Design and optimization of chlordiazepoxide solid self-microemulsifying drug delivery system

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ABSTRACT

The objective of the present study was to design and optimized chlordiazepoxide solid microemulsifying drug delivery system prepared via spray drying for oral administration. Four formulations were selected from pseudoternary phase diagram (ethyl oleate-labrasol+cremophor RH40-water) of highest microemulsion region and exposed to spray drying. Reconstitution properties (dilution studies, globule size and zeta potential) and solid state characterization (PXRD, DSC and SEM) of formulations were investigated. The dissolution characteristics of solid SMEDDS and commercial formulation (Librium®) were indicated statistically significant drug release in discriminating dissolution medium. Thus, this solid SMEDDS may provide useful solid dosage form to improve solubility and dissolution rate of chlordiazepoxide and concomitantly bioavailability.

Key words: Chlordiazepoxide, self microemulsifying, solid SMEDDS, spray drying.

INTRODUCTION

Oral route is the easiest and most convenient way of non-invasive administration. Oral drug delivery systems being the most cost effective have always contributing major role in the worldwide drug delivery market. Approximately 40% of new drug candidates are frequently associated with low oral bioavailability, high intra- and inter-subject variabilility, and a lack of dose proportionality. Often poor aqueous solubility and dissolution rate in water rather than permeability though gastrointestinal epithelial barrier associated with low oral drug bioavailability. To overcome this barrier, various formulation strategies are exploited including the use of surfactants, lipids, permeation enhancers, micronisation, salt formation, cyclodextrins, nanoparticles, liposomants and solid dispersions12. Recently, much attention has been paid to lipid-based formulations with particular emphasis on self-microemulsifying drug delivery systems (SMEDDS) after the commercial success of Sandimmune Neoral16 (Cyclosporine A), Fortovase (Saquinavir) and Norvir (Ritonavir) to improve oral bioavailability of lipophilic drug17. SMEDDS are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactnants, or alternatively, one or more hydrophilic co-solvent and co-surfactants or surfactants that have a unique ability of forming fine oil-in-water (o/w) microemulsion upon mild agitation followed by dilution in aqueous media, such as GI fluids18. The digestive motility of stomach and intestine provides the agitation required for self-emulsification in vivo. SMEDDS offer advantages over emulsion are thermodynamic stability, globule size (surface area), flexibility of reconstitution properties (dilution studies, globule size and zeta potential) and solid state characterization (PXRD, DSC and SEM) of formulations were investigated. The dissolution characteristics of solid SMEDDS and commercial formulation (Librium®) were indicated statistically significant drug release in discriminating dissolution medium. Thus, this solid SMEDDS may provide useful solid dosage form to improve solubility and dissolution rate of chlordiazepoxide and concomitantly bioavailability.

2. MATERIAL AND METHODS

2.1 Materials

Chlordiazepoxide was gift sampled from Centaur chemicals Pvt. Ltd., Goa. Captex 355 and 800 were procured from Abitech corporation, USA. Labrasol, maisine 1-35, capryol 90 were obtained from Gattefosse Pvt. Ltd., Mumbai. Cremophor RH40 was obtained from Libraw pharma, New Delhi. Ethyl oleate, Tween80, PEG400, Maltodextrin and Methanol (HPLC grade) were purchased from Lotachemie Pvt. Ltd., Mumbai. All other chemicals and reagents were used as per analytical grade.

2.2 Methods

2.2.1 Saturation solubility studies for screening of excipients

In order to find out appropriate solvents with good solubilizing capacity of CDP, the saturation solubility of CDP was investigated in some oils and surfactants by shake flask method. An excess amount of CDP was added to vial containing 5 ml of each solvent. After sealing, the mixture was vortexed using a cyclomixer for 10 min in order to facilitate proper mixing of CDP with the vehicles. Mixtures were kept for 72 hr at ambient temperature to attain equilibrium, and afterwards, mixtures were centrifuged at 2000 rpm for 15 min. Aliquots of supernatant was filtered through membrane filter (0.45 µm) and diluted with mobile phase (methanol:water). Drug content was quantified directly by using high performance liquid chromatography (HPLC) technique.

