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Innovative topical niosomal gel formulation containing diclofenac sodium (niofenac)

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Abstract

The purpose of this research was to enhance the transdermal delivery of diclofenac sodium niosomal formulations. To characterise the obtained niosomes, SEM, XRPD, DSC and ATR-FTIR were employed. The size of the niosomes increased from 158.00±6.17 to 400.87±4.99 nm when cholesterol was incorporated into the formulations. It was observed that the zeta potential of niofenac varies from -25.40±1.352 to -43.13±1.171 mV when the cholesterol percentage decreased from 2% to 0.2%. The higher entrapment efficiency percentage (63.70±0.18%) was obtained for the formulations with larger particle sizes and higher cholesterol content. The optimised niofenac formulation showed a controlled release fashion where 61.71±0.59% of the drug released within 24 h. The results showed that the value of permeated diclofenac sodium through the skin layers was higher for the niofenac gel formulation (242.3±31.11 μg/cm^2) compared to simple gel formulation (127.40±27.80 μg/cm^2). Besides, niofenac formulation outperformed the anti-inflammatory activities in the formalin test compared to the control and diclofenac simple gel group. The licking time was significantly lower in both early (40.2±7.3 s) and late stages (432.4±31.7 s) for niofenac compared to conventional formulation (early stage 130.4±8.73 s and late stage 660.6±123.73 s). This study indicates that niosomal formulations can improve drug therapeutic effects by increasing drug delivery to specific sites.

Keywords: Niosomes, Green technology, Diclofenac sodium, Solid-state analysis, Anti-inflammatory

Introduction

Critical goals to reduce the severity of inflammation symptoms are comprised of pain relief and inflammation reduction [1]. To achieve this goal, generally, non-steroidal anti-inflammatory drugs (NSAIDs) have been proposed among the most commonly applied therapeutics to reduce pain and inflammation [2,3].

Diclofenac sodium (DS), known as a potent NSAID with pronounced analgesic properties, has been so far utilised in the prolonged treatments of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. The drug is considerably metabolised in the liver and has to be administered frequently since its biological half-life is only 1 to 2 hours [4]. Gastrointestinal consequences such as ulcer, bleeding, or perforation of the intestinal walls have thus been reported with the long term use of DS [5]. Because of its shorter biological half-life as well as adverse effects, it is considered as a perfect candidate for controlled transdermal drug delivery [6-8].

It should be noted that the transdermal application of drugs has many benefits over the oral and intravenous routes. These include the absence of first-pass metabolism and the incidence of systemic toxicity [9]. In addition, transdermal patches showing the systemic effect that can be used to treat inflammation in the body. Moreover, these systems have demonstrated improved patient compliance because of easy application and the ability to discontinue treatment one's whenever being requested [10].

Different nanotechnology-based formulations are available today for the delivery of drugs and cosmetics. Proniosomes [11], solid lipid nanoparticles [12], nano lipid carrier [13], nanoemulsion gels [14] and bilosome [15], as well as transdermal matrix patches [16], have been previously studied and reported. Despite these advances, vesicular systems such as

liposomes and niosomes represent an essential drug delivery system [17,18]. The advantages of niosomes over liposomes include enhanced chemical and physical stability [19], lower costs, and the easy use of surfactants [20]. Niosomes refer to vesicular systems composed of non-ionic surfactants. In aqueous media, these non-ionic surfactants self-assemble to produce bilayer structures [21,22]. Thus, they can encapsulate both hydrophilic and hydrophobic drugs into bilayer structures [23]. Niosomes are therefore assumed as a beneficial drug delivery system with several applications [23].

The present study aimed to develop niofenac gel via a green technology (e.g., ultrasonication method) where the use of harmful additives or organic solvents are avoided. Furthermore, the developed formulation was designed to improve the permeation of an ionic drug, diclofenac sodium, increasing its anti-inflammatory and antinociceptive potential.

Materials and Methods

Materials

Diclofenac sodium was a gift from Darou Pakhsh Pharma Chem Co. (Tehran, Iran) and the non-ionic surfactants (cholesterol, Tween 20, and Span 20) were purchased from Merck KGaA, (Darmstadt, Germany). Carbopol 941 polymer was obtained from B.F Goodrich Chemical Co (UK).

Niosome preparation using Ultrasonic Processing (UP) Technique

Niosomes loaded with DS (niofenac) were prepared using the ultrasonic technique [24]. Briefly, an accurate amount of cholesterol, Span 20, and Tween 20 (as shown in Table 1) were added to a glass vial by magnetic stirring at 75 °C. The aqueous phase (water and DS) was heated to the same temperature. The two phases were mixed using a hot plate magnetic stirrer

in order to form a pre-emulsion. Afterwards, a probe sonicator was used to sonicate the blend (Bandelin; 3100; Germany) with an amplitude of 20% for 3 min, followed by immediate cooling by immersing the mixture in an ice bath to obtain niofenac formulation.

Preparing niofenac gel and DS-simple Gel

Carbopol (0.75%w/v) was dispersed in the deionised water for preparing the plain gel base. Triethanolamine was added dropwise to carbopol solution until pH values adjusted between 6 and 6.5 and resulted in clear gel. To prepare the niofenac gel, 12.5 g of niofenac (containing 250 mg DS) was combined with 12.5 g of the plain gel base under stirring conditions. The DS-simple gel was prepared using a 12.5 g solution of DS (containing 250 mg of DS) mixed with 12.5 g of the plain gel base under stirring conditions.

Characterisation of niosomes

To determine particle diameter and size distribution of the niosomes, the dynamic light scattering (DLS) technique was used with the Zetasizer Nano ZS device (Malvern Instruments, Worcestershire, UK) in angle 90° at 25 °C. The polydispersity index (PDI) was also utilised as one of the measures of the size distribution. The zeta potential of a vesicle was measured through laser Doppler electrophoretic mobility measurements [25].

Drug Entrapment evaluation

The niosomal formulations were ultracentrifuged at 18,000 rpm for 30 min at 4 °C to determine DS entrapment. The ultracentrifugation (Sigma, Germany) was used to isolate the loaded DS from the dispersion. The amount of drug in the supernatant was then measured by ultraviolet-visible (UV-vis) spectroscopy (UV-Vis Jasco V-630, UK) at a wavelength of the drug (DS)

(276 nm). The entrapment efficiency percentage (EE%) was determined according to equation 1 [26]:

$$EE\% = \left(\frac{Winitial - Wfree}{Winitial}\right) \times 100 \qquad \text{(Equation. 1)}$$

Where $W_{initial}$ is the quantity of total drug initially added to the formulations and W_{free} is the quantity of the drug in the supernatant.

Morphology

The SEM analyses were carried out to inform on the morphology of niosomes. To this end, the formulations were coated with gold, and SEM was used to observe the morphology of the samples (FE SEM: Tescan Mira3, Czech Republic) at the pre-determined acceleration voltage of 20.0 kV and a magnification of 15,000 X.

Transmission electron microscopy (TEM)

TEM (Philips CM 120 KV, Amsterdam: The Netherlands) was used to examine the morphology of niofenac 2. To this end, some drops of niofenac 2 were placed on carbon-coated copper grids. Then, a 2% (v/v) phosphotungstic acid solution was prepared to negatively stain the nanoparticles for 30 seconds. Finally, the solvent was removed by drying the sample overnight at room temperature, and TEM visualization was captured.

***Attenuated total reflectance-Fourier transform infrared (ATR-FTIR)* spectroscopy**

Chemical interaction between DS and other formulation components were examined via a Cary 630 FTIR spectrophotometer (Agilent Technologies Inc., CA: USA) with a diamond as an ATR. The formed niofenac were separated by an ultracentrifuge as described earlier in the 'Entrapment evaluation' section and subsequently were freeze-dried using a freeze dryer (alpha 1-2 LDplus, martin Christ GmbH, Germany). The ATR-FTIR spectra of DS, cholesterol, Span

20, Tween 20, and the respective freeze-dried niofenac powder were recorded in the range of 400 to 4000cm^{-1} with 2 cm^{-1} of resolution [27].

