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The aggregation of sodium dehydrocholate in water

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Abstract We used a battery of different methods to study the association in aqueous sodium dehydrocholate (NaDHC) solutions. This salt associates by a stepwise mechanism. Below (9.6 ± 4.2) × 10^{-4} mol dm^{-3} there is a molecular solution with some strongly insoluble dehydrocholic acid produced by hydrolysis. Between (9.6 ± 4.2) × 10^{-4} and (5.2 ± 2.2) × 10^{-3} mol dm^{-3}, an aggregate similar to acid soap (NaDHC.HDHC) appears and its amount and the aggregate's size increase with concentration. At = (2.20 ± 0.85) × 10^{-2} mol dm^{-3} the aggregates formed have properties usually associated with true micelles, such as solubilisation of water-insoluble dyes. These aggregates increase in size with concentration and change their shape at 8 × 10^{-2} mol dm^{-3}, giving nonsymmetrical aggregates. The changes in the solution physicochemical properties at these concentrations may be misinterpreted and this explains the different values of the critical micelle concentration reported in the literature for substances with similar structure, such as bile salts.

Keywords Sodium dehydrocholate · Micelles · Stepwise association · Bile salts · Critical micelle concentration

Introduction

Bile salts are natural surfactants mainly stored in the gall bladder. Their function is essentially the emulsification and transport of food fats and lipids. The physicochemical properties of bile salts are of interest because of their very important role in the metabolic process of absorption at the intestinal level.

Bile salts are different from common surfactants, which in general have their polar headgroup attached to a flexible hydrocarbon chain. Bile salts have a rigid steroid backbone, having up to three hydroxyl groups and a branched linear chain ended by a carboxylate group which may or may not be conjugated with glycine or taurine. This difference in structure generates a atypical aggregation behaviour.

From the point of view of the surfactant physical chemistry, bile salts are far from being as well studied as the more traditional structured surfactants. For the same bile salt there is a large dispersion of data leading to contradictory conclusions. As an example, for aqueous sodium cholate Fontell [1] found by vapour pressure osmometry that the aggregation number was 3–4, whereas by X-ray spectrometry [2] he deduced a value of 16. There is also no agreement in the number of counterions bound to aggregates. Many authors deduced values of $\beta = m/n$ below 0.3 (m being the number of counterions in an aggregate whose aggregation number is n), but Sugihara et al. [3] found $\beta = 0.7$. There is also a controversy about the critical micelle concentration (cmc) value, ranging from 0.003 mol dm^{-3} [4–7] to 0.018 mol dm^{-3} [8]. Besides, the cmc values depend on the method used in their determination. [9–11]. Coello et al. [12] and Norman [13] said that sodium cholate behaves as a normal 1:1 electrolyte up to 0.014–0.015 mol dm^{-3}, whereas Carpenter and Lindenbaum [14] found “very important deviations from the Debye–Hückel theory even at the lowest concentrations used, typically 0.0025 moldm^{-3}”. The existence of a cmc is also controversial [15]. Many authors reported values of the cmc [7, 16, 17]. Ultrasonic relaxation measurements suggest that the association of bile salts into aggregates occurred for a range of concentrations and not at a cmc [18]. Some authors indicate that “association starts at…” [16] without use of the concept of a cmc. The structure of the aggregates and the association driving force are also under discussion [15].

Other authors found that most of the physicochemical properties of aqueous bile salts undergo important changes at several concentrations. These changes have been interpreted as arising from a stepwise aggregation process. As an example, at least three aggregation steps were reported in sodium cholate aqueous solutions [1, 2].

In this work we studied the aggregation of sodium dehydrocholate (NaDHC), which is a derivative of cholic acid by oxidation. The structure of this substance is shown in Fig. 1, and shares with bile salts the hydrocarbon backbone; however, this hydrocarbon backbone is more hydrophobic than that of cholic acid because the alcoholic hydroxyl groups were replaced by carbonyl groups. This change in structure must affect the association mechanism. In particular, both sides of the hydrocarbon backbone are hydrophobic, and the mechanism of dimer formation via hydrogen bonds among the backbone hydroxyl groups [19] is not possible.

This study was carried out by a battery of methods involving effects depending on the monomer behaviour (ion-selective electrodes, surface tension), on large aggregates (dye solubilisation, changes in fluorescence and colour of rhodamine 6G, viscosity), and the whole system (density, conductivity, pH) to elucidate how the NaDHC aggregation behaves. NaDHC is a scarcely studied bile salt, which adds interest to this work.

