

Dynamic viscoelastic behavior of triple helical Lentinan in water: Effect of temperature

Yangyang Zhang, Xiaojuan Xu ^{*}, Lina Zhang

Department of Chemistry, Wuhan University, Wuhan 430072, China

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Abstract

This study deals with the dynamic viscoelastic property for Lentinan fractions with different molecular weight in water under different constant temperatures and polysaccharide concentrations. The results revealed that a transition exists between a sol-like and a weak gel behavior for the Lentinan/water system. The gel point, T_{gel} , was determined from the point of intersection in $\tan \delta$ vs. temperature (T) for different constant frequencies, indicating the validity of Winter–Chambon criteria. The T_{gel} decreased with decreasing polymer concentration and molecular weight. Moreover, the value of exponent n at the gel point decreased with increasing polymer concentration but exhibited an independence of molecular weight, indicating that the gels of Lentinan had similar fractal structure. The dynamic strain sweep measurements proved that the gelation of Lentinan in water is induced by the extremely entangled and stiff triple helices forming continuous network, and the Lentinan gel is structurally more like a solution that is unable to flow within a timescale of usual observation. The heating–cooling process proved that the sol–gel transition of Lentinan in water was thermally reversible.

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Keywords: Triple helical β -(1 \rightarrow 3)-D-glucan; Lentinan; Cold-set gelation; Gelation temperature

1. Introduction

More than 30 years ago, Chihara et al. (Chihara, Hamuro, Maeda, Arai, & Fukuoka, 1970; Chihara, Maeda, Hamuro, Sasaki, & Fukuoka, 1969) isolated and purified Lentinan, a polysaccharide possessing significant antitumor activity, from hot water extracts of the fruiting bodies of *Lentinus edodes*, one kind of mushroom. The structure of Lentinan was reported as β -1,3-linked-D-glucan with β -1,6 branches (Saito, Ohki, & Sasaki, 1979; Saito, Ohki, Takasuka, & Sasaki, 1977; Sasaki & Takasuka, 1976). Since then, polysaccharides have attracted much attention of many investigators in the world because they are not only a source of dietary fiber but also linked with certain biomedical effects. Several investigators have reported that Lentinan showed strong host-mediate antitumor activities

against various tumors, via so-called activations of T-cells, NK cells, and macrophages (Freunhauf, Bonnard, & Heberman, 1982; Gergely, Vallent, Bodo, Feher, & Kaneko, 1988; Hamuro, Rölinghoff, & Wagner, 1980; Ladányi, Tímár, & Lapis, 1993; Maeda & Chihara, 1973). *L. edodes* is now cultivated and is the second most commonly produced edible mushroom in the world (Chang, 1993). Furthermore, Lentinan has been used clinically in Japan as a host immunopotentiator for cancer therapy (Chihara et al., 1969). Therefore, Lentinan has potential wide application in food and medical fields.

Recently, we have also isolated and purified Lentinan with relatively high yield from sodium hydroxide/water extracts of fruiting bodies of *L. edodes*, and first proved that it exists as triple helical chains in aqueous solution, while a single chain in dimethyl sulfoxide (DMSO) by using viscometry, light scattering combined with size exclusion chromatography (LS-SEC), NMR and so forth (Zhang, Li, Zhou, Zhang, & Chen, 2002; Zhang et al., 2001). Moreover, we find from the experiments that Lentinan is gelled

^{*} Corresponding author. Tel.: +86 27 87219274; fax: +86 27 68754067.
E-mail address: xuxj626@263.net (X. Xu).

easily at room temperature with low concentration in water. Interestingly, it is different completely from the non-gelling double helical Xanthan gum and triple helical Schizophyllan. The later two form gels only under condition of addition of certain ions or other non-gelling polysaccharides (Annable, Williams, & Nishinari, 1994; Fang & Nishinari, 2004). This suggests there are stronger intermolecular interactions among Lentinan chains than in Xanthan and Schizophyllan. In our previous work, it was found that the sol–gel transition of Lentinan with different molecular weight in water at 25 °C occurred at low concentration, and the critical gelation concentration c_{gel} increased with a slight decrease of molecular weight. Furthermore, the gelation mechanism was revealed as the extremely entangled Lentinan chains make a continuous network, and the structure of Lentinan gel has been proven not to contain junction zones (Zhang, Xu, Xu, & Zhang, 2007).

As mentioned above, Lentinan is one of the important members among the bioactive branched (1 → 3)-β-D-glucan family. Although the gelation of some structure-like polysaccharides such as Schizophyllan (Fang & Nishinari, 2004) and Scleroglucan (Aasprong, Smidsrød, & Stokke, 2003) have been extensively studied, few work dealt with Lentinan gels and most of the work on Lentinan is related to its structure, conformation and bioactivity. It is well known that the sol–gel transition is an important physical phenomenon and a basically scientific topic in the study of physical hydrogels. In this paper, the aim is to study the cold-set gelation behavior of Lentinan with different molecular weight by using rheometer in order to present a thorough investigation of the dynamic viscoelastic properties of Lentinan aqueous systems. This work will contribute to the application of natural gelling polysaccharides in food and pharmaceutical industries.

