Ionization and Solubilization of 4 Alkyl Benzoic Acids and 4 Alkyl Anilines in Sodium Taurodeoxycholate Solutions

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Purpose. The aqueous solubility and the extent of solubilization and ionization constant in sodium taurodeoxycholate (NaTDC) solutions of a series of benzoic acid and aniline derivatives were measured as a basis to characterize and thereby help predict the nature of the interaction of drugs with bile aggregates.

Methods. The aqueous solubility and the solubilization of two series of compounds, 4-alkyl benzoic acids and 4-alkyl anilines, was measured as a function of NaTDC in 0 and 150 mM NaCl. The ionization constants were determined in water and in 50 mM NaTDC at sodium chloride concentrations of 0, 75 and 150 mM by spectrophotometric titration. The diffusion coefficients of NaTDC and the solutes were measured by pulsed-field gradient spin echo NMR spectroscopy.

Results. The aqueous solubilities decreased with increasing alkyl chain length in both series, and the aniline derivatives had larger solubilities than the benzoic acid derivatives. The number of moles of solute solubilized per mole of bile salt ranged from 0.17 to 0.31 for the benzoic acid derivatives and from 1.3 to 3.0 for the aniline derivatives. The pKa values of the benzoic acid derivatives in the presence of NaTDC were higher relative to the controls, and the difference in the pKa (ΔpKa,obs) increased with increasing chain length. With the aniline derivatives, the pKa values were also shifted to higher values in NaTDC relative to the control but only in the absence of salt. The presence of the solute caused a decrease in the diffusion coefficient of NaTDC, and the diffusion coefficients of the solutes decreased with increasing alkyl chain length. With the hexyl derivatives, the diffusion coefficient of the solute was smaller than the diffusion coefficient of the bile salt. The chemical shift of the protons attached to carbon 18 and 19 of the bile salt were decreased to a greater extent in the presence of the solutes than the protons attached to carbon 26.

Conclusions. Both the solubilization and ionization behavior of solutes were affected by the presence of bile salt aggregates. The surface potential and effective polarity of NaTDC aggregates were found to be dependent on the alkyl chain length for these two homologous series of solutes. The solubilization ratio was largely independent of alkyl chain length, but the unitary partition coefficient was dependent on both the alkyl chain length as well as ionization state. The derivatives reduced the diffusivity of the micelles suggesting the formation of larger sized aggregates and the solutes (hexyl derivatives) appear to favor association with the larger sized aggregates. The phenyl ring of the solutes appears to be oriented parallel to the plane of the steroid frame with preferential positioning near the hydrophobic rings.

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INTRODUCTION

The paradigm for oral drug absorption requires that drugs first dissolve in the intestinal lumen before undergoing transport across the epithelial surface to the systemic blood stream. This has led to a great deal of effort being devoted to investigating the water solubility and the influence of pH and pKa on the amount of drug in solution. However, the intestinal lumen contains other constituents in addition to water that influence the amount of drug in solution. In particular, bile salt aggregates are present and are known to be critical for the absorption of fat and fat soluble compounds (1). These aggregates can affect the amount of neutral drug in solution (2) and can be expected to influence the pH-dependent solubilization of ionizable drugs as well.

Recently, there has been a number of investigations of the solubilization of neutral compounds with bile salt aggregates where hydrophobicity was identified as the most important factor in determining the extent of solubilization (2–7). While Dressman and coworkers have suggested that bile salt solubilization may be predicted from the octanol/water partition coefficient (4), in other studies of low molecular solutes, no definitive correlation was identified (8,9). In our laboratory, hydrophobic interactions were also found to be important for solubilization as well as the number of hydroxyl groups on the steroid (7).

As opposed to neutral compounds, little effort has been directed towards the understanding of the interaction of charged solutes with bile salt aggregates (10–13). Because of their dietary importance, fatty acids have been studied and have been shown to form mixed micelles with bile salts (10). Other negatively charged compounds, as represented by retinoids, are also solubilized by bile salts (12). In a study of weak bases, Carey *et al.* (11) found that the amount of chlorpromazine in solution and its pKa are substantially altered by the presence of bile salts. To date, little information of the effect of bile salts on the pH-dependent-solubilization of charged compounds is available. Thus, there is a poor understanding concerning the magnitude of the charge interaction on the observed extent of solubilization.

In this study, the extent of solubilization and effective acid ionization constants of two homologous series of weak acids and weak bases with sodium taurodeoxycholate (NaTDC) have been measured. In addition, the diffusion coefficients of the solute and NaTDC have been determined to examine the effect of the solute on the bile salt aggregation. The results of this study provide quantitative characterization of the interaction of neutral as well as negatively and positively charged solutes with bile salt aggregates.

THEORY

For weak acids, the process of ionization and dissociation are influenced by the unique properties of the micelle (14–16). In addition to specific interactions, the two general properties of the micelles that affect the ionization constant are the effective polarity and the electrical potential of the surface, both of which can be derived from Coulomb's law (16). For the effective polarity, the force between charges at a specified distance is

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inversely related to the dielectric constant of the medium. Thus, the energy required to ionize a weak acid will be greater in the relatively nonpolar environment of a micellar solution in comparison to the polar, aqueous solution. For the electrical potential, the energy to dissociate a proton from a weak acid in a micelle is a function of the charge of the surface. In bile salt aggregates, movement of a proton away from a negatively charged surface makes an unfavorable contribution to the energy. Therefore, these unfavorable energetic contributions to the ionization and dissociation of a weak acid in a bile salt solution will result in a decrease in the ionization constant or equivalently an increase in the pKa relative to an aqueous solution.

While for a weak base the effect of the negatively charged surface on the dissociation process is the same as that of a weak acid, the lower polarity has the opposite effect. A decrease in the pKa is predicted, since the conjugate acid of a weak base is ionized. For example with an amine, increasing the pH causes the proton to be separated from the positively charged acid which yields an electrically neutral free base. The lower effective polarity in a micelle leads to a more favorable energetic contribution for the dissociation, since the charge is moved from a relatively nonpolar environment to water.

