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Formulation of Microemulsion Systems for Transdermal Delivery of Aceclofenac

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An O/W microemulsion system was developed to enhance the skin permeability of aceclofenac. Of the oils studied, Labrafil® M 1944 CS was chosen as the oil phase of the microemulson, as it showed a good solubilizing capacity. Pseudo-ternary phase diagrams were constructed to obtain the concentration range of oil, surfactant, Cremophor® ELP, and co-surfactant, ethanol, for micoemulsion formation. Eight different formulations with various values of oil of 6-30%, water of 0-80%, and the mixture of surfactant and co-surfactant (at the ratio of 2) of 14-70%. The *in vitro* transdermal permeability of aceclofenac from the microemulsions was evaluated using Franz diffusion cells mounted with rat skin. The level of aceclofenac permeated was analyzed by HPLC and the droplet size of the microemulsions was characterized using a Zetasizer Nano-ZS. Terpenes were added to the microemulsions at a level of 5%, and their effects on the skin permeation of aceclofenac were investigated. The mean diameters of the microemulsions ranged between approximately 10~100 nm, and the skin permeability of the aceclofenac incorporated into the microemulsion systems was 5-fold higher than that of the ethanol vehicle. Of the various terpenes added, limonene had the best enhancing ability. These results indicate that the microemulsion system studied is a promising tool for the percutaneous delivery of aceclofenac.

Key words: Aceclofenac, Microemulsions, Permeation enhancer, Transdermal delivery, Terpenes

INTRODUCTION

Aceclofenac is one of the non-steroidal anti-inflammatory drugs (NSAIDs) used for the treatment of rheumatoid arthritis and osteoarthritis (Yamazaki *et al.*, 1991; Gonzalez *et al.*, 1994), which reduces levels of prostaglandin E_2 in the synovial fluid and suppresses its production by blood polymorphonuclear leukocytes or mononuclear cells. The oral administration of aceclofenac has often resulted in side effects, including gastrointestinal ulcer and anemia due to gastrointestinal bleeding. As an alternative route for the drug, transdermal administration can eliminate these side effects, which also offer many advantages, such as increased patient compliance and the possibility for continuous and controlled drug absorption.

A microemulsion is defined as a dispersed system consisting of an oil, surfactant, co-surfactant and an aqueous phase, which is a single optically isotropic and thermodynamically stable solution, with a droplet diameter usually within the range of 10~100 nm (Tenjarla *et al.*, 1999). Microemulsions have several advantages as drug delivery systems, such as enhanced drug solubility, good thermodynamic stability, ease of manufacturing and enhancement of drug permeation effects upon transdermal administration (Lawrence *et al.*, 2000; Gasco *et al.*, 1997).

Therefore, a transdermal therapeutic system for aceclofenac was formulated using microemulsion systems. In transdermal drug delivery, the goal of dosage form design is to maximize flux through the skin into the systemic circulation. A useful strategy for improving the percutaneous flux is the selection of an appropriate vehicle for transdermal delivery (Lawrence *et al.*, 2000).

Terpenes constitute a heterogeneous class of natural odoriferous compounds that contain repeating isoprene units comprised of carbon, hydrogen and sometimes oxygen, but have no aromatic character. Due to their extensive use in foods, cosmetics and pharmaceutical products, terpenes are considered a less toxic alternative than many other chemical classes of permeation enhancers.

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The enhancement of skin penetration of drugs can be explained as being a result of the increased drug solubility in the stratum corneum treated with terpenes. Terpenes interact with intercellular lipids, which perturb their lamellar packing. With lipophilic drugs, terpenes increase the partition coefficient between the stratum corneum and the vehicle (Williams *et al.*, 1991; Kobayashi *et al.*, 1993; Cornwell *et al.*, 1996; Moghimi *et al.*, 1997).

In this study, O/W microemulsions containing 1.5% aceclofenac have been developed after screening of oils and obtaining optimum concentration ranges of components for microemulsion formation to provide maximal skin permeation rate of aceclofenac. Terpenes were also evaluated as potential permeation enhancers.

MATERIALS AND METHODS

Materials

The aceclofenac was supplied by Daewoong Pharmaceutical Co., Ltd. (Kyeonggi, Korea), and the linoleic acid, oleic acid, (*R*)-(+)-limonene, menthol, carvone, nerolidol, cineole, fenchone, and thymol were from Sigma Chemical Co. (St. Louis, U.S.A.). The Labrafil® M 1944 CS (oleoyl macrogol-6-glycerides) and Cremophor® ELP were supplied by Gattefosse (France) and BASF (Wyandotte, Germany), respectively. The isopropyl myristate (IPM) and triacetin were from Kanto Chemical Co., Inc. (Tokyo, Japan). All other chemicals and solvents were of analytical grade, and used with no further purification.

