

# Structural Study of the Micellar Aggregates of Sodium Taurodeoxycholate

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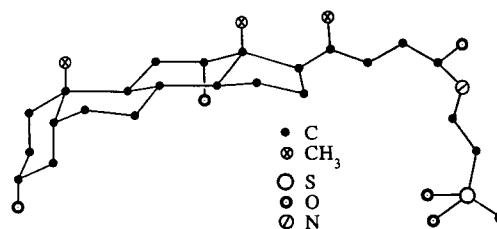
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Previously, fibers of sodium and rubidium salts of glycodeoxycholic and taurodeoxycholic acids have been drawn from aqueous micellar solutions. Their X-ray patterns have been interpreted by means of very similar unit cell parameters and helical structures, formed by trimers, having a 3-fold rotation axis, arranged in 7/1 helices. Micellar aggregates with an aggregation number  $N \leq 12$  and  $\geq 15$ , having oblate and cylindrical (7/1 helix) shape, respectively, have been proposed for sodium taurodeoxycholate (NaTDC) and satisfactorily verified by means of electromotive force and quasi-elastic light-scattering (QELS) measurements. The aim of this paper is to further check this two-structure model by means of QELS, circular dichroism (CD), and dielectric measurements on NaTDC aqueous solutions within concentration and temperature ranges 10–100 mM and 5–45 °C. The average intensity scattered by the NaTDC samples does not depend on the temperature and can be fitted by two straight lines, which intersect at a concentration about 40 mM. Two structures seem to be present, one prevailing above and the other below this concentration. The change of structure, which occurs during the growth of the aggregates, has been monitored by means of CD spectra increasing the ionic strength. The CD data agree with the two-structure model assuming that the cylindrical aggregates have an enantioselective ability toward bilirubin-IX $\alpha$  lower than that of the oblate aggregates. The average electric dipole moment  $\mu$  of a NaTDC monomer has been calculated from dielectric data. The  $\mu$  values, plotted as a function of NaTDC concentration within the temperature range 5–45 °C, can be fitted by two straight lines and show a break at a concentration that is, once more, about 40 mM. The  $\mu$  values vary from 33 to 68 D, depending on the temperature and NaTDC concentration. These high values can be justified by a remarkable hydration of the NaTDC aggregates. The moderate decrease of  $\mu$  when the populations of the aggregates with  $N \geq 15$  increase can agree with the formation of 7/1 helices, but disagrees with the formation of disordered structures, especially when they have a pseudo center of symmetry. The temperature dependence of the relaxation time follows an Arrhenius law. The molar enthalpy of activation vs NaTDC concentration for the relaxation process can be fitted by two straight lines, which show a break point at a concentration about 40 mM and could be connected with two different structures.

## Introduction

Aqueous solutions and fibers of sodium and rubidium salts of 3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -cholanoyltaurine (NaTDC and RbTDC, Figure 1) and 3 $\alpha$ ,12 $\alpha$ -dihydroxy-5 $\beta$ -cholanoylglycine have been previously studied mainly by means of X-ray, quasi-elastic light-scattering (QELS), circular dichroism (CD), extended X-ray absorption fine structure (EXAFS), and electromotive force (emf) measurements.<sup>1–4</sup> The very similar X-ray patterns of their fibers have been interpreted by means of nearly equal unit cells and structures, formed by 7/1 helices. These structures have been proposed as representative of those of the corresponding micellar aggregates in aqueous solutions. The 7/1 helices are built up using as repetitive unit a trimer having a 3-fold rotation axis.<sup>1,2</sup>

This model has been supported by emf measurements, accomplished on aqueous micellar solutions of NaTDC as a function of pH and ionic strength, applying the method of the constant ionic medium.<sup>3</sup> The emf data have provided the distribution of the micellar aggregation numbers and have shown that each aggregation number ( $N$ ) is almost always a multiple of 3. From the distribution the mean hydrodynamic radius  $\langle R_h \rangle$



**Figure 1.** Structure formula of the taurodeoxycholate anion.

has been calculated and compared with the apparent hydrodynamic radius of NaTDC aqueous solutions obtained from QELS measurements. Two types of structure with an approximate shape of oblate and prolate ellipsoid have been proposed. The aggregates with  $N \leq 12$  and those with  $N \geq 15$  are represented by an oblate and a cylindrical structure (the 7/1 helix), respectively. The satisfactory agreement achieved with this two-structure model is a further proof in favor of the 7/1 helix.<sup>2</sup>

Moreover, the CD study of the interaction complexes between bilirubin-IX $\alpha$  (1,3,6,7-tetramethyl-4,5-dicarboxyethyl-2,8-divinyl-(b-13)-dihydrobilenone, BR) and NaTDC or RbTDC has pointed out that very similar enantioselective complexes are formed. This result is an evidence of the chirality and similarity

