Solgel transition in dye fluorescence measurements

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The results of fluorescence quenching measurements, obtained for a dye-substituted aqueous gelatin solution during the sol–gel transition, are presented. Simultaneous optical polarization rotation and fluorescence studies allow us to find the dependence of the fluorescence yield on the amount of helix created in the medium. The exact gel point has been found as the point of inflection on the fluorescence yield versus the helix amount plot. The second-order character of the sol–gel transition is shown, which is in agreement with the theoretical predictions obtained by use of the percolation theory. © 1997 Optical Society of America

Key words: Fluorescence, gelation process, sol–gel transition, gelatin, gel, self-ordering systems.

1. Introduction

The gel state is a novel condensed state of matter in which a minor amount of solute molecules in solution can form a tenuous, macroscopic network to hold the solution in a solidlike phase. Gels are found in many significant applications, including numerous structures in living organisms, foods, and new low-density materials. For physicists and chemists, gels are also interesting to investigate as an example of self-organizing systems. In particular, gelatin and gelatin gels deserve special attention because of their importance to biological and food industries. Gelatin is a protein that is derived from naturally occurring collagen by a denaturating process, which depends on breaking the hydrogen bonds stabilizing the collagen triple helixes. The gelatin molecule is a single chain made of a succession of approximately 1000 amino acid monomers with a total molecular weight of $M_W = \sim 95.000$. In an aqueous solution above the temperature $T = 35\ °C$ the gelatin molecules are separate, randomly oriented coils. When the medium is cooled below this temperature, the gelatin chains undergo a conformation change; they start to rebuild the hydrogen junctions and, in an attempt to renature themselves back to the helix form, they start to construct the network of the chains.[1] This is the process of gelation. In the sol state the aggregates of gelatin molecules, which we call clusters, are local. In the gel state the network of the gelatin chains becomes macroscopic, and it fills the whole volume of the medium. In gelatin this process is reversible, which means that the junctions are formed and broken again when subjected to temperature variations. There is an ever-present question: Is the phase transition from the sol to the gel state in gelatin of the first order, like a crystallization process, or of the second order, like a liquid-to-glass transition?[2] Our aim in this paper is to discuss this and to find the exact gel point for the sol–gel transition.

There are a number of papers that have investigated the above-mentioned problem by means of different experimental methods, e.g., optical polarization rotation and rheological measurements,[1,3] viscosity measurements,[4] and static and dynamic light scattering,[5] which suggest that the passage from the sol to the gel state in gelatin is a second-order transition. In this paper we propose a method that so far has not been used for investigation of self-organizing systems, which depends on the registration of the probe dye fluorescence yield time evolution during the gelation process. We have studied the fluorescence of dye molecules dissolved in a gelatin aqueous solution. It appears that during the gelation process, the total fluorescence intensity falls, and we believe that this behavior is due to the formation of a network of gelatin molecules linked by hydrogen bonds. The theoretical explanation based on the percolation theory is presented. Additional

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measurements of the optical polarization rotation allow us to find the dependence of the fluorescence yield on the number of existing hydrogen bonds and establish the peculiar character of this dependence near the gel point.

2. Experimental

The experiment was performed with a 100-mW power Ar-ion laser at the 488-nm wavelength. The gelatin sample, from a pig skin, bloom test 150, was provided by the British Drug House Ltd., Poole, England. The measurements were made for a fluorescein dye diluted in 3% aqueous gelatin solution at a low concentration ($c = 2 \times 10^{-6}$ mg/100 cm^3) at three temperatures, $T = 20$, 22, and 24 °C. To obtain a high pH of the solution and in this way ensure good efficiency of the dye fluorescence, we added 0.01 M of NaOH. The sample was kept for 1 h in the bath at $T = 50$ °C and then switched to the second bath at a lower temperature. The stabilization of the temperature was ensured by use of a jacketed cell. The temperature was directly measured in the cell by a thermocouple. The fluorescence spectra, resolved by a 0.5-m monochromator, were acquired every 3 or 4 min by a photon-counting technique. Simultaneously, the measurements of the optical activity of the medium were made by a polarimeter.