Selection of oils for microemulsions

To find suitable oils with a good solubilizing capacity for aceclofenac, which can also be used as the oil phase in microemulsions, the solubilities of aceclofenac in various oils were measured. The oils selected were vegetable oils (soybean oil, cotton seed oil; olive oil), oleic acid, IPM and Labrafil® M 1944 CS. An excess amount of aceclofenac was added to 2 mL of each selected oil, and shaken at 20°C for 24 h. The suspensions were centrifuged at 5000 rpm for 10 min, and aceclofenac solubility in the supernatant determined using an HPLC method, following appropriate dilution with the mobile phase.

Construction of phase diagram

The pseudo-ternary phase diagram of the oil (Labrafil® M 1944 CS), water and surfactant (S, Cremophor® ELP) and co-surfactant (Co-S, ethanol) mixture was constructed at room temperature. At the surfactant to co-surfactant (S/Co-S) ratio of 2, oily mixtures of oil, surfactant and co-surfactant, which are known to be self-microemulsifying drug delivery system (SMEDDS), were able to be prepared. Water was added to the SMEDDS to form microemulsions. Transparent, low viscous single phases were identified as microemulsions. Based on the phase diagram, seven microemulsions, with fixed ratios of oil/surfactant-co-surfactant (3:7), and a wide range of water contents, ranging 20 to 80% (w/w), were prepared for further characterization, which included the measurement of the droplet size, zeta potential and viscosity (Table II).

Preparation of O/W microemulsions loaded with aceclofenac

Appropriate quantities of Labrafil® M 1944 CS, Cremophor® ELP and ethanol, together with water, were weighed into a screw-capped glass vial. The mixtures were stirred with a magnetic bar, at room temperature, until the formation of a transparent system. Transparent, single-phased microemulsions were formed within a few seconds. Furthermore, in order to evaluate their drug delivery potential, aceclofenac was dissolved into pre-weighed vehicles, at a concentration of 1.5 % (w/w).

Measurement of droplet size, zeta-potential and viscosity

The mean diameter and zeta-potential of the microemulsions were measured, at 20°C, using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK). The viscosities of the microemulsions were evaluated using a viscometer (Brookfield Viscometer, model LVT, U.S.A.), with the apparent viscosity data obtained at 20 ± 1°C.

In vitro skin permeation study

Dorsal skin, excised from male Sprague Dawley rats (7-8 weeks old, 140-160 g) was mounted in Franz diffusion cells, with a diffusional surface area of 1.76 cm^2 and a volume of receptor cells of 11 mL. After the hair on the dorsal skin had been removed with animal hair clippers, the subcutaneous tissue was surgically removed, and the dermis side wiped with isopropyl alcohol to remove the residually adhered fat. The receptor compartments were filled with ethanol: pH 7.4 Tris buffer (30:70 v/v), to ensure perfect sink conditions, with the receptor phase stirred with a small magnetic bar for uniform mixing of the contents. In order to attain the skin surface temperature, 32°C, the receptor phase was maintained at 37 ± 0.5°C. The mounted skin tissues were equilibrated for 1 h, and the air bubbles were then removed. A 2 g of sample of each formulation; E, F, and G, was placed on the skin surface, with the donor compartment covered by paraffin film. Samples, 20 μL of the receiving solution, were withdrawn at appropriate time intervals over a 12 h period. All experiments for each sample were carried out in triplicate, with results presented as the mean ± S.D.

To improve the skin permeation rate of the aceclofenac, the selected terpenes (carvone, cineole, fenchone, limonene, menthol, nerolidol and thymol) were further added

to the final microemulsion formulations, at a concentration of 5%w/w, and their effects on the skin permeation of aceclofenac were evaluated.

HPLC analysis of aceclofenac

The cumulative amount of aceclofenac that permeated through the excised rat skins into the receptor medium was determined by high-performance liquid chromatography (HPLC). The HPLC system consisted of a quaternary pump (Hitachi, L-7100), an autosampler (Hitachi, L-7200), a UV/Vis detector (Hitachi, L-7400) set at 205 nm and an integrator (Hitachi, D-7000). Chromatography was performed on a Capcell pak C18 UG column (5 μm, 4.6 mm I.D. × 250 mm, Shiseido), using a sodium acetate buffer (20 mM) and acetonitrile (64:36) mobile phase, at a flow rate and injection volume of 1 mL/min and 20 μL, respectively.

