Development, Optimization and Evaluation of Glibenclamide Gastroretentive Tablet Formulation

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Dedication

To my family;
To parents;
To Faculty of Pharmacy, University of Khartoum;
And To Wafra Pharm.
Content

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Preface

This work was carried out in the laboratories of Department of Pharmaceutics (faculty of Pharmacy, University of Khartoum) and, in part, in the laboratories of Wafra Pharma Pharmaceuticals Plant and Omdurman Army Hospital
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ملخص الأطروحة

مرض السكري من الأمراض الخطيرة التي تنتشر بسرعة في المجتمع نسبة لتغير أنواع الطعام والاستعمال الأفراد لأنواع من الطعام تحتوي على سعرات حرارية عالية وطبيعة الأعمال التي يؤديونها، وهذه الخطورة تتأرجح بين الغيوبية في حالتها إرتفاع تركيز السكر في الدم وانخفاضه بشدة في حالة استعمال عقار القلايكنكلاميد أحيانا.

من هنا وتلك الأسباب بنىت هذه الدراسة على تقنية حديثة تعتمد على زيادة زمن بقاء هذه الأقراس في داخل المعدة، عن طريق عمل أقراس تتميز بخصائص الطفو في السائل المعدوي وبزيادة زمن البقاء في المعدة تتم زيادة زمن الامتصاص وفترة قد تمتد طويلا ومن ثم زيادة نسبة التكافؤ الحيوي (أقراس مميتة المفعول).

في هذه الدراسة توصلنا إلى أن المواد التالية: الهيدروكس بروبيل ميثيل سيليلوز و ستيريل الكحول و البولي فينيل بيروليدون و كالسيوم هيدروكسيد ذات اثر فعال على خاصية الطفو وبالتالي ذوبانية المادة الفعالة، وقد أثبتت التجارب أن درجة صلابة الحبة لها تأثير مباشر علي تلك الخاصية.

في هذه الدراسة كان التحكم بواسطة كل من التصميمين (المتكامل والبوكس ويلسون) علي التوالي وتم التوصل إلى التركيبة المتوازنة عن طريق التصميم المركزي لكل الخواص المطلوبة (الطفو والذوبانية).

زيادة نسبة التكافؤ الحيوي كانت موقع المقارنة بين التركيبة المستدامة والقديمة المتوفرة حاليا في الأسواق وقد أثبتت الدراسة ان استعمال الأقراس التي تتميز بخصائص الطفو في السائل المعدوي قد ادى إلى زيادة نسبة التكافؤ الحيوي لعقار القلايكنكلاميد بنسبة 200-25% مقارنة بالأقراس العادية.
Abstract

Hydroxypropyl methylcellulose (HPMC-4000cps, fixed amount), various contents of calcium hydroxide, stearyl alcohol, magnesium stearate, different drug: PVP ratios and altered tablet hardness were used to design floating tablet formulation capable to deliver glibenclamide in a sustained manner. Both full factorial and Box-Wilson designs, in consecutive manner, were used to investigate for the influences of these formulation variables on the developed dosage form performance and drug release.

Responses selected for optimization of the dosage form using central composite design were tablet swelling, onset of buoyancy, buoyancy duration, time for 50% drug release ($T_{50\%}$), percent of drug released after 1 hour (Rel$_{%-1}$) and percent of drug released after 6 hours (Rel$_{%-6}$).

Although both drug: PVP ratio and hardness were shown to influence the swelling ability of the developed tablets, only tablet hardness has revealed an influential effect on tablet onset of floating. Whilst reduction in drug: PVP ratio resulted in terminal acceleration of drug release, the effect of tablet hardness on drug release was shown to be more evident in the initial phase of drug release (p<0.05). Loading level of calcium hydroxide, stearyl alcohol and tablet swellability all have showed profound enhancing effects on duration of tablet buoyancy and drug release.

Glibenclamide release from tablets of the invention was shown to follow the anomalous type ($n = 0.8212-1.0244$). Application of statistical and mathematical modeling has enabled the optimization of the developed tablet formulation to meet selective constraints for floating, hardness and drug release.

The optimized buoyant tablet formulation with highest CI revealed hardness of 50 N, immediate onset of floating and floating duration >6hours. Concerning drug release, the formula showed evidence of 25 and 84% drug release after 1 and 6 hours, respectively, with $T_{50\%}$ of 3 hours. Moreover, release kinetics of the drug from the optimized formula was shown to be near the desired zero order type of release ($n =0.8897 \pm 0.0132$; $r^2 = 0.9993$). The in vivo dosage form residence time study in six human subjects demonstrated that the developed tablet for-
mulation retained in the stomach for more than four hours under fasting conditions.
Comparative bioavailability study revealed that floating tablets showed 2-2.5 times increase in AUC (p≤0.1). The attained average drug C_{max} of the two tablet formulations was found to be comparable (p≥0.1). However, elimination half-life (t_{1/2}) and mean residence time (MRT) of the drug were greater with the floating tablet formulation (p≤0.1) indicating the sustained release tendency of the drug from the floating tablet formulation.
The three months-based stability study indicated that the drug and the dosage form retained their initial physical characters in both accelerated and normal conditions for the test duration as far as blister pack is considered.
1. Literature Review

1.1. Oral Solid Dosage Forms:
Drug substances most frequently are administered orally by means of solid dosage forms such as tablets and capsules. Large-scale production methods used for their preparations necessitate the presence of other materials in addition to the active ingredients. Additives also may be included in the formulations to facilitate handling, enhance the physical appearance, improve stability, and aid in the delivery of the drug to the bloodstream after administration. These supposedly inert ingredients, as well as the production methods employed, have been shown in some cases to influence the absorption or bioavailability of the drug substances. Therefore, care must be taken in the selection and evaluation of additives and preparation methods to ensure that the drug delivery goals and therapeutic efficacy of the active ingredient will not be diminished.

1.1.1. Tablets:
Tablets comprise 63-70% of the solid dosage forms that present in the drug market. Among the reasons behind this popularity are the enhanced product stability, the ease of administration, the simplicity of manufacturing, the controlled drug toxicity, the economical acceptance and the non-sophisticated dose adjustment according to the need. Tablets may be defined as solid pharmaceutical dosage forms containing drug substances with or without additives and prepared by either compression or molding methods. Although tablets frequently are discoid in shape, they also may be round oval, oblong, cylindrical, or triangular. They may differ greatly in size and weight depending on the amount of drug substance present and the intended method of the administration. They are divided into two general cases by whether they are made by compression or molding. Compressed tablets usually are prepared by large-scale production methods, while molded tablets generally involve small-scale operations. The various tablet types and abbreviations used in referring to them are listed below.
1.1.1.1. Compressed Tablets (CT):
These tablets are formed by compression and contain no special coating. They are formed from powder, crystalline, or granular material, alone or in combination with binders, disintegrants, controlled release polymers, lubricants, diluents, and in many cases colorants (Rudnic and Schwartz, 2000).

1.1.1.2. Sugar Coated Tablets (SCT):
These are compressed tablets containing a sugar coating. Such coating may be colored and are beneficial in covering up drug substances containing objectionable tastes or odors and in protecting materials sensitive to oxidation (Rudnic and Schwartz, 2000).

1.1.1.3. Film Coated Tablets (FCT):
These are compressed tablets that are covered with a thin layer or film with water-soluble material. A number of polymeric substances with film forming properties may be used. Film coating imparts the same general characteristics as sugar coating, with added advantage of a greatly reduced time period required for coating operation (Rudnic, and Schwartz, 2000).

1.1.1.4. Enteric Coated Tablets (ECT):
These are compressed tablets coated with substances that resist solution in gastric fluid but disintegrate in the intestine. Enteric coating can be used for tablets containing drug substances that are inactivated or destroyed in the stomach, for those that irritate the mucosa, or as a means for delayed release of the medication.

1.1.1.5. Multiple Compressed Tablets (MCT):
These are compressed tablets made by more than one compression cycle.

1.1.1.6. Layered Tablets:
Such tablets are prepared by compressing additional tablet granulation on a previously compressed granulation. The process may be repeated to produce multiple-layer tablets of two or three layers.
1.1.1.7. Press Coated Tablets:
Such tablets, also referred to as dry coated, are prepared by feeding previously compressed tablets into a special tableting machine and compressing another granulation layer around the preformed tablets. They have all the advantage of a compressed tablet, i.e. slotting, monogramming, speed of disintegration, etc., while retaining the attributes of sugar coated tablets in masking the taste of the drug substance in the core tablets (Rudnic and Kottke, 1996).

1.1.1.8. Controlled Release Tablets:
Compressed tablets can be formulated to release the drug slowly over a prolonged period of time. Hence these dosage forms have been referred to as prolonged release or sustained release dosage forms as well. These tablets can be categorized into three types a) Those that respond to some physiological conditions to release the drug, such as enteric coating; b) Those that release the drug in a relatively steady controlled manner; and c) Those that combine combination of mechanisms to release pulses of drug, such as repeat-action tablets (Jantzen and Robinson, 1996).

1.1.1.9. Tablets for Solution:
Compressed tablets to be used for preparing solution or imparting given characteristics to solutions must be labeled to indicate that they are not to be swallowed.

1.1.1.10. Effervescent Tablets:
In addition to the drug substance, these tablets contain sodium bicarbonate and an organic acid such as tartaric or citric acid, where in the presence of water, these additives react, to generate carbon dioxide which acts as a disintegrating agent (Rudnic and Schwartz, 2000).

1.1.1.11. Compressed Suppositories or Inserts:
Occasionally, vaginal suppositories, such as metronidazole tablets are prepared by compression. Tablets for this use usually contain lactose as the diluent. The label must indicate the manner to which it is to be used.
1.1.1.12. Buccal and Sublingual Tablets:
These are small flat oval tablets. Tablets intended for buccal administration by inserting into the buccal pouch may dissolve or erode slowly; therefore they are formulated and compressed with sufficient pressure to give a hard tablet.

1.1.1.13. Molded Tablets or Tablet Triturates:
Tablet triturates usually are made from moist materials, using a triturate mold that gives them the shape of cut sections of a cylinder. Such tablets must be completely and rapidly soluble. The problem arising from the compression of these tablets is failure to find a lubricant that is completely water-soluble.

1.1.1.14. Dispensing Tablets (DT):
These tablets provide a convenient quantity of potent drug that can be incorporated readily into powders and liquids, thus circumventing the necessity to weigh small quantities. These tablets are supplied primarily as a convenience for extemporaneous compounding and should never be dispensed as a dosage form (Rudnic and Schwartz, 2000).

1.1.1.15 Hypodermic Tablets:
Hypodermic tablets are soft, readily soluble tablets and originally were used for the preparation of solutions to be injected. Since stable parenteral solutions are now available for most drug substances, there is no justification for the use of hypodermic tablets for injection. Their use in this manner should be discouraged since the resultant solution is not sterile. Nowadays these kinds of tablets do not exist.

1.1.2. Tablet Ingredients:
In addition to the active or therapeutic ingredient, tablets contain a number of inert materials which are collectively known as additives or excipients. They may be classified according to the part they play in the finished tablets. The first group contains those that help to impart satisfactory processing and compression characteristics to the formulation. These include diluents, binders, glidants, and lubricants. The second group of added substances helps to give additional desirable physical characteristics to the finished tablet. Included in this group are
disintegrants, colors, and in the case of chewable tablets, flavors and sweetening agents, and in the case of controlled release tablets, polymers or waxes or other solubility retarding materials (Rubinstein, 1988).

1.1.3. Powder Compaction:
The process of compaction has several identifiable phases. When powders undergo compression (a reduction in volume), the first process to occur is a consolidation of the powders. During this consolidation phase, the powder particles adopt a more efficient packing order. The second phase of the compaction process is elastic or reversible deformation. If the force were to be removed during this phase, the powder would recover completely to the efficiently packed state. For most pharmaceutical powders, this phase is very short in duration and very difficult to identify on most instrumented tablet presses. The third phase of compaction is plastic, or irreversible, deformation of the powder bed. It is this phase of the compaction process that is the most critical in tablet formation. If too much force is applied to the powder, brittle fracture occurs. If the force was applied too quickly, fracture and de-bonding during stress relaxation can occur. It has been reported that if a material has significant plastic flow under compression, it will be more likely to form a compact. (Rudnic and Schwartz, 2000).

1.1.3.1. Tablet Strength- Compression Pressure Profile:
Most formulators use tablet hardness, or tensile strength, as a measure of the cohesiveness of a tablet. With even the simplest of instrumented tablet presses, it is possible to plot tensile strength versus the force applied to the tablet. These plots can be useful in identifying forces that can cause fracture and can lead to a quick, tangible assessment of the compactibility of the formulation. However, there are many limitations to this method, as these plots cannot predict lamination or capping. In fact, bulk powders change their state of packing during compaction and individual particles fracture and/or plastically deform. During this process the surface area of the powders and the compact in whole, changes. What is worth mentioning is that increasing the duration of compression period (dwell time), plastic flow is maximized, and tablet strength in-
creases (Rudnic and Schwartz, 2000). On the other hand, the cohesiveness of a tablet can change upon storage, in either positive or negative direction.

1.1.4. **Methods of Tablet Manufacture:**
Based on the nature of the powder blend, physicochemical characters of the drug and the desired manufacturing processing, tablets could be prepared by any of the following four different methods. These are:

1.1.4.1. **Wet Granulation:**
The most widely used and most general method of tablet preparation is wet granulation method. The steps in wet methods are weighing, mixing, granulation, screening the damp mass, drying, dry screening, lubrication, and compression (Rudnic and Schwartz, 2000).

1.1.4.2. **Double Compression:**
These are compressed tablets made by two compression cycles to prepare slugs, which are to be re-sized further, lubricated and compressed into tablets (Rudnic and Schwartz, 2000).

1.1.4.3. **Direct Compression:**
As the name implies, direct compression consists of compressing tablets directly from powdered material without modifying the physical nature of the material itself (Rudnic and Schwartz, 2000).

1.1.4.4. **Moist Granulation:**
This is a newly developed technique where it resembles wet granulation in that powder blend is wetted but using only aqueous solution. However, drying of the sized granules is carried out using sodium carboxy methyl cellulose as water withdrawing agent. The technique is suitable for heat sensitive drugs (Rudnic and Kottle, 1996).

1.2. **Diabetes Mellitus:**
Diabetes mellitus is the condition arising due to abnormal metabolism of carbohydrates, fats and proteins. It is characterized mainly by an unusually high sugar level in the blood and the presence of sugar in urine (glucosuria). The normal blood glucose level in human plasma is 70-90
mg per 100 ml. Hyperglycemia is characterized by more than normal concentration of the blood sugar, and hypoglycemia develops when the blood sugar level falls below the normal range.

In the absence of insulin a much higher concentration of blood glucose is required before it can cross the cell surface. Glucose is produced first from glycogen reserves in the liver and the resulting hyperglycaemia can be regarded as a response directed towards increasing the extracellular–intracellular glucose gradient and hence the passage of glucose into the cell. The compensatory value of this mechanism is limited, for as soon as the renal threshold is reached glucose overflows into the urine, preventing any further rise in blood sugar and leading to a depletion of the liver stores of glycogen. Insulin inhibits the formation of cyclic adenosine monophosphate (cAMP) and hence the breakdown of glycogen (Nolte and Karam, 2001).

