ENHANCEMENT OF BIOAVAILABILITY OF CARBAMAZEPINE THROUGH FORMULATION OF A SOLID DISPERSION BASED SUSPENSION

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Summary

The objective of the work was to enhance the bioavailability of carbamazepine (CBZ), a poorly soluble drug, through formulation of a suspension using its solid dispersions. The solid dispersions were formulated with hydrophilic carriers like Polyethylene glycol 6000 (PEG 600), Polyvinylpyrrolidone (PVP), Hydroxypropyl methylcellulose (HPMC) by solvent evaporation method. They were characterized by infrared (IR) spectroscopy and Differential scanning calorimetric (DSC) studies. Based on the in vitro dissolution studies, the best solid dispersion was formulated into a suspension. It was further evaluated for drug content, particle size, drug release, sedimentation, and in vivo bioavailability. A marked increase in dissolution and bioavailability was exhibited by CBZ suspension made from its solid dispersion with HPMC, in the ratio of 1: 0.5. AUC was increased about 4.3 folds; C_{max} increased about 3.2 folds and t_{max} reduced by 2.3 times, when compared to the conventional suspension. The solid dispersion system of carbamazepine with a hydrophilic polymer HPMC has provided a simple method of preparing a suspension of carbamazepine with increased bioavailability.

Key words: Solid dispersion, carbamazepine, hydroxypropyl methylcellulose, bioavailability

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Introduction

Oral bioavailability of drugs is affected by a variety of factors, which influence their absorption from gastrointestinal tract. One determinant factor for absorption is drug dissolution, which is influenced by solubility of drugs in gastrointestinal fluids [1]. Nearly 40% of the new chemical entities currently being discovered are poorly water soluble and are associated with slow drug absorption leading eventually to inadequate and variable bioavailability [2]. Varieties of methods have been developed over the years to improve the release and dissolution of such drugs. Carbamazepine (CBZ) is an antiepileptic drug and considered to be a primary drug for the treatment of generalized tonic-clonic, simple and complex partial seizures. It is absorbed slowly and erratically after its oral administration due to its limited aqueous solubility. Peak concentration in plasma is usually observed 4 to 8 hours after oral ingestion and may be delayed as much as 24 hours [3]. It belongs to the class II drugs, according to the biopharmaceutic classification system. Compounds of this category have high intestinal permeability and low water solubility. Subsequently the bioavailability of such compounds is limited by their solubility in water [4]. Hence, improving the aqueous solubility should be able to enhance the bioavailability of this drug. Among the various approaches available to increase the aqueous solubility of drugs, solid dispersion technique has been used extensively [5]. But limited work has been reported on the application of solid dispersion in the formulation of suspensions. So, an attempt was made to prepare solid dispersions of CBZ with hydrophilic polymers like HPMC, PEG 6000 and PVP [5], and then formulate into suspensions, with an objective of enhancing bioavailability.

Materials and Methods

Materials

Carbamazepine (gift sample from M\S, Micro Labs., Bangalore) Polyethylene glycol 6000 (PEG-600) (Loba Chemie Pvt. Ltd., Mumbai) Polyvinylpyrrolidone (PVP) (Ozone International, Mumbai) Hydroxypropyl methylcellulose (HPMC) (Loba Chemie Pvt. Ltd., Mumbai)

