SYNTHESIS AND CHARACTERIZATION OF MCM-BASED COMPOSITE FOR BIOMEDICAL APPLICATION

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CERTIFICATE

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DEDICATION

With profound reverence,

We dedicate this project to our **beloved parents** and **respected teachers** whose guidance and unflinching support helped us throughout the process.

ACKNOWLEDGEMENTS

Praise be to Almighty Allah who made us capable to do our best and get through this project.

For the successful completion and execution of our final year project, we sincerely acknowledge all the advice and support we have received from our Supervisor Dr. Usman Liaqat. Without his diligence, we would not have been able to project our true capabilities for this strenuous task.

Furthermore, all the guidance our Co-Supervisor Dr. Zakir Hussain provided us with, helped us achieve a better understanding and problem-solving approach. We thank him for the provision of all the required material samples.

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We pay our sincerest regard to all the staff and faculty members of the Materials Engineering department for guiding and helping us at various stages throughout the process.

ABSTRACT

This study focuses on the synthesis of material for glucose detection. Diabetes is a major concern worldwide and therefore constant research is going on to develop methods for its better and cheap detection.

Initially, the glucose sensors developed were focused on developing a material that could detect glucose concentration through its enzymatic breakdown. However, recent developments in glucose sensors have started employing non-enzymatic techniques. Therefore, materials with greater surface areas and better electrochemical activities are being tested.

In this experiment, we used Mobil Composition of Matter No.40 (MCM-41), an amorphous material with a high surface area. To further enhance the electrochemical activity, its composite was formed with copper oxide (CuO) nanoparticles which are known for high surface area.

This material MCM-41 has already been reported for glucose sensing however, used in enzymatic glucose sensing only and with the use of Selenium nanoparticles.

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CHAPTER 1

Introduction

1.1 Major Health Issues

The world is expanding day by day with an increase in the world population. Due to the increase in the world population, the number of the people that are getting effected by certain diseases such as Cancer, Diabetes, Tuberculosis, Alzheimer's, etc. is also increasing. The World Health Organization (WHO) is working effortlessly to make sure that the number of the people affected can be bought down and the diagnosis of such diseases can be made and can be treated effectively.

The first chapter discusses the disease which is diabetes, its different definitions, how it effects the people of this world and how far they have come in terms of advancement and technology today to perform the diagnosis and treatment of diabetes. The classification of diabetes that people are suffering from will be reviewed next, with the focus being diabetes type 1 and diabetes type 2. Diabetes type 2 will be elaborated on in detail, followed by the problem statement and the major issues faced due to diabetes type 2. Various generalized terms will be explained along with the problems being faced by a patient that is having diabetes.

1.1.1 Diabetes – A Major Health Issue

Diabetes is a major disease that is spread worldwide. According to the report issued by the World Health Organization (WHO) in the year 2020, around 422 million people in the world have diabetes [1]. The majority of the people that are suffering from the disease are residents of low- and middle-income countries. The number of deaths that are attributed to the disease yearly is around 1.5 million. The rise in the number of cases of people suffering from diabetes and the prevalence of diabetes is steadily increasing over the past few decades.

The report by the World Health Organization (WHO) also tells that the patients that are affected by diabetes, access to affordable treatment which includes insulin is very much critical for their survival. A globally agreed target was made to halt the rise in diabetes and obesity by the year 2025 [1]. Figure 1 tells us about the comparative prevalence of diabetes mellitus in adults (20-79 years) in countries with high prevalence (≥10%).

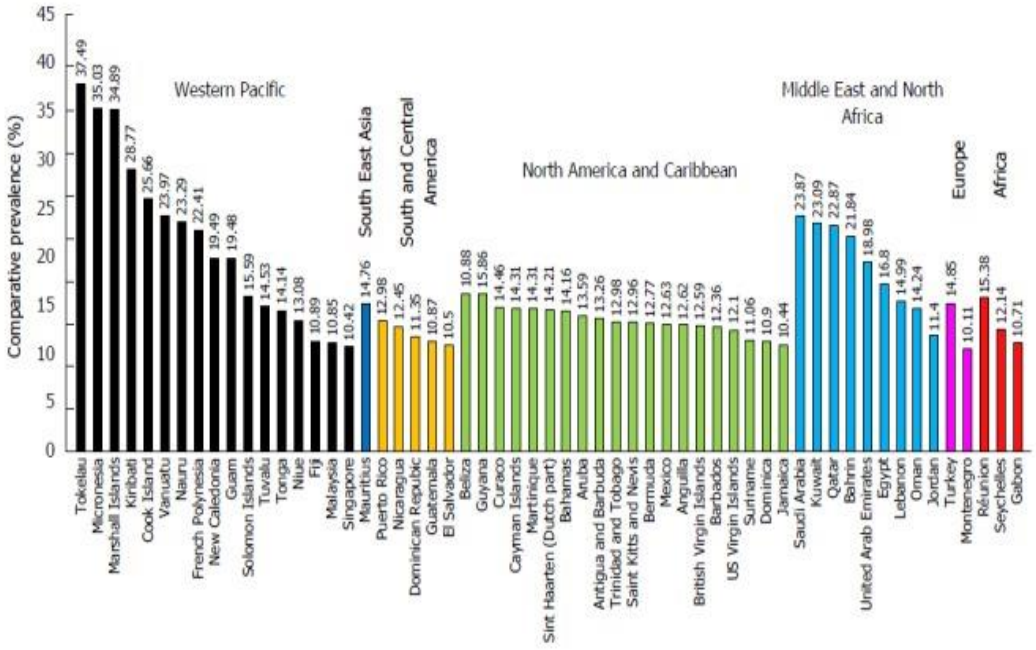


Figure 1: Comparative prevalence of diabetes in adults (20-79 years) in countries with high prevalence (≥10%)

1.1.2 Definition of Diabetes

Diabetes, also known as diabetes mellitus, represents a set of metabolic, autoimmune, and genetic disorders that has one common characteristic that is hyperglycemia [2]. Hyperglycemia is a term that is used for high blood glucose (blood sugar). Diabetes mellitus is not a single disorder, and the definition of the disease is dependent on the perspective considered. Considering it from the medical perspective, the disease is a representation of a series of metabolic conditions associated with hyperglycemia; the cause of which is partial or total insulin sufficiency. It

can also be defined as a chronic disorder of glucose metabolism with serious clinical issues [3].

If defined from a social perspective, the inclusion of the burden the disease puts on the economies, regarding both the costly treatment and associated with premature mortality and morbidity [2]. The definition of diabetes from a patient's perspective can be considered as a life-long condition that requires daily consideration of diet, lifestyle, and inspecting blood glucose, along with regular supervision of medication. It can also be associated with varying degrees of anxiety, depression, and multiple visits to healthcare providers according to the patient's perspective [2].

Diabetes mellitus can also be defined as a group of metabolic diseases that are characterized by chronic hyperglycemia resulting from defects in insulin action, insulin secretion, or both [4]. The results obtained from the importance of insulin as an anabolic hormone are metabolic abnormalities in carbohydrates, lipids, and proteins. The factors that can be held responsible for these metabolic disorders can be the low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly the skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes [4]. The severity of the symptoms can base on the type and the duration of diabetes one is having to face.

1.2 Classification of Diabetes

Although classification of diabetes, also known as diabetes mellitus, is very important and has effects for the treatment methods that are to be used but it is not an easy task to do so and many of the patients do not fit easily into a single class. The classical classification of diabetes as proposed by the American Diabetes Association (ADA) in the year 1997 included the following types [5]:

1. Type 1 diabetes
2. Type 2 diabetes

3. Gestational diabetes mellitus (GDM)
4. Other types

This classification of diabetes is still the most accepted and adopted by American Diabetes Association (ADA). A proposition was made by Wilkins [6] in the accelerator hypothesis that argued that "type 1 and type 2 are the same disorder of the insulin resistance that is just set against different genetic backgrounds". Figure 2 tells us about the different disorders of glycemia and their etiologic types and stages.

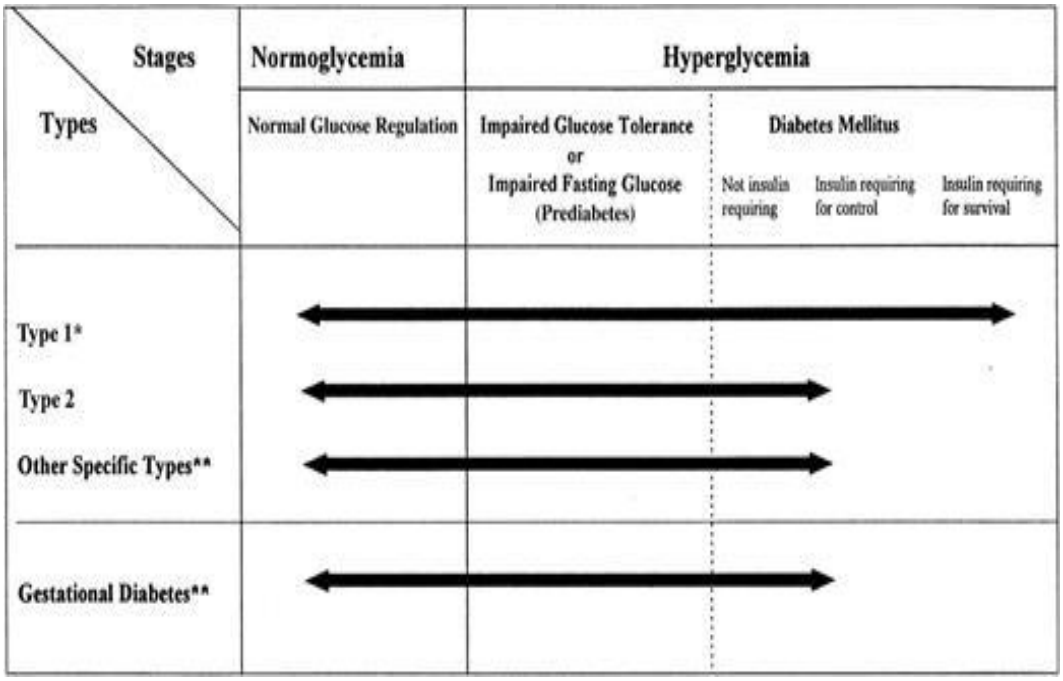


Figure 2: Disorders of glycemia: etiologic types and stages

1.2.1 Type 1 Diabetes

The type 1 diabetes also known as juvenile diabetes or insulin-dependent diabetes can be regarded as a chronic condition in which little or no insulin is produced by the pancreas in the human body. Insulin can be defined as a hormone that is needed to allow sugar (glucose) to enter the cells to result in the production of energy. Different factors that can include certain genetics and some viruses may play a role in contributing to the type 1 of diabetes. The exact cause of this type is unknown. Usually,

the body's immune system which normally fights harmful bacteria and viruses starts destroying mistakenly the insulin-producing cells in the pancreas. Other possible causes that are mentioned above can include genetics and exposure to viruses and other environmental factors. The period where this type of diabetes appears can be the childhood or adolescence, but it can also develop in adults. Despite all the active research on this type, there isn't a cure possible for this disease. The treatment of this disease focuses on managing the blood sugar levels with the usage of insulin, a proper diet, and a healthy lifestyle to prevent complications.

The type 1 diabetes signs and symptoms that can appear suddenly may include increased level of thirst, frequent urination, bed wetting in case of children who didn't previously wet the bed at night, extreme level of hunger, unintentional weight loss, irritability and irrational mood changes, tiredness, fatigue and weakness and blurred vision. The further types that have been researched on of type 1 diabetes include autoimmune type 1 diabetes, Idiopathic type 1 diabetes, and Fulminant type 1 diabetes [4].

1.2.2 Type 2 Diabetes

422 million people live in the world with diabetes and an estimated 193 million people have diabetes that is undiagnosed. Type 2 diabetes accounts for more than ninety percent of the patients and this diabetes leads to micro vascular and macro vascular complications that can cause profound psychological and physical distress to both the patients and the caretakers. This also puts a burden on the healthcare systems of the countries [9].

Type 2 diabetes is exemplified by the relative insulin insufficiency that is caused by the pancreatic β-cell dysfunction and insulin resistance in the target organs [9]. It is an intensifying global problem, closely linked to the epidemic of obesity. The individuals that are having type 2 diabetes mellitus are at a higher risk for micro vascular complications that include

retinopathy, nephropathy, and neuropathy and at a higher risk for macro vascular complications that include cardiovascular comorbidities [10]. These complications owe to the hyperglycemia and individual components of the insulin resistance (metabolic) syndrome. Certain ecological factors including obesity, an unhealthy diet and physical inactivity and certain genetic factors make a contribution to the several pathophysiological disturbances that are responsible for impaired glucose homeostasis in type 2 diabetes. Insulin resistance and impaired insulin secretion remain the major defects in the type 2 diabetes but at least six other pathophysiological malformations contribute to the dysregulation of the glucose metabolism [10].

According to the International Diabetes Federation, the highest prevalence of type 2 diabetes is in Saudi Arabia where the wealth derived from oil results in frequent obesity which is one of the major causes of type 2 diabetes. A dramatic increase in obesity in the United States of America resulted in 11% of the adults being diabetics. In Europe, most of the type 2 diabetes is in the central region and Scandinavians are the ones least affected. Figure 3 represents the diabetes prevalence in adult population.

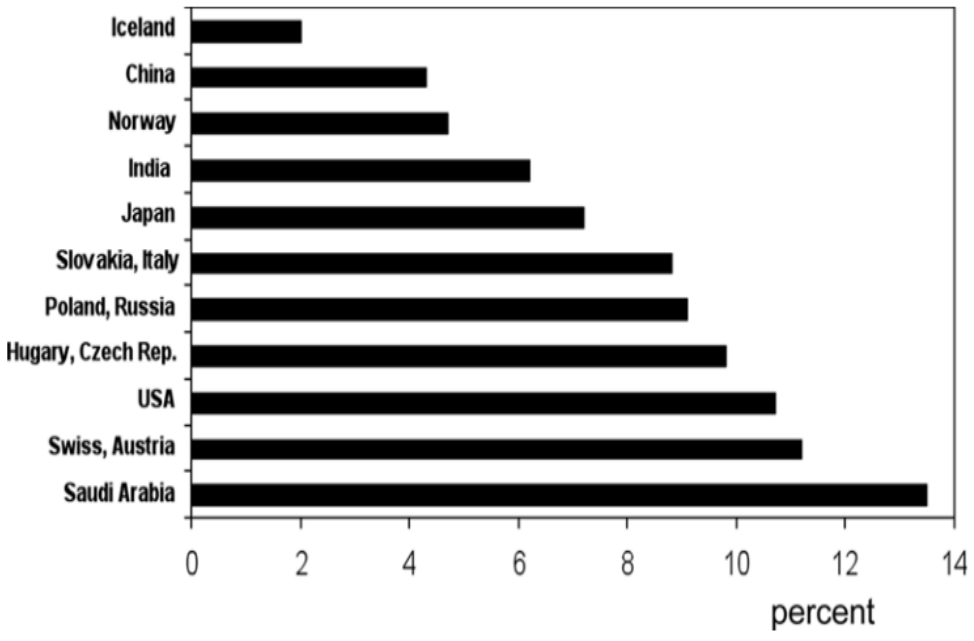


Figure 3: Type 2 diabetes prevalence in adult population in the world

1.2.2.1 Symptoms of Type 2 diabetes

The symptoms of type 2 diabetes are so mild that many people can't notice them. Around 8 million people that have it don't know that they are having this disease. The symptoms of type 2 diabetes include:

- Being very thirsty
- Peeing a lot
- Blurry vision
- Being in a state of cranky mood
- Numbness or tingling in your feet or hands
- Fatigue/feeling tired or worn out
- Wounds that are unhealable
- Yeast infections that don't go away and keep coming back
- Feeling hungry or an increased appetite for food
- Loss in weight without trying
- Getting more and more infections

1.2.2.2 Causes of type 2 diabetes

The pancreas in a human body is the producer of a hormone called insulin. The insulin helps the human body cells to convert the glucose into energy. The pancreas in the body of patients of type 2 diabetes makes insulin but their cells don't use it well as they should be doing it. At first, the pancreas makes more amount of insulin to try to get the glucose in your cells but eventually it can't keep up and the glucose builds in your blood instead. Usually, a combination of things is the reason for type 2 diabetes. They might include the genes, the extra body weight, the metabolic syndrome, too much glucose from the liver and bad communication between the cells of the body.

1.2.3 Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is a condition in which a hormone that is made by the placenta prevents the body from using insulin

effectively. Glucose is built up in the blood instead of being absorbed by the cells [7]. Unlike the type 1 diabetes, gestational diabetes is not triggered by a lack of insulin, but by other hormones that are produced during pregnancy which can result in making the insulin less effective; a condition that is referred to as insulin resistance. The symptoms of gestational diabetes disappear after the delivery. The numbers show that approximately three to eight percent of all pregnant women in the United States of America (USA) are diagnosed with this type of diabetes [7]. The cause of this type like diabetes type 1 is not known but there are certain theories that depict why the condition occurs.

The placenta provides a growing fetus with the nutrients and water it requires and produces several hormones to maintain the pregnancy. Some of the hormones that include estrogen, cortisol, and human placental lactogen can have a blocking effect on the insulin that is being produced. This blocking effect on the insulin is called contra-insulin effect which usually starts about twenty to twenty-four weeks into pregnancy. As the placenta keeps on growing, the number of hormones produced keeps on increasing and the risk of insulin resistance becomes greater. Normally, the pancreas can make additional insulin to tackle the insulin resistance, but when the additional insulin isn't able to tackle the insulin resistance and to overcome the effect of the placental hormones, gestational diabetes mellitus occurs. Although, any woman can develop this disease during their pregnancy, some of the factors that can increase the risk of getting this diabetes are [7]:

1. Obesity or overweight
2. Having given birth to a child before that weighed more than 9 pounds
3. The race of the woman
4. Prediabetes, also known as impaired glucose tolerance
5. The age of the woman (women who are above twenty-five years of age are at a greater risk of getting this type)
6. Family history of diabetes

Although, increase amount of glucose in the urine often makes to the list of risk factors, it is not considered to be a reliable indicator of Gestational Diabetes Mellitus (GDM).

1.2.4 Other types:

The other types of diabetes include Maturity onset diabetes of the young (MODY), Neonatal diabetes, Type 3C diabetes, Steroid-induced diabetes, and Cystic fibrosis diabetes [8]. These types of diabetes are briefly explained to get an idea that what are they about.

