Hindered Diffusion in Agarose Gels: Test of Effective Medium Model

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ABSTRACT The diffusivities of uncharged macromolecules in gels (D) are typically lower than in free solution (D_∞), because of a combination of hydrodynamic and steric factors. To examine these factors, we measured D and D_∞ for dilute solutions of several fluorescein-labeled macromolecules, using an image-based fluorescence recovery after photobleaching technique. Test macromolecules with Stokes-Einstein radii (r_s) of 2.1–6.2 nm, including three globular proteins (bovine serum albumin, ovalbumin, lactalbumin) and four narrow fractions of Ficoll, were studied in agarose gels with agarose volume fractions (ϕ) of 0.038–0.073. The gels were characterized by measuring the hydraulic permeability of supported agarose membranes, allowing calculation of the Darcy permeability (κ) for each gel sample. It was found that κ, which is a measure of the intrinsic hydraulic conductance of the gel, decreased by an order of magnitude as ϕ was increased over the range indicated. The diffusivity ratio D/D_∞, which varied from 0.20 to 0.63, decreased with increases in r_s or ϕ. Thus as expected, diffusional hindrances were the most severe for large macromolecules and/or relatively concentrated gels. According to a recently proposed theory for hindered diffusion through fibrous media, the diffusivity ratio is given by the product of a hydrodynamic factor (F) and a steric factor (S). The functional form is $D/D_\infty = F(r_s/\kappa^{1/2})\,S(f)$, where $f = [(r_s + r_f)/r_f]^2\phi$ and r_f is the fiber radius. Values of D/D_∞ calculated from this effective medium theory, without use of adjustable parameters, were in much better agreement with the measured values than were predictions based on other approaches. The strengths and limitations of the effective medium theory for predicting diffusivities in gels are discussed.

INTRODUCTION

The widespread use of hydrogels in areas such as liquid chromatography, drug delivery, and therapeutic implants, and the existence of various body tissues with gel-like characteristics (e.g., connective tissue and basement membranes) make it important to understand the rates of diffusion of proteins and other macromolecules through these materials. In gels and in other porous media where the pore diameters, interfiber spacings, or other dimensions of the microstructure are comparable to the size of a diffusing macromolecule, the diffusivity tends to be lower than that in free solution, and the percentage of the reduction increases with molecular size. One approach for interpreting such diffusion data is to assume that the porous material consists of an array of cylindrical or other regularly shaped pores, and to apply a hydrodynamic theory that extends the Stokes-Einstein equation to account for the effects of the pore walls (Deen, 1987). This theory, which is most completely developed for spherical molecules in long, straight pores, includes two factors that influence the average diffusivity of a neutral macromolecule in a pore. There is an increased hydrodynamic drag on the molecule (and a consequent reduction in its mobility) caused by the pore walls, and there are steric restrictions on the positions that can be occupied by a molecule of finite size. Diffusion data obtained in track-etch membranes (which have straight, uniform pores) are generally consistent with the theory (Deen, 1987), so that the pore model is often a good choice for correlating hindered diffusion results in membranes. However, an array of straight pores bears little resemblance to the microstructure of a cross-linked, polymeric gel.

A more realistic model of the microstructure of gels, at least for those with relatively stiff polymer chains, is a randomly oriented array of straight, cylindrical fibers of radius r_f and fiber volume fraction ϕ. This model was proposed by Ogston et al. (1973) to describe diffusion of spherical macromolecules through solutions of linear polymers. An expression for the diffusivity in the polymer solution was derived from stochastic arguments, by considering the probability that a molecule of radius r_s would encounter spaces of sufficient size to permit its movement. The result, which is equally applicable to a fiber-matrix model for diffusion in gels, was

$$\frac{D}{D_\infty} = \exp\left(-\phi^{1/2}\frac{r_s}{r_f}\right) \tag{1}$$

where D and D_∞ are the diffusivities in the gel (or polymer solution) and in free solution, respectively. As used in Eq. 1 and throughout this paper, D is the macroscopically observable diffusivity defined for solute concentrations based on the total gel volume. Macroscopic (or effective) diffusivities in structured media are sometimes defined using concentrations based on the volume of the continuous phase (fluid), and the definition used is not always stated, causing much confusion in the literature. The distinction between these definitions is discussed by Ogston et al. (1973) and by Johansson and Löfroth (1993).

