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Interactions between bovine serum albumin and sodium taurodeoxycholate: thermodynamic properties

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Abstract

The interaction between bovine serum albumin (BSA) and sodium taurodeoxycholate (NaTDC), a component of the bile in mammals, have been investigated in a wide range of experimental conditions and for several protein to surfactant ratios. The solution region has been investigated by surface tension σ, freezing point depression ΔT, as well as by integral heat of dilution data $\Delta H_{i,dil}$. The corresponding properties of NaTDC in aqueous solutions have been investigated too.

Surfactant binding onto the protein is presumably controlled by a delicate balance of hydrophobic and electrostatic contributions, responsible for the adduct stability, but does not give rise to precipitation or coacervate formation. This behaviour is in line with current knowledge on the solution behaviour of BSA–TDC complexes occurring in hepatic and gallbladder bile.

Keywords: BSA; NaTDC; Surface and colligative properties; Solution calorimetry; Interactions

1. Introduction

The interactions between macromolecules and surface-active agents are the subject of noticeable interest in recent years. Investigations reported so far deal with mixtures containing neutral homo-polymers and ionic surfactants [1,2], block co-polymers (or hydrophobically modified ones) and surface-active agents [3,4], polyelectrolytes and surfactants having unlike charge [5], proteins (usually globular) and surfactants [6–10]. Applications of all the above systems as gelling agents, thickeners, solubilisers, etc., are possible [11].

As far as protein–surfactant systems (PSS), most studies focus on practical applications, oriented toward protein recovery from biological matrices [12]. Unfortunately, a detailed knowledge of the forces responsible for protein stabilisation, or precipitation, by surfactants is not at hand. A complete understanding of the forces controlling the interactions between proteins and surfactants requires a detailed knowledge of the related electrostatic and hydrophobic contributions. The combination of the above effects, in fact, is important in the organisation and structure of protein–surfactant complexes [13].

Not much is known on the details of protein–surfactant interactions and studies on the stability, dynamics and structure of PSS are few [14,15]. This is surprising, since protein–surfactant complexes play a key role in bio-chemically relevant processes. Significant examples are the lung surfactants, mixtures of lipids and proteins responsible for the pulmonary expansion and contraction [16] and the complexes formed by albumin and bile salts in entero-hepatic and gallbladder bile [17]. The present work focuses on the dilute region of a system containing albumin and bile salts.

Bile salts, by-products of the cholesterol pool in vivo, are characterised by the presence of a polar and a non-polar surface, allowing the formation of aggregates [18–20] and liquid crystalline phases [21]. Micelles formed by the above surface-active agents are small and their aggregation features strongly depend on surfactant content [22].

The interactions of bile salts with proteins are presumably modulated by bile salt structure. In the case of albumin, for instance, the stability constant of the adducts formed with bile salts is, at least, two orders of magnitude lower than *n*-alkyl chain surfactants, fatty acids and soaps [23]. In addition, no albumin precipitates from solutions and media

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containing bile salts, at least in the present experimental conditions (pH close to 6, when the duodenal physiological value is about 6.3).

Significant changes in physico-chemical properties are associated to the interactions between bile salts and albumin. To get reasonable hypotheses on the adduct(s) stability detailed knowledge of their thermodynamic properties is required. For this purpose we present and discuss the thermodynamic behaviour observed in ternary system water–bovine serum albumin (BSA)–sodium taurodeoxycholate (NaTDC), at 25 °C. We report on surface tension, colligative properties and calorimetric findings. Comparison shall be made between the behaviour in water and in some water–BSA pseudo solvents. When possible, data shall be analysed in terms of transfer functions.

The results we report indicate, in perspective, possible links between the observed behaviour and the formation of TDC–BSA adducts occurring in entero-hepatic and gallbladder bile.

2. Experimental

2.1. Materials

Bovine serum albumin fraction *V*, referred to as BSA, lot no. 90 K1352, 99% nominal purity was from Sigma. The average molal mass of the monomer is about 66 kD and its iso-electric point is close to 4.7 [24]. BSA was desiccated under vacuum, at room temperature and used as such. Density, viscosity and ionic conductivity measurements on its aqueous solution [17,23] confirmed the protein purity.

Sodium taurodeoxycholate (NaTDC), 97% purity was from Sigma. NaTDC was dissolved in hot ethanol, filtered by fritted funnels (to remove dust and particles) and mixed with cold acetone. The whole procedure was repeated twice. The precipitate was vacuum dried at 70 °C for 2 days. The product purity was confirmed by comparison with data by Sesta et al. [19]. The agreement with CMC values and surface pressure at the CMC, $\Pi_{CMC} = \sigma^{\circ} - \sigma_{CMC}$ is within ±2%.

