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Investigation of the Interaction between Simple Micelles and Mixed Micelles in Taurocholate−Lecithin Solutions by Laser Light Scattering

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A laser light-scattering technique was employed to investigate simple micelles and mixed micelles thermodynamically coexisting in various taurocholate (TC)−lecithin (L) solutions. When the nonmixed micellar TC (NMTC) concentrations were higher than 25 mM for L = 12 mM and 21 mM, the sizes of simple TC micelles (∼10 Å) and mixed TC−L micelles (∼24 Å) as well as the polydispersity (∼45%) of these micelles were nearly unchanged. At a constant concentration of L, the average size decreased and, more notably, the total scattered intensity also decreased with increasing concentrations of TC. A modified physical equation successfully modeled the scattered intensity versus different concentrations of simple TC micelles and mixed TC−L micelles. Moreover, a strong interaction between simple TC micelles and mixed TC−L micelles was found which appeared to be a process for the transfer of cholesterol molecules in the cholesterol gallstone dissolution.

Introduction

The molecular weights and interaction coefficients of simple taurocholate (TC) micelles and mixed TC−lecithin (L) micelles have been determined recently[1] using laser light-scattering measurements together with a physical model proposed by Flory and Krigbaum.[2] These results not only showed the consistent molecular masses of simple TC micelles as well as mixed TC−L micelles[3−5] but also provided a basis for further investigation of the thermodynamic interaction between simple micelles and mixed micelles.

Because these two kinds of micelles coexist and, moreover, the L concentration is always greater than 12 mM in human bile,[4−7] equilibrium dialysis studies have shown that the TC to L molar ratio (*K*) for mixed TC−L micelles is nearly constant (∼2) if the L concentration is greater than 12 mM and the nonmixed micellar taurocholate (NMTC) concentration is greater than 25 mM.[6,8,9] Furthermore, the fact that the size of simple TC micelles is unchanged with increasing concentrations of TC supports the theory that simple TC micelles are of a single size.[3,4,5,10] Based on a monomer−micelle model, the aggregation number of simple TC micelles has been estimated to be 5 and the critical micellar concentration (cmc) has been estimated to be 10 mM.[1,11,12,13] The estimated value of cmc is very close to the previously determined TC monomer concentrations in TC−L solutions containing simple micelles and mixed micelles.[12]

The previously determined values of *K* and cmc[1,6,8,9] reveal the concentrations (g/dL) of simple TC micelles and mixed TC−L micelles coexisting in the TC−L system. Interestingly, the value of *K* in the TC−L solutions containing mixed TC−L micelles and TC monomers has been determined to be ∼2 also.[9] Moreover, in contrast to the result from Mazer et al.,[4] a previous study showed that the hydrodynamic radius (∼24 Å) of mixed TC−L micelles was unchanged with total lipid concentrations, indicating that mixed TC−L micelles are also all of a single size.[1] Therefore, further investigation of the interaction between simple TC micelles and mixed TC−L micelles using light-scattering measurements of the scattered intensity can be facilitated by employing previous results of the molecular masses and the interaction coefficients of simple TC micelles and mixed TC−L micelles.

Although Mazer et al. measured the sizes and polydispersity of TC−L solutions containing simple TC micelles and mixed TC−L micelles, the total scattered intensity with increasing concentrations of TC, under a constant concentration of L, was not mentioned.[4,5]

In this study, a quantitative investigation of the interaction between simple TC micelles and mixed TC−L micelles was carried out by employing the light-scattering technique to measure the scattered intensity and by modifying a physical model originally proposed by Flory and Krigbaum.[2] A nonlinear least-squares program was used to determine the best fit curve of the scattered intensity and the concentrations of simple TC micelles and mixed TC−L micelles. As will be seen, according to the magnitudes of the resulting parameters from the modified physical model, the thermodynamically stable interaction between simple micelles and mixed micelles in TC−L solutions is successfully estimated.

Experimental Section

Materials. Egg yolk lecithin (L) was purchased from Lipid Products, Surrey, U.K. Its purity was checked with thin layer chromatography (TLC) and found to be greater than 97%.[14] Sodium taurocholate (TC) was purchased from Calbiochem Corporation, San Diego, CA. Its purity (>97%) was also checked by TLC.[15] Other chemicals were analytical grade and were used as received. The toluene used in the light-scattering measurements was obtained from EM Science, Gibbstown, NJ. Distilled deionized water was used to make electrolyte, buffer, and TC−L solutions.

