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Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: Effects of cosurfactants

Gamal M. El Maghraby *

Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, P.O. Box 2457, Saudi Arabia

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Abstract

This study investigated the effects of cosurfactants on the transdermal delivery of hydrocortisone (model drug) from eucalyptus oil microemulsion. Eucalyptus oil which was successfully employed for steroidal drugs was used as the oil. Tween 80 which was readily miscible with eucalyptus oil was used as surfactant. Ethanol, isopropanol and propylene glycol which are relatively tolerable by the skin were employed as cosurfactants. Pseudo-ternary phase diagrams were constructed in the presence and absence of cosurfactants. Microemulsion formulations containing 20% oil, 20% water and 60% of either Tween 80 or 1:1 surfactant/cosurfactant mixture were compared. Incorporation of cosurfactants expanded the microemulsion zone. The cosurfactant free microemulsion was viscous showing pseudo-plastic flow. The cosurfactant containing preparations were less viscous with Newtonian flow. The drug loading and release rate were increased in the presence of cosurfactants with the release depending on the viscosity. Incorporation of hydrocortisone in microemulsion increased the transdermal flux compared to saturated aqueous solution. The presence of cosurfactants increased the transdermal drug flux compared to the cosurfactant free formulation. Ethanol produced the greatest effect followed by propylene glycol and isopropanol. The presence of cosurfactant and its type can thus affect both the phase behavior and the transdermal delivery potential of microemulsion.

Keywords: Transdermal microemulsion; Cosurfactants; Hydrocortisone; Eucalyptus oil

1. Introduction

The skin provides the formulator with a vast area for drug application. Transdermal drug delivery offers many advantages over other traditional routes of drug delivery. Unfortunately, the barrier nature of the skin made it difficult for most drugs to be delivered into and through it (Barry, 1983). Many strategies have been employed to enhance dermal and transdermal delivery. These include the use of chemical penetration enhancers (Goodman and Barry, 1988; Williams and Barry, 2004), preparation of supersaturated drug delivery systems (Megrab et al., 1995), electrically driving molecules into or through the tissue employing iontophoresis (Miller et al., 1990), physically disrupting the skin structure, for example, by electroporation or sonophoresis (Kost et al., 1996; Banga et al., 1999) or encapsulating the drug in vesicular delivery systems (Mezei and Gulasekharam, 1980; Cevc and Blume, 1992; Schreier and Bouwstra, 1994; El Maghraby et al., 1999, 2006). Microemulsion provides another promising alternative for dermal and transdermal delivery of both hydrophilic and lipophilic drugs (Boltri et al., 1994; Delgado-Charro et al., 1997; Kreilgaard, 2002; Kogan and Garti, 2006).

Microemulsion is a thermodynamically stable transparent, single optically isotropic liquid system of water, oil and surfactants (Danielsson and Lindman, 1981). Microemulsions can be considered as ideal liquid vehicles for drug delivery as they have most of the requirements for this including the thermodynamic stability, ease of formulation, low viscosity, high solubilization capacity and small droplet size. The latter characteristic provides better chance for adherence to biological membranes transporting drugs in controlled manner (Kogan and Garti, 2006). The use of microemulsions in skin drug delivery has been reviewed and it was noted that although many reports of cutaneous drug delivery potential of topical microemulsions have been published recently, most of the studies have not been very systematic or consecutive which hampered drawing general conclusions on the

* Tel.: +966 1 4677363.
E-mail address: gmmelmag@yahoo.com.

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interrelations between the microemulsion properties or composition and the drug delivery rate (Kreilgaard, 2002). The effect of cosurfactant on the transdermal drug delivery from microemulsion is one of the factors which need to be considered.

Accordingly, the objective of this study was to investigate the effects of cosurfactants on the transdermal delivery of hydrocortisone (model drug) from eucalyptus oil microemulsion. Cosurfactants are included in the microemulsion formulations to provide further reduction in surface tension and to fluidize the interfacial surfactant film. This can expand the area of existence of microemulsion system (Stilbs et al., 1983; Aboofazeli and Lawrence, 1994). Short and medium chain alcohols were successfully tested as cosurfactants (Aboofazeli and Lawrence, 1994; Stilbs et al., 1983; Aboofazeli et al., 1995). Accordingly, ethanol, isopropanol and 1,2-propanediol (propylene glycol) were selected as cosurfactants in this study. This selection was based on the fact that they are relatively tolerable by the skin if compared with other alcohols. The study employed hydrocortisone as a model lipophilic drug with eucalyptus oil as the oil phase. Eucalyptus oil was selected in this study as it has been reported to enhance the transdermal delivery of steroidal drugs (Biruss et al., 2007).

2. Materials and methods

2.1. Materials

Hydrocortisone and Tween 80 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Eucalyptus oil and isopropanol were from Winlab Laboratory Chemicals, Leicestershire, UK. Methanol (HPLC grade), acetonitrile (HPLC grade), ethanol (96%), propylene glycol and propylparaben were purchased from BDH, England.

