Development and Characterization of Eucalyptol Microemulsions for Topic Delivery of Curcumin

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Microemulsions have received great attention for applications in transdermal drug delivery. The use of curcumin for treating various skin diseases like scleroderma, psoriasis, and skin cancer was extensively reported. The solubility of curcumin in various oils, surfactants, and cosurfactants was studied herein in order to find the optimal components for a transdermal delivery vehicle. Microemulsion systems composed of eucalyptol, polysorbate 80, ethanol, and water were developed as transdermal delivery vehicles for curcumin. Effects of the microemulsion composition on transdermal curcumin delivery were studied using Franz diffusion cells. The transdermal curcumin flux, permeability coefficient, and enhancement ratio were analyzed to evaluate the effects of eucalyptol/water ratios in the microemulsions. Pseudo-ternary phase diagrams of the eucalyptol microemulsions with various surfactant/cosurfactant ratios (1 : 1—1 : 3) were constructed to investigate their phase behaviors. Conductivity, interfacial tension, size, and viscosity data of the microemulsions were used to characterize the physicochemical properties of transdermal vehicles. The influence of the microemulsions on skin histology and on the delivery route was analyzed using hematoxylin/eosin staining and confocal laser scanning microscopy. In conclusion, microemulsions were successfully developed for transdermal curcumin delivery after screening various components and adjusting the oil/water ratios. The curcumin permeation rate of the microemulsion developed was 15.7-fold higher than that of the control (eucalyptol only). These results indicate that an eucalyptol microemulsion system is a promising tool for the percutaneous delivery of curcumin.

Key words eucalyptol microemulsion; transdermal delivery; curcumin

Transdermal drug delivery has many advantages over other administration routes: for example, avoidance of hepatic metabolism, convenient administration for the patient, and easy withdrawal of treatment if necessary. Despite extensive studies of transdermal drug delivery, only a few drug formulations are commercially available.[1] One of the reasons for this is the permeation barrier by the stratum corneum to exogenous substances. Skin penetration enhancement can be chieved using appropriate physical and chemical means to overcome the skin's barrier function. Chemical penetration enhancers such as fatty acids, fatty esters, surfactants, and terpenes have received considerable attention due to their low cost, ease of use, safety, and efficacy.[2] Terpenes are lipophilic ingredients of essential oils which can disturb intracellular lipids and increase the fluidization of the stratum corneum, thereby enhancing drug penetration.[3] Eucalyptus oil (eucalyptol, a terpene from trees) was adopted as the oil phase to design transdermal vehicles for various drugs including estradiol, progesterone, cyproterone acetate, finasteride, and hydrocortisone.[4,5] Investigations of microemulsions composed by eucalyptus/peppermint oil and water were reported to have many potential applications.[6,7] Lipid-based systems such as microemulsions, nanoemulsions, solid lipid nanoparticles, and liposomes were recently applied to transdermal drug delivery.[8] Microemulsions are single phase, optically isotropic nano-structured solutions composed of a surfactant, cosurfactant, oil, and water. The ability of microemulsions to deliver drugs through a transdermal route in a controlled manner was recently reviewed.[9] Advantages of microemulsion delivery systems include spontaneous formation, easy preparation, low viscosity, high surface area, high drug solubilization, optical transparency, and thermodynamic stability.[10] Microemulsions were applied to deliver different lipophilic drugs including fluconazole,[11] penciclovir,[12] quercetin,[13] diclofenac,[14] and progesterone[15] through the transdermal route. Those *in vitro* and *in vivo* studies demonstrated that lipophilic drugs incorporated into microemulsions can efficiently penetrate the skin.

Curcumin and curcuminoids are yellow polyphenolic compounds extracted from the spice turmeric (*Curcuma longa*). Recent studies demonstrated that curcumin has the potential to protect skin by reducing inflammation through nuclear factor (NF)-κB inhibition and to reduce wound-healing time by improving collagen deposition.[16] Curcumin can regenerate muscle cells after traumatic injury by modulating NF-κB activity within muscle tissues. Additionally, the use of curcumin for the chemoprevention and treatment of various skin diseases like scleroderma, psoriasis, and skin cancer was reviewed.[16] Patel *et al.* demonstrated that curcumin-containing films have good anti-inflammatory activity against carrageenan-induced edema in rats similar to a standard formulation through the transdermal route.[17] Those studies suggested the beneficial effects of curcumin for treating skin and muscle diseases. Additionally, the poor bioavailability of curcumin through the oral route was reported.[18—20] Although topical curcumin delivery for treating skin and muscle disorders is promising, little work has focused on researching curcumin's transdermal delivery using a terpene microemulsion system. Using terpene microemulsion vehicles, curcuminoids can be solubilized to attain a high concentration gradient for transdermal delivery. At the same time, terpene can act as an enhancer to accelerate the dermal administration of curcumin. The aim of this study was to characterize eucalyptol microemulsions and evaluate their permeation characteristics with curcumin across porcine skin. Pseudo-ternary phase diagrams were used to investigate the constituents of oils, surfactants, and cosurfactants in stable microemulsions. The transdermal flux, permeability coefficient, and curcumin

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retained in the skin were evaluated using neonate pig skin mounted on a Franz diffusion cell. The fluorescence of curcumin was used to trace its transdermal route using confocal microscopy. Finally, the effects of the drug payload and water ratios on the physicochemical characteristics of the microemulsions were studied to understand the relation between transdermal delivery efficiency and the formulation.

