Structural Changes in Polymer Gels Probed by Fluorescence Correlation Spectroscopy

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Introduction

Previous studies, e.g., by NMR,[1,2] fluorescence photobleaching,[3] and dynamic light scattering (DLS),[4,5] have shown that the diffusion of noninteracting probe particles in polymer solutions and gels primarily depends on the polymer concentration and the size of the probe. In interpreting these results, it is widely assumed that polymer gels behave like semidilute polymer solutions, while the structural differences due to the presence of permanent cross-links are ignored. These differences have been revealed and characterized by scattering experiments, such as small-angle neutron scattering (SANS) and light scattering, which show significant structural rearrangement of the polymer chains upon gelation.[6] This observation is corroborated by results from elasticity measurements, indicating that gelation is accompanied by an increase in the elastic modulus of the samples.[7] However, the effect of cross-linking on the diffusion of small particles in a gel has yet to be fully elucidated and understood.[4,7]

In this paper, we demonstrate how fluorescence correlation spectroscopy (FCS) can provide quantitative measurements on the diffusion of particles in polymer systems. We apply FCS to measure the diffusion time of fluorescent TAMRA molecules in poly(vinyl alcohol) (PVA) solutions and gels prepared at various polymer concentrations and cross-link densities. The measurements indicate that the diffusion of probe particles is affected not only by the polymer concentration but also by the cross-link density of the gel. Remarkably, we find a simple linear relation between the diffusion times and the elastic moduli of the same gels.

Experimental Section

Sample Preparation. PVA (Sigma Aldrich, M_w = 85 000 Da, degree of hydrolysis > 99%) was first dissolved in deionized water at 95 °C. Solutions having PVA concentration ranging from 1% to 8.6% (w/v) were then prepared at room temperature. Cross-linking was performed with glutaraldehyde at pH = 2, forming gels with molar ratios of 1/400, 1/200, 1/100, and 1/50 cross-links per monomer units (cross-link density, defined by stoichiometric ratios). Under these experimental conditions, no gelation was observed below 3% PVA concentration. The fluorescent probe was carboxytetramethylrhodamine (TAMRA, Molecular Probes, M_w = 430 Da), which was mixed at nanomolar concentration with the PVA solutions prior to cross-linking. The PVA gels were formed in FCS sample chambers (60 μL). For the elastic modulus measurements, gel cylinders (1 cm height, 1 cm diameter) were made in a special mold. Uniaxial compression measurements were collected using a TA.XT2I HR texture analyzer (Stable Micro Systems, U.K.), and the data were analyzed according to the method described in ref 8. All the experiments were performed at 22 °C.

FCS. Our custom-built FCS setup has been described elsewhere.[9] FCS measures the intensity correlation function[10,11]

$$\boldsymbol{F}(\tau) = 1 + \frac{\langle \delta I(t)\delta I(t+\tau)\rangle}{\langle I(t)\rangle^2} \qquad (1)$$

where $\delta I(t) = I(t) - \langle I(t)\rangle$ denotes the deviation of the intensity $I(t)$ emitted by the fluorescent particles at time t from the average intensity, $\langle I(t)\rangle$. For monodisperse particles diffusing freely in a solution, eq 1 can be written as[9,11]

$$F(\tau) = 1 + \frac{1}{N}\frac{1}{\left(1+\dfrac{\tau}{\tau_d}\right)}\frac{1}{\left(1+p\dfrac{\tau}{\tau_d}\right)^{1/2}} \qquad (2)$$

Here $p = (r_0/z_0)^2$ is a constant, where r_0 and z_0 characterize the Gaussian profile ($W(r,z) = Ae^{-2(r/r_0)^2}e^{-2(z/z_0)^2}$) of the excitation beam. In eq 2 N denotes the average number of particles in the excitation volume, and $\tau_d = r_0^2/4D$ is the diffusion time, where D is the translational diffusion coefficient. On the basis of the known value of the diffusion coefficient of rhodamine R6G,[12] we estimate z_0 to be about 0.2 μm.

Equation 2 can be readily applied to fit data obtained in solutions. However, for analysis of correlation functions measured from gels, one needs to derive an equation equivalent to eq 2 to account for the inhomogeneous structure of gels, a daunting task since this structure generally is difficult to describe by a simple expression. Recently, Fatina-Rouge et al.[12] validated FCS for use in gels and provided an expression for the correlation function of particle in a fractal gel. Our PVA gels are not fractal, as indicated in ref 6. For the present purposes, we use eq 2 to fit the data and extract the apparent diffusion times, τ_d, allowing us to compare the motion of particles in solutions to that in gels. We emphasize that we apply eq 2 merely to quantify the changes in the behavior of the particles when sample conditions are changed.

