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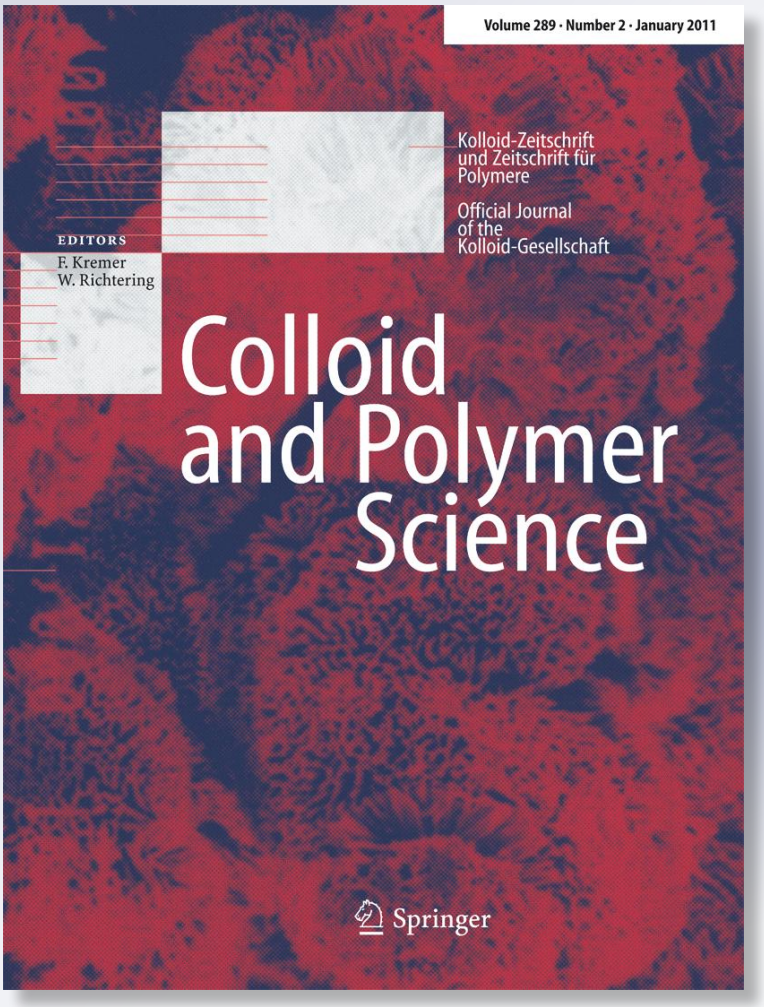

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ORIGINAL CONTRIBUTION

Bile salt structural effect on the thermodynamic properties of a catanionic mixed adsorbed monolayer

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Abstract The interfacial effects of two bile salts (sodium deoxycholate (NaDC) and sodium dehydrocholate (NaDHC)) in a catanionic mixed adsorbed monolayer have been investigated at 25 °C. The surfactant interfacial composition, the interfacial orientation of the molecules and the energy changes are analysed to show a thermodynamic evidence of the hydrophobic BSs effect during its intercalation into interfacial adsorbed didodecyldimethyl ammonium bromide (DDAB) molecules. Both mixed systems (NaDC–DDAB and NaDHC–DDAB) have analogous adsorption efficiencies, which are similar from a pure DDAB monolayer and superior to that obtained for both bile salts molecules. Nevertheless, their adsorption effectiveness is different: NaDC causes an increment of Γ while NaDHC produces the opposite effect. The adsorption efficiency in surface tension reduction is due to the existence of interfacial synergistic interactions (confirmed by the analysis of β^{γ} and $\Delta G_{ad}{}^{0}$ values). Maximum synergistic interaction is seen for α_{BSs}= 0.4. The hydrophobic steroid backbone of NaDHC molecule presents a deep interfacial penetration than NaDC. This fact causes a great disturbance of DDAB hydrocarbon tails and conduces to a large separation of molecules (high A_m values) which explains the reduction of adsorption effectiveness (low Γ_m values).

Keywords Bile salts · Adsorbed monolayers · Thermodynamic · Membrane disturbance

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Introduction

The surface-active agents used in a multitude of industrial products, processes and other applications usually consist of a mixture of surfactants. Therefore, the influence of the interactions between the components and their physico-chemical properties, including adsorption behaviour and micelle formation are of fundamental importance [1] Mixed surfactant systems are also very interesting from theoretical point of view. The effectiveness of a mixed surfactant system is related to specific interactions between molecules of different surfactants, which can enhance or deteriorate the action of a mixture with respect to some property of single systems. In contrast, some effects that are not expected in pure systems can take place in aqueous solution containing mixed surfactants, for example, synergism in surface tension reduction [2].

A particular type of mixed system is the so-called catanionic surfactant mixture, a mixture of an anionic and a cationic amphiphile where their respective counterions are also present [3].

Cationic surfactants have wide applications as disinfectants, antiseptic agents, as antistatic agents (hair conditioners), textile softeners, corrosion inhibitors, foam depressants, biocides and emulsifiers [4]. Because of their positive charge, they are able to interact strongly with DNA helices. Alkyltrimethylammonium salts are used together with other lipids in transfection experiments or for DNA extraction from cells. For cationic surfactant–lipid mixtures, DNA compaction at vesicles is documented [5]. Membrane structures formed in the presence of surfactants, such as lipid domains, lipid clusters, or so-called surfactant resistant membrane patches, were intensively studied in recent years [6]. Studies of the interaction of lipids and surfactants are of great relevance for the understanding

of solubilization of membrane proteins and their purification as well as the reconstitution of membrane proteins in a biological environment [7]. In addition, intense research was conducted on surfactants in combination with phospholipids for pharmaceutical formulations as drug delivery systems for slightly soluble therapeutic agents [8]. The wide field of different applications is the reason for the intensive study of cationic surfactants, especially their mixtures with biological surfactants as bile salts (BSs).

The BSs are surfactants of vital biological importance [9–11]. They play an active role in the emulsification of fats in the gut and aid in the excretion process of lecithin and cholesterol. Unlike conventional surfactants molecules, BSs posses a rigid steroid backbone having 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 an aggregation in water different from that of usual surfactants [9]. Due to the natural occurrence of BSs in complex mixtures with other compounds, phase behaviour investigations have naturally extended to ternary and quaternary systems where bile salts are mixed with phospholipids [12, 13], monoglycerides [14] or several common synthetic surfactants [15, 16]. Various types of micellar and vesicular aggregates have been directly imaged in human bile [10, 11]

The phase and aggregation behaviour of aqueous catanionic mixtures of BSs have been studied extensively [17–22]. Swanson-Vethamuthu et al. [18–22] showed large differences in the mixed system behaviour depending on the bile salt molecular structure. Both CTAB–NaC and CTAB–NaDOC mixtures were non-ideal at and below the critical micellar concentration, owing to the strong interaction between the oppositely charged surface-active ions and the dissimilar molecular structures of the hydrophobic moieties in the participating components. Fluorescence quenching studies on the aggregation behaviour have clearly demonstrated that the micelles were small for the CnTAB–NaC system over the entire concentration range studied but tended to grow into rod-like cylindrical micelles for the CnTAB–NaDOC system close to the equimolar concentrations where phase separation into two isotropic phases occurred. No coacervation region was found for the trihydroxy bile salt, NaC, thus showing a major difference in phase behaviour. Experiments based on cryo-TEM and viscosity consistently showed differences in micelle microstructure and relative viscosity between the two systems. Static and dynamic light scattering studies showed the presence of strong intermicellar interactions and large structures in the CnTAB–NaDOC system but gave poor scattering intensity from the corresponding CnTAB–NaC mixtures. Furthermore, when NaC promoted the formation of globular aggregates with high curvature, NaDOC induced a formation of cylinder micelles. At high concentrations of an added electrolyte, however, NaC also gave long micelles. These differences were awarded to the mode of insertion of the bile ions into the aggregates. Cholate ions predominantly adsorb flat onto the hydrophobic core/water interface of the micelle, promoting highly curved aggregates whereas desoxycholate ions, with one OH group less, have a larger tendency to be inserted into the core, and allow aggregates of less curvature to form. The head group interactions between the oppositely charged surfactants are of course strongly attractive and would allow a tight packing at the surface.

