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A Study of Deoxycholate Micellar Solutions as a Function of the Ionic Medium Concentration

EMILIO BOTTARI*,
Dipartimento di Chimica, Università di Roma 'La Spaienza', P. le A. Moro 5, 00185 Roma, Italy

MARIA ROSA FESTA, and RENATA JASIONOWSKA
Università del Molise, Via Cavour 50, 86100 Campobasso, Italy

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Abstract. The behaviour of sodium deoxycholate aqueous solutions at 25°C, using different concentrations of $N(CH_3)_4Cl$ as the ionic medium, was studied. Electromotive force measurements of galvanic cells containing electrodes reversible to sodium deoxycholate (DC) and hydrogen ions were performed. Moreover, the influence of sodium and DC ions on the solubility of lead (II) deoxycholate was investigated. Experimental data were explained by assuming the formation of several species of the type $Na_qH_p(DC)_n$. The general trend is that the q, p, n values increase with increasing concentration of $N(CH_3)_4Cl$. The observed aggregation numbers of the largest micellar aggregates satisfactorily agree with those obtained by small-angle X-ray scattering measurements.

Key words. Deoxycholate micellar aggregates, electromotive force measurements.

1. Introduction

The salts of bile acids, like sodium deoxycholate (NaDC), play an important role in many physiological and biological systems, due to their detergent-like and surface active properties. Bile salts can form molecular aggregates (micelles) in aqueous solutions that are capable of solubilizing many water-insoluble compounds. Knowledge of the structure of bile salts micelles is crucial for understanding their physical-chemical behaviour.

Some models have been proposed. Small *et al.* [1] accounted for the micellar association by invoking intermolecular hydrophobic interactions among the β faces of the steroid molecules and hydrophilic interactions with the water molecules of the α faces. Oakenfully and Fisher [2, 3] suggested that the driving force of the aggregation is due to the formation of intermolecular hydrogen bonds. More recently a study of the structure of NaDC and rubidium deoxycholate micellar aggregates was undertaken. Structural determinations were made by means of small-angle X-ray scattering (SAXS), extended X-ray absorption fine structure, nuclear magnetic resonance, electron spin resonance and circular dichroism techniques [4–7]. The structure, which appears to be helical, is apolar on the outer lateral surface, whereas the interior of the helix is filled with cations and water molecules. The helix is strongly stabilized by ion–ion and ion–dipole interactions as well as by a complicated network of hydrogen bonds. The solubility of this helical structure in water can be explained, since the hydroxyl and carboxyl polar groups

* Author for correspondence.

of the DC ions can be approached by water molecules perpendicular both to the bases and to the lateral surface of the helix, the separation between two adjacent DC ions being sufficiently large.

Experimental data concerning the aggregation number [6, 8] and the geometry [6, 7] of the NaDC micelles, as well as the critical micellar concentration (c.m.c.) values [9] are available in the literature. Therefore, it is interesting to establish by electromotive force (e.m.f.) measurements the aggregation numbers of the aqueous micellar solutions containing DC ions and to compare them with those observed by means of other techniques. Moreover, the knowledge of the q, p, n values sheds light on the polydispersity of the micellar solution and on the amount of surfactant present as monomer in solution, which can be related to the c.m.c.

A previous paper [10] reported a comparison between the behaviour of deoxycholate ions in 0.50 M aqueous solutions of $N(CH_3)_4Cl$ and $N(butyl)_4Cl$. The change of the ionic medium gave information on the influence of different ions on the cation–deoxycholate association. Experimental data were accounted for by means of species with different nuclearity, some of which were characterized by the presence of protons. Several species of the type $Na_qH_p(DC)_n$ were found. The largest aggregate had $n_{max} = 90$, $p_{max} = 21$; 22 and $q_{max} = 30$, and 60 for $N(CH_3)_4Cl$ and $N(butyl)_4Cl$, respectively.

Since the aggregate species formed are complicated, it seemed reasonable to try to measure more parameters at equilibrium. Recently, a method for measuring the free concentration of deoxycholate was reported [11].

The aim of this paper is to study how changes in the ionic medium concentration affect the q, p and n values. The free concentration of sodium, hydrogen and DC ions was measured with suitable electrodes as a function of the reagent concentrations. The experimental data can be explained by assuming species different from those previously proposed [10].

