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Research Article

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 with a hydrophilic carrier viz., poloxamer 407 (PXM) by lyophillisation 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 1:5w/w ratios were prepared by physical mixing and lyophillisation (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 (C_{max}) 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 (T_{max}) 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 lyophillisation 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, Lyophillisation, 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[1]. 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 retinopathy[2] . 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 fibrinolysis[3]. However, the drawback of this potentially useful hypoglycemic agent is that it is highly hydrophobic and practically insoluble in water[4]. 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[5]. 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 [6,7]. 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 [8].

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 [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 fluids[15]. 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 commercially[16, 17].

Many different water-soluble carriers have been employed for preparation of solid dispersion of poorly water soluble drugs [18-20]. 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 forms[27]. Poloxamer 407 (PXM) is specifically one of the tri block copolymer grade consisting of a central hydrophobic 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. PXMs 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 cyclodextrin[28]. In the present study, PXM was thus empirically selected as a hydrophilic carrier for its excellent surfactant properties and oral safety. The lyophillisation (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 [29-31].

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 rate[32,33]. 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[34]. 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-1601, 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. The standard curve of GLC in distilled water over a concentration range of 0 to 20 µg/mL at 227 nm was plotted. The apparent 1:1 ratio stability constant (K) and the Gibbs free energy (ΔF^o) were calculated from the phase-solubility diagram using equations (I) and (II) respectively.

$$K = \frac{\text{slope}}{\text{y intercept (1-slope)}} \quad \text{.....(I)}$$

where the y- intercept corresponds to the intrinsic solubility of GLC in the absence of PXM at 25±1°C.

$$\Delta F^o = - RT \ln K \quad \text{.....(II)}$$

where, R is the ideal gas constant and K is the absolute temperature.

Preparation of solid complexes

Physical mixtures

Physical mixtures (PM) of GLC and PXM in 1:1, 1:3 and 1:5 w/w ratios were prepared by blending the two components in geometric proportion in a mortar for 10 minutes in order to obtain a homogeneous mixture. The resulting mixtures were sieved through 60 mesh sieve (Endecott's, London) and stored in air-tight containers until further evaluation.

Freeze dried (lyophilized) solid dispersions

Each SD preparation containing different ratio (1:1, 1:3 and 1:5 w/w) of GLC and PXM was prepared by the freeze drying method. GLC was weighed and dispersed into 100 mL of PXM solution, the dispersion being stirred with the help of a magnetic stirrer. 25% liquid ammonia was added drop wise and stirred until a clear solution was obtained. The sample was freezed to a temperature of -45˚C (Freezer Unicryo) and lyophilized in a freeze dryer (Vertis Sentry, Freeze Mobile, 25SL, Gardiner, NY, USA) at a temperature of - 40˚C and vacuum of 90 x 10^{-3} Mbar. The freeze dried mass was then sifted through 60 mesh sieve and stored in air-tight containers until further evaluation.

Analysis of solid dispersions

Drug content

Samples of physical mixtures and solid dispersions containing an equivalent of 40 mg of GLC were dispersed in a suitable quantity of methanol and sonicated (Elma Transonic, 460/H, Germany) for 15 minutes. The drug content of the filtered samples was determined at 227 nm by UV spectrophotometry after suitable dilution with methanol.

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) [35]. 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 227nm 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 f_2 was used [36]. The similarity factor f_2 is a measure of similarity in the percentage dissolution between two dissolution curves and is defined by following equation:

$$f_2 = 50 \log \{[1+(1/n) \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} 100\}$$

where; n is the number of withdrawal points, R_t and T_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 f_2 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 (f_2) 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 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 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 5µ Zorbax Eclipse XDB-C8 column [250 x 4.6 mm (l 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 228 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) whose temperature was maintained constant at 40±1˚C. The dry residue was reconstituted with 1ml of acetonitrile, diluted with 1 ml of internal standard (1.6 µg/ml glibenclamide in acetonitrile) and vortexed for 30 seconds. The resulting solution having a final internal standard concentration of 800 ng/ml, was filtered through 0.45µ syringe filter (Millipore) and 100 µl of the sample was injected and analyzed for GLC content using the HPLC method mentioned above.

