Formulation and Evaluation of Piroxicam Loaded Ethosomal Gel for Transdermal Delivery

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Abstract

This research study was in aim to formulate and evaluation of ethosomes containing piroxicam by using phospholipid (1-3%), ethanol (20-40%), propylene glycol (10%) and distilled water by cold method. Prepared ethosomal vesicles evaluated for vesicular size, shape, entrapment efficiency; in vitro skin diffusion, skin irritation and stability studies were carried out. Scanning electron microscopy and size analyser results showed that ethosomes were in spherical, unilamellar, nanometric size. The formulation EF5 showed highest entrapment efficiency of 73.59%. Then, optimized formulation of ethosomal vesicles was further formulated to gel by using carbopol. EFG2 was found to have shown excellent invitro drug release comparing gel containing free piroxicam drug. The kinetic study was found to be fit in first order model and observed no remarkable symptoms on skin from skin irritation study.

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Key Words
Piroxicam, Ethosomal gel
Carbopol, Phospholipid,
Vesicles, lecithin
INTRODUCTION (1, 2)
Piroxicam is a non steroidal anti-inflammatory drug (NSAID) used in treatment of pain and inflammatory disorders, such as rheumatoid arthritis. Oral administration of this drug is associated with severe gastrointestinal side effects like ulceration and gastrointestinal bleeding. Further as it is required for chronic use in the condition like rheumatoid arthritis, these drawbacks become disabling factors of such therapy. The solution of this problem lies in the fact that, topically applied NSAIDs are safer than and as efficacious as oral NSAIDs. Problem of drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs. It is the most preferred route for systemic delivery of drugs to paediatric, geriatric and patients having dysphasia. More recently such dosage forms have been developed and modified in order to enhance the driving force of drug diffusion thermodynamic activity and increase the permeability of the skin3. These approaches include the use of penetration enhancers, supersaturated systems, prodrug, liposome and other vesicles. One of the major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin4. The interest of both the pharmaceutical and cosmetic industry for skin delivery has prompted the development and investigation of a wide variety of vesicular systems. Therefore this study was focused to prepare vesicle such as ethosomes and further formulated to gel form using carbopol.

MATERIALS AND METHODS
Piroxicam was received a gift sample from Chandra labs Hyderabad, India. Phospholipon 90, Propylene glycol, Carbopol-934 and Triethanolamine were purchased from Research lab fine Chem Industries (Mumbai); Alcohol from Jiangsu Huaxi International Trade Co.Ltd (CHINA); Cholesterol- Virat lab (Mumbai); ultrapure water from Cortex laboratories (Hyderabad).

Preparation of ethosomal vesicles
Ethosomal formulation was prepared according to the cold method5. Phospholipid and Piroxicam was dissolved in ethanol in a covered vessel at room temperature by
vigorously stirring. Propylene glycol was added during stirring. This mixture was heated to 30°C in a separate vessel and was added to the mixture dropwise in the centre of the vessel, which was stirred for 5 min at 700 rpm in a covered vessel. The vesicle size of ethosomal formulation can be decreased to the desired extent using sonication or extrusion method. Finally, the formulation is stored under refrigeration. Ethosomes were formed spontaneously by the process with variable composition of ethosomal formulations (Table 1.)

Based on the results of ethosomal vesicle evaluation, the best achieved ethosomal vesicles formulation was prepared into gel formulation by incorporated into carbopol gel (1%, 1.5%, 2% w/w). The specified amount of carbopol 934 powder was slowly added to ultrapure water and kept at 100°C for 20 min and triethanolamine was added to it dropwise as shown in Table 2.

**Table 1.** Shows variable composition of ethosomal formulations

<table>
<thead>
<tr>
<th>Ethosomal formulation</th>
<th>Lecithin (Soya lecithin) (%)</th>
<th>Ethanol (%)</th>
<th>Propylene glycol (%)</th>
<th>Drug (g)</th>
<th>Cholesterol (g)</th>
<th>Water (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF1</td>
<td>2</td>
<td>20</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF2</td>
<td>3</td>
<td>20</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF3</td>
<td>4</td>
<td>20</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF4</td>
<td>5</td>
<td>20</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF5</td>
<td>2</td>
<td>30</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF6</td>
<td>2</td>
<td>40</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF7</td>
<td>2</td>
<td>50</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
</tbody>
</table>