2. Methods

Two independent approaches were adopted to study the association in the micellar aggregates. In the text the following symbols are used: A = total concentration of deoxycholate (DC) ions, B = total concentration of sodium ions, H = analytical excess of hydrogen ions; b, a, h refer to the free concentrations of sodium, DC and hydrogen ions, respectively. In addition $\eta = \log(B/b)$; K = protonation constant of DC and $\beta_{q,p,n}$ = stability constant of $Na_qH_p(DC)_n$, defined as $\beta_{q,p,n} = [Na_qH_p(DC)_n]b^{-q}h^{-p}a^{-n}$. Charges are omitted.

2.1. APPROACH 1

The e.m.f.s of the following galvanic cells were measured at 25°C:

$$(-)\ \text{R.E./ Solution T/G.E.}\ (+) \tag{1}$$

$$(-)\ \text{R.E./ Solution T/Na E.}\ (+) \tag{2}$$

$$(-)\ \text{Pb E./ Solution T/R.E.}\ (+) \tag{3}$$

where G.E. is the glass electrode, Na E. is the sodium electrode, Pb E. is the lead electrode and R.E. is the reference electrode Ag, AgCl/X M $N(CH_3)_4Cl$ saturated with AgCl/X M $N(CH_3)_4Cl$, X being 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 in connection with the variable ionic medium concentration. Solutions T, saturated by lead (II) deoxycholate ($Pb(DC)_2$), had the general composition:

$$B \text{ M in } Na^+; \quad H \text{ M in } H^+; \quad (X - H - B) \text{ M in } N(CH_3)_4^+;$$

$$A \text{ M in DC}; \quad (X - A) \text{ M in } Cl^-.$$

Thus, according to Biedermann and Sillén [12], activity coefficients could be assumed to be constant and concentrations could replace activities in the calculations.

The measurement of the e.m.f. of cells (I), (II) and (III) allows us to know h, b and a, respectively. These values, together with known values of A, B and H, represent the basis for the evaluation of q, p, n and $\beta_{q,p,n}$ of the prevailing species. For each value of the ionic medium concentration, differing series of measurements were performed by keeping $-\log a$ and $-\log h$ constant and by increasing B. The measurements of the e.m.f. of cells (I) and (III) allowed us to check h and a, respectively, whereas those of cell (II) gave b. Experimental data can be expressed as $\eta(B)_{h,a}$.

2.2. APPROACH 2

The solubility (S) of $Pb(DC)_2$ in $N(CH_3)_4Cl$ solutions was studied as a function of sodium ion concentration and compared with that previously estimated in the absence of sodium ions [11]. The solubility was determined by bringing solid $Pb(DC)_2$ into contact with a solution of composition:

$$B \text{ M in } Na^+; \quad A \text{ M in DC}; \quad (X - B) \text{ M in } N(CH_3)_4^+; \quad (X - A) \text{ M in } Cl^-.$$

The solubility was measured by a DP50 pulse polarograph [13] after the equilibrium was reached and b, h and a were measured by direct potentiometry [14] using galvanic cells similar to (I), (II) and (III). However, this second approach was used as a rough test to confirm the results obtained from the first one. Since the occurrence of species with high q, p and n values is expected [6], small errors in S, b, h and a can generate large errors on the $\beta_{q,p,n}$s.

3. Experimental

All reagents were prepared and analyzed as previously described [10, 11]. All measurements were carried out at 25°C and the experimental apparatus was similar to that already described [10, 11]. The slope of the sodium electrode (Radelkis, Hungary, or Radiometer, Denmark) and that of the lead electrode (Metrohm, Switzerland) was checked [10, 11]. Reproducibility of the e.m.f. measurements of the cells indicated above as (I), (II) and (III) was within ± 0.3 mV in the first approach and within ± 0.5 mV in the direct measurements.

4. Results

4.1. APPROACH I

Solutions of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 M $N(CH_3)_4^+ + Na^+$ were examined. More concentrated solutions were not used since they did not provide reliable and reproducible data, owing to the inability to reach a real equilibrium in a reasonable time ($\approx$4 hr).

The measurements were performed by using the ionic medium concentrations and the selected values of $-\log h$ and $-\log a$ collected in Table I.

The addition of sodium ions to solution T was interrupted when a stable e.m.f. value was not obtained within $\approx$4 hr in one of the cells (I), (II) or (III).

