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# Protein/Polysaccharide Cogel Formation Based on Gelatin and Chemically Modified Schizophyllan

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In the work, aldehyde groups were quantitatively introduced into schizophyllan (SPG) side chains through periodate oxidation. The periodate-oxidized SPG (POSPG) forms an elastic gel with gelatin. The cogel formation is based on the Schiff-base reaction between the amino groups of gelatin chains and the aldehyde groups of POSPG chains. The POSPG/gelatin cogel has an elastomeric character with a very small value of loss tangent. The gelation kinetics and gel properties were discussed as a function of POSPG concentration, gelatin concentration, oxidation degree, temperature, and pH. This method can be used to design a large variety of cogels between SPG and proteins.

## Introduction

For years, there has been an impressive growth of activity and interest in the discovery and design of two-component biopolymer systems capable of cogelling.<sup>1–3</sup> There are several reasons for this. In the pharmaceutical area, biopolymer cogel formation is one of the important methods to control drug release and design new drug matrices. In the food industry, biopolymer cogel formation is widely employed to modify food texture or structure, design functional or healthy food, and reduce food cost. Of course, the studies on biopolymer cogels also have important academic significance. For most biopolymer cogels, gel characteristics result from the synergistic interaction or covalent bonding between two or more components. The former case includes xanthan/locust bean gum,<sup>4–6</sup> xanthan/konjac glucomannan,<sup>7</sup>  $\kappa$ -carrageenan/konjac glucomannan,<sup>8,9</sup> and  $\kappa$ -carrageenan/gellan,<sup>10</sup> and so forth, and the latter case is such as the chemical cogels formed between chitosan and scleroglucan and so forth.<sup>11,12</sup>

Schizophyllan (SPG) is a neutral extracellular polysaccharide first discovered by Kikumoto and co-workers.<sup>13,14</sup> Its chemical structure consists of linearly linked  $\beta$ -(1 $\rightarrow$ 3)-D-glucose residues substituted with a  $\beta$ -(1 $\rightarrow$ 6)-D-glucose side chain for every three backbone residues. SPG is nontoxic and biocompatible and has the ability to enhance the immune system sensitivity, resulting in improved antitumor, antibacterial, antiviral, anticoagulatory, and wound healing response.<sup>15,16</sup> Because of these properties, SPG has been a quite promising biomaterial or drug in biomedical and pharmaceutical areas. However, SPG has a poor gelling ability, which limits its further applications. It was reported that only in the presence of sorbitol or Borax can SPG form a gel, which is relatively weak and brittle.<sup>17–24</sup>

We show here that, after some of the side chains are locally modified by periodate oxidation,<sup>25–27</sup> SPG can form an elastic gel with gelatin. The cogel formation is based on the Schiff-base reaction between the amino groups of gelatin chains and the aldehyde groups of the periodate-oxidized SPG (POSPG) chains (Figure 1). Since most proteins have free amino groups, this method can be extended to prepare a wide range of POSPG/protein cogels, which would be helpful in broadening the applications of SPG. On the other hand, gelatin is a well-known protein extracted from mammalian or fish tissues, which has numerous applications in the photographic and food industries.<sup>28,29</sup> Cross-linking gelatin with some biocompatible agents is crucial and necessary in tissue repair.<sup>30,31</sup> Thereby, the present cogel formation of gelatin with the biocompatible POSPG may also provide a possible way for tissue repair. In this work, we will discuss the gelation kinetics and gel properties of the POSPG/gelatin systems.

