

Original Research Article

Solubility and Bioavailability Improvement of Gliclazide by Solid Dispersions Using Novel Carriers

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Abstract

Solid dispersions (SD) of Gliclazide (Glc); a poorly water soluble drug and Vitamin E TPGS (Vit E), Gelucire 44/14 (Gel44) and Gelucire 50/13 (Gel50) prepared by fusion method. Prepared solid dispersions were subjected for solubility studies in various buffers. Best formulation was selected based on solubility and a fast dissolving tablet was developed, dissolution was performed in 7.4 pH buffer. SD s was characterized by X-RD and DSC. After ageing for three months, fast dissolving tablets were again tested for dissolution. Formulation F3 was selected for in vivo bioavailability study. Solubility and dissolution was proportionately improved for all the formulations with the carrier. X-RD and DSC studies revealed that the crystal nature of the drug has been transformed to amorphous after preparing SD. *In vivo* study in albino rabbits has justified the improvement of solubility by enhancing the bioavailability of the SD. Vit E TPGS has been proved to be effective in improving the solubility and in vivo bioavailability of Gliclazide.

Keywords: Solid dispersion, Vitamin E TPGS, Gelucires, Fusion, bioavailability.

Introduction

Gliclazide (Glc) is a second-generation sulphonyl urea oral hypoglycemic agent used in the treatment of non-insulin dependent diabetes mellitus (NIDDM). It acts by stimulating insulin secretion from pancreatic beta cells. Gliclazide appears to be a drug of choice in prolonged therapy for the control of NIDDM. In the long-term, it reduces hepatic gluconeogenesis and increases insulin effects by acting at receptor or post-receptor sites. It also inhibits platelet aggregation and increases fibrinolysis [1]. However, the drawback of this potentially useful hypoglycemic agent is that it is highly hydrophobic and practically insoluble in water [2]. In general, rapid gastrointestinal (GI) absorption is required for oral hypoglycemic drugs, in order to prevent a sudden increase in blood glucose level after food intake in patients with diabetes mellitus [3]. However, the GI absorption rate of Gliclazide in conventional dosage form appears to be rather slow. Slow absorption of a drug usually originates from either its poor dissolution from the formulation or poor permeability across the GI membrane. This eventually limits its oral bioavailability and therapeutic efficacy [4]. Various techniques have been used to improve the solubility and dissolution rate of poor water soluble drugs. Among them, the solid dispersion method is the most frequently and effectively used one

[5-8]. Solid dispersions (SDs) of poorly soluble drugs in hydrophilic carrier matrix have been reported to improve their solubility and dissolution rate [9, 10]. Moreover, they are also proven to enhance their bioavailability by increasing their dissolution in gastrointestinal fluids [11]. Many different water - soluble carriers have been employed for preparation of solid dispersions of poorly water soluble drugs [12 - 14]. The most common ones are various 711 β - cyclodextrin, and hydroxypropyl methylcellulose [15 - 18]. But they required to be used at higher concentrations which is a limitation for their commercial usage. The use of lipid-based amphiphilic carriers with solubilizing properties like Gelucire 44/14 and Vit E has recently attracted much interest [19]. Gelucire 44/14 is a saturated polyglycolized glyceride consisting of a well-defined mixture of mono-, di and tri-glycerides and mono- and di-fatty acid esters of polyethylene glycol. It has a hydrophilic-lipophilic balance (HLB) value of 14. Vit E (d_-tocopheryl polyethylene glycol 1000 succinate) is a water-soluble derivative of Vitamin E consisting of a hydrophilic polar head group (polyethylene glycol) and a lipophilic tail (tocopherol succinate) resulting in amphiphilic properties (HLB value = 13). Vit E has a relatively low critical micelle concentration of 0.02 wt.% above which this carrier offers the advantage of spontaneously solubilizing lipophilic drugs upon contact with an aqueous medium to form a fine emulsion that, in turn, further



facilitate drug absorption. Gelucire 44/14 and TPGS have relatively low melting temperatures of 44 °C and a range of 37–41 °C, respectively [20]. They can be used at very low concentration to get same magnitude of solubility which was exhibited by the earlier carriers [21]. In the present study, Gelucires and Vit E were used as hydrophilic carriers for their excellent surfactant properties and oral safety. The present research work aimed to increase the gastric absorption and to minimize the inter-individual variability in bioavailability of Gliclazide due to its hydrophobic nature and poor dissolution rate.

