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Hydrodynamic Properties of Micelles of Dihydroxy Bile Salts: Sodium Taurodeoxycholate and Sodium Glycodeoxycholate*

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The dependence of the mutual translational diffusion coefficient and the sedimentation coefficient on concentration of sodium taurodeoxycholate in aqueous 0.15 m NaCl solutions at 25 °C indicates a pronounced increase of the micelle size in the region between the critical micelle concentration, equal to 0.00082 gcm^{-3}, and approximately ten times higher concentration. These results were substantiated by the variation of the Rayleigh ratio of scattered light. At concentrations of bile salt higher than about 0.008 gcm^{-3} the hydrodynamic and the thermodynamic interactions dominate the measured quantities. The quasielastic light scattering measurements provided the estimates of the polydispersity of the micelles. The diffusion coefficient of sodium glycodeoxycholate varied with concentration in a manner similar to that for the taurine conjugate.

INTRODUCTION

Until recently investigations of the hydrodynamic properties of the micellar particles of dihydroxy bile salts were mostly limited to a few unrelated viscosity measurements on solutions of sodium deoxycholate.[1–7] The gel filtration,[8] the rate of sedimentation and the mutual translational diffusion,[9] and the intrinsic viscosity[10,11] of micelles of sodium taurodeoxycholate (NaTDC) were studied at several solvent compositions. More recently, the mutual diffusion coefficients of the micelles of NaTDC and several other bile salts were determined by means of the quasi-elastic light scattering technique (QELS).[12–16] The self-diffusion was also investigated in solutions of two taurine conjugates.[17]

We present data for the mutual translational diffusion coefficient, D, and for the sedimentation coefficient, S, over a broad range of concentrations of

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NaTDC in 0.15 m NaCl solutions at 25 °C. These results, which are supplemented by the measurements of the absolute intensity of scattered light, reveal features of the reversible aggregation of NaTDC not observed in earlier studies. A comparison has been made of the value of D measured by the boundary spreading and by QELS techniques. To a lesser extent, the diffusion of sodium glycodeoxycholate (NaGDC) micelles was also investigated.

MATERIALS AND METHODS

Several samples of NaTDC and NaGDC, purchased from Calbiochem, La Jolla, CA or Sigma, St. Louis, MO, were purified by repeated charcoal treatment and recrystallization. For purified samples no minima were found in the surface tension--concentration curves in the vicinity of the critical micelle concentration (cmc), even for solutions without added electrolyte. The concentration dependence of the Rayleigh ratio of scattered light, R_{90}, did not exhibit any erratic behavior in the region of the cmc. Below the cmc the variation of excess R_{90} closely corresponded to the molar mass of the monomer of NaTDC (521.7 g mole^{-1}). The properties of solutions of unpurified or inadequately purified samples varied from batch to batch, particularly at low concentrations. Such samples were not suitable for the purpose of this investigation. Full details will be provided in a forthcoming thesis.[18]

Quasi-elastic light scattering measurements were performed at State University of New York at Stony Brook. The details of the experimental techniques and the treatment of data were previously described.[19-22] The incident beam from a Spectra Physics model 165 argon ion laser was focused to the center of a 1-cm o. d. cylindrical sample cell. The scattered light with $\delta\Theta < 0.1^0$ was detected using a ITT FW130 photomultiplier tube. The pulse signal was amplified, discriminated, and then fed to a single-clipped 96-channel Malvern correlator. The time correlation functions were analyzed using a single exponential fit and the method of cumulants for the evaluation of the mean decay rate, $\bar{\Gamma}$, and the normalized z-average variance of the distribution function, $\mu_2/\bar{\Gamma}^2$, at several scattering angles Θ. $\bar{\Gamma}$ is related to the z-average value of D by $\bar{\Gamma} = D\,Q^2$ where Q is the scattering vector.

