Mixed Adsorbed Film and Micelles of Sodium Taurocholate and Sodium Taurodeoxycholate

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The surface tension of an aqueous mixture of sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC) in the presence of 0.1 mol kg^{-1} sodium chloride was measured as a function of the total concentration and composition of the bile salts at 25 °C. The result, processed by a thermodynamic treatment based on excess thermodynamic quantities, showed that NaTC and NaTDC are freely miscible in an adsorbed film, while mixing in a limited composition region of the micelle.

The sterol nucleus constitutes a hydrophobic group of a variety of surfactants having a biological origin. In our previous papers we have shown the strong lateral attractive force of the sterol nucleus and how it mixes with a hydrocarbon chain in a mixed adsorbed film.[1-3] Sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC) are typical surfactants which have the sterol nucleus as a hydrophobic group, and in which the hydrophobic property is changed accounting to the number of hydroxyl groups. For NaTDC, dihydoxy bile salt, the characteristic behavior (such as their small aggregation number and unusual shapes of the micelle) have been shown.[4-6] For NaTC, trihydoxy bile salt, the critical formation of a micelle has been questioned because of its broad critical concentration region.[4-8]

In order to understand the properties of bile salts better, such as micelle formation and lateral interaction arising from the difference in the number of hydroxyl groups, we have chosen an NaTC–NaTDC mixed system in an aqueous solution with 0.1 mol kg^{-1} sodium chloride added. This mixed system is suitable for differentiating between trihydoxy and dihydoxy bile salts, since NaTC and NaTDC have chemically the same structure, except for their 7 position. We have measured the surface tension of the aqueous mixtures as a function of the concentration and composition of bile salts at 25 °C. It has been shown that the thermodynamic properties of synthetic surfactants in micelles are quite similar to that in an adsorbed film.[9-11] A thermodynamic treatment seems to be applicable to the NaTC–NaTDC system, although the micelle structure of the bile salts is very different from that of synthetic surfactants. Special interest is put on the relation of the compositions between the bulk, the adsorbed film, and the micelle. We hope to demonstrate some of the characteristics of the lateral interaction of bile salts in the micelle and the adsorbed film using thermodynamic analysis.

Materials and Method

Sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC) were purchased from Sigma Chem. Co. and used without purification. As shown in the result section, the measured surface tension vs. concentration curves for NaTDC and their mixtures show no minimum around their critical micell concentration (cmc). Aqueous solutions of bile salts were prepared with a 0.1 mol kg^{-1} sodium chloride solution. Sodium chloride was used as received, and water was twice distilled from an alkaline permanganate solution. The surface tension was measured by a drop volume technique, described elsewhere.[12] The drop was permitted to stand for 15 min in order to confirm the adsorption equilibrium.

Result

Mixed Adsorbed Film of NaTC and NaTDC. In order to analyze the mixed adsorbed film of NaTC and NaTDC, we measured the surface tension of aqueous solutions of the mixture as a function of the total concentration of bile salts (m_t) in a molality scale defined by

$$m_t = m_1 + m_2. \tag{1}$$

The composition of the bile salts (X_2^W) was defined as

$$X_2^W = m_2/m_t \tag{2}$$

at constant pressure and temperature, 25 °C.[13] Here, subscripts 1 and 2 refer to NaTC and NaTDC, respectively, and superscript W refer the aqueous phase. The results are shown in Fig. 1. The surface tension decreases steeply with increasing total concentration, representing the high surface activity of bile salts.

In the solution measured, the ith salts completely dissociated into $\nu_{i,k}$ k-ions. Then, the variation of the surface tension (γ) at constant temperature and pressure is given as[9-14]

$$d\gamma = -\sum_k\sum_i \nu_{i,k}\Gamma_k^H d\mu_k, \tag{3}$$

where Γ_k^H and μ_k refer to the surface densities and chemical potential of the kth ions. Subscript i counts for NaTC, NaTDC, and NaCl, respectively, and k counts for taurocholate anion (TC), taurodeoxycholate anion (TDC), Cl$^-$, and Na$^+$, respectively. In the present system all ions behave almost ideally up to the cmc,[6] the chemical potential of ion k is thus given as

