slope at lower concentrations and a gradual slope at higher concentrations in the double logarithmic plot of the elastic modulus against concentration C. The power law dependence of modulus on the KGM concentration, $G'_{sat} \sim C^n$, where the value of n for a KGM sample with a molecular weight of 2.56×10^5 in the concentration range 1.0−3.0 wt % has been reported as 2.55 previously.[46] The linear relation of log G'_{sat} and log C was found for Rs in the present work in the concentration ranges 0.5−1.0 and 1.2−3.0 wt %, respectively (Figure 2b). From the slope of the straight lines, values of 5.37 and 2.27 were obtained for n for KGM gels at low and high concentration ranges, respectively. The value of the exponent 2.27 at high concentration is close to 2.55 which was reported previously.[46] A larger value of the exponent 5.37 at a low concentration range is consistent with the prediction of the above theories.

Hirai[49] reported that alkali was not necessary to form a gel if the KGM concentration was higher than 8 wt %. Dave et al.[19] observed that concentrated (>7 wt %) KGM in aqueous media exhibited a liquid crystalline behavior demonstrated by polarized optical microscopy and circular dichroism. Since the concentrated KGM aqueous dispersion shows a very high viscosity, it will take a long time to obtain homogeneous samples with concentrations higher than 3 wt %. For example, all the KGM dispersions used by Dave et al.[19] were allowed to equilibrate for about 1 month to prepare homogeneous samples prior to analysis. Both of Hirai[49] and Dave et al.[19] carried out their measurements under the condition that alkali was free. Because of the very rapid gelation process of the concentrated KGM solution (>3 wt

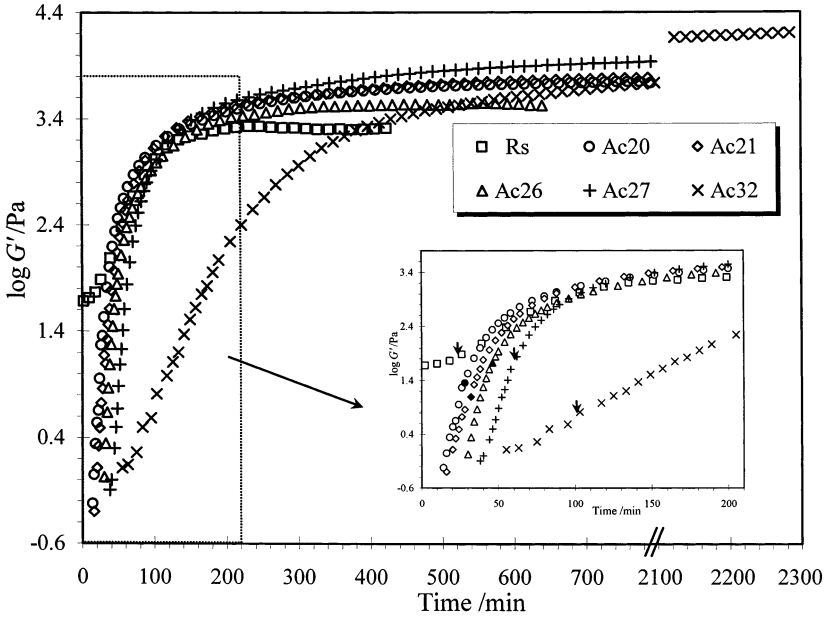

Figure 3. Time dependence of G' of 2.0 wt % KGM aqueous dispersions in the presence of Na_2CO_3 at 45 °C. The concentration of Na_2CO_3 was 0.2 wt %. The arrows or the closed symbols represent the crossover points of G' and G'' (corresponding to t_0, G'' not shown).

%) in the presence of alkali, it is almost impossible to get enough time to pursue the gelation process so that alkali is homogeneously distributed in KGM dispersion, so the highest concentration used in the present work is set as 3 wt %.