2.2. Construction of pseudo-ternary phase diagrams

Eucalyptus oil was selected as the oily phase as it was successfully used in transdermal delivery of steroidal drugs similar to hydrocortisone from microemulsion (Biruss et al., 2007). Tween 80 was selected as the surfactant in this study as it was readily miscible with the eucalyptus oil. When cosurfactants were used the surfactant/cosurfactant ratio was 1:1 (w/w). This ratio was selected on the bases that this ratio solubilized the greatest amount of water on titration compared to various surfactant/cosurfactant ratios when mixed with the oil at 1:1 weight ratios (Alany et al., 2001).

The pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature (Chen et al., 2004). 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 dropwise with water under gentile magnetic stirring. After being equilibrated the systems were visually characterized. Transparent fluid systems were characterized as microemulsion. Highly viscous systems that did not show a change in the meniscus after being tilted to an angle of 90° were considered as gel.

Table 1
The composition of the selected microemulsion formulations

Material	TW ME	TW ETH ME	TW ISO ME	TW PG ME
Eucalyptus oil	20	20	20	20
Tween 80	60	30	30	30
Ethanol	–	30	–	–
Isopropanol	–	–	30	–
Propylene glycol	–	–	–	30
Water	20	20	20	20

2.3. Preparation of microemulsions

Microemulsion formulations selected from the constructed phase diagrams were prepared according to the composition presented in Table 1. The formulations were selected so that the concentrations of oil and water are the same. The variables were the presence or absence of cosurfactants and the type of cosurfactant. The microemulsions were prepared by mixing the oil with the surfactant or surfactant/cosurfactant mixture before adding the required amount of water under magnetic stirring. Excess drug was added to prepare saturated drug solutions with excess crystals to maintain saturation. These were equilibrated by continuous mixing in water bath maintained at 32 °C for 72 h before application to the skin. No phase change was noted after addition of the drug or after equilibration in the water bath. These formulations were evaluated for transdermal drug delivery potential with saturated aqueous drug solution which also included excess drug crystals being used as the control.

2.4. Characterization of the selected microemulsion formulations

The flow properties and the viscosity of the formulations were determined at 32 ± 1 °C. Viscosity determinations employed a DV II+ rotating Brookfield viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA).

The droplet size values of the microemulsion formulations were measured using photon correlation spectroscopy. These measurements employed a 90Plus Brookhaven instrument (Brookhaven Instrument Corp., Holtsville, NY, USA).

The saturation solubility of the drug in different formulations was determined at 32 °C. Excess drug was added and the mixtures were equilibrated by continuous mixing in water bath maintained at 32 °C for 72 h. The excess drug was removed by centrifugation and the supernatant was suitably diluted before HPLC analysis.

2.5. In vitro drug release

The release experiments employed the FDC-6 Transdermal Diffusion Cell Drive Console (Logan Instrument Corp., NJ, USA). The system is fitted with VTC-200 heater circulator with jacketed vertical glass Franz diffusion cells being the main unit. The artificial membrane (Cellulose tubing, Sigma diagnostics,

St. Louis, MO, USA) was mounted between the donor and receptor compartments of the diffusion cells. These cells provided a diffusional area of 1.7 cm^2 and the receptor compartment was 12 ml. To maintain sink conditions 40% (v/v) propylene glycol in water was used as receptor. This receptor was employed to monitor skin delivery of steroidal drugs from microemulsion (Biruss et al., 2007). The system was adjusted to ensure that membrane surface was at $32 \pm 1\,^{\circ}C$ to mimic in vivo conditions. The tested formulations (2 ml) were loaded into the donor compartment before occluding the donor compartments using a parafilm. Receptor samples were taken periodically and replaced with fresh receptor. These were analyzed for the drug content by HPLC (see below). The cumulative amount of drug released was calculated as a function of time and the release rate was determined.

2.6. Preparation of skin samples

Due to the difficulty of obtaining human skin samples, the rabbit ear model was used. This model was adopted to monitor the skin delivery of a variety of drugs including the lipophilic ones similar to our drug (Corbo et al., 1990; Touitou et al., 2000). Full thickness skin obtained from the inner side of freshly excised ears of 10 male rabbits, weighing 2–3 kg was used. The skin was peeled from the underlying cartilage after cutting along the tips of the ears. The skin samples were mounted immediately on the diffusion cells (see below).

2.7. Skin permeation studies

As for the release experiments skin permeation studies employed the FDC-6 Transdermal Diffusion Cell Drive Console (Logan Instrument Corp., NJ, USA). The skin was mounted with the stratum corneal side uppermost on the vertical glass diffusion cells. These cells provided a diffusional area of 1.7 cm^2 and the receptor compartment was 12 ml. To ensure sink conditions, 40% (v/v) propylene glycol in water was employed as a receptor. This receptor was employed to monitor skin delivery of steroidal drugs from microemulsion (Biruss et al., 2007). The system was adjusted to ensure that the skin surface was maintained at $32 \pm 1\,^{\circ}C$ to mimic in vivo conditions. The mounted skin was equilibrated overnight. The tested formulations were applied to skin surface before occluding the donor compartments with parafilm. Receptor samples were taken periodically and replaced with fresh receptor fluid. These samples were analyzed for the drug content by HPLC. The control was saturated aqueous solution of the drug with excess drug crystals being included to maintain saturation throughout the experiments (maximum thermodynamic activity). All the tested formulations contained the drug at saturation with excess crystals present to maintain saturation.

