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Research Paper

Mass Transport Properties of Progesterone and Estradiol in Model Microemulsion Formulations

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Purpose. The purpose of these studies was to determine the extent to which drug loading influences the mass transport characteristics of poorly soluble steroids from model microemulsion formulations *in vitro*.
Methods. Two conditions of drug loading in the microemulsions were tested, "near saturation" and "constant loaded." Mass flux of ^{3}H-labelled progesterone or estradiol was measured in a side-by-side diffusion chamber from microemulsions consisting of Brij 97, Miglyol 812 and water. Pulsed gradient NMR was used to measure the diffusivities of all components. The thermodynamic activity and fraction of free drug in the formulations were measured by polymer uptake. Solute flux was calculated employing an aqueous boundary layer model.
Results. Under near saturation loading, all microemulsion formulations showed significantly increased flux of steroids compared to the saturated aqueous solution. For both steroids flux values in the 0.5 and 1% systems were significantly lower for the 3% oil formulation, despite the observation that the 3% formulation held significantly more drug. On the other hand, for all the formulations under constant drug loading, solute flux was only moderately increased for progesterone and not at all for estradiol when compared to the saturated aqueous solution. Under both loading conditions, thermodynamic activities did not correlate to flux indicating some other factor was modulating mass transport. Effective diffusivities of the steroids in formulations as determined by NMR were significantly reduced compared to those of the monomer drug in aqueous solution. In both near-saturated and constant-loaded conditions, the calculated values for progesterone flux were markedly similar to those observed experimentally suggesting solubilization and diffusion events in the aqueous boundary layer had a strong influence on mass transport. In contrast, calculations for estradiol were less successful in modeling the observed flux values.
Conclusions. In systems nearly saturated with drug, the microemulsion formulation leads to a greatly enhanced rate of steady-state mass transport while in systems with drug loading far from saturation, the microemulsion formulation appears to have a minimal ability to promote mass transport. The aqueous boundary layer diffusion model was successful in fitting progesterone results but was not successful for estradiol.

KEY WORDS: aqueous boundary layer; mass transport; microemulsions; steroid drugs.

INTRODUCTION

Any new chemical entity that may be classified as poorly water-soluble will present a major challenge during formulation (1,2). One alternative formulation approach for poorly water-soluble compounds is the lipid-based drug delivery systems, including, but not limited to, self-emulsifying and self-microemulsifying systems (1,3,4). Some lipid-based systems have been shown to improve the rate and extent of absorption of water-insoluble drug compounds, as well as resulting in more reproducible blood-time profiles (1–5). Although the utility of lipid-based formulations for oral delivery of poorly water-soluble compounds has been recognized for many years, only a few lipid-based products have been commercialized to date (3) with others soon to be introduced. One reason that lipid-based oral systems have not yet achieved their full potential may be a poor understanding of the fundamental mechanisms, either biochemical (6,7) or physicochemical (8,9) responsible for absorption enhancement *in vivo*. In a practical sense, the selection and formulation process is not always perfect. Some water-insoluble drug molecules formulated in a lipid-based system have not shown improved bioavailability (1).

From a physicochemical standpoint it has been suggested that lipid-based delivery systems may result in enhanced mass transport of drug across a biological membrane by solubilizing greater amounts of drug in the donor

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Table I. Formulations and Solubilities of Steroids in Formulations at 27°C

Formulation	Miglyol % w/w	Brij 97 %w/w	Deionized water	Solubility Progesterone[a] ($\times 10^{-3}$ mole/l)	Solubility Estradiol[a] ($\times 10^{-3}$ mole/l)
3ME	3	22	75	18.6 ± 0.3	11.4 ± 0.1
1ME	1	10	89	7.6 ± 0.1	7.2 ± 0.2
0.5ME	0.5	10	89.5	7.4 ± 0.2	6.8 ± 0.1
Deionized water	0	0	100	0.038 ± 0.001	0.011 ± 0.001

[a] Mean ± S.D., $n = 3$.

phase (1,10–12). At first glance, this proposed mechanism would appear to be a reasonable one, but the details and physicochemical implications remain unexplored, most notably whether partitioning and diffusion of drug and oil droplets in the aqueous boundary layer can modulate the mass transport. The current study is designed to characterize the mass transport properties of poorly water-soluble compounds from a model lipid-based system. Toward that end, we have employed an approach that combines flux studies with characterization of the thermodynamic activities of the solutes, the ability of the solutes to partition into the oily droplet and the diffusivities of the drugs both in the monomer and in the droplet. The effect of partitioning and diffusivity was probed by studying formulations that were loaded with drug to varying extents. Model calculations were carried out to probe the extent to which an aqueous boundary layer model can explain experimentally observed flux values. The overall goal is to obtain a better understanding of the physicochemical mechanisms involved in mass transport from lipid-based systems with the hope that such information may be helpful to formulation scientists in the rational and cost-effective design of optimized lipid-based drug delivery systems.

MATERIALS AND METHODS

Formulations

As listed in Table I, three model microemulsion formulations consisting of Brij 97 (Aldrich, Inc) as the surfactant, Miglyol 812 (medium chain triglyceride oil; Sasol, Germany) as the oil phase and water (de-ionized) as the aqueous phase were prepared. In addition to the surfactant and oil components many lipid-based delivery systems also contain other ingredients, including co-surfactants and co-solvents. We have chosen to examine the simple Brij 97-Miglyol-Water-steroid formulations without additional ingredients because this system presented less experimental difficulties in the NMR and thermodynamic activity experiments. Preparation of each microemulsion was carried out by first combining the surfactant and oil components followed by slow addition of the aqueous phase and mixing well. Model steroids progesterone and 17-β-estradiol (Sigma Chemical) were added to the formulations at the appropriate concentrations either by dissolving in the oil phase prior to microemulsification, or alternatively, by dissolving in the microemulsion after formation. The order of addition of the model solutes had no effect on the outcomes of the experiments. Systems were permitted to age overnight at 27°C under stirring before additional experiments were carried out. Phase diagrams of the three microemulsion systems in the absence of model steroids (not shown) were consistent with those published by Malcolmson and Lawrence (13). The choice of 27°C as the temperature of the study was dictated by the capability of the NMR spectrometer, outlined below.

