Tianjin Key Laboratory of Modern drug Delivery and High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, PR China

Microemulsion: a novel transdermal delivery system to facilitate skin penetration of indomethacin

LIANGMEI CHEN, FENGPING TAN, JINFENG WANG, FENG LIU

Received November 11, 2011, accepted December 20, 2011

Liangmei Chen, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, PR China
cliangmei@gmail.com

Pharmazie 67: 319–323 (2012) *doi: 10.1691/ph.2012.1150*

In this study, we aimed to develop thermodynamically stable microemulsion formulations of indomethacin with lower surfactant and cosurfactant contents, to improve drug permeability. Formulations were based on the oil/water microemulsion region of pseudo-ternary phase diagrams. The characteristic parameters (viscosity, diameter, and polydispersity) of the microemulsion formulations were then determined. *In vitro* permeation studies were performed using Franz diffusion cells. Permeation through mouse skin and skin retention of indomethacin microemulsions and ointment were tested. The cumulative amount of permeated indomethacin and its skin retention were significantly higher in microemulsion formulations compared with ointment. Drug flux and skin retention improved with decreasing droplet diameter of the microemulsions. On the basis of these results, we suggest some possible mechanisms for the enhanced transdermal permeation of drugs in microemulsions, including high drug-loading capacity, permeation enhancement by surfactants and cosurfactants, and smaller droplet diameter. In conclusion, microemulsions represent a novel transdermal delivery vehicle for increasing the solubility and permeability of indomethacin.

1. Introduction

Microemulsions are transparent, optically isotropic, thermodynamically stable systems composed of water, oil, surfactant and cosurfactant (Wu et al. 2001). They have been studied as drug delivery systems because of their capacity to increase the solubility of poorly water-soluble drugs, as well as their ability to improve topical and systemic drug availability (Lee et al. 2005, 2002). Microemulsions are spontaneously formed, single-phase colloidal dispersions of either oil-in-water (O/W) or water-in-oil (W/O), stabilized by an interfacial film of surfactants and cosurfactants (Gupta et al. 2008). There are several mechanisms whereby microemulsions could enhance drug permeation, such as through increased drug-loading capacity and through the permeation-enhancing abilities of the microemulsion components (Kweon et al. 2004; Peltola et al. 2003).

Indomethacin (IMC) is a poorly water-soluble, non-steroidal anti-inflammatory drug, which is effective for managing conditions including rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis (Chauhan et al. 2003). Oral therapy with indomethacin is very effective, but its clinical use is often limited by its potential adverse effects, such as nephrotoxicity, and irritation and ulceration of the gastrointestinal mucosa (Beetge et al. 2000). Transdermal administration of indomethacin bypasses disadvantages associated with the oral route, and can result in the maintenance of relatively consistent long-term plasma levels following a single dose. Several commercial products have been developed to enhance the permeation of indomethacin, including ointment, patches and liniment. In this study, indomethacin was incorporated into a microemulsion system to improve its low solubility and permeability, which are key issues in the design of transdermal delivery systems.

The present study thus aimed to develop a novel microemulsion containing biocompatible soybean oil and nonionic mixed surfactants as a transdermal delivery vehicle to enhance the permeation of indomethacin.

2. Investigations, results and discussion

2.1. Phase diagrams

Figures 1, 2 and 3 show the pseudo-ternary phase diagrams with various weight ratios of oil:Smix, with DGME, ETOH or PG as cosurfactants. The concentration range of components for O/W microemulsions could easily be determined using these phase diagrams. In systems using DGME as cosurfactant (Fig. 1), the area of the O/W microemulsion was larger when the ratios of oil:Smix were 1:3 and 1:4. Figure 2 indicates that the area of the O/W microemulsion was larger when the ratio of oil:Smix was 1:4 in the systems using ETOH as cosurfactant, while Fig. 3 shows that a 1:5 ratio of oil:Smix had the largest O/W microemulsion region when PG was used as the cosurfactant. Five microemulsion formulations were selected on the basis of these phase diagrams, as described in Table 1.

