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The Second CMC of an Aqueous Solution of Sodium Dodecyl Sulfate. IV. Fluorescence Depolarization

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The depolarization of the fluorescence emitted from a dye solubilized in the micelle was measured over a wide concentration range of sodium dodecyl sulfate (SDS). The polarization of the fluorescence showed an abrupt increase at the 1st CMC and at about 70 mM of SDS; the latter agrees very closely with the 2nd CMC revealed by conductivity and viscosity measurements. This phenomenon implies that there is a certain change in the micelle structure at the 2nd CMC. The micellar size was calculated from the fluorescence depolarization data using Perrin's equation. The results showed that: (1) the micellar volume below the 2nd CMC is in good agreement with that determined by light-scattering, (2) the micellar volume gradually increases with an increase in the concentration of SDS, and (3) the micellar volume increases abruptly at the 2nd CMC.

In our previous papers, the micelle structure of aqueous solutions of sodium dodecyl sulfate (SDS) was investigated by systematic studies including measurements of the conductivity,[1] the density,[2] the viscosity,[2] and the light-scattering.[3] It was revealed by these measurements that, in addition to the 1st critical micelle concentration (CMC), there exists a 2nd CMC, where a change in the micelle structure takes place. Further, the viscosity and light-scattering measurements suggested that the size of the micelle increases at the 2nd CMC.

The micellar sizes of the surfactants in the neighborhood of the 1st CMC have been determined by such methods as light-scattering and osmotic pressures. However, little is known about the micellar size in the high-concentration region because some complications arise upon the application of the above methods to a concentrated solution.[4] Since the theoretical basis of the fluorescence depolarization method has been established by Perrin,[5,6] it has been used successfully in the study of the Brownian motion of a macromolecule in solution.[7-9] This method will provide a powerful tool for the determination of the size of a micelle if we obtain a suitable fluorescent molecule which can tightly

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combine with the micelle.[10-12] Very recently, we succeeded in preparing a derivative of acridine orange (AO) which possesses a dodecyl group; this dye can be expected to bind firmly to the SDS micelle, because the dye molecule has a long hydrocarbon chain and its charge is opposite to that of the micelle. The present paper will report some information about the size of the SDS micelle, particularly in the high-concentration region above the 2nd CMC.

Experimental

Materials. The SDS used in this study was the same as that described in a previous paper.[1] AO-10-dodecyl bromide (Fig. 1) was prepared by modifying the method of Miethke and Zanker;[13] a mixture of AO and 1-bromododecane (ten times the molar quantity of AO) was heated in an oil bath at 110—120°C for 12 hr. The dye thus produced was recrystallized from ethanol–ether and then subjected twice to chromatography on alumina, using ethanol as the eluent.

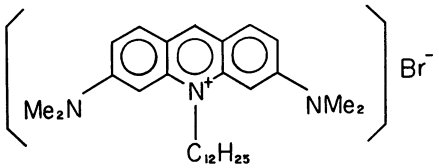

Fig. 1. AO-10-dodecyl bromide.

Methods. The absorption spectra were recorded with a Hitachi ESP-3T spectrophotometer.

The fluorescence spectra were measured with a Hitachi MPF-2A fluorescence spectrophotometer, using an R-106 photomultiplier. They were corrected for the spectral sensitivity of an optical system consisting of lenses, a monochromator, and a photomultiplier.

The degree of polarization of the fluorescence light (P) was measured with a Shimadzu light-scattering photometer equipped with a pair of polaroid dichroic filters to serve as the polarizer as well as the analyzer, and with suitable filters for the incident and fluorescence lights. A 436 nm mercury line was used as the exciting light; it was plane-polarized. The value of P was calculated by means of the following equation:

$$P = (I_{//}-I_{\perp})/(I_{//}+I_{\perp}) \quad (1)$$

where $I_{//}$ and $I_{\perp}$ are, respectively, the components of the fluorescence light parallel to and perpendicular to the direction of the polarization of the exciting light.

