DEVELOPMENT AND CHARACTERIZATION OF ETHOSOMES BEARING LOSARTAN POTASSIUM FOR TRANSDERMAL DRUG DELIVERY

RAVINDRA BHANA*, AJAY VERMA, SANJAY JAIN

Smriti College of Pharmaceutical Education, Indore-452010, India. Email: vermaajay77m@gmail.com

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ABSTRACT

The aim of the current investigation was to evaluate the transdermal potential of novel vesicular carrier, ethosomes, bearing Losartan Potassium, an anti-hypertensive, hydrophilic agent, loaded ethosomal formulation and optimized. Losartan Potassium loaded ethosomal formulations were prepared, optimized, and characterized for vesicular shape and surface morphology, vesicular size, entrainment efficiency, zeta potential, fluorescence microscopy, and stability. The optimized ethosomal formulation revealed an enhanced permeation of calcein loaded ethosomal formulation to the deeper layers of the skin. Skin irritation study revealed that the system is free of irritation. Stability study was performed for 60 days, which revealed no significant change in the entrapment efficiency and vesicular size. The result of in vitro drug permeation studies of different formulations across the pig ear skin showed transdermal flux of ethosomal gel (0.194±0.12 mg/cm²/h) 4-fold higher than that of the hydroethanolic solution of drug (0.048±0.07 mg/cm²/h), and 2.7-fold higher than that of the hydroethanolic solution of drug (0.071±0.05 mg/cm²/h). In light of the data obtained from experimental work we can expect the ethosomal formulation to be safe and very efficient as a drug carrier for transdermal delivery of drug, holding future in effective transdermal delivery.

Keywords: Transdermal Permeation, Ethosomes, Losartan Potassium, Zeta Potential.

INTRODUCTION

Losartan Potassium is a highly selective, non-peptide angiotensin II receptor antagonist indicated for the treatment of hypertension [1]. Losartan Potassium available in market as tablet form, following oral administration, bioavailability is approximately 33%, due to extensive first pass metabolism. When given orally it has a bigger disadvantage of hepatotoxicity. The distribution volume of Losartan Potassium is 34 litres. The terminal half-life of Losartan Potassium is 2 hours. Losartan Potassium also causes gastrointestinal disorders, neutropenia, pancreatitis and migraine.

Transdermal drug delivery offers many advantages as compared to traditional drug delivery systems, including oral and parenteral drug delivery system [2,3).Advantages claimed are increased patient acceptability (non invasiveness), avoidance of gastrointestinal disturbances and first pass metabolism of the drug. The traditional transdermal drug delivery systems involve a patch, in which the drug permeates through various layers of skin, via a passive diffusion pathway. However, this limits the basic potential of these systems, as stratum corneum is the most formidable barrier to the passage of most of the drugs, except for highly lipophilic, low molecular weight drugs [5,6]. To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier [7,8,9].

The ethosomes have been well known for their importance in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicles structure for use in better drug delivery within their cavities, which would help to target the vesicle for cell specificity. One of the major advances in vesicle research was the finding a vesicle derivatives, known as an ethosomes [11,12,13].

Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation [14]. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed mainly of phospholipids, (phosphatidylycholine, phosphatidylethanolamine, phosphatidic acid), high concentration of ethanol and water [15,16]. 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 lipid layers [17-20].

The objective of the present work is to develop and characterize ethosomes bearing Losartan Potassium for transdermal delivery so as to avoid extensive first pass metabolism, leading to improved bioavailability, and minimum side effects.

MATERIALS AND METHODS

Losartan Potassium (LP) was obtained as a gift sample from Ipca Laboratories Ltd., Ratlam. Soya lecithin, ethanol and propylene glycol was obtained from Himedia Laboratory Pvt. Ltd. Mumbai (India). Carbopol 934, disodium hydrogen phosphate, potassium dihydrogen orthophosphate, sodium chloride obtained from Loba Chemie Pvt. Ltd. Mumbai (India). All other reagents and solvents were of Analytical grade. Other reagents and solvents were of Analytical grade.

Preparation of Ethosomes

Ethosomes were prepared by “Hot” method with required modification reported by United State Patent – 5, 540, 934 [28]. The ethosomal system prepared here comprised of 50 mg drug (Losartan Potassium), 2-4% (w/v) Soya lecithin, 20-40 % (v/v) ethanol, 20 % (v/v) propylene glycol and water up to 100%. Drug and phospholipid were dispersed in water by heating in a water bath at 40°C until a colloidal solution was obtained. In a separate vessel ethanol and propylene glycol were mixed and heated to 40°C. Once both mixtures reach 40°C, the organic phase was added to the aqueous phase with continuous stirring on magnetic stirrer for 10 minutes. Finally preparation was sonicated by using probe sonicator for time 5 min. 
Characterization of Ethosomes

Vesicle morphology

Optical microscopy

A drop of ethosomal suspension (before sonication) was spread on a slide and covered with cover slip and observed by optical microscopy (LABOMED) and photomicrographs were taken.