Experimental

Dehydrocholic acid (HDHC) was from Dr. Theodor Schuchardt, Munich, and was of analytical grade. A concentrated NaDHC aqueous solution was prepared by weighing a quantity of HDHC and dissolving it in an appropriate amount of concentrated NaOH solution. The solution was then diluted to obtain the desired concentrations. Only double-distilled water was used.

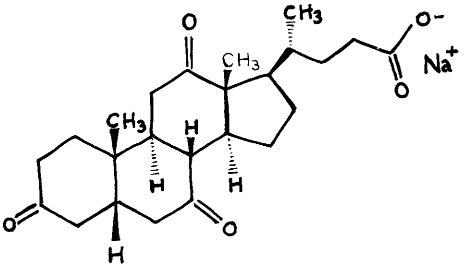

Fig. 1 Structure of dehydrocholic acid

Conductivity measurements were made using an immersion cell and an automatic conductimeter, namely an Antares II from Instrumentalia.

The surface tension was measured with a ring tensiometer (Krüss).

Dye solubilisation experiments were performed by two batteries of tubes having different bile salt concentrations. Small Sudan III crystals were added to the tubes of one battery, and small Sudan black B crystals to the other. The tubes were sealed and left for a week with periodic stirring. Then, the tubes were centrifuged and the supernatant absorbance was measured with a Spectronic-20 UV–vis spectrophotometer at 600 nm (Sudan III) and 490 nm (Sudan black B).

A drop of dilute solution of rhodamine 6G was added to tubes with solutions of different surfactant concentration and the cmc was determined by the changes in fluorescence and colour.

The pH determinations were made with a millivoltmeter and a CRIBABB pH meter and a Broade and James glass electrode. The same millivoltmeter was used with an Orion 8411 Ross Na^+-ion-selective electrode and a DHC^--ion-selective electrode. Both ion-selective electrodes were used against a saturated calomel electrode.

The DHC^--ion-selective electrode was made by gluing at one extreme of a poly(vinyl chloride) (PVC) tube a membrane made with 300 mg PVC dissolved in 50 ml tetrahydrofuran (THF)/0.2 ml dibutylphthalate (plasticiser) and 0.167 g $\mathrm{Pd(DHC)_2}$. $\mathrm{Pd(DHC)_2}$ was made by mixing the appropriate amounts of $\mathrm{PdCl_2}$ and NaDHC aqueous solutions. The insoluble $\mathrm{Pd(DHC)_2}$ salt was filtered and washed several times with double-distilled water and then left to dry. The crystals were powdered and suspended in the solution. Then, the PVC mixture was left in a Petri dish to let the THF evaporate, and the resulting membrane was cut and glued to the tube. The tube was filled with an aqueous solution of 0.01 moldm^{-3} NaDHC/0.1 moldm^{-3} KCl with a small amount of solid AgCl. An Ag/AgCl electrode made with a silver wire was placed into the tube and this was sealed with a rubber plug.

Viscosity measurements were made with an Ostwald viscometer. Water was used as a reference.

Density measurements were made with a Beckers Sons density balance.

All the determinations were made at 25.0 °C.

Mean values and variances were computed by the minimum variance linear unbiased method [20] and the Student t function was employed to compute the error intervals. The confidence level was 0.90. Errors of data derived were computed with the error expansion method.

Results

Solubilisation results for Sudan III and Sudan black B are shown in Fig. 2. Sudan black B is much less solubilised than Sudan III; however, both dyes began to be solubilised at the same concentration of $(3\text{–}4) \times 10^{-2}$ moldm^{-3}.

The specific conductivity (Fig. 3) shows breaks at concentrations of 9×10^{-4}, 6×10^{-3}, 2×10^{-2} and 8×10^{-1} moldm^{-3}. The equivalent conductivity plot is shown in Fig. 4.

