Letters to the Editor

A Hydrodynamic Model for Hindered Diffusion of Proteins and Micelles in Hydrogels

Recently, several papers have been published that address the important topic of hindered diffusion of macromolecules in hydrogels. Two papers in particular, by Johnson et al. (1996) and Clague and Phillips (1996), present models of hindered diffusion that account for hydrodynamic interactions, albeit in different ways. The model of Johnson et al. (1996) makes use of an effective medium approach based on Brinkman's equation, whereas Clague and Phillips (1996) explicitly calculate those interactions for a spherical solute suspended in a liquid-filled, three-dimensional medium of randomly placed cylindrical fibers. It is the purpose of this note to combine elements from those two papers to form a hydrodynamic model of hindered diffusion that does not rely on an effective medium approximation, and to evaluate the new model by comparing its predictions with published data.

Both Johnson et al. (1996) and Clague and Phillips (1996) adopt the proposition of Brady (1994) that the diffusivity in a gel can be written as a product of factors F and S, where F accounts for hydrodynamic effects and S for steric or tortuosity effects. The resulting expression is

$$\frac{D}{D_0} = FS(f), \tag{1}$$

where D/D_0 is the ratio of the diffusivity in the gel to that in solution at infinitely dilute solute concentrations. In Eq. 1, the parameter f is an adjusted volume fraction given by

$$f = \left(1 + \frac{r_s}{r_f}\right)^2 \phi, \tag{2}$$

where ϕ is the actual fiber volume fraction and r_s and r_f are the radius of the solute and fibers, respectively.

The Brinkman or effective medium result for the hydrodynamic factor F is

$$F = \left(1 + \frac{r_s}{\sqrt{k}} + \frac{1}{9}\left(\frac{r_s}{\sqrt{k}}\right)^2\right)^{-1}, \tag{3}$$

where k is the hydraulic permeability. As discussed by Solomentsev and Anderson (1996), the factor of 1/9 in Eq. 3 is correct when it is the friction on a sphere moving through an effective medium that is of interest. The factor of 1/3 that is more commonly used (cf. Phillips et al., 1989) pertains to the friction on a stationary sphere in the midst of an imposed flow and contains a contribution from the pressure gradient needed to drive that flow. It is therefore correct to use Eq. 3 in effective medium models of hindered diffusion.

To provide an alternative to Eq. 3, Clague and Phillips (1996) performed numerical simulations in which the frictional drag on a sphere suspended in a disordered array of cylinders is calculated and ensemble-averaged over many fiber configurations. They correlated their results for F with a stretched exponential of the form

$$F(\lambda, \phi) = e^{-a\phi^b}. \tag{4}$$

Results for the parameters a and b are given in Table 1 for a range of values of λ, where λ is the ratio of fiber radius to solute radius, $\lambda = r_f/r_s$. To avoid the need for interpolation between the values in the table, one can find a and b by using the expressions

$$a = 3.727 - 2.460\lambda + 0.822\lambda^2 \tag{5}$$

and

$$b = 0.358 + 0.366\lambda - 0.0939\lambda^2. \tag{6}$$

Eqs. 5 and 6 were obtained by fitting the results in Table 1 to a quadratic polynomial in λ. When used to evaluate F in Eq. 1, they yield predictions for D/D_0 that are nearly indistinguishable from those obtained by interpolating between the tabulated results. Clague and Phillips (1996) note that Eqs. 3 (with the factor of 1/3) and 4 give very similar results when λ is comparable to unity (see their Fig. 9). However, in general, Eq. 3 predicts values of F that are too low.

The steric factor $S(f)$ can be calculated in a number of ways. Clague and Phillips (1996) use an expression derived by Tsai and Strieder (1985) by using the method of volume averaging. However, that expression is limited to lower volume fractions than the result used by Johnson et al. (1996), which is

$$S(f) = e^{-0.84f^{1.09}}. \tag{7}$$

Eq. 7 provides a convenient representation of stochastic simulations performed by Johansson and Löfroth (1993), and it also agrees well with the result of Tsai and Strieder (1985) for small enough f, or when $f < 0.7$. Combining Eqs. 4 and 7 as suggested by Eq. 1 then yields

$$\frac{D}{D_0} = e^{-0.84f^{1.09}} e^{-a\phi^b}, \tag{8}$$

a result that is a product of stretched-exponential terms of the form often found useful in describing hindered diffusion data. One advantage of Eq. 8 over previous models is that it does not rely on an effective medium or Brinkman approximation, and hence there is no need to estimate the hydraulic

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TABLE 1 Hindered diffusion parameters for Eq. 8

λ	a	b
0.1	3.483	0.354
0.2	3.248	0.434
0.29	2.871	0.477
0.4	3.146	0.532
0.6	2.526	0.518
0.75	2.500	0.600
1.0	1.900	0.600
2.0	2.114	0.719

permeability k; good estimates for the ratio $\lambda = r_f/r_s$ and the fiber volume fraction ϕ are all that is needed.