2. Experimental

2.1. Sample preparation

Lentinan coded as LF-1 was isolated from fruiting bodies of *L. edodes* cultivated in Fujian of China by extraction with 5% NaOH/0.05% NaBH₄ two times, and precipitation with 36% acetic acid to remove α-(1 → 3)-D-glucan, according to previously reported method (Zhang et al., 2001). The supernatant was subjected to the Sevag method (Whistler, 1965) to remove proteins, and treated with 30% H₂O₂ to decolorize. Aqueous solution of the Lentinan was dialyzed against distilled water for 4 days, and concentrated by rotary evaporator at reduced pressure below 45 °C, and finally lyophilized to obtain colorless flakes (LF-1). In order to investigate the effect of molecular weight on the sol–gel transition, LF-1 was dissolved in water with concentration of 1.5 wt%, and degraded into three fractions by ultrasonic with different time, respectively. The sample solutions were dialyzed, and lyophilized to get the final fractions coded as LF-2, LF-3, LF-4, respec-

Table 1

Molecular characteristics of Lentinan fractions in water at 25 °C (Zhang et al., 2007)

Samples	$[\eta]$ (mL/g)	$M_w \times 10^{-4}$	c_{gel} (% w/w)
LF-1	906.9	160.1	0.38
LF-2	820.8	141.2	0.69
LF-3	744.3	137.0	0.70

tively. The three samples LF-1–LF-3 were used in the following experiments. The molecular characteristics of the fractions are summarized in Table 1 (Zhang et al., 2007).

2.2. Rheological measurements

The dynamic viscoelastic behaviors of Lentinan fractions were carried out on ARES-RFS III rheometer (TA Instruments, USA). A double-concentric cylinder geometry ($R_1/R_2 = 32 \text{ mm}/34 \text{ mm}$) was used to measure dynamic parameters such as the shear storage modulus (G') and loss modulus (G'') as functions of angular frequency (ω) at different temperature. The rheometer was equipped with two force transducers allowing the torque in the range from 0.004 to 1000 g cm. Dynamic strain sweep measurements were carried out at 1 rad/s to determine the linear viscoelastic regime of Lentinan fraction in water with a strain range from 0.3% to 900% for Lentinan fractions in water. Dynamic frequency sweep measurements of G' and G'' were performed in a controlled-strain mode. For each dynamic frequency sweep measurement, a fresh Lentinan fraction solution was prepared and poured into the couette geometry instrument. Temperature control was established by a julabo FS18 cooling/heating bath kept within ± 0.4 °C over an extended time. The dynamic temperature sweep measurements were conducted from 10 to 60 °C at an angular frequency of 1 rad/s and with heating or cooling rates of 1 °C/min. The solution was covered with a thin layer of low-viscosity silicone oil in order to prevent dehydration during rheological measurements.

3. Results and discussion

3.1. Dependence of viscoelastic behavior on temperature

Fig. 1 shows the storage shear modulus G' and loss modulus G'' as a function of angular frequency for LF-1 solution with a concentration of 0.3%w/w at various temperatures. Clearly, G' and G'' showed frequency dependence at all temperatures examined. At higher temperatures G' and G'' curves intersected at the middle of the frequency range, indicating an entanglement network system (Clark & Ross-Murphy, 1987; Ross-Murphy, 1984), and the solutions exhibited the liquid-like behavior that G' scaled approximately with ω by $G' \sim \omega$ and $G'' \sim \omega^{0.78}$ in the range of low frequency, but the typical terminal behavior $G' \sim \omega^2$ and $G'' \sim \omega$ for a Newtonian fluid (Kobayashi, Huang, & Lodge, 1999; Li & Aoki,

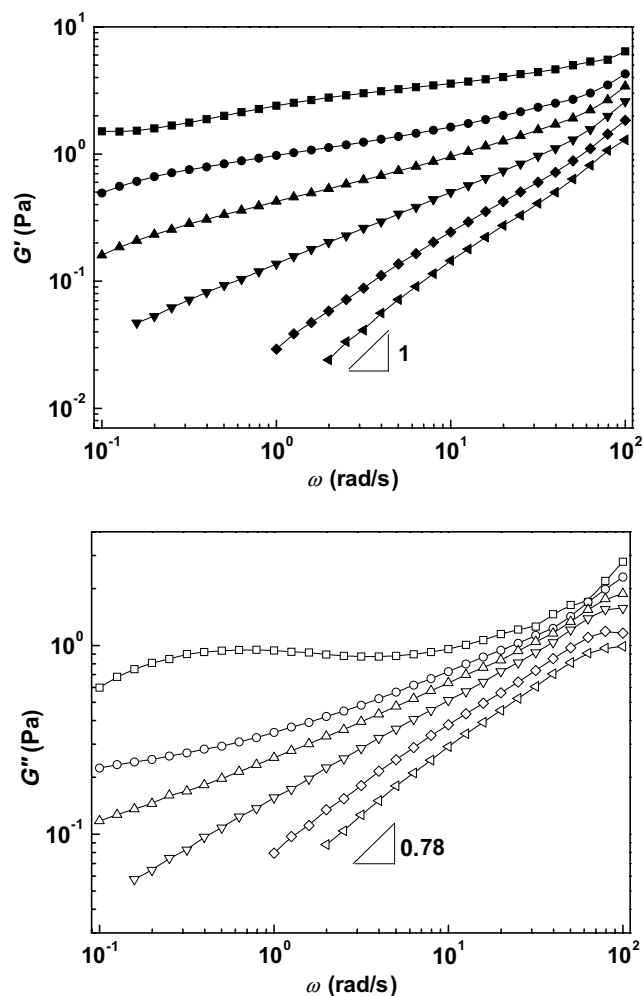


Fig. 1. Shear storage modulus G' (top) and loss modulus G'' (bottom) as a function of angular frequency ω for LF-1 solutions at concentrations of 0.3%w/w at various temperatures. The temperature is 5.4, 10.2, 15.1, 20.0, 24.9 and 44.6 °C from top to bottom.

