



Niosomes loaded with diclofenac for transdermal administration: Physico-chemical characterization, *ex vivo* and *in vivo* skin permeation studies

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ABSTRACT

The objectives of this study were to prepare and characterize niosomes loaded with diclofenac (DiC), to evaluate skin permeation of DiC niosomes by *ex vivo* and *in vivo* studies. DiC niosomes were prepared by ethanol injection method using nonionic surfactant Span 80 and cholesterol in different molar ratios. The vesicle size and polydispersity index (PDI) of DiC niosomes were determined by dynamic light scattering method. Niosomal vesicles were also investigated by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and field emission scanning electron microscopy studies. The transdermal permeation of niosomal hydrogel was assessed in both *ex vivo* and *in vivo* studies on rats. The results showed that DiC niosomes were spherical particles in shape with the average vesicle size of less than 100 d.nm; PDI was smaller than 0.2. Results of DSC and FTIR showed that the drug no longer remained in its crystalline form after niosomes had been formed; it might disperse in the bilayer of niosomes. The data of *ex vivo* and *in vivo* experiments suggested that DiC niosomal hydrogel not only improved the amount and rate transport of DiC through the skin but also increased drug concentration in the muscle in comparison to the commercial drug.

INTRODUCTION

Transdermal drugs have high bioavailability on the site of action because of absence of the first-pass metabolism of oral drugs (Davies and Anderson, 1997). Importantly, transdermal drug deliveries of non-steroidal anti-inflammatory drugs (NSAIDs) were developed to decrease the side effects, associated with oral NSAIDs, on gastrointestinal, cardiovascular, and renal systems (Altman *et al.*, 2015; Evans *et al.*, 1995). Although some of the NSAIDs are absorbed into the systemic circulation from the dermal site of application, systemic exposure to drugs is decreased. Transdermal NSAID is commonly used to relieve pain and inflammation. Therefore, efficacy of therapy by transdermal drug is dependent on its ability to penetrate the skin and permeate to the target tissues. Different approaches were applied for their enhancer action, such as phospholipid micelles (Duan

et al., 2015), liposomes (Cosco *et al.*, 2015), niosomes (Tavano *et al.*, 2013), polymers, lipotropic liquid crystals (Estracanholli *et al.*, 2014), and surfactants (Som *et al.*, 2012).

Vesicular systems such as liposomes and niosomes represent an important drug delivery system. The advantages of niosomes over liposomes include enhanced chemical and physical stability, lower cost, and ease of use of surfactants (Moghassemi and Hadjizadeh, 2014). Niosomes are classified as vesicles containing the bilayer of nonionic surfactants around the aqueous core (Sankhyan and Pawar, 2012; Onochie *et al.*, 2013). The vesicular structure of niosomes presents an advantage with ability to load both hydrophilic and lipophilic drugs (Alomrani *et al.*, 2015; Manca *et al.*, 2014; Pando *et al.*, 2015; Tavano *et al.*, 2013). Niosomes is a very beneficial drug delivery system with several applications (Mahale *et al.*, 2012; Moghassemi and Hadjizadeh, 2014). Niosomal vesicles were investigated to enhance the transdermal drug permeation for several active pharmaceutical ingredient (Auda *et al.*, 2016; El-Ridy *et al.*, 2017; Hagen and Baker, 2017; Ioele *et al.*, 2015).

Diclofenac sodium (DiC) is an NSAID, which is administered by the oral, intravenous, intramuscular, and topical

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routes to relieve pain and inflammation in humans (Hagen and Baker, 2017). Oral dosage forms of DiC have been proven to be less effective and tolerated than transdermal DiC in the treatment of osteoarthritis (OA) (Lin et al., 2004; Mason et al., 2004; Rannou et al., 2016). Therefore, percutaneous dosage forms of DiC are recommended before the use of oral NSAIDs in certain international guidelines for OA of the knees or hands (Bruyère et al., 2014; Zhang et al., 2007). In a preference study, the amount of OA patients who choose to use a topical rather than oral NSAID was almost three times higher; particularly elderly were more concerned about side effects related to oral drugs (Carnes et al., 2008; Underwood et al., 2008).

The objective of the present study is to develop transdermal niosomal hydrogel of DiC to improve the skin permeation of drug. The current research work comprises an *in vitro* evaluation including preparation and characterization of DiC niosomes, as well as *ex vivo* and *in vivo* skin permeation studies.

