Formulation and evaluation of ciclopirox using ethosomal gel

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ABSTRACT

The present study focuses on the formulation of ethosomal gel of ciclopirox, an anti-fungal agent, for delivery as a carrier for transdermal application. The ethosomes were prepared using different concentrations of phospholipids (2-4 % w/v), ethanol (20-50 % w/v). They were formulated into 12 different formulations with different concentrations of phospholipids and ethanol. The drug release profile exhibited Higuchi's and zero order kinetics. The optimized formulation was found to be EF7 containing 40 % w/v ethanol and 3% w/v lecithin. The optimized formulation was evaluated for particle characteristics and zeta potential. Ethosomal gel was prepared by incorporation of optimized ethosomal suspension into gel base. The ethosomal gel was characterized for physical appearance, pH, content uniformity, rheological behaviour, skin-retention, in-vitro drug release and stability. From the results it can fairly be concluded that ethosomes are capable of delivering ciclopirox into systemic circulation by transdermal route.

Keywords: Ciclopirox, Ethosomal gel, Rheological behavior.

INTRODUCTION

Optimization of drug delivery through human skin is important in modern therapy. Recently, the transdermal route vied with oral treatment as the most successful innovative research area in drug delivery [1]. Transdermal delivery is an important delivery route that delivers precise amount of drug through the skin for systemic action. Improved methods of drug delivery for biopharmaceuticals are important for two reasons; these drugs represent rapidly growing portion of new therapeutics, and are most often given by injection. Discovery of new medicinal agents and related innovation in drug delivery system have not been only enabled the successful implementation of novel pharmaceutical, but also permitted the development of new medical treatment with existing drugs. Throughout the past two decades, the transdermal patches have become a proven technology holding the promise that new compound could be delivered in a safe and convenient way through the skin. Since the first transdermal patch was approved in 1981 to prevent nausea and vomiting associated with motion sickness, the FDA has approved through the past 22 years more than 35 transdermal patch products spanning 13 molecules [2].

Routes of penetration

At the skin, molecules contact cellular debris, microorganisms, sebum and other materials, which negligibly affect permeation. The penetration has three potential pathways to the viable tissue - through hair follicles with associated sebaceous glands, via sweat ducts, or across continuous stratum corneum between these appendages (Fig 1,3).

Liposomes were discovered in the early 1960’s by Bangham and colleagues (Bangham et al., 1965) and subsequently became the most extensively explored drug delivery system5. In early 1960’s a great
knowledge of vesicle derivatives have been tested for their abilities. Most experiments, however, have centered on liposomes, since derivations only add to their basic properties. Vesicles are closed, spherical membrane that separate a solvent from the surrounding solvent. Possible use of liposomes in topical drug delivery vehicles for both water and lipid soluble drug has been investigated. While it has been suggested that the external envelop of a liposomes would allow it to pass through lipophilic skin, most researches show that liposomal vesicles become trapped within the top layer of the stratum corneum cells. Generally liposomes are not expected to penetrate into viable skin, although occasional transport processes were reported. This behavior is useful both for local treatment of skin disorders and for cosmetic formulations. Specific drug accumulation at the site of action and decreased systemic drug absorption can impart increased efficiency as well as decreased side effect to a compound applied topically [6].

**Ethosomes**

The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers have been understanding the properties of vesicle structures for use in better drug delivery within their cavities that would allow to tag the vesicle for cell specificity. Vesicles would also allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and would be able to release just the right amount of drug and keep that concentration constant for longer periods of time. One of the major advances in vesicle research was the finding a vesicle derivative, known as an ethosomes [4, 6]. Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipid (Phosphotidyl choline; PC), ethanol at relatively high concentration and water. It was found that ethosomes penetrate the skin and allow enhanced delivery of various compounds to the deep strata of the skin or to the systemic circulation.

**Characterization**

Various methods of characterization of ethosomes are as follows

**Visualization**

For the initial characterization of the vesicles, ethosomal preparation can be examined by negative stain transmission electron microscopy (TEM). It also visualize the lamellar character of ethosomes. The three dimensional nature of phospholipid vesicle can be confirmed by further analysis by scanning electron microscopy (SEM) [7].

**Assay**

HPLC Assay of drug. Drug can be quantified by a modified HPLC method using a UV detector, column oven, auto-sample, pump and computerized analysis program [9].

