Novel Microemulsion Enhancer Formulation for Simultaneous Transdermal Delivery of Hydrophilic and Hydrophobic Drugs

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Purpose. Microemulsion (ME) systems allow for the microscopic co-incorporation of aqueous and organic phase liquids. In this study, the phase diagrams of four novel ME systems were characterized.
Methods. Water and IPM composed the aqueous and organic phases respectively, whereas Tween 80 served as a nonionic surfactant. Transdermal enhancers such as n-methyl pyrrolidone (NMP) and oleyl alcohol were incorporated into all systems without disruption of the stable emulsion.
Results. A comparison of a W/O ME with an O/W ME of the same system for lidocaine delivery indicated that the O/W ME provides significantly greater flux ($p < 0.025$). The water phase was found to be a crucial component for flux of hydrophobic drugs (lidocaine free base, estradiol) as well as hydrophilic drugs (lidocaine HCl, diltiazem HCl). Furthermore, the simultaneous delivery of both a hydrophilic drug and a hydrophobic drug from the ME system is indistinguishable from either drug alone. Enhancement of drug permeability from the O/W ME system was 17-fold for lidocaine free base, 30-fold for lidocaine HCl, 58-fold for estradiol, and 520-fold for diltiazem HCl.
Conclusions. The novel microemulsion systems in this study potentially offers many beneficial characteristics for transdermal drug delivery.

KEY WORDS: microemulsion; transdermal drug delivery; chemical enhancers; n-methyl pyrrolidone.

INTRODUCTION

Microemulsions (ME) are thermodynamically stable emulsions with droplet sizes in the sub-micron range. They typically consist of an aqueous phase, an organic phase, and a surfactant/cosurfactant component. Microemulsion systems have been studied extensively with regards to pharmaceutical applications (1). There are two basic types of ME systems: water-in-oil (W/O) and oil-in-water (O/W). In each case, it is believed that the minority phase is encapsulated by the continuous bulk phase. Surfactants are necessary to reduce the hydrophobic interactions between the phases and maintain a stable emulsion. Typical properties of ME include optical transparency, thermodynamic stability, and solubility of both hydrophobic and hydrophilic components.

Microemulsions have been proposed to offer enhanced drug delivery properties for transdermal transport (2,3). Flux enhancement from these formulations was primarily due to an increase in drug concentration. In these studies, it was concluded that drug transport occurs only from the continuous (outer) phase. By this account, hydrophobic drugs show greater flux from W/O emulsions, whereas O/W systems provide controlled release of drug that is dependant on the partitioning of drug into the outer phase. This pathway of drug release from ME systems is supported by work with a hydrophilic molecule (glucose) where it parallels the diffusion of water from the bulk phase (4). The stability and encapsulation properties of emulsions make the transdermal delivery of protein drugs a useful application (5–8).

In this study, multiple features are incorporated into a ME formulation. A nonionic surfactant was selected to minimize skin irritation and charge disruption of the system. The surfactant studied, Tween 80 (Polysorbate 80) has previously been used in transdermal formulations (9,10). A key feature of the ME systems studied is incorporation of the transdermal chemical enhancers oleyl alcohol and n-methyl pyrrolidone (NMP). Oleyl alcohol is a cis-unsaturated C_{18} fatty acid that is believed to reduce the barrier properties of the skin by disrupting lipid bilayers within the stratum corneum (11,12). NMP has been used as a transdermal enhancer for multiple drugs and formulation compositions, but never in conjunction with a ME (13–15). We selected NMP based on our earlier studies showing that it is capable of significantly enhancing drug transport from both the organic and aqueous phases. Through IR spectroscopy, we determined that NMP is capable of hydrogen bonding with drugs such as lidocaine. The high flux of NMP through human skin (~10 mg/cm^2/h) thus provides a driving force for drug molecules (as well as certain chemical enhancers) to transport through the skin barrier. We propose that this enhancing ability should occur in ME systems as well.

In this study we evaluated the transdermal transport of several hydrophobic and hydrophilic drug moieties from novel ME systems that incorporate chemical enhancers. Drug molecules investigated include lidocaine free base (16,17) and HCl salt, estradiol (18,19) and diltiazem HCl, a drug which to our knowledge has not been previously studied in the transdermal literature due to its large molecular weight (415 Da) ionic, and hydrophilic nature.

