RESEARCH PAPER

Evaluation of an innovative, green, and eco-friendly baclofen-loaded niosome (Baclosome) formulation for pain management by transdermal delivery

*Jafar Akbari [1], Majid Saeedi [1, 2], Katayoun Morteza-Semnani [3], Seyede Nastaran Mousavi [4], Seyyed Mohammad Hassan Hashemi [5, 6] *, Seyyed Mobin Rahimnia [1, 2] **

[1]Department of Pharmaceutics, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[2]Pharmaceutical Sciences Research Centre, Haemoglobinopathy Institute, Mazandaran University of Medical Sciences, Sari, Iran
[3]Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[4]Student Research Committee, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[5]Food Health Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[6]Department of Pharmaceutics, Faculty of Pharmacy, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

ABSTRACT

Objective(s): Baclofen, a muscle relaxant, exhibits limited dermal penetration, restricting its therapeutic efficacy. This study aimed to optimize baclofen delivery using ultrasonically manufactured niosomes (Baclosomes) for improved antinociceptive and anti-inflammatory activity.

Materials and Methods: The effect of cholesterol:surfactant (Chol:Surf) ratio on Baclosome characteristics was investigated using powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. Dermal permeation studies were conducted in rats to assess baclofen delivery to the dermis and recipient compartment. Antinociceptive and anti-inflammatory effects were evaluated using a rat model.

Results: Increasing Chol content significantly increased Baclosome particle size. The Chol:Surf ratio also influenced zeta potential (ZP), ranging from -9.10 ± 1.81 to -28.81 ± 1.3mV. DSC and PXRD analyses confirmed the amorphous nature of baclofen within the Baclosomes. There was no chemical interaction between the drug and the excipients which supported by ATR-FTIR analysis. Dermal permeation studies showed higher baclofen levels in the dermis and recipient compartment for Baclosome gel compared to plain baclofen gel, without inducing dermal irritation. Baclosome gel demonstrated significant antinociceptive and anti-inflammatory effects compared to control groups (baclofen gel and diclofenac gel).

Conclusion: This study demonstrated that ultrasonically manufactured Baclosomes effectively enhance baclofen delivery to the skin, resulting in improved antinociceptive and anti-inflammatory activity. Optimization of the Chol:Surf ratio significantly influenced Baclosome characteristics, highlighting the potential of this formulation approach for enhancing drug efficacy. This approach could offer a promising strategy for improving the therapeutic benefits of baclofen in treating musculoskeletal pain and inflammation.

Keywords: *Analgesic, Baclofen, Green synthesis, Niosome, Transdermal*

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INTRODUCTION

Pain is an unpleasant sensation that is typically caused by an internal or external acute stimulus and has a powerful mental response [1]. Despite the fact that a variety of treatments, including nonsteroidal anti-inflammatory drugs (NSAIDs), are used to treat chronic and acute pain, none have been shown to be especially helpful [2]. Since NSAIDs induce numerous side effects, including prevention of platelet aggregation, and liver and renal problems, other analgesic medications with minimum adverse effects and an effective

* *Corresponding authors: Emails: mobin.rahimnia@yahoo.com; smhhashemipharma@gmail.com*
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therapeutic index have been explored as NSAID substitutes [3].

It has been observed that baclofen retains anti-inflammatory and analgesic assets due to its potent inhibition of (GABA)-B receptors [4]. Baclofen has exhibited analgesic effects in the treatment of persistent spinal cord damage pain and post-hemorrhoidectomy pain while maintaining a satisfactory safety profile [5, 6]. The principal barrier to use baclofen as an analgesic is its limited half-life, which also has a variety of side effects when administered orally [4]. To conquer these insufficiencies, it appears critical to select an adequate path of drug delivery. Transdermal delivery of baclofen is one of the most effective and convenient options due to the various advantages described elsewhere [7]. Nevertheless, the level of dermal absorption is extremely dependent on the substance's physicochemical features and the carrier's type (e.g., the polarity of the solvent, particle size, and type of vehicle) [8, 9]. Baclofen is an ideal option for transdermal application due to its molecular weight of 250.12 Da, melting point of 220 °C, and partition coefficient of 1.42 [7]. The nano preparation of pharmaceuticals has been advocated to improve skin absorption, bioavailability, and therapeutic effects [10].

Colloidal drug vehicle platforms such as niosome appear to be promising drug delivery systems (DDSs) and have garnered a rising amount of interest over the past two decades [11]. Niosomes, have been assumed hopeful formulations for transporting larger quantities of medications via the dermal path which will be released for systematic absorption at controlled manner [12]. Niosomes are typically innocuous, have a minimal manufacturing cost, and are appropriate for long stability [13]. Niosome were discovered and developed for cutaneous uses in 1975 and since then, numerous preparations relying on niosome technology have been developed and introduced to the market [14]. Several medications incorporating niosomes have also been identified for skin permeation administration, such as lidocaine, diclofenac, arbutin, niosome applying nonionic surfactant, and Chol [15-17]. Various techniques for the preparation of niosomes have been documented in the literature, including thin film hydration, organic solvent injection, reverse phase evaporation, and the bubble method [18]. One of the most common and easy experimental methods for niosome preparation is the melted dispersion method followed by ultrasonication [19, 20]. The principal benefits of this procedure are the lack of using hazardous, highly volatile, and harmful chemical solvents, that are prohibitively priced, challenging to eliminate entirely, and unsafe. Another benefit of this technique is its single stage and simple scaling up manufacture of nanoparticle. In addition to these advantages, this approach is not appropriate for substances that are sensitive to heat [13].