MODY is a rare form of diabetes which is different from that of type 1 and type 2 diabetes. It runs strongly in families and is caused by a mutation in a single gene. If the parent is having a mutated gene, any offspring that they have, would be having a fifty percent chance of getting it from them. If the child inherits the mutated gene, they will go on to have MODY before they reach twenty-five years of age irrespective of their weight, lifestyle or race they belong to [8].

Neonatal diabetes is a type of diabetes that is diagnosed under the age of six months. It's different from type 1 diabetes as it is not an autoimmune condition in which the body destroys its insulin producing cells. This type is different from that of type 1 diabetes because type 1 doesn't affect anyone under the age of six months. The cause of this type is a change in a gene that affects the insulin production which means that an increase in the levels of blood sugar would be happening, and the levels would be very high [8].

Type 3C diabetes is the type of diabetes that is developed when another disease damages the pancreas in the human body. The conditions that are related to this type of diabetes are pancreatic cancer, pancreatitis, and cystic fibrosis. A person can also develop type 3C diabetes if any part or whole of the pancreas is removed from the human body because of any other damage [8].

Steroid-induced diabetes is developed in the people that take steroids. Steroids can cause high blood glucose levels which is why the people that

take steroids can go on to develop this type of diabetes. This type is more common in the people that are at a higher risk of type 2 diabetes. [8]

Cystic fibrosis is considered as a genetic condition with which some people are born with. The diagnosis of this condition happens before a child turns one and can be caused by both parents passing on a faulty CFTR gene [8]. The people that have this disease produce thick, sticky, mucus which can build up in the organs such as lungs, pancreas, and others and all of this leads to a range of symptoms that can include problems with breathing, infections in lungs, and food digestion problems. This build-up of mucus caused by the cystic fibrosis can lead to the inflammation and damaging of the pancreas. This can lead to a damage to the cells that produce insulin and cause high blood glucose levels. As the pancreas can't produce enough insulin due to being scarred, the blood glucose levels may keep on increasing and the disease is incurred. This is known as cystic fibrosis diabetes.

1.3 Problem Statement

The problem identified at the beginning of our project was that diabetes is a major concern worldwide and has been the reason for many health issues and reasons leading to deaths. Once thought of a disease of the West, the prevalence of diabetes mellitus, commonly known as diabetes is increasing at alarming rates in many areas of the world. This disease promises to become an even larger public health issue with significant social and economic burden with clinical practice and public health policy implications. Around 18 million people die every year from cardiovascular disease for which diabetes is a major disposing factor [11]. Also, today more than 1.7 billion adults worldwide are overweight, and 312 million of them are obese. All these facts lead the focus on diabetes becoming a global health issue and a diabetic epidemic underway. All of this makes diabetes a major concern and to put the focus on diabetes mellitus and the problems regarding it.

1.4 Objectives

The main objectives of this project were the synthesis of a material for glucose detection and to measure the response of material against different analyte concentrations. The synthesis of the material for glucose detection would be able to provide us with a better material for usage and the measuring of the response of the synthesized material against different analyte concentrations will enable us to get the required information that at which concentrations the synthesized material can detect the analyte and is it doing the job for which it has been made. This will also make sure that whether a new type of material can be made by synthesis to use it for the purpose of glucose detection.

1.4.1 Glucose

Glucose, that is also called dextrose, is one of the group of carbohydrates known as simple sugars. Glucose has the molecular formula $C_6H_{12}O_6$. It can be found in the fruits and honey and is the major free sugar circulating in the blood of humans and higher animals. It is the source of energy in cell function and the regulation of its metabolism is very much important.

It is the most important carbohydrate fuel in the human body. In the fed state, most of the glucose circulating in the human body comes from the diet and in the fasting state, gluconeogenesis and glycogenolysis maintain the glucose concentrations. Very little amount of glucose is found in the diet as glucose is mostly found in the more complex carbohydrates that are broken down to monosaccharides through the digestive process. Glucose is classified as monosaccharide because it cannot be further broken down by hydrolysis. Glucose is further classified as hexose because of the six-carbon skeleton and as an aldose, because of the presence of an aldehyde group on carbon 1. The aldehyde group condenses with a hydroxyl group so that the glucose exists as a hemiacetal ring structure. The ring structure explains many of the reactions of glucose.

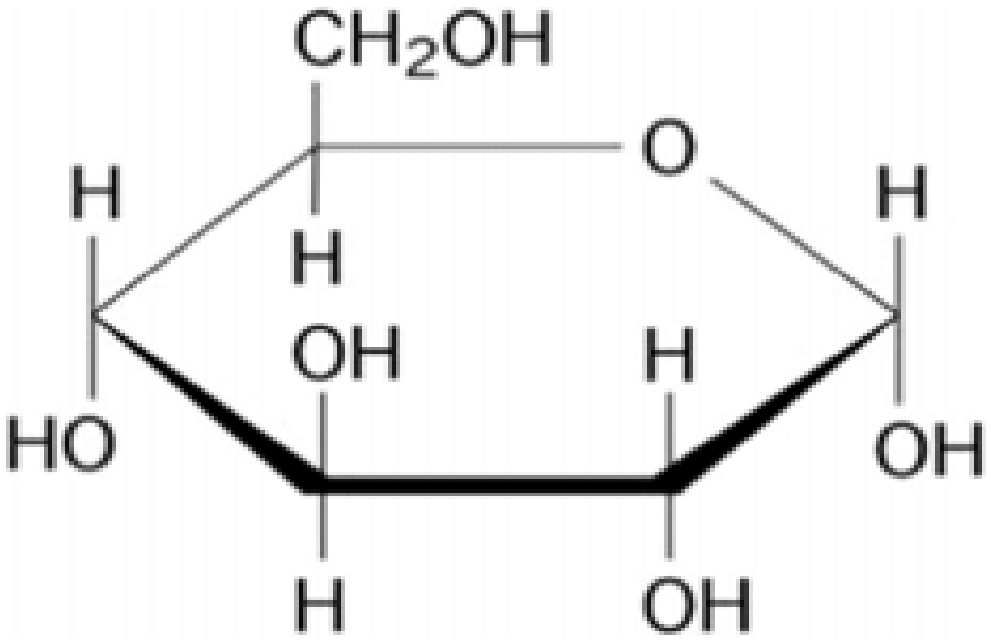


Figure 4: Molecular structure of Glucose

1.4.2 Glucose detection

Over the years, many diverse methods for detecting and quantifying glucose have been developed. The different methods that have been developed over the years for the detection of glucose include copper-iodometric methods, enzymatic methods, glucose meters, non-enzymatic glucose sensors, High performance liquid chromatography (HPLC), Capillary zone electrophoresis, Gas chromatography (GC) and certain other forms of Fourier transform (FT) spectroscopy. Different types of materials with different types of combinations are also being used to detect and quantify the glucose that is present in the human body over the period of research.

1.4.3 Need for Glucose detection

Checking your blood sugar level is one of the best ways to obtain information about diabetes and one's health. The checkup helps a person to obtain information about what changes are required in their diet, activities, and lifestyle to stay away from the risk of severe consequences

caused by diabetes. Keeping a track of a person's blood glucose can help them and their doctor in managing the disease.

Blood glucose monitoring or detection has been established as a valuable tool in managing diabetes. As the maintenance of blood sugar levels is recommended, a series of different types of sensing materials and biosensors have been established to perform the task of keeping a check of the blood sugar levels. During the last few decades, the glucose biosensor technology that includes point-of-care devices, continuous and non-invasive glucose monitoring systems has considerably improved. The challenges that are still there regarding glucose detection are further technological developments in the biosensors, development of biosensors for everyday use by patients in personalized monitoring, calibration of the analytical goals for their performance, and continuously evaluating and training lay users.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The major emphasis of this chapter is the elaboration on Mobil Composition of Matter (MCM), Mobil Composition of Matter-41 (MCM-41), Nanoparticles and Copper oxide (CuO) nanoparticles, the synthesis methods that are present there for both MCM-41 and CuO nanoparticles. The elaboration of the sensors i.e., the enzymatic sensors and the non-enzymatic sensors that are present there for the glucose detection will also be done.

2.2 Mobil Composition of Matter

The ordered mesoporous materials arose from the efforts to obtain materials with larger pores, which can process larger molecule than the microporous channels of the zeolites synthesized so far. This strategy led to the formation of Mobil Composition of Matter by the Mobil group. Mobil Composition of Matter (MCM) was the initial name that was given to this series of mesoporous materials that were first synthesized in the year 1992 [11]. The two of the most popular mesoporous molecular sieves that are keenly studied by the researchers are MCM-41 (Mobil Composition of Matter No.41) and MCM-48 (Mobil Composition of Matter No.48). This family of nano-structured mesoporous materials was also known as M41S.

2.2.1 Types of Mobil Composition of Matter

The two of the most prevalent types of Mobil Composition of Matter (MCM) are MCM-41 (Mobil Composition of Matter No.41) and MCM-48 (Mobil Composition of Matter No.48) [11]. Another type that is there is MCM-50 (Mobil Composition of Matter No.50).

2.2.2 Structure of Mobil Composition of Matter

Among the types of Mobil Composition of Matter, the most studied material and the material that got the most attention was the MCM-41. The structures of the types mentioned above were hexagonal array of unidirectional and non-interconnecting pores for MCM-41, three-dimensional cubic pore structure for MCM-48 and unstable lamellar structure with the presence of surfactant molecules between the lamellae for MCM-50 [12]. The structures can be very well observed in the figure 5 shown below.

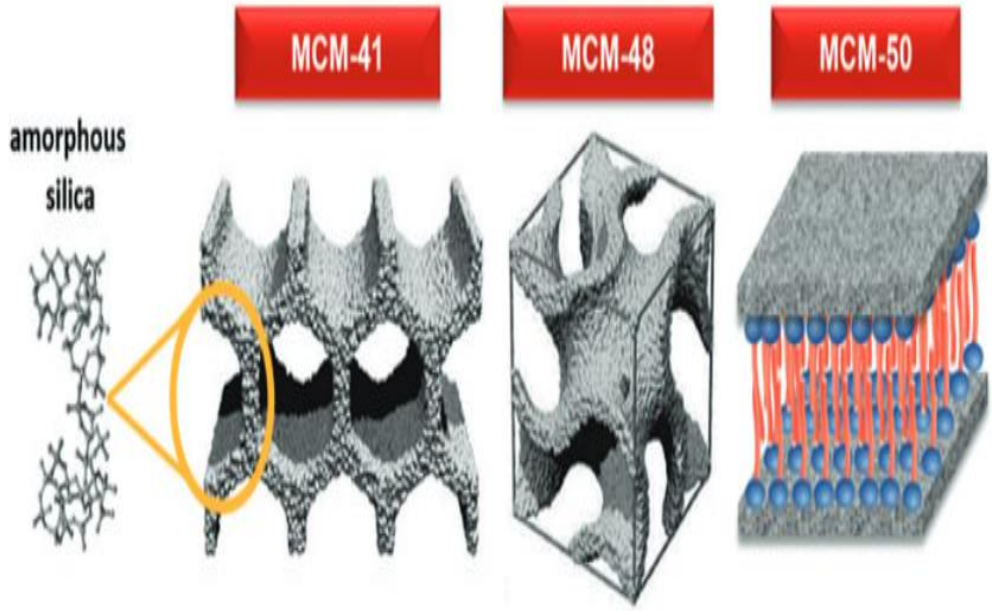


Figure 5: Structures of the members of M41S family

2.2.3 Properties of Mobil Composition of Matter

The properties of Mobil Composition of Matter that have made them quite useful these days and being used in different types of applications are their mesoporous structure, high surface area and high surface energy, high thermal stability and the pore size distribution which is very much uniform. The most remarkable factor about the two most popular types mentioned above is that although, they are made of amorphous silica wall, the possession of long range ordered framework with uniform mesopores

is present there. Large surface area is also occupied by these materials which can be up to 1000 m^2g^{-1} [13]. Moreover, through the modification of the synthesis conditions and/or by utilizing surfactants with different chain lengths in the preparation of these materials, the pore diameter of these materials can be nicely regulated within the mesoporous range between 1.5 nm and 20 nm. The two materials have been also applied as catalysts for various chemical reactions, as a support for drug-delivery system and as an adsorbent in wastewater treatment [11]. Other properties of the members of the Mobil Composition of Matter include relative non-toxicity, non-corrosiveness, non-air sensitivity and the MCMs' being highly reusable materials.

2.2.4 Applications of Mobil Composition of Matter

The unique and excellent properties of Mobil Composition of Matter have made the researchers and the scientists to make its use in different fields and different applications. The applications that these mesoporous materials have made their way into are nanomaterials, biotechnology, adsorption technology, usage of the M41S family members along with inorganic compounds and organic compounds in environmental applications. The usage of these mesoporous materials for the remediation of carbon dioxide (CO_2), mainly because CO_2 is one of the major gases that plays a role in the greenhouse effect, which has been released into the atmosphere because of burning of fossil fuels and /or other sources of energy by food, fuel, petrochemical, automotive, steel, and mineral industries [14].

These functionalized mesoporous materials are widely used in approaches that aim at the pre-concentration of organic and inorganic compounds from their use as a sorbent in extraction methods. These are also used as catalysts in different reactions and as the heterogenous catalysis is one of the most researched applications in materials science focused on the environmental issues, the use of such materials is very much usable [14]. The members of the M41S family of mesoporous materials are also promising candidates for hybrid catalysis because they

are relatively non-toxic, non-corrosive, non-air sensitive, highly reusable, completely pollution-free, and environmentally benign supports for catalytic transformation in the liquid phase [14]. Figure 6 shown below provides us with the information about the evolution of the number of catalytic applications using mesoporous materials that include the members of the M41S family i.e., MCM-41 and MCM-48.

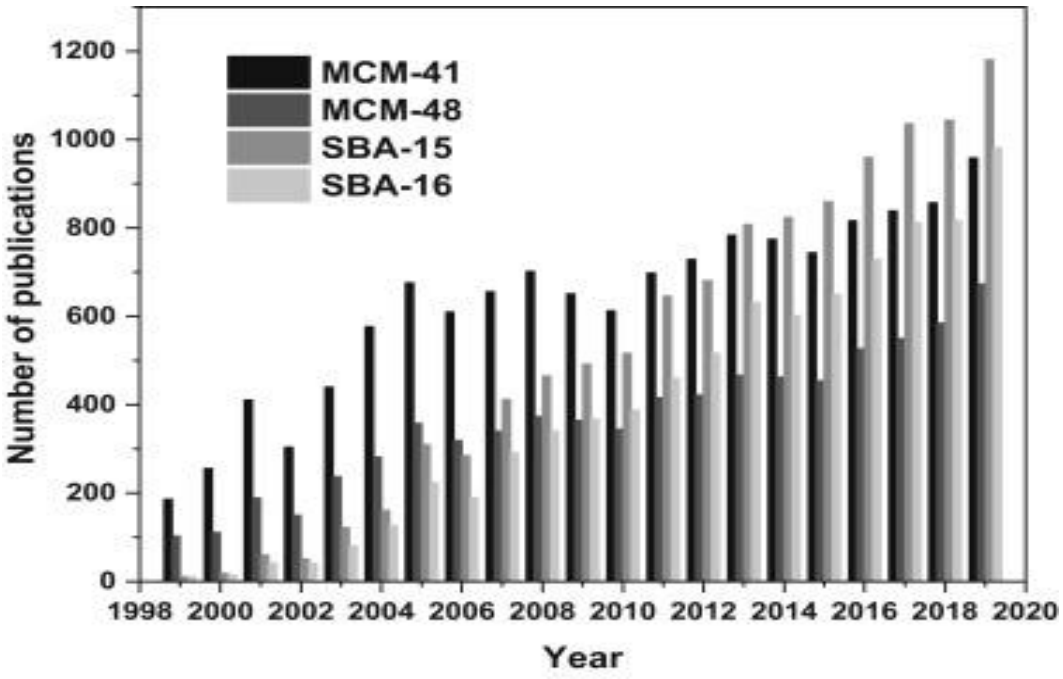


Figure 6: Evolution of the number of catalytic applications using mesoporous materials

2.3 Mobil Composition of Matter No.41

Mobil Composition of Matter No.41 (MCM-41) is a mesoporous material that was created by the Mobil Oil Corporation. MCM-41 is part of a family of silicate and alumo-silicate solids that are very well suited for use as catalysts and catalytic supports. MCM-41 has a hierarchical structure that has an ordered arrangement of cylindrical mesopores that range in diameter from 2nm to 6.5nm as its base. The independently adjustable mesopores form a unique, one-dimensional pore system that has sharp, well-defined pore distribution and large surface area and volume [15].

2.3.1 Synthesis of Mobil Composition of Matter No.41

The synthesis of Mobil Composition of Matter No.41 can be done by using various synthesis methods; few of which are mentioned in the details below.

1. **Microwave synthesis method**

   In this method, a certain precursor was added into deionized water at the room temperature. Under strong mixing, another precursor was slowly added to the solution and the mixed solution was agitated for a certain amount of time. The pH of the mixture was altered to a certain amount using a molar solution [16]. After all of this, the gel that was obtained was placed in a microwave vessel and heated under certain conditions. The resulted solid was then centrifuged at high speed, washed with distilled water, dried in air overnight, and calcinated at high temperature for a certain period [16]. By doing the steps mentioned above, the synthesis of MCM-41 was done, and the material was obtained.

Figure 7: Schematic diagram of synthesis of MCM-41 by the Microwave synthesis method

2. **Hydrothermal method**

   In this synthesis method, a precursor as the template was added to deionized water and agitated to form a uniform solution. After this, another precursor was introduced in the solution and the solution was agitated for some time. Another precursor after all of this was added into the solution and was agitated for certain period [17]. The solution was then moved to a steel autoclave and stored at a fixed temperature for a fixed time. The pH was adjusted typically during the time to attain stability. The obtained product was then filtered, washed, and stored at fixed temperature for a fixed time. The final step of the method was calcination of the final product at a high temperature for a period and MCM-41 was synthesized [17].

3. **Modified conventional synthesis methods:**

   MCM-41 was synthesized through a modified conventional synthesis method by adding a precursor A to a solution containing precursor B, a base, and deionized water. For the classical method, after the further agitation without the ultrasound irradiation for short time at low temperature, the pH of the solution was fixed by the addition of an acid [18]. For the ultrasound method, the gel mixture was stirred with ultrasound irradiation for some time at low temperature and the pH of the mixture was fixed. The mixture was kept at room temperature for a period. The resulting powder was filtered, washed, and dried at a temperature for a certain time. The synthesized material was then calcinated to remove the surfactant. MCM-41 was obtained by using the above synthesis methods [18].