Two sets of formulations based on drug content were prepared (Table II). In the first set, known as "near-saturation loaded," the concentration of drug in each formulation was set at 90% of saturation solubility in that system. The second set, termed "constant loaded" employed the same concentration of steroid in each formulation (progesterone, 1.4×10^{-3} mol/l; estradiol 1.21×10^{-3} mol/l). The concentrations employed in the constant loading set were chosen to be about 100 × that of the saturated aqueous solution.

Table II. Observed Steady-State Flux and Concentrations of Model Steroids in Donor Compartment at 27°C

	Near saturation condition		Constant loaded condition	
Estradiol	Flux[a] ($\times 10^{-13}$ mole/cm^2-s)	Concentration in donor ($\times 10^{-2}$ mole/L)	Flux[a] ($\times 10^{-14}$ mole/cm^2-s)	Concentration in donor ($\times 10^{-3}$ mole/L)
3ME	1.35 ± 0.28	1.03	1.20 ± 0.02	1.2
1ME	2.60 ± 0.07	0.66	3.25 ± 0.01	1.2
0.5ME	3.07 ± 0.01	0.62	3.55 ± 0.2	1.2
Deionized water	0.37 ± 0.08	0.00092	–	–
Progesterone	Flux[a] ($\times 10^{-11}$ mole/cm^2-s)	Concentration in donor ($\times 10^{-2}$ mole/L)	Flux[a] ($\times 10^{-12}$ mole/cm^2-s)	Concentration in donor ($\times 10^{-3}$ mole/L)
3ME	1.10 ± 0.10	1.68	0.80 ± 0.10	1.4
1ME	2.01 ± 0.06	0.68	2.83 ± 0.05	1.4
0.5ME	2.47 ± 0.08	0.67	3.50 ± 0.01	1.4
Deionized water	0.06 ± 0.03	0.0034	–	–

[a] Mean ± S.D., $n = 4$.

Solubility of Steroids in Microemulsion Formulations

Solubilities of progesterone and estradiol in microemulsion formulations 3ME, 1ME and 0.5ME and in water were determined as outlined previously (14). The various formulations were exposed to excess solid progesterone or estradiol (Sigma Chemical) with an appropriate amount of ^{3}H-labeled drug in 4 ml glass vials with Teflon lined caps. Samples were blanketed with nitrogen gas, sealed, and rotated at 27°C. At predetermined time points, samples were withdrawn, filtered using a 0.2 μm nylon syringe filter and the filtrate assayed for ^{3}H-radioactivity by liquid scintillation counting (LSC).

Mass Transport

Temperature-controlled (27°C) side-by-side diffusion cells consisted of two glass chambers with a 4 ml volume separated by a silicone rubber membrane (Samco Silicon Products, U.K.) of area 0.95 cm^2 and thickness 300 μm. Although silicone rubber is a poor model of a biological membrane, it does afford several advantages including chemical stability and impermeability to surfactant and oil components. In double membrane studies, a very thin coating of laboratory silicone grease was employed to ensure good membrane contact. Donor solutions were prepared as listed in Table II. In all cases, the formulations contained a known trace amount of ^{3}H-labeled drug. Receiver solutions were prepared with the identical formulation, except no drug or radioactivity was added. The bulk solutions on each side of the membrane were well-mixed employing star-head magnetic bars and 300 rpm stirring. Appearance of ^{3}H labeled drug in the receiver chamber was monitored by LSC. Sink conditions were maintained at all times in the receiver solutions (<10% of drug transported). Samples removed from the receiver chamber were replaced with fresh, drug-free solution. Accumulated amount of drug in the receiver chamber was plotted as a function of time and steady-state flux was calculated as the slope from the linear part of the curve divided by the area of the membrane available for transport. Typically, the curves exhibited an initial non-linear region associated with a time period necessary to establish steady state flux, followed by the linear steady state region.

Thermodynamic Activity

The silicon rubber uptake method (15,16) was employed in the determination of thermodynamic activity (A_T) of each steroid in the microemulsion formulations and in deionized water. Briefly, a series of steroid solutions were prepared with tracer levels of ^{3}H-labeled solute. To each mixture, a know mass of minced silicone rubber membrane was added. After equilibration at 27°C, the polymer was separated from the supernatant, gently washed with water and assayed for radioactivity by liquid scintillation counting. The supernatant was similarly assayed for radioactivity. The concentration of steroid in both the supernatant ($C_{aq,eq}$) and the polymer ($C_{sp,eq}$) at equilibrium was expressed in terms of moles of drug per gram of sample. $C_{sp,eq}$ was plotted as function of SSI (saturation index),

$$SSI = C_{aq,eq}/C_{s,eq} \tag{1}$$

$C_{s,eq}$ is the solubility of the drug in the absence of the polymer in the corresponding formulation. Extrapolation of this plot to SSI = 1 resulted in a value for the concentration of steroid in the polymer at unit activity, C^O_{sp}. Linearity of this plot shows that the partitioning of lipophilic drug into silicone polymer occurs in direct proportion to thermodynamic activity of the solute in the formulation.

