Solubilization of Drugs by Physiological Mixtures of Bile Salts

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Purpose. The solubilization of a number of steroids was determined in bile salt simple micelles and a bile salt/phospholipid micellar system to provide a better basis to predict the extent of drug solubilization *in vivo*.

Methods. Excess solid drug was dispersed in taurodeoxycholate or mixed micelle solutions prepared with fixed mole ratios of taurocholate, taurodeoxycholate, taurochenodeoxycholate, glycodeoxycholate, glycocholate, and glycochenodeoxycholate with egg phosphatidylcholine. Drug concentrations were determined from the absorbance following centrifugation. Using NMR spectroscopy, the diffusivities of the simple and mixed micelles were 2×10^{-6} and 8×10^{-7} cm^2/s, respectively.

Results. From the change in the concentration of drug in solution with a change in the lipid concentration, the solubilization ratio (SR) was calculated. The SR and aqueous solubility were used to calculate the micelle/aqueous partition coefficients ($K_{m/w}$). $K_{m/w}$ was correlated with octanol/water partition ($P_{o/w}$) for the TDC and mixed micelle data sets with correlation lines of $\log K_{m/w} = 0.74 \log P_{o/w} + 1.55$ ($r^2 = 0.91$) and $\log K_{m/w} = 0.61 \log P_{o/w} + 2.44$ ($r^2 = 0.95$), respectively.

Conclusions. With such data, a refined, predictive relationship between the *in vitro* and the *in vivo* solubilization with additional information concerning the bile salt/lipid concentration in the human intestine appears possible.

KEY WORDS: bile salt; steroids; solubilization; micelle.

INTRODUCTION

Bile salts are surface-active agents that aggregate in aqueous solutions (1). These aggregates are known as simple micelles. Bile salts also form mixed micelles with phosphatidylcholines, glycerides, and fatty acids in the intestine (2). These micelles play an important role in the emulsification, solubilization, and absorption of ingested fat and lipid-soluble vitamins. Many investigators have examined the effects of bile salt micelles on the solubilization and dissolution of drugs (3). These studies have demonstrated the importance of the bile salt micelles for the dissolution and thereby the absorption of orally administered, poorly water soluble compounds (4). However, accurately predicting the dissolution or even the solubilization of drugs in the human intestine remains difficult. This effort will be enhanced by additional measurements of the solubilization of drugs in bile salt micellar systems that more closely reflects the reported composition of bile salts in the human intestine (5,6).

Such an approach has been initiated in the recent studies of Petersen *et al.* (5,6). The solubilization and dissolution of hydrocortisone (5) and danazol (6) were determined in human intestinal aspirates. The results were also compared with micellar systems that were prepared *in vitro*. While the solubilization of hydrocortisone, a relatively polar steroid, was not correlated with the concentration of bile salts, the results with danazol did follow expectations.

In this study, a series of steroid hormones was the focus. The steroids are poorly water-soluble drugs that represent a structurally related, neutral group of compounds. In addition, many of these compounds have been studied with micelles containing taurocholate, cholate, and glycocholate (7–10). Thus, the steroids represent a good set of compounds in which the solubility and solubilization in physiologically relevant mixtures of bile salt micelles may be determined. With such measurements, a database will be provided to investigate the most appropriate system for predicting the solubilization of drugs *in vivo*.

MATERIALS AND METHODS

Materials

The bile salts used in this study were taurocholate (TC), taurodeoxycholate (TDC), taurochenodeoxycholate (TCDC), glycocholate (GC), glycodeoxycholate (GDC), and glycochenodeoxycholate (GCDC) and were purchased from Sigma Chemical Co, St. Louis, MO, USA. Egg phosphatidylcholine was purchased from Avanti Polar Lipids, Alabaster, AL, USA, and was used as received. The steroids used in this study were progesterone; deoxycorticosterone; 11α-hydroxyprogesterone; 11-ketoprogesterone; 4-pregnene-11β-hydroxy-17α, 20-dione; cortexolone; cortisone; corticosterone; prednisolone; hydrocortisone; dexamethasone; flunisolide; and triamcinolone acetonide, which were purchased from Sigma Chemical Co. All chemicals were reagent grade or better and were used as received.