Data treatment

The skin flux can be experimentally determined from the following equation:

$$J_{ss} = (dQ/dt)_{ss} \cdot 1/A$$

where J_{ss} is the steady-state flux (μg/cm^2 per h), A the area of skin tissue (cm^2) through which drug permeation takes place and $(dQ/dt)_{ss}$ the amount of drug passing through the skin per unit time at a steady-state (μg/h).

The cumulative amount of aceclofenac permeating through the rat skin was plotted as a function of time. The permeation rate of aceclofenac through rat skin at a steady-state (J_{ss}, μg/cm^2 per h) 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.

The following equation:

$$K_p = J_{ss}/C_0$$

was used to calculate the permeability coefficient, K_p (cm/h), where, K_p is the permeability coefficient and C_0 represents the drug concentration, which remains constant in the vehicle.

RESULTS AND DISCUSSION

Solubilities of aceclofenac in various oils

The solubilities of aceclofenac in various oils at room temperature are presented in Table I. Labrafil® M 1944 CS was chosen as the oil phase as aceclofenac solubility in this medium was greater (29.2 ± 1.2 mg/mL) than in the other oils investigated, resulting in the desired amount of solubilized aceclofenac.

Phase behavior

The pseudo-ternary phase diagram of the investigated quaternary system water/Cremophor® ELP(S)/ethanol (Co-S)/Labrafil® M 1944 CS is presented in Fig. 1. The formation of microemulsion systems (the dark area) was observed at room temperature. The investigation of the phase behavior of this system was shown to be suitable for the determination of transparent, one-phase and low-viscous systems. During the addition of water to the selected oily mixtures (i.e., a mixture of oil, surfactant and co-surfactant), a continuous transition from water in oil systems (W/O right side of phase diagram) to oil in water systems (O/W left side of phase diagram) was observed.

To differentiate the O/W from the W/O type of microemulsions, the changes in the viscosities and particle sizes of the selected systems, with increasing water content, were investigated against a mixture of oil/surfactant-co-surfactant with a constant ratio of 3:7 (Table II).

Table I. Solubility of aceclofenac in various oils at 20°C (Mean ± S.D., n=3)

Oil	Solubility (mg/mL)
Labrafil® M 1944 CS	29.15±1.17
Oleic acid	3.03±0.32
IPM	2.83±0.37
Soy bean	1.11±0.12
Cotton seed oil	1.48±0.09
Olive oil	1.27±0.22

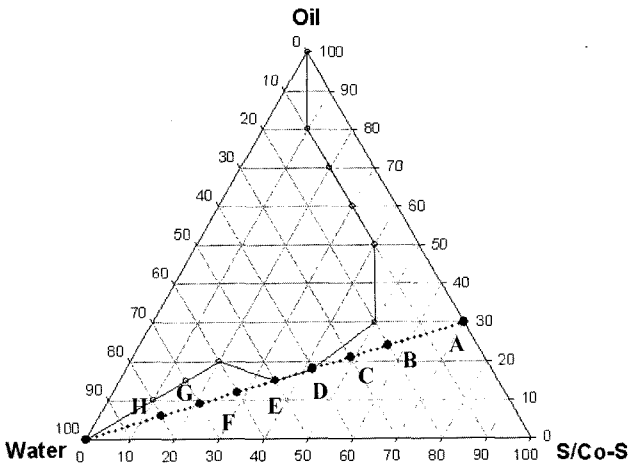

Fig. 1. Pseudo ternary phase diagram of a microemulsion composed of oil (Labrafil® M 1944 CS), surfactant (S, Cremophor® ELP), co-surfactant (Co-S, ethanol) and water.