3.3. Effects of Degree of Acetylation (DA) on Gelation. Figure 3 shows the time dependence of G' of 2.0 wt % KGM aqueous dispersions in the gelation process at 45 °C in the presence of Na_2CO_3. The concentration of Na_2CO_3 was fixed as 0.2 wt %. The value of G' for Rs at the initial stage was far larger than that for acetylated KGM samples. However, G' of Rs was overtaken by that of acetylated KGM samples finally with time elapsing. The gelation time t_0 became longer with increasing DA. It is expected that the deacetylation reaction and further aggregation process for KGM samples with higher DA need longer time than those for KGM with lower DA. G' of all the samples increased rapidly in the beginning of gelation and attained the plateau values finally. It took a longer time for KGM with higher DA to reach the saturated value of G'. G' of the native KGM, Rs attained plateau values in ca. 245 min (ca. 4 h), while G' of Ac 32 still continued to increase even after 2300 min (ca. 38 h). The reason Ac32 showed a different behavior is interpreted as follows: the DA of Ac32 is the highest, and the largest amount of alkali is required. The present concentration of Na_2CO_3 solution was insufficient for 2 wt % Ac32 dispersion to form a gel in a short time. It was difficult to observe the exact plateau values of G' for some acetylated KGM samples under this experimental condition due to the extremely long measuring time.

Table 2 shows the dependence of t_0 on the DA at different temperatures (60 and 45 °C) and alkaline concentrations (Na_2CO_3 were 0.2 and 0.4 wt %). The gelation time t_0 became longer with increasing DA at a fixed alkaline concentration. This tendency is more remarkable at lower temperature or lower alkaline concentration.

The native KGM, Rs, showed a gelation behavior different from other acetylated samples. The plateau value of G' was

Table 2. Dependence of Gelation Time t_0 (min) of 2.0 wt % KGM Aqueous Dispersions on the Degree of Acetylation at Various Temperatures and Alkaline Concentrations

	Rs	Ac20	Ac21	Ac26	Ac27	Ac32
Na_2CO_3 = 0.2 wt %, 45 °C	24.6	28.9	33.0	46.1	60.2	102.0
Na_2CO_3 = 0.2 wt %, 60 °C	12.0	14.1	16.1	23.5	31.1	73.1
Na_2CO_3 = 0.4 wt %, 45 °C	8.5	9.7	11.6	14.8	19.5	27.5
Na_2CO_3 = 0.4 wt %, 60 °C	4.6	5.9	6.6	7.7	10.5	14.2

previously found to increase with increasing molecular weight of KGM for native KGM samples without acetylation.[46] In the present study, the molecular weight of acetylated KGM decreased to about half of that for native Rs after acetylation. So it is expected that the G'_{sat} for acetylated KGM should be far smaller than that for Rs. However, Figure 3 shows clearly that G'_{sat} of acetylated samples is larger than that of Rs. It implies that the presence of acetyl groups plays an important role in the gelation behavior of KGM. Since there are more acetyl groups in acetylated KGM than the native KGM, the gelation takes a longer time for KGM with higher DA if the alkali concentration is fixed. Acetyl groups are believed to confer the solubility to KGM and prevent the aggregation of KGM chains. It was shown previously[46] that KGM with higher molecular weight forms a gel with larger elastic modulus at the same alkali concentration and temperature. It was also shown[46] that the gelation proceeds faster at higher alkaline concentration and that the saturated modulus tends to decrease in a too rapid gelation. Therefore, molecular weight and alkali concentration both influence the saturated value of elastic modulus of KGM gels of a fixed concentration. It should be kept in mind that saturated moduli of KGM dispersions with lower degrees of acetylation including a native KGM Rs, which has the lowest degree of acetylation, cannot reach a larger value than that with higher degrees of acetylation as shown in Figure 3 although the molecular weight of Rs is about twice higher than that of acetylated samples. One possible explanation for the larger elastic moduli of gels of KGM with higher

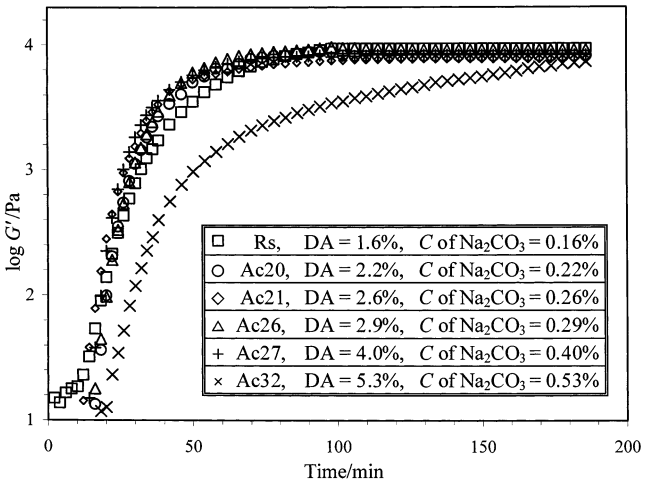

Figure 4. Time dependence of G' of 2.0 wt % KGM aqueous dispersions in the presence of Na_2CO_3 at 60 °C. The ratio of alkaline concentration to the degree of acetylation was fixed to a constant (0.1).

