Probe Diffusion in Polyacrylamide Gels As Observed by Means of Holographic Relaxation Methods: Search for a Universal Equation

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ABSTRACT: Probe diffusion in polyacrylamide (PA) gels was investigated with holographic relaxation methods. The probes were a benzospiropyran (SP) dye and bovine serum albumin labeled with *p*-(isothiocyanato)azobenzene (BSA-ABITC). It was found that the reduced translational diffusion coefficients D/D_0 of probes in this gel system are well represented by the empirical stretched exponential equation, $D/D_0 = \exp(-3.03R_h^{0.59}C^{0.94})$, where D and D_0 are diffusion coefficients of the probe particle in the gel and in the pure solvent, respectively, R_h (Å) is the hydrodynamic radius of the probe, and C (g/mL) is the PA concentration. The smaller probe (SP) diffused more slowly than the larger one (BSA-ABITC) at the gel concentration which gave the same ratio of probe radius R_h to mesh size ξ, where ξ (Å) $= 12.6C^{-0.64}$ from dynamic light scattering. Literature values of D/D_0 for D_2O, urea, and sucrose in PA gels are described by the same empirical equation. Reduced probe diffusion coefficients do not scale with single-variable R_h/ξ but do scale approximately with the combination R_hC/ξ. No theoretical justification is currently available for the latter scaling parameter.

Introduction

Probe diffusion in polymer solutions, gels, and other porous media has received considerable experimental and theoretical attention in recent years.[1-19] Theoretical treatments by de Gennes,[2] Langevin et al.,[3] Cukier,[4] and Altenberger et al.,[5] though based on different physical models, lead to formulas in which the reduced diffusion coefficients of the probe particles have simple exponential dependences on the product of the probe radius R and an inverse hydrodynamic screening length k. Thus

$$D/D_0 = \exp(-kR) \tag{1}$$

where D and D_0 are the diffusion coefficients of the probe particle in the gel and in the pure solvent in the absence of the gel network, respectively. According to Cukier,[4] the inverse hydrodynamic screening length k, in semidilute polymer solutions, has a power law dependence ($k = AG^{\upsilon}$) on the concentration of polymer matrix. Therefore, eq 1 becomes the "stretched exponential" function:

$$D/D_0 = \exp(-\alpha C^{\upsilon}) \tag{2}$$

where $\alpha = AR$ and in general the prefactor α is a function of R. In solutions of rod-like polymers, $\upsilon = 1/2$, and eq 2 reverts to the form proposed by Ogston.[6] The simple universal equation, eq 1, has been found to agree with some experimental cases.[3,7-10]

Another hydrodynamic scaling approach to this problem has been presented by Phillies,[15] who suggested the more general scaling equation

$$D/D_0 = \exp(-aR^uM^xC^y) \tag{3}$$

with $u = \pm 0.2$, $x = 0.8$, $y = 0.5$–1.0. M is the molecular weight of the polymer matrix. This equation was proposed as an alternative to reptation theory for the self-diffusion of polymer chains in polymer solutions, but it was also claimed that this model is applicable to all probe diffusion, including the diffusion of globular proteins and polystyrene latex particles, in polymer solutions. A significant difference between eqs 1 and 3 is the dependence on probe size. The ratio of the probe size R to the hydrodynamic screening length (k^{-1}) of the polymer solution is the most important factor in eq 1, while in eq 3 there is almost no probe size dependence; the transient pseudogel characteristic of polymer solutions does not appear to influence probe diffusion.

Most of the experimental data that have been analyzed in terms of eqs 1–3 have been obtained with polymer solutions, and little data for probe diffusion in real gels has been analyzed in terms of scaling concepts. Real gels have permanent networks of cross-links, and these matrices may exhibit quite different barriers to probe diffusion than do polymer solutions. For example, it is well-known that gels can act as size filters in which probe particles larger than the pore size are immobilized. Our aim in this work was to search for a universal equation to relate probe diffusion rates in gels to easily measured parameters. Our method was to measure tracer diffusion coefficients for probes in a gel system and to analyze the new data as well as data from the literature in terms of scaling equations.

