Introduction

The purpose of preparing Self microemulsifying drug delivery system in this work is to enhance the solubility and oral bioavailability of poorly water soluble drug, Glimepiride. SMEDDS are the isotropic mixture of surfactant, co-surfactant and oil incorporated with drug. In the aqueous media, gastrointestinal motility emulsification takes place. Glimepiride was undergone solubility studies in various surfactants, co-surfactants and oils. Pseudo ternary titrations were done using surfactants/co-surfactants and oil in different ratios against water. By these titrations pseudo ternary phase diagrams are constructed through which self emulsifying region are identified Cremophor EL and Transcutol as surfactant and co-surfactant with sunflower oil are identified as optimized SMEDDS formulation for Glimepiride. FTIR analysis was done for investigating the drug excipient compatibility. Poorly water soluble new drug candidates are approximately 40%, whose oral delivery is frequently associated with implications of low bioavailability, high intra and inter subject variability and lack of dose proportionality. These drugs come under hydrophobic nature which is often dissolved in SMEDDS.

Keywords: SMEDDS, Glimepiride, Cremophor EL, Transcutol, Phase diagrams.
1. Introduction

One of the modern oral hypoglycaemic agent is Glimepiride (1-[[P-2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamide)ethyl phenyl]sulfonyle]-3-(trans-4-methylcyclohexyl)urea Which belongs to the group of sulfonyl derivatives which is used for the treatment of non-insulin dependent diabetes mellitus (NIDDM) to achieve exact control of blood glucose level. Instead Glimepiride shows the principal mechanism of action with other drugs of this class like the stimulation of insulin secretion from pancreatic β-cell, it has multiple pharmacological benefits (1,2,3). Glimepiride is the first III generation sulphonyl urea. It is a very potent sulphonyl urea with long duration of action. Chemical formula of Glimepiride drug is C24H34N4O5S.

Glimepiride is a poorly water soluble and highly permeable active pharmaceutical product belonging to BCS class II (4). To improve the solubility, chemical stability and oral bioavailability of several poorly water soluble drug, self microemulsifying drug delivery systems have been successfully emerged in recent years. The SMEEDS are isotropic mixtures of oils, surfactants, co-surfactants incorporated with drug these SMEEDDS mostly forms oil-in-water microemulsions whose size would be less than 100nm (i.e. <100nm-50nm). This droplet size can be measured when this microemulsion undergoes gastrointestinal tract (5,6). The large interfacial surface area is provided due to small droplet size of microemulsion which is useful for drug release and absorption (7,8).

The purpose of the work done was to develop a SMEDDS formulation of Glimepiride to improve its oral bioavailability. Glimepiride was tested for its solubility behavior in various vehicles and an optimized SMEDDS containing Glimepiride was formulated. To evaluate the improved solubility and dissolution profile of Glimepiride loaded SMEDDS in comparison with 2mg glimepiride marketed tablet, dissolution was performed.

The pharmacokinetics of Glimepiride loaded SMEDDS and Glimepiride suspension after oral administration in male wistar rats had been characterized and compared in order to evaluate the increased bioavailability of formulated SMEDDS. The plasma concentration of formulated SMEDDS and pure drug suspension was simultaneously determined by a modified HPLC method.

The solubility of Glimepiride in various oils, surfactants and co-surfactants was measured respectively. An excess amount of Glimepiride (50mg) was added into each vehicle and stirred continuously for 48 hours at 37°C under Orbital shaking incubator (Remi electrotechnic ltd) to facilitate solubilisation. The mixtures were centrifuged at 12,000 rpm for 20 mins after equilibrium was achieved. The supernatant was diluted appropriately and the concentration of Glimepiride in the solution was assayed by U.V spectroscopy at 230nm.

Table 1: Formulations of Glimepiride SMEDDS.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
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</thead>
<tbody>
<tr>
<td>% Cremophor EL</td>
<td>54.1</td>
<td>50.8</td>
<td>51.6</td>
<td>55.4</td>
<td>50.5</td>
<td>55.2</td>
<td>54.4</td>
<td>56.2</td>
<td>56.1</td>
<td>54.7</td>
</tr>
<tr>
<td>% of transcutol</td>
<td>27</td>
<td>30.4</td>
<td>25.2</td>
<td>22</td>
<td>23.9</td>
<td>17.6</td>
<td>17.2</td>
<td>32.5</td>
<td>12.3</td>
<td>11.3</td>
</tr>
<tr>
<td>% of sunflower oil</td>
<td>18</td>
<td>18.6</td>
<td>23</td>
<td>22.4</td>
<td>10.5</td>
<td>27.1</td>
<td>28.3</td>
<td>31.5</td>
<td>31.4</td>
<td>33.9</td>
</tr>
</tbody>
</table>
Characterization for Glimepiride loaded SMEDDS:
Droplet size analysis studies, zeta potential, TEM, SEM, self emulsification time, visual observation, cloud point measurement, FTIR, invitro drug release studies, exvivo drug release studies were carried out for Glimepiride loaded SMEDDS.

