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Bicontinuous Water-AOT/Tween85-Isopropyl Myristate Microemulsion: A New Vehicle for Transdermal Delivery of Cyclosporin A

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ABSTRACT The purpose of this study was to investigate the influence of structure and composition of microemulsions (AOT/Tween85/isopropyl myristate/water) on their transdermal delivery potential of a lipophilic model drug (Cyclosporin A), and to compare the drug delivery potential of microemulsion to the suspension of drug in normal saline containing 20% ethanol. Their type and structure were examined by measuring surface tension, density, viscometry, and electric conductivity; the degree of agreement between the techniques was assessed. Transdermal flux of Cyclosporin A through rat skin was determined in vitro using Franz-type diffusion cells. Results of conducting, viscosity, and surface tension measurement confirmed the prediction transition to a bicontinuous structure. The microemulsions increased transdermal drug delivery of Cyclosporin A up to 10 times compared to the suspension. The increased transdermal delivery was found to be due mainly to water concentration and appeared to be dependent on the structure of the microemulsions.

KEYWORDS Bicontinuous Microemulsion, Cyclosporin A, Transdermal delivery, AOT

INTRODUCTION

Cyclosporin A (CyA) is a nonpolar cyclic oligopeptide consisting of 11 amino acids. It is a well-established immunosuppressive that has been used clinically for the treatment of inflammatory management and skin disorders like psoriasis. However, long term systemic adminstration of CyA has been noted to produce harmful effects such as hypochromic, granulomatous, hepatitis, and proximal renal tubular cell damage (Tran et al., 1999). This drawback has led to the exploration of investigations using CyA to achieve local immune suppression. Topical delivery of CyA is hindered by its physicochemical properties and the barrier property of stratum corneum (SC) (Duncan et al., 1990; Choi et al., 1995).

Several formulations of topical CyA are being tested for superior efficacy and reduced side effects (Verma & Fahr, 2004; Boinpally et al., 2004; Wang et al.,

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1998). A useful stragegy for improving percutaneous flux is to choose an appropriate vehicle for the transdermal delivery (Hilton et al., 1994; Oh et al., 2001).

Microemulsions and other related colloidal systems have received increased attention during the past few years. Formulations based on microemulsions have several interesting characteristics, namely: enhanced drug solubilization, good thermodynamic stability, and ease of manufacturing (Constantinides, 1995; Gasco, 1997). Many studies have shown that microemulsion formulations possessed improved transdermal and dermal delivery properties (Schmalfuss et al., 1997; Kreilgaard et al., 2000; Rhee et al., 2001). The microemulsion structure is important for the rate of drug release. The wide range of possible structures means that the microemulsions can release the solubilized drug at different rates. In an o/w microemulsion, hydrophobic drugs, solubilized mainly in oil droplets, experience hindered diffusion and therefore release rather slowly. Although the reverse behavior is expected in w/o type, low hydration levels of the SC will decrease the permeation of the microemulsions. For balanced microemulsions, due to the bicontinuous structure, relatively fast diffusion and release occur for oil-soluble drugs (Podlogar et al., 2004). Consequently, bicontinuous microemulsion should be an appropriate vehicle for hydrophobic drug in the transdermal delivery. An excellent and comprehensive review on the role of microemulsion in percutaneous penetration of drugs was recently published by Kreilgaard (2002).

The aim of this work was to formulate a new bicontinuous microemulsion system for transdermal delivery of CyA. The stable microemulsion systems consisting of AOT (aerosol-OT), Tween85, IPM (isopropyl myristate), and water were prepared, and their physicochemical properties, structure types and transdermal ability of CyA were also evaluated.

MATERIALS AND METHODS

Materials

Aerosol-OT (AOT) and CyA were procured from Sigma Chemical, IPM was obtained from Shanghai Chemical Reagent Corporation (Shanghai, China), Tween85 as co-surfactant was from Tianjin Bodi Chemical Company (Tianjin, China). The chemicals were used without any further purification. Distilled water was used in preparing microemulsion.

Construction of Pseudo-ternary Phase Diagrams

In order to find out the concentration range of components for the existing range of the microemulsions, pseduo-ternary phase diagrams were constructed using oil titration method at ambient temperature (25°C). Three phase diagrams were prepared with 1:1, 2:1, and 3:1 weight ratio of AOT to Tween85, respectively. For each phase diagram at a specific surfactant/co-surfactant weight ratio, the ratio of water to the mixture of surfactant and co-surfactant was varied at 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The mixture of water, surfactant, and co-surfactant at certain weight ratios was diluted with oil dropwise under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions or crude emulsions. No attempt was made to distinguish between oil-in-water, water-in-oil, or bicontinuous type microemulsions.