2.2. Characterization of the selected microemulsion formulations

The characteristic parameters of the microemulsions are reported in Table 2. The average diameter of all microemulsions ranged from 104–305 nm, and the polydispersity was < 0.5 in all cases. The viscosities of the microemulsions E and F were 526.62 ± 25.47 mPa.s and 436.47 ± 19.61 mPa.s, respectively, which were higher than those of formulations A–D

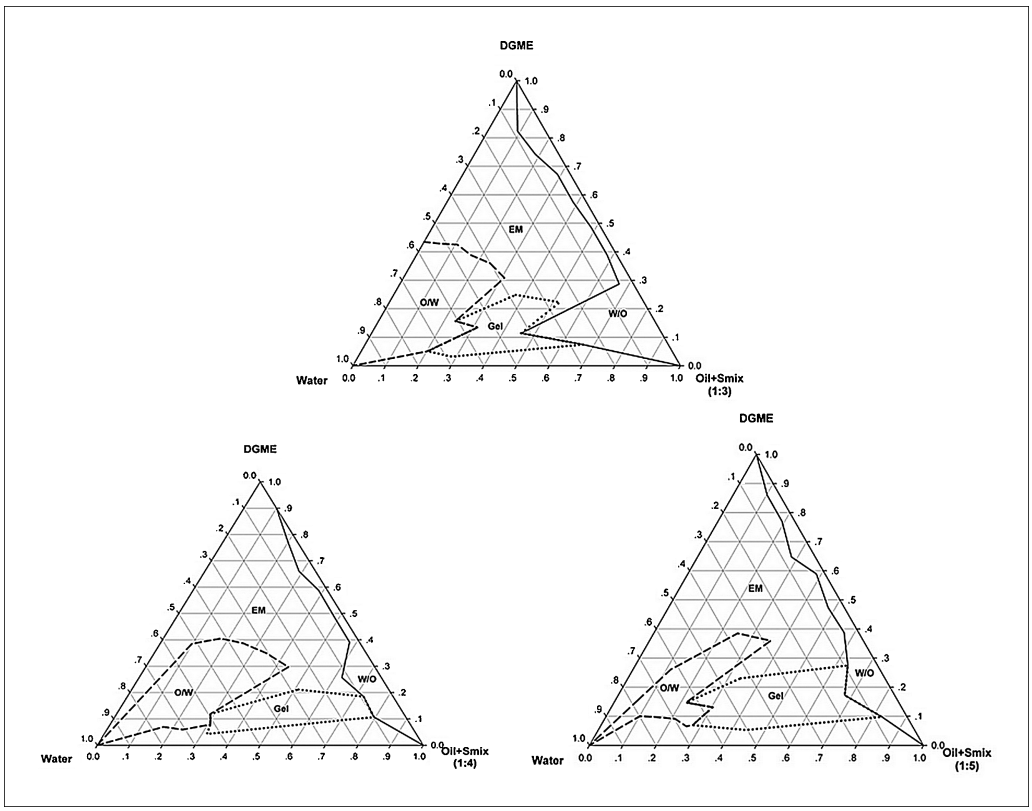


Fig. 1: Pseudo-ternary phase diagrams of systems with DGME as cosurfactant (oil:Smix were 1:3, 1:4 and 1:5, respectively.)

(Table 2). Comparing the microemulsions E and F with the other formulations (A–D), it was obvious that the viscosity increased significantly with increasing oil and surfactant contents (Tables 1, 2).

