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Spread mixed monolayers of deoxycholic and dehydrocholic acids at the air–water interface, effect of subphase pH. Characterization by axisymmetric drop shape analysis

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Abstract

Bile acids (deoxycholic and dehydrocholic acids) spread mixed monolayers behavior at the air/water interface were studied as a function of subphase pH using a constant surface pressure penetration Langmuir balance based on the Axisymmetric Drop Shape Analysis (ADSA). We examined the influence of electrostatic, hydrophobic and hydration forces on the interaction between amphiphilic molecules at the interface by the collapse area values, the thermodynamic parameters and equation of state virial coefficients analysis.

The obtained results showed that at neutral (pH = 6.7) or basic (pH = 10) subphase conditions the collapse areas values are similar to that of cholanoic acid and consistent with the cross-sectional area of the steroid nucleus (≈ 40 Å^2). The Gibbs energy of mixing values ($\Delta G_{\mathrm{mix}} < 0$) and the first virial coefficients of the equation of state ($b_0 < 1$) indicated that a miscible monolayer with laterally structured microdomains existed. The aggregation number ($1/b_0$) was estimated within the order of 6 (pH = 6.7) and 3 (pH = 10).

At pH = 3.2, acidic subphase conditions, no phase separation occurs ($\Delta G_{\mathrm{mix}} < 0$) but a high expanded effect of the monolayer could be noted. The mixed monolayer behavior was no ideal and no aggregates were formed ($b_0 \geq 1$). Such behavior indicates that the polar groups of the molecules interacts each other more strongly by repulsive electrostatic forces than with the more hydrophobic part of the molecule.

Keywords: Bile salts; Spread monolayers; ADSA; Thermodynamic parameters; Interactions; Langmuir balance

1. Introduction

Many phenomena that occur in nature take place at interfaces and involve only surface regions while the bulk almost does not participate in the overall process. The air/water interface is of particular relevance, due to its abundance; however, it remains one of the most difficult to investigate.

Many substances with the general formula $C_nH_{2n+1}X$, where X denotes a polar group, are characterized by their surface activity. Small molecules of such compounds dissolve in water and undergo adsorption from bulk solution into the interface to form so-called Gibbs (or adsorbed, soluble) monolayers.

However, with an increase in hydrocarbon chain length, the solubility decreases. Molecules of non-ionized carboxylic acids, amines or alcohols possessing more that 12 carbon atoms in the hydrophobic chain are practically insoluble in water. Nevertheless, when dissolved in an appropriate volatile organic solvent (for example chloroform) and placed on the free water surface, they spread and cover the entire surface [1]. A one-molecule thick film, termed Langmuir (or insoluble, spread, floating) monolayers is thus formed provided that the area of the surface is sufficient enough to accommodate all molecules spread.

Spread monolayers of biologically important molecules, such as bile acids, are useful models to study physicochemical properties of physiological active materials [2,3]. Bile acids from

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different species chemically differ in three aspects: (i) side-chain structure, (ii) stereochemistry of the A/B ring fusion, and (iii) the distribution of the number, position and stereochemistry of hydroxyl groups in the steroid nucleus.[4] These facts can alter notably their behavior at the interfaces in both spread and soluble monolayers. Shibata et al. [5] have reported a study on the monolayer properties of cholic acid (CA) and β-muricholic acid (β-MCA) spread on concentrated NaCl solutions. Subsequently, Miyoshi et al. [6] have studied the mixed monolayer formation of bile acids mixtures chenodeoxycholic acid (CDA) with usodeoxycholic acid (UDA) and CA with β-MCA. These studies revealed that the main molecular surface area and the tilt plane of the steroid skeleton of the molecule floating on the water surface are dependent on the magnitude of the hydrophilicity of the α-plane of the steroid skeleton [6].

In a previous work we investigated the sodium dehydrocholate (NaDHC)–sodium deoxycholate (NaDC) mixed system behavior at aqueous solution [7]. These two bile salts are very similar to each other except for the presence of the hydroxyl groups, i.e. NaDC has two hydroxyl groups (3 and 12 α-OH) while NaDHC has three carbonile groups at positions 3, 7 and 12 of the steroid backbone. This difference leads to a great divergence in interfacial and colloidal properties. The obtained results showed that micellar interaction was repulsive when the aggregates were rich of 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 the adsorbed mixed monolayer at the air–solution interface at the critical micellar concentration (cmc) was also investigated. The soluble mixed monolayer is 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 supposed due to structural factor in their hydrocarbon backbone.

Here we extend the studies of bile acids (deoxycholic and dehydrocholic acids) insoluble mixed monolayers at the air–solution interface as a function of pH subphase by the employ of a constant surface pressure penetration Langmuir balance based on the Axisymmetric Drop Shape Analysis (ADSA).

Among the interfacial tension techniques, ADSA is one of the most precise and versatile [8]. It fits experimental drop profiles, obtained from digital drop micrographs, to the Laplace equation of capillarity, and provides the interfacial tension γ and area A as outputs. It is non-invasive; i.e. the measuring device is not in direct contact with solvent or the adsorbate and does not interact with them.

The understanding of the behavior of bile acids spread mixed monolayers at the aqueous solution–air interface is an additional step towards cholanology (bile acid science). The studied system could be considered the simplest model to get molecular information and hence, their study is the first step to understand the properties and the behavior of bile acid macromolecular assemblies with future biological or medical applications, for example: bile acids constitute one of the most prominent classes in the study of molecular recognition, host–guest chemistry and biomimetic chemistry [9]. Some of bile acids and their analogues act as potent gelators (gel-forming agents) in both organic and aqueous media [10]. These gels may hold promise for futures biomaterials.

