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FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACEUTICS

PHYSICOCHEMICAL AND IN VITRO DISSOLUTION PROPERTIES OF SOME METFORMIN TABLET PREPARATIONS ON THE GHANAIAN MARKET

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN PHARMACEUTICS

BY

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DECLARATION

"I, Akwasi Sougi, declare that I have fully undertaken the study reported herein under the supervision of Prof. K. Ofori-Kwakye and that except portions where references have been duly cited, this dissertation is the result of my research".

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(Head of Department) (Signature) (Date)

DEDICATION

This work is dedicated to my sweet mother, Delali Gbenyo and to my only uncle, Mr. Charles Gbenyo for their unending love and support. Lastly, to all my siblings and friends.

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I lifted up my eyes unto the hills and indeed my help came from the Lord who made the heavens and the earth. Glory be to His Name for His faithfulness.

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ABSTRACT

Metformin hydrochloride is an oral hypoglycaemic agent belonging to the class of drugs called biguanides and the first line drug for the management of type 2 diabetes particularly in overweight and obese patients. There are numerous generics of metformin hydrochloride tablets available within the health delivery system in Ghana and globally, which places medical practitioners in a dilemma of generic substitution. The study sought to determine the physicochemical equivalence and to establish whether or not the selected brands were interchangeable based on *in vitro* dissolution properties.

Fourteen brands of metformin hydrochloride tablets plus the innovator brand, Glucophage were purchased from selected licensed pharmacies within the country and they were all conventional, immediate-release oral dosage forms. Coding of all the brands was done to avoid bias and the genuineness of the samples was determined using infra-red spectroscopy and thin layer chromatography. Pharmacopoeia tests such as friability, hardness, uniformity of weight, disintegration and assay were used to assess the physicochemical equivalence of the various brands of metformin hydrochloride tablets. In vitro dissolution testing was conducted using the paddle method at six time points to obtain their dissolution profiles which were subjected to analysis involving dissolution efficiency, model-independent and ANOVA-based methods. Lastly, Biopharmaceutics classification system based biowaiver conditions were applied to the various brands as a surrogate for bioequivalence studies.

All the brands of metformin hydrochloride tablets sampled complied with the official specifications for identification, disintegration, uniformity of weight, hardness and thickness tests. However, for the friability test, brand M9 failed to meet the required specification. Both UV spectroscopy and HPLC were used for the assay analysis and all the brands except three

(M5, M9 and M12) had values which fell within the specification range for assay in the British Pharmacopoeia. All the brands met the pharmacopoeia criterion for dissolution rate test for conventional immediate release tablets. All the brands with the exception of three (M5, M9 and M12) passed all the official tests and therefore, they could be regarded as having the same physicochemical properties. Brands M6 and M11 had the highest dissolution efficiency of 95% while M9 had the lowest dissolution efficiency of 83.8%. From f_1 and f_2 analysis, the dissolution profiles of seven brands were similar to that of the reference brand which indicated that they could be used interchangeably in clinical practice. However, using one-way ANOVA analysis followed by Dunnett's multiple comparison test, only the dissolution profiles of five brands were significantly different from that of the reference drug. None of the brands including the reference drug was able to meet the criteria for BCS-based biowaiver for very rapidly or rapidly dissolving tablets and therefore, the need for *in vivo* studies to establish the bioequivalence of these brands cannot be overemphasized.

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CHAPTER ONE

1.1 General introduction

Diabetes mellitus is a group of metabolic diseases characterized by elevated levels of glucose in the blood (hyperglycemia) resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2003).There are two main types of diabetes mellitus (type 1 and type 2 diabetes mellitus) though there are other rare forms of diabetes mellitus. Type 1 diabetes (formerly known as insulin-dependent diabetes) is characterized by insulin deficiency resulting from pancreatic beta cell destruction. However, the most prevalent form of diabetes, type 2 diabetes (which accounts for over 90% of all diabetes cases) presents as a spectrum of metabolic abnormalities with prominent insulin resistance and relative insulin deficiency (WHO**,** 1999**).** The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs.

Globally, 347 million people have diabetes (WHO, 2013**).** In 2004, an estimated 3.4 million people died from consequences of fasting high blood sugar. Although type 2 diabetes mellitus is more prevalent in developed countries, the increase in incidence seems to be more pronounced especially in populations that are experiencing rapid westernization **(**Zimmet *et al,* 2002). Data on the prevalence of diabetes in Ghana is scanty and unreliable. However, a recent study (Amoah *et al,* 2002)**;** reported a significant prevalence rate of 6.3%.

Metformin is an oral hypoglycemic agent which belongs to the class of drugs called biguanides. It is the first line drug for treatment and management of Type 2 diabetes mellitus particularly in overweight and obese people (International Diabetes Federation, 2005). Metformin exerts its effect mainly by decreasing gluconeogenesis and by increasing peripheral utilisation of glucose

in skeletal muscles. It has a half-life of about 3 hours and is excreted unchanged in the urine. It is also characterized by high solubility, low intestinal permeability and bioavailability of 50-60%.

There are numerous generics of metformin hydrochloride tablets available within the health delivery system globally as well as in Ghana after the expiration of patent on Glucophage, the innovator brand. They are promoted for use in practice because they are usually less expensive than the innovator products. Regardless of price, generic drug quality should be comparable to that of the innovator product. Generic drug products can only be interchangeable with innovator products when they are pharmaceutically and therapeutically equivalent. The first stage in establishing the therapeutic equivalence of any drug product involves ascertaining the chemical and biopharmaceutical equivalence of such drug products **(**Olaniyi *et al.*, 2001**)**. Drug products that are chemically and biopharmaceutically equivalent must be identical in strength, quality, purity, active ingredient release profile and must be in the same dosage form and for same route of administration.

The introduction of generic drug product from multiple sources into the health care delivery system of many developing countries is aimed at improving access to life-saving drugs in such countries **(**Adegbolagun *et al.*, 2007). However, this has been bedeviled with widespread distribution of fake and substandard drug products and therefore the quality of medicinal drugs in many developing countries is inadequate. This revelation reiterates the importance of monitoring drug quality in order to safeguard the health of patients.

Drug dissolution testing is routinely used in the pharmaceutical industry to provide critical *in vitro* drug release information for both quality control i.e., to assess batch-to-batch consistency of solid oral dosage forms such as tablets and drug development, i.e., to predict *in vivo* drug release

profile (Bai *et al*., 2011).It serves as a quality control test in support of routine manufacture to establish batch-to-batch performance consistencies. Any substantial variations in the dissolution rate among same generics indicate deficiency in the entire drug formulation and the delivery system. To this extent, manufacturing methods coupled with excipients used in the production, could contribute to the overall quality and release proficiency of medicament.

Therefore, in order to ensure the requisite quality, drug manufacturers are required to test their products during and after manufacturing and at various intervals during the shelf life of the product (Chow, 1997). As such the need to ensure that the generic drugs are pharmaceutically equivalent cannot be overemphasized and the necessity to select one product from several generic drug products of the same active ingredients during the course of therapy is always a cause for concern to healthcare practitioners.

1.2 Justification

It has been estimated that about 30% of the medicinal products on sale for consumption in many countries in Africa and parts of Asia and Latin America are counterfeit and substandard **(**WHO, 2006). Counterfeiting can apply to both branded and generic products and could include products with the correct ingredient or with the wrong ingredient, without active ingredient, with insufficient active ingredient, or with fake packaging **(**WHO, 1999**)**. While substandard drugs are genuine drug products that upon laboratory testing do not meet the quality specification claimed by their manufacturers **(**Taylor *et a*l., 2009**)**.

There are several generics of metformin hydrochloride tablets available within the health delivery system globally as well as Ghana after the expiration of patent on Glucophage, the innovator brand. Evidence point to the fact that different products with the same amount of

active ingredient have shown distinct differences in their therapeutic effects **(**Fujii *et al*., 2009**).** This places health practitioners in a dilemma of generic substitution. The increasing level of use of metformin hydrochloride tablets in clinical setting creates the need to monitor and ascertain the quality and drug release proficiency of the various brands available in the drug market for quality control assessment and for purpose of generic substitution.

1.3 Aim of study

To carry out a comparative evaluation of the physicochemical and in- vitro dissolution properties of some metformin tablet preparations on the Ghanaian market.

1.4 Specific objectives

- To sample the various metformin hydrochloride tablet preparations on the Ghanaian market.
- To perform identification test on the various brands of metformin tablets sampled using infrared spectroscopy and TLC.
- To determine the physicochemical equivalence of the metformin tablet brands that would be sampled using both compendia and non- compendia methods.
- To assay the sampled metformin hydrochloride tablets using both HPLC and UV.
- To perform a comparative in-vitro dissolution study of the tablets.
- To ascertain whether the various brands of metformin tablet sampled would meet BCS Biowaiver criteria.
- To determine the similarity of the dissolution profiles of the metformin hydrochloride tablets by using appropriate models.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

2.1 Substandard and counterfeit medicines

2.1.1 Definition of substandard and counterfeit medicines

Substandard medicines are products whose composition and ingredients do not meet the correct scientific specifications and which are consequently ineffective and often dangerous to the patient. Substandard products may occur as a result of negligence, human error, insufficient human and financial resource or counterfeiting (WHO, 2003).

Counterfeit medicines are part of the broader phenomenon of substandard pharmaceuticals. The difference is that they are deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit medicines may include products with the correct ingredients but fake packaging, with the wrong ingredients, without active ingredients or with insufficient active ingredients (WHO, 2003).

2.1.2 Drug counterfeiting

The phenomenon of using medicinal agents to improve healthcare delivery has existed for thousands of years, but it was not until the last century that the manufacturing and distribution of pharmaceutical products became widespread. The last few decades alone have seen an exponential increase in the number of commercially available pharmaceutical products. Along with the multitude of health benefits that have resulted from this phenomenon are unique challenges. The most pressing issue for many people is that of access; despite amazing advancements in medicine, the potential health benefits of drugs are lost if people do not have access to them because of cost or other constraints. Because so many people are afflicted by

untreated health conditions worldwide, the drugs that can provide benefit have become an extremely sought-after commodity. The market for pharmaceuticals is extremely competitive since the sale of such products has become so lucrative globally. As with the sale of any type of goods, individuals and companies are always looking for ways to maximize profits, and one of the simplest ways to do this is to decrease costs. One way to achieve this goal, albeit illegal, is to manufacture or distribute drugs that contain substandard amounts of the active ingredient. This dimension of counterfeiting is possible because methods of detecting such drugs are difficult for the end user. Furthermore, drug counterfeiters are often able to make products that are incredibly similar in terms of shape, color, and likeness, and it can sometimes require very sensitive scientific means to determine a counterfeit product. Creating products with none or a decreased amount of the labeled active ingredient is not the only form of counterfeiting. Mislabelling a drug product with a different brand name or expiration date are other examples of this phenomenon.

Public health is at increasing risk because of an apparent growing global epidemic of the manufacture and trade of counterfeit pharmaceuticals. For example, in Haiti, India, Nigeria, and Bangladesh some 500 children died of acute renal failure after ingesting counterfeit paracetamol (acetaminophen) and cough syrup made using diethylene glycol, a renal toxin (O'Brien *et al*., 1998; Hanif *et al*., 1995).

2.1.3 Available data on counterfeit and substandard drugs

The prevalence of counterfeit drugs in the global marketplace is reaching epidemic proportions; with developing countries disproportionately affected to a greater degree (Quick *et al.,* 1997). Due to the difficult nature of identifying counterfeit drugs, estimates on prevalence are not precise, but they give valuable insight to the scope of the problem. The World Health

Organization has estimated that approximately 10 percent of the global pharmaceuticals market consists of counterfeit drugs, but this estimate increases to 25% for developing countries, and may exceed 50% in certain countries (WHO, 2006). Considering potential outcomes of receiving counterfeit drugs, including lack of successful treatment, adverse effects, and even death, these numbers are staggering. Increasing international and regional free trade, high demand for curative and preventive drugs and vaccines, proliferation of small pharmaceutical industries, and insufficient regulation of drug manufacture and trade are some of the factors that have contributed to the wide distribution of low-quality medicines, particularly in developing countries. Although practically all types of pharmaceutical products have been shown to be involved, the existing data suggest that anti-infectious agents, in particular antibiotics and anti-parasitic agents, are the most counterfeited products in developing countries (Wondemagegnehu, 1999). A recent study that analyzed samples of the two most common anti-malarial drugs in Kenya,sulphadoxine-pyrimethamine (SP) and amodiaquine (AQ), found that about 40% of these drugs available for sale were of substandard quality (Amin *et al.*, 2005). In a controlled study done in Nigeria and Thailand, samples of selected antibiotics from pharmacies were analyzed using appropriately validated methods based on High- Performance Liquid Chromatography. The results indicated that 36.5% of the samples were sub-standard with respect to pharmacopoeial limits (Shakoor *et al*., 1997).

2.1.4 Substandard drugs in developing countries

Developing countries often do not impose the same level of regulations of pharmaceutical product qualities in the same extent as developed countries do. Furthermore, the security measures and tests that can be employed to identify counterfeit drugs can be extremely costly and difficult to implement, and many of these countries simply do not have enough resources to

use these techniques consistently. In addition to these issues, the general demand for pharmaceuticals can be much higher in developing countries than in developed countries, particularly for acute life-threatening conditions. These needs can create a sense of urgency and desperation, causing people to sacrifice more to attain the treatments that they believe will save their lives and those of their families. The combination of these factors ripens the atmosphere in developing countries for drug counterfeiting.

Circulation of substandard drugs is further encouraged by the fact that drugs manufactured for export are often not regulated to the same standard as those manufactured for domestic use. An analysis done by the European Union and the French Ministry of Cooperation (Andriollo *et al*., 1997) revealed many problems in the export legislation from European countries to developing countries, including imprecise controls regarding good manufacturing practices for exported products, lack of quality control of products that have not been marketed in Europe, and discordant information between drugs to be exported and drugs for European use (Andriollo *et al.*, 1997).

2.1.5 Causes and methods of drug counterfeiting

If drug counterfeiting is not curbed in some way, the problem is likely to continue or worsen because of its highly profitable nature. Profits are the single most important motivating factor for drug counterfeiters, and so far they have proven many of their endeavors not only worthwhile but also capable of expanding. According to the Centre for Medicines in the Public Interest, counterfeiting represented approximately 10% of the global pharmaceuticals market, with sales exceeding $35 billion (Pitts, 2005).

Poor compliance with GMP standards can lead to substandard production. This may be accidental (such as human error) or the result of insufficient resources (expertise, appropriate

manufacturing infrastructure, or human and financial resources). Other deliberate causes are often ignored or underestimated. Quality audits of manufacturing sites done by MSF pharmacists (180 sites visited over the last 4 years) have found that manufacturers that regularly pass the most stringent inspections adjust their standards to that of the recipient country. In our observations, parallel productions can exist in the same GMP compliant' facilities: a high standard of production for the strictly regulated markets and for exacting clients such as UN organizations and international aid agencies; an intermediate standard of production for middle-income countries; and a much lower standard for poorly regulated countries (Caudron *et al*., 2008).

There is a wide range of methods of counterfeiting pharmaceutical products, ranging from little or no active ingredient to the wrong ingredient, or mislabelling. Perhaps the most traditional method of counterfeiting drugs is to create a product that looks similar to a particular popular selling brand name medication, but containing none of the active ingredient. While many counterfeiters employ this method, it may be detected somewhat easily, and therefore more advanced methods have been developed. Another method is to include substandard amounts of the active ingredient, or for a generic manufacturer to make a product and sell it as the brand name in order to increase revenues. Sometimes counterfeiters use one active ingredient and label it as another, or change some other part of the labelling such as the expiration date for products that are beyond their initial expiration date. As these methods and technologies become more advanced, it becomes increasingly difficult to detect a fraudulent medication. Patients may not ever realize that they are taking a fake, or they may only realize after taking it for some time and experiencing either lack of treatment or an adverse event.

2.1.6 Global implications of counterfeiting

While the data on prevalence of counterfeiting drugs is startling, the real issues are the global health implications that are a result. One may argue that if it is so difficult to identify the counterfeits, then patients must not be suffering. The reality, however, is that millions of people worldwide have been affected by the perils of fraudulent pharmaceuticals, particularly in developing countries. One of the biggest disasters related to counterfeit pharmaceuticals was a case of vaccines given as a gift to the country of Niger from neighbouring Nigeria in 1995 to help contain the meningitis epidemic there (Cockburn *et al.*, 2005). The vaccines did not contain any active ingredient, but more than 50,000 people received the vaccine and this subsequently led to 2,500 deaths, according to the World Health Organization (WHO, 2006)). This is one of the few documented cases of counterfeited products causing deaths on such a large scale, but there are other cases of large-scale counterfeiting schemes in which it is more difficult to estimate directly related deaths.

Whether people learn about counterfeit drugs from the media, the government, or have a personal experience with such products, there is another very detrimental consequence that may often be overlooked. People may begin to lose faith not only in certain brand names, but also in the value of taking drugs altogether to treat their illnesses. This could be especially dangerous in developing countries, where infectious diseases are much more prevalent. If patients decide not to take medications due to distrust of manufacturers, governmental agencies, or health care professionals, they not only endanger their own health, but it also becomes a greater public health concern if they remain infectious. This can also be a bigger problem in developing countries since people often spend more of their income on pharmaceuticals, and they may be

less likely to spend their already limited income on drugs that may be ineffective or potentially harmful.

2.1.7 Tackling counterfeit and substandard medicines

Significant resources have been devoted to tackle counterfeit medicines, but very little specific attention has been given to the far more serious and widespread problem of substandard medicines. This is partly a consequence of the poor differentiation made between these two distinct problems. However, reducing the problem of substandard medicines to a consequence of counterfeiting skews resources towards legal action alone, complicating efforts to define targeted strategies to specifically address the problem of the substandard medicines. The focus of attention should rather be on the detection and removal of poor quality medicines, whether they are counterfeit or not, while at the same time assisting legitimate manufacturers to improve the quality of their pharmaceutical production. The limited resources available for the development of efficient pharmacovigilance systems in developing countries compound the problem. Because the consequences of substandard medicines, both on individuals and on public health, often go unreported, there is no stimulus to intervene. The pre-qualification programme has recently been expanded but capacity remains limited, and the majority of essential drugs remains outside of the scope of the programme and are still purchased without a proper evaluation. Other recent initiatives by the WHO are important but remain financially fragile; moreover, these measures will be only successful if other actors involved in drug procurement assume their responsibilities. Donors have an important role to play by strengthening quality clauses based on WHO standards in the tender mechanisms they impose on non-governmental organizations. Likewise, drug purchasers (NGOs, international organizations, charities, and national purchase centres in resource-limited countries) should assume their responsibility towards protecting patients‘ health

and insist that producers and distributors supply drugs that meet WHO standards. Quality assurance is a mandatory preliminary to drug purchases in the West, and there is no rationale for this procedure to be any different when drugs are exported to poor populations. Governments could act now to reduce this problem granting export authorization only to pharmaceutical products that comply with the WHO standards for quality, efficacy and safety (Caudron *et al.*, 2008).

2.2 Diabetes Mellitus

2.2.1 Definition of diabetes mellitus

Diabetes mellitus describes a heterogenous metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with distortions in carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both **(**WHO, 1998**).**

2.2.2 Types of diabetes mellitus

There are two main types of diabetes mellitus (type 1 and type 2 diabetes mellitus) though there are other rare forms of it. Type 1 diabetes (formerly known as insulin-dependent diabetes) develops when the body's immune system destroys pancreatic beta cells, the only cells in the body that make the hormone insulin that regulates blood glucose. This form of diabetes usually strikes children and young adults, although disease can occur at any age.

Type 2 diabetes (formerly known as non-insulin dependent diabetes) which accounts for over 90% of all diabetes cases presents as a spectrum of metabolic abnormalities with prominent insulin resistance and relative insulin deficiency **(**WHO, 1998**)**. It is associated with older age, obesity, family history of diabetes, history of gestational diabetes and physical inactivity.

2.2.3 Global prevalence of diabetes mellitus

In the year 2000, the global number of individuals with diabetes was estimated to be 171 million (2.8% of the world's population), and this figure has been projected to increase in 2030 to 366 million (6.5%), 298 million of whom will be living in developing countries **(**WHO, 1998). Type 2 diabetes mellitus has reached epidemic proportions with explosive increase in incidence worldwide over the past few decades. Although type 2 diabetes mellitus is more prevalent in developed countries, the increase in incidence seems to be more pronounced especially in populations that are experiencing rapid westernization **(**Zimmet *et al,* 2002**).** Apart from microvascular complications, cardiovascular disease, with its attendant morbidity and mortality, is on the rise in the developing countries. Current evidence suggests that environmental factors are major determinants of the increasing rates of diabetes **(**WHO, 1998**).** Overweight and obesity are increasing dramatically and contribute to the burden of diabetes mellitus and other chronic health conditions. Indeed, the modern environment promotes behaviors that cause obesity.

2.2.4 Prevalence of diabetes mellitus in Ghana

Although diabetes was thought to be rare in sub-Saharan Africa, recent studies from some countries suggests that the disease may now be more common in sub-Saharan Africa than previously thought **(**Cooper *et al*, 1997, Mbanya *et al*, 1999, Aspray *et al,* 2000). Though epidemiological data on the prevalence of diabetes in Ghana is scanty, evidence suggests that it is on the increase. In the 1950s, the prevalence of diabetes among an outpatient urban population in Accra was estimated at less than 0.5% (Dodu, 1958). The impression was therefore created among policy makers that diabetes is rare in Ghanaians. However, a recent study (Amoah *et al,* 2002); reported a high prevalence rate of 6.3%.

2.2.5 Overview of the development of type 2 diabetes mellitus

Under normal physiological conditions, the plasma glucose concentrations are maintained within a narrow range, despite the wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially in the liver) and insulin secretion **(**DeFronzo, 1997). The ingestion of carbohydrates causes a momentary increase in blood glucose concentration, resulting in a rapid release of insulin by the ß-cells within the islets of Langerhans. Insulin then binds to specific receptors in target peripheral tissues to produce its effects. Under normal conditions, insulin promotes the transport of glucose into the skeletal muscle and adipose tissue. In the liver, insulin acts by suppressing glycogenolysis and gluconeogenesis, and in adipose tissue, it inhibits lipolysis. Through the mechanism of decreasing hepatic and adipose glucose production, and by accelerating the uptake of glucose into peripheral tissues, the net effect of insulin action is to lower blood glucose concentration **(**DeFronzo, 1997**)**. In people with type 2 diabetes mellitus (type 2 DM) however; there is a gradual change in glucose homeostasis manifested as glucose intolerance and inefficient uptake of glucose from the blood by the peripheral tissues. The glucose intolerance is caused, in part, by an attenuated biological response to normal concentrations of insulin, a condition known as insulin resistance. In addition, type 2 DM is often associated with a progressive decrease in the sensitivity of the pancreatic ß-cells to glucose stimulation, with a subsequent decrease in insulin secretion. In time, there may be an increased demand for insulin due to worsening of the insulin resistance. Eventually, the combined effects of increased insulin resistance and inadequate insulin secretion in response to a glucose challenge will result in hyperglycaemia, which is a significant and prolonged increase in blood glucose concentration (Bao *et al.,* 1996). Although the central figure is hyperglycaemia, the effect of diabetes mellitus is not limited to carbohydrate

metabolism. Lipid and protein metabolism also play an important role in the progression of the disease. The abnormal glucose metabolism accounts for poorly regulated biochemical processes that glycosylate haemoglobin and other proteins and lipids throughout the body (Bao *et al.*, 1996). The effects of the dysmetabolism in carbohydrates, lipids, and proteins include long-term damage dysfunction and failure of various organs. The symptoms are often not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may persist for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific microvascular complications including retinopathy with potential blindness, nephropathy that leads to renal failure, and/or neuropathy with risk of foot ulcers and potential amputation and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of macrovascular damage including cardiovascular, peripheral vascular and cerebrovascular disease (WHO, 1998, Freedman *et al.*, 2001, Ford *et al.*, 2002).

Type 2 DM is a heterogeneous syndrome that results from an interaction between *1)* a genetic predisposition and 2*)* environmental factors. In genetically predisposed individuals, the development and progression of type 2 diabetes mellitus appears to be facilitated by factors such as obesity, lack of physical activity, cigarette smoking, high intake of calorie-rich diets and low intake of fruits and vegetables **(**WHO, 1998, Halliwell, 2000, Hu *et al.*, 2001)**.**

Type 2 diabetes mellitus has a gradual and insidious onset, and some degree of hyperglycaemia may have been present for several years (10 – 20 years) before the diagnosis is confirmed **(**WHO, 1998). The pathogenesis of diabetes mellitus, regardless of its aetiology, progresses through several clinical stages during its natural history. Moreover, individuals may move from stage to stage and persons who have, or who are developing diabetes mellitus can be categorized

by stage according to the clinical characteristics, even in the absence of information concerning the underlying aetiology (Bao *et al.,* 1996).

2.2.6 Diagnosis of diabetes mellitus

The diagnostic criteria of hyperglycaemia for diabetes mellitus have recently been revised by comprehensive reviews of the world's research findings by the Expert Committees of the American Diabetes Association (ADA) and the World Health Organization (WHO). The reviews have led to a lowering of the fasting glucose diagnostic criteria from ≥7.8 mmol/l to ≥7.0 mmol/l. The 2-hour criterion (≥ 11.1 mmol/L) however was unchanged. Additionally, a new category, impaired fasting glucose (IFG), was established and defined as fasting plasma glucose of 6.1 – 6.9 mmol/l (ADA, 1997 and WHO, 1998).

2.2.7 Signs and symptoms of diabetes mellitus

The signs and symptoms of diabetes are related to hyperglycemia, hypoglycemia (temporarily low glucose levels), complications associated with diabetes. Type 1 diabetics are often diagnosed with acute severe symptoms that require hospitalization while in early type 2 diabetes, there are usually no symptoms. Symptoms of type 1 and type 2 with hyperglycemia include:

- Increased thirst
- Increased urination
- Increased appetite (with type 1, weight loss is also seen)
- Numbness, tingling and pain in the feet
- Slow-healing infection
- Blurred vision
- Vomiting and nausea
- Erectile dysfunction in men

- Absence of menstruation in women (STG, 2010).

2.2.8 Complications of diabetes mellitus

Much of the concern for optimal treatment of diabetes is focused around the long-term complications of the disease which include:

- Hyperglycemia
- Diabetic Ketoacidosis; this is a condition characterized by hyperglycemia and metabolic acidosis due to ketone body accumulation. Signs and symptoms include dehydration with dry skin, reduced skin turgor or sunken eyes, ketone odour in breath and hyperventilation. Other symptoms of diabetic ketoacidosis include altered consciousness and abdominal pain.
- Blindness
- Heart attacks
- Strokes
- Gangrene and subsequent amputation
- Kidney disease
- Impotence
- Infertility
- Lipid disorders
- Recurrent stillbirths (STG, 2010)

2.2.9 Management of diabetes mellitus

The main aims of diabetes management are:

- To achieve a normal glycaemic state

- To reduce the risk of long-term damage to organs and tissues from sustained hypoglycemia
- To enable the patient to maintain as near normal lifestyle as possible while ensuring adequate control of his or her diabetes.
- To establish compliance with the patient relating to his or her management plan (STG, 2010).

