Colloid Polym Sci 273:392–398 (1995)
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ORIGINAL CONTRIBUTION

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A potentiometric titration study on the dissociation of bile acids related to the mode of interaction between different head groups of nonionic surfactants with free bile salts upon mixed micelle formation in water

Received: 8 June 1994
Accepted: 13 September 1994

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Abstract By constructing an elaborate set of potentiometric titration together with data analysis system, apparent acid dissociation indices (pK_a^{app}) for two bile acids were determined in the mixed surfactant system of bile salts (Sodium Deoxycholate, NaDC, and Sodium Chenodeoxycholate, NaCDC) with nonionic surfactants (Hexaethyleneglycol mono *n*-dodecylether, $C_{12}E_6$, Decanoyl-*N*-methylglucamide, MEGA-10) in aqueous solution at ionic strength 1.5 as a function of mole fraction in the surfactant mixture. It was found that with increasing the bile salt concentration, pK_a^{app} as well as pH showed an abrupt rise at a certain concentration of the bile salt being regardable as a critical micellization concentration (CMC) and reached a constant value at the range sufficiently higher than CMC for each pure bile salt system, meaning that the dissociation degree of carboxyl group in micelle is smaller than that in bulk. In the mixed systems of free bile salts with nonionic surfactants, the dissociation state of carboxyl groups in mixed micelles depends on the species of hydrophilic group of nonionic surfactants as well as on mole fraction in the surfactant mixture.

Key words Potentiometric titration – bile acid – nonionic surfactant – mixed micelle formation – MEGA-10

Introduction

Studies of mixed surfactant systems have so far attracted much research of pure scientific interest concerning the intermolecular interaction between hydrophilic groups as well as hydropohobic groups in addition to application interest to improve the surfactant performance. Recently, as for the "mixed surfactant systems" two books collecting different authors' review works edited systematically, have been published by Rubingh and Holland [1] and Ogino and Abe [2]. According to Nishikido and Sugihara, it is estimated that more than 700 papers have been published up to the present (until the first half of 1990, 642 papers appeared) and their review offers information on conveniently classified papers [3].

The present authors also have been interested in the mixed surfactant systems, especially in the mixture of bile salts with respect to their mixed micelle formation, monolayer formation and solubilization of biological lipids such as cholesterol and glycerides [4–6]. Additionally, in our research series on mixed systems of hydrocarbon-fluorocarbon surfactant mixtures, we found that the combination of sodium perfluorooctanoate (SPFO) and a nonionic surfactant MEGA-10 showed a negative deviation from ideal mixing upon mixed micelle formation [7–9], indicating that the stronger interaction between carboxyl group of SPFO and the hydrophilic group of

MEGA-10 (glucamide) can override the mutual-phobic interaction acting between hydrocarbon and fluorocarbon chains of hydrophobic groups. As for the latter, a mutual-phobic effect on micelle formation has been found by several authors [10–17], while the effect of the interaction between hydrophilic groups on micelle formation has been extensively studied by Ogino and Abe [18].

In the present paper, two combinations of bile acids (sodium deoxycholate, NaDC and sodium chenodeoxycholate, NaCDC) with nonionic surfactants (decanoyl-*N*-methylglucamide, MEGA-10 and hexa-ethyleneglycol mono *n*-dodecylether, $C_{12}E_6$) are studied in order to examine the effect of both differences in chemical structure of hydrophobic groups and in hydrophilic groups on mixed micelle formation by means of measurement of the pK_a value of bile acid (BA) species. In addition, the present result will be discussed by comparing with the previous work [19], in which mixed systems of sodim cholate (NaC), sodijm glycocholate (NaGC) and NaDC with $C_{10}E_8$ were treated.

Experimental

Materials

Sodium deoxycholate (NaDC) and sodium chenodeoxycholate (NaCDC) were purchased from Calbiochem, USA. The purity was checked by TLC; only one spot with no tailing was found. Hexa-ethyleneglycol mono *n*-dodecylether ($C_{12}E_6$) was supplied from Nikko Chemicals, Japan and used as received. Decanoyl-N-methylglucamide (MEGA-10) was obtained from Dojindo Laboratories, Japan and recrystallized three times from 9:1 diethylether-ethanol mixture. NaCl, HCl, and NaOH obtained from Nacalai Tesque, Japan were of analytical grade and used as received.