Table II. Compositions of the microemulsion vehicles for aceclofenac (%, w/w)

	A	B	C	D	E	F	G	H
Water	0	20	30	40	50	60	70	80
Labrafil® M 1944 CS	30	24	21	18	15	12	9	6
Cremophor® ELP/EtOH	70	56	49	42	35	28	21	14

Determination W/O or O/W type microemulsions by measurements of droplet size and apparent viscosity

The droplet sizes and viscosities of the oily mixture (A) and microemulsions (B-H) are presented in Table III and Fig. 2. Photon correlation spectroscopy indicated the mean droplet sizes of the above seven formulations were within the 5.0~143.1 nm range. The droplet sizes were found to increase with increasing water content, showing a maximum droplet size of 143.1 nm with formulation E, followed by a decrease in the droplet size with an increase in the water content. The trend of the viscosity measurements was found to be similar to the changes in the droplet size, with the exception of the maximum observed viscosity (1170 ± 232 mPa·s) for formulation D. An increase in the dispersed phase of microemulsions is known to increase the viscosity and droplet size (Bennett *et al.*, 1982). Thus, a water content of around 40~50%, may cause a transformation of the microstructures within the current system. With a water content of less than 50%, W/O microemulsions might be formed (i.e., formulations B, C, and D). Conversely, with water contents exceeding 50%, O/W microemulsions might be formed (formulations F, G, and H). From these results, the identified O/W microemulsion formulations were selected as transdermal vehicles for aceclofenac, with 1.5% (w/w) aceclofenac loaded, which were subsequently evaluated in an *in vitro* skin permeation study.

Table III. Droplet size and apparent viscosity of the selected microemulsion vehicles (Mean±S.D., *n*=3)

Formulation	Viscosity (mPa·s)	Droplet size (nm)
A	70.3 ± 7.5	-
B	240.4 ± 32.5	5.0 ± 2.2
C	465.0 ± 63.2	22.8 ± 1.9
D	1170.2 ± 232.1	29.3 ± 1.8
E	452.1 ± 38.7	143.1 ± 20.0
F	60.4 ± 9.3	36.6 ± 5.6
G	10.2 ± 3.2	28.7 ± 2.4
H	6.3 ± 2.5	6.2 ± 0.3

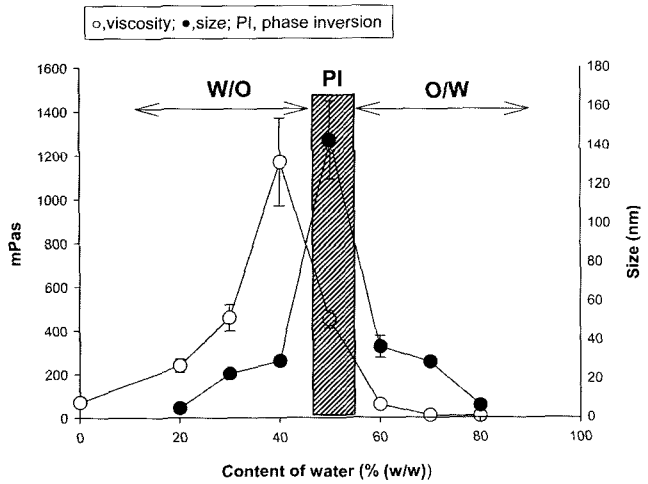

Fig. 2. Changes in the apparent viscosities and droplet sizes of microemulsions as a function of the water content in the oil(O)/surfactant(S)-co-surfactant (Co-S) system at a 3/7 ratio, with S/Co-S = 0.5

In vitro skin permeation study

Based on the constructed phase diagram, O/W microemulsions, containing 1.5% aceclofenac, were prepared for an *in vitro* skin permeation study. The selected formulations contained 60, 70, and 80% water, respectively (Formulations F, G, and H). The permeation rates of aceclofenac in selected microemulsions increased about 5~6 fold compared to those of the control (aceclofenac in ethanol). However, there were no significant differences in the skin permeation rates of the drug between the microemulsions with water contents of 60, 70, and 80%, which resulted in flux volumes of 11.98 ± 1.25, 12.35 ± 1.14, and 13.38 ± 1.02 (μg/h/cm^2), respectively (Table VI, Fig. 3). There were also no significant changes in the lag time, K_p

Table IV. *In vitro* percutaneous permeation parameters for the transmission of aceclofenac contained within the microemulsions through excised rat skin (Mean±S.D., *n*=3)

Microemulsion formulations	Permeation parameter			
	J_{ss} (μg/h/cm^2)	T_L (h)	K_p (× 10^3 cm/h)	Q_{12} (μg/ cm^2)
Control (aceclofenac in ethanol)	2.68±0.25	5.05±0.32	0.04±0.01	22.35± 3.24
F	11.98±1.25	4.48±0.82	0.79±0.08	101.02±15.99
G	12.35±1.14	4.39±1.11	0.82±0.07	94.75±15.34
H	13.38±1.02	4.53±0.32	0.89±0.07	90.46±13.24

