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Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with diclofenac diethylammonium

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ABSTRACT

The objective of this study was to develop a novel elastic bilayer vesicle entrapped with the non-steroidal anti-inflammatory drug (NSAID), diclofenac diethylammonium (DCFD) for topical use. Eighteen bilayer vesicular formulations composing of DPPC or Tween 61 or Span 60 mixed with cholesterol (at 1:1, 3:7 and 1:1 molar ratios, respectively) and ethanol at 0–25% (v/v), by chloroform film method with sonication were developed. The elastic Tween 61 niosomes which gave no sedimentation, no layer separation, unchanged particle sizes (about 200 nm) were selected to entrap DCFD. The entrapment efficiency of the drug in the conventional and elastic Tween 61 niosomes was 65 and 93%, respectively. At least 87% of DCFD determined by HPLC remained in elastic Tween 61 niosomes when kept at 4, 27 and 45 °C for 3 months. The deformability index values of the elastic niosomes were 13.76 and 3.44 times higher than the conventional niosomes entrapped and not entrapped with the drug, respectively, indicating the higher flexibility of the elastic vesicle especially, when entrapped with the drug. Transdermal absorption through excised rat skin was performed by vertical Franz diffusion cell at 32 ± 2 °C for 6 h. Gel containing elastic niosomes exhibited fluxes of DCFD in the stratum corneum (SC), deeper skin layer (viable epidermis and dermis, VED) and receiver chamber at 191.27 ± 9.52, 16.97 ± 2.77 and 3.76 ± 0.54 μg/(cm^2 h), whereas the commercial emulgel, containing an equivalent DCFD, gave 60.84 ± 13.63, 7.33 ± 1.70 and 0.14 ± 0.01 μg/(cm^2 h), respectively. The *in vivo* anti-inflammatory activity was evaluated by ethyl phenylpropiolate (EPP)-induced rat ear edema (n = 3). DCFD entrapped in the developed elastic niosomes and incorporated in gel gave the same ear edema inhibition percentages of 23.81% at 30 min, but 2 and 9 times more inhibition percentages at 45 and 60 min than the commercial emulgel, respectively. This result has not only demonstrated the enhancement of transdermal absorption through rat skin, but also the *in vivo* anti-inflammatory effect of DCFD when entrapped in the developed novel elastic Tween 61 niosomes, as well.

1. Introduction

Traumatic conditions such as strains and sprains can occur in any location of human body. Diclofenac is a widely used anti-inflammatory drug for this purpose. It causes local mucosal irritations and metabolism in the first passage after oral administration, which occurs in the liver and leads to partial inactivation. Thus, only 50% of the drug reaches the circulation. Topical dosage forms are desirable for the chronic use of this drug, especially in the case of rheumatic symptoms. The efficacy of topical diclofenac sodium depends greatly on the capacity of the preparation to allow the drug penetrates through the skin. Since the permeability of intact skin for diclofenac sodium is low, various salts, such as diclofenac diethylammonium (DCFD) have been used due to its amphiphilic nature (Fig. 1). This compound is more lipophilic than diclofenac sodium and can permeate the skin at a higher rate. In addition, many diclofenac formulations have been developed, such as an aqueous gel of diclofenac (Nishihata et al., 1987), niosomal diclofenac (Raja-Naresh et al., 1993) and pluronic lecithin organo-gel (plo) of diclofenac (Burnham et al., 1998; Grace et al., 1999). The latter has been reported to be effective for short-term reduction in elbow pain and wrist extensor weakness. In order to increase skin permeability of diclofenac, the promoting effect of ethanol on percutaneous absorption of diclofenac (Nishihata et al., 1987) and the combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac (Obata et al., 1993) have been performed. However, these approaches do not

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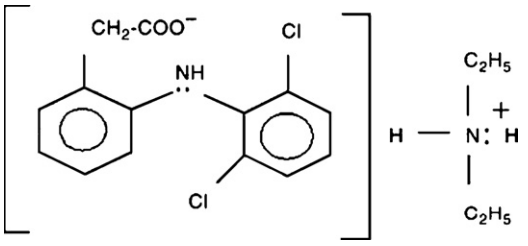

Fig. 1. Chemical structure of diclofenac diethylammonium (DCFD).

only have limited skin permeability, but also cause skin irritation as well.

Stratum corneum (SC) is a main barrier of many compounds passing through the skin. Several approaches have been developed to weaken this skin barrier. One possibility for increasing the penetration of drugs and many cosmetic chemicals is the use of vesicular systems, such as liposomes and niosomes. Conventional liposomes are usually not efficient to transdermally delivery across the skin, because they do not deeply penetrate the skin, but rather remain on the upper layer of SC. Several researchers have developed novel elastic vesicles in order to deeply and easily penetrate through the skin (Dayan and Touitou, 2000; Valjakka-Koskela et al., 1998). Ethanol is known as an efficient permeation enhancer and is added to the vesicular system to prepare the elastic vesicles. It can interact with the polar head group region of the lipid molecules, resulting in the reduction of the melting point of the SC lipid, thereby increasing lipid fluidity, and cell membrane permeability. The high flexibility of the vesicular membrane from the added ethanol permits the elastic vesicles to squeeze themselves through the pores which are much smaller than their diameters (Cevc, 1996; Van and Bergh, 1999). Thus, elastic vesicles could overcome the limitation of low penetration ability of the conventional liposomes or compounds in the commercial formulations across the skin. Ethosomes are vesicular systems composed mainly of phospholipids together with the high contents of ethanol (20–45%) (Touitou et al., 2000). However, ethosomes have problems of variable purity and high cost of phospholipids. In the present study, the novel elastic vesicles (niosomes) composing of non-ionic surfactants have been developed. An *in vivo* anti-inflammatory activity of gel containing DCFD entrapped in elastic niosomes was compared with the commercial formulation.

