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FORMULATION AND EVALUATION OF BUCCOADHESIVE SUSTAINED-RELEASE DISCS OF GLIPIZIDE

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Glipizide is an oral hypoglycemic agent used in the treatment of type II diabetes mellitus. It is characterized by its poor aqueous solubility and delayed absorption with concomitant food intake. The objective of the present study was to enhance the absorption rate of glipizide and avoid its side effects on stomach by formulating it into buccoadhesive sustained release disc formulations. The discs were prepared by direct compression method. Hydroxypropyl methylcellulose (HPMC 15000), was used as the main hydrophilic matrix forming polymer either alone or in combinations in two ratios (3:2 & 4:1) with various mucoadhesive polymers namely; Sodium alginate (NaAlg), Sodium carboxymethyl cellulose (SCMC), Hydroxyethyl cellulose (HEC), Hydroxypropyl cellulose (HPC) and Chitosan. The discs were evaluated for weight variation test, thickness, diameter, drug content, hardness, friability, swelling index, surface pH, in-vitro bioadhesion, in-vitro release studies and in-vivo bioavailability studies. In-vitro release studies demonstrated that formulation F8 which contains HPMC / SCMC (40%: 10%) has sustained the drug release up to 8 hrs which was considered an optimum pattern of drug release. The kinetic studies revealed that all formulations follows zero order release kinetics except F3, F4 and F11 which fitted well in first order release model. Bioavailability parameters including C_{max}, T_{max}, and $AUC_{0-24\ h}$ of F8 and the commercial oral tablets of glipizide (Minidiab® 5 mg) were compared. The selected formulation F8 produced higher C_{max} and extended T_{max} ($P<0.05$).

INTRODUCTION

Oral transmucosal drug delivery has been the focus of attention of many formulation scientists for several years.

Buccal mucosa appears to be better suited to the use of retentive systems, such as a mucoadhesive tablet or patch system, in that it has an expanse of smooth and relatively immobile surface for placement of such systems. These attributes make the buccal mucosa more suitable for sustained-delivery applications, delivery of less well permeating molecules, and perhaps peptide drugs[1].

Harsh environmental factors that exist in oral delivery of a drug are circumvented by buccal delivery. Avoiding acid hydrolysis in the gastrointestinal (GI) tract and bypassing the first-pass effect are some of the advantages of this route[2&3].

Glipizide is a second generation sulphonylurea that is commonly used in the pharmacological treatment of type 2 diabetes mellitus[4]. It acts by increasing the release of endogenous insulin as well as its peripheral effectiveness; but it has been associated with gastric disturbances like nausea, vomiting, heartburn, anorexia and increased appetite after oral therapy in the normal doses[5].

Accordingly, there is a strong clinical need and market potential for a dosage form that will deliver glipizide in a controlled manner to a patient needing this therapy which in turns could circumvent the aforementioned problems associated with oral administration of glipizide, thereby resulting in a better patient compliance. For the aforementioned reasons, the study was developed to formulate sustained release buccoadhesive discs of glipizide as a

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promising alternative to the conventional oral tablets.

MATERIALS AND METHODS

Materials

Glipizide (GPZ) was kindly donated from Pharco Corporation, Alexandria, Egypt. Glibenclamide, Glimipride and Hydroxyethyl cellulose (HEC) was kindly donated from T3A Industrial, Assiut, Egypt. Hydroxypropyl methylcellulose 15000 (HPMC 15000) and Mannitol was supplied from El-Gomhouria Co., Cairo, Egypt. Sodium alginate (NaAlg) (general chemical & pharmaceutical Co. Ltd, Sudbury Middlesex, England), Sodium carboxymethyl cellulose (SCMC) (El-Nile Co., for pharmaceutical and chemical industry, Egypt), Polyethylene glycol 6000 (PEG 6000) (Merck, Germany), Colloidal silicon dioxide (Aerosil 200) (Evonik GmbH, Germany), Magnesium stearate (MgSt) (El-Nasr Pharmaceutical Chemicals Co., Egypt), Sodium hydroxide (El-Gomhouria Co., Cairo, Egypt), Potassium dihydrogen phosphate (El-Nasr Pharmaceutical Chemicals, Cairo, Egypt), Agar (Chemi-search for chemi-trade & laboratory supplies, Egypt) and Porcine stomach mucin was purchased from Sigma Aldrich Chem., Germany. Diethyl ether HPLC grade (Aldrich, USA), Streptozotocin (Sigma Aldrich, USA).

Preparation of sustained release muco-adhesive buccal discs of GPZ

Glipizide 5 mg discs were prepared by the direct compression technique. All ingredients of the discs were passed through 100 µm sieve, weighed using electric sensitive balance (Sartorius A200S, Germany) and mixed by trituration using a pestle and mortar to obtain uniform mixing. Powder blends weighing 200 mg each were compressed by a single punch tablet machine (Korsch-Berlin, Ek/0, Frankfurt, Germany) using flat faced 13 mm tablet tooling. HPMC was considered the main polymer and was used as a single polymer or in combination in certain ratios (3:2 & 4:1) with other polymers. Mannitol was added as a diluent to obtain the desired weight of each disc (200 mg). PEG 6000 was used as a solubility enhancer. Aerosil 200 was used as a glidant and as an anti-adherent. Magnesium stearate (1% w/w) was added as a lubricant. The compositions of all formulations are presented in table 1.

Table 1: Composition of the formulated buccoadhesive discs of GPZ.