2.3.2 Properties of Mobil Composition of Matter No.41

The interest of researchers in MCM-41 since the time it was discovered is due to the properties that it offers. The properties that are provided by MCM-41 are large internal surface, high thermal and hydrothermal stability, possibility of controlling the pore dimensions, and potential

acidity [19]. The material also displays the property of biocompatibility [20]. The material also provides highly ordered mesoporosity (pores with diameter between 2 and 50 nm) and surface roughness. MCM-41 also provides certain degradability and meostructure stability depending on the application the material is being used in [21]. All these properties lead to the usage of this material in certain applications that require these properties such as in-situ synthesis of a composite that comprises of MCM-41 and certain nanoparticles.

2.3.3 Applications of Mobil Composition of Matter No.41

The important applications of Mobil Composition of Matter No.41 due to the properties that it provides is the usage of it in adsorption, CO_2 capture, catalysis, drug delivery and biomedical applications in the last few years [19]. Other applications that MCM-41 finds its use in are semiconductors, biofluids and production of novel materials by the encapsulation of metals. The porosity of MCM-41 makes it an ideal candidate for the loading and encapsulation of metals, metal oxides, semiconductors and molecular liquids e.g. water. Recently, composites of MCM-41 with metals are used as catalytic templates to synthesize single-wall or multi-wall nanotubes of uniform diameter. These are some of the applications that MCM-41 finds its use in, and research is still going on to use this material in other applications.

2.4 Nanoparticles

Nanoparticles are being explored due to their unique properties as compared to the bulk materials. The properties of these materials are dependent on the reduction in the size. Nanoparticles are the class of materials that include particulate materials having one dimension less than 100nm.

Depending upon their size, shape, and chemical composition, nanoparticles have got a broad classification. The interest in these materials is due to the unique properties that arise due to the size reduction of the materials which provides them with unique properties

such as chemical reactivity due to high surface area and energy, electrical conductivity or electrical resistivity, strength, hardness, and biological activity.[22]

Nanoparticles come in the category of 0D nanomaterials. These materials can have different shapes and morphology depending upon the synthesis technique used to synthesize them. Nanoparticles are not just simple substances as they consist of three layers. i) The surface layer which can be functional for specific surfactants, metallic ions, polymers, and small molecules, ii) The shell layer, which is completely chemically different from the core material, iii) The core, which is the center of the nanoparticle, also known to be the nanoparticle itself. [23]

2.4.1 Types of Nanoparticles

Depending upon the composition of the nanoparticles it is classified into various types which include the following:

2.4.1.1 Carbon-based Nanoparticles

There are two major classes of carbon-based nanoparticles that include fullerenes and carbon nanotubes. Fullerenes are nanomaterials comprised of globular hollow cage while the carbon nanotubes are materials having one dimension greater than 100nm. Fullerenes are characterized by their high strength, electrical conductivity, and versatility. [24] Similarly, the carbon nanotubes are formed by rolling a single sheet of graphene in such a way that it forms an elongated and tubular structure. Typically, they have a diameter of the order 1-2nm and are characterized by their unique mechanical, chemical, and physical properties. Due to the unique properties, they offer, they can be used in multiple applications like fillers, supporting materials, or gas absorbents. The figures below show the fullerenes and carbon nanotubes. [23]

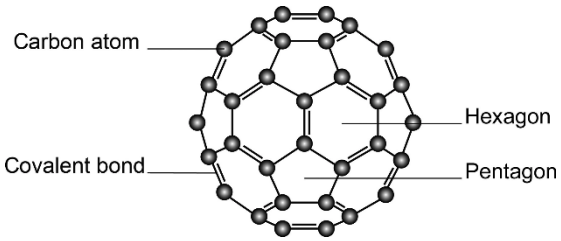


Figure 8: Fullerene (buckyball)

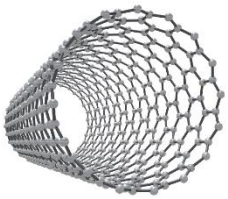


Figure 9: Carbon-nanotube

2.4.1.2 Metal Nanoparticles

These are the nanoparticles that are purely synthesized from the precursors of metals. These particles have unique optoelectrical properties and a wide absorption band for the visible region that allows them to give away different colors according to their sizes. Due to their properties, they are being used in multiple applications like sensors, catalysts, medical applications, microelectronics, etc. One of the examples of the applications is the sputtering of gold nanoparticles onto non-conductive samples being characterized through a scanning electron microscope (SEM) to enhance the electronic stream which provides us with a better-quality image.

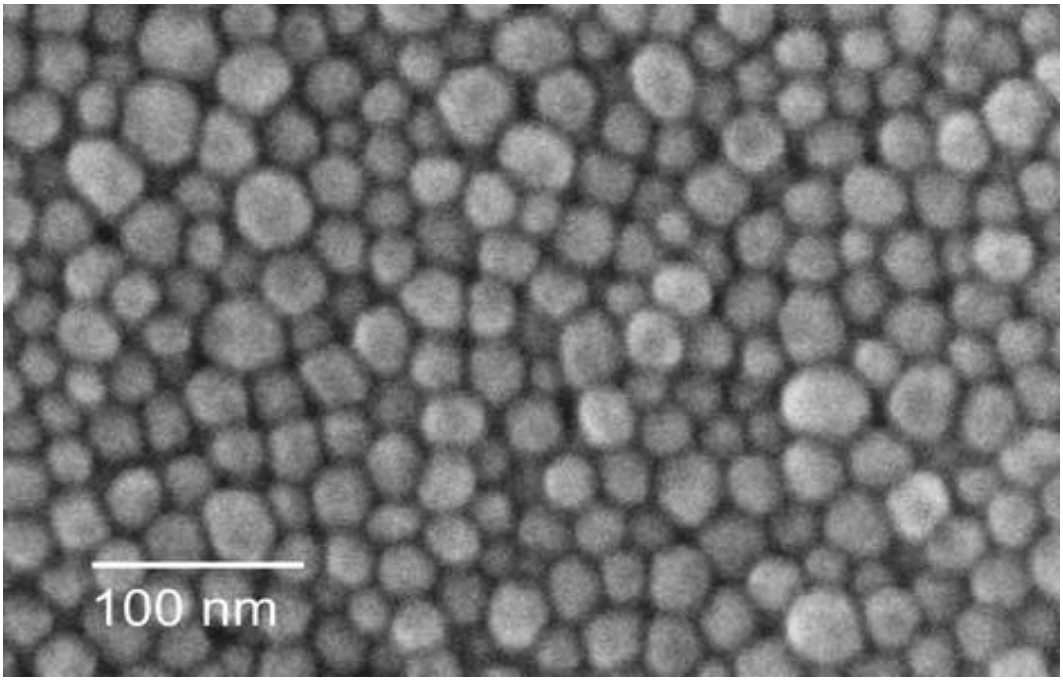


Figure 10: SEM image of Gold Nanoparticles

2.4.1.3 Ceramic Nanoparticles

These are the non-metallic and inorganic solid particles that can be crystalline or amorphous, dense, or hollow [25]. The properties that these provide due to their shape and size makes them available in vast applications i.e., photocatalysis, imaging applications, etc.

2.4.1.4 Semiconductor Nanoparticles

These are the nanoparticles that lie in between metal and non-metals. Due to the band gap present in them, they possess various unique properties. These properties can be altered by tuning the band gap.[26] Due to this reason, they can be used in various applications like electronic devices, photocatalysis, etc.

2.4.1.5 Polymeric Nanoparticles

These are organic-based nanoparticles which exist in the shape of nanospheres. In nanospheres, there are matrix particles that have solid mass, and the molecules are absorbed in the surface of the sphere while in the nano-capsular shape the solid mass is encapsulated in the particle fully which acts as a reservoir system.[27] The figure shows the difference between both.

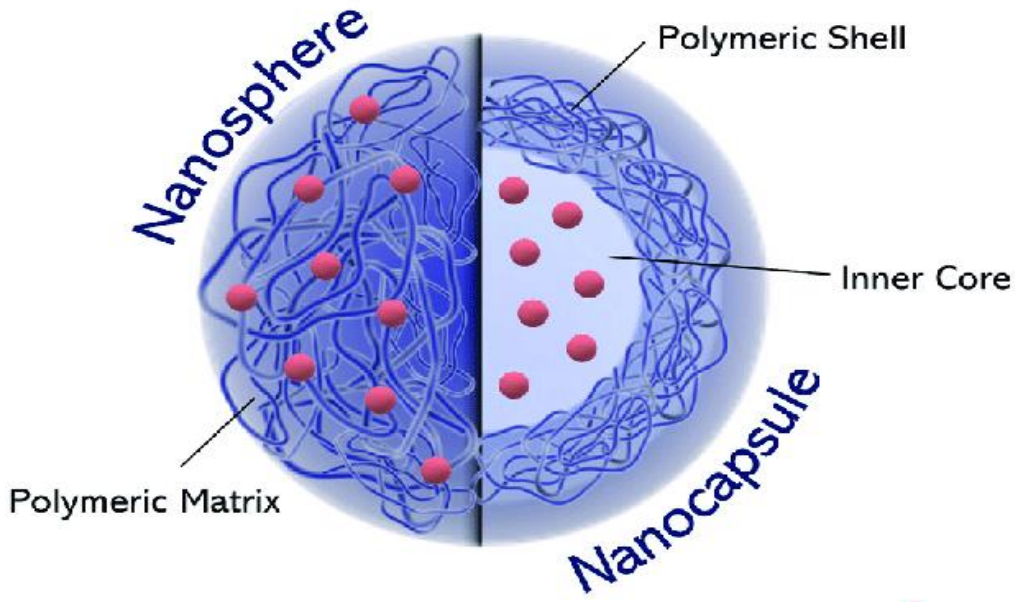


Figure 11: Polymeric NPs

2.4.1.6 Lipid-based Nanoparticles

These particles have a constituent of lipid. They have a spherical shape that has a core and a matrix surrounding them. The core consists of a lipid and the matrix contains soluble molecules.[28] Due to a core present, they have wide applications in biomedical applications like drug delivery, etc.

2.4.2 Synthesis of Nanoparticles

There are various methods being used to synthesize nanoparticles. The methods used to synthesize nanoparticles decide the uniformity of size, morphology, and size. There are two main classifications for the synthesis methods that are discussed below.

- Top-down approach
- Bottom-up approach

2.4.2.1 Top-down Approach

This is a destructive technique in which the bulk is broken down into smaller parts which are then converted into required nanoparticles. There are various techniques present that fall under this category which are mentioned below.

2.4.2.2 Optical Lithography

This is the most used technique to synthesize the pattern on the electronic devices. This technique produces nano-electronic devices. This technique is photon based in which the photons fall onto the substrate that produces a specific pattern of order in nanoscale. The resulting product is long-lasting and is an accepted nanofabrication tool.

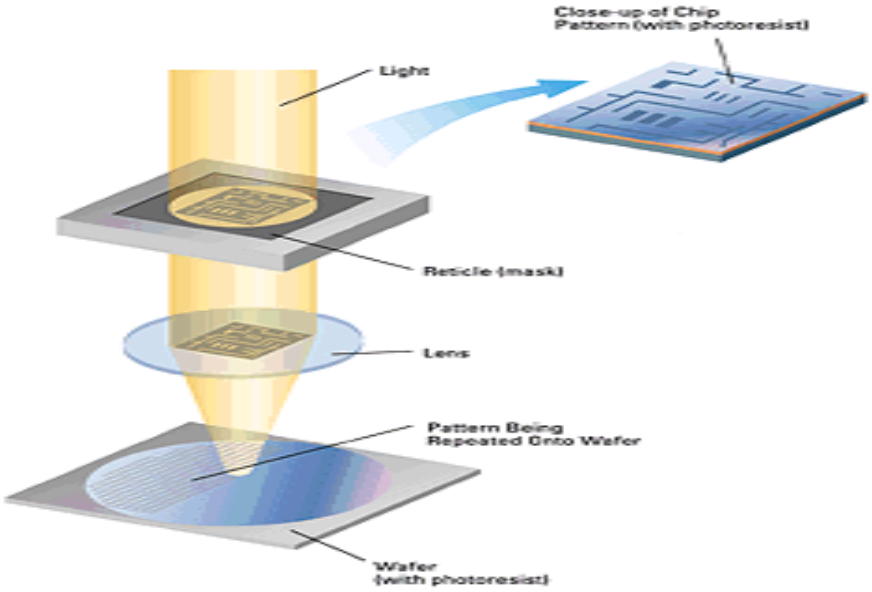


Figure 12: Schematic of Optical Lithography

2.4.2.3 E-beam Lithography

This is another approach of lithography to produce a pattern with a resolution of about 10nm. In this process, a highly focused electron beam is allowed to strike the substrate producing the designed pattern. This technique is very well used for research purposes as this is highly effective to produce the required pattern.

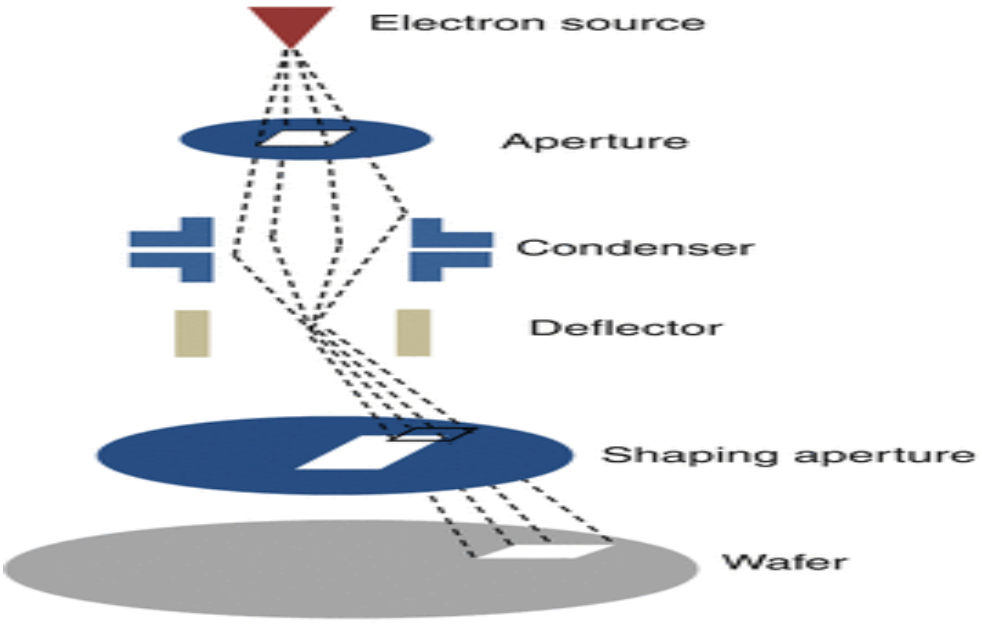


Figure 13: Schematic of E-beam Lithography

2.4.2.4 Soft and nanoimprint Lithography

Another type of lithography used to produce ultra-low size features which are less than 10nm. In this lithography technique, the pattern is transferred onto the substrate. It falls under the category of mechanical lithography in which the designed pattern is imprinted onto the substrate through pressing.

2.4.2.5 Block copolymer Lithography

This is an emerging technique of lithography based on the self-assembly of the polymers that are used to produce the designed pattern at a relatively larger area than others, but the features produced are on the nanoscale. Various shapes can be formed through this technique including spherical, cylindrical, or lamellar.

2.4.2.6 Grinding/Milling

This is another technique to reduce the size of bulk materials to the required nano size. These large materials are broken down through balls to convert them into small parts which are then further processed to reduce the size at the nanoscale. The size depends upon the milling time. As the time increases, the size reduces further.[29]

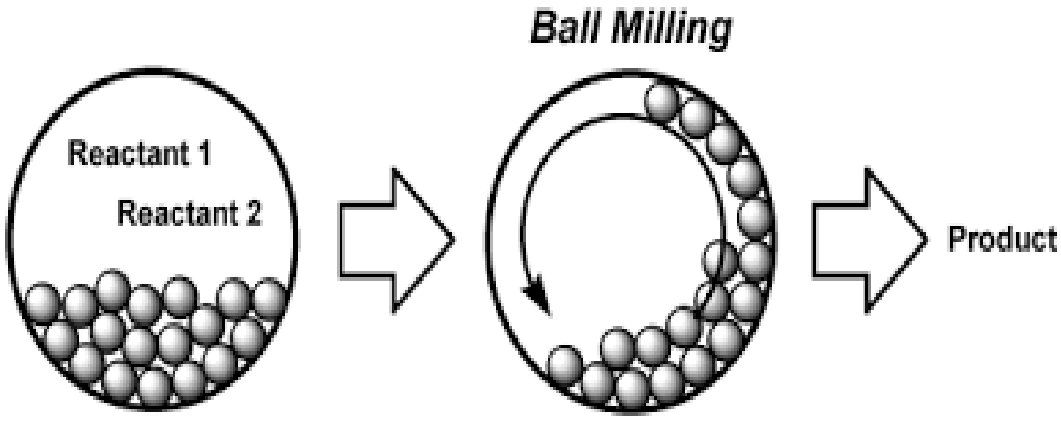


Figure 14: Schematic of Milling

2.4.3 Bottom-Up Approach

This is another method for the synthesis of nanoparticles. In this approach, nanoparticles are synthesized by binding atoms together until the required size is achieved. This includes various techniques which are discussed below.

2.4.3.1 Atomic Layer Deposition

This is the technique that uses the vapor phase of the precursors to produce the required product. Atom by atom is deposited onto the substrate which produces a very fine and uniformed thick product. This technique has good reproducibility and adhesion obtained is strong due to formation of bonds.

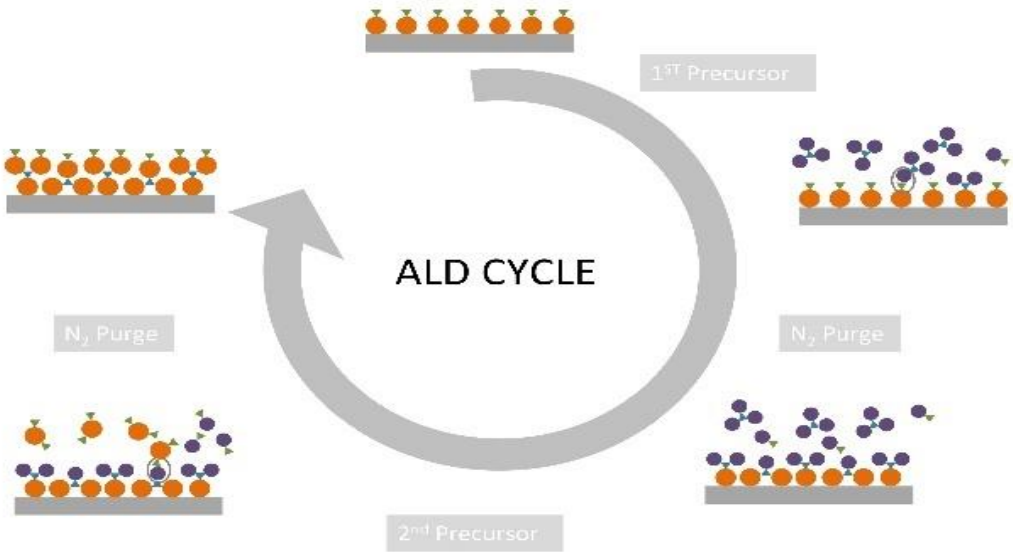


Figure 15: Schematic for atomic layer deposition

2.4.3.2 Sol-gel Nanofabrication

This is another very simple and old method used to produce the nanoparticles. It is a chemical reaction-based process in which the precursors are allowed to react to produce the required product. This method is used to produce various nanomaterials and nanocomposites.

