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ORIGINAL CONTRIBUTION

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Potentiometric studies on sodium dehydrocholate micelles

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Abstract The scarcely studied sodium dehydrocholate–water system was investigated at low concentration by using H^+-, Na^+- and dehydrocholate-ion-selective electrodes. The findings at concentrations below the critical micelle concentration are consistent with those of previous work. A dimeric chelate structure entrapping Na^+ ions at very low concentration is proposed. The composition of the micelles and the degree of ionisation were studied and explained as the result of the possibility of a back-to-face mechanism of aggregation of this steroid salt. This mechanism also explains the possibility of formation of acid soaplike aggregates at intermediate concentrations.

Keywords Sodium dehydrocholate · Micelles · Acid soap · Degree of micellar ionisation · Sodium chelates

Introduction

Bile salts are biologically important surfactants whose micellar behaviour is considerably different from that of ordinary surfactants having a long alkyl chain and a hydrophilic head group [1, 2, 3]. The bile salts consist of a steroid nucleus having two or three hydroxyl groups which are situated in the concave side (face or α side) of the steroid backbone. This side is hydrophilic, whereas the convex side (back or β side) is hydrophobic. Besides, the hydroxyl groups may form intermolecular links via hydrogen bonds. Attached at the C-24 position there is a short aliphatic chain terminated by a hydrophilic group. This structure promotes the formation of unusual aggregates whose structure and properties are still controversial [4]. To elucidate the structure of these aggregates it is interesting to know their composition and degree of ionisation and the composition of the intermicellar solution. There is some information in the literature concerning these points. Funasaki et al. [4] obtained the free surfactant ion concentration in equilibrium with micelles of sodium taurochenodeoxycholate (NaTCDC) and sodium tauroursodeoxycholate (NaTUDC) by frontal chromatography. Other authors found values of the degree of ionisation of bile salts by indirect methods. In some cases, sodium-ion-selective electrodes have been used [5]. However, we have not found a complete potentiometric study on steroid surfactants. Dehydrocholic acid (HDHC) is a derivative of cholic acid by oxidation. Its sodium salt (NaDHC) has been scarcely studied. We investigated the aggregation of this surfactant by studying all the free ions in solution (H^+, DHC^- and Na^+). As far as we know, this is the first study of steroid surfactants of this kind. The information derived from these measurements may be useful to complete the knowledge of the complex aggregation behaviour of bile salts and related compounds.

Experimental

HDHC (analytical grade) was from Dr. Theodor Schuchardt. A concentrated NaDHC aqueous solution was prepared by weighing a quantity of HDHC and adding an appropriate amount of concentrated NaOH solution. Then, the solution was diluted with doubly distilled water to obtain the desired concentrations.

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 Na^+-ion-selective electrode was calibrated with a series of NaCl solutions with concentration ranging from 9.7×10^{-5} to 0.2 $moldm^{-3}$.

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) and 0.2 ml dibutylphtalate (plasticiser) and 0.167 g $Pd(DHC)_2$. This salt was made by mixing the appropriate amounts of $PdCl_2$ and NaDHC aqueous solution. The insoluble $Pd(DHC)_2$ salt was filtered and washed several times with double-distilled water and then left to dry. The crystals were powdered and suspended in the PVC solution. Then, the suspension 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 and 0.1 $moldm^{-3}$ KCl containing a small amount of solid AgCl. An Ag/AgCl electrode made with a silver wire was placed into the tube and connected to the millivoltmeter by a copper wire passing through a rubber plug.

The ion-selective electrodes are sensitive to the respective free (nonaggregated) ion activity, $a_{i,\text{free}}$. The activity coefficients were computed using the Davies equation [6]:

$$\log\gamma_i = -0.5091\sqrt{I}\Big/\left(1+\sqrt{I}\right)+0.15I, \qquad (1)$$

where I is the ionic strength. In the NaCl solutions I is equal to the concentration of NaCl Then, the E versus the logarithm of the NaCl concentration straight line was transformed into a $\log a_{Na^+}$ versus E straight line. This calibration curve was employed to obtain the $\log a_{Na^+,\text{free}}$ versus the logarithm of the total NaDHC concentration, c_T, curve from the E versus the $\log c_T$ curve obtained with the Na^+-ion-selective electrode. The very low concentration zone of the E versus $\log c_T$ curve obtained with the DHC^--ion-selective electrode was employed to obtain the $\log a_{DHC^-}$ versus E calibration straight line. This line was then used to obtain the complete $\log a_{DHC,\text{free}}$ versus $\log c_T$ curve from the remainder of the experimental data. With both $\log a_{i,\text{free}}$ versus $\log c_T$ curves, the ionic strength of the aqueous interaggregate solution was computed by using the free-ion activities instead of their concentrations, at each c_T. This I value was employed to compute γ_i, which was in turn used to compute the concentration of free Na^+ and DHC^- ions. Then, a new I value was computed with these concentrations. The iteration procedure was stopped when the difference between two successive γ_i values was less than 0.1%. Then, the concentrations of free Na^+ and DHC^- ions were plotted versus c_T.

