

ENHANCEMENT OF ANTI-DIABETIC EFFECTS OF GLICLAZIDE USING IMMEDIATE RELEASE TABLETS IN STREPTOZOTOCIN-INDUCED DIABETIC AND NORMAL RATS

JALEH VARSHOSAZ*, NASER TAVAKOLI, SAIDEH ENTESHARY

Department of Pharmaceutics, Faculty of Pharmacy and Novel Drug Delivery Systems Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

**corresponding author: varshosaz@pharm.mui.ac.ir*

Abstract

Gliclazide is widely used in the treatment of non-insulin dependent diabetes mellitus. Its major drawback is the hydrophobic nature, low dissolution rate and inter-individual variation in bioavailability.

The objective of the present study was to enhance dissolution rate and antidiabetic effect of gliclazide using immediate release tablets. Tablets containing wetting agents like: propylene glycol (PG), ethanol (Et) and Solutol H15 (S) in ratios of 1:1, 2:1, 4:1 to drug and mixed with ProSolv (A) as filler in concentrations of 10, 15, 20% were prepared. Sodium starch glycolate as disintegrating agent was added 5% w/w. The dissolution of gliclazide in tablets was investigated in HCl 0.1 N. The blood glucose lowering effect of tablets was studied in normal and streptozotocin-diabetic rats.

The developed tablets caused significantly higher drug release rate than conventional tablets due to increased wetting properties and increased surface of drug available for dissolution. Increasing the ratio of ProSolv from 10 to 20% caused to increase drug dissolution rate.

The area under the normalized blood glucose level vs. time curve up to 25 hr after administration of the optimized formulation (PG)₄A₂₀ showed significant decrease in blood glucose level in both normal and diabetic rats compared to conventional tablets. The developed tablets enhance water solubility of gliclazide and its efficacy.

Rezumat

Gliclazida este un medicament frecvent administrat la pacienții cu diabet zaharat tip II.

Obiectivul studiului a constat în evaluarea metodelor de îmbunătățire a ratei de dizolvare și a efectului anitdiabetic al gliclazidei prin utilizarea tabletelor cu eliberare imediată.

În acest scop, s-au folosit diferiți agenți de umectare: propilenglicol, etanol, solutul H15 în diferite proporții și ProSolv drept agent de umplere în diferite concentrații. Tabletele obținute au prezentat solubilitate crescută în mediu apos și o eficacitate terapeutică accentuată.

Keywords: gliclazide; dissolution rate; streptozotocin-induced diabetic rat; blood glucose levels.

Introduction

A drug should be appropriately dissolved within the gastrointestinal tract in order to be absorbed. In general drug dissolution can be defined by the extent and the rate of dissolution and involves 2 steps: 1) drug release from the dosage form and 2) drug transport within the dissolution medium [1]. According to the biopharmaceutical classification system (BCS) drug substances are classified upon their solubility and permeability to 4 classes [2]. For poorly soluble, highly permeable drugs (class II), the rate of oral absorption is often controlled by dissolution rate in gastrointestinal tract [3]. Therefore together with the permeability, the solubility and dissolution behavior of a drug are key determinants of its oral bioavailability [3]. There have been numerous efforts to improve drugs dissolution rate [4].

Gliclazide 1-(3-azabicyclo (3.3.0) oct-3-yl)-3-p-tolysulphonylurea a second generation hypoglycemic agent used in the treatment of non-insulin dependent diabetes mellitus is a white crystalline powder. Its plasma half-life is 6-14h. It is rapidly absorbed from the gastro-intestinal tract and extensively metabolized in the liver by hydroxylation, N-oxidation and oxidation to a number of inactive metabolites. Approximately 60-70% of a dose is excreted in the feces as metabolites. It binds to plasma proteins in a proportion of 89-95% [4] and its duration of action is 12h or more. As its effects are less prolonged than those of chlorpropamide or glibenclamide it may be more suitable for elderly patients, who are prone to hypoglycemia with longer-acting sulfonylureas [5].

Gliclazide is a poorly soluble, highly permeable drug (class II of BCS). The problem with this widely used hypoglycemic drug is that it is practically insoluble in water (40-55 $\mu\text{g/mL}$ at 37°C) [6]. Its low solubility and low dissolution rate in gastric fluids may be the reason for its delayed and unpredictable absorption. So its bioavailability is limited and variable [7, 8]. The slow dissolution can be attributed, at least in part, to hydrophobicity of gliclazide powder as evidence by poor wetting of powder surface by water [9, 10].

Numerous attempts have been made to improve the dissolution rate of gliclazide to obtain more rapid and complete absorption. Seedher and Kanojia [6] used co-solvent crystallization of gliclazide for enhancing its solubility. The same researchers used five surfactant systems; a cationic (CTAB), anionic (SDS) and a non-ionic (Tween-80) surfactant as well as equimolar mixtures of cationic + non-ionic (CTAB + Tween-80) and anionic + non-ionic (SDS+Tween-80) surfactants for enhancing the solubility of this drug [6]. Hong *et al.* [11] used a soft gelatin capsule

containing PEG 400, PEG 4000, Tween 20 and glycerin as a formulation that may accelerate dissolution of gliclazide and Varshosaz et al. [12] used *in-situ* micronization by solvent change method for dissolution enhancement of gliclazide.

The aim of this study was to enhance the dissolution rate of gliclazide by formulating immediate release tablets in which wetting agents are added. To our knowledge this method has not been used for gliclazide yet. Then the lowering blood glucose effect of optimized tablet formulation was tested in normal and streptozotocin (STZ) induced diabetic rats.

Materials and Methods

Materials

Gliclazide powder was obtained from Labochim Company (Italy), ProSolv (JRS. Germany), sodium starch glycolate, propylene glycol (PG), ethanol (Et), glacial acetic acid, HCl, and other reagents were all analytical grade reagents from Merck Chemical Company (Germany). Solutol H15 (S) was obtained from BASF (The Chemical Company, Ludwigshafen, Germany) and streptozotocin from Sigma, USA. Gliclazide conventional tablet (Zircon[®]) 40 mg was obtained from Bristol Laboratories.

