Effect of SDS Micelles on Rhodamine-B Diffusion in Hydrogels

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The diffusion process involves random molecular motion. A difference in solute concentration between parts of a solution or a gel may give rise to a flow, J, leading towards complete mixing. The rate of such transport is related to the solute diffusion coefficient, D, defined by Fick's first law:

$$J = -D\,(dC/dx) \qquad (1)$$

Since the direction of diffusion is opposite to that of increasing concentration, a negative sign is given in the equation. The change in solute concentration with time is described by Fick's second law:

$$\partial C/\partial t = D\,(\partial^2 C/\partial x^2) \qquad (2)$$

The solution to this second-order differential equation depends on the concentration and spatial conditions of the system.

The free diffusion model can be visualized utilizing a cylinder with pure solvent carefully layered on top of a solution of a dye. The concentration profile (C vs. x) of the spreading dye is illustrated in Figure 1. Initially, there is a sharp boundary at $x = 0$, but with time a smooth S-shaped, time-dependent curve develops. The equation for the concentration profile (eq 3) is a solution to Fick's second law under the assumption that the liquid column is so long that the dye concentrations at the ends do not change during the experiment (*1*).

$$C = \frac{C_o}{2}\left(1 - \frac{2}{\sqrt{\pi}}\int_0^z e^{-y^2}\mathrm{d}y\right) \qquad (3)$$

The exponent y in eq 3 is a dummy variable of integration.

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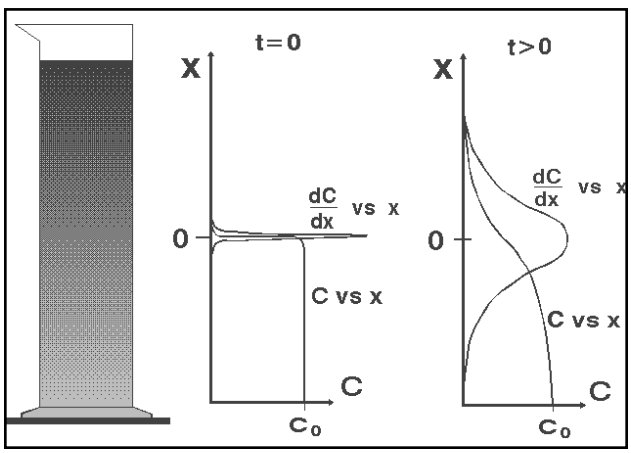

Figure 1. The free diffusion model.

The integral term is the error function, erf (Z), where $Z = x/(4Dt)^{1/2}$ and t is the time of the experiment (*2–3*). At the boundary the concentration (C_B) is constant during the experiment and equals half of the initial dye concentration (C_0).

$$C = C_B\,[1 - \mathrm{erf}(Z)] \qquad (4)$$

Figure 1 also shows how the derivative of the concentration profile (dC/dx vs x) develops from a sharp peak to a bell-shaped Gaussian "error" curve (eq 5).

$$\frac{\partial C}{\partial x} = \frac{C_o}{2\sqrt{\pi Dt}}e^{-x^2/4Dt} \qquad (5)$$

Diffusion cells where a solvent is sheared in place over a solution of diffusant have been designed and used with viscous solutions (*4–6*). For measurements in water, however, very strict precautions must be exercised to avoid convection currents when the boundary is formed. These problems can be avoided by substituting the "pure solvent part" of the liquid column with a hydrogel. The method is based on the principle of a constant diffusant concentration (C_B) at the water/gel interface. A substance is allowed to diffuse from a solution, endwise, into a gel cylinder. The part of the concentration profile corresponding to ($x \geq 0$) develops in the gel, and consequently the upper half of the free diffusion model becomes applicable to the gel cylinder (*7, 8*).

Methodology

A number of 5-mL plastic syringes, with cut fronts and the plungers in the pulled-out position, were filled with a 1% (w/w) hot agarose solution (Fig. 2) and allowed to set and cool. The protruding gel was trimmed flush with the syringe openings. The syringes were then arranged to dip endwise into a solution of the dye. Due to the diffusion into the gel, a small amount of dye was lost from the solution, but using a large solution volume allowed the approximation of a constant dye concentration.

