Pulsed Field Gradient NMR Study of Probe Motion in Polyacrylamide Gels

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ABSTRACT: Probe diffusion and electrophoresis in polyacrylamide (PA) gels have been studied by means of pulsed field gradient (PFG) NMR methods. The probes were HDO, tetramethylammonium ion, *N,N,N',N'*-tetramethylethylenediamine, and tetrahexylammonium ion. Reduced tracer diffusion coefficients of the probes are well correlated by $D/D_0 = \exp(-3.2R_h^{0.53}C)$, where D and D_0 are the probe tracer diffusion coefficients in the presence and absence of the gel, respectively, R_h (Å) is the hydrodynamic radius of the probe, and C (g/mL) is the PA concentration. The electrophoretic mobility of the tetramethylammonium ion scales with gel concentration in the same fashion. These results support the conclusion of Park et al.[7] that R_hC/ξ, where ξ is the mesh size, is a useful scaling parameter.

Introduction

Diffusion in polymer solutions, gels, and other porous media is important in a wide variety of contexts including biological transport, separations, and polymer dynamics.[1,2] Many experimental and theoretical studies[3-5] have led to the conclusion that the frictional coefficient for probe motion, f, in many such systems can be correlated by an equation of the form

$$f/f_0 = \Psi(\kappa R_h) \quad (1)$$

where R_h is the hydrodynamic radius of the probe, κ is an inverse hydrodynamic screening length characteristic of the porous matrix, and f_0 is the frictional coefficient in the absence of the porous matrix. The precise form of the function is not known, but many have argued,[4] on both experimental and theoretical grounds, that eq 1 can be adequately represented by

$$f/f_0 = \exp(\kappa R_h) \quad (2)$$

In addition, for polymer solutions and gels, κ^{-1} has been identified with the mesh size of the polymer network, ξ.

Support of eqs 1 and 2 by experimental data is, however, not universal,[6] and Park et al.[7] have recently presented evidence that eq 2 is not applicable for probe diffusion in polyacrylamide gels. Their experimental data as well as data available in the literature[8] lead rather to an empirical scaling relationship of the form

$$D/D_0 = \exp[-\beta(R_hC/\xi)^{\zeta}] \quad (3)$$

where D_0 is the diffusion coefficient of the probe in the absence of gel, and D is the probe diffusion coefficient in a gel of polymer concentration C. For C measured in grams per milliliter, β and ζ have the approximate values 13.5 and 0.6, respectively. For these incompletely swollen gels, estimates of the mesh size ξ were provided by photon correlation spectroscopy, and it was found that $\xi = 12.6C^{-0.64}$ where ξ is measured in angstroms.

Measurements of D/D_0 as a function of polymer concentration were made for the probes benzospiropyran (SP) and labeled bovine serum albumin (BSA) by holographic relaxation spectroscopy[7] and for D_2O, sucrose, and urea by a macroscopic boundary-relaxation technique.[8] A theoretical basis for the success of this empirical correlation is at present not obvious, but we believe that the failure of eq 3 to meet the form of eq 1, where κ^{-1} is taken to be ξ, is not particularly disturbing. It is rather quite plausible that the scaling relationship for probe diffusion in a gel or polymer solution should also include a dependence on relative sizes of the probe and the fibers composing the mesh. Such a model has indeed been proposed by Ogston[9] and has been widely used to correlate electrophoretic mobilities of proteins in polyacrylamide gels.[10-12] In addition, Phillips et al.[13] have recently shown that diffusion coefficients in an ordered array of fibers significantly depend on both the interfiber spacing and the fiber size.

The experimental evidence presented previously for eq 3 is, however, not completely convincing for primarily two reasons. First, the data of White[8] were obtained for extensively swollen gels, wheras the measurements of SP and BSA diffusion coefficients and the determinations of the values of ξ were performed with incompletely swollen gels.[7] It is known that ξ can be different for swollen and unswollen gels.[14] Further, the low accuracy of the data of White and the limited range of gel concentrations examined make quantitative interpretation difficult. Second, the data used to generate eq 3 correspond to four small probes, D_2O, urea, sucrose, and SP, and one large probe, BSA. It is expected that interpolation of the behavior between such extremes and extrapolation to larger probes may not be reliable.

