Abstract:

Diabetes is a disorder of metabolism, the way the body uses digested food for growth and energy. The solubility of glibenclamide (GLB), metformin and acarbose were determined in pH 1.2, pH 5.5 and pH 6.8 in triplicate using the shake-flask method using water bath shaker at 37°C. In vitro dissolution release showed that GLB showed higher release in the duodenum and small intestine (pH5.5 and pH6.8) than acidic stomach pH (pH1.2) due to the weak acid nature of the drug and this the opposite for metformin and acarbose because of high pKₐ value for these drugs.

Keywords: Acarbose, Glibenclamide, Metformin, Diabetes, PH-solubility profile.

1. Introduction

Almost everyone knows someone who has diabetes. An estimated 23.6 million people in the United States—7.8 percent of the population—have diabetes, a serious, lifelong condition. Of those, 17.9 million have been diagnosed, and 5.7 million have not yet been diagnosed. In 2007, about 1.6 million people ages 20 or older were diagnosed with diabetes. (1)

1.1 What is diabetes?

Diabetes is a disorder of metabolism—the way the body uses digested food for growth and energy. Most of the food people eat is broken down into glucose, the form of sugar in the blood. Glucose is the main source of fuel for the body. After digestion, glucose passes into the bloodstream, where it is used by cells for growth and energy. For glucose to get into cells, insulin must be present. Insulin is a hormone produced by the pancreas, a large gland behind the stomach.
When people eat, the pancreas automatically produces the right amount of insulin to move glucose from blood into the cells. In people with diabetes, however, the pancreas either produces little or no insulin, or the cells do not respond appropriately to the insulin that is produced. Glucose builds up in the blood, overflows into the urine, and passes out of the body in the urine. Thus, the body loses its main source of fuel even though the blood contains large amounts of glucose.

1.2 What are the types of diabetes?

- Type 1 diabetes
- Type 2 diabetes
- Gestational diabetes

1.2.1. Type 1 Diabetes

Type 1 diabetes is an autoimmune disease. An autoimmune disease results when the body’s system fighting infection—the immune system—turns against a part of the body. In diabetes, the immune system attacks and destroys the insulin-producing beta cells in the pancreas. The pancreas then produces little or no insulin. A person who has type 1 diabetes must take insulin daily to live.

At present, scientists do not know exactly what causes the body’s immune system to attack the beta cells, but they believe that autoimmune, genetic, and environmental factors, possibly viruses, are involved. Type 1 diabetes accounts for about 5 to 10 percent of diagnosed diabetes in the United States. It develops most often in children and young adults but can appear at any age.

Symptoms of type 1 diabetes usually develop over a short period, although beta cell destruction can begin years earlier. Symptoms may include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue. If not diagnosed and treated with insulin, a person with type 1 diabetes can lapse into a life-threatening diabetic coma, also known as diabetic ketoacidosis. 

1.2.2. Type 2 Diabetes

The most common form of diabetes is type 2 diabetes. About 90 to 95 percent of people with diabetes have type 2. This form of diabetes is most often associated with older age, obesity, family history of diabetes, previous history
of gestational diabetes, physical inactivity, and certain ethnicities. About 80 percent of people with type 2 diabetes are overweight or obese.

Type 2 diabetes is increasingly being diagnosed in children and adolescents, especially among African American, Mexican American, and Pacific Islander youth.

When type 2 diabetes is diagnosed, the pancreas is usually producing enough insulin, but for unknown reasons the body cannot use the insulin effectively, a condition called insulin resistance. After several years, insulin production decreases. The result is the same as for type 1 diabetes—glucose builds up in the blood and the body cannot make efficient use of its main source of fuel.

The symptoms of type 2 diabetes develop gradually. Their onset is not as sudden as in type 1 diabetes. Symptoms may include fatigue, frequent urination, increased thirst and hunger, weight loss, blurred vision, and slow healing of wounds or sores. Some people have no symptoms.

1.2.3. Gestational Diabetes

Some women develop gestational diabetes late in pregnancy. Although this form of diabetes usually disappears after the birth of the baby, women who have had gestational diabetes have a 40 to 60 percent chance of developing type 2 diabetes within 5 to 10 years. Maintaining a reasonable body weight and being physically active may help prevent development of type 2 diabetes.