2. Experimental

2.1. Materials

Dehydrocholic acid (HDHC) was from Dr. Theodor Schuchardt (Munich) and Deoxycholic acid (HDC) was obtained from Sigma. Both acids were of analytical grade (99% pure) and were used as purchased, as a reference their structures are shown in Fig. 1. pH subphase was controlled by using different buffer solutions: (i) pH = 3.2: 50×10^{-3} mol dm^{-3} Glycine plus hydrochloric acid. (ii) pH = 6.7: NaH_2PO_4 (50×10^{-3} mol dm^{-3}) plus Na_2PHPO_4. (iii) pH = 10: 50×10^{-3} mol dm^{-3} glycine plus sodium hydroxyde.

2.2. Apparatus and operation conditions

The experiments were performed with a constant surface pressure penetration Langmuir balance based on Axisymmetric Drop Shape Analysis (ADSA) [11]. The whole setup, including the image capturing, the micro-injector, the ADSA algorithm, and the fuzzy pressure control, is managed by a Windows integrated program (DINATEN). A solution droplet is formed at the tip of a coaxial double capillary, connected to a double micro-injector. The program fits experimental drop profiles, extracted from digital drops micrographs, to the Young–Laplace equation of capillarity by using ADSA, and provides as outputs the drop volume V, the interfacial tension γ and the surface area A. Area control uses a modulated fuzzy logic PID (proportional, integral and derivative control) algorithm and is controlled by changing the drop volume in a controller manner. During the experiment, the drop is immersed in a glass cuvette (Hellma®) that is kept in a temperature controlled cell. The surface pressure is obtained from the relationship $\pi=\gamma_0-\gamma$, where π is the surface pressure; γ and γ_0 are the surface tension of the subphase liquid covered with and without the monolayer. All experiment were performed at (25 ± 0.1) °C. Temperature was maintained by a thermostat bath with recycling water throughout all the experiment.

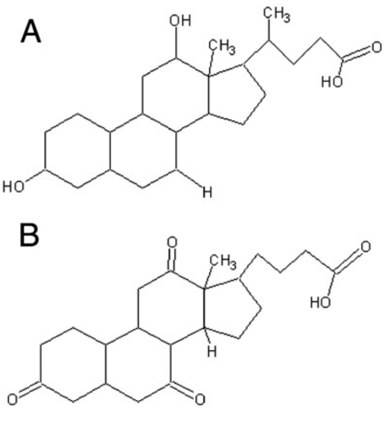

Fig. 1. Bile acids molecular structures (A) deoxycholic acid (HDC) and (B) dehydrocholic acid (HDHC).

2.3. Monolayers

Spreading solution of HDHC and HDC and their mixtures were prepared dissolved in a methanol: chloroform mixture (1:4) to obtain solutions of (7×10^{-5} M) total concentration. Then an aliquot of 1–1.2mL was spread on the subphase using a micro syringe. Four minutes were allowed for solvent evaporation before starting the expansion until a volume of 25 μL at a rate of 0.2 μL/s. When expansion was finished the program maintain the drop area constant for 118 s to reach the monolayer equilibrium and then the compression started at the same rate of expansion.

3. Results and discussion

3.1. π–A isotherms

Monolayer characterization at air/water interface can be directly obtained thought the variation of pressure surface (π) *vs.* the occupied molecular area (A) at the monolayer. Fig. 2A, B and C show π–A isotherms for mixed and pure HDHC/HDC monolayers at pH subphase of 3.2; 6.7 and 10 respectively. The obtained isotherm of pure bile acids agrees well with those found in literature [12]. It can be seen that pure spread monolayers ($X_{\mathrm{HDHC}}=0$ and $X_{\mathrm{HDHC}}=1$), at pH subphase values of 3.2 and 6.7, does not shown the liquid expanded (LE)–liquid condensed (LC) phase transition in opposition with the behavior of mixed monolayers. At pH = 3.2, the mixed monolayers composed by $X_{\mathrm{HDHC}}=0.4$ and $X_{\mathrm{HDHC}}=0.8$ show a LE monolayer without LE–LC transition similarly to the pure surfactant monolayers. On the other hand, the mixed system $X_{\mathrm{HDHC}}=0.6$ presents a LE monolayer until $A\approx4.5$ nm^2 molec^{-1} followed by a LE–LC transition and finally a LE phase from $A\approx4$ nm^2 molec^{-1}. At neutral pH subphase conditions (pH = 6.7), the mixed $X_{\mathrm{HDHC}}=0.2$ monolayer shows a behavior similar to that show by pure surfactants monolayers (a completely LE monolayer exist at all surface pressures). On contrary for $X_{\mathrm{HDHC}}=0.4$ and $X_{\mathrm{HDHC}}=0.6$ a LE phase exists until $A\approx0.46$ nm^2 molec^{-1} and $A\approx0.41$ nm^2 molec^{-1} respectively followed by a LE–LC phase transition and finally a LC phase appeared from $A\approx0.44$ nm^2 molec^{-1} and $A\approx0.38$ nm^2 molec^{-1} respectively. At pH = 10, all monolayers (formed by pure and mixed surfactant systems) present LE phase until $A\approx0.68$ nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=0$); ≈0.82 nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=0.2$); $A\approx0.61$ nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=0.4$); $A\approx0.51$ nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=0.8$) and $A\approx0.76$ nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=1$). Then a LE–LC phase transition appeared followed by a final LC phase from $A\approx0.64$ nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=0$); ≈0.76 nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=0.2$); $A\approx0.60$ nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=0.4$); $A\approx0.47$ nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=0.8$) and $A\approx0.72$ nm^2 molec^{-1} ($X_{\mathrm{HDHC}}=1$). All monolayers at all pH subphase do not show a visible transition to the solid (S) state.

