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Tawfeek, HM, Roberts, M, El Hamd, MA, Abdellatif, AAH and Younis, MA (2018) Glibenclamide Mini-tablets with an Enhanced Pharmacokinetic and Pharmacodynamic Performance. AAPS PharmSciTech. ISSN 1530-9932

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Glibenclamide Mini-tablets with an Enhanced Pharmacokinetic and Pharmacodynamic Performance

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ABSTRACT:

In an attempt to decrease the dose, anticipated side effects and decrease the cost of production of glibenclamide, GLC, a potent oral hypoglycemic drug, the enhancement of the dissolution and hence the oral bioavailability were investigated. Adsorption and co-adsorption techniques using carriers having a very large surface area and surface active agents were utilized to enhance the drug dissolution. Moreover, the Langmuir adsorption isotherms were constructed to identify the type and mechanism of adsorption. The optimized formulation showing the highest *in vitro* release was compressed into mini-tablet to facilitate drug administration to elderly patients and those having swallowing difficulties. The produced mini-tablets were tested for their mechanical strength and *in vitro* release pattern. In addition, the pharmacodynamic and pharmacokinetic studies in New Zealand rabbits were performed using the optimized mini-tablet formulation. Mini-tablets containing GLC co-adsorbate with Pluronic F-68 and Laponite RD showed 100±1.88% of GLC released after 20 min. Pharmacodynamics studies in rabbits revealed significantly higher ($p \leq 0.05$) hypoglycemic effect with the optimized mini-tablets at a lower GLC dose compared to mini-tablets containing the commercial GLC dose. Moreover, Pharmacokinetic analysis showed significantly higher ($p \leq 0.05$) AUC, C_{max} and shorter T_{max} . The optimized mini-tablet formulation showed 1.5 fold enhancement of the oral bioavailability compared to mini-tablets containing untreated GLC. It could be concluded that the co-adsorption technique successfully enhanced the oral bioavailability of GLC. Furthermore, the produced mini-tablets have a higher oral bioavailability with a lower GLC dose, which could offer economic benefit for industry as well as acceptability for patients.

KEYWORDS: Glibenclamide, co-adsorbates, mini-tablets, pharmacodynamics, oral bioavailability.

INTRODUCTION

Glibenclamide (GLC, glyburide) is a potent oral hypoglycemic drug which is categorized under the second generation sulphonylureas. It is widely used in the treatment of type II diabetes mellitus and available at doses of 1.25, 2.5 and 5 mg [1]. It is practically-insoluble in water with a pKa value of 5.3, hence it is considered a weakly-acidic drug with a very low *in-vitro* dissolution rate at the gastric pH (1.2) [2]. According to Biopharmaceutical Classification System (BCS), GLC is categorized under class II drugs which are poorly water-soluble and highly-permeable [2]. Therefore, the dissolution of GLC is considered to be the rate-limiting step for its absorption, hence, it has a low oral bioavailability of approximately 45% [3]. In addition, as a drug of choice for diabetes mellitus, so the administration of conventional tablets could be an issue especially with patients suffering from dysphagia which affects significant percent of population [4, 5]. Different approaches have been developed to solve the problem of low oral bioavailability including the use of surfactants [6], cosolvents [6], inclusion complexes [7], solid dispersions [8], self-emulsifying systems [9], prodrugs [10], solid lipid nanoparticles [11] and micro-environmental buffering systems [12]. Formulation of drug adsorbates and co-adsorbates are among the most promising techniques adopted for dissolution enhancement [13-15]. Adsorbents with large surface areas have the potential to carry different drugs onto their surfaces hence, increasing their dissolution and bioavailability [13, 16]. Mini-tablets with their unique size have been introduced into the field of oral delivery to address problems such as dose accuracy and swallowing difficulties [17-19]. Previously, Tawfeek *et al.* [20] successfully prepared rapid-release mini-tablets containing small doses of lornoxicam compared with conventional tablets with a high dose which was more economic and suit patients with swallowing difficulties.

Generally, a reduction in drug dose will be beneficial to decrease the potential of any side effects for patients and to decrease the cost of producing and exporting expensive API for manufacturers [20, 21]. In an attempt to decrease the amount of GLC contained in tablets, our study focused on enhancing the GLC dissolution. Numerous studies have been performed to enhance the dissolution of GLC using solid dispersion technique with hydrophilic carriers or cyclodextrin inclusion complexation. However, to date, there has been no attempt to use water insoluble adsorbents having very high surface area and surface active agents. Adsorption and co-adsorption techniques were utilized in our study to enhance the dissolution and hence the oral bioavailability of GLC. The adsorption nature was studied using the Langmuir adsorption isotherm and the optimized formulation in terms of *in vitro* release was incorporated into mini-tablets. The produced mini-tablets were evaluated for their physical properties and pharmacodynamic performance after induction of diabetes in New Zealand rabbits using a lower dose of GLC compared to a commercial product. Furthermore,

the pharmacokinetic parameters were investigated in rabbits and compared to mini-tablets containing untreated GLC.

MATERIALS AND METHODS

Materials:

GLC and glipizide (used as internal standard, IS, in the HPLC method) were kindly gifted by T3A company for pharmaceutical industries, Assiut, Egypt. Laponite RD and Laponite FP were obtained from Rockwood Ltd, UK. Neusilin US2 was obtained from Fuji Chemical Industry, Japan. Florite R was supplied by Tokuyama Soda, Tokyo, Japan. Tween 80, Pluronic F-68 and Pluronic F-127 were supplied by Sigma Aldrich Co, St. Louis, MO, USA. StarLac® was supplied by Meggle BG excipients and technology, Germany. Streptozotocin was obtained from Sigma Aldrich, UK. HPLC-grade acetonitrile and methanol were purchased from BDH, Poole, UK. Aerosil 200, magnesium stearate, dipotassium hydrogen phosphate and potassium dihydrogen orthophosphate powders were obtained from El-Nasr Chemical Co., Abu Zaabal, Egypt. Heparin was purchased as Cal-Heparine® ampoules manufactured by Amoun pharmaceutical Co., Egypt. All other chemicals used were of analytical or pharmaceutical grades.

Drug-excipient compatibility studies:

The incompatibility between GLC and the excipients used were studied using a computer interfaced differential scanning calorimeter (DSC-50, Kyoto, Japan). Samples of about 5 mg from GLC and its physical mixtures (1:1 w/w ratio) with Laponite RD, Pluronic F-68, StarLac® and magnesium stearate were placed in an aluminum pan of 50 µl and covered with 0.1 mm thickness aluminum cover after being hermetically sealed. The run was adjusted from 30 to 300 °C using a rate of 10 °C min⁻¹ and a flow of nitrogen gas of 25ml/min. During the run, a reference sealed empty pan was used and the equipment was calibrated using indium. The obtained thermograms were interpreted to determine the melting temperature and heat of fusion (ΔH) for the corresponding peak.

Adsorption studies:

Determination of Equilibrium Adsorption of GLC:

The adsorption of GLC onto five different adsorbents namely; Laponite RD, Laponite FP, Neusilin US2, Florite R and Aerosil 200 was investigated. Briefly, a buffer solution of pH 1.2 containing 20 µg/ml of the drug was prepared and added to 100 mg of each of the investigated adsorbents in clean dry 100 ml volumetric flasks. The flasks were firmly closed and shaken at a rate of (40±2.0) stroke/minute in a thermostatically controlled water bath (DAIHAN scientific company, Model WSB-45, Korea) at 37±0.5 °C. After suitable time intervals (1, 2, 4, 6, 8, 12, 18 and 24 hours), samples of 1

ml were withdrawn from each test solution, filtered immediately and assayed spectrophotometrically at λ_{\max} of 230 nm for the remaining GLC content. Control test solution, containing an identical concentration of GLC without adsorbent, was treated similarly to check for any drug loss. Blank solutions containing only adsorbents without drug were also treated similarly.

Construction of Langmuir adsorption isotherms:

Buffer solutions of pH 1.2 containing different concentrations of GLC (16, 20, 24, 30 and 34 $\mu\text{g/ml}$) were added to 100 mg of each of the investigated adsorbents in clean, dry 100 ml volumetric flasks. All samples were subjected to the same conditions and treated as previously mentioned in the equilibrium adsorption study. All samples were left for 12 hours to ensure equilibrium. The adsorption of GLC onto the investigated adsorbents was evaluated using the Langmuir adsorption model according to equation 1 [13].

$$Y = \frac{X}{m} = \frac{KnC_{eq}}{1+nC_{eq}} \quad (\text{Equation 1})$$

Where, (y) is the amount of drug in millimoles (X) adsorbed per (m) grams of adsorbent, (C_{eq}) is the equilibrium concentration of drug (m.mole/L), (K) is the association constant (L/m.mole), and (n) is the maximum amount of drug adsorbed to form a monolayer under experimental conditions (limiting adsorption capacity) (m.mole/g). The data revealed that both grades of Laponite as well as Neusilin US2 showed the most promising results. Therefore, they were selected for further adsorption studies.

