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Determination of the Critical Micelle Concentrations and Microviscosity with a Fluorescence Probe, Auramine

Shigeyoshi Miyagishi,* Hirotaka Kurimoto, Yuuhichi Ishihara, and Tsuyoshi Asakawa
Department of Chemistry and Chemical Engineering, Faculty of Technology, Kanazawa University,
2-40-20, Kodatsuno, Kanazawa 920

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A new method based on the fluorescence intensity of bis[4-(dimethylamino)phenyl]methanimine (auramine) was proposed in order to determine the critical micelle concentration (cmc). The cmc values of the following surfactants were determined by the present method, and were compared with those by a surface-tension method. The nonionic surfactants used were homogeneous polyethylene glycol monododecyl ether ($C_{12}E_5$, $C_{12}E_6$, $C_{12}E_7$, and $C_{12}E_8$) and *N*-acyl-*N*-methylglucamine (MEGA-7, -8, -9, -10, and -12). The cationic surfactants were dodecyltrimethylammonium chloride and bromide (DTAC, DTAB), tetradecyltrimethylammonium bromide (TTAB), hexadecyltrimethylammonium chloride and bromide (CTAC, CTAB). The potassium salt of *N*-acylalanines, the sodium salt of *N*-acylvalines, and sodium dodecyl sulfate were used as anionic surfactants. The cmcs measured by both methods were in good agreement with each other in all systems of surfactants used. The effects of added salt, temperature, and size of the acyl group on the microviscosity were also estimated.

Surfactant molecules aggregate to micelles above a critical concentration which depends on their molecular structure and environment. The concentration is wellknown as the critical micelle concentration (cmc), which is one of significant properties of micellar solutions. For example, various thermodynamic quantities of micellization can be determined based on this concentration.[1)] A significant number of reports have been published about the determination of the cmcs.[2)] It is wellknown that not only the macroscopic properties of a micellar solution, but also its microscopic properties, change rapidly at the cmc; such changes have been used to measure the cmc. Micropolarity probes[3—9)] have often been utilized for determining the cmc, though some of them have various weak points. However, 1-pyrenecarbaldehyde[9)] is better among the widely useful fluorescence probes for determing the cmc; unfortunately, there is some difficulty in preparing its solution.

The utilization of a microviscosity probe for determining the cmc has been very rare.[10)] Micro-viscosity changes at the cmc and its change can be detected by using some viscosity probes. We previously reported[10)] that auramine can be a good probe for determining the microviscosity in micelles, since it exhibits intense fluorescence in viscous solutions.[11)] We have also indicated that the microviscosity in a micelle depends on both its external enviroment and micellar shape.

The purpose of this report is to clarify that auramine is a useful probe for determining the cmc, and to estimate the microviscosity. Some of the characteristics of this method are as follows: 1) it is applicable to many kinds of surfactants, being independent of their ioninc properties; 2) it can be very easily dissolved in surfactant solutions; 3) one can measure the fluorescence spectrum immediately after the probe is dissolved; 4) stock solutions of the probe are very stable, and 5) the cmcs determined with this method are in good agreement with those determined by other methods.

Experimental

Auramine (guaranteed reagent, Kanto Chemical Co.) was used as received. An aqueous solution of 1×10^{-4} mol dm^{-3} auramine was prepared. This stock solution was stable for at least one month. Pentaethylene glycol monododecyl ether ($C_{12}E_5$), hexaethylene glycol monododecyl ether ($C_{12}E_6$), heptaethylene glycol monododecyl ether ($C_{12}E_7$), and octaethylene glycol monododecyl ether ($C_{12}E_8$) were purchased from Nikko Chemicals Co., and were used as received. *N*-acyl-*N*-methylglucamine (MEGA-n) surfactants were prepared as descibed before,[12)] and were recrystallized from diethylether–acetone or acetone. Dodecyltrimethylammonium chloride and bromide (DTAC, DTAB, from Tokyo Kasei Ind., Co.) were recrystallized from diethylether–ethanol. Tetradecyltrimethylammonium bromide, cetyltrimethylammonium chloride and bromide (TTAB, CTAC, CTAB, from Tokyo Kasei Ind., Co.) were recrystallized from acetone. *N*-Acyl amino acids were the same as those used previously.[13)] *N*-Acylalanine and *N*-acylvaline were used as potassium and sodium salts, respectively. Sodium dodecyl sulfate (SDS, special prepared reagent for reseach of protein, Nacalai Tesque) was recrystallized from ethanol after washing with diethylether in a Soxhlet's extractor.