2. Materials and methods

2.1. Materials

DCFD was a gift from Siam Pharmaceutical Co., Ltd., Thailand (90.0–110.0% label amount, USP 25). The commercial diclofenac emulgel containing 1.16% (w/w) of DCFD salt equivalent to 1% (w/w) of diclofenac sodium was purchased from the local pharmacy in Chiang Mai, Thailand. Tween 61 (polyoxyethylene sorbitan monostearate), Span 60 (sorbitan monostearate), DPPC (dipalmitoyl phosphatidylcholine), cholesterol, Sephadex-G-50 and phenylbutazone were purchased from Sigma Chemicals, U.S.A. Ethyl phenylpropiolate (EPP) was purchased from Fluka. Ethanol, methanol and chloroform were analytical grade solvents.

2.2. Preparation of DCFD-loaded nanovesicular systems

Eighteen elastic niosomal formulations were prepared by chloroform film method (Bangham et al., 1965) with sonication. DPPC, Span 60 or Tween 61 mixed with cholesterol (at 1:1, 1:1 and 3:7 molar ratios, respectively) of 20 mM were placed in a clean, dry round bottom flask. The mixture was dissolved in chloroform. The organic solvent was removed by a rotary evaporator under vacuum (R-124 Buchi, Switzerland). The resulting film was dried overnight under vacuum at room temperature ($27 \pm 2\,^{\circ}C$). The film was hydrated with an aqueous 0–50% ethanolic solution. The resulting dispersion in a beaker put in an ice bath ($4\,^{\circ}C$) was sonicated by a microtip probe sonicator (Vibra Cell™, Sonics & Materials, Inc., Newtown, CT, USA) at pulse on 3.0 and pulse off 1.0, 33% amplitude for 15 min. The vesicular dispersions were kept in transparent vials tightly covered with aluminum caps. The most physical stable vesicles at 4, 27 and $45\,^{\circ}C$ for 3 months was selected to entrap DCFD. To entrap DCFD in the select vesicles, DCFD was dissolved in chloroform together with other vesicular components and followed the preparation produce as aforementioned.

2.3. Morphology, vesicle size and zeta potential determination

A drop of nanovesicular dispersion was applied on a 300-mesh formvar copper grid on paraffin and allowed the sample to adhere on the formvar for 10 min. The remaining dispersion was removed and a drop of 2% aqueous solution of ammonium molybdate was applied for 5 min. The remaining solution was then removed. The sample was air dried and examined with a transmission electron microscope (TEM 1200S JEOL, JEOL Ltd., Tokyo, Japan). The morphology and lamellarity of the bilayer vesicles were observed. The diameter of empty and drug loaded nanovesicles were determined using dynamic light scattering (DLS), Zetasizer 300HSA (Malvern Instruments, Malvern, UK) based on photon correlation spectroscopy. Analysis ($n = 3$) was carried out for 100 s at room temperature ($27 \pm 2\,^{\circ}C$). The charges on empty and drug loaded vesicular surface were determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 s. The average zeta potential and charges were determined. The time-dependent correlation function on the scattered light intensity was measured at a scattering angle of 90°. All samples were diluted 30 times with freshly filtrated Millipore water for the particle size and zeta potential measurement.

2.4. Stability studies

The selected elastic vesicles entrapped with DCFD were stored in transparent vials covered with aluminum cap at 27 ± 2, 45 ± 2 and $4 \pm 2\,^{\circ}C$ for 3 months. The physical characteristics (color, sedimentation, morphology and particle size by DLS) of the dispersions and the remaining DCFD contents at 1, 2, 3, 4, 8, 12 weeks were determined by HPLC.

2.5. HPLC assay

The quantitative determination of DCFD were performed by HPLC (AS 1000, Thermo Finigan, USA) using a H5ODS-Hypersil, Hichrom (250 mm × 4.6 mm, 5 mm) column and a phosphate buffer/methanol (3:7, v/v) as a mobile phase delivered at 1 ml/min. An amount of 20 ml of the injection volume was eluted in column at $27 \pm 2\,^{\circ}C$ and monitored at 254 nm UV-detector. Under this condition, the retention time of the DCFD was 12.7 min. Every sample was filtered through a 0.45-μm membrane filter, prior to injection onto the HPLC column. The peak areas of samples were calculated and the concentrations of DCFD in the samples were determined from the standard curve. The experiment was done in triplicate. The standard curve of DCFD demonstrated linear with high correlation ($r^2 = 0.9989$). The following regression equation was obtained: $y = 1.84 \times 10^4 x + 9427$, where y is the peak area and x is the quantity of DCFD (μg).

2.6. Quantitative analysis of ethanol by GC

The gel containing DCFD elastic niosomes at 4 ± 2, 27 ± 2 and 45 ± 2 °C were quantitatively analyzed for ethanol content at initial and after 3 months. Ethanol content was analyzed by GC (Shimadzu GC-8A) equipped with flame-ionization detector and Restex Chromosorb (1.2 m × 5 mm) column; carrier gas: nitrogen gas 1.5 kg/cm^2. The temperature of the column was 270 °C while the injector and detector were at 300 °C. Under this condition, the retention time of ethanol was 3.0 min. The experiment was done in triplicates. The standard curve of ethanol demonstrated linear with high correlation (r^2 = 0. 9988). The following regression equation was obtained: $y = 13623x - 163.06$, where y is the detector response (mV) and x is the quantity of ethanol (μl).

