9000-Gent, Belgium.
Accepted for publication September 5, 1975.
The authors thank Mrs. A. Cruyl, recipient of a fellowship from the National Research Foundation (N.F.W.O.), for obtaining the mass spectra. They also are indebted to the National Foundation for Medical Research (F.G.W.O.) for supporting this work through Grants 20007 and 20210.
[x] To whom inquiries should be directed.

Solubilization as a Method for Studying Self-Association: Solubility of Naphthalene in the Bile Salt Sodium Cholate and the Complex Pattern of Its Aggregation

PASUPATI MUKERJEE [x] and JOHN R. CARDINAL [*]

Abstract □ Solubilization of uncharged, slightly soluble solutes is shown to be a useful approach for investigating patterns of self-association. The solubility of naphthalene in aqueous solutions of sodium cholate was determined over the concentration range of 0–0.20 mole/liter at 25°. Bile salts such as sodium cholate have many detergent-like properties and exhibit hydrophobic self-association in aqueous solutions. It has become customary to describe this aggregation using the model of micelle formation. The naphthalene solubility data show that the CMC for sodium cholate is not well defined. Comparison with solubilization in a typical micelle-forming system, sodium decanesulfonate, shows clearly that sodium cholate does not resemble a micelle-forming system. Further examination of the solubility data in terms of mutual association of naphthalene with aggregate species shows that the self-association of sodium cholate is not consistent with the formation of (*a*) only large micelles containing 10 or more monomers, (*b*) only dimers, (*c*) dimers and large micelles, and (*d*) any unique oligomer or multimer. A complex pattern of association, including the formation of dimers and one or more higher oligomers, is indicated.

Keyphrases □ Solubilization—method for study of self-association, naphthalene in aqueous solutions of sodium cholate compared to sodium decanesulfonate □ Micelle formation—solubilization of naphthalene in aqueous sodium cholate solutions compared to sodium decanesulfonate, method for study of self-association □ Naphthalene—solubility in aqueous sodium cholate solutions, method for study of self-association □ Sodium cholate—solvent for naphthalene, method for study of self-association

Bile salts are considered to be physiological surfactants (1–3). In common with ordinary surfactants or detergents, they contain a large hydrophobic moiety, which is responsible for their ability to emulsify and solubilize fats and lipids. Like detergents, they exhibit a tendency toward hydrophobic self-association (aggregation) in aqueous solution (1–8).

It has become customary to describe this aggregation using the model of detergent micelles (1–3). As discussed recently (9), hydrophobic self-association in aqueous solution can have very different patterns, depending upon the structure of the hydrophobic solutes. Fundamental requirements of an extensive cooperativity of self-association in the early stages of growth for a micelle-forming pattern of self-association and the existence of a critical micellization concentration (CMC) are not satisfied by all molecular structures.

The purposes of the present work were to examine how well the micellar model applies to the self-association of sodium cholate and to investigate the more general problem of how the solubilization by aggregates of a slightly soluble, uncharged solute can be used to provide information about self-association of aggregating solutes.

EXPERIMENTAL

Materials—Cholic acid[1] was recrystallized according to the method of Hofmann (10). Naphthalene[2] was purified by sublimation before use.

Apparatus—Absorbance measurements were made using silica cells of 1-cm path length in a spectrophotometer[3]. The constant-temperature bath for the solubilization studies was equipped with a thermoregulator[4], and the temperature was maintained at 25 ± 0.1°.

Solubility Experiments—Stock solutions of sodium cholate were obtained by titration of weighed quantities of cholic acid to pH 8–9 with sodium hydroxide solutions. These solutions were then brought to volume with double-distilled water. The desired concentrations of sodium cholate were prepared by volumetric dilution of this stock solution.

For the solubilization studies, 5 ml of a solution of sodium cholate was placed in a 2-dram vial to which naphthalene crystals were added in amounts more than sufficient to produce saturation. The vials were covered[5], sealed with parafilm, capped, placed in a water bath, and rotated at 25 ± 0.1° for 3 days. After this time, an appropriate volume, usually 2 ml, was withdrawn with a pipet whose tip had been covered with glass wool to filter excess crystals of naphthalene remaining in the solution.

The sample of cholate solution was quickly diluted to an appropriate volume with double-distilled water. The samples were always added to a volume of water close to that needed for the final volume to prevent precipitation of solid naphthalene crystals and to reduce loss of naphthalene by evaporation. Because of the volatility of naphthalene and its low solubility in water, losses due to evaporation on extensive handling of aqueous solutions can be serious.

