# Solubilization of Retinoids by Bile Salt/ Phospholipid Aggregates

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***Purpose.*** The capacity and specificity of bile salt (BS)/phosphatidylcholine (PC) mixed lipid aggregated systems in solubilizing four structurally related retinoids, etretinate, motretinid, fenretinide and N-ethyl retinamide, were determined.

***Methods.*** Excess solid drug was dispersed into sodium taurocholate (NaTC)/egg PC systems at lipid ratios of 10:0, 10:2 and 10 mM:10 mM in isotonic HEPES buffer, pH 6.5. A sensitive HPLC method was used to quantify the amount solubilized. The melting point and associated enthalpy change as well as the aqueous solubilities were also measured.

***Results.*** The retinoids had aqueous solubilities of less than 25 nM. The predicted aqueous solubility was less than 0.01 nM. The amount of retinoid in 10 mM NaTC was increased from three to four orders of magnitude relative to the aqueous solubility. Further increases in the amount solubilized were observed in the 10:10 mixed micelle dispersion. Fenretinide and N-ethyl retinamide were particularly well solubilized by BS and BS/PC aggregated systems which may be related to the presence of a cyclohexenyl ring.

***Conclusions.*** The discrepancy between the observed and predicted aqueous solubility may be due to self-association of the retinoids. Micellar/aqueous distribution ratios appear to be dominated by the hydrophobic effect, although specific interactions also are important. In considering intestinal absorption, the large increase in solubilization with BS/PC micelles would be capable of dramatically increasing the bioavailability in spite of the smaller effective diffusivity of the solubilized retinoid.

**KEY WORDS:** retinoids; bile salt; solubilization, phospholipids; micelle.

## INTRODUCTION

The naturally occurring mixed lipid aggregates in the intestine are referred to as bile salt mixed micelles (1,2). It is believed that these colloids interact with emulsified and enzymatically hydrolyzed dietary lipids and thereby are responsible for the absorption of ingested fat (3,5). In addition, this natural colloidal system has long been an important concern in the analysis of the intestinal absorption of drugs (6). Recently, efforts have been made to identify more clearly the effect of bile salt and bile salt/phospholipid systems on the dissolution rates of poorly water soluble drugs (7,8). Furthermore, there are extensive studies addressing the dissolution of cholesterol by these systems as it relates to the treatment of gall stones (9,10). Although these studies indicate the importance of surface interactions on the dissolution rates, the capacity and specificity of bile salt mixed lipid systems to solubilize drugs are not known.

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Retinoids are analogs of Vitamin A (retinol) which may be useful in cancer chemoprevention and treatment (11). Because of their low water solubility, the oral absorption is of particular concern since the structural modifications which enhance pharmacological activity (12) may lead to a decrease in aqueous solubility with a resultant decrease in bioavailability. However, bile salt micelle solubilization and transport is known to be important for the absorption of retinol as well as other water-insoluble dietary compounds (13,14). A better understanding of the structural requirements for solubilizing drugs in bile salt micelles represents the first step in investigating the broader issue of intestinal absorption of poorly water-soluble drugs (15). Therefore, the capacity and specificity of bile salt mixed lipid systems in solubilizing retinoids have been investigated.

## EXPERIMENTAL

### Solid State Characterization of Retinoids

The structures of the retinoids are given in Figure 1. Motretinid (MOE), etretinate (ET), and N-ethyl retinamide (RET) were received as gifts from Hoffmann-La Roche Inc., (Nutley, NJ) while fenretinide (4-HPR) was obtained from R. W. Johnson Pharmaceutical Research Institute (Raritan, NJ). All of the retinoids were used as received. Differential scanning calorimetry (DuPont 910, Wilmington, DE) was used to measure the melting point and heat of fusion of each retinoid both before and after the solubilization study to detect the occurrence of solid state polymorphic transitions during the solubility study. The scanning rate was 10 °C/min. Hot stage microscopy (WILD, Heerbrugg, Switzerland, M3Z stereomicroscope with a Mettler FP80 central processor for programmed temperature control and a Mettler FP82HT hot stage) was used to determine the nature of the phase change.