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Equation 1 is based on the probability distribution of fiber spacings used by Ogston (1958) to predict equilibrium partition coefficients for macromolecules in fiber arrays. The fiber-matrix concept is supported by partitioning data for proteins in cross-linked dextran gels (Laurent and Killander, 1964) and agarose gels (Laurent, 1967; Dubin and Principi, 1989; Boyer and Hsu, 1992; Moussaoui et al., 1992; Johnson et al., 1995). Equation 1 successfully correlated diffusion data for macromolecules in various polymer solutions (Ogston et al., 1973), although it was found that the values of r_f needed to fit diffusion data were usually larger than those obtained from partitioning data. This suggests that Eq. 1 may have a tendency to overestimate D/D_∞.

One factor not considered in the derivation of Eq. 1 is hydrodynamic interactions, analogous to those seen in pores, between the fixed fibers and the diffusing macromolecule. Phillips et al. (1989, 1990) addressed this issue by using Stokesian dynamics and generalized Taylor dispersion theory to compute the long-time (macroscopic) diffusivity of a sphere moving through a viscous fluid contained within a periodic array of parallel fibers. It was proposed that the diffusivity could be estimated for other fiber arrangements by treating the fiber array as an effective medium characterized only by its Darcy permeability, κ. The Darcy permeability is the intrinsic conductance of the fiber array for the pressure-driven flow of water, and $\kappa^{1/2}$ is a hydrodynamic screening length or correlation length that is of the order of magnitude of the fiber spacing. Using Brinkman's equation (Brinkman, 1947) to compute the drag on a sphere moving through such a medium, it was suggested that $D/D_\infty \cong F$, where

$$F(r_s/\sqrt{\kappa}) = \left[1 + (r_s/\sqrt{\kappa}) + \frac{1}{3}(r_s/\sqrt{\kappa})^2\right]^{-1}. \qquad (2)$$

Kosar and Phillips (1995) have shown that the Brinkman model for describing screened hydrodynamic interactions gives results equivalent to models of the Kirkwood-Riseman type, in which the effects of fixed polymer chains are described using a distribution of immobile point forces. The only structural information in Eq. 2 is that embedded in κ. Thus, the model implies that macromolecular diffusivities in gels or other fibrous media can be predicted from a single, macroscopic (flow vs. pressure) measurement; the values of r_f and ϕ, and the details of fiber spacing and orientation are not needed. Comparisons of Eq. 2 with the results of rigorous calculations showed fairly good agreement for parallel fibers in square arrays, with $r_s/r_f = 1$ (Phillips et al., 1989). However, subsequent results for smaller or larger values of r_s/r_f and for less uniform fiber arrangements were not as promising (Phillips et al., 1990). Overall, Eq. 2 consistently overestimated D/D_∞.

On the basis of hydrodynamic arguments, Brady (1994) has proposed that the hydrodynamic and steric effects that influence the diffusivity of a macromolecule in a fibrous medium can be separated into two multiplicative factors. According to this approach, the hydrodynamic effect of the fibers can be approximated using the Brinkman result, the function F in Eq. 2. The other factor is a steric or tortuousity effect, given by a function that we term S. The overall functional dependence is of the form

$$\frac{D}{D_\infty} = F(r_s/\sqrt{\kappa})S(f) \qquad (3)$$

$$f = \left(1 + \frac{r_s}{r_f}\right)^2 \phi. \qquad (4)$$

The steric factor S is calculated from the effective diffusivity of a point-size molecule in an array of fibers whose centers are positioned as in the actual system, but that have a radius of $r_f + r_s$. In other words, the volume fraction of fibers is augmented, according to the size of the actual molecule of interest. This adjusted volume fraction of fibers is given in Eq. 4 as f. Results are available to calculate $S(f)$ for various regular or random arrays of fibers (Perrins et al., 1979; Johansson and Löfroth, 1993; Tomadakis and Sotirchos, 1993). Brady (1994) noted that for diffusion normal to the axis of regular arrays of parallel fibers, evaluating S using the results of Perrins et al. (1979) provided excellent agreement between Eq. 3 and the rigorous calculations of Phillips et al. (1989, 1990). Thus, Eq. 3 may provide a way to obtain simple and accurate predictions of macromolecular diffusivities in gels.

