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RESEARCH ARTICLE

Proniosomal gel for transdermal delivery of lornoxicam: optimization using factorial design and in vivo evaluation in rats

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Abstract

Objective Clinical utility of lornoxicam in oral therapy is primarily restricted by the low solubility and gastric adverse effects. This study evaluated the prospective of optimized proniosomal gel to improve the clinical efficacy of lornoxicam and compare with oral therapy.

Methods Proniosomes were formulated by coacervation phase separation technique using span 60, lecithin and cholesterol. A four-factor three-level Box-Behnken design was used to evaluate the effect of amount of four independent variables; span 60 (X_1), cholesterol (X_2), lecithin (X_3) and lornoxicam (X_4) on response variables; vesicle size (Y_1), entrapment efficiency (Y_2) and transdermal flux (Y_3). The selected proniosomal gel (F19) was characterized, and evaluated for the transdermal efficacy by ex vivo and in vivo experiments.

Results Optimization study signifies that amount of formulation components (span 60, cholesterol, lecithin and lornoxicam) influence the vesicle size, entrapment efficiency and/or transdermal flux. Optimized formulation F19 exhibited nano size with high entrapment efficiency, adequate zeta potential, greater transdermal flux and better stability (at refrigerated conditions). The entrapment of lornoxicam in the bilayers of proniosome vesicles was confirmed by differential scanning calorimeter. Release profile of F19 was distinct ($p < 0.001$) from gel prepared using hydroxypropyl methylcellulose (control) and displayed steady lornoxicam release by Fickian diffusion. Transdermal administration of F19 significantly inhibited the carrageenan induced hind-paw edema in rats as compared to oral lornoxicam group.

Conclusions The data observed in this study demonstrated that the developed proniosomal gel (F19) improved the clinical efficacy of lornoxicam as compared to oral therapy.

Keywords Lornoxicam · Transdermal · Edema · Proniosome · Box-Behnken design · In vivo

Introduction

Lornoxicam belongs to oxicam category of non-steroidal anti-inflammatory drug (NSAID) with potent analgesic, anti-inflammatory and antipyretic effects. It is primarily indicated in pain, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and shown proven efficacy as opioid analgesics in the treatment of postoperative pain associated to gynaecological, orthopaedic, dental and abdominal surgeries [1]. The mechanism of action of lornoxicam, is mainly because of its potential to inhibit prostaglandin biosynthesis by blocking the cyclooxygenase enzymes (COX-1 and COX-2). Lornoxicam is commercially available as tablets (immediate/rapid release) and injection for intravenous and intramuscular administration [2]. Even though lornoxicam possess potent analgesic and anti-inflammatory actions, its short duration of action (3–5 h; due to fast elimination) leads to frequent oral administration. Being a biopharmaceutics classification system class II drug, it exhibits low solubility in stomach pH and remains insoluble for long

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time. Therefore, the oral administration of lornoxicam generally leads to gastric irritation, peptic ulcers and gastrointestinal hemorrhage, which often leads to discontinuation of therapy [3]. In this context, transdermal delivery of lornoxicam could be a possible alternative to avoid issues associated with oral route as well as to target the inflammatory region.

Delivery of drug into and through the skin to achieve therapeutic activity has gained acceptance primarily because of the high adaptability and versatility [4]. The efficiency of transdermal delivery largely depends on the potential to overcome the stratum corneum (SC), a major barrier, which essentially limits the transport of molecules through the skin. Various transdermal systems and enhancement approaches are used to achieve effective drug delivery across the skin [5]. The choice of transdermal system depends on the drug's physicochemical properties as well as the therapeutic use [6]. The characteristics of lornoxicam such as low molecular weight (371 Da), good log P (2.62), low dose (8 mg) and short half-life (3–5 h) appears suitable for transdermal administration. Different transdermal systems (emulsions, lotions, gels, patches, nano-vesicles) were developed to deliver lornoxicam though the skin [7–9]. Among these, gel based formulation could be most ideal for transdermal delivery of NSAIDs as it offers higher patient compliance, greater efficiency, tailored pharmacokinetics, increased residence time, faster drug release and low skin irritation [10, 11]. On the other hand, nanocarriers have demonstrated their potential to enhance the percutaneous absorption of drugs [12, 13]. Among the nanocarriers, niosomes, a versatile delivery approach has the prospective to deliver therapeutic actives into the systemic circulation. These vesicle systems owns amphiphilic moieties and can accommodate both hydrophilic and hydrophobic components as well as enhance drug delivery [14]. Proniosomes, a niosome hybrid, is another promising means of percutaneous drug delivery which offers better suitability for application on dry skin, convenience of transportation, distribution, storage, dosing and secured industrial acceptance [15, 16]. The prospective of transdermal therapy of lornoxicam by proniosomal gel over conventional gel has been demonstrated [17]. Different from earlier studies, the objective of the current study was to design and optimize lornoxicam loaded proniosomal gel using factorial design to improve the percutaneous absorption and compare the clinical efficacy with marketed oral therapy in rat model. Optimization was carried out by assessing concentration effect of formulation components (span 60, cholesterol, lecithin, and lornoxicam) on vesicle size, entrapment, and transdermal flux. The optimized proniosomal gel (F19) was further characterized, evaluated for the transdermal efficacy by ex vivo and in vivo studies and compared with marketed oral tablet.

Materials and methods

Materials

Lornoxicam was gifted by Zydus Cadila, Ahmedabad, India. Soya lecithin and cholesterol were gifted by CP Kelco UK Ltd., Surrey, England. Hydroxypropyl methylcellulose (HPMC), and span 60 were purchased from Central Drug House Pvt. Ltd., Mumbai, India. Syringe filter, dialysis membrane and membrane filter were purchased from Merck India Ltd., Mumbai, India.