2.4.3.3 Physical and chemical Vapor Deposition

This is another synthesis method which is used to produce nanoparticles. In this method, controlled deposition of materials is done and allowed to settle to form the required product. It is a versatile tool for the synthesis of nanomaterials as well as complex nanocomposites. It is a scalable process.

2.5 Copper oxide Nanoparticles

CuO nanoparticles fall in the category of metallic oxide nanoparticles. Metallic nanoparticles with unique properties of electrical conductivity, optical, chemical, and physical play very important roles depending on their size and shape.[30] Copper oxide (CuO) has a semiconductor nature with a narrow band gap which makes it very suitable for photothermal and photoconductive applications.[31]

Copper oxide (CuO) with a one-to-one ratio of copper and oxygen is very difficult to synthesize because maintaining the ratio is a difficult task. There are various methods to produce copper oxide (CuO) nanoparticles with the required ratio. Below are some methods discussed that are used to synthesize copper oxide (CuO) nanoparticles.

2.5.1 Synthesis of Copper oxide Nanoparticles

There are various methods to synthesize the copper oxide (CuO) nanoparticles which include the following methods depending on the size requirements.

- Precipitation synthesis
- Sol-gel synthesis
- Electrochemical method
- Microwave irradiation

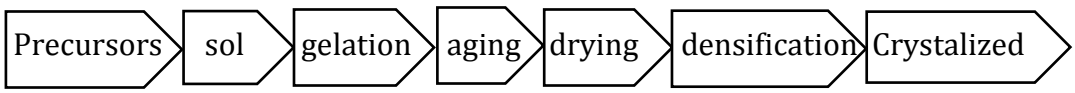
2.5.1.1 Precipitation Method

It is a very simple method to produce copper oxide (CuO) nanoparticles. In this method, the precursors are mixed to form a uniform solution. After

this, the temperature of the solution is raised. When the temperature reaches a specific range, a base is added to normalize the pH of the solution. This produces brownish particles. After the production of the brownish particles, the solution is stirred at a constant speed and temperature. The temperature allows the precursors to react together to provide uniform-sized nanoparticles. After the reaction ends, it is allowed to cool down. The precipitates settle down which are then washed, dried, and annealed.[31] Through this process, copper oxide (CuO) nanoparticles are obtained which are of the size of 4nm.

2.5.1.2 Sol-gel Synthesis

This is an old, simple, and fast method to produce nanoparticles. That's why it has been used widely to produce copper oxide (CuO) nanoparticles. It is a chemical process in which the precursors are dissolved in a solvent-producing sol. Through heating, the reaction occurs (hydrolysis and polycondensation) and the sol converts into gel. The process is called gelation which is then followed by aging, drying, densification, and crystallization. In the end, the required nanoparticles are obtained in the size of the order 7-9nm. [22]. The flow diagram drawn below shows the steps that are performed in this technique.



2.5.1.3 Electrochemical Method

This is another method that is used to produce required nanoparticles which are copper oxide (CuO) nanoparticles. This method works on the reaction between the electrode and the electrolyte being used. When the reaction occurs, there is some deposition on the surface of the electrode due to the developed potential. By changing various parameters like temperature, voltage, current density, and time, this method helps to control the size and morphology of the required copper oxide (CuO)

nanoparticles. The size obtained of the nanoparticles from this method is of the order 4nm.

2.5.1.4 Microwave Irradiation

This is another method which is used to synthesize copper oxide (CuO) nanoparticles. In this process, microwaves are used to heat the solution. It is a relatively fast process of producing the nanoparticles. The homogeneous heating of precursors due to penetration into the solution allows this technique to produce the homogenized nanoparticle with controlled size. Through this method, the size of the obtained nanoparticles is of the order 3-5 nm.

Other methods to produce copper oxide (CuO) nanoparticles include sono-chemical synthesis, thermal oxidation method, thermal plasma technique, green synthesis, and hydrothermal synthesis.[22]

2.5.2 Properties of Copper oxide Nanoparticles

Nanoparticles have unique properties due to their very small size. Similarly, copper oxide (CuO) nanoparticles possess unique properties due to their size. Following are the properties possessed by the copper oxide (CuO) nanoparticles [30].

- They are semiconductors with a narrow band gap.
- They have a monoclinic crystal structure.
- Exhibition of high potential in metal oxides due to optical and antimicrobial activity.
- They are also photocatalysts due to low band gap.
- They have high thermal stability due to the stability they display at high oxidation states.
- Low toxicity and easy availability.
- High surface area allows the enhancement of its absorbing capability towards oxygen.
- High surface energy makes it an excellent catalyst.

- Size and morphology influence optical properties of copper oxide (CuO) nanoparticles.
- Magnetic properties depend on the morphology of the copper oxide (CuO) nanoparticles.
- Electrical conductivity of copper oxide (CuO) nanoparticles depends on the synthesis temperature.
- They provide an improvement in the thermal conductivity.
- They have an endothermic nature.

The properties offered by the copper oxide (CuO) nanoparticles make it an ideal choice for the application such as glucose sensing through a composite of MCM-41 and copper oxide (CuO) nanoparticles.

2.5.3 Applications of Copper oxide Nanoparticles

Due to various properties depending on their size and morphology, copper oxide (CuO) nanoparticles are being used in applications that include the requirement of doping material for semiconductors. Due to their good antimicrobial activity, they are being used as an antimicrobial agent, and due to their high surface energy, they are being used as a catalyst for the reactions too. Anti-cancer formulation and coating materials are also the applications they find a use in. They are used as an antioxidant because they absorb molecular oxygen due to high surface area [30]. They are also put to a use in thermoelectric and superconducting applications. A low band gap allows them to be used in solar energy conversions for solar cell window preparation. Semiconductor nature makes them a good choice for electronic and optoelectronic devices. They are also used in gas sensors [31].

2.6 Biosensors

A sensor is a device that displays a response to a physical stimulus, such as heat, light, sound, pressure, magnetism, or movement and then transmits the resultant electrical impulse as a means of the measurement of change in any intrinsic property of the constituent material.

The simplest definition of a biosensor that one can obtain is an analytical device that can perform the detection of changes in certain biological processes and do the conversion of the changes into electrical signals. The definition of the term biological process can be narrated as any biological element or material e.g., tissues, cells, acids, microorganisms etc. [46]

Biosensors have been under development for the last thirty-five years and the research in this field has become very popular over the last fifteen years. A biosensor can be defined as a device that causes the production of a measurable signal proportional to the concentration of the target analyte that usually incorporates a biological sensing element and measures the signals that are derived from the biological interactions [32]. Biosensors are the oldest of them all, yet sensors for only one analyte which is glucose have achieved widespread success. Several biosensors have been developed to provide diagnostic information regarding the health of a patient. Many different types of biosensors have been developed and an insight into the types of biosensors provide us the information regarding the enzymatic and non-enzymatic glucose sensing approaches over the past decade [47]. The figure shown below gives a pictorial representation of the important components of a biosensor.

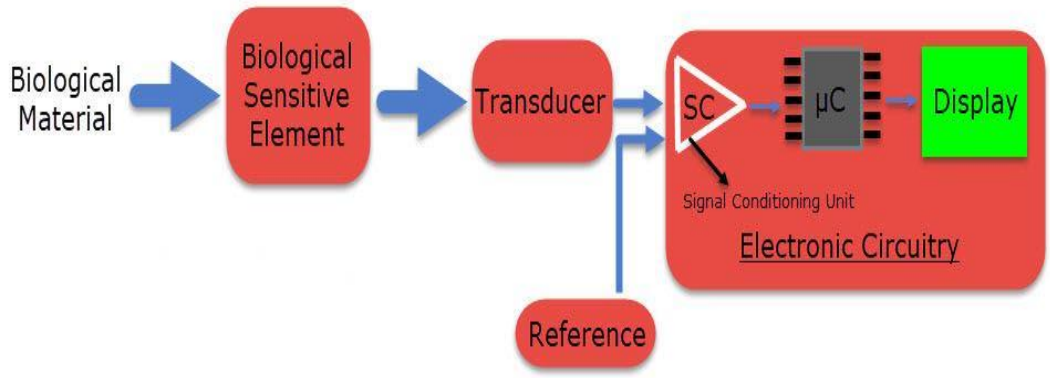


Figure 16: Important components of a biosensor

2.6.1 Principle and working principle of a Biosensor

The principle and working of a biosensor will be discussed in this section.

2.6.1.1 Principle of a Biosensor

The desired biological material is usually obtained in the form of an enzyme. The process of conversion of the enzymes into the corresponding electrical signals which is usually current using a transducer is known as Electroenzymatic approach. The process is a chemical one [47]

One of the biological responses commonly used is the oxidation of the certain enzyme that is being used. The oxidation of the enzyme performs the act of catalysis and results in the alteration of pH of the biological material. The alteration in the pH will be directly affecting the current carrying capacity of the enzyme which is again in a direct relationship to the enzyme being measured [46]. The output given by the transducer is the direct representation of the enzyme being measured. The current generally is then converted into voltage to get the proper analysis and make a representation of it.

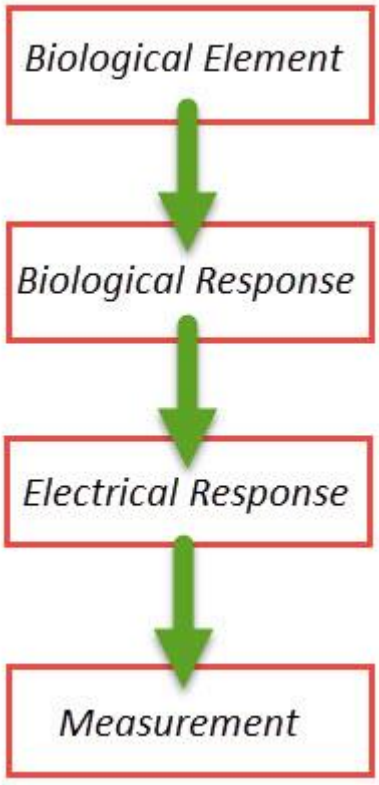


Figure 17: Principle of operations of a biosensor

2.6.1.2 Working principle of a Biosensor

In this part, the working principle of a biosensor will be discussed. The conversion of the biological material into a corresponding electrical response which exists in the form of signal is performed by the union of the biological sensitive element and the transducer [47]. The output provided by the transducer will be either current or voltage and is dependent on the type of enzyme being used. If the obtained output is voltage, the resulting data is fine but if the obtained output is current, then it needs to be converted into the equivalent voltage. The conversion is done by using an Op-Amp based current to voltage converter before the proceedings are moved further. The amplitude of the output voltage signal is very low and superimposed on a high frequency noise signal. Thus, this demands the signal to be amplified before passing it through a low pass RC filter by the usage of an Op-Amp based Amplifier. The component that can be held responsible for the process of amplification and filtration of the signal is the Signal Processing Unit or Signal Conditioning Unit. The terminology used for the output of the signal processing unit is known as an analog signal [47]. The output obtained here is equivalent to the biological quantity that is being measured. LCD display can be directly used to exhibit the analog signal, but this analog signal is delivered to a microcontroller, where the conversion of the analog signal into a digital signal takes place. This is done because it is easy to analyze, process or store a digital signal. The figure 18 below shows the schematic diagram of a biosensor's working principle.

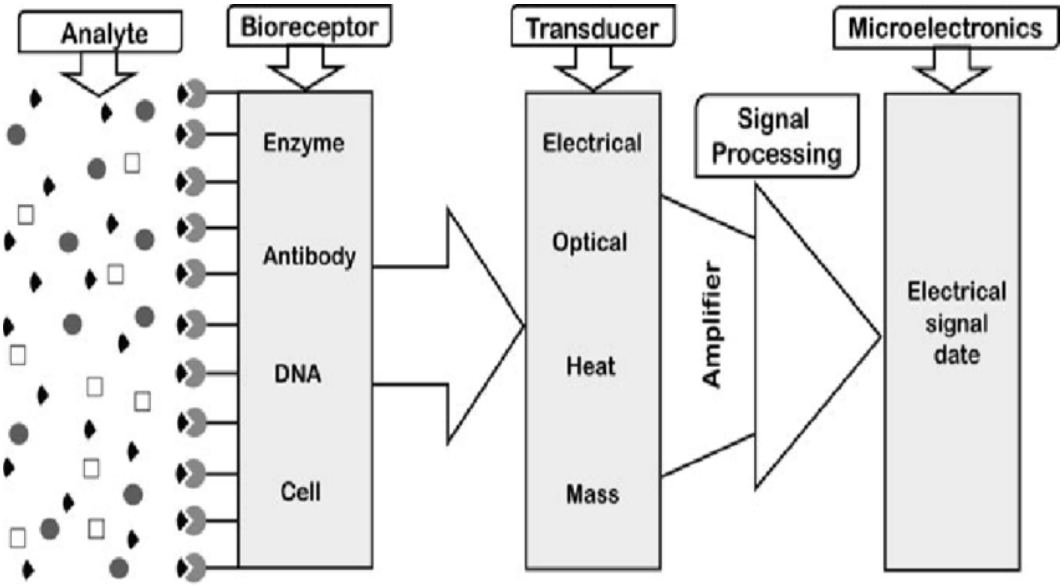


Figure 18: General schematic diagram of a biosensor's working principle

2.6.2 Types of Biosensors

The biosensors are classified into two different groups i.e., either based on the biological element that is being used in the analysis or the method of transduction implemented. As mentioned above, some of the common usable biological elements or one can say bio-recognition elements are the enzymes, antibodies, tissues, and cell receptors etc. [47]

The second group of biosensors that are based on the type of transduction used in the sensor. The transduction can be defined as the physiochemical resulting data from the sensing event. The biosensors that are based on the type of transduction are further divided into three types which include mass-based biosensors, electrochemical biosensors, and optical biosensors. There are further subtypes present in each of the mentioned sensors. These types are discussed below.

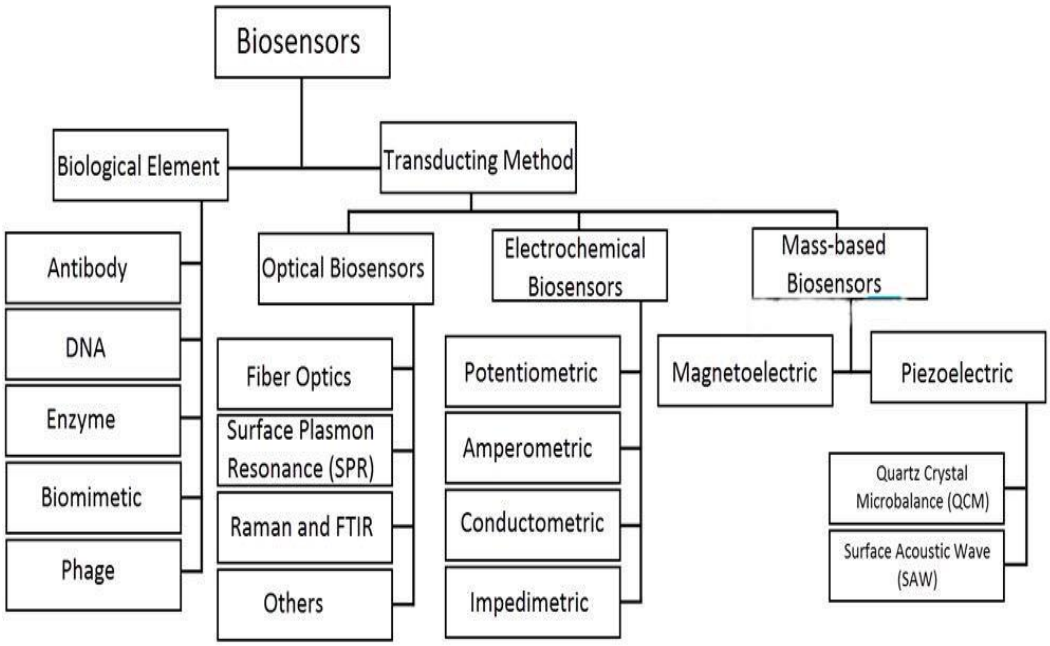


Figure 19: Different types and subtypes of biosensors

2.6.2.1 Piezoelectric Sensors

These are the subdivision of mass-based biosensors. They are also known as acoustic based biosensors because of them being based on the principle of acoustic vibrations. The thing that they perform is that when a mechanical load is applied on them, they produce an electric signal. The biological elements that are to be used are attached to the surface of the piezoelectric biosensor [46]. The sensor is a mass to frequency converter which converts the mechanical vibrations of the sensing molecules into the electric signals that are proportional to the mechanical vibrations of the sensing molecules.

2.6.2.2 Optical Biosensors

The importance of role of optical fibers in the case of optical biosensors can't be neglected. These optical fibers that are present in the optical biosensors allow the detection of the sensing elements based on their different properties of light which include properties like absorption, scattering, and fluorescence [35]. The reaction that occurs causes changes in either of the above-mentioned properties because of a change in the refractive index of the surface that is interacting. The example of this can

be taken as that if the biological elements being used are antibodies and are bounded with a metal layer, the refractive index that will be varied will be of the medium that encounters this layer. One of the advantages provided using these sensors is the non-electrical of them. This advantage allows them to perform the analysis of multiple elements present on a single layer just by varying the wavelength of the light [46].

2.6.2.3 Electrochemical Sensors

In this type of biosensors, a probing surface is coated with the biological molecules. The molecules that are performing the function of being the sensing molecules, are held in place with the help of a non-interfering membrane. After this, the sensing molecules react appropriately to the compound whose detection is to be done and the production of an electric signal that is proportional to the quantity being measured happens. These biosensors can employ different types of transducers that include potentiometric, amperometric, impedimetric etc. leading to the conversion of the chemical signal into an electrical signal that can be measured [47].

