Diffusion of Proteins and Nonionic Micelles in Agarose Gels by Holographic Interferometry

Donna D. Kong and T. Fettah Kosar
Dept. of Chemical Engineering and Materials Science, University of California at Davis, CA 95616

Stephanie R. Dungan
Dept. of Chemical Engineering and Materials Science and Food Science and Technology,
University of California at Davis, CA 95616

Ronald J. Phillips
Dept. of Chemical Engineering and Materials Science, University of California at Davis, CA 95616

Diffusion coefficients measured by holographic interferometry are presented for two globular proteins and three nonionic surfactant species in agarose gels. The cloud points for the surfactants in agarose solutions are also given. In all cases, rates of diffusion in the gel are smaller than those in bulk solution, with the hindering effect of the gel increasing both with increasing gel concentration and with increasing solute size. It is shown that the diffusion rate of the surfactant micelles is very similar to that of proteins having similar hydrodynamic radii; it would therefore appear that the size and shape of the micelles in the solution and gel phases are similar. In addition, the measured rates of hindered diffusion agree very well with rates predicted by a rigorous hydrodynamic theory in which the solutes are modeled as hard spheres and the gel fibers are modeled as straight, cylindrical fibers. Hence, for the range of conditions considered, rates of hindered diffusion for both the globular proteins and the micellar species are determined primarily by hard-sphere hydrodynamic and steric interactions between the solute and the gel matrix.

Introduction

The interaction between micelles and polymer fibers that are cross-linked to form a hydrogel is an important area of study that has received relatively little attention to date. In addition to obvious applications in separations such as sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE), possibilities exist to use such systems for novel extraction and controlled release applications. For example, we have shown that immobilization of block copolymer surfactants in alginate gel beads results in a material that readily extracts hydrophobic toxins from water (Calvert et al., 1994), and similar surfactants have shown to have a beneficial impact on the rate and duration of controlled release of drugs from a polymer matrix (Park et al., 1992). Similarly, properties of proteins in gels are directly relevant to many controlled release applications as well as most bioseparation methods, including gel chromatography, ultrafiltration, and electrophoresis.

The rate of diffusion of proteins and surfactants in gels is an important property both for the characterization of solute–gel interactions and for the design of novel applications of such materials. Rates of protein diffusion in liquids have been measured by a variety of standard techniques, with dynamic light scattering being perhaps the most common one at present. Rates of micelle diffusion in liquids have also been measured by many methods, including Taylor tube dispersion (Weinheimer et al., 1981; Evans et al., 1983; Leaist, 1991), dynamic light scattering (Corti and Degiorgio, 1981; Kato et al., 1989, 1990, 1993), and pulsed field-gradient nuclear magnetic resonance (PFGNMR) (Kato et al., 1989, 1993; Asakawa et al., 1991; Penders et al., 1993).

With some modification, many of the techniques just mentioned and some others, such as forced Rayleigh scattering (FRS) (Park et al., 1993), can be applied to measuring rates of diffusion in gels (Pajevic et al., 1993; Gibbs et al., 1992). However, the gel measurement introduces complications suf-

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Correspondence concerning this article should be addressed to R. J. Phillips.

ficient to warrant the development of new techniques specifically tailored to use in a heterogeneous medium of fibers. Fluorescence recovery after photobleaching (FRAP), which has recently been used to measure diffusion coefficients for several proteins in gels, is one example of such a method (Johnson et al., 1996). We have recently shown that the technique of holographic interferometry (HI) can be used to measure protein diffusion in polymer solutions (Kosar and Phillips, 1995), and it has been shown by others that HI can be readily adapted to measure diffusion of low-molecular-weight molecules in polymer hydrogels (Ruiz-Bevia et al., 1989; Gustafsson et al., 1993). In this article we report new HI results for the diffusion in agarose gels of two globular proteins and three polyoxyethylene dodecyl ether surfactants (i.e., $C_{12}H_{25}(OC_2H_4)_mOH$ or $C_{12}E_m$). The two proteins are bovine serum albumin (BSA) and myoglobin, and the surfactants are $C_{12}E_6$, $C_{12}E_8$, and $C_{12}E_{10}$.