2.8. Chromatography

The drug concentrations in all samples were determined using HPLC analysis. This employed a high pressure liquid chromatograph (Waters^{TM} 600 controller, USA) equipped with a variable wavelength detector (Waters^{TM} 486, Tuneable Absorbance Detector, USA) and an automatic sampling system (Waters^{TM} 717 Plus Autosampler, USA). This was under computer control. Separation was accomplished on a reversed phase column 15 cm × 3.9 mm (i.d.) C_{18}, μ Bondapak^{TM}, Waters, with an average particle size of 10 μm.

The mobile phase was a mixture of methanol, acetonitril and water (50:10:40) flowing at 1 ml/min, with propylparaben employed as internal standard. The column effluent was monitored at 238 nm and the chromatographic data analysis was performed with the Millinium^{TM} Program (Waters, USA).

The samples were suitably diluted with mobile phase before addition to test tubes spiked with the internal standard in an amount sufficient to produce a concentration of 2 μg/ml. The tubes were vortex mixed for 2 min before loading into the HPLC vials and injecting 30 μl into the HPLC.

2.9. Data analysis

The cumulative amounts of the drug permeated with time produced the permeation profiles. These were typical steady-state profiles which are expected after occlusive application of saturated systems (Fig. 1). These profiles 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. Extrapolation of this line will intercept with the *x*-axis at a time equal to the lag time.

The Student's *t*-test was used for statistical analysis.

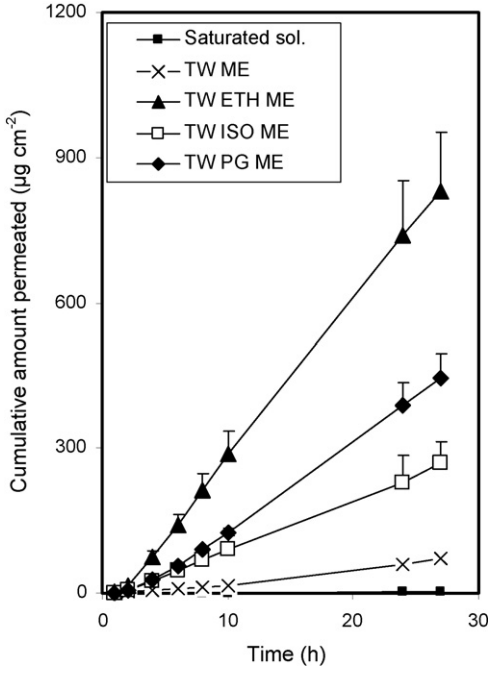

Fig. 1. The hydrocortisone transdermal permeation profiles obtained after application of different microemulsion formulations or saturated aqueous drug solution to rabbit ear in vitro. Formulation details are presented in Table 1.

3. Results and discussion

3.1. Pseudo-ternary phase diagrams

Fig. 2 shows the pseudo-ternary phase diagrams of eucalyptus oil, Tween 80, water systems in the presence and absence of different cosurfactants. In the absence of cosurfactants Tween 80 was able to form microemulsion over a range of surfactant–oil–water ratios (Fig. 2a). The microemulsion zone occupied about 20% of the total area of the pseudo-ternary phase diagram. At very low oil concentration a maximum of 40% water was solubilized in the surfactant oil blend. The amount of incorporated water was reduced progressively with increasing oil concentrations. It should be noted that within the formed microemulsion zone the fluidity of the microemulsion reduced with increasing water content. Outside the microemulsion zone especially at high surfactant concentrations the system changes into gel structure by increasing the water contents. This gel structure was broken down upon further dilution with water before transformation into coarse emulsion.

Incorporation of cosurfactants increased the maximum amount of incorporated water in the oil–surfactant system with the microemulsion zone being increased in all cases compared to the cosurfactant free system (Fig. 2). Incorporation of ethanol increased the water incorporation to a maximum of 86% compared to 40% in the cosurfactant free system, with the microemulsion zone occupying about 40% of the total area of the pseudo-ternary phase diagram compared to 20% in absence of ethanol. As for ethanol, incorporation of isopropanol increased the maximum amount of incorporated water to 87% with the area occupied by the microemulsion zone reaching 42%. For systems containing propylene glycol the maximum water content was also 86% at very low oil concentrations. This was reduced sharply with increasing oil concentration. The microemulsion zone occupied 27% of the phase diagram which was again higher than that obtained in cosurfactant free system. The presence of any of the tested cosurfactants abolished the region of the gel from the phase diagrams compared to the cosurfactant free system (Fig. 2). Breaking of the gel and liquid crystalline structure was reported after introduction of short chain alcohol with up to 4 carbon atoms (Alany et al., 2000).

The main factor determining the range of formation of microemulsion zone is the physicochemical properties of the oil phase, aqueous phase and surfactant with some essential conditions required for microemulsion formation. These include the existence of a very low surface tension at the oil–water interface, the presence of highly fluid interfacial film of surfactant and the penetration and association of oil molecules with the interfacial surfactant film (Schulman et al., 1959). Surfactant–oil miscibility can thus give initial indication on the possibility of microemulsion formation with this system. For a single surfactant to form a microemulsion its lipophilic chains should be short or at least containing a fluidizing group such as double bonds (Lawrence, 1994). This explains the ability of Tween 80 to form microemulsion with eucalyptus oil as it contains double bond in its lipophilic chain in addition to its complete miscibility with the oil.