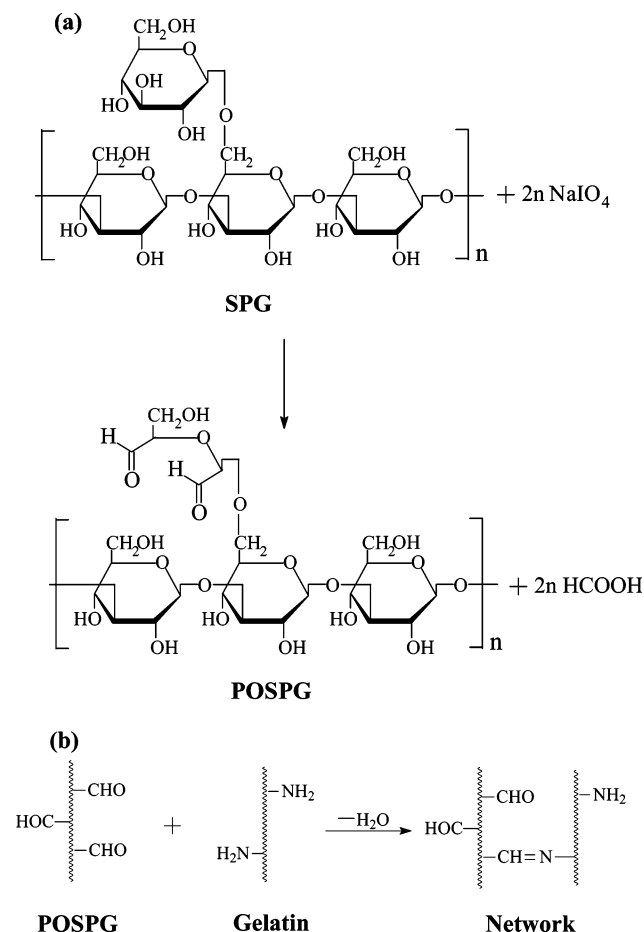
## Materials and Methods

**Materials. 1. Gelatin.** Commercial bovine bone gelatin with a Jelly strength of about 250 g/cm<sup>2</sup> was purchased from Wako Pure Chemical Industries, Ltd. (Japan). It was characterized by multiangle static light-scattering measurements at 50 °C using a multiangle light-scattering detector of DAWN EOS (Wyatt Technology) with a vertically polarized GaAs laser ( $\lambda_0 = 684$  nm). The photometer, which was calibrated using pure toluene and aqueous solutions of low molar mass dextran, was connected to a size exclusion chromatography column of GMPW<sub>XL</sub> (Tosoh) and a differential refractive index detector OPTILAB DSP (Wyatt Technology), which was used to determine gelatin concentration at each position of the elution peak. Temperature of the flow cell was controlled with the constancy of 0.1 °C. Test solutions were filtered through 0.2- $\mu$ m omnipore hydrophilic PTFE membrane filters (Millipore) before measurements. Phosphate buffer solution ( $1/15$  mol/dm<sup>3</sup>, pH 7.0)

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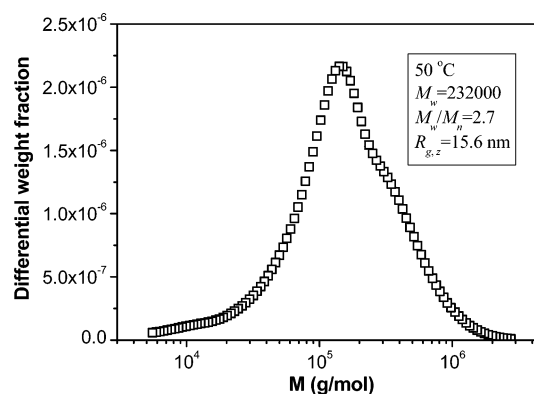
**Figure 1.** (a) Introduction of aldehyde groups into SPG by periodate oxidation. The periodate-oxidized SPG is denoted POSPG. (b) The cross-linking of POSPG with gelatin via the Schiff-base reaction. The zigzag line represents the backbone of POSPG or gelatin (virtually, POSPG and gelatin have different backbone structures).

was used as both solvent and eluent. Scattered light intensities at the scattering angles over 15–163° were measured. The angular dependence of the scattering intensity was analyzed using the following Berry's square-root plot to determine the  $z$ -average square radius of gyration  $\langle R_g^2 \rangle_z$  and the weight-average molar mass  $M_w$  at each position of the elution peak<sup>32</sup>

$$(Kc/R_\theta)^{1/2} = M_w^{-1/2} (1 + \langle R_g^2 \rangle_z k^2/6 + M_w A_2 c) \quad (1)$$

where  $K$ ,  $c$ ,  $\theta$ ,  $R_\theta$ , and  $A_2$  are the optical constant, the polymer mass concentration, the scattering angle, the reduced excess scattering intensity at  $\theta$ , and the second virial coefficient, respectively.  $k$  is the momentum transfer vector defined as  $k = (4\pi n_0/\lambda_0) \sin(\theta/2)$ ,  $n_0$  being the refractive index of a medium. Concentration effects on  $(Kc/R_\theta)^{1/2}$  were always negligible.

Figure 2 shows the differential weight distribution curve of the gelatin sample measured at 50 °C, with the corresponding molecular characteristics listed beside. Gelatin at 50 °C is assumed to exist as a random coil.<sup>28,29,33</sup> For the present bovine bone gelatin at 50 °C,  $M_w$  is about 232 000, which is significantly higher than the known molar mass of  $\alpha$ -chain (a single and linear coil,  $M_w \approx 125$  000).<sup>28</sup> The differential weight distribution curve at this temperature reveals the presence of a high molar mass component. This



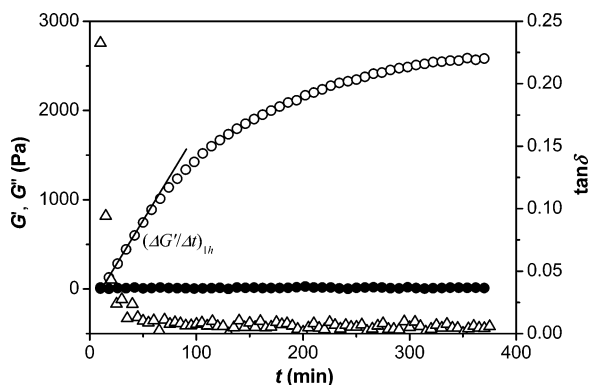
**Figure 2.** The differential weight distribution curve of gelatin measured at 50 °C. The corresponding molecular characteristic is listed beside. Note that the differential weight fraction is not normalized.

high molar mass component should be the  $\beta$ -chains of gelatin (a double form of  $\alpha$ -chain cross-linked with covalent bonds; these cross-links were not totally broken during extraction).<sup>33</sup>

**2. Schizophyllan.** Schizophyllan (SPG) with  $M_w = 450$  000 was kindly supplied by Taito Co., Ltd. (Kobe, Japan). Its intrinsic viscosity in water at 25 °C is 490 cm<sup>3</sup>/g as determined using a Zimm-Crothers rotating cylinder viscometer.