A similar procedure is employed for each solute to determine the thermodynamic activity of steroids in formulations employed in mass transport studies. The concentration of drug in the polymer is determined and the thermodynamic acidity is calculated by Eq. (2).

$$A_T = C_{sp,eq}\Big/C^O_{sp} \tag{2}$$

These experiments were also employed to calculate the silastic membrane-aqueous phase partition coefficient (K) to be employed in mass transport calculations.

$$K = C_{aq,eq}/C_{sp,eq} \tag{3}$$

Determination of Free Fraction of Steroid

The equilibrium dialysis method of Yamaguchi *et al.*, (17) was employed to determine the fraction of steroid-free in the aqueous phase of each of the microemulsion formulations. A silicone membrane was placed between the donor and receiver chambers of the side-by-side diffusion cell holding equal volumes of donor and receiver phases at 27°C. The donor compartment held steroid-containing microemulsion formulation while the receiver compartment held buffer. Once equilibrium had been established, donor and receiver chambers were each sampled in triplicate and assayed by LSC. The fraction of drug residing in the aqueous continuous phase of the microemulsions (F_f) was calculated by Eq. (4) (17,18).

$$F_f = \frac{2C_{R,E}}{(C_{D,i}-2C_{R,E})} \tag{4}$$

$C_{R,E}$ is the concentration of drug in the receiver chamber at equilibrium, and $C_{D,i}$ is the concentration of drug in the donor phase initially.

The effective partition coefficient describing the distribution of steroid between the microemulsion lipid aggregates and the aqueous phase (k^*) can be defined as in Eq. (5).

$$k^* = C_{me}/C_{aq} = F_{me}C_T/F_fC_T \tag{5}$$

C_{me} is the concentration of drug in the microemulsion aggregates and is the product of the fraction of drug in the aggregate (F_{me}) and the total drug concentration (C_t). C_{aq} is the concentration of steroid in the aqueous phase and is the product of F_f and C_T. If it is assumed that drug is present either in the aqueous phase or in lipid aggregates, k^* can be calculated from Eq. (6).

$$k^* = (1-F_f)/F_f \tag{6}$$

Mass balance with respect to steroid content was achieved for each system (mean 99.1 ± 1% recovery of progesterone; mean 99.6 ± 1.3% recovery of estradiol).

Pulsed Gradient Spin Echo Nuclear Magnetic Resonance

The Pulsed Gradient Spin Echo Nuclear Magnetic Resonance (PGSE NMR) method has been applied to a variety of lipid assembly systems providing the diffusivities of individual components (19,20). It was assumed that diffusivity measured in the solution contained in the NMR tubes approximates the diffusivity that would be found in the aqueous boundary layer of the side-by-side diffusion cell. A 400 MHz Varian Fourier Transform NMR spectrometer (S/N S010883) equipped with a Highland, Performa II gradient probe (S/N P003732) and the "Diffusion Software Package" was used for all measurements. Three millimeter, thin-walled glass sample tubes were used and the sample volume fixed at 150 μl to contain the sample within the linear region of the gradient coil. Diffusion measurements were performed at 27 ± 0.01°C with temperature controlled by a variable temperature controller (Varian, Inc.).

Chemical shifts of all components were identified from literature reports (21,22). The same values were adopted for the PGSE NMR spectra of microemulsion systems with the –OH peak of the solvent (4.7 ppm) used as a reference (small amounts of ^1H will be present in all samples of D_2O due to exchange with the environment). The unique NMR bands at 3.6, 4.2, 5.8 and 6.5 ppm were assigned to the ethylene oxide group of Brij 97, glycerol protons on the Miglyol 812 triglyceride backbone, the proton on the carbon in position 4 of the progesterone steroid ring structure (alpha to the carbonyl), and the protons on the 1 and 2 positions of the estradiol aromatic ring of the steroid ring structure, respectively. The magnetic field was locked by an internal deuterium lock signal for all samples containing D_2O.

Diffusion coefficients were measured by using the stimulated spin echo pulse sequence, modulating the strength of fixed-length gradient pulses in an array of 21 steps. Experiments were carried out by varying G between 6.0 E-4 T/cm and 3.5 E-3 T/cm and keeping all other timing parameters constant. The translational diffusion coefficient of each component was calculated by fitting the Stejskal-Tanner equation (Eq. 7) to the obtained integral for the area under each peak.

$$\mathrm{Ln}I_g = \mathrm{Ln}I_0 - G^2\left[\gamma^2 g^2 D_{obs}(\Delta - g/3)\right] \tag{7}$$

where I_g is the echo intensity following the application of a field gradient, I_0 is the echo intensity in the absence of a field gradient, γ is the proton gyromagnetic ratio (2.68×10^8 $s^{-1}T^{-1}$), G is the strength of magnetic field gradient, g is the field gradient pulse length, D_{obs} is the diffusion coefficient, and Δ is the diffusion time (typically 7.8 ms).

As established by the calibration samples (D_2O and dioxane), the error between replicate measurements of the same sample in the same NMR tube was less than 1%. With the experiments conducted in this manner, standard deviations between identical samples prepared multiple times were typically less than 2% and never exceeded 20% consistent with what has been reported in the literature (21). For all experimental samples 64 replicates were accumulated for each gradient strength, in order to maximize signal to noise ratio, while still maintaining a reasonable run time.

