ELECTROCHEMICAL CONVERSION OF EXCESS SUGAR IN THE SIMULATED BODY FLUID OVER A FEW ELECTROCATALYTIC MATERIALS IN FUEL CELL TO USEFUL RENEWABLE ELECTRICAL ENERGY

Thesis submitted in partial fulfilment of the requirement for the degree of

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By

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CERTIFICATE

This is to certify that the thesis entitled "E*lectrochemical Conversion of Excess Sugar in the Simulated Body Fluid over a few Electrocatalytic Materials in Fuel Cell to Useful Renewable Electrical Energy*" has been carried out by **Mr. Arnab Dutta (Roll No: 002111303002, Registration No: 160307 of 2021- 2022)** under my guidance and supervision and accepted in partial fulfillment for the degree of Master of Technology in Material Engineering from Jadavpur University. To the best of my knowledge the contents of this thesis or any part thereof have not been previously submitted for the award of any degree or diploma.

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I do hereby solemnly declare that the work embodied in this thesis entitled **"*Electrochemical Conversion of Excess Sugar in the Simulated Body Fluid over a few Electrocatalytic Materials in Fuel Cell to Useful Renewable Electrical Energy*"** is the original investigation carried out independently by me under the supervision of Prof. Subir Paul, Department of Metallurgical and Material Engineering, Jadavpur University, Kolkata, India for the award of the degree of Master of Technology (Material Engineering) of Jadavpur University. To the best of my knowledge and belief, this work has not been presented for any degree or distinction under any other university.

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Certificate of Approval

The forgoing thesis **"*Electrochemical Conversion of Excess Sugar in the Simulated Body Fluid over a few Electrocatalytic Materials in Fuel Cell to Useful Renewable Electrical Energy*"** by ARNAB DUTTA is hereby approved as credible study of an engineering subject carried out and represented in a manner satisfactorily to warrants its acceptance as a prerequisite to the degree for which it has been submitted. It is to be understood that by this approval the undersigned does not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn Therein but approved the thesis only for which it has been submitted.

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Abstract

Diabetes, a chronic metabolic disorder, continues to pose a significant global health challenge, affecting millions of individuals worldwide. The prevalence of diabetes has reached epidemic proportions, with both developed and developing countries experiencing a dramatic increase in cases. Untreated or poorly treated diabetes accounts for approximately 1.5 million deaths per year. Such a situation calls for a novel and alternative route to deal with this epidemic problem, that can be used by patients worldwide. This thesis paper attempts to find a solution to this problem, by taking the route of electrochemistry. It was attempted to demonstrate that the excess sugar (glucose) in the blood stream of a diabetic patient, can be lowered by electro-oxidising the excess sugar in Simulated Body Fuid (SBF) and convert it into energy. Initially, one of the most popular as well as costly electrocatalytic material i.e Platinum was used to electro-oxidise the excess sugar. Upon its success, some highly eletrocatalytic but cheap electrode materials were developed and they also successfully electro-oxidised the excess glucose in SBF solution. Thus a potentially novel route to deal with the epidemic problem of diabetes, has been proposed through this research work.

Chapter 1
Introduction

1. Introduction

Diabetes is a chronic metabolic disorder that affects millions of people worldwide. It is characterized by elevated blood glucose levels resulting from insufficient production or ineffective utilization of insulin, the hormone responsible for regulating blood sugar levels. With its increasing prevalence and impact on public health, acknowledging diabetes and its implications is crucial for individuals, healthcare professionals, and society as a whole. A big challenge that a diabetic person faces is about how his/her/their everyday lives get limited in many ways. For example, a diabetic patient has a lot of restrictions on his diet. He cannot consume the food items that he might have cherished till now. His everyday activities get restricted in various ways.

There are several ways to deal with diabetes in today's time. Most of them are based on leading a healthy lifestyle. Medical interventions are also available for those who need help from outside the body. But wouldn't it be great if a way is found to totally cure the continuous high blood glucose condition or hyperglycemia, which happens to be the biggest diabetic problem in the world? Through this project work, an attempt has been to find a practical solution or cure of this problem.

Glucose is nothing but a form of fuel. And as it is known, any fuel can be burnt or oxidised to get energy from it, not to mention that the fuel gets consumed and depleted in the process. So it is very possible to think that, one of the ways to reduce the glucose concentration in a chemical solution, is to oxidise the glucose and convert it into energy. This particular idea forms the bedrock of this project work. In this project, attempt has been made to electro-oxidise the glucose molecules in Simulated Body Fluid (Phosphate Buffer Solution), which has the exact ionic composition as the human blood, in order to find out whether the glucose levels can be lowered by this method or not. It is needless to say, if this experiment becomes successful, it can open a whole new way of treating and dealing

with diabetes, thereby giving diabetic patients a big hope of living a normal healthy lifestyle, without any kind limitations and restrictions.

The project work starts with identifying a chemical solution that can be used as a substitute of human blood. This was important because, it is not possible to work with real human blood, since this lab has neither access to any medical college lab, nor any authorisation or permission to work with blood. After a preliminary research, Phosphate Buffer Solution (PBS) was found, which have the exact same ionic composition as human blood. Its pH value and conductivity values also match exactly with blood. Thus, Phosphate Buffer Solution (PBS) was chosen as the substitute solution of blood, for this project work.

Once the solution was confirmed, effort was made to look for a way to detect the precise concentration of glucose in the PBS solution. Now, there are a lot of glucometers in the market which could have been used for the purpose, but relying on them is difficult since most of the available glucometers in the market lack in accuracy and also they are quite expensive to use. Thus a novel way was devised to measure glucose. And that was via Cyclic voltammetry(CV) test.

CV tests gave a certain peak current density value, for a particular glucose concentration. CV tests were run to make a calibration chart of glucose concentrations versus peak current density. Then, a linear regression analysis of the calibration data was done, and a linear equation was obtained, through which an unknown glucose concentration can be found out from their peak current density value, from the CV test. So, the new glucose detection system was also ready.

Now, attempt was made to try to lower the glucose level in the PBS solution, by electro-oxidising glucose. The work started with Platinum as the working electrode. From the calibration curves, the potential value was obtained at which the electro-oxidation of glucose was occurring. Simulated Body Fluid(SBF) solution was taken with a known concentration of glucose and then Chronoamperometry (CA) procedure was run in it,

around that potential value, for a certain time duration. This CA procedure is supposed to electro-oxidise glucose, and thereby lower its concentration in the SBF solution. To check that, again CV test was run in that solution, to detect the glucose levels. And it worked! The glucose level indeed was lowered. The experiment was repeated, with different glucose concentrations.

Now that the experiment was a total success, attempt was made to push the envelope a bit further. Till now, all the experiments were done with Platinum as the working electrode. Now it was decided to develop new materials and try to lower the glucose level with them. Four materials were developed, namely Nickel (Ni), Manganese dioxide (MnO_2), Nickel with nanocarbon (Ni C) and Manganese dioxide with nanocarbon(MnO_2 C). All these materials were developed by the process of electrodeposition over Stainless Steel (SS) substrate. After their synthesis, the materials were electrochemically tested, as well as microscopically characterised. After that, attempt was made to electro-oxidise glucose in SBF solution, with these developed materials as the working electrode, just like it was done with Platinum. And again success! All four materials successfully lowered glucose level. Again the experiments were repeated with varying conditions, and each time the results were positive. Thus, through this project work, it has been conclusively demonstrated that reduction of glucose levels by electro-oxidation is a genuine phenomena, and it holds the potential to absolutely revolutionise the diabetic treatment regime throughout the world.

Chapter 2
Literature Review

2. Literature Review

For an effective output in any research field, literature review plays a vital role for foundation of the future work. So the purpose of this chapter is to record briefly the established knowledge and the findings of earlier researches, conducted on related problem areas.

2.1 Diabetes and its natural control(insulin)

Diabetes, also known as **diabetes mellitus**, is a group of common endocrine diseases characterised by sustained high blood sugar levels [16] [14]. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced [17]. Diabetes, if left untreated, leads to many health complications [18]. Untreated or poorly treated diabetes accounts for approximately 1.5 million deaths per year [14].

The classic symptoms of untreated diabetes are unintended weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger)[19]. Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes [20].
Several other signs and symptoms can mark the onset of diabetes although they are not specific to the disease. In addition to the known symptoms listed above, they include blurred vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. Long-term vision loss can also be caused by diabetic retinopathy. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes[21].

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20) but may be the first symptom in those who have otherwise not received a diagnosis before that time[22].

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in people with diabetes are due to coronary artery disease. Other macrovascular diseases include stroke, and peripheral artery disease. These complications are also a strong risk factor for severe COVID-19 illness.

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and eventual blindness Diabetes also increases the risk of having glaucoma, cataracts, and other eye problems. It is recommended that people with diabetes visit an optometrist or ophthalmologist once a year. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplantation. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, sudomotor dysfunction, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle atrophy and weakness.

There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function. Having diabetes, especially when on insulin, increases the risk of falls in older people.

