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Research Article

IN VITRO ABSORPTION STUDY OF CARBAMAZEPINE SOLID DISPERSION USING EVERTED GUT SAC METHOD

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ABSTRACT

The oral Bioavailability of BCS (Bio Pharmaceutical Classification System) class II drug with poor solubility and reasonable permeability is limited by drug dissolution. In order to improve the aqueous solubility of the drug and dissolution of the drug, the solid dispersion was prepared and evaluated for its absorption in intestine using modified everted gut sac method. The solid dispersion of carbamezepine (CBZ) was prepared using polaxomer and guargum by kneading method. The CBZ and CBZ-SD (Solid Disposisi) shows 2.329% and 3.948% drug absorption, respectively. The data show that solid dispersion increase the absorption of the CBZ in CBZ-SD is more than 70% in comparison to pure CBZ. The increase in CBZ solubility of the SD could be attributed to several factors such as improved wettability, local solubilization, drug particle size reduction and crystalline or, interstitial solid solution reduction.

Key words: Everted gut sac method, solid dispersion, absorption, solubility

INTRODUCTION

A drug that is completely but slowly absorbed may fail to show therapeutic response as the plasma concentration for desired effect is never achieved. On the contrary, a rapidly absorbed drug attains the therapeutic level easily to elicit pharmacological effect. Thus, both the rate and extent of drug absorption are important (Ayyanna *et al.,* 2012). The absorption of drugs via the oral route is a subject of intense and continuous investigation in the pharmaceutical industry since good bioavailability implies that the drug is able to reach the systemic circulation by mouth. Oral drug absorption is affected by both drug properties and the physiology of the gastrointestinal tract (GIT1), or patient properties, including drug dissolution from the dosage form, the manner in which drug interacts with the aqueous environment and membrane, permeation across membrane, and irreversible removal by first-pass organs such as the intestine, liver, and lungs (Pang, 2003).

With rapid advances in drug design technologies, druggable compounds have dramatically been introduced. However, many of these molecules have suffered from low bioavailability upon oral administration. Therefore, improving oral drug absorption and bioavailability of drugs has become an important issue within the pharmaceutical industries. There are numerous approaches to enhance the intestinal absorption. Attempts have been made to increase the gastrointestinal absorption of water-soluble drugs, water-insoluble drugs, ionophores, and a variety of polypeptides and proteins (Chiang and Weiner 1987).

With the advent of high throughput screening (HTS) for agents with potential therapeutic value, the number of candidate drugs which are poorly soluble in water has increased considerably (Choud, 2010). Developing novel techniques to improve dissolution and bioavailability is of great importance in the development of pharmaceutical formulations, particularly those containing an active ingredient that is poorly soluble in water.

Several methods have been employed to improve the solubility of poorly water soluble drugs. Solid dispersions are the effective method to increase solubility profiles of BCS-2 class drug (Torrado *et al.,* 1996; Kushida *et al.,*

2002, Gohel *et al*., 2003) and they have proven to increase the amount of dissolved drug at the absorption site some time to supersaturated concentrations and consequently increase the bioavailability (Fawaz *et al.,*1996; Kai *et al.,*1996; Kohri *et al.,* 1999).

A solid dispersion can be defined as "A dispersion of one or more active ingredients in an active carrier or matrix at solid state prepared by the melting (fusion), solvent, or melting solvent method, or some time by kneading". In solid dispersion system, drug and carrier matrix are mixed. Generally a hydrophilic coat is developed upon the drug. The solid dispersion system has lower energy so that it dissolves easily and effectively as compared to the pure drug. The solid dispersion represents the system with lower crystallanity (Chiou *et al.,* 1971). Solid dispersions proved to be a system to increase the solubility of drug by various mechanisms like particle size reduction, change in drug crystallanity, and solubilising effect of hydrophilic carriers and better wettability of drug surrounded by carriers (Kyong *et al.,* 2011).

Carbamazepine (CBZ) is an antiepileptic with different crystalline forms, all of which have variable dissolution leading to irregular and delayed absorption. CBZ has partition coefficient of 2.45 and is practically insoluble in water (around 113μg/mL at 25°C). CBZ and similar drugs of low solubility and high permeability i.e. "Class II" in the Biopharmaceutical Classification System are more likely to display dissolution-dependent oral bioavailability (Sethia and Squillante 2004).

