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On the Composition of Sodium Glycodeoxycholate Micellar Solutions

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Summary. The behaviour of sodium glycodeoxycholate solutions at 25 °C, in different concentrations of $N(CH_3)_4Cl$ as ionic medium, was investigated by performing electromotive force measurements of galvanic cells containing electrodes reversible to sodium, glycodeoxycholate (*GDC*) and hydrogen ions Experimental data were explained by assuming the formation of species of the kind $Na_qH_p(GDC)_n$, where $q \geqslant 1$, $p \geqslant 0$, $n \geqslant 1$, and the relative constants were determined, as well. The values of *q*, *p* and *n* increase by increasing the concentration of the ionic medium. A comparison with the composition of solutions of sodium deoxycholate in the same experimental conditions was proposed and discussed.

Keywords. Bile salts; Glycodeoxycholate; Deoxycholate; Micelles.

Über die Zusammensetzung von micellaren Lösungen von Natrium-Glycodeoxycholat

Zusammenfassung. Das Verhalten von Natrium-Glycodesoxycholat-(Na*GDC*)-Lösungen wurde bei 25 °C in verschiedenen Konzentrationen von $N(CH_3)_4Cl$ als ionischem Milieu untersucht. Die elektromotorische Kraft (EMK) wurde mit Elektroden für Natrium, Glycodesoxycholat (*GDC*) und Wasserstoff-Ionen gemessen. Die experimentellen Daten wurden durch die Annahme der Bildung von Aggregaten des Typs $Na_qH_p(GDC)_n$ erklärt, wobei $q \geqslant 1$, $p \geqslant 0$ und $n \geqslant 1$. Die entsprechenden Konstanten wurden bestimmt. Die Werte *q*, *p*, *n* steigen mit der Konzentration des ionischen Milieus an. Ein Vergleich zwischen der Zusammensetzung der Lösungen von Na*GDC* und Natrium-Desoxycholat unter denselben experimentellen Bedingungen wird angeführt und diskutiert.

Introduction

The common naturally occurring bile acids are C_{24} saturated acids and belong to the series of cholanic acids. Their sodium salts play an important role in many physiological and biological systems, due to their detergent-like and surface active properties. Among them the sodium deoxycholate free or conjugated with glycine or taurine is present in the bile of man [1].

Bile salts can form molecular aggregates (micelles) in aqueous solutions that are capable of solubilizing many water-insoluble compounds. Many studies have been performed using different methods of investigation to indicate molecular association in bile salt solutions and to determine c.m.c. (critical micellar concentration) [2–8].

The mass action model develops from the application of the mass action law to the overall aggregation process: $m M \Leftrightarrow M_m$ connected with an association constant of the global process. In opposition with this mechanism, Mukerjee and Cardinal [9], Djanvanbakht et al. [10] and Norman [11] by studying self-association of sodium cholate (NaC), sodium deoxycholate (Na*DC*) and their taurine and glycine conjugates explained their experimental data by means of a stepwise association process and the assumption of the existence of a complex pattern of dimers and one or more higher oligomers.

O'Connor et al. [12] suggested that the real interpretation lies somewhere between the two extremes and that the aggregation is very sensitive to the ionic strength, *pH* and the nature of the bile salt.

Although many values exist for the c.m.c. of the bile salts depending often on the used techniques for their determination, few data are reported on their behaviour in aqueous solutions and analytical properties in solution [2]. The knowledge of the composition of bile salt micelles, their stability and the influence of the ionic medium and of other thermodynamic parameters are essential to explain their behaviour.

Previously, investigations on the behaviour of Na*DC* in different ionic media such as $N(but)_4Cl$, $N(CH_3)_4Cl$ and NaCl at different concentration were carried out [13–15], by measuring the electromotive force (e.m.f.) of suitable galvanic cells containing glass electrodes able to supply free sodium and hydrogen ions in micellar solutions. Recently, a study of deoxycholate micellar solutions as a function of the ionic medium concentration was performed by measuring the e.m.f. of galvanic cells containing, together with two glass electrodes (for Na^+ and H^+, respectively), a deoxycholate electrode able to furnish the deoxycholate ion (DC^-) free in micellar solutions [16]. The results obtained in Ref. [16] were good and agreed with those obtained in a SAXS (small-angle X-ray scattering) study [17] so that we were induced to carry out a similar investigation on aqueous micellar solutions of sodium glycodeoxycholate (Na*GDC*). A potentiometric electrode able to give the free concentration of GDC^- ions was developed as Pb, $Pb(GDC)_2/GDC^-$ and tested with success as a function of concentration of glycodeoxycholate [18].

The aim of this work is to study the composition of the species $\{Na_qH_p(GDC)_n\}$ present in aqueous micellar solutions of glycodeoxycholate, to determine the prevailing values assumed by q, p and n, the relative stability and the range of existence of the found species.

Since for these purposes it was necessary to vary the concentration of the reagents within a wide range, the constant ionic medium method proposed by Biedermann and Sillén [19] was adopted so that it was possible to assume that the activity coefficients remained constant in spite of the variation of the concentration of the reagents. In this connection it was necessary to establish how changes in the ionic medium concentration could affect q, p and n and then the formation of aggregates between glycodeoxycholate, sodium and hydrogen ions was investigated at 25 °C and in 0.100, 0.200, 0.300, 0.400, 0.500, 0.600 and 0.750 *M* $N(CH_3)_4Cl$. The results will be discussed and compared with those relative to Na*DC*.

