

## Research Paper

# A Novel Solid-in-oil Nanosuspension for Transdermal Delivery of Diclofenac Sodium

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**Purpose.** We formulated a solid-in-oil nanosuspension (SONS) as a novel transdermal delivery carrier for diclofenac sodium (DFNa). The basic transdermal characteristics of the SONS were evaluated using a Yucatan micropig (YMP) skin model.

**Methods.** DFNa-sucrose erucate (i.e. surfactant) complexes were prepared via the formation of a water-in-oil emulsion. The complexes were suspended in isopropyl myristate (IPM) to form a SONS. The basic transdermal characteristics of the SONS were examined using full-thickness YMP dorsal skin in a Franz-type diffusion cell. DFNa powder suspended in IPM without complex formation was used as a control. The effect of the weight ratio of surfactant to DFNa on DFNa penetration of the skin was evaluated.

**Results.** DFNa was successfully dispersed into IPM as a nanosized suspension via complex formation with sucrose erucate. The resultant SONS increased the permeability flux of DFNa across the YMP skin by up to 3.8-fold compared with the control. The size of the SONS depended on the weight ratio of the surfactant to DFNa. The optimal weight ratio for the highest DFNa permeation was 8.8, at which point the mean diameter of the SONS was 14.4 nm.

**Conclusion.** The SONS formulation can enhance the percutaneous absorption of DFNa.

**KEY WORDS:** diclofenac sodium; isopropyl myristate; solid-in-oil nanosuspension.; sucrose ester; transdermal delivery; Yucatan micropig skin.

## INTRODUCTION

Diclofenac sodium (DFNa) (1) is a representative non-steroidal anti-inflammatory drug (NSAID) that is widely used for long-term pain control. However, DFNa-induced gastric damage is known to be a major problem following its oral administration (2). Topical transdermal administration of NSAIDs provides the advantage of achieving a therapeutic effect without the risks of the potentially severe side effects that may occur after oral administration (3).

For topical transdermal administration, the selection of a suitable drug carrier is very important since it can affect the percutaneous absorption. Factors that contribute to the selection of a suitable drug carrier for a transdermal delivery system are: (a) solubility of the drug in the carrier; (b) release of the drug from the carrier into the skin; and (c) enhance-

ment of drug penetration through the stratum corneum (SC). DFNa is a hydrophilic charged drug with a high melting point (280°C). Due to its physicochemical characteristics, it does not easily penetrate the lipophilic SC (4). Although DFNa is not a good candidate for transdermal application (5), the permeability flux of DFNa through skin may be sufficiently enhanced to reach a topical therapeutic effect if a suitable drug carrier can be identified. In fact, a number of water-based transdermal delivery systems for DFNa have been reported, including hydrophilic gels with suitable penetration enhancers such as monoterpenes (6) or fatty acid esters (7,8), lecithin vesicles (9) and microemulsions (10,11). Due to the limitation of the above-mentioned factor (a), there have been few reports of oil-based transdermal formulations of DFNa. Takahashi *et al.* reported that fatty acid diesters, lipophilic base materials, were more effective for enhancing the penetration of DFNa across rat skin than for penetration of lipophilic NSAIDs (12). However, in this system, DFNa powder was directly suspended in the base materials. The potential enhancement of DFNa penetration with oil-based formulations has yet to be examined.

Solid-in-oil (S/O) suspensions are unique formulations for improving the dispersibility (or solubilization) of hydrophilic drugs into an oil phase. In our previous work, we applied an S/O suspension system to intestinal delivery of DFNa (13), and found that DFNa-induced gastric damage was markedly reduced following oral administration, even at the LD<sub>50</sub> level in rats (~50 mg/kg DFNa). The results

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obtained also indicated that the selection of a suitable oil phase can increase the drug delivery ability and targeting characteristics of S/O suspensions.