**Periodate Oxidation of Schizophyllan.** SPG was hydrated in distilled water overnight and then stirred for 24 h to obtain a homogeneous solution. To a 2 wt % SPG aqueous solution was added a weighed amount of NaIO<sub>4</sub>. The solution was kept in the dark under stirring for 24 h to ensure complete reaction. During the oxidation, 1 mol of SPG side groups consume 2 mol of NaIO<sub>4</sub>, as shown in Figure 1. It was demonstrated that the oxidation degree of SPG side groups can be quantitatively manipulated by controlling the stoichiometric ratio of NaIO<sub>4</sub> to SPG side group, and the molar mass is only slightly altered by modification.<sup>12,34</sup> In this work, four periodate-oxidized SPG (POSPG) samples with different oxidation degrees, 6%, 29%, 58%, and 93%, were prepared. These oxidation degrees were based on reaction stoichiometry and can represent the actual modification degree.<sup>12</sup> The POSPG solutions were sealed into cellulose tubing membrane and dialyzed extensively against distilled water at 5 °C to remove smaller molecules introduced during the oxidation. The dialyzed POSPG solutions were adjusted to desired concentrations and directly used for preparing the cogels with gelatin. The concentration of POSPG was determined by weighing residues after drying the solutions.

**Preparation of Cogel.** A weighed amount of gelatin was swollen in a phosphate buffer solution (pH = 6.4, 6.8, 7.0, 7.2, or 7.4) at 5 °C overnight. The sample was then dissolved at 50 °C for 30 min using a magnetic stirrer. Note that it is crucial to use the same protocol of dissolution to prepare gelatin solutions, i.e., the same dissolution temperature and duration time, because of the risk of hydrolysis that could modify the molar mass of gelatin.<sup>28</sup> The prethermostated gelatin and POSPG solutions were rapidly mixed under vigorous stirring. After being quickly degassed by centrifugation (shorter than 5 min), the sample was loaded onto the rheometer preset at a desired temperature for rheological



**Figure 3.** A typical gelation process of POSPG/gelatin system: storage modulus  $G'$  (○); loss modulus  $G''$  (●);  $\tan \delta$  (△).  $C_{\text{POSPG}} = 5$  g/L,  $C_{\text{gelatin}} = 30$  g/L,  $D = 58\%$ , pH = 7.0,  $T = 50$  °C,  $\omega = 1$  rad/s, strain = 0.5%. The solid line represents the linear least-squares fit to  $G'$  during the first 1 h.

examinations. The time when the mixing of POSPG and gelatin is started is taken as  $t = 0$ .

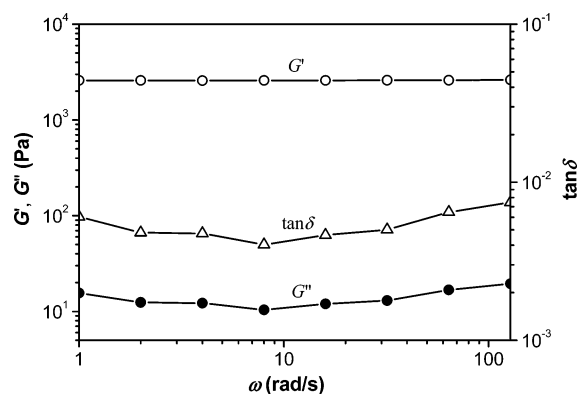
**Rheological Measurement.** The time dependence of the viscoelastic properties of POSPG/gelatin mixtures during gelation was examined on a Fluids Spectrometer RFSII (Rheometrics Far East, Tokyo). A parallel plate geometry of 25-mm diameter and 0.5-mm gap was used. A small strain of 0.5% was applied in all the measurements in order to minimize the structural damage caused by mechanical oscillations. The exposed surface of samples was covered with a layer of light silicon oil to avoid evaporation. Experimental temperature was controlled at a constant value within  $\pm 0.1$  °C by a refrigerated circulator (Julabo, Germany). After the completion of gelation, the formed POSPG/gelatin gels were subjected to a cooling/heating cycle to investigate the temperature dependence of viscoelasticity. The temperature was first lowered to 5 °C and then immediately raised to 80 °C at a rate of 0.5 °C/min.

In this work, the oxidation degree of POSPG is denoted  $D$ , and the concentrations of POSPG and gelatin in grams per liter are denoted  $C_{\text{POSPG}}$  and  $C_{\text{gelatin}}$ , respectively.