Materials and Methods

Gliclazide (Glc) was obtained as a gift sample from Aurabindo Pharma Ltd, Hyderabad, India. Vit E was gifted by Dr.Reddy's Laboratories, Hyderabad, India. Gelucires were obtained as gift samples from Gattefosse. All other reagents used were of analytical grade and were obtained from S.D. Fine Chemicals, Mumbai, India. Animal studies were approved and conducted in accordance to the Institutional Animal Ethics Committee.

Preparation of solid dispersions

Fusion (Melt) Method

Each SD preparation containing different ratio (1:0.1, 1:0.2 and 1:0.3 w/w) of Glc and respective carriers (Vit E TPGS, Gelucire 44/14 and Gelucire 50/13) were prepared by fusion method. Weighed amount of Glc (table 1) was transferred into molten carrier in china dish which was heated to melt on a heating mantle, mass was then mixed thoroughly until entire drug get dissolved in the molten carrier, allowed to cool then sifted through 60 mesh sieve and stored in air - tight containers until further evaluation.

Preparation of physical mixtures

Physical mixtures (PM) of Glc and Vit E TPGS in 1:0.1 and 1:0.3 w/w ratios were prepared by blending the two components in geometric proportion in a mortar for 5 minutes in order to obtain a homogeneous mixture. The resulting mixtures were sieved through 60 mesh sieve and stored in air-tight containers until further evaluation.

Phase solubility studies

Excess amount of drug was added in screw-capped conical flasks containing 50 mL of aqueous solution each of different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5% w/v) of Vit E TPGS in double distilled water [22]. The suspensions were continuously stirred on an orbital shaker (Remi, India, Mumbai), at room temperature with 200 rpm for 48 hours. The suspensions were filtered through 0.45µ Millipore membrane filter. The filtrates were suitably diluted with water and analyzed, spectrophotometrically (Shimadzu UV-1800, UV/Vis spectrophotometer, Shimadzu Corp, Japan), for the dissolved drug at 230 nm. Respective

concentrations of Vit E aqueous solution used as blank in the study. All assays were performed in triplicate. The standard curve of Glc in distilled water over a concentration range of 0 to 20 µg/mL at 230 nm was plotted.

Differential scanning calorimetry

The DSC Thermograms were recorded for drug, carrier and solid dispersions. About 2–4 mg of sample was taken in an open aluminium standard pan, heated at a scanning rate of 10 C /min from a temperature 0 to 300 °C under a nitrogen gas flow. The heat of fusion of pure drug, carriers, and solid dispersions were measured separately.

X-ray diffraction Analysis

X-ray diffraction patterns of the samples (drug, Solid dispersions) were obtained using X-ray powder diffractometer. The scanning angle ranged from 10⁰-80⁰ of 2θ.

Preparation of Fast Dissolving tablets

Weighed amount of Gliclazide/SD was mixed with excipients in the order of aerosol, avicel, croscopolvidone and talc in a mortar (table 2). Mixed portion was passed through sieve #40, compressed as 150 mg flat tablet on a rotary tablet compression machine with an average hardness of 2-3 kg/cm².

Drug content

Samples of physical mixtures and solid dispersions containing an equivalent of 80 mg of Glc were dispersed in a suitable quantity of methanol and sonicated for 10 minutes. The drug content of the filtered samples was determined at 230 nm by UV spectrophotometer after suitable dilution with methanol.

In vitro dissolution studies in 7.4 pH Phosphate buffer

Tablets prepared from pure Glc, physical mixture and solid dispersions equivalent to 80 mg of Glc were used for the dissolution studies. The study was performed in 900ml phosphate buffer pH 7.4 using USP XXV Type II apparatus (Electrolab, India) with a paddle speed of 50 rpm at 37.5±2 °C (18). Aliquots of 5ml, withdrawn at different time intervals, filtered and measured at 230 nm spectrophotometrically, after suitable dilution with the dissolution medium if needed, to determine the percent drug released. An equal volume of fresh dissolution medium was replaced after each sampling to maintain the sink conditions. All studies were performed in triplicate.

In vivo bioavailability studies

Based on the in-vitro dissolution profile, an optimum solid dispersion Glc:Vit E (1:0.3w/w) prepared by fusion method was selected for comparison of in-vivo performance against pure Gliclazide. A two-way crossover study was done as follows. Nine