Differential scanning calorimetry (DSC)

DSC was employed to study the thermal behaviour of cholesterol, DS and the freeze-dried niofenac powder using a Pyris 6, PerkinElmer (US). Around 5 mg of the sample of interest was placed in an aluminium pan and was sealed. To reach the equilibrium condition, the sealed pans were kept at 20 °C for 30 min. The DSC pans were heated from 20 °C to 250 °C with a heating rate of 20 °C/min [28]. Indium was utilised to calibrate DSC before performing experiments on the samples.

X-ray diffraction (XRD) studies

XRD (PHILIPS-PW1730, Netherland) was used to determine the solid-state of all chemicals used in the preparation of niosomes and the freeze-dried niofenac powder. The samples were subjected to Copper K-α radiation with a wavelength of 1.5406 Å, at 40 kV, and 30 mA. The scanning was performed from 5.000° to 80.000°, a step time of 1 s, and 2Θ at a step size of 0.050°.

Drug release

In vitro release studies were performed using immersion cells with acetate cellulose membrane (cut-off 12 kDa). The samples (5 ml of 1% drug concentration) were placed in the cells, and an acetate cellulose membrane was placed on the cells followed by closing the cells by a cap. The cells were placed in the dissolution apparatus, and 900 ml water was added to dissolution vessels as a dissolution medium. At different time intervals (2, 4, 6, 8, and 24 h), 5 ml of the dissolution medium were removed and filtered with a 0.22 µm filter. A UV spectrophotometer

at 276 nm was used to analyse the drug content in the withdrawn samples. All experiments were performed three times, and the data were presented as the mean±standard deviation (SD)

In Vitro Skin Permeation Examination

This research was approved by the ethical committee of Mazandaran University of Medical Sciences according to the Ethical Guidelines in the examination of laboratory animals (approval code: IR.MAZUMS.REC.1399.7144). Male Wistar rats weighing between 120 and 150 g were anaesthetised with 87 mg ketamine/kg body weight and 13 mg xylazine/kg. Electric hand razors were then used to shave the abdominal skins. After 48 h, chloroform was used to sacrifice the rats followed by a surgical operation to remove the abdominal skin. The skins removed from the adherent subcutaneous fats were washed carefully. The washed skins were put in a saline solution for 24 h before being positioned in the Franz cells with a diffusion region of 3.8 cm^2 where the dermis meets the receptor fluid [24,29-31]. The receiver compartment was then filled with DI water and the diffusion cells held at 32 ± 0.5 °C with thermostatically regulated water circulating during the experiment by a jacket around the cell bodies. Stirring with the magnetic stirring bars was kept at 150 rpm. On the shaved dorsal surfaces in the donor compartment, 1 g of niofenac gel (containing 0.01 g of DS) was evenly distributed by mild rubbing of a spatula. 5 ml of samples were removed from the receiver medium at different time intervals and analysed using UV-vis spectrophotometry [32] at a wavelength of 276 nm. The amount of sample taken out was replaced by the same volume of the fresh DI water each time to keep the volume constant throughout the experiment. The plain or simple gel formulation of DS prepared as reported earlier was tested with the same concentration of the DS in the formulation. All results reported were the mean±standard deviation (SD) of at least three determinations.

Diclofenac sodium retention by the skin

To explore the amount of drug retained in the skin at the end of the permeation studies, the skins were removed from Franz cells and washed three times by DI water. The washed skins were then cut into small fragments using a pair of scissors, placed into a tube, digested for 24 h in water and sonicated for 1 h at room temperature in a bath sonicator (Bandelin, Germany). The supernatant was withdrawn and filtered using a syringe filter (pore size: 0.22 μm) followed by the measurement of absorbance using UV-vis spectrophotometry at a wavelength of 276 nm.

In vivo biological evaluation of niofenac

Animals

This study utilised male Swiss-Webster mice weighing between 25 to 30 g. Six mice in each group were housed in each plastic cage in an animal room kept at 21 ± 2 °C on a 12 h light/dark cycle (light on 07:00-19:00 h). Food and water were always provided excluding during the experiment. The research was accepted by the Animal Research Ethics Review Committee, associated with the Mazandaran University of Medical Sciences, Iran.

Formalin Test

The formalin test described by Dubuisson and Dennis was used to assess antinociceptive behaviour [33]. The nociceptive response stimulated by the formalin test provides a biphasic pain response which is very helpful for pain-relieving effectiveness assessments [34,35]. A mild rubbing (50 times with the index finger) of 1% niofenac gel was used on the dorsal surface of the left hind paw (n = 6). The plain gel base without any drug was used as the control for the control group mice. The mice with the DS-simple gel administered were used as a reference. 50 μl of formalin (2.5%) was injected into the right hind paw 5 min later, and pain-related

responses immediately analysed. The mice were monitored for 60 min after the formalin was injected. The length of time taken for the licking of the injected hind paw by the mice was then recorded. It should be noted that the first 5 min after injecting formalin refers to the early phase and the interval between 15 and 60 min is termed the late phase [29,36].

Data Analyses and Statistics

SPSS 22 (IBM Co., USA) was employed to perform the statistical analyses. The analysis of variance (ANOVA) and Tukey's post-hoc test were performed. The data were significant when p-value was < 0.05.

Results and Discussion

Analysis of Niosome Characteristics

The particle size of the nanoparticles (NPs) is reported in Table 1. The polydispersity index (PDI) represents the dispersion quality (as the index of the width of the particle distribution) [28]. PDI-values are commonly in the range between 0 and 1. PDI values greater than 0.7 indicate a very wide distribution of the particle size [28].

During the preparation of the diclofenac vesicles, it was observed that the solution was opalescent, but became milky and cloudy when the concentration of cholesterol increased. The particle size of the NPs increased following increases in the concentration of cholesterol in the formulation (Table 1). For instance, when the concentration of cholesterol increased from 0.2% (niofenac 1) to 2% (niofenac 6), their particle size significantly increased from 158.00±6.17 nm to 401 ± 5 nm ($p<0.05$). Studies by Varshosaz *et al*. and Moazeni *et al*. have confirmed this phenomenon of the size of the vesicles being increased by an increase in the quantity of cholesterol [37,38]. The PDI values also increased with an increase in the concentration of

cholesterol. This was observed as an increase in PDI value from 0.342±0.009 (cholesterol concentration of 0.2%) to 1±0 (cholesterol concentration of 2%) (Table 1). The PDI results, therefore, suggest that when the concentration of cholesterol increases, the size distribution becomes wider and less uniform.

The zeta potential data demonstrated that although the presence of cholesterol seems to be necessary, care and consideration must be taken as higher concentrations of cholesterol decrease the zeta potential. This was observed as a change in value from -43.13±1.171 mV (niofenac 1) to -25.40±1.352 mV (niofenac 6) which indicates that lower concentrations of cholesterol produced relatively more stable vesicles when compared to vesicles made from a higher concentration of cholesterol (Table 1). It was thus suggested that the zeta potential of the vesicle could be dependent on the structure of the DS and cholesterol in the bilayers which potentially changes the surface charge [32].

It was interesting to note that the non-ionic surfactants used produced a negative zeta potential surrounding the colloidal particles. This may be attributed to the dipole nature of the ethoxy groups of non-ionic surfactants [28]. The higher zeta potential values were, therefore, necessary for electrostatic stabilisation, thereby making it easier to suspend the niosomes in water, a property essential for their administration and storage [39]. Moreover, by using the binary mixtures of surfactants (Span 20 and Tween 20) which regulates the HLB value, the properties of the DS vesicles can be modified to achieve an optimum value in terms of size, PDI, EE% and stability. Surfactants with the high and low HLB value could also be distributed in the aqueous and oily phase respectively thus leading to the better physical stability of the surfactant films at the interface [28]. The presence of cholesterol could also enhance the cohesion and rigidity of the niofenac bilayer, causing a significant increase in the vesicle diameter [32].

