Aqueous sodium dehydrocholate–sodium deoxycholate mixtures at low concentration

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Abstract

The behavior of the sodium dehydrocholate (NaDHC)–sodium deoxycholate (NaDC) mixed system was studied by a battery of methods that examine effects caused by the different components of the system: monomers, micelles, and both components. The behavior of the mixed micellar system was studied by the application of Rubingh's model. The obtained results show that micellar interaction was repulsive when the aggregates were rich in NaDHC. The gradual inclusion of NaDC in micelles led to a structural transformation in the aggregates and the interaction became attractive. The bile salts' behavior in mixed monolayers at the air–solution interface was also investigated. Mixed monolayers are monotonically rich in NaDC, giving a stable and compact adsorbed layer. Results have shown that the interaction in both micelles and monolayer is not ideal and such behavior is assumed to be due to a structural factor in their hydrocarbon backbone.
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1. Introduction

Despite having a history of more than a century, bile-acid science (cholanology) continues to have importance in biology and medicine [1–5]. Bile salts are steroidal surfactants, which in aqueous environment aggregate to form micelles [6]. Under physiological conditions these micelles are transformed into mixed micelles, which are responsible for fat/cholesterol solubilization in the small intestine.

In addition to their physiological roles, bile salts' mixed micelles are promising systems for drug delivery. The solubilization of drugs by bile salts micelles (through the formation of mixed micelles) has been examined [7]. It has recently been realized that the high specificity and capacity of bile acid transport systems during their enterohepatic circulation might form the basis of research on drug–bile acids conjugates for specific drug targeting to the liver and on improving the intestinal adsorption of poorly adsorbed or nonadsorbed drugs, such as peptides [8,9].

The physical chemistry of bile salt micellization has been, and still is, an active area of research. A variety of techniques have been employed to gain more insight into the structure/size/shape of bile salts micelles.

Unlike conventional surfactant molecules, bile salts possess a rigid steroid backbone having polar hydroxyl groups on the concave α-face and methyl groups on the convex one. This arrangement creates a unique facial amphiphilicity for this class of molecules, causing aggregation in water different from that of conventional amphiphiles.

In a previous work we studied the physicochemical aggregation properties of sodium dehydrocholate in aqueous solution [10–13] and its behavior in combination with other surfactants [14] or proteins [15].

The purpose of this research was better knowledge about the formation of bile salts mixed micelles and mixed monolayers. The aim is to understand the behavior of such systems in the biological environment and the optimization of applications of these surfactant mixtures. We paid special attention to the electrostatic and steric interaction of the two surfactants in mixed micelles and in the mixed air–solution monolayer, with the goal of understanding the interplay of forces that govern the phase behavior.

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The understanding of the mixed monolayers' behavior at the aqueous solution–air interfaces may be of fundamental importance in the formulation of a general theory for the cholelithiasis processes. The investigation of mixed monolayer properties has great interest because the interactions between the components in the monolayer play an important role in some biological processes.

2. Experimental

2.1. Materials

Dehydrocholic acid (HDHC) was obtained from Dr. Theodor Schuchardt (Munich) and was of analytical grade. Sodium deoxycholate (NaDC) was obtained from Aldrich, 99%, and used as purchased.

2.2. Solutions

Dehydrocholic acid sodium salt (NaDHC) solution was prepared by weighing a quantity of HDHC and by dissolution in an appropriate amount of concentrated NaOH.

Stock NaDHC and NaDC solutions (0.1 mol dm^{-3}) were prepared and diluted as required for each experiment. The appropriate amounts of NaDHC and NaDC stock solutions were mixed to obtain the different NaDHC–NaDC mixture solutions.

Only double-distilled water was used.

2.3. Methods

Conductivity measurements were performed with an immersion cell and an automatic conductimeter, namely an Antares II from Instrumentalia. Surface tension was measured with a ring tensiometer from Krüss. pH determinations were performed with a millivoltmeter and a pH-meter from CRIBABB with a Broade and James glass electrode.

Density and viscosity measurements were performed in an Anton Paar DMA 35 N densimeter and an AND SV-10 Vibro viscosimeter, respectively.

All determinations were performed by titration of 50 ml of a stock solution (0.1 mol dm^{-3}) of each pure surfactant and their mixtures with water at $25.0 \pm 0.1\,°C$.