Lornoxicam analysis

The concentrations of lornoxicam in samples were determined by high performance liquid chromatography (HPLC) using a reported method with minor modifications [18]. The HPLC system consists of UV-2075 Plus ultraviolet absorbance detector, MD-2015 Plus PDA detector and PU-2080 Plus pump (LC-2000 Plus, Jasco, Japan). A Lichrosphere 100 RP-18 (250 mm × 4.6 mm × 5 μm; Merck, Germany) column was used to separate lornoxicam. The mobile phase made up of methanol and distilled water containing 0.5% acetic acid (60:40, *v*/v) and the pH was adjusted to 6.8 with triethylamine. The mobile phase was allowed to flow at 1 ml/min and the absorbance were measured at 292 nm. Calibration curve was linear in nano-concentrations (100–1000 ng/ml) with higher correlation coefficient ($r^2 = 0.991$). Samples (20 μl) were injected and the retention time was found to be 5.5 min. The internal standard used was methyl paraben.

Formulation of optimized proniosomal gel (F19)

Coacervation phase separation method reported earlier with minor modifications was used to prepare proniosome formulations [19]. For F19 preparation, span 60 (90 mg), lecithin (90 mg), cholesterol (10 mg), and lornoxicam (5 mg) were allowed to mix with absolute ethanol (2.5 ml) in a glass tube. After mixing, the tube was sealed to avoid solvent evaporation and warmed the mixture at 65 ± 3 °C to dissolve surfactants (~5 min). Further, phosphate buffer (1.6 ml; pH 7.4) was incorporated and warming was continued for ~2 min and then cooled to room temperature to obtain proniosomes. The final ratio of surfactant: alcohol: aqueous phase in proniosomes was 1:10:8 *w*/w/w. The proniosomes were then mixed with polymeric gel (HPMC 2% w/w) to get proniosomal gel containing 0.5% (w/w) lornoxicam. The control gel was prepared using HPMC (2% w/w) containing 0.5% (w/w) lornoxicam.

Box-Behnken design

A four-factor three-level Box-Behnken design was used to assess the influence of independent variables on dependent variables and to get optimized formulation using Design Expert software 9.0.3 (Stat-Ease, Minnesota, USA). The design matrix displays 29 experimental runs, for which the non-linear software generated quadratic model as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2 + b_{44}{X_4}^2$$

where Y indicate the response affiliated with each factor level combination; b_0 is a constant; b_1, b_2, b_3 and b_4 are linear coefficients, b_{12}, b_{13}, b_{14}, b_{23}, b_{24} and b_{34} are interaction coefficients; b_{11}, b_{22}, b_{33} and b_{44} are quadratic coefficients; and X_1, X_2, X_3 and X_4 are the coding for independent variables. The terms X_1X_2 and ${X_1}^2$ (I = 1, 2, or 3) symbolize the interaction and quadratic terms, respectively. The amount of span 60 (X_1), cholesterol (X_2), lecithin (X_3), and lornoxicam (X_4) used in 29 formulations and the respective values are shown in Table 1. Three formulations (F30, F31 and F32) were taken as check point batches. The coded and uncoded values of independent variables are given in supplementary Table 1.

Characterization

Entrapment efficiency

Proniosomes (0.2 g) was reconstructed with phosphate buffer (pH 7.4; 10 ml) and the aqueous suspension was sonicated (Transonic T460/H, Elma, Germany) for 30 min. Centrifugation (9000 rpm at 4 °C) was done to separate the drug from other components for 45 min. Subsequently, the supernatant was analyzed for lornoxicam concentration. The entrapment efficiency was calculated by the equation [20]:

Percentage entrapment efficiency

= (Amount of drug entrapped/Amount of drug incorporated) × 100

Table 1 Variables in Box–Behnken design for preparation of lornoxicam proniosomal gels

Factors	Level used (actual coded)		
	Low (−1)	Medium (0)	High (+1)
Independent variables (mg)			
X_1 = Span 60	70	90	110
X_2 = Cholesterol	5	10	15
X_3 = Lecithin	90	100	110
X_4 = Lornoxicam	5	10	15
Dependent variable	Targets		
Y_1 = Vesicle size (nm)	Minimum		
Y_2 = EE %	Maximum		
Y_3 = Flux (μg/cm^2/h)	Enhancement		

Vesicle size and zeta potential

The mean vesicle size, size distribution and zeta potential of proniosomes were measured by a dynamic light scattering technique using Zetasizer (Nano ZS90, Malvern, UK). The prepared proniosomes was diluted and the size measurements were done at 25 ± 0.5 °C.

Microscopic studies

Proniosomes (0.2 g) was reconstituted with phosphate buffer (pH 7.4; 10 ml) and viewed under optical microscopy. The images were captured using a digital camera. Transmission electron microscopy (Tecnai 20, Philips, Holland) was used to determine the morphological features of the vesicles. A drop of diluted dispersion (deionized water; 10 fold dilution) was placed on a carbon-coated grid and analyzed.

Differential scanning calorimeter

To check the presence of any interactions between lornoxicam and other ingredients, samples (4 mg) of lornoxicam, span 60, empty and F19 were analyzed by differential scanning calorimeter (DSC 60, Shimadzu, Japan). The weighed amount of sample were kept in sealed aluminium container and heated between 25 °C to 300 °C at a rate of 10 °C/min.

In vitro release

The release study was carried out using vertical Franz diffusion cells which has surface area of 1.13 cm^2. The cellulose dialysis membrane (MW cut off 12,000; 2.4 nm pore size; Himedia, India) soaked in distilled water (12 h) was mounted between the donor and receptor sections (10 ml) and the later was filled with phosphate buffer (pH 7.4) [21]. The receptor compartment was kept at 37 ± 0.5 °C and the receptor medium was constantly stirred. Weighed amount of F19 (equivalent of 5 mg lornoxicam) was loaded into the donor compartments and was occluded. At scheduled time intervals (1, 2, 3, 4, 6, 8, 12, 18 and 24 h) samples (1 ml) were withdrawn and replaced immediately by fresh buffer. Similarly, control experiments were run in parallel for lornoxicam gel prepared using HPMC. The samples were then assayed by HPLC. The release of lornoxicam from prepared proniosomes were further assessed for kinetics using various mathematical models [22];

a. Zero order model $Q = Q_0 + kt$
b. First order model $Q = Q_0 \times e^{kt}$
c. Higuchi model $Q = k \times t^{0.5}$
d. Hixson-Crowell model $Q^{1/3} = kt + Q_0^{1/3}$
e. Korsmeyer–Peppas model $Q = k \times t^n$
f. Weibull model $Q = 1\text{-}exp\ [-(t)^{b/a}]$

where Q represents quantity of drug released in time t, Q_0 represents value of Q at zero time, k represents the rate constant, n represents the diffusional exponent, a represents the time constant and b represents the shape parameter. The correlation coefficient (r^2) and the order of release pattern was calculated in each case.