### Aqueous solubility

Aqueous solubilities were determined by dispersing 1–5 mg of solid drug in dialysis bags (Spectra, molecular weight cut-off: 3,500) containing 4–5 ml of 10 mM HEPES buffer, pH 6.5, in 0.9% NaCl with 2 mM EDTA. For RET and 4-HPR, 2 mM vitamin C was added to the buffer. All solutions in this study were repeatedly exposed to reduced vacuum followed by bubbling with argon. These were then placed in Pyrex test tubes with Teflon-lined screw caps, and the tubes were filled with about 10 ml of buffer. After equilibration in a shaking water bath for 24 hrs at 37 °C in the dark, the bags were removed. The remaining solution volume was determined by weight. An internal standard (20 μl) was added to each sample which was then extracted twice with hexane/isopropanol (3/1 v/v). The internal standard depended on the compound under study. 13-*cis*-retinoic acid was the internal standard (IS) for motretinid; motretinid was the IS for N-ethyl retinamide and fenretinide; and retinyl acetate was the IS for etretinate. The organic solution was dried under argon, reconstituted with 200 μl of acetone and placed into an amber autoinjection vial for HPLC assay.

The HPLC set-up consisted of a Waters (Milford, MA) Model 6000A pump, a WISP 710B autoinjector module, a Shimadzu (Kyoto, Japan) Chromatopac C-R6A detector, and a



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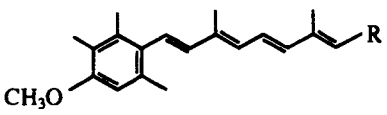
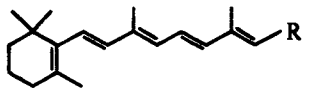
(A)

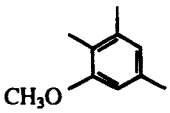



| | | | |
|---|---|---|---|
| $R = CONHC_2H_5$ | Motretinid | R = CONH–⬡–OH | Fenretinide |
| $R = COOC_2H_5$ | Etretinate | $R = CONHC_2H_5$ | N-ethyl retinamide |

(B)

$R_1$:




$= ⬡ + 2\,[o\text{-}CH_3] + [m\text{-}CH_3] + [p\text{-}OCH_3]$

$\Sigma \pi_{o/w} = 2.13 + 2\,(0.37) + 0.50 + (-0.02) = 3.35$

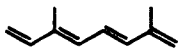
$\Sigma \pi_{hc/w} = 2.75 + 2\,(0.65) + 0.74 + (-0.10) = 4.69$

$= ⬡ + 3\,[o\text{-}CH_3]$

$\Sigma \pi_{o/w} = 2.86 + 3\,(0.37) = 3.97$

$\Sigma \pi_{hc/w} = 4.05 + 3\,(0.65) = 6.00$

$R_2$:

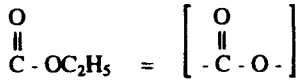


$= 4\,[\text{-CH=CH}] + 2\,[\text{-}CH_3] + 2\,[\text{branch}]$

$\Sigma \pi_{o/w} = 4\,(1.27) + 2\,(0.41) + 2\,(-0.20) = 5.50$

$\Sigma \pi_{hc/w} = 4\,(1.30) + 2\,(0.65) + 2\,(-0.20) = 6.10$

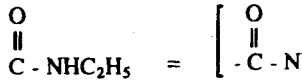
$R_3$:



$= [\text{-C(=O)-O-}] + [\text{-}C_2H_5]$

$\Sigma \pi_{o/w} = (-1.19) + 2\,(0.41) = -0.37$

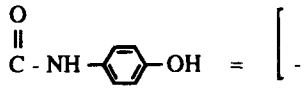
$\Sigma \pi_{hc/w} = (-0.80) + 2\,(0.65) = 0.50$



$= [\text{-C(=O)-NH-}] + [\text{-}C_2H_5]$

$\Sigma \pi_{o/w} = (-1.92) + 2\,(0.41) = -1.10$

$\Sigma \pi_{hc/w} = (-4.65) + 2\,(0.65) = -3.35$



$= [\text{-C(=O)-NH-}] + [⬡] + [\text{-OH}]$

$\Sigma \pi_{o/w} = (-1.92) + 2.13 + 0 = 0.21$

$\Sigma \pi_{hc/w} = (-4.65) + 2.75 + (-3.50) = -5.40$

**Fig. 1.** (A) Structures of the retinoids; (B) $\pi$ values for $R_1$, $R_2$, and $R_3$ groups in the octanol-water and cyclohexane-water systems.

