ready discussed by several authors.[24,25] Conversely, the formation of the weaker links is solvent-dependent. Tabb and Koenig[26] have shown in PVC plasticized by bis(2-ethylhexyl) phthalate that there is a strong interaction between C=O and Cl—C, which (according to these authors) form a complex. A similar result is reported for PVC in methyl ehter ketone.[27] We may accordingly infer that the same type of interaction takes place with diethyl malonate and that while one carbonyle interacts with one chain, the other one can interact with another chain, thus forming a bridge. This may help the ordering of the less stereoregular parts and form the weak links. Solvents possessing only one site of interaction such as bromonaphthalene or isoamyl acetate should therefore not be able to bridge two chains together. This does agree with the results detailed above.

A negative influence of the solvent is also possible. The solvation of the PVC chains by large molecules would take them apart, thus impeding the formation of the weakest links.

Additional experiments are now needed to confirm or invalidate the above explanations.

Registry No. PVC, 9002-86-2; DES, 123-25-1; diethyl malonate, 105-53-3; cyclohexanone, 108-94-1; hexanol, 111-27-3; benzyl alcohol, 100-51-6; bromonaphthalene, 27497-51-4; bis(2-ethylhexyl) phthalate, 117-81-7; diethyl oxalate, 95-92-1; dibutyl oxalate, 2050-60-4; dimethyl adipate, 627-93-0; diethyl adipate, 141-28-6; isoamyl acetate, 123-92-2; ethyl heptanoate, 106-30-9.

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Polyelectrolyte Tracer Diffusion in a Thermoreversible Gel: Structural Probe across the Gelation of Gelatin

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ABSTRACT: Structural changes in gelatin gel across its gel temperature are probed by examining the diffusion coefficient D_{tr} of a tracer polyelectrolyte with the technique of forced Rayleigh scattering. The tracer polymer was poly(2-vinylpyridine), quaternized partially with bromoethane and labeled randomly with 4-[(bromomethyl)azo]benzene. The temperature dependence of D_{tr} exhibited a significant retardation below the gel temperature, which spans over 3 orders of magnitude, and the retardation is interpreted as a consequence of the reduced effective channel size due to larger crystallite formation at lower temperatures. Further D_{tr} was found to be free of temperature hysteresis, provided a sufficient time was allowed for the gel to equilibrate at a given temperature. There appeared two components of D_{tr} below the gel temperature. The slow component becomes increasingly prominent at lower temperatures and the corresponding diffusion coefficient was found to be 5–10 times smaller than that of the fast component over the temperature range 0–30 °C. A kinetic study at 5.7 °C showed that the gelation process is of second order and it reached an equilibrium state within 10 h after quenching. The observed second order of the reaction is tentatively ascribed to the crystallites growth and annealing processes in the context of the mechanism proposed by Hauschka and Harrington.

Introduction

In this study, the forced Rayleigh scattering (FRS) technique was used to determine the tracer diffusion coefficient of a linear polyelectrolyte diffusing in a 10% solution of gelatin and the corresponding gel which is thermally induced upon cooling below the gel temperature (33 °C). The FRS technique has an unique advantage of following only the tagged molecule, a linear polyelectrolyte in this study, and this was exploited to probe the dynamic processes taking place in the matrix of gelatin solution and its thermoreversible gel by tracer diffusion coefficient D_{tr}.

Gelatin, derived from naturally occurring collagen, has been well-known for its gelation behavior.[1-3] This is attributed to the renaturation of gelatin molecules into

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collagen-like structures, through triple-stranded helix formation.[4] There exists a vast literature dealing with gel structures of the gelatin by various physical techniques.[5-7] The renaturation is strongly dependent on temperature and concentration of the gelatin.[8] While, the "pore size" of the amorphous region of gelatin gel is known to remain constant with temperature as will be described below, how the gel network affects the translational diffusion of tracer polymer chain is an important point to focus upon. This is our object in this paper. It is to make use of the tracer diffusion coefficient as a diagnostic probe to delineate the dynamic structure of the gel network. Our hope is to gain insight into network dynamics in the length scale of macromolecular dimension, 10–100 nm, with gelatin gel.