The fluorescence lifetimes were measured by means of a JASCO FL-10 phase fluorometer.[14]

All the measurements were carried out at temperatures from 17 to 40°C for SDS solutions containing constant amounts of the dye, $(1—2.0)\times10^{-6}$ M. In order to control the temperature of the solution, water from a thermostat was circulated through a water jacket surrounding the cell; the temperature was measured with a calibrated thermistor. One-centimeter quartz cells were used in all the measurements.

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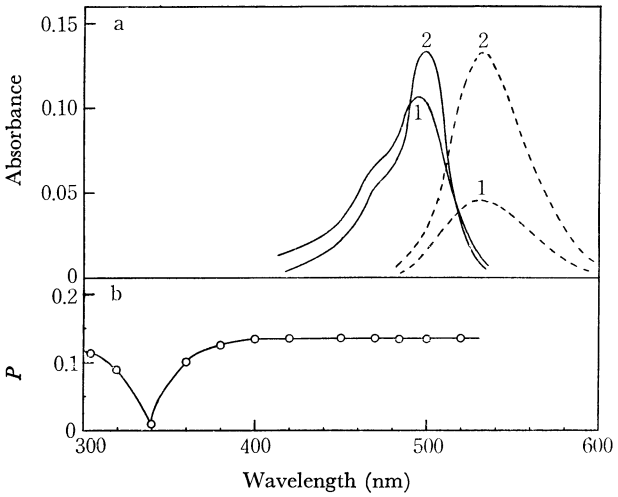

Fig. 2. Absorption (—), fluorescence (---), and fluorescence-polarization (-o-) spectra of the SDS-dye systems in aqueous solutions at 25°C. Fluorescence spectra were obtained with excitation at 460 nm. Fluorescence spectrum (1) is enlarged to a scale 10 times that of (2).
1: dye only (1.59×10^{-6} M), 2: SDS (0.1 M)

Results and Discussion

Figure 2 shows some typical findings on the absorption and fluorescence spectra of the SDS-dye systems. As may be noted from Fig. 2a, there occurred a many-fold enhancement in the fluorescence intensity of the dye above the 1st CMC. In addition, the dye fluorescence in the SDS solutions above the 1st CMC was markedly polarized (Fig. 2b), though that in water showed no polarization. The absorption spectra of the dye in SDS solutions had a peak at 500 nm corresponding to a monomeric species of the dye; this peak was shifted by a few nm towards longer wavelengths compared with that in water (Fig. 2a). The absorbance at 500 nm is plotted against the concentration of SDS in Fig. 3; the absorbance increases abruptly at about the 1st CMC, and then remains constant above that point. These spectral phenomena are rather common results in studies of the association of the dye with the micelle[10,11] and may imply that most of the dye molecules are tightly solubilized in the SDS micelles as a monomeric form. Hence, it is expected that the

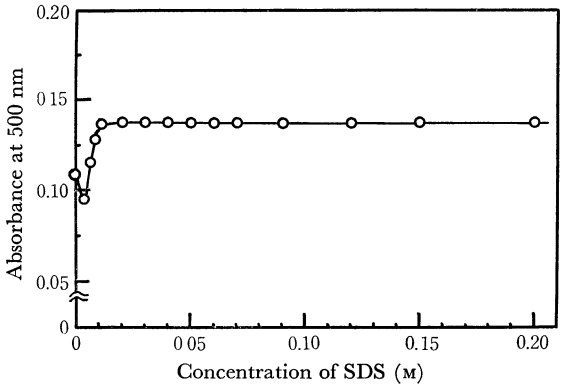

Fig. 3. Absorbance of the SDS-dye systems at 500 nm as a function of the concentration of SDS at 25°C.
Dye concn.: 20×10^{-6} M