Previous studies of the complex behavior exhibited by solutions of surfactants and freely mobile polymers suggest that interactions in micelle/gel systems may provide a rich subject for study (see, for example, Robb, 1981; Saito, 1987; Piculell and Lindman, 1992; Brackman and Engberts, 1993; Goddard and Ananthapadmanabhan, 1993). In addition, the few existing studies of micelles in gels have suggested that properties such as the critical micelle concentration (cmc) and aggregation number are affected by the gel environment. Philippova and Stardoubtzev (1993) found that the cmc of SDS in poly(diallyldimethylammonium bromide) gels that are more than 99% water is approximately tenfold lower than those in solution. Penders et al. (1993) have used PFGNMR to measure rates of self-diffusion of $C_{12}E_6$ and $C_{12}E_8$ in 1% agarose gels as a function of temperature, and found that the surfactants diffused faster in gelled agarose than in agarose solutions, possibly because of changes in the fiber conformation that occur during gelation. Johansson et al. (1993) measured aggregation numbers and rates of diffusion of $C_{12}E_6$ and $C_{12}E_8$ in κ-carrageenan solutions and gels. The authors concluded that the micelle diffusion for $C_{12}E_8$ is well-described by hard-sphere theories, while for $C_{12}E_6$ more complex diffusive behavior is observed. The aggregation numbers for both surfactants were observed to increase in the presence of the charged gel, with the increase being fairly small in the case of $C_{12}E_8$.

By measuring the diffusion of both globular proteins and micellar aggregates of comparable size, our study provides additional insight into the mechanism by which micelles diffuse through gels. We have chosen uncharged C_nE_m surfactants and an uncharged agarose gel to avoid complicating factors associated with electrostatic interactions or the effect of ionic strength on micellar properties. Similarly, the protein measurements are made at ionic strengths high enough to eliminate electrostatic effects. We can therefore make a direct comparison between micelle diffusion and the diffusion of sphere-like, globular proteins with comparable dimensions, and determine by direct experimental observation the extent to which micelles diffusing in gels can be modeled as spherical solutes.

In addition to this experimental comparison between globular proteins and micellar aggregates, we have recently developed a hydrodynamic theory for hindered diffusion of spherical solutes in porous media comprised of straight, cylindrical fibers (Clague and Phillips, 1996). The theory accounts rigorously both for the enhanced hydrodynamic drag on a solute that is caused by the presence of a surrounding fibrous medium and for the tortuosity or steric effect on the path of diffusion. Given information on the solute radius, fiber radius, and fiber volume fraction of the gel, the theory predicts rates of protein diffusion in agarose accurately with no adjustable parameters. It therefore provides an additional tool that one can use in conjunction with diffusion data to infer structural information about micelles in gels.

Experimental Materials and Methods

Agarose with a low gelling temperature (Type VII, No. A-4018) and the proteins BSA and myoglobin were obtained from Sigma (St. Louis, MO) and used without further purification. The temperature for gelation of this agarose is approximately 27°C. The use of agarose with a low gelation temperature allows for the dissolution of proteins in agarose solutions at temperatures above that for gelation, but low enough so as not to alter the conformation of the protein.

The three surfactants used here are hexaethylene, octaethylene, and decaethylene glycol monododecyl ether, or $C_{12}E_6$, $C_{12}E_8$ and $C_{12}E_{10}$. $C_{12}E_6$ and $C_{12}E_{10}$ were obtained from Sigma (St. Louis, MO) and $C_{12}E_8$ from Fluka (Ronkonkoma, NY). All surfactants were used as supplied. The clouds points for these surfactant molecules in agarose solutions of varying concentrations are given in Table 1, where it is seen that the cloud point decreases with increasing agarose concentration. These temperatures were obtained by direct visual observation; the range given reflects the temperature change over which the solution changes from being slightly cloudy to very cloudy. The trend of the cloud point decreasing with agarose concentration has been reported previously (Penders et al., 1993), although the actual cloud point temperatures reported here are a few degrees lower, presumably because we have used low-gelling-temperature agarose and have obtained our surfactants from different sources (see also Mitchell et al., 1983). Depression of surfactant cloud points in agarose solutions has been attributed to repulsive interactions between the polysacchride and the surfactant, which promote phase separation (Penders et al., 1993). The results of Penders et al. (1993) indicate that agarose gel also lowers the cloud point, although to a lesser extent than does agarose solution.