In a separate study, baclofen-loaded niosomes were produced with the type of Span and Chol to enhance baclofen skin penetration via the lipid film hydration method which leaves organic solvent residues that could induce some toxic and adverse effects on healthy tissues alongside environmental concerns [7]. Various techniques, such as patch, iontophoresis, and microneedles, have been used by some investigators to enhance the dermal delivery of baclofen [21, 22].

In the present study, the baclofen-loaded niosomes (Baclosomes) prepared by an ultrasonication method (an eco-green preparation for topical delivery) were characterized. Then, Baclosomes were incorporated into a gel (Baclosome gel) for the next *in-vivo* studies. This study is the first attempt to evaluate the efficacy of Baclosome gel as an analgesic and anti-inflammatory substance.

MATERIALS AND METHODS

Materials

Baclofen was presented by Chemidarou Co. (Iran) [23]. Also, Span 60 and Tween 60 were provided from Merck (Germany). Chol was acquired from Sigma (Germany). Carbopol 941 was supplied from BF Goodrich) USA.

Fabrication of baclofen niosome via ultrasonic method

Baclosome was formulated by ultrasonic method. The required amounts of baclofen, Chol, Tween 60, and Span 60 were provided to the beaker while stirring at 150 rpm at 75 °C. The watery stage was warmed to an identical temperature as a mixture of surfactants (Table 1). Subsequently, the two mixtures were blended with an electromagnetic stirrer to produce a course-emulsion. Afterward, the primary niosome was subjected to ultrasound probe with 30% amplitude for 5 min prior to being quickly cooled in an ice immersion to acquire Baclosomes.

Table 1. Constituent and physicochemical description of observed Baclosome

Formulation	baclofen (mg)	Chol [a] (mg)	Span60 (mg)	Tween60 (mg)	Chol: Surf ratio [b]	Water up to (mL)	Particle size (nm)	PDI [c]	ZP [d] (mv)	EE [e] (%)	Appearance
Baclosome 1	125	50	300	200	1:10	25	315.68 ± 15.42	0.426 ± 0.04	-28.81 ± 1.3	61.33 ± 0.33	Milky and homogenous
Baclosome 2	125	125	300	200	1:4	25	383.35 ± 28.61	0.466 ± 0.04	-23.81 ± 1.61	64.51 ± 1.16	Milky and homogenous
Baclosome 3	125	250	300	200	1:2	25	422.33 ± 6.06	0.552 ± 0.05	-20.16 ± 4.47	67.2 ± 0.34	Milky and homogenous
Baclosome 4	125	500	300	200	1:1	25	599.76 ± 39.30	0.557 ± 0.04	-9.10 ±1.81	68.1 ± 1.79	Milky and homogenous

[a] Cholesterol; b Cholesterol: surfactant ratio; c Poly dispersity index; d Zeta potential, e Encapsulation efficiency

Description of Baclosomes

The mean diameter, polydispersity index (PDI), and ZP of niosomes were analyzed via a Zetasizer Nano-ZS device and dynamic light scattering (DLS) (Malvern Instruments Ltd., UK). The homogeneity and precipitation were determined by visual inspection for the appearance of Baclosomes and the presence of any aggregates. A Cary 630 FTIR spectrometer (Fourier Transform Infrared Spectrometer, Agilent Technologies Inc., CA; USA) with a diamond ATR (Attenuated Total Reflectance) was applied to assess the potential interactions between the ingredients. ATR-FTIR spectra were recorded in the range of 4000-400 cm^{-1} with a resolution of 2 cm-1. DSC assessments were conducted with a pyris6 (PerkinElmer, Norwalk, USA). The diffraction graph (XRD) was produced by an X-ray diffractometer (PHILIPS-PW1730; Netherlands) at 40 kV, 30 mA, an angle range of 5 to 50°, and a scan speed of 0.05°/min. The morphology aspects of formulation were examined via SEM (HITACHI S-4160).

Encapsulation efficiency (EE%)

The amount of entrapped baclofen in niosome nanoparticles was determined by centrifugation (SIGMA 3-30KS refrigerated centrifuge, Germany). The Baclosome specimen was centrifuged for 45 min at 21000 rpm at 4 °C. The sample was then retracted and assessed with a UV-Vis Spectrophotometer at 264 nm (Jasco V-630, Japan). The percent of pharmaceutical encapsulation (EE%) was calculated using the succeeding equation:

$$EE\% = \left(\frac{Winitial - Wfree}{Winitial}\right) \times 100$$

where W initial is the amount of total baclofen originally inserted to the formulation and W free is the amount of unentrapped baclofen in the supernatant.