The diffusion of BSA-ABITC in fibrin gels has previously been studied by means of holographic relaxation spectroscopy (HRS).[19,20] However, the retardation of BSA in fibrin gels is too small to permit the determination of quantitative scaling relations. With the BSA–fibrin gel combination, the minimum value of D/D_0 was around 0.4 even at the highest useable gel concentrations and in the presence of additives thought to tighten the gel structure.[13] For the present work, we chose polyacrylamide (PA), which gives much smaller pore sizes than can be obtained with fibrin. We note that PA is perhaps the most important gel used in gel electrophoresis. An added advantage of this transparent, elastic gel is that the hydrodynamic screening length ξ can easily be measured by means of photon correlation spectroscopy (PCS). According to de Gennes,[2,21] ξ can be interpreted as the distance between two cross-linked points, i.e., the mesh size. Our

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analysis is based on the assumption that the pore size of a PA gel is at least proportional to ξ.

In the following sections, we discuss the characterization of PA gels by means of PCS and the measurement of tracer diffusion rates for benzospiropyran (SP) and BSA-ABITC in PA gels by means of HRS. These diffusion data as well as literature values for D_2O, urea, and sucrose in PA gels are analyzed by means of plots of $-\log (D/D_0)$ versus C and R_h/ξ to establish the power law dependences of D/D_0 on R_h, ξ, and C. Here R_h is the hydrodynamic radius of the probe. To anticipate the results, we have found that the reduced diffusion coefficients do not scale with the single reduced variable R_h/ξ; i.e., eq 1 is not a universal equation for the PA gel system. There is a dependence on the gel concentration C, or the volume fraction of gel, in addition to the dependence on R_h/ξ. It turns out that the combination R_hC/ξ is an approximate scaling variable for the PA gel with the probes used in this study and provides the basis for a universal diffusion equation for probes in PA gels. No theoretical justification is presented for this scaling variable, but in the Discussion section we point out that this type of dependence on the gel volume fraction is consistent with a local viscosity model for the solvent in a gel.

Experimental Section

Materials. Highly purified, sulfhydryl blocked monomer standard bovine serum albumin (BSA) was purchased from Miles Laboratories. Amino azobenzene-*p*-isothiocyanate (ABITC) was purchased from Trans World Co., and spiropyran (1-(carboxyethyl)-3,3-dimethyl-6′-nitrospiro[indoline-2,2′-2*H*-benzopyran]) was custom synthesized by Chroma Chemicals. Acrylamide (AA) and *N,N′*-methylenebisacrylamide (BIS) were obtained from Biorad and Aldrich, respectively. Ammonium persulfate and *N,N,N′,N′*-tetramethylethyleneamine were also obtained from BioRad.

Sample Preparation. Gel samples were prepared by polymerizing AA and BIS in a buffer solution (0.1 M NaCl, 0.05 M Tris, pH 7.4) at room temperature (22 °C). The weight ratio of BIS to AA was held constant at 1:19 in all samples. Constant amounts of ammonium persulfate (4×10^{-4} g/mL) and *N,N,N′,N′*-tetramethylethyleneamine (0.8 μL/mL) were used as initiator and catalysis, respectively. The samples were polymerized in the light-scattering cuvettes and were allowed to stand overnight at room temperature before use. No special efforts were made to prepare extremely swollen gels. The probes (SP or BSA-ABITC) were directly added to the monomer solution before gelation. The concentration of SP was held constant at 0.05 mM when SP was used as the probe.[22]

The procedure for labeling BSA with ABITC has been described elsewhere.[20] The concentration of BSA-ABITC was determined by means of optical absorption measurements at 280 and 360 nm. A correction for the absorbance of the dye at 280 nm was made with the relation $(OD_{280})_{BSA} = (OD_{280})_{total} - A(OD_{360})_{ABITC}$ where A, the ratio of absorbance of ABITC at 280 nm to its maximum value at 360 nm, is approximately equal to 0.32.[18]

PCS Measurements. The collective diffusion coefficients D_c of the elastic PA gels were measured by means of PCS. It has been shown that this diffusion coefficient is in exact correspondence with the Stokes–Einstein formula:

$$D_c = k_BT/(6\pi\eta\xi) \tag{4}$$

where k_B is the Boltzmann constant, T is the absolute temperature, η is the solvent viscosity, and ξ ($=k^{-1}$) is the hydrodynamic screening length. In the following analyses, the screening length ξ is interpreted as the mesh size of the gel. This provides an estimate of the "pore" size of the gel. The vertically polarized scattered light from the pure PA gel network was measured with a BI-200 laser light scattering system (Brookhaven Instruments Corp.), and the time correlation function of the scattered intensity was acquired by a 64-channel Langley-Ford correlator (Model DC64; 56 data channels and 8 delayed channels).