Results

The pH–c_T curve is shown in Fig. 1 and the E versus $\log c_T$ curves for both ion-selective electrodes are shown in Figs. 2 and 3.

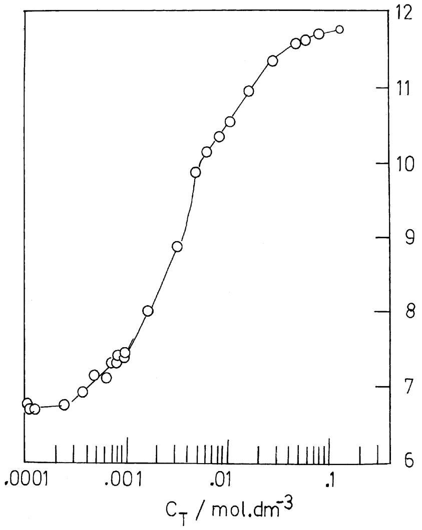

Fig. 1 pH versus total sodium dehydrocholate concentration curve

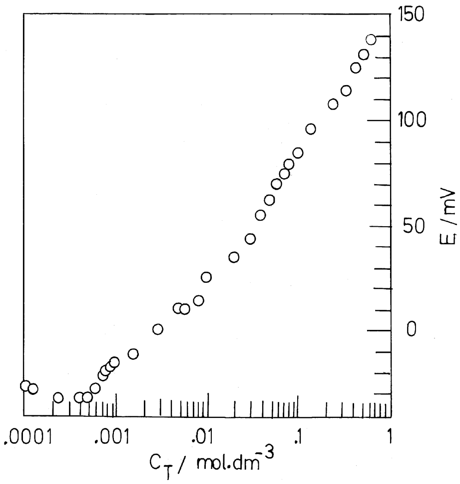

Fig. 2 Potential versus total concentration, Na^+-ion-selective electrode

Discussion

pH measurements

Accordingly, to the theory presented by Lucassen [7] for the dependence of the pH on the total concentration of the surfactant salt, c_T, which is applicable at c_T lower than the critical micelle concentration (cmc), we obtained the following results in previous work [8]. HDHC solubility in water $S_{HDHC} = (5.6 \pm 0.7) \times 10^{-10}$ $moldm^{-3}$, acidity constant $K_a = 0.0708 \pm 0.0055$. Below $c_1 = (9.6 \pm 4.2) \times 10^{-4}$ $moldm^{-3}$ the system is basically a monomer solution having some insoluble HDHC in suspension, between this concentration and $c_2 = (5.2 \pm 2.2) \times 10^{-3}$ $moldm^{-3}$ an acid soaplike aggregate (NaDHC.HDHC) appears, the proportion (and the size of aggregates) of which increases with total concentration above this concentration, and at $c_3 = (2.20 \pm 0.85) \times 10^{-2}$ $moldm^{-3}$ micelles appear. We refer to the $(NaDHC.HDHC)_n$ aggregates as "acid soaplike aggregates", to c_3 as the cmc and to the aggregates formed at c_3 as "micelles".

The theory of Stainsby and Alexander [9] mainly deals with the increased hydrolysis when micelles appear. The curve of degree of hydrolysis $\beta = [OH^-]/c_T$

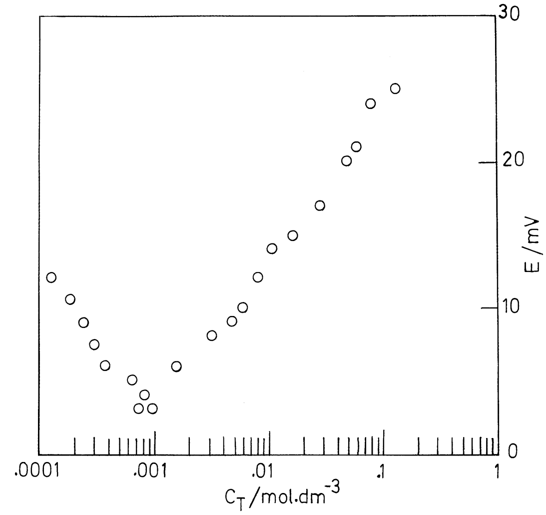

Fig. 3 Potential versus total concentration, dehydrocholate-ion-selective electrode