[1] Aldrich Chemical Co., Milwaukee, WI 53233
[2] Baker and Adamson quality, Allied Chemicals, Morristown, N.J.
[3] Cary 16.
[4] Model 20, Bronwill Scientific, Rochester, N.Y.
[5] With a liner of Teflon (du Pont).

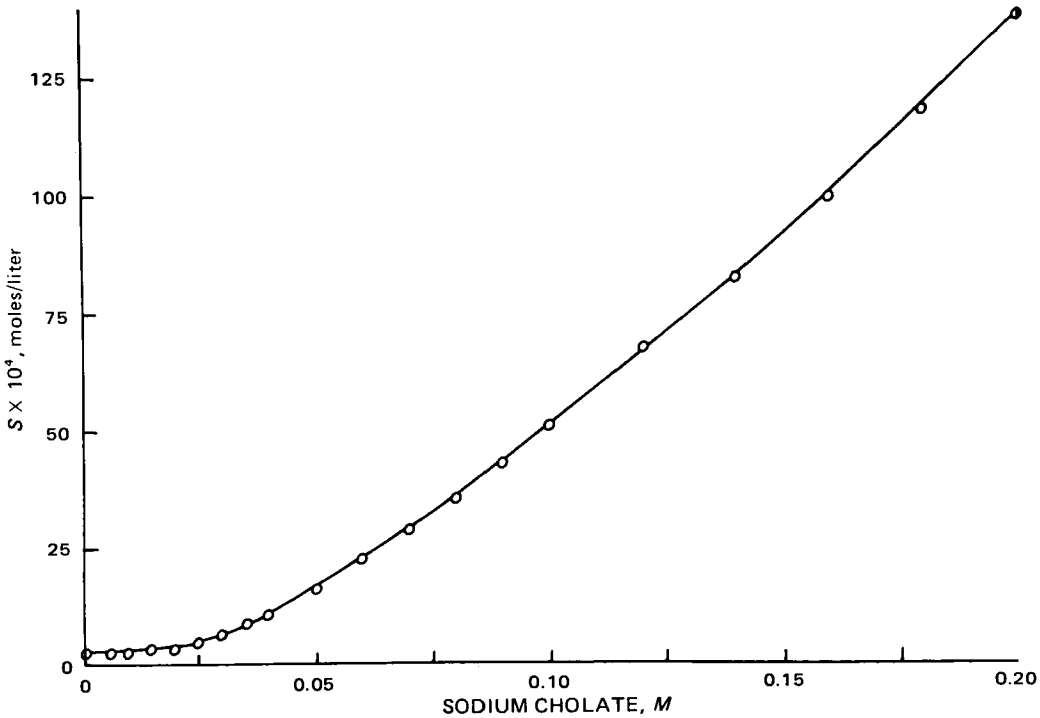

Figure 1—*Solubility of naphthalene, S, in aqueous solutions of sodium cholate at 25°.*

The concentration of naphthalene was determined by absorbance measurements at 276.0 nm. The wavelength of maximum absorbance of naphthalene changes with the increasing concentration of sodium cholate. This effect is negligible, however, when the concentration is low. Solutions at high concentrations of sodium cholate were always diluted to concentrations less than 0.007 *M* before absorbance measurements were performed.

The molar absorptivity of naphthalene was determined at 276.0 nm by dilution of a concentrated solution of naphthalene in a 50% water–methanol (v/v) mixture with sufficient water to reduce the methanol concentration to less than 0.5%. The value of the absorptivity was 5.070×10^3 liters/mole cm. This value compares favorably with the values of 4.946×10^3 and 4.927×10^3 reported (11) for two different samples of naphthalene determined by essentially the same technique.

RESULTS

The solubility, *S*, in moles per liter of naphthalene in water and sodium cholate solutions up to 0.2 *M* is shown in Fig. 1. Each value is the average of three to six determinations. The solubility in water was 2.55×10^{-4} *M*, as compared to the 2.62×10^{-4} *M* value found by Gordon and Thorne (11). This small difference appears to be due mainly to different estimates of molar absorptivities. The average absorbance measurements of saturated solutions of naphthalene in water agree within 0.2%.