Through all the experiments, the twice distilled water useα was boiled to exclude dissolved CO_2 and cooled down to room temperature in a bottle with a column of $CaCl_2$ to avoid the invasion of CO_2.

Potentiometric titration

Potentiometric titration to measure H^+ ion concentration was carried out by using a combination electrode (ROSS pH electrode) and a potentiometer (made by Orion Research Incorporated Laboratory Products Groups, Models 81-03 and EA940).

The standard electrode potential (SEP) (E'_0 in Eq. (1)) was determined by the well-known Gran's plot method [20] in aqueous 0.15 M (M = mol dm^{-3}) NaCl solution just before and after each titration. The SEP data averaged before and after tiration were used for calculating H^+ ion concentration. The procedure employed is as follows. 5 ml of solution of ionic strength fixed at 0.15 M containing desired concentration of sodium salt of bile acid (NaBA) together with NaCl (i.e., the total concentration of Na^+ was always kept at 0.15 M) was exposed in a flow of N_2 gas (super extra pure grade, 99.995%) during the operation. The pH of sample solution was adjusted to ca. 10 with 0.001 M NaOH solution. Then the solution was very slowly titrated with aqueous 0.02 M HCl solution containing 0.13 M NaCl, and the HCl-NaCl solution was added step wise into the cell after sufficient settling of the potential indication. Titration was made from pH 10 down to about pH 7. The blank solution (0.15 M NaCl solution with no NaBA) was also titrated in the same manner.

The electric potential of solution (NaBA/nonionic surfactants/NaCl/NaOH mixed systems) was measured to determine H^+ concentration, $[H^+]$, according to the following equation.

$$[H^+] = 10^{[(E-E'_0)/g]}\,, \tag{1}$$

where E'_0 is SEP, E the measured e.m.f., and g = $(RT/nF)\ln 10 = 60.15$ at 30 °C. Equation (1) is expressed in terms of titer (X in ml) of 0.15 M titrant (0.02 M NaOH + 0.13 M NaCl) whose NaOH concentration is referred to as C_{NaOH}; the volume of 0.15 M NaCl solution, V_A; and that of 0.15 M HCl-NaCl solution (0.02 M HCl + 0.13 M NaCl) whose HCl concentration is referred to as C_{HCl}, V_B.

$$[H^+] = \frac{C_{HCl} \cdot V_B - C_{NaOH} \cdot X}{(V_A + V_B) + X}\,. \tag{1'}$$

In practice, V_A and V_B were 50 ml and 10 ml, respectively and thus when $X = 10$ ml the neutralization should be established. Each time, 0.5 ml of the titrant was added dropwise based on the following relation:

$$((V_A + V_B) + X) \cdot 10^{E/g} = (C_{HCl} \cdot V_B - C_{NaOH} \cdot X) \cdot 10^{E'_0/g}\,. \tag{1''}$$

E'_0 was determined by a personal computer and the calculated left-hand side was plotted against $C_{NaOH} \cdot X$; this so-called Gran's plot should give a linear relation with a slope of $-10^{E'_0/g}$. In fact, a very good linearity was obtained over 21 measured points for every titration. Usually, the E'_0 values calculated at 14 measured points excluding six points of both ends were averaged.

The concentration of H^+ ion bound to bile acid ion (BA^-), i.e., the concentration of hydrolyzed bile acid species ([HBA]) should be determined to evaluate the dissociation constant of a bile acid (HBA) or the pK_a. This concentration is calculated according to the following.

$$[HBA] = [A_1] - ([A_2] + [A_3])\,, \tag{2}$$

where $[A_1]$ is the concentration of H^+ ion expected from the titrant HCl-NaCl solution; $[A_2]$, that of free H^+ ion; and $[A_3]$, that of H^+ ion exhausted for neutralization of OH^- ion coming from added NaOH and water. $[A_3]$ at each pH was preliminarily determined from 100 ml of the blank solution in which pH was raised up to ca 10 with NaOH.