***In-vitro* drug release experiment**

The *in-vitro* release investigation was conducted with immersion cells containing an acetate cellulose dialysis bag. Formulas were inserted in the cell, and an acetate cellulose membrane was applied before capping the cell with a seal. The cells were immersed in the USP dissolution equipment Number II, and 900 mL of purified water was inserted into the dissolution vessel to produce the dissolution media [24]. 5 mL of the dissolving medium was collected at the various periods (2, 4, 6, 8, and 24 hr) and filtered by a 0.22 µm syringe filter. The concentration of the baclofen in the discarded specimens was assessed via a UV spectrophotometer at 264 nm. The following equation calculates the cumulative amount of drug released.

$$Cumulative\ amount\ of\ drug\ released\ (\%) = \left(\frac{W2}{W1}\right) \times 100$$

where W_1 denotes to the quantity of the pharmaceutical substance inserted to the immersion cells and W_2 showed the cumulative pharmaceutical substance released in the dissolution media.

Baclosome gel and baclofen simple gel preparation

A specific amount of Baclosome 2 (as the selected formulation) freeze-dried powder, which contains 200 mg of baclofen, was scattered in preservation agent aqua (0.1% w/v sodium benzoate in distilled water). After that, Carbopol 941 pharmaceutical standard (0.75% w/v) was dispersed into the mixture and left overnight to hydrate the polymer. After that, the mixture was neutralized with 200 mg of triethanolamine to form a gel. To manufacture baclofen plain-gel, a propeller homogenizer at 400 rpm was used to blend 10 g baclofen solution (200 mg baclofen in preserving water) with 0.75% w/v Carbopol 941. Then, according to the same procedure, the gel was formed.

In-vitro studies of dermal penetration

Male Wistar rats (weighing 120-150 g) were sedated with 87 mg ketamine/kg body mass and 13 mg xylazine/kg, before trimming their abdomen cutaneous with an electronically controlled scissors. The animals were euthanized via chloroform breath after 48h, and the abdominal cutaneous was separated. The adhering abdominal fats and other subcutaneous tissue were omitted carefully. The dermis was initially immersed in 0.9% NaCl for 24 hr to remove excess debris and enzymes. The dermis was placed among the donating and receiving compartments of the Franz cell, with the dermal surface in direct connection with the receiving media. The receiver part was loaded with approximately 33 mL of deionized water. A thermal-control water chamber was utilized to maintain a temperature of 32 ± 0.5 oC, and a magnetic stirrer was employed to mix the solution at 200 rpm throughout the assessment [25]. The number of preparations employed in the penetration investigation was equivalent to 1 g of Baclosome 2 gel comprising 20 mg of baclofen, and the concentration of baclofen simple gel was identical to that of Baclosome gel. 0.5 mL of aliquots were collected at predetermined intervals (1, 2, 4, 6, 8, and 24 hr) and analyzed using a UV-spectrophotometer at 264 nm. Consequently, an equivalent amount of the fresh dissolution media was replaced in the recipient section.

Dermal remaining test of baclofen

The dermis was removed once the penetration experiment was finalized. The dermis was cleansed 3 times with aqua and then drained before calculating the extent of baclofen deposited in the dermis. The detached skin was clipped into slight fragments with scissors, placed in a tube, digested in aqua, and then, sonicated in a bath sonicator for 1 hr. The samples were further filtered with filter paper (Whatman filter paper grade 591) and a syringe filter (pore size 0.22 mm) and analyzed with a UV spectrophotometer at 264 nm wavelength [25].

In-vivo Investigation on Baclosome

Animals

Male Swiss-Webster mice weighing 25 to 30 g were used throughout this examination. Six mice were located in each plastic cabin of the animal department with a 12-hr light phase at 21 ± 2°C (light on 08:00–20:00 h). Mice always had access to waters and nutrition, except during the trial.

Formalin test

Baclosome 2 gel was administered to the behind regions of the cutaneous of the left paw (n = 6) by gently applying 50 folds with the pointer-finger. The gel base was applied identically to the control group. The baclofen simple gel, diclofenac gel, and bare-niosomal gel were all applied similarly. 50 µL of 2.5% formalin solution was subcutaneously injected into the plantar region of the left rear toe for formalin evaluations. The mice were placed in a plastics observing box following the formalin injection. The duration of licking, shaking, and biting the injected fingers was analyzed with a stopwatch and reflected a sign of analgesia. The initial step of the nociceptive reaction lasted between 0 and 5 min, whereas the final stage lasted between 15 and 60 min after formalin injection [26].