Here we report an attempt to clarify the situation for probe diffusion in polyacrylamide gels by determining the gel concentration dependence of probe diffusion coefficients for a wide range of probe sizes in unswollen gels. The experimental technique chosen for these studies, pulsed field gradient (PFG) NMR, is economical and reliable, especially for small probes with their characteristically long nuclear relaxation times, but becomes increasingly time consuming and unreliable for larger probes.[15] We therefore limited the scope of our study to four relatively small probes. Recent improvements in PFG NMR hardware and experimental procedure will permit accurate study of larger probes in the future.[16,17] In addition, we apply the newly developed electrophoretic NMR (ENMR) method[18] to determine the dependence of probe electrophoretic mobility on gel concentration.

For the probes studied, we find that the empirical correlation in eq 3 is supported. Further, electrophoretic mobility measurements for one of the probes provide

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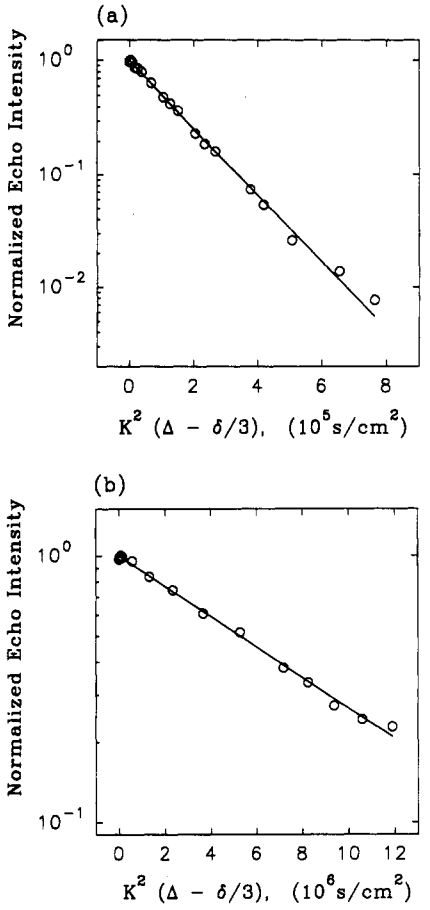

Figure 1. PFG echo attenuation plots for (a) HDO and (b) tetrahexylammonium ion in 0.4 g/mL of polyacrylamide gel. The normalized experimental echo intensities (O) are shown as functions of gradient-pulse current and the solid lines are nonlinear least-square fits of eq 4 to the data (see text).

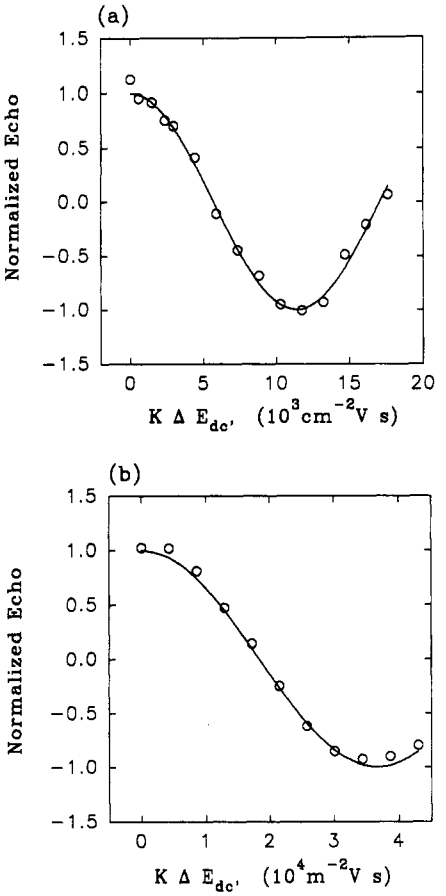

Figure 2. Electrophoretic NMR of tetramethylammonium ion in polyacrylamide gel. Experimental data (O) and fits of eq 5 for (a) 0.05 and (b) 0.30 g/mL gels.

evidence that electrophoretic mobility scales with gel concentration in the same fashion as the tracer diffusion coefficient.