In order that these aims are achieved it is important the following points are observed; educate the patient about

- Control of diet
- Therapy with oral hypoglycemic agents or insulin
- Frequent monitoring of diabetic control by attending diabetic clinic
- Correction of other cardiovascular risk factors (e.g. hypertension)
- Early detection of signs and symptoms of complication

2.2.9.1 Non pharmacological treatment

Exercise: Although physicians as part of the treatment of diabetes nearly always recommend exercise, it is seldom prescribed. Exercise improves insulin sensitivity, or the ability of insulin to be used to drive glucose into the cell. Exercise lowers blood glucose by allowing glucose to penetrate the muscle cell and be metabolized without the assistance of insulin. Exercise improves circulatory function, an important factor in diabetic management. It also helps maintain normal body weight, aids breathing, digestion and metabolism. An exercise programme should be prescribed for both type 1 and type 2 patients. The exercise should be structured to suit the individual's condition.

Diet: Patient must be referred to a dietician or diet nurse for individualized meal plans. In general, patients must avoid free or refined sugars, such as in soft drinks or adding sugar to their beverages. Diet, soft drinks, which contain a sweetener and not glucose, are preferred. Complex carbohydrates are to be encouraged. Most of a day's diet must consist of carbohydrate (60%), protein (15%) and fat (25%), mostly of plant origin and low in animal fat (STG, 2010). The total caloric content of meals must be reduced in those who are also overweight.

Daily Foot Care: As one grows older, the circulation to the extremities decreases. This is especially true for diabetics (National Diabetes Advisory Board, 1982**).** With decreased circulation, the body cannot heal injuries to the feet. An ingrown toenail or blister can lead to an infection. The nerves in the feet that tell if something is too cold or too hot may also be affected. You may burn yourself without knowing it, so taking care of the feet every day is important to prevent problems.

Alcoholism: Alcohol can make certain health problems such as diabetic nerve damage or high blood pressure worse. Even if you don't have additional health problems, alcohol can cause hypoglycemia almost immediately and up to 12 hours after drinking (National Diabetes Advisory Board, 1982) .In general, alcohol use is discouraged in diabetic patients.

2.2.9.2 Pharmacological treatment

There are two main classes of drugs used in the management of diabetes mellitus. These are oral hypoglycemic agents and insulin.

There are two major classes of oral hypoglycemic drugs: the sulfonylureas and the biguanides (Craig, 1994). They should be prescribed only if the patient fails to respond adequately to at least 3 months' restriction of energy and carbohydrate intake and an increase in physical activity. They should be used to augment the effect of diet and exercise and not to replace them (British

National Formulary, 2013). Sulfonylureas are considered for patients who are not overweight while the biguanides are considered in obese patients (Craig, 1994).

Insulin is usually indicated in a patient who has been in ketoacidosis and young patients who usually have type 1 diabetes mellitus. It is also indicated in older or type 2 patients when oral ant-diabetic drugs cease to be effective. Oral anti-diabetic drugs should be avoided in type 1 patients and should not be used during pregnancy and breastfeeding (British National Formulary, 2013).

The sulfonylureas act mainly by augmenting insulin secretion and consequently are effective only when some residual pancreatic beta-cell activity is present. Several sulfonylureas are available and choice is determined by side effects and the duration of action as well as the patient's age and renal function (British National Formulary, 2013). Commonly used sulfonylureas are glibenclamide, gliclazide, glimepiride, glipizide and tolbutamide. They are best taken with meals. Tolbutamide and gliclazide are short-acting and are preferred in the elderly and those with mild kidney disease. In general sulfonylureas should be avoided in all patients with liver disease and used with care in kidney disease.

The only available biguanide, metformin has a different mode of action from the sulfonylureas and is not interchangeable with them. It exerts its effect mainly by decreasing gluconeogenesis and by increasing peripheral utilisation of glucose (Craig, 1994). Metformin is the drug of choice in overweight patients in whom strict dieting has failed to control diabetes; if appropriate it may also be considered as an option in patients who are not overweight. It is also used when diabetes is inadequately controlled with sulfonylurea treatment.

Exogenous insulin is given as replacement therapy to compensate for the lack of endogenous insulin in type 1 diabetes and the relative lack of endogenous insulin (due to insulin resistance or

a defect in the insulin release mechanism) in types 2 diabetes. There are three types of insulin available from different species: beef, pork and human. Insulin acts by promoting cellular uptake of glucose form the blood stream. Insulin may be categorized into three groups based on their time-action as short acting, intermediate-acting and long-acting (British National Formulary, 2013). The short-acting insulin includes semilente, regular and regular buffered insulin. Regular and buffered regular insulin formulations are clear and contain solubilized crystalline insulin. Regular insulins are the only insulin products that may be administered by the intravenous route because all other insulin formulations are suspension.

Intermediate-acting insulins include lente insulin and NPH (neutral protamine hagedorn). Lente insulin is a suspension composed of a 30:70 mixture of semi-lente and ultralente.

The long-acting insulins include protamine zinc insulin and ultralente insulins (British National Formulary, 2013).

2.2.10 Side effects of drugs for diabetes mellitus management

Sulfonylurea: Side effects of sulfonylureas are generally mild, infrequent and include:

- Gastrointestinal disturbances such as nausea, vomiting, diarrhea and constipation.
- Blood disorders such as haemolytic anaemia, agranulocytosis, pancytopenia, thrombocytopenia, leucopenia and aplastic anaemia.
- Cutaneous rashes and generalized hypersensitivity reactions
- Dizziness, Muscular weakness and mental confusion
- Disturbance in liver function which may lead to cholestatic jaundice, hepatitis and hepatic failure and
- Hypoglycemia (British National Formulary, 2013).

Biguanides: Side effects of the biguanide include:

- Anorexia ,nausea, vomiting, diarrhea
- Abdominal pain, metallic taste and
- Lactic acidosis (British National Formulary, 2013).

Insulin: Side effects of insulin include:

- Hypoglycemia, which result in CNS symptoms such as tremors, nervousness, convulsions, sweating, headache, double vision and weakness.
- Localized infection at the site of injection
- Lipodystrophy and
- Insulin lipoma (British National Formulary, 2013).

2.3 Metformin Hydrochloride

2.3.1 Description of metformin hydrochloride

Metformin hydrochloride is an oral antihyperglycemic drug used in the management of type 2 diabetes.Metformin hydrochloride (N, N-dimethylimidodicarbonimidic diamide hydrochloride) is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. It was approved by FDA in December, 1994. The chemical structure is as shown:

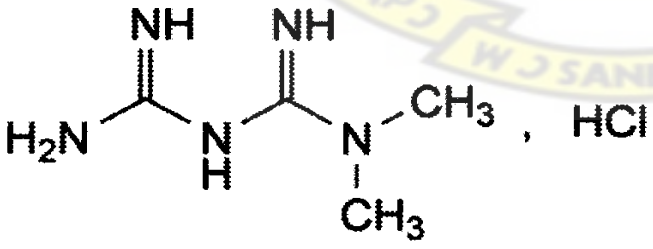

Fig 2.1: Chemical structure of metformin hydrochloride (British Pharmacopoeia, 2013)

Metformin hydrochloride is a white to off-white crystalline compound with a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether and chloroform. The pK, of metformin is 12.4.

2.3.2 Mechanism of action of metformin

Metformin is antihyperglycemic, not hypoglycemic (Bailey, 1992). It does not cause insulin release from the pancreas and generally does not cause hypoglycemia, even in large doses. Metformin has no significant effects on the secretion of glucagon, cortisol, growth hormone, or somatostatin. Metformin reduces glucose levels primarily by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. At a molecular level, these actions are mediated at least in part by activation of the cellular kinase AMP-activated protein kinase (AMP kinase) (Zhou et al., 2001).

2.3.3 Pharmacokinetics of metformin

Metformin is absorbed mainly from the small intestine. The absolute bioavailability of a Metformin hydrochloride 500 mg tablet given under fasting conditions is approximately 50% to 60%. It has a half-life of about 2 hours. The drug is stable, does not bind to plasma proteins, and is not metabolized, and is excreted by the kidneys as the active compound. As a consequence of metformin's blockade of gluconeogenesis, the drug may impair the hepatic metabolism of lactic acid. In patients with renal insufficiency, biguanides accumulate and thereby increase the risk of lactic acidosis, which appears to be a dose-related complication.

2.3.4 Clinical uses and dosing of metformin

Metformin hydrochloride is the drug of choice for treating type 2 diabetes mellitus which is associated with obesity. It is also being used increasingly in polycystic ovary syndrome (Lord et

al., 2003), non-alcoholic fatty liver disease (Marchesini et al., 2001) and premature puberty (Ibanez et al., 2006). The dosage of metformin is from 500 mg to maximum of usually 2 g daily, with the lowest effective dose being recommended **(**British National Formulary, 2013). A common schedule would be to begin with a single 500 mg tablet given with breakfast for several days. If this is tolerated without gastrointestinal discomfort and hyperglycemia persists, a second 500 mg tablet may be added with the evening meal. If further dose increases are required after 1 week, an additional 500 mg tablet can be added to be taken with the midday meal, or the larger (850 mg) tablet can be prescribed twice daily or even three times daily (the maximum recommended dosage) if needed. Dosage should always be divided, since ingestion of more than 1000 mg at any one time usually provokes significant gastrointestinal side effects.

2.3.5 Precautions and contraindications of metformin

Patients with renal impairment should not receive metformin. Other contraindications include hepatic disease, a past history of lactic acidosis (of any cause), cardiac failure requiring pharmacological therapy, or chronic hypoxic lung disease. The drug also should be discontinued temporarily prior to any surgical procedure. The drug should not be readministered any sooner than 48 hours after such procedure and should be withheld until renal function is determined to be normal. These conditions all predispose to increased lactate production and hence to the potentially fatal complication of lactic acidosis.

2.4 Dissolution

2.4.1 Theory of dissolution

For a drug to be absorbed it must first be dissolved in the fluid at the site of absorption. For example, an orally administered drug in the tablet form is not absorbed until drug particles are dissolved in the fluid at some point along the gastrointestinal tract depending on pH-solubility

profile of the drug substance. Thus, dissolution is a principal factor in dosage form design. The process by which drug particles dissolve is termed dissolution (Aulton, 2002).

The dissolution of a solid in a liquid is composed of two consecutive stages:

- First is an interfacial reaction that results in the liberation of the solute molecules form the solid phase. This involves a phase change, so that molecules of the solid become molecules of solute in the solvent in which the crystal is dissolving. The solution in contact with the solid will be saturated. The concentration of this solution is a saturated solution.
- Secondly, the solute molecules must migrate through the boundary layers surrounding the crystal to the bulk of the solution. This step is under the influence of diffusion or convection. Boundary layers are static slow-moving layers of liquid that surround all wetted solid surfaces. Mass transfer takes place more slowly through these static or slow-moving layers which inhibit the movement of the solute molecules from the surface of the solid to the bulk of the solution. The concentration of the solution in the boundary layers changes from being saturated at the crystal surface to being equal to that of the bulk of the solution at the outmost limit (Aulton, 2002).

In dissolution, the interfacial step is virtually instantaneous and so the rate of dissolution will be determined by the rate of the slower step (step 2).

2.4.2 Factors affecting dissolution rate

Scientists have reviewed the factors which can affect the dissolution of tablets and these include the stirring speed, temperature, viscosity, pH, composition of the dissolution medium and the presence or absence of wetting agents (Singhvi and Singh, 2011). The rate of dissolution can

also be affected by variables which include characteristics of the active pharmaceutical ingredient (API) e.g. particle size, crystal form, bulk density, drug product composition (e.g. drug loading and the identity, type and levels of excipients), the product manufacturing process (e.g. compression forces) and the effects of stability storage conditions (Tadey and Carr, 2009).

2.4.3 Significance of dissolution testing

Over the last quarter century, dissolution testing has emerged as a highly valuable *in vitro* test to characterize the performance of a dosage form. The popularity of dissolution testing is based on the fact that solubilization of a drug in gastrointestinal fluid is a prerequisite for a drug to be absorbed and be available to the systemic circulation. The dissolution testing is performed as a relatively fast and inexpensive technique to evaluate pharmaceutical dosage forms before they are tested in clinical trials. It is prudent to have extensive dissolution data to maximize the chances for success in bioavailability testing in humans (Gray and Grady, 1997). Dissolution testing can be used:

- To detect the influence of critical formulation and manufacturing variables in Formulation and Development as well as Research and Development
- To assist in selection of a best formulation
- To check the changes during stability studies
- To establish final dissolution specifications for the pharmaceutical dosage form
- To develop *In vitro In vivo* Correlation, (Swarbrick, 1997)
- As a quality control tool and
- To establish the similarity of pharmaceutical dosage forms, for which composition, manufacturing site, scale of manufacture, manufacturing process and/or equipment may have changed within defined limits (FDA, 1995a).

2.4.4 Dissolution methods

A number of official and unofficial methods exist for dissolution testing. The main test methods are based on based on forced convection of the dissolution medium and can be classified into two groups. There are continuous-flow methods and stirred-vessel methods.

The most important stirred-vessel methods are the rotating-basket method and the paddle method. With the continuous-flow method, the preparation is held within a flow cell, through which the dissolution medium is pumped at a controlled rate from a large reservoir. The liquid which has passed the flow cell is collected for analysis of drug content. The continuous-flow cell method may have advantages over stirred-vessel methods, in that, it maintains sink conditions throughout the experiment and avoids floating of the preparation (Aulton, 2002).

2.4.5 Dissolution profile specification

In vitro dissolution specifications are established to ensure batch-to-batch consistency and to signal potential problems with *in vivo* bioavailability. Once a dissolution specification is set, the drug product should comply with that specification throughout its shelf life. Three categories of dissolution test specification for immediate release drugs products are described in the guidance for industry. These include single-point specifications, two-point specification and dissolution profile comparison (FDA, 1995b).

2.4.5.1 Single-point specifications

This is usually set for highly soluble and rapidly dissolving drug products. Single-point involves determining the amount of drug (Q) dissolved within a particular time point (FDA, 1995b). The Q value obtained is compared with standard Q values stated in official compendia within certain tolerances. Single-point specification is used as a routine quality control test.

2.4.5.2 Two-point specification

This specification involves the use of two points in characterizing the quality of the drug product. Two-point specification are also employed as routinely quality control test for certain type drugs such as slow dissolving or poorly water soluble drug product (FDA, 1995b).

2.4.5.3 Dissolution profile comparison

The dissolution profile comparison is developed in connection with observations taken on tablets or capsules over time. This provides conditions for accepting product sameness under Scale-up and Post-Approval Changes (SUPAC). It is also applied in the waiving bioequivalence requirement for lower strength of dosage form. More so dissolution profile comparison is used to support waivers for other bioequivalence requirement (FDA, 1995b).

2.4.6 Methods for dissolution profile comparison

To compare dissolution profiles between two drug products, several methods have been proposed. These methods, by nature of the procedures, could be classified as Model Dependent Method (curve fitting), Model Independent Method and Statistical Methods (Demirturk, 2006).

2.4.6.1 Model Dependent Method

The commonly used models in the curve fitting procedure include exponential probit, Gompertz, logistic, Weibull and the three control factor models. Others include Zero order, First order, Hixson-Crowell and Higuchi (Demirturk, 2006). For the curve fitting procedure, it is assumed that the dissolution profiles can be expressed by the selected curve. The procedure is first to fit the curve of the response of the experiment and compute the associated parameters of the model for both reference and test, respectively. It is then followed by comparing the corresponding parameters from the two curves.

2.4.6.2 Model Independent Methods

Model independent methods involve comparison of the two profiles only at the observed time points. Examples include Difference factor (f_1) and similarity factor (f_2) (Moore and Flanner, 1996). In the SUPAC guidance on immediate release (1997), FDA indicates that dissolution profiles could be compared based on a similarity factor (FDA, 1997b). The difference factor (f_1) calculates the percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves: The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves.

$$f_2 = 50 \times \log \{[1 + (1/n) S_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$$

$$f_1 = \{[S_{t=1}^{n} |R_t - T_t|] / [S_{t=1}^{n} R_t]\} \times 100$$

Where, n is the number of dissolution sample times, Rt and T_t are the individual or mean percent dissolved at each time point, t, for the reference and test dissolution profiles, respectively. A specific procedure to determine difference and similarity factors is as follows:

- Determine the dissolution profile of two products of the test and reference products.
- Using the mean dissolution values from both curves at each time interval, calculate the difference factor and similarity factor using the above equations.
- For curves to be considered similar, f_1 values should be close to 0, and f_2 values should be close to 100. Generally, f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves and, thus, of the performance of the test and reference products.

2.4.6.3 Dissolution Efficiency

Dissolution efficiency (DE) on the other hand is a parameter for evaluation of in-vitro dissolution data. It is expressed as the area under dissolution curve up to a certain time 't' expressed as percentage of the area of the rectangle described by 100 percent dissolution at the same time (Mauralidhar et al,2011).

This concept was proposed by khan and Rhodes and is defined as follows

$DE = \{({}_0\int^t Y.dt) / Y_{100}.(t_2-t_1)\}\times 100$

Where y is the percentage of dissolved product. DE is then the area under the dissolution curve between time points t_1 and t_2 expressed as a percentage of the curve maximum dissolution. Y_{100} over the same period (Costa and Sausa lobo, 2001).

DE= Dissolution efficiency = $\{({}_0\int^t Y.dt) / Y_{100}.(t_2-t_1)\}\times 100$

$({}_0\int^t Y.dt)$ = area under the dissolution curve (AUC)

Y= the percentage dissolved at t_2

t_2= time for all active ingredient to dissolve

t_1= time at which first sample was withdrawn

2.4.6.4 Statistical methods

The most commonly used statistical methods include exploratory data analysis methods, moment based comparison, individual time point test, two-way analysis of variance (ANOVA) and analysis of covariance (Montgomery, 1991). Other known methods are analysis of first difference, repeated measurement split-plot (Gill, 1988) and multivariate analysis. These

methods do not require the preset curve of the profiles. Some analyses, however, have underlying assumption that requires the responses to be independent. This assumption could not be applied to dissolution testing since the amount dissolved over time within the same drug product is in fact correlated.

2.5 Biopharmaceutics Classification System

Biopharmaceutics classification system (BCS) is a scientific approach for classifying drug substances based on specific criteria namely, the permeability, solubility, and dissolution of the drug. These characteristics include the in vitro dissolution of the drug product in various media, drug permeability information, and assuming ideal behavior of the drug product, drug dissolution and absorption in the GI tract. For regulatory purpose, drugs are classified according to BCS in accordance with the solubility, permeability and dissolution characteristics of the drug **(**FDA, 2000). According to the BCS, drug substances are classified as follows:

- Class 1: High Solubility-High Permeability
- Class 2: Low Solubility-High Permeability
- Class 3: High Solubility-Low Permeability
- Class 4: Low Solubility-Low Permeability (Amidon, 1995)

The solubility of a drug is determined by dissolving the highest unit dose of the drug in 250 ml of buffer adjusted between pH 1.0 and 8.0. A drug substance is considered highly soluble when the dose/solubility volume of solution is less than or equal to 250 ml. High-permeability drugs are generally those with an extent of absorption that is greater than 90% in the absence of documented instability in the gastrointestinal tract or those whose permeability has been determined experimentally (FDA, 2000). The BCS suggests that for high solubility, high permeability (class 1) drugs and in some instances for high solubility, low permeability (class 3)

drugs, 85% dissolution in 0.1N HCl in 15 minutes can ensure that the bioavailability of the drug is not limited by dissolution. In these cases, the rate limiting step for drug absorption is gastric emptying. The mean gastric residence (emptying) time is 15-20 minutes under fasting conditions. Based on this information, a conservative conclusion is that a drug product undergoing 85% dissolution in 15 minutes under mild dissolution test conditions in 0.1N HCl behaves like a solution and generally should not have any bioavailability problems. If the dissolution is slower than gastric emptying, a dissolution profile with multiple time points in multimedia is recommended.

In the case of low solubility/high permeability drugs (case 2), drug dissolution may be the rate limiting step for drug absorption. A dissolution profile in multiple media is recommended for drug products in this category. In the case of high solubility/low permeability drugs (case 3), permeability is the rate controlling step. Drugs in case 4 (i.e., low solubility/low permeability drugs) present significant problems for oral drug delivery.

BCS is employed to waive *in vivo* bioequivalence testing for new and generic drugs. Granting biowaiver under systems such as the BCS, eliminates unnecessary drug exposures to healthy subjects and provides economic relief while maintaining the high public health standard for therapeutic equivalence.

2.6 Biowaiver

In vivo bioequivalence studies are commonly used to assess therapeutic equivalence, but these studies are often costly and involve procedures. The Biopharmaceutics classification system can be used to reduce in-vivo bioequivalence requirements (Lobenberg and Amidon, 2000). In-vitro dissolution tests based on BCS are acceptable surrogates for establishing the bioequivalence of generics with the innovator products. The concept is applicable to immediate release solid

pharmaceutical dosage forms for oral administration and systemic action having the same pharmaceutical form. However, it is not applicable for sublingual, buccal and modified release formulations. According to FDA and WHO, a biowaiver for *in vivo* studies can be obtained for BCS class 1 drugs that are very rapidly dissolving (≥85% dissolved within 15 minutes) or are rapidly dissolving (≥85% dissolved within 30 minutes) with f2≥50 in three dissolution media ranging from 1.2 to 6.8. WHO also recommends biowaiver for class 2 and 3 drugs that are very rapidly dissolving.

2.6.1. Criteria for BCS-based biowaiver

The *in vivo* bioavailability or bioequivalence of the drug product for certain drug products may be self-evident. FDA waives the requirement for the submission of evidence obtained during i*n vivo* demonstrating the bioavailability or bioequivalence of these drug products. The *in vivo* bioavailability or bioequivalence of such products may be considered self-evident if the product meets one of the following criteria:

- If the drug product is a parenteral solution intended solely for administration by injection or an ophthalmic or otic solution.
- If the drug product contains same active and inactive ingredients in the same concentration as a drug product that is the subject of an approved full new drug application (Smetanova *et al*., 2011).

Additionally, FDA may waive the requirement for the submission of evidence obtained *in-vivo* demonstrating the bioavailability or bioequivalence of the drug products:

- If they are highly soluble: Highest dose is soluble in 250 ml at pH 1.2-6.8
- If they are highly permeable: the extent of absorption is greater than 85%.

- If they are rapidly dissolving: 85% or greater by basket method at 100 rpm or by paddle method at 50 rpm in 900 ml at pH 1.2, 4.5 and 6.8.

Moreover, for waiver of bioequivalence test and reference products, they should exhibit similar dissolution profile ($f_2 \geq 50$) (Kortejarvi *et al.*, 2005).

2.6.2 Requirement for a biowaiver study

There have been certain requirements for a biowaiver study that include allowance of regulatory authorities like FDA and WHO etc. The drugs should have high solubility and high permeability according to BCS. However, other classification systems are part of current investigations. The other requirements for a biowaiver study include:

- Dissolution test in 3 different media which are:
    - ✓ Buffer pH 1.2, simulated gastric fluid (SGF) without enzymes or 0.1N HCl
    - ✓ Buffer pH 4.5
    - ✓ Buffer pH 6.8 or simulated intestinal fluid (SIF) without enzymes, all in 900 ml and at 37℃
- 12 samples in each media, paddle rotating at 50 rpm or basket at 100 rpm
- Sampling times are 10, 15, 20,30, 45 and 60 minutes
- The profile of the test and reference product must be similar in all three media.
- The products are similar if the similarity factor $f_2 \geq 50$ and both products show ≥ 85% dissolution in 15 minutes (Seema *et al.*, 2012).

2.7 Tablet characteristics

Tablets as a dosage form should meet certain specific requirements. The diameter, shape, thickness, uniformity of content and weight, hardness, disintegration time and dissolution of a

tablet all have to conform to certain parameters.

2.7.1 Tablet thickness

Thickness can vary with no change in weight due to the difference in the density of the granulation and the pressure applied to the tablets, as well as the speed of the tablet compression. Tablet thickness may be determined using caliper or thickness gauge which measures the thickness in millimeters. A plus or minus 5% may be allowed, depending on the size of the tablet (Sandell, 1983).

2.7.2 Uniformity of dosage forms

A fundamental quality for all pharmaceutical preparations is the requirement for a constant dose of the drug between individual tablets. For tablets, uniformity of dose or dose variation is tested in two separate tests; uniformity of weight and uniformity of content.

2.7.3 Tablet weight

The volumetric fill of the die cavity determines the weight of the compressed tablet. The weight of the tablet is the quantity of the granulation which contains the labelled amount of the therapeutic agent. The tablet weights must conform to the set standards as in the USP or BP. As stated in the pharmacopoeia when twenty tablets are selected at random and a uniformity weight test is performed, not more than two tablets should deviate from the average weight by a greater percentage as illustrated below and not even one should deviate by twice that value (United States Pharmacopoeia, 2007).

Table 2.1 Permissible percentage deviation of tablet for uniformity of weight test

Average weight	Percentage deviation
130 mg or less	±10
More than 130 mg but less 324 mg	±7.5
More than 324 mg	±5

2.7.4 Content uniformity

The test for uniformity of drug content is carried out by collecting a sample of tablets, normally ten, followed by a determination of the amount of drug in each. The average drug content is calculated and the content of the individual tablets should fall within specified limits in terms of percentage deviations from the mean (Harold et al., 1961).

2.7.5 Resistance to abrasion (friability)

It is unlikely that a tablet would be subjected to a compressive load large enough to fracture it. It may however, be subjected to tumbling motion. e.g. during coating, packaging or transportation, which whilst not severe enough to break the tablet, may abrade small particles from its surface. Friability measurement is made by use of the friabilator. The instrument is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping. In the tumbling apparatus the tablet is exposed to rolling and repeated shocks resulting from free falls within the apparatus. A number of tablets are weighed and placed in the friabilator and the machine allowed to operate for 4 minutes at a total of 100 revolutions. For tablets with a unit weight equal to or less than 650 mg, a sample of whole tablets corresponding as near as possible to 6.5 g is used. For those higher than 650 mg, 10 whole tablets are used. A maximum loss of weight obtained from a single test not greater than one percent is considered acceptable for most

products (British Pharmacopoeia, 2013).

2.7.6 Tablet hardness

The resistance of the tablet to chipping, abrasion or breakage under conditions of storage, transportation and handling before usage depends on its hardness (Sandell, 1983). Hardness determinations are made throughout the tablet runs to determine the need for pressure adjustment on the tableting machine. If the tablet is too hard, it may not disintegrate in the required period of time or meet the dissolution specifications. If too soft, it will not withstand the handling during subsequent processing such as coating or packaging and shipping operations. Quantitatively, hardness of tablet is determined either in N (Newton) or kg. The minimum satisfactory value for tablet hardness is 4kg or 40N (Alfonso, 1990).

2.7.7 Tablet disintegration

For a drug to be absorbed it must be in solution and the disintegration test is a measure only of the time required under a given set of conditions for a group tablets to disintegrate into particles. The test is useful as a quality assurance tool for conventional dosage forms.

The disintegration test is used as a control for tablets intended to be administered by mouth, except where tablets are intended to be chewed before being swallowed or where the tablets are designed to release the drug substance over a period of time. The apparatus consists of a basket rack holding six plastic tubes, open at the top and the bottom; the bottom of the tubes is covered with 10-mesh screen. The basket rack is immersed in a bath of suitable liquid held at 37℃ preferable in a 1 liter beaker. The rack moves up and down in the fluid at a specified rate. The volume of the fluid is such that on the upward stroke the mesh remains at least 2.5cm below the surface of the fluid and descends to not less than 2.5cm from the bottom on the downward stroke. Tablets are placed in each of the six cylinders along with a plastic disc over the tablet

unless otherwise directed in the monograph. The endpoint of the test is indicated when any residue remaining in a soft mass having no palpably soft core. For most uncoated tablets the time is 15 minutes and for coated tablets up to one hour may be required (British Pharmacopoeia**,** 2013). The test is useful to assess the potential importance of formulation and as a control procedure to evaluate the quality of the tablet during production.