The interaction between amphiphilic molecules at the air-aqueous solution interface is composed by a contributions of electrostatic, hydrophobic and hydration forces [1,13]. For mixed systems, we will examine the influence of such forces, analyzing their effect on the collapse area values.

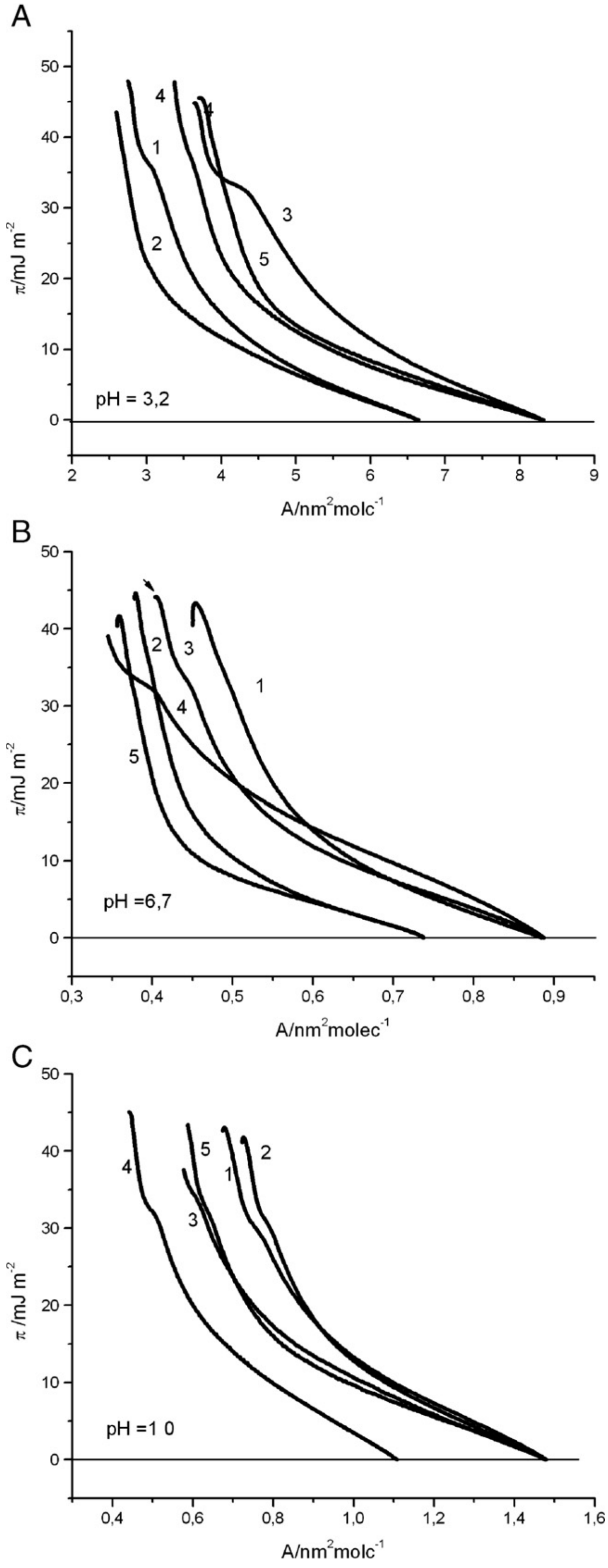

Fig. 2. π–A isotherms for HDHC/HDC systems at different pH subphase: (A) pH = 3.2; (1) $X_{\mathrm{HDHC}}=1$, (2) $X_{\mathrm{HDHC}}=0.4$, (3) $X_{\mathrm{HDHC}}=0.6$, (4) $X_{\mathrm{HDHC}}=0.8$, (5) $X_{\mathrm{HDHC}}=0$. (B) pH = 6.7; (1) $X_{\mathrm{HDHC}}=1$, (2) $X_{\mathrm{HDHC}}=0.2$, (3) $X_{\mathrm{HDHC}}=0.4$, (4) $X_{\mathrm{HDHC}}=0.6$, (5) $X_{\mathrm{HDHC}}=0$. (C) pH = 10; (1) $X_{\mathrm{HDHC}}=1$, (2) $X_{\mathrm{HDHC}}=0.2$, (3) $X_{\mathrm{HDHC}}=0.4$, (4) $X_{\mathrm{HDHC}}=0.8$, (5) $X_{\mathrm{HDHC}}=0$.

3.2. Surface thermodynamic analysis

In previous literatures [1,5,14], it is well established that the miscibility in mixed monolayers consisting of binary components can be simply and indirectly determined by means of the thermodynamically interfacial phase rule. An ideal mixed monolayer and a completely immiscible monolayer are absolute opposite. However, both follow Eq. (1). In an ideal mixed monolayer of component 1 and 2, the intermolecular force $F_{11}=F_{12}=F_{22}$ whereas in a completely immiscible monolayer $F_{11}>>F_{12}<<F_{22}$, where F_{12} are the net attractive forces between molecules of the two components.

The collapse area per molecule for an ideal two-component mixed monolayer, A_c^{id}, can be calculated from:

$$A_c^{id} = X_1 A_{1,c} + X_2 A_{2,c} \tag{1}$$

where X_1 and X_2 are the mole fractions of the components 1 (HDHC) and 2 (HDC) at the mixed spread monolayer, $A_{1,c}$ and $A_{2,c}$ are the collapse area per molecule of the pure components.