Scanning electron microscopy (SEM)

For examination by SEM, one drop of ethosomal system was mounted on a stub covered with clean glass. The drop was spread out on the glass homogeneously. A polaron ES100 sputter coat the samples with gold and the samples were examined under a Philips 505 scanning electron microscope (Philips, Eindhoven, Netherlands) at an accelerating voltage of 20 KV.

Determination of entrapment efficiency

The total volume of the ethosomal suspension was measured and 5ml of this formulation was transferred to 15 ml centrifuge tube. The suspension was diluted with distilled water up to 8 ml. Ethosomes were separated by ultra centrifugation at 15,000 rpm for 2hr (Remi Instruments Ltd., Mumbai). Supernatant and sediment was recovered and their volume was measured.

The unentrapped and entrapped drug contents were analyzed by estimating drug in supernatant by spectroscopic method.

Percentage entrapment efficiency = \( \frac{(T - C) \times 100}{T} \)

Where "C" is amount of drug that is detected in the supernatant layer and "T" is the total amount of drug used in preparation.

Determination of vesicle size and zeta potential

The average diameter, size distribution and zeta potential of all the batches of ethosomes were measured by Particle size analyzer (Malvern Zetasizer) Malvern Instrument Ltd., Model S, Ver.5.03, Malvern, UK.

Preparation of ethosomal gel

Carbopol 934 (0.75% w/v) was soaked in minimum amount of water for an hour. Ethosomal suspensions 20 ml containing Losartan Potassium (100mg) was added to the swollen polymer under stirring. Stiring was continuous at 700 rpm in a closed vessel and maintained at temperature 30°C until homogeous ethosomal gels were achieved. The pH was then adjusted to neutral using triethanol amine and stirred slowly till a gel was obtained.

In-Vitro Drug permeation studies

In-vitro drug permeation of ethosomal gel formulation, plain drug gel formulation and hydroethanolic solution were studied using a Franz glass diffusion cell. The effective release area of the diffusion cell and receptor cell volume was 1 cm² and 10 ml respectively. The receptor compartment contained phosphate buffer saline (pH 7.4) maintained at 37°C±1°C by magnetic stirrer. Pig ear skin was mounted between the donor and receptor compartment. Ethosomal gel containing drug equivalent to 5 mg was applied on the skin surface for any visible change such as erythema (redness) after 24, 48 and 72 hr. of the formulation application.

Draize patch test was used on rats to evaluate the mean erythemal and edema scores. The mean erythemal scores were recorded depending on the degree of erythema: no erythema = 0, very slight erythema (barely perceptible-light pink) = 1, well defined erythema (dark pink) = 2, moderate to severe erythema (light red) = 3 and severe erythema (extreme redness) = 4. The mean edema scores were recorded depending on the degree of edema: no edema = 0, very slight edema (barely perceptible) = 1, slight edema (edges well raise) = 2, moderate edema (raised approx 1mm) = 3 and severe edema (raised more than 1mm) = 4.

Average score = \( \sum \) erythema grade at 72hr + \( \sum \) edema grade at 72hr

No. of subject

Stability Studies

Optimized ethosomal formulation was selected for stability studies of vesicles. The vesicular suspension was stored in amber colored glass bottles at 5±3°C and room temperature for a period of 60 days. Ethosome formulation was evaluated for change in residual drug content and vesicle size at different time intervals, the initial drug concentration of the formulation was taken to be 100%.

RESULTS AND DISCUSSIONS

Ethosomal carrier is a system containing soft vesicles and composed mainly of phospholipids, ethanol in relatively high concentration and water. Optimization of formulation and process variables was done on the basis of vesicle size and entrapment efficiency. Different ethosomal formulations were prepared by hot method using different concentration of lecithin and ethanol in this investigation (Table 1). With phospholipids concentration range of 1-4 % w/v, the size of the vesicles increased with increasing phospholipids concentration, the largest vesicle size in preparation containing 4% w/v phospholipids 31±13 nm (F=4) and smallest in preparation containing 1% w/v phospholipids 179± 06 nm (F=9) were observed during study. The entrapment efficiency of ethosomal preparations was determined by ultracentrifugation method. By increasing the ethanol concentration up to 30% (w/v) the entrapment efficiency also increases and with further increasing the ethanol concentration (>30%w/v), the vesicle membrane becomes more permeable and that may have led to decrease in the entrapment efficiency of ethosomal formulation. Formulation batch F=7 (70.66± 2.2%) exhibited high entrapment efficiency and formulation F=1 (40.02 ± 2.5%) exhibited low entrapment efficiency. The data indicate that both ethanol and lecithin amounts, used for ethosomal preparation influenced the entrapment efficiency of the ethosomal carrier. Formulation code F=7 were found to be optimized, the ethosomal formulation (F=7) prepared with 30% (w/v) ethanol and 3% (w/v) phospholipids show an average vesicle size of 202 ±66 nm and high entrapment efficiency ethosomes was found to be 70.66± 2.2%.