Alcohol quantification Touitou et al. reported quantitative determination of ethanol using enzymatic diagnostic kit based on the oxidation of alcohol to acetaldehyde. The reduction of nicotinamide adenine dinucleotide (NAD) was followed in a UV spectrophotometer as an increase in absorbance at 340 nm. The increase in absorbance is directly proportional to alcohol concentration in the sample [7].

**Vesicle stability**

The stability of vesicles can be determined by assessing the size and structure of vesicles overtime. Mean size is measured by DLS and structural changes are observed by TEM [4]. Touitou et al. performed stability study by...
Solubility measurement

The solubility of drug in ethosomal medium can influence its entrapment capacity, vesicular structure and consequently permeation of drug through the skin. Dayan and Touitou found the solubility of drug in water, phosphate buffer and 30% hydroethanolic solution at 22°C and 37°C by solubility method [11].

Penetration and permeation studies

Depth of penetration of ethosomes can be visualized by confocal laser scanning microscopy (CLSM) [4]. The ability of ethosomes to deliver lipophilic molecules to the deep layers of the skin was investigated using a lipophilic fluorescent probe, Rhodamine red and confocal laser scanning microscopy [7].

The dermal and intracellular delivery of Bacitracin, a model polypeptide antibiotic, from ethosomes. Efficient delivery of antibiotics to deep skin strata from ethosomal applications was reported to be highly beneficial, reducing possible side effects and other drawbacks associated with systemic treatment [16].

Osmotics inc., U.S.A., reported a new cellulite creach celled lipoduction cream which smoothes the skin and has a break through permeation technology called ethosomes that penetrate skin lipid barrier and deliver cocktail of fat metabolizing ingredient directly into fat cells [4].

MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Supplier</th>
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<tr>
<td>Ciclopirox</td>
<td>Chandra Labs, Hyderabad</td>
</tr>
<tr>
<td>Soya lecithin</td>
<td>Research lab fine chem. Industries(Mumbai)</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>Research lab fine chem. Industries(Mumbai)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Jiangsu Huaxi International Trade Co.Ltd(CHINA)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Viratlab(Mumbai).</td>
</tr>
<tr>
<td>Carbopol-934</td>
<td>Research lab fine chem. Industries(Mumbai)</td>
</tr>
<tr>
<td>Triethanol amine</td>
<td>Research lab fine chem. Industries(Mumbai)</td>
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Preparation of ciclopirox ethosomes (by cold method)

Preparation of Ciclopirox ethosomes was followed by method suggested by Touitou et al., with little modification. [7] The ethosomal system of Ciclopirox comprised of 2-4 % phospholipids, 20-50 % ethanol, 10 % of propylene glycol, 0.005g of cholesterol and aqueous phase to 100 % w/w. Ciclopirox 0.25 gm was dissolved in ethanol in a covered vessel at room temperature by vigorous stirring. Propylene glycol was added during stirring. This mixture was heated to 30°C in a separate vessel and was added to the mixture drop wise in the center of the vessel, which was stirred for 5 min at 700rpm in a covered vessel the vesicle size of ethosomal formulation can be decreased to desire extend using sonication [29] or extrusion method. Finally, the formulation is stored under refrigeration [31]. Ethosomes were formed spontaneously by the process. The formulation table was shown in table 5.

Characterization of ethosomes

Entrapment efficiency

The entrapment efficiency of Ciclopirox by ethosomal vesicle was determined by ultracentrifugation 10 ml of ethosomal suspension [18] Each sample was 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 226 nm in both vortexed and unvortexed samples. The entrapment efficiency was calculated as follows

Entrapment Efficiency = \frac{T - CX}{100}
CHARACTERIZATION OF GEL

Surface morphology
The surface morphology of the ethosomal was determined by using scanning electron microscope using gold sputter technique. The system was vacuum dried, coated with gold palladium, and observed microscopically.

Organoleptic Characteristics
The formulations were tested for its psycho rheological properties like color, odor, texture, phase separation and feel upon application (grittiness, greasiness)

Wash ability
A small quantity of gel was applied on the skin. After washing with water, checked for whether the gel was completely washable or not.

Spread ability
It was determined by modified wooden block and glass slide apparatus. A measured amount of gel was placed on fixed glass slide, the movable pan with a glass slide attached to it and was placed over the fixed glass slide, such that the gel was sandwiched between the two glass slides for 5 min. The weight was continuously removed. Spread ability was determined using the below given formula:

\[ S = \frac{M}{T} \]

Where,

- \( S \) is the Spreadability in g/s,
- \( M \) is the mass in grams &
- \( T \) is the time in seconds.

pH
Solution of 1gm of gel dissolved in 30ml of distilled water (pH 7) was prepared. The pH of the ethosomal gel was determined by using digital pH meter, measured by bringing the probe of the pH meter in contact with the samples.