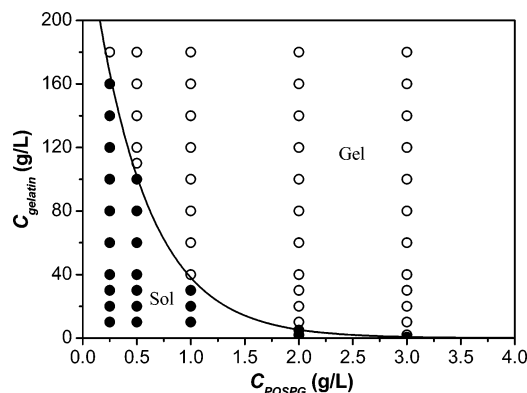
## Results and Discussion

**Gelation Process.** Figure 3 shows a typical gelation process of POSPG/gelatin system at 50 °C with  $C_{\text{POSPG}} = 5$  g/L,  $C_{\text{gelatin}} = 30$  g/L, and  $D = 58\%$ . Obviously, gelation indeed occurs between gelatin and the chemically modified SPG. It was confirmed that no gelation occurs between gelatin and unmodified SPG. During the gelation, the storage modulus  $G'$  increases with time and levels off at a sufficiently long time.  $\tan \delta$  decreases very quickly at the initial stage of gelation. However, the loss modulus  $G''$  does not show a considerable change during the gelation. This behavior differs from most observations on gelation processes in which  $G''$  was always found to increase during gelation. Moreover,  $G'$  increases almost linearly with time during the first 1 h. The slope of the increase,  $(\Delta G'/\Delta t)_{1h}$ , can be used to describe the gelation rate.<sup>12</sup>  $(\Delta G'/\Delta t)_{1h}$  is obtained from the linear least-squares fit of  $G'$  using the data during the first 1 h.

Figure 4 shows the frequency dependence of  $G'$  and  $G''$  of the formed POSPG/gelatin cogel with  $C_{\text{POSPG}} = 5$  g/L,



**Figure 4.** Dynamic mechanical spectra of a POSPG/gelatin cogel taken after curing for 300 min: storage modulus  $G'$  (○); loss modulus  $G''$  (●);  $\tan \delta$  (△).  $C_{\text{POSPG}} = 5$  g/L,  $C_{\text{gelatin}} = 30$  g/L,  $D = 58\%$ , pH = 7.0,  $T = 50$  °C, strain = 0.5%.

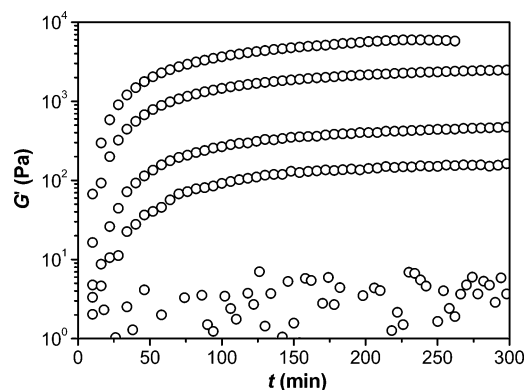


**Figure 5.** The phase diagram of POSPG/gelatin system: gel state (○); sol state (●).  $D = 58\%$ , pH = 7.0,  $T = 50$  °C. The solid line represents the sol-gel boundary. The sol and gel states are judged by whether they can flow in an inverting tube.

$C_{\text{gelatin}} = 30$  g/L, and  $D = 58\%$ .  $G'$  and  $G''$  are almost independent of frequency in the examined frequency range. Moreover,  $G'$  is far larger than  $G''$ , with  $\tan \delta (=G''/G')$  being a very small value ( $< 0.01$ ). It means that the POSPG/gelatin gel has an elastomeric character.<sup>35</sup> The pure gelatin gels, both chemical and physical ones, were also reported to have a small  $\tan \delta$  value,<sup>29,36</sup> which could be attributed to fewer entanglements of relatively compact gelatin coils.<sup>37</sup> In contrast, the SPG gels formed in the presence of sorbitol or Borax have a relatively larger  $\tan \delta$  value ( $> 0.1$ ).<sup>23,24</sup> It is interesting to see that the incorporation of SPG chains into the gelatin network does not increase the loss tangent of the gelatin network.

**Effect of Polymer Concentration.** A phase diagram of POSPG/gelatin system with  $D = 58\%$ , measured at pH = 7.0 and  $T = 50$  °C, is illustrated in Figure 5. The solid line is the sol-gel boundary of the system. Either POSPG or gelatin exhibits a critical concentration for gelation. This behavior is common with most chemically cross-linked gel systems.<sup>38</sup> It can be seen that a very small amount of POSPG (i.e., 0.25 g/L) is enough to gel a gelatin solution. Note that the pH susceptibility and the uncertainty of the concentration of the free amino groups on gelatin chains and the formation of hemiacetals of aldehyde groups on POSPG chains make it quite difficult to quantitatively analyze the critical gelation behavior as well as the gelation kinetics to be discussed.





**Figure 6.** Time dependence of the storage modulus  $G'$  during the gelation of POSPG/gelatin systems with different POSPG concentrations  $C_{\text{POSPG}}$ : 1, 2, 3, 5, 7 g/L (from bottom to top).  $C_{\text{gelatin}} = 30$  g/L,  $D = 58\%$ ,  $\text{pH} = 7.0$ ,  $T = 50^\circ\text{C}$ ,  $\omega = 1$  rad/s, strain = 0.5%.