MATERIALS AND METHODS

Materials

DiC sodium was purchased from Sinochem Ningbo (China); cholesterol was purchased from MP Biomedicals North America (US); Span 80 was purchased from Spectrum Chemical (US); ethanol 96% (*v/v*) was purchased from DG Chemical Company (Vietnam). Carboxymethylcellulose sodium (CMC sodium) was purchased from Sigma Aldrich (US); glycerin was purchased from Puyer Biopharma (China). Methanol for high performance liquid chromatography (HPLC) was purchased from J.T. Baker (US).

Animal

Adult male rats were used; each weighed between 200 and 250 g [the National Institute of Drug Quality Control (Vietnam)]. The rats were housed on a 12 hours light/dark cycle in a temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and relative humidity of $55\% \pm 15\%$ in controlled room with commercial food and water *ad libitum*. The animals were allowed to acclimatize for at least 7 days before the start of the experiment. The protocol of the animal experiments was approved by the Animal Care and Use Committee of the Hanoi University of Pharmacy, Vietnam.

Method preparation of DiC niosomes

DiC niosomes were prepared by ethanol injection method. The solvent phase was prepared by dissolving of Span 80, cholesterol, and DiC in the 10 ml of ethanol 96% and then heated up to 65°C . Solvent phase was injected in the purified water at 65°C with magnet stirring. The mixture was evaporated under reduced pressure at 40°C using rotary evaporator R25 (Buchi, Germany) to remove ethanol to form niosomal suspension.

Drying. The suspension of DiC niosomes was dried in the vacuum oven at 40°C for 48 hours for differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) characterization. Dry samples less than 2% moisture were stored in the refrigerator at $2^{\circ}\text{C}-8^{\circ}\text{C}$.

DiC niosomes characterization

Size analysis

Vesicle size and distribution of vesicle size were evaluated by dynamic light scattering method using Zetasizer

ZS90 (Malvern, UK). Each sample was diluted with purified water until the count rate in the range of 200–300 kcps. The results of analysis give a mean vesicles size and a polydispersity index (PDI) and characterized the width parameter of size distribution.

Determination of DiC content in the niosomal suspension

Quantitative analysis of DiC in the niosomal suspension was determined by UV-visible spectrography at 276 nm, the maximum wavelength of DiC aqueous solution. Linearity was determined from six working standard solutions of DiC. The correlation R^2 and the slope of standard curve were calculated.

Determination of entrapment efficiency

Free DiC was removed from niosomal suspension by filtration through Vivaspin 50 kDa molecular weight cut off (GE Healthcare, US). Accurately, 1 ml of DiC niosomal suspension was poured in the vivaspin and then was centrifuged at 5,000 rpm for 30 minutes. The filter solution at the bottom was collected to determine the free amount of DiC by UV-visible spectrography as described above. The total amount of DiC was determined in the niosomal suspension prior filtration. The entrapment efficiency (EE%) was calculated by the following equation:

$$\text{EE (\%)} = (m - m_1) / m \times 100\%,$$

where m is the total amount of DiC and m_1 is the unencapsulated amount of DiC.

Morphology of DiC niosomes

Morphology of DiC niosomes was observed by Field Emission Scanning Electron Microscope. The sample was put on a plate and continued drying in vacuum oven at 40°C for 48 hours. The dried sample was coated with gold and observed in the field emission scanning electron microscopy (FESEM) S4800 (Hitachi, Japan).

Evaluation of DiC niosomal hydrogel

Preparation of DiC niosomal hydrogel and DiC hydrogel

DiC niosomal hydrogel 1% (*w/v*): CMC sodium was put in the suspension of DiC niosomes to form gel. Methylparaben was dissolved in the glycerin and then continued mixing with the gel.

DiC hydrogel 1% (*w/v*): CMC sodium was put in the water to form gel. Methylparaben and DiC were dissolved in the glycerin and then continued mixing with the gel.

Placebo hydrogel: CMC sodium was put in the water to form gel. Methylparaben was dissolved in the glycerin and then continued mixing with the gel.

Ex-vivo skin permeation study

Skin preparation. To evaluate the skin permeation of DiC niosomal hydrogel, formulations were subjected to *ex vivo* permeation study. Back skins were obtained from rats. A 2.0 cm \times 2.0 cm patch of hair-removal skin was excised, continuing removing subcutaneous fat and connective tissue. To achieve higher reproducibility, the integrity of the excised skins was confirmed under an optical microscope before experiment to verify that there was no scratch or injury on the stratum corneum-epidermis layer. Then, the excised skins were stored at $2^{\circ}\text{C}-8^{\circ}\text{C}$ for later use (Butani et al., 2014; El-Ridy et al., 2017).

