Fluorescence Correlation Spectroscopy Study of Probe Diffusion in Poly(vinyl alcohol) Solutions and Gels

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Summary: In this study, we demonstrate how the diffusion of probe particles in aqueous poly(vinyl alcohol) (PVA) solutions and gels is affected by: (i) the presence of cross-links, (ii) the cross-link density, (iii) the polymer concentration. We apply fluorescence correlation spectroscopy (FCS) to measure the diffusion time of a rhodamine-based fluorescent particle (TAMRA) and TAMRA-labeled dextran in PVA solutions and gels prepared at various polymer concentrations (1% to 8.6% w/v) and cross-link densities (1/400 to 1/50 cross-link monomers per PVA monomers). The measurements indicate that the probe particles are slowed down with increasing polymer concentration and with increasing cross-link density. Also, FCS can detect differences in the diffusion times measured in "fresh" and "aged" PVA solutions. We find that FCS provides a quantitative measure of network inhomogeneities.

Keywords: cross-linking; diffusion; fluorescence correlation spectroscopy; inhomogeneities; poly(vinyl alcohol) gel

Introduction

How drugs get encapsulated and released from hydrogels and how nutrients move within a tissue scaffold are examples of the general problem of particle transport in seemingly random, inhomogeneous media or networks. Elucidating the factors affecting this transport is of interest to both fundamental research and applied engineering. Poly(vinyl alcohol) (PVA)-based gels are commonly studied as model gel systems for drug delivery and tissue engineering matrices[1,2,3] because of their high water content and structural and mechanical similarity to biological tissues. Their structures have been characterized extensively by scattering techniques (light and neutron scattering)[4] and the diffusion of particles through their interiors has been investigated by NMR[5,6] and dynamic light

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scattering (DLS)[7,8]. However, a consistent and clear picture on the diffusion processes of particles in these gels has yet to emerge.

In this Paper, we describe results from fluorescence correlation spectroscopy (FCS) measurements on the diffusion of two kinds of fluorescent particles in "fresh" and "aged" PVA solutions and in chemically cross-linked PVA-gels prepared at various polymer concentrations and cross-link densities. FCS is a non-invasive optical technique that can be used to monitor a small amount (~ nanomoles) of fluorescent particles diffusing through a small detected volume (~ femtoliter) of the PVA solution or gel. By measuring changes in the temporal behavior of the fluorescence intensity of the probe particles, it is, in principle, possible to monitor changes in the structure of the samples or the interactions of the probes with the surrounding medium.

Sample Preparation

PVA (Sigma Aldrich, M_w = 85,000 Da) was first dissolved in de-ionized water at 95 °C and kept at this temperature for several hours. Solutions with this "fresh" PVA were then prepared at room temperature with concentrations ranging from 1% to 8.6% (w/v). The "aged" sample (H-PVA) was a batch of PVA solution that had been left at room temperature for approximately 9 months, and, hence, hydrogen bonds were expected to form between chains. At fixed polymer concentration, 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). Under these experimental conditions, no macroscopic gelation was observed below 3% PVA concentration. Each fluorescent probe – carboxytetramethylrhodamine (TAMRA, M_w = 430 Da, Molecular Probes, Oregon, USA) and rhodamine-labeled dextran (M_w = 10^4 Da, Molecular Probes, Oregon, USA) – was mixed at nanomolar concentration with the PVA solutions and gels. The cross-linked gels were formed in FCS sample chambers (60 μL) and all the experiments were performed at 24 °C.

FCS Aparatus

The experimental setup has been described previously[9]. We used a 543 nm laser to illuminate the sample through a 60X objective (NA=1.2 water) in an Olympus IX70 inverted microscope (Olympus, Japan). The 25-μW incident beam was expanded and

focused onto a small spot of radius r_0 (< 1 micron). The emitted fluorescent light was collected by the same objective and focused onto an optical fiber with a core diameter of 10 or 25 microns. This small diameter ensured the confocal detection necessary for delimiting small volumes of interest. For signal detection, two avalanche photodiodes (PerkinElmer, EG&G, Vandreuil, Canada) were used in cross-correlation mode to reduce the effects of spurious detector afterpulsing on the correlation function, which is important at short time scales (< 10 μs.). The pulses of the photodiodes were processed by a digital correlator (Brookhaven Instrument, Holtsville, NY, USA), yielding the time-correlation functions.

