ELSEVIER

European Journal of Pharmaceutical Sciences 12 (2001) 195–203

EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES

www.elsevier.nl/locate/ejps

# Capsaicin and nonivamide as novel skin permeation enhancers for indomethacin

Jia-You Fang[a,b,*], Chia-Lang Fang[c], Chi-Tzong Hong[d], Hsiang-Yin Chen[a,b], Tzu-Ying Lin[a], Hsiu-Mei Wei[a]

[a]*Graduate Institute of Pharmaceutical Sciences, Taipei Medical College, 250 Wu-Hsing Street, Taipei, Taiwan*
[b]*Research Center for Clinical Pharmacy, Taipei Municipal Wan Fang Hospital, Taipei, Taiwan*
[c]*Department of Pathology, Taipei Medical College, Taipei, Taiwan*
[d]*Department of Neurology, Taipei Municipal Wan Fang Hospital, Taipei, Taiwan*

Received 22 February 2000; received in revised form 15 May 2000; accepted 23 June 2000

**Abstract**

The study was conducted in vitro to investigate the changes of indomethacin transdermal permeation pretreated by capsaicin and nonivamide, two compounds chemically similar to Azone. The combined effect of low frequency ultrasound (20 kHz) and enhancers on the indomethacin permeation was also evaluated. The experimental data demonstrated that capsaicin and nonivamide significantly enhanced the flux of indomethacin across nude mouse skin. Enhancement effects of both analogues were very similar and depended predominantly on the concentration tested. Histological examination coupled with visual scores indicated the safety of capsaicin and nonivamide on skin structure. Simultaneous application of ultrasound and enhancers significantly increased skin permeation of indomethacin compared with either ultrasound or enhancers alone. Better effect was obtained by the combination with capsaicin than nonivamide. 

*Keywords:* Indomethacin; Capsaicin; Nonivamide; Skin permeation; Enhancer; Ultrasound

## 1. Introduction

Many investigators have examined the possibility of the percutaneous delivery of drugs, but a major difficulty is the impermeability of stratum corneum. An effective method employed to reduce this effect of diffusional barrier is to use permeation enhancers. One of the most promising permeation enhancers is Azone (laurocapram, 1-dodecylazacycloheptan-2-one). It is non-irritant to human skin and hardly absorbed through skin (Niazy, 1996). Azone consists of a seven-membered ring and a long hydrocarbon side chain which appears to act by reducing the diffusional barrier of the stratum corneum by inserting itself into the structured lipids located in the intercellular routes. This fluidity of microenvironment of lipids is reduced and the permeation of drugs enhanced (Phillips and Michniak, 1995).

Capsaicin (8-methyl *N*-vanillyl-6-nonenamide; $C_{18}H_{27}NO_3$), the pungent principle of red pepper, has a variety of therapeutic advantages such as antinociceptive, hypotension and hypolipidemia activities (Monsereenusorn et al., 1982; Fang et al., 1995). Besides the pharmacological actions, capsaicin itself can act as a skin permeation enhancer for naproxen because of the similarity of chemical structure to Azone (Degim et al., 1999). Nonivamide (*N*-nonanoyl vanillylamide; $C_{17}H_{27}NO_3$) is one of the synthetic analogues of capsaicin which has the similar chemical structure and pharmacological profiles to those of capsaicin (Hayes et al., 1984). The main difference in structures of capsaicin and nonivamide is in the long alkyl chain. A side chain of $-CH_3$ and a double bond in alkyl chain is observed in capsaicin but not in nonivamide. It can be a substitute of capsaicin because of the lower cost than capsaicin extracted from natural products. In our previous study, the incorporation of nonivamide could increase the in vivo transdermal permeation of sodium nonivamide acetate (Fang et al., 1996a,b).

The aim of this study is to investigate the effect of capsaicin and nonivamide on the in vitro transdermal permeation of indomethacin. The present study also quan-

*Corresponding author. Tel.: +886-2-2736-1661; fax: +886-2-2707-4804.

*E-mail address:* fajy@ms9.tisnet.net.tw (J.-Y. Fang).

0928-0987/01/$ – see front matter 
PII: S0928-0987(00)00118-4

tifies the skin irritation and damage produced by application of these enhancers on skin. The developed method presented in this study is able to distinguish between epidermal and dermal changes in histological structure, thereby allowing differentiation between mild and moderate irritants. This study also examines the combined effect of low frequency ultrasound (20 kHz) application together with capsaicin or nonivamide on the skin permeation of indomethacin. Different methods of transdermal enhancement have been found to increase drug delivery via different mechanisms. Since ultrasound and enhancers can each individually increase transdermal drug delivery by different mechanisms (Johnson et al., 1996), it is hypothesized that the combination of ultrasound and enhancers may result in a greater enhancement than that resulting from each method alone.

## 2. Materials and methods

### 2.1. Materials

Indomethacin was purchased from Sigma Chemical Co. (USA). Capsaicin was supplied by Wako Chemical Co. (Japan). Nonivamide was obtained from Tokyo Kasei Co. (Japan). Propylene glycol (PG) was supplied by Nihon Shiyaku Co. (Japan). All other chemicals and solvents were of analytical grade.

### 2.2. In vitro permeation experiments

The diffusion cell used in present in vitro study was Franz vertical diffusion assembly. The dorsal skin of female nude mouse (10–12 weeks old) was used as the barrier membrane. One milliliter of enhancer with different concentrations (1, 3 and 5%) in ethanol/water or PG/water (1:1, v/v) vehicle was applied to the skin surface for 2 h by the occlusion dressing technique. The applied area was then gently swabbed clean with cotton and rinsed with distilled water. One milliliter of indomethacin (1%, w/v) in ethanol/pH 7.4 buffer (1:1, v/v) was then applied to the treated skin. The area of the skin available for permeation was 2 $cm^2$. The donor compartment was covered with parafilm. The receptor medium (10 ml) was composed of 50% ethanol and 50% pH 7.4 buffer. The receptor compartment was kept at a constant temperature of 37°C and stirred by a magnetic stirrer at 600 rpm. Samples (0.3 ml) were withdrawn from the receptor at regular intervals and an equal volume of fresh receptor solution was added. Samples were analyzed using HPLC system described previously (Huang et al., 1995).