Ex vivo permeation

Intact full thickness skin of Albino rats were prepared by removing the fat adhering to it by surgical treatment. The treated skin was further washed and used within a week [23]. The skin was mounted with the SC side facing the donor compartment. All the experimental conditions were maintained same as in vitro release studies. The concentration of lornoxicam in the receiver media was determined using HPLC. The steady state flux, Jss (μg/cm^2/h), was determined by measuring the slope from the plot of the cumulative amount permeated versus time [24].

Physical stability

The stability of F19 was evaluated by keeping in sealed vials at 37 °C or in refrigerator (4–8 °C) for 3 months. The amount of drug retained in the entrapped vesicles, the mean particle size and drug crystallization were observed after preparation and then after every month during storage. The formulation is considered stable when the proniosomes shown greater entrapment efficiency (>60%) and high lornoxicam retention value (>90%), at every time interval.

Anti-inflammatory effect

The anti-inflammatory activity of F19 was assessed using the carrageenan-induced hind-paw edema method [25, 26]. Albino rats weighing 150–250 g were housed in cages for a week to adjust animal house conditions (25 ± 2 °C with at 12 h light / dark cycle) and were accessed to water and food. Experiments were performed in agreement with ethical standards of Institutional Animal Ethics Committee guidelines (ASP&BRI/AH/2013/07) for animal care at Arihant School of Pharmacy & BRI, Gujarat, India. The inflammation in the local area was produced by subplantar injection of carrageenan suspension (0.1 ml; 1% *w/v* in pyrogen free saline solution) into the left footpad of the rat's hind paw, 60 min after the therapy. Rats were then randomly distributed into four groups each containing six animals.

Group I: Negative control
Group II: Positive control (Marketed tablet as standard)
Group III: Placebo (Tested proniosomal gel without lornoxicam)
Group IV: Treated group (Tested proniosomal gel with lornoxicam; F19)

Group I did not receive any medications. For group II, oral marketed tablet (weight equivalent to 0.57 mg of lornoxicam) was dispersed in phosphate buffer saline (2 ml) and administered orally to rats via intra-gastric gavage. The dose of lornoxicam administered was calculated according to the equation described in the literature [27]. For groups III and IV, the back side of rats was shaved and gel was applied. Group III was applied with placebo gel, while group IV received the F19 (weight equivalent to 0.57 mg of lornoxicam). The edema volume was measured immediately (0 h) and at 1, 2, 3, 4, 5, 6 and 8 h after injection using a plethysmometer and the percentage inhibition was determined as;

$$Inhibition\ (\%) = Sc{-}St/Sc \times 100$$

where, Sc is the swelling of the control paw, St is that of the test gel treated paw.

Data analysis

Statistics of data was carried out by one-way analysis of variance (ANOVA) and a *t*-test to evaluate the effects of different treatments. The statistical differences between values showing $p < 0.05$ were considered as significant while $p < 0.001$ were considered highly significant.

Results and discussion

Preparation of proniosomes

In general, proniosomes can be prepared by mixing three basic components i.e. surfactant, alcohol and water, which further converted to noisome dispersion spontaneously on further dilution with aqueous phase. Typically, the proniosome formation happens when the surfactants make contact with water and the lipophilic chains of it get transform into lyotropic liquid crystals. This transformation can be accomplished by rising the temperature at Kraft point (Tc) and/or adding solvent that dissolves the lipid. Hence, these type of arrangements were in structured lattice form known as gel phase or liquid crystalline proniosomal gel form [28]. In the current study, proniosomes were prepared by coacervation phase

using span 60, lecithin and cholesterol. Initial studies were carried out using span 20, 40 or 60 to select suitable nonionic surfactant for preparing lornoxicam proniosomes. Based on vesicle size (small) and entrapment efficiency (high), span 60 was selected for further optimization. Lecithin and cholesterol are used to increase the system stability and rigidity of proniosomes, respectively. Incorporation of span 60 and cholesterol produces white semi-solid form and addition of lecithin results in a gel-like appearance in the current experimental conditions. Optimization of lornoxicam proniosomes was carried out by varying the ratio of surfactant: alcohol: aqueous phase from 1:8:7 to 1:12:10 *w*/w/w, respectively. Similarly, the concentration of lornoxicam was varied from 5 mg to 15 mg. Polymer was included in the final product to maintain effective consistency. The selection of polymer for lornoxicam proniosomal gel was carried by preparing three different gels using HPMC, sodium carboxymethyl cellulose and carbopol. Then the proniosomes were incorporated in all three prepared gels and evaluated for drug release. It was observed that lornoxicam release from proniosomal gel contain HPMC polymer and proniosomes (without polymers) were comparable and were statistically insignificant. This is probably because the drug is hydrated to the same extent in both formulations and therefore the addition of proniosomes into HPMC gel did not influence the drug release rate. However, the release rate of lornoxicam from other polymers like sodium carboxymethylcellulose and carbopol were significantly different ($p < 0.05$). Similar data was also observed in earlier studies [29]. Based on the release data, HPMC gel was selected and included in all further studies.