Fluorescence measurements were carried out on a Hitachi fluorescence spectrophotometer (F-3010) equipped with a temperature control unit. The excitation and emission wavelengths were 410 and 490 nm, respectively; however, 440 and 500 nm were used in alkaline solutions for excitation and emission. The concentration of auramine in sample solutions was usually maintained at 1×10^{-5} mol dm^{-3}. The surface tensions of solutions were measured using of a Wilhelmy plate technique (Kyowa Kagaku Model A-3 surface-tension meter). The unit of (M) in each figure corresponds to mol dm^{-3}

Results and Discussion

Dependence of the Fluorecence Intensity on the Surfactant Concentration and Microviscosity. The fluorescence intensity of auramine was al-

Table 1. The Values of K/N and I_m/I_0

Surfactants		$K/N/(\mathrm{dm^3\,mol^{-1}})$	I_m/I_0
MEGA-8	(25°C)	17.5	13.2
MEGA-9	(25°C)	39.6	15.0
MEGA-10	(25°C)	77.8	16.4
MEGA-12	(25°C)	272	19.5
$C_{12}E_7$	(25°C)	117	8.65
	(50°C)	65.9	5.38
$C_{12}E_8$	(25°C)	129	8.48
	(50°C)	74.3	5.01
DTAB	(25°C)	9.59	7.00
	(NaBr 1.0 mol dm^{-3})	158	7.33
CTAB	(35°C)	7.49	5.08
	(NaBr 0.03 mol dm^{-3})	20.6	5.99
CTAC	(35°C)	9.07	10.9

most constant at lower surfactant concentrations than the cmc, began to sharply increase at the cmc, and then approached a constant value at higher concentrations. Typical examples are given in Fig. 1, where I and I_0 are the fluorescence intensities of auramine in the presence and absence of a surfactant, respectively. This figure depicts the tendency that the variation of I/I_0 at the cmc is larger in a system having a lower cmc than that in a system having a higher cmc. Such a tendency may result from the greater solubilization power of the former system, compared with that of the latter and/or a higher fluorescence intensity (I_m) in the former micelle (described below).

Since auramine is soluble in aqueous solutions, its partition between the aqueous and micelle phases may significantly contribute to its fluorescence intensity in micellar solutions. The binding coefficient (K) is defined as $K=[SM]/[S][M]$, where [S] and [SM] are the concentrations of nonbinding and binding probes, respectively, and [M] is the concentration of a micelle containing no probe molecule. The value of K can be determined as reported in a previous paper.[10] That is, the fluorescence intensity (I) increases above cmc with increasing surfactant concentration (C), and approaches a saturated value (I_m). These data are used to determine the binding coefficient of a probe (K), as follows:

$$\frac{1}{(I-I_0)}=\frac{1}{(I_m-I_0)}+\frac{1}{(I_m-I_0)}\frac{N/K}{(C-\mathrm{cmc})}, \qquad (1)$$

where I_m is the fluorescence intensity in the micelle phase, and N is the aggregation number of a micelle. The value of the cmc can be determined more easily with increasing difference between I_0 and I_m, as well as with increasing value of K.

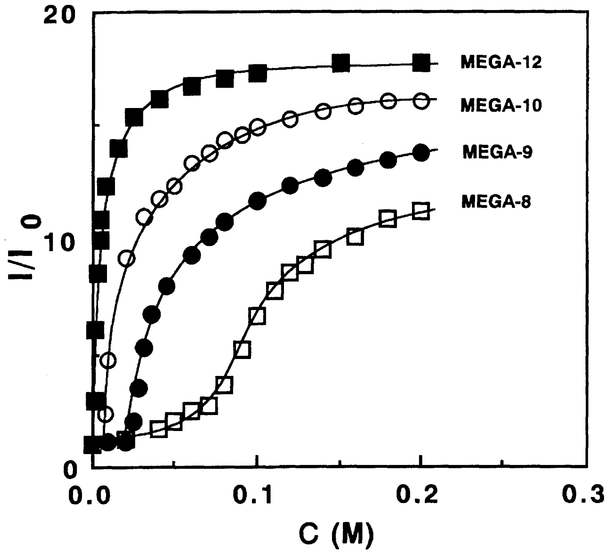

Fig. 1. Dependence of fluorescence intensity of auramine on concentration of MEGA-n at 25 °C.