Polyoxyethylene forms the hydrophilic head group of these molecules and the monododecyl chain forms the hydrophobic tail group. Since all the surfactants have the same tail group, varying the length of the head group allows us to study these size effects systematically. Both agarose gel and the surfactants used are nonionic, so that no charge effects are present in the system. In the case of proteins, charge effects have been studied by varying the salt concentration, and it was found that the electrostatic effects are generally negligible at

Table 1. Cloud Point (°C) of 1% Surfactant in 0–4% Agarose Solutions

	Agarose Concentration				
	0%	1%	2%	3%	4%
$C_{12}E_6$	45 ± 2	< 35			
$C_{12}E_8$	71 ± 2	63 ± 1	56 ± 3	51 ± 1	46 ± 1
$C_{12}E_{10}$	75 ± 1	67 ± 3	58 ± 2	52 ± 2	48 ± 2

salt concentrations above 0.01-M KCl (Kosar and Phillips, 1995). Measurements of protein diffusion reported here were performed at 0.15-M KCl.

Diffusion of proteins and surfactants in aqueous solution and in agarose gels was measured by using holographic interferometry. This method involves contacting a solution or gel that contains solute (surfactant or protein) with an identical, solute-free phase. In each case the solute-containing medium is formed at the bottom of a spectrophotometric cuvette, with the solute-depleted solution or gel on top. Diffusion of solute from the lower region of the cuvette to the upper region results in a gradient of refractive index that changes in time. By storing the image of the cell at a time t_1 on a holographic plate, changes in the refractive index at a series of later times can be monitored via interference fringes, which are analyzed to determine the diffusion coefficients. Details for performing measurements in solutions and analyzing the interference fringes are given by Kosar and Phillips (1995).

In order to prepare the gel phases for diffusion measurements, a pure agarose solution at a specified weight percentage (1 to 4%) is first prepared by mixing agarose powder and deionized water while slowly heating to the solution boiling temperature. The solution is kept at this temperature until the agarose is completely dissolved. The solution is then cooled down to approximately 40°C, and additional deionized water is added to compensate for the loss due to evaporation. The solution is stirred on a warm plate until it appears homogeneous, then transferred using a syringe to a quartz spectrophotometric cuvette until the cuvette is half filled. The first few drops of the solution are purged from the syringe so that no air bubbles are transferred to the cuvette and left trapped when the solution starts to gel. The cuvette is then cooled at room temperature (21 ± 2°C) for at least an hour to ensure complete gelation.

The portion of gel that contains surfactant is prepared as follows: 5 wt. % surfactant solutions are made by stirring surfactant and deionized water at room temperature for 20 min. Agarose solutions (1.25 to 5%) are prepared as described above and added to the surfactant solution at approximately 40°C. Because the surfactant solutions phase separate at temperatures below the boiling temperature of the agarose solution, care is taken to cool the agarose solution down below this cloud point temperature before it is added to the surfactant. The concentrations and ratios of the agarose and surfactant solutions are chosen to yield the appropriate final surfactant and gel concentration, and the surfactant–agarose mixtures are stirred in a water bath at approximately 40°C for 20 min. In order to prepare protein-containing gels, protein powder is added directly to the cooled agarose solution in the appropriate amount and stirred until the protein is dissolved and thoroughly mixed.

The solute–agarose mixture is transferred to the cuvette by using a prewarmed syringe so that gelation does not take place in the needle. The first few drops of solution are purged as described above. Since the solute-containing gel must be added *below* the solute-depleted gel to ensure a flat interface, the syringe needle is pushed into the solute-free gel already in the cuvette by sliding the needle along one corner. Injection of the solute–gel mixture begins after the tip of the needle reaches the bottom of the cuvette, the injection being performed rapidly and carefully in order to achieve a high-quality gel–gel interface. The interface between the two gel phases is pushed up until it reaches the middle of the cuvette, at which point the syringe needle is slowly removed. Finally, the samples are cooled in the air at room temperature. After 3 h, a holographic exposure is taken, and is used to form fringes that are photographed at several later times, the entire experiment lasting from 8 to 20 h. Further details of the holographic interferometry measurements are given by Kosar and Phillips (1995).