Solubilization

The extent of solubilization was measured in two different series of solutions, a TDC series ranging from 10 to 50 mM and a bile salt/phospholipid series. The TDC solutions were prepared in 0.9% sodium chloride, and solubilization was determined at room temperature. The bile salt/phospholipid micellar solutions were prepared in a buffer solution consisting of 10 mM HEPES, pH of 6.5, in 0.9% NaCl, and the experiments were conducted in a shaking water bath at 37.0 ± 0.5°C. For the mixed micellar system, egg PC was lyophilized overnight under high vacuum (11). The dried egg PC was reconstituted to a concentration of 7.5 mM with a solution containing 2.17 mM TC, 1.45 mM TDC, 2.17 mM TCDC, 4.16 mM GC, 2.89 mM GDC, and 4.16 mM GCDC for a total concentration bile salt of 17 mM. A 2 mM solution containing the same molar ratio of bile salts was used to dilute the 7.5 mM egg PC/17 mM bile salt solution to yield four additional solutions. Thus, solubilization was determined in a

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series of six solutions that ranged from 17 mM total bile salt/7.5 mM egg PC to 2 mM total bile salt/0 mM egg PC.

The extent of solubilization was determined by placing about 1 mg steroid in microcentrifuge tubes and adding 0.5 to 1 ml of bile salt solution. After equilibration for 2 days in a shaking water bath, the tubes were centrifuged in a temperature controlled, tabletop ultracentrifuge (Marathon 26KMR, Fischer Scientific) at 13,000 xg to sediment the solid phase. An aliquot of the supernatant was withdrawn, diluted, and then analyzed by ultraviolet spectrophotometry (Beckmann Model 7400, Fullerton, CA, USA). The absorbance was determined at the wavelength of maximum absorbance for the steroid under study, and the correction for the background absorbance was never more than 10% of the total absorbance.

The solubilization ratio, SR, is defined as (12):

$$SR = N_D/N_L$$

where N_D is the moles of drug in solution and N_L is the moles of surfactant in solution. The SR was calculated from the linear portion of the slope of a plot of the total amount of steroid in solution as a function of total surfactant concentration (bile salt + egg PC for the mixed system). Linearity was visually judged. The mole fraction solubilized, X_m, was obtained from the solubilization ratio as:

$$X_m = SR/(1 + SR) = N_D/(N_D + N_L)$$

The micelle/aqueous partition coefficient, $K_{xm/a}$ was calculated as follows:

$$K_{xm/a} = X_m/X_a$$

where X_a is the mole fraction aqueous solubility, which is defined as the moles of drug in solution per mole of solution.

Diffusion Measurements

The Fourier-transform pulsed-field gradient spin-echo (PFG-SE) ^{1}H NMR diffusion coefficients of bile salt and phospholipids were measured using a stimulated spin echo pulse sequence preceded by a water suppression pulse as previously described (13). The resulting transformed area under the peak was analyzed on line by the following linearized equation:

$$Ln[A(\tau_1 + \tau_2)] = \text{constant} + \{-(\gamma G\delta)^2 D_{obs}(\Delta - \delta/3)\}$$

where $Ln[A(\tau_1+\tau_2)]$ is the natural logarithm peak area at the time of the echo, $\tau_1+\tau_2$, γ is the gyromagnetic ratio, G is the strength of magnetic field gradient, δ is the field gradient pulse length, D_{obs} is the diffusion coefficient, and Δ is the diffusion time, which is equal to the time interval between the first and second gradient pulses.