male albino rabbits of average weight 1.8 ± 0.10 kg were used for the study. The rabbits were divided into three groups of three rabbits in each ($n=3$). All the rabbits were fasted overnight with ad libitum access to water during the experiment. In first level of study, group one animals received a single dose of Gliclazide (40 mg/kg), formulated as aqueous suspension in 0.5% w/v sodium carboxy methyl cellulose. The second group was administered a suspension containing solid dispersion Glc:Vit E (1:0.3w/w) at the same dose. Third group was administered with 0.5% w/v of sodium carboxy methyl cellulose aqueous suspension without drug, which serves as control. The suspensions were administered orally through a sterile pediatric feeding tube (size 8) followed by 2 ml of distilled water to wash off any drug remaining in the feeding tube and upper alimentary tract. 1mL of blood sample was collected using 22 gauge needle from the shaved marginal ear vein into heparinized Eppendorf micro-centrifuge tubes at time intervals of 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours. Xylene was applied to the marginal ear vein before withdrawal, which causes blood vessel to dilate. The blood samples were immediately centrifuged at 6000 rpm for 10 minutes (Remi Centrifuge, India) to separate the plasma and stored at -40°C (Blue Star, India) until further analysis. In crossover study, solid dispersion was given to first group and pure drug was given to second group and the same experimental procedure was repeated (23). A two week washout period was maintained between first and second level of study. The experimental procedure was approved by institutional animal ethical committee.

Analysis of Glc by HPLC

The concentration of Glc in the plasma samples was analyzed by an established standardized reverse phase HPLC method (23). Mobile phase was comprised a mixture of pH 3.6 Phosphate buffer: Acetonitrile (40:60) which was run at a flow rate of 0.8 ml/min. 20 μ l sample was injected and the eluting peaks were monitored at a λ_{max} of 228 nm. The plasma samples were spiked with known concentrations of Glc in acetonitrile so as to obtain plasma concentrations of 0, 1, 2, 3, 4, and 5 μ g/ml. To 0.5 ml of the spiked plasma sample taken in a polypropylene centrifuge tube, 0.5 ml of acetonitrile was added and the samples were vortexed for 30 seconds to precipitate plasma proteins. 2 ml of chloroform was added and the samples were vortexed again for 2 minutes to extract Glc into the organic layer. The mixture was then centrifuged for 15 minutes at 3000 rpm (Remi centrifuge). Then, 1 ml of the organic layer was transferred to a clean glass vial and evaporated in a vacuum oven whose temperature was maintained constant at $40 \pm 1^{\circ}\text{C}$. The dry residue was reconstituted with 1ml of acetonitrile. The resulting solution was filtered through 0.45 μ syringe filter (Millipore) and 20 μ l of the sample was injected and analyzed for Glc content.

Determination of PK parameters

The pharmacokinetic parameters were calculated using PK Solver 2.0, One-compartmental Pharmacokinetic Data Analysis Software.

The area under the plasma concentration time curve (AUC₀₋₂₄), the maximum plasma concentration C_{max} and the time to achieve the maximum concentrations T_{max} , the elimination rate constant (Kel) and absorption rate constant (K_a) were directly determined from the resulting concentration-time profile. The data from different formulations was presented as mean ($n=3$) \pm standard deviation.

Results and Discussion

Phase solubility studies

The solubility of drug against increasing concentration of carrier was observed in all formulations. Solubility of pure Glc in water at $37 \pm 1^{\circ}\text{C}$ was found to be 758 $\mu\text{g/ml}$. The order of solubility in various pH buffers was found to be 7.4 pH > Distilled water > 6.5 pH > 0.1 N HCL. This clearly indicates that a pH dependent solubility behavior was exhibited by Gliclazide. Out of the three carriers employed, solid dispersions prepared with Vit E TPGS were exhibited highest solubility than other. Among various concentrations, 1:0.3 % w/w of drug: Vit E was projected as a best concentration. The solubility values were revealed that the fusion process is a better approach rather simple physical mixture. Because the solubility values of all carriers at 1:0.3% w/w were superior to that of physical mixtures of same concentration.

Differential scanning calorimetry studies for Gliclazide

The thermograms of the Gliclazide, Vit E TPGS, Gelucire 44/14, solid dispersion of Gliclazide Vit E TPGS, Gelucire 44/14 were shown in figure 1.