Method

In this investigation we consider glycodeoxycholate (GDC), sodium and hydrogen ions as three independent reagents which react according to the following general equilibrium,

$$q\mathrm{Na}^{+} + pH^{+} + nGDC^{-} \Leftrightarrow \mathrm{Na}_q\mathrm{H}_p(GDC)_n, \qquad (1)$$

where $q \geqslant 1$, $p \geqslant 0$, $n \geqslant 1$. Generally charges are omitted. By applying the method of constant ionic medium [19], it was possible to substitute the concentrations to the activities so that the constant of equilibrium (1) can be expressed as $\beta_{q,p,n} = [\mathrm{Na}_q\mathrm{H}_p(GDC)_n]b^{-q}h^{-p}a^{-n}$, where b, h and a represent the free concentration of sodium, hydrogen and glycodeoxycholate ions, respectively. The values of b, h and a for each point were obtained by measuring the e.m.f. of the following cells:

$$(-)\ \mathrm{R.E./Solution\ S/G.E.}\ (+), \qquad \mathrm{(I)}$$

$$(-)\ \mathrm{R.E./Solution\ S/Na\ E.}\ (+), \qquad \mathrm{(II)}$$

$$(-)\ GDC\ \mathrm{E./Solution\ S/R.E.}\ (+), \qquad \mathrm{(III)}$$

where G.E. is the glass electrode, Na E. is the sodium electrode, GDC E. is the electrode Pb, $\mathrm{Pb}(GDC)_2/GDC$ built as described previously [18] and R.E. is the reference electrode Ag, $\mathrm{AgCl}/XM\ \mathrm{N(CH_3)_4Cl}$ saturated with $\mathrm{AgCl}/XM\ \mathrm{N(CH_3)_4Cl}$, being $X = 0.100$, 0.200, 0.300, 0.400, 0.500, 0.600 and 0.750 M in connection with the variable ionic medium concentration. Solutions S, saturated with lead(II) glycodeoxycholate, $\mathrm{Pb}(GDC)_2\cdot(1/2)\mathrm{H_2O}$, had the following general composition: BM in Na^+, HM in H^+, $(X\text{-}H\text{-}B)\ M$ in $\mathrm{N(CH_3)_4^+}$, AM in GDC^-, $(X\text{-}A)\ M$ in Cl^- where X has the same meaning as before, B and A indicate the total concentration of sodium and glycodeoxycholate ions, respectively, and H represents the analytical excess of hydrogen ions.

The analytical values of B and A combined with the values of h, b and a obtained as previously described [18] from cells (I), (II) and (III) constitute the basis for the evaluation of q, p, n and $\beta_{q,p,n}$ of the prevailing species.

A second approach was used as a rough test to confirm the results obtained from the above described one. The solubility (S) of $\mathrm{Pb}(GDC)_2$ at 25 °C and in different concentration of ionic medium was determined as a function of sodium ion concentration and compared with that estimated in the absence of sodium ions [18]. Values of S were determined by DP50 pulse polarography on solutions of the following general composition: BM in Na^+, AM in GDC^-, HM in H^+, $(X\text{-}B\text{-}H)\ M$ in $\mathrm{N(CH_3)_4^+}$, $(X\text{-}A)M$ in Cl^- equilibrated with solid $\mathrm{Pb}(GDC)_2(1/2)\mathrm{H_2O}$. Such values together with b, h and a measured by direct potentiometry [20] were used to confirm the results of the main approach.

Experimental

All reagents were prepared and analysed as previously described [18]. All measurements were carried out at 25 °C and the experimental apparatus was similar to that already described [18]. The response of sodium, glass and lead electrode (Metrohm, Switzerland) was checked as before described [18]. E.m.f. measurements expressed as $E - E^\circ$ of cells (I), (II) and (III) could be considered reproducible within ±0.3 mV in the main approach, whereas their reproducibility could be considered within ±0.5 mV in the direct measurements.

Results

Solutions at 25 °C and 0.100, 0.200, 0.300, 0.400, 0.500, 0.600, and 0.750 *M* in $N(CH_3)_4Cl$ as ionic medium were investigated. More concentrated ionic media could not be used because we were not able to obtain reliable and reproducible data, since it was hard to reach a real equilibrium in a reasonable time (~5 h).

For each ionic medium concentration, measurements were performed in the range $7 \leqslant -\log h \leqslant 10$ and for each selected $-\log h$ value *B* and *A* were increased stepwise from 0.5×10^{-3} *M* to different concentrations depending on the concentration of the ionic medium. The reached A_{max} and B_{max} for each concentration of ionic medium are collected in Table 1. Higher *A* and *B* produced no stable e.m.f. values within ~5 h at least in one of the cells (I), (II) or (III). Probably the slow drift of e.m.f. values depend on a structural change in the tested solutions. Micellar solutions turn into gel that cannot more be considered a real solution.

From the material balance of *B*, by taking into account the mass action law, the following equation can be written,

$$B = b + \Sigma_q \Sigma_p \Sigma_n q\, \beta_{q,p,n} b^q h^p a^n, \tag{2}$$

where $q \geqslant 1$, $p \geqslant 0$ and $n \geqslant 1$.

In the following the protonation of GDC^- can be neglected at $-\log h \geqslant 7$, on the basis of the value of its protonation constant [21]. Moreover, it is important to remember that since e.m.f. measurements of cell (III) involve the use of the elctrode Pb, $Pb(GDC)_2/GDC^-$ (according to Ref. [18]), it was proved that, in the studied concentration range of the reagents, hydrolytic species of lead(II) could be neglected by taking into account the results obtained by Carell and Olin [22].

Equation (2) represents the basis for the elaboration of the data to find the prevailing values assumed by *q*, *p*, *n* and the corresponding constants $\beta_{q,p,n}$.

Since measurements were carried out at selected $-\log h$, the resulting data were treated as described previously [16], while the refined values of the constants were obtained by using the program ECORM [23], written for a personal computer.

