

# Improvement of Dissolution Rate of Gliclazide Using Solid Dispersions with Aerosil 380 and Its Effect on Alloxan Induced Diabetic Rats

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## Abstract

The main objective of this research is to conduct a comprehensive study for enhancing the aqueous solubility of poorly water soluble gliclazide using hydrophilic fumed silica particles (Aerosil® 380) and evaluating the influence of silica on drug release profile and pharmacological activity on alloxan induced diabetic rats. Solid dispersions (SD's) of gliclazide were prepared using solvent evaporation method. The dissolution profiles and solid state characterization of the SD's prepared were all evaluated. The dissolution rate of gliclazide in the SD's with fumed silica (weight ratio, 1:1) was approximately 38%, which is about 10 fold higher than that of the pure drug after 30 min. After forming the SD's, gliclazide changed into an amorphous state, which can infer from differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). Fourier transform infrared spectroscopy (FTIR) also revealed the formation of weak hydrogen bonding through the interactions between the secondary amine groups of gliclazide and silanol groups of silica particles in the SD's. The rapid dissolution rate from the SD's might be attributed to the amorphization of drug, improved specific surface area and wettability than the original drug crystals. Further, we investigated the antidiabetic effects of SD's of gliclazide in alloxan induced diabetic rats. The SD's of gliclazide decrease the blood glucose level 64% whereas the conventional gliclazide decreases only 37% in diabetic rats. Lipid profiles, kidney and liver functions are remarkably improved in diabetic rat treated with SD's of gliclazide than that of conventional gliclazide. These results suggest that SD's of gliclazide have much more bioavailability and hence are more pharmacologically active than that of conventional gliclazide form.

\*Contributed equally.

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## Keywords

*In-Vitro* Dissolution, Fumed Silica, Solid Dispersions, Gliclazide, Alloxan, Diabetes

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## 1. Introduction

The dissolution rate of active ingredients exhibiting poor aqueous solubility is a fundamental determinant of rate of absorption, and hence oral bioavailability [1]. According to the Biopharmaceutics Classification System (BCS), the dissolution of drugs exhibiting low solubility-high permeability (Class II) may be judged as the rate-limiting step through which possible *in vivo* performance may be predicted in terms of onset of action and intensity of pharmacological effect [2] [3]. It is, therefore, recommended to carry out the *in vitro* assessment of dissolution of Class II drugs in multiple media as an indicative test of their *in vivo* effect.

Gliclazide, a class-II drug, is an oral hypoglycemic sulfonylurea derivative that is commonly used for the treatment of non-insulin dependent diabetes mellitus (NIDDM). Due to possessing low aqueous solubility [4] [5] [6], gliclazide exhibits an unpredictable and slow absorption rate with considerable intra and inter subject variability [7]. Therefore, the enhancement of oral bioavailability of gliclazide remains one of the most challenging aspects of formulation development. Last few years, a remarkable number of approaches aimed at improving solubility and/or dissolution rate of gliclazide have been reported [8]-[17]. One approach involves the preparation of solid dispersions of gliclazide with hydrophilic carriers such as polyethylene glycols through applying different methodologies including fusion techniques [10] [11] [12] [13], co-grinding methods [14], and solvent evaporation methods [15] and solvent melting [16]. Furthermore, salt formation of gliclazide with sodium hydroxide has been reported to improve solubility and drug dissolution rate [18].

Dissolution rate as well as bioavailability enhancement by increasing the surface area is also well documented [19] [20]. In recent times, porous and mesoporous silica materials, characterized by the large specific surface area, have been reported to be a step ahead for enhancing drug dissolution and oral bioavailability [21]-[26]. It had been reported that SD particles with colloidal silica or porous silica prepared by the spray-drying method improve the dissolution properties of poorly water soluble drugs [27] [28] [29] [30]. Some studies reported preparation of SD's of indomethacin with silica particles using the co-grinding and melt-quenching method [31] [32] [33]. According to a number of earlier reports, drug molecules adsorbed onto the surface of the porous materials, due to the mixing or heating method were found to exist in the amorphous state [6] [34] [35] [36] [37].

To gain a clear understanding of gliclazide-aerosil (GA) interactions and their dynamics in the release rate of drug, we prepared their solid dispersions (SD's) using solvent evaporation method and a possible scenario for drug dissolution rate has been discussed in this study. The first part of the study consisted to examine the dissolution behavior of the SD's of GA and compared it to that of pure gliclazide (G) and their physical mixing systems (PMs). Subsequently, a set of complementary techniques (differential scanning calorimetry [DSC], scanning electron microscopy [SEM], X-ray powder diffraction [PXRD], and Fourier transform infrared spectroscopy [FTIR]) was used to monitor the physical changes of gliclazide in the SD's.

Therefore, in the present study, the physicochemical investigation is subsequently followed by pharmacological study of the formulation. In this portion, the hypoglycemic activity of the formulation was evaluated in a separate animal study. Moreover, biochemical investigations were carried out for further confirmation of activity and seeking possible side-effects associated with the formulation.

## 2. Materials & Methods

### 2.1. Drugs and Chemicals

Gliclazide was generously gifted by Square Pharmaceutical Ltd., Pabna, Bangladesh. Silica (Aerosil 380) was obtained from Evonik Pvt. Ltd., Hanau, Germany. Ethanol and methanol were purchased from Hong Yang Chemical Corporation, China and Merck, Germany, respectively. Potassium dihydrogen phosphate, sodium hydroxide and hydrochloric acid (35% - 38%) were purchased from Scharlab S. L. Spain; Merck Specialities Private Ltd India; and Merck, Germany; respectively. Alloxan was purchased from Loba Chemiie, Bombay, India. Blood glucose level was measured by using a glucose test meter (CLEVER CHECK-TD 4226, Germany) during the course of treatment. In-house distilled water from Millipore was used for all the experiments. All other reagents and solvents used were of analytical grades.

### 2.2. Preparation of SD's and PMs

The SD's (solid dispersions) of gliclazide with silica particles (Aerosil 380) were prepared using the solvent evaporation method. The drug-to-carrier ratios used in this study were 3:1, 2:1, 1:1, 1:2 and 1:3 (by weight) and denoted as GA (3:1), GA (2:1), GA (1:1), GA (1:2) and GA (1:3) respectively. The PMs (physical mixtures) were prepared by mixing gliclazide with silica particles at ratios of 1:3 (weight by weight) denoted as PMs (1:3) and then grinding them thoroughly using a mortar and pestle until a homogeneous mixture was obtained.

### 2.3. *In Vitro* Dissolution Studies

The pharmaceutical performance of pure gliclazide in powder form, SD's and its PMs were evaluated using *in vitro* dissolution tests. Dissolution tests were per-

formed using USP apparatus 2 (paddle method) in a Tablet Dissolution Tester, India [38]. The two media employed for testing were distilled water and pH 7.4 phosphate buffer. Samples equivalent to 15 mg gliclazide were spread onto the surface of the dissolution medium (900 ml), which was thermostatically maintained at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  and stirred at 75 rpm for 240 min. At the specified times (5, 15, 30, 60, 90, 120 and 240 min), 10 ml samples were withdrawn from dissolution vessel and filtered through a  $0.45\ \mu\text{m}$  membrane filter (Toyo Roshi Kaisha Ltd. Japan) and then assayed for gliclazide content by measuring the absorbance at 229 nm using a UV-Visible spectrophotometer (UV-1200, UV-Visible spectrophotometer, Shimadzu, Japan).

## **2.4. Solid-State Characterization of SD's of GA (Gliclazide-Aerosil® 380)**

### **2.4.1. Characterization by DSC**

Thermograms of gliclazide, aerosil and the different SD's of GA were obtained from DSC (Exstar SII DSC7020, Hitachi High-Tech Science Corporation, Tokyo, Japan). SD's samples (3 - 5 mg) were placed in sealed standard aluminium pans that heated from temperature between  $0^{\circ}\text{C}$  to  $300^{\circ}\text{C}$ , at a scanning rate of  $10^{\circ}\text{C}/\text{min}$ , under nitrogen purge, with an empty aluminium pan as reference.

### **2.4.2. Characterization by PXRD**

An X-ray diffractometer (RAD-C, Rigaku Denki Co. Ltd., Tokyo, Japan) was used for the diffraction studies. The samples were exposed to Cu-K $\alpha$  radiation (30 kV, 50 mA) and scanned from  $2^{\circ}$  -  $40^{\circ}$ ,  $2\theta$  (scanning rate of  $5^{\circ}/\text{min}$ ). Samples used for XRD analysis were free gliclazide, and the SD's of GA.

### **2.4.3. Characterization by SEM**

The cross-sectional morphology, surface, and shape of gliclazide, aerosol and the SD's of GA were observed using a scanning electron microscope (SSX-500, Shimadzu, Tokyo, Japan) after platinum metallization. An accelerating voltage of 15 kV was used.