Drug content and content uniformity
1g of gel was dissolved in a 100ml of phosphate buffer pH 7.4 for 48 hrs with constant stirring using magnetic stirrer. Solution was filtered and observed with UV spectrophotometer at \( \lambda_{\text{max}}226\text{nm} \). the measurements were made in triplicate.

In-vitro release kinetics (Harris shoaiie et al., 2006) [29]
To analyze the in vitro release data various kinetic models were use to describe the release kinetics. The zero order rate Eq. (2) describes the systems where the drug release rate is in-dependent of its concentration. The first order Eq.(3) describes the release from system where release rate is concentration dependent. Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on

Korsmeyer equation / Peppa’s model
To study the mechanism of drug release from the liposomal solution, the release data was also fitted to the well-known exponential equation (Korsmeyer equation/ Peppa’s law equation), which is often used to describe the drug release behavior from polymeric systems.

\[ \frac{M_t}{M_\alpha} = K t^n \]

Where

- \( \frac{M_t}{M_\alpha} = \) The fraction of drug released at time ‘\( t ' \).
- \( K = \) Constant incorporating the structural and geometrical characteristics of the drug / polymer system.
- \( n = \) Diffusion exponent related to the mechanism of the release.

Above equation can be simplified as follows by applying log on both sides,

\[ \log \frac{M_t}{M_\alpha} = \log K + n \log t \]

Stability studies
Stability study was carried out for Ciclopirox ethosomal preparation at two different temperature i.e. refrigeration temperature (4 ± 2° C) at room temperature (27 ± 2° C) for 8 weeks (as per ICH guidelines). The formulation was subjected to stability study and stored in borosilicate container to avoid any sort of interaction between the ethosomal preparation and glass of container, which may affect the observations.
RESULTS AND DISCUSSIONS

Preformulation studies

Solubility studies of Ciclopirox

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Ciclopirox (mg/mL)</th>
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<tbody>
<tr>
<td>Water</td>
<td>4.5</td>
</tr>
<tr>
<td>Methanol</td>
<td>9.2</td>
</tr>
<tr>
<td>0.1N Hcl</td>
<td>5.6</td>
</tr>
<tr>
<td>pH 6.8 buffer</td>
<td>7.3</td>
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Ciclopirox pure drug was scanned in methanol between 200 nm and 400 nm using ultraviolet spectrophotometer. Ciclopirox was identified by its light absorption pattern which follows the absorption of light in the range 220 to 300 nm and a maximum absorbance at about 212.5 nm. A broad shoulder at about 212.5 nm was also observed which confirm the Ciclopirox presence. Ciclopirox spectrum gave a highest peak at 212.5 nm and same was selected for further evaluations.

Calibration curve in methanol (make up with pH 7.4 phosphate buffer)

Standard solutions of different concentration were prepared and their absorbances were measured at 212.5 nm (Table10). Calibration curve was plotted against drug concentrations versus absorbance’s as given in the (Figure no 7.).

Size and shape analysis

Microscopic analysis was performed under different magnification to visualize the vesicular structure, lamellarity and to determine the size of ethosomal preparations.

Scanning electron microscope (SEM)

![Fig 1: Scanning electron microscope image](image)

Size distribution of ciclopirox ethosomal formulations

Each value is an average of 3 replications.

Average diameter ($d_{avg}$) = \frac{\sum_{i=1}^{n} d_i}{n}

\[= \frac{585.73}{150} = 3.904 \mu m\]
The results obtained by vesicular size analysis showed concentration of ethanol affect vesicular size. The size of ethosomes decrease as the concentration of ethanol increased with the largest vesicles size 5.89 µm containing 20% ethanol and smallest 3.26 µm containing 50% ethanol. Results obtained in the present investigation are in conformity with the results of Touitou E et al., 2007. Result obtained here showed the maximum vesicular size is 5.89 µm for formulation containing 20% ethanol (EF3) and minimum is 3.26 µm for formulation containing 50% ethanol (EF7), (figure). Results obtained here are in same relation with concentration of ethanol. In average sonication found to reduce the size to three or more times of initial.