Modeling of Mass Transport

Stead-state flux per unit area (J) under sink conditions for a drug in aqueous solution through a series of barriers consisting of one membrane and two aqueous boundary layers can be described by Eq. (8).

$$J = \frac{D_m K D_a C_T}{h_a D_a + 2 h_a D_m K} \tag{8}$$

D_m and D_a are the diffusivities of the drug in the membrane and in the aqueous phase, respectively. K is the membrane-aqueous distribution coefficient. The thickness of the membrane and the aqueous boundary layer are h_m and h_a, respectively, and C_T is the drug concentration in the aqueous phase. Eq. (8) can be expressed in terms of effective permeability coefficient (P_{eff})

$$J = P_{eff} C_T \tag{9}$$

The effective permeability coefficient can be defined by Eq. (10).

$$P_{eff} = \frac{1}{(1/P_m)+(2/P_a)} \tag{10}$$

where P_m is the permeability of the silicone rubber membrane to the monomer drug, and P_a is the permeability of the aqueous boundary layer to both monomer and solubilized drug (17). P_m and P_a may be determined from Eqs. (11) and (12), respectively (23).

$$P_m = \frac{K D_m}{h_m (k^* + 1)} \tag{11}$$

$$P_a = \frac{D_{eff}}{h_a} \tag{12}$$

Values for P_a and P_m were calculated from NMR and partitioning data as outlined above. Equation (9) was then used to calculate the expected flux values, which could then be compared to the experimental flux values. We were unable to determine directly D_m and have employed the value of progesterone from Roseman, 2.5×10^{-7} cm^2/s, for both steroids (24). Although the two steroids possess similar molecular weights, the error associated with applying the value to estradiol is unknown. The value of h_a for the side-by-side diffusion cell was estimated to be 0.02 cm, as determined by fitting transport data from saturated solutions to Eq. (8).

RESULTS AND DISCUSSION

Solubility of Steroids in Microemulsion Formulations

The solubilities of progesterone and estradiol in the three microemulsion formulations 3ME, 1ME and 0.5ME and in water are shown in Table II. Not surprisingly, in all cases, solubilities in the microemulsion formulations were several orders of magnitude greater than those found in deionized water. For both steroids, solubility in the 3ME

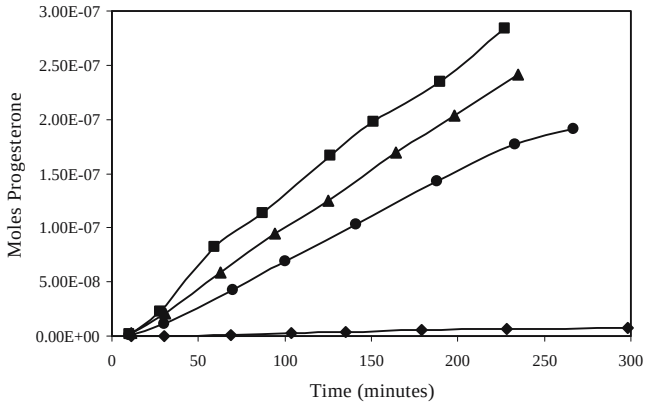

Fig. 1. Moles of progesterone appearing in the receiver chamber under near saturation loading of each formulation. Key: Aqueous solution, donor 3.4×10^{-5} moles/L, (*diamonds*); 0.5ME, donor 0.67×10^{-2} moles/L, (*squares*); 1ME, donor 0.68×10^{-2} moles/L (*triangles*); 3ME, donor 1.68×10^{-2} moles/L (*circles*).

formulation was much greater than that observed in the 1ME or 0.5ME formulations. The solubilities of each steroid in the 1ME and 0.5ME formulations were identical within experimental error. As confirmed previously, the progesterone was in the anhydrous form, while the estradiol was in the hemihydrate form (14). There was no evidence of polymorph change (14).

MASS TRANSPORT

Examples of flux curves for progesterone and estradiol formulations, under both near-saturation and constant drug loading, are presented in Figs. 1–4. Flux values in the steady state region are tabulated in Table II. In all cases, the molar flux values of progesterone were significantly greater than those of estradiol. These results were consistent with previous findings where the permeabilities of steroids through silicone membranes were found to be inversely proportional to the number of –OH groups (25).

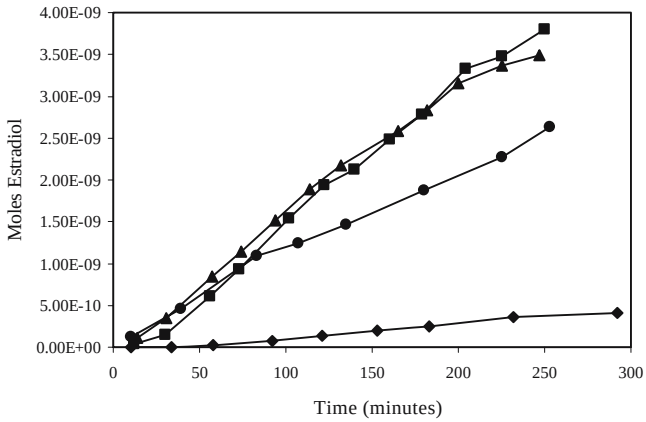

Fig. 2. Moles of estradiol appearing in the receiver chamber under near saturation loading of each formulation. Key: Aqueous solution, donor 9.2×10^{-6} moles/L, (*diamonds*); 0.5ME, donor 0.62×10^{-2} moles/L, (*squares*); 1ME, donor 0.66×10^{-2} moles/L (*triangles*); 3ME, donor 1.03×10^{-2} moles/L (*circles*).