A Beckman Model E analytical ultracentrifuge and a synthetic boundary cell were used for the determination of D from the boundary spreading and S from the preformed boundaries. S was also measured in a standard 12-mm cell from boundaries evolving from the meniscus. Identical values of S were obtained in both cells. The use of the synthetic boundary cell allows the determination of S to much higher concentrations. The description of the data collection and analysis is given elsewhere.[23] The experiments on the micellar solutions of sodium dodecyl sulfate in water and in 0.1 m NaCl over a broad range of the surfactant concentrations established that the boundary spreading and the QELS techniques lead to practically identical values of D at all concentrations.[23]

A Brice-Phoenix light scattering photometer was used for measurements of R_{90} at the vacuum wavelength $\lambda_0 = 436$ nm from below the cmc (equal to 0.00082 gcm^{-3} = = 0.00157 M) to 122 times the cmc. The techniques were similar to the earlier ones[24] with substantial improvements in the stabilization of the instrument signals, the temperature control, and the clarification of solutions.[18] The values of excess R_{90} (above the cmc) are treated in the form of Debye plot, i. e., as Kc_2/R_{90} versus c_2, where $K = 2\,\pi^2\,n^2\,(dn/dc_2)^2\,(N_\Lambda\,\lambda_0^4)^{-1}$, n is the refractive index of solution of micellar concentration c_2 (equal to the total bile salt concentration, c_2^t, minus the cmc), dn/dc_2 is the refractive index increment of micelles (0.179 cm^3 g^{-1}) determined at a constant molality, m_3, of the electrolyte, and N_Λ is Avogadro's number. In Figure 1 (upper panel) the ordinate on the right gives the values of the *apparent* aggregation number of micelles, N_a^*, equal to $M_0^{-1}\,(Kc_2/R_{90})^{-1}$, M_0 being the molar mass of the NaTDC monomer. The asterisk superscript signals that the effects of the preferential interactions between the micelles and the solvent components[10,18,25-27] on R_{90} and, consequently, on the measured aggregation numbers are not accounted for (the corrected values are 11% higher; the details are given in Reference 18). The subscript a in N_a^* designates that the effects of the second and possibly higher virial coefficients on R_{90} at the finite values of c_2 are neglected.