**Entrapment efficiency**

Once the presence of bilayer vesicles was confirmed in the ethosomal system, the ability of vesicles for entrapment of drug was investigated by ultracentrifugation. Ultra-centrifugation was the method used to separate the ethosomal vesicles. The maximum entrapment efficiency of ethosomal vesicles as determined by ultracentrifugation was 95.21% for ethosomal formulation containing 50% ethanol (EF8). As the ethanol concentration increased from 20% to 50% w/w, there was increase in the entrapment efficiency. Results of entrapment efficiency also suggest that 3% phospholipid is optimal concentration for entrapment efficiency and hence increased or decreased in concentration of phospholipid reduces the entrapment efficiency of vesicles. These result further supported by observation made by Jain NK et al. 2014. Entrapment efficiency of ethosomal formulations are significantly different and are reported in Table 17. Increase in entrapment efficiency may be due to the possible reduction in vesicle size. The detrimental effect on the vesicle during ultra-centrifugation which are larger in size. Sonication gives the more uniform lamellae, smaller vesicle and uniform size and hence it may be the reason for higher vesicular stability and lesser vesicular disruption during ultra-centrifugation.

**In vitro release studies**

In-vitro skin permeation study or in-vitro diffusion study have been extensively studied, developed and used as an indirect measurement of drug solubility, especially in preliminary assessments of formulation factors and manufacturing methods that are likely to influence bioavailability. The objectives in the development of in-vitro diffusion tests are to show the release rate and extent of drug from the dosage form. The in-vitro skin permeation study of Ciclopirox from ethosomal formulation was studied using franz diffusion cell and method described in methodology chapter.
Inferences

In trails EF4,EF8,EF12, the phospholipids was increased from (2%-4%) but ethanol concentration was kept constant at 50%, with increase the concentration of the lecithin the drug release also increased gradually. This may be due to decrease in vesicle size which contributed to increase in drug release. There was a slight decrease in drug release observed at 4% lecithin.

Inference

Optimized formulation showed good fit for Higuchi (r²=0.972) kinetic models (Table 29; fig 30). From Higuchi model it is evident that ciclopirox is released by diffusion process from the vesicles. Lecithin controls the release of soluble drugs by diffusion process. This diffusion is probably due to the presence of gel barrier of lecithin. The optimized formulation EF7 follows zero order and higuchi kinetic model.

Measurement of zeta potential

![Zeta Potential Table and Graph]

**Fig 4:** Zeta potential result of optimized Ethosomal suspension was found to be -32.4mV.

Inferences

Zeta potential can be termed as an important parameter to predict the physical stability of the prepared ethosomes. Higher the electrostatic repulsions between the particles higher the stability. Ethosomes with a zeta potential more than +20 mV or less than -20 mV can be termed as physically stable dispersion. The zeta potential of the optimized formulation EF7 was found to be -32.4 mV, from which it can be inferred that the ethosomes has good physical stability which prevents aggregation with aging.

CONCLUSION

It is well known that if drug molecules presenting any difficulties in its solubility and bioavailability along the GI tract, are candidates for other routes of administration and if the site of action for drug candidate is subdermal, an effective penetration enhancers are required to provide the drug molecule...
deeper into skin tissue for optimized therapeutic delivery of drug. It is generally agreed that classic liposomes are of little or no value as carriers for transdermal drug delivery because they do not penetrate the skin. Recently derived ethosomal system can deliver drug molecules into and through the skin. An attempt was made to formulate the highly efficient ethosomal drug delivery system using Ciclopirox as model drug. The cold method was employed with little modification for the preparation of various ethosomal formulations containing different concentration of ethanol (20 % to 50 %) with sonication. The techniques used were simple and reproducible. The prepared ethosomes were spherical and discrete in shape. The size of vesicles were found to be in the range of 3.26 μm – 5.89 μm sonicated ethosomes. Entrapment efficiency of ethosomal formulations are significantly different and are reported in range of 81.21 – 95.21% for EF1 and EF8 formulations respectively. The concentration of the drug in all the formulations with different polymers was found to be 95.3 – 99.7%. Based upon the drug release studies and all other evaluations EF7 formulation with 3% lecithin and 40% of ethanol is optimized. Zeta potential value had suggested good particle stability. Controlled release achieved by these formulations may reduce the dose frequency and improves patient compliance.

Thus, the specific objectives namely design; characterization and release studies of Ciclopirox ethosomal formulations were achieved. Certainly these finding can be applied for transdermal drug delivery of Ciclopirox for treatment of superficial mycoses. Further these findings may help the industry for development and scaling up a new formulation.

REFERENCES