About 3 to 8 percent of pregnant women in the United States develop gestational diabetes. As with type 2 diabetes, gestational diabetes occurs more often in some ethnic groups and among women with a family history of diabetes. Gestational diabetes is caused by the hormones of pregnancy or a shortage of insulin. Women with gestational diabetes may not experience any symptoms.\(^{(1)}\)

1.3. How is diabetes diagnosed?

The fasting blood glucose test is the preferred test for diagnosing diabetes in children and non-pregnant women. The test is most reliable when done in the morning. However, a diagnosis of diabetes can be made based on any of the following test results, confirmed by retesting on a different day:

1- A blood glucose level of 126 milligrams per deciliter (mg/dL) or higher after an 8-hour fast. This test is called the fasting blood glucose test.
2-A blood glucose level of 200 mg/ml or higher 2 hours after drinking a beverage containing 75 grams of glucose dissolved in water. This test is called the oral glucose tolerance test (OGTT).

3-A random—taken at any time of day—blood glucose level of 200 mg/ml or higher, along with the presence of diabetes symptoms.

Gestational diabetes is diagnosed based on blood glucose levels measured during the OGTT. Glucose levels are normally lower during pregnancy, so the cutoff levels for diagnosis of diabetes in pregnancy are lower. Blood glucose levels are measured before a woman drinks a beverage containing glucose. Then levels are checked 1, 2, and 3 hours afterward.

If a woman has two blood glucose levels meeting or exceeding any of the following numbers, she has gestational diabetes: a fasting blood glucose level of 95 mg/ml, a 1-hour level of 180 mg/ml, a 2-hour level of 155 mg/ml, or a 3-hour level of 140 mg/ml.

1.4. Treatment of diabetes

Currently, one goal for diabetics is to avoid or minimize chronic diabetic complications, as well as to avoid acute problems of hyperglycemia or hypoglycemia. Adequate control of diabetes leads to lower risk of complications associated with unmonitored diabetes including kidney failure (requiring dialysis or transplant), blindness, heart disease and limb amputation. The most prevalent form of medication is hypoglycemic treatment through either oral hypoglycemics and/or insulin therapy. There is emerging evidence that full-blown diabetes mellitus type 2 can be evaded in those with only mildly impaired glucose tolerance.\(^{(2)}\)

Patients with type 1 diabetes mellitus require direct injection of insulin as their bodies cannot produce enough (or even any) insulin. As of 2010, there is no other clinically available form of insulin administration other than injection for patients with type 1: injection can be done by insulin pump, by jet injector, or any of several forms of hypodermic needle. Non-injective methods of insulin administration have been unattainable as the insulin protein breaks down in the digestive tract. There are several insulin application mechanisms under experimental development as of 2004, including a capsule that passes to the liver and delivers insulin into the bloodstream. There have also been proposed vaccines for type I using glutamic acid decarboxylase (GAD), but these are currently not being tested by the pharmaceutical companies that have sublicensed the patents to them.\(^{(2)}\)
For type 2 diabetics, diabetic management consists of a combination of diet, exercise, and weight loss, in any achievable combination depending on the patient. Obesity is very common in type 2 diabetes and contributes greatly to insulin resistance. Weight reduction and exercise improve tissue sensitivity to insulin and allow its proper use by target tissues. Patients that have poor diabetic control after lifestyle modifications are typically placed on oral hypoglycemics. Some Type 2 diabetics eventually fail to respond to these and must proceed to insulin therapy. A study conducted in 2008 found that increasingly complex and costly diabetes treatments are being applied to an increasing population with type 2 diabetes. Data from 1994 to 2007 was analyzed and it was found that the mean number of diabetes medications per treated patient increased from 1.14 in 1994 to 1.63 in 2007.

Patient education and compliance with treatment is very important in managing the disease. Improper use of medications and insulin can be very dangerous causing hypo- or hyper-glycemic episodes.\(^{(3)}\)

1.4.1. Insulin

Insulin therapy requires close monitoring and a great deal of patient education, as improper administration is quite dangerous.