In the present study, we first investigated the potential of an S/O suspension for transdermal delivery of DFNa. Isopropyl myristate (IPM) was selected as an oil phase for preparing the S/O suspension, since it can disrupt the lamellar bilayer structure of the lipids in the SC and reduce the resistance to the permeability flux of a hydrophilic drug (14) without serve skin irritation (15). Although the solubility of DFNa in IPM is too low to sufficiently enhance its permeation flux across the skin, DFNa was highly dispersed in IPM after complex formation with a lipophilic surfactant to yield a solid-in-oil nanosuspension (SONS). In order to evaluate the SONS as a novel transdermal delivery system for DFNa, its basic percutaneous characteristics were investigated using a Yucatan micropig (YMP) skin model.

## MATERIALS AND METHODS

### Materials

DFNa was supplied by ASPION Co. (Tokushima, Japan). Sucrose esters ER190 (HLB=1) and ER290 (HLB=2) (Fig. 1) were kindly provided by Mitsubishi-Kagaku Foods Co. (Tokyo, Japan). IPM was purchased from Tokyo Kasei Co. (Tokyo, Japan). Frozen skin sections from 5-month-old male YMPs were purchased from Charles River Japan Inc. (Tokyo, Japan).

### Preparation of a SONS Containing DFNa

A 15 ml aqueous solution containing 10 mg/ml DFNa and a 30 ml cyclohexane solution containing a sucrose ester (i.e. surfactant) were poured into a round-bottom flask (50 ml) and mixed with a homogenizer at 26,000 rpm for 2 min to form a water-in-oil (W/O) emulsion. The resulting emulsion was rapidly frozen in liquid nitrogen, and lyophilized using a freeze-drying machine (FD5N; EYELA, Tokyo, Japan) for 24 h. IPM was added to the resulting viscous solid, and thoroughly dispersed by ultrasonication (140 W for 30 min). The resulting nanosized suspensions were designated SONS<sub>ER190</sub> and SONS<sub>ER290</sub>, respectively, with the subscript abbreviation

indicating the type of surfactant employed to prepare the SONS. A physical mixture of the surfactants and DFNa in IPM with the same composition as the SONS was prepared by ultrasonication (140W for 30 min). The resultant mixture was designated M<sub>ER290</sub>. The DFNa concentration in all formulations was set at 15 mg/ml.

### Measurement of the Particle Size of the SONS

The size distribution in the SONS was determined by dynamic light scattering (DLS) using a computerized inspection system (Nano-ZS; Malvern, Worcestershire, UK). The viscosity and refractive index of each formulation was measured using an automatic microviscometer (AWVn; Anton Parr GmbH, Graz, Austria) and a refractive-index detector (RA-500; Kyoto Electronics Manufacturing Co. Ltd., Kyoto, Japan), respectively. The mean particle size of the SONS was measured using a laser diffraction particle size analyzer (SAID-200 V; Shimadzu, Tokyo, Japan) when the size of the SONS was larger than 10  $\mu\text{m}$ .

### Measurement of Residual Cyclohexane in the Formulations

After the lyophilization process, acetone instead of IPM was added to the resultant complexes, and the resultant mixture was dispersed completely by ultrasonication (140 W for 10 min). The acetone phase was filtered through a 0.22- $\mu\text{m}$  pore-size membrane (Millex; Millipore Corporation, Bedford, MA), and the samples were analyzed by gas chromatography (HP6890, Hewlett Packard, Palo Alto, California, USA) equipped with an FID detector and a 30 m $\times$ 0.25 mm capillary column (HP-INNOWAX, J&W scientific, California, USA). Toluene was used as an internal standard.

### In Vitro Studies

#### Preparation of Full-Thickness YMP Skin

The frozen skin sections (approximately 8 $\times$ 10 cm) were stored at  $-80^{\circ}\text{C}$  prior to use. When needed, the skin samples were allowed to thaw at room temperature. The full-thickness skin was prepared by carefully removing the subcutaneous fat, and then immediately used for permeation studies.