### 2.3. Application of low frequency ultrasound

The nude mouse skin was also pretreated by low frequency ultrasound for 2 h prior to in vitro skin permeation experiment. Ultrasound was applied with a

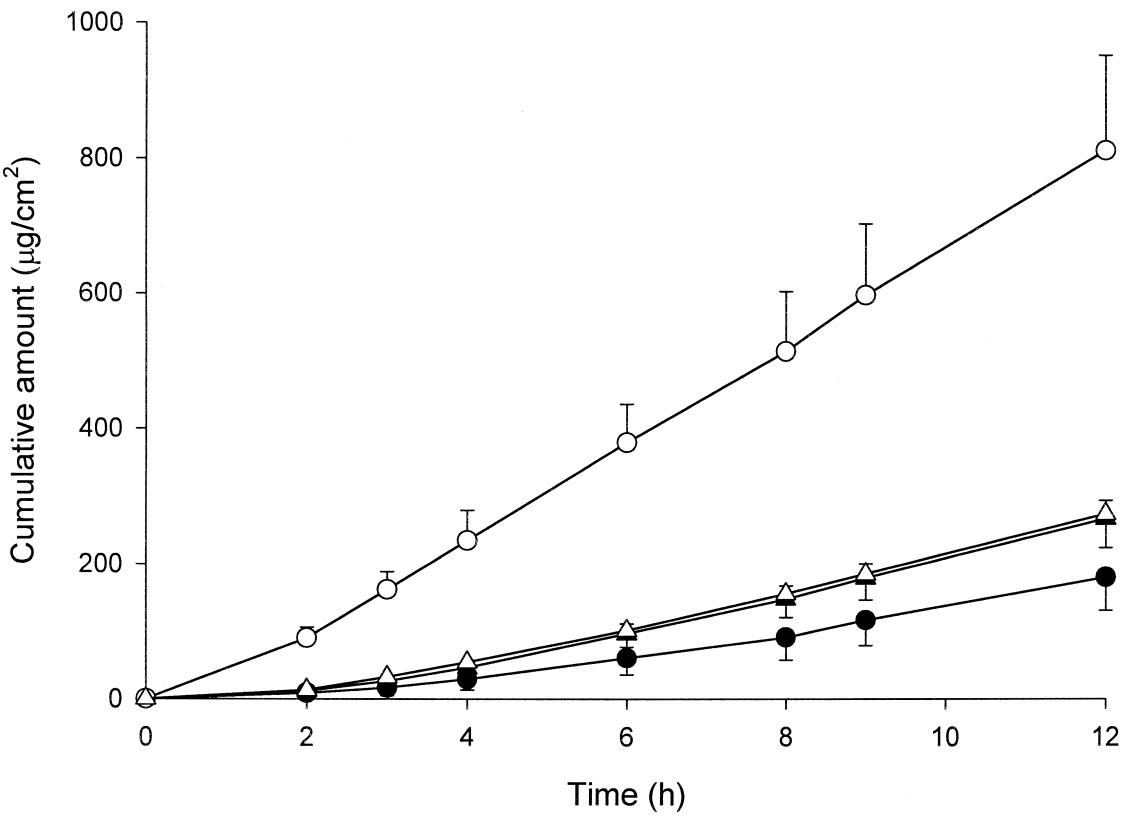


Fig. 1. Cumulative amount of indomethacin detected in the receptor compartment versus time following pretreatment with 3% enhancers in ethanol/water (1:1, v/v) vehicle: (●) control group, (○) Azone, (▲) capsaicin, (△) nonivamide. All data represent the means of three experiments±S.D.

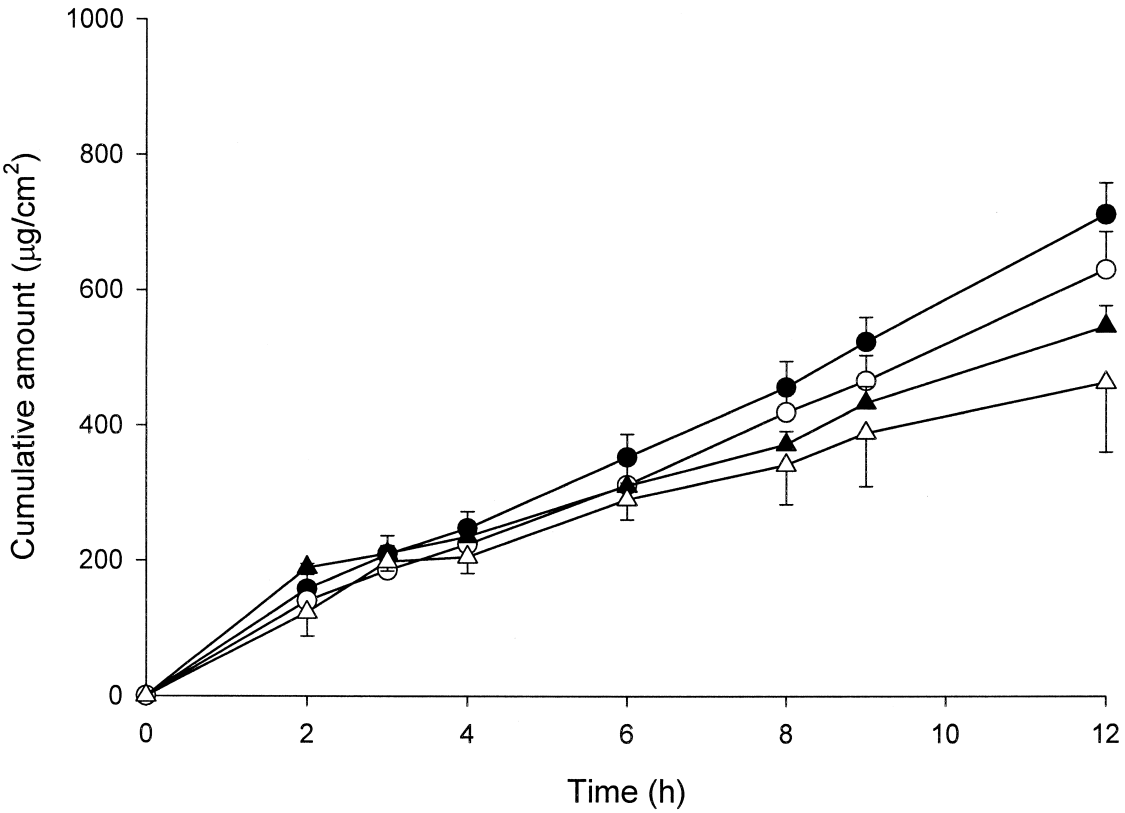


Fig. 2. Cumulative amount of capsaicin and nonivamide in the receptor compartment versus time after pretreatment in various vehicles: (●) 3% capsaicin in ethanol/water (1:1, v/v), (○) 3% nonivamide in ethanol/water (1:1, v/v), (▲) 3% capsaicin in PG/water (1:1, v/v), (△) 3% nonivamide in PG/water (1:1, v/v). All data represent the means of three experiments±S.D.

sonicator (VCX 600, Sonics and Materials, USA) with a transducer. The radiating diameter of transducer was 13 mm. The frequency was set at 20 kHz, and the estimated skin intensity was 0.2 W/cm$^2$. The ultrasound transducer was located approximately 0.5 cm from stratum corneum.