MATERIALS AND METHODS

Materials

Drugs

Lidocaine free base, Lidocaine HCl, Estradiol, and Diltiazem HCl were purchased from Sigma (St. Louis, MO).

Chemicals

NMP was a generous gift from ISP Technologies, Inc. (Wayne, NJ). Polysorbate 80 NF, HLB = 15.0 (Tween 80) was purchased from Advance Scientific & Chem. (Ft. Lauderdale, FL). Isopropyl myristate (IPM), oleyl alcohol (99%), anhydrous ethyl alcohol, sorbitan mono-oleate (Span 20), HLB = 8.6, and phosphate buffered saline tablets (PBS) were purchased from Sigma (St. Louis, MO). HPLC grade solvents were used as received.

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Skin

Human cadaver skin from the chest, back, and abdominal regions was obtained from the National Disease Research Institute (Philadelphia, PA). The skin was stored at –80°C until use.

Methods

Microemulsion Phase Diagrams

Four microemulsion (ME) systems were investigated to determine their ternary phase diagrams. Since 4 chemical species were incorporated in the ME, one of the components (ethanol) was in fixed ratio with either water or Tween 80. All percentages are given as mass ratios (Table I).

Each of the three components for a system was titrated until a phase change between microemulsion and two-phase mixture was observed. The boundary of this transition was recorded over the entire concentration range. A microemulsion was determined as a miscible, optically clear, stable solution. At the transition to a two-phase regime, there is an unmistakable clouding of the mixture as well as an eventual separation of the phases. All microemulsion systems were stable for over 6 months.

Preparation of Formulations

Sample solutions were prepared in 20 ml glass vials and saturated with drug. Saturation was observed when drug precipitation occurred after 24 h. Drug flux was tested through ME system 1 at two selected concentrations, one in the W/O region, and the other in the O/W. The W/O system consisted of H_2O:IPM:Tween 80 (10:52:38 w/w) whereas the O/W system contained H_2O:Ethanol:IPM:Tween 80 (27:18:16:39 w/w). These mixtures were selected because they maximized the concentration of the "encapsulated" phase while maintaining a suitable excess of the "bulk" phase. Both systems stably incorporated 10% w/w NMP and 10% w/w oleyl alcohol. Drug concentration in the formulation was generally 4% for lidocaine, 2% for diltiazem HCl, and 0.4% for estradiol. The "water phase" sample consisted of the aqueous elements H_2O:Ethanol:NMP (51:31:18) in the same relative proportions as if the organic components were removed. All vehicles studied formed miscible, single-phase liquids.

Lidocaine Partitioning

The logarithm of the relative partition coefficient between IPM and water (log[IPM/H_2O]) was determined for NMP concentrations of 0–35% (v/v). In a micro-centrifuge tube, 500 μl of IPM was added to 500 μl of ddH_2O with the addition of the appropriate amount of NMP. Lidocaine free base was included at 1.0 mg/ml in the organic (IPM) phase.

Table I.

System	Aqueous phase	Organic phase	Surfactant phase
1	H_2O:Ethanol (1:1)	IPM	Tween 80
2	H_2O:Ethanol (1:1)	IPM	Tween 80:Span 20 (49:51)
3	H_2O	IPM	Tween 80:Ethanol (1:1)
4	H_2O	IPM	Tween 80:Ethanol (2:1)

For lidocaine HCl samples, the drug was dissolved in the aqueous phase at 1.0 mg/ml. The two-phase system was thoroughly vortexed and allowed to equilibrate. The samples were then centrifuged at 14,000 rpm for 6 min to separate the phases. The concentration of lidocaine in each phase was determined by HPLC.

Preparation of Skin Samples

Human cadaver skin was thawed at room temperature. The epidermis-SC was separated from the full thickness tissue after immersion in 60°C water for 2 min. Heat stripped skin was immediately mounted on diffusion cells.