Effect of different wetting agents on the saturation solubility of glyclazide

Solubility measurements were performed according to the method of Higuchi and Connors [13]. In brief, solubility studies of pure glyclazide and its 1:1 to 1:4 mixture with PG or Et or S were carried out at pH 1.2 containing 0.1% Tween 20. Saturated solutions were prepared by adding excess drug to the medium and shaking on the shaker (Velp, Italy) for 48 h at $25 \pm 0.5^\circ\text{C}$ under constant vibration. After this period the solutions were filtered, through a 0.22μ membrane filter and the filtrate was assayed spectrophotometrically (Shimadzu, Japan) at 227.4 nm. Three determinations were carried out for each sample to calculate the solubility of gliclazide.

Particle size analysis

The size distribution of pure drug powder was measured with a laser diffraction particle size analyzer (SALD-2101 Shimadzu, Japan). The particle size distribution and mean particle size diameter were automatically calculated using the software provided. The size distribution was evaluated with the span value defined as follows: $\text{Span} = (\text{D90\%}-\text{D10\%})/\text{D50\%}$.

Where, DN% (N= 10, 50, 90) means the volume percentage of microparticles with diameters up to DN%. The smaller span value indicates the narrower particle size distribution.

Compressibility percent or Carr's Index

About 1mL of drug powder was carefully filled into a mounted measuring cylinder with known tare. The powder bed was leveled with a spatula, and the maximum bulk volume was read. A single tap was employed, and the volume was read again. This procedure was repeated, thereby gradually increasing the number of taps between individual readings, until three consecutive replicates of 100 taps did not reduce the powder volume further. Hence the minimum powder volume (to give the maximum bulk density) had been reached. The measuring cylinder was then weighed to determine the powder mass. Compressibility percent [14] was calculated using equation 1:

$$\text{Compressibility percent} = 100 \times (\rho_t - \rho_a) / \rho_t \quad \text{Eq. 1}$$

where ρ_t is the maximum bulk density, ρ_a is the minimum bulk density. All experiments were performed in triplicate.

Preparation of tablets

Three different types of wetting agents (PG, Et or S) were used for the preparation of tablets. Gliclazide was dispersed in PG, Et or S with different ratios ranging from 1:1 to 1:4 (the ratio of drug was 1 in all mixtures). Then, ProSolv (A) powder in different concentrations of 10, 15 and 20% was added to a mixture containing the drug and the wetting agent under continuous mixing in a mortar. Sodium starch glycolate (5% w/w) as disintegrant was mixed with other ingredients for 10 min.

The final mixture was compressed using the single punch press machine (Kilian Co, KS 43373-202, Germany) to achieve tablet hardness of 6-7 kp (Table I).

Disintegration time of tablets

The disintegration time of the tablets was determined in distilled water at $37 \pm 0.5^\circ\text{C}$ in a six station disintegration test unit (a Pharma-test (PTZE) disintegration tester). Tablets were placed on the wire mesh just above the surface of the distilled water in the tube. The time taken for each tablet to disintegrate was recorded. The end point of the test was indicated when the remaining residue had a soft mass with no palpable soft core. Results were expressed as the mean of three determinations.

Table I
Formulation of different studied tablets

| Formulation code | Gliclazide amount (mg) | Wetting agent type | Wetting agent (mg) | ProSolv (%) | Sodium starch glycolate (mg) |
|-----------------------------------|------------------------|--------------------|--------------------|-------------|------------------------------|
| (PG) ₁ A ₁₀ | 40 | Propylene glycol | 40 | 10 | 10 |
| (PG) ₁ A ₁₅ | 40 | Propylene glycol | 40 | 15 | 10 |
| (PG) ₁ A ₂₀ | 40 | Propylene glycol | 40 | 20 | 10 |
| (PG) ₂ A ₁₀ | 40 | Propylene glycol | 80 | 10 | 10 |
| (PG) ₂ A ₁₅ | 40 | Propylene glycol | 80 | 15 | 10 |
| (PG) ₂ A ₂₀ | 40 | Propylene glycol | 80 | 20 | 10 |
| (PG) ₄ A ₁₀ | 40 | Propylene glycol | 160 | 10 | 10 |
| (PG) ₄ A ₁₅ | 40 | Propylene glycol | 160 | 15 | 10 |
| (PG) ₄ A ₂₀ | 40 | Propylene glycol | 160 | 20 | 10 |
| (Et) ₁ A ₁₀ | 40 | Ethanol | 40 | 10 | 10 |
| (Et) ₁ A ₁₅ | 40 | Ethanol | 40 | 15 | 10 |
| (Et) ₁ A ₂₀ | 40 | Ethanol | 40 | 20 | 10 |
| S ₁ A ₁₀ | 40 | Solutol H15 | 40 | 10 | 10 |
| S ₁ A ₁₅ | 40 | Solutol H15 | 40 | 15 | 10 |
| S ₁ A ₂₀ | 40 | Solutol H15 | 40 | 20 | 10 |