Statistical analysis of pharmacokinetic data

The pharmacokinetic parameters were calculated using PK Solutions 2.0™, Non-compartmental Pharmacokinetic Data Analysis Software (Windows 2.0.6, Summit Research Services, USA). The area under the plasma concentration time curve (AUC_{0-24}) was determined by the linear trapezoidal rule. The area from last data point to infinity ($AUC_{0-\infty}$) was determined by dividing the last plasma concentration by elimination rate constant. The maximum plasma concentration C_{max} and the time to achieve the maximum concentrations T_{max} were directly determined from the resulting concentration time profile. The elimination rate constant (K_{el}) 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 15.0Software 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 $r^2 = 0.9999$. The plot of drug solubility against increasing PXM concentrations investigated at 25±1°C is represented in figure 1.

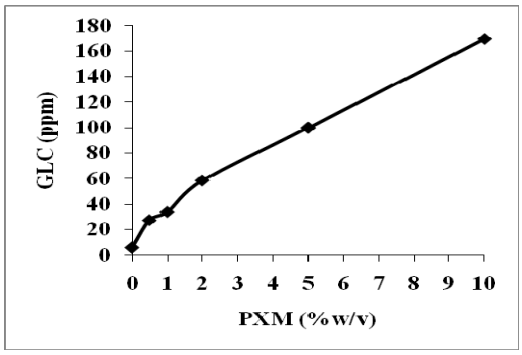

Fig. 1: Phase solubility studies of GLC with PXM

The solubility curve was classified as A_L type according to Higuchi and Connors. Solubility of pure GLC in water at 25°C was found to be only 6.14 µg /mL. The extent of interaction between the drug and the carrier in aqueous media characterized by the apparent stability constant $K_{1:1}$, calculated according to the equation given by Higuchi and Connors, was found to be 56.246 M^{-1} whereas the Gibbs free energy (ΔF^o) was - 9.9845 KJ/mole. The results showed that the solubility of GLC increased linearly with increasing carrier concentration. The negative nature of the Gibbs free energy is indicative of the spontaneous process of solid solution formation process.

Drug content of solid dispersions

Content analysis of the freeze dried products and physical mixtures were carried out using UV Spectrophotometry. The mean calibration curve of GLC (regression equation: $y = 0.0424x - 0.0054$) in methanol 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 $r^2 = 0.9998$ and hence it could be employed for routine assay. Analysis of the freeze dried products and physical mixtures confirmed that GLC could be found to a level of 99.15 to 102.85% of the theoretically added amount in the various dispersions.

In vitro dissolution studies in distilled water

The dissolution profiles of GLC, physical mixture (PM) and the solid dispersions prepared by freeze drying method (SDL) in different drug-polymer w/w ratios are shown in figure 2 and 3 respectively. The initial dissolution rate of GLC was extremely slow and erratic with only 2.91(± 1.87) % of the drug dissolved in 15 minutes and 6.02 (± 3.39) % dissolution at the end of one hour. This could be due to its highly hydrophobic nature and poor wettability. Rapid dissolution is a characteristic behavior observed for various solid dispersions. It was noted that the freeze dried products at all the three ratios showed a higher release as compared with the pure drug and the corresponding physical mixtures. The results in terms of percent of active ingredient dissolved at 15 and 60 minutes (DP_{15} and DP_{60}) are presented in table 1.

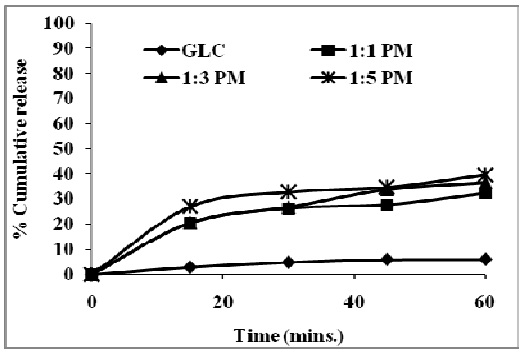

Fig. 2: In vitro release profile in distilled water of GLC and physical mixtures in different GLC:PXM w/w ratios

