Solubilization of Naphthalene by Sodium Cholate and Pattern of Self-Association of Sodium Cholate in 0.15 *M* Sodium Chloride

JOHN R. CARDINAL ˣ, YUNIK CHANG *, and DARRYL D. IVANSON

Received August 18, 1977, from the *Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT 84112.* Accepted for publication October 19, 1977. *Present address: College of Pharmacy, University of Kansas, Lawrence, KS 66044.

Abstract □ Naphthalene solubility was determined in aqueous 0.15 *M* NaCl containing sodium cholate in the 0–0.05 *M* concentration range at 25 ± 0.1°. Sodium cholate tends to self-associate in aqueous solutions. Most often, the association pattern has been described in terms of a monomer–micellar model in which it is assumed that no association occurs below the critical micelle concentration. By comparison of the experimental solubilization curve with curves calculated on the basis of the monomer–micellar model, it was shown that this model is inappropriate for the self-association pattern of sodium cholate. The solubility data were consistent with a model that assumes that sodium cholate associates to form dimers, trimers, and higher aggregates with an average aggregation number of 7.63. Model calculations suggest that naphthalene is solubilized by dimers and higher aggregates. Solubilization of naphthalene by trimers appears to be negligible.

Keyphrases □ Naphthalene—solubilization by sodium cholate in 0.15 *M* NaCl □ Solubilization—of naphthalene by sodium cholate in 0.15 *M* NaCl □ Sodium cholate—solubilization of naphthalene and self-association in 0.15 *M* NaCl □ Association, self—sodium cholate in 0.15 *M* NaCl, multiple aggregate model □ Bile salts—sodium cholate, solubilization of naphthalene and self-association in 0.15 *M* NaCl

Bile salts are physiological surfactants that play an important role in the solubilization of lipids such as cholesterol, fatty acids, and lecithin (1–3). In the presence of lecithin and/or fatty acids, complex mixed micelles are formed that have a high capacity for the solubilization of lipophilic species such as cholesterol (4–6). In the absence of lecithin and fatty acids, the bile salts also can solubilize lipophilic species such as cholesterol in water; however, the extent of solubilization is markedly decreased (5, 6). In spite of this decrease, it has been shown from *in vitro* studies that pure bile salt solutions are more effective in the dissolution of cholesterol gallstones than are the mixed micellar solutions (7). Pure bile salt solutions seem to be more effective clinically in cholesterol gallstone dissolution by T-tube infusion (8, 9).

The monomer–micellar model is often utilized to describe the solubilization of lipophilic solutes by the bile salts (1–3). This model is analogous to that used for flexible chain surfactants and assumes that the solute partitions into the micelle formed by the bile salts. Below the critical micellization concentration (CMC), this model assumes that surfactant association and, therefore, solubilization of lipophilic solutes do not occur. Above the CMC, it is assumed that solubilization is proportional to the concentration of micellized species. More complex models of the association of the bile salts also have been suggested. For example, Ekwell *et al.* (10) and Fontell (11–13) argued that discrete solubilization limits exist and that the average size of the bile salt aggregates undergoes discrete changes at these solubilization limits.

More recently, it was argued, using data on the solubilization of naphthalene by sodium cholate in the absence of added electrolytes, that both of the discussed models are inconsistent with the experimental data and that a complex association pattern exists (14). It was concluded that the solubilization data were consistent with a model that includes dimers and one or more higher oligomers. Support for this model was obtained from studies on the light scattering of the bile salts in the presence of added electrolytes (15). It was concluded that the trihydroxy bile salts associate to form dimers, trimers, and a higher aggregate containing approximately eight monomeric units. The association pattern was not strongly influenced by the concentration of added electrolytes or by the degree of conjugation of the bile salt.

In the present study, the solubilization of naphthalene by sodium cholate in aqueous 0.15 *M* NaCl was determined in the range from 0 to 0.05 *M* sodium cholate. The experimental solubilization curve was compared with curves calculated on the basis of various models for the self-association of sodium cholate. The solubilization data were consistent with the multiple aggregate model for the self-association of sodium cholate.

EXPERIMENTAL

Materials—Cholic acid[1] was purified as previously described (15). Sodium cholate was obtained from cholic acid by titration of aqueous solutions of the free acid to pH 10.0 with sodium hydroxide. The solid salts were obtained by flash evaporation of the solvent, followed by drying overnight in a vacuum oven at 45°. Naphthalene[2] was recrystallized four times from methanol and sublimed before use.

Apparatus—Absorbance measurements were made using silica cells of 1-cm pathlength in a spectrophotometer[3]. Samples for the solubilization studies were maintained at 25 ± 0.1° in a constant-temperature water bath[4].