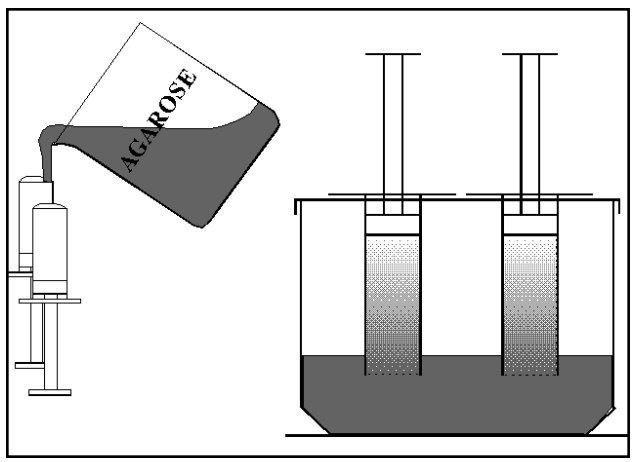

Figure 2. Left: Filling of syringes with hot agarose solution. Right: Experimental arrangement.

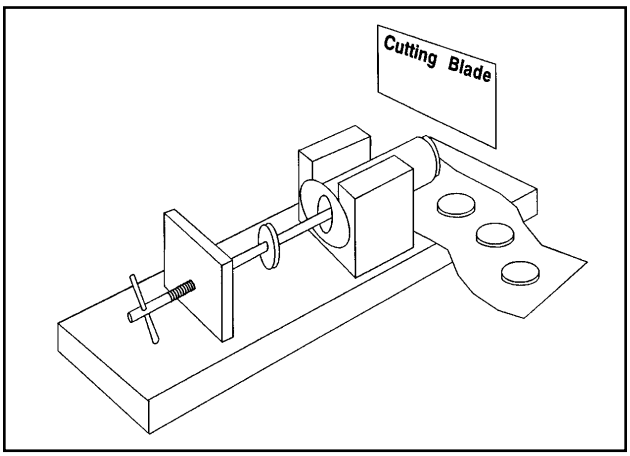

Figure 3. Calibrated device for cutting disks of uniform thickness (one full turn of the screw is equal to 1.0 mm).

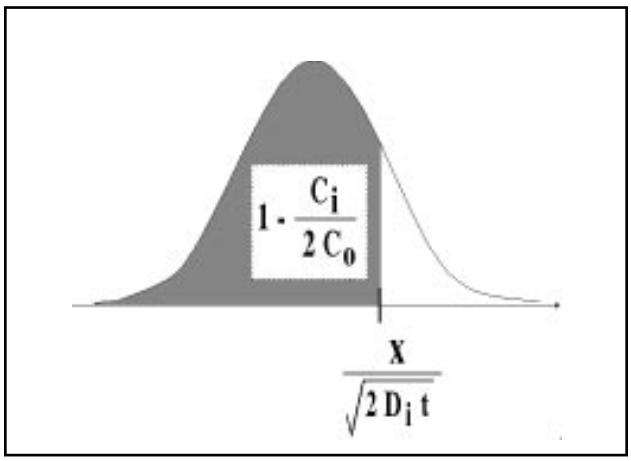

Figure 4. Illustration of the use of the normal distribution.

When the dye had diffused a few centimeters through the gel, the syringe was removed and 20 disks of uniform thickness (2.0 mm each) were cut from the gel cylinder. This was accomplished using a calibrated device operating on the plunger (Fig. 3). Each disk was placed in a vial containing 10 mL of buffer, into which the dye was released. After two days of equilibration, the samples were analyzed spectrophotometrically.

To facilitate comparison between concentration profiles for different compounds and investigation of solution/gel partitioning phenomena, the relative concentration for each disk was calculated using eq 6:

$$C_i = \frac{\text{Dye concentration in } i\text{th disk}}{\text{Dye concentration in sink solution}} \qquad (6)$$

Concentration profiles were obtained by plotting C_i versus the distance x from the gel surface to the center of the ith disk. For calculation of diffusion coefficients from the profile, eq 4 is used in the form:

$$\frac{C_i}{C_B} = 1 - \text{erf}\left(\frac{x}{2\sqrt{D_i t}}\right) \qquad (7)$$

C_B is the relative dye concentration at the gel surface. Unless the diffusant is appreciably excluded or adsorbed by the gelling polymer, the relative concentration in the solution/gel interface, C_B, should be close to unity. The error function can be translated into terms of the more well-known normal distribution using eq 8, where the function Φ represents the normal distribution integrated up to the argument given in parentheses.

$$\text{erf}\,(a) = 2\Phi\left(a\sqrt{2}\right) - 1 \qquad (8)$$

Substituting the argument of the error function in eq 7 into eq 8 gives:

$$\text{erf}\left(\frac{x}{2\sqrt{D_i t}}\right) = 2\Phi\left(\frac{x\sqrt{2}}{2\sqrt{D_i t}}\right) - 1 \qquad (9)$$