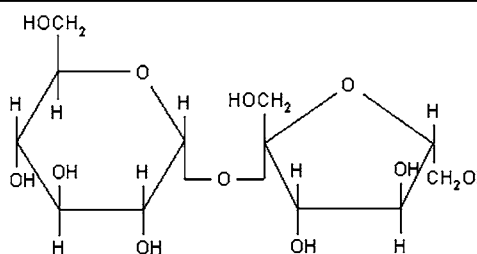
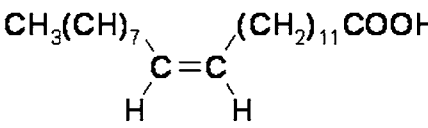
Hydrophilic head	Lipophilic groups	Commercial name
 <p>Sucrose</p>	$\text{CH}_3(\text{CH}_2)_7$  <p>Erucic acid</p>	ER190 (HLB=1)
		ER290 (HLB=2)

Fig. 1. Chemical compositions of the sucrose esters used in this study.

### Permeation Studies

*In vitro* permeation studies were performed using a modified home-made Franz-type diffusion cell with a diameter of 10 mm. YMP full-thickness dorsal skin was sandwiched between the upper donor compartment and lower receiver compartment, and 0.5 ml of the formulation was placed in the donor compartment of the skin. The acceptor compartment contained 5 ml of phosphate-buffered saline (PBS) that was thermostatically maintained at 32°C by a circulating water bath (NTT-20S; Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The acceptor solution was stirred at 1000 rpm with a Teflon magnetic stirrer. An aliquot (0.5 ml) of the receptor phase was withdrawn at predetermined time intervals, and analyzed by HPLC. Each sample removed was replaced by an equal volume of fresh PBS solution. DFNa suspended in IPM at the same DFNa concentration as in the SONS was employed as a control. To quantify the DFNa concentration in the skin after 24 h, the skin under the donor compartment was thoroughly washed with methanol and shredded with scissors. The shredded skin was dispersed in 10 ml of methanol by ultrasonication (140 W for 4 h) to extract DFNa from the skin. The methanol phase was filtered through a 0.22- $\mu$ m pore-size membrane (Millex; Millipore Corporation, Bedford, MA), and the samples were analyzed by HPLC. The experimental conditions for the HPLC analysis were: column, Shiseido CAPCELL PAK C18 MG (4.6 $\times$ 250 mm); mobile phase, 50 mM acetic acid/methanol=25:75; flow rate, 0.7 ml/min. DFNa was detected by monitoring the absorption at 270 nm. For histopathological studies, the skin was fixed in 10% buffered formalin saline (pH 7.4) after permeation studies. Paraffin tissue sections with a thickness of 20  $\mu$ m were cut and stained with the haematoxylin solution (Muto Pure Chemicals Co. Ltd., Tokyo, Japan). The skin section was observed by a light microscope (IX70, Olympus, Tokyo, Japan) compared with untreated skin.

### Skin Permeation Parameters

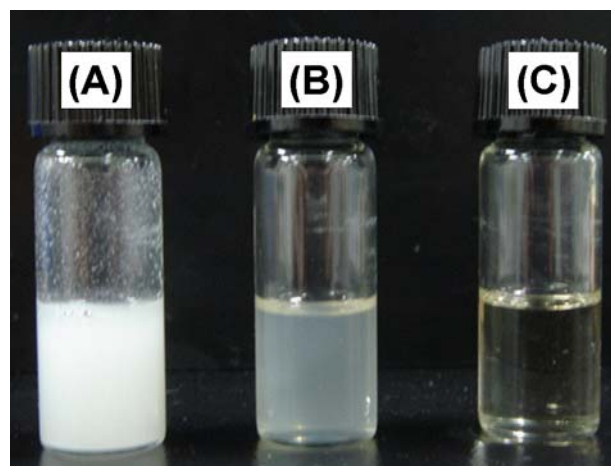
The cumulative drug permeation ( $Q_t$ ) was calculated using Equation 1:

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i \quad (1)$$

where  $C_t$  is the drug concentration of the receiver solution at each sampling time,  $C_i$  is the drug concentration of the  $i$ th sample, and  $V_r$  and  $V_s$  are the volumes of the receiver solution and the sample, respectively. Data were expressed as the cumulative drug permeation per unit of skin surface area,  $Q_t/A$  ( $A=0.785$  cm<sup>2</sup>). The theoretical penetration curve can be approximated by Equation 2 (4):

$$Q_t = \frac{KDC_0}{L} \left( t - \frac{L^2}{6D} \right) \quad (2)$$

Where  $C_0$  is the initial DFNa concentration of the formulation (15 mg/ml),  $K$  is the skin/vehicle partition coefficient,  $D$  is the diffusion coefficient and  $L$  is the thickness of the skin.