Solubility Experiments—Stock solutions of sodium cholate in 0.15 *M* NaCl were adjusted to pH 10.0. The desired concentrations of sodium cholate were prepared by volumetric dilution of this stock solution. For the solubilization experiments, 5 ml of a sodium cholate solution was placed in a 2-dram vial to which an excess of naphthalene crystals had been added. The vial was sealed with parafilm and allowed to rotate in the water bath at 25 ± 0.1° for 3 days. An appropriate volume of solvent, usually 2 ml, was then withdrawn with a pipet whose tip had been covered with glass wool to filter excess naphthalene crystals from the samples.

This solution was quickly added to a volumetric flask containing a volume of water nearly sufficient to yield the desired final volume of solution. This step was required to prevent the precipitation and sublimation of naphthalene. The dilutions were made in such a fashion that the final concentrations in the diluted samples were far from saturation. Each reported naphthalene solubility value is an average of at least four samples.

The naphthalene concentrations were obtained by absorbance measurements at 276 nm. The extinction coefficient of naphthalene was 5.00 × 10^3 liters/mole cm, in good agreement with the values of 5.07 × 10^3 and 4.946 × 10^3 liters/mole cm reported previously (14, 16).

[1] J. T. Baker Chemical Co., Phillipsburg, N.J.
[2] Amersham/Searle Corp., Des Plaines, Ill.
[3] Model 240, Gilford Instrument Co., Oberlin, Ohio.
[4] Model 2095, Forma Scientific Marietta, Ohio.

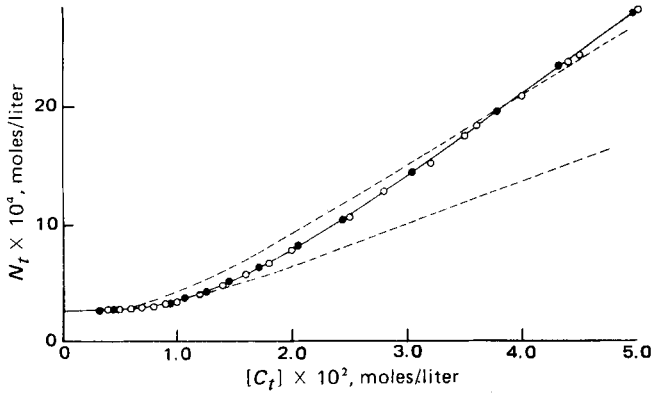

Figure 1—*Solubility of naphthalene,* N_t, *in aqueous 0.15* M *NaCl solutions of sodium cholate at 25°. Key: — and* O, *experimental values;* ●, *multiple aggregate model; lower dashed (– –) line, monomer–micellar model,* K = *150; and upper dashed (– –) line, monomer–micellar model,* K = *250.*

RESULTS AND DISCUSSION

The solubility of naphthalene, N_t, in sodium cholate solutions varying in concentration from 0 to 0.05 M is shown in Fig. 1. The solubility of naphthalene in 0.15 M NaCl, N_0, was 2.615×10^{-4} M. This value was higher than expected based on previously reported values of 2.55×10^{-4} M (14) and 2.62×10^{-4} M (16) for naphthalene solubility in water. For comparison, naphthalene solubility in water was found to be 2.79×10^{-4} M. When using this value for the water solubility and 0.22 for the salting coefficient in the Setschenow equation (16), naphthalene solubility in 0.15 M NaCl was calculated to be 2.59×10^{-4} M. Therefore, the results obtained for naphthalene solubility in water and 0.15 M NaCl in the present study appear to be internally consistent. The reasons for the discrepancy between the present values and those reported previously are not known. Presumably, a small amount of impurity exists in naphthalene; however, it could not be removed by the recrystallization and sublimation steps used in the purification of this sample.

The curve shown in Fig. 1 is similar to that reported previously (14) for naphthalene solubility in sodium cholate in the absence of added electrolytes. At low sodium cholate concentrations, naphthalene solubility increased slowly with increasing concentrations of sodium cholate. This increase was followed by a region of mild curvature. Then at high concentrations of sodium cholate, naphthalene solubility increased nearly linearly with increases in the sodium cholate concentration. This curve shows no distinct "kinks" or "breaks," which are required of both the monomer–micellar model (1–3) and the model of Ekwell *et al.* (10) and Fontell (11–13), and, therefore, appears to be inconsistent with this model (14).

To demonstrate this point more clearly, model calculations, according to the monomer–micellar model, of the variation of naphthalene solubility as a function of the sodium cholate concentration are presented in Fig. 1 as dashed lines. According to the monomer–micellar model, the variation in the total concentration, C_t, as a function of the monomer concentration, b_1, is given by:

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

where n is the aggregation number and β_n is the association constant for micelle formation. The association constant, β_n, is defined as:

$$\beta_n = \frac{[b_n]}{[b_1]^n} \qquad \text{(Eq. 2)}$$

where b_n represents the concentration of micellized species. Equations 1 and 2 are strictly valid only for uncharged species under the assumption that activity coefficients for monomers and micelles are constant. For ionic micelles, counterion binding becomes important (17). However, for the systems investigated here, the counterion concentration is large and effectively constant, so Eqs. 1 and 2 should be valid without correction (18).