Eq. 8 also provides better agreement with experimental data than does the corresponding effective medium model, particularly when $\lambda < 1$. In Figs. 1 and 2, diffusivities of the proteins RNAse and bovine serum albumin (BSA) in polyacrylamide gel are plotted for a range of gel volume fractions. These data were obtained by Tong and Anderson (1996) by using fluorescence recovery after photobleaching (FRAP) and by Park et al. (1990) by using holographic relaxation spectroscopy. The results of Park et al. (1990) shown in Fig. 2 were obtained by using Eq. 17 in Tong and Anderson's (1996) paper. The parameters r_f and r_s used in the calculations for Figs. 1 and 2 are shown in Table 2 and are the same as those given by Tong and Anderson (1996). Also like Tong and Anderson (1996), the hydraulic permeability was calculated from

$$k = (2.64 \times 10^{-6}\ \text{cm}^2)\phi^{-1.42}, \qquad (9)$$

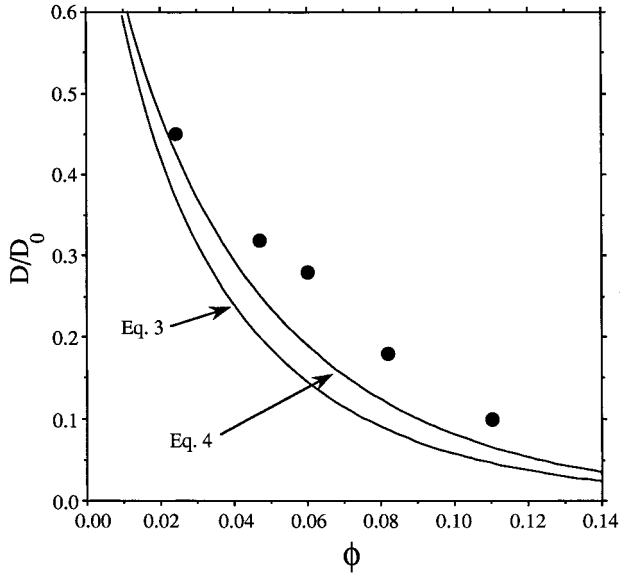

FIGURE 1 The normalized diffusivity D/D_0 is plotted versus volume fraction ϕ for the solute RNAse in polyacrylamide gel. The lower curve is obtained by using Eq. 3 and the upper curve by using Eq. 4 to calculate the hydrodynamic factor F in Eq. 1. ●, data reported by Tong and Anderson (1996).

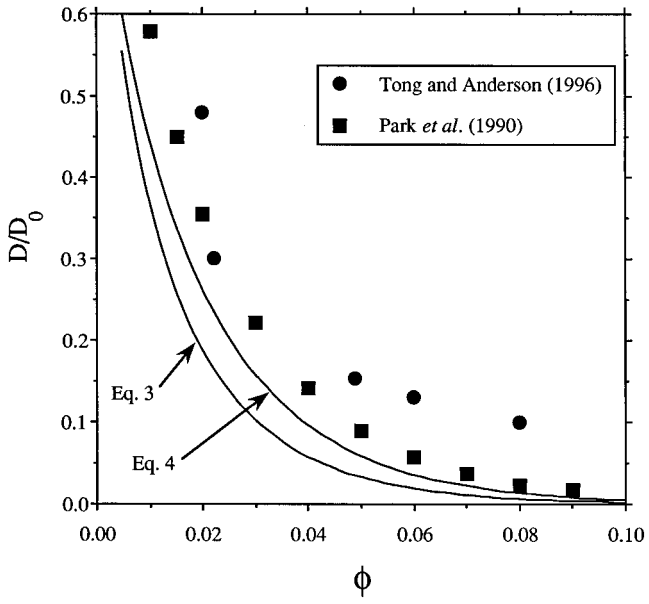

FIGURE 2 Same as Fig. 1 for the solute BSA in polyacrylamide gel. ● and ■, data reported by Tong and Anderson (1996) and Park et al. (1990), respectively.

which they obtained by using the measurements of Tokita (1993). In both figures, the lower curves show the prediction of Eq. 1 when the hydrodynamic factor is calculated by using the Brinkman result (Eq. 3) with the steric factor given by Eq. 7. The upper curves, which are in better agreement with the data, were calculated by using Eq. 8.