Samples for Light-Scattering Measurements. Acid-cleaned glassware was used for the preparation of solutions for light-

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scattering measurements. The TC−L stock solution was prepared by adding the appropriate amounts of TC and L in 0.01 M phosphate buffer (pH = 7.4) with 0.1 M sodium chloride (NaCI). Then, the TC−L admixtures were sonicated (Bransonic Ultrasonics Co., Danbury, CT) until transparent solutions were obtained. The temperature of the solutions was maintained at 37 °C by using a water bath. A 2 mL aliquot was filtered through a 0.22 μm filter (Millipore, Bedford, MA). After voiding 5 drops, the remaining filtered solution was collected into a light-scattering cuvette for the light-scattering measurements.

Light-Scattering Measurements. The light-scattering apparatus consisted of a model 95 argon ion laser (Lexel Co., Palo Alto, CA), a Brookhaven goniometer (Brookhaven Instruments Corporation, Holtsville, NY) with a temperature-controlled light-scattering cuvette holder with toluene index matching bath, and a Brookhaven multibit correlator/computer (model BI-2030, extendable 72 channel) for autocorrelation and data analysis. Light-scattering measurements were done at a wavelength of 5145 Å and a sample temperature of 37 °C.

Light-scattering measures the fluctuations in the intensity of scattered light and yields information regarding sizes and polydispersity of the particle populations.[16] When the total lipid concentration was lower than 10 g/dL, the total scattered intensity of each TC−L solution was measured and normalized by the scattered intensity from toluene. As described previously,[5,16−18] the measured autocorrelation functions were analyzed by two techniques: (i) analysis of cumulants; and (ii) two-component analysis. The model with the lowest order of cumulants was chosen when the value of the root mean square (rms) was unchanged. From cumulant analysis, the mean diffusion coefficient ($\bar{D}$) and index of polydispersity (V) were obtained. The mean hydrodynamic radius ($\overline{\mathrm{Rh}}$) of each particle population could then be derived from the respective $\bar{D}$ based on the Stokes−Einstein equation

$$\overline{\mathrm{Rh}} = \frac{kT}{6\pi\eta\bar{D}} \tag{1}$$

where k is Boltzmann's constant (1.38×10^{-16} erg/K), T is the absolute temperature (K), and η is the viscosity of the solvent. Moreover, the V was calculated based on the following equation:

$$V\,(\%) = 100\frac{(\overline{D^2} - \bar{D}^2)^{1/2}}{\bar{D}} \tag{2}$$

When a solution contained monodispersed particles only, the V was ~20%.[4] In cases where the V of TC−L micellar solutions was high (40−50%), two-component analysis revealed the hydrodynamic radii (R_1, R_2) and the fractions of the scattered intensity (G_1, G_2) for two coexisting discrete components.[4,5,18] When two-component analysis yielded a lower rms value, the $\overline{\mathrm{Rh}}$ and V values calculated from deduced parameters (R_1, R_2, G_1, G_2) were selected for data analysis. From two-component analysis,[4] the $\overline{\mathrm{Rh}}$ and V values were also calculated based on the following equations:

$$\frac{1}{\overline{\mathrm{Rh}}} = \frac{G_1}{R_1} + \frac{G_2}{R_2} \tag{3}$$

$$V\,(\%) = 100\left\{\frac{[G_1R_2^{\ 2} + G_2R_1^{\ 2} - (R_1G_2 + R_2G_1)^2]^{1/2}}{R_1G_2 + R_2G_1}\right\} \tag{4}$$

Curve Fitting and Parameter Estimation. A least-squares technique was used to minimize the sum of squared deviation between the experimental data and the interaction model presented here. Based on the least-squares minimization, a computational program was designed for curve fitting, parameter estimation, and statistical analysis using MINSQ software (MicroMath, Salt Lake City, UT). In the statistical output, the model selection criterion (MSC) was normalized and, therefore, was independent of the scaling of the data points. From a plot of the data and the best curve fitting, the resulting values of parameters were obtained with an MSC value; the higher the MSC value, the better the curve fitting and the better the resulting values of the parameters.

