

Interactions and Molecular Weights of Simple Micelles and Mixed Micelles in Taurocholate and Taurocholate–Lecithin Solutions

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A quasielastic light-scattering (QLS) technique was employed to investigate simple micelles and mixed micelles formed in taurocholate (TC) and TC–lecithin (L) solutions. When the size and polydispersity of simple TC micelles were nearly unchanged with total TC concentrations, the simple TC micelles were suggested to be all of a single size. According to the relationship between the total TC concentration and the TC monomer concentration, a monomer–micelle model predicted the aggregation number (n) and the critical micellar concentration (cmc) of simple TC micelles to be 5 and 10 mM. Based on Flory's theory, the scattered intensity, the TC monomer concentration, the molecular weights of simple TC micelles, and mixed TC–L micelles were estimated to be 2138 Da and 35 840 Da. Moreover, the interaction coefficients of simple TC micelles and mixed TC–L micelles were found to be 1.30×10^{-5} dL mol g⁻² and 5.09×10^{-6} dL mol g⁻². According to Flory's equation, the estimated interaction coefficients and the concentrations of simple TC micelles and mixed TC–L micelles resulted in different values of the proportionality constant. When the proportionality constant values were assumed to be unchanged, significant errors in the determined molecular weights of simple TC micelles and mixed TC–L micelles occurred.

Introduction

The most abundant lipid in bile, bile salt (BS), possesses not only the self-association property by forming simple BS micelles^{1–3} but a remarkable ability to solubilize insoluble lecithin (L) bilayers by forming mixed BS–L micelles.^{4–6} The fact that mixed BS–L micelles are in equilibrium with simple BS micelles and BS monomers in physiological bile provides a basis for physicochemical investigations.^{7–10}

Previously, an equilibrium dialysis method was employed to study the coexistence between simple micelles and mixed micelles.^{7–9} The outcome showed that the taurocholate (TC):L molar ratio (K) for mixed micelles is nearly constant (~ 2) when (a) the total L concentration is greater than 12 mM and the nonmixed micellar bile salt (NMBS) concentration is greater than 20 mM and (b) the NMBS concentration is around 10 mM. Moreover, the TC monomer concentration required for the formation of simple TC micelles and mixed TC–L micelles has been determined by employing the Sephadex G-10 beads uptake method.¹⁰ Based on the results of equilibrium dialysis and application of the Sephadex G-10 bead method, the concentration (g/dL) of simple TC micelles and mixed TC–L micelles could be estimated for quasielastic light-scattering (QLS) investigation.

QLS measurements have determined the mean hydrodynamic radius (R_h) and the polydispersity (V) of simple micelles in a TC system as well as mixed micelles in a TC–L system.^{3,11} In addition, when the molecular weight of simple BS micelles was estimated by measurements of scattered intensity with increasing concentrations (g/dL) of simple BS micelles according to Flory's model, the second virial coefficient represented an interaction between simple BS micelles.¹² However, the values of the molecular weights and second virial coefficient were dependent on the model of micellar formation.¹²

Although the self-association of BS has been suggested to occur in a stepwise fashion,¹³ it is very likely that the monomer–

micelle model was based on the fact that the particle size is unchanged with increasing concentrations of total lipid.^{3,11,14} Nevertheless, Chang and Cardinal did not show a quantitative agreement between the light-scattering data and the monomer–micelle model in BS solutions.¹² Therefore, from a physicochemical standpoint, further investigation of molecular weights and interactions of different micellar aggregates formed in BS and BS–L solutions is necessary.

The present study was performed to model the previously determined relationship between total TC concentrations and TC monomer concentrations¹⁰ and to determine the aggregation number and the critical micellar concentration required for the formation of simple TC micelles. QLS measurements were carried out to measure and analyze the size, polydispersity, and average scattered intensity of different TC and TC–L solutions. The QLS results were compared with those found previously.^{7–9} As will be seen, based on Flory's theory¹⁵ and results of QLS measurements and the monomer–micelle model,^{7–11} the thermodynamically stable interactions and molecular weights for simple micelles and mixed micelles in TC and TC–L solutions have been successfully estimated.