2.3. In vitro skin permeation studies

Figure 4 shows the permeation profiles of indomethacin through mouse skin using the selected microemulsion formulations, with commercial ointment as a control. A steady increase in cumulative permeated indomethacin with time was observed, with the permeation profiles following zero order kinetics. The cumulative permeated amounts from microemulsions A–F at 12 h application ranged from 107.79–197.31 $\mu g.cm^{-2}$, while that from the control (ointment) was only 16.07 $\mu g \cdot cm^{-2}$. These results indicate that a microemulsion system was able to significantly ($P < 0.05$) improve the permeability compared with the ointment.

Figure 5 shows the permeation flux and skin retention of indomethacin in relation to the droplet diameter of the selected

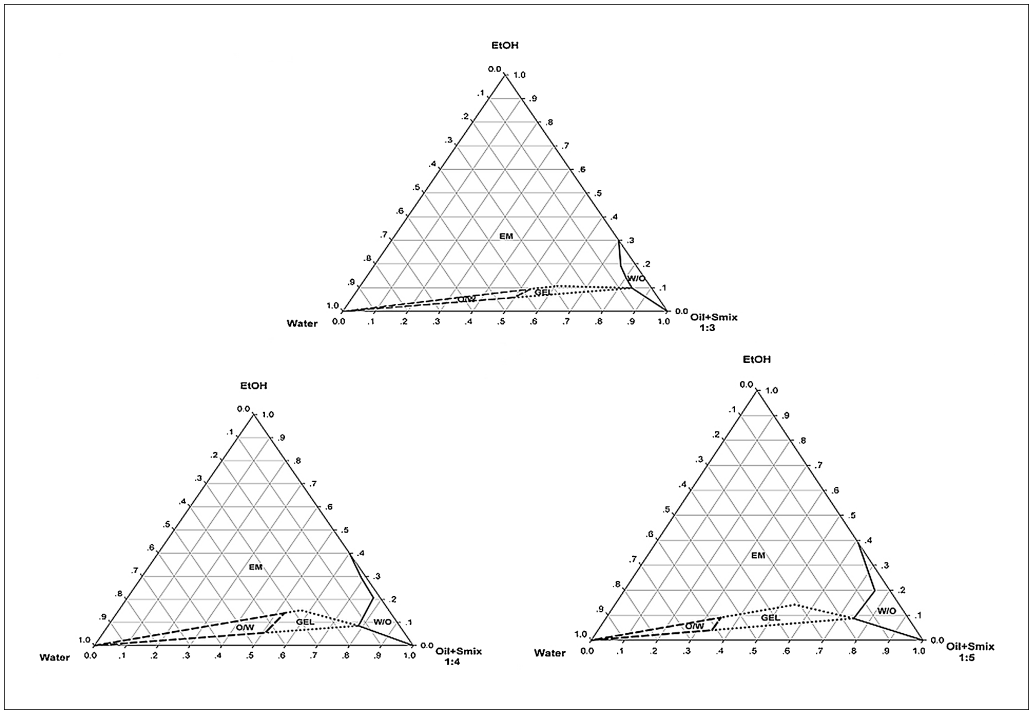


Fig. 2: Pseudo-ternary phase diagrams of systems with ETOH as cosurfactant (oil:Smix were 1:3, 1:4 and 1:5, respectively.)