Results and Discussion

Modified Interaction Model. Based on the statistical mechanics of dilute polymer solutions proposed by Flory and Krigbaum,[2] the scattered intensity at different particle concentrations of light-scattering measurements has the following relation:

$$\frac{HC}{I_{\mathrm{sol}}/I_{\mathrm{tol}}} = \frac{1}{M_{\mathrm{w}}} + 2A'C \tag{5}$$

where H is the light-scattering constant (dL mol/g^2); C is the concentration of particles (g/dL); $I_{\mathrm{sol}}/I_{\mathrm{tol}}$ is the total scattered intensity (turbidity) with respect to toluene; M_{w} is the average molecular mass of particles (daltons); and A' is the total interaction coefficient (dL·mol/g^2). A' has been reported as[2]

$$A' = \left(\frac{J}{N}\right)\left(\frac{1}{M_{\mathrm{w}}}\right)^2 \sum i \sum j\ F(J\zeta_{ij}^{\ 3})w_iM_iw_jM_j \tag{6}$$

where J is a constant; N is Avogadro's number; ζ_{ij} is a coefficient; and w_i (or w_j) is a weight fraction of particles.

If the solution contains two kinds (small and large) of particles with concentrations (g/dL) of C_1 and C_2 and molecular masses of M_1 and M_2, the concentration (C) and molecular mass (M_{w}) of the solution can be derived as

$$C = C_1 + C_2 \tag{7}$$

$$M_{\mathrm{w}} = \frac{C_1M_1 + C_2M_2}{C_1 + C_2} \tag{8}$$

Furthermore, w_1 and w_2 in the solution containing two kinds (small and large) of particles can be simply derived as

$$w_1 = \frac{C_1}{C_1 + C_2} \tag{9}$$

$$w_2 = \frac{C_2}{C_1 + C_2} \tag{10}$$

Substituting eqs 8−10 into eq 6, eq 6 can be expressed as

$$A' = \frac{J(C_1 + C_2)^2}{N(C_1M_1 + C_2M_2)^2}\left\{2F(J\zeta_{12}^{\ 3})\frac{C_1}{C_1 + C_2}M_1\frac{C_2}{C_1 + C_2}M_2 + F(J\zeta_{11}^{\ 3})\left(\frac{C_1}{C_1 + C_2}\right)^2 M_1^{\ 2} + F(J\zeta_{22}^{\ 3})\left(\frac{C_2}{C_1 + C_2}\right)^2 M_2^{\ 2}\right\} \tag{11}$$

To simplify eq 11, the following assumptions were made:

$$A_{11} = \frac{J}{N} F(J\zeta_{11}^{\ 3}) \quad (12)$$

$$A_{22} = \frac{J}{N} F(J\zeta_{22}^{\ 3}) \quad (13)$$

$$A_{12} = \frac{J}{N} F(J\zeta_{12}^{\ 3}) \quad (14)$$

where A_{11} is the interaction coefficient for small particles; A_{22} is the interaction coefficient for large particles; and A_{12} is the interaction coefficient of small particles and large particles coexisting in the solution. Substituting these equations into eq 11, the total interaction coefficient, A', can be expressed as

$$A' = \frac{2A_{12}C_1C_2M_1M_2 + A_{11}C_1^{\ 2}M_1^{\ 2} + A_{22}C_2^{\ 2}M_2^{\ 2}}{(C_1M_1 + C_2M_2)^2} \quad (15)$$

Substituting eq 7, eq 8, and eq 15 into eq 5, the following equation is obtained:

$$I_{\mathrm{sol}}/I_{\mathrm{tol}} = \frac{(H_1C_1M_1 + H_2C_2M_2)(C_1M_1 + C_2M_2)}{C_1M_1 + C_2M_2 + 2A_{11}C_1^{\ 2}M_1^{\ 2} + 2A_{22}C_2^{\ 2}M_2^{\ 2} + 4A_{12}C_1M_1\,C_2M_2} \quad (16)$$

where the light-scattering constants H_1 and H_2 are given by[19-21]

$$H_1 = \frac{4\pi^2 n_{\mathrm{tol}}^{\ 2}(\partial n_1/\partial C)^2}{R_{\mathrm{v,tol}}\lambda^4 N_{\mathrm{A}}} \quad (17)$$

$$H_2 = \frac{4\pi^2 n_{\mathrm{tol}}^{\ 2}(\partial n_2/\partial C)^2}{R_{\mathrm{v,tol}}\lambda^4 N_{\mathrm{A}}} \quad (18)$$

