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Enthalpies of Dilution of Bile Salt Solutions: Sodium Taurodeoxycholate and Sodium Taurocholate*

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The enthalpies of dilution of solutions of two bile salts in water and aqueous NaCl were measured over a range of solute concentrations encompassing the critical micellization concentrations (c.m.c.). The compounds were sodium taurodeoxycholate and sodium taurocholate. An isoperibolic reaction calorimeter was used. The observed changes of enthalpies of dilution as a function of surfactant and NaCl concentrations were related to the c.m.c. values, micellar aggregation numbers and counterion binding.

INTRODUCTION

In this communication we report the results of an investigation of the enthalpies of dilution of solutions of two bile salts. The physical chemistry of solutions of these biological micelle-forming surfactants is a rather popular subject of research[1-4] which is also beset with many unresolved problems and controversies.[5] However, there are very few reports dealing with the thermochemical measurements on bile salt solutions. Rajagopalan *et al.*[6] measured heat capacity at three temperatures and enthalpy of dilution at 25 °C of solutions of four unconjugated bile salts (sodium cholate, sodium deoxycholate, sodium chenodeoxycholate and sodium ursodeoxycholate) by means of flow microcalorimeters. The solvent was aqueous 0.02 mol dm^{-3} Tris buffer (pH = 9.0) and the bile salt concentrations varied over a broad range but were considerably above the critical micellization concentrations (c.m.c.) of the surfactants. The enthalpies of dilution were combined with the osmotic

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and activity coefficients to obtain the excess thermodynamic functions. The data were interpreted in terms of various aspects of hydrophobic interactions. Correlations were also made with the extent of counterion binding by the micelles of bile salts. Another paper from the same laboratory[7] dealt with the enthalpies of dissolution of lecithin in bile salt solutions and the enthalpies of mixing of bile salt and other surfactant solutions. The enthalpies of mixed micelle formation in solutions containing varying proportions of sodium deoxycholate and sodium dodecyl sulfate were measured by Birdi.[8]

In our calorimetric studies two taurine-conjugated bile salts, sodium taurodeoxycholate (NaTDC) and sodium taurocholate (NaTC) were used. The solution and micellar properties of these two compounds were extensively studied at Clarkson University[5,9–13] by means of surface tension, light scattering, translational diffusion, sedimentation velocity, and fluorescence techniques over broad regions of surfactant concentrations and at different counterion (Na^+) concentrations. Particular attention was given to the region of low surfactant concentrations in order to resolve the question of the existence of the c.m.c.'s. It was established that for the more hydrophobic dihydroxy compound, NaTDC, of sufficient purity, there is a critical concentration below which the aggregates are not detectable.[5,10,11] The critical concentrations, identified then as the c.m.c.'s, decrease with increasing counterion concentrations. For the more hydrophilic trihydroxy NaTC, aggregates were detectable in the presence of NaCl even at surfactant concentrations lower than the apparent c.m.c. values as determined, for instance, from surface tension measurements.[5,11] A pronounced concentration-dependent growth of the size of NaTDC aggregates above the c.m.c. was observed. The main purpose of the present study was to establish how the above observations correlate with the calorimetric data also obtained at low bile salt concentrations.

Since the pK_a of the sulfonate head groups of taurine-conjugated bile salts is very low ($\simeq 2$), there is no need to control pH of solutions of these surfactants. A drawback in the work of Rajagopalan *et al.*[6] on unconjugated bile salts, with carboxylate as the head group, was the use of Tris buffer at $pH = 9.0$ to prevent the protonation of the bile salt anions and the concomitant loss of solubility. Tris forms a large organic cation which serves as another counterion type in addition to Na^+. Since micelle formation, in general, is affected by the nature of counterions as well as by their concentration the use of mixed electrolytes in surfactant solutions could lead to ambiguities in the interpretation of the experimental results.

