Dissolution and Bioavailability Enhancement of Carbamazepine

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ABSTRACT

Relatively insoluble candidate drug like carbamazepine (CBZ) often exhibit incomplete or erratic absorption; and hence wide consideration is given to improve aqueous solubility of such compound. Dissolution of carbamazepine was improved in the present work using novel swellable hydrogel (mucilage) isolated from the seeds of Ocimum basilicum (Gel). Solid dispersion of carbamazepine was prepared by adopting co grinding/comminization method. In vitro dissolution studies revealed increase in the dissolution efficiency of CBZ in its solid dispersion form when compared to the neat plain CBZ as from 30.84%±1.9 to 77.26%±0.32 in first 30 minutes. There was improved in vivo bioavailability of CBZ determined by using male Sprague Dawley rats from its solid dispersion form than its plain form which was statistically significant at p< 0.01. Inhibition of the formation of (less soluble) dihydrate form of CBZ (in aqueous environment) when it is in its solid dispersion form was found to be the major cause of its improved dissolution and bioavailability.

Keywords: Carbamazepine, Solid dispersion, Gel, Dried Mucilage, Ocimum basilicum

INTRODUCTION

Poorly soluble drugs exhibit erratic dissolution and hence variable bioavailability. Thus, improvement in their dissolution characteristics is prerequisite of formulation development [1]. Many methods are reported in the literature for the preparation of solid dispersions like solvent evaporation method, meltting method, spray drying etc. One of the important causes of increase in rate of dissolution of the drug when these strategies are followed; is the amorphization of the drug. This makes commercialization of products containing solid dispersions difficult because of possibility of recrystallisation of drug during processing or its shelf life [2]. Moreover many of the polymers used in such preparations of solid dispersions can absorb moisture as they are hydrophilic; promoting recrystallisation of the drug [3]. Even then solvent evaporation method is widely accepted approach to prepare solid dispersions. The general method involves solubilization of drug and carrier polymer in common solvent and eventual evaporation of the solvent. Two major difficulties to be faced while following this method are- i) availability of suitable solvent since the solubility characteristics of drug (hydrophobic) and carrier polymer (hydrophilic) are fundamentally different. Hence organic solvent alone or their admixture is preferred to prepare the solution of drug and carrier polymer. In such cases failure in complete removal of solvent/s and higher preparation cost makes it unsuitable for large scale production, ii) small variations in processing conditions (particularity when organic solvents are used) lead to differences in performance of the product, lacking batch to batch consistency [4]. So, researchers suggested adoption of combination of approaches to overcome these barriers to marketing success of solid dispersions [3].

Water soluble carriers like polyethylene glycols (PEGs) and polyvinyl pyrrolidion (PVP) when used to prepare solid dispersions of poorly soluble drugs by solvent evaporation method they should be added in high proportions. In addition to this fact they themselves get preferentially dissolved in dissolution media owing to their high solubility; leaving the drug in undissolved state. Hence certain hydrophilic swellable polymers were tried to form solid dispersion of poorly soluble drugs like carbamazepine by modified solvent evaporation technique. The results of this study clearly showed remarkable increase in percent drug dissolution [5]. Carbamazepine (CBZ) is well established anticonvulsant antiepileptic drug. It is very well researched molecule and most of research was demanded due to its erratic dissolution behavior and hence bioavailability. It’s four anhydrous polymorphs and a dihydrate form are reported in the literature. Among these; form III is thermodynamically stable form of commercially available CBZ at room temperature. It converts to its enantioform i.e. form I above its transition temperature (70°C). Compositions of the dissolution medium influence the polymorphic transformations of CBZ. Anhydrous CBZ transforms into its dihydrate form in aqueous medium; particularly at acidic pH [6-9]. This fact critically affects its dissolution and bioavailability when given orally, because solubility of dihydrate form of CBZ is one third to that of Form I or form III [10]. These transformations in commercially available CBZ samples with and without the presence of excipients were thoroughly studied and reported by N. Sandler et al in 2006. They demonstrated the potential of Scanning Electron Microscopy (SEM) to investigate such polymorphic conversions. According to them there was clear evidence of inhibition of polymorphic transformation of CBZ (form III to dihydrate form) in its solid dispersion form prepared with excipients having strong hydrogen bonding groups [11].