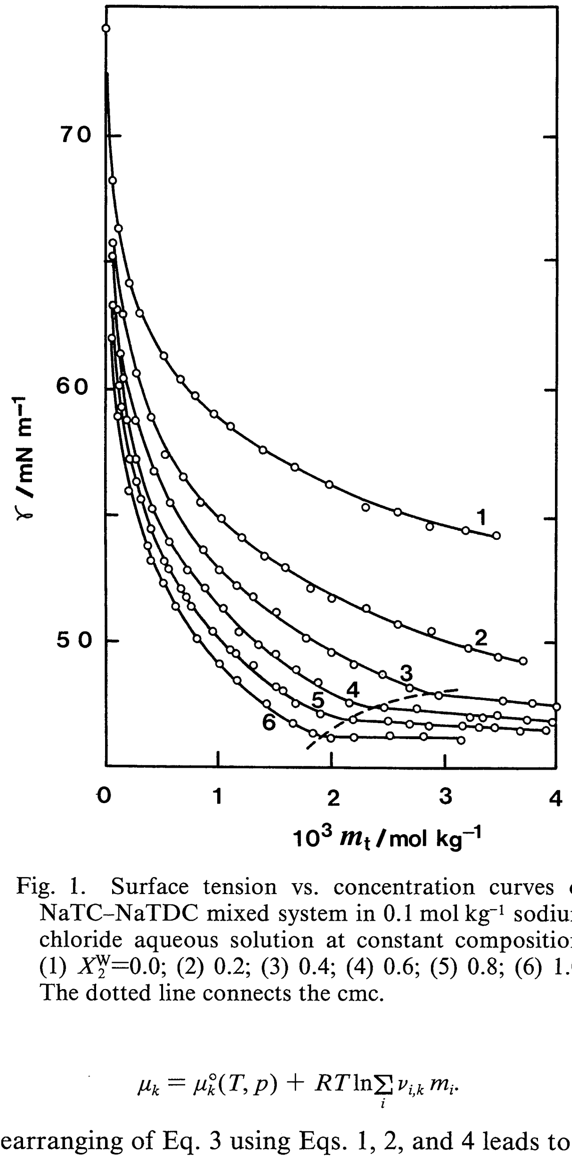

Fig. 1. Surface tension vs. concentration curves of NaTC–NaTDC mixed system in 0.1 mol kg^{-1} sodium chloride aqueous solution at constant composition: (1) X_2^W=0.0; (2) 0.2; (3) 0.4; (4) 0.6; (5) 0.8; (6) 1.0. The dotted line connects the cmc.

$$\mu_k = \mu_k^\circ(T,p) + RT\ln\sum_i \nu_{i,k}\, m_i. \tag{4}$$

Rearranging of Eq. 3 using Eqs. 1, 2, and 4 leads to

$$\mathrm{d}\gamma = -RT\left(\frac{\Gamma_t^H}{m_t} + \frac{\Gamma_t^H}{m_t+m_3} + \frac{\Gamma_{Cl}^H}{m_t+m_3}\right)\mathrm{d}m_t - RT\left(\frac{\Gamma_2^H}{X_2^W} - \frac{\Gamma_1^H}{X_1^W}\right)\mathrm{d}X_2^W, \tag{5}$$

where subscript 3 refers the added NaCl and Γ_t^H refers to the total interfacial density defined as

$$\Gamma_t^H = \Gamma_1^H + \Gamma_2^H. \tag{6}$$

Taking into account the fact that Γ_{Cl}^H is smaller than Γ_t^H as well as the high concentration of NaCl, the third term within the first brackets in Eq. 5 is negligible compared to the other terms. Then, the Γ_t^H and the composition of the adsorbed film (X_2^H) are evaluated, respectively, using the relations

$$\Gamma_t^H = -\frac{m_t(m_t+m_3)}{RT(2m_t+m_3)}\left(\frac{\partial\gamma}{\partial m_t}\right)_{T,p,X_2^W}, \tag{7}$$