Animals

New Zealand white rabbits (1.5-2.5 kg) maintained at 25 ± 1ºC was used for pharmacokinetic study. The animals were housed in steel metabolic cages and provided standard diet and water *ad libitum*. Preparation and characterization [6,7,8, 9] of solid dispersion and its suspension. The method of solvent evaporation was employed for the preparation of solid dispersions. Hydrophilic polymers such as PEG 6000, PVP, and HPMC were used as the carriers. Among these polymers, HPMC was selected for further studies based on the preliminary evaluation for drug release. The details of preparation[4] and analysis are as follows: The carbamazepine (CBZ) and HPMC were dissolved in a mixture of methanol: dichloromethane (2:1) to get a clear solution. The solvent was then evaporated at room temperature, the mass obtained was pulverized and sifted through mesh number 100. Three different batches were prepared with CBZ:HPMC ratios of 1:0.25, 1:0.5, and 1:1. The drug content was estimated spectrophotometrically at 285 nm [1,8]. IR spectroscopic analysis was used for studying drug - carrier interactions. Differential scanning calorimetry (DSC) was employed using Shimadzu DSC-50 thermal analyzer in the temperature range of 0- 300ºC, to confirm the formation of a solid dispersion[2]. The best product among these ratios was further formulated into a suspension[6,7,8,9] using sodium carboxy methylcellulose as suspending agent (1%), by simple dispersion method. During dispersion, the carrier is supposed to dissolve leaving behind very fine precipitate of the drug, giving rise to a suspension. A conventional suspension (CBZ conventional) was also prepared using pure drug along with the suspending agent by the method

of trituration and homogenization, to be used as the reference product. These suspensions were evaluated for drug content, particle size by optical microscopy, sedimentation and redispersibility, and *in vitro* drug release. *In vitro* release studies were carried out using paddle type USP XXIII dissolution apparatus, at 100 rpm in 0.1 N HCl[1].

Pharmacokinetic study in rabbits [1, 10]

The best formulation as per *in vitro* drug release profile was selected for *in vivo* pharmacokinetic study. Pharmacokinetic studies were conducted in rabbits as a single dose study using four animals each for conventional CBZ suspension and CBZ solid dispersion. All animals were fasted for 24 hours prior to the study. At 8.00 AM, conventional CBZ suspension (dose: 100 mg contained in 5 mL of suspension) and CBZ solid dispersion (e.g., 100 mg CBZ contained in 5 mL of suspension) were administered orally. Blood samples (2 mL) were collected from ear artery[3] into sterilized glass tubes at pre-dose (15 minutes before administration) and 1, 2, 3, 4, 5, 6, 7, 8 ,9 ,10, and 12 hours after the administration of the drug. The blood samples were then subjected to extraction procedure.

This study was conducted in accordance with the latest CPCSEA guidelines and the experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC/024/2004-05).

Extraction of drug from blood sample and estimation by HPLC method[11] 1.0 mL of blood was taken in a 15 mL centrifuge tube and 1.5 mL of ammonium chloride buffer (pH 9.5) and 5 mL of the extraction solvent [chloroform: propan-2-ol: n-heptane (10:14:26 V/V/V)] were added to this. The contents were then vortex mixed for 1 minute and centrifuged at 2000 rpm for 5 minutes. Aqueous layer was removed by suction and the organic layer was transferred into a clean glass test tube and evaporated under vacuum to dryness. The residue was reconstituted with 0.1 mL of the mobile phase [methanol: water (68:32)]. 60 µL of reconstituted solution was injected into HPLC column (Shimadzu-1601, C18-column and UV detector) for analysis.

Pharmacokinetic analysis [1,12]

Pharmacokinetic parameters such as peak plasma concentrations (C_{max}) and time of its occurrence (t_{max}) were read directly from the individual plasma concentration-time profiles. The area under the curve (AUC 0-12 hour) was calculated using GraphPad Prism 4.0 (GraphPad, Inc.) software, from plasma concentration- time plot.

Statistical analysis

Values are in mean ± SEM, statistical significance of the difference observed in various pharmacokinetic parameters were analyzed by unpaired Student t-test, using GraphPad Prism 4.0 (GraphPad, Inc.) software, $p \leq 0.05$ was considered to be statistically significant. The relative bioavailability of CBZ:HPMC suspension to that of conventional suspension was calculated using the formula,

$$\text{Relative bioavailability} = \left[\frac{AUC_{test}}{AUC_{reference}}\right] \times \left[\frac{Dose_{reference}}{Dose_{test}}\right]$$

Results

The formulated solid dispersions exhibited drug content of more than 90 %(91-97 %) indicating the suitability of the method of preparation. In the IR spectra, the principal peaks of

