A pH and pNa Study of Aqueous Solutions of Sodium Deoxycholate

Gohsuke Sugihara and Mitsuru Tanaka

Department of Chemistry, Faculty of Science, Fukuoka University, Nishi-ku, Fukuoka 814

(Received July 7, 1976)

The pH and pNa of aqueous solutions of sodium deoxycholate (SDC) were measured at various temperatures. The critical micelle concentrations (CMC) were determined by means of the crooked points on the plot of pH or pNa *vs.* the SDC concentration. The data obtained were interpreted thermodynamically, and changes in the enthalpy and entropy of micellization were evaluated, from which it was concluded that the hydrogen bond plays a very important role in the micelle formation.

Sodium deoxycholate (SDC), a bile salt, shows unique properties in its physicochemical and biological aspects, and SDC behaves as a surfactant. Although the micellar behavior in an aqueous solution of SDC has been studied in connection with various physicochemical properties,[1–9] the peculiarity with respect to pH and pNa has not been examined sufficiently.

As SDC is a salt of the "weak acid-strong base" type, the solution is basic due to hydrolysis. Though fatty acid soaps are also salts of the "weak acid-strong base" type, they are different from SDC in terms of the architecture of the hydrophobic part of the molecule, the balance of hydrophilicity and lyophilicity, and especially the ability to form intermolecular hydrogen bonding. In this paper the unique behavior of the hydrogen-ion and sodium-ion activity in an aqueous solution of SDC will be examined and discussed.

Experimental

Materials. SDC (Merck Co.) was recrystallized several times from a mixed solution of methanol and ethanol and then dried *in vacuo* at 110 °C. Sodium chloride, of a guaranteed reagent grade, was recrystallized from thrice-distilled water and dried *in vacuo*. The distilled water used for the preparation of solutions was previously freed from CO_2 by passing sufficient CO_2 free air through a soda lime column.

Apparatus and Procedure. a) *pH Measurement*: pH measurements were performed for the determination of the CMC, which was given by the break in the SDC concentration *vs.* pH curve at each temperature. The measurements were carried out using a Hitachi-Horiba pH meter, model F-7. The influence of CO_2 during the measurements was avoided by using an air current freed from CO_2 by means of a soda lime column. The solution to be measured was stirred by means of a magnetic stirrer (Acrobat Stirrer, made by the MS-Kiki Co.) which could be immersed in a water bath. The hydrolysis equilibrium was checked by means of the curve on the recorder for each measurement. The apparatus is shown schematically in Fig. 1.

b) *Activity Measurement of the Sodium Ion*: The measurements were carried out using a Hitachi-Horiba pNa meter, model N-5. The apparatus was almost the same as that of the pH measurements. The calibration curve was made by correlating the observed value with the literature value[10] for the activity of sodium ions in an aqueous solution of NaCl.

Results and Discussion

The pH change with the SDC concentration at various temperatures is shown in Fig. 2, while the relations of the sodium-ion activity *vs.* the SDC concentration are shown in Fig. 3. Every curve in both figures shows a break in its trend at the same concentration at the same temperature. These significant points correspond to the CMC values reported in other papers.[1–7] Accordingly, these methods can be used for the determination of the CMC of SDC. Table 1 lists the values of CMC obtained by both methods at each temperature.

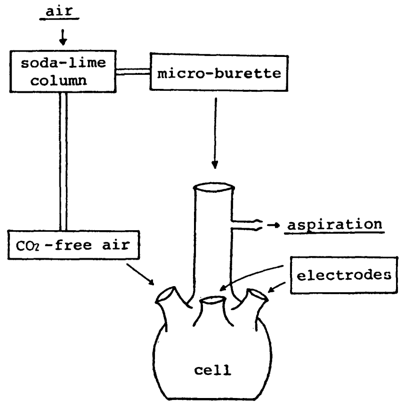

Fig. 1. The apparatus of measurement for the relation of SDC concentration *vs.* pH.