The diffusion coefficients of the TDC solutions were analyzed as follows. Assuming a two state model for the observed diffusion coefficient of the bile salt, D_{obs}, then:

$$D_{obs} = f_i D_i + f_m D_m$$

where f_i the fraction of bile salt as monomer, f_m is the fraction of bile salt in micellar form, and D_i and D_m represent the corresponding diffusion coefficients. The fractions may be written in terms of the total bile salt concentration, $[TDC]_t$, and monomer concentration, cmc, as follows

$$D_{obs} = (cmc/[TDC]_t)D_i + \{1 - (cmc/[TDC]_t)\}D_m$$

This may be rearranged as

$$D_{obs}[TDC]_t = cmc(D_i - D_m) + D_m[TDC]_t$$

so that a plot of the product of the observed diffusion coefficient and total bile salt concentration as a function of the total bile salt concentration would yield a straight line with a slope equal to the diffusion coefficient of the bile salt micelle.

RESULTS AND DISCUSSION

Prediction of the oral absorption of poorly water-soluble compounds remains a significant challenge in the drug development process (4). The underlying premise of the present work is that measurement of the solubilization of drugs in a milieu that most closely matches the intestinal fluid will be of greatest relevance. Therefore, solubilization studies were carried out with a two-fold purpose. First, it is clear that measurements need to be made in a mixture of bile salts and phospholipids that reflects the intestinal composition. However, measurements were also made in the TDC simple micelle system. These latter results add to the existing database and can be used for identifying a simpler model system, which will allow prediction of the extent of solubilization of drug in the intestine. For these measurements, a series of steroids were chosen, since a sizeable literature base was already available where the solubilization has been determined in number of bile salt micellar solutions.

Beginning with the simple micelle system, the concentration of TDC ranged from 10 to 50 mM. Although the concentrations exceed the bile levels in the intestine, it encompasses a range where the solubilization ratio can be approximated by a linear function of bile salt concentration. The results from the solubilization of four of the steroids in solutions of TDC are shown in Fig. 1. Progesterone had the lowest extent of solubilization at all concentrations of TDC. The amount for progesterone, dexamethasone and prednisolone increased linearly with increasing concentration of TDC. Deoxycorticosterone was poorly solubilized at low bile salt levels; however, the concentration rose significantly with increasing TDC concentration. This increase was clearly non-linear.

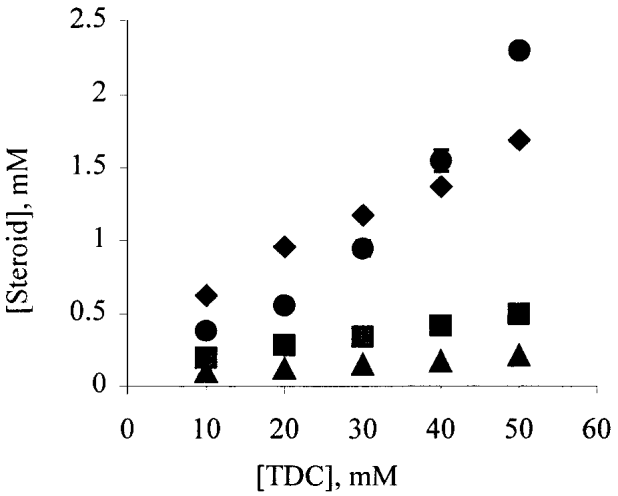

Fig. 1. A plot of the total concentration of steroid in solution as a function of TDC concentration for (▲) progesterone, (●) deoxycorticosterone, (◆) prednisolone, and (■) dexamethasone. Each point represents the mean ± SD, n = 3.

Table I. Solubilization Ratios (SR, Mean ± 95% CL) in the TDC Solutions and Mixed Bile Salt Phospholipid Solutions, Aqueous Solubility (Caq), Molecular Weight (Mwt), and Logarithm of the Octanol/Water Partition Coefficient ($LogP_{o/w}$) for Each Steroid in the Study