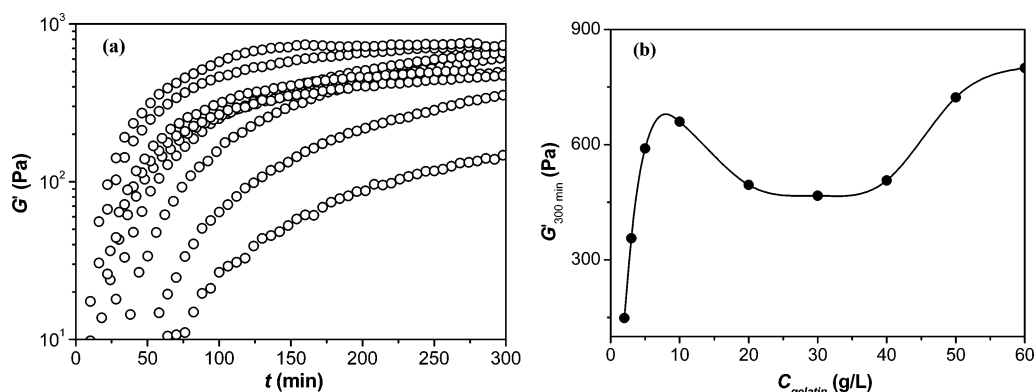
Figure 6 shows the effect of POSPG concentration  $C_{\text{POSPG}}$  on the gelation process of POSPG/gelatin systems. With increasing  $C_{\text{POSPG}}$ , gelation proceeds more rapidly. When  $C_{\text{POSPG}} \leq 1$  g/L, no gelation occurs. Moreover, the final strength of the obtained gels increases with increasing  $C_{\text{POSPG}}$ . The plot of  $(\Delta G'/\Delta t)_{1h}$  against  $C_{\text{POSPG}}$  also shows that the gelation rate is greatly influenced by POSPG concentration (data not shown). Moreover,  $(\Delta G'/\Delta t)_{1h}$  is not linearly related to  $C_{\text{POSPG}}$  as expected from the cross-linking mechanism (Figure 1b). This may be due to the fact that the studied POSPG concentration range is close to the critical regime.

Figure 7a,b show the effect of gelatin concentration  $C_{\text{gelatin}}$  on the gelation of POSPG/gelatin systems. The final gel strength largely depends on the gelatin concentration. In region I where  $C_{\text{gelatin}} \leq 10$  g/L,  $G'_{300\text{min}}$  increases with increasing  $C_{\text{gelatin}}$ . In region II where  $C_{\text{gelatin}} = 20, 30$ , and 40 g/L,  $G'_{300\text{min}}$  does not seem to increase with increasing  $C_{\text{gelatin}}$ . Moreover,  $G'_{300\text{min}}$  values in these systems are even smaller than that for  $C_{\text{gelatin}} = 10$  g/L. In region III where  $C_{\text{gelatin}} \geq 50$  g/L, the increase of  $C_{\text{gelatin}}$  again leads to an increase in  $G'_{300\text{min}}$ .

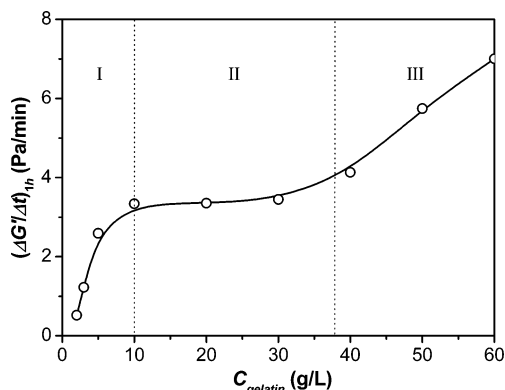
Figure 8 gives the plot of  $(\Delta G'/\Delta t)_{1h}$  against  $C_{\text{gelatin}}$ . Similar to the final gel strength, the gelation rate  $(\Delta G'/\Delta t)_{1h}$  also exhibits three apparently different regions depending on  $C_{\text{gelatin}}$ . In region I,  $(\Delta G'/\Delta t)_{1h}$  increases approximately linearly with increasing  $C_{\text{gelatin}}$ . In region II,  $(\Delta G'/\Delta t)_{1h}$  does not change too much with  $C_{\text{gelatin}}$ . In region III,  $(\Delta G'/\Delta t)_{1h}$

again increases linearly with increasing  $C_{\text{gelatin}}$ . At the present, we cannot give an explanation as to why the gel strength and the gelation rate show such different behaviors in different gelatin concentration ranges. However, it is indeed found that the nature of POSPG/gelatin cogel changes from enthalpic network to entropic network when  $C_{\text{gelatin}}$  passes through regions I, II, and III. Figure 9 shows the temperature dependence of the storage modulus  $G'$  of the POSPG/gelatin gels in the temperature range between 30 and 50  $^\circ\text{C}$  where the coil-helix transition of gelatin does not happen. Note that, if temperature is further changed below 30  $^\circ\text{C}$ , the gelation of gelatin induced by the coil-helix transition will occur. We only discuss the network nature at temperatures above 30  $^\circ\text{C}$  in order to avoid the effect of the coil-helix transition of gelatin. When  $C_{\text{gelatin}} \leq 10$  g/L (region I),  $G'$  decreases with increasing temperature, indicating the enthalpic nature of the gel networks.<sup>39</sup> When  $20 \leq C_{\text{gelatin}} \leq 40$  g/L (region II),  $G'$  is almost independent of temperature. When  $C_{\text{gelatin}} \geq 50$  g/L (region III),  $G'$  increases with increasing temperature, indicating the entropic nature of the gel networks.<sup>39</sup>