Table 1. Survey of used A_{max} and B_{max} values for Na*GDC* and Na*DC* [16] with regard to the different ionic medium concentrations

Ionic medium *XM*	Na*GDC* $A_{max} = B_{max}(M)$	Na*DC*	
		$A_{max}\,M$	$B_{max}\,M$
0.100	0.015	0.020	0.010
0.200	0.030	0.040	0.025
0.300	0.040	0.060	0.040
0.400	0.050	0.080	0.060
0.500	0.050	0.100	0.060
0.600	0.050	0.060	0.040
0.750	0.025	–	–

Table 2. Proposed values for the aggregation constants of the species $\mathrm{Na}_q\mathrm{H}_p(GDC)_n$ at 25 °C and at different concentration (XM) of the ionic medium $\mathrm{N(CH_3)_4Cl}$

Ionic medium XM	Species ($\log \beta_{q,p,n}$)
0.100	$\mathrm{Na}GDC$ (2.56 ± 0.08); $\mathrm{NaH}(GDC)_2$ (11.3 ± 0.1); $\mathrm{Na}_3(GDC)_3$ (12.85 ± 0.15); $\mathrm{Na}_3(GDC)_5$ (17.8 ± 0.1); $\mathrm{Na}_3\mathrm{H}_2(GDC)_7$ (37.7 ± 0.2); $\mathrm{Na}_3\mathrm{H}_3(GDC)_8$ (47.8 ± 0.3); $\mathrm{Na}_4(GDC)_9$ (32.1 ± 0.15)
0.200	$\mathrm{Na}GDC$ (1.95 ± 0.2); $\mathrm{NaH}(GDC)_2$ (11.5 ± 0.1); $\mathrm{Na}_2(GDC)_3$ (9.0 ± 0.15); $\mathrm{Na}_3(GDC)_3$ (12.2 ± 0.1); $\mathrm{Na}_4\mathrm{H}_4(GDC)_{10}$ (62.5 ± 0.3); $\mathrm{Na}_7(GDC)_{10}$ (40.7 ± 0.3); $\mathrm{Na}_7(GDC)_{13}$ (49.1 ± 0.8)
0.300	$\mathrm{Na}GDC$ (2.1 ± 0.1); $\mathrm{NaH}(GDC)_2$ (12.2 ± 0.1); $\mathrm{Na}_2(GDC)_3$ (10.4 ± 0.2); $\mathrm{Na}_3(GDC)_3$ (13.0 ± 0.25); $\mathrm{Na}_4\mathrm{H}_2(GDC)_7$ (41.9 ± 0.2); $\mathrm{Na}_{12}\mathrm{H}_7(GDC)_{25}$ (153.5 ± 3); $\mathrm{Na}_{17}(GDC)_{20}$ (96.0 ± 2); $\mathrm{Na}_{19}(GDC)_{25}$ (116 ± 5)
0.400	$\mathrm{Na}GDC$ (2.5 ± 0.3); $\mathrm{NaH}(GDC)_2$ (12.0 ± 0.1); $\mathrm{Na}_3(GDC)_3$ (12.8 ± 0.2); $\mathrm{Na}_5(GDC)_7$ (30.0 ± 0.1); $\mathrm{Na}_8\mathrm{H}_7(GDC)_{32}$ (165.3 ± 5); $\mathrm{Na}_{22}(GDC)_{27}$ (130.6 ± 3.5); $\mathrm{Na}_{15}\mathrm{H}_{10}(GDC)_{35}$ (211 ± 7); $\mathrm{Na}_{36}(GDC)_{44}$ (215 ± 4)
0.500	$\mathrm{Na}GDC$ (2.4 ± 0.1); $\mathrm{NaH}(GDC)_2$ (12.5 ± 0.1); $\mathrm{Na}_3(GDC)_3$ (14.0 ± 0.1); $\mathrm{Na}_4\mathrm{H}_3(GDC)_9$ (57.1 ± 0.2); $\mathrm{Na}_{30}(GDC)_{45}$ (218.4 ± 3.5); $\mathrm{Na}_{30}\mathrm{H}_{25}(GDC)_{63}$ (459 ± 5); $\mathrm{Na}_{30}\mathrm{H}_{30}(GDC)_{75}$ (543 ± 8); $\mathrm{Na}_{35}(GDC)_{58}$ (274 ± 3); $\mathrm{Na}_{50}(GDC)_{70}$ (350 ± 5)
0.600	$\mathrm{Na}GDC$ (2.4 ± 0.1); $\mathrm{NaH}(GDC)_2$ (12.7 ± 0.1); $\mathrm{Na}_3(GDC)_3$ (13.4 ± 0.3); $\mathrm{Na}_6\mathrm{H}_4(GDC)_{15}$ (90.4 ± 0.4); $\mathrm{Na}_{20}\mathrm{H}_{20}(GDC)_{50}$ (356.8 ± 3.5); $\mathrm{Na}_{35}(GDC)_{60}$ (283 ± 7); $\mathrm{Na}_{45}(GDC)_{65}$ (325 ± 6); $\mathrm{Na}_{48}\mathrm{H}_{20}(GDC)_{73}$ (505 ± 8); $\mathrm{Na}_{50}(GDC)_{83}$ (396 ± 9)
0.750	$\mathrm{Na}GDC$ (2.45 ± 0.15); $\mathrm{NaH}(GDC)_2$ (13.5 ± 0.2); $\mathrm{Na}_3(GDC)_3$ (15.9 ± 0.2); $\mathrm{Na}_9\mathrm{H}_7(GDC)_{18}$ (136.6 ± 0.3); $\mathrm{Na}_{25}\mathrm{H}_{21}(GDC)_{55}$ (416 ± 6); $\mathrm{Na}_{45}(GDC)_{69}$ (360 ± 10); $\mathrm{Na}_{53}\mathrm{H}_{25}(GDC)_{88}$ (639 ± 15); $\mathrm{Na}_{56}(GDC)_{92}$ (470 ± 20)

As the agreement between the results achieved from the graphic [16] and computerized procedures is satisfactory, the refined values of $\beta_{q,p,n}$ together with the found species are collected in Table 2 for each value of the concentration of ionic medium.

The limits of error of Table 2 were deduced from the reproducibility of e.m.f. measurements and by assuming that analytical values of A, B and H are known within ±0.1%.