2.7.8 Dissolution

For certain tablets the monographs direct compliance with limits on dissolution rather than disintegration. Since drug absorption and physiological availability depend on having the drug in dissolved state, suitable dissolution characteristics are an important property of a satisfactory tablet. Like the disintegration test, the dissolution test for measuring the amount of time required for a given percentage of the drug substances in a tablet to go into solution under a specified set of conditions, is an in vitro test. It is intended to provide a step towards the evaluation of the physiological availability of the drug but is not designed to measure the efficacy or safety of the tablet being tested (Sandell, 1983).

2.8 Identification tests

Identification of the test sample is one important preliminary test that needs to be carried out. The identity of the sample under test needs to be ascertained before further experimental work could be done. The identification of the state of purity of the pure sample is also of prime importance. Three common methods used to carry out identification are;

- Infrared spectroscopy
- Thin-layer chromatography
- Melting point determination

2.8.1 Infrared spectroscopy

Infrared spectroscopy determines the absorption recorded by the samples under the infrared portion of the electromagnetic spectrum. Vibrational frequencies registered by the various functional groups (usually very characteristic) are recorded as spectra.

2.8.2 Thin layer chromatography

Thin-layer chromatography is a separation technique in which a stationary phase consisting of an appropriate material is spread as a uniform thin layer on a support of glass, metal or plastic. The separation is based on adsorption, partition, ion-exchange or combinations of these mechanisms. The separation is carried out by migration of solutes in a solvent or a suitable mixture of solvents (mobile phase) through the thin-layer (stationary phase). When a mixture of analytes is spotted and dried on the plates, the drugs move across the plate at different rates depending on the extent of adsorption or partitioning on the plates and its solubility in the mobile phase (Clarke, 1986). Some of the stationary phases used for TLC include silica gel, cellulose, alumina (aluminum oxide), magnesium silicate, ion exchange resins and reversed phases like paraffin and octadecyl silane ODS. TLC is one of the most widely used techniques for the separation of pharmaceutical products and their identification. This method of characterization has gained popularity as an analytical method because of its simplicity, reliability as well as the simple method location procedures.

2.8.2.1 Location of spots

As most organic compounds are colourless, they must be made visible, preferably by a non-destructive technique. Most compounds can be located by examining plate containing a chromophore under a 254nm wavelength. In this process the absorbing compounds are seen as dark spots. These spots can be ringed up with a pencil. Compounds that naturally fluoresce can

be located under UV lamp as coloured spots. Another very important but destructive test is to spray ethanolic sulphuric acid on the plate and gently warm in an oven. Organic material when treated in such manner, char-up and are seen as dark spots.

2.8.2.2 Retention factor (Rf)

The basic chromatographic measurement of a substance on TLC is the Retention factor (Rf). The distance travelled by the substance is measured from the centre of the round spot. However if the spot is tailing, it is measured from the middle of the dense area of that spot. Rf values needed for the identification of samples are to be run at the same time and on the same plate with both known and unknown side by side. The RF value if quoted as a fraction ranges between 0 and 1, and if quoted as a percentage ranges between 0 and 100 (Clarke, 1986**).**

$$Rf = \frac{\text{Distance travelled by solute from origin}}{\text{Distance travelled by solvent from origin}}$$

2.8.3 Melting point determination

It is an important criterion to know the purity of a substance; however, it has a few limitations. The accuracy and precision of melting point is dependent on a number of factors such as capillary size, sample size, initial temperature of heating-block and the rate of rise of temperature per unit time (minutes). Keeping in view the different manufacturing processes available for a particular drug the melting point has a definite range usually known as the melting range. Thus the melting range takes care of the variance in manufacture together with the storage variance over a stipulated period of time (Ashutosh Kar, 2005).

2.9 Theory and instrumentation of analytical methods

2.9.1 UV visible Spectrophotometric analysis

The technique of Ultraviolet- visible spectrophotometry is one of the most frequently employed technology employed in pharmaceutical analysis. The wavelength used ranges from 190 nm – 380 nm for ultraviolet radiation and 380 nm–800 nm for visible radiation (Beckett and Stenlake, 1988). Different sources of light are needed for the generation of the radiation needed. Hydrogen discharge lamps and xenon arc lamps are needed for ultraviolet radiation generation, and tungsten filament lamps and deuterium discharge lamps generate the radiation in the visible region. Since these light sources generate large range of wavelengths, there are monochromatic filters incorporated in the machine that is able to filter and produce light of specified range of wavelength needed by the user. Examples of monochromator include prisms and diffraction gratings. Light from the monochomator passes through the cuvette containing the sample to the light detector system. The signals generated are compared with the incident light and the amount of light absorbed displayed by the machine. The Beer-Lambert law is the basis for all analytical absorption spectrophotometry. The law states that, the absorbance of a solution of a substance is related to the path length of the solution through which the light passes and to its concentration (Beckett and Stenlake, 1988). Mathematically: A= a*b*c

A = Absorbance

a= specific absorbance if concentration is in %w/v

b= Path length in cm

c= concentration in %w/v

Or

$A = \varepsilon bc$

A= Absorbance

ε = molar extinction coefficient

b = Path-length in cm

c = concentration in g/L

The law holds when monochromatic light is used and the solution used is diluted and stray light is excluded. Therefore, the plot of absorbance against varying concentration for a cell of unit thickness, usually 1cm should give a straight line passing through the origin. This is termed the calibration curve. The calibration curve can be used for the determination of the concentration of an unknown sample when the absorbance has been determined. However, for a solution containing a mixture of compounds with each having a different maximum absorbance with spectral overlaps, the overall absorbance at their maximum wavelength will be equal to the summation of the specific absorbance of their product times the concentration.

2.9.2 High performance liquid chromatography (HPLC)

HPLC is now the most widely used method of assay and separation technique. The simple high performance liquid chromatographic method developed in the late 1960s has evolved into high pressure and high speed chromatography. HPLC has many advantages over the classical column chromatography. With the packed stationary phase made of smaller particle size, there are improved resolution of substances, faster separation with increased precision and accuracy. The separation principles used for effective separation involves adsorption, partition, ion exchange and gel permeation (Watson, 1999).

2.9.2.1 Instrumentation of HPLC

The basic instruments consist of mobile phase reservoir, a high–pressure pump, an injector, a stationary phase embedded in a stainless steel column, a detector and a chart recorder.

2.9.2.1.1 High Pressure Pumps

High pressure pump is an important part needed to deliver a constant flow of the mobile phase with a decisive pressure. Most pumps are able to deliver a constant pressure of -600bar. A dual – Piston reciprocating pump is performed due to its pulse-free flow. In this system as one shaft phase is filling the valve another phase is pumping the mobile phase. Unlike a single piston pump a damping device is required to smoothen out the flow. This is necessary so as to avoid excessive noise at high level of sensitivity causing high base line noise preventing small quantities of substances to be detected **(**Beckett and Stenlake, 1988).

2.9.2.1.2 Injector system

The sample solution is introduced into the flowing mobile phase at or near the head of the column using an injection system which can operate at high pressure. They contain Fixed- loop and variable volume devices which are operated manually or by an auto-sampler are used. Manual partial filling of loops may lead to poorer injection volume precision. The sample is introduced into the loop when the valve is in the load position. At this stage the eluent flows from the pump to the column through another passage. When the valve is switched to inject, the loop is redirected to flow into the column conveying the sample into its destination.

2.9.2.1.3 Column

The columns are made of highly polished stainless steel usually having a column length of 10 to 30cm and an internal diameter of 4.5 to 5mm. Longer and larger pore size columns are available and are used usually for commercial purposes. The most widely used stationary phase is silica ($SiO_2.XH_2O$). The stationary phase consists of a network of siloxane linkages (Si-O-Si) in a rigid three dimensional structure containing interconnecting pores. The pore size and the amount of silanol groups are controlled in the manufacturing process. In a straight stationary phase

column the silanol groups are vital as they are involved in adsorption chromatography. Silica can be modified to the reversed stationary phase. This is done by a controlled reaction of organochlorosilanes with the silanol groups or the use of organoalkoxysilanes which modifies the surface of the silica. The linkage of these hydrocarbons to the surface impacts a non polarity to the surface and enhances partitioning, thus the separation of lipophilic compounds. The most popular stationary phase material used is the (ODS) Octadecyl-silica C18.Others include octyl (C8), Phenyl (C6H5), Cyanopropyl (CH2) 3-CN and aminopropyl (CH2) 3-NH2 groups. Pharmaceutical products contain both lipophilic and polar groups. These groups are exploited during separation on columns (Watson, 1999**)**.

2.9.2.1.4 Detector

Four main types of detectors are frequently used in High performance liquid chromatography. These are the electrochemical detectors, Fluorescent detector, Refractive index detector, Mass spectrometers, Radioactivity detectors and the Ultra-Violet visible detectors. Among these, the most widely used is the Ultra-Violet Visible detectors.

CHAPTER THREE

MATERIALS, EQUIPMENT AND METHODOLOGY

3.1 Materials and equipment

3.1.1 Equipment and Apparatus

Erweka Dissolution Apparatus (Type DT6, GmbH Heusenstamm, Germany Nr 68045), Erweka Disintegrating Test Apparatus (Type ZT 3/1, GmbH Heusenstamm, Germany Nr 68318), UV spectrophotometer (T90 UV/VIS spectrometer, PG Instruments Ltd., UK), Analytical balance (SN: AE 436647 Adam Equipment, UK), DBK Hardness Tester (SR. No 123), Interspectrum Fourier Transform Infra-red spectrometer (200-X, SN: 200043), Erweka Friabilator (Type TA 20, Germany), Shimadzu HPLC, Agilent Zorbax SB column (4.6 x250 nm), Fused silica cuvettes Hewlett Packard, HP 3396 Series II integrator, LC-10AT Shimadzu Liquid chromatograph Pump, Applied Biosystems 783 A Programmable absorbance detector, Shimadzu CR 501 Chromatopac, Eutech pH meter (SN: 2025520, Singapore), Electronic Vernier caliper, Stuart melting point apparatus (SN: R000105350, Bibby Scientific Ltd., UK), TLC silica gel G plate, Whatman filter papers, Number 4 sintered glass filter and general-purpose glassware.

3.1.2 Chemicals and Reagents

All the reagents used for the experiment were of analytical grade and obtained from the Chemical Store of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, KNUST-Kumasi. Hydrochloric acid fuming GR (by Merck, Germany), sodium hydroxide (BDH, PROLABO), potassium dihydrogen orthophosphate (Fisons Laboratory), potassium ferricyanide (BDH, Poole England), ortho-phosphoric acid (Phillip Harris, England), glacial acetic acid (BDH, PROLABO), butanol (BDH, Poole England), absolute ethanol, sodium nitroprusside,

acetonitrile (BDH, PROLABO), potassium bromide (BDH) and pure metformin hydrochloride (RS) were obtained from the Department of Pharmaceutics-KNUST.

Table 3.1 Profile of sampled metformin hydrochloride tablets (M1-M8)

CODE	**TRADE NAME/ TYPE OF TABLET**	**BATCH NUMBER**	**MANU. DATE**	**EXPIRY DATE**	COUNTRY OF ORIGIN	MANUFACTURER
M1	Enaphage film-coated	2909M	-	09/16	Ghana	Ernest Chemist Ltd.
M2	Metformin film-coated	MEAG016	-	06/14	U. K	Winthrop Pharmaceuticals UK Ltd.
M3	Metformin Denk film-coated	81R	08/12	07/16	Germany	Denk Pharma GmbH & Co. KG
M4	Kinamet uncoated	12018	05/12	05/16	Ghana	Kinapharma Ltd.
M5	Metformin film-coated	BS1579	05/11	05/16	Germany	Salutas Pharma GmbH (1A Pharma)
M6	Diabetmin film-coated	BC06712	06/12	06/15	Malaysia	Hovid Bhd
M7	Metformin film-coated	MH008	12/10	11/13	India	Umedica Laboratories PVT. Ltd.
M8	Metformin uncoated	NP11061	05/11	04/14	India	Nauketan Pharma PVT. Ltd.

Table 3.2 Profile of sampled metformin hydrochloride tablets (M9-M15)

CODE	TRADE NAME/ TYPE OF TABLET	BATCH NUMBER	MANU. DATE	EXPIRY DATE	COUNTRY OF ORIGIN	MANUFACTURER
M9	Metformin film-coated	1012	-	11/14	Ghana	Pharmanova Ltd.
M10	Metformin film-coated	B344	12/11	12/14	India	XL Laboratories PVT. Ltd.
M11	Glucophage film-coated	108007	04/12	03/17	France	Merck santé s.a.s.
M12	Metformin film-coated	BUH022139	-	11/16	U.K	Bristol Laboratories Ltd.
M13	Metformin film-coated	RM1001	-	12/13	U.K	RelonChem
M14	Metformin film-coated	RM1286	-	07/14	U.K	Actavis (RelonChem)
M15	Metformin uncoated	E07515	01/11	12/13	India	Ciron Drugs & Pharmaceuticals Pvt. Ltd.

3.2 Methodology

3.2.1 Sampling of metformin hydrochloride tablets from the market

Fourteen brands of metformin hydrochloride tablets, each having label strength of 500 mg plus the innovator brand were identified. Samples of the identified brands were purchased from different registered retail pharmacies in Kumasi, Accra, Sunyani, Sekondi Takoradi and Koforidua. The selection of retail pharmacies was done with reference to an updated list of all registered pharmacies within the aforementioned cities in the country obtained from the office of the Pharmacy Council. There was an even selection of facilities from each of the five places. The products were coded for purposes of the research and the study was performed before product expiration dates.

3.2.2 Tests for identification of metformin hydrochloride RS

The metformin hydrochloride reference standard was subjected to identification tests as specified in BP (2013) namely; melting point determination and infra-red spectroscopy.

3.2.2.1 Infra-red spectroscopy

The wavenumber scale of the infra-red spectrophotometer was calibrated with the aid of a strip of polystyrene film fixed on a frame. Twenty milligram of metformin hydrochloride RS was triturated with 200 mg of dry, finely powdered KBr of spectroscopic grade using agate pestle and mortar. The mixture was ground thoroughly, spread uniformly in a suitable die and compressed into a disc. The resultant disc was mounted in suitable holder in the spectrophotometer and IR spectrum was then obtained at wavenumber range of 600-4000 cm^{-1}.

3.2.2.2 Melting point determination

The melting point of the metformin hydrochloride RS was determined by open capillary method using Stuart melting point apparatus. An open capillary was sealed by inserting the tip into a Bunsen flame near the base of the flame and turning the tube in the fingers. To pack the tube, the open end was pressed gently into a small amount of the sample of the crystalline material on a weighing paper. To transfer the crystal from the open end to the bottom of the tube, the bottom of the tube was tapped gently on the bench top. The sample tube was inserted into the Stuart melting point apparatus and the melting point determined.

3.2.3 Extraction of metformin hydrochloride from tablets

A quantity (0.0758g) of the powdered tablet equivalent to 60 mg of metformin hydrochloride (M1) was shaken with 60 ml of ethanol and filtered with Whatman filter paper (No 5). The filtrate was evaporated to dryness on a water bath and the residue dried at 105℃ for one hour (British Pharmacopoeia, 2013). The experimental procedure was repeated for the other brands. The residue of each brand of metformin hydrochloride tablet was used for subsequent infra-red and thin layer chromatography analysis.

3.2.4 Identification of extracted metformin hydrochloride

3.2.4.1 Identification of extracted metformin hydrochloride using TLC

TLC silica gel G plate was used. The solvent system: the upper layer of a mixture of glacial acetic acid, butanol and water in the following proportion was used-10:40:50. Twenty milligram of the extracted residue of each metformin tablet brand as well as 20 mg of reference metformin hydrochloride was dissolved in distilled water and diluted to 5 ml with same solvent. The test solutions and that of the reference were spotted on the plate and immersed in the solvent system

contained in the chromatank. The plate was removed when the solvent had moved three-fourths of the length of the plate. The solvent front was then marked, allowed to evaporate from the plate and the spots were detected by spraying with a mixture of equal volumes of sodium nitroprusside, potassium ferricyanide and sodium hydroxide solution all of 100g/L. The Rf value of each spot was then determined **(**British Pharmacopoeia, 2013**)**.

3.2.4.2 Identification of extracted metformin hydrochloride using IR

Two hundred milligram of spectroscopic grade KBr was ground with 20mg of the test sample (extracted residue) using agate pestle and mortar (Ashutosh Kar, 2005). The resulting mixture was compressed into KBr discs which were used for the analysis. The infra-red spectrum of each brand of metformin tablet at 600-4000 $cm^{-1}$was then compared with the reference spectrum.

3.2.5 Thickness test

An Electronic Vernier caliper was used to measure the thickness of ten tablets from each brand. The mean and standard deviation were then determined for all brands.

3.2.6 Weight uniformity test

Twenty (20) tablets from a particular brand were randomly selected and weighed collectively to obtain a mean weight. The tablets were then weighed individually and the percentage deviation of each tablet was then calculated **(**British Pharmacopoeia, 2013). This method was repeated for the other brands. The percentage deviations of the tablets from the mean were calculated using the formula below;

Percentage deviation = $\frac{A-B}{B} * 100$

Where, A= weight of a tablet, B = Average weight of 20 tablets

3.2.7 Friability test

Ten tablets were selected from a particular brand, dusted and weighed together and then placed in the friabilator. The friabilator was operated for 4 minutes at 25 revolutions per minute. The tablets were dedusted and reweighed. The percentage weight loss was then calculated (British Pharmacopoeia, 2013). This method was repeated for the other brands. The percentage weight loss was calculated using the formula below

Percentage weight loss = $\frac{Wi-Wf}{Wi} * 100$

Where, Wi = initial weight, Wf = final weight

3.2.8 Hardness test

Ten tablets were selected at random from each brand to perform this test. DKB hardness tester was used to measure the hardness. A tablet was placed between the spindle and anvil of the tester and the calibrated scale adjusted to zero. Compression force was applied on the tablet and the position on the calibrated scale at which the tablet broke was recorded in kgf units. A mean hardness with standard deviation was calculated for each brand.

3.2.9 Disintegration test

Six tablets selected randomly from a brand were placed individually in each of the six cylindrical tubes of the basket rack of the disintegration apparatus. The bottom of the basket rack was positioned such that it was at least 15mm below the surface of the distilled water and the experiment was conducted at 37℃. The disintegration time was taken to be the time no granule of any tablet was left on the mesh. Two determinations were done and the mean disintegration time was determined for each brand.

3.2.10 Assay of metformin hydrochloride tablets

The assay of the various metformin tablet brands was carried out by both UV and HPLC analytical methods.

3.2.10.1 UV analysis

3.2.10.1.1 Calibration curve

A stock solution of metformin hydrochloride of concentration 0.1% w/v was prepared by dissolving 0.1g of pure metformin hydrochloride powder in small volume of distilled water and made up to 100 ml. The following concentrations: 0.0001, 0.00025, 0.0003, 0.0005 and 0.0007 % w/v were then prepared form the stock solution. The absorbance of these solutions was determined by ultraviolet spectrophotometry at a wavelength of 232 nm. A calibration curve showing the relationship between concentration and absorbance was plotted and the equation and correlation values of the curve generated from the scatter plot.

3.2.10.1.2 Assay procedure

Twenty tablets of metformin hydrochloride (M1) were weighed and powdered. A quantity (0.0126g) of the powder equivalent to 0.1 g of metformin hydrochloride was shaken with 70 ml of distilled water for 15 minutes, diluted to 100 ml with water and filtered with Whatman filter paper (No 5), discarding the first 20 ml. Ten milliliters of the filtrate was diluted to 100 ml with distilled water and 10 ml of the resulting solution to 100 ml with distilled water. The absorbance of the resulting solution was measured at a wavelength of maximum absorption of 232 nm. The content of metformin hydrochloride was then calculated using the calibration curve (British Pharmacopoeia, 2013). The experimental procedure was repeated for the other brands.

3.2.10.2 HPLC method

The content of active ingredient (assay) for each of the fifteen brands was also determined by HPLC method, using the Shimadzu HPLC equipment. The HPLC method used was a reverse phase chromatographic technique which was developed and validated by Kar and Choudhury (2009).

3.2.10.2.1 Mobile phase preparation (for use as diluent)

Acetonitrile of volume 650 ml and 350 ml phosphate buffer (pH 5.75) were measured with a measuring cylinder into a 1000 ml standard volumetric flask and mixed thoroughly to obtain the specified ratio of 65: 35 of acetonitrile and phosphate buffer (pH 5.75) respectively (Kar and Choudhury, 2009).

3.2.10.2.2 Calibration curve

Standard stock solution of metformin hydrochloride with concentration of 100 μg/ml was prepared separately in the mobile phase. For preparation of drug solutions for the calibration curve, a series of 10 ml volumetric flasks containing 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of standard stock solution of metformin hydrochloride were made up to the volume with the mobile phase. Each solution was then filtered using sintered glass filter. Each solution was loaded in the injector of the Shimadzu HPLC fitted with 20 µl fixed volume loop, injected by rheodyne at a flow rate of 1.0 ml/min and the chromatogram for each injection was then recorded. The calibration curve was plotted between concentration of drug and peak area of metformin hydrochloride.

3.2.10.2.3 Assay preparation

A quantity (0.0632g) of powdered metformin hydrochloride tablets (M1) equivalent to 10 mg of metformin hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask containing about 75 ml of mobile phase. The powder mixture was dissolved in the mobile phase with the aid of sonication and then made up to the 100 ml mark with the mobile phase. The solution was filtered through Whatman filter paper (No 5) into another 100 ml volumetric flask. From the above filtrate, 1 ml was taken in a 10 ml volumetric flask and volume was made up to the mark with mobile phase, the solution was then filtered using sintered glass filter. After setting the chromatographic conditions and stabilizing the instrument, the sample solution was injected at flow rate of 1ml/min and a chromatogram was recorded. The injection was repeated three times and the peak area of metformin hydrochloride were recorded. The average peak area and calibration curve were then used to calculate the amount of drug present (Kar and Choudhury, 2009). The experimental procedure was repeated for the other brands.

3.2.10.2.4 Chromatographic conditions for assay

Shimadzu HPLC that consisted of LC-10A Shimadzu pump with programmable absorbance detector and Shimadzu CR50 Chromatopac was used. The column used was Agilent Zorbax SB C-18 (4.6x 250nm). Flow rate employed was 1.0 ml/min, an injection volume of 20 µl and the detection of eluent was carried out at 232 nm. The pump was set up to deliver a mobile phase which was filtered and degassed automatically by the in-built degasser of the HPLC equipment. After the chromatographic conditions were set, the instrument was stabilized to obtain a steady base line.

3.2.11 In vitro dissolution study

3.2.11.1 Calibration curve

A stock solution of metformin hydrochloride of concentration 0.1% w/v was prepared by dissolving 0.1g of pure metformin powder in small volume of distilled water and made up to 100 ml. The following concentrations: 0.0002, 0.0004, 0.0005, 0.0006, 0.0008 and 0.0009 %w/v were then prepared from the stock solution. The absorbance of these solutions was determined spectrophotometrically at 232 nm. A calibration curve showing the relationship between concentration and absorbance was plotted and the equation and correlation values of the curve generated from the scatter plot.

3.2.11.2 Dissolution test procedure

Nine hundred milliliters of each of the following media was employed: 0.1N hydrochloric acid, phosphate buffer (pH 4.5) and phosphate buffer (pH 6.8). Dissolution testing was performed using Erweka Dissolution Apparatus (USP Apparatus 2) at 50 rpm. Six dosage units of each brand were evaluated in the three different dissolution media.

Nine hundred millilitres of 0.1M HCl was placed in each of the six vessels of the dissolution machine. The dissolution medium was then equilibrated to 37 ± 0.5℃, and the paddle speed set to 50 revolutions per minute. One tablet was placed in each of the vessels of the dissolution machine. At specified time intervals of 10, 15, 20, 30, 45, and 60 minutes, 10 ml samples were withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating paddle blade, not less than 1 cm from the vessel wall. To replace the 10 ml sample withdrawn, 10 ml of fresh dissolution medium was added to the vessel from which the volume was withdrawn. The vessel was kept covered for the duration of the test and the temperature of

the medium maintained at 37 ± 0.5 ℃ at all times. The withdrawn samples were filtered with Whatman filter paper (No 5) and diluted 100 times with distilled water. The diluted filtrates were analysed by UV spectrophotometer at a wavelength of 232 nm. Using the equation obtained from the calibration curve, the concentration of metformin hydrochloride in samples taken at times 10, 15, 20, 30, 45, and 60 minutes were calculated and the percentage release values were then calculated. A plot of mean cumulative percentage drug release against time was established. The experiments were repeated using phosphate buffer, pH 4.5 and phosphate buffer, pH 6.8 as dissolution media.

3.2.11.3 Dissolution data comparison

A graph of mean percentage amount of drug dissolved against respective time points was plotted for each brand to obtain the release profiles. An ANOVA based method was also used to statistically analyze the dissolution data obtained at the various time points. One-way ANOVA followed by Dunnett's multiple comparison tests was also used to establish similarity or dissimilarity. The profiles were also compared to that of the reference drug (Glucophage) using simple model independent approach (similarity factor (f_2) and difference factor (f_1) (FDA, 1995). Lastly, the dissolution efficiency (DE) was calculated for each brand.

3.2.11.3.1 Dissolution efficiency

The dissolution efficiency was calculated using the equation below;

DE= Dissolution efficiency = $\{(0\int^{t} Y.dt) / Y100.\ (t_2\text{-}t_1)\}\times 100$

$(0\int^{t} Y.dt)$ = area under the dissolution curve (AUC)

Y= the percentage dissolved at t_2

t_2= time for all active ingredient to dissolve

$t_{1=}$ time at which first sample was withdrawn.

3.2.11.3.2 Difference and Similarity factors

Dissolution data obtained from the dissolution profiles were fitted into equations to determine the difference and similarity factors of the various brands compared to a standard.

$f_1 = \{[S_{t=1}^{n} |R_t - T_t|] / [S_{t=1}^{n} R_t]\} \times 100$..... (1)

$f_2 = 50 \times \log \{[1 + (1/n) S_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$..... (2)

Where f_1 = difference factor

f_2 = similarity factor

n = time points

Rt = cumulative percentage dissolved at time t for the reference

Tt = cumulative percentage dissolved at time t for the test

3.2.11.3.3 ANOVA-based method analysis

The area under dissolution curve (AUC) were determined from the cumulative percentage drug release versus time curves using Graphpad prism and were compared by one-way analysis of variance (one-way ANOVA) followed by Dunnett's test using Graphpad prism (version 5). 'P' Values were obtained after performing ANOVA test. P value gives an idea of whether the means of the paired samples are actually significantly different.

Table 3.3 Interpretation of 'P' values

P value	Meaning
< 0.001	Extremely significant
0.001 to 0.01	Very significant
0.01 to 0.05	Significant
>0.05	Not significant

3.2.11.4 BCS-based biowaiver

The dissolution data of metformin tablets (BCS class 3 drug) in the three dissolution media were subjected to BCS-based biowaiver under WHO criteria (i.e. very rapidly dissolving). The percentage drug dissolution achieved in 15 minutes in the respective dissolution media were estimated.

