LYOPHILIZED GLICLAZIDE-POLOXAMER SOLID DISPERSIONS FOR ENHANCEMENT OF IN-VITRO DISSOLUTION AND IN-VIVO BIOAVAILABILITY

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ABSTRACT
Gliclazide (GLC), an oral hypoglycemic agent, is characterized by low solubility in gastric fluid, low dissolution rate and inter-individual variability in bioavailability. The objective of this study was therefore to design optimized solid dispersions (SD) of GLC and hydrophilic carriers viz., poloxamer 407 (PXM) by lyophilization method in an attempt to enhance the aqueous solubility and therapeutic efficacy of the drug. Phase solubility study with increasing PXM concentrations (0.5 to 10% w/v) was done to study the influence of polymer concentration on solubility of GLC SD’s of GLC and PXM in 1:1, 1:3 and 1:5 w/w ratios were prepared by physical mixing and lyophilisation (freeze drying) method, followed by dissolution studies. A comparative in vivo study between optimized SD and GLC was conducted on twelve healthy New Zealand rabbits. The dissolution rate of GLC from the lyophilized dispersions was greatly enhanced as compared to those from physical mixtures and pure drug. The in vivo studies indicated that the pharmacokinetic parameters following oral administration of the optimized SD and pure GLC were significantly different (P < 0.05). The peak serum concentration (Cmax) for the lyophilized SD and GLC were found to be 3.01±0.42µg/mL and 2.27 ± 0.39µg/mL respectively, whereas the time required to reach the peak serum concentration (Tmax) for the optimized SD was significantly shorter (2.16±0.41h) compared to that for GLC (4.33±0.52h). The relative bioavailability of the SD under in-vivo test was found to be 158.52%. These results demonstrate that the use of a suitable hydrophilic carrier like PXM to formulate SDs by the lyophilisation technique can rapidly accelerate the solubility and in vitro dissolution of a lipophilic drug like GLC. The suggested method also provides good signs of improvement in the rate of absorption as well as bioavailability of gliclazide following oral administration.

Keywords: Gliclazide, Poloxamer 407, Lyophilisation, 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 cells4. Prior research work revealed that it has good general tolerability, low incidence of hypoglycemia and low rate of secondary failure. In addition, it has the potential for slowing the progression of diabetic retinopathy4. For these reasons, 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 fibrinolysis5. However, the drawback of this potentially useful hypoglycemic agent is that it is highly hydrophobic and practically insoluble in water4. 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 mellitus5. However, the GI absorption rate of gliclazide, in conventional dosage form appears to be rather slow. Several studies using healthy volunteers or patients revealed that the time to reach peak serum GLC concentration ranged from 2 to 8 hr following oral administration of a conventional tablet 4,6. 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 7.

For decades, various techniques have been used to improve the solubility and dissolution rate of poorly water soluble drugs. Among them, the solid dispersion method is the most frequently and effectively used one 5,9-12. Solid dispersions (SDs) of poorly water-soluble drugs in hydrophilic carrier matrix have been reported to improve their solubility and dissolution rate 13,14. Moreover, they are also proven to enhance their bioavailability by increasing their dissolution in gastrointestinal fluids31. Since long, many investigators have studied SDs of poorly water-soluble drugs with various pharmacologically inert carriers to increase the dissolution and oral absorption of poorly water-soluble drugs; however, only a few systems are useful commercially9,16,17. Many different water-soluble carriers have been employed for preparation of solid dispersion of poorly water soluble drugs 10-20.

The most common ones are various grades of polyethylene glycols, polyvinyl pyrrolidone, lactose, β-cyclodextrin, and hydroxypropyl methylcellulose 21-24. Recently, poloxamers, a group of block copolymer nonionic surfactants, have attracted considerable attention for application in preparation of solid dispersions 25-26. These polymers are widely used as emulsifiers, solubilizing agents, and suspension stabilizers in liquid, oral, topical, and parenteral dosage forms and also act as wetting agents and plasticizers, and have been reported for enhancing the solubility and bioavailability of sparingly soluble drugs in solid dosage forms27. Poloxamer 407 (PXM) is specifically one of the tri block copolymer grade consisting of a central hydrophilic block of polypropylene glycol flanked by two hydrophilic blocks of polyethylene glycol (PEG). The approximate lengths of the two PEG blocks is 101 repeat units while the approximate length of the propylene glycol block is 56 repeat units. PXM’s have been recently widely used as wetting and solubilizing agents as well as surface adsorption excipients. They have been employed to enhance the solubility, dissolution and bioavailability of many hydrophobic drugs using various techniques. For some drugs, the improvement in solubility using PXM was higher compared to the other meltable polymers such as PEGs and complex forming agents such as cyclodextrins28. In the present study, PXM was thus empirically selected as a hydrophilic carrier for its excellent surfactant properties and oral safety. The lyophilisation (freeze drying) method was used to prepare GLC-PXM SDs in a relatively easy, simple, rapid and reproducible manner and the formulated SDs were evaluated for their in vitro and in vivo performances 29,30.