Determination of apparent pK_a values

In this experiment, the titration was started, as mentioned above, from about pH 10 at which NaDC is regarded as being completely dissociated. However, since the protonated species of HBA lacks solubility in water, the measurement was restricted within a narrow range of the degree of dissociation: $1 > \alpha > 0.7$.

The α is given by:

$$\alpha = \frac{[BA]_t - [HBA]}{[BA]_t}, \tag{3}$$

where $[BA]_t$ denotes the total concentration of NaBA. Then, we have

$$-\log[H^+] = pKa + \log\frac{\alpha}{1-\alpha}, \tag{4}$$

According Eq. (4), the plot of $\log\{(1-\alpha)/\alpha[H^+]\}$ vs. α is considered to lead to a linear with a slope of zero. But since BA salts form micelles above around 3 mM and produce an electric potential Ψ due to an increase in negative charge on micellar surface. This will result in an apparent increment of pK_a so that the linear relation with a slope of zero will not be obtainable. Therefore, Eq. (4) should be arranged as follows.

$$-\log[H^+] - \log\left(\frac{\alpha}{(1-\alpha)}\right) = pK_a^{int} - 0.4343\frac{\varepsilon\Psi}{kT} \tag{5}$$

$$= pK_a^{app}. \tag{6}$$

Here, pK_a^{int} denotes an intrinsic acid dissociation index which is independent of electric field due to micelles; pK_a^{app}, an apparent pK_a value depending on the micellar electric field; ε, the dielectric constant, k, the Boltzman constan and T, the Kelvin temperature.

Since Ψdiminishes as α approaches zero, we have the following relation:

$$\lim_{\alpha\to 0}\left(-\log[H^+] - \log\left[\frac{\alpha}{1-\alpha}\right]\right) = pK_a^{int}. \tag{7}$$

From this equation pK_a^{int} is, in principle, determinable by exptrapolating α to zero. However, since the range of determinable α value is so restricted (1 ~ 0.85, practically) it did not allow us to extrapolate α to zero.

As a result, the determination of the apparent acid dissociation index, pK_a^{app} was performed by the following procedure. First, the coordination number of H^+ onto a carboxyl group of BA is referred to as $\bar{n}$ as follows.

$$\bar{n} = \frac{[HBA]}{[HBA] + [BA^-]}. \tag{8}$$

Since the dissociation constant K_a is defined as $K_a = \frac{[H^+]\cdot[BA^-]}{[HBA]}$, $\bar{n}$ is rewritten as:

$$\bar{n} = \frac{\frac{[H^+]}{K_a}}{1 + \frac{[H^+]}{K_a}}. \tag{9}$$

From this, the following relation is derived.

$$\frac{\bar{n}}{1-\bar{n}} = \frac{[H^+]}{K_a}. \tag{10}$$

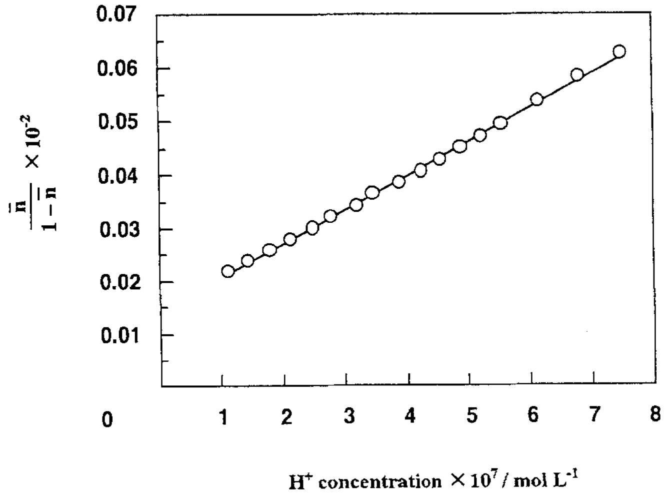
This equation tells us that plotting the left-hand side against $[H^+]$ gives a curve with a slope of $1/K_a$; this logarithm corresponds to pK_a^{app}.