Experimental

Materials Eucalyptol and ethanol were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). Propylene glycol, isopropanol, and tetrahydrofuran were from Echo Chemicals (Taipei, Taiwan). Curcumin was procured from Masterasia (Taipei, Taiwan). Polysorbate (Tween) 20, 40, 60, 80, 85 were purchased from IL-Shin Emulsifier (Seoul, Korea). All reagents were used without further purification. The water used in this study was freshly purified by the Milli-Q Gradient A10 system (Millipore, Molsheim, France).

Construction of the Pseudoternary Phase Diagrams Eucalyptol, polysorbate 80, and ethanol were selected as the oily phase, surfactant, and cosurfactant, respectively. Surfactant/cosurfactant mixtures were tested at a ratio of 1 : 1 (w/w). The pseudoternary phase diagrams were constructed using the water titration method at room temperature.[5] For each phase diagram, mixtures of oil and surfactant or surfactant/cosurfactant mixtures were prepared at weight ratios of 0.5 : 9.5, 1 : 9, 1.5 : 8.5, 2 : 8, 2.5 : 7.5, 3 : 7, 3.5 : 6.5, 4 : 6, 5 : 5, 6 : 4, 7 : 3, 8 : 2, and 9 : 1. These mixtures were titrated drop-wise with water under gentle magnetic stirring. After being equilibrated, the systems were visually characterized. Single-phase, transparent mixtures were designated microemulsions.

Preparation and Characterization of Microemulsions Microemulsions were prepared by mixing the oil with the surfactant or surfactant/cosurfactant mixture before adding the required amount of water under magnetic stirring. Curcumin (0.4 wt%) was then added to the prepared microemulsion. No phase change was noted after addition of the drug or after equilibration in the water bath. The flow properties and viscosity of the formulations were determined at 32±1 °C. Viscosity determinations employed a Brookfield viscometer (DV II+, Brookfield Engineering Laboratories, Stoughton, MA, U.S.A.). The pH and conductivity of the microemulsions were measured with a pH meter (WTW, Inolab 720, Burladingen, Germany) and conductivity meter (Con 500, Eutech Instruments, Singapore). Interfacial tension measurements were carried out at room temperature using a thin platinum plate attached to a transducer amplifier (Kyowa CBVP-A3, Saitama, Japan). During the measurement, the plate is dipped into the test microemulsions. The tensiometer measures the pulling force of the liquid on the plate and calculates the interfacial tension by dividing by the known plate size. The average particle size was characterized using a Zetasizer Nano ZS 90 (Malvern, Worcestershine, U.K.) at a fixed angle of 90° and a temperature of 25 °C. Microemulsion samples were measured without dilution with water due to the immiscible property of the microemulsion and water.

***In Vitro* Drug Release** Full-thickness skin from the porcine ear is a generally accepted model of permeation for human dermatological research. Researchers use pig ear skin for *in vitro* transdermal diffusion experiments.[21,22] In this study, the skin of the outside of the ears of corpses of new-born piglets was used to study the permeation of curcumin in a Franz diffusion assembly. The skin was peeled from the underlying cartilage after cutting along the tips of the ears. The ear skin was mounted between the donor and receptor compartments with the stratum corneum side facing the donor compartment. The donor medium consisted of 0.5 ml of vehicle containing curcumin. The receptor medium (5.5 ml) was obtained by mixing 1 : 1 ethanol and pH 7.4 phosphate-buffered saline (PBS) in order to maintain sink conditions. Similar solution was successfully applied to monitor the skin delivery of steroidal drugs from microemulsions.[23] Other studies also employed 40% propylene glycol solution as the receptor solution.[5] The available diffusion area between the cells was 0.785 cm^2. The stirring rate and temperature were respectively kept at 600 rpm and 32 °C. At appropriate intervals, all of the receptor medium was withdrawn and immediately replaced with equal volumes of fresh buffer. The permeated amount of curcumin was determined by high-performance liquid chromatography (HPLC). The curcumin retained in the skin was measured after the permeation experiment (24 h). The skin was washed three times using a cotton cloth containing ethanol. A skin sample with the 0.785 cm^2 permeated area was cut, weighed, and then homogenized in a 50% ethanol solution. The resulting solution was centrifuged, and the surpernatant was analyzed by HPLC. The permeated skin (0.785 cm^2) was equivalent to 0.042±0.008 mg (n=10).

Curcumin Analysis Quantification of curcumin was achieved using an HPLC system (Jasco, Tokyo, Japan) consisting of a pump, a UV detector, and a Microsorb-C18 column (Varian, Lake Forest, CA, U.S.A.). The mobile phase consisted of a 1% (w/v) citric acid solution, adjusted to pH 3.0 using a 45% (w/v) potassium hydroxide solution, and tetrahydrofuran in the ratio of 50 : 50 (v : v). The flow rate of the mobile phase was 1.0 ml/min. The column effluent was monitored at 430 nm, and the chromatographic data analysis was performed with the Borwin Program (vers. 1.5, Jasco).

Curcumin Solubility We screened oil, cosolvent, and surfactant with a good solubilizing capacity for curcumin which can also be used as the component in microemulsions. The solubilities of curcumin in various vehicles were measured in the following procedure. An excess amount of curcumin was added to 5 ml of each selected vehicle, and stirred at 25 °C for 14 h. The suspensions were centrifuged at 10000 rpm for 3 min, and curcumin solubility in the supernatant determined using an HPLC method, following appropriate dilution with the mobile phase.