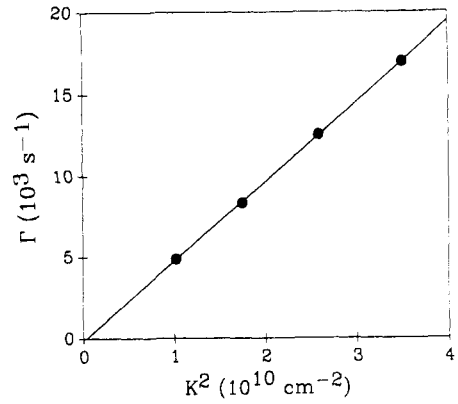

Figure 1. $\langle\Gamma\rangle$ versus K^2 for a PA gel at 21 °C with C = 0.18 g/mL.

The intensity autocorrelation function has the form

$$G^{(2)}(\tau) = B(1 + \beta|g^{(1)}(\tau)|^2) \tag{5}$$

where B and β are background and coherence parameters, respectively. In eq 5, $g^{(1)}(\tau)$ is the normalized electric field correlation function. For scattering by monodisperse solute particles in an isotropic solution, $g^{(1)}(\tau) = \exp(-\Gamma\tau)$ where $\Gamma = DK^2$ is the characteristic line width, D is the mutual diffusion coefficient, and K is the magnitude of the momentum transfer vector. Polydisperse systems are treated by expressing the electric field correlation function $g^{(1)}(\tau)$ as

$$g^{(1)}(\tau) = \int_0^\infty G(\Gamma)\exp(-\Gamma\tau)\,d\Gamma \tag{6}$$

where $G(\Gamma)$ is the normalized line width distribution.

The PCS data for the PA gels were analyzed by means of the second-order cumulant expansion method.[23] This leads to the expansion

$$\ln [g^{(1)}(\tau)] = -\langle\Gamma\rangle\tau + \frac{1}{2!}\left(\frac{\mu_2}{\langle\Gamma\rangle^2}\right)(\langle\Gamma\rangle\tau)^2 + \ldots \tag{7}$$

where

$$\langle\Gamma\rangle = \int_0^\infty \Gamma\, G(\Gamma)\,d\Gamma$$

$$\mu_2 = \int_0^\infty [\Gamma - \langle\Gamma\rangle]^2\,d\Gamma$$

are the first and second cumulants in this expansion. The collective diffusion coefficients D_c of the PA gels were obtained from the initial slopes of plots of $\langle\Gamma\rangle$ versus K^2 as illustrated in Figure 1.

The mesh sizes were calculated directly from corrected values of D_c by means of eq 4. A correction was necessary because of the relative displacement of the solvent resulting from polymer motion in a volume fixed frame. This correction consists of dividing D_c by the volume fraction of the solvent, i.e., by $(1 - \phi)$ where ϕ is the volume fraction of the gel.[24] The volume fraction of the gel was calculated by multiplying the concentration of PA by its specific volume, 0.91 mL/g.[25] At gel concentrations above 0.05 g/mL, the normalized variance $\mu_2/\langle\Gamma\rangle$ was approximately 5%, indicating a narrow distribution of mesh sizes.

HRS Measurements. The spectrometer and the methodology of HRS have been described in detail in previous papers.[26,27] A Spectra Physics 2020 argon ion laser operating at λ = 488 nm supplied the coherent writing beams that were used to form an intensity grating in the sample. The fringe spacing Λ of this grating is related to the crossing angle of the beams by $\Lambda = \lambda/[2\sin(\theta_w/2)]$, where the wavelength λ and the crossing angle θ_w are measured in the same medium (in this case air). In these experiments, Λ was typically about 6 μm and $K = 2\pi/\Lambda$ was about 10^4 cm^{-1}. The duration of the writing laser pulse was approximately 2 ms, and the average power was less than 100 mW. The reading beam was provided by a low-power He–Ne laser (λ = 633 nm). Electromechanical shut-

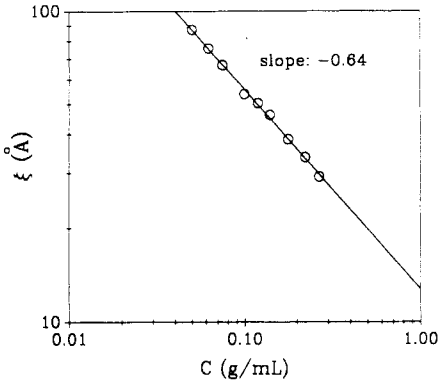

Figure 2. Hydrodynamic screening length ξ versus the concentration C for PA gels at 20 °C.