**Fig. 2.** Photograph of the solid-in-oil nanosuspensions prepared in this study. (A) DFNa suspended into IPM (Control), (B) SONS<sub>ER190</sub> and (C) SONS<sub>ER290</sub>.

The skin permeation parameters were calculated by the following equations. The steady-state fluxes ( $J_{ss}$ ) were calculated by linear regression interpolation for the experimental data at steady state using Equation 3:

$$J_{ss} = \frac{dQ_t}{dt} = \frac{KDC_0}{L} \quad (3)$$

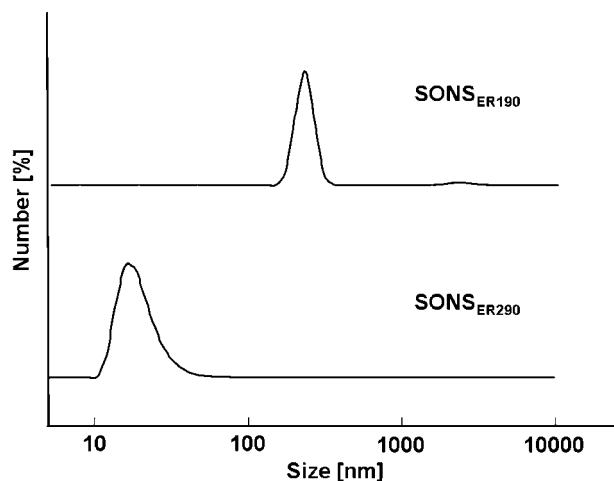
The apparent permeability coefficients ( $P$ ) were calculated according to Equation 4:

$$P = \frac{J_{ss}}{C_0} = \frac{KD}{L} \quad (4)$$

The penetration-enhancing activities were expressed by defining the enhancement ratio ( $ER$ ), which was determined using equation 5:

$$ER = \frac{J_{ss}(\text{formulation})}{J_{ss}(\text{control})} \quad (5)$$

where  $J_{ss}(\text{formulation})$  and  $J_{ss}(\text{control})$  represent the  $J_{ss}$  values obtained with the formulation and control samples, respectively.



**Fig. 3.** Size distributions of the solid-in-oil nanosuspensions (SONSs) containing DFNa determined by DLS analysis.

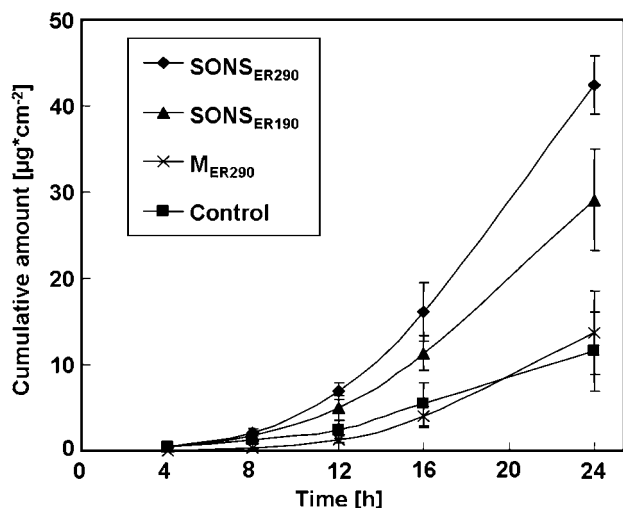


Fig. 4. Permeation profiles of DFNa across full-thickness YMP dorsal skin. Each point represents the mean  $\pm$  S.D. of three independent experiments.