FCS Theory

Fluorescence correlation spectroscopy utilizes the fluctuations in emission from fluorescent particles moving through a system. These fluctuations are typically caused by changes in the number of fluorescent particles in a small illuminated volume or changes in the emission quantum yield of the particles. The illuminated volume is made small (~ femtoliters) by confocal setup or by two-photon emission.[10] The detected intensity, *I(t)*, of the fluorescent particles in the sample volume at time, *t*, is time-correlated to generate a correlation function defined as:

$$F(\tau) = 1 + \frac{< \delta I(t) \delta I(t+\tau) >}{< I(t) >^2} \qquad (1)$$

where $\delta I(t) = I(t) - < I(t) >$ denotes the deviation of the measured intensity from the average intensity, $<I(t)>$. Analysis of this correlation function can reveal information about the mechanisms responsible for the intensity fluctuations, such as diffusion, particle interaction, and electronic transitions within the molecules.

For the ideal case of freely diffusing monodisperse and uniformly bright fluorescent particles, the correlation function can be written as:[11]

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

when the excitation beam is a 3-D Gaussian beam, with an intensity profile given by:

$W(r,z) = Ae^{-2\left(r/r_0\right)^2} e^{-2\left(z/z_0\right)^2}$ where r_0 and z_0 characterize the width of the focused beam and the length along the optical axis defined by the size of the pinhole, respectively. We define a characteristic time for a particle to diffuse along the lateral width (r_0) of the focused incident beam as $\tau_d = r_0^2/4D$, where D is the translational diffusion coefficient of the particle. In Equation 2, N denotes the average number of particles in the excitation volume and p is an instrumental constant equal to $\left(r_0/z_0\right)^2$.

While the expression in Equation 2 can be readily applied to fit FCS data measured on dilute solutions, its applicability to gels is still unclear. In general, gels are non-uniform systems in which the fixed network prevents the probe particles from exploring the entire excitation volume, whereas unhindered movement is assumed in the derivation of Equation 2. For simplicity, though, we use Equation 2 to fit the data and extract apparent diffusion times, τ_d, that would quantify possible changes of the probe diffusion in solutions and in gels.

Results and Discussion

Figure 1 shows normalized correlation functions, $(F(\tau)-1)/(F(0)-1)$, of TAMRA molecules in water, in 6% PVA solution, and in 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 using the TAMRA probe. Note the systematic shift of the curves with increasing cross-link density, indicating slowing down of the particles. The solid lines are the fits of the expression in Equation 2 to the data, showing 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 the probe particle in water) for TAMRA and dextran probes as a function of concentration of PVA solutions. For both probes, Figure 2 shows that τ_d increases linearly with the polymer concentration, which can be, at first glance, attributed to changes of the viscosity of the polymer solution according to the Stokes-Einstein relation, $D = \frac{k_B T}{6\pi\eta r_h}$, where k_B is the Boltzmann constant, T is the temperature, η is the viscosity, and r_h is the hydrodynamic radius. Using the Stokes-Einstein equation, the scaled diffusion time is expected to be a ratio of viscosities of the

solution and water, independent of the probe size. However, the data in Figure 2 show a dependence on probe size, indicating an apparent dependence of the viscosity of the solution on the size of the probe particle.

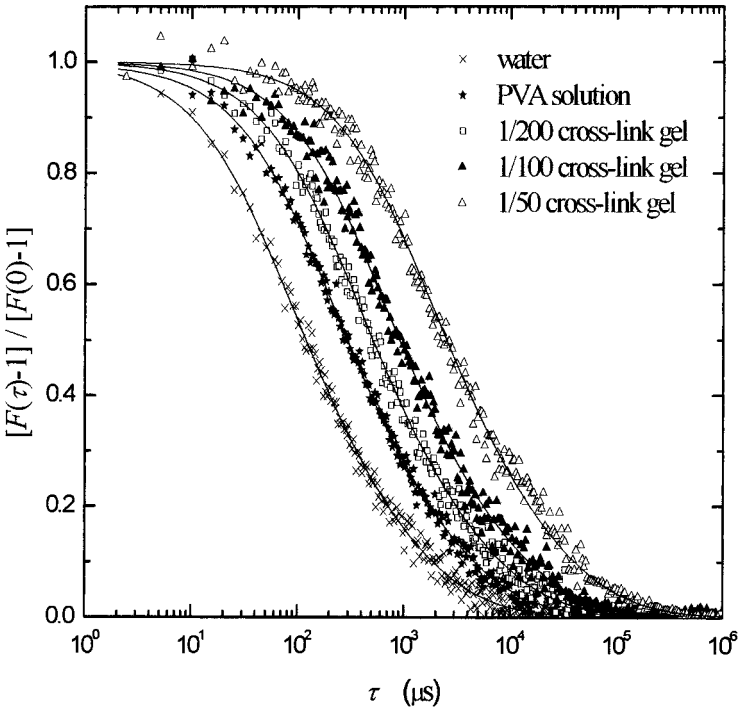

Figure 1. Normalized correlation functions for fluorescent TAMRA molecules in water, 6% (w/v) PVA solution, and 6% (w/v) gels at several cross-link densities (see labels on plot) as a function of delay time, τ. The solid lines are fit to the data with Equation 2.

