ETODOLAC LOADED ETHOSOMES: DESIGN AND IN VITRO CHARACTERIZATION

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ABSTRACT
The present work deals with the preparation of Etodolac ethosomes and study of effect of alcohol and phospholipid on transdermal delivery. Etodolac is a nonsteroidal anti-inflammatory drug used for the management of mild to moderate pain, fever, and inflammation. They work by reducing the levels of prostaglandins, which are chemicals that are responsible for pain and the fever and tenderness that occur with inflammation. Etodolac blocks the cyclooxygenase (COX) enzymes which form prostanoids, resulting in lower concentrations of prostaglandins. As a consequence, inflammation, pain and fever are reduced. Skin is the main target of topical and transdermal preparations. Major aim of transdermal drug delivery system is to cross stratum corneum. Ethosomal carriers are system containing soft vesicles, composed of hydro alcoholic or hydro/glycolic phospholipid in which the concentration of alcohol is relatively high. Etodolac loaded ethosomes were prepared by hot method by using different concentrations of Alcohol and phospholipids in different ratios and propylene glycol. The prepared ethosomal formulations were evaluated for Vesicle size analysis, Morphological studies, Entrapment efficiency, In vitro release, Stability studies, In vitro permeation study.

KEY WORDS: Etodolac ethosomes, Inflammation, Transdermal Drug Delivery, Method of preparation.
INTRODUCTION

Transdermal drug delivery is gaining importance due to its noninvasive procedure for administration. The transdermal drug delivery overcomes a number of limitations of oral drug delivery such as degradation of drugs by digestive enzymes, irritation of gastrointestinal mucosa and first pass effect. Also due the pain on administration associated with parenteral route, patients highly prefer transdermal route. Hence transdermal dosage forms enjoy being the most patient compliant mode of drug delivery.

Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed of mainly phospholipids (phosphatidylcholine, phospholipids, phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids.

Proposed figure of Ethosome

Ethosomes systems were found to be significantly superior at delivering drugs through the skin in terms of both quantity and depth when compared to liposomes and to many commercial transdermal and dermal delivery systems. Ethosomes are sophisticated vesicular delivery carriers that are capable of delivering various chemical applications. Visualization by dynamic light scattering showed that Ethosomes could be unilamellar or multilamellar through to the core. The size of Ethosomes can be modulated to range anywhere from 30 nm
to a few microns. These novel delivery systems contain soft phospholipid vesicles in the presence of high concentrations of ethanol.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation. Etodolac, an NSAID, has been recommended orally for the treatment of rheumatoid arthritis and osteoarthritis. It also has antiinflammatory, antipyretic, and analgesic activities. The short biological half-life (about 4 h) and a higher dosing frequency make Etodolac an ideal candidate for sustained release. The oral administration of Etodolac causes gastrointestinal ulcers and gastrointestinal bleeding with chronic use. Because of gastrointestinal bleeding, it also causes anemia. Using the transdermal route eliminates these side effects, increases patient compliance, avoids first-pass metabolism, and maintains the plasma drug level for a longer period of time. Therefore, an improved Etodolac formulation with a high degree of permeation could be useful in the treatment of locally inflamed skin and inflammatory and painful states of supporting structures of the body.

Advantages of Ethosomal transdermal drug delivery system

1. Delivery of large molecules is possible (proteins and peptides)
2. It contains non-toxic raw material in formulation. Enhanced permeation of drug through skin for transdermal drug delivery.
3. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
4. High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
5. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods
6. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.

EXPERIMENTAL DETAILS

MATERIALS

Etodolac obtained as gift sample from Hetero Pharmaceuticals Pvt. Ltd, Hyd. Phospholipid, Cholesterol, Propylene Glycol and Alcohol were procured from S.D Fine Chemicals Pvt Ltd, Hyd.
METHODS OF PREPARATION

**Etodolac Ethosomes:** The ethosomal system of Etodolac were comprised of 1.5-2.5 % phospholipids, 20-40 % ethanol, 0.4 % of Etodolac and aqueous phase to 100 % w/w. Phospholipid and drug were dissolved in ethanol. In this solution double distilled water was added slowly in a fine stream with constant mixing at 700 rpm in a closed vessel. The temperature was kept 30°C throughout the experiment. The mixing was continued for addition of five minutes. The preparation was stored at 4°C.