Recently, Cooper and Litster[9] studied the reorientation of laser dye, oxazine-4-perchlorate, dissolved in gelatin gels of concentrations from 2% to 10%. They deduced the interstitial pore size based on the decay time of the reorientation of dye in free solutions and gels at various temperatures over a range 20–50 °C. They conclude that the longer decay times in gelatin solutions and gels, compared to that in pure water, are caused by polymers disturbing the Stokes velocity field around the interstitial pore size, which does not depend on temperature over the range studied but does decrease with increasing gelatin concentration. The absence of temperature dependence of the effective Stokes radius of an azo dye, methyl red, in a given concentration of gelatin was similarly established in this laboratory recently;[10] here the viscosity-corrected tracer diffusion coefficient at a given temperature is used to deduce the effective Stokes radius. In a somewhat similar vein, Amis et al.[11] with the mutual diffusion coefficient measurements have established that the size of elastic strands between adjacent junction points in gels remains constant within experimental error over a temperature range of 5–20 °C. On the other hand, Harrington and von Hippel showed some time ago that the degrees of crystallization at low temperatures are significantly higher than those obtained at high temperatures.[12] There are two possible ways to explain this. The first one is that crystallites of roughly similar size increase in number and the second is that they become larger in size. The first is inconsistent with the findings that the pore size or size of the elastic strands in the amorphous region is independent of temperature. This is because the increase in the number of crystallites necessarily makes the pore size or strand size smaller in the amorphous region of the gel. The second is more plausible at the moment, and this is in keeping with the general acceptance that the "gel structure" in aqueous gelatin gel increases with decreasing temperature.[13]

If so, the diffusion coefficient of a tracer chain D_{tr} should become an efficient diagnostic probe for the intercrystallite channels in the gel in the length scale of chain dimension, e.g., 10–100 nm. Here, the intercrystallite channels are considered to be delineated by crystallites and filled by amorphous domain polymer strands. Thus, we may think of two length scales, one in the amorphous domain designated as "pore" or "mesh" as in the hydrodynamic screening length of any semidilute solutions, which remains invariant with temperature at a given gel concentration, and the other designated as "channels", which becomes narrower with decreasing temperature. Thus our study focuses on the retardation effect of the channel size with respect to gel temperature at a constant gelatin concentration, e.g., 10%. Such a study then should be analyzed in the context of the diffusion through coarse meshes[14] where $D_{tr} \propto \exp(-R/\xi)$, with R being the size of the diffusant and ξ being the channel size, which can be smaller or larger than R by an order of magnitude.

There are two prerequisites for D_{tr} to be an effective probe for ξ. The first one is that the gelatin chains should not diffuse at all. According to Chang and Yu,[15] the self-diffusion of gelatin chains is quenched in the gel, presumably since the majority of gelatin chains are incorporated into the gel network via the crystallites. Since they did not extend the scanning time beyond 10 s, it was necessary to confirm the absence of self-diffusion for much longer periods, comparable to those required for the tracer diffusion process. This we have done for periods extending up to 28 h to confirm that the self-diffusion remains quenched for the duration of the tracer diffusion. The other prerequisite is that the gel should be at an equilibrium state, one at which time dependence for a process of interest is assured to be leveled out. In order to determine how long it takes to reach the equilibrium, we performed a kinetic study with use of D_{tr} upon quenching a gelatin solution from some point above the gel point to another point below the gel point and followed the time evolution of D_{tr}. Additionally, we could deduce the order of gelation reaction. Hauschka and Harrington[16] proposed that the renaturation process consists of three processes: nucleation, growth of crystal, and annealing. Each of these processes is of first order and their sum is equal to the overall order of the reaction. The overall order of 3 was confirmed by several other investigators.[17,18]

