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Concentration-dependent aggregation patterns of conjugated bile salts in aqueous sodium chloride solutions

A comparison between sodium taurodeoxycholate and sodium taurocholate

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Abstract: Based on light scattering intensity measurements, a critical concentration for micelle formation can be assigned to sodium taurodeoxycholate in aqueous electrolyte solutions. For sodium taurocholate a progressive aggregation even at very low concentrations of bile salt is indicated. Surface tension and diffusion coefficients are also reported.

Key words: Bile salts, Sodium taurodeoxycholate, Sodium taurocholate, Micelles, Critical micellar concentration.

The detergent-like properties and the propensity to form micellar aggregates are related to the most importance physiological functions of bile salts. Yet, in spite of numerous investigations, partly summarized in several review articles [1–3], there is a great deal of disagreement even about such common parameters as the critical micellar concentration (cmc) and the micellar aggregation number which, for specified temperature and solvent composition, are characteristic for any micelle-forming amphiphilic compound. This lack of agreement among many experimental observations, not unusual for surfactants [4, 5], can be ascribed to such obvious causes as the presence of impurities in the samples of bile salts and the use of inappropriate experimental techniques, or to the more subtle factors, for instance, those related to the inadequate or incomplete treatment of the experimental data [4, 6, 7]. Notably lacking are the systematic, comparative studies on carefully purified materials using several complementary experimental techniques. In this report we compare the reversible aggregation behaviour of a dihydroxy bile salt, sodium taurodeoxycholate (NaTDC) and a trihydroxy compound, sodium taurocholate (NaTC), in aqueous electrolyte solutions over a broad range of solute concentrations. The light scattering intensity measurements at 25 °C were supplemented by surface tension and diffusion experiments. The state of aggregation of two bile salts at low concentrations was explored in detail. For this purpose we made use of two favorable properties of bile salts for the light scattering measurements, namely, a relatively high molar mass of the monomer and the large refractive index increment. Consequently, our results are particularly relevant to the currently debated question of the existence of cmc in solutions of bile salts [8–19].

The results of the light scattering experiments are presented in figure 1 as Debye plots in terms of the *total* bile salt concentration, c_2^t, and the Rayleigh ratio in excess of the *solvent* (0.15 m or 0.60 m NaCl), R_{90}. The logarithmic scale is used in order to incorporate in a single diagram the data over almost three orders of magnitude in c_2^t and to emphasize those at low c_2^t. These plots are different from the customary Debye plots for treatment of light-scattering intensity measurements on surfactant micelles, in which the micellar concentration, $c_2 = c_2^t - \text{cmc}$, and Rayleigh ratio in excess of that at the cmc are used. Consequently, the *apparent* aggregation numbers, N_a^*, indicated on the right, represent the mass-average values for all sufactant solute species, monomers and aggregates, present in solution. These apparent values are too low when compared to the true, anhydrous aggregation numbers obtained after correcting N_a^* for the preferential interaction and the virial coefficient effects on R_{90} [6, 7, 21–24]. For the purpose of this report a detailed analysis of these corrections is not needed. Several observations emerge from figure 1.

For NaTDC in both solvents there is a clearly pronounced concentration below which only mono-

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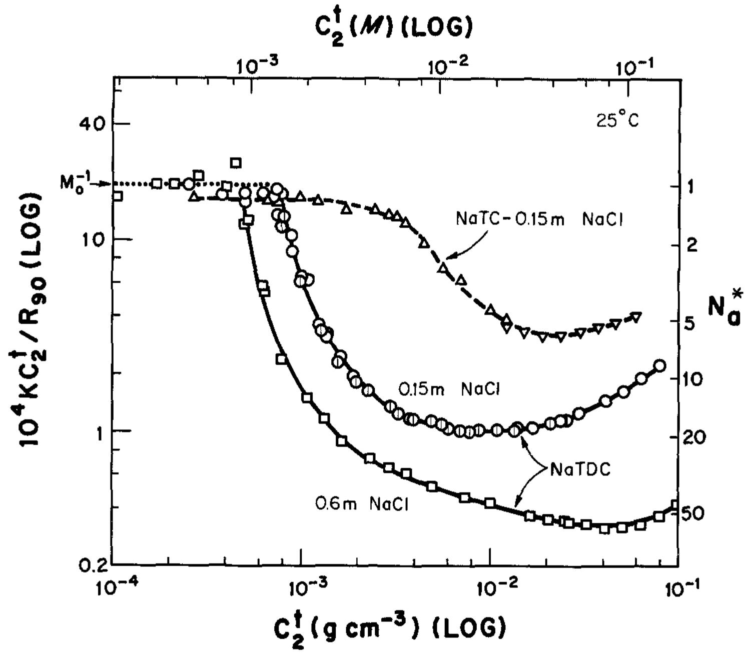