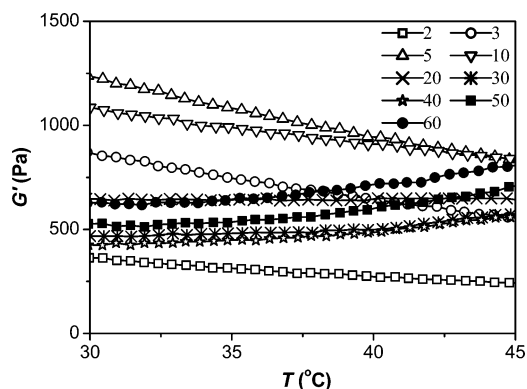
As mentioned above, gelatin surely exists as a flexible coil in the examined temperature range.<sup>28</sup> However, the conformation of POSPG is not straightforward, although its mother analogue, schizophyllan (SPG), proved to have a rigid rodlike triple helical conformation.<sup>40</sup> By theoretically modeling small-angle X-ray scattering (SAXS) data, Maeda et al.<sup>34</sup> found that POSPG with an oxidation degree lower than 20% retains the rigid triple helical conformation; at higher oxidation degrees, POSPG can be viewed as a broken rod with some parts of helices dissociated into coils. Moreover, the dissociated parts increase with increasing oxidation degree. For a POSPG sample with an oxidation degree of 58%, it can be roughly estimated that more than 50% of triple helices are dissociated into coils. The present POSPG/gelatin cogel can be visualized as a network composed of broken POSPG rods and gelatin coils. Note that, although the POSPG helix is broken somewhere along the backbone, it is still significantly more rigid than the random coil of gelatin. When the ratio of the two polymer chains, i.e.,  $C_{\text{gelatin}}/C_{\text{POSPG}}$ , is low, the network is made from long and relatively rigid POSPG segments connected with a small number of gelatin segments. The network elasticity mainly comes from



**Figure 7.** (a) Time dependence of the storage modulus  $G'$  during the gelation of POSPG/gelatin systems with different gelatin concentrations  $C_{\text{gelatin}}$ : 2, 3, 5, 10, 20, 30, 40, 50, 60 g/L (from right to left).  $C_{\text{POSPG}} = 3$  g/L,  $D = 58\%$ ,  $\text{pH} = 7$ ,  $T = 50^\circ\text{C}$ ,  $\omega = 1$  rad/s, strain = 0.5%. (b) The plot of  $G'_{300\text{min}}$  against gelatin concentration  $C_{\text{gelatin}}$ ;  $G'_{300\text{min}}$  is the storage modulus at 300 min.



**Figure 8.** The plot of  $(\Delta G'/\Delta t)_0$  against gelatin concentration  $C_{\text{gelatin}}$ .  $(\Delta G'/\Delta t)_0$  is the initial rate of gelation, which is obtained from the linear fit of  $G'$  during the first 1 h.

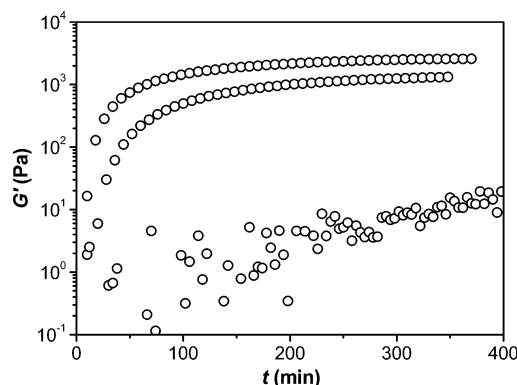


**Figure 9.** Temperature dependence of the storage modulus  $G'$  of the POSPG/gelatin gels with different gelatin concentrations  $C_{\text{gelatin}}$ .  $C_{\text{POSPG}} = 3$  g/L,  $D = 58\%$ ,  $\text{pH} = 7$ ,  $\omega = 1$  rad/s, strain = 0.5%.