The estimated values of K/N and I_m/I_0 are given in Table 1. Both values of the surfactant which exhibited a greater change of I/I_0 at the cmc in Fig. 1 are also larger in Table 1. In the systems of $C_{12}E_n$, K/N and I_m/I_0 decreased along with increasing temperature, while K/N increased and I_m/I_0 decreased with an addition of one unit of oxyethylene to a $C_{12}E_7$ molecule. In the MEGA series, a surfactant with a longer acyl group gave larger values for K/N and I_m/I_0. Both K/N and I_m/I_0 in cationic surfactant systems increased upon the addition of salt. Taking account of the aggregation number of each micelle (N), the binding coefficient (K) may be higher than several hundred; that is, most of the probe molecules exist in the micelles, rather than in a bulk solution. Therefore, the value of I in a high surfactant concentration can probably be used as a measure of the microviscosity in a micelle, instead of I_m.

Since the microviscosity can be followed by the fluorescence intensity of auramine,[10,11] I_m/I_0 is a measure of the microviscosity in a micelle, and can be related to the bulk viscosity using the calibration curve of Fig. 1 in Ref. 10. An increase of the microviscosity upon increasing the acyl chain of the MEGA series, its increase upon an addition of salt in cationic surfactant systems, and its decrease upon increasing temperature in the $C_{12}E_n$ series seem to be a reasonable trend. As shown in Table 1, cationic surfactant systems contain-

Table 2. cmc of Ionic Surfactants (25 °C)

	cmc/mmol dm^{-3}	
Surfactants	Auramine method	Other methods
DTAB	15	14.8[c)]
DTAC	19	19.5[d)]
TTAB(35°C)	3.5	3.3[a)]
CTAC(35°C)	1.3	1.3[e)]
CTAB(35°C)	0.91	0.91[a)]
Lau-DL-Val	6.3	6.5[a)]
NaCl 0.02 mol dm^{-3}	3.3	3.3[a)]
0.1 mol dm^{-3}	1.3	1.2[a)]
Myr-DL-Val	1.5	1.4[a)]
NaCl 0.05 mol dm^{-3}	0.23	0.49[a)]
Pal-DL-Val	0.16	0.16[a)]
NaCl 0.05 mol dm^{-3}	0.016	0.018[a)]
Lau-DL-Ala[f)]	1.7	1.7[a)]
Myr-DL-Ala[f)]	0.13	0.14[a)]
SDS (1 mmol dm^{-3} NaOH)	7.6	7.8[a)]

a) by surface tension method, b) from Ref. 19, c) from Ref. 20, d) from Ref. 21, e) from Ref. 22, f) potassium salt.

ing the bromide ion gave smaller I_m/I_0 than did CTAC, because the bromide ion acted as a quencher. However, attention should be given to the fact that the probe is solubilized in each site having a different property in the case of comparing different homologous surfactants.[14)] The microviscosity estimated by a probe method can be a relative measure, but not the absolute viscosity. This is because the viscosity is usually defined in a macroscopic media, while the microviscosity is a measure of the molecular motion of a probe, and depends strongly on not only its quenching mechanism,[16—18)] but also its solubilized site. The values of I_m/I_0 should be explained based on the strength of the molecular interaction between the probe and the hydrophilic group of a surfactant molecule, and from its molecular motion at its site. A further investigation is required for this purpose.

cmcs of Ionic Surfactants. As shown in Fig. 2, the value of I/I_0 in each system of DTAC and DTAB increased steeply above a surfactant concentration corresponding to each cmc. A similar behavior was also observed in each system TTAB and CTAC (Fig. 3). The cmc values of these cationic surfactants were compared to those obtained by a surface-tension method, or values from the literature (Table 2). Both methods provide almost the same cmcs. Although auramine is cationic, it seems to be an excellent probe for the cmc determintion of cationic surfactants. Figure 4 shows that this probe can also be useful for determining the cmcs of anionic surfactants. In systems of acylvalinates, the fluorescence intensity ratio (I/I_0) varied little until its concentraiton reached its cmc, then increased steeply above that point with increasing concentration. The increment in I/I_0 at the cmc was much larger in these systems than that in the systems of cationic surfactants. The cmcs of several anionic surfactants derived from amino acids were determined with both methods. Excellent agreement was obtained (Table 2).