Effect of surfactant addition on the adsorption of GLC onto the investigated adsorbents:

To assess the effect of surfactant addition on the adsorption process, three surfactants were investigated; Tween 80, Pluronic F-127 and Pluronic F-68. Each of the investigated surfactants was added to the drug solutions to which adsorbent (Laponite RD or Neusilin US2) was added and proceeded as mentioned before in Langmuir adsorption study. Tween 80 was added in different concentrations (2, 5 and 10 % v/v) while Pluronics were added in concentrations of (2, 5 and 10% w/v). Tween 80 and Pluronics were chosen as non-ionic surfactants known to enhance the solubility and dissolution of many water insoluble drugs as reported previously [13, 20, 22, 23].

Preparation of GLC adsorbates and co-adsorbates:

Physical mixtures of GLC and Laponite RD were prepared in 1:1, 1:3 and 1:5 w/w ratios, respectively. Briefly, the calculated amounts of both GLC and Laponite RD were gently blended using a mortar and a pestle.

Solvent evaporation technique was used to prepare the loaded mixtures of GLC with Laponite RD in 1:1, 1:3 and 1:5 w/w ratios. Briefly, the calculated amount of Laponite RD was added to the

methanolic solution of GLC with sufficient stirring on a magnetic stirrer (Gallenkamp, UK). Further, methanol was removed under reduced pressure at 40 °C until a constant weight was obtained.

Co-adsorbates of GLC with Pluronic F-68 and Laponite RD were prepared in weight ratios of 1:1:1, 1:3:3 and 1:5:5 using solvent evaporation technique. The desired amounts of GLC and Pluronic F-68 were dissolved in methanol, then Laponite RD was added to the solution of GLC and Pluronic F-68 with sufficient stirring on a magnetic stirrer, further samples were obtained similarly to the loaded mixture. Finally, for all the previous preparations, the prepared samples were pulverized, sieved to obtain a particle size range of 125-250 μm and stored in a desiccator over calcium chloride for further analysis.

Characterization of the prepared systems:

GLC content:

An accurately weighed amount of the prepared systems equivalent to 1.25 mg GLC was added to 100 ml volumetric flask, dissolved in a minimum amount of methanol and the volume was completed to 100 ml with a buffer solution of pH 1.2. After suitable dilutions, GLC content was determined spectrophotometrically at λ_{max} of 230 nm (UV-VIS spectrophotometer, Jenway, Japan). It was found that Laponite RD and Pluronic F-68 did not interfere with the UV-absorbance of GLC at the λ_{max} . Only those samples containing 100 \pm 5.0% of the claimed amounts of GLC were considered for further studies.

DSC study:

DSC thermograms of pure GLC, Laponite RD, their physical mixture, adsorbate (GLC: Laponite RD; 1:5 wt. ratio) and co-adsorbates (GLC: Laponite RD: Pluronic F-68; 1:5:5 wt. ratio) were investigated as described previously in drug – excipients compatibility study section.

In vitro dissolution studies:

The *in vitro* dissolution performance the prepared samples was performed using the USP dissolution apparatus II (paddle type, Erweka, USA). Powdered samples from the previously prepared systems equivalent to 1.25 mg GLC were sprinkled into 900 ml buffer solution of pH 1.2, kept at 37 \pm 0.5 °C. Un-treated GLC powder was used as for a comparison after being sieved to obtain a size range of 125-250 μm . At predetermined time intervals of 5, 15, 30, 45, 60, 90 and 120 minutes, samples were withdrawn using a volumetric pipette with 0.45 μm membrane filter (Agilent Technologies, Santa Clara, CA) and replaced with an equal volume of fresh buffer equilibrated at the same temperature. The collected samples were analyzed using UV/VIS spectrophotometer at λ_{max} of 230 nm. Three runs

were performed and the mean values were used to calculate the percentage GLC dissolved. Dissolution profiles were compared by calculating the similarity factor (f_2 , Equation 2) [24]. A high degree of similarity is indicated by values in the range of $50 \leq f_2 \leq 100$.

$$f_2 = 50 \times \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (\text{Equation 2})$$

Where; $R_t - T_t$ are the cumulative percentage dissolved at each time point (n) of the two profiles being compared. Calculations of f_2 values were based on comparisons between the dissolution profiles for pure GLC, adsorbates, co-adsorbates and mini-tablet formulations. The degree of similarity between dissolution profiles was also confirmed by calculating the f_2 values based on the bootstrap statistical approach using the PhEq_bootstrap v 1.2 software (number of bootstraps = 5000 and Confidence Interval = 90%) [25].

Formulation and in vitro evaluation of GLC mini-tablets:

Pre-tableting evaluation of GLC co-adsorbate– excipients blend:

The selected GLC co-adsorbate, representing 37.5 or 62.5 %w/w of the mini-tablet weight, was mixed with 61.5 or 36.5 %w/w ratio, respectively of StarLac® using a turbula mixer (W.A. Bachofen, Switzerland) for 15 min and subsequently with 1 %w/w magnesium stearate for further 5 min. The bulk and tapped density properties of GLC formulations were determined. Bulk density (ρ_B) was calculated by measuring the volume of a known weight of powder mixture in a measuring cylinder. Tapped density (ρ_T) was calculated using the volume of the powder after tapping the cylinder 200-250 times, after which there was no further reduction in the volume of powder. Compressibility (Carr's Index) and Hausner ratios were determined according to equation 3 and equation 4, respectively [20].

$$\text{Carr's Index (\%)} = \frac{\rho_T - \rho_B}{\rho_T} \times 100 \quad (\text{Equation 3})$$

$$\text{Hausner Ratio} = \frac{\rho_T}{\rho_B} \quad (\text{Equation 4})$$

Production and testing of GLC mini-tablets:

Formulations were compressed into mini-tablets over a range of compression pressures using a Stylcam® 100R rotary press simulator (Medel Pharm, France) fitted with flat-faced 3 mm tooling at a speed of 20 rpm, which is equivalent to a rotary press production rate of approximately 80,000 tablets per hour [26]. Mini-tablet thickness, T (mm) and diameter, D (mm) were measured using a micrometer (Mitutoyo, Japan). Crushing strengths, F (N), were determined using a model 6D tablet tester (Dr. Schleuniger, Germany) and tensile strengths, σ_t (MPa) were calculated [27] according to Equation 5:

$$\sigma_t = \frac{2F}{\pi DT} \quad (\text{Equation 5})$$

Compression profiles (pressure vs. strength) were used to characterize two GLC mini-tablet

formulations and subsequent mini-tablets were produced at compression pressures of 250 – 350 MPa which will be used for *in vitro* dissolution, content uniformity and *in vivo* studies. Target mini-tablet weight was 22 mg to provide GLC doses of 0.75 or 1.25 mg from the formulations comprising 37.5 or 62.5 %w/w of the co-adsorbate. Further mini-tablets with a target weight of 11 mg were produced comprising 9.25 and 5.78 %w/w of co-adsorbate to provide doses of 92.5 and 57.8 µg, respectively for the *in vivo* studies on rabbits which represents the rabbit equivalent dose. The GLC content in the mini-tablets was determined according to the US Pharmacopeia 36 (2013). The drug analysis was performed similarly to the GLC content previously described section and the acceptance value was calculated according to the US Pharmacopeia equation for uniformity of drug content [28].

In vitro dissolution of GLC from the prepared mini-tablets:

The dissolution rate of GLC from the prepared mini-tablets was investigated as previously described in the characterization of adsorbates and co-adsorbates.

In vivo evaluation of GLC mini-tablets:

Pharmacodynamic study:

This study aimed to compare the hypoglycemic effect of mini-tablets containing the optimized GLC formulation in an amount equivalent to 0.75 mg human dose of GLC and those containing the non-adsorbed GLC in a dose equivalent to the standard commercial dose of 1.25 mg. The Medical Ethics Committee in Faculty of Medicine, Assiut University, Egypt proved the *in vivo* study protocol. Fifteen healthy adult male New Zealand rabbits weighing 1.25-1.75 Kg (average body weight= 1.5 Kg) were used and housed at room temperature. Diabetes was induced in the tested rabbits by the intraperitoneal injection of streptozotocin (STZ) at a dose of 65.0 mg/kg. The calculated amount of STZ was dissolved in 0.01M citrate buffer of pH 4.5. Seven days after the injection, the blood glucose levels were measured using Glucometer (ACCU-CHECK Compact Plus®, Roche Diagnostics, Japan). Each animal with a blood glucose level above 200 mg/dl was considered to be diabetic [29, 30]. Animals were restricted access to food for 24 hours before the experiment but had access to tap water. Rabbits GLC dose was calculated as previously published [31]. Three groups were used in this study, each consisting of five rabbits. The first group was designated as a control experiment which did not receive any tablets. The 2nd and 3rd groups were given mini-tablets containing pure GLC in a dose of 92.5 µg (equivalent to 1.25 mg GLC human dose) and mini-tablets containing the optimized GLC co-adsorbate in a dose of 57.8 µg (equivalent to 0.75 mg GLC human dose), respectively using a stomach tube. The blood glucose levels were measured at time intervals of 0.5,1,2,3 and 4 hours following mini-tablets administration.