Formula	Ingredients (mg/Disc)											total
	GPZ	HPMC 15000	SCMC	NaAlg	HEC	HPC	Chitosan	Mannitol	PEG 6000	Aerosil 200	MgSt	
F1	5	60	-	-	-	-	-	86	45	2	2	200
F2	5	80	-	-	-	-	-	66	45	2	2	200
F3	5	60	40	-	-	-	-	46	45	2	2	200
F4	5	60	-	40	-	-	-	46	45	2	2	200
F5	5	60	-	-	40	-	-	46	45	2	2	200
F6	5	60	-	-	-	40	-	46	45	2	2	200
F7	5	60	-	-	-	-	40	46	45	2	2	200
F8	5	80	20	-	-	-	-	46	45	2	2	200
F9	5	80	-	20	-	-	-	46	45	2	2	200
F10	5	80	-	-	20	-	-	46	45	2	2	200
F11	5	80	-	-	-	20	-	46	45	2	2	200
F12	5	80	-	-	-	-	20	46	45	2	2	200

Physical evaluation of glipizide bucco-adhesive disc formulations

Uniformity of weight (B.P. 2009)[6]

For determination of tablet weight variation, twenty discs were individually weighed. The average weight was determined and the standard deviation was calculated.

Disc thickness and diameter

For each formulation, 5 discs were selected and their thickness and diameter was determined using DR. Schleuniger Tablet tester (Model 6D, Pharmatron, Incorporated, USA).

Disc hardness

For each formulation, 5 discs were selected and examined using DR. Schleuniger Tablet tester (Model 6D, Pharmatron, Incorporated, USA).

Disc friability

The percentage weight loss was calculated using the following equation:

$$\%F = \frac{(W_{initial}) - (W_{final})}{(W_{initial})} \times 100$$

Where $W_{initial}$ is the intial weight of discs and W_{final} is the weight of discs after 100 revolutions inside the tester.

Uniformity of drug content

For each formulation, one accurately weighed disc (n=5) was powdered and transferred in 100 ml volumetric flask containing 30 ml of ethanol, the sample was sonicated in an ultrasonic bath (Retsch GmbH, Model UR 1, Germany) for 15 mins and the volume was made up to 100 ml by phosphate buffer pH 6.8, then mixed and filtered through 0.45 µm nylon filter. The filtered solution, after appropriate dilution with Phosphate buffer pH 6.8 was analyzed by UV spectroscopy at 276 nm using UV-visible Spectrophotometer (JENWAY-Model 6305, England). The concentrations were calculated from the calibration curve.

Swelling study of mucoadhesive buccal discs using agar-gel plate method[7]

The swelling behavior of all disc formulations were evaluated using the agar-gel plate method, by placing individually weighed buccal discs (n=3) of each formulation on 1% (w/v) agar plates and incubated at 37ºC. The discs were removed at time intervals 0.5, 1, 2, 3, 4, 5, 6 and 8 hrs; excess water on the surface was carefully removed using filter paper, and the swollen discs were reweighed.

The swelling index (SI) was calculated according to the following formula:

$$SI = \frac{W_2 - W_1}{W_1} \times 100$$

where W_1 and W_2 are the initial weight of buccal discs and the weight of the swollen discs at different time intervals respectively.

Surface pH study of the discs

The surface pH of the buccal discs was determined in order to investigate the possibility of any side effects *in-vivo*. As an acidic or alkaline pH may irritate the buccal mucosa, it was proposed to keep the surface pH as close to neutral as possible. The method adopted by Bottenberg *et al.*[8] was adopted to determine the surface pH of the disc. A combined glass electrode was used for this purpose. The discs were allowed to swell by keeping it in contact with 1 ml of distilled water (pH 6.5 ± 0.05) for 2 hrs at room temperature. The pH was identified by bringing the electrode of a pH meter (JENWAY-Model 3310, England) into contact with the discs surface and allowing the surfaces to equilibrate for 1 min.

***In-virto* bioadhesion**

In-vitro bioadhesion of the prepared disc formulations was examined by measuring the force required to detach the formulation from a mucin disc as a model mucosal substrate using a locally assembled device (Fig. 1) which is a modification of the reported method[9]. The mucin discs 200 mg each were compressed using a hydraulic press (Perkin Elmer, USA), equipped with a 13-mm die by applying a force of 30 kN for 30 seconds. The mucin disc was glued with cyanoacrylate adhesive on the ground surface of one of the two holders made of Plexiglas and its surface was hydrated with 30µl phosphate buffer pH 6.8. The buccal disc was glued to the other holder and put in contact with each other. A preload of 20 g for 3 mins was applied on the upper holder after getting them together into contact to ensure the formation of adhesive bond. The whole assembly was allowed to hang on an iron stand

with the help of an aluminum wire fastened with a hook provided on the back of the upper holder. A pre-weighed light weight polypropylene bag was attached to the hook on the back of the lower holder with aluminum wire. Water was added to the polyethylene bag through an intravenous infusion set at a rate of 2.0 drops per second until the buccal disc detached due to the heavy weight of water infused. The weight of the empty bag plus the weight of water collected in the bag expressed as weight (gram force) required for the detachment.

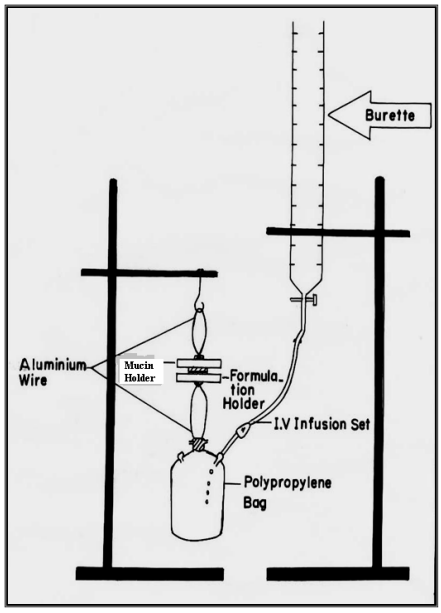

Fig. 1: Modified apparatus for discs bioadhesion test.

Each experiment was carried out in triplicate and the mean values were calculated. The detachment force was determined using the following equation:

$$\text{Detachment Force (dyne/cm}^2\text{)} = [\text{m. g/A}]$$

Where, m is the weight of empty bag and of water infused at detachment, g is the acceleration due to gravity considered as 980 cm/s^2, and A the area of disc exposed (cm^2).