Introduction of cosurfactants provides further reduction in the surface tension and fluidizes the interfacial surfactant film which can expand the area of existence of microemulsion system (Stilbs

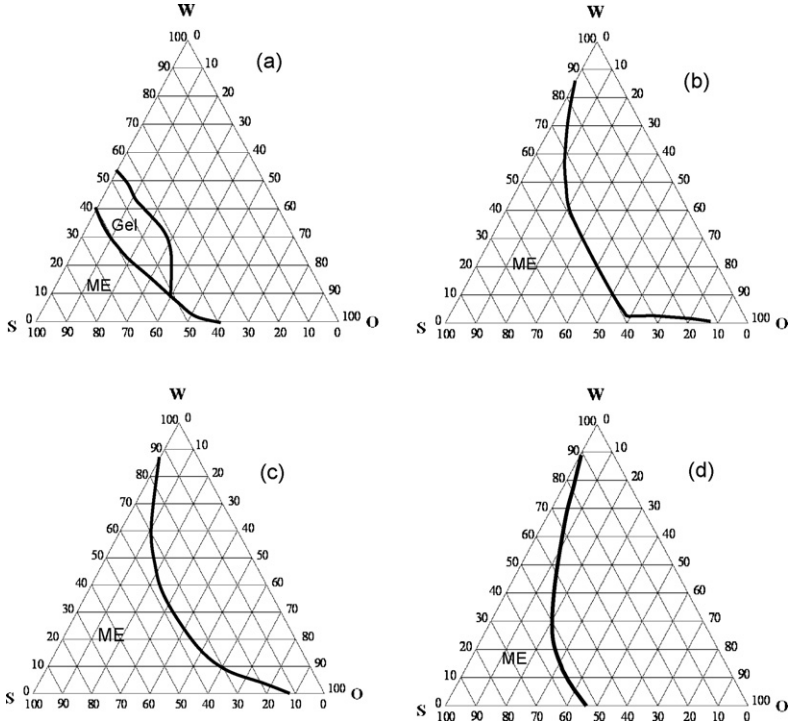

Fig. 2. The pseudo-ternary phase diagrams of eucalyptus oil, Tween 80 and water system in presence and absence of different cosurfactants. (a) Cosurfactant free system, (b) ethanol containing system, (c) isopropanol containing system and (d) propylene glycol containing system. W means water, O means oil and S means surfactant/cosurfactant mixture.

Table 2
The characteristics of the selected microemulsion formulations

Parameter	TW ME	TW ETH ME	TW ISO ME	TW PG ME
Viscosity (CP)	19738 (2392)	5.3 (0.5)	5.8 (0.3)	60.3 (5.5)
Droplet size (nm)	ND	41.8 (6.2)	37.2 (9.7)	170.5 (17.8)
Drug solubility[a] (mg/ml)	4.3 (0.3)[b]	23.7 (1.1)	22.9 (0.6)	8.5 (0.4)

Values between brackets are S.D. ($n = 3$).
[a] The saturation solubility of hydrocortisone in water was 1.8 mg/ml at 32 °C.
[b] The drug solubility in the TW ME was determined microscopical examination of replicates of this system containing increasing amounts of the drug. Formulation details are in Table 1.

et al., 1983; Aboofazeli and Lawrence, 1994). Short and medium chain alcohols were successfully tested as cosurfactants (Stilbs et al., 1983; Aboofazeli and Lawrence, 1994; Aboofazeli et al., 1995). This can explain the increase in the area occupied by the microemulsion zone after incorporation of ethanol, isopropanol or propylene glycol compared to the cosurfactant free system. Incorporation of ethanol or isopropanol as cosurfactants was shown to increase the microemulsion zone (Yuan et al., 2006).

Comparing the effects of the tested cosurfactants, it should be pointed out that increasing the chain length as you move from ethanol to isopropanol increased the area of existence of microemulsion. However, increasing the number of hydroxyl groups as we move from isopropanol to propylene glycol reduced the area of existence of microemulsion but remained larger than that obtained in cosurfactant free system. Similar trend was obtained when comparing *n*-propanol with propylene glycol but with different oil and surfactant system (Alany et al., 2000). It should be noted that replacing propylene glycol with glycerol abolished the microemulsion zone completely.

3.2. Characterization of the selected microemulsion formulations

The tested microemulsion formulations were selected so that all formulations contained the same concentration of eucalyptus oil and water (20% each) with 60% of the system being either pure surfactant or surfactant/cosurfactant mixture. This selection will thus allow the investigation of the effect of cosurfactants on the microemulsion characteristics and hydrocortisone skin delivery from microemulsion.

Table 2 presents the characteristics of the tested formulations. The cosurfactant free formulation showed a high viscosity with a pseudo-plastic flow behavior. Incorporating the cosurfactant resulted in a significant reduction in the viscosity of the formulations with the flow changing to a simple Newtonian flow. Comparing the viscosity of formulations containing different cosurfactants, propylene glycol containing microemulsion produced the highest viscosity among the cosurfactant containing microemulsions with no significant difference between ethanol and isopropanol containing formulation (Table 2). Reduction in the viscosity of microemulsion was reported after incorporation of short chain alcohol of 3–4 carbon atoms (Alany et al., 2000).