2.2.2 Construction of pseudoternary phase diagram

From these, the extent of microemulsion region can be identified and its relation to other phases can be established. The pseudo-ternary phase diagrams were constructed by drop wise addition of double distilled water to homogenous liquid mixture of oil, surfactant, and co-surfactant, at ambient temperature (water titration method). From the result of solubility studies ethyl oleate, labrasol and cremophor RH40 were selected as oily phase, surfactant and co-surfactant respectively. At desired Km (ratio of surfactant to co-surfactant) value (1:1, 2:1, 3:1, 4:1 and 5:1), Smax and oil were mixed at ratio of 1:1, 1:5:1, 2:1, 2:5:1, 3:1, 3:5:1, 4:1, 4:5:1 and 5:1 in pre-weighed test tube. To the resultant mixtures, double distilled water was added dropwise till the first sign of turbidity in order to identify the end point and after equilibrium; if the system became clear then the water addition was continued. After complete equilibrium was reached, the mixtures were checked visually for phase clarity and flow ability. The resultant emulsion with a clear or slightly bluish appearance, exhibiting good stability (being stable after centrifugation for 10 min at 2000 rpm) and flow ability was defined as a microemulsion. A slightly less clear system, which had a bluish white or bright white appearance, was defined as an emulsion. No attempt was made to find out other region except boundary of microemulsion region in the ternary phase diagram. After identifying highest microemulsion region at desired Km value, randomly four formulations were selected to prepare liquid SMEDDS. Phase diagram was constructed by using Chemix School (evaluation copy) software.

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2.2.3 Preparation of solid SMEDDS
Maltodextrin (10.0 gm) was dissolved in 100 ml bidistilled water by magnetic stirring and sonicated for 5 min to completely dissolve. This solution was filtered through whatman filter paper to remove any undissolved particles. The liquid SMEDDS (10.0 gm) was then added with constant stirring, and the solution was kept at 40 °C to obtain fine o/w emulsion. The emulsion was spray dried with lab scale spray dryer (Labuthina LU-222, Mumbai) under the following optimized condition given as follows: inlet temperature-120 °C, outlet temperature-80 °C, aspirator speed-70%, feed rate-75 ml/hr and compressed air flow-rate-3 bar.

2.2.4 Reconstitution properties of solid SMEDDS

2.2.4.1 Dilution study by visual observation
Dilution study was done to study the effect of dilution on solid SMEDDS, because dilution may better mimic the condition of stomach after oral administration. In this method, solid SMEDDS and liquid SMEDDS (100.0 mg) were introduced into 100 ml of double distilled water at 37 °C, the formulations were mixed gently using a magnetic stirrer. The tendency to emulsify spontaneously and progress of emulsion droplets were observed with respect to time. The emulsification ability of SMEDDS was judged qualitatively “good” when clear microemulsion formed and “bad” when there was turbid or milky white emulsion formed after stopping of stirring[14].

2.2.4.2 Globule size and zeta potential determination
Solid SMEDDS formulations (10 mg) were diluted with 10 ml bidistilled water in a beaker with constant stirring on a magnetic stirrer. A visual test was carried out to assess self emulsification of solid SMEDDS and liquid SMEDDS in 100 ml bidistilled water at 37 °C. The samples were filtered through 0.45 µm pore size nylon filter. The amount of drug dissolved at 20, 25 and 30 min with replacement by an equal volume of temperature-equilibrated media and filtered through 0.45 µm pore size nylon filter. The liquid SMEDDS (10.0 ml) was prepared by the addition of uniform droplets of solid SMEDDS in 100 ml bidistilled water at 37 °C under gentle agitation. All solid SMEDDS formulations were shown in Table2.