Shimadzu CR501 integrator. A reversed-phase $C_{18}$ Supelcosil, 4.6 mm I.D., 5 μm column preceded by a guard column containing LC-18 pellicular packing (both from Supelco, Bellefonte, PA) was used with UV detection at 350 nm. A 15 cm column was used for etretinate and motretinid whereas a 25 cm column was used for N-ethyl retinamide and fenretinide. The mobile phase was delivered at a flow rate of 1.5 ml/min. For etretinate the mobile phase was prepared by adding 0.8 g ammonium acetate and 10 ml acetic acid to a liter of 84% (v/v) acetonitrile in water; for motretinid, 0.86 g ammonium acetate and 2.1 ml acetic acid were added to a liter of 65% acetonitrile in water; for N-ethyl retinamide and fenretinide, 0.8 g ammonium acetate and 10 ml acetic acid were added to a liter of 78% acetonitrile in water (16).

### Aggregate Preparation

Solubilization experiments were conducted with sodium taurocholate (NaTC) (Sigma Chemical Co.), which was recrystallized from ethanol/ethyl acetate (17). Egg phosphatidylcholine (egg PC) was obtained from Avanti Polar Lipids. Both NaTC and egg PC moved as single spots on silica plates with a mobile phase of 65:35:5 chloroform:methanol:water. The NaTC/egg PC solutions were prepared in the same buffer as indicated in the aqueous solubility studies. For solubilization, as much as 10% degradation occurred based on the relative peak areas of the HPLC. The addition of 1:1000 mole ratio of BHT to phospholipid was found to improve the stability (< 2% decomposition) of the retinoids with no detectable effect on the solubility results. In the micellar solubility studies, the concentration of NaTC was kept constant at 10 mM, and the concentration of egg PC was varied to achieve the molar ratios of 10:0, 10:2, and 10:10. Experiments were conducted by first lyophilizing egg PC from an ethanol/cyclohexane mixture. From the measured dry weight, the appropriate weight of 10 mM NaTC was added to achieve a 10:10 molar ratio of NaTC:egg PC. The 10:2 ratio was prepared by combining the 10:10 and 10:0 in a 1:4 weight ratio. The mixed lipid solutions were allowed to equilibrate in excess of 24 hours at room temperature before use. Aggregates were characterized by pulsed-field gradient spin echo NMR diffusion measurements as described elsewhere (18).

### Solubilization Measurement

For determining the micellar solubility, 1–5 mg of solid drug was added to the mixed lipid solutions. The suspensions were placed in Teflon-lined screw-cap test tubes and then saturated with argon. After the tubes were covered with foil, they were placed in a shaking water bath at 37°C. The amount of etretinate in solution was monitored for five days, and equilibrium was attained in 12 hours. Therefore, the time for equilibration was set at 24 hours. After the test tubes were centrifuged in a table top centrifuge for about 15 minutes to sediment solid drug, aliquots were taken. The sample preparation for HPLC involved an addition of 20 μl of internal standard and a simple dilution by a factor of 10 with acetone, centrifugation and direct injection of the supernatant on a reversed-phase column. The HPLC assay conditions were identical to those described above. Standard curves of six different concentrations prepared in triplicate were used to determine the recovery for each retinoid at each of the three NaTC/egg PC compositions. The mean percentage recovered was approximately 100%, and the standard deviations ranged from 0.3 to 8%.

