Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 335 (2007) 97–105
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

The effect of terpene concentrations on the skin penetration of diclofenac sodium

A. Nokhodchi [a,*], K. Sharabiani [b], M.R. Rashidi [b], T. Ghafourian [a,b]

[a] *Medway School of Pharmacy, Central Ave., University of Kent and Greenwich, Chatham, Kent ME4 4TB, England, United Kingdom*
[b] *School of Pharmacy and Drug Applied Research Center, Tabriz Medical Sciences University, Tabriz 51664, Iran*

Received 23 June 2006; received in revised form 5 October 2006; accepted 31 October 2006
Available online 3 November 2006

Abstract

Terpenes and sesquiterpenes have been suggested as promising non-toxic, non-irritating transdermal penetration enhancers. This investigation aimed to study the effect of terpene concentration on the transdermal absorption of diclofenac sodium from ethanol:glycerin:phosphate buffer solution (60:10:30). Therefore, enhancing effects of various terpenes (menthone, limonenoxide, carvone, nerolidol and farnsol) with different concentrations (0.25, 0.5, 1, 1.5 and 2.5%, v/v) on the permeation of diclofenac sodium were evaluated using Franz diffusion cells fitted with rat skin. Furthermore, solubility of diclofenac sodium in the vehicle in presence of different concentrations of terpenes was determined. The results showed that despite the negligible effect of terpenes on the drug solubility, there was a profound skin penetration enhancement effect, although the terpene enhancers varied in their ability to enhance the flux of diclofenac sodium. The results showed that at the highest concentration of terpene (2.5%, v/v) the rank order of enhancement effect for diclofenac sodium was nerolidol > farnesol > carvone > methone > limonenoxide, whereas at the low concentration of 0.25% the rank order was farnesol > carvone > nerolidol > menthone > limonenoxide. No direct relationship existed between terpene concentration and the permeation rate. The most outstanding penetration enhancer was nerolidol, providing an almost 198-fold increase in permeability coefficient of diclofenac sodium, followed by farnesol with a 78-fold increase.

Keywords: Terpenes; Diclofenac sodium; Skin permeation; Enhancer; log *P*

1. Introduction

Diclofenac sodium is a non-steroid-type anti-inflammatory agent and is used widely and clinically because of its strong analgesic, antipyretic and anti-inflammatory effects. It is known that this drug inhibits biosynthesis of the prostaglandin in vivo and in vitro and the drug is considered to have only a slightly adverse effect on the stomach and intestines (Manasse et al., 1978). It is extensively metabolized in the liver and because of its short biological half-life, the drug has to be given frequently. Therefore, developing a therapeutic system to provide a transdermal delivery is beneficial.

Transdermal delivery of drugs promises many advantages over oral or intravenous administration, however, the success of a transdermal drug delivery system depends on the ability of the drug to penetrate the skin in sufficient quantities to maintain therapeutic level. The principal barrier to most transdermal drug delivery is the stratum corneum, the outermost layer of the skin comprising keratin-rich cells embedded in multiple lipid bilayers. Many strategies have been suggested in order to overcome the low permeability of drugs through the skin. A popular approach is the use of penetration enhancers (or accelerants), which enhance the permeability of the stratum corneum (Barry, 1983). These agents partition into, and interact with, the stratum corneum constituents to induce a temporary, reversible increase in skin permeability.

As diclofenac sodium is not absorbed easily by transdermal application (Nishihata et al., 1987) the unionized form of the drug, diclofenac diethylamine, has been used in some preparations. On the other hand, it is possible to increase the skin absorption of diclofenac sodium by the use of penetration enhancers in the topical formulations. Many compounds, such as isopropyl myristate (Naito and Tominagaa, 1985), nicotinic acid esters (Yasukawa et al., 1985), hydrogenated soya phspholipid (Nishihata et al., 1987), ethanol (Nishihata et al., 1988; Obata et

* Corresponding author. Tel.: +44 1634 8833846; fax: +44 1634 883927.
E-mail addresses: a.nokhodchi@kent.ac.uk, phaanokhodchi@yahoo.com (A. Nokhodchi).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.10.041

al., 1993), *n*-octanol and decanol (Takahashi, Tamagawa, Katagi, Yoshitomi, Kamada, Rytting, Iwasa et al., 1991; Takahashi, Tamagawa, Katagi, Yoshitomi, Kamada, Rytting, Nishihata et al., 1991), non-ionic surfactants (Iwasa et al., 1991) and terpenes (Arellano et al., 1996) have been reported to enhance the permeability of diclofenac sodium.

With the safety of permeation enhancers being of a prime consideration, the search continues for an ideal enhancer that is pharmacologically inactive, non-irritant, non-damaging for the skin, potent, and cosmetically acceptable (Pfister et al., 1990). Many of the chemical enhancers such as dimethyl sulfoxide (Kurihara-Begstrom et al., 1987), surfactants (Ashton et al., 1986; Shokri et al., 2001; Nokhodchi et al., 2003), alcohols (Tsuzuki et al., 1988), and urea and its derivatives (Wong et al., 1989) have been screened for their penetration enhancement. The adverse effects caused by some of these enhancers restrict their widespread use. Currently, there has been an upsurge in the use of naturally occurring chemicals such as terpenes as enhancers (Moghimi et al., 1996, 1997; Nokhodchi et al., 2002). Terpenes, naturally occurring volatiles oils, appear to be promising candidates for clinically acceptable enhancers (Williams and Barry, 1989). They were reported to have good toxicological profiles, high percutaneous enhancement abilities, and low cutaneous irritancy at low concentrations (1–5%), (Opdyke, 1979; Okabe et al., 1990; Obata, 1991). Moreover, a variety of terpenes have been shown to increase the percutaneous absorption of both hydrophilic and lipophilic drugs (Cornwell and Barry, 1991; Moghimi et al., 1996, 1997; Gao and Singh, 1998; Ghafourian et al., 2004).

Arellano et al. (1996) studied the effect of 1% concentration of several terpenes in a carbopol gel formulation on the skin penetration of diclofenac sodium. They found promising enhancement results for some of the terpenes, with the most effective terpenes being geraniol and nerolidol. In a previous investigation we found a good correlation between hydrogen bonding ability of the terpenes and diclofenac sodium skin penetration enhancement ratios reported by Arellano et al.; the effect of partition coefficient was inconclusive (Ghafourian et al., 2004). Furthermore, there is little information in literature regarding the effect of enhancer concentration on skin penetration of drugs. For example surfactants have been shown to have an optimum concentration for the skin penetration enhancement of diazepam and lorazepam (Shokri et al., 2001; Nokhodchi et al., 2003). In this investigation the effect of some other terpenes with varying partition coefficient, including farnesol which has a hydroxyl group capable of hydrogen bonding was investigated on the skin penetration of diclofenac sodium. Moreover the influence of terpene concentration on the permeation enhancement of diclofenac sodium was examined.