### **2.4.4. Characterization by FTIR**

Drug-carrier interactions in the SD's were monitored using IR spectra measured by the diffuse reflection method using an FTIR spectrometer (IR-Prestige 21, Shimadzu Co. Japan). At first samples were ground and mixed properly with potassium bromide. The scanning range was  $4000$  to  $400\ \text{cm}^{-1}$ .

## **2.5. Experimental Animals and Treatment Protocol**

Male Wistar rats were purchased from ICDDR, B, Dhaka, Bangladesh weighing about 120 - 130 gm and housed in colony cages (four rats per cages) under standard environmental conditions ( $22^{\circ}\text{C}$  -  $25^{\circ}\text{C}$ ) and relative humidity  $55\% \pm 10\%$  with 12 h light and dark cycles having proper ventilation in the room. The rats were fed with standard rat diet (formulated by ICDDR, B) and water *ad libitum*. The experiment was conducted in accordance with the international principles

for laboratory animals' use and care as found in the guidelines [Communities, C. o. E. (1986). "Council instructions about the protection of living animals used in scientific investigations." Official Journal of the European Communities, (JO86/609/CEE), L358; Brussels: 1 - 18]. The study protocol was approved by Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) at the Institute of Biological Sciences, University of Rajshahi, Bangladesh. The animals were fasted overnight and intraperitoneally injected with alloxan monohydrate (Loba Chemiie, Bombay, India) dissolved in 0.9% v/v cold normal saline solution at a dose of 100 mg/kg body weight [39] [40]. The rats were then kept for the next 24 hours on 5% w/v glucose solution bottles in their cages to prevent hypoglycemia [41]. Blood glucose level higher or equal to 250 mg/dl are considered as diabetic rats. [42] [43] [44]. In our experiment, rats were randomly assigned into 5 groups (n = 4): NC (Normal Control), DC (Disease Control), GA/H (Diabetic, given GA high dose; 4 mg/kg), GA/L (Diabetic, given GA low dose; 2 mg/kg) and CG (Diabetic, given Conventional Gliclazide; 4 mg/kg). Drugs were administered orally via gastric gavage once a day for 15 days.

## 2.6. Measurement of Blood Glucose Level

At day 0, 3, 7, 10, 12, 15 the blood glucose level of rats were measured on overnight fasting by using a glucose test meter. Blood samples were withdrawn by pricking with sharp needle from the tail of rat.

## 2.7. Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test were performed according to the standard method [45]. In short, the treatment and control group were selected for OGT test after starving at water for 16 hours. The baseline glucose level was measured by glucometer. After 30 minutes of drug administration (4 mg/kg), the rats were orally treated with 2 g/kg of glucose. At 0, 30, 60, 90, 120 minutes, blood samples were collected from tail and measured. Male Wistar rats were divided into 3 groups consisting of 4 rats in each group.

Groups:

NC-Received only glucose.

GA/H-Received glucose and Gliclazide-Aerosil SD.

CG-Received glucose and conventional Gliclazide.

## 2.8. Collection and Storage of Blood and Serum

After the end of experiments, the rats were anesthetized, chest opened and blood samples were withdrawn directly from abdominal aorta and poured into Eppendorf tube by syringes [46]. It was then allowed to clot for 30 min. After that serum was collected by micropipette after separation by centrifugation (Ultra-centrifuge, Centurion, UK) at 4000 rpm for 10 min at 4°C and serum samples were preserved for the determination of biochemical parameters.

## 2.9. Measurement of Body Weight, Organ Weight and Organ Weight to Body Weight Ratio

The body weight of rats of each group was measured at day 0, 3, 7, 10, 12, 15 during treatment period. Rats were sacrificed; heart, liver, kidney and pancreas were removed and cleaned of the surrounding tissues. The organ weights were measured immediately and the ratio of organ weight to body weight (g/kg) were calculated.

## 2.10. Estimation of Lipid Profile, SGOT, Bilirubin and Creatinine

Total cholesterol (TC), Triglycerides (TG), high density lipoprotein (HDL) levels in serum were determined by the method described by Allain *et al.* [47], Foot and Principe [48] and Bergmenyer [49] respectively. Serum low-density lipoprotein cholesterol (LDL) was calculated according to Friedewald *et al.*, [50] with the following equation:

$$\text{LDL} = \text{TC} - \left( \text{HDL} + \frac{\text{TG}}{5} \right)$$

Serum glutamic oxaloacetic transaminase (SGOT) were measured according to Reitman and Frankel by using Randoxkit, Uk [51].

Total bilirubin and creatinine levels were estimated by Pearlman and Lee [52] and Henry *et al.* [53] respectively.

## 2.11. Statistical Analysis

Data are expressed as mean  $\pm$  standard error of mean (SEM). Statistical comparisons were performed by t test: two sample assuming equal variances. Results are considered as significant when  $p < 0.05$ .

# 3. Results

## 3.1. SD's Has a Markedly Higher Dissolution Rate of Gliclazide Than That of Pure Gliclazide

The dissolution profiles of the gliclazide and the SD's composed with different ratios in the first 5, 15, 30, 60, 90, 120 and 240 min are shown in **Table 1**. The dissolution profile of pure drug indicates very slow dissolution rate with less than 4% of the drug was dissolved in the first 15 min. The total amount of drug dissolved in 60 min was just over 8%. Nevertheless, the SD's of GA (such as 1:3 ratio) showed significantly higher dissolution rate (5 - 10 fold) of gliclazide than that of pure gliclazide in respect to every time point.

## 3.2. Presence of Free Drug in SD's Retards the Dissolution Rate

At 30 minutes, the release of drug from SD's of the GA ratio of 3:1 and 2:1 was about 20% and 28% respectively, which was approximately a 2 fold and 1.4 fold lower compared to that of 1:1 ratio (38%) (**Table 1**). This result is reflecting that large amount of drug in comparison to content of silica carrier affects the dissolution rate of drug. This is probably; the SD's of 1:1 is the optimized ratio to

**Table 1.** % release of drug from gliclazide and various ratios of gliclazide-aerosil SD's.

| Method         | Time (min) | Pure Gliclazide (% release) | % Release of Drug from SD's (Gliclazide: Aerosil) in Water |                             |                             |                             |                             |
|----------------|------------|-----------------------------|------------------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                |            |                             | 3:1                                                        | 2:1                         | 1:1                         | 1:2                         | 1:3                         |
| Solvent method | 5          | 0.96 ± 0.06                 | 15.85 ± 0.05 <sup>+++</sup>                                | 20.39 ± 0.06 <sup>+++</sup> | 30.97 ± 0.06 <sup>+++</sup> | 33.85 ± 0.07 <sup>+++</sup> | 38.05 ± 0.07 <sup>+++</sup> |
|                | 15         | 3.65 ± 0.06                 | 18.21 ± 0.06 <sup>+++</sup>                                | 24.71 ± 0.08 <sup>+++</sup> | 35.71 ± 0.08 <sup>+++</sup> | 39.89 ± 0.08 <sup>+++</sup> | 39.98 ± 0.09 <sup>+++</sup> |
|                | 30         | 4.19 ± 0.07                 | 20.13 ± 0.07 <sup>+++</sup>                                | 27.60 ± 0.08 <sup>+++</sup> | 37.90 ± 0.08 <sup>+++</sup> | 41.05 ± 0.08 <sup>+++</sup> | 41.15 ± 0.09 <sup>+++</sup> |
|                | 60         | 8.10 ± 0.07                 | 22.31 ± 0.08 <sup>+++</sup>                                | 29.93 ± 0.09 <sup>+++</sup> | 39.31 ± 0.09 <sup>+++</sup> | 41.86 ± 0.10 <sup>+++</sup> | 41.90 ± 0.10 <sup>+++</sup> |
|                | 90         | 9.48 ± 0.08                 | 23.55 ± 0.10 <sup>+++</sup>                                | 31.29 ± 0.10 <sup>+++</sup> | 39.64 ± 0.10 <sup>+++</sup> | 42.50 ± 0.10 <sup>+++</sup> | 42.58 ± 0.11 <sup>+++</sup> |
|                | 120        | 11.20 ± 0.10                | 24.57 ± 0.10 <sup>+++</sup>                                | 31.69 ± 0.11 <sup>+++</sup> | 39.68 ± 0.11 <sup>+++</sup> | 42.62 ± 0.11 <sup>+++</sup> | 42.65 ± 0.12 <sup>+++</sup> |
|                | 240        | 14.65 ± 0.11                | 28.24 ± 0.11 <sup>+++</sup>                                | 34.43 ± 0.11 <sup>+++</sup> | 39.72 ± 0.12 <sup>+++</sup> | 42.68 ± 0.12 <sup>+++</sup> | 42.72 ± 0.13 <sup>+++</sup> |

SD's: Solid Dispersions. Data are expressed as mean ± SEM. (n = 3), \*p < 0.05, \*\*p < 0.01 and \*\*\*p < 0.001 versus % release of pure gliclazide.

consume all the drug molecules to be adsorbed on its surfaces and accordingly increase the release of drug from the silica surfaces that were saturated with dispersed drug. Further increasing the amount of drug may be in the form of unabsorbed and agglomerated free drug particles, which ultimately retards the rate of drug release from SD's.