### Unitary Distribution Ratio

The unitary distribution ratio, K, was calculated by dividing the mole fraction solubility in the micelle by the mole fraction solubility in the water.

$$K = \frac{X^{mic}_{solub}}{X^{aq}_{solub}} = \frac{\dfrac{n^{mic}_{solub}}{n^{mic}_{solub} + n^{mic}_{lipid}}}{\dfrac{n^{aq}_{solub}}{n^{aq}_{solub} + n^{aq}_{lipid} + n_{H_2O}}} \quad (1)$$

where X is the mole fraction solubility of the solubilizate, n is the number of moles in the solution, the superscript, aq, and, mic, refer to the aqueous and micellar phases, respectively, the subscript, solub, and, lipid, refer to solubilizate and bile salt and/or phospholipid.

### Estimation of Octanol-Water and Cyclohexane-Water Partition Coefficients

The group contribution approach was used to estimate the octanol-water, $P^c_{o/w}$, and cyclohexane-water partition coefficients of retinoids, $P^c_{hc/w}$, in terms of the molar concentration scale (19–22). For consistency, the retinoid molecule was divided into three parts: $R_1$ (ring portion), $R_2$ (middle portion, i.e., alkene linkage) and $R_3$ (terminal ester or amide group) as shown in Figure 1. The contributions to the free energy of transfer for $R_1$, $R_2$ and $R_3$ were calculated based on the additive-constitutive properties of all the functional groups in each part (19–22). The logarithm of the partition coefficient of retinoid was then calculated as the algebraic sum of the $\pi$ values of the component groupings of the molecule (22):

$$\log P^c = m[\Sigma\, \pi(R_1, R_2, R_3) + \Sigma\, \pi(\text{interaction}(R_1, R_2, R_3)] + b \quad (2)$$

where $\pi$ interaction accounts for the interaction between two polar groups attached to the same carbon atom or to adjacent carbon atoms, m is equal to 1, and b is 0 for the octanol-water and 1.3 for cyclohexane-water partition coefficients. When expressed in terms of mole fraction concentration, $P^x$, the partition coefficient is related to that expressed in terms of molar concentration, $P^c$, by $P^x = P^c\,(V_s/V_w)$, where $V_s$ and $V_w$ are the molar volume of the organic solvent and water, respectively (19).

### Estimation of Aqueous Solubility

The aqueous solubilities of the retinoids $C^s$ were estimated using the equation of Yalkowsky and Valvani (23):

$$\log C^s = -1.00 \log P^c_{o/w} - 1.11 \cdot \frac{\Delta S_f(\Theta_m - 37)}{2.303\ R\ 310.15} + 0.54 \quad (3)$$

where $\Delta S_f$ is the entropy of fusion, which was calculated by dividing the heat of fusion by the absolute melting temperature, $\Theta_m$ is the melting temperature (°C), and R is the gas constant.

## RESULTS AND DISCUSSION

Whereas extensive studies have been conducted addressing aqueous solubility, there are few studies that systematically address the solubilization of drugs by bile salt/phospholipid aggregates (15). Since these aggregates are present in the intestine, a better understanding of their influence on the solubility and solubilization of drugs would be useful for improving oral drug delivery. The retinoids serve as model compounds that demonstrate poor water solubility. In addition, they are structural analogs of the naturally occurring compound vitamin A. The results obtained demonstrate that the extent of solubilization of retinoids in bile salt/phospholipid aggregates is very sensitive to relatively minor structural changes.

Aside from the implications for oral drug delivery, retinoids represent a challenging series of drugs for determining solubility and solubilization. The poor chemical stability coupled with their poor wetting characteristics and extensive loss due to surface adsorption all cause technical difficulties. The use of dialysis membranes in the measurement of the aqueous solubility led to relatively rapid equilibration, and thus the chemical stability problem was minimized. It was also superior to approaches involving filtration which resulted in extensive adsorption of the drug to the filter. During dialysis any drug loss of due to adsorption was apparently compensated by additional dissolution of the excess solid drug. The addition of bile salt eliminates the wetting problem, therefore, centrifugation was sufficient to sediment the solid drug in the solubilization measurements.

