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Development and Evaluation of Ethosomal Gel of Lornoxicam for Transdermal Delivery: In-Vitro and In-Vivo Evaluation

Ankit Acharya, Mohammed Gulzar Ahmed*, Baba Datteshwara Rao, Vinay C H

Abstract

Abstract: Lornoxicam is a non-steroidal anti-inflammatory BCS-II class drug, having low solubility and high permeability. The aim of present investigation was to develop ethosomal gel of lornoxicam, for its transdermal delivery. The ethosomal formulations were prepared by hot method using phospholipid and ethanol (20% to 40%) and then evaluated for entrapment efficiency, vesicular size, shape, in-vitro skin permeation, skin retention, drug-membrane component interaction and stability. FT-IR studies revealed no interaction between the drug and excipients. Transmission electron microscopy (TEM) confirmed the three dimensional nature of ethosomes. The sonicated ethosomal formulation ET7 was selected for further skin permeation studies as it exhibited highest percentage of drug entrapment (93.96%) and small particle size (100 ± 3.9 nm). Formulation ET7 containing 2% w/w phospholipid and 30% alcohol showed highest percentage of drug permeation (74.18%) at the end of 24 hours. The ethosomal vesicles were incorporated in carbopol gel base and its anti-inflammatory efficiency was compared with the plain lornoxicam gel. The pharmacodynamic studies showed the enhanced anti-inflammatory activity of ethosomal gel compared to the plain gel formulation. Stability studies carried out at two different temperatures, showed no significant change in entrapment efficiency of vesicles at the end of 3 months, indicating that all the formulations were physiochemically stable. The results obtained suggested that the ethosomes could be an efficient carrier for transdermal delivery of lornoxicam in the treatment of inflammation compared to plain gel.

Keywords: Lornoxicam, phospholipid, ethanol, ethosomes, sonication.

Introduction

Transdermal drug delivery systems are designed to deliver a therapeutically effective amount of drug across a patient's skin for prolonged period of time¹. In the present scenario, around 40% of the drug candidates are under clinical evaluation for their transdermal delivery. Transdermal route offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life

drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter and intra patient variations, and most importantly, it provides patient convince. However, a major problem in transdermal route is the low penetration rate of the drug through the outer most layer of skin². The non-invasive approaches like usage of permeation enhancers, vesicular approach, ablation, microneedle array, ultrasound, iontophoresis, electroporation, etc. is being used for transdermal drug delivery of various therapeutic substances³. Among them, we choose vesicular drug delivery approach. The application of vesicular system in drug delivery has changed the definitions of diagnosis and treatment in different aspects of biomedical field. The vesicular system such as liposomes, niosomes, ethosomes, sphinosomes, transfersomes and pharmacosomes are used to improve the therapeutic index of both

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existing and new drug molecules by encapsulating drug inside vesicular structure in one such system².

Ethosomes “soft vesicles” represents novel vesicular carriers for enhanced delivery to or through skin. The size of ethosomes vesicles can be modulated from 10 nm to microns. Ethosomes are the modified forms of liposomes that are high in ethanol content. The ethosomal system is composed of phospholipid, ethanol and water. They can penetrate the skin and enhance compound delivery both to deep skin strata and systemically. Ethanol present in ethosome fluidizes both ethosomal lipids and bilayer of the stratum corneum intercellular lipid. The soft, malleable vesicles, penetrate the disorganized lipid bilayers^{3, 4}.

Lornoxicam is a potent non-steroidal anti-inflammatory drug (NSAID), used for the variety of inflammatory conditions such as inflammation, pain, edema, rheumatoid arthritis. Its half-life is 3 to 5 hours and peak plasma concentration is attained within 2.5 hours. Lornoxicam is ten times more potent than other derivatives. The daily dose of lornoxicam is 8-16 mg taken before meal but dose above 8 mg should be divided into two or more doses. The mechanism of action of lornoxicam is primarily due to inhibition of prostaglandin synthesis through the inhibition of cyclooxygenase (COX-1 and COX-2) enzymes. Like other NSAIDs, common side effect of lornoxicam is gastrointestinal irritation^{5, 6}.