Water was distilled over alkaline $KMnO_4$. Its ionic conductivity at 20 °C is 1 μS cm^{-1}. The solutions were prepared by weight, corrected for buoyancy and allowed to equilibrate before use for 2 days. Surface tension and calorimetric findings have observed no kinetic effects on the system properties.

2.2. Methods

2.2.1. Surface tension

A digital Kruss unit, Mod. K10T, measured the surface tension, σ of the systems. Measurements are accurate to ±0.2 mN m^{-1}. The temperature is controlled to ±0.1 °C by an external water circulation jacket. The solutions were equilibrated for 20 min before running the experiments. Each datum is the mean value of five or more independent measurements. Details on the apparatus setup are given elsewhere [25].

2.2.2. Colligative properties

A Knauer cryoscopic unit, Model 24.00, measured the freezing point depression of the solutions. It is equipped with a sample holder, connected to a peltier unit and a measuring readout. Preliminary calibration was performed on NaCl solutions. Information on the apparatus setup and calibration may be found elsewhere [26].

The following empirical relation calculated the freezing point depression of the solutions ΔT:

$$\Delta T = K_c \nu m \quad (1)$$

where K_c is the freezing point depression constant of the solvent, ν the number of ions and m is the solute molality.

In the ternary H_2O–BSA–NaTDC systems, the protein content in the solvent was fixed. It is less always than 1.0 wt.%, to minimise undesired effects on K_c value, i.e. on the freezing point depression of the H_2O–BSA solution with respect to water.

2.2.3. Potentiometry

Additional e.m.f. studies by sodium ion electrodes were performed. A Philips commercial potentiometric unit equipped with an ion selective electrode, Model 561, was used. Measurements were performed at 5.0, 15.0 and 25.0 °C. They were used to estimate sodium ion activity γ_+, to extrapolate the corresponding values to 0.0 °C and to determine counter–ion binding to micelles. The latter quantity β is the ratio of e.m.f. slopes versus $\log m$ above and below the CMC. In water β was found to be 0.65 ± 0.03, at 25.0 °C. A similar value, but with much higher uncertainty was found in the 0.50 wt.% water–BSA system.

2.2.4. Dilution enthalpy

The batch calorimeter is a heat conduction type LKB unit [27,28] (Model 2107) operating at 25.00 ± 0.01 °C. It is equipped with two gold vessels of about 7 cm^3 total volume, a multi-temperature cooling circulator (LKB 2210), a control unit (LKB 2107-350) and a potentiometric recorder (LKB 2110). Each vessel consists of a chamber divided in two compartments by a wall. The reactants are introduced separately in each compartment. When the experiment is started and the calorimetric unit is rotated, the reactants are mixed and the process takes place, giving a curve reporting a voltage versus time.

In enthalpies of dilution, as well as in all other experiments, the amount of BSA in the (pseudo) solvent is fixed to 0.20, 0.50 and 1.00 wt.%. Eventual effects due to the supra-molecular association of BSA have not been observed in the concentration range between 0 and 2.0 wt.% of protein, at 25 °C. This is in line with the fact that albumin association is essentially thermal in origin and occurs at much higher temperatures [29].

A calibration constant, η (J cm^{-2}), was obtained for each instrumental sensitivity range. It is the average value of, at least, 10 independent measurements (performed at different current intensities) and was obtained by using the Joule equation to calculate the calibration heat. Accordingly

$$Q_i{}^* = r{I_i}^2 t \quad (2)$$

where r is the value in Ω of the resistance inside the measuring vessel, I_i the intensity of the calibration current used for each measurement and t is the calibration time (20 s). For each $Q_i{}^*$ value an area $A_i{}^*$ was measured. From the ratio $Q_i{}^*/A_i{}^*$, the value of η was obtained for each sensitivity range. The integral heat of dilution, Q_{meas}, was calculated by

$$Q_{meas} = \eta A_{meas} \quad (3a)$$

where A_{meas}, the mean area obtained by the recorder, represents the average value of at least three independent measurements.

The integral enthalpies of dilution, $\Delta H_{i,dil}$, were obtained by the equality

$$\frac{Q_{meas}}{n} = \Delta H_{i,dil} \quad (3b)$$

where n is the number of moles of NaTDC.

The apparatus was calibrated with aqueous solutions of sucrose [27]. The uncertainty on the heat of dilution values $\Delta H_{i,dil}$ is lower than 1%.