In eqs 17 and 18, $R_{\mathrm{v,tol}}$ is the absolute Rayleigh ratio for toluene (3.2×10^{-5} cm^{-1} for a polarized light source at 90° scattered angle and depolarization ratio = 0.47), λ is the wavelength (5145 Å), N_{A} is Avogadro's number, n_{tol} is the refractive index of toluene (1.494), and $\partial n_1/\partial C$ (and $\partial n_2/\partial C$) are the refractive index increments of the small and large particles, respectively. Based on the refractometer measurements of $\partial n_1/\partial C$ and $\partial n_2/\partial C$, the values of H_1 and H_2 have been estimated to be 2.00×10^{-4} dL·mol/g^2 for simple TC micelles and 1.71×10^{-4} dL·mol/g^2 for mixed TC−L micelles.[1]

Interaction between Simple TC Micelles and Mixed TC−L Micelles. Dialysis studies have quantitatively investigated the coexistence equilibria of simple micelles and mixed micelles in the TC−L system.[6,8,9] The results indicated that, when the nonmixed micellar taurocholate (NMTC) concentration is greater than 25 mM and the L concentration is greater than 12 mM, mixed micelles are stable in a solution with a nearly constant K value of about 2. Physiologically, mixed TC−L micelles are thermodynamically stable because the L concentration is greater than 12 mM and NMTC is also greater than 25 mM.[4-7] Therefore, in the present study, the mixed micelle concentration (C_2) was estimated, based on a K value of 2, for systems where (a) the NMTC concentration was greater than 25 mM in all cases and (b) the total L concentration employed was 12 or 21 mM. The value of K (=2) from equilibrium dialysis studies was indirectly confirmed by light-scattering measurement of micellar sizes. Table 1 includes the light-scattering results that indicate the presence of constant-sized simple micelles (R_1 = 9−10 Å) and results that indicate the presence of constant-sized mixed micelles (R_2 = 23−25 Å). Although polydispersity values were slightly low (∼45%) in the two-component analysis, the results were consistent with previous light-scattering studies.[1] Moreover, the average particle size and the polydispersity from cumulant analysis were consistent with those calculated from eqs 3 and 4 (within ±14% error), as shown in Table 1. This consistency further validates the results from the two-component analysis. In addition, according to their hydrodynamic radii, the molecular masses of simple TC micelles and mixed TC−L micelles can be roughly estimated to be 2500 and 35 000 Da, given the density of 1 g/cm^3 and assuming that they are spherical. These estimated values are consistent with the previously determined molecular masses of simple TC micelles and mixed TC−L micelles obtained using Flory's theory.[1] Therefore, the present light-scattering measurements are reliable.

Similar to the estimation of mixed micelle concentration described above, simple micelle concentration (C_1) was further estimated based on the value of the aggregation number (∼5) and, more importantly, the concentration of TC monomers (∼10 mM) reported previously.[1,12] The results of estimation for C_1 and C_2 are summarized in Table 1 for later analysis.

From Table 1, it is noteworthy that the percentage (G_1) of intensity from simple micelles increased because the simple micelle concentration increased and mixed micelle concentration was unchanged with increasing TC concentrations. Then, when the total concentrations of micelles increased, the total scattered intensity from both simple micelles and mixed micelles was supposed to increase. However, Table 1 shows that the total intensity decreased with increasing TC concentrations. Therefore, further investigation of the interaction between simple micelles and mixed micelles is necessary.