Experimental Section

Materials. Bone gelatin (Type IV) was supplied by Eastman Kodak Co. and it was used without further purification. The tracer polyelectrolytes, poly(2-vinylpyridine)s partially quaternized with bromoethane (P2VP-EtBr), were made by heating poly(2-vinylpyridine) (P2VP) solutions in dry *N,N*-dimethylformamide (DMF) at 65 °C with excess bromoethane after dye labeling. The vapor-phase anionic polymerization tecnnique was used to make two P2VP samples (I, M_w = 33000, M_w/M_n = 1.13; II, M_w = 96000, M_w/M_n = 1.16), which were synthesized by Dr. Markus Antonietti of University of Mainz. Dye labeling was effected in DMF solution with an excess amount of 4-[(bromomethyl)azo]benzene and the dye-labeling ratios were determined to be one in every 300–400 monomer units. Conductometric titration with silver nitrate gave the degree of quaternization of 38% and 32% for samples I and II, respectively. The matrix samples for FRS measurements were 10 wt % aqueous gelatin solutions, without correcting for the generally accepted moisture content of 12% in solid gelatin.[5] The dye-labeled P2VP-EtBr consisted of 1% of whole sample. The hot solutions at 65 °C were filtered into 5-mm path length standard spectrophotometer cells in a warm oven and sealed under vacuum.

The self-diffusion of gelatin chains in gel state was probed by FRS at 30 °C with a fluorescein-labeled gelatin sample. The labeling was carried out according to the procedure described by Chang and Yu.[15] The gel was aged for about 30 h at room temperature before continuous FRS measurements were effected for 28 h.

Methods. For the kinetic study of gelation, the gelatin solution at 65 °C was quenched to 5.7 °C and the diffusion coefficients were measured continuously at a fixed crossing angle of 131.6 mrad, i.e., grating spacing d = 3.71 μm. Following this observation, all measurements in the gel state were performed after the resulting gels were aged for 24 h or longer at each measurement temperature.

FRS instrument, the data acquisition, and the analysis scheme are described elsewhere.[14,19] When needed, the photomultiplier output $V(t)$ was analyzed by a double-exponential model function with the fast and slow time constants represented by τ_1 and τ_2, respectively

$$V(t) = |Ae^{-t/\tau_1} + Be^{-t/\tau_2}|^2 + C^2 \quad (1)$$

Having determined the FRS decay profiles at four different crossing angles, amounting to the fringe spacing range of 5–12

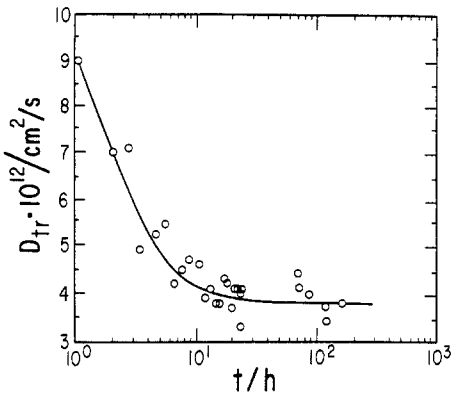

Figure 1. Plot of D_{tr} versus log t for the time-dependence measurement at $T = 5.7$ °C with crossing angle of 131.6 mrad, amounting to the fringe spacing of 3.71 μm, for P2VP-EtBr derived from sample II of P2VP with molecular weight $M_w = 96\,000$g/mol, as the tracer. The time asymptotic value of tracer diffusion coefficient of $(4.1 \pm 0.5) \times 10^{-12}$ cm^2/s was obtained.

μm, we obtained the tracer diffusion coefficient in each case from the slope of a least-squares fit to the linear relation[20] between $1/\tau$ and q^2

$$1/\tau = 1/\tau_{life} + D_{tr}q^2 \qquad (2)$$

where τ is the FRS signal decay time constant, τ_{life} the lifetime of the photochromically shifted state of the photolabel, and q the scattering wave vector that is inversely proportional to the transient grating spacing d, i.e., $q = 2\pi/d$. The reported errors in D_{tr} stand for a 95% confidence interval of the slope determination of $1/\tau$ versus q^2 with the Student's t distribution. With the azo dye used here as the photolabel, we could determine τ_{life} as about 2000 s; such a determination was possible only when D_{tr} was small enough that both terms in the right-hand side of eq 2 were comparable, and such was the case at 0 °C. Otherwise, τ_{life} is indeterminate since the second term becomes so predominant that the intercept of a straight line according to eq 2, $1/\tau$ versus q^2 plot, is not accessible.