DISCUSSION

The variation in the solubility of naphthalene with sodium cholate concentration (Fig. 1) is qualitatively similar to the results of numerous investigations reporting the solubilization of slightly soluble, uncharged, hydrophobic solutes by aggregating micelle-forming surfactants (12–14). The solubility changes relatively little below a certain concentration range and increases comparatively rapidly above this range. This rapid increase is generally attributed to the formation of micelles.

Such solubilization data are frequently used to determine CMC values (3, 12). This discussion will be concerned primarily with the meaning and significance of any such CMC value for sodium cholate and how solubilization data of the kind presented can be used to derive limited information on the pattern of self-association exhibited by aggregating hydrophobic solutes.

Limiting Association Concentrations—Some investigators (5, 7) suggested that bile salts, including sodium cholate, have three limiting association concentrations. According to this picture, the association begins above limit 1, but all of the added bile salt does not associate to form higher aggregates until above limit 2. Limits 2 and 3 are associated with changes in the aggregation numbers. Below limit 2, the aggregation numbers for sodium cholate fall in the 2–5 range; between limits 2 and 3, the aggregation number is about 5; above limit 3, the aggregation number is in the 15–20 range. For sodium cholate, these limiting concentrations are: limit 1, 0.013–0.015 *M*; limit 2, 0.045–0.050 *M*; and limit 3, 0.09–0.11 *M*.

The solubility data in Fig. 1 appear to be best represented by a smooth curve, with little evidence of any distinct "kink" or "break" at the limiting concentrations mentioned previously. To illustrate this point further, the ratio of the increment in solubility, $\Delta S = S_2 - S_1$, between adjacent concentrations and the increment in concentration, $\Delta C = C_2 - C_1$, were calculated, and $\Delta S/\Delta C$ was plotted against the mean concentration, $\bar{C} = (C_2 + C_1)/2$ (Fig. 2).

The value of $\Delta S/\Delta C$ thus calculated is *approximately* equivalent to the rate of change of *S* with *C*; $\Delta S/\Delta C$ measures the average value of the rate of change of *S* with *C* over the concentration range $C_2 - C_1$. It is subject to the error inherent in small increments such as ΔS or ΔC. It avoids, however, the uncertainties of differentiation by graphical or other means, which sometime involve subjective interpolations of data. The procedure is similar to that used previously (15, 16) for specific conductance (κ) data of ionic surfactants, in which 10^3

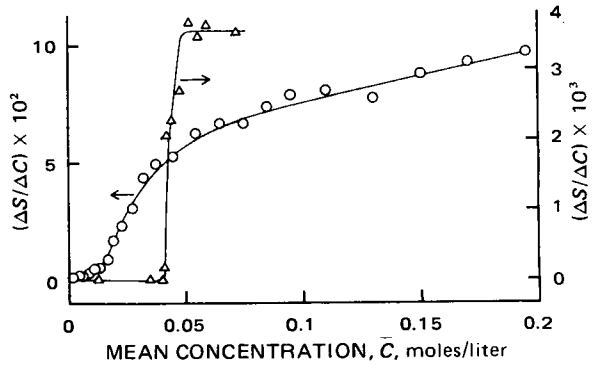

Figure 2—*The* ΔS/ΔC versus C̄ *plots (see text for definition). Key:* ○, *naphthalene in sodium cholate; and* △, *Orange OT in sodium decanesulfonate [based on data from Schott (14)].*