Data Analysis Cumulative amounts of curcumin permeated with time were used to calculate the transdermal drug flux, which was obtained from the slope of the regression line fitted to the linear portion of the profile. Student's t-test was used for the statistical analysis. A 0.05 level of significance ($p<0.05$) was considered significant. The skin flux can be experimentally determined from the following equation[23]:

$$J=(dQ/dt)/A$$

where J is the steady-state flux ($\mu g/cm^2/h$), A is the diffusion area of skin tissue (cm^2) through which drug permeation takes place, and dQ/dt is the amount of drug passing through the skin per unit time at a steady-state ($\mu g/h$). The cumulative amount of curcumin permeating through the rat skin was plotted as a function of time. The permeation rate of curcumin through piglet ear skin was calculated from the slope of the linear portion of the plot. The intercept of the extrapolated linear region with the x-axis gave the lag time. Enhancement ratio (Er) was calculated by dividing the flux of respective formulation with flux of control (pure eucalyptol). The following equation was used to calculate the permeability coefficient (Kp):

$$Kp=J/Co$$

where Kp has the units of cm/h, and Co represents the drug concentration.

Histological Examination of Skin Specimens Porcine skin samples were treated with PBS and optimized microemulsion (ME4) respectively for 24 h on a Franz diffusion cell. Thereafter, skin samples were fixed in PBS solution containing 10% formalin. Skin was cut vertically, dehydrated using ethanol, embedded in paraffin, and stained with hematoxylin and eosin (H&E) staining. These samples were then observed under light microscope (Olympus BX51, Tokyo, Japan) using 40× magnification. For confocal laser scanning microscopy observation, the skin was removed from the diffusion cell, rinsed with 50% ethanol and then the surface of the skin was wiped gently. The skin was directly sandwiched between a glass slide and a coverslip in a 1 : 1 solution of PBS-glycerol, and examined confocal microscopy without additional tissue processing. We used a Leica TCS SP2 confocal laser scanning microscope (Leica, Heerbrugg, Switzerland). The fluorescence of curcumin was excited at a wavelength of 420 nm by means of an argon laser. The skin sample was scanned from the skin surface (0 μm) to a depth of 162 μm at a 9.7-μm interval.

Results

Effects of Oils, Surfactants, and Cosurfactants on the Solubility of Curcumin Since the transdermal transport of a drug involves interactions among solubility, partition, and diffusion processes, in the present study relationships between the vehicle composition and curcumin solubility were investigated. The solubility of curcumin in various oils, surfactants, and cosurfactants was studied in order to determine the optimal components for a transdermal delivery vehicle. The solubility, flux, and permeability coefficient are shown in Table 1. Different oils including oleic acid (a fatty acid), estasan (a triglyceride), and eucalyptol (a terpene) were evaluated. Among the tested oils, curcumin in eucalyptol had the

Table 1. Effects of Ingredients on the Curcumin Solubility, Permeability, and Flux[a)]

Components	Saturated solubility of curcumin (mg/l)	Permeability coefficient (cm/h)	Flux (μg/cm^2 h)
Oil			
Oleic acid	90	0	0
Eucalyptol	1090	3.39±0.64	0.37±0.07
Estasan	400	0	0
Surfactants			
Polysorbate 20	105000	0	0
Polysorbate 40	82000	0	0
Polysorbate 80	208000	0	0
Polysorbate 85	34000	0	0
Co-surfactants			
Ethanol	4524	0.27±0.18	0.12±0.08
Isopropanol	1372	0.22±0.29	0.03±0.04
Propylene glycol	1863	0	0
Water	7	0	0

a) Permeability and flux are measured by using the diffusion cells in the 24-h peroid.

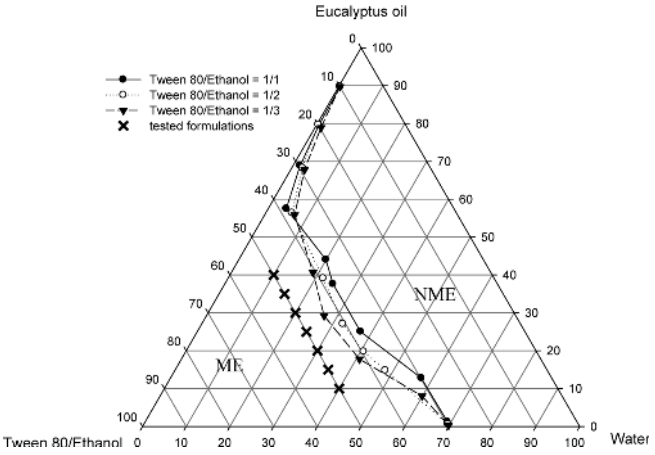

Fig. 1. The Pseudo-Ternary Phase Diagrams of Microemulsion Composed of Oils, Polysorbate80/Ethanol, and Water

ME represents the microemulsion region. NME stands for the non-microemulsion region.