Despite many investigations dealing with such type of catanionic mixtures, the problem of interactions in the surface film and micelle in the composed systems still has some unclear points. The aim of this article is to show a thermodynamic evidence of the hydrophobic BSs effect [23] during its intercalation into interfacial adsorbed cationic didodecyldimethyl ammonium bromide (DDAB) molecules. The interfacial chemistry created by adsorbed molecules, both biological and synthetic, dominates the end-use properties of the material in many different applications. Surface tension has proved to be a valuable tool to evaluate the thermodynamics of solute and detergent partitioning into interfaces. Adsorption is undoubtedly one of the most important manifestations of surface and interfacial energies. We paid special attention to determine the surfactant interfacial composition, the interfacial orientation of the molecules and the energy changes to gain more insight into the structure-activity of BSs. The two molecules studied here, sodium dehydrocholate (NaDHC) and sodium deoxycholate (NaDC), were selected because are very similar to each other except for the presence of hydroxyl groups. NaDC has two hydroxyl groups (3 and 12 α-OH) while NaDHC has three carbonyl groups at positions 3, 7 and 12 of the steroid backbone. This difference leads to a great divergence in their interfacial [24, 25] and solution properties [26]. We expect that the information obtained here will shed light to a better understanding of the complex aggregation behaviour of bile salts and related compounds in catanionic mixtures. NaDHC is a scarcely studied bile salt derivative, which ads interest to this work.

Experimental section

Materials

Dehydrocholic acid (HDHC) was obtained from Dr. Theodor Schuchardt (Munich) and was of analytical grade. DDAB and NaDC were obtained from Aldrich, 99%, and used as purchased. As a reference, their structures are shown in Fig. 1.

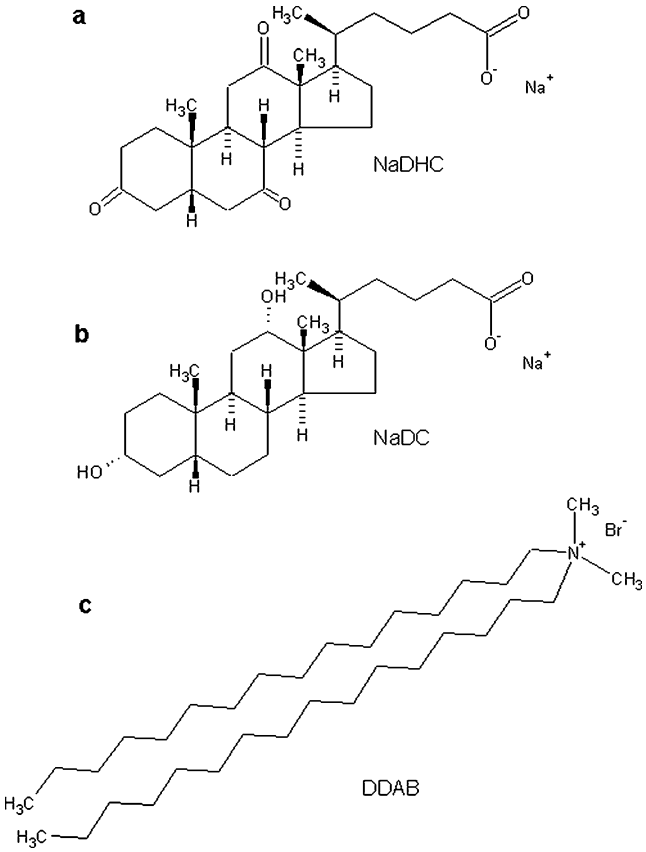

Fig. 1 Surfactant molecular structures: **a** sodium dehydrocholate (*NaDHC*); **b** sodium deoxycholate (*NaDC*); and **c** didodecyldimethylammonium bromide (DDAB)

Solutions

NaDHC solution was prepared by weighing a quantity of HDHC and by dissolution in an appropriate amount of concentrated NaOH. Stock NaDHC, NaDC and DDAB solutions (0.1 mol dm^{-3}) were prepared and diluted as required for each experiment. The appropriate amounts of NaDHC, NaDC and DDAB stock solutions were mixed to obtain the different NaDHC–DDAB and NaDC–DDAB mixture solutions. All surfactants solutions were prepared using double-distilled water.

Methods

Experimental techniques

Surface Tension measurements were made with a Krüss Easy Dyn tensiometer under atmospheric pressure by the ring method. The platinum ring was thoroughly cleaned and dried to the flame before each measurement. The measurements were done in such a way that the vertically hung ring was dipped into the liquid to measure its surface tension. It was then subsequently pulled out. The maximum force needed to pull the ring thought the interface was then expressed as the surface tension, γ (mN m^{-1}). Measurements of the surface tension of the pure water at 298 K were performed to calibrate the tensiometer and to check the cleanliness of the glassware. In all cases, more than ten successive measurements were carried out and the standard deviation did not exceed ±0.2 mN m^{-1} [27]. All experiments were performed at (298±0.1) °K. Temperature was maintained by a thermostat bath with recycling water throughout all the experiment. Equation fitting were done from non-linear procedures using ORIGIN® computer package (release 7.0).

Theoretical methods

The surface excess concentration of surfactants at the water–air interface can be determined on the basis of the adsorption isotherm using Gibbs equation [28]. For dilute solutions (10^{-2} mol dm^{-3} or less) containing a 1:1 strong electrolyte type surfactant (AB) that dissociates in aqueous solution to A^+ and B^- ions, the appropriate form of the Gibbs is:

$$d_\gamma = -RT\,(\Gamma_{A^+}\, d\ln a_{A^+} + \Gamma_{B^-}\, d\ln a_{B^-}) \tag{1}$$

where d_γ is the change in surface or interfacial tension of the solvent, $\Gamma_A{}^+$ and $\Gamma_B{}^-$ are the surface excess concentration and $a_A{}^+$, $a_B{}^-$ are the activity of ion A^+ and B^-, respectively.

Since $\Gamma_A{}^+ = \Gamma_B{}^- = \Gamma$ to maintain electro neutrality and $a_A{}^+ = a_B{}^- = C \times \rho_{AB}$ without a significant error, then:

$$d_\gamma = -2RT\ \Gamma d\ln(C \times \rho_{AB}) \tag{2}$$

where C represents the total surfactant concentration, ρ_{AB} is the mean activity coefficient of the surfactant, log ρ_{AB} can be assumed to equal (log $\rho_A{}^+$ + log $\rho_B{}^-$)/2 and calculated on the basis of the Debye–Hückel equations [29].