Two types of diabetes mellitus do exist; these are Type I diabetes mellitus which is insulin-dependent and is due to deficiency of insulin following autoimmune destruction of pancreatic beta–cells. Patients with type 1 require administration of insulin. Type II diabetes mellitus is a non-insulin dependent and is due to reduced secretion of insulin or to peripheral resistance to the action of insulin. Although patients may be controlled on diet only, they may require administration of oral antidiabetic drugs or insulin to maintain satisfactory controlled treatment of diabetes mellitus aimed at alleviating symptoms and minimizing the risk of long-term complications.

The Objective of the therapy is to maintain glucose levels as normal as possible throughout the day without producing hypoglycemia or severely restricting the patient life (Lebovitz and Melander, 1992).

1.2.1. Antidiabetic Agents:
For the treatment of type II diabetes mellitus (NIDDM), oral hypoglycemic agents are generally used. These agents are categorized into salicylates, diguanides, biguanides, alpha–glycosidase inhibitors, thiazolidinediones and sulphonylureas. Among these agents, sulphonylureas are the most frequently utilized.
1.2.1.1. Sulphonylureas:
Sulfonylurea antidiabetic agents are used to treat type II diabetes mellitus in which insufficient amount of insulin is being produced by the pancreas (Lebovitz and Melander, 1992). These agents work by two approaches one of them is to stimulate the pancreas to release more insulin into the blood stream. All of the body cells need insulin to help turn the food into energy. This is done by either utilizing sugar (or glucose) in the blood as a quick energy source or the sugar may be stored in the form of fats, sugars, and proteins for use later, such as for energy between meals. The other approach is that sulfonylurea helps insulin get into the cells where it can work properly to lower blood sugar and in this way, sulfonylurea will assist in lowering blood sugar and help restore the way the food is used to make energy (Nolte and Karam, 2001).

The sulfonylureas are considered to be subdivided into two subcategories: the first generation agents, e.g. tolbutamide, chlorpropamide, tolvazamide, acetohexamide, and the second generation agents, e.g. glyburide (glibenclamide), glipizide and gliclazide.

1.2.2. Glibenclamide:
It is one of the most commonly applied second generation sulfonylurea as an oral Antidiabetic agent (Sullivan and Cashman, 1970; Lebovitz and Melander, 1992). Chemically, it is 1-[4-[2-chloro-2-methoxybenzamido] ethylbenzosulphonyl]-3-cyclohexylurea]. Physically, glibenclamide is a white, crystalline powder, insoluble in water, sparingly soluble in methylene chloride, slightly soluble in alcohol and it dissolves in dilute solution of alkali hydroxides. The drug has a melting point range from 169 to 174 °C and in solution, it has two absorbance maxima at 300 nm and a less intense at 275 nm with a specific absorbance of 61—65 and 27—32, respectively (USP 1995).

1.2.2.1. Sulphonylurea's Structure-Activity Relationship:
The relationship between chemical structure and hypoglycemic activity of glibenclamide is as follows: the benzene ring should contain one substituent preferably in the para-position. Some substituents that enhance hypoglycemic activity are methyl, amino, acetyl, chloro, bromo, iodo, methyl thio and trifluoromethyl groups. The high activity of these derivatives is a function of the specific distance between the nitrogen atom
of the substituent and the sulfonamide nitrogen atom. The group attached to the terminal urea nitrogen should be of certain size and should impart lipophilic properties to the molecule. Optimal activity is found in compounds containing three to six carbons in the nitrogen substituent, while the activity is lost if the nitrogen substituent contains twelve or more carbon.

1.2.2.2. Glibenclamide's Mechanism of Action:
The principal action of the drug is on the beta cells of islets of Langerhans where it stimulates insulin secretion and thus reducing plasma glucose concentration. The drug reduces the potassium channels causing depolarization, calcium ion entry and hence insulin secretion. Moreover, glibenclamide reduces the serum glucagon concentration, which contributes to the hypoglycemic effect of the drug. Furthermore, the drug potentiates insulin action on target tissues, on the liver, muscles and adipose tissue by increasing insulin receptor number and by enhancing the post receptor complex enzyme reaction mediated by insulin. The principal result is decreased hepatic glucose output and increased glucose uptake in muscle (Lebovitz and Melander, 1992). Glibenclamide is effective in insulin deficient patients, and for successful therapy probably requires about 30% of normal beta cell function to be present.

1.2.2.3. Glibenclamide's Pharmacokinetics:
Following oral administration, the drug is absorbed rapidly from the gastrointestinal tract and transported in the blood as highly protein bound complex. Owing to its low aqueous solubility, the drug is characterized by dissolution-limited absorption and is metabolized in the liver into products with low hypoglycemic activity. The drug has a half-life of 1.5-3 hours and is excreted in the faeces and, as metabolites, in the urine (El-Sayed et al., 1989; Coppack et al., 1990).

1.2.2.4. Glibenclamide's Therapeutic Dose:
Glibenclamide is an oral hypoglycemic agent for treatment of non-insulin-dependent diabetes which is not controlled by diet alone. For non-micronized glibenclamide tablet the starting dose is 2.5 mg/day or less, and the average maintenance dose is 5-10 mg/day given as single
morning dose. For micronized glibenclamide tablets, the adult dose is 1.5 mg–3 mg/day. The elderly may need a low dose of about 0.75-3 mg and not more than 12 mg/day (USP, 2003), because their poor eating habits and decreased renal function predispose them to hypoglycemic reactions. Low doses are given once daily before breakfast. Increasing the dose above maximum levels may result in an increased incidence of adverse effects without producing any further decrease in blood glucose and is not recommended. (Eric T. Herfindal, Pharm.D., M.P.H.)

1.2.2.4. Glibenclamide's Side Effects and Drug Interactions:
Among the reported side effects of glibenclamide are hypoglycemia, stimulation of appetite and weight gain, gastrointestinal upset, allergic skin rashes, bone marrow damage, cardiovascular problems, hepatoxicity, transient cholestatic jaundice, leukopenia, granulocytosis and fatal aplastic anemia. Hypothyroidism after long term administration was also reported (Davis and Granner, 1996). A variety of drugs are known to augment the hypoglycemic effect of glibenclamide. These are non-steroidal anti-inflammatory drugs (NSAID’s), monoamine oxidase (MAO) inhibitors, sulphonamides, tetracycline, phenylbutazone, beta-adrenergic blocking agents, chloramphenicol, biguanides, and salicylates. On the other hand, drugs that decrease the action of glibenclamide also do exist. Among them are thiazides, loop diuretics, corticosteroids, oral contraceptives, beta-adrenergic stimulants and thyroid hormones. This is beside chronic alcohol intake and abuse of laxatives.

1.2.2.5. Glibenclamide's Contra-indications and Precautions:
The drug is contraindicated to patients with known hypersensitivity to glibenclamide; patients suffer from or with a history of diabetic ketoacidosis, those who have insulin-dependent diabetes mellitus, those with serious impairment of renal, hepatic, or adrenocortical function, in surgical condition, during pregnancy and for children. However, in the case of surgery, trauma, fever, or infection, glibenclamide is discontinued and insulin is prescribed.

1.2.2.6. Glibenclamide's Dosage, Packaging and Storage:
Glibenclamide is available as tablets of 1.25 mg, 2.5 mg, and 5 mg strengths for oral administration and is typically administered twice a day. The tablets are blister-packed and since both humidity and tempera-
ture are known to affect glibenclamide and dosage form stability, tablets are to be stored at relative humidity beyond 30% and temperature not exceeding 25ºC.

1.3. Drug Delivery with Enhanced Gastrointestinal Residency:

Oral delivery of drugs is by far the most preferable route of drug delivery due to the ease of administration, patient compliance and flexibility in formulation, etc. From immediate release to site-specific delivery, oral dosage forms have really progressed. It is evident from the recent scientific and patent literature that an increased interest in novel dosage forms that are retained in the stomach for a prolonged and predictable period of time exists today in academic and industrial research groups. One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the GI tract is to control the gastric residence time (GRT). Dosage forms with a prolonged GRT, e.g. gastroretentive dosage forms (GRDFs), will provide prescribers and patients with new and important therapeutic options (Katayama et al., 1999; Whitehead et al., 2000; Özdemir et al., 2000).

GRDFs extend significantly the period of time over which the drugs may be released. Thus, they not only prolong dosing intervals, but also increase patient compliance beyond the level of existing controlled release dosage forms. This application is especially effective in delivery of sparingly soluble and insoluble drugs. It is known that, as the solubility of a drug decreases, the time available for drug dissolution becomes less adequate and thus the transit time becomes a significant factor affecting drug absorption. To address this, oral administration of sparingly soluble drugs is carried out frequently, often several times per day. As a mechanism to override this problem, erodible, gastroretentive dosage forms have been developed that provide continuous, controlled-administration of these drugs at the absorption site. In addition, these dosage forms are useful for delivering drugs incorporated into vesicles such as liposomes, nanoparticles, proteinoid microspheres and pharmacosomes, etc… (Singh and Kim, 2000).

Compared with other applications, the frequency of dosing may be the same, but the gastroretentive dosage forms will alter beneficially the absorption profile of the active agent, thus enhancing its bioavailability.
For example, a significant increase in the bioavailability of furosemide from a floating dosage form (42.9%) has been reported, compared with commercially available tablets (Lasix® (33.4%)) and enteric products (29.5%) (Klausner et al., 2003b; Özdemir et al., 2000).

GRDFs greatly improve the pharmacotherapy of the stomach through local drug release, leading to high drug concentrations at the gastric mucosa (eradicating Helicobacter pylori from the submucosal tissue of the stomach), making it possible to treat stomach and duodenal ulcers, gastritis and oesophagitis, reduce the risk of gastric carcinoma and administer non-systemic, controlled-release antacid formulations (calcium carbonate) (Singh and Kim, 2000).

GRDFs can be used as carriers for drugs with so-called absorption windows. These substances, for example antiviral, antifungal and antibiotic agents (sulphonamides, quinolones, penicillins, cephalosporins, aminoglycosides and tetracyclines, etc.), are taken up only from very specific sites of the GI mucosa.

In addition, by continually supplying the drug to its most efficient site of absorption, the dosage forms allow for more effective oral use of peptide and protein drugs such as calcitonin, erythropoietin, vasopressin, insulin, low-molecular-weight heparin, protease inhibitors and luteinising hormone-releasing hormone analogues.

Various attempts have been made to retain the dosage form in the stomach as a way of increasing the retention time. These attempts include introducing floating dosage forms (gas-generating systems and swelling or expanding systems), mucoadhesive systems, high-density systems, modified-shape systems, gastric-emptying delaying devices and co-administration of gastric-emptying delaying drugs. Among these, the floating dosage forms have been used most commonly. However, most of these approaches are influenced by a number of factors that affect their efficacy as a gastroretentive system (Singh and Kim, 2000; Li et al., 2002; Nur et al., 2005).

1.3.1. Buoyant Drug Delivery Systems:
Floating drug delivery systems (FDDS) have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the
desired rate from the system. After release of drug, the residual system is emptied from the stomach. This results in an increased GRT and a better control of the fluctuations in plasma drug concentration. However, besides a minimal gastric content needed to allow the proper achievement of the buoyancy retention principle, a minimal level of floating force (F) is also required to keep the dosage form reliably buoyant on the surface of the meal. The FDDS can be divided into gas-generating and non-effervescent systems (Singh and Kim, 2000).

1.3.1.1. Gas Generating Floating Delivery Systems:
These buoyant systems utilize matrices prepared with swellable polymers like methocel, polysaccharides like chitosan, effervescent components like sodium bicarbonate, citric acid and tartaric acid, or chambers containing a liquid that gasifies at body temperature. The optimal stoichiometric ratio of citric acid and sodium bicarbonate for gas generation is reported to be 0.76:1.

The common approach for preparing these systems involves resin beads loaded with bicarbonate and coated with ethylcellulose. The coating, which is insoluble but permeable, allows permeation of water. Thus, carbon dioxide is released, causing the beads to float in the stomach. Other approaches and materials that have been reported are highly swellable hydrocolloids and light mineral oils, a mixture of sodium alginate and sodium bicarbonate, multiple unit floating pills that generate carbon dioxide when ingested, floating minicapsules with a core of sodium bicarbonate, lactose and polyvinyl pyrrolidone coated with hydroxypropyl methylcellulose (HPMC), and floating systems based on ion exchange resin technology (Talwar et al., 2001; Choi et al., 2002; Moursy et al., 2003; Garg and Sharma, 2003).

1.3.1.2. Non-effervescent Floating Delivery Systems:
This type of system, after swallowing, swells un-restrained via imbibition of gastric fluid to an extent that it prevents their exit from the stomach. These systems may be referred to as the ‘plug-type systems’ since they have a tendency to remain lodged near the pyloric sphincter. One of the formulation methods of such dosage forms involves the mixing of drug with a gel, which swells in contact with gastric fluid after oral administration and maintains a relative integrity of shape and a bulk den-
sity of less than one within the outer gelatinous barrier (Nur and Zhang, 2000). The air trapped by the swollen polymer confers buoyancy to these dosage forms.

Other approaches reported in the literature are hydrodynamically-balanced systems developed by Sheth and Tossounian (1984), which contain a mixture of drug and hydrocolloids, sustained-release capsules containing cellulose derivatives like starch and a higher fatty alcohol or fatty acid glyceride, bilayer compressed capsules, multilayered flexible sheet-like medicament devices, hollow microspheres of acrylic resins, polystyrene floatable shells, single and multiple unit devices with floatation chambers and microporous compartments and buoyant controlled-release powder formulations (Streubel et al., 2003; Arora et al., 2005). Oral drug delivery formulations made from the gels would swell rapidly in the stomach, causing medications to move more slowly from the stomach to the intestines and be absorbed more efficiently by the body.

Drugs reported to be used in the formulation of floating dosage forms are floating microspheres (aspirin, griseofulvin, p-nitroaniline, ibuprofen, terfenadine and tranilast), floating granules (diclofenac sodium, indomethacin and prednisolone), films (cinnarizine), floating capsules (chlordiazepoxide hydrogen chloride, diazepam, furosemide, misoprostol, L-Dopa, benserazide, ursodeoxycholic acid and pepstatin) and floating tablets and pills (acetaminophen, acetylsalicylic acid, ampicillin, amoxycillin trihydrate, atenolol, diltiazem, fluorouracil, isosorbide mononitrate, para-aminobenzoic acid, piretamide, theophylline and verapamil hydrochloride) (Arora et al., 2005). Excipients used most commonly in these systems include HPMC, polyacrylate polymers, polyvinyl acetate, Carbopol®, agar, sodium alginate, calcium chloride, polyethylene oxide and polycarbonates.