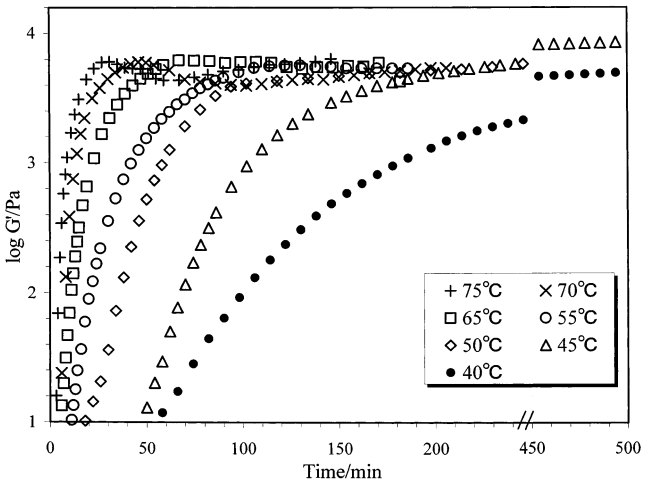

Figure 5. Time dependence of G' of 2.0 wt % Ac20 aqueous dispersions in the presence of Na_2CO_3 at different temperatures. The concentration of Na_2CO_3 was fixed to 0.2 wt %.

DA is that there is a longer time for molecular chains to rearrange to form more ordered structures than in KGM with lower DA. Consequently, the distribution of junction zones may become more homogeneous and the number of junction zones becomes more numerous, which would lead to more elastic gels with larger G'_{sat} values. This should be explored urgently by atomic force microscopy.

Where the alkaline concentration (C_{Al}) to DA was fixed to a constant (0.1), a similar time course of G' for all the samples except Ac32 was observed as shown in Figure 4. It indicates that C_{Al}/DA plays a crucial role in the gelation process. Hata et al.[50] have speculated that gelation is accompanied by a chemical change in KGM caused by alkalis, but the alkalis cannot be incorporated into the gel as a constituent. Sakurada et al.[51] and Ridout et al.[38] have maintained the idea on the basis of X-ray diffraction analysis and concluded that the gelation was induced by the conformational transformation of KGM, from an amorphous form to an ordered form.

3.4. Effects of Heating Temperature on Gelation. Figure 5 shows the time dependence of G' for 2.0 wt % Ac20 aqueous dispersions in the presence of Na_2CO_3 at different temperatures ranging from 40 to 75 °C. The concentration of Na_2CO_3 was 0.2 wt %. Values of G' observed at temperatures below 55 °C were found to increase monotonically with temperature. At temperatures higher than 55 °C, different rheological phenomena were observed; G' increased quickly and then decreased, namely, significant initial peaks in G' were seen, and the size and shape of the peaks changed with temperature. Values of G'_{sat} at lower temperatures (40, 45, and 50 °C) were found to be larger than those at higher temperatures, indicating the possibility that a slower gelation process can form more elastic gels, which is consistent with the situation observed in Figure 3.

The appearance of initial peaks of G' in the gelation process of KGM has been reported previously[46] and was worked out further by Zhang et al.[52] It was suggested that the initial peak results from the wall slip between sample and measuring geometry owing to a rapid gelation process with syneresis and/or disentanglement of molecular chains adsorbed on the surface of parallel plate from those located in the solution. The slippage observed by Zhang et al.[52] was related to the temperature, applied strain, gap size, and the molecular weight and concentration of KGM. In the present work, the degree of acetylation and alkaline concentration were also found to influence the slippage, due to their effect on the gelation process. Generally, the degree of syneresis seems to increase with increasing rate of gelation, and thus induces the slippage. It should be explored in the near future.