2. Materials and methods

Diclofenac sodium was provided by Industrial Sobhan Pharmaceutical (Rasht, Iran). Farnesol, menthone, nerolidol, carvone, limonenoxide, acetonitrile, phosphoric acid, ethanol, glycerin and phosphate buffer were obtained from Merck (Darmstdt, Merck, Germany).

2.1. Preparation of the vehicles

The terpenes, nerolidol, farnesol, carvone, menthone and limonene oxide were used as the skin penetration enhancers of diclofenac sodium. Preliminary studies showed that some terpenes were insoluble in phosphate buffer and that a solvent mixture containing ethanol, glycerin and phosphate buffer (K_2HPO_4, pH 7.4) was able to dissolve all the five terpenes at the maximum required concentration. Therefore, the solvent mixture containing ethanol, glycerin and the phosphate buffer with the ratio of 60:10:30, respectively, was prepared and used to dissolve 0, 0.25, 0.5, 1, 1.5 and 2.5% (w/v) of the terpenes. These were then used as the vehicles for the delivery of diclofenac sodium.

2.2. Solubility studies

Saturated solubility of diclofenac sodium in the vehicles (explained above) was evaluated. Saturated solutions were prepared by adding excess drug to the vehicles and shaking for 48 h at 25 °C (preliminary studies showed that 48 h is enough to reach the equilibrium solubility). After this period the solutions were filtered, diluted with the vehicle and analyzed by HPLC. Three determinations were carried out for each sample to calculate the solubility of diclofenac Na. The enhancement ratio for apparent solubility of diclofenac sodium in the vehicle was calculated using the following equation: $ER_{Sol} = C_t/C_s$, where C_t is diclofenac concentration in the presence of terpene and C_s is the saturation solubility of sodium diclofenac in the control sample (no terpene).

2.3. Preparation of diclofenac sodium solutions

The saturated solutions of diclofenac sodium in the vehicles (terpene solutions (0–2.5%, w/v) in the solvent mixture containing 60:10:30 ratio of ethanol: glycerin: phosphate buffer) were prepared according to the technique explained above. Five millilitres of the saturated solution was used for in vitro permeation through the rat skin. The pH of the receptor phase solution was adjusted at 7.4 by phosphate buffer. As the saturated solution of diclofenac sodium was used, depending on the formulation the amount of diclofenac sodium in samples was varied between 245 and 351 mg/5 ml.

2.4. Viscosity measurements

A Brookfield RVT viscometer was used to measure the viscosities (in cP) of the vehicle and gels prepared. A spindle (no. 2) was rotated at 20 rpm. Samples of the gels and vehicle were left to settle over 30 min at the assay temperature (37 °C) before measurements were taken.

2.5. Preparation of diclofenac sodium gels

The composition of the diclofenac sodium gel formulations used in this study was diclofenac sodium 1 g, sodium carboxymethylcellulose (NaCMC) 3 g, propylene glycol 20 g,

water up to 100 g. Gels were prepared by dispersing 3% (w/w) NaCMC in water for a period of 2 h. Diclofenac sodium was dissolved in propylene glycol and the solution was added gently to NaCMC dispersion under continuous stirring (200 rpm). The same method was employed for the preparation of gels containing nerolidol as the penetration enhancer. In this case the enhancer (2.5 g) was dissolved in propylene glycol.

2.6. In vitro permeation through rat skin

After killing the animal (Wistar male rats, weighing 160 ± 25 g) with pentobarbital, given intraperitoneally, hair on dorsal skin of animal was removed with animal hair clipper (Aesculp, Germany), the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. The skin was excised and washed with distilled water and allowed to equilibrate for 1 h before experimentation. This protocol was approved by TUMS's Institutional Animal Ethics Committee. A system employing improved Franz diffusion cells with a diffusional area of 5.3 cm^2 was used for permeation studies. The excised rat skin was set in place with stratum corneum facing the donor compartment and the dermis facing the receptor. Five milliliters of the saturated solution or gel formulation was placed on the skin surface in the donor compartment that was sealed from the atmosphere using a plastic film. The receptor compartment of the cell was filled with 25 ml of the vehicle without drug (pH 7.4). During the experiments, the solution in the receptor phase was maintained at 37 °C and stirred at 800 rpm with Teflon-coated magnetic stirring bars. After application of the test formulation on the donor side, 0.25 ml aliquots were collected from the receptor side at designated time intervals (0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h) and 0.25 ml of the phosphate buffer was added into the receptor side immediately after each sample collection. The drug concentration in collected samples was determined by HPLC. The results are the mean and standard deviations of at least three determinations.

2.7. Analytical method

Diclofenac sodium in samples was determined using HPLC apparatus (Ceceil 1100, UK) equipped with a variable-wavelength UV detector. The column was Spherisorb C18 (150 mm × 4 mm, 5 μm, hichrom). Elution was carried out at room temperature with a mobile phase consisting of acetonitrile and water (50:50, v/v) adjusted to pH 2.2 with phosphoric acid; the flow rate was 2 ml/min. Detection was performed at 276 nm (Arellano et al., 1996).

2.8. Data treatment

According to Fick's second law of diffusion, the total amount of drug (Q_t) appearing in the receptor solution in time t is expressed as:

$$Q_t = AKLC_0 \left[(Dt/L^2) - (1/6) - (2/\pi^2) \sum \frac{(-1)^n}{n^2} \right) \times \exp \left(\frac{D^n 2\pi^2 t}{L^2} \right) \right] \quad (1)$$

where A is the effective diffusion area, C_0 represents the drug concentration which remains constant in the vehicle, D is the diffusion coefficient, L denotes the thickness of the membrane and K is the partition coefficient of the drug between membrane and vehicle. At steady-state, Eq. (1) is expressed as follows:

$$\frac{Q_t}{A} = KLC_0 \left[\left(\frac{Dt}{L^2} \right) - \left(\frac{1}{6} \right) \right] \quad (2)$$

The flux, J, was determined from the slope of the steady-state portion of the amount of the drug permeated divided by A versus time. The lag time values were determined from the x-intercept of the linear region at steady-state.