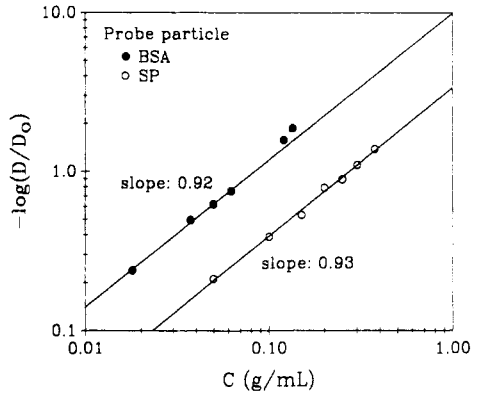

Figure 3. Reduced tracer diffusion coefficients D/D_0 for the probes BSA (●) and SP (○) versus the gel concentration C at 20 °C.

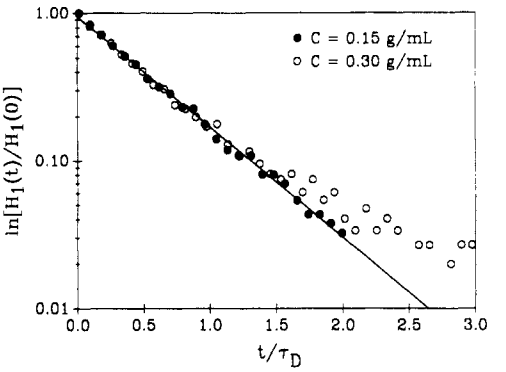

Figure 4. Reduced decay function $H_1(t)/H_1(0)$ versus t/τ_D for the SP probe in PA gels.

ters were used to control the laser exposure times of the sample to both writing and reading beams.

As previously described, the phase (or position) of the laser-induced grating was accurately controlled, and the "phase shift and add" method was used to accumulate signals so that the cross-term between the diffracted signal and the coherent stray light could be eliminated.[19] For a monodisperse sample, the accumulated transient signal (minus background) can be represented under certain conditions by[17]

$$H_1(t) = a^2 \exp(-2\Gamma t) \tag{8}$$

where a is proportional to the scattering amplitude, $\Gamma = DK^2$ is the decay constant, and here D is the tracer diffusion coefficient of the diffracting species. At low gel concentrations, both probes accurately obeyed eq 8, but at high concentrations, where the mesh size ξ was comparable to the probe size, the signals could no longer be represented by single-exponential functions of t. When nonexponential signals were encountered, the HRS data were analyzed by the second-order cumulant method as described in the PCS section. The tracer diffusion coefficient and photochromic lifetime of the probe molecules were obtained from the slope and intercept, respectively, of a plot of Γ versus K^2. It turned out that the photochromic lifetimes of both probes in PA gels were sufficiently long that there were no significant nondiffusive contributions to the decay rates when K^2 was in the range 10^7–10^8 cm^{-2}.

Results

Characterization of Mesh Size of PA Gels. The mesh size ξ of each PA gel in absence of probe molecules was calculated from the collective diffusion coefficient D_c obtained by PCS. A log–log plot of ξ versus the PA concentration C is shown in Figure 2. The concentration dependences of the corrected diffusion coefficient D_c and the mesh size ξ, obtained by analysis of this data, are given by

$$D_c = 1.70 \times 10^{-6} C^{0.64} \tag{9}$$

$$\xi = 12.6 C^{-0.64} \tag{10}$$

where D_c, ξ, and C are measured in units of cm^2/s, Å, and g/mL, respectively. In these experiments, D_c ranged from 2.39×10^{-7} to 7.46×10^{-7} cm^2/s as C was increased from 0.05 to 0.267 g/mL. The corresponding values of ξ ranged from 85.7 to 29.2 Å, and as previously mentioned the small variance in the PCS experiments implies a narrow distribution of ξ values. We do not have verification of the mesh sizes by an independent experimental method. However, it is interesting to note that, if the cross-linking molecules (BIS) are assumed to be randomly distributed in space, the average nearest-neighbor distances between BIS molecules is the same order of magnitude as ξ.[28] Of course, the average nearest-neighbor distance is proportional to $C^{-1/3}$ in contrast to eq 10 for ξ.

