

## Dynamic Viscoelastic Study on the Gelation of Acetylated Konjac Glucomannan

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Gelation kinetics of acetylated konjac glucomannan in the presence of an alkaline coagulant was studied by dynamic viscoelastic measurements. The critical gelation point  $T_{cr}$  was determined as time at which the mechanical loss tangent  $\tan \delta (= G''/G')$  is independent of frequency. At gel point a power law dependence of the storage and the loss shear moduli,  $G'$  and  $G''$  in the double logarithmic plot against frequency was observed. Increasing concentration of KGM or temperature shortened the gelation time, but increasing acetylation degrees of KGM delayed it.

Key words: Acetylated konjac glucomannan, sol-gel transition, dynamic viscoelasticity, alkaline coagulant.

### 1. INTRODUCTION

Konjac glucomannan (KGM) is a neutral polysaccharide derived from the tuber of *Amorphophallus konjac* C. Koch. KGM consists of  $\beta$ -1-4-linked glucose and mannose units. It is known that the glucose: mannose ratio is between 1:1.6[1] and 1:1.4[2] and there are some branching points at the C-3 position of the mannoses. The glucomannan backbone possesses 5-10% acetyl substituted residues[3,4], and it is widely accepted that the presence of this group confers solubility on the glucomannan in aqueous solution.

KGM forms a thermally stable gel on heating in the presence of an alkaline coagulant. The addition of alkali plays an important solubilizing role in addition to facilitating the deacetylation of the chain[5]. Deacetylation is important both in reducing the inherent aqueous solubility of the polymer and in progressively negating the alkali-induced polyelectrolytic nature of the polysaccharide chain via reaction induced pH changes. It is proposed that the induction periods following alkali addition are not simply deacetylation delays but are related to the aggregation kinetics of the deacetylated material[5]. However, as refined KGM has a few or no acetyl groups, the mechanism of the gelation still remains problems to be studied, especially the quantitative analysis for the effect of acetyl groups on the gelation process. In the present study, the rheological properties during KGM gelation on addition of an alkaline coagulant as a function of degrees of acetylation and concentration of KGM were investigated by observing dynamic viscoelasticity. The

effects of heating temperature on the gelation process of the KGM with high degree of acetylation were also investigated.

### 2. EXPERIMENTAL

#### Materials

Six fractions of KGM with different degrees of acetylation were prepared by Mitsukan Group Co. Ltd. (Nagoya, Japan). The native KGM, RS was treated with acetic anhydride for different reaction temperatures and different quantities of catalyst (zinc chloride), and Ac20, Ac21, Ac26, Ac27 and Ac32 fractions with different acetylation levels were obtained. The degrees of acetylation (%) and the degrees of substitution of RS, Ac20, Ac21, Ac26, Ac27 and Ac32 fractions were listed in Table I.

Pre-treatment: 20ml of 50% acetic acid was added into 10 gram of RS (native KGM) and mixed sufficiently, then was dried for 30min.

Acetylation: 50 ml of acetic anhydride was added into the pre-treated materials at different temperatures (Ac20 and Ac21: 50°C, Ac26 and Ac27: 70°C, Ac32: 90°C), then mixed for 30 min. Zinc chloride as catalyst with different quantities (Ac20 and Ac26 and Ac32: 0.1g, Ac21 and Ac27: 0.2g) was added then mixed for 2 hours. 50 ml of deionized water was added and mixed for 10 min. The precipitate was obtained by adding 100 ml of ethanol then filtered by using a glass filter G3. 100ml 60% ethanol was added then mixed for 30 min and filtered, this process was repeated twice. 100ml 100% ethanol was added then

Samples	RS	Ac20	Ac21	Ac26	Ac27	Ac32
Degrees of acetylation (%)	1.6	2.2	2.6	2.9	4.0	5.3
Degrees of substitution	0.06	0.09	0.10	0.11	0.16	0.21

Table I Molecular characteristics of KGM

filtered. After drying by draft, the material was evacuated at 60°C for 4 hours.

#### Preparation of KGM dispersion

Powders of KGM fractions (RS, Ac20, Ac21, Ac26, Ac27 and Ac32) were dispersed in distilled water at room temperature for 1 hour and were heated to 80°C and then maintained at 80°C for 1 hour and cooled to room temperature. The KGM concentrations used were 1-3 w/w%.