2.2.5 Solid state characterization of solid SMEDDS
2.2.5.1 Powder X-ray diffraction (PXRD)
To obtain the changes in the crystallinity of the components of formulation prepared, the PXRD study was carried out by using X ray diffractometer (Philips PW-3710, Holland). The samples of pure drug, solid SMEDDS and physical mixture were taken and irradiated with monochromatised CuKa radiation and analyzed between from 10° to 60° (2θ).

2.2.5.2 Differential scanning calorimetry (DSC)
Thermograms of CDP, solid SMEDDS batches and physical mixtures were obtained using DSC instrument (TA Instruments SDT-2960, USA) equipped with an intracooler. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The samples of pure drug, solid SMEDDS and physical mixture were taken and irradiated with monochromatised CuKa radiation and analyzed between from 10 °C to 350 °C.

2.2.6 Morphological analysis of solid SMEDDS
The outer macroscopic shape (morphology) of the solid SMEDDS was investigated by SEM (JEOL, Japan), operating at 20 kV. The sample was fixed on SEM stub and then coated with thin layer of gold or platinum.

2.2.7 In vitro dissolution studies
To understand the characteristics of drug release from solid SMEDDS, an in vitro release test was carried out. Dissolution studies were performed for the solid SMEDDS form and conventional tablet form (Librium®). The dissolution test was performed in USP type II apparatus (Electrolab, Mumbai) according to United State Pharmacopeia dissolution procedure. The solid SMEDDS equivalent to 10 mg of CDP was fixed into hard gelatin capsules (capsule no. 00). Solid SMEDDS hard gelatin capsule put into sinker and sinker was loaded with 900 ml of simulated gastric fluid without pepsin at 37 ± 0.5 °C with paddle speed of 100 rpm. Each sample (5 ml) was withdrawn at 5, 10, 15, 20, 25 and 30 min with replacement by an equal volume of temperature-equilibrated media and filtered through 0.45 µm pore size nylon filter. The amount of drug dissolved determined by UV spectrophotometer at λmax of 309 nm for simulated gastric fluid. Dissolution studies were also performed in other media (phosphate buffer pH 5.8 and 7.0 at λmax of 245 nm) to examine the effect of pH on drug release.

2.2.8 HPLC analysis of CDP
HPLC was carried out using HPLC system consisted of Jasco PU 2080 Plus intelligent HPLC Pump (Jasco, Japan), equipped with HIQ SIL, C18 HS (250 x 4.6 mm) (KYA technology, Japan), 5 µm particle size column and Jasco UV 2075 Intelligent UV-VIS detector (Jasco, Japan), with 20 µl sample injector. It was operated by Jasco Borwin Chromatography software version 1.5. The mobile phase15 (methanol/water in the ratio of 97:03) was run at a flow rate of 1 ml/min and detection was carried out at 245 nm.

2.2.9 Statistical analysis
All data was expressed as a mean ± SD (n=3) and in vitro drug release were performed by unpaired t-test by Graphpad Instat demo version. Difference were considered significant when p < 0.05.

3. RESULT AND DISCUSSION

3.1 Saturation solubility studies for screening of excipients
Based on results of solubility studies ethyl oleate, labrasol and cremophor RH40 were selected for microemulsion formulation. The boundary layer of microemulsion for each desired Km value was observed at ambient temperature, due to there was no distinct conversion of w/o to o/w emulsion observed. The region of microemulsion existence for ethyl oleate-labrasol+cremophor Rh40-water (Km=4) was determined by pseudoternary plot shown in Figure 1 since microemulsion region at Km of 4 was bigger than 1, 2 and 3.

3.2 Construction of pseudoternary phase diagram
Based on results of solubility studies ethyl oleate, labrasol and cremophor RH40 were selected for microemulsion formulation. The boundary layer of microemulsion for each desired Km value was observed at ambient temperature, due to there was no distinct conversion of w/o to o/w emulsion observed. The region of microemulsion existence for ethyl oleate-labrasol+cremophor Rh40-water (Km=4) was determined by pseudoternary plot shown in Figure 1 since microemulsion region at Km of 4 was bigger than 1, 2 and 3.