CBZ found at wave numbers 1677 cm-1, 1600 cm-1, 1298 cm-1, 800 cm-1, 765 cm-1, corresponded to the theoretical peaks [11], which were also found in the IR spectrum of solid dispersions. This reveals no interaction between the drug and the carrier. In the DSC thermogram (Figure 1), the DSC curve for CBZ alone shows two melting endotherms at 177ºC and at 193ºC. They correspond to the crystalline forms III and I of CBZ respectively[4]. The DSC curve for the solid dispersion shows the absence of peak at 177ºC and a small peak at 193ºC. A lack of drug melting endotherm in case of solid observed in the DSC for solid dispersion under study reveals conversion of most of the drug to amorphous form and only a small amount still remaining in the crystalline form[13]. A pronounced enhancement of both rate and extent of drug dissolution was observed with the solid dispersion i.e., CBZ:HPMC in 1:0.5 ratio, showing almost complete dissolution product was further formulated as a suspension using sodium carboxy methyl cellulose as the suspending agent. This suspension also exhibited still a better dissolution profile in comparison to conventional reference suspension, as shown in Figure 2. Microscopic evaluation of the suspension revealed considerable reduction in particle size. The mean size of particles in conventional suspension was 30.07 µm ± 0.606 whereas it was only 6.03 µm ± 0.196 in case of suspension of solid dispersion. Though there was complete sedimentation of particles in 48 hours, the sediment was easily redispersible on moderate shaking. The mean plasma concentration- time profiles of CBZ conventional and solid dispersion suspension are shown in Figure 3. The pharmacokinetic parameters calculated are given in Table 1. Pharmacokinetic parameters such as C_{max}, t_{max} and area under the curve (AUC) were significantly ($p<0.001$) higher in case of carbamazepine solid dispersion suspension. The C_{max} has been increased from 3.07 to 9.86 µg/mL; the t_{max} has been reduced from 8.5 to 3.75 hours and AUC has been raised from 17.3 to 74.62 µg.hour/mL with a relative bioavailability of 4.31.These results imply a marked increase in both the rate and extent of absorption.