### *Aqueous Solubilities of Retinoids*

The measured aqueous solubilities are given in Table I. The values were very low, and the rank order of the aqueous solubilities was N-ethyl retinamide > fenretinide > motretinid > etretinate. Motretinid and etretinate are the ethyl amide and ethyl ester pair possessing an aromatic ring with a methoxy moiety. Thus, the only structural difference is the amide as opposed to an ester, yet there is a two-fold difference in the observed aqueous solubility. N-ethyl retinamide and fenretinide had higher aqueous solubilities in comparison to motretinid and etretinate, and they contain a cyclohexenyl ring in place of the aromatic ring.

### *Predicted Aqueous Solubilities*

To obtain a prediction of the aqueous solubility, thermal analysis was performed and the octanol/water partition coefficients were estimated. The melting points and associated enthalpies are given in Table I. Both etretinate and fenretinide had a single, well-defined phase change. For motretinid, there was a small initial endotherm, followed by a smaller exotherm, and finally another large endotherm. This suggests the presence of a metastable solid. With visual inspection of the transition, liquid was only observed with the final endotherm indicating that the earlier transition was a solid-solid phase change. N-ethyl retinamide underwent decomposition.

The method to estimate of the partition coefficients is shown in Figure 1, and the values are given in Table I. The $P^c_{o/w}$ of fenretinide was higher than that of motretinid by about two orders of magnitude and was about 16–20 times higher than those of etretinate and N-ethyl retinamide. The polar amide group and hydroxyl moiety are largely responsible for these differences. The estimated aqueous solubilities based on Eq. 3 are also given in Table I, and the rank order is different from that observed with the measured solubilities.

A decrease in the melting point, entropy of fusion or octanol/water partition coefficient should lead to a higher solubility as indicated in Eq. 3 (23,24). However, etretinate had the lowest melting point and entropy of fusion but still had the lowest aqueous solubility. While the solvation term leads to a less favorable interaction of etretinate with water in comparison to that predicted for the amides, it represents a relatively small contribution to the predicted solubility (Table I). Not only do the predicted values suggest that there should be a greater difference in the observed solubilities for motretinid and etretinate, but also the observed values for these compounds are more than an order of magnitude greater than the predicted solubilities.

For motretinid, there exists the possibility that the aqueous solubility was determined with a metastable polymorphic form, since a solid-solid phase transition was observed with DSC. This other solid phase may also be more stable at the temperature of the solubility measurement. The use of a metastable polymorphic solid phase may have caused some discrepancy between the predicted and observed solubility for motretinid as well as the differences between etretinate and motretinid. Of course, if equilibrium was achieved in the samples, then there would be conversion from the unstable to the stable polymorph. However, there was no change in the thermal properties of recovered motretinid which may indicate that equilibrium was not achieved probably due to the expected slow nucleation rate.

While polymorphism may account for motretinid, large discrepancies remain between the observed and predicted solubilities for etretinate and especially fenretinide. The thermal behavior of the recovered solid of the other retinoids was unchanged suggesting that the solid state phase was unaltered. Analysis of the specific functional moieties does not provide a rationale for the failure of the theoretical predictions. This is

**Table I.** Melting Points, Enthalpies of Fusion, Octanol-water Partition Coefficients (Molar Concentration Scale), Predicted Aqueous Solubilities (nM) of Retinoids, and Assayed Concentrations (Mean ± SD, n ≥ 3, in nM) of the Aqueous Solubilities

| Retinoid | Tm (°C) | ΔH (J/g) | $P^c_{o/w}$ | Aq. solubility (predicted) | Aq. solubility (measured) |
|---|---|---|---|---|---|
| Motretinid | 169, 184 | 146 | $5.62 \times 10^7$ | 0.0832 | 6.5 ± 0.3 |
| Etretinate | 106 | 95.4 | $3.02 \times 10^8$ | 0.813 | 3.4 ± 0.3 |
| Fenretinide | 175 | 115 | $6.46 \times 10^9$ | 0.00186 | 12.8 ± 2.0 |
| N-ethyl retinamide | dec | — | $3.16 \times 10^8$ | — | 23.5 ± 2.4 |

in spite of the general reliability of the approach for other compounds with equally poor water solubility (1). One possibility is that these compounds form aggregates in solution. Although aggregation has also been suggested to occur with retinoic acid (25), further study of the aggregation of these compounds is hampered by the low total concentration in solution. In summary, the discrepancy between the observed and predicted solubilities may be a result of self-association of the retinoids.