The EE% value is quite varied (Table 1), ranging from 35.04±1.55 (niofenac 1) to 63.70±0.18% (niofenac 6). The results suggest that cholesterol associates with the surfactant molecules, which in turn changes their physical characteristics and vesicular structures via the regulation of bilayer strength and cohesion, which induces an increase in EE% [40]. The addition of cholesterol reduces the gel-liquid transition temperature of the vesicles, thus rendering them more stable and less permeable to drug leakage, which can increase the EE% [41]. DS is considered as an amphiphilic molecule, which tends to self-associate and interacts with biological membranes similar to classical surfactants, hence why it is defined as a surface-active drug. Increasing the amount of cholesterol in the formulation may progressively convert the diclofenac (as surface active drug) in the gel phase to a liquid state, whose properties are intermediate between those of the solid and of the liquid fluid in the bilayer membrane [32]. It is clear that the maintained DS into the system increases as the amount of cholesterol augments. Such a condition is possibly caused by the competition between the two amphiphilic molecules in order to form a bilayer. In addition, the presence of the growing content of cholesterol can restrict retaining DS in the lipophilic lamellae [32].

The EE% in this study also improved when the ratio of cholesterol:surfactant increased to 1:1 because of the decline in the DS leakage. The increase in cholesterol concentration makes the niosome bilayer stronger. This bilayer can also act as a barrier for drug release thereby reducing the leakage of the drug. The strong bilayer could also reduce the permeability which may, in turn, result in a slower release rate from the vesicles [42]. A similar observation was reported by Mokhtar *et al*., who investigated the effects of a number of formulation factors such as the amount of cholesterol in niosomes on encapsulating flurbiprofen and the release rate of the niosomes procured from proniosomes [43]. Another factor that should be considered is the

impact of the vesicle size (Table 1). It was observed that EE% reduces as particle size decreases. This trend has also been reported elsewhere (Table 1) [44,45].

The benefit of the proposed technique employed for preparing niofenac compared to the other observed methods described by Ghanbarzadeh *et al*., and Bhattacharya *et al*., is that no organic solvent was utilised [46,47]. The niosomes can therefore be incorporated into a gel base and considered a finished product. The niofenac 2 formulation was selected as an optimised formulation for further experiments based on the particle size (smallest particle size), a satisfactory zeta potential, mid-range PDI and high EE%. The particle size distribution and the intensity zeta potential of the optimised formulations is shown in Figures 1B and 1C. The optimised niofenac prepared by the ultrasonic processing technique had a particle size of 142.43±12.45 nm, PDI of 0.593±0.003, zeta potential of -38.47±1.320 mV, and EE% of 47.16±1.60.

There are other process variables investigated that can change the EE% and the size of niosomes such as stirring rate, stirring time and amplitude [48]. Tavano *et al*. loaded DS in niosomes through a thin-film hydration method. The authors obtained a larger mean particle size for the diclofenac niosome (547.0 ± 12 nm) and a lower EE% (35.19± 1.30%) [32]. In another study, Ioele *et al*. loaded DS in a nano-vehicle by a lipidic film method. In their study, the size of niosome particles varied and ranged from 392.32±11.00 nm to 495.67±10.00 nm with the greatest EE% of 68.11±4.81% [49].

SEM

SEM was used to examine the morphologies of the niosomes. According to the SEM image shown in Figure 2 for niofenac 2, the niosomes were mostly spherical with discrete particles and relatively uniform in size.

TEM

TEM image of the niofenac 2 revealed that the nanoparticles are approximately in a spherical shape (Figure 1A). As can be seen, no sign of aggregation was observed in the image.

ATR-FTIR analysis

ATR-FTIR spectroscopy investigated possible interactions between the drug and the niosomal components (Figure 3). The ATR-FTIR spectrum of DS presented a characteristic broad peak at 3388 cm^{-1} (N-H stretching), also main peaks at 1304 cm^{-1} & 1281 cm^{-1} (C-N stretching), 1573 cm^{-1} (C=O stretching of carboxylate group), and 744 cm^{-1} (C-Cl stretching). The spectrum of cholesterol showed characteristic bands at 3402 cm^{-1} (O-H stretching**)**, 3000-2850 cm^{-1} (C-H of CH_2 and CH_3 groups, asymmetric and symmetric stretching**),** 1463-1457 cm^{-1} (C-H bending), and 1054 cm^{-1} (C-O stretching**).** The spectrum of Span 20 exhibited main peaks at 3392 cm^{-1} (O-H stretching**)**, 2923 cm^{-1} (-CH_2- asymmetric stretching), 2854 cm^{-1} (-CH_2- symmetric stretching), and 1738 cm^{-1} (C=O stretching). The spectrum of Tween 20 showed diagnostic peaks at 3488 cm^{-1} (O-H stretching**)**, 2920 cm^{-1} (-CH_2- asymmetric stretching), 2860 cm^{-1} (-CH_2- symmetric stretching), and 1734 cm^{-1} (C=O stretching).

The ATR-FTIR results ruled out any chemical interactions between DS and the excipients used in the preparation of formulations since the main peaks of the drug, including the peak of C=O stretching vibration can be seen in the spectrum of the selected formulation without shift.

DSC

DSC was employed to study the thermal behaviour of DS, cholesterol and the niofenac 2 powder (Figure 4). DS and cholesterol thermograms revealed a single intense endothermic peak at ~178 °C [50] and 150 °C [28] respectively. These peaks corresponded to their melting points, indicating their highly crystalline nature. The DSC thermograms of the examined niofenac formulation consisted of an endothermic peak close to the melting peak of cholesterol (149.2 °C). DSC traces of niofenac also showed that the endothermic peak of the drug disappeared (Figure 4), suggesting that the DS in niofenac was in an amorphous state or was molecularly dispersed in the niosome.

XRD

Figure 5 presents the XRD pattern of DS, cholesterol, and niofenac 2. The XRD of DS indicated peaks at 2θ values of 10.7°, 13.5°, 15.4°, 17.7°, 18.9°, 20.5°, 21.6°, 23.5, 24.5°, 25.5°, and 28.7° indicative of DS [50]. The XRD of cholesterol showed peaks at 2θ of 5.27°, 10.60°, 12.83°, 15.50°, 16.97°, 17.39°, 18.15°, 23.56°, 26.24°, 37.15° and 42.40° with very low intensities. These peaks suggest that cholesterol and DS are crystalline in nature. The XRD pattern of niofenac 2 also demonstrated the peaks at 2θ of 5.27°, 10.60°, 12.83°, 15.50°, 16.97° and 18.15°. These are the diagnostic peaks of the cholesterol, which seem to be present in the niosome although at a relatively lower intensity compared to the pure cholesterol. A reduction in the intensity of the peaks could be due to the lower concentration of cholesterol in the niosome formulation. This is in agreement with the DSC results where the intensity of the cholesterol peak in noisome was reduced which could be an indication of a reduction in the crystallinity of the cholesterol upon incorporation into the niosome formulation. Furthermore, the incorporation of cholesterol and DS into the segments of the niosome membrane can lead to changes in the crystallinity of cholesterol. The lack of the diclofenac diagnostic peaks in

niofenac (2θ of 21.6°, 23.5, 24.5°, 25.5°, and 28.7°) confirms that DS in the niosome is in an amorphous phase or molecularly dispersed. This result is also consistent with the DSC studies.