Skin Transport Experiments

The skin was mounted onto a side-by-side glass diffusion cell with an inner diameter of 5 mm. The two halves of the cell were clamped shut and both reservoirs were filled with 2 ml of phosphate buffered saline (PBS, 0.01 M phosphate, 0.137 M NaCl, pH 7.4). The integrity of the skin was verified by measuring the electrical conductance across the skin barrier at 1 kHz and 10 Hz at 143.0 mV (HP 33120A Waveform Generator). Skin samples measuring 4–14 μA at 1 kHz were used for the diffusion studies. Prior to introducing the donor solution, the skin sample was thoroughly rinsed with PBS to remove surface contaminants. At $t = 0$, the receiver compartment was filled with 2.0 ml of PBS, and 2.0 ml of sample was added to the donor compartment. Both compartments were continuously stirred to maintain uniform concentrations. At regular time intervals, 1.0 ml of the receiver compartment was transferred to a glass HPLC vial. The remaining solution in the receiver compartment was thoroughly aspirated and discarded. Fresh PBS (2.0 ml) was dispensed into the receiver compartment to maintain sink conditions. At 21 h, the experiment was terminated. After both compartments were refilled with PBS, the conductance across the skin membrane was again checked to ensure that the skin was not damaged during the experiment. All flux experiments were conducted in triplicate at room temperature.

Drug Quantification

Lidocaine was assayed by high pressure liquid chromatography (Shimadzu model HPLC, SCL-10A Controller, LC-10AD pumps, SPD-M10A Diode Array Detector, SIL-10AP Injector, Class VP v.5.032 Integration Software) on a reverse phase column (Waters μBondapak™ C_{18} 3.9 × 150 mm) using ddH_2O (5% acetic acid, pH 4.2)/acetonitrile (35:65 v/v) as the mobile phase, under isocratic conditions (1.6 mL/min) by detection at 237 nm. The retention time of lidocaine under these conditions was between 3.4 and 4.3 min. Standard solutions were used to generate calibration curves. Diltiazem HCl was quantified on a Waters Symmetry® C_{18} 5μm, 3.9 × 150 mm column (WAT046980). The mobile phase consisted of aqueous phase:acetonitrile:methanol (50:25:25) where the aqueous phase consisted of 1.16 g/L *d*-10-camphorsulfonic acid, 0.1 M sodium acetate, pH 6.2. The system ran at a flow rate of 1.6 ml/min. Chromatograms were integrated at a peak of 240 nm. Estradiol was quantified on a Waters 4.6 × 250 mm C_{18} column. The mobile phase consisted of acetonitrile:water (55:45) at a flow rate of 2.0 ml/min. Chromatograms were integrated at a peak of 280 nm.