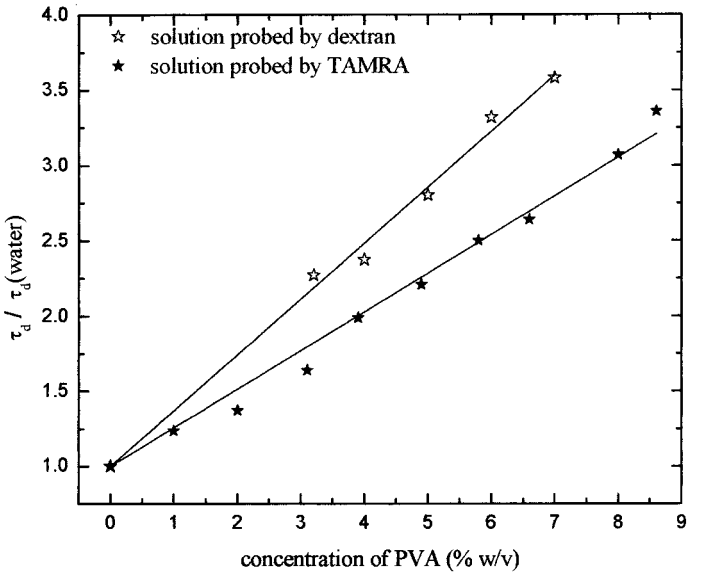

Figure 2. Scaled characteristic diffusion times of fluorescent TAMRA molecules and TAMRA-labeled dextran polymers in PVA solutions as a function of concentration, with linear fits. The times are scaled by the diffusion time of the respective probe in water.

Figure 3 shows τ_d (scaled by the diffusion time of TAMRA molecules in water) as a function of the PVA concentration for solutions and gels. 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 TAMRA particles remain unchanged even in the presence of cross-linker in the polymer solution, indicating the absence of observable interactions between the cross-linker and the TAMRA particles. 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.[12]

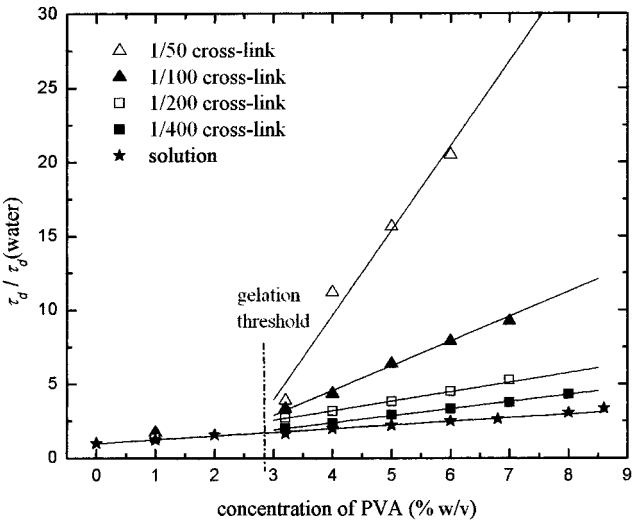

Figure 3. Scaled characteristic diffusion times of fluorescent TAMRA molecules in PVA solutions and gels at several cross-link densities (as labeled on plot) as a function of polymer concentration, with linear fits. The times are scaled by the diffusion time of the probe in water, τ_d(water).

In polymer solutions, simple scaling theory predicts that the characteristic length scale (correlation length, ξ) decreases with increasing polymer concentration $\xi \sim c^{-m}$ where m = 0.75 (good solvent condition) or m = 1 (theta condition).[13] When a semi-dilute polymer solution is cross-linked, the resulting gel contains structural regions of differing sizes and the simple scaling relation may no longer be valid. Small-angle neutron scattering measurements performed on a variety of weakly cross-linked gels indicate that cross-linking produces structural changes at larger length-scales ($>> \xi \sim 5$ nm in similar PVA samples), whereas at shorter length-scales ($\leq \xi$) the polymer configuration is only slightly modified.[4] 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 3 should reflect changes in the large-scale structure of the system due to cross-linking.