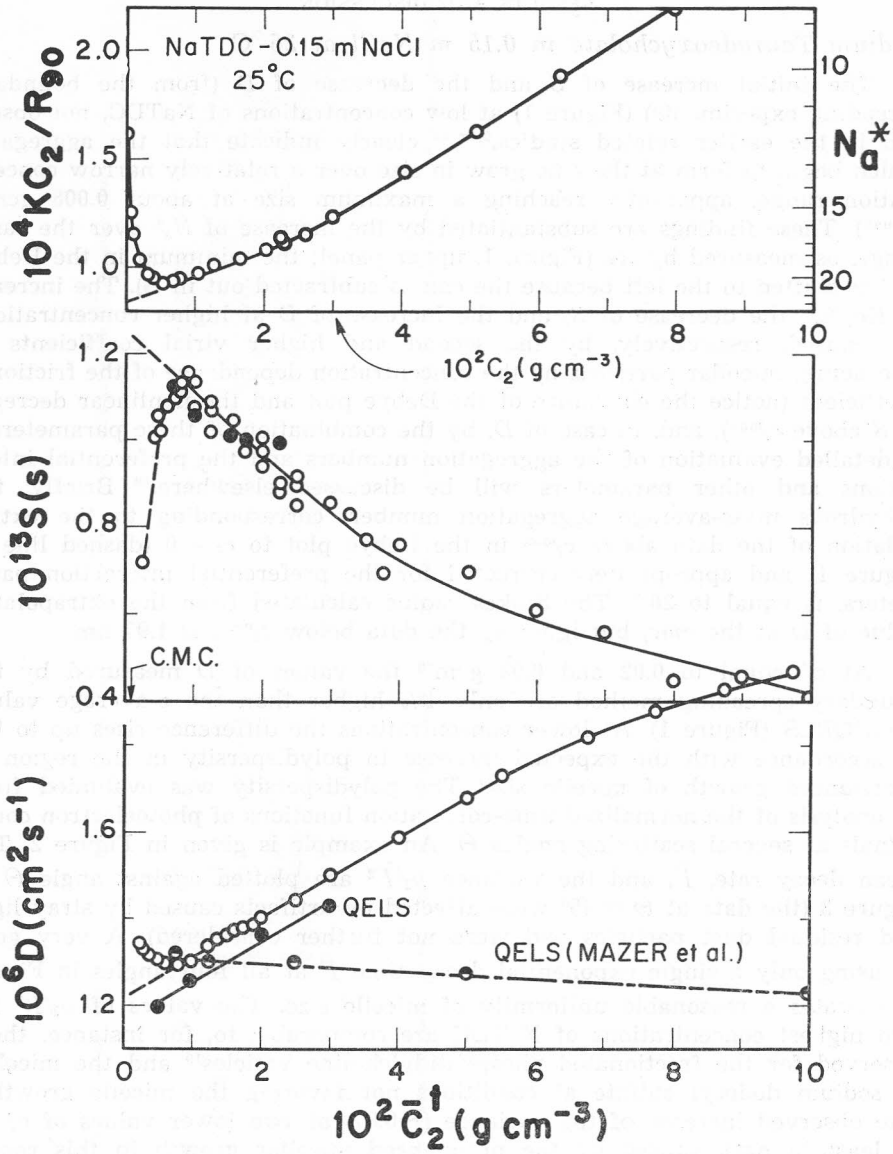

Figure 1. *Lower panel:* Diffusion coefficient, D, measured by the boundary spreading method (open circles) and QELS method (filled circles) versus the concentration of NaTDC. The values of D from QELS are obtained from the average decay rates at the scattering angles other than 45° (see Figures 2 and 3). Also included are QELS results of Mazer et al.[14,15] (half-filled circles) measured at 20 °C and converted to 25 °C disregarding a small effect of temperature on aggregation number. The abscissa scale for QELS data is the total concentration, c_2^t, of NaTDC in solution. For the experiments with the synthetic boundary cell, the mean concentration, $\bar{c}_2^t$, is used on the abscissa.[23] *Middle panel:* Sedimentation coefficient, S, versus $\bar{c}_2^t$ measured in the synthetic boundary cell (open circles) and in the standard cell (filled circles). *Upper panel:* The Debye plot for the intensity of scattered light, *i. e.*, Kc_2/R_{90} versus the micellar concentration, $c_2 = c_2^t$-cmc. The ordinate on the right refers to the apparent aggregation numbers, N_a^* (see the text).

RESULTS AND DISCUSSION

Sodium Taurodeoxycholate in 0.15 m NaCl at 25 °C

The initial increase of S and the decrease of D (from the boundary spreading experiments) (Figure 1) at low concentrations of NaTDC, not observed in the earlier related studies,[9,12-15] clearly indicate that the aggregates which begin to form at the cmc grow in size over a relatively narrow concentration range, apparently reaching a maximum size at about 0.008 gcm^{-3} (c_2^{max}). These findings are substantiated by the increase of N_a^* over the same range, as measured by R_{90} (Figure 1, upper panel; the minimum in the Debye plot is shifted to the left because the cmc is subtracted out in c_2). The increase of Kc_2/R_{90}, the decrease of S, and the increase of D at higher concentrations are caused, respectively, by the second and higher virial coefficients of interacting micellar particles, by the concentration dependence of the frictional coefficient (notice the curvature of the Debye plot and the nonlinear decrease of S above c_2^{max}), and, in case of D, by the combination of these parameters.[28] A detailed evaluation of the aggregation numbers and the preferential interactions and other parameters will be discussed elsewhere.[18] Briefly, the anhydrous mass-average aggregation number, corresponding to the extrapolation of the data above c_2^{max} in the Debye plot to $c_2 = 0$ (dashed line in Figure 1) and appropriately corrected for the preferential interaction parameters, is equal to 26.8. The Stokes radius calculated from the extrapolated value of D at the *cmc*, but ignoring the data below c_2^{max}, is 1.97 nm.

At c_2^t equal to 0.02 and 0.03 gcm^{-3} the values of D measured by the boundary spreading method are only 4% higher than the z-average values from QELS (Figure 1). At lower concentrations the difference rises up to 8% in accordance with the expected increase in polydispersity in the region of pronounced growth of micelle size. The polydispersity was evaluated from an analysis of the normalized time-correlation functions of photoelectron count signals at several scattering angles Θ. An example is given in Figure 2. The mean decay rate, $\bar{\Gamma}$, and the variance $\mu_2/\bar{\Gamma}^2$ are plotted against angle Θ in Figure 3 (the data at $\Theta = 45^0$ were affected by artifacts caused by stray light and residual dust particles and were not further considered). A very good fit using only a single exponential decay time $\bar{\Gamma}$ at all four angles in Figure 2 indicates a reasonable uniformity of micelle size. The values of $\mu_2/\bar{\Gamma}^2$ for two highest concentrations of NaTDC are comparable to, for instance, those observed for the fractionated phosphatidylcholine vesicles[19] and the micelles of sodium dodecyl sulfate at conditions not favoring the micelle growth.[29] The observed increase of the variance ($\sim$0.37) at two lower values of c_2^t is, at least in part, caused by the pronounced micellar growth in this range, although in part it may also be a consequence of stray light and heterodyning of the signal with dust particles.