Enhancement ratio (Er) was calculated by dividing the Jss of respective formulation with Jss of control formulation by using the following equation:

Er = \( \frac{J_{ss \text{of formulation}}}{J_{ss \text{of control}}} \)
Table 1: Effect of ethanol and lecithin concentration

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Lecithin (%) w/v</th>
<th>Ethanol (%) v/v</th>
<th>Vesicle size (nm)</th>
<th>Polydispersity Index</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>1</td>
<td>20</td>
<td>193</td>
<td>0.33 ± 0.05</td>
<td>48.82 ± 2.5</td>
</tr>
<tr>
<td>F-2</td>
<td>2</td>
<td>20</td>
<td>211</td>
<td>0.28 ± 0.06</td>
<td>56.50 ± 3.0</td>
</tr>
<tr>
<td>F-3</td>
<td>3</td>
<td>20</td>
<td>267</td>
<td>0.34 ± 0.06</td>
<td>67.35 ± 3.1</td>
</tr>
<tr>
<td>F-4</td>
<td>4</td>
<td>20</td>
<td>312</td>
<td>0.41 ± 0.03</td>
<td>67.97 ± 2.6</td>
</tr>
<tr>
<td>F-5</td>
<td>1</td>
<td>30</td>
<td>185</td>
<td>0.27 ± 0.05</td>
<td>58.77 ± 2.8</td>
</tr>
<tr>
<td>F-6</td>
<td>2</td>
<td>30</td>
<td>196</td>
<td>0.32 ± 0.07</td>
<td>61.16 ± 3.5</td>
</tr>
<tr>
<td>F-7</td>
<td>3</td>
<td>30</td>
<td>202</td>
<td>0.23 ± 0.06</td>
<td>70.66 ± 2.2</td>
</tr>
<tr>
<td>F-8</td>
<td>4</td>
<td>30</td>
<td>255</td>
<td>0.31 ± 0.05</td>
<td>68.35 ± 2.9</td>
</tr>
<tr>
<td>F-9</td>
<td>1</td>
<td>40</td>
<td>179</td>
<td>0.20 ± 0.06</td>
<td>56.02 ± 3.0</td>
</tr>
<tr>
<td>F-10</td>
<td>2</td>
<td>40</td>
<td>185</td>
<td>0.23 ± 0.04</td>
<td>58.66 ± 2.2</td>
</tr>
<tr>
<td>F-11</td>
<td>3</td>
<td>40</td>
<td>195</td>
<td>0.25 ± 0.01</td>
<td>66.16 ± 2.8</td>
</tr>
<tr>
<td>F-12</td>
<td>4</td>
<td>40</td>
<td>240</td>
<td>0.34 ± 0.04</td>
<td>68.03 ± 2.0</td>
</tr>
</tbody>
</table>

(*50 mg drug and 20% v/v propylene glycol kept constant) Values represented as mean ± SD, n=3

Formulation code F-7 were found to be optimized, the ethosomal formulation (F-7) prepared with 30%(w/v) ethanol and 3% (w/v) phospholipids showed an average vesicle size of 202 ±06 nm and high entrapment efficiency ethosomes was found to be 70.66± 2.2%.

Vesicle Morphology Study

Optical and Scanning Electron Microscopy

To confirm the presence of vesicular structure, formulations were visualized under optical microscope at different magnified fields, which showed presence of spherical structure of vesicles (Figure 1). Further investigation of formulation by SEM showed presence of lipid bilayer, spherical structure of vesicles with a smooth surface (Figure 2).

Vesicle Size and Zeta Potential

The vesicle size, size distribution and zeta potential of formulation was determined by light scattering method Malvern Zetasizer (Malvern Instrument Ltd., Model S., Malvern, UK). This investigation showed that with lecithin concentration range of 1-4 % w/v, the size of the vesicles increased with increasing phospholipids concentration, the largest particles in preparation containing 4% w/v phospholipids 312±13 nm (F-4) and smallest in preparation containing 1% w/v phospholipids 179± 06 nm (F-9) were observed during study (Table 1). Size distribution curve (Figure 3) confirms the normal size distribution of the vesicles of optimize batch.