Pharmacokinetic study:

Treatment of animals:

The protocol was similar to that used in the pharmacodynamic study. Three groups were chosen, and each has five rabbits. Control group, did not receive any dosage forms. The 2nd and 3rd groups were given an oral dose 57.8 µg of GLC (equivalent to 0.75 mg human dose) from mini-tablets containing pure GLC and those containing the optimized co-adsorbate, respectively using a stomach tube. Blood samples with a volume of approximately of 1-2 ml were withdrawn via an indwelling catheter in the marginal ear vein into a 5 ml screw-capped heparinized centrifuge tubes at the following time points: pre-dose, 0.5, 1, 2, 4, 6, 12 and 24 hours following drug administration. The samples were centrifuged at 5000 rpm for 15 min using Centurion Scientific Ltd, UK centrifuge. The supernatant was removed and transferred into a new screw-capped centrifuge tube. Further, the separated plasma was deep frozen at -20°C until analysis.

Assay of GLC in plasma:

A validated reversed-phase HPLC method was developed for analysis of GLC. Glipizide was used as internal standard (IS). The mobile phase (isocratic) consisted of acetonitrile-phosphate buffer (20 mM, pH 3.5) (60:40, v/v), degassed and filtered through a 0.45 µm pore size membrane filter. A pure standard stock solution of GLC was prepared in methanol, 1mg/ml and stored at 4 °C. This stock solution was diluted with mobile phase to obtain the concentrations required for preparation of standard working solutions in the range of 0.1-4 µg/ml. The IS solution was prepared in methanol at a concentration of 10 µg/ml. Samples for the determination of recovery, precision, and accuracy were prepared by spiking quality control sample (QC) with standard GLC concentrations of 0.1, 0.8 and 4 µg/ml, then stored at 4 °C. To each sample of 1ml of rabbit plasma, 1 ml of IS (10 µg/ml) and 5 ml methanol were added to precipitate the plasma proteins. GLC was extracted by vortex mixing the samples for 10 min followed by centrifugation at 5000 rpm for further 10 min. The organic layer was removed after precipitation of plasma proteins and then transferred into a Pyrex conical tube. Further, a complete evaporation of the organic solvent was performed by application of a stream of N₂ gas. The remaining solid residues were reconstituted into 100 µl of mobile phase and 20 µl sample was injected into HPLC column. The HPLC system consisted of a PerkinElmer 200 series system with a 200-series programmable absorbance UV detector operated at λ_{max} of 254 nm (710 Bridgeport Avenue Shelton, CT 06484-4794, USA). The separation was performed on Brownlee analytical RP-C₁₈ (250 × 4.6 mm i.d, 5 µm, PerkinElmer). The data were collected using TotalChrom 4.1 software. The mobile phase flow rate was 1 ml/min and all analysis was performed at room temperature. Triplicate measurements for each sample were performed and the amount was represented as means ± SD.

HPLC method validation:

The recovery of GLC from rabbit's plasma was evaluated in triplicate at three QC samples (0.1, 0.8 and 4 µg/ml). Blank rabbit plasma was spiked with known amounts of GLC and 10 µg/ml of IS. Absolute recovery was calculated by comparing the peak area ratios for direct injection of pure GLC and IS in methanol with those obtained by methanol extracted plasma samples containing the same amount of drugs.

Calibration curves were constructed in rabbit plasma samples spiked with GLC in the concentration ranges of 0.1- 4 µg/ml (seven points, 0.1, 0.2, 0.4, 0.8, 1, 2 and 4 µg/ml) and a fixed concentration of IS (10 µg/ml). Linearity was calculated from the correlation coefficient of the curve, while limits of detection (LOD) and quantitation (LOQ) were calculated using equations 6 and 7, respectively: [32, 33]

$$\text{LOD} = 3.3 \sigma/S \quad (\text{Equation 6})$$

$$\text{LOQ} = 10 \sigma/S \quad (\text{Equation 7})$$

Where, σ is the residual standard deviation of the regression line and S is the slope of the standard plot.

Inter-assay precision and accuracy were determined using QC samples spiked with three concentrations of GLC. For inter-assay, three replicates of each QC sample were assayed over five consecutive days. The QC concentrations were determined from three calibration curves which were run along with the QC samples to determine the accuracy of the developed HPLC-UV method and methanol extraction procedure. Precision was expressed as RSD% and accuracy was measured as the bias from the theoretically-expected value. The external standard method at three levels was used in the calibration and evaluation of the unknown samples.

Pharmacokinetic study:

The GLC concentrations in blood at different time intervals were used to obtain the GLC pharmacokinetics. (C_{max}) and (T_{max}) values were obtained directly from the obtained plasma concentration-time curve, whereas, the method of residual was adapted to calculate the absorption rate constant (K_{abs}). The elimination rate constant (K_{el}) and the apparent half-lives of absorption and elimination ($t_{1/2}$) were calculated as previously reported [34, 35]. In addition both the area under plasma concentration-time curve and the area under the first-moment curve, (AUC_{0-t}) and ($AUMC_{0-t}$),

from zero to end time were calculated by using linear trapezoidal rule. Equations 8 and 9 were used to calculate the values of both AUC and AUMC from zero time to infinity ($AUC_{0-\infty}$ and $AUMC_{0-\infty}$).

$$AUC_{(0-\infty)} = AUC_{(0-t)} + \frac{C_t}{K_{el}} \quad (\text{Equation 8})$$

$$AUMC_{(0-\infty)} = AUMC_{(0-t)} + \frac{t \cdot C_t}{K_{el}} + \frac{C_t}{K_{el}^2} \quad (\text{Equation 9})$$

Where, C_t is the last measured concentration at the last time point (t), K_{el} is the elimination rate constant of the drug. GLC mean residence time (MRT) was calculated via equation 10.

$$MRT = \frac{AUMC_{(0-\infty)}}{AUC_{(0-\infty)}} \quad (\text{Equation 10})$$

In addition, GLC clearance and volume of distribution was also calculated by dividing the dose by $AUC_{(0-\infty)}$ and by extrapolation method, respectively. The percentage relative bioavailability F_R was calculated using equation 11.

$$F_R(\%) = \frac{AUC_{(0-\infty)} \text{ for mini-tablets containing optimized co-adsorbate}}{AUC_{(0-\infty)} \text{ for mini-tablets containing pure GLC}} \times 100 \quad (\text{Equation 11})$$

The data were presented as mean values \pm SD and the significance of the pharmacokinetic results between mini-tablets containing the optimized formulation and the commercial GLC product was tested through student's t-test and the level of significance was set as ($p \leq 0.05$).

RESULTS AND DISCUSSION

Compatibility study:

Table I shows the temperature and heat of fusion (ΔH) obtained from DSC thermograms of GLC with the investigated excipients. Pure GLC showed a sharp melting endothermic peak at 175.55 °C with a ΔH value of (-110 J/g) which indicated the crystalline state of GLC. Each of the individual excipients showed a melting endothermic peak corresponding to its melting point except Laponite RD which has a melting point outside of the used scale (higher than 300 °C) [36]. Physical mixtures at 1:1 w/w ratio showed the same characteristic melting endotherm of the drug however, it was reduced in intensity concomitant with a reduction in the heat of fusion which could be possibly attributed to the dilution effect [8]. These results confirming the absence of any physical and chemical interaction between GLC and the used excipients, indicated the suitability of their further use in the GLC formulations.

Adsorption studies:

Construction of Langmuir adsorption isotherms:

The equilibrium time for adsorption of the drug onto the surface of all the investigated adsorbents was achieved within 4-6 hours. There was no decrease in drug concentration in the control experiment confirming the absence of any drug loss due to degradation or adsorption to glass utensils during equilibration.

Langmuir isotherms were obtained by plotting (X/m) versus (C_{eq}) . The curves showed typical type I Langmuir isotherms proving the formation of an adsorbed monolayer of GLC onto the investigated adsorbents [10]. Figure (1) represents typical Langmuir isotherm for adsorption of GLC onto the surface of Laponite RD as an example.