1.3.2. Mucoadhesive Drug Delivery Systems:
Bioadhesive drug delivery systems (BDDS) are used to localize a delivery device within the lumen to enhance the drug absorption in a site-specific manner. This approach involves the use of bioadhesive polymers, which can adhere to the epithelial surface in the stomach (Ponchel and Irache, 1998). Gastric mucoadhesion does not tend to be strong enough to impart to dosage forms the ability to resist the strong propulsion forces of the stomach wall. The continuous production of mucous
by the gastric mucosa to replace the mucous that is lost through peristaltic contractions and the dilution of the stomach content also seems to limit the potential of mucoadhesion as a gastroretentive force. Some of the most promising excipients that have been used commonly in these systems include polycarbophil, carbopol, lectins, chitosan, CMC and gliadin. Some investigators have tried out a synergistic approach between floating and bioadhesion systems (Nur and Zhang, 2000).

1.3.3. Expanded Drug Delivery Systems:
These dosage forms are larger than the pyloric opening and so are retained in the stomach. There are some drawbacks associated with this approach. Permanent retention of rigid large-sized single-unit forms can cause bowel obstruction, intestinal adhesion and gastroplasty (Klausner et al., 2003a).

1.3.4. High Density Drug Delivery Systems:
Sedimentation has been employed as a retention mechanism for pellets that are small enough to be retained in the rugae or folds of the stomach body near the pyloric region, which is the part of the organ with the lowest position in an upright posture. Dense pellets (approximately 3g/cm$^3$) trapped in rugae also tend to withstand the peristaltic movements of the stomach wall. With pellets, the GI transit time can be extended to an average of 5.8–25 hours, depending more on density than on diameter of the pellets, although many conflicting reports stating otherwise also abound in literature. Commonly used excipients are barium sulphate, zinc oxide, titanium dioxide and iron powder, etc. These materials increase density by up to 1.5–2.4 g/cm$^3$. However, no successful high density system has made its way to the market.

1.4. Experimental Design and Formulation Optimization:
Experimental Design (or DOE) economically maximizes information. In an experiment, one or more process variables (or factors) are changed in order to observe the effect the changes have on one or more response variables. The statistical design of experiments (DOE) is an efficient procedure for planning experiments so that the data obtained can be analyzed to yield valid and objective conclusions. DOE begins with determining the objectives of an experiment and selecting the process factors for the study.
An Experimental Design is the laying out of a detailed experimental plan in advance of doing the experiment. Well-chosen experimental designs maximize the amount of "information" that can be obtained for a given amount of experimental effort. The statistical theory underlying DOE generally begins with the concept of process models (Fisher, 1926; Myers, 1990).

1.4.1. Full Factorial Designs:
It is one of the DOE in which every setting of every factor appears with every setting of every other factor in a full factorial design. Full factorial designs not recommended for five or more factors since the design under such condition requires a large number of runs and is not very efficient.

1.4.1.1. Three-level Full Factorial Designs:
Three-level designs are useful for investigating of the main effects and to some extent the quadratic interaction effects and it is written as a $3^k$ factorial design. It means that $k$ factors are considered, each at 3 levels. These are (usually) referred to as low, intermediate and high levels. These levels are numerically expressed as 0, 1, and 2. One could have considered also the digits -1, 0, and +1. The reason that the three-level designs were proposed is to model possible curvature in the response function and to handle the case of nominal factors at 3 levels. A third level for a continuous factor facilitates investigation of a quadratic relationship between the response and each of the factors. The simplest form of the three level full factorial design is The $3^2$ design which has two factors, each at three levels (Myers, 1990).

1.4.2. Box-Wilson (Central Composite) Design:
It is a classical quadratic design most often used for response surfaces. It is a combination of a factorial and axial design with experiments at a distance of along each axis (thus, the name). It enables to study the system (formulation) by varying the parameters around a point of interest, the domain is a sphere, and the coordinates of axial experiments are outside those of the factorial ones. $\alpha$ is chosen to give the best statistical properties (e.g., constant prediction precision). This design provides a check for both process stability and possible curvature and could be ap-
plied for screening and/or optimization processes (Box and Wilson, 1951; Box and Meyer, 1986).

1.5. Controlled Oral Delivery of Glibenclamide:
Glibenclamide is the most extensively used sulphonylurea in many parts of the world for the management of non-insulin-dependent diabetes mellitus (NIDDM) (Lebovitz et al., 1992). Following oral administration, the drug is documented to possess low aqueous solubility, possible content uniformity problems, and dissolution rate-limited bioavailability (Chalk et al., 1986; Varma et al., 1992; USP 1995). Moreover, large inter- and intra-individual responses following administration of glibenclamide preparations have also been reported (El-Sayed et al., 1989; Marchetti and Navalesi, 1989; Coppack et al., 1990). Such variations are undesirable and may expose susceptible patients to the danger of hypoglycaemia or other hazards when changing a patient's therapy from one preparation to another.

Several techniques have been reported to enhance the dissolution rate of the drug. Solid dispersion technique has been successfully applied by Geneidi, et al. (1980a) to enhance the in vitro dissolution of glibenclamide where the authors utilized sorbitol and/or manitol as solid dispersion agent for the drug. The same authors applied the same technique with poloxamer and polyvinyl pyrrolidone (PVP) as solid dispersion agents for glibenclamide and the results were comparable to that of the previous study (Geneidi et al., 1980b). Betageri and Makaria (1995, 1996) have reported the efficiency of co-melt solid dispersion technique of glibenclamide with polyethylene glycol (PEG) 6000 and/or 4000 and lyophilization to enhance the solubility of the drug.

The ability of liquid and semi-solid matrix filling capsule technology to improve the dissolution rate of glibenclamide has been investigated by Galal et al. (2003). In the conducted study, the drug was formulated in different concentrations as solutions in tetruglycol or tetruglycol/PEG 6000 blend and as suspensions in semi-solid matrix composed of Gelucire44/14 as a base. The authors applied the semi-quantitative estimation of glibenclamide solubility in various vehicles and were able to show that tetruglycol is the most efficient solubilizer for the drug.

A significant problem facing the pharmaceutical formulator attempting to prepare a bioavailable oral sustained release dosage form of gliben-
clamide relates to the ability of the dosage form to release the drug over the desired period of time to such an extent that the drug content of the dosage form will be effectively bioavailable. One aspect of this problem is the fact that glibenclamide is relatively insoluble and therefore inherently difficult to be solubilized from an oral dosage form in the gastrointestinal tract and then be absorbed through the walls of the gastrointestinal tract. This solubility and bioavailability problem has been overcome with respect to immediate release oral drug dosage form by utilizing a solubilizing agent, as discussed above. However, such agents are expected to cause the fast, i.e., immediate release of all of the glibenclamide when orally administered. Therefore, the use of such solubilizing agents would not necessarily be considered desirable in sustained-release oral dosage forms, where the goal is to slow the release of drug from the dosage form over an extended period of time. Thus, there is a continuing need in the art for a relatively simple and economical controlled-release glibenclamide formulation for oral administration that is fully bioavailable and suitable for administration once every 24 hours.

It is worth mentioning that most of the supportive in vivo data concerned with efficiency of solid dispersion as a tool for solubility enhancement of glibenclamide is either not held or not available due to the nature of the patent publication. However, few reports showed that solid dispersion of glibenclamide results in increase in total fraction of the drug that reaches the general circulation without affecting the time to reach the maximum attained drug concentration in the blood (Tashtoush et al., 2004).

Since glibenclamide is usually intended to be taken for a long period, enhancement of patient compliance is very important (Faber et al., 1990; Bitzen et al., 1992). In fact, it is the target upon which the strategy of drug controlled-release is based. Few attempts have been made to develop glibenclamide controlled-release delivery systems in order to enhance patient compliance, reduce fluctuation of drug plasma concentration and to increase drug bioavailability.

Many of the cited reports focused on the skin delivery of glibenclamide claiming the reduction of the in vivo drug level fluctuation with enhanced input duration. Mutalik and Udupa (2005) have investigated the influence of transdermal glibenclamide delivery on the hyperglycemia
control in mice where the authors applied membrane-moderated transdermal systems using drug-containing carbopol gel as reservoir and ethyl cellulose, Eudragit RS-100, Eudragit RL-100 and Ethylene vinyl acetate (EVA) (2%, 9% and 19% vinyl acetate content) as rate controlling membranes. They were able to show that membrane-controlled transdermal systems of glibenclamide exhibited better control of hyperglycemia and more effectively reversed the diabetes mellitus complications than oral glibenclamide administration in mice. The authors have attributed the results to the constant drug delivery that characterized the transdermal delivery system. Extended-release glibenclamide formulation with improved dissolution properties is therefore a desirable addition to the medical treatment of diabetes, including type II diabetes.

The majority of the cited reports on the development of oral controlled-release of antidiabetics are concerned with glipizide and very few (if any) are related to glibenclamide. However, in most of these glipizide-based researches, authors generally claimed that glibenclamide could also be used as a model drug for the respective developed formulations. Nevertheless, supportive data for such claim is always not available due to the nature of the patented formulations (Dileep et al., 2005)
2. Scope of the Work

Glibenclamide, a potent hypoglycemic agent, is used to reduce glucose concentration in diabetic human patients to the normal level. Fluctuation of dose delivery of this drug can result in serious hazards, specifically, hypo- or hyper-glycemia and accordingly, accurate dose delivery of the drug seems to be essential. The scope of the present study is to develop and optimize a buoyant tablet formulation capable to deliver glibenclamide in a sustained manner and suitable for once day administration using consecutive application of full factorial, Box-Wilson and composite index experimental designs. Moreover, the study is designed in a way that explored the influence of different formulation variables on physical characters of the developed dosage form and to evaluate the pharmacokinetics of the drug from the developed dosage form compared to Daonil® tablets as a marketed reference drug product.
3. Experimental

3.1. Materials:
The following materials have been utilized during the experimental part of the research:
Glibenclamide reference standard and raw material (G. Amphray Laboratories, Mumbai, India) were obtained from Wafra Pharma Lab. Sudan and used as received.
HPMC (pharmaceutical grade, 4000cps) is a product of Horst G.F. von Valtier, Hamburg, Germany.
Polyvinyl pyrrolidone (PVP, DC grade) was a product of FMC, Ireland, and supplied by Wafra-pharma lab. (Crosslinked homopolymer of N-vinyl-2-pyrrolidinone)
Stearyl Alcohol (Shing Poong Pharmaceutical Co., Seoul, Korea) received as a gift sample from G.M.C. (Sudan).
Isopropanol, Methanol and Acetonitrile were HPLC grade products of Scharlau, Spain.
Calcium Hydroxide, Magnesium stearate, Dihydrogen Phosphate, Sodium Hydroxide, Barium Sulfate, Hydrochloric Acid and Ethylenediamine-tetra-acetic acid are pharmacopoeial grade and were provided by Wafra Pharma lab (Sudan).
Ortho-phosphoric acid (analytical grade, Merk) and Dichloromethane (analytical grade, Loba Chemie, India) were also supplied by Wafra Pharma.

3.2. Instruments and Apparatus:
The following instruments and equipment were used during the course of the work:
Tablet compression machine (Manesty, 15D4B3/16, England);
Analytical balance (Digital Mettler tolledo, Switzerland);
Monsanto tablet hardness tester; Tablet friability tester; Disintegration tester (Erweka, Germany);
Tablet dissolution tester (DT700 Erweka, Germany) and
U.V Spectrophotometric device (Perkin Elmer Spectrometer, Lambda 11, Germany).
Utilized HPLC was comprised of variable wave length UV detector (Knauer, Germany); Pump (64, Knauer, Germany); Column (250x4.6mm ID, Eurospher-100 C8, 5µm, Berlin, Germany).

3.3. Methods:

3.3.1. Experimental Design and Dosage Form Formulations:
The screening design initially selected in this study was $3^2$ full factorial experimental design in which two factors namely, drug: PVP ratio and tablet hardness each at three levels were investigated within 9 formulation runs for their main and interactive effects on tablet swelling, floating capability and drug release behavior. The design is described fully in the introduction section (under 1.4.1.1.) and presented in Table 1. In these preliminary batches, direct compression was adopted where drug (10 mg/tablet), HPMC (fixed load of 4000cps grade), PVP (different amounts) were passed through 50 mesh, thoroughly mixed for 10 min using mortar and pestle, lubricated with Mg stearate (1% w/w) and compressed into tablets using Manesty tablet presser (15D4B3B/16, equipped with 8mm flat punch). Various compression loads were adopted to produce tablets of three hardness ranges for each formulation (40—70N). Summary of the results concerning the physical characters of tablets within these preliminary batches are tabulated in Table I and figures 1—3.

3.3.2. Buoyancy Studies:
Tablets within all formulations in the design were tested for floating capability where six tablets from each formula were investigated. Except for the basket, USP apparatus 2 of dissolution was used where time between introduction of the dosage form and its buoyancy on simulated gastric fluid (0.1 N HCl, pH 1.2, enzyme-free) and phosphate buffer, pH 5.8 at 37° C ± 0.5, rotated at 50 rpm, and the time during which the dosage form remained buoyant were measured. Results were summarized in Table I and figures 1—2.

3.3.3. Swelling Studies:
To verify for the influence of drug: PVP ratio and hardness on aqueous fluid uptake by tablet and consequently, the dosage form buoyancy and
drug release, tablets within all formulations showing floating tendency were subjected to swelling studies where medium uptake into tablet preparations and their weight gaining at pre-selected time point was determined. In this study weighed tablet samples were placed in USP apparatus 2 dissolution baskets at 50 rpm, mounted in simulated gastric fluid (0.1 N HCl- enzyme- free, and/or phosphate buffer pH 5.8, 500 ml), maintained at 37°C ± 0.5. At pre-determined time point, each basket was withdrawn, and the enclosed wetted tablets were wiped with a piece of paper tissue and weighed.

The percentage increase in weight due to the absorbed media (Q) was estimated according to the equation:

\[ Q = 100 \left( \frac{W_f - W_i}{W_i} \right) \]  

where \( W_i \) and \( W_f \) are the initial mass of the tablet and the final hydrated mass of the same tablet after the designated time point (Efentakis, et al 2000). Results were summarized in Table II and figure 3.

3.3.4. In vitro Drug Release Studies:

In vitro release of the model drug, glibenclamide, from floating tablets within selected formulations tested was determined using USP paddle method. Tablets were investigated in 900 ml 0.1N HCl, pH 1.2 and phosphate buffer pH 5.8 at 37.0°C ± 0.5 with stirring speed of 50 rpm. Samples of six tablets for each formula were examined and five ml dissolution samples were withdrawn at intervals filtered (membrane pore filter 0.45µm) and replaced by an equivalent volume of fresh dissolution medium, kept at the same temperature. Dissolution samples were immediately assayed for glibenclamide.

3.3.4.1. Calibration Curve for Reference Standard Glibenclamide:

Different dilutions of glibenclamide in phosphate buffer pH 5.8 and 0.1N HCl were carried out to obtain different solutions of varying concentrations. Absorbance of each solution was measured at 229 nm against blank. Sample absorbance is an indication for the concentration of glibenclamide in that solution. From the data obtained, the calibration curve was generated, and the linear regression equation was found out (Fig. 4 and 5) using software package Microcal origin (V.6, Microcal Inc. USA). Validation of the analytical process was assessed using values of coefficient of variation (CV%) in and between days (Table III).
### 3.3.4.2. Determination of Glibenclamide in Dissolution Samples:

The filtered samples were measured for glibenclamide concentration using UV spectrophotometry at 229 nm. The obtained absorbance values were converted into concentration values using a general regression equation generated for standard curve run prior to the experiment. Drug released was determined using the regression equation obtained from the calibration curve thus generated (Fig. 5). Results of the *in vitro* glibenclamide release from formulations tested were presented in figure 6 and Table IV.