Using eq 16 and the estimated values of H_1 (2.00×10^{-4} dL·mol/g^2), H_2 (1.71×10^{-4} dL·mol/g^2), M_1 (2138 daltons), M_2 (35 840 Da), A_{11} (1.30×10^{-5} dL·mol/g^2), and A_{22} (5.09×10^{-6} dL·mol/g^2) determined previously,[1,4,5] the interaction between simple micelles and mixed micelles was estimated from the best curve fitting by MINSQ. A_{12} was determined to be 8.28×10^{-5} dL·mol/g^2 with an MSC of 3.49. In this case, it is difficult to compare values from the best curve fitting with experimental data in a two-dimensional plot because of the two independent variables (C_1 and C_2). Nevertheless, the C_2 values used in this case were 2.23 g/dL (i.e., L = 12 mM) and 3.90 g/dL (i.e., L = 21 mM) only. To simplify this result, a constant value of C_2 (2.23 g/dL for Figure la and 3.90 g/dL for Figure 1b) was maintained when plotting the intensity versus C_1. Figure 1 shows that although there is a slight deviation due to a low MSC value, the curve fitting is quite satisfactory.

Because of the two values of C_2 (2.23 g/dL and 3.90 g/dL), another approach to determine the value of A_{12} was to measure the scattered intensity at different values of C_1; that is, C_2 was maintained constant as either 2.23 or 3.90 g/dL. When C_2 was equal to 2.23 g/dL, the value of A_{12} was estimated to be 7.52×10^{-5} dL·mol/g^2, as shown in Table 2, with a higher value of MSC (5.79). Similarly, the estimated value of A_{12} was 9.05×10^{-5} dL·mol/g^2 at a C_2 of 3.90 g/dL with an even higher MSC (6.22). Figure 2 shows better curve fittings compared to Figure 1. Interestingly, the average value of A_{12} (7.52×10^{-5} dL·mol/g^2) from two different curve fittings was exactly the same as the value of A_{12} obtained with two independent variables. The error of A_{12} due to different curve fittings was within a range of 10%.

Estimation of Molecular Masses for Coexisting Micelles. The value of A_{12} obtained was based on eq 16, scattered intensity

TABLE 1: Light-Scattering Results, Simple Micelle Concentrations, and Mixed Micelle Concentrations in the TC−L System

TC−L concentration (mM)	$\overline{\mathrm{Rh}}$ (Å)	$\overline{\mathrm{Rh}}^a$ (Å)	V (%)	V^a (%)	R_1 (Å)	R_2 (Å)	G_1 (%)	G_2 (%)	I_{sol}/I_{tol}	C_1 (g/dL)	C_2 (g/dL)
60−12	19.7	19.6	47.9	41.6	9.0	23.0	11	89	5.37	1.39	2.23
90−12	15.9	17.1	48.4	48.0	9.0	23.0	22	78	4.18	3.00	2.23
110−12	14.5	14.7	44.9	47.9	9.0	23.0	36	64	3.75	4.08	2.23
118−12	13.8	14.2	45.6	46.1	9.0	22.5	39	61	3.65	4.51	2.23
128−12	13.0	13.5	46.8	45.5	9.0	23.0	45	55	3.41	5.05	2.23
70−21	19.5	19.7	43.8	43.7	10.0	24.5	17	83	7.72	0.95	3.90
90−21	17.7	18.0	47.7	46.1	10.0	24.5	25	75	6.39	2.03	3.90
112−21	16.2	16.4	43.6	46.0	10.0	24.5	34	66	5.31	3.21	3.90
133−21	14.3	14.4	45.8	42.7	10.0	24.5	48	52	4.70	4.34	3.90

[a] Indicates the values obtained from two-component analysis.

