Journal of Fluorescence, Vol. 3, No. 3, 1993

Cationic Surfactant/Bile Salt Interaction Studied by Fluorescence Quenching

Martin Swanson Vethamuthu,[1] Mats Almgren,[1,2] Emad Mukhtar,[1] and Pratap Bahadur[1]

Received October 18, 1993

Fluorescence quenching measurements were performed on aqueous solutions of the cationic surfactant cetyltrimethylammonium halide (CTAX) and two bile salts, sodium cholate (NaC) and sodium deoxycholate (NaDC), to study the state of aggregation in the mixtures. Pyrene was used as a photoluminiscence probe in the study, and dimethylbenzophenone (DMBP) as the quencher. Analysis of time-resolved decay data with and without quencher using a simple kinetic model gave information of the different aggregation characteristics in the above two cases. Mixed micelles of CTAX/NaC were small and spherical at all compositions, while those of CTAX/NaDC tended to grow from spherical micelles to larger rod-like mixed aggregates at equimolar and close-to-equimolar concentrations. In the latter case more complex kinetics ensues and the fluorescence decays were treated using a generalized model for diffusion-controlled quenching along one dimension for infinitely long rod-like micelles. The mutual diffusion coefficient for the probe–quencher pair was determined.

KEY WORDS: Cationic surfactant; bile salt; fluorescence quenching; mixed micelles.

INTRODUCTION

Fluorescence and fluorescence quenching methods have been used extensively in the last 15 years to provide significant information on the structure, size, and dynamics of micellar systems at the molecular level [1]. Although it has been an important tool in characterizing micellar systems, limited work has been reported on the application of the technique to pure bile salt micelles and bile salt/surfactant mixed aggregates.

Bile salts are classified as important biological detergents and are widely known for their pivotal role in the solubilization of dietary lipids in the digestive tract and the transport of lipophilic products from the liver to the small intestine [2]. Unlike conventional surfactants, bile salts do not have a hydrophobic tail but, instead, a hydrophobic face made up of the steroidal core with the methyl groups at the C-18 and C-19 positions (β-moieties). The two or three hydroxyl groups at the C-3, C-7, and C-12 positions (α-moieties) with a terminal carboxylic headgroup attached to a short and flexible aliphatic tail renders some degree of hydrophilicity to the other face.

In this study we have investigated the interaction and mixed micelle formation in two simple bile salts, sodium cholate (NaC) and sodium deoxycholate (NaDC), with a long-chain cationic surfactant, cetyltrimethylammonium bromide (CTAB), (Fig. 1). Static or wavelength-resolved fluorescence and the dynamic fluorescence decay characteristics of pyrene quenched by dimethylbenzophenone (DMBP) in aqueous mixed micellar solutions have been analyzed to show similarities and differences in the aggregation behavior of trihydroxy bile salt (NaC) and dihydroxy bile salt (NaDC) with CTAB, respectively.

[1] University of Uppsala, Department of Physical Chemistry, Box 532, S-75121 Uppsala, Sweden, and Department of Chemistry, South Gujarat University, Surat 395007, India.

[2] To whom correspondence should be addressed at Department of Physical Chemistry, Box 532, S-75121 Uppsala, Sweden.

