Fluorescence Technique for the Determination of Low Critical Micelle Concentrations

Ammanuel Mehreteab* **and Betty Chen**[1]

Colgate-Palmolive Co., Piscataway, New Jersey 08854

ABSTRACT: A technique for determining low critical micelle concentrations (CMC) by means of a hydrophobic fluorescence probe has been developed. The amount of the fluorescent probe at the CMC is so small that the effect of the probe on micelle formation is negligible. The fluorescence intensity was measured at fixed dye/surfactant ratios, and it decreased with concentration. A quantity proportional to fluorescent quantum yield was calculated and found to be high for concentrations of surfactant above the CMC and almost zero below the CMC, giving a distinct break in the quantum yield vs. the concentration curve.

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KEY WORDS: Critical micelle concentration, 1,6-diphenylhexatriene (DPH), fluorescence, fluorescent probe.

The critical micelle concentration (CMC) is an important parameter used to characterize surfactants. Many properties of surfactant solutions have different rates of change above and below the CMC (1). The concentration at which the rate changes has been associated with the CMC. Properties such as surface tension have been commonly used to determine CMC (2). Fluorescence techniques have also been used to determine CMC. Some fluorescence techniques are based on the fact that the various emission bands of some fluorescent probes (e.g., pyrene) change differently in intensity or wavelength, depending on the viscosity and polarity of the probe's environment, i.e., the surrounding solvent (3–12). Below the CMC, the fluorescent probe will be mainly in aqueous medium, whereas above the CMC it will be inside a micelle—a less polar medium (13). A plot of the ratio of peak heights of two bands in an emission spectrum vs. surfactant concentration is used to determine CMC (12,14). Some fluorescence techniques make use of excimer formation of the fluorescent probe (15) or take advantage of the fact that the emission quantum yield of some fluorescence probes is higher in a nonpolar medium than in a polar solvent such as water (16,17). In such cases, however, the probe has to be used in quantities greater than 10^{-5}M (18).

Most fluorescence techniques require the presence of the same amount of dye in the solutions with different surfactant concentrations. As the dye is generally dissolved in an organic solvent, such as alcohol, the ratio of the dye (and organic solvent) to the surfactant increases as the concentration of the surfactant decreases. This ratio would become very large at the CMC of surfactants with a low CMC. Thus, it is necessary to have a low dye/surfactant ratio in order not to disturb the micellization and, consequently, change the CMC. As a result, most fluorescence techniques have been used for surfactants with CMCs of 1×10^{-4}M or higher. In this paper we present a simple method that can be used to determine the CMC of any surfactant. The dye/alcohol solution is initially added to the surfactant stock solution. A serial dilution is made from the resulting dye/alcohol/surfactant aqueous solution. The ratio of the dye and alcohol to the surfactant is kept constant during dilution. This has two advantages: (i) the ratio of the dye to surfactant (less than 10^{-2}) is low enough that micellization is not perturbed by the presence of the dye; and (ii) around the CMC, the alcohol has been diluted significantly so that it does not change the solvent character of the water. Lastly, simple data manipulation of the results is used to increase the sensitivity of fluorescence change at the CMC. [In the present study, the ratio is kept constant, the solvent in which the dye is dissolved (e.g., ethanol) is minimized, and the detection of the point where micelles are formed is made sensitive by simple data manipulations.]

EXPERIMENTAL PROCEDURES

Materials. The dye used as a fluorescent probe, 1,6-diphenylhexatriene (DPH), was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Its purity was 98%. Sodium lauryl sulfate (99% purity) was obtained from BDH Chemicals Ltd. (Poole, England). Tetradecyltrimethylammonium bromide (TTAB) (99% purity) was obtained from Sigma Chemical Co. (St. Louis, MO). AEOS (Alfonic® 1214-65 ethoxylate), a sodium salt of an alkylpoly(oxyethylene)sulfate (20.4% activity) with a carbon chainlength of 12–14 and 65% degree of ethoxylation, was obtained from Vista Chemical Co. (Houston, TX). Nonionic surfactants, Neodol® 23-6.5 and Neodol® 23-6.5 topped, were obtained from Shell Chemical Co. (Houston, TX). Variquat® 50MC, obtained from Sherex

*To whom correspondence should be addressed.

[1]Present address: Robert Wood Johnson Medical School, Rutgers University, Piscataway, NJ 08854.

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Chemical Co. (Dublin, OH), is composed of 50% alkyl (50% C_{14}, 40% C_{12}, 10% C_{15}) dimethylbenzylammonium chloride, 7.5 % isopropyl alcohol and 42.5% water.

Method. Aqueous stock surfactant solutions (about 0.2M) and ethanol stock solutions of DPH (about 0.06%) were prepared. About 2 mL of the DPH solution is diluted with the surfactant solution to 25 mL total volume. Five mL of the resulting solution is further diluted to 25 mL with filtered deionized water. This serial dilution is repeated until the concentration of the surfactant is below its suspected CMC or, as will be discussed later, until the fluorescence intensity of the solution divided by the concentration of the DPH in the solution is less than that of the solution from which it was diluted.