The density measurement results are shown in Fig. 5; these, have a break at 7×10^{-3} $\mathrm{mol\,dm}^{-3}$. The effect of surfactant concentration on the solution viscosity (Fig. 6) begins at a very low concentration

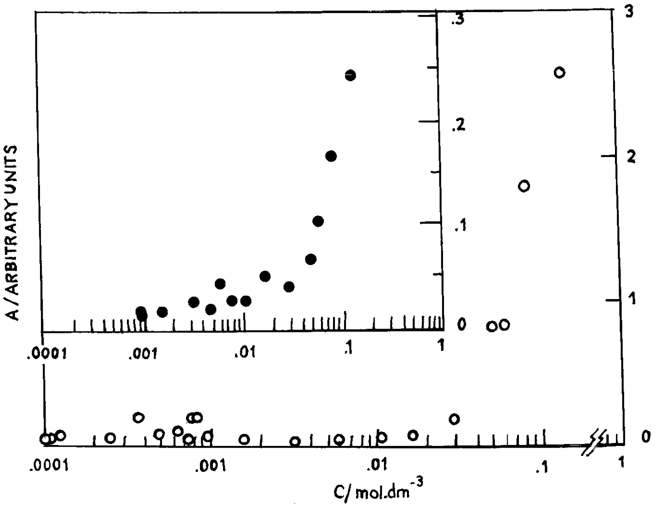

Fig. 2 Sudan III (*open circles*) and Sudan black B (*filled circles*) solubilisation

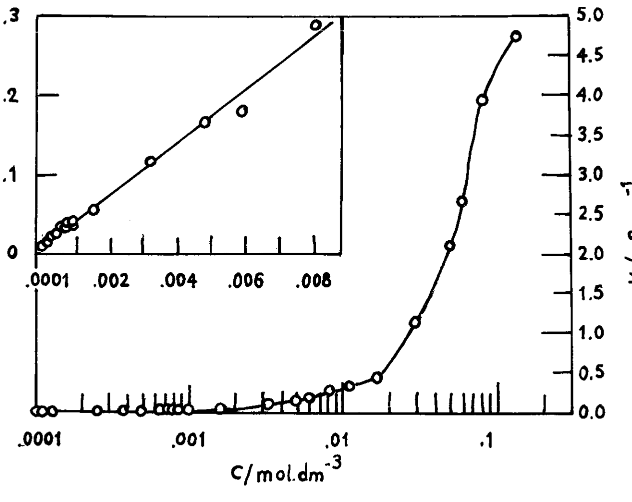

Fig. 3 Specific conductivity of aqueous sodium dehydrocholate (*NaDHC*) solutions. The *insert* is the very low concentration zone

(7 × 10^{-4} mol dm^{-3}), and this effect increases with concentration. Below the concentration mentioned, a diminution of viscosity with respect to that of the solvent (water at 25 °C) can be seen. At 2 × 10^{-2} mol dm^{-3} a break appears giving higher viscosity, and at 8 × 10^{-2} mol dm^{-3} there is a sudden increase in this property.

Potentiometric measurements with the DHC^{-}-ion-selective electrode show that aggregation begins between 8 × 10^{-4} and 9 × 10^{-4} mol dm^{-3} (Fig. 7). The Na^{+}-ion-selective electrode measurements shown a sharp break at 7 × 10^{-4} mol dm^{-3} and a more gradual break between 1 × 10^{-2} and 2 × 10^{-2} mol dm^{-3}.

The pH–log*c* curve (Fig. 8) has four straight sections, represented by the equations:

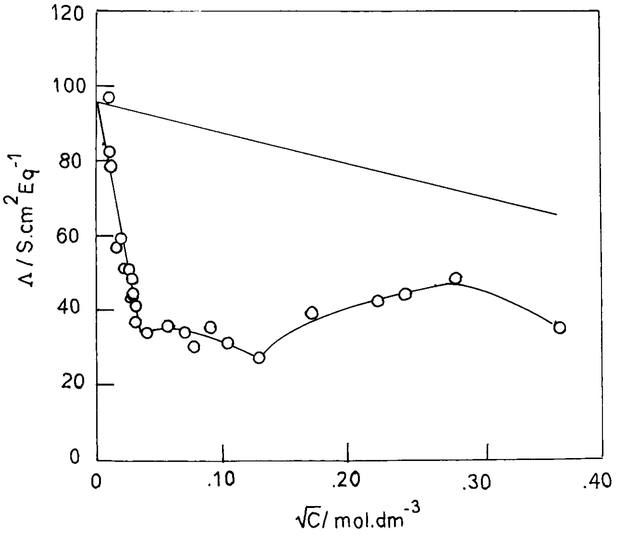

Fig. 4 Equivalent conductivity versus $\sqrt{c}$. The *straight line* corresponds to the Debye–Hückel–Onsager slope