Fig. 1. Debye plot of the light scattering data at the vacuum wavelength λ_0 = 436 nm and 25 °C for solutions of sodium taurodeoxycholate (NaTDC) and sodium taurocholate (NaTC). $K = 2\pi^2 n^2 (dn/dc_2^t)^2/N_A\lambda_0^4$, n is the refractive index of solution, dn/dc_2^t is the refractive index increment measured at constant molality of NaCl, N_A is Avogadro's number, R_{90} is the Rayleigh ratio of light scattered at angle of 90° in excess of that of the solvent (0.15 m or 0.60 m NaCl), and c_2^t is the total concentration of bile salt in solution (both molarity and mass-volume concentration scales are given). N_a^* (the right-hand ordinate) is the *apparent* mass-average aggregation number equal to $M_0^{-1}(Kc_2^t/R_{90})^{-1}$, where M_0 is the molar mass of the monomer. The arrow on the left-hand ordinate and the dots correspond to the reciprocal of M_0 (or to the aggregation number of 1) for NaTDC (M_0 = 522 g mole^{-1}). For NaTC (M_0 = 537 g mole^{-1}) the arrow would be slightly lower by a factor 522/537 = 0.972. The same factor applies to the molar scale for the concentration. Two sets of experimental points for solutions in 0.15 m NaCl refer to different purified samples. A Brice-Phoenix light scattering photometer was used following the procedure described earlier [20] with improved stabilization of the instrument signals, the temperature control, and the clarification of solutions [21]. The values of dn/dc_2^t (cm^3 g^{-1}) are: 0.179 for NaTDC in 0.15 m NaCl, 0.175 for NaTDC in 0.6 m NaCl, and 0.174 for NaTC in 0.15 m NaCl.

mers appear to be present. This "critical" concentration which mimics the commonly defined cmc is lower at higher content of NaCl. In contrast, small aggregates of NaTC in 0.15 m NaCl are present in solutions even at the lowest concentration used and the transition to somewhat larger values of N_a^* is gradual.

Ekwall and Fontell [25–28] suggested that the aggregation of bile salts occurs over three distinct steps each characterized by its "critical" concentration (so-called limits, 1, 2, and 3). Limit 1 should correspond to the usually measured "cmc". We find no evidence for the existence of limits 2 and 3. They are not evident even in the results of light scattering studies of Fontell [27]. Neither is there any indication of a sharp transition between the so-called primary and secondary micelles in a model introduced by Small [2, 29] and refined by Mazer et al. [30]. However, the growth of the aggregates of NaTDC in both solvents from the "critical" concentration to the values of N_a^* often assigned to the primary micelles (for instance, 4 to 8) occurs over a narrow range of c_2^t.

At intermediate concentrations of NaTDC the values of N_a^* approach those that are typical for other surfactants, particularly, of shorter n-alkyl chain. In view of these results, frequent references in the literature to the notion that bile salts in general form only small micelles are unfounded and puzzling. The decrease of R_{90} or N_a^* at the highest concentrations, particularly in 0.15 m NaCl, is a consequence of the positive second and possibly higher virial coefficients caused by intermicellar interactions.