### 3.3. Enhancement in Dissolution Rate of Gliclazide Reaches a Limit Role

However, the dissolutions of GA (at 30 minutes) prepared at ratios of 1:1, 1:2 and 1:3 are very similar, which are 38%, 41.05% and 41.15%, respectively (**Table 1**). This result is reflecting that when the ratio of gliclazide to silica increased from 1:1 to 1:2 and then to 1:3, the dissolution rate did not show a significant improvement, indicating that the improvement in dissolution rate of gliclazide reached a limit role when the ratio is 1:1. Therefore, no more significant improvement in the dissolution rate was observed. This suggests that, as the content of silica surface area increases; the drug release increases to a limiting value, where in further increase in the silica surface area may not increase the drug release linearly [54].

### 3.4. Dissolution Profiles of Drugs in Water and Phosphate Buffer

The prime goal of the dissolution study was to improve the dissolution rate of various SD's over plain gliclazide and physical mixtures (PMs). Release pattern of gliclazide was conducted by performing dissolution studies in both distilled water (W) and phosphate buffer (P) pH 7.4. The release was compared with gliclazide loaded fumed silica (Aerosil® 380) (A) in a ratio 1:3 by solvent evaporation method; and their physical mixtures (PMs). The following rank order with a decrease in drug release rate was observed; GAP > GAW > PMsP > GP > PMsW > GW, **Table 2**. The drug release from SD's of gliclazide-silica was significantly faster. This significant enhancement in dissolution clearly confirms the superiority of fumed silica carriers for improving the dissolution rate in all media. However, no significant difference was observed for release of drug from SD's with silica in both water and phosphate buffer medium.



**Table 2.** Comparison of drug release from SD's in water medium and phosphate buffer medium.

| Time (min) | % Release of Drug in Water Medium |                                 |                                 | % Release of Drug in Phosphate Medium |                                 |                                 |
|------------|-----------------------------------|---------------------------------|---------------------------------|---------------------------------------|---------------------------------|---------------------------------|
|            | Pure Gliclazide                   | Gliclazide + Aerosil, PMs (1:3) | Gliclazide + Aerosil, SDs (1:3) | Pure Gliclazide                       | Gliclazide + Aerosil, PMs (1:3) | Gliclazide + Aerosil, SDs (1:3) |
| 5          | 0.96 ± 0.06                       | 11.4 ± 0.06                     | 38.05 ± 0.07                    | 8.10 ± 0.08 <sup>ΨΨΨ</sup>            | 21.79 ± 0.06 <sup>†††</sup>     | 40.99 ± 0.07 <sup>***</sup>     |
| 15         | 3.65 ± 0.06                       | 19.92 ± 0.07                    | 39.98 ± 0.09                    | 12.55 ± 0.09 <sup>ΨΨΨ</sup>           | 29.45 ± 0.08 <sup>†††</sup>     | 42.16 ± 0.09 <sup>***</sup>     |
| 30         | 4.19 ± 0.07                       | 24.13 ± .09                     | 41.15 ± 0.09                    | 26.32 ± 0.10 <sup>ΨΨΨ</sup>           | 29.57 ± 0.08 <sup>†††</sup>     | 42.53 ± 0.10 <sup>***</sup>     |
| 60         | 8.10 ± 0.07                       | 26.86 ± 0.09                    | 41.90 ± 0.10                    | 29.51 ± 0.11 <sup>ΨΨΨ</sup>           | 29.68 ± 0.09 <sup>†††</sup>     | 42.68 ± 0.10 <sup>**</sup>      |
| 90         | 9.48 ± 0.08                       | 28.44 ± 0.10                    | 42.58 ± 0.11                    | 30.77 ± 0.11 <sup>ΨΨΨ</sup>           | 29.70 ± 0.10 <sup>††</sup>      | 42.72 ± 0.11                    |
| 120        | 11.20 ± 0.10                      | 29.20 ± 0.10                    | 42.65 ± 0.12                    | 31.02 ± 0.12 <sup>ΨΨΨ</sup>           | 29.70 ± 0.11 <sup>†</sup>       | 42.75 ± 0.12                    |
| 240        | 14.60 ± 0.11                      | 29.25 ± 0.11                    | 42.72 ± 0.13                    | 31.69 ± 0.12 <sup>ΨΨΨ</sup>           | 29.77 ± 0.12 <sup>†</sup>       | 43.04 ± 0.12                    |

PMs: Physical Mixtures; G: Gliclazide; A: Aerosil; P: Phosphate; W: Water. Data are expressed as mean ± SEM (n = 3). <sup>Ψ</sup>p < 0.05, <sup>ΨΨ</sup>p < 0.01 and <sup>ΨΨΨ</sup>p < 0.001 versus % release of gliclazide in water medium. <sup>†</sup>p < 0.05, <sup>††</sup>p < 0.01 and <sup>†††</sup>p < 0.001 versus % release of drugs from PMs of GA (1:3) in water medium. \*p < 0.05, \*\*p < 0.01 and \*\*\*p < 0.001 versus % release of drugs from SDs of GA (1:3) in water medium.

### 3.5. Solid-State Characterization of SD's of GA

#### 3.5.1. Particle Morphology by SEM

To observe the differences in the surface conditions of gliclazide, silica and SD's of GA, SEM measurements were performed. The SEM photographs of gliclazide, silica and SD's of GA are shown in **Figure 1**. Gliclazide was observed as crystalline like structure in **Figure 1(a)** depicts that silica (Aerosil® 380) **Figure 1(b)** appears as cotton like substance with small pores and smooth surfaces which may facilitate the adsorption of drug on the surface/pores of silica particles in the SD's of GA at various degree (**Figures 1(c)-(e)**). On the other hand, when amount of silica (Aerosil® 380) was increased in the SD's of GA (1:2 and 1:3), the silica particles formed more agglomerates (**Figure 1(f)** and **Figure 1(g)**).

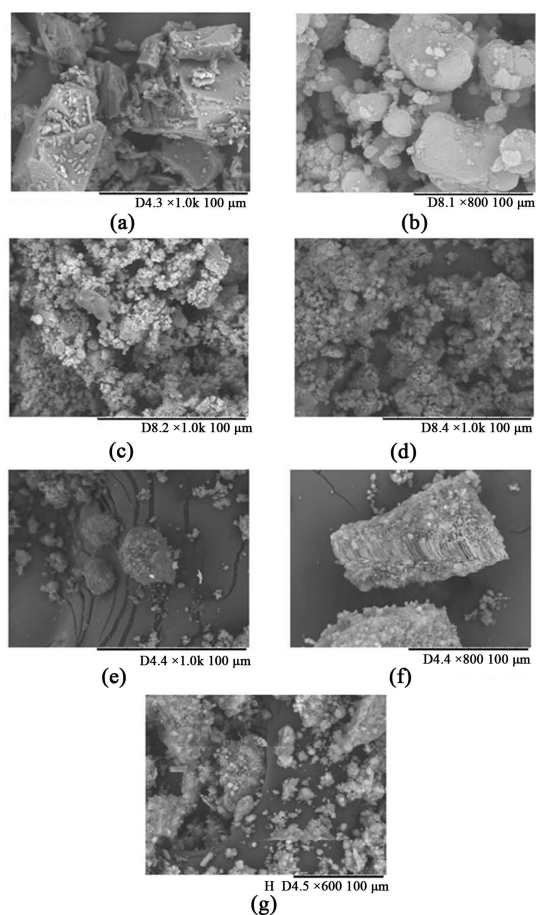
#### 3.5.2. Confirmation of Interactions by FTIR Spectrum

The major adsorption sites on a silica surface are hydroxyl groups. The interaction between drug molecules and silanol groups of silica has already been reported [30] [31] [32] [33]. From the FTIR spectra shown in **Figure 2**, gliclazide is characterized by the absorption of carbonyl (C=O) sulphonyl urea group at 1706 cm<sup>-1</sup> [55]. In case of SD's, this band was shifted towards higher frequencies with decreased intensities significantly. Gliclazide alone showed N-H stretching vibrations due to its secondary amine groups (-NH-) at 3262 cm<sup>-1</sup>, which was disappeared in the FTIR spectra of all SD's of GA which indicates a possibility of weak hydrogen bonding between gliclazide NH and CO group and silanol groups of silica formed during the formation of SD's [56].