Drug release

It has been reported that the controlling drug release rate from nanoparticles have an impact on drug bioavailability [47,48]. In this research, the drug release from niosomal formulation and DS solution were carried out and their release profiles are shown in Figure 6. The results represented that up to 30.64±0.03% of DS was released from niofenac 2 within the first 2 h, followed by a prolonged release pattern for the 22 h (61.71±0.59%). The release results indicated that the noisome released DS in a controlled behaviour could be related to the diffusion of the drug from the core of the niosmes to the environment [49]. In contrast, DS solution formulation released the drug in a faster manner where around 45% of the drug released within the first 2 h.

***In vitro* percutaneous absorption study**

Drug penetration into and through the skin defines which formulation is appropriate for applications in transdermal delivery (across the skin) or dermal delivery (into the skin). In most experiments, rat skins were commonly used in percutaneous absorption models, even though there are comprehensive publications that indicate the skin of humans to have more reliable skin absorption outputs than that of the skins of rats. Human skin is also less permeable than rat skin [54-57]. Nevertheless, since the purpose of the present investigation was to determine the performance of niosomes and their comparison with published articles that have used rat skins in their studies, the authors, therefore, found the rat skin models useful in this study. Figures 7 and 8 are a representation of the cumulative plots of the amounts of DS permeated across the rat skin (i.e., transdermal delivery) as the time function and the content of DS

penetrated to the layer of skin (that is, dermal delivery) for the gel formulations of niofenac 2 and DS-simple. According to the results, niofenac had the capability of penetrating into the various layers of the skin (Figures 7 and 8). The greater degree of penetration into and through the skin layers was also shown by the niofenac gel formulation, thus indicating that it was more acceptable as a transdermal delivery for the green niofenac 2 gel formulation in comparison with DS-simple gel *($p<0.05$)*. The maximum amount of DS measured in the receptor chamber for DS-simple gel (4.83±1.47% or 127.40±27.80 µg/cm^2) was much lower than that of the niofenac 2 gel formulation (9.20±1.44% or 242.3±31.11 µg/cm^2) ($p < 0.05$). The amount of DS deposited however in the skin for DS-simple gel (18.6±2.09% or 489.77±55.54 µg/cm^2) was significantly ($p<0.05$) higher than that of the niofenac 2 (14.04±0.54 % or 369.63±10.04 µg/cm^2). The results indicated that for transdermal delivery purposes, a niosomes formulation could be a better option than a conventional gel formulation. The opposite is however observed if dermal delivery is the preferred route.

The concentration of the cholesterol in the niosomes seemed to have played a significant role in the DS penetration into the skin. It has already been demonstrated that cholesterol can affect and modulate the fluidity, phase behaviour and stability of membrane permeation and trafficking [32]. Cholesterol and its esters have also been widely used as penetration enhancers for topical delivery [58, 59]. The HLB value of the carrier has also been known to be a crucial parameter in the evaluation of the percutaneous permeability of medications, according to published reports [24,32].

There are many several reported studies on DS niosomes or proniosomes, where researchers have attempted to improve the transdermal distribution of DS in each experiment [32,49,60]. DS niosomes have been applied to rabbit ear skin with findings revealing that about 0.1 µg/cm^2

DS is carried through the skin for 24 h [49]. As different DS concentrations have been reported in various studies, it is very difficult to say which of the niosome formulations shows greater permeability at the same concentrations. In either case, relative to other traditional formulations, it seems that niosome-formulated drugs might have greater permeation.

In general, researchers have regarded the niosomes as the best vesicular systems for topical delivery of drugs because of their biocompatible, biodegradable, and nonimmunogenic structure. It should be noted that their versatility also relates to their capability for controlling the release of a medicinal agent based on certain requirements of treatment. However, the vesicular systems can also present a localised depot for drugs into the skin, assuring the lengthy release of the drug over time, which reduces the systemic adsorption and lessens the complications [61]. As an alternative, the vesicles can be developed for enhancing the transdermal drug delivery, hence enhancing the systemic drug absorption [62]. Evidently, a more pronounced drug reservoir within the upper layers would be prioritised in a case of the need for the local effect.

Formalin Test

Figures 9A and 9B depict the formalin test results in the early and late phases of pain. The total licking time in early and late stages, respectively reported as follow: niofenac 2 gel (40.2±7.3 and 432.4±31.7 s), control (115.4±28.9 and 900±64.2 s) and the diclofenac simple gel formulation (130.4±8.73 and 660.6±123.73 s). A greater analgesic impact was observed for the niofenac 2 gel containing 1% DS compared to the DS-simple gel in the early phase (Figure 9(A)) *($p < 0.05$)*. Results of the formalin test in the late phase similarly confirmed that the niofenac 2 had a remarkable impact on the antinociceptive activities of DS. In addition, the antinociceptive enhancing effect of the niosome was observed *($p<0.05$)* in the late phase of the

formalin test (Figure 9(B)). Niosome-encapsulated DS correspondingly revealed a higher anti-inflammatory effect when compared to the simple gel formulation of DS with a similar dose.

There are several reports on the anti-inflammatory activity of niosomes or proniosomes and their pharmacological effects [29,63,64]. Akbari *et al*. studied the anti-inflammatory effect of curcumin loaded niosomes and revealed a higher anti-inflammatory and antinociceptive activity of the curcumin niosome formulation in the formalin test [29]. Marzoli *et al*. also reported ibuprofen-loaded niosomes to demonstrate peripheral antinociceptive action and to be more potent than free ibuprofen in the reduction of inflammatory pain in the formalin test [645]. Shah *et al*. reported an increase in the anti-inflammatory effects of proniosome carriers, suggesting its ability to be a good complement to tramadol oral therapy [63].

Conclusion

In this research, diclofenac sodium (a hydrophilic drug) was successfully incorporated into niosomes made of cholesterol and surfactants mixture for transdermal delivery. According to the solid-state analyses of niofenac, diclofenac sodium loaded into niosome was in an amorphous state without any chemical interaction with the other components of the niosomes with the average particle size, polydispersity index, encapsulation efficiency, and zeta potential of 142.43±12.45 nm, 0.593±0.003, 47.16±1.60%, and -38.47±1.320 mV, respectively. *In vitro* drug release showed that niofenac 2 had controlled release behaviour. Finally, niofenac showed significant antinociceptive and anti-inflammatory activities compared to the control group in animal models. It can be concluded that this strategy could be regarded as one of the effective alternatives for the transdermal delivery of diclofenac sodium.

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Disclosure of interest

The authors declare no conflict of interest.

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Table 1. Formulation component and physicochemical characteristics

Formulation	DS (%)	Cholesterol (%)	Span 20 (%)	Tween 20 (%)	HLB*	Water Up to 100%	Particle size (nm)	PDI	Zeta potential (mv)	EE (%)
niofenac 1	1	0.2	1	1	12.65	100	158.00±6.17	0.342±0.009	-43.13±1.171	35.04±1.55
niofenac 2	1	0.6	1	1	12.65	100	142.43±12.45	0.593±0.003	-38.47±1.320	47.16±1.60
niofenac 3	1	1	1	1	12.65	100	189.67±6.54	0.676±0.012	-34.50±0.264	49.99±0.51
niofenac 4	1	1.4	1	1	12.65	100	321.07±1.92	0.857±0.016	-29.53±3.265	53.18±0.50
niofenac 5	1	1.8	1	1	12.65	100	325.87±7.37	0.904±0.011	-29.67±1.361	57.38±0.99
niofenac 6	1	2	1	1	12.65	100	400.87±4.99	1±0	-25.40±1.352	63.70±0.18