Results and Discussion

The interpretation of data for the diffusion of surfactants is complicated somewhat by the dynamic nature of micelles, which exist in equilibrium with surfactant monomers. For the relatively simple nonionic micelles of interest here, we can analyze the diffusion process by using the approach described by Turq et al. (1983). We assume that the monomer and micellar species diffuse according to Fick's law,

$$J_m = -D_m \nabla C_m, \tag{1}$$

and

$$J_M = -D_M \nabla C_M. \tag{2}$$

Here J, D, and C are the flux, diffusivity, and concentration, respectively, and the subscripts m and M refer to monomer and micelle, respectively. Monomer and micelle concentrations are also related through a formation/dissolution process in which N surfactant molecules associate to form a micelle. For diffusion over length scales of millimeters such as exist in our holographic interferometry experiments, this micelle "reaction" can be assumed to be fast (Weinheimer et al., 1981), yielding the equilibrium condition

$$C_m^N = K C_M, \tag{3}$$

where N is the aggregation number of the micelles, and K is an equilibrium constant. From this condition and the definition of the total surfactant concentration C as

$$C \equiv N C_M + C_m, \tag{4}$$

one can readily show that

$$\frac{\nabla C}{N C_M + \dfrac{C_m}{N}} = \frac{\nabla C_M}{C_M} = \frac{\nabla C_m}{\dfrac{C_m}{N}}. \tag{5}$$

From Eqs.1 and 2 the total flux of surfactant is then

$$J = -\left[\frac{D_m C_m + N^2 D_M C_M}{C_m + N^2 C_M}\right] \nabla C. \tag{6}$$

Our experiments are carried out at a total surfactant concentration of 1 wt. %. For nonionic surfactants such as those used here, a typical critical micelle concentration is that of $C_{12}E_8$, which is 3.7×10^{-3} wt. % (Kato et al., 1989), and a typical aggregation number is 79 (Johansson et al., 1993).

Thus, even if the monomer diffusion coefficient D_m is ten times the micelle diffusion coefficient D_M, it is true that

$$N^2 D_M C_M \gg D_m C_m \tag{7}$$

and

$$N^2 C_M \gg C_m, \tag{8}$$

so that Eq. 6 reduces to

$$J = -D_M \nabla C. \tag{9}$$

Although the values of the transport coefficients are different in the gel and solution phases, the derivation above can be applied to either phase. Under the conditions of interest, the total flux of surfactant is therefore expected to be governed by the diffusion coefficient of the micelles.

Diffusion in solution

Our measured values for the diffusion coefficients D_0 of the proteins and the C_nE_m micelles in pure solution are given in Table 2. The values shown are adjusted to 20°C. The hydrodynamic radius R_h is calculated from the Stokes-Einstein equation,

$$D_0 = \frac{kT}{6\pi\mu R_h}, \tag{10}$$

by using published values of the solvent viscosity μ (Weast and Astle, 1980). In Eq. 10, kT is the product of Boltzmann's constant and absolute temperature. Values for the hydrodynamic radius reported in the literature obtained by alternative methods for measuring diffusion are given in Table 2 for purposes of comparison. Clearly the agreement between the various techniques is quite good.

As explained above, in its initial stage a holographic interferometry experiment consists of a lower, solute-rich solution in contact with an upper, solute-depleted solution. The initial exposure of the holographic plate is taken several hours after the start of the experiment, and hence the average concentration in an experiment is somewhat between the initial values in the lower and upper solutions. In Figure 1 we show results obtained for $C_{12}E_8$ obtained by using different concentrations in the lower, solute-rich solution, while keeping the upper solution solute-free. The error bars on these data and on the other diffusion data discussed below are 99% confidence intervals calculated from what is typically 6–10 data points. For a small number of conditions there are less than four data points, and in those cases the error bars are estimated from the errors calculated for the same solute-gel combination at the other gel concentrations.

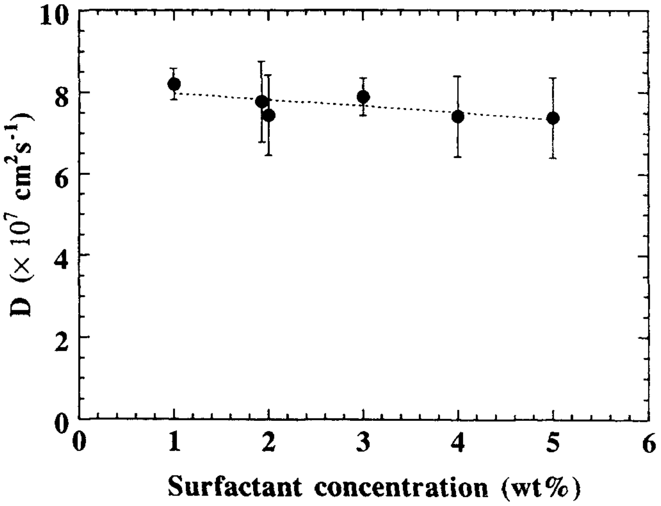

Figure 1. Diffusion coefficients for $C_{12}E_8$ micelles in solution at 21°C as a function of surfactant concentration.