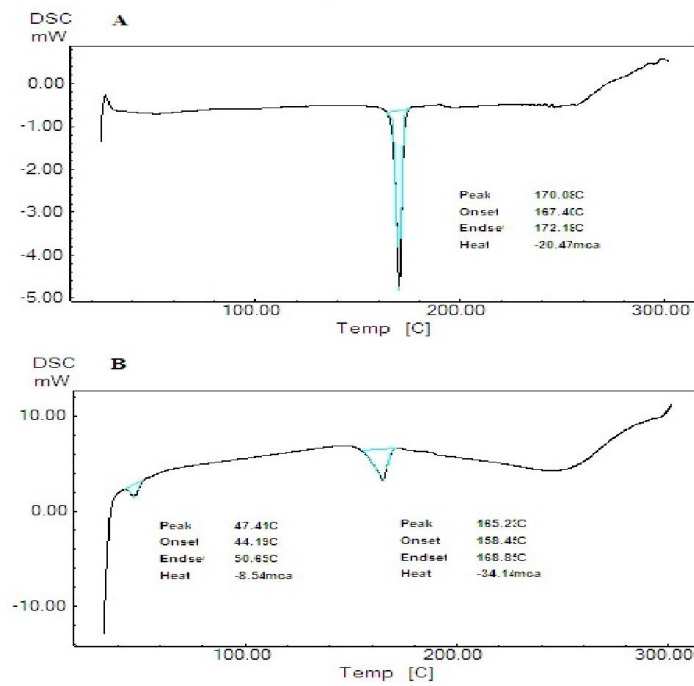
The DSC thermograms of Gliclazide exhibited a sharp endothermic peak around 170°C corresponding to melting point. The DSC thermograms of Vit E TPGS, Gelucire 44/14 exhibited a broad endothermic peak around 37.9°C , 49°C corresponding to their melting point. The broad peak may be due to loss of water in the carrier. The thermograms of SDs of Vit E TPGS, Gelucire 44/14 showed a short endothermic peak of drug at 165°C , 168.2°C respectively. It is clearly evidenced that there was a change in the intensity of the peak in solid dispersions compared to that of pure drug which indicates the dispersion of drug in polymeric matrix.

X-RD

There was significant decrease in intensity of some major Gliclazide crystalline peaks in diffractograms of SDs. The lack of numerous distinctive peaks of the drug in SDs demonstrated that high concentration of the drug was dissolved in the solid-state carrier matrix in an amorphous structure suggesting the transformation of crystalline form of Gliclazide to amorphous form in the solid dispersions (fig 2).

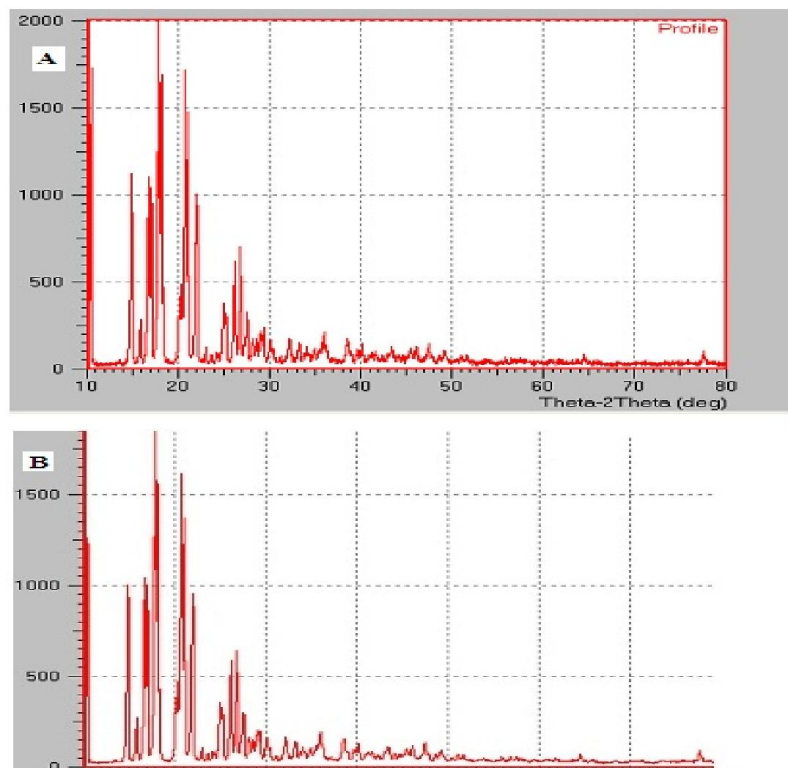


Figure 1: DSC Thermograms of Gliclazide pure drug and solid dispersions



*A - Pure drug, B- Glc-Vit E

Figure 2: X-RD of Gliclazide pure drug and solid dispersion



A- Pure drug, B- Glc- Vit E

Figure 3. Mean dissolution rate of Gliclazide Fast dissolving tablets in 7.4pH phosphate buffer