Discussion

The dominating species found in solutions at different concentration of ionic medium $\mathrm{N(CH_3)_4Cl}$ collected in Table 2 with the relative formation constants represent the most important result of this work. The proposed species and their constants account for the experimental data within the studied range of concentration of the reagents (Table 1) assuming the most simple hypothesis involving the minimum number of species. Since the studied micellar system is very complicated and lot of possibilities can exist, the species proposed in Table 2 represent the set of species and constants that in our opinion is the best fit with the experimental data. Although several other trials, with different types and number of species, gave less reliable results, the proposed $Na_qH_p(GDC)_n$ with the relative constants were assumed as predominant species in the investigated concentration range of the reagents. However, other sets of species cannot be excluded, but their existence seems unlikely.

From an inspection of Table 2, it is easy to remark that in all cases polynuclear ($q \geqslant 1$) and protonated ($p \geqslant 0$) species take place and also the value assumed by

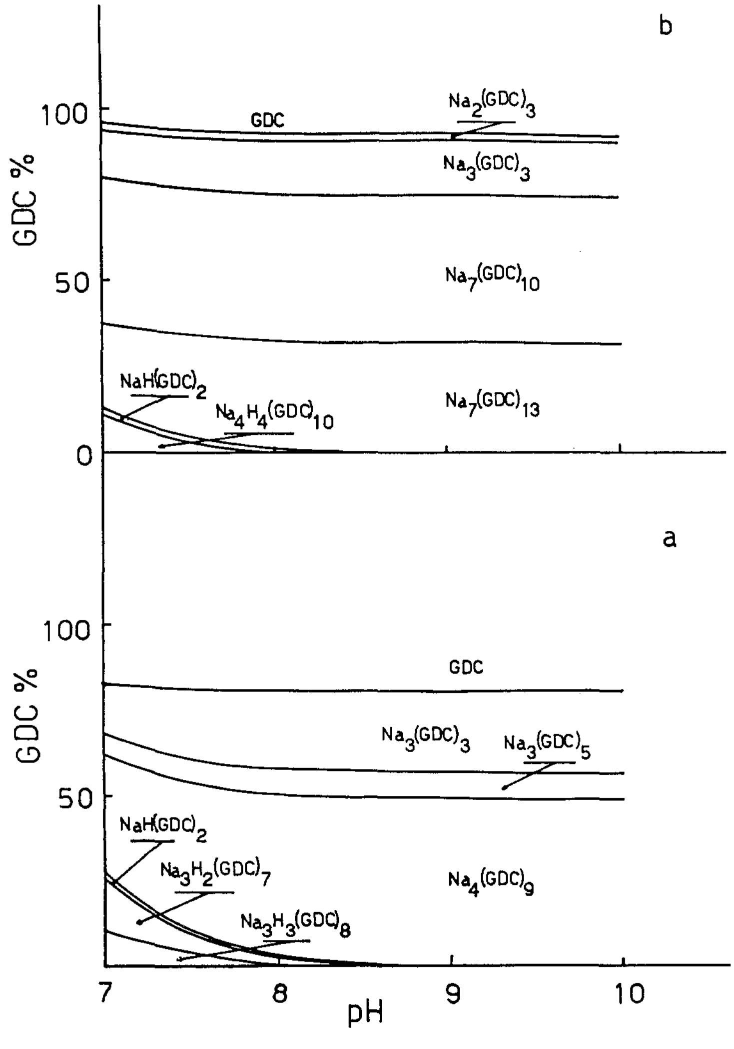

Fig. 1. Distribution curves of the found species as a function of *pH* for ionic medium concentration $N(CH_3)_4Cl$ 0.100 *M* (**a**) and 0.200 *M* (**b**)

$n \geqslant 1$, while species formed with loss of protons cannot be detected, as expected. The nuclearity of the aggregates (with regard to both q and n) increases by increasing the concentration of $N(CH_3)_4Cl$, as expected for micellar aggregates found for bile salts.

The value of n_{max} increases from 9 (for $XM = 0.100\ M$) to 83 (for 0.600 M) and to 92 (for 0.750 M). For all the concentrations of ionic medium the species Na*GDC*, $NaH(GDC)_2$, $Na_3(GDC)_3$ are always found although with different $\beta_{q,p,n}$. Protonated species are present in any case.

Depending on the presence of the ionic medium we find for the other species of Table 2 that $n > (q + p)$, because, as explained previously [16], the general formulation of the proposed species must be correctly written as follows: $Na_qH_p(GDC)_n[N(CH_3)_4]_xCl_y[H_2O]_w$, where x, y, and w cannot be determined.