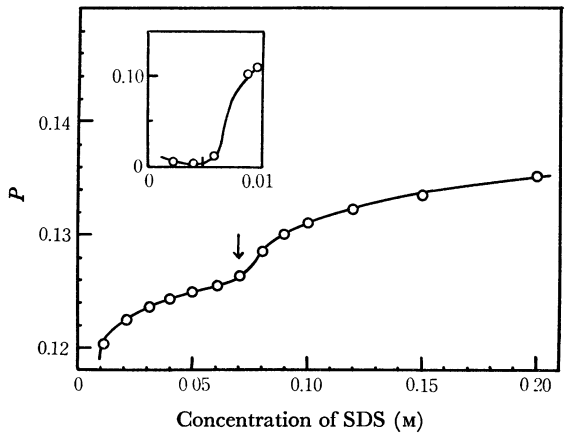

Fig. 4. A plot of the P value against the concentration of SDS at 25°C. The result in the neighborhood of the 1st CMC is shown under enlargement.

value of P reflects exactly the Brownian rotation of the SDS micelle in the solution.[10,11]

Figure 4 shows the relation between the P value and the concentration of SDS at 25°C. It may be seen in Fig. 4 that the P value increases abruptly at the 1st CMC and, in addition, at about 70 mM of SDS; the latter agrees very closely with the 2nd CMC revealed by other measurements.[1-3] This phenomenon may be attributed to a certain change in the micelle structure at the 2nd CMC.

According to Perrin,[5,6] the P value is related to the molar volume of the fluorescent molecule (V) by the following equation, on the assumption that the molecule undergoing rotation is spherical:

$$1/P - 1/3 = (1/P_0 - 1/3)(1+\tau RT/\eta V) \qquad (2)$$

where P_0 is the limiting value of P when $T/\eta=0$; R, the gas constant; T, the absolute temperature; τ, the lifetime of the fluorescence, and η, the viscosity of the solvent. The rotational relaxation time of this molecule (ρ) equals $3V\eta/RT$. In the micelle-dye systems, V in Eq. (2) is taken as the effective volume of the micelle in which the dye is solubilized.

The V value is dependent not only on the measured values of P, η, and T, but also on the values of τ and P_0. Singleterry *et al.*[10-12] developed an elegant technique for the determination of the effective micellar volume from the fluorescence-depolarization data. Their method, however, was incomplete because the fluorescence lifetimes had never been measured directly. In the present study, the values of τ in the SDS-dye systems were determined directly by means of a phase fluorometer.[14] The results at 25°C were as follows: (1) τ in water was 0.37 nsec and (2) τ in SDS solutions was constant (2.0 nsec) over a wide concentration range above the 1st CMC. According to Eq. (2), a straight line which cuts the $1/P$ axis at $1/P_0$ should be obtained when $1/P$ is plotted against T/η. Thus, the values of P_0 in the SDS-dye systems were determined experimentally by measuring the P value in the temperature range from 17 to 40°C and by then extrapolating the straight line to $T/\eta=0$. Some results are shown in Fig. 5, where the plots of $1/P$ *vs.* T/η at each concentration of SDS give the concordant value of $P_0=0.167$.

The effective micellar volumes of SDS at 25°C were calculated by introducing the quantities obtained above into Eq. (2), on the assumption that the micelle is spherical. In view of the findings on small-angle X-ray

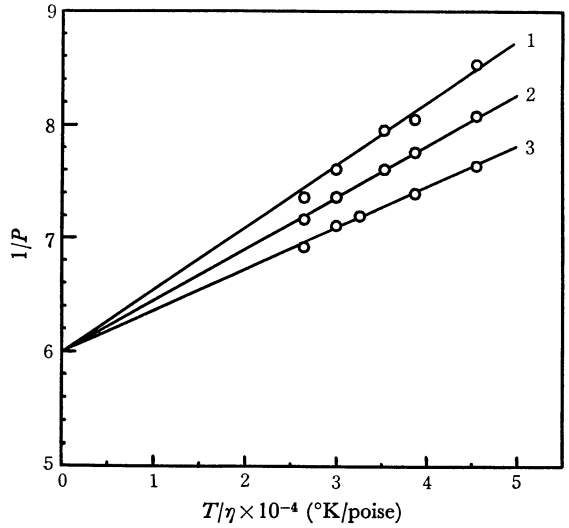

Fig. 5. Typical plots of $1/P$ *vs.* T/η for the SDS-dye systems at 25°C.
Dye concn.: 1.59×10^{-6}M
SDS concn.: 1: 0.05M, 2: 0.10M, 3: 0.20M