best skin permeability and solubility. In addition to the oil phase, a cosolvent and surfactant were also used to create the microemulsion. The cosolvents tested in this study included the short-chain alcohols of ethanol, isopropanol, and propylene glycol which are relatively tolerated by the skin.[5)] Ethanol had the best transdermal curcumin flux and solubility as it was used as the cosolvent. Polysorbates are one of the most popular non-ionic surfactants used in the pharmaceutical industry.[24)] Interestingly, curcumin was highly soluble in the tested polysorbates compared to oil and solvent. However, the transdermal flux of curcumin in surfactants was not detectable among the four surfactants. Polysorbate 80 had the highest curcumin solubility at a level of about 20 wt%. The development of transdermal vehicles with high curcumin solubility has many advantages. One of the most important is that increasing the drug solubility by lipid-based formulations can enhance drug absorption.[25)] Therefore eucalyptol, polysorbate 80, and ethanol, which exhibited better curcumin solubility, were combined to develop a microemulsion formulation. The phase behavior of microemulsions composed of four ingredients was investigated using pseudo-ternary phase diagrams with the water titration method at room temperature. Pseudo-ternary phase diagrams are frequently used to obtain concentration ranges of the oil, surfactant, and cosurfactant for clear microemulsion formation.[26)] The polysorbate 80/ethanol ratios in the microemulsions were fixed at 1 : 1, 1 : 2, and 1 : 3. Figure 1 exhibits the phase diagrams composed of water, surfactant (polysorbate 80), cosurfactant (ethanol), and eucalyptol. In the triglyceride microemulsions, polysorbate/ethanol ratios significantly affected the phase behavior of the microemulsions.[27)] However, the polysorbate 80/ethanol ratios tested in this study did not efficiently affect the transparent region formed in the phase diagrams (Fig. 1). About 36% of the total area of the phase diagram was a transparent region independent of the surfactant/cosurfactant ratios. A stable microemulsion was formed when the contents of polysorbate 80 and ethanol were more than 40% as indicated by our pseudo-ternary phase diagrams. A milky colloid solution (non-microemulsion zone, NME) appeared for the tested terpenes when the concentration of polysorbate 80/ethanol exceeded 38 wt%. Eucalyptol emulsions within this area were not stable, and phase separation occurred when mixing stopped. Microemulsions have advantages as delivery vehicles such as high drug solubility, optical transparency, and thermodynamic stability.[28)] Recently, oil/water ratios were found to be important for the stability of microemulsion systems prepared from water, isopropyl myristate, Tween 40, and Imwitor 308.[29)] Formulations with various eucalyptol/water ratios were evaluated to study their effects on curcumin delivery in the next section.

Effects of Eucalyptol/Water Ratios on Transdermal Curcumin Delivery When the concentrations of polysorbate 80/ethanol exceeded 40 wt%, eucalyptol formed a stable microemulsion. In order to investigate the delivery effects of the eucalyptol/water fraction, the detailed characterization and delivery of seven potential vehicles (compositions referred to as formulations ME1—ME7 in Table 2) were evaluated. The formulations were selected so that all of them contained the same concentrations of polysorbate 80 and ethanol (12.5, 37.5% respectively) with the remaining 50% being eucalyptol and water. This selection allowed us to investigate the effects of the eucalyptol and water contents on the microemulsion characteristics and curcumin skin delivery from the microemulsions. Furthermore, 0.4 wt% curcumin was simultaneously added to the microemulsions to evaluate their potential for dermal delivery. Curcumin retention in the skin was slightly affected by the eucalyptol/water ratios in the microemulsions (Fig. 2). The microemulsion containing 25% water and 25% eucalyptol showed the highest curcumin retention in the skin (Table 2). Lipid extraction and osmotic expansion by the microemulsion may account for the increased drug retention in the skin.[23)] Importantly, transdermal curcumin permeation of the test microemulsions was also affected by the eucalyptol/water ratios. The microemulsion containing 25% water and 25% eucalyptol had the best delivery efficiency, followed by the microemulsion containing 20% water and 30% eucalyptol. For transdermal delivery to take place, the drug encapsulated in the vehicles has to be released from the microemulsion, partitioned into the skin, and then diffused into the receptor solution. The results of curcumin retained in the skin indicated that the skin plays a role as a reservoir for curcumin, which then sequentially diffuses

Table 2. Compositions and Characteristics of the Microemulsions for Curcumin[a)]

No.	Eucalyptol	Water	Curcumin in skin (μg/mg)	Flux (μg/cm^2 h)	Kp ($\times 10^4$cm/h)	Enhancement ratio
ME1	40	10	0.53±0.18	0.17±0.03*	0.43±0.07	0.5
ME2	35	15	0.56±0.11	1.44±1.15	3.61±2.88	3.9
ME3	30	20	0.69±0.08	2.20±0.06*	5.49±0.15	5.9
ME4	25	25	0.73±0.17	5.81±0.64*	14.54±1.60	15.7
ME5	20	30	0.72±0.06	1.59±0.03*	3.97±0.06	4.3
ME6	15	35	0.69±0.09	1.45±0.56*	3.64±1.41	3.9
ME7	10	40	0.59±0.19	0.05±0.01	0.13±0.03	0.1

a) Concentrations for polysorbate 80 and ethanol were fixed at 12.5 and 37.5%, respectively. Enhancement ratio was calculated by dividing the flux of respective formulation with flux of curcumin in pure eucalyptol (0.37±0.07 μg/cm^2 h). * $p<0.05$ compared with the curcumin in pure eucalyptol.

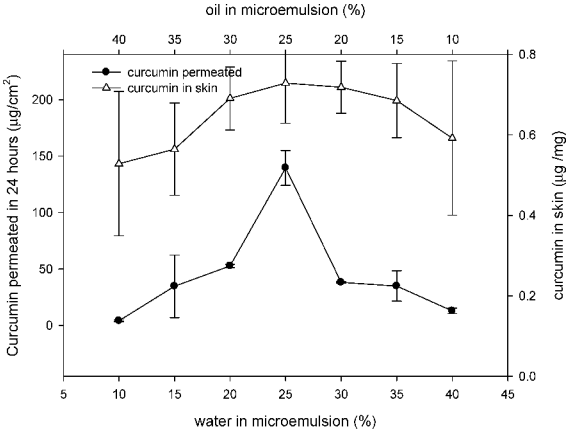

Fig. 2. Effects of Oil/Water Concentration on Curcumin Permeated and Retained in the Excised Pig Ear Skin with Fixed Water (10%) and Polysorbate 80/Ethanol Ratio (1 : 1)

The error bar stands for S.D. ($n=3$).