For example, when food intake is reduced, less insulin is required. A previously satisfactory dosing may be too much if less food is consumed causing a hypoglycemic reaction if not intelligently adjusted. Exercise decreases insulin requirements as exercise increases glucose uptake by body cells whose glucose uptake is controlled by insulin, and vice versa. In addition, there are several types of insulin with varying times of onset and duration of action.

Insulin therapy creates risk because of the inability to continuously know a person's blood glucose level and adjust insulin infusion appropriately. New advances in technology have overcome much of this problem. Small, portable insulin infusion pumps are available from several manufacturers. They allow a continuous infusion of small amounts of insulin to be delivered through the skin around the clock, plus the ability to give bolus doses when a person eats or has elevated blood glucose levels. This is very similar to how the pancreas works, but these pumps lack a continuous “feed-back” mechanism. Thus, the user is still at risk of giving too much or too little insulin unless blood glucose measurements are made.
A further danger of insulin treatment is that while diabetic microangiopathy is usually explained as the result of hyperglycemia, studies in rats indicate that the higher than normal level of insulin diabetics inject to control their hyperglycemia may itself promote small blood vessel disease.\(^{(4)}\)

While there is no clear evidence that controlling hyperglycemia reduces diabetic macrovascular and cardiovascular disease, there are indications that intensive efforts to normalize blood glucose levels may worsen cardiovascular and cause diabetic mortality.\(^{(5)}\)

1.4.2. Oral hypoglycemic agents

Table (1-1) compares some common anti-diabetic agents, generalizing classes, although there may be substantial variation in individual drugs of each class. When the table makes a comparison such as "lower risk" or "more convenient" the comparison is with the other drugs on the table.\(^{(6)}\)

Table 1-1. Comparison of anti-diabetic medications.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-glucosidase</td>
<td>Reduces glucose absorbance by acting on small intestine to cause decrease in production of enzymes needed to digest carbohydrates</td>
<td>• slightly decreased risk of hypoglycemia as compared to sulfonylurea</td>
<td>• less effective than most other diabetes pills in decreasing glycated hemoglobin</td>
</tr>
<tr>
<td>inhibitor (acarbose,</td>
<td></td>
<td>• not associated with weight gain</td>
<td>• increased risk of GI problems than other diabetes pills except metformin</td>
</tr>
<tr>
<td>miglitol, voglibose)</td>
<td></td>
<td>• decreases triglycerides</td>
<td>• inconvenient dosing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• no effect on cholesterol</td>
<td>• expensive</td>
</tr>
</tbody>
</table>
| Metformin | Acts on liver to cause decrease in insulin resistance |  • not associated with weight gain  
• low risk of hypoglycemia as compared to alternatives  
• Good effect on LDL cholesterol  
• Decreases triglycerides  
• no effect on blood pressure  
• inexpensive |  • increased risk of gastrointestinal problems  
• Contraindicated for people with moderate or severe kidney disease or heart failure because of risk of lactic acidosis  
• increased risk of Vitamin B12 deficiency[^3]  
• less convenient dosing  
• Metallic taste |
|---|---|---|
| Sulfonylurea (glyburide, glimepiride, glipizide) |  • Inexpensive  
• Fast onset of action  
• No effect on blood pressure  
• No effect on low-density lipoprotein  
• lower risk of gastrointestinal problems than with metformin  
• more convenient dosing |  • causes an average of 5–10 pounds weight gain  
• Increased risk of hypoglycemia  
• Glyburide has increases risk of hypoglycemia slightly more as compared with glimepiride and glipizide  
• Higher risk of death compared with metformin[^4] |

| Thiazolidinediones (Pioglitazone, Rosiglitazone) | Reduce insulin resistance by activating PPAR-γ in fat and muscle | • Lower risk of hypoglycemia  
• Slight increase in high-density lipoprotein  
• Actos linked to decreased triglycerides  
• Convenient dosing | • increased risk of heart failure  
• causes an average of 5–10 pounds weight gain  
• associated with higher risk of edema  
• associated with higher risk of anemia  
• increases low-density lipoprotein  
• Avandia linked to increased triglycerides and risk of heart attack  
• Actos linked to increased risk of bladder cancer  
• slower onset of action  
• requires monitoring for hepatotoxicity |

### 1.5 Properties and action of some oral hypoglycemic agents

#### 1.5.1. Glibenclamide (GLB)

Chemically Glibenclamide is 5-chloro-n-[2[4][[(cyclohexylamino)carbonyl]-amino]sulphonyl[phenyl]-ethyl]-2-methoxy benzamide is oral hypoglycaemic drug sulphonyl ureas – second generation) act by inhibiting ATP-sensitive potassium channels in pancreatic beta cells. This inhibition
causes cell membrane depolarisation, which causes voltage dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release.