The main objective of the present study was to prepare solid dispersion by kneading method and evaluate the influence of solid dispersion on the solubility, dissolution rate and intestinal absorption of Carbamazepine.

METHODOLOGY

Materials

CBZ was obtained as gift sample from Pschyo excipients, Ludhiana, Punjab, India. Polaxamer-407 were obtained from BASF Corporation Mumbai. Guar gum was obtained from Yarrow Chem. Mumbai. All other chemicals and regent were of analytical grade and used as received.

Preparation of solid dispersion

Solid dispersion of carbamazepine was prepared with a hydrophilic carrier polaxamer and guragum (Table I) by kneading method. A selected amount of drug, polaxamer and guar gum was weighed and passed to the pestle and mortar then adequate amount of water was added to it. Then the paste was kneaded and allowed to dry for 24hrs. The dried mixture was crushed and passed through sieve no.60. The solid dispersion was placed over anhydrous calcium chloride in the dessicator until use.

Table I. Formula for solid dispersion

	Amt. (mg)	Category
CBZ	200	Active Ingredient
Polaxomer	840	Polymer
Guar gum	160	Co-polymer

Preparation of phosphate buffer pH 7.4

Potassium dihydrogen 26.67g phosphate was dissolved in 500mL of distilled, water and 390.10mL of dibasic sodium hydroxide solution was added to it. Then the final volume was made up to 2L.

Preparation of stock and standard solution of carbamazepine in phosphate buffer

100mg of drug was dissolved in 10mL of methanol and volume was made up to 100mL with phosphate buffer (Stock Solution 1000μg/mL). Then 10mL of stock solution was taken and diluted to 100mL with buffer to prepare solution of 100μg and further dilution ranging from 1μg to 10μg were made by suitable dilutions from this solution using phosphate buffer.

Everted sac modification method

Intestinal permeability studies using everted gut sac were performed using established methods adopted from literature after some modifications. The freshly excised goat intestine was collected from the local slaughter house immediate after slaughtering the goat and transfer to the laboratory in ice-cold normal saline. Remove the small intestine by cutting each end. The middle small intestine

Table II. The relationship between concentration and absorbance value

S.No.	Conc (µg)	Abs ±*S.D**
1	1	0.06152 ±0.001
2	2	0.12352 ±0.005
3	3	0.18304 ±0.002
4	4	0.24557 ±0.001
5	5	0.30440 ±0.001
6	6	0.36708 ±0.001
7	7	0.41708 ±0.005
8	8	0.49208 ±0.005
9	9	0.54955 ±0.001
10	10	0.61478 ±0.006

*n=3

Table III. *In vitro* absorption using everted gut sac method

S.No	Time (Mins)	%Drug absorbed ±*S.D** (CBZ)	%Drug absorbed ±*S.D** *(CBZ-SDs)*
1	10	0.629±0.10	1.224±0.08
2	30	1.003±0.39	2.156±0.51
3	60	1.321±0.45	2.965±0.65
4	90	1.663±0.26	3.139±0.25
5	120	2.329±0.24	3.948±0.24

*n=3

was obtained from the proximal end (Mahmood, 2004). The excised pieces of intestinal segments were immediately flushed with ice-cold normal saline to clean it from intestinal contents and removed the underlying mesenterium, blood and debrise (Ling 2007).

A pieces of small intestine having 25±2cm length was taken. Intestinal segments were gently everted over a glass rod (Li 2008). Tie a ligature over the thickened part of the glass rod and evert the sac by gently pushing the rod through the whole length of the intestine. Remove the rod and place the intestine in a ice cold normal saline solution at room temperature. After eversion the mucosal side comes out and serosal side is present inside, tied the one end of the intestine and the other end of the intestine is connected to a cannula. Take 45mL of plain buffer solution inside the intestine and the intestine is immersed in a jar containing 900mL buffer solution (pH 7.4). The medium was kept under mild agitation while oxygenation was maintained by an oxygen pump. The oxygen concentration of the medium was controlled with an oximeter. The temperature was maintained at 37°C±0.5°C. Peristaltic movements are provided with stirrer. The buffer solution contains the drug present in outside (mucosal side) and plain buffer present in the intestine (serosal side). In this technique repeated sampling is possible (Ayyanan, 2012). The study was carried out by taking 40mg of pure drug and dispersion containing the same amount of drug (Naima Zerrouk *et al.,* 2001). At predetermined intervals, 1mL aliquots of sample was withdrawn from the sac and same volume was replaced with fresh buffer. The study was performed upto two hours. The concentration of drug that traversed intestinal surface was analysed using UV Spectro-photometer at 285nm. All experiments were conducted in triplicate.

