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Assessment of the percutaneous penetration of indomethacin from soybean oil microemulsion: effects of the HLB value of mixed surfactants

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The objective of this study was to evaluate the influence of the ratios or the hydrophile-lipophile balance (HLB) values of Cremophor EL and Span 80 on the phase behavior of the O/W microemulsions and the percutaneous absorption and penetration of indomethacin microemulsions. The existence of microemulsion regions is investigated in quaternary systems composed of soybean oil/Cremophor EL and Span 80 (mixed surfactants)/diethylene glycol monoethyl ether (cosurfactant)/water by constructing pseudo-ternary phase diagrams at various Cremophor EL/Span 80 ratios. In addition, five microemulsion formulations with various mixed surfactants HLB values were evaluated by *in vitro* penetration experiments using mouse skin and Franz diffusion cells. The flux and amount of indomethacin penetration from 5 microemulsion formulations were significantly different from the control, and the enhance ratios ranged from 2.38 to 4.68 and 2.11 to 4.23, respectively. The HLB value of mixed surfactants in the formulations was a principal factor in determining the percutaneous penetration of the drug. The flux and amount of drug penetration increased gradually with increasing content of the lipophilic surfactant Span 80 and skin retention was highest for mixed surfactants with a HLB value of 7.6. Therefore, it is suggested that the presence of mixed surfactants was beneficial in the formation of O/W microemulsions and enhanced percutaneous penetration of indomethacin.

1. Introduction

Microemulsions are transparent, optically isotropic and thermodynamically stable systems of water, oil, surfactant and cosurfactant (Wu et al. 2001), which have several significant advantages as transdermal drug delivery vehicles, including low irritation, powerful permeation ability and high solubilization for both hydrophilic and hydrophobic drugs when compared with other conventional vehicles (Sintov et al. 2004; Bolzinger et al. 2008; Shakeel et al. 2008). The enhanced absorption is typically associated with the high surface area of microemulsion aggregates and the presence of surfactants which minimize hydrophobic interactions between the phases and maintain the stable formulations as well as act as permeation enhancers (Biruss et al. 2008; Yuan et al. 2009). Unfortunately, a limitation of microemulsions is the requirement for pharmaceutically acceptable ingredients that are safe, non-irritating and non-toxic to the skin. This can be overcome by the use of biocompatible oil and nonionic surfactants (Warisnoicharoen et al. 2000). Therefore, in this study a variety of O/W microemulsions were prepared using pharmaceutical ingredients such as soybean oil and combinations of the nonionic surfactants, Cremophor EL (polyoxyethylene 35 castor oil) and Span 80 (sorbitan monooleate), which have been widely used in topical administration (Bachhav et al. 2006; Wu et al. 2001). To date, most studies on microemulsions as transdermal drug delivery vehicles have used low molecular weight oil such as isopropyl myristale (IPM) (Lee et al. 2003; Huang et al. 2008) and ethyl oleate (Chen et al. 2006) which are generally acknowledged as penetration enhancers. However, for the purpose of pharmaceutical formulation, the large molecular volume polar oils such as soybean oil are preferred (Kantaria et al. 2003). Soybean oil is commonly called 'vegetable oil' and is composed mainly of long-chain triglycerides and natural antioxidants which remain in the oil even after extraction. Compared with the low molecular volume oils, soybean oil has better biocompatibility with skin and increases the rigidity of the interfacial surfactant film (Lui et al. 1998).

In recent years, the widespread use of surfactants in microemulsion formulations has promoted considerable interest in the enhancement effect of these agents on the percutaneous penetration of drugs. In order to develop microemulsion formulations, the right blend of low and high HLB surfactants is necessary for the formation of a stable microemulsion (Constantinides and Scalart 1997; Wu et al. 2001). The HLB value of mixed surfactants, therefore, plays an important role in determining the formation of a microemulsion and transdermal drug delivery from the microemulsion.