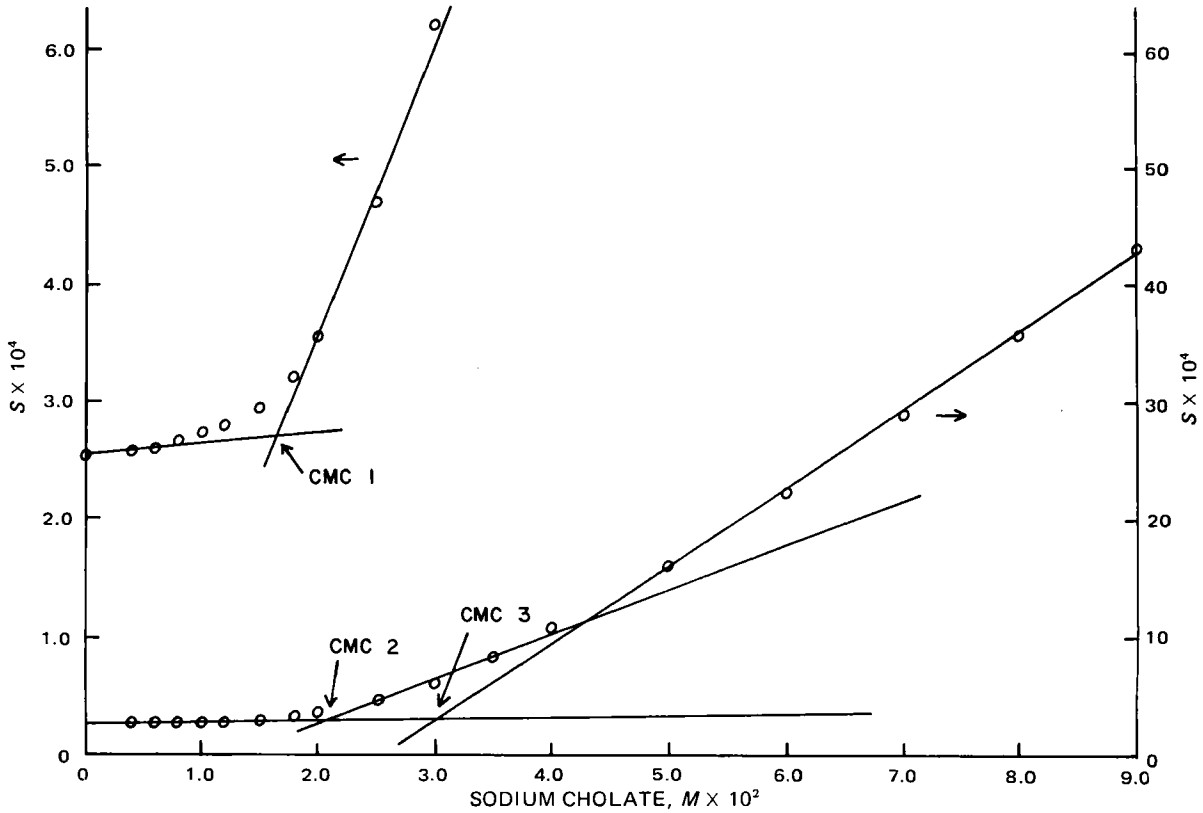

Figure 3—*Three different apparent CMC values from naphthalene solubilization data in sodium cholate* (S *in moles per liter*).

$\Delta\kappa/\Delta C$, the differential equivalent conductivity, is plotted against the mean concentration to demonstrate the abrupt transition brought about by micelle formation at the CMC in solutions of ionic surfactants containing flexible chains.

For comparison with a typical ionic detergent, Fig. 2 includes a $\Delta S/\Delta C$ *versus* $\bar{C}$ plot from the careful measurements of the solubility of Orange OT, a slightly soluble uncharged dye, in sodium decanesulfonate reported by Schott (14). The nearly vertical rise in $\Delta S/\Delta C$ for Orange OT in a typical detergent, sodium decanesulfonate, in the CMC region shows the effect of highly cooperative self-association, which leads to the beginning of the formation of large micelles at about the CMC (9, 15). The $\Delta S/\Delta C$ of naphthalene in sodium cholate shows no such abrupt transition. The mild upturn at about 0.017 *M* is followed by a continuous rise in $\Delta S/\Delta C$ over nearly a factor of 10 in concentration. In particular, there is no evidence of any other limiting association concentration across which $\Delta S/\Delta C$ shows any sharp rise. Therefore, it was concluded that the postulate of several limiting association concentrations is not tenable for sodium cholate.

CMC—Solubilization data of the kind presented in Fig. 1 are used to determine the CMC's of bile salts (3). In Fig. 3, the data below 0.09 *M* sodium cholate are plotted to examine the significance of this CMC. If the CMC is estimated by the usual procedure of linear extrapolations of data below and above the CMC (12), very different CMC values are obtained, depending upon what sections of the data are used. Three such apparent CMC values are indicated in Fig. 3. Depending upon which portion of the solubility curve is used, apparent CMC values can be obtained at 1.6, 2.0, or 3.0×10^{-2} *M*.

For surfactants with flexible chains forming micelles, such solubilization data do not allow a variation of more than 1–2% in the CMC value when using data over roughly a factor of 1.5–2 in concentrations below and above the CMC to locate the CMC (13, 14). Although many CMC data for sodium cholate and other bile salts are reported in the literature from similar studies (3), their significance is clearly rather limited. In particular, if some data in the transition region are absent, the CMC determination may *appear to be* quite reliable.