From Eq. (2) the flux is expressed as:

$$J = \frac{C_0 KD}{L} = C_0 K_p \quad (3)$$

where K_p is the permeability coefficient.

The enhancement ratios (ER) were calculated from following equation (Williams and Barry, 1989).

$$\text{ER} = \frac{K_p \text{ with pretreatment}}{K_p \text{ without pretreatment}} \quad (4)$$

The values reported are mean ratios from a minimum of three replicates.

2.9. Statistical analysis

All the data were statistically analyzed by analysis of variance (ANOVA) or Turkey's multiple comparison test. Results were quoted as significant where $P < 0.05$.

3. Results and discussion

Terpenes are naturally occurring volatile oils that appear to be promising candidates for clinically acceptable enhancers (Williams and Barry, 1991). They have been reported to have good toxicological profiles, high percutaneous enhancement abilities, and low cutaneous irritancy at low concentrations (Okabe et al., 1990). In this study skin penetration of diclofenac sodium in presence of five different terpenes (one ether, two ketones and two alcohols) was investigated. Five different concentrations of each terpene were studied. Therefore, different concentrations of terpenes (0, 0.25, 0.5, 1, 1.5 and 2.5%, w/v) were prepared in a solvent mixture comprising ethanol, glycerin and phosphate buffer (60:10:30 ratios, respectively) which were then used as carriers of the model drug diclofenac sodium at the saturated state.

The $\log P$ values and the chemical structures of terpene enhancers tested are shown in Fig. 1. Table 1 shows the solubility of diclofenac sodium in presence of various terpenes in the mixtures of ethanol:glycerin:phosphate buffer (60:10:30). The drug has a relatively high solubility in ethanol:glycerin:phosphate buffer (48.52 mg/ml); the addition

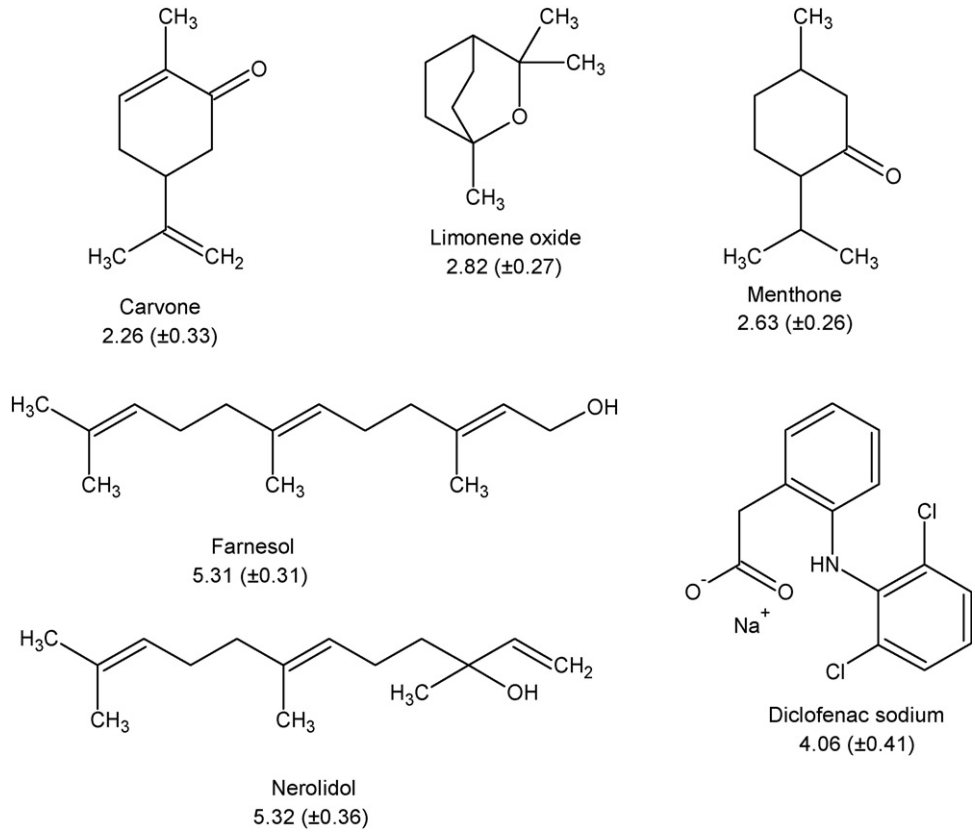

Fig. 1. Molecular structures of the terpenes studied and diclofenac sodium together with the corresponding log *P* values and standard deviation calculated by ACD/log *D* suite (Advanced Chemistry Development Incorporated, Ontario, Canada).

of terpenes slightly increased the solubility of diclofenac sodium in ethanol:glycerin:phosphate buffer with an exception of the samples containing 0.25% menthone (Table 1). The vehicle containing the highest concentration of menthone (2.5%, v/v) showed the highest solubility.

The permeation profiles of diclofenac sodium through rat skin from the vehicle in presence of menthone, carveone, nerolidol, limonenoxide and farnesol are shown in Figs. 2–6. The results show that terpene concentration plays an important role in the ER. The flux, *J*, permeability coefficient, K_p, lag time and ER for each of the different concentrations of the enhancer according to equations (2)–(4) are listed in Table 2. The table shows that, generally, all the terpenes used in the study can promote the skin transport of diclofenac sodium. Figs. 2–6 indicate that, as a general rule, the highest permeation rate is achieved with the solution containing the highest concentration (2.5%) of terpenes

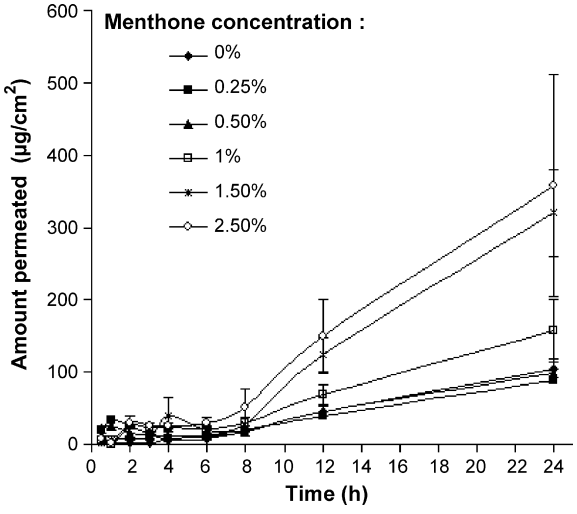

Fig. 2. Permeation profiles of diclofenac sodium in presence of different concentration of menthone (%, v/v) through rat skin.