Results and Discussion

Figure 1 shows normalized correlation functions, $(F(\tau) - 1)/(F(0) - 1)$, of TAMRA molecules in water, 6% PVA solution, and 6% PVA gels (cross-link density = 1/200, 1/100, and 1/50) as a function of delay time, τ. Each correlation function was collected over a 45 min period. Note the systematic shift of the curves with increasing cross-link density. The solid lines are the fits of the expression in eq 2 to the data, indicating that each of the solutions and gels can be satisfactorily described by a single characteristic time, τ_d.

Figure 2 shows τ_d (scaled by the diffusion time of TAMRA molecules in water, τ_d(water) = 35 μs) as a function of the PVA concentration for all systems. For both solutions and gels, the increase in τ_d appears to be linear with the concentration. Below the threshold PVA concentration (approximately 3% w/v), the diffusion times of the probe particles remain unchanged even in the presence of the cross-linker, indicating the absence of observable interactions between the cross-linker and the fluorescent probe. Above the threshold, however, a change in the slope is visible, reflecting the transition from the solution to the gel state. The diffusion times in gels exceed those in the corresponding solutions and increase with cross-link density.

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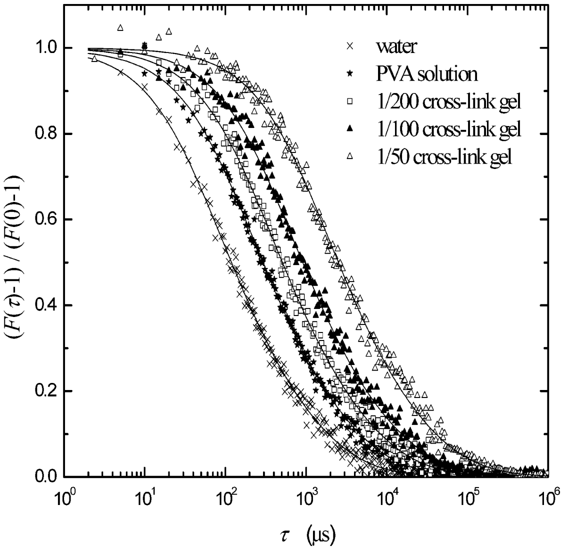

Figure 1. Normalized correlation functions for fluorescent TAMRA molecules in water, 6% (w/v) PVA solution, and 6% (w/v) gels at several cross-link densities (see labels on plot) as a function of delay time, τ. The solid lines are fit to the data with eq 2.

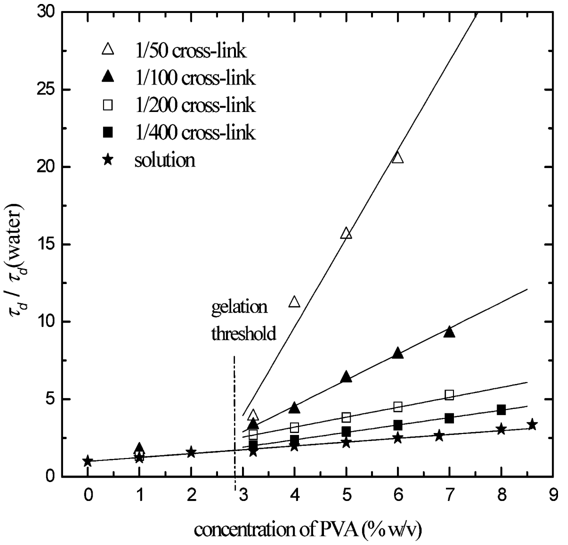

Figure 2. Scaled characteristic diffusion times of fluorescent TAMRA molecules in PVA solutions and gels at several cross-link densities (as labeled on plot) as a function of polymer concentration with linear fits. The times are scaled by the diffusion time of the probe in water, τ_d(water). The vertical dashed line indicates the approximate gelation threshold.

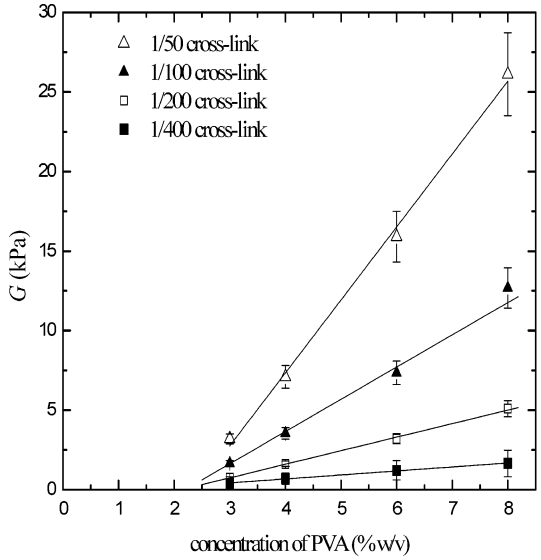

Figure 3. Elastic modulus, G, is shown as a function of polymer concentration for gels at several cross-link densities (as labeled on plot) with linear fits.