(Fig. 4) in which [OH$^-$] is the hydroxide ion concentration in moles per cubic decimetre, shows the typical shape when surfactant hydrolysis occurs and the acid molecules formed are solubilised into micelles. This curve has a minimum at $c_{T,\min} = 5\times10^{-3}$ moldm^{-3} ($\beta_{\min} = 2.3\times10^{-4}$) and a maximum at $c_{T,\max} = 4\times10^{-2}$ moldm^{-3} ($\beta_{\max} = 7.8\times10^{-2}$). At $c_T <$ cmc, this theory gives the hydrolysis constant of NaDHC as

$$K_h = \beta^2 c_T / (1 - \beta). \qquad (2)$$

Because this derivation ignores the activity coefficients, the value of K_h is dependent on c_T. To obtain the thermodynamic hydrolysis constant the logarithm of K_h was plotted as a function of the square root of the total concentration, and extrapolated to $c_T = 0$ This procedure gave p$K_{h,ter} = 12.85 \pm 0.09$, $K_{h,ter} = (1.40 \pm 0.01)\times10^{-13}$.

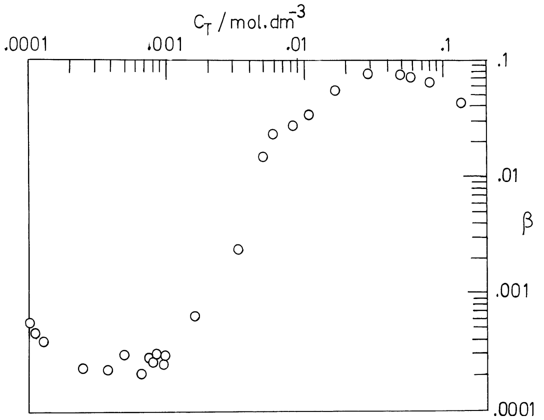

Fig. 4 Degree of hydrolysis versus total concentration

The theory of Stainsby and Alexander gives a concentration $c_F <$ cmc at which the acid molecules begin to aggregate. The value of c_F is assumed to be constant and may be computed by [9]

$$c_F = K_{h,ter} / \beta_{\min}, \qquad (3)$$

giving $c_F = 6.13\times10^{-10}$ moldm^{-3}, which is (within the error limits) the same value of the HDHC solubility [$S_{HDHC} = (5.6 \pm 0.7)\times10^{-10}$ moldm^{-3}] [8] as obtained from Lucassen theory.

The constant $K_D = [\mathrm{HDHC}]_{\mathrm{micelles}}/[\mathrm{HDHC}]_{\mathrm{water}}$ which describes the distribution of HDHC molecules between micelles and water at the cmc was computed by [9]

$$K_D = 4\rho\beta^2_{\max} / K_{h,ter}, \qquad (4)$$

in which ρ is the density of NaDHC micelles. There is no experimental value of the NaDHC partial molar volume, but that of KDHC is 320.6 ± 0.3 cm^{3}mol^{-1} [10]. By subtraction of the K$^+$ ion partial molar volume (4.5 cm^{3}mol^{-1}) [11] and addition of that of the Na$^+$ ion (–5.7 cm^{3}mol^{-1}) [11], we obtained the partial molar volume of NaDHC (310.5 ± 0.5 cm^{3}mol^{-1}) and then its density ($\rho = 1.418 \pm 0.001$ gcm^{-3}). We thus obtained $K_D = 5.77\times10^{-11}$.

From the β (c_T) plot the ratio, r, of the number of HDHC molecules in the aggregates per unit volume to the number of HDHC molecules in water per unit volume can be obtained:

$$r = [\mathrm{HDHC}]_{\mathrm{agg}} \Big/ [\mathrm{HDHC}]_{\mathrm{w}} = ([\mathrm{OH}^-] - c_F)/c_F. \qquad (5)$$

This value is plotted in Fig. 5. It can be seen that the number of acid molecules in the aggregates increased above 3×10^{-4} moldm^{-3}. Between this concentration and $c_T \approx 3\times10^{-3}$ moldm^{-3}, at which the acid soap appeared, the solid acid was in equilibrium with the dissolved acid. When the acid soap appeared, there was a sudden increase in r. There is only a slight change in the dependence of r on c_T at the cmc [$(2.28 \pm 0.55)\times10^{-2}$ moldm^{-3}]. This may mean that the transition among premicelles and micelles was gradual and did not imply a dramatic change in structure. This agrees with a stepwise aggregation process, which was proposed in previous work [8].