Glibenclamide is an oral hypoglycemic agent of the sulphonylurea group used in the treatment of noninsulin dependent diabetes. It has a history of low bioavailability, which is attributed to its poor dissolution properties.\(^8\) Glibenclamide lowers high blood glucose by increasing the amount of insulin released by your pancreas.

Glibenclamide belongs to a group of medicines called sulphonylureas.

If your blood glucose is not properly controlled, you may experience hypoglycaemia (low blood glucose) or hyperglycaemia (high blood glucose). High blood glucose can lead to serious problems with your heart, eyes, circulation or kidneys.\(^9\)

Low blood glucose can occur suddenly. Signs may include: \(^9\)

1-Weakness, trembling, shaking
2-Sweating
3-Light headedness, dizziness, headache or lack of concentration
4-Tearfulness, crying or depression
5-Irritability
6-Hunger

High blood glucose usually occurs more slowly than low blood glucose. Signs of high blood glucose may include:

1-Lethargy or tiredness
2-Headache
3-Severe thirst
4-Passing large amounts of urine and more often
5-Blurred vision
6-Dry mouth or dry skin

Glibenclamide is a weak acid (pKa = 5.3) practically insoluble in water and acidic environment but highly permeable (class 2) according to the Biopharmaceutical Classification System (BCS). The oral absorption is
uniform, rapid and complete with nearly 100% bioavailability. Therapy with Glibenclamide is usually initiated with 2.5mg given once daily. The maximal recommended daily dose is 20mg.\(^{(10)}\)

1.5.2 Metformin HCL

Metformin hydrochloride (MH), an antidiabetic drug, lowers both basal- and postprandial-elevated blood glucose in patients with non-insulin-dependent diabetes mellitus (NIDDM or type 2 diabetes) whose hyperglycemia cannot be satisfactorily managed by diet alone. Some high incidence of concomitant GI symptoms, such as abdominal discomfort, nausea, and diarrhea, may occur during the treatment. Gastrointestinal absorption of metformin is incomplete with an absolute bioavailability of 40–60% (under fasting conditions) in combination with rapid elimination and 20–30% of an oral dose is recovered in feces. It decreases as the dose increases, suggesting some form of saturable absorption or permeability/transit time-limited absorption and the negligible hepatic metabolism of metformin happened in humans. Side effects and the need for twice to three times a day administration when larger doses are required can also reduce patient compliance and hinder more successful therapy. Administration of a sustained-release, once-a-day MH dosage form could reduce the dosing frequency and improve patient compliance.\(^{(11)}\)

Metformin HCl is an anti-hyperglycemic drug used to manage type-2 diabetes. It is a unique and widely used anti-hyper glycemic drug throughout the world. It is not chemically or pharmacologically related to the other classes of oral anti-hyper glycemic agents. Inhibition of glyconeogenesis appears to be an important component of the drug’s activity. The IUPAC name for Metformin HCl is 1,1—Dimethyl biguanide hydrochloride with molecular formula, C4H11N5.HCl.\(^{(13)}\)

1.5.3. Acarbose

Acarbose is produced by certain strains of Actinoplanes utahensis. Acarbose contains not less than 95.0 % and not more than 102.0 % of acarbose (C25H43NO18), calculated on the anhydrous basis.\(^{(14)}\)