Various methods have been employed to measure D for proteins and other compact macromolecules in gels and polymer solutions. Techniques used in recent years include fluorescence recovery after photobleaching (FRAP) (Hou et al., 1990; Jain et al., 1990; Moussaoui et al., 1992; Wattenbarger et al., 1992; Berk et al., 1993; Saltzman et al., 1994; Johnson et al., 1995), pulsed-field-gradient NMR (Gibbs et al., 1992), and holographic interferometry (Kosar and Phillips, 1995). Methods involving transient diffusion into a thick slab of gel (Cameron et al., 1994; Leloup et al., 1990) and dispersion in a chromatography column (Boyer and Hsu, 1992) have also been employed; these approaches are somewhat less direct, in that they measure only the product of D and the gel-to-free-solution partition coefficient. Despite the amount of experimental activity in this area, there is little data with which to test the hydrodynamic theories for hindered diffusion in gels. In particular, there is a paucity of information on the values of κ in gels where diffusion measurements have been made. The purpose of this study was to provide such data by measuring the diffusion coefficients of several well-characterized macromolecules in agarose gels, varying both r_s and ϕ. The Darcy permeability was determined for each gel sample, and r_f for agarose was obtained independently from the literature, so that all of the parameters needed to apply Eqs. 1–3 could be evaluated. We employed the FRAP method to measure diffusivities, as in a recent study of charge effects on partitioning and diffusion in sulfated agarose gels (Johnson et al., 1995). In our previous study, however, there were no measurements of κ.

MATERIALS AND METHODS

Macromolecules

Three fluorescein-labeled proteins, bovine serum albumin (BSA), ovalbumin, and lactalbumin were obtained from Molecular Probes (Eugene, OR). Size-exclusion chromatography indicated that there was no free fluorescein present, so the proteins were used without further purification. Four narrow fractions of Ficoll were obtained by special order from Pharmacia LKB (Piscataway, NJ). The Ficoll samples were labeled with dichlorotryazinyl amino fluorescein (DTAF) (Sigma, St. Louis, MO) using a procedure described by De Belder and Granath (1973). Samples were purified from unreacted label using desalting chromatography columns (Bio-Rad, Hercules, CA) and freeze-dried until used. Fresh aqueous samples were prepared by dissolving the fluorescent macromolecule in a buffer consisting of 0.01 M sodium phosphate and 0.1 M potassium chloride at pH 7.0. In each case the macromolecule concentration was 1 mg/mL.

It should be noted that native agarose has very little net charge and that the amount of fluorescein label attached ($\cong$ 1 fluorescein per Ficoll molecule) was insufficient to give Ficoll a significant charge. Moreover, in highly charged, sulfated agarose gels the diffusivities of the three proteins have been shown to have little or no dependence on ionic strength in the range 0.1–1 M (Johnson et al., 1995). Thus, there should be no significant effects of charge in the experiments reported here.

The characteristics of the seven fluorescein-labeled macromolecules are summarized in Table 1. For Ficoll, the weight-average molecular weight (M_w) and polydispersity index (M_w/M_n, where M_n is number-average molecular weight) are values supplied by the manufacturer. The diffusivities and Stokes-Einstein radii were obtained from the present data, as described below.

Preparation of gels

Gels were made by first adding 10 ml of the phosphate-KCl buffer to a measured amount of agarose powder (Type VI; Sigma, St. Louis, MO) in a 20 ml glass vial, and then placing the resulting slurry in a 90°C oven for 3 h. The vial, which was sealed to prevent evaporation, was rotated by hand periodically to ensure adequate mixing. Two glass plates and several rectangular glass microslide chambers with dimensions of 0.3 × 3 × 50 mm (Vitro Dynamics, Rockaway, NJ) were also heated. To cast membranes for hydraulic permeability measurements, a 2.5 cm diameter piece of woven polyester mesh (Spectrum Medical, Houston, TX) was placed on one of the glass plates. The mesh formed a square pattern with a fiber radius of 20 μm, a center-to-center fiber spacing of 93 μm, and a thickness of 70 μm. The hot agarose was quickly poured onto the mesh, and the second plate used to form a sandwich. The glass plates were then clamped together, taking care to ensure that air bubbles were not trapped in the gel. To form a gel for diffusion measurements using the same batch of agarose, the heated solution was drawn into one end of a microslide by capillarity; this was done immediately after casting the membrane. Separate microslide gels were prepared for use with each of the seven test macromolecules. After the gels (in membrane or microslide) cooled to room temperature, they were immersed in the phosphate-KCl buffer and stored overnight at 4°C. Samples were prepared with agarose concentrations ranging from 3.9 to 7.5% (w/v). Weight fractions were converted to volume fractions by dividing by 1.025 (Johnson et al., 1995).