For the calculations, it was necessary to know the values of the protonation constant of DC, and the hydrolysis constants of lead(II). The pK_a value, 5.3,

Table I. Survey of selected $-\log a$ and pH values at different molar concentrations of the ionic medium

	$-\log a$						
	pH 7.75	7.80	8.00	8.50	9.00	9.50	10.0
$[N(CH_3)_4^+] + [Na^+]$							
	3.00		3.00	3.00	3.00	3.00	3.00
	2.70		2.70	2.70	2.70	2.70	2.70
0.1	2.52		2.52	2.52	2.52	2.52	2.52
	2.30		2.30	2.30	2.30	2.30	2.30
	2.00		2.00	2.00	2.00	2.00	2.00
	3.00		3.00	3.00	3.00	3.00	3.00
	2.70		2.70	2.70	2.70	2.70	2.70
0.2	2.52		2.52	2.52	2.52	2.52	2.52
	2.30		2.30	2.30	2.30	2.30	2.30
	2.00		2.00	2.00	2.00	2.00	2.00
	3.00		3.00	3.00	3.00	3.00	3.00
	2.52		2.52	2.52	2.52	2.52	2.52
0.3	2.30		2.30	2.30	2.30	2.30	2.30
	2.00		2.00	2.00	2.00	2.00	2.00
	3.00		3.00	3.00	3.00	3.00	3.00
	2.70		2.70	2.70	2.70	2.70	2.70
0.4	2.52		2.52	2.52	2.52	2.52	2.52
	2.30		2.30	2.30	2.30	2.30	2.30
	2.00		2.00	2.00	2.00	2.00	2.00
	3.00		3.00	3.00	3.00	3.00	3.00
	2.70		2.70	2.70	2.70	2.70	2.70
0.5	2.52		2.52	2.52	2.52	2.52	2.52
	2.30		2.30	2.30	2.30	2.30	2.30
	2.00		2.00	2.00	2.00	2.00	2.00
		3.00	3.00	2.30	2.30	2.30	2.30
0.6		2.70	2.70	2.00	2.00	2.00	2.00
		2.52	2.52				

previously determined [15] at 25°C and 0.5 M $N(CH_3)_4Cl$ allowed us to neglect the protonation of DC within the investigated $-\log h$ range. Moreover, hydrolytic species of lead (II) were neglected on the basis of our e.m.f. measurements and of the results of Carell and Olin [16].

From the material balance of B, by taking into account the mass action law, the following equation can be written without preliminary hypotheses:

$$\eta = \log\left(1 + \sum_q \sum_p \sum_n q\beta_{q,p,n} b^{q-1} h^p a^n\right) \tag{1}$$

where $q \geqslant 1$; $p \gtrless 0$; $n \geqslant 1$.

At $-\log a$ and $-\log h$ = constant, equation (1) can be written as follows:

$$\phi = (10^{\eta} - 1) = \sum_q q\delta_q b^{q-1}, \quad \text{where} \quad \delta_q = \sum_p \sum_n \beta_{q,p,n} h^p a^n$$

By plotting ϕ against b, the prevailing values of q and the corresponding values of δ_q were obtained.

Subsequently, the dependence of δ_q on h and a was studied. The prevailing q, p, n values and the corresponding $\beta_{q,p,n}$ values were found. In the case of high q, p, n values, the procedure of a previous work was adopted [10].

4.2. APPROACH 2

The solubility of $Pb(DC)_2$ was determined in solutions containing different concentrations of sodium and deoxycholate ions under the same experimental conditions of approach 1. Selected values of B and A are collected in Table II. It was assumed

Table II. A and B values at 25°C and at different molar concentrations of the ionic medium of solutions equilibrated with solid $Pb(DC)_2$ used to perform solubility measurements

$[N(CH_3)_4^+] + [Na^+]$	A M × 10^3 (B M × 10^3)
0.1	5.0(0.5, 1.0, 2.0, 3.0); 10(0.5, 1.0, 2.5, 5.0); 15(1.0, 2.5, 5.0, 7.5, 10); 20(2.5, 5.0, 7.5, 10).
0.2	5.0(0.5, 1.0, 2.0, 3.0); 10(0.5, 1.0, 2.5, 5.0); 20(2.5, 5.0, 10, 15); 30(5.0, 10, 15, 20); 40(10, 15, 20, 25).
0.3	10(0.5, 1.0, 2.5, 5.0); 20(2.5, 5.0, 10, 15); 30(5.0, 10, 15, 20); 40(10, 15, 20, 25); 60(10, 20, 30, 40).
0.4	10(0.5, 1.0, 2.5, 5.0); 20(2.5, 5.0, 10, 15) 40(10, 15, 20, 25); 60(10, 20, 30, 40); 80(10, 25, 40, 50, 60).
0.5	10(0.5, 1.0, 2.5, 5.0); 25(1.0, 2.5, 5.0, 10, 15); 50(5.0, 10, 15, 25, 35); 100(10, 25, 35, 50, 60).
0.6	10(0.5, 1.0, 2.5, 5.0); 20(2.5, 5.0, 10, 15); 40(10, 15, 20, 25); 60(10, 20, 30, 40).