The calorimetric experiments were conducted at the University of Zagreb using a calorimeter of isoperibolic type designed by Simeon *et al.*[14] The same instrument was employed recently in the studies of enthalpies of precipitation of metal-surfactant salts in aqueous solutions[15,16] and enthalpy of dilution of sodium dodecyl sulfate solutions.[16]

EXPERIMENTAL

Samples of bile salts were obtained from Sigma Chemical Company, St. Louis. Except for the drying to constant weight they were used as received. Sufficient quantities of purified materials were not available for these exploratory experiments. Previously it was established that samples from all commercial sources contain small amounts of strongly surface-active impurities of limited solubility.[5,10,12] The effects of these impurities on surface tension and light scattering of NaTDC and

NaTC solutions were particularly noticeable for solutions without added electrolyte in the vicinity of the c.m.c. In the presence of NaCl at concentrations used in this study (0.15 and 0.6 mol dm^{-3}) these effects were less pronounced or completely absent due to the decreased c.m.c. values and the enhanced degrees of aggregation at increased counterion concentration. In our experience the samples provided by Sigma Company were, in general, among the highest-quality commercially available compounds of this type.

An isoperibolic reaction calorimeter, designed and tested by Simeon *et al.*[14], was operated at 20 °C. The calorimeter was initially charged with 70 cm^3 of water or NaCl solution (0.15 or 0.5 mol dm^{-3}). The bile salt stock solution (NaTDC, 0.2 mol dm^{-3} NaTC, 0.3 mol dm^{-3}) was contained in a micrometer burette of 2 cm^3 capacity. The experiments were designed as calorimetric titrations: small known portions of the bile salt stock solution were successively delivered from the burette into stirred 70 cm^3 batch of the solvent. Each delivery was preceded and followed by a rating period so that the corresponding heat change, ΔQ (positive for an endothermic process), could be determined. Most of the experiments were made in triplicate. The precision of the calorimetric data can be estimated from the typical noise level of about ± 30 mJ (peak-to-peak). Combined with the mid-range values of concentration and solution volume added, this figure yields ± 0.15 kJ mol^{-1} as the maximum calorimetric uncertainty in $\Delta Q/\Delta n$, where Δn is the number of moles of bile salt added in one portion. In all cases the dilution process was fast; therefore, the thermograms were evaluated by using a simple graphical method.

RESULTS

The molar enthalpies of dilution, $\Delta Q/\Delta n$, of the stock solutions with water or NaCl solutions at 20 °C as a function of the NaTDC concentration in diluted solutions are presented in Figures 1. and 2. The abscissa values are the averages of the initial and the final concentrations of an interval for successive additions of stock solution from the microburette. In order to indicate the range of the total counterion concentration (i. e., Na$^+$ from NaCl and NaTDC) in each run, the respective values (in mol dm^{-3} Na$^+$) are provided at selected points. Because of high dilution ratios in all cases there were only relatively small variations of the total Na$^+$ concentration at increasing NaTDC concentrations.

For the data in Figure 1. the stock solution contained 0.2 mol dm^{-3} NaTDC and 0.6 mol dm^{-3} NaCl. In each case $\Delta Q/\Delta n$ changes abruptly over a narrow concentration range in the vicinity of the c.m.c. The arrows at each line correspond to the c.m.c. values for purified NaTDC samples determined by light scattering[11,12] at 25 °C and confirmed by surface tension[12] and fluorescence of solubilized pyrene.[13] The c.m.c. values are 0.0040, 0.0016 and 0.00096 mol dm^{-3} in water, 0.15 and 0.6 mol dm^{-3} NaCl, respectively. The measured values of $\Delta Q/\Delta n$ below and above the transition concentrations become more endothermic on dilution from 0.6 mol dm^{-3} to 0.15 mol dm^{-3} NaCl or to water, the latter values being the largest. Dilution in 0.6 mol dm^{-3} NaCl produced no measurable heats above the c.m.c. The reported values of $\Delta Q/\Delta n$ in Figure 1. for dilutions of the stock solution with 0.15 mol dm^{-3} or with water contain also the heats of dilution of 0.6 mol dm^{-3} NaCl solution itself. However, the contributions of the dilution heats[17] are far too small to account for the differences among the three $\Delta Q/\Delta n$ plateaus above the c.m.c.

The data shown in Figure 2 were obtained in an experiment where the stock solution of 0.2 mol dm^{-3} NaTDC in water was diluted with water or NaCl solution (0.15 or 0.6 mol dm^{-3}). Again there were sharp changes in the dependence of $\Delta Q/\Delta n$ on the concentration of NaTDC in the respective c.m.c.