de Gennes predicted that the exponent in eq 9 should be 0.75 for a swollen gel in the good solvent limit.[21] The exponent (0.64 ± 0.01) obtained here probably means that our aqueous PA gel cannot be considered a perfectly swollen gel. Hechst et al.[29] and Sellen[30] have reported similar values (~0.63) for the exponent for PA gels. It should be noted that the prefactor in eq 9 is only about one-half the value reported by Sellen. This discrepancy may result from different BIS concentrations in the two studies. In our study, [BIS] was 5% of C versus 1.4–2% of C in Sellen's work. The diffusion data reported by Gelman et al.,[31] with C = 0.05–0.075 g/mL and [BIS] = 5% of C, agreed with our results within 5–12%, but their concentration exponent was 0.7.

SP in PA Gel. Tracer diffusion coefficients were measured for SP by means of HRS for PA gels having concentrations in the range 0.05–0.38 g/mL. All diffusion data were converted to 20 °C by simple correction of the temperature and the viscosity of water. In Figure 3, the values of $-\log(D/D_0)$ for SP (open circles) versus C are displayed in a log–log plot. This linear plot implies that the relationship between D/D_0 and the PA concentration C can be expressed with the stretched exponential function, and a nonlinear regression analysis yields

$$D/D_0 = \exp(-7.75 C^{0.93}) \tag{11}$$

In Figure 4 we show selected data points from the HRS signals at two PA concentrations. The complete data set included 1000 points for each decay. Here the logarithm of the normalized diffraction intensity $H_1(t)/H_1(0)$ is plotted versus the reduced sweep time t/τ_D where $\tau_D = (DK^2)^{-1}$ is the decay constant obtained from the initial slope. This figure illustrates a correlation between

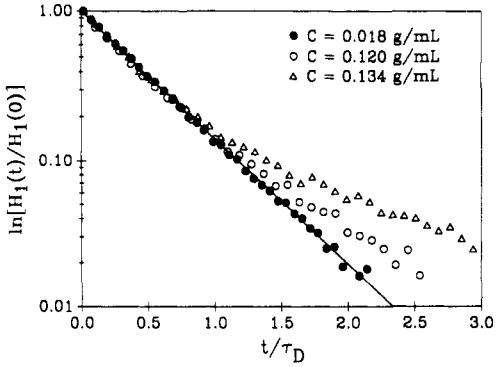

Figure 5. Reduced decay function $H_1(t)/H_1(0)$ versus t/τ_D for BSA-ABITC in PA gels.

the deviation from linearity and the gel concentration. At the higher gel concentration, we found a very small value of the reduced SP diffusion coefficient (D/D_0 = 0.08) and a significant deviation from single-exponential character. It is interesting to note that at this gel concentration (0.3 g/mL), where ξ = 27 Å, the SP molecule with R_h = 5.3 Å experiences severe retardation. Here, the hydrodynamic radius of the probe was calculated from the tracer diffusion coefficient in buffer solution. Since strong retardation and nonexponential decays occurs with ξ/R_h = 5.1, we conclude that this ratio is not be the most important factor in determining the D/D_0 ratio.

BSA-ABITC in PA Gel. Diffusion coefficients were measured for BSA-ABITC in PA gels with concentrations in the range 0.025–0.134 g/mL. The results are displayed in Figure 3 (filled circles). The shapes of the decay signals are shown in Figure 5. At $C = 1.8 \times 10^{-2}$ g/mL, where the ξ/R_h is only 2.7, we observed single-exponential character. Also, at this concentration the retardation was not severe (D/D_0 = 0.58). This stands in strong contrast to the situation for SP, where severe retardation and nonexponential decays were observed with ξ/R_h = 5. Thus, it appears that the deviation from single-exponential character is more directly related to the retardation factor (D/D_0) rather than the length ratio R_h/ξ. We also note that the strong nonexponential character in Figure 5 occurs for C = 0.134 g/mL where ξ = 47 Å and ξ is only 1.27 times the radius of the BSA probe (R_h = 37 Å). Of course, there is a distribution of pore sizes in the gel. Some of the pores are undoubtedly smaller than the BSA probe, and the fraction of pores available to the diffusing probe particle decreases with increasing gel concentration.

