Composition of sodium taurocholate micellar solutions

The Analyst

Emilio Bottari,* Maria Rosa Festa and Magda Franco

Dipartimento di Chimica, Università "La Sapienza", P. le A. Moro 5, 00185 Rome, Italy

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The composition and range of existence of aggregates formed by sodium taurocholate in aqueous micellar solutions were studied. Electromotive force measurements were used to obtain concentrations of free hydrogen and sodium ions. Experimental data obtained at 25 °C and at three $N(CH_3)_4Cl$ concentrations, used as an ionic medium, can be explained by assuming the presence of aggregates with different compositions depending on the reagent concentrations and the ionic strength. Comparison with taurodeoxycholate shows wide differences. Protonated species of taurocholate are observed only at pH $\leqslant$ 5. At higher pH, the micellar aggregate distribution remains nearly constant at a given ionic medium concentration. As expected, the size of the micellar aggregates increases on increasing the ionic strength. A dimer is found at all concentrations of the ionic medium. All species found have aggregation numbers of anions in multiples of two. The affinity of sodium ions for micellar aggregates is greater than that of $N(CH_3)_4^+$ ions.

Introduction

Bile salts play an important role in many physiological and biological systems, because of their detergent-like and surface-active properties.[1] Sodium cholate (NaC) and deoxycholate (NaDC) and their conjugates with glycine and taurine are present in human bile.[2] Most of these bile salts form molecular aggregates (micelles) in aqueous solution and readily solubilize many water-insoluble compounds, such as cholesterol and lecithin.[3]

The behaviour of some sodium salts of dihydroxycholanic acids such as sodium deoxycholate (NaDC),[4] glycodeoxycholate (NaGDC)[5] and taurodeoxycholate (NaTDC),[6] was studied previously. Experimental data, obtained from electromotive force (e.m.f.) measurements, were explained by assuming the presence of several species with different aggregation numbers and with the presence of species formed with the uptake of protons. For such bile salts and particularly for NaTDC, a trimer, observed at all ionic medium concentrations, seems to constitute the building unit of the micellar aggregates, which in most cases have aggregation numbers which are a multiple of three.[6] A helical model formed by an assembly of trimers, inferred from X-ray diffraction analysis of NaTDC, RbTDC, NaGDC and RbGDC fibres and from quasi-elastic light scattering (QELS) measurements,[7] supported the validity of our results.

Trihydroxycholanic bile salts, such as sodium taurocholate (NaTC), form micellar aggregates which are much smaller than those formed under the same conditions by dihydroxy compounds and generally they show different chemical physical properties.[8]

Many studies have been performed on the structure, size and shape of bile salt micelles and on their aggregation number and critical micellar concentration (c.m.c.).[9,10]

Although many studies have been carried out on the structure of bile salts and many c.m.c. values of their micellar aggregates determined, only a few data have been reported on their properties in aqueous solutions. The knowledge of the composition of bile salt micellar and premicellar solutions is very limited. The composition of the species formed in such solutions, their range of existence, the effect of ionic strength and other parameters have to be explained from many points of view.

The aim of this work was to establish the composition of the species formed in aqueous micellar and premicellar solutions of NaTC and their relative stability. For this purpose, the concentration of the reagents had to be changed over a wide range. The constant ionic medium method proposed by Biedermann and Sillén[11] was adopted in order to minimize the variation of the activity coefficients in spite of the changes in the concentration of the reagents. All experiments were performed at 25 °C using $N(CH_3)_4Cl$ aqueous solutions as an ionic medium. Hereafter, the ionic medium concentration in mol L^{-1} will be indicated as *W*. By adopting the constant ionic medium method, it was possible to substitute concentrations for activities in all calculations.

Previously, research on the behaviour of NaDC, NaGDC and NaTDC in $N(CH_3)_4Cl$ at various concentrations was carried out[4–6] by measuring the e.m.f. of suitable galvanic cells with glass electrodes sensitive to free hydrogen and sodium ion concentrations in micellar solutions. The results of these potentiometric studies agreed with those of a small-angle X-ray scattering study.[12]

The valuable results obtained for NaDC, NaGDC and NaTDC motivated us to undertake a similar study on NaTC in order to find which species $Na_qH_p(TC)_r$ are present in aqueous micellar solutions of NaTC, and to determine the values of *q*, *p* and *r* and the relative range of existence of the various species. As experiments were carried out at three concentrations of the ionic medium, it was possible to evaluate the influence of the ionic medium concentration on the *q*, *p* and *r* values.

The results of this work were compared with those obtained for NaDC, NaGDC and NaTDC under the same experimental conditions.