Lornoxicam satisfied all the criteria required for transdermal drug delivery. Hence, for the treatment of inflammation, lornoxicam was selected as model for its delivery through the skin.

Materials and Methods

materials and reagents used

Lornoxicam was procured from Yarrow Chem Products, Mumbai, India. Phospholipid was procured from S.D. Fine Chem. Ltd, Mumbai, India. All other reagents used were analytical grade.

Animal used

Male albino rats weighing 180-220 g were used for skin permeation studies and to study anti-inflammatory activity.

Ethical approval

The study protocol was approved by Institutional Animal Ethics Committee, SAC College of Pharmacy, BG Nagara-571 448, Karnataka (Reg. no. SACCP/IAEC/271(b)/2012-13).

Compatibility studies

FT-IR spectrophotometer (Thermo Nicolet FT-IR system) was used to observe possible interaction between drug and excipients⁴.

Preparation of ethosomes

Hot method

Procedure: In hot method, phospholipid was dispersed in water and dispersion was heated in a water bath at 40° C until a colloidal solution is obtained. In a separate vessel, ethanol and propylene glycol are mixed and heated to 40° C. Once both mixtures reach 40° C, the organic phase was added to the aqueous phase. The drug was dissolved in ethanol due to its hydrophobic property. The drug solution was then added to the phospholipid dispersion with continuous stirring on magnetic stirrer (1500 rpm) for 10 minutes. Finally, the prepared formulations were stored in refrigerator⁷.

Preparation of sonicated lornoxicam ethosomes

Ethosomes prepared by the above procedure were subjected to sonication using ultrasonic bath sonicator (ICH sonicator, Mumbai) for an hour with a cycle of 10 minutes⁸.

Incorporation into Gel

Carbopol 1% w/v was soaked in minimum amount of water for an hour. 20 ml of ethosomal suspensions containing lornoxicam (100mg) was added to the swollen polymer under the continuous stirring at temperature of 30°C until homogeneous gels were achieved. The pH was then adjusted to neutral using triethanolamine and stirred slowly until a clear transparent gel was obtained^{9, 10}.

Table 1: Composition of different un-sonicated ethosomal formulations

Formulation code	Phospholipid (%w/w)	Ethanol (%w/w)	Propylene Glycol (%w/w)	Drug (%w/w)	Water
ET ₁	2.0	20	20	0.4	q.s.
ET ₂	2.0	30	20	0.4	q.s.
ET ₃	2.0	40	20	0.4	q.s.
ET ₄	1.0	30	20	0.4	q.s.
ET ₅	4.0	30	20	0.4	q.s.

ET₁, ET₂, ET₃, ET₄ and ET₅= formulation code of un-sonicated ethosomal formulation

Table 2: Composition of different sonicated ethosomal formulations

Formulation code	Phospholipid (%w/w)	Ethanol (%w/w)	Propylene Glycol (%w/w)	Drug (%w/w)	Water
ET ₆	2.0	20	20	0.4	q.s.
ET ₇	2.0	30	20	0.4	q.s.
ET ₈	2.0	40	20	0.4	q.s.

ET₆, ET₇ and ET₈= formulation code of sonicated ethosomal formulation

Preparation of plain drug in gel base

Plain gel of lornoxicam was prepared by similar method applied for preparation of ethosomal gel. In this method, required quantity of lornoxicam was taken and triturated into water miscible gel base. Final concentration of lornoxicam in formulations was fixed to 0.4%¹¹.

Evaluation of prepared formulation

Visualization: The initial characterization of the ethosomal vesicles can be examined by transmission electron microscopy (TEM). It also visualizes the lamellar character of ethosomes⁶.

Vesicle size: The size of the vesicles can be characterized by optical microscopy with a calibrated stage micrometer¹¹.