The presented research work thus deals with the erratic gastric absorption and inter-individual variability in bioavailability of gliclazide due to its hydrophobic nature and poor dissolution rate29,30. The formulation of solid dispersion of gliclazide with poloxamer by the freeze drying technique was never cited before. The reported method overcomes these problems which is evident from the pharmacokinetic studies using a non-tedious analytical method and holds good potential for commercial scale up.
MATERIALS AND METHODS

Gliclazide (GLC), Glibenclamide (GLB) and poloxamer 407 (PXM) were purchased from Sigma Aldrich (Germany) and BASF respectively. All other reagents used were of analytical grade and were obtained from S.D. Fine Chemicals (India). Ultrapure double distilled water (Millipore) was used throughout the study. Animal studies were approved and conducted in accordance to the Institutional Animal Ethics Committee.

Phase-solubility studies

Phase solubility studies were carried out according to the method reported by Higuchi and Connors.\textsuperscript{4} Excess amount of drug (25 mg) was added in screw-capped conical flasks containing 50 mL of aqueous solution each of different concentrations (0.5, 1, 2, 5 and 10% w/v) of PXM in double distilled water. The suspensions were continuously stirred on an orbital shaker (maxQ 3000, Barnstead Lab-Line, Thermo Scientific, USA) at 25±1°C and 200 rpm for 48 hours (this duration was previously tested to be sufficient to reach equilibrium). The suspensions were filtered through 0.45μ Millipore membrane filter (Agilent, USA). The filtrates were suitably diluted with water and analyzed, spectrophotometrically (Shimadzu UV-vis spectrophotometer, Shimadzu Corp, Australia), for the dissolved drug at 227 nm. Blank samples of PXM at the different concentrations used in the study were analyzed to rule out polymer interference. All assays were performed in triplicate.

Dissolution

Dissolution conditions. All studies were performed in triplicate. The drug content of the filtered samples was determined at 227 nm by UV spectrophotometry after suitable dilution with water and analyzed, spectrophotometrically (Shimadzu UV-Vis spectrophotometer, Shimadzu Corp, Australia), for the dissolved drug at 227 nm. Blank samples of PXM at the different concentrations used in the study were analyzed to rule out polymer interference. All assays were performed in triplicate.

MATERIALS

Distilled water (Millipore) was used throughout the study. Animal studies were approved and conducted in accordance to the Institutional Animal Ethics Committee.

In vitro dissolution studies in distilled water

Pure GLC, physical mixture and the freeze dried solid dispersions equivalent to 80 mg of GLC were used for the dissolution studies. The study was performed in 900ml distilled water using USP XXV Type II eight station dissolution apparatus (Erweka DT 80, GmbH, Germany).\textsuperscript{25} The stirring speed employed was 100 rpm and the temperature was maintained at 37°C ± 0.5°C. Powdered samples of each preparation, equivalent to 80 mg of gliclazide, were placed in the dissolution medium. Samples (5ml) withdrawn at different time intervals were filtered and measured at 227 nm spectrophotometrically, after suitable dilution with the dissolution medium if needed, to determine the amount of drug released. An equal volume of fresh dissolution medium kept at the same temperature was replaced after each sampling to maintain the sink conditions. All studies were performed in triplicate.

Dissolution test in phosphate buffer (pH 7.4)

Based on the dissolution results in distilled water, the optimum solid dispersion was further studied for release in phosphate buffer (pH 7.4) which is the dissolution medium recommended by USP XXV for GLC. The test conditions maintained were same as those mentioned above. Subsequently, the aliquots (5ml) withdrawn at different time intervals were analyzed spectrophotometrically at 228nm for drug release after suitable dilution with the dissolution medium. All assays were performed in triplicate.