3. Results

3.1. Surface tension

Data were analysed by the Gibbs adsorption equation, according to

$$d\sigma = -\Gamma_2{}^*(RT\, d\ln a_2) \quad (4a)$$

where the solute activity, a_2, is replaced by the corresponding molality, m_2R the gas constant, T the temperature and Γ_2 is the surface excess concentration.

CMC values are the intersection point of two straight lines in surface tension versus $\ln m_2$ plots. As can be seen in Table 1, addition of BSA has a small effect on CMC values of sodium taurodeoxycholate. A plot of surface tension findings is reported in Fig. 1. Unexpectedly, no critical association concentration threshold [11] was observed, whereas the CMC regularly increases with protein content. Relevant data are reported in Table 1.

Table 1
The amount of BSA in the water–protein solvent (wt.%), the critical micellar concentration of NaTDC, CMC, in molality (mol kg^{-1}), the Gibbs energy of micelle formation, ΔG_{mic} (kJ mol^{-1}), the surface pressure at the CMC, Π_{CMC} (mN m^{-1}) and the area per molecule A (Å^2), at 25 °C

BSA (wt.%)	CMC	ΔG_{mic}	Π_{CMC}	A
0.00	$2.1 \pm 0.2 \times 10^{-3}$	−20.6	18.5	60 ± 6
0.21	$2.4 \pm 0.1 \times 10^{-3}$	−20.1	16.4	92 ± 4
0.50	$2.5 \pm 0.2 \times 10^{-3}$	−20.0	14.6	102 ± 6
1.01	$2.8 \pm 0.2 \times 10^{-3}$	−19.2	12.6	118 ± 8

The area per molecule at the interface $A_{2,min}$ was calculated by

$$A_{2,min} = \frac{10^{20}}{N\,\Gamma_{2,max}} \quad (4b)$$

where N is Avogadro's number and $\Gamma_{2,max}$ is the maximum surface excess concentration below the CMC (Table 1).

The Gibbs energy of adsorption, ΔG°_{ads}, was inferred by the relation [25]:

$$\Delta G^\circ_{ads} = \Delta G^\circ_{mic} - \frac{\Pi_{CMC}}{\Gamma_{2,max}} \quad (5)$$

where G°_{mic} is the Gibbs energy of micelle formation. The dependence of G°_{ads} on BSA content is reported in Fig. 2.

3.2. Colligative properties

The practical osmotic coefficients, Φ, were calculated from freezing point depression, according to [30]

$$\Phi = \left(\frac{0.1278\,\Delta T}{m\nu}\right)(4.207 + 2.1^* \times 10^{-3}\,\Delta T) \quad (6)$$

where the meaning of symbols is as before. Errors on Φ values are to ±1%. Selected osmotic coefficients are reported in Table 2. The mean activity coefficients of NaTDC, $\gamma_\pm$, were calculated according to

$$\ln\gamma_\pm = (\Phi - 1)\left[1 + \frac{1}{M^\circ}\int_0^m \frac{d\sqrt{m}}{\sqrt{m}}\right]$$

where M° is the solvent molecular mass and $\sqrt{m}$ is the square root of solute molality. Since the integral diverges as $\sqrt{m}$ approaches zero, the lower limit of the integrand was

Table 2
The practical osmotic coefficients, Φ (in °C kg mol^{-1}), the molal concentration, m (mol kg^{-1}), of NaTDC in water and in 1 BSA wt.% in water solutions as inferred from freezing point depression data

Water		1 BSA wt.%	
m	Φ	m	Φ
0.0011	0.989	0.0015	0.908
0.0013	0.988	0.0020	0.873
0.0015	0.985	0.0026	0.852
0.0017	0.980	0.0034	0.829
0.0020	0.971	0.0055	0.794
0.0023	0.961	0.0073	0.772
0.0027	0.947	0.0134	0.708
0.0030	0.935	0.0231	0.607
0.0041	0.891	0.0332	0.495
0.0055	0.835	0.0403	0.430
0.0072	0.770	0.0499	0.400
0.0096	0.687	0.0592	0.407
0.0131	0.585		
0.0170	0.492		
0.0222	0.417		