Ex-vivo permeation study. The *ex vivo* permeation study of DiC niosomal hydrogel was evaluated using hair-free back skin of the rat. Dynamic Franz diffusion cell was used for skin permeation study of DiC niosomal hydrogel; the effective diffusion area was 1.767 cm². The phosphate buffer solution (7 ml) was used as a receptor in continuous stirring condition. The prepared skin was placed at the diffusion area between donor and receptor compartments. Experiments were carried out at 37°C to guarantee that the temperature of skin surface was 32°C ± 0.5°C. About 0.5 g of the experiment hydrogel (corresponding to 5 mg DiC) was applied on the epidermal side of the rat skin and 0.5 ml of the receptor medium was got after predetermined period of time at 1, 2, 3, 4, 5, 6, 7, and 8 hours for DiC determination by HPLC method.

In vivo skin permeation study

Experimental groups for determination of concentrations of DiC in rats. Before the initiation of the *in vivo* skin permeation study, rats were divided into three groups; each group contained eight rats. The animals were fed three times a day and had a free access to water. Before experiments, rats were inhaled with ether, and then hair of back thigh was removed, where rats were applied experiment hydrogels on. For the first group, rats were applied DiC niosomal N hydrogel 1%; rats of the second group were applied commercial prodrug of DiC sodium, Voltaren Emulgel 1%; rats in the third group were applied placebo hydrogel. DiC at dose 50 mg/kg was applied topically in rats of the first and second groups.

Thigh muscle and thigh skin were collected at the 1.5 hours after administration from four rats of each group. Blood was collected 1.5 hours after hydrogel application from four rats of each group. DiC concentrations in tissue and blood were quantified by HPLC in all rats of three groups.

Blood sampling. At the 1.5 hours moment after hydrogel application, the blood samples from the rats were collected retroorbitally. About 1 ml of blood was withdrawn with capillary and placed in the clean heparinized tube. Blood samples were centrifuged for 10 minutes at 4,000 rpm (Hanza, Korea) to receive the plasma samples, which were stored in a freezer at -20°C for later analysis.

Muscle/skin sampling. At the 1.5 hours moment after the application of hydrogels, rats were sacrificed with chloroform. Thigh muscle and skin were excised from the appropriate site using sterile surgical equipments. Skin was stripped and cleaned from subcutaneous fat. The excision of the tissue sample was weighed and stored at -20°C to be further homogenized for drug analysis.

Muscle sample homogenization

Muscle sample was thawed before the experiment and then was exactly weighed 1 g. The tissue was homogenized using Wise StirHS 30 E (Germany), following the addition of 0.9% sodium chloride solution to form 10 ml suspension. Later, the sample was sonicated for 10 minutes by Q 500 Sonicators (Qsonica, US) and then was centrifuged at 10,000 rpm by Hanil Supra R22 (Korea). DiC in the supernatant was extracted by a liquid-liquid extraction method using n-hexane as extract solvent.

Skin extraction. The cumulated amount of DiC in the rat skin was determined by cutting the striped skin into small pieces and put in a 5 ml vial, which contained absolute ethanol, with 5 minutes sonication for complete extraction of DiC. The validated extraction method showed that the recovered amount of DiC from the stripped skin was more than 90%.

Extraction of muscle samples. The tissue supernatant (500 µl) of each animal was added to each tube; 500 µl of phosphoric acid 1 M was added in each tube; and the sample was vortexed for 2 minutes; 500 µl of acetone was added, and the sample was vortexed for 2 minutes; 5 ml of n-hexane was added, extraction was carried out by homogenization (vortex) for 5 minutes, and then the tube was centrifuged at 5,500 rpm for 10 minutes to extract upper organic phase to another clean glass tube. The sample was dried by evaporation under nitrogen flow at 45°C; the dried residue was diluted with 1 ml of the mobile phase to form a sample solution, which was analyzed to determine the concentration of DiC by HPLC method.

Extraction of plasma samples. The plasma was extracted by the method like the extraction method of muscle samples. The plasma (500 µl) of each animal was added to each tube; 500 µl of phosphoric acid 1 M was added in each tube; the sample was vortexed for 2 minutes; 500 µl of acetone was added, and the sample was vortexed for 2 minutes; 5 ml of n-hexane was added, extraction was carried out by homogenization (vortex) for 5 minutes, and then the tube was centrifuged at 5,500 rpm for 10 minutes to extract upper organic phase to another clean glass tube. The sample was dried by evaporation under nitrogen flow at 45°C; the dried residue was diluted with 1 ml of the mobile phase to form sample solution, which was analyzed to determine the concentration of DiC by HPLC method.