1997) is not observed. The deviations from the expected slopes may be attributed at least in part, to the rod-like chain conformation of Lentinan and to the relatively high concentration, which resulted in the non-Newtonian behavior even at low frequency. With decreasing temperature, the value of G' increased significantly, and the frequency dependence of G' and G'' became weaker and weaker. Furthermore, G' became larger than G'' with moduli almost parallel to each other indicating formation of weak gels (Clark & Ross-Murphy, 1987). Especially at 10.2 °C, a plateau (slope = 0) of the G' curves became apparent at low frequency, and G'' displayed a minimum at intermediate frequencies suggesting that Lentinan formed relatively strong gels possessing long-lived cross-links or supermolecular network structure (Clark & Ross-Murphy, 1987; Morris, 1982; Richardson & Norton, 1998). From table 1, it is known that the critical gelation concentration of LF-1 in water at 25 °C is higher than the concentration of 0.3%w/w, that is, LF-1 solution with concentration of 0.3%w/w will not form gels at 25 °C.

However, when decreasing temperature, Lentinan solution was changed from entanglement network (G' and G'' curves crossed over at certain frequency) to weak gels (G' curve paralleled to G'' curve with $G' > G''$ in the entire experimental frequency range), that is, decreasing temperature induced the formation of gels.

Fig. 2 shows G' and G'' as a function of angular frequency ω for LF-1, LF-2 and LF-3 solutions at concentrations of 0.6%w/w at various temperatures. G' and G'' increased with decreasing temperature. At relative high temperature, G' and G'' curves crossed-over at intermediate frequency, indicative of concentrated solution or viscoelastic fluids or entangled system (Clark & Ross-Murphy, 1987). This frequency dependence of G' and G'' can be explained as follows. At relatively low frequencies, where substantial disentanglement of polymer chains can occur easily during the long period of oscillation, the predominant response to the imposed deformation is characterized by the viscous storage G'' ; at relatively high frequencies, as the rate of oscillation exceeds the time scale of molecular rearrangements, entanglement coupling becomes less distinguishable from “permanent” association of chains in the gel network, and plays a role of knots and forms a temporary three-dimensional network, resulting in the overall response approaching that of a gel where G' predominates (Clark & Ross-Murphy, 1987; Cui, 2001; Morris, 1982; Nijenhuis, 1990). Moreover, the crossover frequency decreased with decreasing temperature, showing the ability of temporary network was enhanced by an increase in temperature. Then G' became larger than G'' , and finally G' curve paralleled to G'' curve with $G' > G''$ in the entire experimental frequency range with decreasing temperature. But both moduli still show frequency dependence, indicative of an obvious weak gel-like character (Clark & Ross-Murphy, 1987). Based on the above experimental results, it can be concluded that the solution–gel transition occurred with decreasing temperature.

Fig. 3 depicts directly the evolution of the exponents n' ($G' \propto \omega^{n'}$) and n'' ($G'' \propto \omega^{n''}$) determined from Fig. 2c. for Lentinan fraction LF-3 at concentration of 0.6%w/w with temperature. The values of n' and n'' decreased with decreasing temperature, i.e. the frequency dependence of G' and G'' became weaker and weaker. Moreover, n' and n'' inclined to close to each other with decreasing temperature and overlapped at ~ 20 °C, suggesting the curves of G' and G'' are parallel and formation of gel.

$\tan \delta$ is a measure of the relative contribution of viscous components to the mechanical properties of the material. For a conventional elastic gel, $\tan \delta < 0.1$, while for a typical weak gel, the moduli exhibit relatively large frequency dependence accompanied by a $\tan \delta$ value > 0.1 (Chronakis, Piculell, & Borgström, 1996; Ikeda & Nishinari, 2001; Ross-murphy, Morris, & Morris, 1983). The G' in Fig. 2 and the corresponding G'' values have been used to estimate the values of $\tan \delta$ for Lentinan fraction LF-1, LF-2 and LF-3 at various temperatures. Fig. 4 shows frequency dependence of $\tan \delta$ at various temperatures for the three