3. Results and discussion

3.1. Micellar behavior

In order to perform a better analysis of conductivity data, $\Delta\kappa = \kappa - \kappa_{\text{extrapolated}}$ versus concentration method was used [16]. The $\kappa_{\text{extrapolated}}$ values were found by extrapolation of pre-cmc specific conductivity data by fitting them to a least-squares straight line. This method magnifies the slope difference between pre- and post-cmc data. The cmc values were corroborated by the employment of other techniques such as pH, density, and viscosity measurements (results not shown).

The dependence of the cmc on the surfactant mixture composition is shown in Fig. 1, in which α_{NaDC} is the mole fraction of NaDC in the surfactant mixture, without considering the sol-
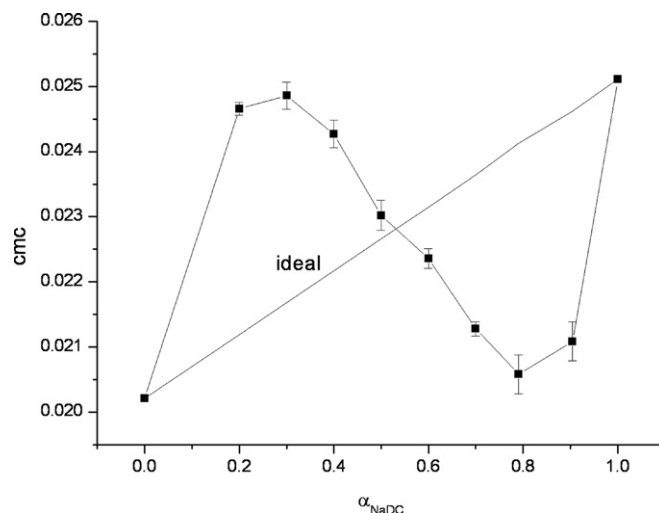

Fig. 1. Critical micelle concentration (CMC) dependence on the mole fraction of NaDC (α_{NaDC}) in the mixed surfactant system. Error bars were computed with a 0.90 confidence level. The straight line represents the ideal behavior computed with the following equation: $\text{cmc}_M = [(\alpha_i/\gamma_{i,M}\,\text{cmc}_i) + (\alpha_j/\gamma_{j,M}\,\text{cmc}_j)]$.
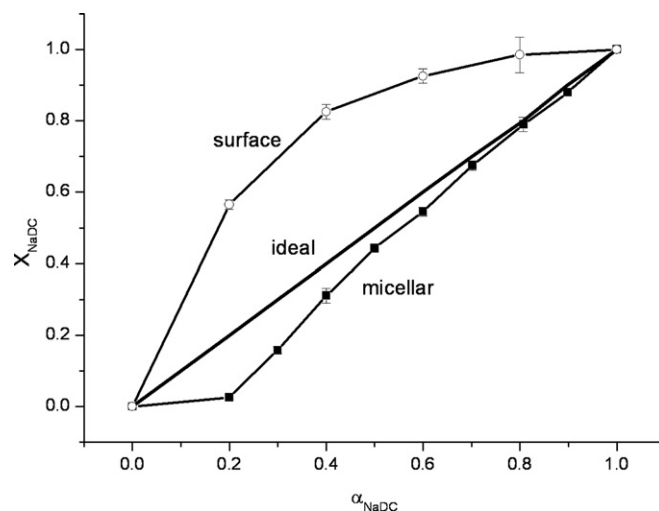

Fig. 2. Variation of the mole fraction of NaDC in mixed micelles and in the mixed monolayer at the air–solution interface (X_{NaDC}) versus surfactant mixture composition (α_{NaDC}).

vent. The micelle composition was computed with Holland and Rubingh's model [17] and is plotted in Fig. 2 together with the ideal behavior. Here X_{NaDC} is the mole fraction of NaDC in micelles, on a surfactant-only basis.