Ion-selective electrodes

The dependence of the free Na$^+$ and DHC$^-$ ions on the total concentration together with the concentration of aggregated surfactant (on a monomer basis) are presented in Fig. 6. It may be seen that at there is an increase in the total free Na$^+$ above $c_T \approx 4.9\times10^{-4}$ moldm^{-3}, which may be attributed to liberation of Na$^+$ ions by precipitation of acid. The dependence of the

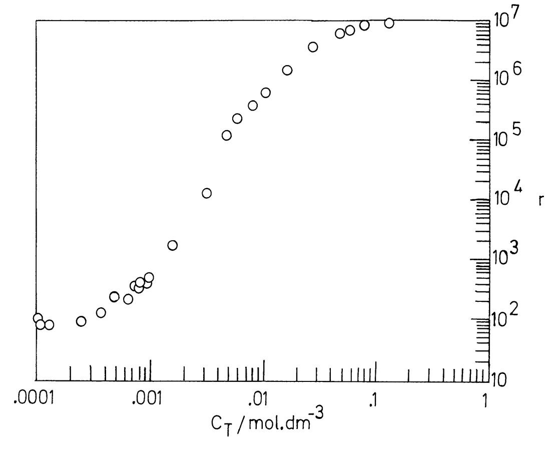

Fig. 5 Dependence of the ratio, *r*, of the number of dehydrocholic acid molecules in the aggregates per unit volume to the number of dehydrocholic acid molecules in water per unit volume on the total salt concentration

total free Na^+ concentration on c_T changes at the concentration at which acid soaplike aggregates form ($c_T \approx 3\times10^{-3}$ moldm^{-3}) but there is not a significant change in this dependence at the cmc. It is interesting that the concentration of free Na^+ ions is almost constant between 1×10^{-4} and 4.9×10^{-4} moldm^{-3}. There is information about the formation of alkaline-metal chelates in the literature [12, 13, 14, 15, 16]. Na^+ ions involved in chelates are not free to affect the Na^+-ion-selective electrode potential. From the shape of DHC molecules, the chelate structure may be that shown in Fig. 7. Although similar formation has been found in other steroid salts [17, 18], the structure of bile salts is different from that shown in Fig. 7. Kano et al. [17] suggested a hydrogen-bonded structure of a dimer of NaDC.

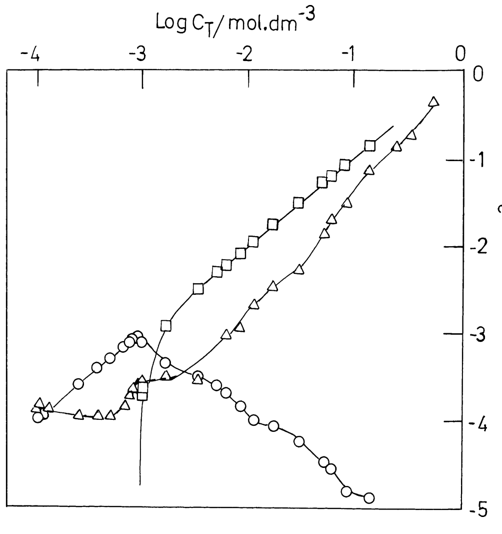

Fig. 6 The dependence of free Na^+ ions (*triangles*), free dehydrocholate ions (*circles*) and aggregated dehydrocholate ions (*squares*) on the total salt concentration

When other kinds of aggregates form, such as acid soaplike ones, premicelles or micelles, the sodium ions which are not involved in these aggregates behave as free ions and affect the electrode potential, thus giving the increase in concentration seen in Fig. 6. Moroi and Okabe [19] studied sodium ursodeoxycholate with a Na^+-ion-selective electrode and found more conventional behaviour. Below the cmc they found the common Nernstian response indicating the full dissociation of the bile salt monomer. Above the cmc this salt has a very high degree of ionisation.