The linear form of Langmuir equation is:

$$\frac{C_{eq}}{y} = \frac{C_{eq}}{n} + \frac{1}{n \cdot K} \quad (\text{Equation 12})$$

When $(\frac{C_{eq}}{y})$ was plotted against $(\frac{C_{eq}}{n})$, a straight line was obtained indicating that adsorption of GLC onto the surface of the investigated adsorbents was a continuous function of the initial concentration of GLC [37] (Fig. 2).

(Insert Figs. 1 and 2, here)

The maximum adsorption capacity (n) and the association constant (K) can be calculated from the slope and intercept of the linear plots:

$$n = \frac{1}{\text{slope}} \quad (\text{Equation 13})$$

$$K = \frac{1}{n \cdot \text{intercept}} \quad (\text{Equation 14})$$

The limiting adsorption capacities and association constants of GLC onto the investigated adsorbents are listed in Table II. The results indicated that the adsorption capacities of the investigated adsorbents could be arranged in the following descending order:

Laponite RD > Laponite FP > Neusilin US2 > FloriteR > Aerosil 200.

These results can be explained on the basis of differences in the relative adsorption power of each of the investigated adsorbents. Laponites have the highest adsorption power among the investigated adsorbents due to their extensive specific surface area (surface area per unit mass) reaching 900 m²/g

[36] followed by Neusilin US2 (300 m²/g) [38] and finally, Aerosil 200 and Florite R (135-200 m²/g) [39, 40]. The superiority of Florite R over Aerosil 200 can be attributed to its extensive internal pore structure [40] as well as its basic nature giving it better affinity towards acidic drugs like GLC [41]. Similar results were reported by Makhlof [41] using glipizide as a model drug. It is worth noting that the porosity of adsorbents could also affect the adsorption process [42]. The investigated adsorbents have pore sizes ranging from nano/microporous like (Laponite and Neusilin) to mesoporous size for Aerosil 200, hence the lowest adsorption capacity [43].

Effect of surfactant addition on the adsorption of GLC onto the investigated adsorbents:

The results revealed that addition of surfactant lead to a reduction in the fraction of GLC adsorbed onto the investigated adsorbents due to the enhancement of GLC solubility in the medium (pH 1.2) [44]. Pluronic F-68 showed the most adsorption lowering effect indicating its highest solubilizing effect on drug followed by Pluronic F-127 and finally, Tween 80. Meanwhile, increasing surfactant concentration increased the drug solubility in the medium and thus, reduced the adsorbed fraction of drug onto the investigated adsorbents (Table III). The results suggested that combining both adsorption and surfactant-induced wetting effects would be beneficial in the enhancement of drug dissolution. Accordingly; formulation of co-adsorbates was tried using the best performing adsorbent (Laponite RD) and surfactant (Pluronic F-68).

Characterization of the prepared systems:

Drug content:

UV spectroscopic analysis confirmed the homogeneity of GLC content in all the investigated samples. The differences between theoretical and actual drug contents were negligible in physical mixtures, but there were slight differences between them in loaded mixtures and co-adsorbates (typically 98.0±2.0%) which could be possibly attributed to the processing steps.

DSC Study:

GLC alone showed its characteristic melting endotherm at 175.55 °C, which is indicative of the drug crystallinity, whereas Laponite RD did not display any melting during the DSC scan, from room temperature to 250 °C as depicted in Fig. 3, traces A and B, respectively. The broad shallow peak from 70 – 90 °C could be possibly due to evaporation of unbound moisture within the Laponite powder sample. The physical Mixture of GLC with Laponite at 1:5 weight ratio showed the characteristic peak of GLC, but was slightly shifted to 173.22 °C, which could be attributed to the mixing process and reduced in its intensity. Adsorbates of GLC onto Laponite and co-adsorbates with both Laponite and Pluronic F-68 at 1:5:5 weight ratio showed a complete disappearance of the GLC melting endotherm

(Fig. 3, traces D and E, respectively). This observation was due to the conversion of GLC from crystalline to amorphous state. Similar behavior was also recorded from other researchers [13, 20, 45].

(Insert Fig. 3, here)

In vitro dissolution studies:

The dissolution profiles of GLC from the various prepared systems are shown in Figs. (4-6). It was clear that the prepared adsorbates and co-adsorbates showed higher dissolution rates compared to untreated GLC which released only $26.69 \pm 1.25\%$ after 2 hrs. The F_2 similarity factor values for 1:5 adsorbate and 1:5:5 co-adsorbate vs untreated GLC were < 50 and the F_2 bootstrap values were confirmed as 20.42 and 3.31 respectively. Both indicate no degree of similarity between the dissolution profiles [24, 25]. The order of drug release was as the following: co-adsorbates > loaded mixtures (adsorbates) > physical mixtures. In addition, increasing drug: adsorbent weight ratio from 1:1 to 1:5 lead to an increase in the dissolution rate of GLC. These results confirmed that the adsorption process effectively participated in the enhancement of drug dissolution rate. This could be explained by drug deposition on more extensive surface areas of the loaded mixtures compared with the physical mixtures [12, 46]. As well as the drug conversion from crystalline state to amorphous state as observed from the DSC study. Similar results were reported by Ismail [46] who studied the effect of Florite R adsorbates on the dissolution rate of naproxen from emulgel. It is obvious that co-adsorbates showed the highest dissolution rates among all prepared systems which can be attributed to the combination of adsorption effect of the adsorbent in addition to the wetting effect of surfactant, which was responsible for the enhancement of solubility and dissolution of GLC compared with adsorbates with adsorption effect only [47]. Moreover, increasing amount of surfactant in the system, the release of drug was enhanced. The results revealed that GLC- Pluronic F-68-Laponite RD co-adsorbate in a weight ratio of 1:5:5 showed the best release profile ($100 \pm 1.88\%$ after 20 mins) and therefore, this formulation was considered as an optimum formulation and selected for further production of GLC mini-tablets.

(Inserts Figs. 4-6, here)

Formulation and in vitro evaluation of GLC mini-tablets:

Pre-tableting evaluation of GLC co-adsorbate– excipients blend:

The Carr's Index and Hausner's ratio of the GLC co-adsorbate together with the two formulations comprising different levels of StarLac® are shown in Table IV. Although the GLC co-adsorbate displayed reasonable flowability, as expected the presence of StarLac® in the formulations improved the densification properties of the powder and the flowability especially with increasing its amount.

StarLac[®] is a co-processed filler-binder consisting of 85% α -lactose monohydrate together with 15% corn starch [48]. The improved flowability and compressibility of StarLac[®] in comparison to the equivalent physical mixtures of its components is due to the spray-drying process employed during processing [49].

Production and testing of GLC mini-tablets:

GLC was uniformly distributed within the mini-tablets as shown from the calculation of the USP acceptance value. The mean of individual GLC contents expressed as a percentage of label claim was found to be 98.8 ± 1.4 . GLC mini-tablets have an acceptance value of 13.4 which is less than the maximum allowed acceptance value of 15 (L1). The compression profiles of the formulations comprising different levels of the GLC co-adsorbate and StarLac[®] are shown in Fig. (7). An initial increase in compression pressure resulted in significant increase in mini-tablet strength, but little further increase was observed beyond 200 MPa for both formulations. A higher level of Starlac[®] in the formulation comprising 37.5 %w/w of GLC co-adsorbate corresponded to greater tensile strengths at the higher compression pressures. Mini-tablets were produced for further testing at the optimum range of compression pressures of 250 – 350 MPa. The mini-tablets manufactured for *in vitro* dissolution studies were uniform in weight (22 ± 0.5 mg) and thickness (2.2 ± 0.1 mm). In a previous study, StarLac[®] was utilized in the formulation of rapid-release lornoxicam mini-tablets and in comparison, to other co-processed directly compressible excipients, mini-tablets produced with StarLac[®] provided optimal properties of superior flowability, good tensile strength, weight and content uniformity [20]. In addition, the presence of starch also imparts rapid disintegration properties [48] and leads to rapid drug release [20].

(Insert Fig. 7, here)

Dissolution of GLC from the prepared mini-tablets:

It was found that both mini-tablets containing either 37.5 % w/w or 62.5% w/w co-adsorbate formulations showed nearly identical release profiles due to the similarity of excipients used in the formulations. The F_2 similarity factor value for comparison between the two mini-tablet formulations was >50 and the F_2 bootstrap values was confirmed as 72.13 indicating a high degree of similarity between the dissolution profiles [24, 25]. Both formulations achieved approximately $100 \pm 2.74\%$ drug release after 15 mins which were slightly better than the release from 1:5:5 co-adsorbate mentioned before. This can be attributed to the presence of StarLac[®] leading to rapid drug release due to its α -lactose monohydrate content [48] which probably, enhanced GLC solubility as well as starch which imparts fast disintegration [20].