CHAPTER FOUR

RESULTS

After conducting the appropriate tests on the selected brands of metformin hydrochloride tablets, the following results were obtained;

4.1 Identification test

4.1.1 Identification of metformin hydrochloride RS

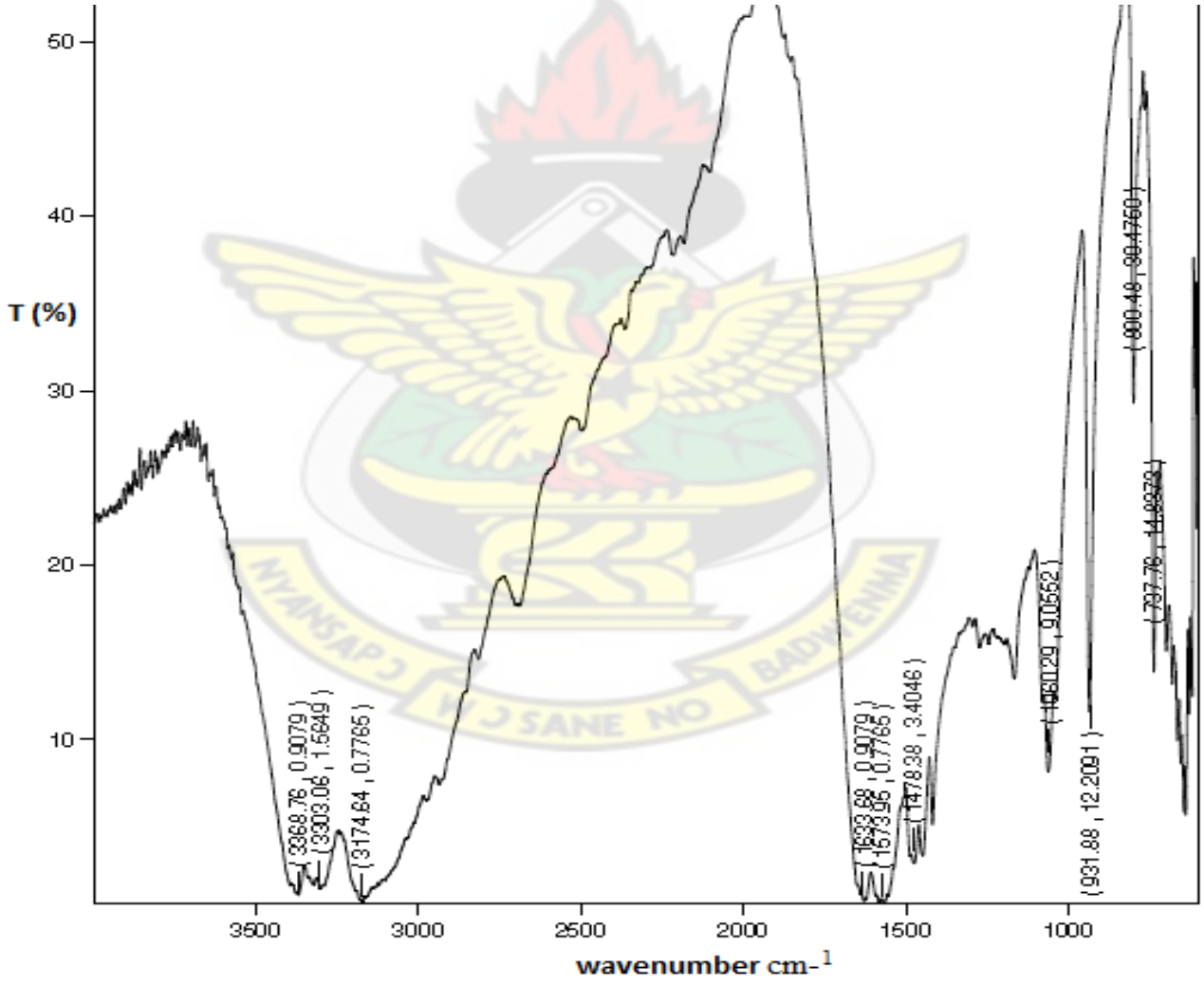

Fig 4.1 IR spectrum of metformin hydrochloride RS

The melting point range of the metformin hydrochloride RS was found to be 223-225℃.

Fig 4.2 Chromatogram of TLC analysis

4.1.2 Identification of metformin hydrochloride tablets

Table 4.1 Rf values of various metformin hydrochloride tablets

Code	Rf value
M1	0.24
M2	0.24
M3	0.24
M4	0.24
M5	0.24
M6	0.24
M7	0.23
M8	0.24
M9	0.24
M10	0.25
M11	0.23
M12	0.24
M13	0.24
M14	0.25
M15	0.25

*Rf value of standard = 0.25, Rf= Retention factor and *standard*= Pure metformin hydrochloride*

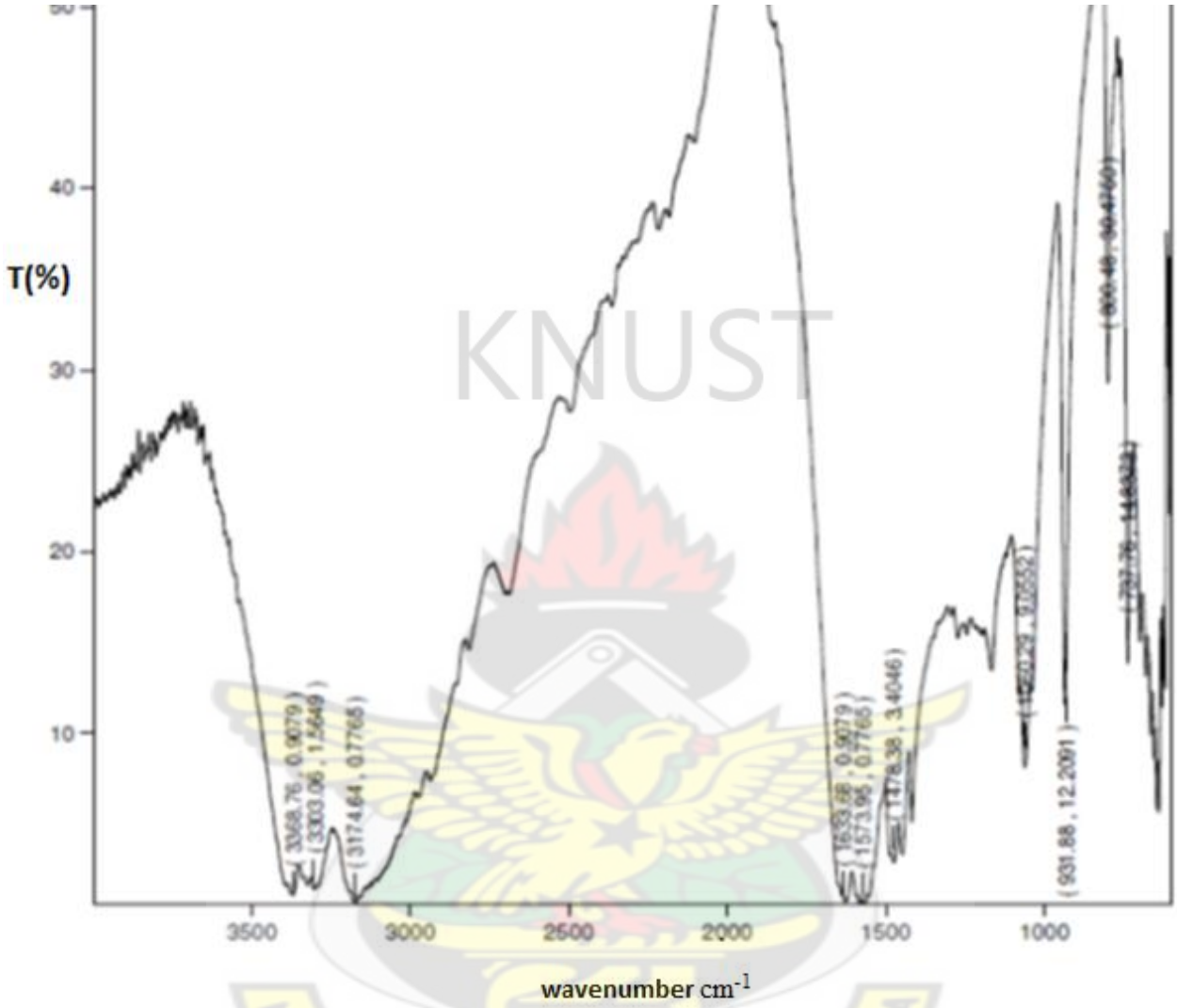

Fig 4.3 IR spectrum of M1

Please, refer to appendix II for the IR spectra for the other brands.

4.2 Tablet thickness

Table 4.2 Thickness of the various brands of metformin hydrochloride tablets, (n=10)

Code	Thickness(mm)
M1	5.18 ±0.015
M2	6.14 ±0.014
M3	6.08 ±0.021
M4	5.60 ±0.035
M5	5.88 ±0.010
M6	5.88 ±0.016
M7	5.67 ±0.016
M8	5.18 ±0.006
M9	5.25 ±0.011
M10	4.74 ±0.02
M11	5.62 ±0.017
M12	6.00 ±0.009
M13	5.93 ±0.008
M14	6.05 ±0.052
M15	5.33 ±0.01

4.3 Weight uniformity test

Table 4.3 Uniformity of weight of the different brands of metformin hydrochloride tablets

Code	Total weight (g)	Mean weight (g)	NO. of tablets deviating by ±5%	NO. of tablets deviating by ±10%	Inference
M1	12.6307	0.6315 ±0.013	nil	nil	passed
M2	11.8328	0.5916 ±0.008	nil	nil	passed
M3	12.9925	0.6496 ±0.006	nil	nil	passed
M4	12.2347	0.6117 ±0.015	one	nil	passed
M5	12.9642	0.6482 ±0.006	nil	nil	passed
M6	11.3571	0.5679 ±0.008	nil	nil	passed
M7	12.3154	0.6158 ±0.009	nil	nil	passed
M8	11.8979	0.5949 ±0.006	nil	nil	passed
M9	12.7870	0.6394 ±0.017	one	nil	passed
M10	12.7326	0.6366 ±0.009	nil	nil	passed
M11	10.6271	0.5314 ±0.004	nil	nil	passed
M12	11.971	0.5986 ±0.007	nil	nil	passed
M13	11.7867	0.5893 ±0.011	nil	nil	passed
M14	11.6205	0.581 ±0.01	nil	nil	passed
M15	12.1866	0.6093 ±0.009	nil	nil	passed

4.4 Friability test

Table 4.4 Friability of the metformin hydrochloride tablets

Code	initial weight (Wi/g)	final weight (Wf/g)	weight loss	% weight loss
M1	6.36	6.32	0.04	0.63
M2	6.49	6.43	0.06	0.93
M3	6.50	6.49	0.01	0.15
M4	6.09	6.05	0.04	0.66
M5	6.47	6.46	0.01	0.15
M6	6.25	6.22	0.03	0.48
M7	6.11	6.10	0.01	0.16
M8	6.56	6.55	0.01	0.15
M9	6.44	6.36	0.08	1.24
M10	6.37	6.31	0.06	0.94
M11	6.38	6.32	0.06	0.94
M12	6.56	6.55	0.01	0.15
M13	6.45	6.44	0.01	0.16
M14	6.31	6.30	0.01	0.16
M15	6.06	6.02	0.04	0.66

4.5 Crushing strength (hardness)

Table 4.5 Hardness of the metformin hydrochloride tablets

Code	Mean force applied (Kgf)
M1	12.16 ±0.85
M2	18.76 ±0.04
M3	14.64 ±0.73
M4	8.24 ±0.97
M5	11.26 ±0.77
M6	18.78 ±0.05
M7	8.34 ±0.99
M8	15.52 ±1.44
M9	6.22 ±0.45
M10	14.62 ±0.79
M11	17.08 ±0.92
M12	13.64 ±0.52
M13	12.2 ±0.42
M14	10.62 ±0.47
M15	14.88 ±0.3

4.6 Disintegration test

Table 4.6 Disintegration time of the metformin hydrochloride tablets

Code	1ST Determ./min	2ND Determ./min	Ave. disintegration time/min
M1*	13.82	13.12	13.47 ±0.495
M2*	12.17	11.56	11.87 ±0.431
M3*	17.07	17.21	17.14 ±0.099
M4	7.38	7.34	7.36 ±0.028
M5*	9.25	9.19	9.22 ±0.042
M6*	8.27	8.35	8.31 ±0.057
M7*	16.50	16.98	16.74 ±0.283
M8	19.45	25.18	22.32 ±4.052
M9*	21.25	20.92	21.09 ±0.233
M10*	16.47	15.82	16.15 ±0.46
M11*	8.05	7.57	7.81 ±0.339
M12*	9.1	8.45	8.78 ±0.46
M13*	8.51	9.12	8.82 ±0.431
M14*	8.12	7.98	8.1 ±0.099
M15	9.4	9.20	9.3 ±0.141

* Film-coated tablets

4.7 Assay

4.7.1 Assay (UV)

Table 4.7 Conc. against absorbance for calibration curve for metformin hydrochloride

Concentration %w/v	Mean absorbance
0.0001	0.283 ±0.010
0.00025	0.385 ±0.005
0.0003	0.466 ±0.004
0.0005	0.651 ±0.002
0.0007	0.779 ±0.006

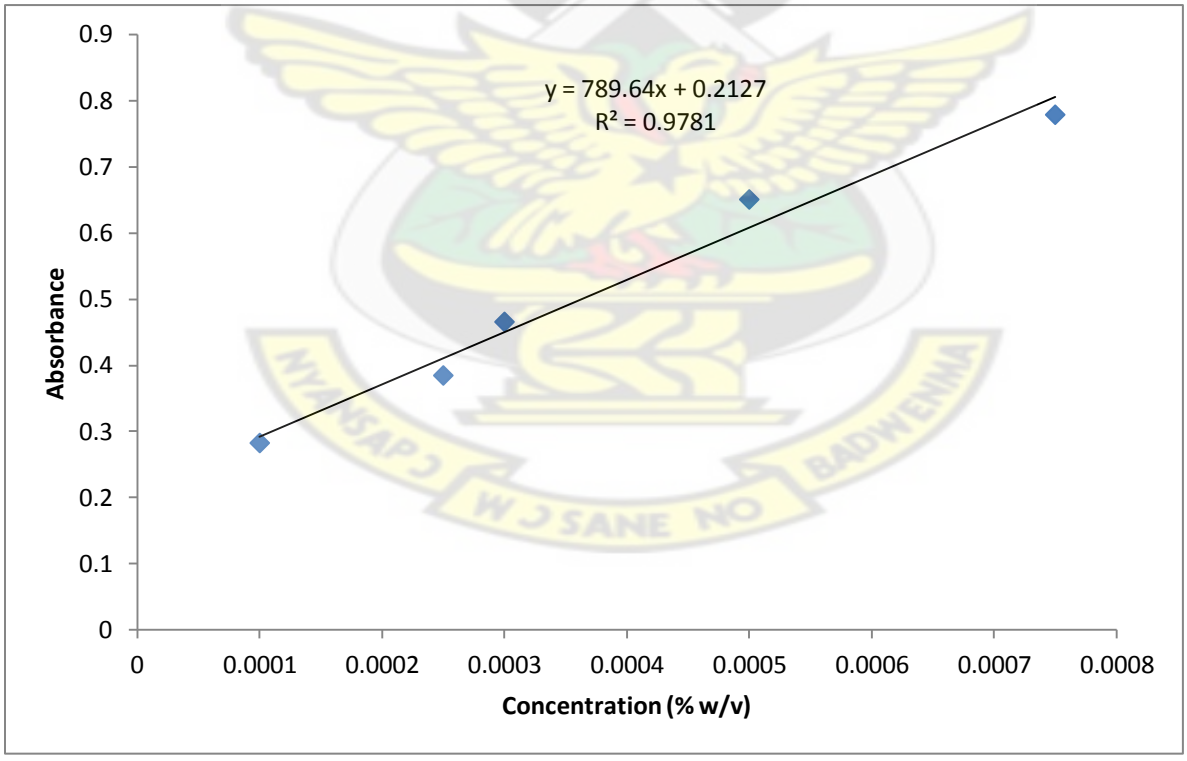

Fig 4.4 Calibration curve for metformin hydrochloride (UV)

Table 4.8 Assay of metformin hydrochloride tablets (UV)

Code	Mean Absorbance	Assay (%)
M1	0.994	98.94
M2	0.984	97.68
M3	1.004	100.21
M4	1.005	100.34
M5	1.058	107.05
M6	1.036	104.26
M7	1.007	100.59
M8	1.000	99.70
M9	1.068	108.32
M10	1.011	101.10
M11	1.031	103.63
M12	1.084	110.34
M13	0.998	99.45
M14	0.992	98.69
M15	0.989	98.31

(BP Range = 95.0-105 %)

4.7.2 Assay (HPLC)

Table 4.9 Conc. against peak area for calibration curve for metformin hydrochloride

Concentration %w/v	Mean Peak Area
0.00025	461253
0.001	1215559
0.0015	1757046
0.002	2358730
0.0025	2918570
0.005	4844899

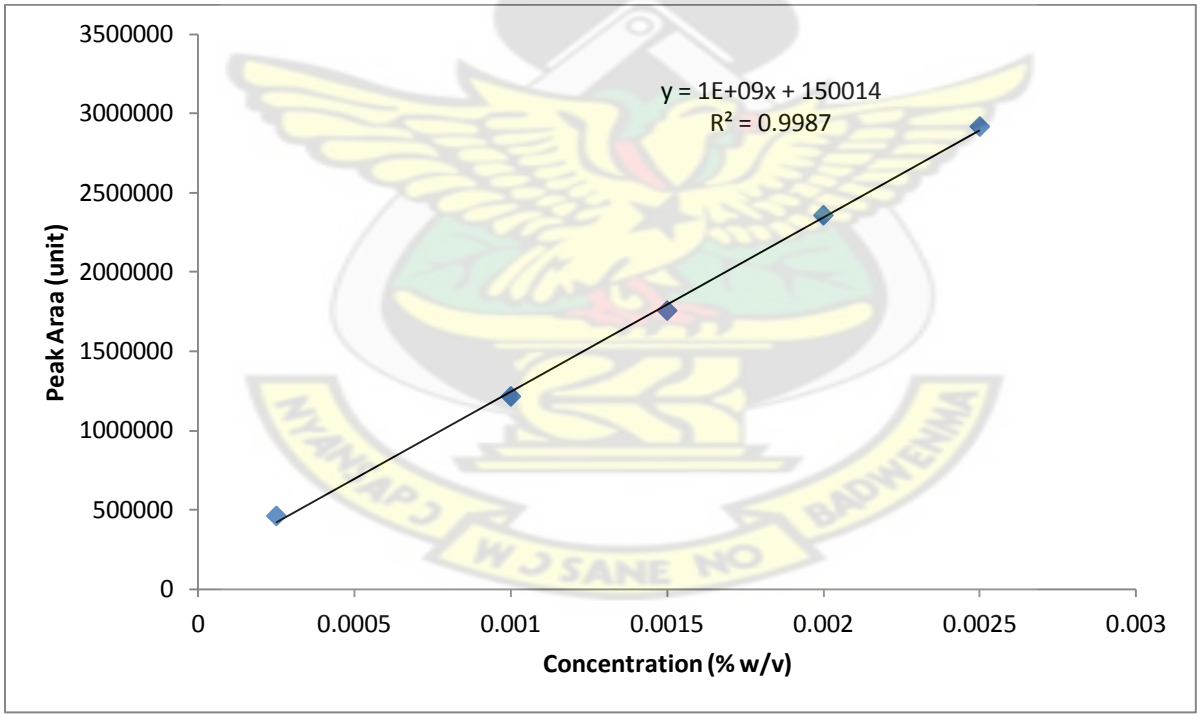

Fig 4.5 Calibration curve for metformin hydrochloride (HPLC)

Mobile phase= acetonitrile: phosphate buffer (65:35) pH adjusted to 5.75

Table 4.10 Assay of metformin hydrochloride tablets (HPLC)

Code	Mean Peak Area	Assay (%)
M1	1147377	99.74
M2	1145257	99.52
M3	1192084	104.21
M4	1160672	101.01
M5	1228364	107.84
M6	1170495	102.05
M7	1173944	102.39
M8	1159302	100.93
M9	1259945	110.99
M10	1182586	103.26
M11	1184686	103.47
M12	1236548	108.65
M13	1154962	100.49
M14	1149348	99.93
M15	1139967	98.99

(BP Range = 95.0-105 %)

4.8 In vitro dissolution studies

Calculation of percentage drug release

Strength of metformin hydrochloride = 500mg

Volume of dissolution medium (phosphate buffer, pH= 6.8) = 900ml

From the calibration curve, the equation of the graph of pure metformin hydrochloride powder dissolved in water:

$y = 832.5x + 0.1523$

Where, y= mean absorbance at time t, and x = concentration

Hence $x = y-0.1523/832.5$

Thus for M1 (A1), at 10 minutes, the mean absorbance was 0.409, therefore,

$x = (0.409 – 0.1523/ 832.5)$

$x = 0.0003084$ % w/v

Multiplying 'x' by the dilution factor of 100 gives the concentration of drug dissolved.

$x = 0.03084$ % w/v

Thus, 100ml of solution = 0.03084 g of metformin hydrochloride

900ml = (0.03084 x 900/100) = 0.2776 g

Amount of drug released at the 5^{th} minute = 0.2776 g

% drug release = (amount of drug released/ amount of drug expected)*100

Hence, % drug release = (0.2776/0.5)*100 = **55.51%**

At 15^{th} minute, y = 0.469, therefore,

x at 15^{th} minute = (0.469-0.1523/832.5) = 0.0003804%

Multiplying 'x' by the dilution factor of 100 gives the concentration of drug dissolved

x = 0.03804%

Thus, 100ml of solution = 0.03804 g of metformin hydrochloride

900ml = (0.03804 x 900/100) = 0.3424 g

Amount of drug released at the 15^{th} minute = 0.3424 g

But weight of metformin hydrochloride in 10ml aliquot pipetted at the 10^{th} minute;

900ml = 0.2776 g

Therefore, 10ml = (0.2776x10)/900 = 0.00308 g

Thus, total amount of metformin hydrochloride released at the 15^{th} minute = 0.3424+0.00308

= 0.3455 g

Hence, % drug release = (0.3455/0.5)*100 = **69.1%**

The calculations were repeated for the percentage drug release at various times for other brands.

4.8.1 Percentage drug release

Table 4.11 Percentage drug release of M1, M2, M3, M4 and M11 in 0.1N HCl, (n=6)

	M11 (R)	M1	M2	M3	M4
Time (min)		**Percentage Drug Release**			
10	71.83 ±1.68	49.33 ±2.69	63.00 ±2.71	46.17 ±2.72	71.43 ±2.80
15	77.99 ±1.32	68.21 ±3.01	80.37 ±1.52	58.85 ±6.24	84.56 ±1.99
20	84.12 ±1.53	79.97 ± 2.02	83.09 ±0.85	69.43 ±6.19	87.16 ±1.01
30	91.91 ±2.26	84.24 ±1.95	86.09 ±0.85	85.25 ±1.61	89.04 ±0.71
45	92.11 ±2.33	84.92 ±1.33	86.35 ±0.77	85.55 ±1.52	89.15 ±0.85
60	93.05 ±1.48	85.36 ±0.78	86.59 ±1.13	86.05 ±0.92	89.17 ±1.76

Table 4.12 Percentage drug release of M5, M6, M7, M8 and M9 in 0.1N HCl, (n=6)

	M5	M6	M7	M8	M9
Time (min)		**Percentage Drug Release**			
10	70.77 ±1.97	79.5 ±0.99	66.07 ±2.5	41.33 ±2.5	62.5 ±2.05
15	85.19 ±2.19	85.82 ±1.29	77.2 ±2.36	56.26 ±6.36	70.46 ±3.46
20	87.89 ±0.73	88.56 ±0.65	82.95 ±1.23	73.21 ±3.78	77.64 ±2.66
30	88.26 ± 1.37	88.06 ±1.08	88.52 ±1.51	82.91 ±2.66	87.32 ±1.28
45	88.35 ±1.31	88.57 ±0.96	88.51 ±1.4	87.88 ±1.36	89.23 ±1.61
60	88.77 ±1.21	90.97 ±0.64	90.42 ±1.1	89.23 ±1.61	90.73 ±0.83

Table 4.13 Percentage drug release of M10, M12, M13, M14 and M15 in 0.1N HCl, (n=6)

	M10	M12	M13	M14	M15
Time (min)			**Percentage Drug Release**		
10	44.8 ±2.12	61.1 ±2.11	69.83 ±2.55	72.9 ±3.97	73.83 ±2.86
15	57.47 ±2.34	72.51 ±2.69	81.52 ±1.29	83.91 ±4.64	83.59 ±1.69
20	70.23 ±5.86	79.38 ±1.47	85.93 ±1.48	88.29 ±1.93	86.61 ±1.07
30	85.17 ±1.78	85.04 ±1.29	87.4 ±1.93	90.03 ±1.39	90.02 ±3.81
45	87.69 ±0.95	87.23 ±1.69	89.48 ±0.39	90.07 ±1.48	89.04 ±1.32
60	90.03 ±1.62	88.66 ±1.39	91.26 ±0.33	90.39 ±1.08	89.72 ±1.52

Table 4.14 Percentage drug release of M1, M2, M3, M4 and M11 in Phosphate buffer (pH 4.5), (n=6)

	M11 (R)	M1	M2	M3	M4
Time (min)			**Percentage Drug Release**		
10	71.47 ±2.13	66.94 ±2.52	69.56 ±2.07	66.49 ±2.29	54.96 ±3.23
15	79.17 ±1.91	75.53 ±2.42	78.98 ±1.62	76.03 ±2.32	70.17 ±4.38
20	84.30 ±1.86	81.09 ±1.60	82.48 ±1.57	80.89 ±1.25	78.22 ±2.19
30	87.78 ±1.47	85.82 ±1.77	86.94 ±1.88	85.78 ±1.37	83.76 ±2.02
45	89.52 ±0.82	89.34 ±0.91	89.43 ±0.99	89.15 ±0.97	86.98 ±1.55
60	90.29 ±0.88	90.55 ±0.55	90.31 ±0.72	89.91 ±0.76	88.55 ±1.17

Table 4.15 Percentage drug release of M5, M6, M7, M8 and M9 in Phosphate buffer (pH 4.5), (n=6)

	M5	M6	M7	M8	M9
Time (min)			**Percentage Drug Release**		
10	69.67 ±2.79	68.84 ±2.16	53.45 ±3.66	44.77 ±3.71	65.34 ±2.31
15	78.63 ±2.52	75.19 ±2.73	67.14 ±4.22	62.09 ±5.23	71.55 ±2.53
20	83.16 ±2.48	82.26 ±1.21	78.25 ±2.26	73.08 ±5.79	80.47 ±2.22
30	87.53 ±1.75	87.41 ±1.32	83.96 ±1.77	79.54 ±3.57	85.28 ±1.34
45	89.55 ±1.12	89.54 ±0.41	86.85 ±1.09	83.46 ±2.11	87.79 ±1.16
60	90.69 ±0.88	90.32 ±0.43	87.89 ±1.18	85.29 ±1.30	88.87 ±1.10

Table 4.16 Percentage release of M10, M12, M13, M45 and M15 in Phosphate buffer (pH 4.5), (n=6)

	M10	M12	M13	M14	M15
Time (min)			**Percentage Drug Release**		
10	64.77 ±1.72	66.31 ±2.08	71.25 ±2.89	66.71 ±2.42	52.12 ±2.97
15	74.28 ±1.54	77.10 ±2.41	78.38 ±2.16	76.03 ±1.99	66.78 ±2.24
20	81.0 ±1.50	83.65 ±1.54	83.21 ±1.44	82.49 ±1.25	70.19 ±4.65
30	86.32 ±1.51	88.70 ±0.54	88.02 ±1.41	87.85 ±1.69	84.22 ±1.14
45	88.87 ±0.42	89.79 ±0.59	89.64 ±0.99	89.27 ±0.89	88.45 ±0.93
60	89.89 ±0.99	90.22 ±0.33	90.06 ±0.71	89.87 ±1.16	89.32 ± 0.74