Fig. 1: Photomicrographs of ethosomes (before sonication) observed by optical microscopy (magnification 100X)

Fig. 2: Photographs of ethosomes observed by SEM
The charge of the ethosomal vesicles is an important parameter that can influence both vesicular properties such as stability, as well as skin-vesicle interactions. Ethanol causes a modification in net charge of the system and confers it some degree of stearic stabilization that may lead to decrease in mean vesicle size [18]. Zeta potential of optimize formulation F-7 was found to be -48.5±2 mV which shows good stability of formulation.

**In-Vitro Drug Permeation Studies**

The in-vitro drug permeation of various formulations was performed in phosphate buffered saline (pH 7.4). The cumulative drug permeation (mg/cm²) of the ethosomal gel was 3.632±0.338 mg/cm² in 24 hrs, which is greater than that of plain drug gel and hydroethanolic solution which showed 0.947±0.139 mg/cm² and 1.247±0.145 mg/cm² in 24 hrs respectively (Table 2). Ethanol provides the vesicles with soft flexible characteristics which allow them to more easily penetrate into deeper layers of the skin. Propylene glycol also acts as permeation enhancer, which increases permeability of vesicle through biological membrane due to synergistic effect with ethanol on bilayer of the vesicles.

Transdermal flux for different formulations across pig ear skin was calculated. The flux from ethosomal gel (0.194±0.12 mg/cm²/h) was 4-fold higher than that obtained after application of plain drug gel (0.048±0.05 mg/cm²/h), and 2.7-fold higher than that of the hydroethanolic solution of drug (0.071±0.07 mg/cm²/h) (Table 2). Data indicated that the ethosomal system was more effective in delivering losartan potassium than hydroethanolic solution alone or plain drug gel. Furthermore, better permeation of losartan potassium from ethosomes than from ethanol alone suggested some kind of synergistic mechanism between ethanol, vesicles, and skin lipids.

### Table 2: Permeability parameters of different formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Transdermal flux (J0) (mg/cm²/h)</th>
<th>Enhancement ratio (Er)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethosomal gel</td>
<td>0.194±0.12</td>
<td>4.04</td>
</tr>
<tr>
<td>Hydroethanolic solution</td>
<td>0.071±0.07</td>
<td>1.47</td>
</tr>
<tr>
<td>Plain drug gel</td>
<td>0.048±0.05</td>
<td>-</td>
</tr>
</tbody>
</table>
Skin Irritation Studies

A primary skin irritation test of ethosomal gel on rat was studies. No signs of erythema and edema were observed on the skin of albino rats after 72 hr. Irritation score (primary skin irritation index) for ethosomal gel was zero which indicated its safety and acceptability (Table-3).

Stability studies

The stability study was performed on optimized losartan potassium loaded ethosomal formulations which was evaluated for change in residual drug content and vesicle size at different time intervals at various temperatures [refrigeration (5±3°C) and room temperature (25±2°C)]. Data of the stability study indicate that there was no significant change in residual drug content and vesicle size of ethosomal formulation (Table-4&5). Ethanol provides a net negative surface charge, which avoids aggregation of vesicles due to electrostatic repulsion [23]. This finding indicated good storage stability of ethosomal formulation. The results shows that formulation store at refrigerated conditions was more stable than room temperature because greater drug loss was observed from the formulation store at room temperature (Table 4), which might be ascribed to the effect of temperature on the gel to liquid transition of lipid bilayers together with possible chemical degradation of the phospholipids, leading to defects in the membrane packing.

CONCLUSION

The presence of ethanol in the aqueous compartment of the ethosomal vesicles favored the encapsulation of Losartan Potassium and enhanced its permeation via the pig ear skin because of the synergistic effect of ethanol, vesicles, and skin lipids. The data indicate that the entrapment efficiency depends on ethanol concentration. By increasing the ethanol concentration up to 30% (w/v) the entrapment efficiency increases. Data of the stability study indicate that there was no significant change in residual drug content and vesicle size of ethosomal formulation. Ethanol provides a net negative surface charge, which avoids aggregation of vesicles due to electrostatic repulsion. This indicates good storage stability of ethosomal formulation. The result of in vitro drug permeation studies of different formulation showed transdermal flux of ethosomal gel (0.19±0.12 mg/cm²/h) was 4-fold higher than that obtained after application of plain drug gel (0.048±0.07 mg/cm²/h), and 2.7-fold higher than that of the hydroethanolic solution of drug (0.071±0.05 mg/cm²/h). Data of in vitro drug permeation studies indicated that the ethosomes are a very promising carrier for the transdermal delivery due to the enhanced delivery of drug through the skin.

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REFERENCES