Preparation of Microemulsions

The microemulsion formulations were selected at different component ratios as described in Table 1. In order to prepare the drug-loaded microemulsions, a stock solution containing CyA was prepared with IPM. Aerosol-OT (AOT) and Tween85 were mixed in a required mass ratio

TABLE 1 Composition of the Microemulsion Studied (See Fig. 1)

Components	ME1	ME2	ME3	ME4	ME5	ME6	ME7	ME8	ME9	ME10
CyA (mg/ml)	4.5	4.5	4.5	4.5	4.5	14.5	4.5	4.5	2.25	13.5
IPM (%)	75	70	65	60	55	50	45	40	50	50
AOT (%)	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3
Tween85 (%)	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7
Water (%)	5	10	15	20	25	30	35	40	30	30

to obtain surfactant mixture at room temperature for 24 h. The stocking solution was then added and after brief mixing, the required amount of water was added. Components were blended using a magnetic stirrer at ambient temperature (25 ± 1°C). The resulting microemulsion remained stable over a period of several months.

The concentration of surfactant mixture was kept constant at 20wt% while the ratio of water varied between 5% and 40% weight fraction. The microemulsion formulations were selected at different component ratios as described in Table 1.

Physicochemical Characterization of Microemulsions

Viscosity

The viscosities of microemulsions were measured with a NDJ-8S digital viscometer (Shanghai Precision and Scientific Instrument, Shanghai, China) with a No. 1 rotor set at 60 rpm. Temperature was controlled at 25 ± 0.5°C.

Electrical Conductivity Measurements

Conductances were measured using a Mettler Toledo MC 226 (Mettler, Zurich, Switzerland) conductivity meter with a Mettler Toledo Incab 730 electrode. Temperature was controlled at 25 ± 0.5°C.

Surface Tension and Density

The density of microemulsions was measured with a digital precision density meter DMA 600 (Anton Paar, Graz, Austria).

Surface tension was measured with a tensiometer JK99C (Shanghai Zhongchen Digital Instrument, Shanghai, China) using Wilhemy's plate method. A square platinum plate was cleaned, washed off twice with distilled water, and heated in a reductive flame to purge all impurities. This cleaning procedure was repeated before every measurement. During the measurement the plate is dipped into the liquid. The tensiometer measures the pulling force of the liquid on the plate and, with known plate size, calculates the surface tension.

Both of measurements were made at 25 ± 0.5°C.

Cyclosporin A Determination

Cyclosporin A (CyA) was analyzed by reversed phase HPLC using JASCO 1500 series. The column was a Diamond C_8 column (5 μm, 4.6 × 150 mm). The mobile phase was a acetonitrile–water–H_3PO_4 (750:250:1) mixture with a flow rate of 1.0 mL/min. The detection wavelength was set at 210 nm and oven at 70°C. The retention time of CyA was 6.8 min. The area under the peak was used to calculate the concentration of CyA and linearity was achieved over the concentration range of 0.2–4.0 μg/mL with a limit of detection of 0.1 μg/mL. No interference of the other formulation components was observed. All samples filtered through an aqueous 0.45 μm pore size membrane filter before injection.

In Vitro Studies of Permeation of CyA

The abdominal skins were obtained from male rats weighing 200 ± 20 g. After hair was removed with a depilatory, the skins were excised. The subcutaneous fat was removed, and then the skins were washed and examined for integrity. The skins were placed in a refrigerator at 4°C overnight, and then used for the experiments. The permeation experiments were performed using modified Franz diffusion cells with diffusion area of 0.785 cm^2 and a receiver volume of 8.0 mL. Normal saline containing 20% ethanol was used as the receiver medium. The receptor chambers were thermostated at 32°C and the solution in the receptor chambers was stirred continuously at 300 rpm. The formulations (100 μL) containing CyA were gently placed in donor chamber. At 2, 4, 6, 8, 10, and 12 h, all of the solution in the acceptor chamber was removed for HPLC determination and replaced immediately with an equal volume of fresh receiver medium. Cumulative corrections were made to obtain the total amount of CyA permeated at each time interval.

The cumulative amount of CyA permeated through rat skin was plotted as a function of time. The permeation rate of CyA at steady state (J_s, μg/cm^2 per h) through rat skin was calculated from the slope of linear portion of the cumulative amount permeated through the mouse skins per unit area vs. time plot.

In order to obtain the permeability coefficient K_p (cm/h), we used the equation:

$$K_p = J_s / C_0$$

where K_p is the permeability coefficient, J_s is the flux calculated at steady state, and C_0 represents the drug concentration that remains constant in the vehicle.

Skin Irritation Studies

ME6 were selected as tested formulations for skin irritation studies. All samples were applied to the shaved skin on the back of six New Zealand rabbits. On one side of the back, the suspension and on the other side ME6 was applied for each day. The animals were observed and evaluated for any sign of erythema or edema for a period of 7 days.