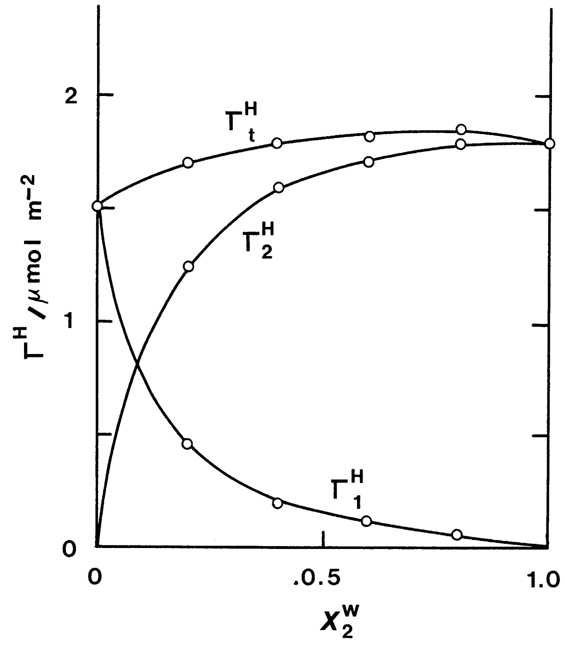

Fig. 2. Surface density vs. composition curves at constant total concentration of 1.0×10^{-3} mol kg^{-1}.

$$X_2^H = X_2^W - (X_1^W X_2^W / RT\Gamma_t^H)(\partial\gamma/\partial X_2^W)_{T,p,m_t}, \tag{8}$$

and

$$X_2^H = X_2^W - [1/m_t + 1/(m_t+m_3)]X_1^W X_2^W \times (\partial m_t/\partial X_2^W)_{T,p,\gamma}. \tag{9}$$

The adsorption isotherms for both bile salts rise sharply at low concentrations and almost reach a plateau over a total concentration of about 0.5×10^{-3} mol kg^{-1}. In Fig. 2, the evaluated total surface density is plotted against the bulk composition at a total concentration of 1×10^{-3} mol kg^{-1}. Here, the total surface density shows increases in going from pure TC and a plateau in going to pure TDC over an X_2^W of about 0.4. Applying Eqs. 8 and 9 for graphs of m_t vs. X_2^W at constant γ and γ vs. X_2^W at constant m_t (obtained from the Fig. 1), the values of the composition in a mixed adsorbed film were calculated and plotted against the bulk composition at a constant surface tension value of 55 mN m^{-1} (Fig. 3). It can be seen that the composition in the adsorbed film lies close to TDC compared with that of a bulk solution. Using this value, the surface densities of TC and TDC are also plotted in Fig. 2. The surface density of TC drops steeply, while that of TDC increases rapidly with increasing X_2^W. These results agree well with previous work in that the adsorbed mixed film is enriched by the component which shows a stronger cohesive interaction in the film.[2,3,12,13]

The interaction in the mixed film can easily be seen in a plot of the mean area per molecule (A) ($A=1/N_A\Gamma_t^H$, where N_A is the Avogadro's number) against the com-

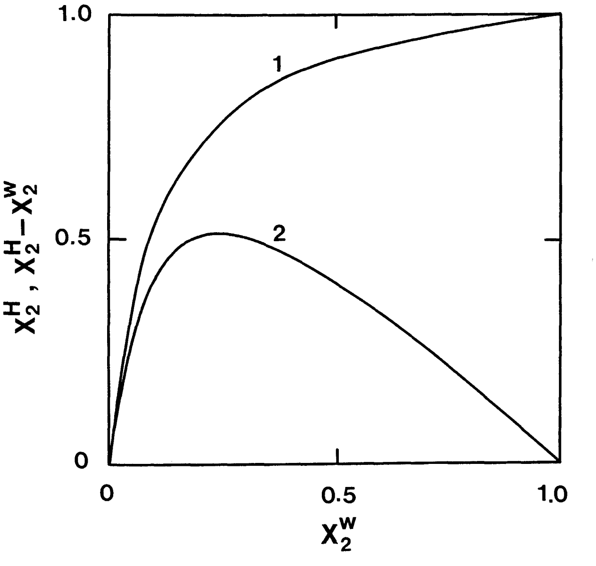

Fig. 3. Relation between compositions in the adsorbed film and aqueous solution in equilibrium at constant surface tension of 55 mN m^{-1}: (1) X_2^H vs. X_2^W; (2) $X_2^H - X_2^W$ vs. X_2^W.