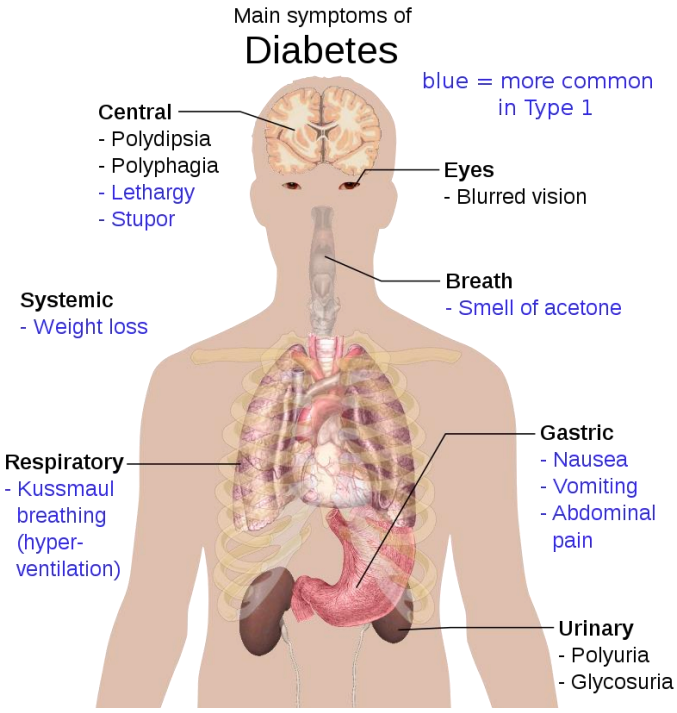


Fig 2.1 Overview of the most significant symptoms of diabetes

In order to ensure normal body function, the human body is dependent on a tight control of its blood glucose levels. This is accomplished by a highly sophisticated network of various hormones and neuropeptides released mainly from the brain, pancreas, liver, intestine as well as adipose and muscle tissue. Within this network, the pancreas represents a key player by secreting the blood sugar-lowering hormone insulin and its opponent glucagon.

Through its various hormones, particularly glucagon and insulin, the pancreas maintains blood glucose levels within a very narrow range of 4–6 mM[34]. This preservation is accomplished by the opposing and balanced actions of glucagon and insulin, referred to as glucose homeostasis. During sleep or in between meals, when blood glucose levels are low, glucagon is released from α-cells to promote hepatic glycogenolysis. In addition, glucagon drives hepatic and renal gluconeogenesis to increase endogenous blood glucose levels[23] during prolonged fasting. In contrast, insulin secretion from β-cells is stimulated by elevated exogenous glucose levels, such as those occurring after a meal[24]. After docking to its receptor on muscle and adipose tissue, insulin enables the insulin-

dependent uptake of glucose into these tissues and hence lowers blood glucose levels by removing the exogenous glucose from the blood stream(Figure 2.2)[25]. Furthermore, insulin promotes glycogenesis[26], lipogenesis[27] and the incorporation of amino acids into proteins[28]; thus, it is an anabolic hormone, in contrast to the catabolic activity of glucagon.

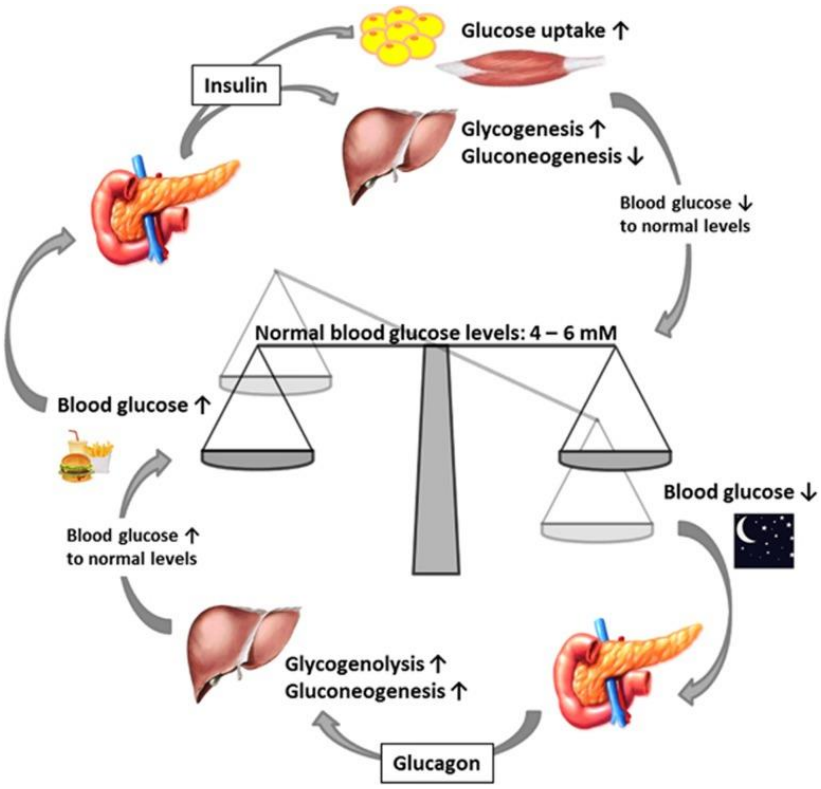


Fig 2.2 Maintenance of blood glucose levels by glucagon and insulin.

Endocrine cells secrete their respective hormones in response to external signals, such as nutrient intake or stress, via humoral, neural or hormonal signaling pathways. The underlying molecular process that translates the stimulus into the actual hormone release is called stimulus-secretion coupling which is known as the stimulus-dependent exocytosis of a particular substance, such as glucose-stimulated β-cell insulin release[29].

In β-cells, the main stimulus for insulin release are elevated blood glucose levels following a meal[30]. The circulating blood glucose is taken up by the facilitative glucose transporter GLUT2 (SLC2A2), which is located on the surface of the β-cells. Once inside the cell, glucose undergoes glycolysis, thereby generating adenosine triphosphate (ATP), resulting

in an increased ATP/ADP ratio. This altered ratio then leads to the closure of ATP-sensitive K+-channels (KATP-channels). Under non-stimulated conditions, these channels are open to ensure the maintenance of the resting potential by transporting positively charged K+-ions down their concentration gradient out of the cell. Upon closure, the subsequent decrease in the magnitude of the outwardly directed K+-current elicits the depolarization of the membrane, followed by the opening of voltage-dependent Ca+-channels (VDCCs). The increase in intracellular calcium concentrations eventually triggers the fusion of insulin-containing granules with the membrane and the subsequent release of their content[31]. The whole secretory process is biphasic with the first phase peaking around 5 minutes after the glucose stimulus with the majority of insulin being released during this first phase. In the second, somewhat slower, phase, the remaining insulin is secreted[32]. Insulin is stored in large dense-core vesicles that are recruited to the proximity of the plasma membrane following stimulation such that insulin is readily available[33].

2.2 Medical control of diabetes

Treatment for type 1 diabetes involves insulin injections or the use of an insulin pump, frequent blood sugar checks, and carbohydrate counting. For some people with type 1 diabetes, pancreas transplant or islet cell transplant may be an option.

Treatment of type 2 diabetes mostly involves lifestyle changes, monitoring of blood sugar, along with oral diabetes drugs, insulin or both.

Depending on the treatment plan, one may check and record the blood sugar as many as four times a day or more often if insulin is being taken. Careful blood sugar testing is the only way to make sure that blood sugar level remains within the target range. People with type 2 diabetes who aren't taking insulin generally check their blood sugar much less often.

Insulin Therapy

People with type 1 diabetes must use insulin to manage blood sugar to survive. Many people with type 2 diabetes or gestational diabetes also need insulin therapy [4].

Many types of insulin are available, including short-acting (regular insulin), rapid-acting insulin, long-acting insulin and intermediate options. Depending on ones needs, the provider may prescribe a mixture of insulin types to use during the day and night.

Insulin can't be taken orally to lower blood sugar because stomach enzymes interfere with insulin's action. Insulin is often injected using a fine needle and syringe or an insulin pen — a device that looks like a large ink pen.

An insulin pump also may be an option. The pump is a device about the size of a small cellphone worn on the outside of the body. A tube connects the reservoir of insulin to a tube (catheter) that's inserted under the skin of the abdomen.

A continuous glucose monitor, on the left, is a device that measures blood sugar every few minutes using a sensor inserted under the skin. An insulin pump, attached to the pocket, is a device that's worn outside of the body with a tube that connects the reservoir of insulin to a catheter inserted under the skin of the abdomen. Insulin pumps are programmed to deliver specific amounts of insulin continuously and with food.

A tubeless pump that works wirelessly is also now available. One can program an insulin pump to dispense specific amounts of insulin. It can be adjusted to give out more or less insulin depending on meals, activity level and blood sugar level.

A closed loop system is a device implanted in the body that links a continuous glucose monitor to an insulin pump. The monitor checks blood sugar levels regularly. The device automatically delivers the right amount of insulin when the monitor shows that it's needed.

The Food and Drug Administration has approved several hybrid closed loop systems for type 1 diabetes. They are called "hybrid" because these systems require some input from the user. For example, It may be necessary to tell the device how many carbohydrates are eaten, or confirm blood sugar levels from time to time.

A closed loop system that doesn't need any user input isn't available yet. But more of these systems currently are in clinical trials.

Oral or other drugs

Sometimes the provider may prescribe other oral or injected drugs as well. Some diabetes drugs help the pancreas to release more insulin. Others prevent the production and release of glucose from the liver, which means the patient needs less insulin to move sugar into their cells.

Still others block the action of stomach or intestinal enzymes that break down carbohydrates, slowing their absorption, or make the tissues more sensitive to insulin. Metformin (Glumetza, Fortamet, others) is generally the first drug prescribed for type 2 diabetes.

Another class of medication called SGLT2 inhibitors may be used. They work by preventing the kidneys from reabsorbing filtered sugar into the blood. Instead, the sugar is eliminated in the urine [3].

Transplantation

In some people who have type 1 diabetes, a pancreas transplant may be an option. Islet transplants are being studied as well. With a successful pancreas transplant, one would no longer need insulin therapy[1].

But transplants aren't always successful. And these procedures pose serious risks. One needs a lifetime of immune-suppressing drugs to prevent organ rejection. These drugs can have serious side effects. Because of this, transplants are usually reserved for people whose diabetes can't be controlled or those who also need a kidney transplant.