Zeta potential: Particle size of vesicle can be determined by dynamic light scattering (DLS). The charge of the ethosomal vesicle is an important parameter that can influence both vesicular properties such as stability, skin-vesicle interactions and its zeta potential can be determined using a computerized inspection system¹².

Entrapment efficiency: Separation of un-entrapped drug and evaluation of entrapment efficiency can be measured by ultracentrifugation. The entrapment efficiency was calculated using following formula.

$$\text{Entrapment efficiency} = (T - C) / T \times 100$$

Where, 'T' is total amount of drug that is detected both in the supernatant layer and resident layer and 'C' is the amount of drug detected only in the supernatant¹¹.

In vitro diffusion study using cellophane membrane

The experiments were conducted in Franz diffusion cells having donor compartment and a receiver compartment. A suitable size of pre-treated cellophane membrane was mounted between donor and receptor cells of the Franz diffusion cells (locally fabricated). The receiver contained 15 ml phosphate buffer solution (PBS) at pH 6.8, was constantly stirred by magnetic stirrer at 100 rpm and was maintained at a temperature of 32 ± 1 ° C throughout the experiments. Formulations equivalent to 20 mg lornoxicam ethosomal gel was applied homogenously in the donor compartments; 1ml samples were withdrawn from receiver at predetermined time intervals over 24 hours and immediately replenished with an equal volume of fresh PBS. Samples were assayed for drug content spectrophotometrically at 376 nm¹³.

Kinetic Analysis

The results of *in-vitro* release profile were plotted in modes of data treatment as follows:-

Zero-order Kinetic model – Cumulative percentage drug released versus time.

First-order Kinetic model – Log cumulative percentage drug remaining versus time.

Higuchi's model- Cumulative percentage drug released versus square root of time.

Korsmeyer equation / Peppas's model- Log cumulative percentage drug released versus log time¹⁴.

***In-vitro* skin permeation study using rat skin**

In-vitro skin permeation studies of ethosomal gel formulations were carried out on Franz diffusion cell with an effective diffusion area of 60 mm² and 15 ml of receptor compartment capacity, using rat abdominal skin. Firstly, 6 to 8 weeks old male albino rats weighing 180 to 220g were killed by giving excess of anesthesia (ether). Skin was excised from the rat abdominal region, entire hair were removed with electrical razor and the dermal side of the skin was wiped with isopropyl alcohol to remove adhering fat. Then, the skin was washed properly with distilled water, wrapped in double folded aluminum foil and finally stored at -20 °C ±1 until further use. The skin was brought to room temperature and treated with 0.5 M NaOH solution for 1-1.5 hours. Pre-treated skin was cut into appropriate size and mounted between the two half of Franz diffusion cell where dermis faced towards the receptor compartment and the stratum corneum faced towards donor compartment. Initially, receptor compartment was filled with phosphate buffer (pH 6.8) while donor compartment was kept empty. The temperature of the apparatus was maintained 32±0.5 °C throughout the study period. The receiver fluid was stirred with magnetic rotor at a speed of 100rpm. The whole receiver fluid was replaced with fresh buffer solution after every 20 minutes to stabilize the skin. It was found that the receiver fluid showed a negligible UV absorption after 3 hours indicating the complete stabilization of the skin membrane. The formulation equivalent to 20 mg of lornoxicam was applied on the skin in donor compartment. Samples were withdrawn at predetermined time intervals over 24 hours, and suitably diluted with phosphate buffer to analyze the drug content⁶.

***In-vivo* anti-inflammatory activity**

Carrageenan induced paw edema method was used to compare the anti-inflammatory activity of plain gel and ethosomal gel. The animals were divided into three groups, each group having four animals. First group served as normal control receiving normal gel containing saline. Second group received lornoxicam plain gel, and third group received lornoxicam ethosomal gel. 30 min after formulation application (0.5 g), rats of both treated groups were challenged by a subcutaneous injection of a 1% (w/v) solution

of carrageenan in saline (0.1 ml) into plantar site of right hind paw and the percentage inhibition of paw edema was measured by plethysmometer (IITC)¹⁵.