Our determination of D by two techniques agrees with those of Mazer et al.[14,15] from QELS only at $c_2^t \sim 0.01$ gcm^{-3}. At higher concentrations their results are drastically different from ours (Figure 1, lower panel). At 0.0975 gcm^{-3} we measured $D = 1.97 \times 10^{-6}$ cm^2 sec^{-1} compared to 1.23×10^{-6} cm^2 sec^{-1} of Mazer et al. Positive slopes of $D - c_2^t$ plots for the same system were obtained by Laurent and Persson[9] at 20 °C and by Holzbach et al.[12,13] at 37 °C, although the latter data led to an improbably large Stokes radius of 2.7 nm

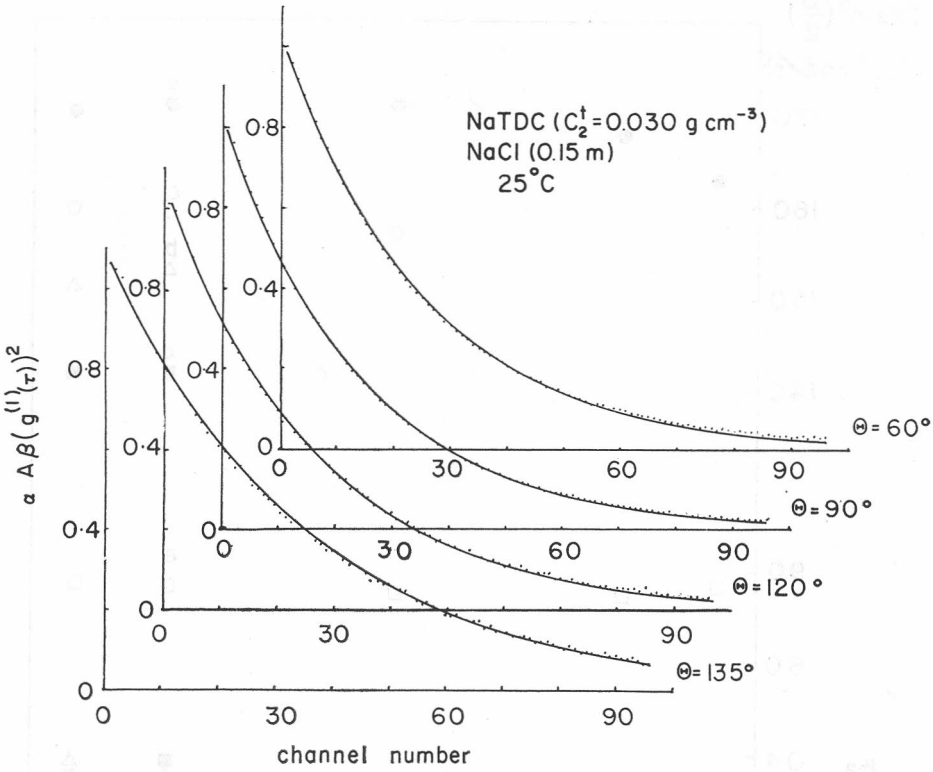

Figure 2. An example of the normalized time-correlation functions of photoelectron count signals at four scattering angles for NaTDC in 0.15 m NaCl. Dots represent the measured values and the solid lines correspond to the least squares fits of single exponentials.

at the cmc. Because of the curvature in Debye plot and the nonlinear dependence of S on c_2^t above c_2^{max}, the quantitative analysis of the slope of $D - c_2^t$ curve, of the kind performed for other systems,[23,28] is not feasible. However, the large slope of the Debye plot and the large drop of S at increasing concentrations strongly indicate that the concentration dependence of D observed by Mazer et al. is unlikely.

According to the theory of bile salt aggregation proposed by Mazer et al., the variance $\mu_2/\bar{\Gamma}^2$ should be zero at the Stokes radius $R_h = 1.5$ nm, corresponding to the size of the so-called primary micelles (Figure 5 in Reference 15). For increasing values of R_h there should be a gradual increase of the variance. Our results for the dependence of $\mu_2/\bar{\Gamma}^2$ on c_2^t and, therefore, on micelle size (Figure 3) do not support this picture.