The excess area, A_c^E, for a binary monolayer can be expressed as:

$$A_c^E = A_{1,2,c} - \left(X_1 A_{1,c} + X_2 A_{2,c}\right) \tag{2}$$

where $A_{1,2,c}$ is the collapse area per molecule of the mixed monolayer. $A_{1,2,c}$, $A_{1,c}$ and $A_{2,c}$ can be obtained from the corresponding π–A isotherms and were taken as the point where the upper part of the curve starts to deviate from the straight line [15].

There are always net intermolecular forces between the molecules in a monolayer. It is a matter of magnitude of forces that determines A_c^E. For a mixed monolayer, if net attractive intermolecular forces exist, A_c^E will be negative. If A_c^E is positive, it suggests that the net interactions between two components are repulsive. The net interaction between two components in a mixed monolayer, at a constant surface pressure π and temperature T, can be evaluated from the calculation of excess Gibbs energy (ΔG_{mix}^E) [1,13,14], which is given by:

$$\Delta G_{mix}^E = \int_0^{\pi} \left[A_{1.2} - (X_1 A_1 + X_2 A_2)\right] d\pi \tag{3}$$

The Gibbs energy of mixing is given by:

$$\Delta G_{mix} = \Delta G_{mix}^{id} + \Delta G_{mix}^E \tag{4}$$

where the first term, the ideal Gibbs energy of mixing (ΔG_{mix}^{id}), can be calculated from the equation:

$$\Delta G_{mix}^{id} = RT(X_1 \ln X_1 + X_2 \ln X_2) \tag{5}$$

where R is the universal gas constant and T is the absolute temperature.

Fig. 3 shows the obtained collapse areas for the different HDHC/HDC mixtures spread on different pH subphase. Straight lines represent the ideal composition dependence of the collapse molecular area calculated by Eq. (1). The mean mixed monolayers molecular area was ideal at neutral subphase pH (pH=6.7). The variation of subphase pH notably altered the mixed monolayer behavior. The effective molecular collapse areas of pure HDC and HDHC at neutral pH subphase are similar to that of cholanoic acid [16] and consistent with cross-sectional area of the steroid nucleus which is ≈40 Å^2 [17]. We infer that at low surface pressures the steroid nucleus of bile acids lies parallel to the aqueous interface but assumes a vertical position upon compression. When subphase had pH=10 or 3.2 there was an increase of the collapse area (A_c) values, showing an expanding effect. At pH=10, because of the lift-off area for both bile acids and their mixtures are slightly greater than the cross-sectional area of the steroid nucleus, we can conclude that the hydrocarbon backbone appears to remaining approximately perpendicular to the aqueous interface. The mixed A_c persist in the ideal behavior whilst at pH=3.2 the collapse molecular areas are significantly different from those ideally computed. For such conditions, A_c is highly superior to the cross-sectional area of steroid group (≈40 Å^2) and to the bile acid molecular length [18] (≈1.3 nm) implying very poorly condensed monolayers. In this last case, for $X_{HDHC}=0.2$ and 0.4 (when the monolayer is rich in HDC) A_c shows a significant negative deviation from ideality. The difference between the theoretical and experimental molecular areas of the bile acids molecules in the condensed region can be explained by a loss of molecules from the monolayer. The HDC molecules (more soluble than HDHC) may desorb from the monolayer [19,20], alternatively some of the molecules may be squeezed out of the monolayer [21,22]. Banghan [21] suggest that over-compression of surfactant monolayers leads to a loss of surface molecules either into a reversible dry multilamellar phase or to an irreversible collapse phase. We speculate that these monolayers can tolerate compression with a rapid, reversible loss of molecules from the surface; this may explain the observation of smaller than theoretical molecular areas. On contrary, at $X_{HDHC}=0.6$ and 0.8 (when the monolayer is rich in HDHC) a significant expanding effect can be noticed. Such positive deviation indicates that the polar groups of the molecules interact more strongly by repulsive electrostatic forces with each other than with the more hydrophobic part of the molecules. Also, the hydration forces

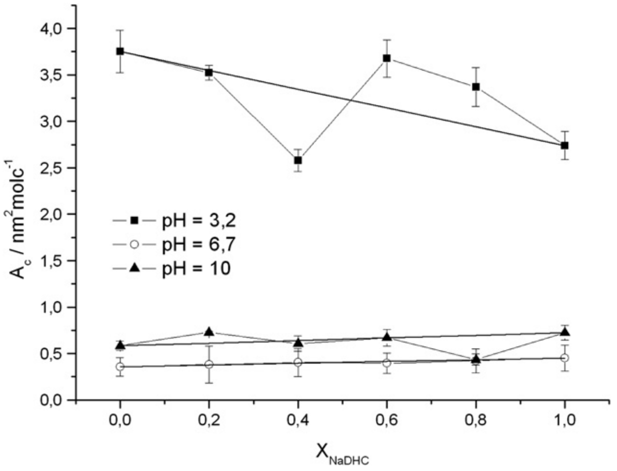

Fig. 3. Collapse areas values (A_c) obtained for the different HDHC/HDC mixtures spread on different subphase pH: 3.2, 6.7 and 10.

can result in the formation of H-bonding due to the presence of carboxyl and hydroxyl groups in the molecules.

The values of excess area (A_c^E) obtained by using Eq. (2) versus surface pressures at various compositions are shown in Fig. 4A, B and C.