2.7. Measurement of elasticity value

Elasticity of the empty and drug loaded of both conventional niosomes and elastic niosomes was carried out by the extrusion measurement (Cevc et al., 1997; Jain et al., 2003). Briefly, the vesicles were extruded through polycarbonate membrane filter with a pore size of 50 nm (Millipore, USA) at constant pressure (2.5 bar). The elasticity of the vesicles was expressed in terms of deformability index according to the following equation:

$$\text{deformability index} = j\left(\frac{r_v}{r_p}\right)^2$$

where j is the weight of dispersion, which was extruded in 10 min through a polycarbonate filter of 50 nm pore size, r_v is the size of the vesicle after extrusion and r_p is the pore size of the filter membrane.

2.8. Entrapment efficiency determination of the drug-loaded bilayer vesicles

The entrapped DCFD in the vesicles was separated from the not entrapped drugs by gel filtration using Sephadex-G-50 as a packing material and purified water as an eluent. Eluates were collected in fractions using a fractional collector (Foxy JR, Isco Inc., Lincoln, USA) at the flow rate of 10 ml/min. The fractions containing the drug-loaded vesicles which were detected at 470 nm (Castanho et al., 1997), were pooled, collected and dried with a Centrivap Console (Labconco, Kansas City, MO). The remaining residues were dissolved in absolute ethanol and assayed for the drug contents by HPLC. The entrapment efficiency was calculated according to the following equation:

$$\text{entrapment efficiency (\%)} = \frac{\text{DCFD entrapped in niosomes}}{\text{total DCFD in the niosomal dispersions}} \times 100$$

2.9. Preparation of DCFD nanovesicular gels

The elastic and conventional Tween 61 niosomes entrapped with DCFD were incorporated into a gel base containing Carbopol® 980. Briefly, gel containing 0.2% Carbopol® 980 was dispersed in the niosomal dispersion with gentle stirring to obtain the total DCFD equivalent to diclofenac sodium 1% (w/w).

2.10. In vitro skin permeation study

Rat skin permeation of DCFD from various gel formulations were studied using vertical Franz diffusion cells having the area between the donor and the receiver chamber of 2.46 cm^2 and the volume of the receptor compartment of 13 ml. The receptor compartment contained phosphate-buffered saline (PBS, pH 6.5) which was constantly stirred at 100 rpm with small magnetic bar and controlled temperature at 32 ± 2 °C throughout the experiment. The skin was prepared from the abdominal skin of male rats (Sprague–Dawley strain, 150–200 g). After the abdominal skin of rat was shaved and separated, subcutaneous fat was carefully removed using a scalpel. The skin was mounted with the SC side facing upwards to the donor compartment, and the subcutaneous side was in contact with the receiver medium. One gram of gel was placed into the donor compartment and covered with paraffin film. Experiments were done in triplicate. The cells were stopped at 15, 30 min 1, 3 and 6 h. Samples in the receiver chamber were analyzed by HPLC. Drug on the skin surface was removed by 3M Scotch Magic™ tape (1 cm × 1 cm) using the stripping method described by Plessis et al. (1992). After stripping, the skin was cut into small pieces and the DCFD in the skin was extracted by absolute ethanol under sonication and the drug contents were assayed by HPLC.

2.11. In vivo anti-inflammatory assay

The anti-inflammatory activity of various DCFD gel formulations were studied by EPP-induced rat ear edema model (Brattsand et al., 1982; Young et al., 1983). The male rats (Sprague–Dawley strain, 30–40 g) were fed with a standard diet ad libitum and housed in a temperature-controlled room (25 ± 2 °C), in accordance to the European Union regulations (CEC council 86/809). The animals were divided into 6 different groups of three each. The first and the second group served as the positive (phenylbutazone) and negative (gel base) control, respectively. The third group received the gel containing the unentrapped drug. The fourth, fifth and sixth groups received the commercial DCFD emulgel, conventional niosomes entrapped with DCFD in gel and the elastic niosomes entrapped with DCFD in gel, respectively. Ear edema was induced by the topical application of EPP at the dose of 1 mg/20 μl per ear to the inner and outer surfaces of both ears by an automatic microliter pipette. An amount of 0.02 g of the samples were applied topically just before EPP application. Before and at 15, 30, 45 min and 1 h after edema induction, the thickness of each ear was measured by a vernier caliper. The inhibition percentages of the edema formation of the test samples were calculated and statistically evaluated (t-test, $p < 0.05$) according to the following equation:

$$\text{percentages of edema inhibition} = \left[\frac{T_c - T_t}{T_c}\right] \times 100$$

where T_c is the mean edema thickness of rat ear in the gel base group and T_t is the mean edema thickness of rat ear in the test sample.