Contact angle of tablets with water

For assessment of wettability, contact angle was measured between the surface of immediate release or conventional tablets of gliclazide and 25 μ L of simulated gastric fluid (HCl 0.1N solution) saturated with gluclazide or gliclazide and wetting agents. The commonly used imaging method is to measure contact angle directly for a drop of liquid resting on a plane surface of the solid, the so-called imaging method. An excess amount of glyclazide and in the case of formulations wetting agents were added to this media and shaken for 24 h with constant rate. The upper solution was centrifuged. A drop of this solution was put on the surface of tablets, and pictures were taken by using a digital camera (Nikon 2200, Japan). The contact angles were calculated by measuring the height (H) and diameter (D) of sphere drop on the tablet (Figure 1) using AdobePhotoshop [15] and the equation 2:

$$\theta = 2 \tan^{-1} (2H/D). \quad \text{Eq. 2}$$

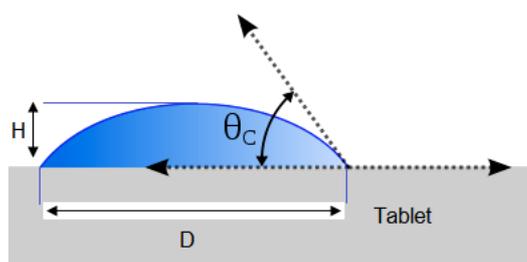


Figure 1

Schematic representation of contact angle measurement using imaging method

Drug content of tablets

10 tablets of each batch were selected, weighed and powdered. A quantity of the powder containing 10 mg of gliclazide was shaken with 30 mL of phosphate buffer (pH 7.4) for three times and then was diluted up to 100 mL with phosphate buffer. One mL of the filtrate was diluted to 5 mL and then the drug content was determined spectrophotometrically at 227.8 nm [16].

In vitro drug release studies

The USP paddle method (Erweka, Germany) was used for all *in vitro* dissolution studies. In this method HCl 0.1N containing 0.1% Tween 20, which simulated gastric fluid (pH 1.2) was used as dissolution medium. The rate of stirring was 100 rpm. The drug powder or tablets were placed in 900 mL dissolution medium (37 ± 0.5 °C). At appropriate time intervals of 10, 20, 30, 60 min 1 mL samples were taken and filtered through a 0.45µm filter and diluted with dissolution medium to 5 mL. The dissolution medium was then replaced by 1 mL of fresh dissolution fluid to maintain a constant volume. The samples were centrifuged (Hettich, Germany) and then analyzed at 227.4 nm by UV/visible spectrophotometer (Shimadzo, Japan). The *in vitro* release studies were performed in triplicate for each sample, and drug free tablets were used as the blank. To compare the dissolution data, dissolution efficiency (DE) and mean dissolution time (MDT) were used [4].

Analysis of release data

All data were statistically analyzed using analysis of variance (ANOVA) and *post hoc* test of Dunckan by SPSS software version 12. DE [17] after 30 minutes of release test was used to compare the results of release tests of different formulations:

$$DE_{30} \% = \frac{\int_0^t y dt}{y_{100} t} \times 100 \quad \text{Eq. 3}$$

where y is the percent of drug released as a function of time, t is the total time of drug release and y_{100} is 100% of drug release.

MDT [18] is calculated from the amount of drug released to the total cumulative drug, measure of the release process. The lower the MDT, the faster the release rate.

$$MDT = \frac{\sum_{i=1}^{i=n} t_{mid} \times \Delta M}{\sum_{i=1}^{i=n} \Delta M} \quad \text{Eq. 4}$$

where i is the release sample number, n is the number of release sample time, t_{mid} is the time of the midpoint between i and $i-1$ and ΔM is the additional amount of drug released between i and $i-1$.

In vivo studies

Animals

36 Male albino Wistar rats with a body weight of 180-250g (70-90 days aged) were selected for all the experiments. Animals were kept in the animal house at 23-30 °C and 45-55% relative humidity and were kept fasted 12-14 h before blood sampling. The Isfahan University of Medical Sciences Ethical Committee approved all animal experiments in the present study.

Induction of diabetes in rats

Diabetes was induced in rats by a single intraperitoneal injection of STZ as much as 55 mg/kg body weight in freshly prepared citrate buffer (25 mg/mL pH 5). Control rats received only carboxy methyl cellulose 1% (CMC 1%) suspension in citrate buffer. The diabetic status of rats was assessed by the determination of fasting glucose concentration on the third day post-administration of STZ [19].

Experimental Design

Rats were randomly divided into the following six groups: each group included six animals (n=6).

Group I: consisted of normal control (untreated) rats that received 3 mL of CMC 1% suspension in normal saline using gastric tube.

Group II: consisted of normal rats that received 3 mL suspension of conventional gliclazide tablet (10 mg/kg b.w.) in CMC 1% using a gastric tube.

Group III: consisted of normal rats that received 3 mL suspension of optimized immediate release tablets (PG)₄A₂₀ (10 mg/kg b.w. of drug) in CMC 1% using a gastric tube.

Group IV to VII were exactly similar to group I to III expect that included diabetic animals. The animals were carefully monitored every day [20].

Blood collection and biochemical analysis

Three days after injection of STZ, when the fast blood glucose level of rats was 300 mg/dL, the rats were fasted (12-14 h) and received drugs as mentioned above. Then 0.3 mL of blood was withdrawn after 0, 0.25, 0.5, 1, 1.5, 2, 5, 8 and 25 h through retro-orbital-plexus using a glass capillary and collected in ependorf tubes containing 1 μ L of 10% EDTA. Collected blood was centrifuged for 15 min at 8000 rpm. The plasma thus obtained, was

used for glucose measuring (analyzed at 500 nm by UV/visible spectrophotometer). Blood glucose concentration (mg/dL) was determined using a commercial kit based on the glucose oxidase method [21].

$$\text{sample glucose concentration(mg / dL)} = \frac{\text{optic absorption of sample}}{\text{optic absorption of standard solution}} \times \text{concentration of standard solution(100mg / dL)} \quad \text{Eq. 5}$$

Normalization of blood glucose levels was carried out using Eq. 6

$$\text{Normalized blood glucose\%} = \frac{\text{Blood glucose level at each time point} - \text{blood glucose level at time zero}}{\text{Blood glucose level at each time point}} \times 100 \quad \text{Eq. 6}$$

Analysis of blood glucose data

The 25-h area under the normalized percentage of blood glucose level-time (h) curve (AUC_{0-25}) was used for comparison of blood glucose lowering effect of gliclazide developed tablets with conventional tablets of gliclazide [22].

$$AUC_{0-t} = \int_0^t y dt \quad \text{Eq. 7}$$

Results and Discussion

Considering low water solubility of gliclazide, different techniques have been developed to increase its dissolution rate and consequently its bioavailability. Although micronization using milling process may enhance dissolution rate extremely, it is inefficient due to a high energy input, and disruptions in the crystal lattice which can cause physical or chemical instability. Therefore, in the present study we tried to enhance antidiabetic effect of this drug by a non-invasive method for the crystalline lattice of the drug that doesn't cause instability. The technique used in this regard is using wetting agents.