Table1: Solubility of CDP in various oils and surfactants

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Oils and Surfactants</th>
<th>Solubility (mg/ml) at 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethyl oleate</td>
<td>1.60±0.09</td>
</tr>
<tr>
<td>2</td>
<td>Captex 355</td>
<td>1.37±0.05</td>
</tr>
<tr>
<td>3</td>
<td>Captex 800</td>
<td>0.93±0.13</td>
</tr>
<tr>
<td>4</td>
<td>Marisile 35-1</td>
<td>1.49±0.06</td>
</tr>
<tr>
<td>5</td>
<td>Captex 90</td>
<td>1.35±0.04</td>
</tr>
<tr>
<td>6</td>
<td>Labrasol</td>
<td>28.73±0.16</td>
</tr>
<tr>
<td>7</td>
<td>Tween 80</td>
<td>22.4±0.06</td>
</tr>
<tr>
<td>8</td>
<td>Cremophor RH40</td>
<td>95.5±0.11</td>
</tr>
<tr>
<td>9</td>
<td>PEG 400</td>
<td>19.9±0.18</td>
</tr>
</tbody>
</table>

Figure1: Pseudoternary phase diagram of ethyl oleate-labrasol+cremophor RH40-water at Km = 4 (Km = surfactant to cosurfactant ratio). Shaded area indicate microemulsion region.

Table2: Composition of CDP SMEDDS formulation

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Formulation</th>
<th>Drug (mg/10 gm)</th>
<th>%Composition (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oil</td>
<td>Labrasol</td>
</tr>
<tr>
<td>1</td>
<td>ME1</td>
<td>350</td>
<td>12.1</td>
</tr>
<tr>
<td>2</td>
<td>ME2</td>
<td>350</td>
<td>15.0</td>
</tr>
<tr>
<td>3</td>
<td>ME3</td>
<td>350</td>
<td>16.0</td>
</tr>
<tr>
<td>4</td>
<td>ME4</td>
<td>350</td>
<td>19.9</td>
</tr>
</tbody>
</table>

3.3 Reconstitution properties of solid SMEDDS
3.3.1 Dilution study by visual observation
A visual test was carried out to assess self emulsification of solid SMEDDS and liquid SMEDDS in 100 ml bidistilled water at 37 °C under gentle agitation. All solid SMEDDS...
formulations showed spontaneous microemulsification (< 1 min) as same as liquid SMEDDS and there was no sign of phase separation or phase inversion of microemulsion.

3.3.2. Globose size and zeta potential determination
The droplet size of the emulsion is the crucial factor in the self-emulsification performance because it determines the rate and extent of drug release as well as drug absorption. The mean droplet size of all reconstituted solid SMEDDS were very low and all were found to be in the nanometric range (<100 nm). The drop size in nano or micron range gives good transparency and increase surface area for partitioning of drug between oil and water. Result of globule size and zeta potential were summarized in Table 3. There was no major difference in zeta potential of the tested SMEDDS formulations. The zeta potential values of all solid SMEDDS were found to be in between -19 to -23. As the proportion of surfactants decreases and oil increases, the zeta potential decline while globule size increases. The zeta potential showed charge on the internal phase, which will decide the stability of the system. An increase in the electrostatic repulsive forces between microemulsion droplets prevents coalescence of microemulsion droplet, and a decrease of electrostatic repulsive forces will result in phase separation.