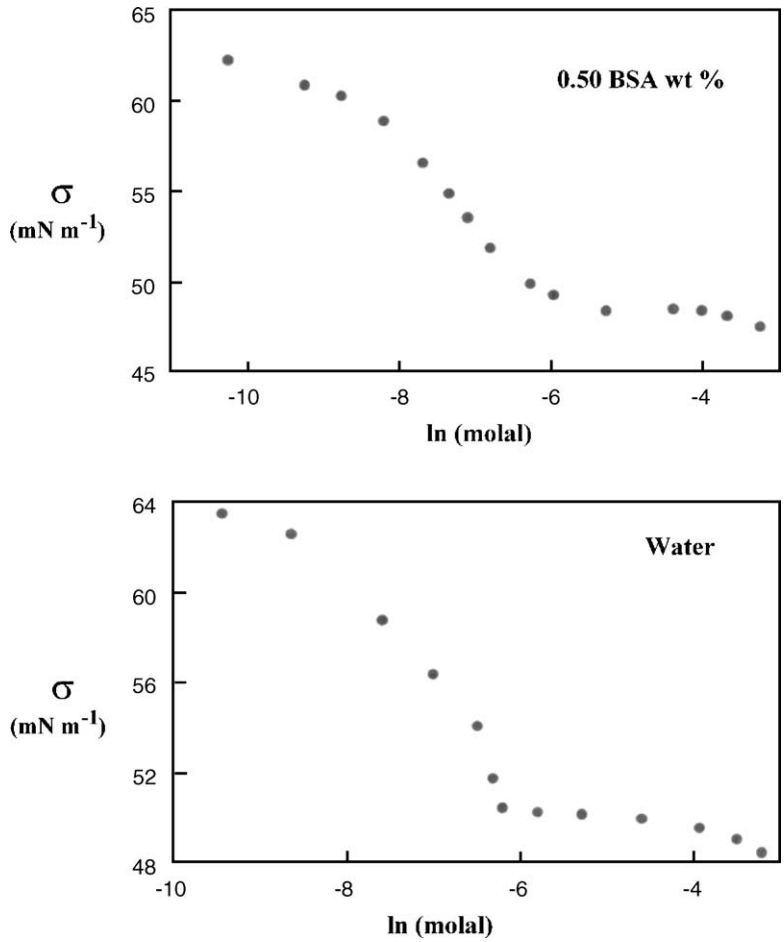

Fig. 1. Plot of the surface tension, σ (mN m^{-1}), as a function of NaTDC molality, in logarithmic form at 25 °C. Data refer to 0.50 BSA wt.%, up and water, down.

replaced by a reference value $\neq 0$ [31]. In this way $\gamma_{\pm}$ data are self-consistent in the whole concentration range.

The dependence of activity coefficients on bile salt molality is reported in Fig. 3. The activity coefficients in the ternary systems can be measured with good accuracy when the amount of BSA in the solvent medium is $\leq$1.0 wt.%.

3.3. Calorimetric data

The integral heat of dilution, $\Delta H_{\mathrm{i,dil}}$ is related to the relative apparent molal enthalpy of dilution, Φ_{L}, by the equality

$$\Delta H_{\mathrm{i,dil}} = \frac{Q_{\mathrm{meas}}}{n} = \Phi_{\mathrm{L,fin}} - \Phi_{\mathrm{L,in}} \quad (8)$$

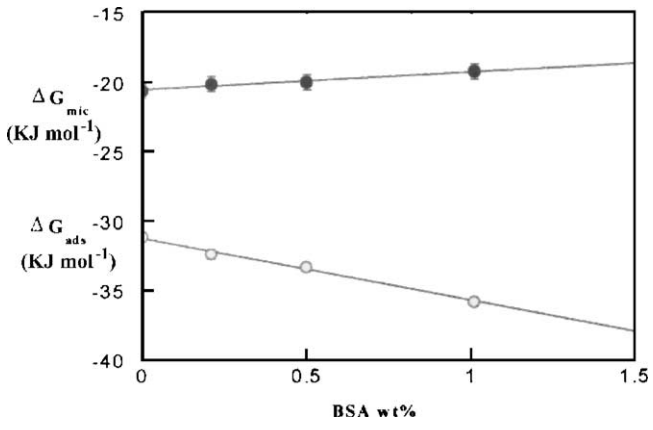

Fig. 2. The Gibbs energy of adsorption, ΔG_{ads} (kJ mol^{-1}) (empty symbols) and of micelle formation, ΔG_{mic} (kJ mol^{-1}) (full symbols) as a function of BSA wt.%, at 25.0 °C.