*Micelle Aggregation*

Prior to discussing the solubilization results, the salient features of the bile salt/phospholipid aggregates should be given. For sodium taurocholate aggregates, it has been suggested there is a broad distribution of sizes with the aggregation process occurring in a stepwise fashion (2,26). That is, the primary micelle, consisting of 2 to 8 monomers, forms from back-to-back association of the non-polar surfaces of the bile salt molecules. The polar surface containing the three α-hydroxyl groups remains in contact with water. At higher bile salt concentrations, larger secondary micelles are formed from the association of the primary micelles. This association is believed to be a result of hydrogen bonding among the primary micelles. For 10 mM NaTC, most of the BS is in primary micelles with an aggregation number of 4 (1).

With the addition of phosphatidylcholine, a unique aggregate is suggested to exist in solution which is referred to as a mixed micelle. The accepted structure of the mixed micelle has changed over the years. Small's bilayer disk model (27) was superseded by the mixed disk model introduced by Mazer *et al.* (28). More recently, a rod model has been proposed (29). In this model, bile salt and phospholipid molecules are oriented radially to the long axis of the rod with their head groups facing outward toward the water. This proposed structure is analogous to a rod-shaped surfactant micelle. Bile salts insert between and separate the phospholipid head groups to provide sufficient curvature for the radial orientation. The hydrophobic ends of the rods are presumably covered by bile salt-rich caps.

*Solubilization: Simple Micelles*

The results for the solubilization study in the simple BS system are given in Table II. For the 10:0 system, very unusual results were obtained. The extent of solubilization ranged from 1.8 μM to over 42 μM. The cyclohexenyl ring of fenretinide and N-ethyl retinamide appeared to correlate with the higher solubilization.

The micelle solubilization process can be conceptualized as a result of three factors (30). One is the transfer of the solute from water to a hydrophobic environment. The second is a correction which arises from the small radius of curvature of the micelle. The third is the change in the interfacial energy brought about by the adsorption of the solute to the micelle/aqueous interface. To interpret the solubilization results, the distribution ratios between the micellar and aqueous phase as well as the hydrocarbon/water partition coefficient are needed.

The distribution ratios between water and simple micelles are shown in Table III. They ranged from $0.621 \times 10^7$ for etretinate to $3.88 \times 10^7$ for fenretinide. The calculated cyclohexane-water partition coefficients of retinoids in the mole fraction scale are also given in Table III. The correspondingly large values of both the micellar distribution ratios and the partition coefficients indicate the importance of the hydrophobic environment of the micelle for solubilization. Thus, even in the simple micelle solution which has an aggregation number of four, the hydrophobic effect appears to be an important factor in the solubilization.

However, the hydrocarbon-water partition coefficient of retinoids failed to correlate with the distribution ratios between the simple micelle and aqueous phases (Table III). These inconsistencies may be due to the rigid environment formed by the bile salt molecules as compared to the flexible structure of hydrocarbon molecules. The hydrocarbon-water partition coefficients typically underestimate the micelle-water distribution ratios, which has been used as support for the existence of a Laplace pressure (30). Alternatively, Moroi (31) has suggested that micelles restrict the mobility of the solubilizate leading to a less favorable transfer to micelles in comparison to bulk hydrocarbons. It is also noteworthy that the estimated octanol/water partition coefficient given in Table II also failed to correlated with the distribution ratios between the simple micelle and aqueous phases.

Because of the small size of the micelle, the extent of micelle solubilization is generally inversely related to molecular size (30). However, all the retinoids have similar molecular weights, thus little difference among the retinoids would be anticipated. For the 10:0 system, even with the most extensively solubilized retinoid, fenretinide, the molar ratio of micelle to solubilized drug is larger than 110. Thus, many simple micelles are needed to solubilize one retinoid. In this respect, retinoids are comparable to other drugs in studies of solubilization by the bile salt micelle (15,32).