Table II summarizes the results of the study on the saturated solubility of pure gliclazide or its mixture with different wetting agents. As this table indicates all used wetting agents have enhanced gliclazide solubility the highest solubility is seen in Glyclazide /PG with the ratio of 1:4 while its 1:1 ratio has nearly enhanced solubility as much as other wetting agent. However, the differences are significant in all of them ($p < 0.05$). The mixture of gliclazide/Et showed lower solubility compared to PG and S ($p < 0.05$).

Table II

Solubility of gliclazide and its mixture with different wetting agents in HCl 0.1 N containing 0.1% Tween 20.

| Substance | Solubility |
|---------------------|-------------|
| Gliclazide powder | 142.88±0.01 |
| Gliclazide /PG 1:1 | 700.43±0.05 |
| Gliclazide /PG 1:4 | 812.59±0.02 |
| Gliclazide / Et 1:1 | 670.99±0.04 |
| Gliclazide /S 1:1 | 688.22±0.06 |

The micromeritic behavior (mean particle size, span and Carr's Index) and of untreated gliclazide powder including particle size diameter, compressibility percent and dissolution efficiency (DE) after 15 of dissolution test were studied and the results are shown in Table III. It is obvious that the original gliclazide (untreated drug) has large mean particle size. Carr's index or compressibility percent which is an indication of powder flow behavior was low. A Carr index greater than 25 is considered to be an indication of poor flowability, and below 15, of good flowability [14].

Table III

Micromeritic properties of gliclazide powder drug and its dissolution efficiency till 15 min

| Mean Particle size (μm) \pm SD | Span | Compressibility Carr's Index (%) \pm SD | DE ₁₅ % \pm SD |
|--|------|--|-----------------------------|
| 290 \pm 36.05 | 2.1 | 20 \pm 0.10 | 23.85 \pm 4.13 |

By considering all data obtained in the present study it can be postulated that there should be two main factors responsible for an increase in dissolution rate of gliclazide powder; (a) an increase in wettability of drug particles and (b) an increase in saturated solubility of the treated drug powder. According to Noyes and Whitney equation [23], $DR = [(DS(C_s - C))/h]$, the drug dissolution rate (DR) is directly proportional to its concentration gradient ($C_s - C$) in the stagnant diffusion layer, diffusion coefficient (D) and its surface (S) available for dissolution. C_s is the saturation solubility of the drug in the dissolution medium which is enhanced in the dissolving media (Table II). Since all of dissolution tests for formulations were carried out at a constant rotational paddle speed (100 rpm) and identical dissolving media, we can assume that the thickness (h) of the stagnant diffusion layer and the diffusion coefficient (D) of the drug molecules remain almost identical. Since treated drug powders with wetting agents showed higher wettability (Table IV) (or lower contact angle) and consequently higher solubility than untreated drug therefore, the observed higher dissolution rates of gliclazide fast releasing tablets are possibly due to the significant increase in the wettability (Table IV) of the treated drug powders.

Figure 2 shows the effect of the type of wetting agent and ratio of drug to wetting agent on the release profile of gliclazide from immediate release tablets in comparison to the conventional tablet used. As it is evident from this figure PG has caused higher dissolution rates than Et or S specially when the ratio of these wetting agents to the drug are higher (1:1 vs 1:4). To compare the dissolution data, $DE_{30}\%$ and MDT were used (Table IV).

Table IV
Contact angle with water, disintegration time, MDT, $DE_{30}\%$ and assay of gliclazide immediate release tablets and conventional tablets (n=3).

| Formulation code | Contact angle (°) | Disintegration time (sec) | $DE_{30}\% \pm SD$ | MDT $\pm SD$ (min) | Drug content $\pm SD$ (mg) |
|-----------------------------------|-------------------|---------------------------|--------------------|--------------------|----------------------------|
| Conventional tablet | 80.0 \pm 3.0 | 100.5 \pm 9.1 | 49.50 \pm 2.42 | 9.64 \pm 0.70 | 40.15 \pm 0.37 |
| (PG) ₁ A ₁₀ | 42 \pm 0 | 44.0 \pm 3.6 | 58.63 \pm 0.75 | 10.10 \pm 0.27 | 40.37 \pm 0.89 |
| (PG) ₁ A ₁₅ | 42 \pm 1 | 43.6 \pm 2.1 | 66.82 \pm 0.77 | 8.57 \pm 0.08 | 40.12 \pm 0.58 |
| (PG) ₁ A ₂₀ | 42 \pm 1 | 42.3 \pm 1.5 | 68.67 \pm 2.48 | 8.24 \pm 1.33 | 41.50 \pm 1.16 |
| (PG) ₂ A ₁₀ | 40 \pm 1 | 47.0 \pm 2.0 | 69.28 \pm 3.6 | 9.17 \pm 1.03 | 41.11 \pm 1.05 |
| (PG) ₂ A ₁₅ | 40 \pm 1 | 48.0 \pm 2.0 | 71.43 \pm 0.81 | 8.57 \pm 0.24 | 39.34 \pm 0.58 |
| (PG) ₂ A ₂₀ | 40 \pm 0 | 50.0 \pm 1.0 | 74.40 \pm 2.72 | 7.68 \pm 0.18 | 34.93 \pm 1.18 |
| (PG) ₄ A ₁₀ | 35 \pm 1 | 53.0 \pm 2.0 | 73.55 \pm 1.41 | 7.53 \pm 0.42 | 41.14 \pm 0.94 |
| (PG) ₄ A ₁₅ | 35 \pm 1 | 50.0 \pm 5.0 | 81.66 \pm 1.01 | 5.49 \pm 0.29 | 40.66 \pm 1.23 |
| (PG) ₄ A ₂₀ | 35 \pm 1 | 59.0 \pm 3.6 | 83.03 \pm 0.52 | 5.09 \pm 0.15 | 40.45 \pm 0.91 |
| (Et) ₁ A ₁₀ | 52 \pm 2 | 55.0 \pm 9.2 | 51.58 \pm 2.7 | 11.52 \pm 0.8 | 39.4 \pm 1.27 |
| (Et) ₁ A ₁₅ | 52 \pm 2 | 61.3 \pm 3.2 | 53.97 \pm 2.16 | 10.06 \pm 0.37 | 38.77 \pm 0.69 |
| (Et) ₁ A ₂₀ | 51 \pm 2 | 63.6 \pm 3.0 | 56.13 \pm 2.05 | 10.08 \pm 0.90 | 39.56 \pm 0.39 |
| S ₁ A ₁₀ | 45 \pm 2 | 54.6 \pm 8.7 | 46.51 \pm 1.77 | 13.05 \pm 0.30 | 39.67 \pm 0.48 |
| S ₁ A ₁₅ | 44 \pm 1 | 47.3 \pm 5.5 | 49.07 \pm 2.13 | 11.88 \pm 0.92 | 38.27 \pm 0.33 |
| S ₁ A ₂₀ | 45 \pm 2 | 53.3 \pm 6.0 | 56.49 \pm 2.57 | 10.88 \pm 1.30 | 37.37 \pm 0.07 |