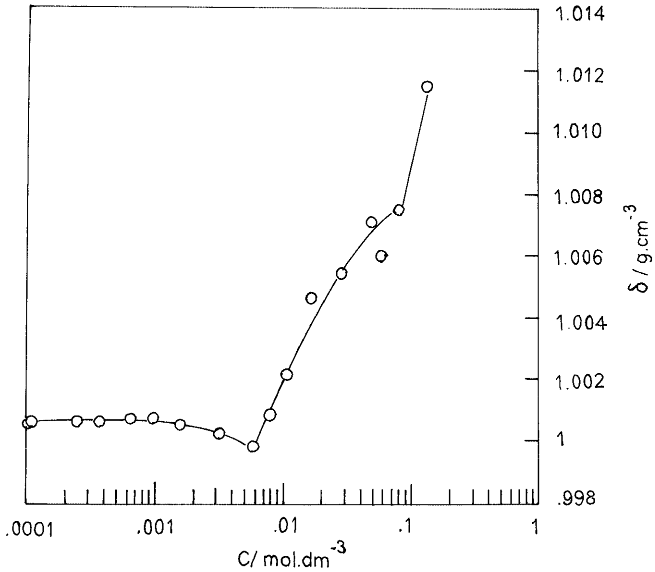

Fig. 5 NaDHC aqueous solutions density

1. Section 1: pH = 10.91 ± 0.23 + (1.155 ± 0.073) log *c*, where $r = 0.9741$ (correlation coefficient).
2. Section 2: pH = 15.7 ± 1.1 + (2.7 ± 0.4) log *c*, where $r = 0.9997$.
3. Section 3: pH = 22.8 + 5.4 log *c*.
4. Section 4: pH = 14.05 ± 0.14 + (1.766 ± 0.074) log *c*, where $r = 0.9986$.

The intersection between lines 1 and 2 is at (7.8 ± 2.0) × 10^{-4} mol dm^{-3}, that of lines 2 and 3 is at 2.3 × 10^{-3} mol dm^{-3}, that of lines 3 and 4 is at 0.0039 mol dm^{-3}, and section 4 deviates from linearity at 0.02 mol dm^{-3}.

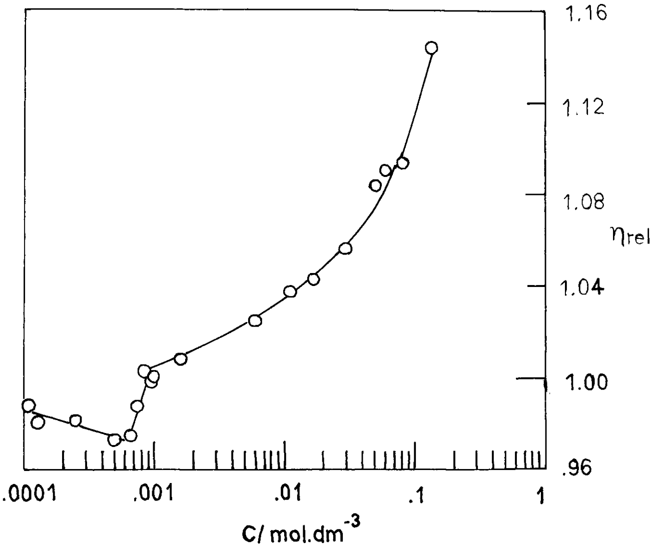

Fig. 6 Relative viscosity of NaDHC aqueous solutions

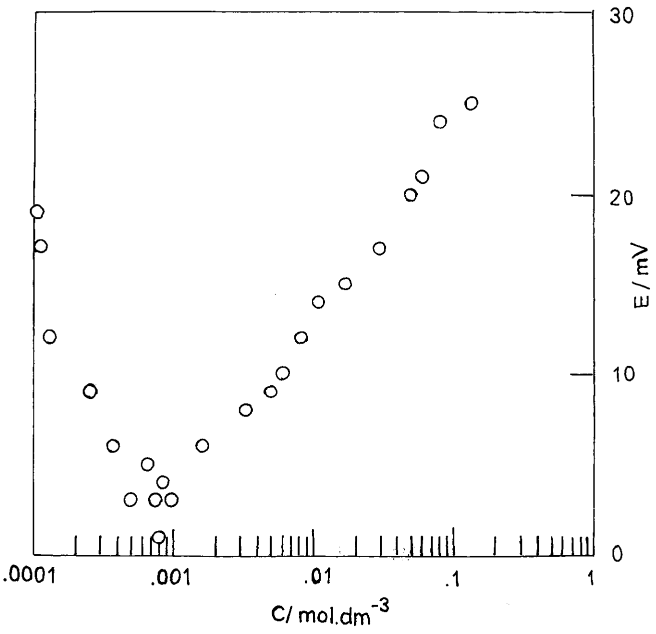

Fig. 7 Response of the dehydrocholate-ion-selective electrode to concentration changes

The degree of hydrolysis ($\beta = [OH^-]/c$) versus c curve (Fig. 9) shows a minimum at about 5×10^{-4} moldm^{-3}, followed by extremely high hydrolysis. The maximum is at about 4×10^{-2} moldm^{-3}.

