

Study on Intermicellar Interactions and Micellar Size in Aqueous Solutions of Sodium Taurocholate by Measurements of Collective Diffusion and Self-Diffusion Coefficients

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Aqueous micellar solutions of sodium taurocholate (NaTC) have been studied by using quasi-elastic light-scattering (QELS) and ^1H pulse-gradient-stimulated-echo NMR (PGSTE-NMR). The collective and self-diffusion coefficients have been obtained as a function of NaTC concentration (0.05–0.20 M) and the ionic strength of the solution (0–0.8 M NaCl added). The comparison of the two techniques' data in each solution has been performed in the context of micellar growth and intermicellar interactions. The model of monodisperse Brownian particles with hydrodynamic interaction and hard core repulsion gives rise to a satisfactory agreement with the experimental data. The translational diffusion coefficient of the single micellar aggregate has been calculated for each solution. The size of the micellar aggregates depends on the ionic strength and bile salt concentration.

Introduction

Bile salts are biological amphiphiles that give rise to very important aggregation and solubilization processes.^{1,2} They can solubilize in water, by formation of micellar aggregates, important biological compounds as, for example, cholesterol, bilirubin-IX α , phospholipids, and fatty acids. The determination of typical parameters of bile salt micelles such as size and shape, and of their sensitivity to thermodynamic variables such as composition and temperature is a subject of considerable interest for understanding their physicochemical and biological properties. Interesting data can be obtained by means of quasi-elastic light-scattering (QELS) and pulse-gradient-stimulated-echo NMR (PGSTE-NMR) measurements, which can provide information on the collective and self-diffusion coefficients, respectively, in a system of diffusing particles. QELS results on bile salt micelles show that their diffusivities vary by changing both the bile salt concentration and the ionic strength.^{3–10} This variation, which is observed also for other ionic micelles,^{11–14} can be due to the combination of two effects: intermicellar interaction and change of micellar size. Therefore, reliable information on micellar size and shape can be obtained only for very diluted micellar solutions where the influence of intermicellar interactions is minimized. A direct proof of micellar growth as a function of bile salt concentration and ionic strength has been obtained by electromotive force measurements (EMF) for sodium deoxycholate,¹⁵ glycodeoxycholate (NaGDC),¹⁶ taurodeoxycholate (NaTDC),¹⁷ and taurocholate (NaTC).^{18,19} Moreover, in the case of NaGDC, the dependence on ionic

strength (NaCl added) of micellar diffusivities, extrapolated at the critical micellar concentration, has been observed,⁵ thus suggesting that a micellar growth occurs by increasing the ionic strength.

In this paper a comparison between diffusion coefficients measured by QELS and PGSTE-NMR on D_2O solutions of NaTC has been performed. Analysis of the data by using a simple model of monodisperse Brownian particles with hydrodynamic interaction and hard core repulsion²⁰ allowed us to separate the effects of micellar growth and particle interactions, and to determine the micellar size without extrapolating the data to the critical micellar concentration.

Experimental Section

Materials. D_2O (100%, Aldrich) and NaCl (Merck, suprapur) were used. NaTC (Sigma) and NaTDC (Sigma) were twice lyophilized from a D_2O solution before the sample preparation.

QELS Measurements. The normalized field autocorrelation function $g_1(\tau)$, which has been derived from the intensity autocorrelation function $g_2(\tau)$ by Siegert's relation^{21,22}

$$g_2(\tau) = \frac{\overline{I(t)I(t+\tau)}}{\overline{I(t)}^2} = 1 + Bg_1^2(\tau)$$

where B is an instrument factor, has been fitted by the following second-order cumulant analysis

$$\ln g_1(\tau) = -q^2 D_{\text{app}} \tau + \frac{\mu_2}{2} \tau^2$$

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where D_{app} is the so-called apparent diffusion coefficient and μ_2 is the second cumulant. 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 Lexel Corp. 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 cm³. Nuclepore filters with a pore size of 0.1 μm have been used. The samples have been placed in the cell for at least 30 min prior the measurement to allow for thermal equilibration. Their temperature has been kept constant within 0.5 °C by a circulating water bath. The time-dependent light scattering correlation function has been analyzed only at the 90° scattering angle. The apparent diffusion coefficients do not depend on the exchanged wave vector in the range 30–150° in our experimental conditions.