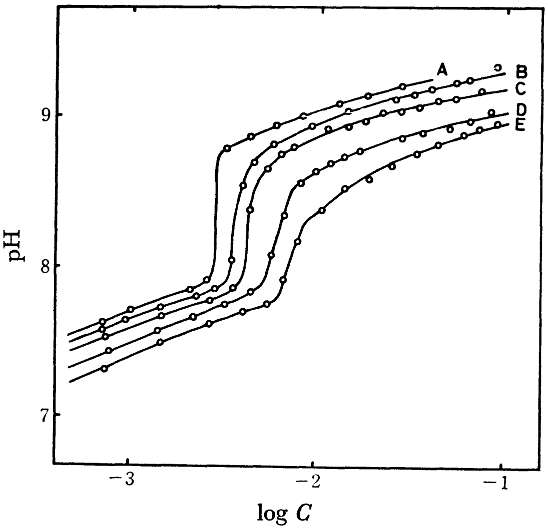

Fig. 2. Hydrolysis curves of SDC solution at various temperatures.
Key A: 10 °C, B: 15 °C, C: 20 °C, D: 30 °C, E: 40 °C.

In the concentration range of single dispersion, SDC molecules will dissociate almost perfectly, and

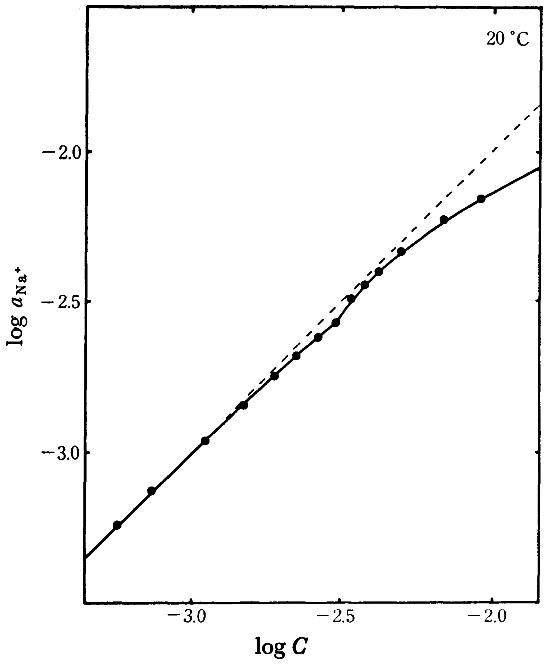

Fig. 3(a).

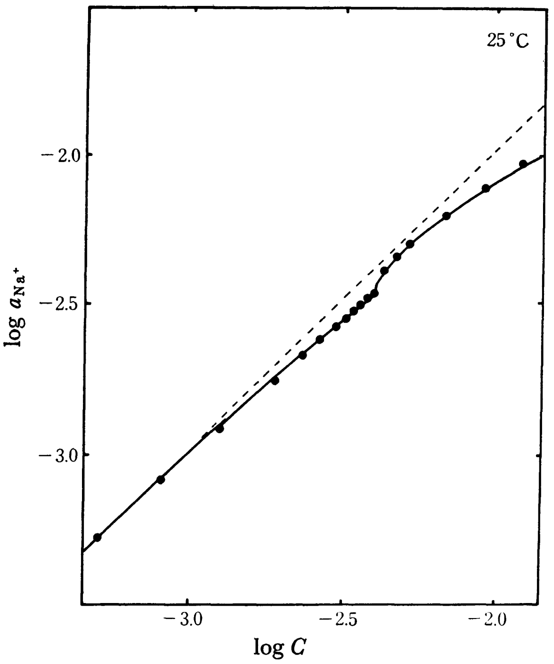

Fig. 3(b).

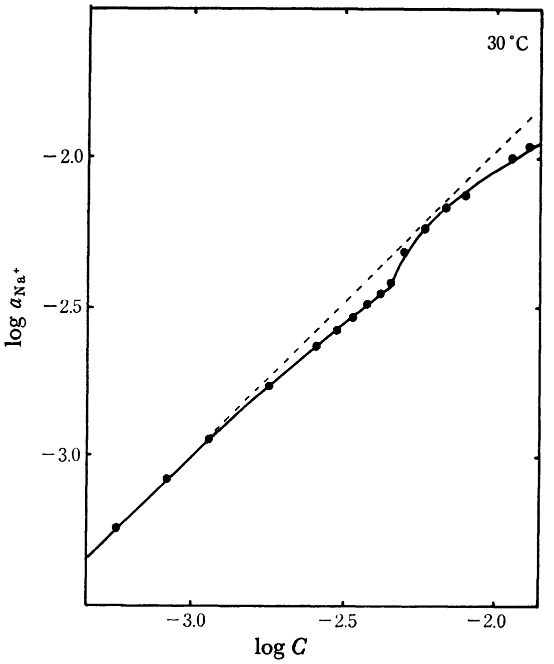

Fig. 3(c).