Acarbose, an a-glycosidase inhibitor, treats diabetes mellitus by delaying the digestion and intestinal absorption of dietary carbohydrates. In effective doses, acarbose induces some passage of carbohydrates into the colon. The effect of such chronic carbohydrate transfer on colonic structure and function is unknown. We studied the effects of 1 year of acarbose administration in diabetes mellitus on fecal energy, protein, and fat, including short-chain
fatty acids (SCFA) output, fecal pH, and several metabolizing bacterial species. Changes in colonic histology and epithelial cell proliferation were investigated in rectal biopsies. Fecal macronutrient output was unaffected by acarbose, but pH decreased and total SCFA, butyrate, and acetate output were markedly greater. Breath hydrogen output increased after acarbose, but digoxin-metabolizing bacteria and diacylglycerol (DAG) production were unaltered. Compared with the control, acarbose did not induce hyperplasia or change rectal proliferation. However, total fecal SCFA and butyrate output correlated inversely with proliferation in the rectal upper crypt—a biomarker of risk for colonic neoplasia. In conclusion, long-term acarbose administration does not adversely affect colonic function or fecal nutrient output. If increased fecal SCFA and butyrate reduces upper-crypt proliferation, then acarbose may reduce the risk of colonic neoplasia.\(^{(15)}\)

Acarbose inhibits enzymes (glycoside hydrolases) needed to digest carbohydrates, specifically, alpha-glucosidase enzymes in the brush border of the small intestines and pancreatic alpha-amylase. Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine, whereas the membrane-bound intestinal alpha-glucosidases hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of complex carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effect of these drugs therapies is to decrease current blood glucose levels; the long-term effect is a reduction in HbA1c level. This reduction averages an absolute decrease of 0.7%, which is a decrease of about 10% in typical HbA1c values in diabetes studies.\(^{(16)}\)

In the small intestine, starch is digested to oligosaccharides by amylase, and further digested by membrane-bound alpha-glucosidases (glucoamylase, dextrinase isomaltase, maltase, sucrase) to glucose. The alpha-glucosidase inhibitors competitively bind to the oligosaccharide binding site of the alpha-glucosidases, which prevents the binding and enzymatic hydrolysis of the oligosaccharide substrate(Figure 1-5 ). In this way, alpha-glucosidase inhibition represents a pharmacologic approach for modifying the digestion and absorption of dietary carbohydrates as an adjunct to dietary changes. Owing to their competitive mechanism of action, alpha-glucosidase inhibitors need to be taken at the start of a meal.(17)
2. Materials and methods

2.1 Materials

Table 2-1. List of Materials.

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamide 5mg (Daonil®)</td>
<td>Sanofi Aventis, France</td>
</tr>
<tr>
<td>Metformin HCL 500mg (Glucophage®)</td>
<td>Merck, Germany</td>
</tr>
<tr>
<td>Acarbose 50mg (Prandase®)</td>
<td>Schering Pharma AG, Germany</td>
</tr>
<tr>
<td>Disodium hydrogen orthophosphate anhydrous</td>
<td>Sd-Fine-Chem limited, Mumbai, India</td>
</tr>
<tr>
<td>Potassium dihydrogen orthophosphate anhydrous</td>
<td>Thomas Baker, Mumbai, India</td>
</tr>
<tr>
<td>0.1N HCL</td>
<td>GCC, UK</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>GCC, UK</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>Himedia, India</td>
</tr>
<tr>
<td>KMNO₄</td>
<td>Merck, Germany</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>BDH, UK</td>
</tr>
</tbody>
</table>

Table 2-2. List of Instruments

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolution apparatus type -II</td>
<td>Copley dissolution tester DIS 8000, UK</td>
</tr>
<tr>
<td>UV-VisibleSpectrophotometer</td>
<td>Carry UV, Varian, Australia</td>
</tr>
<tr>
<td>Electronic balance</td>
<td>A &amp; D company Ltd. Tokyo, Japan</td>
</tr>
<tr>
<td>Water bath shaker</td>
<td>Memmert, England</td>
</tr>
</tbody>
</table>

2.2 Methods

2.2.1. Determination of λmax and calibration curves

2.2.1A. Glibenclamide(GLB)

The prepared solution of 100μg/ml Glibenclamide stock solution in 3 pHs (pH 1.2, pH 5.5 and 6.8) were scanned by a UV spectrophotometer at wave lengths ranging from 200-400nm and the λmax of the drug was determined.
Standard curves of GLB in 0.1N HCL (pH 1.2), pH 5.5 and 6.8 was obtained by preparing different concentrations of GLB from stock solution, (10µg/ml), (20µg/ml), (40µg/ml) and (80µg/ml). The prepared samples were analysed spectrophotometrically at $\lambda_{\text{max}}$ 229 nm. The measured absorbance of each sample was plotted versus concentration.