that the difference between the solubility measured with and without sodium ions was due to the formation of associated species of the type $Na_qH_p(DC)_n$. This difference, together with b, h and a measured in the same solution, was used to verify the results obtained from approach 1. The results of approach 2 agree with those of approach 1 within experimental error.

4.3. REFINED VALUES OF $\beta_{q,p,n}$

This system is very complicated, owing to the formation of several species, which implies a wide change of q, p, n values with the corresponding constants. Therefore, it is necessary to refine the values of the constants by means of a published iterative procedure [10].

Independently, refined values of the constants were obtained by treating the experimental data b, a, h, B, A and H using the program ECORM [17], written for a personal computer. The results are shown in Table III. The agreement between the results achieved from the graphic and computerized procedures is satisfactory. The limits of error on the values of the constants were estimated from the reproducibility of e.m.f. measurements and by assuming that A, B and H are known to within $\pm 0.1\%$.

Table III. Proposed values for the stability constants of the species $Na_qH_p(DC)_n$ at 25°C and at different molar concentrations of the ionic medium

$[N(CH_3)_4^+] + [Na^+]$	Species ($\log \beta_{q,p,n}$)
0.1	$NaDC(0.8 \pm 0.3)$; $NaH(DC)_2(11.6 \pm 0.5)$; $Na_2(DC)_2(7.1 \pm 0.3)$; $Na_2(DC)_3(9.3 \pm 0.6)$; $Na_3H_3(DC)_8(45.9 \pm 2)$; $Na_5(DC)_{10}(34.1 \pm 0.8)$.
0.2	$NaDC(0.7 \pm 0.3)$; $NaH(DC)_2(11.8 \pm 0.5)$; $Na_2(DC)_3(9.9 \pm 0.6)$; $Na_3(DC)_3(12.3 \pm 0.7)$; $Na_4H_5(DC)_{12}(74.3 \pm 3)$; $Na_6(DC)_{12}(42.3 \pm 0.6)$; $Na_8(DC)_{15}(55.7 \pm 0.5)$.
0.3	$NaDC(0.7 \pm 0.3)$; $NaH(DC)_2(11.6 \pm 0.5)$; $Na_2(DC)_3(10.5 \pm 0.6)$; $Na_2H(DC)_6(25.5 \pm 2)$; $Na_5H(DC)_{10}(43.1 \pm 3)$; $Na_{10}H_6(DC)_{32}(150.7 \pm 5)$; $Na_{19}(DC)_{28}(122.2 \pm 0.7)$.
0.4	$NaDC(0.5 \pm 0.3)$; $NaH(DC)_2(11.5 \pm 0.5)$; $Na_2(DC)_3(10.5 \pm 0.5)$; $Na_4H(DC)_6(32 \pm 0.8)$; $Na_7H_{10}(DC)_{40}(196.1 \pm 5)$; $Na_{35}(DC)_{52}(233.9 \pm 0.5)$.
0.5	$NaDC(0.4 \pm 0.25)$; $NaH(DC)_2(11.2 \pm 0.5)$; $Na_2(DC)_3(9.8 \pm 0.5)$; $Na_3H_2(DC)_{10}(47.3 \pm 2)$; $Na_{10}H_8(DC)_{30}(167.5 \pm 5)$; $Na_{30}H_{20}(DC)_{90}(479.7 \pm 10)$; $Na_{30}(DC)_{65}(261.9 \pm 1)$; $Na_{50}(DC)_{80}(363 \pm 2)$.
0.6	$NaDC(0.2 \pm 0.2)$; $NaH(DC)_2(10.2 \pm 0.6)$; $Na_2(DC)_3(9.0 \pm 0.6)$; $Na_4H_4(DC)_{12}(71.1 \pm 2)$; $Na_{10}H_{20}(DC)_{60}(337.8 \pm 8)$; $Na_{30}H_{20}(DC)_{90}(480.2 \pm 10)$; $Na_{40}(DC)_{70}(305.6 \pm 2)$; $Na_{55}(DC)_{95}(418 \pm 5)$.