In considering these factors, the difference in the negative of the logarithm of the acid dissociation constant, ΔpKa,obs, between a micellar solution, pKa,m, and an aqueous solution, pKa,w, has been postulated to take the quantitative form as follows (14,15):

$$\Delta pKa,obs = pKa,m - pKa,w \quad (1)$$

$$\Delta pKa,obs = (\Delta pKa)_{polarity} + (\Delta pKa)_{\Psi} + (\Delta pKa)_{specific} \quad (2)$$

where $(\Delta pKa)_{polarity}$ accounts for the difference in the polarity, $(\Delta pKa)_{\Psi}$ accounts for the surface electrical potential, and $(\Delta pKa)_{specific}$ accounts for specific micelle-solute interactions. The polarity term has been referred to as the electrostatic contribution and can be related to the dielectric constant of the micelle by the Born equation (16,17). The surface electrical potential term is always positive for negatively charged micelles and is given by

$$(\Delta pKa)_{\Psi} = |F\Psi/2.303RT| \quad (3)$$

where F is Faraday's constant, Ψ is the surface potential, R is the gas law constant and T is the absolute temperature.

This approach has been used to characterize the properties of a number of micellar systems (14,15,18). Specifically, if the assumption is made that the interactions among the solutes and surfactants are the same for a weak acid and weak base pair, then

$$\Delta pKa(\text{weak acid}) - \Delta pKa(\text{weak base}) = 2(\Delta pKa)_{polarity} \quad (4)$$

$$\Delta pKa(\text{weak acid}) + \Delta pKa(\text{weak base}) = 2(\Delta pKa)_{\Psi} \quad (5)$$

This allows estimation of the polarity and surface potential of the micelle.

The ΔpKa,obs may also be used in conjunction with solubilization studies to calculate the distribution of the charged species. The original derivation was provided by Rippie *et al.* (19) and is briefly recounted here. In considering the ionization of weak acids in micellar and aqueous solutions, there are two equilibrium expressions written as follows:

$$HA_m \Leftrightarrow H_m^+ + A_m^-$$

$$HA_w \Leftrightarrow H_w^+ + A_w^-$$

where the subscript, m, refers to the micellar solution and subscript, w, refers to the aqueous solution. These expressions can be linked by the distribution of each component between a micellar solution and aqueous solution:

$$HA_w \Leftrightarrow HA_m$$

$$A_w^- \Leftrightarrow A_m^-$$

$$H_w^+ \Leftrightarrow H_m^+$$

The three expressions describe the micelle/aqueous distribution of a nonionized and ionized weak acid and a proton. Equilibrium constants may be defined for the ionization processes as well as for the distribution of each component between the micellar and aqueous phases as follows:

$$\begin{aligned} Ka,m &= \{H_m^+\}\{A_m^-\}/\{HA_m\} \\ Ka,w &= \{H_w^+\}\{A_w^-\}/\{HA_w\} \\ K_{HA} &= \{HA_m\}/\{HA_w\} \\ K_{A^-} &= \{A_m^-\}/\{A_w^-\} \\ K_{H^+} &= \{H_m^+\}/\{H_w^+\} \end{aligned} \quad (6)$$

where brackets have been used to represent activities to simplify the notation. Of these five expressions, only four are independent by the following relationship:

$$1 = (Ka,m/Ka,w)(K_{HA}/K_{A^-}K_{H^+}) \quad (7)$$

While the ionization constant in the aqueous environment may be readily obtained, the ionization constant in the micellar environment, as defined above, can not be directly obtained, since the activity of the proton in the micellar environment cannot be measured. Rather, the usual experimental approach is to measure the ionized and nonionized species associated with the micelle spectroscopically and the bulk pH potentiometrically. Thus, the observed ionization constant is given as follows:

$$Ka,obs = \{H_w^+\}[A_m^-]/[HA_m] \quad (8)$$

This constant and the ionization constant obtained in water yield the following relationship assuming ideality:

$$Ka,obs/Ka,w = ([A_m^-]/[A_w^-])/([HA_m]/[HA_w]) \quad (9)$$

or reexpressing this equation in terms of the negative logarithms of the ionization constants

$$pKa,obs - pKa,w + \log K_{A^-} = \log K_{HA} \quad (10)$$

Therefore, the relationship of the ionization constants is found to be dependent on the value of the distribution coefficients. By measurement of the acid dissociation constants in water and in the presence of micelles as well as the distribution coefficient of the nonionized weak acid, the distribution coefficient of the

ionized weak acid may be estimated. The analogous expression for a weak base is given as

$$pKa,obs - pKa,w - \log K_{BH+} = -\log K_B \quad (11)$$

EXPERIMENTAL

Materials

Sodium taurodeoxycholate (NaTDC) was purchased from Sigma Chemical Co. (St. Louis, MO) and was used as received. Benzoic acid (BA) and its derivatives, 4-ethyl benzoic acid (eBA), 4-butyl benzoic acid (bBA), and 4-hexyl benzoic acid (hBA), were purchased from Aldrich Chemical Co. (Milwaukee, WI. These had stated purities of 99% and were used as received. Aniline 99.5% (A) and its derivatives, 4-ethyl aniline 98% (eA), 4-butyl aniline 97% (bA), and 4-hexylaniline 90% (hA) were purchased from Aldrich, but only aniline was used as received. The remaining aniline derivatives were vacuum distilled. The middle, colorless fraction of the distillate was collected and stored in seal test tubes under argon in the dark. All other chemicals were reagent grade or better. The water for preparing solutions was double-distilled in an all-glass apparatus.

