Monitoring nanoprobe diffusion in osmotically-stressed hydrogels

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ABSTRACT

We have developed an optical chamber for studying the effect of swelling or shrinking of a gel on the translational diffusion of fluorescent nanoprobes embedded in the gel. On one side of the chamber, the gel is in contact with a hydrating or dehydrating polymeric solution through a porous membrane, allowing control of the rate of hydration or dehydration of the gel. On the other side, a laser beam is focused into the gel to excite the fluorescence of the nanoprobes, which is continuously monitored to reveal possible structural changes of the stressed gel. Using fluorescence correlation spectroscopy we measure correlation functions of the nanoprobes at various times following the contact of the gel with the hydrating or dehydrating solution, and determine changes of both the average concentration and the apparent diffusion time of the nanoprobes as the gel is shrinking or swelling. We have tested the chamber using the fluorophore, TAMRA (MW = 430 Da), embedded in a poly(vinyl-alcohol) gel that is being dehydrated by a solution of poly(vinyl-pyrrolidone) (28% w/w). As expected TAMRA moves slower as the gel shrinks. However, the changes in the diffusion time of TAMRA as a function of the PVA concentration of the shrinking gel appear to be different than those measured on TAMRA diffusing in PVA gels prepared at different PVA concentrations but with the same cross-link density.

I. INTRODUCTION

Understanding probe diffusion in gels in general and biological gels in particular remains a subject of prime interest to both basic research and applied engineering such as drug delivery and tissue engineering [1-3]. For most applications, the main goal is to understand the relationship between structure and dynamic properties under various conditions (concentration, cross-linking, pH, salt, temperature,..). Recently, we demonstrated that fluorescence correlation spectroscopy (FCS), an optically non-invasive technique, could be successfully used to measure the diffusion of nanoprobes in polymeric solutions and cross-linked hydrogels [4,5]. In particular, we investigated the effects of the concentration and cross-link density in poly(vinyl-alcohol) (PVA) gels on the diffusion of various nanoprobes. In the case of the gels, we found remarkable correlation between the macroscopic elastic modulus and the diffusion of the fluorescent nanoprobe, TAMRA (430 Da) [4]. The mechanisms behind this correlation have yet to be elucidated.

In the present study we focus on the effect of swelling and shrinking on the diffusion of the same nanoprobe, TAMRA, in similar PVA gels. Dehydration can cause structural changes, ranging from simple shrinking to cracking. In principle, these changes are likely to affect not only the mechanical properties of the gel (e.g., its elastic modulus) but also the diffusion of the embedded nanoprobes. It is unclear whether there is a correlation between the changes of these properties.

To study these changes, we have designed an optical chamber that can be used to measure changes of the diffusion of nanoprobes in a gel. We have tested the chamber using a chemically cross-linked PVA gel containing nanomolar concentration of TAMRA in indirect contact (through air) with poly(vinyl pyrrolidone) (PVP) solution of known osmotic pressure [6]. We apply fluorescence correlation spectroscopy (FCS) to monitor changes in the concentration and diffusion of TAMRA as the gel de-swells slowly and continuously. We find monotonic increase of both the average concentration and the diffusion time of TAMRA with time. This increase is consistent with the underlying shrinkage of the gel. More importantly, we use the increase of the concentration of TAMRA to calculate the volumetric changes of the gel with time, assuming a one-to-one relation between changes of TAMRA concentration and volumetric changes. It is then possible to relate changes in the diffusion of the embedded nanoprobe, TAMRA,with the volumetric changes of the host PVA gel.