Electrochemical sensors perform the detection from the chemical reactions and then perform the measurements by using electrodes which is due to the interaction between the sensing surface and the analytes. The detection and the measurement coverts the responsive information to qualitative and quantitative electric signals. This is based on amperometry, potentiometry and conductometry measurements [32]. These sensors provide a method for detecting diseases that are simpler, faster, and more accurate than the conventional laboratory methods [33].

These biosensors are the most extensively studied biosensors since they provide the advantages of low detection limit, specificity, simplicity of construction and the ease of usage. Due to the recent advancements in electronic instrumentations, these biosensors can be miniaturized as lab-on-chip devices for the purpose of in-vivo monitoring or as a handheld device for on-site monitoring. One of the major applications of these type

of biosensors is their use in glucose detection. These are both scientifically and commercially used for the purpose of glucose detection to measure the blood sugar levels. The inspection of the blood sugar level is very important for the patients that are having diabetes and due to this very reason, electrochemical biosensors find their use in the application very widely spread. Electrochemical sensors that are mainly based on amperometric methods, represent the most relevant group of glucose biosensors, and comprise of both enzymatic and non-enzymatic sensors. Non-enzymatic amperometric glucose sensors have been based on the direct electrochemical oxidation of glucose [47]. Figure 20 displays the types of materials that are being used in electrochemical biosensors.

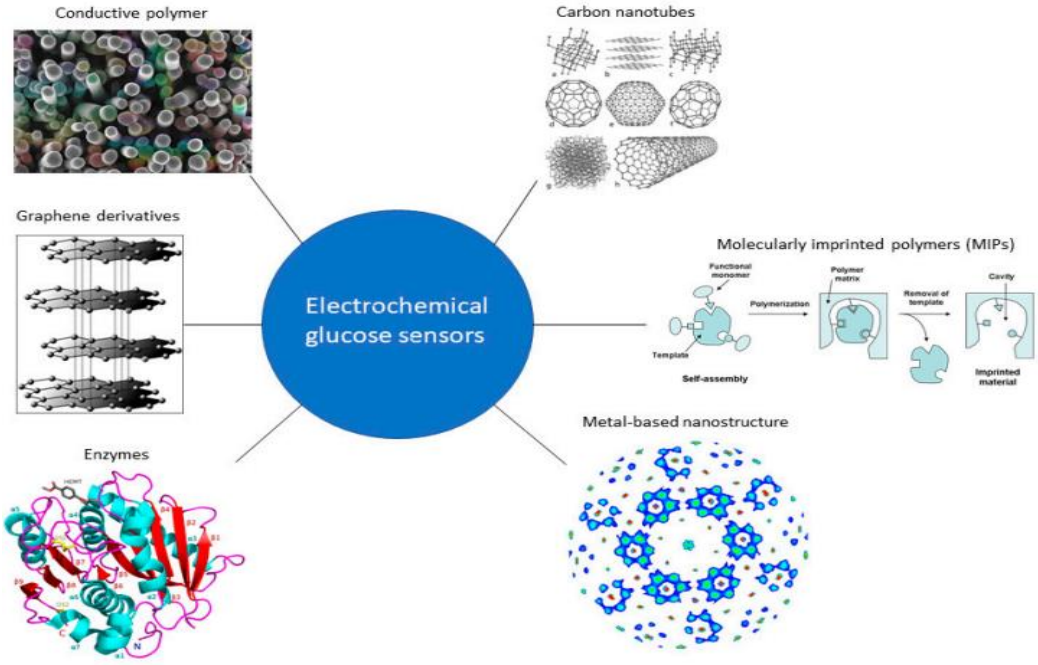


Figure 20: Materials used in electrochemical biosensors

The different reported glucose sensors, both enzymatic and non-enzymatic have been developed over the past few decades and research is still being done to move from the enzymatic glucose biosensors to non-enzymatic glucose sensors. Both have been discussed in the sections below.

2.6.2.3.1 Enzymatic Glucose Biosensors

Each body fluid in which the amount of glucose is proportional to its concentration in blood might be analytically useful; the only prerequisite here is that the other elements present in the sample should not affect the measurement either by the passivation of the electrode or by being acting as an electroactive interference [35]. Highly selective enzymatic reactions can be used to diminish the effect of the influence of those interfering species. Glucose oxidase (GO_X) is one of the most popular enzymes that is used for the glucose detection, and it can reduce the oxygen to hydrogen peroxide while at the same time causing the transformation of glucose into D-glucono-1,5-lactone. Quantification of glucose can be achieved on either the detection of the hydrogen peroxide or the oxygen consumed [35].

Different types of materials that have been developed to perform the role that is played by an enzymatic biosensor over the period have helped the researchers to bring in enhanced selectivity and sensitivity but still not on the level of the one that is provided by the non-enzymatic glucose sensors. Some of the materials that have been developed for the enzymatic glucose sensing include Fe-MIL-88B-NH_2 with glucose oxidase that represented enhanced activity of the nanozyme, excellent reusability, tolerance to temperature and acid-base and improved glucose detection. Another enzymatic glucose sensing material that was developed for this purpose was MCM-41@Se with glucose oxidase that displayed an increased sensitivity and selectivity. These were the few enzymatic glucose sensing materials that were developed recently but the level of sensitivity and selectivity provided by them was still not enough as compared to the non-enzymatic glucose sensing materials.

2.6.2.3.2 Non-Enzymatic glucose sensors

Non-enzymatic sensing could provide with an alternative to enzymatic methods which can be affected by many factors such as temperature, pH, and the presence of detergents [35]. It is quite a difficult task to sterilize

the enzyme-based electrodes, which is one of the requirements in the case of implantable sensors. Additionally, the first-generation GO_X sensors which rely on oxygen as a mediator can be inefficient when applied to the body fluid samples that are oxygen deficient.

There are other challenges present in the development of non-enzymatic glucose sensors which include the difficult process of cleaning the electrodes, selection of the binders, and their respective usage. In addition, the preparation of electrode materials for non-enzymatic glucose sensors and their loading activity results in increased time consumption and overall expenses [36].

The enzymatic glucose sensors require an intermediary enzyme which can be glucose oxidase or glucose dehydrogenase to detect and quantify the glucose samples accurately while the non-enzymatic glucose sensors do not require an intermediary enzyme; instead, they make the direct use of the glucose available in the sample to quantify their levels [37]. Another thing that the enzymatic glucose sensors suffer from are the consequences of enzyme denaturation, inefficiency in the transfer of electrons within electrode surface and enzymes, inconvenient immobilization techniques, inability to reproduce results, deformation due to heat and other external chemical molecules in the vicinity of the samples. The non-enzymatic glucose sensors help in tackling these challenges and solving them. They are cheaper than that of the enzymatic glucose sensors and the lack of enzymes provide them with better stability and leaves them unaffected by external conditions such as temperature, ionic strength, pH etc. [37]. Moreover, the non-enzymatic glucose sensors have got the ability to provide results quicker than those of the enzymatic glucose sensors.

The materials that have been developed over the period in the last few years to be used as non-enzymatic glucose sensors or for the sensing of the glucose include Ni-MOF with Au nanoparticles (enhanced the non-enzymatic glucose sensing), ZIF-67 synthesized, and Ag doped, TIO_2 nanoparticles on ZIF-67, Ag@ZIF-67/MWCNTs and Fe-MOF (where MOF

is Metal-Organic Frameworks) with Pt nanoparticles [37]. These are some of the few that have been named here under the heading of non-enzymatic glucose sensing materials or as sensors because the perform the job that is done by the sensors. Researchers are still working on the development of non-enzymatic glucose sensors because of the advantages they provide over the enzymatic sensors.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter focuses on the experimentation that was carried out for the synthesis of MCM-41, copper oxide (CuO) nanoparticles, and composite after they were synthesized successfully. Electrode preparation for the electrochemical testing was done for the detection of glucose.

3.2 Synthesis of MCM-41

The synthesis of MCM-41 was carried out through a hydrothermal process [34]. The apparatus, chemicals, and process being used are discussed in this section.

3.2.1 Chemicals and materials used:

- Cetyltrimethylammonium bromide (CTAB)
- Tetraethyl orthosilicate (TEOS)
- Ammonium hydroxide (NH4OH)
- Deionized water (DI water)

CTAB acts as a surfactant and is a structure-directing agent in this process while TEOS is the source of silica [17], Deionized water is used as a solvent, and NH_4OH was used to maintain the pH of the solution throughout the process.

3.2.2 Apparatus Used:

The following apparatus was used to carry out the synthesis:

- Drying Oven
- 50 ml Measuring cylinder
- 250 ml media bottles
- Pipettes of 10 ml
- Petri dishes
- Hot Plate
- Magnetic stirrer
- Spatula
- Weighing balance
- Aluminum foil
- Teflon Mold
- Autoclave
- Hydrothermal Reactor
- Centrifuge Machine
- Crucible
- Muffle Furnace

3.2.3 Procedure

As mentioned above, the hydrothermal method was used which was proposed by Kamarudin [34] for the synthesis so the procedure/experimentation for the process is as follows:

- 120 ml of DI water was measured using a measuring cylinder and put in the media bottle.
- Using weighing balance, 2.4 g of CTAB was measured and added to the DI water present in the media bottle.
- Stirred it using a magnetic stirrer on a hot plate until a uniform solution was formed.

- Using a pipette, measured 8 ml of ammonium hydroxide (NH_4OH), and added it to the above solution, and stirred it for 5 minutes.
- Using a pipette again, measured 10 ml of Tetraethyl orthosilicate (TEOS) and added it to the solution.
- Stirred the solution for 24 hours.

Figure 21: Stirring of Solution

- Moved the solution to the autoclave in a Teflon mold and the autoclave was placed in the hydrothermal reactor.
- The solution was kept at 145 ^{0}C for 48 hours.

Figure 22: Hydrothermal Reactor

- After cooling, the product was centrifuged and washed with DI water several times.
- The obtained product was moved to the oven for drying at a temperature of 60 ^{0}C for 24 hours
- Finally, calcination of powder obtained was performed in a muffle furnace at 600 ^{0}C for 5 hours.
- MCM-41 was produced which was ready for testing through different characterization techniques.

Figure 23: MCM-41 Sample

3.3 Synthesis of Copper oxide Nanoparticles

The synthesis of copper oxide (CuO) nanoparticles is the most important as it is very difficult to obtain the required copper (Cu) and oxygen (O) ratio. The process used for the synthesis was a simple precipitation method [17] which is discussed in detail below. The apparatus and chemicals & materials being used are also mentioned.

3.3.1 Chemicals and Materials

- Copper Nitrate (Cu $(NO_3)_2$)
- Polyvinylpyrrolidone (PVP)
- Sodium hydroxide (NaOH)
- Deionized water (DI water)

PVP was used as a surfactant, reducing agent, and shape controlling agent, NaOH was used to maintain the pH and DI water was used as a solvent.

3.3.2 Apparatus used

The following apparatus was used for the synthesis:

- 500 ml and 100 ml Beakers
- Pipettes of 10 ml
- Thermometers
- Magnetic stirrer
- Paraffin films
- Hot plate
- Aluminum foil
- Spatula
- Weighing balance
- Measuring cylinders
- Petri dishes
- Muffle furnace
- Crucible

3.3.3 Procedure

The procedure for the synthesis of copper oxide (CuO) nanoparticles was the precipitation method as mentioned above but some changes were made according to the literature. The precursors were used according to Mayekar, J. [35] but the temperature at which the reaction proceeded was according to A. Muthuvel [30]. So, the procedure is as follows:

- Using a measuring cylinder, took 100 ml of DI water in a beaker.
- Took 2.9 g of copper nitrate in water and stirred it to form a uniform solution.
- Took 1.2 g of polyvinylpyrrolidone into solution and stirred it to form a uniform solution

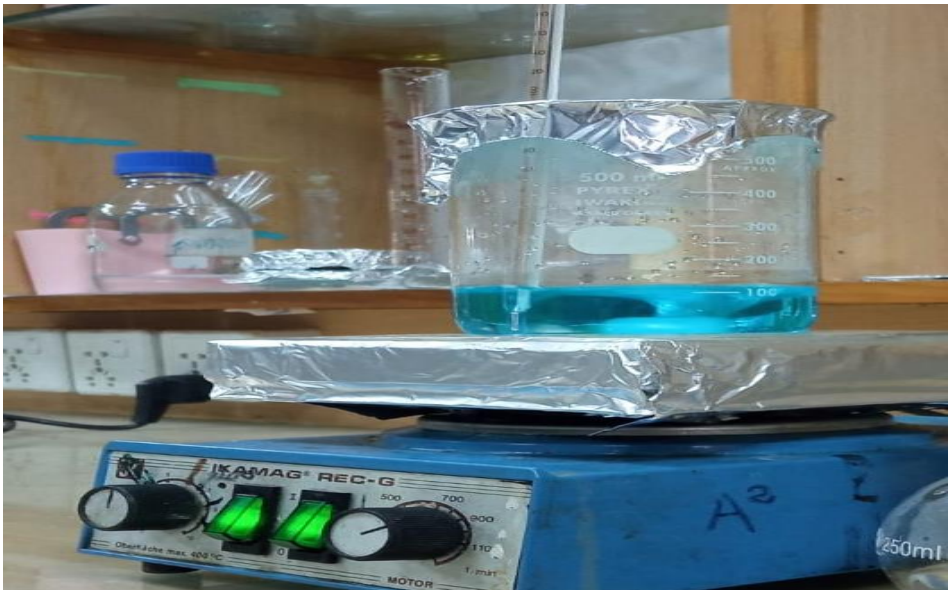


Figure 24: Stirring of solution

- Raised the temperature to 85 ^{0}C with constant stirring having a speed of about 800-850 rpm and kept the beaker covered with an aluminum foil to avoid vapor escape.
- Formed 1 M NaOH solution in DI water
- As the temperature reached 85 ^{0}C, a pipette was used to add NaOH dropwise to the stirring solution until the precipitates are completely formed and the solution changed its color from blue to brownish.
- Kept the temperature at 85 ^{0}C and stirred it for 2.5 hrs.
- The beaker was then removed from the hot plate and put aside to cool down.
- As the temperature reduced it was observed that the precipitates started to settle down at the bottom of the beaker.
- The clear water above the precipitates was removed by tilting the beaker and precipitates were washed several times with DI water.
- The precipitates were allowed to settle overnight

- After that, the precipitates were removed in a petri dish and dried in the oven until all the moisture was removed.
- The dried product was removed from the petri dish using a spatula

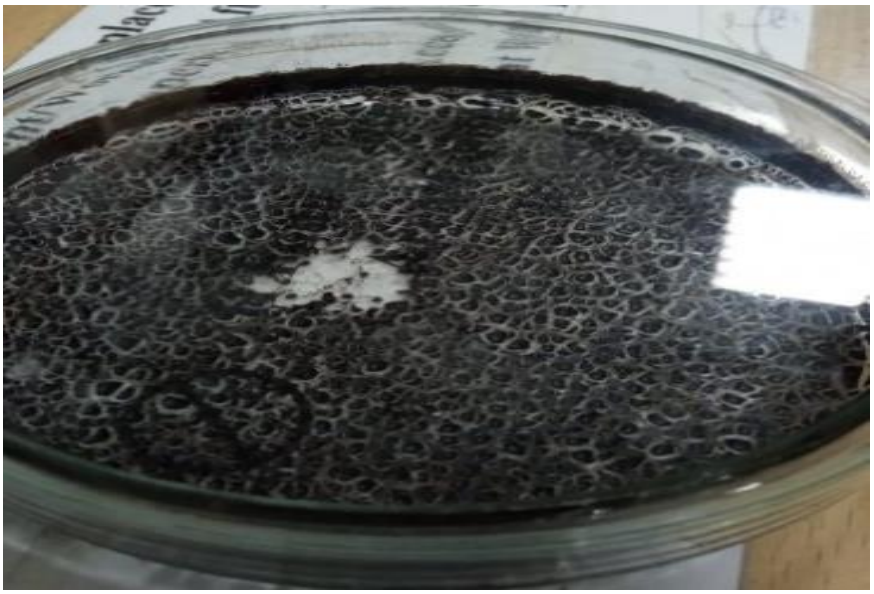


Figure 25: Dried CuO nanoparticles

- The product was ground using mortar and pestle
- Ground powder was annealed at 400 ^{0}C for 4 hours in a muffle furnace.
- Two samples were collected that were annealed and unannealed. Both were characterized to observe the changes that occurred due to annealing.

Figure 26: CuO Nanoparticles Sample

3.4 Synthesis of Composite

After the successful synthesis of MCM-41 and CuO nanoparticles, they were both combined to produce the composite. The synthesis of the composite was carried out in situ. While the synthesis of MCM-41 was proceeding, CuO nanoparticles that were already prepared were added. After the process was completed, we got the desired composite of MCM-41 and CuO nanoparticles. The chemicals & materials, and apparatus which were used are as follows:

3.4.1 Chemicals and Materials

The chemicals and materials used for this process are mentioned as follows.

- Cetyltrimethylammonium bromide (CTAB)
- Tetraethyl orthosilicate (TEOS)
- Ammonium hydroxide (NH4OH)
- Deionized water (DI water)

- Copper Oxide nanoparticles (CuO NPs)

3.4.2 Apparatus used

The following apparatus was used to carry out the synthesis of composite

- Drying Oven
- 50 ml Measuring cylinder
- 250 ml media bottles
- Pipettes of 10 ml
- Petri dishes
- Hot Plate
- Magnetic stirrer
- Spatula
- Paraffin films
- Glass vials
- Weighing balance
- Aluminum foil
- Teflon Mold
- Autoclave
- Hydrothermal Reactor
- Centrifuge Machine
- Crucible
- Muffle Furnace

3.4.3 Procedure

- 120 ml of DI water was measured using a measuring cylinder and put in the media bottle.
- Using weighing balance, 2.4 g of CTAB was measured and added to the DI water present in the media bottle.
- Stirred it using a magnetic stirrer on a hot plate until it formed a uniform solution.

- Using a pipette, measured 8 ml of ammonium hydroxide (NH_4OH) and added it to the above solution, and stirred it for 5 minutes.
- Using a pipette again, measured 10 ml of Tetraethyl orthosilicate (TEOS) and added it to the solution.
- Stirred the solution for 24 hours.
- Moved the solution to the autoclave in a Teflon mold and the autoclave was placed in the hydrothermal reactor.
- CuO nanoparticles were also added to the solution while moving it to autoclave by the ratio of 0.1 CuO:1 MCM-41.
- The solution was kept at 145 ^{0}C for 48 hours.
- After cooling, the product was centrifuged and washed with DI water several times.
- The obtained product was moved to the oven for drying at a temperature of 60 ^{0}C for 24 hours
- Finally, annealing and calcination of powder obtained were performed in a muffle furnace at 400 ^{0}C and 500 ^{0}C for 4 hours.
- This was done to check the effect of temperature on the composite.
- Composite of MCM-41 and CuO NPs was produced, and it was ready for testing through different characterization techniques.