Bariatric surgery

Some people with type 2 diabetes who are obese and have a body mass index higher than 35 may be helped by some types of bariatric surgery. People who've had gastric bypass have seen major improvements in their blood sugar levels. But this procedure's long-term risks and benefits for type 2 diabetes aren't yet known [2].

2.3 Electrooxidation and its application

Electro-oxidation(EO or EOx), also known as anodic oxidation or electrochemical oxidation (EC), is a type of advanced oxidation process (AOP)[35]. The most general layout comprises two electrodes, operating as anode and cathode, connected to a power source. When an energy input and sufficient supporting electrolyte are provided to the system, strong oxidizing species are formed, which interact with the contaminants and degrade them.

Apparatus

The set-up for performing an electro-oxidation treatment consists of an electrochemical cell. An external electric potential difference (aka voltage) is applied to the electrodes, resulting in the formation of reactive species, namely hydroxyl radicals, in the proximity of the electrode surface[36]. To assure a reasonable rate of generation of radicals, voltage is adjusted to provide current density of 10-100 mA/cm^2 [37]. While the cathodes materials are mostly the same in all cases, the anodes can vary greatly according to the application, as

the reaction mechanism is strongly influenced by the material selection[38]. Cathodes are mostly made up by stainless steel plates, Platinum mesh or carbon felt electrodes.

Working principle

Direct Oxidation - When voltage is applied to the electrodes, intermediates of oxygen evolution are formed near the anode, notably hydroxyl radicals. Hydroxyl radicals are known to have one of the highest redox potentials, allowing the degrading many refractory organic compounds. A reaction mechanism has been proposed for the formation of the hydroxyl radical at the anode through oxidation of water[39]:

$$S + H_2O \longrightarrow S[\cdot OH] + H^+ + e^-$$

Where S represents the generic surface site for adsorption on the electrode surface. Then, the radical species can interact with the contaminants through two different reaction mechanisms, according to the anode material[40]. The surface of "active" anodes strongly interacts with hydroxyl radicals, leading to the production of higher state oxides or superoxides[41]. The higher oxide then acts as a mediator in the selective oxidation of organic pollutants. Due to the radicals being strongly chemisorbed onto the electrode surface, the reactions are limited to the proximity of the anode surface, according to the mechanism[42]:

$$S[\cdot OH] \longrightarrow SO + H^+ + e^-$$
$$SO + R \longrightarrow S + RO$$

Where R is the generic organic compound, while RO is the partially oxidized product.
If the electrode interacts weakly with the radicals, it is qualified as a "non active" anode. Hydroxyl radicals are physisorbed on the electrode surface by means of weak interaction forces and thus available for reaction with contaminants[42]. The organic pollutants are converted to fully oxidized products, such as CO_2, and reactions occur in a much less selective way with respect to active anodes:

$$S[\cdot OH] + R \longrightarrow S + mCO_2 + nH_2O + H^+ + e^-$$

Both chemisorbed and physisorbed radicals can undergo the oxygen evolution competitive reaction. For this reason, the distinction between active and non active anodes is made according to their oxygen evolution overpotential. Electrodes with low oxygen overpotential show an active behavior, as in the case of Platinum, graphite or mixed metal oxide electrodes. Conversely, electrodes with high oxygen overpotential will be non-active[36]. Typical examples of nonactive electrodes are lead dioxide or boron-doped diamond electrodes[42]. A higher oxygen overpotential implies a lower yield of the oxygen evolution reaction, thus raising the anodic process efficiency[36].

Mediated Oxidation - When appropriate oxidizing agents are dissolved into the solution, the electro-oxidation process not only leads to organics oxidation at the electrode surface, but it also promotes the formation of other oxidant species within the solution. Such oxidizing chemicals are not bound to the anode surface and can extend the oxidation process to the entire bulk of the system[36]. Chlorides are the most widespread species for the mediated oxidation. This is due to the chlorides being very common in most wastewater effluents and being easily converted into hypochlorite, according to global reaction[35]:

$$Cl^- + H_2O \longrightarrow ClO^- + 2\,H^+ + 2\,e^-$$

Although hypochlorite is the main product, chlorine and hypochlorous acid are also formed as reactions intermediate. Such species are strongly reactive with many organic compounds, promoting their mineralization, but they can also produce several unwanted intermediates and final products[35]. These chlorinated by-products sometimes can be even more harmful than the raw effluent contaminants and require additional treatments to be removed[43]. To avoid this issue, sodium sulfate is preferred as electrolyte to sodium chloride, so that chloride ions are not available for the mediated oxidation reaction.

Although sulfates can be involved in mediated oxidation as well, electrodes with high oxygen evolution overpotential are required to make it happen[44].

Applications Of Electroxidation

Perhaps the biggest application of Electro-oxidation is in the operations of Batteries and fuel cells. The anode/fuel gets electro-oxidised and generates energy in the process. Other than that, Electro-oxidation has recently grown in popularity thanks to its ease of set-up and effectiveness in treating harmful and recalcitrant organic pollutants, which are typically difficult to degrade with conventional wastewater remediation processes[45]. Also, it does not require any external addition of chemicals (contrarily to other processes like in-situ chemical oxidation), as the required reactive species are generated at the anode surface.

Electro-oxidation has been applied to treat a wide variety of harmful and non-biodegradable contaminants, including aromatics, pesticides, drugs and dyes[46]. Due to its relatively high operating costs, it is often combined with other technologies, such as biological remediation. Electro-oxidation can additionally be paired with other electrochemical technologies such as electrocoagulation, consecutively or simultaneously, to further reduce operational costs while achieving high degradation standards.

Other than Waste water treatment, another major area of electro-oxidation application is Medical industry, in the development of bio fuel cells and in detection and monitoring the blood sugar level. Both enzymatic and non enzymatic blood glucose sensors have been developed over the past few decades[47]. All these biofuel cells and glucose sensors typically use the process of electro-oxidation for their operations.

2.4 Electrochemical Procedures

In this topic, the fundamental electrochemical tests and procedures will be discussed, that are used to find out the electrochemical behaviour of various materials in different medium and supporting electrolyte solutions.

2.4.1 Cyclic Voltammetry

In electrochemistry, **cyclic voltammetry** (**CV**) is a type of potentiodynamic measurement. In a cyclic voltammetry experiment, the working electrode potential is ramped linearly versus time. Unlike in linear sweep voltammetry, after the set potential is reached in a CV experiment, the working electrode's potential is ramped in the opposite direction to return to the initial potential. These cycles of ramps in potential may be repeated as many times as needed. The current at the working electrode is plotted versus the applied voltage (that is, the working electrode's potential) to give the cyclic voltammogram trace. Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution[48] or of a molecule that is adsorbed onto the electrode[49].

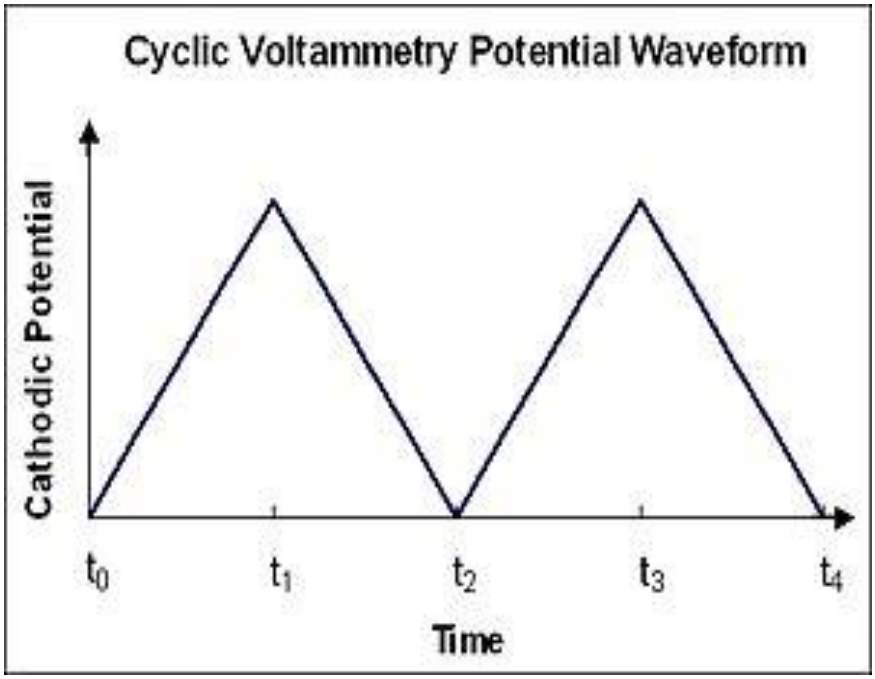


Fig 2.3 Cyclic Voltammetry waveform

In cyclic voltammetry (CV), the electrode potential ramps linearly versus time in cyclical phases (as shown in Figure 3). The rate of voltage change over time during each of these phases is known as the experiment's scan rate (V/s). The potential is measured between the working electrode and the reference electrode, while the current is measured between the working electrode and the counter electrode. These data are plotted as current (*i*) versus applied potential (*E*, often referred to as just 'potential'). In Figure 3, during the initial forward scan (from t_0 to t_1) an increasingly reducing potential is applied; thus the cathodic current will, at least initially, increase over this time period, assuming that there are reducible analytes in the system. At some point after the reduction potential of the analyte is reached, the cathodic current will decrease as the concentration of reducible analyte is depleted. If the redox couple is reversible, then during the reverse scan (from t_1 to t_2), the reduced analyte will start to be re-oxidized, giving rise to a current of reverse polarity (anodic current) to before. The more reversible the redox couple is, the more similar the oxidation peak will be in shape to the reduction peak. Hence, CV data can provide information about redox potentials and electrochemical reaction rates. For instance, if the electron transfer at the working electrode surface is fast and the current is limited by the diffusion of analyte species to the electrode surface, then the peak current will be proportional to the square root of the scan rate. This relationship is described by the Randles–Sevcik equation. In this situation, the CV experiment only samples a small portion of the solution, i.e., the diffusion layer at the electrode surface.