				TDC		Mixed micelles	
	Caq, mM[a]	Mwt	LogP[a]	SR	95% CL	SR	95% CL
Progesterone	0.0353	314.45	3.87	0.0032	0.0008	0.0262	0.0007
Deoxycorticosterone	0.4599	330.45	2.9	0.0481	0.0066	0.0735	0.0052
11α-hydroxyprogesterone	0.361	330.45	2.36	0.0144	0.0016	0.0258	0.0024
4-pregnene-11β-hydroxy 17α,20 dione	0.2361	346.45	1.937			0.0140	0.0007
Cortexolone	0.1273	346.45	1.937			0.0195	0.0027
Cortisone	0.532	346.45	1.89			0.0220	0.0017
Corticosterone	0.695	346.45	1.937			0.0538	0.0048
Prednisolone	0.4695	360.44	1.62	0.0254	0.0015	0.0260	0.0034
Hydrocortisone	1.078	362.47	1.55			0.0409	0.0034
Dexamethasone	0.2344	392.45	1.83	0.0073	0.0004	0.0157	0.0007
Flunisolide	0.2761	434.5	1.025	0.0015	0.0059	0.0173	0.0013
Triamcinolone acetonide	0.0495	434.5	1.025			0.0133	0.0007

[a] Taken from Reference 3.

A non-linear increase in the extent of solubilization was also observed with 11-ketoprogesterone (data not shown). For each compound, a plot of the total concentration of drug in solution as a function of TDC concentration was regressed for the linear portion of the curve. The slope, which equals the solubilization ratio, and the 95% confidence limits were calculated and are given in Table I.

The results for the solubilization in the mixed bile salt system are shown in Fig. 2. The mixed micellar system was chosen such that the solubilization ratio could be determined with a series of solutions in which the size of the micelle was constant but the micelle concentration varied. This can be achieved if the monomer concentration of bile salt is the same in each solution (1). It was also desired that the bile salt composition reflect that reported for the human intestine (5,6,14,15). Finally, the concentrations of bile salt and phospholipid should span the reported levels for the fed and fasted state of the intestine (4). Based on the literature reports of mixed bile salt systems (1), a total monomer bile salt concentration of 2 mM was expected to be the appropriate monomer concentration given the level of PC present. Thus, the stock bile salt/phospholipid solution was diluted with a 2 mM bile salt solution to yield micellar solutions with the desired properties. The diffusion coefficients of the micelles were determined as given below, which substantiated that each solution contained micelles of the same size.

For the solubilization in the mixed micellar system, the total concentration of drug in solution rose with the total lipid concentration (Fig. 2). In general, the plots were linear, although there was somewhat greater scatter in the data in comparison to the TDC system. For a number of compounds, the concentration of drug in solution for the 2 mM lipid concentration exhibited was higher than that calculated from the regression line. This may indicate that the nature of the solubilization is different in the simple micelles containing only bile salts in comparison to mixed micelles that also contained phosphatidylcholine.

In examining the data for TDC, the solubilization ratios are seen to vary from 0.0015 for the polar flunisolide to 0.0481 for the relatively nonpolar deoxycorticosterone. A much larger data set was examined with the mixed micellar system. Here, the range of solubilization ratios was from a low value of 0.0133 observed with triamcinolone acetonide to the high value of 0.0735 observed again with deoxycorticosterone. Thus, simple micelles exhibited a larger relative increase in the solubilization, although the values were lower than those obtained with the mixed micellar system.

In each case, the solubilization ratio was greater in the mixed micellar system in comparison to the TDC system, although the values for prednisolone were not statistically different. To better characterize the properties of these micellar systems, the diffusion coefficients of TDC and the mixed micelles were determined by pulsed-field gradient NMR spectroscopy. In this method, the diffusion coefficient of each molecule may be measured if the resonances of the protons are resolved by a difference in the chemical shift. In Fig. 3, the observed diffusion coefficients of TDC in the micellar system as well as the bile salts and phosphatidylcholine in the mixed micellar system are given as a function of surfactant concentration.