The addition of rhodamine 6G gave changes in colour and fluorescence between 1.68×10^{-4} and 2.94×10^{-4} moldm^{-3}.

The points in which changes in the dependence of the properties studied on concentration occurred are summarised in Table 1.

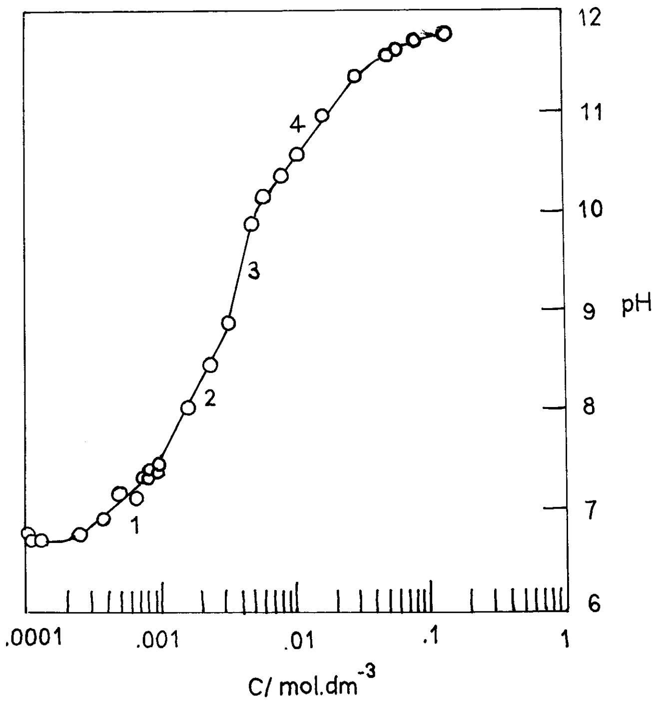

Fig. 8 pH versus total bile salt concentration

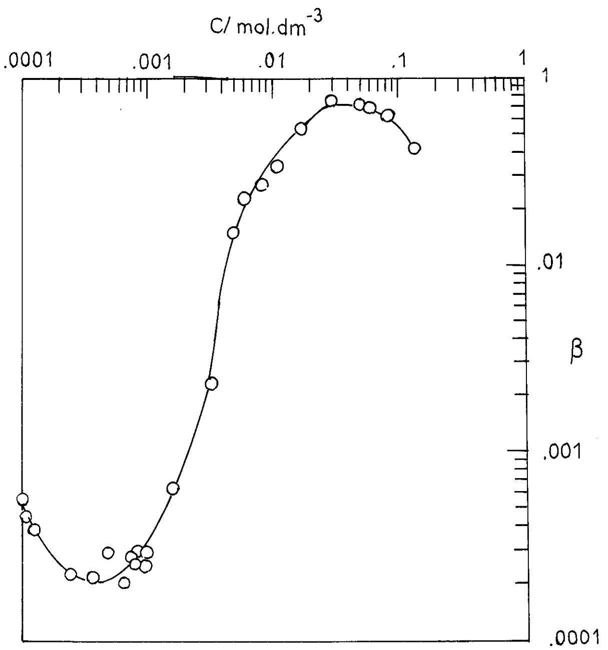

Fig. 9 Dependence of the degree of hydrolysis (β) on the total concentration of NaDHC

Discussion

The preceding results show that there are four concentrations at which there are structural changes in

Table 1 Concentrations at which changes in the dependence of different solution properties on total concentration of sodium dehydrocholate occur