Solubilization

The aqueous solubility and extent of solubilization in bile salt solutions of the benzoic acid, aniline, and their derivatives were measured by adding each compound to Pyrex test tubes along with 0.5 to 1 ml of water, saline or sodium taurodeoxycholate at concentrations ranging between 10 and 50 mM. For the benzoic acid derivatives, the samples were equilibrated for 48 hrs at room temperature (22 ± 1°C). The solid was pelleted by centrifugation in a table top centrifuge for 5 min, and aliquots were taken and diluted with ethanol. The absorbance was converted to concentration using appropriate standard curves. For the aniline derivatives, samples were vigorously vortexed and then centrifuged. The upper layer was carefully removed by gentle aspiration. From the lower phase, aliquots were taken, diluted with ethanol, and the concentration was interpolated from appropriate standard curves.

Titrations

Titrations were conducted with the compounds at a concentration of 0.1 mM. The absorption spectra were determined with a Beckman DU Series 70 spectrophotometer (Fullerton, CA), and the addition of bile salt did not appreciably affect the wavelength of maximum absorption nor the absorptivity. Three different sodium chloride concentrations of 0, 75 and 150 mM were used. The effect of NaTDC was tested at a concentration of 50 mM. The actual titrations involved the measurement of the pH followed by measurement of the absorbance at four wavelengths. The pH meter (Model 611, Orion Research) had a narrow diameter, combination glass electrode (Model Z11341-7, Aldrich Chemical Co.) thereby allowing the pH of the solution to be determined in the quartz cuvettes (Helma).

The apparent negative logarithm of the ionization constant, pKa,obs, was obtained by nonlinear regression of the absorbance, A_λ, expressed as a function of the pH as follows (20):

$$pH = pKa,obs + \log[(A_\lambda - A_{\lambda min})/(A_{\lambda max} - A_\lambda)] \quad (12)$$

where $A_{\lambda max}$ and $A_{\lambda min}$ are the maximum and minimum values of the absorbance. Plots of the residuals as a function of the predicted absorbance yielded variances that were randomly distributed.

Diffusion

The Fourier-transform pulsed-field gradient spin-echo (PFG-SE) ^1H NMR diffusion measurements were performed on nonspinning samples in thin-wall 5-mm tubes on a Varian 500 MHz spectrometer. The sample temperature was controlled at 28 ± 0.2°C. A stimulated spin echo pulse sequence was used, and the transformed intensity was analyzed by the following equation (21):

$$A(\tau_1 + \tau_2) = (1/2)A(0)\exp(-2\tau_1/T_2)\exp[-(\tau_2 - \tau_1)/T_1] \times \exp\{-(\gamma G\delta)^2 D(\Delta - \delta/3)\} \quad (13)$$

where $A(\tau_1 + \tau_2)$ is the peak intensity at time, $\tau_1 + \tau_2$, A(0) is the peak intensity at time, 0, T_2 is the spin-spin relaxation time, T_1 is the spin-lattice relaxation time, γ is the gyromagnetic ratio, G is the strength of field gradient, δ is the duration of field gradient, D is the diffusion coefficient and Δ is the time interval between the first and second gradient pulses. The diffusion experiments were performed at constant τ, Δ and G value. A series of ten δ values were used.

To obtain absolute values for the self-diffusion coefficients, the field gradient strength was calibrated from measurements on reference H_2O, D_2O and sodium dodecyl sulfate samples. The spectra were evaluated off-line utilizing nonlinear least-squares fitting of the peak heights as a function of the time parameter, $\delta^2(\Delta - \delta/3)$, using KaleidaGraph on a personal computer. The mean value obtained from averaging at least three decay curves from different proton groups in the molecule is reported. The standard deviations were typically at 1% and never exceeded 3%. The hydrodynamic radius was estimated using the Stokes-Einstein equation, and the aggregation number was estimated by assuming spherical shape, unit density of the aggregate, and 20 water molecules per bile salt molecule. The diffusion coefficients were first corrected for the excluded volume effect as follows (22,23):

$$Do = Dobs/(1 - 1.5\phi) \quad (14)$$

where Do is the diffusion coefficient corrected for the excluded volume effect, Dobs is the observed diffusion coefficient, and ϕ is the volume fraction of the micelles which was calculated from the molar concentrations assuming unit density. The diffusion coefficient of the micelle, D_{mic}, was taken to be equal to the diffusion coefficient of the bile salt, D_{BS}, after correction for the intermicellar concentration (IMC) as (22,23):

$$D_{mic} = [D_{BS} - (IMC/C_{tot})D_{monomer}]/[(C_{tot} - IMC)/C_{tot}] \quad (15)$$

where the IMC was taken to be 1.0 and 1.8 mM at 0 and 150 mM NaCl (24), and the monomer diffusion coefficient, $D_{monomer}$ was measured to be 3.2×10^{-6} cm^2/s (23).

The measurements were carried out in sodium taurodeoxycholate at a concentration of 50 mM and a sodium chloride concentration of 0 or 150 mM. The solute was added in excess of the solubilization, and the tubes were positioned such that neither the solid for the benzoic acids nor the liquid from the

aniline derivatives was within the coil of the NMR spectrometer probe.

RESULTS

The aqueous solubilities of the benzoic acid derivatives are given in Table I. The solubility was determined at pH 3.0 in the absence of salt and in 150 mM sodium chloride. The aqueous solubility determined for benzoic acid is in good agreement with the literature value (25). At both salt concentrations, there was a decrease in the aqueous solubility with an increase in the alkyl chain length. Also in Table I, the results for the aniline derivatives are given. The values also decreased with increasing alkyl chain length but are much larger than those observed with the benzoic acid derivatives.

In Figures 1A–D and 2A–D, the total concentration of solute solubilized as a function of sodium taurodeoxycholate concentration is given at 0 and 150 mM sodium chloride. For benzoic acid, the presence of salt increased the concentration of solute at all bile salt concentrations whereas for aniline, the presence of salt decreased the concentration at all bile salt concentrations. For the derivatives, the presence of 150 mM NaCl resulted in a lower aqueous solubility, but there was more solute solubilized with bile salt in the presence of salt. Overall, the curves were generally linear above 10 mM NaTDC, with the exception of hexyl benzoic acid, where appreciable solubilization did not occur until a bile salt concentration of 50 mM was reached.