Table IV shows that although all studied wetting agents have decreased the contact angle compared to the conventional tablets ($p < 0.05$), but the least contact angle was seen in tablets prepared by PG in the ratio of 4:1 to the drug. This result confirms the results of saturation solubility shown in table II. The $DE_{30}\%$ value for the conventional tablet is 49.50% whereas this value changes to 58.62, 51.88, 46.51% for tablets containing PG, Et, S, respectively in ratio of 1:1 to drug and ProSolv 10%. The differences are significant ($p < 0.05$) in all cases except for the conventional tablets and the (Et)₁A₁₀ ($p > 0.05$). A similar trend was obtained with the same wetting agents in other ratios of wetting agent to drug. The results show that within each formulation series, $DE_{30}\%$ values increased significantly ($p < 0.05$) as the concentration of wetting agent increased. Table IV also shows that MDT was considerably lower for the developed tablet formulations compared to the conventional tablet, indicating a faster dissolution rate. However, differences between conventional tablet and

(PG)₁A₁₀, (PG)₁A₁₅, (PG)₂A₁₀, (PG)₂A₁₅, (Et)₁A₁₅, (S)₁A₁₀ were not significant ($p > 0.05$).

Among different wetting agent ratios used in production of tablets it was seen that as the concentration of wetting agents increased, DE₃₀% values increased significantly (Table IV) that may be due to higher gliclazide solubility at higher concentrations of wetting agents. One may also find that PG contributes to a higher dissolution rate (Fig. 2) than other agents ($P < 0.05$) that is probably due to higher solubility of gliclazide in PG and its hydrophilicity.

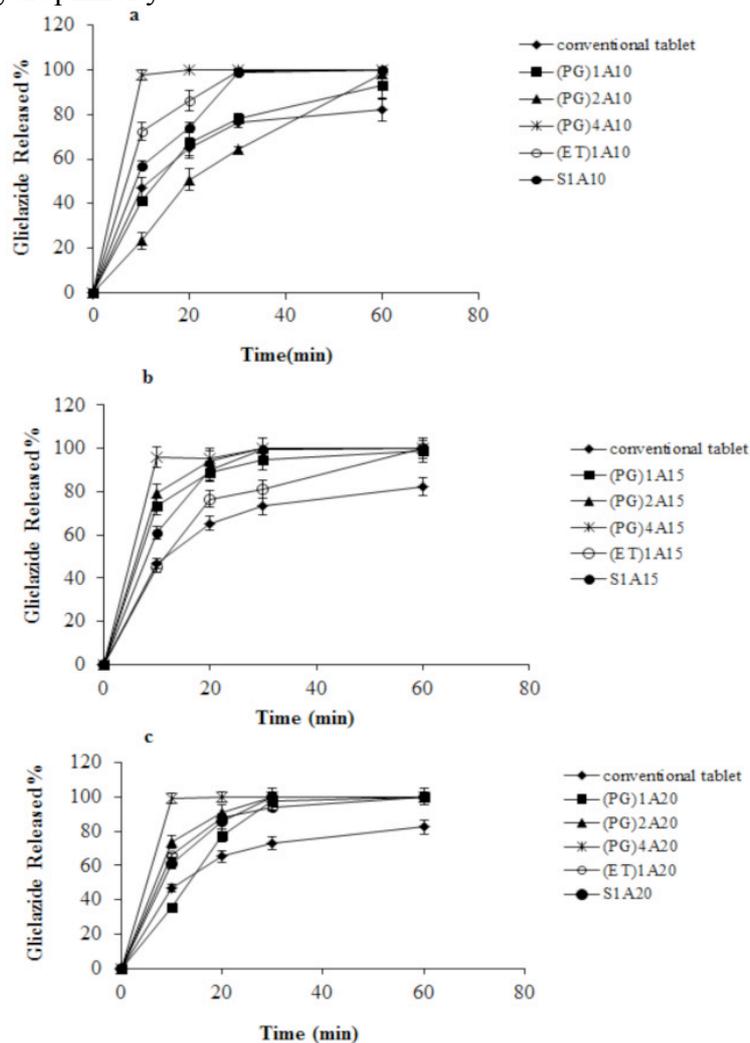


Figure 2

Effect of type of wetting agent and the ratio of drug:wetting agent on the release profile of gliclazide from newly formulated tablets compared to the conventional tablet ($n=3$).