Stability studies

Stability study was carried out by storing the ethosomal formulations at two different temperatures 4°C and 25±2 °C. The drug content was estimated for 3 months to identify any change in the entrapment efficiency of ethosomal formulation¹⁶.

RESULTS

Compatibility Study

Physical mixture of drug and polymer was characterized by FT-IR spectral analysis for any physical as well as chemical alteration of the drug characteristics. Results of FT-IR spectroscopy are shown in Table 3 and Figures 1 and 2.

Table 3: Interpretations of IR-spectrum

Functional group	Wave number (cm ⁻¹)	
	Pure Drug	Drug and physical mixture
OH	3437	3393
NH	3101	3141
CH-Ar	3067	3021
C=N	1649	1735
C=O	1595	1576
C=C	1425	1437
S=O	1039	1091
C-Cl	704	749

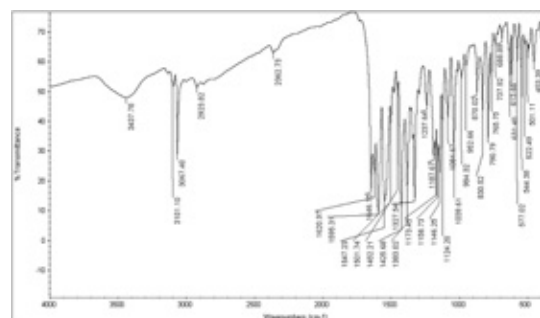


Figure 1: IR Spectrum of Lornoxicam

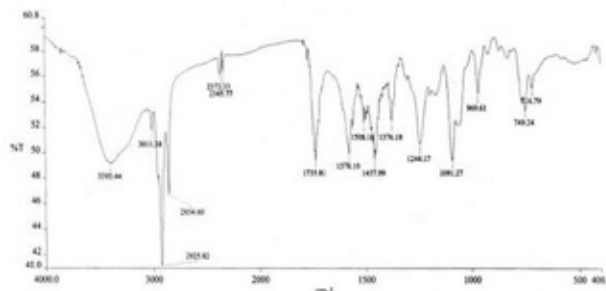
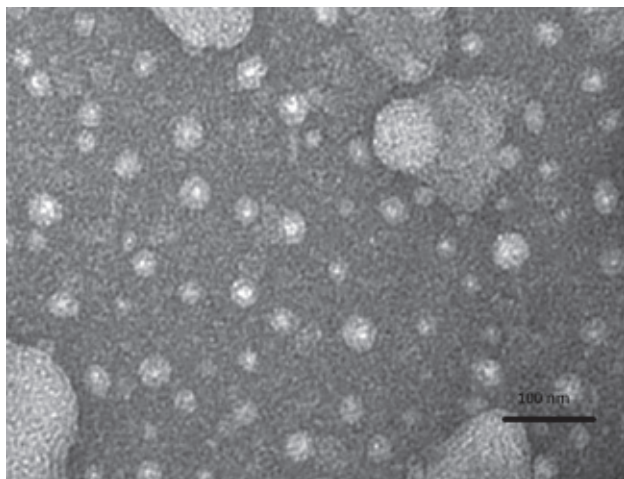


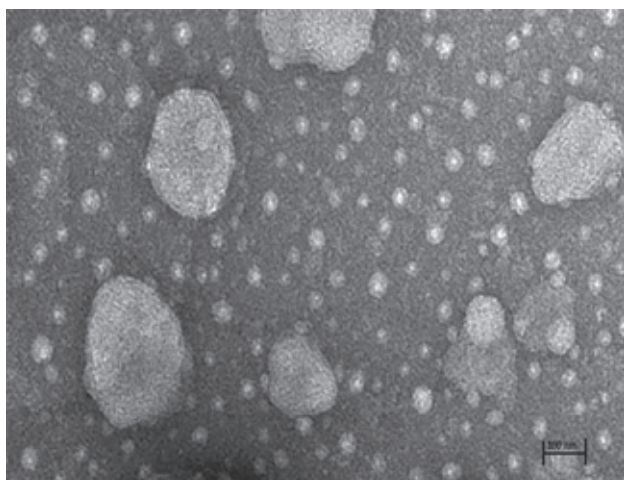
Figure 2: FT-IR spectra of drug and physical mixture