Determination of self emulsifying time:
The foremost means of self microemulsification assessment is through visual evaluation. 100ml water and 0.1N Hcl solution is taken as medium which is stirred under magnetic stirrer with 100rpm at 37±0.5°C and pour 1ml of formulated SMEDDS into the medium and the contents being mixed gently at 100rpm and determining the time required to form microemulsions upon dilution of SMEDDS with water (10,11).

Visual observation, phase separation:
200ml of distilled water is taken in a beaker and each Glimepiride loaded SMEDDS formulation was dropped into water and this is maintained at 37°C, and the diluted preparation was vortexed for 1min. This preparation was stored for 24 hours and then phase separation and precipitation can be observed visually. Mixtures exhibiting a negligible phase separation during the 2 hr period were used for subsequent studies. It gives the information about stability and viability of the formed microemulsion (12).

Cloud point measurement:
The formulated SMEDDS was diluted with 50ml water in a beaker which is placed on a water bath with gradually increasing temperature until the diluted formulation turned cloudy. It mainly insists about the stability of microemulsion at body temperature (13).

Droplet size analysis(Zeta sizer):
The Glimepiride SMEDDS was diluted with distilled water and stirred slowly under magnetic stirrer to mix thoroughly. Zeta potential was used to determine the droplet size of resulting microemulsion. Size of droplet is measured by photon correlation using Malvern zeta sizer.

Transmission electron microscopy(TEM):
SMEDDS was diluted with distilled water 1:30 and mixed by gentle shaking. Copper grids are allowed to stand on for 60 seconds on which one drop of sample obtained after dilution was deposited. Filter paper is used to remove excess fluid and then the grid was stained in 1% phosphotungstic acid solution for 30 seconds. By following the above method which is transmission electron microscopy (TEM) the morphology of Glimepiride loaded SMEDDS was provided by giving information on the porosity and microstructure.

Fourier transform infra red spectroscopy(FTIR):
Fourier transform infrared studies for SMEDDS can be known taking spectra of FT-IR. (Bruker-alpha T)FTIR is used to determine any type of chemical interactions between the drug and excipients (15).

Invitro drug release studies:
Invitro drug release studies were obtained using USP dissolution apparatus typeII. 900ml of 6.8 pH phosphate buffer was placed in the dissolution vessel and the SMEDDS formulation was filled into hard gelatin capsule and placed in the dissolution medium and was stirred at 50rpm at 37°C. 5ml of samples were withdrawn at pre determined time intervals of 5,10,20,30,40,50,60,70,80 and 90 minutes. And the drug concentration was determined under UV spectrophotometer at 230nm wavelength. The withdrawn volume of samples was replaced by fresh dissolution medium every time. Cumulated released amounts were plotted as a function of time(16).

Ex vivo intestinal diffusion studies:
The institutional animal ethics committee approved protocols were followed, male wistar rats were sacrificed and intestine was collected and used for diffusion study. The intestine was suspended in phosphate buffer solution and studies were done for 8 hrs, with sampling at intervals sufficient to estimate the amount of drug diffused by using U.V. spectrophotometer.

3. Results & Discussion
Solubility of the drug plays a vital role in the formulation of SMEDDS. Glimepiride was solubilised in various oils, surfactants and co-surfactants whose results were given below in the graph. Glimepiride drug was highly solubilised in sunflower oil, Cremophore EL and Transcutol as shown in figure 1. Phase diagrams were constructed to obtain optimized concentrations of oil, surfactant and co-surfactant in the presence of Glimepiride and microemulsifying region is identified. Water/Sunflower oil/cremophore EL/Transcutol was shown as investigated as a quaternary system of pseudo ternary diagram which is shown in Fig 2. Formation of the emulsion was done under normal room temperature. As seen in the diagrams maximum microemulsion area was observed for phase diagrams involving sunflower oil, cremophor EL and trancutol in the ratio 2:1, hence the formulations were planned using different points in this area.
In the aqueous media, due to gastro intestinal motility self emulsification takes place. Six formulations are subjected to self emulsifying time test in which all the six has showed their tendency for emulsification in 0.1N HCL and phosphate buffer(6.8) in seconds which were found to be optimized. The time taken for self emulsification is shown in Table 2. Optical clarity of the selected six formulated SMEDDS were seen in 0 hours and after 24 hours. The values obtained both times were seen to be very near. The values obtained for optical clarity were shown in Table 3. No phase separation, no precipitation and clear solution of Glimepiride SMEDDS were obtained. The cloud point for the formulations was observed to be 75-800C confirming the stability of formulation at body temperature.