The gelation time t_0 of aqueous dispersions of 2.0 wt % KGM samples with different DA in the presence of 0.2 wt % Na_2CO_3 was examined as a function of temperature, and it was shown that t_0 became shorter with raising temperature (data not shown), indicating that the gelation was promoted remarkably by raising temperature as observed previously.[46] For example, G' of Rs increased almost immediately when the measurement was performed at 80 °C and attained the plateau values after only ca. 20 min; however, the gelation time t_0 of Rs at 45 °C was 78 min, and it took ca. 8 h to attain the plateau value. The Arrhenius plot is shown in Figure 6, and the apparent activation energy E_a for each KGM sample was obtained from the slopes of the straight lines. Values of apparent activation energy of Rs, Ac20, Ac21, Ac26, and Ac27 were calculated to be 81.3, 99.6, 92.7, 80.4, and 79.1 kJ·mol^{-1}, respectively. Values of E_a for Ac20 and Ac21 were slightly larger than the others, but the reason is still unclear. The Arrhenius relationship was also used to describe the temperature dependence of gelation for some polysaccharides and proteins, such as methylcellulose,[53] κ-carrageenan,[54] β-lactoglobulin,[55] and sunflower globulin.[56] The activation energy was related to the formation and rupture of linkages in "junction zones" during the gelation process in which several kinds of intermolecular interactions are involved.[57] The different temperature dependencies of these intermolecular interactions lead to the different effects of temperature on the gelation process, which may be reflected in the Arrhenius plot of $\ln(1/t_0)$ vs $1/T$.[58] The gelation of KGM is characterized by a single activated process between 40 and 80 °C (Figure 6), and the apparent activation energy E_a was almost independent of DA. Hydrogen bond was supposed to be the most important

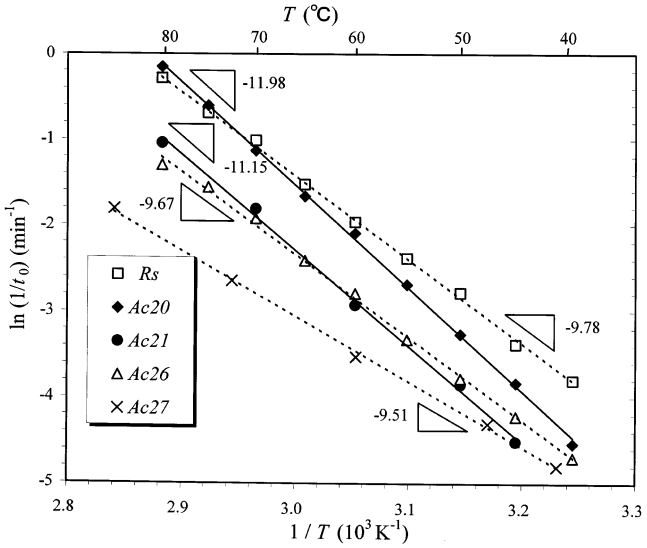

Figure 6. Arrhenius treatment for the effect of temperature on the gelation rate ($1/t_0$, min^{-1}) of 2.0 wt % KGM aqueous dispersions in the presence of Na_2CO_3. Concentration of Na_2CO_3 fixed to 0.2 wt %. The number near each line represents the slope of that line.

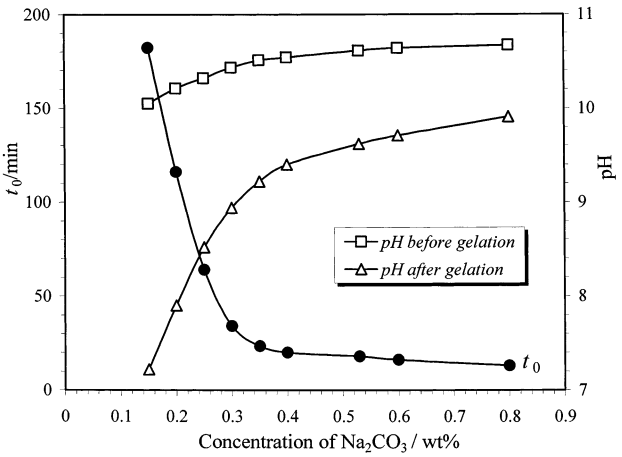

Figure 7. Alkaline concentration dependence of gelation time t_0 (●) and pH of 2.0 wt % Ac32 aqueous dispersions in the presence of Na_2CO_3 at 60 °C.

intermolecular interaction, which mainly contributes to the buildup of gel network of KGM. KGM forms a thermoirreversible gel in the presence of an alkali. The KGM molecules, which lost their acetyl groups with the aid of alkali, aggregated with one another through a linkage such as the hydrogen bond, and then the gel is formed. Maekaji studied the role of hydrogen bonds in the gel formation by examining that KGM gel was peptised under a mild condition with reagents such as salicylate and urea which disrupt the hydrogen bond of the polymers.[13,58] In his studies, Maekaji used the word "peptization" of KGM gels to mean the dissolution of KGM gels once formed by using various reagents. The peptization of KGM gels was not followed by the significant change in the structure of the polymer, and the peptized sol was different from the sol of native KGM before the addition of alkali.[13,58] Further studies based on the dynamic viscoelastic or other physicochemical measurements are required for the quantification of the intermolecular interactions involved in KGM gels.