It should be stressed that the pattern of aggregation presented in figure 1, particularly at low c_2^t (the quasi-cmc region), has been observed only for the bile salt samples of high purity [21]. For inadequately pure samples a much more pronounced aggregation at low c_2^t is indicated and the values of N_a^* even for NaTDC below the quasi-cmc values are larger than one. This point is illustrated in figure 2 which also serves to re-

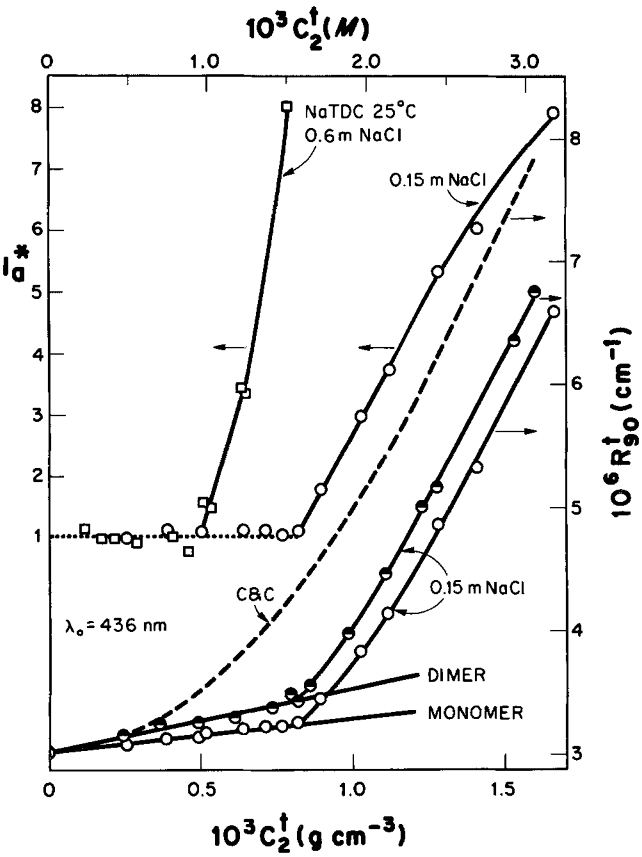

Fig. 2. The total Rayleigh ratio, R_{90}^t, of solutions of sodium taurodeoxycholate (NaTDC) in 0.15 m NaCl (lower curves and right ordinate) for a highly-purified sample [21] (open circles) and for a sample (Calbiochem, San Diego) used as received (half-filled circles). The dashed curve labeled C & C was taken from an enlargement of figure 5 of Chang and Cardinal [11]. The straight lines labeled *Monomer* and *Dimer* correspond to the expected increase of R_{90}^t, starting with that of the solvent (0.15 m NaCl), if all of the added NaTDC would be present in solution only in the monomeric or only in the dimeric form, respectively (an ideal solution behaviour is assumed). The upper part and left ordinate give the *apparent* aggregation number, N_a^*, for pure samples of NaTDC calculated from the Rayleigh ratio in excess of the solvent (0.15 m or 0.60 m NaCl). At low concentrations the nonideal effects on R_{90} are negligible [21] and N_a^* values are equal to the true aggregation numbers.

emphasize the patterns at low c_2^t of NaTDC. These data suggest that in the unpurified sample (half-filled circles) there is a self-association of the contaminant moieties (for instance, bile pigments) or/and an association between the latter and the bile salt itself. The precise pattern varies from batch to batch. It is not clear why the values of R_{90}^t obtained by Chang and Cardinal [11] on a purified sample are higher than ours.

Our samples were purified by repeated charcoal treatment and recrystallization from a suitable solvent [21]. The charcoal treatment and avoidance of diethylether in the recrystallization were crucial steps. As already noted in figure 1, the results for the purified sample follow closely the behaviour predicted for monomeric ideal solutions. Note the precision of our experiments: over the concentration range in figure 2 Rayleigh ratio increased only 2.25 times above that of the solvent and the value of N_a^* corresponding to the monomer, obtained from small excess scattering, does not show any trend with c_2^t. We conclude that for NaTDC the aggregates are not detectable below $c_2^t = 0.00082\ gcm^{-3} = 0.00167\ M$ in 0.15 m NaCl and below $c_2^t = 0.00050\ gcm^{-3} = 0.00096\ M$ in 0.60 m NaCl at 25 °C. *In this sense* these concentrations define the cmc values. In view of a pronounced increase of aggregation numbers at concentrations just above the cmc values of NaTDC, or because of a continuous growth of aggregates in solutions of