The diffusion data for the BSA probe are shown in Figure 3 (filled circles). Analysis based on the low-concentration regime (C < 0.07 g/mL) yields the following stretched exponential function for the reduced diffusion coefficient:

$$D/D_0 = \exp(-27.3C^{0.92}) \quad (12)$$

The exponent (0.92 ± 0.02) in eq 12 is in good agreement with the exponent (0.93 ± 0.01) obtained for the SP/PA gel system. The three data points with C > 0.07 g/mL, taken separately, give an exponent of 1.1.

There remains a question concerning the possible binding of BSA to the PA gel network. To investigate this point, we measured the diffusion coefficients for BSA-ABITC as a function of its concentration while holding C constant at 0.0375 g/mL. The results of these measurements are shown in Figure 6. From the small dependence of D on the protein concentration, we concluded that there is little or no binding between BSA-ABITC

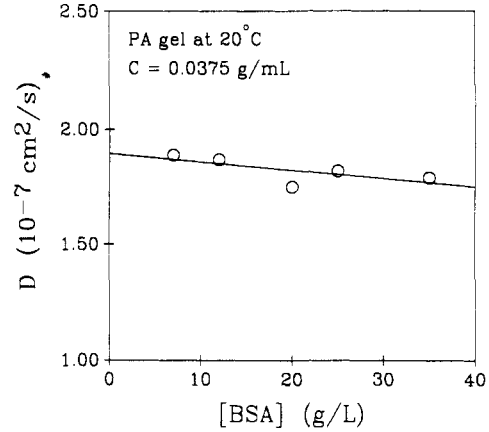

Figure 6. Tracer diffusion coefficient D of BSA versus the concentration of BSA in a PA gel.

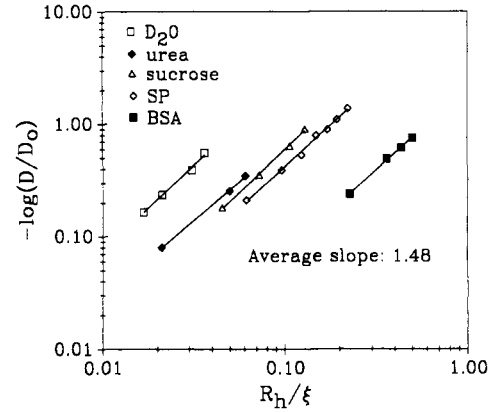

Figure 7. Reduced tracer diffusion coefficients (D/D_0) versus the ratio of probe radius to mesh size (R_h/ξ) for five probe molecules.

and the PA gel and that interactions between BSA-ABITC molecules in the gel are negligible in the concentration range [BSA-ABITC] = 20–30 g/L. Also, photoinduced binding of BSA-ABITC to the gel lattice would produce a permanent grating and a corresponding offset in the base line for the HRS signal.[19] We accumulated the HRS signal without phase shifting to look for this effect but could detect no change in the base line. This experiment also demonstrates that there is no detectable contribution to the diffracted signal from trapped BSA-ABITC molecules.

Analysis and Discussion

Langevin et al.[3] proposed that the retardation of probe diffusion in a gel network is a function only of the ratio of probe size R to pore size ξ and that the retardation can be expressed as

$$D/D_0 = \exp[-(R/\xi)^\delta] \quad (13)$$

for $R < \xi$. In order to compare our experimental results with this prediction, we have plotted D/D_0 for SP and BSA versus R_h/ξ (Figure 7). Throughout this discussion, we have used the hydrodynamic radius R_h of the probe as the probe size. In addition, we have plotted data obtained by White et al.[32] for D_2O, urea, and sucrose in the same figure. For all five probes, the slopes are close to the average value (1.48 ± 0.06), but no data points for any pair of probes fall on the same line; i.e., the lines are parallel but not collinear. Furthermore, at a given value of R_h/ξ, the smaller probe diffused more slowly than the larger one. For example, at ξ/R_h = 10, the extrapolated retardation for D_2O was extremely large (D/D_0 =

Table I
Parameters α and ν[a]

probe	R_h, Å	α	ν	comment
SP	5.27	7.75	0.93	this work
BSA	37.4	37.4	0.92	this work
D_2O	1.04	4.26	0.98	ref 32
urea	1.90	2.80	0.89	ref 32
sucrose	4.09	8.17	0.97	ref 32

[a] These parameters were obtained by fitting diffusion data for various probes to the function $D/D_0 = \exp(-\alpha C^{\nu})$.