In electrochemistry, the **Randles–Ševčík equation** describes the effect of scan rate on the peak current i_p for a cyclic voltammetry experiment. For simple redox events such as the ferrocene/ferrocenium couple, i_p depends not only on the concentration and diffusional properties of the electroactive species but also on scan rate[50].

The Randles–Ševčík equation is given by

$$i_p = 0.4463\, nFAC\left(\frac{nFvD}{RT}\right)^{\frac{1}{2}}$$

Where,

- i_p = current maximum in amps
- n = number of electrons transferred in the redox event (usually 1)
- A = electrode area in cm^2
- F = Faraday constant in C mol^{-1}
- D = diffusion coefficient in cm^2/s
- C = concentration in mol/cm^3
- v = scan rate in V/s
- R = Gas constant in J K^{-1} mol^{-1}
- T = temperature in K
- The constant with a value of $2.69x10^5$ has units of C mol^{-1} $V^{-1/2}$

For novices in electrochemistry, the predictions of this equation appear counter-intuitive, i.e. that i_p increases at faster voltage scan rates. It is important to remember that current, i, is charge (or electrons passed) per unit time. In cyclic voltammetry, the current passing through the electrode is limited by the diffusion of species to the electrode surface. This diffusion flux is influenced by the concentration gradient near the electrode. The concentration gradient, in turn, is affected by the concentration of species at the electrode, and how fast the species can diffuse through solution. By changing the cell voltage, the concentration of the species at the electrode surface is also changed, as set by the Nernst equation. Therefore, a faster voltage sweep causes a larger concentration gradient near the electrode, resulting in a higher current.

2.4.2 Chronoamperometry

In electrochemistry, **chronoamperometry** is an analytical technique in which the electric potential of the working electrode is stepped and the resulting current from faradaic processes occurring at the electrode (caused by the potential step) is monitored as a function of time. The functional relationship between current response and time is measured after applying single or double potential step to the working electrode of the electrochemical system. Limited information about the identity of the electrolyzed species can be obtained from the ratio of the peak oxidation current versus the peak reduction current. However, as with all pulsed techniques, chronoamperometry generates high charging currents, which decay exponentially with time as any RC circuit. The Faradaic current - which is due to electron transfer events and is most often the current component of interest - decays as described in the Cottrell equation. In most electrochemical cells, this decay is much slower than the charging decay-cells with no supporting electrolyte are notable exceptions. Most commonly a three-electrode system is used. Since the current is integrated over relatively longer time intervals, chronoamperometry gives a better signal-to-noise ratio in comparison to other amperometric techniques[51].

In electrochemistry, the **Cottrell equation** describes the change in electric current with respect to time in a controlled potential experiment, such as chronoamperometry. Specifically it describes the current response when the potential is a step function in time. It was derived by Frederick Gardner Cottrell in 1903[52]. For a simple redox event, such as the ferrocene/ferrocenium couple, the current measured depends on the rate at which the analyte diffuses to the electrode. That is, the current is said to be "diffusion controlled". The Cottrell equation describes the case for an electrode that is planar but can also be derived for spherical, cylindrical, and rectangular geometries by using the

corresponding Laplace operator and boundary conditions in conjunction with Fick's second law of diffusion[48].

The Cottrell equation is given by

$$i = \frac{nFAc_j^0\sqrt{D_j}}{\sqrt{\pi t}}$$

Where,

- *i*= current, in units of Amps
- *n* = number of electrons (to reduce/oxidize one molecule of analyte *j*, for example)
- *F* = Faraday constant, 96485 C/mol
- *A* = area of the (planar) electrode in cm^2
- C^0_j = initial concentration of the reducible analyte j in mol/cm^3
- D_j = diffusion coefficient for species *j* in cm^2/s
- *t* = time in s.

Deviations from linearity in the plot of *i* vs. $t^{-1/2}$ sometimes indicate that the redox event is associated with other processes, such as association of a ligand, dissociation of a ligand, or a change in geometry.

2.4.3 Electrochemical Impedence Spectroscopy (EIS)

The electrochemical impedance spectroscopy (EIS) technique is an electrochemical method used in many electrochemical studies, which is based on the use of an alternating current (AC) signal that is applied to the working electrode, WE, and determining the corresponding response. In the most common experimental procedures, a potential signal (E) is applied to the WE and its current response (I) is determined at different frequencies. However, in other cases, it is possible to apply a certain current signal and determine the potential response of the system. Thus, the potentiostat used processes the measurements of potential *vs.* time and current *vs.* time, resulting in a series of impedance values corresponding to each frequency analyzed. This relationship of impedance and

frequency values is called the “impedance spectrum.” In studies using the EIS technique, the impedance spectra obtained are usually analyzed using electrical circuits, made up of components such as resistors (R), capacitances (C), inductances (L), combined in such a way as to reproduce the measured impedance spectra. These electrical circuits are called “equivalent electrical circuits.” [15]

2.4.4 Potentiodynamic Polarization

Potentiodynamic is a term describing the measured change in the electrical potential (voltage) of a system. In electrochemistry, this term is used in describing polarization methods in the corrosion industry. Potentiodynamic polarization refers to a polarization technique in which the potential of the electrode is varied over a relatively large potential domain at a selected rate by the application of a current through the electrolyte. Potentiodynamic polarization is often used for laboratory corrosion testing. It can provide significant useful information regarding corrosion mechanisms, corrosion rate and susceptibility of specific materials to corrosion in designated environments. Tafel extrapolation and polarization resistance are two methods to measure corrosion rates. Polarization methods are faster experimental techniques compared to classical weight loss estimation. Tafel relationship with respect to activation controlled anodic and cathodic processes has been discussed earlier. For an electrochemical reaction under activation control, polarization curves exhibit linear behaviour in the E Vs log (i) plots called Tafel behaviour.

2.5 Electrochemical Enzymatic and Non-enzymatic detection of glucose

Enzymatic detection of glucose

Enzymes have long been key components for building a glucose sensor, and their well-known mechanism of glucose processing constitutes the chemical principle of a glucose sensor. Biological enzymes react with glucose rapidly owing to their catalytic turnover, and the direct oxidation of glucose is energetically favorable. Among the families of enzymes

associated with glucose metabolism, glucose oxidase (GO_x) is widely employed. In principle, GO_x oxidizes glucose to yield gluconic acid and hydrogen peroxide (H_2O_2) via the following reaction

$$\text{Glucose} + H_2O + O_2 \xrightarrow{GO_x} \text{Gluconic acid} + H_2O_2$$

The first generation of GO_x-based sensors monitors the O_2 consumed or H_2O_2 produced, which react with electrodes to indicate the amount of glucose consumed . A high positive overpotential above 1 V versus Ag/AgCl is applied to detect the analytes directly, but the high overpotential also facilitates side reactions such as oxidation of ascorbic acid, acetaminophen, uric acid, and lactic acid, thereby hampering the selectivity toward the target analytes. Additional mediators have been devised to increase the selectivity of the assay by lowering the overpotential and minimizing the oxidation of interfering species. The incorporation of platinum nanoparticles (Pt NPs) increases the sensitivity of glucose detection by favoring the selective oxidation of H_2O_2. Although Pt is highly reactive toward H_2O_2, the high reaction potential of the electrode remains a drawback. To resolve the overpotential issue, Prussian blue (PB) is incorporated as an electrocatalytic mediator to lower the reaction potential to near 0 V versus Ag/AgCl. Unlike Pt-based glucose sensors, PB-based glucose sensors utilize the reduction of H_2O_2. In the cyclic voltammograms (CVs), H_2O_2 is reduced at negative potentials, and the current increases proportionally to the increasing concentration of H_2O_2.

The second-generation GO_x-based sensors feature redox mediators that interact directly with enzymes. For example, ferrocene is incorporated as a diffusional electron mediator that contacts GO_x in the electron exchange process. Electrodes containing ferrocene and GO_x experience an increase in current as glucose is introduced. However, common mediators of ferrocene or ferricyanide derivatives are not recommended for in vivo devices because of their potential leaching and the resulting toxicity issues.

The third-generation GO_x-based sensors use engineered enzymes whose structures are modified to facilitate the direct electron exchange between electrodes and embedded enzymes. Nanostructured electrodes, such as electrodes treated with carbon nanotubes, can be coupled to GO_x so that the electrochemically active flavin adenine dinucleotide (FAD; subunit of the enzyme) can transfer electrons directly to the electrodes. For example, the 1D hierarchically structured TiO_2 (1DHS TiO_2) electrode exhibits redox peaks of FAD/$FADH_2$ in CV, resulting from the direct electron transfer between the 1DHS TiO_2 electrode and coupled enzymes. Although a considerable amount of progress has been made since the introduction of electrocatalytic mediators and structurally modified enzymes for the direct electron transfer, it may be noted that the electrochemical detection of byproducts remains the foundation of glucose sensing. A majority of commercial sensors and related studies rely on the first- and second-generation GO_x-based techniques, and the requirement for an O_2 supply has been resolved with semipermeable membranes, which can be manufactured easily and inexpensively. Nevertheless, advanced sensor designs capable of oxygen-free, selective, and energetically efficient detection of glucose are highly desired.