The droplet size of the prepared microemulsion formulations was monitored by photon correlation spectroscopy. The cosurfactant free formulation was so viscous that it was difficult to measure the droplet size. For the cosurfactant containing formulations the droplets were in the nano-size range with no significant difference between ethanol and isopropanol containing formulations with propylene glycol containing formulation showing larger droplet size.

The selected microemulsion formulation showed a high loading capacity as evidenced by greater solubility of hydrocortisone in these formulations compared to the drug solubility in water. Comparing between the tested formulations with respect to the saturation solubility of the drug (Table 2), it was noted that incorporation of cosurfactants significantly increased the loading capacity of the microemulsion with ethanol and isopropanol containing formulation providing the greatest loading. The data in Table 2 might suggest a dependence of the solubility on both the composition and the viscosity of the formulation. In addition to the solubilizing power of the microemulsion components, the unique structural organization of the microemulsion can add solubility regions and thus increasing the loading capacity of microemulsion (Danielsson and Lindman, 1981; Malcolmson and Lawrence, 1990).

3.3. Effects of cosurfactants on the in vitro release of hydrocortisone from microemulsion

The in vitro release of hydrocortisone was studied at the same skin permeation experimental conditions with the full thickness skin being replaced by the artificial semi-permeable membrane. This study was designed so as to correlate the release data with the skin permeation data. The in vitro release profiles of hydrocortisone obtained from different formulations are shown in Fig. 3 which revealed a typical zero order release in all tested formulations. The calculated release rates are presented in Table 3. The rate of drug release depended on the formulation with the cosurfactant free microemulsion producing the lowest release

Table 3
The in vitro drug release rate and the transdermal permeation parameters of hydrocortisone obtained from different microemulsion formulations

Formulation	Release rate ($\mu g\,cm^{-2}\,h^{-1}$)	Flux ($\mu g\,cm^{-2}\,h^{-1}$)	Lag time (h)
Control	52.5 (1.7)	0.097 (0.01)	1.94 (0.19)
TW ME	40.2 (2.1)	3.17 (0.27)	3.41 (0.68)
TW ETH ME	114.9 (5.5)	32.92 (5.02)	2.28 (0.29)
TW ISO ME	103.2 (3.0)	13.19 (1.83)	2.31 (0.85)
TW PG ME	87.6 (3.1)	18.11 (2.48)	2.75 (0.42)

The control was saturated aqueous drug solution.
Values between brackets are S.D. ($n = 4$). Formulation details are in Table 1.

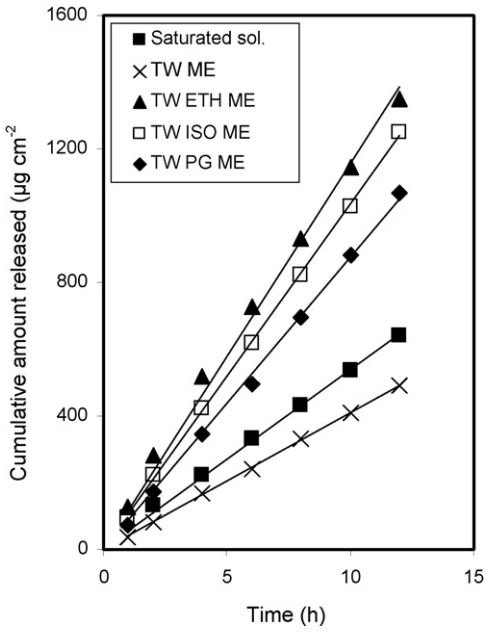

Fig. 3. The in vitro release profiles of hydrocortisone obtained after application of different microemulsion formulations or saturated aqueous drug solution to semi-permeable membrane. Formulation details are presented in Table 1.

rate which was even lower than that obtained from saturated drug solution in water. Incorporation of cosurfactants increased the rate of drug release with the formulations ranked as ethanol containing microemulsion slightly higher than isopropanol containing microemulsion which was higher than the propylene glycol containing formulation. Correlating these release results with the viscosity of the formulations (Table 2) it is clear that the release rate depended on the viscosity of the microemulsion formulations.

3.4. Effect of cosurfactants on skin permeation of hydrocortisone from microemulsion

The skin permeation studies employed full thickness skin obtained from the inner side of freshly excised rabbit ears. Rabbit skin has been successfully used to study skin permeation of a variety of drugs from various vehicles including microemulsion (Corbo et al., 1990; Touitou et al., 2000; Zhao et al., 2006). To ensure sink conditions 40% (v/v) propylene glycol in water was used as receptor fluid. This receptor has been successfully employed to monitor skin delivery of steroidal drugs from microemulsion (Biruss et al., 2007). Other studies also employed aqueous ethanol (El Maghraby et al., 1999, 2000) or even a mixture of ethanol, polyethylene glycol 200 and water (Machet et al., 1998). The transdermal permeation profiles were typical steady-state profiles with a lag time (Fig. 1). These profiles are expected with occlusive application of excess formulations containing drug at saturation. The calculated permeation parameters are presented in Table 3. The results of the statistical analysis of the obtained transdermal flux values are presented as P values in Table 4.