Excitation and emission fluorescence intensities of the above solutions were measured with a Perkin-Elmer (Norwalk, CT) fluorescence spectrophotometer model MPF-3. Emission spectra were obtained by exciting (irradiating) the solution at a wavelength corresponding to one of the dye's absorption peaks while scanning its emission wavelength. For DPH, either the 358- or 378-nm absorption peak can be used as the exciting wavelength. The excitation spectrum can be obtained by measuring the emission intensity at the wavelength of one of the emission peaks while scanning the wavelength of the exciting beam. The excitation and emission wavelengths used to obtain the emission and excitation spectra, respectively, were chosen from the main peaks of DPH's excitation and emission spectra (shown in Fig. 1). The emission intensity at wavelength 430 nm was used when obtaining the excitation spectra. Peak intensities at 358 and 378 nm of the excitation spectra were then recorded for each of the surfactant/DPH solutions Note: It is important to measure the fluorescence of the samples as soon as possible. Some fluorescence probes may aggregate with time which changes their fluorescence and absorption spectra.

RESULTS AND DISCUSSION

Figure 2 shows a plot of fluorescence intensity vs. log of surfactant concentration. No sudden break in the curve was noticed in this case. Therefore, the CMC cannot be determined. However, if the measured fluorescence intensity is normalized by dividing it by a number proportional to the concentration of the DPH, the effect of concentration on the fluorescence intensity is eliminated. Because the ratio of a DPH to surfactant is the same for all samples, "normalized" fluorescence values were obtained by dividing the measured fluorescence intensity by the surfactant concentration for each sample. Figure 3 shows a comparison of the "normalized" fluorescence intensity vs. surfactant concentration to a simple fluorescence vs. concentration curve for AEOS/TTAB complex. The curve passes through a maximum and shows a sudden drop at the lower concentration side, below which the normalized fluorescence drops significantly. The concentration below which the "normalized" fluorescence intensity significantly drops can be associated with the CMC, since the fluorescence probe, DPH, is water-insoluble. In the absence of micelles, it must fall out of solution and, therefore, does not fluoresce.

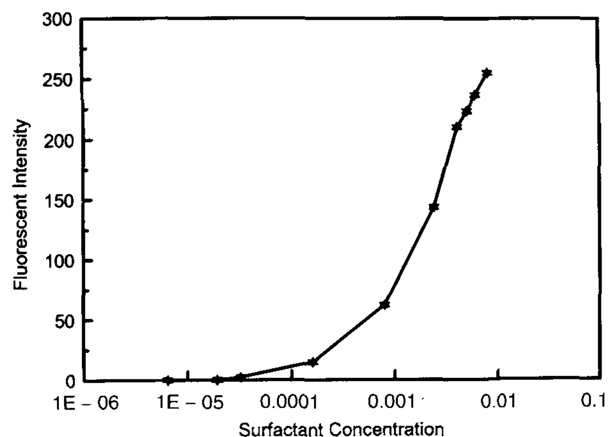

FIG. 2. Fluorescent intensity vs. concentration of fluorescent dye-containing surfactant solution. The dye/surfactant concentration ratio is constant for all solutions.

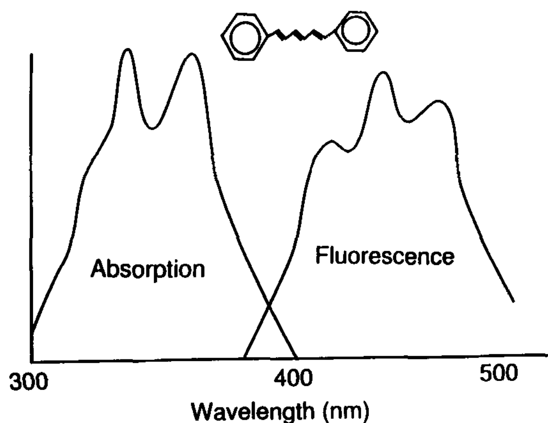

FIG. 1. Absorption and fluorescent spectra of diphenylhexatriene. The intensities (y axes) are normalized so that the absorption and fluorescent structures and wavelength locations could be compared.