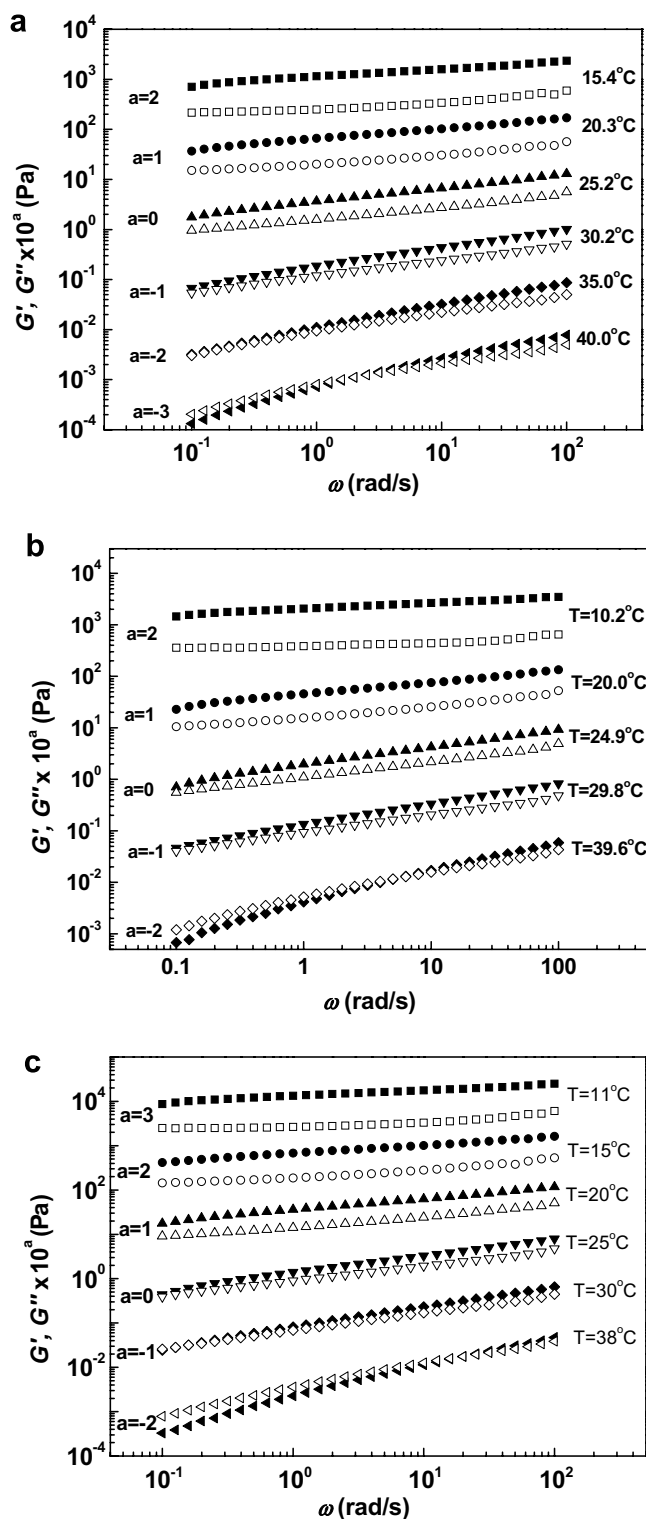


Fig. 2. Shear storage modulus G' (solid symbols) and loss modulus G'' (open symbols) as a function of angular frequency ω for LF-1(a), LF-2(b) and LF-3(c) solutions at concentrations of 0.6%w/w at various temperature. The data were shifted along vertical axes by 10^3 to avoid overlapping.

samples in water. With decreasing temperature, the $\tan \delta$ shows weaker and weaker frequency dependence and even frequency independence almost in the whole frequency

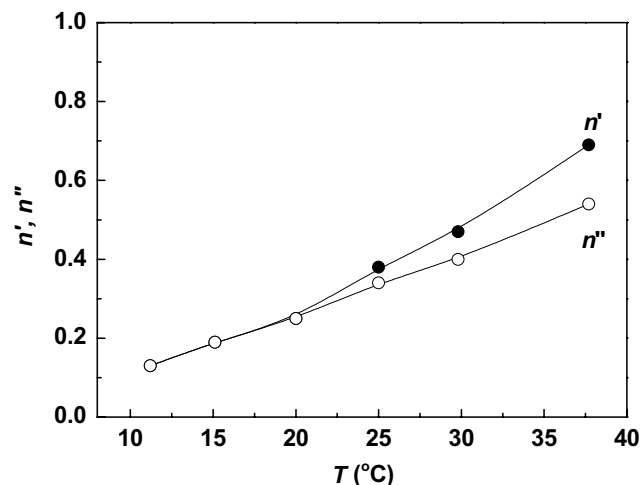


Fig. 3. Exponents n' and n'' of $G' \sim \omega^{n'}$ and $G'' \sim \omega^{n''}$ as a function of temperature for LF-3 in water at concentration of 0.6%w/w.

range examined, revealing formation of gels (Bromberg, 1998; Chambon & Winter, 1987). The $\tan \delta$ values are smaller than 1.0 but slightly higher than 0.1 over the entire frequency range below gelation temperature, suggesting that Lentinan fractions formed weak gels where the elasticity dominates these systems (Tuinier, Oomen, Zoon, Cohen Stuart, & de Kruif, 2000).

Fig. 5 shows the state of Lentinan fraction LF-1/water system with concentration of 0.3%w/w at different temperatures. At 25 °C, the concentration of 0.3%w/w is lower than the critical gelation concentration (Zhang et al., 2007). Therefore, the LF-1/water system exhibits solution state (picture a). With decreasing temperature, the flow ability of the system became weaker and weaker, and gel formed. For example, the gel is so weak that it cannot support the weight of itself at 16 °C as shown in picture b. With decreasing temperature, the strength of the gel increased and could support itself as shown in the picture c and d. These pictures gave us a direct expression that Lentinan/water solution could form gels at low temperature, and the gel strength increased with decreasing temperature.