In vivo evaluation of GLC mini-tablets:

Pharmacodynamic study:

Table V shows the effect of mini-tablets containing the optimized co-adsorbate (in a dose equivalent to 0.75 mg human GLC dose) on the blood glucose level of diabetic rabbits in comparison with mini-tablets containing the non-adsorbed drug (in a dose equivalent to 1.25 mg human GLC dose). It was obvious that the mini-tablets containing the optimized co-adsorbate in a lower GLC dose resulted in more hypoglycemic effect than those containing the non-adsorbed drug in a higher dose. These results proved that the optimized co-adsorbate formulation showed superior pharmacodynamics which would be beneficial in using GLC in a lower dose to reduce the potential of any side effects and to be more economic. Moreover, to confirm the bioavailability enhancement, pharmacokinetic studies have also been investigated.

Pharmacokinetic study:

HPLC Method validation:

Figure (8) shows the HPLC chromatograms of rabbit plasma after 0.5 hour of administration of a mini-tablet containing GLC co-adsorbate (mini-tablets containing 57.8 µg GLC which is equivalent to 0.75 mg human GLC dose). It was obvious that the method gave well-defined peaks with a good resolution between IS and GLC peaks indicating the suitability of the method for accurate pharmacokinetic determinations. Table VI shows the recovery efficiency of GLC from rabbit plasma samples; the average extraction efficiency (mean ± SD) was found to be 93.3 ± 2.2%. Also, the % of RSD for area response for GLC was 2.2% which falls within the reported acceptance value [31] indicating the system repeatability.

(Insert Fig. 8, here)

Calibration curves of GLC were constructed in rabbit plasma samples in the concentration range of 0.1-4 µg/ml. GLC/IS peak area ratio was found to have a good linear relationship with the selected concentration range for all the tested GLC concentrations (correlation coefficient, $r = 0.9905$). The calibration curve equation for CLG in the developed HPLC-UV measurements was $y = 0.7092x + 0.3393$ (where, y = peak area ratio and x = GLC concentration, µg/ml). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.033 and 0.10 µg/ml, respectively.

The mean parameters of intra- and inter-assay precision and accuracy are summarized in Table VII. The mean average of the calculated GLC accuracy was found to be 92.0 ± 6.0 % indicating acceptable accuracy for the developed method [32].

Pharmacokinetic analysis:

Table VIII shows the pharmacokinetic parameters of the optimized GLC mini-tablets compared with those containing untreated GLC. The mean GLC plasma concentrations profiles against time obtained after oral administration of mini-tablets containing optimized co-adsorbate and those containing non-adsorbed GLC at a dose level of 57.8 µg GLC are shown in Fig. (9). The results revealed significant ($p \leq 0.05$) improvement in GLC pharmacokinetic parameters after administration of mini-tablets containing the optimized co-adsorbate. Significantly higher peak plasma concentration (C_{max}) and area under curve (AUC) were achieved compared with mini-tablets containing the non-adsorbed GLC (C_{max} values were 1.66 ± 0.13 and 1.20 ± 0.12 µg/ml for mini-tablets containing optimized co-adsorbate and mini-tablets containing untreated drug, respectively and AUC values were 14.25 ± 4.61 and 9.99 ± 2.28 µg.hr/ml, respectively). The relative bioavailability value comparing the bioavailability of optimized mini-tablets relative to those containing non-adsorbed GLC was $142.67 \pm 11.53\%$ indicating nearly 1.5 fold enhancement of oral bioavailability. These results confirmed that formulation of co-adsorbate resulted in enhancement of GLC bioavailability through enhancement of drug release by the combined effect of adsorption and wetting [50]. In addition, mini-tablets containing co-adsorbate showed significantly ($p \leq 0.05$) shorter T_{max} and $t_{1/2(abs)}$ values than those containing non-adsorbed GLC indicating faster absorption [51]. The mean residence time (MRT) of GLC was also significantly ($p \leq 0.05$) increased from 10.08 ± 0.59 hours for mini-tablets containing untreated GLC to 12.73 ± 0.57 hours for mini-tablets containing optimized co-adsorbate. The elimination half-lives of GLC from mini-tablets containing optimized co-adsorbate and mini-tablets containing untreated drug were 9.50 ± 0.28 and 9.39 ± 0.63 hours, respectively and the apparent volume of distribution values were 0.024 ± 0.02 and 0.015 ± 0.03 L/kg for co-adsorbate-containing mini-tablets and those containing the non-adsorbed GLC, respectively which were in concordance with the reported values of GLC [1].

(Insert Fig. 9, here)

CONCLUSIONS

Adsorption studies confirmed the adsorption of GLC onto the investigated adsorbents following type I Langmuir adsorption model. Laponite RD showed the highest adsorption capacity to GLC. In addition, formulation of co-adsorbates resulted in significant enhancement ($f_2 < 50$) of GLC dissolution rate $100 \pm 1.88\%$ after 20 mins compared to $26.69 \pm 1.25\%$ after 2 hrs from untreated GLC. Furthermore, GLC co-adsorbate with Pluronic F-68 and Laponite RD in a weight ratio of 1:5:5 showed the best dissolution results and was selected in the formulation of GLC mini-tablets. The prepared mini-tablets showed acceptable results regarding their physical properties and *in vitro* release profiles. In addition, *in vivo* studies in rabbits revealed that co-adsorbate-containing mini-tablets in a lower dose resulted

in more hypoglycemic effect than those containing the non-adsorbed GLC in a higher dose. Pharmacokinetic testing of the produced mini-tablets revealed the enhancement of GLC bioavailability about 1.5 folds higher than those containing untreated GLC. Finally, it could be concluded that the optimized GLC mini-tablets will be promising for patients with swallowing difficulties especially geriatric patients. In addition, the lower GLC dose of 0.75 mg saving about 40% of API could be advantageous in terms of lowering any potential side effects and the production cost, hence it could be preferred in industry.

LIST OF ABBREVIATIONS

AUC_(0-24 hr): Area under drug plasma concentration versus time curve from zero time to the end of the experiment.

AUC_(0-∞): Area under drug plasma concentration versus time curve from zero time to infinity.

AUMC_(0-24 hr): Area under first moment curve from zero time to the end of the experiment.

AUMC_(0-∞): Area under first moment curve from zero time to infinity.

BCS: Biopharmaceutical classification system.

C_{max}: Maximum (peak) drug concentration in plasma.

Cl_T: Total drug clearance.

DSC: Differential Scanning Calorimetry.

F_R: Relative bioavailability.

GLC: Glibenclamide.

IS: Internal Standard.

K_{abs}: Absorption rate constant.

K_{el}: Elimination rate constant.

LOD: Limit of detection.

LOQ: Limit of quantitation.

MRT: Mean residence time.

NUS: Neusilin.

PEG: Polyethylene glycol.

RSD: Relative standard deviation.

STZ: Streptozotocin.

$t_{\frac{1}{2}(\text{abs})}$: Absorption half-life.

$t_{\frac{1}{2}(\text{el.})}$: Elimination half-life.

T_{max} , time to achieve peak drug concentration in plasma.

CONFLICT OF INTEREST

The authors report no conflict of interest related to this work.

ACKNOWLEDGMENTS

The authors are grateful to Faculty of Pharmacy, Assiut University, Egypt for supporting and facilitating the research. The authors are also grateful to T3A Company for Pharmaceutical Industries, Assiut, Egypt for gifting GLC and glipizide. The authors are grateful thankful to Dr. Hamzah Maswedeh, Department of Pharmaceutics, Faculty of Pharmacy, Qassim University, KSA.

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Table I. Peak temperatures and enthalpy changes (ΔH) for DSC thermograms of GLC alone and its physical mixtures with the investigated excipients at 1:1 weight ratio

Samples	Peak temperature of GLC ($^{\circ}\text{C}$)	ΔH (J/g)
GLC alone	175.55	-110.0
GLC:Laponite RD	174.30	-56.33
GLC:Pluronic F-68	175.22	-57.62
GLC:Starlac [®]	175.23	-68.36
GLC:Magnesium stearate	175.10	-58.81

Table II. limiting adsorption capacities and association constants of GLC adsorption onto the investigated adsorbents in buffer solution of pH 1.2 at 37 $^{\circ}\text{C}$

Adsorbent	Limiting adsorption capacity, n	Association constant, K
	(m.mole/g) ^a	(L/m.mole) ^a
Laponite RD	0.022 \pm 0.001	32.08 \pm 7.51
Laponite FP	0.021 \pm 0.003	21.75 \pm 5.56
Neusilin US2	0.018 \pm 0.002	22.08 \pm 4.08
Florite R	0.017 \pm 0.008	31.58 \pm 5.77
Aerosil 200	0.014 \pm 0.005	50.80 \pm 8.49

^a Results are expressed as average \pm standard deviation (SD).