An analogous comparison to that in Figs. 1 and 2 is made in Fig. 3 for the case of BSA diffusing in a calcium alginate gel, a system studied by Amsden (1998). Calcium alginate is comprised of polymers that are blocks of poly(mannuronic acid), or M-blocks, and blocks of poly(guluronic acid), or G-blocks. During gelation the G-blocks bind together, and x-ray analysis has yielded a dimension of 5.41 Å for the G-G dimers (Nilsson, 1992). Using this result and the reasonable assumption that the M-block fibers would be half as large as the G-G dimers, Amsden (1998) estimates a

TABLE 2 Radii of solutes and gel fibers

Gel or Solute	Radius (Å)	References
RNAse	20	Tong and Anderson (1996)
Myoglobin	20	Kong et al. (1997)
Lactalbumin	21	Johnson et al. (1996)
Ovalbumin	30	Johnson et al. (1996)
BSA	36	Johnson et al. (1996)
$C_{12}E_6$	35	Kong et al. (1997)
$C_{12}E_8$	28	Kong et al. (1997)
$C_{12}E_{10}$	31	Kong et al. (1997)
Calcium alginate	3.6	Amsden (1998)
K^+-κ-carrageenan	5.1	Johansson et al. (1993)
Polyacrylamide	6.5	Tong and Anderson (1996)
Agarose	19	Johnson et al. (1996)

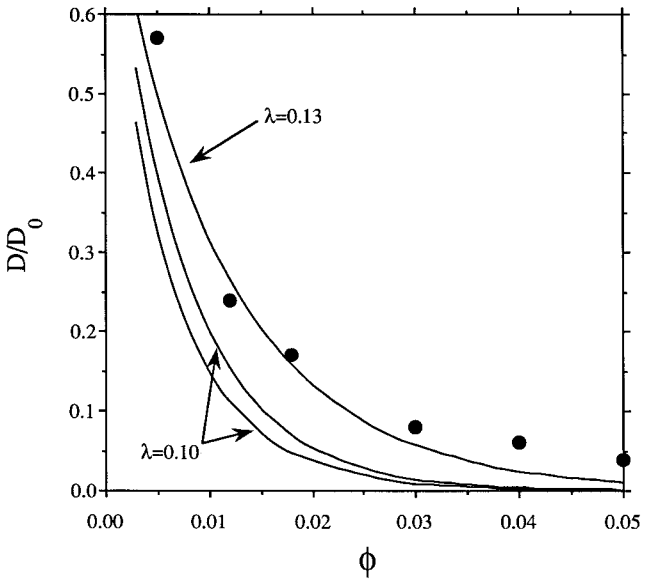

FIGURE 3 Same as Figs. 1 and 2 for the solute BSA in calcium alginate gel. The two upper curves are obtained by using Eq. 4 to calculate F with $\lambda = 0.10$ or $\lambda = 0.13$; the lower curve pertains to Eq. 3 with $\lambda = 0.10$. ●, data reported by Amsden (1998).

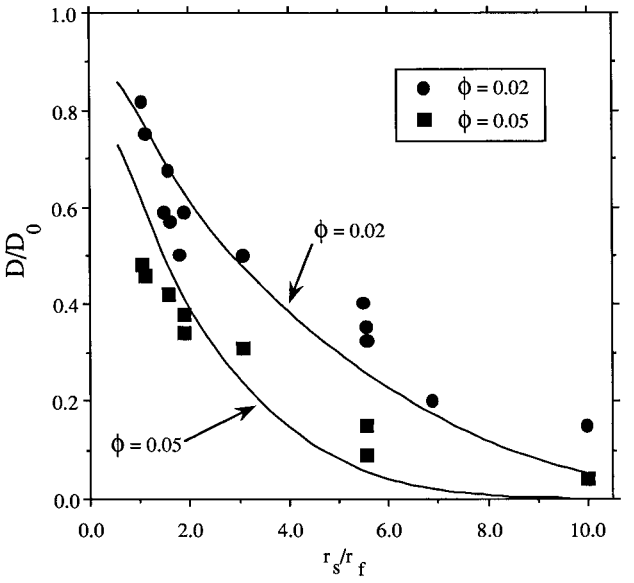

FIGURE 4 The normalized diffusivity D/D_0 is plotted versus normalized solute radius r_s/r_f for the gel/solute systems listed in Table 3. Solid curves are the predictions of Eq. 8.