PGSTE-NMR Measurements. The self-diffusion coefficient of the solutes in the different samples was determined by ¹H PGSTE-NMR measurements. In this technique a 90°–90°–90° pulse sequence is performed under the presence of two magnetic field gradient pulses of magnitude G , duration δ , and separation Δ applied during the dephasing (first 90°–90° interval) and rephasing (90°–echo interval) periods, respectively. In the present study the interval Δ between the magnetic field gradient pulses was kept constant and equal to the sum of the periods τ_1 and τ_2 corresponding to the first and the second 90°–90° radio frequency pulse intervals, respectively. In these conditions the height of the echo amplitude $A(2\tau_1 + \tau_2)$ for a nucleus with self-diffusion coefficient D_s is given by²³

$$A(2\tau_1 + \tau_2) = 0.5A(0) \exp\left[-\frac{\tau_2}{T_1} - \frac{2\tau_1}{T_2} - \gamma^2 G^2 D_s \delta^2 \left(\Delta - \frac{\delta}{3}\right)\right]$$

where T_1 and T_2 are the longitudinal and transversal relaxation times, respectively, and γ is the magnetogyric ratio. In a typical experiment $A(2\tau_1 + \tau_2)$ was measured at $\Delta = 40$ ms and $G = 66.1$ G/cm by varying δ up to 3 ms. The gradient strength was calibrated to values of the self-diffusion coefficient of pure water. The self-diffusion coefficient was obtained from the slope of the logarithmic plot of $A(2\tau_1 + \tau_2)$ vs $\delta^2(\Delta - \delta/3)$.

The spectra were recorded on a Stellar Spinmaster NMR spectrometer operating at 21 MHz for protons. The temperature of the sample during the measurements was controlled by a variable temperature unit Brüker-VT 100 to an accuracy of ± 0.10 °C.

Results and Discussion

For a system of monodisperse noninteracting particles both D_{app} and D_s obtained from QELS and PGSTE-NMR measurements, respectively, are equal to the translational diffusion coefficient D_o of the single particle. In the case of interacting particles, D_{app} and D_s assume different concentration dependent values and approximate D_o for very dilute systems. In suspensions of interacting Brownian particles, two types of interactions can be identified. These are hydrodynamic interactions, which are transmitted by the solvent flow and depend on friction matrix, and direct interactions, which can be expressed by long-range Debye–Hückel and van der Waals potentials. To consider the effect of particles interactions, a model of identical spherical particles with stick boundary conditions at a hydrodynamic radius a and with a hard core repulsion at a larger radius b has been assumed in this paper. Two time-dependent self-diffusion coefficients are defined by the behavior at short and long time.

The long-time self-diffusion coefficient D_s is provided by the PGSTE-NMR technique, which probes times $t \gg \tau_1$, where τ_1 is the velocity fluctuation time associated with the interaction forces. To first-order approximation, D_s is given by

$$D_s = D_o[1 + (\lambda_A + \alpha_s)\phi] \quad (1)$$

where ϕ is the volume fraction of particles. λ_A depends on direct and hydrodynamic pair interactions^{20,24} and is given by

$$\lambda_A = 8 \int_{x_1}^{\infty} [A_{11}(x) + 2B_{11}(x)]x^2 dx$$

where $x = r/2a$ is the dimensionless distance between centers of two spheres, $A_{ij}(x) = 6\pi\eta a\alpha_{ij}(r)$ and $B_{ij}(x) = 6\pi\eta a\beta_{ij}(r)$ (r is the distance between the centers of the two spheres and η is the viscosity). Functions $\alpha_{ij}(r)$ and $\beta_{ij}(r)$ contribute to define the tensorial elements of the 2×2 mobility matrix, which relates the velocity of particle i to the forces acting on the particles i and j . As far as α_s is concerned, it was shown by Batchelor²⁵ that this coefficient requires the solution of the two-body Smoluchowski equation and may be expressed as