3.5. Effects of Alkaline Concentration on Gelation. Figure 7 shows the alkaline concentration dependence of gelation time t_0 and pH of 2.0 wt % Ac32 aqueous dispersions in the presence of Na_2CO_3 at 60 °C. The pH values after gelation decreased with decreasing alkaline concentrations. The gelation time t_0 at the concentration of Na_2CO_3 lower than 0.3% was quite a bit longer than that at higher alkaline concentrations. It seems that there is a critical alkaline concentration below which the gelation does not occur within an accessible experimental time.

It was reported that the specific volume of KGM in aqueous solution was almost constant between pH 3 and 11 and then increased steeply at above pH 11 with increasing pH.[59] It was also reported that the gelation of KGM occurred at a pH range from 11.3 to 12.6. It was suggested that the change in molecular structure is necessary for the gelation of KGM. In the present work, the pH range for the possible gelation of Ac32 was found to be from 10.0 to 10.7 as shown by square symbols in Figure 7, and that for Ac26 was 10.1 to 10.8 (data not shown). Maekaji[12] suggested that the gelation occurs after a certain induction period, and the concentration of hydroxide ion $[OH^-]$ ($mol \cdot L^{-1}$), independent of the kind of alkali, governs the induction reaction. He found that the induction reaction corresponded to deacetylation, and the deacetylation ratio, i.e., (acetyl groups removed)/(total acetyl groups) decreased with increasing concentration of KGM. The deacetylation ratio was also found to increase with the "peptizing power" (dissolving ability of KGM gels once formed) of coexisting ions[12,58] Since the pH value of KGM aqueous dispersion immediately after the addition of Na_2CO_3 solution reflects the initial interaction between the acetyl groups and the alkali, the pH range for the possible gelation should change with DA, and then the lower pH range for acetylated samples in this work was observed, in comparison with that for native sample in the previous work.[59] It was proposed that the induction periods following alkali addition not only are simple deacetylation delays but also are related to the aggregation kinetics of the deacetylated material.[14]

In conclusion, it was suggested that the deacetylation leads to the aggregation of stiffened molecular chains. In the presence of excessive alkali, the gelation proceeds too fast resulting a gel with smaller elastic modulus. The saturated elastic modulus of gels depends strongly on the gelation rate. The KGM gel is thermoirreversible, and the rearrangement of network chain such as in helix−coil transition does not seem to occur as in cold-set gels. It was reported recently that the helix−coil transition occurs in a self-supporting gels of gellan induced by temperature change,[60] which also occurs in κ-carrageenan gels by the immersion in salt solutions as reported previously.[61] In the gelation of KGM, the gelation rate was shown to be governed by the DA and alkaline concentration as well as by temperature, and it was also shown that the slower gelation leads to the more elastic gels. The experimental finding that the saturated elastic modulus of gels of acetylated KGM was larger than that of native KGM suggests that the gelation rate is more important in the present case than the molecular weight difference (about twice) as a determining factor for the saturated elastic modulus. It seems to be contradictory to previous reports that the elastic modulus of polymer gels is an increasing

function of molecular weight as in gels of agarose[62] and gelatin[63] and even for KGM.[46] Gels formed in a fast process may be inhomogeneous; i.e., the dense junction zones and sparse area are separated, while the distribution of junction zones is more homogeneous in gels formed slowly. Too rapid gelation does not permit some molecular chains, which might be incorporated as elastically active chains in a slower gelation process, to become elastically active chains. Although molecular forces responsible for gel formation in both cold-set gels and in KGM gels are believed to be hydrogen bonds, tighter stacking occurs in KGM gels than in other thermoreversible gels such as gellan, carrageenan, agarose, etc. The AFM observations will be useful to get some clues on these problems.

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