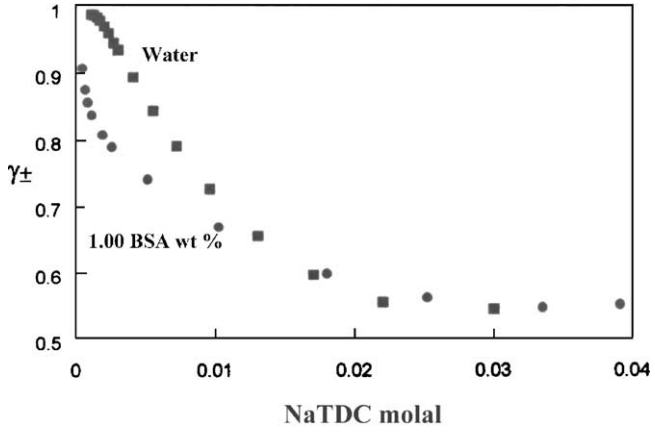

Fig. 3. The average activity coefficient, $\gamma_{\pm}$, of NaTDC as a function of its molality (in mol kg^{-1}), at 0.0 °C. Data in water (squares) and 1.00 BSA wt.% in water (circles).

where $\Phi_{L,in}$ and $\Phi_{L,fin}$ are the corresponding enthalpies before and after dilution, respectively. They can be expressed in terms of a power law equation in $\sqrt{m}$, according to

$$\Phi_L = \sum_{i=1} A_i (\sqrt{m})^i \quad (9)$$

where A_1 is Debye's term (1973 J l$^{1/2}$ mol$^{-3/2}$, at 25 °C, in water) and other constants were obtained by fitting the data into the equation:

$$\frac{[\Delta H_{i,dil} - A_1(\sqrt{m}_{fin} - \sqrt{m}_{in})]}{(m_{fin} - m_{in})} = A_2 + A_3(\sqrt{m}_{fin} - \sqrt{m}_{in}) \quad (10)$$

from which A_2 and A_3 were obtained.

In the case of water–BSA mixtures the A_1 constant was calculated from the dielectric permittivity, ε of water–BSA mixtures according to [32]

$$A_1 = \left(\frac{1.67 \times 10^7}{\varepsilon}\right) \frac{1}{\sqrt{T\varepsilon}} \left[1 + \frac{T}{\varepsilon}\frac{\partial \varepsilon}{\partial T}\right] \quad (11)$$

where ε was experimentally determined as a function of protein content [33]. At low protein content the effect of ε on A_1 is moderate. In 0.2 BSA wt.% solution, for instance, A_1 is 2013 J l$^{1/2}$ mol$^{-3/2}$.

Eq. (10) was used in the molecular region, below the CMC. Data above that threshold were calculated by properly combining Eqs. (8)–(10) up to convergence.

The relative partial molal enthalpies of dilution, hereafter referred as L_2 values, were calculated by

$$L_2 = \frac{\partial \left(\Phi_L \sqrt{m}\right)}{\partial \sqrt{m}} \quad (12)$$

where the meaning of symbols is as above. Plots of relative apparent, Φ_L and partial, L_2, molal enthalpies of dilution for binary and ternary systems as a function of surfactant molality are reported in Figs. 4 and 5, respectively. The small discontinuity in L_2 values occurring around 0.003 m, slightly above the CMC value, obtained by calorimetry is presumably related to small changes in the solution properties, once micelles are formed. A similar behaviour was formerly observed in the water–sodium deoxycholate system [34].

4. Discussion

Due to the presence of polar and non-polar moieties, the association features of bile salts are peculiar. It is extremely difficult to assemble the bile salt units together and form micelles with distinct polar/non-polar domains. The structural peculiarities of bile salts are reflected in the formation of unconventional supra-molecular aggregates [22], unusual gels [35] and complex liquid crystalline phases [21]. The reciprocal arrangement of bile salt ions in three dimensions gives rise to helical steroidal complexes, whose axial ratios increase in proportion to bile salt content, pH and/or medium ionic strength [36].

The thermodynamic forces driving the association of bile salts are a combination of electrostatic, hydrophobic and hydrogen bond contributions. Hence, classical approaches to micelle formation give a non-satisfactory description of the phenomena associated to the self-organisation of bile salts.

To rationalise the aggregation of NaTDC we discuss its thermodynamic properties. The part relative to the interactions between BSA and NaTDC and a comparison between the two sets of data complete the discussion.

The Gibbs energy of micelle formation, ΔG_{mic}, was calculated from a charged (pseudo) phase separation approach [37], according to

$$\Delta G_{mic} = KT(2 - \beta) \ln \mathrm{CMC} \quad (13)$$

where the CMC is in mole fraction units and β is the counter–ion binding [38].