Since this is a gradient diffusion process, hard-sphere interactions between the micelles would normally cause the diffusion coefficient D to increase linearly with the average concentration according to (Batchelor, 1983)

$$\frac{D}{D_0} = 1 + 1.45\phi, \tag{11}$$

where D_0 is the diffusion coefficient at infinite dilution, and ϕ is the volume fraction of micelles. Assuming an aggregation number of 79.4 and a micellar radius of 3 nm (Johansson et al., 1993), Eq. 11 predicts an increase in the diffusion coefficient of approximately 5% for the concentration variation shown. If anything, the data in Figure 1 show a slight *decrease* in D as surfactant concentration increases, indicating a possible slight increase in aggregation number, or a small attractive micelle-micelle interaction. However, the observed and expected trends are so slight compared with experimental uncertainty that any conclusions to be drawn regarding concentration effects are tenuous at best. Owing to the weak concentration dependence shown in Figure 1, we accept val-

Table 2. Diffusion Coefficients D_0 and Hydrodynamic Radii R_h for Proteins and $C_{12}E_m$ Micelles

Solute	D_0 (×10^7 cm^2/s) at 20°C* (Measured)	R_h (nm) (From Eq. 10)	R_h (nm) (Literature)	Reference
BSA	6.5	3.3	3.45	Wattenbarger et al., 1992
Myoglobin	10.7	2.0	2.12	Lehninger, 1975
$C_{12}E_6$	6.2	3.5	3.4–3.7	Brown et al., 1983 Kato and Seimiya, 1986
$C_{12}E_8$	8.0	2.7	2.8–3.1	Nilsson et al., 1983 Matsumoto and Zenkoh, 1990
$C_{12}E_{10}$	6.9	3.1		

*Protein diffusion was measured at 23 ± 2°C; micelle diffusion was measured at 21 ± 2°C. All values were adjusted to 20°C by assuming that D_0T/μ is constant.

ues of D_0 at 1 wt. % as infinite dilution diffusion coefficients, suitable for use in calculating hydrodynamic radii.

The results in Table 2 indicate that $C_{12}E_6$ forms larger micellar aggregates in aqueous solution than does $C_{12}E_8$. This difference between these two surfactants is generally ascribed to a difference in aggregate geometry: while $C_{12}E_8$ is believed to form spherical micelles, the smaller size of the $C_{12}E_6$ headgroup relative to its hydrocarbon tail allows it to form more asymmetric, prolate spheroids or rods. The larger aspect ratio associated with the latter geometries would result in a large hydrodynamic radius for the aggregate, as well as a tendency toward aggregate growth as a function of concentration or temperature (Brown et al., 1983; Kato and Seimiya, 1986; Magid, 1987; Johansson et al., 1993). The size and structure of $C_{12}E_8$, on the other hand, is expected to be relatively insensitive to concentration (at low concentrations) and temperature (well below the point of phase separation), consistent with the results shown in Figure 1. The less well-studied $C_{12}E_{10}$ has a still larger headgroup than the other two surfactants discussed here, and might be expected to form spherical aggregates that are somewhat larger than $C_{12}E_8$. Table 2 shows that the measured hydrodynamic radius for $C_{12}E_{10}$ is 0.4 nm larger than that for $C_{12}E_8$, consistent with the expected trend. Finally, we note that the size of the $C_{12}E_6$ micellar aggregate is very close to that of the protein BSA, allowing for a direct comparison of the importance of hard-sphere hydrodynamics on the diffusion of these two types of particles in gels.

Diffusion in gels

When the solutes discussed above are placed within an agarose hydrogel, the presence of the gel fibers influences their rates of diffusion. For macromolecular solutes as well as ideal, point solutes, there are steric or obstruction effects owing to the impenetrability of the fibers. Also, in the case of the globular BSA and myoglobin, hydrodynamic interactions are expected to hinder substantially rates of diffusion through gels. In spite of the more complicated fibrous geometry, such interactions are analogous to those that occur when globular solutes diffuse through cylindrical pores of comparable dimension, a problem that has been well-studied both theoretically and experimentally (Deen, 1987). In the case of surfactant micelles comparable in size to the proteins, those hydrodynamic interactions are still present, along with the possibility of additional, nonhydrodynamic micelle–fiber interactions. However, unlike the proteins, micelles are relatively free to change conformation in response to changes in their environment. The extent to which such changes occur in various types of gels has not been well-studied to date, and can be probed by diffusion measurements such as those presented here.