In acidic subphase conditions (pH = 3.2), Fig. 4A, positive A_c^E values can be appreciated (at all surface pressures) when the monolayer is rich in HDHC (X_{HDHC} = 0.6 and 0.8). At X_{HDHC} = 0.2, when the monolayer is almost composed by HDC molecules, A_c^E values tend to zero from π = 20 mJ m^{-2}. Finally at X_{HDHC} = 0.4, A_c^E have negative values at all π indicating a high monolayer condensation for such composition.

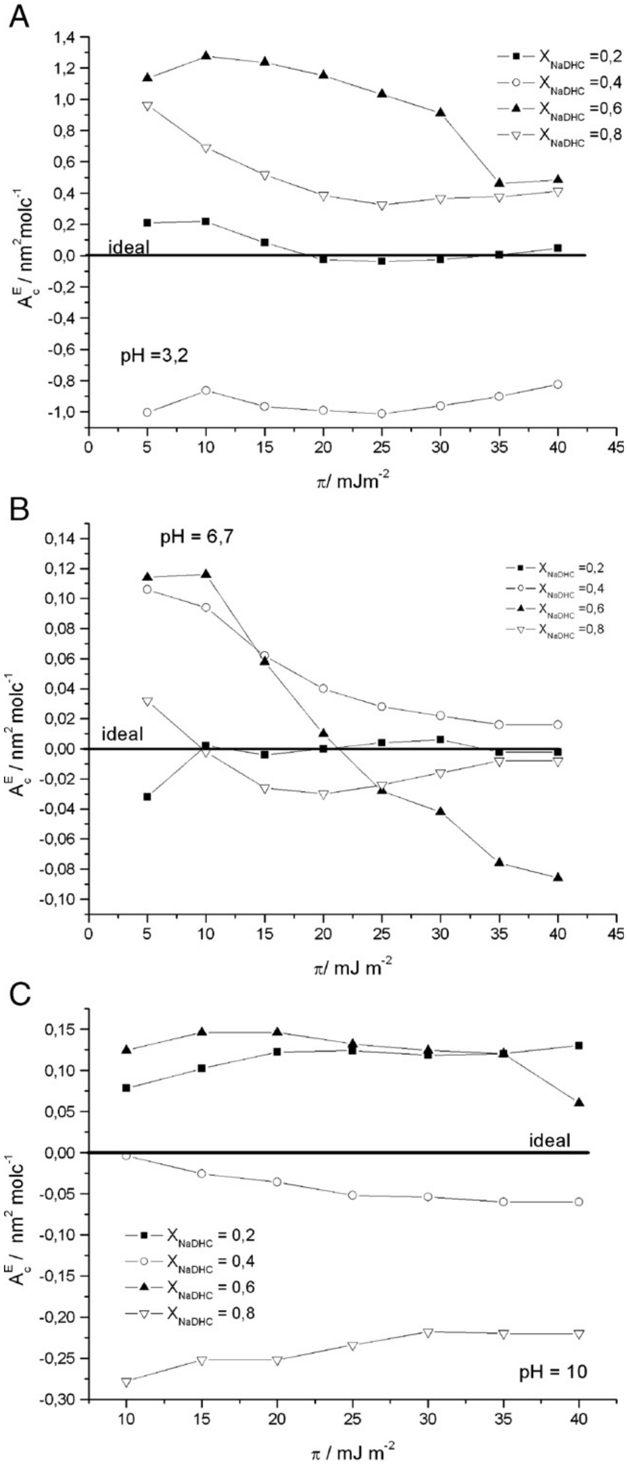

Fig. 4. Excess areas values (A_c^E) obtained for the different HDHC/HDC mixtures spread on different subphase pH (A) 3.2, (B) 6.7 and (C) 10.

When subphase pH is about 6.7, Fig. 4B, $A_c^E \approx 0$ at almost all π for X_{HDHC} = 0.2. At X_{HDHC} = 0.4, $A_c^E > 0$ until a higher decrease from π = 20 mJ m^{-2} when X_{HDHC} augmented (X_{HDHC} = 0.6). Once the monolayer is almost occupied for HDHC molecules (X_{HDHC} = 0.8), $A_c^E < 0$ and tend to ideality at high π values.

Basic subphase pH (pH = 10), Fig. 4C, provokes that $A_c^E \neq 0$ at every composition or pressure surface values. When monolayer composition is X_{HDC} = 0.4; 0.8, $A_c^E > 0$ and when is X_{HDHC} = 0.4 and 0.8, $A_c^E < 0$. The nonzero values of A_c^E and their dependence with the monolayer composition would indicate that miscible monolayers are formed at all π and subphase pH. A monolayer in which two components are immiscible will show two separate collapse surface pressures corresponding to individual pure components. When, $A_c^E \approx 0$ nearly ideal interactions are present in the monolayer.

Another way to estimate the monolayer behavior during compression is by the evaluation of the values of ΔG_{mix}^E at different pressures calculated according to Eq. (3), Table 1.

At pH = 3.2 and when the monolayer is rich in HDHC (X_{HDHC} = 0.8) or in HDC (X_{HDHC} = 0.2), $\Delta G_{mix}^E < 0$. The ΔG_{mix}^E maximum absolute value (4.3 ± 0.5 kJ mol^{-1}) corresponds with a surface pressure of 40 mJ m^{-2} (X_{HDHC} = 0.8) and 25 mJ m^{-2} (X_{HDHC} = 0.2). On the other hand ΔG_{mix}^E values changed from negative to positive at π = 30 mJ m^{-2} when X_{HDHC} = 0.4 and from positive to negative at π = 20 mJ m^{-2} when X_{HDHC} = 0.6.