Hot-plate examination

Mice were divided into five groups of six for the hot-plate assessment. The 1st group served as the control and received base gel. The 2nd group received a placebo (niosome without baclofen), whereas the 3th and 4th groups, respectively, received plain baclofen (2%) gel and diclofenac gel. The 5th group was given Baclosome gel containing 2% baclofen. The evaluation was conducted on a hot-plate apparatus heated to 52 ±1°C. The mice were situated on a device and reactions durations for licking the paw or jumping were measured in seconds [26].

Dermatological irritation evaluations

The dorsal hair of Wistar rats was shaved off using a razor one day before the research. There were five groups of six rats each (n = 6). Group, I served as the control, Group II attained Baclosome 2 gel (optimal niosome), Group III earned the plain baclofen gel, Group IV attained the placebo gel (niosome without drug), and Group V obtained a 0.8% (v/v) aqueous solution of formalin as the normal itching. The formulations (Baclosome gel, baclofen simple gel, placebo, and formalin solution) were applied to the animal dermis daily for 3 days, and the special site was inspected eyesight-vision [27].

Statistical evaluation

SPSS 22 was used to conduct the statistical analyses (IBM Co., USA). Tukey's post hoc analysis and the analysis of variance [28] were used. The outcomes were deliberated substantially while the P<0.05.

RESULTS AND DISCUSSIONS

The characteristics of manufactured baclosome

In this study, stable vesicle formations

incorporating baclofen were manufactured using an ultrasonic approach while adjusting the Chol: Surf ratio (a binary blend of Tween 60 and Span 60) to optimize baclofen niosomal composition. The application of blends of surfactants with minimum and maximum Hydrophilic-Lipophilic Balance (HLB) and Chol often results in improved niosome stability [17]. Furthermore, minimum and maximum HLB surfactants can be spread in the aquatic and organic stages, owing to enhanced surfactant film stability at the interface [29]. All the Baclosome formulations were homogenous and milky, and no evidence of aggregation and precipitation was observed (Table 1).

Table 1 depicts the niosome constitution and their characteristics, indicating that by changing the Chol: Surf relation from 0:10 (Baclosome 1) to 10:10 (Baclosome 4), the particle diameter of Baclosome enhanced substantially from 315.68 ± 15.42 to 599.76 ± 39.30 nm (P<0.05). Chol may multiply the extent of bi-layers, leading to an enhancement in dimension [30]. It was determined that the addition of Chol in niosomes improved bilayer cohesiveness and hardness [31]. Various researches have also demonstrated that a higher Chol concentration in niosomes can lead to a greater size of the niosome [16, 17, 32].

Table 1 also included the percentage of baclofen incorporated in the niosomes platform, which ranges from 61.33 ± 0.33% to 68.1 ± 1.79%. Chol has been demonstrated to affect EE% and membrane permeability, resulting in a change in vesicle membrane fluidity[33]. It was additionally demonstrated that the EE% increased substantially while the Chol content in niosomal preparation of diclofenac [16], arbutin [17], and curcumin [34] expanded.

Table 1 illustrated additionally that the Baclosome ZP was diminished when Chol was integrated into the niosome. In this circumstance, the ZP of Baclosome 1 was -28.81± 1.3 mV, but after increasing the ratio of Chol: Surf from 1:10 to 10:10, it declined to -9.10±1.81mV. Baclosome 1 with the maximum ZP must be extra stable than other preparations throughout storing or when vesicles are combined with aqua [35]. Moreover, it was displayed that the ZP of the niosome increased due to the decreased percent of drug encapsulation, which may be related to the dispersion of the available medication in the watery phase or the potential diffusion barrier [36].

Normally, the PDI score must be between 0 and 1, with results around 0, suggesting homogenous dispersal [30]. In Table 1, the PDI results diverse from 0.426 ± 0.04 to 0.557 ± 0.04 ($P < 0.05$). In overall, PDI numbers greater than 0.7 indicate a wide-ranging particle diameter dispersal [37]. As per Table 1, Baclosome with the smallest quantity of Chol had the minimum PDI mean (0.426 ± 0.04). The greatest mean PDI (0.557 ± 0.04) was reported in Baclosome with the largest Chol content (Baclosome 4). In addition, as noted recently, the addition of more Chol molecules in Baclosome may produce a lesser electric charge, which may enhance the agglomeration partiality of vesicles, leading to a superior PDI number. Some reported research involving various medicines validated these outcomes [38].

Despite earlier stated techniques, the proposed approach does not involve the application of a chemical-solvents in the production of baclosome, and the manufactured niosomes can be simply blended into the gel matrix to form a final dosage form. In addition, none of the prior reports have examined the effects of the Chol: Surf proportion, which is essential for enhancing Baclosome effectiveness. Depending on the outcomes shown in Table 1 and the size requirements for dermal preparations, suitable particle diameter for local administration is between 200 and 700 nm [39]. Baclosome 2 was regarded as the ideal preparation for further research because it exhibited an adequate ZP (-23.81 ± 1.61 mV), PDI number (0.466 ± 0.04), and EE% (64.51 ± 1.16).