1053-0509/93/0900-0185$07.00/0

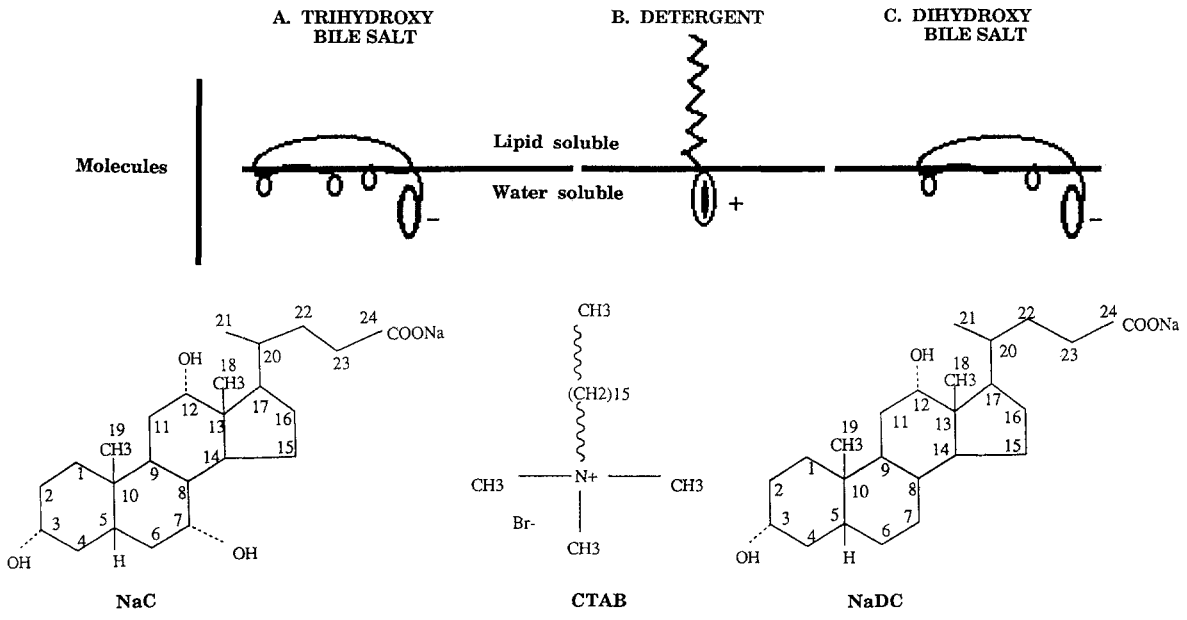


Fig. 1. Molecular structures of the bile salts and cationic detergent: (A) 3α, 7α, 12α-trihydroxy-5β-cholan-24-oic acid, Na Salt (NaC); (B) cetyltrimethylammonium bromide (CTAB); (C) 3α,12α-dihydroxy-5β-cholan-24-oic acid, Na Salt (NaDC).

EXPERIMENTAL

Static fluorescence measurements were made with a SPEX Fluorolog 1680. Time-resolved fluorescence decay data were collected with the single-photon counting technique. Fluorescence quenching data were fitted to a generalized model for fluorescence deactivation proposed by Infelta *et al.* [7] and, in some cases, to a generalized model for diffusion-controlled fluorescence quenching in infinitely long rod-like micelles [8,9].

Fluorescence Decay Equations

The Infelta Model. The fluorescence decay, F_t, of an excited probe molecule subject to quenching by a quencher, both confined to the hydrophobic domain of monodisperse micelles, is described by

$$F_t = A_1 \exp\{-A_2 t + A_3 [\exp(-A_4 t) - 1]\} \quad (1)$$

where the expressions for the parameters A_1–A_4 are given by

$$A_1 = F_o \quad (1a)$$
$$A_2 = k_o + \langle x \rangle_s k_q \quad (1b)$$
$$A_3 = \langle n \rangle (1 - \langle x \rangle_s / \langle n \rangle)^2 \quad (1c)$$
$$A_4 = k_q / (1 - \langle x \rangle_s / \langle n \rangle) \quad (1d)$$

F_o is the fluorescence intensity at time $t = 0$, k_o is the first-order decay constant, of pyrene in the absence of quencher, kq is the first order quenching constant, $\langle x \rangle_s$ is the average number of quenchers in micelles with a surviving excited probe during the stationary part of the fluorescence decay, and $\langle n \rangle$ is the average number of quenchers in a micelle. The aggregation number is obtained from

$$N_{agg} = \langle n \rangle S_m / Q_m \quad (2)$$

where S_m and Q_m are the concentrations of the aggregated surfactants and quenchers in the micellar phase.

Model for Fluorescence Quenching in Infinite Cylinders, One-Dimensional Quenching. A general description of a fluorescence decay curve after excitation with an infinitely narrow δ-pulse in the one-dimensional case is given by

$$\ln \frac{F(t)}{F(0)} = -k_0 t - a^3 c_q Q_1 \left(ha, \frac{t}{\tau_q}\right) \quad (3)$$

with

$$Q_1(ha, t/\tau_q) = \frac{4\pi}{ha}\left\{\sqrt{\left(\frac{ha}{\sqrt{\tau_q}}\right)^2 \frac{t}{\pi}} + \frac{3}{4}\left[\exp\left\{\left(\frac{ha}{\sqrt{\tau_q}}\right)^2 \frac{4t}{9}\right\} \operatorname{erfc}\left\{\left(\frac{ha}{\sqrt{\tau_q}}\right)\sqrt{\frac{4t}{9}}\right\} - 1\right]\right\}$$