Visualization:

Visual characterization of the vesicles was examined by transmission electron microscopy (Tecnai 200 with CCD, Philips Instruments, Holland). In TEM image analysis, both the sonicated and un-sonicated ethosomes are having spherical bilayer vesicles with a smooth surface (figure 3 A, B).



A



B

Figure 3: Transmission electron microscopy (TEM) of (i) un-sonicated ethosomes, (ii) sonicated ethosomes

Vesicles size analysis:

The vesicle size and diameter of sonicated ethosomes was found in the range of 13.9 nm and 30.7 nm respectively, whereas un-sonicated formulation exhibited vesicle size 33.7 nm and 53.0 nm average diameter. Vesicular size distribution of the sonicated product was measured with special software developed by “ZETASIZER NS 300 HS_A”.

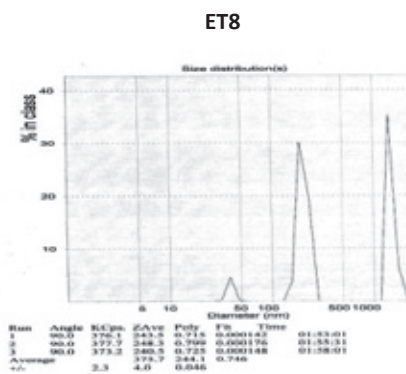
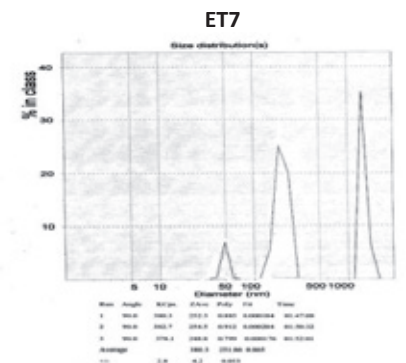
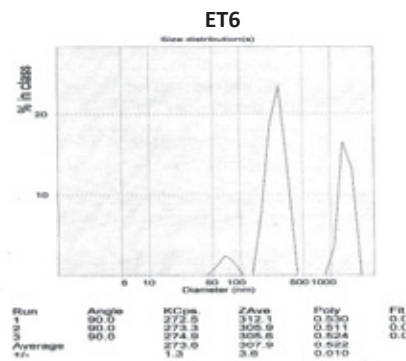


Figure 4: Vesicular size distribution of sonication formulation ET₆, ET₇ and ET₈

Comparison of zeta potential:

Zeta potential analysis was done by using Malvern Zetasizer (Malvern Instrument Ltd., Model S., Ver.5.03, Malvern, UK) in order to determine the surface charge of the vesicles. The zeta potential of un-sonicated formulation ET₂ (-48.23±1.9mV) was high when compared with sonicated formulation ET₇ (-45.35±2.9mV).

Entrapment efficiency

The ability of vesicles to entrap lornoxicam was investigated by ultracentrifugation method (Remi Elektrotech, Limited, Vasai, India, centrifuge). Ultracentrifugation was the method used to separate

vesicles containing drug and free drug and to find out the entrapment efficiency. The sonicated ethosomes formulation ET₇, containing 2% phospholipids and 30% ethanol showed highest entrapment efficiency i.e. 70.25% (Figure 5).