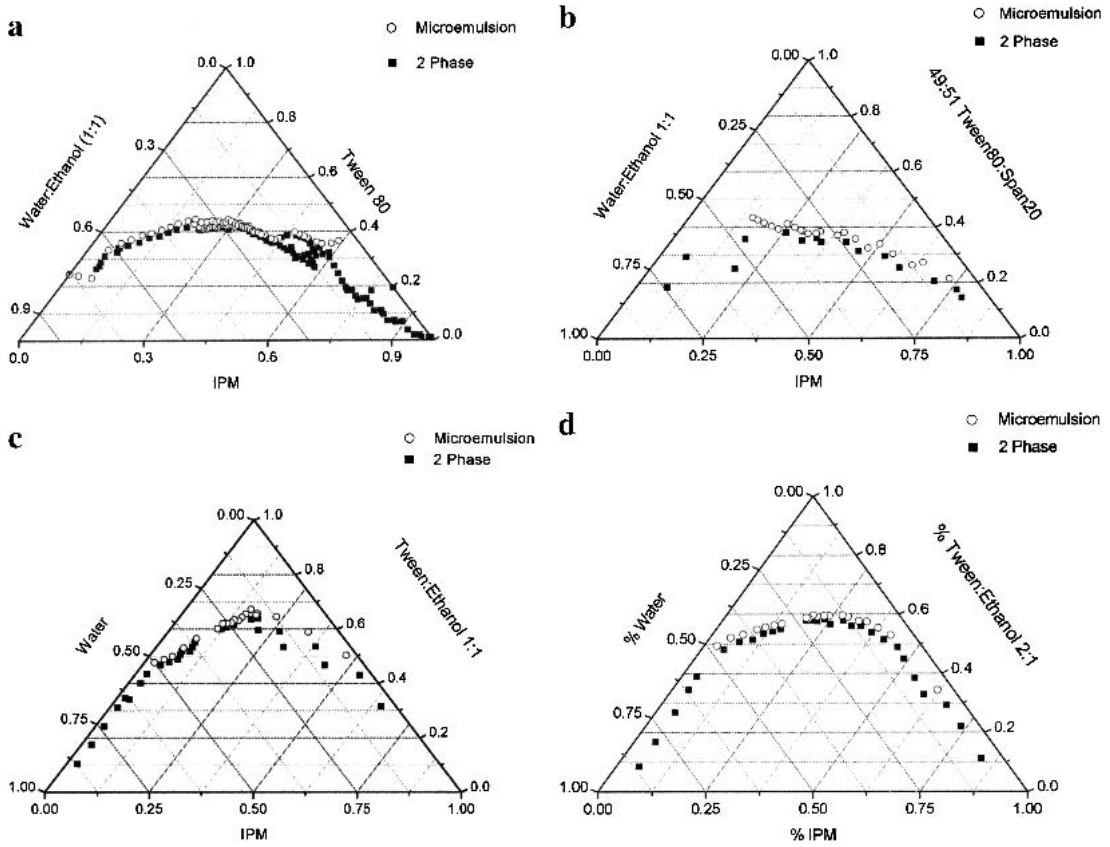

Fig. 1. Phase diagrams of microemulsion systems. Phase miscibility boundaries of (a) System 1: Water:Ethanol:IPM:Tween 80, (b) System 2: Water:Ethanol:IPM:Tween 80:Span 20, (c) System 3: Water:IPM:Tween 80:Ethanol, and (d) System 4: Water:IPM:Tween 80:Ethanol. All ratios w/w.

Calculations

The total mass of drug transported across the skin was determined by HPLC. The flux equation gives:

$$J = \frac{1}{A}\left(\frac{dM}{dt}\right) = P\Delta C$$

where J is flux ($\mu g\ cm^{-2}\ hr^{-1}$), A is cross-sectional area of the skin membrane (cm^2), P is the apparent permeability coefficient ($cm\ hr^{-1}$), and ΔC is the concentration gradient. In this experiment, ΔC is taken as the saturation concentration (given infinite dose and sink conditions), and dM/dt is averaged as the total mass transport over the steady state portion of the transport curve. Statistical analyses were performed by the Student's *t* test.

RESULTS AND DISCUSSION

Microemulsion Systems

Thermodynamically stable, optically transparent, single phase, liquid formulations were created with the four systems (Figs. 1 a–d). An ethanol co-surfactant is necessary to maintain stable O/W emulsions. This is consistent with previous work with ME systems where co-surfactants (usually short chain alcohols) are necessary to maintain a single phase (5). In system 2, a combination of two nonionic surfactants was used. The mixture of 49:51 w/w Tween 80 (HLB = 15) and Span 20 (HLB = 8.6) has been reported to act in synergy to maximize water uptake (20). Although this system was not tested for transdermal transport, the phase diagram does indeed indicate that ME formation occurs at lower surfactant concentrations. The phase diagrams in Figs. 1 c–d contain the same components as Fig. 1 a. In these two diagrams, the surfactant/cosurfactant (Tween80/ethanol) ratio is fixed over the entire range. It is apparent that having too much ethanol is detrimental to ME formation (Table II). The maximum IPM uptake in O/W ME systems occurs at Tween 80/ethanol ratio of 1:1. Furthermore, it was observed that the cosurfactant was necessary primarily to stabilize ME formulations with high water content. Systems with too little ethanol were unable to form stable O/W microemulsions.