For dilute solutions, $\rho_{AB} \approx 1$, Eq. 2 assumes the form:

$$\Gamma = -\frac{1}{2RT}\frac{d_\gamma}{d\ln C} = -\frac{1}{4.606RT}\frac{d_\gamma}{d\log C} \tag{3}$$

The Gibbs adsorption equation for a multicomponent system can be written as [30]:

$$-\frac{d_\gamma}{d\ln C} = RT\sum_i \Gamma_i \tag{4}$$

For a mixture of two ionic surfactants, the equation takes the form [30]:

$$-\frac{d_\gamma}{d\ln C} = 2RT\,\Gamma_m \tag{5}$$

where $\Gamma_m = \Gamma_{1,m} + \Gamma_{2,m}$ is the surface excess concentration for the mixed system, $\Gamma_{1,\,m}$ and $\Gamma_{2,\,m}$ are the surface

excess concentration of surfactant 1 and 2 in the mixed adsorbed monolayer respectively. Therefore, the concentration of each surfactant at the interface can be calculated from the slope of γ vs. log C plot of each surfactant or their mixtures. It is convenient if the dependence between the surface tension and the concentration of the aqueous surfactant solution can be expressed by a known mathematical function.

The surface excess concentrations of mixed systems were also computed on basis of the interfacial composition:

$$\Gamma_m = \Gamma_1 X_1 + \Gamma_2 X_2 \tag{6}$$

and on basis of solution surfactant compositions:

$$\Gamma_m = \Gamma_1 \alpha + \Gamma_2 (1-\alpha) \tag{7}$$

where α is the mole fraction of surfactant 1 in the mixture of two surfactants, X_1 is the mole fraction of surfactant 1 in the mixed monolayer, Γ_1 and Γ_2 are the maximal excess values of a single surfactant concentration at the water–air interface computed by Eq. 3.

X_1 can be obtained from:

$$\frac{(X_1)^2 \ln\left(\alpha\, C_{1,2}/X_1 C_1^0\right)}{(1-X_1)^2 \ln\left[(1-\alpha)\, C_{1,2}/(1-X_1)\, C_1^0\right]} = 1 \tag{8}$$

where $C_1{}^0$ and $C_{1,\,2}$ are the molar concentration in the bulk of surfactant 1 and of the mixtures of surfactants 1 and 2, respectively, required to produce a given surfactant tension value.

The molecular interaction parameter, β^γ, for the monolayer can be evaluated by using the equation derived by Rubingh and Rosen [31, 32]:

$$\beta^\gamma = \frac{\ln\left(\alpha\, C_{1,2}/X_1 C_1^0\right)}{(1-X_1)^2} \tag{9}$$

With the knowledge of the interaction parameters for the mixed monolayer, it is possible to determine the activity coefficient of the surfactants in the mixture. From the non-ideal solution theory, the activity coefficients of surfactants 1 and 2 (f_1 and f_2) in the mixed film can be obtained from the following equations:

$$\ln(f_1) = \beta^\gamma (1-X_1)^2 \tag{10}$$

$$\ln(f_2) = \beta^\gamma (X_1)^2 \tag{11}$$

The adsorption efficiency is related to the standard Gibbs free energy of adsorption, $\Delta G_{ad}{}^0$. In literature different equations are found which may be used to evaluate this parameter. If the surfactant concentration corresponding to the saturated monolayer at interface is lower than 1×10^{-2} M, the Rosen and Aronson equation can be expressed in the form [33]:

$$\Delta G_{ad}^0 = 4.606RT \log\left(\frac{C}{w}\right) - N\pi A_m \tag{12}$$

where N is Avogadro's number, w is the number of water moles per cubic decimetre, and π is the surface pressure corresponding to the surfactant concentration, C, at which A_m (minimal area per molecule at the interface) is achieved.

$$A_m = \frac{1}{N\Gamma_m} \tag{13}$$

Standard Gibbs free energy of adsorption can also be calculated based on monolayer surfactant composition and the mixture excess Gibbs free energy [34]:

$$\Delta G_{ad}^0 = X_1 \Delta G_{ad1}^0 + X_2 \Delta G_{ad2}^0 + g \tag{14}$$

where g is the excess Gibbs free energy:

$$g = RT[X_1 \ln(f_1) + X_2 \ln(f_2)] \tag{15}$$

Results and discussion

Adsorption isotherms

The measured values for the surface tension (γ) of aqueous solution of NaDC, NaDHC, DDAB and their mixtures as a function of the total surfactant concentration (C) are presented in Fig. 2. All time stable adsorbed monolayers are obtained in agreement with previous works [24, 35, 36]. From Fig. 2, it appears that the shape of obtained curves for NaDC, NaDHC and DDAB (Fig. 2, inset) are somewhat different from those obtained for their mixtures; however, for all surfactants and their mixtures a linear dependence exists from γ and log C near the critical micellar concentration, Table 1. To show the influence of the mixture compositions on the water surface tension, the dependence between surface tension and monomer mole fraction of BSs, α, in the bulk phase is plotted in Figs. 3 and 4.

For the NaDC–DDAB system, Fig. 3a, it is seen that at all tested concentrations (even at very low concentration, 1×10^{-6} mol dm^{-3}) of surfactant mixture there is a non-linear dependence with a negative deviation between the surface tension and α_{NaDC}. Therefore, non-ideal mixing of surfactant is evident. It suggests that the composition of the saturated monolayer at the water–air interface should be different from that of the surfactant in the bulk phase. This fact is confirmed by the data shown in Fig. 3b, which presents the relationship between the mole fraction of NaDC (X_S^{NaDC}, computed by Eq. 8 in the mixed monolayer

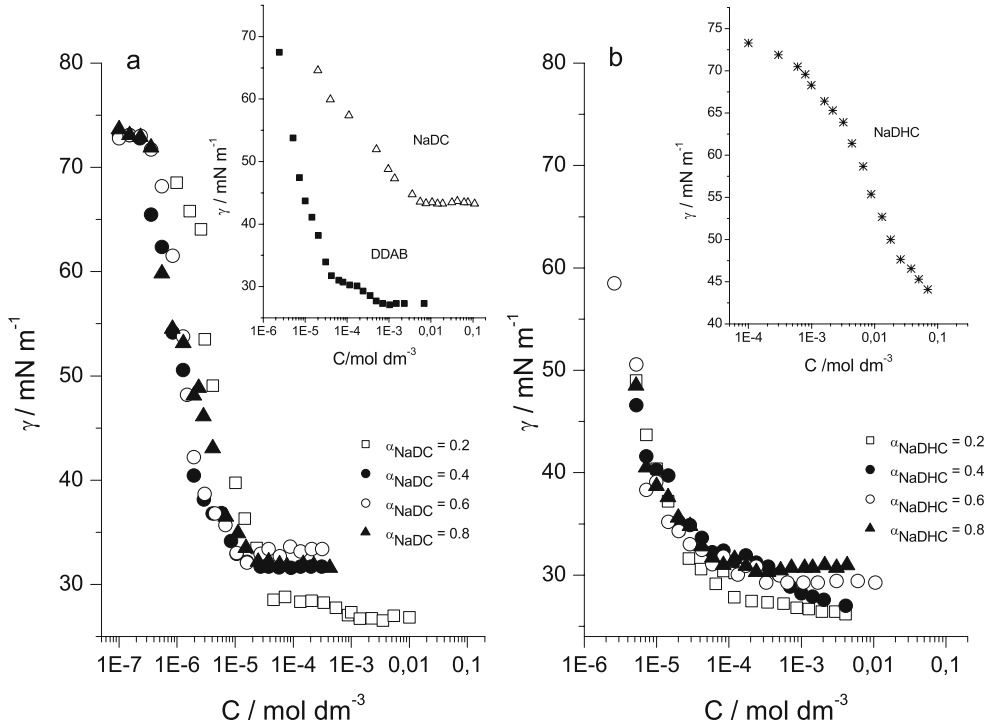

Fig. 2 Dependence of the surface tension of aqueous **a** NaDC–DDAB and **b** NaDHC–DDAB systems on total surfactant concentration. **c** Inset adsorption isotherms of pure NaDC, NaDHC and DDAB