Experimental Methods

Sample Preparation. Gel samples were prepared as described previously.[7] Acrylamide monomer (AA), bisacrylamide (BIS), *N,N,N′,N′*-tetramethylethylenediamine (TMEDA), ammonium persulfate, deuterium oxide (99.9 atom % D), tetramethylammonium iodide (TMA), and tetrahexylammonium chloride (THA) were all obtained from Aldrich (Milwaukee, WI). Reagents were used without further purification.

Gels were prepared by mixing an appropriate amount of stock solution containing 19:1 AA to BIS by weight (0.5 g/mL) with deuterium oxide, ammonium persulfate (4.0×10^{-4} g/mL), TMEDA (0.4 μL/mL) in a 10-mm-o.d. NMR tube. Probes were added to the mixture before gelation to make final concentrations of 2 mM except for HDO and TMEDA, which were already present in the mixture and have higher concentrations. For the electrophoresis experiments, gel preparations were immediately transferred, upon addition of ammonium persulfate, to a U-tube having a cross-sectional area of 0.09 cm^2.[18] Gels were allowed to stand overnight at room temperature before NMR experiments were performed. No efforts were made to prepare completely swollen gels, and the gel-forming solutions were not routinely buffered. However, diffusion experiments on HDO, TMA, and TMEDA for unbuffered gels and buffered gels (100 mM phosphate; pH 7.0) indicate that, at least for these probes, the diffusion coefficients are not sensitive to the presence of the buffer.

PFG and Electrophoretic NMR. The ^1H PFG and ENMR experiments were performed on Brüker spectrometers at 250 MHz and 25 °C with pulsed magnetic field gradient and electrophoresis apparatuses that have been described previously.[19] The field gradient coil was calibrated by means of sample imaging[20] and by diffusion experiments on the reference samples of water, *n*-butanol, *n*-decanol, and glycerol. Both types of experiments gave 24.2 G cm^{-1} A^{-1}. Diffusion and electrophoresis experiments were performed with either the standard Stejskal–Tanner sequence[21] or the stimulated echo variant.[22] The stimulated echo experiment was found to be useful for the THA experiments because of homonuclear scalar coupling and the resulting *J*-modulation effects. Care was taken in the selection

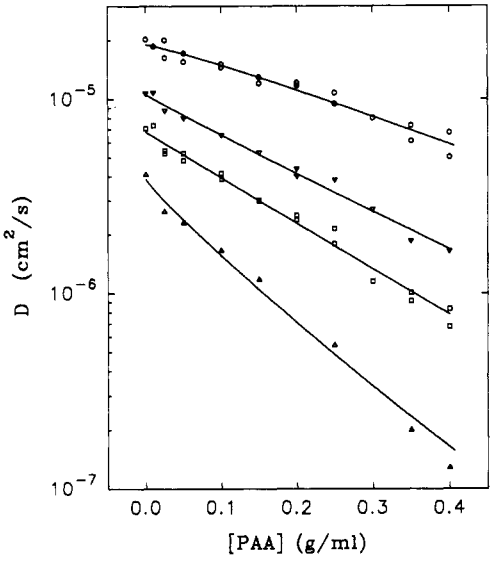

Figure 3. Probe diffusion coefficients in polyacrylamide gels. Shown are both experimental data for HDO (O), TMA ion (▽), TMEDA (□), THA ion (△), and nonlinear least-squares fits of the stretched exponential eq 6 to the data.

