Kinetics of Ion-Induced Gelation in Carrageenan Gels Under Physiologic Conditions

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SYNOPSIS

The kinetics of gelation of an ungelled carrageenan solution exposed to a gel-inducing ionic solution was studied using a turbidity technique. Alkali metal ions were allowed to diffuse through a dialysis membrane into the solution of biopolymer. Optical transmission was measured as a function of distance away from the membrane. In the early stage of 12 h, the transmission increases with distance. After 96 h, the transmission is independent of distance. This indicates that gelation is completed everywhere inside the gel and the gel's structure is homogeneous, in agreement with previous results. Time-dependent transmission indicates the presence of two relaxation processes occurring during ion-induced gelation: a primary relaxation process related to the gelling zone movement, and a secondary relaxation process related to local diffusion of polymer, bound ions, and water molecules.

INTRODUCTION

The sol–gel transition in carrageenan gels has attracted considerable attention because many physical and chemical properties of the polymer solutions are influenced by gelation.[1,2] It is known that gelation of carrageenan can be induced by lowering the temperature or by adding cations above certain concentrations.[3–5] However, there are few reports concerning the kinetics of the transition because of the lack of an appropriate method.[6] Recently, the turbidity technique was used for studying the spinodal phase transition in *N*-isopropylacrylamide gels[7] and the statics of the sol–gel transitions in carrageenan gels.[8] The gelation of carrageenan solutions on cooling is related to the transformation of carrageenan molecules, at least locally, from a coil to helical configuration and the subsequent helix association.[9] It is the spatial variation of the aggregation and temporal/spatial fluctuations that result in strong scattering of light.[10–12] Therefore, optical transmission is drastically reduced below the gelling point. To our knowledge this is the first time such a turbidity technique has been used to study and monitor the kinetics of the sol–gel transition in carrageenan gels. The turbidity A is defined as the reduction in fractional light intensity per unit penetration length in the sample, $A = -(1/I)(\delta I/\delta x)$, with I being the light intensity in the sample. For sufficiently small δx, no multiple scattering will occur in δx and the δI is equal to I_s,

$$A = -\frac{1}{\delta x}\left(\frac{I_s}{I}\right). \tag{1}$$

The turbidity is therefore directly related to the scattering function I_s that does not involve multiple scattering. The transmitted light intensity I_t and the incident intensity I_0 are related by the sample turbidity A and thickness L,

$$\frac{I_t}{I_0} = e^{-AL}. \tag{2}$$

Therefore, turbidity can be obtained from the ratio of the transmitted light intensity to the incident intensity, $A = -(1/L)\ln(I_t/I_0)$.[7,8]

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EXPERIMENTAL

The biopolymer investigated was a low-sulfated κ-carrageenan known to gel on exposure to monovalent alkali metal ions.[2] The biopolymer was isolated from selected fractions of seaweed of the class *Rhodophyceae,* genus *Eucheuma,* and species *gelatinae.*[13] The species κ carrageenan is known to gel on exposure to potassium ions; *E. gelatinae* is known to possess lower charge density and to gel on exposure to sodium ions. The ionic composition (0.832% NaCl and 0.14% KCl) of this study represents the level of exposure expected under physiological conditions. The biopolymer solutions were prepared using standard methods at two concentrations: 0.8 and 1.2 wt %. Below 0.8% the gel is weak and is more difficult to study. The polymer solution was placed in a plastic box ($8.8 \times 1.9 \times 1.2$ cm^3) capped at one end with a dialysis membrane that can retain protein with molecular weight greater than 12,000 (Sigma Chemical Company). The box was immersed in a second solution containing the gel-inducing salt (0.832% NaCl and 0.14% KCl). Alkali metal ions diffused through the membrane, gelling the confined polymer solution. The dialysis was carried out at 40°C in order to increase the rate of ion diffusion (relative to the diffusion at room temperature), and thereby hasten the rate of the sol–gel transition. After a designated time, the gelled polymer was taken out the box at room temperature and sliced into 3.7-mm thin samples, which were marked by their distance from the membrane. The samples were then placed in a glass cell with dimensions of $1 \times 1 \times 5$ cm^3 for measurement of optical transmission.

The kinetics of gel formation was further investigated by diffusion of alkali ions into carrageenan without membranes. In this measurement, the original carrageenan solution was placed in the bottom of the optical sample cell with the depth of 8 mm at room temperature. The solution containing gel-inducing salt (0.832% NaCl and 0.14% KCl) was then put at the top of the solution of biopolymer in the pregelled state. The volume ratio between the ionic solution and the polymer sample was about 1.5. Optical transmission was then measured as a function of time.

The optical transmission of gel samples was monitored by a spectrophotometer operating at the wavelength of 555 nm. For convenience, all data are presented using transmission ($I = I_t/I_o$). The turbidity can be inferred from the transmission using eq. (2), with samples of known thickness of 1 cm. All measurements were performed at room temperature, a temperature at which both polymer solutions exist as gels when equilibrated with the solution of alkali metal ions.

