THE VARIANCE FUNCTION OF THE DIFFERENCE AND THE DIFFERENCE OF THE VARIANCE FUNCTIONS BETWEEN TWO ESTIMATED RESPONSES FOR ROTATABLE DESIGNS

BY

KARANJAH ANTHONY N

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Declaration

I the undersigned declare that this thesis is my original work and to the best of my knowledge has not been presented for the award of a degree in any other University.

KARANJAH ANTHONY N

Reg. No I80/81661/2011

....

Signature Date

Declaration By Supervisor

This thesis has been under our supervision and has our approval for submission as supervisors

DR. F NJUI

Department of Mathematics,

University of Nairobi,

P.O. Box 30197 Nairobi,

KENYA.

....

Signature Date

Prof. G P POKHARIYAL

Department of Mathematics,

University of Nairobi,

P.O. Box 30197 Nairobi,

KENYA.

....

Signature Date

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half the course but inspired me with the line:

"It is not the years in your life that counts,
rather it is the life in your years that matters"

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giving me reason to move on with life even in the toughest of times

Contents

Abstract

Medicinal herbs constitute an important source of raw materials for both the traditional and the conventional medicine. Due to their availability all over the world, they play a key role in world health as complement if not substitute to conventional medicine mainly due to lack of suitable, effective, cheap and reliable drugs at the time they are required and in many cases in the remotest places of the world.

Over reliance on herbal drugs whose active ingredients have not been quantified resulting to different herbalist prescribing different concoctions depending on the flora availability, may lead to resistance development, overdose or under dose which may lead to negative repercussion.

For these reasons there is need to standardize commonly used herbal drugs, by formulating a mathematical model that can be used to determine the best combination of herbs and best preparation practices in order to achieve the optimal response. By so doing, useful results and conclusions can be drawn by planned and designed experiment.

Response surface methodology as a statistical technique is useful in modeling and analysis of problems in which response of interest is influenced by several variables where the objective is to optimize the response. This is equivalent to locating feasible treatment combinations for which the mean response is optimized.

This excursion yields interesting patterns of the response surface where ridges are mapped with a view to identifying combinations which give optimal response. It is of interest to note how to discriminate amongst the various points on the response surface which the yield is the same for different combinations of the predictor variables and to isolate the ones which are identified as parsimoniously feasible.

This research employs response surface methodology to investigate effectiveness of herbal medicine in reducing the blood sugar level of a diabetic to a level that is acceptable. In this setup, observations are made to investigate effectiveness for particular dosage at reducing the blood sugar level with time.

The variance function comes in handy as a tool for discrimination between two points on the identified response surface. The most feasible of all the identified points of equal yield is the one in which the variance function is minimal. In this

research we use the variance function of the difference as well as the difference of the variance functions between two points to provide reliable advice on the range around which the dosage is desirable and time required to effectively reduce the blood sugar level to acceptable range.

Key words: *Response surface; Rotatable design; Variance function ; diabetic; Herbal-Medicine; treatment.*

Chapter 1

Introduction

1.1 Herbal Medicine

Herbal practice is a comprehensive term that is used to refer to both the traditional method and systems such as traditional Chinese medicine, Indian ayurveda, Arabian unani-medicine and other forms of indigenous medicine. This is inclusive of diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and or mineral based medicine, spiritual therapies, manual techniques and exercises that are applied on their own or in combination to maintain the well being, of an individual or to treat, manage, diagnose or prevent illness.

Medicinal plants have been used to treat various diseases since time immemorial and have propelled the field of traditional herbal medicine and herbal drugs today, Evans et al (2007). In the last decades, the use of herbal medicine in treatment has increased in popularity round the globe and it is still estimated to increase in future to 80 percent of the world's population , Mosihuzzanman et al (2008). All modern medicine was derived originally from ancient herbal traditions (Heinrich et al., 2004).

To some extent the term complementary and alternative Medicine is used to describe a group of diverse medical and health care systems, practices and products that are not generally considered part of the convention medicine

The traditional, complementary and alternative medicines attract the full spectrum of criticism as well as reactions and skepticism. In many countries the health professionals and to some extent, the public are critical of the efficacy, safety,

quality availability, preservation and further development of these products and the health care they ought to provide.

Herbalist uses the leaves, flowers, stems, berries, and roots of plants to prevent, relieve, and treat illness. From a scientific perspective, many herbal treatments are considered experimental. The reality is, however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, where their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. Some of these plant components give rise to vincristine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) just to mention a few.

Knowledge of the extraction, usage and preservation of medicinal herbs was passed from one generation to the other by traditional practitioners by family lineage and therefore, traditional medicine is as old as mankind. People in pre-historic times used selected plants in many ways; for food, shelter and for curative aspect for identified ailments and certain disorders. It is believed that the later usage may be attributed to greater life span that the older generations can be proud of to this day. When used as food and prepared according to the traditional ways, some of these plants can be attributed to maturing the traditional man through the nutritional value that is extracted from these foods during digestion. However there is no genesis as to when and how the herbs were identified as far as their medicinal value are concerned. By trial and error, ancient people learned that eating various fruits-berries, roots, leaves and use of other parts of plants, there was either discomfort or death or safe ingested. In this way they were able to identify with parts of plant that could also be used not only as food but to arrest or treat an ailment. Gradually a body of knowledge built that was diverse among different culture and thereafter passed through generations to create the rich cultural heritage that we are proud of today.

The history of usage of plants depends on the culture that is considered. In China, medicinal properties and nutritional value of plants were known around 4500 BC, while the use of medicinal plants in India, Greece and the Arab countries dates back to many thousands years. Hippocrates (460-337 BC) wrote important works on the value of herbal medicine.

The world health Organization encourages and supports member states to integrate traditional and complementary medicine into their national health care system and to ensure that they are used or utilized in a rational manner. Traditional medicine started being incorporated in Kenya's national health policy framework in the late 1970's. Kenya's 1989-1993 development plan recognized traditional medicine and made commitment to promoting the welfare of traditional medicine practioners. The ministry of health and provincial authorities require the registration of traditional medicine practitioners. In 1999, Kenya's patent law was revised to include protection for traditional medicine.

There has been a widespread and growing use of traditional medicine in the health system and the economic importance is appreciated. It is noted that upto 80 percent of the population uses Traditional medicine to help meet their health care need. In Asia and Latin America, people continue to use traditional medicine as a result of historical circumstances and cultural beliefs. In China 40 percent of all the health care needs are accounted for by traditional medicine.

According to Cable News Network (CNN), 50 percent of all medical schools in the US (among them Harvard,Yale, Johns Hospkins and Georgetown Universities) now offer courses in alternative medicine. The World Health Organization estimates that between 65 and 80 percent of the world's population (about 3 Billions) rely on alternative medicine (traditional) as their primary form of health care. Approximately 22 million US dollars have been spent on alternative medicine research in USA since 1992. Traditional Chinese medicine has been chosen by World Health Organization (WHO) for worldwide propagation to meet the health care needs for the twenty-first century. It is in record that in Germany out of three drugs prescribed, one is a herb.

1.2 Diabetes

In the simplest terms, diabetes mellitus (commonly referred to as ”diabetes”) is a blood sugar disease, a disease in which the body either does not produce or does not properly utilize insulin. Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. Due to the fact that diabetes patients have problem with insulin, which cannot use glucose (blood sugar) for energy, which results into elevated blood glucose levels (hyperglycaemia) and the eventual reduction of sugar level through urine. There are three main types of diabetes:

1. **Type 1** *(insulin-dependent, previously called ”juvenile diabetes”).* This type of diabetes is associated with a malfunctioning pancreas which does not produce adequate amounts of insulin. It develops most often in children and young adults. It is traditionally treated with insulin.

2. **Type 2** *(non insulin-dependent, sometimes called ”adult-onset diabetes”).* This type diabetes is known to develop commonly in older adults, however it is now being found to affect younger ages including children.

3. **Gestational** *(pregnancy-related).* Some women develop diabetes during pregnancy, usually toward the end of pregnancy. It affects approximately 3 to 5 percent of all pregnant women. Although it naturally cures itself after pregnancy, these women have a higher risk for developing type 2 diabetes later in life.

1.2.1 Causes of Diabetes

1.2.1.1 Type 1

There is an early interspersed tissue throughout the pancreas which contain cells that make and secrete hormones. This tissue, called the ”Islets of Langerhans” is named after the German pathologist Paul Langerhans, who discovered them in 1869. Through a microscope, Langerhans observed that these cells cluster in groups, which he likened to little islands in the pancreas.

One such group of cells, the beta cells, produce insulin in response to blood glucose. These beta cells are tiny insulin pockets that sense the level of glucose in the blood stream, and produce insulin in precise proportion to that level. Therefore, following a meal, blood sugar levels will rise significantly, and the beta cells will release a large amount of insulin. This insulin will cause body cells to take up the sugar, causing blood sugar to quickly return to its normal range. Once blood sugar is in the normal range, the beta cells will reduce the output of insulin to an idling state. In this way, the beta cells adjust their production of insulin as per demand, always producing just enough insulin to deal with the amount of blood sugar presently in the blood stream.

In type 1 diabetes, the islets are destroyed by the person's own immune system, which mistakenly identifies these essential cells as foreign invaders. This self-destructive mechanism is the basis of many so-called auto-immune diseases. Once the islets are killed, the ability to produce insulin is lost, and the overt symptoms and consequences of diabetes begin.

1.2.1.2 Type 2

The most common causes of type 2 diabetes are poor diet and/or lack of exercise, both of which can result in insulin resistance. This is a condition where the cells in our bodies are not sensitive enough to react to the insulin produced by our pancreas.

Insulin is a chemical messenger that signals proteins called GLUT-4 transporters (residing within the cell) to rise up to the cell's membrane, where they can grab on to glucose and take it inside the cell. For patients with insulin resistance, the cells do not get the message. They simply cannot hear insulin ”knocking” on the door, which results in elevated blood levels of both insulin and glucose.

In the early stages of insulin resistance, the pancreas compensates by producing more and more insulin, and so the ”knocking” becomes louder and louder. The message is eventually ”heard”, enabling glucose transportation into the cells, resulting in the eventual normalization of blood glucose levels. This is known as ”compensated insulin resistance”.

Over time, the stress of excessive insulin production wears out the pancreas

and it cannot keep up this accelerated output. As a result, glucose levels remain elevated for prolonged periods. This is called ”uncompensated insulin resistance” and is the essence of advanced type 2 diabetes. Type 2 diabetes is characterized by a series of chain reactions as follows:

1. The ingestion of too many carbohydrates leads to a rise in blood sugar levels.
2. This is followed by a corresponding rise in insulin.
3. This in turn causes blood sugar to drop.
4. Eventually, this drastic up-and-down activity begins to take its toll on the body’s ability to use insulin and thus metabolize sugar.
5. Over time, the pancreas ”wears out” and can no longer pump out enough insulin to overcome this insulin resistance.
6. This results in a decreased insulin production and/or increased insulin resistance which propagates the cycle and leads to the onset of diabetes.

In medical world it is known that insulin resistance and obesity are correlated, particularly the type where the weight seems to collect around the middle (like an apple). It is also known that physical inactivity contributes to insulin resistance, as excessive feeding on too much of carbohydrate.

1.2.2 Symptoms of Diabetes

Diabetes symptoms vary somewhat, depending on the type of diabetes a patient has. A patient might experience some or all of the symptoms of type 1 and type 2 diabetes generally listed as:

1. Increased thirst.
2. Frequent urination.
3. Extreme hunger.
4. Unexplained weight loss.

5. Fatigue.

6. Blurred vision.

7. Slow-healing sores.

8. Frequent infections, such as gum or skin infections and vaginal or bladder infections.

1.2.3 Risk Factors of Diabetes

The two major factors contributing to today's alarming rise in diabetes are: poor diet and lack of exercise. In today's fast paced culture, with its emphasis on "fast foods" and its silence on exercise, more people are eating unhealthy diets and choosing poor lifestyles.

Our typical diet has become way out of balance. We eat too many simple sugars too often. Most people consume candy, French fries, potato chips, ice cream, pasta etc on a regular basis. We eat twice the calories we need, twice the protein we need and each year an average person consumes over 160 pounds of sugar and sweeteners which is not needed at all.

When we consider that so many of us are overfed and so few get regular exercise and the fact that many of us overuse alcohol and nicotine which increase oxidative stress, it's no wonder that millions of us suffer from diabetes, or are at great risk of developing diabetes in the near future.

The ever-increasing number of overweight, out of shape, oxidatively stressed people in today's societies around the world, is directly proportional to the epidemic rise of diabetes.

The following is a list of risk factors for getting diabetes:

1. Being more than 20 percent overweight.

2. Physical inactivity.

3. Having a first degree relative with diabetes (parents or siblings) .

4. Having an "Impaired Fasting Glucose" (IFG) or "Impaired Glucose Tolerance" (IGT) on previous blood tests.

5. Having Triglycerides (blood fats) which are more than 250 mg/dl.
6. Having HDL cholesterol (”good” cholesterol) which is less than 35 mg/dl.
7. Having a history of hypertension (high blood pressure) .
8. Having a history of gestational (pregnancy-related) diabetes or giving birth to a baby which weighed more than 9 pounds .

1.2.4 Complications of Diabetes

The most important health impacts of diabetes are the long-term complications it can cause. Most of these long-term complications are related to the adverse effects diabetes has on arteries and nerves.

1.2.4.1 Complications related to artery damage

Diabetes causes damage to both large and small arteries. This artery damage results to medical problems that are both common and serious. The most common of complications resulting from artery damage includes;

1. **Cardiovascular disease.** The burden of Cardiovascular risks is high in the diabetic population, Otieno et al (1974). Diabetics have up to a 400 percent greater chance of heart attack or stroke. Heart disease and stroke cause about 65 percent of deaths among people with diabetes. These deaths could be reduced by 30 percent with improved care to control blood pressure and blood glucose and lipid levels.
2. **Amputations.** Diabetic foot ulcers and diabetic foot with peripheral vascular disease also contribute significantly to lower limb amputation, Awali (2007). Over 60 percent of non-traumatic lower limb amputation are diabetes related. Foot care programs that include regular examinations and patient education could prevent up to 85 percent of these amputation.
3. **Kidney disease.** About 38,000 people with diabetes develop kidney failure each year. Treatment to better control blood pressure and blood glucose levels could reduce diabetes-related kidney failure by about 50 percent.

4. **Eye disease and blindness.** Each year, 12,000-24,000 people become blind because of diabetic eye disease, including diabetic retinopathy. Diabetes is the leading cause of new cases of blindness among adults 20-74 years old. Screening and care could prevent up to 90 percent of diabetes-related blindness.

5. **Sexual Dysfunction.** Approximately 70 percent of all adult males with diabetes suffer or experience sexual dysfunction or impotence.

1.2.4.2 Complications related to nerve damage

Between 60 to 70 percent of people with diabetes have mild to severe forms of nervous system damage. This diabetic neuropathy may result in numbness, tingling, and paresthesia in the extremities. Some examples of diabetic neuropathy are as follows.

1. **Peripheral neuropathy.** The feet and legs can develop tingling, pain or some numbness. This problem makes foot ulcers and foot infections more common, adding to the possibility that an amputation may be needed.

2. **Stomach and bowel problems.** The nerves that trigger normal movements of the stomach and intestines can become less active or less predictable. This can result in nausea, constipation or diarrhoea. A stomach that is slow to empty its content has a diabetes condition called gastroparesis.

3. **Dizziness when standing.** Blood circulation has to make some adjustments to move blood from toes to the torso when one is standing up, since it is moving against gravity. When the body is working correctly, this adjustment includes tightening of blood vessels to prevent pooling of blood in the lower body. The circulation relies on nerve signals to know when to make this adjustment. These signals can fail in diabetes, leaving one with low blood pressure and lightheadedness when one is standing.

4. **Sexual-function problems.** Impotence is especially common in people with nerve damage from diabetes. Artery damage also contributes to impotence.

5. **Localized nerve failures.** A nerve that controls a single muscle can lose its function. Examples of problems that might result are eye movement problems with double vision, or drooping of the cheek on one side of the head (commonly known as Bell's palsy).

Many of these potential complications can significantly interfere with the quality of life and to some extent, shorten the life of a person with diabetes. Diabetes complications are primarily caused by two factors. These are:

1. Excessive Glycosylation.

Glycosylation is the process by which the sugar molecule binds irreversibly to a protein molecule. This process takes place in all humans, but because diabetics have higher levels of glucose in their blood and for longer durations than non diabetics, they have a much higher degree of glycosylation occurring

Excessive glycosylation results in abnormal protein structures which lead to a host of cellular dysfunction such as: inactivation of enzymes, inhibition of regulatory molecule binding, decreased susceptibility to proteolysis, abnormalities of nucleic acid function, altered macromolecular recognitions and increased immunogenicity.

In diabetics, glucose binds to proteins in the blood, nerves and the eyes. This pathological process causes much of the damage in the complications of diabetes.

2. Sorbitol Accumulation

Sorbitol is the by-product of glucose metabolism and is produced through the action of the enzyme aldose reductase. In non-diabetics, sorbitol is converted to fructose and is easily excreted from the cell. However, inside the cells of diabetics, when glucose levels become elevated (even after glucose levels outside of the cell return to normal), sorbitol is produced faster than it can be broken down. Since it cannot permeate the cell membrane, it builds up to a toxic level inside the cells, creating an imbalance and causing a loss of electrolytes and other minerals. This accumulated sorbitol draws water in to the cell, by the process known as osmosis,

and ultimately leads to the collapse of its formulation and loss of its function.

Sorbitol-induced osmotic swelling is believed to be one of the main causes of tissue damage in diabetics. This condition seems to target organs and tissues that are not dependent on insulin for their absorption of glucose. Elevations of sorbitol levels are a major problem in peripheral nerves, blood vessels, the cells of the retinal blood vessels, the lens of the eye, the pancreas, kidneys and other organs due to their lack of insulin dependence.

1.2.5 Diabetes: Preventive Measures

Diabetes and it's complications can be treated and/or prevented safely without prescription drugs (contemporary medicine) from the herbalist point of view. If one has diabetes or any of the risk factors for diabetes or is just concerned about diabetes, the herbalists recommend that one start with a natural treatment plan to reduce the risk. There are three components to a natural diabetes cure for which most herbalists are in agreement about. These are:

1. **Diet:** The single most important change any diabetic or person at risk can make is to improve the diet. A proper diet for a diabetic should have a low glycemic index (that is containing low simple carbohydrates), moderate protein and high fibre. This diet reduces blood sugar, reduces insulin levels, and reduce the need for medications. It will also help to reduce weight, reduce blood pressure and support overall health and energy.

2. **Exercise:** Studies have shown that exercise is of great benefit to diabetics and can significantly reduce the risk of developing type 2 diabetes. Regular physical activity helps reduce weight, lower blood sugar, improve insulin sensitivity, strengthen the immune system, improve circulation, lower blood pressure, lower LDL (”bad”) cholesterol, raise HDL (”good”) cholesterol, and reduce risk of heart disease.

3. **Nutritional Supplements:** There are a number of nutritional supplements that every diabetic should be taking on a daily basis. These supplements are very effective in helping to lower blood sugar and insulin levels, reduce

cholesterol levels, reduce triglyceride levels, reduce blood pressure, improve energy, and reduce the risk of heart disease. These supplements can also protect body tissues (eyes, kidneys, blood vessels) from the damage diabetes often causes. They can also support body immune system, protect the heart, and improve circulation.

1.2.6 Commonly used Diabetic Herbs

1.2.6.1 Cinnamon

Cinnamon is the brown bark of the cinnamon tree, which, when dried, rolls into a tubular form known as a quill. It is available in either its whole quill form (cinnamon sticks) or as ground powder. It is one of the oldest spices known but is much more than just a spice. Cinnamon has demonstrated great medical application in preventing and combating diabetes, Anderson et al (2010). According to cellular and molecular studies conducted at the University of California, Santa Barbara, Iowa State University and the U.S. Department of Agriculture, Cinnamon plays the role of an insulin substitute in type 2 diabetes. This initial discovery was made quite accidentally, by Richard Anderson. He investigated the effects of common foods on blood sugar, one was the American favourite, apple pie, which is usually spiced with cinnamon. With his team they found that people who eat apple pie had a significantly lower probability of getting Type II diabetes. Upon further examination, he isolated cinnamon as the substance in the apple pies that was preventing the diabetes. They recently completed a study with associates in Pakistan using cinnamon on human beings. Their study included 60 Pakistani volunteers (30 men and 30 women ranging in age from 44 to 58 years) with type 2 diabetes, who were not taking insulin. Subjects were divided into six groups. For 40 days, groups 1, 2 and 3 were given 1, 3 or 6 grams of cinnamon per day, while groups 4, 5 and 6 received placebo capsules. All the three groups given cinnamon showed reduced blood sugar levels. When daily cinnamon was stopped, blood sugar levels began to increase. No significant changes were seen in those groups receiving placebo.

Their conclusion was that including cinnamon in the diet of people with type 2

diabetes reduces risk factors associated with diabetes and cardiovascular diseases. Anderson cautioned that the most important thing is to add cinnamon to what one would eat normally, since cinnamon triples insulin's efficiency and at least a half teaspoon is critical to "soften" the cell membranes. Further studies by the Anderson team have collaborated cinnamon's ability to improve insulin activity. Their study has led to the discovery of cinnamon's active ingredient, as well as an understanding of its structure and the mechanism by which it enhances insulin activity. Using nuclear magnetic resonance and mass spectroscopy, the Anderson team was able to describe the chemical structure of a molecule with "insulin-like" activity in cinnamon as a water-soluble polyphenol compound called methylhydroxychalcone polymer (MHCP).

Anderson (2003) discovered that MHCP not only stimulates glucose uptake by the cells, but it can even help in the synthesis of glycogen, a polymer of glucose that our bodies produce as a means of storing energy for later use when it is depolymerized back to glucose. Producing adequate amounts of glycogen is a principal function of blood sugar metabolism and MHCP can help.

Anderson, concluded that MHCP mimics insulin, has effects similar to that of insulin and works almost as well as insulin. He asserts that both of these substances work by chemically modifying our cells' insulin receptors in a manner that activates them to do their job, which is to allow glucose molecules to pass through the cell wall into the insulin cascade. He also discovered that when MHCP and insulin act together, the effect is synergistic, i.e., the total effect is greater than the sum of its parts." They characterize the insulin-enhancing complexes in cinnamon as *a collection of catechin/epicatechin oligomers that increase the body's insulin-dependent ability to use glucose roughly 20-fold*.

However some scientists have been concerned about potentially toxic effects of regularly consuming cinnamon. Ongoing (no publications) research suggest that the potentially toxic compounds in cinnamon bark are found primarily in the lipid (fat) soluble fractions and are present only at very low levels in water soluble cinnamon extracts, which are the ones with the insulin-enhancing compounds.

1.2.6.2 Alpha Lipoic Acid (ALA)

Alpha Lipoic Acid (also known as thioctic acid or lipoic acid) is a very powerful natural antioxidant and is the single most important supplement one can take to treat diabetes.

ALA was first isolated in 1953 and was quickly discovered to be a very important cofactor in the Krebs cycle (the body's main process for converting carbohydrates into energy). ALA and its 'cousin' DHLA are often referred to as the "ultimate universal antioxidants". They (referred to collectively as LA) are the only antioxidants that are both fat and water soluble. Both can actually cross the blood/brain barrier to enter the brain. These unique qualities are important, because it means that LA can access all parts of all cells, giving it tremendous ability to scavenge free radicals wherever they may be. Additionally, LA can also recharge other antioxidants that have been used up. In the body, LA helps regenerate other antioxidants such as vitamin C, vitamin E and glutathione. And, because LA functions much like a B-vitamin, it also helps convert food into energy.

Though the body makes some alpha lipoic acid, it is not enough for optimal nutrition. Likewise, there are only very small amounts of ALA found in some of our daily foods such as broccoli, potatoes, and liver. In these foods, it actually occurs as lipolylysine though, and not actual lipoic acid itself. One can never get any useful amount of ALA from a broccoli diet alone. This means one would have to eat over two pounds of broccoli to get one single milligram of lipolylysine to convert into alpha lipoic acid.

Eberhard-Karls University (1999), in their study that was an advancement of studies carried out before them, (Diabetologica 1995 and Arzneimittelorschung 1995), real adult human diabetics were given various doses of ALA. The doctors found that in just 10 days, ALA helped cure insulin resistance, normalize blood sugar levels and cure diabetes. The researchers pointed out that Thioctic acid is a co-factor of key mitochondrial enzymes, involved in the regulation of glucose oxidation, such as the pyruvate dehydrogenase and the alpha-ketoglutatarate dehydrogenase, both enzyme complexes which are known to be diminished in diabetes. This means ALA works with our bodies' enzymes to prevent glucose from being oxidized. They concluded tha the clinical and experimental data indicate that this

compound has beneficial effects on insulin sensitivity, correcting several metabolic pathways known to be altered in type 2 diabetes, such as insulin stimulated glucose uptake, glucose oxidation and glycogen synthesis.

Other studies have shown that ALA Increases glucose effectiveness. When ingested, ALA decreases serum lactate and pyruvate concentrations improving glucose effectiveness in both lean and obese patients with type 2 diabetes. Additionally, because ALA inhibits glycosylation and peroxidation of nervous tissues and increases the levels of intra-cellular glutathione, it has been used to improve diabetic nerve damage and reduce pain associated with that nerve damage. Nerve damage or neuropathy affects over 50 percent of diabetics and is one of its most damaging complications.

In 2001, a study which was conducted at the University of Southern California on Molecular Aspects of Lipoic Acid in the Prevention of Diabetes Complications availed data that strongly suggest that ALA, because of its antioxidant properties, is particularly suited to the prevention and/or treatment of diabetic complications. In addition, ALA increases glucose uptake, increases glucose disposal in type 2 diabetics and remarkedly reduces the symptoms of diabetic pathologies, including cataract formation, vascular damage and polyneurpathy. These are rather powerful statements coming from very well respected research groups.

1.2.6.3 Banaba Leaf

Banaba (Lagerstroemia speciosa) is a plant native to India, Southeast Asia and the Philippines and has several medicinal uses. In many cultures the banaba leaf is brewed into a tea and used as a treatment for diabetes and as a weight loss aid. Banaba Leaf Extract provides a blood sugar lowering effect similar to that of insulin in that it induces glucose transport from the blood into body cells.

Recently, researchers have isolated an active ingredient in the banaba leaf called corosolic acid which was originally thought to be ”the” blood sugar regulating substance in the leaf. Other researchers have found that corosolic acid may not be the only active ingredient in banaba leaves. A study in 2001 compared a whole-leaf extract of banaba with insulin in cell cultures. The researchers concluded that the whole herb has a glucose lowering effect. Another study reported that banaba

leaf extract contains at least three active ingredients that affect blood sugar.

In animal studies, administration of banaba leaf extract resulted in a significant decrease of blood glucose. The same studies suggest that corosolic acid may stimulate glucose transport into tissue. In other animal studies, administration of banaba leaf extract resulted in reduced weight gain, reduced triglyceride accumulation and reduced adipose tissue, with no changes in diet. In non insulin-dependent animals, administration of banaba leaf extract resulted in suppressed blood plasma glucose, lower serum insulin and lower urinary excretion of glucose.

In clinical studies conducted by William Judy and associates at the Southeastern Institute of Biomedical Research in Bradenton, Florida, a one per cent corosolic acid extract of banaba leaf reportedly reduced serum glucose 20-30 percent in people with type 2 diabetes, but did not reduce serum glucose in healthy individuals. In a prior study, some of the same researchers observed that individuals receiving the corosolic acid extract also had an increased tendency toward weight loss with an average of about 3.2 pounds.

1.2.6.4 Momordica

Bitter melon is the common name for Momordica charantia, also known as African cucumber, balsam pear and bitter gourd. The plant is aptly named, as all parts of the plant, including the fruit, taste bitter. Widely sold in Asian groceries as a vegetable, bitter melon is employed as a folk remedy primarily for regulating blood sugar in cases of diabetes, as well as for colitis and dysentery, intestinal worms, jaundice and fevers. Current understanding of the phytochemicals in bitter melon suggests that these multiple uses are well founded.

Among the constituents in bitter melon, charantin is identified as a primary agent for blood-sugar regulation. Charantin demonstrates hypoglycemic (blood sugar lowering) or other actions of potential benefit in diabetes. The fruits also contain insulin-like peptides, including one known as polypeptide P, and alkaloids. It is likely that several substances in bitter melon contribute to its blood sugar-modifying effects. In human studies, bitter melon demonstrates significant blood-sugar control after food intake and overall blood sugar-lowering effects.

Bitter melon has potent anti-diabetes effects melon known as cucurbitane triter-

penoids. Harvard University Medical School (2003) established their effects on glucose (sugar) and fat metabolism in cells and in mice. When tested in muscle and fat cells, the researchers found, the compounds stimulated the glucose receptor GLUT4 to move from the cell interior to the cell surface, thus promoting more effective glucose metabolism. Several of the tested compounds had effects comparable to those of insulin. Tests in mice of two of the compounds found that they promoted both glucose tolerance and fat burning, and one was particularly effective in promoting glucose tolerance in animals consuming high fat diets. The researchers note that there may be as many as 70 active compounds in bitter melon, this is an important basis for further analysis of structure-activity relationship to develop optimized leads from (bitter melon) for the treatment of insulin resistance and obesity.

Research at the Harvard University Medical School (2003) established that, Momordica is just as effective as glibenclamide in reducing blood sugar levels. In fact, a large study at Harvard University Medical School concluded that mormodica is one of the best natural remedies for diabetes . It appears that mormodica contains compounds similar in structure to insulin, which have the same effects in regulating blood sugar levels. There is also evidence that mormodica can prevent the release of excess glucose into the bloodstream from the liver.

1.2.6.5 Gymnema Sylvestre

Gymnema Sylvestre is another herb, whose traditional use in treating diabetes, has been backed up by recent medical research. Originating from India, Gymnema Sylvestre is known as gur-mar, or ”sugar destroyer.” When gymnema leaf is placed directly on the tongue, it eliminates the sensation of sweetness, even if sugar is put in the mouth immediately. When taken internally, it helps to control blood-sugar levels in diabetes.

The leaves of Gymnema sylvestre perform two significant functions relative to diabetes. First, they suppress blood glucose, especially after eating. Secondly, they are insulinotropic and promote insulin secretion. By this two-pronged approach, Gymnema sylvestre proves a valuable aid in diabetes control. Scientists believe that its active ingredients (gymnemic acids) protect the cells of the pancreas from

free radical damage, so allowing them to regenerate and produce insulin more effectively.

Studies have shown that gymnema can also reduce glucose absorption from the intestine, so helping to regulate blood sugar levels. A recent Harvard study indicates that the Gymnema lowers blood sugar levels in Type 1 and Type 2 diabetics. A recent King's College, London, study states that Gymnema acts by increasing cell permatibility, therefore reducing insulin resistance.

1.2.7 Diagnose for Diabetes

Diabetes is diagnosed by evaluating both symptoms and laboratory test results. There are two commonly used laboratory tests:

1. **Fasting Plasma Glucose test (FPG):** With the FPG test, the blood glucose level is measured after an 8 hour fast. If glucose is higher than normal (100 mg/dl),a person is said to have "Impaired Fasting Glucose" (IFG), which suggests pre-diabetes. A diagnosis of Diabetes is made when an FPG level of greater than 125 mg/dl is measured in two different occasions, for confirmation.

2. **Oral Glucose Tolerance Test (OGTT):** An OGTT may be helpful in diagnosing type 2 Diabetes in patients whose FPG is between 115 and 125 mg/dl. During an OGTT test, the blood sugar is measured after a fast and then again 2 hours after drinking a beverage containing a large amount of glucose. If the glucose is higher than normal (140 mg/dl), two hours after the drink, a person is said to have "Impaired Glucose Tolerance" (IGF), which suggests pre-diabetes. A diagnosis of Diabetes is made when an OGTT level is greater than 200 mg/dl

1.3 Response surface

Experimentation plays an important role in Science, Engineering, and Industry, this is an application of treatments to experimental units and then measurement of one or more responses. It requires observing and gathering information about

how a process or a system works. In an experiment, some inputs $x_1, x_2, ..., x_k$ transform into an output that has one or more observable response variables y. Therefore, useful results and conclusions can be drawn from the analysis of data in the experiment. In order to obtain an objective conclusion an experimenter needs to plan and design an experiment and analyze the results.

As an important subject in the statistical design of experiments is the Response Surface Methodology (RSM) which is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery 2005). For example, the growth of a plant is affected by a certain amount of water x_1 and sunshine x_2. The plant can grow under any combination of treatment x_1 and x_2 among other variables. Therefore, water and sunshine can vary continuously. When treatments are from a continuous range of values, then a Response Surface Methodology is useful for developing, improving, and optimizing the response variable. In this case, the plant growth y is the response variable, and it is a function of water and sunshine. It can be expressed as

$$y = f(x_1, x_2) + \epsilon \tag{1.1}$$

The variables x_1 and x_2 are independent variables where the response y depends on them. The dependent variable y is a function of x_1, x_2, and the experimental error term, denoted as ϵ. The error term ϵ represents any measurement error on the response, as well as other types of variations not counted in f. It is a statistical error that is assumed to distribute normally with zero mean and variance σ^2. In most RSM problems, the true response function f is unknown. In order to develop a proper approximation for f, the experimenter usually starts with a low-order polynomial in some small region. If the response can be defined by a linear function of independent variables, then the approximating function is a first-order model.