#### Dynamic viscoelasticity

Dynamic viscoelastic measurements were carried out using RFS II (Rheometrics Co. Ltd., NJ, USA) with a parallel plate geometry ( $D = 25$  mm, 1.5 mm gap). The strain is set as 0.5%. The KGM dispersion of 1 ml was poured directly onto the plate of the instrument which had been kept at each measurement temperature from 45°C to 80°C. Twenty microliters of 1mol/L  $\text{Na}_2\text{CO}_3$  solution as an alkaline coagulant was added at time  $T = 0$  to 1 ml 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. Frequency sweep was performed in the angular frequency range from 0.2 to 25 rad/s, which can be completed within 72 seconds.

### 3. RESULTS AND DISCUSSION

#### 3.1 Determination of sol-gel transition points

Fig. 1 shows the time evolution of the storage shear modulus  $G'$ , the loss shear modulus  $G''$  for 2% Ac21

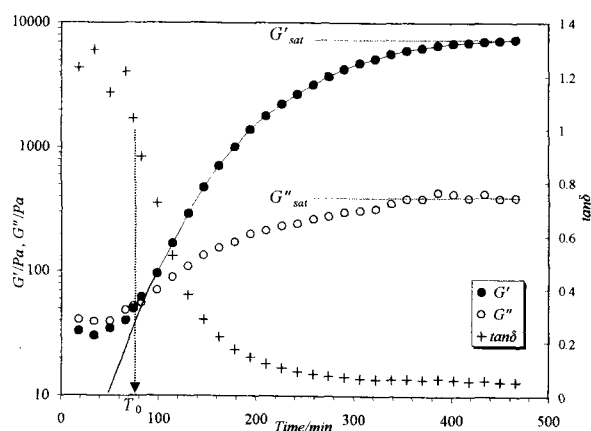


Fig. 1 Time dependence of  $G'$ ,  $G''$ ,  $\tan\delta$  of 2% Ac21 aqueous dispersion at 45°C. Symbols represent the experimental values and the solid line represents the calculated curve.

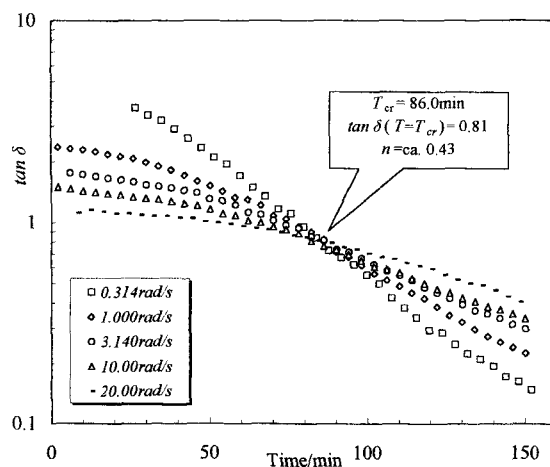


Fig. 2. Loss tangent  $\tan\delta$  against time at 45°C at different frequencies for the gelation process of 2% Ac21 aqueous dispersions

aqueous dispersions with a constant frequency of 1 rad/s at 45°C.  $G'$  and  $G''$  increased with time and attained the plateau values,  $G'_{sat}$  and  $G''_{sat}$ , respectively. The mechanical loss tangent  $\tan\delta = G''/G'$  decreased with time. The gelation time  $T_0$  is defined as the time at which  $G'$  and  $G''$  intersected each other, i.e.  $\tan\delta=1$ . The gelation processes was treated by an equation of first order kinetics or other modified equations[6]. The evolution of  $G'$  against time could be well approximated by the following equation:  $G'(T) = G'_{sat}(1 - e^{-k(T-T_0)})$ , where  $k$  is the rate constant of gelation of KGM. For 2% Ac21 aqueous dispersions at 45°C,  $G'_{sat} = 9200$  Pa,  $k = 0.00045$   $\text{min}^{-1}$ ,  $T_0 = 78$ min were determined from the best fitting of the experimental and calculated values of  $G'$ .

Tung and Dynes[7] suggested that the intersection of  $G'$  and  $G''$  (corresponding to  $T_0$  in this work) marks the instant of gelation point. However, the time of intersection was found to be a function of frequency.  $T_0$  fluctuated around a constant value of  $T_{cr}$  with increasing frequency (Data not shown), where  $T_{cr}$  is critical gelation time determined by using Winter & Chambon's method[8] described as follows.