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of the NaTDC and RbTDC micellar structures, and supports the chiral 7/1 helix as model of the micellar aggregates.<sup>1</sup> On the other hand, an EXAFS study of RbTDC aqueous solutions containing aggregates of small size has shown that the  $\text{Rb}^+$  ions have a liquidlike coordination as that of  $\text{RbOH}$  in water and, hence, are exposed to or dipped into the solvent. Their EXAFS spectra satisfactorily fit a theoretical signal calculated for a coordination model of a  $\text{Rb}^+$  ion surrounded by water molecules by using a  $\text{Rb}-\text{O}$  radial distribution function derived from molecular dynamics simulations.<sup>4</sup>

The coexistence of two structures for the NaTDC micellar aggregates in aqueous solutions agrees with the results of a spin-label study.<sup>5</sup> It has been inferred that at least two kinds and one kind of micelles are present in the aqueous solutions of dihydroxy and trihydroxy bile salts, respectively. In fact, a strongly and a weakly immobilized probe molecule have been observed in dihydroxy salts, whereas only the weakly immobilized component has been observed in trihydroxy salts. A possible explanation is that the strongly immobilized component interacts with cylindrical micellar aggregates, since it is considered to result from solubilization of the spin probe by the larger micelles,<sup>5</sup> and that the weakly immobilized component interacts with monomers and oblate oligomers.<sup>2</sup>

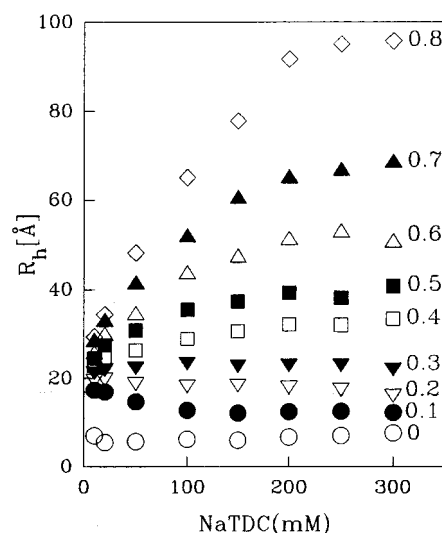
This paper deals with the interpretation of QELS, CD, and dielectric measurements in order to verify the two-structure model for the aqueous solutions of NaTDC.

## Experimental Section

**Materials.** NaTDC (Sigma) has been twice crystallized from a mixture of water and acetone. NaCl (Merck, Suprapur) and BR puriss. (about 99%, Fluka) have been used. HPLC measurements have shown that the commercial sample contains at least 96% of BR. BR has been added to NaTDC aqueous solutions by using a 0.01 M aqueous NaOH vehicle. Octaethylene glycol monododecyl ether ( $\text{C}_{12}\text{E}_8$ ) from Fluka (purity > 98%) has been used. Phosphate buffer, pure reagent, 0.050 M, is from Carlo Erba.

**QELS Measurements.** A Brookhaven instrument constituted by a BI-2030AT digital correlator with 136 channels and a BI-200SM goniometer has been used. The light source is an argon ion laser model 85 from Lexcel Corporation operating at 514.5 nm. Dust has been eliminated by means of a Brookhaven ultrafiltration unit (BIUU1) for flow-through cells, the volume of the flow cell being about 1.0  $\text{cm}^3$ . Nuclepore filters with a pore size of 0.1  $\mu\text{m}$  have been used. The samples have been placed in the cell for at least 30 min prior to measurement to allow for thermal equilibration. Their temperature has been kept constant at 25  $^\circ\text{C}$  within 0.5  $^\circ\text{C}$  by a circulating water bath. The scattered intensity and the time-dependent light scattering correlation function have been analyzed only at the 90 $^\circ$  scattering angle. The observed intensity and the apparent diffusion coefficients do not depend on the exchanged wave vector in the range 30–150 $^\circ$  in our experimental conditions. The scattering decays have been analyzed by means of cumulant expansion up to second order, because higher order contributions do not improve the statistics. The results are reported in terms of the apparent hydrodynamic radius obtained by the Stokes–Einstein relationship. The scattered intensity has been calibrated by using the well-known standard  $\text{C}_{12}\text{E}_8$  at 25  $^\circ\text{C}$ .<sup>6</sup>

**CD Measurements.** CD spectra have been recorded on a JASCO J-500A spectropolarimeter at 25  $^\circ\text{C}$  by using quartz cells with path lengths of 1.0 cm and by flushing with dry ultrapurified nitrogen before and during the experiments. A slit program that gives a wavelength accuracy better than 0.5



**Figure 2.**  $R_h$  values of NaTDC aqueous solutions as a function of bile salt and NaCl concentrations. The NaCl molar concentration is given on the right side. The average standard deviation is  $\pm 0.3$  Å.

nm has been used. The instrument has been calibrated with androsterone ( $1.69 \times 10^{-3}$  M in dioxane) on the basis of a molar ellipticity  $[\theta]_{304} = 11\,180 \text{ deg cm}^2 \text{ dmol}^{-1}$ . All the CD spectra have been recorded starting from 3 min of solution preparation, and refer to a 1.0 cm path length. The concentration of BR is always  $4.6 \times 10^{-6}$  M. The pH of the aqueous solutions ranges from 6.9 to 7.1 so that BR is mainly in the biacid form.