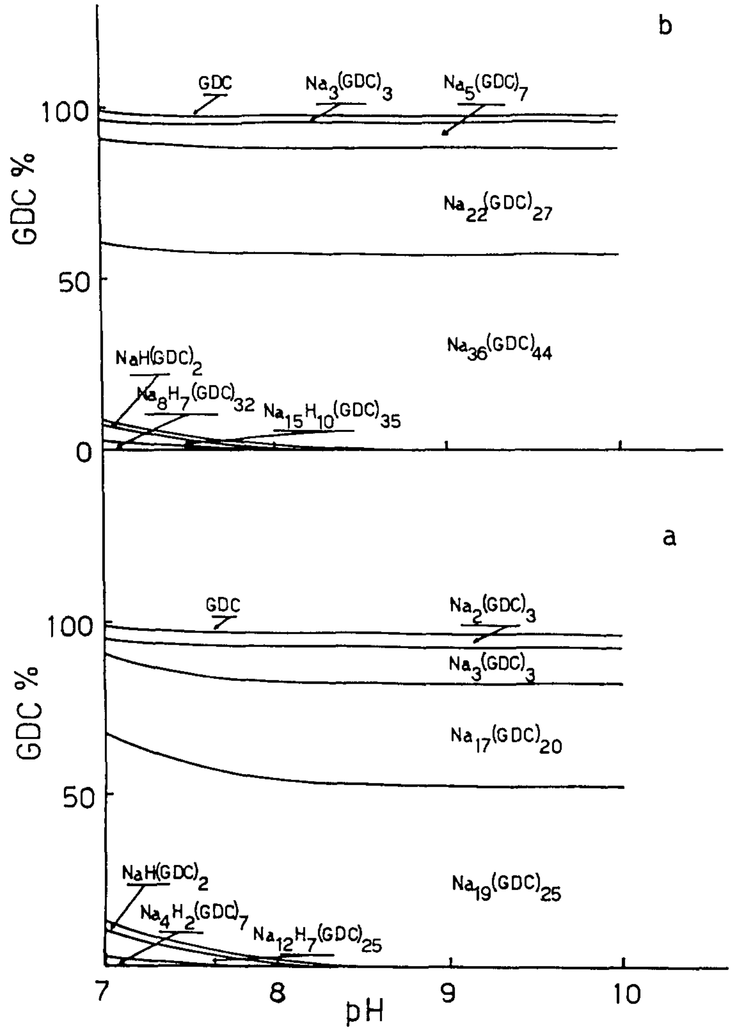

Fig. 2. Distribution curves of the found species as a function of *pH* for ionic medium concentration $N(CH_3)_4Cl$ 0.300 M (**a**) and 0.400 M (**b**)

From the results collected in Table 2, distribution curves of the species as a function of *pH* can be calculated as shown in Figs. 1–4 for the different $N(CH_3)_4Cl$ concentrations. From the figures, it can be seen that for each ionic medium concentration, the formation of protonated species takes place in the range $7 \leqslant -\log h \leqslant 8.3$. Beyond $pH \sim 8.3$, species with $p = 0$ prevail and the composition of the solution remains the same in the range $8.5 \leqslant -\log h \leqslant 10$. In this field of *pH*, the presence of more than one species with different values of *q* and *n* must be assumed. At higher concentration of ionic medium even three species $Na_{30}(GDC)_{45}$, $Na_{35}(GDC)_{58}$ and $Na_{50}(GDC)_{70}$ for 0.500 *M* $N(CH_3)_4Cl$ and $Na_{35}(GDC)_{60}$,

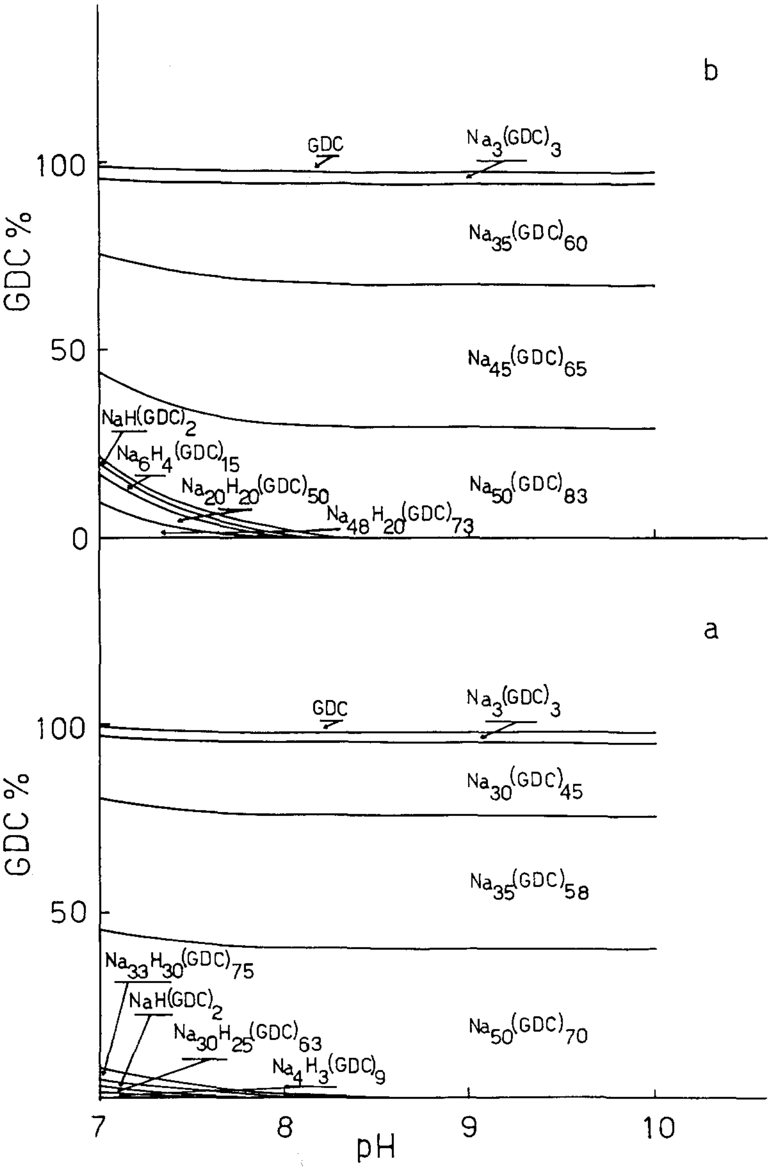

Fig. 3. Distribution curves of the found species as a function of *pH* for ionic medium concentration $N(CH_3)_4Cl$ 0.500 *M* (**a**) and 0.600 *M* (**b**)