In the actual treatment, the values of H^+ ion concentration determined from Eq. (1) were processed through a personal computer in which Eqs. (1), (2), (8), and (10) were programmed.

Results and discussion

An example of the relation in Eq. (10) is shown in Fig. 1, where $\bar{n}/(1-\bar{n})$ is plotted against $[H^+]$ determined for

Fig. 1 An example of the plot of $\bar{n}/(1-n)$ against $[H^+]$ (the relation in Eq. (10) determined for a pure NaDC system of 10 mM at 30 °C

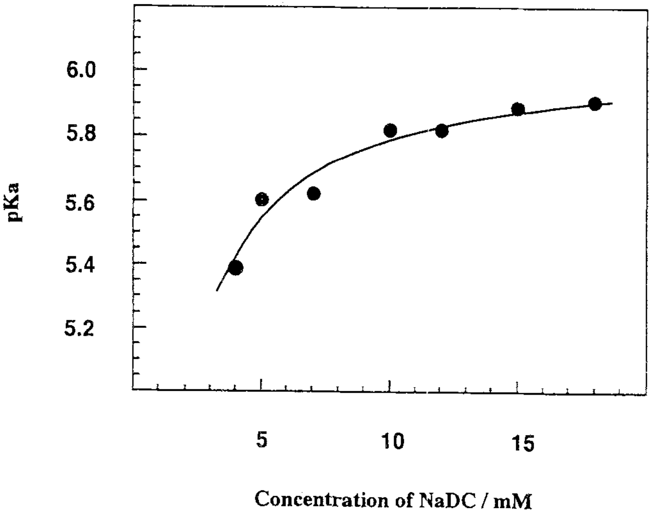


a pure NaDC system of 10 mM at 30 °C. The logarithmic value of the slope corresponds to an apparent pK_a value.

The apparent pK_a values thus obtained for pure NaDC system are plotted against concentration, as is shown in Fig. 2, indicating that with increase in NaDC concentration, pK_a value starts to abruptly increase and then reaches a constant. A similar behavior of pK_a was observed even for NaCDC (not shown here). Such a hyperbolic increase in pK_a was first observed for sodium cholate (NaC) and NaDC, by Ekwall et al. [21]., and for NaCDC and their glycine and taurine conjugates by Dowling and Small [22, 23]. In their studies BS solution at each fixed concentration of BS was titrated with hydrochloric acid and from the obtained titration curves (plots of HCl conc. vs. pH) they determined pH of precipitation and the maximum solubility of the acid in the BS solution in addition to apparent pK_a (pK'_a in their notation) at each given BS concentration, where the pK'_a was calculated based on a simplification of the formula proposed by Back and Steenberg [24].

On the other hand, considering that BS's are salts of the type of "weak acid-strong base," Sugihara and Tanaka [25] have measured the pH change with increase in BS concentration in pure and saline water and found that the pH is abruptly raised at the critical micellization concentration (CMC), suggesting that micelle formation is initiated with intermolecular hydrogen bonding as well as hydrophobic interaction. This hydrogen bonding accompanied by the aggregate formation was confirmed by means of NMR [26] and ESR [27*, 28].

Fig. 2 The apparent pKa values determined as a function of NaDC concentration at 30 °C