HPLC method for determination of DiC concentration in biological tissue muscle. Quantitative analysis of DiC in the biological tissue was performed by high-performance liquid chromatography (HPLC-Agilent, US). The analytical column was C18 (150 × 4.6 mm, 5 µm) (Cosmosil, Japan); the operation was carried out at ambient temperature. The mobile phase was a mixture of methanol and phosphate buffer pH 2.5 in a ratio of 80:20 (v/v), the flow rate was 1.0 ml/minute, the detector was UV-visible at 254 nm, and the volume of injection was 50 µl. The sample was filtered through nylon membrane with pore size of 0.45 µm before injection to the column. Under the HPLC condition, the retention time of DiC was about 7.1 minutes. HPLC linearity was determined from six working standard solutions of DiC. HPLC method was validated by specificity, accuracy, precision, linearity, lower limit of detection, and lower limit of quantification.

Statistical analysis

The cumulative amount of DiC permeated (mg.cm⁻²) through excised rat skin was calculated by (Zhu et al., 2008)

$$Q_n \text{ (mg. cm}^{-2}\text{)} = \frac{V_0 \times C_n + \sum_{i=1}^{n-1} C_i \times V_i}{S}, \quad (1)$$

where C_n (mg/ml) is the drug concentration of the solution in the receptor compartment at each sampling time (minimum $n = 1$; maximum $n = 8$), C_i is the drug concentration of the i th sample (minimum $i = 1$; maximum $i = 7$; $i = n-1$), and V_i (ml) and V_0 (ml) are the volumes of the sample and the solution in

the receptor compartment, respectively. S is the effective diffusion area ($S = 1.767 \text{ cm}^2$).

The permeation rate of DiC at a steady state (J_{ss} , mg. $\text{cm}^{-2}.\text{hours}^{-1}$) through the rat skin was calculated from the slope of linear portion of the plots of Q_t versus time (Butani et al., 2014; Das et al., 2017).

The results of all experiments were presented as an average \pm SD. The data of all *in vivo* skin permeation experiments were analyzed by one-way analysis of variance with Tukey's post hoc test. A p -value of < 0.05 was considered to be significant.

RESULTS AND DISCUSSIONS

Characterizations of DiC niosomes

Characterization of DiC niosomes is composed of Span 80 and cholesterol (chol) at molar ratios of 5:5 and 6:4 shown in Table 1.

As the results shown in Table 1, mean diameters of niosomal vesicles formulations M and N are smaller than 100 nm; they are distributed in the narrow range with PDI smaller than 0.2. FESEM image of DiC niosomes in Figure 1 shows that DiC niosomes are spherical; they have diameter in the range of 80–150 nm. Ethanol injection method was used to prepare niosomes loaded DiC to form small vesicles with diameter less than 100 nm, distributed in the narrow range with PDI smaller 0.2. Entrapment efficiency of niosomes of N formulation was higher than M formulation. But both N and M DiC niosomes had entrapment efficiency less than 50%, which is correlated with the solubility of drug in the aqueous phase. In some different researches, encapsulation of DiC increased by incorporation of positive-charge agents likely stearylamine to form lipophilic ion-pair between DiC and stearylamine. However, in this case, entrapment efficiency was not higher than 50% (Abdallah et al., 2013).

FTIR

Figure 2 presents the IR spectra of Span 80, cholesterol, sodium DiC, and niosomes of M and N formulations.

The DiC spectrum (Fig. 2E) exhibits broad NH peaks at 3,440.39 cm^{-1} . The Span 80 spectrum (Fig. 2A) presents peak at 3,438.46 cm^{-1} (O-H stretching) and 1,737.55 cm^{-1} (C=O stretching). The broad peak at 3,438.46 cm^{-1} shows intermolecular hydrogen bond (Pretsch et al., 2009). The cholesterol exhibits broad OH peak at 3,422 cm^{-1} . The peak at 1,737.55 (C=O stretching) is changed in the niosomal samples, indicating their involvement in the formation of niosomal vesicles. This could be due to hydrogen bond between C=O of Span 80 and N-H and O-H groups of DiC and cholesterol, respectively.

Differential scanning calorimetry

Figure 3 presents the DSC thermograms of DiC, cholesterol, and DiC niosomes of M and N formulations. DiC exhibits characteristic endotherm peaks at 89.58°C, indicating its crystal nature. The cholesterol displays an endotherm at 133.23°C corresponding to its melt temperature. The DiC and cholesterol had peak at 52.79°C, indicating the vaporizing process of water. The DSC curve of DiC niosomes of M and N formulations showed that endothermic peaks of cholesterol and drug have disappeared, which indicated that the DiC and cholesterol dispersed in the niosomal membrane. The DSC results suggested that some weak hydrogen bonds or Vander Waals forces are formed between DiC, Span 80, and cholesterol (Jena et al., 2014; Ma et al., 2014; Singh et al., 2014, 2012; Yanyu et al., 2006).