Our holographic interferometry measurements indicate that, for all species considered, rates of diffusion in gels are significantly lower than those in pure solution. In Figure 2 we show results for diffusion of BSA and myoglobin in agarose for gel concentrations up to 7 wt. %. The diffusion coefficients D are normalized by their values D_0 in bulk solution. Clearly the diffusion of both proteins is significantly hindered, with the larger BSA hindered relatively more than the smaller myoglobin. Also shown in Figure 2 are predictions from the theory of Clague and Phillips (1996), which as stated above contains no adjustable parameters, and requires a knowledge only of the fiber volume fraction and the radii of the solute and fibers. As shown by Johnson and Deen (1996), values of weight percent and volume percent are approximately equal for agarose, and hence are known for each experiment, and values of R_h for the two proteins are taken from diffusion measurements in solution. As is also discussed by Johnson and Deen (1996), the distribution of fiber radii in agarose is bimodal, with 87% of fibers having a radius of 1.5 nm and 13% having a radius of 4.5 nm. To obtain our theoretical predictions, we therefore add 0.87 times the diffusion coefficient corresponding to a fiber radius of 1.5 nm to 0.13 times that for a fiber radius of 4.5 nm. These relatively large values for the sizes of agarose fibers make agarose gels quite porous—that is, at a given volume fraction of gel the spacing between fibers is large relative to many other hydrogels. Yet even for such a porous gel, clearly the protein diffusion is hindered significantly, an effect that is captured accurately by the hydrodynamic theory.

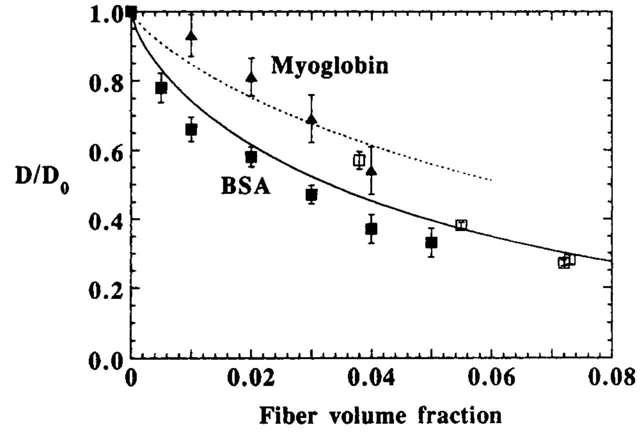

Figure 2. Normalized diffusion coefficients of BSA and myoglobin as a function of agarose gel volume fraction.

Solid squares and triangles are HI data for BSA and myoglobin, respectively; open squares are BSA data reported by Johnson et al. (1996). Curves are predictions from the theory of Clague and Phillips (1996).

Data for BSA diffusion in agarose have also been reported by Johnson et al. (1996), who used the method of fluorescence recovery after photobleaching (FRAP). These data are included in Figure 2, and are in good agreement with our HI results and with the theory. One concludes therefore that to a good approximation, rates of hindered diffusion of globular proteins in agarose gels are determined by a combination of hydrodynamic and steric interactions. These interactions can be evaluated by modeling the proteins as spherical solutes and the gel as a medium of straight cylindrical fibers.

The micelles exhibit diffusive behavior that is qualitatively and quantitatively similar to the protein solutes. In Figure 3 the normalized diffusion coefficients for $C_{12}E_8$ and $C_{12}E_{10}$ are plotted as a function of gel concentration. For purposes of comparison we also show the predictions of the hydrodynamic theory of Clague and Phillips (1996), using the hydrodynamic radii given in Table 2. Clearly the hydrodynamic theory for hindered diffusion of spherical solutes is nearly as

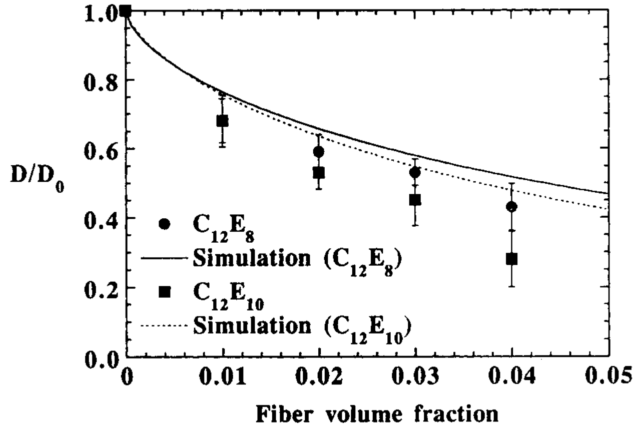

Figure 3. Normalized diffusion coefficients of $C_{12}E_8$ and $C_{12}E_{10}$ micelles as a function of agarose gel volume fraction.