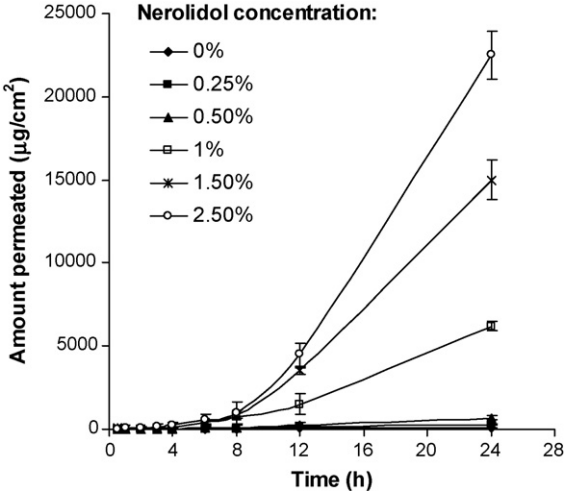

Fig. 3. Permeation profiles of diclofenac sodium in presence of different concentration of nerolidol (%, v/v) through rat skin.

Table 1
Effect of terpenes on the Diclofenac sodium solubility in the vehicle at 25 °C (values are the mean and standard deviations of five determinations)

Terpene	Apparant solubility (mg/ml)	ER_{sol}[a]
Carvone		
0%	48.95 ± 2.11	1.0000
0.25%	50.75 ± 2.50	1.0367
0.5%	54.61 ± 2.40	1.1155
1.0%	60.26 ± 2.85	1.2311
1.5%	62.32 ± 3.11	1.2731
2.5%	64.89 ± 1.96	1.3257
Menthone		
0%	48.95 ± 2.11	1.0000
0.25%	40.20 ± 1.75	0.8213
0.5%	51.78 ± 1.99	1.0577
1.0%	53.58 ± 2.10	1.0945
1.5%	57.95 ± 2.15	1.1838
2.5%	70.29 ± 3.15	1.4360
Nerolidol		
0%	48.95 ± 2.11	1.0000
0.25%	53.58 ± 1.45	1.0945
0.5%	54.35 ± 2.34	1.1103
1.0%	64.12 ± 3.42	1.3099
1.5%	62.32 ± 1.80	1.2731
2.5%	63.35 ± 1.90	1.2941
Farnesol		
0%	48.95 ± 2.11	1.0000
0.25%	59.75 ± 4.21	1.2206
0.5%	59.23 ± 3.18	1.2101
1.0%	57.98 ± 2.11	1.1681
1.5%	62.27 ± 3.78	1.2679
2.5%	64.63 ± 1.56	1.3204
Limonenoxide		
0%	48.95 ± 2.11	1.0000
0.25%	54.86 ± 3.55	1.1208
0.5%	51.00 ± 2.87	1.0420
1.0%	54.61 ± 2.33	1.1155
1.5%	57.43 ± 2.89	1.1733
2.5%	53.58 ± 1.99	1.0945

[a] ER_{sol} is the solubility enhancement ratio of diclofenac sodium.

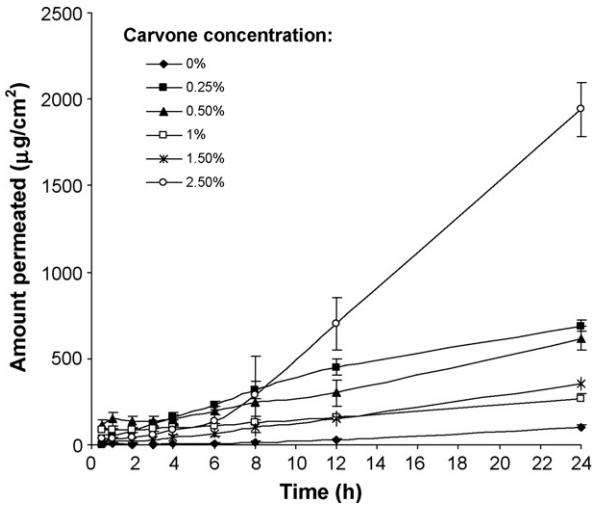

Fig. 5. Permeation profiles of diclofenac sodium in presence of different concentration of carvone (%, v/v) through rat skin.

(see also Table 2). The plot of ER versus concentration of the terpenes is shown in Fig. 7. The figure shows that although in all cases the greatest enhancement of the skin transport occurs at the highest concentration of the enhancer, there is no direct linear relationship between terpene concentration and the permeation rate. When the concentration of farnesol and carvone was increased from 0 to 0.5% the flux of diclofenac sodium increased. This was then followed by a decline in the activity at the higher concentration of 1% and a second increase when concentration was increased further to 2.5%. A similar trend has been observed for penetration enhancement activity of surfactants (Shokri et al., 2001; Nokhodchi et al., 2003) where the optimum concentration was attributed to the critical micelle concentration of surfactants. Okabe et al. (1989) observed similar abnormalities for the skin penetration of indomethacin where no explanation could be provided as to why there was a reduction in penetration enhancement at higher concentration of menthol. Abnormal changes in enhancement ratios have also been

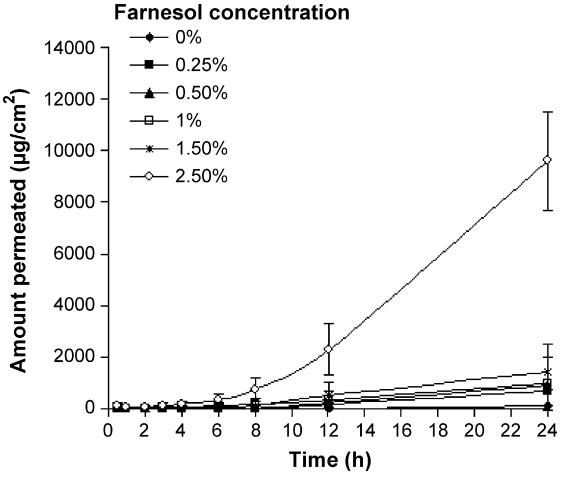

Fig. 4. Permeation profiles of diclofenac sodium in presence of different concentration of farnesol (%, v/v) through rat skin.