Winter & Chambon[8] have proposed a method to determine the gelation point from mechanical spectra. According to their proposition, the gelation point corresponding to the specific instant  $T_{cr}$  is defined as a point at which

$$G'(\omega) \sim G''(\omega) \sim \omega^n, 0 < n < 1 \quad (1)$$

and

$$\tan\delta = G''/G' = \tan(n\pi/2) \quad (2)$$

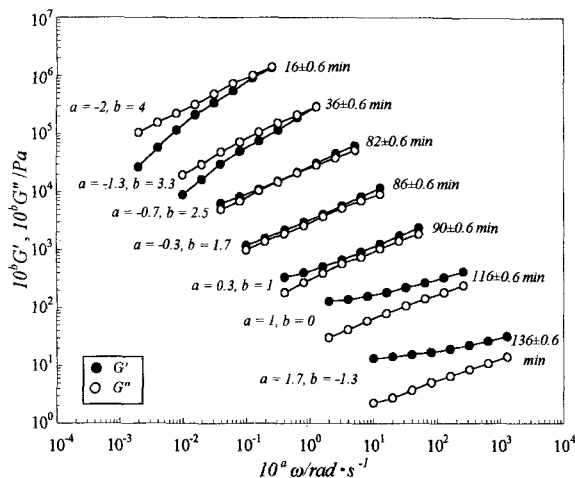


Fig. 3 Frequency dependence of  $G'$  and  $G''$  of 2% Ac21 aqueous dispersions at various times at 45°C. The data are shifted along both of horizontal and vertical axes by shift factor  $10^a$ ,  $10^b$  to avoid overlapping.

hold simultaneously, where  $n$  is the relaxation exponent. The condition  $\tan \delta = \text{const.}$  means that  $T_{cr}$  is independent of frequency.

Fig. 2 shows the gelation process of Ac21 aqueous dispersions.  $\tan \delta$  was plotted against time at 45°C at different frequencies. The critical gelation time  $T_{cr}$  was determined as 86.0 min, and  $n$  was calculated to be 0.43.

Fig. 3 shows the frequency dependence of  $G'$  and  $G''$  of 2% Ac21 aqueous dispersions at various times at 45°C. The frequency range is 0.2~25.6 rad/s<sup>-1</sup>, and the data are shifted along both of horizontal and vertical axes by shift factor  $10^a$  and  $10^b$  to avoid overlapping. Both of  $G'$  and  $G''$  became proportional to  $\omega^n$  at  $T = 86.0$  min, where  $n \approx 0.46$  for both of  $G'$  and  $G''$ . The slight difference between each exponent  $n$  calculated from eq.(1) and (2) was believed due to the experimental error. For KGM, the behaviour in the sol state is typical of a macromolecular solution, and, in the gel state, the structured network exhibits an elastic behaviour:  $G' > G''$  and  $G'$  is almost frequency independent. Anyway, to obtain such experi-

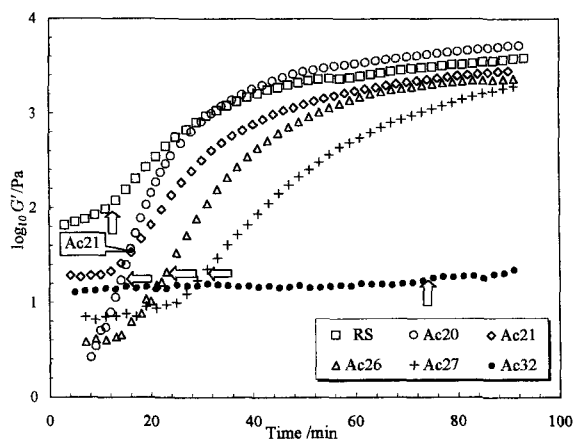


Fig. 4 The time dependence of  $G'$  of 2% KGM aqueous dispersions at 60°C. The arrows represent the intersecting points of  $G'$  and  $G''$  (corresponding to  $T_0$ ,  $G''$  not shown).

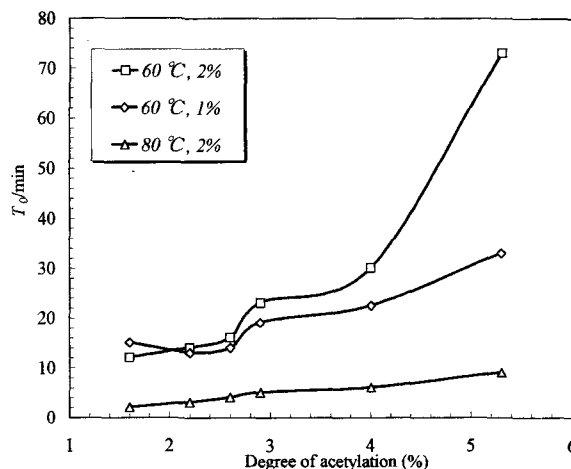


Fig. 5 The dependence of gelation time  $T_0$  of KGM aqueous dispersions on the degree of acetylation at various temperatures and concentrations.

mental results in a wide frequency range, it is necessary that the gelation system itself does not evolve significantly during the whole frequency sweep[8].