II. FLUORESCENCE CORRELATION SPECTROSCOPY

Fluorescence Correlation Spectroscopy (FCS) utilizes the fluctuations in emission from fluorescent particles moving in and out of a focused beam, yielding information about the diffusion, interaction, and photodynamics of the particles. These fluctuations are typically caused by changes in the number of fluorescent particles in the small-illuminated volume or changes in the emission quantum yield of the particles. The illuminated volume is made small (~femptoliter) by confocal setup or by two-photon emission [9-10]. 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 τ is the delayed time and $\delta I(t) = I(t) - < I(t) >$ denotes the deviation of the measured intensity from the time average intensity, $<I(t)>$. For the ideal case of freely diffusing monodisperse and uniformly bright fluorescent particles, the correlation function can be written as [9-10]

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

where the excitation beam is a 3-D Gaussian beam, with an intensity profile given by: $W(r,z) = Ae^{-2\left(r/r_0\right)^2} e^{-2\left(z/z_0\right)^2}$. Here 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. τ_d is a characteristic time for a particle to diffuse along the lateral width (r_0) of the focused incident beam, viz., $\tau_d = r_0^2 / 4D$, where D is the translational diffusion coefficient of the particle. In Eq.2 N denotes

the average number of particles in the excitation volume and $p = \left(r_0 / z_0 \right)^2$ is an instrumental constant.

III. EXPERIMENTAL DETAILS

III.1 CHAMBER DESIGN

Figure 1 shows the schematic diagram of the chamber. All parts were custom-made. Aqueous PVP solution (MW=28 kDa, Sigma-Aldrich) was loaded in the top container. The porous membrane (Spectra/Por, Spectrum Labs) had a small molecular weight cut-off (MWCO: 6000-8000 Da). The silicon-rubber spacers were cut from double adhesive sheets purchased from Grace-Bio-Labs.

III.2 SAMPLE PREPARATION

PVA ($M_w \approx 85$ kDa, Sigma-Aldrich) was dissolved in de-ionized water at 95^o C and kept at this temperature for several hours. PVA solutions were prepared at room temperature with concentrations ranging from 3% to 8.6% (w/v). The fluorescent probe, carboxytetramethylrhodamine (TAMRA) ($M_w = 430$ Da, Molecular Probes) was mixed at nanomolar concentration with PVA and the whole mixture was let to stabilize overnight. The cross-linker (glutaraldehyde) was added to the PVA-TAMRA solution at pH = 2. The cross-linking solution was loaded into the chamber for the completion of the gelation. The cross-link density of the gel is defined by the ratio of the number of moles of glutaraldehyde and that of the monomers of the PVA molecule. We typically wait 30 minutes before placing the PVA gel in contact with the PVP solution (see Fig.1) and collecting FCS correlations.

III.3 FCS APPARATUS and EXPERIMENTAL PROCEDURE

The experimental setup has been described elsewhere [10]. We use a 543 nm He-Ne laser (JDS Uniphase) to illuminate the sample through a 60X objective (NA=1.4, Oil) in an Olympus IX70 inverted microscope. The 10-μW incident beam is expanded and focused onto a small spot of radius r_0 (< 1 micron). The emitted fluorescent light is collected by the same objective and focused onto an optical fiber with a core diameter of either 10 or 25 microns. This small diameter ensures the confocal detection necessary for delimiting small volumes of interest. For signal detection, two avalanche photodiodes (PerkinElmer, EG&G, Vandreuil, Canada) are 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 are processed by a digital correlator (Brookhaven Instrument, Holtsville, NY, USA), yielding the time-correlation functions. Concurrently, an MCS+ acquisition card (Ortec, Oak Ridge, TN, USA) is used to collect the fluorescence time (1-5 s bin time), allowing the monitoring of changes of the fluorescence intensity over time.

After placing the chamber in the FCS instrument measurements are made over several days as the PVA-TAMRA gel slowly shrinks. We monitor continuously the average fluorescence intensity of TAMRA, which provides first indications on the status of the sample (absence of leaks and other artifacts). Because of the slow rate of deswelling (several hours), one can consider the gel in a steady-state over the 10-15 minute period used to measure an FCS

correlation function. We collect several FCS correlations over the duration of the experiment as the gel keeps shrinking. All the experiments are performed at ambient temperature (~25 ^{o}C).

IV. EXPERIMENTAL RESULTS

In Fig.2 we show correlation functions of TAMRA diffusing in the PVA gel ([PVA] = 4%; [cross-link density = 1/200]) measured at various times during the shrinking of the gel. The decrease of the amplitude of the correlation functions over time indicates increase of the concentration of TAMRA as the gel shrinks, an observation consistent with Eq.2. In the same figure we plot the curve fits (solid lines) obtained from fitting the measured correlation functions to Eq.2, where the fitting parameters are the diffusion time, τ_d, and the amplitude of the correlation (1/N). Each of the curves can be satisfactorily described by a single characteristic time, τ_d, i.e., no anomalous diffusion process is observed.