Poorly water-soluble indomethacin (IMC), a non-steroidal anti-inflammatory drug, is effective in the management of rheumatoid arthritis and osteoarthritis (Chauhan et al. 2003). The transdermal delivery of IMC provides numerous advantages compared with other routes of administration, especially with regard to systemic side effects and patient compliance, and several commercial products in the form of ointment, gel, patches and liniment have been explored. In this study, IMC was incorporated into microemulsion carriers which were composed of

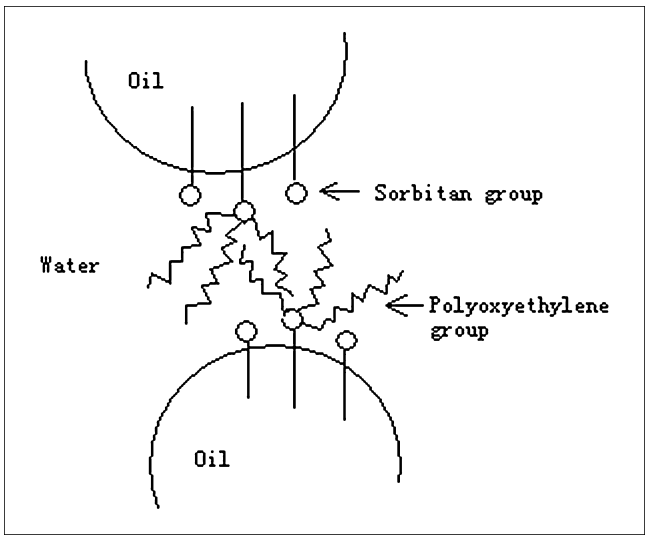


Fig. 1: Schematic diagram of the mixture of Cremophor EL and Span 80 at the oil-water interface

soybean oil, mixed surfactants of Cremophor EL and Span 80 and an aqueous phase in the presence of cosurfactant (diethylene glycol monoethyl ether). The objective of this work was to investigate the effects of the HLB values of mixed surfactants and the structure of the nonionic surfactants at the interfacial film on phase behavior and the transdermal penetration of IMC through mouse skin *in vitro*.

2. Investigations, results and discussion

2.1. Selection of surfactants

The HLB value is commonly used to express the relative degree of hydrophilic and lipophilic characteristics possessed by the hydrophilic and lipophilic parts of a surfactant molecule, respectively (Verdinelli et al. 2008). High HLB values indicate surfactants exhibiting mainly hydrophilic or polar properties, whereas low values represent lipophilic or non-polar characteristics (Schmidts et al. 2009). Each type of oil used in the microemulsion formulation will require a surfactant with a particular HLB value in order to ensure a stable product, however, a single surfactant usually cannot satisfy this condition with regard to soybean oil. Therefore, blending surfactants with two extreme HLB values would result in a strongly hydrophilic surfactant dissolving mostly in the water phase, conversely the strongly lipophilic surfactant would dissolve mostly in the oil phase (Gullapalli et al. 1999). This could strengthen the stability of the oil-water interfacial film and weaken the insufficient nature of the single surfactant.

Cremophor EL (polyoxyethylene 35 castor oil), is a nonionic solubilizer and hydrophilic surfactant with a high HLB value (HLB = 13.3). It forms clear solutions in water and is also miscible with fatty acids, fatty alcohols and certain animal and vegetable oils. Span 80 (Sorbitan monooleate), is a nonionic lipophilic surfactant with a low HLB value (HLB = 4.3). Both of these nonionic surfactants were selected to minimize skin irritation and charge disruption of the system (Lee et al. 2003). In our preliminary studies, Cremophor EL and Span 80 were used as surfactants in the microemulsion formulation, respectively, but it was not easy to form an O/W microemulsion when each surfactant was used alone. Therefore, in the present work, Cremophor EL and Span 80 were employed as mixed surfactant formulations and a series of mixtures of Cremophor EL and Span 80 were screened to determine the mixture with the required HLB value. It is well known that the presence of a lipophilic surfactant can enhance the blending interaction of surfactant molecules which are adsorbed in the oil-water interfacial film and make the film more stable (Hou and Papadopoulos 1997). Fig. 1 is a schematic diagram of the mixture of Cremophor EL and Span 80 at the oil-water interfacial film. This may be the arranged state of mixed surfactant molecules at the oil-water interface. Compared with a single surfactant, the intense interaction of the mixed surfactants may lead to a lower interfacial tension and increased interfacial adsorption. The arrangement of molecules then becomes more compact and enhances the rigidity of the interfacial film and stability of the microemulsion. Studies were carried out to evaluate the effect of the ratios or HLB values of the mixed surfactants on the phase behavior of the O/W microemulsions and the percutaneous absorption and penetration of IMC microemulsions.