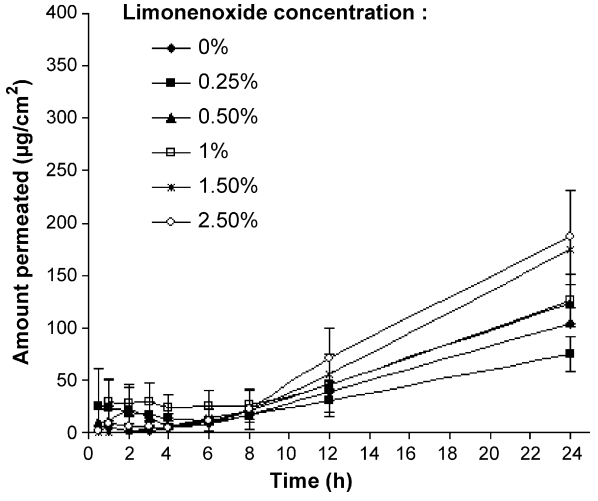

Fig. 6. Permeation profiles of diclofenac sodium in presence of different concentration of limonenoxide (%, v/v) through rat skin.

Table 2
Diclofenac sodium skin permeation parameters at the presence of each enhancer

Terpene (%, v/v)	Steady-state flux ($\mu g\,cm^{-2}\,h^{-1}$)	Kp ($\times 10^3\,cm\,h^{-1}$)	ER (k_{pa}/k_{pb})	Lag time (h)
Carvone				
0%	5.37 ± 1.07	0.110 ± 0.002	1.00	4.8
0.25%	24.50 ± 2.14	0.483 ± 0.010	4.40	0.0
0.5%	25.09 ± 2.45	0.459 ± 0.008	4.17	0.0
1.0%	22.09 ± 3.11	0.367 ± 0.006	3.34	0.0
1.5%	28.20 ± 4.22	0.453 ± 0.007	4.12	2.1
2.5%	101.24 ± 7.87	1.560 ± 0.024	14.18	5.0
Menthone				
0%	5.37 ± 1.07	0.110 ± 0.002	1.00	4.8
0.25%	4.27 ± 0.99	0.106 ± 0.003	0.96	3.2
0.5%	4.98 ± 1.21	0.096 ± 0.002	0.87	4.8
1.0%	8.04 ± 2.45	0.150 ± 0.003	1.36	4.3
1.5%	18.27 ± 4.23	0.315 ± 0.005	2.86	7.2
2.5%	21.67 ± 4.76	0.309 ± 0.004	2.81	5.2
Nerolidol				
0%	5.37 ± 1.07	0.110 ± 0.002	1.00	4.8
0.25%	15.23 ± 3.45	0.284 ± 0.005	2.58	5.8
0.5%	36.26 ± 8.42	0.667 ± 0.012	6.06	5.0
1.0%	353.85 ± 50.12	5.519 ± 0.090	50.17	4.7
1.5%	903.85 ± 385	14.501 ± 0.278	131.82	8.9
2.5%	1379 ± 422	21.780 ± 0.320	198.00	7.8
Farnesol				
0%	5.37 ± 1.07	0.110 ± 0.002	1.00	4.8
0.25%	43.12 ± 6.23	0.722 ± 0.012	6.56	7.0
0.5%	55.68 ± 6.76	0.940 ± 0.016	8.54	8.0
1.0%	48.54 ± 10.11	0.837 ± 0.014	7.61	4.0
1.5%	74.95 ± 18.54	1.220 ± 0.021	11.09	3.2
2.5%	552.64 ± 100.12	8.549 ± 0.137	77.72	6.8
Limonenoxide				
0%	5.37 ± 1.07	0.110 ± 0.002	1.00	4.8
0.25%	3.58 ± 0.74	0.065 ± 0.001	0.59	3.7
0.5%	6.35 ± 1.99	0.124 ± 0.002	1.13	4.1
1.0%	6.17 ± 1.92	0.113 ± 0.002	1.03	3.9
1.5%	9.53 ± 1.89	0.166 ± 0.003	1.51	5.8
2.5%	10.29 ± 3.11	0.192 ± 0.004	1.75	6.0

The values are mean and standard deviations of 3–6 determinations.

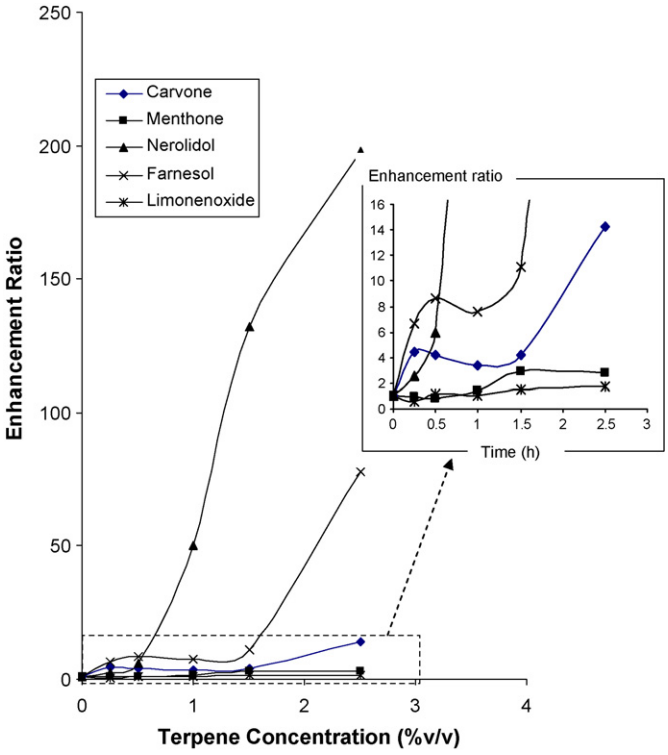

Fig. 7. The effect of terpenes concentration on the ER of diclofenac sodium through rat skin. The internal window is the higher magnification of the first small part of enhancement ratio (ER) vs. time.

observed for menthol and limonene in the skin transport of propranolol hydrochloride (Kunta et al., 1997). However, this was attributed to the limited solubility of hydrophobic terpenes in 40% (v/v) of ethanol solution used in the experiments. In our study we deliberately selected a vehicle that could dissolve all the terpenes.

With all the terpenes studied, the transport of diclofenac sodium was affected by increasing the enhancer concentration from 1.5 to 2.5%, with the exception of menthone and limonenoxide. It can be concluded from Fig. 7 that, in comparison with the other three terpenes, menthone and limonene oxide could be regarded as inactives. For carvone, nerolidol and farnesol, there is a slight enhancement at low concentrations followed by a rapid increase that starts at 1% for nerolidol and 1.5% for carvone and farnesol.