Results and Discussion

The quenched self-diffusion of gelatin chains was ascertained as stated in the Introduction. The intensity of diffracted signal output from the PMT upon writing a fringe at a crossing angle of 104.04 mrad, i.e., $d = 4.69$ μm, at 30 °C remained constant up to 28 h without any detectable decay. On this basis, we can claim that there exists only a negligible amount of sol fraction in our gel, if any, although this is contrary to the findings by Russo et al.[21] with another gelatin source. We take this as evidence that the diffusion coefficients reported here are purely from the tracer P2VP-EtBr, not influenced by any diffusive contributions from gelatin itself. It is possible, however, that our labeling yield is so low that a sol fraction, even if it were present, could not have contributed to the FRS signal if it consisted primarily of short chains; since our labeling frequency is one dye per 300–400 repeating units and if the sol fraction consists of short chains with less than say 100–200 repeating units, we could not have detected any FRS signal decay attributed to such a short-chain sol fraction.

Next, we show first the results of a kinetic study at 5.7 °C. In Figure 1 is displayed a semilogarithmic plot of D_{tr} versus t, obtained at a constant fringe spacing of 3.71 μm. The solid curve is drawn merely to indicate the trend, and it does not stand for any attempt to fit the data. As seen clearly from the figure, the tracer diffusion gets retarded rapidly at the beginning and slowly reaches a time asymptotic value of $(4.4 \pm 0.5) \times 10^{-12}$ cm^2/s after about 10 h. This finding is used subsequently to age all gel samples for 24 h or more before any FRS measurements were undertaken. Parenthetically, we should note that a larger error in D_{tr} in this case, compared those that will be reported below, comes about by virtue of the single crossing angle measurements since the focus here is on the time evolution of D_{tr}, so that angle scan was not effected.

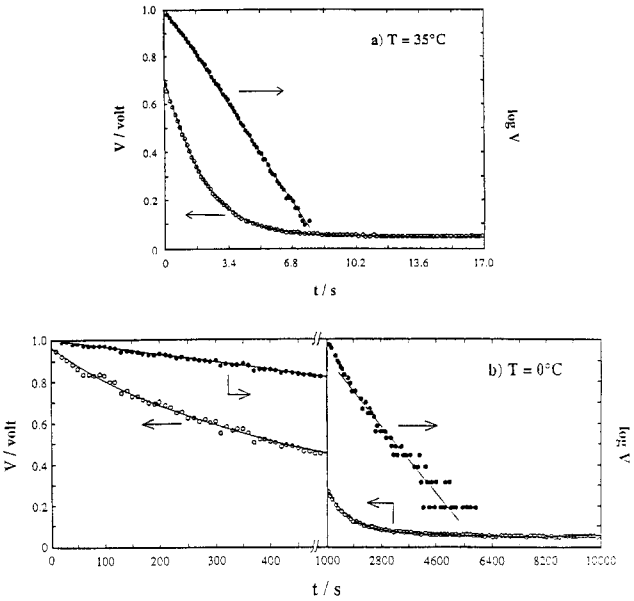

Figure 2. Signal decay profiles (open circles) and their semilogarithmic plots (filled circles) at two different temperatures: (a) at 35 °C which is above the gel temperature and (b) at 0 °C which is below the gel temperature. At 35 °C, log V follows a single straight line against t, showing only one component in the diffusion process. But, at 0 °C, two components appear; the fast one in a short time scale and the slow one in a long time scale are juxtaposed in two different time scales.