for each α_{NaDC} and the solution surface tension. From this figure, it is evident that change direction of X_S^{NaDC} with γ is the same for each α_{NaDC}. In the whole range of the presented surface tension values, the monolayer is always rich in DDAB $\left(X_s^{DDAB}0.65\right)$ and it composition augments as surface tension decrease and α_{NaDC} augment. At α_{NaDC}=0.2 (high content of DDAB at the bulk solution), the mole fraction of NaDC at the mixed monolayer is always bigger than in the bulk phase. The same behaviour can be seen for α_{NaDC}=0.4, at $\gamma \geq 60$ mNm^{-1}.

Table 1 Values of critical micelle concentration (CMC) and negative logarithm of the concentration of surfactant or their mixture in the bulk phase required to produce a 20 mJm^{-2} reduction in the solvent surface tension, pC_{20} for NaDC–DDAB and NaDHC–DDAB mixtures

α	CMC/moldm^{-3}	pC_{20}
NaDC–DDAB		
0	(4.17±0.50)×10^{-5}	5.35±0.50
0.2	(4.59±0.55)×10^{-5}	5.37±0.50
0.4	(1.09±0.23)×10^{-5}	6.02±0.52
0.6	(1.06±0.11)×10^{-5}	5.90±0.51
0.8	(1.59±0.31)×10^{-5}	5.94±0.51
1	(7.90±0.83)×10^{-3}	3.42±0.19
NaDHC–DDAB		
0	(4.17±0.50)×10^{-5}	5.31±0.45
0.2	(4.16±0.46)×10^{-5}	5.29±0.40
0.4	(1.19±0.34)×10^{-4}	5.45±0.34
0.6	(1.18±0.33)×10^{-4}	5.40±0.33
0.8	(8.35±0.76)×10^{-5}	5.40±0.33
1	(2.50±0.13)×10^{-2}	1.86±0.20

The composition behaviour of NaDHC–DDAB mixed adsorbed monolayers is similar to those obtained for NaDC–DDAB systems, Fig. 4b. Nevertheless, a different tendency can be seen for the γ vs. α_{NaDHC} dependence, Fig. 4a. Generally, there is a negative deviation from linear dependence at all tested concentration, except for C=1×10^{-6} mol dm^{-3}. The α_{NaDHC}=0; 0.2; 0.4; 0.6 and 0.8 mixed systems present similar γ values at all concentration.

The differences between NaDC–DDAB and NaDHC–DDAB mixtures would be possible due the existence of a dissimilar adsorption "efficiency" and "effectiveness". The surface excess concentration at the surface saturation, Γ, is a useful measure of the adsorption effectiveness of the surfactant at the water–air interface [34], since it is the maximum adsorption value possible. Whereas, the efficiency of the adsorption is related to the negative logarithm of the surfactant concentration (or their mixture) in the bulk phase required to produce a 20 mNm^{-1} reduction in the solvent surface tension, pC_{20} [34].

On the basis of the adsorption isotherms, Fig. 2, the surface excess concentration at the water–air saturation are computed for pure NaDC, NaDHC and DDAB monolayers (Eq. 3) and for their mixtures (Eq. 5). Then the minimal area (A_m) per molecule at the interface is obtained by Eq. 13. The obtained results are shown in Figs. 5 and 6, respectively. DDAB showed high adsorption effectiveness and efficiency than NaDC and NaDHC molecules. The

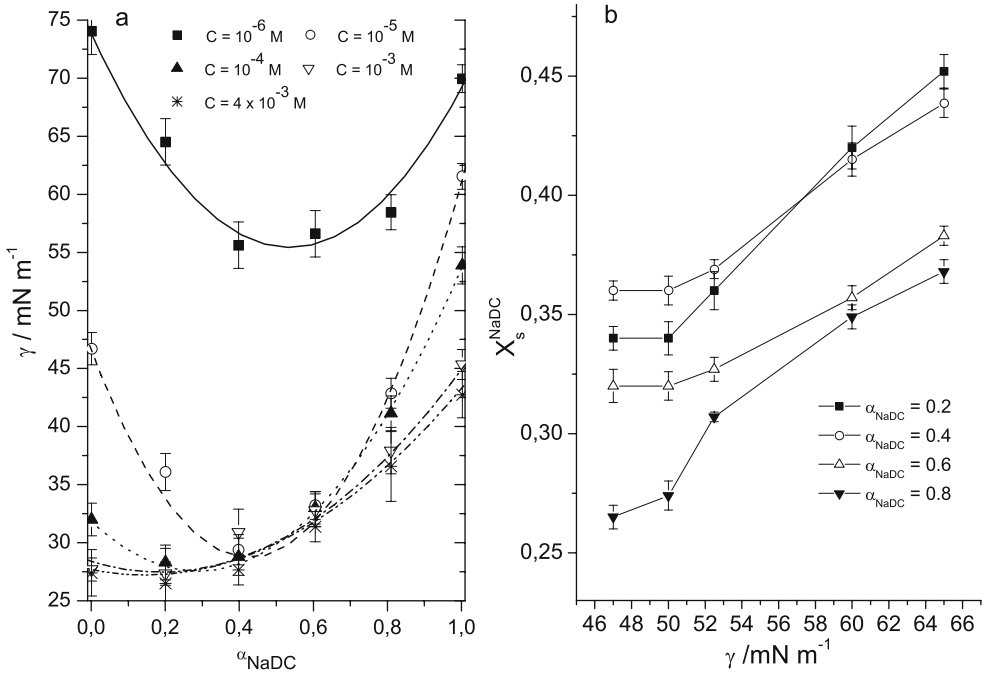

Fig. 3 **a** Dependence of the surface tension of aqueous NaDC–DDAB mixtures on the monomer fraction of NaDC, α, at different total surfactant concentration. **b** Variation of NaDC interfacial mole fraction, X_s^{NaDC}, with the surface tension of the mixed NaDC–DDAB aqueous solution at different NaDC bulk mole fraction, α_{NaDC}.

NaDC–DDAB and NaDHC–DDAB mixed systems show similar adsorption efficiency than pure DDAB monolayer and superior to the obtained for bile salts molecules, Table 1. However, their adsorption effectiveness is very different. The presence of NaDC in the mixed monolayer causes an increment of Γ values, while NaDHC produces the opposite effect.

There are also essential differences among the values of surfactant concentrations in mixed monolayers computed based on adsorption isotherms (Eq. 5), interfacial composition (Eq. 6) and bulk phase composition (Eq. 7). The Γ values computed by Eqs. 6 and 7 are similar for both mixed systems. Nevertheless, for NaDC–DDAB system the Γ values obtained by application of Eq. 5 are superior than those obtained using Eqs. 6 and 7 whereas for NaDHC–DDAB are inferior. This fact, suggest that the interfacial interactions between molecules play an important role in the packaging of the mixed monolayers and these interactions are influenced by the structure of the bile salt molecule.