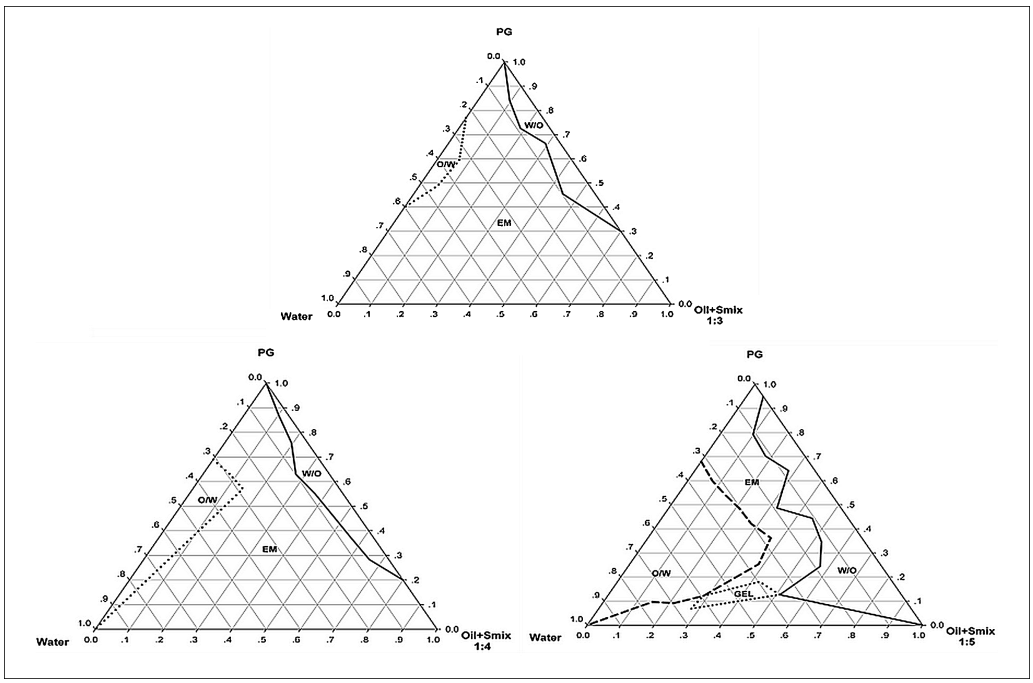


Fig. 3: Pseudo-ternary phase diagrams of systems with PG as cosurfactant (oil:Smix were 1:3, 1:4 and 1:5, respectively.)

Table 1: Compositions of the microemulsion formulations (w/w, %)

	IMC	SO	Smix	DGME	EtOH	PG	Water
A	1	6.60	19.80	24.75	–	–	47.85
B	1	5.45	21.78	27.22	–	–	44.55
C	1	7.26	21.78	27.22	–	–	42.74
D	1	5.94	23.76	29.70	–	–	39.60
E	1	10.0	40.0	–	11.43	–	37.57
F	1	6.0	30.0	–	–	30	33.0

formulations. The data for flux and skin retention are listed in Table 2. Both flux (Fig. 5a) and skin retention (Fig. 5b) decreased with increasing droplet diameter of the formulation. The diameters of the formulations were ranked as follows: B < E < A < C < F < D, while they were ranked inversely, in terms of flux and skin retention: B > E > A > C > F > D. The permeation flux, skin retention and diameter for each microemulsion formulation are shown in Table 2. Formulation B had the highest flux and skin retention, and the smallest diameter of all the formulations. These results indicate that a small droplet diameter is preferable in terms of skin penetration, and that cumulative permeation and flux were improved with decreasing droplet diameter. Droplet diameter is thus an important characteristic of microemulsion formulations that directly affects the transdermal skin delivery of indomethacin.

There are several possible mechanisms whereby microemulsions could enhance drug permeation for transdermal delivery. Firstly, the mechanism may be related to the high drug-loading capacity of microemulsions (Kreilgaard 2002). The solubility of indomethacin in the surfactants and cosurfactants was higher than in water and soybean oil (data not shown). It is possible that most indomethacin accumulated at the droplet interfaces, rather than staying in the oil or continuous phase. The indomethacin content in the selected microemulsion formulations was about 340 times higher than that in water. This high drug concentration would result in a high concentration gradient, which might account for its increased skin permeation.