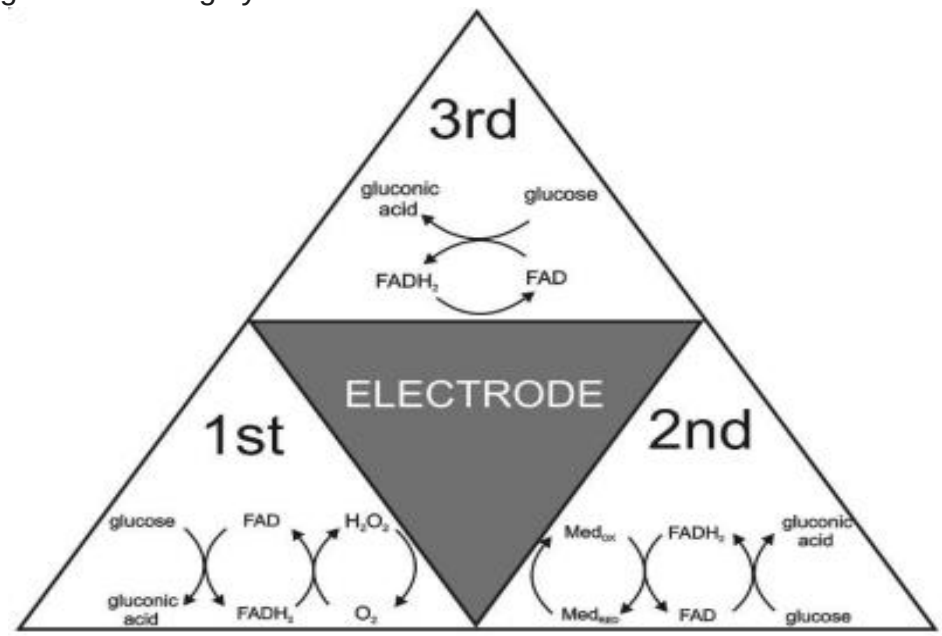


Fig 2.4 Summary of enzymatic glucose oxidation mechanisms, presented as first, second and third generation sensors

In addition to GO_x, another enzyme family of glucose dehydrogenases (GDHs) has been used as a key material of the electrochemical reaction for glucose sensors. In terms of the reaction mechanism, GDH-based sensors can be classified by their various cofactors, ranging from tightly bound FAD or pyrroloquinoline quinone (PQQ) to unbound nicotinamide adenine dinucleotide (phosphate) [NAD(P)]. While GO_x utilizes O_2 as an external electron acceptor, GDHs are unable to utilize O_2, thereby avoiding the issue of O_2 supply. Therefore, GDH-based amperometric detection is independent of the O_2 concentration However, FAD/PQQ-GDH has a broad substrate specificity and catalyzes other analytes in the blood such as maltose. Protein engineering of the enzymes has helped eliminate the enzymes' activity toward other species and improve substrate specificity. Because the cofactor NAD(P) is not structurally bound to GDH, exogenous NAD(P) needs to be supplied .However, NAD(P)H should not be oxidized directly within the sensor because the direct oxidation of NAD(P)H may beget spontaneously polymerized oxidation products, which would increase the overpotential and fouling of the electrodes significantly. Therefore, electrocatalytic mediators are employed to lower the applied potential to the extent that NAD(P)H is not oxidized. Although GDH has the advantage of operating independently of the O_2 concentration, it is strictly limited by its substrate specificity and often prone to reaction with other biomarkers (e.g., maltose), resulting in the overestimation of glucose readings. Therefore, advanced protein engineering of GDH is required to minimize such potential errors from intrinsic enzymatic reaction pathway and to increase the reliability of glucose sensing via GDH.

Non-enzymatic detection of glucose

Non-enzymatic sensors will probably become the fourth generation glucose sensors for analytical applications. Considering the flaws of former-generation glucose sensors, this is an ideal system that was first investigated a century ago by Walther Loeb [53]. As reported, glucose has three aqueous isomers denoted as α-glucose (α-G), β-glucose (βG) and γ-glucose (γ-G), as shown in Figs. 1 and 2. α glucose and β-glucose are converted through

acid-catalyzed hydrolysis via aldehyde-type glucose (γ-glucose). When at equilibrium in water at room temperature these isomers are present at the ratio of 37:63:0.003 for α-, β-, and γ-glucose respectively [54], thus indicating that glucose is most stable in its cyclic form.

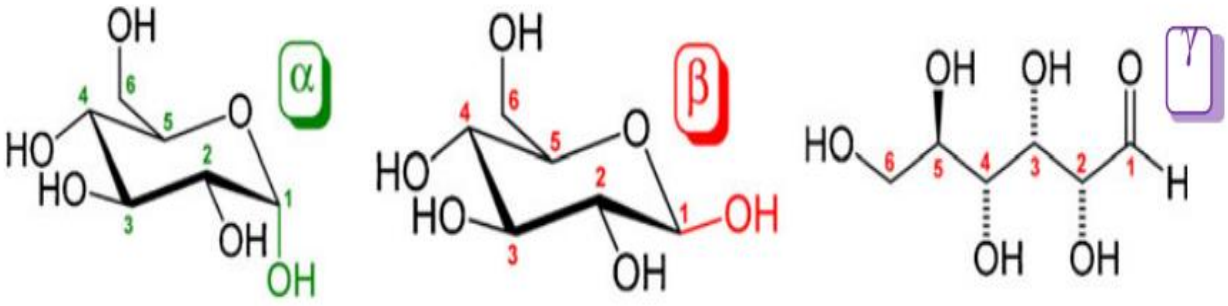


Fig 2.5 The interconversion of glucose anomers (α-, β-, and γ-) and their relative ratio at pH 7

Theories of non-enzymatic electrocatalysis

Non-enzymatic electrocatalysts come in a number of forms, specifically metals, alloys and bimetallic, carbon-based materials, metal-metal oxide heterogeneous based nanocomposites, layered double hydroxide. Apart from the latter, all the catalysts listed are dependent on a transition metal centre. The process of electrocatalysis is generally observed to occur via the adsorption of the analyte to the electrode surface, a process that presumably involves the d-electrons and d-orbitals of the metal substrate that allows it to form a suitable bond with the adsorbate[55]. The proposal of active transition metal centers across the electrode only explains the process of adsorption onto the surface, yet fails to consider the oxidative role of hydroxyl radicals. It has been evident in numerous publications [56] that electrooxidation of glucose and many other organic molecules coincide with the onset of adsorbed OHads. Burke [57] discussed the importance of this hydrous oxide layer on the electrocatalytic process, and proposed the 'Incipient Hydrous Oxide Adatom Mediator' model (IHOAM)[57]. This was based on the observation that 'active' surface metal atoms undergo a pre-monolayer oxidation step that forms an incipient hydrous oxide layer of reactive OHads mediating oxidation and inhibiting reduction of kinetically slow electrode reactions. Considering this effect, both the activated

chemisorption model [55] and the IHOAM model will be considered in the following sections regarding each main metal electrode individually.

2.6 Electroxidation of glucose by Platinum

A large amount of the work first investigating the direct electrooxidation of glucose was performed at platinum electrodes[58][59]. Researchers have explored the behaviour of glucose at a platinum electrode in acid[60], neutral[58] and alkali [58] conditions. A common conclusion from a number of authors was that the sole product of oxidation is gluco-δlactone, which hydrolyses to gluconic acid on standing, regardless of the solution pH (Scheme 3)[58]

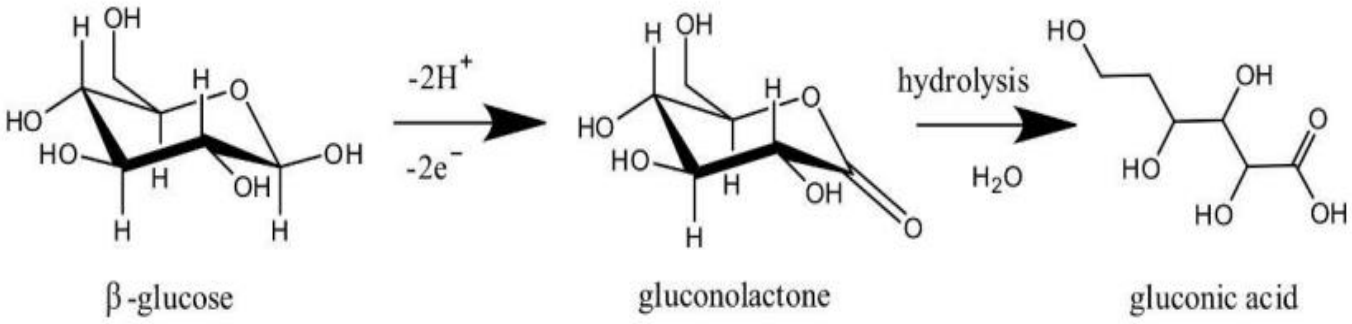


Fig 2.6 (Scheme 3) The 2e- oxidation of glucose to gluconolactone and further hydrolysis to gluconic acid

However, spectrochemical evidence regarding intermediate adsorbates has frequently disagreed, suggesting reduced CO_2, CO_{ads} and fragments of the glucose molecule to also be present as oxidation products[61].

The cyclic voltammetry of glucose at a platinum electrode is reflective of the three distinct areas associated with platinum voltammetry, though it may vary significantly depending on electrolyte and temperature conditions. Investigation by Vasil'ev et al[58]and numerous other authors[59][61][62][63] all conclude that in the anodic sweep three oxidation peaks are observed. A typical cyclic voltammogram of glucose on a platinum electrode at pH 7 is shown in Figure 7[59] with each peak numbered, including the two cathodic oxidation peaks

4 and 5. Platinum and platinum group metals electrocatalyse glucose oxidation in a manner that is, for the most part, analogous to the electro oxidation of methanol, methanal and methanoic acid[58].