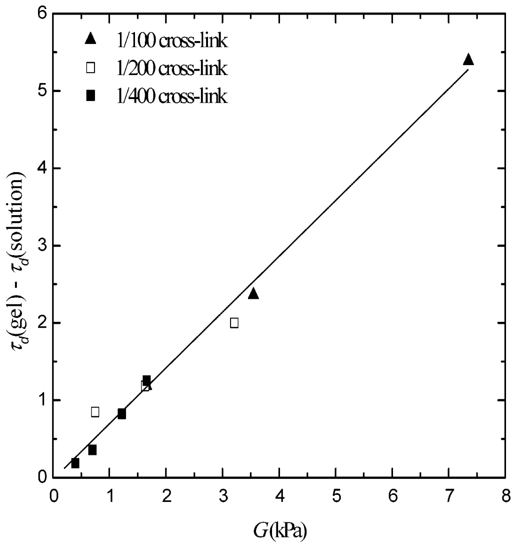

Figure 4. Differences between characteristic probe diffusion times [τ(gel) − τ(solution)] in PVA gels plotted versus the elastic modulus, G, of the gels. These times are scaled by τ_d(water). The line is a guide to the eye.

In polymer solutions simple scaling theory predicts that the characteristic length scale (correlation length, ξ) decreases with increasing polymer concentration $\xi \approx c^{-m}$, where $m = 0.75$ (good solvent condition) or 1 (theta condition).[13] When a semidilute polymer solution is cross-linked, the resulting gel contains structural regions of differing sizes and the simple scaling relation is no longer valid. SANS measurements performed on a variety of weakly cross-linked gels indicate that cross-linking produces changes at larger length scales ($\gg \xi \approx$ 5 nm in similar PVA samples), whereas at shorter length-scales ($\leq \xi$) the structure is only slightly modified.[6] In the present FCS measurements the TAMRA probe (~1 nm) is expected to explore structures ranging from nanometers to micrometers in extent. Therefore, at a fixed polymer concentration, the changes in the characteristic time, τ_d, in Figure 2 should reflect changes in the large-scale structure of the system due to cross-linking.

Since gels exhibit finite elasticity and the elastic modulus depends on both the polymer concentration and the cross-link density, it is natural to compare the characteristic diffusion time of the probe particles with the elastic modulus, G, of the gel. Figure 3 shows G as a function of the PVA concentration at different cross-link densities. Similar to the behavior of τ_d, G increases linearly with concentration, suggesting the existence of a simple relation between these two quantities. Thus, in Figure 4 we plot [τ_d(gel) − τ_d(soln)] versus G for several PVA gels. The data appear to fall on the same straight line, independent of the cross-link density and the polymer concentration, indicating that diffusion of the probe particles is strongly correlated with the gel elasticity.

In summary, we applied FCS to measure the diffusion of small fluorescent probe particles (TAMRA) in non-fluorescent—hence invisible—PVA solutions and cross-linked PVA gels. The present measurements indicate that for the same polymer concentration diffusion of the particles slows down when the polymer solution is cross-linked. Further, the more the polymer chains are cross-linked, the slower the probe particles diffuse. We attribute this effect to the formation of large-scale structural changes caused by cross-linking of the PVA chains. These results suggest that cross-link density is an important parameter when assessing and analyzing probe diffusion data in gels. Measurements of the elastic modulus support this conclusion, as indicated by the linear correlation between the diffusion times of the particles and the elastic modulus of the weakly cross-linked gels.

References and Notes

(1) Petit, J.-M.; Zhu, X. X.; Macdonald, P. M. *Macromolecules* **1996**, *29*, 70.
(2) Tokita, M.; Miyoshi, T.; Takegoshi, K.; Hikichi, K. *Phys. Rev. E* **1996**, *53*, 1823.
(3) Furukawa, R.; Arauz-Lara, J. L.; Ware, B. R. *Macromolecules* **1991**, *24*, 599.
(4) Narita, T.; Knaebel, A.; Munch, J.-P.; Candau, S. J. *Macromolecules* **2001**, *34*, 8224.
(5) Reina, J. C.; Bansil, R.; Konak, C. *Polymer* **1990**, *31*, 1038.
(6) Horkay, F.; Hecht, A. M.; Geissler, E. *Macromolecules* **1994**, *27*, 1795.
(7) Nisato, G.; Hebraud, P.; Munch, J.-P.; Candau, S. J. *Phys. Rev. E* **2000**, *61*, 2879.
(8) Horkay, F.; Tasaki, I.; Basser, P. J. *Biomacromolecules* **2000**, *1*, 84.
(9) Boukari, H.; Nossal, R.; Sackett, D. L. *Biochemistry* **2003**, *42*, 1292.
(10) Webb, W. W. *Appl. Opt.* **2001**, *40*, 3969.
(11) Aragon, S. R.; Pecora, R. *J. Chem. Phys.* **1976**, *64*, 1791.
(12) Fatin-Rouge, N.; Starchev, K.; Buffle, J. *Biophys. J.* **2004**, *86*, 2710.
(13) De Gennes, P. G. *Scaling Concept in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.

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