In Fig.3 is plotted the apparent diffusion time, τ_d , of TAMRA (scaled by the diffusion time of TAMRA in water) as a function of the PVA concentration of the shrinking gel. We assume that the increase of the fluorescence intensity is linearly proportional to the PVA concentration, and calculate accordingly the PVA concentration of the gel. For comparison, we plot in the same figure (Fig.3) the changes of the apparent diffusion times of TAMRA for PVA gels prepared at different PVA concentrations but with the same cross-link density (1/200). We also show data obtained for the solutions of the uncross-linked PVA. In all gels the apparent diffusion time increases with increasing PVA concentration. It can also be seen that TAMRA nanoprobes move slower in the gel than in the corresponding solution as reported earlier [4]. However, the concentration dependence of the diffusion of TAMRA is different in gels made at different initial polymer concentrations. Although, at low polymer concentration (4% < [PVA] < 5.5 %) the slope of the apparent diffusion times in all gels appears similar, there is systematic deviation of the slopes at higher polymer concentrations (> 5.5%). This deviation may reflect differences in the structure of the equilibrated gels and the shrunken gel, which can be characterized by high-resolution techniques, such as small-angle scattering [11].

V. Summary:

In this paper we describe an optical chamber designed and built to study diffusion of fluorescent molecules in gels in the course of the swelling/shrinking process. We use FCS to simultaneously monitor changes of the fluorescence intensity as well as changes of the concentration and diffusion time of nanoprobes. We have tested the chamber in a study of the diffusion of TAMRA in a PVA gel subjected to dehydration by osmotic stress induced by a PVP solution.

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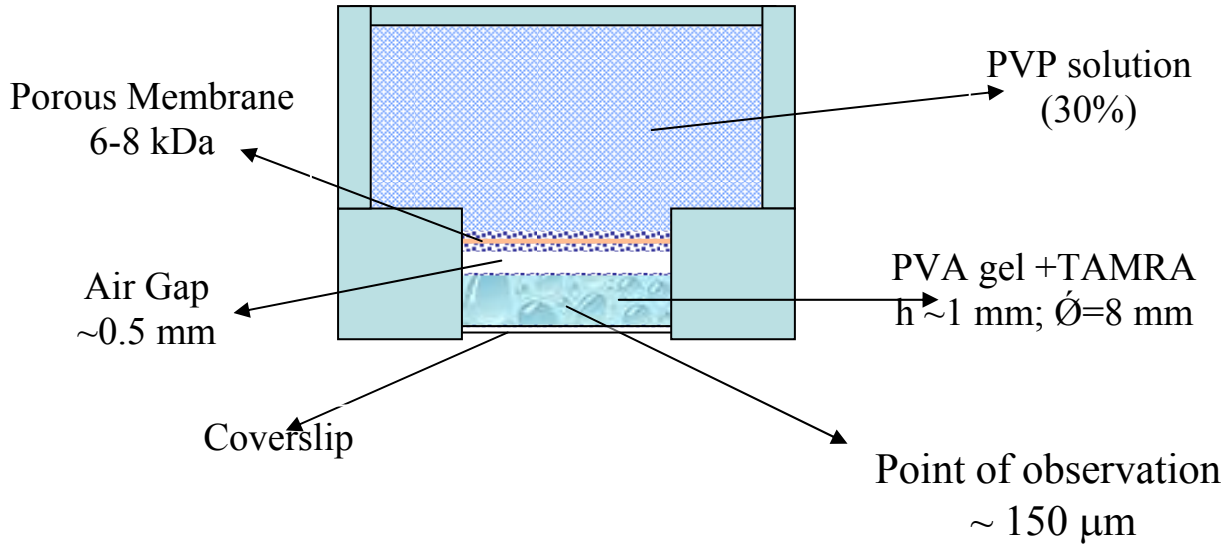

Figure1: Schematic diagram of the optical chamber designed to study the effects of drying on nanoprobe diffusion in a gel.