Property	c_1 (mol dm^{-3})	c_2 (mol dm^{-3})	c_3 (mol dm^{-3})	c_4 (mol dm^{-3})
Sudan black B solubilisation			4×10^{-2}	
Sudan III solubilisation			2.4×10^{-2}	
Dehydrocholate ion electrode potential	8.5×10^{-4}	5×10^{-3}	1×10^{-2}	8×10^{-2}
Na$^+$ electrode potential	7×10^{-4}		1.5×10^{-2}	
Density		7×10^{-3}		
Specific conductivity	9×10^{-4}	8×10^{-3}	2×10^{-2}	8×10^{-2}
Hydrolysis			2×10^{-2}	
pH	7.8×10^{-4}	$(2.3\text{–}3.9) \times 10^{-3}$	2×10^{-2}	
Viscosity	7×10^{-4}	3×10^{-2}	8×10^{-2}	
Surface tension	1.8×10^{-3}		1.8×10^{-2}	
Rhodamine 6G colour and fluorescence changes			2.3×10^{-2}	
Average values	$(9.6 \pm 4.2) \times 10^{-4}$	$(5.2 \pm 2.2) \times 10^{-3}$	$(2.20 \pm 0.85) \times 10^{-2}$	8×10^{-2}

solution. These are $c_1 = (9.6 \pm 4.2) \times 10^{-4}$ mol dm^{-3}, $c_2 = (5.2 \pm 2.2) \times 10^{-3}$ mol dm^{-3}, $c_3 = (2.20 \pm 0.85) \times 10^{-2}$ mol dm^{-3} and $c_4 = 8 \times 10^{-2}$ mol dm^{-3} (Table 1). Like other surfactants such as soaps [21] and hydroxylated cationic surfactants [22], NaDHC undergoes a stepwise association process. In many surfactant systems, there is premicellar aggregation followed by micelle formation and structural changes of micelles at a higher concentrations. In particular, the equivalent conductivity curve (Fig. 4) is very similar to that found in the literature for such association [21, 22].

The use of a battery of methods to study the association process enabled us to obtain information about the phenomena occurring when the concentration was raised.

Some information may be obtained on low-concentration aggregates from the Lucassen theory [23] applied to the pH versus log$c$ curve (Fig. 8). Line 1 has a slope of about 1. This means that small amounts of suspended, water-insoluble acid were produced by hydrolysis. From line 1 the solubility of HDHC, S_{HDHC}, may be estimated by [23]

$$S_{\mathrm{HDHC}} = (c[\mathrm{H}^+] - K_{\mathrm{W}})/K_{\mathrm{a}} \ ,$$

where c is the total concentration of surfactant, [H$^+$] that of the hydrogen ion, K_{W} the self-dissociation constant of water and K_{a} the acidity constant of the acid. From hydrolysis data the K_{a} values were obtained and plotted against $\sqrt{c}$. The extrapolation to $\sqrt{c} = 0$ gave $K_{\mathrm{a}} = 0.0708 \pm 0.0055$. Then, $S_{\mathrm{HDHC}} = (5.6 \pm 0.7) \times 10^{-10}$ mol dm^{-3}.

Line 2 has a slope of about 3, which corresponds to the formation of small amounts of an acid soap in soap solutions [23]. In this system an association compound of composition NaDHC.HDHC may be formed, like the aggregates known as "acid soap" in soap solutions. The slope is ($\alpha + 1$), where α is the sum of the salt and acid molecules in the NaDHC.HDHC unit [23]. However, nothing is said about the size of the aggregates; they may be (NaDHC.HDHC)$_n$, n being an integer. Both HDHC and NaDHC.HDHC are water-insoluble, but they were so finely divided that no precipitation occurred in 1 month.

Line 4 has a slope of about unity, which occurs at higher pH values when almost complete formation of NaDHC.HDHC occurs [23]. Figure 10 shows that hydrolysis is very high, about 10% of the total surfactant content, in agreement with this interpretation. Line 3 is a transition between lines 2 and 4. The curvature at $c = 0.02$ mol dm^{-3} is interpreted in Lucassen theory [23] as the cmc in solutions of hydrolysable surfactants.

