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The hydration of sodium dehydrocholate micelles

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Abstract The hydration of micellised sodium dehydrocholate molecules was determined by viscosity measurements. It was found that there are 39 water molecules for each micellised surfactant molecule. About ten water molecules may be attributed to the hydration of the sodium carboxylate group. By assignation of two water molecules to each of the three carbonyl groups, the total hydration of a

micellised sodium dehydrocholate molecule was estimated as about 16 water molecules. The remaining 23 water molecules per micellised sodium dehydrocholate molecule may be attributed to water trapped in the structure of micelles.

Keywords Micelles · Sodium dehydrocholate · Micelle hydration · Viscosity · Bile salts

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 head group 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. This carboxylate group may be conjugated with glycine or taurine. Bile salts associate in water to form micelles [1]. However, there are many open questions on the structure, size and shape of these aggregates and also on parameters such as the aggregation number and critical micellar concentration (cmc) [2, 3]. In order to clarify one of the characteristics of micelles, their hydration, we studied the viscosity of sodium dehydrocholate (NaDHC) aqueous solutions. NaDHC is a derivative of cholic acid by oxidation. It has three

carbonyl groups in the backbone instead of the hydroxyl groups of cholate (Fig. 1).

NaDHC is a scarcely studied surfactant. We studied NaDHC micelle formation previously [4, 5]. Micelle hydration is information of interest to know the micelle structure. It can be obtained from viscosity data. Hydration of bile salt micelles is presumably very different to that of common surfactant micelles. However, as far as we know, there is no work in the literature dealing with micelle hydration of bile salts.

Experimental

Dehydrocholic acid (HDHC) was from Dr. Theodor Schuchardt, and was of analytical grade. A concentrated NaDHC aqueous solution was made 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.

Viscosity measurements were made with an Ostwald viscometer. Water was used as a reference. All determinations were made at 25.0 °C.

A solution of 0.03 mol dm⁻³ NaDHC was treated with a solution of uranyl acetate following the staining technique to study the micelles by transmission electron microscopy as described in the

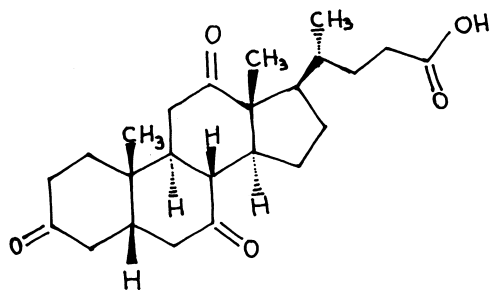


Fig. 1 The structure of dehydrocholic acid

literature [6, 7, 8]. Because of the dilution with uranyl acetate solution, the final concentration was slightly above the cmc, giving a dilute solution of micelles. The confidence level of errors was 0.90.

Theory

The reduced specific viscosity of micelles is defined by [9]

$$\eta_{sp}/c_M = (\eta/\eta_0 - 1)/c_M, \quad (1)$$

where η and η_0 are the viscosity of the micellar solution and of the solvent. The concentration of the micellised surfactant, c_M , in grams per cubic centimetre, is taken as

$$c_M = \frac{(c_T - [\text{DHC}^-]_{\text{free}})M}{1000}, \quad (2)$$

where c_T is the total concentration and $[\text{DHC}^-]_{\text{free}}$ is the concentration of free DHC^- ions, which was determined with a DHC^- ion-selective electrode [5], and M is the molecular weight of the surfactant.

By extrapolation to $c_M = 0$ the intrinsic viscosity may be obtained [10]:

$$[\eta] = vE(v_2 + wv_1), \quad (3)$$

where v is the shape factor, v_1 and v_2 are the specific volumes of the solvent (water) and the dry surfactant, and w is the ratio of the number of grams of water per gram of surfactant in the micelles. E is the electroviscous effect correction, which may be computed by Booth's theory [11]:

$$E = 1 + \frac{\pi(\sum c_i z_i^2 u_i^{-1})(\sum c_i z_i^2 u_i)(e\zeta/2\pi)^2(\kappa a)^2(1 - \kappa a)^2 Z}{(\sum c_i z_i^2)^2 \kappa_{sp} \eta_0}, \quad (4)$$

where c_i and z_i are the concentration and the charge (in e units) of the ion i , whose electrophoretic mobility is u_i , e , κ_{sp} and η_0 are the dielectric constant of the solvent, the specific conductivity and the viscosity of the solution at

the cmc, ζ is the zeta potential of the micelles, whose radius is a , and κ^{-1} is the Debye distance, computed by the Debye-Hückel equation [12]. Z is a function of κa which may be obtained from Ref. [13].