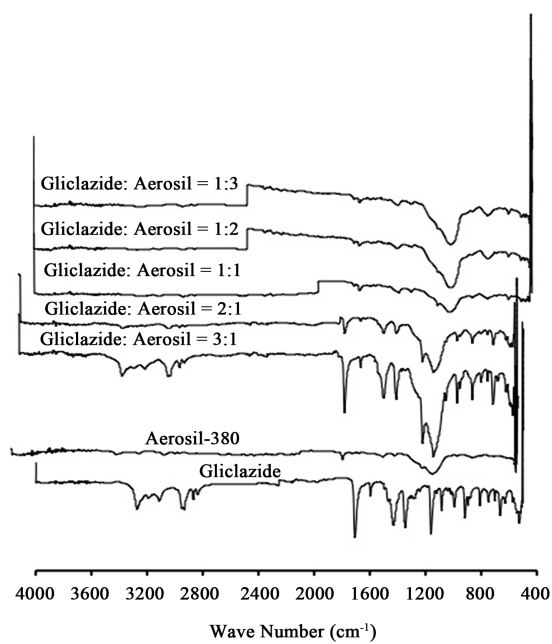
#### 3.5.3. Changes in Crystallinity Confirmed by DSC

The DSC thermogram of pure gliclazide (**Figure 3(a)**) showed a sharp endothermic peak with an onset melting temperature of 175.6°C indicating the crystallinity, whereas Aerosil® 380 (**Figure 3(b)**) showed a melting endotherm at 77.5°C. Thermograms of SD's of GA ratio 3:1 and 2:1 (**Figure 3(c)** and **Figure 3(d)**) showed the shifted endothermic peak for crystalline gliclazide. However,

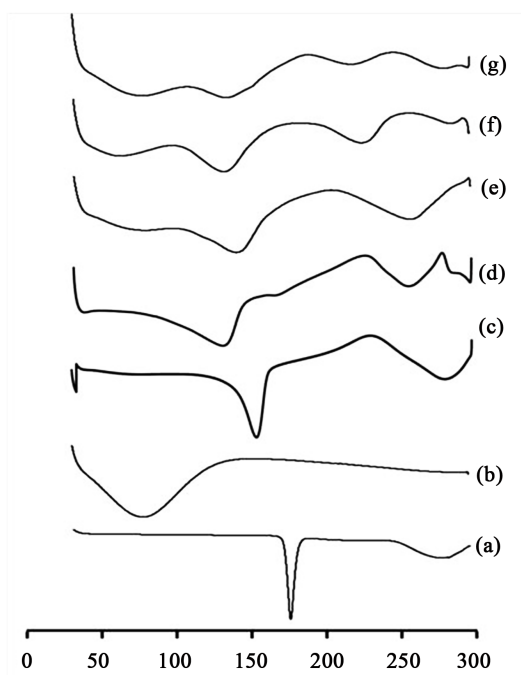




**Figure 1.** SEM photographs of (a) Gliclazide; (b) Aerosil; (c) GA (3:1); (d) GA (2:1); (e) GA (1:1); (f) GA (1:2); (g) GA (1:3).



**Figure 2.** FTIR spectrum of Gliclazide, Aerosil 380 and SD's of Gliclazide with Aerosil 380 in different ratio.



**Figure 3.** DSC thermogram of (a) Gliclazide; (b) Aerosil; (c) GA (3:1); (d) GA (2:1); (e) GA (1:1); (f) GA (1:2); (g) GA (1:3).

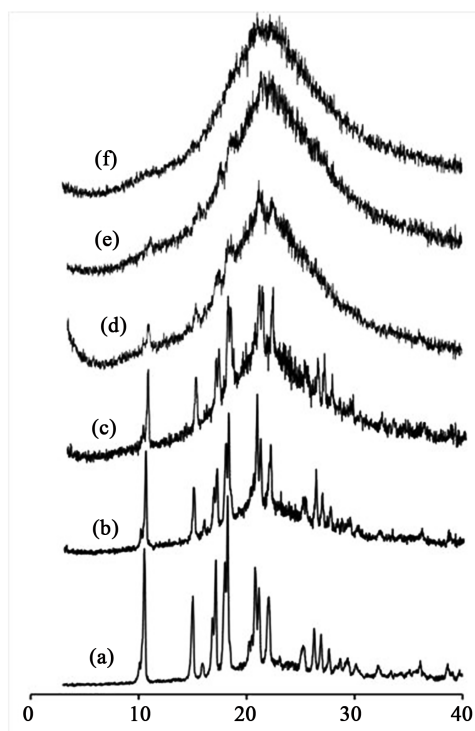
the thermograms of SD's of GA ratio 1:1, 1:2 and 1:3 (**Figures 3(e)-(g)**) showed the broad curves with no endothermic peak corresponding to the melting of pure crystalline gliclazide. This observation suggests that gliclazide is transformed from crystalline nature to an amorphous state at the formation of SD's with the solvent evaporation method, which is consistent with the previous suggestion on the absence of an endothermic peak of drug in SD's [36] [57] [58].

#### 3.5.4. Changes in Crystallinity Confirmed by PXRD

Powder X-ray diffraction patterns of gliclazide and SD's of GA (ratio 3:1, 2:1, 1:1, 1:2, and 1:3) are shown in **Figure 4**. In the gliclazide-Aerosil SD's (**Figure 4(b)** and **Figure 4(c)**), the distinctive diffraction peaks of gliclazide persisted with a marked decrease in their intensity compared with original gliclazide crystals (**Figure 4(a)**). We observed further decreased intensity of gliclazide peaks in SD's (**Figures 4(d)-(f)**) when prepared by using more amount of Aerosil 380. This observation suggests that the crystalline nature of the gliclazide was still maintained with GA ratio 3:1 and 2:1, but the relative reduction of diffraction intensity of gliclazide in Aerosil 380 starts at increased silica concentration (GA ratio 1:1, 1:2, 1:3), reflecting the transformation of the crystals to an amorphous state [59].

### 3.6. Anti-Diabetic Studies

Intraperitoneal injection of alloxan induced marked diabetes in rats was compared with normal rats. After 15 days treatment with SD's significantly decreases in blood glucose level in alloxan induced diabetic rats. (**Table 3**). High dose of



**Figure 4.** PXRD diffraction patterns of (a) Gliclazide; (b) GA (3:1); (c) GA (2:1), (d) GA (1:1); (e) GA (1:2); (f) GA (1:3).

**Table 3.** Time course of changes in blood glucose levels (mmol/L) after 15 days treatment protocol in alloxan-induced diabetic rats.

|      | Day 0        | Day 3        | Day 7        | Day 10                    | Day 12                    | Day 15                    |
|------|--------------|--------------|--------------|---------------------------|---------------------------|---------------------------|
| NC   | 6.35 ± 0.15  | 6.45 ± 0.44  | 6.3 ± 0.25   | 6.45 ± 0.06               | 6.6 ± 0.24                | 6.55 ± 0.19               |
| DC   | 19.65 ± 1.97 | 18.55 ± 2.81 | 18.9 ± 2.97  | 18 ± 2.27                 | 17.75 ± 1.43              | 17.9 ± 1.93               |
| GA/H | 21.75 ± 1.47 | 18.45 ± 1.72 | 15.15 ± 1.04 | 11.35 ± .685 <sup>†</sup> | 8.85 ± 0.82 <sup>††</sup> | 7.7 ± 0.62 <sup>††</sup>  |
| GA/L | 18.6 ± 1.37  | 16.5 ± 0.842 | 14.4 ± 0.825 | 12.35 ± 0.78              | 10.95 ± 0.87 <sup>†</sup> | 8.15 ± 0.858 <sup>†</sup> |
| CG   | 17.75 ± 0.88 | 16.8 ± 0.522 | 14.75 ± 0.77 | 12.85 ± 0.88              | 12.2 ± 1.33 <sup>†</sup>  | 11 ± 1.03 <sup>†</sup>    |

NC: Normal Control; DC: Disease Control; GA/H: Gliclazide-Aerosil (High dose); GA/L: Gliclazide-Aerosil (Low dose); CG: Conventional Gliclazide. Data are expressed as mean ± SEM; n = 4 in each group. <sup>†</sup>p < 0.05, <sup>††</sup>p < 0.01 and <sup>†††</sup>p < 0.001 versus Group DC.

GA-380 decreases the blood glucose levels to a great extent and it returns the blood glucose levels almost to the normal level ( $p < 0.01$ ). Low dose of GA-380 decreases the blood glucose levels from  $18.6 \pm 1.37$  to  $8.15 \pm 0.85$  mmol/L. On the other hand conventional dose of gliclazide showed less decrease of blood glucose levels compared to SD's of gliclazide ( $p < 0.05$ ).