TABLE 1 Properties of test macromolecules

Molecule	M_W	M_W/M_N	D_∞ (10^{-7} cm^2/s)	r_s (nm)
Lactalbumin	14,200		10.10 ± 0.70	2.12
Ovalbumin	45,000		7.15 ± 0.77	3.00
BSA	68,000		5.97 ± 0.44	3.59
Ficoll 21K	21,300	1.22	7.06 ± 0.42	3.03
Ficoll 37K	37,400	1.18	5.63 ± 0.18	3.80
Ficoll 61K	60,700	1.15	4.45 ± 0.18	4.82
Ficoll 105K	105,000	1.13	3.44 ± 0.14	6.23

Diffusion coefficients are given as mean ± SD for 5 measurements, corrected to 20°C.

Hydraulic permeability measurements

The Darcy permeability of each agarose sample was obtained by measuring the hydraulic permeability of the mesh-reinforced membrane, as described by Johnson and Deen (1995). Briefly, the gel membrane was mounted on a porous frit inside a 3 mL ultrafiltration cell (Model 3; Amicon, Beverly, MA), and the phosphate-KCl buffer forced through the membrane at a constant pressure drop of 17 kPa using compressed nitrogen. The transmembrane pressure drop was monitored using a pressure transducer (Model DP15, Validyne Engineering, Northridge, CA) and the flow rate was determined by collecting and weighing the filtrate. The thickness of the hydrated membrane was measured using a micrometer, by placing the membrane between two microslides of known thickness. The Darcy permeability was calculated as

$$\kappa = \frac{\mu Q L}{\beta A \Delta P} \tag{5}$$

where μ is the viscosity of water, Q is the filtrate volume per unit time, L is the membrane thickness, β (= 0.495) is a correction factor to account for the presence of the polyester mesh support, A (= 1.5 cm^2) is the exposed area of the membrane, and ΔP is the pressure drop.

Diffusion measurements

Samples for diffusion measurements were prepared by drawing a solution of the fluorescent macromolecule into a microslide containing the agarose gel, using a syringe attached to the microslide by silicon tubing. The gel and solution were allowed to equilibrate for 2 h, which was ample time to achieve diffusional equilibrium at the test locations in the gel (typically centered ~100 μm from the gel-solution interface). Diffusion coefficients were determined using an image-based FRAP technique (Berk et al., 1993; Johnson et al., 1995). The image-based FRAP technique using a spatial Fourier transform has the advantage of giving results that are insensitive to the actual radius of the bleached spot. This is especially important for measurements in gels, such as agarose, that scatter light, because the bleached radius changes as a function of depth in the sample. The consequent uncertainties in the true bleached radius make it difficult to obtain reliable results using a direct photometric analysis. For each test macromolecule, five measurements each were made of the diffusion coefficient in the gel (D) and in free solution (D_∞). To allow for complete recovery of the bleached areas (~20 μm initial radius), the gel measurements were alternated with the free solution measurements. The room temperature was recorded (23 - 29°C), and all diffusion coefficients were corrected to 20°C by assuming that $D\mu/T$ (or $D_\infty\mu/T$) is constant, where T is absolute temperature.

RESULTS

The free-solution diffusivities measured for the three proteins and four Ficoll fractions are given in Table 1. Also shown is the corresponding Stokes-Einstein radius (r_s), calculated as

$$r_s = \frac{k_B T}{6\pi\mu D_\infty} \tag{6}$$

where k_B is Boltzmann's constant. The Stokes-Einstein radii shown for the proteins are very close to those obtained previously using various methods, as summarized by John-

son et al. (1995). Likewise, the values of r_s for Ficoll are all within 6% of those measured by quasielastic light scattering using unlabeled samples (Oliver et al., 1992). Overall, the test macromolecules had radii ranging from 2.1 to 6.2 nm.