3. Results and discussion

3.1. Characteristics of the vesicles

Liposomes with 0–5% ethanol, Tween 61 niosomes with 0–25% ethanol and Span 60 niosomes with 0–20% ethanol gave no sedimentation, no layer separation and unchanged particle sizes at 4, 27 and 45 °C within 3 months (data not shown). The maximum loading of ethanol at 27 ± 2 °C for Tween 61 niosomes was higher than DPPC liposomes and Span 60 niosomes which gave 25, 5 and 20%, respectively. Precipitation was observed with high ethanol contents. This may be due to the high hydrophilicity of the polar head group of Tween 61 which is more compatible with ethanol. When the ethanol content was higher than 25%, aggregation of the Tween 61 vesicles was observed. This may be resulting from the salting out effect of the lipid or non-ionic surfactants from ethanol,

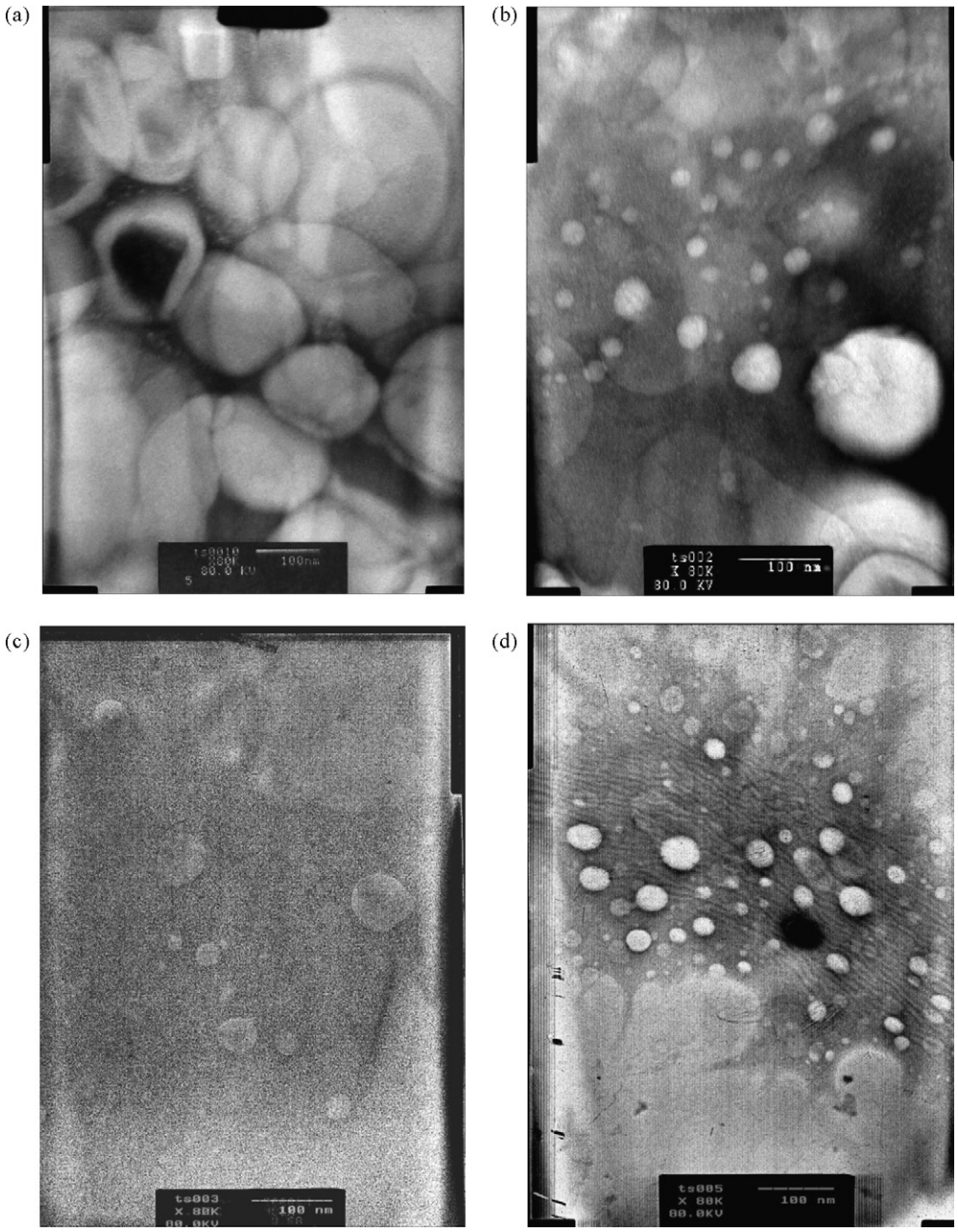

Fig. 2. Negative-staining TEM images of conventional niosomes and elastic niosomes entrapped with DCFD: (a) conventional Tween 61 niosomes (80,000×); (b) elastic Tween 61 niosomes (80,000×); (c) conventional Tween 61 niosomes entrapped with DCFD (80,000×); (d) elastic Tween 61 niosomes entrapped with DCFD (150,000×).

thereby decreasing the water to lipid or surfactants ratio which was not suitable to form the physical stable vesicles. This result agreed with the previous study that vesicles cannot coexist with high concentrations of ethanol (Namdeo and Jain, 1996). Tween 61 niosomes was selected to entrap DCFD because of its highest ethanol loading contents. The effects of ethanol on the mean vesicle size of the Tween 61 niosomal vesicles investigated using DLS, entrapment efficiency, zeta potential and deformability index were demonstrated in Table 1. A decrease in vesicular size was observed when 25% ethanol was incorporated. It has been reported that the

Table 1
Effects of ethanol contents on size, entrapment efficiency, zeta potential and deformability index of niosomes entrapped and not entrapped with DCFD

Formulations	Initial size (nm)	Size after filtration (nm)	Entrapment efficiency	Zeta potential	Deformability index
Niosomes	395.05 ± 14.38	83.55 ± 11.91	–	−48.57 ± 0.38	1.65 ± 0.46
Elastic niosomes (25% ethanol)	256.20 ± 12.73	185.65 ± 13.18	–	−18.37 ± 1.04	5.67 ± 0.88
DCFD niosomes	327.15 ± 6.72	37.82 ± 3.13	64.98 ± 4.49	−32.17 ± 0.81	0.30 ± 0.05
DCFD elastic niosomes (25% ethanol)	224.45 ± 11.95	207.90 ± 13.44	93.15 ± 2.87	−17.73 ± 0.32	4.13 ± 0.53

Values represent mean ± S.D. ($n = 3$).