Table 1: Effects of HLB values on O/W microemulsion region

HLB value of S_{mix}	Area of O/W nanoemulsion region in phase diagrams (%)	S_{mix} concentration range over which the O/W nanoemulsion was formed (% w/w)	Maximum amount of oil solubilized (S_{mix} concentration) (% w/w)
8.4	15.84	21 ~ 100	17 (40)
8.0	16.56	24 ~ 82	18 (40)
7.6	21.42	24 ~ 81	27 (40)
7.2	20.76	28 ~ 73	28 (40)
6.8	19.74	33 ~ 70	40 (40)

2.2. Phase behavior studies and formulations

The pseudo-ternary phase diagrams of five systems are shown in Fig. 2 and the area of the O/W microemulsion region is listed in Table 1. Different pseudo-ternary phase diagrams with various weight ratios of Cremophor EL to Span 80 were constructed to investigate the effect of the HLB value of the mixed surfactants on the area of the O/W microemulsion region. It was concluded from inspection of these phase diagrams, that the area of the O/W microemulsion isotropic region increased to a maximum (21.42%) when the mixed surfactants had a HLB value of 7.6. The results indicated that at a HLB value of 7.6, the affinity between the mixed surfactants, the oil phase, and the aqueous phase was greatest i.e., the balance of Span 80 dissolving in the oil phase and Cremophor EL dissolving in the aqueous phase was optimal when the mixed surfactant molecules decreased the interfacial tension to a low level and the O/W microemulsion was formed. Furthermore, the effect of HLB value on the level of soybean oil uptake at a mixed surfactants concentration of 40% and the mixed surfactants range in O/W microemulsions are given in Table 1, where the concentration of surfactants and the maximum level of soybean oil incorporation at which this occurred are reported. These findings suggested that the S_{mix} concentration range and the maximum amount of oil solubilized increased with increasing content of Cremophor EL and Span 80, respectively. These results demonstrated that Cremophor EL had better hydrophilicity and Span 80 had higher lipophilicity in order to enlarge the O/W microemulsion region and simultaneously dissolve more oil due to blending of the two surfactants at particular HLB values which made the system more stable.

Based on the above pseudo-ternary phase diagrams, five formulations were selected and are shown in Table 2. The formulations contained IMC, soybean oil, DGME, water and a combination of Cremophor EL and Span 80 at various weight ratios.

Since the combination of Cremophor EL (high HLB value) and Span 80 (low HLB value) determined the properties of the interfacial film structure, the difference in HLB values may be significant. This was basically the reason for changing the mixed