Patil and Gaikwad [7] enhanced the dissolution of gliclazide using hydrophilic carrier of polyethylene glycol 4000 (PEG 4000) in its solid dispersions. Dissolution enhancement was attributed to decreased crystallinity of the drug and to the wetting and solubilizing effect of the carrier. PEG 6000 also has been used in preparation of solid dispersions of gliclazide and has increased dissolution rate of this drug [9]. In other words hydrophilic carriers can enhance dissolution properties of this drug. In this regard complexation of gliclazide with beta-cyclodextrin in the presence of hydroxypropylmethylcellulose (a hydrophilic polymer) also has enhanced the rate and extent of gliclazide dissolution to 2.5 fold [24]. This technique produced not only an early onset but also more intense hypoglycemic effect as compared to pure drug powder and commercial tablets. The use of *in situ* micronization process through pH change method in the presence of hydrophilic stabilizers like, HPMC, PEG and Brij has also been reported to produce micron-size gliclazide particles for fast dissolution and hence better bioavailability [25, 26].

Figure 3 shows the effect of increase in the ratio of ProSolv on the release profile of gliclazide prepared tablets in comparison to conventional tablets. As this figure shows increased ratio of ProSolv has elevated the dissolution efficiency of gliclazide from the tablets significantly ($p < 0.05$). For instance in Fig. 3c the amount of $DE_{30}\%$ (Table IV) for conventional tablet is 49.50% and for formulated tablets with drug: wetting agent ratio of 1:4 and ProSolv percentages of 10, 15, 20 are 73.55, 81.66, 83.03%, respectively. A similar trend was obtained for other ratios.

Results show that in general, increasing the ratio of ProSolv causes an increased drug dissolution rate (Figure 3c and Table IV) which may be due to disintegrating effect of ProSolv. So increasing the amount of ProSolv, causes faster disintegration of the tablets and consequently faster drug dissolution in medium [27]. Possibly the presence of Aerosil as a co-processed ingredient in ProSolv powder effectively adsorbs wetting agent during mixing the powders and prevents its enhancing solubility effect on the drug. However, disintegration effect of ProSolv has more pronounced effect than decreasing effect of Aerosil on dissolution rate and consequently ProSolv has enhanced the dissolution rate of the drug.

Considering the higher $DE_{30}\%$ and lower MDT values of developed tablets of $(PG)_4A_{20}$, which contained 1:4 drug/PG with 20% ProSolv, this formulation was chosen for *in vivo* studies.

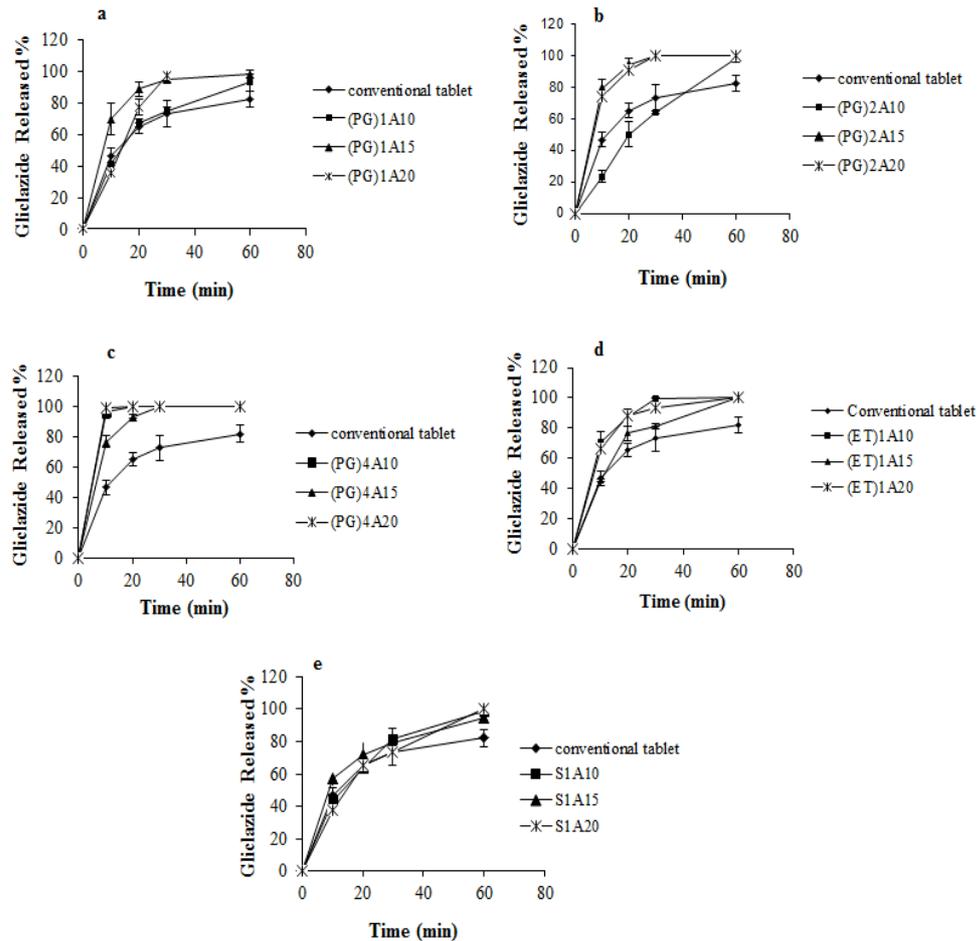


Figure 3

Effect of different concentrations of ProSolv on the release profile of gliclazide developed tablets compared to the conventional tablet (n=3)

Figure 4 shows normalized percentage of blood glucose level and effect of gliclazide conventional and developed tablets in normal (Figure 4a) and STZ-induced diabetic (Figure 4b) rats. These two groups both showed significant ($p < 0.05$) lower normalized blood glucose levels compared to the control group. As figure 4a indicates, in normal rats the blood glucose levels in immediate release tablets was significantly ($p < 0.05$) lower at 0.25, 0.5, 1, 1.5 and 2 h compared with conventional and untreated group but after that the differences between immediate release tablets was not significant with conventional tablets at 5, 8 and 25 h ($p > 0.05$). In the diabetic rats (Fig. 4b) in all studied time points the difference in blood glucose level was

significantly different in the immediate release tablets with conventional and untreated group ($p < 0.05$). Only after 25 h the difference between immediate release tablets with conventional ones was not significant ($p > 0.05$).