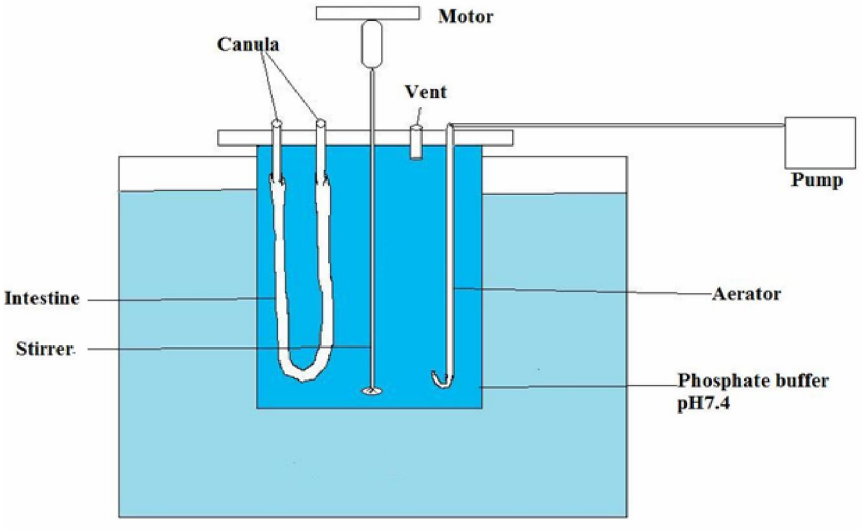

Figure 1. Dramatically diagram of assembly used

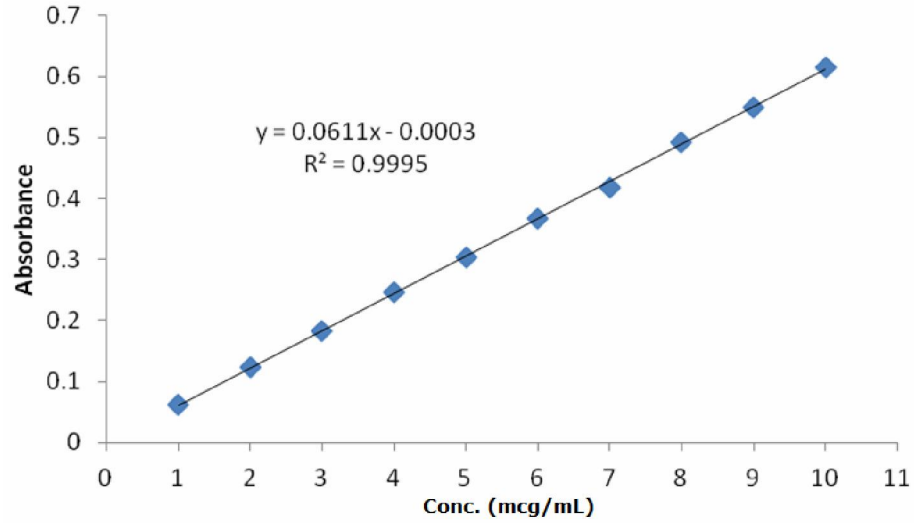

Figure 2. Standard curve of carbamazepin in phosphate buffer

RESULT AND DISCUSSION

Standard curve

The Carbamazepine was found to be soluble in organic solvents such as methanol. The Methanol was used as cosolvent for dissolving the drug as per USP (1% Co solvent can be used for dissolution).The standard plot was found to be linear for the concentration studied with a linear regression coefficient R^2 of 0.999 (Figure 2).