Under saturation conditions and the assumption that the increase in the naphthalene concentration arises from solubilization by the cholate micelles, the amount of naphthalene solubilized, defined as $\Delta N = N_t - N_0$, where N_t is the total solubility and N_0 is the solubility in the absence of cholate, can be written as (14):

$$\Delta N = KN_0n\beta_n[b_1]^n \qquad \text{(Eq. 3)}$$

where K is a proportionality constant. In the presence of naphthalene, Eq. 1 must be rewritten to include a term that accounts for the aggregates containing naphthalene. Under these conditions, Eq. 1 becomes (14):

$$C_t = [b_1] + n\beta_n[b_1]^n + KN_0n\beta_n[b_1]^n \qquad \text{(Eq. 4)}$$

Equations 3 and 4 can be utilized to calculate the variation in the naphthalene solubility as a function of the cholate concentration provided that estimates of n and β_n are available. (The value of the proportionality constant, K, can be obtained by fitting the calculated curve to the experimental values.) Literature estimates of the aggregation number, n, for sodium cholate in 0.15 M NaCl are generally from about 4 to 6 (1–3). Recently, an indepth analysis of light-scattering data according to the monomer–micellar model found an aggregation number of 5.83 and an association constant of 8×10^8 liters/mole (19). Since these values were corrected for the nonideality effects in light scattering as discussed by Vrij and Overbeek (20), they were utilized for the present calculation.

In an attempt to fit the experimental solubility curve according to this model, two values of the proportionality constant, K, were used. The lower dashed curve in Fig. 1 was obtained with a value of 150 for K. This curve is in good agreement with the experimental curve at low cholate concentrations but in rather poor agreement at high concentrations. The upper dashed curve in Fig. 1 was calculated with a value of 250 for K. In this case, the agreement is improved at high concentrations but is poor at the lower concentrations.

These results suggest that the monomer–micellar model is inappropriate for describing the self-association pattern of sodium cholate. However, this lack of agreement possibly might arise from a poor choice of assumptions concerning the model rather than from the failure of the model itself. For example, the presence of a solubilized species can lead to an alteration in the micellar properties and to low estimates of the CMC. This lowering of the CMC is proportional to the mole fraction of the solubilized species (21). In the present case, even at the highest concentrations considered, the term $KN_0n\beta_n[b_1]^n$, *i.e.*, the concentration of aggregates containing naphthalene, represents only 5% of the total. Therefore, this small concentration of aggregates containing naphthalene should have relatively minor effects on the overall association pattern.

A second point is the validity of the estimates of the aggregation number and the association constants used in the model calculations. These estimates were obtained from a light-scattering study in which it was concluded that the monomer–micellar model gives a poor fit to the experimental light-scattering curves (19). In that study, a similar conclusion was reached about the necessary variation of the association constant required to fit the experimental curve in the low and high concentration regions. Finally, an independent study (22) using an ultrasonic absorption method also concluded that the monomer–micellar model does not describe adequately the overall self-association pattern of sodium cholate. Based on these studies, the conclusion that the monomer–micellar model does not describe adequately the overall self-association pattern of sodium cholate in 0.15 M NaCl seems justified.

Previous investigators (14, 15, 22) argued that the self-association pattern of the trihydroxy bile salts is complex and involves small aggregates together with higher oligomers. A specific model for sodium cholate self-association was proposed (15), and it was suggested that sodium cholate associates to form dimers, trimers, and larger aggregates with an average aggregation number of 7.63. This model yielded good agreement with the light-scattering curves for sodium cholate in various 0.15 M sodium halides. Since the association constants for the formation of the various aggregates are known from this study, it is possible to apply this model to the solubilization data obtained in the current study.

According to this multiple aggregate model, the total amount of naphthalene solubilized by cholate can be written as:

$$\Delta N = K_2'N_02K_2[b_1]^2 + K_3'N_03K_2K_3[b_1]^3 + K'_{7.63}N_07.63\beta_{7.63}[b_1]^{7.63} \qquad \text{(Eq. 5)}$$

where K_2, K_3, and $\beta_{7.63}$ are the association constants for the formation of the dimer, trimer, and large aggregate, respectively; and K_2', K_3', and $K'_{7.63}$ are the proportionality constants relating the amounts solubilized by the dimer, trimer, and large aggregate to the monomer equivalent concentration of these aggregates, respectively. The total concentration of cholate, C_t, at any given monomer concentration, b_1, can be defined as:

$$C_t = [b_1] + 2K_2[b_1]^2 + 3K_2K_3[b_1]^3 + 7.63\beta_{7.63}[b_1]^{7.63} + K_2'N_02K_2[b_1]^2 + K_3'N_03K_2K_3[b_1]^3 + K'_{7.63}N_07.63\beta_{7.63}[b_1]^{7.63} \qquad \text{(Eq. 6)}$$