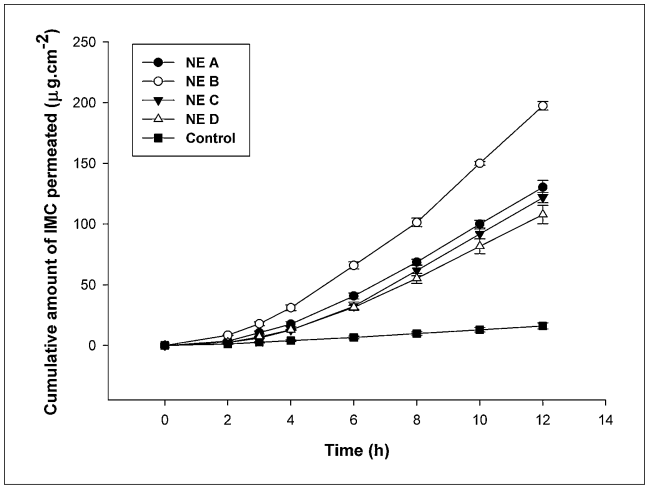
Secondly, surfactants and cosurfactants in the microemulsion may reduce the diffusional barrier of the stratum corneum by acting as permeation enhancers (Huang et al. 2008; Bolzinger et al. 2008). Figure 4 and Table 2 present the effects of the microemulsion compositions on indomethacin permeation profiles and parameters. The enhancement ratios of the six microemulsions were 8.15–13.66-fold higher than that of the control. These results demonstrate that microemulsions can potently enhance transdermal delivery. Cosurfactants, as well as surfactants, can also enhance transdermal drug delivery.

Thirdly, it is possible that drug transfer from the microemulsion droplet to the stratum corneum could enhance permeation

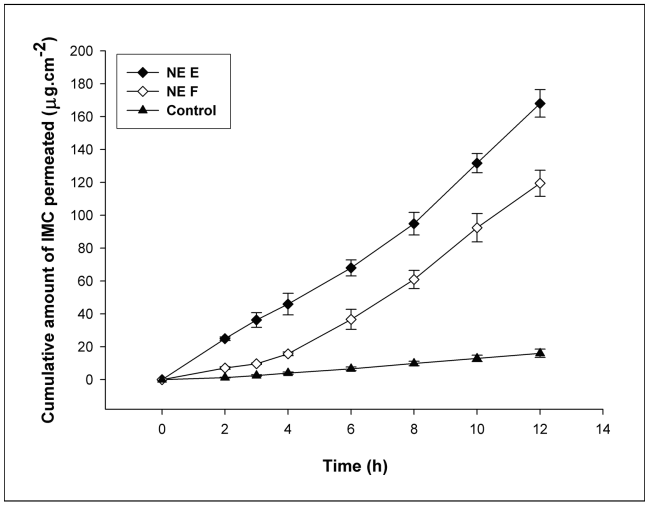
Table 2: Characteristics and permeation parameters of various formulations

Formulation	Viscosity[a] (mPa.s)	Diameter[a] (nm)	Polydispersity[a]	Flux[b] ($\mu g.cm^{-2}.h^{-1}$)	Skin Retention[b] ($\mu g.mg^{-1}$)
A	64.45 ± 3.13	184.7 ± 1.9	0.318 ± 0.007	$14.99 \pm 0.61^*$	$0.65 \pm 0.036^*$
B	104.84 ± 4.38	104.0 ± 0.4	0.375 ± 0.004	$21.45 \pm 1.88^*$	$0.72 \pm 0.035^*$
C	95.46 ± 4.21	227.0 ± 1.2	0.289 ± 0.01	$14.31 \pm 0.37^*$	$0.61 \pm 0.032^*$
D	110.90 ± 5.52	305.3 ± 4.0	0.306 ± 0.011	$12.79 \pm 0.98^*$	$0.55 \pm 0.025^*$
E	526.62 ± 25.47	137.3 ± 0.5	0.285 ± 0.004	$16.85 \pm 0.36^*$	$0.67 \pm 0.034^*$
F	436.47 ± 19.61	229.1 ± 3.1	0.242 ± 0.006	$13.99 \pm 0.42^*$	$0.59 \pm 0.026^*$
Control	–	–	–	1.57 ± 0.24	0.18 ± 0.011

a: mean ± SD, n = 3; b: mean ± SD, n = 6.
* $P < 0.05$, when compared with control.