***** the ratio of the span and tween used generated this calculated HLB value

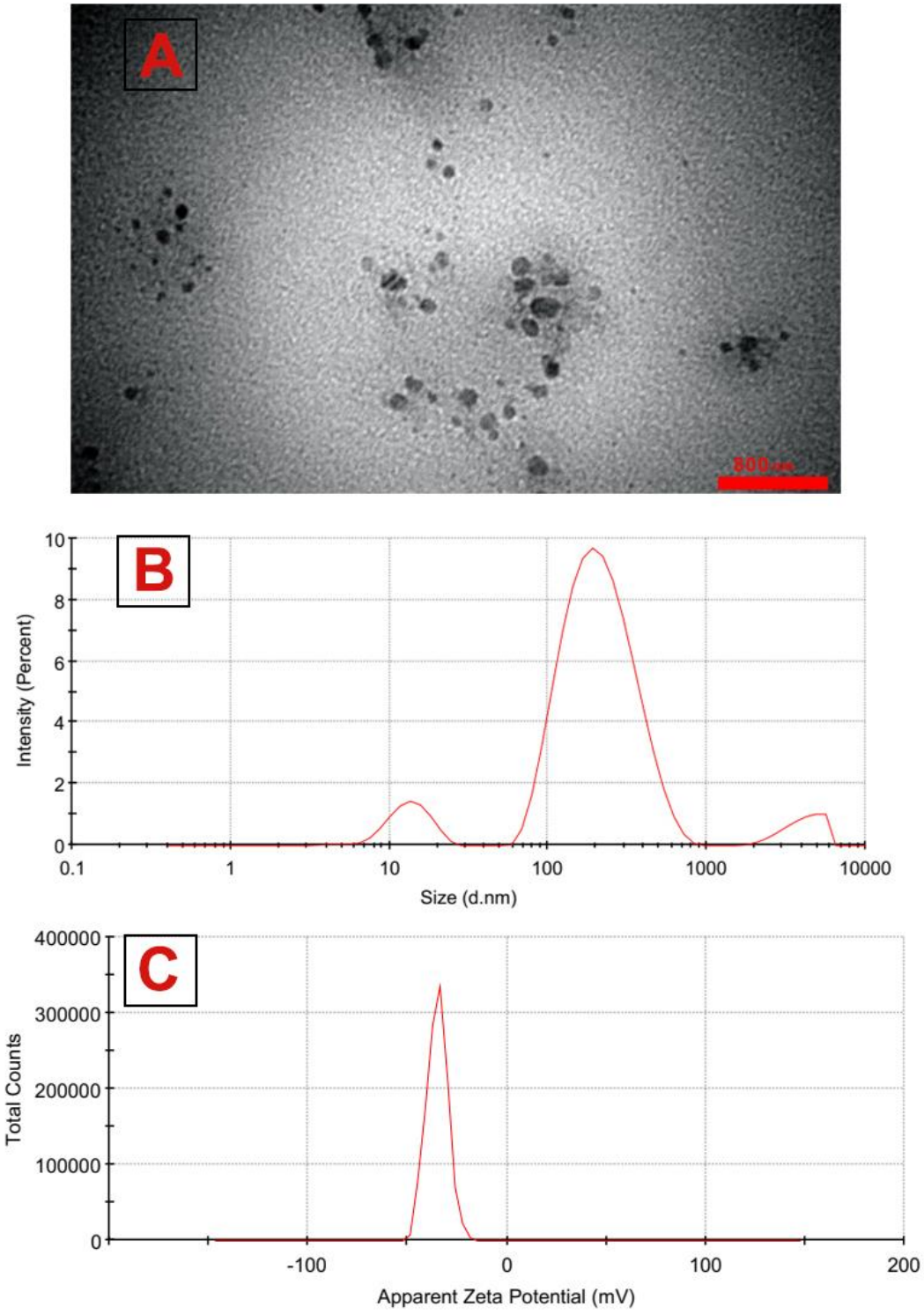

Figure 1. (A) TEM micrograph, (B) size distribution by the intensity and, (C) zeta potential of the niofenac 2 formulation.

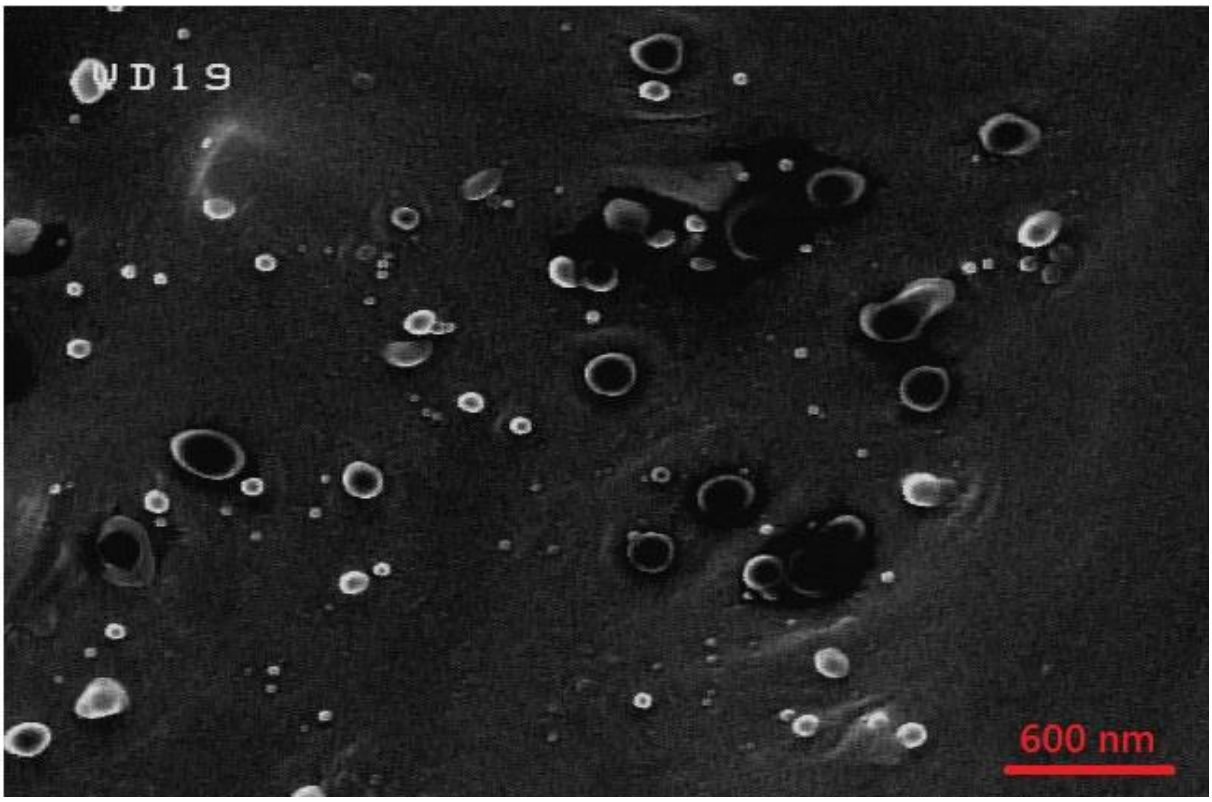

Figure 2. SEM micrographs of the niofenac 2 formulation.