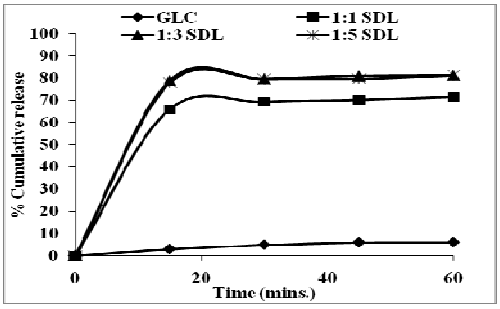

Fig. 3: In vitro release profile in distilled water of GLC and freeze dried SD'S in different GLC:PXM w/w ratios

Table 1: DP_{15} and DP_{60} parameters for pure GLC and various solid dispersions

Compound†	Dissolution Parameters ††	
	DP_{15}	DP_{60}
Pure GLC	02.91 ± 1.87	06.02 ± 2.39
GLC:PXM (1:1 w/w) PM	20.37 ± 2.19	32.19 ± 2.63
GLC:PXM (1:3 w/w) PM	20.53 ± 1.74	36.49 ± 1.98
GLC:PXM (1:5 w/w) PM	27.01 ± 1.28	39.70 ± 3.04
GLC:PXM (1:1 w/w) SDL	66.05 ± 2.71	71.51 ± 2.79
GLC:PXM (1:3 w/w) SDL	78.75 ± 1.95	81.21 ± 1.32
GLC:PXM (1:5 w/w) SDL	77.85 ± 0.66	81.19 ± 1.48

† PM and SDL indicate physical mixtures and lyophilized solid dispersions respectively; †† n = 3

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 lyophillisation 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 (f_2). Calculated f_2 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 f_2 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 (f_2 = 95.10); therefore, the 1:3 w/w SD of GLC with PXM was selected for further studies.

Table 2: Similarity factors (f_2) for release profiles of GLC from physical mixtures (PM) and lyophilized solid dispersions (SDL) in different w/w ratios

Test No.	Sample		f_2 values
	Test	Reference	
1.	GLC	1:1 PM	32.96
2.	GLC	1:3 PM	30.12
3.	GLC	1:5 PM	27.01
4.	GLC	1: 1SDL	09.55
5.	GLC	1:3 SDL	06.23
6.	GLC	1:5 SDL	06.34
7.	1:5 PM	1:1 SDL	22.31
8.	1:1 PM	1:3 PM	70.78
9.	1:1 PM	1:5 PM	57.68
10	1:3 PM	1:5 PM	65.67
11.	1: 1 SDL	1:3 SDL	48.02
12.	1:1 SDL	1:5 SDL	49.18
13.	1:3 SDL	1:5 SDL	95.10

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 a f_2 test ,which was irrelevant in this case, was not performed.

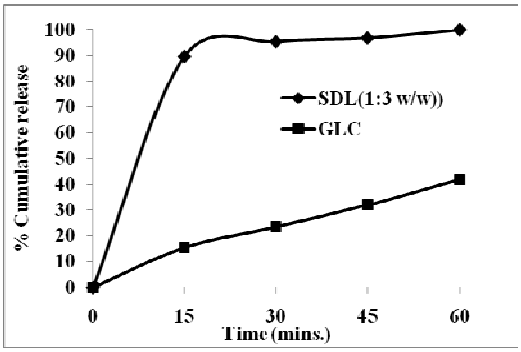

Fig. 4: In vitro release profile in phosphate buffer (pH 7.4) for GLC and optimized freeze dried SD (GLC:PXM - 1:3 w/w)

In vivo pharmacokinetic studies

HPLC analysis of GLC

The mean calibration curve (n=6) of GLC (regression equation: $y = 1627811x - 3254.4$) was obtained by plotting the peak area ratios of GLC and I.S. (internal standard) versus concentration. It was found to be linear in the concentration range of 0.1 to 10 µg/ml (r^2=0.9999). Figure 5 shows a typical chromatogram of the extracted plasma sample. The retention times of GLC and internal standard (GLB) were 6.745 and 8.893 min respectively.