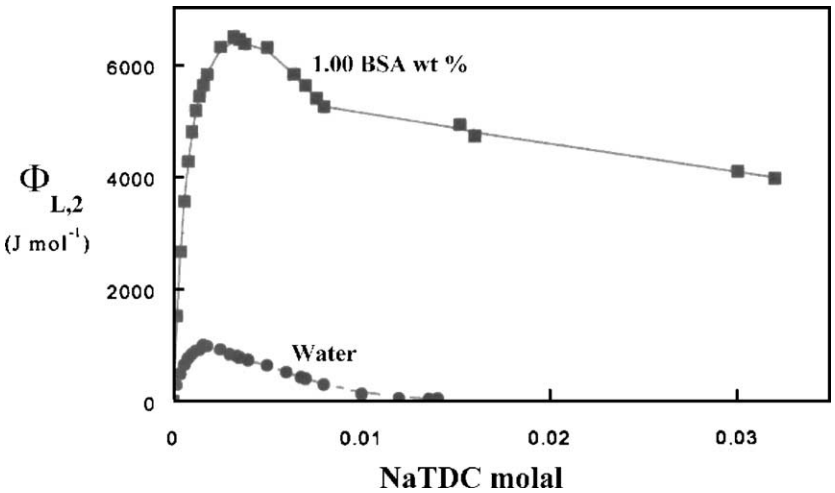

Fig. 4. The apparent molal enthalpy of dilution, Φ_L (J mol^{-1}), as a function of NaTDC molality, at 25.0 °C. Data refer to water (squares) and 1.00 BSA wt.% in water (circles).

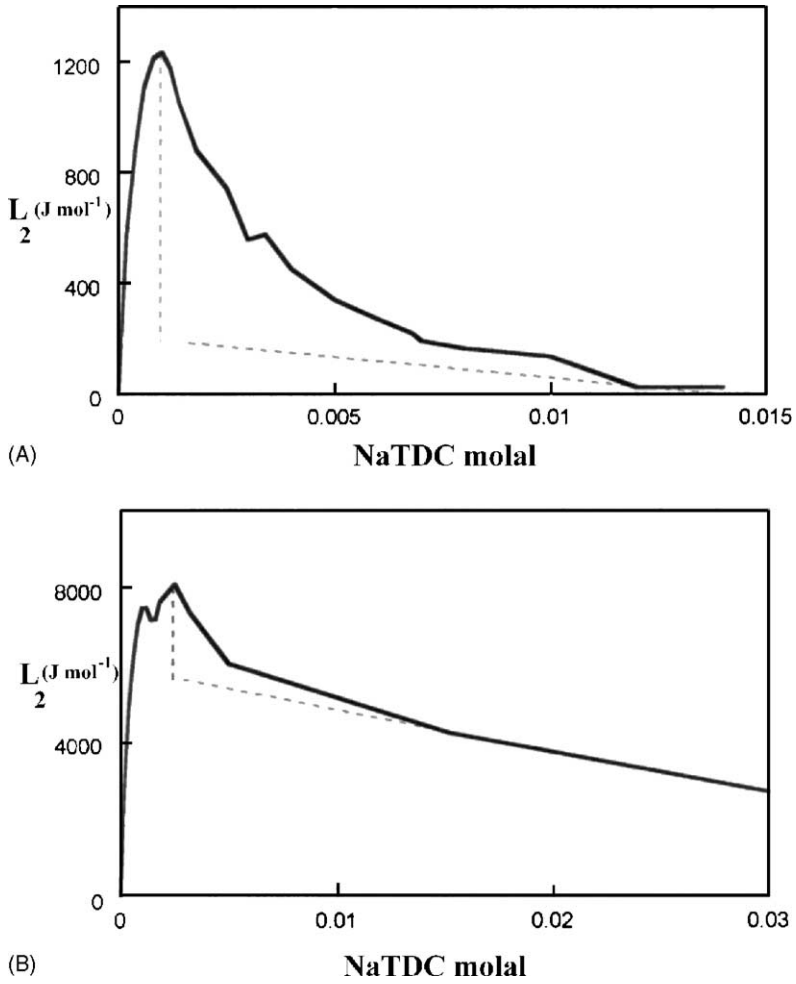

Fig. 5. The partial molal enthalpy of dilution, L_2 ($J\,mol^{-1}$), as a function of NaTDC molality, at 25.0 °C. Data refer to water (A) and 1.00 BSA wt.% in water (B). The CMC is indicated by vertical lines. The concentration at which the interactions between BSA and NaTDC begin to occur is close to the secondary maximum of the curve, below the CMC.

The reasons for using the phase separation approach in the evaluation of thermodynamic data are:

1. Micelle formation in bile salts is questionable, because of the limited association [39].
2. It is unrealistic to rationalise the association features of BS in terms of an average micelle aggregation number, $\langle N\rangle$, when the aggregation is a continuous process.
3. In the mass action approach to micelle formation, ΔG_{mic} is related to the transfer of a surfactant molecule from water to a micelle-like interior and depends on $1/\langle N\rangle$.