where $t_q = a^2/D$, and k_o, the natural decay rate constant,

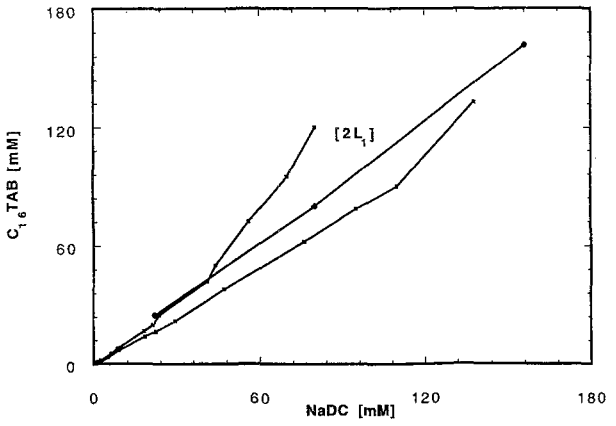


Fig. 2. Partial phase diagram at 23°C for the NaDC/CTAB system.

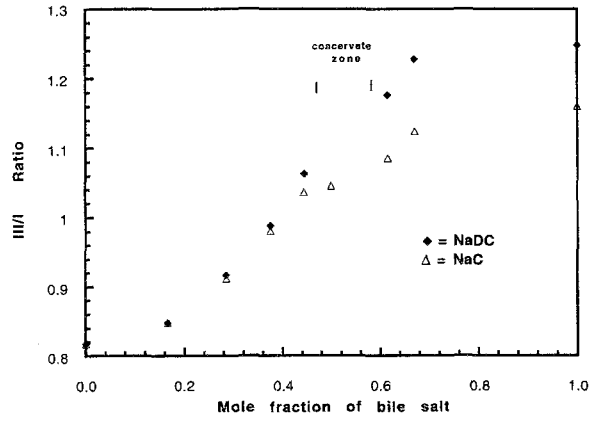
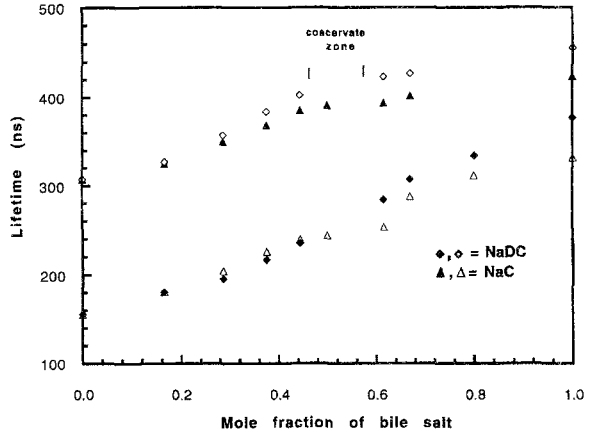


Fig. 3a. Dependence of III/I ratio of pyrene in CTAC/bile salt micelles on the mole fraction of bile salt, T=25°C.



b

Fig. 3b. Pyrene fluorescence lifetimes in CTAC/bile salt mixtures (aerated (△, ◆)/deaerated (▲, ◇), 25°C.

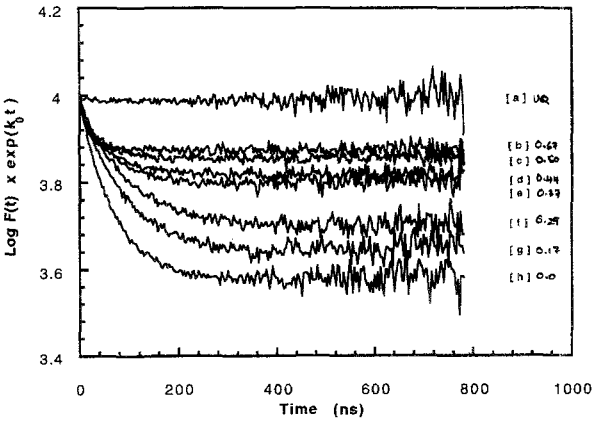


Fig. 4. Fluorescence quenching curves of CTAC/NaC mixed micelles. The mole fraction of NaC was (a) unquenched (b) 0.67 (c) 0.50 (d) 0.44 (e) 0.37 (f) 0.29 (g) 0.17 (h) 0.0.

is determined in a separate experiment and kept fixed in the analysis. *a* is the radius of the cylinder and c_q is the quencher concentration per unit hydrophobic volume of micelle. *ha* is a parameter that weights diffusion against reaction. The first-order quenching rate constant of the probe/quencher pair in the rod-like micelle $k_{q(cyl)}$ is related to the *ha* parameter and is defined as ha/t_q. The probe–quencher pair was assumed to be confined to the hydrophobic domain of the rod aggregate.