SEM evaluation

Based on the SEM photos in Fig. 1 for the improved preparation (Baclosome 2), the nanoparticles are divided, dispersed, and round shape. The photograph also indicated that the

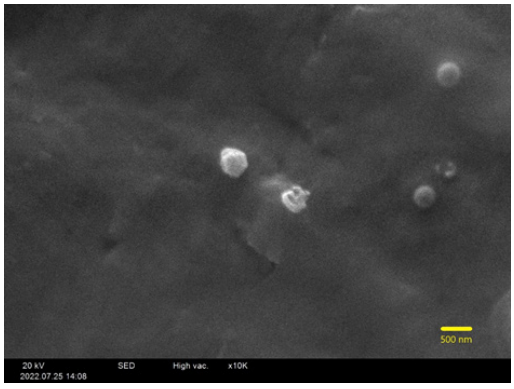

Fig. 1. SEM image of Baclosome 2

produced nanovesicles are sufficiently uniform in terms of niosomes' structural properties.

ATR-FTIR spectroscopy analysis

ATR-FTIR spectroscopy was applied to investigate the constituents included in the formula and to explore the feasible chemical interaction between baclofen and ingredients present in the formulation. Fig. 2 depicted the ATR-FTIR spectra of Baclosome 2, pure baclofen, Chol, Tween 60, and Span 60. The ATR-FTIR spectrum of baclofen showed the characteristic peaks at 3325-3305 cm^{-1} (N-H stretching), 3200-2400 cm^{-1} (O-H of carboxylic acid stretching), 2984 cm^{-1} & 2858 cm^{-1} (C-H stretching), 1527 cm^{-1} (C=O stretching), and 1247 cm^{-1} (C-O stretching). The ATR-FTIR spectrum of Chol showed peaks at 3401 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 ATR-FTIR spectrum of Tween 60 indicated peaks at 3498 cm^{-1} (O-H stretching), 2922 cm^{-1} (C-H asymmetric stretching), 2856 cm^{-1} (C-H symmetric stretching), and 1735 cm^{-1} (C=O stretching). The ATR-FTIR spectrum of Span 60 displayed peaks at 3385 cm^{-1} (O-H stretching), 2917 cm^{-1} (C-H asymmetric stretching), 2850 cm^{-1} (C-H symmetric stretching), and 1736 cm^{-1} (C=O stretching). As shown by the ATR-FTIR outcomes, any chemical interaction did not occur between baclofen and the other materials in the selected formulation (Baclosome 2).

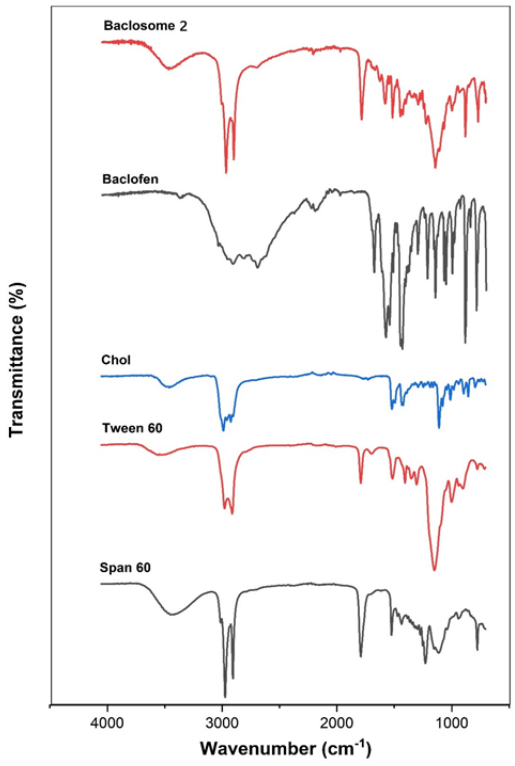

Fig. 2. ATR-FTIR spectra of Baclosome 2, baclofen, Chol, Tween 60, and Span 60

DSC investigation

DSC was utilized to examine the thermal reactions of powdered baclofen, Chol, and Baclosome (Fig. 3). Baclofen and Chol presented a pointed endothermic melting trace at approximately 216 °C and 150 °C, respectively. When comparing the DSC graph of Baclosome to the DSC patterns of each ingredient employed to manufacture formulated, the endothermic spike for Chol was observed, but there was no indication of an endothermic spike for baclofen. This reveals that the baclofen in Baclosome is possibly amorphous.