The effect of bile salts on monolayer packing also can be appreciated from the analysis of effective interfacial molecular areas, Fig. 6. The obtained A_m value for DDAB pure monolayer is about (0.70 nm^2 molec^{-1}), which is in

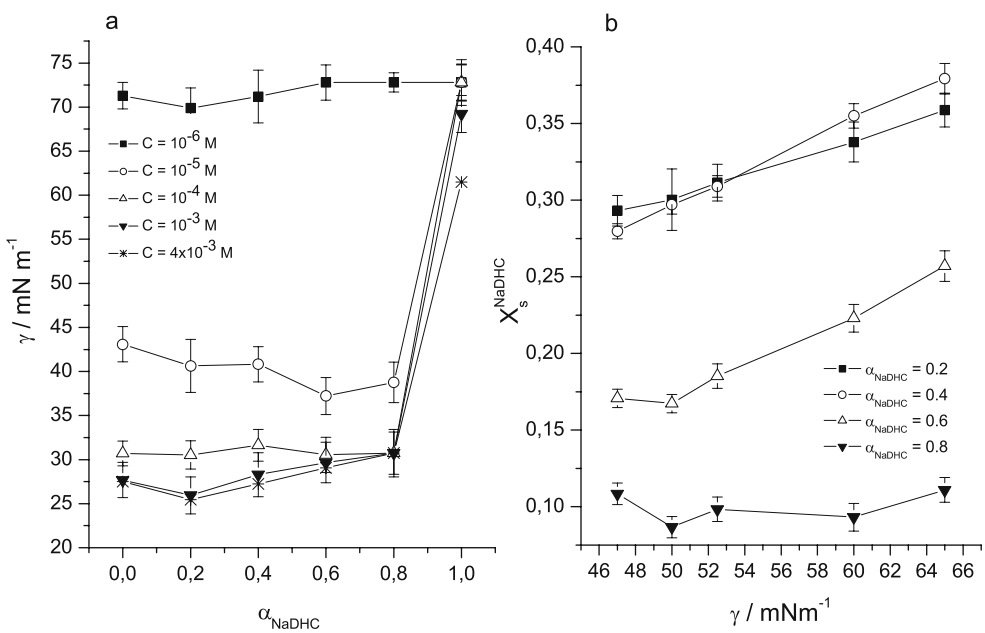

Fig. 4 **a** Dependence of the surface tension of aqueous NaDHC–DDAB mixtures on the monomer fraction of NaDHC, α, at different total surfactant concentrations. **b** Variation of NaDHC interfacial mole fraction, X_s^{NaDHC}, with the surface tension of the mixed NaDHC–DDAB aqueous solution at different NaDHC bulk mole fraction, α_{NaDHC}.

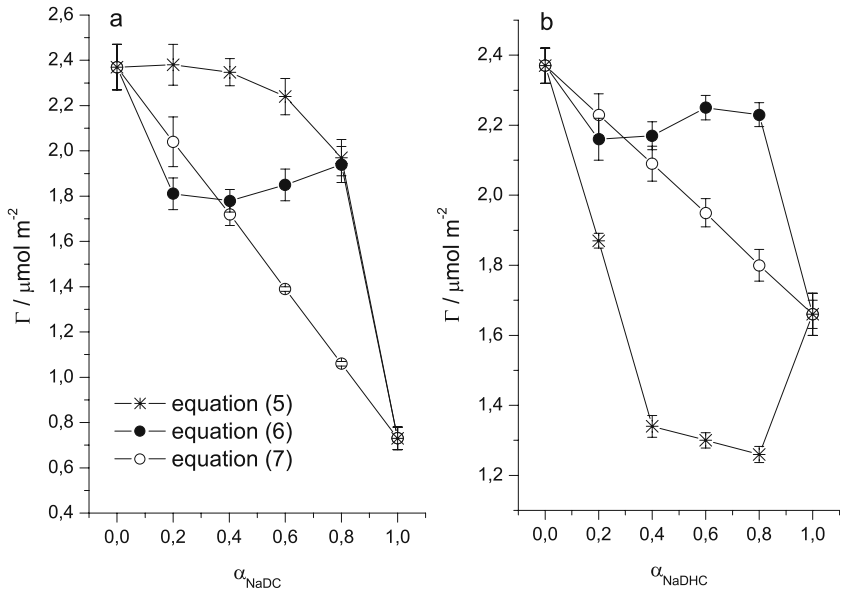

Fig. 5 Surface excess concentration (Γ) variation of **a** NaDC–DDAB and **b** NaDHC–DDAB systems with the solution molar fraction, α

agreement with the collapse area value obtained for dialkyldimethylammonium bromide spreaded monolayers [35]. Comparing the obtained A_m^{DDAB} with the molecular cross-sectional area (c.a. 0.40 nm^2 molec^{-1} [37]) of the close packed double chain quaternary ammonium salts; we assumed that the DDAB molecules take a conformation with both alkyl chains lying with a certain angle at the surface as found in single tailed surfactants by neutron reflection measurements [38]. The effective molecular interfacial area for pure NaDC (A_m=2.28 nm^2 molec^{-1}) and NaDHC (A_m=0.99 nm^2 molec^{-1}) was similar to those obtained in literature [39]. Differences between NaDC and NaDHC interfacial area is related to the high hydration of polar groups present in NaDC molecule. In a previous work [39], it was determined that there is about of 39 water molecules for each micelliced NaDHC molecule. Ten water molecules were assigned to the hydration of the sodium carboxilate group, the hydration of two water molecules for each carbonyl group may be supposed regarding the two free electron pair of the oxygen atom. The other 23 molecules were attributed to water trapped in the structure of the micelles. A similar behaviour would be expected for NaDC. Garidel et al. [40] studied the bile salt aggregation from a thermodynamic point of view and determined by isothermal titration calorimetry (ITC) that hydration of polar parts of the bile salts molecules did not change to a large extent into the aggregates. 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 [39], the computed value of NaDHC

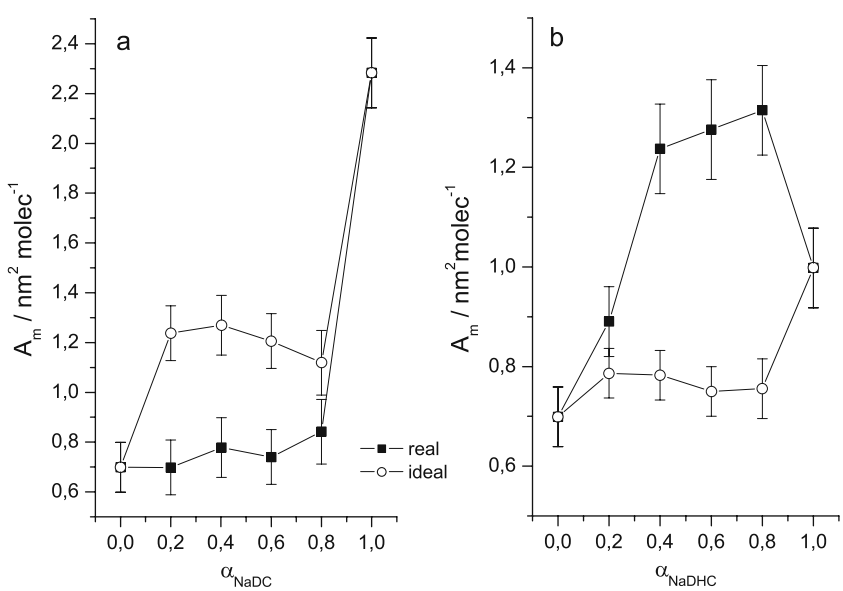

Fig. 6 Dependence of the interfacial molecular area of **a** NaDC–DDAB and **b** NaDHC–DDAB systems on the solution molar fraction, α

area is a_{NaDHC}=0.734 nm^2. Therefore, the experimental value (a_{NaDHC}=0.99 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. The major solubility of NaDC respect to the NaDHC [41] let us to infer that the last one is less hydrated, probably due to the presence of carbonyl instead hydroxyl groups. The presence of −OH groups also favours the formation of intra H-bonds among NaDC molecules that is not possible for NaDHC [24, 26].