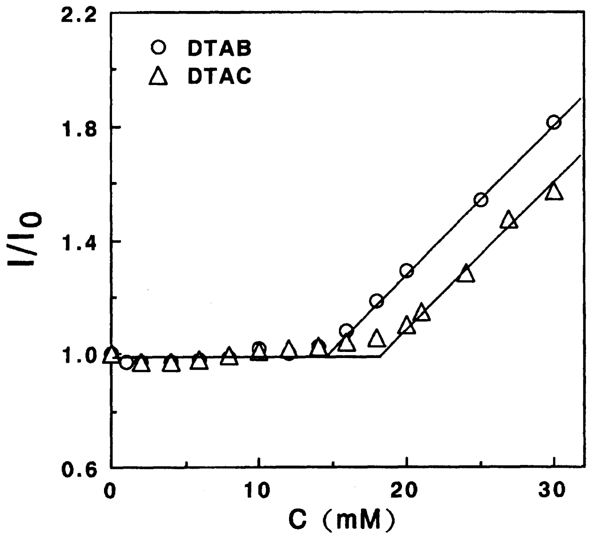

Fig. 2. Determination of cmc of DTAC and DTAB at 25 °C.

The value of the cmc in an SDS solution containing 1 mmol dm^{-3} NaOH was determined as shown in Fig. 5, where the fluorescence intensity of auramine began to increase just above the cmc. This value was also the same as the cmc determined by the surface tension method. However, in an SDS system containing no NaOH, the fluorescence intensity increased very steeply within a very narrow concentration range just below its cmc, and became nearly constant above this point.[10)] Such a difference in the fluorescence behavior between systems with and without NaOH results from the following: Although auramine is a neutral molecule in an

Table 3. cmc of Nonionic Surfactants (25 °C)

Surfactants	cmc/mmol dm^{-3}	
	Auramine method	Other methods
MEGA-8	64.0	67.7$^{a)}$
MEGA-9	22.5	23.2$^{a)}$
MEGA-10	5.9	5.9$^{a)}$
MEGA-12	0.48	0.50$^{a)}$
$C_{12}E_5$	0.065	0.065$^{b)}$
$C_{12}E_6$	0.070	0.068$^{b)}$
$C_{12}E_7$	0.071	0.069$^{b)}$
$C_{12}E_8$	0.085	0.072$^{b)}$

a) by surface tension method, b) from Ref. 19.

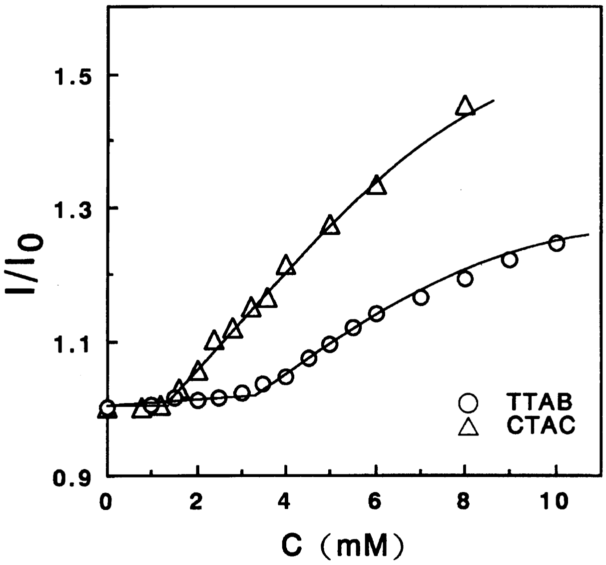

Fig. 3. Determination of cmc of TTAB and CTAC at 35 °C.

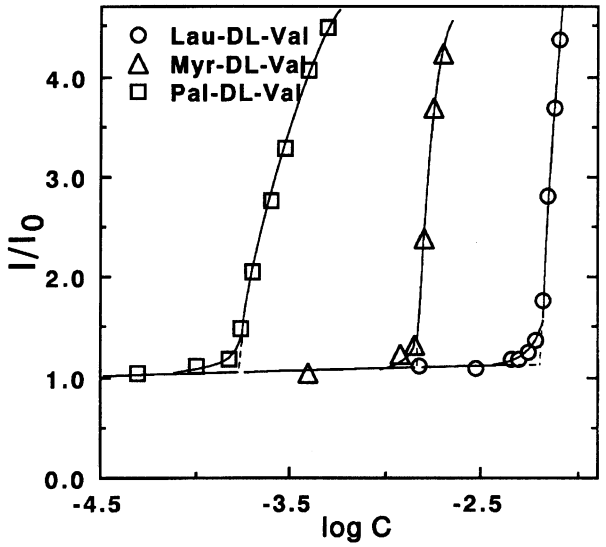

Fig. 4. Determination of cmc of sodium salt of *N*-acylvalines at 25 °C.