Before proceeding with the results for the temperature dependence, it would be appropriate to show what the signal profile looks like. In Figure 2, two typical signal decay profiles are shown: (a) The FRS decay profile shown in the upper portion is obtained at $T = 35$ °C (above the gel temperature) and (b) that in the lower portion at $T = 0$ °C (below the gel temperature). At 35 °C (upper), the semilogarithmic plot of log V versus t (filled circles) gives a single straight line over the whole time scale, representing only a single component in the diffusion process. In contrast, the two components are apparent at 0 °C (lower). For clear comparison, the fast and slow ones are juxtaposed with differing time scales together with the semilog plots with filled circles; the onset of the slow process is seen to take place over the time interval of 1000–2000 s. At all temperatures below the gel temperature, the slower component appeared along with the fast component. The second component was also seen to give a good deal of scatters in the data set, resulting in larger errors in the diffusion coefficient determinations, and these are made obvious by the error bars in Figure 3. Figure 2 is merely to illustrate the appearance of two components and not to show how we have analyzed the data. The analysis method has been detailed in the Experimental Section.

Next we report the results for the temperature dependence. We show a plot of D_{tr} versus $1/T$ in Figure 3. P2VP-EtBr used for this study was sample I, that with parent molecular weight of 33 000 g/mol, and the temperature range was from 0 to 50 °C. The gel temperature is indicated by an arrow. There are three specific points of observation to be made from the results:

1. Above the gel temperature (33 °C), the change is rather small, mainly attributable to the temperature dependence of the solution viscosity.

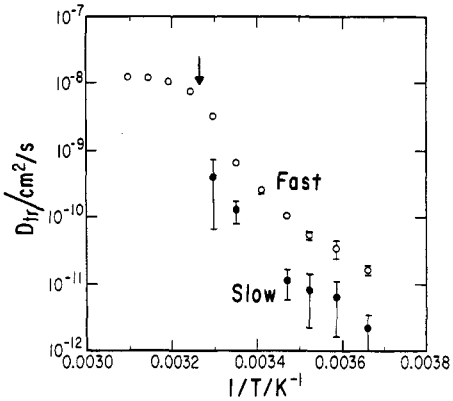

Figure 3. D_{tr} versus $1/T$. The tracer was P2VP-EtBr from sample I of P2VP with molecular weight of 33 000 g/mol. Above the gel temperature at 33 °C, indicated by an arrow, D_{tr} changes only slightly, reflecting the temperature dependence of gelatin solution viscosity. Below the gel temperature, it decreases precipitously over some 3 orders of magnitude, accompanied by the slow component which is found to be 5–10 times slower than the fast one.

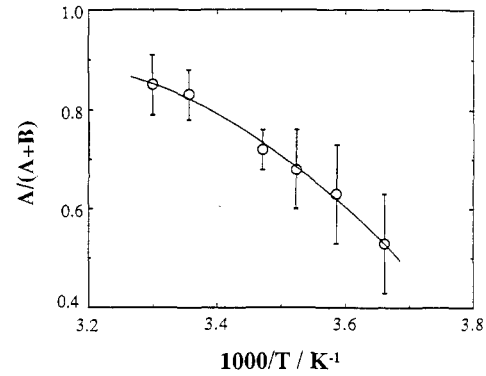

Figure 4. Plot of $A/(A + B)$ versus $1/T$, where A and B are the intensity factors of the FRS signal in the gel state for the fast and slow components, respectively, and the latter becomes increasingly prominent as the temperature is lowered.

2. Below the gel temperature, D_{tr} falls precipitously with temperature and spans over 3 orders of magnitude. Further, there emerges a second, slower component in the diffusion process, which becomes increasingly prominent as the temperature is lowered (see below).

3. Although it is not shown specifically in the figure, we have confirmed that the D_{tr} values contain no temperature hysteresis effect; as long as we allowed the aging of the gel to extend 24 h or more at a given measurement temperature, the temperature ascending side or descending side did not provide different values of D_{tr}. Under such a condition, we claim to have established the thermoreversibility of our gel samples.