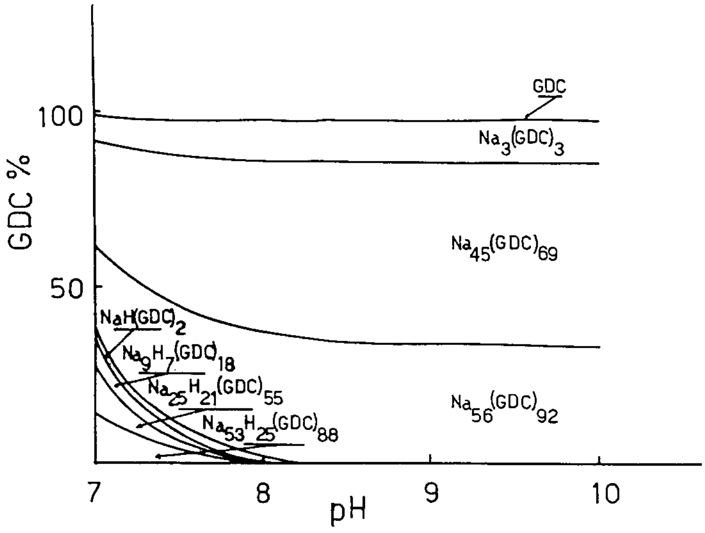

Fig. 4. Distribution curves of the found species as a function of *pH* for ionic medium concentration $N(CH_3)_4Cl$ 0.750 *M*

$Na_{45}(GDC)_{65}$ and $Na_{50}(GDC)_{83}$ for 0.600 *M* $N(CH_3)_4Cl$ had to be assumed to explain the experimental data.

The percentage of *GDC* (as monomer) decreases by increasing the concentration of ionic medium, in correspondence of the decreasing of the value of c.m.c. At 0.100 *M* $N(CH_3)_4Cl$, it is about 18%, at 0.200 *M* 8% and it decreases till about 1–2% when the concentration of ionic medium increases till 0.600–0.750 *M*.

A comparison between the results obtained in this work for glycodeoxycholate and those previously obtained for deoxycholate [16] seems interesting.

Also in the case of deoxycholate, *q* and *n* increased by increasing the concentration of the ionic medium, but the composition of the found species shows some differences.

First of all, in the case of Na*GDC* it is possible to analyse solutions prepared in more concentrated ionic medium (till 0.750 *M*) than in the case of sodium deoxycholate, whereas it was very hard to obtain real equilibria for Na*GDC* at the same concentration of ionic medium but at higher concentration of the reagents.

For all the studied concentrations of ionic medium, the percentage of deoxycholate in monomeric form is higher than the corresponding one of the glycodeoxycholate and this result agrees with the proposed c.m.c. value reported in the literature of the two bile acids. Ekwall et al. [24] proposed the value 5×10^{-3} *M* for Na*DC* and the value 4×10^{-3} *M* for Na*GDC* in water at 25 °C and many other researchers agree about the decreasing of the c.m.c. value by increasing the ionic strength.

It seems interesting to compare the behaviour of deoxycholate and glycodeoxycholate towards the formation of protonated aggregates. In Fig. 5 the percentages of protonated species are reported in form of a histogram and by considering the weight of the aggregates. The white column refers to glycodeoxycholate and the shaded one to Na*DC*. Three *pH* values are considered: 7.7, 8.0 and 8.2, because only in the range $7.7 \leq pH \leq 8.5$ we were able to obtain reliable data relative to

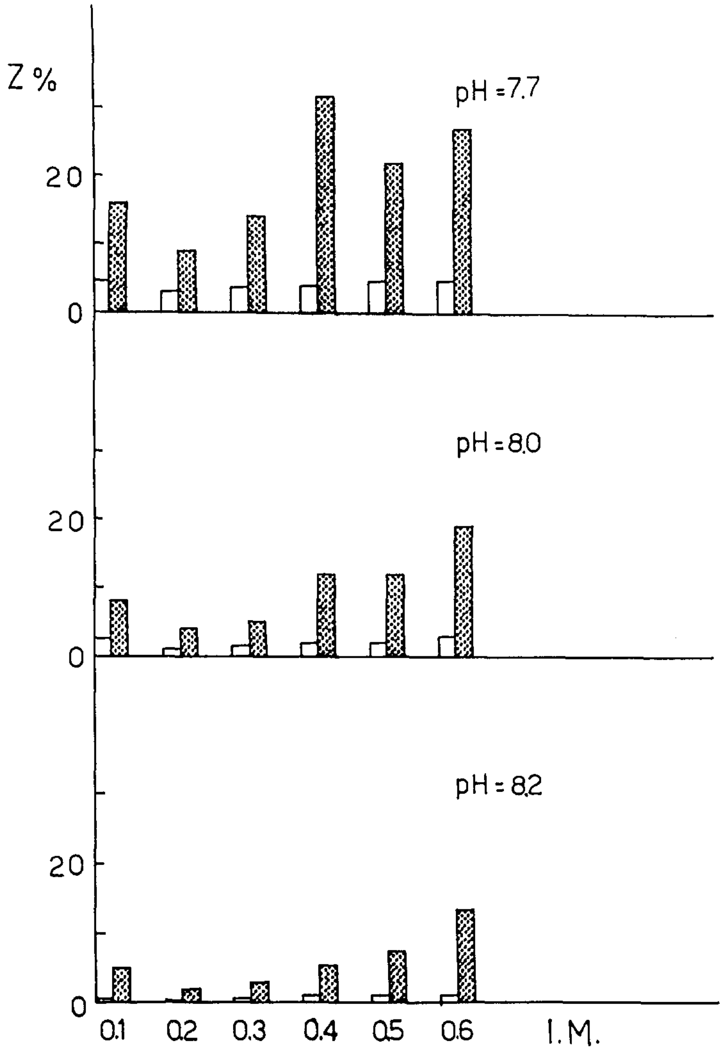

Fig. 5. Percentage of protonated species ($Z\%$) versus ionic medium concentration (I.M.) at three values of *pH*; white and shaded columns refer to glycodeoxycholate and deoxycholate, respectively

protonated species for Na*DC*. As expected, the quantity of protonated species increases for both systems by increasing the concentration of the reagents including $N(CH_3)_4Cl$ and by decreasing *pH*.

The general trend relative to the formation of protonated species shows a predominance of the protonated species for deoxycholate with respect to the glycodeoxycholate and this is explainable by taking into account that the existence of protonated species takes place already at *pH* values ($7.7 \leqslant pH \leqslant 8.5$) higher for Na*DC* than for Na*GDC* ($7.0 \leqslant pH \leqslant 8.3$).