3. Theoretical

The theoretical approach to the presented problem is based on the simplified Flory–Stockmayer bond percolation model,[6] which does not take into account the presence of the solvent. In view of the percolation theory, below the gel point, molecules group in clusters of different sizes. Thus we denote by n_s the probability per bond that a molecule belongs to the cluster that contains s hydrogen bonds created between monomers, which we refer to as the s cluster. The probability p that the bond between two adjacent monomers exists is temperature dependent[7]:

$$p = 1 - \exp(-E/kT), \qquad (1)$$

where E is the energy of the hydrogen bond. The probability p describes the fraction of reacted bonds, which corresponds to the experimentally measurable quantity, the helicity χ of the solution. When the fraction of bonds increases, the average size of the clusters also increases. The number n_s of s clusters per bond has the known form[8]

$$n_s = (b_s/s)p^s(1-p)^{f+(f-2)s}, \qquad (2)$$

where b_s is the total number of possible configurations of monomers creating an s cluster and f is the number of the nearest neighbors of each monomer in the cluster.

We assume that, in the sol phase, a certain number of dye molecules are imprisoned in the clusters of gelatin molecules. As the process of gelation develops, the number and the sizes of the clusters grow and thus the number of captured dye molecules increases. The probability that the dye molecule is conjugated to the finite s cluster is[8]

$$P_s = sn_s. \qquad (3)$$

In the sol state below a certain critical value $p = p_c$, the probability that the molecule is attached to a finite cluster is

$$F(p) = \sum_s sn_s = p \qquad \text{for } p \le p_c. \qquad (4)$$

Above the gel point, the infinite cluster appears with a maximum number of bonds $s_{\max}$. The probability that the molecule belongs to this cluster,

$$R(p) = p - \sum_s sn_s \qquad \text{for } p > p_c, \qquad (5)$$

is called the gel fraction and is equal to the fraction of bonds between the molecules belonging to it, once it is formed.

The number of dye molecules embedded in an s cluster is $N_s = N_d P_s$, where N_d denotes total number of dye molecules. N_d is assumed to be very small compared with the number of monomers N_m, $N_d \ll N_m$, so that there is no energy transfer between dye molecules. The dye molecules are resonantly excited by cw laser light. The stationary fluorescence yield has the form

$$I = \Gamma_R N^*, \qquad (6)$$

where N^* is the total number of excited dye molecules, and

$$N^* = N_F^* + \sum_s N_s^*, \qquad (7)$$

where N_F^* is the number of excited free dye molecules and $\sum_s N_s^*$ is the number of dye molecules embedded in clusters. Our assumption is that part of the excitation energy of the dye molecules embedded in clusters is dissipated in a nonradiative process into the manifold of vibrational states of the cluster[9] with a dissipation rate that is dependent on cluster size, k_{NR}^s. The numbers N_F^* and N_s^* have been found as stationary solutions of rate equations and are given in the form

$$N_s^* = I_0 P_s / \Gamma_R + k_{\mathrm{NR}}^s, \qquad N_F^* = I_0 \left(1 - \sum_s P_s\right)/\Gamma_R, \quad (8)$$

where I_0 is the absorbed light intensity.

Thus, by substituting Eq. (8) into Eq. (6), we obtain the following expression for the relative fluorescence intensity in the sol state,

$$I/I_0 = 1 - \sum_s P_s \frac{k_{\mathrm{NR}}^s}{\Gamma_R + k_{\mathrm{NR}}^s}, \qquad (9)$$

and in the gel state,

$$I/I_0 = 1 - R(p)\frac{k_{\mathrm{NR}}^{s_{\max}}}{\Gamma_R + k_{\mathrm{NR}}^{s_{\max}}} - \sum_s P_s \frac{k_{\mathrm{NR}}^s}{\Gamma_R + k_{\mathrm{NR}}^s}. \quad (10)$$

According to the percolation theory,[7,8] $R(p) = 0$ in the sol state, and it increases rapidly in the gel state. In the vicinity of the gel point, the dependence of the gel fraction on the fraction of bonds p is given by the following power law[7,8]:

$$R(p) \propto (p - p_C)^{\beta} \qquad \text{for } p > p_c, \qquad (11)$$

where β is the universal critical exponent.

