The present work deals with the preparation of Glimeperide (GP) ethosomes and study of effect of alcohol and phospholipid on systemic as well as topical delivery of drug. Glimepride loaded ethosomes were prepared by cold method by using different concentrations of alcohol (20-30% w/w) and soya lecithin (3.5-4% w/w) in different ratios and propylene glycol (10% w/w). In the present work ethanol and isopropyl alcohol both alcohol are used at different concentration. Total eight formulations (F1-F8) of ethosomes were prepared. They were evaluated for FT IR, vesicular shape, size, entrapment efficiency, turbidity, zeta potential, \textit{In-vitro} and stability studies. Vesicle size and of the ethosomal formulation was found to be range between 22 to 105 nm and the Entrapment efficiency for Glimepiride in ethosomal formulation in ethanol was found to be 47.91±0.3 to 58.4±0.3 and in Isopropyl alcohol was found to be 43.4±0.5 to 57.6±0.5 % respectively. FT-IR and zeta potential studies revealed the integrity of the drug in the formulations. The cumulative percentage of drug release of prepared formulation F1 to F8 was in range 34.34±0.01% to 49.01±0.03% respectively at end of 24 h. The value for drug permeation (release) for optimized formulation F4 and F8 through the egg membrane after 24 h was found to be 49.01±0.03% and 47.03±0.02%. Stability studies indicated that, the prepared ethosomes remained stable at refrigeration (4±2°C) and room (25±2°C) temperature. The prepared ethosomes showed promising results under \textit{in-vitro} conditions.
INTRODUCTION:
Diabetes mellitus is a heterogeneous group of metabolic disorder characterized by chronic hyperglycemia with disturbance of carbohydrate, protein and fat metabolism. There are two major types of diabetes mellitus - Type 1: Insulin dependent diabetes mellitus (IDDM) and Type 2: Non-insulin dependent diabetes mellitus (NIDDM) [1].

Glimepride is a sulfonyl urea used to treat type–II diabetes mellitus. Molecular formula of glimepiride is C_{24}H_{34}N_{4}O_{5}S with a molecular mass of about 490.617g/mol [2]. It belongs to class –II of Biopharmaceutical classification system. It is completely insoluble in water, acidic media and slightly soluble in various buffers and organic solvents [3]. The drug shows low, pH dependent solubility. In acidic and neutral aqueous media, Glimepiride exhibits very poor solubility at 37°C (<0.004 mg/mL). In media pH>7, solubility of drug is slightly increased to 0.02 mg/mL. This poor solubility may cause poor dissolution and unpredicted bioavailability. Glimepiride is a white powder and is insoluble in water, soluble in dimethylformamide, and slightly soluble in methylene chloride and methanol [4].

A number of techniques and efforts have been targeted to weaken and disrupt the highly organized intercellular lipids in an attempt to improve drug transport across the whole skin or to increase the permeation of drugs across this skin barrier. The soft, malleable vesicles adapt for superior delivery of active agents. To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers, such as sonophoresis, iontophoresis, etc [5]. To overcome problems of poor skin permeability Cevc et al. [6] and Touitou et al. [7] recently introduced two new vesicular carrier systems transfersomes and ethosomes, respectively for non-invasive delivery of drugs into or across the skin. Transfersomes and ethosomes incorporated edge activators (surfactants) and penetration enhancers (alcohols and polyols), respectively, to influence the properties of vesicles and stratum corneum. One of the major advances in vesicle research was the finding a vesicle derivatives, known as ethosomes [8]. Permeation enhancers increase the permeability of the skin, so that the drugs can cross through the skin easily. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes. Ethosomes (Fig. 1) are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to microns (µ) [9].