5. Discussion

It must be stressed that the micellar systems studied are very complicated owing to polydispersity so that the determination of the species together with the relative constants is, obviously, less accurate than in the case of 'classic' complexes.

The most important results of this work are summarized in Table III, where the prevalent species with the relative constants are listed. These species account for the experimental data within the investigated range of the reagent concentrations, assuming the minimum number of species, which, as a consequence, can represent average populations. Numerous trials, in which the type and the number of the species were varied, gave less reliable results. However, 'better' sets of species cannot be excluded, owing to the high number of possible combinations. As expected for micellar aggregates of bile salts, the nuclearity of species (represented by the q and n values) increases with ionic strength. The highest values assumed by q, p and n are different ranging from $N(CH_3)_4^+ + Na^+$ concentration of 0.1 M (4, 3 and 10, respectively) to 0.6 M (30, 20 and 95, respectively). Unfortunately, this last solution scarcely reached a real equilibrium, especially within the range $7.8 \leqslant pH \leqslant 8.0$. On the other hand, even though equilibrium was reached, the reproducibility of the e.m.f. values was worse than ± 0.3 mV.

By using the data of Table III, distribution curves of the species as a function of pH were calculated at constant A and B values and plotted in Figures 1, 2 and 3. Species at low concentration ($<1\%$) are not considered. In all cases $q < n$, so that the stoichiometric ratio between sodium and deoxycholate ions is always less than 1, since the ionic medium must be considered. A general formulation of a species, $Na_qH_p(DC)_n$, has to be written correctly $Na_qH_p(DC)_n[N(CH_3)_4]_xCl_y[H_2O]_w$. The formation of aggregates containing tetramethylammonium and DC ions was previously demonstrated [10]. In the hypothesis of a neutral micelle with $p = y = 0$, it follows that the difference $n - q$ equals x, the number of tetramethylammonium ions.

Species containing protons are formed approximately within the investigated range $7.75 \leqslant pH \leqslant 8.50$, thus explaining the buffer capacity of NaDC solutions below the upper value [16]. This may be due, at low pH, to the preferential uptake from the NaDC micellar aggregate of hydrogen ions with respect to other cations, mainly on the bases of the helix containing monomers at the end-points with unsaturated interactions, if those with the solvent medium are neglected. Moreover, by inspection of Figures 1, 2 and 3 it is clear that the monomers and some small oligomers tend to disappear by decreasing the pH and by increasing the ionic strength.

At pH values greater than ≈ 8.5–9.0 the aggregation number of the largest aggregate increases with the ionic strength and is comparable with that observed in a SAXS study [6] carried out on NaDC solutions with variable NaCl concentrations (see Figure 4).

Unfortunately, there are three major differences in the aqueous micellar solutions investigated by the e.m.f. and SAXS methods. The first one regards the DC concentration, which is only approximately equal in the two types of measurements (0.05–0.15 M for SAXS and about 5×10^{-3}–0.10 M for e.m.f. measurements). However, the SAXS data depend very slightly on the NaDC concentration [6] and