$$\alpha_s = 4 \int_{x_1}^{\infty} W(x)f(x)x^2 dx$$

where x_1 is b/a , $W(x)$ is a combination of hydrodynamic interaction functions $A_{ij}(x)$ and $B_{ij}(x)$, and $f(x)$ is the radial part of the solution of the two-sphere Smoluchowski equation. However, we have chosen a form for α_s following Cichocki and Felderhof,²⁴ who expressed it by use of a dielectric analogy as

$$\alpha_s = \frac{3\alpha}{2a^3} + J_s$$

where α is the electric polarizability of a related dielectric problem and J_s depends on hydrodynamic interaction functions. This relationship is of great help in practical calculations.

On the other hand, to first order in volume fraction, D_{app} can be expressed by equation

$$D_{\text{app}} = D_o(1 + \lambda_C\phi) \quad (2)$$

where λ_C can be estimated in terms of direct and hydrodynamic pair interactions and is given by a sum of five contributions:^{20,24}

$$\lambda_C = \lambda_v + \lambda_O + \lambda_D + \lambda_s + \lambda_A$$

where for the present model the virial correction λ_v , the Oseen contribution λ_O , and the dipole contribution λ_D are given by

$$\lambda_v = 8x_1^3 \quad \lambda_O = -6x_1^2 \quad \lambda_D = 1$$

and the term λ_s is expressed as

$$\lambda_s = 8 \int_{x_1}^{\infty} \left[A_{12}(x) + 2B_{12}(x) - \frac{3}{2x} \right] x^2 dx$$

Equations 1 and 2 are also expressed in terms of the effective volume fraction ϕx_1^3 , keeping the same forms with the introduction of new parameters to replace λ_A , α_s , and λ_C .²⁰

Actually, micellar systems are inherently polydisperse. In this case D_s and D_{app} are different also because they are differently related to the micellar size distribution. In particular, if X_n is the fraction of micelles with aggregation number n and self-diffusion coefficient D_n , the PGSTE-NMR self-diffusion coef-

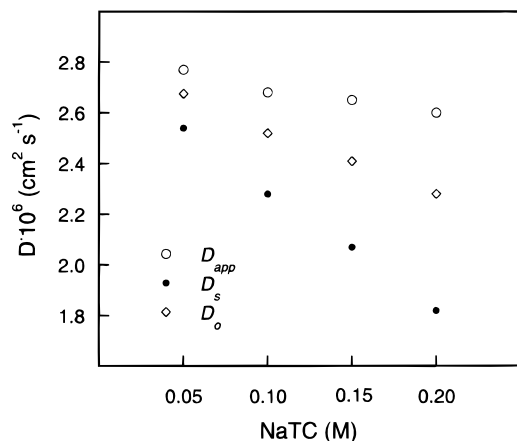


Figure 1. D_{app} , D_s , and D_o as a function of NaTC concentration for D_2O solutions at NaCl 0.8 M and $T = 65^\circ C$.

ficient is represented by the following average

$$\bar{D}_s = \frac{\sum_n n X_n D_n}{\sum_n n X_n}$$

whereas, if no interaction among the micelles is considered, the QELS diffusion coefficient is given by

$$\bar{D}_{app} = \frac{\sum_n n^2 X_n D_n}{\sum_n n^2 X_n}$$

Moreover, for a polydisperse system, the influence of particle interactions on D_{app} and D_s remains an unresolved theoretical problem. For this reason, by following a common assumption in the study of intermicellar interaction, the simplification of a monodisperse system will be considered in this paper.^{4,5,11}

