Preparation, In Vitro and In Vivo Characterization of Solid Dispersions of Lamotrigine Using Solvent Evaporation Technique

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Abstract: Lamotrigine is one of the newer antiepileptic drugs and low aqueous solubility of Lamotrigine is responsible for its poor dissolution and delayed onset of action. The purpose of the present investigation is to increase the dissolution rate of Lamotrigine by preparing its solid dispersions with PEG 6000 using solvent evaporation technique and subjecting them to drug-carrier interaction, dissolution and stability studies and it was found that the dissolution rate was improved for Lamotrigine in its solid dispersion. As indicated from XRD and DSC studies, Lamotrigine was in the amorphous form in the solid dispersions, which confirmed the better dissolution rate of prepared stable solid dispersions. Pharmacokinetic profiles of Lamotrigine and solid dispersion were compared by one way ANOVA followed by Dunnett Post Hoc test which indicated higher attainable plasma concentrations. Solid dispersion showed a difference with the pure drug in its pharmacokinetic profile which may be attributed to better dissolution rate of Lamotrigine from its solid dispersion.

Keywords: Lamotrigine, Solubility, Physical Mixture, Solid dispersions, Solvent evaporation, Pharmacokinetic.

I. INTRODUCTION:

Lamotrigine is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder [1]. Lamotrigine also acts as a mood stabilizer [2]. Lamotrigine is rapidly and completely absorbed after oral administration with negligible first-pass metabolism (absolute bioavailability is 98%). Common oral dosage is 25 mg/day (dose/solubility ratio ≥250 ml; class II drug according to the BCS). Peak plasma concentrations occur anywhere from 1.4 to 4.8 hours following drug administration. This delay in the onset of action in spite of good bioavailability is because of its low aqueous solubility which is only 0.17 g/litre. The most promising method for promoting dissolution is the formation of solid dispersion in a proper carrier [3-4]. Polyethylene glycol (PEG) is amongst the most frequently investigated hydrophilic polymeric carriers [5-6]. The purpose of the present investigation is to increase the solubility and dissolution rate of Lamotrigine by preparing its solid dispersions with polyethylene glycol (PEG) 6000 using solvent evaporation technique.

II. MATERIAL AND METHODS:

Lamotrigine was a gift sample from Jubilant Organosys Ltd, Noida, U.P, India and Polyethylene Glycol 6000 was purchased from Oxford Laboratory, Mumbai, India. All other chemicals and reagents used were of analytical grade.

Phase solubility studies: Solubility measurements were performed according to method reported by Higuchi and Connors [7]. Both PEG 4000 and 6000 were assessed for solubility enhancement. Various (1%, 2%, 5% and 10% w/v) aqueous solutions of PEG 6000 and PEG 4000 were prepared and transferred to volumetric flasks. An excess amount of drug was added to each flask. The contents of each flask (10 ml) were equilibrated by shaking for 48 hours in a thermostatically controlled water bath at 37±0.1°C. After 48 hours, samples were analyzed at 304nm for Lamotrigine. Solubility studies were performed in triplicate (n=3).

Preparation of physical mixtures and solid dispersions: For Lamotrigine, the physical mixtures and solid dispersions were prepared by solvent evaporation technique in three different ratios by using PEG 6000 as a hydrophilic carrier. The following combination of drug and carrier were used. PEG 6000 was chosen as it was found to give a better dissolution profile of Lamotrigine.