Solid curves are predictions from the theory of Clague and Phillips (1996).

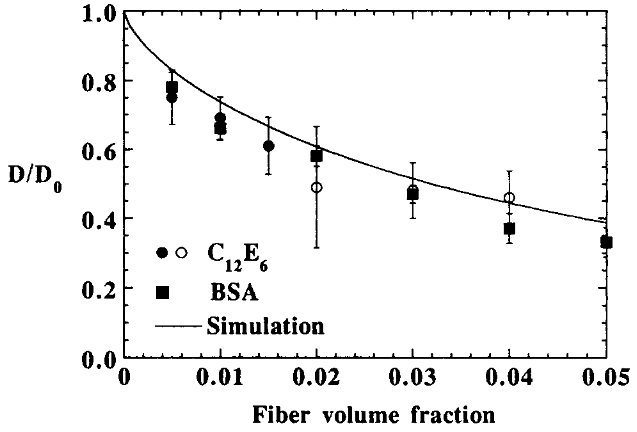

Figure 4. Normalized diffusion coefficients of $C_{12}E_6$ micelles and BSA molecules as a function of agarose gel volume fraction.

Solid curve is prediction from the theory of Clague and Phillips (1996).

successful in predicting D/D_0 for the $C_{12}E_8$ micelles as it is for BSA and myoglobin. The agreement between theory and experiment is quite good over the entire range of agarose concentrations studied. The slightly larger size of the $C_{12}E_{10}$ micelles in solution apparently results in more hindered diffusion in the gel, an effect that is captured qualitatively, although underestimated, by the theory.

Interestingly, the diffusion coefficients for the $C_{12}E_8$ and $C_{12}E_{10}$ micelles and the BSA are all slightly overpredicted by the theory. However, the theory is based on fundamental hydrodynamic calculations for a hard-sphere solute in a medium of straight cylindrical fibers, an idealized geometry that is no doubt a significant simplification of the real situation. In addition, there may be nonhydrodynamic interactions at work that are not included. Given these factors and the absence of adjustable parameters in the theory, the data in Figure 3 can be interpreted as evidence in support of the observation that, for purposes of describing their hindered diffusion in gels, these nonionic micelles can be considered to be hard-sphere solutes with sizes and aggregation numbers that do not differ substantially in solution and agarose gel phases. This contention is supported by the work of Johansson et al. (1993), who report less than a 20% increase in aggregation number when $C_{12}E_8$ micelles are formed within a κ-carrageenan gel. Since κ-carrageenan gels are charged, and are less porous than agarose gels as a result of their smaller fiber radii, we expect that such increases would be even less in our system.

Experimental data and theoretical predictions for the largest of the micelles studied, those formed by $C_{12}E_6$, are shown in Figure 4. Because these micelles are so similar in size to BSA, diffusion coefficients for that protein are also plotted for purposes of comparison. Again data are shown for the full range of concentrations, from 0 to 5%. However, gels containing $C_{12}E_6$ at agarose concentrations exceeding 1.5% exhibit a milky white color, a feature that is absent in the other systems studied. This attribute may indicate that some phase separation of surfactant is being promoted by the agarose fibers (see the second section). These data are therefore given an open symbol to differentiate them from the data taken for the clearer gels at the lower concentrations. Like $C_{12}E_8$, the data for $C_{12}E_6$ are in good agreement with the hydrodynamic theory, its diffusive properties therefore conforming to those predicted for a hard-sphere solute. Furthermore, there appears to be little difference between the hindered diffusion of these micelles and the globular protein BSA, which has a very similar hydrodynamic radius in pure solution.