### *2.4. Histological examination of skin*

Histological changes in the nude mouse skin were examined after enhancers pretreatment. A hemispherical glass reservoir with the available diffusion area of 2 cm$^2$ was placed on the donor side of nude mouse skin adhered by Superglue. One milliliter of enhancer formulation was added into the reservoir. Immediately after pretreatment, specimen of the exposed area was taken for histological examination. The adjacent untreated skin area was also examined as the control group. Each specimen was fixed in 10% pH 7.4 buffered formaldehyde solution for at least 48 h. The specimen was cut vertically against skin surface. Each section was dehydrated using ethanol and then embedded in paraffin wax, stained with hematoxylin and eosin. In each skin samples, three different sites were examined and evaluated under an optiphoto light microscopy. A scoring system as described previously was employed (Lashmar et al., 1989; Phillips and Michniak, 1995).

## 3. Results

### *3.1. The permeation of indomethacin after pretreatment of azone, capsaicin and nonivamide*

The enhancing effect of 3% Azone, capsaicin and nonivamide on the permeation of indomethacin is shown in Fig. 1 as the cumulative amount–time profiles. The slopes of the resulting plot from 0 to 12 h was computed and the flux (μg/cm$^2$/h) was calculated from the slope. The skin flux of indomethacin pretreated by Azone, capsaicin and nonivamide in ethanol/water (1:1, v/v) vehicle was 69.32±11.35, 23.24±2.47 and 23.74±4.35 μg/cm$^2$/h individually, which was significantly higher (*t*-test, $P<0.05$) than that of control group (15.47±4.13 μg/cm$^2$/h). The cumulative amounts of capsaicin and nonivamide in the receptor compartment after pretreatment are shown in Fig. 2. It indicated that capsaicin and nonivamide greatly permeated across the skin. The flux of capsaicin (56.54±3.33 μg/cm$^2$/h) was slightly higher than that of nonivamide (50.41±4.73 μg/cm$^2$/h) which was similar to our previous report (Tsai et al., 1994).

The permeation of indomethacin after pretreatment by Azone, capsaicin and nonivamide in PG/water (1:1, v/v) vehicle was examined. It was demonstrated obviously in Fig. 3 that capsaicin and nonivamide exhibit no enhancing effect on indomethacin permeation when using PG/water

(1:1, v/v) was used as the pretreatment vehicle. Fig. 2 also shows that the permeation of capsaicin and nonivamide in PG/water is significantly lower (*t*-test, $P<0.05$) than that in ethanol/water.

### 3.2. Pretreatment of capsaicin and nonivamide with various concentrations

The flux of indomethacin pretreated by ethanol/water vehicle in the presence of 1, 3 or 5% (w/v) capsaicin or nonivamide is shown in Table 1. The permeation of indomethacin increased as the concentration of enhancers increased. The flux of capsaicin and nonivamide across skin after pretreatment at different concentrations was also shown in Fig. 4. There was an ascendant trend for capsaicin or nonivamide to increase its flux from the concentration of 1–5%, however, no significant change (*t*-test, $P>0.05$) in the flux from 3 to 5% was observed.

The microscopic appearance of nude mouse skin treated with 3% capsaicin and nonivamide was shown in Fig. 5. There was almost no change observed in the anatomical structure of skin after treatment as compared with control group.

### 3.3. The permeation of indomethacin incorporated with capsaicin or nonivamide in donor

To measure the influence of enhancers on the thermodynamic activity and permeability of drug across skin, transdermal permeation of indomethacin was determined by incorporating 3% capsaicin or nonivamide in the donor of in vitro diffusion cell. The indomethacin flux was significantly enhanced after incorporating capsaicin or nonivamide into the donor cell as compared to the control group (Fig. 6). However, no significant difference (*t*-test, $P>0.05$) was detected between the permeation flux enhanced by capsaicin and nonivamide.

Table 1
Flux of indomethacin after pretreatment of capsaicin or nonivamide with various concentrations (mean±S.D., $n=3$)

| Enhancer concentration (%) | Capsaicin | | Nonivamide | |
|---|---|---|---|---|
| | Flux | ER | Flux | ER |
| 0 | 15.47±4.13 | – | 15.47±4.13 | – |
| 1 | 17.88±6.43 | 1.16 | 19.87±2.48 | 1.28 |
| 3 | 23.24±2.47 | 1.50 | 23.74±4.35 | 1.53 |
| 5 | 34.47±11.51 | 2.23 | 31.57±4.77 | 2.04 |

### 3.4. Combined effect of ultrasound and enhancers

Table 2 shows the effect of pretreating the skin with enhancer and 0.2 W/cm$^2$ ultrasound followed by application of the indomethacin-containing vehicle. The result demonstrated that the low frequency ultrasound alone was effective to enhance the permeation of indomethacin, however, significant difference (*t*-test, $P>0.05$) was ob-