### 3.7. Effects on Body Weight and Organ Weight to Body Weight

Effect of SD's of gliclazide and conventional gliclazide on body weight and organ weight to body weight ratios in alloxan induced diabetic rats are summarized in **Table 4**. The body weights were lower in diabetic rats on the other hand organ

**Table 4.** Effects of SD's of gliclazide and conventional gliclazide on body weight and organ weight to body weight ratios in alloxan-induced diabetic rats.

|      | Initial body wt. (g) | Final body wt. (g) | LW/BW                     | KW/BW                       | HW/BW                     | PW/BW                     |
|------|----------------------|--------------------|---------------------------|-----------------------------|---------------------------|---------------------------|
| NC   | 185.35 ± 3.7         | 214.6 ± 6.03       | 33.98 ± 0.7               | 6.57 ± 0.18                 | 3.11 ± 0.06               | 2.3 ± 0.04                |
| DC   | 161.55 ± 3.7         | 123.2 ± 3.17       | 41.83 ± 2.76              | 9.07 ± .49                  | 3.22 ± 0.07               | 3.34 ± 0.07               |
| GA/H | 186.9 ± 3.60         | 201.55 ± 4.6       | 33.75 ± 0.12 <sup>†</sup> | 5.22 ± 0.111 <sup>†††</sup> | 2.70 ± 0.160 <sup>†</sup> | 3.02 ± 0.05 <sup>†</sup>  |
| GA/L | 181 ± 4.19           | 174.55 ± 6.3       | 32.40 ± 0.44 <sup>†</sup> | 6.52 ± 0.29 <sup>††</sup>   | 2.73 ± 0.15 <sup>†</sup>  | 2.91 ± 0.04 <sup>††</sup> |
| CG   | 171.95 ± 1.4         | 154.2 ± 4.9        | 35.97 ± 0.244             | 7.75 ± .42                  | 3.05 ± 0.07               | 3.06 ± 0.21               |

BW: Body Weight; LW: Liver Weight; KW: Kidney Weight; HW: Heart Weight; PW: Pancreas Weight. Data are expressed as mean ± SEM; n = 4 in each group. <sup>†</sup>p < 0.05, <sup>††</sup>p < 0.01 and <sup>†††</sup>p < 0.001 versus Group DC.

weight to body weight ratio (LW/BW, KW/BW, HW/BW, and PW/BW) were higher in Group DC than in Group NC rats.

Induction of diabetes induces loss in body weight. In group DC the body weight decreases 23.73%. In case of conventional dosage form the rate of weight loss is reduced but it is not satisfactory. On the other hand in group GA/H, the% of body weight gain is 7.83%.

### 3.8. Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test (OGTT) was performed in order to evaluate the effects of SD's of gliclazide and conventional gliclazide on blood glucose level with respect to NC group in glucose induced hyperglycemic rats (**Figure 5**). Most significant decrease in blood glucose level had been observed in group treated with GA.

### 3.9. Effects on Lipid Profile

Both total cholesterol and triglycerides levels were significantly elevated in Group DC comparable to Group NC. Both the levels of total cholesterol and triglycerides were significantly reduced among the treatment groups (**Table 5**). The plasma HDL level was lower and the levels of LDL and LDL/HDL ratio were significantly higher in Group DC as compared to Group NC. Although drug treatment compensated the level of HDL, LDL and LDL/HDL ratio but most satisfactory result found in group GA/H.

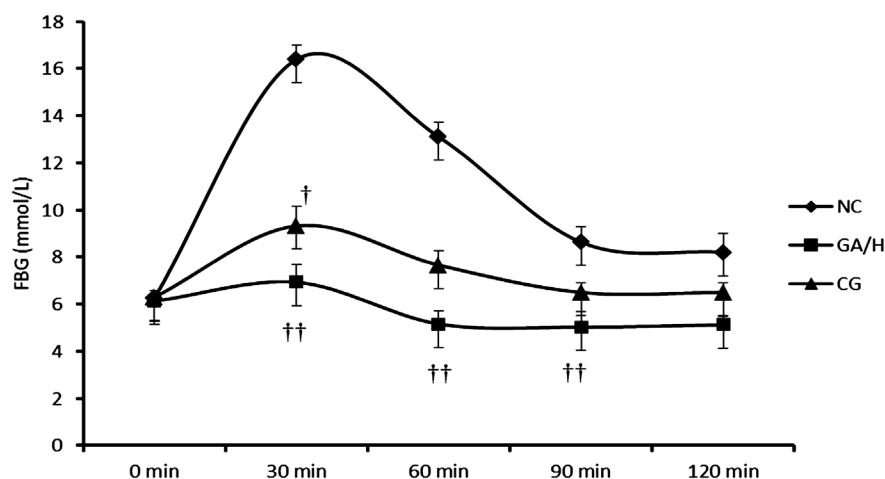
### 3.10. Effects on Serum SGOT, Bilirubin and Creatinine

The levels of SGOT, bilirubin and creatinine are shown in **Table 5**. The increased level of SGOT indicates the damage or inflammation of liver. Treatment with conventional dosage form reduced SGOT levels less significantly but, high dose of GA/H cause greater reduction in SGOT levels. In alloxan induced diabetic rats both the serum bilirubin and creatinine levels increased almost two fold which were normalized after fifteen days treatment protocol.

**Table 5.** Effects of SD's of gliclazide and conventional gliclazide on serum lipid profile, SGOT, bilirubin and creatinine in alloxan-induced diabetic rats.

|      | TC (mg/dl)                  | TG (mg/dl)                  | HDL (mg/dl)            | LDL (mg/dl)                | LDL/HDL                    | SGOT (U/L)             | Bilirubin (mg/dl)          | Creatinine (mg/dl)        |
|------|-----------------------------|-----------------------------|------------------------|----------------------------|----------------------------|------------------------|----------------------------|---------------------------|
| NC   | 89 ± 5.11                   | 84 ± 3.21                   | 35 ± 2.66              | 37 ± 2.08                  | 1.06 ± 0.02                | 31 ± 2.01              | 0.63 ± 0.07                | 0.71 ± 0.07               |
| DC   | 146.05 ± 7.05               | 160.25 ± 6.32               | 23 ± 2.5               | 93.9 ± 1.62                | 4.22 ± 0.43                | 75 ± 4.45              | 1.18 ± 0.03                | 1.42 ± 0.10               |
| GA/H | 90.25 ± 4.64 <sup>†††</sup> | 107.75 ± 6.47 <sup>††</sup> | 31 ± 1.63 <sup>†</sup> | 37.7 ± 4.57 <sup>†††</sup> | 1.24 ± 0.19 <sup>†††</sup> | 42 ± 3.8 <sup>††</sup> | 0.71 ± 0.05 <sup>†††</sup> | 0.81 ± 0.06 <sup>††</sup> |
| GA/L | 103.5 ± 6.07 <sup>††</sup>  | 111.7 ± 7.18 <sup>††</sup>  | 28 ± 2.41              | 53.16 ± 9.6 <sup>†</sup>   | 2.02 ± 0.53 <sup>†</sup>   | 46 ± 4.14 <sup>†</sup> | 0.76 ± 0.07 <sup>††</sup>  | 0.89 ± 0.09 <sup>†</sup>  |
| CG   | 115 ± 5.11 <sup>†</sup>     | 127 ± 6.12 <sup>†</sup>     | 26 ± 3.13              | 63.6 ± 3.04 <sup>†</sup>   | 2.56 ± 0.34 <sup>†</sup>   | 53 ± 4.81              | 0.89 ± 0.07 <sup>†</sup>   | 1.07 ± 0.1                |

TC: Total Cholesterol; TG: Triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; SGOT: Serum Glutamic Oxaloacetic Transaminase. Data are expressed as mean ± SEM; n = 4 in each group. <sup>†</sup>p < 0.05, <sup>††</sup>p < 0.01 and <sup>†††</sup>p < 0.001 versus Group DC.

**Figure 5.** Effects of SD's of Gliclazide and conventional Gliclazide on OGTT. Data are expressed as mean ± SEM; n = 4 in each group; <sup>†</sup>p < 0.05, <sup>††</sup>p < 0.01 and <sup>†††</sup>p < 0.001 versus NC.

#### 4. Discussion

The low aqueous solubility not only lowers the bioavailability but also hinders its clinical application. Low bioavailability may result in the precipitation of high doses of an active pharmaceutical ingredient in order to reach the therapeutic level. This is associated with toxicity issues and high manufacturing and therapeutic cost. Therefore, the main focus of the pharmaceutical field in drug formulation is to improve bioavailability by improving drug solubility and dissolution rate.

In this research, preparation of SD's using Aerosil® 380 as a carrier was successfully applied to increase the dissolution rate of poorly water-soluble gliclazide. Various possible mechanisms may involve in this improvement. Decreases in particle size and increases in surface area were confirmed using SEM, amorphous state of gliclazide in SD's, which was confirmed using DSC and PXRD analyses. The others effect, such as hydrogen bonding between the silanol groups of silica and both carbonyl groups and amino groups of gliclazide were demonstrated using FTIR, suggesting that this interaction could improve the

wettability properties of the drug and further increase drug dissolution rates [60] [61] [62].

Moreover, the particles of pure gliclazide and PMs were found to be floating on the surface of the dissolution medium during the dissolution experiment. Conversely, the SD's were well dispersed as soon as they were added to the dissolution medium. Accordingly, both the amorphization of the drug and the good wettability of SD's might account for the faster dissolution rate of SD's compared with PMs.