The aim of present work was to investigate the potential of hydrogel (mucilage) isolated from the seeds of Ocimum basilicum (Gel) to improve dissolution of CBZ, as it is water swellable material [12]. Its water swelling property was due to the fact that there is presence of moderately strong hydrogen bonding groups (confirmed by its ATR-FTIR spectrum which exhibited peak with centre around 3410 cm⁻¹) in its dried mucilage form (DM) [12,13]. In the present study solid dispersion of carbamazepine (CBZ) was prepared by using mucilage isolated from the seeds of Ocimum basilicum (Gel) and eventual removal of water by evaporation. CBZ was mixed and milled in colloid mill with Gel. Since the method of isolation of hydrogel (mucilage) used in this study entailed deminerlized water alone, there was no possibility of presence of traces of organic solvent [14]. The mucilage forming material is present in the pericarp of the seeds and swells immediately in the water. It was tried as solubility enhancing swellable polymer expecting that it would retard/impede dihydrate formation of CBZ. In vitro dissolution studies exhibited increased dissolution efficiency of solid dispersion of CBZ (77.26%±0.32) than that of plain CBZ (30.84%±1.9). The SEM images of solid dispersion of CBZ when immersed in 0.1 N HCl buffer did not reveal its conversion to dihydrate form at least up to 5 hours which was found to be the major cause for its enhanced dissolution. This fact was further supported by the results of in vivo bioavailability studies carried out by using Sprague Dawley rats. Thus the Gel was found to be the efficient solubility and bioavailability enhancer for carbamazepine.

MATERIALS AND METHODS

Materials

Carbamazepine was received as a gift sample from Cipla Pvt. Ltd. Mumbai. The Gel was isolated in the laboratory from the seeds of Ocimum basilicum collected from regional sources. All the chemicals used in bioavailability studies were of HPLC grade. All other
chemicals and solvents were of analytical reagent grade and used without further purification.

**Methods**

**Preparation of solid dispersion of Carbamazepine**

Mucilage (Gel) was isolated from the seeds of *Ocimum basilicum* adopting the procedure described in our earlier paper, dried and used in the compatibility study as dried mucilage (DM) [12,15]. Solid dispersion of carbamazepine was prepared by adopting co grinding/ co micronization method [16]. Carbamazepine was co-ground in colloid mill with Gel (in suitable proportion to obtain solid dispersion of CBZ, DM in 1:1 ratio). It was dried in microwave at 350 mW for 20 minutes.

**Compatibility and stability studies of Carbamazepine with DM**

The interaction study of dried mucilage and CBZ was done by Fourier transform infra red spectroscopy (FTIR) and Differential scanning calorimetry (DSC). The physical compatibility of CBZ with DM as a novel excipient in the form of solid dispersion was carried out with an aim to develop a stable and robust formulation. Solid dispersion was kept in glass vials previously sterilized by dry heat method and exposed to 40°C/75% RH for three months according to the guidelines from International Conference on Harmonization [17]. The samples were withdrawn at initial, 1 month, 2 month and 3 month duration and evaluated for following parameters.

**Fourier Transform Infrared spectroscopy**

FT-IR spectrum of CBZ and its solid dispersion was scanned with potassium bromide powder over frequency range 4000-500 cm⁻¹ at resolution of 4 cm⁻¹ to study principle peaks using FT-IR spectrophotometer (FT-IR 8400S, Shimadzu). The identified peaks were compared with the principle peaks of reported IR spectrum [18].

**Differential scanning calorimetry**

The DSC study was carried out for pure CBZ, its physical mixture with DM in 1:1 proportion and its solid dispersion. The DSC patterns were recorded by heating 5-10 mg material in crimped aluminum pans at a scanning rate of 10°C/min in an atmosphere of nitrogen using the range of 40-240°C (Model TA 4000, METTLER TOLİDO). The temperature calibrations were performed periodically using indium as a standard.