Ex-vivo skin permeation study

M hydrogel 1%, N hydrogel 1%, DiC hydrogel 1%, and Voltaren Emulgel 1% (all hydrogels contained 1% DiC w/v) were used to evaluate *ex-vivo* skin permeation of drug. M, N, and DiC hydrogels 1% were prepared following formulations, shown in Table 2. All hydrogels were homogenized and had an elegant appearance. The cumulative amount of DiC, permeated through rat skin and permeation rate on steady state of the evaluated hydrogels, is given in Figure 4.

As illustrated in Figure 4A, cumulative amounts of DiC, released from the N and M niosomal hydrogels after 8 hours, were 0.74 and 0.44 mg/cm², respectively, which were higher than those of Voltaren Emulgel and DiC hydrogel (0.15 and 0.02 mg/cm², resp.). As reported in Figure 4B, the rates permeation (flux) of N and M hydrogels was higher than that of DiC hydrogel and Voltaren Emulgel. Specifically, the flux of DiC from N hydrogel (0.1017 mg.cm⁻².hours⁻¹) was about thirty times and five times that obtained with DiC hydrogel (0.0034 mg.cm⁻².hours⁻¹) and commercial formulation (0.0218 mg.cm⁻².hours⁻¹), respectively. These results have explained that the lower drug permeation observed from DiC hydrogel and commercial product in comparison with niosomal hydrogel proved the permeation enhancing effects of niosomal vesicles on drug delivery. Several excipients, such as surfactants, polyol, and alcohol in the Voltaren Emulgel®, play as percutaneous permeation enhancers. For this reason, the cumulative permeated DiC reached intermediate values, in comparison to the lower value of the DiC hydrogel prepared with only glycerol and CMC sodium. However, the presence of a raised amount of surfactant in the form of vesicular systems leads to the higher percutaneous penetration of DiC (Iole et al., 2015). Enhancing skin permeation of drug from niosomal hydrogel was observed in the different studies (Iole et al., 2015;

Table 1. Particle size, distribution, and entrapment efficiency of DiC niosomes at different molar ratios of Span 80 and cholesterol ($n = 4$).

Formulations	Molar ratio Span 80: chol:DiC	Z_{average} (d.nm)	PDI	Entrapment efficiency (%)
M	6:4:7	97.72 10.2	0.198 \pm 0.011	35.58 \pm 1.1
N	5:5:7	81.82 \pm 9.1	0.184 \pm 0.009	42.38 \pm 1.3