The drug peaks were well resolved (resolution = 3.97) and symmetrical with a tailing factor of 1.08 and 1.19 for GLC and GLB respectively. No endogenous interference was observed with both GLC and I.S. The developed HPLC method showed good reproducibility with the mean C.V of 2.8 and 3.1% for within-day and between-day precision, respectively. The analyte sample were stable when stored for three days under refrigeration (8 ± 0.5°C) with a mean % RSD < 3. The limit of quantification (LOQ) of GLC was 60 ng/ml (C.V < 5%) and the minimum detectable level (LOD) was 35 ng/ml. The recovery was obtained by comparing the peak area of known serum samples spiked with GLC to those of their respective aqueous solutions.

The results showed an absolute recovery of 95.37 ± 2.16%. These results ensure that the analytical method developed could be appropriately used for the pharmacokinetic analysis of GLC with good sensitivity and precision.

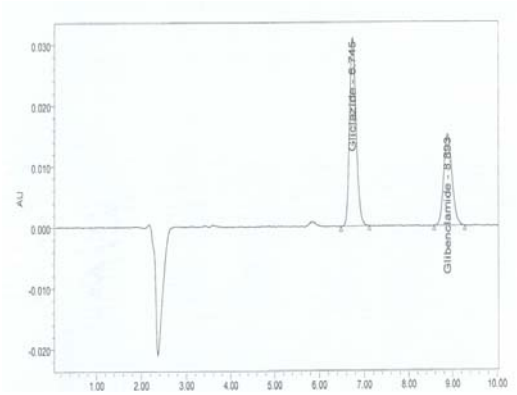

Fig. 5: Chromatogram of extracted plasma samples (GLC – 10 µg/ml and I.S. – 0.8 µg/ml)

In vivo pharmacokinetic studies

Figure 6 shows the mean serum levels of MX following the oral administration of 40 mg dose of GLC or an equivalent of its 1:3 lyophilized SD to 6 healthy rabbits and the mean pharmacokinetic parameters after administration of the drug and the solid dispersion are listed in table 3. It is evident that there exists a clear difference between the biological performance of both the pure drug and the solid dispersion under test, which reflects itself by the significant difference ($p<0.05$) in the peak concentrations between both of them, determined using one way ANOVA. The maximum plasma level (C_{max}) of 2.27 ± 0.39 µg/mL was attained after 4.33 ± 0.52 hours. On the other hand, the solid dispersion resulted in significantly rapid appearance of GLC in plasma, showing C_{max} value of 3.01 ± 0.42 µg/mL after 2.16 ± 0.41 hours. The C_{max} value of the SDL was found to be 1.91 times greater than that of the pure drug. The average area under the plasma concentration time curve (AUC_{0-24}) of the SDL complex up to 24 hours post administration was 20.18 ± 4.77 µg.h/mL, while that of GLC was 12.73 ± 3.95 µg.h/mL, demonstrating that the bioavailability of the solid dispersion under test is significantly higher than that of the original drug. The relative bioavailability of the lyophilized solid dispersion was found to be 158.52% depicting an improvement in the efficacy of the drug.

Table 3: Pharmacokinetic parameters for pure GLC and GLC:PXM (1:3w/w) lyophilized solid dispersion after administration of a 40 mg single oral dose in rabbits (p < 0.05)

Pharmacokinetic parameter	Sample name	
	GLC	1:3 SDL
C_{max} (µg/mL)	2.27 ± 0.39	3.01 ± 0.42
T_{max} (h)	4.33 ± 0.52	2.16 ± 0.41
K_{el} (h^{-1})	0.186 ± 0.073	0.282 ± 0.091
$t_{1/2}$	8.15 ± 1.95	6.28 ± 0.83
AUC0-24 (µg.h/mL)	12.73 ± 3.95	20.18 ± 4.77
AUC0-∞ (µg.h/mL)	14.56 ± 2.36	23.76 ± 4.25
Relative Bioavailability	-	158.52 %