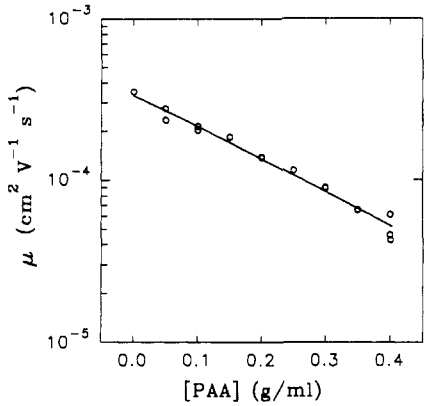

Figure 4. Electrophoretic mobility of TMA in PA gels. The solid line is a fit of the stretched exponential function to the experimental data.

of experimental timing parameters to ensure that sufficient time was allowed after a gradient pulse to allow the decay of resulting field transients before the refocussing radio-frequency pulse or the acquisition period.[15]

Diffusion experiments were typically performed by varying either the gradient pulse amplitude or the duration with constant echo time and diffusion time. Data were analyzed by nonlinear least-squares regression of the equation[21]

$$A/A_0 = \exp[-K^2(\Delta - \delta/3)D] \quad (4)$$

to the resulting echo amplitudes with A_0 and D as parameters. In eq 4, A is the echo amplitude, $K = \gamma g\delta$, γ is the magnetogyric ratio of the observed nucleus, δ is the duration of the field gradient pulse, g is the pulsed field gradient intensity, Δ is the time interval between the leading edges of the field gradient pulses, and D is the tracer diffusion coefficient of the observed species. Echo amplitudes were measured by integration over a well-resolved region of the Fourier transformed echo.

As has been previously described,[18] ENMR experiments were performed in a U-tube with platinum electrodes. Gels were formed directly in the U-tubes, and 10 mM tetraethylammonium chloride in D_2O was layered above the gel sample to provide electrical contact between the gel and the platinum electrodes. The electric field E_{dc} in the U-tube was determined by measuring the potential difference between the electrodes and dividing by the path length in the gel.

In the type of 2D ENMR experiment employed here, the echo intensity is monitored as a function of the amplitude of the applied electric field, E_{dc}, and the migration time Δ is held constant.[23] For a species exhibiting a single electrophoretic mobility μ, the echo signal can be fit by

$$S/S_0 = \cos[KE_{dc}\mu\Delta] \quad (5)$$

where S is the observed signal, and S_0 is the echo intensity given by A in eq 4.

Typical results of PFG NMR experiments are shown in Figure 1, where we plot the normalized echo intensity versus $K^2(\Delta - \delta/3)$ for HDO and THA in a 0.4 g/mL gel sample. The data in Figure 1a were collected with the standard Stejskal–Tanner pulse sequence with Δ = 250 ms and δ = 1 ms, while the data in Figure 1b were collected with the stimulated echo sequence with Δ = 350 ms and δ = 1 ms. The solid curves are nonlinear least-squares fits of the date to eq 4. In both experiments the gradient pulse duration was fixed. Typical results of the ENMR experiments are shown in Figure 2, where we plot echo amplitude and sign as a function of $K\Delta E_{dc}$ for (a) 0.05 and (b) 0.03 g/mL gels.

Results and Discussion

Representative PFG echo attenuation plots for the smallest and largest probes used are shown in Figure 1. Note that the data in both sets are well fit by single exponentials. None of our data sets showed significant deviation from single-exponential behavior. This type of behavior is expected for the long diffusion times (>100 ms) and thus long diffusion distances (approximately microns) compared to probe size and gel mesh size, which are both on the order of nanometers. In addition, the results of our ENMR experiments on TMA are well fit by single cosines as shown in Figure 2. We note that Park et al.[7] found significant deviations from single-exponential behavior in HRS experiments for large values of R_h/ξ.

Table I

probe	R_H, Å	α	ν
HDO	1.0	3.3 ● 0.5	1.13 ± 0.15
TMA[a]	1.8	4.4 ± 0.4	0.97 ± 0.08
TMA[b]	1.8	4.8 ± 0.4	1.03 ● 0.09
TMEDA	2.8	5.4 ± 0.5	0.99 ± 0.08
THA	4.9	7.2 ± 0.8	0.89 ● 0.09
SP[c]	5.3	7.9 ± 0.5	0.95 ± 0.04
BSA[c]	37.4	21.7 ± 2.5	0.91 ± 0.05

[a] Results from regression of D. [b] Results from regression of μ. [c] From ref 7.