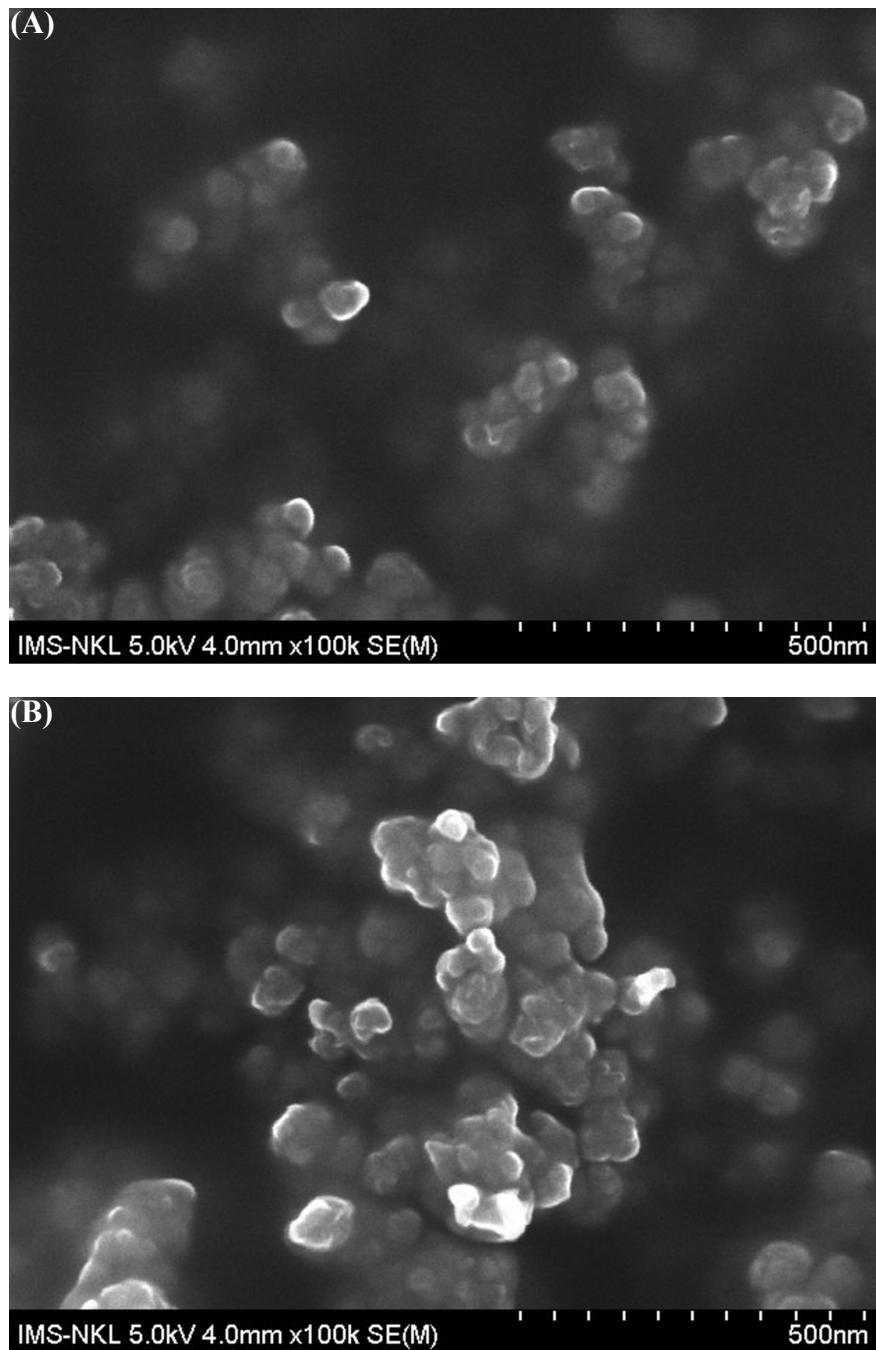


Figure 1. FESEM of DiC niosomes of M formulation (A) and N formulation (B).

Junyaprasert *et al.*, 2012; Manca *et al.*, 2014; Pando *et al.*, 2015; Tavano *et al.*, 2013).

***In vivo* skin permeation study**

Data in Table 3 shows that DiC from Voltaren Emulgel and N niosomal hydrogel could be depth permeated through skin and subcutaneous tissue and finally was observed in the blood after dermal administration. In Voltaren Emulgel, diethylammonium salt of the drug and several skin permeation enhancers, such as ethanol, polyalcohol, and surfactant, are used to penetrate the skin barrier. Several pieces of literature discussed that topical dosage

form containing DiC overcomes through the skin and permeates to the muscle (Azevedo *et al.*, 2013; Brun *et al.*, 2016; Hagen and Baker, 2017).

In our study, T_{\max} (blood concentration) of N niosomal hydrogel and Voltaren Emulgel was observed at 1.5 hours after hydrogel application in rats (data not public). Therefore, at the moment of 1.5 hours after dermal administration of N niosomal hydrogel and Voltaren Emulgel, concentrations of DiC in the skin, muscle, and blood were shown in Table 3. The data show that drug concentration in the rat skin of the N hydrogel group was statistically higher by about three times than that in the Voltaren