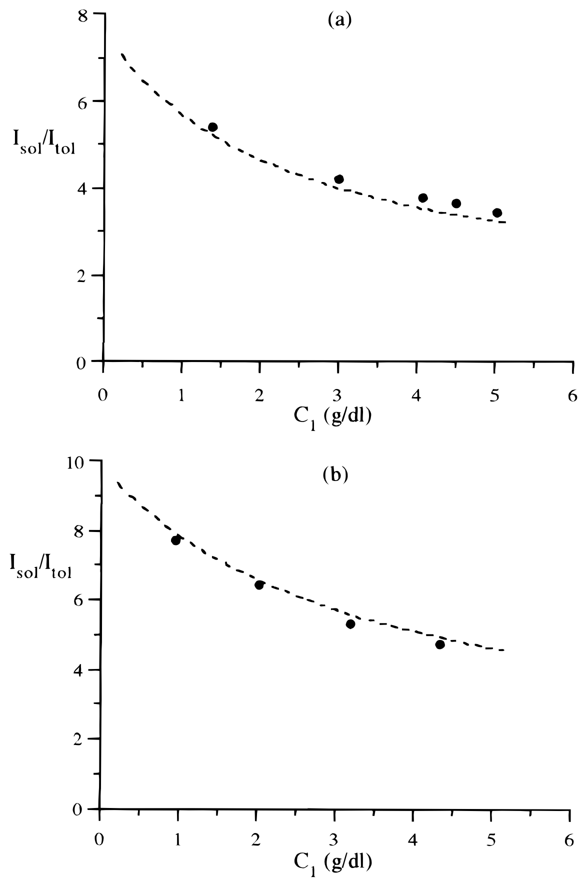

Figure 1. Scattered intensity with respect to toluene (I_{sol}/I_{tol}) vs simple micelle concentration (C_1) based on eq 16 for $A_{12} = 8.28 \times 10^{-5}$ dL·mol/g^2, $M_1 = 2138$ Da, and $M_2 = 35\,840$ Da. (a) The resulting plot when $C_2 = 2.23$ g/dL. (b) The plot when $C_2 = 3.90$ g/dL. The dotted line indicates the prediction from the best curve fitting of a parameter A_{12} with an MSC value of 3.49. (●) Experimental data.

by light-scattering measurements, and values of H_1, H_2, M_1, and M_2. By employing a refractometer, values of H_1 and H_2 as estimated in a previous study[1] were used in the present study. However, previous estimations[1,3−5] of the values of M_1 and M_2 were made with solutions that contained only one kind of particle (simple micelles or mixed micelles), and these values had to be reestimated for the present study since, in the coexistence region, the values of M_1 and M_2 may be different. From eq 16, three independent variables of M_1, M_2, and A_{12} were assumed for the best curve fitting. For simplicity, C_2 was maintained constant (2.23 or 3.90 g/dL). When C_2 was 2.23 g/dL, the resulting values of M_1, M_2, and A_{12} were 2574 Da, 36 386 Da, and 6.74×10^{-5} dL·mol/g^2 and the MSC value was 5.14, as shown in Table 2. Further, the estimated values of

TABLE 2: Interaction Coefficients and Molecular Masses of Simple Micelles and Mixed Micelles, and the Value of MSC As Determined by a Least-Squares Technique under Different Sets of Data and Curve-Fitting Parameters in the TC−L System

condition	A_{12} (dL·mol/g^2)	M_1 (Da)	M_2 (Da)	MSC
$C_2 = 2.23$ and 3.90 g/dL, parameter A_{12}	8.28×10^{-5}	2138[a]	35 840[a]	3.49
$C_2 = 223$ g/dL, parameter A_{12}	7.52×10^{-5}	2138	35 840[a]	5.79
$C_2 = 3.90$ g/dL, parameter A_{12}	9.05×10^{-5}	2138	35 840[a]	6.22
$C_2 = 2.23$ g/dL, parameters A_{12}, M_1, M_2	6.74×10^{-5}	2574	36 386	5.14
$C_2 = 3.90$ g/dL, parameters A_{12}, M_1, M_2	7.23×10^{-5}	2830	35 926	5.15

[a] Indicates the molecular mass estimated previously by Liu.[1]

M_1, M_2, and A_{12} were 2830 Da, 35 926 Da, and 7.23×10^{-5} dL·mol/g^2 and the MSC value was 5.15 when C_2 was 3.90 g/dL. Figure 3 shows good curve fittings because of the high values of MSC obtained. Table 2 shows that the values of M_1 and M_2 obtained from different curve fittings were consistent. Moreover, the resulting values of M_1 and M_2 were nearly the same as those reported previously.[3−5] This correspondence further validates the present results. Although the resulting A_{12} values were slightly different under different conditions for curve fittings, Tables 2 and 3 clearly show that the magnitude of A_{12} was much higher than that of both A_{11} and A_{22}.