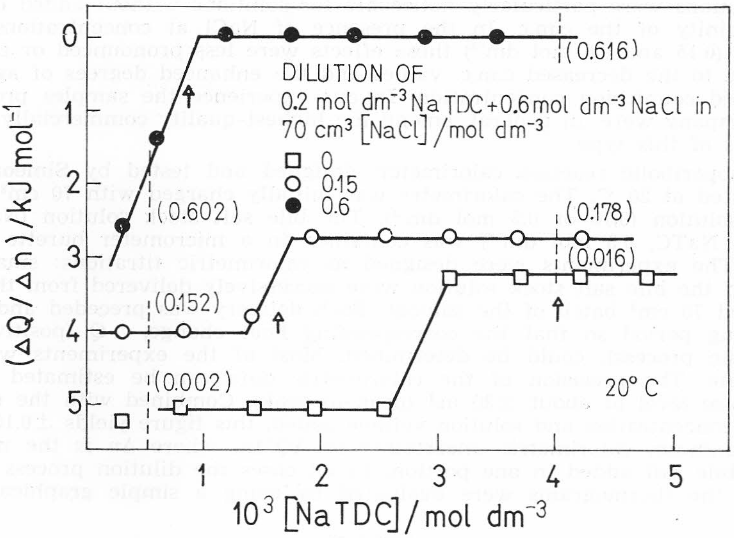

Figure 1. Heats of dilution of a solution containing 0.2 mol dm^{-3} NaTDC and 0.6 mol dm^{-3} NaCl in 70 cm^3 of aqueous NaCl. c_{NaCl}/(mol dm^{-3}): 0 (□), 0.15 (○), 0.6 (●). The arrows indicate the c.m.c. values of NaTDC. The numbers at selected NaTDC concentrations equal the total counterion molar concentrations (Na$^+$ from NaTDC and NaCl).

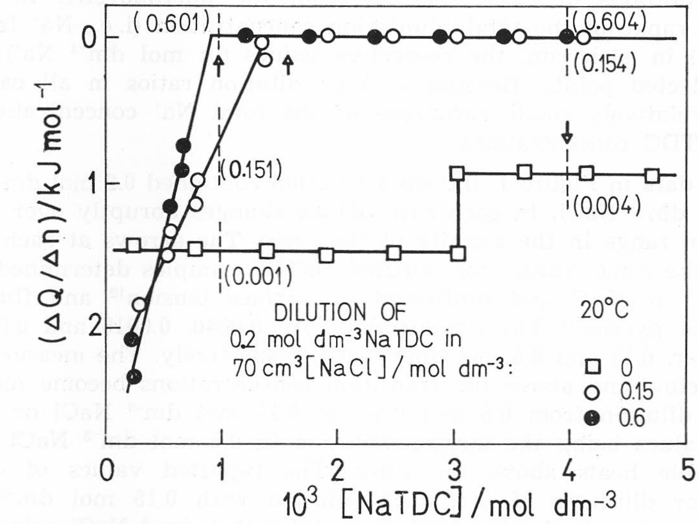

Figure 2. Heats of dilution of aqueous 0.2 mol dm^{-3} NaTDC in 70 cm^3 of aqueous NaCl. The symbols, the arrows and the numbers along the lines have the same meaning as in Figure 1.

regions. However, zero values of $\Delta Q/\Delta n$ were obtained above the c.m.c. for solutions diluted with 0.6 and 0.15 mol dm^{-3} NaCl, whereas the values for aqueous solutions at all NaTDC concentrations were still positive but smaller than those in Figure 1. The same trend was observed for NaCl containing solutions below the c.m.c. Thus, the general data pattern was roughly the same in both experiments although there were some quantitative differences which could be tentatively attributed to the interactions of micelles with Na^+ and Cl^- ions.