**Dielectric Measurements.** The dielectric measurements have been carried out at 5, 15, 25, 35, and 45  $^\circ\text{C}$  on 10, 20, 30, 40, 50, 80, and 100 mM NaTDC aqueous solutions by means of an Impedance Analyzer HP 4191 A in the frequency range from 1 MHz to 1 GHz. The measured reflection coefficient and phase angle at the interface with the sample have been converted to the real and imaginary part of the dielectric constant by an interpolation method based on dielectric measurements of electrolyte solutions which have conductivities near those of the samples investigated.<sup>7</sup> The dielectric loss values have been obtained by subtraction of the ionic conductivity contribution according to the known formula:

$$\epsilon'' = \epsilon''_T - \sigma_0/(\epsilon_0\omega)$$

where  $\epsilon''$  is the dielectric loss,  $\epsilon''_T$  is the observed imaginary part of the complex dielectric constant,  $\sigma_0$  is the ionic conductivity of the solution,  $\epsilon_0$  is the dielectric constant in vacuum, and  $\omega$  is the angular frequency. The conductivity  $\sigma_0$  has been measured by an Impedance Analyzer HP 4194 A at the frequency of 1 kHz. The sample holder has been thermostated within 0.1  $^\circ\text{C}$ .

## Results

**QELS Study of NaTDC Aqueous Solutions.** The apparent hydrodynamic radius  $R_h$  of NaTDC aqueous solutions is reported as a function of bile salt and NaCl concentrations in Figure 2. The results agree with previous data but extend to higher NaTDC concentrations and lower ionic strengths.<sup>2,8,9</sup> The  $R_h$  values practically do not change as a function of NaTDC concentration (0.01–0.30 M) within the range of NaCl concentration 0–0.3 M. This finding seems to be in contrast with the distribution of micellar aggregation numbers for NaTDC aqueous solutions, containing  $\text{N}(\text{CH}_3)_4\text{Cl}$ , obtained from emf measurements<sup>2,3</sup> by means of the method of the constant ionic medium.<sup>10</sup> The data show<sup>3</sup> that the proportions of the aggregates

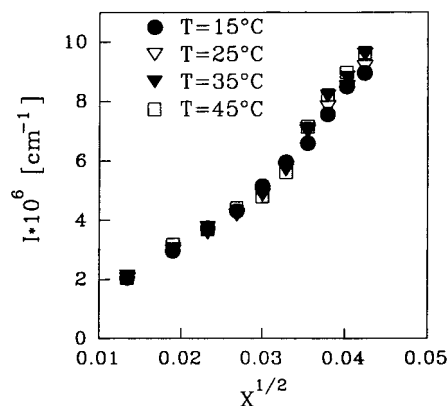
**TABLE 1:**  $[N(\text{CH}_3)_4^+ + \text{Na}^+]$  ( $c_s$ ) and NaTDC ( $c_T$ , mM) Concentration, Aggregation Number ( $N$ ) of the Taurodeoxycholate Aggregates, Percentages of the Taurodeoxycholate Aggregates,<sup>a</sup> Experimental  $R_h$  (Å),<sup>b</sup> and Calculated  $\langle R_h \rangle$  (Å)

$c_s$	$c_T$	$N$	%	$R_h$	$\langle R_h \rangle$
0.10	10	1	29.3	15.8	16.2
		3	29.3		
		6	39.6		
		15	1.7		
0.10	20	1	14.7	16.3	16.5
		3	32.3		
		6	41.0		
		15	12.0		
0.20	10	1	26.0	17.6	17.7
		3	25.3		
		6	17.5		
		12	24.9		
		15	5.1		
		18	1.1		
0.20	20	1	13.9	19.0	18.2
		3	18.1		
		6	16.5		
		12	35.7		
		15	11.6		
		18	3.2		
		23	0.3		
		27	0.7		
		36	0.7		
0.20	50	1	3.5	16.5	17.0
		3	16.4		
		6	16.1		
		12	14.7		
		15	30.0		
		18	9.9		
		23	6.9		
		27	2.5		
		36	2.5		
0.30	10	1	17.5	19.9	18.8
		3	19.5		
		12	35.2		
		15	16.2		
		24	0.9		
		27	1.8		
		32	2.2		
		36	6.7		
		36	6.7		
0.30	20	1	9.2	21.0	19.7
		3	12.0		
		12	29.5		
		15	17.6		
		24	1.6		
		27	3.9		
		32	6.0		
0.30	50	1	2.1	20.0	19.9
		3	8.3		
		12	2.9		
		15	12.9		
		24	3.0		
		27	26.7		
		32	24.5		
		36	19.6		

<sup>a</sup> Percentages expressed as a function of the monomer concentration.