Box-Behnken design

The four-factor three-level Box-Behnken design was used and best lornoxicam proniosomal gel was chosen on the basis of various criteria like attaining small vesicles size, highest entrapment efficiency and better transdermal flux, by using the point prediction method [30]. The observed response are summarized in supplementary Table 2. The optimized level of proniosomal gel by point prediction method is summarized in Table 2. Upon studying diverse response variables and viability search, the proniosomal gel (F19) containing span 60 (90 mg), cholesterol (10 mg), lecithin (90 mg), and lornoxicam (5 mg) was seen to match with required attributes of an optimized formulation. The F19 had average vesicles size of 485.08 ± 24.12 nm, entrapment efficiency of 92.33 ± 2.37%, and the skin permeation flux of 52.15 ± 3.01 μg/cm^2/h (Table 2). Further, it was observed that the best-fitted model for the response variable could be the quadratic model when assessed with different models (Table 3). It is established that a positive sign in the regression equation will favor the response while a negative will oppose the effect [31]. Hence it is also proved from Table 3 that the all four independent variables i.e. span 60, cholesterol, lecithin, and lornoxicam have positive effects on the vesicle size, and entrapment efficiency. However, the flux (Y_3) shown positive response with span 60 concentration, while concentration of cholesterol, lecithin, and lornoxicam had a parabolic effect on the flux.

Table 2 Optimized level of proniosomal gel by point prediction method

Composition	Optimized level (mg)	Response	Predicted value	Experimental value
Span 60	90	Vesicle size (nm)	489.0	485.08 ± 24.12
Cholesterol	10	Encapsulation efficiency (%)	89.57	92.33 ± 2.37
Lecithin	90	Flux (μg/cm^2/h)	52.58	52.15 ± 3.01
Lornoxicam	5			

Vesicles size

The size of vesicles in proniosomal gels was found in the range of 401.04 ± 14.70 nm (F7) to 550.81 ± 31.13 nm (F3). The polydispersity index values of prepared proniosomes were in the range of 0.110–0.267, indicates monodispersibility. Concentration of surfactant is an important factor responsible for vesicle formation. The experimental design also signified that the proniosome size has a positive relationship with concentration of span and cholesterol as shown in the below equation:

$$\begin{aligned}\text{Size} = {} & +415.34 + 40.80\,X_1 + 32.89\,X_2 \\ & + 6.82\,X_3 - 8.69\,X_4 - 2\,X_1X_2 \\ & + 1.05\,X_1X_3 - 4.9\,X_1X_4 - 5.13\,X_2X_3 \\ & + 11.85\,X_2X_4 - 5.77\,X_3X_4 + 40.19\,X_1^2 \\ & + 32.60\,X_2^2 + 47.69\,X_3^2 + 30.73\,X_4^2\end{aligned}$$

Cholesterol and span 60 in formulation affect vesicle size by interacting with lipid bilayers as shown in Fig. 1a and supplementary Fig. 1A. On increasing amount of span (70 to 90 mg), the average vesicle size was found decreasing but further increase in amount (from 90 to 110 mg), it shows enlargement in size, which may be as a result of creation of micellar structure. Increasing the cholesterol content (5–15 mg) contributed to enhancement in the hydrophobicity which in turn leads to minor reduction in vesicle size. However,

Table 3 Summary of results of regression analysis for responses Y_1, Y_2, and Y_3 for fitting to quadratic model

Quadratic model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	% CV
Response (Y_1)	0.9695	0.9389	0.8604	17.57	11.66	2.44
Response (Y_2)	0.9554	0.9108	0.7467	17.223	3.74	4.83
Response (Y_3)	0.9638	0.9275	0.8123	18.812	2.56	7.34

from the equation, it was observed that lecithin and lornoxicam have minor effect on vesicle size and is evident in Fig. 1b and supplementary Fig. 1B.

Entrapment efficiency

The Fig. 2a indicates that the amount of span 60 and cholesterol have significant effect on entrapment efficiency. A direct correlation was observed between concentration of span 60 and percentage entrapment efficiency of the drug containing vesicles (Supplementary Fig. 2A). The increase in concentration of span 60 improved the percentage entrapment efficiency significantly ($p < 0.05$). This is probably due to the large number of proniosomes formed which increases the dimensions of hydrophobic bilayer domain, and makes space for entrapment of lornoxicam, which is generally observed with hydrophobic drugs [32]. The lowest entrapment efficiency was found in F23 (53.54%) while it was maximum in F8 (94.05%).

On increasing concentration of cholesterol from 5 mg to 10 mg shows substantial improvement ($p < 0.05$) in the drug entrapment. But further increasing the cholesterol content (10–15 mg) shows decrease in drug entrapment, which could be due to following reasons; (1) the initial improvement in drug entrapment may be due to increase in bilayer hydrophobicity and stability with decrease in permeability which in turn leads to entrapment of drug inside bilayers and (2) in the later part, as increase in concentration of cholesterol would have occupied the space in bilayer while excluding drug out of the vesicles [33].

The experimental design also signified that the percentage entrapment efficiency has a positive correlation with amount of lecithin as indicated in the below equation:

$$\text{Entrapment efficiency} = +92.66 + 5.97\,X_1 + 5.72\,X_2 + 1.62\,X_3 + 0.22\,X_4 - 4.08X_1X_2 + 4.43\,X_1X_3 - 3.86\,X_1X_4 - 0.39\,X_2X_3 - 3.33\,X_2X_4 + 5.84\,X_3X_4 - 20.18\,X_1^2 - 9.77X_2^2 - 1.69\,X_3^2 - 5.40\,X_4^2$$

A significant enhancement ($p < 0.05$) in percentage entrapment efficiency was witnessed with increasing lecithin content. This might be due to the potential of lecithin to rigidify cholesterol which in turn makes bilayers membrane less leaky and therefore the drug entrapped inside membrane is unable to exit from vesicles, as described previously [34]. The responses of increasing amount of lornoxicam from 5 to 15 mg on the entrapment efficiency are shown in Table 3. As lornoxicam amount increased from 5 to 10 mg, the entrapment efficiency increased (Fig. 2b and supplementary Fig. 2B), this is probably due to the saturation of drug in media which helps drug remain encapsulated inside proniosomes [35]. However, same relationship was not observed on further rise in drug concentration from 10 to 15 mg. In this case, entrapment efficiency ($p < 0.05$) decreased after achieving saturation and any further increase in drug concentration leads to precipitation of excess drug from the formulation.