Table III. Effect of addition of various concentrations of different surfactants on the limiting adsorption capacities of GLC onto investigated adsorbents in buffer solution of pH 1.2 at 37 $^{\circ}\text{C}$

Surfactant concentration ^a	Limiting adsorption capacity, n (m.mole/g) ^b					
	Laponite RD			Neusilin US2		
	Pluronic F-68	Pluronic F-127	Tween 80	Pluronic F-68	Pluronic F-127	Tween 80
0	0.022 \pm 0.001	0.022 \pm 0.001	0.022 \pm 0.001	0.018 \pm 0.002	0.018 \pm 0.002	0.018 \pm 0.002
2	0.015 \pm 0.001	0.018 \pm 0.002	0.020 \pm 0.004	0.013 \pm 0.002	0.014 \pm 0.003	0.017 \pm 0.006
5	0.013 \pm 0.003	0.015 \pm 0.002	0.016 \pm 0.001	0.009 \pm 0.001	0.012 \pm 0.002	0.014 \pm 0.005
10	0.008 \pm 0.002	0.011 \pm 0.003	0.013 \pm 0.002	0.006 \pm 0.004	0.008 \pm 0.003	0.011 \pm 0.004

^a Pluronics concentrations are expressed as % w/v while Tween 80 concentrations are expressed as % v/v.

^b Results are expressed as average \pm standard deviation (SD).

Table IV. The percentage of compressibility (Carr's index) and Hausner ratio for GLC formulations

System	Carr's Index (%) ^a	Hausner ratio ^a
GLC co-adsorbate alone	16.70±1.10	1.20±0.06
62.5 % w/w co-adsorbate formulation ^b	16.0±0.90	1.19±0.03
37.5 % w/w co-adsorbate formulation ^b	15.40±0.82	1.18±0.02

^a Number of samples (n)=5; results are expressed as average ± standard deviation (SD).

^b The formulations are composed of optimized GLC co-adsorbate in the specified % w/w and Starlac[®] as a filler.

Table V. Effect of GLC mini-tablets optimum formulation on the blood glucose level of diabetic rabbits in comparison with mini-tablets containing untreated GLC

Time after administration (hours)	Blood glucose level (mg/dL) ^a	
	Mini-tablets optimum formulation	Mini-tablets containing untreated GLC
0	282 ± 9	261 ± 4
0.5	143 ± 9	176 ± 5
1	122 ± 10	131 ± 10
2	110 ± 11	118 ± 6
3	102 ± 10	114 ± 2
4	90 ± 7	101 ± 8

^a Number of samples (n)=5; results are expressed as average ± standard deviation (SD).

Table VI. The percentage recovery of GLC from rabbit plasma QC samples spiked with different concentrations of GLC

QC sample (µg/mL)	Recovery (%) ^a	RSD (%)
0.1	90.2 ± 3.5	3.9
0.8	93.6 ± 1.8	1.9
4.0	96.1±1.3	1.4

^a Number of samples (n)=5; results are expressed as average ± standard deviation (SD).

Table VII. Accuracy and precision (RSD %) for GLC assayed in spiked rabbit plasma samples

GLC spiked concentration ($\mu\text{g/mL}$)	Intra-day ^a		Inter-day ^a	
	GLC measured concentration ($\mu\text{g/mL}$)	RSD(%)	GLC measured concentration ($\mu\text{g/mL}$)	RSD (%)
0.1	0.09 \pm 0.01	11.11	0.08 \pm 0.01	12.50
0.8	0.76 \pm 0.06	7.89	0.75 \pm 0.08	10.66
4.0	3.85 \pm 0.10	2.59	3.91 \pm 0.20	5.11

^a Number of samples (n)=5; results are expressed as average \pm standard deviation (SD).

Table VIII. Pharmacokinetic parameters of mini-tablets containing optimized GLC co-adsorbate and those containing untreated GLC after oral administration in rabbits at dose level of 57.8 μg

Pharmacokinetic parameters ^a	Mini-tablets containing optimized GLC co-adsorbate	Mini-tablets containing untreated GLC	Significance of the difference ^b
C_{max} ($\mu\text{g/ml}$)	1.66 \pm 0.13	1.20 \pm 0.12	significant
T_{max} (hr)	1.5 \pm 0.16	2 \pm 0.21	significant
K_{abs} (hr^{-1})	2.58 \pm 0.90	0.82 \pm 0.16	significant
$t_{1/2(\text{abs})}$ (hr)	0.27 \pm 0.06	0.84 \pm 0.27	significant
$AUC_{(0-24 \text{ hr})}$ ($\mu\text{g}\cdot\text{hr/ml}$)	10.73 \pm 2.61	8.69 \pm 1.48	significant
$AUC_{(0-\infty)}$ ($\mu\text{g}\cdot\text{hr/ml}$)	14.25 \pm 4.61	9.99 \pm 2.28	significant
$AUMC_{(0-24 \text{ hr})}$ ($\mu\text{g}\cdot\text{hr}^2/\text{ml}$)	96.94 \pm 13.19	69.46 \pm 11.17	significant
$AUMC_{(0-\infty)}$ ($\mu\text{g}\cdot\text{hr}^2/\text{ml}$)	181.44 \pm 24.19	100.72 \pm 18.16	significant
MRT (hr)	12.73 \pm 0.57	10.08 \pm 0.59	significant
Cl_T (ml/min)	0.07 \pm 0.03	0.10 \pm 0.05	significant

^a Number of experiments; n=5, results are expressed as mean \pm SD.

^b Statistically-significant when (* $p \leq 0.05$), statistically non-significant when (* $p > 0.05$).

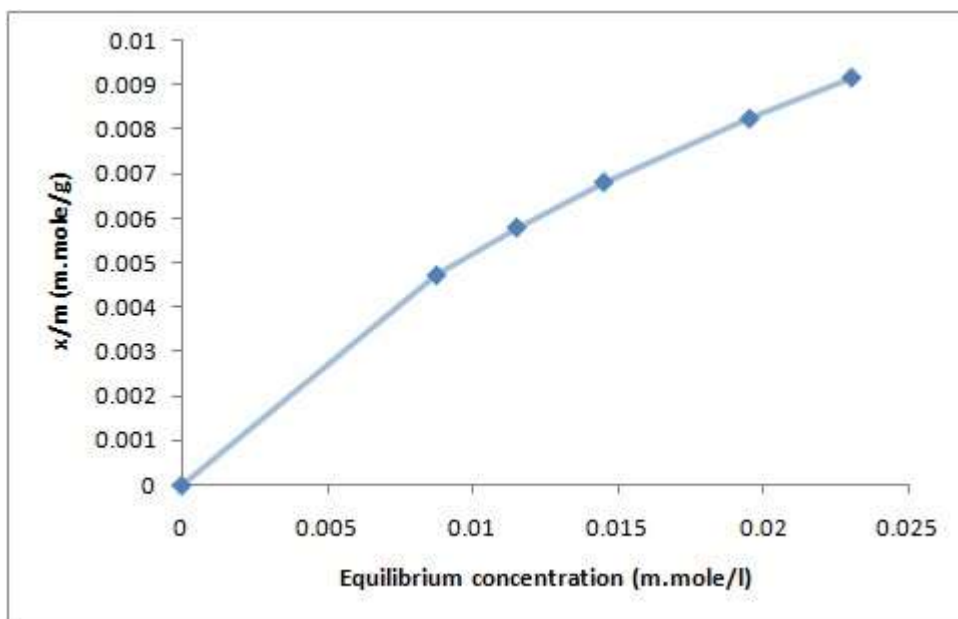


Fig. 1: Typical Langmuir isotherm of GLC onto Laponite RD in a buffer solution of pH 1.2 at 37°C