Holland and Rubingh's model is the first model developed for nonideal mixed systems [17]. It is based on a regular solution approach to the treatment of nonideal mixing and due to its simplicity; it has been the main model used, even after the development of more complex models. The nonideality is introduced with the inclusion of the activity coefficients γ_i, into the relationship between the critical micellar concentration of the mixed system (cmc_M) and of the i pure components (cmc_i):

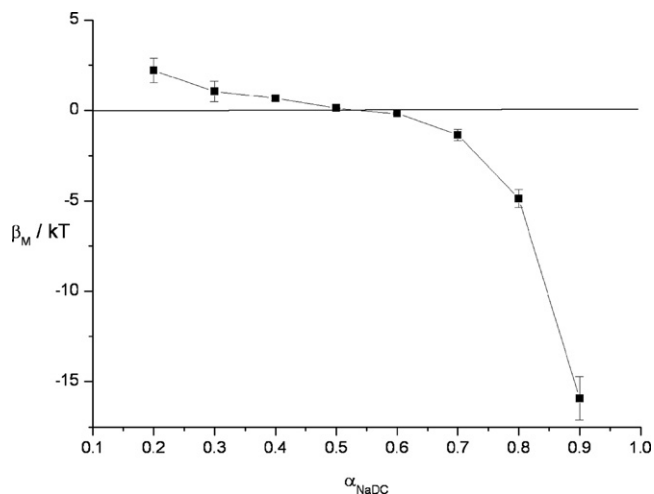$$\frac{1}{\text{cmc}_M} = \sum_i \frac{\alpha_i}{\gamma_i \text{cmc}_i}. \tag{1}$$

Fig. 3. Micellar interaction parameter (β_M) plotted as a function of surfactant mixture composition.

For a binary solution, for example, according to this model we have

$$\gamma_i = \exp\left[\beta_M(1 - X_i)^2\right] = \exp\left[\beta_M(X_j)^2\right], \quad (2)$$

$$\gamma_j = \exp\left[\beta_M(X_i)^2\right]. \quad (3)$$

Here X_i is the molar fraction of the ith surfactant in the micelle and the β_M parameter (in kT units, where k is the Boltzmann constant and T the absolute temperature) can be interpreted in terms of an energetic parameter that represents the excess Gibbs free energy of mixing. The β_M parameter can be determined from experimental values of cmc_M using the following expressions:

$$\beta_M = \frac{\ln(\frac{\alpha_i cmc_M}{X_i cmc_i})}{X_j^2} = \frac{\ln(\frac{\alpha_j cmc_M}{X_j cmc_j})}{X_i^2}. \quad (4)$$

The micellar composition X_i and the activity coefficients γ_i can be obtained from β_M by using Eqs. (2) and (3). β_M quantitatively captures the extent of nonideality. The larger the negative values of β_M, the stronger the attractive interaction between the two different surfactant molecules. Repulsive interactions yield a positive β_M value, whereas $\beta_M = 0$ indicates an ideal mixture.

The cmc values for the different NaDC–NaDHC mixtures show a transition from repulsive (at $\alpha_{NaDC} < 0.51$) to attractive (at $\alpha_{NaDC} > 0.51$) interaction. On the other hand, Fig. 2 shows that in micellar aggregates the NaDC content was lower than that in the bulk solution (i.e., $X_{NaDC} < \alpha_{NaDC}$). Nevertheless, micelle compositions tend to ideal behavior at high α_{NaDC} values.

The β_M values (Fig. 3) are positive at low X_{NaDC}, zero at $X_{NaDC} = 0.51$, and negative at higher X_{NaDC} values. The highest positive β_M value was about $2.21kT$, which is typical for hydrocarbon–fluorocarbon surfactant mixtures. As an example, $\beta_M = 2.2kT$ for a mixture of lithium dodecyl sulfate–lithium perfluorooctanoate [18]. Furthermore, the highest negative β_M value was $-15.92kT$, which is a typical value for catanionic mixtures [18].

The activity coefficients of both surfactants in mixed micelles (γ) are shown in Fig. 4 as functions of the square root
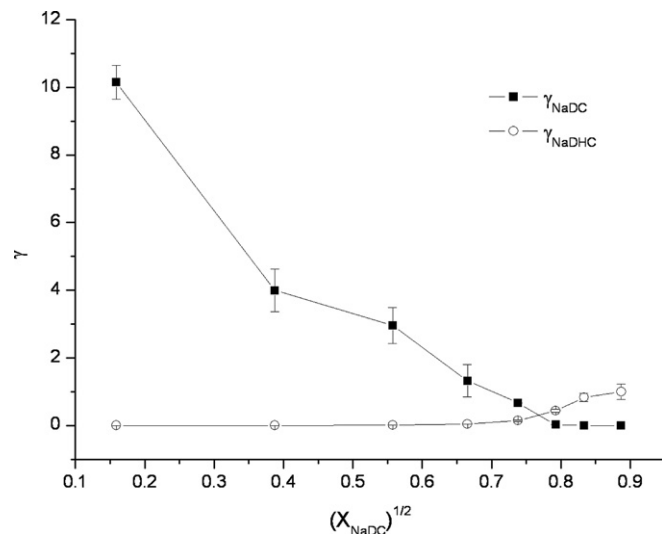