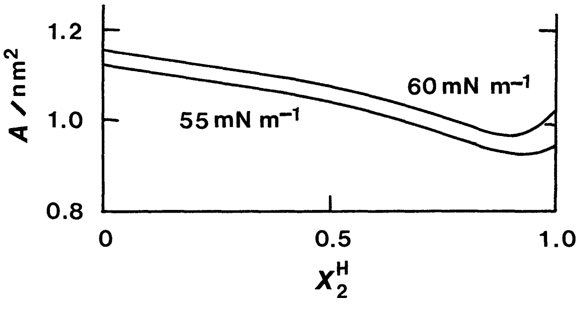

Fig. 4. Mean area occupied per molecule vs. composition in the adsorbed film curves at constant surface tension values.

position of the mixed film, as shown in Fig. 4. In the TC-rich region the mean area shows almost ideal mixing, while a slight attractive interaction in the TDC-rich region can be seen.

Mixed Micelle of NaTC and NaTDC. In order to obtain the cmc of a mixture of NaTC and NaTDC, the surface tension of the aqueous mixtures was measured at a high total concentration under a fixed NaCl concentration of 0.1 mol kg^{-1} at 25 °C. For NaTDC the slope of the plot changes markedly at 1.89×10^{-3} mol kg^{-1}. Above this concentration the surface tension remains constant, giving evidence that the aggregation number is sufficiently large to determine the cmc by surface tension measurements. The value of the cmc agrees well with the interpolated value from emf measurements in several added NaCl concentrations reported by Ryu et al.[6] For NaTC the surface tension gradually decreases with m_t over the entire concentration range measured. For mixtures, there are sharp breaks

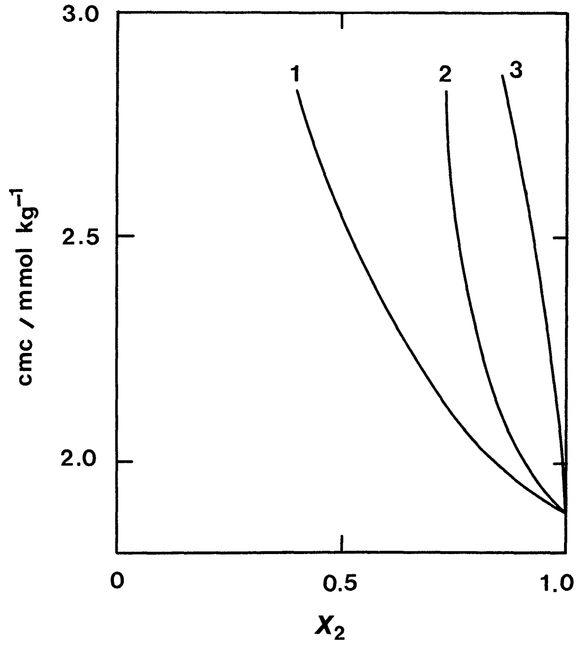

Fig. 5. Critical micelle concentration vs. composition curves. (1) cmc vs. X_2^W; (2) cmc vs. X_2^M; (3) cmc vs. $X_2^{H,\mathrm{cmc}}$.

in the plots, except for X_2^W=0.2. However, the changes of the slopes at the break points become less sharp in going to pure TC, and at concentrations above the break point the surface tension gradually decreases with increasing concentration. We can assume that the aggregations which can be designated as micellization occur at these break points, since the curve connecting these break points is monotonous. The cmc's, thus obtained, are plotted against X_2^W in Fig. 5. It is important to note that the cmc depends strongly on the bulk composition and is difficult to extrapolate to the pure NaTC axis, indicating that a pure NaTC micelle can not be formed in a moderate concentration region.

By a thermodynamic treatment based on the excess thermodynamic quantities, similar to those used for the adsorbed film, the Gibbs–Duhem equation of the mixed micelle is given as[15]

$$\sum_k \sum_i \nu_{i,k} N_k^M \mathrm{d}\mu_k = 0. \tag{10}$$

Denoting the composition of a mixed micelle by X_2^M ($X_2^M = N_1 + N_2$, where N is the number of k-ions in a mixed micelle) and using Eqs. 4 and 10 yields

$$X_2^M = X_2^W - X_1^W X_2^W [1/\mathrm{cmc} + 1/(\mathrm{cmc} + m_3)] \times (\partial \mathrm{cmc}/\partial X_2^W)_{T,p}, \tag{11}$$

where assumptions that the condition of electroneutrality holds for a micelle, the solution can be treated ideally, and N_{Cl^-}/N_t smaller than unity are achieved.[15] Applying this relation to the curve in Fig. 5, the composition of a micelle at the cmc is shown as cmc vs.