Statistical Analysis

All skin permeation experiments were repeated six times and data were expressed as the mean value ± S.D. Statistical data were analyzed by one way analysis of variance (ANOVA). A multiple comparison test was used to compare different formulations, and a P value of 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Phase Studies

Aerosol-OT (AOT) or sodium bis-(2-ethylhexyl) sulphosuccinate is a well-known anionic medicinal surfactant. The chemical structure of AOT(HLB = 10.5) imparts a well-balanced hydrophilic-lipophilic property. This unique feature of AOT allows the formation of alcohol-free reverse micellar and normal micellar aggregates in nonaqueous and aqueous mediums, respectively, without any co-surfactant. When Tween85(HLB = 11.0) was added to the systems, the area of microemulsion isotropic region was increased (the ternary of AOT–IPM–water was not dated). Aerosol-OT (AOT) micellar system was found to produce moderate irritation, while AOT reverse micellar system having a high ratio of AOT was found to be safe to use as a vehicle for transdermal delivery (Johan et al., 1996). The construction of phase diagrams makes it easy to find out the concentration range of components for the existence range of microemulsions. The pseduo-ternary phase diagrams with various weight ratios of AOT to Tween85 are described in Fig. 1. The translucent microemulsion region is presented in phase diagrams. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) microemulsions was observed. Dermal administration requires a low content of surfactants. Therefore, a system of low content of surfactant/co-surfactant was selected for investigations on rat skin with regard to a potential skin irritation risk of these amphiphilic compounds. It has been concluded from the inspection of these phase diagrams that in the case of S / CoS ratio = 2, the microemulsion has the maximum water solubilization at surfanctant concentration of 20%.

Surface Tension and Density

Surface tension vs. water weight ratio is presented in Fig. 2. Up to 15wt% of water, the surface tension of the system increases slowly. In the interval of 15~25wt% water content, it does not change. With further increases of water, surface tension first decreases sharply until 30wt%, and then an increase is observed. At similar composition of water, breaking points of the lines in the densities could be observed (see Fig. 3).

As we know, the middle-phase microemulsion is an interesting system because there is a self-assembled organized microstructure in it. The microemulsion shows ultra-low interfacial tensions between it and water and oil-rich phases (Leser et al., 1996). It is stabilized by a surfactant layer which is very flexible and which has a zero mean curvature (bicontinuous microstructure) (Mehta & Bala, 2000). Therefore, we propose that the phase behavior changed from w/o-type to o/w-type by means of the middle-phase microemulsion.

Viscosity and Conductivity

Mobility properties expressed with viscosity and electrical conductivity are presented in Figs. 4 and 5, respectively. The viscosity increases sharply until 20wt% water content and then decreases till 30wt% water content; after 30wt% the viscosity remains almost constant. This implies that the microemulsion system inverted from oil-in-water (o/w) to water-in-oil (w/o) or changed to bicontinuous.

The drastic changes in conductivity around a given water volume fraction Φ_p can be attributed to the formation of an infinite transient cluster of the aqueous microemulsion nanodroplets. The transition marks the onset of the so-called "percolated state" of the microemulsion where both oil and nanodroplet network are continuous (Gennes, 1976).

The interpretation of the curves requires the concept of percolation transition proposed by

