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Evaluation of Dye–Micelle Binding Constants Using Diffusion Sensitive Band Broadening Effects

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The diffusion coefficients of small solutes can be significantly altered by the presence of association colloids such as micelles. A relationship is utilized that relates the diffusion coefficient of a solute to its partitioning or binding behavior to a micellar pseudophase. The Taylor–Aris dispersion method was used to evaluate the diffusion coefficient of several dyes in the presence and absence of sodium dodecyl sulfate micelles. With this approach, all binding constants can be determined easily and reproducibly. The theory, experimental approach, and advantages of this technique are discussed.

INTRODUCTION

The diffusion coefficients of small solutes in aqueous solution can be affected by the presence of colloidal-sized particles or aggregates. The change in diffusional behavior is dependent on a number of factors including the size difference between the solute and colloid (i.e., the difference in their diffusion coefficients), the concentration of the colloid, and whether or not the solutes bind or associate with the colloidal particles. In the case of solutes that do not associate or bind to colloids, there is a relatively small decrease in the diffusion coefficient associated with the presence of the colloid. This is due to the fact that the small solute cannot diffuse through the colloidal particle and must take a relatively longer path around it. This effect can be increased by increasing the concentration of the colloid. A somewhat greater effect can be observed if the solute binds to the colloid. For example, if the diffusion coefficient of a colloidal particle is 10 times less than that of a small solute and the solute binds completely to the colloid, then the diffusion coefficient of the small solute and colloid will appear to be the same. Solutes that are partially associated with a colloid will have diffusion coefficients somewhere in magnitude between that of the free solute and the colloid. The exact value of the observed coefficient depends on the degree of solute association (i.e., binding constant or partition coefficient) with the colloid as well as the concentration of the colloid.

A theoretical relationship relating observed diffusional behavior to the association behavior between small solutes and aqueous micelles was recently derived by Armstrong *et al.* (1). Analogous expressions can be used for most colloidal systems. It was assumed that the observed diffusion coefficient (D_{obs}) of a solute in micellar solution is given by

$$D_{obs} = \beta D_m + (1 - \beta) D_{aq}, \qquad [1]$$

where D_m is the diffusion coefficient of the micelle, D_{aq} is the diffusion coefficient of a solute in aqueous surfactant solution just below the critical micelle concentration (e.g., no micelles present), β is the mole percent of solute in the micelle, and $(1 - \beta)$ is the fraction located in the nonmicellar solution (which also contains surfactant monomers, etc.).

The partition coefficient of a solute between the micelle and bulk aqueous solution (P_{mw}) is given by

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$$P_{mw} = \frac{N\beta C_t / CV}{(1 - \beta)C_t/(1 - CV)}, \quad [2]$$

where C_t is the total solute concentration, V is the molar volume of the micellar surfactant, and C is the concentration of micellized surfactant, which is obtained from

$$C = C_s - \text{CMC}, \quad [3]$$

where C_s is the total surfactant concentration, CMC is the critical micelle concentration, and N is the aggregation number. It is apparent that CV is the volume percent of the micellar phase and $(1 - CV)$ is the volume percent of the bulk aqueous phase. Simplifying and rearranging Eq. [2], one obtains a relationship independent of C_t:

$$\frac{1}{\beta} = 1 + \frac{N(1 - CV)}{P_{mw}CV}. \quad [4]$$

Equation [4] can be solved for β or $(1 - \beta)$ and substituted into Eq. [1] to give

$$D_{obs} = \frac{D_m}{1 + N(1 - CV)/P_{mw}CV} + \frac{D_{aq}}{1 + P_{mw}CV/(1 - CV)N}. \quad [5]$$