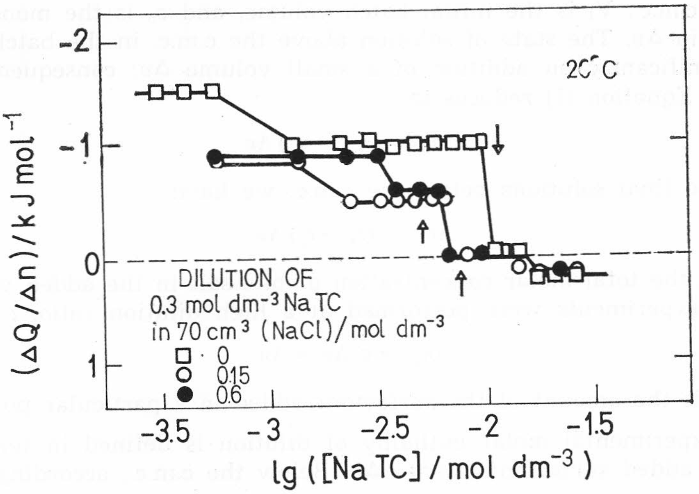

Figure 3. Heats of dilution of aqueous 0.3 mol dm^{-3} NaTC in 70 cm^3 of aqueous NaCl. The symbols are same as in Figure 1 and the arrows correspond to the quasi-c.m.c. values of NaTC.

Calorimetric results for the dilution of an aqueous 0.3 mol dm^{-3} stock solution of NaTC with water or NaCl solutions (Figure 3.) demonstrate exothermic enthalpies of dilution which abruptly change to less negative or even slightly positive quantities at characteristic concentrations. These aproximately coincide with the generally indicated quasi-c.m.c. values for this trihydroxy bile salts, i. e. 0.012, 0.006 and 0.005 mol dm^{-3} in water, 0.15 and 0.6 mol dm^{-3} NaCl, respectively, as obtained from surface tension measurements.[10,12]

DISCUSSION

The experimental data presented above will be interpreted in terms of the behavior of an ideal surfactant system and the deviations from ideal behavior will be related to the changes in the counterion binding and nonmicellar association. In the context of this discussion an ideal system is described as follows. Below the c.m.c. the monomer concentration is equal to the total surfactant concentration. Above the c.m.c. in the presence of micelles the monomer concentration is constant and equal to the c.m.c. The activity coefficient of monomer is constant and equal to one and no premicellar association is assumed.

In the calorimetric experiments small volumes, Δv, of concentrated stock micellar solution are added to a relatively large volume (70 cm^3) of the solvent or NaCl solution. The extent of the dissociation of micelles on dilution is equal to the increase in the monomer amount (number of moles, Δn_m), i. e., the monomer amount in the final batch volume minus the monomer amount in the initial batch volume and in the added portion Δv. Then Equation (1) is valid:

$$\Delta n_{\mathrm{m}} = c_{\mathrm{o,f}} (V_{\mathrm{i}} + \Delta v) - c_{\mathrm{o,i}} V_{\mathrm{i}} - c_{\mathrm{o}} \Delta v \tag{1}$$

where $c_{\mathrm{o,f}}$ and $c_{\mathrm{o,i}}$ are the final and the initial molar monomer concentrations above the c.m.c., V_{i} is the initial batch volume, and c_{o} is the monomer concentration in Δv. The state of solution above the c.m.c. in the batch does not change significantly on addition of a small volume Δv; consequently, $c_{\mathrm{o,f}} = c_{\mathrm{o,i}}$, and Equation (1) reduces to

$$n_{\mathrm{m}} = (c_{\mathrm{o,f}} - c_{\mathrm{o}}) \Delta v \tag{2}$$

For the final solutions below the c.m.c. we have

$$\Delta n_{\mathrm{m}} = (c_{\mathrm{t}} - c_{\mathrm{o}}) \Delta v \tag{3}$$

where c_t is the total molar concentration of bile salt in the added volume Δv. Since the experiments were performed at a high dilution ratio, $c_{\mathrm{t}} \gg c_{\mathrm{o}}$, and

$$\Delta n_{\mathrm{m}} = c_{\mathrm{t}} \Delta v = \Delta n \tag{4}$$

where Δn is the amount of the surfactant added in a particular portion.