The six agarose samples used, identified as A-F, had the properties summarized in Table 2. Two samples of each were made with agarose volume fractions (ϕ) of 0.038, 0.055, and ~0.072. The measured Darcy permeability (κ) varied by an order of magnitude over this range of gel concentrations, decreasing as gel concentration was increased. Individual measurements of κ were reproducible to within 1–2%, so that the differences in Table 2 between nominally identical gels were evidently due to differences in the gel microstructure. This variability in κ among agarose samples, noted previously in Johnson and Deen (1995), is the reason care was taken to measure Darcy permeabilities in gels made from the same agarose solutions as the samples used for the diffusion experiments. Although the gel concentration is the main determinant of the Darcy permeability for agarose, there is also a significant effect of the applied pressure, κ decreasing in an approximately linear manner with increasing ΔP (Johnson and Deen, 1995). Values of the Darcy permeability extrapolated to $\Delta P = 0$ are shown in Table 2 as κ_0. The extrapolation of κ to zero applied pressure was done by using values of $\partial\kappa/\partial(\Delta P)$ estimated previously at the respective gel concentrations (Johnson and Deen, 1995). The uncertainty in κ_0 was calculated using a propagation-of-errors analysis, based on the uncertainties in κ and $\partial\kappa/\partial(\Delta P)$. The uncertainty in κ was estimated as 1%, whereas the uncertainty in $\partial\kappa/\partial(\Delta P)$ was 12–23%, based on the standard errors of the slopes of linear regressions of κ versus ΔP for the various gel concentrations (Johnson and Deen, 1995). As shown in Table 2, the estimated uncertainties in κ_0 were 6–14%.

Also shown in Table 2 are the values of κ predicted from a correlation given by Jackson and James (1986) for fibrous media,

$$\frac{\kappa}{r_f^2} = -\frac{3}{20\phi}(\ln\phi + 0.931) \tag{7}$$

where r_f is the fiber radius. For these calculations we assumed an average fiber radius for agarose of 1.9 nm, which is the number-average value obtained from small angle x-ray scattering data (Djabourov et al., 1989). (This and other measurements of r_f for agarose are discussed by Johnson et al., 1995.) It is seen that Eq. 7 is in excellent agreement with the values of κ_0 at the highest gel concentration, but the agreement worsens as gel concentration is decreased, the correlation underpredicting the (extrapolated) experimental results by a factor of 3–4 at the lowest gel concentration. Previous measurements of Darcy permeability for agarose show similar deviations from Eq. 7 (Johnson and Deen, 1995); the reason for these deviations is not clear.

TABLE 2 Properties of agarose gels

Gel	ϕ	κ (data) (nm^2)	κ_0 (nm^2)	κ (eq. 7) (nm^2)
A	0.038	107	134 ± 8	34
B	0.038	88	115 ± 7	33
C	0.055	30	53 ± 6	19
D	0.055	17	40 ± 6	20
E	0.072	5.0	13 ± 1	13
F	0.073	7.8	16 ± 1	13

The values of κ_0 are extrapolations of the measured values of κ to zero pressure difference. The uncertainties shown for κ_0 are based on a propagation-of-errors analysis, as described in the text.

Table 3 and Figs. 1-3 show the results for the gel-to-free solution diffusivity ratio, D/D_∞. Values of D and D_∞ measured in succession were paired to compute an individual value for the ratio, and the results averaged over the five repetitions to obtain the mean ± SD values shown in Table 3. For each test macromolecule, the diffusivity ratio decreased as the gel concentration increased. For any given gel sample, the diffusivity ratio generally decreased as the probe radius increased. In other words, as one would expect, the hindrances to diffusion were most severe for large macromolecules and/or relatively concentrated gels. The significance of the theoretical curves in Figs. 1–3 is discussed below.

DISCUSSION

The present experiments were designed to test the diffusivity predictions given by Eqs. 1–3. As mentioned earlier, the function S in Eq. 3 may be evaluated using available results for various arrangements of cylindrical barriers. Two such arrangements are considered here. Using the result of Perrins et al. (1979) for transport perpendicular to the axis of a square array of cylinders, one gets

$$S(f) = \frac{1}{1-f}\left\{1 - 2f\left[1 + f - \frac{0.305827f^4}{1 - 1.402958f^8 - 0.013362f^8}\right]^{-1}\right\}. \tag{8}$$

Equation 8 was obtained by dividing Eq. 14 of Perrins et al. (1979) by 1 - f. (The result of Perrins et al. is analogous to a transmembrane diffusivity based on external concentrations, so that it must be divided by the partition coefficient, which for a point-size molecule in a fiber array with volume fraction f is 1 - f.) Johansson and Löfroth (1993) used Brownian dynamics simulations to calculate diffusivities for spherical macromolecules moving through random, overlapping arrays of polymer chains. Their calculations were for diffusing molecules of finite r_s, but without hydrodynamic interactions, so that their diffusivity ratios were equivalent to S. Using a curve fit to the simulations for straight polymer chains with $f < 3$, their results are expressed as

$$S(f) = \exp(-0.84f^{1.09}). \tag{9}$$

Tomadakis and Sotirchos (1993) reviewed many other results that could be used to evaluate S, including their own