Fig. 4. Activity coefficient (γ) of NaDC and NaDHC in micelles vs the square root of the micelle composition $(\alpha_{NaDC})^{1/2}$.

of micelle composition. In the repulsion zone γ_{NaDHC} remained constant and near to the ideal value ($\gamma_{NaDHC} = 1$). This fact implies that the mixed aggregate neighborhoods for these surfactant molecules are not very different from those in pure NaDHC micelles. In contrast, γ_{NaDC} is higher than unity, indicating a high repulsion for such surfactant to integrate the mixed micelles, which are basically of NaDHC (as shown in Fig. 2). The gradual inclusion of NaDC molecules inside NaDHC micelles gives rise to a structural change that modifies the interactions between surfactants from repulsion to attraction. This last fact was reflected in the γ values, which show mutual miscibility behavior. In fact, the composition becomes almost ideal. An explanation for such behavior would be the following: when aggregates are rich in NaDHC, the interaction between NaDHC and –NaDHC molecules is stronger than between NaDHC and NaDC, and consequently the NaDC molecules are rejected from micelles. Nevertheless, when NaDC predominates in micelles, aggregates of this salt act as a good solvent for NaDHC molecules, giving rise to an attractive interaction.

The effect may also be explained by the high water solubility of NaDC, which interacts with water via the hydroxyl groups. The inclusion of NaDC in NaDHC micelles may hinder this interaction by replacing C–OH≡OH$_2$ hydrogen bonds with C–OH≡O=C bonds. But these latter hydrogen bonds are less favored than the former, because they depend on the mutual orientation of the two bile salt backbones. Thus, the inclusion of NaDC molecules in NaDHC-rich micelles may be disfavored, while that of NaDHC molecules in NaDC-rich ones may be favored by the formation of C–OH≡O=C bonds stabilizing the dissolution in micelles. Since micelles are rich in NaDC molecules, only a few (those with the appropriate molecular orientation) of them may interact with NaDHC, the remaining ones maintaining the interactions existing in pure NaDC micelles.

The micellar ionization degree (α) was computed with the equation proposed by Evans [19],

$$1000(d\kappa/dC)_2 = \frac{(n - m)^2}{n^{3/4}}\left[1000(d\kappa/dC)_1 - \lambda_X\right] + \alpha\lambda_X, \quad (5)$$

Table 1
Micellar ionization degree (α)

α_{NaDC}	$X_{NaDC,M}$	α
0	0	0.43168
0.2	0.0239	0.42947
0.3	0.1313	0.4307
0.4	0.2705	0.43098
0.5	0.4395	0.42329
0.6	0.5527	0.42596
0.7	0.6809	0.41755
0.8	0.7917	0.42883
0.9	0.8934	0.4205
1	1	0.41673

Fig. 5. Surface tension at the critical micelle concentration versus α_{NaDC}.

where $(d\kappa/dC)_1$ and $(d\kappa/dC)_2$ are the slopes of the specific conductivity curves before and after the cmc, n is the aggregation number, m is the number of counterions attached to the micelle, and λ_X is the equivalent conductivity of the counterion ($\lambda_{Na^+} = 50.9$ S cm^2 mol^{-1} [20]). Equation (5) is a quadratic function of α, because $(n - m) = n\alpha$. It is also dependent on n; however, this dependence is not strong and any reasonable value of n gives a good estimation of α [19]. We employed $n = 10$, which is a common value for n in bile salt micelles [21].

The dependence of α on micelle composition and on total mixture composition is shown in Table 1. It may be seen that mixed micelles had ionization degrees slightly lower that those of pure surfactant micelles. This is consistent with a closely packed micellar head group layer. High α values are consistent with a low surface potential arising from a low surface charge density in a naked micelle (i.e., a micelle without attached counterions). Since α in mixed micelles was smaller than that in both pure NaDHC and pure NaDC micelles, it may be concluded that the carboxylate groups appertaining to both surfactants must be in the same region of the mixed aggregates, forming a compact ionic layer. If the carboxylate groups were distributed on the whole surfaces of mixed micelles, the surface density of charge might be smaller than that of pure surfactant micelles and α might be higher than for pure NaDC or NaDHC aggregates.