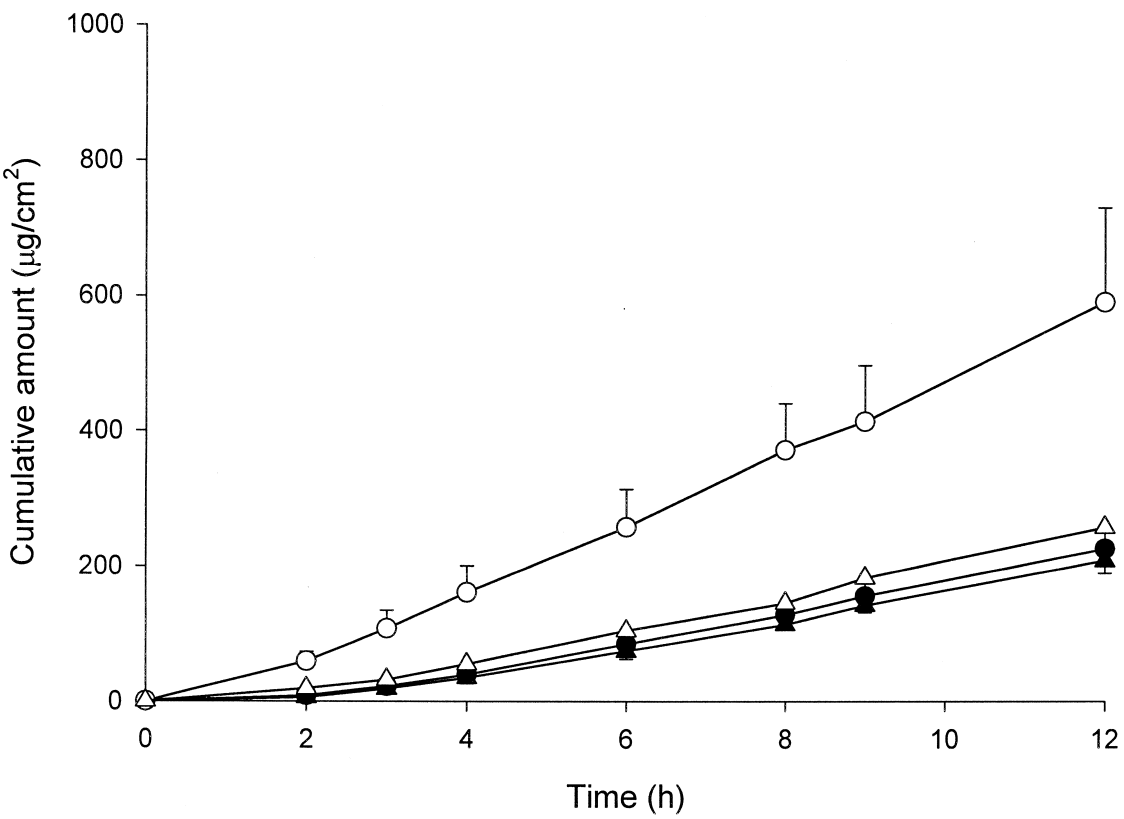


Fig. 3. Cumulative amount of indomethacin detected in the receptor compartment versus time following pretreatment with 3% enhancers in PG/water (1:1, v/v) vehicle: (●) control group, (○) Azone, (▲) capsaicin, (△) nonivamide. All data represent the means of three experiments±S.D.

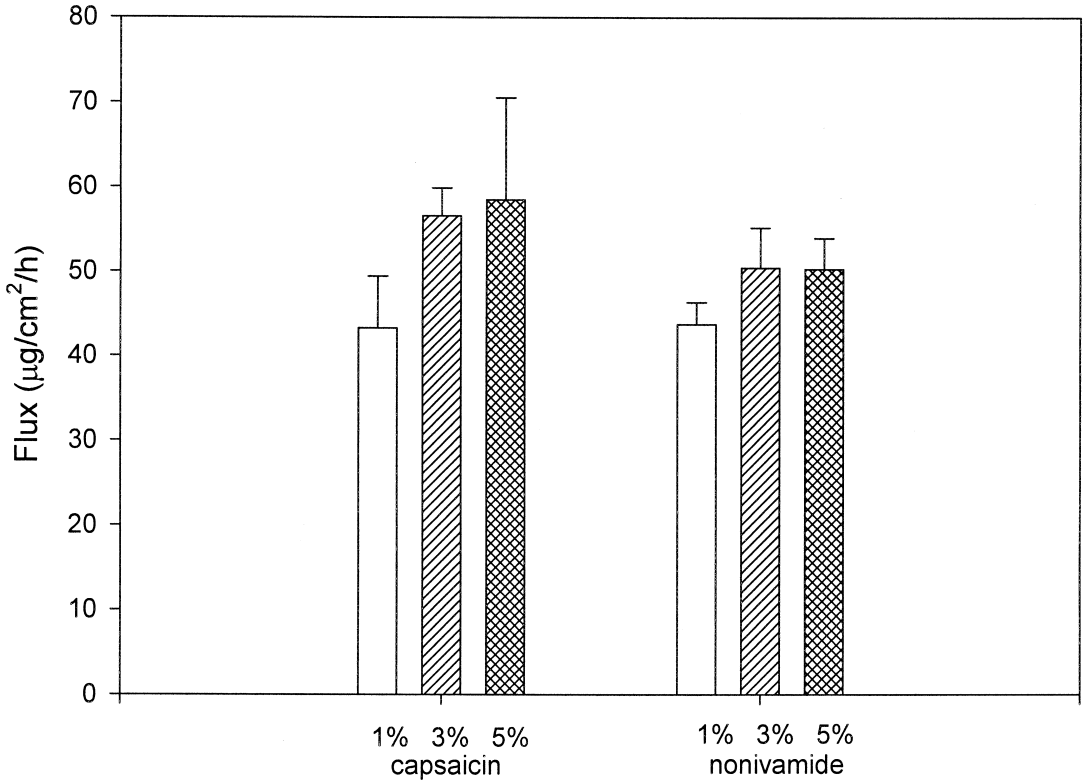


Fig. 4. Flux of capsaicin and nonivamide after pretreatment with various concentrations. All data represent the means of three experiments±S.D.

served. Ultrasound also increased the indomethacin permeation when combined with all the enhancer formulations examined. As shown in Fig. 7, both of the flux of capsaicin and nonivamide was increased after simultaneous application of ultrasound.

## 4. Discussion

### *4.1. The permeation of indomethacin after pretreatment of Azone, capsaicin and nonivamide*

The enhancing effect of Azone, capsaicin and nonivamide on the permeation of indomethacin is shown in Fig. 1 as the cumulative amount–time profiles. The result demonstrated that the three compounds were capable of enhancing the permeation of indomethacin across nude mouse skin. Because of the structural likeness in molecular size, it has been suggested that the enhancers may share with a similar mechanism (Degim et al., 1999). It was thought that Azone might intercalate into the ceramide matrices and create disruption in their stacking (Phillips and Michniak, 1995; Hadgraft, 1999). It consequently decreased the diffusion resistance of the stratum corneum. Lower enhancing effect of capsaicin and nonivamide may be due to the different partition behavior with Azone. Even though with similar chemical structures, the *n*-octanol/water partition coefficient of Azone was significantly higher than that of capsaicin and nonivamide (Tsai et al., 1994; Degim et al., 1999). Thus it was deduced that the amount of Azone retained in the stratum corneum might be far more than that of capsaicin and nonivamide, resulting in a greater disruption of lipid matrices. Previous studies had demonstrated that Azone did not permeate deeply into the dermis (Ogiso et al., 1992; Phillips and Michniak, 1995). The data in current study showed that capsaicin and nonivamide greatly permeated across the skin (Fig. 2).