### 3.3.4.3. Determination of *in vitro* Drug Release Kinetics:

*In vitro* release data of glibenclamide from floating tablet formulations of the preliminary batches (namely formulations 1, 2, 4, 5, 7, and 8) were subjected to analysis in order to verify the release kinetics. Model used for the fit was the power law introduced by Korsmeyer *et al.* (1983) in which release data were fitted to the equation:

\[
\frac{M_t}{M_\infty} = k t^n
\]

Where \( \frac{M_t}{M_\infty} \) is the fraction of drug released, \( t \) the release time, \( k \) a constant including the geometric and structural characteristics of the controlled-release device, and \( n \) the diffusional release exponent that characterizes the drug release mechanism.

Fractions of the drug release (\( \frac{M_t}{M_\infty} < 0.6 \)) were evaluated according to the shown equation in a search for the value of the diffusional exponent \( n \). Moreover, values of \( t_{50\%} \) for the different formulations tested were calculated using the following equation:

\[
t_{50\%} = \sqrt{\frac{0.5}{k}}
\]

The process of data fitting and parameters’ estimation described above was aided by using software package Microcal origin (V.6, Microcal Inc. USA). Best fit was characterized by the comparative values of the correlation and determination coefficient \( (r) \) and \( (r^2) \), respectively. Fitting data were summarized in Table IV.

### 3.3.5. Formulation Optimization:

Composite index-based statistics was selected and applied for the data derived from floating tablet formulations in the \( 3^2 \) full factorial design.
for selection of the optimized floating formulation. This was done in two consecutive steps based on pre-set constraints. Parameters for selection of the optimum formulation among the floating tablet batches tested in the factorial design were constrained in two steps as follows:

**Step 1:** where constraints selected were: % drug released in the first hour ($\text{Rel}_{5\%} = 12.5—32.5\%; \text{ average 22.5\%}$); onset of tablet floating ($F_{\text{onset}} = 0—30\text{min}; \text{ average 15\text{min}}$); and diffusional exponent, ($n = 0.8—1.2; \text{ average 1.00}$).

**Step 2:** in which selected constraints were: time for 50\% drug release ($T_{50\%} = 2—4 \text{hrs}; \text{ average 3 hrs}$); % swelling at 6 hr interval ($S_{\%} = 300—500\%; \text{ average 400\%}$); and % drug released after 6 hrs ($\text{Rel}_{\%6} = 80—100\%; \text{ average 90\%}$).

In both steps, a weighted composite index was generated in order to designate a single score utilizing the six constraints (in separate steps each of three constraints) shown above. Since the relative contribution of each individual constraint to the true composite score within each step was unknown, decision was made to assign an arbitrary value of $1/3$ to each of the three dependent variables and, accordingly, each test result was transformed to a value between 0 and 33.33.

Within each separate step, multi-linear regression equations (Appendix-1) were applied for the three constraints in order to generate the composite index (CI) for each selected formulation variable including higher than and lower than ideal values. The batch having a highest composite index would be considered as a batch fulfilling the constraints and consequently would be considered as optimized one. The method was also applied to detect the most optimized glibenclamide floating tablet formulation after conducting Box-Wilson set of formulations. The process of statistical composite index application was aided by the computer package S-Plus 2000 professional 1 (Lucent Technologies, Inc., USA). Results are summarized in Tables V, VI, IX and X and figure 7.

**3.3.6. Central Composite Box-Wilson Design**

Based on findings of the composite index analysis of the data concerning glibenclamide floating formulations in the $3^2$ factorial design, Box-Wilson design was selected to screen for and optimization of the possible effects (main and interactive) of three targeted formulation variables.
Target variables were content of stearyl alcohol, content of Mg stearate, and tablet hardness. Responses measured were buoyancy and the drug release behavior of the developed floating formulations. Each variable was investigated at five different levels (17 formulations). The model includes three runs of central points and two axial levels for each factor investigated as shown in Table VII. The benefit behind this was summarized in the introduction section under 1.4.2. and the obtained results were tabulated in Table VIII.

3.3.6.1. Preparation of Floating Tablets in Box-Wilson Design:
Wet granulation was adopted in this section where drug and fixed load of HPMC-4000cps were thoroughly mixed for 10 min using mortar and pestle, wetted with specific constant volume of Ca (OH)$_2$ solution and forced through 12 mesh screen. Granules thus obtained were dried overnight at 50ºC (specification of the oven).
To the 12/16 mesh fraction of the granules PVP (different amounts) and stearyl alcohol (if any, different amounts) were added and the blend was mixed for 5 min. The blend was then lubricated with Mg stearate (different % content of the formula) and compressed into tablets under various compression loads to produce tablets of different hardness level on Monsanto hardness tester. Tablets thus obtained were characterized by same drug and HPMC content (10 and 70mg, respectively) and vary with respect to content of PVP, stearyl alcohol, Mg stearate and hardness (Table VII).

3.3.6.2. Mathematical Modeling for Drug Release Data Derived from Box-Wilson Design:
Full quadratic mathematic equation was written to include all possible main, interaction and quadratic effects of the variables in the Box-Wilson design as follows:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_1X_2 + B_5X_1X_3 + B_6X_2X_3 + B_7X_1^2 + B_8X_2^2 + B_9X_3^2$$

Where $Y$ stands for specific response, $B_0, B_1...B_9$ are the regression coefficients, $X_1...X_3$ are the main effect, $X_1X_2...X_2X_3$ are the interactions between the main effects and $X_1^2...X_3^2$ are the quadratic terms. Results obtained from the three central points concerned with the drug release were compared for determination of lack of fit of the proposed
model and to assure the absence of the pure error in the design (Fig. 8). Analysis was carried out using step-regression and significance of the effect was verified using the probability value ($p \leq 0.05$). Stepwise regression to achieve an optimized mathematical model for $t_{50\%}$ is contained in Appendix-2.

3.3.7. Comparative Bioavailability Study:
To examine the comparative bioavailability of the drug from the newly developed formulation, optimized floating formulation of glibenclamide (formulation 19) was tested against the immediate release marketed formula (Daonil® (Hockest) 5-mg tablets, batch No. 13E32, expiry date 7/2006).

3.3.7.1. Study Design:
The study design is a fasting, single-dose, two-treatment, two-period, two-sequence crossover with a 1-week washout period between Phase I and Phase II dosing. Such design is selected to eliminate the influence of subject variation and biased assessment on the pharmacokinetic parameters of the drug (USP, 2003).

3.3.7.2. Drug Content Assurance:
Before administration to volunteers, drug content in both conventional and developed tablet formulations was assured. At least ten tablets from each formulation were crushed down and fraction equivalent to content of one tablet were dissolved in phosphate buffer pH 5.8 and subjected to serial dilutions. Referring to the generated calibration curve of the UV drug absorbance described under section 3.3.4.1, drug content in each fraction was calculated after using suitable dilution. Tablets used for the in vivo investigations were within the labeled drug potency.

3.3.7.3. HPLC Method for Quantification of Glibenclamide in Human Plasma:
A high-performance liquid chromatographic (HPLC) method reported by Al-Hazayamah and Abu Lubbad (2001) has been adopted, validated and applied with some modifications in this study for the assay of glibenclamide in human plasma. One-ml human plasma samples were placed in ten ml conical glass tubes and spiked with 100 µl of gliben-
clamide methanolic solutions of different concentrations to produce drug plasma concentration in the range of 0.010-10µg/ml. To these samples, 100 µl of Ibuprofen as internal standard (1 mg/ml), three ml of 0.1 % orthophosphoric acid as a buffer and four ml Dichloromethane as extracting solvent were added. Samples were vortex-mixed for ten seconds, shaken on a rotary mixer for ten min., and centrifuged at 3000 rpm for five min. The organic phase was transferred to a glass centrifuge tube and evaporated to dryness under N₂-gas. The residue was reconstituted in 100 ml mobile phase (0.1% ortho-phosphoric acid pH 2.7: isopropanol: acetonitrile, (45: 25: 30) and injected into a reversed phase C-8 column of the HPLC at ambient temperature. The eluted drug was monitored by UV detector at 235 nm with sensitivity set at 0.01 AUFS. Mobile phase pump delivery was 1 ml/min. Under such conditions no peak corresponding to glibenclamide was observed in blank drug-free plasma samples treated similarly (Appendix-3)

3.3.7.4. Calibration Curve for Reference Standard Glibenclamide in Human Plasma:
Glibenclamide ten mg was dissolved in 100 ml of methanol. This stock solution was diluted ten-fold in methanol to give a working standard solution of one mg/ml. The internal standard Ibuprofen was treated similarly and both solutions were found to be stable at 20⁰ C for at least one month. Calibration curve was constructed from drug-free human plasma spiked with increasing concentrations of glibenclamide (0.01-4µg/ml), fixed concentration of the internal standard and processed as described under 3.3.7.3. Peak area ratios of the drug to the internal standard and drug concentrations were used for quantification. Validation of the method was verified using the criteria of RSD and the % recovery values of the drug within and between days, respectively. Results of calibration study were depicted in figure 11. Parameter values for method validation were summarized in Table XI.

3.3.7.5. Subjects for the Comparative Bioavailability Study:
Sixteen informed, healthy, non-smoking adult male volunteers (aged 24—44 years and within 12±2% of ideal body weight for height) participated in this study (Metropolitan Life Insurance Company Statistical Bulletin, 1983). Subjects’ selection was based on an acceptable medical history, physical examination, and clinical laboratory test results. Writ-
ten, informed consent forms have been signed by all participants upon agreeing before they were accepted into the study.

3.3.7.6. Dosage Forms Administration and Samples Collection:
Volunteers were randomly assigned into two groups (each of eight subjects). Following an overnight fast of at least ten hours, subjects have administered (based on their categorization) a single dose of ten mg of glibenclamide contained in the test (1 floating tablet) or reference product (two tablets) with 240 ml of a 40% glucose solution in water. After dosing, subjects have received 60 ml of a 40% glucose solution in water every 15 minutes for four hours. No additional water or fluids, except for the glucose solution, were allowed from one hour pre-dose to one hour post-dose. Fasting condition was maintained for at least four hours after administration of the test or reference treatment. Food and fluid intake were standardized according to a strict protocol. A sandwich of house-prepared and cooked meat, and two cakes were provided at 5, 8, and 12 hours post—administration for all subjects during both phases of the study. The water intake was 250, 250, and 400 ml at 5, 8, and 12 hours post—administration. No Rx or OTC medication was allowed beginning 2 weeks before drug administration and until after the study is completed. Following a 1-week washout period, subjects began the second phase of the study where those that had received the test products in the first run received the reference products in this phase and vice versa.

Venous blood samples were collected according to the following time pattern pre—dose (0 hours) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 30 and 36 hours post-dose. To plasma samples obtained upon immediate centrifugation, 100 µl of internal standard was added and samples were then kept frozen at the temperature below – 20 °C for subsequent analysis for glibenclamide. The time elapsed between sample collection and its assay was documented.

3.3.7.7. Determination of Glibenclamide in Human Plasma:
Frozen plasma samples were drawn out of the fridge, allowed to thaw at room temperature and processed for glibenclamide analysis as described before. Drug plasma time profile plots were then generated for each subject. The average drug plasma-time profiles of the two drug products were plotted in figure 12.
3.3.7.8. Determination of Pharmacokinetic Parameters of Glibenclamide:

The individual pharmacokinetic parameters of glibenclamide were derived by non-compartmental analysis (Gibaldi and Perrier, 1982) using the Statistica 6 (StatSoft, Inc., OK, USA). The following parameters were derived: the peak plasma concentration $C_{\text{max}}$ and the time to reach peak plasma concentration $t_{\text{max}}$ (both were observed values); the apparent elimination half-life of glibenclamide $t_{1/2}$ as determined by log-linear regression analysis of the terminal portion of the plasma concentration-time curve of the drug; The two zero moments $\text{AUC}_{0-24}$ and $\text{AUC}_{0-\infty}$ by the linear trapezoidal rule and extrapolation to infinity, respectively. The first moment curve was constructed by the time course data obtained by multiplying the plasma concentration $C_p$ with the corresponding time point. The trapezoidal rule was then used to obtain the area under the first moment curve ($\text{AUMC}$). The tail area of moment curve (beyond the last data point) was estimated by the equation: tail $\text{AUMC} = C_p t/k + C_p k^2$. Mean residence time (MRT) was calculated by dividing total $\text{AUMC}$ by $\text{AUC}_{0-\infty}$. Results are summarized in Table XII.

3.3.7.9. Statistical Data Analysis:

The results of the pharmacokinetic parameters ($C_{\text{max}}$, $T_{\text{max}}$, $\text{AUC}_{0-\infty}$, $\text{AUC}_{0-24}$) are given as mean ± standard deviation (SD). All data were analyzed by paired t-test and two one-sided t-tests at 90% confidence interval (CI$_{90\%}$) (S-Plus 2000 professional 1, Lucent Technologies, Inc., USA). A result of $p$ <0.10 was regarded as significant. Summary of the analysis is contained in Appendix-4 and the final statistical conclusion is presented in Table XII.

3.3.8. In vivo Dosage Form Localization Study:

In order to correlate the drug level attained in the plasma with dosage form location in the gastrointestinal tract, six out of the 16 subjects participating in the bioavailability study were randomly selected to conduct dosage form localization study one week after phase II of the bioavailability study.
3.3.8.1. Formulation of Contrast-Containing Floating Tablets:
Contrast-containing floating tablets were produced using the same procedure described under section 3.3.6.1. The formulation being the same as that of formulation 19 which has been subjected to the comparative bioavailability study except for the drug where it was replaced with Ba SO₄ as a contrast in an allowable amount (10 mg) (Eytan et al., 2003).

3.3.8.2. Dosage Form Administration:
Extended informed consent form was applied in this study. The six subjects participating in this study were randomly assigned into two groups each of three subjects. In one group, dosage forms were administered under fasting condition with 150 ml water. Fasting conditions were maintained four hours post-administration. In the other group, dosage forms were administered under non-fasting condition with the same amount of water. In both groups, X-Ray pictures were taken two and five hours post-administration to evaluate the location of dosage form in the gastrointestinal tract. Results were summarized in figures 13a and b.

3.3.9. Stability Study:
Stability of the drug in the developed floating tablets and the physical specifications of the floating tablets were tested according to the known quality control testing and stability protocol. Test environments were temp 45±5 ºC and 75%RH in presence of light. Floating tablets were investigated in the final pack where blister pack was tested as packaging systems. Real time study was simultaneously conducted for the same batches where test temp was 31ºC and 30%RH. Responses measured were drug content, drug release profile, tablet hardness, tablet floating capability and tablets discoloration. Results were summarized in Table XIII.
4. Results and Discussion

The high volume of the obtained result compared to the limited total numbers of formulations conceived in this study clearly reflect the potential role of the experimental design in development, evaluation and optimization of pharmaceutical formulations. In fact, application of such design has result in saving considerable amount of raw material and shortening the time for this study to complete.