<table>
<thead>
<tr>
<th>Code</th>
<th>Quantity of Drug</th>
<th>Quantity of carrier (PEG 6000)</th>
<th>Ratio (Drug:Carrier)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1</td>
<td>150 mgs</td>
<td>150 mgs</td>
<td>(1:1)</td>
</tr>
<tr>
<td>SD2</td>
<td>150 mgs</td>
<td>300 mgs</td>
<td>(1:2)</td>
</tr>
<tr>
<td>SD3</td>
<td>150 mgs</td>
<td>750 mgs</td>
<td>(1:5)</td>
</tr>
</tbody>
</table>

Table1: Ratio of drug and carrier used for preparation of solid dispersion
Preparation of solid dispersions by solvent evaporation method: Accurately weighed quantity of polyethylene glycol 6000 was dissolved in 10 ml of acetone. To these solutions, accurately weighed quantities of drug was added and allowed to dissolve. The solution was transferred to a petridish and the solvent was allowed to evaporate at room temperature for 1 hour and then was kept in desiccators for 48 hours. Solid dispersions thus obtained were crushed, pulvèrised, sifted and stored in desiccators for further evaluation.

Characterization of physical mixture and solid dispersions of Lamotrigine 

Fourier transforms infrared spectroscopy (FTIR): Fourier transform infrared spectra were obtained using Thermo Nicolet 380 FTIR. The scanning range was 40 to 4000 cm\(^{-1}\) and the resolution was 4 cm\(^{-1}\).

Differential scanning calorimetric (DSC): The DSC thermograms of samples were recorded on a DSC (SISI Nanotheke). The samples (6.5-10 mg) were heated under nitrogen atmosphere in hermetically sealed aluminium pans over a temperature range 20°C to 350°C at a constant rate of 20°C/min under nitrogen purge (10 ml / min).

Powder X-ray diffraction (PXRD): The powder X-ray diffraction patterns were determined. The scanning rate was 1°/min over a 2θ range of 1-50° C.

Dissolution studies: The solid dispersions were subjected to dissolution studies using USP Paddle Type II apparatus. The dissolution medium used was 0.1 N HCL (PH 1.2)\(^{19}\) temperature 37±0.5°C and paddles rotated at 50 rpm. Samples of 150 mg of pure drug and solid dispersion samples equivalent to 150 mg of drug (Table 1) were filled inside muslin cloth pouches and dropped inside 900 ml of dissolution medium. 10 ml of samples were withdrawn every 10 minutes, filtered through membrane filter (pore size 0.45 μm) and analyzed at 304 nm for Lamotrigine.

Accelerated stability studies for solid dispersions: Accelerated stability studies were performed according to ICH guidelines at 40°C±2°C, 75±5% RH for a 6 months period. Solid dispersions were removed at the end of three and six months and evaluated for drug release. A paired ‘t’ test was applied to tablet dissolution initial and after 6 months results in order to study the effect of storage on the solid dispersion.

Comparison of oral absorption between Lamotrigine pure drug and solid dispersions: The in vivo absorption studies of pure Lamotrigine and solid dispersions were carried out using male Wistar rats (250-300 g). The animals were fasted for 12 hours prior to commencement of the study as well as during the study and had access to water ad libitum. The institutional animal ethical clearance was obtained from (Reg No. CPCSEA/MRC/2008/1217) before conducting the studies. Animals were divided into three groups (six in each group); Control group, Pure drug, Solid dispersion. The plasma concentration of drug was determined by High Performance Liquid Chromatography (HPLC) \(^{10}\)

HPLC Analysis: The HPLC system consisted of a system controller (M-721), a data module (M-730), a solvent delivery pump (M-501), an auto sampler (WISP-712) and a variable wavelength U.V. detector (M-481). Chromatographic separations were performed using a symmetry C\(_{18}\) stainless steel column (150 x 3.9 mm i.d., 5 μm). A mobile phase consisting of 0.01 M potassium phosphate–acetonitrile–methanol (70:20:10% v/v/v) at a pH adjusted to 6.7 was used using a flow rate of 1.3 ml/min and monitored at 214 nm with a sensitivity of 0.01 absorbance units full scale (AUFS) and a chart speed of 0.5 cm/min.