The enhancing effects of capsaicin and nonivamide on indomethacin permeation are comparable (Fig. 1). It is because the similarity of their chemical structures. The same result was observed in the permeation of capsaicin and nonivamide after pretreatment, although the flux of capsaicin was slightly higher than that of nonivamide.

The contents of pretreatment vehicles used in this present study, water, ethanol and PG, have been reported and demonstrated partial effect on skin permeation (William and Barry, 1992; Hadgraft, 1999). The indomethacin fluxes in this study showed no significant difference (ANOVA test, $P>0.05$) with or without pretreatment with vehicles (Table 3), indicating water, ethanol and PG did not exert the enhancing effect. The permeation of capsaicin and nonivamide when using PG/water (1:1, v/v) as the pretreatment vehicle was significantly lower (*t*-test, $P<0.05$) than that from ethanol/water. This result may verify the inactivity of capsaicin and nonivamide in PG/water on permeation of indomethacin. It may be due to the lower solubility of capsaicin and nonivamide in PG/water vehicle (suspension type) than that in ethanol/water vehicle (solution type), resulting in the poorer efficiency of capsaicin and nonivamide partitioned from PG/water vehicle into the skin.

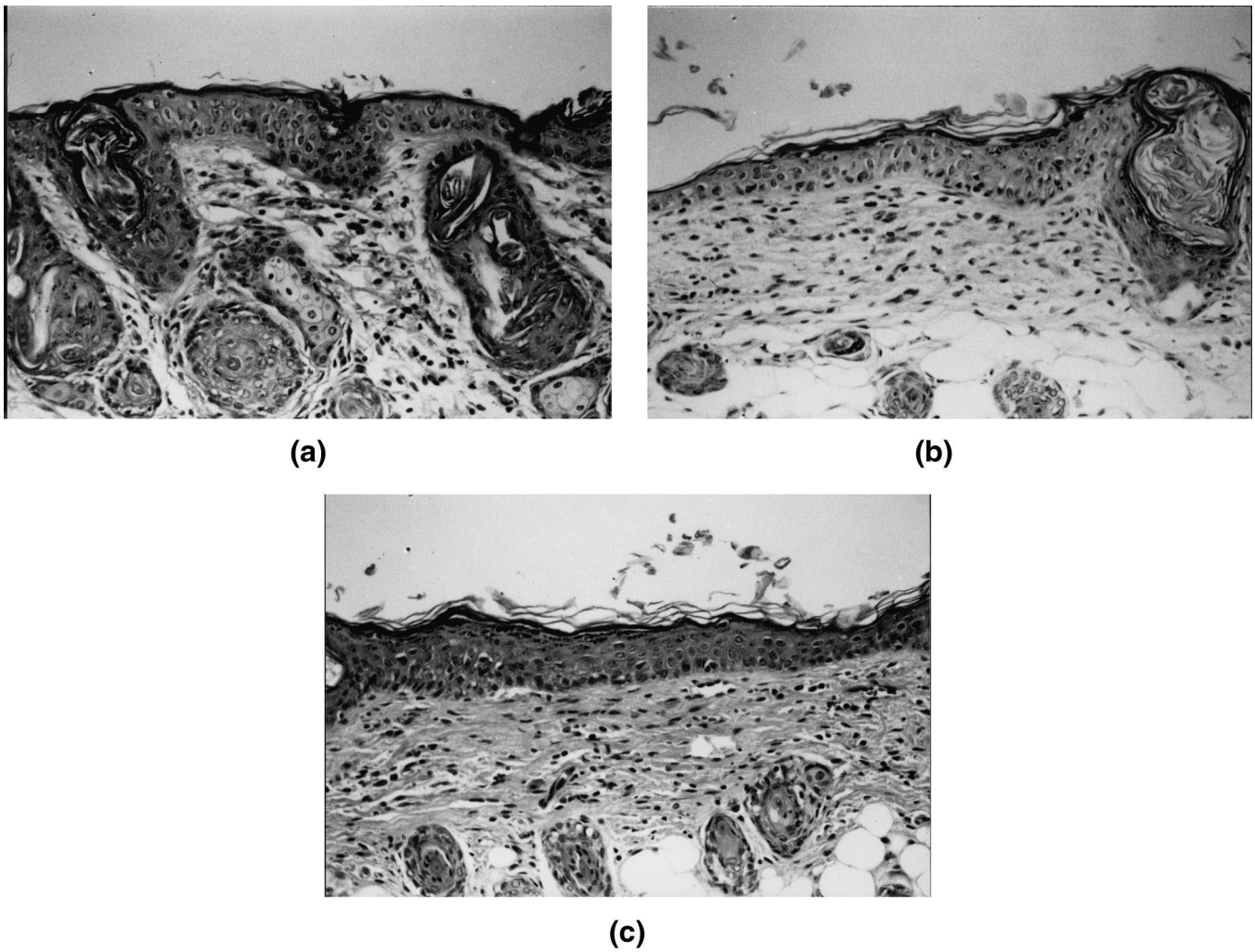


Fig. 5. Microscopic photographs of nude mouse skin after treatment of 3% enhancers in ethanol/water (1:1, v/v) vehicle for 2 h: (A) non-treatment, (B) capsaicin, (C) nonivamide (original magnification ×200).

### *4.2. Pretreatment of capsaicin and nonivamide with various concentrations*

The permeation of indomethacin increased with increasing the concentration of enhancers. After calculating the correlation coefficient ($r$) between enhancer concentration and indomethacin flux, a linear relationship ($r=0.98$ for capsaicin; $r=0.99$ for nonivamide) was observed. It can be concluded that the concentration of capsaicin and nonivamide used is extremely important in enhancing indomethacin permeation.

The degree of skin irritation caused by the topical application of capsaicin and nonivamide was evaluated in this study. Initial histological examination coupled with visual observation was performed to preliminarily assess the safety of enhancers. The histology scores provide a means of comparing the irritant effects of the different enhancers (Lashmar et al., 1989). Scores from 0 to 10 were regarded as not causing undue reactions in skin. Scores from 11 to 20 caused skin reactions, which alone are not sufficiently extensive to exclude their potential use. Preparations scored above 21 were considered to cause unacceptably severe damage. The histology scores of skin after pretreated with 1, 3 and 5% capsaicin were 0, $1.33\pm0.58$ and $1.67\pm0.58$, respectively. The scores of 1, 3 and 5% nonivamide concentrations were $3.67\pm0.58$, $3.33\pm1.15$ and $3.67\pm0.58$, respectively, which were slightly higher than those of capsaicin. The data were significantly lower than those of Azone-treated nude mouse skin as previously reported (Lashmar et al., 1989).