For TDC, the diffusion coefficient was near 4×10^{-6}

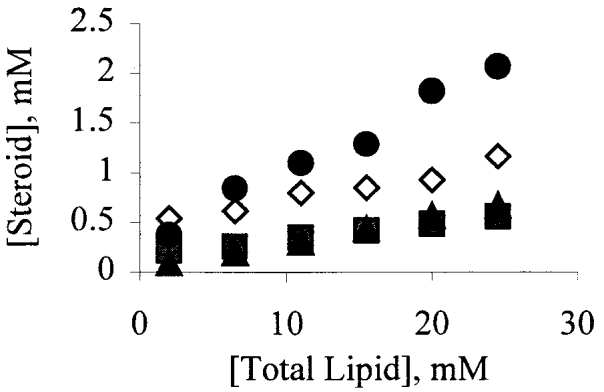

Fig. 2. A plot of the total concentration of steroid in solution as a function of total bile salt and phospholipid concentration for (▲) progesterone, (●) deoxycorticosterone, (◇) prednisolone, and (■)dexamethasone. Each point represents the mean ± SD, n = 3.

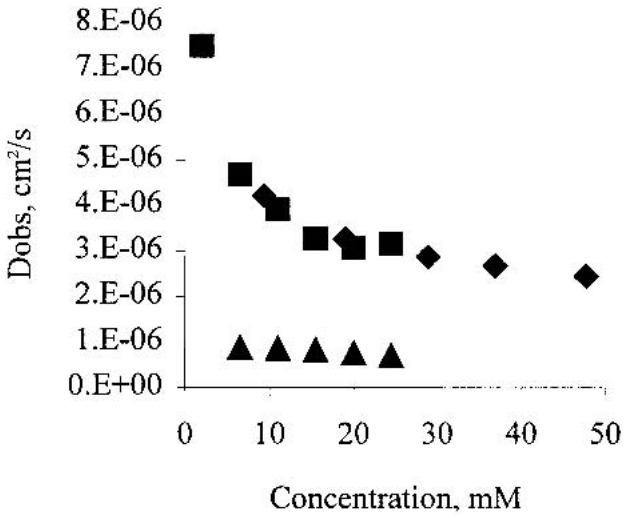

Fig. 3. A plot of the diffusion coefficient as a function of the total surfactant concentration for (■) simple micelles of TDC and mixed micelles of (▲) bile salts and (▲) PC. The size of the point reflects the typical fitting error.

cm^2/s at a concentration of 10 mM. As the concentration increased, the diffusion coefficient progressively fell and ultimately reached a value less than 3×10^{-6} cm^2/s at a concentration near 50 mM (Fig. 3). The change in the observed diffusion coefficient is related to the fact that TDC exists in solution as a monomer and in an aggregate (16). Moreover, the pulse sequence used in the NMR spectroscopic method allows for a diffusion time of 240 ms, during which TDC can exchange between the monomer and micelle. As such, the observed diffusion coefficient is the number weighted average diffusion coefficient of the monomeric and micellar TDC in solution. As the concentration increases, the time-averaged fraction of total TDC that exists in solution as a monomer decreases, and therefore the observed diffusion coefficient decreases from the value near the monomer in favor of the smaller micelle diffusivity. Fitting a plot of the product of the observed diffusion coefficient and total concentration of TDC as a function of TDC yields a slope that reflects the diffusion coefficient of the micelle. In doing so, the plot was linear, and the estimated diffusion coefficient of the micelle was 2.0×10^{-6} cm^2/s. Since TDC micelles have been reported to be polydispersed and the distribution depends on concentration (17), the micellar diffusion coefficient is perhaps best viewed as an average of all micelles in solution.

For the mixed micellar system, the presence of six different but structurally related bile salts along with phosphatidylcholine presents an added complication. While the resonances of the bile salts were clearly distinct from the resonance of the choline methyls arising from the PC, individual resonances arising from each bile salt could not be resolved. Therefore, only an average diffusion coefficient of some or all of the bile salts could be obtained. Like TDC, the observed diffusion coefficient fell with increasing concentration of lipid. In an analogous manner, this observation reflects the falling fraction of bile salt that is in solution as a monomer. However, because of the presence of six different bile salts, the fitting procedure failed to yield a reasonable diffusivity of the micelle.