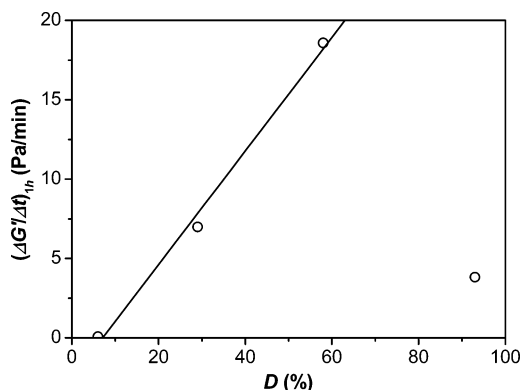
the enthalpy change in the bending of the POSPG segments.<sup>41</sup> It should be pointed out that in this situation the entropic term of the elasticity coming from the extension of gelatin coils is less pronounced. On the contrary, if the ratio  $C_{\text{gelatin}}/C_{\text{POSPG}}$  is high, the POSPG/gelatin network is made from shorter rigid POSPG segments connected with a larger number of gelatin segments. The network elasticity is mainly entropic and contributed by the conformational change of gelatin chains in extension.<sup>35,42</sup> In the situation, the role of POSPG chains is just like a small cross-linking agent.

**Effect of Oxidation Degree.** Figure 10 shows the time dependence of the storage modulus  $G'$  during the gelation of POSPG/gelatin systems with three different oxidation degrees:  $D = 6\%$ ,  $29\%$ , and  $58\%$ . Note that the data for  $D = 93\%$  are not included, because a POSPG sample at such a high oxidation degree is heterogeneous because of its poor water solubility. In fact, we observed a white fiberlike precipitate suspended in the POSPG solution of  $D = 93\%$ . From Figure 10, obviously, the increase in oxidation degree greatly increases the gelation rate. It also remarkably improves the final strength of the POSPG/gelatin gels. Figure 11 plots  $(\Delta G'/\Delta t)_0$  against oxidation degree  $D$ . Except for the data point of  $D = 93\%$ ,  $(\Delta G'/\Delta t)_0$  is almost a linear function of  $D$ . This means that the gelation rate of POSPG/gelatin depends only on the actual concentration of aldehyde groups in the solutions.

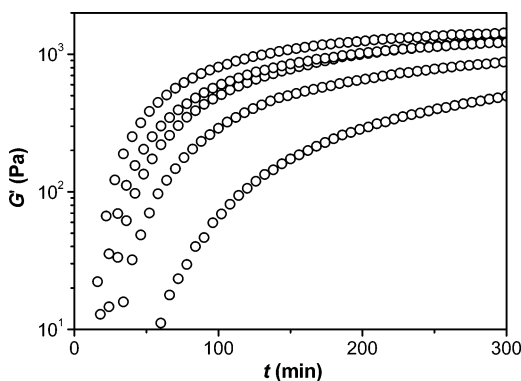
**Effect of pH.** The change of pH is expected to affect the gelation of POSPG/gelatin systems in the following two



**Figure 10.** Time dependence of the storage modulus  $G'$  during the gelation of POSPG/gelatin systems with different oxidation degrees of POSPG  $D$ :  $6\%$ ,  $29\%$ ,  $58\%$  (from bottom to top).  $C_{\text{POSPG}} = 5$  g/L,  $C_{\text{gelatin}} = 30$  g/L,  $T = 50$  °C,  $\text{pH} = 7$ ,  $\omega = 1$  rad/s, strain = 0.5%.

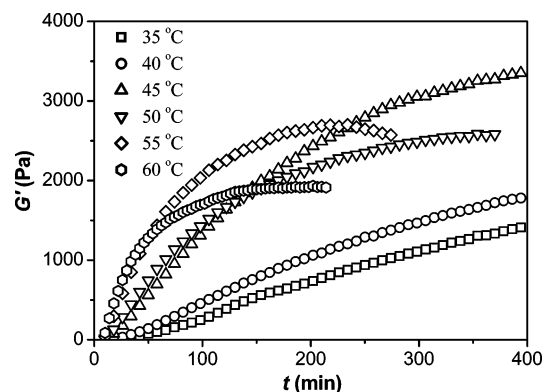


**Figure 11.** The plot of  $(\Delta G'/\Delta t)_0$  against oxidation degree of POSPG  $D$ .  $(\Delta G'/\Delta t)_0$  is the initial rate of gelation, which is obtained from the linear fit of  $G'$  during the first 1 h.

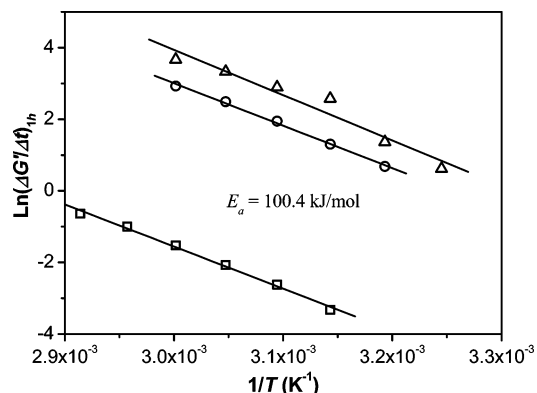


**Figure 12.** Time dependence of the storage modulus  $G'$  during the gelation of POSPG/gelatin systems at different pH values:  $6.4$ ,  $6.8$ ,  $7.0$ ,  $7.2$ ,  $7.4$  (from bottom to top).  $C_{\text{POSPG}} = 5$  g/L,  $C_{\text{gelatin}} = 30$  g/L,  $D = 29\%$ ,  $T = 50$  °C,  $\omega = 1$  rad/s, strain = 0.5%.