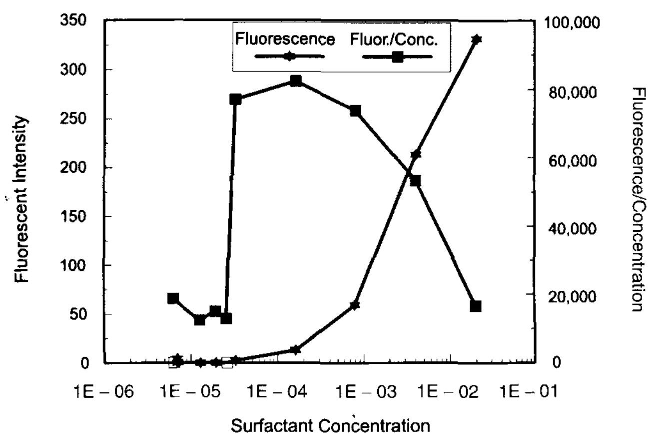

FIG. 3. Effect of data manipulation on the location of critical micelle concentration (CMC). The Fluor./Conc. curve shows a significant drop at surfactant concentration of 3 x 10^{-5} M, which corresponds to its CMC.

The normalized fluorescence intensity decreases at higher surfactant concentration. This is due to an attenuation of the beam. The fluorescence intensity decreases with reduction in the intensity of the exciting beam. At higher dye concentration, the intensity of the exciting beam is attenuated due to absorption by the dye. This is known as inner filter effect. When the absorption and emission spectra of a dye overlap, part of the emitted beam can be absorbed while it is leaving the cuvette. For the DPH, this latter phenomena can be neglected because its emission and absorption spectra are significantly separated, where it cannot be neglected, inner filter effects due to absorption can be corrected by using the following equation:

$$I(corr.) = I \times 10^{(0.5 \times \text{o.d.})} \quad [1]$$

where $I(corr.)$ is the intensity corrected for inner filter effect, I is the intensity of the incoming beam, and o.d. is the optical density of the solution in a 1-cm pathlength. The factor 0.5 is the distance (in cm) the exciting beam has to travel to the center of the cuvette from which the fluorescence intensity is monitored. Because o.d. is proportional to the dye concentration which, in this case, is proportional to the surfactant concentration, o.d. in Equation 1 can be replaced with the surfactant concentration or (for more accuracy) can be measured experimentally with an ultraviolet/vis spectrophotometer. Figure 4 shows the curve for the "normalized" fluorescence intensity vs. surfactant concentration, along with the one corrected for the inner filter effect. This yields a curve that shows no change in fluorescence (and therefore, no change in probe environment) above the CMC.

Table 1 (Refs. 19–22) shows a comparison of CMC values (in dynes/cm) of several surfactant systems obtained by the above method and literature values derived from the surface tension method. There is good agreement between the two methods.

TABLE 1
Comparison of Critical Micelle Concentrations Values of Several Surfactants Obtained by the New Method and the Literature Values by the Surface Tension Method

Surfactant	New method (dynes/cm)	Literature value (dynes/cm)
SLS	0.008	0.0082[a]
TTAB	0.0034	0.0035[b]
AEOS	0.0001	0.0003[c]
AEOS/TTAB	0.000022	0.00004[c]
NEODOL® 23-6.5	0.000015	
NEODOL® 23-6.5 Topped	0.000035	0.00005[d]

[a]TTAB, tetradecyltrimethylammonium bromide; AEOS, Alfonic® 1214-65 ethoxylate (Vista Chemical Co., Houston, TX); NODOL® from Shell Chemical Co. (Houston, TX).
[b]Reference 19.
[c]Reference 20.
[d]Estimated from Figure 2 in Reference 21.
[e]Value is for n-$C_{12}H_{25}(OC_2H_4)_7OH$ where the hydrophitic group is not homogeneous, but distribution of polyoxyethylene chains was reduced by distillation (Ref. 22).

The main advantage of the present method is that it detects the presence of micelles directly, whereas the surface tension method detects formation of micelles indirectly by measuring the degree of surface coverage by the surfactant. The advantage over the other fluorescence techniques is that micelle formation is minimally affected by ethanol and the probe because their concentrations are minimized by this method. This is in contrast to the high concentrations of alcohol and dye which must be used in other methods.

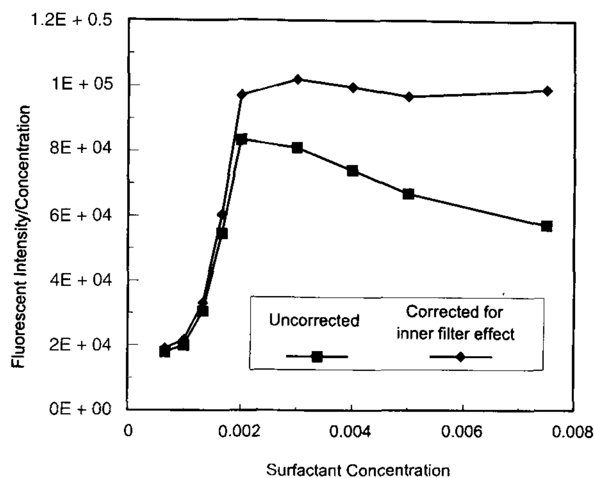

FIG. 4. The effect of correction for inner filter effect. At concentrations above the CMC, the fluorescent quantum yield should remain constant if corrections for inner filter effect are made.

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