<sup>b</sup> The average standard deviation is  $\pm 0.4$  Å.

with  $N \geq 15$  increase both with the NaTDC and  $N(\text{CH}_3)_4\text{Cl}$  concentration. Probably, the addition of the same amount of  $N(\text{CH}_3)_4\text{Cl}$  or NaCl in the concentration range 0–0.3 M has a nearly equal effect on the  $R_h$  value on the basis of previous results.<sup>2</sup> Thus, it is possible to calculate the  $\langle R_h \rangle$  values, which depend on the mole fraction, the aggregation number, and the calculated  $R_h$  of each micellar aggregate for NaTDC aqueous solutions containing  $N(\text{CH}_3)_4\text{Cl}$ .<sup>3</sup> The  $R_h$  value of each micellar aggregate has been computed using the same rules of ref 2, assuming that the aggregates with  $N \leq 12$  are oblate ellipsoids,<sup>11</sup> and those with  $N \geq 15$  are prolate ellipsoids.<sup>12</sup> The comparison



**Figure 3.** Rayleigh ratio of NaTDC aqueous solutions as a function of the square root of the NaTDC mole fraction at different temperatures.

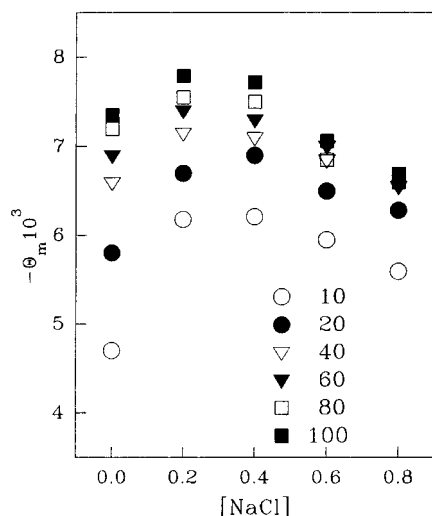
of the calculated  $\langle R_h \rangle$  values with the experimental  $R_h$  values, obtained from QELS measurements of NaTDC solutions containing NaCl, is reported in Table 1.

A check of the presence of two structures, at least, can be provided by the average intensity scattered by the NaTDC solutions with concentrations 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mM at the temperatures of 15, 25, 35, and 45 °C (Figure 3). Since the light-scattering form factor is in the limit where approximates unity,<sup>8,9</sup> the experimental points of the intensity vs the square root of the mole fraction should lie on a straight line<sup>13</sup> if the micellar aggregates have just one type of structure and grow along one direction (as, for example, in a helix). The NaTDC intensity data can be fitted by two straight lines, which intersect at a concentration about 40 mM, and are practically independent of the temperature. When 0.8 M NaCl is added the intensity decreases by increasing the temperature (data not shown).

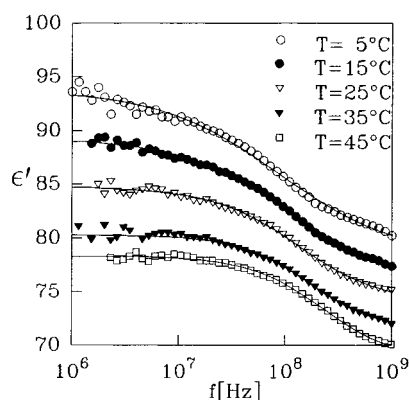
**CD Study of NaTDC–BR Aqueous Solutions.** Aqueous solutions of dihydroxy and trihydroxy salts of bile acids containing BR have been previously investigated.<sup>1,14–17</sup> The CD spectra and the structures of the micellar aggregates of the dihydroxy salts differ remarkably from those of the trihydroxy ones.<sup>1,16</sup> These results, together with potential energy calculations performed on the interaction complexes between BR and sodium glycodeoxycholate<sup>14</sup> or glycocholate,<sup>16</sup> strongly suggest that the chiral recognition of BR is sensitive to the structure of the micellar aggregates. Their structure could be helical since a helix is a chiral object. A preferential complexation of the left-handed BR enantiomer has been generally found for the dihydroxy salts on the basis of an observed (–) longer wavelength Cotton effect followed by a (+) shorter wavelength Cotton effect.<sup>1,14,15</sup>

CD measurements have been accomplished as a function of ionic strength within the NaTDC and NaCl concentration ranges 10–100 mM and 0–0.8 M, respectively, to check the enantioselective ability of small (low ionic strength) and big (high ionic strength) aggregates. The ellipticity values at 465 nm (wavelength of the minimum in the CD spectrum) are reported as a function of NaCl concentration in Figure 4. A preferential complexation of the left-handed BR enantiomer has been always observed in the NaTDC and NaCl concentration ranges investigated.