Fig. 3. The plot of SDC concentration *vs.* logarithm of sodium ion activity.
(a) 20 °C, (b) 25 °C, (c) 30 °C.

a hydrolysis equilibrium will be established. The concentrations of the undissociated species, sodium deoxycholate (NaDC), and the hydrolyzed species, deoxycholic acid (HDC), will increase with an increase in the SDC concentration. The associations of NaDC and HDC are expected to be easier than that of deoxycholate ions (DC^-) because of the smaller coulombic repulsion among them. Especially, HDC molecules are likely to form stable associates by hydrogen bonding. The micellization will be initiated with the association of HDC, and then accompanied by the participation of NaDC and DC^-. As soon as the micelles are formed, a new reaction of hydrolysis will begin abruptly on the surface of the micelles and a new equilibrium of hydrolysis will be established. Figure 2 shows that the pH increases abruptly from the CMC with an increase in the SDC concentration. This means that the degree of hydrolysis of DC^- in micelles is larger than that in single dispersion.

It can be seen from Fig. 3 that SDC dissociates perfectly in the dilute range of concentration below CMC as a 1-1 electrolyte, and that the degree of dissociation seems to decrease gradually up to the CMC as the concentration increases. Above the CMC the curve shows an abrupt increase in the activity coefficient. This behavior is different from that of sodium dodecylsulfate or sodium tetradecylsulfate.[11] Figures 2 and 3 tell us that the change in the sodium-ion activity in the vicinity of the CMC with an increase in the SDC concentration is contrary to that of the hydrogen-ion activity. This can be interpreted by thermodynamic considerations as follows.

We assume that the composition of the micelles is $DC^-\cdot\alpha H^+\cdot\beta Na^+$, where α and β are the numbers of the H^+ ion and the Na^+ ion respectively, per DC^- ion in the micelle.

Then, the equilibrium of micelle formation is expressed as follows:

$$DC^- + \alpha H^+ + \beta Na^+ \rightleftharpoons DC^-\cdot\alpha H^+\cdot\beta Na^+ \qquad (1)$$

The chemical potentials of the components in the solution are, respectively:

$$\mu_{DC^-} = \mu^0_{DC^-} + RT\ln a_{DC^-} \qquad (2)$$

$$\mu_{H^+} = \mu^0_{H^+} + RT\ln a_{H^+} \qquad (3)$$

$$\mu_{Na^+} = \mu^0_{Na^+} + RT\ln a_{Na^+} \qquad (4)$$

The chemical potential of the micelle-forming species ($DC^-\cdot\alpha H^+\cdot\beta Na^+$) in the micelle can be expressed by the following equation, if the micelle is regarded

Table 1. The CMC values determined by pH and pNa measurements

Temperature (°C)	CMC (mol/l) × 10^3	
	by pH measurement	by pNa measurement
10	2.95	
15	3.35	3.24
20	3.55	3.47
25	4.01	4.05
30	4.80	4.57
35	5.35	5.19
40	6.10	

as a pseudo phase:

$$\mu_\mathrm{m} = \mu^0_\mathrm{m}\ (T, P) \tag{5}$$

From Eqs. 2—5 we obtain the free energy change of micelle formation per mole of the micelle-forming species, $\Delta\overline{G}_\mathrm{m}$:

$$\begin{aligned}\Delta\overline{G}_\mathrm{m} &= \mu_\mathrm{m} - \mu_{\mathrm{DC}^-} - \alpha\mu_{\mathrm{H}^+} - \beta\mu_{\mathrm{Na}^+} \\ &= \Delta\overline{G}{}^0_\mathrm{m} - RT\ln a_{\mathrm{DC}^-} - \alpha RT\ln a_{\mathrm{H}^+} - \beta RT\ln a_{\mathrm{Na}^+}\end{aligned} \tag{6}$$

where

$$\Delta\overline{G}{}^0_\mathrm{m} = \mu^0_\mathrm{m} - \mu^0_{\mathrm{DC}^-} - \alpha\mu^0_{\mathrm{H}^+} - \beta\mu^0_{\mathrm{Na}^+} = \mathrm{const}\ (T, P).$$

As $\Delta\overline{G}_\mathrm{m}$ is equal to zero at equilibrium, Eqs. 7—9 hold:

$$\ln a_{\mathrm{DC}^-} = \mathrm{const}(T, P) - \alpha\ln a_{\mathrm{H}^+} - \beta\ln a_{\mathrm{Na}^+} \tag{7}$$