**Table 2.** Composition of different ethosomal gel formulation

<table>
<thead>
<tr>
<th>Gel formulation</th>
<th>Piroxicam ethosomal suspension (ml)</th>
<th>Carbopol (%)</th>
<th>Triethanolamine (ml)</th>
<th>Phosphate buffer (pH 6.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFG1</td>
<td>20</td>
<td>1</td>
<td>0.5</td>
<td>q.s</td>
</tr>
<tr>
<td>EFG2</td>
<td>20</td>
<td>1.5</td>
<td>0.5</td>
<td>q.s</td>
</tr>
<tr>
<td>EFG3</td>
<td>20</td>
<td>2</td>
<td>0.5</td>
<td>q.s</td>
</tr>
<tr>
<td>PG4* (g)</td>
<td>0.025</td>
<td>1.5</td>
<td>0.5</td>
<td>q.s</td>
</tr>
</tbody>
</table>
Analysis of drug excipients interaction
The compatibility between drug and polymers was detected by IR spectra. FTIR analysis was carried out on Corporation, Japan in the region of 400 to 4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\) and the data was analyzed.

Evaluation of Ethosomes

Characterisation of ethosomes
Microscopic analysis was performed to determine the average size of ethosomes. A sample of ethosomes was diluted with distilled water and a drop of diluted suspension was placed on a glass slide covered with cover slip in order to observe individual vesicle and examined under microscope and average diameter was calculated.

\[
\text{Average diameter} = \frac{nd}{n}
\]
\(n = \text{number of vesicles}
\)
\(d = \text{diameter of vesicles}
\)

Scanning Electron Microscopy\(^9\)
Determination of surface morphology (roundness, smoothness and formation of aggregates) of piroxicam ethosomal gel with polymer was mounted on a clear glass stub. It was then air dried and gold coated to visualize under scanning electron microscopy (SEM).

Entrapment Efficiency\(^{10}\)
The entrapment efficiency of ethosomal vesicle was determined by ultracentrifugation\(^{11}\). 10 ml of (ethosomal suspension) 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 334 nm in both vortexes and unvortexed samples. The entrapment efficiency was calculated as follows:

\[
\text{Entrapment Efficiency} = \frac{T-C}{T} \times 100
\]

\(T\) is total amount of drug that detected from supernatant of vortexed.

Evaluation of ethosomal Gel

Surface morphology\(^{12}\)
The surface morphology of the ethosomal was determined by scanning electron microscope using gold sputter technique. The system was vacuum dried, coated with gold palladium, to visualize under scanning electron microscope at 10\(\times\)60SE magnifications.

Organoleptic Characteristics
The formulations were tested for its psycho rheological properties\(^{13}\) like color, odor, texture, phase separation and feel upon application (grittiness, greasiness).
**Washability**

A small quantity of gel was applied on the skin and washed with water in order to check the washability of gel.

**Spreadability**

Spreadability of gel 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. Spreadability was determined using the 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 measurement**

1 gm of gel dissolved in 30 ml of distilled water (pH 7.0). 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**

One gram of gel was dissolved in a 100 ml of phosphate buffer pH 6.8 stirred constantly for 2 days using magnetic stirrers. The resultant solution was filtered and content was analysed by U.V spectrophotometer.

**Skin irritation test**

The abdominal skins of the male rats (wistar rat) were shaved the hair 24 h prior to the gel application. 0.5 g gel was applied on the hair-free skin of rat by uniform spreading over an area of 4 cm². The skin surface was observed for any visible change such as erythema (redness) after 24, 48 and 72 h of the formulation application. The mean erythema scores were recorded depending on the degree of erythema: no erythema = 0, slight erythema (barely perceptible-light pink) = 1, moderate erythema (dark pink) = 2, moderate to severe erythema (light red) = 3 and severe erythema (extreme redness) = 4.

**In-Vitro Release Studies**

**Drug release study from dialysis membrane**

The skin permeation of Piroxicam from ethosomal vesicles and gels were studied using dialysis membrane and open ended diffusion cell specially designed in our laboratory according to the literates. The effective permeation area of the diffusion cell and receptor cell volume was 2.4 cm² and 200 ml respectively. The temperature was maintained at 37 ± 0.5°C.

The receptor compartment contained 200 ml of pH 6.8 buffer and was
constantly stirred by magnetic stirrer at 100 rpm. Dialysis membrane was mounted between the donor and the receptor compartments. Ethosomal formulation was applied to the dialysis membrane and the content of diffusion cell was kept under constant stirring then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analysed by spectrometric method at 334 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh pH 6.8 buffer. Triplicate experiments were conducted for drug release studies.

**Drug release kinetic modelling**

The kinetics of piroxicam release from the ethosomal vesicles and from the gels formulated was determined by finding the best fit kinetic model by fitting the release data into various kinetic equations such as Zero order, First-order, Higuchi, and Korsemeyer–Peppas and finding the $R^2$ values of the release profile corresponding to each model.