We now turn to the discussion of these points. Above the gel point, the tracer diffusion in a 10% gelatin solution is no more than the usual polymer solution matrix effect.[22] In the case of bone gelatin as the matrix, which is known to have various kinds of amino acid residues[5] and a cationic polyelectrolyte (P2VP-EtBr) as the diffusant, one should expect many specific (ionic, hydrogen bonding, and hydrophobic) interactions between the diffusant and matrix as contrasted to the case of polystyrene as both the diffusant and matrix.[22] Despite such suspected interactions, the fact that the temperature dependence of D_{tr} follows more or less the solution viscosity indicates relatively benign effects posed by the gelatin solution toward P2VP-EtBr.

As for the temperature-mediated retardation of D_{tr} in the gel state, we return to the postulate alluded to in the Introduction. As the temperature is lowered, the amorphous domain decreases and intercrystallite channel narrows, whereby the effective channel size ξ decreases. Although we have no independent quantitative measure of ξ, D_{tr} reflecting its temperature dependence appears plausible. Our suggestion is that the observed retardation of the diffusion coefficient with temperature is a consequence of the reduced effective channel size due to larger crystallite formation at lower temperatures. In the context of the diffusion through coarse meshes, the results displayed in Figure 3 may be taken as the inverse temperature profile of ξ, if we accept $D_{tr}(T) \propto \exp[-R/\xi(T)]$.

Turning to the second, slow component of D_{tr} in the gel state, the values are shown to be 5–10 times smaller than those of the fast component within the temperature range studied, except at 20 °C where the slow component could not be separated. Given the difference, the separation when feasible is readily effected, though the error range of the slow one is bound to be larger. Why there should be only two separable diffusion processes is difficult to answer at this point. From the decay profiles of FRS signals, we could not conclude specific departure from exponentiality in either the fast or the slow component. On the other hand, it is not surprising to observe different diffusion pathways since the interactions among the tracer molecules and gelatin chains are to be expected; the interactions should include Coulombic interactions among the charged moieties and secondary interactions such as hydrogen bonding and hydrophobic interaction. The hydrogen bonding should be efficiently operative between nitrogen atoms on free (unquaternized) pyridine units of the diffusant and hydrogens on hydroxyproline residues of gelatin, and similarly we should expect the hydrophobic interactions between pyridine rings of P2VP-EtBr and hydrophobic portions on gelatin. Without speculating further on the specifics of the interactions, we note that the separation is effected with increasing ease as the temperature is lowered. The relative contribution of the two components was plotted in Figure 4 in the form of $A/(A + B)$ versus $1/T$. The A and B are preexponential amplitude factors in eq 1. The slow component becomes increasingly prominent as the temperature is decreased. The temperature dependences of the two are remarkably parallel each other. Hence we are tempted to ascribe the slow component to a diffusion process mediated by the crystallite–diffusant interactions. Why there exists no exchange mechanism between fast diffusing and slow diffusing species is a puzzle, and we are not able to decipher it at this point.

We now return to the results of the kinetic study displayed in Figure 1. P2VP-EtBr with higher parent molecular weight (M_w = 96 000) was used for this measurement, with an expectation that the longer chain should be more sensitive to structural changes in gelatin gels during gelation. In several reports,[23,24] the equilibration time varied from a few hours to pratically infinity. Taking account of concentration and temperature effects, it is reasonable to assume that the relatively high concentration (10%) and low temperature (5.7 °C) chosen in this study simultaneously should have accelerated the gelation process, resulting in a short equilibration time. It is entirely possible that the tracer diffusion coefficient is not sensitive enough to detect large-scale structural changes in gelatin gels unlike some mechanical measurements. After all there is a vast literature dealing with slow growth of "gel structure" as detected by various macroscopic probes.[6,7]

Finally we come to the last point of the kinetic study. If we accept that the intercrystallite channel size ξ is detected by D_{tr} and its size is somehow inversely related to