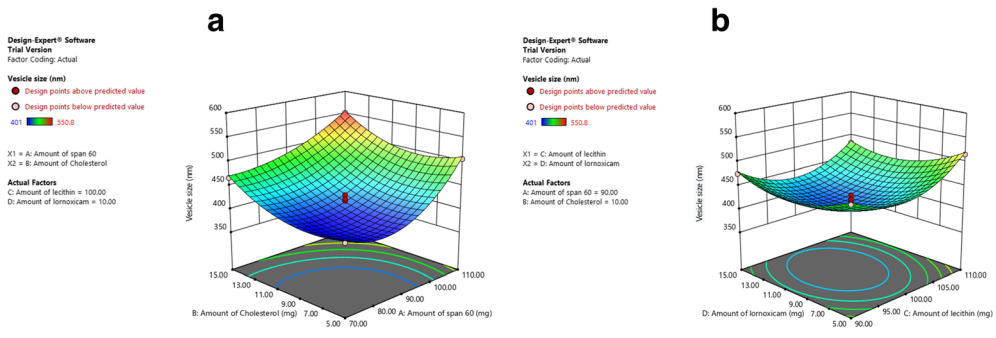

Fig. 1 3D surface plots showing relative effects of (**a**) amount of span 60 and cholesterol and (**b**) amount of lecithin and lornoxicam on vesicle size

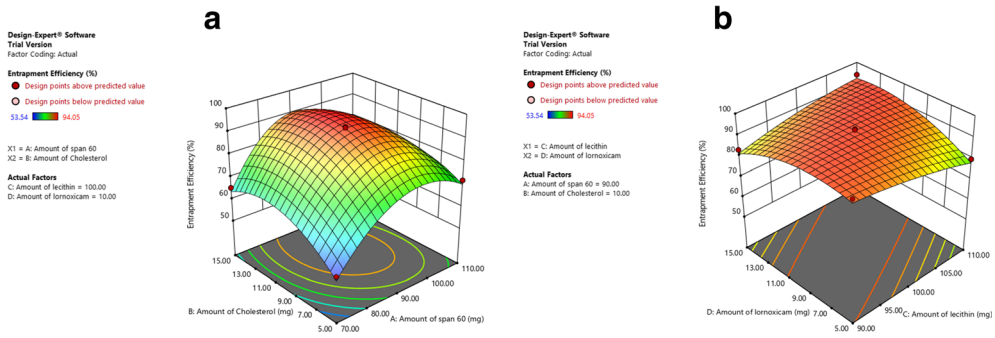

Fig. 2 3D surface plots showing relative effects of (**a**) amount of span 60 and cholesterol and (**b**) amount of lecithin and lornoxicam on entrapment efficiency

Drug release

Figure 3 compares the cumulative percentage of lornoxicam released at different time intervals in F19 and control gel. Two distinct release profiles were observed for F19 and control. In case of F19, the release increased steadily over time (Fig. 3). The controlled drug release observed in F19 could be probably due to the effect of proniosome components (span 60, lecithin and cholesterol). In contrast, the control gel showed a rapid release with ~90% of drug being released in 6 h ($p < 0.001$). The ideal proniosomal gel should show prolonged release in order to avoid frequent application and for better patient compliance. The release mechanism for F19 was studied using various models. Values of Sum of Square of Residuals (SSR) were found to be 298.10, 214.03, 36.38, 66.26, 31.33 and 238.66 for Zero order, First order, Higuchi, Korsmeyer-Peppas, Weibull and Hixon-Crowell model, respectively. Release of lornoxicam from F19 was fitted into Weibull model showing high r^2 value (0.9825), least SSR value (31.33) and F value (3.92) as compared to Higuchi and is summarized in supplementary Table 3. Thus, the release of lornoxicam from F19 was Weibull diffusion controlled mechanism. Further, the n value is less than 0.5 which indicates drug release mechanism in F19 is Fickian diffusion.

Permeation studies

Permeation studies mostly focus on the drug behavior in vivo. The below equation indicate that the flux of lornoxicam across the skin from proniosomes was initially improved with increase in amount of span 60 (up to 90 mg) and then decreased with further increase to 110 mg (Fig. 4a).

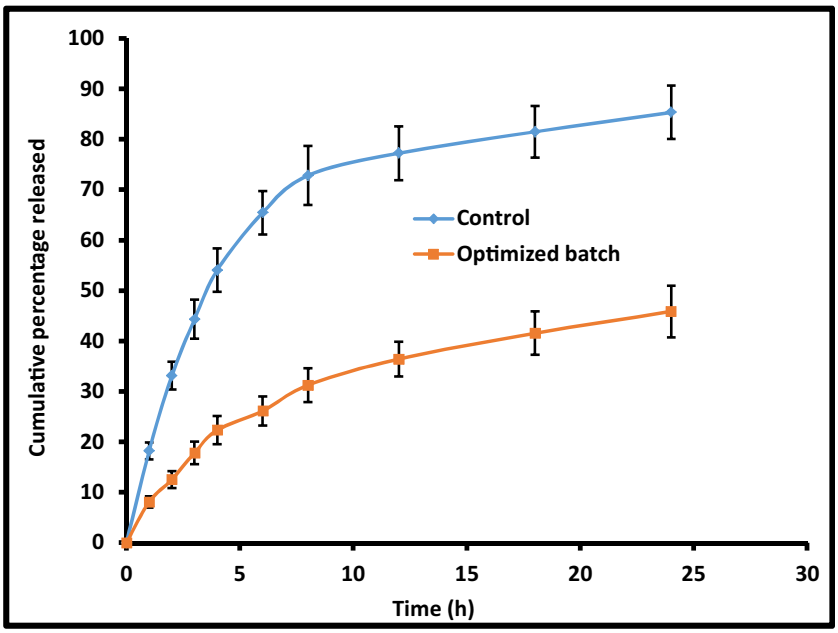

Fig. 3 Comparison of percentage lornoxicam release profiles of optimized proniosomal gel (F19) and control. The data represents average ± SD of six trials