Because the value of the form factor is close to 1 for spherical micelles based on the Guinier equation, the scattered intensity has been suggested to be proportional to molecular mass and concentration if there is no interaction between the spherical micelles.[4,5,18] This suggestion is consistent with the interaction model represented in eq 5. In contrast, because of the strong interaction seen in this study, the scattered intensity decreased with increasing concentration of TC, as shown in Table 1. Hence, the previous technique[4,5,18] cannot be used for the estimation of molecular mass and concentration of micelles when the interaction is too strong. Rather, the original equation (eq 5) or the modified equation (eq 16) should be used when estimating the molecular mass and interaction of particles.

Analysis of Interaction Coefficients. In comparing the interaction coefficients of A_{11}, A_{22}, and A_{12} in Table 3, the large A_{12} value that indicates a strong interaction between simple micelles and mixed micelles is of particular interest. Moreover, the value of A_{11} is about 2 times larger than that of A_{22} because a hydrophobic interaction, in addition to the hydrogen bonding, between simple micelles has been suggested.[3,11]

The TC molecule contains three hydroxyl groups as compared to bile salts with two hydroxyl groups; simple TC micelles, therefore, possess more hydroxyl groups.[3,11] However, due to

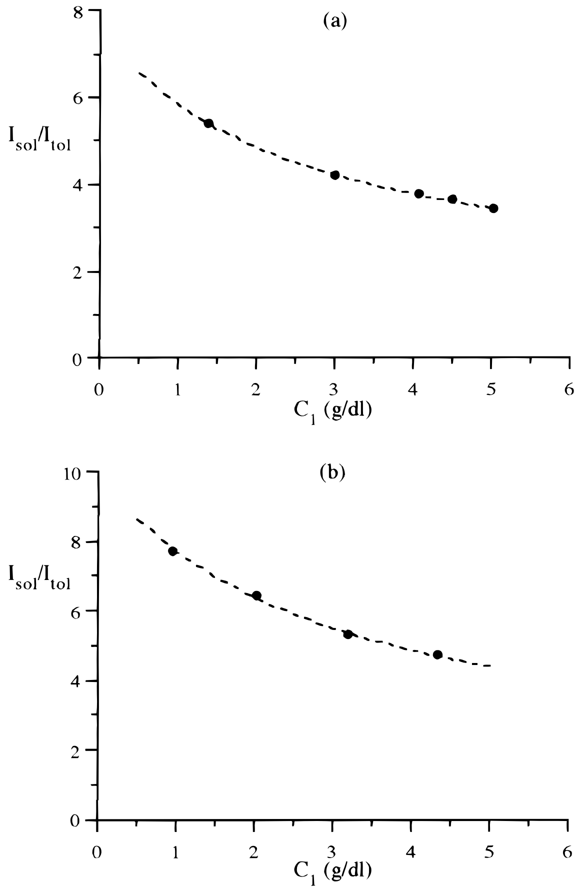

Figure 2. Scattered intensity with respect to toluene (I_{sol}/I_{tol}) vs simple micelle concentration (C_1) based on eq 16 for (a) $A_{12} = 7.52 \times 10^{-5}$ dL·mol/g^2, $M_1 = 2138$ Da, $M_2 = 35\,840$ Da, and $C_2 = 2.23$ g/dL and (b) $A_{12} = 9.05 \times 10^{-5}$ dL·mol/g^2, $M_1 = 2138$ Da, $M_2 = 35\,840$ Da, and $C_2 = 3.90$ g/dL. The dotted line indicates the prediction from the best curve fitting of a parameter A_{12} with MSC values of (a) 5.79 and (b) 6.22. (●) Experimental data.