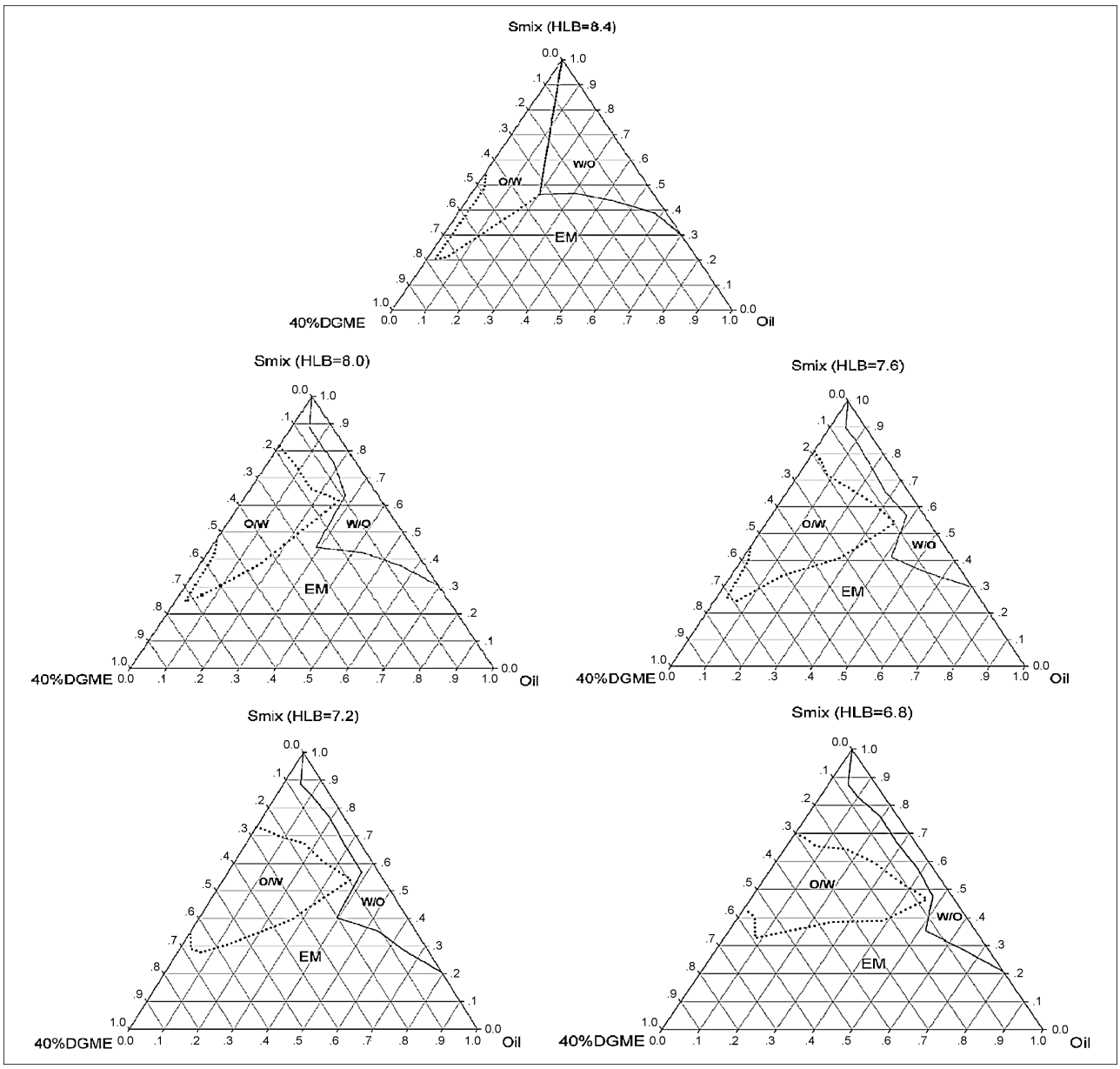


Fig. 2: Pseudo-ternary phase diagrams of microemulsions composed of soybean oil, mixed surfactants (Cremophor EL and Span 80) with various HLB values, cosurfactant (DGME) and aqueous solution. O/W: oil in water microemulsion; W/O: water in oil microemulsion; EM: crude emulsion

surfactants ratios to evaluate the influence of different HLB values on the efficacy of the microemulsion as a transdermal drug delivery vehicle.

2.3. Skin penetration flux and skin retention

The effects of various HLB values on the cumulative delivery of IMC across mouse skin versus time are plotted in Fig. 3, and the corresponding penetration values are summarized in Table 3. The flux of IMC from the five microemulsion formulations (from 3.73 ± 0.17 to $7.35 \pm 0.71\ \mu g \cdot cm^{-2} \cdot h^{-1}$) was significantly higher than that of the control ($1.57 \pm 0.24\ \mu g \cdot cm^{-2} \cdot h^{-1}$), and the enhance ratio (ER_1) ranged from 2.38 to 4.68. It was apparent that the flux value gradually increased when the mixed surfactants HLB value in the formulations (F1-F5) decreased. A significantly higher level of IMC was collected in the receptor medium at all time points for the five microemulsions compared

Table 2: Composition of the microemulsion formulations

Formulation	HLB value of S_{mix}	IMC (w/w, %)	Ingredient (w/w, %)				
			SO	EL 35	Span 80	DGME	Water
F1	8.4	1.00	9.90	18.04	21.56	19.80	29.70
F2	8.0	1.00	9.90	16.30	23.30	19.80	29.70
F3	7.6	1.00	9.90	14.56	25.04	19.80	29.70
F4	7.2	1.00	9.90	12.77	26.83	19.80	29.70
F5	6.8	1.00	9.90	11.00	28.60	19.80	29.70

Table 3: Penetration parameters of IMC microemulsions and ointment (control), mean ± SD (n = 6)