The experimental molar enthalpy of dilution is defined in terms of the amount of added surfactant, $\Delta n = c_{\mathrm{t}}\Delta v$. Below the c.m.c., according to Equation (4)

$$\Delta Q/\Delta n = \Delta Q/\Delta n_{\mathrm{m}} = \Delta_{\mathrm{diss}} H \tag{5}$$

where $\Delta_{\mathrm{diss}} H$ is the enthalpy change for the complete dissociation of micelles per mol of the monomer. Above the c.m.c., according to Equation (2)

$$\Delta Q/\Delta n = \Delta_{\mathrm{diss}} H \, \Delta n_{\mathrm{m}}/\Delta n = \Delta_{\mathrm{diss}} H \, (c_{\mathrm{o,f}} - c_{\mathrm{o}})/c_{\mathrm{t}} \tag{6}$$

Since for the assumed ideal system $c_{\mathrm{o,f}} = c_{\mathrm{o}} =$ c.m.c., $\Delta Q/\Delta n$ should be zero. Even if there are differences between $c_{\mathrm{o,f}}$ and c_{o}, in the experimental design of this study they must be small compared to c_{t}, and $\Delta Q/\Delta n \simeq 0$. Positive or negative values of the heats of dilution are indicative of other processes such as changes in the degree of counterion binding.

For further consideration of the results of this study it should be useful to enumerate the available information on the micellar aggregation numbers, N. The mass-average values of N, as determined by light scattering[5,9–12] are collected in Table I. In case of NaTDC the tabulated values were corrected for the preferential interaction effects and, if appropriate, for the intermicellar interactions; those for NaTC are only the apparent values. The concentration of bile salt of 0.003 mol dm^{-3} above the c.m.c's corresponds approximately to the highest concentrations of solutions in Figures 1—3. Between the c.m.c.'s and higher concentrations the size of micelles of NaTDC increases monotonically to the values indicated in Table I. For aqueous solutions of NaTDC (without added NaCl) N is known only at the c.m.c.; it does not appear to increase substantially at higher concentrations,[12] but the precise information is lacking. Micelles of NaTC are smaller than those of NaTDC, particularly in the presence of NaCl. Their size also varies with the surfactant concentration; however, the details are sketchy.[5,10,12]

TABLE I

Mass-Average Micellar Aggregation Number, N, *of Sodium Taurodeoxycholate (NaTDC) and Sodium Taurocholate (NaTC) at Different NaCl Concentrations and* 25 °C[5,9–12]

[NaCl]/(mol dm^{-3}):	0.6	0.15	0
0.2 mol dm^{-3} NaTDC	160	27	—
At c.m.c. of NaTDC	14	10	4
At 0.003 mol dm^{-3} NaTDC above the c.m.c.'s	44	19	—
At 0.003 mol dm^{-3} NaTC above the quasi-c.m.c.'s[a]	9	6	2.5

[a] Apparent values (not corrected for interaction effects); true values would be approximately 10—30% larger (see the text).

It was already mentioned in the introduction that small aggregates were detectable in solutions of NaTC containing NaCl below the commonly determined c.m.c.'s from surface tension measurements.[5,11,12] A corrollary of this observation is the finding in several studies[18–20] that the concentration of NaTC monomer increases monotonically with the total surfactant concentration. In contrast, above the c.m.c. the monomer concentration of NaTDC was found to be constant and equal to the c.m.c.;[18,19] at concentrations lower than the c.m.c. the solute is in monomeric form.

Several points can be made based on the results of this preliminary calorimetric study. Considering the data in Figures 1. and 2. for solutions of NaTDC $\Delta Q/\Delta n$ is zero above the c.m.c. irrespective of whether the stock solutions containing large micelles (in 0.6 mol dm^{-3} NaCl) or small micelles (in water) were diluted into 0.6 mol dm^{-3} NaCl. Thus, the changes in the degree of aggregation of micelles above the c.m.c. (see Table I) do not produce measurable enthalpy changes in the range of NaTDC concentrations in our experiments. The same conclusion appears to be valid for dilutions into 0.15 mol dm^{-3} NaCl.