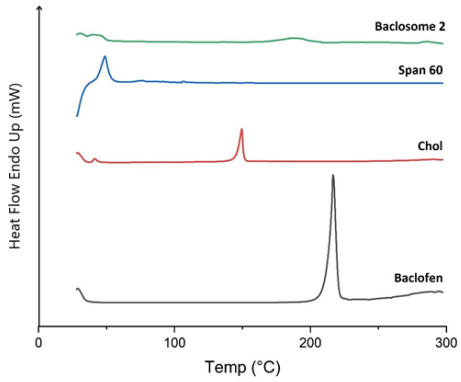

Fig. 3. DSC traces of Baclosome 2, Span 60, Chol, and Baclofen

XRD analysis

Fig. 4 exhibits Chol, baclofen, and Baclosome 2 XRD graphs. The baclofen XRD exhibited the distinct peaks at 2θ of 17.455, 19.205, 21.505, 23.505, 25.85, 29.05, and 38.70°. Chol XRD confirmed traces at 2θ

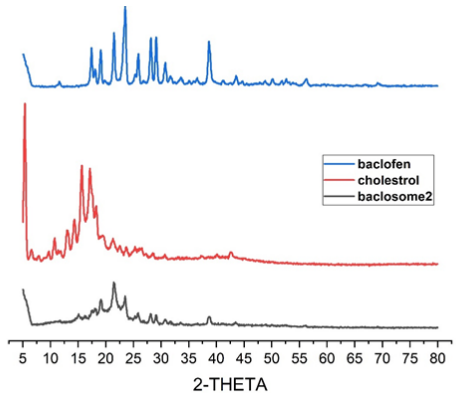

Fig. 4. XRD peak of Baclofen, Cholesterol, and Baclosome 2

of 5.30°, 10.60°, 12.83°, 15.55°, 17.05°, 17.4°, 18.15°, 23.55°, 26.2°, 37.15°, and 42.40°. The presence of pointed spikes for Chol and baclofen revealed that they are both extremely crystalline compounds. While the XRD of Baclosome 2 was contrasted to the XRD of pure baclofen and Chol, the crucial distinguishing spikes of Chol were detectable in Baclosome (2 of 5.3°, 15.55°, 17.05°, and 23.55°), but not in baclofen in niosomal preparation. This is consistent with the Baclosome 2 DSC patterns (Fig. 3).

Drug release

Fig. 5 illustrates the influence of preparation form (niosomal dispersion and simple solution) on the baclofen release pattern. It demonstrated that the dissolution rate of baclofen from niosome formulations increased in general. The release of baclofen from niosomes occurred in the biphasic model. Initially, more than 50% of the drug was released rapidly in the first 6 hr. This was followed by sustained release that continued for up to 24 hr. This biphasic release pattern is a typical characteristic of bilayer vesicles [40]. Drug release rates from Baclosome were substantially greater than from baclofen simple solution. The drug release percentages from Baclosome and simple solution are 100.312±8.081 and 73.505±5.170, respectively, during 24 hr. These observations demonstrated that the niosome could improve the dissolving rate, bioavailability, and continuous drug release of the poorly soluble medication. Other investigators have reached the same outcome when using niosomal preparations of ketoconazole and curcumin [34, 41].

Skin penetration evaluation

Fig. 6 and 7 depict the accumulated amount of baclofen that passed the rat dermis (transdermal administration, Fig. 6) and the amount of baclofen that absorbed the cutaneous layers (dermal delivery, Fig. 7) for Baclosome gel and baclofen simple gel. The Baclosome gel displayed more permeation into and throughout the epidermal layers than the baclofen simple gel, indicating that it was more suitable for transdermal application ($P<0.05$). The quantity of baclofen in the recipient section was substantially lower for baclofen simple gel (697.948±55.009 µg/cm^2) than for Baclosome gel (992.733±53.596 µg/cm^2) ($P<0.05$). Moreover, the concentration of baclofen that was retained in the skin after administration of Baclosome gel (286.085±18.695 µg/cm^2), meaningfully greater than that of baclofen plain gel (225.825±4.331 µg/cm^2) ($P<0.05$). These outcomes revealed that a niosomes product would be desirable to a typical gel product for cutaneous and transdermal delivery.

Chol levels in niosomes proved to have a significant effect on baclofen penetration into the dermis. Chol may change the mobility,

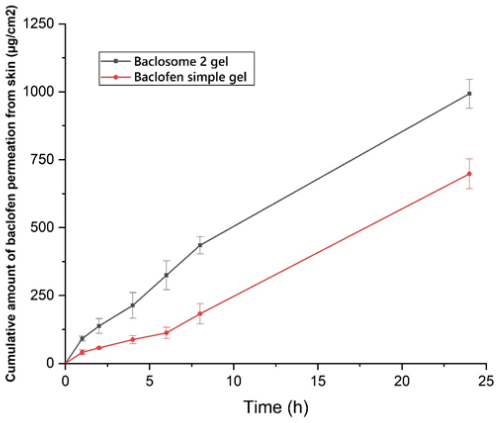

Fig. 6. The extent of baclofen absorbed via rat skin (average±SD, n=3)

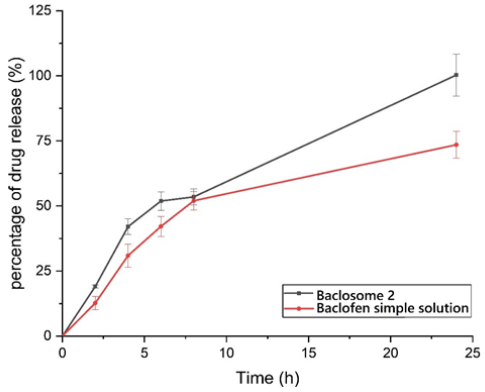

Fig. 5. Cumulative drug release of baclofen simple gel and Baclosome gel (mean±SD, n=3)