![Structure of Ethosome](Fig 1: Structure of Ethosome)

Ethosomal drug delivery is noninvasive and delivers the drug to the deep skin layers or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents [10]. Ethosomes are the modified forms of liposomes that are high in ethanol content (Fig. 1). The high concentration of ethanol makes ethosomes unique because ethanol causes disturbance of skin lipid bilayer organization, hence when incorporated into a vesicle membrane, it enhances the vesicle’s ability to penetrate the stratum corneum [11]. Skin acts as a major target as well as a principal barrier for topical/transdermal drug delivery. Ethosomes have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier [12].
MATERIALS AND METHODS

Materials: Glimeperide was obtained as gift sample from Dr. Reddy’s laboratories, Hyderabad, India. Soya lecithin was purchased from Jeetraj corporation, Allahabad, India. Ethanol, Isopropyl alcohol, and Propylene glycol was used of analytical grade.

METHODS

Preparation of ethosomes of Glimeperide: The ethosomal formulation was prepared according to the method reported by the Touitou et al (2000) with slight modification. The ethosomal system prepared here comprised of 3.5-4% phospholipids, 20-30% ethanol, 20-30% isopropyl alcohol, drug, 10% propylene glycol and water to 100% w/w.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Phospholipid (% w/w)</th>
<th>Ethanol (% w/w)</th>
<th>Isopropyl alcohol (% w/w)</th>
<th>Propylene glycol (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>3.5</td>
<td>20</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>F2</td>
<td>4</td>
<td>20</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>F3</td>
<td>3.5</td>
<td>30</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>F4</td>
<td>4</td>
<td>30</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>F5</td>
<td>3.5</td>
<td>-</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>F6</td>
<td>4</td>
<td>-</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>F7</td>
<td>3.5</td>
<td>-</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>F8</td>
<td>4</td>
<td>-</td>
<td>30</td>
<td>10</td>
</tr>
</tbody>
</table>

CHARACTERIZATION OF ETHOSOMAL FORMULATION

Fourier transform-infrared ray spectroscopy (FT-IR) Studies
The interaction studies between drug, phospholipid and formulations (F4 and F8) were studied using FT-IR spectroscopy.

Vesical size and size distribution
Vesical size and size distribution were measured by using Malvern Zetasizer (Malvern Instruments) equipped with a 4.0 mW He-Ne red laser (633 nm).

Optical microscopy
A drop of ethosomal suspension before sonication was spread on a slide and covered with cover slip. Then it was observed by optical microscope and photomicrographs were taken.

Vesicles morphology
Ethosomes vesicles were visualized using Transmission Electron Microscopy (TEM). TEM was done by using Tecnai 12, 120 KV, FEI Company, Eindhoven, Netherlands. Selected formulations (1 mL) were diluted with 500 mL of phosphate buffer of pH 7.4. A drop of emulsion was spread on a copper grid coated with carbon film and excess droplets were instantly removed using a filter paper. After a while, a drop of 2% (w/v) of phosphotungstic acid solution was dripped on the copper grid for about 60 sec and excess solution was removed. Then the grid was dried in the air at room temperature before loading in the microscope.

Turbidity and zeta potential determination
Turbidity of all ethosomal vesicular suspensions was measured by Digital Turbidity Meter. In this method, 500 NTU (Nephelometric Turbidity Units) range is set. Then zero reading is set with Millipore water. After this, formulation is transferred to glass cuvettes of capacity 50 mL and placed in the holder inside the instrument. The method is repeated for each formulation and measurement of turbidity is displayed on the screen and expressed as (NTU). Zeta potential of the vesicles was determined using Malvern Zetasizer (Malvern Instruments) equipped with a 4.0 mW He-Ne red laser (633 nm). For measurement of zeta potential 1 mL of each formulation was diluted with milli Q water (100 mL).

Entrapment efficiency
The entrapment efficiency of the vesicles was determined by ultracentrifugation method (Micro...
Refrigerated Centrifuge) one ml of the formulation was centrifuged at 4°C at 6000 rpm for 1 h.