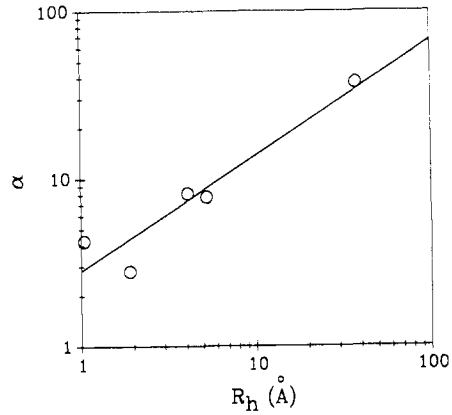

Figure 8. Prefactor α for five probes versus the mesh size ξ (see Table I).

0.007) compared to the value ($D/D_0 = 0.87$) for BSA at same ratio. This difference is outside the error limits of the various experimental techniques. White et al. used both the diaphragm cell method and a nuclear magnetic resonance (NMR) technique in their measurements. We note that the pore size estimated by White et al. is much smaller than our value, 15 versus 54 Å at $C = 0.1$ g/mL, and is probably too small to permit BSA to diffuse in the gel.

Similar trends have been reported for probe diffusion in random porous glass matrices.[33,34] For example, Bishop et al.[33] obtained $D/D_0 = 0.69$ and 0.63 for polystyrene probes with $\xi/R_h = 10$ and 18, respectively, at $\xi = 745$ Å. In contrast, Dozier et al.[34] reported retardation by 2 orders of magnitude ($D/D_0 = 0.016$) for an azobenzene probe with $\xi/R_h = 7.1$ at $\xi = 30$ Å. The ξ/R_h values are not identical in these two studies, but on the basis of eq 13 with $\delta = 1$ the expected retardations for $\xi/R_h = 7.1$ and 10 differ by less than 5% and would be experimentally indistinguishable. We also note that the values of D/D_0 in both of these studies are much smaller than expected on the basis of eq 13.

Before discussing physical models for the retardation, we consider types of scaling equations that are consistent with our data. In this analysis, we assumed a stretched exponential function of the form $D/D_0 = \exp(-\alpha C^{\nu})$, and we used log–log plots of $-\log (D/D_0)$ versus C to obtain the parameters α and ν. The values obtained for all five probes are listed in Table I. The values of ν obtained from different experiments showed good agreement, but α was found to depend on the probe size. This dependence was investigated by means of a log plot of α versus R_h as shown in Figure 8. A least-squares fit of this data yielded the following empirical relation between α and R_h:

$$\alpha = 3.03 R_h^{0.59} \tag{14}$$

where R_h is measured in Å and the standard deviations in α and ν are 0.80 and 0.12, respectively. By combining eqs 2 and 14, we obtain the general stretched exponential equation for D/D_0:

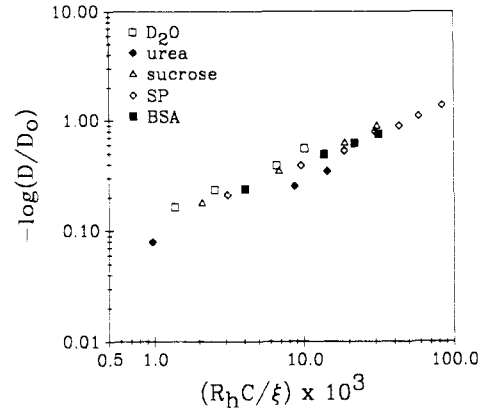

Figure 9. Reduced diffusion coefficients D/D_0 for five probes versus the proposed scaling parameter (R_hC/ξ).

$$D/D_0 = \exp(-3.03 R_h^{0.59} C^{0.94}) \tag{15}$$

On the basis of this analysis, we conclude that eq 15 can be used to calculate the diffusion coefficient of any probe in a PA gel if the probe size and the PA concentration are known.