In general all RSM problems use either one or a combination of RSM models. In each model, the levels of each factor are independent of the levels of other

factors. In order to get the most efficient result in the approximation of polynomials, the proper experimental design must be used to collect data. Once the data are collected, the Method of Least Squares is used to estimate the parameters in the polynomials. The response surface analysis is performed by using the fitted response surface. The Response surface designs are types of designs for fitting response surface. Thus, the objective of studying RSM can be accomplished by;

Understanding the topography of the response surface (local maximum, local minimum, ridge lines),

Finding the region where the optimal response occurs. The goal is to move rapidly and efficiently along a path to get to a maximum or a minimum response so that the response is optimized,

Estimation of the optimum conditions, and

Verification of the optimum conditions.

Some notable features will emerge, where the objective of the experiment is not to investigate the functional relationship $f(x_1, x_2, \cdots, x_k)$ over the whole factor space but to locate the region in which the response is at its highest and map it. Regarding the response surface as a mountain, the objective of an excursionist is to find the peak of the mountain and explore the area in which the peak is found. Analogously, this is equivalent to locating feasible treatment combinations for which the mean response is optimized (maximized/minimized or equal to a specific target value) and to estimate the response surface in the vicinity of this good location to better understand the local effects of the factors on the mean response.

This excursion will yield interesting patterns of the response surface where we might map peaks and ridges (series of contours of equal measure) in which we may identify with optimal yield. Of interest to note then is how to discriminate amongst these various points at which the yield is the same for different combinations of the predictor variables and isolate that one which we identify as parsimoniously feasible. The variance function comes in handy as a tool for this aforementioned discrimination. The most feasible of all the identified point (peaks or ridges) of equal yield is one in which the variance function is minimal.

A special case of interest is when the design is rotatable for which the variance function of the estimated response will remain constant on the sphere about the centre of the design.

1.3.1 Response surface

By application of the same line of thought on the growth of a plant, one can model or optimize any response that is affected by the levels of one or more quantitative factors. Generally the response is a quantitative continuous variable (e.g. yield, growth, purity, cost, e.t.c) and the mean response is a smooth but unknown function of the one or more factors say k, whereas the levels of a factor are real valued and controllable.

Suppose we wish to investigate the plant growth where two factors, say, amount of water x_1 and sunshine x_2 are involved. The experimental procedure will be to make several trials (runs) on the pilot plant at different levels of water x_1 and sunshine x_2 and to make observations, on each run a response say the plant growth y is the response variable. Here we have a two-dimensional factor space of the qualitative factors (i.e. measurable on a continuous scale). We can think therefore of the expected response $E(y)$ as a function of the levels of the factors. Let x_1 and x_2 denote the levels of the factors, we have,

$$y = f(x_1, x_2) + \epsilon \tag{1.2}$$

where, $\epsilon \sim N(0, \sigma^2)$

The function is single valued and the expected value of (1.2) is

$$E(y) = f(x_1, x_2) \tag{1.3}$$

which is called a $Response\ Surface$.

The reason why we call it a response surface is that though we have $E(y) = f(x_1, x_2)$ if the x_1 and x_2 axes are taken in their usual way and the y axis is drawn perpendicular to the plane of the paper, the response can be represented on the surface of the paper just like altitude is represented on a map using lines of equal response (height) as contours. In general therefore for k factors, mean response plotted as a function of the treatment combinations will yield a surface in $k + 1$ dimensions called the response surface.

Prior knowledge of the process involved will enable us know the form of $f(x_1, x_2, \ldots, x_k)$ for k factors. However with lack of this knowledge, we consider a situation in which $f(x_1, x_2, \ldots, x_k)$ is approximated by a polynomial of degree say d for which the model will be referred to as the d^{th} order model. The set of points $(x_1, x_2, \ldots, x_k)$ at which trials are made is referred to as the ***Variable Factor Space***.

With reference to a d^{th} order design (model) the general design is represented as;

$$y_i = f(x_i)\beta + \epsilon_i \tag{1.4}$$

whose matrix equivalent is,

$$Y = X\beta + \varepsilon \tag{1.5}$$

where,

Y is an $n \times 1$ vector of observations,

X is an $n \times k$ matrix,

β is a $k \times 1$ vector of unknown parameters, and

ε is a $n \times 1$ vector of independently identically distributed random variables with mean zero and variance σ^2.

Consider a second order model written as,

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \varepsilon, \tag{1.6}$$

where, $\beta_0, \beta_1, \beta_2, ..., \beta_k, \beta_{11}, ..., \beta_{kk}, \beta_{12}, ..., \beta_{k-1,k}$ are constants and ε is normal with mean zero and variance σ^2 and $x_1, x_2 ..., x_k$ are the levels of the predictor (independent) variables.

Let x'_{a} and x'_{b} be the transpose of two vectors of the matrix X but which arises from two distinct points in the predictor variable space. Then,

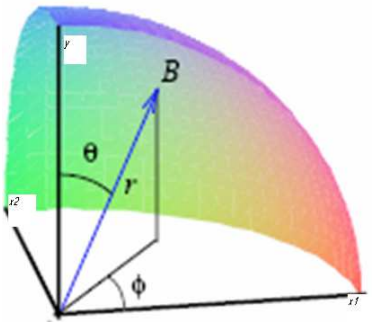
$$\hat{y}(\mathrm{x}'_{\mathrm{a}}) = \mathrm{x}'_{\mathrm{a}}\hat{\beta} \tag{1.7}$$

and

$$\hat{y}(\mathrm{x}'_{\mathrm{b}}) = \mathrm{x}'_{\mathrm{b}}\hat{\beta} \tag{1.8}$$

where, the vector $\hat{\beta}$ is the Least Square Estimate of β, describe two points on the response surface. If for example $k = 2$,

then the response surface in three dimensions is presented as,



The rotation of the two points through any angle will be such that their distance from the centre as well as between them is maintained to be constant for an evenly shaped response surface from the centre.

Likewise for a third order design

$$y_u = \beta_0 x_{0u} + \sum_{i=1}^{k} \beta_i x_{iu} + \sum_{i=1}^{k} \beta_{ii} x_{iu}^2 + \sum_{i=1}^{k}\sum_{j=1}^{k} \beta_{ij} x_{iu} x_{ju} + \sum_{i=1}^{k}\sum_{j=1}^{k}\sum_{l=1}^{k} \beta_{ij} x_{iu} x_{ju} x_{lu} + \varepsilon_u, \tag{1.9}$$

where

$x_{0u}, x_{1u}, ..., x_{ku}, , x_{1u}^2, ..., x_{ku}^2, x_{1u}x_{2u}, ..., x_{k-1,u}x_{ku}, x_{k-2,u}x_{k-1,u}x_{ku}$ are variables or input levels,and

$\beta_0, \beta_1, \beta_2, ..., \beta_k, \beta_{11}, ..., \beta_{kk}, \beta_{12}, ..., \beta_{k-1,k}, \beta_{123}, ..., \beta_{k-2,k-1,k}$ are parameters to be estimated, with $E(\varepsilon_u) = 0,\ Var(\varepsilon_u) = \sigma^2$ (unknown constant), $Cov(\varepsilon_u \varepsilon_u') = 0,\ u \neq u' = 1, 2, ..., N$

1.3.2 Rotatability

The property of rotatability as a desirable quality of an experimental design was first introduced by Box and Hunter (1957). This property states that; *The variance of the estimated response is constant on circles spheres or hyper-spheres about the centre of the design*. Thus a rotatable arrangement which achieves this property could be rotated through an angle around the centre and the variance of the estimated response would remain invariant. That is, there is same amount of information about the response surface at the same distance say ρ in any direction from the centre of the design. This is a reasonable requirement of a design since the data are generally collected without knowing in which direction from the design centre the stationary points of the fitted surface will be located.

1.3.3 Moment Conditions and Rotatability

1.3.3.1 First-Order Model

The moment conditions which are necessary for a set of N points $(x_{1u}, x_{2u}, \ldots, x_{ku})$, $u = 1, 2, ..., N$ to form a first order rotatable design are;

1. The sum of the powers or sum of product of powers of the x_{iu}'s with at least one power odd is zero, that is;

   (i) $\sum_{u=1}^{N} x_{iu} = 0$

   (ii) $\sum_{u=1}^{N} x_{iu}x_{ju} = 0$

2. the sum of product of powers of the x_{iu}'s with even powers is constant

   (iii) $\sum_{u=1}^{N} x_{iu}^2 = N\lambda_2$

   this condition is just but the orthogonality condition.

1.3.3.2 Second-Order Model

The moment conditions which are necessary for a set of N points $(x_{1u}, x_{2u}, \ldots, x_{ku})$, $u = 1, 2, ..., N$ to form a second order rotatable design are;

1. The sum of the powers or sum of product of powers of the x_{iu}'s with at least one power odd is zero, that is;

   (i) $\sum_{u=1}^{N} x_{iu} = 0$

   (ii) $\sum_{u=1}^{N} x_{iu}x_{ju} = 0$

   (iii) $\sum_{u=1}^{N} x_{iu}x_{ju}^2 = 0$

   (iv) $\sum_{u=1}^{N} x_{iu}^3 = 0$

   (v) $\sum_{u=1}^{N} x_{iu}x_{ju}x_{lu} = 0$

   (vi) $\sum_{u=1}^{N} x_{iu}x_{ju}x_{lu}^2 = 0, \quad i \neq j \neq l$

2. the sum of the powers or sum of product of powers of the x_{iu}'s with even powers are constant

(i) $\sum_{u=1}^{N} x_{iu}^2 = N\lambda_2$

(ii) $\sum_{u=1}^{N} x_{iu}^4 = 3\sum_{u=1}^{N} x_{iu}^2 x_{ju}^2 = 3N\lambda_4 \quad i \neq j \neq l$

In addition to these conditions we must ensure that $X'X$ is non singular for which the non-singularity condition for a second order design is that λ_4 and λ_2 must satisfy;

$$\frac{\lambda_4}{\lambda_2^2} > \frac{k}{k+2} \tag{1.10}$$

1.3.3.3 Third-Order Model

The moment conditions which are necessary for a set of N points $(x_{1u}, x_{2u}, \ldots, x_{ku})$, $u = 1, 2, ..., N$ to form a third order rotatable design are;

1. The sum of the powers or sum of product of powers of the x_{iu}'s with at least one power odd is zero, and

2. the sum of the powers or sum of product of powers of the x_{iu}'s with even powers are constant, that is;

(i) $\sum_{u=1}^{N} x_{iu}^2 = N\lambda_2$

(ii) $\sum_{u=1}^{N} x_{iu}^4 = 3\sum_{u=1}^{N} x_{iu}^2 x_{ju}^2 = 3N\lambda_4$

(iii) $\sum_{u=1}^{N} x_{iu}^6 = 5\sum_{u=1}^{N} x_{iu}^2 x_{ju}^4 = 15\sum_{u=1}^{N} x_{iu}^2 x_{ju}^2 x_{lu}^2$
$= 15N\lambda_6 \; i \neq j \neq l$

Further to these two conditions we must also ensure that $X'X$ is non singular for which the non-singularity condition for a third order design is that λ_6,λ_4 and λ_2 must satisfy;

$$\frac{\lambda_4}{\lambda_2^2} > \frac{k}{k+2} \tag{1.11}$$

$$\frac{\lambda_6\lambda_4}{\lambda_4^2} > \frac{k+2}{k+4} \tag{1.12}$$

for which in this case if they are not satisfied, then the addition of N_0 central points (0,0,...,0) can make condition 1.10 satisfied. However addition of centre points does not make or satisfy 1.11. This can be satisfied by combining at least two spherical set of points with different radii.

1.3.3.4 Fourth-Order Model

The moment conditions which are necessary for a set of N points $(x_{1u}, x_{2u}, \ldots, x_{ku})$, $u = 1, 2, ..., N$ to form a fourth order rotatable design are;

1. The sum of the powers or sum of product of powers of the x_{iu}'s with at least one power odd is zero, and

2. the sum of the powers or sum of product of powers of the x_{iu}'s with even powers are constant, that is;

(i) $\sum_{u=1}^{N} x_{iu}^2 = N\lambda_2$

(ii) $\sum_{u=1}^{N} x_{iu}^4 = 3\sum_{u=1}^{N} x_{iu}^2 x_{ju}^2 = 3N\lambda_4$

(iii) $\sum_{u=1}^{N} x_{iu}^6 = 5\sum_{u=1}^{N} x_{iu}^2 x_{ju}^4 = 15\sum_{u=1}^{N} x_{iu}^2 x_{ju}^2 x_{lu}^2 = 15N\lambda_6$

(iv) $\sum_{u=1}^{N} x_{iu}^8 = 7\sum_{u=1}^{N} x_{iu}^2 x_{ju}^6 = \frac{35}{3}15\sum_{u=1}^{N} x_{iu}^4 x_{ju}^4$

$$= 105\sum_{u=1}^{N} x_{iu}^2 x_{ju}^2 x_{lu}^2 x_{ru}^2 = 105N\lambda_8$$

while the necessary and sufficient conditions for which a set of points to form a rotatable design of order four are,

$$\omega_1 > \omega_2 - \omega_3 \tag{1.13}$$

$$\frac{\lambda_4\lambda_8}{\lambda_6^2} > \frac{k+4}{k+6} \tag{1.14}$$

$$\frac{\lambda_2\lambda_6}{\lambda_4^2} > \frac{k+2}{k+4} \tag{1.15}$$

Where,

$$\omega_1 = (k+2)(k+4)[(k+6)\lambda_4\lambda_8 - (k+4)\lambda_6^2]$$
$$\omega_2 = k(k+4)\lambda_2[(k+6)\lambda_2\lambda_8 - (k+2)\lambda_4\lambda_6]$$
$$\omega_3 = k(k+2)\lambda_4[(k+4)\lambda_2\lambda_6 - (k+2)\lambda_4^2]$$

these three later conditions (1.13), (1.14) and (1.15) ensures that the moment matrix $X'X$ is non singular.

1.4 Variance Function in Response surface Designs

Consider a general model $\boldsymbol{f(x_1, x_2, ..., x_k)}$ and its matrix equivalent as presented in (1.4). Assuming that the least square estimates of $\boldsymbol{\beta}$ are to be obtained by use of the normal equation,

$$\hat{\boldsymbol{\beta}} = (\boldsymbol{X'X})^{-1}\boldsymbol{X'y} \tag{1.16}$$

the variance of $\hat{\boldsymbol{\beta}}$, is the variance-covariance matrix and is given as

$$\boldsymbol{Var}(\hat{\boldsymbol{\beta}}) = (\boldsymbol{X'X})^{-1}\boldsymbol{X'}\ \boldsymbol{Var}(\boldsymbol{y})\boldsymbol{X}(\boldsymbol{X'X})^{-1} = (\boldsymbol{X'X})^{-1}\boldsymbol{\sigma^2} \tag{1.17}$$

Thus the variance of estimated response $(\hat{\boldsymbol{y}})$ at a point on the sphere of radius $\boldsymbol{\rho}$ where, $\hat{\boldsymbol{y}} = \boldsymbol{X'}\hat{\boldsymbol{\beta}}$ is

$$\begin{aligned} \boldsymbol{Var}(\hat{\boldsymbol{y}}) &= \boldsymbol{X'}\ \boldsymbol{Var}(\hat{\boldsymbol{\beta}})\boldsymbol{X} \\ &= \boldsymbol{X'}(\boldsymbol{X'X})^{-1}\boldsymbol{\sigma^2}\boldsymbol{X} \\ &= \boldsymbol{\sigma^2}\boldsymbol{f'}(\boldsymbol{x})(\boldsymbol{X'X})^{-1}\boldsymbol{f}(\boldsymbol{x}) \end{aligned} \tag{1.18}$$

here $\boldsymbol{\sigma^2}$ is assumed to be unknown constant and $\boldsymbol{x_i},\ \boldsymbol{i = 1, 2, ..., k}$ are taken to be non-stochastic.

The variance function of the fitted model in a general case can be used to evaluate competing designs whereby the best design is one which has the smallest possible variance. The prediction variance of the estimated response at a point say $\mathbf{x}$ is given by,

$$\boldsymbol{Var}(\hat{\boldsymbol{y}}(\mathrm{x})) = \boldsymbol{N^{-1}}\mathrm{x}'^{(\boldsymbol{m})}(\boldsymbol{X'X})^{-1}\mathrm{x}^{(\boldsymbol{m})}\boldsymbol{\sigma^2} \tag{1.19}$$

where $\mathrm{x}^{(m)}$ is the vector of co-ordinates of a point in the design space expanded to model form. Mostly, experimenters opt to use scaled predicted variance (SPV) arrived at by multiplying (1.18) by the design size and then dividing through by the process variance $\boldsymbol{\sigma^2}$, that is

$$\frac{\boldsymbol{N}\ \boldsymbol{Var}(\hat{\boldsymbol{y}}(\mathrm{x}))}{\boldsymbol{\sigma^2}} = \mathrm{x}'^{(\boldsymbol{m})}(\boldsymbol{X'X})^{-1}\mathrm{x}^{(\boldsymbol{m})} \tag{1.20}$$

This scaling is widely used to facilitate comparisons among designs of various sizes and eliminates the need to know the value of $\boldsymbol{\sigma^2}$.

Chapter 2

Literature Review

2.1 Herbal Medicine

2.1.1 General Herbal Medicine

Herbal medicine, which is sometimes referred to as botanical medicine or herbalism, is the use of plants, or parts of plants, to treat injuries or illnesses. The field also covers the use of herbs or botanicals to improve overall health. Seeds, leaves, stems, bark, roots, flowers, and extracts of all of these have been used in herbal treatment. These supplemental treatments have been delivered raw, in tea and tinctures, as topical applications, in liquid form and in pills and capsules. In the beginning the plants were consumed raw or combined with hot water as soup or tea. Later, the plants were dried and crushed for other uses. The plants were found in the wild and uses were often based on superstitious or visual clues. Plants were often used to treat body systems because they were shaped like that body part or because they grew in a particular area. As science began to take a closer look at herbal remedies, their use became more refined. Herbs, and other plants, are actually the precursors to many of today's medicinal drugs. Some of the pharmaceutical medications on the market are extracts of some of these traditional herbs.

2.1.2 History of Herbal Medicine

It is not known, for sure, when humans began using herbs for medicinal purposes. The first written record of herbal medicine use showed up in 2800 B.C. (Titled the Pen Ts'ao by Shen Nung). The Greeks (400 B.C.) joined the field of herbal medicine where Hippocrates stressed the ideas that diet, exercise and overall happiness formed the foundation of wellness. In 50 A.D. the Roman Empire spread herbal medicine around the Empire, and with it the commerce of cultivating herbs, whereas in 200 A.D the first classification system that paired common illnesses with their herbal remedy appeared. This was prepared by the herbal practitioner Galen.

In 600 A.D Herbs were used in treating the poor, while extracts of plant, minerals, and animals "the drugs", were used for the rich. *The English Physician* and Herbal medicine, explaining the practice of herbal medicine was written during this time. In 800 A.D. Monks took over the herbal field with herbal gardens at most monasteries and infirmaries for the sick and injured while in 1100 A.D, the Arab world became a centre of medicinal influence during which time the Physician Avicenna wrote the Canon of Medicine, which gave mention to herbal medicines. In 1500 A.D., herbal medicine and herbalists were promoted and supported by King Henry VII and the Parliament, due to the large number of untrained apothecaries giving substandard care.

By the seventeenth century, the knowledge of herbal medicine was widely disseminated throughout Europe. In 1649, Nicholas Culpeper wrote A Physical Directory, and a few years later produced The English Physician. This respected herbal pharmacopoeia was one of the first manuals that the layperson could use for health care, and it is still widely referred to and quoted today. Culpeper had studied at Cambridge University and was meant to become a great doctor, in the academic sense of the word. Instead, he chose to apprentice to an apothecary and eventually set up his own shop. He served the poor people of London and became known as their neighbourhood doctor, the herbal he created was meant for the layperson. It was not until 1700 A.D. that Herbal medicine got high profile endorsement from Preacher Charles Wesley who advocated for sensible eating, good hygiene and herbal treatments for healthy living.

In 1800 A.D. Pharmaceuticals emerged and herbal treatments took a back seat, but as side effects from the drugs began to be documented, herbal remedies came into favour again. This led to the formation of The National Association of Medical Herbalists , later renamed the National Institute of Medical Herbalists (NIMH.)

The first United States of America Pharmacopoeia was published in 1820. This volume included an authoritative listing of herbal drugs, with descriptions of their properties, uses, dosages, and tests of purity. It was periodically revised and became the legal standard for medical compounds in 1906. As Western medicine evolved from an art to a science in the nineteenth century, information that had at one time been widely available became the domain of comparatively few. Once scientific methods were developed to extract and synthesize the active ingredients in plants, pharmaceutical laboratories took over from providers of medicinal herbs as the producers of drugs. The use of herbs, which for most of history had been mainstream medical practice, began to be considered unscientific, or at least unconventional and to fall into relative obscurity.

During World War One, lack of drugs increased the use of herbal medicine again. However after the war pharmaceutical production increased and penicillin was discovered. Herbal practitioners had their rights to dispense their medications taken away and then reinstated.

Thus Herbal medicines have been documented for almost 4000 years. These medicines have survived real world testing and thousands of years of human testing. Some medicines have been discontinued due to their toxicity, while others have been modified or combined with additional herbs to offset side effects. Many herbs have undergone changes in their uses. Studies conducted on the herbs and their effects keep changing their potential uses.

Herbal medicines are still in use today, in some respects they have gained a new momentum in the medical field. As many people seek alternative treatments and begin to check out traditional medicine, herbs are becoming more popular. As physicians seek new treatments for many common illnesses they are beginning to revisit the traditional remedies, using herbal medicines.

Pharmaceutical medications, with their potential for harmful side effects and addiction, are becoming less popular. People are seeking alternatives to the modern

medical interventions. Improving and maintaining health naturally is a very popular approach to overall health of an individual.

The herbs used today are generally cultivated for those purposes. Very few herbs are harvested from the wild, with the exception of a few still found in the rainforest and higher elevation. The cultivation of herbs for medicinal uses is a large field and more people are beginning to have their own herb gardens.

Herbal medicine has enjoyed a long, and colourful, history. From the early Chinese Empires to modern physicians' offices, herbal medicine has continued to be a part of the medical field. Herbal treatments have matured throughout history, along with the methods of delivering them. In the beginning, the herbs were used in a hit or miss method and required major events to change their use. Research and clinical trials have helped to shape the field of medicine, and the future for herbal medicine looks bright.

In the local setting (Kenya) the herbalists information is not that well organized, however there is indication of treatment of diabetes by use of herbs, wide spread and mostly concentrated in the rural setup. The only shortcoming from the Kenyan case is that the herbalists are suspicious of any scientific investigation into their work and due to this reserved view, there is not so much in published or documented therapy on common herbs.

2.2 Response surface

Response Surface Methodology is a common framework for many industrial experiments which is useful in aiding statistical analysis of experimental work in the yield of a product that depends on some functional relationship with one or more predictor variables. This was a concept outlined by chemical engineers and statisticians in the Imperial chemical industries in Great Britain with the first major paper published by Box and Wilson (1951). However before the data analysis can be carried out, experiments must be carried out at predetermined levels of the predictor factors. This requires that an experimental design should be selected before experimentation, Bose and Draper (1959).

Box and Wilson (1951) described the experimental attainment of optimum con-

ditions (in their answers to problems of determining optimum conditions) in chemical investigations, but they believed that the method would also be useful in other fields where experimentation is sequential and the error fairly small. Grandage and Harder (1959), considered the problem arising in the design of experiments of empirically investigating the relationship between a response and several predictor variables, when all variables are continuous. They made the assumption that the form of the functional relationship is unknown but within the range of interest, the function may be approximated by a Taylor series expansion of moderately low order.

Box and Hunter (1957), introduced the concept of rotatability as a desirable property of an experimental design and gave the necessary and sufficient conditions for a set of points to form a second order rotatable design. Gardiner, Grandage and Harder (1959), derived out the necessary and sufficient conditions for a set of points to form a third order rotatable design. Patel and Koske (1985), gave the necessary and sufficient conditions for a set of points to form a fourth order rotatable design. Njui and Patel (1988), with illustrations and examples, gave the estimates of coefficients and their variance covariance matrix together with the variance function for an estimated response of the fifth order response surface design.

Rotatability is one of the desirable characteristics of a response-surface design. Draper and Guttman (1988) and also Khuri (1988) provided ways to measure ”how rotatable” a design may be when it is not perfectly rotatable. This had previously been assessed by the viewing of tediously obtained contour diagrams for a 3-dimension design. Their paper provides a criterion that is easy to compute and is invariant under design rotation. It can also be extended to higher dimension models.

In selecting on an experimental design, Box and Hunter (1957) discussed the use of the variance function and in particular, the spherical variance function which leads to rotatable designs. They commented that the problem of choosing a best design has usually been interpreted as being equivalent to choosing a design matrix $\boldsymbol{D}$ so that the coefficients in the model are estimated with minimum variance. Herzberg (1967 b), argued that in some cases the variance of the difference between

two estimated responses at two different points is of more interest than the simple variance function. She outlined the variance function of the difference between two estimated responses for first and second order rotatable designs in two dimensions. Atkinson (1970 b), in a slightly different context, remarks that experimenters often wish to consider not the absolute response in the factor space but the difference in responses. He observed that the difference in response at points close together in the factor space can be used to estimate the local slope of the response function.

In fitting a parameter-based regression model, there arises several objectives which require to be addressed by the design of the experiment. One of the concerns is the adequacy of a model, Box and Draper (1959). Usually, designs are desirable if on one hand they are efficient for discriminating between several competing models and on the other hand they portray good properties for enabling the estimation of the parameters that are in the identified model.

Box and Draper (1980), reviewed Herzberg's work and discussed some additional features of the variance function of the difference between two estimated responses, for first, second and third order rotatable designs. Huda and Mukerjee (1984) gave an overview on minimizing the maximum variance of the difference between two estimated responses over all pairs of points in the design space being taken as a criterion for selecting optimal designs. They derived optimal designs for second order polynomial models when the design spaces are spherical.

Meyers, Vinning, and Giovanniti-Jansen (1992) presented an extensive study of the prediction variance property of second order designs. Their work focused on spherical regions of interest and evaluated the rotatable and spherical Central Composite Designs (CCD), the Box-Behnken designs, small composite designs (SCD) and hybrid designs. Recent surveys of response surface methodology by Meyers, Montgomery, Borris and Kowalski (2004) show that second order models and designs play a central role in design of experiments. A second order response surface design is often chosen based on consideration of several criterion. Among the most important of these is the stability of the prediction variance over the region of interest.

Njui et al (2008), considered the difference of the variance functions between two estimated responses for a fourth order design at any two points in the factor

space. In particular, they considered variance function in two dimensions when the design used is rotatable. The variance function in this situation is a function of the distances of the points from the origin of the design and the angle subtending the points at the origin. In particular the variance function of this approach is discussed in detail when the two points are equidistant from the origin of the design. They also gave criterion for the choice of an optimal design. Karanjah et al (2008), extensively looked at the variance function of the difference between two estimated responses for a fourth order rotatable design at any two points in the factor space and specifically detailed their work in consideration of the main effects when the number of factors are $\boldsymbol{k = 2}$.

With the growth of computing software's, the graphical techniques that are useful in evaluating experimental designs for any region of interest have been put to use. These are the Variance Dispersion Graphs (VDG) and the Fraction of Design Space (FDS) plot. the VDG was developed by Giovannitti-Jabsen and Meyers (1989) and displays the minimum, maximum and average prediction variance for specific designs through the region of interest. The plots concentrate on examining prediction variance for spheres moving from the overall centroid of the design space. The VDG's illustrate how well the design can predict response throughout the design space.

The field of response surface methodology is enormously dynamic with research and publications being made as a contribution to the increasing demand of the subject in our everyday life.

2.3 Statement of the Problem

It has been recognized in recent years that even in response surface designs the response at individual locations on the response surface may not always be the main interest. Often the difference between estimated responses at two points may be of greater interest. Often, the response at a range of points (ridge) may be the same but resulting from different factors level combinations. Minimization of the variance of the difference between estimated responses at two points over all pairs of points in the design space taken as a criterion will help in selecting optimal

designs.

This research uses the variance function of the difference as well as the difference of the variance functions between estimated responses at various points with a view to identify the points that will yield an optimal design. Response surface design is used to identify the range within which the factors can be varied to effectively manage the blood sugar level in a diabetic patient using identified herbal medicine.

2.4 Aims and Objectives

The work of Herzberg, (1967 b) outlined the variance function of the difference between two estimated responses for a first order and a second order rotatable design. Box and draper, (1980) reviewed Herzberg's work and also worked out the variance function of the difference between two estimated responses for a third order rotatable design.

To demonstrate the use of these two functions in application, we shall consider their role in selecting an appropriate herbal treatment combination for treating diabetes using a second order rotatable design.

To achieve this stated aim we have the following specific objectives;

(i) Identify and fit the appropriate model for the test in order to get the best estimated response

(ii) Identify the variance function of the estimated responses

(iii) Find the variance of the difference between two estimated responses

(iv) Find the difference of the variances of two estimated responses

(v) Compare (iii) and (iv) and conclude

2.5 Significance of the Study

In any treatment arrangement, we seek a treatment or treatment combination that can be used to either reverse a condition, eradicate or arrest a condition in order

to minimize suffering or to help the patient bear a condition with less pain. In this research we highlight that the investigation is not about to eradicate diabetes via this treatment. We seek to reduce the blood sugar level of a patient at that particular time to a level that is acceptable according to medical standards. Again the treatment as suggested here is based on a one dosage treatment model, then observations are made so as to note how effective the particular amount is at reducing the blood sugar level with time. This study will therefore be used to determine;

(i). The best possible level of the identified herbal medicine to reduce the blood sugar level in diabetics, and

(ii). Time taken to achieve (i)

This study will be used to provide the most reliable advice (on the basis of the findings) on the range around which the dosage is desirable so as to make use of the information in maintenance of the desired level of glucose in a diabetic patient.

Chapter 3

Experimental Setup and Data Collection

3.1 Experimental -Study Design

Prior to any test being carried out on the Albino rats, the rats was grouped into six equal groups. Group sizes depended on the total number of rats available and also the consumables. The rats were subjected to treatment as follows:

Group A: The rats in this group were not induced with Diabetes, this group of rats served as control group for rats in the blood sugar test.

Group B: The rats were induced with diabetes but were not subjected to any drug as a treatment therefore served as a negative control for rats induced with diabetes.

Group C: The rats were induced with diabetes and subjected to treatment using conventional drug Metformin. This group served as a positive control group for rats induced with diabetes.

Group D: The rats were induced with diabetes and treated with the herbal drugs under the test at a dose rate of 500 mg/Kg

Group E: The rats were induced with diabetes and treated with the same herbal drug under the test at a dose rate of 1000 mg/Kg.

Group F: The rats were induced with Diabetes and treated with the same herbal drug at a dose rate of 1750 mg/Kg.

In any clinical analysis some of the cases (animals) may be lost due to factors such as death, failing to be diabetic after inducement, thus resulting from their exclusion from the test or they may be replaced if time and other necessary conditions do allow. Therefore there may be some variation in the number of animals per grouping and hence the total in the specific test run. However this is not likely to affect the test results since procedure of the test is done per animal in a group.

3.2 Inducement of Diabetes

Animals were made to fast by depriving food for 18 hours before treatment but allowed access to water. The concentration of blood glucose was also recorded before treatment. A single dose (150 mg/Kg) of 5 percent alloxan monohydrate dissolved in normal saline was administered using insulin needles to induce diabetes to the rats. The induction of diabetes mellitus was confirmed after the following day of alloxan treatment by estimation of elevated random blood glucose (RBG) level as described by Trivedi et al. (2004).

3.3 Treatment arrangement

The groups that did not receive treatment were given 2 ml of distilled water which was to act as a negative control group. Those that received treatment with conventional diabetic medicine were subjected to 2 ml of 10 mg/Kg of a diabetic drug (Glucophage) crushed into a fine powder, weighed and then dissolved in distilled water. The group receiving herbal drug treatment were given 500 mg/Kg, 1000 mg/Kg and 1750 mg/Kg of the herbal drug extract as per the grouping.