**In-vitro drug release through egg membrane**
The *In-vitro* skin permeation of glimeperide from ethosomal formulation was studied using egg membrane. The *In-vitro* diffusion of the drug through semipermeable membrane was performed. Suitable size of egg membrane was cut and was kept in saline solution for 1 h before dialysis to ensure complete wetting of the membrane. It was clamped carefully to one end of the hollow glass tube of (dialysis bag). This acted as donor compartment. One ml of drug loaded vesicles was placed in the dialysis bag, which was then transferred into 50 ml of phosphate buffer saline (PBS) (pH 6.8) was taken in a beaker which was used as a receptor compartment. The receiver medium was stirred with a magnetic stirrer which is thermostatically controlled. Sample was withdrawn after 0.5, 1, 1.5, 2, 3, 4, 6, 12 and 24 h time intervals and replace with equal volumes of PBS.

**Stability studies**
Stability testing of drug products begins as a part of drug discovery and ends with the commercial product. To assess the drug and formulation stability, stability studies were done. The stability studies were carried out for the most satisfactory formulations F4 and F8. The formulation was kept in a borosilicate container to avoid the interaction between ethosomal formulation and glass of container and kept at 4±2°C and at 25±2°C for 2 months. At the end of 2 months, the sample was analyzed for the entrapment efficiency percentage, and *In-vitro* diffusion study.

**RESULT AND DISCUSSION**

Fourier Transform-Infrared Ray Spectroscopy Studies (FT-IR) Studies:
Drug polymer compatibility studies were carried out using FT-IR spectroscopy to establish any possible interaction of Glimeperide with the excipients used in the formulation. The *FT-IR* spectra of pure drug, excipients and optimized formulation were shown in (Fig: 2A, 2B, 2C, 2D). The data shows that pure drug has 1238.76, 1689.29, 3281.89, 1712.53, 2927.74 wave number as major peaks. The results indicated that mixture of pure drug and excipients has no major change in the position of peaks. This shows that there is no possible interaction between drug and excipients.
Figure 2: FT-IR Spectra of 2A) Glimeperide, 2B) Soya Lecithin, 2C) F4, 2D) F8

Vesicle size:
The vesicle size of ethosomes ranges from 22 to 105 nm. On increasing the concentration of phospholipid from 3.5 to 4% w/w, the size of vesicle was increased and on increasing the concentration of ethanol and isopropyl alcohol from 20 to 30% w/w, the size of vesicle decreases. From F1 to F8 the size of vesicle increases on increasing the concentration of phospholipid (Table 2). From F1 to F4 in ethanol and F5 to F8 in isopropyl alcohol shows that on increasing the concentration of ethanol and isopropyl alcohol from 20 to 30% w/w the size of vesicle decreases (Fig. 3). This may be due to the formation of a phase with interpenetrating hydrocarbon chain. The decrease in particle size is due to the ethanol and isopropyl alcohol causing a modification of the net charge of the system and some degree of steric stabilization.
Fig. 3: Size Distribution of 3A) F4, 3B) F8

Vesicle Morphology:

The vesicle shape of all the ethosomal formulations was found to be spherical with the smooth surface as shown in the Fig. 4.

Fig. 3B

Fig. 4: Photomicrograph of 4A) F4, 4B) F8, and TEM of 4C) F4, 4D) F8

Turbidity and zeta potential determination:

The turbidity of ethosomal formulation in ethanol and isopropyl alcohol was found to be in the range of 60±0.1 to 110±0.05 and 102±0.20 to 171±0.20 NTU (Fig. 5). The data shows that on increasing the
ethanol concentration from 20 to 30% w/w the turbidity increases from F1 to F8. Zeta potential measurement study was observed as shown in the Table 2.

Table 2: Characteristic Parameters of Ethosomal Formulation

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Formulation code</th>
<th>Vesicle Size(nm)</th>
<th>Zeta Potential</th>
<th>Turbidity</th>
<th>% Entrapment Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>53±2.5</td>
<td>-40.6±0.5</td>
<td>78±0.15</td>
<td>47.91±0.3</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>72±2.0</td>
<td>-41.6±1.5</td>
<td>60±0.1</td>
<td>55.3±0.7</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>22±2.0</td>
<td>-22.3±2.0</td>
<td>110±0.05</td>
<td>49.27±0.7</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>40±2.5</td>
<td>-82.3±2.0</td>
<td>92±0.11</td>
<td>58.46±0.3</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>99±2.3</td>
<td>-21.6±1.5</td>
<td>139±0.11</td>
<td>43.4±0.5</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>105±1.5</td>
<td>-24.6±0.5</td>
<td>102±0.20</td>
<td>51.88±0.7</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>30±2.5</td>
<td>-21.6±1.5</td>
<td>171±0.20</td>
<td>45.19±0.8</td>
</tr>
<tr>
<td>8</td>
<td>F8</td>
<td>101±1.1</td>
<td>-25.6±2.0</td>
<td>141±0.20</td>
<td>57.63±0.5</td>
</tr>
</tbody>
</table>