Nevertheless, when PC is present, an alternative and more direct means of determining the diffusion coefficient of the micelle is available (16). Since the aqueous solubility of PC is extremely low, PC can be assumed to reside exclusively within the micelle. As such, the diffusion coefficient of PC can be equated to the diffusion coefficient of the micelle, since the free fraction is essentially zero. As can be seen in Fig. 3, the diffusion coefficient of PC was nearly constant over the entire concentration with an average value of 8.0×10^{-7} cm^2/s. From the Stokes-Einstein equation, the ratio of the diffusion coefficients of the TDC and mixed micelle yields the ratio of their sizes. Thus, the diameter of the mixed micelle is expected to be about 2.5 times larger than that of the TDC micelle.

Returning to the solubilization ratios, the greater solubilization of solutes in the mixed micelle may be related to its larger size. Nevertheless, other factors may be more important in determining the extent of solubilization. Specifically, the acyl chains of the phospholipid are much more flexible in comparison to the rigid frame of the steroid in the bile salt. This greater flexibility would be expected to accommodate the solubilizates better by providing more molecular contacts among the molecules (12).

Although the above theoretical considerations are important in examining the solubilization, the predictive power is of greater practical value. Recently, the pertinent literature of drug solubilization by bile salt simple and mixed micelles was reviewed (3). From that analysis, there appears to be a reasonable correlation between of micelle/water and octanol/water partition coefficients. Thus, the micelle/water partition coefficients were calculated from the solubilization ratios. The logarithm of the TDC micelle aqueous partition coefficients obtained in this study and those available from the literature are plotted as a function of the octanol/water partition coefficients (literature values, 3) as shown in Fig. 4.

The results obtained from the steroids fit reasonably well with the results available from the literature, which were largely obtained from a series of para-alkyl benzoic acid and para-alkyl aniline derivatives (13). From linear regression, the best fit line had a slope of 0.7375 and an intercept of 1.55 as shown in the figure. The correlation coefficient, r^2, was 0.91.

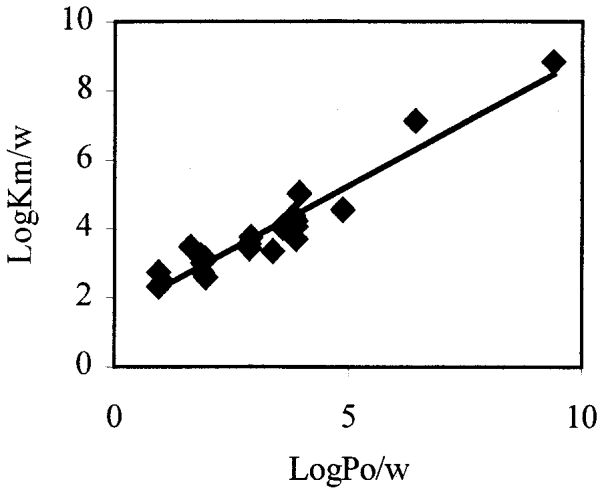

Fig. 4. A plot of the logarithm of the micelle/aqueous partition coefficient as a function of the logarithm of the octanol water partition coefficient for the steroids determined in TDC. Pooled data from present study and reference (3).

Thus, the objective of providing a larger data set for TDC was accomplished.

The analogous partition coefficients for the mixed micelles of this study were calculated along with the available literature values and were plotted as a function of the octanol/water partition coefficient. The results are shown in Fig. 5. The best fit line for the data set of the mixed bile salt system had a slope of 0.61 and an intercept of 2.44. The slopes and intercepts were compared in a pair-wise fashion using the *t* test at 95% confidence and were found statistically different. Although there is considerable scatter about the line, it is perhaps less than expected given the variability in terms of the composition of the mixed micelles used. There are other factors that can be included in future analyses, such as molecular size or surface activity to suggest a few possibilities, to further refine the correlation.