Experimental

Apparatus

E.m.f. measurements were performed by means of Radiometer (Copenhagen, Denmark) Model pHM4 and pHM64 and Metrohm (Herisau, Switzerland) Model 654 and 605 potentiometers, as explained in the Method section. Glass electrodes from Metrohm (Nos. 6.0102.000 and 6.0501.100 for H^+ and

Na^+ respectively) and Radiometer (Nos. G 202 C and G 502 Na for H^+ and Na^+, respectively) were used. The reference electrode [Ag, AgCl/*W* mol L^{-1} $N(CH_3)_4Cl$ saturated with AgCl] was prepared according to Brown.[13] Details concerning e.m.f. measurements were similar to those described previously.[14]

A stream of nitrogen was bubbled into the solutions during the e.m.f. measurements and all the experiments were carried out at 25.0 ± 0.1 °C.

Reagents

Tetramethylammonium taurocholate [$N(CH_3)_4TC$] was prepared in two independent ways.

In the first, a slight excess of $N(CH_3)_4OH$ was added to taurocholic acid (HTC). This compound, was obtained by diffusion of HCl saturated vapour into a 10% suspension of NaTC in propan-1-ol. The propanolic NaTC suspension and 37% HCl were mixed in a glass box. After 2 weeks, the propanolic suspension, now containing HCl, NaCl and HTC, with a small amount of NaTC, was evaporated in a Rotavapor, to obtain a solid. Since the solubility of NaCl in absolute ethanol was slight, the solid obtained was treated with absolute ethanol and centrifuged. The solid was eliminated, while the ethanolic phase was again evaporated in a Rotavapor. About 50 g of solid HTC were obtained each time. A slight excess of a standard solution of $N(CH_3)_4OH$ (~ 1 mol L^{-1}) was slowly added to the HTC with stirring and under a strong stream of nitrogen. The solution obtained was analysed for its content of Cl^-, Na^+ and OH^-. The residual chloride, determined by argentometric titration according to Mohr, was always < 1%. The Na^+ and OH^- concentrations were determined potentiometrically on very dilute solutions of $N(CH_3)_4TC$ by using glass electrodes for sodium and hydrogen ions and evaluating the equivalence according to Gran.[15] The Na^+ and OH^- contents were taken into account in the preparation of working solutions.

The second method to prepare $N(CH_3)_4TC$, was similar to that described by Norman[16] for NaTC synthesis. Instead of NaOH, $N(CH_3)_4OH$ was added to a mixture of cholic acid, tributylamine, ethyl chorocarbonate and taurine in dioxane. The mixture was evaporated *in vacuo* to a syrup and dissolved in boiling ethanol. After cooling to room temperature, isoamyl acetate was added to the filtered solution to incipient cloudness. Often, no crystals of $N(CH_3)_4TC$ were obtained, but again a syrup. This was solubilised at room temperature in the minimum amount of ethanol. Acetone was added until cloudness occurred. On leaving this mixture at room temperature for about 1 week, white crystals of $N(CH_3)_4TC$ were obtained.

Sodium taurocholate (Sigma, St. Louis, MO, USA) was recrystallized, according to Pope,[17] by adding to an NaTC ethanolic (80%) solution an excess of diethyl ether and keeping this mixture for 3–4 days at 3–4 °C. Most of the solvent remaining in NaTC, obtained from the filtration, was removed by drying under vacuum. The last traces were eliminated by heating in an oven at 70 °C until constant mass was reached.

$N(CH_3)_4TC$ and NaTC were determined by TLC according to Hofmann[18] and no detectable spots of cholate were revealed. Thermogravimetric analysis of the prepared $N(CH_3)_4TC$ showed a profile similar to that of NaTC.

Working solutions of hydrochloric acid, tetramethylammonium chloride, sodium chloride and tetramethylammonium hydroxide were prepared and analysed as previously described.[19]

Method

The reagents sodium, hydrogen and taurocholate ions can form the species $Na_qH_p(TC)_r$. By taking into account the presence of the ionic medium, a more general species is $Na_qH_p(TC)_r[N(CH_3)_4]_xCl_y[H_2O]_z$. However, in the following equilibrium, the former is considered since there are no experimental data on *x*, *y* and *z*:

$$q\ Na + p\ H + r\ TC \rightleftharpoons Na_qH_p(TC)_r \qquad (1)$$

where charges are omitted and $q \geq 1$, $p \geq 0$ and $r \geq 1$. Values of $p > 0$ correspond to protonated species, while values of $p < 0$ correspond to species formed with OH^- uptake.The equilibrium constant, $\beta_{q,p,r}$, is defined according to the relationship

$$c_{Na_qH_p(TC)_r} = \beta_{q,p,r}\, c_{Na}{}^q\, c_H{}^p\, c_{TC}{}^r \qquad (2)$$

In eqn. (2) and subsequent expressions, a small or a capital *c* indicates the free or total concentration, respectively, with the exception of C_H, which refers to the analytical excess of hydrogen ions.