Finally, the lag time was determined as the value of  $t$  at  $Q_t=0$  when the steady-state line was extrapolated to the time axis using Equation 6.

$$t_L = L^2/6D \quad (6)$$

## RESULTS

### SONS Preparation

Sucrose esters (Fig. 1) were selected as surfactants for the present study. The physical appearances of the SONSs are shown in Fig. 2, while their size distributions measured by DLS are shown in Fig. 3. The mean particle size of SONS<sub>ER190</sub> was about 220 $\pm$ 23 nm, while that of SONS<sub>ER290</sub> was about 14.4 $\pm$ 4.9 nm. The polydispersity index of SONS<sub>ER190</sub> and SONS<sub>ER290</sub> were 0.618 $\pm$ 0.047 and 0.355 $\pm$ 0.018, respectively.

### Percutaneous Studies with YMP Skin

The DFNa permeation parameters determined from the permeation profiles of DFNa through full-thickness YMP dorsal skin (Fig. 4) are summarized in Table I. The cumulative DFNa permeation ( $Q_{24h}$ ) values were in the following order: SONS<sub>ER290</sub> > SONS<sub>ER190</sub> > M<sub>ER290</sub>  $\approx$  control. Marked penetration enhancement was observed with SONS<sub>ER290</sub>, producing an approximately 3.8-fold increase in the DFNa permeability flux compared with the control. SONS<sub>ER190</sub> showed a 2.3-fold increase, while the physical mixture (M<sub>ER290</sub>) exhibited a comparable permeability flux ( $J_{ss}$ ) of

DFNa to the control. Both SONS samples showed identical lag times (Table I).

Table II shows that the SONS size was significantly reduced with increasing weight ratio in the range of 2.2–8.8, while it remained constant at about 14 nm when the weight ratio was larger than 8.8.

Figure 5 shows the effects of the weight ratio on the topical (to the skin) and transdermal (across the skin) delivery of DFNa. The permeation of DFNa ( $Q_{24h}$ ) was in the following order:  $F_{8.8} \approx F_{6.6} \geq F_{13.2} > F_{17.6} > F_{3.3} \approx F_{2.2} \approx control$ . The amount of DFNa extracted from the skin was in the following order:  $F_{8.8} > F_{6.6} \approx F_{13.2} > F_{17.6} > F_{3.3} \approx F_{2.2} \approx control$ . Based on statistical analysis, at weight ratios lower than 3.3, the DFNa penetration levels both into and across the skin were comparable to the control levels ( $P > 0.05$ ). At weight ratios higher than 6.6, significantly different enhancement (at least  $P < 0.01$ ) in the penetration of DFNa both across and into the skin compared with the control were observed. Additionally,  $F_{8.8}$  was significantly different ( $P < 0.001$ ) compared with  $F_{13.2}$  and  $F_{17.6}$ , but no significant difference was shown with  $F_{6.6}$  ( $P > 0.05$ ) in the penetration of DFNa across the skin. The results suggested that, at weight ratios between 6.6 and 8.8, DFNa permeation reached its maximum value. When the weight ratio was larger than 8.8, decreases in the penetration of DFNa both into and across the skin were observed ( $P < 0.05$  compared with  $F_{8.8}$ ).

## DISCUSSION

### Characteristics of the SONS

To obtain a SONS containing DFNa, DFNa complexes with lipophilic surfactants were first prepared via the formation of W/O emulsions (12). Both the sucrose esters used were nonionic surfactants with low HLB values and formed stable W/O emulsions. First, the weight ratio between each surfactant and drug for complex formation was set at 8.8 according to our previous study (13). The viscous solids obtained after lyophilization of the resulting W/O emulsions were readily and highly dispersed in IPM as nanosized suspensions. With respect to the stability of suspension, SONS<sub>ER290</sub> showed little precipitates at least in 3 weeks after preparation. In the case of SONS<sub>ER190</sub>, the slight aggregation of nanosuspension was observed when the formulation was stored at room temperature for 3 weeks. However, no crystal deposition of DFNa in the SONS<sub>ER190</sub> was found, and the aggregates were readily re-suspended to yield the original state. For estimating residual amount of cyclohexane in the formulations, acetone was selected as a disperse medium instead of IPM because DFNa and the surfactants showed little solubility in acetone, so that