In-vitro drug release from the prepared disc formulations

The drug release from the prepared disc formulations was determined using a modified USP dissolution apparatus (Erweka DT-D6, Germany); a rotating paddle-rotating basket[10]. Phosphate buffer solution of pH 6.8 (500 ml) was used as the dissolution medium which was maintained at 37±0.5ºC and stirred at a rate of 50 rpm. Aliquots (5 ml each) were withdrawn after 15, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 mins, filtered through 0.45 µm nylon syringe filters and analyzed spectrophotometrically at 276 nm for drug content. An equal amount of fresh dissolution medium, kept at the same temperature, was replaced immediately after withdrawal of the test sample. The release studies were conducted in triplicates and the mean was considered.

Kinetic analysis of the drug release data

The data of drug release from disc formulations were analyzed to determine the order of kinetic release according to the following models (zero order, first order and Higuchi diffusion).

Then, the release data were analyzed using the equation proposed by Peppas[11]:

$$M_t / M_\infty = Kt^n$$

Where M_t / M_∞ is the fractional release of the drug at time t, K is the release rate constant and n is the diffusional exponent that characterizes the type of release mechanism during the dissolution process. For non-Fickian release, the value of n for buccoadhesive tablets or discs (cylindrical sample) falls between 0.45 and 0.89; while in case of Fickian diffusion, n= 0.45; for zero order release (case II transport), n= 0.89 and for supercase II transport, n > 0.89.

For the determination of the exponent of n the portion of the release curve where $M_t / M_\infty < 0.6$ should only be used[12]. The values of n and k were estimated by linear regression of log (M_t / M_∞) versus log t.

In-vivo evaluation of adhesion behavior of the selected plain disc formulation

The bioadhesion of the drug free discs was tested in six healthy male volunteers (aged 22-32 years).

The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and followed the ICH-GCP guidelines, and was in compliance with local regulatory requirements. All subjects were completely informed concerning the pertinent details and the purpose of the study.

A written consent form was supplied, understood and signed by each subject prior to dispensing test materials[13].

The participants were instructed to press the discs against the cheek for about 30 seconds without moistening before application. Then the disc and the upper lip were moistened with saliva to prevent the sticking of the disc to the lip[14] Volunteers were allowed to drink during the study, while food intake and smoking was prohibited.

The duration of mucosal adhesion was the time required for complete wash-off of the disc.

At the end of the test period, the volunteers were asked to record:

A- The adhesion time, time of detachment or complete erosion of discs.

B- Side effects e.g. irritation (severe, moderate, slight or non-irritant), hinderance, bad taste, dry mouth, and increase in salivary flux or mucosal lesions.

Accelerated stability testing of glipizide in the selected formulae of buccoadhesive discs

Samples from the selected disc formulation F8 were stored in amber colored glass bottles in closed desiccators containing saturated solution of sodium chloride to attain 75% relative humidity (RH). The desiccators were kept at temperatures of 30, 40, and 50±2.0°C in thermostatically controlled hot air ovens (Binder, Germany) for six months. Samples from each of the selected stored formulae were withdrawn after time intervals of 1, 2, 3, and 6 months. The drug content was determined using HPLC assay method.

Chromatographic conditions for glipizide assay[15]

The drug and the internal standard were separated on C18 column, Nucleosil, C-18 column (250 x 4.60 mm, 7 µm) using High Performance Liquid Chromatographic system (HPLC, HP 1100 equipped with G1322A Degasser, G1311A Quaternary pump, G1313A ALS (autosampler), G1316A column oven, G1314A 1100 variable wavelength Detector and hp ChemStation for LC 3D Rev. A. 06.03 [509] computer software.

The mobile phase consisted of filtered and degassed mixture of methanol and water (80:20 v/v), pH of which was maintained at 3.5 using phosphoric acid (85% w/w).

The drug was eluted isocratically at a mobile phase flow rate of 1.0 ml/min and monitored with a UV detector operating at 230 nm. Glimipride was used as internal standard.

The eluent peaks were integrated using area under curve (AUC) ratio. The column and the mobile phase were used at ambient conditions.

Determination of glipizide in the stored discs

Assay of GPZ in the stored discs was determined using HPLC method.

At the specified time intervals, three randomly selected discs were finely powdered. An accurately weighed amount of powder equivalent to 5 mg of GPZ was transferred into 100 ml volumetric flask and dissolved in mobile phase. The solution was subjected to vigorous shaking, and then allowed to stand for 1 h with intermittent sonication for complete extraction of the drug. 10 ml of the internal standard stock solution (0.5 mg/ml) was pipetted into the volumetric flask containing the powdered formula and the volume was brought to mark with the mobile phase. The obtained solutions were filtered through 0.45 µm disk filter, degassed and 20 µl were injected onto the HPLC column. GPZ concentration in each sample was determined utilizing the constructed calibration curve.

Bioavailability study of glipizide from the selected prepared buccoadhesive discs

The study was carried out to compare the pharmacokinetics of the marketed oral tablets Minidiab® with the selected buccoadhesive discs formula (F8).

According to Paget and Barners table which related the animal dose to the daily human dose[16], dose of rabbit (1.5 kg) = maximum daily human dose (15-40 mg) × 0.07 = (1.05-2.8 mg). So, for rabbits weighing 2 kg; the drug doses were (1.4-3.73 mg). For both oral and buccal administrations, the dose level of 2.5 mg was used to ensure obtaining detectable plasma drug concentrations.

Treatment of experimental animals

The study was conducted using placebo-controlled study. Nine healthy rabbits,

weighing 1.8-2.0 kg, were divided into three groups; each group consists of three animals.