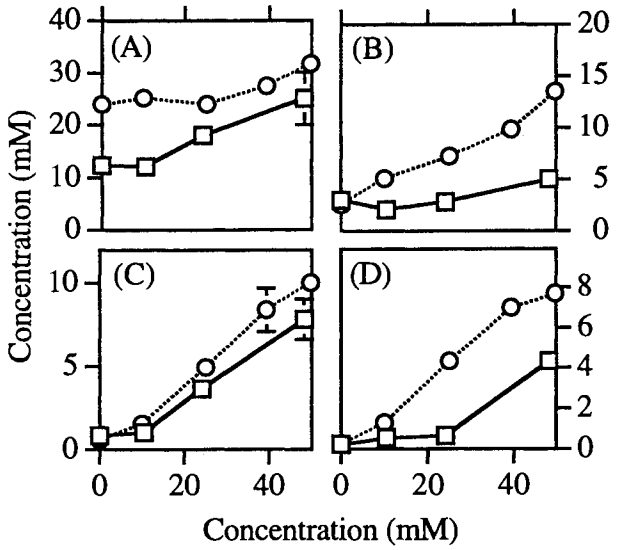

Fig. 1. Concentration of solute solubilized as a function of concentration of sodium taurodeoxycholate for (A) benzoic acid, (B) ethyl benzoic acid, (C) butyl benzoic acid, and (D) hexyl benzoic acid in (□) 0 mM NaCl and (○) 150 mM NaCl.

Table I. Solubility and Solubilization of Benzoic Acid (BA) and Its Ethyl (eBA), Butyl (bBA) and Hexyl (hBA) Derivatives and Aniline (BA) and Its Ethyl (eA), Butyl (bA) and Hexyl (hA) Derivatives

Solute	0 mM Sodium chloride			
	Cs(mM)[a]	SR[b]	Log $K_{o/w}$ (HA)[c]	Log $K_{o/w}$ (A−)[d]
BA	12.3 ± 1.2	0.29	3.01	2.86
eBA	3.01 ± 0.15	0.17	3.43	2.83
bBA	0.827 ± 0.065	0.20	4.05	2.67
hBA	0.224 ± 0.001	0.16	4.53	2.63
	150 mM Sodium chloride			
BA	23.97 ± 0.51	0.31	2.74	2.44
eBA	2.57 ± 0.14	0.20	3.56	2.83
bBA	0.600 ± 0.081	0.22	4.22	2.84
hBA	0.224 ± 0.027	0.17	4.56	2.79
Solute	0 mM Sodium chloride			
	Cs(mM)[a]	SR[b]	Log $K_{o/w}$ (B)[c]	Log $K_{o/w}$ (BH+)[d]
A	195.2 ± 9.2	3.0	2.33	2.28
eA	65.0 ± 1.7	0.86	2.60	2.92
bA	2.46 ± 0.55	0.98	4.05	4.33
hA	0.266 ± 0.032	0.90	5.00	5.02
	150 mM Sodium chloride			
A	75.6 ± 2.5	3.0	2.74	2.73
eA	29.4 ± 3.6	2.3	3.12	3.14
bA	2.66 ± 0.27	1.0	4.02	3.96
hA	0.294 ± 0.0064	1.3	5.03	4.48

[a] Aqueous solubility (mean ± standard deviation, n ≥ 3).
[b] Solubility ratio expressed as moles of solute dissolved per mole of added sodium taurodeoxycholate.
[c] Logarithm of the partition coefficient expressed as a ratio of mole fraction in the micellar lipid and the mole fraction in the aqueous phase for the nonionized derivatives.
[d] Logarithm of the calculated partition coefficient expressed as a ratio of mole fractions for the ionized derivatives.

The solubility ratios for the benzoic acid derivatives were obtained from linear regression of a plot of the total amount of solute in solution as a function of sodium taurodeoxycholate concentration (two to four data points). The values were generally near 0.2 with no evident trend with respect to chain length or salt concentration. However, the unitary partition coefficients (Table I) increased with increasing alkyl chain length. In addition, the partition coefficients are somewhat higher when deter-

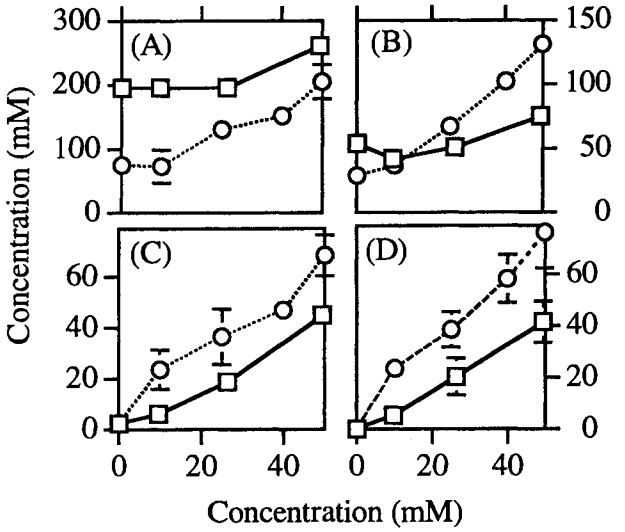

Fig. 2. Concentration of solute solubilized as a function of concentration of sodium taurodeoxycholate for (A) aniline, (B) ethyl aniline, (C) butyl aniline, and (D) hexyl aniline in (□) 0 mM NaCl and (○) 150 mM NaCl.