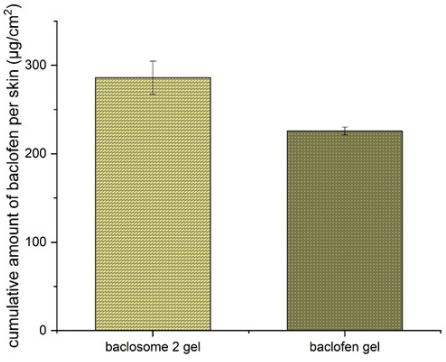

Fig. 7. The extent of baclofen deposited in the skin from Baclosome 2 gel and baclofen simple gel ($P<0.05$), (mean±SD, n=3)

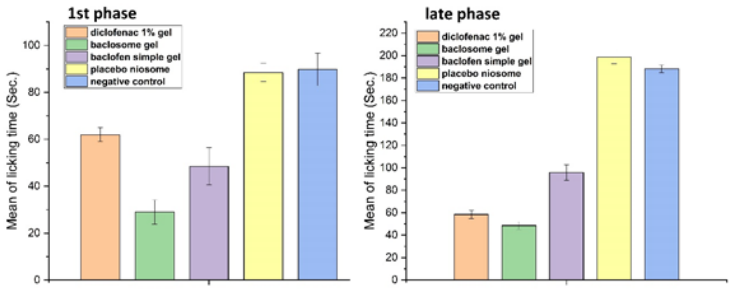

Fig. 8. Evaluation of the anti-inflammatory and analgesic impact of baclofen in the formalin test (average±SD, n=5)

phase behavior, and persistence of membrane permeability to increase the absorption of medicines across the dermis, according to reports [31]. Chol and its esters are frequently applied for local delivery as permeability facilitators [32, 42]. It is widely accepted that nonionic surfactants have the lowest cytotoxicity and cutaneous sensitization potential, which is why they have been widely researched as cutaneous absorption enhancer [43]. Surfactants improve cutaneous absorption by directly influencing the skin's membrane qualities and indirectly influencing the permeate's thermodynamic activities in the carrier. Permeate diffusion into the dermis is propelled by the thermodynamic activities of a permeate in a carrier. The cutaneous 's membrane qualities can be altered by the surfactant monomers' absorption and interaction with the cutaneous tissue, making it simpler for permeate to infiltrate the cutaneous [44]. The synergistic skin penetration effect of both combined surfactants has been stated [45]. These could be the explanation for why niosomal compositions penetrate the skin more effectively. In consequence, it has been demonstrated that the HLB ratio of the surfactants is an essential element in determining the dermal permeation of medication [17, 36]. The HLB of a surfactant plays a significant role in determining its interaction with the skin and subsequent drug absorption. Surfactants with the different HLB values have varying affinities for water and lipid phases, which can influence their ability to penetrate the skin barrier and facilitate drug absorption. Also, surfactants with HLB values that match the lipophilicity of the pharmaceutical agent or the components of the cutaneous barrier can enhance medicine penetration. For instance, if the drug is lipophilic, surfactants with a lower HLB value (more lipophilic) may be more effective in solubilizing the drug and facilitating its penetration through the lipid-rich stratum corneum. In addition, surfactants with HLB values appropriate for the drug and skin composition can improve drug solubility in the vehicle applied to the skin. This enhanced solubility can increase the concentration gradient driving drug diffusion into the skin. At this end, surfactants with higher HLB values (more hydrophilic) tend to have greater affinity for water and may disrupt the skin barrier by interacting with intercellular lipids, facilitating drug permeation through the skin [19, 26].

Formalin examination

The formalin research assesses the effect of different therapies on earlier and late-stage pain. The formalin study results are shown in Fig. 8. During the initial stage of the formalin experiment, the Baclosome gel containing 2 % baclofen produced a greater analgesic impact compared to the baclofen simple gel and diclofenac gel (Fig. 8, 1st phase; $P<0.05$). The late-stage formalin assay illustrated that Baclosome gel had a substantial influence on the antinociceptive properties of baclofen. Moreover, the analgesic augmenting reaction of the niosome was verified ($P<0.05$) in the last stages of the formalin study (Fig. 8, late phase). Comparing Baclosome 2 gel to baclofen simple gel (at the same dose) and diclofenac 1% gel, Baclosome gel exhibited the improved anti-inflammatory efficacy. Similar outcomes were observed when diclofenac [34], venlafaxine [11], and curcumin [34] were incorporated in niosomes constituted of Chol and surfactants for dermal application and revealed that the niosome-integrated gel exhibited stronger anti-inflammatory and analgesic action in the formalin assay.