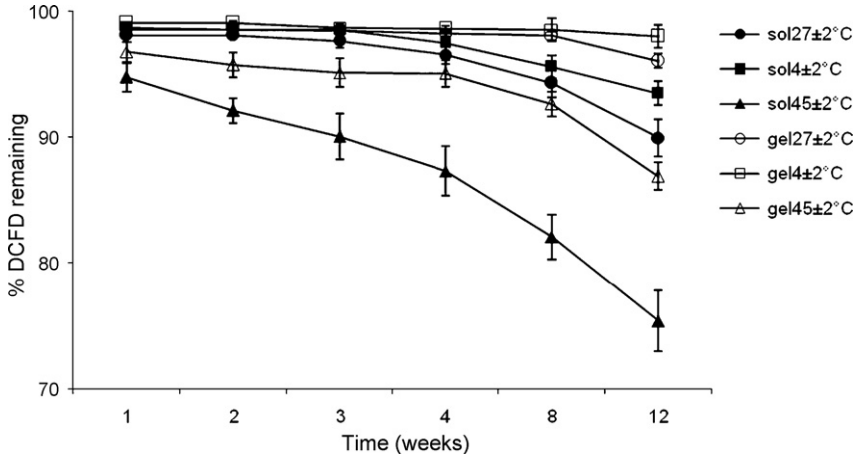

Fig. 3. The percentages of DCFD remaining in the elastic niosomal dispersion (sol) and the gel containing DCFD entrapped in elastic niosomes (gel) at different storage temperatures (27 ± 2, 4 ± 2 and $45 \pm 2\,^{\circ}C$) versus times (weeks).

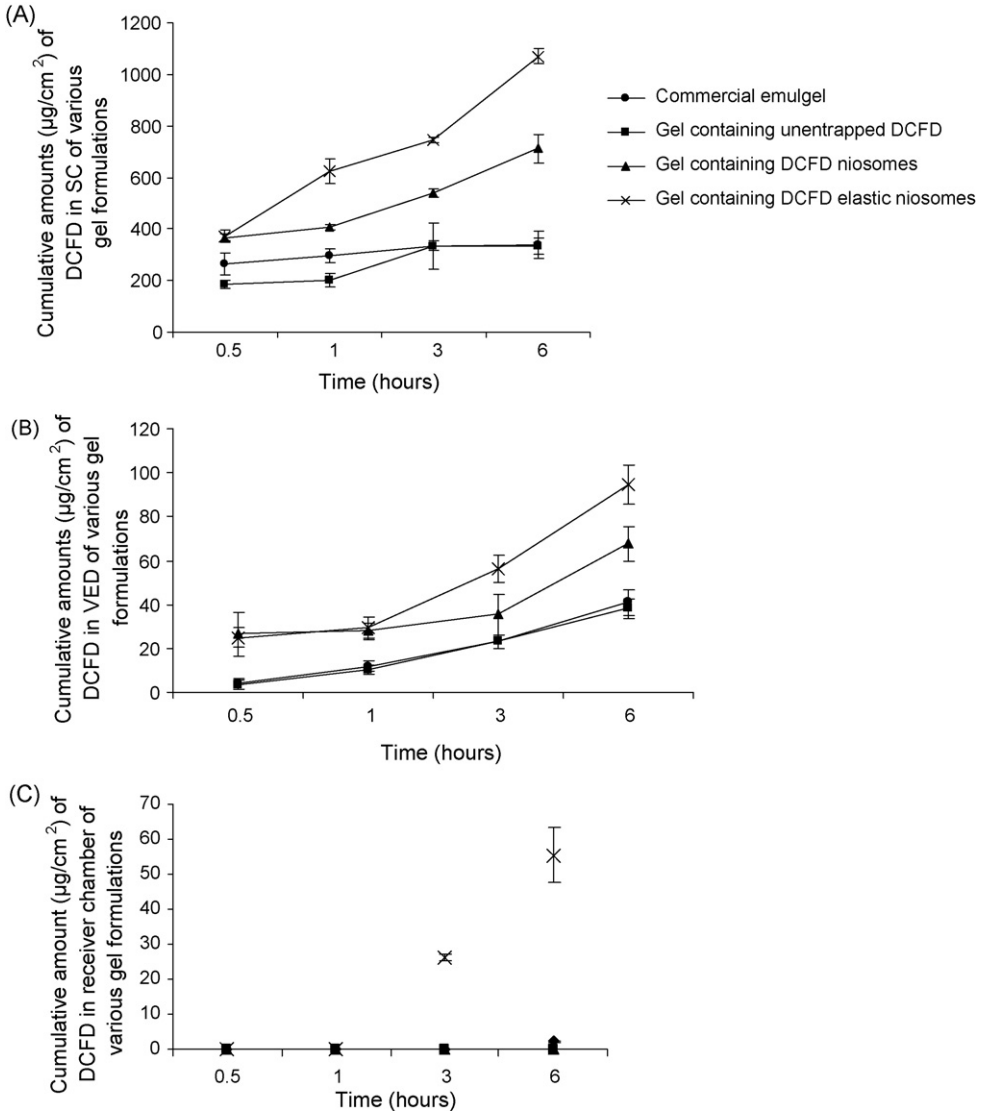

Fig. 4. Cumulative amounts ($\mu g/cm^2$) of DCFD versus time (hours) in SC (stratum corneum) (A), VED (viable epidermis and dermis) (B) and receiver chamber (C) following transdermal absorption across excised rat skin by vertical Franz diffusion cells from various gel formulations. Each value represents the mean ± S.D. ($n = 3$).