Diabetes was induced by injecting streptozotocin (80 mg/kg; intraperitoneal), dissolved in citrate buffer (3 mM; pH 4.5), to overnight fasted rabbits[17]. Seven days later, rabbits became hyperglycemic with blood glucose levels between 136–189 mg/dL. Blood glucose levels were determined using Bionime GS100 Glucometer (Bionime GmbH, Switzerland).

The first group was fasted and received oral GPZ (2.5 mg), half a tablet of (Minidiab® 5 mg). The second group received the selected prepared medicated buccal disc formulation (F8) which contains 2.5 mg of the drug. The remaining group was kept as control which had received equal volume of citrate buffer without streptozotocin[17]. The selected formula F8 was applied by attaching this mucoadhesive disc on the cheek pouch of rabbits. The rabbits were fasted for 24 hrs with free access to water before drug administration and anaesthetized with intraperitoneal injection of thiopental to allow adhesion of the tablets to the buccal mucosa.

Blood sampling

Blood samples of about 1 ml were withdrawn via an indwelling catheter from the marginal ear vein into a 5 ml screw-capped centrifuge tubes at the following time points: predose, 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hrs following drug administration. The samples were centrifuged at 5000 rpm for 15 mins in a bench top centrifuge Z200A (Hermla Labortechnik Gm bH, Germany).

The supernatant was removed and transferred into a new screw-capped centrifuge tube. This separated plasma was stored at -20°C until analysis[17&18].

Extraction and analysis of the drug from blood samples[19]

Five hundred microliters of plasma or calibration standards, 50 µL of internal standard solution (10 mcg/ml glibenclamide), and 850 ml of 0.05M HCl were added to a glass tube. After mixing using and MS2 Minishaker (IKA® Works, INC., Wilmington, NC, USA), 5 ml of diethyl ether was added and the mixture was stirred for 30 seconds. Each sample was centrifuged at 2500 rpm for 10 mins. The organic layer was transferred to a new tube and evaporated to dryness under a nitrogen stream at 50°C. The residue was reconstituted with 500 µL of 50% methanol and an 80 µL aliquot was injected to HPLC for analysis.

Chromatographic conditions[17]

The drug and the internal standard were separated on C18 column (250×4.6 mm, 5 µm), Ace, Advanced Chromatography Technologies Limited, Aberdeen, Scotland. The Mobile phase consisted of a mixture of acetonitrile: 2 mM phosphate buffer (50:50% v/v), adjusted to pH 3.5 with orthophosphoric acid. The drug was eluted isocratically at a mobile phase flow rate of 1.0 ml/min and monitored with a UV detector operating at 225 nm. Glibenclamide was used as internal standard. The run time for the assay was 15 mins, and the retention time for the drug was 5.6±0.1 mins and glibenclamide retention time was 12.8±0.1 mins.

RESULTS AND DISCUSSION

Table 2 illustrates the physical properties of the prepared GPZ buccoadhesive discs. Direct compression technique produced discs of uniform weight according to B.P (2009).

The diameter and thickness of the prepared discs were also uniform with low standard deviation values (Table 2).

GPZ buccoadhesive discs prepared by direct compression technique showed acceptable hardness values ranged from 2.10 kg ± 0.436 to 3.83 kg ± 0.289 (Table 2). Discs containing sodium alginate showed the lowest hardness values. The percent weight loss (friability) of the prepared discs was also in the acceptable range (< 1%) of B.P. 2009 (0.311% - 0.682%) which indicates that all formulations are mechanically stable and have acceptable physical characteristics.

The percent of the total drug content of the prepared discs was found to be within the range from 98.67% ± 2.99 to 103.70% ± 1.48. These values indicate that all the prepared discs are uniform in drug content according to B.P (2009) requirements.

Table 2: Physical properties of the formulated GPZ buccoadhesive discs.

Formula no.	Weight (mg) n= 20	Drug content (%) n= 5	Thickness (mm) n= 5	Diameter (mm) n= 5	Hardness (kg) n= 5	Friability (% loss) n= 10
F1	200.47±1.01	98.80±2.64	1.32±0.062	12.91±0.006	2.13±0.321	0.412
F2	200.15±1.79	99.52±1.71	1.30±0.017	12.92±0.012	2.17±0.252	0.367
F3	201.42±1.69	99.19±1.29	1.32±0.035	12.92±0.012	2.73±0.907	0.393
F4	200.12±2.45	101.70±1.54	1.29±0.006	12.90±0.012	3.57±1.823	0.682
F5	201.38±2.36	100.76±3.24	1.40±0.006	12.86±0.006	2.13±0.451	0.458
F6	202.52±1.31	102.33±1.99	1.34±0.053	12.87±0.017	3.67±1.012	0.311
F7	202.05±1.70	101.41±2.11	1.38±0.074	12.88±0.01	2.17±0.503	0.594
F8	201.25±1.10	100.70±1.77	1.34±0.006	12.93±0.021	2.57±0.611	0.467
F9	200.38±1.42	103.70±1.48	1.34±0.015	12.91±0.006	2.10±0.436	0.549
F10	202.78±1.61	101.93±1.76	1.39±0.058	12.90±0.006	2.27±0.473	0.521
F11	200.37±0.92	99.45±2.36	1.34±0.021	12.88±0.01	3.83±0.289	0.353
F12	200.95±1.91	98.67±2.99	1.34±0.01	12.89±0.012	2.20±0.361	0.558

Swelling studies of buccoadhesive discs

The rate of swelling of HPMC increases with an increase in the concentration and the viscosity grade of the polymer. The swelling of matrix depends very much on the rate of water entry into the matrix. When the water uptake into matrices is enhanced with a greater amount of HPMC, the swelling of the polymer is increased.