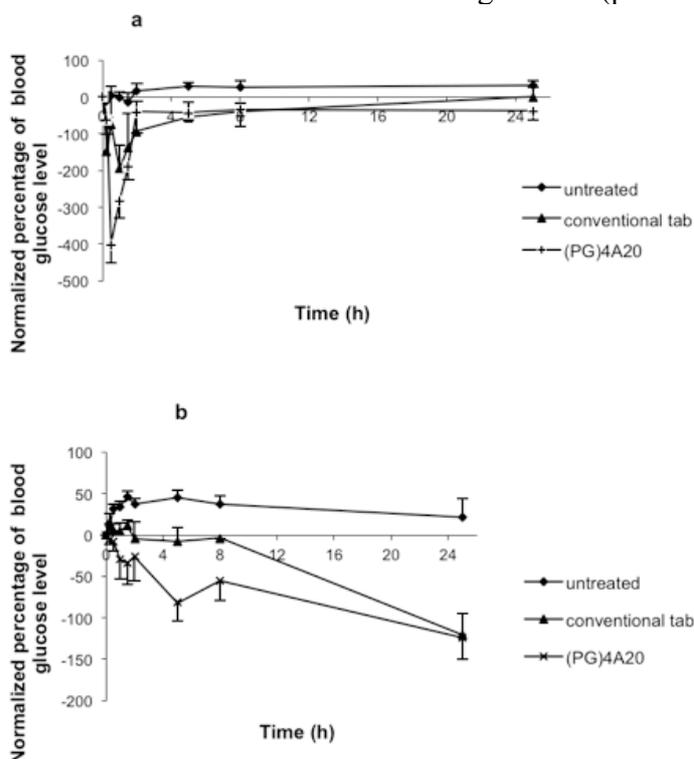


Figure 4

Normalized percentage of blood glucose level after administration of conventional tablets and immediate release tablets of gliclazide in a) normal and b) streptozotocin-diabetic rats ($n=6$)

As figure 4 and table V indicate the reducing blood glucose effect was registered in both normal and STZ-diabetic rats. The (PG)₄A₂₀ tablets caused about 7 folds different AUC_{0-25} compared to traditional tablets in STZ-diabetic rats. Although this effect was more pronounced in diabetic rats than normal ones, the reduction in normalized blood glucose levels for normal rats was also significant ($p < 0.05$). There are different reports indicating blood glucose reduction in short term consuming of sulfonylurea drugs that may induce more insulin secretion even in normal rats [28-31].

Table V displays AUC_{0-25} of normalized percentage of blood glucose-time curve that shows more lowering effect of optimized tablet formulation (PG)₄A₂₀ on blood glucose level in normal and diabetic rats compared to the control and conventional tablet groups ($p < 0.05$).

Table V

AUC₀₋₂₅ of normalized percentage of blood glucose level-time (h) curve for normal and STZ-diabetic rats (n=6)

| Groups | AUC ₀₋₂₅ (mg/dL.h) ±SD | |
|-----------------------------------|-----------------------------------|----------------|
| | Diabetic rats | Normal rats |
| Untreated (control) | 804.50±34.02 | 64.65±30.04 |
| Conventional tablet | 270.39±29.50 | 1166.22±101.91 |
| (PG) ₄ A ₂₀ | 1936.39±71.30 | 1197.26±47.46 |

Conclusions

The use of wetting agents is a promising alternative for the formulation of water insoluble drugs such as gliclazide into immediate release tablets. The higher dissolution rates displayed by them may also imply enhanced oral bioavailability due to the increased wetting properties and solubility of drug in the wetting agents. Also it has been shown that the dissolution rate of gliclazide increases with increasing the ProSolv that may be due to its disintegrating effect. The DE₃₀% and MDT from optimized formulation (PG)₄A₂₀ show that the dissolution rate becomes 1.7 times more than those of conventional tablets. This optimized formulation could decrease blood glucose level in both normal and STZ-diabetic rats compared to conventional gliclazide tablet and control groups significantly (p< 0.05).

The developed tablets may be considered for further human evaluation as a promising rapid release dosage form for gliclazide.

Acknowledgments

The authors wish to thank the Vice Chancellery for Research of Isfahan University of Medical Sciences that supported this work financially.

References

1. Jamzad S., Fassihi R. Role of surfactant and pH on dissolution properties of fenofibrate and glipizide-A technical note, *APPS PharmSciTech*, 2006; 7(2): E33.
2. Yu L.X., Amidon G.L., Polli J.E., Zhao H., Mehta M.U., Conner D.P. Biopharmaceutics classification system: the scientific basis for biowaiver extensions, *Pharm Res*, 2002; 19(7): 921-925.
3. Löbenberg R., Amidon G.L. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards, *Eur J Pharm Biopharm*, 2000; 50(1): 3-12.
4. Glowka F.K., Hermann T.W., Zabel M. Bioavailability of Gliclazide from some formulation tablets, *Int J Pharm*, 1998; 172 (1-2): 71-77.
5. Sweetman S.C. (34th ed), *Martindale The Extra Pharmacopeia, Complete Drug Reference*, Pharmaceutical Press, London, 2007.