### 3.2 Effects of acetylation degrees of KGM

Fig.4 shows the time dependence of  $G'$  of 2% KGM aqueous dispersions at a constant frequency 1 rad/s at 60°C.  $G'$  increased with time and attained the plateau value finally. There was a slight frequency dependence of  $T_0$  that are approximately equal to  $T_{cr}$ . In the following parts of this paper,  $T_0$  at 1 rad/s was used to substitute the  $T_{cr}$  to represent the onset time of gelation for convenience.  $T_0$  became longer with increasing acetylation degree of KGM, especially that of Ac32 which has the highest acetylation degree was far longer than the others. However, the plateau values of  $G'$  after gelation, i.e.  $G'_{sat}$ , did not change so much with the order of the acetylation degrees at all. It can be interpreted that  $G'$  has not attained the real plateau values due to the short observation time; or a gel was broken partially after its formation, or slippage occurred and then led to smaller values in long term. It is proposed that the acetylation degrees for an unknown KGM, can be identified by the observation of the gelation time  $T_0$ .

Fig. 5 shows the dependence of  $T_0$  on the degree of acetylation at different temperatures (60°C and 80°C) and concentrations (1% and 2%). Gelation time was delayed by increasing acetylation degree of KGM. This tendency is more remarkable at lower temperature or higher concentration of KGM.

Maekaji[10] suggested that deacetylation occurs on alkaline treatment, and that deacetylation is a necessary reaction for gel formation of KGM. The gelation occurred after a certain induction period, and the concentration of hydroxide ion  $[\text{OH}^-]$  (mol/L), instead of the kind of gelling agent, 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. It also increased with the

'peptising power' (dissolving ability of KGM gels once formed) of coexisting ions[10]. As shown in Fig. 4,  $G'$  remained a constant value, i.e. was independent of time, for a short period right after alkaline treatment, then increased with time. It is supposed that the induction reaction corresponding to deacetylation occurred in this period. The longer the induction period, the larger gelation time  $T_0$ . The deacetylation ratio of 2% KGM aqueous dispersion was lower than that of 1% dispersion, and then led to a longer  $T_0$  (Fig. 5). The added volume of alkaline coagulant  $\text{Na}_2\text{CO}_3$  solution was same as  $20 \mu\text{l}$  for every 1ml KGM dispersions with different degrees of acetylation. Because the KGM with high degrees of acetylation need more alkaline coagulant in induction reaction, the insufficiency of hydroxide ion led to a far longer  $T_0$  of Ac32 than other KGM samples (Fig. 4 & 5).

#### 4. CONCLUSION

The gelation process of acetylated konjac glucomannan studied exhibits a typical viscoelastic behaviour at a critical state between sol and gel, characterized by a power law dependence of loss and storage moduli on frequency, with the same exponent. Besides the gelation process, the mechanical properties of KGM are also important for konjac products, these properties will be studied in the

near future.

#### 5. REFERENCES

- [1] M. Maeda, H. Shimahara, N. Sugiyama, *Agr. Biol. Chem.*, **44** (2), 245-252 (1980).
- [2] J. D. Bewley, J. S. G. Reid, "Biochemistry of Storage Carbohydrates in Green Plants", Ed. By P. M. Dey, R. A. Dixon, Academic Press, New York (1985) pp 289-304.
- [3] I. C. M. Dea, A. Morrison, *Adv. Carbohydr. Chem. Biochem.* **31**, 241-312 (1975).
- [4] K. Maekaji, *Agr. Biol. Chem.* **42** (1), 177-178 (1978).
- [5] M. A. K. Williams, T. J. Foster, D. R. Martin, I. T. Norton, M. Yoshimura, K. Nishinari, *Biomacromol.*, **1**(3), 440-450 (2000)
- [6] K. Nishinari, *Colloid Polym. Sci.*, **275**, 1093-1107 (1997).
- [7] C. Y. M. Tung, P. J. Dynes, *J. Appl. Polym. Sci.*, **27**, 569-578 (1982)
- [8] H. H. Winter, F. Chambon, *J. Rheol.*, **30**, 367-382 (1986).
- [9] G. Cuvelier, B. Launay, *Makromol. Chem. Macromol. Symp.*, **40**, 23-31 (1990).
- [10] K. Maekaji, *Nippon Nogeikagakuishi*, **52**, 251, 485, 513 (1978).

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