RESULTS AND DISCUSSION

The phase separation diagram for mixtures of dihydroxy bile salt (NaDC) and CTAB is shown in Fig. 2. Mixtures whose composition fell on or inside the boundaries of the curves were turbid and separated into two distinct liquid phases. The tie-line in this region is also shown. The phase separation, called coacervation, is reversible and is suppressed by increasing temperature. Similar mixtures with trihydroxy bile salt (NaC) showed no coacervation but a single isotropic micellar phase.

Figure 3a shows the ratio of the vibronic peaks III/I in the pyrene fluorescence spectra as a function of the mole fraction of bile salt, which is often used as a measure of the polarity of the microenvironment around the probe. The measured lifetime as a function of the mole fraction is shown in Fig. 3b for aerated (△, ◆) and deaerated (▲, ◇) samples of bile salt/CTAC mixtures. In both cases the lifetime and III/I ratio increases with increasing mole fraction of bile salt. However, in the

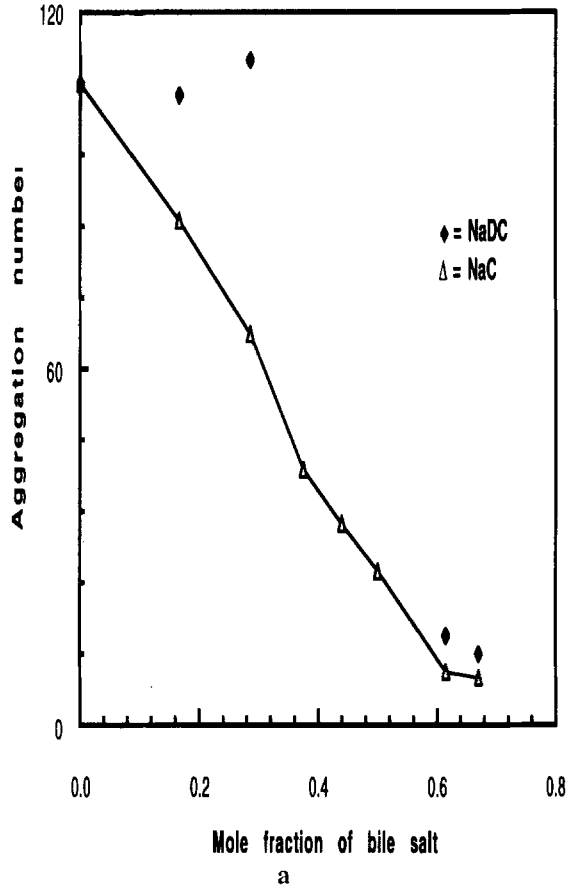


Fig. 5a. Aggregation number of CTAB in CTAB/bile salt mixed micelles as determined from fluorescence quenching, T = 25°C.

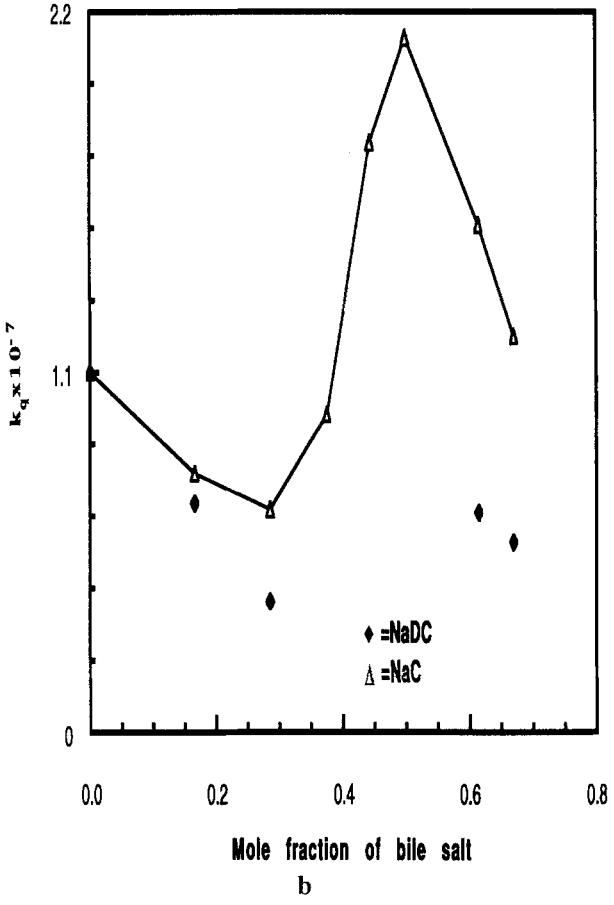


Fig. 5b. First-order quenching rate constant for pyrene in CTAB/bile salt mixed micelles.