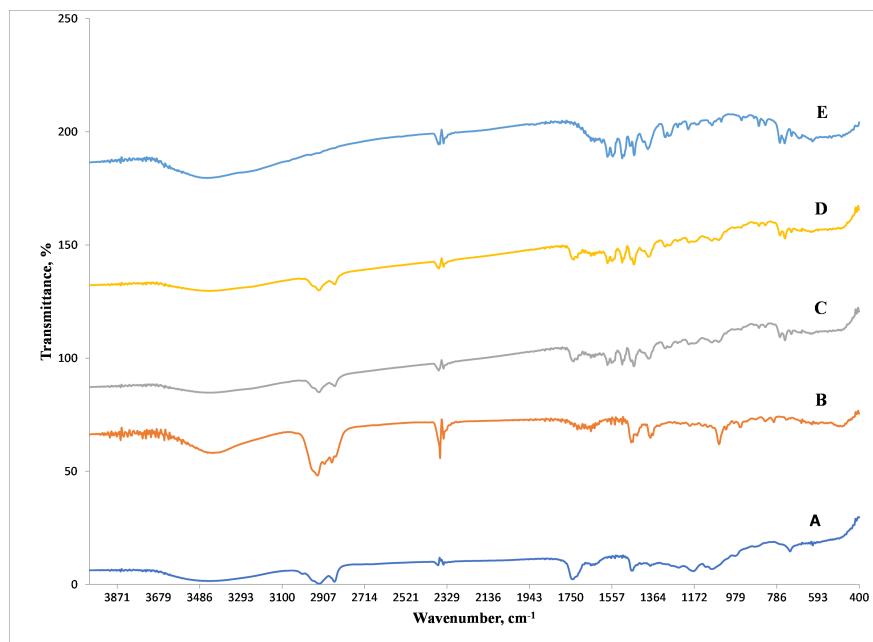


Figure 2. FTIR spectra of Span 80 (A), cholesterol (B), DiC niosomes of M formulation (C), DiC niosomes of N formulation (D), and diclofenac sodium (E).

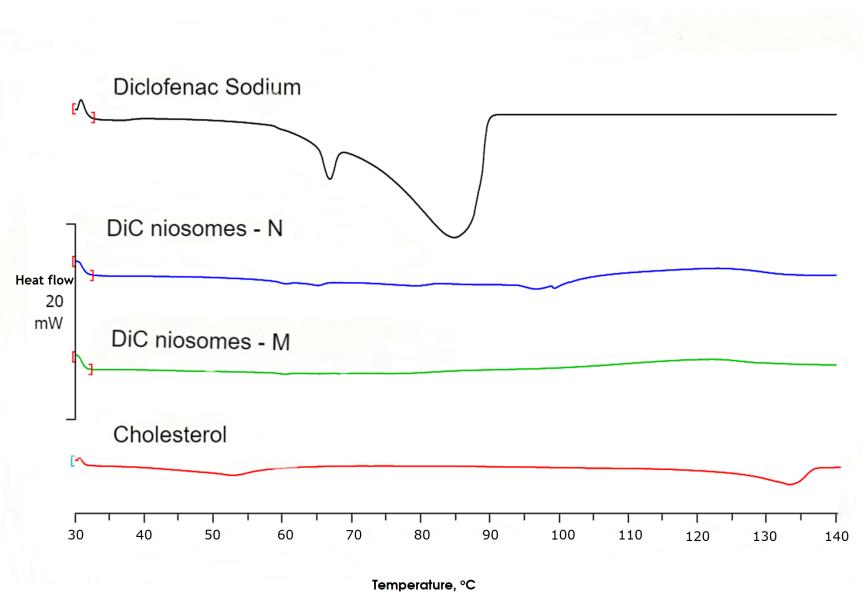


Figure 3. DSC thermograms of DiC (A), cholesterol (B), DiC niosomes of M formulation (C), and DiC niosomes of N formulation (D).

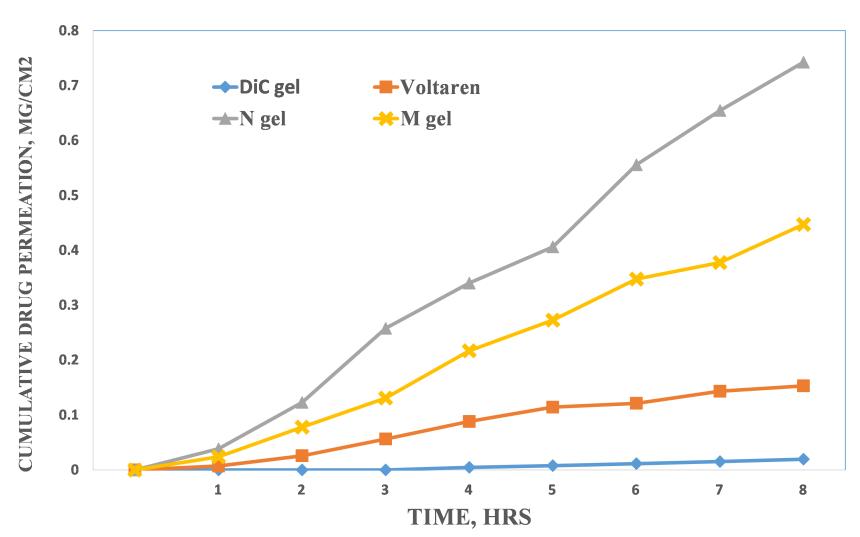
Emulgel group, *p*-value < 0.005. DiC concentration in the rat muscle of Voltaren Emulgel group was statistically lower by about 1.8 times than that of N niosomal hydrogel group, *p*-value < 0.005. Blood concentrations of drug in rats of Voltaren Emulgel and N niosomal hydrogel groups were not statistically different (21.24 µg/ml of Voltaren Emulgel group and 26.02 µg/ml of N niosomal group), *p* > 0.05.