Incorporation of hydrocortisone in different microemulsion formulations increased the transdermal drug flux significantly,

Table 4
The statistical analysis data of the obtained transdermal flux

	Solution	TW ME	TW ETH ME	TW ISO ME
TW ME	0.001			
TW ETH ME	0.007	0.009		
TW ISO ME	0.005	0.01	0.023	
TW PG ME	0.005	0.008	0.052	0.165

Data are presented as P values. The difference is statistically significant at $P < 0.05$.

compared with the saturated aqueous control (Tables 3 and 4). This effect was evident even with the basic formulation (TW ME) which contains no cosurfactant. Investigating the effects of incorporation of cosurfactants on the hydrocortisone skin delivery from microemulsion, the permeation parameters obtained in presence and absence of cosurfactants were compared. Incorporation of any of the tested cosurfactants in the microemulsion significantly increased the transdermal flux of the drug compared to the cosurfactant free formulation (TW ME). Comparing between different cosurfactants, ethanol produced the greatest enhancement in transdermal delivery followed by propylene glycol and isopropanol.

Ethanol, isopropanol and propylene glycol have been previously employed as cosurfactants but none of the studies have compared between them in one study using the same oil and surfactant system. Ethanol and isopropanol containing microemulsions were investigated for skin delivery of estradiol in one study (Peltola et al., 2003). The study revealed greater transdermal fluxes of estradiol from ethanol containing microemulsions compared to isopropanol containing microemulsions (Peltola et al., 2003). This is in agreement with our results but it should be noted that the authors used different oil for each cosurfactant. Propylene glycol has been successfully used as cosurfactant in skin delivery of drugs like ibuprofen and triptolide from microemulsion (Chen et al., 2004, 2006).

Correlating the skin permeation results with the release results (Table 3), the formulations were ranked in terms of release rate as TW ETH ME > TW ISO ME > TW PG ME > control > TW ME. In terms of skin permeation they were ranked as TW ETH ME > TW PG ME > TW ISO ME > TW ME > control. This indicates that the transdermal skin delivery of hydrocortisone from microemulsion is not dependent on the rate of drug release from the formulation. This is also evident taking into consideration that the release rate was greater than the transdermal flux in all cases. In addition, there were no significant differences between the lag time values obtained after application of different formulations. The lag time is a permeation parameter depending mainly on the diffusivity of the drug through the skin with the lag time being reduced with increasing diffusivity. However, for diffusion to take place the drug has to release from the applied formulation and partition into the upper layers of the skin. The lag time can thus indirectly depend on the drug release. Considering this with no significant differences between the lag time values obtained after application of different formulations will further indicate that the skin delivery did not depend on the drug release. Accordingly, we can point out that the effect of the formulation on the skin may play a

major role in the improved skin permeation. The composition of microemulsion was found important in determining the efficiency of microemulsion as skin drug delivery system (Changez et al., 2006; Yuan et al., 2006). Liquid microemulsion system was more efficient than eutectic mixture local anesthetic cream (EMLA) (Sintov and Shapiro, 2004).

Alternative mechanisms were reported for the enhanced transdermal drug delivery from microemulsion (Kreilgaard, 2002). The first possible mechanism was related to the high drug loading capacity of microemulsion (Kreilgaard et al., 2000). This possibility can explain the superiority of various microemulsion formulations (with or without cosurfactants) over the saturated aqueous solution. However, it cannot explain the superiority of cosurfactant containing formulations over the TW ME system because the microemulsion formulations were ranked in terms of transdermal drug flux as TW ETH ME > TW PG ME > TW ISO ME > TW ME (Table 3) but they were ranked in terms of drug loading as TW ETH ME > TW ISO ME > TW PG ME > TW ME (Table 2). The second possibility is the penetration enhancing effect of the microemulsion components (Dreher et al., 1997). In our case eucalyptus oil was employed as an integral component of microemulsions. This oil contains cineole, a well-known skin penetration enhancer as the main component. This can suggest a possible penetration enhancing effect of various microemulsion systems. Cineole is known to produce greater skin penetration enhancement when applied in combination with either ethanol or propylene glycol (Yamane et al., 1995; Narishetty and Panchangnula, 2005). Considering this with the penetration enhancing effect of the employed cosurfactants, the superiority cosurfactant containing formulations over TW ME can be explained. However, if this mechanism is the main mechanism operating, we must have an increase in the diffusivity (reduction in the lag time) and this was not the case in our study which revealed no significant difference between the lag time values obtained with various formulations (Table 3). Accordingly, the penetration enhancing mechanism of the microemulsion components cannot be considered as the main mechanism operating. The third mechanism depends on the possibility that the microemulsion components can enter the skin as monomers with the result that the solubility of the drug in the skin is increased (Kreilgaard, 2002). This process will increase the partitioning of the drug into the skin creating 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 mechanism can provide an explanation for the enhanced transdermal delivery of hydrocortisone from the cosurfactant free system (TW ME), compared with the saturated aqueous control. Incorporation of cosurfactants in the formulation can provide further increase in drug partitioning into the skin which will result from the additional effect arising from penetration of the cosurfactant molecules into the skin. This additional effect may be responsible for the superiority of the cosurfactant containing formulations. The fourth mechanism depends on the possibility of direct drug transfer from the microemulsion droplet to the stratum corneum (Peltola et al., 2003). As the drug molecules are distributed in the microstructure of the microemulsion system with its very small droplet size, a very large surface area for drug transfer to skin will be created. This can explain the superiority of microemulsion formulations over the saturated aqueous solution. Addition of the cosurfactant can increase the flexibility of the surfactant film, reduce the droplet size and contact angle between the microemulsion and skin. This will provide further increase in the surface area with better spreading on the skin surface leading to further enhancement in drug transfer to skin. The last possibility is the supersaturation process which increases the thermodynamic activity and the driving force for the transdermal drug transfer (Kemken et al., 1992). This process can result from the fact that microemulsion systems usually undergo phase transition upon dilution with aqueous phase or evaporation of any volatile constituents which can influence the drug loading with the possibility of formation of supersaturated systems. This process is more likely to occur after open application on a relatively large surface area. Accordingly, the impact of this mechanism will be minor in the current study as evaporation is inhibited by the occlusive application protocol and the impact of back diffusion of the receptor will be minor due to application of 2 ml of the formulation on a small surface area of the donor compartment.