Since equilibrium (1) is very complicated and from preliminary attempts it can be deduced that *q*, *p* and *r* can assume different values, it was necessary to study solutions in which the concentration ratios of sodium and taurocholate ions vary within wide limits and to investigate a large range of hydrogen ion concentrations. However, it was expected from previous studies[9] and more recent work[20] that taurocholate is able to form smaller aggregates than those formed by deoxycholate, glycodeoxycholate and taurodeoxycholate. The measure of only c_H and c_{Na}, together with the knowledge of the analytical concentrations of the reagents, can be sufficient to find the predominant values of *q*, *p* and *r* and the relative constants $\beta_{q,p,r}$.

At equilibrium, c_H and c_{Na} were obtained by measuring the e.m.f. of the following galvanic cells:

(I) (−) R.E./Solution S/G.E. (+)
(II) (−) R.E./Solution S/NaE (+)

where R.E. is a reference electrode and G.E. and NaE are two glass electrodes for hydrogen and sodium ions, respectively. Solution S had the following general composition: C_{Na} mol L^{-1} in Na^+; C_H mol L^{-1} in H^+; C_{TC} mol L^{-1} in TC^-; $(W - C_{Na} - C_H)$ mol L^{-1} in $N(CH_3)_4{}^+$; and $(W - C_{TC})$ mol L^{-1} in Cl^-. In this investigation, experiments were carried out at W = 0.100, 0.500 and 0.800 mol L^{-1}.

At 25 °C and in mV units, the e.m.f. of cells I and II can be expressed as follows:

$$E_I = E^\circ_I + 59.16 \log c_H + E_j$$
$$E_{II} = E^\circ_{II} + X \log c_{Na} + E_j$$

The constants E°_I and E°_{II} were determined when $C_H = c_H$ and $C_{Na} = c_{Na}$, at the beginning of each series of measurements.

For $-\log c_H \leq 4$, the NaE response was not only dependent on c_{Na}, but was also influenced by c_H. However, the E°_{II} values were different at different $-\log c_H$ but, as the e.m.f. measurements were performed at constant $-\log c_H$, they were constant during each series of measurements. On this basis, it can be assumed that the obtained c_{Na} values are correct, without loss of accuracy.

The liquid junction potential is $E_j = -jc_H$, where *j*, expressed in mV L mol^{-1}, depends on the ionic medium. Since E_j depends only on c_H, it is negligible in the range $4 \leq -\log c_H \leq 11$. *X* shows the dependence of E_{II} on c_{Na}. It is fairly close to the theoretical value (59.16 mV), but it changes with the value of *W*. At W = 0.100 mol L^{-1}, *X* was 59.2 mV, whereas it progressively decreased to 57.5 mV at W = 0.800 mol L^{-1}. From the knowledge of E°_I and E°_{II} and the measurement of E_I and E_{II}, c_H and c_{Na} could be obtained for each point.

After the determination of E°_I, E°_{II} and E_j, two different approaches were carried out. In the first, E_{II} was measured in solutions where C_{TC} was gradually increased by keeping C_{Na} as low as possible and $-\log c_H$ constant at a selected value. In the second approach, E_{II} was measured in solutions where C_{Na} was gradually increased by keeping constant both c_{TC} and $-\log c_H$.

Measurements were interrupted when C_{Na} reached approximately c_{TC}.

In Table 1 the values of c_{TC} used for each concentration of ionic medium, W, are indicated by +.

Results and discussion

The e.m.f. measurements provide c_{Na} and c_H for the studied solutions.

From the material balance of sodium and hydrogen ions, by taking into account the mass action law, we can write:

$$C_{Na} = c_{Na} + \Sigma\Sigma\Sigma\, q\, \beta_{q,p,r}\, c_{Na}^{q}\, c_H^{p}\, c_{TC}^{r} \quad (4)$$

$$C_H = c_H + \Sigma\Sigma\Sigma\, p\, \beta_{q,p,r}\, c_{Na}^{q}\, c_H^{p}\, c_{TC}^{r} \quad (5)$$

where the sums are over q, p and r. In eqn. (5), the protonation of taurocholate ions is neglected on the basis of the investigated range of $-\log c_H$, and of preliminary measurements, carried out in the absence of sodium ions and under the same experimental conditions.

From a preliminary inspection of the experimental data, it was evident that q, p and r could assume different series of values and that the aggregation numbers of the micellar aggregates increase on increasing the concentration of the reagents and the ionic medium.

The data corresponding to each concentration of $N(CH_3)_4Cl$ were manipulated in order to obtain the composition and the distribution of the micellar aggregates as a function of pH and bile salt concentration.[21]

The minimum number of species necessary to fit the experimental data satisfactorily was determined and are reported later (see Table 3 and Figs. 3–5). Species with a negligible percentage (< 1%) were omitted.

It seems useful to describe briefly the first stage of the manipulation of the experimental data for the discussion of the results. As an example, the data corresponding to W = 0.100 mol L^{-1} within the range $C_{Na} \leq 0.01$ mol L^{-1} are processed starting from eqn. (4).