bile-rich region the III/I ratio and lifetimes were slightly lower in the case of the NaC/CTAC system. An important conclusion is that in both cases the probe pyrene migrates from the palisade layer to the interior of the mixed micelle with increases in bile salt concentration.

Typical results of time-resolved fluorescence quenching for mixed micelles of NaC/CTAC are shown in Figs. 4, 5a and b. The fluorescence quenching curves shown in Fig. 4 are freed from the influence of the natural decay by multiplication of the measured intensities by an appropriate factor of $\exp(k_o t)$. The results obtained by fitting the decay curves to the Infelta model [Eqs. (1) and (2)] are also presented. Good agreement with the model was obtained, and the aggregation number with respect to CTA+ and the quenching rate constant k_q as a function of the mole fraction of bile salt are shown in Figs. 5a and b. The monotonic decrease in aggregation number in the above case is strikingly different from that

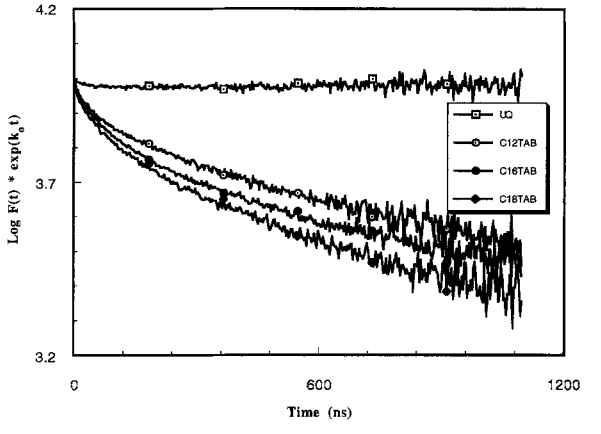
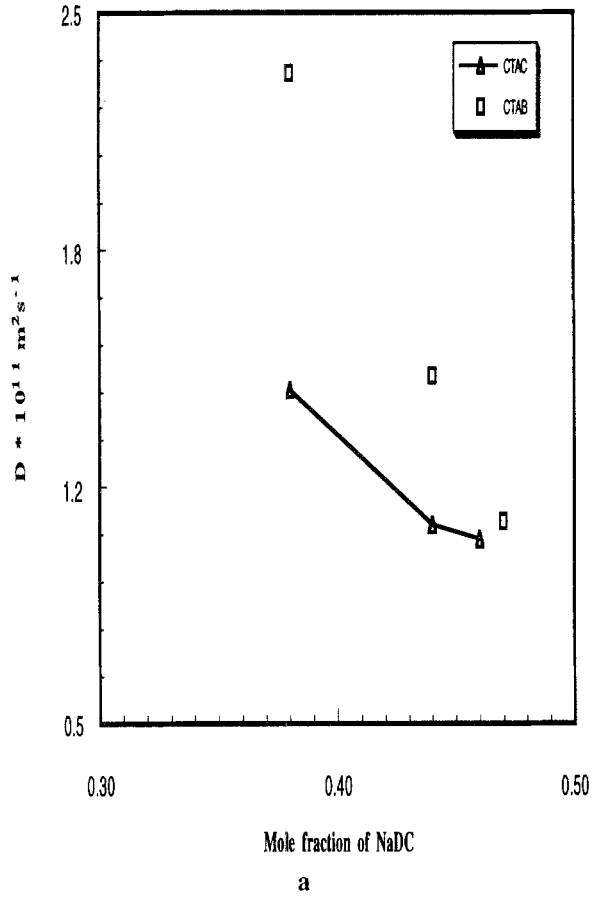
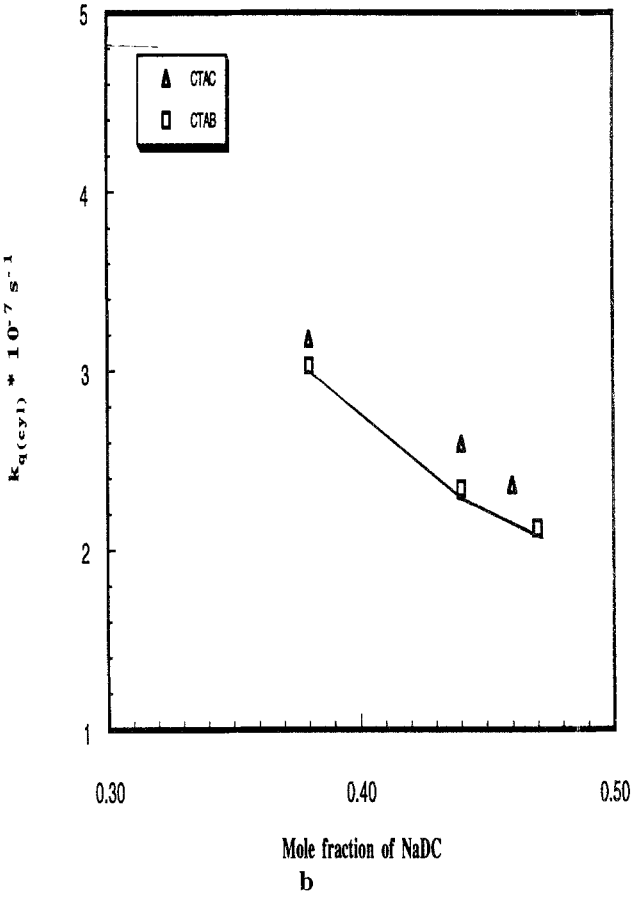