CHARACTERIZATION OF ETHOSOMES

**Size and Shape Analysis:** Microscopic analysis was performed to determine the average size of ethosomes. A sample of ethosomes was suitably diluted with distilled water in order to observe individual vesicle and a drop of diluted suspension was put on a glass slide covered with cover slip and examined under microscope (magnification 15 × 45 X). The diameters of 150 vesicles were determined randomly using calibrated eyepiece micrometer with stage micrometer.

The average diameter was calculated using the formula,

\[ \text{Average diameter (dav)} = \frac{nd}{n} \]

Where, \( n \) = number of vesicles and \( d \) = diameter of the vesicles

**Surface Morphological Study:** The morphology of vesicles derived from ethosomal preparation was studied using Scanning Electron Microscopy. SEM revealed that the vesicles formed were spherical, smooth, and there was no formation of aggregates.

**Entrapment efficiency:** The entrapment efficiency of Etodolac by ethosomal vesicle were determined by ultracentrifugation, 10ml of ethosomal formulation is vortexed for 2 cycles of 5 min with 2 minutes rest between the cycles. 1.5ml of each vortexed sample and fresh untreated ethosomal formulations were taken into different centrifugal tubes. These samples were centrifuged at 20,000 rpm for 3 hours. The supernatant layer was separated, diluted with water suitably and drug concentration was determined at 206 nm in both vortexed and unvortexed samples. The entrapment efficiency was calculated as follows,

\[ \% \text{ Entrapment Efficiency} = \left[ \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \right] \times 100 \]

**In Vitro Release:** In vitro release studies on ethosomal preparation were performed using Franz-diffusion cell. The capacity of receptor compartment was 15 ml. The area of donor
compartment exposed to receptor compartment was 1.43 cm. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of ethosomal preparation was placed on one side of the dialysis membrane. The receptor medium was phosphate saline buffer of pH 6.8. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1°C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 206 nm.

**In vitro permeation studies:** The permeation of Etodolac from ethosomal formulations was determined by using Franz diffusion cell. The shaved abdominal skin of mice (0.5±0.1 mm thickness and 3.17 cm exposed surface areas) was mounted on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment. The receptor compartment was filled with 15 ml of pH 6.8 phosphate buffer maintained at 37.8°C and stirred by a magnetic bar at 600 rpm. One ml of ethosomal formulation was placed on the skin and the top of the diffusion cell was covered with paraffin paper. At appropriate time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 h), 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain sink conditions samples withdrawn were analyzed spectrophotometrically at 206 nm.\[^9\]

**RESULTS AND DISCUSSION**

**Vesicle size analysis:** Results of Vesicle size of Etodolac ethosomal formulations are presented in Table, which indicated that Vesicle formed with 40% alcohol are smaller in size than vesicle formed with 20% alcohol this is due to increase in the alcohol content. It is indicated that increase in alcohol content as well as decreased in the concentration of the phospholipid content resulting in smaller vesicle size. Size of vesicle was reduced when dispersion was sonicated. The reason for this is attributed to increase in alcohol concentration reduces the strength of vesicular layer due to perforation which results in breakage of larger vesicles to smaller vesicles. The size range was found to be 1.62 ± 1.31 µm to 4.56 ± 0.08 µm. Vesicle size of liposomal formulation was found to be 5.21 ± 0.02 µm. Alcohol used in ethosomes has a great effect on vesicle size. Vesicles formed from alcohols are of different size and they follow the order of 20% > 30% > 40%.
Surface morphological studies: Surface morphological studies mainly done with the help of scanning electron microscopy (SEM) which indicated that vesicle formed in ethosomal formulation was spherical, rounded, smooth and there was no formation of any aggregates.

Entrapment efficiency (%): Vesicle entrapment efficiency mainly dependent on the amount of phospholipid forming the bilayers and intrinsic properties of chemical structure, liphophilicity, phase transition temperature, alkyl chain length and alcohol concentration. It was found that phospholipid content which having higher liphophilicity, higher phase transition temperature and longer alkyl chain length shows higher entrapment. Thus depending upon these properties ethosomal formulations prepared with 2-3% of phospholipid and 30% alcohol shows higher entrapment efficiency than other formulations. The entrapment efficiency of formulations with 1-2% phospholipid and more than 30% of alcohol shows less than those of 2-3% of phospholipid and 30% of alcohol. This is due to reason that not uniform vesicle formation and more permeation of vesicle layer due to increased alcohol concentration. Values for entrapment efficiency were ranging from 54.81±0.30 to 78.04±0.30 (%) for different formulations.