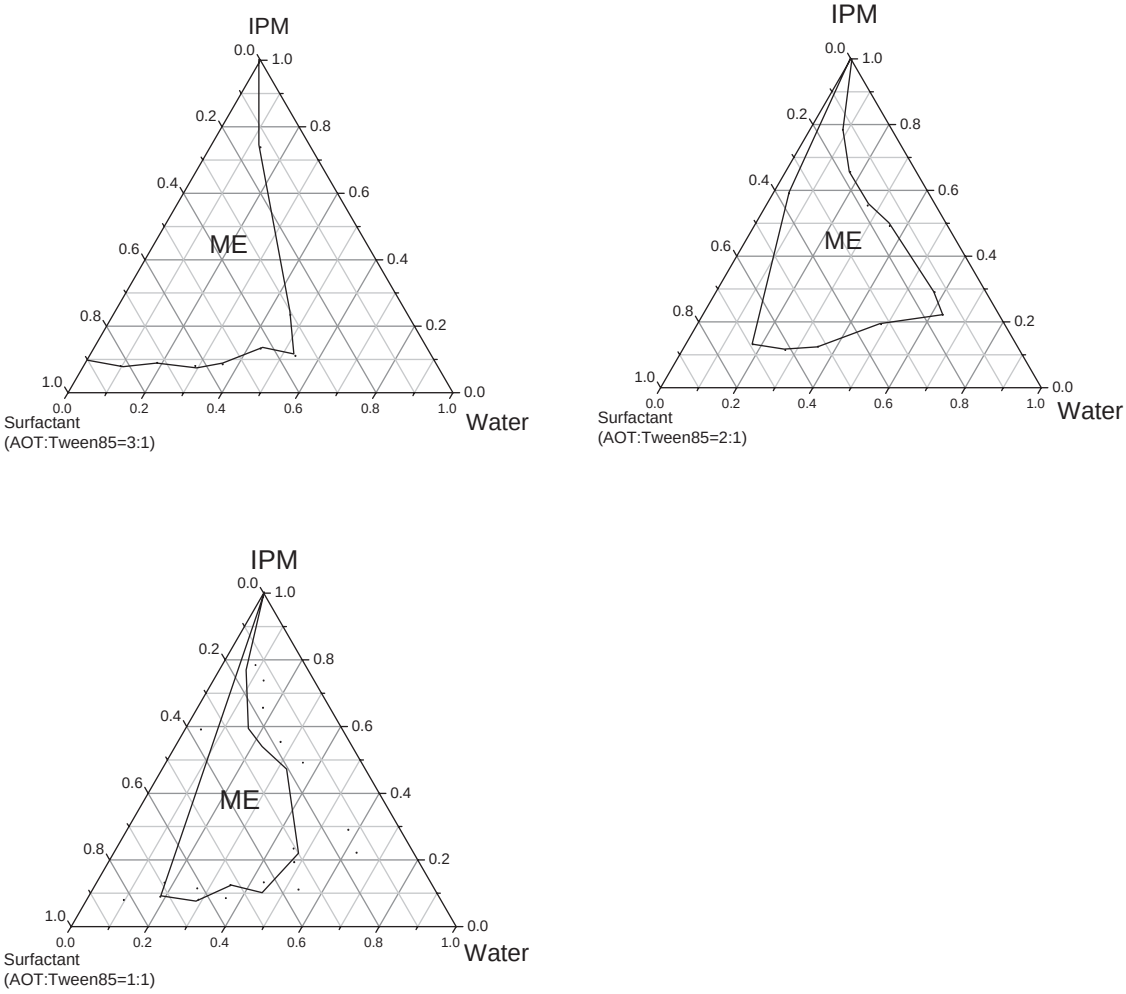

FIGURE 1 **Pseudo-ternary Phase Diagrams of Oil-Surfactant-Water System at 1:1, 2:1, and 3:1 Weight Ratios of AOT to Tween85 at 25°C. ME Represent Microemulsion Regions.**

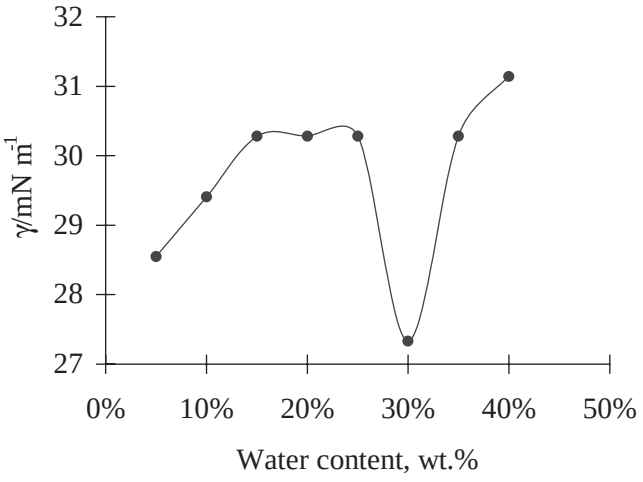

FIGURE 2 **The Variation of Surface Tension as a Function of Water Weight Ratio (wt%) in Water-AOT/Tween 85-IPM Microemulsions.**

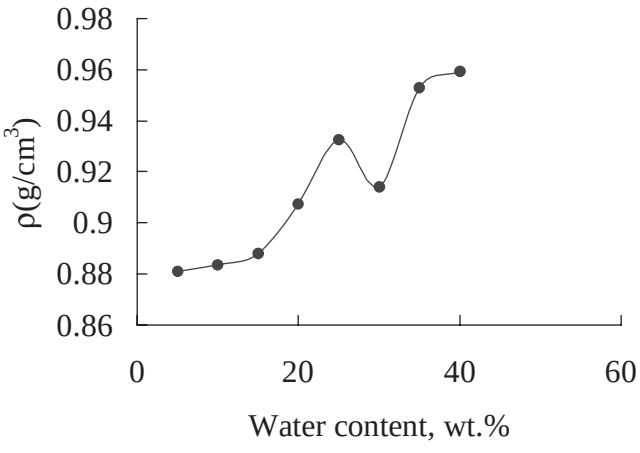

FIGURE 3 **The Variation of Density as a Function of Water Weight Ratio (wt%) in Water-AOT + Tween 85-IPM Microemulsions.**