2.2.1. B. Metformin

The prepared solution of 100µg/ml metformin stock solution in 3 pHs (pH 1.2, pH 5.5 and 6.8) were scanned by a UV spectrophotometer at wave length ranging from 200-400 nm and the $\lambda_{\text{max}}$ of the drug was determined. Standard curves of GLB in 0.1N HCL (pH 1.2), pH 5.5 and 6.8 was obtained by preparing different concentrations of metformin from stock solution, (10µg/ml), (20µg/ml), (40µg/ml) and (80µg/ml). The prepared samples were analysed spectrophotometrically at $\lambda_{\text{max}}$ 237 nm. The measured absorbance of each sample was plotted versus concentration.

2.2.1. C. Acarbose

The prepared solution of 50µg/ml metformin stock solution in 3 pHs (pH 1.2, pH 5.5 and 6.8) were scanned by a UV spectrophotometer at wave length ranging from 300-700 nm and the $\lambda_{\text{max}}$ of the drug was determined. Calibration curve was established by using alkaline potassium permanganate as an oxidizing agent. The method involves determination of acarbose by kinetic studies of their oxidation at room temperature for a fixed time of 15 minutes. The absorbance of the colored manganate ion was measured at 610 nm. The absorbance concentration plot was rectilinear over the concentration range (4 µg/ml, 5 µg/ml, 10 µg/ml and 15 µg/ml) for Acarbose. The method was successfully applied for the determination of drug in dosage forms. The results obtained were in good agreement with those obtained with the reference methods.

2.2.2. Determination of PH-solubility profile of selected drugs

The solubility of GLB, metformin and acarbose were determined in pH 1.2, pH 5.5 and pH 6.8 in triplicate using the shake-flask method using water bath shaker at 37°C.

An excess amount of drug was added to 10ml of the buffer in small glass vial in order to get a complete saturated solution. Phosphate buffer 1.2, pH 5.5 and pH 6.8 flasks were sealed and shaken at 37°C in a shaker for 48 hr. Samples withdrawn, filtered and then the solubility determined by UV scan at specified $\lambda_{\text{max}}$ for each drug.
2.2.3. **In-vitro drug release**

In-vitro release of each drug from formulation was performed using USP drug dissolution apparatus II (paddle type).

Tablet for each drug put in dissolution flask and 900ml volume of dissolution media was added and was used for 3 pH conditions. (pH 1.2, pH 5.5 and pH 6.8)

10ml Samples for each dissolution procedure were withdrawn every 10 minutes and replaced by the same amount of fresh buffer (1.2 or 5.5 or 6.8 according to the type of buffer), to maintain sink condition.

### 3. Results and discussion

#### 3.1. Characterization of drugs

##### 3.1.1. Determination of $\lambda_{\text{max}}$ of drugs

**3.1.1.A. Glibenclamide**

UV scans for solution contain 100µg/ml of GLB in pH 1.2, pH 5.5 and pH 6.8 by UV spectrophotometer at 200-400nm gave the same peak as shown in figure (3-1) with a $\lambda_{\text{max}}$ at 229nm for all pH values. The result is in agreement with the reported one.\(^{(19)}\)

**3.1.1.B. Metformin**

UV scans for solution contain 100µg/ml of GLB in pH 1.2, pH 5.5 and pH 6.8 by UV spectrophotometer at 200-400nm gave the same peak as shown in figure (3-2) with a $\lambda_{\text{max}}$ at 237nm for all pH values. The result is in agreement with the reported one.\(^{(20)}\)

**3.1.1.C. Acarbose**

UV scans for solution contain 100µg/ml of GLB in pH 1.2, pH 5.5 and pH 6.8 by UV spectrophotometer at 300-700nm gave the same peak as shown in figure (3-3) with a $\lambda_{\text{max}}$ at 610nm for all pH values. The result is in agreement with the reported one.\(^{(19)}\)

#### 3.1.2. UV calibration curves

##### 3.1.2.1. UV calibration curves for GLB

Figures (3-4), (3-5) and (3-6) showed the calibration curves of GLB insolutions of 0.1N HCL (pH 1.2), pH 5.5 and pH 6.8. A straight line was obtained by plotting the absorbance versus concentration with high regression.
coefficient. This indicates that calibration curve obeys Beer-lambert's law at $\lambda_{\text{max}}$ 229 nm within the range of concentrations used.