**Entrapment efficiency:**
The entrapment efficiency for glimepiride in ethosomal formulation in ethanol was found to be 47.91±0.3% to 58.4±0.3% and in isopropyl alcohol was found to be 43.4±0.5% to 57.6±0.5% respectively. Increasing the concentration of ethanol and isopropyl alcohol from 20% to 30% increased the entrapment efficiency owing to increase in fluidity of membranes. The entrapment efficiency of isopropyl alcohol was found to be less as compared with ethanol. The different concentration of ethanol, isopropyl alcohol and phospholipid on the ethosomal preparation influenced the size of vesicles. The data shows that the entrapment efficiency of vesicle increases on increasing the concentration of ethanol and isopropyl alcohol from 20 to 30% w/w and phospholipid concentration 3.5 to 4% w/w (Fig. 6).
**In-vitro drug release through egg membrane**

The In-vitro drug release of Glimeperide ethosomes was done for 24h in Egg membrane in PBS 6.8 pH at 37ºC. The cumulative percentage of drug release of prepared formulation F1 to F8 was in range 34.34±0.01% to 49.01±0.03% respectively at end of 24h (Figure 7). The value for drug permeation (release) for optimized formulation F4 and F8 through the egg membrane after 24 hrs was found to be 49.01±0.03% and 47.03±0.02%. There was increase in the amount of drug release with the gradual increase in concentration of ethanol and isopropyl alcohol from 20 to 30 % w/w. Among all the formulations F4 was found to be the best formulation as drug release was 49.01±0.03% in 24 hrs. This may be due to the effect of ethanol, which acts as penetration enhancer and more effective than isopropyl alcohol.

**Stability studies**

The stability study was done on most satisfactory formulation F4 and F8 at 25± 2°C and 4 ± 2°C for period of 2 months and was analyzed for drug release and entrapment efficiency at the end of 2 month. The residual drug release of F4 and F8 was found to be 47.23±0.02% and 44.33±0.02 % up to 24 hours respectively at 25± 2°C (Fig. 8). The drug release of F4 and F8 was found to be 47.68±0.03% and 45.72±0.04% respectively at 4± 2°C (Fig. 9). The entrapment efficiency of drug of F4 and F8 was found to be 58.23±0.45% and 57.29±0.46 % at 25°C.
whereas at 4°C it was found to be 58.39±0.40% and 57.44±0.49% (Fig. 10 and 11). The results showed that there is not much significant difference in entrapment efficiency and *in vitro* of drug for the period of 2 months. It was observed that the ethosomal system was more stable at 4 ± 2°C showed in (Table 3).

**Table 3: Stability studies of Glimeperide Ethosomal Formulation**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>25±2°C for 2 months</th>
<th>4±2°C for 2 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>In vitro</em> drug release (%)</td>
<td>Drug entrapment efficiency (%)</td>
</tr>
<tr>
<td>F4</td>
<td>47.23±0.02</td>
<td>58.23±0.45</td>
</tr>
<tr>
<td>F8</td>
<td>44.33±0.02</td>
<td>57.29±0.46</td>
</tr>
</tbody>
</table>
CONCLUSION
In this study, total eight ethosomes of Glimeperide by cold method and were prepared and evaluated for different parameters. On the basis of different parameters like vesicle shape, vesicle size and entrapment efficiency, F4 was selected as the best formulation. Thus F4 showed maximum release. The results showed the potential of ethosomes of being a safe and very efficient drug carrier for systemic as well as topical delivery of drug.

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REFERENCES

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