Statistical analysis of dissolution profiles

For comparison between dissolution profiles of different samples, a model independent mathematical approach of calculating a similarity factor f2 was used.\textsuperscript{36} The similarity factor f2 is a measure of similarity in the percentage dissolution between two dissolution curves and is defined by following equation:

\[
f_2 = 50 \log \left( \frac{1}{n} \sum_{i=1}^{n} \left( R_i - T_i \right)^2 \right) + 100
\]

where, n is the number of withdrawal points, R, and T are the cumulative percentage of the drug dissolved at each of the selected n time points of the comparator (reference) and test product respectively. The dissolution time points for both the profiles should be the same, e.g., for immediate release products 15, 30, 45 and 60 minutes. Because f2 values are sensitive to the number of dissolution time points, only one measurement should be considered after 85% dissolution of the product. For products which are rapidly dissolving, i.e., more than 85% in 15 minutes or less, a profile comparison is not necessary. A value of 100% for the similarity factor (f2) suggests that the test and reference profiles are identical. Values between 50 and 100 indicate that the dissolution profiles are similar, whereas smaller values imply an increase in dissimilarity between release profiles.

In vivo pharmacokinetic studies

Based on the in-vitro dissolution profile, an optimum solid dispersion GLC:PXM (1:3w/w) prepared by freeze drying technique was selected for comparison of in-vivo performance against plain GLC.

Study design

12 male albino New Zealand rabbits of average weight 3.5 ± 0.010 kg were used for the study. The rabbits were divided into 2 groups of 6 rabbits each (n=6). All the rabbits were fasted overnight with ad libitum access to water during the experiment and the animals were fed 4 hours after the oral dose. One group of animals received a single dose of GLC (40 mg/2ml), formulated as a suspension containing sodium carboxy methyl cellulose (equivalent to 0.5%w/w of the drug). The second group was administered a suspension containing solubility enhanced lyophilized GLC:PXM (1:3w/w) at the same dose. The suspensions were administered orally through a sterile pediatric feeding tube (size 6) 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
dilute. The blood samples was immediately centrifuged at 6000 rpm for 10 minutes (Eppendorf Centrifuge, Germany) to separate the plasma and stored at –40˚C (Freezer Unicryo) until further analysis.

**Analysis of GLC by High Pressure Liquid Chromatography (HPLC)**

The concentration of GLC in the plasma samples was analyzed by a standardized reverse phase HPLC method. The system consisted of a Waters 2690 Separation Module with a multi-solvent delivery pump, an in-line degasser and an auto-sampler programmable PDA detector coupled to a personal computer. Data and system management was handled by Millennium 32 chromatography manager software. The separation was performed at 30˚C using a 5 µm Zorbax Eclipse XDB-C8 column [250 x 4.6 mm (1 x i.d.)], supplied by Agilent Technologies, USA. The mobile phase comprised of acetonitrile : water : trifluoroacetic acid : triethylamine (55:45:0.1:0.1 v/v) and was run isocratic at a flow rate of 1 ml/min.

The aliquots were loaded in an auto sampler tray in glass vials, 100µl sample was injected and the eluting peaks were monitored at a λmax of 227 nm. The developed HPLC method was validated for linearity (100 to 10,000 ng/ml), repeatability, precision (intra-day and inter-day), accuracy, analyte stability, asymmetry, limit of quantification (LOQ), limit of detection (LOD), resolution and extraction efficiency.

**Extraction of GLC from plasma**

The plasma samples were spiked with known concentrations of GLC in acetonitrile so as to obtain plasma concentrations of 0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/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 (Harrier Bench Top Centrifuge, Sanyo). Then, 1 ml of the organic layer was transferred to a clean glass vial and evaporated in a vacuum oven (Gallenkamp, UK). The area under the plasma concentration time curve (AUC0‐∞) was determined by dividing the last plasma concentration by the terminal elimination rate constant (Kt). The elimination rate constant (Kt) was determined from log linear least square regression of the terminal phase data points. The data from different formulations was presented as mean (n=6) ± standard deviation (SD) and compared for statistical significance at p<0.05% level by one-way analysis of variance (ANOVA) using SPSS 15.0 software for Windows (SPSS Inc., Chicago, Illinois, USA).