Results and computations

The viscosity-concentration curve for the NaDHC-water system is shown in Fig. 1. NaDHC associates in water by a stepwise mechanism. Below $(9.6 \pm 4.2) \times 10^{-4} \text{ mol dm}^{-3}$, there is a molecular solution with some strongly insoluble HDHC produced by hydrolysis. Between $(9.6 \pm 4.2) \times 10^{-4}$ and $(95.2 \pm 2.2) \times 10^{-5} \text{ mol dm}^{-3}$, an aggregate similar to acid soap ($\text{NaDHC} \cdot \text{HDHC}$) appears and its amount and the aggregate's size increase with concentration. At $c = (2.2 \pm 0.85) \times 10^{-2} \text{ mol dm}^{-3}$, 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 $8 \times 10^{-2} \text{ mol dm}^{-3}$, giving asymmetrical aggregates [4].

It may be seen in Fig. 2 that the acid-soap-like aggregates formed below the cmc affected the solution viscosity. Above the second critical concentration, a large increase in viscosity indicates that surfactant aggregates became strongly asymmetrical. We studied the region between the cmc and the second critical concentration.

The concentration of aggregated surfactant was computed with Eq. (2) and the values of $[\text{DHC}^-]_{\text{free}}$ obtained from previous work [5]. Then, this concentration was expressed in grams per cubic centimetre with the molecular weight of NaDHC, $M_{\text{NaDHC}} = 440.289 \text{ g mol}^{-1}$. The

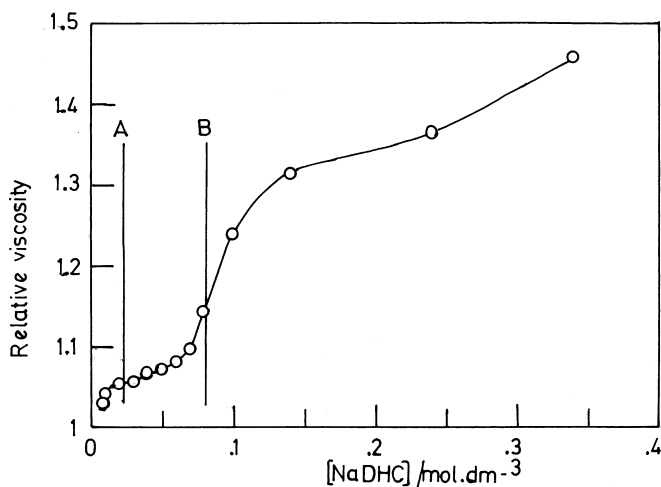


Fig. 2 Relative viscosity of sodium dehydrocholate (NaDHC) aqueous solutions. Line A is the critical micellar concentration, line B is the transformation from spheroidal micelles to anisometric ones [4]

reduced specific viscosity of the micelles was then computed with Eq. (1), and is plotted in Fig. 3.

The data fit the equation

$$\eta_{sp}/c_M = 5.4114 - 45.743c_M - 5753.1c_M^2 + 157808c_M^3, \quad (5)$$

with the correlation coefficient being $r=0.9997$. The strong negative slope indicates that there is an attractive interaction between the micelles.

To compute the electroviscous effect correction, the micelle radius, a , must be known. There is no literature value of a for NaDHC; however, there are some literature values of the hydrodynamic radius, R_h , of bile salt micelles at the cmc. For sodium chenodeoxycholate, sodium deoxycholate and sodium taurodeoxycholate, $R_h=0.5$ nm [14] and the same value was reported for sodium taurocholate [15]. For sodium deoxycholate and sodium taurocholate it was also reported that $R_h=0.8$ nm [16] and $R_h=1.1$ nm [17], respectively.

Cholic acid molecules may be roughly considered as banana-shaped with a diameter of 0.607 nm and a length of 1.321 nm [18]. Sugihara and Tanaka [18] proposed bile salt micelle structures with an aggregation number, n , between 2 and 10. These structures have an average radius of about 1 nm. For aqueous sodium cholate, Fontell [19] found by vapour pressure osmometry that the aggregation number was 3–4, whereas by X-ray spectrometry [20] he deduced a value of 16.

The microphotography of the uranyl-stained NaDHC micelles showed them as small granules. By using a low-amplification microscope, we measured 30 of these granules and obtained $a=1.00 \pm 0.06$ nm. The granules