On the basis of previous studies relating to the aggregates of NaDC, NaGDC and NaTDC, the hypothesis can be formulated that, in a wide range of $-\log c_H$, the function $\eta = \log (C_{Na}/c_{Na})$ was independent of c_H. To verify this hypothesis, several series of measurements were performed according to the first approach, for a wide range of hydrogen ion concentrations. In Table 2 the values of $-\log c_H$ studied for each concentration of ionic medium are indicated by +. From the measurement of E_{II} the values of c_{Na} and then η could be obtained for each point. Values of c_{TC} were obtained as described below. Plots of η *versus* $-\log c_{TC}$, at constant values of c_H, showed that the points were independent of the hydrogen ion concentration above a determined limit of $-\log c_H$, and deviations were evident at lower $-\log c_H$. The limit depended on W. An asterisk in Table 2 indicates the begining of the deviations from the normal trend for the participation of protons in the formation of aggregates. In Fig. 1, examples of plots of η *versus* $-\log c_{TC}$ are shown for W = 0.100 mol L^{-1} at three different $-\log c_H$ values. Points obtained in the range $11 \leq -\log c_H \leq 7$ fall on the same curve, whereas those relative to $-\log c_H \leq 4$ deviate from that curve.

From the inspection of such plots, it can be deduced that species formed with the assumption that OH^- ions are not present in appreciable amounts (*i.e.* $p \geq 0$). As shown in Table 2, η is independent of $-\log c_H$ over a wide range where eqn. (4) can be written as follows:

$$C_{Na} = c_{Na} + \Sigma\Sigma\, q\beta_{q,p,r}\, c_{Na}^{q}\, c_{TC}^{r} \quad (6)$$

Eqn. (6) shows that η still depends on c_{Na} and c_{TC} and to process the experimental data, the c_{TC} values for each point are necessary. These values can be calculated from the material balance of taurocholate:

$$C_{TC} = c_{TC} + \Sigma\Sigma\, r\, \beta_{q,p,r}\, c_{Na}^{q}\, c_{TC}^{r} \quad (7)$$

where the mass action law was taken into account. As the last term of eqn. (7) is not known *a priori*, first approximate values of c_{TC} were calculated as follows. Since the aggregation number r depends on the ionic medium concentration and in the case of NaTC it is expected that r does not assume high values, approximations to eqn. (7) were applied in different ways for different W. The c_{TC} values used for each W are reported in Table 1.

***W* = 0.100 mol L^{-1} data.** In such solutions, it is assumed that the dimer is the predominant species (r = 2) and with this hypothesis, the c_{TC} values for each point can be calculated with the following equation:

$$c_{TC} = C_{TC} - 2\,(C_{Na} - c_{Na}) \quad (8)$$

As will be verified below, the approximation introduced in eqn. (8) does not involve a loss of accuracy, because the refined $-\log c_{TC}$ and the first approximation values agree to within ±0.01.

To investigate the dependence of η on c_{TC} and c_H, e.m.f. measurements of E_{II} were performed by keeping E_I and $-\log c_{TC}$ constant and by gradually increasing C_{Na}. With $-\log c_H$ and $-\log c_{TC}$ constant, eqn. (6) can be written as follows:

$$\varphi = 10^{\eta'} - 1 = \Sigma q\gamma_q\, c_{Na}^{q-1} \quad (9)$$

where η' indicates η at c_H and c_{TC} constant and γ_q is a conditional constant depending on c_{TC} according to the equation $\gamma_q = \Sigma\, \beta_{q,0,r}\, c_{TC}^{r}$

Table 1 W and c_{TC} values of the investigated solutions

	$c_{TC} \times 10^3$/mol L^{-1}										
W/mol L^{-1}	5	10	15	20	25	30	35	40	50	65	80
0.100	+	+	+	+	+						
0.500	+	+		+	+	+	+	+	+		
0.800	+	+		+	+	+	+	+	+	+	+

Table 2 W and $-\log c_H$ values of the investigated solutions

	$-\log (c_H$/mol $L^{-1})$											
W/mol L^{-1}	11	10	9	8	7	6	5.5	5	4.5	4	3.5	3
0.100	+	+	+	+	+	+	+	*	*	*	*	*
0.500	+	+	+	+	+	+	*	*	*	*	*	*
0.800	+	+	+	+	+	+	*	*	*	*	*	*

Eqn. (9) was applied in the range $5.5 \leq -\log c_H \leq 11$, where the absence of protonated species was already verified, then $p = 0$. As described below, at $-\log c_H \leq 5$, the participation of protons must be considered. In the range $p = 0$ and c_{TC} = constant, eqn. (9) can give information on q and γ_q.