When the aggregation numbers do not change too much, conversely, it is possible to determine the number of molecules involved in micelle formation from thermodynamic methods. In the concentration range from the CMC to 0.04 m in NaTDC (where changes in aggregation numbers are moderate [22]), we assumed the validity of a mass action model for micelle formation [30] and imposed that a single micellar species dominates. In this case, the concentration of surfactant in molecular, X_1 and micellar form, X_{mic} (in mole fraction units), obey the equation:

$$\Delta G_{mic} = \frac{KT}{\langle N\rangle} \ln K_{mic,\langle N\rangle} = \frac{KT}{\langle N\rangle}\{\ln[X_{mic,\langle N\rangle}\gamma_{mic,\langle N\rangle}] - \langle N\rangle \ln[X_{1\gamma 1}]\} \quad (14)$$

where $K_{mic,\langle N\rangle}$ is the thermodynamic constant (at P and T fixed). The activity of molecular surfactant does not vary independently from the micellar one, i.e. the derivative of $K_{mic,\langle N\rangle}$ with respect to the overall surfactant content, X_{tot} is null.

The analysis of solute activity and aggregation numbers is based on previously developed models. A treatment of the links between activity, concentration and aggregation features in micelle forming systems is reported elsewhere [26,40].

Above the CMC the activity coefficients of bile salt anions, γ_-, are average values arising from the contributions of molecular and micellar form and depend on counter–ion binding. $\gamma_- = {\gamma_{expt}}^2/\gamma_+$ can be related to the concentration

of micelles according to

$$\gamma_- = (\gamma_{-,\mathrm{mic},\langle N\rangle})\left[\frac{X_{\mathrm{mic},\langle N\rangle}}{X_{\mathrm{tot}}}\right] = (\gamma_{-,\mathrm{mic},\langle N\rangle})\left[\frac{X_{\mathrm{tot}} - \mathrm{CMC}}{X_{\mathrm{tot}}}\right] \quad (15a)$$

$$X_1 = \frac{X_{\mathrm{tot}}[\gamma_{-,\mathrm{mic},\langle N\rangle} - \gamma_-]}{\gamma_{-,\mathrm{mic},\langle N\rangle}} \quad (15b)$$

which introduced in Eq. (14), allows getting $\langle N\rangle$ values as linear plots of $\ln[X_{\mathrm{tot}}\gamma_-]$ versus $\ln[X_{\mathrm{tot}}\ (\gamma_{-,\mathrm{mic},\langle N\rangle} - \gamma_-)]$.

The micelle aggregation numbers inferred by Eqs. (15a) and (15b) are low, between 3 and 5, both in water and water–BSA. They are in good agreement with values from other sources, spanning between 2 and 10 units (at 25 °C) [18,20,41]. It must be pointed out, however, that $\langle N\rangle$ values depend on the overall amount of surfactant in micellar form, i.e. on the concentration limits to which the mass action approach is applied, as mentioned previously. This is in line with studies stating that the aggregation numbers of BS micelles depend on concentration and/or ionic strength [22].

Surface adsorption and micelle formation can be analysed as a function of temperature. In this way, the entropic contributions to micelle formation can be evaluated. From the above comparison it results that ΔG_{mic} is not much sensitive to the presence of albumin. From the analysis of the Gibbs energy of adsorption, conversely, it comes out that surface adsorption of NaTDC is noticeably sensitive to the amount of albumin in the medium. For instance, areas per molecule change significantly in presence of BSA. The same holds for the enthalpy and entropy of adsorption at the interface. This may be due to the competition between the adsorption of NaTDC and BSA at the air–solution interface. The possibility to have the formation of interfacial (NaTDC + BSA) complexes cannot be ruled out a priori. In absence of more detailed information on this regard, however, this is only a working hypothesis.

Calorimetric data, reported in Fig. 4, indicate the occurrence of significant thermal effects. The reported behaviour is different from that observed in *n*-alkyl chain surfactants [30]. In water, the enthalpy of micelle formation, ΔH_{mic} is about 1.2 kJ mol^{-1}, in good agreement with previous findings [42]. The shape of the L_2 function versus concentration, Fig. 5A, resembles the one formerly observed in sodium deoxycholate [34]. According to calorimetric data, the association process extends in a wide concentration range. The above data support the hypothesis of a continuous association for bile salts [43,44].