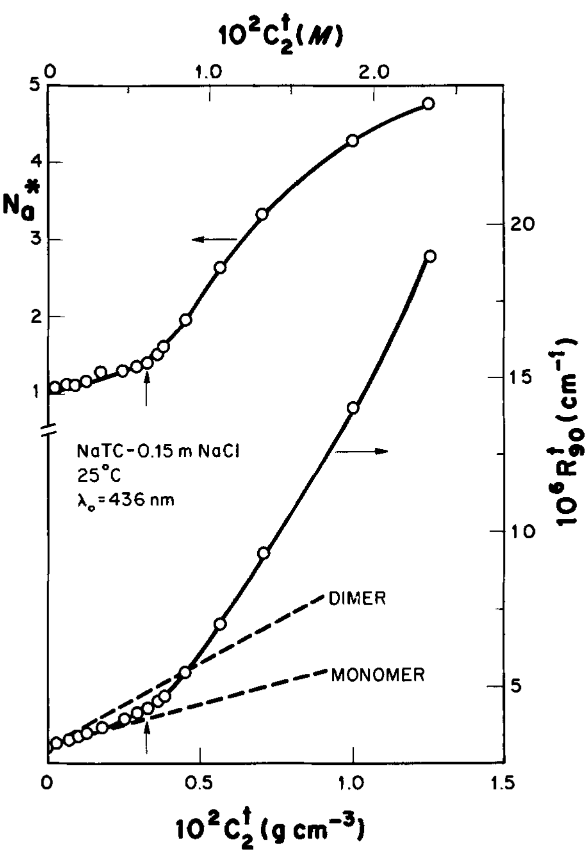

Fig. 3. Total Rayleigh ratio, R_{90}^t, and the corresponding *apparent* aggregation number, N_a^*, versus concentration of pure sodium taurocholate (NaTC) in 0.15 m NaCl. Note that the concentrations covered are roughly an order of magnitude higher than for NaTDC. Vertical arrows at $c_2^t = 0.0032\ gcm^{-3} = 0.0060\ M$ refer to the middle concentration of NaTC in the region in which the slope of the surface tension – log c_2^t plot changes markedly (fig. 4). See legend of figure 2 for other details.

NaTC, there is no physical justification for using the quasi-cmc values of bile salts as a basis of evaluating the thermodynamic quantities for micelle formation or the fraction of bound counterions [2, 3, 31].

In order to emphasize the contrast between the aggregation patterns for two bile salts, the results at lower concentrations of NaTC in 0.15 m NaCl are replotted in figure 3. A progressive aggregation begins at very low c_2^t values. Comparing the break in the surface tension (γ) – log c_2^t plot (fig. 4) with the light scattering curves in figure 3 clearly shows that a cmc value in the sense defined for NaTDC cannot be assigned to NaTC. It is apparent that surface tension is not sensitive to the presence of relatively few aggregates of small size. Our light scattering results for NaTC cast doubt on the validity of the interpretation of the self-diffusion coefficients of taurocholate at 37 °C in the work of Lindheimer et al. [32] who arrived at a constant aggregation number of 7 at all c_2^t above 0.008 M.

Figure 4 also contains the data for 0.60 m NaCl which suggest a more pronounced aggregation of NaTC in this solvent [30]. In addition, the surface tension of solutions without added NaCl reveals a great effect of surface-active impurities present even in the best commercially available samples of bile salts.