In considering the interfacial energy, the extent of micelle solubilization is generally enhanced by the adsorption of the solubilizate. The hydroxyl groups of the bile salts may make the polarity and hydrogen bond-forming properties of the solubilizates critical. The ethyl amide group of motretinid has one

**Table II.** Assayed Concentrations (mean ± SD, n ≥ 6, in μM) of the Solubilization Study of Retinoids in Simple and Mixed Micellar Systems at 37 °C and Calculated Results for the 10:2 Mixed Micellar System

| | NaTC:Egg PC ratio (mM:mM) | | | Calculated |
|---|---|---|---|---|
| Retinoid | 10:0 | 10:2 | 10:10 | 10:2 |
| Motretinid | 10.2 ± 2.5 | 16.7 ± 3.1 | 32 ± 4 | 14.5 |
| Etretinate | 1.8 ± 0.6 | 31.9 ± 2.5 | 163 ± 17 | 34.0 |
| Fenretinide | 42.4 ± 4.9 | 95.4 ± 3.1 | 870 ± 31 | 208 |
| N-ethyl retinamide | 18.0 ± 1.4 | 220.0 ± 8.0 | 771 ± 28 | 167 |

**Table III.** Hydrocarbon-water Partition Coefficients, $P^x_{hc/w}$, (Mole Fraction Concentration Scale) Predicted by the Group Contribution Method, and Distribution Ratios Calculated from Mole Fraction Solubilities in the Micellar and Aqueous Phases

| | Motretinid | Etretinate | Fenretinide | N-ethyl retinamide |
|---|---|---|---|---|
| $P^x_{hc/w}$ | $0.83 \times 10^7$ | $5.89 \times 10^{10}$ | $1.51 \times 10^6$ | $1.70 \times 10^8$ |
| K, simple micelle/aqueous | $1.84 \times 10^7$ | $6.21 \times 10^6$ | $3.88 \times 10^7$ | $8.98 \times 10^6$ |
| K, mixed micelle/aqueous | $1.58 \times 10^7$ | $1.56 \times 10^8$ | $2.20 \times 10^8$ | $1.06 \times 10^8$ |

proton to donate as well as two proton-accepting sites; however, the ethyl-ester group of etretinate acts only as a proton acceptor. Thus, motretinid is expected to be more surface active than etretinate. The cyclohexenyl ring of N-ethyl retinamide is less polar than the aromatic ring of motretinid, therefore, N-ethyl retinamide is also less surface active than motretinid. Fenretinide has a phenyl-amide group which possesses three proton-accepting sites as well as two protons to donate. The phenyl-amide group gives fenretinide more surface activity than motretinid in spite of the cyclohexenyl ring. Thus, the expected surface activity of the retinoids, 4-HPR > MOE > RET ≈ ET, gives a better indication of the observed distribution ratios. This also correlates somewhat with the dramatic solubilization of bile salts observed with the surface active fatty acids and even phosphatidylcholines (1).

*Solubilization in Mixed Micelles*

The distribution ratios between the mixed micellar and aqueous phases are shown in Table III. They ranged from $1.58 \times 10^7$ to $2.2 \times 10^8$. The mixed lipid aggregate greatly enhances the extent of solubilization of the retinoids. The first important point to note is the difference in the total lipid concentration used in these experiments of 10:0 and 10:10. Although the presence of more lipid will lead to greater solubilization, the increase in the concentration solubilized ranged from a factor of three for motretinid to over a factor of 90 for etretinate (Table II). The larger distribution ratios of the retinoids in mixed micelles compared to the simple micelles further demonstrate that BS/PC mixed micelle is a better solubilizer than the BS simple micelle. In addition, fenretinide and N-ethyl retinamide have much greater solubilities in the mixed micelles than motretinid and etretinate. It would seem that the presence of the cyclohexenyl ring is associated with greater solubilization.