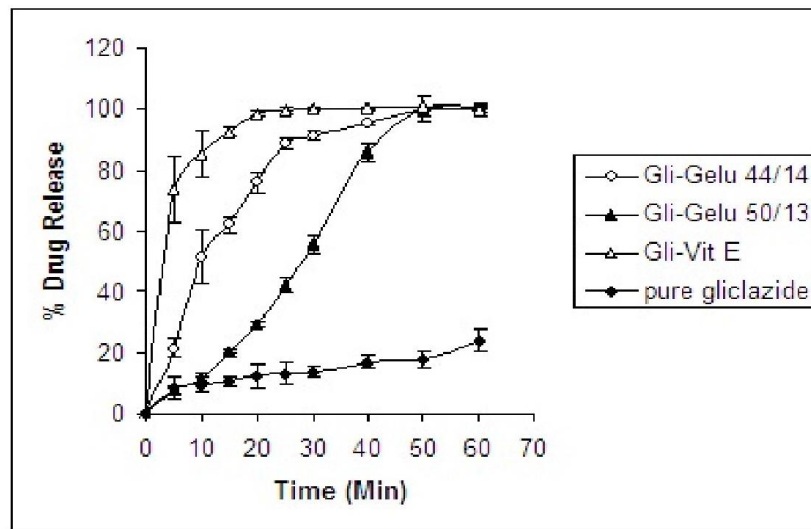
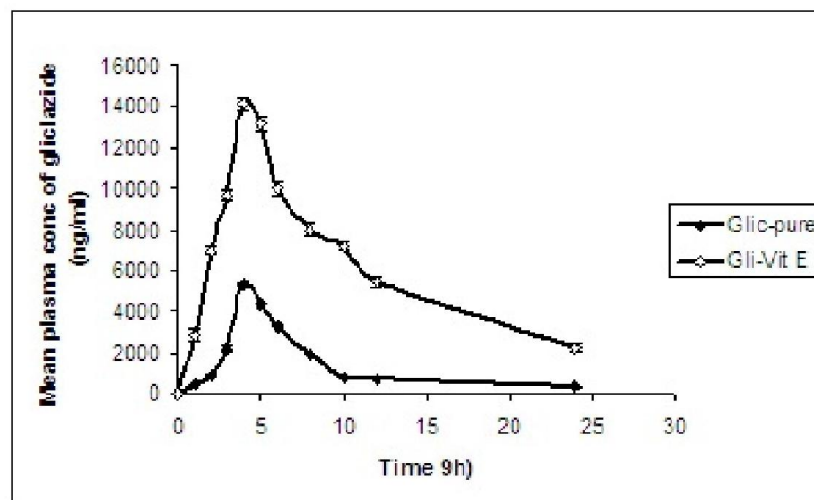


Figure 4: Mean plasma concentration of Gliclazide



Drug content of solid dispersions

Content uniformity for all tablet formulations was done using UV Spectroscopy. The values were found to be (table 4) in the range of 98.44 ± 0.12 to 102.0 ± 0.20 % w/w of drug.

Dissolution test in phosphate buffer (pH 7.4)

The in vitro release study for fast dissolving tablets of pure Glic and solid dispersions were performed in phosphate buffer pH 7.4. The drug: polymer ratio 1:0.3 w/w was considered to be optimum for prepared solid dispersions at solubility studies for all carriers. Hence the comparative dissolution was carried out in pH 7.4 dissolution media for the formulations contain 0.3% w/w of carriers

with that of formulation contain pure drug. The results of dissolution studies were supporting the results found at solubility study. The dissolution profiles of F3, F6, F9 and Glic-P were compared in figure 3.

It was observed that after 20 minutes of dissolution <20 % of drug was released in Glic-P, whereas in case other three formulations the order of drug release was in the order of F3>F6>F9 by releasing 100%, 85%, 55% of the drug respectively after 30 minutes.

Hence F3 was selected for comparing the bioavailability with that of pure drug.

In vivo bioavailability studies

An in vivo absorption study was performed by taking best SD (F3) to determine whether the improvement in solubility and dissolution can enhance the bioavailability when compared with that of pure drug. The mean plasma concentration – time profile of Gliclazide after a 40mg/kg single oral dose in rabbits was shown in figure 4. Pharmacokinetic parameters were listed in table 5. It shows that the AUC 0-t values of Gliclazide following the oral administration of F3 and Glc-P was 136740.81 ± 228.26 ng/ml*h and 34831.32 ± 321.23 ng/ml*h respectively. These values revealed that there is a significant improvement in the bioavailability of the drug in the form of solid dispersion compared to pure form of drug. Similar findings were reported by Hisham S et al (24).

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Conclusion

Development of solid dispersions to improve the solubility of poorly soluble drugs will become the prime choice rather other techniques. Carriers like Vitamine E TPGS, gelucire 44/14 and gelucire 50/13 were able to improve the solubility of Gliclazide even at very lower concentrations. Finally solubility improvement by Vitamin E TPGS can also helps in the improvement of bioavailability.

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