number-average fiber radius of 3.6 Å for alginate. With the Stokes-Einstein radius of 36 Å for BSA, this yields $\lambda = 0.10$. Under these conditions, Eq. 1 underpredicts the data when either Eq. 3 (lower curve) or Eq. 4 (middle curve) is used to calculate the hydrodynamic factor F. Here the permeabilities needed in Eq. 3 were calculated by using the same expression given by Amsden (1998):

$$k = 0.31 r_f^2 \phi^{-1.17}. \quad (10)$$

The closeness of the results obtained using Eqs. 3 and 4 to calculate F is partly a result of the very strong steric contribution to D/D_0 at this small value of λ. The steric contribution is independent of hydrodynamic interactions.

Also because $\lambda \ll 1$ here, the predicted diffusivities are very sensitive to the value used for the fiber radius. Increasing the estimated radius r_f from 3.6 Å to 4.6 Å, for example, increases λ from 0.10 to 0.13, and the agreement between theory and experiment at $\lambda = 0.13$ is excellent. Because 4.6 Å is well below the measured dimension of 5.41 Å for the G-G dimers, and because the appropriate radius for calculating either steric or hydrodynamic effects is not necessarily the number-average radius, the theory and data may be considered consistent. However, given the sensitivity to the choice of λ when $\lambda \ll 1$, it would be difficult to make quantitatively accurate, a priori predictions at these conditions.

Because the theory depends on both the dimensionless fiber radius λ and the volume fraction ϕ, the effect of varying $\lambda = r_f/r_s$ is also of interest. In Fig. 4, results for D/D_0 are plotted for a range of r_s/r_f at two volume fractions, $\phi = 0.02$ and $\phi = 0.05$. The data shown were collected from a variety of systems, including four gels and seven solutes (proteins and micelles), and these are listed along with appropriate references in Table 3. The theory (Eq. 8)

TABLE 3 Sources of data in Figure 4

Gel	Solute(s)	r_s/r_f	ϕ	References
Agarose	Myoglobin	1.1	0.02,0.05	Johnson et al. (1996)
	Lactalbumin	1.1	0.02,0.05	Kong et al. (1997)
	Ovalbumin	1.6	0.02,0.05	Pluen et al. (1999)
	BSA	1.9	0.02,0.05	
	$C_{12}E_6$	1.8	0.02	
	$C_{12}E_8$	1.5	0.02	
	$C_{12}E_{10}$	1.6	0.02	
Calcium alginate	BSA	10	0.02,0.05	Amsden (1998)
κ-Carrageenan	$C_{12}E_6$	6.9	0.02	Johansson et al. (1993)
	$C_{12}E_8$	5.5	0.02	
Polyacrylamide	RNAse	3.1	0.02,0.05	Park et al. (1990)
	BSA	5.5	0.02,0.05	Tong and Anderson (1996)

captures the overall behavior reasonably well over a full order-of-magnitude change in the ratio r_s/r_f, at both volume fractions. We note that the data of Johnson et al. (1996) were actually obtained at $\phi = 0.055$ instead of $\phi = 0.05$. Also, the heterogeneous nature of the fiber radii in agarose is not taken into account in Fig. 4, nor is the nonspherical nature of $C_{12}E_6$ micelles (Johansson et al., 1993). These simplifications were needed to facilitate the comparison. In all cases, the solute and fiber radii used in conjunction with Eq. 8 are those given in Table 2. No adjustable parameters are needed to make the predictions.

The hydrodynamic model given by Eq. 8 is a simple result that is relatively easy to use and requires only basic geometric information about a particular gel-solute system. Comparisons with data taken in agarose gels, provided by Clague and Phillips (1996), Johnson et al. (1996), and Pluen et al. (1999), combined with the comparisons with polyacrylamide, alginate, and carrageenan gels given here, demonstrate that Eq. 8 captures much of the basic physics that affect hindered diffusion in gels. However, with the exception of agarose, there does appear to be a consistent tendency of the theory to underpredict the actual rate of diffusion. Because the theory is based on a physical model that consists of a monomodal, homogeneous distribution of immobile, rigid fibers, it is not surprising that it tends to yield a lower bound for D/D_0. A relaxation of these features, allowing consideration of microstructural heterogeneity, fiber flexibility and motion, and nonsteric fiber-solute interactions will likely lead to better predictions, albeit at the cost of greater complexity.