The safety of the transdermal delivery vehicle is an important factor in formulation development. The safety of the microemulsion formulations will depend mainly on the irritancy of the individual microemulsion components. It was recently observed that a formulation containing triptolide in aqueous solution containing 20% propylene glycol was irritating but no irritation was observed after loading the same drug in a microemulsion with propylene glycol used as cosurfactant (Chen et al., 2004). This finding may suggest the possibility that microemulsion can reduce the irritant effect of some materials. However, this requires extensive toxicity studies to investigate the effect of formulation type and composition on the skin irritation.

4. Conclusion

Microemulsion is a promising transdermal drug delivery vehicle. Incorporation of cosurfactant in the microemulsion formulations will not only affect the physical characteristics of the microemulsion but will also influence the transdermal drug delivery efficacy. The physical effects range from reduced viscosity, reduced droplet size to increased drug loading capacity. The presence of cosurfactants enhanced the transdermal drug delivery from microemulsion. The overall effects depended on the type of cosurfactant with ethanol being the most efficient among the tested cosurfactants.

References

Aboofazeli, R., Lawrence, M.J., 1994. Investigations into the formation of and characterization of phospholipids microemulsion. II Pseudo-ternary phase diagrams of systems containing water-lecithin-isopropyl myristate and alcohol: influence of purity of lecithin. Int. J. Pharm. 106, 51–61.

Aboofazeli, R., Patel, M., Thomas, M., Lawrence, M.J., 1995. Investigations into the formation of and characterization of phospholipids microemulsion. IV Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol and oil; the influence of oil. Int. J. Pharm. 125, 107–116.

Alany, R.G., Rades, T., Agatonovic-Kustrin, S., Davies, N.M., Tucker, I.G., 2000. Effects of alcohols and diols on the phase behaviour of quaternary systems. Int. J. Pharm. 196, 141–145.

Alany, R.G., Tucker, I.G., Davies, N.M., Rades, T., 2001. Characterizing colloidal structures of pseudoternary phase diagrams formed by oil/water/amphiphile systems. Drug Devel. Ind. Pharm. 27, 31–38.

Banga, A.K., Bose, S., Ghosh, T.K., 1999. Iontophoresis and electroporation: comparisons and contrasts. Int. J. Pharm. 179, 1–19.

Barry, B.W., 1983. Dermatological Formulations: Percutaneous Absorption. Marcel Dekker, New York and Basel.

Biruss, B., Kahlig, H., Valenta, C., 2007. Evaluation of an eucalyptus oil containing topical drug delivery system for steroid hormones. Int. J. Pharm. 328, 142–151.

Boltri, L., Morel, S., Trotta, M., Gasco, M.R., 1994. In vitro transdermal permeation of nifedipine from thickened microemulsion. J. Pharm. Belg. 49, 315–320.

Cevc, G., Blume, G., 1992. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim. Biophys. Acta 1104, 226–232.

Changez, M., Varshney, M., Chander, J., Dinda, A.K., 2006. Effect of the composition of lecithin/*n*-propanol/isopropyl myristate/water microemulsions on barrier properties of mice skin for transdermal permeation of tetracaine hydrochloride: in vitro. Colloids Surf. B: Bioenterf. 50, 18–25.

Chen, H., Chang, X., Du, D., Li, J., Xu, H., Yang, Z., 2006. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int. J. Pharm. 315, 52–58.

Chen, H., Chang, X., Weng, T., Zhao, X., Gao, Z., Yang, Y., Xu, H., Yang, X., 2004. A study of microemulsion systems for transdermal delivery of triptolide. J. Control. Rel. 98, 427–436.

Corbo, M., Liu, J.C., Chien, Y.W., 1990. Bioavailability of propranolol following oral and transdermal administration in rabbits. J. Pharm. Sci. 79, 584–587.