The problems associated with the determination of precise CMC values of bile salts and similar rigid molecules were pointed out previously (9, 12). In their compilation and evaluation of CMC data (12), Mukerjee and Mysels chose not to include any value for the bile salts. They pointed out "that the existence of a c.m.c. requires that higher multimers be relatively more stable than the small oligomers and that this requirement is met most commonly when the monomeric unit is a flexible chain."

Hydrophobic solutes with rigid monomers, such as bile salts and fused ring aromatic or heteroaromatic compounds, are expected to have rather diffuse and extended concentration ranges over which the degree of aggregation increases from low to high values (9, 12). The patterns of self-association they exhibit are different from micellar self-association involving extensive cooperativity in the early stages of growth (9). Nevertheless, such systems often lend themselves to the application of the micellar hypothesis if an apparent CMC value is obtained from limited data used in an uncritical fashion (9). If the CMC values are used to estimate monomer and micelle concentrations and thermodynamic quantities associated with micelle formation, rather serious errors of interpretation become likely (9).

ASSOCIATION MODELS

The preceding discussion of the nature of the solubilization of naphthalene by sodium cholate indicates strongly that the pattern of its self-association does not resemble that of micelle-forming surfactants containing flexible chains. Further examination of the solubility data provide some additional insight into the nature of the self-association. In this analysis, the solubilization of naphthalene as a typical uncharged, slightly soluble solute is used as a marker for the extent of association. If the monomer concentration is denoted by $[b_1]$ and an aggregate, b_n, forms according to Scheme I:

$$nb_1 \rightleftharpoons b_n$$

Scheme I

the formation of b_n is governed by the equilibrium constant β_n:

$$\beta_n = \frac{[b_n]}{[b_1]^n} \qquad \text{(Eq. 1)}$$

Then the amount solubilized, defined as $\Delta N = S - S_0$, where S is the solubility in the presence of sodium cholate and S_0 is the solubility in pure water, can be written as:

$$\Delta N = Kn[b_n] \qquad \text{(Eq. 2)}$$

where $n[b_n]$ is the monomer-equivalent concentration of the aggregate, and K is a proportionality constant. Equation 2 is valid because the activity of the solubilizate, naphthalene, is constant in these experiments under saturation conditions. Thus, if a series of mutual association equilibria between b_n and naphthalene, N, are postulated (Scheme II):

$$b_n + N \overset{K_1}{\rightleftharpoons} b_nN,\ b_n + 2N \overset{K_2}{\rightleftharpoons} b_nN_2, \ldots$$

Scheme II

the total amount of naphthalene solubilized is given by:

$$\Delta N = \sum_{i=1}^{\infty} i[b_nN_i] = \sum_{i=1}^{\infty} iK_i[b_n][N]^i \qquad \text{(Eq. 3)}$$

At saturation:

$$\Delta N = [b_n] \sum_{i=1}^{\infty} iK_i[S_0]^i \qquad \text{(Eq. 4)}$$

i.e., ΔN is proportional to $[b_n]$ (Eq. 2). The postulated equilibria assume ideality and neglect charge effects and counterion binding (17–20). These effects will be discussed later. For approximate treatment, the contribution of the $[b_nN_i]$ species to the total concentration, C, also is neglected so that:

$$C = [b_1] + n[b_n] \qquad \text{(Eq. 5)}$$

The ratio $\Delta N/C$ has values of 0.005 at 0.02 M, 0.021 at 0.04 M, 0.033 at 0.06 M, 0.049 at 0.1 M, and 0.068 at the highest concentration 0.2 M. The neglect of the aggregates containing naphthalene is not a serious approximation, particularly in dilute solutions (see later discussion).

To examine some specific models of association, log ΔN was plotted against log C and compared with predictions of the models (Fig. 4). The data at the lowest concentrations are rather uncertain because small differences in solubilities are involved. Some estimated error bars are indicated. The uncertainty of ΔN decreases rapidly with an increasing concentration of sodium cholate. At the higher concentrations, if C is corrected for the $[b_nN_i]$ species, the experimental curve would become steeper or, conversely, the calculated curves would be less steep.