The mean molecular areas of all tested mixtures were strongly dependent on composition as well as surface tension, which is an indication of a miscible monolayer [42]. Mixed NaDC–DDAB systems show slightly values of interfacial molecular area (A_m) than A_m^{DDAB} and inferior than ideal computed values, $A_{m,\ ideal}=X_S^{NaDC}\times A_{m,NaDC}+X_S^{DDAB}\times A_{mDDAB}$. Therefore, it is supposed that there is not a great disruption of DDAB packed hydrocarbon chains at the air–water interface saturation due to NaDC intercalation. In contrast, the analysis of A_m for NaDHC–DDAB systems (which are always higher than A_m^{DDAB} and than ideal computed values) shows an expansion effect. Therefore, the presence of NaDHC molecules (even in small proportions) causes a great disruption of DDAB monolayer.

Molecular interaction parameter

The above results prove that mutual interactions among molecules or ions at the interface play an important role in the composition and packing of the mixed monolayer. The composition has a valid influence on the monolayer properties but does not give an answer about synergistic or antagonistic properties of the mixture. To explain these effects, the interaction parameters (β^γ) should be computed from Eq. 9. The obtained values are presented in Table 2. It is seen that for both NaDC–DDAB and NaDHC–DDAB mixed systems at all solution composition, β^γ has a negative value that becomes more negative as surface tension decreases. The negative values of β^γ suggest that the introduction of BSs molecules into DDAB monolayer cause a synergism in mixed systems which may confirm the efficiency of surface tension reduction. However, the second condition for the existence of negative synergism must be fulfilled. This condition is that the absolute value of the β^γ parameter should be greater than $\left|\ln\left(C_1^0/C_2^0\right)\right|$, where C_1^0 and C_2^0 are the concentration of a simple surfactant at a given surface tension. From inspection of Table 2, it is seen that such condition is satisfied. Higher negative values exist at α_{BSs}=0.4. The presence of synergism is accompanied by a minimum in the curve corresponding to the variation of the total mixed surfactant concentration, C (selected at a fixed γ), versus the molar fraction in solution (α), Fig. 7. This minimum corresponds to the condition for maximal synergism. At the point of maximum synergism according to Gonzales-Caballero et al. [43], the mole fraction of surfactant in solution (α*) and at the interface, (X*) are equal. In both systems, this minimum corresponds to a BSs molar fraction in solution of 0.4, which explain the minimum β^γ values. According to theory, at this α the total concentration of the mixed surfactant required for obtaining a selected surface tension value is lower than the concentration of the individual surfactants.

By knowing the X and β^γ values, it is possible to calculate the surfactants activity coefficients in the mixed film (f_{DDAB}, f_{NaDHC} and f_{NaDC}) from Eqs. 10 and 11. From the analysis of activity coefficients for NaDC–DDAB system, Fig. 8, it appears that all their values are smaller than 1, which according to literature [44], indicates that interactions between NaDC and DDAB molecules are stronger than

Table 2 Values of the $-\beta^\gamma$ and $\left|\ln\left(C_1^0/C_2^0\right)\right|$ for NaDC–DDAB and NaDHC–DDAB mixtures

γ/mN m^{-1}	$-\beta^\gamma$/k$_B$T				$\left\|\ln\left(C_1^0/C_2^0\right)\right\|$
	0.2	0.4	0.6	0.8	
α_{NaDC}					
47	9.737±1.101	15.232±2.201	14.468±1.801	12.491±1.365	5.596±0.732
50	7.983±0.852	13.667±1.522	12.879±1.307	12.908±1.308	4.969±0.621
52.5	8.492±0.951	13.719±1.632	12.378±1.165	13.362±1.387	4.572±0.598
60	5.826±0.755	11.153±1.365	8.759±0.742	11.277±1.356	2.657±0.102
65	5.231±0.701	11.616±1.402	8.504±0.659	10.863±1.010	2.081±0.089
α_{NaDHC}					
47	15.244±2.201	16.378±2.760	11.221±1.201	12.005±1.201	8.572±0.960
50	15.515±2.321	17.331±2.987	10.676±1.006	8.904±0.547	8.309±0.879
52.5	15.839±2.232	18.175±3.014	11.202±1.182	8.926±0.550	8.131±0.803
60	16.673±2.560	22.118±4.392	11.628±1.254	8.138±0.500	7.469±0.765
65	16.770±2.870	24.276±4.845	12.465±1.348	7.557±0.432	6.770±0.731

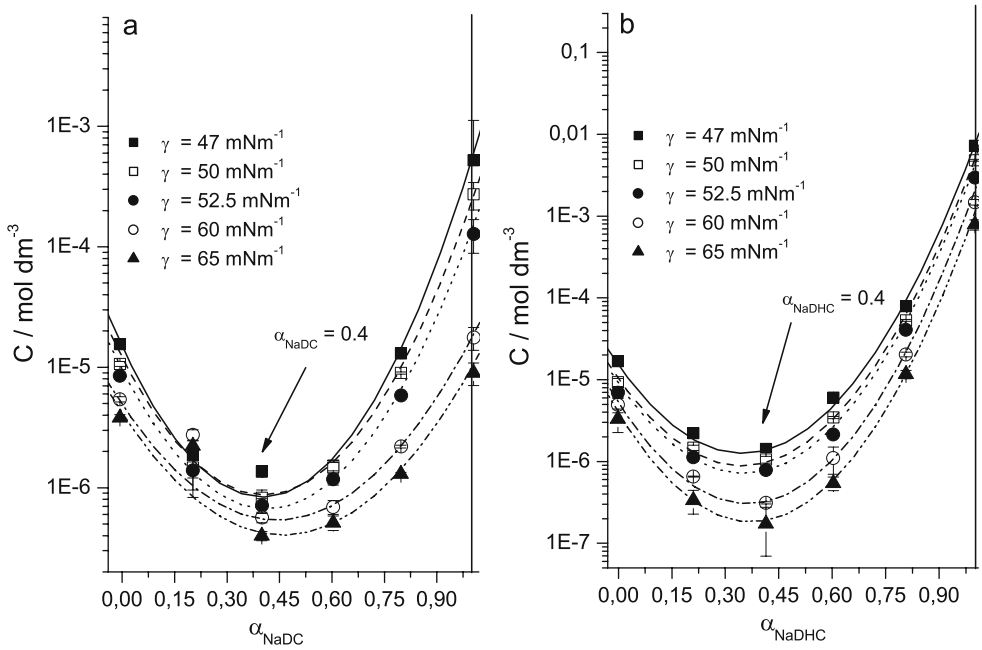

Fig. 7 Logarithm of total surfactant concentration as a function of the molar fraction of **a** NaDC and **b** NaDHC in the mixed aqueous solution

between single surfactants. For NaDHC–DDAB mixtures, it is seen that f_{DDAB} >>>f_{NaDHC}, Fig. 9, which imply that NaDHC acts as a highly soluble solute in the DDAB monolayer. As the content of NaDHC in the bulk solution augments, f_{DDAB} tends to 1, showing that interactions are similar to those that existed in pure DDAB molecules.