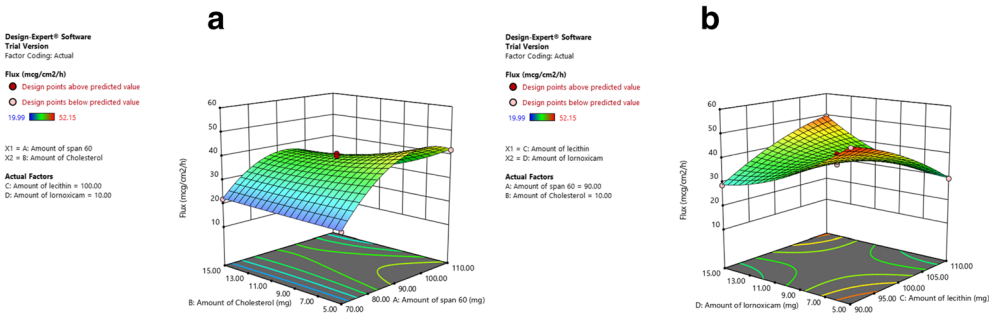

Fig. 4 3D surface plots showing relative effects of (**a**) amount of span 60 and cholesterol and (**b**) amount of lecithin and lornoxicam on flux

$$\text{Flux} = +38.60 + 5.03\,X_1 - 3.66\,X_2 - 0.99\,X_3 - 0.41\,X_4 - 4.18\,X_1X_2 - 1.7\,X_1X_3 + 5.2\,X_1X_4 + 3.16\,X_2X_3 + 1.16\,X_2X_4 + 10.9\,X_3X_4 - 12.27\,X_1^2 + 1.77\,X_2^2 - 2.7\,X_3^2 + 4.39\,X_4^2$$

However, the flux decreased on increasing the total lipid concentration, probably because the high lipid content might have disturbed the proniosome membrane which eventually leads to the leakage of entrapped drug. The drug quantity also influences the transdermal flux of lornoxicam (Fig. 4a and supplementary Fig. 3A). On increasing drug concentration from 5 to 10 mg shows enhancement in the flux while further increase led to reduction in flux value (Fig. 4b and supplementary Fig. 3B). Thus these results suggest that careful consideration of drug concentration in proniosomes is required for effective transdermal therapy.

Figure 5 compares the concentration of lornoxicam permeated across the rat skin membrane from F19 and control. It is apparent from Fig. 5 that the rate of lornoxicam permeation was significantly high ($p < 0.001$) from F19 in comparison to control. The average steady state flux values of lornoxicam from F19 and control were 52.15 μg/cm^2/h and 8.27 μg/cm^2/h, respectively, signifies 6.3 folds enhancement in flux values with F19. The main mechanism for the enhancement in transdermal flux by proniosomes involves disruption of the densely packed lipids that fill the extra cellular spaces of the SC. Many researchers demonstrated that the proniosomal treatment can improve the permeability of the SC [36]. Furthermore, the adsorption and fusion behavior of proniosomes onto the skin surface can enhance thermodynamic activity which in turn helps the drug permeation [37].

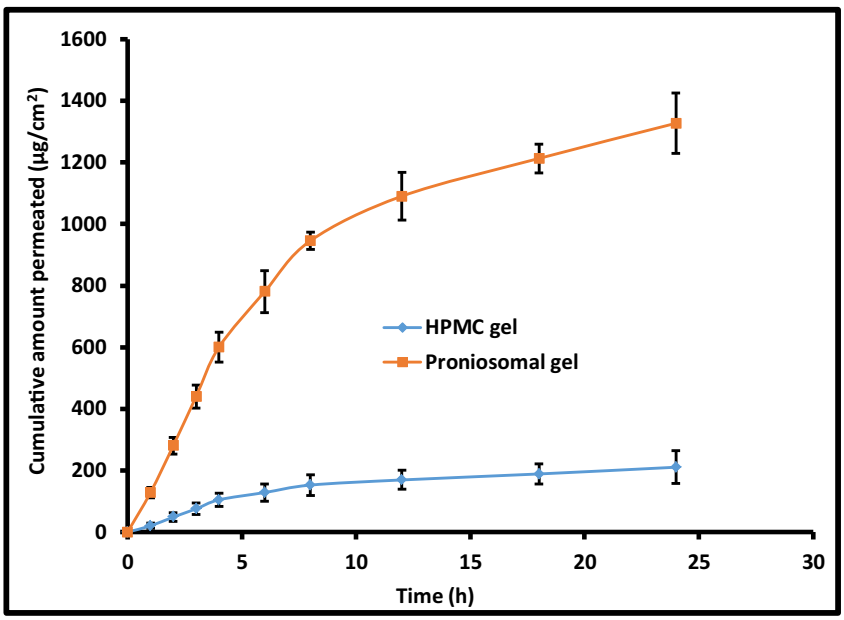

Fig. 5 Comparison of permeation profiles of lornoxicam from optimized proniosomal gel (F19) and control across the rat skin membrane. The data represents average ± SD of six trials

Table 4 Estimated and observed values of check point formulations

Formulation No.	Y_1 (vesicle size) nm		Polydispersity index		Y_2 (EE) %		Y_3 (flux) μg/cm^2/h	
	Estimated value	Observed value	Estimated value	Observed value	Estimated value	Observed value	Estimated value	Observed value
F30	488.26	488.78 ± 25.81	0.211	0.247 ± 0.04	89.55	89.08 ± 2.41	38.68	37.79 ± 2.24
F31	396.29	397.13 ± 13.74	0.110	0.145 ± 0.03	78.31	77.92 ± 1.89	34.25	35.13 ± 1.67
F32	432.61	432.89 ± 17.44	0.178	0.201 ± 0.04	93.26	92.79 ± 2.87	41.05	40.69 ± 3.02

Check point analysis

Table 4 shows estimated and observed values of check point formulations. Results indicate that the observed values were as expected, and the difference between estimated and observed values was found to be statistically insignificant for vesicle size, entrapment efficiency and flux. Therefore, the mathematical equation is effective in predicting the proniosome vesicle size, entrapment efficiency and transdermal flux.