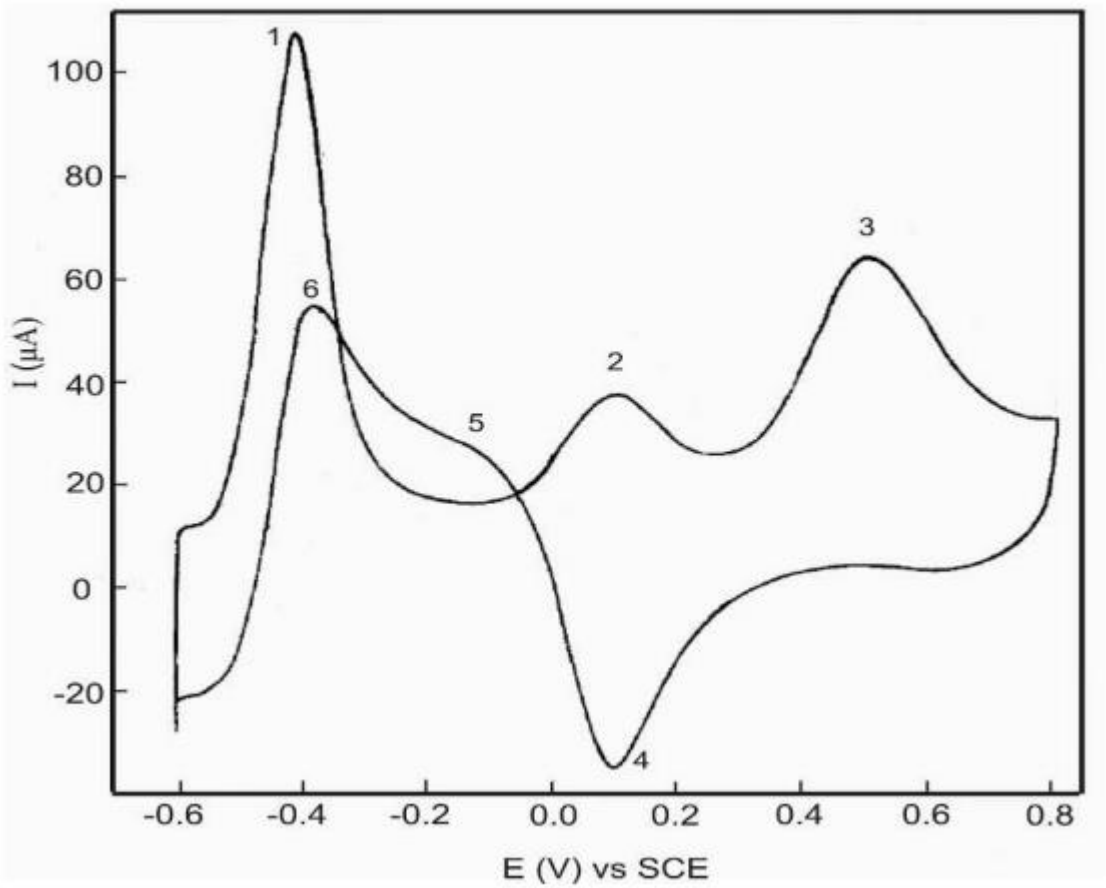


Fig 2.7 Typical cyclic voltammogram of glucose oxidation (0.1M) taken at 30°C on a bright platinum electrode in pH 7 phosphate buffer (0.2 M). Taken from ref [59]

Scheme 4 illustrates the 3 oxidation mechanisms associated with each potential region of the platinum electrode. These are as follows.

Peak 1 – The chemisorption and dehydrogenation of glucose in the hydrogen region. This initial step is the dehydrogenation of the glucose molecule at the hemiacetalic carbon 1 atom (C1)[61], and adsorption of the glucose molecule onto the platinum surface (Sch. 4a). This occurs in the potential region of > 0.3 V vs. RHE, with the removal of the first hydrogen atom considered the rate determining step[58][64].

Peak 2 – Electrooxidation of the chemisorbed species occurs in the double layer region spanning <0.3 to <0.6 V vs. RHE. At increasingly anodic potentials, the abundance of OHads species increases, as the fast dissociation of water reaction (b(i)), and subsequent adsorption of the hydroxide anion occurs. In accordance to the IHOAM model, the incipient hydroxide is catalytic to the oxidation of the adsorbed glucose, thus accelerates electro oxidation by following reaction b(ii). Thus the maxima for Peak 2, and surface bound glucose oxidation is observed at the potential onset for pre monolayer oxygen adsorption on platinum[57][58[63].

Peak 3 – The oxygen region from ca. >0.7 V vs. RHE to the onset of bulk oxygen evolution. Here the platinum surface is covered by a monolayer of adsorbed oxygen, initially inhibiting glucose oxidation as OHads is desorbed and replaced by less catalytically active Oads. However, as soon as a suitable PtO film has formed, direct catalytic oxidation of the bulk glucose solution may occur. This is observed experimentally as the reaction kinetics become diffusion controlled rather than surface bound[60][65], and inhibition of the reaction is now observed as other oxy-compounds are formed at the platinum instead of platinum oxide. This reaction is shown in Scheme 4c, and is followed by the immediate regeneration of the PtO layer.

In the above approach it is assumed that the glucose oxidation pathway follows Scheme 3, i.e. glucose to gluconolactone to gluconic acid. However spectrochemical evidence of glucose oxidation suggests otherwise. Bolzan et al [66] used online mass spectrometry to explore the oxidation intermediates and products observed during glucose oxidation on platinum.

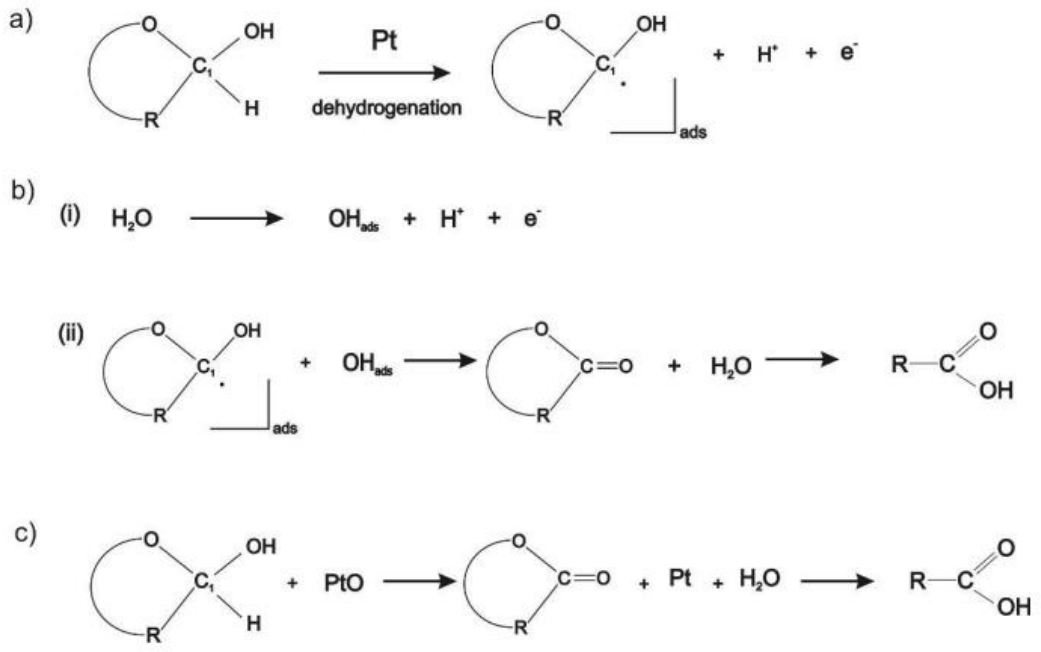


Fig 2.8 Scheme 4. A possible mechanism for oxidation of glucose at a platinum electrode. Glucose is adsorbed onto the platinum surface following hydrogen abstraction at the C1 position. This dehydrogenation process is observed in peak 1 of Figure 4. b) (i) the dissociation of water to produce hydroxide anions (ii) the subsequent oxidation of adsorbed glucose by the adsorbed hydroxide ions c) Oxidation of glucose by PtO in oxygen region of the scan in Figure 2.7, peak 3

They found that CO_2 was formed in the hydrogen region via the hemiacetalic C atom, via decarboxylation of a gluconate intermediate. In the double layer region a number of strongly adsorbed intermediates are formed bound at the C1 and other C atoms which then oxidise to CO_2. Beden et al[61] used Fourier transform infrared reflectance spectroscopy (FTIRS) to discern the structures of the adsorbed intermediates. They claimed that in the double layer region the dehydrogenated glucose species was adsorbed as the gluconate bound at one or two oxygen atoms, but at E< 0.6 V the intermediate was adsorbed gluconolactone. The latter investigation again stressed the importance of 'active' OH- anions in the vicinity of electrode surface to drive the double layer oxidation by breaking the C-O-C bond. However, Beden makes no claim to have observed the formation of CO_2 at the surface. Skou[60] investigated the oxidation of glucose in the PtO region (i.e. Peak 3) in both acidic and neutral conditions. It was observed that relative to the blank solution, surface oxide formation was inhibited in the region of Peak 2, presumably due to the presence of adsorbed glucose, yet a large oxidation peak (Peak 3)

was observed at higher anodic potentials. The peak current of Peak 2 was found not to alter with increasing scan rate, yet Peak 3 was scan rate dependent.