Combination of eqs 7 and 9 with algebraic rearrangement gives:

$$\Phi\,[x/(2D_i t)^{1/2}] = 1 - C_i/2C_B \qquad (10)$$

The left side of eq 10 is represented by the shaded area in Figure 4, and the right side of eq 10 states that the same area can be calculated from the experimental parameters C_B and C_i. Hence the corresponding numeric value of the argument can be looked up in tables of the normal distribution, after which D_i can be calculated since x and t are known. C_B was not directly measurable, but for low values of x, the concentration profile was nearly linear (Figs. 6 and 10); hence, C_B could be extrapolated from the C_i values of the first five disks.

Prediction of Rhodamine-B Diffusivity

With a very low concentration of uncharged gelling polymer (agarose), the importance of interactions between the diffusant molecules and the polymer is negligible, and although the macroscopic viscosity of the gel is very high, the microenvironmental transport conditions for the diffusant will be the same as in water (*7–9*). The diffusion coefficient is determined by the linear size of the penetrant molecule via the Stokes–Einstein relationship:

$$D = kT/6\pi\eta R \qquad (11)$$

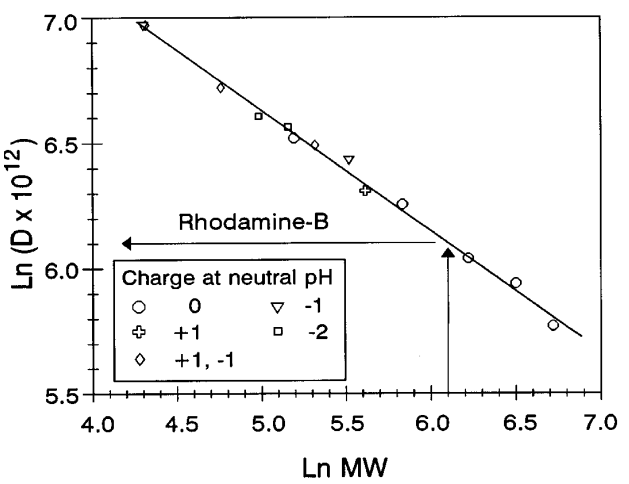

Figure 5. Logarithms of literature data $D/(10^{-12}$ m^2/s) versus logarithms of MW. Key: substances given in order of increasing molecular weight, ln MW values in parentheses: propionic acid (4.30), glycine (4.32), valine (4.76), adipic acid (4.98), octanedioic acid (5.16), glucose (5.19), tryptophan (5.32), dodecyl sulfate (5.52), chlorpheniramine (5.62), sucrose (5.82), tri-hexose (6.22), tetra-hexose (6.50), penta-hexose (6.72).

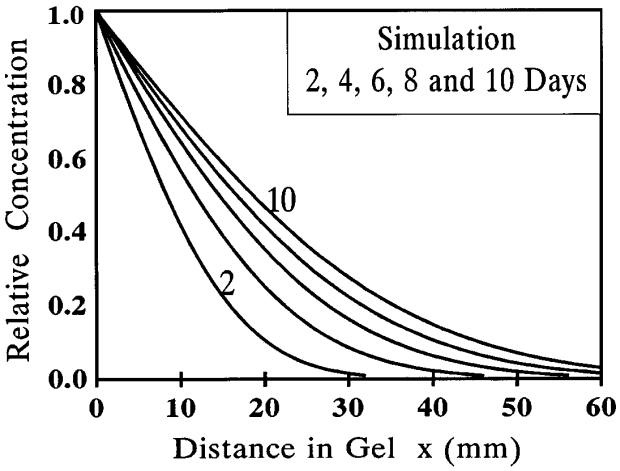

Figure 6. Simulated concentration profiles for rhodamine-B (after 2, 4, 6, 8, and 10 days).

where R is the equivalent hydrodynamic radius of the diffusant, k is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent (water). For prediction of diffusivity and comparison of results with those in the literature, it may be sufficient to use molecular weight as a measure of molecular size. Since the diffusant molecular volume is proportional to its molecular weight, the linear size (R) is proportional to $(MW)^{1/3}$.