ways: (1) the Schiff-base reaction is catalyzed by the presence of  $\text{H}^+$ ;<sup>43</sup> and (2) the number of free amino groups on gelatin chains is reduced when the pH value is lowered.<sup>33</sup> The gelation kinetics of POSPG/gelatin systems is determined by these two competitive factors. Therefore, the presence of an optimum pH value is expected for the POSPG/gelatin system. Figure 12 shows the effect of pH on the gelation of POSPG/gelatin systems at five pH values,  $\text{pH} = 6.4$ ,  $6.8$ ,  $7.0$ ,  $7.2$ , and  $7.4$ , using different phosphate buffers. On the whole, the gelation proceeds more rapidly at a higher pH value. Moreover, the final gel strength increases with increasing pH value. However, we cannot observe an



**Figure 13.** Time dependence of the storage modulus  $G'$  during the gelation of POSPG/gelatin systems at different temperatures.  $C_{\text{POSPG}} = 5$  g/L,  $C_{\text{gelatin}} = 30$  g/L,  $D = 58\%$ ,  $\text{pH} = 7$ ,  $\omega = 1$  rad/s, strain = 0.5%.



**Figure 14.** Arrhenius plots of the gelation rate  $(\Delta G'/\Delta t)_{\text{th}}$  against  $1/T$  for the gelation processes of POSPG/gelatin systems with different oxidation degrees  $D$ : 6% ( $\square$ ); 29% ( $\circ$ ); 58% ( $\triangle$ ).  $C_{\text{POSPG}} = 5$  g/L,  $C_{\text{gelatin}} = 30$  g/L,  $\text{pH} = 7$ .

optimum pH value as expected. This means that, in the examined pH range, the gelation is dominated by the second factor pointed out above. The optimum pH may appear in a higher pH range. Indeed, it can be found that, starting from  $\text{pH} = 7.0$ , the effect of pH becomes blunt (judging from the increasing tendency of the saturated storage modulus).

**Effect of Temperature.** Figure 13 shows the effect of temperature on the gelation of POSPG/gelatin systems with  $C_{\text{POSPG}} = 5$  g/L,  $C_{\text{gelatin}} = 30$  g/L,  $D = 58\%$ , and  $\text{pH} = 7$ . The higher temperature is favorable for the gelation, which results in a faster gelation. This is in accordance with most reports about the effect of temperature on heat-set gelations.<sup>44–46</sup> Moreover, it can be seen that, for the gelation processes at temperatures higher than  $55^\circ\text{C}$ , the storage modulus  $G'$  first increases and then decreases with time. The occurrence of the  $G'$  maxima could be interpreted by one or two of the following reasons: (1) the higher gelation temperatures cause slippages between the measured samples and the geometry;<sup>47</sup> (2) the gelations are so fast that syneresis occur.<sup>20</sup>

The Arrhenius treatments for the gelation processes of POSPG/gelatin systems with different oxidation degrees of POSPG are displayed in Figure 14. For all three oxidation degrees, we can obtain fairly good linearity. Additionally, the three linear lines give approximately the same slope, suggesting that the activation energy,  $E_a$ , of the gelation is

independent of the oxidation degree of POSPG.  $E_a$  obtained here is  $100.4 \pm 4$  kJ/mol. This value is significantly larger than that of 43.3 kJ/mol reported for the Schiff-base reaction of scleroglucan and chitosan.<sup>12</sup>

## Conclusion

We introduced a method to prepare schizophyllan (SPG)/protein gels. This method would be helpful in widening the application of SPG as gel matrices. In the method, some aldehyde groups are introduced into SPG side chains by periodate oxidation, and then, the periodate-oxidized SPG (POSPG) forms a cogel with any protein that contains free amino groups. The cogel is chemically cross-linked via the Schiff-base reaction of the aldehyde groups with the free amino groups.

This method was practiced using a well-known protein, gelatin. It was found that POSPG indeed forms a gel with gelatin. The obtained POSPG/gelatin cogel has an elastomeric character with a very small loss tangent value. This could be attributed to the presence of fewer entanglements of compact gelatin coils. We examined the effect of polymer concentration, oxidation degree, pH, and temperature on the gelation kinetics and gel properties. There exists a critical gelation concentration for each of the two polymers, and the higher POSPG or gelatin concentration leads to a faster gelation. In the examined pH range (6.4–7.4), the gelation proceeds more rapidly at a higher pH value. The increase of temperature on one hand increases the gelation rate; however, it could bring the risk of syneresis. It was also found that, when the ratio of the two polymer chains, i.e.,  $C_{\text{gelatin}}/C_{\text{POSPG}}$ , is low, the elasticity of the cogel is enthalpic. The cogel could be visualized as a network made from long and relatively rigid POSPG segments connected with a small number of gelatin segments. Oppositely, the higher ratio  $C_{\text{gelatin}}/C_{\text{POSPG}}$  results in an entropic network, which is made from shorter rigid POSPG segments connected with a large number of gelatin segments.

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