Although no skin damage occurred, the clinical value of capsaicin and nonivamide might be limited by the heat sensitization following in vivo topical application of these two analogues (Culp et al., 1989; Crimi et al., 1992). In our previous report (Fang et al., 1996b), the healthy volunteers could undergo pungent sensation when applied capsaicin and nonivamide topically. On the other hand, the volunteers could not distinguish the degrees of heat sensation of various concentrations. Capsaicin and nonivamide also cause marked vasodilation and ascendant skin temperature (Fang et al., 1997). It would be expected that the skin permeation-enhancing effect of these two

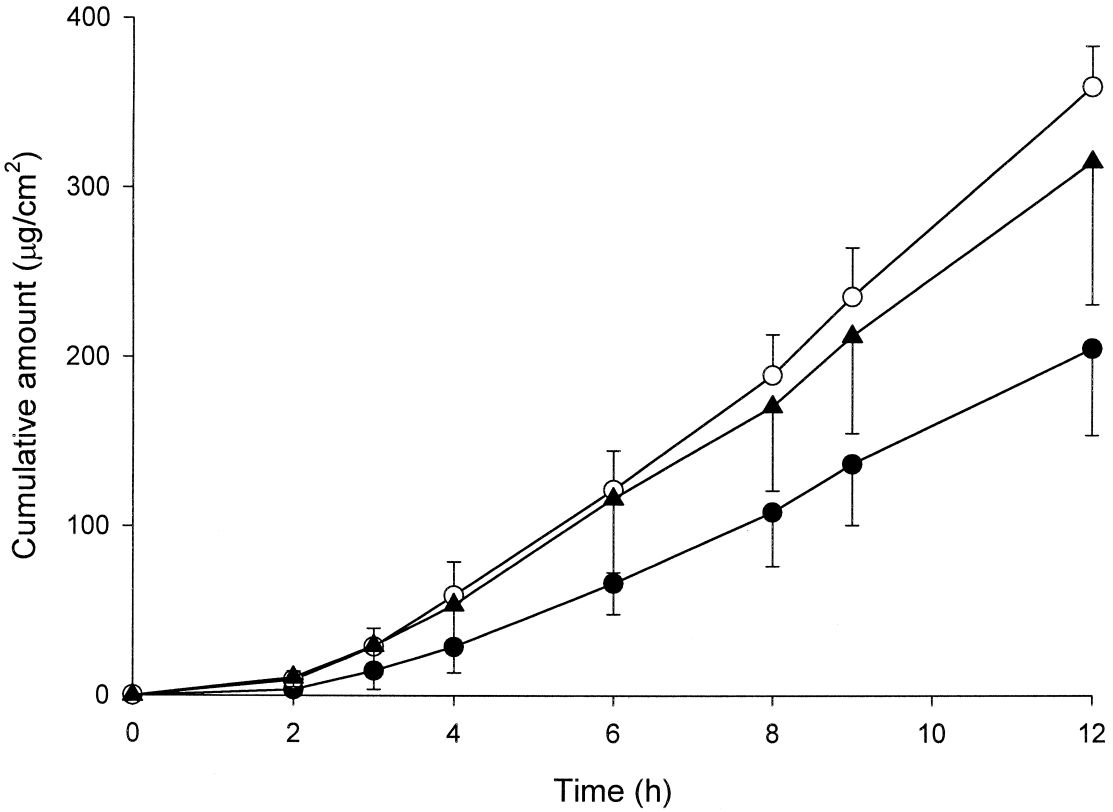


Fig. 6. Cumulative amount of indomethacin detected in the receptor compartment versus time incorporated with 3% enhancers: (●) control group, (○) capsaicin, (▲) nonivamide. All data represent the means of three experiments±S.D.

analogues is more significant in the in vivo status because of increased blood flow and temperature in skin surface.

### *4.3. The permeation of indomethacin incorporated with capsaicin or nonivamide in donor*

The indomethacin flux was significantly enhanced after incorporation of capsaicin or nonivamide in donor compared to control group, but no significant difference (*t*-test, $P>0.05$) between the permeation of the two enhancers (Table 1). This indicated that both analogues showed similar enhancing effect either by the pretreatment method or by the partitioning into drug vehicle. The flux of capsaicin or nonivamide itself was also determined (Table 4). A consideration of the physicochemical properties suggests that capsaicin and nonivamide will permeate the skin more readily than Azone. Since both analogues act as enhancers on indomethacin permeation and also have antinociceptive activities, it may be possible to create topical analgesic formulations in which there are two active ingredients (Degim et al., 1999). As shown in Table 4, the formulations with enhancer attain greater therapeutic effects after calculation of the therapeutic index. The therapeutic index was determined by the result of the steady-state flux multiplied by the potency ratio (Chen et al., 1992). The result suggests a synergistic effect was achieved and the formulation may be created which is more effective than indomethacin or enhancer alone.

Table 2
The flux and enhancement ratio of indomethacin by combined pretreatment of 0.2 W/cm$^2$ ultrasound and 3% enhancers[a]

| Pretreatment mode | Flux (μg/cm$^2$/h) | $ER_{US+E}$ | $ER_E$ |
|---|---|---|---|
| No pretreatment | 18.07±4.25 | – | – |
| US only | 22.00±2.10 | 1.22 | – |
| US+capsaicin | 37.18±8.53 | 2.06 | 1.69 |
| US+nonivamide | 29.98±6.68 | 1.66 | 1.36 |

[a] US, ultrasound; $ER_{US+E}$, flux of indomethacin by combined pretreatment of ultrasound and enhancers/flux of indomethacin without pretreatment of ultrasound and enhancers; $ER_E$, flux of indomethacin by combined pretreatment of ultrasound and enhancers/flux of indomethacin by pretreatment of ultrasound only. Each data represent the mean±S.D. ($n=3$).