Table 4.17 Percentage drug release of M1, M2, M3, M4 and M11 in Phosphate buffer (pH 6.8), (n=6)

	M11 (R)	M1	M2	M3	M4
Time (min)			**Percentage Drug Release**		
10	65.45 ±2.84	53.70 ±2.32	60.40 ±3.49	57.95 ±1.59	54.82 ±2.68
15	77.42 ±1.64	66.23 ±2.83	69.58 ±3.67	65.80 ±2.58	64.41 ±2.18
20	87.24 ±1.37	75.71 ±3.11	78.09 ±2.80	77.74 ±2.31	76.46 ±2.88
30	91.22 ±1.42	83.35 ±1.73	85.50 ±2.01	86.77 ±1.64	85.51 ±1.83
45	92.29 ±0.57	86.66 ±1.56	86.35 ±0.79	88.69 ±0.58	87.80 ±0.77
60	93.16 ±0.59	86.74 ±0.69	87.18 ±1.0	88.87 ±1.31	88.99 ±1.15

Table 4.18 Percentage drug release of M5, M6, M7, M8 and M9 in Phosphate buffer (pH 6.8), (n=6)

	M5	M6	M7	M8	M9
Time (min)			**Percentage Drug Release**		
10	62.99 ±3.18	67.14 ±2.88	60.22 ±2.65	49.23 ±1.69	43.21 ±4.60
15	73.89 ±1.87	74.78 ±2.64	70.23 ±3.04	67.94 ±2.63	50.43 ±3.17
20	80.19 ±1.86	82.12 ±1.33	81.74 ±1.49	71.57 ±3.0	57.91 ±2.72
30	86.15 ±1.69	87.33 ±1.51	86.41 ±1.07	81.18 ±1.53	73.68 ±4.72
45	88.15 ±1.10	89.09 ±1.40	88.61 ±0.44	85.16 ±1.89	83.37 ±2.46
60	88.69 ±0.76	89.63 ±1.09	89.63 ±0.65	87.12 ±1.08	86.15 ±1.30

Table 4.19 Percentage release of M10, M12, M13, M14 and M15 in Phosphate buffer (pH 6.8), (n=6)

	M10	M12	M13	M14	M15
Time (min)	**Percentage Drug Release**				
10	54.71 ±2.24	65.48 ±2.84	67.87 ±2.21	67.50 ±2.66	52.80 ±2.45
15	66.35 ±2.03	77.42 ±1.64	77.95 ±1.44	75.86 ±2.37	65.93 ±2.81
20	76.84 ± 1.89	87.24 ±1.37	84.07 ±1.41	82.82 ±1.80	76.64 ±2.12
30	83.81 ±1.55	91.22 ±1.42	88.80 ±1.23	88.69 ±1.25	82.99 ±1.57
45	87.28 ±1.46	92.29 ±0.57	91.21 ±0.91	91.09 ±0.97	87.63 ±1.38
60	89.07 ±0.99	93.16 ±0.59	91.89 ±0.65	92.03 ±0.79	88.64 ±1.35

4.8.2 Dissolution profiles

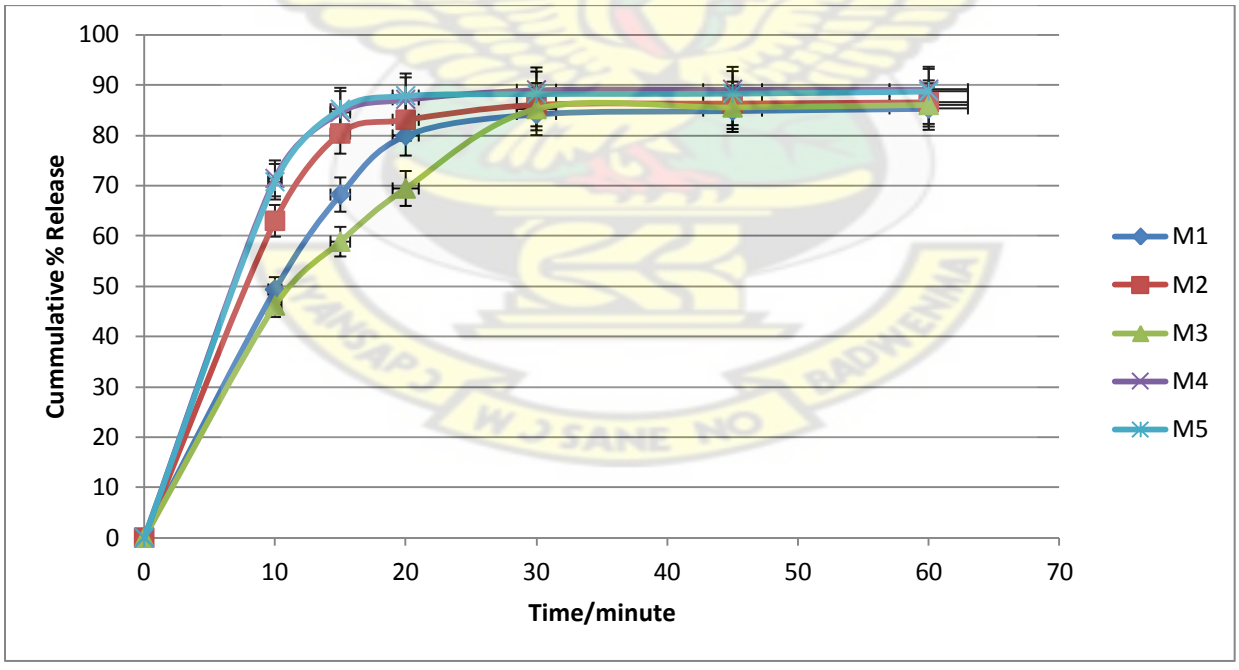

Fig 4.6 Dissolution profiles of M1, M2, M3, M4 and M5 in 0.1N HCl

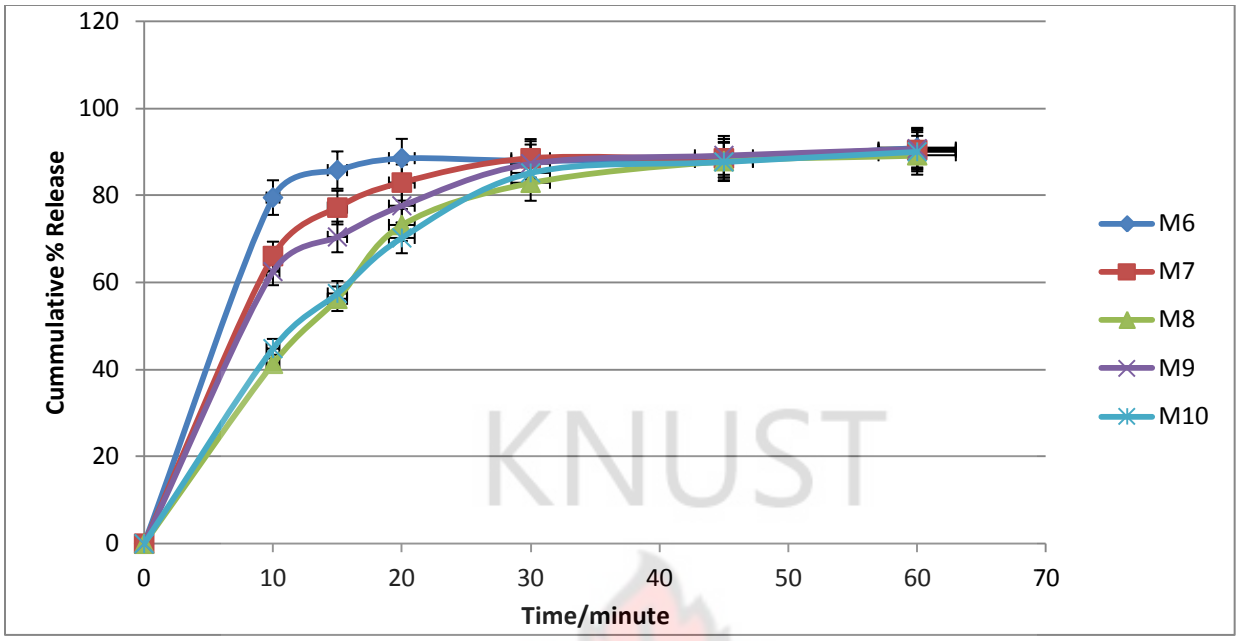

Fig 4.7 Dissolution profiles of M6, M7, M8, M9 and M10 in 0.1N HCl

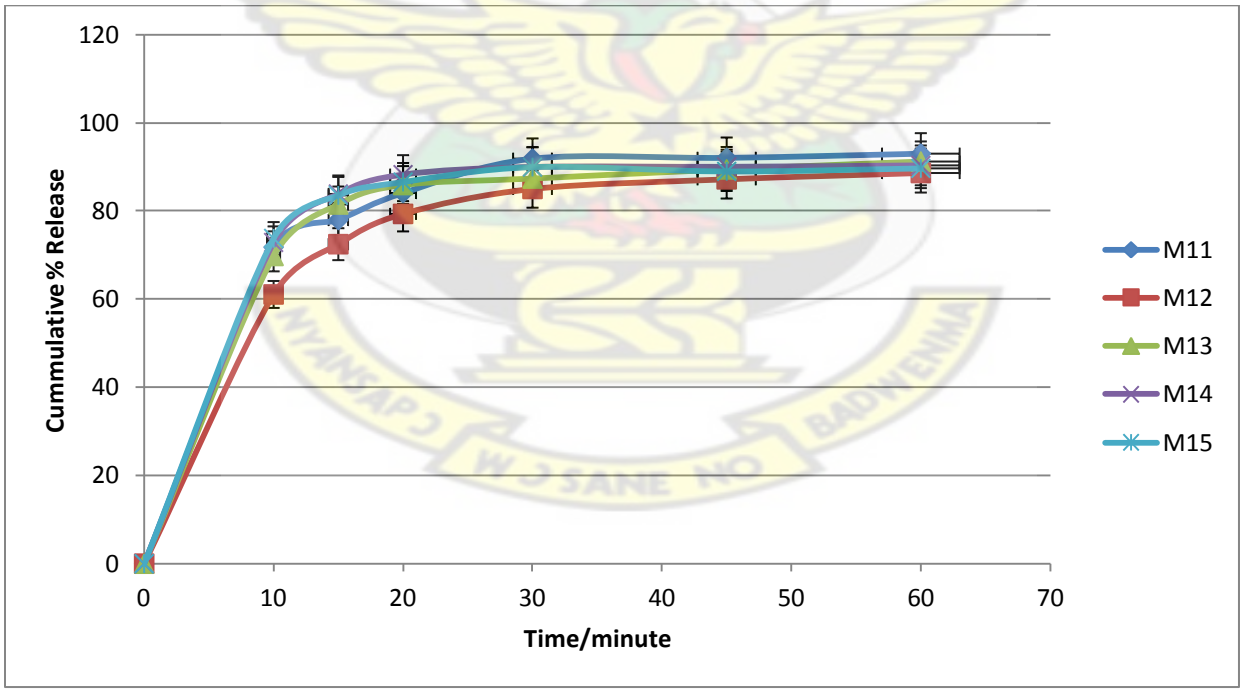

Fig 4.8 Dissolution profiles of M11, M12, M13, M14 and M15 in 0.1N HCl

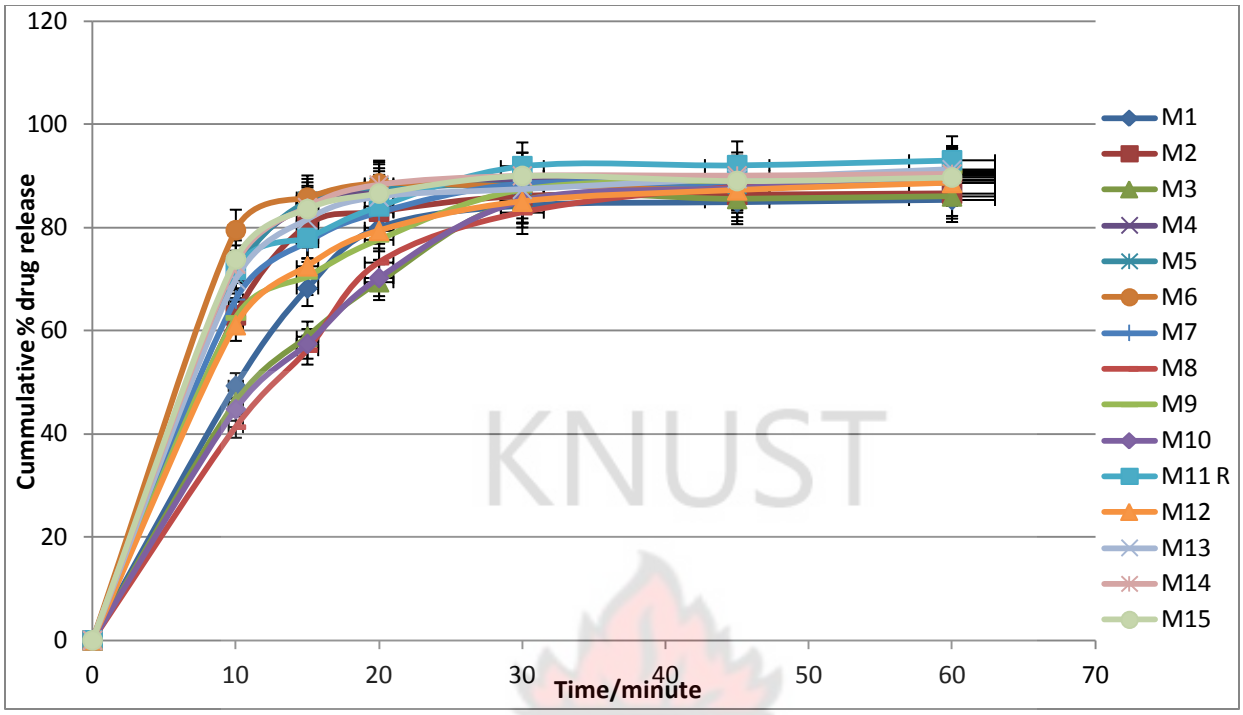

Fig 4.9 Dissolution profiles of all the metformin brands in 0.1M HCl

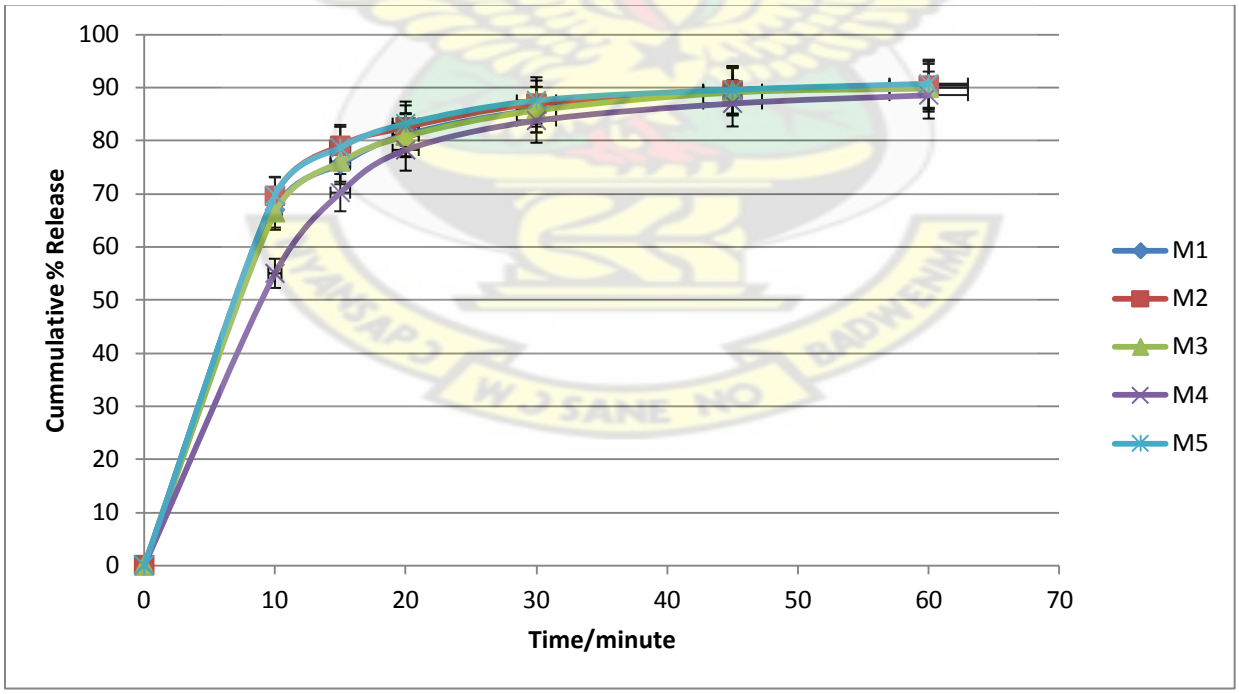

Fig 4.10 Dissolution profiles of M1, M2, M3, M4 and M5 in phosphate buffer (pH 4.5)

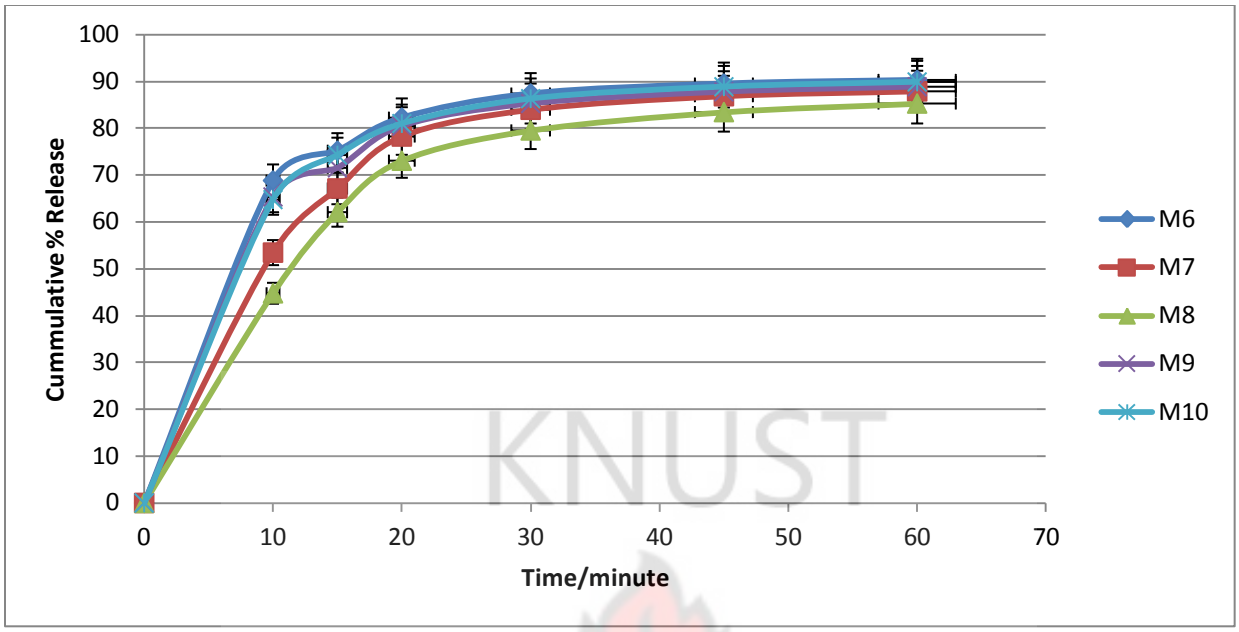

Fig 4.11 Dissolution profiles of M6, M7, M8, M9 and M10 in phosphate buffer (pH 4.5)

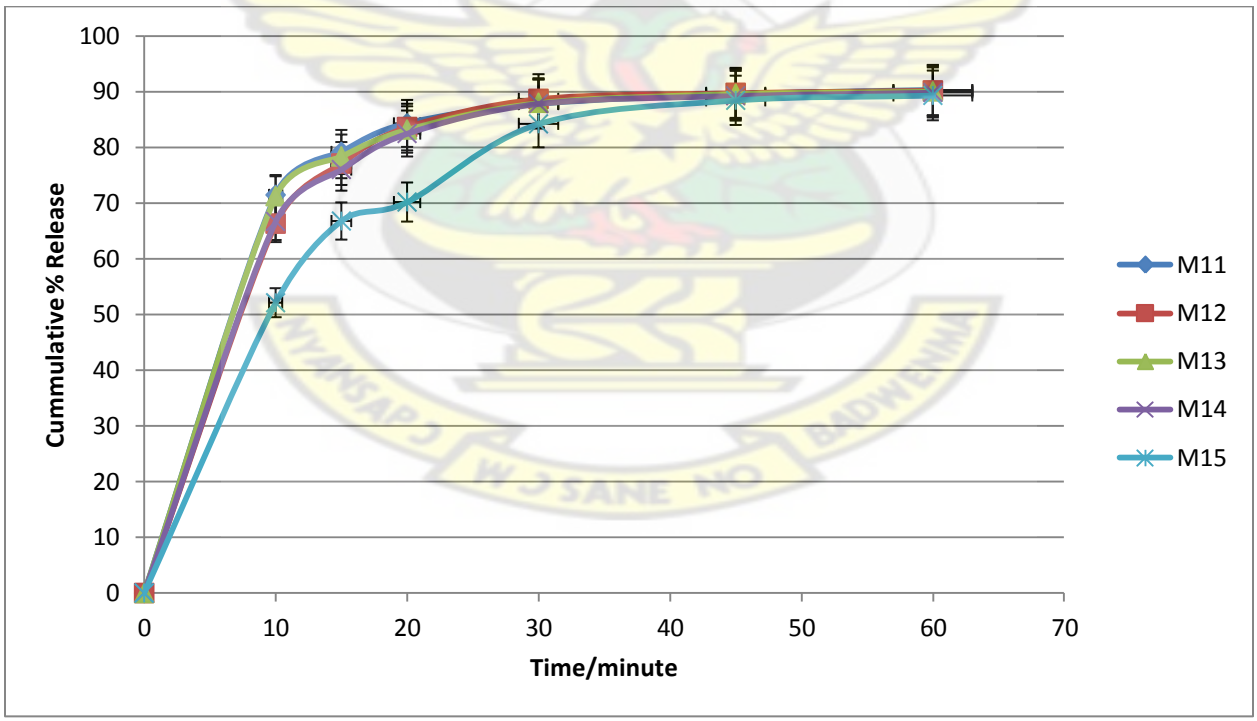

Fig 4.12 Dissolution profiles of M11, M112, M13, M14 and M15 in phosphate buffer (pH 4.5)

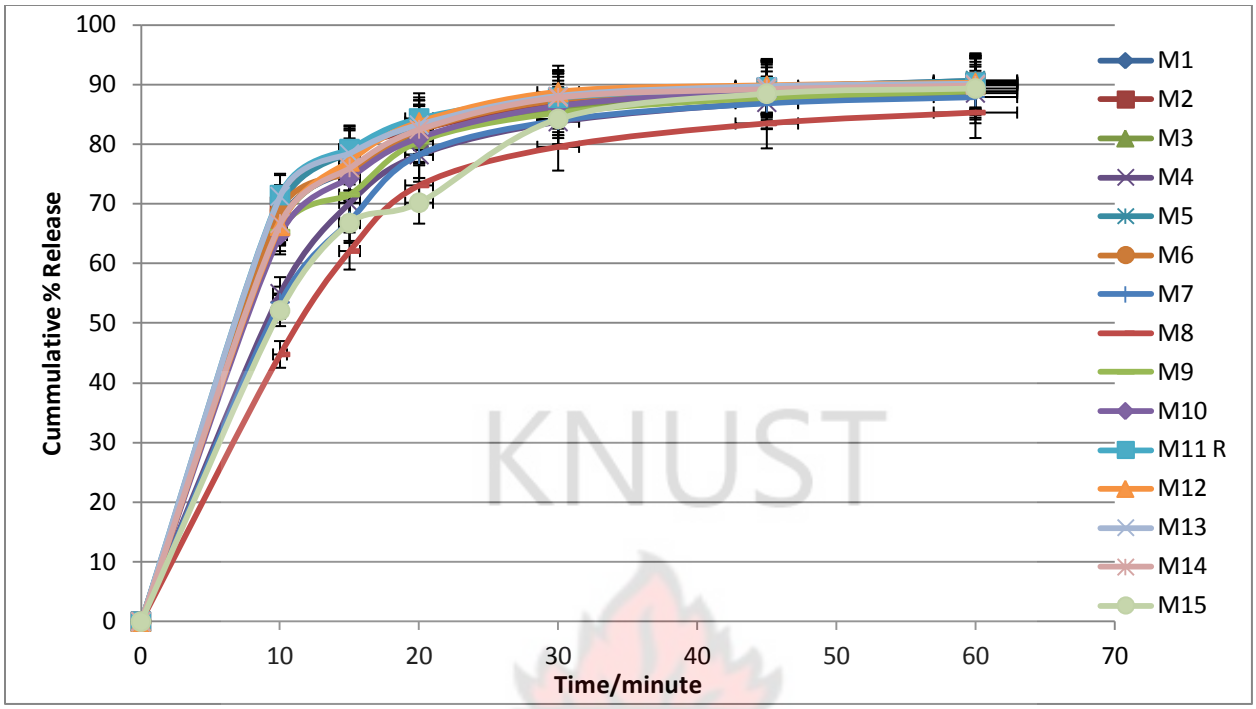

Fig 4.13 Dissolution profiles of all the metformin brands in phosphate buffer (pH4.5)

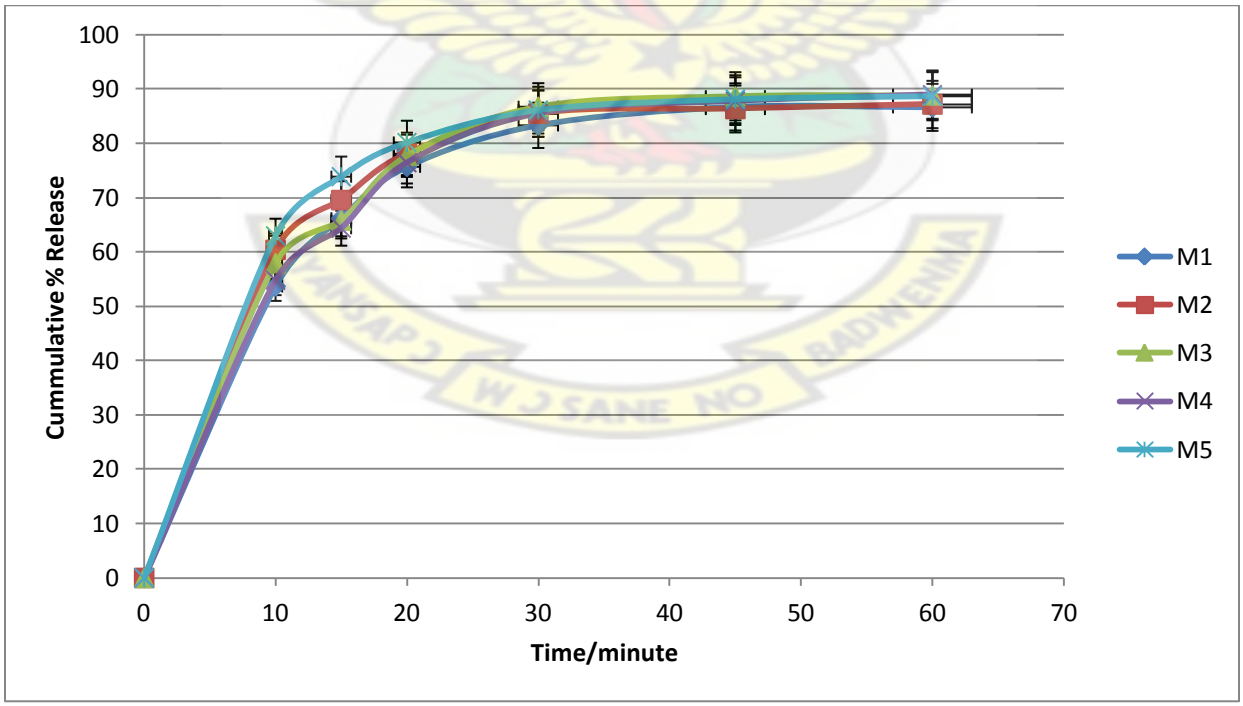

Fig 4.14 Dissolution profiles of M1, M2, M3, M4 and M5 in phosphate buffer (pH 6.8)