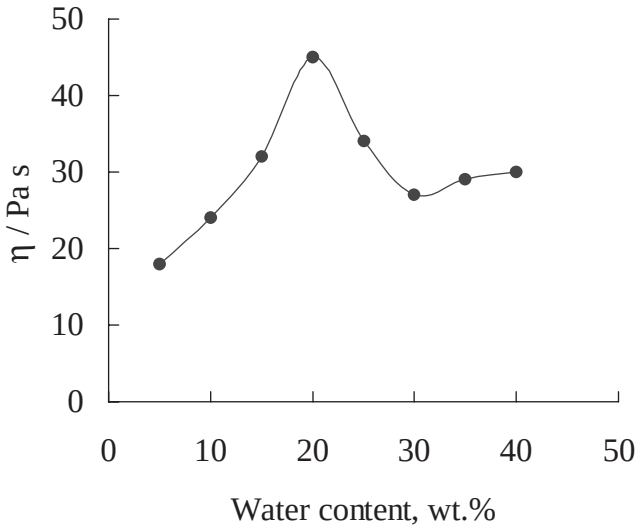

FIGURE 4 **Viscosity as a Function the Water Weight Ratio in Water-AOT + Tween 85-IPM Microemulsions.**

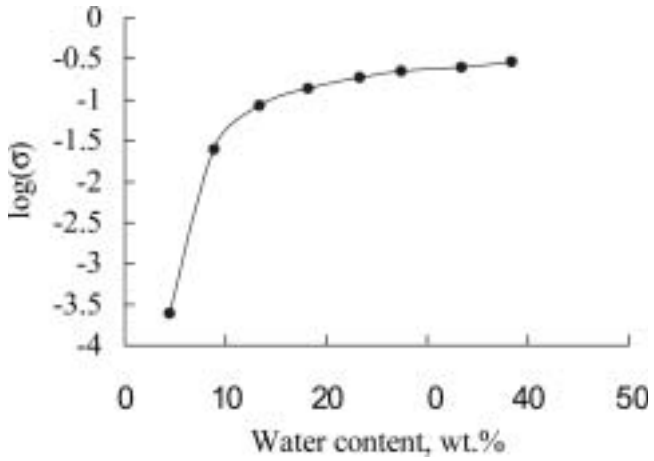

FIGURE 5 **Electrical Conductivity as a Function of the Water Weight Ratio (wt%) in Water-AOT + Tween 85-IPM Microemulsions.**

Gennes, 1976 (Laguës et al., 1978). This concept is used for interpreting the conductivity of disordered media such as microemulsions (Gradzielski & Hoffman, 1999). The percolation transition signifies the first emergence of an infinite cluster for a critical value of the water volume fraction, Φ_p, called the percolation threshold. The clustering of the droplets at the percolation threshold typically leads to a sharp increase in conductivity (Brian et al., 1997). According to the theory:

$$\sigma \propto \left|\Phi_p - \Phi\right|^{-s} \quad \Phi < \Phi_p$$

$$\sigma \overset{\infty}{\Delta} \left|\Phi_p - \Phi\right|^{t} \quad \Phi_p < \Phi$$

where Φ is the fraction of bonds below and above Φ_p, and s and t are two critical exponents. Since percolation is an exclusively geometry defined problem, the above exponents should be constant and independent of the chemical nature of the system. However, they depend on whether the charge carriers are able to diffuse or not. The classical dynamic percolation limit typically predicts t ringing between 1.5 and 1.9 and s about 1.2.

The sharply increased conductivity indicates the infinite cluster of the microemulsions. The σ values checked in our systems follow this law. In order to determine Φ_p and t independently, $\sigma^{1/t}$ was plotted vs. Φ; $\sigma^{1/t}$ is linear in Φ for $t = 1.6$ as shown:

$$\sigma^{1/t} = 0.0176\Phi - 0.072$$

Our system undergoes a percolation transition (see Fig. 6) and it is possible to calculate the percolation threshold that corresponds to the beginning of the bicontinuous phase.

$$\Phi_p = 0.072 / 0.0176 = 4.1\%$$

The low value of the threshold is the consequence of the attractive interactions of the conductive species in the system.