**Determination of zeta potential of solid dispersion of Carbamazepine**

Zeta potential measurement of plain CBZ (treated similarly to experimental process adopted for preparation of its solid dispersion without Gel) and its Solid dispersion was performed in order to investigate the surface properties. The zeta potential was measured by using aqueous suspensions containing 1% of CBZ or solid dispersion (Model-Beckman coulter).

**Uniformity of content of carbamazepine in solid dispersion**

Weighed accurately solid dispersion equivalent to about 100 mg of CBZ and dissolved in sufficient ethanol (95%) to produce 100 mL. It was diluted sufficiently and measured the absorbance of the resulting solution at 285 nm. Calculated the content of CBZ taking 490 as the value of A [%], 1cm at the λₘₐₓ at 285 nm [19].

**Determination of dissolution efficiency of solid dispersion of Carbamazepine**

The dissolution efficiency of solid dispersion is the area under the dissolution curve of the rectangle described by 100% dissolution in the same time. It was calculated by the Equation (1).

\[
\text{D.E} = \frac{\int_0^T y(t) \, dt \times 100}{y_{100} \times T} \quad \text{Eq. [1][20]}
\]

Where \( y \) is the drug percent dissolved at time \( t \).

Scanning Electron Microscopy

SEM micrographs were taken of plain CBZ and its solid dispersion as it is and recovered after immersing in 0.1 N HCl periodically. Samples were sputter coated with a thin layer of gold-palladium under argon vacuum prior to analysis. SEM was performed using a 5 kV beam acceleration voltage.

**In vivo bioavailability study of solid dispersion of Carbamazepine**

The experimental protocol was carried out in accordance with Organization for Economic Cooperation and Development (OECD) guidelines (OECD, 2001). All animal experiments were performed in accordance with institutional guidelines and the procedure was approved by the Committee for the purpose of Control and Supervision of Experiments on Animals. Bioanalytical method was developed and validation was carried out following the guidelines given by the Food and Drug Administration (FDA) [21]. Efforts were made to minimize the number of animals for experimentation purpose. Male Sprague Dawley rats were used this study. The treatments consisted of a single oral dose of plain CBZ and its solid dispersion equivalent to 50 mg was administered with 20mL water to each rat. Blood samples of 2.0mL were withdrawn from retro orbital cavity in 6 X 50 mm glass test tubes containing disodium edetate and centrifuged at 4500 rpm for 15 minutes at 4°C. In order to extract the drug form plasma sample, 0.5 mL of supernatant was transferred to centrifuge tube and 1.5 mL of mixture was chloroform and ethyl acetate (1:1) was added, vortexed and again centrifuged at 4500 rpm for 15 minutes at 4°C. Organic layer was separated after centrifugation and dried at 60°C in water bath. At last, 2000 µL acetonitrile was added to centrifuge tube and 20 µL samples was injected for HPLC analysis.

**Analysis of CBZ concentration in the plasma**

The high performance liquid chromatogram used was Agilent 1120 Compact LC with UV Detector. Column used was C-18 column with internal diameter of 2.1 and 150 mm of length with average particle size of 5 µm. Mobile phase used was water: acetonitrile (70:30). The column temperature was kept at 25°C. Flow rate was maintained at 1 mL/min. Aliquot of 20 µL was injected into column for HPLC analysis. Monitoring was performed at 220 nm.

**Pharmacokinetic and statistical analysis**

The maximum plasma concentration (Cₘₐₓ) and the time to reach peak concentration (Tₘₐₓ) were obtained directly from the concentration-time data. The area under the curve to the last measurable concentration (AUCₜₙ) was calculated by the linear trapezoidal rule. The significance of the differences observed for the mean pharmacokinetic parameters of solid dispersion of the CBZ and pharamacokinetic parameters of plain CBZ and were evaluated using t test.