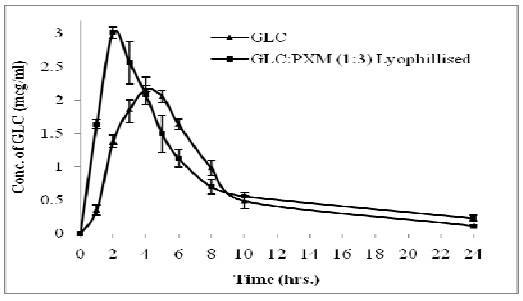

Fig.6: Mean plasma concentration-time curve for GLC and 1:3 SDL after oral administration of a single oral dose equivalent to 40 mg of drug in fasted rabbits (n = 6)

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 lyophillisation 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

1. Palmer KJ, Brogden RN. Gliclazide. An update of its pharmacological properties and therapeutic efficacy in non-insulin-dependent Diabetes Mellitus. Drugs 1993; 46 (1): 92–125.
2. Chazan ACS, Gomes MB. Gliclazide and bedtime insulin are more efficient than insulin alone for type 2 diabetic patients with sulfonylurea secondary failure. Braz J Med Biol Res 2001; 34(1): 49-56.
3. Gillmann AG, Rall TW, Nies AS, Taylor P, In: Goodman and Gillmann's The Pharmacological Basis Therapeutics. 8th ed., Pergamon, Maxwell House, New York, U.S.A, 1990; 1485-1486.
4. Chawdhary KPR, Vijayasrinivas S. Biopharmaceutical classification system. Indian Pharmacist 2004; 2: 7-10.
5. Vikas S, Vipin K, Mahesh K, Manoj G, Pratim C. Dissolution Enhancement of Drugs. Part I: Technologies and Effect of Carriers. International Journal of Health Research 2009; 2 (2): 107-124.
6. Mohammad-Reza R, Afshin M, Mohammad-Hosein T. A simple and sensitive HPLC method for determination of gliclazide in human serum. Chem Pharm Bull 2002, 50(11): 1495 - 1498.
7. Park JY, Kim KA, Kim SL, Park PW. Quantification of gliclazide by semi micro HPLC: application to a bioequivalence study of 2 formulations in healthy subjects. Journal of Pharmaceutical and Biomedical Analysis 2004; 35: 943-949.
8. Overhoff KA, Engstrom JD, Chen B. Novel ultra rapid freezing particle engineering process for enhancement of dissolution rates of poorly water soluble drugs. Eur J Pharm Biopharm 2007; 65: 57-67.
9. Ahuja N, Katare OP, Singh B. Studies on dissolution enhancement and mathematical modeling of drug release of a poorly water-soluble drug using water-soluble carriers. Eur J Pharm Biopharm 2007; 65: 26-38.
10. Friedrich H, Fussnegger B, Kolter K, Bodmeier R. Dissolution rate improvement of poorly water soluble drugs obtained by adsorbing solutions of drugs in hydrophilic solvents onto high surface area carriers. Eur J Pharm Biopharm 2006; 62: 171-177.
11. Varshosaz J, Talari R, Mostafavi SA, Nokhodchi A. Dissolution enhancement of gliclazide using in situ micronization by solvent change method. Powder Technology 2008; 187(3): 222-230.
12. Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm 2000; 50: 47-60.
13. Duncan QMC. The mechanism of drug release from solid dispersions in water-soluble polymers. Int J Pharm 2002; 231: 131-144.
14. Sharma DK, Joshi SB. Solubility enhancement strategies for poorly water-soluble drugs in solid dispersions. Asian journal of Pharmaceutics 2007; 1(1): 9-14.
15. Asyarie S, Rachmawati H. In vivo and in vitro evaluation of a solid dispersion system of gliclazide: PEG 6000. PDA J Pharm Sci Technol 2007; 61(5): 400-410.
16. Ghebremeskel AN, Vemavarapu C and Lodaya M. Use of surfactants as plasticizers in preparing solid dispersions of poorly soluble API: Selection of polymer surfactant combinations using solubility parameters and testing the process ability. Int J Pharm, 2007; 328: 119-129.