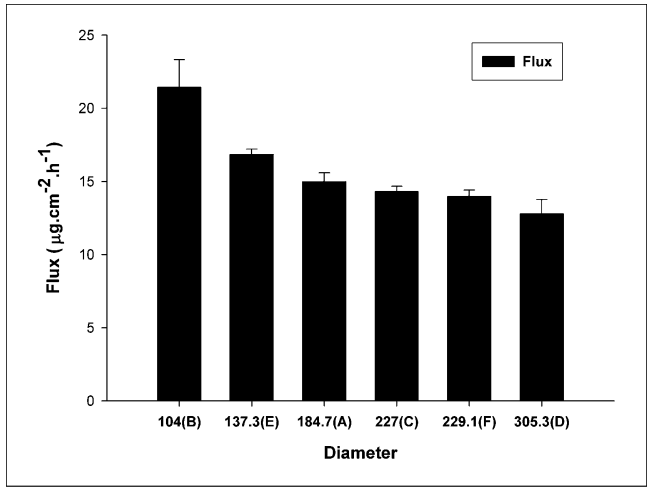


(A)

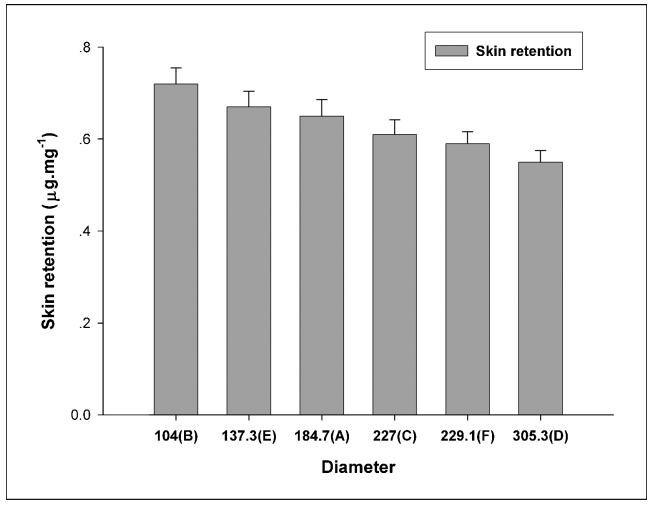


(B)

Fig. 4: Permeation profiles of indomethacin through mouse skin using various formulations (ointment), mean ± SD, n = 6



(a)



(b)

Fig. 5: Relationships between permeated flux (a) and skin retention (b) of indomethacin and microemulsion diameter, mean ± SD, n = 6

(Gamal et al. 2008). The small droplet size produced by the surfactants and cosurfactants means that the microemulsion provides a very large area for the drug, which is distributed within the nanostructure of the system. Usual surfactants are unable to lower the interfacial tension between the oil and water to such ultra-low values, and a cosurfactant is frequently necessary (Trotta 1999). Incorporation of cosurfactant can further reduce the interfacial tension between the oil and water in the microemulsion, adjust the flexibility of the interfacial membrane, and reduce the amount of surfactant required (Lee et al. 2003). Among the six microemulsion formulations in this study, DGME, ETOH and PG were investigated as alternative cosurfactants. The solubility of indomethacin with DGME as cosurfactant was 133.12 ± 2.63 mg·ml^{-1}, which was much higher than with EtOH or PG. Thus, as indicated in Table 1, DGME reduced the amount of surfactant required significantly more than EtOH or PG. Meanwhile, DGME could also help the surfactant to reduce the interfacial tension between the oil and water in the microemulsion more efficiently than EtOH or PG. Formulation B had the lowest contents of surfactant and cosurfactant and the highest permeation flux (21.45 ± 1.88 μg·cm^{-2}·h^{-1}) of the six microemulsion formulations (Table 2).