All systems could stably incorporate 10% w/w of the transdermal enhancers NMP, oleyl alcohol, oleic acid, or decanoic acid. Drug solubility reached ~30% w/w lidocaine free base in the W/O system and ~25% lidocaine HCl in the O/W

Table II. Maximum IPM Uptake in O/W ME Systems

Tween 80/ethanol ratio	% IPM uptake (w/w)	% Tween 80/ethanol (w/w)
1:2	8.3	66
2:3	8.1	65
1:1	47	50
2:1	42	53
4:1	4.5	56
9:1	1.6	58

Table III. Lidocaine Free Base and Lidocaine HCl Transport from ME Systems

	Lidocaine free base		Lidocaine HCl	
Formulation	$Flux_{ss}$ ($\mu g/cm^2/h$)	Permeability ($cm/hr \cdot 10^5$)	$Flux_{ss}$ ($\mu g/cm^2/h$)	Permeability ($cm/hr \cdot 10^5$)
Water	6.0 ± 1.0	133 ± 23	0.61 ± 0.38	0.61 ± 0.38
W/O ME	16.5 ± 1.8	40.2 ± 4.5	2.1 ± 0.2	3.5 ± 0.3
O/W ME	23.3 ± 1.3	75.8 ± 4.1	10.2 ± 3.9	18.1 ± 6.9

n = 3.

system. With such high tolerance for the addition of both hydrophilic and hydrophobic molecules, the ME systems studied are robust vehicles for transdermal drug delivery.

Transdermal Transport

Although each system offers an unlimited number of sample formulations, we selected a representative W/O and O/W formulation from system 1 to test transdermal delivery of hydrophilic and hydrophobic drugs across stripped human skin. The results (Table III) indicate that the O/W system provided significantly better flux for all the drugs studied ($p < 0.025$). The simultaneous delivery of estradiol with diltiazem HCl from the ME system did not affect the transport of either drug ($p > 0.5$, Table IV). Interestingly, the drug permeability from a homogenous single phase composed of all the water-soluble components is similar to drug flux from O/W ME ($p > 0.25$). This suggests that the oil phase in our formulation serves as a depot for the drug, while the drug transport occurs primarily from the water phase. This phenomenon could have significant implications for the development of transdermal systems for long-term sustained delivery.

For all the drugs tested, the ME systems provided significant enhancement (Table V). The finding that flux is improved in O/W formulations as compared with W/O systems even for the hydrophobic drugs suggests that transport from the aqueous phase is key. When the organic phase and surfactants were removed from the ME, leaving only the water phase components (H_2O, ethanol, NMP), the flux was comparable to that from the O/W ME (Table IV). Previous work indicates that the H_2O/NMP synergy provides greater transdermal flux enhancement than H_2O/ethanol. Although the complexity of the multiple components in the system makes it difficult to determine the exact molecular interactions, it appears that the presence of NMP in the water phase plays a key role in the transport of hydrophobic drugs from an O/W ME.

It has been suggested that ME transdermal enhancement is a result of increasing drug concentration in the donor phase (2). In our systems containing the chemical enhancer NMP, we believe that the effective permeability of the membrane is also affected. If enhancement is merely a concentration effect, then the permeability of drug across human skin should remain constant. The permeability of all four drugs was compared from the ME systems against the solvent (IPM or H_2O) in which they were most soluble (Table V). There is a clear permeability enhancement for both hydrophilic and hydrophobic drugs from the ME systems ($p < 0.001$). This finding agrees with previous work where we found that NMP is capable of improving permeability of drugs from both IPM and H_2O.

Effect of NMP on Lidocaine Partitioning

NMP is freely miscible in both H_2O and IPM. It is also capable of improving lidocaine partitioning into the phase where the drug is less soluble (Fig. 2). The hydrophobic lidocaine free base partitions 2.6 times more in the aqueous phase with the addition of 33% v/v NMP. Similarly, the hydrophilic lidocaine HCl partitions 6.5 times more favorably in the IPM phase with the addition of 33% v/v NMP. The concentration of drug in the minority phase is improved 1.9-fold for lidocaine free base and 5.7-fold for lidocaine HCl. From these results, we conclude that NMP can act as a partition enhancer in ME systems. For a hydrophobic drug to transport from an O/W ME, the drug (e.g., lidocaine free base) must first partition from the "encapsulated" organic phase into the aqueous phase to reach the skin. The presence of NMP in the system is able to increase the concentration of the hydrophobic drug in the water phase, making it available for transport. Data from Fig. 2 indicates that NMP is also capable of improving the partitioning of hydrophilic drugs to the IPM phase in a W/O ME.