**RESULTS AND DISCUSSION**

**Phase solubility studies**

The mean calibration curve of GLC in distilled water (regression equation: y = 0.0417x - 0.006) over a concentration range of 0 to 20 µg/mL at 227 nm was found to be linear (n=6) with a correlation coefficient of r2 = 0.9999. The plot of drug solubility against increasing PXM concentrations investigated at 25±1°C is represented in figure 1.
Dissolution

respectively; †† n = 3

and SDL indicate physical mixtures and lyophilized solid material due to lyophillisation and the surface acting property of the samples formulated were made by similarity factor (f2). Comparisons between the release profile of GLC from different aqueous solutions.

The comparative dissolution profiles at pH 7.4 indicate that the release of the active material was strongly affected by the pH of the dissolution medium in case of both the solid dispersion and the pure drug. An overall increase in the dissolution rate was noted in the alkaline medium as compared to that in distilled water, which may be due to the ionization of the drug as it is a weak acid. It was observed that at pH 7.4, after 15 minutes only 15.35 % of pure GLC was dissolved and at the end of 60 minutes it reached 41.99%. However, in the case of 1.3 GLC:PXM solid dispersion prepared by freeze drying method, 89.69% drug release was observed at the end of 15 minutes itself followed by 100 % release at the end of 1 hour (figure 4). For products which are rapidly dissolving, i.e., more than 85% in 15 minutes or less, a profile comparison is not necessary, and hence f2 test, which was irrelevant in this case, was not performed.

The sequence of improved dissolution rate at the end of 15 minutes for the three ratios tested was found to be in the following order: GLC < 1:1PM = 1:3PM < 1:5PM < 1:1SDL < 1:3SDL = 1:5SDL; indicating that increasing the drug polymer ratio from 1:3 to 1:5 did not further enhance the dissolution, but remained constant and hence higher concentrations of PXM were not tried out. The improvement in the dissolution rate of the dispersed systems may be attributed to the decrease in degree of crystallinity of the active material due to lyophilisation and the surface acting property of the carrier which together attributed to the increase in both wettability and solubility of the drug.

Comparisons between the release profile of GLC from different samples formulated were made by similarity factor (f2). Calculated f2 values are presented in table 2. It is evident from the similarity value studies that the release profile of GLC from all the freeze dried SD samples and from pure GLC are dissimilar because f2 values for all these comparisons were less than 50. Release of GLC from SDs are also significantly different from PMs at different concentration levels. The dissolution profiles of 1:3 SDL and 1:5 SDL were almost identical (f2 = 95.10); therefore, the 1:3 w/w SD of GLC with PXM was selected for further studies.

Table 2: Similarity factors (f2) for release profiles of GLC from physical mixtures (PM) and lyophilized solid dispersions (SDL) in different w/w ratios

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</tr>
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<td>22.31</td>
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Dissolution test in phosphate buffer (pH 7.4)

The in vitro release study of GLC and 1:3 SDL was performed in phosphate buffer pH 7.4 as it is the medium of dissolution recommended in US Pharmacopeia for GLC. The standard plot of GLC in phosphate buffer (pH 7.4) over a concentration range of 0 to 20 μg/ml at 227 nm was linear with a correlation coefficient of 0.9998 (regression equation: y = 0.0402x + 0.0087). The drug: polymer ratio 1.3 w/w was considered to be optimum for lyophillisation as there was no further significant increase in dissolution observed in water beyond this ratio.

The comparative dissolution profiles at pH 7.4 indicate that the release of the active material was strongly affected by the pH of the dissolution medium in case of both the solid dispersion and the pure drug. An overall increase in the dissolution rate was noted in the alkaline medium as compared to that in distilled water, which may be due to the ionization of the drug as it is a weak acid. It was observed that at pH 7.4, after 15 minutes only 15.35 % of pure GLC was dissolved and at the end of 60 minutes it reached 41.99%. However, in the case of 1.3 GLC:PXM solid dispersion prepared by freeze drying method, 89.69% drug release was observed at the end of 15 minutes itself followed by 100 % release at the end of 1 hour (figure 4). For products which are rapidly dissolving, i.e., more than 85% in 15 minutes or less, a profile comparison is not necessary, and hence f2 test, which was irrelevant in this case, was not performed.

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CONCLUSION

With the increasing number of drug candidates which are poorly soluble, solid dispersions with hydrophilic carriers play an increasingly important role in pharmaceutical development, which was depicted in the present study. Successful solubilization of GLC was achieved using the lyophilisation technique and poloxamer (water soluble carrier). Based on the rapidly improved in vitro dissolution and in vivo absorption rate, it is very much evident that such formulations would be highly advantageous for the use of oral drug therapy, with faster onset of action and better therapeutic efficacy.

REFERENCES