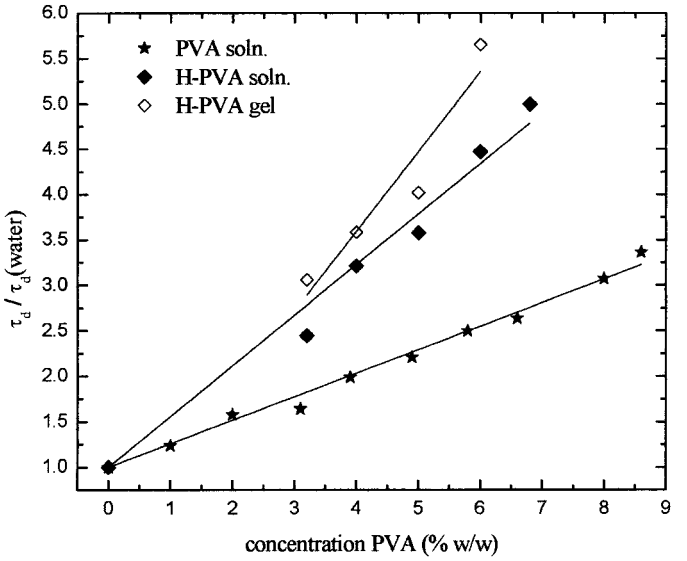

Figure 4. Scaled characteristic diffusion times of fluorescent TAMRA molecules in "fresh" PVA solution (PVA soln.), "aged" PVA solution (H-PVA soln.), and "aged" PVA gel (H-PVA gel) as a function of polymer concentration, with linear fits. The times are scaled by the diffusion time of the TAMRA probe in water, τ_d(water).

We also observed a similar behavior in "aged" PVA samples. Figure 4 compares diffusion times measured on "fresh" PVA solutions, H-PVA solutions ("aged" solution), and H-PVA gels (gels made from the H-PVA solution). The H-PVA solutions exhibit longer diffusion times than those of the "fresh" PVA solutions. We attribute this difference to the formation of hydrogen bonds between the polymer chains over time, which change the overall structure of the PVA solutions. In H-PVA gels, there is only a slight increase in the diffusion times when compared with those obtained from the corresponding H-PVA solution, unlike the clear difference between the diffusion times measured from the "fresh" PVA solution and their corresponding cross-linked gels (see Figure 3). It appears that the H-bonds alter the gelation process of the polymer solution, though a detailed description on how is not clear.

Conclusion

In this experimental study, we have demonstrated how FCS can provide insight into the structural changes occurring in PVA solutions and gels. We have successfully applied the technique to investigate the diffusion of small fluorescent probe particles in non-fluorescent – hence invisible – PVA solutions and chemically cross-linked PVA gels. Using two different sized probes, TAMRA and TAMRA-labeled dextran, we have compared the diffusion times of the probes as a function of polymer concentration. Although the measurements indicate a monotonic, linear increase of the diffusion time with the polymer concentration, it appears that the Stokes-Einstein relation cannot account consistently for this behavior (i.e. the slopes in Figure 2 should not depend on the probe size). We plan to explore further the size effects on the diffusion of particles in concentrated solutions.

The measurements on gels indicate that for the same polymer concentration, the 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. In a 9-month aged PVA solution, the formation of H-bonds between the polymer chains appears to affect the dynamical behavior of the probe, showing the sensitivity of FCS in detecting such structural changes.

[1] A.S. Hoffman, *Advanced Drug Delivery Review* **2002**, *43*, 3.
[2] C. R. Nuttelman, S. M. Henry, K. S. Anseth, *Biomaterials* **2002**, *23*, 3617.
[3] R. H. Schmedlen, K. S. Masters, J. L. West, *Biomaterials* **2002**, *23*, 4325.
[4] F. Horkay, A. M. Hecht, E. Geissler, *Macromolecules* **1994**, *27*, 1795.
[5] J-M. Petit, X. X. Zhu, P. M. Macdonald, *Macromolecules* **1996**, *29*, 70.
[6] M. Tokita, T. Miyoshi, K. Takegoshi, K. Hikichi, *Phys. Rev. E* **1996**, *53*,1823.
[7] T. Narita, A. Knaebel, J-P. Munch, S. J. Candau, *Macromolecules* **2001**, *34*, 8224.
[8] J. C. Reina, R. Bansil, C. Konak, *Polymer* **1990**, *31*, 1038.
[9] H. Boukari, R. Nossal, D. L. Sackett, *Biochemistry* **2003**, *42*, 1292.
[10] W. W. Webb, *Appl. Optics* **2001**, *40*, 3969.
[11] S. R. Aragon, R. Pecora, *J. Chem. Phys.* **1976**, *64*, 1791.
[12] A. Michelman-Ribeiro, H. Boukari, R. Nossal, F. Horkay, *Macromolecules* **2004**, *37*, 10212.
[13] P. G. de Gennes, *Scaling Concepts in Polymer Physics*; Cornell University Press; Ithaca, NY, 1979.