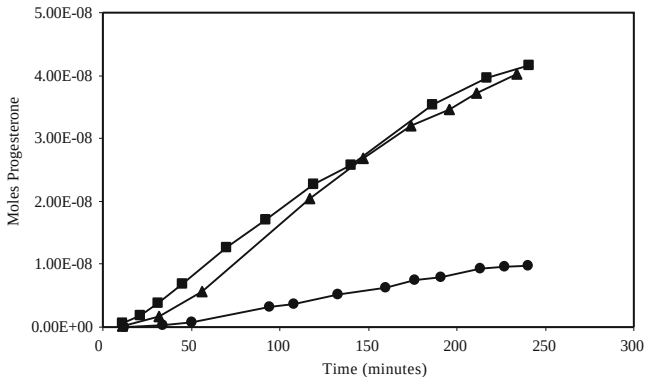

Fig. 3. Moles of Progesterone appearing in the receiver chamber under constant loading conditions. Each donor formulation contained 1.4×10^{-3} moles/L. Key: 0.5ME (*squares*); 1ME (*triangles*); 3ME (*circles*).

It has been long recognized that solubility of the drug in each phase of the microemulsion would likely be a critical factor in controlling absorption from such formulations (26). We have tested the hypothesis that concentration directly influences drug flux. Under near-saturation levels of drug loading, progesterone flux was up to 41-fold greater and estradiol flux was about eight-fold greater from the microemulsion formulations, compared to the aqueous solutions. Of particular interest was the rank-order of flux when comparing the various formulations. In the 0.5ME and 1ME systems the flux values of both steroids was significantly greater than that of the 3ME formulation, despite the fact that the 3ME systems contained significantly more drug than either the 0.5ME or 1ME formulations.

In an attempt to better understand this apparent anomaly of the relationship of flux and drug concentration, the thermodynamic activity of drug in the formulations was examined. Activities for both solutes in the microemulsion formulations are listed in Tables III and IV. As expected, in the case of near-saturation conditions, both solutes exhibit thermodynamic activities near 1 in all microemulsion systems tested. These results indicate that the thermodynamic activity of the

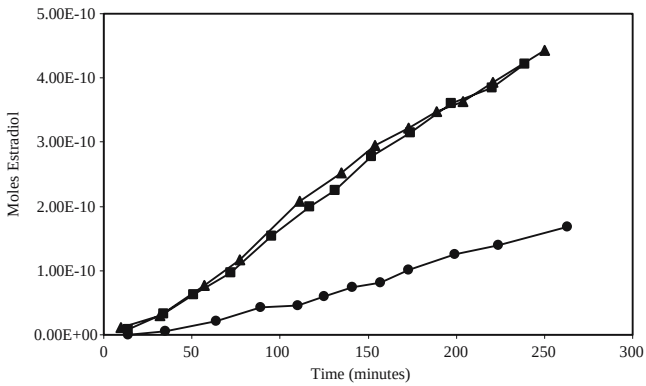

Fig. 4. Moles of Estradiol appearing in the receiver chamber under constant loading conditions. Each donor formulation contained 1.2×10^{-3} moles/L. Key: 0.5ME (*squares*); 1ME (*triangles*); 3ME (*circles*).

Table III. Characteristics of Progesterone in Microemulsion Formulations and Solution at 27°C

Formulation	Observed diffusivity[b] ($\times 10^{-8}$ cm^2/s)	Fraction of progesterone in aqueous phase[b]	Thermodynamic activity at near saturation[a]	Thermodynamic activity at constant loading[a]	k^*
3ME	4.90 ± 0.2	0.0018 ± 0.0001	0.99	0.07	555
1ME	20.5 ± 0.3	0.0044 ± 0.0001	0.99	0.18	232
0.5ME	21.8 ± 0.3	0.0047 ± 0.0001	1.00	0.19	212
Deionized water	556 ± 7	1.0	0.98	–	–

[a] In all cases, standard deviations did not exceed 0.03.
[b] Mean ± S.D., $n = 3$.

steroid in the aqueous phase, the driving force for diffusive mass transport, is independent of microemulsion formulation. This suggests that there must be some other physical reason to explain why the flux of steroids from the 0.5ME and 1ME formulations was greater than that from the 3ME.

For the set of microemulsion systems in the constant loaded condition the relationship of flux from the formulations was also complex. For progesterone, flux from the 3ME microemulsion was essentially equal to that observed for the aqueous solution, despite the much higher steroid concentration in the microemulsion. On the other hand, flux values from the 0.5ME and 1ME formulations were only slightly greater than that of the aqueous solution. For estradiol, flux from 0.5ME and 1ME were essentially equal to that of the aqueous solution, while flux from the 3ME system was significantly less. Compared to the near-saturation set, thermodynamic activities of steroids in the constant loaded series were significantly reduced. On the other hand, A_T values varied little; ranging from 0.10 to 0.17 for estradiol and from 0.07 to 0.19 for progesterone. Thus, in the constant loaded series of microemulsions, thermodynamic activity alone of the steroids in the bulk state appears not to be an accurate predictor of the flux of the steroids.

To gain a better physical understanding of the factors that govern mass transport in this microemulsion system, a series of calculations employing the model of Amidon *et al*., (23) were carried out. Independent experiments were conducted first to characterize the critical physical parameters of the model, including the diffusivity of the steroids in the microemulsion formulations, the silicone rubber-aqueous distribution coefficient of the monomer drug and the oil droplet-aqueous distribution coefficient. Other mass transport models have been proposed (for example, (27)), but the Amidon model has the advantage of employing parameters (such as diffusion coefficient) that may be obtained independently.