### *4.4. Combined effect of ultrasound and enhancers*

Sonophoresis (phonophoresis), the movement of drug molecules through skin in coupling medium under influence of ultrasound, has been extensively studied for over 30 years, and well documented to practically increase skin permeation of indomethacin (Miyazaki et al., 1992b; Meidan et al., 1995). Since physical ultrasound and chemical enhancers individually can increase transdermal drug transport, it is hypothesized that the combination of ultrasound and enhancers may result in a greater degree of enhancement than that resulting from each enhancement method alone (Johnson et al., 1996). The flux of in-

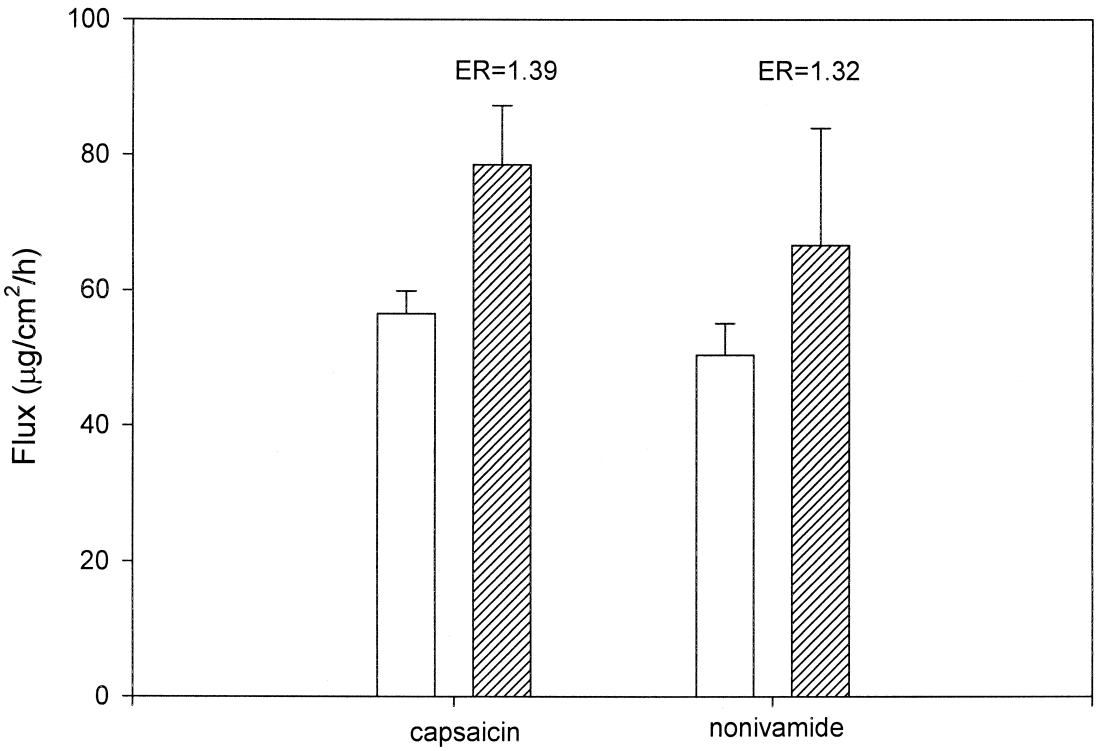


Fig. 7. Flux of capsaicin and nonivamide after simultaneous pretreatment with ultrasound and enhancers. All data represent the means of three experiments±S.D.

Table 3
Flux of indomethacin after pretreatment of various vehicles[a]

| Pretreatment vehicle | Flux (μg/cm²/h) |
|---|---|
| No pretreatment | 18.07±4.25 |
| $H_2O$ | 14.28±4.84 |
| Ethanol/$H_2O$ (1:1, v/v) | 15.47±4.13 |
| PG/$H_2O$ (1:1, v/v) | 19.97±2.82 |

[a] Each data represent the mean±S.D. ($n$=3).

domethacin was significantly strengthened by the combined method (Table 2), indicating that the combination of both methods is more effective than either method alone on the skin permeation of indomethacin.

Various mechanisms have been suggested to explain the enhancement of drug permeation via ultrasound. They include: radiation pressure, temperature increase and cavitation. Simonin (1995) has ruled out the involvement of radiation pressure after using mathematical calculations recently. Little increase in skin temperature (<5°C) was measured under the present experimental condition which is the same with our previous investigation (Fang et al., 1999). The heating effect on skin can be discounted as a mechanism of increasing indomethacin permeation. Miyazaki et al. (1992a) also illustrate that heat alone has no effect on the transdermal absorption of indomethacin.

Cavitation consists of creation and subsequent collapse of microbubbles from dissolved gas. Application of low frequency ultrasound has been suggested to enhance skin permeability due to cavitation near the keratinocyte–lipid bilayer interfaces which induces structural disorder of the stratum corneum lipid bilayers (Mitragotri et al., 1995, 1996). This cavitation may further reduce the barrier properties of enhancer-pretreated skin, resulting in the synergistic effect on enhancing drug permeation. Ultrasound may drive enhancers into the skin over time (Johnson et al., 1996; Ueda et al., 1996). This would

Table 4
Flux and therapeutic of indomethacin incorporated with enhancers[a]

| Donor formulation | Indomethacin alone | | Indomethacin+capsaicin | | Indomethacin+nonivamide | |
|---|---|---|---|---|---|---|
| | Flux | TI | Flux | TI | Flux | TI |
| Indomethacin | 18.07±4.25 | 18.07 | 31.29±2.26 | 31.29 | 27.57±5.27 | 27.57 |
| Capsaicin | – | 0 | 122.32±8.32 | 1921.65 | – | 0 |
| Nonivamide | – | 0 | – | 0 | 110.122±0.39 | 1514.15±15 |
| Total TI | | 18.07 | | 1952.94 | | 1541.72 |

[a] Antinociceptive potency ratio: indomethacin: 1; capsaicin: 15.71; nonivamide: 13.75 (courtesy of Chen et al., 1992). TI (therapeutic index) is calculated by the antinociceptive potency ratio multiplied by flux. Each data represents the mean±S.D. ($n$=3).

increase the capsaicin or nonivamide levels in the stratum corneum, which would likely result in increased bilayer disorder relative to the passive case. Without stir in the donor of in vitro Franz diffusion cell in this present study, ultrasound-induced cavitation may produce significant agitation in aqueous systems. Ultrasonic disruption of stagnant aqueous diffusion layers in poorly stirred diffusional systems would reduce the boundary layer thickness and decrease the total barrier resistance, leading to the higher amount of permeant or enhancer in skin (Julian and Zentner, 1986; Simonin, 1995).