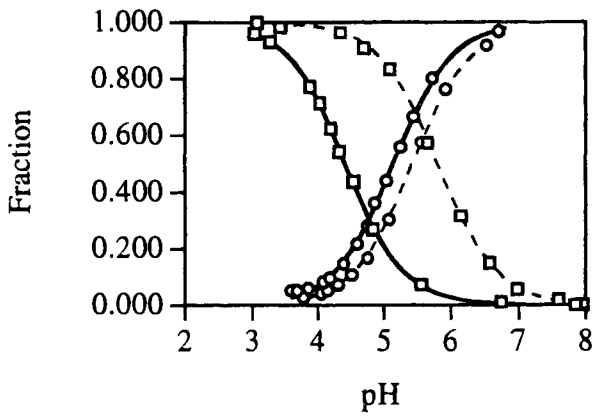

Fig. 3. Fraction of nonionized given as a function of pH in distilled water at 19°C for (□) 4-butyl benzoic acid and (O) 4-butyl aniline where the solid line is the control and the dotted line is in the presence of 50 mM sodium taurodeoxycholate. The lines are the result of nonlinear least squares regression analysis.

mined in the presence of 150 mM NaCl. The solubility ratios for the aniline derivatives ranged from 0.86 to 3.0 and were much higher than those observed with the benzoic acid derivatives. As with the benzoic acid derivatives, there was an increase in the unitary partition coefficient with an increase in the alkyl chain length.

Figure 3 is a plot of the nonionized fraction of butyl benzoic acid and butyl aniline in 0 mM and 50 mM sodium taurodeoxycholate at 0 mM sodium chloride. As is evident, the data are well-fit by the sigmoidal, theoretical line which represents the best fit to equation 12. At the midpoint of the titration curve, or when the fraction equals 0.5, the pH is equal to the observed negative logarithm of the acid dissociation constant, pKa,obs. In the presence of sodium taurodeoxycholate, the plots of the nonionized fraction as a function of pH retained their sigmoidal shape, but the curves for both bBA and bA were shifted to a higher pH. Moreover, the magnitude of the shift was much greater for butyl benzoic acid in comparison to butyl aniline.

The values of the observed pKa of the parent compounds are in very good agreement with the literature (26) and are given Table II with the other observed pKa's. The values are reported as the mean ± standard deviation of the nonlinear least squares fit with all of the titrations being well fit by the Henderson-Hasselbalch relationship. For the controls determined in the absence of bile salts, the values of the pKa's of the 4-alkyl derivatives of both benzoic acid and aniline were higher than for the parent compound. For the benzoic acid derivatives in the presence of 50 mM NaTDC, there is a clear trend of an increasing pKa,mic with increasing alkyl chain length. This trend appears to be relatively insensitive to the concentration of sodium chloride. In contrast, there does not appear to be any trend with respect to the alkyl chain length or salt concentration for the pKa,mic of the aniline derivatives, although the values of the derivatives are higher than the control values.

The surface potential and polarity were determined by first calculating the ΔpKa,obs, the difference between the pKa values obtained in the presence and absence of NaTDC. Most of the values of the pKa's in the presence of sodium chloride were estimated from the Debye-Huckel equation (27), since the measurements of butyl aniline and butyl benzoic acid in 150 mM sodium chloride were in excellent agreement with the theoretical predictions. The ΔpKa,obs for each derivative pair were combined by addition and subtraction as given in equations 4 and 5. The results are plotted in Figures 4A and B. The estimated surface potential changed from a small negative value of about −7 mV for the parent compounds to a relatively large negative value of −55 mV for the hexyl derivatives as reflected in the increasing value of the ΔpKa. The estimated surface potential had the largest absolute value in the absence of salt and often had the smallest absolute value in presence of the highest sodium chloride concentration, although the difference was at most 15 mV.

In Figure 4B, the ΔpKa,obs attributed to the effective polarity of the environment is plotted as a function of the alkyl chain length. For the parent derivatives, the change in the pKa was less than 0.25. However, with increasing alkyl chain length there was an increase in the ΔpKa,obs such that with the hexyl derivatives, the ΔpKa,obs approached one unit.

From the ΔpKa,obs and the partition coefficient of the nonionized form of the benzoic acid and aniline derivatives, the unitary micelle/aqueous partition coefficients of the ionized forms were calculated and are given in Table I. The values of

Table II. Values of the pKa obtained for the Benzoic Acid and Aniline Derivatives in the Presence and Absence of Sodium Taurocholate at the Three NaCl Concentrations of 0, 75 and 150 mM

[NaTDC] mM	[NaCl] mM	Benzoic acid	Ethyl benzoic acid	Butyl benzoic acid	Hexyl benzoic acid
0	0	4.188 ± 0.032[a]	4.438 ± 0.032	4.421 ± 0.002	4.371 ± 0.034
50	0	4.373 ± 0.043	5.035 ± 0.050	5.811 ± 0.015	6.275 ± 0.003
50	75	4.386 ± 0.022	4.986 ± 0.087	5.733 ± 0.011	6.170 ± 0.095
50	150	4.346 ± 0.031	5.029 ± 0.088	5.654 ± 0.086	5.999 ± 0.076
[NaTDC] mM	[NaCl] mM	Aniline	Ethyl aniline	Butyl aniline	Hexyl aniline
0	0	4.686 ± 0.005[a]	5.096 ± 0.055	5.141 ± 0.021	5.145 ± 0.051
50	0	4.638 ± 0.071	5.411 ± 0.028	5.427 ± 0.006	5.361 ± 0.15
50	75	4.821 ± 0.023	5.450 ± 0.015	5.176 ± 0.025	5.295 ± 0.014
50	150	4.82 ± 0.020	5.259 ± 0.016	5.227 ± 0.026	5.151 ± 0.014

[a] Values represent the mean ± standard deviations with n ≥ 3.