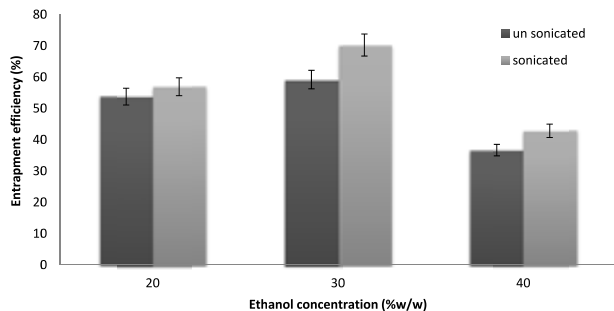


Figure 5: Comparative entrapment efficiency of ethosomes before and after sonication

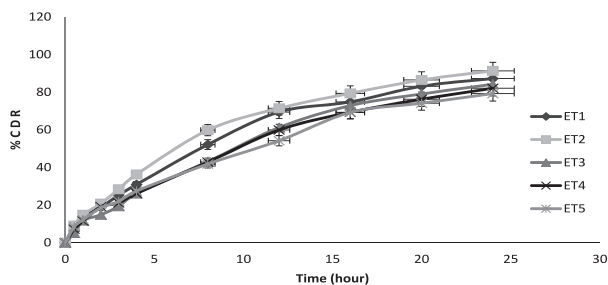


Figure 6: Comparative drug release profile of un-sonicated ethosomal formulation

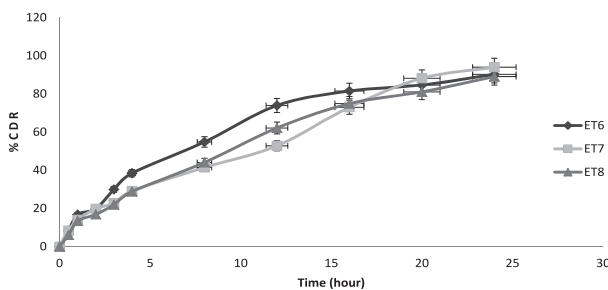


Figure 7: Comparative drug release profile of sonicated ethosomal formulation

In-vitro drug release studies:

For *in-vitro* drug release studies, cellophane membrane having MW cut-off range 12,000-14,000 was used. This type of cellophane membrane only allows the diffusion of drug molecule in solution form while it does not allow lipid vesicles to pass through it. Therefore, to diffuse the entrapped drug through cellophane membrane, first it should release from the vesicles to surrounding liquid medium. In this present study, a drug release study was performed for both sonicated and un-sonicated ethosomal

formulation. According to the *in-vitro* drug release profiles, the best release in un-sonicated ethosomes was shown by ET₂ i.e., 91.32% drug release. In case of sonicated formulations best release was exhibited by formulation ET₇ i.e., 93.96% drug release at the end of 24 hours.

In-vitro skin permeation study

In-vitro skin permeation was conducted for plain lornoxicam gel and ethosomal gel (ET₂ and ET₇) for a period of 24 hours using rat skin. It has been observed that the ethosomal system showed higher permeation compared to plain gels (Figure 8).

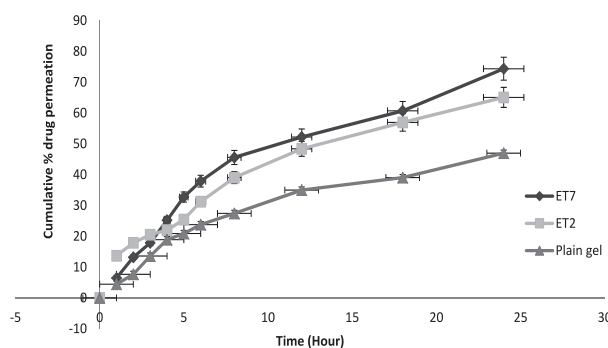


Figure 8: Comparative skin permeation study of three different formulation

Drug release kinetics

The release obeyed zero, first order kinetics, the results of this investigation showed high correlation coefficient among the formulation for mixed order release and the probable release mechanism was initial diffusion. The value of release exponent (n) was found to be a function of the polymer used and the physicochemical properties of the drug molecule itself and the 'n' values was found to be in the range of 0.410 to 0.5463 followed with non-Fickian release.