3.1.2.2. UV calibration curves for Metformin

Figures (3-7),(3-8) and (3-9) showed the calibration curves of metformin in solutions of 0.1N HCL (pH 1.2),pH 5.5 and pH 6.8.A straight line was obtained by plotting the absorbance versus concentration with high regression coefficient. This indicates that calibration curve obeys Beer-lambert's law at $\lambda_{\text{max}}$610 nm within the range of concentrations used.

3.1.2.3. UV calibration curves for Acarbose

Figures (3-10),(3-11) and (3-12) showed the calibration curves of acarbose insolutions of 0.1N HCL (pH 1.2),pH 5.5 and pH 6.8.A straight line was obtained by plotting the absorbance versus concentration with high regression coefficient. This indicates that calibration curve obeys Beer-lambert's law at $\lambda_{\text{max}}$ 237 nm within the range of concentrations used.

3.1.3. Determination of PH-solubility profile of the drugs

According to the result obtained as shown in table (3-1).GLB can be classified as very slightly soluble drug in pH 1.2 and pH 5.5 and the solubility increase in the alkaline pH 6.8, since 3mg of GLB in 10 ml at pH 1.2 and 5mg of GLB in 10 ml at pH 5.5and slightly soluble in pH 6.8 (20mg in 10ml).

Table 3-1. Solubility of GLB, metformin and acarbose.(n=3), SD: Standard deviation.

<table>
<thead>
<tr>
<th>Drug</th>
<th>pH=1.2</th>
<th>pH=5.5</th>
<th>pH=6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLB</td>
<td>0.3mg/ml</td>
<td>0.5mg/ml</td>
<td>2mg/ml</td>
</tr>
<tr>
<td>Metformin</td>
<td>17.5mg/ml</td>
<td>7mg/ml</td>
<td>0.4mg/ml</td>
</tr>
<tr>
<td>Acarbose</td>
<td>7.5mg/ml</td>
<td>3.3mg/ml</td>
<td>0.56mg/ml</td>
</tr>
</tbody>
</table>

Glibenclamide is a weak acid (pKa = 5.3) practically insoluble in water and acidic environment but highly permeable (class 2) according to the Biopharmaceutical Classification System (BCS). (10)

The ionization and hence the solubility was increased in the higher pH medium, while started to decrease as the pH became closer to the pKa. (23)
Metformin can be classified as sparingly soluble drug in pH 1.2 and slightly soluble in pH 5.5 because it is weak base and very slightly soluble in pH 6.8, since 175 mg of metformin in 10 ml at pH 1.2 and 70 mg of metformin in 10 ml at pH 5.5 and very slightly soluble in pH 6.8 (4mg in 10ml).

Acarbose can be classified as slightly soluble drug in pH 1.2 and slightly soluble in pH 5.5 because it is weak base and very slightly soluble in pH 6.8, since 75 mg of acarbose in 10 ml at pH 1.2 and 33 mg of acarbose in 10 ml at pH 5.5 and very slightly soluble in pH 6.8 (5.6mg in 10ml).

3.2. In-vitro drug release

In vitro release gave precious information about the product performance in vivo. The drug release dictates the amount of drug available for absorption. The release pattern of the drugs from the dosage form was studied using USP dissolution apparatus II. Each drug evaluated in 3 pHs and determine the effect of pH on release and study the best pH for each one as shown in the following figures.

From these data results showed that the physiochemical properties of each drug affects largely on its dissolution as the pH changes along the G.I.T.

GLB showed higher release in the duodenum and small intestine (pH5.5 and pH6.8) than acidic stomach pH (pH1.2) due to the weak acid nature of the drug and this the opposite for metformin and acarbose because of high pK_a value for these drugs than GLB i.e. metformin and acarbose gave higher release in pH1.2 than pH5.5 and pH6.8.

Results in some papers showed that GLB in the stomach the drug is actuallyor even exclusively absorbed from the stomach.