Table I. Effects of S/O Suspensions on DFNa Skin Permeation Parameters

	$P$ ( $\times 10^{-3}$ cm h <sup>-1</sup> )	$J_{ss}$ ( $\mu$ g cm <sup>-2</sup> h <sup>-1</sup> )	$L_t$ (h)	ER
Control	0.05 $\pm$ 0.01	0.76 $\pm$ 0.2	9.0 $\pm$ 0.7	–
M <sub>ER290</sub>	0.10 $\pm$ 0.06	1.5 $\pm$ 0.9	11.6 $\pm$ 1.7	1.6 $\pm$ 0.6
SONS <sub>ER190</sub>	0.15 $\pm$ 0.10	2.1 $\pm$ 1.6	9.9 $\pm$ 0.5	2.7 $\pm$ 0.5
SONS <sub>ER290</sub>	0.20 $\pm$ 0.01	2.9 $\pm$ 0.2	9.6 $\pm$ 0.7	3.8 $\pm$ 0.3

**Table II.** Effects of the Weight Ratio of ER290 to DFNa on the Size and Viscosity of the SONS

Sample	DFNa (mg)	ER290 (mg)	IPM (ml)	Viscosity (mPa s)	Size (nm)
Control	150	–	10	5.5	57,200±5,000
F <sub>2.2</sub> <sup>a</sup>	150	330	ad.10	11.9±0.5	19,300±1,300
F <sub>3.3</sub>	150	660	ad.10	13.5±0.5	2,100±500
F <sub>6.6</sub>	150	990	ad.10	15.8±0.1	800±100
F <sub>8.8</sub> <sup>b</sup>	150	1320	ad.10	16.7±0.2	14.4±4.9
F <sub>13.2</sub>	150	1980	ad.10	20.3±0.5	14.0±5.4
F <sub>17.6</sub>	150	2640	ad.10	28.8±0.2	13.3±6.4

<sup>a</sup>The subscript in the sample name represents the weight ratio of ER290 to DFNa in the formulation.

<sup>b</sup>F<sub>8.8</sub> corresponds to the formulation designated as SONS<sub>ER290</sub> throughout the main text.

cyclohexane was easily separated from the complexes. From the results obtained, the residual amount of cyclohexane in the DFNa-ER190 complexes and DFNa-ER290 complexes were estimated to be 613±11 ppm and 631±30 ppm, respectively. These values correspond to the presence of about 60 ppm cyclohexane in the final formulations, suggesting that almost all of cyclohexane (>99.99 %) was removed in the lyophilization process.

### Percutaneous Characteristics of SONS

YMP skin was selected as a skin model in the present study because it has previously been employed as a potent model for transdermal studies without overestimation (16). The results of percutaneous studies with different formulations (Fig. 4) clearly demonstrated that the SONSs were effective accelerants for the permeability flux of DFNa through the skin. It was noted that M<sub>ER290</sub> showed little enhancement of the permeability flux compared with the control, suggesting that the nanosized formulations promoted the permeation of DFNa. According to equation 6, the comparable lag times between SONS<sub>ER190</sub> and SONS<sub>ER290</sub> (Table I) suggest that the diffusion coefficient (*D*) of DFNa in the skin was not altered, implying that the surfactants may dissociate from DFNa after penetrating through the skin. According to equation 3 with constants *C*<sub>0</sub> and *L*, the different permeation characteristics between the SONSs are likely to be due to differences in the skin/vehicle partition coefficient (*K*).