Dimer Model—Several investigations (7, 21) indicate that the degree of aggregation for sodium cholate in the absence of electrolyte is about two. This dimer model can be shown to be inconsistent with our data. If a low value of 1.0 liter/mole is used for the dimerization constant, β_2 ($n = 2$ in Eq. 1), and the ΔN value at 0.015 M is fitted with the appropriate K value (Eq. 2) of 0.092 liter/mole, curve 2 is obtained. This curve is consistent with the data at the lowest concentrations but falls considerably below the experimental curve at higher concentrations. When using a higher value of 20.0 liters/mole for β_2 and fitting again at 0.015 M with the appropriate K value, curve 3 is obtained, which deviates more than curve 2 at the higher concentrations from curve 1.

The calculations have so far neglected charge effects, which are certainly important for self-association of ions. Upon applying corrections for activity coefficient variations using the Debye–Hückel limiting law for dimerization of monovalent ions according to Ghosh and Mukerjee (20), it is found that β_2 increases by a factor of 2.5 as the ionic strength increases from 0.01 to 0.25. If a further correction is applied for the shielding of the charges in the dimer, *i.e.*, reduction of the charge–charge repulsion of the dimer on increasing the ionic strength (22), β_2 can increase even more at higher ionic strengths. However, if it is assumed that these factors increase β_2 by a factor as large as 10, from 20 to 200 liters/mole as the concentration changes from 0.015 to 0.2 M, point 4 is obtained (Fig. 4). The improvement is only very slight.

Unique Oligomer Model—The possibility of the formation of any other unique oligomer, such as a tetramer, can be tested in a similar manner. Curve 5 in Fig. 4 shows, for example, that a 10-mer model ($n = 10$ in Eqs. 1 and 2 and Scheme I) fitted at the 0.015 M concentration is unsatisfactory, particularly in dilute solutions. A more general approach is outlined here and should apply to a variety of systems.

By neglecting charge effects and assuming ideality, *i.e.*, the applicability of Schemes I and II and Eqs. 1–5, one has:

$$C = [b_1] + n\beta_n[b_1]^n \qquad \text{(Eq. 6)}$$

and:

$$\Delta N = Kn\beta_n[b_1]^n \qquad \text{(Eq. 7)}$$

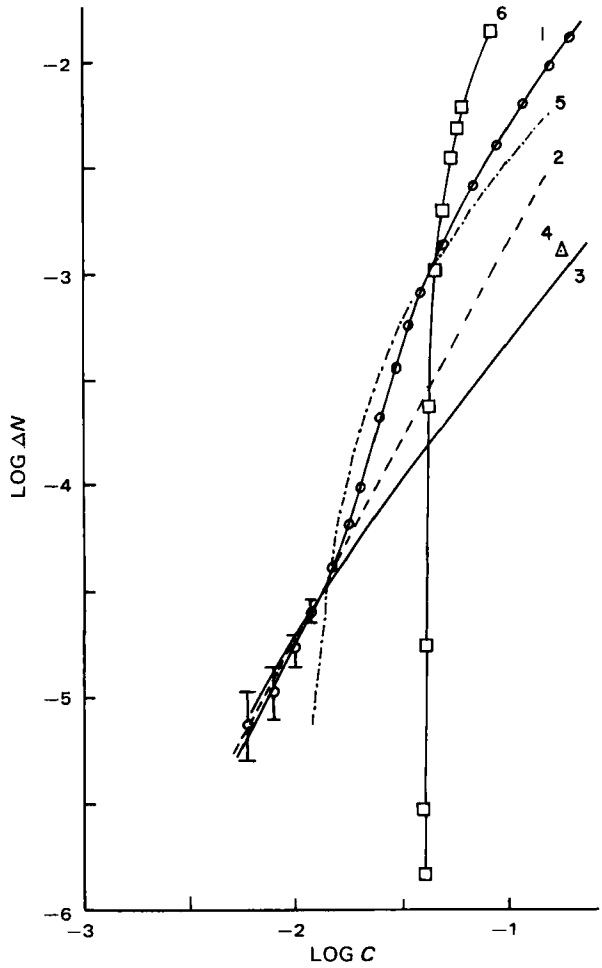

Figure 4—*Logarithm of the increase in concentration of the solubilizate, $\Delta N = S - S_0$, where S_0 is the solubility in the absence of surfactant, plotted against logarithm of the surfactant concentration. Key: ○, curve 1, naphthalene in sodium cholate; □, curve 6, Orange OT in sodium decanesulfonate [based on data from Schott (14)]; curve 2, dimer model with a low dimerization constant (1.0 liter/mole); curve 3, dimer model with a high dimerization constant (20 liters/mole); Δ, point 4, effect of applying several corrections (see text) on the dimer model of curve 3; and curve 5, the 10-mer model. All theoretical models (curves 2, 3, and 5) are fitted to the experimental curve (curve 1) at about 0.015* M.