RESULTS AND DISCUSSION

Optical Transmission Versus Distance Away from Ion/Gel Interface

Figure 1 shows optical transmission ($I = I_t/I_o$) as a function of distance, with the zero point at the membrane position, for the 0.8 and 1.2% samples, respectively. The samples of biopolymer solution were placed in the ionic solution at 40°C for 48 h. The ion-induced gelation process involves the random coil to double helix transition.[1] The variation of aggregation of helix domains causes reduction of transmitted light. The optical transmission is lowest at the membrane position and highest at the other end. This is expected because diffusion of alkali metal ions in the sol is slow and diffusion of the ions through an already gelled site is even slower.[10] After 48 h, the gelation is still not complete away from the ion/gel interface. The transmission for the 1.2% sample is lower than that for the 0.8% sample at any given distance. The lower transmission is indicative of increased scattering from the stronger gels composed of higher concentration of biopolymer and having greater local concentration fluctuations.[7]

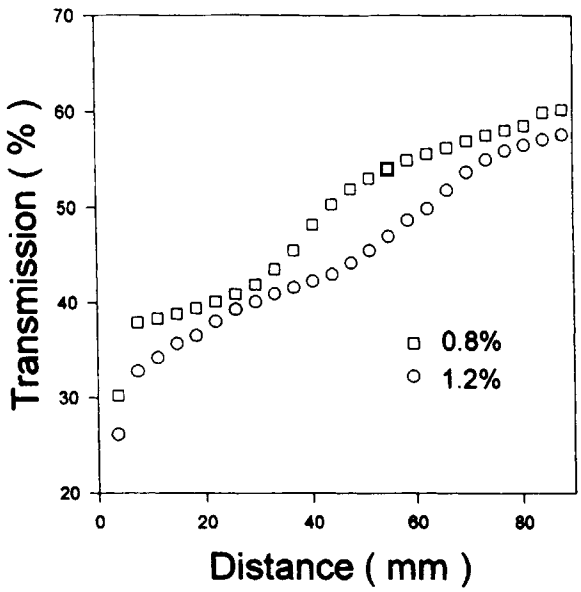

Figure 1 Optical transmission of the (□) 0.8% and (○) 1.2% gels is plotted as a function of distance starting from the potassium/gel interface. The sample cell with one end sealed by a dialysis membrane was immersed in the gel-inducing ionic solution for 48 h at 40°C.

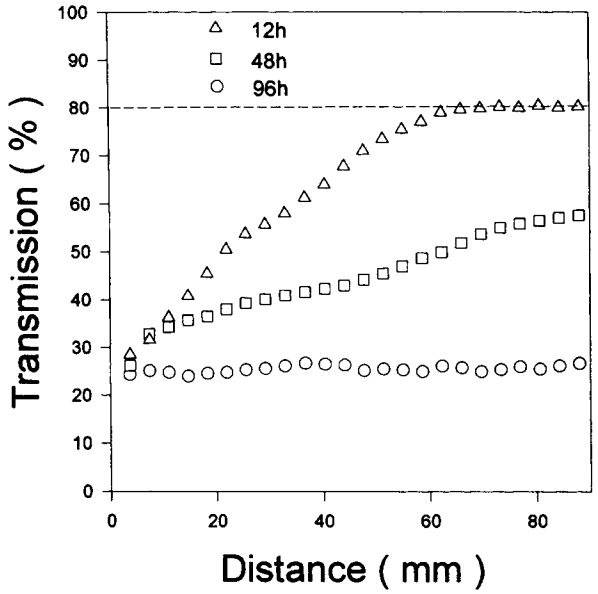

Figure 2 Optical transmission of the 1.2% gel as a function of distance for three different times when they were immersed in the gel-inducing ionic solution at 40°C: (△) 12 h, (□) 48 h, and (○) 96 h.

Optical transmission of the 1.2% samples is shown in Figure 2 as a function of distance after they were immersed in the gel-inducing ionic solution for 12, 48, and 96 h. The dashed line in the figure represents values at the zero time when the sample cell was immersed in the ionic solution. In the early stage (12 h), the gradient of transmission with respect to distance (dI/dx) is large. This is because the alkali metal ions do not have enough time to diffuse from the dialysis membrane to the other end of the box. As the time increases, the gelling zone moves forward and the ions gradually diffuse into the carrageenan solution. After 92 h, migration of the gelling zone has extended to the other end, and the optical transmission becomes independent of the distance. This indicates that the gelation is complete everywhere in the sample cell.

It was recently found that gels with a nonuniform distribution of polymer are formed when calcium ions are allowed to diffuse into solutions of sodium alginate or pectate.[14] This was in contrast to the uniform gels obtained when carrageenan was dialyzed against potassium ions.[14] The observed inhomogeneity of polymer concentration was attributed to the difference in the gelling mechanisms of carrageenan from the alginate systems.[14] Our turbidity measurements confirm that the diffusion of alkali metal ions into carrageenan solution indeed produces a homogeneous gel. This may be because there is reversible binding of alkali ions to the polymer chains, while the binding is nearly irreversible for the calcium/alginate system.[14] Furthermore, our experiment revealed the early and intermediate stages of the transition from a solution state to the gelled state under the exposure of the alkali ions. We have found that in the early and the intermediate stages the gel is not homogeneous. This points to simultaneously monitoring the gelation process as a function of time and distance using the turbidity technique.