Table 2
The cumulative amounts (μg/cm^2) and fluxes (μg/(cm^2 h)) in SC (stratum corneum), VED (viable epidermis and dermis) and receiver chamber following transdermal absorption across excised rat skin by vertical Franz diffusion cells from various gel formulations

Formulation	Amount of DCFD (μg/cm^2)			Flux (μg/(cm^2 h))		
	SC	VED	Receiver chamber	SC	VED	Receiver chamber
Commercial emulgel	340.18 ± 53.52	40.96 ± 5.84	0.81 ± 0.12	60.84 ± 13.63	7.33 ± 1.7	0.14 ± 0.01
Gel containing the unentrapped DCFD	333.62 ± 31.37	38.24 ± 4.54	0	59.67 ± 14.32	6.84 ± 1.46	0
Gel containing the entrapped DCFD in conventional niosomes	711.22 ± 55.35	67.84 ± 7.86	0	127.21 ± 13.75	12.13 ± 1.21	0
Gel containing the entrapped DCFD in elastic niosomes	1069.39 ± 28.14	94.81 ± 8.90	21.01 ± 7.92	191.27 ± 9.52	16.96 ± 2.77	3.76 ± 0.54

Each value represents mean ± S.D. (n = 3).

higher ethanol concentration in the vesicles, the lesser membrane thickness was observed owing to the formation of a phase with interpenetrating hydrocarbon chains (Dubey et al., 2007; Barry and Cullis, 1995). Also, ethanol may cause a modification of the net charge of the system resulting in some degree of steric stabilization that may finally lead to a decrease in the mean particle size (Lasic et al., 1998). Both conventional (327.50 ± 6.72 nm) and elastic niosomes (224.45 ± 11.95 nm) entrapped with DCFD gave smaller mean vesicle sizes than their corresponding unentrapped niosomes (395.05 ± 14.38 and 256.20 ± 12.73 nm, respectively). DCFD which located within the hydrophobic tails of Tween 61 in the vesicles may tighten the bilayer membranes (Friberg and Osborne, 1985). All niosomal formulations exhibited negative charges. The larger the size of the vesicles, the higher the number of charges was observed. Visualized by negative-stain TEM in Fig. 2, both conventional and elastic niosomes entrapped or not entrapped with DCFD were in unilamellar structure. In fact, the presence of a hydrophilic surfactant in the bilayer structure has been reported to form unilamellar vesicles (Cevc et al., 1996). The entrapment efficiency of DCFD in the elastic niosomes (93.15 ± 2.87%) was higher than in the conventional niosomes (64.98 ± 4.49%). This may be due to the solubility enhancement of DCFD by ethanol that facilitates the entrapment of DCFD in the vesicular membrane.

For the effect of ethanol on vesicular deformability, the elastic niosomes entrapped and not entrapped with DCFD showed deformability index of 4.13 ± 0.53 and 5.67 ± 0.88 which were higher than the conventional niosomes entrapped and not entrapped with DCFD (0.30 ± 0.05 and 1.65 ± 0.46) of about 13.76 and 3.44 times, respectively. After 3 months, the percentages of ethanol remained at 4 ± 2, 27 ± 2 and 45 ± 2 °C were 97.98 ± 0.92, 97.24 ± 2.19 and 94.70 ± 0.13, respectively. This has indicated the stability and content uniformity of ethanol in the formulation. Ethanol in the elastic niosomes may interact with the surfactant molecules in the polar head group region, resulting in a reduction in the melting point, thereby increasing the fluidity of the vesicles (Touitou et al., 2000; Dayan and Touitou, 2000). The conventional niosomes (without ethanol) still showed some deformability. This can be explained that, when a surfactant is present in an adequate concentration, it can accommodate to particle shape deformation of bilayer vesicles under stress. The location of a proper amount of surfactant within the lipid bilayer has been shown to provoke a disruption and fluidization of the bilayer itself (Touitou et al., 2000).

The elastic niosomes entrapped with DCFD in the form of dispersion and those incorporated in a gel base gave good physical stability with no sedimentation, no layer separation and no color change at 4 ± 2, 27 ± 2 and 45 ± 2 °C for 3 months. The amounts of DCFD remaining in the elastic niosomal dispersion and those incorporated in the gel were 76 and 87% when stored at 45 ± 2 °C for 3 months, respectively (Fig. 3). At 4 ± 2 °C, no loss of DCFD in both samples was observed. At elevated temperatures, leakage of the drug from the vesicles was due to the fluidity of the vesicular membrane and the chemical degradation of the drug. DCFD entrapped in the vesicles and incorporated in the gel appeared to be more protected against thermal degradation than in the form of vesicular dispersion. The gel structure may retard the leakage of the drug from the vesicles, thereby decreasing the thermal effects on the drugs (Bochot et al., 1998; Ruel-Gariepy et al., 1994; Glavas-Dodov et al., 2002).