At pH = 6.7, $\Delta G_{mix}^E < 0$ for almost all mixture compositions except for X_{HDHC} = 0.2 where almost all ΔG_{mix}^E values are positives.

Basic subphase causes that $\Delta G_{mix}^E > 0$ for almost all mixtures. The exception was X_{HDHC} = 0.4 where $\Delta G_{mix}^E < 0$ at all surface pressures.

Finally, the thermodynamic stability of the mixed monolayers composed of two miscible materials at the air/liquid interface was determined by the free energy of mixing, ΔG_{mix}. This parameter indicated the energetic tendency to form a mixed monolayer from an unmixed.

The values of ΔG_{mix} are shown in Fig 5A, B and C. It can be seen that ΔG_{mix} are all negative, suggesting that no phase separation occurs in the mixed monolayers and confirming that a miscible monolayer exists. Minimum values of such parameter are found at X_{HDHC} = 0.6 at all subphase pH values for all the studied surface pressures (5–40 mJ m^{-2}). As it can be observed, the absolute value of ΔG_{mix} increases as the surface pressure is augmented for X_{HDHC} = 0.4, 0.6 and 0.8 at pH = 6.7; X_{HDHC} = 0.6 and 0.8 at pH = 3.2 and X_{HDHC} = 0.4 and 0.6 at pH = 10. Negative values of ΔG_{mix} indicate the presence of attractive intermolecular interactions. The existence of a minimum will indicate a mixture composition of the greatest thermodynamic stability in comparison with the pure component monolayer. It is also observed that the values of ΔG_{mix} become progressively more negative as surface pressure increases. This may be due to shorter intermolecular distances at higher surface pressure as the monolayer becomes more condensed [23].

Table 1
Excess Gibbs energy of mixing of HDHC:HDC mixtures at different surface pressure and pH values

π (mJ m^{-2})/ X_{HDHC}	ΔG^{E} (kJ mol^{-1}) 0.2	0.4	0.6	0.8
pH=3.2				
5	−0.41±0.02	−0.08±0.02	0.31±0.02	−0.36±0.02
10	−1.49±0.05	−0.21±0.02	0.57±0.02	−0.94±0.05
15	−3.3±0.10	−0.65±0.03	1.65±0.05	−0.56±0.02
20	−4.11±0.20	−1.10±0.24	0.57±0.02	−1.68±0.11
25	−4.99±0.65	−2.37±0.32	−1.97±0.08	−4.11±0.13
30	−4.44±0.26	−1.26±0.07	−4.00±0.23	−4.02±0.02
40	−0.43±0.01	2.69±0.12	−1.31±0.15	−4.33±0.26
pH=6.7				
10	0.03±0.01	−0.13±0.02	−0.08±0.01	−0.14±0.01
15	0.11±0.01	−0.27±0.03	−0.34±0.08	−0.44±0.02
20	0.20±0.02	−0.51±0.02	−0.96±0.03	−0.51±0.03
25	0.38±0.03	−0.57±0.02	−1.43±0.12	−0.65±0.03
30	0.47±0.01	−0.68±0.02	−1.83±0.12	−0.61±0.07
35	0.55±0.01	−0.87±0.06	−2.48±0.23	−0.57±0.05
40	0.72±0.03	−0.85±0.06	−2.64±0.15	−0.52±0.02
pH=10				
10	0.09±0.02	−0.07±0.02	0.45±0.05	0.53±0.05
15	0.23±0.05	−0.19±0.01	0.70±0.07	0.76±0.08
20	0.33±0.02	−0.34±0.03	0.68±0.02	0.89±0.07
25	0.44±0.06	−0.50±0.05	0.35±0.04	0.83±0.07
30	0.69±0.08	−0.60±0.07	0.41±0.01	0.99±0.09
35	0.65±0.10	−0.50±0.02	0.09±0.01	0.81±0.06
40	0.71±0.02	−0.58±0.03	−1.59±0.13	0.88±0.03

3.3. Equation of state analysis

The equation for a two-dimensional ideal is:

$$\pi A = \kappa T \tag{6}$$

where κ is the Boltzmann constant.

If it is assumed that the molecules at the monolayers can not be compressed to a zero area, the equation of state can be expressed [24] as:

$$\pi(A - A_0) = \alpha \kappa T \tag{7}$$

or in its linear form:

$$\frac{\pi A}{\kappa T} = \alpha + \frac{\pi A_0}{\kappa T} \tag{8}$$

where α is a factor related to the aggregation between molecules in the monolayer and A_0 is the minimum area per molecule occupied by the monolayer molecules. A value of $\alpha=1$ is attributed to the ideal gas monolayer behavior.

The surface pressure, area and temperature behavior of non-ideal monolayers have been accurately represented using the virial equation of state [25–27]. Thus, the two-dimensional virial equation of state can be written as:

$$\frac{\pi A}{\kappa T} = b_0 + b_1 \pi + b_2 \pi^2 \tag{9}$$

where b_0, b_1 and b_2 are the virial coefficients. The value of b_0 is attributed to the aggregation state of the film-forming molecules. When π tends towards zero, the πA product would tend towards κT, or a fraction of κT for an aggregated system [14]. On the other hand, the value of b_1 provides information about the exclusion volumes and the interaction between the molecules in the film. Negative or positive values indicate attractive or repulsive interaction between the molecules, respectively. The rest of the virial coefficients are not significant.