Formulation	Flux ($\mu g.cm^{-2}.h^{-1}$)	ER_1	Amount in collection medium (μg)	ER_2	T_{lag} (h)
F1	3.73 ± 0.17	2.38	60.02 ± 1.74	2.11	2.97
F2	3.97 ± 0.24	2.53	61.31 ± 1.84	2.16	3.31
F3	6.75 ± 0.68	4.30	107.52 ± 9.75	3.78	2.98
F4	7.04 ± 0.57	4.48	114.00 ± 9.16	4.01	2.89
F5	7.35 ± 0.71	4.68	120.25 ± 8.22	4.23	2.77
Control	1.57 ± 0.24	/	28.44 ± 4.46	/	1.77

ER_1: enhance ratio of flux; ER_2: enhance ratio of amount in collection medium

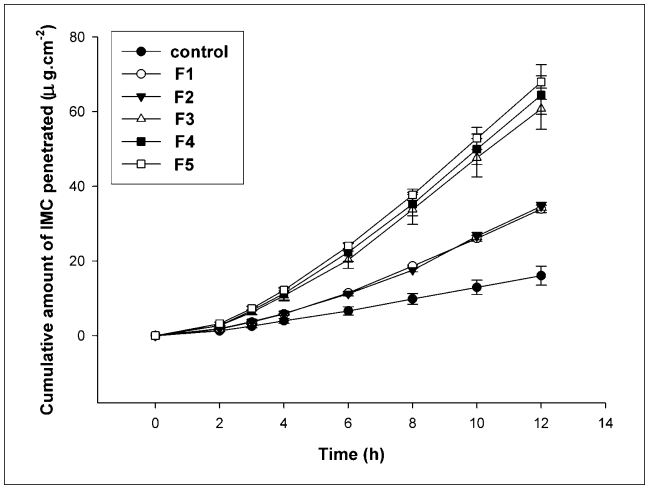


Fig. 3: Percutaneous penetration profiles of IMC from microemulsions and commercial ointment, mean ± SD, n = 6

with that of the control (ointment), and the enhance ratio (ER_2) ranged from 2.11 to 4.23. These findings showed that the content of Span 80 in the formulations affected the skin penetration of IMC.

Fig. 4 (A) shows the total amount of IMC penetration over 12 h after application. The values of F3, F4 and F5 were significantly higher than that of F1 and F2. A similar trend in the amount applied was observed in Fig. 4 (C). Fig. 4 (B) also shows that the amount of skin retention was highest with F3 compared with the other formulations. The surfactants HLB value in F3 was 7.6. These results further demonstrated that the microemulsion enhanced drug penetration and that the F3 surfactants interfacial film may be the most stable.

Many studies have reported on the possible mechanisms of enhanced percutaneous penetration from microemulsions. These have included the high drug loading capacity of microemulsions (Kreilgaard et al. 2000), the penetration enhancement of surfactants and cosurfactants which may reduce the diffusional barrier of the stratum corneum (Huang et al. 2008; Gamal and Maghraby 2008), and the possibility of direct drug transfer from the microemulsion droplet to the stratum corneum (Peltola et al. 2003). However, in this study, we focused on the effects of the ratios of mixed surfactants (Cremophor EL and Span 80) on percutaneous penetration. The five formulations (Table 2), contained the same components and percentages but with various ratios of Cremophor EL and Span 80. It has been suggested that the higher the percentage of Span 80, the higher the penetration flux would be, however, when the content of Span 80 was increased, drug penetration did not increase significantly (Table 3, Fig. 3). The penetration enhancing mechanism of surfactants is related to interference in the stratum corneum lipid barrier. In other words, the penetration ability across lipid bilayers is a combination of partition and passive diffusion, both of which are correlated with lipid fluidity. It was reported that an increase in fluidity was associated with an increase in penetration (Yokomizo and Sagitani 1996). The lipophilic surfactant, Span 80, as a penetration enhancing agent, may have a direct interaction with skin structures and increase the fluidity of lipid bilayers. Thus, it was able to facilitate the transport of drug molecules into cells when the percentage of Span 80 increased. In contrast, the hydrophilic surfactant, Cremophor EL, reduced percutaneous penetration as the surfactant exceeded the limited content. The reason for this may be that an excess of Cremophor EL might form micelles in the microemulsion system and a part of the drug was solubilized into these micelles in which the thermodynamic activity of the drug was weakened and the penetration flux was then decreased significantly. In summary, increasing Span 80 concentration benefited percutaneous penetration and Cremophor EL had the opposite effect.