4.1. The Influence of Drug: PVP Ratio and Tablet Hardness on Dosage Form Buoyancy:

At this level, it is obvious from Table I and figure 1 that the floating ability of the developed dosage form is a function of the dosage form hardness (X₂) where tablet floating ability is significantly enhanced when tablets were compressed to low hardness level (40N). Drug: PVP ratio showed no (or a little effect) on dosage form buoyancy. The same results could be obtained from analysis of regression coefficient accompanying each variable, which appear in the polynomial regression equation attached in figure 1.

Coefficient associated with the variable X₂ (hardness) is greater than that of X₁ (drug: PVP ratio). Moreover, no interaction between the two variables was shown to affect the tablet buoyancy (coefficient associated with the term X₁X₂ in the equation is 0). Furthermore, the positive sign accompanying the terms X₂ and X₂² indicates the positive contribution of the variable X₂ (hardness) in tablets buoyancy.

Fig. 2 is 3D surface plot concerned with onset of tablet floating among different floating tablet formulations. It showed that the variable X₂ (tablet hardness) is a determining factor for the quickness of tablet floating where short time for floating onset is achievable in case of tablet formulations with low hardness level which supports the concept derived before.
Table I

Floating Capability of Tablet Formulations in the $3^2$ Factorial Design

<table>
<thead>
<tr>
<th>Formula No</th>
<th>Drug:PVP X&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Hardness (N) X&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Floating ability</th>
<th>Onset (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+1 (1:2)</td>
<td>-1 (40)</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>+1 (1:2)</td>
<td>0 (60)</td>
<td>+</td>
<td>180</td>
</tr>
<tr>
<td>3</td>
<td>+1 (1:2)</td>
<td>1 (70)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0 (1:3)</td>
<td>-1 (40)</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>0 (1:3)</td>
<td>0 (60)</td>
<td>+</td>
<td>120</td>
</tr>
<tr>
<td>6</td>
<td>0 (1:3)</td>
<td>1 (70)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-1 (1:4)</td>
<td>-1 (40)</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>-1 (1:4)</td>
<td>0 (60)</td>
<td>+</td>
<td>300</td>
</tr>
<tr>
<td>9</td>
<td>-1 (1:4)</td>
<td>1 (70)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) float within 20—300 min; (-) sink.

Evident from the plot, drug: PVP ratio (X<sub>2</sub>) has no (or a little) in the onset of tablet floating; however, the use of medium—high drug: PVP ratio seems to be better than low ratio. The same conclusion could easily be derived from the regression coefficients of the two variables appearing in the accompanying regression equation.

The Table above indicates that the floating tablet formulations differ widely with regard to onset of floating; however, once the tablet floated, it remained buoyant till the end of the experiment run (six hrs).

It is well documented that compression of tablets to high hardness levels significantly reduces tablet porosity which, in turn, affects the initial water penetration and consequently, delays the onset of tablet floating (Nur et al., 2005). However, with time this phenomenon might be offset by the high affinity of the polymer HPMC to water and accordingly tablet achieves a delayed floating as the result implies. Based on these findings on floating ability, the different formulations could be ranked as follow: 1, 4, 7 (were the best), 5, 2, 8, 3, 6, and finally 9.
Fig. 1

Surface Plot for the Influence of Variables $X_1$ (drug: PVP) Ratio and $X_2$ (Tablet Hardness) on Buoyancy of Tablet Formulations in the Factorial Design,

$$Z = 100 + 0X_1 + 0.5X_2 + 9.326e-15X_1^2 + 0X_1X_2 + 0.5X_2^2$$

Consequently, one might conclude that:

a) for rapid onset of floating, low level of hardness (40—50 N) should be adopted and

b) PVP content has no (or a little effect) on tablet buoyancy; however, drug: PVP ratio better to be 1:3—1:2 w/w. Accordingly, formulations 1, 4, and 7 were considered as promising; however, they need to be further differentiated with respect to the floating duration.
Surface Plot for the Influence of Variables $X_1$ (drug: PVP) Ratio and $X_2$ (Tablet Hardness) on Floating Onset of Tablet Formulations in the Factorial Design,

\[ Z = 173.333 - 20X_1 + 40.5X_2 + 40X_1^2 + 0X_1X_2 + 139X_2^2 \]

4.2. The Influence of Drug: PVP Ratio and Tablet Hardness on Tablets Swelling Behavior:

Swelling behavior reflects the ability of dosage form to uptake aqueous fluid and hydrate. Rate of hydration indicates how fast the dosage form might float. On the other hand, the extent of such hydration measures the diffusional path length for the drug and hence determines the rate of drug release from the dosage forms (Nur and Zhang, 2000). All of the investigated tablet batches exhibited floating ability with varying times for floating onset (formulations showing + floating in Table I).

In the light of this scenario, differences in swelling characters among the different floating formulations could be attributed either to the differences in variable $X_1$ (drug: PVP ratio) or $X_2$ (tablet hardness).
Table II

*The Effect of Drug: PVP Ratio and Tablet Hardness on Swelling Behavior of Floating Tablet Formulations in the Factorial Design*

<table>
<thead>
<tr>
<th>Formula No</th>
<th>Drug: PVP $X_1$</th>
<th>Hardness (N) $X_2$</th>
<th>Swelling% after 6 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+1 (1:2)</td>
<td>-1 (40)</td>
<td>660</td>
</tr>
<tr>
<td>2</td>
<td>+1 (1:2)</td>
<td>0 (60)</td>
<td>520</td>
</tr>
<tr>
<td>4</td>
<td>0 (1:3)</td>
<td>-1 (40)</td>
<td>500</td>
</tr>
<tr>
<td>5</td>
<td>0 (1:3)</td>
<td>0 (60)</td>
<td>450</td>
</tr>
<tr>
<td>7</td>
<td>-1 (1:4)</td>
<td>-1 (40)</td>
<td>340</td>
</tr>
<tr>
<td>8</td>
<td>-1 (1:4)</td>
<td>0 (60)</td>
<td>300</td>
</tr>
</tbody>
</table>

![Fig.3](image)

**Fig.3**

*Surface Plot for the Effect of Drug: PVP Ratio and Tablet Hardness on Swelling Behavior of Floating Tablet Formulations in the Factorial Design,

\[(Z = -1.126e5 \cdot 1120X_1 + 3587.76X_2 - 20X_1^2 + 50X_1X_2 + 43.355X_2^2)\]*
Figure 3 clearly demonstrates the dependency of the % swelling on both variables; moreover, to achieve considerable swelling, low to medium level of hardness and high level of drug: PVP ratio are to be considered. The same conclusion could be obtained from values and sign of regression coefficients accompanying the two variables in the best—fit polynomial equation attached to the figure. Moreover, For a rapid initial water uptake by the tablets to initiate the floating, Drug: PVP and tablet hardness are better to be fixed as 1:2 w/w and 40—60N, respectively. This supports the conclusion drawn earlier.

It might be advantageous to have a dosage form with enhanced rate of water uptake since this will aid in the rapidness of floating; however, if water uptake rate is accompanied with an enhanced extent (excessive water being absorbed by the dosage form), this might result in two drawbacks: first, the bulk density of the dosage form starts to increase and this will limit the floating duration; secondly, release-controlling gel will be formed exclusively around the dosage form resulting in significant delay of the drug release characteristics from the developed dosage form.

4.3. In vitro Drug Release Characteristics:

4.3.1. Calibration Curve of Glibenclamide in Phosphate Buffer, pH 5.8:

Figure 4 shows the relation between glibenclamide different concentrations (mg/ml in phosphate buffer) and their respective UV absorbance at 229 nm. The plot reflects an excellent linear correlation between drug concentration and absorbance with high determination coefficient ($R^2 = 1$) as shown below. And the following equation could be applied efficiently to monitor drug concentration in the solution based on the measured solution absorbance where drug concentration (mg/ml) = $(\text{Abs} – 0.0017) / 7.7364$. 
4.3.2. Calibration Curve of Glibenclamide in 0.1N HCl:
The obtained graph (Fig. 5) shows an excellent linear correlation between drug concentration and absorbance with a highly-accepted determination coefficient (R^2 =0.9993) in the concentration range 0.0014—0.0896 mg/ml as shown below. And the following equation could be applied efficiently to monitor drug concentration in the solution based on the measured solution absorbance where drug concentration (mg/ml) = (Abs – 0.0654) / 11.477.

In both of cases, precision, accuracy and reproducibility of the UV method in glibenclamide determination were validated using the criteria of recovery % and coefficient of variation (CV%) inter- and intra-day at three different concentration levels as shown below (Table III). Minimum concentration of glibenclamide that can be determined with high precision and accuracy using this method is 0.0014 mg/ml, which is considered as an acceptable sensitivity limit.

Based on the two criteria shown in Table III, together with other considerations concerning the nature of the developed dosage form as gastroretentive tablets, 0.1 N HCl was selected as dissolution medium to inves-
tigate for the *in vitro* glibenclamide release from the different floating tablet formulations.

![Graph showing the calibration curve of glibenclamide in 0.1N HCl.](image)

**Fig. 5**

*Calibration Curve of Glibenclamide in 0.1N HCl*

**Table III**

*Validation of the UV Method for Glibenclamide Assay in Dissolution Testing*

<table>
<thead>
<tr>
<th>Concentration level (mg/ml)</th>
<th>Recovery %&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CV%&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In buffer</td>
<td>In 0.1N HCl</td>
</tr>
<tr>
<td><em>(Intraday)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0014</td>
<td>101.4%</td>
<td>100.3%</td>
</tr>
<tr>
<td>0.0112</td>
<td>101.8%</td>
<td>101.5%</td>
</tr>
<tr>
<td>0.0896</td>
<td>99.7%</td>
<td>100.1%</td>
</tr>
<tr>
<td><em>(Between days)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0280</td>
<td>101.2%</td>
<td>100.8%</td>
</tr>
<tr>
<td>0.0112</td>
<td>100.4%</td>
<td>101.1%</td>
</tr>
<tr>
<td>0.0448</td>
<td>99.8%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Accuracy in calculation of the concentration (obtained/actual *100%)

<sup>b</sup> Coefficient of variation (standard deviation/mean value *100%)

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4.3.3. Effect of Drug: PVP Ratio on the *in vitro* Drug Release:

Figure 6 shows the release pattern of glibenclamide from different floating tablet formulations of the preliminary batches tested in 0.1 N HCl. Release data of floating tablet formulations characterized by the same hardness level were compared (at two hardness levels 40 and 60N) in order to investigate the influence of Drug: PVP ratio in the drug release. Accordingly, drug release profiles of formulations 1, 4, and 7 (tablet hardness 40N) and formulations 2, 5, and 8 (Tablet hardness of 60N) were separately compared within each group. Criteria for comparison were percent of drug released in the first hour (Rel%-1), time for 50% drug release (t50%) and cumulative amount of drug released after six hours (Rel%-6). Values of Rel%-1 and Rel%-6 were traced directly from the respective release profiles of different formulations tested.

As expected, increase in the amount of PVP has resulted in significant acceleration of the drug release profiles from different formulations compressed to different hardness levels. Decrease in drug: PVP ratio from 1: 2 to 1: 4 has resulted in 1.6-fold increase in drug released at six hrs intervals when tablet hardness were in the range of 40—60N (formulation 1 compared to 7, and 2 compared to 8). This might better be explained in terms of the enhanced drug solubility caused by PVP (Geneidi *et al.*, 1980b).

![Glibenclamide Release Pattern of Floating Tablet Formulations in the Factorial Design Tested in 0.1N HCl, each data point is the average of 6 determinations.](image-url)
4.3.4. Release Kinetics:
In swellable matrix tablets, drug release kinetics are associated with the dynamics of gel layer thickness. The relative contributions of drug diffusion, polymer relaxation and matrix erosion to drug release in HPMC cylindrical matrices produce \( n \) values that range from 0.5 to 1.0.

Based on data in Table IV, both drug diffusion and polymer chain relaxation seem to control the drug release from these floating tablets. Numerous reports, concerned with the contribution of more than one mechanism in the release of sparingly soluble drugs from matrix tablets, are documented in the literature (Ponchel et al., 1987; Baveja et al., 1987; Uko-Nne et al., 1989).

### Table IV

**Drug Release Characteristics and Mechanisms (n) for the Different Floating Formulations in the Factorial Design**

<table>
<thead>
<tr>
<th>Formula</th>
<th>Drug:PVP</th>
<th>Hardness</th>
<th>( \text{Rel}_1% )</th>
<th>( t_{50%} ) (hr)</th>
<th>( \text{Rel}_6% )</th>
<th>( K )</th>
<th>( n )</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:2</td>
<td>40</td>
<td>8</td>
<td>4.7</td>
<td>63</td>
<td>0.1301</td>
<td>0.8761</td>
<td>0.9998</td>
</tr>
<tr>
<td>2</td>
<td>1:2</td>
<td>60</td>
<td>5</td>
<td>5.4</td>
<td>60</td>
<td>0.0783</td>
<td>1.0244</td>
<td>0.9996</td>
</tr>
<tr>
<td>4</td>
<td>1:3</td>
<td>40</td>
<td>19</td>
<td>3</td>
<td>82</td>
<td>0.1998</td>
<td>0.8374</td>
<td>0.9985</td>
</tr>
<tr>
<td>5</td>
<td>1:3</td>
<td>60</td>
<td>13</td>
<td>3.7</td>
<td>78</td>
<td>0.1576</td>
<td>0.8697</td>
<td>0.9989</td>
</tr>
<tr>
<td>7</td>
<td>1:4</td>
<td>40</td>
<td>30</td>
<td>2</td>
<td>98</td>
<td>0.2993</td>
<td>0.8212</td>
<td>0.9999</td>
</tr>
<tr>
<td>8</td>
<td>1:4</td>
<td>60</td>
<td>22</td>
<td>2.5</td>
<td>93</td>
<td>0.2232</td>
<td>0.8598</td>
<td>0.9987</td>
</tr>
</tbody>
</table>

\( ^a \% \) of drug released in 1 hour, \(^b \) Time required for 50% drug release, \(^c \% \) of drug released after 6 hours, \(^d \) Fitting constant, \(^e \) Diffusional exponent, \(^f \) Correlation coefficient of fitting to the release model.

4.3.5. Influence of Hardness on Drug Release from Floating Tablet Formulations:
Drug release of floating tablet formulations characterized by the same drug: PVP ratio was compared at different hardness levels in order to examine the role of the applied compression force (during manufacture of these tablet formulation) on the drug release. Formulations compared were 1 and 2; 4 and 5; 7 and 8 for the drug: PVP ratios of 1:2, 1:3, and 1:4, respectively. Parameters considered in the comparison were those mentioned before.
Based on data derived in Table IV, and at the three drug: PVP ratios, increasing the tablet hardness seems to result in significant delay in the initial drug release as indicated by values of Rel$_{-1}$ accompanying the respective formulations ($p<0.05$). However, no significant effect of hardness variation was revealed on the terminal drug release profiles of the different formulations ($p>0.05$). This could easily be traced from $T_{50\%}$ and Rel$_{-6}$ values of different formulations. In fact, this might be attributed to the lowered tablet porosity caused by the application of relative compression force during manufacture which, in turn, results in delay in the drug release owing to delay in the initial water penetration into the tablets. With time this effect might be offset by the high affinity of HPMC to the aqueous fluid and, consequently, no significant difference in the terminal drug release was traced from different formulations at the three different levels of drug: PVP ratios. These findings correlate well with the relevant published work of Nur et al. (2005).