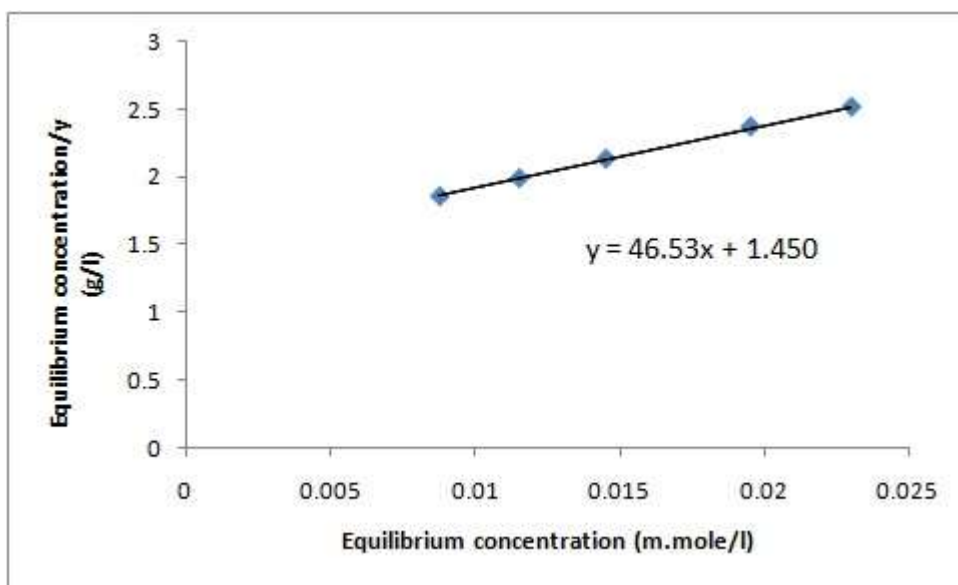


Fig.2: Linear Langmuir plot for adsorption of GLC onto Laponite RD in a buffer solution of pH 1.2 at 37°C

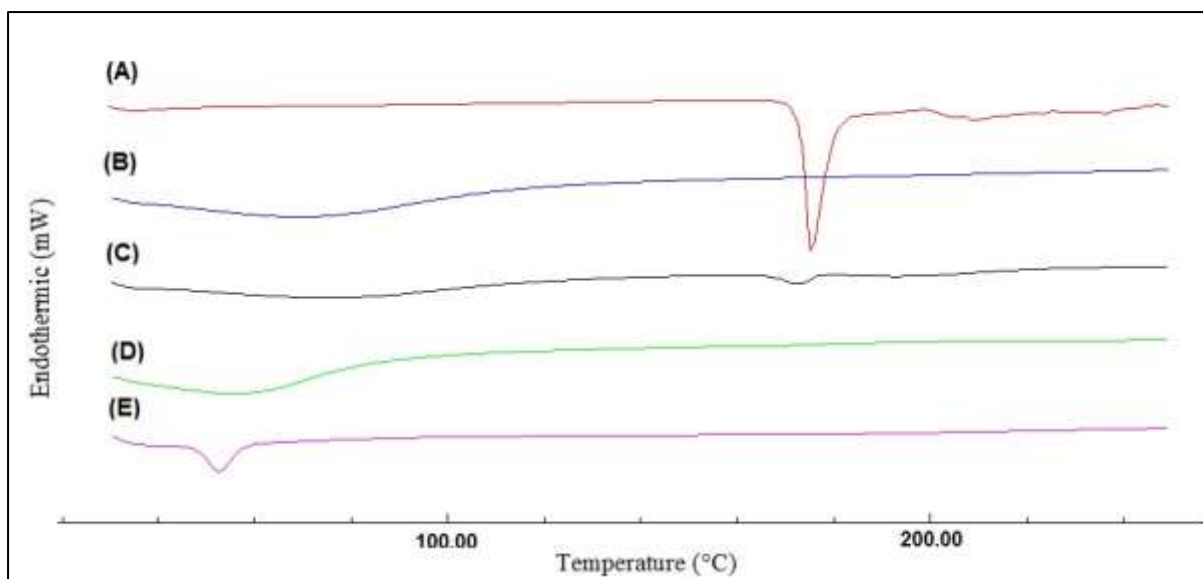


Fig.3: DSC thermograms of (A) GLC alone; (B) Laponite RD alone; (C) Physical mixture of GLC with Laponite RD (1:5 wt. ratio); (D) Adsorbates, GLC with Laponite RD (1:5 wt. ratio); (E) Coadsorbates, GLC to Laponite RD to Pluronic F-68 (1:5:5 wt.ratio).

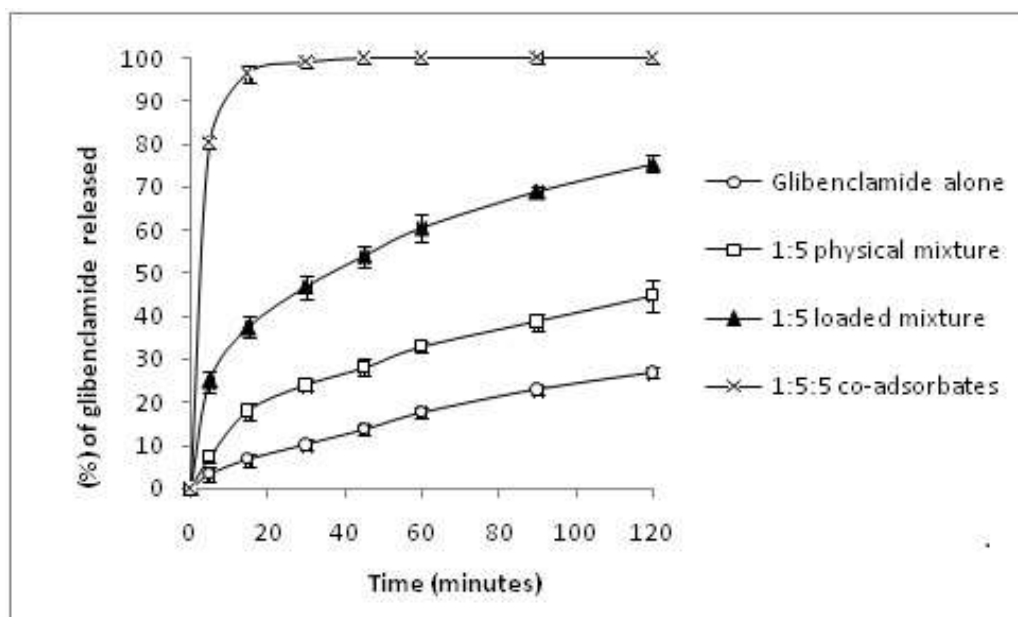


Fig. 4: Comparison of GLC release from its physical, loaded mixtures with Laponite RD and co-adsorbates with Pluronic F-68 and Laponite RD in a buffer solution of pH 1.2

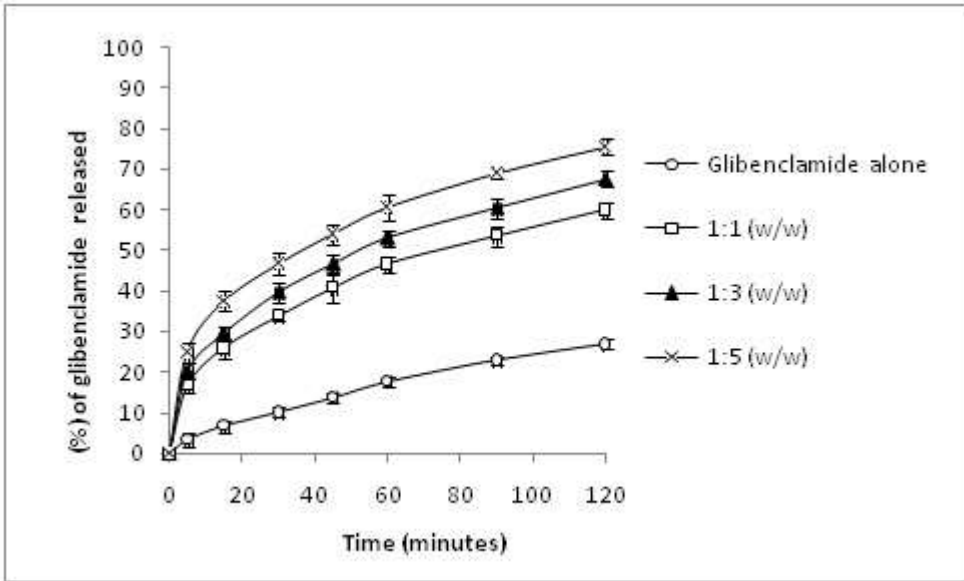


Fig. 5: Effect of different weight ratios of Laponite RD on the release of GLC from its loaded mixtures in a buffer solution of pH 1.2

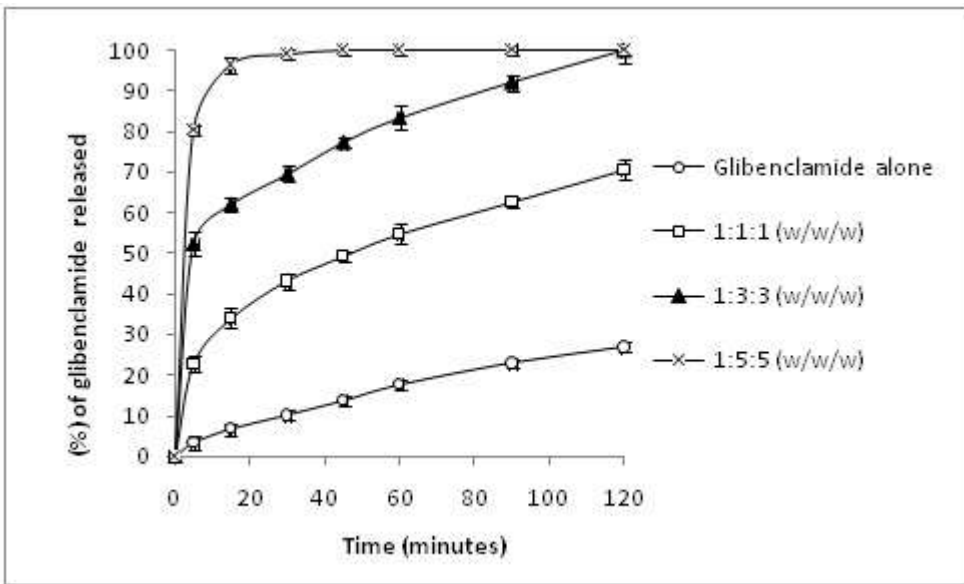


Fig. 6: Release profiles of GLC from the prepared co-adsorbates in a buffer solution of pH 1.2.