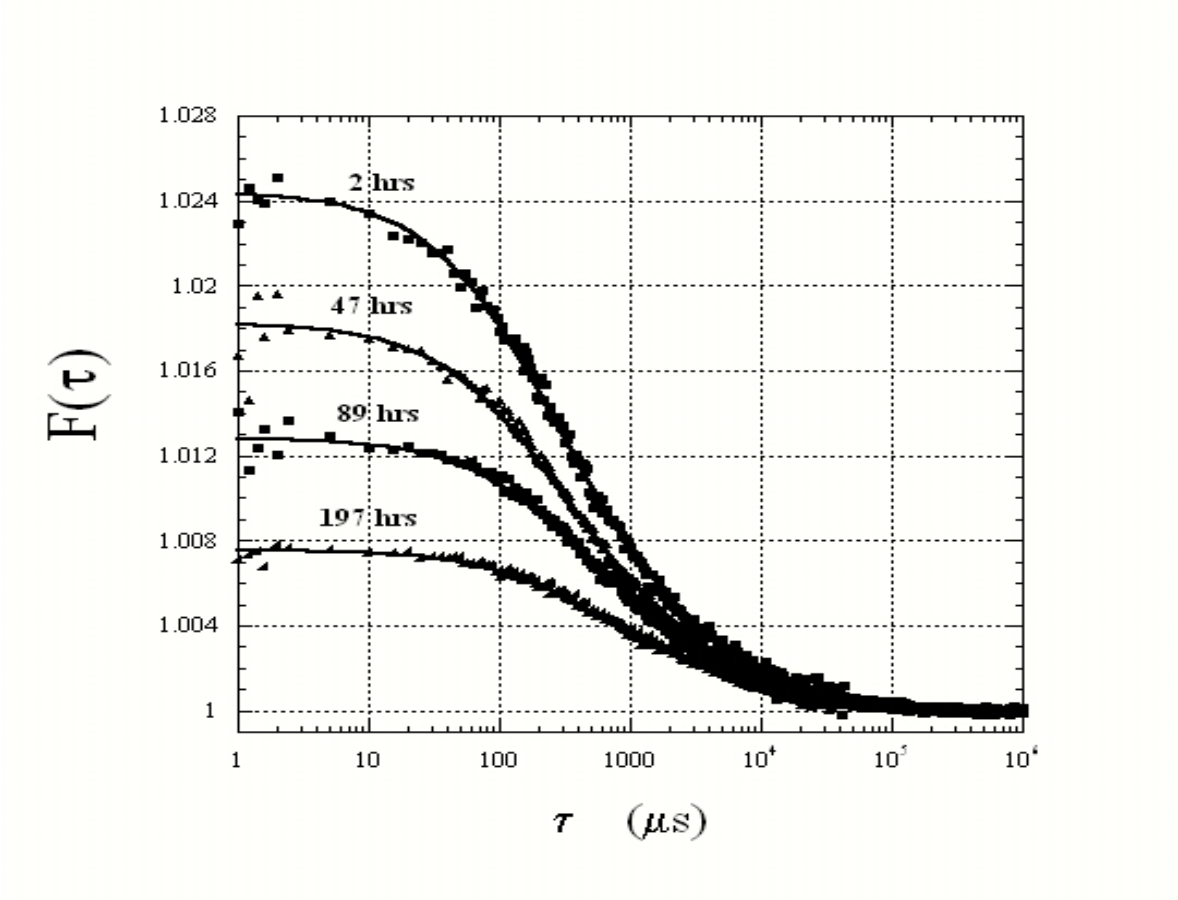

Figure 2: FCS time-correlation functions of TAMRA diffusing in a PVA gel, which is subjected to drying by a PVP sample. Each curve is labeled with the time it is collected (t=0 is the instant the gel is placed in contact with PVP). The solid line is the fit of the measured data with the expression in Eq.2 for the diffusing nanoprobe.