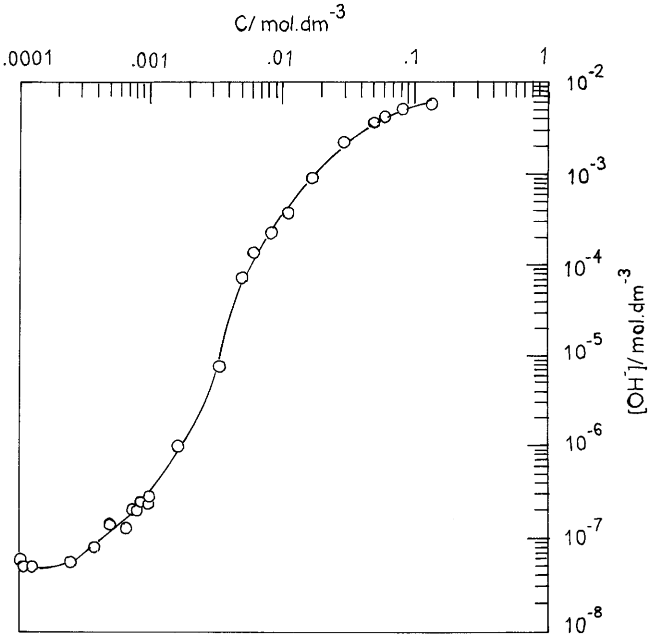

Fig. 10 Hydroxyl ion concentration versus the total NaDHC concentration

In conclusion, before c_1 a certain amount of insoluble acid was formed, between c_1 and c_2 an acid-soap-like compound appeared, whose proportion increased above c_2, and at c_3 micelles were produced. Lucassen theory applies to pre–cmc solutions.

The relative viscosity results are also interesting at concentrations below c_1. At very low concentrations NaDHC behaves as a water structure breaker [24, 25], whereas above this concentration the aggregates are large enough to increase the solution viscosity. Figure 6 shows that at c_3 and c_4 the aggregates have a different shape. Above c_4 anisometric (probably rods or disks) aggregates were formed. This gave a sharp viscosity increase.

The effect below c_1 may be explained on the basis of the theory developed by Némethy and Scheraga for hydrophobic hydration [26–30]. The structure of the hydrocarbon hydration cages is affected by the size and shape of the solubilised hydrocarbon, and some hydrocarbons which may not enter the hydrophobic hydration cages act as structure breakers [31–33]. In addition, the hydration of charged polar groups, that of uncharged polar groups and the hydrophobic hydration are mutually incompatible. Which of them predominates depends on the structure of the solute molecule: the size and shape of the hydrocarbon backbone and the kind and localisation of the hydrophilic groups. The large size and the stiffness of the steroid backbone and the presence of the polar groups (three carbonyl and one carboxyl) in different parts of the molecule hindered the formation of a structured water cage surrounding the surfactant molecule and favoured the destruction of the "icebergs". This increased the solution fluidity, in spite of the structure-making effect of Na^+ and OH^- ions.

Density (δ) measurements (Fig. 4) show that δ is almost constant up to 1×10^{-3} mol dm^{-3} and then there is a slight decrease up to 5×10^{-3} mol dm^{-3}. Above this concentration there is an increase in density up to 2×10^{-2} mol dm^{-3}, in which a change in slope appears. Another change in slope occurs at 8×10^{-2} mol dm^{-3}. The formation of free acid does not seem to affect the density, whereas the formation of acid-soap-like aggregates first decreases δ. The change to an increase in density at c_2 may be interpreted as a change in the structure of the acid-soap-like aggregates, possibly the formation of adducts $(NaDHC.HDHC)_n$, with $n > 1$. The changes at c_3 and c_4 may be interpreted as micelle formation and the change of the micelle structure, which affects the density of the aggregates.

Owing to the structure of NaDHC, a comparison with the behaviour of bile salts may be illustrative. Bile salt aggregates have a size distribution which is highly polydisperse [9, 18, 34–38]. Accordingly to Mukerjee and Cardinal [38], this implies that the mean aggregation number increases with the total concentration of surfactant and that the aggregation process is complex and involves the formation of dimers and higher oligomers, besides micelles [39]. Sesta et al. [40] also found evidence for a progressive aggregation process in bile salt solutions. In particular, they found Λ versus $\sqrt{c}$ curves similar to Fig. 4. This behaviour suggests that the aggregation process is different to that usually found in 1:1 colloidal electrolytes. The data below the Debye–Hückel – Onsager straight line suggest a strong reduction in the number of conductive particles with respect to a common 1:1 electrolyte. This may be due to the capture of counterions by aggregates or to ion-pair formation.

Going back to Fig. 5, it can be seen that aggregates formed at c_2 remarkably affect the density, and the transformations occurring at c_3 and c_4 are changes in degree, instead of character. In other words, these results seem to indicate that aggregates formed at c_2 grew continuously with concentration, and at c_3 and c_4 this growth produced structural changes. Djavanbakht et al. [18], using ultrasound absorption, concluded that in a series of bile salts (including KDHC), there is a continuous aggregation process at concentrations above about 10^{-2} mol dm^{-3}, with a broad micelle size distribution, which is consistent with our results.

Solubilisation experiments with both Sudan III and Sudan black B show that the aggregates have a hydrophobic nucleus large enough to solubilise hydrophobic dyes at c_3. Meyerhoffer and McGown [41], working with sodium taurocholate in water, found that premicellar aggregates do not have an interior hydrophobic enough to solubilise fluorescent probes.