TABLE 3 Diffusivity ratios (D/D_∞) for individual gel samples

Molecule	A	B	C	D	E	F
Lactalbumin	0.62 ± 0.04	0.63 ± 0.03	0.47 ± 0.03	0.45 ± 0.05	0.34 ± 0.03	0.30 ± 0.03
Ovalbumin	0.57 ± 0.05	0.55 ± 0.03	0.38 ± 0.02	0.44 ± 0.02	0.25 ± 0.03	0.32 ± 0.03
BSA	0.61 ± 0.07	0.53 ± 0.04	0.40 ± 0.01	0.36 ± 0.03	0.27 ± 0.02	0.28 ± 0.03
Ficoll 21K	0.62 ± 0.03	0.60 ± 0.06	0.46 ± 0.02	0.47 ± 0.02	0.26 ± 0.02	0.39 ± 0.03
Ficoll 37K	0.55 ± 0.04	0.53 ± 0.03	0.42 ± 0.05	0.40 ± 0.02	0.26 ± 0.01	0.30 ± 0.02
Ficoll 61K	0.48 ± 0.03	0.45 ± 0.03	0.37 ± 0.09	0.33 ± 0.02	0.21 ± 0.00	0.23 ± 0.01
Ficoll 105K	0.43 ± 0.03	0.35 ± 0.03	0.28 ± 0.02	0.25 ± 0.01	0.20 ± 0.02	0.23 ± 0.02

All values shown are mean ± SD for 5 measurements.

Monte Carlo simulations for diffusion of point-size molecules through randomly oriented, overlapping arrays of cylinders. (To obtain S from any of the results summarized by Tomadakis and Sotirchos, one must divide their "inverse formation factor" by $1 - f$, as done above to obtain Eq. 8; f is related to their "matrix volume fraction," ϵ, by $\epsilon = 1 - f$.)

Equation 8 implies that $S \rightarrow 1 - f$ for $f \rightarrow 0$, in agreement with the result for diffusion normal to a dilute array of parallel cylinders (Koch and Brady, 1986). Approximately the same limiting behavior for $f \rightarrow 0$ is exhibited by Eq. 9; the theoretical limit for randomly oriented cylinders is $S \rightarrow 1 - (2/3)f$ (Koch and Brady, 1986). The main distinction between regular and random arrays of fibers is in their behavior at large f. This is illustrated in Fig. 4, which compares the results for S obtained from Eqs. 8 and 9. The results for square and random arrays are very similar for $f < 0.5$, as shown also by Tomadakis and Sotirchos (1993). However, for fibers in a square array there is a critical volume fraction $f_c = \pi/4 = 0.785$ at which all fibers touch, so that for diffusion normal to the fibers $S = 0$ for $f \geq f_c$. For random arrays there is no such sharp cutoff in S. The behavior of $S(f)$ for large f is important for the present data, in that $f = 1.33$ for the largest Ficoll in the most concentrated agarose gel. The fact that there were measurable diffusivities for $f > f_c$ strongly suggests a disordered arrangement of the agarose fibers. Accordingly, Eq. 9 was used for all comparisons with the data.

The measured diffusivity ratios are compared with predictions from Eqs. 1–3 in Figs. 1–3. In calculating the hydrodynamic contribution to the diffusional hindrance, we used the Darcy permeability extrapolated to zero pressure drop, κ_0, because that value should be most representative of the gel microstructure during the diffusion measurements. The differences in κ_0 between pairs of gel samples affected the predicted diffusivities by <5%, so that only the average of the two predictions is shown for each case. The uncertainties in κ_0 reported in Table 2 yielded uncertainties in the theoretical curves from Eqs. 2 and 3 of only 1–6%, depending on gel concentration and molecular size. Thus, the errors introduced in extrapolating the measured Darcy permeabilities to zero pressure drop had little effect on predictions of the hydrodynamic hindrance factor, F. At each gel concentration, the effective medium theory of Brady (1994) yielded much better predictions than the other two approaches, which systematically overestimated D/D_∞. Closer inspection reveals that, although the measured values of D/D_∞ for molecules of intermediate size were predicted