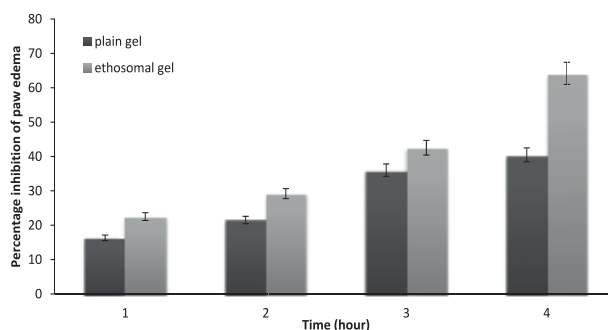


Figure 9: Comparison of *in-vivo* anti-inflammatory activity

Comparison of *in-vivo* anti-inflammatory activity

Carrageenan induced paw edema method was used to compare the anti-inflammatory activity of plain lornoxicam gel and ethosomal gel using albino rats (Figure 9). Results of the anti-inflammatory studies showed that after 4 hours of gel application, ethosomal gel formulation had comparatively higher inhibition of inflammation (64.2%) than plain gel formulation (40.48%) with lesser time for onset of action.

Stability studies

Ethosomal formulations ET₂ and ET₇ were kept for 3- month stability studies for any change in appearance or color for a period of 3 months.

Discussion

FT-IR studies showed there was no interference of the functional group peaks of the lornoxicam in ethosomal formulation, indicating they were chemically compatible. There was no change in functional group peaks (OH, C-Cl, S=O, -C-H, C=N, N-H, C=O, CH-Ar) of lornoxicam in all the IR-spectra, hence compatible. A size of un-sonicated vesicles was comparatively larger than the sonicated ethosomal vesicles and was of multilamellar and giant type. This might be because the drug that was not entrapped properly in vesicles remains free after sonication, which resulted in smooth and smaller size.

Decrease in vesicles size of sonicated ethosomes might be due to the stress exhibited by the ethosomal vesicles after its sonication resulting in removal of un-entrapped drug as well as phospholipids. Among all formulations, formulation ET₁ containing 20% ethanol exhibited vesicle diameter of 53.0 nm and up on increasing concentration of ethanol (from 20%-40%) vesicle size gradually decreases from 53.0 nm to 33.7 nm. Ethanol significantly reduces vesicle membrane thickness, as it provides the softness for vesicle membrane and result in interpenetration of hydrocarbon chain (ethanol) in the vesicular lipid bilayer. Furthermore, ethanol imparts a net negative charge to the ethosomal system and confers it some degree of steric stabilization that may finally lead to a reduction in vesicular size. Zeta potential values are high for ethosomal formulations. A high zeta-

potential value (>-30 mV) is beneficial to vesicles physical stability as it prevents aggregation between vesicles owing to electrostatic repulsion.

Sonicated ethosomes ET₇ exhibited highest percentage of entrapment compared to un-sonicated ethosomal formulation; this might be due to the possible reduction in vesicle size and also due to presence of optimum concentration ethanol, which probably has greater ability to retain lornoxicam in ethosomal core.

The amount of drug release for sonicated formulation was comparatively higher than un-sonicated formulation. High percentage of drug release by the sonicated formulation might be because these formulations have smaller vesicle sizes and highest percentage of drug loading capacity. *In-vitro* drug release results also suggested that the ethosomal formulations of lornoxicam provide a sustained delivery of drug over a period of 24 hour through cellophane membrane. Drug permeation for ethosomal formulation was also higher for ethosomal gel compared to plain gel, this might be due to fact that ethanol present in the ethosomal formulation provides soft and flexible characteristics to the vesicles, which allow them to more easily penetrate into deeper layers of the skin. Finally, results suggested that in ethosomal formulation there was some kind of synergistic mechanism between ethanol, vesicles, and skin lipids, which make it more permeable through skin. Among ethosomal gels, sonicated ethosomal gel showed higher percentage of permeation 74.18 % (ET₇), which was slightly higher than un-sonicated ethosomal gel 65.45% (ET₂). Higher permeation rate of drug from sonicated ethosomes might be smaller size of vesicles compared to un-sonicated vesicle. There was no change in appearance in ethosomal formulations throughout the period of study. Even significant changes were not observed under the magnified view, hence prepared formulation was stable. Thus, ethosomal formulation may be considered as a good choice to improve absorption of the anti-inflammatory drug like lornoxicam.