The diffusivities of the steroids were measured by the PGSE NMR method (Tables III and IV). The diffusion coefficients for progesterone and estradiol in the aqueous solution were 5.56×10^{-6} and 5.18×10^{-6} cm^2/s, respectively, similar to values previously reported (23,28). Solubilization of progesterone and estradiol by microemulsion lipid assemblies decreased diffusivity of the drug by as much as much as 50-fold as compared with that observed in aqueous solutions. If the drug is assumed to be present in two locations, as monomers in the aqueous phase and solubilized in the oil droplets, the observed diffusivity in the microemulsion formulation (D_{eff}) is the weighted average of the two locations and can be expressed as

$$D_{eff} = F_f D_{aq} + F_{me} D_{me} \tag{13}$$

F_f and F_{me} are the fractions of the drug located in the aqueous phase and microemulsion oil droplet, respectively. D_{aq} represents the diffusivity of the monomer drug in the aqueous phase while D_{me} represents the diffusivity of the drug located in the droplet. Since the droplet is so much larger than the monomer drug, and thus diffuses much more slowly, the overall observed diffusivity is much lower than that of D_{aq}. The greater the extent to which the drug can partition into the oil droplet, the lower the value of D_{eff}. Diffusivities of the Brij 97 or Miglyol 812 in these same systems were not affected by solubilization of the steroids (data not shown) suggesting that the steroids had no significant effect on the hydrodynamic radius of the oil droplets.

It should be noted here that it is possible that the microemulsions in the present study may be composed of surfactant micelles as well as oil-surfactant droplets (29). In principle, Eq. (13) could be extended to a three-location model to include directly surfactant micelles. Preliminary calculations suggest that the effect of the three-location model on the fraction of drug-free in solution would be negligible and so in the present study, we have assumed that

Table IV. Characteristics of Estradiol in Microemulsion Formulations and Solution at 27°C

Formulation	Observed diffusivity[b] ($\times 10^{-8}$ cm^2/s)	Fraction of estradiol in aqueous phase[b] ($\times 10^{-3}$)	Thermodynamic activity at near saturation[a]	Thermodynamic activity at constant loading[a]	k^*
3ME	4.96 ± 0.05	0.0012 ± 0.0001	0.99	0.10	832
1ME	19.5 ± 0.3	0.0018 ± 0.0001	1.02	0.17	548
0.5ME	20.5 ± 0.5	0.0020 ± 0.0001	1.01	0.17	497
Deionized water	518 ± 7	1.0	1.02	–	–

[a] In all cases, standard deviations did not exceed 0.03.
[b] Mean ± S.D., $n = 3$.

Table V. Calculated Flux under Near saturation and Constant Loaded conditions for Progesterone

Formulation	Calculated flux ($\times 10^{-10}$ mol/cm^2-min) Near saturation	Ratio Calculated/Observed	Calculated flux ($\times 10^{-10}$ mol/cm^2-min) Constant loading	Ratio Calculated/Observed
3ME	9.4	1.4	0.47	1.6
1ME	14	1.1	1.7	1.6
0.5ME	15	1	2	1.5

any solute not in the aqueous phase is solubilized in a microemulsion droplet.

The diffusivities of progesterone in the 0.5ME and 1ME formulations were found to be equivalent. On the other hand, diffusivity of progesterone in the 3ME system is decreased by about a factor of 4 as compared with the other two formulations. This trend is similar to the results found in the literature (22) for other systems and is thought to be due to the formation of a larger oily droplet in the 3ME formulation. Estradiol behavior in the microemulsion systems was similar to that of progesterone where diffusivity in the 0.5ME and 1ME formulations was constant, but decreased by a factor of about 4 in the 3ME formulation.

Modeling of Mass Transport

The membrane aqueous phase partition coefficient, K, was found to be 318 ± 68, 6.8 ± 0.4 for progesterone and estradiol, respectively. The effective lipid aggregate-aqueous phase partition coefficients, k^*, are listed in Tables III and IV. The values for both K and k^* are strictly valid only under near-saturated loading conditions, but were assumed to be invariant to drug concentration. The magnitude of the error associated with this assumption is not known. Listed in Table V are the expected flux values for progesterone under both near-saturation and constant loading conditions as calculated from Eqs. (9), (10), (11), (12). Also included are the accompanying ratios of the calculated flux values to the experimentally observed values. In all cases, the calculated values for progesterone are markedly similar to those observed experimentally. At most, the calculated flux values overestimate the observed values by less than a factor of 2. It is important to note that the mathematical model was able to fit the experimental results both under near-saturated and constant-loaded conditions. These results suggest that accounting for the altered diffusivity of progesterone in the aqueous boundary layer due to partitioning of the drug into the oil droplets is critical for understanding flux in this model system. In the case of mass transport from simple solutions, the aqueous boundary layer model assumes that drug concentration in the layer decreases in going from the bulk to the membrane face. It is the activity of the drug at the membrane face that drives partitioning into and diffusion through the membrane. If supplying drug to the membrane face is slow compared to movement through the membrane, then increasing the supply of the drug at the face would enhance overall flux. It has been proposed that simple micelles act as a reservoir carrying drug across an aqueous boundary layer allowing a higher concentration of drug to be delivered to the membrane face, thus promoting flux (23). Such a mechanism requires that the drug be in very rapid equilibrium between the aqueous phase and the micelle. It is reasonable to propose that a similar mechanism is in effect in the present study, including a rapid equilibrium between the aqueous phase and the oily droplets. Under this model, despite the fact that the oil droplets and dissolved progesterone diffuse even slowly than monomer drug, the droplet continues to release drug as it approaches the membrane face. Essentially, the concentration gradient within the aqueous boundary layer would be lessened and the activity of progesterone at the membrane face would be greater than that in the case of the saturated aqueous solution. Larger droplets, such as those that exist in the 3ME system, move more slowly across the aqueous layer and so tend to show a lesser ability to enhance transport compared to the smaller, faster-diffusing droplets of the 0.5ME and 1ME formulations.