Figure 6 also shows that the concentration of aggregated DHC increases when acid soaplike aggregates appear, and in solutions above $c_T \approx 3\times10^{-3}$ moldm^{-3} one may consider that all the added salt forms aggregates. Funasaki et al. [4] obtained the free surfactant ion concentration in equilibrium with micelles of NaTCDC and NaTUDC by frontal chromatography. For NaTCDC, the concentration of free ions above the cmc was constant, whereas that for NaTUDC slightly increased above the cmc. This behaviour is similar to that of taurocholate ions, obtained by quasielastic light scattering [20]. The NaDHC behaviour is similar to that of some more conventional surfactants such as alkyltrimethylammonium halides [21, 22, 23].

Figure 8 shows the number of aggregated DHC^- ions to aggregated HDHC molecules, *R*, computed by

$$R = \left(c_T - [DHC^-]_{free}\right) / \left([OH^-] - c_F\right). \quad (6)$$

At $C < 3\times10^{-4}$ moldm^{-3}, $R \approx 2$. Since it is an average ratio over all the aggregates in the solution, it may be interpreted in the sense that there is about one chelate unit for each acid molecule in solution. *R* decreased to about 1 between 3×10^{-4} and 1×10^{-3} moldm^{-3}, which corresponds to HDHC.NaDHC units, i.e., acid soaplike aggregates. However, there was a sudden increase at 0.0016 moldm^{-3} followed by a decreasing curve which reached a minimum at the cmc. This indicates that new

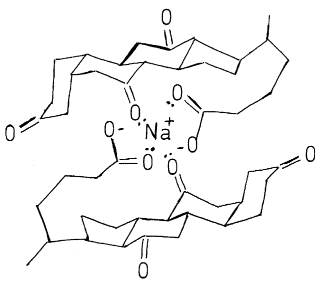

Fig. 7 The proposed structure for the chelate entrapping a sodium ion

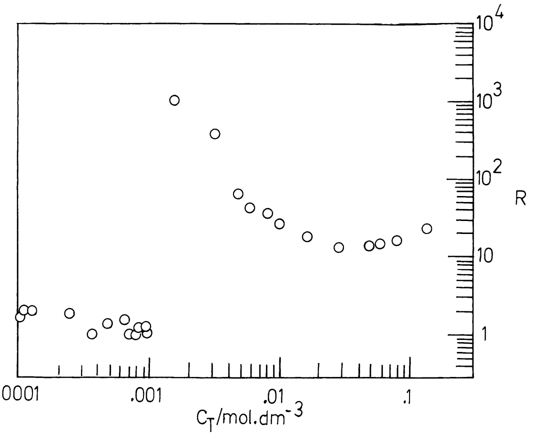

Fig. 8 The number of aggregated dehydrocholate ions to aggregated dehydrocholic acid molecules, R, as a function of total sodium dehydrocholate concentration

kind of aggregates, mainly formed by DHC^- ions, were formed. These aggregates increased [8] in size and captured increasing numbers of HDHC molecules, thus decreasing R. At the cmc, R increased slightly with c_T.

Figure 9 shows the charge per aggregated molecule, which is defined by

$$\alpha = \left([DHC]_{agg} - [HDHC]_{agg} - [Na^+]_{agg}\right) \Big/ [DHC]_{agg}, \quad (7)$$

in which $[HDHC]_{agg} = [OH^-]_{free}$ is the acid in aggregates and $[DHC]_{agg} = (c_T - [DHC^-]_{free})$ is the total concentration of DHC in the aggregates. The value of α was low (0.1–0.16) when premicellar aggregates formed at c_2, but increased with concentration above the cmc up to 0.54. This high value may be due to a large surface area per polar head group at the micelle–water interface. This leads to a reduction in the surface electrical potential and a reduction in the counterion adsorption. Similar high values of α have been found in other steroid surfactants. For sodium cholate (NaC) an α value ranging from 0.86 to 0.6 was found [24, 25, 26, 27, 29, 29]. Sugihara et al. [30] found $\alpha = 0.3$ for the same bile salt. Coello et al. [31] using pNa values together with micelle aggregation numbers obtained by freezing-point depression, found $\alpha = 0.923$ for NaC, which seems an excessively high value. They also found α values between 0.98 and 0.76 for several bile salts [31]. This may be due to an oversimplified model. On the other hand, Bonincontro et al. [32] found a very strong interaction between Na^+ ions and deoxycholate micelles and suggested that α is very low.