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The Model of Snyder et al. Does Not Simulate Graded Ca^{2+} Release from the Cardiac Sarcoplasmic Reticulum in Intact Cells

The recent paper by Snyder et al. (2000) represents a commendable and carefully executed effort to marshal the currently understood mechanisms in cardiac excitation-contraction coupling into a simplified, qualitatively correct macroscopic model. However, one major deficiency of the model needs to be pointed out. Their paper leaves the impression that the feedback interaction between sarcoplasmic reticulum luminal calcium and kinetics of the ryanodine receptor is sufficient to give rise to graded release of sarcoplasmic reticulum calcium in response to the triggering L-type calcium current. This is not the case. The authors base their claim of gradedness on a simulation of the classic experiment of Fabiato, in which calcium is applied to a skinned muscle cell whose "fuzzy space" is not intact (Fig. 3 in Snyder et al., 2000). However, if one uses their model to simulate the experiment in which the L-type current is varied over a wide range in an intact cell (Wier et al., 1994; Cannell et al., 1995; Janczewski et al., 1995), the result is

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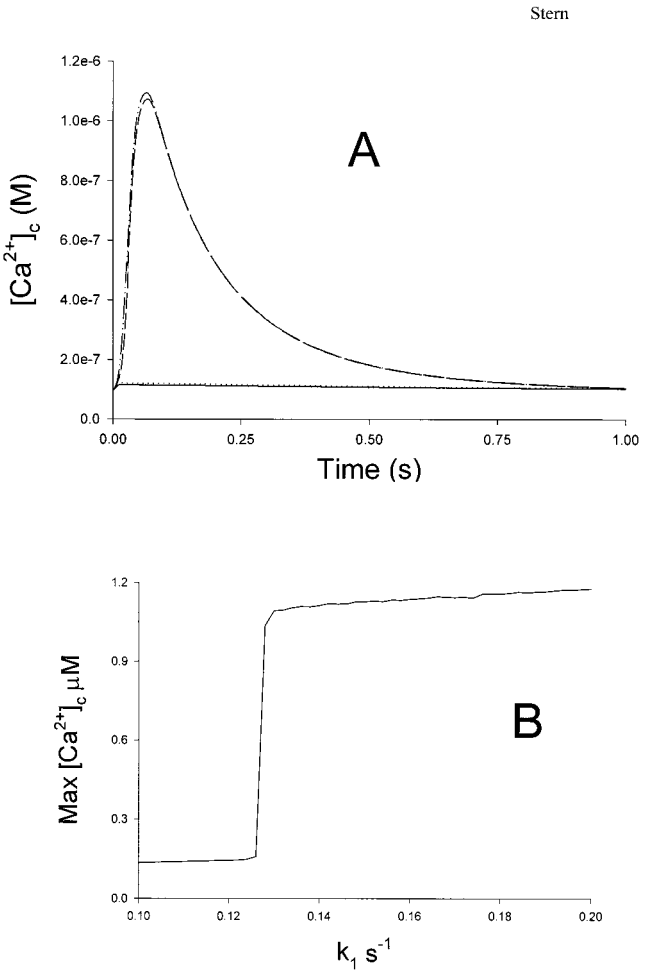

FIGURE 1 Cytosolic calcium transients generated by the model of Snyder et al. (2000) as the L-type current parameter, k_1, is varied. All parameters were the same as in their Fig. 5 and Table 3, for which $k_1 = 0.2$. (*A*) Stack plot of 4 transients corresponding to $k_1 = 0.1$, 0.125, 0.15, and 0.175. The transients fall into two sets of large and small, nearly all-or-none responses (sub- and supra-threshold responses). (*B*) Peak cytosolic [Ca^{2+}] as a function of k_1 showing clear-cut threshold behavior. Simulations were carried out by transcribing the model equations from Snyder et al. (2000) into the modeling language MLAB (Civilized Software, Bethesda, MD), in consultation with the original authors in order to correct minor typographical errors and ambiguities.

entirely ungraded, in disagreement with the experimental findings. In this more physiological context, the model shows nearly all-or-none threshold response (Fig. 1). This is consistent with the idea that such a common-pool compartmental model cannot demonstrate high gain and also robust gradedness (Stern, 1992). Thus, although the sarcoplasmic reticulum luminal feedback mechanism can produce a stable quiescent state together with high gain, it cannot provide a macroscopic approximation of the graded response, which is produced robustly by microscopic stochastic local-control models (Stern et al., 1999).

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