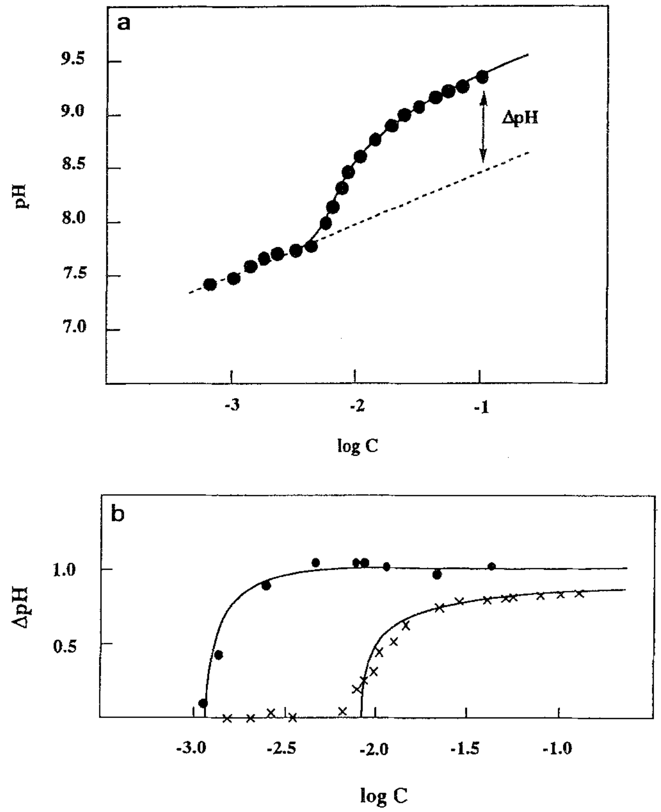


Fig. 3 The pH change with BS concentration observed for NaDC in CO_2-free pure water at 25 °C (NaDC hydrolysis curve). ΔpH corresponds to Eqs. (12) and (13). b) The relations of ΔpH with logarithmic concentration of NaDC at 25 °C. Solid lines indicate the curves calculated for the systems with added 0.75 M NaCl (●) and with no added salt (x), respectively [29]

The abrupt rise of the apparent pK_a or of pH in BS solution at the CMC has been observed as shown in Fig. 3 (given as a relation of pH vs log C) [25] and the pH jump (ΔpH) is interpreted in terms of the ratio of Ka above CMC (k_{a2}) to Ka below CMC (k_{a1}) or the ratio of hydrolysis constants above and below CMC, k_{h1}/k_{h2}, as follows.

$$K_R = k_{a2}/k_{a1} = k_{h1}/k_{h2} \tag{11}$$

$$\Delta pH = \log\left[\sqrt{\frac{(S-1)^2}{SK_R\{K_R+(S-1)\}}}\left\{1+\frac{K_R}{S-1}\right\}\right], \tag{12}$$

where S denotes a reduced concentration C/CMC, i.e., the analytical concentration is divided by CMC. Note that in such a range as $(S-1) \gg K_R$ and $K_R < 1$, S may be much larger than unity. Accordingly, the next approximate equation may be derived, i.e., the ΔpH (or ΔpKa) should approach a constant value as in the following

$$(\Delta pH)_{S \gg 1+K_R} \approx \frac{1}{2}\log\frac{1}{K_R} = \frac{1}{2}(pK_{h1} - pK_{h2})$$

$$= \frac{1}{2}(pK_{a2} - pK_{a1}) \,. \tag{13}$$

In earlier work [29], Sugihara demonstrated that the curve of pH jumping can be calculated according to Eq. (12) (see theoretical curves in Fig. 3b)

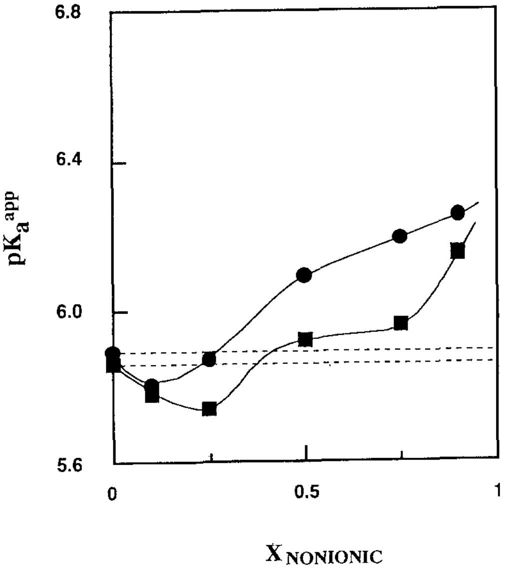
Returning to the results obtained from the present experiment, the pK_a^{app} values showing a constancy at sufficiently high concentration were determined as 5.76 and 5.89 for HDC and HCDC, respectively. This difference must come from the difference in location of hydroxyl group other than 3α OH, i.e., 7α or 12α. The 12α OH which is located closer to the carboxyl group is likely to make it more easily dissociate than 7α OH, so that pK_a^{app} for HDC is a little smaller than that for HCDC. A similar difference has been reported [21, 23].