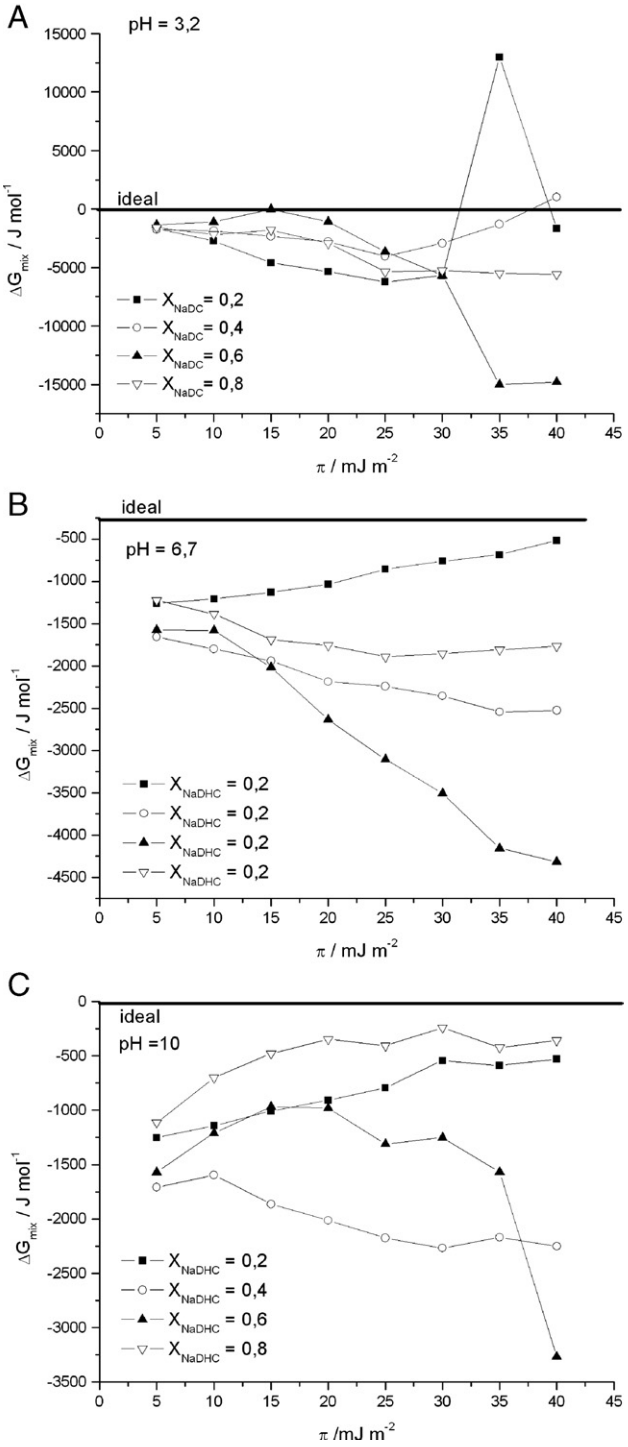

Fig. 5. Gibbs energy of mixing (ΔG_{mix}) obtained for the different HDHC/HDC mixtures spread on different subphase pH (A) 3.2, (B) 6.7 and (C) 10.

Table 2
Virial and correlation coefficient calculated from the fitting values of Eq. (9)

pH=3.2	X_{HDHC}					
	0	0.2	0.4	0.6	0.8	1
b_o	2.08±0.08	2.21±0.09	1.69±0.08	0.99±0.05	2.04±0.08	1.13±0.05
b_1 (m mN^{-1})	1.16±0.01	1.12±0.01	0.89±0.01	1.56±0.01	1.13±0.01	1.07±0.01
b_2 (m^2 mN^{-2})	$(-68\pm2.5)\times10^{-4}$	$(-66\pm2.7)\times10^{-4}$	$(-78\pm3.4)\times10^{-4}$	$(-170\pm2.8)\times10^{-4}$	$(-83\pm2.8)\times10^{-4}$	$(-100\pm2.3)\times10^{-4}$
r	0.995	0.993	0.983	0.998	0.991	0.996
pH=6.7	0	0.2	0.4	0.6	0.8	1
b_o	0.154±0.005	0.118±0.005	0.176±0.006	0.139±0.01	0.231±0.009	0.124±0.006
b_1 (m mN^{-1})	$(102\pm0.9)\times10^{-3}$	$(113\pm0.7)\times10^{-3}$	$(140\pm0.9)\times10^{-3}$	$(175\pm1.6)\times10^{-3}$	$(124\pm1)\times10^{-3}$	$(154\pm0.9)\times10^{-3}$
b_2 (m^2 mN^{-2})	$(-46\pm2.0)\times10^{-5}$	$(-55\pm1.6)\times10^{-5}$	$(-84\pm2.2)\times10^{-5}$	$(-27\pm0.5)\times10^{-4}$	$(-5.6\pm0.3)\times10^{-4}$	$(-11.4\pm0.3)\times10^{-4}$
r	0.998	0.998	0.998	0.992	0.995	0.998
pH=10	0	0.2	0.4	0.6	0.8	1
b_o	0.39±0.07	0.32±0.01	0.31±0.02	0.21±0.01	0.17±0.01	0.38±0.02
b_1 (m mN^{-1})	0.207±0.003	0.236±0.002	0.236±0.005	0.285±0.001	0.196±0.002	0.235±0.002
b_2 (m^2 mN^{-2})	$(-19\pm0.6)\times10^{-4}$	$(-16\pm0.4)\times10^{-4}$	$(-30\pm0.7)\times10^{-4}$	$(-28\pm0.4)\times10^{-4}$	$(-27\pm0.6)\times10^{-4}$	$(-19\pm0.5)\times10^{-4}$
r	0.988	0.997	0.990	0.998	0.994	0.995

The virial coefficients (b_0, b_1, b_2) and the correlation coefficients obtained are listed in Table 2. Since the values of the first virial coefficient (b_0) are lower than 1 for all HDHC/HDC mixtures at neutral or basic subphase conditions, two-dimensional bile acids aggregates could exist at low pressures. Thus, in agreement with the literature [17,18] their aggregation number ($1/b_0$) can be estimated within the order of 6 (pH=6.7) and of 3 (pH=10). This could be related, at least indirectly to the existence of laterally structured microdomains.