According to Table 2 at highest concentration of terpene (2.5%, v/v) the rank order of enhancement effect for diclofenac sodium is nerolidol > farnesol > carvone > methone > limonenoxide, whereas at low concentration (0.25%, v/v) the rank order was farnesol > carvone > nerolidol > menthone > limonenoxide. The most outstanding penetration enhancer was nerolidol, providing an almost 198-fold increase in permeability coefficient of diclofenac sodium, followed by farnesol with a 78-fold. In addition to providing the highest ER, nerolidol also provided the highest Q_{24} ($22{,}510 \pm 1450\,\mu g/cm^2$), followed by farnesol ($9597 \pm 1887\,\mu g/cm^2$). At the very low concentration of 0.25, however, Farnesol was the best enhancer with more than six-fold increase in the permeability coefficient of diclofenac. These enhancements cannot be explained by the solubility enhancements (ER_{sol}) (Table 1) as the enhancement ratios of solubility are quite low and in a similar range for all the terpenes. The enhancements could not be related to skin damage by the terpenes as they have been reported to be safe and non-toxic at high concentrations/or pure form (Goodman and Barry, 1988; Yamane et al., 1995). In experiments where skin was treated with pure terpene for 12 h prior to the permeation studies, Barry and co-workers showed using differential scanning calorimetry that the effect of terpenes (including nerolidol) on skin is reversible (Yamane et al., 1995). Terpenes seem to increase skin permeability mainly through the enhancement of permeant diffusion. Jain et al. (2002) reported that the contribution of partitioning in enhanced permeation of imipramine hydrochloride after terpene treatment at 5% (w/v) was negligible. Yamane et al. (1995), however, reported some increase in partitioning of 5-fluorouracil after 6 or 12 h treatment with certain terpenes (including nerolidol) whereas D-limonene produced no positive effect on the drug partitioning. Arellano et al. (1996) reported a 13.6-fold increase in the permeability coefficient of diclofenac sodium formulated

in carbopol gel when the gel contained 1% nerolidol. Table 2 shows 50.17-fold increase in diclofenac sodium permeability coefficient with the same concentration of nerolidol. The other constituents of the formulation and the enhancement synergism between the terpene and ethanol could be responsible for the different ER values. Ethanol is commonly used in many transdermal formulations and is often the solvent of choice for use in patches. As with water, ethanol permeates rapidly through human skin with a steady-state flux of approximately 1 mg cm^2/h (Berner et al., 1989). Ethanol has been used to enhance the flux of levonorgestrel, estradiol, hydro-cortisone and 5-fluoroucil through rat skin (Friend et al., 1988) and of estradiol through human skin in vivo (Pershing et al., 1990). However, it has been shown that when ethanol was used as a co-solvent with water, the enhancement effect of ethanol appears to be concentration dependent (Berner et al., 1989; Kurihara-Bergstrom et al., 1990; Megrab et al., 1995; Thomas and Panchagnula, 2003). For example, when the ratio of ethanol:water was increased upto 0.63 the permeation of salicylate ion through the rat skin increased, whereas higher levels of the ethanol decreased permeation (Kurihara-Bergstrom et al., 1990). It is possible that great enhancing effect of terpenes in presence of ethanol could be partly due to the enhancing effect of ethanol. The synergy between ethanol and cyclic monoterpene enhancers has been reported previously (Obata, 1991).

In this series of terpenes, nerolidol provided the best enhancement activity for diclofenac sodium permeation. In a study of the effects of terpenes, nerolidol was found to be the most effective enhancer in promoting the permeation of hydrocortisone through the skin among the series. Nerolidol is also an effective enhancer for the permeation of 5-fluorouracil through human abdominal skin (Cornwell and Barry, 1991). Cornwell and Barry (1991) attributed this to nerolidol possessing an amphiphile-like structure (higher partition coefficient) that was appropriate for the disruption of the lipid packing of the stratum corneum. Fig. 1 shows the molecular structure of the terpenes used in this study together with the corresponding logarhitim of partition coefficient (log P) values. It can be seen that nerolidol and farnesol have considerably higher log P values than the other three. It has been suggested that hydrophilic terpenes are more effective in enhancing the permeation of hydrophilic drugs, whereas, hydrophobic terpenes are more effective towards hydrophobic drugs (Moghimi et al., 1997; Gao and Singh, 1998; Okabe et al., 1989; Kunta et al., 1997; El-Kattan et al., 2000; Hori et al., 1991).

Nerolidol and farnesol share another important structural feature that might be the reason behind the higher enhancement activity: the presence of alcoholic OH group that is capable of hydrogen bonding. The possibility of complex formation between penetrant and terpene enhancers has been studied by Moghimi et al. (1998) for 5-fluorouracil as the penetrant. Diclofenac sodium also possesses hydrogen bonding group (carboxylic acid and amine groups, Fig. 1) capable of interaction with OH group of nerolidol and farnesol.

Therefore, two mechanisms of permeation enhancement are possible here: increased lipid disruption in the stratum corneum by terpenes and the complex formation between the enhancer and drug or structures from stratum corneum (Williams and Barry, 1989, 1991).

Table 2 also shows that while the addition of the enhancer increased diclofenac sodium flux, diffusional lag times were not reduced. For example, an increase in concentration of nerolidol in the solution from 0 to 2.5% resulted in 198-fold increase in permeation rate and the lag time increased from 4.8 to 7.3 h. It is likely that increased lag times were due to gradual increases in membrane permeability produced by the distribution of the enhancer within the stratum corneum and consequently a conditioning of the membrane in early stages of the diffusion process. Similar delayed onsets of action have been reported with some other enhancers (Komata et al., 1992; Santoyo et al., 1995).