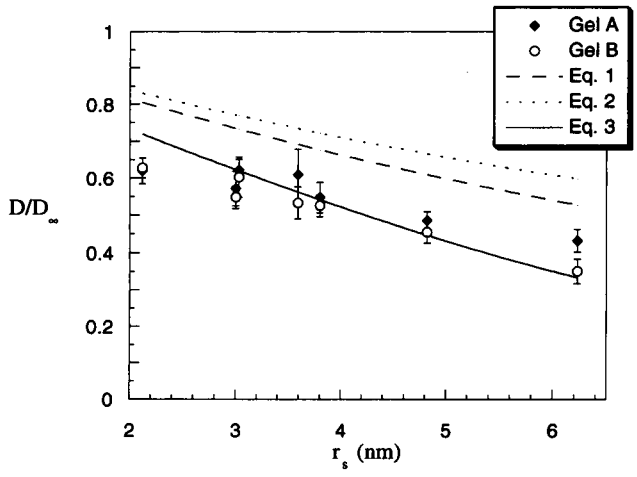

FIGURE 1 The reduced diffusion coefficents, D/D_∞, of proteins and Ficoll in 3.9% agarose gels. "Gel A" and "gel B" are two different samples at this concentration. The data are given as mean ± SD. The three curves represent predictions from the Ogston diffusion theory (Eq. 1), the Brinkman equation (Eq. 2), and the effective medium theory of Brady (Eq. 3). Note that Eq. 3 agrees best with the data.

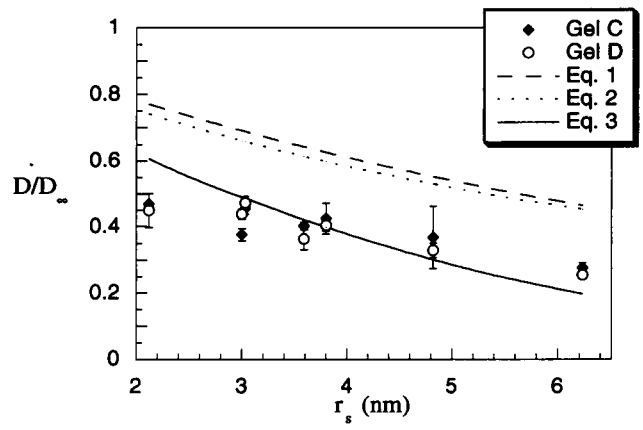

FIGURE 2 The reduced diffusion coefficents, D/D_∞, of proteins and Ficoll in 5.6% agarose gels. "Gel C" and "gel D" are two different samples at this concentration. The data are given as mean ± SD. The three curves represent predictions from the Ogston diffusion theory (Eq. 1), the Brinkman equation (Eq. 2), and the effective medium theory of Brady (Eq. 3). Note that Eq. 3 agrees best with the data.

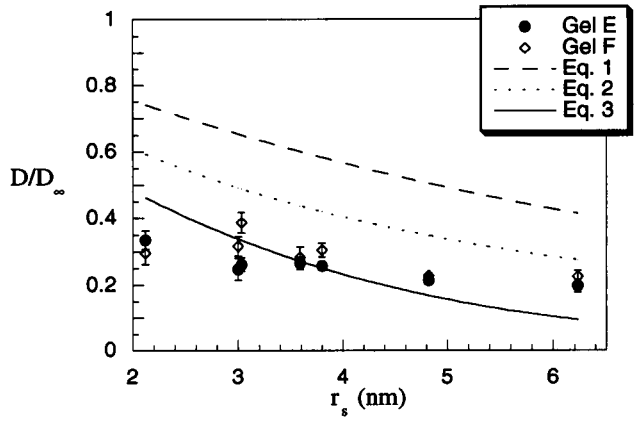

FIGURE 3 The reduced diffusion coefficents, D/D_∞, of proteins and Ficoll in 7.4% (gel E) and 7.5% (gel F) agarose gels. The data are given as mean ± SD. The three curves represent predictions from the Ogston diffusion theory (Eq. 1), the Brinkman equation (Eq. 2), and the effective medium theory of Brady (Eq. 3). Note that Eq. 3 agrees best with the data.

very accurately by Eq. 3, the model gave a stronger dependence of D/D_∞ on r_s than was observed. These differences in slope notwithstanding, the agreement between the data and the predictions of Eq. 3 seems to us very impressive, given that there were no adjustable parameters in the calculations.