By plotting φ *versus* c_{Na}, the experimental points fall on a straight line over the whole ranges $5.5 \leq -\log c_H \leq 11$ and $0.005 \leq c_{TC} \leq 0.025$ mol L^{-1}. In these ranges, q can assume the values of 1 and 2. By introducing $q = 1$ and 2 in eqn. (9), it can be deduced that only species with one or two sodium ions per aggregate are formed. From the extrapolation and from the slope, γ_1 and γ_2, respectively, can be obtained. Fig. 2 shows an example of such plots, for $-\log c_H = 7$ and $c_{TC} = 0.010$ mol L^{-1}.

Thus, the γ_1 and γ_2 dependence on $-\log c_{TC}$, studied for the first time by means of normalised curve methods,[22] shows that r can assume values of 2 and 4 and then the presence of the species $Na(TC)_2$, $Na(TC)_4$, $Na_2(TC)_2$, was found. By taking into account that $\gamma_1 = \Sigma \beta_{1,0,r} c_{TC}^r$ and that $\gamma_2 = \Sigma\beta_{1,0,r} c_{TC}^r$, the functions $\log(\gamma_1 c_{TC}^{-2})$ and $\log(\gamma_2 c_{TC}^{-4})$ were plotted *versus* $-\log c_{TC}$. The former fit with a normalised curve[22] of the equation $Y = \log (1 + \alpha u + u^2)$, while the latter gave constant values independent of $-\log c_{TC}$. The assumption of the presence of the above species explains well the data obtained in the range $5.5 \leq -\log c_H \leq 11$. Data obtained in a more acid range ($-\log c_H \leq 5.5$) can be explained by assuming also the presence of species formed with uptake of protons. It was found that p can assume values of 1 and 2. In such a way all the data obtained at $W = 0.100$ mol L^{-1} can be explained by assuming the following species: $Na(TC)_2$, $NaH(TC)_2$, $Na(TC)_4$, $NaH(TC)_4$, $NaH_2(TC)_4$, $Na_2(TC)_2$ and $Na_2H_2(TC)_2$.

The experimental data for C_{Na}, C_{TC}, C_H, c_{Na}, c_{TC} and c_H were also treated in an independent way by means of the computer program BSTAC[23] and they were explained by assuming the same species as found by graphical methods. The relative $\beta q,p,r$ were close to those calculated by graphical methods, within the experimental errors. The observed species and relative constants are given in Table 3.

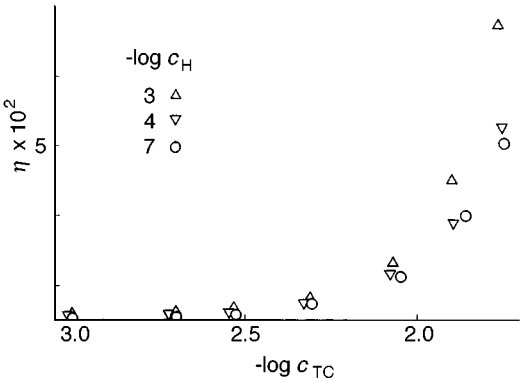

Fig. 1 Trend of η *versus* $-\log c_{TC}$ at different $-\log c_H$. Deviations are evident at $-\log c_H \leq 4$.

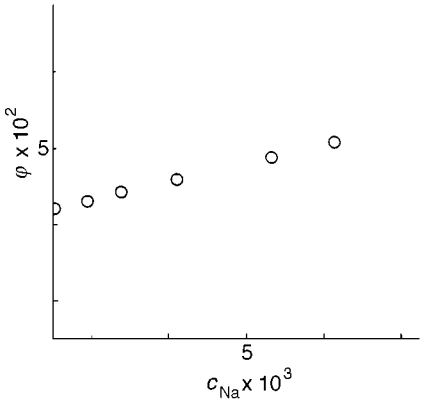

Fig. 2 The dependence of φ on c_{Na} at $-\log c_H = 7$ and $-\log c_{TC} = 2.00$. The trend of the points, well approximated with a straight line, can be explained by assuming the existence of monomeric and dimeric species.

Refined values of $-\log c_{TC}$ and the first approximate values agree to within ±0.01. By means of first approximate values of $\beta_{q,p,r}$ and eqn. (7), more accurate $-\log c_{TC}$ values could be calculated. By using an iterative procedure refined values of $-\log c_{TC}$ were obtained.