3.2. *In vitro transdermal absorption through the excised rat skin of different gel formulations containing DCFD*

The cumulative amounts and the fluxes after 6 h of DCFD per area of different gel formulations investigated by vertical Franz diffusion cells were presented (Table 2 and Fig. 4). The cumulative amounts (μg/cm^2, n = 3) through skin of all DCFD gel formulations increased with times. The gel containing DCFD entrapped in elastic niosomes exhibited the highest amount in SC, VED (viable epidermis and dermis) and the receiving solution comparing to the commercial emulgel and gel containing the unentrapped DCFD and gel containing the DCFD entrapped in conventional niosomes. This formulation showed the amounts of DCFD after 6 h in SC, VED and the receiving solution of 1069.39 ± 28.14, 94.81 ± 8.90 and 21.01 ± 7.92 μg/cm^2 which were 3.14, 2.31 and 25.94 times higher than the commercial emulgel which gave 340.18 ± 53.52, 40.96 ± 5.84 and

Table 3
Effects of various gel containing DCFD on %inhibition of EPP-induced ear edema at various time intervals

Formulations	Time after topical application of EPP							
	15 min		30 min		45 min		60 min	
	T_t (mm)	PI (%)	T_t (mm)	PI (%)	T_t (mm)	PI (%)	T_t (mm)	PI (%)
G1	0.19 ± 0.02	42.42	0.25 ± 0.06	40.48	0.27 ± 0.02	27.03	0.28 ± 0.10	24.32
G2	0.33 ± 0.04	–	0.42 ± 0.03	–	0.37 ± 0.03	–	0.34 ± 0.08	–
G3	0.20 ± 0.05	39.39	0.34 ± 0.03	19.05	0.36 ± 0.07	2.70	0.39 ± 0.04	−5.41
G4	0.22 ± 0.03	33.33	0.32 ± 0.05	23.81	0.34 ± 0.03	8.11	0.36 ± 0.02	2.70
G5	0.22 ± 0.01	33.33	0.32 ± 0.11	23.81	0.33 ± 0.01	10.81	0.34 ± 0.07	8.11
G6	0.20 ± 0.01	39.39	0.32 ± 0.02	23.81	0.31 ± 0.01	16.22	0.30 ± 0.01	18.92

Values are mean ± S.D. (n = 3). T_t: edema thickness; PI: percent inhibition; G1: phenylbutazone; G2: gel base; G3: gel containing the unentrapped DCFD; G4: commercial emulgel; G5: gel containing niosomal vesicles entrapped with DCFD; G6: gel containing elastic niosomal vesicles entrapped with DCFD.

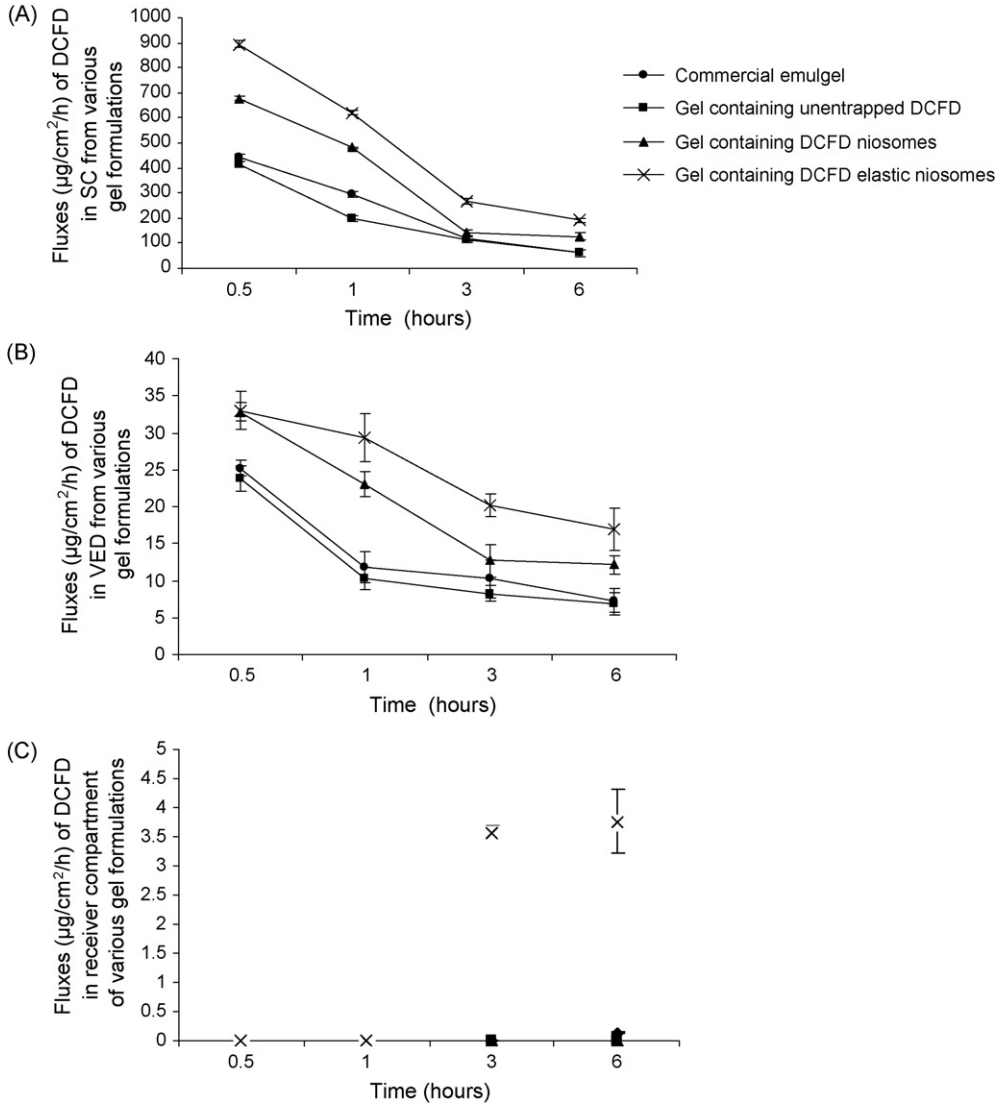

Fig. 5. The fluxes (μg/(cm² h)) of DCFD in SC (stratum corneum) (A), VED (viable epidermis and dermis) (B) and receiver chamber (C) versus time (hours) following transdermal absorption across excised rat skin by vertical Franz diffusion cells from various gel formulations. Each value represents the mean ± S.D. ($n = 3$).