Figure 2 shows the swelling indices of GPZ buccoadhesive discs containing HPMC 30% and 40% as a single polymer while, figures 3&4 represent the swelling indices of GPZ buccoadhesive discs containing different ratios of the mixture of each two polymers. The highest swelling index was seen in the formulation batches F3 which contained HPMC/SCMC (30%:20%) among all buccoadhesive disc formulations containing HPMC: polymer combinations. This greater tendency of water uptake might be attributed to the presence of carboxylic acid groups on the main chain of SCMC which appears to confer this polymer a higher affinity to water compared with HPMC[20]. Formulation F4 which contain HPMC/NaAlg (30%:20%) have demonstrated similar swelling behavior as F3 which could be explained by the increased porosity of discs by increasing the content of hydrophilic alginates[21]. HPMC / HEC formulations displayed a quite lower swelling index values as shown in figures 3&4. It was reported that HEC matrices formed a viscous gel layer immediately after coming in contact with the release medium and this gel layer was durable and resistant to erosion[22]. In general decreasing NaAlg, SCMC or HEC concentration resulted in decreasing the swelling of their respective formulations.

For HPMC / HPC and HPMC / Chitosan formulations, increasing HPC or Chitosan content led to decreased water uptake. The hydrosolubility of HPMC, despite its only moderate swelling properties, promote liquid entry and entrapment in the HPC network. High HPC contents without the initiating action of HPMC produce a smaller swelling effect[23]. On the other hand, Lehr *et al.,*1992 have reported that chitosan underwent minimal swelling in artificial intestinal fluid due to its poor aqueous solubility at neutral pH values[24].

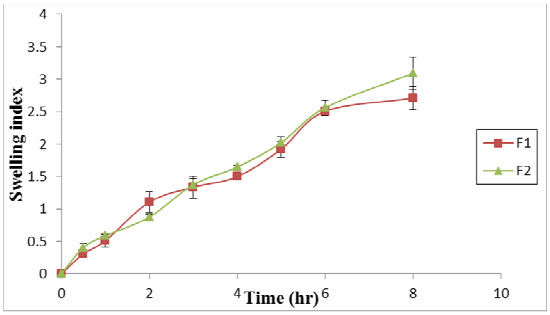

Fig. 2: Swelling indices of GPZ buccoadhesive discs containing single polymer (F1-F2) using agar-gel plate method in pH 6.8.

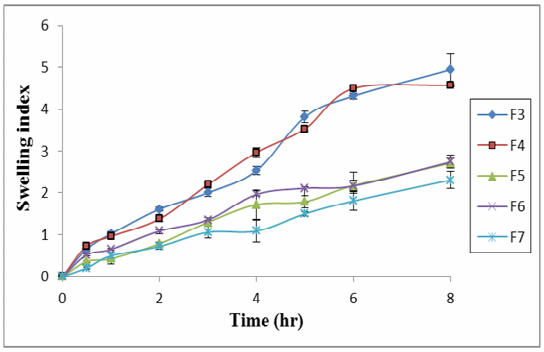

Fig. 3: Swelling indices of GPZ buccoadhesive discs (F3-F7) containing various bioadhesive polymer combinations (HPMC 30%: polymer 20%) using agar-gel plate method in pH 6.8.

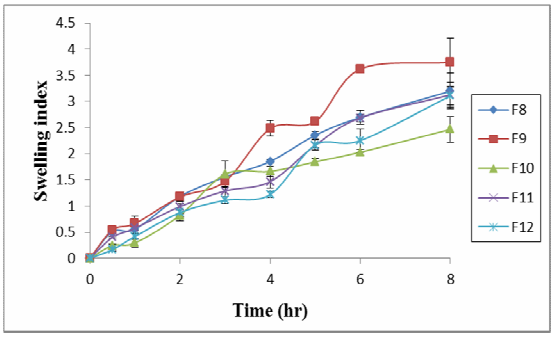

Fig. 4: Swelling indices of GPZ buccoadhesive discs (F8-F12) containing various bioadhesive polymer combinations (HPMC 40%: polymer 10%) using agar-gel plate method in pH 6.8.

Surface pH of the discs

The surface pH of the discs was determined in order to investigate the possibility of any side effects on the buccal mucosa. As an acidic or alkaline pH may cause irritation to the buccal mucosa, it was attempted to keep the surface pH as close to neutral as possible.

The prepared buccoadhesive formulations exhibited surface pH values within satisfactory limits around the neutral pH (6.43±0.021 - 6.88±0.028). These formulations may not cause irritation to the buccal mucosa since their pH values lies within that of salivary pH (5.5-7.0)[25].

In-vitro bioadhesion test

In the present study, all formulations showed good bioadhesion force ranging from 41.53 x 10^3 dyne/cm^2 for formulation F5 to 82.30 x 10^3 dyne/cm^2 for formulation F10. There are two factors which might be contributed to the bioadhesion behavior of all formulations a) the presence of mannitol which is reported to have good bioadhesive properties that could be related to its spatial conformation, and linear configuration, which facilitated interactions between the adhesive sites (-OH groups) and the mucus layer[26] and b) the wide surface area offered for binding to the buccal mucus membranes by the large and flat discs.

It was observed that buccal disc formulation F10 which contain HPMC: HEC 40%:10% has the highest bioadhesive force followed by F2 and F1 which contain HPMC 40% and HPMC 30% as a single polymer, respectively.

Figure 5 depicts an increasing trend in the bioadhesive force with the increase in the concentration of SCMC, NaAlg and HPC from 10% to 20% with subsequent decrease in HPMC concentration from 40% to 30% of the disc weight. Rapid rate of hydration of SCMC led to higher degree of swelling in a short period of time, which improved entanglement of polymer chains with the mucus. This hypothesis was confirmed with that previously reported by Lehr *et al.*[24]. With discs containing NaAlg, the concentration of the polymer had little effect on bioadhesive properties whereas with the HPC containing formulations, the bioadhesion was increased significantly with the increasing HPC concentration in the discs.