Pharmacokinetic Studies: The animals were fasted overnight (water given ad libium) and then given a single oral dose of (10 mg/Kg) Lamotrigine\(^{11}\) and Lamotrigine solid dispersion \(^{11}\) suspended in normal saline with the help of Tween 80. Blood samples were collected through the lateral tail vein of rats before dosing and at 10, 20, 30 minutes followed by 1, 1.5, 2, 3, 4, 6 and 24 hours after dosing. The blood samples were centrifuged at 3000 rpm for 10 mins and 100 μl of plasma samples were stored at -20°C until analysis. The results obtained were analyzed for various non-compartmental pharmacokinetic parameters using PK functions

Statistical analysis: Results are expressed as mean±S.D. ANOVA was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Dunett Post Hoc test for multiple comparison.
III. RESULTS:

![Comparative solubility graph of Lamotrigine](image)

Figure 1: Effect of PEG on the solubility of Lamotrigine

The results of the phase solubility as seen from Figure 1 revealed that PEG 6000 has a more pronounced effect on increasing the solubility of Lamotrigine as compared to PEG 4000. The aqueous solubility of Lamotrigine was found to be 0.17 mg/ml. The solubility of drug was increased up to 35 fold in 10% w/v PEG 6000 aqueous solution at 25°C as compared to pure drug. This may be attributed to more number of ether linkages in case of PEG 6000 and hence greater solubility.

The infrared spectra of the solid dispersions are shown in Figure 2 together with those for Lamotrigine alone and PEG 6000 alone as references.

![FTIR Curves](image)

Figure 2: FTIR Curves a) Pure Drug Lamotrigine b)PEG c)SD1 d)SD2 e)SD3

As seen from Figure 2, the spectrum of Lamotrigine is characterized by the presence of strong absorption band at 3451 cm\(^{-1}\), 3318 cm\(^{-1}\) and 3267 cm\(^{-1}\), which are all indicative of amines (-NH- group). The carbonyl-stretching mode appears as a very strong doublet at 1600 cm\(^{-1}\) (C=O stretching) and at 800 cm\(^{-1}\), which was indicative of presence of aromatic rings. The spectra of PEG 6000 are characterized by the C-H stretching vibrations at 2883 cm\(^{-1}\) and C-O (ether) stretching at 1105 cm\(^{-1}\). The careful observation of the IR spectra reveals that all the major peaks of the pure drug and PEG 6000 appear with negligible variation in the IR spectrum of the solid dispersion, indicating that there is no chemical interaction between the drug and polymer.
Preparation, In Vitro And In Vivo Characterization Of Solid Dispersions Of Lamotrigine Using

The DSC curve of pure drug Lamotrigine shows an endothermic peak at 224.76°C indicating that it has a sharp melting point whereas PEG 6000 displays a peak at 74.79°C (Figure 3). In the solvent evaporation method both the drug as well as the polymer shows a slight shift and broadening in the peaks indicating amorphization of the drug in the polymer\footnote{12} which may be helpful in increasing the solubility of pure drug in the formulation.\footnote{12} The solid state crystallinity of Lamotrigine, PEG 6000 & formulations prepared by solvent evaporation method were studied by PXRD technique illustrated in Figure 4.

The reduction in crystallinity of Lamotrigine in the formulations was observed and it was also noted that the crystallinity was decreased with increase in concentration of PEG 6000 (1:1, 1:2, 1:5).

![Figure 3: DSC Thermogram a) Pure Drug Lamotrigine b) PEG c) SD1 d) SD2 e) SD3](image)

![Figure 4: PXRD a) Pure Drug Lamotrigine b) PEG c) SD1 d) SD2 e) SD3](image)

![Figure 5: Comparative Dissolution Profile Lamotrigine](image)

**Comparative Dissolution data**

- %Drug Release after 30 mins
- %Drug Release after 60 mins

LM- Lamotrigine Pure drug; PM- Physical Mixture; SE- Solvent Evaporation Method; MM – Melting Method.
From Figure 5, it is evident that the dissolution rate of Lamotrigine has improved in the case of solid dispersion and the best dissolution profile was obtained when drug and carrier were combined in 1:1 ratio in the solvent evaporation method. Solid dispersions and physical mixtures prepared in 1:2 and 1:5 ratio did not give a comparable release profile.