Mixed Systems of $C_{12}E_6$ with NaDC and with NaCDC.

The pK_a^{app} values of NaDC and NaCDC in the mixture with $C_{12}E_6$ were determined at different mole fractions of $C_{12}E_6$ as a function of molarity of BS in the range from 4 to 18 mM (above CMC). In the plot of pK_a^{app} against molarity of BS, a good constancy of pK_a^{app} was observed in the region between 10 ~ 18 mM of BS for each mixed systems, so the pK_a^{app} value of BS in mixed micelles was evaluated by averaging four measured points in the region. It was found that pK_a^{app} depends on the mixing ratio. The results are shown for NaDC–$C_{12}E_6$ and NaCDC–$C_{12}E_6$ mixtures in Fig. 4.

In Fig. 4 broken horizontal lines indicate the pK_a^{app} values of the respective pure systems. Looking at the curve for NaCDC–$C_{12}E_6$ system, the pK_a^{app} decreases with increase in mixing ratio of $C_{12}E_6$ followed by its increase once a minimum has been reached at $X_{C_{13}E_6} \approx 0.1$ and then by exceeding the value for pure NaCDC at $X_{C_{12}E_6} \approx 0.25$. On the other hand, the mixed system of NADC–$C_{12}E_6$ exhibits a much larger dependency on mixing ratio than the NaCDC–$C_{12}E_6$ mixture; the curve has a deeper minimum at $X_{C_{12}E_6} \approx 0.25$ and after exceeding the value of pure NaDC at $X_{C_{12}E_6} = 0.35$, the curve shows a slow change followed by again starting to increase at $X_{C_{12}E_6} = 0.75$. Both curves are likely to approach to the same value at $X_{C_{12}E_6} = 1$.

Fig. 4 The apparent pKa values of NaDC (■) and NaCDC (●) in the mixture with $C_{12}E_6$ as a function of mole fraction of $C_{12}E_6$ in the surfactant mixture at 30 °C



Paying attention to the lower region of mole fraction of $C_{12}E_6$ ($0 < X_{C_{12}E_6} < 0.25$ for NaCDC–$C_{12}E_6$ mixture and, $0 < X_{C_{12}E_6} < 0.35$ for NaDC–$C_{12}E_6$ mixture) pK_a^{app} is lower than that of each BS. In this region, the mixed micelles are likely to be formed with less number of $C_{12}E_6$ molecules (1 or 2) being inserted among more number of BS molecules, i.e., one $C_{12}E_6$ molecule is surrounded by several BS molecules. In such a scheme the distance between carboxyl groups of BA becomes farther in the mixed micelles than in pure micelles so that the electrostatic repulsion may be a little reduced and thus H^+ ion bound to the micellar surface as counterion will dissociate more easily from a carboxyl group, meaning that pK_a is lowered more than that of the pure BS. On the other hand, in the region of the curve higher than the broken lines ($0.25 < X_{C_{12}E_6} < 1$ for NaCDC–$C_{12}E_6$, $0.35 < X_{C_{12}E_6} < 1$ for NADC–$C_{12}E_6$), the situation is in contrast to the above, that is, a lesser number of BS molecules form a mixed micelle with a greater number of $C_{12}E_6$ molecules. In such a situation, a carboxyl group of BS must be in contact with oxyethylene chains having a lower dielectric constant compared with water, so that the dissociation of carboxyl groups of BS may be depressed, resulting in the increase of pK_a^{app}.