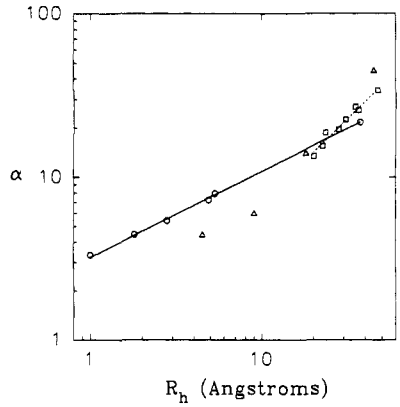

Figure 5. Empirical correlation of the factor α with probe size. Experimental data from Table I (○) as well as a nonlinear least-squares fit (solid line) of the data are shown. For comparison experimental data from Morris and Morris[10] (□) and numerical results of Phillips et al.[13] (△) are displayed.

Figure 3 shows a plot of reduced probe diffusion coefficient versus gel concentration for the four probes used in our study. The lines in Figure 3 are the results of nonlinear least-squares regression of the stretched exponential function

$$D/D_0 = \exp(-\alpha C^\nu) \quad (6)$$

to the data with α and ν as parameters. Figure 4 shows values of the electrophoretic mobility of TMA as a function of gel concentration. The experimental data are also fit by the stretched exponential function in eq 6.

The resulting estimates α and ν and their 95% confidence intervals are listed in Table I. The parameter estimates obtained from the regression of TMA diffusivities and electrophoretic mobilities as a function of gel concentration agree well. This agreement suggests that the frictional coefficients for probe diffusion and electrophoresis scale in the same manner with gel concentration. Also shown in Table I are the values of α and ν for SP and BSA based on a reanalysis of the experimental data of ref 1. There are considerable uncertainties in the values for BSA; the resulting estimates of α and ν are very sensitive to the weighting of the experimental data in the nonlinear regression.

The exponent ν is approximately unity for all of the probes. This value agrees both with previous experimental estimates[7] and with the prediction of Ogston.[9] Also, in agreement with previous results,[7] the estimates of α are strongly correlated with probe size. Figure 5 shows a plot of the estimates of α versus probe hydrodynamic radius,

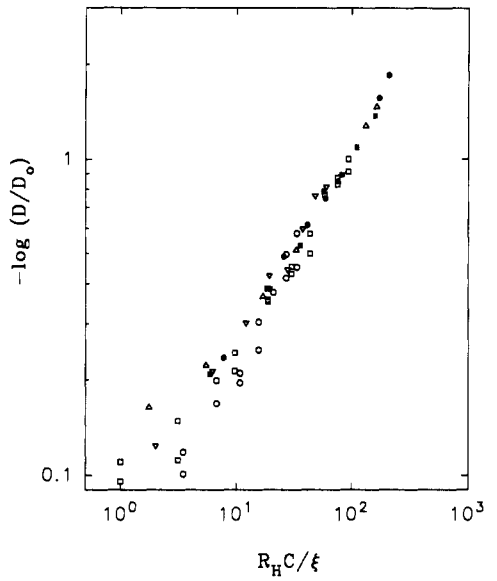

Figure 6. Reduced diffusion coefficients D/D_0 versus the scaling parameter R_hC/ξ for probe diffusion in PA gels: HDO (○), TMA (▽), TMEDA (□), THA (△), SP (■), and BSA (●).

as determined from D_0 with $D_0 = kT/(6\pi\eta R_h)$. The solid line shown in Figure 5 is a nonlinear least-squares fit of

$$\alpha = aR_h^b \tag{7}$$

to the data with a and b as parameters. The resulting estimates of a and b are 3.2 and 0.53, respectively. These values are well within the confidence limits of the previous estimates of 3.03 and 0.59, respectively. Hence, the general form of the empirical correlation of eq 3 is supported.