Table 2: Self emulsification times of SMEDDS formulation.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1N HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emulsification time(secs)</td>
<td>23</td>
<td>28</td>
<td>29</td>
<td>45</td>
<td>67</td>
<td>26</td>
</tr>
<tr>
<td>Tendency for emulsification</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Phosphate buffer(6.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emulsification time(secs)</td>
<td>20</td>
<td>31</td>
<td>34</td>
<td>50</td>
<td>61</td>
<td>22</td>
</tr>
<tr>
<td>Tendency for emulsification</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>

Table 3: Optical clarity values of SMEDDS formulation.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>F1</th>
<th>F4</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
</tr>
</thead>
<tbody>
<tr>
<td>0Hours</td>
<td>0.756</td>
<td>0.234</td>
<td>0.456</td>
<td>0.467</td>
<td>0.045</td>
<td>0.225</td>
</tr>
<tr>
<td>24Hours</td>
<td>0.786</td>
<td>0.245</td>
<td>0.467</td>
<td>0.471</td>
<td>0.047</td>
<td>0.220</td>
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</tbody>
</table>

Four formulated SMEDDS were subjected to droplet analysis, zeta potential and poly dispersity index. Partical size of the SMEDDS should be <100nm which was obtained for four formulations. Zeta potential values obtained are negative due to the presence of oil droplets which seems to be good as observed in values depicted in table 4 and fig 3.

Table 4: Droplet size, zeta potential and zeta sizer values of SMEDDS.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Particle Size(nm)</th>
<th>Zeta Potential(V)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FG7</td>
<td>55</td>
<td>-15.2</td>
<td>0.240</td>
</tr>
<tr>
<td>FG8</td>
<td>19.43</td>
<td>-19.8</td>
<td>0.332</td>
</tr>
<tr>
<td>FG9</td>
<td>176.1</td>
<td>-22.3</td>
<td>0.222</td>
</tr>
<tr>
<td>F10</td>
<td>62.32</td>
<td>-19.4</td>
<td>0.176</td>
</tr>
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</table>
The TEM and SEM photographs show the surface morphology of the optimized SMEDDS as seen in fig 4 & 5, the nanosized droplets as discreet particles can be seen in the TEM analysis and SEM photograph is evidence to show that the adsorption onto solid carrier was good as no oil droplets are visible. The FTIR spectra of the pure drug and solid SMEDDS formulation show almost similar peaks in some areas to prove absence or almost no interaction between the drug and formulation as seen in fig 6 & 7. The invitro drug release studies is observed in fig 8, the optimized formulation is seen to release 99.9% by 45 min as compared to marketed formulation which released only 60% at the end of one hour, this can be due to the high concentration of surfactants used in the formulation which has reduced the particle size of the drug to nano range hence improving the dissolution. The intestinal diffusion results can be seen in fig 9 and as seen in the drug diffusion profiles the pure drug has diffused to a very small extent of 33.6 at the end of 6 hrs whereas the SMEDDS formulation has released 80% at the end of study period.
4. Conclusion
In conclusion the present research was mainly aimed at improving the solubility and bioavailability of otherwise poorly soluble BCS class II drug glimepiride. The phase solubility studies and the phase diagrams were helping tools in designing the optimized formulation, which was evaluated for various tests and proved to be satisfying the required parameters for effective drug dissolution and diffusion. Thus this formulation can be further tested in-vivo in animals to prove the improvement in bioavailability of the anti diabetic drug.

5. References
3. Petra kovarikova, Jiri klimes, Jiri dohnal, Lucie tisovska. HPLCstudy of glimepiride under hydrolytic stress conditions; Journal of
pharmaceutical and biomedical analysis, 2004, 36: 205-209.