### Table 3: Globule size and zeta potential of solid SMEDDS formulation

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Batch code</th>
<th>Globule size (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ME1</td>
<td>56.97</td>
<td>-23.1</td>
</tr>
<tr>
<td>2</td>
<td>ME2</td>
<td>58.64</td>
<td>-22.1</td>
</tr>
<tr>
<td>3</td>
<td>ME3</td>
<td>65.94</td>
<td>-21.1</td>
</tr>
<tr>
<td>4</td>
<td>ME4</td>
<td>72.43</td>
<td>-19.1</td>
</tr>
</tbody>
</table>

3.4. Solid state characterization of solid SMEDDS

3.4.1 Powder X-ray diffraction (PXRD)
In the powder X-ray diffraction studies, the diffractograms of the representative batches were taken to find out the effect on the crystallinity of the drug and excipients. In the diffractograms of physical mixture and solid SMEDDS batches, complete amorphousization of drug along with excipients occurred. There is no intense peak was observed which revealed solubilization of drug in the formulation batches as shown in Figure 2.

3.4.2 Differential scanning calorimetry (DSC)
DSC curves of pure drug, physical mixture and spray dried solid SMEDDS were shown in Figure 3. Pure CDP show sharp endothermic peak at near about 244°C, which may be melting point of drug. The physical mixture didn’t show any obvious peak for CDP, may be due to more dilution by solid carrier or absence in the sample exposed to DSC. Very small broad endothermic peaks (less heat of absorption) for CDP were found in solid SMEDDS batches. It might be explained that CDP was present in the amorphous phase of a molecular dispersion state in the matrix.

3.5. Morphological analysis of solid SMEDDS
The SEM images of solid SMEDDS of batch ME2 were shown in Figure 4. According to SEM images, the solid SMEDDS consisted of well separated particles with no agglomeration. It has satisfactory regular spherical shape with small wrinkled wave like surface. It may be the ability of maltodextrin to inhibit the agglomeration of particles.

3.6. In vitro dissolution studies
The in vitro dissolution comparison of solid SMEDDS formulation in different dissolution media (SGF pH 1.2, phosphate buffer pH 5.8 and 7.0) were shown in Figure 5-7. The drug releases from solid SMEDDS formulations were found to be significantly higher as compared with that of conventional chlordiazepoxide tablet (Librium®). It could be suggested that the solid SMEDDS formulation resulted in spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of conventional chlordiazepoxide tablet (Librium®). Thus, this greater availability of dissolved chlordiazepoxide from the solid SMEDDS formulations could lead to higher absorption and higher oral bioavailability. From Figures 5-7, it was also seen that changes in the dissolution medium had tremendous effect on the drug release of marketed preparation (Librium®), but no effect on solid SMEDDS formulations. Solid SMEDDS formulations shows more than 90% drug release in 30 min in all dissolution media (except ME4 formulation) whereas as marketed preparation shows < 50% and < 40% drug release in phosphate buffer pH 5.8 and 7.0 respectively. Solid SMEDDS shows statistically significant difference (p < 0.05) in percent release of drug when compared with marketed preparation (phosphate buffer pH 5.8).
and 7.0). ME4 formulation failed to show > 90% drug release because of increased globule size but it passes the dissolution test criteria as per USP. This observation can be explained by the fact that CDP has ionizable group and thus its solubility and dissolution is pH dependent. But in same case one can also conclude that in case of solid SMEDDS formulation, dissolution of chlordiazepoxide is pH independent.

4. CONCLUSION
In the present investigation, the solid SMEDDS of chlordiazepoxide was prepared by spray drying, using water-soluble maltodextrin as solid carrier for direct filling into hard gelatin capsule for oral administration. The solid SMEDDS consisted of well-separated spherical particles and maintained the rapid self-emulsifying ability as that of liquid SMEDDS. Both DSC measurements and X-ray diffraction analysis suggested that chlordiazepoxide in the solid SMEDDS was in the amorphous molecular dispersion state. In vitro dissolution test showed that the solid SMEDDS had a faster in vitro release rate than the conventional tablet in discriminating dissolution medium (phosphate buffer pH 5.8 and 7.0). Thus, this solid self-microemulsifying system may provide a useful solid dosage form for oral poorly water-soluble drug such as chlordiazepoxide to enhance solubility and dissolution rate which may improve therapeutic performance.

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