Conclusion

In the present study, lornoxicam entrapped ethosomal gel for transdermal drug delivery was prepared by using various concentrations of phospholipids and ethanol. The prepared formulation showed good entrapment efficiency, particle size and drug release. The result advocates the superiority of ethosomal formulation over the plain gel formulation with respect to skin permeation and anti-inflammatory activity. In conclusion, it can be suggested that ethosomes could be superior drug carrier for topical delivery of lornoxicam in the treatment of inflammation.

Conflict of interest: Nil

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References

1. Shingade GM, Aamer Q, Sabale PM, Grampurohit ND, Gadhave MV, Jadhav SL, Gaikwad DD, et al., A review on recent trend on transdermal drug delivery system. *J Del Ther.* 2012; 2(1):1-12.
2. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. *Eur J Pharma Sci.* 2011;14: 101-14.
3. Nikalje AP, Tiwari S. Ethosomes: a novel tool for transdermal drug delivery. *Int J Res Pharm Sci.* 2012; 2(1): 1-20.
4. Budhathoki U, Thapa P. Effect of chemical enhancers on *in-vitro* release of salbutamol sulphate from transdermal patches. *Kath Uni J Sci Eng Technol.* 2005; 1(1):1-8.
5. Patel MN, Bharadia PD, Patel MM. Skin penetration enhancement techniques: physical approaches. *Int J Pharma App Sci.* 2010; 1(2): 62-72.
6. Tripathi KD. Essentials of medical pharmacology. 6th edition. New Delhi: Jaypee; 2006.174-75.
7. Kumar R, Aslam Md, Tripathi A, Prasad D, Chaudhary V, Jain V, Mishra SK, Singh R. et al., Ethosomes: novel vesicular carriers in transdermal drug delivery. *J Glo Pharma Tech.* 2010; 2(6): 1-7.
8. Kumar KP, Radhika PR, Sivakumar T. Ethosomes-a priority in transdermal drug delivery. *Int J Adv Pharma Sci.* 2010; 1: 111-21.
9. Kumar R, Kumar S, Jha SS, Jha AK. Vesicular system-carrier for drug delivery. *Der Pharmacia Sinica.* 2011; 2(4):192-202.
10. Abdul Hasan SA, Vijayakumar MR, Arun K. Formulation and evaluation of diclofenac potassium ethosomes. *Int J Pharm Pharma Sci.* 2010; 2(4): 82-6.
11. Akiladevi. D, Sachinandan B. Ethosomes a non-invasive approach for transdermal drug delivery. *Int J Curr Pharm Res.* 2010; 2(4):1-4.
12. Touitou. E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J Cont Rel.* 2000; 65:403-18.
13. Vyas SP, Khar RK. Controlled drug delivery concepts and advances. First ed. New Delhi: Vallabh Prakshan; 2002. 173-243.
14. Shah MB, Shah AJ, Shah R. An overview of ethosome as advanced herbal drug delivery system. *Int J Res Rev Pharm App Sci.* 2012; 2(1):1-14.
15. Sivakranth M, Anjuma AP, Krishnaveni C, Venkatesh E. Ethosomes: a novel vesicular drug delivery system. *Int J Adv Pharma.* 2012; 2(1):16-27.
16. Batlouni M. Non-steroidal anti-inflammatory drugs: cardiovascular, cerebrovascular and renal effects. *Arq Bras Cardiol.* 2010; 94(4):522-29.