Table IV. Estradiol and Diltiazem HCl Transport from ME Systems

	Estradiol		Diltiazem HCl	
Formulation	$Flux_{ss}$ ($\mu g/cm^2/hr$)	Permeability ($cm/hr \cdot 10^5$)	$Flux_{ss}$ ($\mu g/cm^2/hr$)	Permeability ($cm/hr \cdot 10^5$)
H_2O	0.015 ± 0.006	460 ± 183	0.05 ± 0.01	0.015 ± 0.004
W/O ME	0.053 ± 0.029	1.1 ± 0.6	0.25 ± 0.13	1.2 ± 0.6
W/O ME Both Drugs	0.12 ± 0.06	2.4 ± 1.2	0.24 ± 0.08	1.2 ± 0.4
O/W ME	0.27 ± 0.07	5.8 ± 1.5	1.6 ± 0.3	7.8 ± 1.3
O/W ME Both Drugs	0.23 ± 0.05	5.0 ± 1.2	1.6 ± 0.4	7.8 ± 1.9
Water Phase		6.5 ± 1.7		6.1 ± 3.7

n = 3.

Table V. Permeability Enhancement of ME Systems

	Permeability ($cm/hr \cdot 10^5$)	Enhancement
Estradiol		
IPM	<0.1	
W/O ME	1.1 ± 0.6	>11
O/W ME	5.8 ± 1.5	>58
Diltiazem HCl		
H_2O	0.015 ± 0.004	
W/O ME	1.2 ± 0.6	80
O/W ME	7.8 ± 1.3	520
Lidocaine Free Base		
IPM	7.2 ± 1.1	
W/O ME	40.1 ± 4.5	5.6
O/W ME	123 ± 36	17
Lidocaine HCl		
H_2O	0.61 ± 0.38	
W/O ME	3.5 ± 0.3	5.7
O/W ME	18.1 ± 6.9	30

n = 3.

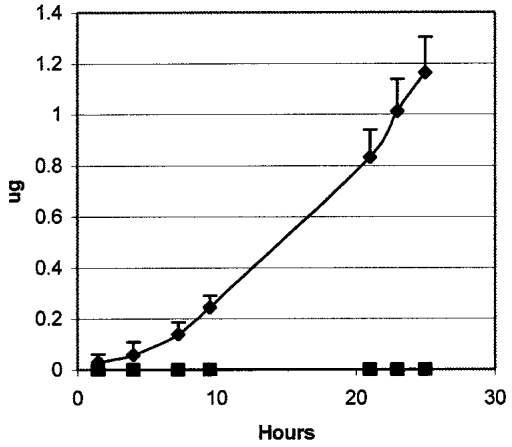

Fig. 3. Estradiol transport across stripped human skin in O/W formulation. *In vitro* transport of the hydrophobic drug estradiol through stripped human skin (5 mm diameter) from the oil-in-water microemulsion (0.4% drug w/w, ♦) and IPM (saturated drug, ■). n = 3

O/W ME Systems

The following mode of enhancement by NMP in the O/W system is supported by our data. A hydrophobic drug will preferentially partition in the encapsulated organic phase, making flux difficult. Since the presence of NMP improves partitioning (and concentration) of a drug in the bulk aqueous phase, a hydrophobic drug can transport across the skin from this phase with the aid of NMP, which has been shown to improve the permeability of human skin. However, the role of the organic phase for hydrophilic drug transport from an O/W ME is unknown.

We hypothesize that NMP is a more effective enhancer from the aqueous phase of a ME than the organic phase. NMP has an IPM/H_2O partition ratio of 0.02. Because NMP resides almost exclusively in the water phase of the system, its enhancing effects from that phase should dominate. In a W/O ME, the NMP is sequestered in the encapsulated phase and unable to interact with the skin. This might explain why both the hydrophilic and hydrophobic drugs transport better from the O/W ME. A second mode of hydrophobic drug flux enhancement by NMP from the water phase is also possible. Hydrophobic molecules will not readily leave an organic phase in which they are soluble. For this reason, the partitioning of lidocaine free base from IPM into the skin is low. However, when lidocaine is in the aqueous phase, it has two partitioning options. It can return to the organic phase, or follow NMP via hydrogen bonding across the skin membrane. This hydrogen bonding between NMP and lidocaine has been shown by IR spectroscopy. By this account, the water phase of an O/W ME provides a favorable environment from which a hydrophobic drug can partition into the skin.