3.2. Determination of the critical gelation temperature

All the while, the determination of gel point of polymer system is a difficult task, and many researchers are trying to explore some methods to determine the gel point. Dynamic rheology is one of the most extensive methods to study rheological properties of polysaccharide gel, and is also the most direct and reliable way for determination of the sol-gel transition and characterization of rheological properties of gels. The rheological characteristics in the vicinity of the sol-gel transition can be described using the power laws or scaling laws (de Gennes, 1979; Flory, 1953; Li & Aoki, 1997; Stockmayer, 1943; Stockmayer, 1944). At the gel point, the dynamic moduli G' and G'' obey the following

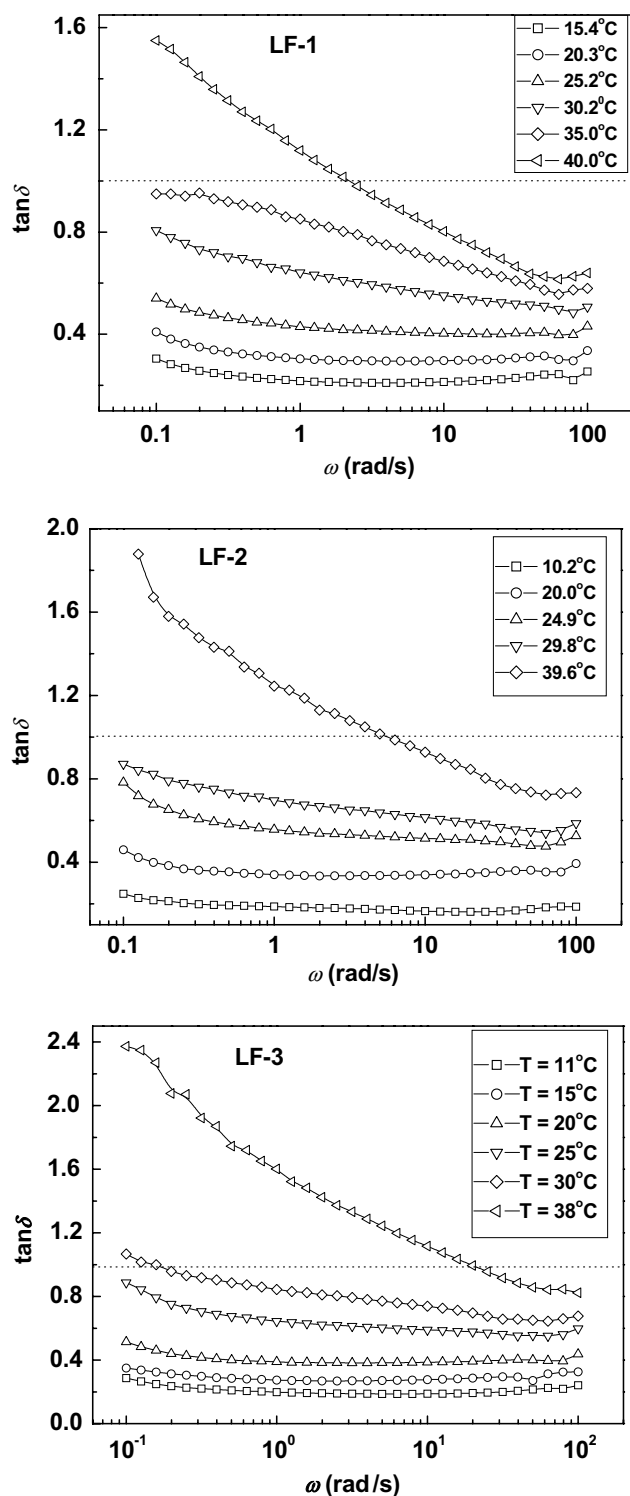


Fig. 4. Loss tangent $\tan \delta$ as a function of frequency for LF-1, LF-2, LF-3 in water with concentration of 0.6%w/w at different temperature.

power law (Chambon & Winter, 1985; Li & Aoki, 1997; Scanlan & Winter, 1991; Winter & Chambon, 1986)

$$G'(\omega) \cong G''(\omega) \propto \omega^n \quad (0 < n < 1) \quad (1)$$

where ω is the angular frequency; n is the critical exponents determining the critical characteristics in the vicinity of the

sol–gel transition, which have been predicted theoretically by many models (Li & Aoki, 1998). Furthermore, Eq. (1) enables establishment of the following relation (Chambon & Winter, 1987)

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

This frequency independence of loss tangent in the vicinity of the gel point has been widely used to determine the gel point for chemical and physical gels, and proven to be reliable and valid.

In our previous work, we have obtained the critical gelation concentration of Lentinan having different molecular weight at 25 °C by using Winter–Chambon method, and proved that it is in validity for this system. In the present work, we also used this method for determining the sol–gel transition temperature of Lentinan gels from a multifrequency plot of $\tan \delta$ versus temperature for Lentinan in water at fixed concentration. Figs. 6 and 7 illustrated the loss tangent $\tan \delta$ as a function of temperature T for LF-1, LF-2 and LF-3 in water at fixed concentration and various angular frequencies. One can observe that all curves in each figure passed through the common point at the certain temperature, which is defined as the gel point or critical gelation temperature T_{gel} . It is apparent that the gel point for each gelling system is accurately determined by using this method and summarized in Table 2. Obviously, T_{gel} increased with an increase of polymer concentration and molecular weight. It can be explained as the higher is the concentration, the collision probability of polymer chains is higher even at high temperature, and as at fixed concentration the higher is molecular weight, the chain is longer and the collision probability of polymer chains is higher resulting in more entanglements and gelation at higher temperature.