Thus, in the gel state, the number of dye molecules embedded in an infinite cluster grows, and at the same time the sum over the finite clusters tends to zero. The probability of nonradiative dye energy dissipation in finite clusters exhibits different behavior on both sides of the gel point. Thus, in the sol state, it increases with $p \rightarrow p_c$, approximately as

$$\sum_s P_s \frac{k_{NR}^s}{\Gamma_R + k_{NR}^s} \propto p \frac{k_{NR}^{s_{max}}}{\Gamma_R + k_{NR}^{s_{max}}}, \qquad (12)$$

whereas in the gel state it decreases, as

$$\sum_s P_s \frac{k_{NR}^s}{\Gamma_R + k_{NR}^s} \propto \frac{k_{NR}^{s_{max}}}{\Gamma_R + k_{NR}^{s_{max}}} [p - R(p)], \qquad (13)$$

where, in this estimation, we have substituted k_{NR}^s by its maximum value in the gel point $k_{NR}^{s_{max}}$. Substituting expression (12) into Eq. (9) and expression (13) into Eq. (10) results in the conclusion that, in vicinity of the gel point $p \rightarrow p_c$, the fluorescence intensity yield becomes the linear function of p,

$$I/I_0(p) = 1 - \frac{k_{NR}^{s_{max}}}{\Gamma_R + k_{NR}^{s_{max}}} p \qquad (14)$$

and that the discussed sol–gel transition is continuous. The condition $(\partial^2/\partial p^2)I/I_0(p)|_{p \rightarrow p_c} = 0$ suggests that at the critical value $p = p_c$ the curve $I/I_0(p)$ should exhibit the inflection point.

The percolation theory[7,9,10] predicts that, according to the Landau theory of continuous phase transitions, the sol–gel transition is analogous to a second-order phase transition. $R(\chi)$ plays the role of order parameter and the helicity χ is equivalent to the temperature in thermal phase transitions.

We have calculated the dissipation rate assuming that the electron excitation energy is transferred from the dye to the adjacent gelatin molecule in a nonradiative process with the rate k_{NR}^0, and then the energy flows to more distant molecules of the cluster through the weak hydrogen bonds in an avalanchelike way with the single transition rate h. The nonradiative transition rate k_{NR}^s for the s cluster, calculated for the Bethe lattice model of functionality f, has the form

$$k_{NR}^s = k_{NR}^0 \left[1 + fh \frac{1 - (2h)^r}{1 - 2h} \right], \qquad (15)$$

where $r = \log_2(s + 1)$. When the system approaches the gel point, $s \rightarrow \infty$, and for $h \ll 1$,

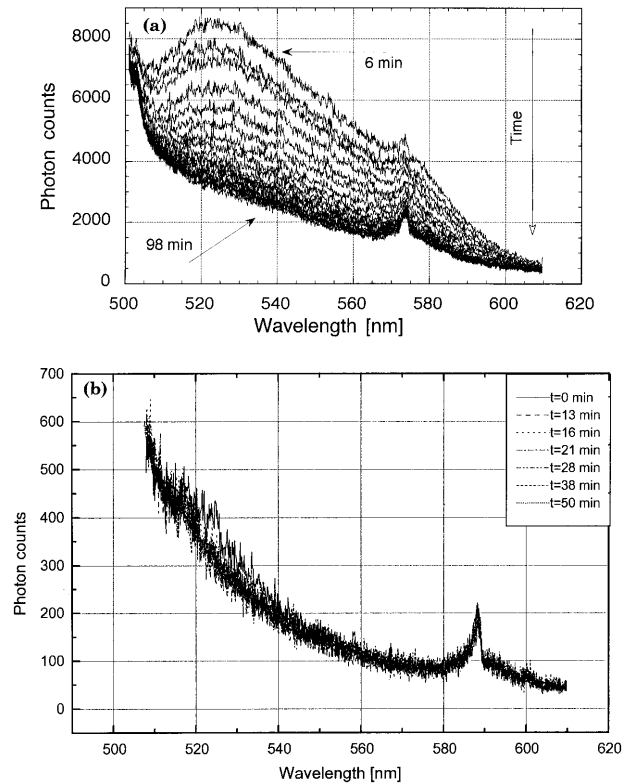$$k_{NR}^{s_{max}} = k_{NR}^0 \left[1 + f \frac{h}{1 - 2h} \right]. \qquad (16)$$