The effects of the microemulsion system on both hydrophobic and hydrophilic drugs were experimentally tested. The hydrophobic steroid estradiol transports significantly better from the O/W ME than from bulk IPM solution (Fig. 3). The *in vitro* transdermal delivery of the hydrophilic diltiazem HCl shows a similar enhancement (Fig. 4). A drug such as diltiazem HCl is normally precluded from transdermal delivery is due to its large molecular weight that greatly diminishes its permeability across the skin (21) and ionic nature (22–24). Ionic drugs have also been proven to be difficult to deliver

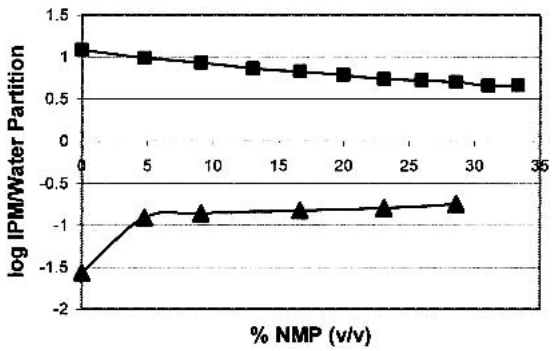

Fig. 2. Effect of NMP on lidocaine partitioning. The partition coefficient of the hydrophobic lidocaine free base (■) and the hydrophilic lidocaine HCl salt (▲) favors the less soluble phase as the NMP concentration increases.

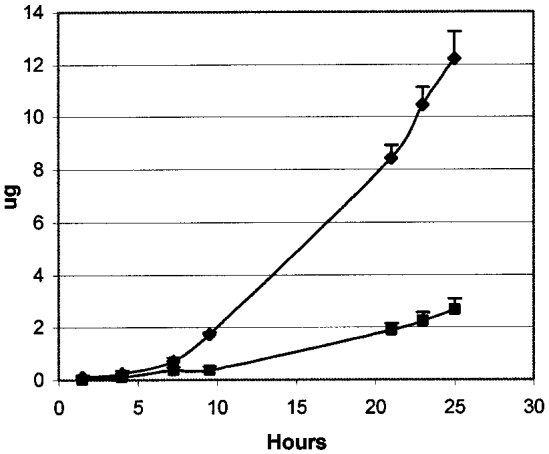

Fig. 4. Diltiazem HCl transport across stripped human skin in ME formulation. Transport of the hydrophilic drug diltiazem HCl through stripped human skin (5 mm diameter) from oil-in-water microemulsion (2% drug w/w, ♦) and water (2% drug w/w, ■). n = 3

transdermally (22–24). Transport of diltiazem HCl from the O/W ME system showed the most dramatic enhancement of the 4 drugs tested (Table V). The systemic concentration range for diltiazem HCl that is considered to be of therapeutic significance is 50–200 ng/ml (25). A 16 × 16 cm patch containing 15% drug in an O/W system would provide a flux of 3.07 mg/h, which corresponds to dose of 120 mg/day (hourly flux ~3 mg) (25). If the observations scale both in terms of surface area and diltiazem concentration, a 16 × 16 cm patch containing 15% drug in an O/W system could provide a flux of 3.07 mg/hr, which corresponds to dose of 120 mg/day (hourly flux ~3 mg)*****. However, locally therapeutic plasma concentrations could be attained with smaller doses. The results presented in this paper may be promising for delivery of other ionic salt drugs as well.

CONCLUSIONS

The systems studied provide many potentially interesting characteristics for a transdermal delivery vehicle. They are robust, and stable to the addition of significant amounts of soluble enhancers or excipients. They are capable of enhancing both hydrophilic and hydrophobic drugs, as well as simultaneous delivery of two drugs without diminished flux. The ME systems are also thermodynamically stable, and transport of lidocaine free base after 6 months storage at room temperature was equivalent to its initial value. Although no *in vivo* testing has been conducted, the components of our ME are common transdermal enhancers and are used at concentrations that should not raise safety issues. We believe further study of the systems proposed may offer a viable vehicle for transdermal drug delivery.

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