Small and coworkers [33, 34] proposed that small micelles of bile salts are formed by hydrophobic interactions among the convex backs of the nuclei and that large micelles are formed by intermolecular hydrogen bonds among the hydroxyl groups of these small micelles. This has been named the back-to-back model. NaDHC molecules have three carbonyl groups and both sides of the molecule are hydrophobic. This means that face-to-back hydrophobic-driven aggregates may form, the structure of which is essentially similar to that of the ordinary micelles. We confirmed this possibility with a molecular model. This situation may explain some differences among our findings and the data obtained from the literature for naturally occurring bile salts. In particular, the formation of acid soaplike aggregates is possible because a face-to-back association situates both carboxylic and carboxylate groups close enough to interact as in the common acid soaps.

The high α value is consistent with a low surface potential arising from a low surface charge density. This in turn may be due to the structure of the hydrophobic part, which hinders the formation of a compact hydrophobic core, even if a back-to-face aggregation structure is accepted.

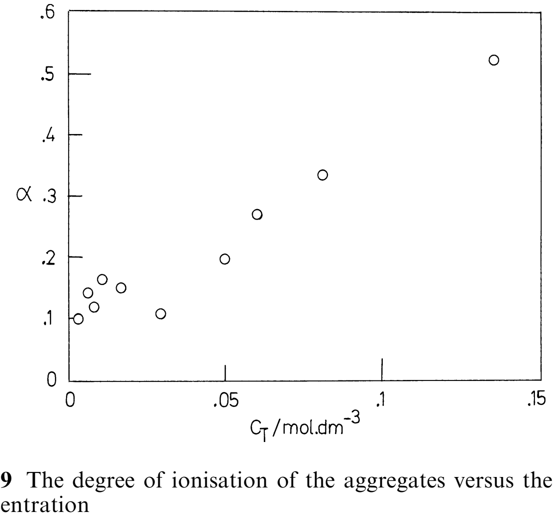

Fig. 9 The degree of ionisation of the aggregates versus the total concentration

Concluding remarks

- The solubility of HDHC found by the Lucassen theory [7] [$S_{HDHC} = (5.6 \pm 0.7) \times 10^{-10}$ moldm^{-3}] in previous work [8] was confirmed by the Stainsby and Alexander [9] treatment ($c_F = 6.13 \times 10^{-10}$ moldm^{-3}).
- The thermodynamic NaDHC hydrolysis constant value was found to be $K_{h,ter} = (1.40 \pm 0.01) \times 10^{-13}$.
- The distribution constant $K_D = [HDHC]_{micelles}/[HDHC]_{water}$ at the cmc, which describes the distribution of HDHC molecules between micelles and water, was 5.77×10^{-11}. This means that the concentration of HDHC in the micelles is very low at the cmc. However a better view of the total concentration of acid in the aggregates compared with the dissolved

HDHC molecules in water is given by $r = [\mathrm{HDHC}]_{\mathrm{agg}}/[\mathrm{HDHC}]_{\mathrm{w}}$, the dependence of which on c_T is presented in Fig. 5. This dependence suggests that the transition among premicelles and micelles was gradual and did not imply a dramatic change in structure.

- The composition of aggregates may be followed by the value of *R*, the number of aggregated DHC– ions to aggregated HDHC molecules. At $c < 3\times10^{-4}$ moldm^{-3}, there is one chelate unit per HDHC molecule. *R* decreased to about 1 between 3×10^{-4} and 1×10^{-3} moldm^{-3}, which corresponds to HDHC.NaDHC units, i.e., acid soaplike aggregates. At the cmc, micelles are formed mainly by DHC$^-$ ions, but when c_T increased, the micelles dissolved HDHC molecules and *R* decreased.
- The concentration of free Na$^+$ ions was constant at very low total concentration (below 4.9×10^{-4} moldm^{-3}) and this may be attributed to the formation of a chelate. The precipitation of acid liberated Na$^+$ ions. When acid soaplike structures formed ($c_T\approx3\times10^{-3}$ moldm^{-3}), the dependence of the Na$^+$ concentration on c_T changed.
- The concentration of free DHC$^-$ ions strongly decreases above the cmc, and above $c_T\approx3\times10^{-3}$ moldm^{-3} almost all the DHC$^-$ ions form micelles.
- The micelles are highly ionised, having α values which increase from the cmc to about 0.54. This is consistent with a low surface potential arising from a low surface charge density. This in turn may be due to the structure of the hydrophobic backbone, which hinders the formation of a compact hydrophobic core.

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