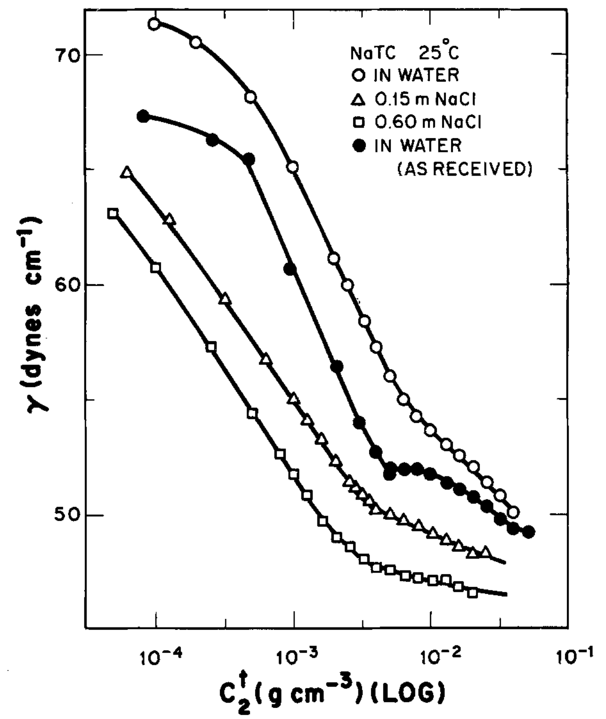

Fig. 4. Surface tension, γ, versus concentration of pure sodium taurocholate (NaTC) in water, 0.15 m, and 0.60 m NaCl, and of an unpurified sample (Calbiochem, San Diego) in water. The experimental technique was similar as in reference [20].

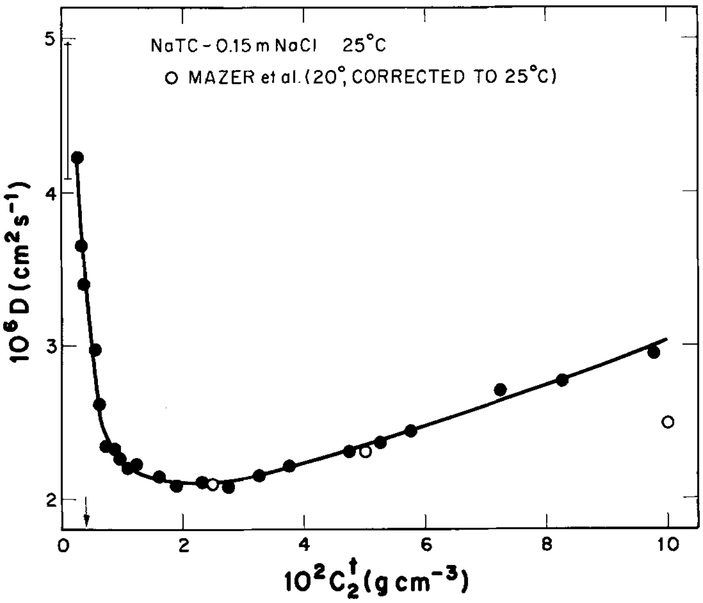

Fig. 5. The concentration dependence of the mutual translational diffusion coefficient, D, of sodium taurocholate (NaTC) in 0.15 m NaCl. D was evaluated from the spreading of the boundary between two solutions of closely spaced concentration intervals [5]. The data of Mazer et al. [30] from quasi-elastic light scattering at three values of c_2^t are also given. The vertical bar drawn close to the ordinate between $D = 4 \times 10^{-6}$ and 5×10^{-6} cm^{2}s^{-1} indicates the range of the literature values [32, 33, 34] of the self-diffusion coefficient of taurocholate anion measured at 37 °C at very low concentrations and extrapolated to $c_2^{t'} = 0$, and reduced to 25 °C. The vertical arrow indicates the quasi-cmc value for this system (as in fig. 3).

Removal of the impurities leads to high values of γ which decrease gradually with a hardly discernible change of the slope around $c_2^f = 0.0063\ \mathrm{gcm}^{-3} = 0.012$ M. We find in general that the inspection of the $\gamma - c_2^f$ curves for solutions *without* an added electrolyte is an excellent criterion of the improved purity of bile salt samples. Many $\gamma - c_2^f$ curves in the literature, including the earlier ones from this laboratory [20], which exhibit sharp breaks mimicking the cmc and zero slopes at higher concentrations must be considered suspect.

Another evidence for the absence of a cmc value for NaTC in 0.15 m NaCl comes from the concentration dependence of the mutual translational diffusion coefficient, D (fig. 5). At the lowest concentration the measured value of D merges with the self-diffusion coefficient of the monomer. The gradual drop of D at increasing c_2^f through the quasi-cmc corroborates the existence of small aggregates at low concentrations as detected by light scattering. A rise of D at c_2^f larger than about $0.03\ \mathrm{gcm}^{-3}$ is a manifestation of the intermicellar interactions already noticed in the Debye plot of figure 1.

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