For aqueous solutions of NaTDC diluted with water the picture is different. As evident in Figure 2, $\Delta Q/\Delta n$ is endothermic at all concentrations. Since large changes in the micellar aggregation numbers encountered in NaCl-containing solutions produce zero values of $\Delta Q/\Delta n$ above the c.m.c., different behavior of aqueous solutions in which micelles are small (Table I) must be related to a greater degree of dissociation of counterions from micelles at lower ionic strength. Similar behavior is apparent in Figure 1 for solutions diluted into water.

Although the total counterion concentrations are somewhat larger for the systems in Figure 1 than for those in Figure 2, the differences are too small to affect any of the conclusions of this paper.

Returning to the question of the effect of the counterion binding, the dissociation of micelles (i. e., their break-up) can be considered as a two-step process: (1) separation of monomers from an existing micelle, and (2) release of counterions. Since electrostatic forces must be overcome the latter step is expected to be endothermic. If the overall heat change is endothermic, the monomer separation process can be either endothermic or exothermic. However, if the overall heat change is exothermic, the separation of monomers must also be exothermic. Such a behavior was observed for the dilution of aqueous NaTC solutions either into water or NaCl solutions (Figure 3). However, above the quasi-c.m.c.'s for this bile salt $\Delta Q/\Delta n$ values are approximately equal to zero, even for solutions diluted into water. The latter observation suggests a significantly weaker counterion binding than for NaTDC, in accordance with smaller size of NaTC micelles.

Transitions in the plots of Figures 1 and 2 for NaCl-containing solutions occur at NaTDC concentrations close to the c.m.c.'s determined independently. A few more experiments in the transition region would probably improve the agreement. For aqueous solutions (without NaCl) this is not the case; the transitions occur at concentrations definitely lower than the c.m.c. The most likely explanation lies in the use of highly purified NaTDC samples for the c.m.c. determination, whereas the calorimetric measurements were done on samples as received from the supplier. These samples contain small amounts of impurities that lower the c.m.c. of NaTDC.[12] The presence of these impurities may also explain the observed changes in $\Delta Q/\Delta n$ below the c.m.c.'s for solutions diluted into NaCl solutions in Figure 2. A gradual release of counterions is indicated due to a low degree of non-micellar association (i. e., association involving impurities) promoted by NaCl, eventually leading to heats of dilution at low NaTDC concentrations more endothermic than for aqueous solutions. The presence of impurities can also explain the shifts and kinks noticed in the plots of Figure 3. for NaTC. In comparing the c.m.c.'s to the concentrations of NaTC at which $\Delta Q/\Delta n$ drops to zero, we again emphasize that for purified samples of NaTC, strictly speaking, there are no c.m.c.'s.[5,10,12] The quasi-c.m.c.'s indicated in Figure 3 refer to the mid-point of the range of concentrations of NaTC at which the slope of the plot of the surface tension versus the logarithm of NaTC concentration is found to change.

A direct comparison of our results with those of Rajagopalan *et al.*[6] is not possible. As pointed out in the introduction they investigated unconjugated bile salts (in sodium form) dissolved in 0.02 mol dm^{-3} Tris buffer. Consequently, their solutions contained two kinds of counterions. The highest final concentrations of NaTDC in our experiments were several times lower than the lowest concentrations of dihydroxy bile salts in the studies of Rajagopalan *et al.* In their experiments the initial and final concentrations differed only by a factor of two. The integral enthalpies of dilution obtained by these authors were endothermic for sodium deoxycholate and sodium chenodeoxycholate at all concentrations. For sodium cholate and sodium ursodeoxycholate exothermic values were obtained at lower concentrations, whereas at higher concentrations enthalpies of dilution became endothermic.

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SAŽETAK

Entalpije razrjeđivanja otopina soli žučnih kiselina: natrij-taurodeoksikolat i natrij-taurokolat

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S pomoću izoperibolnoga reakcijskog kalorimetra izmjerene su entalpije razrjeđivanja natrij-taurodeoksikolata i natrij-taurokolata vodom i vodenim otopinama NaCl, i to u širokom opsegu koncentracija oko kritične micelizacijske koncentracije (c.m.c). Ovisnost entalpije razrjeđenja uspoređena je s vrijednostima c.m.c., s agregacijskim brojem micela te s vezivanjem protuiona.