**RESULTS AND DISCUSSION**

**Preparation of solid dispersion of Carbamazepine**

The resulting particles of solid dispersion of CBZ were spherical in shape with smooth surfaces. As observed in SEM images, the particle size distribution of CBZ was narrowed down because of method adopted for the preparation of its solid dispersion (as shown in Figure 1A, B).

**Compatibility and Stability Studies of Carbamazepine with DM**

Fourier Transform Infrared Spectroscopy

There were two characteristic peaks observed at 3466.20 cm⁻¹ and 1605.79 cm⁻¹ due to a NH₂ bending vibration and at 1386.86 cm⁻¹ due to C-H deformation (as shown in Figure 2A). There was no variation in these peaks when observed in FTIR spectrum of solid dispersion immediately after its preparation and also after 3 months stability studies (as shown in Figure 2B). Hence there was no chemical change in the CBZ when it was in the solid dispersion form.
**Differential Scanning Calorimetry**

DSC of plain CBZ exhibited two endotherms at 175.78°C and 191.47°C corresponding to melting of its two enantiomorphs i.e. Form III and Form I respectively (as shown in Figure 3). At room temperature (below its transition temperature which is 71°C) Form III is more stable; but above its transition temperature, Form I is more stable owing to its lower free energy. Dried mucilage (DM) being an amorphous carrier did not show any endothermic event. There was no evidence of amorphisation of the CBZ during processing. This endothermic pattern remained same, even after 1, 2 and 3 months of stability studies indicating that there was no amorphization of CBZ and no chemical interaction between CBZ and Gel [22,23].

**Determination of zeta potential of solid dispersion of Carbamazepine**

The surface properties of the solid dispersions depend on the method of its preparation [24]. In the present work the CBZ was milled along with the Gel which caused reduction in particle size of CBZ with subsequent adsorption of dried mucilage (DM) on micronized CBZ due to removal of water during microwave drying. Zeta potential is powerful indicator for the characterization of drug molecule adsorption [25]. Zeta potential on the surface of plain CBZ was shifted from -12.43mV to still lower value of -24.39mV in its solid dispersion form; clearly indicating surface coating of carbamazepine by dried mucilage (DM) (as reported in Table 1). The zeta potential is also a measure of the electrostatic stabilization provided by coating material. Though as a thumb rule, zeta potential higher than ±30 mV provides good suspension stability; even if it is >±20 mV it conferred optimum stability to suspended particles preventing agglomeration [26]. This might be the one of the probable reasons for improved dissolution of CBZ from its solid dispersion form. It was reflected in the results of in vivo study as its improved bioavailability in the present study. After administration of the solid dispersion of CBZ in the form of aqueous suspension to rats there would not had been agglomeration (favoring faster dissolution of individual CBZ particle coated by Gel) due to optimum zeta potential on the surface of CBZ in its solid dispersion form [27].
Uniformity of content of carbamazepine in solid dispersion

Uniformity of content of CBZ in its solid dispersion form was found to be 96.12±1.74. It remained above 95% even when the solid dispersion was exposed to 40°C/75%RH for three months.

Determination of dissolution efficiency of solid dispersion of Carbamazepine

There was increase in dissolution efficiency of CBZ in its solid dispersion form when compared to that with plain CBZ as from 30.84±1.9 to 77.26±0.32 in first 30 minutes (as reported in Table 2).

Scanning Electron Microscopy

SEM images of micro particles of plain CBZ and its solid dispersion are shown in Fig. 1. The solid dispersion micro particles appeared with sooth and uniform surface (due to coating of DM) and with reduced particle size than that for plain CBZ (due to colloid milling); while the plain CBZ appeared as irregularly shaped particles, several microns in size. The SEM images of solid dispersion of CBZ recovered after immersing in 0.1 N HCl clearly exhibited inhibition of formation of less soluble dihydrate form of CBZ up to 5 hrs (as shown in Figure 4 b, d and f). Since there was no conversion of CBZ to its less soluble dihydrate form; this was found to be the major cause of dissolution improvement of the CBZ from its solid dispersion form. These results were expected owing to the inherent properties of the Gel, as mentioned in the introduction section of this article.