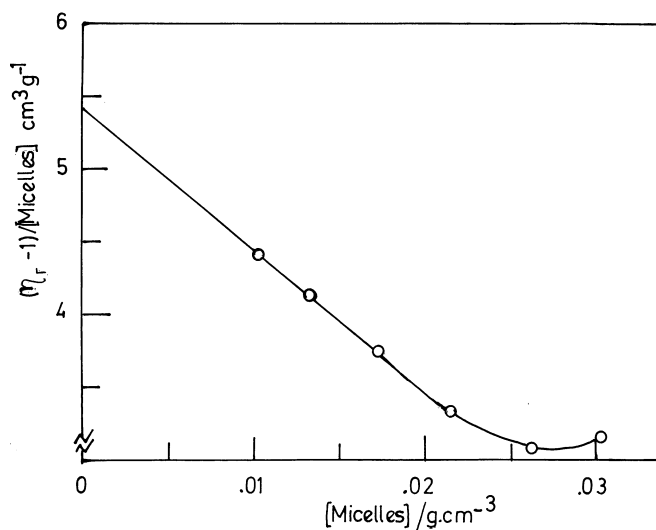


Fig. 3 Reduced specific viscosity of NaDHC micelles versus the concentration of aggregated surfactant

were nearly spherical. Then, the zeta potential of the NaDHC micelles was estimated by [21]

$$\zeta \approx \frac{Q}{4\pi\epsilon\epsilon_0 a(1 + \kappa a)}, \quad (6)$$

where ϵ_0 is the vacuum permittivity. The micelle charge is $Q = ne\alpha$, n being the aggregation number, e the elementary charge and α the degree of ionisation of the micelles. On the basis of the micelle structure of Sugihara and Tanaka for $a=1$ nm, we took $n=8$. From potentiometric data, $\alpha=0.1$ at the cmc [5]. The concentrations of free ions at the cmc were $[\text{Na}^+] = 0.00442$ and $[\text{DHC}^-] = 0.000069$ mol dm⁻³ [5], so the ionic strength at the cmc was $I=0.00224$ mol dm⁻³ and the inverse of the Debye distance at the cmc was $\kappa=0.1557$ nm⁻¹. Then $Q=1.28 \times 10^{-19}$ C and $\zeta \approx 14.4$ mV. From Booth's theory [13], $Z=0.00724$.

From the literature, $\Lambda_{\text{NaDHC}}^0 = 95.4$ S cm² Eq⁻¹ [4]. With this value and the Na⁺ ion conductivity at infinite dilution, $\lambda_{\text{Na}^+}^0 = 50.9$ S cm² Eq⁻¹ [22], we obtained the DHC⁻ ion conductivity at infinite dilution, $\lambda_{\text{DHC}^-}^0 = 44.5$ S cm² Eq⁻¹. From $\lambda_{\text{DHC}^-}^0$ we computed the DHC⁻ ion electrophoretic mobility, $u_{\text{DHC}^-} = 4.61 \times 10^{-4}$ cm² s⁻¹ V⁻¹. The Na⁺ ion electrophoretic mobility ($u_{\text{Na}^+} = 5.275 \times 10^{-4}$ cm² s⁻¹ V⁻¹) was obtained from the literature [22].

The solution relative viscosity at the cmc was 1.055. With the viscosity of water (0.8904 cP [22]), the solution viscosity at the cmc was 0.9394 cP. Using Eq. (4) and the preceding data, the electroviscous effect factor was $E=1.076$. This is a small value, and the influence of the electroviscous effect may be considered smaller than the intrinsic viscosity experimental error.

There is no experimental value of the NaDHC partial molar volume, but that of KDHC is 320.6 ± 0.3 cm³ mol⁻¹ [23]. By subtraction of the K⁺ ion partial molar volume (4.5 cm³ mol⁻¹) [24] and addition of that of Na⁺ ion (-5.7 cm³ mol⁻¹) [24], we obtained the partial molar volume of NaDHC (310.5 ± 0.5 cm³ mol⁻¹) and then its specific volume, $v_2 = 0.7052 \pm 0.0005$ cm³ g⁻¹. The specific volume of water was $v_1 = 0.81659$ cm³ g⁻¹, computed from literature data [25]. Since the electron micrograph showed spheroidal micelles, we took the Einstein shape factor $\nu=2.5$. By application of Eq. (3), the weight of water per gram of surfactant was $w=1.60 \pm 0.02$. This value gives 39 water molecules for each micellised NaDHC molecule. In comparison, dodecyltrimethylammonium hydroxide micelles have 39.2 ± 0.7 water molecules per surfactant molecule [26], alkyltrimethylammonium bromides have 60–70 [27]; sodium soaps have about 10 [28, 29], the nonionic surfactant Triton X-100 has 43 [30] and sodium dodecyl sulfate has 6 [31].

From the sodium soap data, ten water molecules may be attributed to the hydration of the sodium carboxylate group. The hydration of two water molecules for each carbonyl group may be supposed regarding the two free

electron pairs of the oxygen atom. The other 23 water molecules per micellised NaDHC molecule may be attributed to water trapped in the structure of the micelles. Because of the rigid and bulky steroid backbone, the structure of the micelles cannot be very compact and water can intercalate among surfactant molecules. This situation was already seen in the three-dimensional representation of bile salt micelles given by Sugihara and

Tanaka [18]. A noncompact structure was also proposed for sodium taurodeoxycholate [32], sodium taurocholate [33] and sodium deoxycholate micelles [34].

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