The standard Gibbs energy of adsorption

Some explanation of the synergistic behaviour can be found from the analysis of the surface free energy components of each of these bile salts and their relationship with surface tension. Generally, the changes of the standard free energy of adsorption, ΔG_{ad}^{0}, tell us whether adsorption (at standard states) is spontaneous (where ΔG_{ad}^{0} is negative) or not. In our case, all studied systems present high and negative values of ΔG_{ad}^{0}, so interfacial adsorption of molecules is favoured, suggesting that no phase separation occurs in the mixed adsorbed monolayers and confirming that a miscible monolayer exists. However, a previous study [35] showed that in HDHC–DDAB insoluble monolayer existed two-dimensional molecular aggregates at low surface

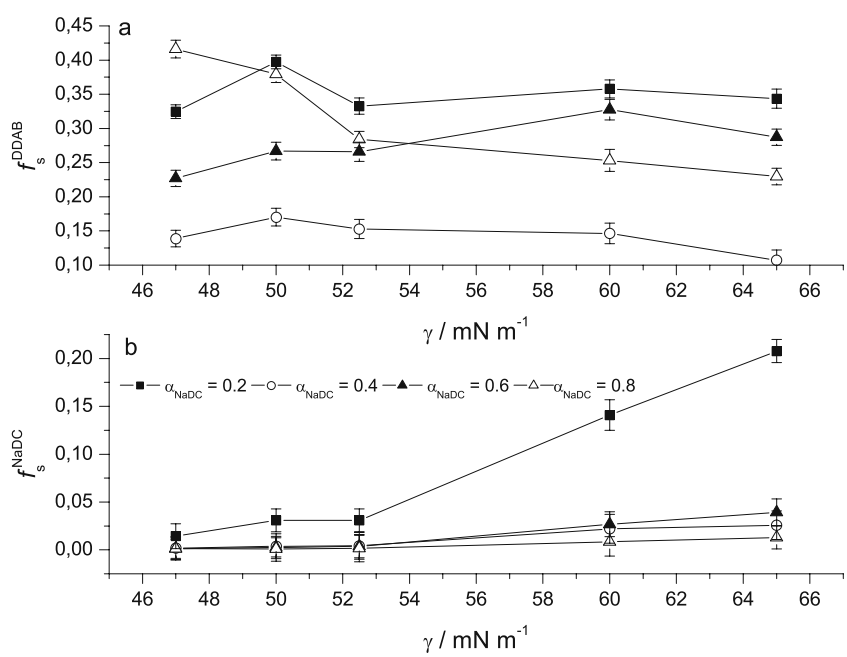

Fig. 8 Dependence of the NaDC and DDAB activity coefficients, f_s^{NaDC} and, f_s^{DDAB} on the surface tension of the mixed NaDC–DDAB aqueous solution at different NaDC bulk mole fraction, α_{NaDC}

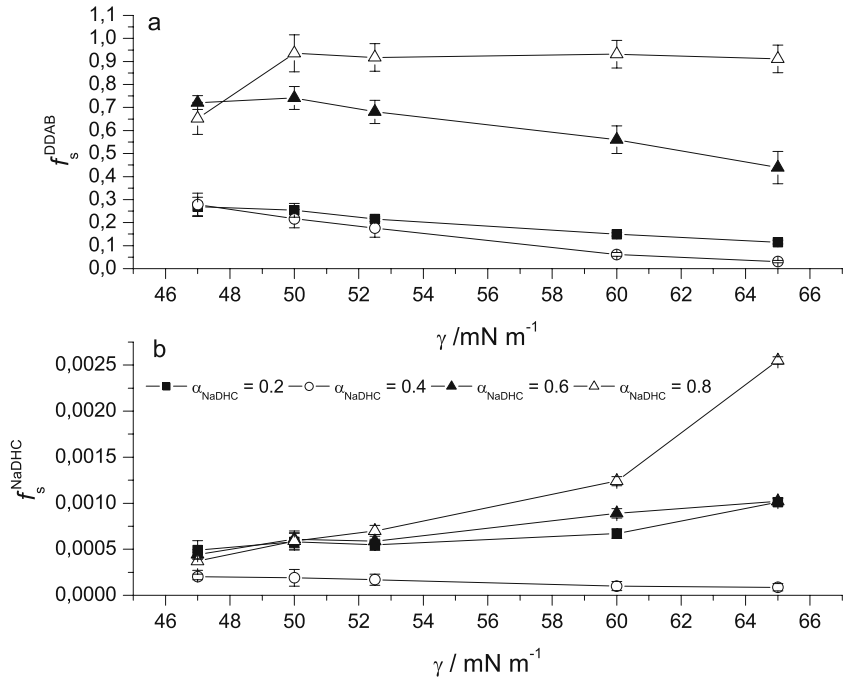

Fig. 9 Dependence of the NaDHC and DDAB activity coefficients, f_s^{NaDHC} and f_s^{DDAB} on the surface tension of the mixed NaDHC–DDAB aqueous solution at different α_{NaDHC} values

pressures. This could be indirectly related to the existence of laterally structured microdomains. Small et al. [45] proposed that ionised bile acids are oriented with their long axis perpendicular to membrane surface and that could then form reverse dimmers or tetramers in biological membranes, with their hydrophilic surfaces in opposition, and their hydrophobic surface interacting with the acyl moieties of long-chain phospholipids. The presence of a few number of HDHC molecules causes the computed aggregation number was about 31 molecules but as the content of HDHC augmented the following HDHC/DDAB mixtures presented an aggregation number of about 6 that corresponded to the aggregation number of HDHC pure monolayers. The great interaction between HDHC and DDAB and the formation of microdomians would explain the observed great disruption of DDAB monolayer and the increment of A_m values.

The NaDC–DDAB and NaDHC–DDAB mixtures present high negative $\Delta G_{ad}{}^0$ values compared with pure surfactants. The $\Delta G_{ad}{}^0$ values calculated using experimental surface tension data corresponding to saturated adsorption monolayer (Eq. 12) and with respect to monomer mole fraction of surfactant in mixed monolayer (Eq. 14) presented a good