Disadvantages of platinum electrodes

The oxidation of glucose on platinum strongly depends on the electrolyte conditions, particularly the nature and the concentration of the ions present[59]. This is due to the dependence of glucose adsorption on the availability of the platinum surface. Competitive adsorption by other anions, especially true of phosphate anions[58][59], the extent of hydrogen and hydroxide adsorption, and the isomeric structure of the glucose molecule (i.e. α, β or γ glucose) [67] all influence the extent of glucose chemisorption, and therefore the extent of glucose oxidation. The HPO_4^{2-} anion (pKa 12.48 at 25ºC) is the most abundant at pH 7.7 to pH 12.03 and is also has the greatest affinity for adsorption onto platinum out of the phosphate anions. It is therefore necessary to consider the competitive adsorption of the anion when investigating glucose electrooxidation on platinum in neutral phosphate buffers that do not reflect human blood conditions[59]. Because of the dependence of glucose oxidation on the degree of adsorption to the electrode surface, direct proportionality between the oxidation current and glucose concentration is lost as soon as the electrode surface is saturated. This is a limiting factor for platinum electrodes, as the linear range for glucose oxidation becomes dependent on the electrode surface area, and this is over the physiological glucose concentration range of 2 to 30 mM. Furthermore, the activity of the platinum electrode largely dictates the extent of the catalytic current for glucose, and is difficult to reproduce from one experiment to the next[58]. The electrode surface structure is paramount to the electroactivity of platinum towards glucose oxidation, and a number of other small organic molecules. Adsorption sites vary across various single crystal and polycrystalline surfaces thus greatly altering adsorption and surface activity, and therefore the fundamental kinetics of glucose oxidation at platinum. The onset and degree of catalytic activity therefore varies depending on surface structure, as researchers have observed[58][62][68]. One of the biggest drawbacks

of using platinum electrodes however, especially in the physiological condition, is its tendency to undergo poisoning from so many species. Within physiological solutions numerous species exist that immediately inhibit the electroactivity of platinum. One of the most significant constituents is chloride anions, which strongly chemisorb to the surface of platinum and thus render the surface inaccessible to glucose, hydrogen, and hydrous oxide, particularly in acidic solutions[58]. Other organic compounds also severely reduce the ability of platinum, in particular amino acids and other blood based proteins, and electroactive compounds such as uric acid (UA), ascorbic acid (AA) and acetaminophen (AP), which also strongly adhere to the surface and react. However, even in the absence of the extraneous species, platinum can undergo self poisoning during the oxidation of glucose, simply due to the adsorption of the oxidation products and intermediates[61][66]. Essentially the surface of a platinum electrode is non selective to what adsorbs onto it, and as such, poorly diffusive molecules such as glucose, cannot compete for electroactive surface over any length of time. Finally, the significant cost of platinum far outweighs its practical use in disposable glucose sensors, and without a doubt their application would vastly increase the cost of the overall product. This is a factor that cannot be ignored in the search for practical, non-enzymatic glucose sensors, as the prevalence of the disease rapidly increases in economically poor areas of the world, and the enzymatic alternatives are screen printed carbon based electrodes.

Chapter 3
Experimental Methods

3. Experimental Methods

After going through the complete literature review, a firm and steady theoretical understanding has been obtained about the basic concepts of electrochemistry and about electro-oxidation of glucose in PBS solution, in particular. Now, the experimental work can be started. This chapter will discuss about the various stages of experimental processes in details , that have been undertaken in this project.

3.1 Making of Solution

The experimental works start with the making of the Stimulated Body Fluid(SFB) solution. The solution we have used in our experiment is the Phosphate Buffer Solution (PBS). PBS or phosphate-buffered saline is a buffer solution that is particularly valuable because it mimic the ion concentration, osmolarity, and pH of human body fluids. In other words, it's isotonic to human solutions, so it's less likely to cause cell damage, toxicity, or unwanted precipitation in biological, medical, or biochemical research. PBS has many uses because it is isotonic and non toxic to cells. It can be used to dilute substances. It is used to rinse containers containing cells. PBS can be used as a diluent in methods to dry biomolecules, as water molecules within it will be structured around the substance (protein, for example) to be 'dried' and immobilized to a solid surface. Then thin film of water that binds to the substance prevents denaturation or other conformational changes [13].

There are several recipes to prepare PBS solution. The essential solution contains water, sodium hydrogen phosphate, and sodium chloride. Some preparations contain potassium chloride and potassium dihydrogen phosphate. EDTA may also be added in cellular preparation to prevent clumping. Phosphate-buffered saline is not ideal for use in solutions that contain divalent cations (Fe^{2+}, Zn^{2+}) because precipitation may occur. However, some PBS solutions do contain calcium or magnesium. Also, it should be kept in mind that phosphate may inhibit enzymatic reactions. One should be particularly aware of this

potential disadvantage when working with DNA. While PBS is excellent for physiological science, be aware the phosphate in a PBS-buffered sample may precipitate if the sample is mixed with ethanol. The recipe for the solution made for this project is mentioned below.

Reagent	**Weight/Volume**	**Final concentration**
Sodium chloride	8.00 grams	137 mM
Potassium chloride	0.20 grams	2.7 mM
Disodium phosphate (Na_2HPO_4)	1.44 grams	10 mM
Monopotassium phosphate (KH_2PO_4)	0.24 grams	1.8 mM
MilliQ water	Up to 1 L	

Table 3.1 Ingredients for making PBS solution

1. Prepare 800 mL of distilled water in a suitable container.
2. Add 8 g of Sodium chloride to the solution.
3. Add 0.2 g of Potassium Chloride to the solution.
4. Add 1.44 g of Sodium Phosphate Dibasic to the solution.
5. Add 0.245 g of Potassium Phosphate Monobasic to the solution.
6. Adjust solution to desired pH with HCL and NaOH (typically pH ≈ 7.4).
7. Add distilled water until the volume is 1 L.

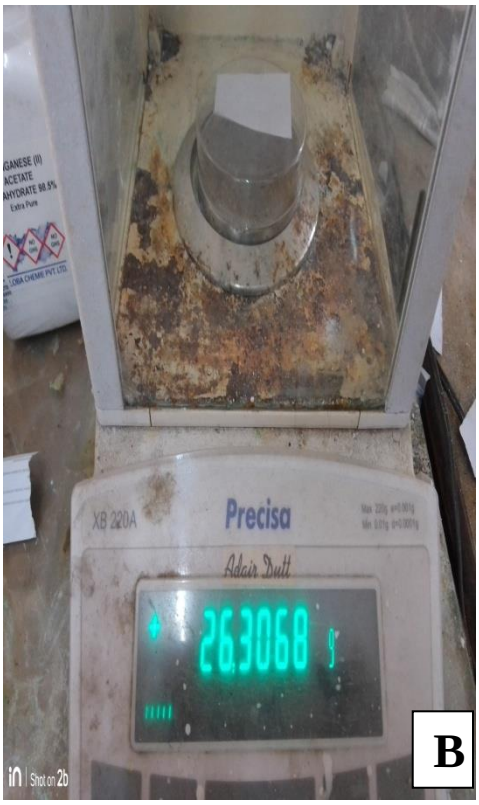
Sterilization isn't necessary for some applications, but if it is sterilized, the solution should be dispensed into aliquots and autoclave for 20 minutes at 15 psi (1.05 kg/cm^2) or filter sterilization.

Phosphate-buffered saline may be stored at room temperature. It may also be refrigerated, but 5X and 10X solution may precipitate when cooled. If a concentrated solution must be

chilled, first it should be stored at room temperature until it is certain the salts have completely dissolved. If precipitation does occur, warming the temperature will bring them back into solution. Shelf life of refrigerated solution is 1 month [10][11][12].

For obtaining the calibration curve, which will be discussed in the subsequent chapters, the PBS solution was slightly modified, by adding 0.01M NaOH in it. This was necessary to increase the conductivity of the solution, so that the electro-oxidation of glucose molecules happen smoothly in the solution. NaOH acts as supporting electrolyte without taking part in the electroactive reactions.