such that:

$$R = \frac{d \ln \Delta N}{d \ln C} = \frac{(Kn^2\beta_n[b_1]^{n-1})([b_1] + n\beta_n[b_1]^n)}{(1 + n^2\beta_n[b_1]^{n-1})(Kn\beta_n[b_1]^n)} \qquad \text{(Eq. 8)}$$

where R measures the slope of the log ΔN *versus* log C curve in Fig. 4. The value of R according to Eq. 8 is maximum in dilute solutions: $R \to n$ as $C \to 0$. The value of R decreases continuously from n as C increases and approaches unity at high concentrations. The experimental curve in Fig. 4 shows a slope of about 2 in dilute solutions, indicating formation of dimers. The slope, however, *increases* with concentration to a maximum of about 3.4 and then decreases at higher concentrations. The data are thus inconsistent with the formation of any unique oligomer or multimer with a constant aggregation number.

In Eq. 6, the contribution of the aggregates containing naphthalene, *i.e.*, the b_nN_i species, are neglected. If these are included, then, by using Eq. 1 and Scheme II, C is given by:

$$C = [b_1] + n\beta_n[b_1]^n + n\sum_{i=1}^{\infty}[b_nN_i] =$$

$$[b_1] + n\beta_n[b_1]^n(1 + \sum_{i=1}^{\infty} K_iS_0{}^i) \qquad \text{(Eq. 9)}$$

Under saturation conditions, $\Sigma_{i=1}^{\infty} K_i S_0^i$ is a constant. If the full equation (Eq. 9) is used to derive R, the limiting values of R at low concentrations ($r \rightarrow n$ as $C \rightarrow 0$) or high concentrations ($R \rightarrow 1$ as $C \rightarrow \infty$) are not affected. However, the values of R at intermediate concentrations are changed.

When ionic surfactants form large micelles, counterion binding becomes very important (17–19). The mass action approach here (18) is represented by Scheme III:

$$nb_1^- + m\mathrm{Na}^+ \rightleftharpoons b_n^{-(n-m)}$$

Scheme III

where b_1^- is the monomer, Na^+ is a representative counterion, and n is the aggregation number, the charge of the micelle, b_n, being $-(n - m)$. If this equilibrium is governed by the equilibrium constant β^*, as given by:

$$\beta^* = \frac{[b_n]^{-(n-m)}}{[b_1^-]^n[\mathrm{Na}^+]^m} \qquad \text{(Eq. 10)}$$

and if Eq. 2 continues to apply, *i.e.*, the solubilization under saturation conditions is proportional to the micelle concentration, then it can be shown by proper substitution in Eqs. 6 and 7 that $(d \ln \Delta N)/(d \ln C) \rightarrow n + m$ as $C \rightarrow 0$. Thus, the solubilization increases more rapidly with concentration because of counterion participation. This limiting slope again remains unaffected if $[b_n N_i]$ species are included in C. When n is large, close to the CMC, under these idealized conditions, $R \rightarrow n + m$ when the solubilization just becomes appreciable.

The data of Schott (14) on the solubilization of Orange OT by sodium decanesulfonate, when plotted on a log–log scale (curve 6, Fig. 4), give a limiting mean value of 80 ± 20 for R at the four lowest concentrations reported. These concentrations are all actually below the reported CMC but are within about 10% of it. Since the value of m is frequently found to be about $2n/3$ (17–19), this would correspond to an aggregation number of about 48 for the micelles of sodium decanesulfonate. Because of the low absorbance measurements involved, this value is unlikely to be accurate. It is, however, consistent with the formation of large micelles by sodium decanesulfonate close to the CMC or below it.