0.81 ± 0.12 μg/cm², respectively. The fluxes (μg/(cm² h), $n = 3$) of all gels containing DCFD decreased with times (Table 2 and Fig. 5). The gel containing DCFD entrapped in elastic niosomes exhibited the highest flux after 6 h in SC, VED and receiving solution at 191.27 ± 9.52, 16.96 ± 2.77 and 3.76 ± 0.54 μg/(cm² h) which were 3, 2.3 and 27 times higher than the commercial emulgel which gave 60.84 ± 13.63, 7.33 ± 1.70 and 0.14 ± 0.01 μg/(cm² h), respectively. The fluxes through rat skin in SC and VED after 6 h of DCFD entrapped in conventional niosomes or elastic niosomes incorporated in gel were significantly different from the unentrapped DCFD in gel and the commercial emulgel ($p < 0.05$). The fluxes of DCFD in the gel containing the unentrapped drug were close to that of DCFD in the commercial emulgel. No DCFD was found in the receiving solution of the gel containing DCFD not entrapped and entrapped in the conventional niosomes. This result has supported that conventional niosomes are usually not efficient to transdermally delivery across the skin, because they do not deeply penetrate the skin, but rather remain on the upper layer of SC and in the skin (VED). This has also indicated that transdermal absorption through rat skin of DCFD was enhanced by entrapping in elastic niosomes, which may be due to not only the suitable polarity for transdermal absorption of the drug modified by the arrangement of the DCFD molecule in the elastic vesicular membrane and in the inner hydrophilic core of the bilayer vesicles, but also the deformability of the vesicles as well. Gel containing DCFD entrapped in the conventional niosomes and elastic niosomes showed 1.77 and 2.45 times higher fluxes in VED, respectively, than the gel containing the unentrapped DCFD. A synergistic mechanism between ethanol, vesicles and the skin lipids was suggested (Touitou et al., 2000). Ethanol may provide with soft flexible characteristics which allow the vesicles to easily penetrate into deeper layers of the skin. It was also proposed that ethanol may penetrate into the skin and influence the bilayer structure of SC leading to the enhancement of drug penetration (Kirjavainen et al., 1999).

3.3. *In vivo anti-inflammatory activity of different gel formulations containing DCFD*

The developed gel containing DCFD entrapped in elastic niosomes showed %inhibition of rat ear edema after 1 h of application higher than the commercial emulgel, gel containing DCFD entrapped in conventional niosomes and gel containing the unen-

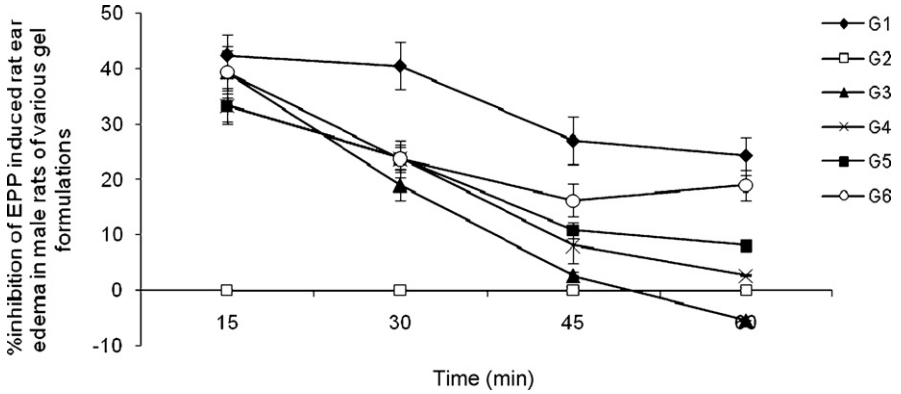

Fig. 6. The plot of the %inhibition of EPP-induced rat ear edema of phenylbutazone (G1), gel base (G2), gel containing the unentrapped DCFD (G3), commercial emulgel (G4), gel containing conventional niosomal vesicles entrapped with DCFD (G5) and gel containing elastic niosomal vesicles entrapped with DCFD (G6).

trapped drug of 16.22%, 10.81% and 24.33%, respectively, but lower than phenylbutazone of 5.4% (Fig. 6 and Table 3). This result was in the same trends as the fluxes of DCFD in this formulation observed in VED (Table 2). This has indicated the *in vivo* anti-inflammatory activity enhancement of DCFD when entrapped in niosomes, especially the elastic niosomes. The commercial emulgel gave a little superior rat skin transdermal flux and % ear edema inhibition than the gel containing the unentrapped drug, since the commercial emulgel which did not contain any bilayer vesicles, may have some transdermal absorption enhancers.

4. Conclusion

This study has demonstrated that the gel containing the novel Tween 61 elastic niosomes entrapped with DCFD did not only show physical and chemical stability for 3 months, but also high fluxes through rat skin and high anti-inflammatory activity in rat ear edema assay. This optimized developed gel can offer a promising formulation for DCFD in the topical non-invasive treatment of inflammation.

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