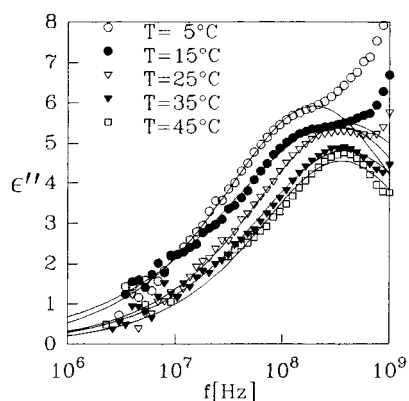
**Dielectric Study of NaTDC Aqueous Solutions.** The complex dielectric constant  $\epsilon^* = \epsilon' - j\epsilon''$  of NaTDC aqueous solutions has been measured as a function of frequency. As an example, the permittivity  $\epsilon'$  and the dielectric loss  $\epsilon''$  of the 80 mM solution are reported as a function of frequency at temperatures of 5, 15, 25, 35, and 45 °C in Figures 5 and 6,



**Figure 4.** Ellipticity values at 465 nm of NaTDC-BR aqueous solutions as a function of added NaCl. The symbols refer to different NaTDC concentrations expressed in millimoles.



**Figure 5.** Permittivity of the 80 mM NaTDC aqueous solution as a function of frequency at 5, 15, 25, 35, and 45 °C.



**Figure 6.** Dielectric loss of the 80 mM NaTDC aqueous solution as a function of frequency at 5, 15, 25, 35, and 45 °C.

respectively. The profiles of  $\epsilon'$  and  $\epsilon''$  clearly show the presence of the dielectric relaxation, usually termed  $\beta$  dispersion, which is found at these frequencies in macromolecular solutions.<sup>18–21</sup> The lines superimposed to the experimental points are the result of a best fit in terms of a Cole–Cole dispersion represented by the known equation:

$$\epsilon^* = \epsilon_{\infty} + \Delta\epsilon/[1 + (j\omega\tau)^{1-\alpha}]$$

where  $\epsilon_{\infty}$  is the high-frequency limit permittivity of the NaTDC solution,  $\Delta\epsilon$  is the dielectric increment,  $\tau$  is the relaxation time,

and  $\alpha$  is an empirical parameter which indicates a spread of relaxation times. The contribution of water to the dielectric loss at the higher frequencies, especially for the lower temperatures, is evident in Figure 6. In fact, the relaxation frequency of water varies from 10 to 20 GHz within the temperature range 5–25 °C. Thus, the contribution of water to the dielectric loss is just appreciable near 1 GHz. The apparent electric dipole moment of the solute can be estimated from the dielectric increment by means of the Oncley formula,<sup>22,23</sup> verified in many solutions with solutes of molecular weights comparable to those of the NaTDC micellar aggregates:<sup>2,3,24–27</sup>

$$\mu^2 = 2KT\epsilon_0 M \Delta\epsilon / N_0 c$$

where  $\mu$  is the “effective” electric dipole moment,  $K$  is the Boltzmann constant,  $T$  is the absolute temperature,  $M$  is the molecular weight of the NaTDC monomer,  $N_0$  is the Avogadro number, and  $c$  is the solute concentration in g/m<sup>3</sup>. Since  $M$  is the molecular weight of the NaTDC monomer, the estimate of the effective  $\mu$  values refers to the monomer and not to the micellar aggregates. The previous relationship has been used in the limit of non interacting micellar aggregates.<sup>26</sup> Depending on the specific aggregation state, different values of  $\mu$  can be obtained. Our results correspond to the average properties of the monomers in the specific aggregation distribution function of the solution. The calculated  $\mu$  values as a function of solute concentration at three of the investigated temperatures are shown in Figure 7. The  $\mu$  value per monomer decreases of about a factor 2 as the concentration increases from 10 to 100 mM. The data can be fitted by two linear trends with different slopes which intersect at a concentration about 40 mM. A similar behavior has been observed at the other temperatures. The dielectric increment and the relaxation frequency  $f_0$ , inferred from the fitting procedure, and the electric dipole moment, estimated by the Oncley formula, are reported in Table 2.