3.4 Herbal Drug Dosage

The various concentrations of herbal drugs were made by dissolving 10 g of the solid plant extracts in 250 ml distilled water and warmed to 40 C with addition

of 5 percent Dimethyl Sulfoxide (DMSO) to form a standard solution of 40 g/ l from which various concentrations were derived. The volume of the dosage as per clinical trial guideline,is calculated taking into account the weight of the animal, dose rate and concentration of the crude herbal drug using the following equation

$$Volume\ of\ Dose = \frac{Body\ \ weight\ (Kg)\ X\ Dose\ rate\ mg/Kg}{Concentration\ mg/ml} \tag{3.1}$$

Reagan et al (2007),

3.5 Analysis of glucose Level

Blood was withdrawn from the retro-orbital sinus under restricted movement in a cage using a clinical glucometer and glucose oxidase - peroxidase reactive strips. Xylene was used to dilate the veins of the rats' tails to collect the blood efficiently. After treatment, 2 g/Kg of glucose was given to the rats and readings were taken in intervals of 30 minutes and recorded to monitor the changes of glucose concentration in the blood of the Rats.

3.6 Experimental Data

3.6.1 First Test run (Pilot Test Run)

3.6.1.1 Treatment arrangement

The Pilot test was carried out on a set of 12 Albino rats. The rats were grouped into six groups of two each. They were then subjected to treatment as earlier described in section 3.1, with treatment effected as follows:

Group 1: The rats in this group were not induced with Diabetes (NID). This group served as control group for rats in the blood sugar test.

Group 2: The rats were induced with diabetes but were not subjected to any drug as a treatment therefore served as a negative control (NC) for rats induced with diabetes.

Group 3: The rats were induced with diabetes and subjected to treatment using conventional drug Metformin. This group served as a positive control (PC) group for rats induced with diabetes.

Group 4: The rats were induced with diabetes and treated with the herbal drugs under the test at a dose rate of 500 mg/Kg

Group 5: The rats were induced with diabetes and treated with the same herbal drug under the test at a dose rate of 1000 mg/Kg.

Group 6: The rats were induced with Diabetes and treated with the same herbal drug at a dose rate of 1750 mg/Kg.

In this Pilot test run the variables of interest were time in minutes after the animals were subjected to the prescribed treatment dependent on the group, that is $(\boldsymbol{t = 0, t = 30, t = 60, t = 90, t = 120, t = 150, t = 180})$ the dosage amount for the herbal drug in the respective test groups (500 mg/Kg, 1000 mg/Kg and 1500 mg/Kg) and at every time interval the blood sugar level was ascertained to monitor the progress and effectiveness.

3.6.1.2 Pilot Data

Using the observations time as indicated above the data resulting from the investigation depicts the blood sugar level after the administration of the treatment as per the procedures laid down was as follows:

FBG Oral Glucose Tolerance Test versus Time

$Group$	$Rat\ label$	$t=0$	$t=30$	$t=90$	$t=120$	$t=150$	$t=180$
$Group\ 1$	Y	5.0	6.4	7.3	9.1	3.3	4.5
	Z	6	6.6	7.0	6.0	6.4	6.3
$Group\ 2$	AF	10.6	23.8	19.3	10.4	5.4	9.0
	J	9.7	17.8	23.1	20.3	21.7	17.6
$Group\ 3$	AA	11.7	19.6	17.4	15.0	13.0	11.1
	AB	11.8	18.7	16.8	15.1	7.6	4.9
$Group\ 4$	AD	11.9	25.1	19.8	18.2	11.9	7.3
	AG	6.2	7.2	3.8	4.5	5.9	5.0
$Group\ 5$	AE	17.3	23.7	22.4	21.8	13.1	9.9
	AL	11.7	31.9	31.9	29.7	17.3	13.8
$Group\ 6$	AI	22.1	26.1	17.6	10.8	17.3	13.8
	L	27.9	15.4	.8	6.1	4.3	4.8

The rats were labelled to facilitate the identification during the entire procedure. However the assignment into the particular group was on random selection.

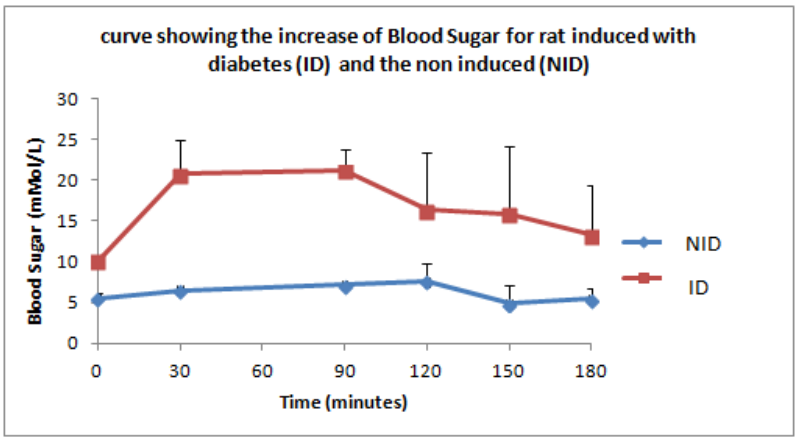
The measurements as per the glucometer reading are in minimolar per litre which can be converted to the international standard in milligrams per deciliter by use of the following equation:

$$18\ Minimolar\ per\ litre = 1\ Milligram\ per\ Deciliter \quad (3.2)$$

3.6.1.3 Excursion for Pilot Data

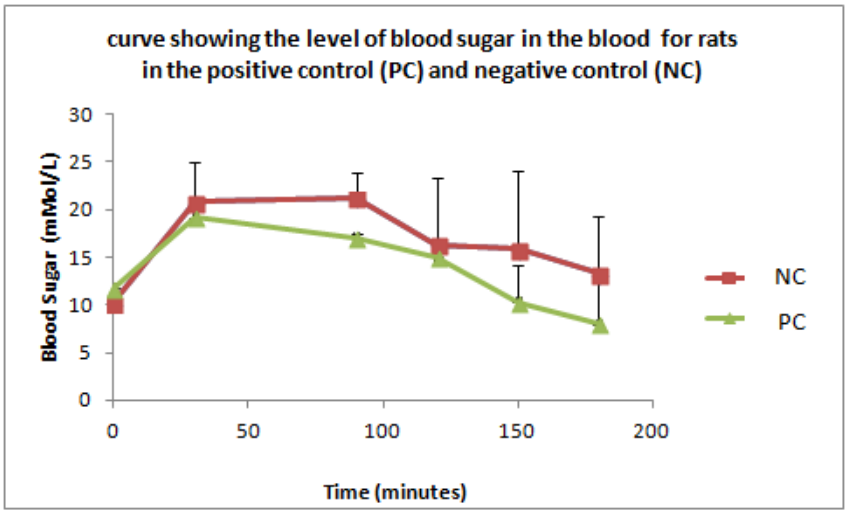
By first computing the average blood sugar levels at every time interval for the animals in the same group, the various treatments are compared as shown in the following graphs:

Graph 3.6.1.1



The curve shows the Blood Sugar for rat induced with diabetes (ID) and the non induced (NID). From the Graph it is evident that the animals that were induced with diabetes exhibit blood sugar level that is higher than those not induced with diabetes.

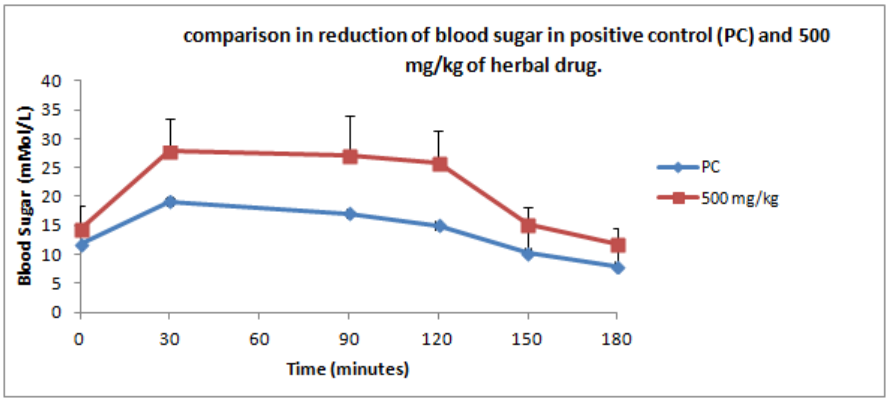
Graph 3.6.1.2



The curve shows the Blood Sugar for rats in the positive control (PC) and negative control (NC). From the Graph we find out that even though all the animals in the two groups are diabetic, the sugar level for those in the positive control

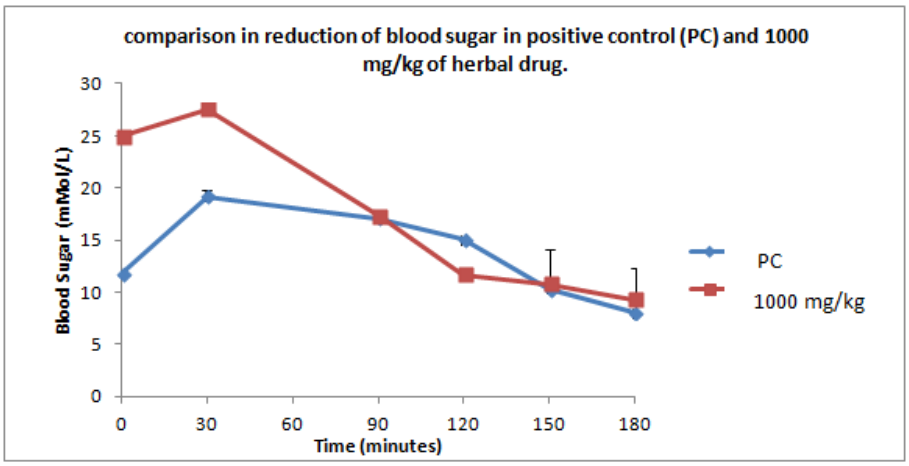
group decreases as a result of treatment using conventional medicine as compared to those in negative control that received no treatment. This is an indication that the treatment is effective in the reduction of sugar level.

Graph 3.6.1.3



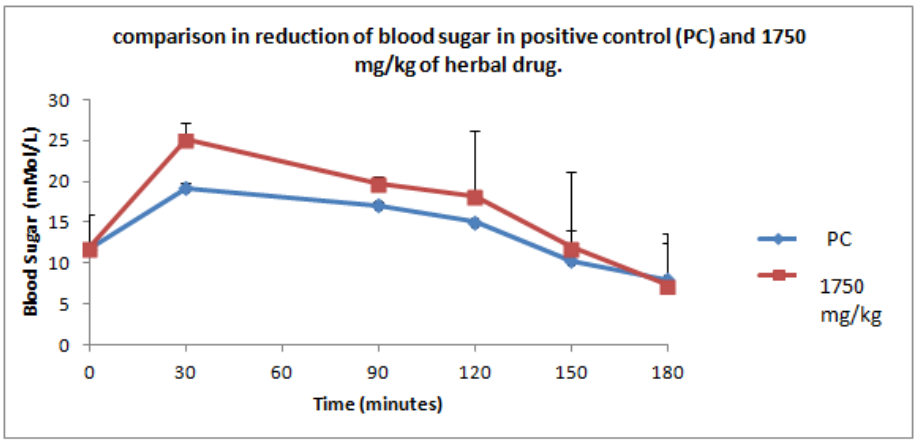
The curve shows the Blood Sugar for rats in positive control (PC) and 500 mg/Kg of herbal drug. From the Graph we find that even though all the animals in the two groups are diabetic, the sugar level for those in the positive control group decreases as a result of treatment using conventional medicine and the sugar level for those treated with herbal medicine 500 mg/Kg also decreased. The graphs are almost replica of each other apart from the fact that the graph of PC is below the one for 500 mg/Kg. This is an indication that both the treatment are almost effective in the reduction of sugar level.

Graph 3.6.1.4



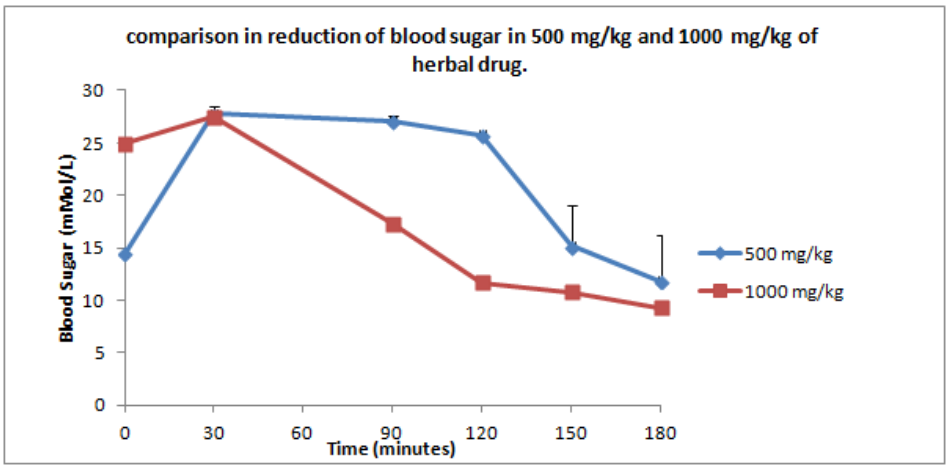
The curve shows the Blood Sugar for rats in positive control and 1000 mg/Kg of herbal drug. At time $t = 0$ the blood sugar level for animals treated with herbal medicine 1000 mg/Kg is higher than that of the animals in PC, but towards time $t = 30$ there is a rise of blood sugar level for both the groups with that of PC being more steady. After 30 minutes the sugar level in both groups is decreasing but the decrease of that in herbal treatment is steady towards time $t = 90$. At time $t = 120$ the sugar level for those treated with herbal medicine is lower than the PC and at time $t = 150$ they have the same sugar level after which those treated with herbal medicine slightly increase compared to that of PC. However we recognize that both the treatments have worked towards reduction of the sugar level.

Graph 3.6.1.5



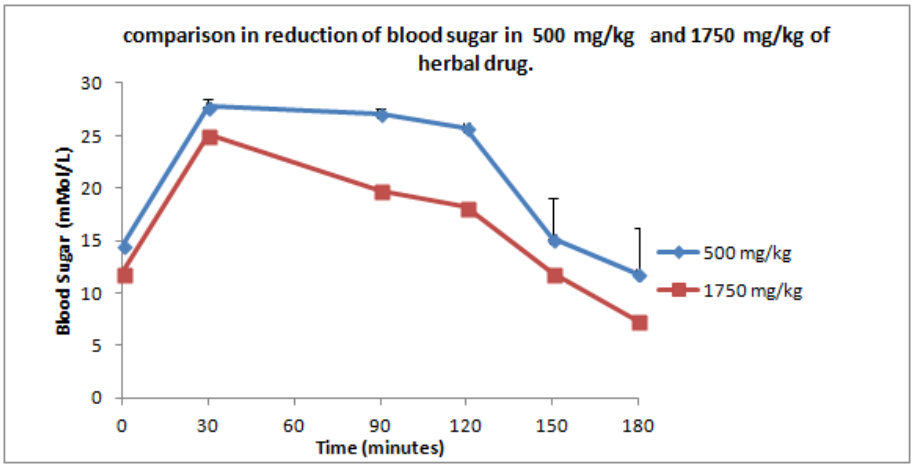
The curve shows the Blood Sugar for rats in positive control and 1750 mg/Kg of herbal drug. At time $t = 0$ the blood sugar level for animals in the two groups is the same, but towards time $t = 30$ there is a rise of blood sugar level for both the groups with that of Herbal treatment being higher. After 30 minutes the sugar level in both groups is decreasing but the decrease of that in herbal treatment is steady towards time $t = 90$. At time $t = 150$ the sugar level for those treated with herbal medicine is slightly higher than the PC and at time $t = 180$ they have the same sugar level. We conclude that both the treatments have worked towards reduction of the sugar level with a variation at the times of measurements.

Graph 3.6.1.6



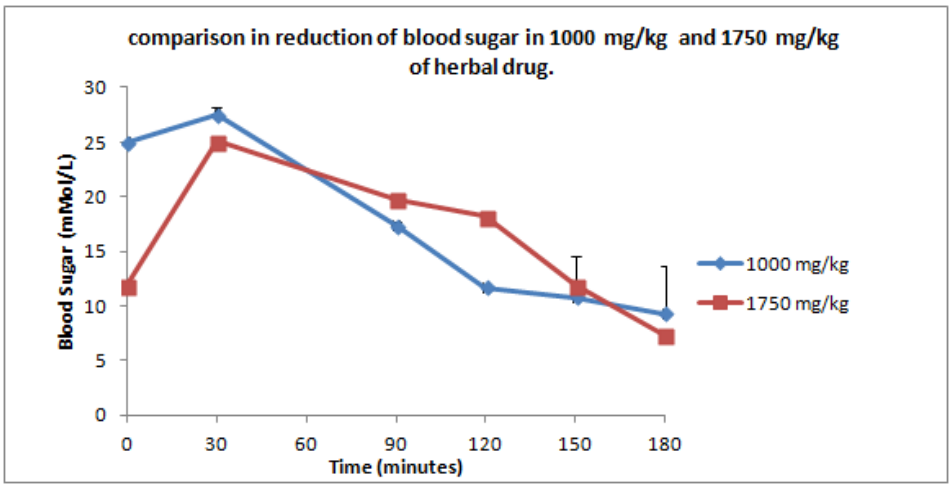
The curve shows the Blood Sugar for rats treated with 500 mg/Kg of herbal drug and those treated with 1000 mg/Kg of herbal drug. At time $t = 0$ the average blood sugar level for animals treated with 1000 mg/kg is higher than for those treated with 500 mg/kg. However the later is increasing and at time $t = 30$, they exhibit the same average blood sugar level after which that of treatment of 1000 mg/kg starts to decrease at a fixed rate till $t = 120$ while that of 500 mg/kg remains on a very slow decreasing path till $t = 120$ after which there is a drastic decrease towards $t = 150$ after which the decrease is relatively lower compared to the other group. The average blood sugar level for animals treated with herbal drug of 1000 mg/kg decreases after $t = 120$ and is lower than that of 500 mg/kg which is also decreasing.

Graph 3.6.1.7



The curve shows the average Blood Sugar for rats treated with 500 mg/Kg of herbal drug and those treated with 1750 mg/Kg of herbal drug. Between time $t = 0$ and $t = 30$ the blood sugar level for animals in the two groups increase at almost same rate. After $t = 30$ there is a decrease in both curves throughout the observation times but the animals treated with 1750 mg/kg of herbal medicine shows a better response in decreasing the average blood sugar level compared to the treatment of 500 mg/kg of the herbal medicine.

Graph 3.6.1.8



The curve exhibits the trend of the herbal treatments at two levels and the corresponding average blood sugar level, 1000 mg/Kg and 1750 mg/Kg of herbal drug. At time $\boldsymbol{t = 0}$ the blood sugar level for animals treated with 1750 mg/kg of the herbal drug is lower but increases faster than that of animals treated with 1000 mg/kg. The curves reaches a maximum at time $\boldsymbol{t = 30}$ and decrease thereafter but those treated with 1000 mg/kg falls faster than that of 1750 mg/kg and remains below upto $\boldsymbol{t = 150}$ after which the decrease trend slows with that of 1750 mg/kg falling below. The trends suggest that both the treatments have worked towards reduction of the sugar level with a variation at the times of measurements.

3.6.2 Second Test Run

The test run was based on Herbal medicine extracted from Medicinal Mushrooms referred to as Aqueous extract of Ganotech. There was a total of 28 test animals divided into four groups all induced with diabetes as per inducement procedures outlined in section **3.2**. The number of rats were 7 per each as seven were lost at the initial stages. The loss was occasioned by the inducement to diabetes after which the rats succumbed to the effect of the inducement while others failed to be diabetic. The remaining rats were subjected to treatment as follows:

3.6.2.1 Treatment arrangement

Group 1: The rats were induced with diabetes and treated with the herbal drugs under the test at a dose rate of 25 mg/Kg

Group 2: The rats were induced with diabetes and treated with the same herbal drug under the test at a dose rate of 50 mg/Kg.

Group 3: The rats were induced with Diabetes and treated with the same herbal drug at a dose rate of 75 mg/Kg.

Group 4: The rats were induced with diabetes and subjected to treatment using conventional drug Metformin 500mg/Kg. This group served as a positive control group for rats induced with diabetes.

Using this test arrangement our desire was to ascertain the effect of herbal drug at different concentration levels and the time taken to control the blood sugar level to within the acceptable range.

3.6.2.2 Collected Data

After the recording of the Fasting Blood glucose the treatments were carried on the animals as per grouping and dosage levels. The readings of the Oral Glucose Tolerance Test was undertaken at $\boldsymbol{t = 30}$, $\boldsymbol{t = 90}$ and $\boldsymbol{t = 150}$ minutes. From this test arrangement the following data was realised.

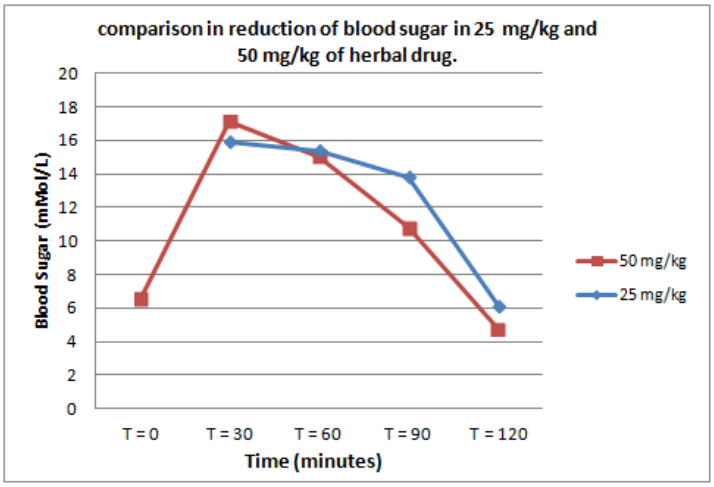
		FBG	Oral Glucose Tolerance Test/Time			
$\boldsymbol{Group}$	$\boldsymbol{Rat}$	$\boldsymbol{t=0}$	$\boldsymbol{t=30}$	$\boldsymbol{t=90}$	$\boldsymbol{t=120}$	$\boldsymbol{t=150}$
$\boldsymbol{Group\ 1}$	1	H	16.1	15.7	13.8	5.3
	2	H	15.1	12.3	16.0	11.3
	3	H	16.7	11.9	11.8	4.6
	4	H	15.7	21.6	13.6	3.3
	5	H	17.5	14.2	14.0	13.9
	6	H	14.2	13.6	13.4	13.0
	7	H	12.8	11.4	12.8	13.4
$\boldsymbol{Grup\ 2}$	2	10.3	17.9	16.7	12.1	3.3
	5	6.3	10.7	13.8	9.9	5.0
	3	4.8	18.8	14.1	10.2	2.4
	4	4.9	21.2	15.6	11.1	8.3
$\boldsymbol{Group\ 3}$	2	4.2	12.4	5.1	9.8	7.2
	3	7.1	17.4	23.9	8.1	5.1
	4	4.0	12.6	13.4	11.8	7.3
	5	3.0	17.6	17.1	10.7	3.7
	1	19.7	17.5	14.2	14.0	13.9
	6	14.5	14.2	13.6	13.7	13.0
$\boldsymbol{Grup\ 4}$	1	5.7	15.7	6.7	2.7	2.5
	2	4.8	13.4	10.1	5.7	3.1
	3	18.3	19.4	19.1	17.2	15.5
	4	8.4	17.3	15.2	13.4	11.2

3.6.2.3 Excursion for Data

Using group average blood sugar levels at every time interval for the animals in the same group, various treatments are compared against the control. In this set up we note that the results of group indicates readings that were high at FBG (H- in the data at firsting), attributed to the concentration of the glucose dosage. However, these readings do not affect the experimental outcome as evidence in the ability of the animals to regulate the sugar level to within the range of the other groups.

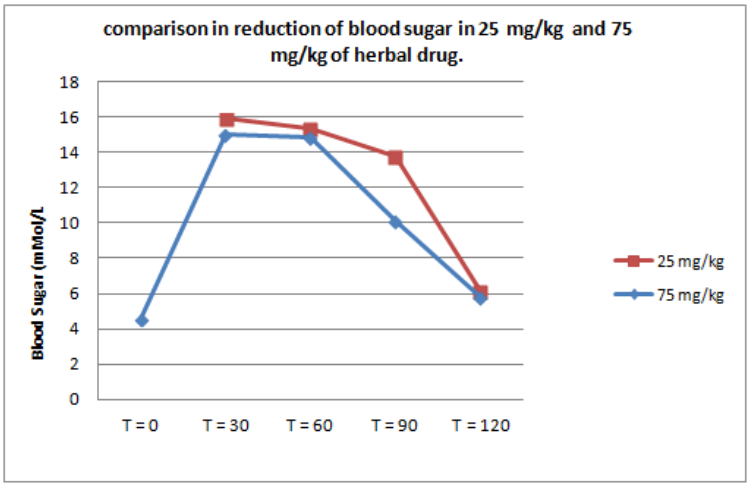
The results obtained are presented graphically as follows:

Graph 3.6.2.1



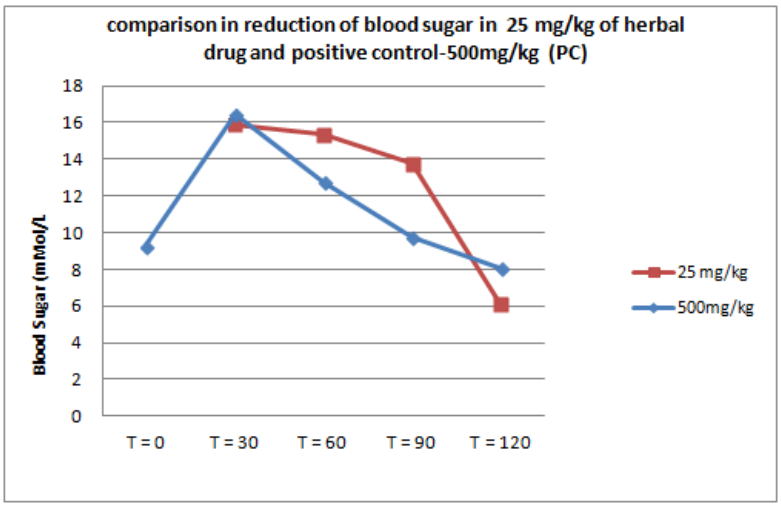
The curve shows the average Blood Sugar levels for rats subjected to herbal medicine 25 mg/kg against rats subjected to herbal medicine 50 mg/kg. At time $\boldsymbol{t = 30}$ the sugar level for 50 mg/kg is above that of 25 mg/kg after which the two curves depict a declining trend to almost being equal at $\boldsymbol{t = 60}$ after which that of 50 mg/kg decreases faster than that of 25 mg/kg throughout the other observation times. At time $\boldsymbol{t = 120}$ both the two groups average sugar levels are within the required limits.

Graph 3.6.2.2



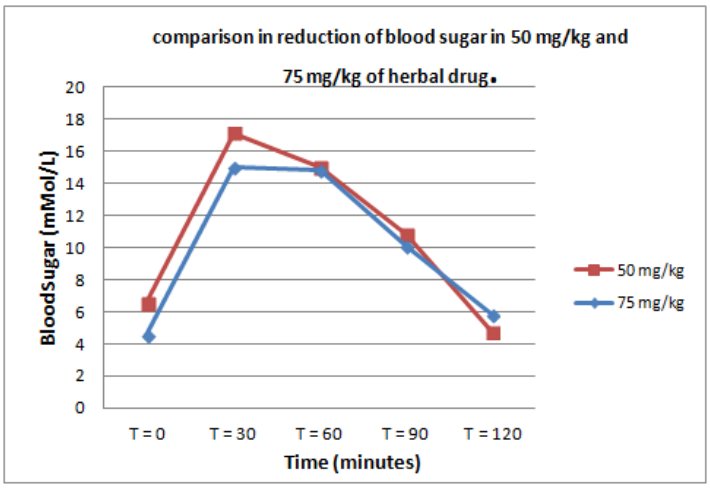
The curve shows the average Blood Sugar levels for rats subjected to herbal medicine 25 mg/kg against rats subjected to herbal medicine 75 mg/kg. From the trend of the two curves it is evident that on average the 75 mg/kg of the herbal drug is performing better in reduction of the blood sugar level compared to the 25 mg/kg since after time $t = 30$ the two curves depict a declining trend with 75 mg below that of 25 mg/kg through the entire observation times. However both the herbal drugs effectively reduces the average sugar levels to within acceptable limits.

Graph 3.6.2.3



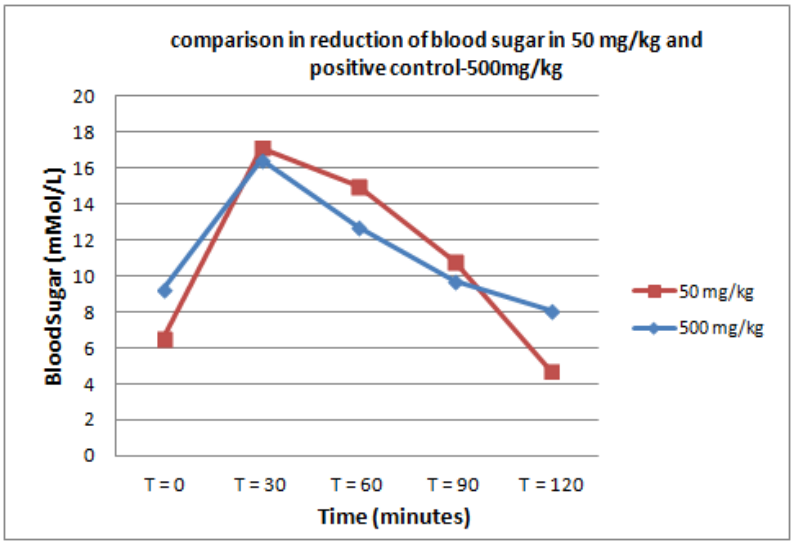
The curve depicts the comparison of the trends of the average blood sugar levels for the positive control group against those treated with the herbal medicine at dosage of 25 mg/kg. From the graph we find out that the blood sugar levels on average are the same at time $t = 0$ after which the PC curve decreases faster than that of 25 mg/kg of herbal medicine. However at the last observation point $t = 120$ the curve for 25 mg/kg is below that of PC. The two treatments effectively lowered the sugar levels to within acceptable limits.

Graph 3.6.2.4



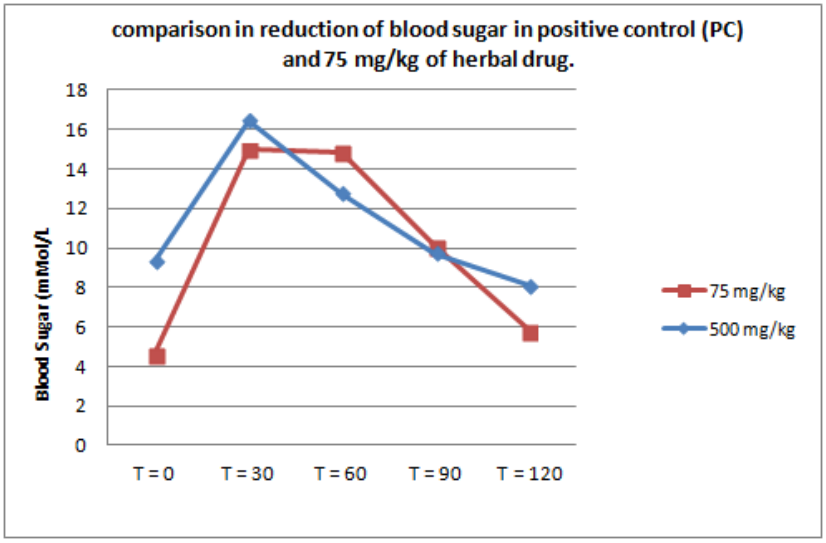
The curve shows the Blood Sugar for rats treated with the herbal medicine 50 mg/kg along side that of those treated with the same herbal medicine but dosage of 75 mg/kg. From the graph we find out that even though all the animals in the two groups are diabetic, the sugar level for those in 50 mg/kg group and 75 mg/kg group responds almost identically to herbal medicine. The graphs are almost replica to each other apart from the fact that that of 75 mg/kg is slightly below the one for 50 mg/kg. This is an indication that both the treatment are almost equally significant in the reduction of average blood sugar levels.

Graph 3.6.2.5



The curve shows the Blood Sugar for rats treated with the herbal medicine 50 mg/kg against those in the positive control group . The average sugar levels start at different point at time $t = 0$ but increases towards time $t = 30$ to almost the same level after which both curves portrays declining trend with the PC below the herbal medicine . At time $t = 120$ the herbal drug indicates the dosage of 50 mg/kg to be more effective in comparison to the PC with the curve remaining below that of PC. The two treatments effectively reduces the average blood sugar level to within acceptable limits.

Graph 3.6.2.6



The curve shows the comparison of the trends of the average blood sugar levels for the positive control group against those treated with the herbal medicine at dosage of 75 mg/kg. The herbal drugs starts at a lower average in comparison to the PC at $t = 0$ with both increasing to their different maximums at time $t = 30$ after which the two curves are on the decline with that of PC falling faster than that of herbal medicine. At $t = 60$ the PC curve is below that of herbal medicine but at $t = 90$ the herbal medicine curve falls below that of PC till the end of observation time. Both treatments on average have reduced the average blood sugar level to the acceptable limits.