Quite different is the behaviour in presence of BSA. Comparison of the data in Fig. 5A and B indicate that the self-organisation of bile acid salts in presence of albumin is concomitant to a much higher overall heat effects, about 8 kJ mol^{-1} (compared to 1.2 kJ mol^{-1} in water). This behaviour is partly due to direct interactions between TDC anions and albumin. A significant effect can be observed, in fact, at concentrations below the CMC, as a secondary maximum in the curve. It is well known that protein–surfactant interactions usually take place at concentrations lower than the critical threshold [45]. The overall heat effect observed in presence of albumin, thus, is presumably due to the combination of binding and micelle formation contributions. The significant differences between L_2 values in water and water–BSA systems must be pointed out. The enthalpy of micelle formation in the two cases is different, as can be seen by comparing the dotted lines reported in Fig. 5A and B. In the water–BSA solvent the enthalpy of micelle formation is 2.3–2.5 kJ mol^{-1}.

Proper de-convolution of the curve reported in Fig. 5B indicates the presence of additional heat effects at low surfactant content. In agreement with finding relative to water–protein–surfactant systems [45,46], they are tentatively ascribed to direct interactions between the bile salt (in molecular form) and the protein.

Differences between the behaviour in water and water–BSA mixtures can also be obtained by comparing

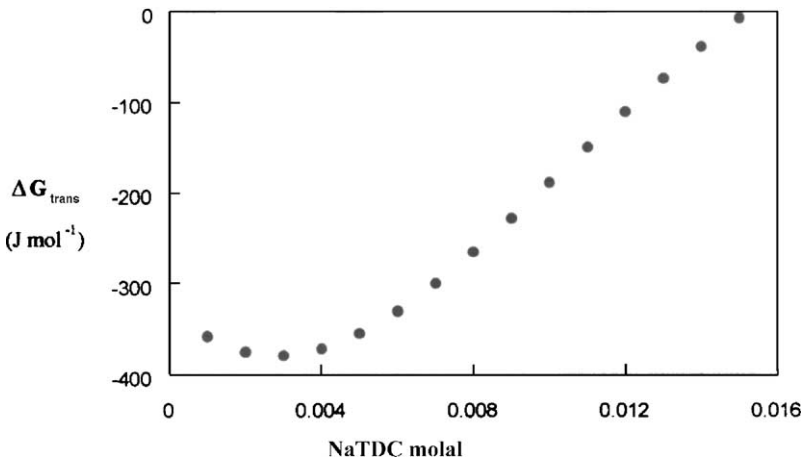

Fig. 6. The Gibbs energy of transfer from water to 1.00 BSA wt.% in water, $\Delta G_{\mathrm{trans}}$ (in J mol^{-1}), as a function of NaTDC molality.

the activity coefficients in the two systems. From a thermodynamic viewpoint, the difference between the activity coefficients of NaTDC in the two solvent systems reflects the Gibbs energy of transfer of the BS from water to the pseudo solvent BSA–H_2O. In Fig. 6 it is indicated that the energy of transfer reaches a minimum (i.e. the maximum stability) at concentrations close the CMC and progressively becomes less negative once micelles are formed. In many aspects, information from activity coefficients supports the ones obtained from calorimetric findings.

The role of electrostatic contributions is, presumably, not much relevant in BSA–NaTDC systems when the pH is above the iso-electric point of the protein. To get more realistic models, able to account for the behaviour occurring in real biological matrices, studies as a function of pH may be important.

As far as the role of hydrophobic and hydrogen bond contributions, it is not possible to quantify the role of hydrophobic interactions to the adduct stability in terms of energy of transfer of methylene units from water to an hydrophobic environment, as commonly done in linear alkyl chain surfactants [47].

The role of hydrogen bond contributions to micelle formation in bile salts has been severely questioned in the past [31,48]. In case of hydrogen bond interactions it is also necessary to have reasonable hypotheses for the organisation of aggregates, since their strength depends on bile salt concentration and on he distance between OH groups located onto different bile salt units [49].

5. Conclusions

Studies on mixtures containing BSA and NaTDC have been performed by different thermodynamic methods. Surface properties do not put in evidence significant interaction between the components, which have been observed, conversely, in calorimetric studies and in colligative properties. Interaction between the component, in particular, give rise to significant heat effects in the region close to the critical concentration.

A combined analysis of the above data suggests the presence of significant interactions between the components and the possible formation of stable adducts, or complexes.

Work in this direction, however, requires the support from other experimental investigation (including electrophoretic mobility, structural and spectroscopic methods) and the extension of experimental studies to a much wider concentration range.

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