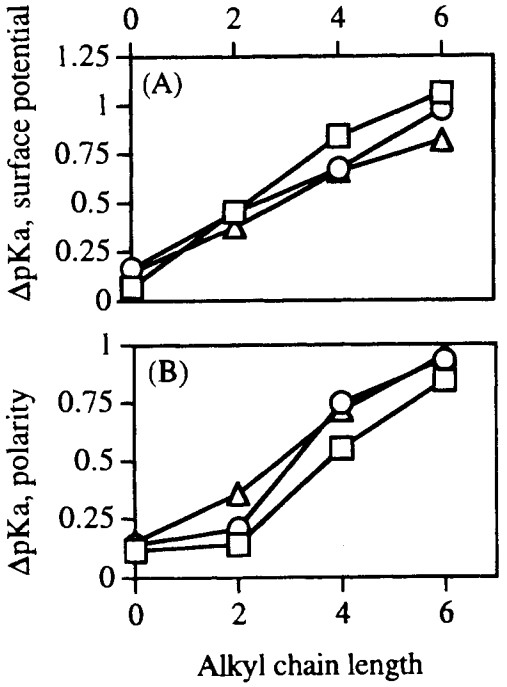

Fig. 4. The difference in the pKa given as a function of alkyl chain length arising from the effect of the (A) surface potential and (B) polarity at salt concentrations of (□) 0 mM, (○) 75 mM, and (△) 150 mM.

the partition coefficients of the ionized forms of the benzoic acids were smaller than the corresponding values of the nonionized forms. These ranged from 2.44 to 2.86 without any evident trend with respect to alkyl chain length. The partition coefficients for the ionized aniline derivatives were slightly larger than the nonionized forms at the low ionic strength, but there was little or no difference in the values at high ionic strength. In addition, there was a strong dependence of the value on the alkyl chain length.

The observed diffusivities for sodium taurodeoxycholate and each of the derivatives are given in Table III. The diffusion coefficient of NaTDC at a concentration of 50 mM in 0 mM NaCl was 1.77×10^{-6} cm^2/s. With the addition of 150 mM NaCl, the diffusion coefficient was reduced to 1.54×10^{-6} cm^2/s. The aggregation numbers were estimated to be 6 and 9 in the low and high salt concentrations, respectively.

With the addition of the solutes, the diffusion coefficients of the bile salt and the solute were simultaneously obtained. For NaTDC, the presence of the benzoic acid derivatives caused about a 5% reduction in the diffusion coefficient. With the addition of the aniline derivatives, the diffusion coefficient underwent a greater reduction. The values for ethyl aniline are questionable, since the tube became cloudy during the NMR run suggesting contamination by larger aggregates which would lead to an underestimate of the diffusion coefficients.

The diffusion coefficients of the solutes are also given in Table III. There was a clear decrease in the diffusion coefficient with an increase in the alkyl chain length of the derivative. This reflects the increasing fraction of solute associated with the aggregate with an increase in chain length. While in principle the distribution coefficient may be obtained from analysis of the diffusion coefficients, the change in the diffusion coefficient of the bile salt introduces an additional unknown parameter. Of interest is the observation that the diffusion coefficient of the hexyl derivatives are all smaller than that of the bile salts.

Finally, in Figure 5A, the change in the chemical shift of carbon 18 NaTDC as a function of solute solubilized is given. The largest effect was observed with carbon 18 in the presence of the aniline derivatives. These shifts were not correlated with the solubilization ratios. However, for the benzoic acid derivatives, the shifts were smaller and correlated with the solubility ratios. Very similar results were also observed with carbon 19

Table III. Observed Diffusion Coefficients ($\times 10^{-6}$ cm^2/s) of NaTDC and Solute

	Diffusion coefficient in 0 mM NaCl		Diffusion coefficient in 150 mM NaCl	
	NaTDC	Solute	NaTDC	Solute
NaTDC control	1.77	—	1.54	—
BA/NaTDC	1.65	9.89	1.45	10.4
eBA/NaTDC	1.66	5.25	1.44	4.46
bBA/NaTDC	1.64	1.67	1.43	1.45
hBA/NaTDC	1.69	1.48	1.51	1.31
A/NaTDC	N.D.[a]	N.D.	0.806	9.95
eA/NaTDC	0.63	2.40	0.356	2.46
bA/NaTDC	1.04	1.25	0.710	1.26
hA/NaTDC	1.19	1.04	0.690	0.688

[a] Not determined.

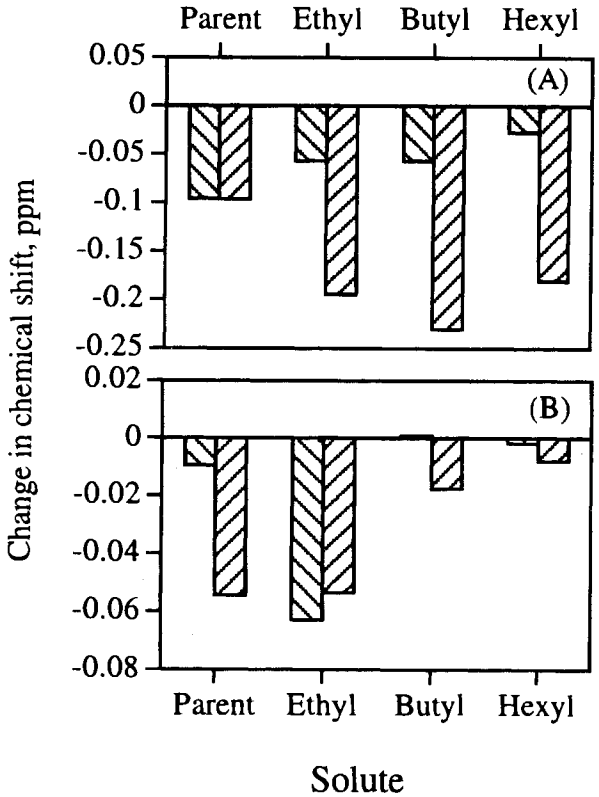

Fig. 5. Change in the chemical shift of (a) carbon 18 and (b) carbon 26 of sodium taurodeoxycholate as a function of added solute for (▨) benzoic acid and (▨) aniline derivatives.

as well. In Figure 5B, the effect of the solute on the observed chemical shift of carbon 26 of NaTDC is given. Here, the shifts are relatively small, although still largely negative.

DISCUSSION

In this study, the interaction of two homologous series of weak acids and weak bases with sodium taurodeoxycholate has been studied. The intent was to develop a better understanding of the charge interaction on the observed extent of solubilization of drugs by bile salt micelles. The aqueous solubility, extent of solubilization and effective acid dissociation constants have been measured. In addition, the diffusion coefficients were also determined to examine the effect of the solute on the aggregation of bile salts. The results of this study provide insight into the nature of the interaction of neutral as well as negatively and positively charged solutes with the negatively charged bile salt aggregates.