Everted gut sac method

The inverted intestinal sac model is useful for studying physiological and molecular processes of absorption, providing physical and chemical information on the properties of the pharmaceuticals, and ascertaining the role of the absorption facilitators and inhibitors of intestinal metabolism. Such study on the intestinal absorption sites would be expected to prove the adsorption improvement effect of

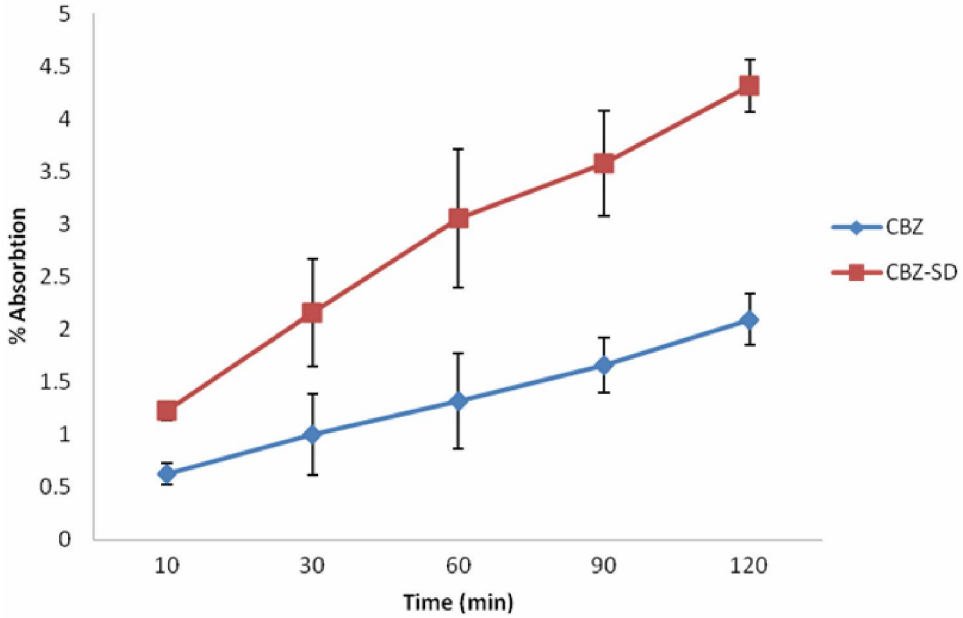

Figure 3. *In vitro* absorption using everted gut sac method

solid dispersion. The absorption data showed that CBZ-SD displayed good absorption in intestinal area as compared to pure CBZ.

The absorption of CBZ in SDs was compared with the absorption of pure CBZ. Figure 3 shows the profile of intestinal absorption of CBZ over a 120-minute period. At the end of the study, solid dispersions showed 3.948% drug absorption and CBZ showed 2.329% drug absorption. The data shows that solid dispersion increases the absorption of the CBZ around 70% higher comparatively than pure. The improvement in the intestinal permeation is related to the increase in the dissolution rate of CBZ caused by Solid Dispersions. Guar gum has hydrophilic nature (Nagesh *et al.*, 2011) and polaxamer has the amphillic nature (Li *et al.,* 2011). Both guar gum and polaxamer has the synergistic effect in enhancement of solubility of the CBZ.The kneaded mass of the solid dispersion i.e. drug and polymer was in close contact. When this mixture comes in the contact of the fluid or buffer medium the polymer particles get hydrated rapidly and solubilising the adjacent drug particles in to the medium (Newa *et al.,* 2007). Polaxamer also acts as the surfactant hence decreases the surface tension between the dissolution media and drug particles thus brings about the additional wettability and solubilising effect (Li *et al.,* 2011). All these factors worked together and increased the solubility of the drug to a remarkable extent.

CONCLUSION

Carbamazepine is practically insoluble in water. However, its very low aqueous solubility and poor dissolution can cause formulation problems and limit its therapeutic application by delaying the rate of absorption and the onset of action. SD is a useful strategy for increasing the bioavailability of CBZ and could prove equally useful for other pharmaceutical compounds with poor water solubility.Present study also established the synergistic effect of natural guar gum and polaxamer in the solubility enhancement. The results of our study showed that CBZ-SD is better absorbed in intestine compared to Pure CBZ. The mechanisms responsible for this improvement could be a solubilization effect of the carrier, and/or conversion of the drug from crystalline to amorphous form.

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