Figure 27: Composite samples annealed at different temperatures

3.5 Preparation of Electrode

For the electrode preparation, a simple slurry of the composite was drop casted on a glassy carbon electrode. [36] The chemicals & materials, apparatus, and procedure used are discussed below.

3.5.1 Chemicals and Materials

The chemicals and materials that were used for this process are as follows.

- Dimethylformamide (DMF)
- Nafion
- Composite (MCM-41@CuO NPs)
- Diamond paste
- Ethanol

DMF was used as a solvent for the slurry, Nafion was used as a binder, and diamond paste was used as a polishing agent.

3.5.2 Apparatus used

- Glassy Carbon Electrode (GCE)
- Weighing balance
- Glass Vials
- Ultra Sonicator
- Micropipette
- Polishing pad
- Aluminum Foil

3.5.3 Procedure

The following procedure was followed for the preparation of slurry and electrode:

- The glassy carbon electrode was polished using diamond paste placed on the polishing pad to remove any contaminates and make the electrode surface completely clean.
- After polishing, it was washed and sonicated in ethanol for 5 minutes to ensure the cleaning.
- For the slurry preparation, 2ml dimethylformamide was taken in a glass vial.
- Using micropipette, 10 µL nafion was added to the DMF
- Then 3 mg of the prepared composite was measured using weighing balance and was added to the suspension in the vial.
- It was ultra-sonicated for about 30 minutes and the slurry was prepared.
- Using micropipette, it was drop casted onto the surface of glassy carbon electrode
- The electrode was allowed to dry overnight.

- Both samples of composite that were annealed at different temperatures (400^{o}C and 500^{o}C) were used one by one for slurry and testing.
- The electrochemical testing was carried out.

3.6 Preparation of Electrolyte for Electrochemical Testing

For the electrochemical testing, different solutions were prepared. The procedure is given below, and the chemicals & materials, and apparatus used are given.

3.6.1 Chemicals and Materials

The chemicals and materials that were used for the preparation of electrolyte for electrochemical testing are discussed below.

- Sodium Hydroxide (NaOH)
- Glucose ($C_6H_{12}O_6$)
- Distilled Water

3.6.2 Apparatus used

- Weighing Balance
- Beakers 100ml
- Measuring cylinder
- Magnetic Stirrers
- Hot Plate
- Aluminum foil
- Spatula
- Drying Oven

3.6.3 Procedure

- Beakers were washed and dried in the oven.

- Using a measuring cylinder, 50ml of distilled water was taken into the beaker.
- 0.1 M NaOH solution was prepared.
- With a similar solution, a 2mM glucose solution was prepared.
- Then the electrochemical testing was performed using Gamry.
- Similarly, different molar solutions of glucose were prepared for the testing which included 2mM, 4mM, 6mM, 8mM and 10mM of glucose solution in 0.1M NaOH.
- They all were tested to get the comparisons.
- After that, 8mM Glucose solution was taken and scanned at different scan rates varying from 5mV/s to 50mV/s.

CHAPTER 4

CHARACTERIZATION TECHNIQUES

4.1 Introduction

To identify the composition, morphology, and structure of our produced samples, several characterization techniques were implied. Then whether our sample detects glucose, electrochemical studies were carried out. The techniques we used are briefly explained below.

4.2 Scanning Electron Microscopy

It is an imaging technique which gives the composition and topography of a conductive sample surface. It uses an incident beam of electrons which interacts with the surface of the sample to produce backscattered and secondary electrons. These electrons are then used in the imaging.

Its working principle is based on hitting of the sample surface with a beam of electrons and secondary electrons are given off as shown in Figure 1. Intensity of these secondary electrons is measured and displayed in the form of a 2D image. The intensity also determines composition as the number of secondary electrons produced is different for each element.

The SEM setup consists of an electron gun, detector, focusing lenses, and sample compartment. Electron gun emits high energy electrons which hit the surface of the sample that is placed in the sample compartment. The focusing lenses focus this electron beam onto the sample while the detector detects the secondary electrons given off. A vacuum pump is used to create a vacuum inside the chamber to prevent the air molecules from deflecting the electron beam.

Before SEM analysis a sample needs to be prepared. Since conductive samples can be imaged only, any sample which is not conductive is covered with a conductive coating using sputter deposition. This causes the excessive electrons to flow through the sample surface and give a high-resolution image. [37]

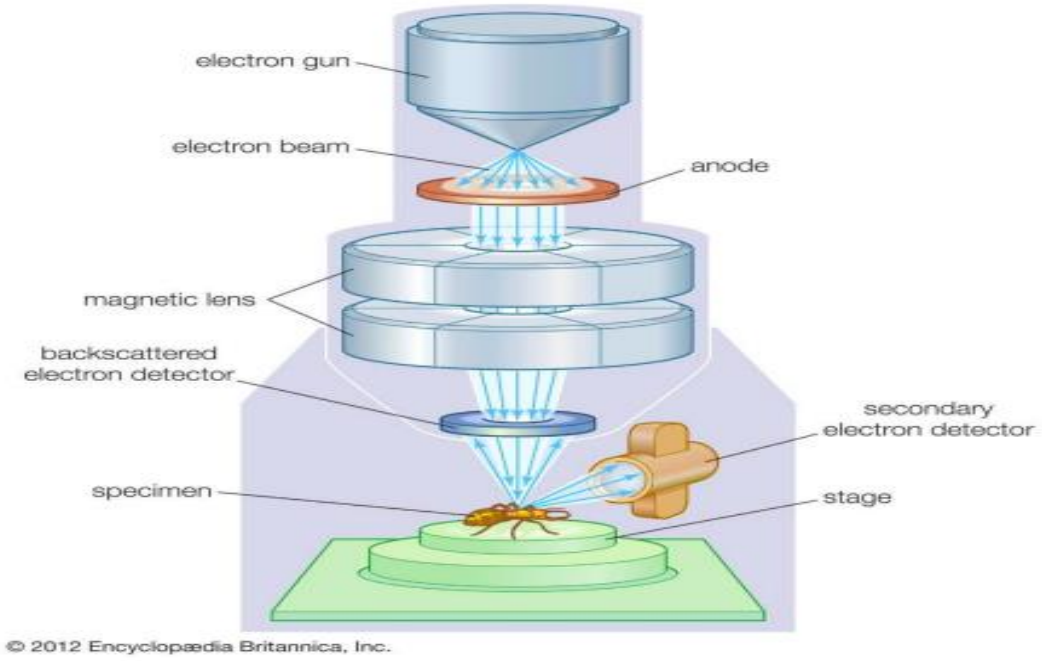


Figure 28: Schematic of Working of SEM [38]

Scanning electron microscopes have been used for various applications. Either its environmental sensitive sensing of material or the normal morphological and compositional studies, SEM can give high magnification and high-resolution imaging results due to its various types of electron interactions. [39]

4.3 Energy Dispersive Spectroscopy/ Energy Dispersive X-Ray analysis

EDX analysis is used along with SEM to give the composition of the sample. It is a highly sensitive technique for compositional analysis working on the principle of characteristic x-rays emission.

When incident electrons hit the atoms on the surface of the sample, electrons are knocked out from it. This causes the atoms to ionize. Now as

they return to their ground state, x-rays are emitted which are characteristic to the orbital energy through which electron transition occurred. Hence each element has different characteristic x-ray energies. A semiconductor detector is used for the detection of these x-rays.

In the resulting spectrum, the peak position determines the element present, while area under each peak gives the number of atoms of the element in the tested area. A software is then used to identify the element and give the results. [40]

The apparatus assembly is just like the scanning electron microscopy only with additional amplifiers and detectors.

4.4 X-Ray Diffraction

X- Ray Diffraction is a non-destructive technique used to get crystal structures of the sample. A beam of x-rays is directed towards the sample from where secondary electrons are ejected and detected by a detector. This can be shown in Figure 2. Some of these secondary electrons come from same planes of a crystal lattice where Bragg's Law is being satisfied. Hence, they interfere constructively to create sharp peaks in the final pattern representing the crystal structure of the material.

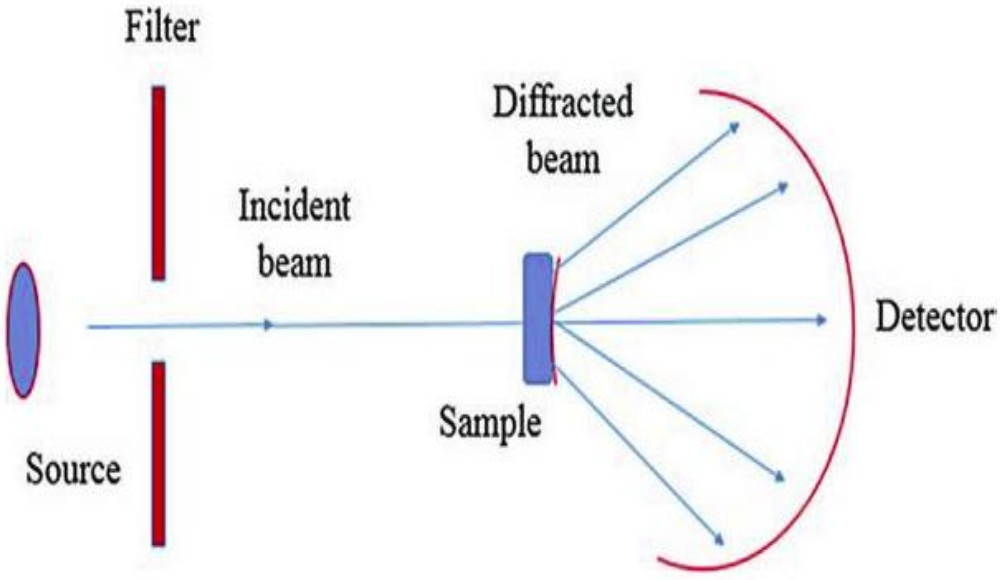


Figure 29: Schematic of working principal of XRD [41]

4.5 Fourier Transmission Infrared Spectroscopy

This is a technique used to identify the bonds present between the atoms of any material. Electromagnetic radiations within the infrared range are used. These are directed towards the surface of the sample. Some of these rays are absorbed while others are reflected by the sample. Each bond has a certain vibrational frequency according to which it absorbs the infrared rays. The reflected light can then be analyzed to create a signal representing the bonds present. The resulting graph is known as an interferogram.

4.6 Cyclic Voltammetry

It is an electrochemical technique used to determine a material's electrochemical activity. This is done by observing the redox reactions that the material can undergo. It consists of a 3-electrode based electrochemical cell. The material to be tested is coated on the working electrode. The counter electrode is used to complete the circuit by drawing in electricity. The reference electrode does not play any role in the reaction directly.

The three electrodes are placed in the analyte and the voltage across the electrode is swept linearly to highest value in the positive direction and then back again. The duck shaped graph gives the results of oxidation and reduction in form of current output. Oxidation reactions show that our material is able to sense properly while the reduction reaction shows whether the material has the ability to carry out more reactions consecutively.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Introduction

Several characterization techniques were used to identify the proper composition and structure of our produced samples. This chapter investigates the results obtained for all the samples that were worked on and the discussion of the results is also present in this chapter.

5.2 Characterization of MCM-41

To characterize MCM-41 FTIR, XRD, SEM, and EDX were performed on our sample.

5.2.1 FTIR of MCM-41

Figure 30 shows the use of FTIR to identify the functional groups in MCM-41 in the range 350–4000 cm-1. The peak at 3430 cm-1 represents the surface hydroxyl groups of SiOH. We can observe that two peaks of Si-O-Si are present which are due to differences in bond stretching energies. The peak at 1079 represents asymmetric stretching vibrations of the Si–O–Si bridges while the peaks at 809 cm-1 represent symmetric stretching vibrations of Si–O–Si. Bending vibrations of Si–O can be observed at 451 cm-1. [42][17]

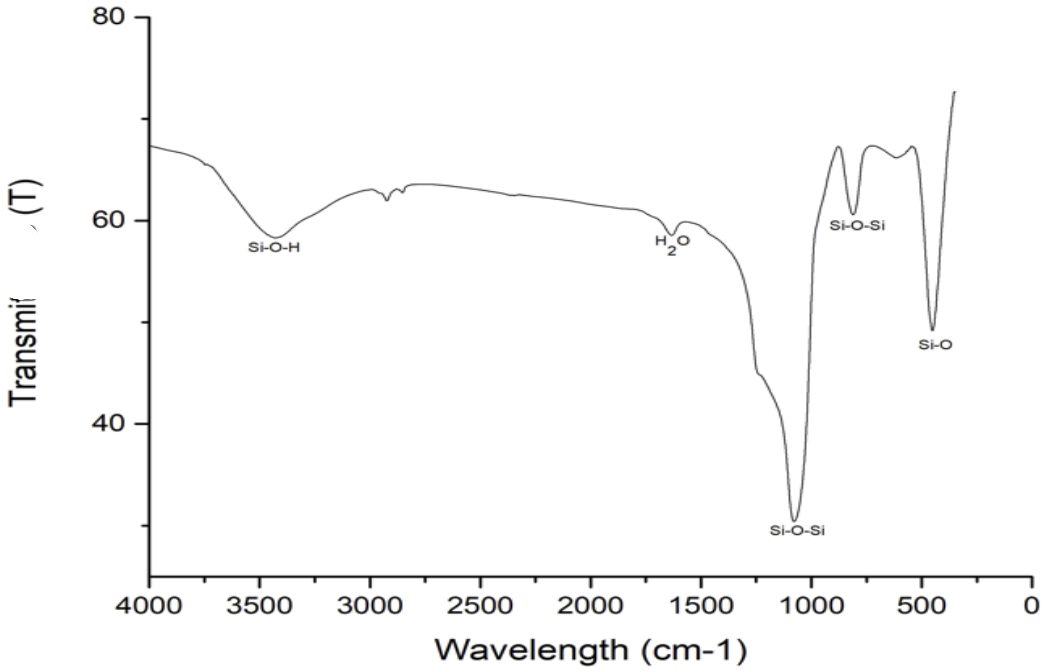


Figure 30: MCM 41 FTIR

5.2.2 XRD of MCM-41

Further XRD was carried out to determine whether the required crystal structure of MCM-41 was obtained. The results are represented in Figure 31. Two sharp peaks were obtained at $3 < 2\theta < 5$ in the XRD pattern which represent (100), (110), and (200) planes. These peaks show presence of a hexagonal unit cell which represents the structure of the MCM-41. [42][17]

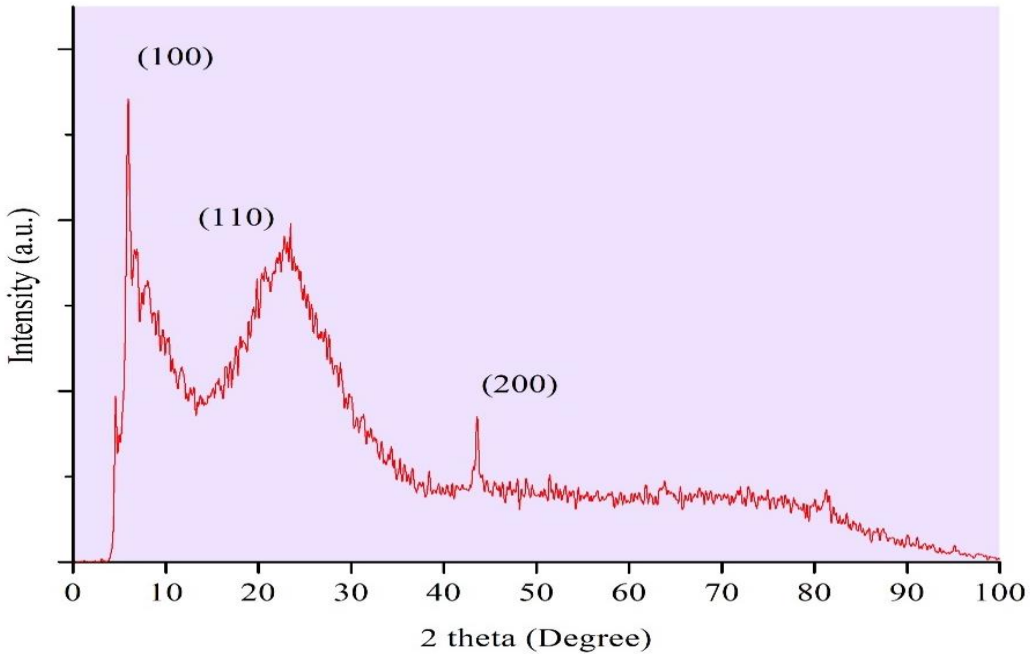
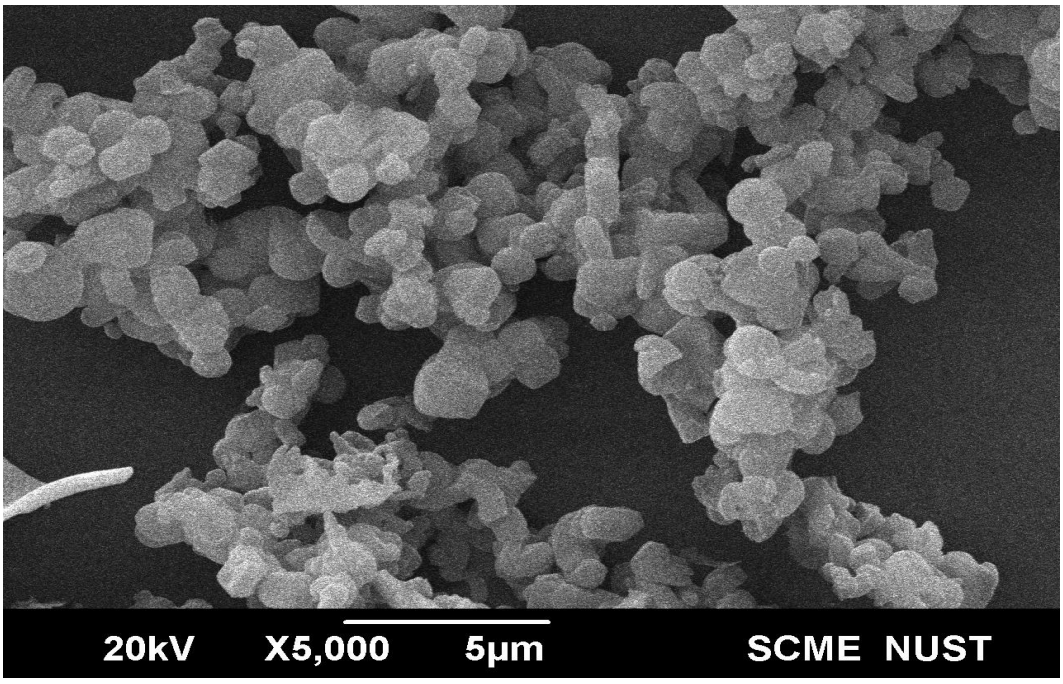


Figure 31: XRD of MCM-41

5.2.3 SEM Analysis of MCM-41

To further analyze the microstructure SEM was performed. The results of SEM as shown in Figure 32 represent the morphology of MCM-41 structure as small, uniformly sized particles. Also, SEM micrograph clearly shows that the structure has a spheroidal shape.