Aqueous Solubility and Solubilization

The solubility of both derivatives decreased with increasing alkyl chain length. This is expected given the theoretical and empirical correlations of a linear relationship between the logarithm of the solubility and the alkyl chain length (28). While the slopes of such plots range from 0.43 to over 0.7, depending whether the series is an aromatic alcohol or straight chain alkanols (28), in this study, the slopes in the absence of salt were 0.30 and 0.316 for the benzoic and aniline derivatives, respectively. The relatively small values may simply reflect the fact that the correlation is based on only four derivatives. Alternatively, this may indicate some degree of self-association with the longer chain derivatives.

The difference in the aqueous solubility between the aniline and benzoic acid derivatives is likely to be related to the difference in the melting point of the derivatives. The anilines are liquids at room temperature, whereas the benzoic acid derivatives are solids and have melting points in the range of 100 to 120°C. For the aqueous solubility, there was a lack of any clear trend of the effect of salt. That is, while increasing the salt concentration generally tends to reduce the solubility of compounds with hydrophobic groups, in the present case, apparently more complicated interactions are occurred

The presence of sodium taurodeoxycholate at concentrations in excess of 10 mM resulted in an increase in the amount of derivative in solution. Given the nature of the data, it is not possible to rigorously determine whether or not the increase in the amount of derivative in solution rises linearly with bile salt concentration. It often appears that in the absence of salt there is an initial decrease in the amount of solute in solution followed by a sharper increase. This has been more carefully studied with steroids solubilized by bile salts and has been explained as a consequence of salting out of the solutes (29).

The solubilization ratios were calculated from the linear portions of the curve which consisted of typically three data points, although there were only two points the solubilization of hexyl benzoic acid in the absence of salt. Coincident with the results from the aqueous solubility determination, the solubility ratios for the aniline derivatives were much higher than those obtained for the benzoic acid derivatives. This result indicates that the extent of micelle solubilization, as measured by the solubilization ratio, is dependent on the properties of the pure solute.

In contrast to the solubilization ratios, the partition coefficients showed a strong dependence on alkyl chain length. This dependence is a consequence of the correlation between the aqueous solubility and alkyl chain length. Also of interest is the close relationship between the partition coefficients of the aniline and benzoic acid derivatives which also reflects the normalizing influence of dividing by the aqueous solubility. As above, no clear influence of ionic strength was noted.

Ionization

The presence of an alkyl chain in the para position of these compounds led to an increase in the pKa of about 0.3 units relative to the parent derivative consistent with the expected inductive effect. The addition of salt caused an increase in the pKa of the butyl derivatives of aniline and benzoic acid which was in very good agreement with that predicted by the Debye-Huckel equation. Thus, the remaining values of the other derivatives were calculated.

The presence of sodium taurodeoxycholate in the solution resulted in an increase in the measured pKa. For the benzoic acid derivatives, the ΔpKa,obs increased with increasing alkyl chain length. This is indicative of different environments. In contrast, the ΔpKa,obs for aniline and hexyl aniline were not appreciably different from zero in the absence of salt, although the values for the ethyl and butyl aniline derivatives were about 0.3 units.

With the assumption that the aniline and benzoic acid derivatives are equivalent except for the sign of the charge, the difference in the ΔpKa,obs of these two series may be added and subtracted to yield estimates of the surface potential and effective polarity (cf Equations 4 and 5). The surface potential and effective polarity are seen to have a strong dependence on the alkyl chain length (Figures 4A and B). This suggests that the properties of the aggregate are specific to the solute being solubilized.

With the results of the solubilization and ionization in the presence and absence of bile salt aggregates, it is possible to estimate the distribution between the aqueous and micellar phase of the ionized form of the solutes. The results for the calculated partition coefficients are given in Table I. Overall, there is a clear dependence of the distribution on the charge of the solute. For the benzoic acids, ionization led to a dramatic decrease in the partition coefficient. Consistent with expectations, introduction of a negative charge has unfavorable consequences for solubilization within bile salt micelles.

For the aniline derivatives which carry a positive charge, the partition coefficient was modestly increased but only at low ionic strength. It would seem that ionization of the aniline derivatives causes an increase in the solute affinity for both water and the aggregate thereby leaving the distribution coefficient largely unaffected by ionization. There was no evidence of complexation between the aniline derivatives and NaTDC as has been observed with the phenothiazines and bile salts (11). The relatively small size and the presence of a flexible alkyl chain would appear to favor a water soluble association.

Diffusion Coefficients

The pulse field gradient spin echo NMR measurements provided the effective diffusion coefficient of the bile salt mono-

mer in dilute solutions and of the bile salt aggregate in more concentrated solutions. With the diffusion coefficient of the bile salt aggregates in 0 and 150 mM NaCl, it was possible to estimate the aggregation numbers. First, the excluded volume effect was accounted for by assuming there was a decrease in diffusivity proportional to the volume fraction of micelles present in solution. Second, the observed diffusion coefficient of the bile salt was corrected by the monomer concentration to yield the diffusion coefficient of the aggregate. The resulting diffusion coefficient was then used to estimate the hydrodynamic radius of the aggregate by the Stokes-Einstein equation, and the hydrodynamic volume was estimated assuming that the aggregate is spherical. Finally the aggregation number was calculated with the assumption of unit density and that the hydration number was twenty leading to a molecular weight of the hydrated bile salt of 881 g/mol (30,31).

While each of these steps involves an approximation, it did allow comparison of the measurements made in this study with the extensive literature (32). From the NMR, the aggregation numbers were found to be 6 and 9 in 0 and 150 mM NaCl. From the literature, the aggregation numbers have been reported to be 6 and 22 at similar temperatures and ionic strengths (33). The values of the present study are reasonably close given the assumptions that are required to make the comparison. It does appear, however, that the effect of ionic strength on the aggregation number was found to be less in the present study than what has been reported in the literature.