The opposite trend was observed with HEC and Chitosan combinations with HPMC as shown in figure 5. The weak mucoadhesive properties of Chitosan may be explained by the poor wetting properties of the polymer. The results obtained with Chitosan support previous findings about its weak and short-lasting mucoadhesion[24].

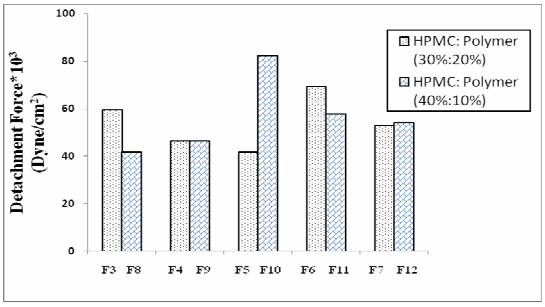

Fig. 5: *In-vitro* bioadhesion force of GPZ buccoadhesive discs containing certain ratios of HPMC/Polymer combinations.

In-vitro drug release studies

Increasing HPMC concentration from 30% to 40% w/w resulted in decreasing the percent of drug released from the prepared discs. This may be attributed to the ascending amount of the polymer which led to lengthen the diffusion path length for GPZ which could reduce the drug release[27]. Also, with a higher polymer concentration per unit area, the resultant gel layer would be more viscous and consequently more resistant to erosion[28].

Figure 6 depicts the release profiles of GPZ from formulations (F3-F7) prepared using HPMC and various bioadhesive polymers at a ratio of (30% : 20%). The release rate of GPZ from these formulations decreased in the following order:

HPMC / Chitosan ≈ HPMC / NaAlg > HPMC / HPC ≈ HPMC / SCMC > HPMC / HEC

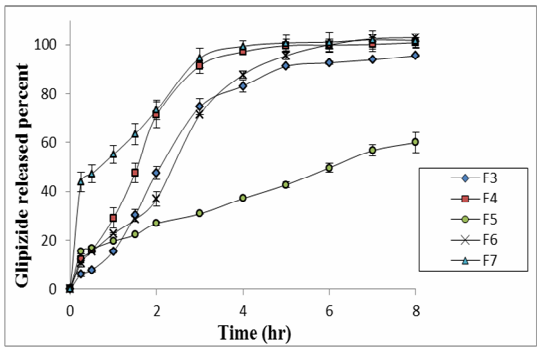

Fig. 6: Release profiles of GPZ from the prepared buccoadhesive discs (F3-F7) containing HPMC 30%: Polymer 20% combinations in pH 6.8.

Figure 7 depicts the release profiles of GPZ from formulations (F8-F12) prepared using HPMC and various bioadhesive polymers at a ratio of (40%: 10%). The release rate of GPZ from these formulations decreased in the following order:

HPMC / Chitosan > HPMC / NaAlg > HPMC / SCMC > HPMC / HPC > HPMC / HEC

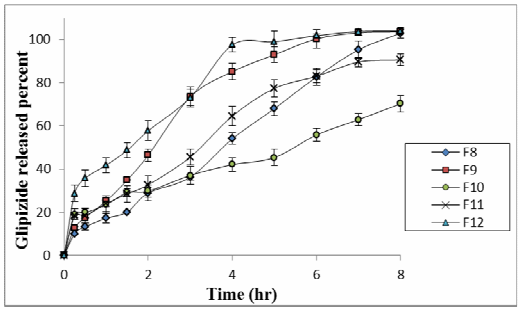

Fig. 7: Release profiles of GPZ from the prepared buccoadhesive discs (F8-F12) containing HPMC 40%: Polymer 10% combinations in pH 6.8.

Among all HPMC based formulations, only formulation F8 which contain HPMC / SCMC (40% : 10%) showed sustained and complete drug release, 102.95 ± 2.53, within the whole dissolution test period (8 hrs). On the other hand, formulations F5 and F10 which contain HPMC / HEC combinations showed marked reduction in the total release of the drug, as the percentage drug released from these formulations were 59.99 ± 4.06 and 70.26 ± 3.80 respectively. Also, formulation F11 which contain HPMC: HPC (40%: 10%) showed incomplete drug release within 8 hrs. All other HPMC based formulations showed complete drug release in 6 hrs and even less.

Generally, increasing the bioadhesive polymer concentration namely; SCMC, NaAlg, HPC and Chitosan from 10 to 20% relative to HPMC concentration resulted in increasing the rate of drug release. In contrast, increasing the concentration of HEC relative to HPMC concentration retarded the drug release from these formulations.

The rapid release of GPZ from HPMC / NaAlg matrices could be attributed to rapid erosion of the resultant gel layer upon increasing sodium alginate concentration. Also, SCMC swells and erodes rapidly, which explain high release rate from formulation batches containing high concentration of that polymer. Due to the erodible properties of SCMC, the discs could not maintain their matrix integrity and the erosion of polymeric matrix in the higher rate than swelling

properties could accelerate the drug release[29]. These results are in agreement with earlier workers [30-32].

The fast dissolution of F7 formulation matrix was due to high percentage of Chitosan, where HPMC was not effective in maintaining matrix cohesiveness. Ionic interaction was absent between HPMC and Chitosan due to the neutral nature of the former polymer. It was reported that the rapid rate of drug dissolution from the Chitosan tablet was due to the poor gel formation ability and easy disintegration characteristics of Chitosan at neutral pH[33].

Also, it has been reported that HEC matrices formed a viscous gel layer immediately after coming in contact with the release medium and this gel layer was durable and resistant to erosion[22] which explains the slower rate of GPZ release from matrices containing HPMC / HEC combinations.

Kinetic analysis of the release data of glipizide from the prepared buccoadhesive discs

Table 3 shows the kinetics of release data of GPZ from discs containing different HPMC / polymer blends with certain ratios in dissolution medium of pH 6.8. The release of GPZ from HPMC-based buccoadhesive disc formulations followed either zero-order release mechanism especially those containing high content of HPMC or first order release mechanism.