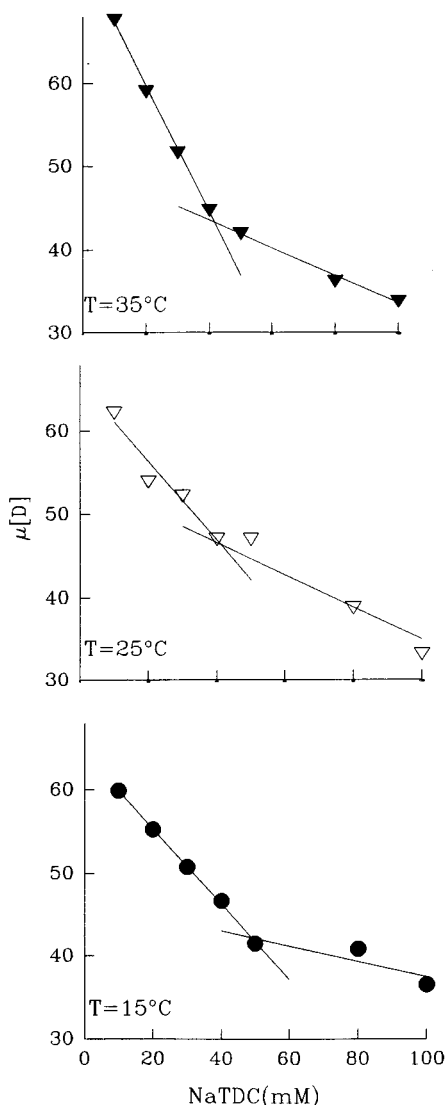
Considering the relaxation process as analogous to a chemical rate process, the temperature dependence of the relaxation time can be expressed according to the equation:

$$(\tau T)^{-1} = (K/h) \exp(\Delta S/R) \exp(-\Delta H/RT)$$

where  $\Delta S$  and  $\Delta H$  are the molar entropy and enthalpy of activation for the relaxation process, respectively. Provided that  $\Delta S$  and  $\Delta H$  are temperature independent, a graph of  $\ln(\tau T)^{-1}$  against  $T^{-1}$  is a straight line of negative slope from which an order of magnitude of  $\Delta H$  can be calculated. The  $\Delta H$  values of the NaTDC solutions, shown in Figure 8, have been inferred from a linear interpolation of  $\tau$  vs NaTDC concentration at each temperature. Also in this case the data can give rise to a break in the linear trend near the 40 mM concentration.

## Discussion

Inspection of Table 1 shows that the calculated  $\langle R_h \rangle$  values at a fixed  $c_s$  agree with the experimental  $R_h$  values, and that there is a slight change of the  $\langle R_h \rangle$  values as a function of NaTDC concentration. This trend is similar to that observed for NaTDC, fixing the NaCl concentration within the range 0–0.3 M, and can explain the practically constant  $R_h$  values of Figure 2. The micellar aggregates (see Table 1) have the following calculated  $R_h$  values (Å) reported as  $N(R_h)$ : 1(7.3), 3(17.4), 6(18.6), 12(21.0), 15(15.3), 18(16.3), 23(18.2), 27(19.2), 32(21.0), 36(21.9). The increase of the NaTDC concentration favors the formation of aggregates with higher  $N$ . However, the  $R_h$  values of oblate aggregates as trimers and hexamers are greater than those of prolate aggregates with higher  $N$  as pentadecamers and octadecamers. Therefore, when the percent-



**Figure 7.** Electric dipole moment per NaTDC monomer as a function of NaTDC concentration at 15, 25, and 35 °C.

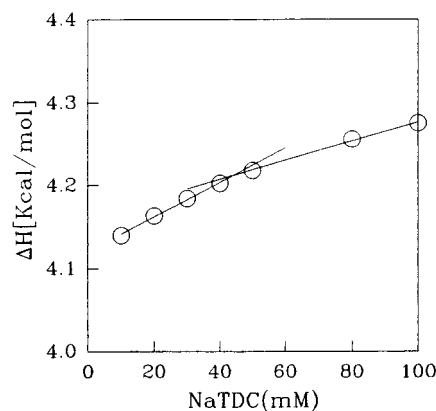
age of trimers ( $R_h = 17.4$  Å) and hexamers ( $R_h = 18.6$  Å) decreases and that, for example, of pentadecamers ( $R_h = 15.3$  Å) and heptaecicosamers ( $R_h = 19.2$  Å) increases, the  $\langle R_h \rangle$  value not necessarily increases.

Sedimentation and diffusion experiments have pointed out that the NaTDC micellar aggregates in salt solutions are highly hydrated.<sup>28</sup> The trimer of the oblate aggregates (radius of about 19 Å) presents between each pair of NaTDC molecules a large empty room which can be filled by water molecules. That of the cylindrical aggregates (7/1 helices, radius of about 11 Å) is more compact and can accommodate a smaller amount of water molecules.<sup>2</sup> Thus, the average intensity scattered by NaTDC could mainly depend on the ratio between these two types of trimer, and each of the two straight lines, which fit the points of Figure 3, could refer to a concentration range where one type of trimer predominates. The scattered intensity is practically independent of the temperature when the  $N$  values are low. When they increase by adding 0.8 M NaCl, the increase of the temperature, which causes the breaking of the polar interactions, gives rise to a more remarkable decrease of the micellar size as well as of the scattered intensity.