In conclusion, the experimental data in this present study have suggested that both capsaicin and nonivamide improved the permeation of indomethacin across skin in a similar degree. Nonivamide caused milder skin erythema than capsaicin in human (Fang et al., 1996b). Synthetic nonivamide may be a good substitute for natural extracted capsaicin due to lower skin pungent sensation and cheaper price to capsaicin. The microscopic observation and histology scores showed that there was no disruption in skin structure after pretreatment of capsaicin and nonivamide. The combination of low frequency ultrasound and enhancer pretreatment increased transdermal indomethacin transport more than each enhancement method. Capsaicin and nonivamide might permeate across the skin much better than Azone, resulting in eliciting the pharmacological effect sufficiently. It is possible to create topical analgesic formulation with indomethacin and capsaicin/nonivamide which is more effective than indomethacin or capsaicin/nonivamide alone, thus reducing the individual applied doses. Further investigation is needed and in progress to study the in vivo drug permeation enhanced by capsaicin and nonivamide.

## References


Chen, I.J., Yang, J.M., Yeh, J.L., Wu, B.N., Lo, Y.C., Chen, S.J., 1992. Hypotensive and antinociceptive effects of ether-linked and relatively non-pungent analogues of *N*-nonanoyl vanillylamide. Eur. J. Med. Chem. 27, 187–192.

Crimi, N., Polosa, R., Maccarrone, C., Palermo, B., Palermo, F., Mistretta, A., 1992. Effect of topical application with capsaicin on skin responses to bradykinin and histamine in man. Clin. Exp. Allergy 22, 933–939.

Culp, W.J., Ochoa, J., Cline, M., Dotson, R., 1989. Heat and mechanical hyperalgesia induced by capsaicin. Brain 112, 1317–1331.

Degim, I.T., Uslu, A., Hadgraft, J., Atay, T., Akay, C., Cevheroglu, S., 1999. The effects of Azone and capsaicin on the permeation of naproxen through human skin. Int. J. Pharm. 179, 21–25.

Fang, J.Y., Wu, P.C., Huang, Y.B., Tsai, Y.H., 1995. In vitro permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types. Int. J. Pharm. 126, 119–128.

Fang, J.Y., Wu, P.C., Huang, Y.B., Tsai, Y.H., 1996a. In vivo percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from ointment bases: pharmacokinetic analysis in rabbits. Int. J. Pharm. 128, 169–177.

Fang, J.Y., Wu, P.C., Huang, Y.B., Tsai, Y.H., 1996b. In vivo percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from ointment bases: skin erythema test and non-invasive surface recovery technique in humans. Int. J. Pharm. 131, 143–151.

Fang, J.Y., Tsai, M.J., Huang, Y.B., Wu, P.C., Tsai, Y.H., 1997. Percutaneous absorption and skin erythema: quantification of capsaicin and its synthetic derivatives from gels incorporated with benzalkonium chloride by using non-invasive bioengineering methods. Drug. Dev. Res. 40, 56–67.

Fang, J.Y., Fang, C.L., Sung, K.C., Chen, H.Y., 1999. Effect of low frequency ultrasound on the in vitro percutaneous absorption of clobetasol 17-propionate. Int. J. Pharm. 191, 33–42.

Hadgraft, J., 1999. Passive enhancement strategies in topical and transdermal drug delivery. Int. J. Pharm. 184, 1–6.

Hayes, A.G., Orford, A., Reynolds, M., Skingler, A.H., Skingler, M., Smith, C., Tyers, M.B., 1984. The effects of a series of capsaicin analogues on nociception and body temperature in the rat. Life Sci. 34, 154–156.

Huang, Y.B., Wu, P.C., Ko, H.M., Tsai, Y.H., 1995. Cardamom oil as a skin permeation enhancer for indomethacin, piroxicam and diclofenac. Int. J. Pharm. 126, 111–117.

Johnson, M.E., Mitragotri, S., Patel, A., Blankschtein, D., Langer, R., 1996. Synergistic effects of chemical enhancers and therapeutic ultrasound on transdermal drug delivery. J. Pharm. Sci. 85, 670–679.

Julian, T.N., Zentner, G.M., 1986. Ultrasonically mediated solute permeation through polymer barriers. J. Pharm. Pharmacol. 38, 871–877.

Lashmar, U.T., Hadgraft, J., Thomas, N., 1989. Topical application of penetration enhancers to the skin of nude mice: a histopathological study. J. Pharm. Pharmacol. 41, 118–121.

Meidan, V.M., Walmsley, A.D., Irwin, W.J., 1995. Phonophoresis. Is it a reality? Int. J. Pharm. 118, 129–149.

Mitragotri, S., Blankschtein, D., Langer, R., 1995. Ultrasound-mediated transdermal protein delivery. Science 269, 850–853.

Mitragotri, S., Blankschtein, D., Langer, R., 1996. Transdermal drug delivery using low-frequency sonophoresis. Pharm. Res. 13, 411–420.

Miyazaki, S., Kohata, Y., Takada, M., 1992a. Effect of ultrasound on the transdermal absorption of indomethacin. Continuous mode and pulsed mode. Yakuzaigaku 52, 264–271.

Miyazaki, S., Mizuoka, H., Kohata, Y., Takada, M., 1992b. External control of drug release and penetration. VI. Enhancing effect of ultrasound on the transdermal absorption of indomethacin from an ointment in rats. Chem. Pharm. Bull. 40, 2826–2830.

Monsereenusorn, Y., Kongsamut, S., Pezalla, P., 1982. Capsaicin. A literature survey. CRC Crit. Rev. Toxicol. 13, 321–339.

Niazy, E.M., 1996. Differences in penetration-enhancing effect of Azone through excised rabbit, rat, hairless mouse, guinea pig and human skins. Int. J. Pharm. 130, 225–230.

Ogiso, T., Iwaki, M., Bechako, K., Tsutsumi, Y., 1992. Enhancement of percutaneous absorption by laurocapram. J. Pharm. Sci. 81, 762–767.

Phillips, C.A., Michniak, B.B., 1995. Topical application of Azone analogs to hairless mouse skin: a histopathological study. Int. J. Pharm. 125, 63–71.

Simonin, J., 1995. On the mechanisms of in vitro and in vivo phonophoresis. J. Control. Release 33, 125–141.

Tsai, Y.H., Huang, Y.B., Fang, J.Y., Wu, P.C., 1994. Percutaneous absorption of capsaicin and its derivatives. Drug. Dev. Ind. Pharm. 20, 719–730.

Ueda, H., Isshiki, R., Ogihara, M., Sugibayashi, K., Morimoto, Y., 1996. Combined effect of ultrasound and chemical enhancers on the skin permeation of aminopyrine. Int. J. Pharm. 143, 37–45.

William, A.C., Barry, B.W., 1992. Skin absorption enhancers. Crit. Rev. Ther. Drug Carrier Syst. 9, 305–353.