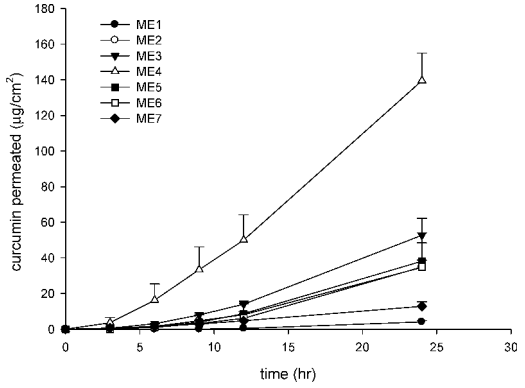

Fig. 3. Curcumin Permeated and Retained in the Excised Pig Ear Skin from ME1—ME7 with Various Eucalyptol/Water Ratios (Mean±S.D., $n=3$)

into deeper tissues. It is important to note that the microemulsion containing 25% water and 25% eucalyptol achieved about 30-fold higher curcumin flux than that with a lower water content (10%) and higher water content (40%). The increased water content may have enhanced the transdermal curcumin delivery before it reached 25%. However, the rate of transdermal curcumin delivery dropped when the water content exceeded 30%. The appropriate amount of water in the microemulsion may have hydrated proteins in the stratum corneum and caused a disordering of the lipid bilayers in corneous cells.[30)] Therefore, the cumulative curcumin permeation of microemulsions containing 25% water was improved compared to the other microemulsions. In addition, the lag time is a permeation parameter that mainly depends on the diffusivity of the drug through the skin.[5)] There were no significant differences in lag times among the six microemulsions indicating that the procedure of curcumin release from the microemulsions was not the limiting step (Fig. 3). The detailed results of curcumin retention in the skin and permeation flux for the microemulsions tested are summarized in Table 2. The fastest curcumin permeation rate was found in microemulsion ME4 (containing 25% water, 25% eucalyptol, 37.5% ethanol, and 12.5% polysorbate 80), which was 15.7-fold higher than that of eucalyptol alone. In order to explain their influences on the transdermal delivery efficiency, the effects of eucalyptol/water ratios on the physicochemical properties of microemulsions were studied. The conductivity and viscosity of the microemulsions were used to describe the colloidal microstructure of the selected formulations. In addition, the drug was reported to affect the microstructure of the delivery vehicles when added to the emulsions.[31)] The conductivity, viscosity, size, and surface tension of both unloaded and curcumin-loaded microemulsions were studied in order to explain why the eucalyptol/water content affected the efficacy of curcumin dermal delivery. The conductivity and viscosity of the unloaded and curcumin-loaded microemulsions are presented in Fig. 4. The conductivity of the microemulsions with increasing water content followed a bell-shaped curve, a phenomenon known as the percolation transition indicating that the system changed from isolated oil droplets to an interconnected bicontinuous structure, and finally to isolated water droplets.[29,31)] In this study, the conductivity of the microemulsions increased from 12 to 30 μS/cm when the water content increased from 10 to 40%. No percolation transition was observed in the eucalyptol-containing microemulsions, indicating that the system consisted of segregated water particles in a continuous oil phase. The increased conductivity levels can be explained by the dilution of the water/oil microemulsion with the added water which increased the concentration of the dispersed water droplets. One of the most special and useful properties of microemulsions is the typically low interfacial tension at liquid–liquid interfaces. The incorporation of curcumin slightly decreased the conductivity of the microemulsions. Furthermore, the interfacial tension of the six formulations slightly increased from 26 to 28 mN/m with an increase in the water content (Fig. 4). Therefore, the interfacial tension was not the major factor determining the transdermal flux because the interfacial tension

of the tested microemulsions was very low, even lower than the interfacial tension of water. In addition, the pH value remained at 7.7 for both unloaded and curcumin-loaded microemulsions. Additionally, the droplet size of the microemulsions with various oil/water ratios remained in the 2—5-nm range. We also found that the viscosity increased with an increase in water in the eucalyptol microemulsion. The viscosity range of the microemulsions prepared was 6—14 cP with an increase in the water content from 10 to 40%. Previously, the viscosity of drug-loaded microemulsions was reported to influence drug partitioning into the skin. The higher the viscosity was, the lower the drug delivery efficacy was.[31] The eucalyptol microemulsion with increased water content (>25%) had low permeation flux which may have been caused by the increased viscosity. However, the viscosity and size results could not fully explain why the microemulsion composed of 25% eucalyptol and water had the best delivery efficacy. Effects of the curcumin concentration (2, 4, 6, 8 g/l) on transdermal delivery were investigated using the optimal formulation, ME4. Curcumin permeating through the excised pig ear skin was significantly affected when curcumin increased from 0.2 to 0.8 wt%. The permeation of curcumin was proportional to the increasing concentration of curcumin (Fig. 5). In addition, the lag time decreased with an increase in the curcumin concentration indicating the elevation of the curcumin driving force on the donor site. In addition, the stability of curcumin microemulsion was also evaluated under two storage conditions. After 2-week storage at 25 °C, the 88.5% of curcumin in the formulation (ME4) could be preserved in the microemulsion. About 96% of curcumin could be preserved in the microemulsion ME4 when stored at 4 °C for two weeks. No phase separation or curcumin precipitation was observed in both conditions. Transdermal curcumin delivery was further observed using confocal laser scanning microscopy. The histological damage to the skin by the microemulsion vehicle was analyzed using HE staining technology in the next section.