Danielsson, I., Lindman, B., 1981. The definition of microemulsion. Colloid Surf. 3, 391–392.

Delgado-Charro, M.B., Iglesias-Vilas, G., Blanco-Mendez, J., Lopez-Quintela, M.A., Guy, R.H., 1997. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur. J. Pharm. Biopharm. 43, 37–42.

Dreher, F., Walde, P., Walther, P., Wehrli, E., 1997. Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. J. Control. Rel. 45, 131–140.

El Maghraby, G.M., Williams, A.C., Barry, B.W., 1999. Skin delivery of oestradiol from deformable and traditional liposomes: mechanistic studies. J. Pharm. Pharmacol. 51, 1123–1134.

El Maghraby, G.M., Williams, A.C., Barry, B.W., 2000. Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. Int. J. Pharm. 196, 63–74.

El Maghraby, G.M., Williams, A.C., Barry, B.W., 2006. Can drug bearing liposomes penetrate intact skin? J. Pharm. Pharmacol. 58, 415–429.

Goodman, M., Barry, B.W., 1988. Action of penetration enhancers on human skin as assessed by permeation of model drugs 5-fluorouracil and estradiol. I. Infinite dose technique. J. Invest. Dermatol. 91, 323–327.

Kemken, J., Ziegler, A., Muller, B.W., 1992. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsion as vehicle. Pharm. Res. 9, 554–558.

Kogan, A., Garti, N., 2006. Microemulsions as transdermal drug delivery vehicles. Adv. Colloid Interf. Sci. 123–126, 369–385.

Kost, J., Pliquett, U., Mitragotri, S., Yamamoto, A., Langer, R., Weaver, J., 1996. Synergistic effect of electric field and ultrasound on transdermal transport. Pharm. Res. 13, 633–638.

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

Kreilgaard, M., Pederson, E.J., Jaroszewski, J.W., 2000. NMR characterization and transdermal delivery potential of microemulsion systems. J. Control. Rel. 69, 421–433.

Lawrence, J., 1994. Surfactant systems: microemulsions and vesicles as vehicles for drug delivery. Eur. J. Drug Metab. Pharmacokin. 3, 257–269.

Machet, L., Cochelin, N., Patat, F., Arbeille, B., Machet, M.C., Lorette, G., Vaillant, L., 1998. In vitro phonophoresis of mannitol, oestradiol and hydrocortisone across human and hairless mouse skin. Int. J. Pharm. 165, 169–174.

Malcolmson, C., Lawrence, M.J., 1990. A comparison between nonionic micelles and microemulsions as a means of incorporating the poorly water soluble drug diazepam. J. Pharm. Pharmacol. 42, 6P.

Megrab, N.A., Williams, A.C., Barry, B.W., 1995. Oestradiol permeation across human skin and silastic membranes: effects of propylene glycol and supersaturation. J. Control. Rel. 36, 277–294.

Mezei, M., Gulasekharam, V., 1980. Liposomes-a selective drug delivery system for topical route of administration. 1. Lotion dosage form. Life Sci. 26, 1473–1477.

Miller, L.L., Kolaskie, C.J., Smith, G.A., Rivier, J., 1990. Transdermal iontophoresis of gonadotropin releasing hormone and two analogues. J. Pharm. Sci. 79, 490–493.

Narishetty, S.T., Panchangnula, R., 2005. Effect of L-menthol and 1,8-cineole on the phase behavior and molecular organization of SC lipids and skin permeation of zidovudine. J. Control. Rel. 102, 59–70.

Peltola, S., Saarinen-Savolainen, P., Kiesvaara, J., Suhonen, T.M., Urtti, A., 2003. Microemulsions for topical delivery of estradiol. Int. J. Pharm. 254, 99–107.

Schreier, H., Bouwstra, J., 1994. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J. Control. Rel. 30, 1–15.

Schulman, J.H., Stoeckenius, W., Prince, L.M., 1959. Mechanism of formation and structure of microemulsions by electron microscopy. J. Phys. Chem. 63, 1677–1680.

Sintov, A.C., Shapiro, L., 2004. New microemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability in vivo. J. Control. Rel. 95, 173–183.

Stilbs, P., Lindman, B., Rapacki, K., 1983. Effect of alcohol cosurfactant length on microemulsion structure. J. Colloid Interf. Sci. 95, 583–585.

Touitou, E., Dayan, N., Bergelson, L., Godin, B., Eliaz, M., 2000. Ethosomes—novel vesicular carriers for enhanced delivery: characterisation and skin penetration properties. J. Control. Rel. 65, 403–418.

Williams, A.C., Barry, B.W., 2004. Penetration enhancers. Adv. Drug Del. Rev. 56, 603–618.

Yamane, M.A., Williams, A.C., Barry, B.W., 1995. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J. Pharm. Pharmacol. 47, 978–989.

Yuan, Y., Li, S., Mo, F., Zhong, D., 2006. Investigation of microemulsion system for transdermal delivery of meloxicam. Int. J. Pharm. 321, 117–123.

Zhao, X., Liu, J.P., Zhang, X., Li, Y., 2006. Enhancement of transdermal delivery of theophylline using microemulsion vehicles. Int. J. Pharm. 327, 58–64.