It is generally recognized that drug release from HPMC matrices follows two mechanisms, drug diffusion through the swelling gel layer and polymer relaxation and erosion[34]. An increase in the quantity of the HPMC resulted in greater swelling and greater water uptake, and more polymer relaxation[35]. Therefore, the drug release became less diffusion governed, and its approach toward zero-order erosional-type release.

It was found that F1 and F2 which contain ascending concentration of HPMC have n values < 0.45 which indicate a fickian drug release pattern i.e., diffusion governed drug release.

Also, It was observed that the obtained values of n (release exponent) of most formulations containing HPMC / SCMC, HPMC / NaAlg and HPMC / HPC combinations lies between 0.45 and 0.89 indicating that the drug release is non-fickian i.e., the mechanism of the drug release is due to polymer relaxation as well as diffusion.. On the other hand, all formulations containing HPMC / HEC or HPMC / Chitosan mixtures have n values < 0.45 indicating fickian drug release.

Table 3: Release kinetics of GPZ from the formulated buccoadhesive discs containing mixtures of HPMC and bioadhesive polymers in pH 6.8.

Formula number	Correlation coefficient (r)			Mechanism of drug release	K-value
	Zero-order	First-order	Higuchi-model		
F1	0.9921	0.9441	0.9613	Zero-order	0.34505
F2	0.9822	0.9306	0.9819	Zero-order	0.25767
F3	0.9248	0.9873	0.9680	First-order	-0.007235
F4	0.9203	0.9874	0.9653	First-order	-0.01894
F5	0.9987	0.9938	0.9815	Zero-order	0.09859
F6	0.9782	0.8858	0.9757	Zero-order	0.28918
F7	0.9882	0.9467	0.9819	Zero-order	0.26658
F8	0.9958	0.9192	0.9653	Zero-order	0.21343
F9	0.9880	0.9844	0.9828	Zero-order	0.30319
F10	0.9958	0.9804	0.9745	Zero-order	0.10710
F11	0.9844	0.9867	0.9793	First-order	-0.00508
F12	0.9891	0.9393	0.9791	Zero-order	0.26127

*The underlined value is the highest correlation coefficient, which indicates the operating release mechanism. * k_0 = ($mg.h^{-1}$).

***In-vivo* bioadhesion of the selected formula (tolerance and residence time)**

Good adhesion and tolerance were used as criteria for selecting the formulation to be used for clinical assessment. The prepared plain buccoadhesive discs formulation (F8) was evaluated for its tolerance and contact time on five male human volunteers.

The results revealed that the selected buccoadhesive discs had an acceptable taste and no signs of local irritation were observed. This is in agreement with many earlier workers[30&36&37]. With respect to contact time, the discs retained readily on buccal mucosa, the mean residence time of F8 was more than 6.5 hrs.

Accelerated stability testing of glipizide in the selected formulae of discs

The chromatograms of GPZ standard and test preparations show a sharp peak, clearly identifiable and well-separated from the internal standard (IS) peak (Fig. 8). The retention time of GPZ and glimipride (as an internal standard) peaks were 4.2 and 5.8 mins, respectively. The chromatograms of different prepared GPZ discs show the same two peaks with the same retention time. This indicates the absence of any detectable degradation products of GPZ after storage for six months at elevated temperatures and humidity indicating the chemical stability of GPZ under these storage conditions.

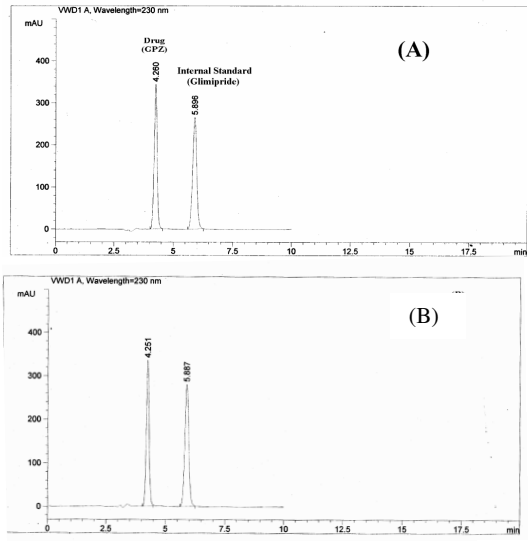

Fig. 8: HPLC chromatograms of GPZ bucco-adhesive discs stored for six months at temperature of 50°C and 75% relative humidity:
A: Standard GPZ B: Formula F8

The chemical stability results of the selected formulation F8 demonstrated that the percentage drug remaining after storage for a period of 6 months was found to be 97.62, 97.27 and 96.83% at the three elevated temperatures 30, 40 and 50°C, respectively. Regression analysis of stability data indicated that the decomposition of the drug followed first-order kinetics.

Bioavailability studies

Figure 9 shows the mean plasma concentration-time profiles of GPZ after buccal application of formula (F8) which was selected due to its optimum drug release profile, good bioadhesion properties and acceptable physical characteristics. For comparison, the plasma concentration-time profile after oral administration of GPZ is also shown in the figure.

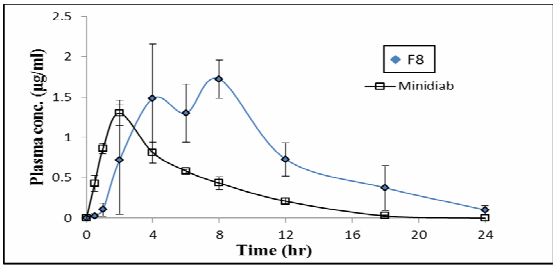

Fig. 9: Plasma concentrations of GPZ at dose level of (2.5 mg) after administration of the commercial oral tablets (Minidiab®) and application of the prepared GPZ buccoadhesive discs (F8). (Error bar represents: mean ± SD for each value).