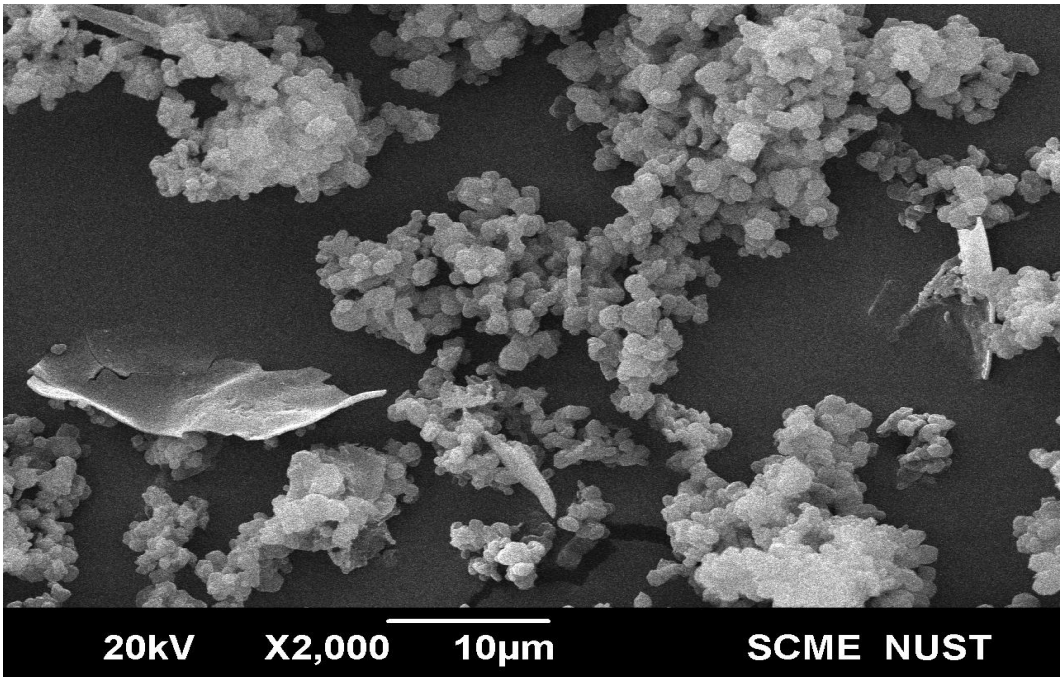
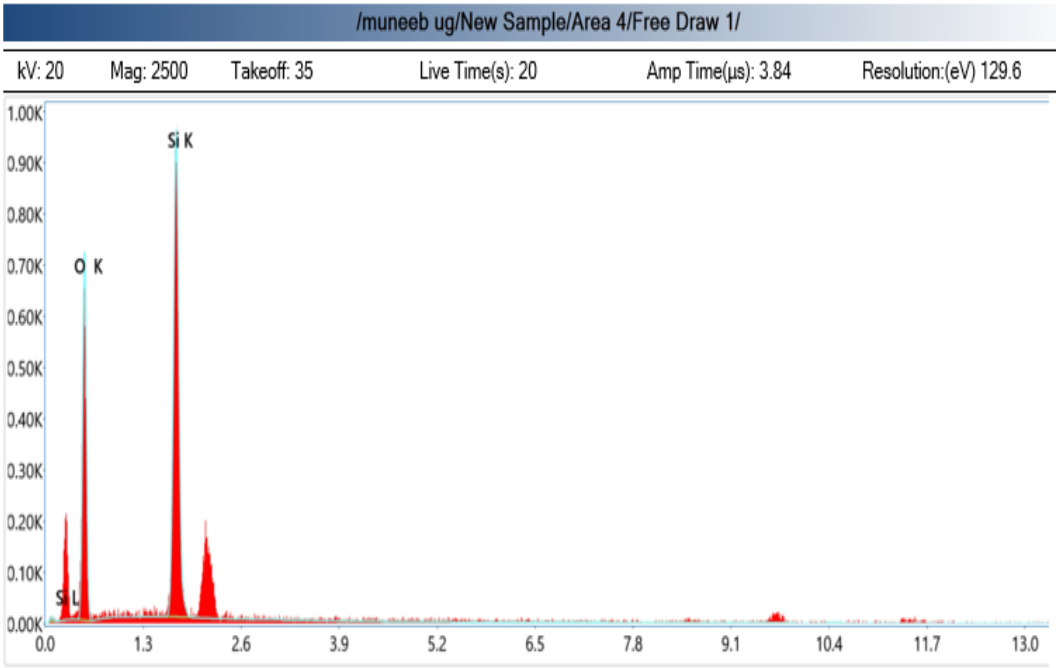


Figure 32: SEM Micrographs of MCM-41

5.2.4 EDX of MCM-41

MCM-41 was also characterized through EDX analysis. The elements present, and their respective percentages were analyzed. Figure 33 shows the results of EDS performed on MCM41. Peaks of silicon and oxygen are clearly visible. Moreover, it was deduced from the results that 73.3% oxygen and 26.7% silicon were present.



Element	Weight %	MDL	Atomic %	Error %
O K	61.0	0.62	73.3	10.7
Si K	39.0	0.42	26.7	5.5

Figure 33: EDX of MCM-41

5.3 Characterization of Copper oxide Nanoparticles

To characterize and identify the formation of copper oxide, CuO nanoparticles in our experiment, XRD, SEM and EDX were carried out.

5.3.1 XRD of Copper oxide Nanoparticles

XRD was used to determine the crystal structure of the nanoparticles produced. Peaks were observed from 30° to around 80°. However, two sharp peaks were obtained at $34 < 2\Theta < 40$ in the XRD pattern followed by sharp peaks with smaller heights. The peaks represent the Miller indices (-110), (002), (111), (-202), (020), (202), (-113), (-311), (-220), (311) and (- 222). This confirms the structure of the sample prepared is monoclinic as required in the CuO structure. [30]

Annealing of the CuO nanoparticles enhanced phase purity. As shown in Figure 34, the peaks are sharp, on the exact angles and showed no other impurity peaks. This indicates that the sample prepared is highly pure and according to that cited in the literature. [43][30]

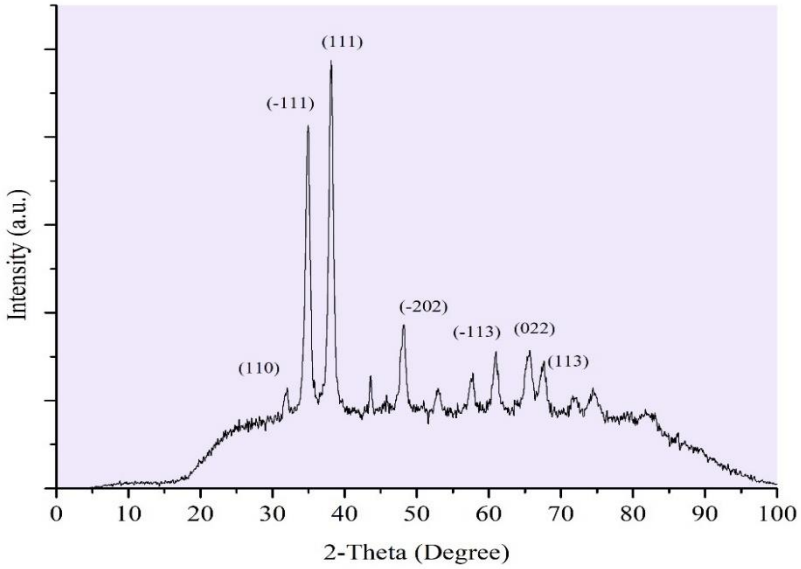


Figure 34: XRD Results of CuO Nanoparticles

5.3.2 SEM and EDX of Copper oxide Nanoparticles

The results of SEM analysis and EDS can be seen in Figure 35 and 36 below. From the SEM micrograph it is seen that the particles are agglomerated. CuO nanoparticles are highly reactive and agglomerate together easily. The results show that a proper dispersion of these particles is needed in future for better imaging results. The EDS analysis matches accurately with the reported literature and hence we can conclude that the required composition of CuO nanoparticles has been achieved. [31]

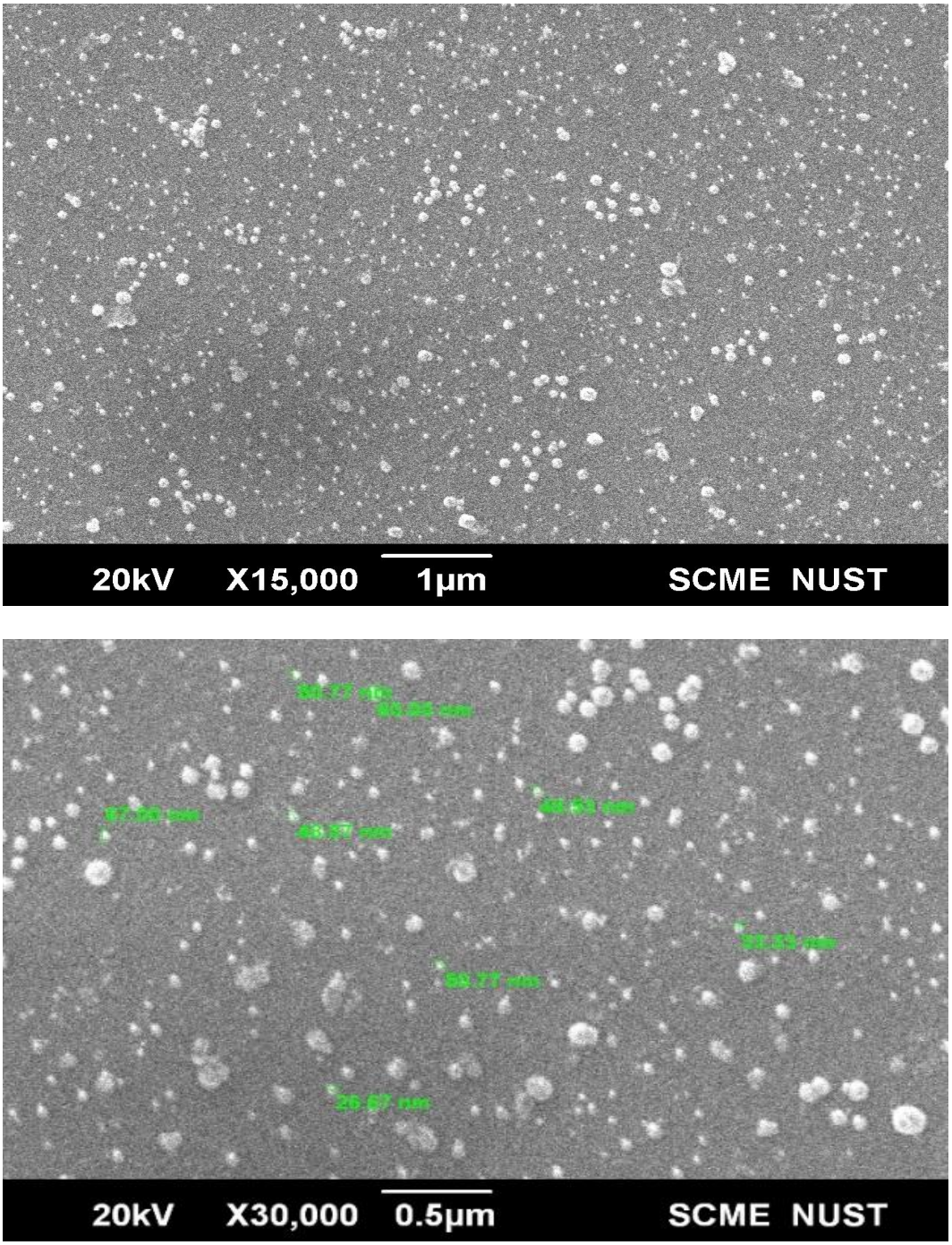
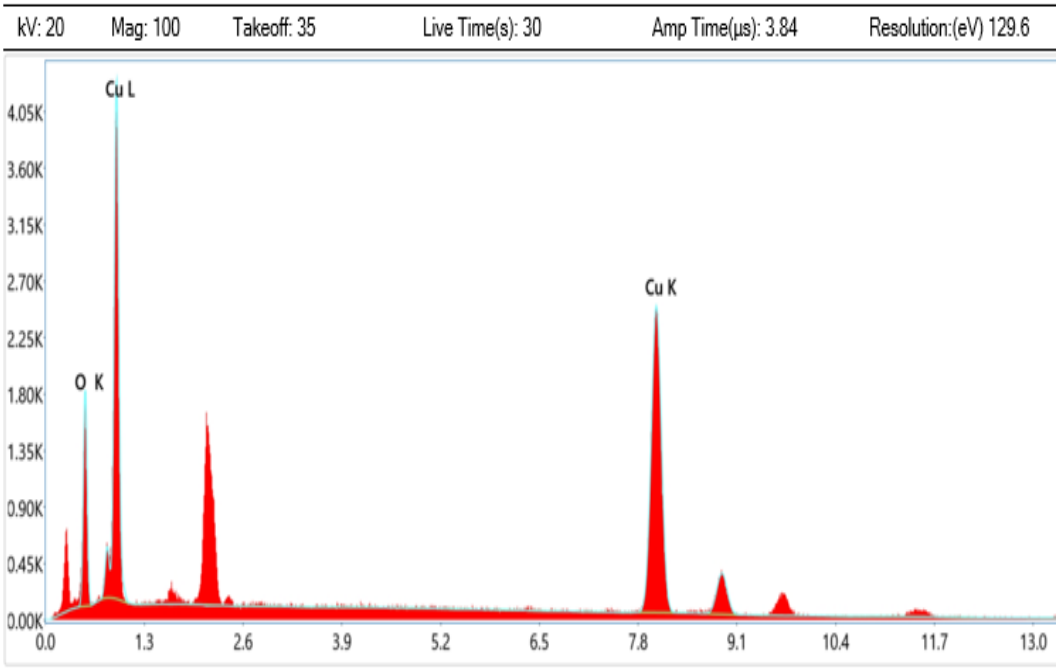


Figure 35: SEM micrograph of CuO Nanoparticles



Element	Weight %	MDL	Atomic %	Error %
O K	13.7	0.24	38.7	10.3
Cu K	86.3	0.74	61.3	2.7

Figure 36: EDS Result of CuO Nanoparticles

All of these results matched with the cited literature and hence show that the experiment was properly carried out to form the required structure of MCM-41 and CuO nanoparticles.

5.4 Characterization of Composite MCM-41@CuO Nanoparticles

Once the structures of MCM-41 and CuO nanoparticles were separately analyzed and the required structures were obtained, structure and composition of the composite made was analyzed using XRD, SEM and EDX analysis.

5.4.1 XRD of the Composite

Figure 37 below shows the superimposed XRD pattern of MCM-41 and CuO nanoparticles. The peaks observed in the composite are the same as those that were obtained in each of the components separately.

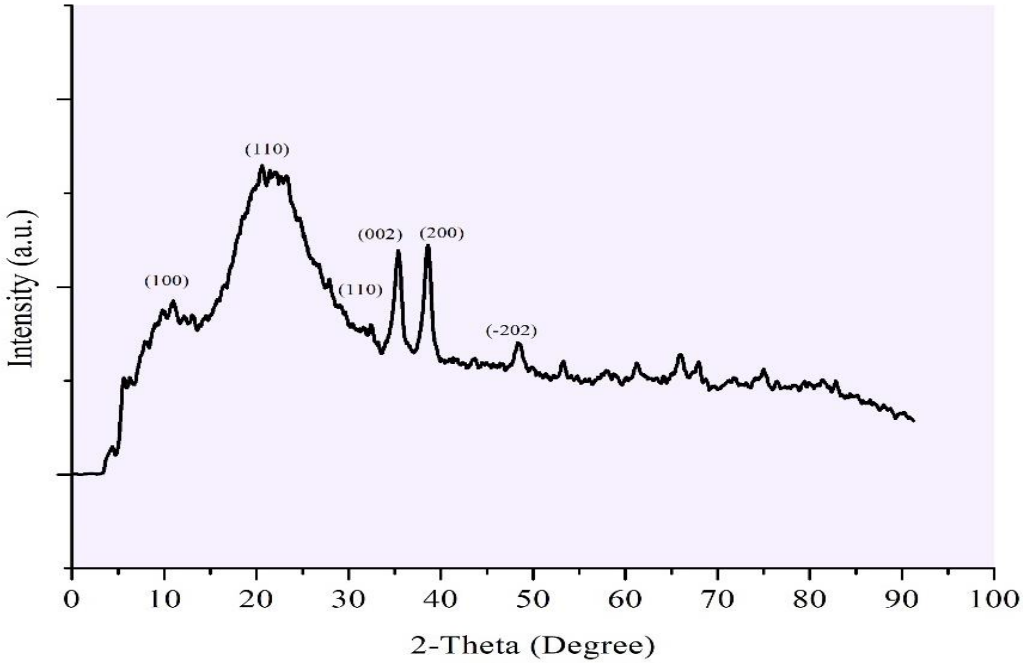


Figure 37: XRD of MCM-41@CuO NPs

The initial graph till around 25^0 represent the peaks obtained from MCM-41. The graph from approximately 34^0 and onwards represent the peaks for CuO nanoparticles. Peaks showing MCM-41 are broader representing the amorphous, porous, hexagonal structure of MCM-41. The peaks representing CuO nanoparticles are sharp, representing pure monoclinic structures of the particles.

5.4.2 SEM Analysis of Composite

Further, to analyze the morphology of the composite, SEM analysis was carried out. Both the micrographs in Figure 38 below clearly show the worm like structure of the MCM-41. The CuO nanoparticles are very small in size i.e., an average diameter of 48.3nm and hence are not visible in the micrographs among the large MCM-41 particles.