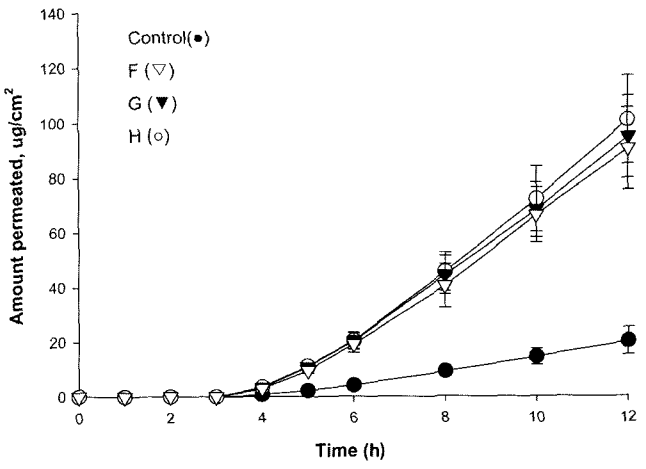

Fig. 3. Permeation profiles of aceclofenac through the excised rat skin from microemulsion formulations F, G and H. The control sample was prepared by solubilizing aceclofenac in ethanol (1.5 w/v%) (Mean±S.D., n=3).

and Q_{12}. This may have been due to the complete solubilization of aceclofenac in the oil phase of the microemulsion systems; no formulation components showed permeation enhancing effects.

Influences of permeation enhancers

To improve the skin permeation rate of aceclofenac from the microemulsions, various terpenes (fenchone, carvone, cineole, limonene, nerolidol, and menthol), as shown in Fig. 4, were added to microemulsion formulation G. When used with at a terpene concentration of 5%, the droplet sizes of the microemulsions were similar (~30 nm), but significantly increased to about 100 nm when menthol was added (data not shown). Likewise, when 5% terpene was added, the zeta-potential values of all the microemulsion formulations tested were also similar (~30 mV) (Fig. 5). Thus, the addition of permeation enhancers to microemulsion formulations caused no change in their physicochemical properties.

The permeation profiles obtained are shown in Fig. 6. The most pronounced enhancing effect on the skin permeation of aceclofenac was shown by limonene. The permeation parameters calculated from the *in vitro* permeation profiles are presented in Table V. While limonene increased the permeation rate of aceclofenac 3-fold compared to the control, which contained no permeation enhancer, the other terpenes did not significantly increase the rate of drug permeation through the skin.

Terpenes have been used to increase the skin permeation of a large number of compounds (Buyutimkin *et al.*, 1997), and have been reported to increase drug diffusivity and partitioning into the stratum corneum due to disruption of the intercellular lipid bilayers. The intensity of their effects mainly depends on the lipophilicities of the drug and vehicle used (Williams *et al.*, 1991a, 1996b).

It appears that the hydrocarbon or 'non-polar group containing' terpenes, such as limonene, provide better enhancing effect for lipophilic drug than 'polar' terpenes. Conversely, the terpenes containing polar groups, such as