In Figure 1 the D_{app} and D_s values as a function of the NaTC concentration are reported for D_2O solutions containing 0.8 M NaCl at $T = 65^\circ C$. The concentration dependence of both the diffusion coefficients is generally due to the combination of two effects: intermicellar interaction and variation of micellar size. The main problem in the interpretation of experimental results is to separate these two effects. For aqueous solutions of sodium dodecyl sulfate (SDS),^{11,12,14} NaTDC,^{3,4} and NaGDC⁵ the addition of NaCl has been found to have a strong influence on the observed D_{app} . At low and high NaCl concentration repulsive electrostatic interactions^{5,11} and the growth of micelles^{3,4,12,14} appear to dominate the concentration dependence, respectively. Models based exclusively on intermicellar interaction using the DLVO interaction potential have been proposed to explain the data at low ionic strength (≤ 0.2 and ≤ 0.6 M NaCl for NaGDC and SDS, respectively),^{5,11} whereas micellar growth together with excluded volume interaction has been considered to interpret the concentration dependence at high ionic strength (≥ 0.8 M NaCl for NaTDC).⁴

The diffusion coefficient values as a function of NaCl concentration in the range 0–0.8 M and $T = 55^\circ C$ are reported for NaTC and NaTDC 0.2 M in D_2O in Figure 2. As commonly observed for bile salts, in both cases D_{app} decreases with increasing ionic strength, the variation being sensitively greater for NaTDC than NaTC. In this range of NaCl concentration variation of both micellar size and intermicellar interaction occurs. This behavior, which is similar to the one observed for water solutions of the same salts, indicates that, at the same ionic strength, much larger aggregates are formed by NaTDC than NaTC.³ Several published results suggest that at high NaCl

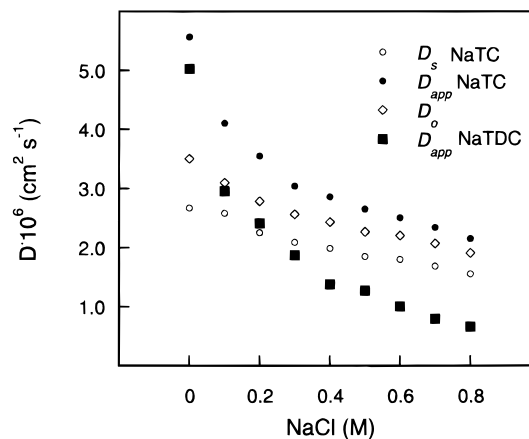


Figure 2. D_{app} , D_s , and D_o of NaTC and D_{app} of NaTDC in D_2O as a function of NaCl concentration. For each sample the bile salt concentration is 0.2 M and $T = 55^\circ C$.

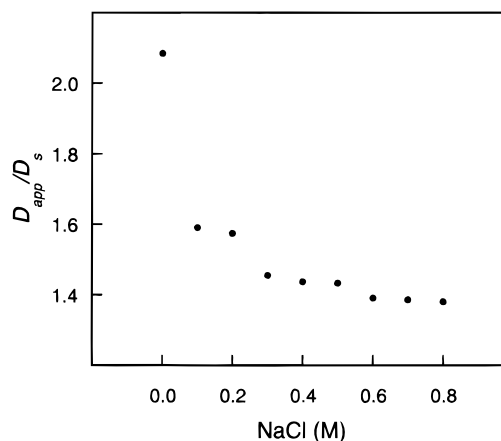


Figure 3. D_{app}/D_s values as a function of NaCl concentration for D_2O solutions of NaTC 0.2 M and $T = 55^\circ C$.

concentration NaTDC gives rise to rod-shaped helical micelles formed by aggregation of trimers.⁶ In the case of NaTC, the information so far available supports the hypothesis that in the same conditions small aggregates, which better approximate the globular shape, are formed by association of dimers.^{18,19} For this reason, the model of spherical particles could be suitable to estimate the effect of interactions on the NaTC experimental data. The attractive interactions among the particles can be neglected. Actually, the hypothesis of intermicellar attractive interactions managed to explain the negative slope in the plot of D_{app} as a function of surfactant concentration for some more conventional amphiphiles, without taking into account any variation of micellar size.¹¹ Nevertheless, since the EMF data showed that at fixed ionic strength the NaTC averaged aggregation number increases by increasing the bile salt concentration,^{18,19} we have chosen to emphasize the effect of micellar growth and to neglect the attractive intermicellar interactions.