Sodium Glycodeoxycholate in NaCl Solutions at 25 °C

The values of $\Gamma/\sin^2(\Theta/2)$ for NaGDC in 0.50 m NaCl at $c_2^t = 0.02$ gcm^{-3} are essentially independent of Θ (Figure 3). The derived average value of D is 7.38×10^{-7} cm^2 sec^{-1} which, disregarding the concentration dependence of D, gives $R_h = 3.32$ nm. Although R_h almost doubled compared to that for NaTDC

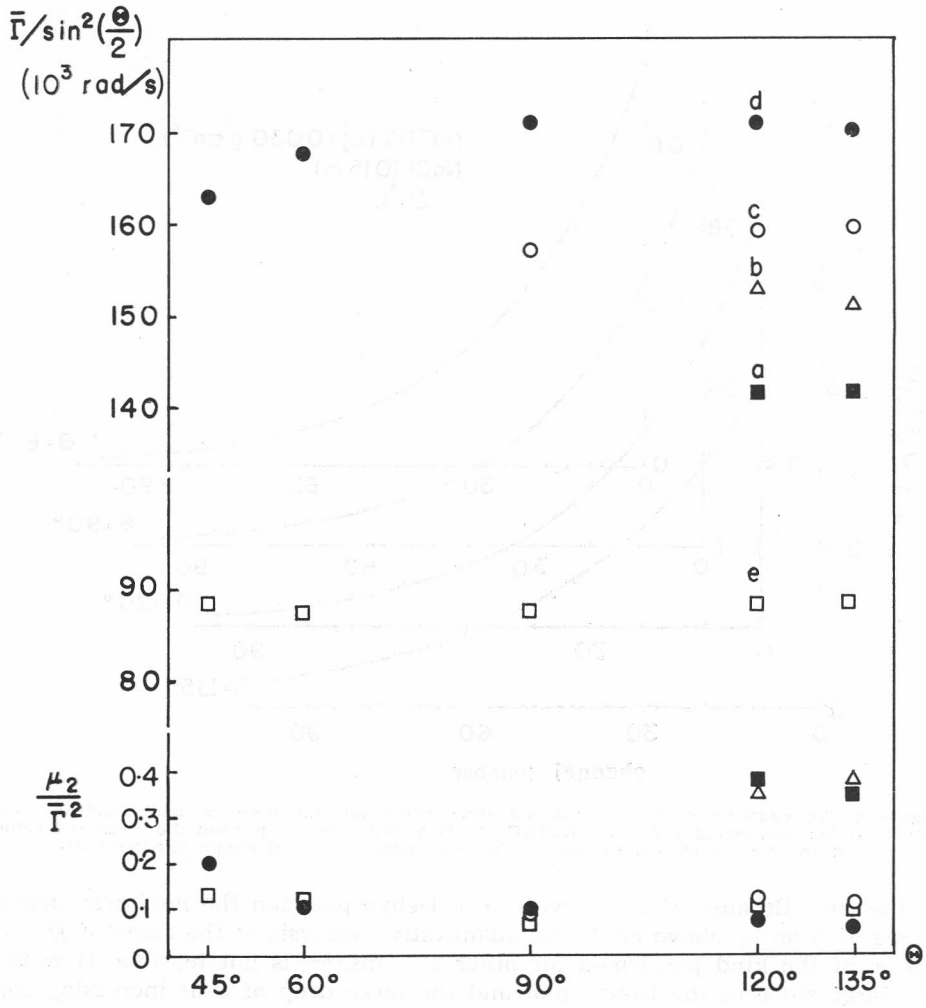

Figure 3. The decay rate, $\bar{\Gamma}$, divided by $\sin^2(\Theta/2)$, and the normalized variance, $\mu_2/\bar{\Gamma}^2$ at various scattering angles, Θ, and concentrations c_2^t of bile salts. NaTDC in 0.15 m NaCl: *a* (filled squares), 0.005; *b* (open triangles), 0.01; *c* (open circles), 0.02; *d* (filled circles), 0.03 gcm^{-3}. NaGDC in 0.5 m NaCl: *e* (open squares), 0.02 gcm^{-3}.