The more important value of the diffusion measurements is that a comparison can be made of the diffusion coefficients of the solute and bile salt in the same solution. Because of the resolution provided by the differences in the chemical shift, the average size of the bile salt aggregate may be compared with the average size of the aggregates solubilizing the solute. The results in Table III allow for some interesting conclusions. First, the addition of the solutes invariably led to a reduction in the diffusion coefficient of the bile salt. For benzoic acid derivatives, the effect is relatively small and may be a result of three factors. A reduction in the intermicellar concentration of the NaTDC, an increase in the size of the aggregate due to the presence of the solute, or an increase in the size of the aggregate due to the presence of more NaTDC molecules.

The diffusion coefficients of the benzoic acid derivatives fall systematically with alkyl chain length. This is primarily a result of the changing distribution ratio. Thus, the observed diffusion coefficient decreases as a greater fraction of solute molecules associate with the bile salt aggregate. Nevertheless, a comparison of the diffusion coefficient of NaTDC and hexyl benzoic acid is of significance. In this case, the diffusion coefficient of hexyl benzoic acid is slightly smaller than NaTDC. Since the estimated distributions of NaTDC and hexyl benzoic acid between the aqueous phase and the aggregate are about the same, the differences in the diffusion coefficient would seem to arise from the benzoic acid derivative being preferentially located in larger aggregates.

In an examination of the diffusion coefficients of aniline derivatives, very similar conclusions are reached as with the benzoic acid derivatives. With the addition of the aniline derivatives, there is a decrease in the diffusivity of NaTDC. This decrease is greater than that observed with the benzoic acid derivatives which reflects the larger solubilization ratios of aniline derivatives. In fact, from the solubilization ratios, the increase in molecular weight from the presence of the anilines correlates reasonably well with the observed decrease in the diffusion coefficient. However as above, the size distribution measured by the NaTDC is smaller than that measured by the hexyl derivative in the low ionic strength solution suggesting that the hexyl derivative tends to sample larger aggregates.

With the size information, it is possible to discuss the dependence of the charge and polarity on the alkyl chain length. Since the properties of the bile salt micelle are dependent on the solute being solubilized, the micelle may be changed by the presence of the solute. Alternatively, the properties of the micelle may remain relatively constant, but the position of the solute within the aggregate depends on alkyl chain length. Specifically, the longer chain length benzoic acid derivatives may be drawn more deeply into the micelle and thereby experience a less polar and more highly charged environment. The latter explanation is more reasonable given the insensitivity of the size of the micelle in the presence of the different solutes.

The final consideration is the change in the chemical shift observed with carbon 18 and 26 of NaTDC due to the presence of the aniline and benzoic acid derivatives. The chemical shift of groups near phenyl rings are susceptible to the induced magnetic field brought about by the circulation of the π electrons. The phenyl group may be considered as a ring of circulating electrons. If the ring is oriented with its plane perpendicular to the spectrometer's field, the induced field will oppose the spectrometer's field in the region within, above, and below the ring. However, the induced field will add to the spectrometer's field in the region outside of the ring (34). Moreover, a reduction in the effective field strength causes a reduction in the observed chemical shift. Thus, the observed reductions in the chemical shifts appear to be a result of the groups of NaTDC being positioned near the phenyl groups of the solubilized solutes.

First, the significant changes in the chemical shift of carbons 18 and 19 were negative. Since these are the side chain methyl groups of the steroid frame, the decrease is consistent with the phenyl group lying in a planar arrangement to the steroid frame of the NaTDC molecule. As with the diffusion coefficients, the magnitude of the change in the chemical shift is theoretically dependent on the solubilization ratio. Nevertheless, there was a considerable decrease in the chemical shift of carbon 18 as well as carbons 19 due to the presence of the solutes. For the benzoic acid derivatives, the magnitude of the shift was correlated with the solubilization ratio which suggests a similar location for all of the solutes. In contrast, the magnitude did not correlate well with the solubilization ratio for the aniline derivatives. This may indicate that the phenyl ring of the longer chain length aniline derivatives are positioned more closely to carbons 18 and 19 of the NaTDC.

The effect of the phenyl groups on the chemical shift of carbon 26 was much smaller. Carbon 26 lies near the amide link which connects the taurine residue. There appears to be fewer molecules near this region or more freedom in the arrangement. The change was generally larger for the aniline derivatives which may reflect the larger solubilization ratio but also may reflect the preferential association of the amine group, as opposed to the carboxylic acid, with the sulfate residue of the taurine moiety.

In summary, the results from this study have shown that the presence of bile salts has a profound effect on the amount of solute in solution as well as the ionization behavior. This

suggests that bile salts present in the intestine can have a significant effect on the dissolution and absorption of neutral and ionizable drugs. Moreover, more careful consideration of the solutes present in the intestine and their effect on the solubility and acid dissociation behavior is warranted.

From the analysis of these measurements, it appears that bile salt molecules provide a range of environments for the solutes that are solubilized. The dependence of the surface electrical potential and effective polarity on the solute is a result of the displacement of the solutes within the aggregate and/or changes in the aggregation number. For the nonionized aniline and benzoic acid derivatives, there was no significant difference in terms of partitioning into the micelle, whereas the large differences in the solubilization ratios are accounted for by their aqueous solubilities. The ionized benzoic acid derivatives had much lower micelle/aqueous partition coefficients presumably due to the unfavorable charge interaction. The partition coefficients of the ionized aniline derivatives were modestly changed at low ionic strength suggesting there is compensation between the affinity of the solute for the aggregate and the tendency to remain in the aqueous phase. Due to the relatively complicated interactions between these solutes and the unique aspects of the bile salt aggregates, caution is needed when using synthetic detergents for estimating the in vivo dissolution rates.

The NMR diffusion coefficient measurements provided a powerful means to independently assess the distribution of the solute and bile salt in aggregated systems. In this study, it appears that the derivatives induced a shift in the size distribution of the bile salt aggregates and may preferentially associate with the larger micelles.

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