Figure 1: DSC thermogram of CBZ, HPMC and CBZ:HPMC

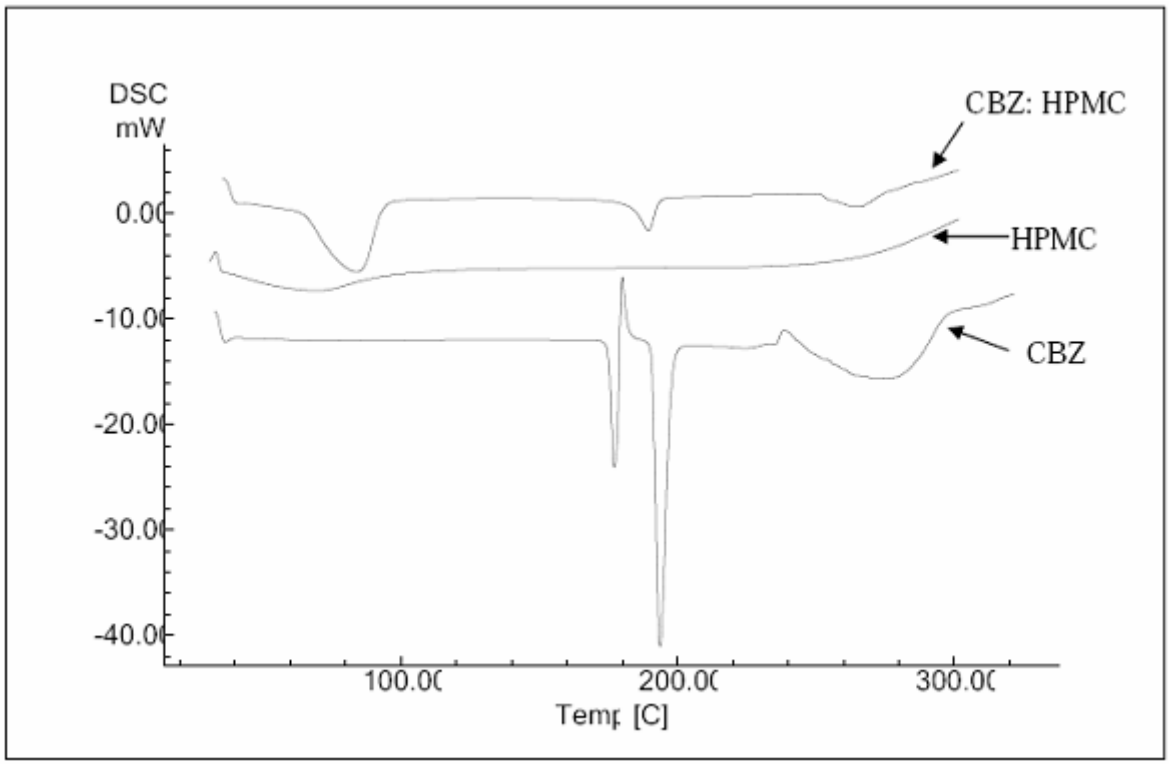

Figure 2: Drug release profile of conventional CBZ suspensions and suspensions formulated from solid dispersions with sodium CMC (1%) as suspending agent

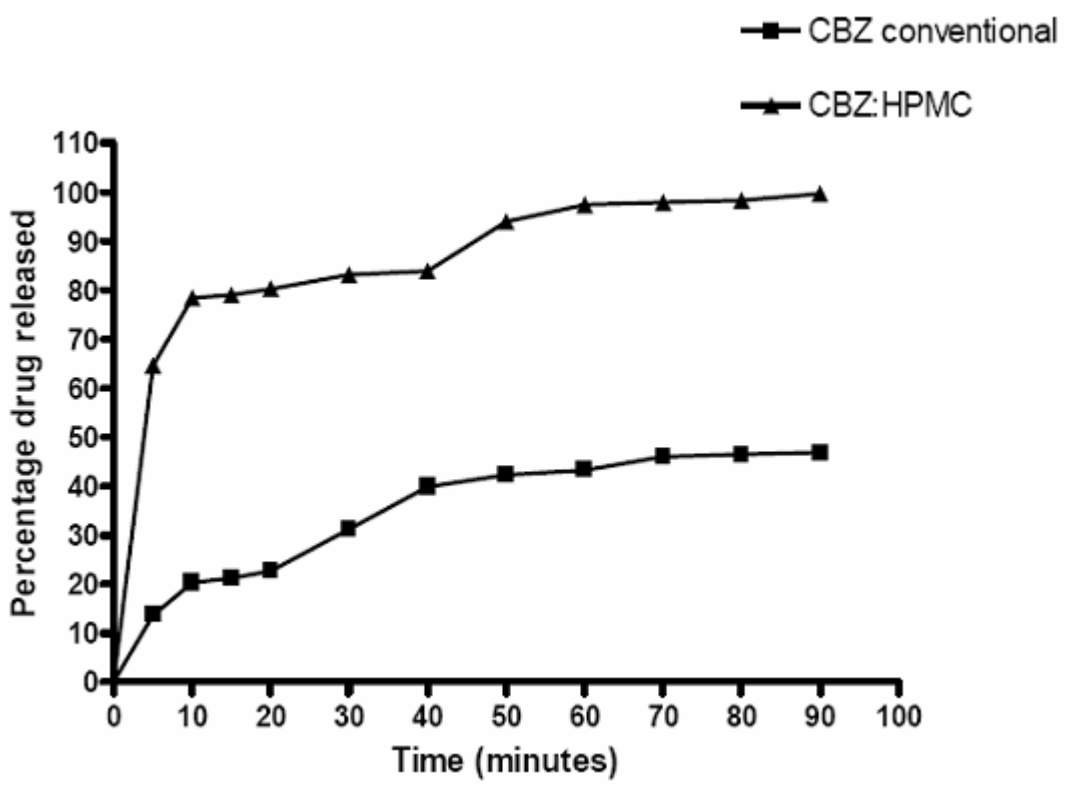

Figure 3: Plasma concentration-time profiles of CBZ conventional and CBZ: HPMC suspensions