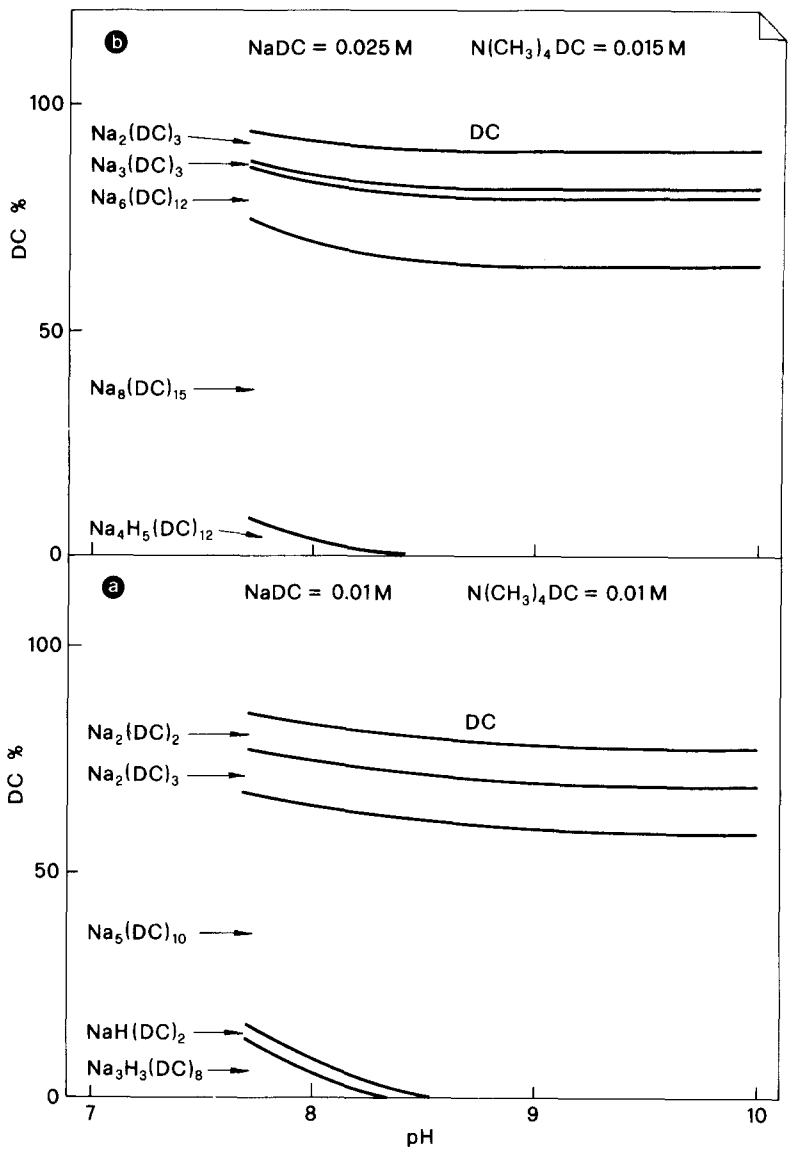

Fig. 1. Distribution curves of the observed species as a function of pH at $N(CH_3)_4Cl$ concentrations of 0.08 M(a) and 0.16 M(b).

the same largest aggregates explain the e.m.f. data (see Table III) in all the studied concentration range. The second difference concerns the pH values of the micellar solutions, included within the range 11–12 in the SAXS measurements and 8.5–10 in the e.m.f. measurements. Nevertheless, the plots of Figures 1, 2 and 3 show that the aggregation number of the species remains constant for at least pH $\geqslant$ 9, thus supporting the hypothesis that the same species are present for pH $\geqslant$11. The third difference is due to the use of different ionic species, namely $N(CH_3)_4^+$ instead