The small droplet diameter of microemulsions means that the oily droplets might enter the stratum corneum, and the drug molecules could thus be delivered directly from these droplets into the stratum corneum, with no transfer via the hydrophilic phase of the microemulsion (Mou et al. 2008), allowing drug molecules to more easily permeate into the stratum corneum. The results of this study demonstrate that the droplet diameter of the microemulsion is a critical factor determining the permeation of indomethacin through mouse skin (Fig. 5, Table 2).

Safety is also a critical factor of transdermal delivery systems. All the components of the microemulsion formulations selected in this study had low irritant and toxicity characteristics; however the content of surfactant mixture was 30–40% in formulations E and F, which would increase the skin irritation. Thus, although E and F demonstrated high permeation abilities, they would be unsuitable for chronic skin application for safety reasons (Chen et al. 2004). Formulation B, which had the highest permeation flux in association with a lower content of surfactant mixture, was selected as the optimum formulation. This formulation should be further investigated in future studies.

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 was purchased from BASF (Germany). Span 80, diethylene glycol monoethyl ether (DGME), absolute ethanol (EtOH), and propylene glycol (PG) were purchased from Jiangtian Chemical Technology Co., Ltd. (Tianjin, China).

3.2. Construction of pseudo-ternary phase diagrams

Soybean oil was selected as the oil phase on the basis of its good biocompatibility with skin. Various surfactants were screened in our preliminary studies, and Cremophor EL/Span 80 was chosen as the mixed surfactant (Smix) for the soybean oil phase.

Pseudo-ternary phase diagrams were constructed by aqueous solution titration at ambient temperature (25 °C) to obtain the concentration ranges of components for the microemulsions, without the drug (Huang et al. 2008). Firstly, oil and Smix were mixed at certain weight ratios (1:3, 1:4, and 1:5) and the mixtures of oil/Smix and cosurfactant were then titrated dropwise with water, under gentle magnetic stirring, at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. After equilibration, the systems were identified visually and determined as transparent fluid microemulsions, crude emulsions, or gels. Based on these phase diagrams, the microemulsion formulations with the desired component ratios were selected and tested as indomethacin-containing microemulsions.

3.3. Preparation of microemulsions

After identification of the microemulsion regions in the phase diagrams, formulations were selected at different component ratios, as described in Table 1. The microemulsions were prepared, briefly as follows: indomethacin was added to mixtures of soybean oil, surfactant, and cosurfactant at various component ratios, and water was then added to the above mixtures drop by drop, with magnetic stirring for 30 min at ambient temperature, until all the microemulsions were transparent.

3.4. Characterization of the selected microemulsion formulations

The average diameters and polydispersity indexes of the microemulsion formulations were measured using ZetaPALS (Brookhaven Instrument Corporation, US) at 25 °C. The viscosities of the microemulsion formulations were determined using an Ubbelohde viscometer (Glass Instrument Factory of Taizhou, Zhejiang Province, China) at 25 °C.

3.5. In vitro skin permeation studies

Abdominal skin was obtained from male Kunming mice weighing 25 ± 2 g. Mice were sacrificed and hair was removed using clippers. Skin was then excised from the abdominal region, and 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. Full-thickness skin was mounted between the donor and receptor compartments of the cells, with the stratum corneum facing the donor compartment. The microemulsion or ointment (containing indomethacin 5 mg) was placed in the donor compartment. The receptor compartment was filled with 16 ml of phosphate buffered saline (pH 7.4), maintained at 32 ± 1 °C in a water bath and stirred continuously with a magnetic bar. The receptor medium was removed and replaced by fresh, preheated medium at time intervals of 2, 3, 4, 6, 8, 10 and 12 h. Samples were analyzed by high-performance liquid chromatography (HPLC). Six parallel experiments were performed for each formulation.