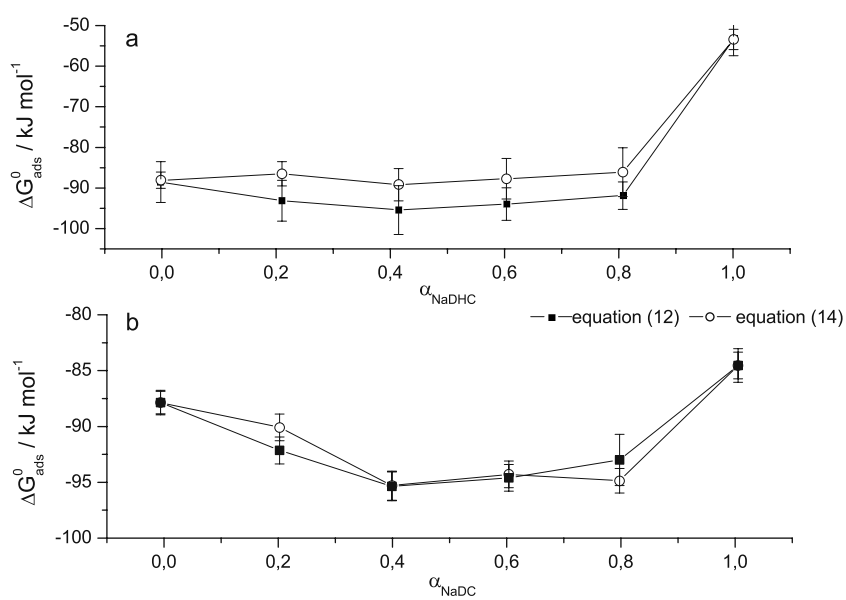

Fig. 10 Gibbs free energy of adsorption $\left(\Delta G^0_{ad}\right)$ dependence on the molar fraction of bile salts in the mixed aqueous solution

agreement, Fig. 10. Such values confirm the synergistic effect in surface tension reduction, which is larger at α=0.4.

It is known from literature that the surface tension of a conventional surfactant molecule can be divided into the surface tension of the tail and head [46]. The surface tension of the hydrocarbon tail results from the Lifshitz-van der Waals intermolecular interactions, and that of the hydrophilic head from Lifshitz-van der Waals, Lewis acid–base and electrostatic interactions.

If we assume that after adsorption at water–air interface, the hydrophobic tail is present at the air phase and the hydrophilic head at the water phase. Then, the transfer of surfactant molecule from bulk water phase to the interface is associated with changes in the interfacial tension of water-tail (γ_{WT}) to the surface tension of the tail (γ_T) and the interfacial tension of the water-head (γ_{WH}) from γ_{WH} to γ_{WH1} because of dehydration of the head during the adsorption process [46]. Thus, the standard free energy of adsorption at water–air interface should fulfil the condition:

$$\Delta G^0_{ad} = (\gamma_T - \gamma_{WT}) \times A_T + (\gamma_{WH1} - \gamma_{WH}) \times A_H \qquad (16)$$

where A_T is the contactable area of the surfactant tail, and A_H is the contactable area of the surfactant head. It is possible to calculate the standard free energy of adsorption from Eq. 16 if we assume that during the transfer of the surfactant molecule from water to water–air interface the surfactant head does not dehydrate. This means that (γ_{WH1}–γ_{WH}) should be nearly zero. A simple relation [47] can express the volume of *n*-alkane in the liquid phase at different temperatures:

$$v = (l + d) \times (w + d)^2 \qquad (17)$$

where v, l and w are the volume, the length and the width of the molecule respectively and d is a constant value for a given temperature corresponding to the intermolecular forces. There is very good agreement between the volume of a *n*-alkane molecule calculated from Eq. 17 and the volume obtained from the density data at a given temperature [46]. From Eq. 17, it follows that the total contactable area of *n*-alkane molecule A is:

$$A = 4 \times (l + d) \times (w + d) + 2 \times (w + d)^2 \qquad (18)$$

In the case of a hydrocarbon tail (T) there are five contactable areas, for example, with water phase: one with the surfactant head and four of them correspond to the proper contactable areas of the alkyl groups. Therefore, the total contactable area of the surfactant tail perpendicular to water is found by the expression:

$$A = 4 \times (l + d/2) \times (w + d) + (w + d)^2 \qquad (19)$$

The free energy of adsorption of surfactant having a straight chain hydrocarbon tail (from hexyl to hexadecyl) was calculated earlier from Eq. 16 by Jańczuk et at [46, 47] on the basis of the proper *n*-alkane—water interfacial tension and *n*-alkane surface tension. From these calculations, it results that the standard free energy of transfer from the bulk phase to the interface a dodecyl tail is equal to −51.04 kJmol^{-1}. Nevertheless, the head of the surfactant molecule should include one or two $-CH_2-$ groups which are immersed in the water phase. Thus $\Delta G_{ad}{}^0$ probably correspond to the standard free energy of adsorption of an undecyl or a decyl group (−48.03 kJmol^{-1} or −45.66 kJmol^{-1}). Using such information and taking into account that DDAB molecule has two dodecyl hydrocarbon tails, the computed value of $\Delta G_{ad}{}^0$ using Eq. 16 was 96.06 kJmol^{-1} or 91.32 kJmol^{-1}. This values are similar to $\Delta G_{ad}{}^0$=87.86±4.01 kJmol^{-1} (computed by Eq. 12) of pure DDAB monolayer.

González-Caballero et al. [43] studied the apolar, Lifshitz-van der Waals contribution to the surface free energy of different BSs. It was found that for all tested BSs (γ_T–γ_{WT}) was nearly identically and about −26 mJm^{-2}. Using such value and calculating A_T by Eq. 19, we determined that for BSs the $\Delta G_{ad}{}^0$ should be −83.5 kJ mol^{-1}. For our calculations we assumed that d=2 Å [46], l= 1.3 nm and w=0.571 [39]. The obtained $\Delta G_{ad}{}^0$=−84.54± 2.3 kJmol^{-1} which is similar to those found for the interfacial adsorption of NaDC molecule by the application of Eq. 12. On the other hand, the correspondent ΔG^0_{ad} for NaDHC adsorption was about −50.0±1.8 kJmol^{-1}. This value is very different from those found by the application of Eq. 16. This fact is assumed to be due to a different location of the steroidal backbone once the molecule migrates to interface.

Concluding remarks

We used surface tension measurements to evaluate the interaction of NaDHC and NaDC with DDAB in a catanionic adsorbed mixed monolayer. We found thermodynamic evidence of the hydrophobic BSs effect during its intercalation into interfacial adsorbed DDAB molecules.

All adsorbed mixed monolayers show a higher dependence of molecular areas on composition and negative ΔG^0_{ad} values. This indicated the formation of a miscible monolayer. The analysis of pC_{20} values shows that both mixed systems (NaDC–DDAB and NaDHC–DDAB) have analogous adsorption efficiency than pure DDAB monolayer and superior to the obtained for both bile salts. Nevertheless, their adsorption effectiveness is different: NaDC causes an increment of Γ while NaDHC produces the opposite effect. The adsorption efficiency in surface tension reduction is due to the existence of interfacial synergistic interactions (confirmed by the analysis of β^γ and ΔG^0_{ad} values). Maximum synergistic interactions are seen for α_{BSs}=0.4. Then low Γ_m values obtained for

NaDHC–DDAB system are related to the deep penetration of the hydrophobic steroid backbone of NaDHC molecules that causes a great disturbance of DDAB hydrocarbon tails (high A_m values). This would be indirectly related to the existence of laterally structured microdomains. The ionised NaDHC oriented with their long axis perpendicular to the interface can form reverse dimmers or tetramers with their hydrophilic surfaces in opposition, and their hydrophobic surface interacting with the acyl moieties of DDAB.

In contrast, the NaDC intercalation disturbs the environment of the DDAB monolayer in a smaller extent (low A_m values). Therefore, it is supposed that this molecule (less hydrophobic and highly hydrated than NaDHC) should be inserted into the polar part of the DDAB monolayer, thereby allowing the formation of H-bond between −OH groups in bile salt molecules and water. This fact causes an increment of adsorption effectiveness for NaDC–DDAB mixtures.

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