Experimental Section

Materials. Sodium taurocholate (TC) was purchased from Calbiochem Corp., San Diego, CA, and its purity ($>97\%$) was checked by thin layer chromatography (TLC).¹⁶ Egg yolk lecithin (L) was purchased from Lipid Products (Surrey, U.K.). Its purity was also checked by TLC.¹⁷ Other chemicals were analytical grade and were used as received. The toluene used in the QLS measurements was obtained from EM Science (Gibbstown, NJ). Distilled deionized water was used to make electrolyte, buffer, TC, and TC–L solutions.

Samples for QLS Measurements. All solutions for QLS measurements were prepared with acid-cleaned glassware. The TC and TC–L stock solutions were prepared by adding the appropriate amounts of TC and L to 0.01 M phosphate buffer (pH = 7.4) with 0.1 M sodium chloride (NaCl). Then the TC and TC–L admixtures were sonicated (Bransonic Ultrasonics

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

TC concn (mM)	Rh (Å)	V (%)	$I_{\text{sol}}/I_{\text{tol}}$ (g/dL)	C_1 (mM)	TC monomer
30	11.0	37.5	0.51	1.16	8.5
60	10.5	39.3	0.98	2.69	10.0
90	9.7	35.6	1.47	4.25	11.0
120	10.2	35.9	1.89	5.82	11.7

Co., Danbury, CT) until transparent solutions were obtained. The temperature of the solutions was maintained at 37 °C. Prior to measurements, an aliquot (~2 mL) was taken and filtered through a 0.22 μm filter (Millipore, Bedford, MA). After ~5 drops, the remaining filtered solution was collected into a light-scattering cuvette for QLS measurements.

QLS Measurements. The light-scattering apparatus consisted of a Model 95 argon ion laser (Lexel Co., Palo Alto, CA), a Brookhaven goniometer (Brookhaven Instruments, Holtsville, NY) with a temperature-controlled light-scattering cuvette holder with toluene index-matching bath, and a Brookhaven multibit correlator (Model BI-2030, extendable 72-channel) for data analysis. In the present study, light-scattering measurements were done at a wavelength of 514.5 nm and sample temperature of 37 °C.

QLS measures the fluctuations of the intensity of scattered light and reveals information about sizes and polydispersities of the particle populations.¹⁸ As described previously,^{18–21} the measured autocorrelation functions were analyzed by a technique of cumulant analysis.^{19–21} From cumulant analysis, the mean diffusion coefficient (\bar{D}) and an index of polydispersity (V) were obtained. In the present study, the model with the lowest order of cumulants was chosen when the value of the root-mean-square (rms) was unchanged. The mean hydrodynamic radius (R_h) of the particle populations was derived from \bar{D} based on the Stokes–Einstein equation

$$R_h = kT/(6\pi\eta\bar{D}) \quad (1)$$

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. The total scattered intensity from TC and TC–L solutions was measured and normalized by the scattered intensity from toluene.

Refractive Index Measurements. Refractive index measurements were carried out in a Model RF-600 differential refractometer (C. N. Wood Co., Newtown, PA) with the temperature maintained at 37 °C. Prior to the measurements, the calibration constant was estimated to be 1.005×10^{-3} at a wavelength of 546 nm.

Results and Discussion

Monomer–Micelle Model. Recently, Funasaki et al.¹³ proposed a stepwise model for the formation of simple TC micelles by employing the Sephadex G-10 uptake method. Interestingly, results showed that one equilibrium constant (k_s) was much higher than the others, indicating that only one type of particle was dominant in the solution.