Table 2: Dissolution parameters of the solid dispersion of CBZ and pure CBZ.

<table>
<thead>
<tr>
<th>Material</th>
<th>DE30 (%)</th>
<th>Q30min (%)</th>
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<tbody>
<tr>
<td>Plain Carbamazepine</td>
<td>30.84±1.9</td>
<td>39.26±0.68</td>
</tr>
<tr>
<td>Solid dispersion of CBZ</td>
<td>77.26±0.32</td>
<td>95.38±1.16**</td>
</tr>
<tr>
<td>*(Mean±SD; n=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>**Indicate significant difference at p ≤0.01 vs. pure drug.</td>
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</table>
In vivo bioavailability study of solid dispersion of Carbamazepine

The area under the plasma conc. time curve from 0 to 8 hrs was determined by the trapezoidal rule. The time to reach maximum conc. ($T_{\text{max}}$) and maximum plasma conc. ($C_{\text{max}}$) was determined by inspection. The results are reported in the Table 3. $C_{\text{max}}$ is one of the important parameter which is the function of both the rate and extent of absorption. Statistically significant difference was observed in $C_{\text{max}}$ obtained after administering plain CBZ and the solid dispersion of CBZ at $**p<0.01$. Area under the curve (AUC) for solid dispersion of CBZ was found to be higher than AUC for plain CBZ; and the difference was found statistically significant at $***p<0.001$. There was considerable difference found in $T_{\text{max}}$ after administration of plain CBZ (0.25 hr) and $T_{\text{max}}$ after administration of solid dispersion of CBZ(3 hrs). The most probable cause for this would be coating of CBZ particles by the Gel in its solid dispersion form. Though there was inhibition of dihydrate form of CBZ, due to the coating, the same might have impeded the rate of diffusion of solubilized CBZ from the interior to the gut content of the rat. This observation is not correlated with the results of in vitro dissolution rate studies; because the ratio of amount of CBZ: Volume of dissolution medium (used in in vitro dissolution study) was much higher than the amount of CBZ: volume of gut content of the rat.

As CBZ belongs to BCS Class II; the volume and the viscosity of the dissolution medium available is the matter of concern; as observed in the present study. Drug particle coating was reported to elongate the dissolution process of the drug. The delayed but improved dissolution of CBZ from its solid dispersion form is attributed due to coating of CBZ by the Gel [28].

<table>
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<tr>
<th>Description</th>
<th>*AUC&lt;sub&gt;0-8&lt;/sub&gt; (µg.hr/mL)</th>
<th>*C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</th>
<th>$T_{\text{max}}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain Carbamazepine</td>
<td>2.095±0.01</td>
<td>1.89±0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Solid dispersion of CBZ</td>
<td>3.579±0.06***</td>
<td>2.42±0.17***</td>
<td>3</td>
</tr>
</tbody>
</table>

*Mean ± SD; n=3

**Indicate significant difference at p ≤0.01 vs. pure drug.

***Indicate significant difference at p ≤0.001 vs. pure drug.

**CONCLUSION**

The solid dispersion of carbamazepine (CBZ) was prepared by using novel material ‘Gel’ with uniformity of content >96%. The results of compatibility studies exhibited no chemical interaction of CBZ with Gel. From the photographs of SEM studies it was concluded that there was no formation of needle shaped dihydrate form when solid dispersion of carbamazepine was kept in aqueous environment of which is less soluble than commercially available form III of carbamazepine.

There was increase in the dissolution as well as bioavailability of CBZ when carbamazepine was in solid dispersion form. In addition to this, the zeta potential on the solid dispersion of CBZ was found to be optimum i. e. between ±20 to ±30 mV which is essential to prevent agglomeration if the solid dispersion is presented in suspension form. Thus the solid dispersion prepared could be presented in the form of dry syrup. Since, adoption of logical approach to product development is utmost important; the methodology followed in the present work was found to be fit for the purpose.
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