In subphase acid conditions (pH=3.2), $b_0 \geq 1$ signifying that no aggregation exists at the monolayer.

The values of the second virial coefficient (b_1) are all positive indicating that the predominant interactions in the monolayer are repulsive. Maximum repulsion can be observed at acid subphase pH in accordance with the obtained A_c values for such pH.

To evaluate the nature of the interactions between two molecules in a mixed monolayer the following equation can be used [28]:

$$(b_1)_m = (b_1)_1 X_1^2 + (b_1)_2 X_2^2 + 2(b_1)_{12} X_1 X_2 \qquad (10)$$

where $(b_1)_1$, $(b_1)_2$ and $(b_1)_{12}$ are the second virial coefficients of the pure components 1 (HDHC), 2 (HDC) and that due to interaction of both components in the mixture respectively. In ideal condition $(b_1)_2=[(b_1)_1+(b_1)_2]/2$ and Eq. (10) becomes:

$$(b_1)_m^{id} = (b_1)_1 X_1 + (b_1)_2 X_2 \qquad (11)$$

where $(b_1)_m^{id}$ is the $(b_1)_m$ value in an ideal mixed monolayer.

Also, by similarity with Eq. (2) we can define the excess second virial coefficient of the mixed monolayer as:

$$(b_1)^E = (b_1)_m - (b_1)_1 X_1 - (b_1)_2 X_2 \qquad (12)$$

The values of $(b_1)^E$, for all HDHC/HDC mixture compositions and all subphase pH, are shown in Fig. 6. For neutral and basic subphase conditions, the values of $(b_1)^E$ are nearly zero indicating an ideal behavior. That fact would imply that there was an ideal mixture at the monolayer or that the components are immiscible.

At pH subphase of 3.2, the behavior is nearly ideal when the monolayer is rich in one of both components (X_{HDHC}=0.2, 0.8), but the value of $(b_1)^E$ is highly negative when X_{HDHC}=0.4 indicating attractive interactions and highly positive for X_{HDHC}= 0.6 implying repulsive interaction.

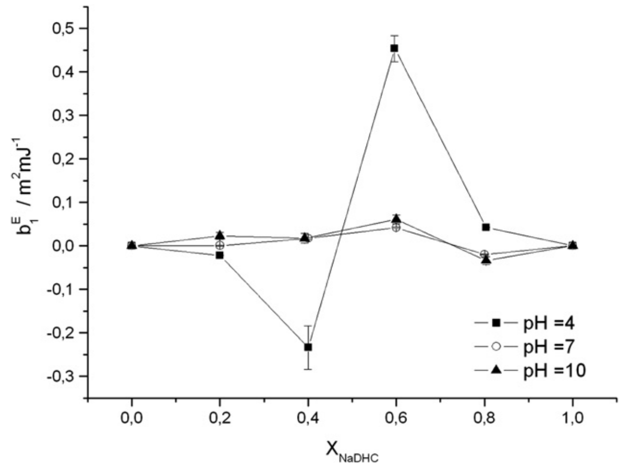

Fig. 6. Excess second virial coefficients, $(b_1)^E$, for different HDHC/HDC mixture composition and subphase pH.

4. Concluding remarks

Bile acids (deoxycholic and dehydrocholic acids) spread mixed monolayers behavior at the air/water interface were studied as a function of subphase pH using a precise, versatile and non-invasive technique based on the Axisymmetric Drop Shape Analysis (ADSA).

The influence of electrostatic, hydrophobic and hydration forces on the interaction between amphiphilic molecules at the interface were evaluated. The investigation of surface thermodynamic parameters together with the equation of state analysis showed that the variation of pH subphase notably altered the mixed monolayer behavior.

At neutral (pH=6.7) or basic (pH=10) subphase conditions the collapse area values are similar to that of the cholanoic acid and consistent with the cross-sectional area of the steroid nucleus (≈40 Å^2). The evaluation of A_c and $(b_1)^E$ values indicated that the corresponding mixed monolayer presented and ideal behavior at such pH conditions. The values of the Gibbs energy of mixing ($\Delta G_{\mathrm{mix}}<0$) and the first virial coefficients ($b_0<1$) indicated that attractive forces existed between the monolayer components giving rise to a miscible monolayer with laterally structured microdomains. The microdomains aggregation numbers ($1/b_0$) were estimated as about 6 at pH = 6.7 and of 3 at pH = 10.

At acidic subphase conditions (pH = 3.2), no phases separation occurred ($\Delta G_{\mathrm{mix}}<0$) but a high expanded effect of the monolayer could be noted by the evaluation of A_c which was superior to the cross section area of the steroid group and to the bile acid molecular length (≈1.3 nm). The mixed monolayer behavior was not ideal and no aggregates were formed ($b_0 \geq 1$). Such behavior indicates that the polar groups of the molecules interact more strongly by repulsive electrostatic forces than with the more hydrophobic part of the molecule. Also hydration forces can act giving rise to the formation of H-bond between carbonyl and hydroxyl groups in bile acids molecules.

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