Figures 6, 7 and 8 show a comparison in form of a histogram between deoxycholate and glycodeoxycholate relative to the weight of the aggregates at *pH* 7.7, 8.0, and 9.0, respectively. The number written in correspondence to each column represents the values of *n* for each aggregate and the height is the total percentage relative to the species with the same n. As earlier, white columns are relative to glycodeoxycholate, whereas shaded ones to deoxycholate.

The contribution of the protonated species is more at $pH = 7.7$ (Fig. 6) than at $pH = 8$ (Fig. 7), as expected. At $pH = 9$, protonated species are not present in appreciable concentrations (Fig. 8). Furthermore, it can be seen from Figures 6, 7 and 8 that $n_{\max}$ for glycodeoxycholate is lower than the corresponding value found for deoxycholate. This evidence could appear in contrast with the results found by other authors [2 and references quoted therein], but it can be explained by taking into account that a greater number of aggregates are present in the case of Na*GDC* than in the case of Na*DC*. As an example at $pH = 9$ (Fig. 8) and in 0.3 M $N(CH_3)_4Cl$, for deoxycholate aggregates with $n = 28$ are present at 28%, while for glycodeoxycholate, aggregates with $n = 25$ arrive to 19% and others with $n = 20$ are present at 12%, as well. At $pH = 8$ (v. Fig. 7) and 0.5 M $N(CH_3)_4Cl$, we have for deoxycholate species with $n = 90$ ($\sim 3\%$), with $n = 80$ ($\sim 21\%$), and $n = 65$ ($\sim 3\%$), while for

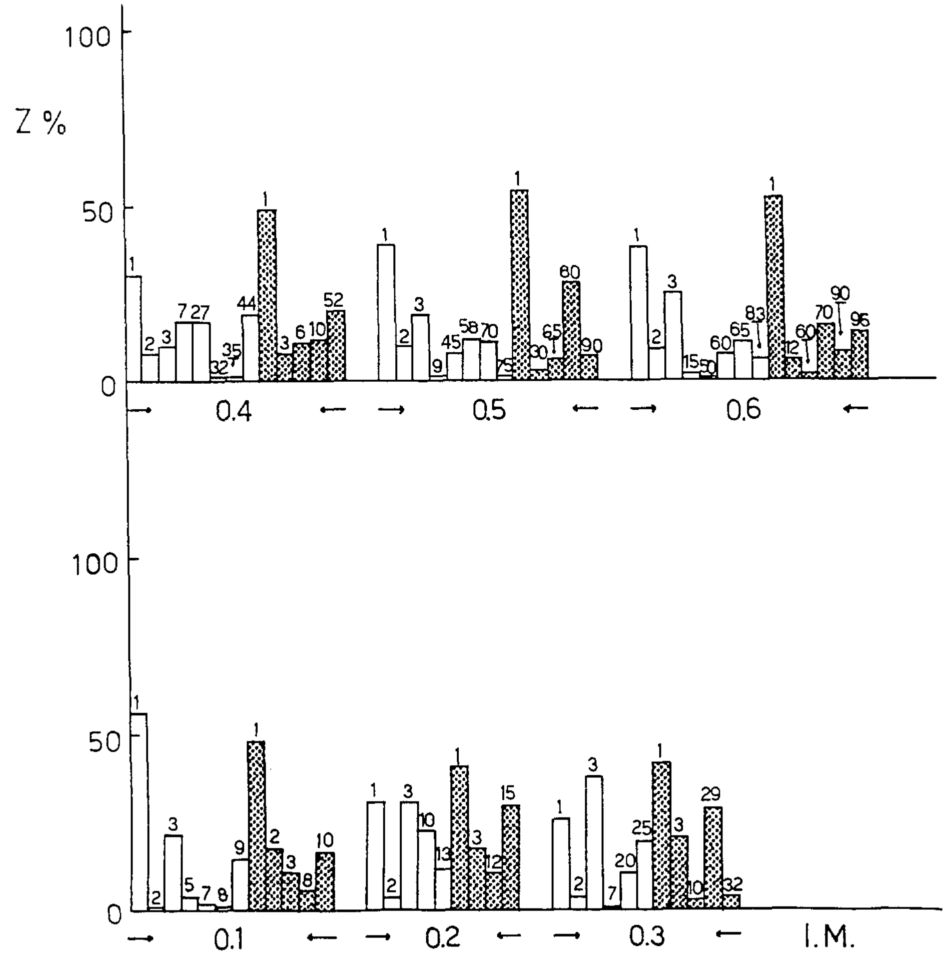

Fig. 6. Comparison between the found percentages ($Z\%$) of aggregates at $pH = 7.7$ for glycodeoxycholate and deoxycholate; I.M. indicates the ionic medium concentration