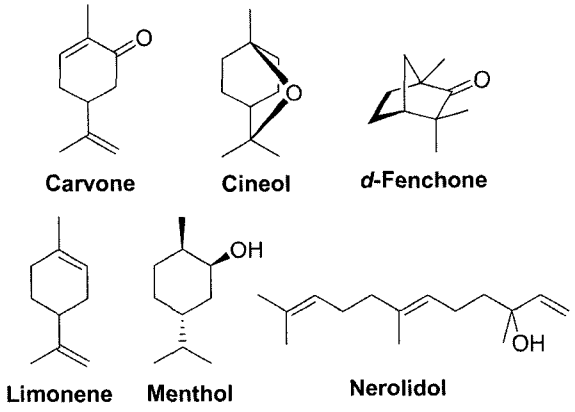

Fig. 4. The chemical structures of the terpenes used in this study

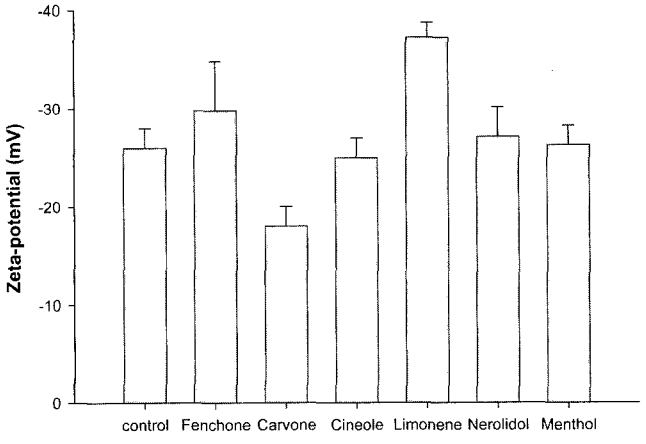

Fig. 5. Changes in the zeta potentials on the addition of 5% of the different permeation enhancers (Mean±S.D., n=3)

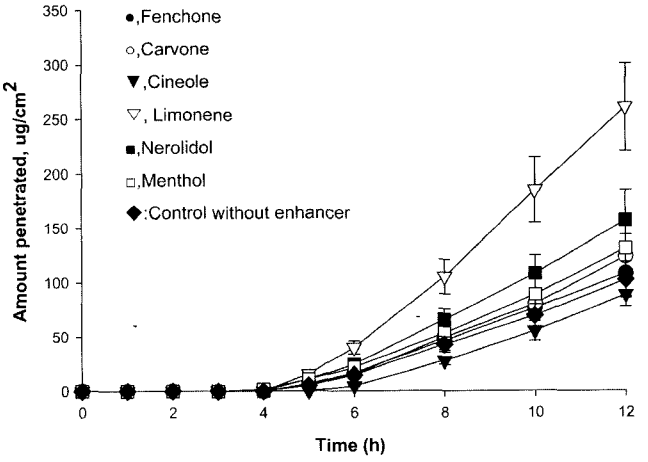

Fig. 6. Permeation profiles of aceclofenac through excised rat skin from microemulsions containing 1.5% aceclofenac and 5% of the different permeation enhancers (Mean±S.D., n=3)

Table V. *In vitro* percutaneous permeation parameters for the transmission of aceclofenac within the microemulsions, containing 5% of various terpene enhancers, through excised rat skin (Mean ± S.D., n=3)

Enhancer	Permeation parameters			
	J_{ss} (μg/h/cm^2)	T_L (h)	K_p (× 10^3 cm/h)	Q_{12} (μg/ cm^2)
Control (Formulation G)	12.35±1.14	4.39±1.11	0.82±0.07	94.75±15.34
Fenchone	15.15±2.64	4.92±0.78	1.01±0.18	107.96±21.22
Carvone	17.75±2.12	5.22±0.96	1.18±0.14	123.47±11.56
Cineole	13.95±3.57	5.84±1.78	0.93±0.25	88.47±20.43
Limonene	37.07±6.78	5.01±1.21	2.47±0.45	260.63±14.57
Nerolidol	21.92±1.98	4.95±0.88	1.46±0.28	156.82±13.14
Menthol	18.14±3.52	4.94±1.98	1.21±0.24	130.83±28.97

menthol and 1, 8-cineole, provide better enhancement for hydrophilic drugs.

Of the alcoholic terpenes, the acyclic terpene, nerolidol, showed the best enhancing effects. Nerolidol has a chemical structure that is suitable for the disruption of the lipid packing of the stratum corneum, due to the presence of definitive hydrocarbon tails, as well as a group with a polar head (Cornwell *et al.*, 1994).

With all of the enhancers studied, no significant change in the lag time for the skin permeation of aceclofenac was noted, unlike other report, where terpenes were found to enhance the permeation rate of aceclofenac, which resulted in a longer lag time than the control that contained no enhancer (Arellano *et al.*, 1996).

CONCLUSIONS

Different microemulsion formulations were designed using pseudo-ternary phase diagrams. Droplet sizes and viscosity data of the microemulsions confirmed the continuous structural transitions during increases in the water phase volume fraction in the oil/surfactant/co-surfactant mixture selected in this study.

The permeation rates of the drug in the microemulsion formulations studied were increased about 5~6 fold compared to that the control. However, there were no differences in the skin permeation rates of the drug from the microemulsion containing different water contents. The addition of limonene to the microemulsion significantly increased the skin permeation rate of aceclofenac, 3-fold, compared to the control with no enhancer. There was no significant change in the lag times for any of the enhancers studied. From these results, the transdermal delivery of aceclofenac was clearly confirmed as being by means of o/w microemulsion systems.

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