The S-shaped initial rise in the blood level curves after instilling the drug into the stomach forseveral individuals indicates that a transport mechanismmay be involved with this site of application.

This may be a process of dissolution, since glibenclamide is insoluble (according to the nomenclature of USP XX) below a pH of 7.0 (Hajdfl et al. 1969), or of protracted transport to the site of absorption within the intestinal tract. However, neither the amount absorbed nor the rate of absorption - as measured by the mean residence time - is significantly different from application in the duodenum. (24)
“Poorly absorbed from the lower part of the gastro-intestinal tract”. This statement is based only on the pH partition hypothesis using an incorrect pKa value of 5.3 instead of 6.3 to 6.8 depending on the evaluation method (Hajd6 et al. 1969). Contrary to their statement, the same amounts are absorbed from the colon as from the upper part of the gastro-intestinal tract, but the rate of absorption is clearly slower. Because of the slower absorption from the colon, it cannot be ruled out that the extent of absorption may also be affected in some cases by the time the drug spends in contact with the absorbing surface. However, the main fraction of an orally administered dose of glibenclamide will be absorbed in the upper part of the gastro-intestinal tract, and even that portion which passes the small intestine unabsorbed has a clear chance of being made available to the body via the large intestine.\(^{(24)}\)

Literature reviews showed that the dissolution of glibenclamide increased in case of magnesium oxide, magnesium trisilicate and sodium bicarbonate as well as retarded by food, small amounts of antacids as aluminum hydroxide, aluminum trisilicate, calcium carbonate, magaldrate and simethicone, these containing polyvalent cations.\(^{(25)}\)

4. Conclusions

1-From all the resulted data one can conclude that the pH of the medium affect greatly on the dissolution and absorption of GLB, metformin and acarbose.

2-The best pH for each one is the pH which gave higher release according to the physiochemical properties for each drug.

3-Food and other drugs that may change the pH of the medium leads to change in the absorption behavior for each drug due to the effect on the dissolution medium as shown when GLB taken with antacid or food. This gave idea about the time for oral administration of drug to achieve higher dissolution and absorption.

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Figures:

**Figure 1-1.** Sites of oral therapies for type 2 diabetes.

**Figure 1-2.** Structure of glibenclamide

**Figure 1-3.** Structure of metformin HCL

**Figure 1-4.** Structure of acarbose
Figure 1-5. Acarbose competitively inhibits the enzymatic hydrolysis of oligosaccharides by α-glucosidases in the small intestine\textsuperscript{(18)}. 

Figure 3-1. UV spectrum of GLB in 0.1N HCL , pH 5.5 and pH 6.8 

Figure 3-2. UV spectrum of metformin in 0.1N HCL , pH 5.5 and pH 6.8
Figure 3-3. Absorption spectra of the studied drug after reaction with KMnO4/NaOH system.

A) The produced manganate ions after the reaction of KMnO4 with acarbose (50 μg/ml), B) Oxidation products of acarbose.

Figure 3-4. Calibration curve of GLB in 0.1N HCL (pH 1.2).

Figure 3-5. Calibration curve of GLB in (pH 5.5).
Figure 3-6. Calibration curve of GLB in (pH 6.8).

\[ y = 0.008x + 0.011 \]
\[ R^2 = 0.998 \]

Figure 3-7. Calibration curve of metformin in 0.1N HCL (pH 1.2).

\[ y = 0.010x - 0.007 \]
\[ R^2 = 0.999 \]

Figure 3-8. Calibration curve of metformin in (pH 5.5).

\[ y = 0.007x - 0.001 \]
\[ R^2 = 0.996 \]
Figure 3-9. Calibration curve of metformin (pH 6.8)

Figure 3-10. Calibration curve of acarbose in 0.1N HCL (pH 1.2)

Figure 3-11. Calibration curve of acarbose in (pH 5.5)
Figure 3-12. Calibration curve of acarbose in (pH 6.8)

\[ y = 0.032x - 0.011 \]

\[ R^2 = 0.997 \]

Figure 3-13. In vitro release of GLB, metformin and acarbose at pH 1.2

Figure 3-14. In vitro release of GLB, metformin and acarbose at pH 5.5
Figure 3-15. In vitro release of GLB, metformin and acarbose at pH 6.8

References


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