4. Air–solution interfacial behavior

The interfacial behavior of the mixed system can be treated by an extension of the pseudophase separation model for micelles, using a nonideal analogue of Butler's equation [22,23], giving [24]

$$\pi^{mx} = \frac{RT}{A_i} \ln(\gamma_{i,M} X_{i,M} / \gamma_{i,S} X_{i,S}) + \pi_i, \qquad (6)$$

in which A_i is the area per mole of pure i surfactant at the air–water interface, π_{mx} and π_i are the surface pressures at the cmc of the surfactant mixture and of the i component, respectively, and $\gamma_{i,S}$ and $X_{i,S}$ are the activity coefficient and mole fraction of i component in the surface adsorbed state, respectively, while $\gamma_{i,M}$ and $X_{i,M}$ are the same properties in the micellized state. When this equation was derived, the assumption that A_i does not change in surfactant surface mixtures was made. The activity coefficients of the adsorbed monolayer are given by

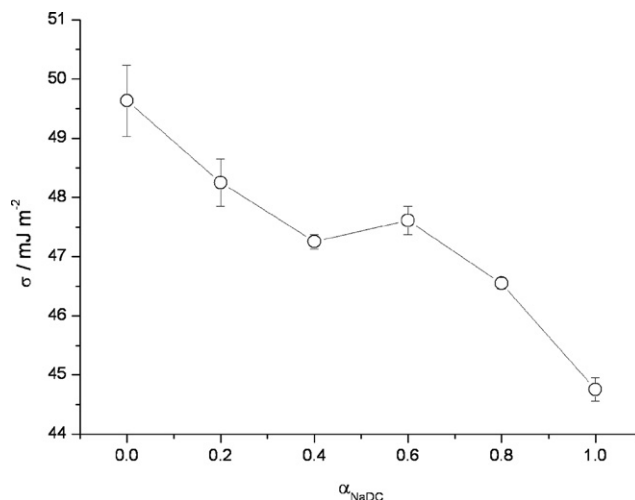$$\gamma_{i,S} = \exp(\beta_S X_{j,S}^2), \qquad (7)$$

where β_S is a dimensionless parameter, interpreted as representing an excess free energy of mixing in the surfactant aggregate at the interface [24]. Equations (6) and (7), together with the constraint that the surface mole fractions $X_{j,S}$ sum to unity and the measured values of π_{mx} and π_i, give the basis for an iterative solution of the model, provided the micellar composition and activity coefficients were previously computed.

The mixed monolayers at the air–solution interface are monotonically rich in NaDC (Fig. 2), leading to stable adsorbed layer formation (probably stabilized by H-bonds between NaDC and NaDHC molecules). This fact reduced the surface free energy excess at the cmc, as can be seen in Fig. 5.

On the basis of dehydrocholate ion structure, which may roughly be considered as a rectangular plate of width 0.571 nm and length 1.286 nm [25], the computed value of NaDHC area was $a_{NaDHC} = 0.734$ nm^2. Therefore, the experimental value ($a_{NaDHC} = 1.07$ nm^2) may be explained by an accommodation of the molecule, with its plane lying parallel to the water surface. This situation allows the three carbonyl groups in the hydrocarbon backbone to form hydrogen bonds with water molecules, as can be seen in Fig. 6. Some literature values of the limiting area at the collapse pressure for bile salt monolayers are 1.08 nm^2 (cholic acid), 0.80 nm^2 (chenodeoxycholic acid), and 0.73 nm^2 (ursodeoxycholic acid) [26]. The similarity of the preceding areas to that of NaDHC suggested that the adsorbed monolayers at the air–solution interface at the cmc were very compact. Other bile salts show less compact monolayers at the cmc, with an area per molecule of 1.48 nm^2 (sodium cholate) and 2.23 nm^2 (sodium deoxycholate) [27].