**Stability Studies**

Stability study was carried out for model drug ethosomal preparation at two different temperature i.e. refrigeration temperature $(4 \pm 2^\circ C)$ room temperature $(27 \pm 2^\circ C)$ 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. Sample was collected for every 2 weeks and content was analysed at 334nm in U.V spectrometer.

**RESULTS AND DISCUSSION**

**Compatibility studies by FTIR**

FTIR spectrum of pure Piroxicam exhibited characterized by the peak at $3340\text{cm}^{-1}$ and $1318 \text{cm}^{-1}$ for pyridine2-yl amino stretching and saturated methyl group respectively (Fig.1A). The presence of those characteristic peaks of drug observed in the FTIR spectra of formulation (Fig 1B), indicates that absence of chemical interaction between the drug and the polymers employed in the study.

**Characterisation of ethosomes**

Prepared ethosomes were found to be spherical vesicles and uniform size distribution when observed under light microscope and also confirmed in the scanning electron micrographs.
The vesicular size of the ethosomes significantly increases with increase in phospholipids concentration and also shows increased with increase in concentration of ethanol, however lesser than former. Amounts of ethanol and Phospholipon 90, used for ethosomes preparation were seemed to influence the entrapment efficiency. The entrapment of drug into ethosomes was found to have decreased with the increase in ethanol concentration appreciably. Reason could be possibly increase in fluidity and formation thinner membrane with higher ethanol concentration which becomes more permeable to the drug and that may have led decreases efficiency of the system to entrap the drug molecule.

**Evaluation of ethosomal gel**

The ethosomal gel was prepared with optimized ethosomal vesicles with various concentrations of carbopol 934 as given in composition table 2. All the three formulation were evaluated for physical properties. All formulations were golden yellow colour. The pH results for all formulation exhibit in the range of 6.82-6.84 which demonstrate that the prepared gels will irritation free to the skin. The spreadability of the gels was found to be in the range of 6.4g.cm/sec conforming that its spread smooth and uniformly. A gel was easily washable with water without leaving any residue on the surface of the skin. The results presented in table 4.
**Drug content**

The drug content of the formulations ranged from 0.384-0.386. The values obtained from the drug content (Tab 5), it was concluded that there was no loss of drug during the gel preparation process.

Tab 3. Various size of Piroxicam Ethosomal formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Average diameter (μm)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF1</td>
<td>3.90</td>
<td>74.62</td>
</tr>
<tr>
<td>EF2</td>
<td>4.92</td>
<td>70.00</td>
</tr>
<tr>
<td>EF3</td>
<td>5.20</td>
<td>65.30</td>
</tr>
<tr>
<td>EF4</td>
<td>5.4</td>
<td>60.01</td>
</tr>
<tr>
<td>EF5</td>
<td>4.5</td>
<td>77.87</td>
</tr>
<tr>
<td>EF6</td>
<td>5.0</td>
<td>53.05</td>
</tr>
<tr>
<td>EF7</td>
<td>5.10</td>
<td>49.30</td>
</tr>
</tbody>
</table>

Fig 3. Variation of size among different ethosomal vesicles of piroxicam

Tab 4. Organoleptic characteristics of ethosomal gel

<table>
<thead>
<tr>
<th>Organoleptic Characteristics</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Color</td>
<td></td>
</tr>
<tr>
<td>Greasiness</td>
<td>Greasiness</td>
<td></td>
</tr>
<tr>
<td>Grittiness</td>
<td>Grittiness</td>
<td></td>
</tr>
<tr>
<td>Ease of application</td>
<td>Ease of application</td>
<td></td>
</tr>
<tr>
<td>Skin irritation</td>
<td>Skin irritation</td>
<td></td>
</tr>
<tr>
<td>Washability</td>
<td>Easily washable without leaving any residue on the surface of the skin</td>
<td></td>
</tr>
<tr>
<td>Spreadability</td>
<td>6.4g.cm/sec</td>
<td></td>
</tr>
</tbody>
</table>

**Skin irritation test**

No any visible change such as erythema (redness) after 24, 48 and 72 h was observed on the skin of the formulation applied.

**Stability studies**

The ability of vesicles to retain the drug was assessed by keeping the ethosomal suspension at different temperature. Optimized ethosomes formulation and gel (EF5 & EFG2) were selected for stability studies. Ethosomal formulations were observed for any change in appearance or color for a period of 8 weeks showed no noticeable changes in drug release profile occurred. It was concluded that the ethosomal system was more stable at specified storage conditions.
Tab 5. Drug content and pH measurements of Ethosomal gel

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>pH</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFG1</td>
<td>6.82</td>
<td>0.384</td>
</tr>
<tr>
<td>EFG2</td>
<td>6.83</td>
<td>0.386</td>
</tr>
<tr>
<td>EFG3</td>
<td>6.84</td>
<td>0.386</td>
</tr>
</tbody>
</table>

In-vitro release studies

The in vitro drug releases of various formulations are shown in (Fig 5 &6). The cumulative release of the drug from ethosomes vesicle and ethosomal gel (EF5 & EFG2) were 95.07±0.98 and 88.72±0.90 respectively in 24 hrs.