Comparing between NaDC and NaCDC in the mixed micelle with $C_{12}E_6$ in more detail, not only pK_a^{app} values of the respective pure systems but also the depth of minimum and the points intersecting with the broken lines are different. These differences may be interpreted in terms of the effective hydrophobic surface area. The limiting surface areas (determined from a monolayer study [5] and unpublished data) for NaDC and NaCDC are 1.17 and 1.40 nm^2 per molecule, respectively. A NaCDC molecule has a wider surface area usable for hydrophobic interaction than NaDC, implying that the magnitude of hydrophobic interaction per BA molecule is different between NaCDC and NaDC. The magnitude difference should affect mixed micelle formation with respect to the binding degree of counterions including H^+ ions as well as the structure and the aggregation number, although the details are not yet well known, therefore, the resultant difference in mixed micelles formed may lead to a different behavior in pK_a.

From the result by the steady-state quenching model [19], it was found that the mixed micelles of bile salt (sodium cholate (NaC), sodium glycocholate (NaGC) and sodium deoxycholate (NaDC)) and nonionic surfactant (octaoxyethylene glycol mono *n*-decyl ether ($C_{10}E_8$)) became dramatically more hydrophobic from a certain mole fraction of the bile salt. This fact means that the mixed micelles contain an excess amount of $C_{10}E_8$ molecules below the mole fraction corresponding to each break point; above the mole fraction at the break point, the bile salts in the mixed micelles increase with an increase in mole fraction of the bile salt in the mixture. In the range of the mole fraction of bile salts from 0 to each break point, bile salts' molecules with a strong cohesion are concluded to be solubilized into the nonionic surfactants micelles. The break point nearly corresponds to the intersecting point of pK_a^{app} curve with the broken line in Fig. 4. From this fact, the intersecting point of pK_a^{app} curve with the broken line is likely to correspond to the micellar structure transition from the type "$C_{12}E_6$ surrounded by BS molecules" to the type of "BS surrounded by $C_{12}E_6$ molecules". The lower intersection point for NaCDC may be related to the wider limiting area or the wider hydrophobic surface area and to the lower molecular number of surrounding $C_{12}E_6$ molecules in a mixed micelle.

NaDC-MEGA-10 and NaCDC-MEGA-10 Mixed Systems.

The pK_a^{app} values of NaDC in mixed micelles with MEGA-10 were measured at discrete mixing ratios as shown in Fig. 5. In the concentration range above 10 mM of NaDC, pK_a^{app} shows a constancy at each mole fraction of MEGA-10, similar to the above BS–$C_{12}E_6$ mixed systems. The values of pK_a^{app} in mixed micelles were obtained by averaging four measured points in the region from 10 to 18 mM.

In Fig. 6, the pK_a^{app} data for the mixed systems of MEGA-10 with NaDC and NaCDC are given as a function of mole fraction of MEGA-10, X_{MEGA} along with the data for two BS–$C_{12}E_6$ mixed systems for comparison. The mixed systems with MEGA-10, interestingly, demonstrate that the pK_a^{app} ranges below the value of the pure NaDC (indicated by broken lines in the figure) up to $X_{MEGA} \approx 0.6$, and beyond the intersection with the broken line the curve sharply goes up, and that the NaCDC–MEGA-10 mixed system also shows a similar trend. This may be ascribed to the difference in nature of hydrophilic group, as is naturally considered. Comparing the hydrophilic groups between MEGA-10 and $C_{12}E_6$, the property of polyoxyethylene chain of $C_{12}E_6$ is a little closer to oil and lower in dielectric constant compared with that of methylglucamide which may be able to more easily incorporate water molecules. In the situation where a carboxyl group exists in the surrounding of oxyethylene chain, the dissociation of H^+ will be reduced even in the mixing ratio of $C_{12}E_6$ is low; in contrast, if it is surrounded by several groups of N-methylglucamide the dissociation