From Table 2, it is apparent that the values of $\tan \delta$ for Lentinan fractions in water system at concentration of 0.6%w/w are almost the same, implying a unique feature of the gelling system at the gel point (Li & Aoki, 1997). The value of $\tan \delta$ at the gel point also provides the information about the scaling exponent n of Eq. (1), and then directly obtained n as shown in Table 2 for Lentinan fractions in water at concentrations of 0.6% and 0.3%w/w by using the Eq. (2). It is apparent that the value of n increased with decreasing concentration. In general, a lower value of n implies formation of a more highly elastic gel (Li & Aoki, 1997). Therefore, the low value of n at 0.6%w/w further proved our previous result that the gel strength increased with increasing concentration. The truth that the n values were independent on molecular weight suggested that the gels of Lentinan fractions exhibit similar fractal structure (Aoki, Li, & Kakiuchi, 1998). It has been reported that the values of n for the chemical gels can be different completely such as 0.5–1 (Chambon & Winter, 1985; Chambon & Winter, 1987; Winter & Chambon, 1986), 0.13–0.92 (Scanlan & Winter, 1991), 0.31–0.91 (Izuka, Winter, & Hashimoto, 1992), 0.45–0.5 (Mours & Winter, 1996) and 0.67 (Koike, Nemoto, Watanabe, & Osaki, 1996), depend-

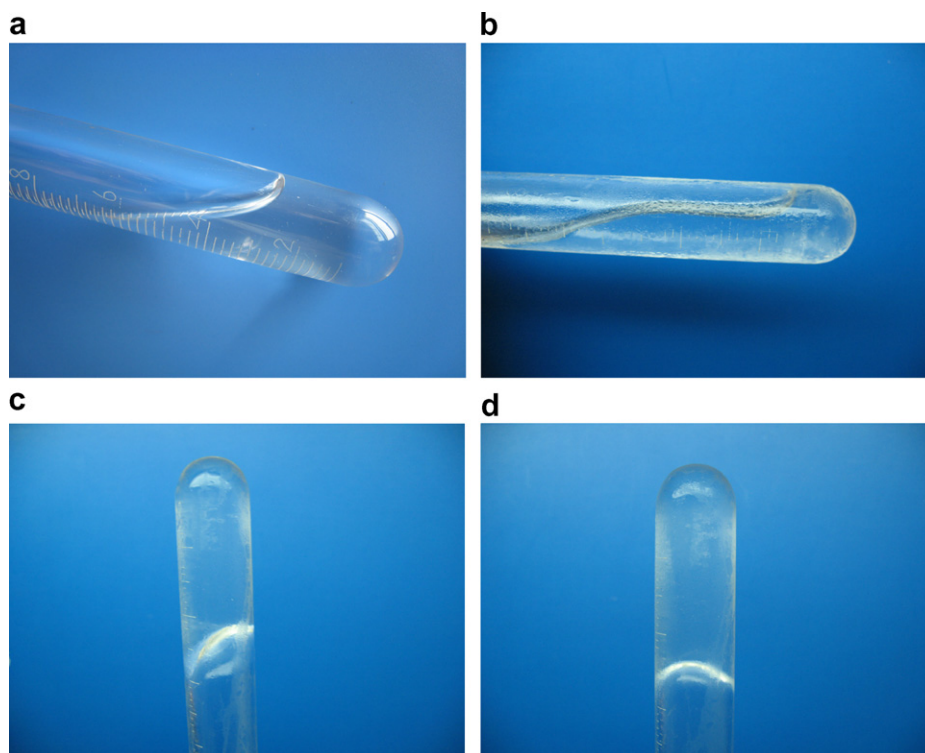


Fig. 5. Photographs of LF-1 with concentration of 0.3%w/w at different temperatures. (a) 25 °C, (b) 16 °C, (c) 13 °C and (d) 9 °C.

ing on the special nature of each gelling system. Whereas for the case of physical gels, the n values were reported to be relatively higher than 0.6 (Li & Aoki, 1997; Te Nijenhuis & Winter, 1989; Schwittay, Mours, & Winter, 1995). In the case of Lentinan gels, the n values are much lower than those of reported physical gels, whereas close to those of some chemical gels. It was also reported that the increasing cross-linking density could decrease the n value (Koike et al., 1996; Li & Aoki, 1998). Therefore, the lower n value at high concentration may be attributed to the relatively high physical cross-linking density of gel network.

3.3. Gelation mechanism of Lentinan aqueous solution

In order to examine the nature of the structure in Lentinan gels and clarify the gelation mechanism, dynamic strain sweep measurements for Lentinan fraction LF-1 in water with concentration of 6.0×10^3 g/mL at 15 °C were repeated for four runs. Moreover, the second, third and fourth runs of dynamic strain sweep measurements were done immediately after the end of the former run during which structures in samples had been broken due to large deformation. Fig. 8 shows the results of the above experiments. At the temperature of 15 °C, the gelation has already happened and the system is in the gel state. From Fig. 8, it can be seen that both G' and G'' keep constant until strain $\approx 20\%$, beyond which G' and G'' decrease sharply resulting from the structure breaking due to large deformation. Therefore, the strain of 20% can be taken as the upper limit of the linear viscoelastic regime of the

present Lentinan gels. As for the results of repeated runs, both G' and G'' are almost the same as those determined in the first run. The good reproducibility of G' and G'' during the repeated experiments reveals that the network structure in Lentinan gels is not permanent and can recover immediately even if the existing structure was disrupted almost completely in the former runs. Additionally, it implies that no junction zones exit in the Lentinan gels and the gel property just comes from the extremely entangled network with a very long relaxation time; otherwise G' and G'' are irreproducible after large deformation, similar to the gelation behavior of Schizophyllan-sorbitol aqueous solution (Fang & Nishinari, 2004).