The area per adsorbed molecule was computed for each α_{NaDC} from surface tension plots using the Gibbs equation,

$$\Gamma_i = \frac{1}{2RT} \frac{\partial \sigma}{\partial \ln C_i}, \qquad (8)$$

and the relationship $a_i = (\Gamma_i N_A)^{-1}$, and plotted in Fig. 7. The solid line was computed, on the supposition that the a_i values of the two pure surfactants do not change in the mixed monolayer, with the following equation:

$$a_{average} = a_{NaDC} X_{NaDC} + a_{NaDHC}(1 - X_{NaDC}). \qquad (9)$$
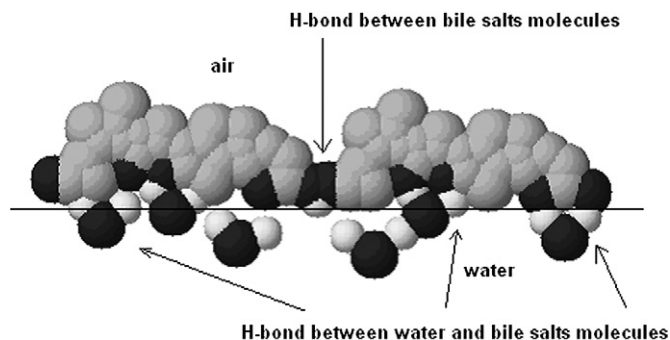
Fig. 6. Proposed accommodation of the NaDHC and NaDC molecules adsorbed at the air–water interface.
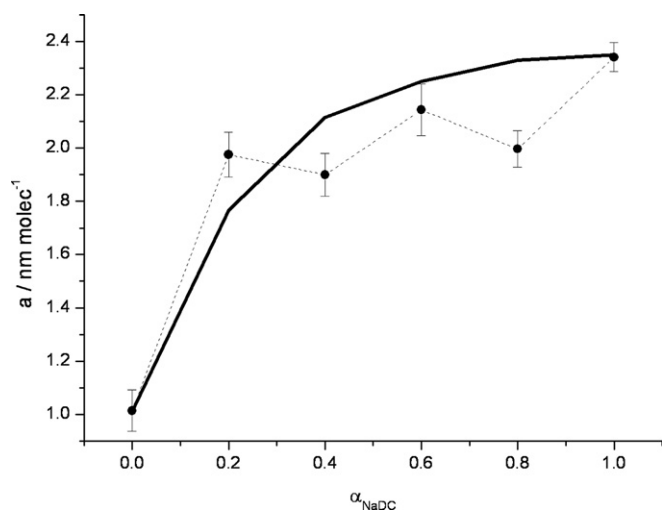

Fig. 7. Average area per molecule at the air–solution interface versus α_{NaDC} computed at the critical micelle concentration. The full line represents the ideal behavior.

It can be seen that the experimental data approximately fit to Eq. (9). The experimental $a_{average}$ values are slightly smaller than the theoretical ones. This can be explained by assuming a lateral attractive interaction (which was corroborated by the obtained negative β_S values, not shown) between the two bile salts at the surface. This fact increased the monolayer compactness. Taking into account the structure of NaDC and NaDHC molecules, such interactions would probably be through H-bonds between C=O and C–OH groups in NaDHC and NaDC molecules, respectively.

5. Concluding remarks

Bile salts molecules form rigid back-to-back micelles in an aqueous solution owing to bulky hydrophobic parts with a strong affinity among steroid rings in the molecules [1–10]. As a previous step in the study of carrier properties, the bile salts mixed micelles and air–water monolayer have been investigated in our laboratory using a battery of experimental methods. In both structures the interaction was not ideal. In mixed micelles, repulsive interaction exists when the aggregates are rich in NaDHC, and consequently the NaDC molecules are expelled from micelles. The gradual micellar inclusion

of NaDC leads to a structural transformation in aggregates and micellar interactions change from repulsive to attractive when $X_{NaDC} > 0.5$. This behavior is also reflected by the activity coefficients' values. When aggregates are rich in NaDC, these molecules act as a good solvent for NaDHC, giving rise to an attractive interaction. At this point the micelle composition is almost ideal.

The monolayer at the air–solution interface is systematically richer in NaDC than the bulk, and a nonideal interaction was found. The presence of NaDHC molecules and their possibility of form H-bonds with NaDC molecules and with water leads to a more compact and stable monolayer than expected from ideal mixing of the pure components.

Acknowledgments

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