4.4. Statistical Optimization of Formulations Using Composite Index Method:

Derringer and Suich (1980) illustrated how several response variables can be transformed into a desirability function. Composite index has been applied for statistical optimization of drug formulations in different cited reports (Gohel et al., 2003). And as the relative contribution of each individual constraint to the true composite score was unknown, a decision was made to assign an arbitrary value of one-third to each of the three dependent variables (Taylor et al., 2000).

Since higher or lower values of floating, swelling and drug release responses investigated in this study may not be desirable, an ideal is most suitable (Tables V and VI). The empirical composite index was devised to yield a score of 100 for an optimum result for each of the three variables in each step and each test result was transformed to a value between 0 and 33.33. The batch having a highest composite index would be considered as a batch fulfilling all the six constraints favorable for a 24-hr sustained release of glibenclamide floating tablets.

It is worth mentioning that composite index statistical technique has been selected in this study in order to reduce the possible increase in the number of formulations run and that was for economical reasons. Moreover, having known the mathematical model that could be applied effi-
ciently to describe the effects of different variables on performance of the best-chosen batch, one easily can optimize further such formulations to meet other constraints. Comparing the results derived from the two separate steps, a decision could easily be made with regard to the best buoyant batch fulfilling all of the six constraints.

Table V
Summary of Composite Index Estimation for Constraints in Step One of Formulations in the Factorial Design

<table>
<thead>
<tr>
<th>Formula</th>
<th>Rel&lt;sub&gt;a&lt;/sub&gt;</th>
<th>F&lt;sub&gt;onset&lt;/sub&gt;</th>
<th>n&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Transformed</th>
<th>Cl&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rel&lt;sub&gt;a&lt;/sub&gt;</td>
<td>F&lt;sub&gt;onset&lt;/sub&gt;</td>
<td>N</td>
<td>Rel&lt;sub&gt;a&lt;/sub&gt;</td>
<td>F&lt;sub&gt;onset&lt;/sub&gt;</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>20</td>
<td>0.8761</td>
<td>0</td>
<td>22.22</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>180</td>
<td>1.0244</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>20</td>
<td>0.6374</td>
<td>21.67</td>
<td>22.22</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>120</td>
<td>0.8697</td>
<td>1.67</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>20</td>
<td>0.8212</td>
<td>8.3</td>
<td>22.22</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>300</td>
<td>0.8598</td>
<td>31.67</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> % of drug released in 1 hour, <sup>b</sup> Time required for onset of tablet floating (min), <sup>c</sup> Diffusional exponent and <sup>d</sup> composite index

Table VI
Summary of Composite Index Estimation for Constraints in Step Two of Formulations in the Factorial Design

<table>
<thead>
<tr>
<th>Formula</th>
<th>t&lt;sub&gt;50%&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S&lt;sub&gt;6h&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Rel&lt;sub&gt;6h&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Transformed</th>
<th>Cl&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t&lt;sub&gt;50%&lt;/sub&gt;</td>
<td>S&lt;sub&gt;6h&lt;/sub&gt;</td>
<td>Rel&lt;sub&gt;6h&lt;/sub&gt;</td>
<td>t&lt;sub&gt;50%&lt;/sub&gt;</td>
<td>S&lt;sub&gt;6h&lt;/sub&gt;</td>
</tr>
<tr>
<td>1</td>
<td>4.7</td>
<td>660</td>
<td>63%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>520</td>
<td>60%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>500</td>
<td>82%</td>
<td>33.33</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3.7</td>
<td>450</td>
<td>78%</td>
<td>10</td>
<td>16.67</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>340</td>
<td>98%</td>
<td>0</td>
<td>13.33</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>300</td>
<td>93%</td>
<td>16.67</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Time for 50% drug release, <sup>b</sup> % swelling at 6hrs time interval, <sup>c</sup> % of drug released after 6 hours, and <sup>d</sup> composite index
From the results of step one shown in Table V, formulation 4 gained the highest score among other formulations and seems to be the best with regard to the specified constraints; whereas, both formulations 4 and 8 were ranked similarly (CI = 40), when the other three constraints evolved as indicated by results of step two (Table VI).

Since the onset of floating is a critical criterion for dosage forms that are claimed to act as a buoyant system, formulation 4 ($F_{onset} = 20\text{min}$) is considered as being better than formulation 8 ($F_{onset} = 300\text{min}$). It is worth mentioning that although formulation 7 exhibited an acceptable floating limitation and optimum swelling character, yet it fails to gain high score with regard to drug release, which might be attributed to the accelerated drug release from this formulation. This clearly demonstrates the power of the composite index in the evaluation of the proposed factorial design.

Nevertheless, based on the attained score of formulation 4 (40—43.89%), it might be necessary and advantageous to optimize further this formula specifically with regard to hardness, onset of floating and $n$ magnitude. In other words, the hardness is to be increased to a relative high hardness level (50N); onset of floating is to be reduced to 0-10 minutes maximally; and magnitude of $n$ is to be increased so as to achieve the desirable uniform zero order release kinetics (>0.89). These are the three new objectives.

Obviously, interaction between formulation variables could possibly results in contradiction between the first and the second objectives since any change in tablet hardness as a result of increase in magnitude of applied force of compression will definitely affect the porosity of the produced tablets which, in turn, would significantly reduce the capability of the tablet with regard to the immediate and prompt floating (floating onset will be increased). In fact, this might be overcome by incorporating a minor fraction of suitable floating enhancer where tablets with both satisfactory hardness and floating characters could be obtained.
Figure 7 is a three-directional surface plot for the influence of tablet hardness and drug: PVP ratio on the magnitude of the diffusional exponent $n$. From the plot it is apparent that both variables affect the value of $n$; moreover, medium tablet hardness (transformed $0 = 50N$) and enhanced ratio of drug: PVP (better is 1:3) are to be applied in order to obtain tablets having greater magnitude for $n$ ($\sim 1$, zero order).

4.5. Box-Wilson Design for Further Optimization:
Respective floating and drug release parameters of floating tablet formulations in Box-Wilson design (Table VII) are shown in Table VIII. In order to select between the 17 formulations, the obtained drug release results were subjected to step one composite index optimization (as described earlier).
Table VII

Lay Out of Variables of Glibenclamide Floating Formulations in Box-Wilson Design

<table>
<thead>
<tr>
<th>Formula code</th>
<th>X1 (Stearyl alcohol) (mg)</th>
<th>X2 (Hardness) (N)</th>
<th>X3 (Mg stearate) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>15</td>
<td>40</td>
<td>1.5</td>
</tr>
<tr>
<td>11</td>
<td>22.5</td>
<td>40</td>
<td>1.5</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>60</td>
<td>1.5</td>
</tr>
<tr>
<td>13</td>
<td>22.5</td>
<td>60</td>
<td>1.5</td>
</tr>
<tr>
<td>14</td>
<td>15</td>
<td>40</td>
<td>4.5</td>
</tr>
<tr>
<td>15</td>
<td>22.5</td>
<td>40</td>
<td>4.5</td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td>60</td>
<td>4.5</td>
</tr>
<tr>
<td>17</td>
<td>22.5</td>
<td>60</td>
<td>4.5</td>
</tr>
<tr>
<td>18</td>
<td>12</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>25</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>18.75</td>
<td>34</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>18.75</td>
<td>66</td>
<td>3</td>
</tr>
<tr>
<td>22</td>
<td>18.75</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>23</td>
<td>18.75</td>
<td>50</td>
<td>5.5</td>
</tr>
<tr>
<td>24</td>
<td>18.75</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>25</td>
<td>18.75</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>26</td>
<td>18.75</td>
<td>50</td>
<td>3</td>
</tr>
</tbody>
</table>

The selected constraints were % drug released in the first hour (Rel₁% = 12.5-32.5%; average 22.5%); % drug released after six hrs (Rel₆%-6 = 80-90%; average 85%); and time for 50% drug release (T₅₀% = 2-4 hrs; average 3hrs). In addition, other constraints concerned with dosage form specifications were also adopted for the selection. These are: onset of tablet floating (Fₜₚ ≤ 10 min); duration of floating (Fₜₙ ≥ 6hrs); and tablet hardness (≥50 N).

Based on values of composite indices shown in Table IX, the best formulations that met the constraint specifications of the drug release could be ranked as formulations 17, 19 and 12 with CI of 71.64, 68.32 and 64.98, respectively.
Table VIII
Results of Floating and Drug Release Parameters for Formulations Prepared from Box-Wilson Design

<table>
<thead>
<tr>
<th>Formula code</th>
<th>$F_{\text{onset}}$ (^a) (min)</th>
<th>$F_{\text{duration}}$ (^b) (min)</th>
<th>Rel(_{\leq 1}) (^c) (%)</th>
<th>Rel(_{\leq 6}) (^d) (%)</th>
<th>$t_{50%}$ (^e) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0 (immediate)</td>
<td>1</td>
<td>18</td>
<td>91</td>
<td>2.7</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>3</td>
<td>28</td>
<td>79</td>
<td>3.1</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>90</td>
<td>28</td>
<td>86</td>
<td>3.3</td>
</tr>
<tr>
<td>13</td>
<td>20</td>
<td>120</td>
<td>24</td>
<td>80</td>
<td>3.6</td>
</tr>
<tr>
<td>14</td>
<td>15</td>
<td>360</td>
<td>26</td>
<td>90</td>
<td>2.7</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>40</td>
<td>19</td>
<td>78</td>
<td>3.4</td>
</tr>
<tr>
<td>16</td>
<td>26</td>
<td>360</td>
<td>27</td>
<td>86</td>
<td>3.5</td>
</tr>
<tr>
<td>17</td>
<td>20</td>
<td>25</td>
<td>25</td>
<td>84</td>
<td>3.4</td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>120</td>
<td>17</td>
<td>76</td>
<td>3.2</td>
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<tr>
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<td>0</td>
<td>360</td>
<td>28</td>
<td>84</td>
<td>3.2</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>180</td>
<td>22</td>
<td>77</td>
<td>2.5</td>
</tr>
<tr>
<td>21</td>
<td>30</td>
<td>50</td>
<td>32</td>
<td>85</td>
<td>4.5</td>
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<tr>
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<td>35</td>
<td>60</td>
<td>33</td>
<td>78</td>
<td>5.1</td>
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<td>23</td>
<td>14</td>
<td>360</td>
<td>31</td>
<td>63</td>
<td>5.8</td>
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<tr>
<td>24</td>
<td>20</td>
<td>150</td>
<td>36</td>
<td>86</td>
<td>4.2</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>150</td>
<td>34</td>
<td>87</td>
<td>4.2</td>
</tr>
<tr>
<td>26</td>
<td>24</td>
<td>120</td>
<td>33</td>
<td>86</td>
<td>4.2</td>
</tr>
</tbody>
</table>

\(^a\) Time required for onset of tablet floating, 
\(^b\) Time during which tablets remain buoyant on 0.1N HC, 
\(^c\) % of drug released in 1 hour, 
\(^d\) % of drug released after 6 hours, 
\(^e\) Time for 50% drug release.

Compared to results obtained with formulations before application of Box-Wilson design (Table V and VI), values of CI have been significantly increased towards the set release constraints (from CI of 40 for formulations 4 and 8 to CI of 71.64 and 68.32 for formulations 17 and 19, respectively). This reflects the effectiveness of the Box-Wilson design in formulations optimization.
Table IX

Summary of Composite Index Estimation for Drug Release Constraints of Formulations in the Box-Wilson Design

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Transformed</th>
<th></th>
<th></th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{50%}$</td>
<td>Rel$_{1%}$</td>
<td>Rel$_{6%}$</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>23.33</td>
<td>18.33</td>
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<td>41.66</td>
</tr>
<tr>
<td>11</td>
<td>29.99</td>
<td>14.99</td>
<td>0</td>
<td>44.98</td>
</tr>
<tr>
<td>12</td>
<td>23.33</td>
<td>14.99</td>
<td>26.66</td>
<td>64.98</td>
</tr>
<tr>
<td>13</td>
<td>13.33</td>
<td>28.33</td>
<td>0</td>
<td>41.66</td>
</tr>
<tr>
<td>14</td>
<td>23.33</td>
<td>21.66</td>
<td>0</td>
<td>44.99</td>
</tr>
<tr>
<td>15</td>
<td>19.99</td>
<td>21.66</td>
<td>0</td>
<td>41.65</td>
</tr>
<tr>
<td>16</td>
<td>16.67</td>
<td>18.33</td>
<td>26.66</td>
<td>61.66</td>
</tr>
<tr>
<td>17</td>
<td>19.99</td>
<td>24.99</td>
<td>26.66</td>
<td>71.64</td>
</tr>
<tr>
<td>18</td>
<td>26.66</td>
<td>14.99</td>
<td>0</td>
<td>41.65</td>
</tr>
<tr>
<td>19</td>
<td>26.66</td>
<td>14.99</td>
<td>26.66</td>
<td>68.32</td>
</tr>
<tr>
<td>20</td>
<td>16.67</td>
<td>31.66</td>
<td>0</td>
<td>48.33</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>1.66</td>
<td>33.33</td>
<td>34.99</td>
</tr>
<tr>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>0</td>
<td>4.99</td>
<td>0</td>
<td>4.99</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
<td>26.66</td>
<td>26.66</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>0</td>
<td>19.99</td>
<td>19.99</td>
</tr>
<tr>
<td>26</td>
<td>0</td>
<td>0</td>
<td>26.66</td>
<td>26.66</td>
</tr>
</tbody>
</table>

*Time for 50% drug release, \(b\) % of drug released in 1 hour, \(c\) % of drug released after 6 hours

Compression of formulations 17 and 12 to a comparatively high hardness level (60N, Table X) disabled the tablets within the two formulations to exhibit a quick floating and to maintain that floating for a considerable length of time. It might be true that presence of stearyl alcohol in these formulations is supposed to offset the influence of the hardness on the tablets floating characters (Nur et al., 2005); however, the included amount of stearyl alcohol in the two formulations was less than that of formulation 19.

From Table X, it appears that only tablets within formulation 19 have met the three required constraints for buoyancy, which might be attrib-
uted to the comparative low hardness of the tablets and the sufficient amount of stearyl alcohol present in the formula.

**Table X**

*Comparative Floating Parameters and the Respective CI Values for Drug Release of Formulations 17, 19 and 12*

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Hardness (Target ≥50 N)</th>
<th>F&lt;sub&gt;onset&lt;/sub&gt; (Target ≤10min)</th>
<th>F&lt;sub&gt;duration&lt;/sub&gt; (Target ≥360min)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>60N</td>
<td>20min</td>
<td>25min</td>
<td>71.64</td>
</tr>
<tr>
<td>19</td>
<td>50N</td>
<td>0min (immediate)</td>
<td>360min</td>
<td>68.32</td>
</tr>
<tr>
<td>12</td>
<td>60N</td>
<td>25min</td>
<td>90min</td>
<td>64.98</td>
</tr>
</tbody>
</table>

4.5.1. **Statistical Analysis of Results Generated from Box-Wilson Design:**
Two-way ANOVA at confidence of 95% revealed no significant difference in composite index between formulation 17 and 19 (p>0.05). Accordingly, formulation 19 is selected as the optimized formula for both dosage form performance and drug release based on the settled constraints.