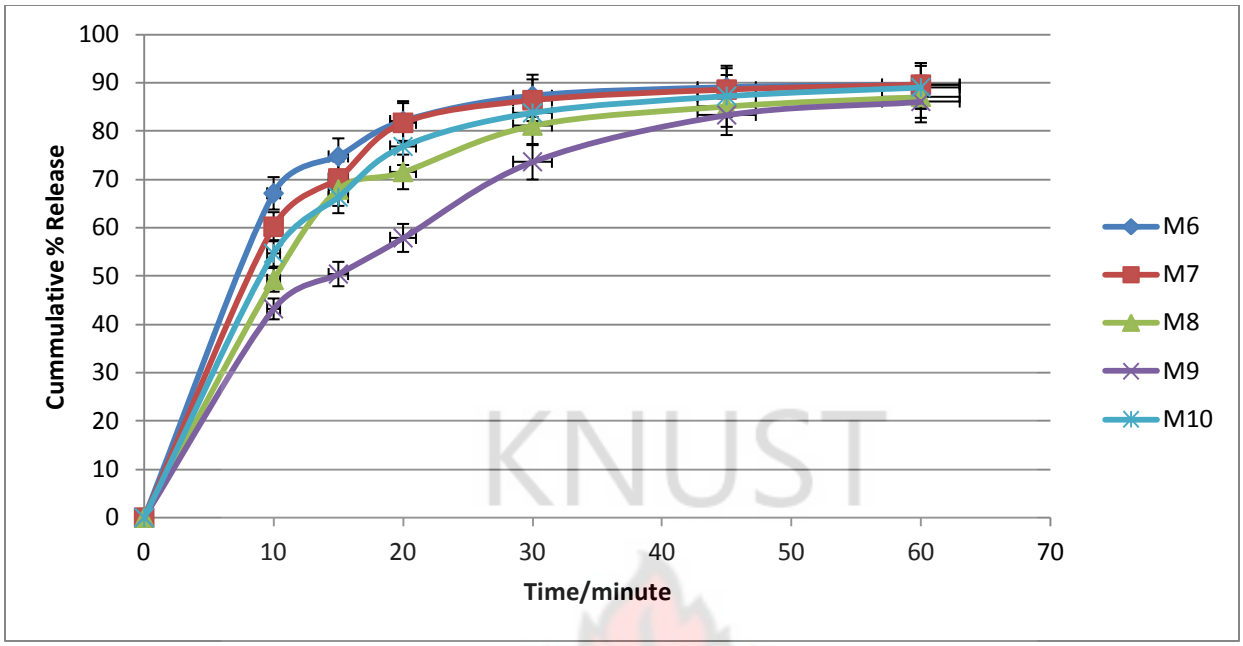

Fig 4.15 Dissolution profiles of M6, M7, M8, M9 and M10 in phosphate buffer (pH 6.8)

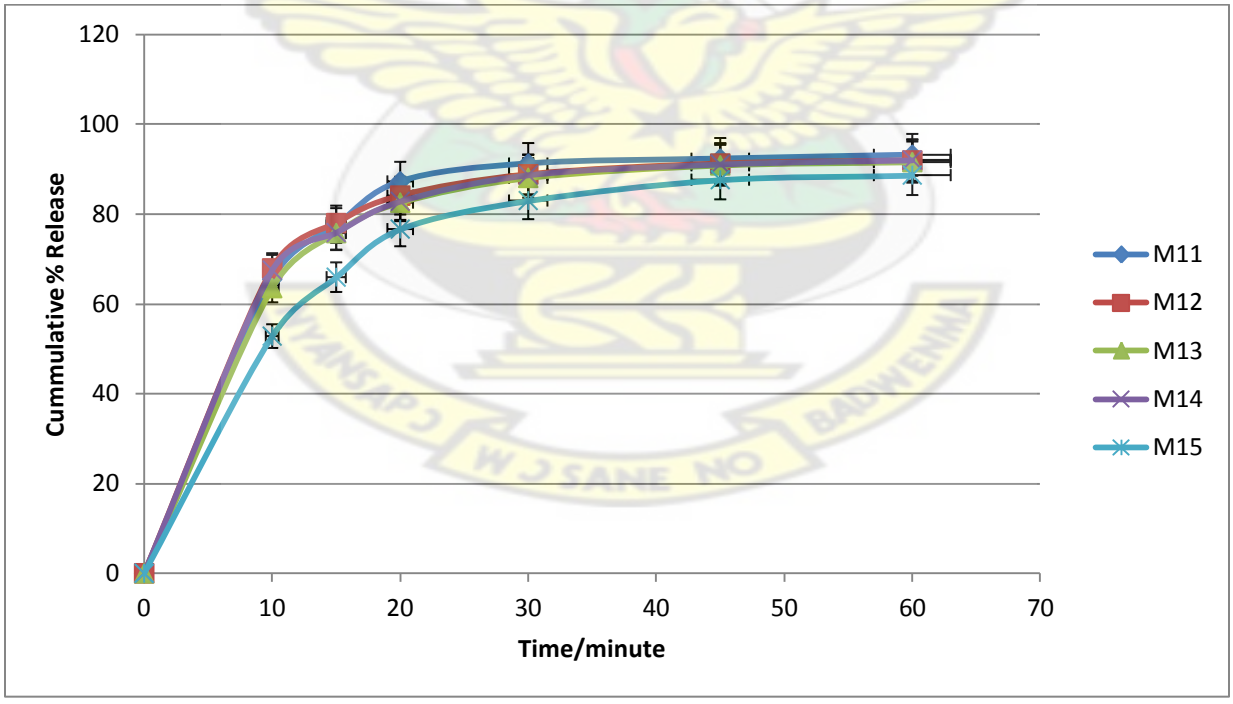

Fig 4.16 Dissolution profiles of M11, M12, M13, M14 and M15 in phosphate buffer (pH 6.8)

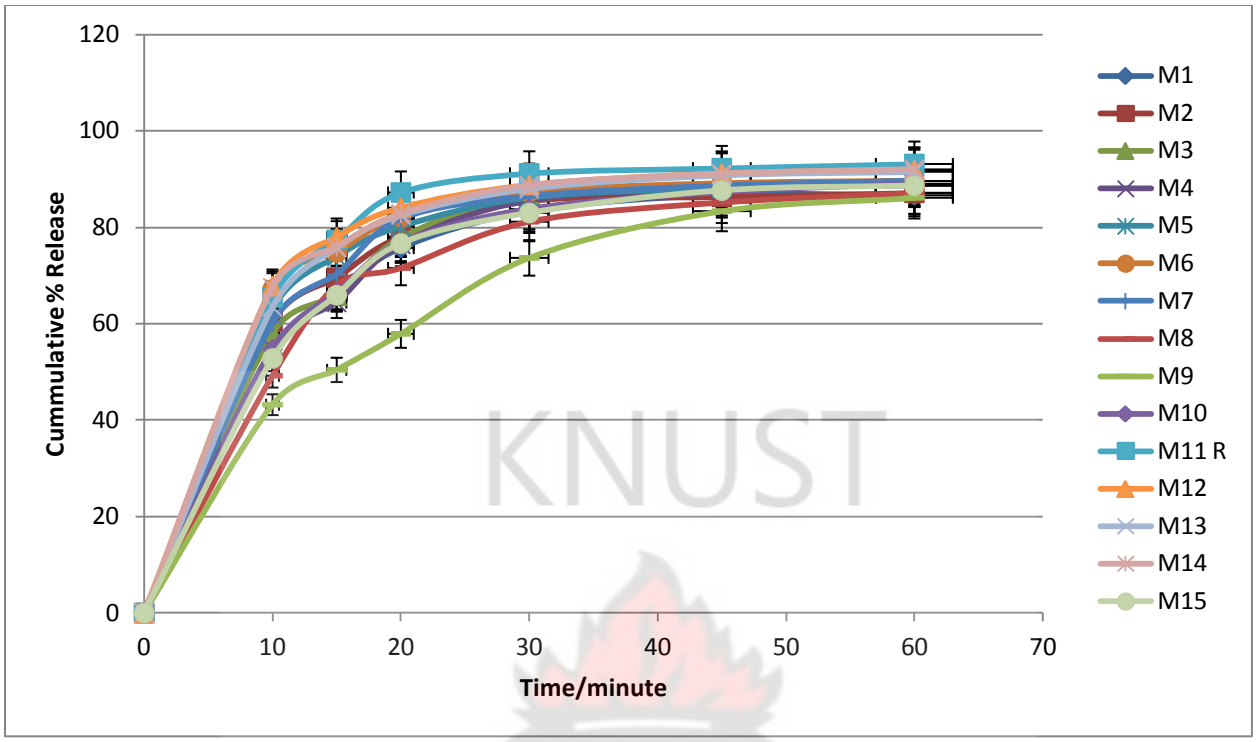

Fig 4.17 Dissolution profile of all the metformin brands in phosphate buffer (pH6.8)

4.8.3 Dissolution profile comparison

4.8.3.1 Dissolution efficiency (DE)

Sample calculation

For M1 (phosphate buffer, pH 6.8)

AUC = 4026

AUC was determined from the mean percentage drug release using Graphpad prism.

Y= 86.7% (total amount of drug dissolved within 60 minutes)

Using the equation, Dissolution efficiency = $\{(0\int^{t} Y.dt) / Y100. (t_2-t_1)\}\times100$

$(0\int^{t} Y.dt)$ = area under the dissolution curve (AUC)

Y= the percentage dissolved at t_2

t_2= time for all active ingredient to dissolve

$t_{1=}$ time at which first sample was withdrawn

$$DE= \frac{4026\times 100}{86.7\ (60-10)}$$

$$= 92.87\%$$

Table 4.20 Dissolution efficiencies of the various brands of metformin hydrochloride tablets

	DE (%)	Dissolution medium	
Sample code	0.1M HCl	phosphate buffer pH(4.5)	phosphate buffer pH(6.8)
M1	94.5	93.8	92.9
M2	97.1	95.1	94.1
M3	91.3	94.3	93.1
M4	98.1	92.5	91.8
M5	98.0	95.0	94.5
M6	96.8	94.3	95.0
M7	94.8	92.6	93.7
M8	88.7	90.4	90.6
M9	88.5	89.9	83.9
M10	93.5	94.0	91.4
M11	95.2	95.8	95.0
M13	95.2	95.8	93.7
M14	97.7	95.2	94.2
M15	97.8	90.5	91.5

4.8.3.2 Similarity factor of the brands using Glucophage as reference product

Table 4.21 Similarity factor (f_2) of metformin tablets using M11 as reference product

	Dissolution medium		
Sample code	0.1M HCl	phosphate buffer pH(4.5)	phosphate buffer pH(6.8)
M1	28	69	39
M2	55	86	51
M3	33	69	49
M4	63	42	45
M5	60	89	59
M6	56	75	65
M7	66	39	57
M8	27	30	35
M9	53	60	15
M10	33	64	41
M12	52	73	76
M13	69	94	70
M14	66	72	72
M15	66	36	40

4.8.3.2 Difference factor of the brands using Glucophage as reference product

Table 4.20 Difference factor (f_1) of metformin tablets using M11 as reference product

	Dissolution medium		
Sample code	0.1N HCl	phosphate buffer pH (4.5)	phosphate buffer pH (6.8)
M1	12	3	11
M2	6	1	8
M3	16	3	8
M4	4	8	10
M5	5	1	5
M6	6	2	4
M7	3	9	6
M8	16	15	13
M9	6	5	22
M10	15	3	10
M12	7	3	2
M13	3	1	3
M14	3	2	3
M15	4	10	10

4.8.3.3 Dissolution profile comparison using one-way ANOVA followed by Dunnett's test

Table 4.21 Comparison of Reference drug, M11 against Test drugs using one-way ANOVA followed by Dunnett's test

Dunnett's multiple comparisons test	phosphate buffer pH (6.8)- SD	phosphate buffer pH (4.5) -SD	0.1N HCl- SD
M11 vs. M1	Ns	Yes (***)	Ns
M11 vs. M2	Ns	Ns	Ns
M11 vs. M3	Ns	Ns	Ns
M11 vs. M4	Yes (***)	Ns	Ns
M11 vs. M5	Ns	Ns	Ns
M11 vs. M6	Ns	Ns	Ns
M11 vs. M7	Yes (***)	Yes (**)	Ns
M11 vs. M8	Yes (***)	Yes (***)	Yes (***)
M11 vs. M9	Yes (**)	Yes (***)	Yes (***)
M11 vs. M10	Ns	Yes (***)	Yes (***)
M11 vs. M12	Ns	Yes (****)	Ns
M11 vs. M13	Ns	Ns	Yes (*)
M11 vs. M14	Ns	Ns	Ns
M11 vs. M15	Yes (***)	Ns	Yes (***)

ANOVA summary, *** P value < 0.0001, ** P value < 0.001,* $P < 0.01$ *and ns* $P > 0.05$, *SD-significance of difference and *Ns-non significant

CHAPTER FIVE

DISCUSSION

5.1 Information gathered on selected metformin hydrochloride tablet brands

Table 3.1 and 3.2 show the information gathered on the fifteen selected brands of metformin hydrochloride tablets. All samples selected were coded and analysis was performed without the packaging. Only codes were used to identify the various samples and thus any possible bias was avoided. Type of tablet, country of manufacture (origin), shelf-life and other relevant information on the packaging were recorded. Each of the samples purchased had at least 6 months left on the shelf-life and all analytical procedures were carried out before product expiration dates. Twelve brands were imported products from foreign countries while three were manufactured locally and this suggests the high prevalence of different brands of metformin tablets in the country.

5.2 Characterization of pure metformin (RS) and metformin hydrochloride tablets

Identification test is one of the important preliminary tests that need to be carried out whenever there is justification for it. The identity of the sample under test as well as the reference sample (pure sample) needs to be ascertained before further experimental work could be done.

The pure metformin hydrochloride (reference standard) was subjected to specific tests namely, melting point determination and infra-red (IR) spectroscopy as stated in literature (British Pharmacopoeia, 2013). The melting point of the pure metformin hydrochloride was found to be 223–225 ^{O}C which complied with the official specification of 222–226 ^{o}C (British Pharmacopoeia, 2013). The reference IR spectrum of pure metformin hydrochloride (Appendix I) is characterized by absorptions bands at the following wavenumbers; 740, 935, 1075, 1063,

1580 and 1620 cm^{-1}. N-H wagging vibrations are assigned to absorption bands occurring at 740 and 935 cm^{-1}; C-N stretch vibrations for 1063 and 1075 cm^{-1} and C=N stretch vibrations accounting for absorption bands at 1580 and 1620 cm^{-1} (Gunasekaran *et al*., 2006). The IR spectrum obtained for the pure metformin hydrochloride (Fig 4.1) exhibited absorption bands around aforementioned wavenumbers. The pure metformin hydrochloride was therefore considered suitable for use as reference standard.

The various brands of metformin hydrochloride tablets were also subjected to both infra-red spectroscopy and thin layer chromatography analysis. The IR spectrum obtained for each metformin hydrochloride tablet brand (Appendix II) had absorption bands at the following wavenumbers 740, 935, 1075, 1063, 1620 and 1580 cm^{-1} similar to that of the reference spectrum. Using thin layer chromatography, the principal spot in the chromatogram obtained for each brand (Fig 4.2) of metformin tablet was similar in position, colour and size to the principal spot in the chromatogram obtained with the pure metformin hydrochloride. The retention factor (Rf value) for each brand (Table 4.1) was almost the same as that of the pure metformin hydrochloride (Rf value of 0.25). From the identification tests, the results indicated that all the brands of metformin hydrochloride tablets did contain metformin hydrochloride as the active pharmaceutical ingredient.

5.3 Quality assessment of metformin hydrochloride tablets

The capacity to identify substandard pharmaceutical products on the market is a crucial component as far as drug quality assurance system is concerned. Quality evaluation studies are primarily important to provide information on the drug content and second, to identify the (cause), if any, of the poor quality on the market. The formulation of the drug product can have a significant effect on the quality parameters such as weight variation, hardness, friability,

disintegration time and dissolution profile. This also includes the physiochemical properties of the active ingredients and excipients as well as the procedures used in the manufacturing process (Ofori-Kwakye *et al.,* 2010; Kalakuntla *et al.,* 2010). Moreover, quality control parameters are useful tools for maintaining consistency in batch-to-batch manufacturing and it should be performed for every drug product. All of these parameters are closely related to each other and have effect on drug absorption, bioavailability etc. (Awofisayo *et al*., 2010; Jain *et al*., 2012)

5.3.1 Thickness of metformin hydrochloride tablets

The thickness of a tablet can vary with no change in weight due to the difference in the density of the granulation and the force of compression applied to the tablets. The tablets had an average thickness ranging from 4.74 ±0.02 mm to 6.14 ±0.014 mm as shown in Table 4.2. Brand M4 had the highest standard deviation value of ±0.035 while M8 had the least value of ±0.006 indicating M8 was the most uniform brand in terms of thickness whereas M4 was the least. The results could be due to the good flow properties exhibited by the granules during compression and the uniform compression force used in tablet compression.

5.3.2 Uniformity of weight of metformin hydrochloride tablets

A fundamental quality attribute for all pharmaceutical tablet preparations is the requirement for a constant dose of drug among individual tablets within a batch. In practice, small variations between individual preparations are acceptable and the limits for these variations are defined as standards in pharmacopoeias. For tablets, uniformity of weight test is one of the tests which are performed to ensure constant dosing among tablets within a batch to prevent the incidence of overdosing or under dosing. The variation of the weight of individual tablet is a valid indication of the corresponding variation in the drug content (Rawlins, 1977).The amount of fill placed in the die of a tablet press will determine the weight of the resulting tablet. The volume of fill

(granulation) or powder permitted to enter the dies adjusted with the first few tablets produced to yield tablets of the desired weight and content.

All the brands of metformin hydrochloride tablets weighed more than 350 mg (Table 4.3). Therefore, for a batch of such tablets to pass the weight uniformity test, not more than two of the individual weight of the tablets should deviate from the average weight by more than percentage deviation of ±5%. Moreover, none of the tablets is to deviate by more than twice the permissible percentage deviation (United States Pharmacopoeia, 2007). From the results (Table 4.3), all the brands of metformin hydrochloride tablets passed the uniformity of weight test. This could be attributed to good flow properties of granules and also even feeding of granules into the die, the uniform compression force used in tablet compression as well as regular movement of the lower punch so as to produce uniform weight distribution of the tablets (Aulton, 2002). Results of the standard deviation (Table 4.3) which gives a measure of the variability around the mean weight of twenty tablets of each brand showed that brand M11which had the least standard deviation value of ±0.004 had the best uniformity of weight variation. However, brand M9 with the highest standard deviation value of ±0.017 indicated a high dispersion of tablet weight from the mean weight making the tablet weights least uniform.

5.3.3 Friability of metformin hydrochloride tablets

Friability test assesses the ability of a tablet to withstand abrasion associated with handling, packaging, transportation and chipping. This property of the tablet is influenced by the nature and amount of binder used and force of compression. A weight loss of not more than 1% of the weight of tablet being tested is generally considered acceptable for pharmaceutical products and hence, values exceeding 1% are considered as unsatisfactory (British Pharmacopoeia, 2013). From the results obtained (Table 4.4), all the brands passed the friability test with the exception

of M9 which had a percentage weight loss of 1.24%. The failure of M9 could be due to the use of inappropriate compaction force and insufficient binder making the tablets friable. The reverse could account for all the brands that passed the test.

5.3.4 Hardness of metformin hydrochloride tablets

The hardness (crushing strength) determinations are made during tablet production run and are used to determine the need for pressure adjustment on tablet machine. If the tablet is too hard, it may not disintegrate in the required period of time to meet the dissolution specifications; if it is too soft, it may not be able to withstand the handling during subsequent processing such as coating or packaging and shipping operations. The resistance of tablets to capping, abrasion or breakage under conditions of storage, transportation and handling before usage depends on its hardness.

The hardness of a tablet depends on the force of compression and the nature and amount of the binder used. A 4 Kgf diametric crushing force is required or considered the minimum force for a satisfactory tablet. Considering this fact, it was observed from Table 4.5 that all the brands of metformin hydrochloride tablets had optimal ability to withstand fracture. M2 had the highest hardness of 18.76 ±0.04 Kgf and M9 having the least hardness of 6.22 ±0.45 Kgf. This observation could be due to incorporation of appropriate amount of binder as well as the right compression force used in the compression of the tablets.

5.3.5 Disintegration time of metformin hydrochloride tablets

Disintegration is a crucial step when it comes to the release of drugs from immediate release dosage forms. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test

apparatus is a soft mass having no palpably firm core. The disintegration of a tablet mostly depends on factors such as the force of compression, the nature and concentration of disintegrant, the temperature of the water in the disintegration apparatus, and nature and amount of binder used. With disintegration test, the British Pharmacopoeia (BP, 2007) stipulates that for uncoated tablets the disintegration time required should fall within 15 minutes and 30 minutes for film coated tablets. It could be seen from the results (Table 4.6) that all the film coated brands passed the test since they all had disintegration time less than 30 minutes. For the uncoated brands, M4 and M15 passed the test while M8 failed. The failure of M8 could be ascribed to the use of high amount of binder, inadequate amount of disintegrant and high compression force.

5.3.6 Assay of metformin hydrochloride tablets

According to a study by Akinleye et al. (2012), one out of eight brands of metformin hydrochloride tablets tested showed unacceptable quantity of active contents against the label claims. However, the study was conducted in Nigeria. Based on this research finding and couple with the fact that about 30% of the medicinal products on sale for consumption in many countries in Africa are counterfeit and substandard (WHO, 2006), it gives rise for concern in the country.

The assay of all the brands of metformin hydrochloride tablets was carried out by both ultra-violet visible spectroscopy and high performance liquid chromatography. According to the standards of the British pharmacopoeia, upon assay of a product of metformin hydrochloride, between 95% and 105% of the label claim should contain the active ingredient. Considering the results obtained from both the UV and HPLC analysis (Table 4.7 and 4.8), all the brands with the exception of M5, M9 and M12 had values which fell within the monograph specifications. The brands M5, M9 and M12 had percentage content of active ingredient above the upper limit of

105%. Brands M5, M9 and M12 contained an overdose of the drug and could be said to be substandard. The deviation from the stated percentage content in drugs M5, M9 and M13 could be attributed to factors involved in the formulation process. Some of the possible factors include inaccuracy in weighing the active ingredient, lack of effective mixing during the granulation and incorporation of excess amount of the active ingredient during the formulation. The content of metformin hydrochloride in all the brands with the exception of M5, M9 and M12 is considered optimal because an effective release *in vivo* would ensure attainment of therapeutic concentration and hence, enhanced therapeutic response.

The content of active ingredient determination of metformin hydrochloride tablets can be carried out officially either by Ultra-violet spectrophotometric (UV) method or High Performance Liquid Chromatography (HPLC) analysis but in this study both analytical procedures were used. The HPLC procedure used was a method developed and validated by Kar and Choudhury (2009) so in order to check the reproducibility and reliability of the method, the metformin hydrochloride tablets were also assayed by UV analysis. Comparing the results obtained from both methods (Table 4.7 and 4.8), it could be seen that both methods gave almost the same results. The fact that brands M5, M9 and M12 failed the test in both methods while the rest of the brands having satisfactory results buttresses this point. However, the percentage contents of active ingredient obtained with HPLC analysis were slightly higher than those obtained with UV analysis buttressing the fact that HPLC as an analytical procedure is more sensitive than UV analysis.

5.4 Dissolution testing

Oral dosage forms only become available for absorption following the process of disintegration and dissolution. Dissolution is pharmaceutically defined as the rate of mass transfer from a solid

surface into the dissolution medium or solvent under standardized conditions of liquid/solid interface, temperature and solvent composition (Singhvi and Singh, 2011). Dissolution testing is employed to distinguish the influence of manufacturing variables such as binder effect, mixing effect, granulation procedure, excipients type, and can be used as a tool to predict product behavior *in vivo* (Papadopoulou et al., 2008). Consequently, dissolution test is currently used as an in vitro bioequivalence test, generally for figuring out dissolution profile and profile comparison, establishing the similarity of pharmaceutical dosage forms (Amidon, et al., 1995; Cheng and Yu, 2004). According to the monographs in British Pharmacopoeia, for each of the tablets tested for dissolution, the amount of active ingredient in solution should not be less than 70% of the prescribed or stated amount within 45 minutes. The results obtained from the study (Table 4.15, 4.16 and 4.17) revealed that all the brands passed the BP general specification standard for dissolution rate test for conventional immediate release tablets since it was observed that for all the brands, at least 80% release in 30 minutes took place. Therefore, all the brands passed this acceptance pharmacopoeia criterion.

5.5 Dissolution profile comparison

Again, according to a study conducted by Akinleye et al. (2012), four out of seven brands of metformin hydrochloride tablets tested had similar dissolution profiles as that of the innovator brand based on f_2 analysis. This revelation necessitates the need to ascertain whether the various metformin tablets sampled would have the same dissolution profiles or not since it would give an indication of whether they can be used interchangeably in clinical practice. Comparison of therapeutic performance of two medicinal products containing the same active ingredient is a critical means of assessing the possibility of an alternative. Pharmacokinetic data instead of therapeutic results may be used to establish bioequivalence (Amidon *et a*l., 1995). A

bioequivalence study is basically a comparative study designed to establish equivalence between test and reference product. In the US Food and Drug Administration (FDA) guidance on Immediate Release, the difference factor (f_1) and similarity factor were adopted in comparing profiles of a Reference and a Test drug. The difference factor (f_1) calculates the percentage difference between the two curves (Reference and test drug) at each time point and is a measurement of the relative error between the two curves. Generally, f_1 values up to 15 (0-15) indicates minor difference between the two products (FDA, 1995a).The difference shows the extent of dissolution in a particular drug sample in comparison to the reference. The greater the difference the lesser the cumulative amount of test drug dissolved across all the time points.

From Table 4.20, all the brands with the exception of M9 had f_1 values which fell within the f_1 range (0-15). Thus, in reality all the brands expect M9 can be said to have minor difference in terms of release of active ingredient with respect to the reference drug. The difference factor for M9 fell out of the required range and thus the drug release profile is different from that of the reference drug.

In dissolution comparison, especially to assure similarity in product performance, regulatory interest is in knowing how similar the two curves are. Such interest also seeks to have a measure which is more sensitive to large differences at any time point. This is the reason why f_2 comparison has been the focus in the various regulatory policies. When two profiles are identical, f_2 is equal to 100. Conventionally, a test brand is considered similar to that of a reference brand if the f_2 value of the two profiles is between 50 and 100 (Mukesh et al., 2005).

From the results (Table 4.19), brands M2, M5, M6, M7, M12, M13 and M14 had f_2 values which fell within the range specified by the FDA (50-100). Thus, it can be concluded that these brands

are similar to the reference (innovator) brand M11 and hence, these brands can be considered to have same drug release performance or bioequivalence with the reference brand, M11. However, brands M1, M3, M4, M8, M9, M10 and M15 had f_2 values outside the accepted range and therefore, these brands cannot be considered to be similar to the reference brand in terms of drug release performance. From the f_1 and f_2 studies conducted, brands M2, M5, M6, M7, M12, M13 and M14 could possibly be used interchangeably with the reference brand (M11) since they had acceptable f_1 and f_2 values.