Fig. 1. (a) Fluorescence spectra of a fluorescein dye with concentration $c = 2 \times 10^{-6}$ mg/cm^3 in 3% aqueous gelatin solution with 0.01M NaOH at $T = 22$ °C. The peak at 585 nm corresponds to the stretch vibration of the OH group in H_2O. (b) Fluorescence spectra of pure H_2O with 0.01-M NaOH under the same conditions.

Substituting Eq. (16) to Eq. (10), we get the following dependence of the fluorescence yield on the helicity in the gel state:

$$I/I_0 = 1 - \frac{k_{NR}^{s_{max}}}{\Gamma_R + k_{NR}^{s_{max}}} (p - p_C)^{\beta}. \qquad (17)$$

The value of critical exponent β, found by the percolation theory, assuming a three-dimensional Bethe lattice model for the medium, equals 0.45.[7] Our aim in this paper is to establish the exponent β in the experimental fluorescence measurements.

4. Results

The experimental results of the fluorescence measurements are presented in Fig. 1. It contains the sequence of the fluorescence spectra of the fluorescein, diluted in 3% aqueous gelatin solution, taken every 3 min during the gelation process. It can be seen that the fluorescence yield falls during the process, until it seems to reach a certain level. The stability of the measuring system is proved in Fig. 1(b), which shows that the spectrum of pure H_2O does not change in time. The time dependence of the fluorescence yield is even better illustrated in Fig. 2, in which, on the vertical axis, we have plotted the values of integrals over the area of the fluorescence peak normalized to the input laser intensity. Rapid decrease of the fluorescence
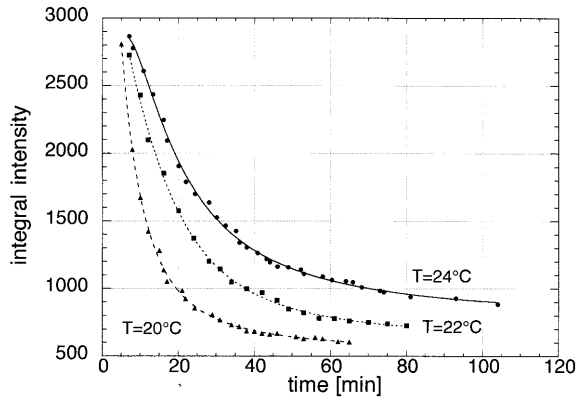
Fig. 2. Time dependence of the normalized fluorescence yield obtained by integration of the spectra [see Fig. 1(a)], for three temperatures, T = 21, 22, and 24 °C.
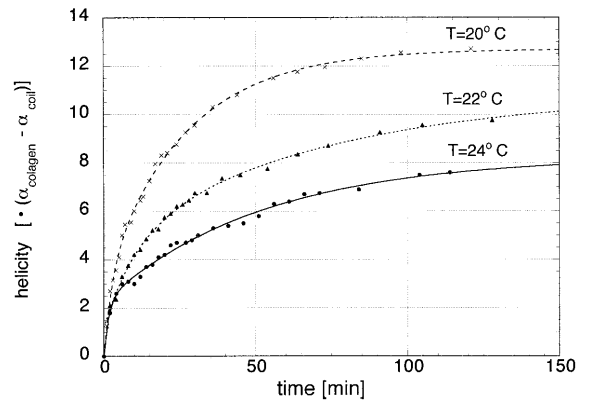

Fig. 3. Time dependence of the helicity of the 3% aqueous gelatin solution at T = 21, 22, and 24 °C, with an accuracy to the multiplicative factor ($\alpha_{collagen} - \alpha_{coil}$).

yield in the sol state becomes slow in the gel state, but it does not have a saturation character. As Fig. 2 shows, the lower the quenching temperature, the bigger the rate of this process and the lower the final fluorescence yield. The explanation of this phenomenon can be provided by the proposed percolation model. In the sol state the separate clusters join together, thus changing their sizes drastically. The nonradiative transition rate, which depends on the number of the hydrogen bonds in the cluster, increases abruptly, resulting in a strong decrease in the fluorescence intensity. On the other hand, in the gel state there is a macroscopic network of a large number of bonds. Thus joining small clusters or single molecules only slightly influences the cluster's size and the value of the transition rate.