In- vitro release study: The Etodolac formulations were prepared by hot method incorporating phsopholipid, alcohol and propylene glycol in different concentrations & in different ratios. In the later studies the effect of these phospholipid and alcohol on the in vitro release of the drug from different ethosomal formulations were carried out in phosphate buffer of pH 6.8 by using Franz diffusion cell. The cumulative percentage drug release from ethosomal formulations ETH1 to ETH6 was in the range of 71.52% to 85.75%. and for LPH it was 50.20±0.23%. From the results obtained it was observed that ethosomes prepared with alcohol 30% and phospholipid 1.5-2.5% showed better release profile when compared to the ethosomes prepared with the same at different concentrations. This is due to fact that uniform vesicle formation with sufficient penetration through the skin.

In- vitro permeation study: Permeation profile of Etodolac from optimized ethosomal formulations ETH3 and ETH6 through the rat abdominal skin after 24 hrs, the value for drug permeation (release) for optimized formulation ETH3 and ETH6 through the rat abdominal skin after 24 hrs was found to be 75.46% and 80.24% which is significantly less as compared to drug permeated through cellophane membrane i.e. 80.97% and 85.75% respectively. The reasons for this is  that skin act as barrier for transport of drug across skin, fusion of
ethosomal vesicle to surface of skin and interaction of ethosomal vesicle with surface of the skin.

Fig- 1: Optical photomicrograph of optimized formulation of Etodolac ethomes (ETH₃)

Fig-2. Scanning electron micrograph of optimized formulation of Etodolac ethosomes

Table 1: Composition of ethosomal formulation

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Phospholipid (% w/w)</th>
<th>Ethanol (% w/w)</th>
<th>Propylene Glycol (% w/w)</th>
<th>Cholesterol (% w/w)</th>
<th>Drug (% w/w)</th>
<th>Distilled Water (% w/w)</th>
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<tbody>
<tr>
<td>ETH₁</td>
<td>1.5</td>
<td>20</td>
<td>20</td>
<td>--</td>
<td>0.4</td>
<td>q.s</td>
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<tr>
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<td>2.5</td>
<td>20</td>
<td>20</td>
<td>--</td>
<td>0.4</td>
<td>q.s</td>
</tr>
<tr>
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<td>1.5</td>
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<td>20</td>
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<td>ETH₄</td>
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<td>ETH₅</td>
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<tr>
<td>ETH₆</td>
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Table 2: Physicochemical characterization of Etodolac ethosomal formulation (ETH₁- ETH₆)

<table>
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<tr>
<th>Sr. no</th>
<th>Formulation Code</th>
<th>Vesicle Size(µm)</th>
<th>% Entrapment Efficiency</th>
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<tr>
<td>1</td>
<td>ETH₁</td>
<td>2.86±0.04</td>
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<tr>
<td>2</td>
<td>ETH₂</td>
<td>2.91±0.04</td>
<td>70.51±0.09</td>
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<tr>
<td>3</td>
<td>ETH₃</td>
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<td>73.67±0.30</td>
</tr>
<tr>
<td>4</td>
<td>ETH₄</td>
<td>2.75±0.02</td>
<td>71.29±0.64</td>
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<tr>
<td>5</td>
<td>ETH₅</td>
<td>1.75±0.97</td>
<td>56.14±0.55</td>
</tr>
<tr>
<td>6</td>
<td>ETH₆</td>
<td>2.06±0.02</td>
<td>65.26±0.26</td>
</tr>
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</table>

CONCLUSION

The aim of the current investigation is to evaluate the transdermal potential of novel vesicular carrier, ethosomes, bearing etodolac, Non-steroidal antiinflammatory drugs (NSAIDs) agents having limited transdermal permeation. The result advocates the potential of ethosome formulation to treat inflammation where facilitated penetration of the drug into muscle and synovial fluid is desirable. In light of the data obtained from experimental work we can expect the ethosome formulation to be safe and very efficient as a drug carrier for systemic as well as topical delivery of drug, holding future in effective transdermal delivery. Ethosome has proven to be superior even to the marketed topical preparations. Further, these finding may help the industry for development and scaling up a new formulation.

REFERENCES