The dissolution efficiency proposed by Anderson et al, (1998) was also determined for the batches to ascertain which batch will be effective in releasing the drug for its therapeutic effect. According to Anderson the higher the dissolution efficiency, the more efficient the tablet is at releasing its embedded drug. It was observed that (Table 4.18) brands M6 and M11 had the highest dissolution efficiency of 95% compared to the other brands while brand M9 had the least value of 83.8%. Based on this analysis, brands M6 and M11 could be considered the most efficient brands when it comes to the release of active ingredient whereas M9 is the least.

Finally, ANOVA-based method was used to compare the metformin products tested. The advantage of using ANOVA is that it is not restricted to any of the requirements suggested for Moore's factor, f_2 and does not depend on data fitting to a specific kinetic model. Since the data were collected as repeated measurement over time on the same experimental unit, a repeated measure analysis was performed in order to compare the complete dissolution profiles for the generic metformin products to that of the reference product, as well as to compare dissolution profiles for the same products.

The ultimate challenge to dissolution testing is its ability to reflect the *in vivo* performance at the dosage unit during its absorption phase. Numerous variables have been derived from *in vitro,* which have been correlated with *in vivo* data. In vitro dissolution profiles can statistically be compared through mean dissolution time, area under dissolution curve (Polli et al., 1997). In the present study AUC was used to estimate the extent of drug dissolved from the dosage form in vitro because this parameter can probably provide a better indication of *in vivo* performance (Banakar, 1992). Analysis of variance using Dunnett's multiple comparison tests showed some significant differences in AUCs for the products studied (Table 4.21). Using M11 as a reference product, a significant difference was observed in the dissolution profiles of brands M4, M7, M8, M9 and M15 whereas the difference was not significant for the other brands (Table 4.21).

5.6 BCS-based biowaiver

A generally accepted practical definition of bioavailability is understood to be the extent and the rate at which a drug is delivered from a pharmaceutical form and becomes available in the general circulation. Two oral dosage forms are considered to be bioequivalent if they have same rate and extent of drug absorption. *In vivo* bioequivalence studies are commonly used to demonstrate bioequivalence, but these studies are often costly (Cook and Bockbrader, 2002) and involve invasive procedures. The Biopharmaceutics Classification System (BCS) can be used to reduce in vivo bioequivalence requirements (Lobenberg and Amidon, 2000). *In vitro* dissolution tests based on BCS are acceptable surrogates for establishing the bioequivalence of generics with the innovator product.

Only generic drug products that are therapeutically equivalent to the innovator products and have been approved by the appropriate regulatory body may be marketed as appropriate substitution (Cheng and Yu, 2004). Metformin hydrochloride is a class 3 drug according to the BCS

(Lindenberg et al., 2004) which is characterized by high solubility and low permeability. For immediate-release products of this class, the assumption is that if their dissolution is very rapid under all physiological pH conditions, they can be expected to behave like oral solutions *in vivo*, since the rate-limiting step in the absorption process is permeability. Class 3 drugs are considered acceptable for biowaiver under WHO criteria (i.e., both the test and reference products are very rapidly dissolving). That is, dissolution of 85% or more of the labeled amount of the active ingredient should be achieved within 15 minutes under all physiological conditions (WHO, 2006). None of the brands including the innovator brand met this requirement since none was able to achieve 85% dissolution in 15 minutes in all the three different dissolution media (Table 4.9 to Table 4.17). The literature, however, shows that class 3 drugs may have similar *in vivo* absorption even when the dissolution is slow. Blume and Schug (1999) and Kortejarvi et al., (2005) advocated biowaiver for class 3 drugs that are rapidly dissolving (85% dissolved within 30 minutes). This advocacy was because bioavailability of this class is independent of drug dissolution; therefore, generic drugs with differing in vitro dissolution will not necessarily exhibit different in vivo performance.

CONCLUSION

From the experiments conducted and the deductions made in the discussion, the following conclusions could be made:

- All the brands of metformin hydrochloride tablet sampled complied with the official specifications for identification, disintegration, uniformity of weight, hardness and thickness tests. However, for the friability test, one of the brands, M9 failed to meet the required specification.
- Both UV and HPLC were used for the assay analysis and all the brands expect M5, M9 and M12 had values which fell within the specification range for assay in the British Pharmacopoeia (2013). Brands M5, M9 and M12 could be said to be substandard tablets.
- All the brands met the pharmacopoeia criterion for dissolution rate test for conventional immediate release tablets.
- Brands M6 and M11 had the highest dissolution efficiency of 95% while M9 had the lowest dissolution efficiency of 83.8%.
- From f_1 and f_2 analysis, the dissolution profiles of M2, M5, M6, M7, M12, M13 and M14 were similar to that of the reference brand (M11) while those of M1, M3, M4, M8, M9, M10 and M15 were not. However, using one-way ANOVA analysis followed by Dunnett's multiple comparison test, only the dissolution profiles of M4, M7, M8, M9 and M10 were significantly different from that of the reference drug (M11).
- None of the brands including the inference drug was able to meet the criteria for BCS-based biowaiver.

RECOMMENDATIONS

Based on the results of the study, the following recommendations may be suggested;

- Further *in vivo* correlation studies could be performed on the selected brands since the drug release performance obtained *in vitro* is a likely prediction but does not necessarily mean that formulations will perform similarly *in vivo*.
- There should be proper validation and authentication of varying manufacturing variables which will help minimize the effect of variations in the manufacturing process and will help attain consistent high product quality.
- Drug regulatory bodies should intensify post market inspection and surveillance on various metformin hydrochloride tablet brands on the market to confirm their quality and disseminate the appropriate information regularly to drug selection committees.
- There should be a drug policy like the "drug interchangeability update" as practiced in some countries like Canada, Japan and Australia, to guide all decisions about drug brand interchangeability.

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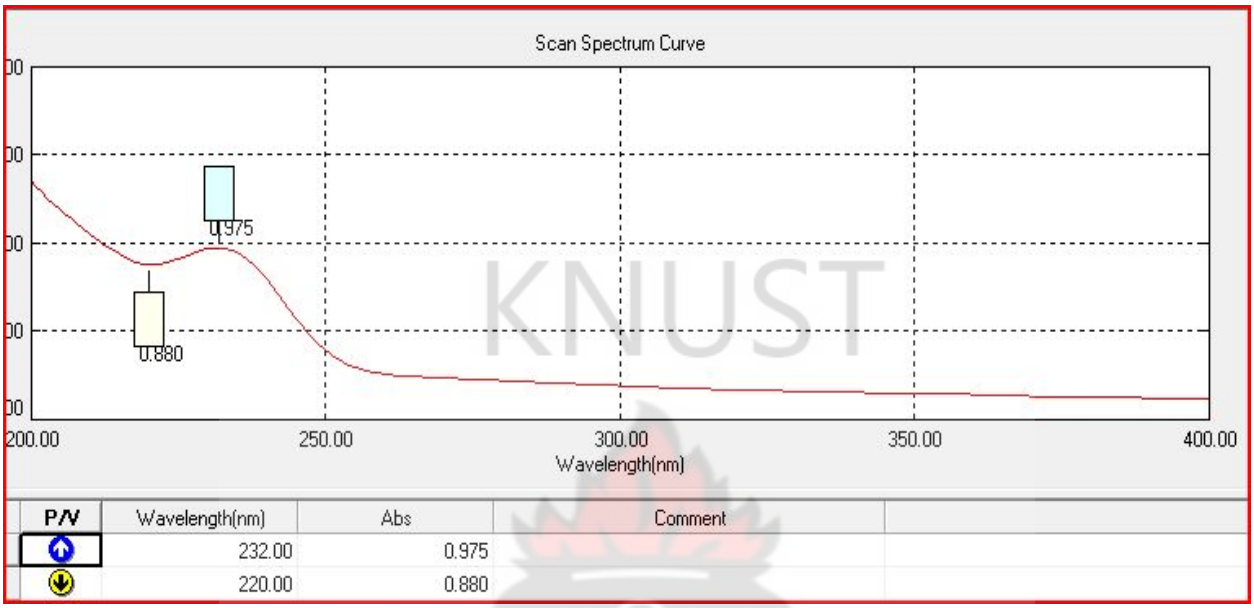
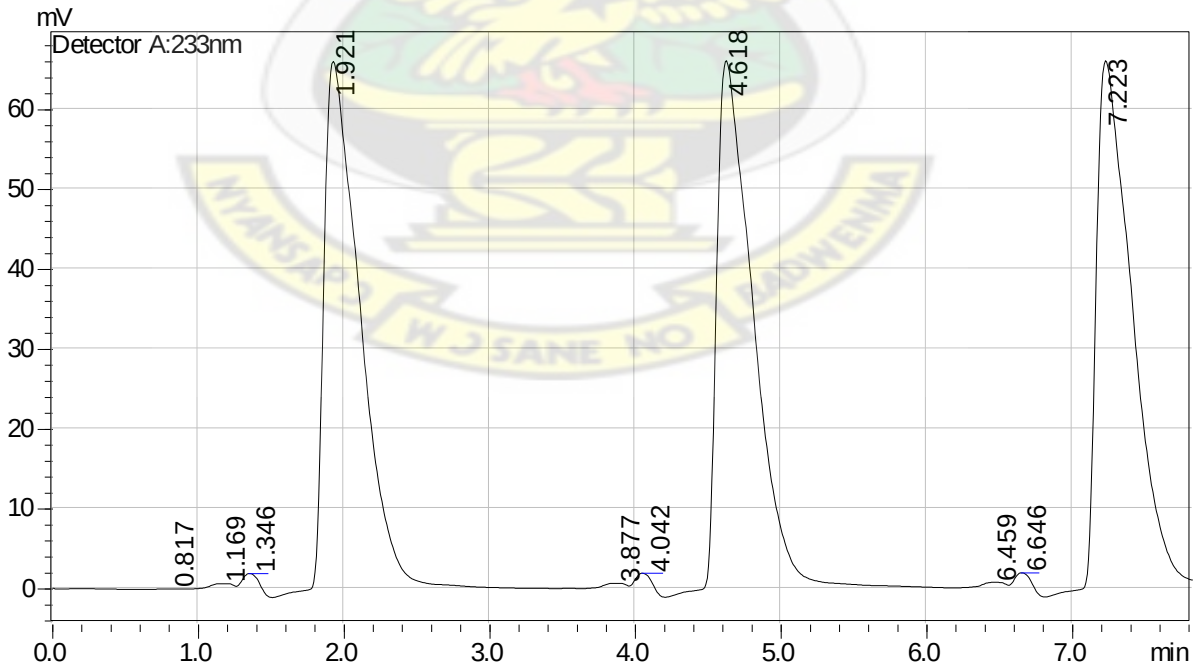
APPENDICES

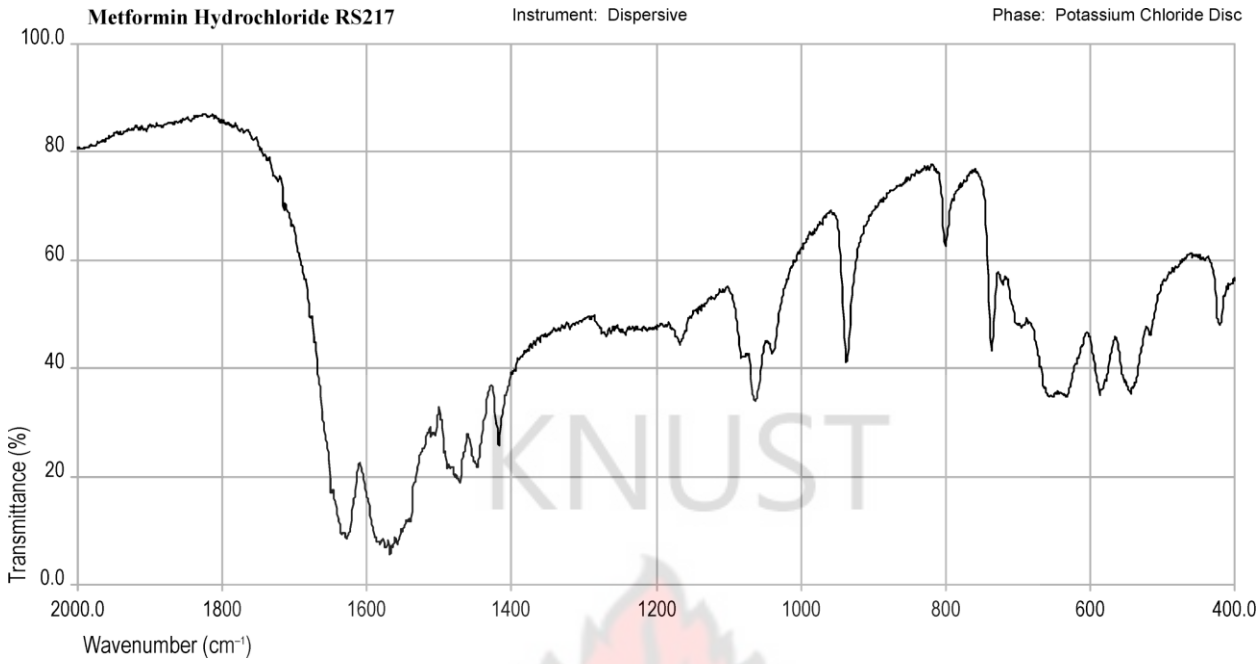
Appendix I: Sample spectra of the various analytical methods



Sample UV spectra of metformin hydrochloride

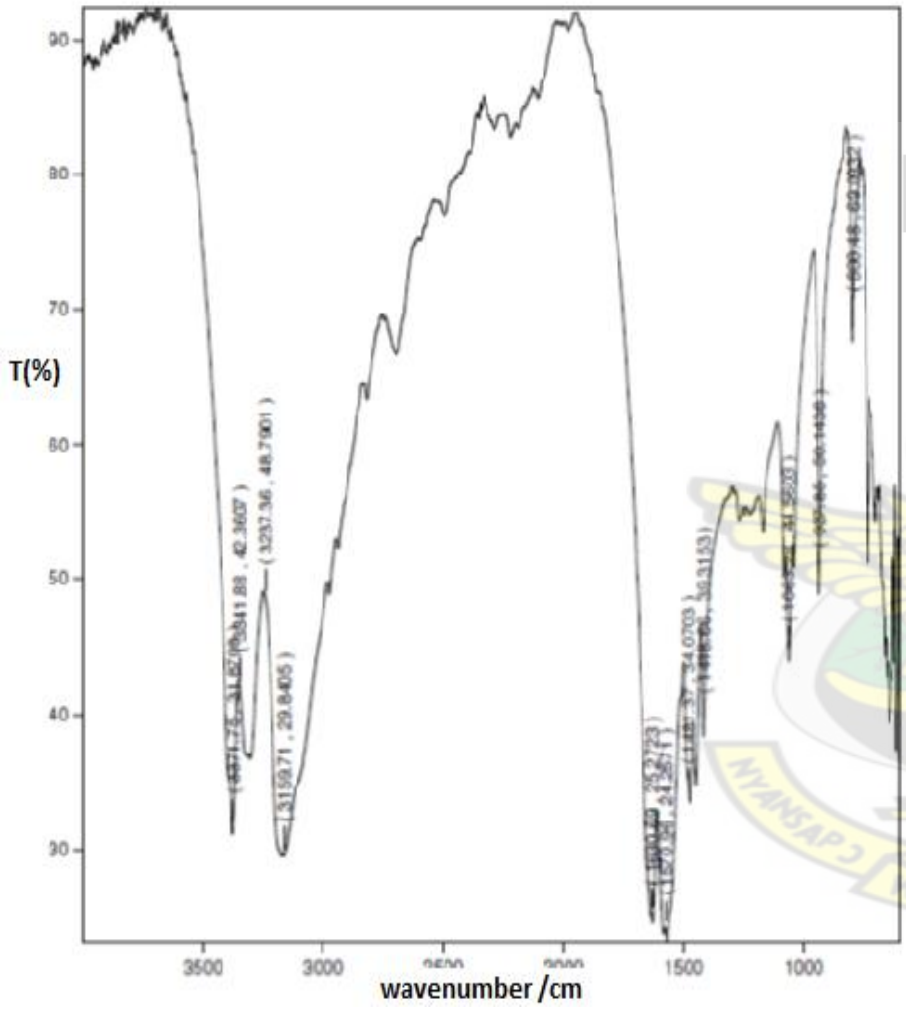


Sample hplc chromatogram of metformin hydrochloride

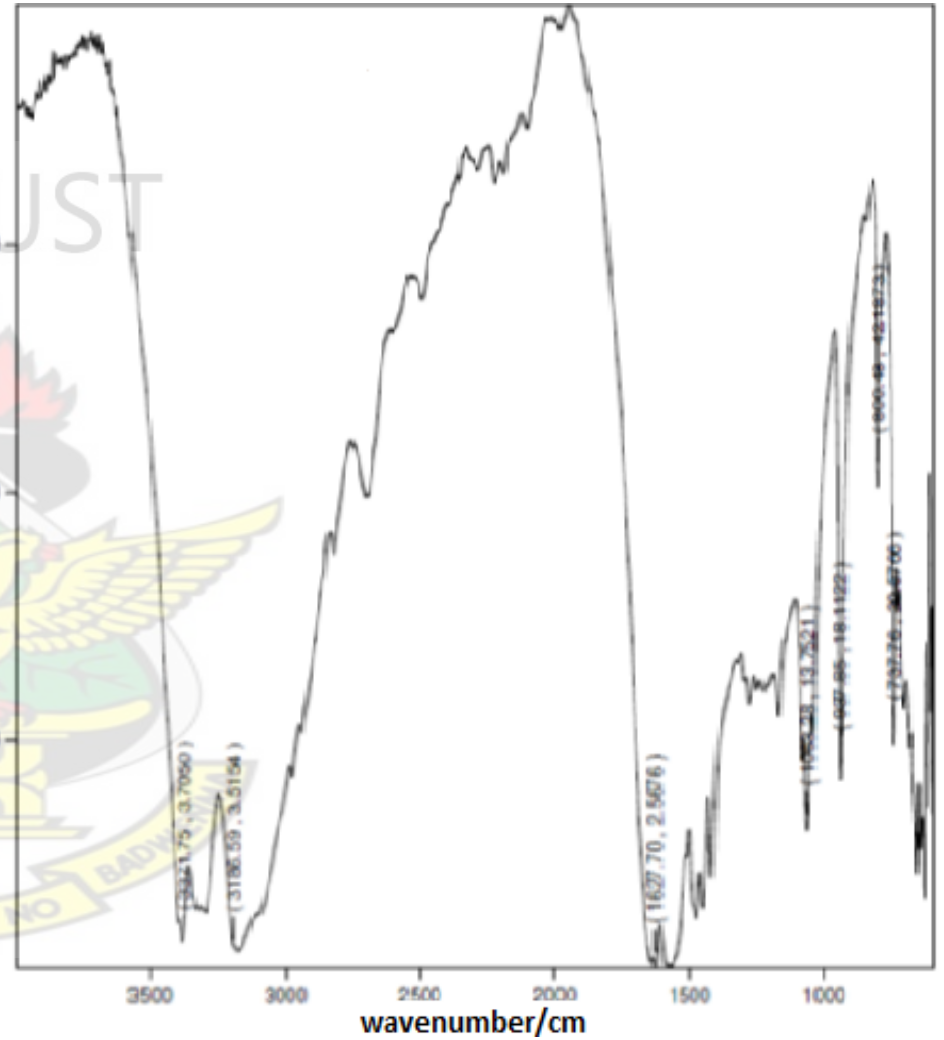


Infrared reference spectrum of metformin hydrochloride (British Pharmacopoeia, 2013)