Confocal Observations and Histological Examination of the Skin Confocal laser scanning microscopy provides the localization and permeation pathway of fluorescent compounds in the tissue without cryo-fixing or embedding procedures. However, it cannot discriminate between intra- and inter-cellular permeation pathways.[32] Curcumin has a light emission of 544 nm after being excited by 420-nm laser light. This fluorescent characteristic is used to visualize the concentration gradient of curcumin in the skin using the microemulsion vehicle (ME4). The 18 confocal curcumin images at various skin depths (from 0 to 162 μm) were obtained from a diffusion cell treated with the ME4 microemulsion (0.4% curcumin) (Fig. 6A). A depth of 0 μm represents the skin surface, whereas a depth of 162 μm represents the deepest dermal site. Curcumin obviously penetrated up to 50 μm into the skin with decreasing fluorescence as indicated in Fig. 6B. This phenomenon was due to curcumin at the donor site diffusing through the skin into the receptor site. Histological examination of treated (ME4) and control (PBS) skin was

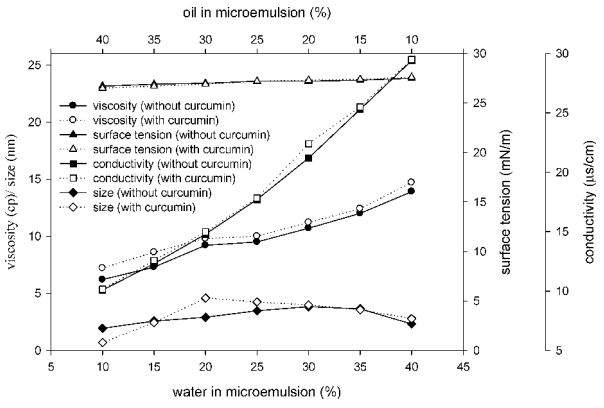

Fig. 4. Conductivity, Viscosity, Size and Surface Tension for ME1—ME7 with Various Eucalyptol/Water Ratios (Mean±S.D., n=3)

Formulation details are presented in Table 2.

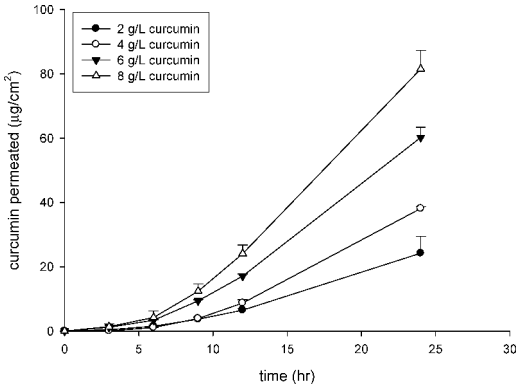

Fig. 5. Effects of Curcumin Concentration on Curcumin Permeated through the Excised Pig Ear Skin with Fixed Water (10%) and Polysorbate 80/Ethanol Ratio (1 : 1)

The error bar stands for S.D. (n=3).

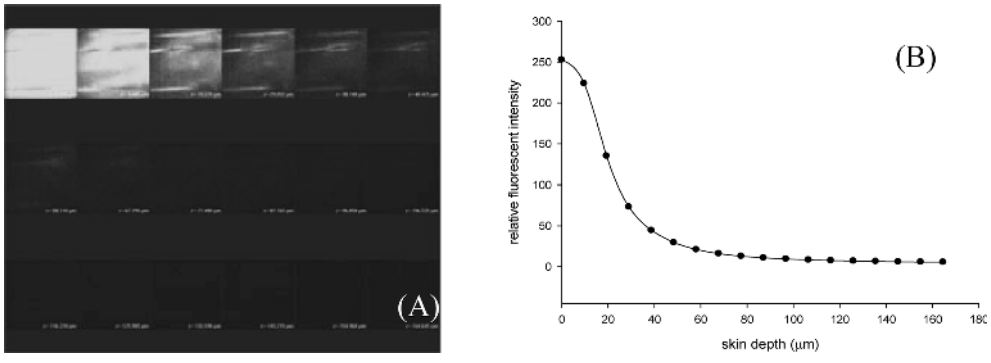

Fig. 6. Laser Scanning Confocal Micrographs of Porcine Skin after 24-h Treatment with Microemulsion ME4 (A) and Their Relative Fluorescence (B)

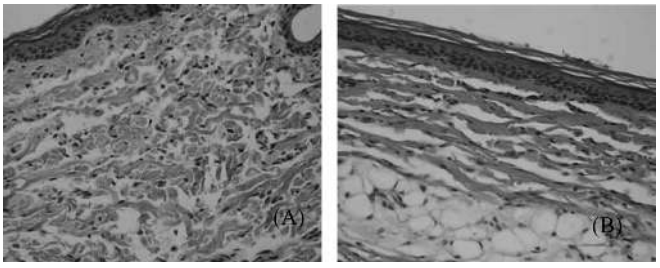

Fig. 7. HE Staining Skin Micrographs after 24-h PBS Treatment (A) and after 24-h ME4 Treatment (×40)

performed using light microscopy to investigate skin changes. Micrographs of control and treated samples demonstrated similar skin layers as shown in Figs. 7A and B. When the skin was treated with the ME4 microemulsion for 24 h, a loose morphology of the stratum corneum was observed in the skin compared to that with PBS treatment, which could have been caused by the action of the microemulsion on the stratum corneum (SC). Some minor changes in epidermis (Figs. 7A, B) were observed in the two samples. A puffy stratum corneum might have contributed to the enhanced effect of curcumin delivery by the ME4 microemulsion.