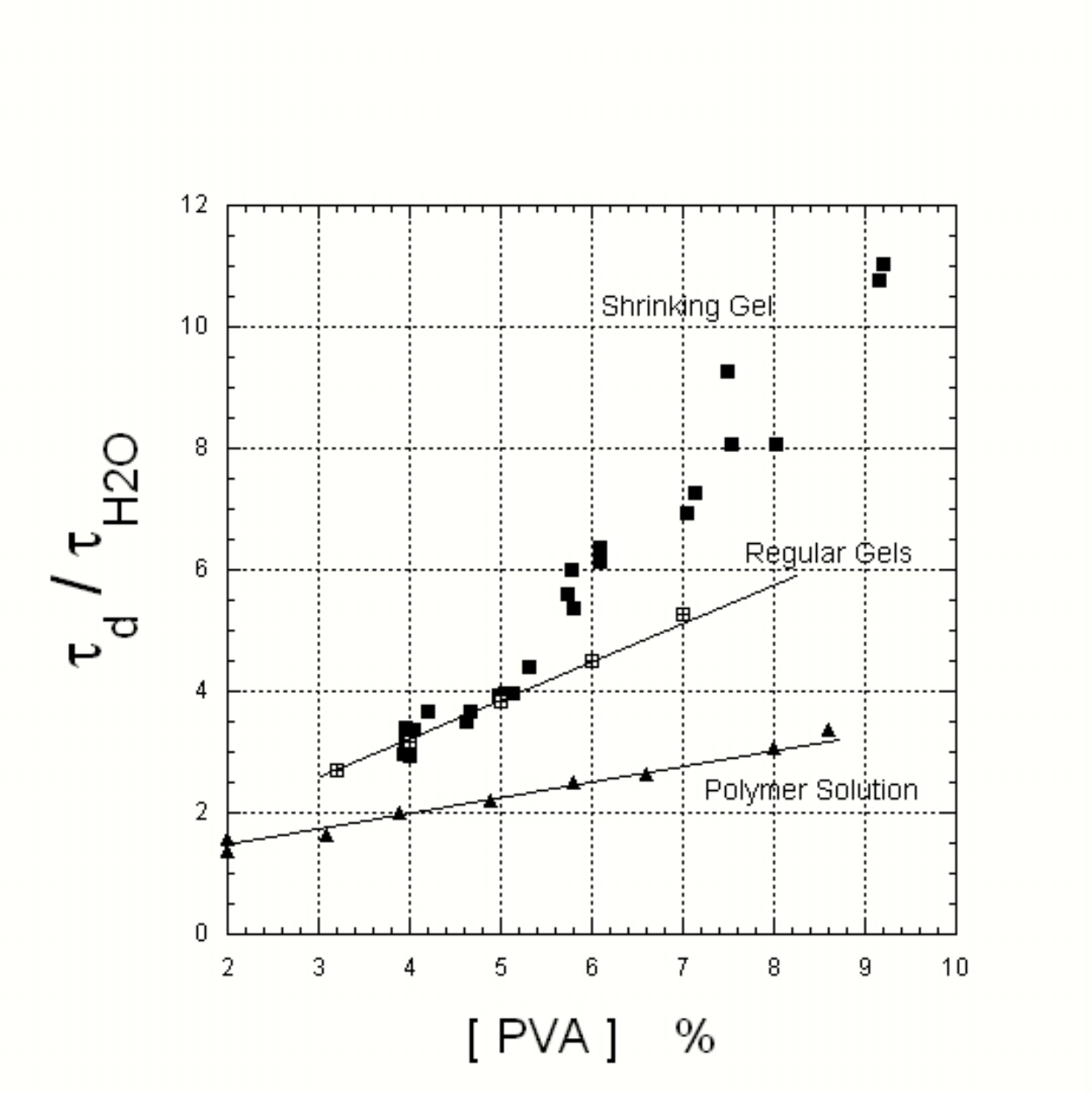

Figure 3: The apparent diffusion time of TAMRA, scaled by its corresponding value in water, is plotted as a function of PVA concentration for different samples: PVA solutions (no cross-linking), PVA gels prepared at different concentration but with the same cross-link density, and PVA gel shrinking under the effect of osmotic pressure of PVP. The shrinking gel was initially prepared at [PVA]=4% by weight and cross-linked with glutaraldehyde at cross-link density of 1/200.