A theory proposed by Nagarajan¹⁴ shows that when the size of simple bile salt micelles cannot increase significantly with increasing total bile salt concentrations, the simple bile salt micelles are all of a single size. As can be seen in Table 1, the size of simple TC micelles was nearly constant with increasing concentrations of total TC. Mazer et al. also found the same result.^{3,11} According to the theory of Nagarajan and the fact of nearly constant size of simple TC micelles, a monomer–micelle model is confirmed.^{22,23} Basically, the monomer–

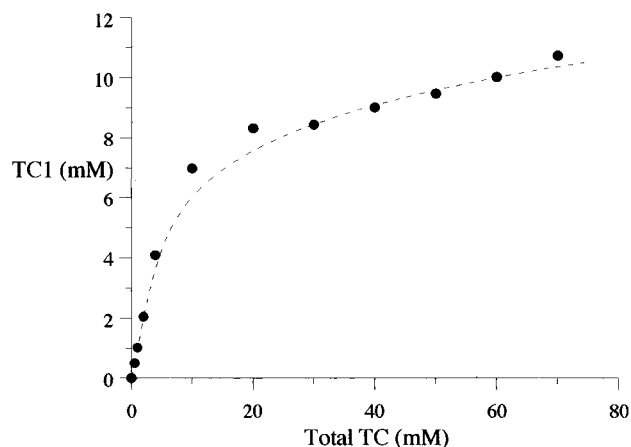


Figure 1. TC monomer concentration vs total TC concentration in the TC system. Dotted line indicates the prediction based on eq 5, aggregation number = 5, and cmc = 10 mM. ● indicates the experimental data determined previously.¹⁰

micelle model can be expressed as



where [TC1] is the TC monomer concentration, [TCn] is the concentration of simple TC micelles, n is the aggregation number of simple TC micelles, and K is the equilibrium constant. The equilibrium constant (K) can be expressed as

$$K = \frac{[\text{TCn}]}{[\text{TC1}]^n} \quad (3)$$

According to a theory proposed previously,^{22,23} the equilibrium constant can also be expressed as

$$K = \text{cmc}^{-n+1} \quad (4)$$

where cmc is the critical micellar concentration.

According to the equilibrium model and the law of mass balance, the total TC concentration ([Total TC]) can be expressed as

$$[\text{Total TC}] = n[\text{TCn}] + [\text{TC1}] = n\{[\text{TC1}]/\text{cmc}\}^n \text{cmc} + [\text{TC1}] \quad (5)$$

Based on eq 5 and the relationship between [Total TC] and [TC1] determined by Lee et al.,¹⁰ the best values of n and cmc were 4.8 and 10.1 mM from a nonlinear least-squares curve-fitting program, SCIENTIST (MicroMath, Salt Lake City, UT). Interestingly, the resulting values are within an acceptable range of n (3–7) and cmc (4–14 mM) reported previously when different techniques were used.^{24,29} Nevertheless, from a physicochemical standpoint, n should be a number close to the value (4.8) of n from the curve-fitting program. Moreover, the resulting value of cmc (10.1 mM) was within ± 0.1 mM error. For modeling purposes, values of n and cmc used in the present study were 5 and 10 mM.

Figure 1 shows that although there was a slight deviation at total TC concentrations between 10 and 20 mM, a good agreement between experimental data by Lee et al. and the results from eq 5 was obtained. The TC monomer concentrations at different concentrations of the total TC were also estimated by this model as shown in Table 1. It is clear that TC monomer concentrations were very close to cmc. Experimental results from Lee et al.¹⁰ also support this finding. Therefore, the TC monomer concentration in the TC–L system was assumed to be equal to the value of cmc (10 mM).

Flory's Model. According to the statistical mechanics of dilute polymer solutions proposed by Flory and Krigbaum,¹⁵ the interaction and molecular weight of particles can be estimated from light-scattering measurements as

$$\frac{HC}{\tau} = \frac{1}{Mw} + 2A'C \quad (6)$$

where H is the light-scattering constant (dL mol g^{-2}), C is the average concentration of particles (g dL^{-1}), τ is the total turbidity (scattered intensity with respect to toluene), Mw is the average molecular weight of particles (Da), and A' is the total interaction coefficient (dL mol g^{-2}).