A glance at Figs. 2, 4, 5, 6 and 8 shows that c_3 may be interpreted as the cmc. All the transformations evidenced at c_3 are commonly associated with the presence of micelles: solubilisation of water-insoluble dyes, changes in the fluorescence of rhodamine 6G, increased hydrolysis. This interpretation depends on what kind of aggregates are considered as micelles. In general, only those aggregates whose size is large enough to solubilise hydrophobic solutes (such as the dyes Sudan III and Sudan black B, and the bile acid formed by hydrolysis) are considered micelles. In this situation, the hydrophobic core is large enough to provide a locus similar to a hydrocarbon microdroplet. These aggregates also have a hydrocarbon–water interface which is different from that of the premicellar aggregates. This difference affects the colour and fluorescence of rhodamine 6G. The aggregates are also large enough to affect the solvent lines of flux and noticeably affect the viscosity dependence on concentration. However, we must point out that in this system, there is not a sudden appearance of micelles at c_3, but an increase in the size of the premicelles until the properties of the aggregates are those usually attributed to micelles.

The transition at c_4 may be interpreted as a change in the shape of the micelles. Figure 6 shows an increase in the slope of the dependence of viscosity on concentration. This is usually interpreted as the transition from spherical, or nearly spherical micelles, to anisometric aggregates, such as disklike or rodlike micelles. This interpretation is supported by the change in slope in the specific conductivity curve (Fig. 3), representing the formation of a less conducting species. The high-concentration zone of Fig. 4 also shows a strong decrease in equivalent conductivity.

This stepwise aggregation process, accompanied by a gradual increase in the size of the aggregates and changes in shape explains why different cmc values were reported in literature for the same bile salt. As an example, for sodium cholate, Kawamura et al. [42] found, using two different methods, cmc values of 5×10^{-3} and 8×10^{-3} mol dm^{-3}, whereas Roda et al. [43] found a value of 1.1×10^{-2} mol dm^{-3}. Fujasaki et al. [44] found evidence of stepwise association in aqueous solutions of other bile salts. Meyerhoffer and McGown [41] found that aqueous sodium taurocholate also undergoes a stepwise association process. These authors found a critical point around $(3–5) \times 10^{-3}$ mol dm^{-3}, but the cmc was $(8–12) \times 10^{-3}$ mol dm^{-3}.

Chiu and Yu [17] found the cmc of several bile salts by dilution titration and a higher concentration transition which they defined as a micelle dissociation concentration, which is interpreted as the beginning of micelle dissociation when the solution is diluted. They did not study NaDHC, but the concentrations interpreted as micelle dissociation concentrations are equivalent to c_4 in our work. In consequence, it is probable that the transition interpreted by Chiu and Yu as the micelle dissociation concentration simply reflects a change in the structure of the micelles.

It is evident that a unique method to detect the cmc by a sharp change in the dependence of a solution property on concentration may lead to a misinterpretation of this change. There are a number of examples in different surfactant systems in which these changes correspond to structural transformations which do not involve micelle formation. The cmc determination of a novel surfactant ought to be made by a battery of methods to avoid misinterpretation of the critical points. Only in well-known surfactant systems may a single method of cmc determination give the cmc without any doubt. The risk of misinterpretation of changes in the dependence of physicochemical properties of surfactant solutions on concentration as the cmc, was emphasised by Mukerjee in 1974 [39].

Concluding remarks

- In aqueous solution, NaDHC aggregates in a stepwise process. At concentrations below $(9.6 \pm 4.2) \times 10^{-4}$ mol dm^{-3}, the system is basically a monomer solution having some insoluble HDHC in suspension. Between $(9.6 \pm 4.2) \times 10^{-4}$ and $(5.2 \pm 2.2) \times 10^{-3}$ mol dm^{-3} acid-soap-like aggregates appear, whose proportion (and the size of aggregates) increase with the total concentration above $(5.2 \pm 2.2) \times 10^{-3}$ mol dm^{-3}. At $(2.20 \pm 0.85) \times 10^{-2}$ mol dm^{-3} micelles appear. The micelle size increases with concentration and at 8×10^{-2} mol dm^{-3} anisometric micelles appear.
- This stepwise aggregation process is reflected by changes in the dependence of different physicochemical properties on total concentration. The significance of these changes may be misinterpreted. In consequence, it is necessary to use a battery of techniques to determine which of the different critical points is the cmc.

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