The weak dependence of the factor α upon probe size found here appears to be unique. For comparison, we show in Figure 5 values of α obtained by Morris and Morris[10] for a number of proteins. These values were obtained by regression of protein electrophoretic mobilities in extensively swollen gels of the same composition as used in our work and show a significantly stronger dependence on probe size. The cause of the difference in observed slopes in the two sets of data is at present not clear. The numerical results of Phillips et al. obtained with eq 9 of ref 13 are also shown in Figure 5. These results for probes in an ordered array of fibers having a radius of 9 Å are not well represented by an equation of the form of eq 7.[9] The numerical values are not significant here, but they suggest that one might expect qualitatively different behavior for probes smaller and larger than the fiber thickness.

The success of the empirical scaling parameter is illustrated by Figure 6 where we show plots of reduced diffusion coefficients D/D_0 versus R_hC/ξ for six probes. The data fall substantially on one curve and suggest that the scaling parameter R_hC/ξ is indeed useful for correlating probe diffusion behavior in incompletely swollen gels.

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References and Notes

(1) Muhr, A. H.; Blanshard, J. M. V. *Polymer* **1982**, *23*, 1012 and references therein.
(2) Deen, W. M. *AIChE J.* **1987**, *33*, 1409 and references therein.
(3) de Gennes, P.-G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.
(4) Cuckier, R. I. *Macromolecules* **1984**, *17*, 252.
(5) Langevin, D.; Rondelez, F. *Polymer* **1978**, *19*, 875.
(6) Ullman, G. S.; Ullman, K.; Linder, R. M.; Phillies, G. D. J. *J. Phys. Chem.* **1985**, *89*, 692.
(7) Park, I. H.; Johnson, C. S., Jr.; Gabriel, D. A. *Macromolecules* **1990**, *23*, 1548.
(8) White, M. L.; Dorion, G. H. *J. Polym. Sci.* **1961**, *55*, 731.
(9) Ogston, A. G.; Preston, B. N.; Wells, J. D. *Proc. R. Soc. London A* **1973**, *333*, 279.
(10) Morris, C. J. O. R.; Morris, P. *Biochem. J.* **1971**, *124*, 517.
(11) Butterman, M.; Tietz, D.; Orban, L.; Chrambach, A. *Electrophoresis* **1988**, *9*, 293.
(12) Rodbard, P.; Chrambach, A. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *65*, 970.
(13) Phillips, R. J.; Deen, W. M.; Brady, J. F. *J. Colloid Interface Sci* **1990**, *139*, 363.
(14) Geissler, E.; Bohidar, H. B.; Hecht, A. M. *Macromolecules* **1985**, *18*, 949.
(15) von Meerwall, E.; Kamat, M. *J. Magn. Reson.* **1989**, *83*, 309.
(16) Gibbs, S. J.; Johnson, C. S., Jr. *J. Magn. Reson.* **1991**, *93*, 395.
(17) Gibbs, S. J.; Morris, K. F.; Johnson, C. S., Jr. *J. Magn. Reson.* **1991**, *94*, 165.
(18) Johnson, C. S., Jr.; He, Q. In *Advances in Magnetic Resonance*; Waugh, J. S., Ed.; Academic Press: San Diego, 1989; Vol. 13, p 131.
(19) Saarinen, T. R.; Woodward, W. S. *Rev. Sci. Instrum.* **1988**, *59*, 761.
(20) Saarinen, T. R.; Johnson, C. S., Jr. *J. Magn. Reson.* **1988**, *78*, 257.
(21) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 282.
(22) Tanner, J. E. *J. Chem. Phys.* **1970**, *52*, 2523.
(23) He, Q.; Hinton, D. P.; Johnson, C. S., Jr. *J. Magn. Reson.* **1991**, *91*, 654.

Registry No. (A)(BIS) (copolymer), 58059-65-7; TMA, 51-92-3; THA, 20256-54-6; TMEDA, 110-18-9.