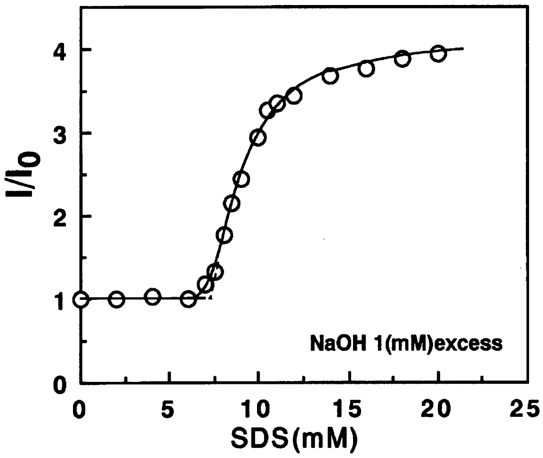

Fig. 5. Determination of cmc of SDS in presence of 1 mmol dm^{-3} NaOH at 25 °C.

alkaline solution, it is a cation under acid or neutral conditions; also the fluorescence spectrum of auramine changes at its pK_a. In addition, the surfactant concentration ranges where the fluorescence intensity steeply varied were very narrow in aqueous anionic surfactant systems compared with the cases of the other surfactant systems.

cmcs of Nonionic Surfactants. The fluorescence intensity ratios (I/I_0) in $C_{12}E_7$ and $C_{12}E_8$ are plotted in Fig. 6. The ratios began to increase rapidly at a certain concentration, which corresponded to the cmc determined by a surface-tension method. A comparison of the cmc between both methods is given in Table 3; both methods gave very similar cmcs with each other. Figure 7 shows the dependence of I/I_0 on the MEGA-n concentration near to the cmc. In every MEGA-n system, the ratio varied little until its concentration reached its cmc, and then increased steeply above its cmc along with increasing concentration. The values of the cmc determined by using auramine are given in Table 3, together with the values based on a surface-tension method. Both values are in good agreement with each other. That is, this result depicts that auramine is an excellent probe for determining the cmc of nonionic surfactants.

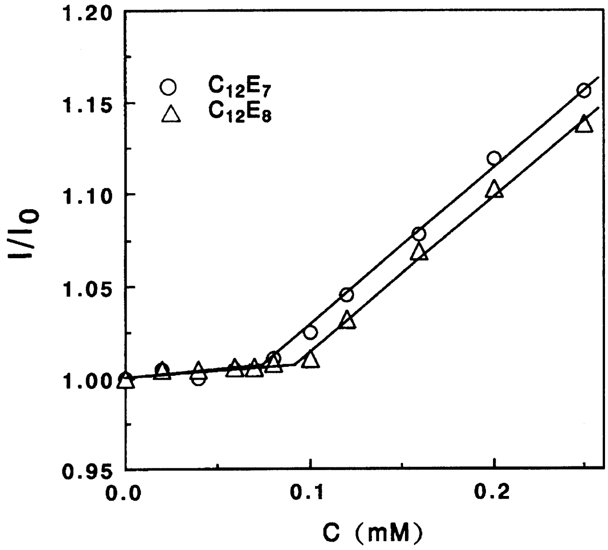

Fig. 6. Determination of cmc of $C_{12}E_7$ and $C_{12}E_8$ at 25 °C.

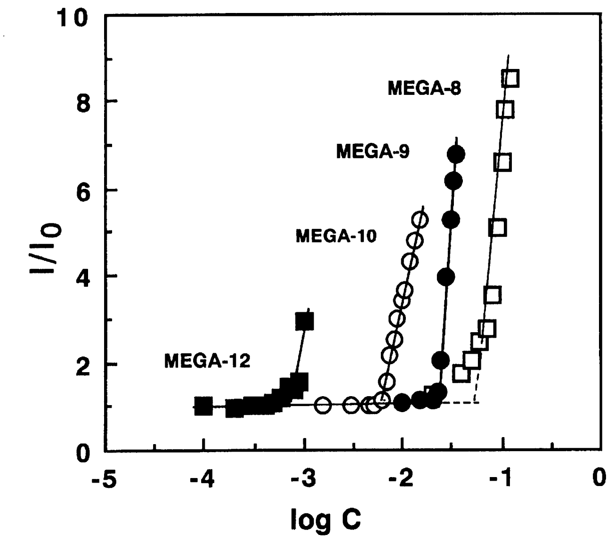

Fig. 7. Determination of cmc of MEGA-n series at 25 °C.