The cumulative amount of indomethacin that permeated through the mouse skin was plotted as a function of time. The steady-state flux (J_s, $\mu g \cdot cm^{-2} \cdot h^{-1}$) through mouse skin was calculated from the slope of the linear portion of a graph plotting the cumulative amount permeated through the skin per unit area, versus time.

At the end of the experiment, the skin samples were carefully washed on both sides with methanol and then dried to remove any traces of drug formulations (Cross et al. 2000, 2001). A defined amount of methanol was then added to each piece of skin, the samples were homogenized, centrifuged at 12,000 g, and the concentrations of indomethacin were analyzed by HPLC. Skin retention experiments were performed to analyze the skin content of indomethacin after 12 h of diffusion.

3.6. HPLC analysis of indomethacin

The concentration of indomethacin was analyzed using an HPLC system consisting of a Series III pump and a Model 201^+ UV detector (LabAlliance, US). The column was a reversed-phase Kromasil C18 column (250 mm × 4.6 mm internal diameter, 5 μm, LabAlliance). The mobile phase was a mixture of 0.1 $mol \cdot L^{-1}$ acetic acid and methanol (20:80, v/v) flowing at 1 $ml \cdot min^{-1}$. The detection wavelength was 228 nm.

References

Beetge E, Plessis J, Muller DG, Goosen C, Rensburg FJ (2000) The influence of the NSAID's on their transdermal absorption. Int J Pharm 193: 261–264.

Bolzinger MA, Briancon S, Pelletier J, Fessi H, Chevalier Y (2008) Percutaneous release of caffeine from microemulsion, emulsion and gel dosage forms. Eur J Pharm Biopharm 68: 446–451.

Chauhan AS, Sridevi S, Chalasani KB, Jain AK, Jain SK, Jain NK, Diwan PV (2003) Dendrimer-mediated transdermal delivery: enhanced bioavailability of indomethacin. J Control Release 90: 335–343.

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

Cross SE, Jiang R, Benson HAE, Roberts MS (2001) Can increasing the viscosity of formulations be used to reduce the human skin penetration of the sunscreen oxybenzone. J Invest Dermatol 117: 147–150.

Cross SE, Roberts MS (2000) The effect of occlusion on epidermal penetration of parabens from a commercial allergy test ointment, acetone and ethanol vehicles. J Invest Dermatol 115: 914–918.

Gamal M, Maghraby E (2008) Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: Effects of cosurfactants. Int J Pharm 355: 285–292.

Gupta S, Moulik SP (2008) Biocompatible microemulsions and their prospective uses in drug delivery. J Pharm Sci 97: 22–45.

Huang YB, Lin YH, Lu TM, Wang RJ, Tsai YH, Wu PC (2008) Transdermal delivery of capsaicin derivative-sodium nonivamide acetate using microemulsions as vehicles. Int J Pharm 349: 206–211.

Kreilgaard M (2002) Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliv Rev 54: S77–98.

Kweon J H, Chi SC, Park ES (2004) Transdermal delivery of diclofenac using microemulsions. Arch Pharm Res 27: 351–356.

Lee J, Lee Y, Kim J, Yoon M, Choi YW (2005) Formulation of microemulsion systems for transdermal deliveryof aceclofenac. Arch Pharm Res 28: 1097–1120.

Lee PJ, Langer R, Shastri VP (2003) Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic Drugs. Pharm Res 20: 264–292.

Li L, Nandi I, Kim KH (2002) Development of ethyl laurate-based microemulsion for rapid-onset intranasal delivery of diazepam. Int J Pharm 237: 77–85.

Mou D, Chen H, Du D, Mao C, Wan J, Xu H, Yang X (2008) Hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs. Int J Pharm 353: 270–276.

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

Trotta M (1999) Influence of phase transformation on indomethacin release from microemulsion. J Control Release 60: 399–405.

Wu H, Ramachandran C, Weiner ND (2001) Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. Int J Pharm 220: 63–75.