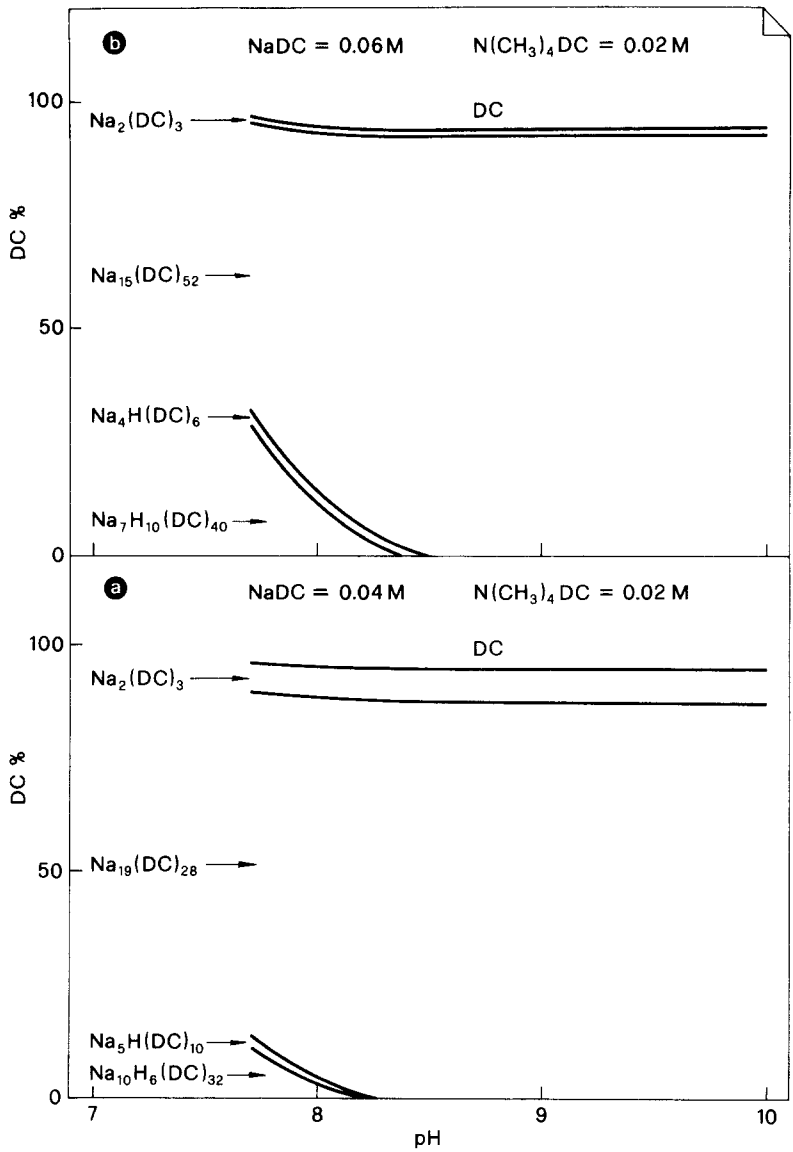

Fig. 2. Distribution curves of the observed species as a function of pH at $N(CH_3)_4Cl$ concentrations of 0.24 M(a) and 0.32 M(b).

of Na^+. In spite of this, the satisfactory agreement (see Figure 4) suggests that the micellar structure does not change considerably.

Another interesting point regards the amount of monomer found as a function of the ionic strength. Generally, the micellar concentration is calculated by subtracting the c.m.c. from the total surfactant concentration. Our results are not in agreement with this assumption. In fact, the concentration of the monomeric species DC, plotted vs. the $N(CH_3)_4Cl$ molar concentration in Figure 5, changes from 5 to

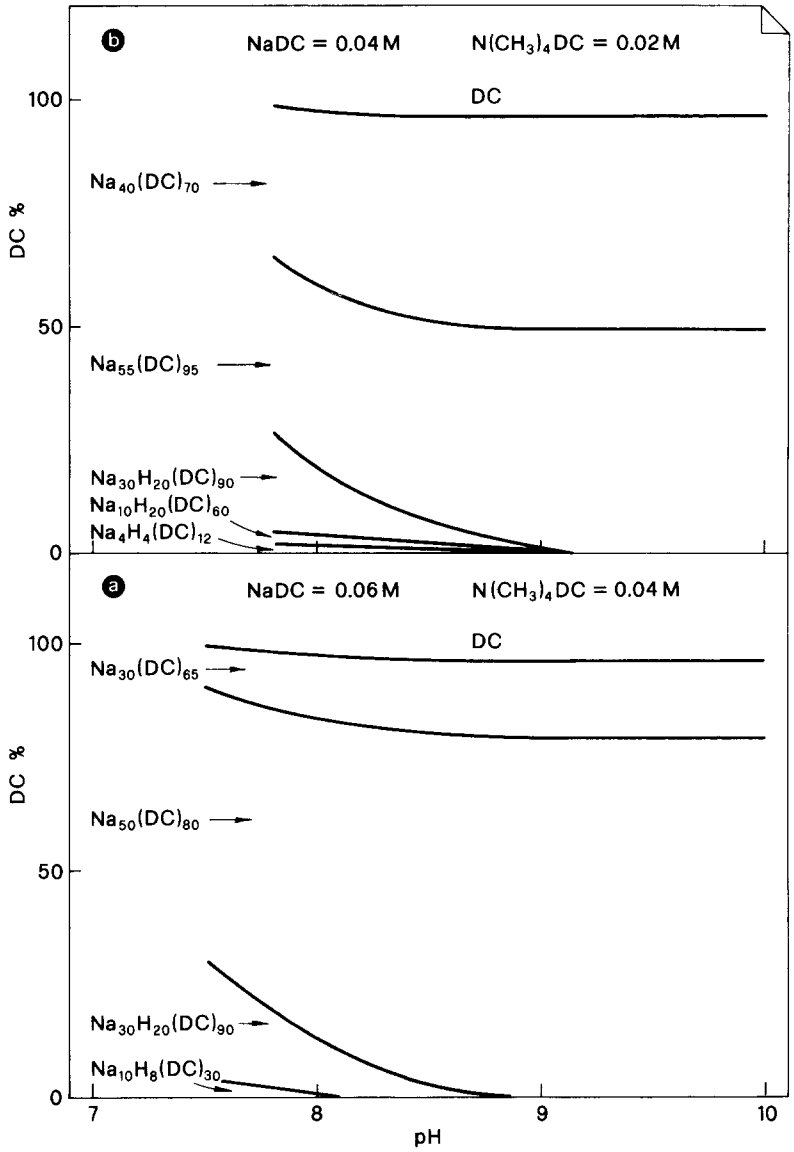

Fig. 3. Distribution curves of the observed species as a function of pH at $N(CH_3)_4Cl$ concentrations of 0.40(a) and 0.54 M(b).