In order to investigate the effect of terpene in real formulation, diclofenac sodium was formulated as gel in presence and absence of 2.5% nerolidol. These were compared with a commercial diclofenac diethylamine product (Fig. 8). The figure shows that the presence of terpene resulted in a profound increase in permeation of diclofenac sodium from the gel formulation. Moreover, gel formulation containing 2.5% (w/w) nerolidol has a higher flux value than the commercial gel. This is despite the fact that the commercial product contains diethylamine salt of diclofenac which, due to the higher lipophilicity of the diethyl amine group, is expected to permeate more readily than the sodium salt used in our formulations. The flux values for the commercial gel and the gel formulation containing nerolidol were 1.48 and 5.96 $\mu g\,cm^{-2}\,h^{-1}$, respectively. These are considerably lower than the flux values of diclofenac from the liquid formulation containing 2.5% (w/v) nerolidol (flux 45.63 $\mu g\,cm^{-2}\,h^{-1}$). The higher flux of liquid over gel formulations could be explained considering the effect of viscosity on drug release. It has been shown that viscosity is one of the most important variables controlling the release rate of drugs from formulations (Fang et al., 2003; Woolfson et al., 2000). Viscosity of the vehicles used in this study was 2.4 and 350 cP for

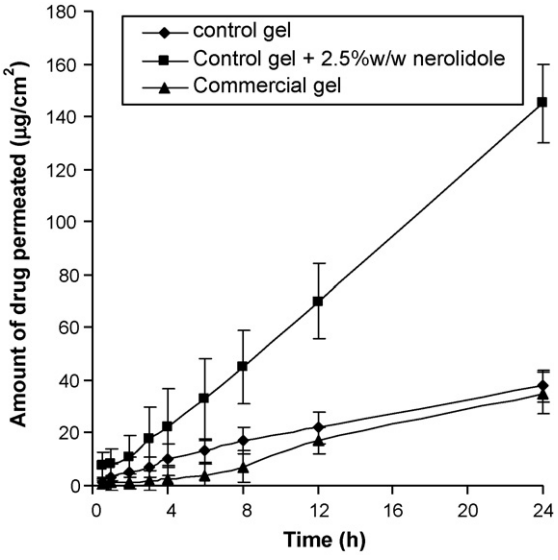

Fig. 8. Permeation profiles of diclofenac sodium from commercial gel, control gel and gel containing 2.5% nerolidol.

the solution and gel products, respectively. The viscosity of the gel matrix may play an important role in controlling the release of the drug from the formulation which precedes the penetration into the receptor phase. It must be emphasized that, apart from viscosity, there are other formulation factors that might be responsible for the differences observed in the flux of drug from the two different vehicles containing similar concentrations of nerolidol. For example absence of ethanol in the gel formulation could have reduced the enhancement ratio by nerolidol due to the reasons explained earlier.

4. Conclusion

The present study showed that the nature of the terpene enhancer exerts an important influence on cutaneous barrier impairment. Significantly high flux values were obtained when using nerolidol as the penetration enhancer. The second most effective enhancer was farnesol. Nerolidol and farnesol share two important structural characteristics; both are alcoholic (as opposed to the ether or keton structures of the other terpenes used in this study) and they are highly lipophilic with log P values of 5.3. Either of these properties or both could be responsible for the high penetration enhancement effect. The effect of terpenes on the drug solubility was not significant suggesting that the enhancement activity can only be attributed to increase in the drug diffusion and partitioning into skin. Although for all the terpenes studied the highest absorption rate was achieved when the highest concentration (2.5%) was used, a direct relationship between terpene concentration and the permeation rate could not be established for some of the terpenes.

References

Arellano, A., Santoyo, S., Martin, C., Ygartua, P., 1996. Enhancing effect of terpenes on the in vitro percutaneous absorption of diclofenac sodium. Int. J. Pharm. 130, 141–145.

Ashton, P., Hadgraft, J., Walters, K.A., 1986. Effect of surfactants in percutaneous absorption. Pharm. Acta Helv. 61, 228–235.

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

Berner, B., Mazzenga, G.C., Otte, J.H., Steffens, R.J., Juang, R.H., Ebert, C.D., 1989. Ethanol: water mutually enhanced transdermal therapeutic system: II. Skin permeation of ethanol and nitroglycerin. J. Pharm. Sci. 78, 402–407.

Cornwell, P.A., Barry, B.W., 1991. Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. J. Pharm. Pharmacol. 46, 261–269.

El-Kattan, A.F., Asbill, C.S., Michniak, B.B., 2000. The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems. Int. J. Pharm. 198, 179–189.

Fang, J.Y., Hwang, T.L., Leu, Y.L., 2003. Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels. Int. J. Pharm. 250, 313–325.

Friend, D., Catz, P., Heller, J., Reid, J., Baker, R., 1988. Transdermal delivery of levonorgestrel I. Alkanols as permeation enhancers. J. Controlled Release 7, 243–250.

Gao, S., Singh, J., 1998. In vitro percutaneous absorption enhancement of lipophilic drug tamoxifien by terpenes. J. Controlled Release 51, 193–199.

Ghafourian, T., Hamishkar, H., Zandasrar, P., Nokhodchi, A., 2004. The effect of penetration enhancers on drug delivery through skin: a QSAR study Skin QSAR. J. Controlled Release 99, 113–125.

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

Hori, M., Satoh, S., Maibach, H.I., Gug, R.H., 1991. Enhancement of propranolol hydrochloride and diazepam skin absorption in vitro: effect of enhancer lipophilicity. J. Pharm. Sci. 80, 32–35.

Jain, A.K., Thomas, N.S., Panchagnula, R., 2002. Transdermal drug delivery of imipramine hydrochloride. I. Effect of terpenes. J. Controlled Release 79, 93–101.

Komata, Y., Inoaka, M., Kaneko, A., Fujie, T., 1992. In vitro percutaneous absorption of thiamine disulfide from a mixture of propylene glycol and fatty acid. J. Pharm. Sci. 81, 744–746.

Kunta, J.R., Goskonda, V.R., Brotherton, H.O., Khan, M.A., Reddy, I., 1997. Effect of menthol and related terpenes on the percutaneous absorption of propranolol across excised hairless mouse skin. J. Pharm. Sci. 86, 1369–1373.

Kurihara-Begstrom, T., Flynn, G.I., Higuchi, W.I., 1987. Physicochemical study of percutaneous-absorption enhancement by dimethylsufoxide mediation of vidarabine (Ara-A) permeation of hairless mouse skin. J. Invest. Dermatol. 89, 274–280.

Kurihara-Bergstrom, T., Knutson, K., De Noble, L.J., Goates, C.Y., 1990. Percutaneous absorption enhancement of an ionic molecule by ethanol–water systems in human skin. Pharm. Res. 7, 762–766.

Iwasa, A., Irimoto, K., Kasai, S., Okuyama, H., Nagai, T., 1991. Effect of nonionic surfactants on percutaneous absorption of diclofenac sodium. Yakuzaigaaku 51, 16–21.

Manasse, R., Hedwall, P.R., Kraetz, J., Pericin, C., Riesterer, J., Sallmann, A., Ziel, R., Jaques, R., 1978. Pharmacological properties of diclofenac sodium aand its metabolites. Scand. J. Rheumatol. Suppl. 22, 5–16.