***W* = 0.500 mol L^{-1} data.** The procedure adopted for the elaboration of the data was similar to that applied for $W = 0.100$ mol L^{-1}. Some differences exist, because the range where protonated species are not found is more limited ($5 \leq -\log c_H \leq 11$), the range of the taurocholate concentration is larger ($0.005 \leq c_{TC} \leq 0.050$ mol L^{-1}) and the $-\log c_{TC}$ values were calculated with different approximations. For this purpose, it was reasonable to take into account the results obtained at $W = 0.100$ mol L^{-1} and to consider the species found. By taking into account that at $W = 0.100$ mol L^{-1} the presence of a tetramer ($r = 4$) was already found, the hypothesis of its presence also at $W = 0.500$ mol L^{-1} was formulated, so that free concentration of taurocolate in the range $5 \leq -\log c_H \leq 11$ was calculated by the following expression:

$$c_{TC} = C_{TC} - 2\,\beta_{1,0,2}\,c_{Na}\,c_{TC}^2 - 4\,\beta_{1,0,4}\,c_{Na}\,c_{TC}^4 - 2\,\beta_{2,0,2}\,c_{Na}^2\,c_{TC}^2 - 4\,(C_{Na} - c_{Na}) \quad (10)$$

The first approximate values of c_{TC} in the range where the formation of protonated species takes place are obtained by considering the presence of the protonated species found at $W = 0.100$ mol L^{-1}. Data treatment was carried out in a similar way to that described above, by graphical methods and by using the program BSTAC.[23] Sets of assumed species with the relative $\beta_{q,p,r}$ values are given in Table 3. Also for $W = 0.500$ mol L^{-1} refined values of $-\log c_{TC}$ and first approximate values, calculated by means of eqn. (10), agree within ±0.02.

***W* = 0.800 mol L^{-1} data.** The procedure for data treatment was similar to that applied for $W = 0.500$ mol L^{-1}. The different ranged where the participation of the protons take place was taken into account and the c_{TC} values were calculated as follows:

$$c_{TC} = C_{TC} - (\Sigma\Sigma\, r\,\beta_{q,p,r}\,c_{Na}^q\,c_{TC}^r)_{W\,=\,0.500} - 8\,(C_{Na} - c_{Na}) \quad (11)$$

Graphical and computer treatment similar to that above described gave the species given in Table 3 with the relative constants.

Refined $-\log c_{TC}$ values and first approximate values, calculated from eqn. (11), gave results in agreement to within ±0.03.

Table 3 Proposed values for the constants of the species $Na_qH_p(TC)_r$ at 25 °C and different *W*

W/mol L^{-1}	Species (log $\beta_{q,p,r}$)
0.100	$Na(TC)_2$ (2.51 ± 0.05); $Na(TC)_4$ (5.18 ± 0.10); $Na_2(TC)_2$ (4.07 ± 0.07); $NaH(TC)_2$ (5.1 ± 0.1); $NaH(TC)_4$ (8.7 ± 0.12); $NaH_2(TC)_4$ (11.4 ± 0.2); $Na_2H_2(TC)_4$ (13.1 ± 0.15)
0.500	$Na_2(TC)_2$ (5.15 ± 0.1); $Na_3(TC)_4$ (10.95 ± 0.15); $Na_4(TC)_6$ (16.2 ± 0.2); $Na_5(TC)_8$ (21.6 ± 0.25); $Na_2H(TC)_4$ (11.6 ± 0.2); $Na_4H_2(TC)_8$ (25.8 ± 0.5); $Na_5H(TC)_8$ (24.7 ± 0.3); $Na_5H_2(TC)_8$ (27.4 ± 0.5)
0.800	$Na_2(TC)_2$ (5.4 ± 0.1); $Na_3(TC)_4$ (10.55 ± 0.15); $Na_5(TC)_8$ (22.7 ± 0.25); $Na_8(TC)_{12}$ (35.6 ± 0.3); $Na_{10}(TC)_{16}$ (47.7 ± 0.4); $Na_{15}(TC)_{24}$ (72.1 ± 0.6); $Na_2H(TC)_4$ (12.5 ± 0.3); $Na_5H(TC)_8$ (25.8 ± 1); $Na_8H(TC)_{16}$(47 ± 1); $Na_{12}H(TC)_{24}$ (71 ± 2); $Na_{13}H_2(TC)_{24}$ (76 ± 3)

As expected, the aggregation numbers of the micellar aggregates increase on increasing the concentration of ionic medium. For instance, the maximum value of r is 4 and 24 at W = 0.100 and 0.800 mol L^{-1}, respectively.

Inspection of Table 3 shows that r for all the species is an even number. TC, $(TC)_2$ and $(TC)_4$ are present for W = 0.100, 0.500 and 0.800 mol L^{-1}, whereas at W = 0.800 mol L^{-1}, r can assume also the values 8, 12, 16 and 24. The percentage of free TC^- decreases with increasing W.

The ratio q/r between sodium and taurocholate ions shows evident regularity. Most of the proposed species for all W could be written in a different way, $Na_m[Na(TC)_2]_n$, where m and n can assume different values, $m \geq 0$ and $n \geq 0$. The single compound not corresponding to this formula is $Na(TC)_4$ and also some protonated species. Inspection of the proposed species shows that m can be 0 or 1 for W = 0.100 and 0.500 mol L^{-1}, while it assumes higher values (up to 3) for W = 0.800 mol L^{-1}. Also, the n values increase with increasing W, reaching a maximum value (12) at W = 0.800 mol L^{-1}. The assumption of the formula $Na_m[Na(TC)_2]_n$ suggests that the aggregates can be formed by assembling units of $[Na(TC)_2]$. The number of $[Na(TC)_2]$, n, increases on increasing the concentration of NaTC or that of the ionic medium.