In a previous paper<sup>17</sup> the enantioselective abilities of NaTDC and sodium taurocholate in aqueous solutions have been studied as a function of ionic strength, using BR and bile salt concentrations  $4.6 \times 10^{-6}$  and 0.1 M, respectively. By

**TABLE 2: Dielectric Data for NaTDC Aqueous Solutions at Different Temperatures**

concn (mM)	$T$ (°C)	$f_o$ (MHz)	$\Delta\epsilon$	$\mu$ (D)
10	5	$37 \pm 2$	$3.7 \pm 0.1$	$62 \pm 1$
	15	$59 \pm 2$	$3.3 \pm 0.1$	$60 \pm 1$
	25	$69 \pm 3$	$3.5 \pm 0.1$	$62 \pm 1$
	35	$72 \pm 5$	$4.0 \pm 0.2$	$68 \pm 2$
	45	$120 \pm 10$	$2.2 \pm 0.2$	$51 \pm 2$
20	5	$48 \pm 1$	$5.9 \pm 0.2$	$55 \pm 1$
	15	$60 \pm 1$	$5.7 \pm 0.2$	$55 \pm 1$
	25	$80 \pm 2$	$5.3 \pm 0.2$	$54 \pm 1$
	35	$81 \pm 4$	$6.1 \pm 0.2$	$59 \pm 1$
	45	$125 \pm 5$	$4.6 \pm 0.2$	$52 \pm 1$
30	5	$48 \pm 1$	$8.1 \pm 0.2$	$53 \pm 1$
	15	$66 \pm 2$	$7.2 \pm 0.2$	$51 \pm 1$
	25	$96 \pm 3$	$7.4 \pm 0.2$	$52 \pm 1$
	35	$110 \pm 4$	$7.0 \pm 0.2$	$52 \pm 1$
	45	$165 \pm 8$	$5.3 \pm 0.2$	$46 \pm 1$
40	5	$55 \pm 1$	$9.4 \pm 0.2$	$49 \pm 1$
	15	$74 \pm 1$	$8.1 \pm 0.2$	$47 \pm 1$
	25	$98 \pm 3$	$8.0 \pm 0.2$	$47 \pm 1$
	35	$112 \pm 5$	$7.0 \pm 0.2$	$45 \pm 1$
	45	$145 \pm 9$	$4.7 \pm 0.2$	$37 \pm 1$
50	5	$54 \pm 2$	$8.6 \pm 0.2$	$42 \pm 1$
	15	$74 \pm 2$	$8.0 \pm 0.2$	$41 \pm 1$
	25	$134 \pm 4$	$10.0 \pm 0.3$	$47 \pm 1$
	35	$149 \pm 4$	$7.7 \pm 0.2$	$42 \pm 1$
	45	$240 \pm 10$	$6.4 \pm 0.4$	$39 \pm 2$
80	5	$80 \pm 1$	$12.0 \pm 0.3$	$39 \pm 1$
	15	$101 \pm 3$	$12.4 \pm 0.3$	$41 \pm 1$
	25	$127 \pm 4$	$10.9 \pm 0.3$	$39 \pm 1$
	35	$166 \pm 6$	$9.2 \pm 0.4$	$36 \pm 1$
	45	$230 \pm 10$	$10.0 \pm 0.4$	$38 \pm 1$
100	5	$82 \pm 2$	$12.7 \pm 0.3$	$36 \pm 1$
	15	$90 \pm 3$	$12.4 \pm 0.3$	$36 \pm 1$
	25	$108 \pm 4$	$10.0 \pm 0.3$	$33 \pm 1$
	35	$163 \pm 5$	$10.0 \pm 0.3$	$34 \pm 1$
	45	$232 \pm 8$	$10.4 \pm 0.3$	$35 \pm 1$



**Figure 8.**  $\Delta H$  values of the NaTDC solutions as a function of concentration.

increasing the ionic strength (and even  $N$ ) the ellipticity absolute values ( $|\theta_m|$ ) at 460 nm (wavelength of the minimum in the CD spectrum) lie on a straight line with negative slope for sodium taurocholate. This result indicates that the enantioselective ability decreases when  $N$  increases, and is not in disagreement with the presence of only one structure of the micellar aggregates, that does not change during their growth. NaTDC behaves in a different way since its  $|\theta_m|$  values at 465 nm show a maximum at 0.3 M NaCl. The CD data shown in Figure 4 confirm this trend and support the coexistence, at least, of two types of structures, as those previously identified.<sup>2,3</sup> That prevailing at low ionic strength should have an enantioselective ability greater than that of the structure prevailing at high ionic strength. The slope of  $|\theta_m|$  decreases by increasing the proportion of the micellar aggregates with  $N \geq 15$  in agreement