The data in Table 3 suggest that niosomal vesicles not only increase the permeation of DiC through the skin but also accumulate into the skin and they also carry a higher drug amount into the tissues (muscle) under the application site. However, the drug concentration of DiC in the blood was high because of good DiC solubility in water. The results of *in vivo* skin permeation study indicated that niosomal vesicles loaded with DiC had magnitude

Table 2. Formulations of M, N, and DiC hydrogels 1% (w/v).

Excipients	Role	M hydrogel	N hydrogel	DiC hydrogel
CMC sodium (g)	Gel formation	1.57	1.57	1.57
Glycerol (g)	Humectant	5.0	5.0	5.0
Methylparaben (g)	Preservative	0.15	0.15	0.15
DiC (g)	API	Niosomal suspension of M formulation, equivalent to 1.0 g	Niosomal suspension of N formulation, equivalent to 1.0 g	1.0
Deionized water (ml)	Solvent	q.s.100	q.s.100	q.s.100

(A)



(B)

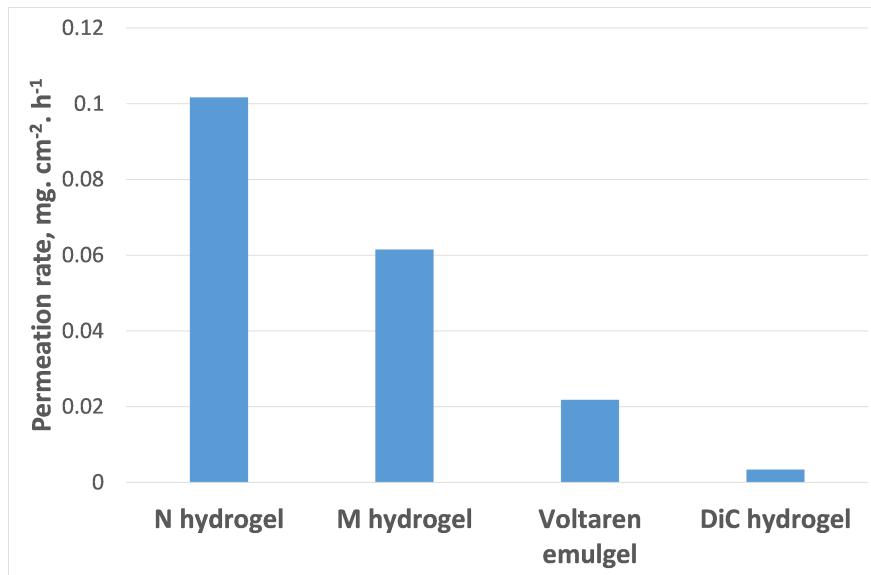
**Figure 4.** (A) Cumulative amount of DiC, permeated through rat skin; (B) permeation rate on steady state (Jss).

Table 3. DiC concentration in the skin, muscle, and blood of rats ($n = 4$).

DiC concentration	Placebo hydrogel	Voltaren Emulgel	N niosomal hydrogel
Skin ($\mu\text{g/g}$)	0.00 ± 0.00	120.4 ± 31.1	$362.4 \pm 43.2^{\text{a,b}}$
Muscle ($\mu\text{g/g}$)	0.00 ± 0.00	0.20 ± 0.04	$0.36 \pm 0.07^{\text{a,b}}$
Blood ($\mu\text{g/ml}$)	0.00 ± 0.00	21.24 ± 3.45	$26.02 \pm 4.56^{\text{a}}$

^aStatistically significant differences of DiC concentrations between Voltaren Emulgel, N niosomal hydrogel, and placebo hydrogel groups, $p < 0.001$.

^bStatistically significant differences of DiC concentrations between Voltaren Emulgel and N niosomal hydrogel groups, $p < 0.005$.

higher DiC concentration in the target tissue (muscle) than using the administration of commercial Voltaren Emulgel.

CONCLUSIONS

DiC niosomes, prepared by simple ethanol injection method, had spherical morphology and small diameter about 100 nm, distributed in a narrow range. Data of *ex vivo* and *in vivo* experiments suggested that DiC niosomal hydrogel not only improved the amount and rate transport of DiC through the skin but also increased drug concentration in the muscle in comparison to the commercial drug. These results strongly emphasized the potential application of DiC niosomes in transdermal drug delivery.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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None.

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