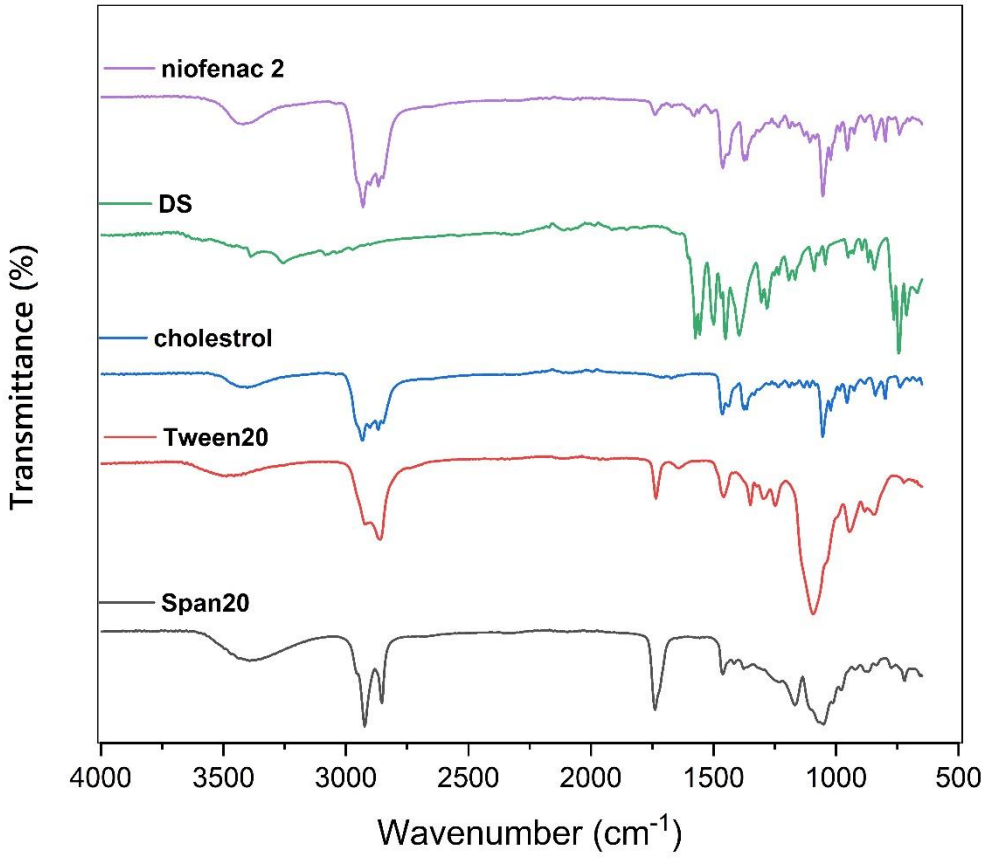

Figure 3. ATR-FTIR spectra of the niofenac 2 formulation, diclofenac sodium (DS), cholesterol, Tween 20 and Span 20.

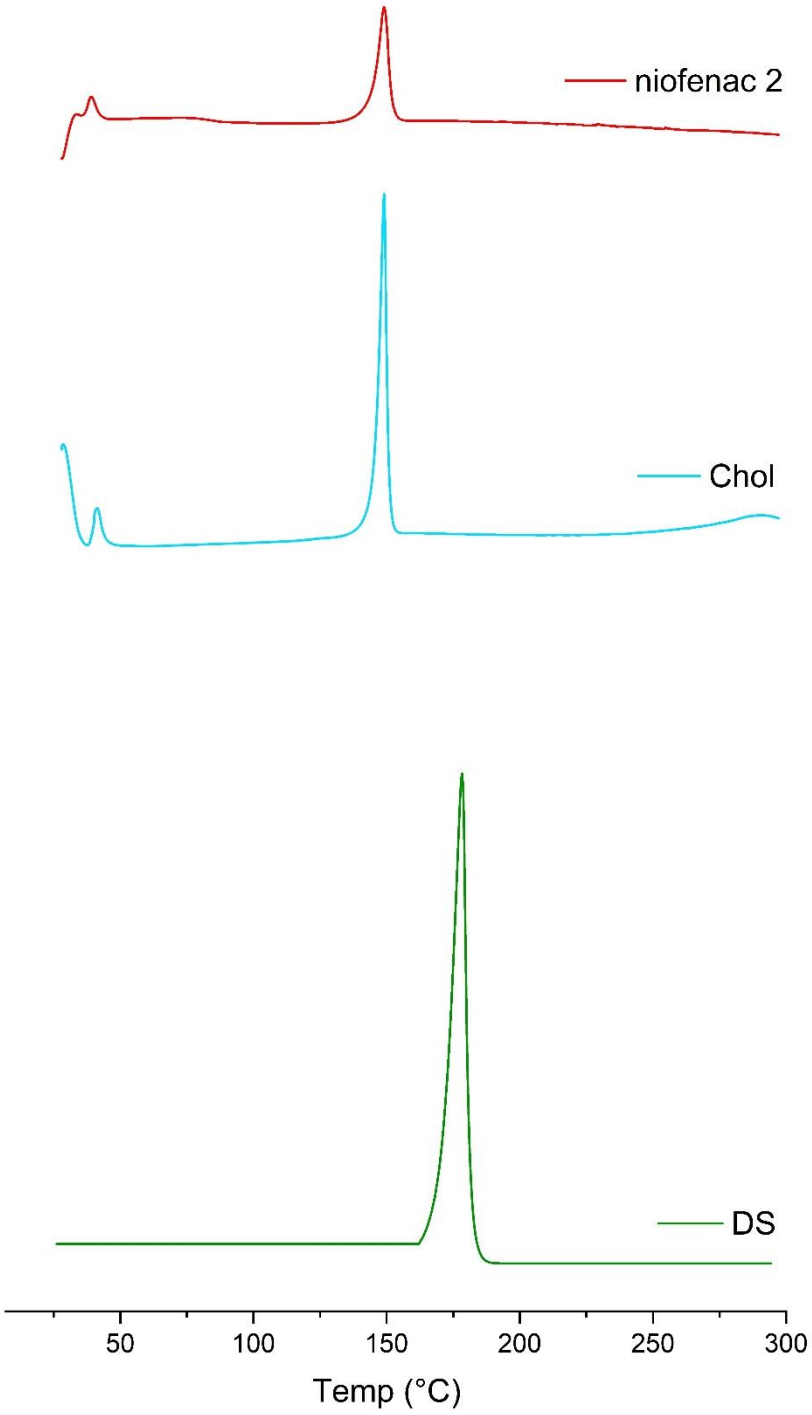

Figure 4. DSC traces of diclofenac sodium (DS), cholesterol (Chol) and the niofenac 2 formulation.

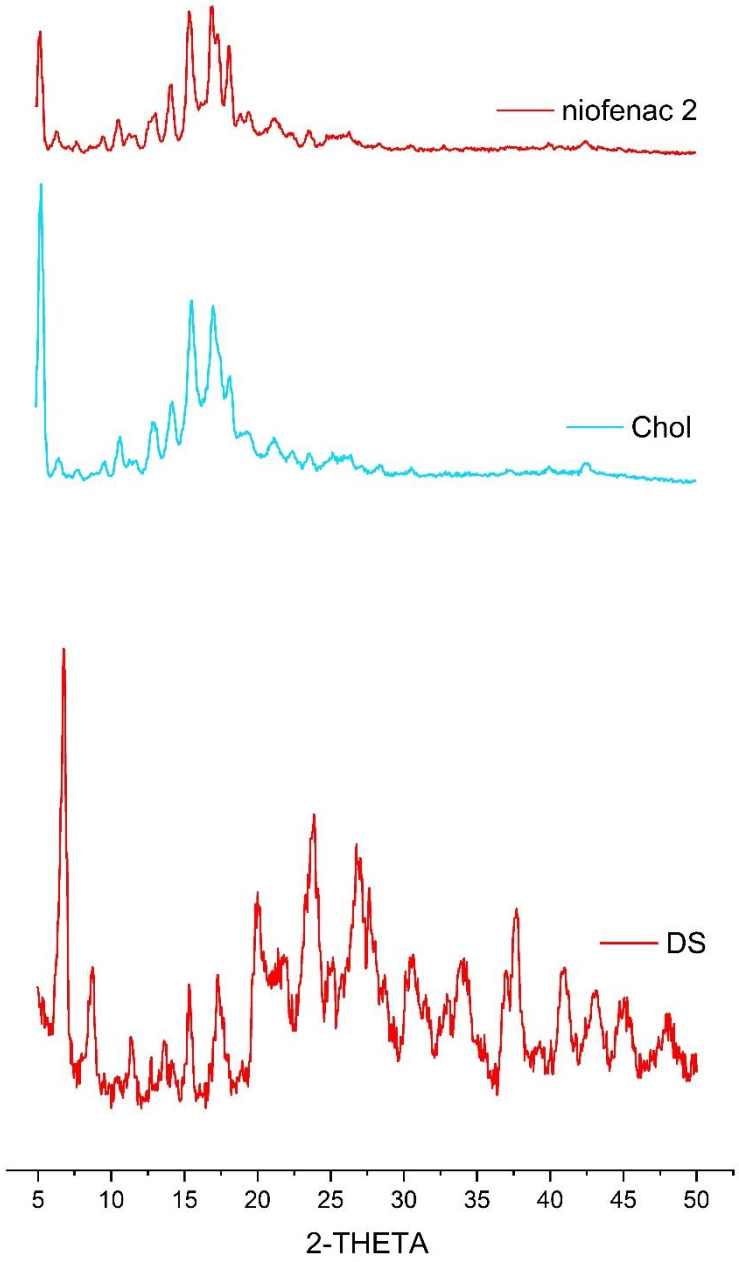

Figure 5. PXRD of cholesterol, diclofenac sodium (DS) and the niofenac 2 formulation.