In-vivo study demonstrates that the prepared buccal disc formula F8 achieved higher C_{max} and AUC values and prolonged T_{max} compared to that of the commercially available tablets (Table 4). The $t_{1/2\ el.}$ was found to be 4.12 ± 0.887 hrs for buccal disc formula F8. However, $t_{1/2\ el.}$ for the commercial oral tablets Minidiab® was found to be 3.025± 0.348 hrs.

GPZ buccoadhesive dosage forms have C_{max} values of 1.723 ± 0.338 mcg/ml for buccal

disc formula F8. While, C_{max} value was 1.302 ± 0.156 mcg/ml for Minidiab® oral tablets. There was a marked increase in the magnitude of peak plasma concentration (C_{max}) after buccal administration of the prepared disc formulation F8 although it was statistically non-significant.

The AUC_{0-24hr} was 18.131 ± 1.059 µg.hr/ml for the selected formulation F8 which is significantly higher than the AUC_{0-24hr} for commercial oral tablets (Minidiab®) which was 8.136 ± 0.048 µg.hr/ml.

These observations clearly indicate that the bioavailability of GPZ from buccoadhesive disc is markedly increased by more than two folds of the oral bioavailability of the drug (Table 4) and so give a useful economical value by reducing the dose of the drug during the manufacturing process which in turns will be favored by patients due to avoidance of GPZ possible side effects on stomach. Also, the ease of removal of these buccoadhesive dosage forms and rapid discontinuation of therapy (i.e. prevention of further drug influx into the circulation through detachment of these dosage forms from their site of application) would be of great benefit as in case of hypoglycemia.

However, these results have revealed that these buccoadhesive formulations suffered from relatively slow rate of absorption. Thus, the incorporation of a second immediate release layer in further studies would be highly recommended.

Table 4: Pharmacokinetic parameters of GPZ following buccal application of the prepared buccoadhesive disc formula (F15) and the commercial oral tablets (Minidiab®) to rabbits.

Pharmacokinetic Parameters	Commercial tablet Minidiab®	Disc formula F8	Significance of the difference*
C_{max} (µg/ml)	1.302 ± 0.156	1.723 ± 0.338	N.S.
T_{max} (hr)	2	8	S.
$K_{el}(hr^{-1})$	0.229 ± 0.027	0.168 ± 0.063	N.S.
$t½_{el.}$ (hr)	3.025 ± 0.348	4.12 ± 0.887	N.S.
AUC_{0-24} hr (µg.hr/ml)	8.136 ± 0.048	18.131 ± 1.059	S.
$AUC_{(0-\infty)}$ (µg.hr/ml)	8.136 ± 0.048	18.746 ± 0.951	S.
$AUMC_{0-24hr}$ ($\mu g.hr^2/ml$)	44.322 ± 3.99	162.861 ± 27.593	S.
$AUMC_{(0-\infty)}$ ($\mu g.hr^2/ml$)	44.322 ± 3.99	181.264 ± 34.34	S.
MRT(hr)	5.447 ± 0.450	9.669 ± 2.269	N.S.
Cl_T (ml/min)	0.307 ± 0.0017	0.133 ± 0.007	S.
F_R (%)	------	222.85	--------

*S. = statistically significant ($p < 0.05$), N.S. = statistically non-significant ($p > 0.05$).

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نشرة العلوم الصيدلية
جامعة أسيوط

تقييم وصياغة أقراص فمية لاصقة تحتوي على عقار الغليبيزيد

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يعتبر عقار الغليبيزيد أحد العقاقير المستخدمة في خفض نسبة السكر في الدم التي تستخدم في علاج داء السكري من النوع II. ومن خصائص هذا العقار قابليته الضعيفة للذوبان في الماء وتأخر إمتصاصه عند تعاطيه مع الأكل. لذا فقد قد كان الهدف من تلك الدراسة هو تحسين معدل إمتصاص عقار الغليبيزيد وتفادي الأعراض الجانبية التي يسببها للمعدة عن طريق صياغته على هيئة أقراص لاصقة فمية ممتدة المفعول. وقد تم تحضير الأقراص عن طريق تقنية الكبس المباشر. وقد استخدم إيدروكسي بروبيل ميثيل السيليلوز ١٥٠٠٠ كمكون أساسي إما منفردا أو في حالة اتحاد مع العديد من البوليمرات تحديدا ؛ ألجينات الصوديوم ، صوديوم كاربوكسي ميثيل السيليلوز ، أيدروكسي بروبيل سيليلوز ، أيدروكسي إيثيل سيليلوز وكيتوزان بالنسب التالية (٣:٢ ، ٤:١). وأخضعت الأقراص المحضرة لاختبارات تجانس الوزن والقطر والسمك والصلابة والهشاشة وتجانس المحتوي الدوائي كما قيمت من حيث معامل الإنتفاخ ، الأس الأيدروجيني السطحي ، قوة الإلتصاق الحيوي ، معدل إنطلاق العقار من هذه الأقراص ودراسات الإتاحة الحيوية للعقار. وقد أوضحت الدراسات أن أقراص المجموعة ف٨ أظهرت أفضل معدل لإنطلاق العقار خلال ثمان ساعات وقد اتضح أن ديناميكية انطلاق العقار من جميع الأقراص تتبع معادلة الرتبة صفر فيما عدا أقراص المجموعات ف٣، ف٤، ف١١ تتبع معادلة الرتبة الأولى. وقد أوضحت مقارنة دلالات الإتاحة الحيوية بين أقراص المجموعة ف٨ ذات الإلتصاق الحيوي بالتجويف الفمي ومثيلاتها من الأقراص التقليدية سريعة الإنطلاق المتوافرة بالسوق المحلي أن أقراص المجموعة ف٨ أظهرت مستويات أعلى من حيث أقصي تركيز للعقار في البلازما والوقت اللازم للوصول إليه.