It was mentioned earlier that Eq. 3 gives values of D/D_∞ that agree very closely with the rigorous computational results of Phillips et al. (1989, 1990) for the diffusion of spheres of finite size normal to the axis of parallel arrays of fibers. One of the requirements in applying Eq. 3 (or Eq. 2) to actual gels is that κ be known. As seen in Table 2, the correlation given by Eq. 7 can be counted on to give only the correct order of magnitude for κ. In a previous study (Johnson et al., 1995) we estimated κ for sulfated agarose beads from Eq. 7 and, because of what seems now to have been a cancellation of errors, obtained fairly accurate predictions of protein diffusivities from Eq. 2. Because κ was

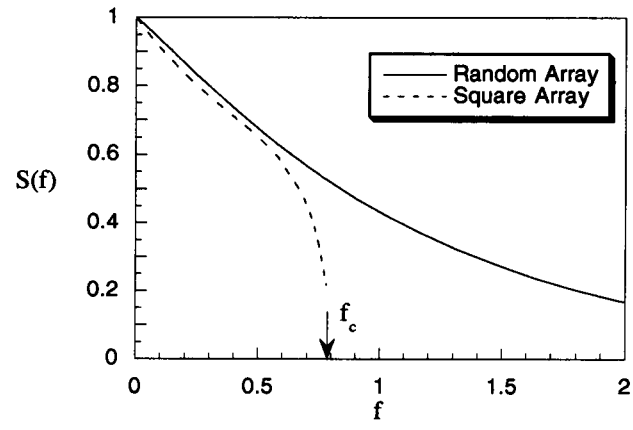

FIGURE 4 The steric factor, $S(f)$, for random (Eq. 9) and square (Eq. 8) arrays of fibers. The critical value of f for a square array is $f_c = 0.785$, as indicated by the arrow. For a square array f cannot exceed f_c.

measured for each gel sample in the present study, we assume that the discrepancies in slope in Figs. 1–3 have more to do with inaccuracies in the steric factor (S) than in the hydrodynamic factor (F). Although, as already discussed, the diffusion data are inconsistent with a regular, parallel arrangement of agarose fibers, the actual arrangement may differ from the random, overlapping network produced by the simulations leading to Eq. 9. In other words, at least for gels with relatively stiff polymer chains, the application of Eq. 3 may be limited more by an inadequate knowledge of the actual microstructure than by any inherent limitations in the effective medium approach.

There are potential effects of polymer structure on the diffusivities of macromolecules in gels. The most successful theory tested here, which uses Eqs. 2 and 9 in Eq. 3, is based on fibers that are straight and immobile. Agarose fibers are expected to be relatively rigid, because they are aggregates formed from multiple polysaccharide chains. Indeed, there is little motion of agarose chains detectable by dynamic light scattering (Mackie et al., 1978), so that assuming that the fibers are stationary on the time scale of macromolecule diffusion should be an excellent approximation. Fibers in alginate gels have similar characteristics. However, the chains in gels formed from cross-linked polymers, such as polyacrylamide and dextran, may exhibit motions comparable to that of a diffusing solute. For example, Tanaka et al. (1973) reported a collective diffusivity for a 5% polyacrylamide gel, by dynamic light scattering, of 2.4×10^{-7} cm^2/s. An effect of fiber curvature on S is revealed by simulations of Johansson and Löfroth (1993) in which chains were constructed having various persistence lengths; when the persistence length was made sufficiently small, there were significant increases in S. However, those simulations were still based on immobile polymer chains. A theory capable of describing the effects of local motions in a polymer chain on F and/or S would be of considerable interest.

There are similarities as well as differences between the diffusion of rigid spheres in gels and in semidilute (entangled) polymer solutions. In extremely dilute gels or solutions, there is negligible hindrance to diffusion, and the diffusivity is given by the Stokes-Einstein equation (a rearrangement of Eq. 6). When the system is not quite as dilute, but the diffusing macromolecule is still smaller than the typical separation between polymer chains ($r_s < \kappa^{1/2}$), the steric and hydrodynamic factors governing diffusion should be very similar in the two situations. Kosar and Phillips (1995) discuss theoretical evidence that hydrodynamic screening in a polymer solution is similar to that for an array of fixed obstacles, both conforming to the Brinkman model. For large macromolecules or particles ($r_s \gg \kappa^{1/2}$), however, a gel and a polymer solution will behave quite differently. Whereas the cross-linked structure of the gel will eventually prevent translation of the particle, entanglements in a polymer solution can break and reform, so that the particle diffusivity does not fall to zero. Instead, as discussed by Kosar and Phillips (1995), there is a second Stokes-Einstein regime, with the applicable viscosity now

being that of the polymer solution, rather than that of the solvent.

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