3.6.3 Third Test Run

3.6.3.1 Treatment arrangement

This test run was based on herbal drug (referred as Herbal Formula) extract sourced from a leading herbalist in Kenya. The herbal formula used, is a mix of six herbs, whose botanical name are:

1. Momodica foetida,
2. Utica Masaica,
3. Cinamon species,
4. Azandracta indica,
5. Moringa Oliefera and
6. gymnema sylvestre.

In this test run 25, albino rats were involved at the start, but one animal in group four was lost before commencement of actual treatment. This loss was insignificant. The treatment procedure was as follows:

Group 1: The rats were induced with diabetes but were not subjected to any drug as a treatment . They served as a Negative Control (NC) for rats induced with diabetes.

Group 2: The rats were induced with diabetes and subjected to treatment using conventional drug Metformin 500 mg/kg. This group served as a Positive Control (PC) group for rats induced with diabetes.

Group 3: The rats were induced with diabetes and treated with the herbal drugs under the test at a dose rate of 125 mg/Kg.

Group 4: The rats were induced with diabetes and treated with the same herbal drug under the test at a dose rate of 250 mg/Kg.

Group 5: The rats were induced with Diabetes and treated with the same herbal drug at a dose rate of 500 mg/Kg.

3.6.3.2 Collected Data

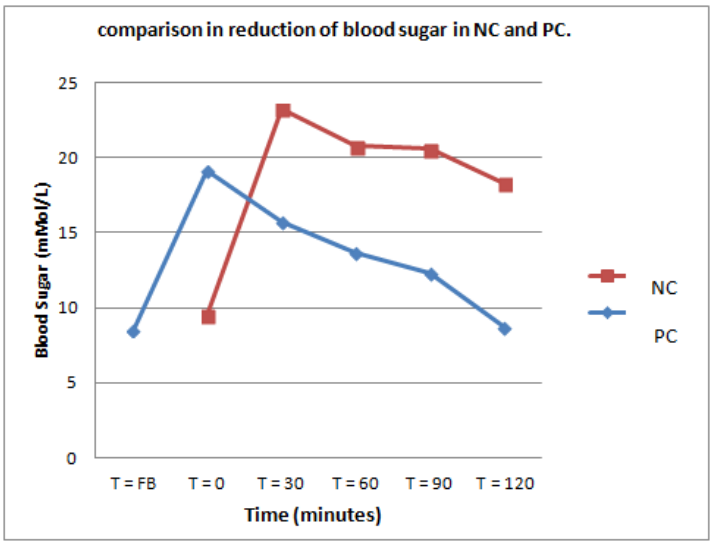
Fasting Blood Glucose level was recorded, following which the treatments were effected as per the schedule of treatment arrangement in subsection 3.6.3.1. The readings of the OGTT was at time interval of 30 minutes resulting in the following data:

			Oral Glucose Tolerance Test/Time				
$\boldsymbol{Group}$	$\boldsymbol{Rat}$	$\boldsymbol{FBG}$	$\boldsymbol{t=0}$	$\boldsymbol{t=30}$	$\boldsymbol{t=60}$	$\boldsymbol{T=90}$	$\boldsymbol{t=120}$
Group 1	1		10.9	26.8	18.7	18.0	17.2
	2		7.4	24.6	24.1	23.0	23.2
	3		9.6	33.0	30.6	27.7	24.4
	4		8.7	13.4	13.2	23.4	12.2
	5		10.8	18.3	17.2	16.7	14.4
$\boldsymbol{Grup\ 2}$	1	6.7	13.7	11.2	8.4	9.9	8.9
	2	9.8	22.7	16.2	15.1	12.7	7.6
	3	7.8	17.4	16.4	12.4	11.9	6.7
	4	8.9	18.3	14.5	13.3	12.8	9.4
	5	9.5	23.4	20.4	19.2	14.4	10.8
$\boldsymbol{Grup\ 3}$	1	7.2	24.5	24.2	23.6	20.8	19.2
	2	7.6	21.7	22.4	21.8	21.2	21.2
	3	6.4	25.5	24.2	21.4	12.4	10.1
	4	4.6	13.5	11.8	13.6	14.9	9.7
	5	5.4	23.4	21.6	19.5	10.5	8.1
$\boldsymbol{Grup\ 4}$	1	17.3	15.8	22.1	19.9	16.7	13.6
	2	9.3	20.4	22.7	10.9	9.8	7.9
	3	7.5	17.3	16.6	9.7	9.4	4.3
	4	8.6	12.8	5.2	3.1	3.1	3.1
$\boldsymbol{Group\ 5}$	1	5.8	17.8	15.6	21.6	22.7	20.4
	2	8.2	33	31.4	28.0	23.0	6.3
	3	11.8	25.5	12.4	9.6	8.9	6.9
	4	6.6	15.3	14.9	12.4	8.7	5.9
	5	13.2	22.4	18.9	13.4	10.9	8.9

3.6.3.3 Excursion for the Data

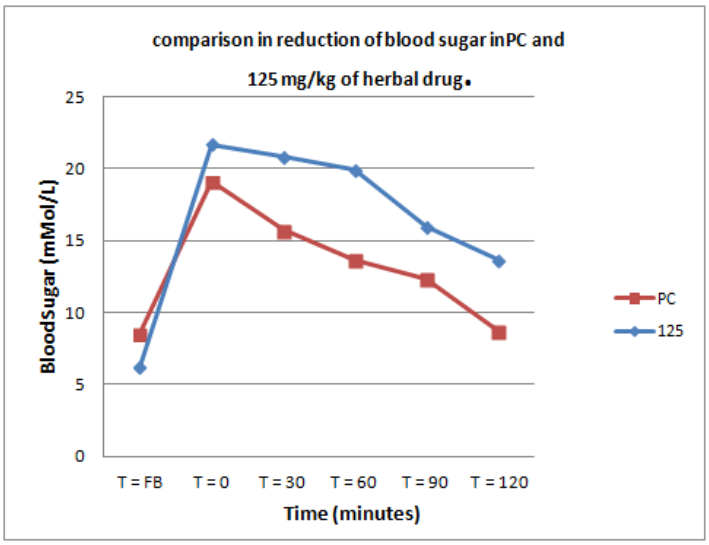
Computing averages in the groups for blood sugar levels at the observation time points for the animals in the same group, we compare the various treatment results and represent this information with following graphs.

Graph 3.6.3.1



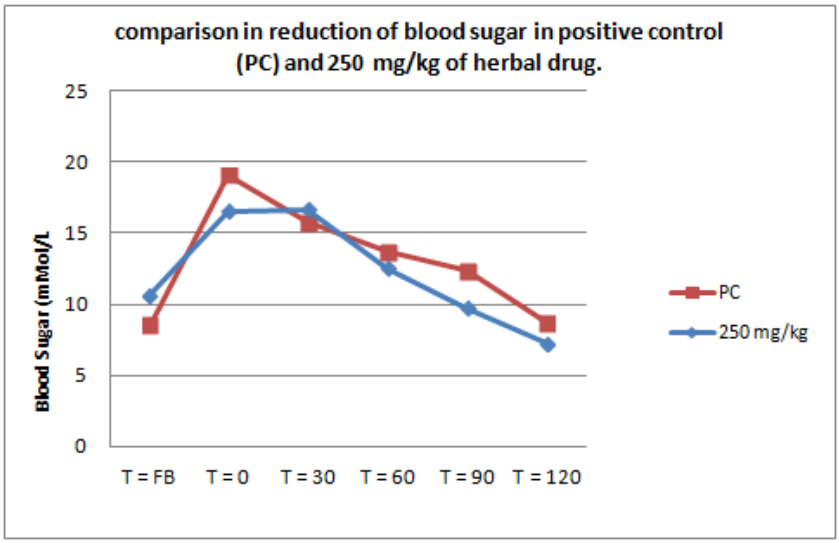
The curve depicts the comparison of the trends of the averages for the negative control group and the positive control group. The trend shows that the animals in the negative control who were not treated hand an increase in the average blood sugar level from $t = 0$ to $t = 30$ and for the positive control which starts at time of fasting also had an increase upto $t = 0$. Both groups exhibit a decrease of the average blood sugar level thereafter, the negative control remains higher than the positive control. The trend portrays a fairly similar trend with the Negative control remaining at a higher level to the end of the observations.

Graph 3.6.3.2



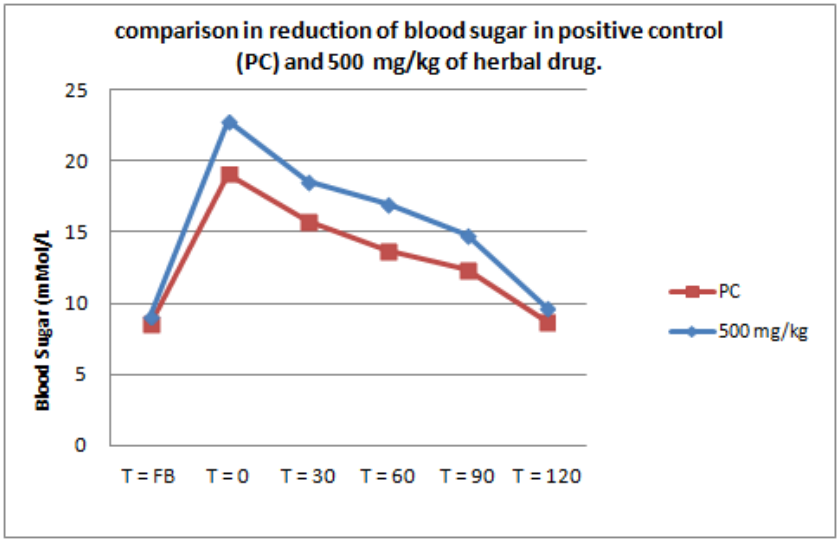
The curves shows the blood sugar for rats treated with the herbal medicine 125 mg/kg compared to those treated with the conventional medicine (PC). At time of fasting the PC has a higher average blood sugar level but at time $\boldsymbol{t = 0}$ the average blood sugar level for the group treated with the herbal medicine is higher. However both groups exhibit a decreasing trend after $\boldsymbol{t = 0}$.

Graph 3.6.3.3



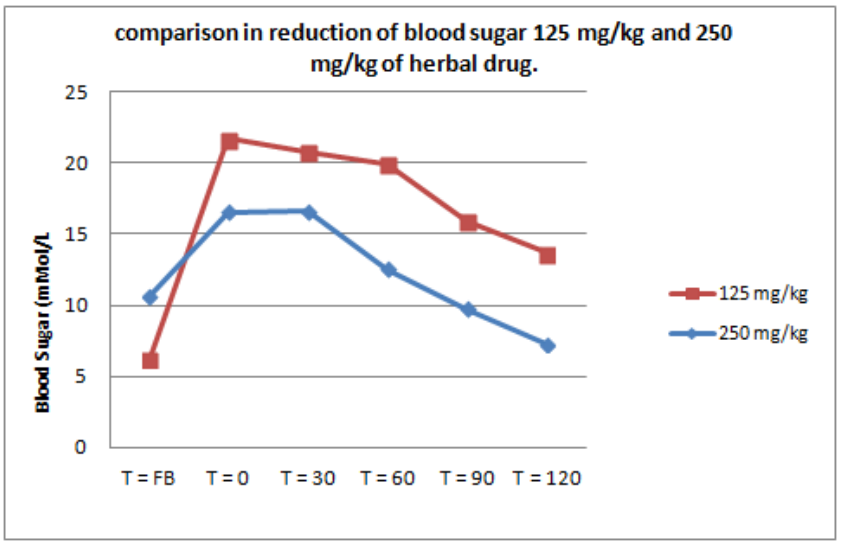
The curves shows the Blood Sugar for rats in positive control (PC) and 250 mg/kg of herbal drug. From the Graph we find out that the sugar level for two groups start at different points with PC below 250 mg/kg and increase, at time $t = 0$ the average blood sugar level is higher for PC compared to the herbal medicine and they both depict a decreasing trend. At $t = 30$ the PC is lower than the herbal medicine however the herbal medicine curve decreases faster than that of PC and at time $t = 60$ PC remains higher upto the end suggesting a better average performance of the 250 mg/kg Herbal medicine compared to PC.

Graph 3.6.3.4



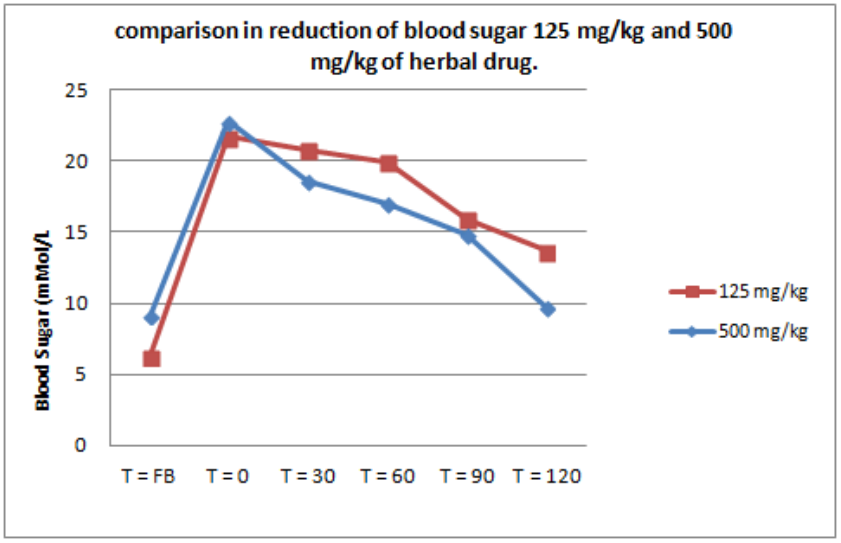
The curves shows the Blood Sugar for rats treated with the herbal medicine 500 mg/kg against and those treated with the Conventional medicine (PC).The average blood sugar level the time of fasting starts almost at the same point and increases until $t = 0$ with that of PC being lower. Thereafter both curves are decreasing at an almost similar pattern until $t = 90$ after which the decrease almost converges to the same point at $t = 120$.

Graph 3.6.3.5



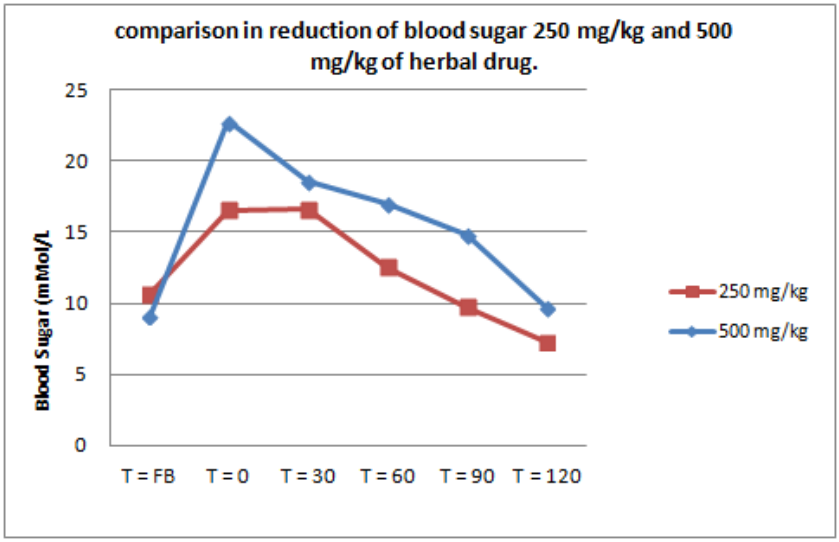
The curves shows the Blood Sugar for rats treated with the herbal medicine 125 mg/kg against those treated with the same herbal medicine but dosage of 250 mg/kg. The two curves start at different averages with the group treated with 250 mg/kg being higher. The one treated with 125 mg/kg is higher after 30 minutes after which they start to fall but that of 250 mg/kg remains below that of the 125 mg/kg treatment, suggesting a better performance in lowering the average blood sugar level.

Graph 3.6.3.6



The curves show the Blood Sugar for the group treated with the herbal medicine 125 mg/kg against those treated with the same herbal medicine but dosage of 500 mg/kg. From the Graph it is evident that for the two groups, average blood sugar level rises on fasting after taking glucose at $t = 0$, the sugar levels reach their maximum with that of 500 mg/kg being higher but both are on a declining path with that of 125 mg/kg being higher but at the time $t = 90$ they almost converge. Thereafter that of 500 mg/kg decrease faster.

Graph 3.6.3.7



The curves show the Blood Sugar for the group of rats treated with the herbal medicine 250 mg/kg against those treated with the same herbal medicine but dosage of 500 mg/kg. The curve depicts a rise in the blood sugar level after fasting blood glucose, with that of 500mg/kg being higher. After time $t = 0$ they both start to decrease throughout the observation times with a better trend and performance of those treatment of 250 mg/kg.

Chapter 4

Data Analysis and Discussions

The desire of any pharmaceutical process is to develop a formulation which is acceptable or effective in the shortest time possible and at the same time using minimum number of man-hours and raw materials. Traditionally, pharmaceutical formulations are developed by changing one variable at a time by trial and error method which is time consuming. Further it requires a lot of imaginative efforts Saeed etal(2013). Moreover, it may be difficult to develop an ideal formulation using this classical techniques, since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using a collection of mathematical and statistical technique which quantifies the functional relationship between a number of measured response variables and several explanatory factors to obtain an optimal response by using a series of tests. The main advantage is to reduce the required experimental runs and to optimize formulation design in pharmaceutics studies.

4.1 Designs for Fitting the First-Order Model

In this research work and in most RSM problems, the true response function $\boldsymbol{f}$ is unknown we therefore need to approximate the function. In order to develop a approprate approximation for $\boldsymbol{f}$, we model the data by starting with a low-order polynomial in some small region. If the response can be defined by a linear function of independent variables, then the approximating function is a first-order model. With reference to any $\boldsymbol{d^{th}}$ order polynomial regression model (design) the

general design for given n observations is given as

$$y_i = f(x_i)\beta + \epsilon_i \qquad i = 1, 2, 3, ...n \tag{4.1}$$

In general, a multiple linear regression model with k independent variable takes the form

$$\begin{aligned} y_i &= \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + ... + \beta_k x_{ik} + \varepsilon_i \qquad (i = 1, 2, 3, ..., n) \\ &= \beta_0 + \sum_{j=1}^{k} \beta_j x_{ij} + \varepsilon_i \end{aligned} \tag{4.2}$$

The parameter β_j measures the expected change in response y per unit increase in x_i when the other independent variables are held constant. The ith observation and jth level of independent variable is denoted by x_{ij}.

The data structure for the general multiple regression model where there are k explanatory variables and one response is as shown in the table below,

Table 4.1 Data for Multiple Linear Regression Model

y	x_1	x_2	...	x_k
y_1	x_{11}	x_{12}	...	x_{1k}
y_2	x_{21}	x_{22}	...	x_{2k}
⋮	⋮	⋮		⋮
y_n	x_{n1}	x_{n2}	...	x_{nk}

The model in equation 4.2 can be represented in matrix form for the data of table 4.1 as

$$Y = X\beta + \varepsilon \tag{4.3}$$

in which

$$\boldsymbol{Y} = \begin{pmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \\ . \\ . \\ . \\ \boldsymbol{y_n} \end{pmatrix}, \qquad \boldsymbol{\beta} = \begin{pmatrix} \boldsymbol{\beta_1} \\ \boldsymbol{\beta_2} \\ . \\ . \\ . \\ \boldsymbol{\beta_p} \end{pmatrix}$$

$$\boldsymbol{X} = \begin{pmatrix} \mathbf{1} & \boldsymbol{x_{11}} & \boldsymbol{x_{12}} & \boldsymbol{\ldots} & \boldsymbol{x_{1k}} \\ \mathbf{1} & \boldsymbol{x_{21}} & \boldsymbol{x_{22}} & \boldsymbol{\ldots} & \boldsymbol{x_{2k}} \\ . & . & . & \boldsymbol{\ldots} & . \\ . & . & . & \boldsymbol{\ldots} & . \\ . & . & . & \boldsymbol{\ldots} & . \\ \mathbf{1} & \boldsymbol{x_{n1}} & \boldsymbol{x_{n2}} & \boldsymbol{\ldots} & \boldsymbol{x_{nk}} \end{pmatrix} \quad \boldsymbol{and} \qquad \varepsilon = \begin{pmatrix} \boldsymbol{\varepsilon_1} \\ \boldsymbol{\varepsilon_2} \\ . \\ . \\ . \\ \boldsymbol{\varepsilon_n} \end{pmatrix}$$

where,

$\boldsymbol{Y}$ is an $(\boldsymbol{n} \times \mathbf{1})$ vector of observations,

$\boldsymbol{X}$ is an $(\boldsymbol{n} \times \boldsymbol{k})$ design matrix,

$\boldsymbol{\beta}$ is a $(\boldsymbol{k} \times \mathbf{1})$ vector of unknown parameters, and

$\boldsymbol{\varepsilon}$ is a $(\boldsymbol{n} \times \mathbf{1})$ vector of independently identically distributed random variables with mean zero and variance $\boldsymbol{\sigma^2}$, (Montgomery 2005).

If $\boldsymbol{X'X}$ has a determinant which is different from zero, then the linear system of **4.3** has a unique least squares solution given by

$$\hat{\boldsymbol{\beta}} = (\boldsymbol{X'X})^{-1}\boldsymbol{X'y} \tag{4.4}$$

The estimated regression equation is

$$\hat{\boldsymbol{y}} = \boldsymbol{X'}\hat{\boldsymbol{\beta}} \tag{4.5}$$

which can also be represented as,

$$\hat{y}_i = \hat{\beta}_0 + \sum_{j=1}^{k} \hat{\beta}_j x_{ij} \qquad (i = 1, 2, 3, ..., n; \quad j = 1, 2, 3, ..., k) \tag{4.6}$$

for particular observations x_{ij}.

4.1.1 First-Order Model Analysis for Test run 2

A first-order model with two independent variables is expressed as:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon_i \tag{4.7}$$

which can be written in coded form as,

$$y_i = \beta_0 x_{0i} + \beta_i x_{i1} + \beta_{ii} x_{i2} + \varepsilon_i \tag{4.8}$$

where:

- y_i represents the response where, in this research, is the amount of blood sugar level in milligrams per decilitre.
- β_0 is the mean response, that is the amount of blood sugar level when all the explanatory factors are zero.
- β_i is the parameter associated with the time taken after the herbal medicine has been used.
- β_{ii} is the parameter associated with the amount of concentration of the herbal medicine.
- $x_{0i} = 1$ represent a vector of one's.
- x_{i1} represent the observation times on the blood sugar level of the animals in minutes at three intervals.
- x_{i2} is the concentration of the herbal medicine in mg/kg which has been controlled at three levels.

If there is a curvature in the response surface, then a higher degree polynomial will be used. In any model, the levels of each factor are independent of the levels of other factors. The Method of Least Square is used to estimate the parameters in the polynomials using Design of Experiments software (DoE) in which the response surface analysis is performed by using the fitted surface.

In order to simplify the calculations, we use appropriately coded variables for describing the explanatory variables. The explanatory variables are centred (rescaled) such that $\mathbf{0}$ is in the middle of the centre of the design, and $+\mathbf{1}$ and $-\mathbf{1}$ are the distances from the centre. The variables time ($\boldsymbol{X_1}$) and Concentration ($\boldsymbol{X_2}$) are called natural variables, because they are expressed in the natural units of measurement. We however note that a considerable improvement in representational capabilities can often be obtained by allowing the possibility of transformations in the response variable. The transformation of these natural variables to coded variables is as follows:

$$\boldsymbol{x_{i1}} = (\boldsymbol{X_{i1}} - \mathbf{90})/\mathbf{60}$$
$$\boldsymbol{x_{i2}} = (\boldsymbol{X_{i2}} - \mathbf{50})/\mathbf{25}$$
$$\boldsymbol{y_i} = \frac{\mathbf{1}}{(\boldsymbol{y}+\boldsymbol{k})^{0.5}}$$

where the constant $\boldsymbol{k} = -\mathbf{3}$.

By employing the above transformations for the data in section 3.6.2.2 the following data is generated from test run two.

4.1.2 Data run from Test 2 in coded form

	NATURAL VARIABLES			CODED VARIABLES		
	Time	Concentration	Response	TIME	CONCENT	Transformed response
RUN	X1	X2	Y	x1	x2	y
1	30	25	16.1	-1	-1	0.2763
2	90	25	15.7	0	-1	0.2806
3	150	25	5.3	1	-1	0.6594
4	30	25	15.1	-1	-1	0.2875
5	90	25	12.3	0	-1	0.3279
6	150	25	11.3	1	-1	0.3471
7	30	25	16.7	-1	-1	0.2702
8	90	25	11.9	0	-1	0.3352
9	150	25	4.6	1	-1	0.7906
10	30	25	15.7	-1	-1	0.2806
11	90	25	21.6	0	-1	0.2384
12	150	25	3.3	1	-1	0.7670
13	30	50	17.9	-1	0	0.2591
14	90	50	16.7	0	0	0.2702
15	150	50	3.3	1	0	0.8771
16	30	50	10.7	-1	0	0.3604
17	90	50	13.8	0	0	0.3043
18	150	50	5	1	0	0.7071
19	30	50	18.8	-1	0	0.2516
20	90	50	14.1	0	0	0.3002
21	150	50	2.4	1	0	0.6455
22	30	50	21.2	-1	0	0.2344
23	90	50	15.6	0	0	0.2817
24	150	50	8.3	1	0	0.4344
25	30	75	12.4	-1	1	0.3262
26	90	75	5.1	0	1	0.6901
27	150	75	7.2	1	1	0.4880
28	30	75	17.4	-1	1	0.2635
29	90	75	23.9	0	1	0.2540
30	150	75	5.1	1	1	0.6901

Data run from Test 2 in coded form continued

	NATURAL VARIABLES			CODED VARIABLES		
	Time	Concentration	Response	TIME	CONCENT	Transformed response
RUN	X1	X2	Y	x1	x2	y
31	30	75	12.6	-1	1	0.3227
32	90	75	13.4	0	1	0.3101
33	150	75	7.3	1	1	0.4822
34	30	75	17.6	-1	1	0.2617
35	90	75	17.1	0	1	0.2663
36	150	75	3.7	1	1	1.1952
37	30	25	17.1	0	-1	0.2663
38	90	25	15.4	1	-1	0.2840
39	150	25	11.4	-1	-1	0.3450
40	30	25	10.4	0	-1	0.3676
41	90	25	9.9	1	-1	0.3807
42	150	25	11.9	-1	-1	0.3352
43	30	25	17.5	-1	-1	0.2626
44	90	25	14.2	0	-1	0.2988
45	150	25	13.9	1	-1	0.3029
46	30	25	14.2	-1	-1	0.2988
47	90	25	13.6	0	-1	0.3071
48	150	25	13	1	-1	0.3162
49	30	25	12.8	-1	-1	0.3194
50	90	25	11.4	0	-1	0.3450
51	150	25	13.4	1	-1	0.3101

4.1.3 Parameter Estimation

On fitting the model in equation (4.1) to the data of section 4.1.2, we derive the parameter estimates, as indicated below.

The design matrix is given as,

$$\boldsymbol{X'X} = \begin{pmatrix} \mathbf{51} & \mathbf{0} & \mathbf{-15} \\ \mathbf{0} & \mathbf{34} & \mathbf{0} \\ \mathbf{-15} & \mathbf{0} & \mathbf{39} \end{pmatrix} \tag{4.9}$$

whose inverse is

$$(\boldsymbol{X'X})^{-1} = \frac{\mathbf{1}}{\mathbf{59976}} \begin{pmatrix} \mathbf{1326} & \mathbf{0} & \mathbf{510} \\ \mathbf{0} & \mathbf{1764} & \mathbf{0} \\ \mathbf{510} & \mathbf{0} & \mathbf{1734} \end{pmatrix} \tag{4.10}$$

Further

$$\boldsymbol{X'y} = \begin{pmatrix} \mathbf{20.0764} \\ \mathbf{4.7222} \\ \mathbf{-4.0504} \end{pmatrix} \tag{4.11}$$

From equation (4.4) we get the parameter estimates as,

$$\hat{\boldsymbol{\beta}} = \frac{\mathbf{1}}{\mathbf{59976}} \begin{pmatrix} \mathbf{1326} & \mathbf{0} & \mathbf{510} \\ \mathbf{0} & \mathbf{1764} & \mathbf{0} \\ \mathbf{510} & \mathbf{0} & \mathbf{1734} \end{pmatrix} \begin{pmatrix} \mathbf{20.0764} \\ \mathbf{4.7222} \\ \mathbf{-4.0504} \end{pmatrix} = \begin{pmatrix} \mathbf{0.4094} \\ \mathbf{0.1389} \\ \mathbf{0.0536} \end{pmatrix} \tag{4.12}$$

Therefore the regression equation is given as

$$\hat{y} = 0.4094 + 0.1389x_1 + 0.0536x_2. \tag{4.13}$$

The interpretation of these parameter estimates are as follows:

$\hat{\beta}_0 = 0.4094$, is the mean response. This is the amount of blood sugar level when all the explanatory factors are zero.

$\hat{\beta}_1 = 0.1389$, shows that when time changes from one observation point to another, the blood sugar level increases by 0.1389 units.

$\hat{\beta}_2 = 0.0536$, indicates that when the concentration of herbal medicine is varied from one level to the other, the blood sugar level increases by 0.0536

The sum of squares due to total is given as

$$\begin{aligned} SST &= y'y - n\bar{y}^2 \\ &= 9.9051 - 51(0.3937)^2 \\ &= 2.0019 \end{aligned} \tag{4.14}$$

and the sum of squares due to regression is give as

$$\begin{aligned} SSR &= \hat{\beta}'X'y - n\bar{y}^2 \\ &= 8.6585 - 51(0.3937)^2 \\ &= 0.7553 \end{aligned} \tag{4.15}$$

The sum of squares due to error is

$$\begin{aligned} SSE &= y'y - \hat{\beta}'X'y \\ &= 9.9051 - 8.6585 \end{aligned}$$

$$= \mathbf{1.2466} \tag{4.16}$$

which verifies that

$$\boldsymbol{SST = SSR + SSE.} \tag{4.17}$$

Using the result of the equations $(\mathbf{4.14})$, $(\mathbf{4.15})$ and $(\mathbf{4.16})$ the analysis of variance table generated for this model is as follows:

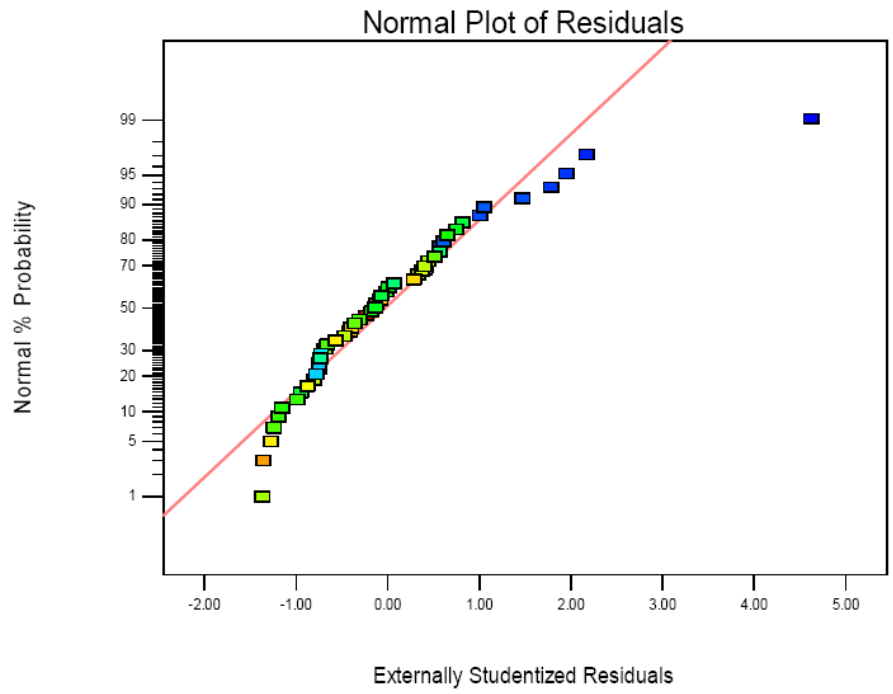
Table 4.2 Analysis of Variance Table

Source	Degrees of freedom	Sum of Squares	Mean SS	F-ratio
Regression	2	0.7553	0.3777	14.5413
Error	48	1.2466	0.0260	
Total	50	2.0019		

4.1.4 The Test for Significance in Regression

We expect a good estimated regression model to explain the variation of the dependent variable in the sample. However there are certain tests of hypotheses about the model parameters that can help the experimenter in measuring the effectiveness of the model. The first of all these tests require the error term $\boldsymbol{e_i}$'s to be normally and independently distributed with mean zero and variance $\boldsymbol{\sigma^2}$. In order to check this assumption, we graph the normal probability of residuals for the described model as shown in Figure 4.1.