If $\psi = N(1 - CV)P_{mw}CV$, then one obtains

$$D_{obs} = \frac{D_m}{1 + \psi} + \frac{D_{aq}}{1 + 1/\psi}. \quad [6]$$

It is apparent that Eq. [6] can be used to determine the partition coefficients of solutes to micelles provided diffusion coefficients can be measured accurately. First one calculates ψ, which is most easily done by rearranging Eq. [6] to

$$\psi = \frac{D_{obs} - D_m}{D_{aq} - D_{obs}}. \quad [7]$$

The partition coefficient then can be determined from

$$P_{mw} = \frac{N(1 - CV)}{\psi CV}. \quad [8]$$

Binding constants (K) can be obtained from partition coefficients using the relationship reported by Berezin *et al.* (2):

$$K_{mw} = (P_{mw} - 1)V. \quad [9]$$

EXPERIMENTAL

Materials. Sodium dodecyl sulfate (electrophoresis purity) was obtained from Bio-Rad Laboratories (Richmond, CA). Phenyl red and HPLC-grade water were obtained from Fisher (Fair Lawn, NJ). Fluorescein and Eosin Y were obtained from Sigma (St. Louis, MO). Eriochrome black T and bromocresol green were obtained from Baker (Phillipsburg, NJ). Bromophenol blue and methyl red were obtained from Matheson, Coleman and Bell (Norwood, OH). Erythrosin was obtained from Pfaltz and Bauer (Stamford, CT) and bromocresol purple was obtained from Eastman–Kodak (Rochester, NY). All chemicals were used without purification.

Methods. All diffusion coefficients were determined by the Taylor dispersion technique (3–6). A 61.0-m length of a stainless-steel tube (0.50 mm i.d., 1.6 mm o.d.) was made into 21-cm-diameter coils and placed in a constant temperature bath (22°C). Four different aqueous carrier solutions were used. They consisted of deionized water and 5.39×10^{-4} M, 0.10 M, and 0.25 M sodium dodecyl sulfate (SDS). A small amount of dilute sample (10 μl of a 0.1% solution) was injected into the capillary tube using a standard LC loop injector. The solutes were dissolved in the same solution that was being used as the carrier. The flow rate was maintained at 0.09 ml/min with an Eldex model A-30-S metering pump. The effluent peak was detected with a Shimadzu SPD-6A UV detector at 254 nm. If the flow is laminar, one obtains a Gaussian peak in which the square of the peak width is inversely proportional to the diffusion coefficient of the solute. The diffusion coefficient can be determined from (3, 4)

$$D = \frac{0.2310r^2t_r}{W_{1/2}^2}, \quad [10]$$

where D is the diffusion coefficient, r is the

radius of the capillary tube (cm), t_r is the residence time of the solute in the tube (s), and $W_{1/2}$ is the peak width at half peak height (s). To avoid secondary flow effects, as discussed by Evans *et al.* (7) and Tijssen (8), one must have a sufficiently long column of the proper diameter, low flow rates, and sufficiently wide diameter coils. The accuracy and performance of this method were checked by measuring the diffusion coefficients of several solutes and comparing them to previously determined values (7, 9) and examining the deviation in a series of replicate analyses. For example, the average deviation and standard deviation of D_{obs} for methyl red in 0.10 *M* SDS was 0.04 and 0.06, respectively (Table I). Literature values for the diffusion coefficients of urea and dextrose in water are 1.382 and 0.6765, respectively (7, 9). Our "system-test" values were 1.39 ± 0.02 and 0.68 ± 0.02, which were determined from four replicate runs. The lack of any peak abnormalities, such as tailing, also indicated that proper conditions were used. All reported data are the mean of three independent determinations.

RESULTS AND DISCUSSION

In a "Taylor–Aris diffusion experiment" (3–6) the band broadening of various solutes, undergoing laminar flow in a narrow-bore tubing, tends to increase as the diffusion coefficient of those solutes decreases Eq. [10]. Figure 1 shows the effect of added sodium dodecyl sulfate (SDS) on the peak width of methyl red. A relatively narrow peak (Fig. 1A) is obtained when no micelles are present in solution. Significant increases in the peak width are observed with the onset of micelle formation (Fig. 1B). As the concentration of micelles is increased, this trend continues until essentially all of the dye is associated with the micelle (Fig. 1C).