From the view of predicting the solubilization, it is evident that the *in vitro* bile salt system used will be important. The most important consideration in predicting solubilization in the intestine is to compare the available results with solubilization values found in the intestinal fluids. Such an effort is given in Fig. 6, where the solubilization of hydrocortisone is given for a number of different micellar systems. The contribution by Petersen *et al.* (5,6) is significant, since the measurement was made with aspirated human intestinal fluid. The observed solubility was greater than the other *in vitro* systems. However, only the total 3α-hydroxy bile salts were analytically determined. Moreover, the presence of bile salts without 3α-hydroxylation as well as neutral/zwitterionic lipids, such as mono-, di-, and tri-glycerides, cholesterol, phospholipids was not determined (14,15), yet these would dramatically affect the solubilization. As such, direct comparison of the present results with those of Petersen *et al.* is difficult. Moreover, it is perhaps evident that additional characterizations of the intestinal composition following normal dietary ingestion would be of value.

Examining the remaining values, there is reasonable agreement, each *in vitro* system will give significantly different predictions. Therefore, it becomes clearer that final selection of a model system will be important in determining the validity of the correlation. In addition, more compounds need to be evaluated in representative intestinal fluid compositions.

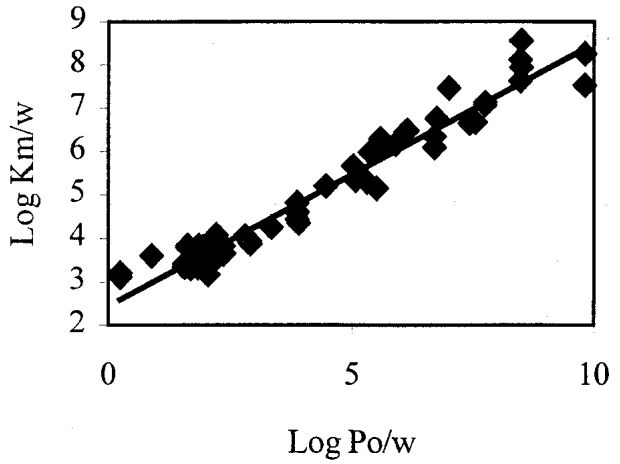

Fig. 5. A plot of the logarithm of the micelle/aqueous partition coefficient as a function of the logarithm of the octanol water partition coefficient for the steroids determined in mixed micelles. Pooled data from present study and reference (3).

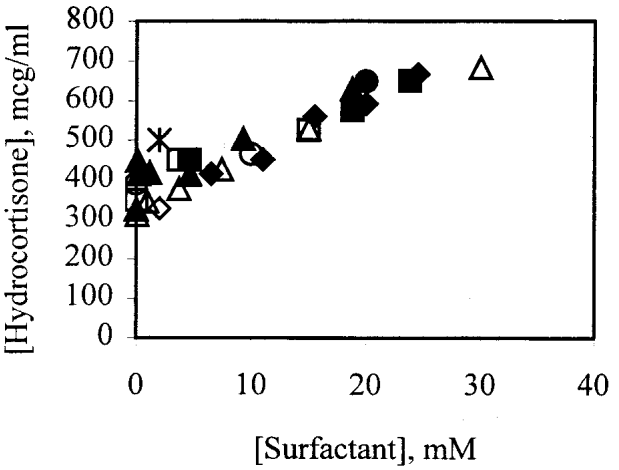

Fig. 6. A plot of the concentration of hydrocortisone in solution as a function of total surfactant concentration for (✳) intestinal solutions (5); (□) GC and (■) GC/PC mixtures (5); (○) TC and (●) TC/PC mixtures (10); (△) TC and (▲) TC/PC mixtures (8,9); and (◇) mixed bile salt and (◆) mixed bile salt/PC mixtures.

To date, there are extensive data sets of solubilization of drugs in simple micellar and mixed micellar systems representative of the intestinal composition. From the present study, the sets for TDC and a mixed micellar system of physiological concentrations of bile salts has been obtained. Thus, it should be possible to obtain a predictive relationship between the *in vitro* and *in vivo* solubilization with additional information concerning the bile salt/lipid concentration in the human intestine.

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