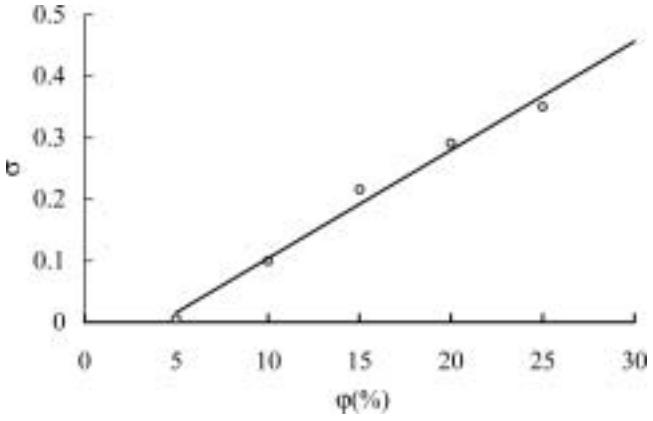

FIGURE 6 **Percolation Threshold Determination.**

In Vitro Studies

The cumulative amount of CyA released over time through the rat skin by AOT + Tween85/water/IPM microemulsions at various water concentrations at fixed surfactant concentration is showed in Fig. 7. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of the drug into skin (Ostrenga et al., 1971). The thermodynamic driving force for release reflects the relative activities of the drug in different phases. In micellar or o/w systems, water diffusion will be rapid, in the same order of magnitude as that of neat water. Oil and surfactant diffusion will be slow and within the same order of magnitude. On the other hand, in inverted micellar or w/o systems, oil diffusion will be rapid, in the same order of magnitude as that of neat oil. Water and surfactant diffusion will be slow and within the same order of magnitude. For bicontinuous systems, both water and oil diffusion will be rapid, only slightly slower than those of the neat liquid. Surfactant diffusion will be low due to the consititution of the interfacial film, but slightly higher than that of o/w and w/o system (Kreilgarrd, 2002). The figure depicts that as the water concentration increases in the microemulsion, permeation of drug through the skin increased until 30wt% water (the microemulsions change from w/o to bicontinuous). It is expected due to increase in hydration level of the SC that facilitates the permeation of drug through skin and fluidization of lipid alkyl chain the SC

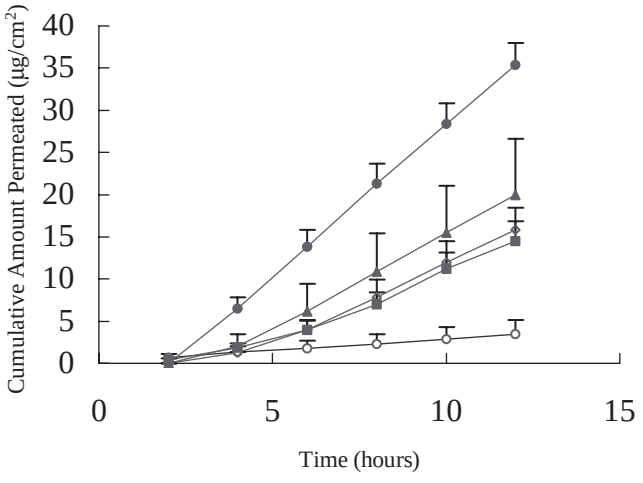

FIGURE 7 **Permeation Profiles of CyA Through Rat Skins from the Microemulsions and the Aqueous Solution. (1) (■) = ME2; (2) (▲) = ME4; (3) (●) = ME6; (4) (◇) = ME8; (5) (○) = the Aqueous Solution.**

(Osborne et al., 1991), although the effective concentrations of drug in w/o and bicontinuous microemulsion could be comparable. However, at 40wt% water the drug through the skin decreased which may due to a decreased thermodynamic activity of drug in microemulsions. For CyA (a hydrophobic drug, log $P_{\text{octanol/water}} = 2.91$), in an o/w microemulsion (40wt% water), since most of the drug dissolves in oil and surfactant, the relative activities may less than that in bicontinuous structure (30wt% water) in which oil and surfactant diffusion will be rapid. The value of skin flux and permeability coefficient are shown in Table 2. The skin flux of drug encapsulated in ME composed of 20wt% mixed surfactant concentration at 10, 20, 30, and 40wt% water gives 4.4-, 6.8-, 10.8-, and 5.6-fold enhancement, respectively, with respect to control at 12 h. The permeation profiles of microemulsions followed zero order release kinetics. Statistical comparison of the flux throughout 12 h showed that most of the microemulsion provided fluxes ($P < 0.05$) higher than the aqueous solution.