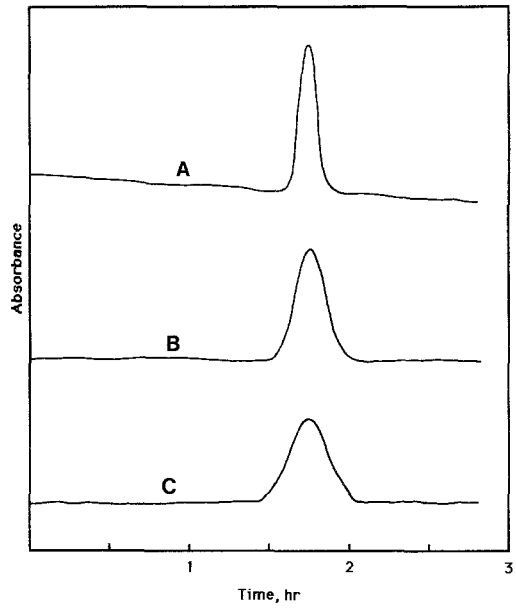

FIG. 1. Elution profiles showing the effect of SDS micelles on the band broadening of methyl red. The greater peak width corresponds to a smaller diffusion coefficient. The concentration of SDS was 5.4×10^{-4} *M* in the experiment that generated curve A. There were no micelles present. In curves B and C, the concentration of SDS exceeded the critical micelle concentration and was 0.1 *M* and 0.25 *M*, respectively.

TABLE I

Diffusion Coefficients (cm^2/s, $\times 10^6$) of Several Dyes in the Presence and Absence of SDS

Dye	[SDS] (*M*)			
	0	5.39×10^{-4}	0.1	0.25
Methyl red	4.55	3.45	1.34	0.97
Fluorescein	3.77	3.66	2.14	1.08
Erythrosin	1.24	1.28	1.02	0.81
Eosin Y	2.80	3.65	2.76	—
Bromocresol purple	3.35	3.89	1.24	—
Bromocresol blue	3.10	4.07	2.54	1.52
Phenol red	4.09	4.33	2.10	—
Bromocresol green	3.27	3.68	2.47	—
Eriochrome black T	3.32	3.75	1.17	—

Using elution profiles such as those in Fig. 1 and Eq. [10], one can obtain all necessary diffusion coefficients. These data are shown in Table I for nine dyes. Using the data from Table I and Eqs. [8] and [9], the desired partition coefficients and binding constants can be determined (see Table II). All nine dyes are water soluble. Eight of the nine dyes are negatively charged while one (methyl red) can exist as a zwitterion. It is apparent from Table

TABLE II

Calculated Values for ψ (Eq. [7]) and the Partition Coefficients (P_{mw}) and Binding Constants (K_b) of Nine Dyes to SDS Micelles

	ψ	P_{mw}	K_b
Methyl red	0.36	7,300	1,800
Fluorescein	1.04	2,600	640
Erythrosin	3.72	720	180
Eosin Y	2.45	1,100	270
Bromocresol purple	0.26	10,400	2,600
Bromophenol blue	1.29	2,100	510
Phenol red	0.68	3,900	960
Bromocresol green	1.57	1,700	420
Eriochrome black T	0.23	11,400	2,800

II that all of the dyes bind to SDS micelles. Those with the largest binding constants (i.e., methyl red, bromocresol purple, and eriochrome black T) tended to be asymmetric in structure. This asymmetry was a result of the charged moiety and the hydrophobic group being located at opposite ends of the molecule, making the molecule somewhat surface active. The more symmetrical dyes had lower binding constants (i.e., erythrosin, bromophenol blue, and bromocresol green).

A variety of techniques have been proposed to evaluate solute micelle binding behavior. Among the first of these were methods that utilized a solute's spectroscopic change upon binding. Virtually any spectroscopic method could be used (e.g., UV–visible, luminescence, NMR, etc.) provided it is sufficiently sensitive and the solute undergoes a sufficient spectral change upon binding. All of these techniques are related to the original Benesi–Hildebrand approach (10–12). A chromatographic technique for evaluating binding constants was first developed for those compounds that didn't undergo a significant spectral change upon binding to micelles (13–15). It has been shown since to have other advantages and is routinely used for a wide variety of compounds. The present diffusion method has several advantages over both the spectral and chromatographic approaches. First, it is independent of the spectral behavior of a solute. The use of a refractive index detector would allow evaluation of spectrally transparent compounds as well as the effect of many additives on binding constants. This method does not require an LC unit or columns, which tend to be expensive and become plugged. Diffusion coefficients can be measured accurately and with a high degree of precision (see Experimental). The apparatus described (a low-pressure pump, stainless-steel tube, and detector) is relatively modest, yet reliable. This theory and experimental approach provides a viable alternative to the well-known, aforementioned methods.

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