TABLE 1. DEPOLARIZATION RESULTS OF THE SDS–DYE SYSTEMS AT 25°C

SDS concn. (M)	P	ρ (nsec)	V (ml per gram micelle)
0.04	0.124_5	16.7	15400
0.05	0.125	17.0	15700
0.06	0.125_5	17.3	16000
0.07	0.126_5	17.8	16500
0.08	0.128	19.1	17700
0.09	0.130	19.9	18400
0.10	0.131	20.4	18800
0.12	0.132	21.4	19800
0.15	0.133	22.7	21000
0.20	0.135	23.9	22000

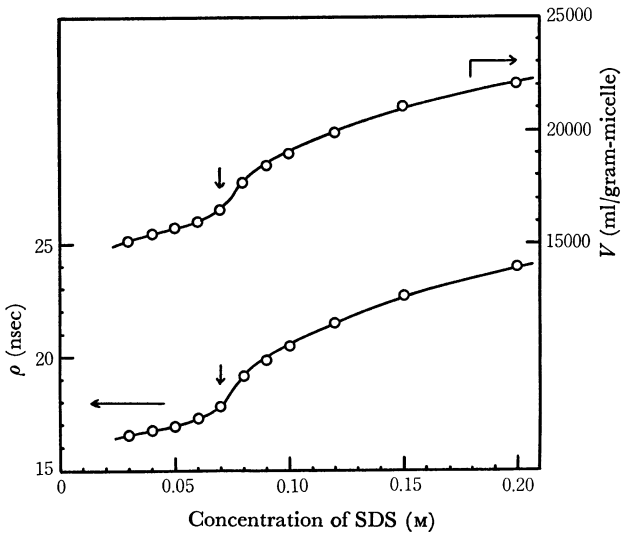

Fig. 6. Plots of ρ and V against the concentration of SDS at 25°C.

scattering,[15,16] this assumption seems reasonable in the concentration range investigated. The results are summarized in Table 1, where ρ and V represent, respectively, the rotational relaxation time of the micelle and the effective micellar volume. Figure 6 shows plots of ρ and V against the concentration of SDS. The depolarization results presented in Table 1 and Fig. 6 can be summarized as follows:

(1) The micellar volumes below the 2nd CMC range from 15500 to 16000 ml/gram-micelle.

(2) The micellar volume increases gradually with an increase in the concentration of SDS below the 2nd CMC.

(3) The micellar volume increases abruptly at the 2nd CMC, and then it increases again gradually with the comcentration.

The micellar density was estimated to be 1.09 g/ml by using the density data[2] obtained according to the method of Mukerjee.[17] Therefore, the micellar weight below the 2nd CMC becomes $(169—174)\times10^2$, which is in good agreement with the value (170×10^2) obtained by light-scattering measurements.[3] This agreement seems to confirm the assumption that the dye has no freedom of rotation with respect to the micelle in which the dye is solubilized. This may be caused by the firm binding of the dye to the SDS micelle, since the dye molecule has not only a charge opposite to the micelle, but also a long hydrocarbon chain. It can be concluded, therefore, that the depolarization of fluorescence observed reflects correctly the Brownian rotation of the SDS micelle and that the fluorescence depolarization method provides a direct means for the determination of the micellar size over a wide concentration range of SDS.

Evidence (2) is consistent with the studies of other investigators;[15,16,18–22] they showed that the micellar size increases with the concentration of the surfactant.

Evidence (3) is a fact which has newly observed in the case of SDS. This fact accounts well for the results of light-scattering, *i.e.*, the marked rise in the reduced intensities at the 2nd CMC. The reason why the micellar volume increases abruptly at the 2nd CMC is still not clear, however; this subject will be dealt with in the future.

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