Figure 4.1 Normal Probability Plot of the Residuals



The residuals plot is approximately along a straight line, thus the normality assumption is satisfied. It is important to note that the error term is the difference between the observed value y_i and the corresponding fitted value $\hat{y}_i$, that is, $e_i = y_i - \hat{y}_i$.

4.1.4.1 The Test for Significance of the model

As a result of the normality assumption being satisfied, observations y_i are also normally and independently distributed. Therefore, the test for the significance of the regression can be applied to determine if the relationship between the dependent variable y and independent variables x_1, x_2, exists. The hypotheses are,

$H_0 : \beta_1 = \beta_2 = 0$, against
$H_1 : \beta_j \neq 0$ for at least one j.

From the analysis of variance table $F_c = 14.5413$. Comparing this with the table value $F_{0.05,2,48} = 3.23$, we find that there is a significant statistical evidence to reject the null hypothesis. It implies that at least one of the independent variables,

time or concentration, contributes significantly to the model, therefore the model is adequate.

We now carry out further tests on the parameters $\boldsymbol{\beta_1}$ and $\boldsymbol{\beta_2}$ in order to identify the variable that significantly contributes to the model.

4.1.4.2 The Test for Significance of parameter Estimates

In order to determine whether given variables are justified to be included or excluded from the model, we undertake the test of hypotheses for the individual regression coefficients as follows:

4.1.4.2.1 Test for β_1

Hypothesis

$$\boldsymbol{H_0 : \beta_1 = 0}, \text{ against}$$
$$\boldsymbol{H_1 : \beta_1 \neq 0} \ .$$

The standard error for $\hat{\boldsymbol{\beta}}_{\mathbf{1}}$, $\boldsymbol{S.E\hat{\beta}_1}$, is found by use of the fact that

$$\boldsymbol{Cov(\hat{\beta}) = MSE(X'X)^{-1}}$$

Thus, using the results in table 4.2 and the diagonal element of $\boldsymbol{(X'X)^{-1}}$ correspondint to this parameter estimate in equation (4.10), the standard error will be,

$$\boldsymbol{S.E\hat{\beta}_1 = [\frac{1764 \times 0.0260}{59976}]^{1/2} = 0.0277.} \tag{4.18}$$

The test statistic will be $\boldsymbol{t_c = \frac{0.1389}{0.0277} = 5.0144}$,

while $\boldsymbol{t_{0.025,48} = 2.011}$.

Since $\boldsymbol{-t_{\alpha/2} < t_c < t_{\alpha/2}}$, we reject the null hypothesis, thus the parameter is significant and the predictor variable-time ($\boldsymbol{x_1}$) is required in explaining the variation of the blood sugar level at $\boldsymbol{\alpha = 0.05}$.

4.1.4.2.2 Test for β_2

Hypothesis

$$\boldsymbol{H_0 : \beta_2 = 0}, \text{ against}$$
$$\boldsymbol{H_1 : \beta_2 \neq 0} \ .$$

Following the same procedure as for $\boldsymbol{\beta_1}$ the corresponding standard error for the parameter estimate, $\hat{\boldsymbol{\beta}}_{\boldsymbol{2}}$ will be,

$$\boldsymbol{S.E\hat{\beta}_2 = [\frac{1734 \times 0.0260}{59976}]^{1/2} = 0.0274,} \tag{4.19}$$

but the test statistic is $\boldsymbol{t_c = \frac{0.0536}{0.1898} = 1.95620}$.

Since $\boldsymbol{-t_{\alpha/2} < t_c < t_{\alpha/2}}$, we accept the null hypothesis. The parameter is not significant and therefore predictor variable-concentration ($\boldsymbol{x_2}$)is not individually important in explaining the variation of the blood sugar level at $\boldsymbol{\alpha = 0.05}$.
The coefficient of multiple determination is given as;

$$\boldsymbol{R^2 = \frac{SSR}{SST} = \frac{0.7553}{2.0019} = 0.3773}$$

which indicates that 37.73 % in the variation of the blood sugar level is accounted for by the model, which is a rather low value to justify the correct relationship between the predictors and the response.

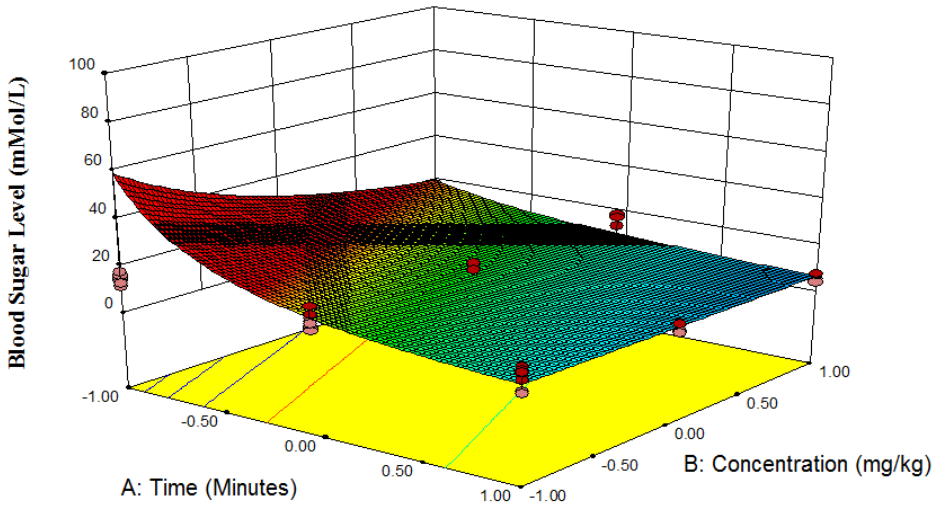
The test of hypotheses have led to the non significance of the parameter associated with the variable concentration which is a key variable for this research. We explore the possibility of fitting a second order model to evaluate whether it is better placed than the first order model in relating the response to the predictor variables.

4.2 Designs for Fitting the Second-Order Model

4.2.1 Justification for the Second-Order Model

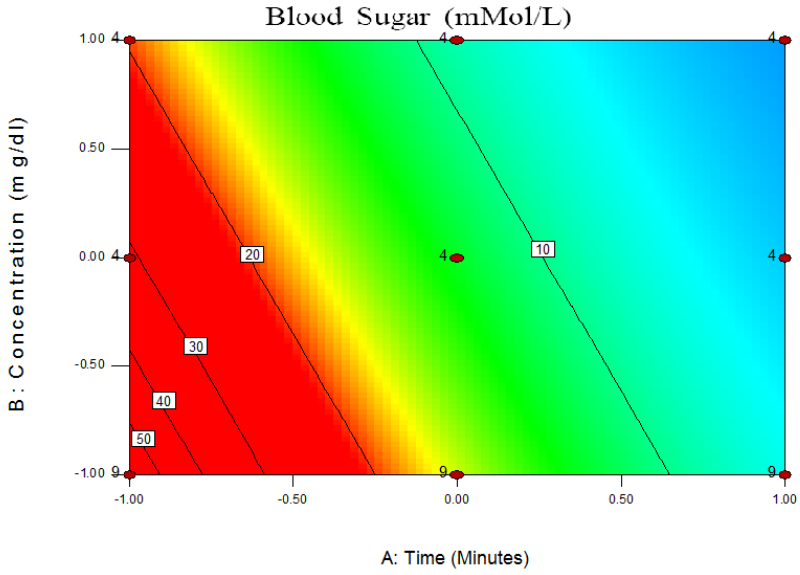
By plotting a three dimensional plot for the variables *time* and *concentration* for the model fitted in section **4.1** for test run 2 we have;

Figure 4.2 Three dimensional surface plot



The contour plot for this graph is as follows:

Figure 4.3 Contour plot



From these two plots, it is evident that the sugar levels are decreasing with respect to time for the albino rats that were used. These are the flat beds that slope downwards or are tilted to portray the sugar level decrease with a slight concave part in figure 4.2.

4.2.2 Fitting of the Second-Order Model

We explore the second order model fit to verify and explore the curvature if it exists. The second-order model includes all the terms in the first-order model plus all quadratic terms and all cross product terms. The model is expressed as;

$$\begin{aligned} y_i &= \beta_0 + \sum_{j=1}^{q} \beta_j x_j + \sum_{i=1}^{q} \beta_{jj} x_{jj}^2 + \sum_i \sum_{j<i} \beta_{ij} x_i x_j + \varepsilon \qquad (4.20) \\ &= \beta_0 + x_i' \beta_i + x_i' \beta_i x_i + \varepsilon_{ij} \qquad (4.21) \end{aligned}$$

Specifically for this investigation equation (4.21) will have two predictor variables which gives the model as,

$$\begin{aligned} y_i &= \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \beta_{11} x_{i1}^2 + \beta_{22} x_{i2}^2 \\ &+ \beta_{12} x_{i1} x_{i2} + \varepsilon \qquad (4.22) \end{aligned}$$

4.2.3 Parameter estimation

The method of least squares can be used to estimate the regression coefficients in (4.22) as described in Section 4.1.3. Using the Design of Experiment (DoE) software for the regression analysis, the same results as those obtained in section 4.1.3 are obtained.

In this case (for a second order model) the parameter estimates, the degrees of freedom, the corresponding standard error of the estimates as well as the 95% confidence interval of the parameters generated by the design of experiment software are as in the following table.

Table 4.3 Parameter Estimates for the Quadratic model

Factor	Coefficient Estimate	df	Standard Error	95% ConfidenceInterval Low	High
Intercept	0.34	1	0.052	0.23	0.44
A-Time	0.16	1	0.027	0.10	0.21
B-Concentration	0.053	1	0.026	1.389E-003	0.11
AB	0.069	1	0.031	6.349E-003	0.13
A^2	0.11	1	0.044	0.021	0.20
B^2	-1.440E-003	1	0.050	-0.10	0.100

The regression equation generated is

$$\hat{y}^* = 0.3371 + 0.1591x_1 + 0.0535x_2 + 0.1102x_1^2 - 0.00144x_2^2 + 0.0687x_1x_2, \tag{4.23}$$

where the variables x_1 as *time* represented by A and the x_2 as *concentration* represented by B in the table 4.3 respectively. The corresponding analysis of variance table generated from this data is as follows:

Table 4.4 ANOVA for Response Surface Quadratic model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	1.00	5	0.20	9.02	< 0.0001
A-Time	*0.76*	*1*	*0.76*	*34.38*	*< 0.0001*
B-Concentration	*0.095*	*1*	*0.095*	*4.28*	*0.0444*
AB	*0.11*	*1*	*0.11*	*4.92*	*0.0316*
A^2	*0.14*	*1*	*0.14*	*6.19*	*0.0166*
B^2	*1.829E-005*	*1*	*1.829E-005*	*8.232E-004*	*0.9772*
Residual	1.00	45	0.022		
Lack of Fit	*0.037*	*3*	*0.012*	*0.53*	*0.6609*
Pure Error	*96*	*42*	*0.023*		
Cor Total	2.00	*50*			

Other statistics computed from the above data are as follows:

Table 4.5 Summaries

Std. Dev.	0.15	R-Squared	0.5006
Mean	0.39	Adj R-Squared	0.4451
C.V. %	37.86	Pred R-Squared	0.3229
PRESS	1.36	Adeq Precision	8.920

4.2.4 Tests of hypotheses

4.2.4.1 Test of hypothesis for the model

Using the results of table 4.4 for the second order modelobtained in (4.22), the test of hypothesis can be carried out for this model as well as the parameter estimates.

The hypothesis for the model is stated as:

$\boldsymbol{H_0 : \beta_1 = \beta_2 = \beta_{11} = \beta_{22} = \beta_{12} = 0}$, against

$\boldsymbol{H_1 : \beta_j \neq 0}$ for at least one $\boldsymbol{j}$.

The calculated value of the test statistic $\boldsymbol{F_c = 9.02 > F_{0.05(5,45)} = 2.45}$ We thus reject the null hypothesis. Therefore model is significant. Alternatively using the generated $\boldsymbol{p}$-value we confidently state that the Model F-value of 9.02 implies the model is significant and there is less tha 0.01% chance that an F-value which is this large could occur due to error (noise).

The coefficient of determination is $\boldsymbol{R^2 = 0.5006}$, indicates that 50.06 % of the variation in the sugar level is accounted for by the model (or is due to the variation in the explanatory variables), which is an improvement from the first order model fitted from equation $\boldsymbol{(4.7)}$ that gave $\boldsymbol{R^2 = 0.3773}$.

4.2.4.2 Test of hypothesis on individual parameter estimates

The test for individual parameters can be performed as was the case in section 4.1.4.2. We compute the test statistic for each parameter estimate and compare the resulting values with the table values at the desired level of significance ($\boldsymbol{\alpha}$) and accompanying degrees of freedom from the model used. However this can equivalently be achieved by using the $\boldsymbol{(1-\alpha)100\%}$ confidence interval in which we find that provided that the confidence interval does not include zero then the parameter estimate is significant otherwise it is not.

The test of hypothesis for individual parameter estimates is stated as

$\boldsymbol{H_0 : \beta_j = 0}$, against

$\boldsymbol{H_1 : \beta_j \neq 0}$ for $\boldsymbol{j = 1, 2, 11, 22, 12}$

Using the confidence interval approach we find that the parameter estimates that are significant are the ones corresponding to the variables, $\boldsymbol{x_1, x_2, x_1^2}$ and $\boldsymbol{x_1 x_2}$. That is to imply that the variables which contribute to the reduction of blood sugar level are time, concentration, time squared and interaction of concentration with time. The parameter estimate corresponding to concentration squared is not significant, which implies that the accompanying variables do not explain the variation of

blood sugar level individually.

4.3 Analysis of the Stationary Point of the Second-Order Model

When there is a curvature in the response surface the first-order model is insufficient. Thus a second-order model becomes useful in approximating a portion of the true response surface with parabolic curvature. Using a statistical software (Design of Experiments-DoE) in analysis of a quadratic response, we get the following three dimension plots for the two continuous factors *time* and *concentration*;

Figure 4.4 Three d-surface plot View 1

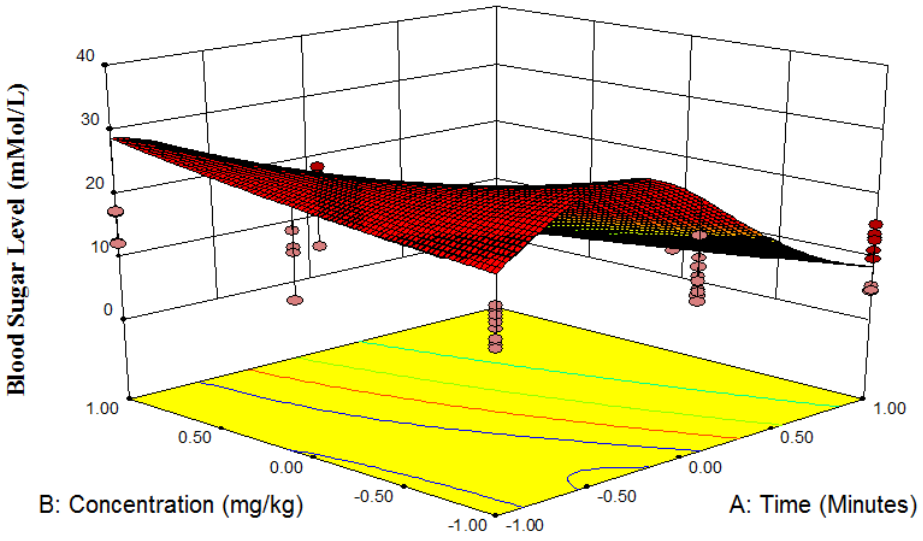


Figure 4.5 Three d-surface plot View 2

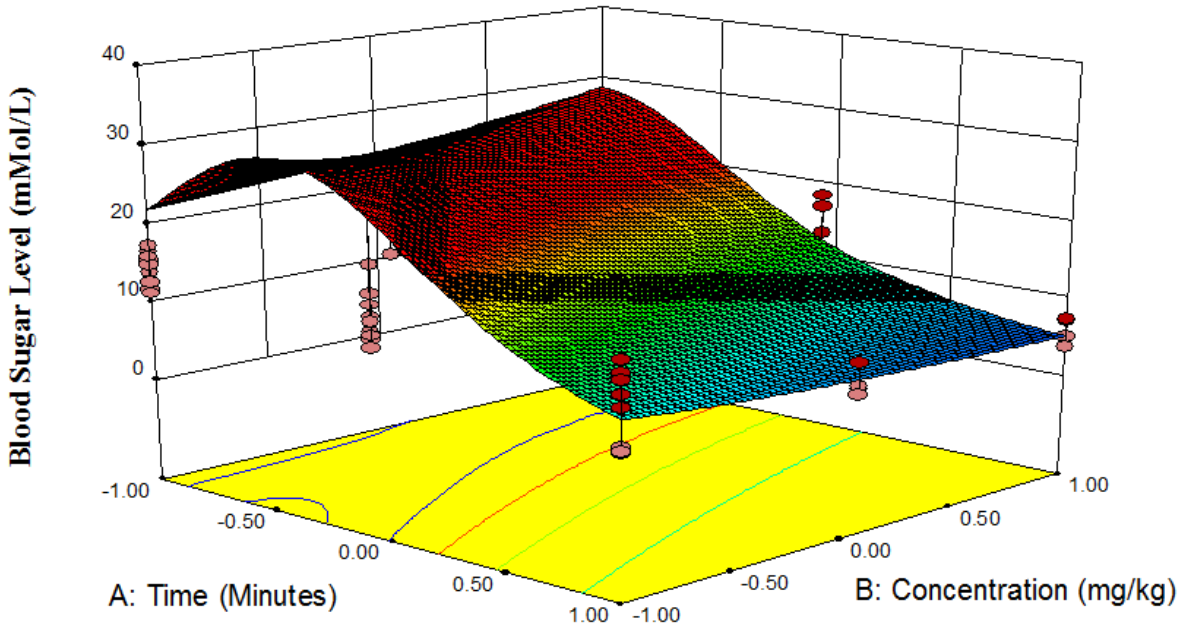
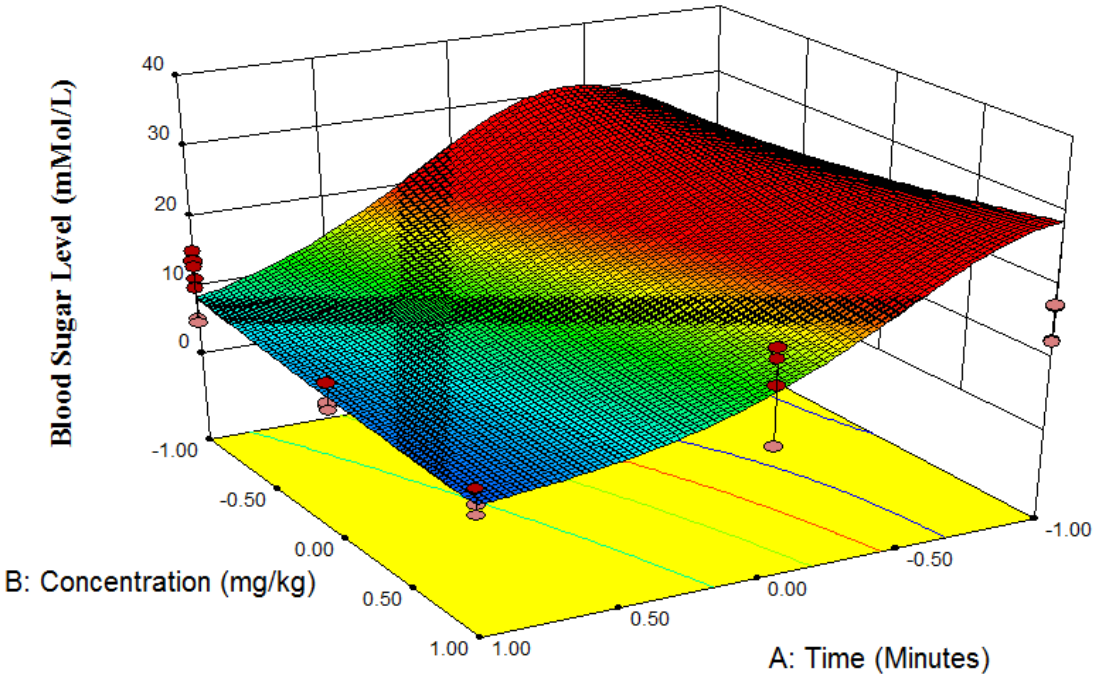
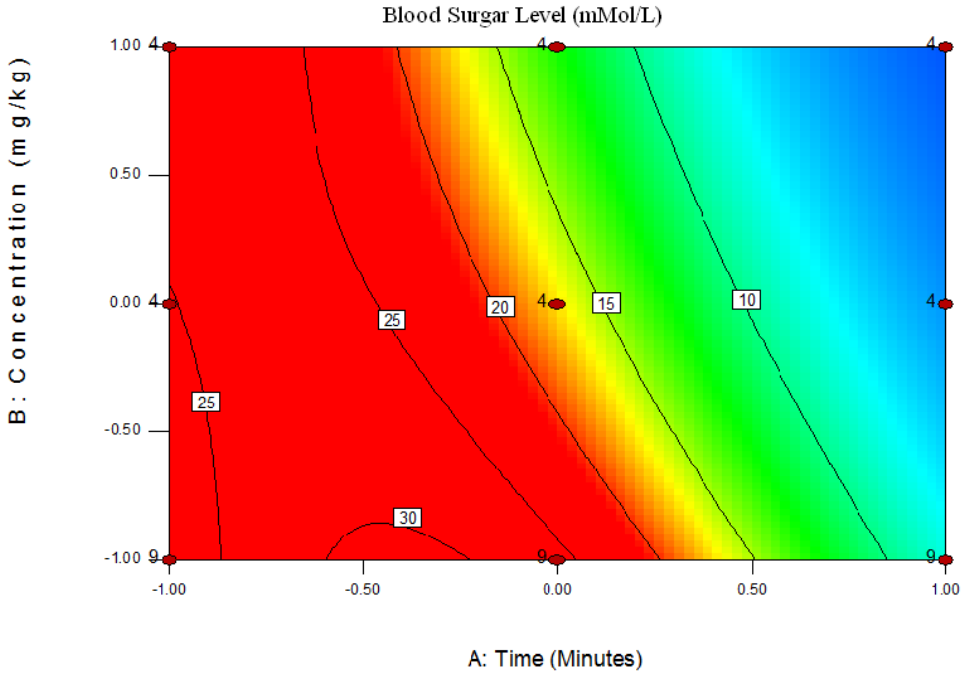


Figure 4.6 Three d-surface plot View 3



The accompanying contour plot for these three dimension views at different orientation is as follows:

Figure 4.7 Contour plot3



All the three dimension plots suggest that curvature exists and hence justification for having fitted the second order model. The second-order model is flexible, as it takes a variety of functional forms and approximates the response surface locally which is a good estimation of the true response surface.

From the above results we conclude that response surface is explained by the second-order model. We now determine the optimum setting and recommed it for the effective management of average sugar level in a diabetic patient. Graphical visualization of contour plots helps in understanding the second-order response surface. Specifically, three dimensional surface plot and their accompanying contour plots help characterize the shape of the surface, and through these we will be able to approximately locate the optimum response.

Using the fit of the second-order models we illustrate quadratic response surfaces such as minimum, maximum, ridge, and saddle point. In the case that an optimum exits, then this point is a stationary point which can result in any of the aforementioned four possibilities. The stationary point in response surface models is the combination of design variables where the surface is at either a maximum or a minimum in all directions. If the stationary point is a maximum in some direction and minimum in another direction, then the stationary point is a saddle

point. When the surface is curved in one direction but is fairly constant in another direction, then this type of surface is called ridge system (Oehlert 2000).

The stationary point is evaluated by use of matrix algebra for which the fitted second order model (4.22) in matrix form is as below:

$$\hat{y} = \hat{\beta}_0 + x'b + x'Bx \tag{4.24}$$

The derivative of $\hat{y}_i$ with respect to the elements of the vector x is

$$\frac{\delta \hat{y}}{\delta x} = b + 2Bx \tag{4.25}$$

Therefore, the solution to stationary point is

$$x_s = -\frac{1}{2}B'b \tag{4.26}$$

where

$$B = \begin{pmatrix} \hat{\beta}_{11} & \hat{\beta}_{12}/2 & \dots & \hat{\beta}_{1q}/2 \\ \hat{\beta}_{21}/2 & \hat{\beta}_{22} & \dots & \hat{\beta}_{2q}/2 \\ . & . & \dots & . \\ . & . & \dots & . \\ . & . & \dots & . \\ \hat{\beta}_{q1}/2 & \hat{\beta}_{q2}/2 & \dots & \hat{\beta}_{qq} \end{pmatrix} \quad and \quad b = \begin{pmatrix} \hat{\beta}_1 \\ \hat{\beta}_2 \\ . \\ . \\ . \\ \hat{\beta}_q \end{pmatrix}$$

b is a $(q \times 1)$ vector of the first-order regression coefficients and B is a $(q \times q)$ symmetric matrix whose main diagonal elements are the quadratic coefficients ($\hat{\beta}_{ii}$) and whose off diagonal elements are one-half the mixed quadratic coefficients ($\beta_{ij(i \neq j)}$), Montgomery (2005). As a result, the estimated response value for the fitted model at the identified stationary point is obtained as:

$$B = \begin{pmatrix} \hat{\beta}_{11} & \hat{\beta}_{12}/2 \\ \hat{\beta}_{12}/2 & \hat{\beta}_{22} \end{pmatrix} = \begin{pmatrix} 0.1102 & 0.0345 \\ 0.0345 & -0.0015 \end{pmatrix} \tag{4.27}$$

while

$$\boldsymbol{b} = \begin{pmatrix} \mathbf{0.1591} \\ \mathbf{0.0535} \end{pmatrix}$$

(4.28)

One of the points of interest in this research is the minimum condition for the explanatory variables. The results used above are for a maximum condition. Modifying equation (4.26) for a minimum condition by negating it so as to achieve our desired results, the stationary point solution with this modification is found to be as follows:

$$\boldsymbol{x_{s2}} = \frac{\mathbf{1}}{\mathbf{2}} \begin{pmatrix} \mathbf{0.1102} & \mathbf{0.0315} \\ \mathbf{0.0345} & \mathbf{-0.0015} \end{pmatrix}^{-1} \begin{pmatrix} \mathbf{-0.1591} \\ \mathbf{0.0535} \end{pmatrix} = \begin{pmatrix} \mathbf{0.76883922} \\ \mathbf{-0.15003135} \end{pmatrix}$$

(4.29)

We now find the stationary point in terms of the natural variables, *time* and *concentration* from the coding concept adopted earlier in subsection 4.1.1. For *time* as a variable, we have

$$\begin{aligned} \boldsymbol{x_1} &= \frac{\boldsymbol{X_1 - 90}}{\mathbf{60}} \\ \boldsymbol{X_1} &= \boldsymbol{x_1 \times 60 + 90} \\ &= \mathbf{0.76884 \times 60 + 90} \\ &= \mathbf{136.1304} \end{aligned}$$

(4.30)

this implies that the time taken to reduce the blood sugar level to within acceptable range is 136.1304 minutes. With respect to *concentration* we have,

$$\begin{aligned} \boldsymbol{x_2} &= \frac{\boldsymbol{X_2 - 50}}{\mathbf{25}} \\ \boldsymbol{X_2} &= \boldsymbol{x_2 \times 25 + 50} \\ &= \mathbf{-0.150031 \times 25 + 50} \end{aligned}$$

$$= \mathbf{46.24923.} \tag{4.31}$$

Thus 46.2492 mg/dl of the herbal formula is to be used to regulate the blood sugar level to within the acceptable range.

As a result, the estimated response value at the stationary point is given as

$$\hat{\boldsymbol{y}} = \hat{\boldsymbol{\beta}}_{\mathbf{0}} + \frac{\mathbf{1}}{\mathbf{2}}\boldsymbol{x}_{\boldsymbol{s}}'\boldsymbol{b} \tag{4.32}$$

which gives us

$$\hat{\boldsymbol{y}} = \mathbf{0.3371} \times \frac{\mathbf{1}}{\mathbf{2}} \begin{pmatrix} \mathbf{0.76883922} & \mathbf{-0.15003135} \end{pmatrix} \begin{pmatrix} \mathbf{0.1591} \\ \mathbf{0.0535} \end{pmatrix} = \begin{pmatrix} \mathbf{0.3942} \end{pmatrix} \tag{4.33}$$

where $\hat{\boldsymbol{\beta}}_{\mathbf{0}}$ is the mean response given in equation $(\mathbf{4.32})$, $\boldsymbol{x}_{\boldsymbol{s}}'$ is as in equation $(\mathbf{4.29})$ and $\boldsymbol{b}$ is provided in equation $(\mathbf{4.28})$. The reversed transformation for the amount of blood sugar level coded in subsection 4.1.1 gives us

$$\begin{aligned} \boldsymbol{y}^{*} &= (\boldsymbol{Y} - \boldsymbol{k})^{\mathbf{-0.5}} \\ \hat{\boldsymbol{Y}} &= \frac{\mathbf{1}}{\boldsymbol{y}^{*\mathbf{2}}} + \boldsymbol{k} \\ &= \mathbf{6.4353 + 3} \\ &= \mathbf{9.4353} \end{aligned} \tag{4.34}$$

This is the estimated minimum blood sugar level for the given predictor variables.

4.4 Analysis for Test run 3

4.4.1 First-Order Model Analysis for Test run 3

Adopting the following transformation (coding) for the data provided in section 3.6.3.2

$$x_{i1} = (X_1 - 60)/30$$

$$x_{i2} = (X_2 - 250)/125$$

we generate the data for the test run three as below.

4.4.2 Data from Test run 3 in coded form

	NATURAL VARIABLES			CODED VARIABLES		
	Time	Concentration	Response	Time	Concentration	Response
RUN	X1	X2	Y	x1	x2	y = Y
1	30	125	24.2	-1	-1	24.2
2	60	125	23.6	0	-1	23.6
3	90	125	20.8	1	-1	20.8
4	30	125	22.4	-1	-1	22.4
5	60	125	21.8	0	-1	21.8
6	90	125	21.2	1	-1	21.2
7	30	125	24.2	-1	-1	24.2
8	60	125	21.4	0	-1	21.4
9	90	125	12.4	1	-1	12.4
10	30	125	11.8	-1	-1	11.8
11	60	125	13.6	0	-1	13.6
12	90	125	14.9	1	-1	14.9
13	30	125	21.6	-1	-1	21.6
14	60	125	19.5	0	-1	19.5
15	90	125	10.5	1	-1	10.5
16	30	250	22.1	-1	0	22.1
17	60	250	19.9	0	0	19.9
18	90	250	16.7	1	0	16.7
19	30	250	22.7	-1	0	22.7
20	60	250	10.9	0	0	10.9

Data run from Test 3 Continued

	NATURAL VARIABLES			CODED VARIABLES		
	Time	Concentration	Response	Time	Concentration	Response
RUN	X1	X2	Y	x1	x2	y = Y
21	90	250	9.8	1	0	9.8
22	30	250	16.6	-1	0	16.6
23	60	250	9.7	0	0	9.7
24	90	250	9.4	1	0	9.4
25	30	250	5.2	-1	0	5.2
26	60	250	9.7	0	0	9.7
27	90	250	3.1	1	0	3.1
28	30	500	15.6	-1	2	15.6
29	60	500	21.9	0	2	21.9
30	90	500	22.7	1	2	22.7
31	90	500	23	1	2	23
32	30	500	12.4	-1	2	12.4
33	60	500	9.6	0	2	9.6
34	90	500	8.9	1	2	8.9
35	30	500	14.9	-1	2	14.9
36	60	500	12.4	0	2	12.4
37	90	500	8.7	1	2	8.7
38	30	500	18.9	-1	2	18.9
39	60	500	13.4	0	2	13.4
40	90	500	10.9	1	2	10.9

4.4.3 Parameter Estimates

Employing equation (4.8) in coded form we regress the data for a first order model using the Design of Experiment (DoE) software.The results generated for the parameter estimates are as follows:

Table 4.6 Parameter Estimates for first order model

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High
Intercept	16.17	1	0.91	14.31	18.02
Time	-1.99	1	1.09	-4.19	0.22
Concentration	-1.06	1	0.71	-2.49	0.37

The corresponding regression equation is obtained as

$$\hat{y} = 16.1667 - 1.8877x_1 - 1.0621x_2. \tag{4.35}$$

The analysis of variance table generated from the regression equation is as follows,

Table 4.7 ANOVA for first order model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	185.92	2	92.96	2.91	0.0669
Time	*106.29*	*1*	*106.29*	*3.33*	*0.0761*
Concentration	*72.04*	*1*	*72.04*	*2.26*	*0.1415*
Residual	1180.79	37	31.91		
Lack of Fit	*233.90*	*6*	*38.98*	*1.28*	*0.2968*
Pure Error	*946.89*	*31*	*30.54*		
Cor Total	1366.71	39			

other summary statistics are;

Table 4.8 Summaries

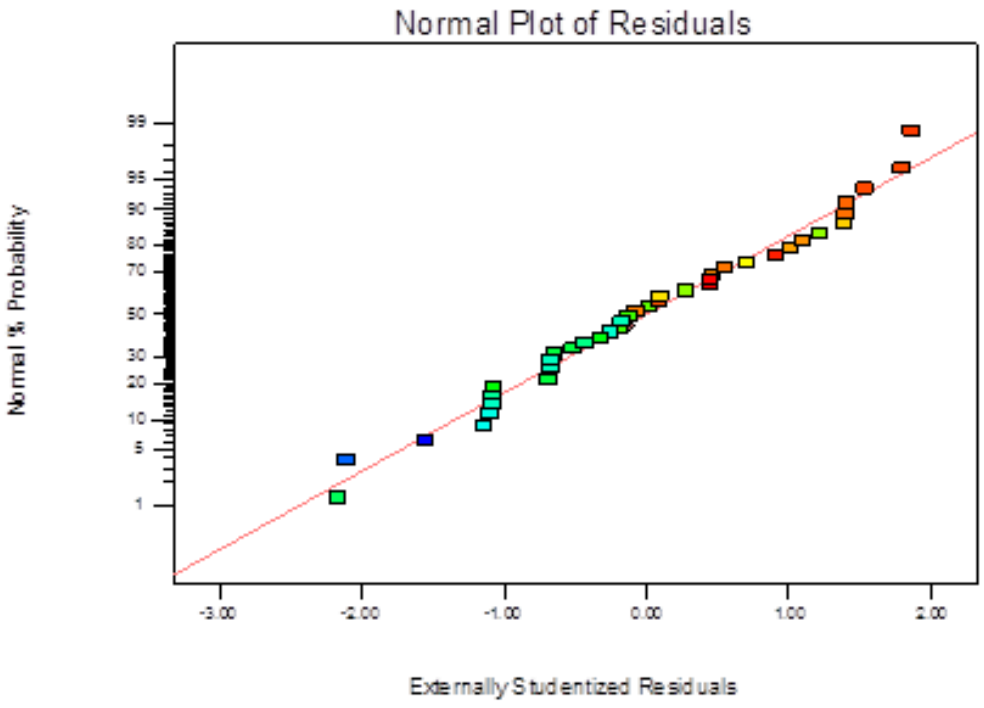
Std. Dev.	5.65	R-Squared	0.1360
Mean	15.82	Adj R-Squared	0.0893
C.V. %	35.70	Pred R-Squared	-0.0076
PRESS	1377.13	Adeq Precision	4.628

4.4.4 Test of hypothesis on regression

4.4.4.1 The Test for Significance of the model

Before undertaking a test of hypothesis we require the error term e_i's to be normally and independently distributed with mean zero and variance σ^2. In order to check this assumption, we draw the normal probability distribution of residuals for the described model as follows:

Figure 4.8 Normal Probability Plot of the Residuals



The residuals plot is approximately along a straight line, which implies that the normality assumption is satisfied. The hypothesis for this test is stated as

$H_0 : \beta_1 = \beta_2 = 0$, against

$H_1 : \beta_j \neq 0$ for at least one j.