with the lower enantioselective ability of the structure prevailing at high ionic strength. Of course, equilibria among monomers, oblate aggregates, and cylindrical aggregates can be conceived. It has been reasonably inferred from CD spectra and potential energy calculations<sup>1,15,16</sup> that the monomers of the dihydroxy and trihydroxy bile salts interact preferentially with the right-handed BR enantiomer. Therefore, a decrease of the monomers in favor of the oblate aggregates causes an increase of  $|\theta_m|$ , as well as a decrease of the oblate aggregates in favor of the cylindrical aggregates causes a decrease of  $|\theta_m|$ . These two processes can explain the trend of  $|\theta_m|$  in Figure 4.

The average values of  $\mu$  reported in Table 2 refer to one NaTDC molecule and are obtained by means of the Oncley formula, which considers that the dielectric relaxation is due to orientational polarizability of polar molecules or assemblies of molecules in solution. The experimental points of  $\mu$  vs NaTDC concentration can be fitted by two straight lines (Figure 7). They intersect at a concentration about 40 mM, as in the case of the average intensity scattered by the same NaTDC samples (Figure 3), reinforcing the hypothesis that there are two structures, each one prevailing in one of the two concentration regions. The  $\mu$  value varies from 33 to 68 Debye units, and seem too high to be considered as an average value of a single NaTDC molecule belonging to the aggregates. In fact, a realistic estimate does not exceed 20 D since the distances between  $\text{Na}^+$  ions and the nearest neighbors oxygen atoms of the sulfonate groups are mainly within the range 2.3–4.5 Å in some crystal structures of NaTDC<sup>29</sup> and sodium taurocholate.<sup>16,30</sup> On the other hand, a value of about 8 Å is obtained for the  $r$  average value of the polar solute, assumed to be a spherical particle, from the equation:

$$\tau = 4\pi\eta r^3/KT$$

where  $\eta$  is assumed as the viscosity of the solvent. Owing to the approximation that the micellar aggregates have spherical shape,  $r$  can be roughly compared with the average  $R_h$  value of about 6 Å observed for the 10–100 mM NaTDC aqueous solutions without added NaCl (Figure 2). The agreement supports that the solute is responsible for the measured dielectric dispersion. It must be noticed also that the  $r$  values for each solution are practically independent of temperature.

The high  $\mu$  values could be justified by a contribution of the hydration water. EXAFS measurements, performed on RbTDC aqueous solutions, have indicated that the cations are coordinated with about nine water molecules in the first hydration shell having a radius of about 4 Å.<sup>4</sup> Sedimentation and diffusion measurements, carried out on NaTDC aqueous solutions, have shown that the micellar aggregates are highly hydrated.<sup>28</sup> The cations, the aggregates, and their coordinated water molecules can move and can be oriented as a rigid body when an electric field is applied. Inspection of Table 2 and Figure 7 shows that  $\mu$  moderately decreases by increasing the NaTDC concentration and, hence, the  $N$  values. Generally, a dramatic decrease of  $\mu$  occurs when the growing aggregates are disordered or with a pseudo center of symmetry. It is interesting to note that the globular and disklike micelles proposed by Small<sup>31,32</sup> and Kawamura et al.,<sup>5</sup> respectively, are disordered systems. The disklike micelle, at least, has also a pseudo center of symmetry since the bile salt molecules are oriented with their long axes parallel or approximately parallel (probably 50% “up-pointing” and 50% “down-pointing” anions). Therefore, these two models do not account for the moderate decrease of  $\mu$  as the NaTDC concentration is increased. On the other hand, a moderate decrease of  $\mu$  can be expected when the growing aggregates are characterized by a screw axis as in the 7/1 helix, which

could have also an amount of hydration water smaller than that of the oblate aggregates.

An additional information comes out from the calculation of  $\Delta H$ , which is a measure of the response of the system to the aligning influence of the electric field. The average scattered intensity and  $r$  are almost independent of temperature within the range 15–45 °C, at least. Hence, each NaTDC sample has, very probably, nearly the same distribution of the micellar aggregation numbers within this temperature range. A further support arises from the narrow range of the  $\alpha$  values (centered around 0.2), calculated for the Cole–Cole dispersion, which result independent of temperature and NaTDC concentration.  $\Delta H$  depends on the distribution of the micellar aggregation numbers, whose average value varies with the NaTDC concentration. Therefore, also  $\Delta H$  must vary with the NaTDC concentration (Figure 8). Since a break point can be identified at a concentration about 40 mM again, the two straight lines suggest the presence of two structures.

Although all these results satisfactorily agree with the coexistence of two structures, at least, further work is in progress with the aim to reach a definite conclusion.

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