It must be stressed that the difference $C_{Na} - c_{Na}$ is assumed as the concentration of the counterions bound to the micellar aggregates. Therefore, the q and p values of the species $Na_qH_p(TC)_r$, reported in Table 3, are given on the basis of this assumption.

Distribution curves of the found species, calculated for W = 0.100, 0.500 and 0.800 mol L^{-1}, are plotted as a function of $-\log c_H$ in Figs. 3–5, where the percentage of each species corresponds to the distance between the two curves which define the range of existence of the species.

Protonated species are present in all the distribution plots, but they reach an appreciable percentage only at $-\log c_H \leq 5$. The narrow $-\log c_H$ range causes a decrease in the accuracy of the corresponding $\beta_{q,p,r}$ values.

Except for $Na_2(TC)_2$ and some protonated species, the composition of the species shows that $r > (q + p)$ and the difference $r - q$ increases in the largest aggregates by increasing the concentration of $N(CH_3)_4Cl$. To account for this finding, it seems reasonable to assume that tetramethylammonium ions also participate in the formation of the aggregates, although no information on their role is available.

Supposing that $(r - q - p)N(CH_3)_4^+$ ions are bound to the micelle, since q is nearly always greater than $r - q - p$ in spite of the excess of $N(CH_3)_4^+$ ions, the free energy gain for the reversible transfer of an Na^+ ion from the bulk solution to the micelle is greater than that of an $N(CH_3)_4^+$ ion.

From the data in Table 3 and Figs. 3–5, it is evident that species with an odd number of aggregations are not observed. This is a substantial difference with respect to the results obtained for dihydroxycholanic bile salts, such as sodium deoxycholate, glycodeoxycholate and taurodeoxycholate.

A similar investigation carried out on NaTDC suggested that a trimer constitutes the building unit of the aggregates of NaTDC, because the species $Na_3(TDC)_3$ was present for all the concentrations of bile salt and ionic medium studied. Furthermore, most of the observed aggregates had an aggregation number that was a multiple of three.[6] The fact that species with r values that are a multiple of three predominated agreed well with the results obtained in a study of fibres of NaGDC, RbGDC, NaTDC and RbTDC[7] by X-ray diffraction analysis. The distributions of the diffracted intensities and the unit cell parameters of these salts are almost identical. The experimental data could be explained satisfactorily by assuming a 7/1 helix formed by trimers. Moreover, e.m.f. measurements on NaTDC solutions with a constant ionic medium provided the distribution of micellar sizes, which allowed the calculation of mean hydrodynamic radii using the helical model. Their values were in good agreement with those measured by QELS over a wide range of ionic strength.[7]

As expected, taurocholate has less affinity than taurodeoxycholate for protons. The number of protons involved in the formation of the aggregates in the acid range is similar for both TC and TDC, but the protonation starts at higher pH for TDC

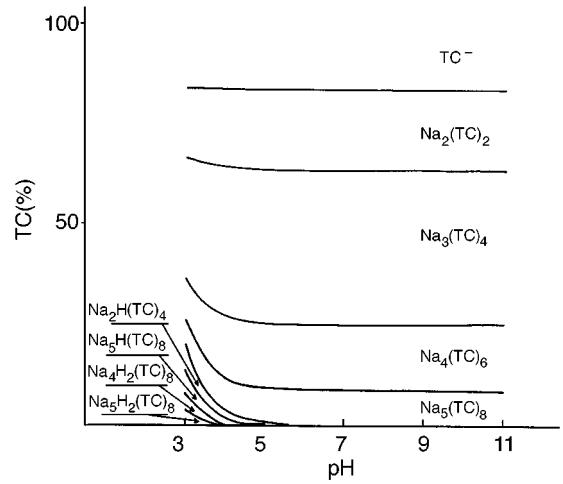

Fig. 4 Distribution curves of the observed species as a function of $-\log c_H$ for W = 0.500 mol L^{-1}. Bile salt concentration, 0.05 mol L^{-1}.