Fig. 6. Fluorescence quenching curves of C_nTAB/NaDC mixed micelles. All the curves are for samples with pyrene concentration of 2.5μM (micro molar) and DMBP concentration of 0.3mM.



a

Fig. 7a. Relative diffusion coefficient for the probe–quencher pair at different compositions.



b

Fig. 7b. First order quenching rate constant for the probe–quencher pair at different compositions.

of the mixed micelles of NaDC/CTAC, where a transition into larger rod-like mixed micelles takes place at close to equimolar composition.

The corresponding fluorescence decay curves for the NaDC/C_nTAB case is shown in Fig. 6 plotted as a function of time for varied alkyl chain lengths. Due to diffusion-influenced quenching, the system did not reach a stationary state within the lifetime of the probe, and hence the Infelta model was inapplicable. The decay curves were fitted to a generalized model of excited-state deactivation in long cylindical micelles without exchange of quenchers [Eq. (3)]. The relative diffusion coefficients of the probe–quencher pair and the rate constant were determined at different compositions using the radius of the hydrocarbon core, r_{hc}, as the interaction distance parameter, and the results are shown in Fig. 7a and b. The effects of structural modifications and changes in the environment such as changes in alkyl chain length or counterion were also investigated.

CONCLUSION

The fluorescence quenching technique has been shown to provide useful information in characterizing bile salt-cationic surfactant interaction. Both micellar size and molecular transport in such microheterogeneous systems could be determined using theoretical advances in the fluorescence quenching in micellar assemblies. The similarities and differences in self-associating systems can be studied by this novel probing method.

ACKNOWLEDGMENTS

This work was supported by the Swedish Natural Science Research Council and the Knut and Alice Wallenberg Foundation. M.S. Vethamuthu is grateful to the Liljewalchs Foundation for receipt of a travel grant.

REFERENCES

1. M. H. Gehlen and F. C. De Schryver (1993) *Chem. Rev.* **93,** 199.
2. D. M. Small (1971) in P. P. Nair and D. Kritchevsky (Eds.) *The Bile Acids*, Plenum Press New York, Vol. 1.
3. B. W. Barry and G. M. T. Gray (1975) *J. Colloid Interface Sci.* **52,** 327.
4. C. La Mesa, A. Khan, K. Fontell, and B. Lindman (1985) *J. Colloid Interface Sci.* **103,** 373.
5. P. K. Jana and S. P. Moulik (1991) *J. Phys. Chem.* **95,** 9525.
6. M. S. Vethamuthu, M. Almgren, E. Mukhtar, and P. Bahadur (1992) *Langmuir* **8,** 2396.
7. P. P. Infelta, M. Grätzel, and J. K. Thomas (1974) *J. Phys. Chem.* **78,** 190.
8. M. Almgren and J. Alsins (1990) *J. Phys. Chem.* **94,** 3063.
9. B. Medhage and M. Almgren (1992) *J. Fluoresc.* **2,** 7.