2 mM by increasing the $N(CH_3)_4Cl$ concentration from 0.08 to 0.40 M. As an example, an accurate c.m.c. value of NaDC from light scattering measurements at a concentration of added NaCl of 0.149 M is 2.4 mM [9]. This value must be compared with that of 4.4 mM shown in Figure 5 for a $N(CH_3)_4Cl$ concentration of 0.16 M. Moreover, if the contribution of dimers and trimers is taken into account the micellar concentration further decreases by 4.3 millimoles. Therefore, inspection of Figure 5 seems to point out that the NaDC micellar concentration, calculated as

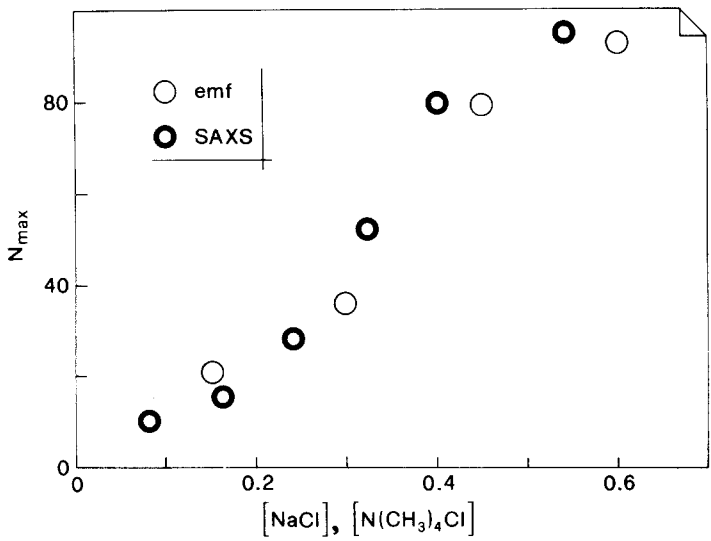

Fig. 4. Comparison between the aggregation numbers of the largest aggregates (N_{max}) observed as a function of the $N(CH_3)_4Cl$ (this work) and NaCl (SAXS study) molar concentration.

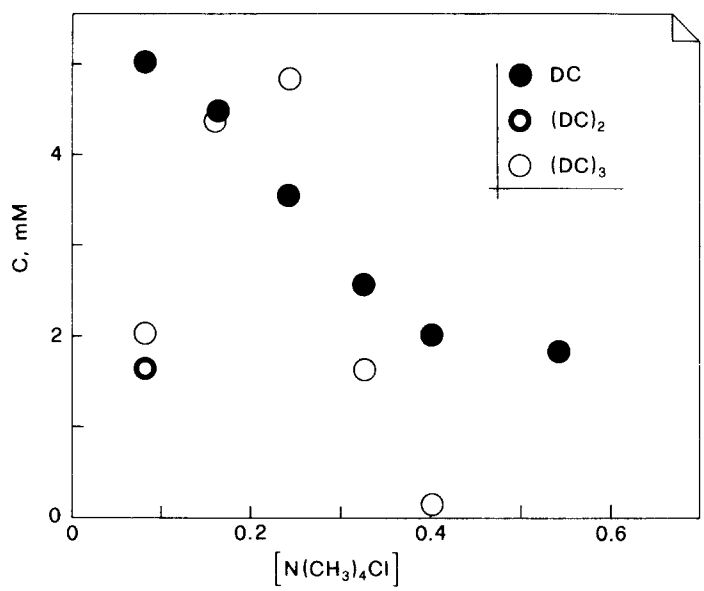

Fig. 5. Concentration (C, mM) of monomer, dimers and trimers as a function of the $N(CH_3)_4Cl$ molar concentration.

the total surfactant concentration minus the c.m.c., is overestimated up to a $N(CH_3)_4Cl$ concentration of about 0.4 M, whereas at greater concentration dimers and trimers disappear and the monomer concentration tends to the c.m.c. (we find a concentration of DC 1.8 mM at 0.54 M $N(CH_3)_4Cl$ against a c.m.c. of 1.45 mM at 0.59 M NaCl [9].

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