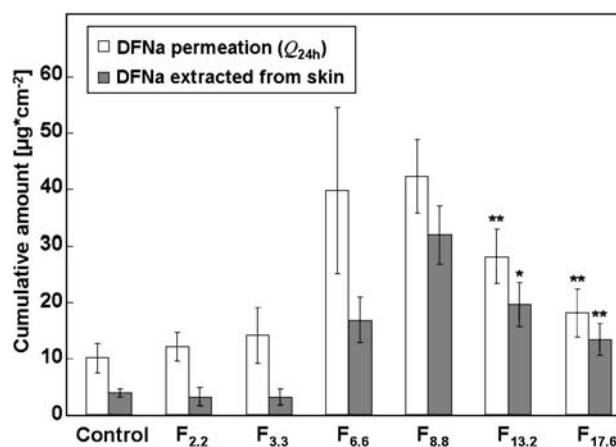
Next, the effect of the weight ratio of ER290 to DFNa on the percutaneous characteristics of the SONS was investigated. No penetration enhancement occurred when the weight ratio was lower than 3.3. Since the size of SONS was larger than 2 μm in these cases, we assume that large SONS sizes hindered the partition of DFNa into the skin. Marked increases in the permeability of DFNa were found upon transdermal application of F<sub>8.8</sub> and F<sub>6.6</sub>. The presence of smaller experimental errors for F<sub>8.8</sub> than for F<sub>6.6</sub> suggested that F<sub>8.8</sub> more stably enhanced DFNa partition into the skin. It should be noted that although the amounts of DFNa crossing the skin with the F<sub>8.8</sub> and F<sub>6.6</sub> formulations were comparable, F<sub>8.8</sub> delivered a higher amount of DFNa in the skin than F<sub>6.6</sub>. These results suggest that smaller SONS sizes can effectively promote DFNa delivery into the SC when the weight ratio of ER290 to DFNa was lower than 8.8.

The question arises as to why further increases in the weight ratio (>8.8) reduced both penetration of DFNa into and across the skin. Similar behavior was reported for

monoolein as a penetration enhancer (17). Briefly, monoolein exhibited its optimal range with respect to transdermal (across the skin) delivery of cyclosporin A (Cys A) in propylene glycol formulations, but increasing the monoolein concentration in the formulation also increased the amount of Cys A that partitioned into the SC. Unlike the lipophilic Cys A, the intrinsically hydrophilic nature of DFNa is likely to decrease its partition into the SC. Thus, when excess surfactant was added, DFNa may partition into the IPM phase (formulation phase) rather than the SC.

Another possibility is increased viscosity of the SONS formulations in the presence of excess surfactant. In fact, the viscosity of the SONS markedly increased with increases in the weight ratio of surfactant to DFNa (Table II). Thus, high viscosity of the SONS cannot be excluded as a possible reason for the decrease in DFNa partitioning to the skin.

With respect to the toxicity potential of SONS<sub>ER290</sub> towards skin, we verified that no potential toxicity such as localized hair loss was observed when SONS<sub>ER290</sub> was applied on a rabbit ear (data not shown). In addition, we confirmed that by histopathological studies, there was no significant change in the skin structure after treating YMP skin for 24 h with SONS<sub>ER290</sub> compared with the untreated one, where SC is intact to the inner layer of skin and not



**Fig. 5.** Effects of the weight ratio between ER290 and DFNa on the percutaneous characteristics of DFNa. Each point represents the mean ± S.D. of six independent experiments. Significantly different from the control at *P*<0.01 in the penetration of DFNa both across and into the skin at weight ratios higher than 6.6. \*Significantly different from F<sub>8.8</sub> at *P*<0.05. \*\*Significantly different from F<sub>8.8</sub> at *P*<0.001.

stripped off (data not shown). Thus, the results suggested that SONS<sub>ER290</sub> system is safe to the skin.

## CONCLUSIONS

In the present study, we first applied a SONS for transdermal delivery of DFNa, a hydrophilic drug. We found that the SONS was a potent candidate carrier for enhancing the percutaneous absorption of DFNa. Due to its unique preparation procedure, the present novel formulation, SONS, is basically applicable to any water-soluble drugs that hardly distribute to the SC. Further research on the SONS is currently underway in our laboratory.

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