Optical Transmission Versus Time

The kinetics of gel formation were further investigated by diffusion of alkali ions into carrageenan without membranes. The gelation is induced by putting the solution containing gel-inducing salt (0.832% NaCl and 0.14% KCl) directly at the top of the solution of biopolymer as explained in the Experimental section. Optical transmission was obtained as a function of time as shown in Figure 3 for the 0.8 and 1.2% samples.

The diffusion of alkali metal ions into the polymer solution gels it and causes the transmission to decrease. The optical transmission (I/I_0) versus time (t) is analyzed in terms of double exponential terms

$$\frac{I}{I_0} = A_1 e^{-(t/\tau_1)} + A_2 e^{-(t/\tau_2)} \qquad (3)$$

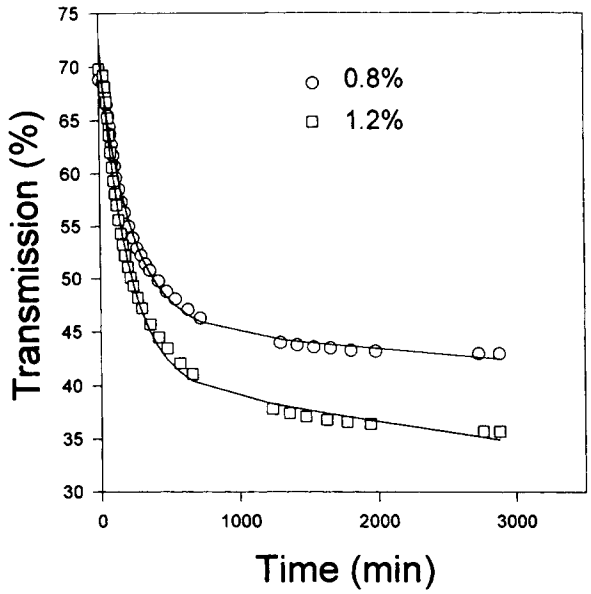

Figure 3 Transmission versus time for (○) 0.8% and (□) 1.2% sample. The samples were under the influence of the gel-inducing ionic solution at room temperature. The solid lines represent the least square fits to the data using eq. (3).

where τ_1 and τ_2 are different relaxation times. A_1 and A_2 are respective contributions. This equation has been previously used to describe the electric birefringence signal of κ-carrageenan gel.[6] The solid lines in Figure 3 are the best fits to the data using eq. (3). τ_1 is 231 and 194 min for the 0.8 and 1.2% samples, respectively. τ_2 is 4.0×10^4 min and 1.8×10^4 min for the 0.8 and 1.2 samples, respectively. It is apparent that there are two different processes associated with these two relaxation times. Let us presume a model in which the K^+ and Na^+ ions from an infinitely large ionic solution reservoir diffuse into an infinite reservoir of the carrageenan polymer solution. Initially the potassium and sodium ions and polymer species diffuse toward each other and the reaction takes place at a plane perpendicular to the direction of the diffusion. Because the polymers diffuse considerably slower than the cations, the gelling zone will move gradually away from the ion/carrageenan interface.[14] The short relaxation time τ_1 may be associated with a primary relaxation process of the movement of the gelling zone, while the longer relaxation time τ_2 with the secondary relaxation (or curing) process involves local diffusion of bound water molecules, or bound ions, or gradual rearrangement of polymer–polymer association.[15] It is known that the water molecules can be either bound to the double helix or trapped in the gel network.[15] The higher polymer concentration results in the higher ion–polymer reaction rate. Therefore, the relaxation times (τ_1 and τ_2) for the 1.2% samples are faster than those of the 0.8% samples, in agreement with experimental observation. Theoretical work is in progress.

CONCLUSION

The kinetics of gelation under the influence of gel-inducing ionic solution was studied using a turbidity technique. The alkali metal ions were allowed to diffuse through dialysis membrane to a predosed gel. The optical transmission was measured as a function of time and distance away from the membrane. It was found that lower transmission corresponded to increased gelation induced by the ions. In the early stage, transmission increased with distance, indicating that the degree of gelation decreased with the distance. After 96 h, the transmission was found to be independent of location and distance from the membrane. By then, the gelation is complete everywhere inside the gel and the gel's structure is homogeneous, in agreement with previous results. The time-dependent transmission data reveals that there may be two relaxation processes occurring during transport mediated gelation: the fast relaxation time related to the gelling zone movement and the slow one related to local diffusion of bound water molecules and ions, polymer rearrangement, or polymer–polymer association. The time dependence of transmission was used as a probe to monitor the gelation process *in situ.*

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