If the solution contains small particles ($C = C_1$) or large particles ($C = C_2$) only, eq 6 can be rearranged and expressed in two different forms

$$\tau = \frac{H_1 C_1 M_1}{1 + 2A_{11} C_1 M_1} \quad (7)$$

$$\tau = \frac{H_2 C_2 M_2}{1 + 2A_{22} C_2 M_2} \quad (8)$$

where A_{11} and A_{22} represent the coefficients of interaction for small and large particles and M_1 and M_2 are molecular weights (Da) for small and large particles. When the total scattered intensity of the solution is normalized by the scattered intensity from toluene, the light-scattering constants H_1 and H_2 are given by^{22,25,26}

$$H_1 = \frac{4\pi^2 n_{\text{tol}}^2 (\partial n_1 / \partial C_1)^2}{R_{v,\text{tol}} \lambda^4 N_A} \quad (9)$$

$$H_2 = \frac{4\pi^2 n_{\text{tol}}^2 (\partial n_2 / \partial C_2)^2}{R_{v,\text{tol}} \lambda^4 N_A} \quad (10)$$

In eqs 9 and 10, λ is the wavelength (5145 Å), $R_{v,\text{tol}}$ is the absolute Rayleigh ratio for toluene ($3.2 \times 10^{-5} \text{ cm}^{-1}$ at $\lambda = 5145 \text{ Å}$, 90° scattered angle, and depolarization ratio = 0.47), N_A is Avogadro's number, n_{tol} is the refractive index of toluene (1.494), and $\partial n_1 / \partial C_1$ (or $\partial n_2 / \partial C_2$) is the refractive index increment of the small or large particles. Based on refractometer measurements, the values of $\partial n_1 / \partial C_1$ and $\partial n_2 / \partial C_2$ were estimated to be $0.175 \text{ cm}^3/\text{g}$ for simple TC micelles and $0.162 \text{ cm}^3/\text{g}$ for mixed TC-L micelles. The calculation results from eqs 9 and 10 showed that H_1 was equal to $2.00 \times 10^{-4} \text{ dL mol g}^{-2}$ and H_2 was equal to $1.71 \times 10^{-4} \text{ dL mol g}^{-2}$.

Interaction and Molecular Weight of Simple TC Micelles. Total scattered intensity with respect to toluene ($I_{\text{sol}}/I_{\text{tol}}$) at different concentrations of TC was measured by QLS. From literature data of TC monomer concentrations,¹⁰ the simple micelle concentration (g/dL), C_1 , was estimated by (a) subtracting TC monomer concentration (mM) from the total TC concentration (mM), (b) making a product of the subtracted TC concentration (mM) and molecular weight of TC, and (c) dividing the product by 10 000. These results are summarized in Table 1.

Interestingly, when the total TC concentration was below 10 mM, the scattered intensity (not shown) was too small (<0.05) for data analysis because TC monomers were dominant in the solution. This result further supports the monomer-micelle model. The slightly higher values of polydispersity (35–40%) shown in Table 1 were due to the TC monomers coexisting with simple TC micelles.

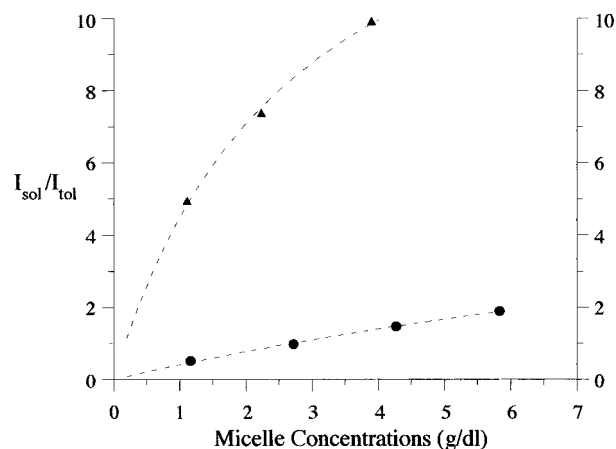


Figure 2. Scattered intensity with respect to toluene ($I_{\text{sol}}/I_{\text{tol}}$) vs micelle concentrations. ● indicates the experimental data of simple TC micelles (C_1). ▲ indicates the experimental data of mixed TC-L micelles (C_2). Dotted lines indicate the prediction from the best curve fittings.