The ability of the hydrodynamic theory to predict the diffusion of $C_{12}E_6$ micelles within a gel is perhaps most intriguing of the three surfactants studied. As discussed above, $C_{12}E_6$ surfactant is believed to form prolate or rodlike micellar aggregates in solution. In cylindrical pores, such nonspherical shapes would influence the extent of hindrance of the aggregate within a gel, generally increasing its diffusion rate relative to a sphere with the same effective radius (Nitsche, 1995). One expects that this effect would become significant only for fairly large aspect ratios, however. In addition, the tendency of this surfactant to grow with changes in surfactant concentration and temperature suggest that $C_{12}E_6$ micellar shapes and sizes might be sensitive to the presence of the gel fibers. We note that aggregation numbers for $C_{12}E_6$ micelles have been measured in κ-carrageenan gels (Johansson et al., 1993), and it was found that the aggregation number for $C_{12}E_6$ increased from 192 in pure water to 250 or 264 in a 5 wt. % gel, depending upon whether it was a K^+-carrageenan or Na^+-carrageenan gel.

In addition, Johansson et al. (1993) noted that the diffusion rates of $C_{12}E_6$ in the κ-carrageenan gel differed qualitatively from that of $C_{12}E_8$, both as a function of surfactant concentration and gel concentration. These differences were attributed to an "increased concentration effect," where the presence of the gel fibers causes the interactions between micelles to be enhanced, as if they were in a surfactant solution with effectively higher concentrations. In the measurements of tracer diffusion conducted by Johansson et al. (1993), more significant hindrance of the micelle diffusion was believed to be the result of these enhanced intermicellar interactions. Because our holographic interferometry experiments measure gradient diffusion, if a similar effect were present in our system it would result in a faster diffusion rate than predicted by hydrodynamic theory. Such an effect is not observed in our agarose gels, as seen in Figure 4.

Comparison of the experimental data reported here with that of Johansson et al. (1993), which were obtained for $C_{12}E_6$ and $C_{12}E_8$ in κ-carrageenan gels at these surfactant concentrations, makes clear the important role that gel type plays in micelle–gel interactions. Although the same micelles are more hindered in a κ-carrageenan gel than in an agarose gel at the same concentration, they are actually less hindered in κ-carrageenan than theory would predict. Perhaps the single most important difference between agarose and κ-carrageenan is the fiber radius. As mentioned above, agarose fibers have a number average radius of 1.9 nm, whereas κ-carrageenan fibers have radii ranging from 0.3 to 0.5 nm, depending on the concentration. This difference in size has as a profound effect on the distribution of space sizes in the matrix. For a medium of straight cylindrical fibers of radius R_f and volume fraction ϕ, Ogston (1958) showed that the probability $P_{R>R_h}$ that a "space size" R is greater than the solute radius is

$$P_{R>R_h} = \exp\left[-\phi\left(1+\frac{R_h}{R_f}\right)^2\right], \tag{12}$$

where a space size at any point is just the distance to the nearest fiber. From Eq. 12, one finds that a 3-nm micelle in a 2% κ-carrageenan gel can access only about 38% of the volume, whereas in agarose that micelle can access 88% of the volume.

In addition, even if a space is labeled "available" by Eq. 12, a solute could be trapped there by being "caged" by surrounding fibers. Since a trapped solute can be released if the fibers are mobile or flexible, this caging effect and the steric effect generally are strongly affected by fiber flexibility, particularly in situations where so little space is available to a solute at any given time. Fiber flexibility is not incorporated into any existing theoretical or simulation results for steric hindrance, including those used in the theory of Clague and Phillips (1996). Also, nonhydrodynamic interactions, which can exist between nonionic micelles and anionic polymers (Saito, 1987, 1990, 1994) may be relatively important in the κ-carrageenan gel system where the fibers are in such close proximity to the micelles. It would therefore seem that an extended theory capable of accurate predictions in the κ-carrageenan system may need to account for hydrodynamic interactions, nonhydrodynamic interactions, and fiber flexibility and motility.

Conclusion

For the systems studied here, it appears that the diffusion processes of nonionic micelles and proteins in nonionic gels are fundamentally similar. Measurements of protein and micelle diffusion in the same agarose gels show that comparably sized micelles and proteins diffuse at essentially the same rate. Furthermore, these rates are determined primarily by hydrodynamic and steric interactions, which can be calculated *a priori* by idealizing the system geometry as a spherical solute diffusing in a medium of straight cylindrical fibers. Although they do not appear to have been significant in this work, changes in the critical micelle concentration and aggregation number are to be expected in a gel environment. However, charged gels as agarose, calculation of effective transport properties may be possible by using the theory of Clague and Phillips (1996) in conjunction with the micellar radius that accurately describes the colloidal aggregate as it exists inside the gel.

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