Especially, at the CMC

$$\ln \mathrm{CMC} = \mathrm{const}(T, P) - \alpha\ln a_{\mathrm{H}^+} - \beta\ln a_{\mathrm{Na}^+} \tag{8}$$

and

$$\log a_{\mathrm{Na}^+} = \mathrm{const}\ (T, P) - \frac{1}{\beta}\log a_{\mathrm{DC}^-} + \frac{\alpha}{\beta}\mathrm{pH} \tag{9}$$

This equation tells us that $\log a_{\mathrm{Na}^+}$ increases with the pH and α, and varies inversely with the β. The rise in $\log a_{\mathrm{Na}^+}$ with the concentration above the CMC just corresponds to the rise in pH, and the decreasing tendency of the increase in a_{Na^+} at higher concentrations may be ascribed to the negative sign of the log a_{DC^-} term.

Since $\Delta\overline{G}_\mathrm{m}$ can be regarded as a function of T, P a_{DC^-}, a_{Na^+} and a_{H^+}, the following equation holds for the perfect differential of $\Delta\overline{G}_\mathrm{m}$:

$$\begin{aligned}\mathrm{d}(\Delta\overline{G}_\mathrm{m}) &= -\Delta\overline{S}_\mathrm{m}\mathrm{d}T + \Delta\overline{V}_\mathrm{m}\mathrm{d}P + \left(\frac{\partial\Delta\overline{G}_\mathrm{m}}{\partial\ln a_{\mathrm{DC}^-}}\right)\mathrm{d}\ln a_{\mathrm{DC}^-} \\ &+ \left(\frac{\partial\Delta\overline{G}_\mathrm{m}}{\partial\ln a_{\mathrm{H}^+}}\right)\mathrm{d}\ln a_{\mathrm{H}^+} + \left(\frac{\partial\Delta\overline{G}_\mathrm{m}}{\partial\ln a_{\mathrm{Na}^+}}\right)\mathrm{d}\ln a_{\mathrm{Na}^+}\end{aligned} \tag{10}$$

The differential coefficients of the third, fourth, and fifth terms in Eq. 10 are obtained from Eq. 6 as $-RT$, $-\alpha RT$, and $-\beta RT$ respectively. Hence, Eq. 11 is derived from Eq. 10;

$$\begin{aligned}\mathrm{d}(\Delta\overline{G}_\mathrm{m}) &= -\Delta\overline{S}_\mathrm{m}\mathrm{d}T + \Delta\overline{V}_\mathrm{m}\mathrm{d}P - RT\,\mathrm{d}\ln a_{\mathrm{DC}^-} \\ &- \alpha RT\,\mathrm{d}\ln a_{\mathrm{H}^+} - \beta RT\,\mathrm{d}\ln a_{\mathrm{Na}^+}\end{aligned} \tag{11}$$

For the reversible micelle formation at a constant pressure, we obtain the following relation:

$$\begin{aligned}\Delta\overline{S}_\mathrm{m} &= \frac{\Delta\overline{H}_\mathrm{m}}{T} = -RT\left(\frac{\partial\ln a_{\mathrm{DC}^-}}{\partial T}\right)_P \\ &-\alpha RT\left(\frac{\partial\ln a_{\mathrm{H}^+}}{\partial T}\right)_P - \beta RT\left(\frac{\partial\ln a_{\mathrm{Na}^+}}{\partial T}\right)_P\end{aligned} \tag{12}$$

Here, Eq. 12 may be rewritten as follows, assuming the temperature dependency of the activity coefficient of the SDC micelle is negligible:

$$\begin{aligned}\Delta\overline{S}_\mathrm{m} &= -2.303\,RT\left\{\left(\frac{\partial\log\mathrm{CMC}}{\partial T}\right)_P - \alpha\left(\frac{\partial\mathrm{pH}}{\partial T}\right)_P\right. \\ &\left. - \beta\left(\frac{\partial\mathrm{pNa}}{\partial T}\right)_P\right\}\end{aligned} \tag{13}$$