$$D \propto (MW)^{-1/3} \qquad (12)$$

Figure 5 is a logarithmic plot of D in water versus MW for a number of substances from the literature (*7, 10*). Although the substances have charges of different sign and magnitudes, a straight line was obtained. Therefore, it was concluded that variation in diffusivity with pH of the system is not to be expected unless the variation in pH also induces other phenomena, such as a decrease in diffusant solubility or interactions with other species in the solution. Figure 5 also shows how the linear relationship obtained is utilized for prediction of rhodamine-B diffusivity. Since rhodamine-B has a molecular weight of 444 (ln MW = 6.10), a diffusivity of 450×10^{-12} m^2/s is estimated.

```
5 CLS : REM Simulation of Profiles
10 Y=EXP(1): A= .31938153
20 B=-.356563782: C=1.78147937
30 D=-1.821255978: E=1.330274429
40 INPUT "Diff.Coeff ="; DC
50 INPUT "Time (Days) ="; T
60 INPUT "Surf.Conc. ="; S
70 T=T*24*3600
80 FOR I=0 TO 60 STEP 5
90 L=I*.001/SQR(2*DC*T)
100 R=1/(1+.2316419*L)
110 Q=1/SQR(2*3.14159)*Y^(-L*L/2)
120 P=1-Q*(A*R+B*R^2+
        C*R^3+D*R^4+E*R^5)
130 F=S*(2-2*P)
140 PRINT I;" mm",F
150 NEXT I: END
```

Figure 7. BASIC program for simulation calculations of concentration profiles.

Simulation of Concentration Profiles

Ideal concentration profiles were calculated by a computer program (Fig. 6) using the estimated dye diffusion coefficient (450×10^{-12} m^2/s). Profiles for 2, 4, 6, 8, and 10 days are shown in Figure 6. For 8 and 10 days, the diffusant has reached the far end of the syringe. Such long times must be avoided, since the free diffusion model is then no longer valid. Hence, if the approximate diffusivity is known, appropriate contact times can be estimated.

The computer program (Fig. 7) is written in Q-Basic and can be run on any IBM-compatible personal computer. It prompts the user to enter a diffusion coefficient in SI units. Type, for example, 450E-12 and press "enter". Enter the time (in days) and the relative surface concentration (unity). The program then calculates the profile for distances into the gel between 0 and 60 mm.

Interactions

Rhodamine-B was chosen because of the evidence for interactions with SDS micelles. Rhodamine-B is a strongly red fluorescent dye and its structure is shown in Figure 8. It is a chloride salt that dissociates in aqueous solution, leaving a positive charge on one of the nitrogens.

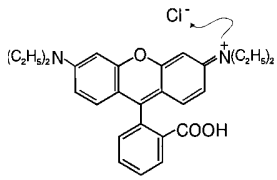

Figure 8. Structure of rhodamine-B.

The system of double bonds can be redistributed with the charge placed on the other nitrogen and thus, due to resonance between the two forms, the positive charge is shared between the two nitrogen atoms. The pK_a for the aromatic carboxylic acid group is 4.2 (*11*). The solubility of rhodamine-B was very low at pH values significantly below the pK_a, and therefore the diffusivity "in water" was measured at pH 7.

Binding of rhodamine-B to SDS micelles is shown in Figure 9. It appears that the ionization state of the carboxyl group may affect the binding, and therefore two pH values, 2.5 and 7, were chosen for investigation. At pH 2.5 the majority of the carboxyl groups are un-ionized, whereas at pH 7 the carboxyl groups are ionized and the molecules are zwitterionic. At both pHs, the positive charge facilitates binding to the negatively charged micelle surface, while at pH 7 the binding presumably is reduced by repulsion of the carboxylate group from the micelle surface (*12*).

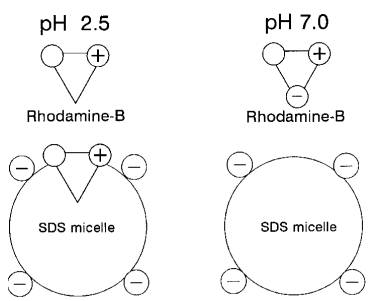

Figure 9. Binding of rhodamine-B to SDS micelles.