Furthermore, the *in-vitro* dissolution result of our study hence justifies the use of effective surface area of carriers for enhancing the dissolution and subsequently its oral bioavailability. Since the relationship between enhancement of dissolution and surface area is not linear, increasing the content of dispersing carriers may not be required to improve the *in vitro* or *in vivo* release property of drugs and it would be a good formulation strategy to optimize the level of carrier surface area or porosity required for the desired release rate.

The latency in achieving maximum concentration after oral administration of gliclazide is long (4 - 8 h) result from low dissolution rate of the drug which could be considered a big shortcoming [7]. It is noteworthy that the percentage release of gliclazide from SD's of GA (1:1) reached its maximum within the first 15 - 30 min, which is about 10 fold higher than that of pure gliclazide in water medium. Thus, the prepared SD's of GA might offer a potential improvement in the onset of action of gliclazide, which might lead to better clinical outcomes. These findings evidenced that nonporous silica (Aerosil® 380) can be a promising carrier to achieve enhanced dissolution rate for drugs with poor solubility.

We designed *in-vivo* study to investigate the antidiabetic effects of SD's of gliclazide in alloxan induced diabetic rats, a representative animal model of antidiabetic animal model as well as comparing the effect with conventional gliclazide to evaluate the antidiabetic efficacy and effect on body weight and several biochemical parameters.

The group GA/H decreases the blood glucose level 64% whereas the conventional group CG decreases only 37% in diabetic rats. These results suggest that SD's of gliclazide have much more bioavailability and hence are more pharmacologically active than that of conventional dosage form.

The decrease in blood glucose level may be due to correcting both defective insulin secretion and peripheral insulin resistance. Unstimulated and stimulated insulin secretions from pancreatic  $\beta$  cells are increased following the administration of gliclazide, with both first and second phases of secretion being affected. This occurs via binding of gliclazide to sulfonylurea receptor (SUR 1) on pancreatic  $\beta$  cells which results in a decrease in potassium efflux and causes depolarization on the cell [63].

Injection of alloxan markedly decreases the body weight in diabetic rats [64]. The body weight was lower in Group DC compared to Group NC rats. In group DC the body weight decreases 23.73%. On the other hand group GA/H prevented weight loss and the% of body weight gain is 7.83. The loss of body weight

may due to lack of sugar in the cells; forcing, the cells to use amino acids and fatty acids as a source of energy.

The marked hyperlipidemia that characterizes the diabetic state may be as a result of the uninhibited actions of lipolytic hormones on the fat depots due to the absence of insulin [65]. The post-treatment of the induced diabetic rats caused reduction in the levels of cholesterol. Furthermore, Neess *et al.* reported that the reduction of cellular cholesterol biosynthesis is associated with increased activity of the LDL receptor, which in turn leads to enhanced removal of LDL from plasma, resulting in reduced serum cholesterol concentration [66]. The present results also suggest that triglyceride levels were improved after fifteen days of oral administration of drugs. The reduction in triglyceride levels may be due to the suppression of triglyceride production without affecting lipoprotein lipase. On the other hand the HDL levels increased slightly compared to the disease control groups.

The increased concentration of creatinine is indicative of impaired renal function induced by alloxan in rats [67]. The effect of drugs on the kidney functions was assessed by the determination of the levels of serum creatinine, and the study revealed that post administration of SD's of gliclazide to the diabetic rats reduced significantly and nearly normalized the levels of serum creatinine compared to the conventional form of gliclazide.

The increase in the activities of serum SGOT indicated that diabetes may induce hepatic dysfunction [68]. In support of this finding, Larcan *et al.* had observed that liver was necrotized in diabetic patients [69]. Therefore, the increment of the activities of SGOT, in serum may be mainly due to the leakage of this enzyme from the liver cytosol into the blood stream, which gives an indication on the hepatotoxic effect of alloxan. On the other hand, treatment of the diabetic rats with SD's of gliclazide caused reduction in the activity of this enzyme in the serum compared to the mean values of disease control group. A possible explanation of the effects of drugs on the SGOT in serum is that these treatments may inhibit the liver damage induced by alloxan.

Furthermore, the improvement of the liver damage by oral administration of SD's of gliclazide could be confirmed by studying their effects on the level of serum bilirubin. The results showed that the experimentally induced diabetes increased the level of plasma bilirubin compare to the normal control. After fifteen days treatment with SD's of gliclazide decrease a significant plasma bilirubin in alloxan-induced diabetic rats when compared to the disease control rats as well as to the conventional treatment group, reflecting that SD's of gliclazide treatment might reduce the liver damage.

## 5. Conclusion

The dissolution rate of SD's of gliclazide with Aerosil 380 (1:1) is considerably improved which denoted faster and enhanced drug release from the formulation. SD's of gliclazide improved blood glucose level, lipid profiles, liver and kidney function better than the conventional gliclazide in alloxan induced diabetic rats.



These findings prefer to conclude that the fumed silica particle based SD's of gliclazide could achieve comprehensive dissolution profiles and pharmacological activities compared to gliclazide alone, which may provide a basis for carrying further research work in future on understanding different kinds of biopharmaceutical profile of BCS Class II drug molecules.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

- [1] Dressman, J.B., Amidon, G. L., Reppas, C. and Shah, V.P. (1998) Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms. *Pharmaceutical Research*, **15**, 11-22. <https://doi.org/10.1023/A:1011984216775>
- [2] Amidon, G.L., Lennernäs, H., Shah, V.P. and Crison, J.R. (1995) A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of *in Vitro* Drug Product Dissolution and *in Vivo* Bioavailability. *Pharmaceutical Research*, **12**, 413-420. <https://doi.org/10.1023/A:1016212804288>
- [3] Löbenberg, R. and Amidon, G.L. (2000) Modern Bioavailability, Bioequivalence and Biopharmaceutics Classification System. New Scientific Approach to International Regulatory Standards. *European Journal of Pharmaceutics and Biopharmaceutics*, **50**, 3-12. [https://doi.org/10.1016/S0939-6411\(00\)00091-6](https://doi.org/10.1016/S0939-6411(00)00091-6)
- [4] Alkhamis, K.A., Allaboun, H. and Al-Momani, W.Y. (2003) Study of the Solubilization of Gliclazide by Aqueous Micellar Solutions. *Journal of Pharmaceutical Sciences*, **92**, 839-846. <https://doi.org/10.1002/jps.10350>
- [5] Varshosaz, J., Talari, R., Mostafavi, S.A. and Nokhodchi, A. (2008) Dissolution Enhancement of Gliclazide Using *in Situ* Micronization by Solvent Change Method. *Powder Technology*, **187**, 222-230.
- [6] Saharan, V.A. and Choudhury, P.K. (2011) Dissolution Rate Enhancement of Gliclazide by Ordered Mixing. *Acta Pharmaceutica*, **61**, 323-334. <https://doi.org/10.2478/v10007-011-0021-7>
- [7] Palmer, K.J. and Brogden, R.N. (1993) Gliclazide: An Update of Its Pharmacological Properties and Therapeutic Efficacy in Non-Insulin-Dependent Diabetes-Mellitus. *Drugs*, **46**, 92-125. <https://doi.org/10.2165/00003495-199346010-00007>
- [8] Varma, M.M. and Kumar, P.S. (2012) Formulation and Evaluation of Gliclazide Tablets Containing PVP-K30 and Hydroxypropyl-B-Cyclodextrin Solid Dispersions. *International Journal of Pharmaceutical Sciences and Nanotechnology*, **5**, 1706-1719.
- [9] Sambath, L., Mathu, A.K., Kumar, M.A., Phaneendra, K. and Shalini (2013) Physicochemical Characterization and *in Vitro* Dissolution Behavior of Gliclazide-Soluplus Solid Dispersions. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5**, 204-210.
- [10] Patil, M.P. and Gaikwad, N.J. (2009) Preparation and Characterization of Glicla-