Hot-plate experiment

The efficacy of a niosomal product of baclofen on severe pain was evaluated, and the outcomes are shown in Fig. 9. The graph displays the response

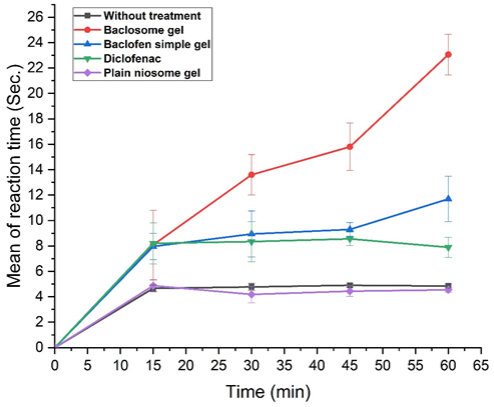

Fig. 9. Hot-plate evaluation of baclofen preparations in pain response (mean±SD, n=5)

period of mice to heating when administered with various baclofen compositions. The Baclosome gel was observed to have the best analgesic efficacy versus heat-induced pain contrasted to the other preparations ($P<0.05$). The diclofenac group's delay duration was also longer than the control groups, which illustrates that this preparation did some quality of pain relief. Nevertheless, the pain-relieving impact of Baclosome was much stronger than that of diclofenac gel. Baclofen simple gel similarly indicated an anti-nociceptive impact that was higher than control groups. Similar outcomes were observed when venlafaxine was incorporated in niosomes constituted of Chol and surfactants for dermal application and revealed that the niosome-integrated gel exhibited stronger anti-nociceptive activity in the hot-plate assay [11].

Dermal sensitivity assessment

The medicine delivery method must not only transfer the pharmaceutical agent but also have no noticeable side effects. As the manufactured niosomal form of baclofen is indicated for the cutaneous applications, it must prevent producing dermal hypersensitive reactions to be considered an harmless preparation [46]. In actuality, one of the most common adverse impact of a dermal remedy is skin discomfort, which has a noticeable association with the quantity of the pharmaceutical substances. Consequently, altering the medication release from the preparations can improve the formula's unfavorable impact by enhancing intra-follicular absorption and declining dermal permeation [46]. Table 2 demonstrates the impact of some baclofen preparation on the dermal sensitivity degree (edema and erythema). Owing to Woodward, Draize, and Calvery, compounds with a rating of two or below (no skin sensitivity) are considered negative. The Baclosome gel formulation exhibited a rating of 0.333 for edema and erythema which was notably lower than the scores recorded for other baclofen formulas and formalin (Table 2). This means that there was no sensitivity and improved the gel's safety and dermatological adaptability. Other investigators have reached the same result when using niosomal gel containing benzoyl peroxide [47], arbutin [17], terbinafine [10], venlafaxine, and curcumin [34].

CONCLUSION

Baclofen was effectively encapsulated in niosomes composed of nonionic surfactants and Chol. Baclosomes were successfully prepared via a green, eco-friendly, and simple method yielding a great medicine EE%. The synthesized Baclosomes werein nano-scale and revealed a spherical morphology. The negative surface charge of Baclosomes produces a repulsive force between nanoparticles, which could hinder their aggregation and promote stability. The crystalline phase of baclofen assumed that the medicine was amorphous and effectively incorporated into the vesicle without chemical interactions with the other niosome constituents supported by DSC and ATFR-FTIR results, respectively. The topical itching test revealed that the applied niosomal gel constituent did not cause dermis sensitivity. Consequently, formalin analysis and

Table 2. The skin sensitivity of Baclosome gel after dermal application

	Control		Baclosome gel		Baclofen simple gel		Blank niosomal gel		Formalin	
Rat num	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	1	0	1	1	1	1	4	3
2	0	0	0	0	1	0	0	0	3	3
3	0	0	0	0	1	1	1	1	3	3
4	0	0	0	0	1	1	1	1	4	3
5	0	0	0	0	1	1	1	0	3	3
6	0	0	0	1	1	1	1	2	3	3
Total score	0	0	0.166	0.166	1	0.833	0.833	0.833	3.333	3

hot-plate assessment experiments confirmed that the Baclosome gel possessed superior anti-inflammatory and antinociceptive activities than the baclofen typical gel and diclofenac gel. It may be stated that Baclosome has the ability to provide improved functionality for both transdermal and local delivery, overcoming the disadvantages of orally administering while retaining its analgesic and anti-inflammatory activities.

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ETHICAL APPROVAL

The Ethical Review Board of Mazandaran University of Medical Sciences granted approval for all animal research studies in accordance with the certification code IR.MAZUMS.4.REC.1400.8959. The research adhered to the ARRIVE standards, the Animals (Scientific Procedures) Act of 1986 and its associated guidelines, as well as the EU Directive 2010/63/EU, which serves as a foundation for the assessment and ethical treatment of animals in experimental procedures.

DATA AVAILABILITY

Data can be available on a reasonable request.

CONFLICTS OF INTEREST

No conflicts of interest were reported by the authors.

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