Conclusions

The fluorescence intensity of auramine increased steeply after the surfactant concentration reached each cmc in every system of the cationic and nonionic surfactants. A similar tendency was also found in anionic surfactant solutions; in addition, such a steep change in the fluorescence intensity occurred over a very narrow concentration range, compared with other surfactant systems. In conclusion, it was found that although this method for determining the cmc is applicable to many kinds of surfactant, it is not always superior to a surface-tension method. Further, the microviscosity increased in DTAB and CTAB micelles along with the addition of salt, and increased in MEGA-n series as the length of the acyl chain increased. An increasing temperature resulted in a lowering of the microviscosity of $C_{12}E_n$ micelles.

References

1) K. Shinoda, "Principles of Solution and Solubility," Marcel Dekker, New York (1978), Chap. 9; C. Tanford "The Hydrophobic Interaction," Wiley, New York (1973), Chap. 7; Y. Moroi, "Micelles," Plenum Press, New York (1992), Chap. 4.

2) P. Mukejee and J. Mysels, *Natl. Stand. Ref. Data Ser.* (*U. S. Natl. Bur. Stand.*) **No. 8.** 36 (1971), and their critical evaluation of the methods for determining cmc.

3) P. Heitmann, *Eur. J. Biochem.*, **3**, 346 (1968).

4) N. Kawashima, N. Fujimoto, and K. Meguro, *J. Colloid Interface Sci.*, **103**, 459 (1985).

5) H. -C. Chlang and A. Lucton, *J. Phys. Chem.*, **79**, 1935 (1975); K. S. Birdi, *J. Phys. Chem.*, **81**, 934 (1977); H. -C. Chlang and A. Lucton, *J. Phys. Chem.*, **81**, 935 (1977).

6) R. C. Mast and L. V. Haynes, *J. Colloid Interface Sci.*, **53**, 35 (1975).

7) K. S. Birdi, T. Krag, and J. Klausen, *J. Colloid Interface Sci.*, **62**, 562 (1977).

8) P. Horowitz, *J. Colloid Interface Sci.*, **61**, 197 (1977).

9) K. P. Ananthapadmanabhan, E. D. Goddard, N. J. Turro, and P. L. Kuo, *Langmuir*, **1**, 352 (1985).

10) S. Miyagishi, T. Asakawa, and M. Nishida, *J. Colloid Interface Sci.*, **115**, 199 (1987). In this reference, term "*N*/*K*" in Table III should be replaced by "*K*/*N*" and term "1/*K*" should be ommited.

11) G. Oster and Y. Nishijima, *J. Am. Chem. Soc.*, **78**, 1581 (1956).

12) E. K. Hildreth, *Biochem. J.*, **207**, 363 (1982).

13) S. Miyagishi, S. Matsumura, T. Asakawa, and M. Nishida, *Bull. Chem. Soc. Jpn.*, **59**, 557 (1986).

14) R. Zana, in "Surfactant Solutions—New Methods of Investigation—," ed by R. Zana, Dekker, New York (1987), p. 273.

15) J. Emert, C. Behrens, and M. Goldenberg, *J. Am. Chem. Soc.*, **101**, 771 (1979).

16) K. Law, *Photochem. Photobiol.*, **33**, 799 (1981).

17) M. Sinitzky, A. -C. Dianoux, C. Gitler, and G. Weber, *Biochemistry*, **10**, 2106 (1971).

18) F. Grieser, M. Lay, and P. J. Thistethwaite, *J. Phys. Chem.*, **89**, 2065 (1985).

19) K. Deguchi and K. Meguro, *J. Colloid Interface Sci.*, **38**, 56 (1972).

20) H. M. Emerson and A. Holtzer, *J. Phys. Chem.*, **71**, 1616 (1950).

21) S. Ozeki and S. Ikeda, *Bull. Chem. Soc. Jpn.*, **54**, 52 (1981).

22) A. W. Ralston, et al., *J. Am. Chem. Soc.*, **69**, 2095 (1947).