The cause of phospholipids concentration in in-vitro release of drug from ethosomes across the membrane shows less when compared to effect of ethanol concentration. Increased in cumulative amount of drug permeation across membrane at higher ethanol concentration upto 30 %, however further increment in it exhibits reduction in permeation. This may due to the disruption of lipid bilayer at higher ethanol concentration. Ethanol provides the vesicles with soft flexible characteristics, which allow them to more easily penetrate into deeper layers on stratum corneum lipids and on vesicle fluidity as well as a dynamic interaction between ethosomes and the stratum corneum all may contribute to the superior skin penetration ability of ethosomes. Phospholipids concentration was as well contributed in drug permeation. The literatures show use of propylene glycol also influenced the amount of drug permeation.

Formulations (EF5) containing 2% Phospholipon and 30 % ethanol showed 95.07 ± 0.98 % drug release within 24 h was higher drug release when compared all other formulation. Because of the average particle size 4.5 and 77% high entrapment efficiency, the ethosomal formulation EF5 was chosen for gel formulation.

Ethosomal gels were prepared with different concentrations of Carbopol. Comparing the permeation profile from various ethosomal gels, it was evident that EFG2 shows maximum permeation (Fig 6). Also it was found out that release from EFG3 was much less as compared to EFG2 probably due to the high viscosity attributed to high carbopol concentration. EFG2 was found to have shown maximum release and good consistency, it was considered favourable for the final development of the formulation.
Fig 4. Effect of lecithin construction in the % of cumulative drug release (EF1, EF2, EF3, EF4)

Fig 5. Effect of ethanol concentration of cumulative drug release of in vitro studies (EF5, EF6, EF7)

Analysis of drug release mechanism

On drug release kinetic modelling and comparison of the release profile, it was found that there was not much difference in the release pattern of drug from all formulation of ethosomes vesicles and gels (Table 5).

All of them were found to release the drug in accordance to first order kinetics. Also Super case II transport (n > 1.0) of drug was involved in all formulations.
### Tab 6. Regression values of pharmacokinetic profiles

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order ((R^2))</th>
<th>First order ((R^2))</th>
<th>Higuchi ((R^2))</th>
<th>Korsemeyer-peppas ((R^2))</th>
<th>N value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF1</td>
<td>0.980</td>
<td>0.914</td>
<td>0.979</td>
<td>0.948</td>
<td>1.48</td>
</tr>
<tr>
<td>EF2</td>
<td>0.972</td>
<td>0.855</td>
<td>0.994</td>
<td>0.980</td>
<td>1.06</td>
</tr>
<tr>
<td>EF3</td>
<td>0.982</td>
<td>0.876</td>
<td>0.995</td>
<td>0.984</td>
<td>1.02</td>
</tr>
<tr>
<td>EF4</td>
<td>0.965</td>
<td>0.862</td>
<td>0.989</td>
<td>0.978</td>
<td>1.04</td>
</tr>
<tr>
<td>EF5</td>
<td>0.938</td>
<td>0.920</td>
<td>0.973</td>
<td>0.957</td>
<td>1.55</td>
</tr>
<tr>
<td>EF6</td>
<td>0.980</td>
<td>0.884</td>
<td>0.992</td>
<td>0.980</td>
<td>1.06</td>
</tr>
<tr>
<td>EF7</td>
<td>0.984</td>
<td>0.876</td>
<td>0.987</td>
<td>0.985</td>
<td>1.03</td>
</tr>
<tr>
<td>EFG2</td>
<td>0.991</td>
<td>0.941</td>
<td>0.961</td>
<td>0.923</td>
<td>1.10</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The studies result advocates the potential of ethosomal vesicles and gel formulation to treat rheumatic disease where facilitated penetration of the drug into muscle and synovial fluid is desirable. In light of the data obtained from this work that a significant amount of piroxicam transported across the skin when entrapped in ethosomes. It can be used for transdermal treatment of the diseases like rheumatoid arthritis, where chronic use is needed. Finally, it can be concluded that ethosomes offers advantages of rapid onset and maximum release of drug with reduction of side effects.

Furthermore, ethosomes do not involve in damage the architecture of skin and hence, drug is transported into the systemic circulation across the intact skin.

**REFERENCES**


**Cited this article as:**