Characterization

Characteristics of F19 are depicted in Fig. 6. A representative size distribution curve of F19 was illustrated in Fig. 6a, which demonstrates average vesicles size of 485.08 ± 24.12 nm and narrow distribution of prepared particles. Figure 6b shows the zeta potential distribution of F19, which was −37.5 ± 8.50 mV. These values are sufficiently high to provide adequate repulsion between the vesicles and electrostatic stabilization [38]. The microscopic examination of F19 revealed vesicular structure with entrapped lornoxicam (Fig. 6c). The TEM micrograph of F19 is illustrated in Fig. 6d, which indicates that the size of prepared vesicles are virtually spherical in shape and are in nanometer size.

Differential scanning calorimeter

Differential scanning calorimeter thermogram of lornoxicam, span 60, cholesterol, empty and loaded proniosomes are illustrated in Fig. 7. Lornoxicam showed exotherm while span 60 and cholesterol showed endotherms at 237.31 °C, 53.21, 148 °C, respectively, consistent to their melting point. Differential scanning calorimeter thermogram of empty proniosomes revealed a sharp endothermic peak at 108.37 °C, might be due to the interaction between span 60, cholesterol, lecithin and double layer structure formation in the vesicle. Conversely, thermogram of lornoxicam loaded proniosomes revealed an absence of drug exothermic peak

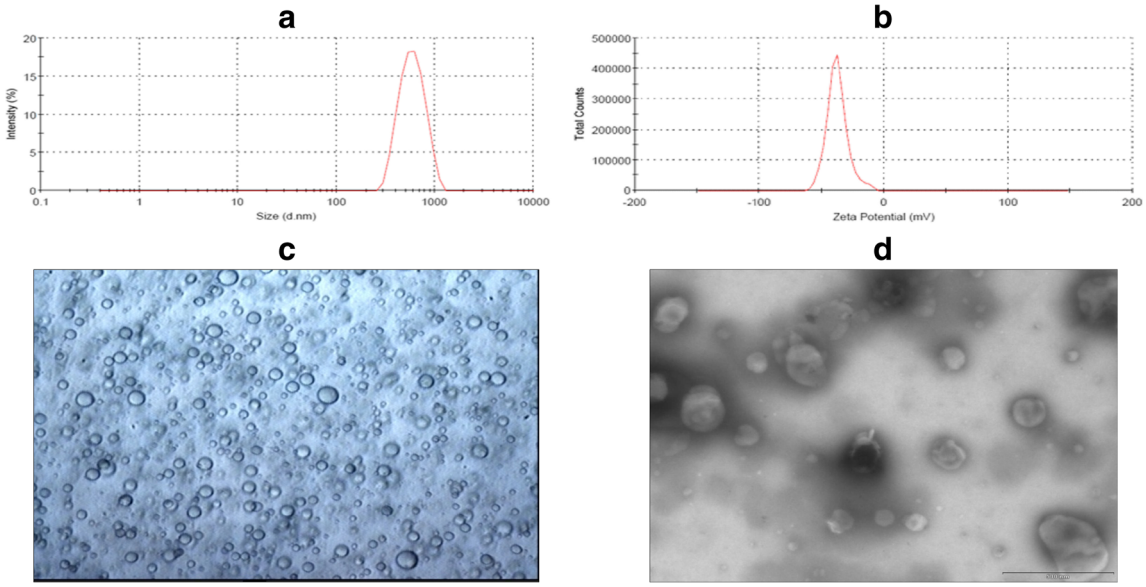

Fig. 6 Characteristics of optimized lornoxicam proniosomal gel (F19). **a**: representative size distribution curve, **b**: zeta potential distribution, **c**: Light microscopy picture (100x), and **d**: transmission electron microscopy image

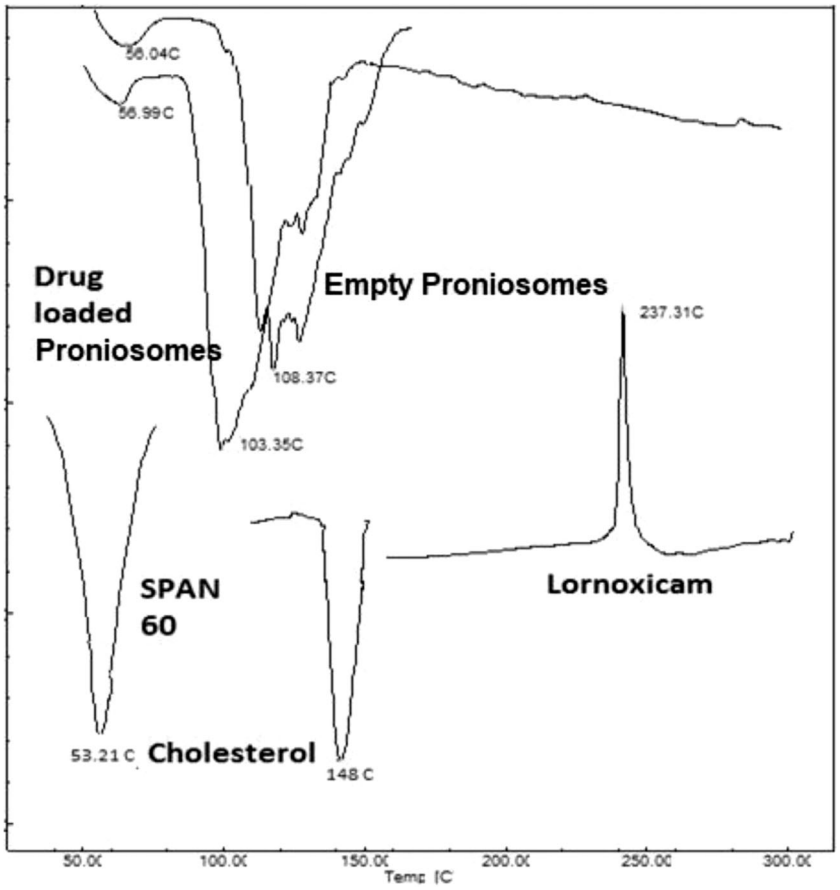

Fig. 7 Differential scanning calorimetry patterns of lornoxicam, span 60, cholesterol, empty (without drug) and optimized proniosomal gel (F19)