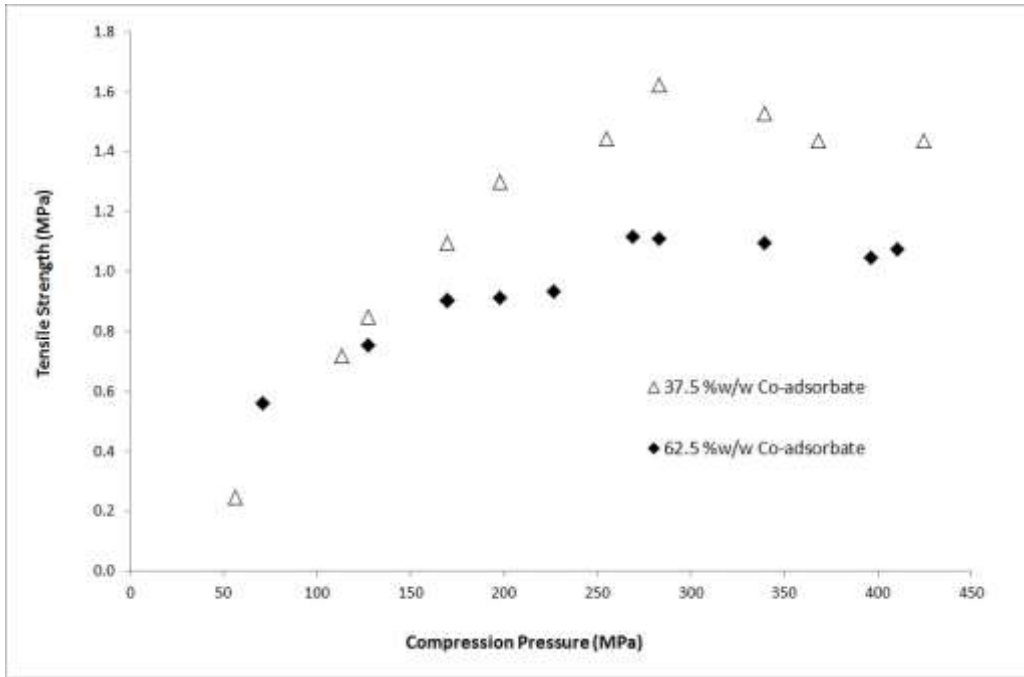


Fig. 7: The effect of compression pressure on the tensile strength of mini-tablets comprising different ratios of GLC co-adsorbate and Starlac®

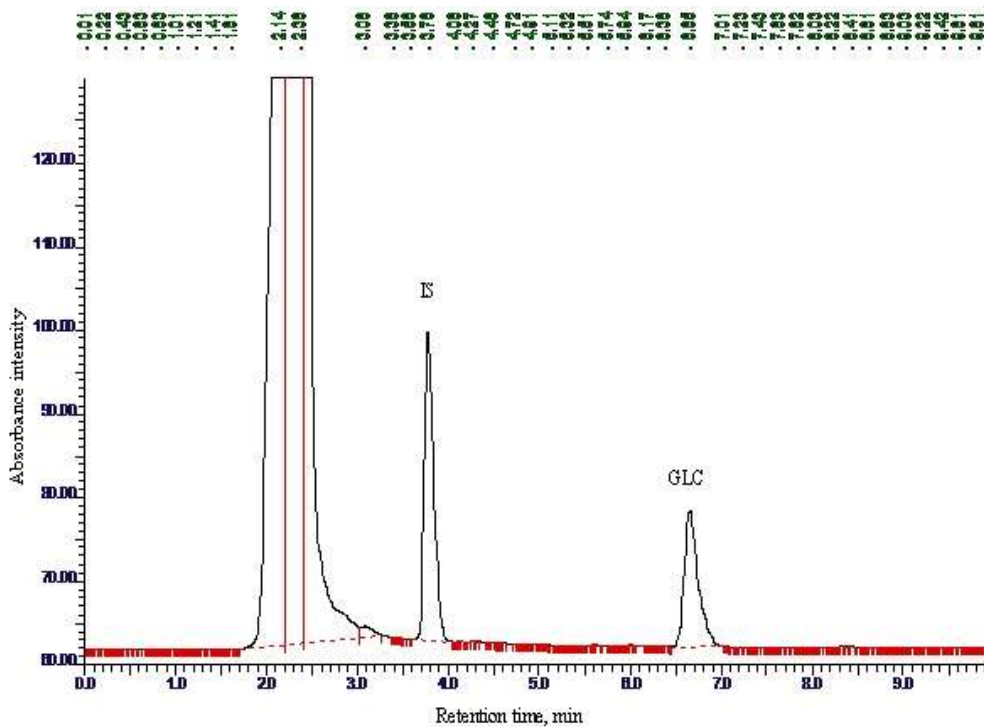


Fig. 8: Representative chromatograms of real rabbit plasma after 0.50 hr of oral administration of a mini-tablet containing optimized co-adsorbate at GLC dose of 57.8 µg (equivalent to 0.75 mg human dose), IS= internal standard (glipizide) at concentration of 10 µg/ml

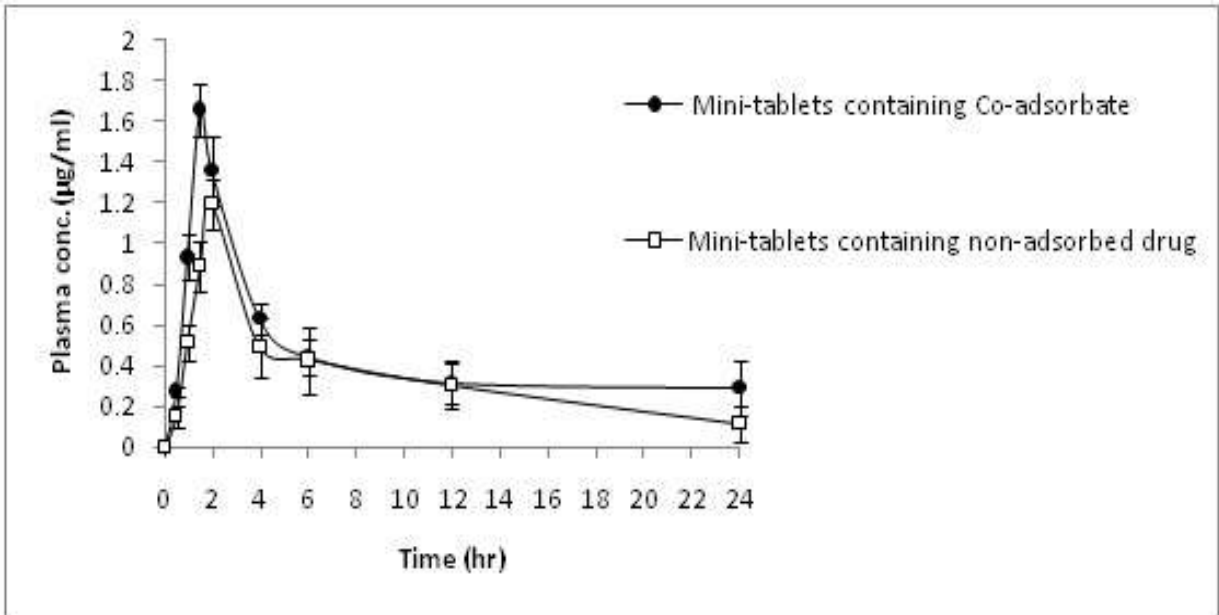


Fig. 9: Plasma concentrations of GLC after oral administration of mini-tablets containing optimized co-adsorbate and those containing untreated GLC at dose level of 57.8 µg (mean ± SD, n = 5)