Listed in Table VI are the calculated flux values for estradiol under both near-saturation and constant loading conditions. In the case of estradiol, the calculated flux values overestimated the observed values by a factor of 5 or less. The greatest deviation between calculation and experiment seemed to arise in the 3ME system under both near-saturated and constant loaded conditions. The reason that the calculated values for estradiol deviate more strongly from experimental values is not clear although there are several possibilities. In the calculation, we assumed that the diffusivity of estradiol in the membrane was equal to that of progesterone. If D_m of estradiol is overestimated by this assumption, flux will also be overestimated.

A second but more important reason for the lesser level of correlation between calculated and observed flux for estradiol could be that the transport of this steroid is not

Table VI. Calculated Flux under Near saturation and Constant Loaded conditions for Estradiol

Formulation	Calculated flux ($\times 10^{-11}$ mol/cm^2-min) Near saturation	Ratio Calculated/Observed	Calculated flux ($\times 10^{-12}$ mol/cm^2-min) Constant loading	Ratio Calculated/Observed
3ME	3.2	4	3.8	5.3
1ME	3.2	2	5.9	3
0.5ME	3.3	1.8	6.5	3.1

aqueous boundary layer controlled under current conditions. The proposed mechanism of flux enhancement requires diffusion through the membrane to be rapid compared to the transport of steroid through the aqueous boundary layer. If the boundary layer is not the rate-controlling barrier to mass transport, no amount of enhancing flux across that barrier will promote appearance of the drug in the receiver compartment. A series of experiments were carried out where the thickness of the silicone rubber membrane was doubled and steroid fluxes from saturated solutions were examined. For progesterone, doubling the thickness of the membrane resulted in a flux that was over 70% of that observed in the single membrane study (data not shown). These results would be in agreement with solute flux that is aqueous boundary layer controlled. Thus, events in the aqueous boundary layer, such as solubilizing a drug in an oil droplet, would likely have a more important effect on transport than events within the single-layer membrane. In contrast, for estradiol, doubling the thickness of the membrane to 0.06 mm resulted in a decrease of flux to 52% of that seen with a 0.03 mm membrane. The sensitivity of estradiol flux to membrane thickness appears to suggest that transport of this steroid from saturated solutions may be membrane controlled. Thus, events in the aqueous boundary layer may be expected to have lesser impact on transport and the calculated flux would over-estimate the observed values. The magnitude of the effect might be expected to be greatest for the 3ME microemulsion where effective diffusivity is the lowest measured.

Overall, Eqs. (11), (12), and (13) showed some success in describing flux through a silicone rubber membrane when the critical parameters of effective diffusivity, membrane-aqueous distribution coefficient and oil droplet-aqueous distribution coefficient were independently determined. It should be kept in mind that increasing the rate of mass transport is only one of a number of possible means by which microemulsions may enhance the oral bioavailability of poorly soluble drugs (6–9). The implications of the findings of the current study toward the enhancement of bioavailability by this highly simplified microemulsion system should not be over-interpreted as the effects of the aqueous boundary layer *in vivo* are not well understood. Further, it is difficult to argue that the impermeability of the silicone rubber membrane to surfactant and oil accurately reflects the complexities of a biological membrane, including the mucin layer that covers the gastrointestinal tract (30,31). Never the less, it is interesting to note that investigators are beginning to systematically probe the effect of mucin on the diffusivity of other lipid aggregates (32). A natural extension of the present work would be to examine diffusivity of the Miglyol-Brij-water microemulsion in more complex biological media, such as mucin. In any event, the current study does raise the possibility that not all poorly soluble drug molecules will benefit from formulation in a microemulsion formulation and that careful consideration of drug loading and diffusivity may be necessary to maximize effectiveness.

CONCLUSIONS

Mass transport studies employing a side-by-side diffusion cell have been used to determine the extent to which solubilization of two model steroids in a microemulsion lipid assembly influences the rate of transport. All microemulsion systems tested showed a greatly enhanced ability to solubilize the model drugs compared to aqueous solution, yet very different mass transport properties were observed, depending upon the level of drug loading. At near saturation levels of drug loading, microemulsion formulations appeared to increase flux of model steroids compared with the saturated aqueous solution, with progesterone showing the greater effect. In contrast, for systems under constant drug loading microemulsion formulations appeared to increase flux only moderately for progesterone and not at all for estradiol. Thus, in systems nearly saturated with drug, the microemulsion formulation leads to a greatly enhanced rate of mass transport of the model steroids while in systems with drug loading far is from saturation, the microemulsion formulation appears to have no effect on mass transport. It was found that mass transport of progesterone, a drug that appears to show aqueous boundary layer control, could be successfully modeled when coupled with pulsed gradient NMR and thermodynamic activity characterization studies. Somewhat less successful was the modeling of mass transport for estradiol, a solute that may not be under aqueous-boundary layer control.

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REFERENCES

1. P. P. Constantinides. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharm. Res.* **12**:1561–1572 (1995).
2. G. L. Amidon, H. Lennernas, V. P. Shah, and J. R. Crison. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm. Res.* **12**:413–420 (1995).
3. R. Gursoy and S. Benita. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomedicine & Pharmacotherapy* **58**:173–182 (2004).
4. S. Tenjarla. Microemulsions: an overview and pharmaceutical applications. *Crit. Rev. in Ther. Drug Carrier Syst.* **16**:461–522 (1999).
5. D. J. Hauss, S. E. Fogal, J. V. Ficorilli, C. A. Price, T. Roy, A. A. Jayaraj, and J. J. Keirns. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB_4 inhibitor. *J. Pharm. Sci.* **87**:164–169 (1998).
6. K. J. MacGregor, J. K. Embleton, J. E. Lacy, E. A. Perry, L. J. Solomon, H. Seager, and C. W. Pouton. Influence of lipolysis on drug absorption from the gastro-intestinal tract. *Adv. Drug Del. Rev.* **25**:33–46 (1997).