Because of the micellar growth that influences the D_o value, intermicellar interactions cannot be treated by considering D_{app} or D_s only. D_o can be eliminated by means of the ratios D_{app}/D_s ,²⁶ which are reported in Figure 3 for NaTC samples. At low ionic strength, where both excluded volume and electrostatic repulsion effects are present, the ratio D_{app}/D_s assumes the highest value. The pattern observed can be attributed to the progressive screening of the Coulomb potential induced by increasing the ionic strength. At high NaCl concentrations, where the screening effect is practically complete, the values of D_{app}/D_s become nearly constant. In this condition, if the

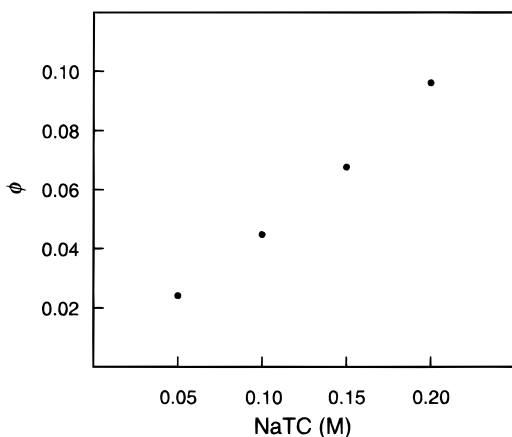


Figure 4. ϕ values as a function of NaTC concentration for D₂O solutions at NaCl 0.8 M and $T = 65^\circ\text{C}$.

attractive interactions are neglected, only the excluded volume effect can be considered ($x_1 = 1$). Values of $\lambda_C = 1.454$, $\lambda_A = -1.8315$, and $\alpha_S = -0.2657^{20}$ can be used in eqs 1 and 2, thus obtaining D_o and ϕ . The ϕ values calculated at 0.7 and 0.8 M NaCl concentration under this assumption are equal to 0.0885 and 0.0875, respectively, and correspond to a volume of one NaTC monomer together with some water molecules in the aggregates equal to 734 and 726 Å³, respectively. The number of water molecules is given by the ratio between those dragged by the aggregate in its motion and the NaTC aggregation number. The above-mentioned values are slightly greater than the molecular volume of 638 Å³ obtained from densimetric measurements on NaTC micellar aggregates in salt (NaCl) solutions.²⁷ This result is reasonable as a first approximation owing to the micellar aggregate hydration and supports the validity of previous assumptions. The procedure described has been used to interpret the data of D_{app} and D_s in Figure 1. For each sample the ϕ and D_o values have been calculated. As shown in Figure 4, a linear dependence of ϕ as a function of NaTC concentration is observed, thus indicating that low and nearly constant polydispersity is present in the samples within the NaTC range 0.05–0.20 M at 0.8 M NaCl. As shown in Figure 1 the calculated D_o slightly decreases by increasing the bile salt concentration.

At lower NaCl concentrations, long-range electrostatic repulsions among the aggregates are reinforced and cannot be discarded. The Cichocki and Felderhof simple model allows us to account for their effect by associating to each particle a hard core sphere with a radius larger than the hydrodynamic one ($x_1 > 1$) and to calculate λ_C , λ_A , and α_S parameters for different x_1 values.

The ϕ values can be affected by the NaCl concentration. This is a consequence of the ionic strength effect on the structure of micellar aggregate (size, shape, monomer packing, hydration, etc.). In the case of NaTC, because of the low micellar growth observed by increasing the ionic strength,^{18,19} it is reasonable to suppose that no dramatic structural change of the aggregates occurs and, consequently, that ϕ is approximately independent of NaCl concentration. Under this assumption, from the ϕ value calculated for the sample with 0.20 M NaTC and 0.8 M NaCl a D_{app}/D_s ratio as a function of x_1 has been derived, considering only the excluded volume effect, and reported in Figure 5. From D_{app}/D_s experimental ratios, x_1 values have been extrapolated, and by using the corresponding λ_C parameters, the single particle diffusion coefficients D_o have been computed from the D_{app} experimental values by means of eq 2 (Figure 2). It should be noted that, while experimental data sometimes show a linear dependence of D_{app} and D_s on ϕ over a quite wide range of ϕ ,