With regard to skin retention, this was highest for F3 in which the HLB value of the surfactants was 7.6 (Fig. 4). At a HLB value of 7.6, the equilibrium between the hydrophilic and lipophilic parts of the mixed surfactant molecules favored a location at the oil-water interface. The arrangement of molecules at the interfacial film became very compact and the F3 system was not destroyed by surrounding factors. Therefore, during the 12 h *in vitro* diffusion period, F3 maintained a stable microemulsion structure to penetrate into the stratum corneum and was absorbed easily by the skin compared with the other formulations.

3. Experimental

3.1. Materials

Indomethacin was purchased from Shijiazhuang Pharmaceutical Group, Huasheng Pharma Co., Ltd. (Herbei, China). Soybean oil (SO) was purchased from Tieling Beiya Medicinal Oil Co., Ltd. (Liaoning, China). Cremophor EL (EL 35) was purchased from BASF (Germany). Span 80 and diethylene glycol monoethyl ether (DGME) were purchased from Jiangtian Chemical Technology Co., Ltd. (Tianjin, China). Indomethacin ointment was purchased from Shenyang Huayi Pharma Co., Ltd. (Liaoning, China).

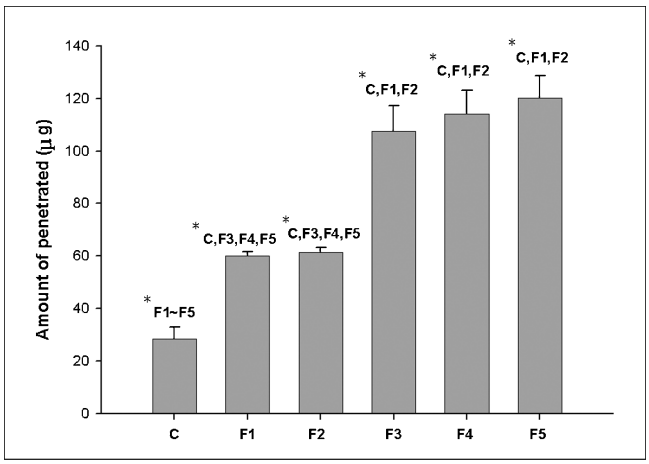
3.2. Construction of pseudo-ternary phase diagrams

Cremophor EL and Span 80 were selected as the mixed surfactants for application to the oil phase (soybean oil). The HLB value has been proved to be very useful in choosing the best type of surfactant for any given oil phase (Wang et al. 2009). The HLB values of the mixed surfactants were calculated using the weight fraction of the corresponding surfactants, and the following equation was employed:

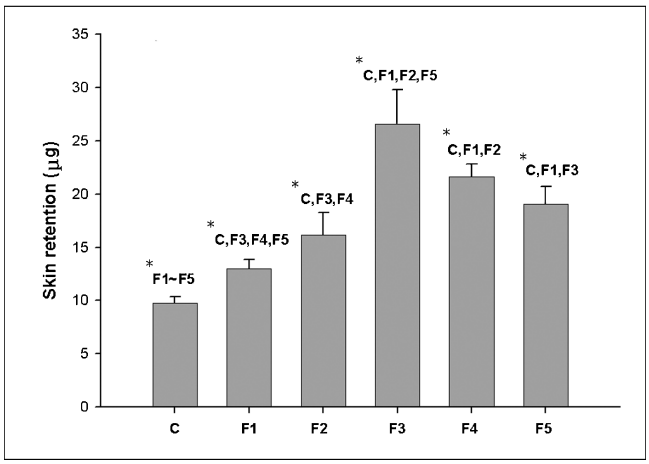
$HLB = f_A HLB_A + f_B HLB_B$ (Griffin 1949)

(HLB_A, HLB_B are the HLB values, and f_A, f_B are the weight fractions of Cremophor EL and Span 80, respectively.) A series of the mixed surfactants (S_{mix}) with different HLB values were prepared in order to find the best HLB value.