The effect of the loading dose of CyA in microemulsions on the permeation rate is shown in Fig. 8. The permeation rate of CyA increased from 1.44 ± 0.08 to 3.58 ± 0.24 μg/cm^2 per h when the loading dose of CyA increased from 2.25 mg/mL to 4.5 mg/mL. The permeation rate of CyA was almost linearly improved as a function of loading dose. However, higher concentrations (13.5 mg/mL or more) have no obvious effect on the transdermal delivery. Cyclosporin A (CyA) is a lipophilic cyclic large molecule (1202Da). These physicochemical properties make CyA a challenge for transdermal delivery. As the cutaneous drug delivery rate of formulations is generally related to the concentration (activity) gradient of the drug towards the skin, the solubility potential of microemulsions may be an important factor in increasing skin absorption of drugs (Kreilgarrd, 2002). The equilibrium solubility of CyA in normal saline containing 20% ethanol was reported to be 60.61 ± 3.96 μg/mL (Guo et al., 2000). Microemulsions in the present study have enhanced its solubility to 12.04 ± 1.75mg/mL. The enhanced permeation of CyA caused by microemulsions may be partly due to the components included in the formulation. Therefore, 13.5 mg/mL (saturated) and higher concentrations did not display a significant increase in the permeation of CyA. This result indicates that to achieve a maximum flux of CyA, the loading drug concentration of 4.5 mg/mL was superior.

TABLE 2 **Permeation of CyA as a Function of Water Concentration in AOT + Tween85/Water/IPM microemulsions Through Depilatory Treated Rat Skin**

System	Water (wt%)	Concentration of CyA(mg mL^{-1})	Steady-state skin flux (J_{ss}) ± S.D. (μg $cm^{-2}h^{-1}$)[a]	Permeability coefficient (P) × 10^{-3} ± S.D. (cm h^{-1})[a]
ME2	10	4.5	1.45 ± 0.24	0.32 ± 0.05
ME4	20	4.5	2.26 ± 0.61	0.45 ± 0.12
ME6	30	4.5	3.58 ± 0.24	0.71 ± 0.05
ME8	40	4.5	1.85 ± 0.34	0.37 ± 0.07
ME9	30	2.25	1.44 ± 0.08	0.57 ± 0.03
ME10	30	13.5	5.38 ± 0.51	0.40 ± 0.04
Controlled		4.5	0.33 ± 0.08	4.82 ± 1.19

[a]Mean ± S.D. of six experiments.

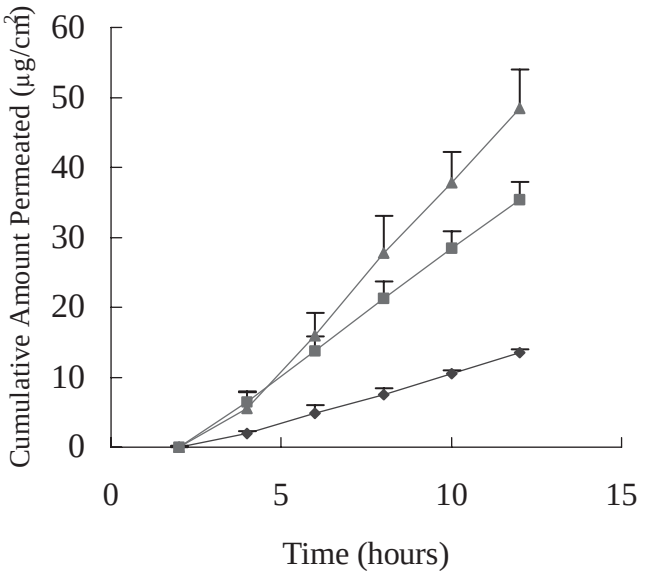

FIGURE 8 **The Effect of the Loading Dose of Skins from the Microemulsions on the Permeation rate. (1) (▲) = ME9; (2) (■) = ME6; (3) (♦) = ME1.**

Skin Irritation Studies

The irritation studies did not show visible irritation after application of ME6 for 7 days on the skin of rabbits. No erythema or edema was observed on the skin of rabbits for ME6. Only rebefaction appeared on the skin of some rabbits occasionally on the third or fourth day and disappeared on the sixth or seventh day. This microemulsion system for the transdermal delivery of CyA is viable.

CONCLUSIONS

The microemulsions containing CyA were studied for transdermal delivery. The different microemulsion formulations were selected using the pseudo-ternary phase diagrams. The components used in this study were shown to enable a broad variety of microemulsion compositions. Comparing the chosen experimental methods, we can conclude that a microemulsion containing roughly between 25 and 35wt% water will be a bicontinuous microemulsion. The microemulsions were shown to increase transdermal delivery of CyA up to 10 times compared to the suspension depending on the microemulsion structure and drug loading.

REFERENCES

Boinpally, R. R., Sen-Lin Zhou; Devraj, G., Anne, P. K., Poondru, S., & Jasti, B. R. (2004). Iontophoresis of lecithin vesicles of Cyclosporin A. *Inter. J. Pharm*., *274*, 185–190.

Brian A., Antony J., & Williams. (1997). Microstructure analysis at the percolation threshold in reverse microemulsions. *Colloids and Surfaces A*. *128*, 1–11.

Choi, H. K., Flynn, G. L., & Amidon, G. L. (1995). Percutaneous absorption and dermal delivery of Cyclosporin A. *J. Pharm. Sci*., *84*, 581–583.