The computed $F_c = 2.91$ is less than $F_{0.05,(2,39)} = 3.32$, we accept the null hypothesis. Thus the model is not significant, with only a 6.69% chance that an F-value this large could occur due to error (noise).

The coefficient of multiple determination $R^2 = 0.1360$, implies that 13.60 % of the variation in the blood sugar level is accounted for by the model. This is a rather low percentage. While the 'Adequate Precision' measures the signal to noise ratio, a ratio greater than 4 is desirable. Our ratio of 4.628 indicates an adequate signal, which gives us reason to use this model as a basis to navigate the design space.

4.4.4.2 The Test for Significance of the parameter estimates

The hypotheses to be tested for the two parameters is stated as follows;

$H_0 : \beta_j = 0$, against

$H_1 : \beta_j \neq 0$, for j=1, 2

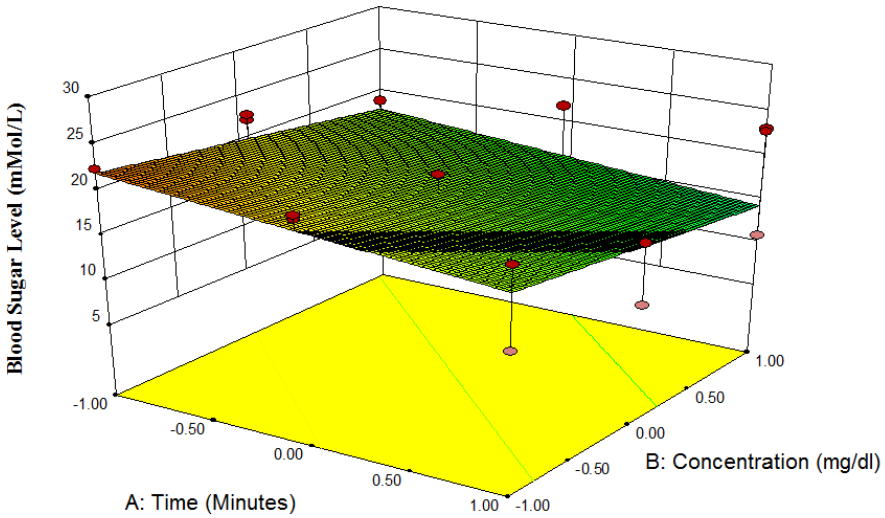
If we were to use the values provided in the ANOVA table 4.7 we find that $p - value$ less than 0.0500 indicate model terms are significant, in this case x_1 and x_2 are not significant model terms. Due to the fact that the model is not significant as well as the parameter estimates, we explore the possibility of fitting a second order model as below.

4.5 Second-Order Model Analysis

4.5.1 Justification for Second-Order Model

We first plot three-dimensional graphs for the variables *time*, *concentration* and *blood sugar levels*

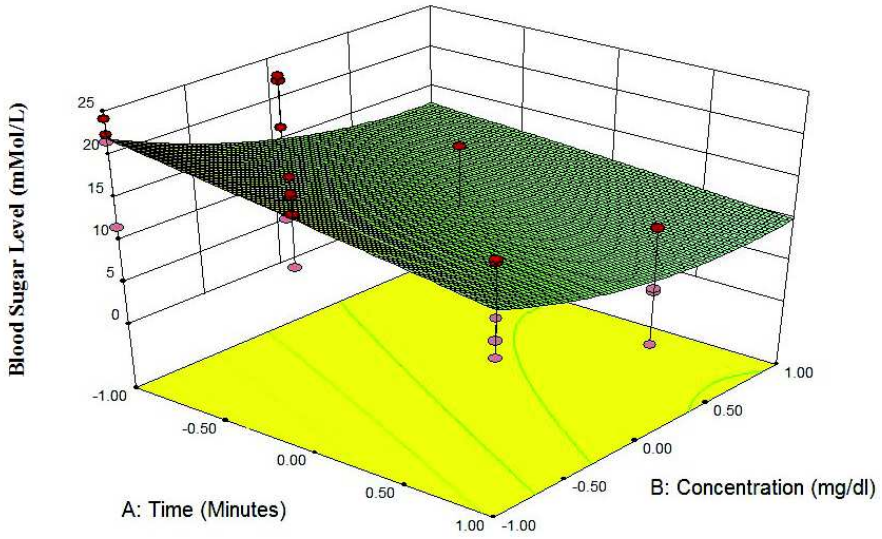
Figure 4.9 Three d-surface plot for blood sugar level



From this plane, we conclude that sugar levels are decreasing with respect to time for the albino rats that were used as earlier described in section 4.2.

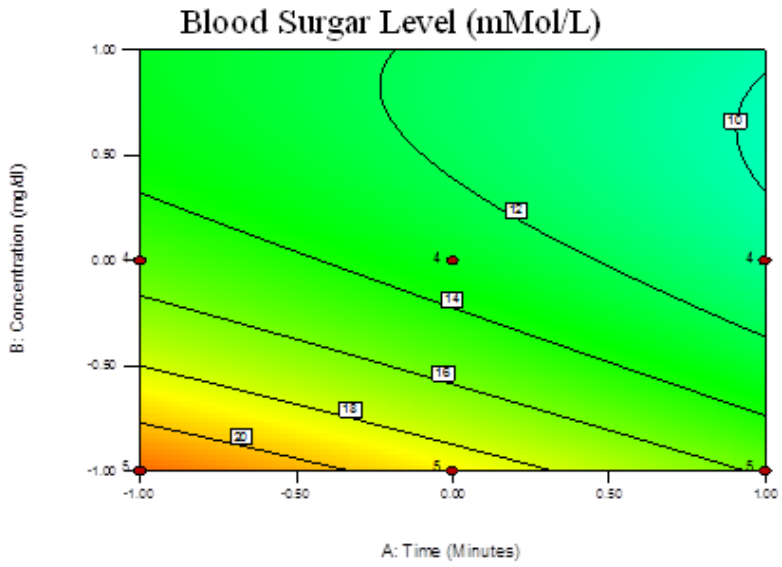
However to explore whether there is any curvature, we use the three dimension plots from a quadratic perspective to get the following plot for the two continuous factors, time and concentration and their corresponding response;

Figure 4.10 Three d-surface plot for quadratic response



The three dimensional plots suggest that curvature exist with a downward sloping concaved curvature, showing decrease of sugar level with time and at varied concentrations of the herbal medicine. This also is evident from the contour plot provided below.

Figure 4.11 Contour plot



Both the three dimension plot and its accompanying contour plot above reveals a trough that suggests minimum function throughout this region described by the

factor combinations. Hence there is need to explore the design space by use of a higher order model for the data.

4.5.2 Parameter Estimates

On fitting the model described in equation (4.23) using the design of experiment software the following parameter estimates are realised.

Table 4.8 Parameter Estimates for second order model

	Coefficient		Standard	95% CI	95% CI
Factor	Estimate	df	Error	Low	High
Intercept	13.07	1	1.96	9.09	17.06
A-Time	-2.26	1	1.06	-4.41	-0.11
B-Concentration	-3.64	1	1.23	-6.14	-1.14
AB	0.82	1	0.81	-0.84	2.47
A^2	-0.13	1	1.81	-3.81	3.54
B^2	2.30	1	0.93	0.41	4.20

The corresponding regression equation is given as

$$\begin{aligned} \hat{y} &= 13.0718 - 2.2593x_1 - 3.6393x_2 - 0.1326x_1^2 + 2.3041x_2^2 \\ &+ 0.8157x_1x_2. \end{aligned} \tag{4.36}$$

The ANOVA table generated from the regression equation is as follows:

Table 4.9 ANOVA for second order model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	392.20	5	78.44	2.74	0.0350
A-Time	*130.53*	*1*	*130.53*	*4.55*	*0.0401*
B-Concentration	*251.71*	*1*	*251.71*	*8.78*	*0.0055*
AB	*28.86*	*1*	*28.86*	*1.01*	*0.3227*
A^2	*0.15*	*1*	*0.15*	*5.377E-003*	*0.9420*
B^2	*174.65*	*1*	*174.65*	*6.09*	*0.0188*
Residual	974.51	34	28.66		
Lack of Fit	*27.62*	*3*	*9.21*	*0.30*	*0.8241*
Pure Error	*946.89*	*31*	*30.54*		
Cor Total	1366.71	39			

other summary statistics are

Table 4.10 Summaries

Std. Dev.	5.35	R-Squared	0.2870
Mean	15.82	Adj R-Squared	0.1821
C.V. %	33.83	Pred R-Squared	0.0150
PRESS	1346.22	Adeq Precision	5.439

4.5.3 Test of hypothesis of the regression model

4.5.3.1 The Test for Significance of the model

The hypothesis for the overall performance of the model is stated as follows

$H_0 : \beta_1 = \beta_2 = \beta_3 = \beta_{11} = \beta_{22} = \beta_{12} = 0$, against

$H_1 : \beta_j \neq 0$ for at least one j.

The computed F_c value of 2.74 in the above ANOVA table compared to

$F_{0.05,(5,34)}$ implies that the model is significant, with only a 3.50% chance that an F-value this large could occur due to error (noise).

Further, considering coefficient of multiple determination from table 4.10 we find that 28.70 % of the variation in the blood sugar level is accounted for by the model, which is a better improvement from the first order model value of 13.60 %. The 'Adequate Precision' measure the signal to noise ratio, of 5.439 indicates an adequate signal.

4.5.3.2 The Test for Significance of the parameter estimates

The hypotheses to be tested for the three parameters can be stated in general as;

$H_0 : \beta_j = 0$, against

$H_1 : \beta_j \neq 0$, for $j = 1, 2, 3, 11, 22, 12$

Using the values provided in the ANOVA table, we find that $p-value$ values which are less than 0.0500 imply that model terms are significant. In this case x_1, x_2 and x_2^2 are significant model terms. This is an indication that the linear terms in the second order model are all significant and the quadratic term associated with concentration is significant. This is an improvement when compared to the first order model. The Lack of Fit F-value of 0.30 implies the Lack of Fit is not significant relative to the pure error. This indicates that there is a 82.41% chance that a 'Lack of Fit F-value' this large could occur due to noise.

4.6 Analysis of the Stationary Point of the Second-Order Model

The stationary point for the second order model is to be evaluated by use of matrix algebra, as described under section 4.3, using equations (4.24) and (4.25). Therefore, the solution to stationary point is,

$$x_{s3} = -\frac{1}{2}B^{-1}b, \tag{4.37}$$

where

$$\boldsymbol{B} = \begin{pmatrix} \hat{\beta}_{11} & \hat{\beta}_{12}/2 \\ \hat{\beta}_{12}/2 & \hat{\beta}_{22} \end{pmatrix} \begin{pmatrix} \mathbf{-0.1376} & \mathbf{0.4079} \\ \mathbf{0.4079} & \mathbf{2.3040} \end{pmatrix} \tag{4.38}$$

and

$$\boldsymbol{b} = \begin{pmatrix} \mathbf{-2.2593} \\ \mathbf{-3.6393} \end{pmatrix} \tag{4.39}$$

Modifying the equation (4.37) for a minimum condition by negating it, the stationary point solution is,

$$\boldsymbol{x_{s3}} = \frac{\mathbf{1}}{\mathbf{2}} \begin{pmatrix} \mathbf{-0.1376} & \mathbf{0.4079} \\ \mathbf{0.4079} & \mathbf{2.3040} \end{pmatrix}^{-1} \begin{pmatrix} \mathbf{-2.2593} \\ \mathbf{-3.6393} \end{pmatrix} = \begin{pmatrix} \mathbf{3.8486} \\ \mathbf{-1.4711} \end{pmatrix}. \tag{4.40}$$

We now can find the stationary point in terms of the natural variables, time and concentration from the coding concept adopted earlier in section 4.4.1. For *time* as a variable, we have

$$\begin{aligned} \boldsymbol{x_1} &= \frac{\boldsymbol{X_1 - 60}}{\mathbf{30}} \\ \\ \boldsymbol{X_1} &= \boldsymbol{x_1} \times \mathbf{30} + \mathbf{60} \\ &= \mathbf{3.8486} \times \mathbf{30} + \mathbf{60} \\ \\ &= \mathbf{175.4580.} \end{aligned} \tag{4.41}$$

This implies that the time taken to reduce the blood sugar to within acceptable range will be 175.4580 minutes. With respect to *concentration* we have

$$x_2 = \frac{X_2 - 250}{125}$$

$$\begin{aligned} X_2 &= x_2 \times 125 + 250 \\ &= -1.4711 \times 125 + 250 \\ &= 66.1125. \end{aligned} \tag{4.42}$$

This indicates that 66.1125 mg/dl of the herbal formula is to be used to regulate the blood sugar level in a diabetic. As a result the estimated response value at the stationary point is calculated as

$$\hat{y} = 13.0718 \times \frac{1}{2} \begin{pmatrix} 3.8486 & -1.4711 \end{pmatrix} \begin{pmatrix} -2.2593 \\ -3.6393 \end{pmatrix} = \begin{pmatrix} 11.3898 \end{pmatrix} \tag{4.43}$$

This is the estimated minimum blood sugar level for the given predictor variables.

Chapter 5

The Variance Function

We have outlined that when a response is fitted to data, the experimenter is more focused in the difference between the estimated responses at two points rather than in the actual responses at a particular location. As a consequence, the variance function is called into play for the achievement of this desire. However there are two approaches to the use of the variance function on the difference between two estimated responses. One of the approaches is to find the variance function of each of the two estimated responses and then find their difference. The second approach is to work out the difference of the two estimated responses and then find the variance function of that difference. In this research work we look at the two approaches after which we compare their results as applied to the herbal medicine data on the treatment of diabetes.

5.1 The Variance function of the Estimated Response

The variance function of the estimated response ($\hat{y}$) is given in general as described in section 1.4. In particular equation (1.18) indicates that the estimated response ($\hat{y}$) at a point on the predictor variable space can be computed for k- predictor variables. With reference to a vector of predictor variables, we use equation (1.20) to compute the scaled predictor variance (SPV) that is to be used to discriminate between competing designs of various sizes. This eliminates the requirement of the knowledge of the value of σ^2.

In this research work the interest is the variance function for the fitted second

order model for the test runs considered. The first order model in both the test runs were found to be inadequate as evidenced by the test of hypothesis procedures carried out on each respectively.

However for us to evaluate the variance function of the estimated response function for the test runs, we call into play the moment conditions for a rotatable design from first order and second order as cited in section 1.33.
For the vector $\boldsymbol{x_{s2}}$ that was used to generate the estimated response, we have the estimated response identified on the two response surfaces which is given as

$$\hat{\boldsymbol{y}}(\mathrm{x}_\mathrm{s}') = \mathrm{x}_\mathrm{s}'\hat{\boldsymbol{\beta}} \tag{5.1}$$

where $\hat{\boldsymbol{\beta}}$ is the Least Square Estimate of $\boldsymbol{\beta}$. The standardized variance of this estimated responses is

$$\boldsymbol{V_s} = \frac{\boldsymbol{N}\hat{\boldsymbol{y}}(\mathrm{x}_\mathrm{s}')}{\boldsymbol{\sigma^2}} = \mathrm{x}_\mathrm{s}'(\boldsymbol{X'X})^{-1}\mathrm{x}_\mathrm{s} \tag{5.2}$$

5.1.1 Variance of the Estimated Response for test run 2

In test run two we have the observations vector for the stationary point given in equation (4.29) as

$$\boldsymbol{x_{s2}} = \begin{pmatrix} \mathbf{0.768839} \\ \mathbf{-0.150031} \end{pmatrix}$$

The corresponding observation vector constructed from $\boldsymbol{x_{s2}}$ for the quadratic model is

$$\boldsymbol{x_r} = \begin{pmatrix} \mathbf{1} \\ \mathbf{0.768839} \\ \mathbf{-0.150031} \\ \mathbf{0.591113} \\ \mathbf{0.022509} \\ \mathbf{-0.115350} \end{pmatrix}. \tag{5.3}$$

From $(\mathbf{5.2})$, the variance is computed using

$$\boldsymbol{X'X} = \begin{pmatrix} \mathbf{51} & \mathbf{0} & \mathbf{-15} & \mathbf{34} & \mathbf{39} & \mathbf{0} \\ \mathbf{0} & \mathbf{34} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{-10} \\ \mathbf{-15} & \mathbf{0} & \mathbf{39} & \mathbf{-10} & \mathbf{-15} & \mathbf{0} \\ \mathbf{34} & \mathbf{0} & \mathbf{-10} & \mathbf{34} & \mathbf{26} & \mathbf{0} \\ \mathbf{39} & \mathbf{0} & \mathbf{-15} & \mathbf{26} & \mathbf{39} & \mathbf{0} \\ \mathbf{0} & \mathbf{-10} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{26} \end{pmatrix}$$

Thus,

$$\boldsymbol{V_r} = (\,\mathbf{1} \quad \mathbf{0.768839} \quad \mathbf{-0.150031} \quad \mathbf{0.591113} \quad \mathbf{0.0225093} \quad \mathbf{-0.115350}\,)$$

$$\times \begin{pmatrix} \mathbf{51} & \mathbf{0} & \mathbf{-15} & \mathbf{34} & \mathbf{39} & \mathbf{0} \\ \mathbf{0} & \mathbf{34} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{-10} \\ \mathbf{-15} & \mathbf{0} & \mathbf{39} & \mathbf{-10} & \mathbf{-15} & \mathbf{0} \\ \mathbf{34} & \mathbf{0} & \mathbf{-10} & \mathbf{34} & \mathbf{26} & \mathbf{0} \\ \mathbf{39} & \mathbf{0} & \mathbf{-15} & \mathbf{26} & \mathbf{39} & \mathbf{0} \\ \mathbf{0} & \mathbf{-10} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{26} \end{pmatrix}^{-1} \begin{pmatrix} \mathbf{1} \\ \mathbf{0.768839} \\ \mathbf{-0.150031} \\ \mathbf{0.591113} \\ \mathbf{0.022509} \\ \mathbf{-0.115350} \end{pmatrix}$$

$$= \mathbf{0.0987.} \tag{5.4}$$

We now show that $\boldsymbol{V_r}$ is minimum by comparing it with variance of another point on the same response surface. We take point $\mathbf{x_q}$ which is different from the stationary point, but in the neighbourhood of this stationary point, where,

$$\mathbf{x'_q} = (\,\mathbf{1} \quad \mathbf{0.9000} \quad \mathbf{-0.2000} \quad \mathbf{0.8100} \quad \mathbf{0.0400} \quad \mathbf{-0.1800}\,)$$

(5.5)

The variance for this estimated response using this vector $\mathbf{x_q}$ is found to be,

$$\boldsymbol{V_{x_q}} = \begin{pmatrix} 1 & 0.9000 & -0.2000 & 0.8100 & 0.0400 & -0.1800 \end{pmatrix}$$

$$\times \begin{pmatrix} 51 & 0 & -15 & 34 & 39 & 0 \\ 0 & 34 & 0 & 0 & 0 & -10 \\ -15 & 0 & 39 & -10 & -15 & 0 \\ 34 & 0 & -10 & 34 & 26 & 0 \\ 39 & 0 & -15 & 26 & 39 & 0 \\ 0 & -10 & 0 & 0 & 0 & 26 \end{pmatrix}^{-1} \begin{pmatrix} 1 \\ 0.9000 \\ -0.2000 \\ 0.8100 \\ 0.0400 \\ -0.1800 \end{pmatrix}$$

$$= 0.1038. \tag{5.6}$$

Comparing the results of equation (5.4) and (5.6), it clearly shows that the variance of the estimated response arising from the vector in equation (5.3) that is generated from the estimated response in (4.46) is a minimum as compared to that generated by the vector of (5.5) on the same response surface. Thus $\boldsymbol{V_r}$ is minimum.

5.1.2 Variance of the Estimated Response for run 3

Using the observations vector for the stationary point given in equation (4.42)

$$\boldsymbol{x_{s3}} = \begin{pmatrix} 3.8486 \\ -1.4711 \end{pmatrix}$$

for which we construct the observation vector for the quadratic arrangement as

$$\boldsymbol{x_t} = \begin{pmatrix} 1 \\ 3.8486 \\ -1.4711 \\ 14.8117 \\ 2.1641 \\ -5.6617 \end{pmatrix} \tag{5.7}$$

The variance is then computed using equation (5.2) where

$$\boldsymbol{X'X} = \begin{pmatrix} \mathbf{40} & \mathbf{1} & \mathbf{11} & \mathbf{27} & \mathbf{67} & \mathbf{2} \\ \mathbf{1} & \mathbf{27} & \mathbf{2} & \mathbf{1} & \mathbf{4} & \mathbf{8} \\ \mathbf{11} & \mathbf{2} & \mathbf{67} & \mathbf{8} & \mathbf{89} & \mathbf{4} \\ \mathbf{27} & \mathbf{1} & \mathbf{8} & \mathbf{27} & \mathbf{46} & \mathbf{2} \\ \mathbf{67} & \mathbf{4} & \mathbf{89} & \mathbf{46} & \mathbf{223} & \mathbf{8} \\ \mathbf{2} & \mathbf{8} & \mathbf{4} & \mathbf{2} & \mathbf{8} & \mathbf{46} \end{pmatrix}$$

With this matrix, we get the variance $\boldsymbol{V_t}$ as,

$$\boldsymbol{V_t} = \begin{pmatrix} \mathbf{1} & \mathbf{3.8486} & \mathbf{-1.4711} & \mathbf{14.8117} & \mathbf{2.1641} & \mathbf{-5.6617} \end{pmatrix}$$

$$\times \begin{pmatrix} \mathbf{40} & \mathbf{1} & \mathbf{11} & \mathbf{27} & \mathbf{67} & \mathbf{2} \\ \mathbf{1} & \mathbf{27} & \mathbf{2} & \mathbf{1} & \mathbf{4} & \mathbf{8} \\ \mathbf{11} & \mathbf{2} & \mathbf{67} & \mathbf{8} & \mathbf{89} & \mathbf{4} \\ \mathbf{27} & \mathbf{1} & \mathbf{8} & \mathbf{27} & \mathbf{46} & \mathbf{2} \\ \mathbf{67} & \mathbf{4} & \mathbf{89} & \mathbf{46} & \mathbf{223} & \mathbf{8} \\ \mathbf{2} & \mathbf{8} & \mathbf{4} & \mathbf{2} & \mathbf{8} & \mathbf{46} \end{pmatrix}^{-1} \begin{pmatrix} \mathbf{1} \\ \mathbf{3.8486} \\ \mathbf{-1.4711} \\ \mathbf{14.8117} \\ \mathbf{2.1641} \\ \mathbf{-5.6617} \end{pmatrix}$$

$$= \mathbf{24.8193}, \tag{5.8}$$

which is the variance of the estimated response in coded variables.

If we select any other point different from the stationary point in the neighbourhood of this stationary point, say $\mathbf{x_h}$ where

$$\mathbf{x_h'} = \begin{pmatrix} \mathbf{1} & \mathbf{4} & \mathbf{-2} & \mathbf{16} & \mathbf{4} & \mathbf{-8} \end{pmatrix}.$$

(5.9)

We then compute the variance function for this estimated response using this vector $\mathbf{x_h}$ and get,

$$\boldsymbol{V_{x_h}} = \begin{pmatrix} \mathbf{1} & \mathbf{4} & \mathbf{-2} & \mathbf{16} & \mathbf{4} & \mathbf{-8} \end{pmatrix}$$

$$\times \begin{pmatrix} \mathbf{40} & \mathbf{1} & \mathbf{11} & \mathbf{27} & \mathbf{67} & \mathbf{2} \\ \mathbf{1} & \mathbf{27} & \mathbf{2} & \mathbf{1} & \mathbf{4} & \mathbf{8} \\ \mathbf{11} & \mathbf{2} & \mathbf{67} & \mathbf{8} & \mathbf{89} & \mathbf{4} \\ \mathbf{27} & \mathbf{1} & \mathbf{8} & \mathbf{27} & \mathbf{46} & \mathbf{2} \\ \mathbf{67} & \mathbf{4} & \mathbf{89} & \mathbf{46} & \mathbf{223} & \mathbf{8} \\ \mathbf{2} & \mathbf{8} & \mathbf{4} & \mathbf{2} & \mathbf{8} & \mathbf{46} \end{pmatrix}^{-1} \begin{pmatrix} \mathbf{1} \\ \mathbf{4} \\ \mathbf{-2} \\ \mathbf{16} \\ \mathbf{4} \\ \mathbf{-8} \end{pmatrix}$$

$$= \mathbf{30.3998.} \tag{5.10}$$

This result (5.10) compared to the result in (5.8) clearly shows that the variance for the estimated response given by the stationary point is a minimum in comparison to the variance using a vector generated from the same response surface. Thus $\boldsymbol{V_t}$ is minimum.

5.2 The Variance functions of the Difference between two Estimated Responses

Suppose that $\mathbf{z}$ and $\mathbf{x}$ are two row vectors of the form of a row of $\boldsymbol{X}$ but which arise from two distinct points identified on two estimated response surfaces of different radii. Then

$$\hat{\boldsymbol{y}}(\mathbf{z}) = \mathbf{z}\hat{\boldsymbol{\beta}} \tag{5.11}$$

which is equivalent to

$$\hat{\boldsymbol{y}}(\mathbf{z}) = \mathbf{z}^{\prime[\mathbf{p}]}\hat{\boldsymbol{\beta}} \tag{5.12}$$

Similarly

$$\hat{\boldsymbol{y}}(\mathbf{x}) = \mathbf{x}^{\prime}\hat{\boldsymbol{\beta}} \tag{5.13}$$

which is equivalent to

$$\hat{\boldsymbol{y}}(\mathbf{x}) = \mathbf{x}^{\prime[\mathbf{p}]}\hat{\boldsymbol{\beta}} \tag{5.14}$$

where $\hat{\boldsymbol{\beta}}$ is the Least Square Estimate of $\boldsymbol{\beta}$. Using the *Schlafflian Vectors* and *Matrices* with $\mathbf{x}' = (\boldsymbol{x_1}, \boldsymbol{x_2}, \cdots, \boldsymbol{x_k})$ for which we define the vector $\mathbf{x}^{[\mathrm{p}]}$ such that

$$\begin{aligned} \mathbf{x}^{[p]'}\mathbf{x}^{[p]} &= [\mathbf{x}'\mathbf{x}]^{\boldsymbol{p}} \\ &= [\boldsymbol{x_0^2} + \boldsymbol{x_1^2} + \boldsymbol{x_2^2} + \cdots + \boldsymbol{x_k^2}]^{\boldsymbol{p}} \\ &= \sum \frac{\boldsymbol{p!}}{\boldsymbol{p_0!p_1!p_2!...p_k!}} (\boldsymbol{x_0^2})^{\boldsymbol{p_0}} (\boldsymbol{x_1^2})^{\boldsymbol{p_1}} (\boldsymbol{x_2^2})^{\boldsymbol{p_2}} \cdots (\boldsymbol{x_k^2})^{\boldsymbol{p_k}} \end{aligned} \tag{5.15}$$

$\boldsymbol{p}$ is the order of the polynomial and the summations are taken over $\boldsymbol{p_0, p_1, p_2, ..., p_k}$ where

$\boldsymbol{p_0 + p_1 + p_2 + \cdots + p_k = p}$ and $\boldsymbol{x_0 = 1}$.

Let

$$\boldsymbol{V}[\hat{\boldsymbol{y}}(\mathbf{z}) - \hat{\boldsymbol{y}}(\mathbf{x})] \tag{5.16}$$

denote the variance of the difference between the two estimated responses $(\mathbf{5.11})$ and $(\mathbf{5.13})$ at the points $\mathbf{z}$ and $\mathbf{x}$. This variance simplifies to

$$\boldsymbol{V_d} = [\mathbf{z} - \mathbf{x}]'(\boldsymbol{X'X})^{-\mathbf{1}}[\mathbf{z} - \mathbf{x}]\boldsymbol{\sigma^2}. \tag{5.17}$$

Further, let

$$\mathbf{z} = \mathbf{AMx} \tag{5.18}$$

where $\boldsymbol{A} = \boldsymbol{diag}(\mathbf{1}, \boldsymbol{a}, \boldsymbol{a...}, \boldsymbol{a})$ is $(\boldsymbol{k}+\mathbf{1})$by $(\boldsymbol{k}+\mathbf{1})$ diagonal matrix and the rotation matrix $\boldsymbol{M} = ((\boldsymbol{m_{ij}}))$ in which $\boldsymbol{m_{11}} = \mathbf{1}$, $\boldsymbol{m_{1j}} = \boldsymbol{m_{i1}} = \mathbf{0}$;
$\boldsymbol{i, j} = \mathbf{2, 3, ..., k+1}$. With this substitution (5.18) becomes

$$\mathbf{z}^{[\mathrm{d}]} = (\mathbf{AM})^{[\mathrm{d}]}\mathbf{x}^{[\mathrm{d}]}. \tag{5.19}$$

Using (5.19) in (5.17), we get

$$\boldsymbol{V_d} = [\mathbf{z}'^{[\mathrm{d}]} - \mathbf{x}'^{[\mathrm{d}]}](\boldsymbol{X'X})^{-\mathbf{1}}[\mathbf{z}^{[\mathrm{d}]} - \mathbf{x}^{[\mathrm{d}]}]\boldsymbol{\sigma^2} \tag{5.20}$$

where $(\boldsymbol{X'X})^{-\mathbf{1}}\boldsymbol{\sigma^2}$ is the variance covariance matrix of equation (4.3).
With reference to section $\mathbf{1.3}$, the design is rotatable, which implies that $\boldsymbol{X'X}$ has a special form (Box and Hunter (1957). Herzberg (1967) pointed out that the

variance as stated in equation (5.13) is invariant under orthogonal rotations in the predictor space. Thus

$$(\boldsymbol{X'X})^{-1} = \boldsymbol{N}'^{[d]}(\boldsymbol{X'X})^{-1}\boldsymbol{N}^{[d]}. \tag{5.21}$$

With this result, we rewrite (5.20) as

$$\boldsymbol{V_d} = \mathbf{x}'^{[d]}(\mathbf{A}'^{[d]}(\mathbf{X'X})^{-1}\mathbf{A}^{[d]} + (\mathbf{I} - \mathbf{2A}'^{[d]}\mathbf{M}'^{[d]})(\mathbf{X'X})^{-1})\mathbf{x}^{[d]}]\boldsymbol{\sigma^2} \tag{5.22}$$

since $\boldsymbol{AM} = \boldsymbol{MA}$.