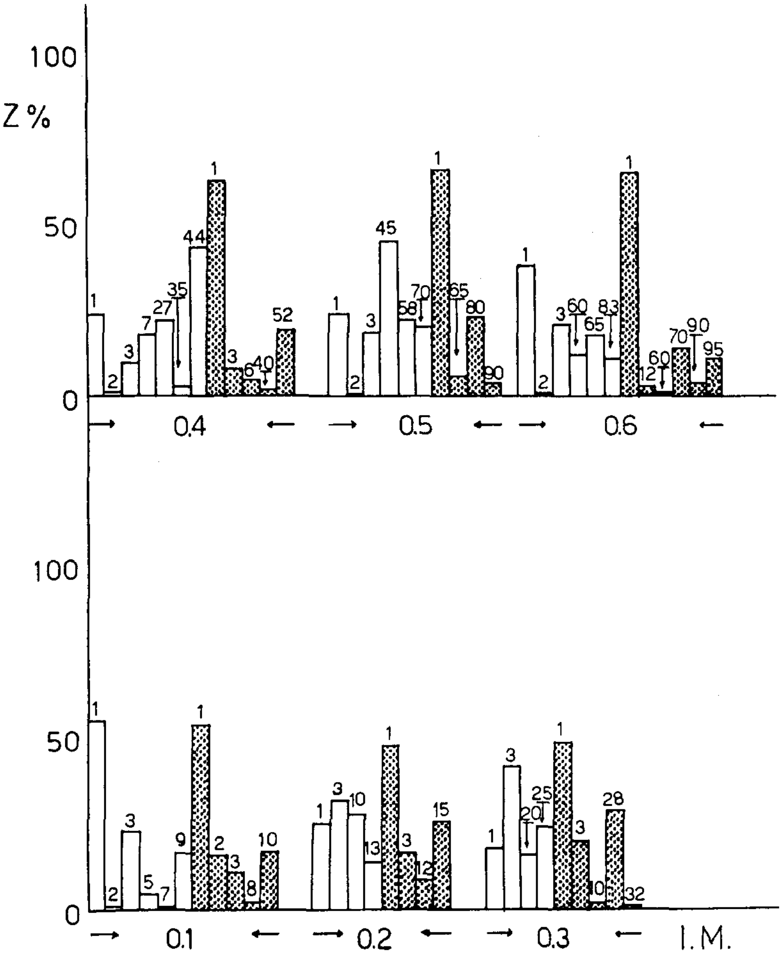

Fig. 7. Comparison between the found percentages ($Z\%$) of aggregates at $pH = 8.0$ for glycodeoxycholate and deoxycholate; I.M. indicates the ionic medium concentration

glycodeoxycholate species with $n = 70$ ($\sim 20\%$), $n = 58$ ($\sim 21\%$), and $n = 45$ ($\sim 45\%$). Another explanation involves the possible more strong affinity of glycodeoxycholate with respect to deoxycholate towards $N(CH_3)_4^+$ present as ionic medium.

However, we suggest that the most probable reason of this relatively low aggregation number for glycodeoxycholate seems to be attributable to the lower investigated concentration range of the reagents. In fact, it results from Table 1 that solutions of sodium glycodeoxycholate more concentrated than 0.050 *M* were not investigated till to obtain a real equilibrium. On the contrary in the case of deoxycholate [16], solutions with $A = 0.100$ *M* and $B = 0.060$ *M* in 0.500 *M* of $N(CH_3)_4Cl$ and $A = 0.080$ *M* and $B = 0.060$ *M* in 0.400 *M* of $N(CH_3)_4Cl$ were studied. One can suppose that in more concentrated solutions of glycodeoxycholate the values of q and n should be increased till values even higher than those found for deoxycholate.

This hypothesis is also supported by the comparison between the values assumed by q_{max} for Na*GDC* and Na*DC* [16]. In correspondence of similar values of B_{max} (Table 1) it was found in 0.300 *M* of $N(CH_3)_4Cl$ $q_{max} = 19$ for both, in 0.400 *M*

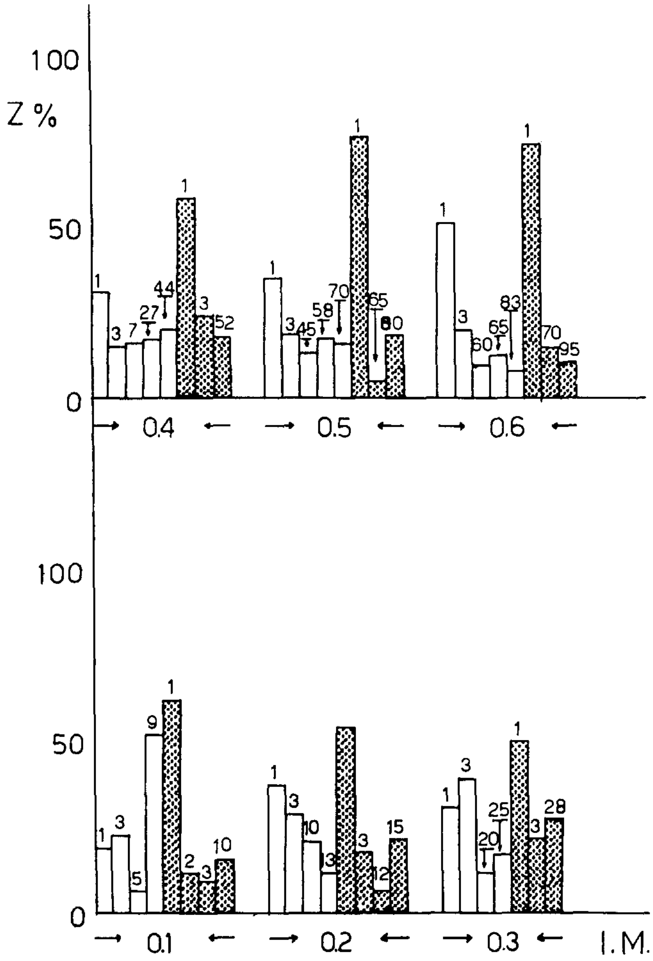

Fig. 8. Comparison between the found percentages ($Z\%$) of aggregates at $pH = 9.0$ for glycodeoxycholate and deoxycholate; I.M. indicates the ionic medium concentration

$q_{\max} = 35$ (Na*DC*) and 36 (Na*GDC*) and in 0.500 *M* of ionic medium $q_{\max} = 50$ in both cases.

In conclusion, the behaviour of Na*GDC* in aqueous solutions at 25 °C and at different concentration of $N(CH_3)_4Cl$ as ionic medium is very similar to that of Na*DC* under the same experimental conditions.

Investigations on Na*DC* and Na*GDC* showed that their crystal structures and the behaviour of micellar aggregates in aqueous solutions is well described by a helical model similar for both compounds [25].

The good results obtained for deoxycholate and glycodeoxycholate encourage us to extend our research to micellar solutions of other bile salts. Under this point of view it seems interesting to undertake an analogous investigation on sodium taurodeoxycholate.

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