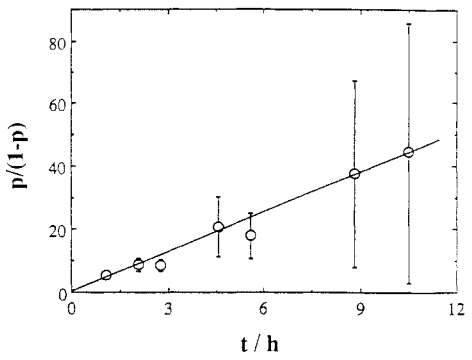

Figure 5. Plot of $p/(1-p)$ versus t, where p is the extent of reaction in terms of the Hauschka–Harrington mechanism for the gelatin gelation and it was calculated from the data in Figure 1 as fully explained in the text. The linear behavior is well borne out, confirming a second-order kinetics of the reaction according to eq 3.

the crystallite size X, the time dependence of D_{tr} should track the gelation process consisting mainly of crystallite growth and annealing. In terms of the Hauschka–Harrington mechanism,[16] this should be a second-order reaction. Implicit in this hypothesis is that the nucleation step is too fast for D_{tr} to probe, hence only the growth and annealing steps are being followed by D_{tr}. In pursuing this point, we start with a general kinetic expression for a single-component nth-order reaction ($n \neq 1$)[25]

$$1/(1-p)^{n-1} = 1 + (n-1)Kt \tag{3}$$

where p is the extent of overall reaction, $K = kc_0^{n-1}$ where c_0 is the initial concentration of reactants, which are the nucleated crystallites in this model, such that $(1-p) = c/c_0$ ($0 \leq p \leq 1$), and t and k are time and the rate constant, respectively. Hence, under an isothermal condition, we can set

$$X \propto 1/\xi \propto -\ln D_{tr} \tag{4}$$

, such that

$$p = [-\ln D_{tr} + \ln D_{tr,i}]/[-\ln D_{tr,f} + \ln D_{tr,i}] \tag{5}$$

since

$$D_{tr}(t,T) \propto \exp[-R/\xi(t,T)] \tag{6}$$

where $D_{tr,i} = D_{tr}(0, T)$ at the time of quenching that can in turn be inferred by extrapolating those values obtained above the gel temperature to 5.7 °C; the value so determined was 7.5×10^{-10} cm^2/s. The final value of the tracer diffusion coefficient $D_{tr,f} = D_{tr}(\infty,T)$ is the time asymptote referred to earlier, i.e., 4.1×10^{-12} cm^2/s, which is just the average value of D_{tr} at times later than 10 h. Because of an indication from the initial behavior of the time dependence, $p \approx Kt$ when $t \geq 0$, it appeared sensible to try this method with a second-order reaction, $n = 2$ in eq 3. Hence, in Figure 5, a plot of $p/(1-p)$ versus t is shown. The linear behavior is well borne out, confirming the second order of the reaction according to eq 3. Plausibility of our model is justified as follows. Since there should be a rapid freeze-in of marginally stable collagen-fold structures which are not to be identified with the true nucleation process[16] at 5.7 °C, we can ignore the nucleation step as a significant component to the overall reaction. Thus the second order arises from the growth and annealing processes in terms of the mechanism of Hauschka and Harrington.

In summary, we conclude that the tracer diffusion should be a sensitive structural probe to examine a complex matrix such as gelatin gel. Sensitive ranges of length and time scale we probe by the diffusion process are respectively 1–10 μm and 0.1–1000 s. Relative to other diffusion studies by label techniques such as (1) quasi-elastic light scattering in an isorefractive index medium of matrix, (2) FRS, and (3) fluorescence recovery after photobleaching, our perspective here differs from those of Ware's group,[26] Langley's,[27] Johnson's,[28] and Lodge's.[29] While ours is to use the tracer to study the matrix, theirs are to delineate the tracer diffusion in a complex but better defined matrix. We have also examine the tracer diffusion with the same perspective before,[14,15,22] but the study here departs from the earlier ones in its focus. In pursuing the point of perspective, the diffusant size could be varied to diagnose the average size and distribution of structural inhomogeneities at different length scales at a given temperature. Such a chain length dependence study will consitute the subject of our forthcoming report.

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