To prove if the gelation of Lentinan in water was thermoreversible, the gel was first developed via keeping at a low temperature of 10 °C, and then a heating process began at a heating rate of 1 °C/min to make the gel be solution, finally cooled slowly to a low temperature at a cooling rate of 1 °C/min. Fig. 9 shows the G' curves for 0.6%w/w Lentinan fractions LF-1 and LF-3 in water under the heating-cooling process with an angular frequency of 1 rad/s. It can be seen that the G' of gel melting curve almost falls on that of gel forming on cooling, implying that no remarkable thermal hysteresis is present between heating and cooling processes. It is proper to say that the gels formed from Lentinan solution are thermally reversible, which is a significant phenomenon with industrial importance (Nishinari & Takahashi, 2003). In general speaking, the thermal hysteresis in a thermalreversible physical gel is often caused by the aggregation of rigid polymer chains (Fang & Nishinari,

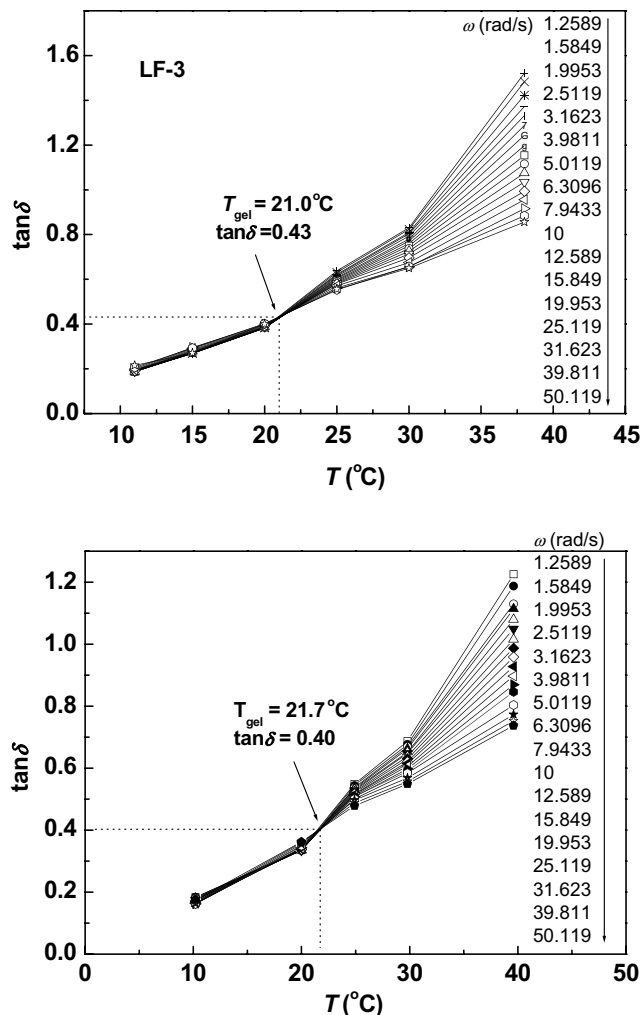
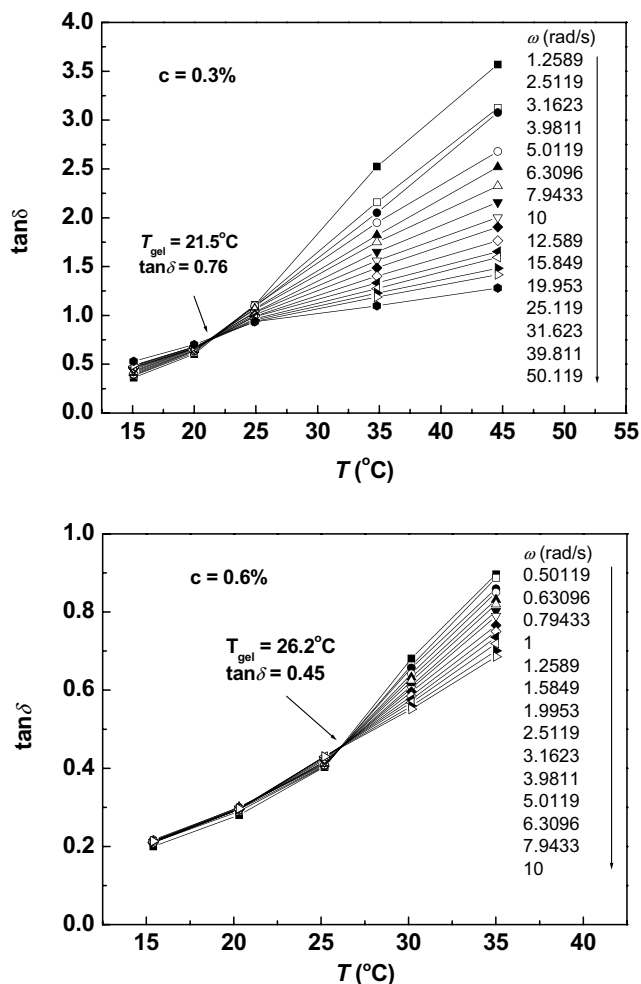


Fig. 6. Loss tangent $\tan \delta$ as a function of temperature T for LF-1 in water with concentration of 0.3%w/w for different constant angular frequencies. T_{gel} is the gel point determined from the intersection point.