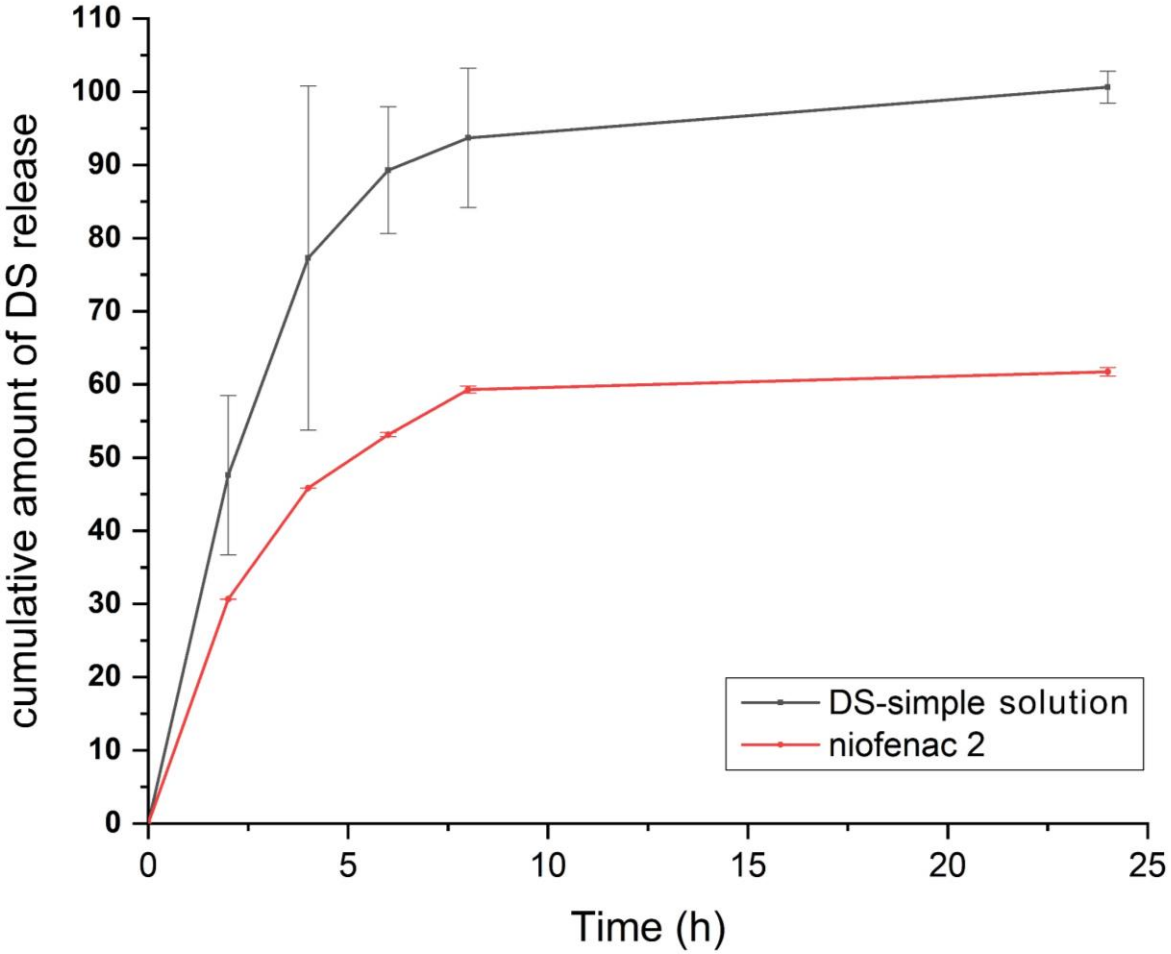

Figure 6. The release profile of diclofenac sodium (DS) from niofenac 2 and DS-simple solution.

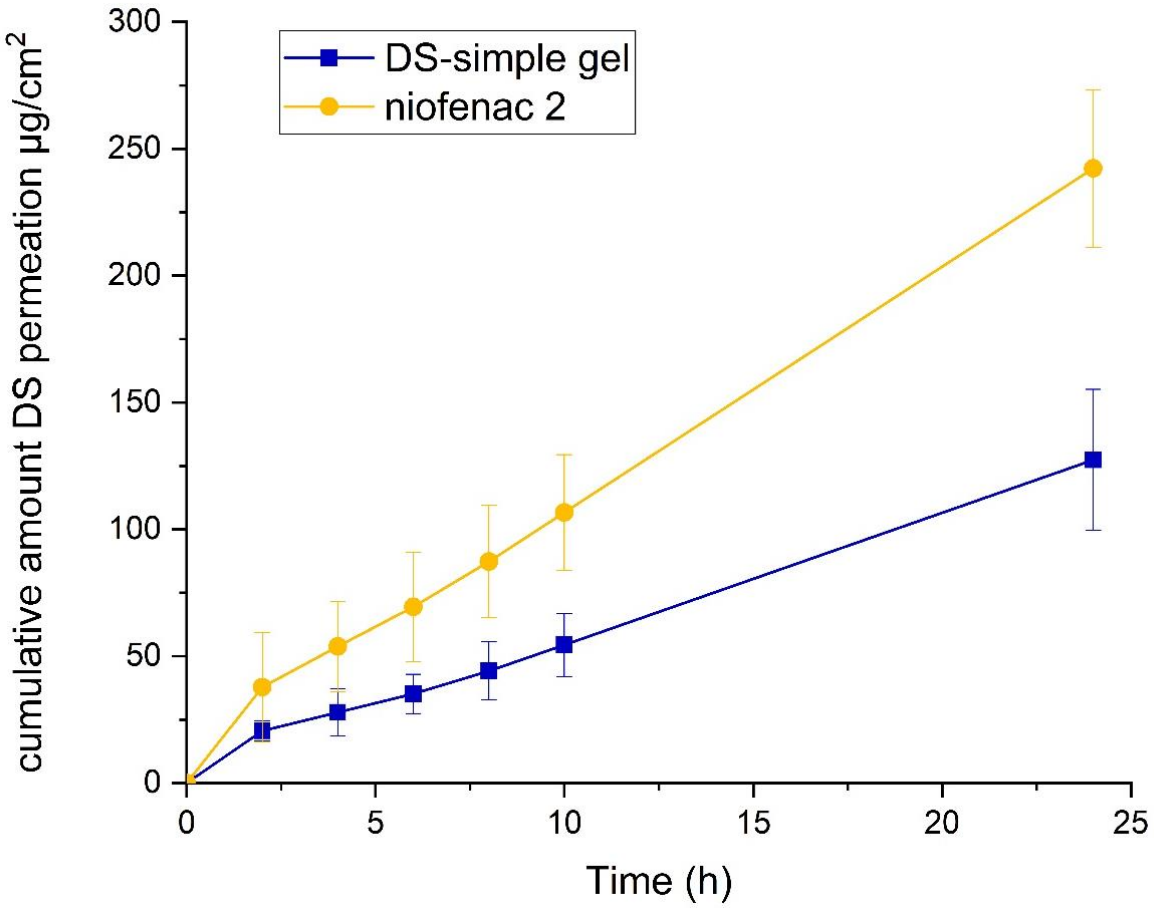

Figure 7. Comparing permeation behaviour (transdermal delivery) of simple gel and niosomal formulation containing diclofenac sodium (DS) through rat skin (n=3).