Since equation (5.13) holds true for rotatable designs, (5.14) is invariant under orthogonal rotation of the points $\mathbf{z}$ and $\mathbf{x}$. The coordinate axes can be rotated in such a manner that $\mathbf{z}$ will be on the first coordinate axis and $\mathbf{x}$ will be in the plane of the first two coordinates axes Herzberg (1967). With that in mind we write,

$$\mathbf{x}' = (\mathbf{1}, \boldsymbol{\rho}, \mathbf{0}, \mathbf{0}, \mathbf{0}, ..., \mathbf{0}) \tag{5.23}$$

where $\boldsymbol{\rho}$ is the distance of $\mathbf{x}'= (\boldsymbol{x_1}, \boldsymbol{x_2}, ..., \boldsymbol{x_k})$ from the design origin. Matrix $\boldsymbol{M}$ is given as

$$\boldsymbol{M} = \begin{pmatrix} \mathbf{1} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{...} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{cos\theta} & \boldsymbol{-sin\theta} & \mathbf{0} & \mathbf{0} & \mathbf{...} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{sin\theta} & \boldsymbol{cos\theta} & \mathbf{0} & \mathbf{0} & \mathbf{...} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} & \mathbf{...} & \mathbf{0} \\ \mathbf{.} & \mathbf{.} & \mathbf{.} & \mathbf{.} & \mathbf{.} & \mathbf{...} & \mathbf{.} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{...} & \mathbf{1} \end{pmatrix}$$

In this case we can assert that the variance function in (5.13) will depend only on the lengths $\boldsymbol{\rho_1^2}$ and $\boldsymbol{\rho_2^2}$ of the straight lines joining these selected points to the origin and on the angle $\boldsymbol{\theta}$ that is between the two lines.

In the special case where $\boldsymbol{A = I}$ then (5.22) is expressed as,

$$\boldsymbol{V_d = 2x'^{[d]}(I - M'^{[d]}(X'X)^{-1})x^{[d]}]\sigma^2} \tag{5.24}$$

Now for a second order response surface model (5.22) reduces to

$$\boldsymbol{V_d = 2x'^{[2]}(I - M'^{[d]}(X'X)^{-1})x^{[2]}]\sigma^2} \tag{5.25}$$

and thus using the equations(5.23) in (5.24), the appropriate variance function for the difference between two estimated responses becomes

$$\boldsymbol{V_d = 2(\frac{sin^2\theta}{2N\lambda_4}\rho^4 + \frac{1 - cos\theta}{N\lambda_2}\rho^2)\sigma^2} \tag{5.26}$$

The standardized variance of the difference between the two estimated responses will be

$$\boldsymbol{V_d = 2(\frac{sin^2\theta}{2\lambda_4}\rho^4 + \frac{1 - cos\theta}{\lambda_2}\rho^2)} \tag{5.27}$$

where $\boldsymbol{\rho}$ is a constant, and is symmetric about angle $\boldsymbol{\theta = 180^0}$.

With regard to rotatability we only need to evaluate $\boldsymbol{\theta}$ for values lying in the first quadrant, since by rotating the points around the sphere the angle remains invariant as well as first quadrant can be used to give values in other quadrants, Karanjah et al (2008). The variance function in this case reaches a minimum value if $\boldsymbol{\rho^2 < \lambda_4/\lambda_2}$ at $\boldsymbol{\theta = 180^0}$. Therefore, for us to minimize the variance function in (5.27) a second order rotatable design should be employed with large values of $\boldsymbol{\lambda_4/\lambda_2}$.

We now can work out the variance function of the difference between two estimated responses for the data used in this discussion. However we bear in mind the excursions above can be called into play if our interest is to minimizing the value of this variance.

5.2.1 Variance function of the Difference for run 2

Taking the expression of (5.11) and that of (5.13) to be the points described in $\boldsymbol{z} = \mathbf{x_r}$ and $\boldsymbol{x} = \mathbf{x_q}$ of (5.3) and (5.5) respectively, then the variance in (5.17) for this specific test run is computed for $\boldsymbol{d} = \boldsymbol{z} - \boldsymbol{x}$ as follows;

$$\boldsymbol{d} = (\,\mathbf{0} \quad \mathbf{-0.1312} \quad \mathbf{0.0500} \quad \mathbf{-0.2189} \quad \mathbf{-0.0175} \quad \mathbf{0.0647}\,) \tag{5.28}$$

$$\boldsymbol{V_d} = (\,\mathbf{0} \quad \mathbf{-0.1312} \quad \mathbf{0.0500} \quad \mathbf{-0.2189} \quad \mathbf{-0.0175} \quad \mathbf{0.0647}\,)$$

$$\times \begin{pmatrix} \mathbf{51} & \mathbf{0} & \mathbf{-15} & \mathbf{34} & \mathbf{39} & \mathbf{0} \\ \mathbf{0} & \mathbf{34} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{-10} \\ \mathbf{-15} & \mathbf{0} & \mathbf{39} & \mathbf{-10} & \mathbf{-15} & \mathbf{0} \\ \mathbf{34} & \mathbf{0} & \mathbf{-10} & \mathbf{34} & \mathbf{26} & \mathbf{0} \\ \mathbf{39} & \mathbf{0} & \mathbf{-15} & \mathbf{26} & \mathbf{39} & \mathbf{0} \\ \mathbf{0} & \mathbf{-10} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{26} \end{pmatrix}^{-1} \begin{pmatrix} \mathbf{0} \\ \mathbf{-0.1312} \\ \mathbf{0.0500} \\ \mathbf{-0.2189} \\ \mathbf{-0.0175} \\ \mathbf{0.0647} \end{pmatrix}$$

$$= \mathbf{0.0049.} \tag{5.29}$$

This is the variance of the difference between two estimated responses for a second order model from the test run involving a herbal medicine extracted from medicinal mushrooms.

5.2.2 Variance function of the Difference for run 3

Using the same approach as used in subsection 5.2.1 above, we have the two points described in $\mathbf{x_t}$ and $\mathbf{x_h}$ of (5.7) and (5.9) to take the expressions of (5.11) and that of (5.13) respectively, whose difference can be expressed as $\boldsymbol{c} = \boldsymbol{z} - \boldsymbol{x}$. With these replacements, the variance function of the difference for run 3 is determined as follows:

$$\boldsymbol{c} = (\,\mathbf{0} \quad \mathbf{-0.1514} \quad \mathbf{0.5289} \quad \mathbf{-1.1883} \quad \mathbf{-1.8359} \quad \mathbf{2.3383}\,) \tag{5.30}$$

$$\boldsymbol{V_c} = (\,\mathbf{0} \quad \mathbf{-0.1514} \quad \mathbf{0.5289} \quad \mathbf{-1.1883} \quad \mathbf{-1.8359} \quad \mathbf{2.3383}\,)$$

$$\times \begin{pmatrix} \mathbf{40} & \mathbf{1} & \mathbf{11} & \mathbf{27} & \mathbf{67} & \mathbf{2} \\ \mathbf{1} & \mathbf{27} & \mathbf{2} & \mathbf{1} & \mathbf{4} & \mathbf{8} \\ \mathbf{11} & \mathbf{2} & \mathbf{67} & \mathbf{8} & \mathbf{89} & \mathbf{4} \\ \mathbf{27} & \mathbf{1} & \mathbf{8} & \mathbf{27} & \mathbf{46} & \mathbf{2} \\ \mathbf{67} & \mathbf{4} & \mathbf{89} & \mathbf{46} & \mathbf{223} & \mathbf{8} \\ \mathbf{2} & \mathbf{8} & \mathbf{4} & \mathbf{2} & \mathbf{8} & \mathbf{46} \end{pmatrix}^{-1} \begin{pmatrix} \mathbf{0} \\ \mathbf{-0.1541} \\ \mathbf{0.5289} \\ \mathbf{-1.1883} \\ \mathbf{-1.8359} \\ \mathbf{2.3383} \end{pmatrix}$$

$$= \mathbf{0.4840.} \tag{5.31}$$

This is the variance of the difference between two estimated responses from the test involving the herbal medicine extracted from the herbal formula.

5.3 The Difference of the Variance functions of two Estimated Responses

In this case we worked out the variance function of the estimated responses for two points in subsection 5.1.1 and 5.1.2 for which we can now find the difference of these variances for the two test in this research work.

5.3.1 The Difference of the Variance function for run 2

Let $\boldsymbol{V_e} = \boldsymbol{V_q} - \boldsymbol{V_r}$ be the difference in the variance functions computed for two points with conditions on their individual computations as outlined there above. With $\boldsymbol{V_q}$ and $\boldsymbol{V_r}$ as given in (5.4) and (5.6) for the points in (5.3) and (5.5) respectively, the difference of the variances $\boldsymbol{V_e}$ is given for the test run 2 as,

$$\boldsymbol{V_e} = \mathbf{0.1038 - 0.0987 = 0.0051} \tag{5.32}$$

5.3.2 The Difference of the Variance function for run 3

Let $\boldsymbol{V_f = V_h - V_t}$ be the difference in the variance with $\boldsymbol{V_t}$ and $\boldsymbol{V_h}$ as given in (5.9) and (5.10) for the points in (5.7) and (5.9) respectively, then $\boldsymbol{V_f}$ for the test run 3 is computed to be,

$$\boldsymbol{V_f = 30.3998 - 24.8193 = 5.5805} \tag{5.33}$$

5.4 Comparison of results in sections 5.2 and 5.3

Equation (5.32) shows the variance of the difference, while (5.33) shows the difference of the variance between two estimated responses for a second order model for the herbal extract drug from medicinal mushrooms and from six different herbs respectively. These results can be used as a basis of identifying the operation region within which the factors t̂ime and *concentration* are more effect by considering the minimum value from a the stationary point and other points on the same response surface.

The desire of every experimental setting in response surface is that of minimizing the variance function be it of the difference between two estimated response or the difference of the variance functions.

With this regard we find that equation (5.29) and (5.32) are the variance function of the difference between two estimated responses and the difference of the variance functions between two estimated responses for the herbal drug extract from medicinal mushrooms respectively. However by selecting the one that provides a minimum we emphasize that the variance function of the difference between two estimated responses should be used in selecting an optimal model in the effective management of diabetes using herbal drug extract from medicinal mushrooms. These results also hold true for the herbal formula extract from six herbs, in which equation (5.31) is a minimum compared to equation (5.33).

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

The desire of any experimenter is that of describing how the response varies as a function of the various treatments combinations as well as determining treatment leves that give optimal responses. Generally factorial experiments (factorial - treatment structures) can be utilized. However when treatment factors are subject to variation across a continuous range of values of the variables, other treatment designs may be more efficient and effective of which Response surface method is considered suitable when finding optimum or describing the response function.

In this research, response surface designs have been used to map the response surface within which the effectiveness of ascertained herbal drug is optimum in regulating the blood sugar level for a diabetic. The tests run using albino rats have successfully provided evidence of the effectiveness of the herbal drug to the treatment of type 2 diabetes. The research has considered two factors of interest, *time* taken to reduce blood sugar level and *concentration* of the herbal drug used. Using the two factors we have been able to map the response surface as the area of operation in management of a diabetic.

This research has successfully undertaken the analysis of stationary points by providing a method of finding the stationary point relating to a minimum. The values generated for the two test runs can be used to give a setting for the utilization by herbalists as a starting point in working the concentration of herbal medicine and with which they have the knowledge of the time it takes to reduce the blood sugar

level for a diabetic patient in the course of treatment. This gives the predictive aspect by the herbalists to be able to ascertain the concentration to use as well as the time it takes to achieve desired results.

In this research work we have been able to successfully utilize response surface methodology to come up with a clear model of the relationship involving time and concentration as factors of a herbal medicine to the blood sugar level of a diabetic by setting up a clinical trial for the arrangement of the variables using albino rats that were carefully considered at the ages of six to eight weeks.

In the test run 2, we have successfully shown that the herbal medicine extracted from medicinal mushrooms should be used at a concentration of 46.2492 mg/dl to maintain the blood sugar level at 9.4353 mMol/L, within 136.1304 minutes from the time of administering the drug to a diabetic.

From the test run 3 the herbal formula extract from a mix of six herbs have been shown in this research to have successfully regulated the blood sugar level in a diabetic to11.3898 mMol/L. This is possible by effecting a treatment of herbal formula at a concentration of 66.1125 mg/dl and the effectiveness is within 175.4580 minutes upon treatment.

Graphics and visualization techniques are some of our best tools for understanding response surfaces, for which this research work has utilized in the expounding of the nature and shape of the response surface generated from fitting a first and a second order model for the two test trials. We thus worked with models for the response and visualize the blood sugar level as a surface of heights over the time $\boldsymbol{x_1}$, herbal concentration $\boldsymbol{x_2}$ plane, like a relief map showing mountains and valleys. There are different perspective plots showing the surface when viewed from different orientations especially for the second order model in both the trials. Contour plots showing the contours of the surface, that is, curves of $\boldsymbol{x_1}$, $\boldsymbol{x_2}$ pairs that have the same response value were generated which depicts the pattern and nature of the combination of the predictive factors, time and concentration of the herbal drug.

The research has been used to find the variance function for the estimated response at a stationary point as well as other points on the response surface. The research has shown that any other point picked that is higher than the stationary

point but on the surface generated by the factors space and the response, yielded a higher variance value for which we verify that the stationary point yields the best possible value of combination of the factors *time* and *concentration* of herbal medicine to give the desired response in terms of the blood sugar level. Thus we are able to discriminate between points on the variable space that we can assert gives the best response thus a way of identifying the best model via the variance function has been utilized and found valid.

The variance function of the difference between two estimated responses was computed, as well as the difference of the variance function for two estimated responses for the two test runs. The comparison of the results of the variance function of the difference between two estimated responses and the difference of the variance function between two estimated responses shows that the first is smaller than the second. Therefore we can use the variance function of the difference between two estimated response to be able to map the range over which we can vary the factors of interest, *time* and *concentration* of herbal medicine to achieve blood sugar level that is within the acceptable range.

6.2 Recommendations

Drug as a relief from an identified condition affect different people differently, especially with regard to gender or age of a recipient. The current work has concentrated on two explanatory variables, *time* and herbal drug *concentration*. There is need to investigate other variables using response surface. New variables may be obtained which may help herbal medicine practionners. This challenge can be an investigation that can yield patterns that might be considered important in the effective treatment and management of diabetes whenever the different sexes are concerned.

Herbalists extract the herbal drug from the different parts of the plant basically by boiling. However the boiling time as well as the temperature at which this boiling is done is undefined in most cases. The current work undertook the extraction of the herbal medicine by following laid out laboratory procedures which might

not be readily available to most if not all herbalists. We therefore recommend a consideration of the exploration into the traditional methods of extraction of the herbal drugs to map the temperature at which the boiling will effectively provide a concentration that is within the range of managing the diabetic condition as well as recommend the amount of time it should take.

Use of a drug may sometimes result to reaction by the body chemical composition. This reaction would be positive if the desired goal is achieved. In the event that we have been able to arrest or eradicate a condition, then the treatment exercise is considered successful. However, the treatment may result in changes in the chemical composition of the body and sometimes the side effects might be more serious to treat than the original ailment. Therefore there is need to investigate the side effects that may arise in the treatment of diabetes by use of these herbs. This way there might be a possibility of investigating the extent to which the concentration may be varied in order to achieve desired results with safety in mind.

In any mix of components our interest to come up with an optimum from the different ingredients used. On the basis of test run 3, we do recommend an investigation into the possible variation of the mix of the six her herbs to evaluate by use of the response surface methodology the best possible mix that is efficient in the treatment and management of diabetes. This will go a long way in setting the standards and procedures of the herbal formula extract for cooperating herbalist.

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The difference of the variance functions between two estimated responses for a fourth order rotatable design in two dimensions

F. Njui, G.P. Pokhariyal. and A. Karanjah

Abstract. In this paper the difference of the variance functions between two estimated responses for a fourth order design at any two points in the factor space is developed. In particular, the variance function is considered in two dimensions when the design used is rotatable. The variance function in this situation is a function of the distances of the points from the origin of the design and the angle subtending the points at the origin. The variance function of this approach is discussed in detail when the two points are equidistant from the origin of the design. The criterion for the choice of an optimal design is given.

M.S.C. 2000: 62K15, 62K20.
Key words: variance functions, estimated responses, rotatable design.

1 Introduction

It is often seen that the difference between estimated responses at two points for a phenomena is a greater interest as compared to the actual response. The variance function and the difference of variances of two estimated responses assist in providing further insight about the criterion under investigation. Herzberg (1967) described the variance function depending on the length of the straight line joining the selected points to the origin and the angle between these two lines. The assumption of rotatability in design helps in determining the appropriate form for the product of the design matrix and its transpose (Box and Draper, 1980).

Huda and Mukerjee (1984) derived optimal design under the criterion for second order polynomial models when the design space is spherical in nature.

Gilmour (2006) provided the summary of use of response surface methodology (RSM) in various biological inductions and discussed in details the applications of RSM to experiments on biotechnological processes. The utility of subset designs is highlighted. In this paper the difference of variance functions between two estimated responses for a fourth order rotatable design has been studied. The extent to which the angle between the lines can be varied is determined.

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2 Fourth order rotatable model

Consider the problem in response surface designs for investigating the relationship between a responce y and two explanatory factors, say x_1 and x_2. Assuming all factors to be continuous in nature and the form of the functional relationship between them as unknown but within the range of interest, such that the function may be represented by a polynomial of moderately low order. In particular, we chose the combinations of levels of independent factors which will:

(i) enable an experimenter to approximate a functional relationship by fitting a polynomial through the terms of order four, and

(ii) have the property of rotatability.

Such a choice of combination of the various levels of the independent factors will provide a fourth order rotatable design.
Let us consider a general model

$$y_i = f'(x_i)\beta + \epsilon_i \tag{2.1}$$

whose matrix equivalent is,

$$Y = X'\beta + \varepsilon \tag{2.2}$$

where, Y is an $(n \times 1)$ vector of observations,
X is an $(n \times k)$ design matrix,
β is a $(k \times 1)$ vector of unknown parameters, and
ε is a $(n \times 1)$ vector of independently identically distributed random variables with mean zero and variance σ^2.

Specifically, for N observations let y_u be the response at the u^{th} run, for a polynomial equation of order four this maybe written as

$$\begin{aligned} y_u &= \beta_0 x_{0u} + \sum_{i=1}^{k} \beta_i x_{iu} + \sum_{i=1}^{k} \beta_{ii} x_{iu}^2 + \sum_{i=1}^{k}\sum_{j=1}^{k} \beta_{ij} x_{iu} x_{ju} \\ &+ \sum_{i=1}^{k}\sum_{j=1}^{k}\sum_{l=1}^{k} \beta_{ijl} x_{iu} x_{ju} x_{lu} + \sum_{i=1}^{k}\sum_{j=1}^{k}\sum_{l=1}^{k}\sum_{r=1}^{k} \beta_{ijlr} x_{iu} x_{ju} x_{lu} x_{ru} + \varepsilon_u \end{aligned}$$

where, $\varepsilon_u \sim N(0, \sigma^2)$, $Cov(\varepsilon_u \varepsilon'_u) = 0,\ u \neq u' = 1, 2, \cdots, N$. The expectation of the response at the u^{th} run is given by $E[y_u] = x'_u\beta$. The estimated response is given by $\hat{y} = X'\hat{\beta}$, with matrix $X = (x_1, x_2, \cdots, x_N)'$ of order $N \times L^*$ (see appendix) and $\hat{\beta}$ is the least square estimate of β.

The algebra of estimating β is involving and tedious, therefore we make use of *Schlafflian Vectors* and *Matrices* to estimate β (([1]). For $k = 2$, $x' = (x_1, x_2)$, and defining the vector $x^{[4]}$, we use the expanded results with,

$$\begin{aligned} \mathbf{x}^{[4]'} x^{[4]} &= x_0^8 + x_1^8 + x_2^8 + 4[x_0^6 x_1^2 + x_0^6 x_2^2 + x_1^6 x_2^2 + x_0^2 x_1^6 + x_0^2 x_2^6 + x_1^2 x_2^6] \\ &+ 6[x_0^4 x_1^4 + x_0^4 x_2^4 + x_1^4 x_2^4] + 12[x_0^4 x_1^2 x_2^2 + x_0^2 x_1^4 x_2^2 + x_0^2 x_1^2 x_2^4], \end{aligned}$$

(2.3)

which implies that

$$\begin{aligned}\mathbf{x}^{[4]'} = \quad & [x_0^8, x_1^8, x_2^8, 2(x_0^3x_1, x_0^3x_2, x_1^3x_2, x_0x_1^3, x_0x_2^3, x_1x_2^3),\\ & \sqrt{6}(x_0^2x_1^2, x_0^2x_2^2, x_1^2x_2^2), 2\sqrt{3}(x_0^2x_1x_2, x_0x_1^2x_2, x_0x_1x_2^2)],\end{aligned}$$

(2.4)

and the parameter β is expressed as

$$\begin{aligned}\beta' = \quad & [\beta_0, \beta_1, \beta_2, \beta_{11}, \beta_{22}, \beta_{12}, \beta_{111}, \beta_{222}, \beta_{112},\\ & \beta_{122}, \beta_{1111}, \beta_{2222}, \beta_{1122}, \beta_{1112}, \beta_{1222}].\end{aligned} \tag{2.5}$$

Applying the model given in (2.1), and Schlafflian vectors approach, the least square estimate is given by

$$\hat{\beta} = (\sum_{u=1}^{N} x^{[4]'}x^{[4]})^{-1}x^{[4]'}y. \tag{2.6}$$

The estimated response $\hat{y}_u$ at any point is given by $\hat{y}_u = x_u^{[4]'}\hat{\beta}$, and the variance of the estimated response will be given by

$$Var(\hat{y}_u) = x_u^{[4]'}\ Var(\hat{\beta})\ x_u^{[4]} = x_u^{[4]'}\ (X'X)^{-1}\ x_u^{[4]}\sigma^2\ . \tag{2.7}$$

We generate vectors D_1, D_2 and D_3, to workout the moment matrix with two predictor variables ([1]). For the design with two predictor variables we write,

$$N^{-1}(X'X) = N^{-1}\sum_{u=1}^{N} x^{[4]}x^{[4]'} = N^{-1}\sum_{=1}^{N}\begin{pmatrix} D_1 \\ D_2 \\ D_3 \end{pmatrix}[D'_1, D'_2, D'_3]$$

(2.8)

Therefore the moment matrix is given by

$$N^{-1}(X'X) = \sum_{u=1}^{N}\begin{pmatrix} D_1D_1' & D_1D_2' & D_1D_3' \\ D_2D_1' & D_2D_2' & D_2D_3' \\ D_3D_1' & D_3D_2' & D_3D_3' \end{pmatrix}$$

(2.9)

Our focus lies on the main diagonal of (2.9) since the off diagonals elements will be 0 with regard to conditions of rotability. That is to say

$$N^{-1}(X'X) = diag\sum_{u=1}^{N}[D_1D_1'\ \ D_2D_2'\ \ D_3D_3'] \tag{2.10}$$

The final form of the moment matrix obtained will be ([1]),

$$N^{-1}(X'X) = diag[\ B\ M\ L\ ]. \tag{2.11}$$

3 Parameter estimates

In order to obtain the paramenter estimate ($\hat{\beta}$) we consider the expression for the inverse of the matrix $X'X$, by rewriting (2.11) as

$$(X'X) = N\ diag[\ B\ \ M\ \ L\ ] \tag{3.1}$$

whose inverse will be of the form

$$(X'X)^{-1} = N^{-1} diag\ [B^{-1}\ \ M^{-1}\ \ L^{-1}], \tag{3.2}$$

obtained by working out the inverses of B, M and L ([1]). Using (2.11) in (2.6) we have

$$\hat{\beta} = N^{-1}\ \ diag\ [B^{-1}\ \ M^{-1}\ \ L^{-1}]\ X'y \tag{3.3}$$

using $x^{[4]'}$ of (4) in (14) we get

$$\hat{\beta} = N^{-1}\ \ diag\ [B^{-1}\ \ M^{-1}\ \ L^{-1}] \begin{pmatrix} D_1 \\ D_2 \\ D_3 \end{pmatrix} y = \begin{pmatrix} \hat{\beta}_{*1} \\ \hat{\beta}_{*2} \\ \hat{\beta}_{*3} \end{pmatrix} \tag{3.4}$$

where

$$\hat{\beta}_{*1} = [\hat{\beta}_0, \frac{1}{\sqrt{6}}\hat{\beta}_{11}, \frac{1}{\sqrt{6}}\hat{\beta}_{22}, \hat{\beta}_{1111}, \hat{\beta}_{2222}, \frac{1}{\sqrt{6}}\hat{\beta}_{1122}]' \tag{3.5}$$

$$\hat{\beta}_{*2} = [\frac{1}{2}\hat{\beta}_1, \frac{1}{2}\hat{\beta}_2, \frac{1}{\sqrt{12}}\hat{\beta}_{122}, \frac{1}{\sqrt{12}}\hat{\beta}_{112}, \frac{1}{2}\hat{\beta}_{111}, \frac{1}{2}\hat{\beta}_{222}]' \tag{3.6}$$

$$\hat{\beta}_{*3} = [\frac{1}{2}\hat{\beta}_{1112}, \frac{1}{2}\hat{\beta}_{1222}, \frac{1}{\sqrt{12}}\hat{\beta}_{12}]' \ . \tag{3.7}$$

The main interest is that of finding the estimates of the coefficients of the general mean and the linear factors β_0, β_1 and β_2 ([1]).

4 The estimated response

The estimated response $\hat{y}_u$ at a point $(x_{0u},\ x_{1u},\ x_{2u})$ from a general situation will be

$$\hat{y}_u = x_u^{[4]'}\hat{\mu}, \tag{4.1}$$

our focus being that of the coefficients of the main effects only where $x_u^{[4]'}$ is as provided in (2.4) and μ giving our parameter system of interest given as $\mu = J'\beta$ [for μ and J see paper1].

Using equation (4.1) we get

$$\hat{y}_u = x_u^{[4]'} J' \hat{\beta} = [D_1' \ D_2' \ D_3'] diag[\ R \ S \ T\ ] \hat{\beta} = [D_1' R \quad D_2' S \quad D_3' T] \hat{\beta} \tag{4.2}$$

we get the expression for the estimated response of a fourth order rotatable design in two dimensions as,

$$\hat{y}_u = \hat{\beta}_0 \sum_{u=1}^{N} x_{0u}^4 + \hat{\beta}_1 \sum_{u=1}^{N} x_{1u}^4 + \hat{\beta}_2 \sum_{u=1}^{N} x_{2u}^4, \tag{4.3}$$

with the variance of estimated response being constant ([1]).

5 Difference of the variance functions of two estimated responses

Suppose that x_a' and x_b' are two distinct points identified on the two response surface of different radii. The two points are given as

$$\hat{y}(x_a') = x_a' \hat{\beta} \ , \qquad \hat{y}(x_b') = x_b' \hat{\beta} \tag{5.1}$$

where $\hat{\beta}$ is the Least Square Estimate of β. The standardized variance of these two estimated responses will be

$$V_a = x_a'(X'X)^{-1} x_a \ , \qquad V_b = x_b'(X'X)^{-1} x_b \tag{5.2}$$

With reference to a rotatable design, $X'X$ has a special form, Box and Hunter (1957). Taking into consideration equation (2.4) where now $x_a' = (\rho_1, 0, 0, ..., 0)$ is taken as a vector of order (15×1) of a row of the design matrix X arising from a point in the predictor variable space. If we express the vector as;

$$x_a' = [d_1' \ \ d_2' \ \ d_3'] \tag{5.3}$$

where

$d_1' = (\rho_1, \ 0, \ 0, \ 0, \ 0, \ 0)$, $d_2' = (0, \ 0, \ 0, \ 0, \ 0, \ 0)$ and $d_3' = (0, \ 0, \ 0)$

then the standardized variance function of the estimated response at x_a' will be given as

$$\begin{aligned} V_a &= x_a' \ J'(X'X)^{-1} J \ x_a \\ &= d_1' \ R' \ B^{-1} R \ d_1 \ + \ d_2' \ S' \ M^{-1} \ S \ d_2 \ + \ d_3 \ T' \ L^{-1} T \ d_3 \\ &= \rho_1^2 \triangle^{-1} \ S_0 = \frac{24 \rho_1^2 \beta_1^*}{\mu^*} \end{aligned} \tag{5.4}$$

which on substituting the values of β_1^*, μ^* and k gives

$$V_a = \frac{24\rho_1^2[8\lambda_4\lambda_8 - 6\lambda_6^2]}{24[8\lambda_4\lambda_8 - 6\lambda_6^2] - 12\lambda_2[8\lambda_2\lambda_8 - 4\lambda_4\lambda_6] + 8\lambda_4[6\lambda_2\lambda_6 - 4\lambda_4^2]} \tag{5.5}$$

Let

$$x_b' = [d_1^{*'}\ d_2^{*'}\ d_3^{*'}] \tag{5.6}$$

be a vector of order (15×1) of a row of the design matrix X arising from a point in the predictor variable space. We observe that this is a particular point on the response surface which must not be along the axes of the predictor variable space. However the vector makes an angle θ with the axis x_1 where
$d_1^{*'} = (\rho_2\cos\theta,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0)$, $d_2^{*'} = (\rho_2\sin\theta,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0)$, and $d_3^{*'} = (0,\ 0,\ 0)$
then the standardized variance of the estimated response at x_b will be expressed as

$$\begin{aligned} V_b &= x_b'\ J'(X'X)^{-1}J\ x_b \\ &= d_1^{*'}\ R'\ B^{-1}R\ d_1^* \ +\ d_2^{*'}\ S'\ M^{-1}\ S\ d_2^* \\ &= \frac{\rho_2^2\cos^2\theta\beta_1^*}{\mu^*} + \rho_2^2\sin^2\theta\beta_3^{*^{-1}}\alpha \end{aligned}$$

(5.7)

which on substituting the values of β_1^*, β_3^*, μ^*, α and k we have

(5.8)

$$V_b = \frac{24\rho_2^2\cos^2\theta[8\lambda_4\lambda_8 - 6\lambda_6^2]}{24[8\lambda_4\lambda_8 - 6\lambda_6^2] - 12\lambda_2[8\lambda_2\lambda_8 - 4\lambda_4\lambda_6] + 8\lambda_4[6\lambda_2\lambda_6 - 4\lambda_4^2]} + \frac{\frac{3}{2}\lambda_6\rho_2^2\sin^2\theta}{6\lambda_2\lambda_6 - 4\lambda_4^2}$$

With reference to conditions of rotatability we have
$\omega_1 = 24[8\lambda_4\lambda_8 - 6\lambda_6^2]$
$\omega_2 = 12\lambda_2[8\lambda_2\lambda_8 - 4\lambda_4\lambda_6]$
$\omega_3 = 8\lambda_4[6\lambda_2\lambda_6 - 4\lambda_4^2]$
then

$$V_a = \frac{\omega_1\rho_1^2}{\omega_1 - \omega_2 + \omega_3} \tag{5.9}$$

and

$$V_b = \frac{\omega_1\rho_1^2\cos^2\theta}{\omega_1 - \omega_2 + \omega_3} + \frac{\frac{3}{2}\lambda_6\rho_2^2\sin^2\theta}{[6\lambda_2\lambda_6 - 4\lambda_4^2]} \tag{5.10}$$

The difference of the variance functions of the two estimated responses will be

$$V_c = V_a - V_b = \frac{\omega_1(\rho_1^2 - \rho_1^2\cos^2\theta)}{\omega_1 - \omega_2 + \omega_3} - \frac{\frac{3}{2}\rho_2^2\sin^2\theta}{[6\lambda_2\lambda_6 - 4\lambda_4^2]} \quad , \tag{5.11}$$

which is a function of θ.

6 Discussion

The results in (5.11) can optimized by finding the first order condition and solving for θ. After which we explore the second order condition to evaluate the nature of the function.