X_2^{M} plots in Fig. 5. The composition in an adsorbed mixed film at cmc is calculated by the following equation[11] (and plotted in Fig. 5):

$$X_2^{\mathrm{H,\,cmc}} = X_2^{\mathrm{M}} - (X_1^{\mathrm{W}}X_2^{\mathrm{W}}/RT\Gamma_{\mathrm{t}}^{\mathrm{H,\,cmc}})(\partial\gamma^{\mathrm{cmc}}/\partial X_2^{\mathrm{W}})_{T,p}. \quad (12)$$

If the aggregation numbers of bile salts are as small as dimers or trimers, a thermodynamic treatment for micelles based on excess thermodynamic quantities is not applicable. The experimental result is that the surface tension vs. concentration curve of pure NaTDC gives constant values after cmc. This confirms an aggregation number which is sufficiently large for a thermodynamic treatment. Furthermore, the evaluated values of the composition in the adsorbed film at the cmc's using Eq. 12 agree well with the values obtained from Eq. 7. These results demonstrate that micelles composed of bile salts can be adequately treated by the thermodynamic treatment employed here, although the treatment assumes a hypothetical dividing surface in the micelle.

Discussion

TC and TDC have the same chemical structure, except for a hydroxyl group in the TC 7 position of the cholanic acid residue. The number of hydroxyl groups results in the difference in their aggregation behavior. It has been shown that TDC forms a micelle while TC does not in a moderate concentration region.[4-6] This behavior is clearly shown in the cmc vs. X_2^{W} graph shown in Fig. 5. For mixtures, the cmc's were observed above about $X_2^{\mathrm{W}}=0.2$. Even at such a low composition of TDC in solution the calculated composition in the mixed micelles shows a value of nearly 0.8; the cmc vs. X_2^{M} curve shows an almost vertical change at that composition, indicating a solubility limit of TC in the TDC micelle.

For synthetic surfactants it has been shown that the molecular interaction in a micelle is quite similar to that in an adsorbed film.[11] For bile salts we can also assume that the molecular interaction in micelles is basically the same as that in the adsorbed film, even though the size and structure of bile salt micelles are unlike that of synthetic surfactants.[15] In the adsorbed film TC and TDC shows a slight attractive interaction, as shown in the A vs. X_2^{H} relations. This result indicates that TC and TDC will attract each other and mix freely in their micelles. However, there is a significant difference in the compositions of the adsorbed film and micelle. A deviation of the compositions from that of a bulk solution is a useful indication of the geometrical packing factor in the micelle, since the interactions between monomeric bile salts in a bulk solution are negligible. In studies of a series of alkyl ammonium salt mixtures,[11,17,18] differences have been clearly shown among compositions of the solution, the adsorbed film, and the micelle arising from the chemical structure of the surfactants and the micelle. For the decylammonium bromide–decylammonium chloride (DeAC) system,[17] in which the difference is their counter ions, both salts mix ideally and the X_2^{W}, $X_2^{\mathrm{H,cmc}}$, and X_2^{M} have almost the same values, even though they differ significantly due to differences in their cmc's and in the surface tension values at the cmc of pure salts. For the dodecylammonium chloride (DAC)–DeAC system,[11] the $X_2^{\mathrm{H,cmc}}$ and X_2^{M} lie close to TDC and $X_2^{\mathrm{H,cmc}}$ is slightly larger than the X_2^{M}, indicating that the stronger cohesive interaction is weakened at the curved interface. On the other hand, the cone-shaped dodecyltrimethylammonium chloride (DTAC) aggregates into a micelle rather than into an adsored film. For the DeAC–DTAC system,[18] X_2^{M} shows a remarkably larger value than does $X_2^{\mathrm{H,cmc}}$.

Together with these findings, the relation among the compositions of the NaTC–NaTDC system indicates that the structure of the bile salt micelle is very different from that of the plane interface; the stronger cohesive force of NaTDC is weakened appreciably in the micelle due to the geometry of the mixed micelle.

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