4.5.2. **Mathematical Modeling and Optimization for Drug Release Data Derived from Box-Wilson Design:**
Results obtained from the three central points concerned with the drug release were comparable (Fig. 8), which, in turn, indicate that there is no pure error due to the experimental procedure. Moreover, this has enabled the determination of lack of fit of the proposed quadratic regression model. Eleven different terms were present in the model initially which were reduced only to six effective terms upon exclusion of unnecessary terms. Although values of probability for the three main effects were much larger than 0.05, they were not excluded based on the expected interaction they might reveal (Appendix-2). Best mathematical model for determination of time for 50% drug release is

\[
T_{50\%} = -26.16 + 1.51(\text{stearyl alcohol}) + 0.61(\text{tablet hardness}) + 0.07(\text{Mg stearate}) - 0.04(\text{stearyl alcohol}^2) - 0.006(\text{tablet hardness}^2).
\]

\[R^2 = 0.9238\]
Verification of the adopted model was established using the analysis of the residual plot, where values of residual obtained upon application of the mathematical model to predict $t_{50\%}$ were shown to distribute normally around zero (see Fig. 8 below).

**Fig. 8**

*Glibenclamide Release from the Three Central Formulations in the Box-Wilson Design*

**Fig. 9**

*Residuals Plot for the Mathematical Model Describing $t_{50\%}$*
Moreover, upon prediction of large values of $t_{50\%}$ using the proposed model, values of residuals showed no increase in their magnitudes (Fig. 9). Based on these facts, the proposed model is considered efficient in the prediction of $t_{50\%}$. From the mathematical methods, it appears that values of correlation coefficient, $R^2$, are high enough to consider the model effective.

Stearyl alcohol, tablet hardness, and content of Mg stearate all affect the value of $t_{50\%}$ where the relation is directly proportional. The relation between $t_{50\%}$ and the three variables seems to be non-linear based on the influence of the quadratic terms in the above equation. Instead, the correlation is of curvature type.

4.5.3. Determination of Release Kinetics of the Optimized Formulation:

General release of glibenclamide from formulation 19 (the optimized formula) in simulated gastric fluid is shown in figure 10. Data derived from such plot is subjected to analysis using the power law of Korsmeyer et al. (1983) as described before, in a search for the diffusional exponent, $n$, that characterizes the drug release mechanism.

![Glibenclamide Release Profile from the Optimized Floating Tablet Formula- tion, each data point is the average of 6 determinations, error bars show ±SEM](image)

Fig. 10
The values of the diffusional exponent, $n$, were found to be in the range of 0.8533—0.9034 (average of 0.8897, $r^2 = 0.9993$). This clearly reflects an improvement in the value of $n$ towards unity ($n=1$) in this formula compared to formulation 4, which indicates the uniformity of the drug release (zero order). This supports the findings concerned with dependency of the magnitude of $n$ on tablet hardness and drug: PVP ratio. In fact this feature is added to the encouraging characters of this formula. Formulation 19 has met all the proposed features and constraints for floating, strength and drug release and, consequently, the formulation is selected for the *in vivo* dosage form localization and drug release in healthy human volunteers.

4.6. *In vivo* Investigations:

4.6.1. Calibration Curve of Glibenclamide in Human Plasma:

The drug concentrations in spiked plasma samples were found to be linearly related with the peak area ratio of the drug to the internal standard within the investigated plasma drug concentration range (0.01-4 µg/ml) (Fig. 11). Regression coefficient of the relation ($r=0.9993$) was an evidence for the linearity and precision. Minimum concentration of glibenclamide that could be determined by this method was 0.015 µg/ml with signal/noise (S/N) ratio of three. However, quantitative detection limit was considered as 0.03 µg/ml, which is an acceptable sensitivity. Table XI shows the values of validation parameters for the HPLC method adopted for glibenclamide assay in human plasma at three different concentration levels. Values of RSD and % recovery in both intra and between days were within the limit to consider the method as efficient, sensitive and reproducible.
Fig. 11

*Calibration Curve for Determination of Glibenclamide in Human Plasma*

Table XI

*Validation of the HPLC Method for Glibenclamide Assay in Human Plasma*

<table>
<thead>
<tr>
<th>Concentration level</th>
<th>% Recovery&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RSD&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraday</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05µg/ml</td>
<td>98.8%</td>
<td>3.4</td>
</tr>
<tr>
<td>1µg/ml</td>
<td>95.7%</td>
<td>7.3</td>
</tr>
<tr>
<td>10µg/ml</td>
<td>101.2%</td>
<td>5.2</td>
</tr>
<tr>
<td><strong>Between days</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05µg/ml</td>
<td>100.4%</td>
<td>8.7</td>
</tr>
<tr>
<td>1µg/ml</td>
<td>97.5%</td>
<td>7.2</td>
</tr>
<tr>
<td>10µg/ml</td>
<td>98.3%</td>
<td>9.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Accuracy in calculation of the concentration (obtained/actual *100%)

<sup>b</sup> Coefficient of variation (standard deviation/mean value *100%)
4.6.2. Comparative Bioavailability Study:

Appendex-4 shows the individual glibenclamide plasma-time profiles among the 16 human subjects examined during the cross-over between the conventional and the developed floating tablet formulation. The average drug plasma-time profiles of the two dosage forms are shown in Figure 12. The results show that in conventional tablets the drug reaches maximum plasma level faster than that of the floating tablets (time ratio is 1: 4).

At the initial four hrs time interval, glibenclamide plasma concentrations from floating tablets were lower than those obtained with the conventional dosage form. Six hrs later, drug levels with conventional tablets start to level off in contrast to those from the floating tablets, where drug levels >0.1 µg/ml were maintained from six hrs and forth going up to 24 hrs. In fact, this might be explained in terms of three assumptions: the enhanced solubility of the drug, the reduced gastric transit time of the
drug and the sustainability of the drug release from the developed floating tablets. It is these assumptions on which the strategy of floating technology is based and such assumptions have been utilized effectively to develop a variety of floating dosage forms (Nur and Zhang, 2000; Özdemir et al., 2000).

Based on the reported minimum effective glibenclamide concentration in the blood (87.5 ng/ml, USP, 2003), the developed floating tablets were able to deliver glibenclamide and to maintain an acceptable plasma drug level over 24 hrs.

4.6.2.1. Pharmacokinetic Parameters Assessment:

The average pharmacokinetic parameters and statistical momentum of glibenclamide from both conventional and floating tablets tested in 16 subjects were summarized in Table XII. Pharmacokinetic parameters of glibenclamide associated with conventional tablets are in agreement with the relevant published data (El-Sayed et al., 1989; Coppack et al., 1990).

Although time to reach peak drug plasma concentration (T\text{max}) for the floating tablets was delayed by three fold of that for the conventional ones, such delay might not significantly affect the efficiency of the developed tablets in glibenclamide delivery (Malinowski, 1983) and even beyond T\text{max} interval, the attained glibenclamide plasma concentrations from the floating tablets were greater than the reported MEC value. This delay might be attributed to the slow drug presentation to absorption characterizing the floating tablet formulation. Nevertheless, the onset of action might not be affected by this delay specifically with this drug (Table XII).

The attained average maximum glibenclamide concentration in the plasma (C\text{max}) in both floating tablets and conventional tablets were comparable (Table XII); however, in the case of the floating tablets, glibenclamide plasma concentrations were maintained with less fluctuations over 24 hrs (Fig. 12).
### Table XII

**Average Pharmacokinetic Parameters of Glibenclamide from Conventional and Floating Tablet Formulation in 16 Healthy Human Subjects**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conventional tablets (mean ± SD)</th>
<th>Floating tablets (mean ± SD)</th>
<th>Floating/conventional ratio (%)</th>
<th>CI90% of Floating/conventional ratio (%)</th>
<th>df</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>3.21±1.25</td>
<td>9.30±1.22</td>
<td>360.71</td>
<td>230.04–491.39</td>
<td>13</td>
<td>0.003672</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>0.29±0.08</td>
<td>0.23±0.04</td>
<td>95.30</td>
<td>84.68–105.91</td>
<td>13</td>
<td>0.446747</td>
</tr>
<tr>
<td>$\ln C_{\text{max}}$</td>
<td>5.62±0.25</td>
<td>5.55±0.16</td>
<td>98.79</td>
<td>96.79–100.79</td>
<td>13</td>
<td>0.310527</td>
</tr>
<tr>
<td>AUC$_{0-24}$ (µg.hr/ml)</td>
<td>2.00±0.61</td>
<td>3.90±0.68</td>
<td>208.34</td>
<td>179.53–237.14</td>
<td>13</td>
<td>0.000016</td>
</tr>
<tr>
<td>$\ln$AUC$_{0-24}$</td>
<td>7.56±0.31</td>
<td>8.26±0.18</td>
<td>109.35</td>
<td>107.41–111.28</td>
<td>13</td>
<td>0.000001</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (µg.hr/ml)</td>
<td>2.08±0.60</td>
<td>5.01±1.26</td>
<td>254.20</td>
<td>217.22–291.18</td>
<td>13</td>
<td>0.000005</td>
</tr>
<tr>
<td>$\ln$AUC$_{0-\infty}$</td>
<td>7.60±0.30</td>
<td>8.49±0.24</td>
<td>111.88</td>
<td>122.96–137.81</td>
<td>13</td>
<td>0.000001</td>
</tr>
<tr>
<td>AUMC (µg.hr$^2$/ml)</td>
<td>17.01±3.26</td>
<td>80.24±8.39</td>
<td>471.45</td>
<td>290.56–524.33</td>
<td>13</td>
<td>0.000427</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>8.20±1.77</td>
<td>16.02±2.34</td>
<td>197.37</td>
<td>162.34–201.38</td>
<td>13</td>
<td>0.003328</td>
</tr>
<tr>
<td>K (hr$^{-1}$)</td>
<td>0.15±0.04</td>
<td>0.10±0.03</td>
<td>72.22</td>
<td>58.28–86.17</td>
<td>13</td>
<td>0.003716</td>
</tr>
<tr>
<td>$T_{\frac{1}{2}}$ (hr)</td>
<td>4.90±1.40</td>
<td>7.43±2.63</td>
<td>160.46</td>
<td>131.75–189.16</td>
<td>13</td>
<td>0.002523</td>
</tr>
</tbody>
</table>

*Data obtained after log transformation; The unit of Cmax was changed to mcg/L before transformation of AUCs & Cmax.*

**p≤0.05 considered as significance level for the difference.

Values of area under drug plasma curves AUC$_{0-24hr}$ and AUC$_{0-\infty}$ associated with the two investigated tablet formulations indicate that there are respective 2 and 2.5-fold increase in these values with floating tablet formulation as shown in Table XII. This could possibly be due to the persistence of drug absorption from floating tablets where prolonged presentation of drug in solution form at absorption site in minor quantities enhances the extent to which the drug was absorbed. This is evident from the large values of elimination half-life ($T_{\frac{1}{2}}$) and mean residence time (MRT) associated with glibenclamide from the floating tablets.
compared to conventional tablets (Table XII). Values of these two parameters are known to reflect the degree of duration tendency of the drug plasma concentration (Yamaoaka et al., 1978; Gibaldi and Perrier, 1982).

4.6.2.2. Statistical Analysis of Pharmacokinetic Parameters:
Statistical analysis showed that, except for the $C_{\text{max}}$, all parameters tested between the two tablet formulations exhibited significant differences as indicated by values of probability (p) associated with each parameter (Table XII and Appendix-5). The parameters $C_{\text{max}}$, $AUC_{0-24\text{hr}}$ and $AUC_{0-\infty}$ were transformed before statistical analysis. In addition to the probability value, confidence interval at $p=0.1$ (90% confidence) was shown to be effective in decision making with regard to the set 100±20% similarity criteria.

4.7. Floating Tablets in vivo Localization Study:

![Localization of Ingested Tablets to Human Subjects; A: 4-hrs Post Administration under Fasting Conditions, and B: 6-hrs Post Administration under Non-Fasting Conditions. The white arrows indicate position of the tablets within the stomach.](image)

Fig. 13
The *in vivo* dosage form residence time study in six human subjects demonstrated that the presence of food has a profound retarding influence on gastric emptying of the developed floating tablets. Expectantly, the presence of food delays the gastric emptying rate and hence the dosage form trapped for prolonged period (six hrs) compared to fasting conditions (3-5 hrs) as the results imply (Fig. 13a and b). Whatever the case may be, developed tablet formulation showed a capability to be retained in the stomach for at least more than 4-5 hours.

When the results of comparative bioavailability and localization studies were simultaneously considered, it was evident that both delayed gastric emptying and the sustained drug release of the developed tablet formulation have contributed to the *in vivo* drug delivery with less fluctuations over 24 hrs. In other words, neither tablet floating alone nor drug sustainability only could enhance bioavailability and *in vivo* durability of glibenclamide and to achieve these targets both factors are to be considered.

### 4.8. Stability Study and Floating Tablets Characterizations:

From Table XIII, it appears that blister packaging is effective in retaining most of the physical characters of the developed floating tablet formulations. However, with time, there was a slight increase in tablet hardness especially at elevated temperature and humidity (accelerated conditions). In fact, this increase could possibly be attributed to the initial HPMC chain relaxation caused by the excess presence of moisture (Hogan, 1989).

Evidently, from results obtained with dosage form buoyancy capability (Table XIII), the buoyancy is not affected by such variation in hardness which might be due to the small magnitude of the variation and, moreover, to the enhanced dosage form porosity as a result of polymer chain disentanglement. The results imply no difference in the mean dissolution time of the drug from the developed floating tablets as a result of change in temperature and humidity as have been set in the test conditions. Moreover, no tablets’ discoloration was detected.

For the benefits behind this stability study, study duration is to be extended for six months to one year and such study now is ongoing.
Table XIII

Physico-chemical Parameters of the Developed Floating Glibenclamide Tablets Packed in Blister Form under Accelerated and Normal Conditions and Tested for Three months

<table>
<thead>
<tr>
<th>Character</th>
<th>Accelerated conditions(^a)</th>
<th>Real-time conditions(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1month</td>
</tr>
<tr>
<td>Drug content (%w/w)</td>
<td>102</td>
<td>102</td>
</tr>
<tr>
<td>MD(^c) (hrs.)</td>
<td>7.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Tablet hardness (N)</td>
<td>40</td>
<td>44</td>
</tr>
<tr>
<td>Onset of floating (min.)</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Floating duration (hrs.)</td>
<td>5</td>
<td>3.5</td>
</tr>
<tr>
<td>Tablet discoloration</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

\(^a\) Temp of 45±5 °C and humidity of 75 %RH, \(^b\) Temp of 31±2°C and humidity of 30%RH, \(^c\) Mean dissolution time.
5. Conclusions

Development of a controlled-release buoyant oral dosage form capable to deliver glibenclamide in a manner suitable for once-day administration is achievable as far as gastrointestinal transit time and sustainability of the drug are simultaneously considered in the formulation design. Both factorial and Box-Wilson designs provide evidence for their effectiveness as tools for formulation optimization. The developed glibenclamide floating formulation showed superiority over the conventional one with regard to bioavailability and persistent blood level durability.