6. Seedher N., Kanojia M. Co-solvent solubilization of some poorly-soluble antidiabetic drugs, *Pharm Dev Technol*, 2009; <http://www.informaworld.com/smpp/title~db=all~content=t713597289~tab=issueslist~branches=14 - v1414> (2): 185-192.
7. Patil M.P., Gaikwad N.J. Preparation and characterization of gliclazide-polyethylene glycol 4000 solid dispersions, *Acta Pharm*, 2009; 59(1): 57-65.
8. Sapkal N.P., Kilor V.A., Bhursari K.P., Daud A.S. Evaluation of some method for preparing gliclazide- β -cyclodextrin inclusion complexes, *Tropical J Pharm Res*, 2007; 6(4): 833-840.
9. Talari R, Varshosaz J, Mostafavi A, Nokhodchi A, Development and validation of a novel RP-HPLC method for pharmacokinetic studies of gliclazide in rat, *Farmacia*, 2011, 59(3), 388-395
10. Palmer K.J., Brogden R.N. Gliclazide on update of its pharmacological properties and therapeutic efficacy in NIDDM, *Drugs*, 1993; 46(1): 92-125.
11. Hong S.S., Lee S.H., Lee Y.J., Chung S.J., Lee M.H., Shim C.K. Accelerated oral absorption of gliclazide in human subjects from a soft gelatin capsule containing a PEG 400 suspension of gliclazide, *J Control Rel*, 1998; 51(2-3): 185-192.
12. Varshosaz J., Talari R., Mostafavi S.A., Nokhodchi A. Dissolution enhancement of gliclazide using in-situ micronization by solvent change method. *Powder Technol*, 2008; 187(3): 222-230.
13. Higuchi T., Connors K.A. Phase-solubility Techniques, *Adv Anal Chem Instr*, 1965; 4: 17-212.
14. El-kamel A.H., Sokar M.S., Al Gamal S.S., Naggar V.F. Preparation and evaluation of ketoprofen floating oral delivery system, *Int J Pharm*, 2001; 220: 13-21.
15. Javadzadeh Y., Siah M.R., Asnaashari S., Nokhodchi A. An investigation of physicochemical properties of piroxicam liquid compact, *Pharm Dev Technol*, 2007; 12(3): 337-43.
16. El-Enany N. Spectrophotometric Determination of gliclazide in pharmaceuticals and biological fluids through ternary complex formation with eosin and palladium (II), *Farmaco*, 2004; 59(1): 63-69. 17. Velescu BŞ, Uivarosi V, Negreş S, Effect of di- μ -hydroxo-bis (quercetinato oxovanadium(iv)) complex on alloxan-induced diabetic rats, *Farmacia*, 2012, 60(5), 696-710
18. Gohe M.C., Panchal M.K. Novel use of similarity factors f_2 and s_d for development of diltiazem HCl modified-release tablets using a 32 factorial design, *Drug Dev Ind Pharm*, 2002; 28(1): 77-87.
19. Prasad S.K., Kulshreshtha A., Qureshi T.N. Antidiabetic activity of some herbal plants in streptozotocin induced diabetic albino rats, *Pak J Nutr*, 2009; 8(5): 551-557.
20. Eidi A., Eidi M., Sokhteh M. Effect of fenugreek (*Trigonella foenum graecum* L) seeds on serum parameters in normal and streptozotocin-induced diabetic rats. *Nutr Res*, 2007; 27(11): 728-733.
21. Thomas L. (1st ed), *Clinical Laboratory Diagnostics*, TH- books, Veriagsgseller Chافت, 1998.
22. Madaras-Kelly K.J., Ostergaard B.E., Baeker H.L., Rotschafer J.C. Twenty-four-hour area under the concentration-time curve /MIC ratio as a generic predictor of fluoroquinolone antimicrobial effect by using three strain of *Pseudomonas aeruginosa* and an in vitro pharmacodynamic model, *Antimicrob Agents Chemother*, 1996; 40(30): 627-632.
23. Noyes A., Whitney W. R. The rate of solution of solid substances in their own solutions, *J Am Chem Soc*, 1897; 19: 930-934.
24. Aggarwal S., Singh P.N., Mishra B. Studies on solubility and hypoglycemic activity of gliclazide beta-cyclodextrin-hydroxypropylmethylcellulose complexes, *Pharmazie*, 2002; 57(3): 191-3.
25. Talari R., Varshosaz J., Mostafavi S.A., Nokhodchi A. Dissolution enhancement of gliclazide using pH change approach in presence of twelve stabilizers with various physico-chemical properties, *J Pharm Pharm Sci*, 2009; 12(3): 250-265.

26. Talari R., Varshosaz J., Mostafavi S.A., Nokhodchi A. Gliclazide microcrystals prepared by two methods of in situ micronization: pharmacokinetic studies in diabetic and normal rats. *AAPS PharmSciTech*, 2010; 11(2): 786-92 .
27. Rowe R.C., Sheskey P.J., Weller P.J. (4th ed), *Handbook of Pharmaceutical Excipients*, American Pharmaceutical Association, Washington DC, 2003; 104.
28. Pulido N., Suarez A., Casanova B., Romero R., Rodriguez E., Rovira A. Gliclazide treatment of streptozotocin diabetic rats restores glut4 protein content and basal glucose uptake in skeletal muscle, *Metabolism*, 1997; 46(Suppl 1): 10- 13.
29. Qiang X., Satoh J., Sagara M., Fukuzawa M., Masuda T., Miyaguchi S., Takahashi K., Toyota T. Gliclazide inhibits diabetic neuropathy irrespective of blood glucose levels in streptozotocin-induced diabetic rats, *Metabolism*, 1998; 47(8): 977-981.
30. Ma A., Kamp M., Bird D., Howlett V., Cameron D.P. The effects of long term gliclazide administration on insulin secretion and insulin sensitivity, *Aust NZ J Med*, 1989; 19(1): 44-9.
31. Della Casa L., del Rio G., Glaser B., Cerasi E. Effect of 6-month gliclazide Treatment on insulin release and sensitivity to endogenous insulin in NIDDM: Role of initial continuous subcutaneous insulin-induced normoglycemia, *Diabetes Res Clin Pract*, 1991; 14(Suppl 2), S69-78.

Manuscript received: October 9th 2012