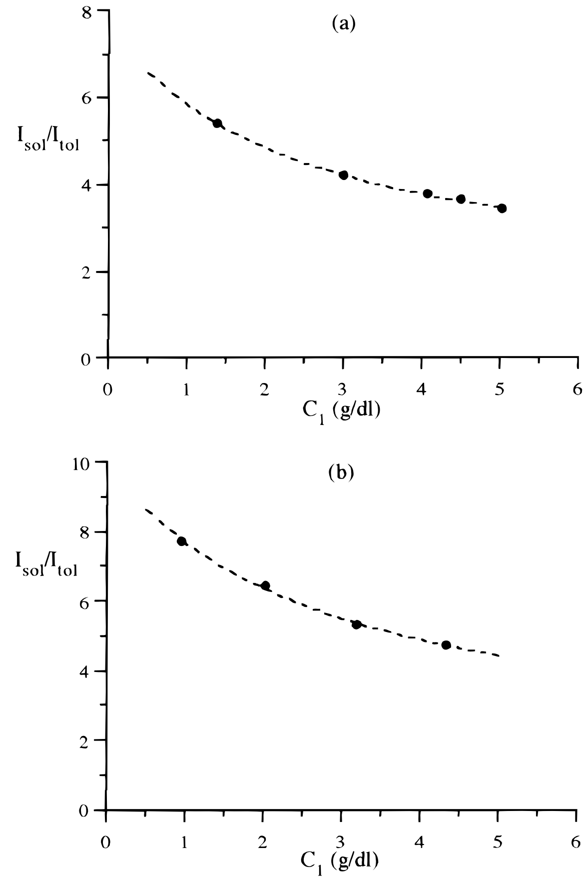

Figure 3. Scattered intensity with respect to toluene (I_{sol}/I_{tol}) vs simple micelle concentration (C_1) based on eq 16 for (a) $A_{12} = 6.74 \times 10^{-5}$ dL·mol/g^2, $M_1 = 2574$ Da, $M_2 = 36\,386$ Da, and $C_2 = 2.23$ g/dL and (b) $A_{12} = 7.23 \times 10^{-5}$ dL·mol/g^2, $M_1 = 2830$ Da, $M_2 = 35\,926$ Da, and $C_2 = 3.90$ g/dL. The dotted line indicates the prediction from the best curve fitting of parameters A_{12}, M_1, and M_2 with MSC values of (a) 5.14 and (b) 5.15. (●) Experimental data.

TABLE 3: Interaction Coefficients for Simple Micelles, Mixed Micelles, and Simple and Mixed Micelles in TC and TC−L Systems

	A_{11} (dL·mol/g^2)	A_{22} (dL·mol/g^2)	A_{12} (dL·mol/g^2)
simple micelles	1.30×10^{-5} [a]		
mixed micelles		5.09×10^{-6} [a]	
simple and mixed micelles			8.28×10^{-5} [b]

[a] Indicates the value estimated previously by Liu.[1] [b] Indicates the value obtained in the present study under the condition of one parameter A_{12} and $C_2 = 2.23$ g/dL and 3.90 g/dL for the curve fitting.

the taurine group in TC, the hydrogen bonding between simple TC micelles was not significantly favored. A similar explanation also applies to mixed TC−L micelles, although the structure of mixed TC−L micelles is somewhat controversial.[4,23] Nevertheless, the small and globular simple TC micelles easily entered the region of the hydroxyl groups in mixed micelles and formed hydrogen bonds;[3,11] a significantly large value of A_{12} was found. The strong interaction was reasonably interpreted by the decrease of the scattered intensity with increasing concentration of TC when the L concentration was unchanged.

Previous studies have indicated that simple TC micelles play an important role in the kinetics of cholesterol gallstone dissolution, although mixed TC−L micelles make a greater contribution to cholesterol solubilization.[6,22] Therefore, it is believed that a transfer of solubilized cholesterol molecules from simple TC micelles to mixed TC−L micelles must take place during the dissolution process before the equilibrium solubilization of cholesterol is reached. Due to the strong interaction between simple TC micelles and mixed TC−L micelles, the transfer process is very likely to occur during the interaction. Nevertheless, further investigations need to be carried out to validate the present interpretation.

Conclusions

A strong interaction between simple TC micelles and mixed TC−L micelles was found in the present study. This important finding provides not only a better way to estimate the molecular mass of micelles but also more information about how micellar lipid interactions should be interpreted. Because conjugated cholate is the only physiological bile salt with three hydroxyl groups, the present result provides a basis for further investigation of the interactions of different simple bile salt micelles, mixed bile salt−L micelles, and coexisting simple micelles and mixed micelles, which may lead to a better understanding of the interaction in physiological bile.

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