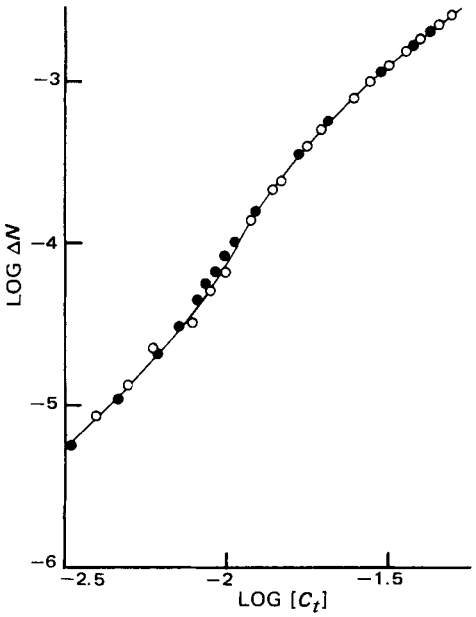

Figure 2—*Logarithm of the excess naphthalene solubility,* ΔN, *plotted against the logarithm of the cholate concentration,* C_t. *Key:* O, *experimental points; and* ●, *multiple aggregate model.*

Two assumptions are important in relating Eqs. 5 and 6 to the solubilization data: (*a*) that the presence of the solute (naphthalene) does not alter the average size of the aggregates formed, and (*b*) that the association constants K_2, K_3, and $\beta_{7.63}$ are not affected by the solute. If assumption *a* is not valid, there should be a poor fit of the calculated curves to the experimental values. If assumption *b* is not valid, the variation in the association constants should not be observable since any changes in the constants would be absorbed in the proportionality constants K_2', *etc.*, needed to fit the solubilization data. However, this assumption ought to be a reasonably good one since the solvent properties of water should not be affected by the small amount of solute present in these solutions.

To compare the experimental solubilization data with this model, log ΔN was plotted *versus* log $[C_t]$ in Fig. 2. By using this figure, K_2' can be obtained by fitting the experimental curve at very low total cholate concentrations. This calculation assumes that the contributions of the larger aggregates to the excess solubility of naphthalene is small in this region. This assumption is reasonable since the initial slope of the plot of log ΔN *versus* log $[C_t]$ is 2, which indicates that the dimer is responsible for solubilization in this region (14). With the value of K_2' already obtained, the value of $K_{7.63}'$ was obtained by fitting the experimental curve at high cholate concentrations. This procedure assumes that K_3' is small, which appears to be reasonable.

Given these values of K_2' and $K_{7.63}'$ and the values of $K_2 = 7.2$, $K_3 = 293$, and $\beta_{7.63} = 2.74 \times 10^{12}$ liters/mole from Ref. 15, the total solubility curve of naphthalene as a function of the total cholate concentration was calculated (Fig. 1). The excess solubility of naphthalene also was calculated and plotted (Fig. 2). From both plots, it can be seen that the calculated values are in good agreement with the experimental curve. The values of the proportionality constants used in fitting the curve are $K_2' = 165$ and $K_{7.63}' = 372$.

As mentioned previously, this calculated curve was obtained by setting $K_3' = 0$. Some attempts were made to improve the fits shown in Figs. 1 and 2 by inclusion of values for K_3', with suitable reductions in the values of K_2' and $K_{7.63}'$. In all cases, however, the fit of the calculated curve with the experimental curve was much worse, especially in the low concentration region. This result seems reasonable when considering molecular models of the cholic acid molecules. Although the arrangement of the monomeric species in the trimer is not known, it can be seen from molecular models that three cholate monomers can be arranged such that a compact cylinder is formed with very little void space. In this arrangement, the hydrophobic faces of the cholate molecules are in contact, and the hydrophilic faces are exposed to water. The inclusion of a naphthalene molecule in this structure would disrupt the compact nature of the aggregate and increase the net exposure of the hydrophobic portions of the cholate molecule to water.

It is concluded, therefore, that the solubilization data are inconsistent with any model of sodium cholate self-association that includes only the monomer and a higher aggregate. The solubilization data can be shown to be in agreement with the multiple aggregate model of Chang and Cardinal (19). It is hoped that the model described in this paper will apply to the solubilization of hydrophobic molecules in general by the trihydroxy bile salts. In addition, this model may be of value in understanding the mechanism by which the bile salts dissolve gallstones clinically.

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