According to the results shown in Figs. 2 and 3, the plot of the pH value at the CMC *vs.* T affords a linear relation with a slope of -5.0×10^{-3}. The plot of the pNa value at the CMC *vs.* T similarly affords a linear relation with a slope of -1.0×10^{-2}. From Table 1, $(\partial\log\mathrm{CMC}/\partial T)_P$ is roughly given as $+1.0\times10^{-2}$ at 20 °C. The α and β are not obtained from our experiment, but the data of Vochten and Joos[12] can be used to determine them approximately. Using Corrin's equation, they estimated β(their notation is K) as 2.0 and 2.5 under the conditions of constant pH values of 7 and 9 respectively at 20 °C. Here, it should be noted that the value of β obtained using Corrin's equation does not account for the contribution of H^+ to the micelle formation, and that β is related only to Na^+. Their data show that the CMC depends strikingly on the pH. The estimation of the α value is possible from the thermodynamic treatment of their data concerning the dependence on pH. Equation 8 is rewritten as follows:

$$\log\mathrm{CMC} = \mathrm{const}\ (T, P) + \alpha\,\mathrm{pH} + \beta\,\mathrm{pNa} \tag{14}$$

From Fig. 7 in their paper, α or $\Delta\log\mathrm{CMC}/\Delta\mathrm{pH}$ at a constant sodium-ion concentration is calculated to be about 0.8, independent of the value of the sodium-ion concentration. Using these values, $\Delta\overline{H}_\mathrm{m}$ and $\Delta\overline{S}_\mathrm{m}$ are evaluated as −6 kcal and −20 e.u. respectively from Eq. 13. On the other hand, the change in the partial molal volume of SDC on micellization, $\Delta\overline{V}_\mathrm{m}$, has been obtained as −11 ml/mol at 30 °C from densitometry.[14] $\Delta\overline{H}$, $\Delta\overline{S}$, and $\Delta\overline{V}$ for the gelation of SDC solution all have negative signs which are characteristic of hydrogen-bond formation.[15] It is, then, reasonable to consider from these data that the micelle formation of SDC is mainly caused by hydrogen bonding, such as that between a hydroxyl group of one molecule and a carboxyl group of the other. This is in contrast to the micelle formation of other surfactants, *e.g.*, sodium dodecylsulfate and sodium tetradecylsulfate, which is mainly caused by hydrophobic interaction. It should be noted that $\alpha+\beta$ is nearly equal to unity, which means that micelles of SDC are electrically neutral. As an SDC molecule has two hydroxyl groups, SDC micelles may exist stably in the solution due to their hydrophilicity, which arises from the hydrogen-bond formation between water molecules and the remaining hydroxyl groups that have not been used for the micelle formation and so are able to contribute to the further association of the micelles.

This interpretation supports the helical structure model proposed by Rich and Blow.[8,9]

We would like to acknowledge the valuable discussions and suggestions by Prof. Dr. Ryohei Matuura, Kyushu University, and Prof. Dr. Iwao Satake, Kagoshima University. We are indebted to Miss Noriko Seike, Miss Mariko Ikeda, and Miss Kazuko Matsuo, Fukuoka University, for their technical assistance.

References

1) P. Ekwall, *J. Colloid Sci. Suppl. I*, **1944**, 66.
2) S. A. Johnston and J. W. McBain, *Proc. R. Soc. London, Ser. A*, **181**, 119 (1943).
3) T. Furusawa, *Fukuoka Acta Medica*, **53**, 124 (1962).
4) K. Fontell, *Kolloid Z. Z. Polym.*, **244**, 246 (1971).
5) K. Fontell, *Kolloid Z. Z. Polym.*, **244**, 253 (1971).
6) K. Fontell, *Kolloid Z. Z. Polym.*, **246**, 614 (1971).
7) K. Fontell, *Kolloid Z. Z. Polym.*, **246**, 710 (1971).
8) A. Rich and D. M. Blow, *Nature*, **182**, 423 (1958).
9) D. M. Blow and A. Rich, *J. Am. Chem. Soc.*, **82**, 3566 (1960).
10) G. J. Janz and A. R. Gordon, *J. Am. Chem. Soc.*, **65**, 218 (1943).
11) I. Satake, T. Tahara, and R. Matuura, *Bull. Chem. Soc. Jpn.*, **42**, 319 (1969).
12) R. Vochten and P. Joos, *J. Chem. Phys.*, **67**, 1373 (1970).
13) M. L. Corrin and W. D. Harkins, *J. Am. Chem. Soc.*, **69**, 679 (1947).
14) G. Sugihara, to be published.
15) G. Sugihara, to be published.