By use of eq 7, data from Table 1, and the SCIENTIST nonlinear curve-fitting program, an excellent fit was obtained with a high value (4.90) of model selection criteria (MSC) under a 95% confidence level (see Figure 2). The resulting values for A_{11} and M_1 were $1.30 \times 10^{-5} \text{ dL mol g}^{-2}$ and 2138 Da (i.e., aggregation number 4–5). It is interesting to note that the M_1 value obtained from the best curve fitting is consistent with the values reported in the literature.^{11,21,24}

The resulting value for the measurement of the hydrodynamic radius of simple TC micelles was $\sim 10 \text{ Å}$, regardless of the TC concentration. This is because the TC molecule contains three hydroxyl groups (3α , 7α , and 12α).³ At high TC concentrations, a formation of rodlike secondary micelles due to hydrophobic interactions of the globular primary micelles is unlikely.³ Based on assumptions of spherical shape and a density of 1 g/cm^3 , the molecular weight of simple TC micelles was estimated to be 2500 Da. The consistent results for the molecular weight of simple TC micelles further confirm the accuracy of the present technique for the estimation of simple TC micelle interaction and the resulting value of A_{11} . Interestingly, the value of A_{11} in the present study was much higher than that ($8.30 \times 10^{-7} \text{ dL mol g}^{-2}$) from Chang and Cardinal,¹² although the value of cmc ($\sim 9 \text{ mM}$) they used was very close to the value in the present study (10 mM) when estimating the concentration of simple TC micelles. Nevertheless, the light-scattering constant term from Chang and Cardinal¹² did not contain the absolute Rayleigh ratio for toluene, which implies that their measured intensity depended on the source or detector but not on the properties of the scatterer.^{25,26} Although the unit of turbidity they used was cm^{-1} ,¹² the magnitude of the parameter close to the absolute Rayleigh ratio was not precisely mentioned, resulting in the lower value of A_{11} . Owing to the inaccuracy of values of H_1 and A_{11} , they could not quantitatively model a plot of τ vs C_1 from light-scattering measurements.¹² On the other hand, because the true value of A_{11} from the present study was much smaller than H_1 , based on eq 7, the relationship between the scattered intensity (i.e., τ) and the simple micelle concentration was almost linear.

Previously, the scattered intensity of micelles (simple and mixed) was suggested to be proportional to molecular weight, concentration, and form factor of micelles.^{11,20} Based on the Guinier equation,¹¹ the value of the form factor was estimated to be ~ 1 for micelles. Therefore, the scattered intensity was linearly proportional to the molecular weight and concentration of micelles. However, because of the interaction of simple TC micelles, the proportionality constant was a function of C_1 also,

TABLE 2: Light-Scattering Results and Mixed Micelle Concentrations in the TC–L System

TC–L concn (mM)	$\overline{R_h}$ (Å)	V (%)	I_{sol}/I_{tol} (g/dL)	C_2 (g/dL)
22–6	25.5	29.3	4.95	1.11
34–12	24.2	31.3	7.39	2.23
52–21	23.8	24.5	9.93	3.90

TABLE 3: Interaction Coefficients and Molecular Weights for Simple TC Micelles and Mixed TC–L Micelles

	A_{11} (dL mol g ⁻²)	A_{22} (dL mol g ⁻²)	M_1 (Da)	M_2 (Da)
simple TC micelles	1.30×10^{-5}		2138	
mixed TC–L micelles		5.09×10^{-6}		35 840

based on eq 7. When C_1 was equal to 5.83 g/dL, the proportionality constant was estimated to decrease ~30%, causing an error in the estimated molecular weight. For this reason, the molecular weight of simple TC micelles might have been estimated accurately if C_1 decreased to 1.16 g/dL. However, this suggestion was considered inappropriate because (a) at low TC concentrations, a minor difference in cmc might cause a high error in the value of C_1 and (b) the scattered intensity from different TC concentrations within a low TC concentration range could not be distinguished easily. Therefore, the present technique seems quite adequate for the estimation of the molecular weight and interaction of micelles.