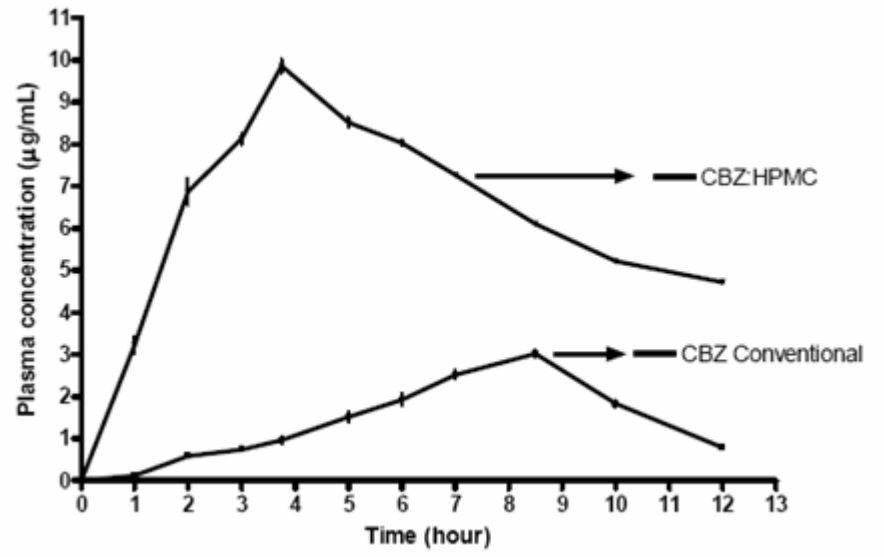

Table 1: Pharmacokinetic parameters of carbamazepine suspensions

Parameters	Conventional	CBZ:HPMC
C_{max} (μg/mL)	3.07 ± 0.094	9.86 ± 0.169 ***
t_{max} (hour)	8.50 ± 0.29	3.75 ± 0.25 ***
AUC (μg.hour/mL)	17.30 ± 0.199	74.62 ± 0.45 ***

Values are in mean ± SEM of 4 animals (n = 4)
*** p<0.001 compared to conventional (Unpaired Student's t-test)

Discussion

CBZ is a poorly soluble antiepileptic drug, facing the problem of low oral bioavailability. Formulation of such poorly soluble drugs for oral delivery presents one of the greatest challenges to formulation scientists in a pharmaceutical industry. Hence the present work was undertaken with the objective of enhancing the bioavailability by formulating a suspension of CBZ, using its solid dispersion. The main reasons postulated for the improvements in dissolution of drugs from solid dispersions are size reduction to a minimum, existence of drug in the form of solid solution in the water soluble carrier, solubilization effect of the carrier, formation of amorphous polymorphs with increased solubility. There have been several reports concerning polymorphism of CBZ and its influence on solubility and bioavailability [4]. The results obtained in the present study revealed a remarkable increase in the rate and extent of dissolution of the drug from its solid dispersion with HPMC. This enhancement can be certainly attributed to the reduction in particle size from 30 μm to 6 μm, as noted from the microscopic analysis. Also conversion of most of the crystalline form to amorphous polymorph is evident from the DSC thermogram. The positive effect of enhanced dissolution from the solid dispersion is further reflected in the outcome of the in vivo bioavailability studies carried out with the corresponding suspension. Since the bioavailability of CBZ is limited only by its dissolution rate, even a small increase in dissolution will result in a large increase in its bioavailability[4]. The results showing about 3.2, and 4.3 folds increase in the C_{max} and AUC respectively, and a decrease of t_{max} by 2.3 folds are very encouraging towards the formulation of such a product which can provide faster onset and higher intensity of action. Thus, the study has illustrated the potential use of a solid dispersion system in the form of suspension, for the delivery of a very poorly soluble drug, CBZ, with a better bioavailability.

Conclusion

The solid dispersion system of carbamazepine with a hydrophilic polymer HPMC has provided a simple method of preparing a suspension of carbamazepine with increased bioavailability.

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