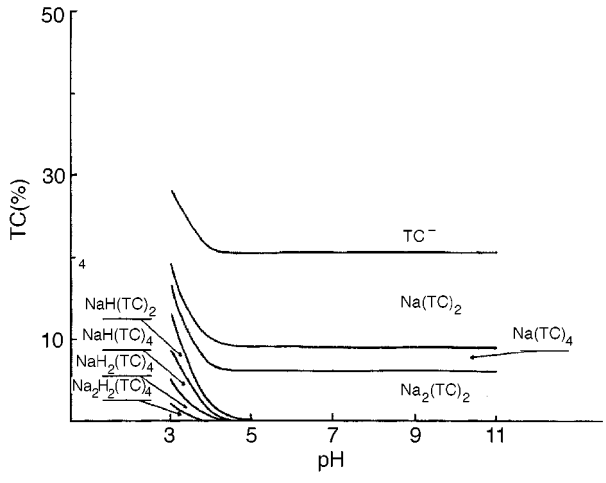

Fig. 3 Distribution curves of the observed species as a function of $-\log c_H$ for W = 0.100 mol L^{-1}. Bile salt concentration, 0.02 mol L^{-1}.

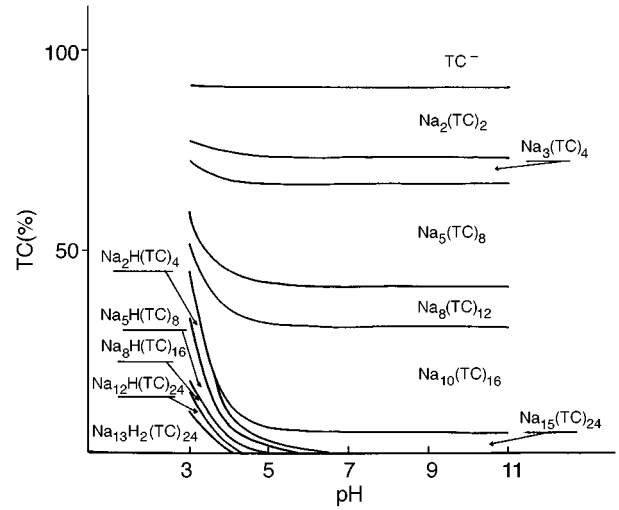

Fig. 5 Distribution curves of the observed species as a function of $-\log c_H$ for W = 0.800 mol L^{-1}. Bile salt concentration, 0.08 mol L^{-1}.

(7.5) than for TC (5.5). Species with $p = 1, 2, \ldots, 8$ are formed in the cases of both taurodeoxycholate and taurocholate. The monoprotonated species is present for both. In particular, from Table 3 and Fig. 5, it can be seen that the presence of protonated species takes place at pH 5, but it increases strongly on decreasing the pH. On going from pH 4 to 3, the level of $Na_2H(TC)_4$ increases from a few per cents to more than 10%, that of $Na_5H(TC)_8$ to more than 15% and that of $Na_{13}H_2(TC)_{24}$ to about 10%.

The fact that in NaTC solutions species with r values that are a multiple of two predominate agreed well also in this case with the results obtained in a study of fibres and crystals of NaTC by X-ray diffraction analysis[24] and QELS and CD.[25]

X-ray diffraction analysis of monoclinic crystals showed the existence of two structural units both constituted by dimers. These models were confirmed by means of QELS measurements performed on aqueous solutions of NaTC as a function of the ionic strength. The hydrodynamic radii calculated for different aggregation numbers on the basis of the geometry of the models agree well with those measured experimentally.[24] CD data obtained from aqueous solutions of NaTC containing bilirubin-IXα confirm the chirality of the models, because an enantioselective complexation for the left-handed or right-handed enantiomer of bilirubin, depending on pH, is observed.[24]

Conclusion

This investigation of aqueous solutions of NaTC permited the determination of the species and their stability constants, as given in Table 3, for different concentrations of $N(CH_3)_4Cl$ at 25 °C.

The measurement of the parameters c_{Na} and c_H of equilibrium (1) is a procedure which ensures high accuracy and provides experimental data suitable for determination of the prevailing values of q, p and r, not reaching high values.

The sets of Table 3, obtained by assuming the minimum number of species with the lowest values of q, p and r, represent the distribution of the aggregates necessary to fit the data satisfactorily. Owing to the accuracy of the measurements, it is reasonable to attribute less confidence to the composition of species with high aggregation numbers and to species involving protons.

A dimeric species, observed in all the samples, seems to constitute the building unit of the micellar aggregates, which in all the cases have aggregation numbers that are a multiple of two.

The high values of q, even in the presence of a large excess of $N(CH_3)_4^+$ ions, indicate an affinity of Na^+ ions for the micellar aggregates greater than that of $N(CH_3)_4^+$ ions. This means that micellar aggregates containing Na^+ ions are preferentially formed compared with those containing $N(CH_3)_4^+$ only.

This work confirms that the behaviour of sodium taurocholate is very different from that of sodium taurodeoxycholate. The former, both in solution and in fibres, forms aggregates with a dimer as the constituent of the building unit, whereas the latter forms aggregates with a trimer as the constituent of the building unit. Furthermore, assemblies of four units exist under conditions which favour aggregates of large size in pre-fibre states.

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