Interaction and Molecular Weight of Mixed TC–L Micelles. Previous dialysis studies have shown that the TC:L molar ratio (K) in a mixed micelle is a function of the total L concentration and NMBS concentration.^{7–9} Nevertheless, when the NMBS concentration is close to the cmc (~10 mM), the K value for systems in which only mixed micelles are present is nearly constant (~2), regardless of the concentration of L.^{7–9} Mazer et al.¹¹ also predicted the same K value, based on a model of mixed disk micelles, at the coexistence boundary of simple and mixed micelles. Therefore, for modeling purposes, TC–L concentrations in this study were selected as 22–6, 34–12, and 52–21 mM, respectively, based on a K value of 2 and a cmc value of 10 mM. QLS results shown in Table 2 are consistent with dialysis studies because the mean hydrodynamic radius ($\overline{R_h}$) was nearly the same (~24 Å), and polydispersity (V) was low (20–30%) in these three cases.

Nichols and Ozarowski later proposed a capped-rod model by using a technique of size-exclusion high-performance liquid chromatography.²⁸ Interestingly, the estimated K value was close to 2. Nevertheless, the hydrodynamic radius (~44 Å) of the capped-rod mixed micelles they measured was much larger than 24 Å. In contrast to the mixed disk micelle and the capped-rod mixed micelle,^{11,28} a rodlike mixed micelle has been recently proposed.²⁷ However, the estimated K values of the rodlike mixed micelles were varied (from 0.2 to 1.2) and significantly different from ~2, indicating that the structure of mixed micelles is still controversial. Further investigation, therefore, needs to be carried out to resolve this important question.

The concentrations of mixed TC–L micelles shown in Table 2 were estimated simply by (a) subtracting the cmc from the total TC concentration, (b) adding the product of the subtracted TC concentration and molecular weight of TC and the product of the L concentration and molecular weight of L, and (c) dividing the sum of the products by 10 000. Based on eq 10 and values from Table 2, a good curve fitting was obtained from SCIENTIST (MSC = 4.48), as shown in Figure 2. The A_{22} and M_2 values obtained from the curve fitting were 5.09×10^{-6} dL mol g⁻² and 35 840 Da, as shown in Table 3. Similar to the procedure for the estimation of molecular weight of simple

TC micelles, when the hydrodynamic radius of mixed TC–L micelles was ~24 Å, the molecular weight of mixed TC–L was estimated to be ~35 000 Da. The consistent M_2 value further validates Flory's model and indicates that the value of A_{22} is reliable. The A_{22} value obtained was 2 times smaller than that of A_{11} . Also, based on eq 10, because the value of H_2 was much greater than that of A_{22} , the scattered intensity increased with increasing C_2 . However, the molecular weight of mixed TC–L micelles was ~15 times higher than that of simple TC micelles when the coefficient of interaction of mixed TC–L micelles decreased only 2 times. According to eq 8, the proportionality constant decreased 50% when C_2 was 3.9 g/dL. Therefore, it appears that the estimated molecular weight of mixed TC–L micelles, based on the technique of an unchanged proportionality constant reported previously,^{11,20,22} was within a 50% error. This finding indicates that the previous technique needs to be reevaluated when estimating the molecular weight of particles.

Conclusions

The monomer–micelle model successfully interpreted the data of simple micelles and monomers in a TC system. The molecular weight of simple TC micelles estimated by this model was consistent with that from both scattered intensity and size of simple TC micelles. This further confirms the interaction model proposed by Flory and Krigbaum.¹⁵ Therefore, according to Flory's model, future investigation of the interaction between simple micelles and mixed micelles needs to be carried out in order to better understand the physiochemical properties of simple micelles and mixed micelles coexisting in physiological bile.

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