Aggregation Model for Sodium Cholate—This analysis indicates that the self-association of sodium cholate involves dimerization as well as the formation of higher aggregates. More detailed analysis of the data rapidly loses significance because of the uncertainties of the assumed models. Thus, if the model of a dimer and one higher multimer is used, the uncertainty due to the unknown counterion binding of the higher multimer becomes serious. If ideality is assumed and counterion participation is neglected, a value of n of about 5 is derived for the inflection point of the log–log plot of Fig. 4, where the maximum slope is 3.4. Because of counterion participation, the true value of n is likely to be lower.

Further evidence that the average value of n remains low even at higher concentrations is indicated by Fig. 2. Thus, whereas $\Delta S/\Delta C$ is nearly independent of concentration for ordinary detergents from a concentration only about 10% above the CMC, as indicated by the results of sodium decanesulfonate in Fig. 2 and many results in the literature (12–14), it increases continuously for naphthalene in sodium cholate solutions by 25% as the concentration increases from 0.1 to 0.2 *M*, *i.e.*, in a range some five to 10 times the apparent CMC values. Thus, even at these high concentrations, the composition of the aggregates, as reflected in their solubilization capacity, changes markedly with concentration.

It was concluded, therefore, that the self-association of sodium cholate, as revealed by the solubilization of naphthalene, is inconsistent with (*a*) a micellar pattern of association involving only large aggregates; (*b*) the formation of any single oligomer, including a dimer; and (*c*) the formation of dimers and large aggregates. A complex pattern of association including the formation of dimers and one or more higher oligomers is indicated. Whether the aggregation involves open-ended continued association of the kind exhibited by the stacking interactions of planar molecules (9) or whether it is more complex remains to be investigated.

REFERENCES

(1) A. F. Hofmann and D. M. Small, *Annu. Rev. Med.*, **18**, 333(1967).

(2) M. C. Carey and D. M. Small, *Am. J. Med.*, **49**, 590(1970).

(3) D. M. Small, in "The Bile Acids," P. P. Nair and D. Kritchevsky, Eds., Plenum, New York, N.Y., 1971, chap. 8.

(4) S. A. Johnston and J. W. McBain, *Proc. R. Soc. London, Ser. A*, **181**, 119(1943).

(5) P. Ekwall, K. Fontell, and A. Sten, *Proc. Int. Congr. Surf. Act., 2nd*, **1957**, 397.

(6) K. Fontell, *Kolloid Z. Z. Polym.*, **244**, 246(1971).

(7) *Ibid.*, **244**, 253(1971).

(8) *Ibid.*, **250**, 333(1972).

(9) P. Mukerjee, *J. Pharm. Sci.*, **63**, 972(1974).

(10) A. F. Hofmann, *Acta Chem. Scand.*, **17**, 173(1963).

(11) J. E. Gordon and R. L. Thorne, *J. Phys. Chem.*, **71**, 4390(1967).

(12) P. Mukerjee and K. J. Mysels, "Critical Micelle Concentrations of Aqueous Surfactant Systems," NSRDS-NBS 36, U. S. Government Printing Office, Washington, D.C., 1971.

(13) R. J. Williams, J. N. Phillips, and K. J. Mysels, *Trans. Faraday Soc.*, **51**, 728(1955).

(14) H. Schott, *J. Phys. Chem.*, **70**, 2966(1966).

(15) G. S. Hartley, "Aqueous Solutions of Paraffin-Chain Salts," Hermann, Paris, France, 1936.

(16) K. J. Mysels and R. J. Otter, *J. Colloid Sci.*, **16**, 462(1961).

(17) K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants," Academic, New York, N.Y., 1963.

(18) P. Mukerjee, *Adv. Colloid Interface Sci.*, **1**, 241(1967).

(19) P. Mukerjee, K. J. Mysels, and P. Kapauan, *J. Phys. Chem.*, **71**, 4166(1967).

(20) A. K. Ghosh and P. Mukerjee, *J. Am. Chem. Soc.*, **92**, 6408(1970).

(21) D. M. Small, *Adv. Chem. Ser.*, **84**, 31(1968).

(22) P. Mukerjee, *J. Phys. Chem.*, **62**, 1397(1958).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 21, 1975, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*

Accepted for publication August 13, 1975.

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, April 1975.

Abstracted in part from a dissertation submitted by J. R. Cardinal to the University of Wisconsin in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported by the Wisconsin Alumni Research Foundation and Public Health Service Research Grant AM 17281-01.

* Present address: College of Pharmacy, University of Utah, Salt Lake City, UT 84112

[x] To whom inquiries should be directed.