We performed the measurements of the optical polarization rotation in the medium in order to obtain information on the temporal evolution of its helicity. The helicity χ is the quantity equivalent to the fraction of reacted bonds in the percolation theory. Figure 3 presents the obtained results. The helicity is defined as the ratio of the present hydrogen bonds to the possible maximum number of bonds. It is connected with the angle of the rotation of the optical polarization as[1,4]

$$\chi = (\alpha - \alpha_{coil})/(\alpha_{collagen} - \alpha_{coil}), \qquad (18)$$

where α is the measured value of the angle, α_{coil} is the value of the angle in the hot completely denatured solution, and $\alpha_{collagen}$ is the value for collagen, which is assumed to have a 100% helix.

By eliminating time from Figs. 2 and 3 we obtained the dependence of the fluorescence yield on the helicity of the medium, which is illustrated in Fig. 4. It can be noted that the shapes of the presented curves are similar in all cases, independently of the temperatures of the medium. Moreover, all curves undergo a smooth change of the slope with the point of inflection in the vicinity of the helicity value equal to 6.5. We interpret this point as the gel point for the sol–gel transition in aqueous solution in gelatin. The transition is smooth, which confirms its continuous char-

acter. The discrepancies between the experimental curves for different temperatures are caused mainly by the difficulty in achieving the identity of temperatures in polarimetric and fluorescence measurements. The results obtained for different temperatures have been averaged in order to fit the theoretical curve to them. The averaged experimental curve obtained in this way and the numerically fitted plot are also shown in Fig. 4. The fit was performed to the theoretical curve defined by Eq. (17) for $\chi > \chi_C$, where $\chi_C = 6.45$. This procedure allowed us to find the critical exponent $\beta = 0.48$, which is in good agreement with the value of this exponent found theoretically[7] by the percolation theory for the three-dimensional Bethe lattice model. Our result proves that the percolation theory well describes the process of gelation in reversible gels and confirms its prediction of a continuous sol–gel transition in these gels. Moreover, we have found the relation $[(k_{NR}^{s_{max}}/(\Gamma_R + k_{NR}^{s_{max}})] = 0.27$, which yields $k_{NR}^{s_{max}} \cong 0.37\Gamma_R$. It re-
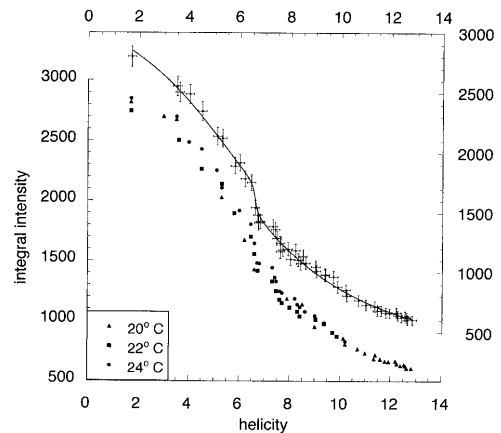

Fig. 4. Fluorescence yield versus the helicity for a fluorescein dye with concentration $c = 2 \times 10^{-6}$ mg/cm^3 in 3% aqueous gelatin solution with 0.01-M NaOH. Lower curve, experimental points obtained at T = 21, 22, and 24 °C. Upper curve, the averaged experimental results and theoretically fitted plot. The error bars correspond to the average of the experimental errors from three measurements.

sults in the conclusion that in the gel state the fluorescence lifetime is less than the radiative lifetime, $(\Gamma_R + k_{NR}^{s_{max}})^{-1} < (\Gamma_R)^{-1}$. It means that the system behaves like large polyatomic molecules.[11]

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