Discussion

Various oil phases, cosolvents, and surfactants were used as carriers to dissolve curcumin. The vehicle composition significantly affects the curcumin solubility and skin permeability. Among the tested oils and cosolvents, curcumin in eucalyptol and in ethanol had the better skin permeability and solubility. Interestingly, curcumin was highly soluble in the tested surfactants compared to oil and solvent. The increase in curcumin solubility by lipid-based formulations can enhance drug absorption by increasing the concentration gradient.[25] The chemicals with better curcumin solubility were combined to develop a stable microemulsion with the aid of pseudo-ternary phase diagrams. Previously, polysorbate/ethanol ratios significantly affected the phase behavior of triglyceride microemulsions.[27] The polysorbate/ethanol ratios did not affect the transparent region of eucalyptol microemulsions which might be due to the difference in the oil phase. In addition, specific physicochemical conditions are accounted for microemulsion formation including the existence of a very low surface tension at the oil–water interface, the presence of a highly fluid interfacial film of the surfactant, and the penetration and association of oil molecules with the interfacial surfactant film.[1]

Formulations (ME1—ME7) with various eucalyptol/water ratios in the pseudo-ternary phase diagrams had great influence on curcumin delivery. It is well known that change in water/oil ratio affects the physicochemical property and microstructure of microemulsions.[1—3,33] The fact that interfacial tension slightly increases with increasing water content might be due to the surfactant partition from oil/water and air/liquid interfaces to the water phase.[34] The change in the microstructure of microemulsion caused by the reorganization of surfactant might contribute to the increase in viscosity after the increasing water content.[33] Similar observations in microemulsions viscosity and interfacial tension elevated by water addition were recently reported.[29,31] Due to their amphiphilic nature surfactants could form various structures in oil and water phases depending on the respective concentrations. Various mechanisms were reported to enhance transdermal drug delivery from terpenes.[1,5,35] The first mechanism is that eucalyptol in the microemulsion can enter the skin as a monomer to increase curcumin's solubility in the skin. Eucalyptol in the skin increases the partitioning of the drug into the skin and creates a high drug concentration within the upper layers of the skin. This results in a higher concentration gradient which is the driving force for transdermal drug delivery. This possibility was supported by our results because a high flux was accompanied by high curcumin retention in the skin. The second possibility is that eucalyptol is a well-known skin penetration enhancer. Eucalyptol is known to produce greater skin penetration enhancement when applied in combination with ethanol as a cosurfactant.[35] The third mechanism depends on the possibility of direct drug transfer from microemulsion droplets to the stratum corneum. As the drug molecules are distributed in the microstructure of the microemulsion system with its very small droplet size (2—5 nm), a very large surface area for drug transfer to the skin is created. This explains the superiority of microemulsion formulations over the curcumin ethanol solution. Accordingly, the effects of the formulation on the skin may play a major role in the improved skin permeation. The compositions of the microemulsions were found to be important in determining the efficiency of microemulsions as skin drug delivery systems. Curcumin permeating through the excised pig ear skin was significantly affected when curcumin increased. Previous researchers showed that an increase in drug solubility enhances the drug delivery rate by increasing the drug gradient.[36] A matrix-type transdermal system was recently developed by Patel *et al.*[17] The potential advantages of the microemulsion over the matrix-type film system included thermodynamic stability, facile preparation, enhanced drug solubility, and low cost as suggested in the review of Azeem *et al.*[30] However, the matrix-type films are one of the most popular transdermal devices. The commercial applications of microemulsion for transdermal curcumin delivery need more investigations and animal tests. Furthermore, the detention range of the size-analyzing instrument (Zetasizer Nano ZS 90) was 0.3 nm to 10 μm by using the technology of dynamic light scattering (DLS). The size distribution for nanoparticles solutions, colloidal dispersions, emulsions, and microemulsions is analyzed by using DLS method.[23,30] Therefore this technology was very suitable for the analysis of droplet distribution in microemulsions. The concentration gradient of curcumin in the skin was visualized using the confocal laser scanning microscopy. Curcumin could penetrate the SC and enter up to 50 μm into the skinaccording to our results (Fig. 6B). However, transdermal delivery by microemulsions was previously demonstrated to be safe for various drugs.[37—40] More animal results for the curcumin microemulsion are needed in order to evaluate whether the developed microemulsion is safe for the transdermal delivery of curcumin.

Conclusion

From the results presented in this paper, we concluded that the transdermal curcumin flux depends upon the composition of the microemulsion including the eucalyptol/water ratio

and curcumin concentration. The polysorbate/ethanol ratio affected the phase behaviors of eucalyptol microemulsions as indicated by the pseudo-ternary phase diagrams. The transdermal curcumin flux was significantly influenced by the eucalyptol/water ratio. Physicochemical properties of microemulsions such as the conductivity, interfacial tension, size, and viscosity influenced curcumin delivery and skin retention. Results of confocal laser scanning microscopy revealed that curcumin was localized in the skin and a linear diffusion pathway existed. No significant damage to the skin was observed using histological examination of the treated skin. Finally, microemulsions containing 25% water, 25% eucalyptol, 37.5% ethanol, and 12.5% polysorbate 80 had the fastest curcumin delivery flux. This eucalyptol microemulsion system was demonstrated to be a promising tool for the percutaneous delivery of curcumin.