With rhodamine-B completely incorporated into continuously intact SDS micelles, the apparent dye diffusivity would decrease by approximately an order of magnitude due to the much greater size of the micelles. The half-life for an intact micelle, however, is quite short; consequently the dye molecules alternate between the micellar and the aqueous environments (*13*). Thus diffusion of rhodamine-B in water occurs, although a continuously intact micelle is a reasonable approximation if the dye-micelle binding constant is high. The prevalence of dye diffusion in water at pH 2.5 was investigated by using higher dye concentrations while the SDS concentration was kept constant. Although the number of SDS molecules per micelle differs somewhat between sources of literature (*12–15*), the dye concentration was adjusted to give about two, four, or eight dye molecules per micelle. As the number of dye molecules per micelle increases, the equilibrium between bound and free dye should shift towards the free form. The dye molecules would thus spend, on the average, more time in the water phase, resulting in a higher apparent diffusivity. At pH 2.5, rhodamine-B has an ionized polar group attached to a large, relatively nonpolar structure, and it is possible that it has sufficient surface activity to allow the formation of mixed micelles. It is also possible that such micelles would be stabilized by interactions between the cationic groups on the rhodamine-B and the anionic groups on the micelle surface (Fig. 9). "Mixed micelles" refers to a special case of solubilization wherein the solubilizate itself becomes an integral part of the micelle structure and larger than normal quantities can be incorporated. In the event of such micelle formation, an increased dye concentration would lead to a higher number of dye molecules per micelle and thus not necessarily to greater dye migration in the aqueous phase.

Experimental Procedure

The gel solution was prepared by adding 1 g of agarose to 99 g of hot water or glycine buffer (pH 7, ionic strength = 0.2 M). The pH was adjusted with HCl. The experiment without SDS was run for 6 days, whereas it was run for 14 days with SDS. The data are summarized in Table 1. To avoid any superimposed gradients in the gel, the pH, ionic strength, and SDS concentration must be homogeneous throughout the system. Although proper amounts of SDS and salt were added to the gelling agarose solution, sufficient time was allowed for equilibration between the aqueous media and the gel before the experiments were started by addition of the dye.

Materials

Agarose (gel point 42 °C, sulfate content < 0.30%, type V), rhodamine-B, and sodium dodecylsulfate were all purchased from Sigma Chemical Company (St. Louis, MO). Deionized water was used.

Results and Discussion

Figure 10 shows the concentration profile for rhodamine-B at pH 7 without SDS. The average diffusivity, 443×10^{-12} m²/s (Table 1), agrees well with that estimated for a substance with a molecular weight of 444 (Fig. 5). Figure 10 also shows the ideal profile, calculated with eq 7 and using the average diffusivity. Good agreement between the experimental and the ideal curves validates the free diffusion model. Except for experimental error, the diffusivity was constant throughout the gel. The relative standard deviation in D_i was ≤ 7%. Results from experiments where SDS was added are shown in Figure 11.

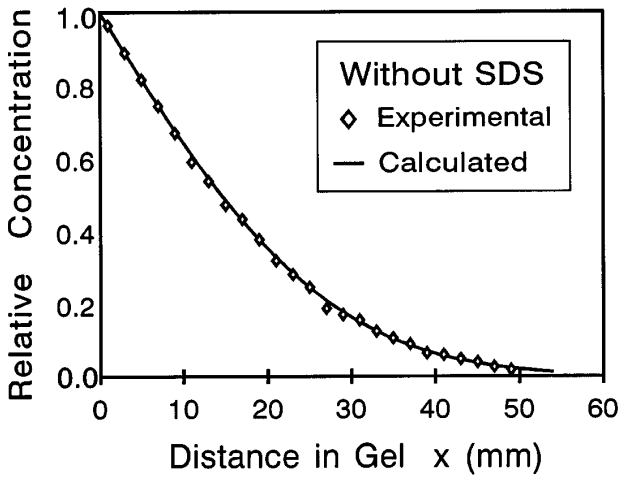

Figure 10. Relative concentration profile for diffusion of rhodamine-B in 1% agarose gel at pH 7 and I = 0.2 M.

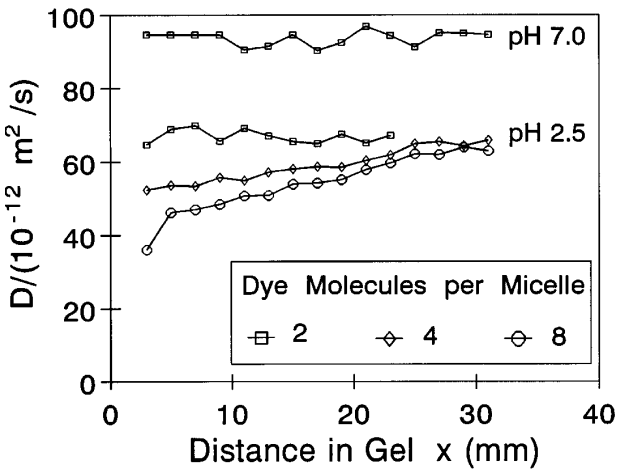

Figure 11. Effect of dye concentration on the relative concentration profiles.