Constantinides, P. P. (1995). Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharm. Res*., *12(11)*, 1561–1572.

Duncan, J. I., Payne, S. N., Winfield, A. J., Ormerod, A. D., & Thomson, A. W. (1990). Enhanced percutaneous absorption of a novel topical Cyclosporin A formulation and assessment of its immunosuppressive activity. *Br. J. Dermatol*., *123*, 631–640.

Gasco, M. R. (1997). Microemulsions in the Pharmaceutical Field: Perspectives and Applications. In *Industrial Applications of Microemulsions*; Gasco, M. R., Ed.; Marcel Dekker, Inc.: New York: 97–132.

Gennes, D, P. G. (1976). La percolation: un concept unificateur. *Recherche*, *72*, 919–927.

Gradzielski, M., & Hoffman. H. (1999). Rheological Properties of Microemulsions. In *Handbook of Microemulsion Science and Technology*; Kumar, P., Mittal, K. L. Eds.; Marcel Dekker, Inc.: New York.

Guo, J., Ping, Q., Sun, G., & Jiao, C. (2000). Lecithin vesicular carriers for transdermal delievery of cyclosporine A. *Int. J. Pharm*., *194*, 201–207.

Hilton, J., Wollen, B. H., Scott, R. C., Auton, T. R., Trbilcock, K. L., & Wilks, M. F. (1994). Vehicles effect on in vitro percutaneous absorption through rat and human skin. *Pharm. Res*., *11*, 1369–1400.

Johan, S., Lindberg, R., & Friberg, S. (1996). Microemulsions—Phase equilibria characterization, structures, applications and chemical reactions. *Adv. Colloid. Inter. Sci*., *95*, 125–287.

Kreilgaard, M. (2002). Influence of microemulsions on cutaneous drug delivery. *Adv. Drug Deliv Rev*., *54*, S77–S98.

Kreilgarrd, M., Pederson, E. J., & Jaroszewski, J. W. (2000). NMR characterization and transdermal drug delivery potential of microemulsion systems. *J. Control. Release*. *69*, 421–433.

Laguës, M., Ober, R., & Taupin, C. (1978). Study of structure and electrical conductivity in microemulsions: evidence for percolation mechanism and phase inversion. *J. Phys. Lett*., *39*, L487-L491.

Leser, M. E., Evert, W. C., & Agterof, W. G. M. (1996). Phase behaviour of lecithin-water-alcohol-triacylglycerol mixtures. *Colloids and Surfaces A*., *116*, 293–308.

Mehta, S. K., & Bala, K. (2000). Tween-based microemulsions: a percolation view. *Fluid Phase Equilibria*., *172*, 197–209.

Oh, H. L., Oh, Y. K., & Kim, C. K. (2001). Effect of vehicles and enhancer on the transdermal delivery of melatonin. *Inter. J. Pharm*., *212*, 63–71.

Osborne, D. W., Ward, A. J., & O'Neill, K. J. (1991). Microemulsions as topical drug delivery vehicles: in vitro transdermal studies of a model hydrophilic drug. *J. Pharm. Pharmacol*., *43*, 450–454.

Ostrenga, J., Haleblian, J., Poulsen, B., Ferrell, B., Mueller, N., & Shastri, S. (1971). Vehicle design for a new topical steroid, fluocinonide. *J. Invest Dermatol*., *56*, 392–399.

Podlogar, F., Gašperlin, M., Tomšic, M., Jamnik, A., & Rogac, M. B. (2004). Structural characterization of Water-Tween40^{R}/Imwitor308^{R}-isopropyl myristate microemulsions using different experimental method. *Inter. J. Pharm*., *276*, 115–128.

Rhee, Y. S., Choi, J. G., Park, E. S., & Chi, S. C. (2001). Transdermal delivery of ketoprofen using microemulsions. *Int. J. Pharm*., *228*, 161–170.

Schmalfuss, U., Neubert, R., & Wohlrab, W. (1997). Modification of drug penetration into human skin using microemulsions. *J. Control. Release*., *46*, 279–285.

Tran, H. S., Malli, D., Chrzanowski, F. A., Puc, M. M., Matthews, M. S., & Hewitt, C. W. (1999). Site-specific immunosuppression using a new formulation of topical Cyclosporin A with polyethylene glycol-8 glyceryl caprylate/caprate. *J. Surg. Res*., *83*, 136–140.

Verma, D. D., & Fahr, A. (2004). Synergistic penetration enhancement effect of ethanol and phosphoilipids on the topical delivery of Cyclosporin A. *J. Control. Release*., *97*, 55–66.

Wang, S., Kara, M., & Krishnan, T. R. (1998). Transdermal delivery of Cyclosporin A using electroporation. *J. Control. Release*, *50*, 61–70.