7. B. Aungst, N. H. Nguyen, N. J. Rogers, S. M. Rowe, M. A. Hussain, S. J. White, and L. Shum. Amphiphilic vehicles improve the oral bioavailability of a poorly soluble HIV protease inhibitor at high doses. *Internat. J. Pharmaceut.* **156**:79–88 (1997).
8. A. Humberstone and W. Charman. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Adv. Drug. Del. Rev.* **25**:103–128 (1997).
9. P. Artursson and J. Karlsson. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* **175**:880–885 (1991).
10. C. Porter, A. Kaukonen, B. Boyd, G. Edwards, and W. Charman. Susceptibility to lipase-mediated digestion reduces the oral bioavailability of danazol after administration as a medium-chain lipid-based microemulsion formulation. *Pharm. Res.* **21**:1405–1412 (2004).
11. M. Kreilgaard, E. Pedersen, and J. Jaroszewski. NMR characterisation and transdermal drug delivery potential of microemulsion systems. *J. Cont. Rel.* **69**:421–433 (2000).
12. P. Lee, R. Langer, and V. Shastri. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. *Pharm. Res.* **20**:264–269 (2003).
13. C. Malcolmson and M. Lawrence. Three-component non-ionic oil-in-water microemulsions using polyoxyethylene ether surfactants. *Colloids and Surfaces B* **4**:97–109 (1995).
14. L. M. Land, P. Li, and P. M. Bummer. The influence of water content of triglyceride oils on the solubility of steroids. *Pharm. Res.* **22**:784–788 (2005).
15. C. L. Liu, U. K. Jain, P. H. Lee, N. A. Mazer, and W. I. Higuchi. Cholesterol thermodynamic activity, quasielastic light scattering, and polarizing microscopy studies in aqueous taurocholate-lecithin solutions supersaturated with cholesterol. *J. Colloid Interfac. Sci.* **165**:411–424 (1994).
16. B. J. Boyd, C. J. H. Porter, and W. H. Charman. Using the polymer partitioning method to probe the thermodynamic activity of poorly water-soluble drugs solubilized in model lipid digestion products. *J. Pharm. Sci.* **92**:1262–1271 (2003).
17. T. Yamaguchi, N. Tanabe, Y. Fukushima, T. Nasu, and H. Hayashi. Distribution of prostaglandin E1 in lipid emulsions in relation to release rate from lipid particles. *Chem. Pharm. Bull.* **42**:646–650 (1994).
18. H. Yamamoto and H. Liljestrand. Partitioning of selected estrogenic compounds between synthetic membrane vesicles and water: effects of lipid components. *Environ. Sci. Technol.* **38**:1139–1147 (2004).
19. P. Stilbs. Fourier transform pulsed-gradient spin-echo studies of molecular diffusion. *Prog. Nucl. Magn. Reson. Spectrosc.* **19**:1–45 (1987).
20. O. Soderman and P. Stilbs. NMR studies of complex surfactant systems. *Prog. Nucl. Magn. Reson. Spectrosc.* **26**:445–482 (1994).
21. C. Ko, Y. Ko, D. Kim, and H. Park. Solution properties and PGSE-NMR self-diffusion study of $C_{18:1}E_{10}$/oil/water system. *Coll. Surf. A: Physicochem. Eng. Aspects* **216**:55–63 (2003).
22. C. Pouchert, and J. Behnke (ed.), The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra, Aldrich Chemical Company, Inc., New York, 1993.
23. G. Amidon, W. Higuchi, and N. Ho. Theoretical and experimental studies of transport for micelle-solubilized solutes. *J. Pharm. Sci.* **71**:77–84 (1982).
24. T. J. Roseman. Release of steroids from a silicone polymer. *J. Pharm. Sci.* **61**:46–50 (1972).
25. S. Friedman, S. Koide, and F. Kincl. Sustained release hormonal preparations. 7. Permeability of three types of silicone rubber to steroids. *Steroids* **5**:679–680 (1970).
26. P. Constantinides. Particle size determination of phase-inverted water-in-oil microemulsions under different dilution and storage conditions. *Internat. J. Pharmaceut.* **115**:225–234 (1994).
27. M. Grassi, N. Coceani, and L. Magarotto. Mathematically modeling of drug release from microemulsions: theory in comparison to experiments. *J. Colloid. Interfac. Sci.* **228**: 141–150 (2000).
28. K. Shikii, S. Sakamoto, H. Seki, H. Utsumi, and K. Yamaguchi. Narcissistic aggregation of steroid compounds in diluted solution elucidated by CSI-MS, PFG NMR and X-ray analysis. *Tetrahedron* **60**:3487–3492 (2004).
29. C. Sirotti, N. Coceani, I. Colombo, R. Lapasin, and M. Grassi. Modeling of drug release from microemulsions: a peculiar case. *J. Memb. Sci.* **204**:401–412 (2002).
30. H. Westergaard and J. Dietschy. Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. *J. Clin. Invest.* **54**:718–732 (1974).
31. H. Westergaard and J. Dietschy. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. *J. Clin. Invest.* **58**:97–108 (1976).
32. T. S. Wiedmann, C. Deye, and D. Kallick. Interaction of bile salt and phospholipids with bovine submaxillary mucin. *Pharm. Res.* **18**:1489–1496 (2001).