in 0.15 m NaCl, the variance stayed low. The concentration dependence of D (from boundary spreading) for NaGDC in 0.15 m NaCl at 25 °C (Figure 4) is very similar to that observed for NaTDC in Figure 1, with a clearly pronounced minimum at $c_2^t \sim 0.008$ g cm^{-3} and the extrapolated (dashed line) Stokes radius of 1.85 nm. These results contradict those obtained by Holzbach et al.[12,13] from QELS between 0.05 and 0.2 gcm^{-3} at 37 °C, also shown in Figure 4. The same authors reported a substantial increase of D with c_2^t for NaTDC under the same conditions. Since the micellar properties of these two bile salts are very similar[24,30] the values of D for NaGDC reported by Holzbach et al. appear to be in error.

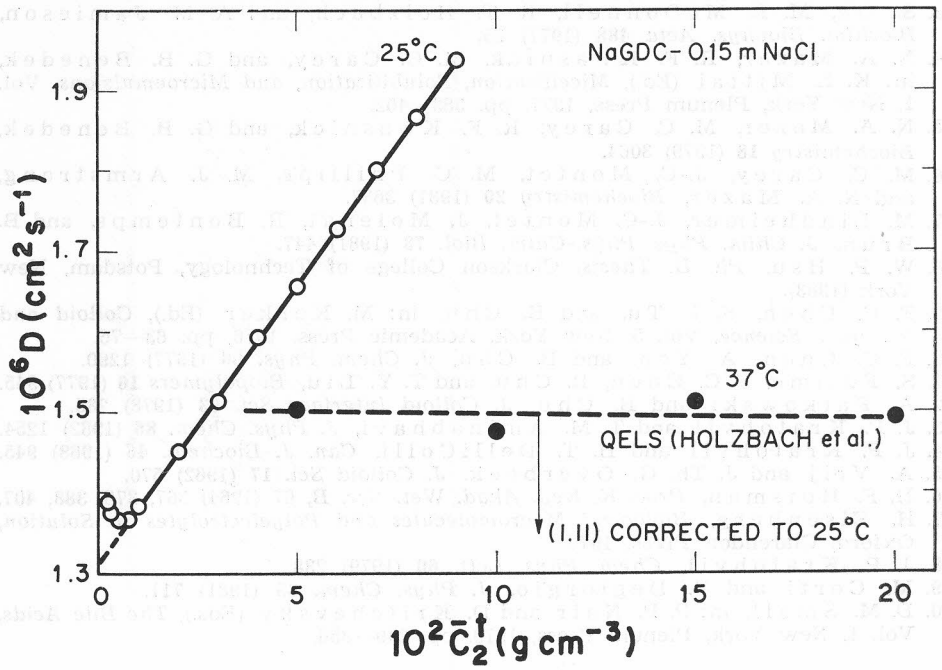

Figure 4. D measured by the boundary spreading method (open circles) at 25 °C for NaGDC in 0.15 m NaCl versus the mean concentration, $\bar{c}_2^t$, in the synthetic boundary cell. Also shown are the results of Holzbach et al.[12,13] (filled circles) from QELS at 37 °C. If corrected to 25 °C disregarding the temperature effect on the micelle aggregation number, the average of the latter data would drop to 1.11×10^{-6} cm^2 s^{-1}.

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SAŽETAK

Hidrodinamička svojstva micela dihidroksižučnih kiselina: Natrijev taurodeoksiholat i natrijev glikodeoksiholat

J. P. Kratohvil, T. M. Aminobhavi, W. P. Hsu, S. Fujime, A. Patkowski, F. C. Chen i B. Chu

Ovisnost međusobnoga translacijskog koeficijenta difuzije o koncentraciji natrijeva taurodeoksiholata u vodenoj otopini natrijeva klorida pri 25 °C upućuje na izrazito povećavanje veličine micela u području koncentracija od kritične micelarne do približno deset puta veće. Ti su podaci potvrđeni varijacijom Rayleighova kvocijenta rasutog svjetla. Pri još većim koncentracijama mjerene veličine određene su hidrodinamičkim i termodinamičkim međudjelovanjem. Kvazielastično raspršenje svjetlosti kao mjerna tehnika dokazuje polidisperznost micela. Podaci za koeficijent difuzije natrijeva glikodeoksiholata pokazuju ovisnost o koncentraciji sličnu kao i za taurinski derivat.