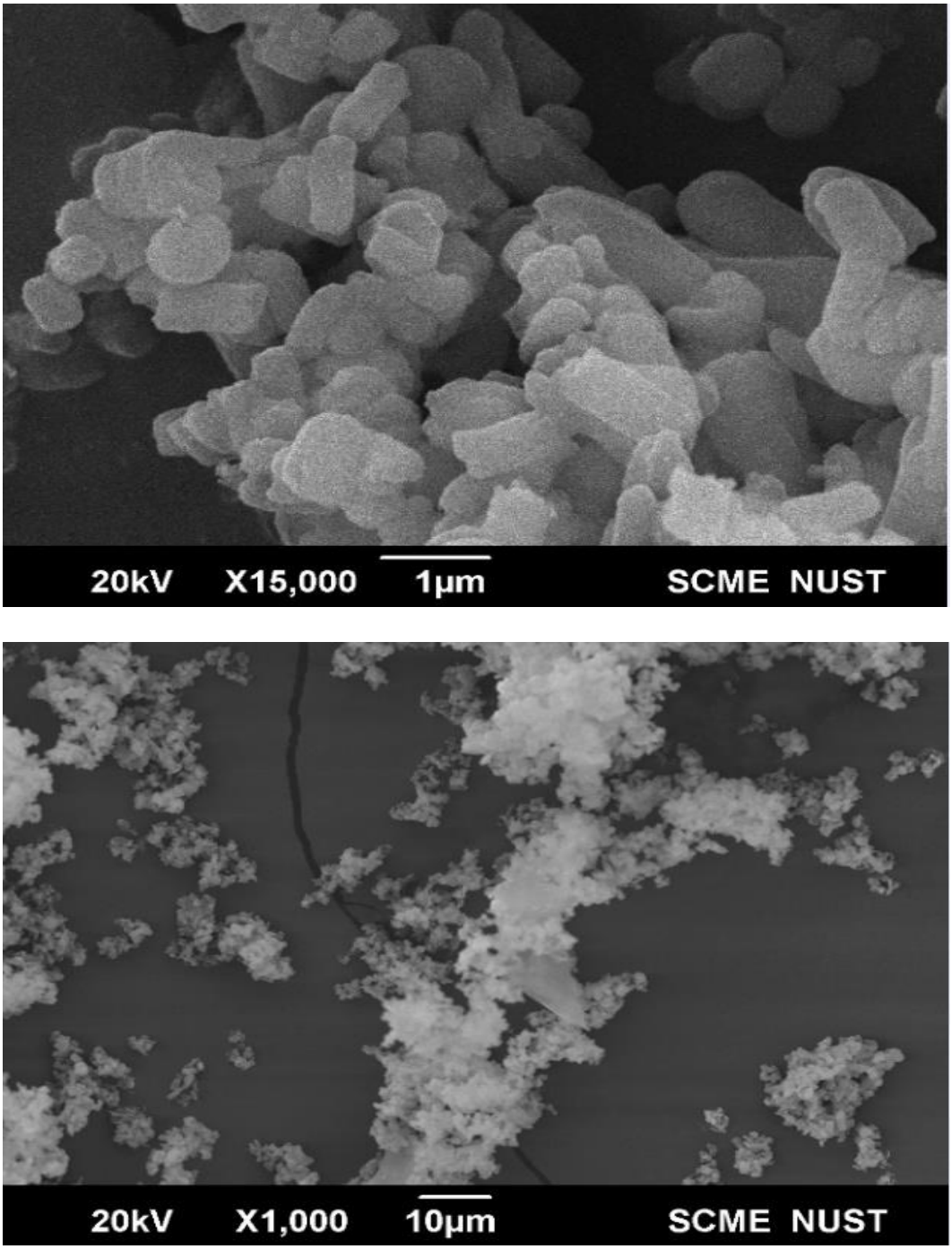


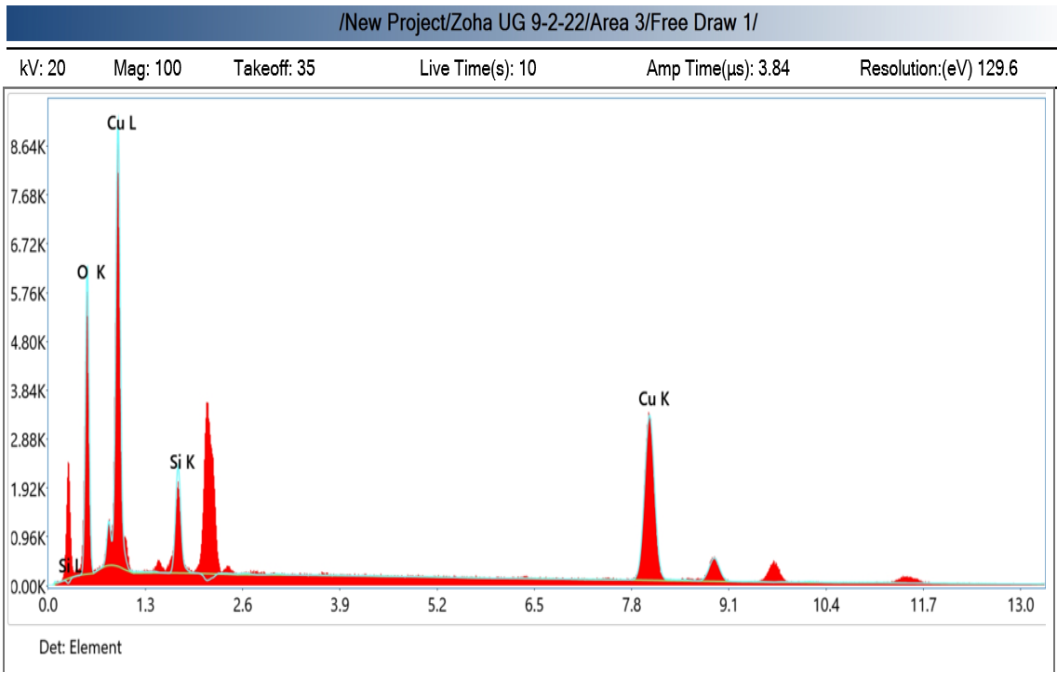
Figure 38: SEM Analysis of composite MCM-41@CuO NPs

From the literature it was deduced that the CuO nanoparticles will be decorated on the MCM-41 as its pore size is 4.5 to 6nm while the average size of CuO nanoparticles is larger.

5.4.3 EDX Analysis of the Composite

Though the CuO nanoparticles were not visible in the SEM analysis but with the use of the EDX technique, we were able to see that both the

MCM-41 and the CuO nanoparticles are present in the sample of the composite that was characterized.



eZAF Quant Result - Analysis Uncertainty: 15.61 %

Element	Weight %	MDL	Atomic %	Error %
O K	26.4	0.18	55.6	9.5
Si K	7.9	0.16	9.5	8.5
Cu K	65.7	0.53	34.9	2.6

Figure 39: EDX of MCM-41@CuO NPs

The figure 39 shows our sample contains silicon and oxygen which are the main elements present in MCM-41 and it also contains copper and oxygen which are present there in copper oxide.

5.5 Electrochemical Study of the Composite

The material MCM-41 that we selected after literature review had been used for enzymatic sensing only using glucose oxidase to facilitate the reaction. [44] However, our approach was to use the same material without glucose oxidase enzyme. Hence to enhance the activity of the

material, composite with CuO nanoparticles was formed. These particles are known for their high electrochemical outputs and hence were considered a better option to compensate for the enzymatic reaction.

For the electrochemical testing, Gamry was used to perform the cyclic voltammetry to check whether our composite is sensing glucose or not.

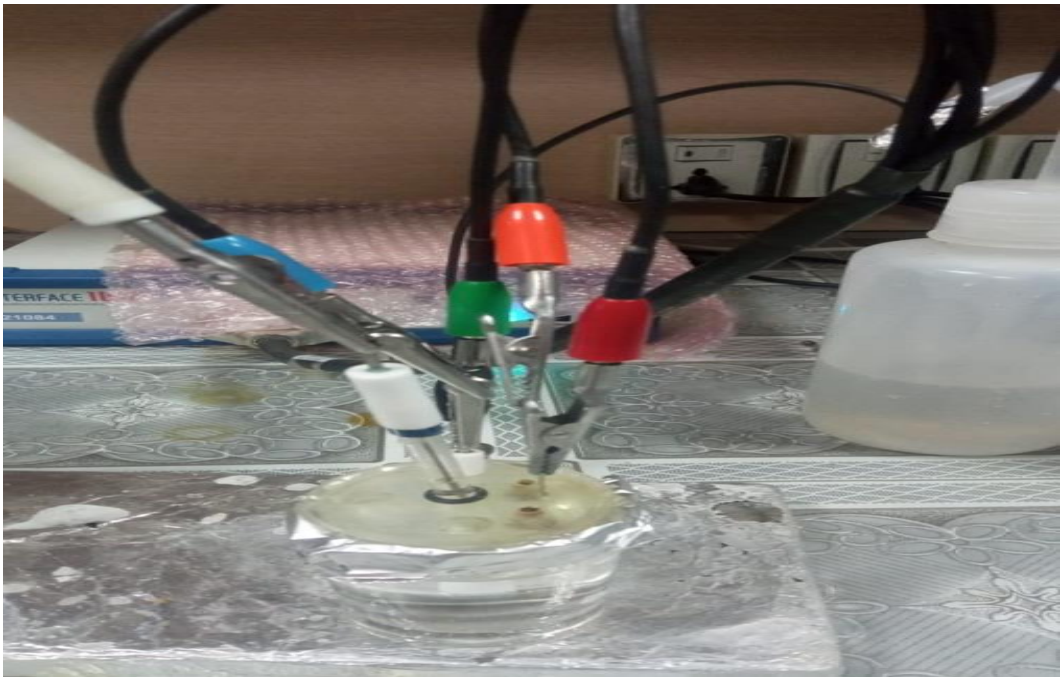


Figure 40: Apparatus for Electrochemical Testing

Initially to obtain better phase purity, samples annealed at two different temperatures were used. Since the composite of MCM-41 with CuO nanoparticles was not reported before, the right annealing temperature was not known. Hence the composite was annealed at 400^0C and 500^0C and both samples were tested for CV. The results obtained are shown in Figure 41 below.

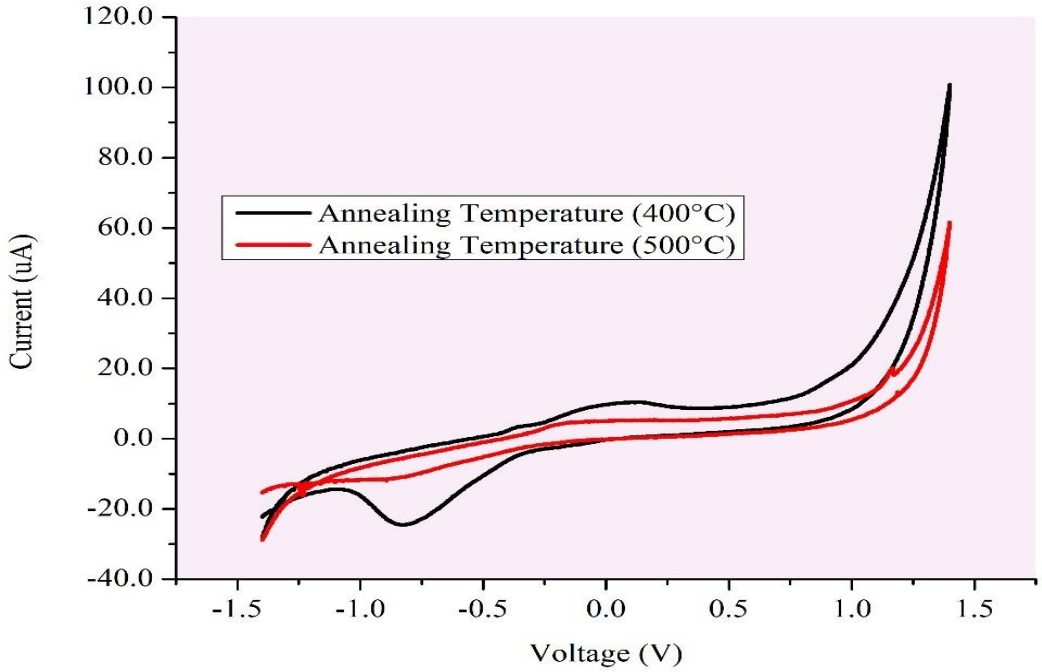


Figure 41: Cyclic Voltammetry with composite annealed at different temperatures

The results show that the sample annealed at 400^0C is better because the area of its curve is greater, and it shows better current output for both oxidation and reduction peaks. The sample annealed at 500^0C shows less current output as higher temperature might have caused over oxidation of CuO nanoparticles or it might have damaged the amorphous structure of MCM-41.

Therefore, the sample annealed at 400^0C was taken and CV was the performed with different concentrations of glucose solutions ranging from 2mM to 10mM, prepared in 0.1M sodium hydroxide solution. Results with different glucose concentrations are shown in Figure 42 below.

As glucose concentration increased, the current output in each sample was increased. This is because more current was now produced as more glucose was now available to be oxidized in the reaction.

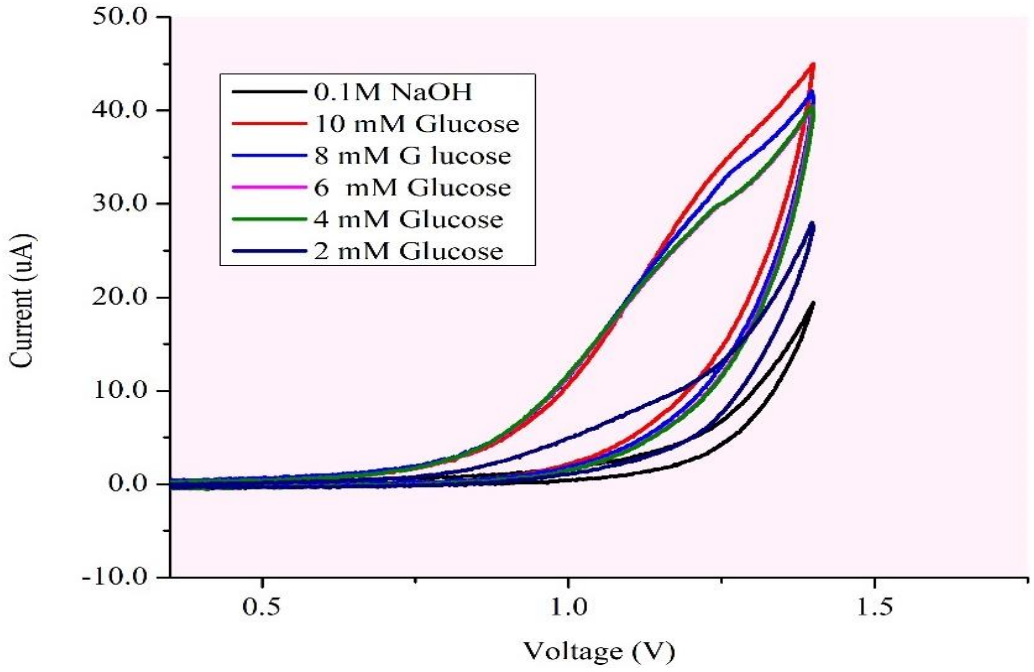


Figure 42: Comparison of Current output with varying Glucose Concentrations

The scan rate of the testing was also varied from 5mV/s to 50mV/s using 2mM glucose solution in 0.1M sodium Hydroxide. The differences in current output were compared by varying scan rates.

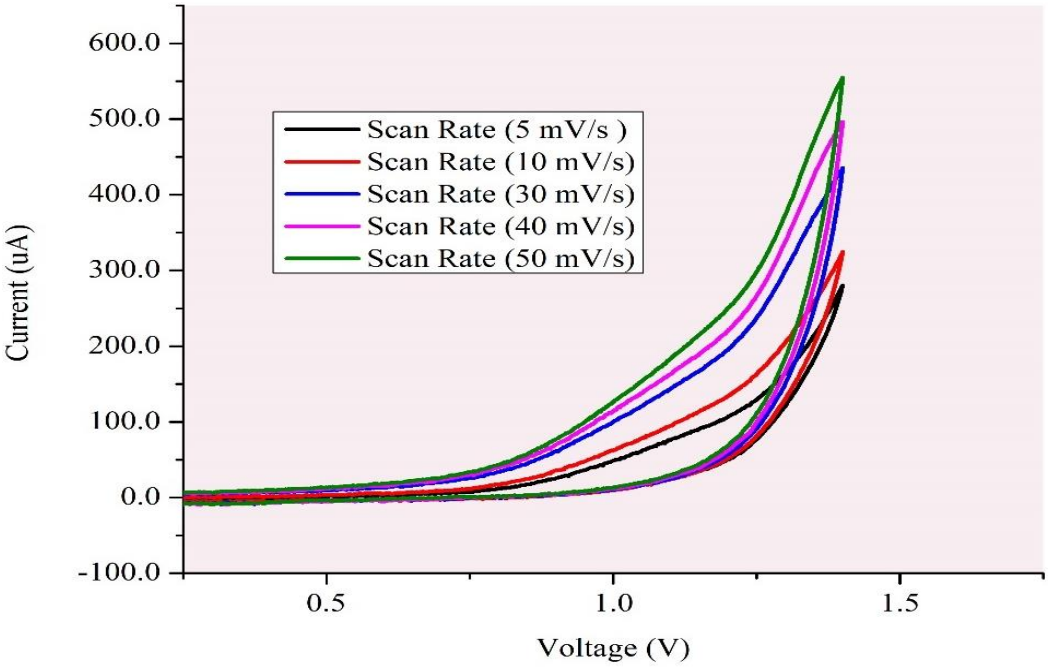


Figure 43: Effect of Scan Rates on Current Output

Figure 43 shows a general trend of increased current output with increasing scan rates. This is because at higher scan rates diffusion is

greater than the rate of reaction and the electrodes have more ions diffused around them, giving higher current output.

Conclusion

Our material is capable of detecting glucose with its varying concentrations in the analyte. It could detect concentrations as low as 2mM whereas the glucose content of blood ranges from 3.9 to 6.9mM. [45] We were successful in synthesizing a composite material which can detect glucose without the presence of glucose oxidase enzyme.

Future Prospects

Some further studies may be carried out on the same material to test its sensitivity and selectivity. Using the Chronoamperometry technique we can get the sensitivity of our sample towards glucose. Further some interfering agents such as uric acid, oxygen and triglycerides may be added in the analyte to test for its selectivity.

Once such results are obtained, the material may be considered for more improvement and use in further applications.

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