In terms of the molecular interactions, phospholipids contain two flexible acyl linked fatty acid chains. As noted with cholesterol, the flexibility of the acyl chains is expected to be able to accommodate solubilizates much better through the formation of close van der Waal contacts (9,10). Another important difference is that the mixed micelle is larger than the simple micelle. Because of the larger radius of curvature, greater solubilization would be expected (30,31). The final point of interest is the similarity of the distribution ratios in the mixed micellar system. Evidently, the mixed micelle system is less discriminating in solubilizing drugs, perhaps due to the presence of two lipid components in the micelle.

*Solubilization in Coexisting Simple and Mixed Micelles*

In the 10:2 system, Mazer *et al.* (28) have proposed that there is coexistence of monomers, simple BS micelles, and BS/PC mixed micelles. Thus, the extent of solubilization of the retinoids in the 10:2 system is given by the weighted arithmetic average of the solubilization in the 10:0 and 10:10 systems.

$$C_{ret(10:2)} = C_{SM}X_{ret(10:0)} + C_{MM}X_{ret(10:10)} \quad (4)$$

where $C_{ret\ (10:2)}$ is the extent of solubilization of retinoid, $C_{SM}$ and $C_{MM}$ are the lipid concentrations of simple, SM, and mixed micelles, MM, and $X_{ret(10:0)}$ and $X_{ret(10:10)}$ are the mole fraction solubilities of retinoid in the 10:0 and 10:10 systems. The lipid concentrations in the 10:2 system was estimated from Mazer *et al.* (28) where the total concentration of bile salt in mixed micelles was estimated to be 3.08 mM and the bile salt monomer concentration was assumed to be 4.75 mM (18). The calculated solubilizations of retinoids in the 10:2 system are reasonably close to the measured solubilities for motretinid, etretinate and N-ethyl retinamide, but are lower by more than a factor of two for fenretinide (Table II). This perhaps is another indication that the presence of fenretinide influences the properties of the lipid aggregates such as the micelle-water interfacial tension.

*Implications for Bioavailability*

Considering the extremely low aqueous solubilities, the retinoids would be expected to have a negligible oral bioavailability; however, the value in humans is approximately 40% for etretinate (33). The effect of bile salt aggregates on the rate of dissolution and the transport across the intestinal diffusional boundary layer can be analyzed by examining the product of the effective diffusivity and the concentration gradient. For etretinate in aqueous solution, the product of the estimated effective diffusivity ($3 \times 10^{-5}$ cm$^2$/s) and solubility (0.0012 μg/ml) is $4 \times 10^{-8}$ μg/cm s (18). In a 10:0 dispersion, the product is $2 \times 10^{-6}$ μg/cm s, and in the 10:10, the product is $5 \times 10^{-5}$ μg/cm s. Therefore, the results of this study provide experimental support for a possible mechanism by which simple and mixed lipid aggregated systems influence the human bioavailability (33) as well as profoundly increase the transport rate of retinoids across the diffusional boundary layer of the intestine (34). For the better solubilized retinoids, N-ethyl retinamide and fenretinide, the effect would be proportionately greater. These calculations are independent of any wetting effect of the simple bile salt micelles on the solid surface that would increase the dissolution rate and thereby the rate of absorption (8,9).

## CONCLUSION

Solubilization studies of four structurally related retinoids have been carried out in simple BS and mixed BS/PC aggregated systems. All of the retinoids had low aqueous solubilities, which was greater than predicted, possibly due to self-association. A

physiologically relevant concentration of 10 mM NaTC consisting of simple BS aggregates markedly increased the amount of drug in solution. Transfer of the solute from the aqueous phase to the hydrophobic micelle appears to be the most important factor in determining the solubilization. The BS/PC mixed lipid system had a more pronounced effect on solubilization. The larger size and the presence of a structurally flexible environment provided by the phospholipids may contribute significantly to the capacity of micelle solubilization. Consideration of both the extent of micelle solubilization as well as the effective diffusivity of the retinoids leads to the conclusion that bile salt/phospholipid aggregates are capable of profoundly increasing the transport rate of retinoids across the diffusional boundary layer of the intestine.

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