Suppose we let

$$\frac{\omega_1}{\omega_1 - \omega_2 + \omega_3} = h_1$$

and

$$\frac{\frac{3}{2}\lambda_6}{[6\lambda_2\lambda_6 - 4\lambda_4^2]} = \frac{12\lambda_4\lambda_6}{\omega_3} = h_2,$$

which we use to re-expressed (5.11) as,

$$V_c = h_1(\rho_1^2 - \rho_1^2\cos^2\theta) - h_2\rho_2^2\sin^2\theta. \tag{6.1}$$

The first order of condition of (6.1) will be

$$f_1'(\theta) = \frac{\partial V_c}{\partial\theta} = 2h_1\rho_2^2\cos\theta\sin\theta - 2h_2\rho_2^2\cos\theta\sin\theta = 0,$$

on solving we get

$$\theta = \{0, 90\}. \tag{6.2}$$

With regard to rotatability we only need to evaluate θ for values of $0^o \leq \theta \leq 90^o$ since by rotating the points around the sphere the angle remains invariant as well as first quadrant can be used to give values in other quadrants. The second derivative of (6.1) will be

$$f_c''(\theta) = \frac{\partial^2 V_c}{\partial\theta^2} = 2[h_1 - h_2]\rho_2^2[1 - 2\sin^2\theta] \tag{6.3}$$

On substitution for values of θ from the set in (6.2) we have two conditions;

(i) for $\theta = 0^o$

$$f_c''(\theta) = 2[h_1 - h_2]\rho_2^2[1 - 0] = 2[h_1 - h_2]\rho_2^2$$

By letting $\lambda_2 < \frac{1}{k}$ and $\lambda_4 \leq \frac{\lambda_2}{k+2}$ from the results of Huda and Mukerjee (1984), while evaluating the values of λ_6 and λ_8 with regard to conditions and same procedures we have, $\lambda_2 < \frac{1}{2}$, $\lambda_4 = \frac{\lambda_2}{4}$;on considering the equality, $\lambda_2\lambda_6 = \frac{\lambda_4}{k+4}$; therefore $\lambda_6 = \frac{\lambda_4}{6\lambda_2}$ and $\lambda_4\lambda_8 = \frac{\lambda_6}{k+6}$ thus $\lambda_8 = \frac{\lambda_6}{8\lambda_4}$ We therefore need only evaluate the values of λ_2 say λ_2^* in order to compute h_1 and h_2. For $\lambda_2^* = 0.4396$ from the results of Huda and Mukerjee we find that

$$h_1 = 34.76694889, \quad h_2 = 1.014808732 \tag{6.4}$$

hence if the vectors are equidistant and the distance is unitary then,

$$f_c''(\theta) = 2[h_1 - h_2]\rho_2^2 = 67.5042805 > 0^o \tag{6.5}$$

which implies that the difference of the variance functions for two estimated responses is minimized when $\theta = 0^o$.

(ii) for $\theta = 90^o$ we have

$$f_c''(\theta) = 2[h_1 - h_2]\rho_2^2[1 - 2\sin 90^o] = -67.50572314 < 0^o \tag{6.6}$$

which implies that the difference of the variance functions for two estimated responses is maximized when $\theta = 90^o$.
We now evaluate the extent to which the angle θ can be varied while still minimizing the functions in V_c of (6.1). We tabulate the results as follows;

Table of Values

θ	$f_c''(\theta)$	θ	$f_c''(\theta)$
0	67.5042805	44	2.355865415
5	66.4787388	45	0
10	63.43327426	46	-2.355865415
15	58.46042178	47	-4.70886057
20	51.71127896	48	-7.056118705
25	43.39091511	49	-9.394780045
30	33.75214025	50	-11.72199529
35	23.08782369	55	-23.08782369
40	11.72199529	60	-33.75214025
41	9.394780045	70	-51.71127896
42	7.056118705	80	-63.43327426
43	4.70886057	90	-67.5042805

Where, $f_c''(\theta)$ is the second derivative of the function V_c, that is the difference of the variance functions of two estimated responses.

7 Conclusions

The aim of every experimenter is to minimize the variance function, therefore we conclude that for the difference of the variance functions for two estimated responses we may achieve a global minimum while others exogenous factors assumed constant by letting the angle between the two vectors θ to be as close as possible to 45^o.

If differences of points close together in the factor space are involved, based on our results, an optimal design for a fourth order rotatable design in two dimensions from the this approaches will be chosen on the basis of minimum variance function criterion as emphasized by Herzberg (1967), Box and Draper (1980), Huda (1985) and Huda and Mukerjee (1984).

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Authors' address:

F. Njui, G.P. Pokhariyal. and A. Karanjah
School of Mathematics, University of Nairobi,
P.O. Box 30197-00200, Nairobi, KENYA.
E-mail: framcis.njui@uonbi.ac.ke, pokhariyal@uonbi.ac.ke, karanjah@yahoo.com

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THE VARIANCE FUNCTION OF THE DIFFERENCE BETWEEN TWO ESTIMATED RESPONSES FOR A FOURTH ORDER ROTATABLE DESIGN IN TWO DIMENSIONS

A. KARANJAH, F. NJUI and G. P. POKHARIYAL

School of Mathematics
University of Nairobi
P. O. Box 30197-00200, Nairobi
Kenya
e-mail: pokhariyal@uonbi.ac.ke

Abstract

In this paper the variance function of the difference between two estimated responses for a fourth order design at any two points in the factor space is developed. Particularly, the variance function is considered in two dimensions when the design used is rotatable. The variance function in this situation is a function of the distances of the points from the origin of the design and the angle subtending the points at the origin. Variance functions of this approach are discussed when the two points are equidistant from the origin of the design. The criterion for the choice of an optimal design is given.

1. Introduction

It has been observed that, by fitting response surface to data, researchers are often more interested in the difference between the

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estimated responses at two points rather than in the actual response at an individual location. It is seen that, the variance function of that difference is obviously of interest.

A case of interest is when the design is rotatable, in which case the product of design matrix and its transpose has a special form (Box and Hunter [2]). Herzberg [5] showed that the variance of difference is invariant under orthogonal rotation in the prediction space.

Herzberg [5] outlined the variance function of the difference between two estimated responses for a first order and a second order rotatable design. Box and Draper [1] reviewed Herzberg's [5] work and gave some additional features of the variance function of the difference between two estimated responses for a first, second and third order rotatable designs. Huda and Mukerjee [6] studied minimization of the variance of the difference between estimated responses at two points maximized over all pairs of points in the design space, taken as the criterion for selecting designs.

Gilmour [4] introduced a much wider class of three level designs, which easily allows a few two and four level factors to be included and provided the practical advice on application. In this paper the variance function of the difference between two estimated responses for a fourth order rotatable design in two dimensions has been studied and the criterion for the choice of an optimal design is also given.

2. Fourth Order Rotatable Model

We consider the problem in response surface designs for investigating the relationship between a dependent and two independent factors, say x_1 and x_2. All factors are assumed to be continuous in nature and the form of the functional relationship between them is unknown but within the range of interest, such that the function may be represented by a polynomial of moderately low order. In particular, we choose the combinations of levels of independent factors which:

(i) enable an experimenter to approximate a functional relationship by fitting a polynomial through the terms of order four, and

(ii) have the property of rotatability.

Such a choice of combination of the various levels of the independent factors provides a fourth order rotatable design. Let us consider the model

$$y_i = f'(x_i)\beta + \varepsilon_i \tag{1}$$

whose matrix equivalent is

$$Y = X\beta + \varepsilon, \tag{2}$$

where Y is an $(n \times 1)$ vector of observations, X is an $(n \times k)$ design matrix, β is a $(k \times 1)$ vector of unknown parameters, and ε is an $(n \times 1)$ vector of independently identically distributed random variables with mean zero and variance σ^2.

Specifically, for N observations let y_u be the response at the uth run, for a polynomial equation of order four this maybe written as

$$y_u = \beta_0 x_{0u} + \sum_{i=1}^{k} \beta_i x_{iu} + \sum_{i=1}^{k} \beta_{ii} x_{iu}^2 + \sum_{i=1}^{k}\sum_{j=1}^{k} \beta_{ij} x_{iu} x_{ju}$$

$$+ \sum_{i=1}^{k}\sum_{j=1}^{k}\sum_{l=1}^{k} \beta_{ijl} x_{iu} x_{ju} x_{lu} + \sum_{i=1}^{k}\sum_{j=1}^{k}\sum_{l=1}^{k}\sum_{r=1}^{k} \beta_{ijlr} x_{iu} x_{ju} x_{lu} x_{ru} + \varepsilon_u,$$

where $\varepsilon_u \sim N(0, \sigma^2)$, $\mathrm{Cov}(\varepsilon_u \varepsilon_{u'}') = 0$, $u \neq u' = 1, 2, \ldots, N$. The expectation of the response at the uth run is given by $E[y_u] = \mathbf{x}_u'\beta$. The estimated response is given by $\hat{y} = X\hat{\beta}$, with matrix $X = (\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_N)'$ of order $N \times L^*$ with

$$L^* = \binom{k+4}{4}$$

and $\hat{\beta}$ is the least square estimate of β.

The algebra of estimating β seems to be involving and tedious, therefore we make use of *Schlafflian Vectors* and *Matrices* to estimate β in the following manner.

For $k = 2$, let $\mathbf{x}' = (x_1, x_2)$. Then we define the vector $\mathbf{x}^{[4]}$ such that,

$$\mathbf{x}^{[4]'}\mathbf{x}^{[4]} = [\mathbf{x}'\mathbf{x}]^4$$

$$= [x_0^2 + x_1^2 + x_2^2]^4$$

$$= \sum \frac{p!}{p_0!\, p_1!\, p_2!} (x_0^2)^{p_0} (x_1^2)^{p_1} (x_2^2)^{p_2}, \tag{3}$$

where summations are taken over p_0, p_1, p_2, and $p_0 + p_1 + p_2 = 4$.

Expanding equation (3), we get

$$\mathbf{x}^{[4]'}\mathbf{x}^{[4]} = x_0^8 + x_1^8 + x_2^8 + 4[x_0^6 x_1^2 + x_0^6 x_2^2 + x_1^6 x_2^2 + x_0^2 x_1^6 + x_0^2 x_2^6 + x_1^2 x_2^6]$$

$$+ 6[x_0^4 x_1^4 + x_0^4 x_2^4 + x_1^4 x_2^4] + 12[x_0^4 x_1^2 x_2^2 + x_0^2 x_1^4 x_2^2 + x_0^2 x_1^2 x_2^4] \tag{4}$$

This implies that

$$\mathbf{x}^{[4]'} = [x_0^8, x_1^8, x_2^8, 2(x_0^6 x_1, x_0^6 x_2, x_1^6 x_2, x_0 x_1^6, x_0 x_2^6, x_1 x_2^6),$$

$$\sqrt{6}(x_0^4 x_1^4, x_0^4 x_2^4, x_1^4 x_2^4), 2\sqrt{3}(x_0^4 x_1^2 x_2, x_0^4 x_1 x_2^2, x_0^2 x_1^4 x_2, x_0^2 x_1 x_2^4)] \tag{5}$$

The parameter β is expressed as

$$\beta' = [\beta_0, \beta_1, \beta_2, \beta_{11}, \beta_{22}, \beta_{12}, \beta_{111}, \beta_{222}, \beta_{112}, \beta_{122}, \beta_{1111},$$

$$\beta_{2222}, \beta_{1122}, \beta_{1112}, \beta_{1222}] \tag{6}$$

Using the model given in (1), by Schlafflian Vectors approach, the least square estimate is given by

$$\hat{\beta} = \left(\sum_{u=1}^{N} \mathbf{x}^{[4]'}\mathbf{x}^{[4]} \right)^{-1} \mathbf{x}^{[4]'}\mathbf{y} \tag{7}$$

The estimated response $\hat{y}_u$ at any point (x_{0u}, x_{1u}, x_{2u}) is given by $\hat{y}_u = \mathbf{x}_u^{[4]'}\hat{\beta}$ and the variance of the estimated response will be given by

$$\mathbf{Var}(\hat{y}_u) = \mathbf{x}_u^{[4]'}\mathbf{Var}(\hat{\beta})\mathbf{x}_u^{[4]} = \mathbf{x}_u^{[4]'}(\mathbf{X}'\mathbf{X})^{-1}\mathbf{x}_u^{[4]}\sigma^2. \tag{8}$$

3. The Moment Matrix with two Predictor Variables

For $k = 2$ it is possible to generate vectors D_1, D_2 and D_3. This is done by arranging these vectors in a particular form so that we obtain sub matrices which will be easy to handle. It is noticed that the non-zero matrices appear along the main diagonal of the moment matrix with elements of the off-diagonal matrices being zero. For the design with two predictor variables we write

$$N^{-1}(XX) = N^{-1}\sum_{u=1}^{N} \mathbf{x}[4]\mathbf{x}[4]' = N^{-1}\sum_{u=1}^{N}\begin{pmatrix} D_1 \\ D_2 \\ D_3 \end{pmatrix}[\mathbf{D}_1', \mathbf{D}_2', \mathbf{D}_3'], \tag{9}$$

where D_1, D_2 and D_3 are given by

$$D_1 = \begin{pmatrix} x_{0u}^4 \\ \sqrt{6}x_{0u}^2 x_{1u}^2 \\ \sqrt{6}x_{0u}^2 x_{2u}^2 \\ x_{1u}4 \\ x_{2u}4 \\ \sqrt{6}x_{1u}^2 x_{2u}^2 \end{pmatrix}, \quad D_2 = \begin{pmatrix} 2x_{0u}^2 x_{1u} \\ 2x_{0u}x_{1u}^3 \\ \sqrt{12}x_{0u}x_{1u}x_{2u}^2 \\ 2x_{0u}^2 x_{2u} \\ 2x_{0u}x_{2u}^3 \\ \sqrt{12}x_{0u}x_{1u}^2 x_{2u} \end{pmatrix}, \quad D_3 = \begin{pmatrix} 2x_{1u}^3 x_{2u} \\ \sqrt{12}x_{0u}^2 x_{1u}x_{2u} \\ 2x_{1u}x_{2u}^3 \end{pmatrix}. \tag{10}$$

Therefore, the moment matrix is given by

$$N^{-1}(XX) = \sum_{u=1}^{N}\begin{pmatrix} D_1D_1' & D_1D_2' & D_1D_3' \\ D_2D_1' & D_2D_2' & D_2D_3' \\ D_3D_1' & D_3D_2' & D_3D_3' \end{pmatrix}. \tag{11}$$

Our interest focuses on the main diagonal of (11) since the off-diagonal elements will be 0 (where 0 is a matrix of corresponding order) with regard to conditions of rotatability. That is to say

$$N^{-1}(XX) = \text{diag}\sum_{u=1}^{N}[D_1D_1' \quad D_2D_2' \quad D_3D_3']. \tag{12}$$

Let

$$\sum_{u=1}^{N} D_1D_1' = B_1 \tag{13}$$

where

$$B = \begin{pmatrix} 1 & \sqrt{6}\lambda_2 & \sqrt{6}\lambda_2 & 3\lambda_4 & 3\lambda_4 & \sqrt{6}\lambda_4 \\ \sqrt{6}\lambda_2 & 18\lambda_4 & 6\lambda_4 & 15\sqrt{6}\lambda_6 & 3\sqrt{6}\lambda_6 & 18\lambda_6 \\ \sqrt{6}\lambda_2 & 6\lambda_4 & 18\lambda_4 & 3\sqrt{6}\lambda_6 & 15\sqrt{6}\lambda_6 & 18\lambda_6 \\ 3\lambda_4 & 15\sqrt{6}\lambda_6 & 3\sqrt{6}\lambda_6 & 105\lambda_8 & 9\lambda_8 & 6\lambda_6 \\ 3\lambda_4 & 3\sqrt{6}\lambda_6 & 15\sqrt{6}\lambda_6 & 9\lambda_8 & 105\lambda_8 & 15\sqrt{6}\lambda_8 \\ \sqrt{6}\lambda_4 & 18\lambda_6 & 18\lambda_6 & 6\lambda_6 & 15\sqrt{6}\lambda_8 & 54\lambda_8 \end{pmatrix}. \quad (14)$$

Further, we let

$$\sum_{u=1}^{N} D_2 D_2' = M = \text{diag}[M_1 \quad M_2]. \quad (15)$$

where

$$M_{i-1-2} = \begin{pmatrix} 4\lambda_2 & 12\lambda_4 & 4\sqrt{3}\lambda_4 \\ 12\lambda_4 & 60\lambda_6 & 12\sqrt{3}\lambda_6 \\ 4\sqrt{3}\lambda_4 & 12\sqrt{3}\lambda_6 & 36\lambda_6 \end{pmatrix} \quad (16)$$

and

$$\sum_{u=1}^{N} D_3 D_3' = L, \quad (17)$$

where

$$L = \begin{pmatrix} 60\lambda_8 & 36\lambda_8 & 12\sqrt{3}\lambda_8 \\ 36\lambda_8 & 60\lambda_8 & 12\sqrt{3}\lambda_8 \\ 12\sqrt{3}\lambda_8 & 12\sqrt{3}\lambda_8 & 12\lambda_4 \end{pmatrix}. \quad (18)$$

Therefore, the final form of the moment matrix obtained will be

$$N^{-1}(XX) = \text{diag}[B \quad M \quad L]. \quad (19)$$

4. Parameter Estimates

We need to obtain the expression for the inverse of the matrix XX, by first expressing the matrix XX obtained in (19) as

$$(XX) = N\,\text{diag}[B \quad M \quad L] \quad (20)$$

whose inverse will be of the form

$$(X'X)^{-1} = N^{-1}\text{diag}[B^{-1} \quad M^{-1} \quad L^{-1}]. \tag{21}$$

This is obtained by working out the inverses of B, M and L.

Using (21) in (7) we have

$$\hat{\beta} = N^{-1}\text{diag}[B^{-1} \quad M^{-1} \quad L^{-1}]X'y. \tag{22}$$

Using $\mathbf{x}^{[4]'}$ of (5) in (22) we have

$$\hat{\beta} = N^{-1}\text{diag}[B^{-1} \quad M^{-1} \quad L^{-1}]\begin{pmatrix} D_1 \\ D_2 \\ D_3 \end{pmatrix} y = \begin{pmatrix} \hat{\beta}_{*1} \\ \hat{\beta}_{*2} \\ \hat{\beta}_{*3} \end{pmatrix}, \tag{23}$$

where

$$\hat{\beta}_{*1} = \left[\hat{\beta}_0, \frac{1}{\sqrt{6}}\hat{\beta}_{11}, \frac{1}{\sqrt{6}}\hat{\beta}_{22}, \hat{\beta}_{1111}, \hat{\beta}_{2222}, \frac{1}{\sqrt{6}}\hat{\beta}_{1122}\right]', \tag{24}$$

$$\hat{\beta}_{*2} = \left[\frac{1}{2}\hat{\beta}_1, \frac{1}{2}\hat{\beta}_2, \frac{1}{\sqrt{12}}\hat{\beta}_{122}, \frac{1}{\sqrt{12}}\hat{\beta}_{112}, \frac{1}{2}\hat{\beta}_{111}, \frac{1}{2}\hat{\beta}_{222}\right]', \tag{25}$$

$$\hat{\beta}_{*3} = \left[\frac{1}{2}\hat{\beta}_{1112}, \frac{1}{2}\hat{\beta}_{1222}, \frac{1}{\sqrt{12}}\hat{\beta}_{12}\right]'. \tag{26}$$

Our main interest is to find the estimates of the coefficients of the general mean and the linear factors, that is, β_0, β_1 and β_2. To do this we let,

$$\sum_{u=1}^{N} x_{0u}y_u = (0y), \quad \sum_{u=1}^{N} x_{0u}x_{iu}y_u = (iy),$$

$$\sum_{u=1}^{N} x_{iu}x_{ju}y_u = (ij)y), \quad \sum_{u=1}^{N} x_{iu}^2 x_{ju}^2 y_u = (iijj)y)$$

and so on.

The least square estimates $\hat{\beta}_0$, $\hat{\beta}_1$ and $\hat{\beta}_2$ for a fourth order rotatable design in two dimensions that we desire to find are contained in $\hat{\beta}_{*1}$ and

$\hat{\beta}_{s2}$ and generally given as

$$\hat{\beta}_0 = S_0\left(\sum_{u=1}^{N} x_{1u}y_u + \sum_{u=1}^{N} x_{2u}y_u\right) + S_1\left(\sum_{u=1}^{N} x_{1u}^2 y_u + \sum_{u=1}^{N} x_{2u}^2 y_u\right) + S_2\sum_{u=1}^{N} x_{1u}^2 x_{2u}^2 y_u, \tag{27}$$

$$\hat{\beta}_1 = 4\alpha\sum_{u=1}^{N} x_{1u}y_u + 4\beta\sum_{u=1}^{N} x_{1u}^3 y_u + 2\sqrt{12}\gamma\sum_{u=1}^{N} x_{1u}x_{2u}^2 y_u, \tag{28}$$

$$\hat{\beta}_2 = 4\alpha\sum_{u=1}^{N} x_{2u}y_u + 4\beta\sum_{u=1}^{N} x_{2u}^3 y_u + 2\sqrt{12}\gamma\sum_{u=1}^{N} x_{1u}^2 x_{2u} y_u. \tag{29}$$

5. The Estimated Response

The estimated response $\hat{y}_u$ at a point (x_{0u}, x_{1u}, x_{2u}) from the generalized situation now is

$$\hat{y}_u = \mathbf{x}_u^{[1]'}\hat{\mu}. \tag{30}$$

Our primary interest is the coefficients of the main effects only where $\mathbf{x}_u^{[1]'}$ is as provided in (5) and μ is the parameter system of interest given as $\mu = J\beta$, where μ and J are given by

$$\hat{\mu} = \begin{pmatrix} \hat{\beta}_0 \\ \hat{\beta}_1 \\ \hat{\beta}_2 \end{pmatrix}, \quad J_{(15\times 15)} = \begin{pmatrix} R & 0 & 0 \\ 0 & S & 0 \\ 0 & 0 & T \end{pmatrix}.$$

Further, we have

$$R_{(6\times 6)} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}, \quad S_{(6\times 6)} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

and $T_{[3*3]}$ is matrix of zero's. In this case 0 refers to a zero matrix of appropriate order with regard to the diagonal elements.

Using equation (30), we get

$$\hat{y}_u = \mathbf{x}_0^{[4]'} J\hat{\beta} = [D_1 \quad D_2 \quad D_3]\,\mathrm{diag}[R \quad S \quad T]\hat{\beta}$$

$$= [D_1 R \quad D_2 S \quad D_3 T]\hat{\beta} \tag{31}$$

That is,

$$\hat{y}_u = \hat{\beta}_0 \sum_{u=1}^{N} x_{0u}^4 + \hat{\beta}_1 \sum_{u=1}^{N} x_{1u}^4 + \hat{\beta}_2 \sum_{u=1}^{N} x_{2u}^4. \tag{32}$$

Hence we are able to express the estimated response $\hat{y}_u$ at a point (x_{0u}, x_{1u}, x_{2u}), that is the estimated response of a fourth order rotatable design in two dimensions.

6. Variance of Estimated Response

With reference to the normal equation using the results obtained in equation (32), we obtain varaiance of the parameter estimates as

$$\mathrm{Var}(\hat{\beta}_0) = S_0(N\Delta)^{-1}\sigma^2, \quad \mathrm{Var}\left(\frac{\hat{\beta}_i}{2}\right) = a(N\beta_3^*)^{-1}\sigma^2, \quad i = 1, 2, \tag{33}$$

the covariances of the coefficients are equal to zero. The standardized variance of the estimated response $(\hat{y})$ is given by

$$\mathrm{Var}(\hat{y}) = \mathbf{x}^{[4]'} J(X'X)^{-1} J\mathbf{x}^{[4]}$$

$$= N^{-1}\sigma^2[24(k+2)(k+4)\delta\beta_3^{*2}\lambda_8\Delta^{-1} + \rho^2\lambda_6(k+4)\beta_3^{*-1}]$$

$$= N^{-1}\sigma^2[576\,\delta\beta_3^{*}\lambda_8\Delta^{-1} + 6\rho^2\beta_3^{*-1}\lambda_6] = \phi(\rho^2)$$

$$= \text{constant.} \tag{34}$$

7. Variances Function of the Difference between two Estimated Responses

Suppose that $\mathbf{x}'_a$ and $\mathbf{x}'_b$ are two distinct points identified on the two response surface of different radii. The two points are given as

$$\hat{y}(\mathbf{x}'_a) = \mathbf{x}'_a\hat{\beta}, \quad \hat{y}(\mathbf{x}'_b) = \mathbf{x}'_b\hat{\beta}. \tag{35}$$

With reference to a rotatable design, $X'X$ has a special form, Box and Hunter [2]. Taking into consideration equation (5) where now $\mathbf{x}'_a = (\rho_1, 0, 0, \ldots, 0)$ is taken as a vector of order (15×1) of a row of the design matrix X arising from a point in the predicter variable space. We express the vector as

$$\mathbf{x}'_a = [\mathbf{d}'_1 \quad \mathbf{d}'_2 \quad \mathbf{d}'_3], \tag{36}$$

where

$$\mathbf{d}'_1 = (\rho_1, 0, 0, 0, 0, 0), \quad \mathbf{d}'_2 = (0, 0, 0, 0, 0, 0) \quad \text{and} \quad \mathbf{d}'_3 = (0, 0, 0).$$

Let

$$\mathbf{x}'_b = [d_1^* \quad d_2^* \quad d_3^*] \tag{37}$$

be a vector of order (15×1) of a row of the design matrix X arising from a point in the predicter variable space. We observe that, this is a particular point on the response surface which must not be along the axes of the predictor variable space. However, the vector makes an angle θ with the axis x_1, where

$$\mathbf{d}_1^* = (\rho_2 \cos\theta, 0, 0, 0, 0, 0), \quad \mathbf{d}_2^* = (\rho_2 \sin\theta, 0, 0, 0, 0, 0) \quad \text{and} \quad \mathbf{d}_3^* = (0, 0, 0).$$

Consider first the difference of the two vectors in equations (36) and (37), that is,

$$\mathbf{x}'_a - \mathbf{x}'_b = [\mathbf{d}'_1 \quad \mathbf{d}'_2 \quad \mathbf{d}'_3] - [\mathbf{d}_1^* \quad \mathbf{d}_2^* \quad \mathbf{d}_3^*] = [\mathbf{e}'_1 \quad \mathbf{e}'_2 \quad \mathbf{e}'_3], \tag{38}$$

where $\mathbf{e}'_1 = (\rho_1 - \rho_2 \cos\theta, 0, 0, 0, 0, 0)$, $\mathbf{e}'_2 = (-\rho_2 \sin\theta, 0, 0, 0, 0, 0)$ and $\mathbf{e}'_3 = (0, 0, 0)$.

The standardized variance function of the difference between the two estimated responses will be

$$V_d = (\mathbf{x_a} - \mathbf{x_b})' J'(X'X)^{-1} J(\mathbf{x_a} - \mathbf{x_b})$$

$$= e_1' R B^{-1} R e_1 + e_2' S' M^{-1} S e_2$$

$$= \frac{24(\rho_1 - \rho_2 \cos\theta)^2 \beta_1^*}{\mu^*} + \frac{a\rho_2^2 \lambda_6 \sin^2\theta}{\beta_3^*}$$

$$= \frac{24(\rho_1 - \rho_2\cos\theta)^2[8\lambda_4\lambda_8 - 6\lambda_6^2]}{24[8\lambda_4\lambda_8 - 6\lambda_6^2] - 12\lambda_2[8\lambda_2\lambda_8 - 4\lambda_4\lambda_6] + 8\lambda_4[6\lambda_2\lambda_6 - 4\lambda_4^2]}$$

$$+ \frac{\frac{3}{2}\lambda_6\rho_2^2 \sin^2\theta}{6\lambda_2\lambda_6 - 4\lambda_4^2}, \qquad (39)$$

With reference to conditions of rotatability, we have

$$\omega_1 = 24[8\lambda_4\lambda_8 - 6\lambda_6^2],$$

$$\omega_2 = 12\lambda_2[8\lambda_2\lambda_8 - 4\lambda_4\lambda_6]$$

and

$$\omega_3 = 8\lambda_4[6\lambda_2\lambda_6 - 4\lambda_4^2].$$

Thus (39) simplifies to

$$V_d = \frac{\omega_1(\rho_1 - \rho_2\cos\theta)^2}{\omega_1 - \omega_2 + \omega_3} + \frac{\frac{3}{2}\lambda_6\rho_2^2\sin^2\theta}{[6\lambda_2\lambda_6 - 4\lambda_4^2]}, \qquad (40)$$

which is a function of θ.

8. Discussions

The results in (40), giving the variance function of the difference between the two estimated response denoted by $[V_d]$ is a function of θ. We optimize the function by finding the first order condition and solve for

9. After which we explore the second order condition to evaluate the nature of the function.

Suppose we let

$$\frac{\omega_3}{\omega_1 - \omega_2 + \omega_3} = h_1 \tag{41}$$

and

$$\frac{\frac{3}{2}\lambda_6}{[6\lambda_5\lambda_6 - 4\lambda_4^2]} = \frac{12\lambda_4\lambda_6}{\omega_3} = h_2. \tag{42}$$

Using (41) and (42) we can re-write (40) as

$$V_{\mathbf{x}_a - \mathbf{x}_b} = V_d = h_1[\rho_1 - \rho_2\cos\theta]^2 + h_2\rho_2^2\sin^2\theta,$$

whose first order condition will be

$$f_2'(\theta) = \frac{\partial V_d}{\partial\theta} = h_1[2(\rho_1 - \rho_2\cos\theta)\rho_2\sin\theta] + 2h_2\rho_2^2\cos\theta\sin\theta = 0.$$

On solving, we get

$$\theta = \left[0, \cos^{-1}\left(\frac{h_1}{[h_2 - h_1]}\rho^*\right)\right], \tag{43}$$

where $\rho^* = \frac{\rho_1}{\rho_2}$.

With regard to rotatability we only need to evaluate θ for values in the interval $0^\circ \le \theta \le 90^\circ$, since by rotating the points around the sphere the angle remains invariant and result of first quadrant can be used to give values in other quadrants.

The second derivative of (40) yields

$$f_2'(\theta) = \frac{\partial^2 V_d}{\partial\theta^2} = 2h_1\rho_1\rho_2\cos\theta + 2\rho_2^2[h_2 - h_1][2\cos^2\theta - 1]. \tag{44}$$

On substitution for the values of θ from (43) in (44) we have two conditions;

(i) for $\theta = 0^\circ$ we have

$$f_2'(\theta) = 2h_1\rho_1\rho_2 + 2\rho_2^2[h_2 - h_1].$$

By letting $\lambda_2 < \frac{1}{k}$ and $\lambda_4 \leq \frac{\lambda_2}{k+2}$ from the results of Huda and Mukerjee [6], while evaluating the values of λ_6 and λ_8 with regard to conditions and same procedures we have, $\lambda_2 < \frac{1}{2}$, $\lambda_4 = \frac{\lambda_2}{4}$; on considering the equality, $\lambda_2\lambda_6 = \frac{\lambda_4}{k+4}$; therefore $\lambda_6 = \frac{\lambda_4}{6\lambda_2}$ and $\lambda_4\lambda_8 = \frac{\lambda_6}{k+6}$ thus $\lambda_8 = \frac{\lambda_6}{8\lambda_4}$. We therefore, need only evaluate the values of λ_2 say λ_2^* in order to compute h_1 and h_2. For $\lambda_2^* = 0.4596$ from the results of Huda and Mukerjee [6], we find that;

$$h_1 = 34.76694889, \quad h_2 = 1.014808732. \tag{45}$$

Hence if the vectors are equidistant and the distance is unitary, then

$$f_2'(0) = 2h_1\rho_1\rho_2 + 2\rho_2^2[h_2 - h_1] = 2.029617464 > 0, \tag{46}$$

which implies that the variance functions of difference between two estimated responses are minimized when $\theta = 0^\circ$.

(ii) for $\theta = \cos^{-1}\left(\frac{h_1}{[h_2 - h_1]}\rho^*\right)$, we have

$$f_2'(\theta) = 2h_1\rho_1\rho_2\cos\theta + 2\rho_2^2[h_2 - h_1][2\cos^2\theta - 1] = -147.3693358 < 0. \tag{47}$$

The results of (47) imply that the variance functions of difference between two estimated responses are maximized when

$$\theta = \cos^{-1}\left(\frac{h_1}{[h_2 - h_1]}\rho^*\right).$$

We now evaluate the extent to which the angle θ can be varied while still minimizing the functions in V_d of (40). We tabulate the results as follows:

Table of values

θ	$f_d''(\theta)$	θ	$f_d''(\theta)$
0	2.029617464	44	47.66263486
5	2.790561691	45	49.16789077
10	5.044247555	46	50.65816906
15	8.704166068	47	52.13086495
20	13.62921185	48	53.58337801
25	19.62819808	49	55.01312162
30	26.46598181	50	56.41752335
35	33.87101097	55	62.97082909
40	41.54406085	60	68.51908923
41	43.0831189	70	75.49327271
42	44.61763776	80	75.50770892
43	46.14501309	90	67.5042805

Here, $f_d''(\theta)$ is the second derivative of the function V_d, that is, the variance function of the difference between two estimated responses.

9. Conclusions

Every experimenter aims at minimizing the variance function. We thus conclude that for the variance functions of difference between two estimated responses, we aspire to achieve a global minimum value for the function while other factors are assumed constant by letting the angle θ between the two vectors be as close as possible to 0°.

If differences of points close together in the factor space are involved, based on our results, an optimal design for a fourth order rotatable design in two dimensions from this approach will be chosen on the basis of minimum variance function criterion as emphasized by Herzberg [5] and Box and Draper [1].

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