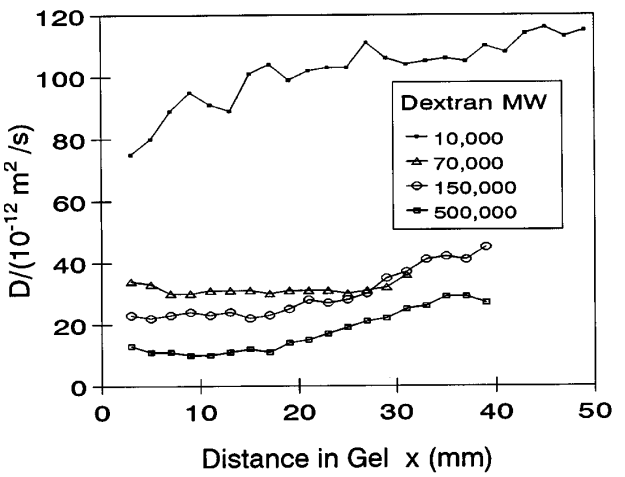

Figure 12. Effect of polydispersity on the diffusion of FITC-labeled dextrans purchased from Sigma Chemical Company (St. Louis, MO). MW 10,000 (FD10S) MW/Mn not given; MW 70,000 (FD70) MW/Mn < 1.25; MW 150,000 (FD150) MW/Mn < 1.35; MW 500,000 (FD500S) MW/Mn not given.

At pH 7 an apparent diffusivity of 94×10^{-12} m²/s was observed. When pH was lowered to 2.5, the binding was evidently facilitated since the diffusivity was further reduced to 65×10^{-12} m²/s. Thus the binding to micelles reduces the apparent dye diffusivity to less than 15% of its value in water. When the number of dye molecules per micelle was increased from two to four, lower diffusivity values were observed close to the gel surface. These values increased along the length of the gel, approaching the diffusivity with two dye molecules per micelle. With eight dye molecules per micelle, the lower diffusivities close to the gel surface were still more pronounced. Similar trends have been observed with FITC-labeled dextrans (Fig. 12) and the increasing diffusivity at distances removed from the gel/water interface was attributed to the inherent polydispersity of the polymer (*8*).

Table 1. Experimental Data

SDS (M)	pH	*I*	Rhodamine-B		$D \times 10^{12}$ m²/s	Hydrodynamic Radius (Å)
			$C \times 1000$ (M)	Molecules per micelle		
0	7	0.2	0.5	–	443	4.8
0.015	7	0.2	0.25	2	94	23.3
0.015	2.5	0.003	0.25	2	118	18.2
0.015	2.5	0.2	0.25	2	65	33.0
0.015	2.5	0.2	0.5	4	52[a]	41.3[a]
0.015	2.5	0.2	1.0	8	37[a]	58.0[a]

[a]Equivalent radius and diffusivity were calculated from the volume segment closest to the gel/water interface (Fig. 7).

The results presented in Figure 11 indicate that larger mixed micelles with four or eight dye molecules form in the solution and that dye diffusion in water (between micelles) is not prevalent. The presence of eight bulky rhodamine-B molecules would increase the size of a micelle even if the number of SDS molecules in the aggregate did not change, and these larger micelles would be expected to have a lower diffusion coefficient, which is seen close to the gel surface. As these mixed micelles spread amongst the pure SDS micelles in the gel, the dye molecules redistribute. As a result, the diffusivity for the mixed micelles gradually approaches that observed for the micelles with two solubilized dye molecules. It is interesting that there is no systematic change in the diffusion coefficient for micelles with two dye molecules. This seems to indicate that incorporation of one or two rhodamine-B molecules into an SDS micelle occurs in the classical solubilization sense; that is, as dissolved guest molecules with little or no influence on the host micelle.

Equivalent hydrodynamic radii, R, for the diffusing units were calculated using the Stokes–Einstein relationship (eq 11). The results are presented in Table 1. For the experiments with trends in diffusivity eq 7 is not valid along the entire length of the gel cylinder. In these cases, radii for mixed micelles were calculated from the observed diffusivity point closest to the gel surface. SDS micelles are known to be larger at higher ionic strength (*12*). This phenomenon was investigated by lowering the ionic strength from 0.2 M to 0.003 M. This resulted in a higher diffusivity and a concomitant decrease in the calculated radius.

Acknowledgment

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