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References

1) Kogan A., Garti N., *Adv. Colloid Interface Sci.*, **123—126**, 369—385 (2006).
2) Trommer H., Neubert R. H. H., *Skin Pharmacol. Physiol.*, **19**, 106—121 (2006).
3) Thong H. Y., Zhai H., Maibach H. I., *Skin Pharmacol. Physiol.*, **20**, 272—282 (2007).
4) Biruss B., Kahlig H., Valenta C., *Int. J. Pharm.*, **328**, 142—151 (2007).
5) El Maghraby G. M., *Int. J. Pharm.*, **355**, 285—292 (2008).
6) Fanun M., *J. Colloid Interface Sci.*, **343**, 496—503 (2010).
7) Mitra R. K., Paul B. K., *J. Colloid Interface Sci.*, **283**, 565—577 (2005).
8) Gershkovich P., Wasan K. M., Barta C. A., *Crit. Rev. Ther. Drug Carrier Syst.*, **25**, 545—584 (2008).
9) Heuschkel S., Goebel A., Neubert R. H. H., *J. Pharma. Sci.*, **97**, 603—631 (2008).
10) Spernath A., Aserin A., *Adv. Colloid Interface Sci.*, **128—130**, 47—64 (2006).
11) Bachhav Y. G., Patravale V. B., *Int. J. Pharm.*, **365**, 175—179 (2009).
12) Zhu W., Yu A., Wang W., Dong R., Wu J., Zhai G., *Int. J. Pharm.*, **360**, 184—190 (2008).
13) Vicentini F. T. M. C., Simi T. R. M., Del Ciampo J. O., Wolga N. O., Pitol D. L., Iyomasa M. M., Bentley M. V. L. B., Fonseca M. J. V., *Eur. J. Pharm. Biopharm.*, **69**, 948—957 (2008).
14) Spernath A., Aserin A., Sintov A. C., Garti N., *J. Colloid Interface Sci.*, **318**, 421—429 (2008).
15) Biruss B., Valenta C., *Int. J. Pharm.*, **349**, 269—273 (2008).
16) Thangapazham R. L., Sharma A., Maheshwari R. K., *Adv. Exp. Med. Biol.*, **595**, 343—357 (2007).
17) Patel N. A., Patel N. J., Patel R. P., *Drug Dev. Ind. Pharm.*, **35**, 234—242 (2009).
18) Bisht S., Feldmann G., Soni S., Ravi R., Karikar C., Maitra A., Maitra A., *J. Nanobiotechnol.*, **5**, 28—45 (2007).
19) Li L., Braiteh F. S., Kurzrock R., *Cancer*, **104**, 1322—1331 (2005).
20) Maiti K., Mukherjee K., Gantait A., Saha B. P., Mukherjee P. K., *Int. J. Pharm.*, **330**, 155—163 (2007).
21) Balaguer-Fernandez C., Femenia-Font A., Rio-Sancho S. D., Merino V., Lopez-Castellano A., *J. Pharm. Sci.*, **97**, 2102—2109 (2008).
22) Heard C. M., Johnson S., Moss G., Thomas C. P., *Int. J. Pharm.*, **317**, 26—31 (2006).
23) Lee J., Lee Y., Kim J., Yoon M., Young W. C., *Arch. Pharm. Res.*, **28**, 1097—1102 (2005).
24) Simoes S. I., Tapadas J. M., Marques C. M., Cruz M. E. M., Martins M. B. F., Cevc G., *Eur. J. Pharm. Sci.*, **26**, 307—317 (2005).
25) O'Driscoll C. M., Griffin B. T., *Adv. Drug Deliv. Rev.*, **60**, 617—624 (2008).
26) Lawrence M. J., Rees G. D., *Adv. Drug Deliv. Rev.*, **45**, 89—121 (2000).
27) Garti N., Yaghmur A., Leser M. E., Clement V., Watzke H. J., *J. Agri. Food Chem.*, **49**, 2552—2562 (2001).
28) Azeem A., Khan Z. I., Aqil M., Ahmad F. J., Khar R. K., Talegaonkar S., *Drug Dev. Ind. Pharm.*, **35**, 525—547 (2009).
29) Podlogar F., Gasperlin M., Tomsi M., Jamnik A., Roga M. B., *Int. J. Pharm.*, **276**, 115—128 (2004).
30) Azeem A., Ahmad F. J., Khar R. K., Talegaonkar S., *AAPS PharmSciTech*, **10**, 1093—1103 (2009).
31) Djordjevic L., Primorac M., Stupar M., Krajisnik D., *Int. J. Pharm.*, **271**, 11—19 (2004).
32) Changez M., Varshney M., Chander J., Dinda A. K., *Colloids Surfaces B Biointerfaces*, **50**, 18—25 (2006).
33) Mehta S. K., Kaur G., Bhasin K. K., *Pharm. Res.*, **25**, 227—236 (2008).
34) Sajjadi S., *Colloids Surf. A Physicochem. Eng. Asp.*, **299**, 73—78 (2007).
35) Fang J. Y., Hung C. F., Chiu H. C., Wang J. J., Chan T. F., *J. Pharm. Pharmacol.*, **55**, 593—601 (2003).
36) El Maghraby G. M., *Colloids Surf. Biointerfaces*, **75**, 595—600 (2010).
37) Shen Q., Li X., Yuan D., Jia W., *Chem. Pharm. Bull.*, **58**, 639—643 (2010).
38) Kitagawa S., Inoue K., Teraoka R., Morita S. Y., *Chem. Pharm. Bull.*, **58**, 398—401 (2010).
39) Han D. H., Jin Z. H., Jin Y. Z., Yin X. Z., Shen Y. Y., Gao Z. G., *Chem. Pharm. Bull.*, **58**, 11—15 (2010).
40) Liu Y., Chen Z. Q., Zhang X., Feng N. P., Zhao J. H., Wu S., Tan R., *Chem. Pharm. Bull.*, **58**, 16—22 (2010).