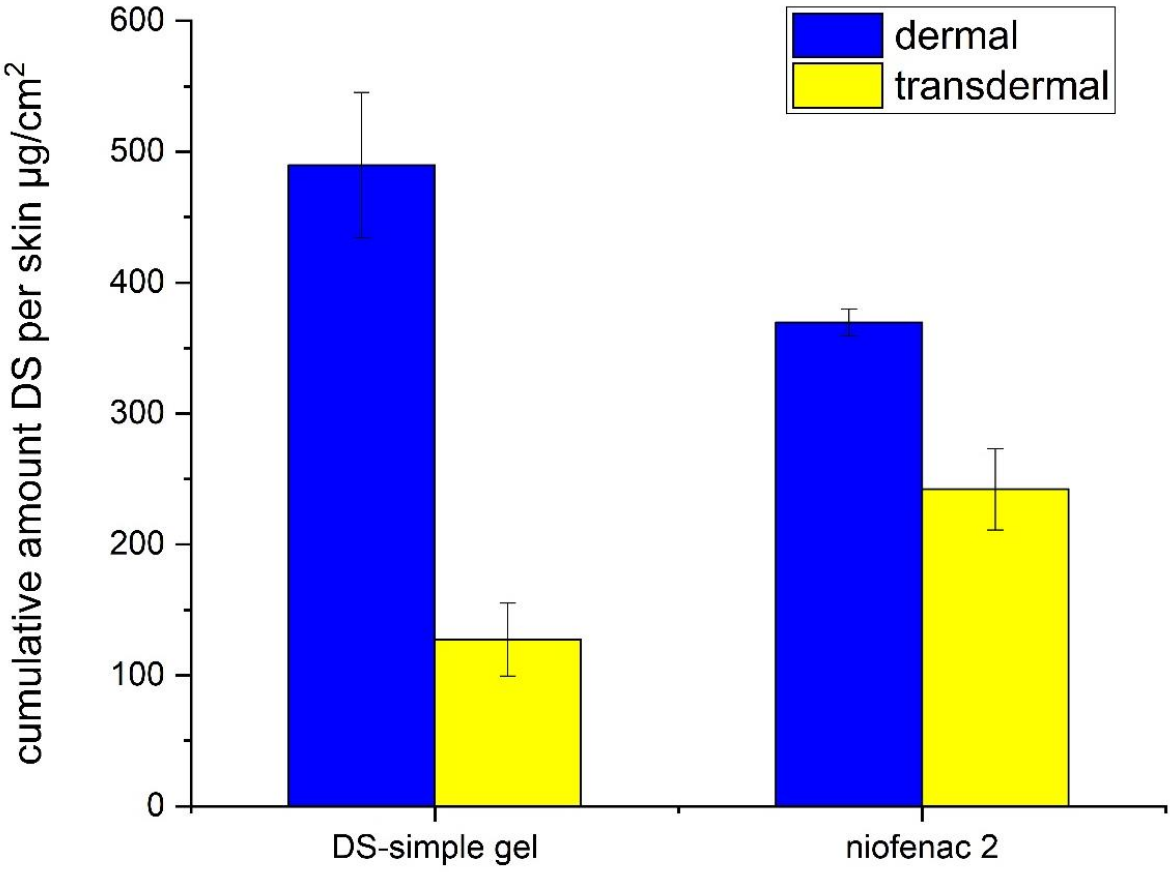

Figure 8. Comparing penetration behaviour (dermal delivery) of simple gel and niosomal formulation containing diclofenac sodium (DS) through rat skin (n=3).

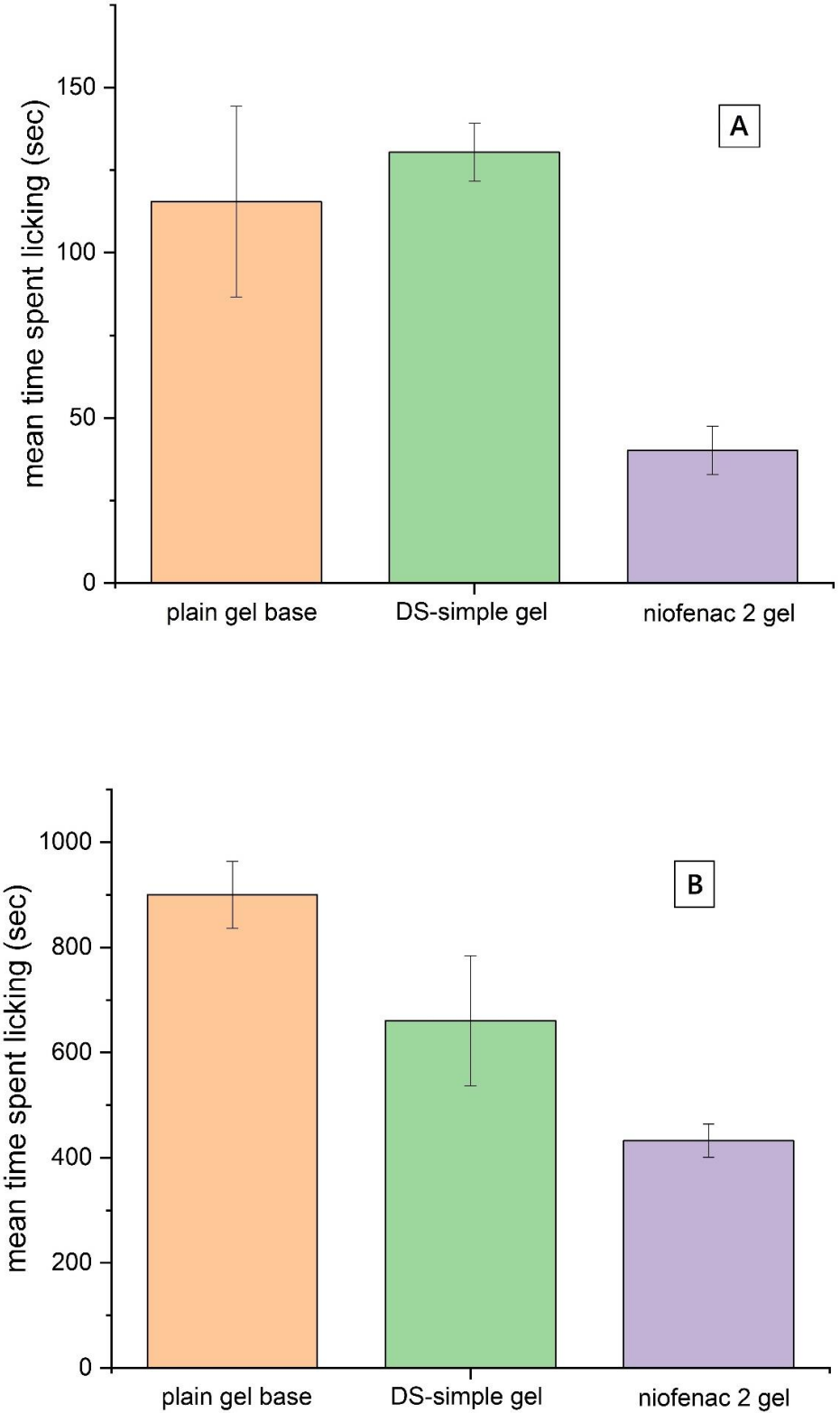

Figure 9. The antinociception performance of various topical formulations on (A) the early phase (0–5 min) and (B) the late phase (15–60 min) obtained in the formalin test (n=5 animals per group).