Fig. 7. Loss tangent $\tan \delta$ as a function of temperature T for LF-2 (bottom) and LF-3 (top) in water with concentration of 0.6%w/w for different constant angular frequencies. T_{gel} is the gel point determined from the intersection point.

2004). Therefore, a conclusion can be drawn properly that no pronounced aggregation of triple helices exists in Lentinan aqueous gel, and the gel property comes from a three-dimensional network constituted by the extremely entangled triple helical Lentinan, similar to the gelation of Schizophyllan-sorbitol aqueous solution (Fang & Nishinari, 2004).

4. Conclusions

The cold-set formation of an elastic fractal gel for triple helical Lentinan in water with different molecular weight and concentration was investigated rheologically from measurements of G' , G'' and $\tan \delta$ over a wide range of frequency under isothermal conditions at different constant temperatures. The results showed that Lentinan fractions formed weak gels with decreasing temperature. The critical gelation temperature T_{gel} was accurately determined by the Winter–Chambon method from the temperature dependence of $\tan \delta$, indicating the validity of the Winter–Chambon criterion. It was found that T_{gel} decreased with

Table 2

The characteristics of Lentinan fractions in water in the vicinity sol–gel transition at fixed concentration

Samples	$c = 0.3\%w/w$			$c = 0.6\%w/w$		
	T_{gel} (°C)	$\tan \delta$	n	T_{gel} (°C)	$\tan \delta$	n
LF-1	21.5	0.76	0.41	26.2	0.45	0.27
LF-2	–	–	–	21.7	0.40	0.24
LF-3	–	–	–	21.0	0.43	0.26

decreasing molecular weight or polymer concentration, and that the exponent (n) values at the gel point decreased with increasing polymer concentration but almost showed an independence of molecular weight. The heating–cooling process proved that the sol–gel transition for Lentinan in water was thermally reversible. The gelation mechanism for Lentinan fractions in water is proposed as follows: the extremely entangled Lentinan chains make a continuous network, conferring to the system the gel-like properties. The structure of Lentinan gel has been proven not

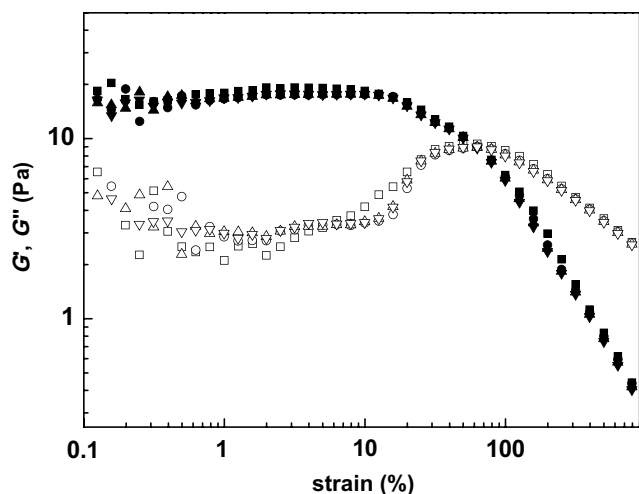


Fig. 8. Strain dependence of G' (solid symbols) and G'' (open symbols) determined by dynamic strain sweep measurement at 1 rad/s for Lentinan fraction LF-2 in water at 15 °C. The polysaccharide concentration is 0.6%w/w. (■, □) the first run, (●, ○) the second run, (▲, △) the third run, (▼, ▽) the fourth run.

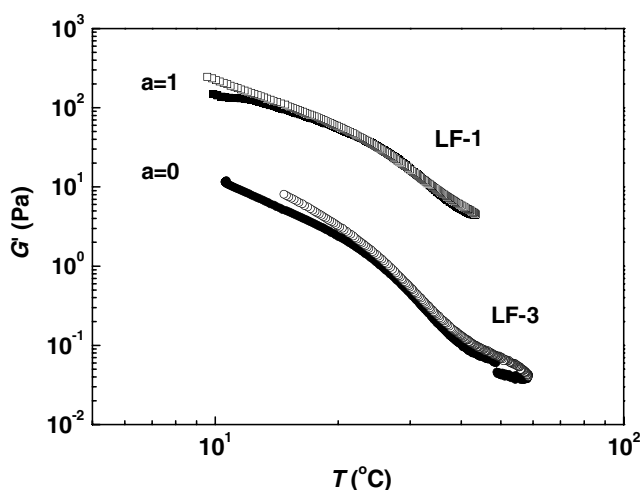


Fig. 9. Comparison of shear storage modulus G' upon heating (solid symbols) and the subsequent cooling (open symbols) for LF-1 (square) and LF-3 (circle) solutions with concentration of 0.6%w/w at a heating/cooling rate of 1.0 °C/min and an angular frequency of 1 rad/s. The data were shifted along vertical axes by 10^a to avoid overlapping.

to contain junction zones and pronounced aggregates. The Lentinan aqueous gel is more like a very concentrated solution that is unable to flow within a timescale of usual observation. The unique rheology of Lentinan in water would have wide application in industries such as food, pharmaceutical, cosmetics, oil recovery and so on.

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