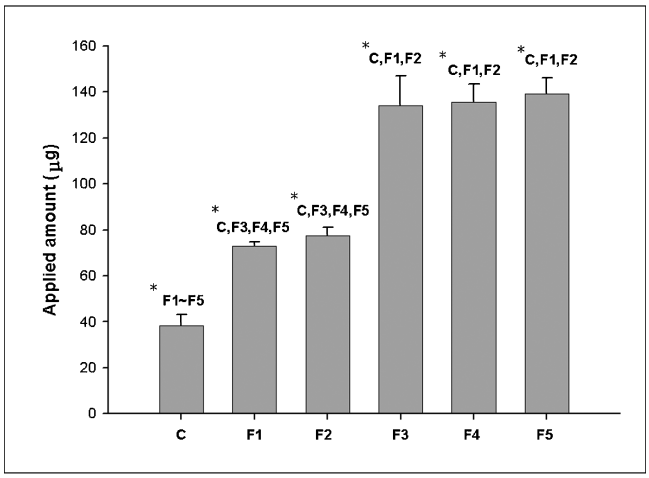
Pseudo-ternary phase diagrams were constructed by the aqueous solution titration method at ambient temperature (25 °C) to obtain the concentration range of components for the range of microemulsions without the drug (Huang et al. 2008). The mixtures of oil and S_{mix} at certain weight ratios (0.5:9.5, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) were titrated dropwise with 40% cosurfactant aqueous solution under gentle magnetic stirring. After equilibration, the systems were identified visually and determined as transparent fluid microemulsions, gel or crude emulsions. Based on these phase diagrams, the microemulsion formulations were selected at the desired



(A)



(B)



(C)

Fig. 4: (A) Total amount of IMC penetration, mean ± SD, n = 6. (B) Total skin retention of IMC, mean ± SD, n = 6. (C) The amount applied, mean ± SD, n = 6 *$P < 0.05$; C: control

component ratios and preparation of the microemulsion containing the drug was performed.

3.3. *Preparation of microemulsion formulation*

IMC was dissolved in DGME, and then mixed with the surfactants (mixture of Cremophor EL and Span 80) and soybean oil, followed by the addition of distilled water drop by drop with magnetic stirring for 30 min at ambient temperature until a transparent solution was obtained. The final concentration of drug in the microemulsion was 1% (w/w).

3.4. *In vitro percutaneous absorption and penetration studies*

Abdominal skin was obtained from a male Kunming mouse weighing 25 ± 2 g. After hair was removed with clippers, the skin was excised from the abdominal region of the sacrificed mouse and the subcutaneous fat and other tissues were trimmed. Skin samples were stored at –20 °C prior to use. The experiments were performed using vertical Franz diffusion cells (Pharmacopoeia Standard Instrument Factory of Tianjin, China) with 1.77 cm^2 of diffusion area. The full-thickness skin was mounted between the donor and the receptor compartments of the cells with the stratum corneum facing the donor compartments. The microemulsion or ointment (containing IMC 5 mg) was placed in the donor compartment, respectively. The receptor compartments were filled with 16 ml of phosphate buffered saline (pH 7.4), maintained at 32 ± 1 °C in a water bath and continuously stirred with a magnetic bar. At defined time intervals of 2, 4, 6, 8, 10 and 12 h, all of the receptor medium was removed and replaced by fresh, preheated medium. Samples were analyzed by HPLC. For all formulations, six parallel experiments were performed.

At the end of the experiments, the skin samples were carefully washed with methanol on both sides and dried to remove any remaining medium on the skin surface (Cross et al. 2000, 2001). A defined amount of methanol was then added to each piece of skin. The samples were homogenited, centrifuged at 12,000 rpm and the concentration of IMC analyzed by HPLC. Skin retention experiments were performed in order to analyze the content of drug stored in skin after 12 h of diffusion.

3.5. *HPLC analysis of IMC*

The IMC content following skin penetration and skin retention was analyzed by the HPLC system which consisted of a Series III pump and a UV detector (Model 201$^+$, LabAlliance, USA). The column was a reverse phase Kromasil C18 column (250 mm × 4.6 mm, 5 μm, LabAlliance). The mobile phase was a mixture of 0.1 mol·L^{-1} acetic acid and methanol (20:80, v/v) flowing at 1 ml·min^{-1}. The detection wavelength was 228 nm.

3.6. *Statistical analysis*

Results were expressed as the mean ± SD. Statistical analysis was performed using the Student's t-test with $p < 0.05$ as the minimum level of significance. The enhancement ratio (ER) for flux or amount of IMC in the collection medium was calculated using the following formula (Huang et al. 2008): ER = flux (or amount in collection medium) from the microemulsion formulation/flux (or amount in collection medium) from control vehicle.

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