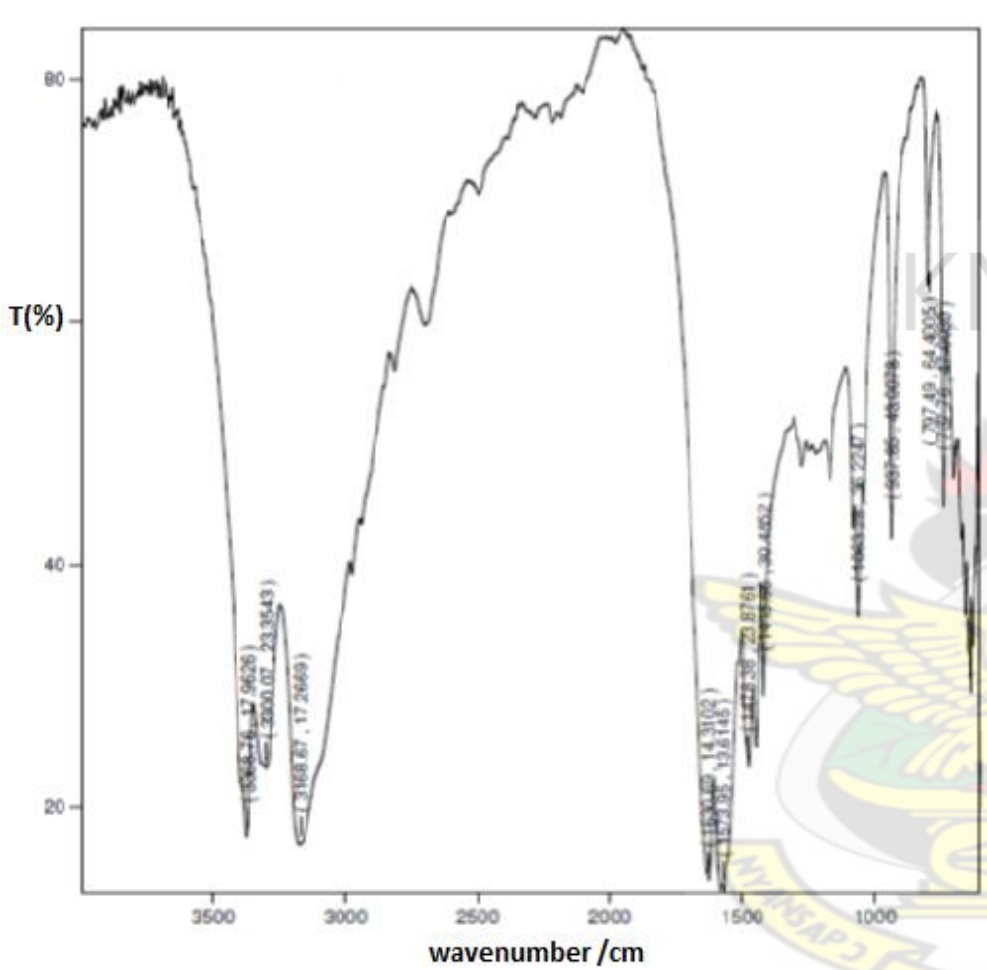
APPENDIX II



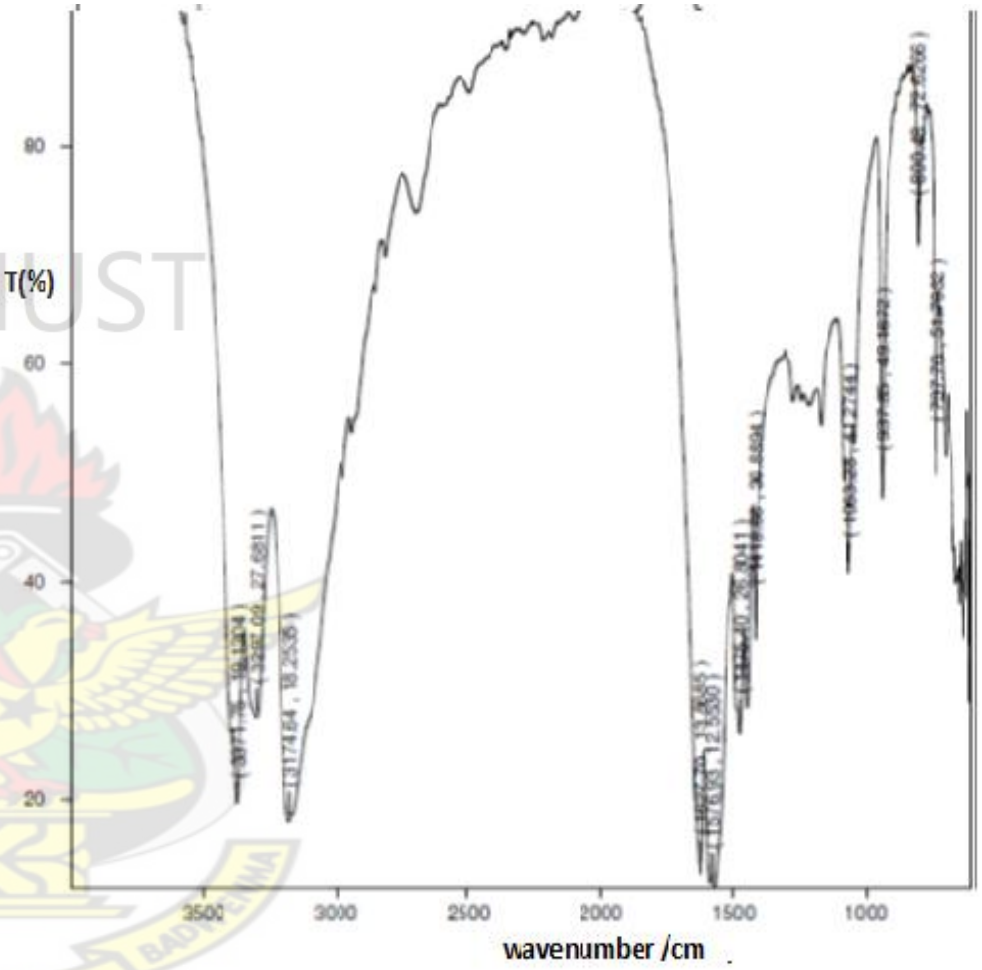
IR spectrum of M2



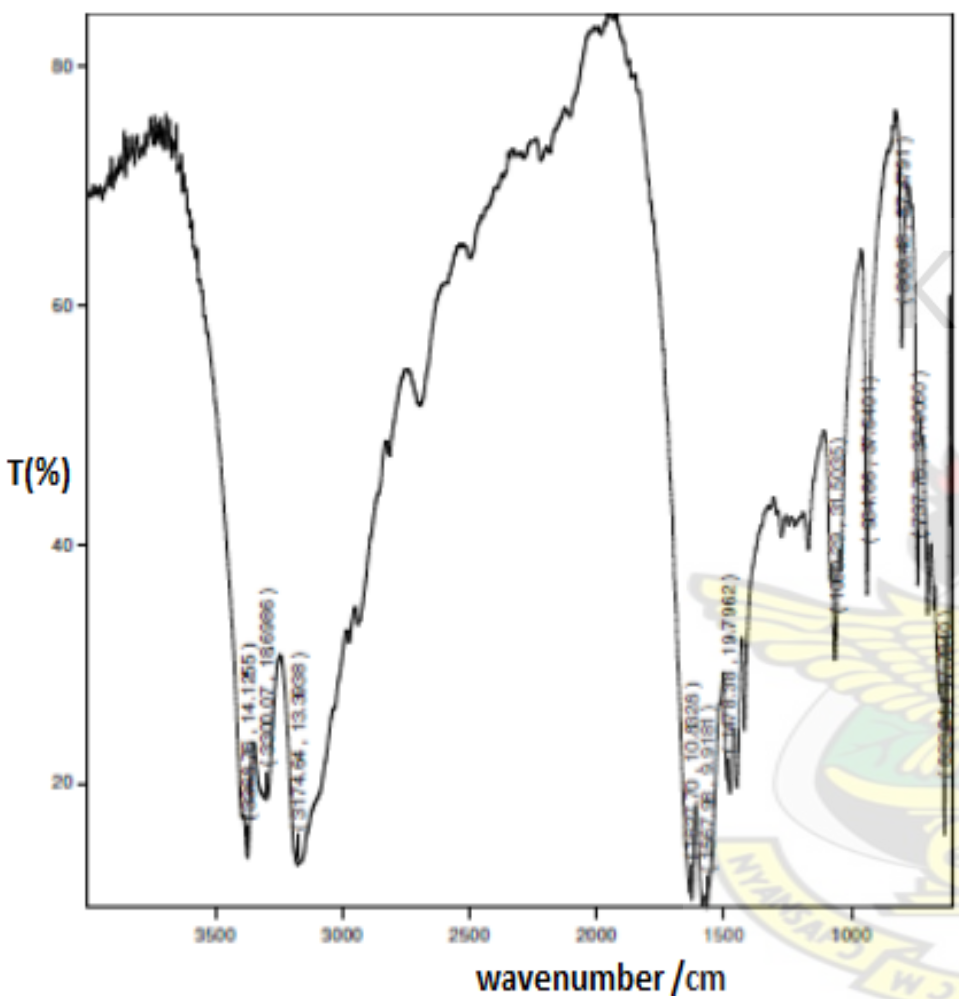
IR spectrum of M3



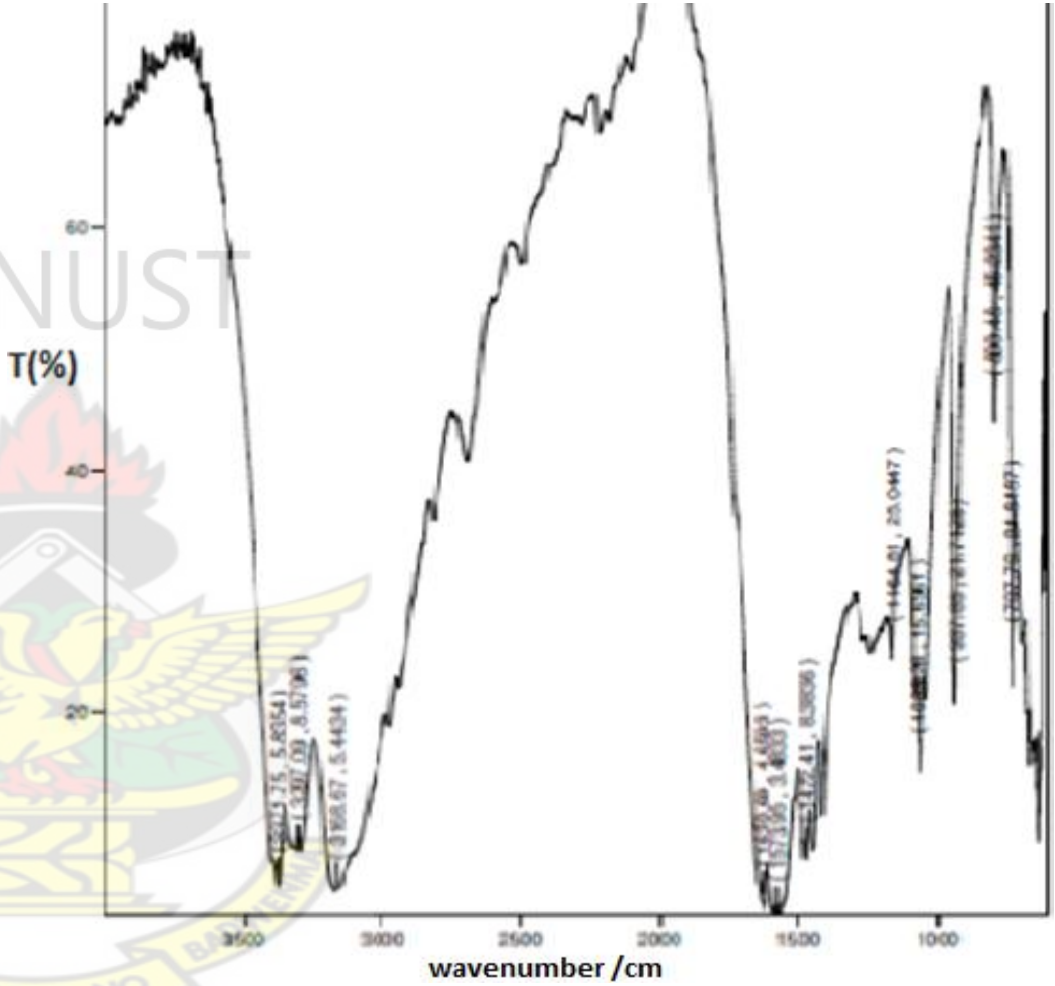
IR spectrum of M4



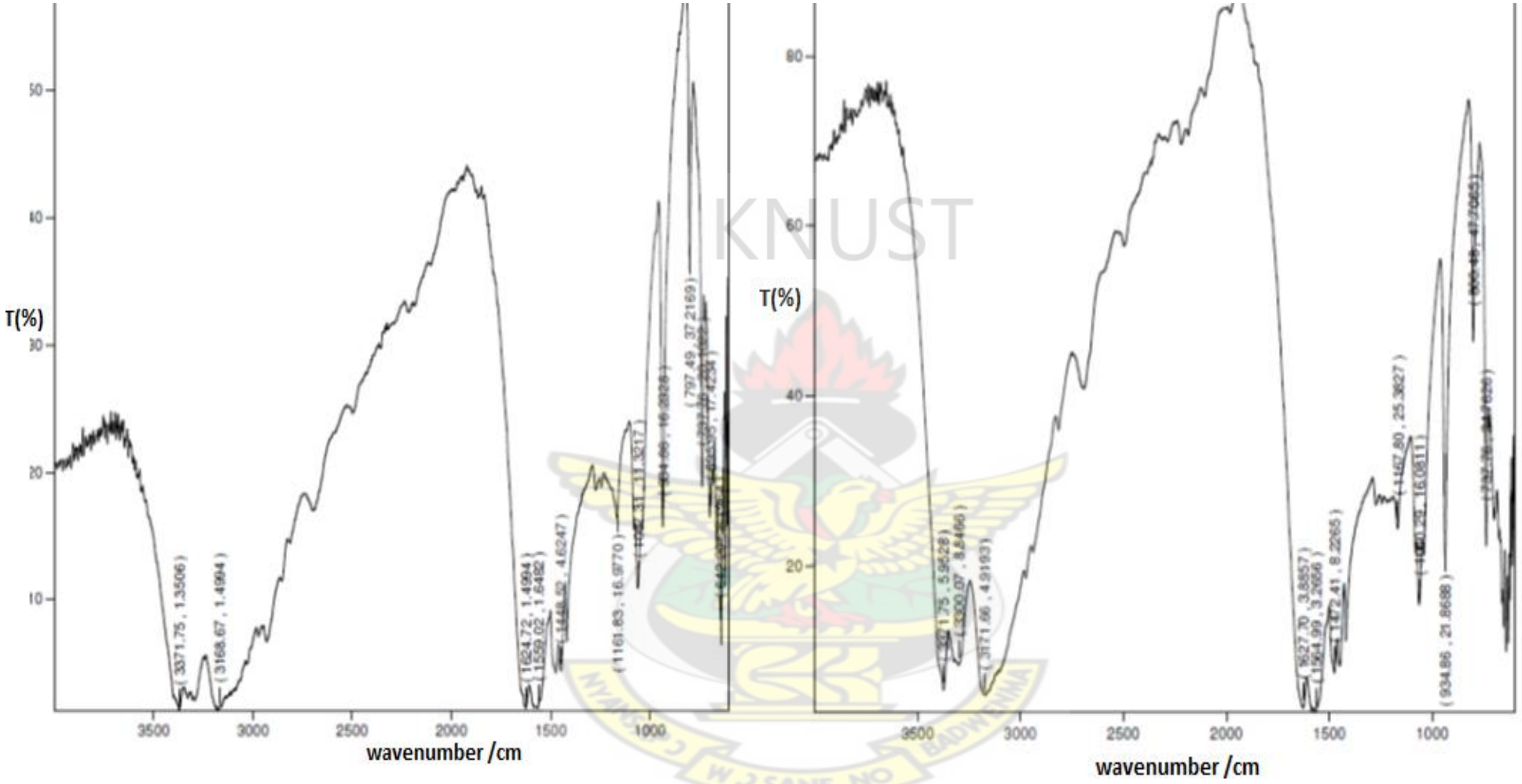
IR spectrum of M5



IR spectrum of M6

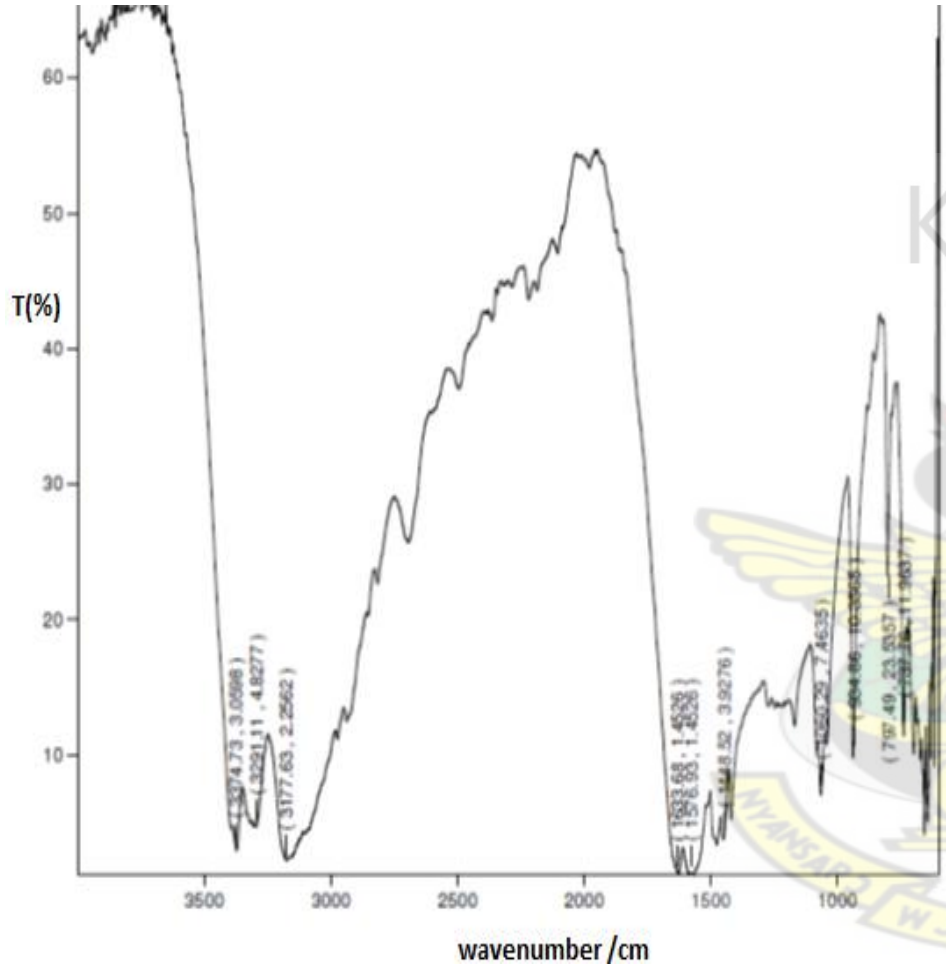


IR spectrum of M7

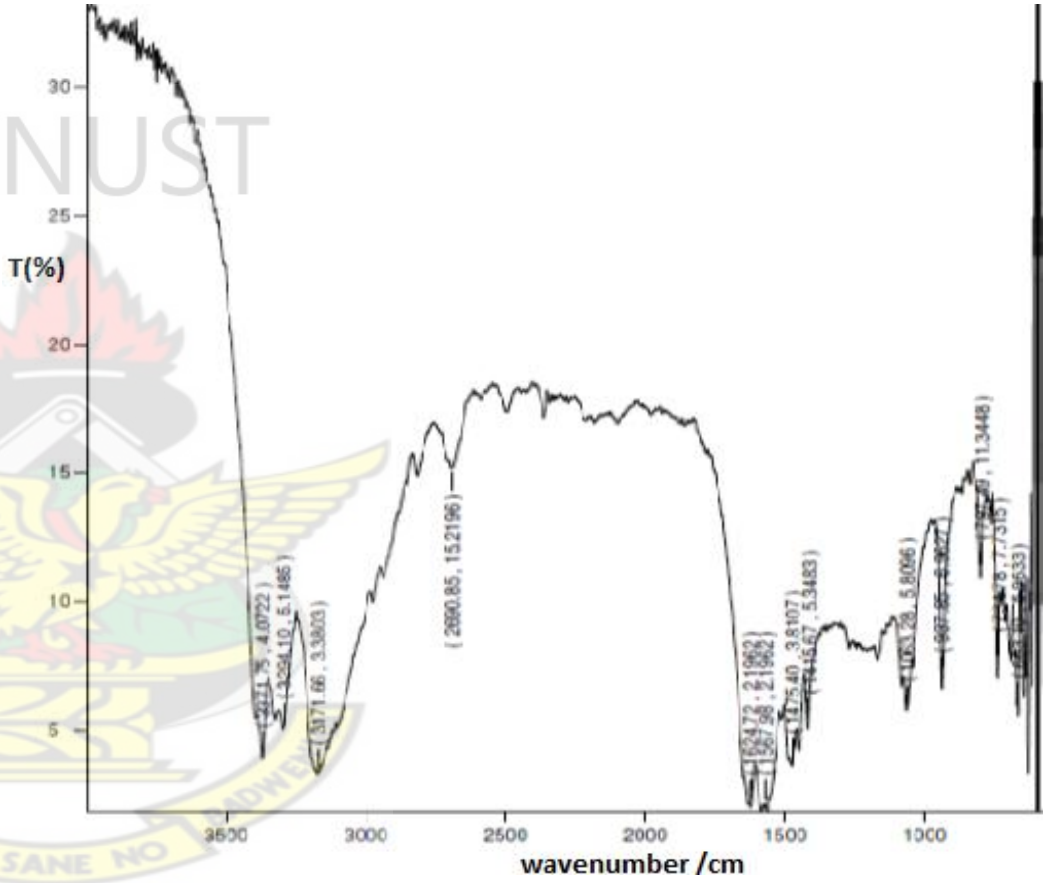


IR spectrum of M8

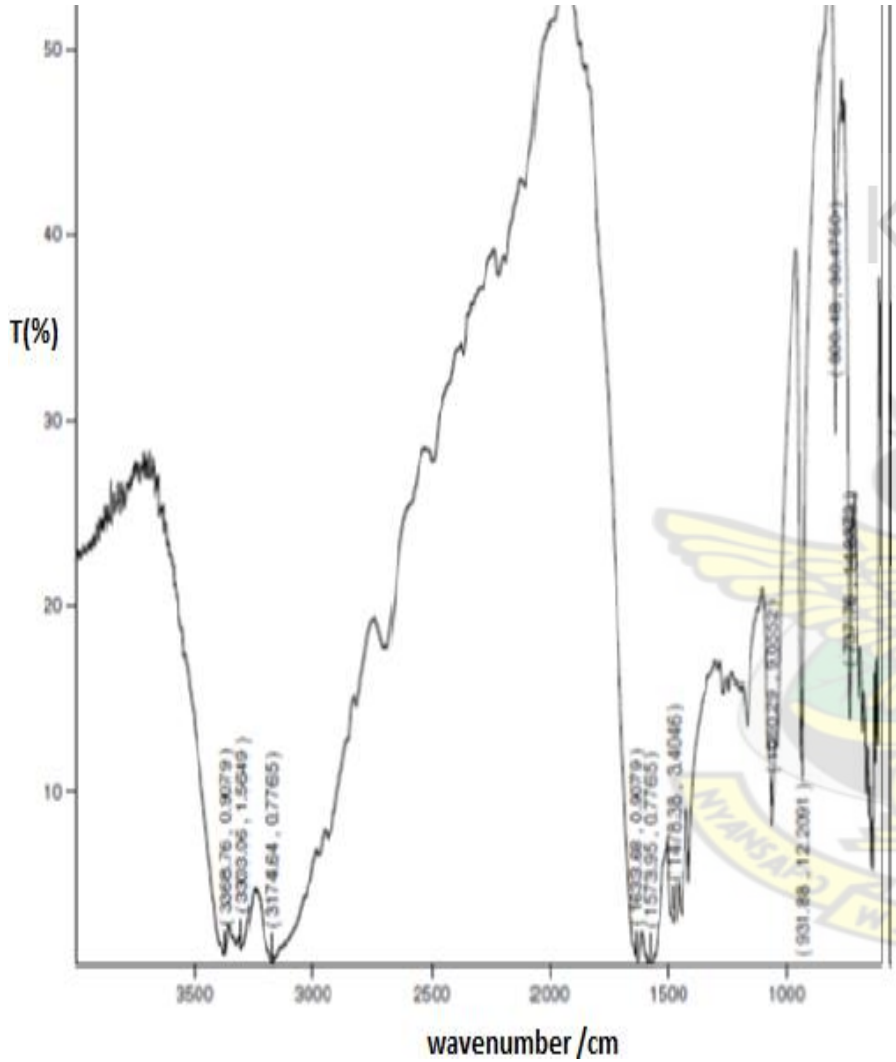
IR spectrum of M9



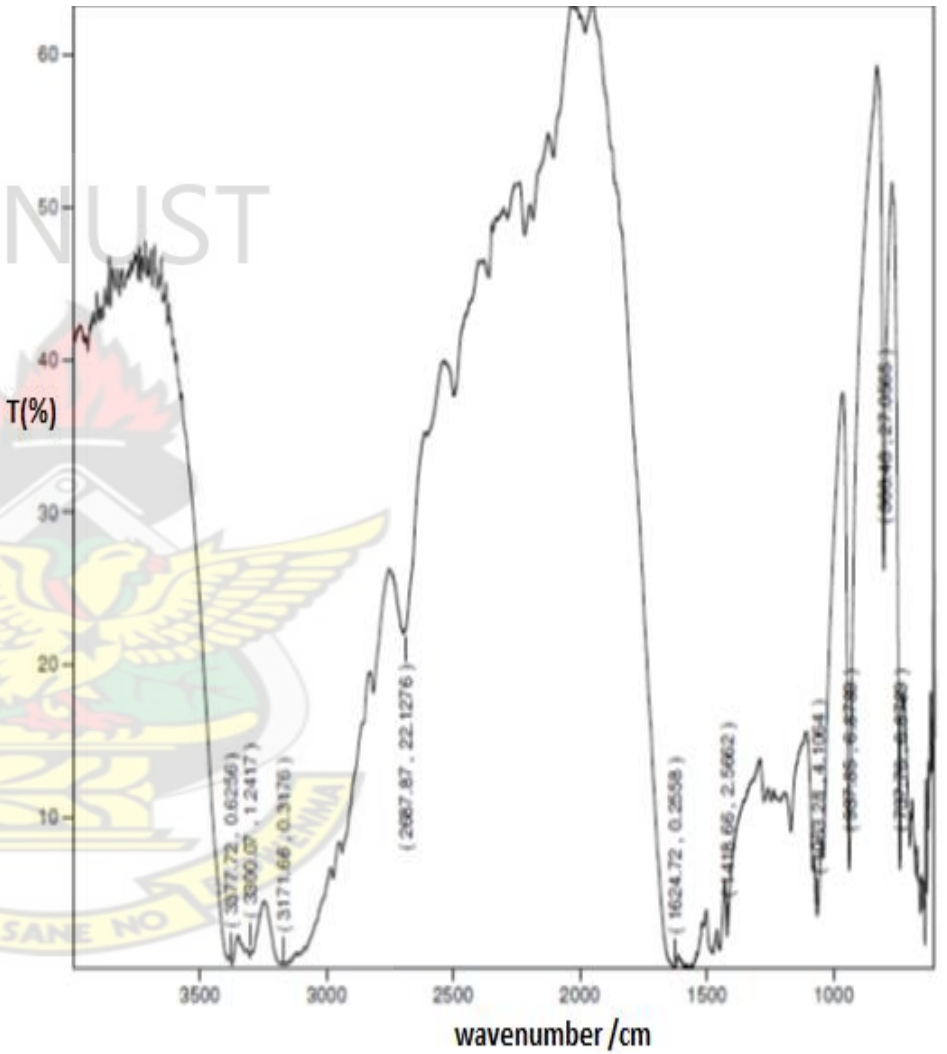
IR spectrum of M10



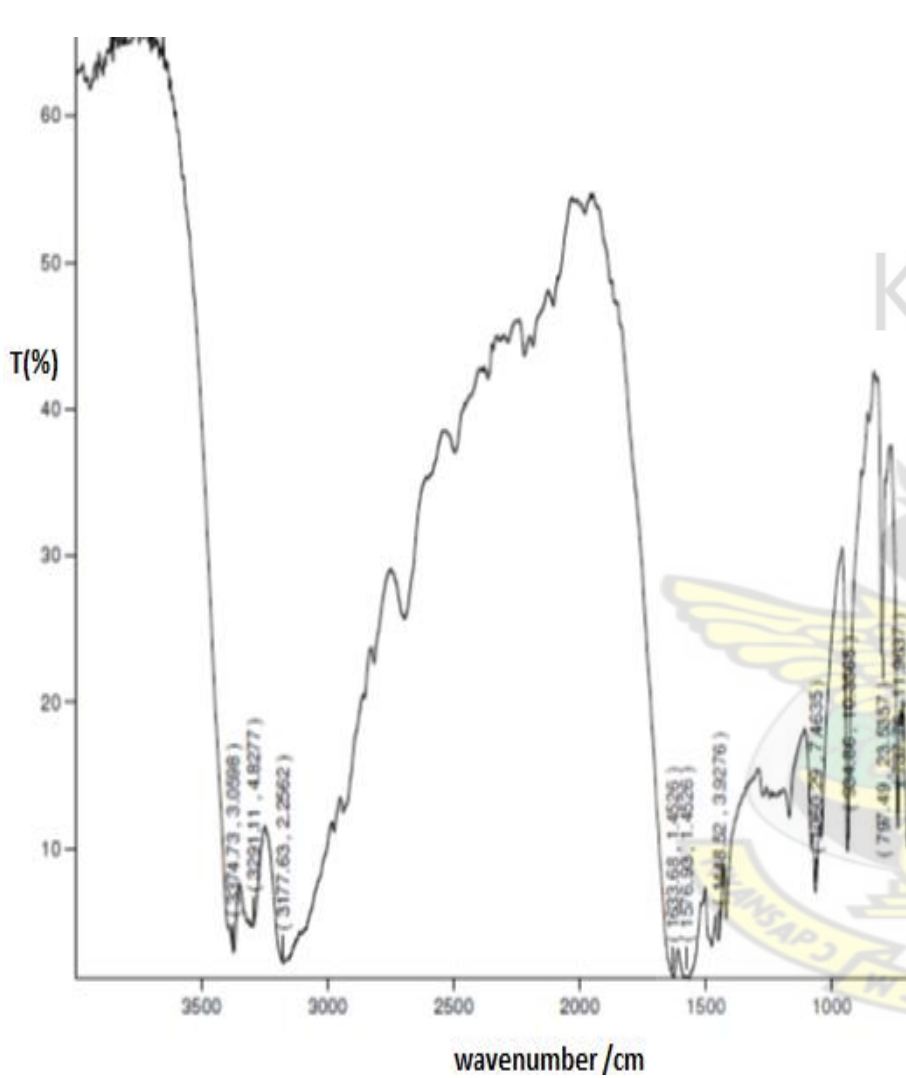
IR spectrum of M11



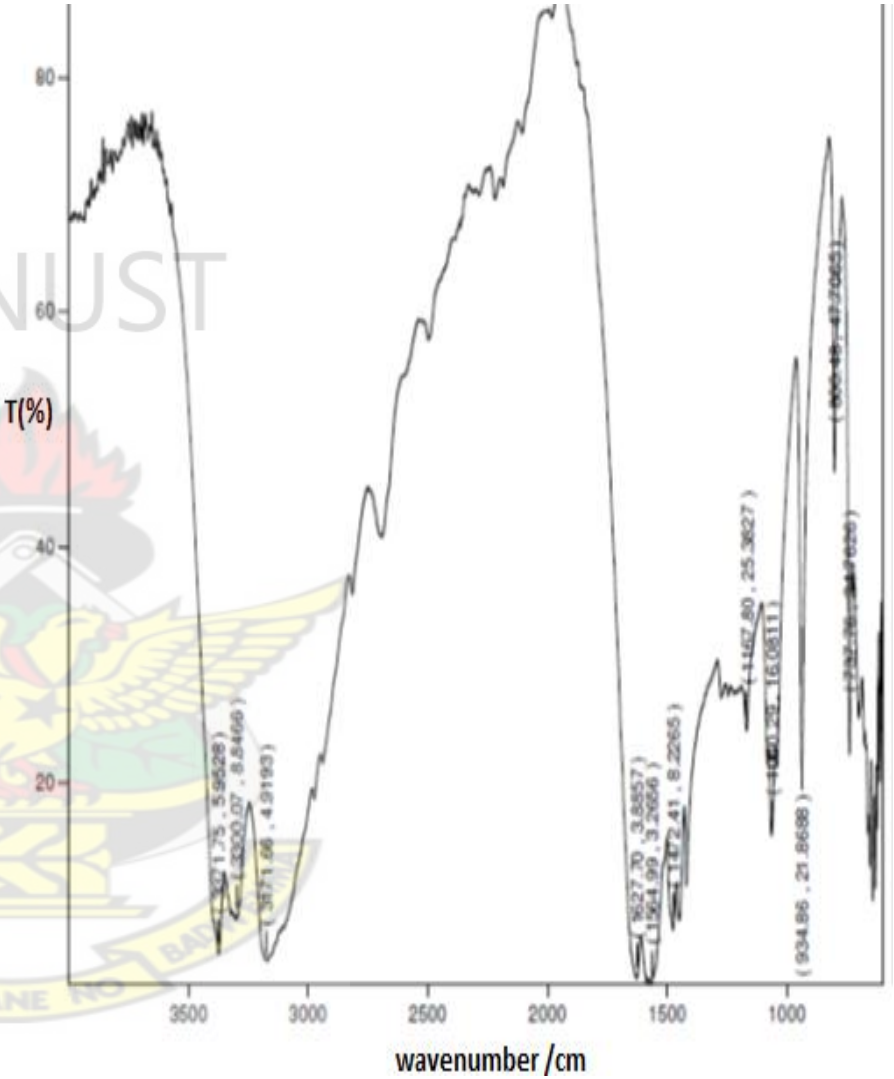
IR spectrum of M12



IR spectrum of M13



IR spectrum of M14



IR spectrum of M15

Appendix III: Preparation of the various dissolution media

- 0.1N HCl solution- 8.9ml 0f conc. HCl (36% purity, 1.18g/ml) was measured into a 1000 ml volumetric already containing some amount of distilled water, the measuring cylinder was rinsed quantitatively into the volumetric flask. The solution was made up to volume to produce 1000 ml.
- Phosphate buffer (pH 4.5)- 13.61 g of potassium dihydrogen orthophosphate was dissolved in 750 ml of distilled water which was adjusted to a pH of 4.5 with 0.1M hydrochloric acid and it was then diluted with distilled water to produce 1000 ml.
- Phosphate buffer (pH 6.8)- A solution of 0.2 M potassium dihydrogen orthophosphate of volume 250 ml was mixed 118.3 ml of 0.2 M sodium hydroxide solution and then diluted with distilled water to produce 1000 ml.