<table>
<thead>
<tr>
<th>TIME IN MINUTES</th>
<th>INITIAL MEAN±S.D.</th>
<th>AFTER 3 MONTHS MEAN±S.D.</th>
<th>AFTER 6 MONTHS MEAN±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>61.6 ± 0.2</td>
<td>61.2 ± 0.2</td>
<td>61.7 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>70.6 ± 0.43</td>
<td>70.8 ± 0.14</td>
<td>70 ± 0.12</td>
</tr>
<tr>
<td>6</td>
<td>81.1 ± 0.09</td>
<td>81.6 ± 0.34</td>
<td>81.9 ± 0.13</td>
</tr>
<tr>
<td>8</td>
<td>88.48 ± 0.00</td>
<td>88.2 ± 0.10</td>
<td>88 ± 0.62</td>
</tr>
<tr>
<td>10</td>
<td>93.8 ± 0.64</td>
<td>93.7 ± 0.38</td>
<td>93.2 ± 0.53</td>
</tr>
</tbody>
</table>

Table:2 In vitro cumulative % drug release at 40 ± 2°C, 75 ± 5% RH for Lamotrigine

Accelerated stability studies dissolution data when subjected to paired ‘t’ test shows that the effect of storage was insignificant at 5% level of F (t stat (0.0128) < t critical (2.3060)) and it can be conclusively stated that the dissolution studies show compliance with the ICH guidelines demonstrating shelf life through curve fitting at 95% confidence limit.

<table>
<thead>
<tr>
<th>PHARMACOKINETIC PARAMETERS</th>
<th>PURE DRUG</th>
<th>SOLID DISPERSION (SD1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak plasma concentration C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</td>
<td>80.44</td>
<td>200.87</td>
</tr>
<tr>
<td>Time to reach peak plasma concentration T&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>1.5 hrs</td>
<td>0.5 hrs</td>
</tr>
<tr>
<td>Biological half life t&lt;sub&gt;½&lt;/sub&gt; (hr)</td>
<td>24.54</td>
<td>24.55</td>
</tr>
<tr>
<td>Elimination rate constant Ke (hr&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.0282</td>
<td>0.028</td>
</tr>
<tr>
<td>Area under the curve AUC&lt;sub&gt;0-24&lt;/sub&gt; (Total) (µg/ml* hr)</td>
<td>251.45</td>
<td>740</td>
</tr>
</tbody>
</table>

TABLE:3 Pharmacokinetic parameters of fast dissolving tablets of Lamotrigine

The average peak plasma concentration obtained for the drug and solid dispersion, indicated an increase in the extent of absorption (AUC<sub>0-24</sub>). The decrease in the t<sub>max</sub> values indicated faster absorption from the solid dispersions and increase in the C<sub>max</sub> values indicated higher attainable plasma drug concentrations with the same dose of the drug. ANOVA followed by Post Hoc Dunnett t3 test indicated that prepared solid dispersion and the pure drug showed a significant difference in their pharmacokinetic profiles. This difference may be attributed to better dissolution rate of Lamotrigine from its solid dispersion

IV. CONCLUSION:

Solid dispersions prepared from hydrophilic polymers using the solvent evaporation technique were effective in improving drug dissolution. The above studies indicated that PEG 6000 inhibited the crystallization of drug, resulting in the amorphous state form of the drug in solid dispersion. Accelerated stability studies of solid dispersion show compliance with the ICH guidelines demonstrating shelf life through curve fitting at 95% confidence limit. Prepared solid dispersion and the pure drug showed a significant difference in their pharmacokinetic profiles which may be attributed to better dissolution rate of Lamotrigine from its solid dispersion. Thus the solid dispersion technique with PEG 6000 as a carrier provides a promising way to enhance the solubility and dissolution rate of Lamotrigine.

REFERENCES:

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