Megrab, N.A., Williams, A.C., Barry, B.W., 1995. Oestradiol permeation across human skin, silastic and snake skin membranes: the effects of ethanol/water co-solvent systems. Int. J. Pharm. 116, 101–112.

Moghimi, H., Williams, A., Barry, B.W., 1996. A lamellar matrix for stratum corneum intercellular lipids. IV. Effects of terpene penetration enhancers on the permeation of 5-fluorouracil and oestradiol through the matrix. Int. J. Pharm. 145, 49–59.

Moghimi, H., Williams, A., Barry, B.W., 1997. A lamellar matrix for stratum corneum intercellular lipids. V. Effects of terpene penetration enhancers on the structure and thermal behaviour of the matrix. Int. J. Pharm. 146, 41–54.

Moghimi, H.R., Williams, A.C., Barry, B.W., 1998. Enhancement by terpenes of 5-fluorouracil permeation through the stratum corneum: model solvent approach. J. Pharm. Pharmacol. 50, 955–964.

Naito, S., Tominagaa, H., 1985. Percutaneous absorption of diclofenac sodium ointment. Int. J. Pharm. 24, 115–124.

Nishihata, T., Kamada, A., Sakai, K., Takahashi, K., Matsumoto, K., Shinozaki, K., Tabata, Y., Keigami, M., Miyagi, T., Tatsumi, N., 1988. Percutanouse absorption of diclofenac in rat and humans: aqueous gel formulation. Int. J. Pharm. 46, 1–7.

Nishihata, T., Kotera, K., Nakano, Y., Yamazaki, M., 1987. Rat percutaneous transport of diclofenac and influence of hydrogenated soya lecithin. Chem. Pharm. Bull. 35, 3807–3812.

Nokhodchi, A., Nazemiyeh, H., Ghafourian, T., Hassan-Zadeh, D., Valizadeh, H., Bahary, L.A.S., 2002. The effect of glycerrhizin on the release rate and skin penetration of diclofenac sodium from topical formulations. Il Farmaco 57, 883–888.

Nokhodchi, A., Shokri, J., Dashbolaghi, A., Hassan-Zadeh, D., Ghafourian, T., Barzegar-Jalali, M., 2003. The enhancement effect of surfactants on the penetration of lorazepam through rat skin. Int. J. Pharm. 250, 359–369.

Obata, Y.T., 1991. Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des. Deliv. 8, 137–144.

Obata, Y., Takayama, M., Maitani, Y., Machida, Y., Nagai, T., 1993. Effect of ethanol on skin permeation of nonionized and ionized diclofenac. Int. J. Pharm. 89, 191–198.

Okabe, H., Takayama, K., Ogura, A., Nagai, T., 1989. Effect of limonene and related compounds on the percutaneous absorption of indomethacin. Drug Des. Deliv. 4, 313–321.

Okabe, H., Obata, Y., Takayama, K., Nagai, T., 1990. Percutaneous absorption enhancing effect and skin irritation of monocyclic monoterpenes. Drug Des. Deliv. 6, 229.

Opdyke, D., 1979. Monographs on fragrance raw materials. Food Cosmet. Toxicol. 1, 11–17.

Pershing, L.K., Lambert, L.D., Knutson, K., 1990. Mechanism of ethanol-enhanced estradiol permeation across human skin in vivo. Pharm. Res. 7, 170–175.

Pfister, W., Dean, S., hsieh, S., 1990. Permeation enhancers compatible with transdermal drug delivery systems. I. Selection and formulation considerations. Pharm. Tech. 8, 132–140.

Santoyo, S., Arellano, A., Ygartua, Y., Martin, C., 1995. Penetration enhancer effects on the invitro percutaneous absorption of piroxicam through rat skin. Int. J. Pharm. 117, 509–511.

Shokri, J., Nokhodchi, A., Dashbolaghi, A., Hassan-Zadeh, D., Ghafourian, T., Barzegar-Jalali, M., 2001. The effect of surfactants on the skin penetration of diazepam. Int. J. Pharm. 228, 99–107.

Takahashi, K., Tamagawa, S., Katagi, T., Yoshitomi, H., Kamada, H., Rytting, J., Iwasa, A., Irimoto, K., Kasai, S., Okuyama, H., Nagai, T., 1991a. Effect of nonionic surfactants on percutaneous absorption of diclofenac sodium. Yakuzaigaaku 51, 16–21.

Takahashi, K., Tamagawa, S., Katagi, T., Yoshitomi, H., Kamada, A., Rytting, J., Nishihata, T., Mizuno, N., 1991b. In vitro percutaneous transport of sodium diclofenac and diclofenac from oleaginous vehicle. Chem. Pharm. Bull. 39, 509–511.

Thomas, N.S., Panchagnula, R., 2003. Transdermal delivery of zidovudine: effect of vehicles on permeation across rat skin and their mechanism of action. Pharm. Sci. 18, 71–79.

Tsuzuki, N., Wong, O., Higuchi, T., 1988. Effect of primary alcohols on percutaneous-absorption. Int. J. Pharm. 46, 19–23.

Williams, A.C., Barry, B.W., 1989. Essential oils as novel human skin penetration enhancers. Int. J. Pharm. 57, R7–R9.

Williams, A.C., Barry, B.W., 1991. Terpenes and the lipid protein partitioning theory of skin penetration enhancement. Pharm. Res. 8, 17–24.

Woolfson, A.D., Malcolm, R.K., Campbell, K., Jones, D.S., Russell, J.A., 2000. Rheological, mechanical and membrane penetration properties of novel dual drug systems for percutaneous delivery. J. Controlled Release 67, 395–408.

Wong, O., Tsuzuki, N., Nghiem, B., Kuehnhoff, J., Itoh, T., Masaki, K., Huntington, J., Konishi, R., Rytting, J.H., Higuchi, T., 1989. Unsaturated cyclic areas as new non-toxic biodegradable transdermal permeation enhancers. 2. Evaluation study. Int. J. Pharm. 52, 191–201.

Yamane, M.A., Williams, A.C., Barry, B.W., 1995. Effects of terpenes and oleic acid as skin penetration enhancers towards 5-fluorouracil as assessed with time; permeation, partitioning and differential scanning calorimetry. Int. J. Pharm. 116, 237–251.

Yasukawa, T., Akiyoshi, Y., Hattori, A., Harada, S. Anti-inflamatory topical formulations containing indomethacin and diclofenac. Jpn. Kokai Tokkio Koho JP 60146823 A2, Aug. 22nd, 1985.