- zide—Polyethylene Glycol 4000 Solid Dispersions. *Acta Pharmaceutica*, **59**, 57-65.
- [11] Khattab, I.S., Nada, A. and Zaghloul, A.A. (2010) Physicochemical Characterization of Gliclazide-Macrogol Solid Dispersion and Tablets Based on Optimized Dispersion. *Drug Development and Industrial Pharmacy*, **36**, 893-902. <https://doi.org/10.3109/03639040903578734>
- [12] Patil, M.P. and Gaikwad, N.J. (2011) Characterization of Gliclazide-Polyethylene Glycol Solid Dispersion and Its Effect on Dissolution. *Brazilian Journal of Pharmaceutical Sciences*, **47**, 161-166.
- [13] Hosmani, A.H. and Thorat, Y.S. (2011) Optimization and Pharmacodynamic Evaluation of Solid Dispersion of Gliclazide for Dissolution Rate Enhancement. *Latin American Journal of Pharmacy*, **30**, 1590-1595.
- [14] Barzegar-Jalali, M., Valizadeh, H., Shadbad, M.R.S., Adibkia, K., Mohammadi, G., Farahani, A., Arash, Z. and Nokhodchi, A. (2010) Cogrounding as an Approach to Enhance Dissolution Rate of a Poorly Water-Soluble Drug (Gliclazide). *Powder Technology*, **197**, 150-158. <https://doi.org/10.1016/j.powtec.2009.09.008>
- [15] Biswal, S., Sahoo, J., Murthy, P.N., Giradkar, R.P. and Avari, J.G. (2008) Enhancement of Dissolution Rate of Gliclazide using Solid Dispersions with Polyethylene Glycol 6000. *AAPS Pharmaceutical Science and Technology*, **9**, 563-570. <https://doi.org/10.1208/s12249-008-9079-z>
- [16] Sudarsan, B., Gouri, S.P., Jagannath, S. and Padala, N.M. (2009) An Approach for Improvement of the Dissolution Rate of Gliclazide. *Dissolution Technologies*. <https://doi.org/10.14227/DT160409P15>
- [17] Talari, R., Varshosaz, J., Mostafavi, S.A. and Nokhodchi, A. (2009) Dissolution Enhancement of Gliclazide using pH Change Approach in Presence of Twelve Stabilizers with Various Physicochemical Properties. *Journal of Pharmacy and Pharmaceutical Science*, **12**, 250-265. <https://doi.org/10.18433/J31P4P>
- [18] Dina, E. and Imad I.H. (2014) Improvement of Dissolution Rate of Gliclazide Through Sodium Salt Formation. *Dissolution Technologies*. <https://doi.org/10.14227/DT210414P49>
- [19] Hu, J., Rogers, T.L., Brown, J., Young, T., Johnston, K.P. and Williams Iii, R.O. (2002) Improvement of Dissolution Rates of Poorly Water Soluble APIs using Novel Spray Freezing into Liquid Technology. *Pharmaceutical Research*, **19**, 1278-1284. <https://doi.org/10.1023/A:1020390422785>
- [20] Mellaerts, R., Aerts, C.A., Van Humbeeck, J., Augustijns, P., Van den Mooter, G. and Martens, J.A. (2007) Enhanced Release of Itraconazole from Ordered Mesoporous SBA-15 Silica Materials. *Chemical Communication*, No. 13, 1375-1377. <https://doi.org/10.1039/b616746b>
- [21] Friedrich, H., Fussnegger, B., Kolter, K. and Bodmeier, R. (2006) Dissolution Rate Improvement of Poorly Water-Soluble Drugs Obtained by Adsorbing Solutions of Drugs in Hydrophilic Solvents onto High Surface Area Carriers. *European Journal of Pharmaceutics and Biopharmaceutics*, **62**, 171-177. <https://doi.org/10.1016/j.ejpb.2005.08.013>
- [22] Horcajada, P., Ramila, A., Perez-Pariente, J. and Vallet-Regi, M. (2004) Influence of Pore Size of MCM-41 Matrices on Drug Delivery Rate. *Microporous and Mesoporous Materials*, **68**, 105-109. <https://doi.org/10.1016/j.micromeso.2003.12.012>
- [23] Sanaa, A., El-Gizawy, S.A., Mohamed, A., Osman, M.F., Arafa, G.M. and El-Maghraby, G.M. (2015) Aerosil as a Novel Co-Crystal Co-Former for Improving the Dissolution Rate of Hydrochlorothiazide. *International Journal of Pharmaceutics*, **478**, 773-778. <https://doi.org/10.1016/j.ijpharm.2014.12.037>

- [24] Angelos, S., Liong, M., Choi, E. and Zink, J.I. (2008) Mesoporous Silicate Materials as Substrates for Molecular Machines and Drug Delivery. *Chemical Engineering Journal*, **137**, 4-13. <https://doi.org/10.1016/j.cej.2007.07.074>
- [25] Wang, S. (2009) Ordered Mesoporous Materials for Drug Delivery. *Microporous and Mesoporous Materials*, **117**, 1-9. <https://doi.org/10.1016/j.micromeso.2008.07.002>
- [26] Wang, L., Cui, F.D. and Sunada, H. (2006) Preparation and Evaluation of Solid Dispersions of Nitrendipine Prepared with Fine Silica Particles Using the Melt-Mixing Method. *Chemical and Pharmaceutical Bulletin*, **54**, 37-43.
- [27] Takeuchi, H., Handa, T. and Kawashima, Y. (1987) Spherical Solid Dispersion Containing Amorphous Tolbutamide Embedded in Enteric Coating Polymers or Colloidal Silica Prepared by Spray-Drying Technique. *Chemical and Pharmaceutical Bulletin*, **35**, 3800-3806.
- [28] Takeuchi, H., Nagira, S., Yamamoto, H. and Kawashima, Y. (2003) Design of Solid Dispersion Particles of Drug with Fine Porous Carriers. *Journal of the Society of Powder Technology, Japan*, **40**, 157-162.
- [29] Takeuchi, H., Nagira, S., Yamamoto, H. and Kawashima, Y. (2004) Solid Dispersion Particles of Tolbutamide Prepared with Fine Silica Particles by The Spray-Drying Method. *Powder Technology*, **141**, 187-195. <https://doi.org/10.1016/j.powtec.2004.03.007>
- [30] Takeuchi, H., Nagira, S., Yamamoto, H. and Kawashima, Y. (2005) Solid Dispersion Particle of Amorphous Indomethacin with Fine Porous Silica Particle by Using Spray-Drying Method. *International Journal of Pharmaceutics*, **293**, 155-164. <https://doi.org/10.1016/j.ijpharm.2004.12.019>
- [31] Watanabe, T., Wakiyama, N., Usui, F., Ikeda, M., Isobe, T. and Senna M. (2001) Stability of Amorphous Indomethacin Compounded with Silica. *International Journal of Pharmaceutics*, **226**, 81-91. [https://doi.org/10.1016/S0378-5173\(01\)00776-1](https://doi.org/10.1016/S0378-5173(01)00776-1)
- [32] Watanabe, T., Ohno, I., Wakiyama, N., Kusai, A. and Senna, M. (2002) Controlled Dissolution Properties of Indomethacin by Compounding with Silica. *STP Pharma Sciences*, **12**, 363-367.
- [33] Watanabe, T., Hasegawa, S., Wakiyama, N., Kusai, A. and Senna, M. (2003) Comparison between Polyvinylpyrrolidone and Silica Nanoparticles as Carriers for Indomethacin in a Solid State Dispersion. *International Journal of Pharmaceutics*, **250**, 283-286. [https://doi.org/10.1016/S0378-5173\(02\)00549-5](https://doi.org/10.1016/S0378-5173(02)00549-5)
- [34] Matsumoto, K., Nakai, Y., Yonemochi, E., Oguchi, T. and Yamamoto, K. (1994) Physicochemical Characteristics of Porous Crystalline Cellulose and Formation of an Amorphous State of Ethenzamide by Mixing. *International Journal of Pharmaceutics*, **108**, 167-172. [https://doi.org/10.1016/0378-5173\(94\)90125-2](https://doi.org/10.1016/0378-5173(94)90125-2)
- [35] Nakai, Y., Yamamoto, K., Terada, K. and Ichikawa, J. (1984) Interaction of Medicinal and Porous Powder. I. Anomalous Thermal Behavior of Porous Glass Mixture. *Chemical and Pharmaceutical Bulletin*, **32**, 4566-4571.
- [36] Matsumoto, K., Nakai, Y., Yonemochi, E., Oguchi, T. and Yamamoto, K. (1998) Effect of Pore Size on the Gaseous Adsorption of Ethenzamide on Porous Crystalline Cellulose and the Physicochemical Stability of Ethenzamide after Storage. *Chemical and Pharmaceutical Bulletin*, **46**, 314-318.
- [37] Kinoshita, M., Baba, K., Nagayasu, A., Yamabe, K., Shimooka, T., Takeuchi, Y., Azuma, M., Houchi, H. and Minakuchi, K. (2002) Improvement of Solubility and Oral Bioavailability of a Poorly Water Soluble Drug, TAS-301, by It's Melt Adsorp-