17. Yung-Kuang L, Chiu-Ju C, Tong-Rong T, Thau-Ming C. Comparison of the Solubility and Dissolution Rate Between Gliclazide Solid Complex and Its Nanospheres. Drug Development and Industrial Pharmacy 2007; 33: 301–309.
18. Neelam S, Mamta K. Micellar Solubilization of Some Poorly Soluble Anti diabetic Drugs: A Technical Note. AAPS Pharm Sci Tech 2008; 9(2): 431-436
19. Khouloud AA, Hussien A, Wafaa YA. Study of the solubilisation of gliclazide by aqueous micellar solutions. Journal of Pharmaceutical Sciences 2003; 92(4): 839-846.
20. Aggarwal S, Singh PN, Mishra B. Studies on solubility and hypoglycemic activity of gliclazide betacyclodextrin-hydroxypropylmethyl cellulose complexes. Pharmazie 2002; 57: 191-203.
21. Hiremath SN, Raghavendra RK, Sunil F, Danki LS, Rampure MV, Swamy PV, Bhosale UV. Dissolution enhancement of gliclazide by preparation of inclusion complexes with β-cyclodextrin. Asian journal of Pharmaceutics 2008; 2(1): 73-76.
22. Sapkal NP, Kilor VA, Bhusari KP, Daud AS. Evaluation of some Methods for Preparing Gliclazide- β-Cyclodextrin Inclusion Complexes. Tropical Journal of Pharmaceutical Research 2007; 6 (4): 833-840
23. Patil MP, Naresh GJ. Preparation and characterization of gliclazide-polyethylene glycol 4000 solid dispersions. Acta Pharm 2009; 59: 57–65.
24. Biswal S, Sahoo J, Murthy PN, Giradkar RP, Avari JG. Enhancement of Dissolution Rate of Gliclazide Using Solid Dispersions with Polyethylene Glycol 6000. AAPS Pharm Sci Tech 2008; 8 (4) Article 108: E1 – E8.
25. Tejal JS, Avani FA, Jolly RP, Rajesh HP. Process Optimization and Characterization of Poloxamer Solid Dispersions of a Poorly Water-soluble Drug. AAPS Pharm Sci Tech 2007; 8 (2), Article 29: E1 – E7.
26. Passerini N, Albertini B, Gonzalez-Rodriguez ML, Cavallari C, Rodriguez L. Preparation and characterization of ibuprofen-poloxamer 188 granules obtained by melt granulation. Eur J Pharm Sci 2002; 15: 71-78.
27. Schmolks IR. Poloxamers in the pharmaceutical industry. In: Tarcha PJ, ed. Polymers for Controlled Drug Delivery, CRC Press Inc, Florida, 2000; 189-214.
28. Chen Y, Zhang G, Neilly J, Marsh K, Mawhinney D, Sanzgiri Y. Enhancing the bioavailability of ABT-963 using solid dispersion containing Pluronic F-68. Int J Pharm 2004; 286: 69-80.
29. Tsinontides SC, Rajniak P, Hunke WA, Placek J, Reynolds SD. Freeze drying-principles and practice for successful scale-up to manufacturing. Int J Pharm 2004; 280(1): 1-16.
30. Bandry MB, Fathy M. Enhancement of the dissolution and permeation rates of meloxicam by formation of its freeze-dried solid dispersions in polyvinylpyrrolidone K-30. Drug Dev Ind Pharm 2006; 32(2): 141-150.
31. Fathy M, Sheha M. In vitro and in vivo evaluation of an amylobarbitone / hydroxypropyl-betacyclodextrin complex prepared by a freeze-drying method. Pharmazie 2000; 55(7): 513- 517.
32. Vijayalakshmi P, Kusum V, Kshama D, Benson MK, Srinagesh S. Formulation Development and in vivo Characterization of Solubility Enhanced Gliclazide Tablets. Current Trends in Biotechnology and Pharmacy; 2008; 2 (3): 456-461.
33. Betageri GV, Makarla KR. Enhancement of dissolution of glyburide by solid dispersion and lyophillisation techniques. Int J Pharm 1995; 126: 155–160.
34. Higuchi T, Connors KA. Phase solubility Techniques. Adv Anal Chem Instrum 1965; 4: 117-212.
35. The United States Pharmacopoeia 25 and National Formulary 20; US Pharmacopeial Convention Inc. 2002; 12th ed. 786: p 204.
36. Moore JW, Flanner HH. Mathematical comparison of curves with an emphasis on in-vitro dissolution profiles. Pharm Tech 1996; 20(6): 64-74.