and proniosome bilayer endotherm was shifted and showed broad wide peak at 102.66 °C. These results signifies the possible contact of drug with bilayer components and also confirm entrapment of lornoxicam into the vesicles [39].

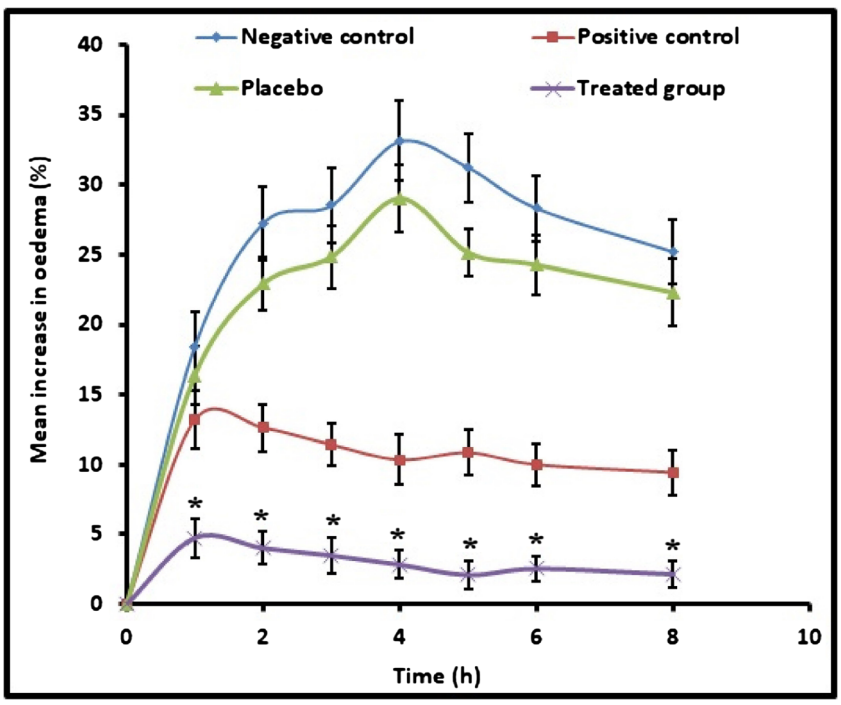

Fig. 8 Comparison of the mean inhibition of edema in the hind paw of rats in various treatments. The data represents average ± SD of six trials. The profiles between negative control and placebo resemble each other and are statistically insignificant. On the other hand, the edema profile of treated group is statistically different as compared to other treatments (control, placebo and positive control groups) at $p < 0.05$

Physical stability

For physical stability of formulation, the vesicle size and entrapment efficiency were determined after storage, specifically for any chance of aggregation and/or fusion of the vesicles. Hydration of proniosomes leads to rapid conversion into niosomes. Furthermore, no significant change was notices in the vesicle size and percentage retention of lornoxicam when kept at refrigerated temperature (data shown in supplementary Table 4). While the case is reversed when proniosomes were stored at room temperature, wherein the entrapment efficiency was decreased with time ($p < 0.05$) probably due to leakage. The stability studies suggest the storage of proniosomes in refrigerated conditions rather than room temperature.

Assessment of anti-inflammatory effect

Figure 8 compares the mean increase in edema after treatment in various groups. It is evident from Fig. 8 that the profile of F19 treated group is very distinct as compared to other treated groups. Transdermal therapy of F19 prominently inhibited the induced inflammation or edema ($p < 0.05$) as compared to other groups. The percentage edema (2.08%–4.75%) was low and consistent throughout the study period (8 h), implies the transdermal application of F19 improved the clinical utility of lornoxicam. Followed by the transdermal application, the positive control (marketed tablet administered by oral route) showed certain inhibition of inflammation. The percentage edema inhibition by oral therapy of lornoxicam with similar dose was 9.44%–13.22% (until 8 h), signifies its moderate efficiency. In contrast, both negative control and placebo groups shown higher edema in rats and were similar and statistically insignificant. The percentage edema observed in control and placebo groups varied from 16.38% to 31.22% during the study period (8 h). Overall, the data observed here indicate the prospective of F19 to rapidly transport into and through the skin and deliver therapeutically required amount of lornoxicam at the target site. Moreover, the dermal delivery of lornoxicam is more advantageous than conventional systemic therapy as it overcomes various issues associated with NSAIDs including gastric irritation.

Conclusion

Optimization study suggest careful consideration is required while preparing lornoxicam proniosomes. Characterization of proniosomes like particle size, entrapment efficiency, zeta potential, thermal behavior, drug release, permeation and stability were assessed at various phases of the study. In vivo data suggests that the anti-inflammatory activity of lornoxicam was significantly improved following transdermal application of optimized proniosomal gel (F19) as compared to oral therapy. Typically, proniosome vesicular systems produce depot in the deeper skin layers and continuously release the drug over time, which is advantageous as it reduces the application frequency and provides sustain therapy in the management of chronic inflammation as well as acute postoperative pain. Moreover, the proniosome approach will be further advantageous for older patients with polypharmacy. In nutshell, these findings indicate that proniosomal gel can be an effective alternative to oral therapy of lornoxicam, however, need to be proved in human.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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