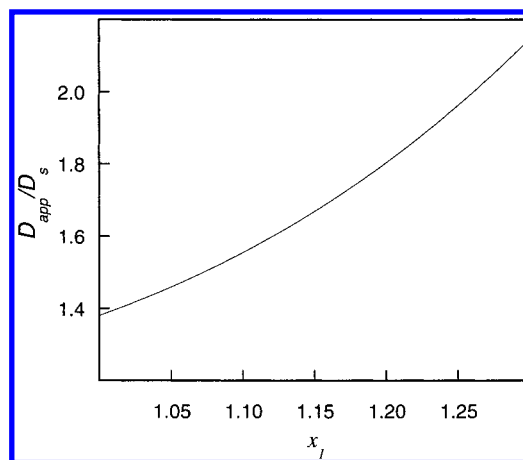


Figure 5. Theoretical D_{app}/D_s as a function of x_1 .

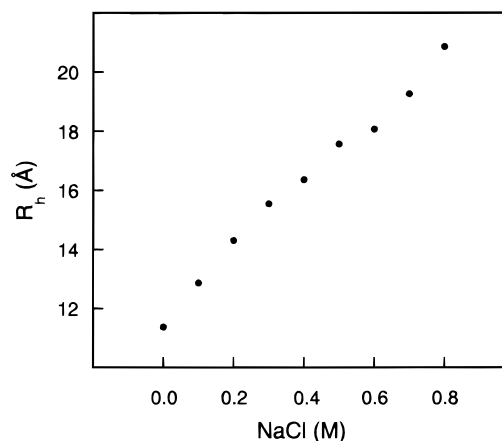


Figure 6. R_h values as a function of NaCl concentration for D₂O solutions of NaTC 0.2 M at $T = 55^\circ\text{C}$.

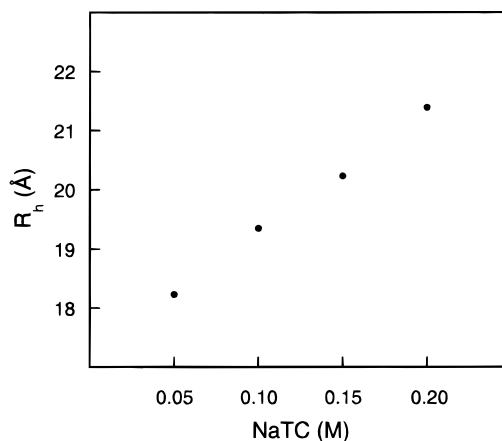


Figure 7. R_h values as a function of NaTC concentration for D₂O solutions at NaCl 0.8 M and $T = 65^\circ\text{C}$.

eqs 1 and 2 are expected to be valid only for small ϕ 's. The ϕ values suggest that the interpretation of the data for the highest NaTC concentration (0.20 M) on the basis of the diluted system approximation could be forced. However, because of the very low echo signal generally observed for NaTC micelles, this concentration has been chosen to analyze the ionic strength effect, to minimize the experimental errors.

By means of D_o the corresponding hydrodynamic radius R_h has been calculated using the Stokes–Einstein equation (Figures 6 and 7). It is important to remark that the patterns shown in these figures reliably represent the aggregate size dependence

on NaTC and NaCl concentration, because of the separation between the intermicellar interaction and the micellar growth effects. In both cases a micellar growth is observed by increasing concentration, the dependence on NaCl concentration being much more pronounced than on the NaTC one.

Conclusions

The NaTC collective and self-diffusion coefficients in D₂O solutions have been measured by using QELS and PGSTE-NMR. The experimental D_{app}/D_s values reported for each sample have been interpreted by using a simple model of monodisperse Brownian particles with hydrodynamic interactions and hard core repulsion. To account for intermicellar interactions, a hard core repulsion sphere radius larger than or equal to the hydrodynamic one has been used and the intermicellar attraction has been neglected. Micellar growth as a function of ionic strength and bile salt concentration has been observed.

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