- tion on a Porous Calcium Silicate. *Journal of Pharmaceutical Sciences*, **91**, 362-370. <https://doi.org/10.1002/jps.10026>
- [38] El-Sabawi, D. and Hamdan, I.I. (2014) Improvement of Dissolution Rate of Gliclazide through Sodium Salt Formation. *Dissolution Technologies*, **21**, 49-55. <https://doi.org/10.14227/DT210414P49>
- [39] Huuponen, R.K., Viikari, J.S. and Saarima, H. (1984) Correlations of Serum Lipids with Diabetes Control in Sulphonylurea Treated Diabetic Patients. *Diabetes Care*, **7**, 575-578. <https://doi.org/10.2337/diacare.7.6.575>
- [40] Nwanjo, H.U. (2005) Efficacy of Aqueous Leaf Extract of Vernoniaamygdalina on Plasma Lipoprotein and Oxidative Status in Diabetic Rat Models. *Nigerian Journal Physiological Science*, **20**, 39-42.
- [41] Dhandapani, S., Ramasamy, S.V., Rajagopal, S. and Namasivayam, N. (2002) Hypo-lipidemic Effect of *Cuminum cyminum* L. on Alloxan-Induced Diabetic Rats. *Pharmacological Research*, **46**, 251-255. [https://doi.org/10.1016/S1043-6618\(02\)00131-7](https://doi.org/10.1016/S1043-6618(02)00131-7)
- [42] El-Demerdash, F.M., Yousef, M.I. and El-Naga, N.I. (2005) Biochemical Study on the Hypoglycemic Effects of Onion and Garlic in Alloxan Induced Diabetic Rats. *Food and Chemical Toxicology*, **43**, 57-63. <https://doi.org/10.1016/j.fct.2004.08.012>
- [43] Kulkarni, J.S., Metha, A.A., Santani, D.D. and Goyal, R.K. (2002) Effects of Chronic Treatment with Cromakalim and Glibenclamide in Alloxan Induced Diabetic Rats. *Pharmacological Research*, **46**, 101-105. [https://doi.org/10.1016/S1043-6618\(02\)00078-6](https://doi.org/10.1016/S1043-6618(02)00078-6)
- [44] Ragavan, B. and Krishnakumari, S. (2006) Antidiabetic Effect of T. ArjunaBark Extract in Alloxan Induced Diabetic Rats. *Indian Journal of Clinical Biochemistry*, **21**, 123-128. <https://doi.org/10.1007/BF02912926>
- [45] Du Vigneaud, V. and Karr, W.G. (1925) Carbohydrate Utilization, Rate of Disappearance of D-glucose from the Blood. *Journal of Biological Chemistry*, **66**, 281-300.
- [46] Morton, D.B., Abbot, D., Barclay, R., Close, B.S., Ewbank, R., Gask, D., Heath, M., Mattic, S., Poole, T., Seamer, J. and Southee, J. (1993) Removal of Blood from Laboratory Mammals and Birds. *Laboratory Animals*, **27**, 1-22.
- [47] Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. and Fu, P.C. (1974) Enzymatic Determination of Total Serum Cholesterol. *Clinical Chemistry*, **20**, 470-475.
- [48] Footsati, S. and Principe, L. (1982) Serum Triglycerides Determined Colorimetrically with an Enzyme that Produces Hydrogen Peroxide. *Clinical Chemistry*, **28**, 2077-2080.
- [49] Bergmenyer, H.U. (1985) Methods of Enzymatic Analysis. 3rd Edition, John Wiley & Sons, New York, 154-160.
- [50] Friedewald, V.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of Low Density Lipoprotein Cholesterol in Plasma, without Use of Preparative Centrifuge. *Clinical Chemistry*, **18**, 499-502.
- [51] Reitman, S. and Frankel, S. (1957) A Colorimetric Method for The Determination of Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases. *The American Journal of Clinical Pathology*, **28**, 56-66. <https://doi.org/10.1093/ajcp/28.1.56>
- [52] Pearlman, F.C. and Lee, R.T. (1974) Detection and Measurement of Total Bilirubin in Serum with Use of Surfactants as Solubilizing Agents. *Clinical Chemistry*, **20**, 447-453.
- [53] Henry, R.J., Cannon, D.C. and Winkelman, J.W. (1974) Clinical Chemistry Principles and Techniques. 11th Edition, Happer and Row Publishers, New York, 1629.
- [54] Ansel, H.C., Popovich, N.G. and Allen, L.V. (1990) Pharmaceutical Dosage Forms

- and Drug Delivery Systems. Lea & Febiger, Philadelphia, PA.
- [55] Damian, F., Blaton, N., Naesens, L., Balzarini, J., Kinget, R., Augustinjs, P. and Mooter, G.V. (2000) Physicochemical Characterization of Solid Dispersions of the Antiviral Agent UC-781 with Polyethylene Glycol 6000 and Gelucire 44/14. *European Journal of Pharmaceutical Sciences*, **10**, 311-322. [https://doi.org/10.1016/S0928-0987\(00\)00084-1](https://doi.org/10.1016/S0928-0987(00)00084-1)
  - [56] Shin, S.C. and Kim, J. (2003) Physicochemical Characterization of Solid Dispersion of Furosemide with TPGS. *International Journal of Pharmaceutics*, **251**, 79-84. [https://doi.org/10.1016/S0378-5173\(02\)00586-0](https://doi.org/10.1016/S0378-5173(02)00586-0)
  - [57] Özkan, Y., Atay, T., Dikman, N., Isimer, A. and Aboul-Enein, Y.H. (2000) Improvement of Water Solubility and *in Vitro* Dissolution Rate Gliclazide by Complexation with B-Cyclodextrin. *Pharmaceutica Acta Helveticae*, **74**, 365-370. [https://doi.org/10.1016/S0031-6865\(99\)00063-1](https://doi.org/10.1016/S0031-6865(99)00063-1)
  - [58] Planinšek, O., Kovačič, B. and Vrečer, F. (2011) Carvedilol Dissolution Improvement by Preparation of Solid Dispersions with Porous Silica. *International Journal of Pharmaceutics*, **406**, 41-48. <https://doi.org/10.1016/j.ijpharm.2010.12.035>
  - [59] Valizadeh, H., Nokhodchi, A., Qarakhani, N.P., Zakeri-Milani, S., Azarmi, D.H. and Lobenberg, R. (2004) Physicochemical Characterization of Solid Dispersions of Indomethacin with PEG 6000, Myrj 52, Lactose, Sorbitol, Dextrin, and Eudragit E100. *Drug Development and Industrial Pharmacy*, **30**, 303-317. <https://doi.org/10.1081/DDC-120030426>
  - [60] Yin, L.F., *et al.* (2012) *In Vitro* and *In Vivo* Studies on a Novel Solid Dispersion of Repaglinide Using Polyvinylpyrrolidone as the Carrier. *Drug Development and Industrial Pharmacy*, **38**, 1371-1380. <https://doi.org/10.3109/03639045.2011.652635>
  - [61] Patel, J.R., *et al.* (2012) Preparation and Structural Characterization of Amorphous Spray-Dried Dispersions of Tenoxicam with Enhanced Dissolution. *Journal of Pharmaceutical Sciences*, **101**, 641-663. <https://doi.org/10.1002/jps.22800>
  - [62] Pahovnik, D., *et al.* (2011) Determination of the Interaction between Glimepiride and Hyperbranched Polymers in Solid Dispersions. *Journal of Pharmaceutical Sciences*, **100**, 4700-4709. <https://doi.org/10.1002/jps.22662>
  - [63] Jackson, J.E. and Bressler, R. (1981) Clinical Pharmacology of Sulphonylurea Hypoglycemic Agents: Part I. *Drugs*, **22**, 211-245. <https://doi.org/10.2165/00003495-198122030-00003>
  - [64] Ojo, R.J., Memudu, A.E., Akintayo, C.O. and Akpan, I.S. (2012) Effects of Pre-induction Administration of Allium Sativum on Some Biochemical Parameters in Alloxan Induced Diabetic Rats. *Research Journal of Applied Sciences, Engineering and Technology*, **4**, 5129-5135.
  - [65] Goodman, L.S. and Gilman, A. (1985) The Pharmacological Basis of Therapeutics. Macmillan, New York, 1490-1510.
  - [66] Neess, G.C., Zha, Z. and Lopez, D. (1996) Inhibitor of Cholesterol Biosynthesis Increase Hepatic Low Density Lipoprotein Degradation. *Archives of Biochemistry and Biophysics*, **325**, 242-248.
  - [67] Karuppasamy, B., Antony, N. and Veerabahu, R.M. (2014) Antidiabetic and Anti-hyperlipidaemic Activity of Ethanol Extract of Melastoma Malabathricum Linn. Leaf in Alloxan Induced Diabetic Rats. *Asian Pacific Journal of Tropical Biomedicine*, **4**, S442-S448. <https://doi.org/10.12980/APJTB.4.2014C122>
  - [68] Navarro, C.M., Montilla, P.M., Martin, A., Jimenez, J. and Utrilla, P.M. (1993) Free

Radicals Scavenger and Antihepatotoxic Activity of Rosmarinus. *Planta Medica*, **59**, 312-314. <https://doi.org/10.1055/s-2006-959688>

- [69] Larcen, A.H., Lambert, M.C. and Delorme, N. (1979) Light and Electron Microscopic Study of Hepatic Lesions in the Course of Hyperlactatemia in Diabetic Patients. *Diabetes and Metabolism*, **5**, 103-112.