For maximum realistic stability of the developed dosage form, final blister pack system is to be considered.

The floating tablet formulation developed in the present study requires more time-based stability monitoring and multiple dose pharmacokinetics investigations in large populations in order to study drug accumulation, to prove safety and to assure the dose regimen.
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### Appendix-1

**Multi-Linear Regression Equations for the Different Constraints in Step ONE**

#### For Rel\% in case of range below ideal

<table>
<thead>
<tr>
<th>Observed Rel%</th>
<th>Transformed Rel%</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td>22.5</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Linear regression revealed:

\[
\text{Transformed Rel\%} = 3.333 \times \text{Observed Rel\%} - 41.6625
\]

#### For Rel\% in case of range above ideal

<table>
<thead>
<tr>
<th>Observed Rel%</th>
<th>Transformed Rel%</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.5</td>
<td>33.33</td>
</tr>
<tr>
<td>32.5</td>
<td>0</td>
</tr>
</tbody>
</table>

Linear regression revealed:

\[
\text{Transformed Rel\%} = -3.333 \times \text{Observed Rel\%} + 108.3225
\]

#### For F\text{onset} in case of range below ideal

<table>
<thead>
<tr>
<th>Observed F\text{onset}</th>
<th>Transformed F\text{onset}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Linear regression revealed:

\[
\text{Transformed F\text{onset}} = 2.222 \times \text{Observed F\text{onset}}
\]

#### For F\text{onset} in case of range above ideal

<table>
<thead>
<tr>
<th>Observed F\text{onset}</th>
<th>Transformed F\text{onset}</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>33.33</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

Linear regression revealed:

\[
\text{Transformed F\text{onset}} = -222.2 \times \text{Observed F\text{onset}} + 66.66
\]
### For n in case of range below ideal

<table>
<thead>
<tr>
<th>Observed n</th>
<th>Transformed n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Linear regression revealed:
\[
\text{Transformed } n = 166.65 \times \text{Observed } n - 133.32
\]

### For n in case of range above ideal

<table>
<thead>
<tr>
<th>Observed n</th>
<th>Transformed n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.33</td>
</tr>
<tr>
<td>1.2</td>
<td>0</td>
</tr>
</tbody>
</table>

Linear regression revealed:
\[
\text{Transformed } n = -166.66 \times \text{Observed } n + 199.98
\]
**Multi-Linear Regression Equations for the Different Constraints in Step TWO:**

Time for 50% drug release ($T_{50\%} = 2—4$ hrs; average 3hrs)
Swelling % at six hrs interval ( $S_{\%} = 300—500$%; average 400%)
% drug released after six hrs ($\text{Rel}_{-%6} = 80—100$%; average 90%)

### For $T_{50\%}$ in case of range below ideal

<table>
<thead>
<tr>
<th>Observed $T_{50%}$</th>
<th>Transformed $T_{50%}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Linear regression revealed:
Transformed $T_{50\%} = 33.33 \times \text{Observed } T_{50\%} - 66.66$

### For $T_{50\%}$ in case of range above ideal

<table>
<thead>
<tr>
<th>Observed $T_{50%}$</th>
<th>Transformed $T_{50%}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>33.33</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Linear regression revealed:
Transformed $T_{50\%} = -33.33 \times \text{Observed } T_{50\%} + 133.32$

### For $S_{\%}$ in case of range below ideal

<table>
<thead>
<tr>
<th>Observed $S_{%}$</th>
<th>Transformed $S_{%}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>400</td>
<td>33.33</td>
</tr>
</tbody>
</table>

Linear regression revealed:
Transformed $S_{\%} = 0.3333 \times \text{Observed } S_{\%} - 99.99$

### For $S_{\%}$ in case of range above ideal

<table>
<thead>
<tr>
<th>Observed $S_{%}$</th>
<th>Transformed $S_{%}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>33.33</td>
</tr>
<tr>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

Linear regression revealed:
Transformed $S_{\%} = -0.3333 \times \text{Observed } S_{\%} + 166.65$

### For $\text{Rel}_{-%6}$ in case of range below ideal

<table>
<thead>
<tr>
<th>Observed $\text{Rel}_{-%6}$</th>
<th>Transformed $\text{Rel}_{-%6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0</td>
</tr>
</tbody>
</table>
Linear regression revealed:
Transformed $\%_\text{6} = 33.33 \times \text{Observed } \%_\text{6} - 266.64$

_For $\%_\text{6}$ in case of range above ideal_

<table>
<thead>
<tr>
<th>Observed $%_\text{6}$</th>
<th>Transformed $%_\text{6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>33.33</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Linear regression revealed:
Transformed $\%_\text{6} = -33.33 \times \text{Observed } \%_\text{6} + 333.3$
Appendix-2

Step Regression for t_{50\%} Model

Call: lm(formula = t50 ~ X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X2:X3:X1 + X2^2 + X3^2 + X1^2, data = DS25, na.action = na.exclude)

Residuals:

<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>-0.6478</td>
<td>-0.3032</td>
<td>-0.06056</td>
<td>0.1958</td>
<td>0.8848</td>
</tr>
</tbody>
</table>

Coefficients:

|    | Value  | Std. Error | t value | Pr(>|t|) |
|----|--------|------------|---------|----------|
| (Intercept) | -20.6975 | 16.4840 | -1.2556 | 0.2559  |
| X1   | 1.2431  | 0.9063   | 1.3716  | 0.2193  |
| X2   | 0.5101  | 0.3577   | 1.4262  | 0.2037  |
| X3   | -1.9131 | 4.2880   | -0.4461 | 0.6711  |
| I(X2^2) | -0.0050 | 0.0022   | -2.2155 | 0.0486  |
| I(X3^2) | 0.1156  | 0.0929   | 1.2445  | 0.2597  |
| I(X1^2) | -0.0348 | 0.0139   | -2.5057 | 0.0462  |
| X1:X2 | 0.0017  | 0.0146   | 0.1139  | 0.9130  |
| X1:X3 | 0.0756  | 0.2224   | 0.3397  | 0.7457  |
| X2:X3 | 0.0267  | 0.0834   | 0.3197  | 0.7600  |
| X2:X3:X1 | -0.0016 | 0.0044 | -0.3566 | 0.7336 |

Residual standard error: 0.694 on 6 degrees of freedom.
Multiple R-Squared: 0.9478, F-statistic: 1.984 on 10 and 6 degrees of freedom, the p-value is 0.2076
**Analysis of Variance Table**  
**Response: t50**  
Terms added sequentially (first to last)

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum of Sq</th>
<th>Mean Sq</th>
<th>F Value</th>
<th>Pr(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>1</td>
<td>0.132795</td>
<td>0.132795</td>
<td>0.275724</td>
</tr>
<tr>
<td>X2</td>
<td>1</td>
<td>1.982470</td>
<td>1.982470</td>
<td>4.116224</td>
</tr>
<tr>
<td>X3</td>
<td>1</td>
<td>0.158689</td>
<td>0.158689</td>
<td>0.329487</td>
</tr>
<tr>
<td>I(X2^2)</td>
<td>1</td>
<td>2.013805</td>
<td>2.013805</td>
<td>4.181287</td>
</tr>
<tr>
<td>I(X3^2)</td>
<td>1</td>
<td>2.068382</td>
<td>2.068382</td>
<td>4.294604</td>
</tr>
<tr>
<td>I(X1^2)</td>
<td>1</td>
<td>3.023826</td>
<td>3.023826</td>
<td>6.278403</td>
</tr>
<tr>
<td>X1:X2</td>
<td>1</td>
<td>0.101250</td>
<td>0.101250</td>
<td>0.210227</td>
</tr>
<tr>
<td>X1:X3</td>
<td>1</td>
<td>0.001250</td>
<td>0.001250</td>
<td>0.002595</td>
</tr>
<tr>
<td>X2:X3</td>
<td>1</td>
<td>0.011250</td>
<td>0.011250</td>
<td>0.023359</td>
</tr>
<tr>
<td>X2:X3:X1</td>
<td>1</td>
<td>0.061250</td>
<td>0.061250</td>
<td>0.127174</td>
</tr>
<tr>
<td>Residuals</td>
<td>6</td>
<td>2.889740</td>
<td>0.481623</td>
<td></td>
</tr>
</tbody>
</table>

**After elimination of unnecessary terms**

Call: lm(formula = t50 ~ X1 + X2 + X3 + X1^2 + X2^2, data = DS25, na.action = na.exclude)

Residuals:

<table>
<thead>
<tr>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.6089</td>
<td>-0.3859</td>
<td>-0.1925</td>
<td>0.1686</td>
<td>1.034</td>
</tr>
</tbody>
</table>

Coefficients:

|          | Value   | Std. Error | t value | Pr(>|t|) |
|----------|---------|------------|---------|---------|
| (Intercept) | -26.1582 | 6.5176     | -4.0135 | 0.0020  |
| X1       | 1.5092  | 0.4206     | 3.5881  | 0.0043  |
| X2       | 0.6105  | 0.1829     | 3.3379  | 0.0066  |
| X3       | 0.0721  | 0.1066     | 0.6768  | 0.5125  |
| I(X1^2)  | -0.0398 | 0.0112     | -3.5420 | 0.0046  |
| I(X2^2)  | -0.0057 | 0.0018     | -3.1378 | 0.0094  |

Residual standard error: 0.5886 on 11 degrees of freedom

Multiple R-Squared: 0.9238. F-statistic: 4.985 on 5 and 11 degrees of freedom, the p-value is 0.01253
## Analysis of Variance Table

**Response: t50**

*Terms added sequentially (first to last)*

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum of Sq</th>
<th>Mean Sq</th>
<th>F Value</th>
<th>Pr(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>1</td>
<td>0.132795</td>
<td>0.132795</td>
<td>0.383327</td>
<td>0.5484360</td>
</tr>
<tr>
<td>X2</td>
<td>1</td>
<td>1.982470</td>
<td>1.982470</td>
<td>5.722614</td>
<td>0.0357222</td>
</tr>
<tr>
<td>X3</td>
<td>1</td>
<td>0.158689</td>
<td>0.158689</td>
<td>0.458072</td>
<td>0.5125078</td>
</tr>
<tr>
<td>I(X1^2)</td>
<td>1</td>
<td>2.949283</td>
<td>2.949283</td>
<td>8.513426</td>
<td>0.0139930</td>
</tr>
<tr>
<td>I(X2^2)</td>
<td>1</td>
<td>3.410770</td>
<td>3.410770</td>
<td>9.845558</td>
<td>0.0094460</td>
</tr>
</tbody>
</table>
Appendix-3:
HPLC chromatogram for human plasma sample spiked with 100µl of 10µg/ml solution of glibenclamide ($R_t=6.78$) and the internal standard Ibuprofen (1mg/ml, $R_t=5.70$).
Appendix-4:

Statistical analysis of glibenclamide pharmacokinetic parameters using paired t test at confidence level of 90% (p=0.1)

**Variable: Ratio of \( T_{\text{max}} \) of the two formulations in 14 tested subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test of means against reference constant (value) (Spreadsheet2 in Workbook2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Var1</td>
<td>360.7143</td>
</tr>
</tbody>
</table>

**Variable: Ratio of \( C_{\text{max}} \) (Ln transformed) of the two formulations**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test of means against reference constant (value) (Spreadsheet2 in Workbook2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Var1</td>
<td>98.7878</td>
</tr>
</tbody>
</table>

**Variable: Ratio of \( \text{AUC}_{0-24} \) of the two formulations**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test of means against reference constant (value) (Spreadsheet2 in Workbook2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Var1</td>
<td>208.3371</td>
</tr>
</tbody>
</table>

**Variable: Ratio of \( \text{AUC}_{0-\infty} \) of the two formulations**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test of means against reference constant (value) (Spreadsheet2 in Workbook2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Var1</td>
<td>254.2023</td>
</tr>
</tbody>
</table>

**Variable: Ratio of \( \text{AUC}_{0-24} \) (Ln transformed) of the two formulations**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test of means against reference constant (value) (Spreadsheet2 in Workbook2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Var1</td>
<td>109.3455</td>
</tr>
</tbody>
</table>
### Variable: Ratio of $AUC_{0-\infty}$ (Ln transformed) of the two formulations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std.Dv.</th>
<th>N</th>
<th>Std.Err.</th>
<th>Confidence</th>
<th>Confidence</th>
<th>Reference</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Var1</td>
<td>111.8843</td>
<td>4.064357</td>
<td>14</td>
<td>1.086245</td>
<td>109.9606</td>
<td>113.8079</td>
<td>100.0000</td>
<td>10.94069</td>
<td>13</td>
<td>0.000000</td>
</tr>
</tbody>
</table>

**For $t_{1/2}$**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std.Dv.</th>
<th>N</th>
<th>Std.Err.</th>
<th>Confidence</th>
<th>Confidence</th>
<th>Reference</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NewVar</td>
<td>160.4570</td>
<td>60.65117</td>
<td>14</td>
<td>16.20971</td>
<td>131.7507</td>
<td>189.1634</td>
<td>100.0000</td>
<td>3.729681</td>
<td>13</td>
<td>0.002523</td>
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</tbody>
</table>

**For $AUC_{0-\infty}$**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std.Dv.</th>
<th>N</th>
<th>Std.Err.</th>
<th>Confidence</th>
<th>Confidence</th>
<th>Reference</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Var1</td>
<td>254.2023</td>
<td>78.12783</td>
<td>14</td>
<td>20.88054</td>
<td>217.2243</td>
<td>291.1804</td>
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<td>7.384978</td>
<td>13</td>
<td>0.000005</td>
</tr>
</tbody>
</table>

**For $AUC_{0-24}$**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std.Dv.</th>
<th>N</th>
<th>Std.Err.</th>
<th>Confidence</th>
<th>Confidence</th>
<th>Reference</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Var1</td>
<td>208.3371</td>
<td>60.85541</td>
<td>14</td>
<td>16.26429</td>
<td>179.5341</td>
<td>237.1401</td>
<td>100.0000</td>
<td>6.661041</td>
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<td>0.000016</td>
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**For $C_{max}$**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std.Dv.</th>
<th>N</th>
<th>Std.Err.</th>
<th>Confidence</th>
<th>Confidence</th>
<th>Reference</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Var1</td>
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<td>14</td>
<td>5.995957</td>
<td>84.67710</td>
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<td>100.0000</td>
<td>-0.784606</td>
<td>13</td>
<td>0.446747</td>
</tr>
</tbody>
</table>

**For $K$**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std.Dv.</th>
<th>N</th>
<th>Std.Err.</th>
<th>Confidence</th>
<th>Confidence</th>
<th>Reference</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Var1</td>
<td>72.22837</td>
<td>29.46128</td>
<td>14</td>
<td>7.873588</td>
<td>58.28429</td>
<td>86.17245</td>
<td>100.0000</td>
<td>-3.52707</td>
<td>13</td>
<td>0.003716</td>
</tr>
</tbody>
</table>