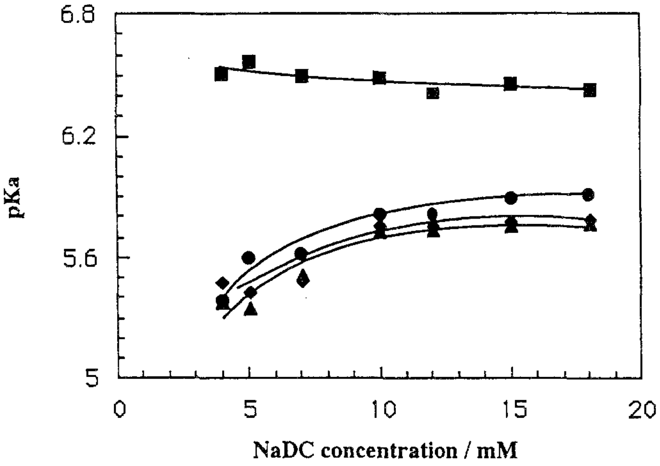


Fig. 5 The pK_a^{app} changes of NaDC in mixed micelles with MEGA-10 measured at discrete mixing ratios accompanied by increasing NaDC concentration at 30 °C. (●): NaDC pure system, (▲): $X_{MEGA-10} = 0.25$, (◆): $X_{MEGA-10} = 0.50$, (■): $X_{MEGA-10} = 0.90$

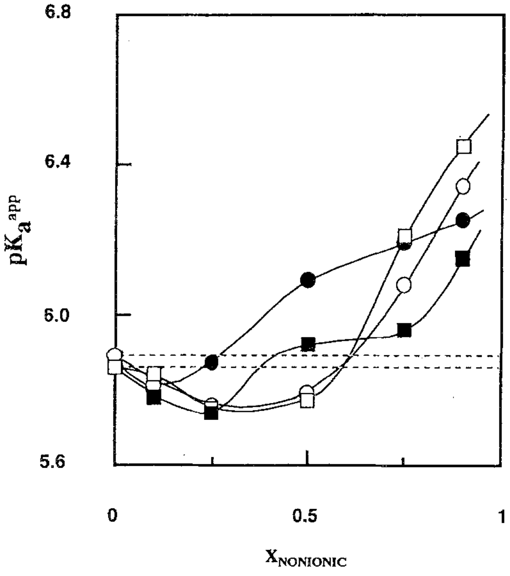


Fig. 6 The pK_a^{app} change of each BS-nonionic surfactant mixed system as a function of mole fraction of nonionic surfactant in each surfactant mixture at 30 °C. (●): CDC-$C_{12}E_6$, (■): DC-$C_{12}E_6$, (○): CDC-MEGA-10, (□): DC-MEGA-10

should be enhanced. The state of water molecules taking part in hydration of the hydrophilic groups is considered to be different between $C_{12}E_6$ and MEGA-10. This implies that the property of palisade layer containing water molecules is not the same due to a difference in hydration mode. When the carboxyl groups of BS are surrounded by a greater number of polyoxyethylene groups as mole fraction of $C_{12}E_6$ becomes more, the surroundings offer the value of pK_a^{app}. In contrast to this, when a carboxyl group of BS is surrounded with the hydrated N-methylglucamide, H^+ ion is more easily taken away because the electron density on hydroxyl groups of glucamide is a little higher than those of water molecule and the higher electron density causes an enhancement of deprotonation. But this interpretation is restricted in the mole fraction range below $X_{nonionic} = 0.6$. The higher pK_a^{app} values of the mixed systems with MEGA-10 at $X_{nonionic} = 0.9$ cannot be well explained in a similar manner. This problem remains unsolved.

In conclusion, two facts have been confirmed in this work: in the pure system of free bile salts, the degree of dissociation of carboxyl group in a micelle is smaller than that in bulk (pK_a^{app} of BA in micellar state in higher than in the single dispersed state), while in the mixed system of free bile salts with nonionic surfactants, the dissociation state of carboxyl group in mixed micelles depends on the species of hydrophilic groups of nonionic surfactants, as well as on the mixing ratio of BS's to nonionic surfactants.

Acknowledgment This material is based on work supported by the Ministry of Education, Culture and Science under Grant No. 05680584, and by funds from Central Research Institute of Fukuoka Univ. and Shionogi Co. Ltd.

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