We are now in a position to express the retardation in terms of the ratio R_h/ξ for comparison with eq 13. By using eq 10 to rewrite eq 15, we obtain

$$D/D_0 = \exp[-13.5(R_h/\xi)^{0.59} C^{0.56}] \tag{16}$$

We find that the nonuniversal behavior of the PA gel system is described by the extra factor $C^{0.56}$ in the exponent of eq 16. Also, by comparison with eq 13 we find that the experimental value of δ is approximately 0.59. The theoretical value is close to unity.[3]

We call attention to the fact that the exponents on R_h/ξ and C in eq 16 are quite similar. It is tempting to use the combination $\Phi = (R_hC/\xi)$, without theoretical justification, as a new scaling variable for diffusion in PA gels. We illustrate this idea by plotting the diffusion data for all of the probes versus (R_hC/ξ) in Figure 9. All of the data fall on a master curve and suggest a new universal equation of the form $D/D_0 = \exp(-a\Phi^b)$.

We briefly recall the important features of our experiments. We have determined the screening lengths for PA gels in the absence of probe molecules by means of PCS. The grating spacings Λ in our HRS experiments were at least a factor of 10^3 larger than the screening lengths ξ. Thus we have observed only the diffusive component of molecular motion. Also, in most of our analyses the molecular radii were much smaller than the screening lengths. There is a distribution of pore sizes, but the PCS experiments indicate that the distribution is narrow at high gel concentrations. The HRS signals for the probe molecules showed exponential decays except when the probe size was comparable to the screening length. For example, as shown in Figures 4 and 5, at a gel concentration of 0.15 g/mL the decay for SP was exponential while at a gel concentration of 0.13 g/mL BSA showed a significant deviation from exponential decay. Even with a narrow pore size distribution, the distribution of apparent diffusion coefficients is large when R_h/ξ is close to unity. The results of our analysis are not affected by the pore size distribution. For example, the exponential data alone in Figures 4 and 5 for SP and BSA reveal the enhanced retardation for smaller probes at constant ξ/R_h. We note that the SP data for $C = 0.15$ g/mL have $\xi/R_h = 8$ and show more retardation than the BSA data for $C = 0.018$ g/mL, with $\xi/R_h = 4.4$.

We suggest that the experiments of Nishio et al.[16] on PA gels formed in the presence of either 500- or 1000-Å-diameter latex spheres are not directly relevant to our work. They attempted to characterize the gels by means of PCS measurements on probe particles for various values of K in situations where the probe and pore sizes were comparable. Also, the diffusion lengths $2\pi/K$ were similar to the pore sizes. In fact, they determined the fraction of trapped particles as a function of K. There is some concern here about the effect of the spheres on the gel structure. In contrast to the situation with small probes, it is impossible to perfuse latex spheres into preformed PA gels. We emphasize that no probe particles were trapped in our experiments with molecular probes and that the average diffusion distances were orders of magnitude larger than the pore sizes.

In searching for physical explanations for the role of the absolute pore size in determining the retardation effect, we have considered among other things the effective pore size for different probes and the local viscosity model. The effective pore size can be defined as $\xi_{eff} = \xi - R_h$ to take into account the excluded volume effect. This correction leads to increased retardation but does not favor larger particles in contradiction to experimental results. An alternative model, based on the idea of local viscosities, has been presented in Yam et al.[36] to explain the effects of polymer chains on the diffusion of small solute molecules. This model postulates two viscosity regions for the solvent. One is a region of higher viscosity close to the polymer chains, i.e., the local viscosity, and the other has the viscosity of the bulk solvent. If the local viscosity increases and/or the volume fraction of the local viscosity region increases when the pore size decreases, the increased retardation for small probes can be explained at least qualitatively. Our data are consistent with this type model but do not prove it.

Conclusions

We have characterized PA gels by means of PCS and have studied the tracer diffusion of SP and BSA-ABITC in these gels by means of HRS. These data and diffusion data for D_2O, urea, and sucrose from the literature are well described by the stretched exponential function in eq 15 that depends only on the probe radius and the gel concentration. An analysis of these data in terms of the parameter R_h/ξ has been attempted on the basis of the assumption that the pore size is similar to the mesh size ξ and that this quantity can be obtained from the collective diffusion coefficient D_c of the gel. We have found that a universal equation depending only on the ratio R_h/ξ cannot fit all of the diffusion data. However, the data do appear to scale with the parameter R_hC/ξ, and we have suggested on empirical grounds a new universal equation for diffusion in PA gels.

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References and Notes

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Registry No. (AA)(BIS) (copolymer), 25034-58-6; (1-(carboxyethyl)-3,3-dimethyl-6′-nitrospiro[indoline-2,2′-2*H*-benzopyran]), 76343-74-3.