Apparatus for solution making



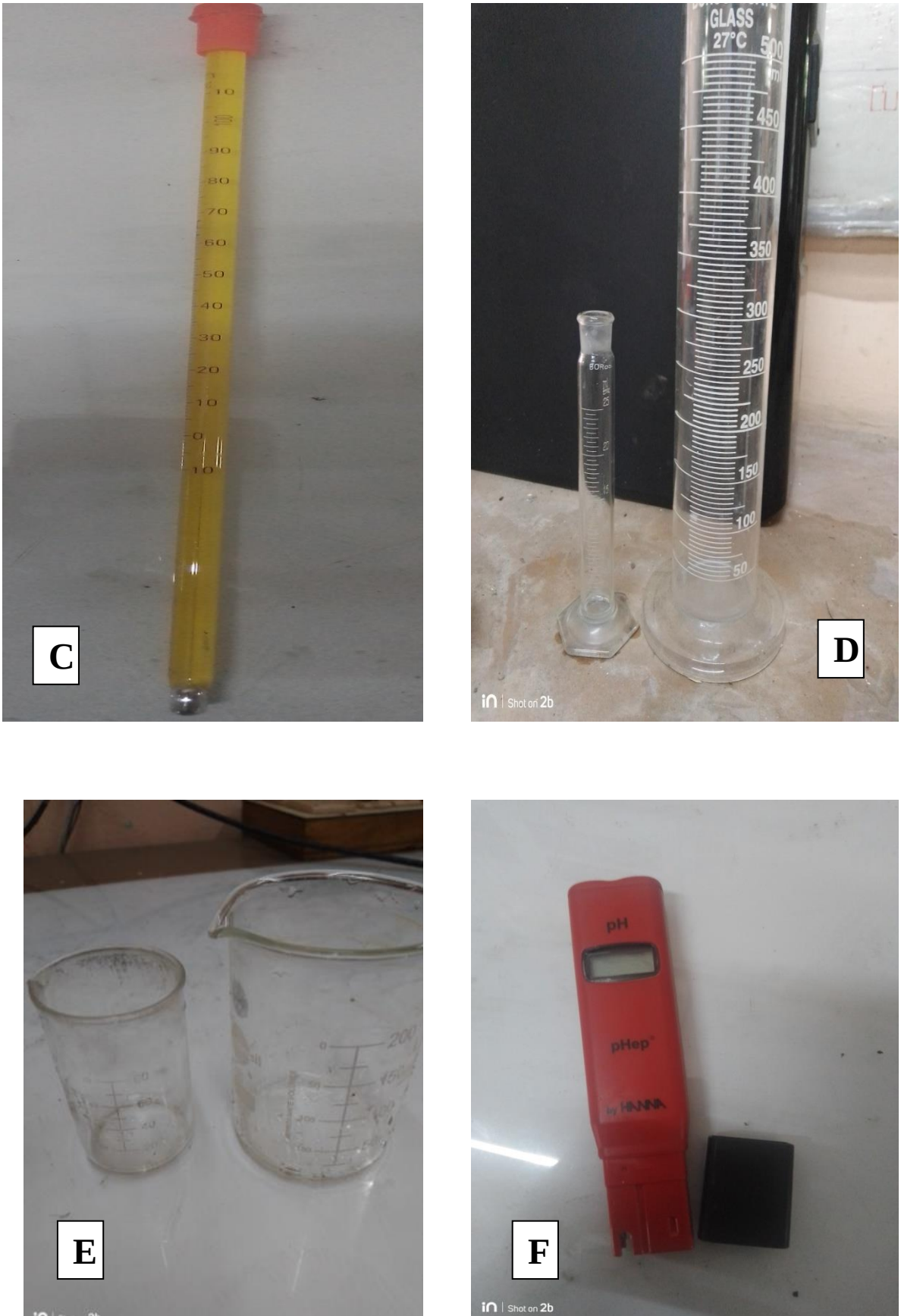


Fig 3.1 Various tools and apparatus for making the required solutions. A. Double Distillation setup B. Weighing scale C. Thermometer D. Conical Flasks E. Beakers F. pH meter

3.2 Electrochemical Setup

In this section, the electrochemical setup will be shown and discussed. The various components of the electrochemical setup are Potentiostat machine, Electrodes, Cell and connecting wires and PC with Nova software. All of these will discussed one by one.

3.2.1 Potentiostat Machine(Autolab)

Fig 3.2 AUTOLAB machine, in the running condition(green light on)

The potentiostat machine used in this project work is AUTOLAB machine, by Metrohm. It is a Netherlands made product. It is having the serial no of MAC90833. It is having two channels.

3.2.2 Electrodes

- Working Electrode

The working electrode (WE) represents the most important component of an electrochemical cell. It is at the interface between the WE and the solution that electron transfers of greatest interest occur. The selection of a working electrode material is critical to experimental success. Several important factors should be considered. Firstly, the

material should exhibit *favorable redox behavior* with the analyte, ideally fast, reproducible electron transfer without electrode fouling. Secondly, the *potential window* over which the electrode performs in a given electrolyte solution should be as wide as possible to allow for the greatest degree of analyte characterization. Additional considerations include the cost of the material, its ability to be machined or formed into useful geometries, the ease of surface renewal following a measurement, and toxicity[9].

The most commonly used working electrode materials are *platinum*, *gold*, *carbon*, and *mercury*. Among these, platinum is likely the favorite, demonstrating good electrochemical inertness and ease of fabrication into many forms. The biggest disadvantage to the use of platinum, other than its high cost, is that the presence of even small amounts of water or acid in the electrolyte leads to the reduction of hydrogen ion to form hydrogen gas (hydrogen evolution) at fairly modest negative potentials (E = -0.059 x pH). This reduction obscures any useful analytical signal.

The technical specifications of the Platinum foil working electrode used in the project is given below.

Purity	99.99%
Length	10 mm
Width	0.75 mm
Thickness	0.25 mm
Application	Laboratory
Brand	Eliteck

Table 3.2 The technical specifications of the Platinum foil working electrode used in the project

Fig 3.3 Platinum Working Electrode (Foil type)

Ideally, a working electrode should behave reproducibly each time that it is used. Factors that affect the electrochemical behavior of a surface are its cleanliness, the kind and extent of chemical functionalities (including oxides) that are present, and the microstructure of the electrode material itself. Generally, a pretreatment step or steps will be carried out prior to each experiment to assure that the surface going into the electrochemical cell can be reproduced experiment-to-experiment. These may be as simple as mechanical polishing, and may include pre-scanning across a certain potential range or exposure to a solvent or chemical species to “activate” the electrode.

Generally, the platinum foil remains clean after the experiments, and the surface looks shiny. However, the appearance of the dull surface indicates the surface contamination. Further, surface contamination can also be detected by performing cyclic voltammetry in a pure electrolyte (e.g., 0.5 mol/L aqueous H_2SO_4). The occurrence of additional peaks other than the traditional voltammogram indicates the presence of surface contamination. In any case, the surface must be cleaned before using it as a working electrode. The following methods can be used for the cleaning of the Pt electrode:

The chemical method for cleaning: Organic impurities¬ can be cleaned with a suitable organic solvent (e.g., ethanol). Protein deposits can be hydrolyzed with a suitable commercial enzyme-based cleaner. Inorganic deposits can be cleaned using dilute acid

and base (0.1 mol/L HCl, HNO_3, NaOH). Hot dilute solutions can be taken if the ambient temperature does not work. In general, hot 10% nitric acid removes most of the inorganic impurities [8].

The electrochemical method for cleaning: Platinum electrode can be cleaned by doing multiple cyclic voltammetry in a clean solvent (10 to 20 cycles). Persistent impurities can be removed by holding the electrode at either a high oxidizing or reducing potential in dilute acid solution (0.1 mol/L sulphuric acid) for few seconds to few minutes depending upon the impurity level. Storing: The exposed surface of the platinum¬ electrode should be kept immersed in clean DI water in an airtight container while not in use.

- Reference Electrode

Voltammetric methods are those in which current passing in an electrochemical cell is measured as a function of the potential applied to the working electrode. Potential, by definition, is not something that can be *directly* measured. Rather, the measurement of applied potential requires that a reference point first be established, and individual potentials be measured relative to that reference point. This is accomplished by placing a second electrode, called the *reference electrode*, in the cell and measuring *potential* as the *energy difference* between the two electrodes. As some famous electrochemists have perfectly expressed, “electrochemistry with a single electrode is like the sound of one hand clapping”.

Reference electrodes should be constructed using half-cell components that are stable over time and with changing temperature, present at well-defined values of activity. They should possess fixed, reproducible electrode potentials. The reference half-cell with which most of us are familiar is the *standard hydrogen electrode* (SHE), composed of an inert solid like platinum on which hydrogen gas is adsorbed, immersed in a solution containing hydrogen ions at unit activity. The half-cell reaction for the SHE is given by

$$2H+(aq)+2e-\rightleftharpoons H2(g)(1)(1)2H+(aq)+2e-\rightleftharpoons H2(g)$$

with a half-cell potential arbitrarily assigned a value of zero (E^0 = 0.000 V). Tables of electrode potential values for many redox couples relative to the SHE are commonly available.

Practical application of the SHE is limited by the difficulties in preparing solutions containing H^+ at unit activity and maintaining unit activity for H_2 (g) in the half-cell. Most experiments carried out in aqueous solutions utilize one of two other common reference half-cells – the *saturated calomel electrode (SCE)* or the *silver-silver chloride electrode (Ag/AgCl)*.

In this project, the reference electrode used is the *saturated calomel electrode (SCE)*.

The SCE is a half cell composed of *mercurous chloride (Hg_2Cl_2, calomel)* in contact with mercury metal, either as a pool or as a paste with calomel. These components are either layered under a saturated solution of potassium chloride (KCl), or within a fritted compartment surrounded by the saturated KCl solution (called a double-junction arrangement). A platinum wire is generally used to allow contact to the external circuit. The half reaction is described by

$$Hg_2Cl_2(s)+2e^-\rightleftharpoons 2Hg(l)+2Cl^-(sat'd)(2)(2)Hg_2Cl_2(s)+2e^-\rightleftharpoons 2Hg+2Cl^-(sat'd)$$

with a potential of 0.241 V with respect to the SHE at 25 ^oC. Contact to the electrochemical cell is made through a porous glass frit or fiber, which allows ions to cross but not bulk mixing of the electrolytes.

Fig 3.4 Saturated Calomel Electrode(SCE) with the connection lead

- Counter/Auxillary Electrode

The purpose of the *auxiliary electrode (AE)* is to provide a pathway for current to flow in the electrochemical cell without passing significant current through the reference electrode. There are no specific material requirements for the electrode beyond it not adversely influencing reactions occurring at the working electrode (WE). Remember that if a reduction occurs at the WE, there must be an oxidation that takes place at the AE. Care should be taken that electrode products formed at the AE do not interfere with the WE reaction. The AE can be physically separated from the WE compartment using a fritted tube, but one should be aware that under certain circumstances this can have a deleterious effect.

The most commonly used material for the auxiliary electrode is *platinum*, due to its inertness and the speed with which most electrode reactions occur at its surface. Other, less expensive materials may also be used as auxiliary electrodes. Choices include *carbon*, *copper*, or *stainless steel* if corrosion is not an issue for a particular electrolyte solution or reaction.

As discussed in the previous section on *electrochemical cells*, the AE should supply current density and potential that is constant across the length of the WE. Many times this means fashioning the AE such that it “mirrors” the shape of the WE, as is the case for a rectangular WE being located near a rectangular AE of similar area. Wire is convenient in that it can be coiled in a symmetrical arrangement around the WE. In some instances, the electrochemical cell can be fashioned from the material chosen for the AE, and the cell itself can serve that purpose.

In this project, Graphite rod has been used as the counter electrode. A large graphite electrode, relative to the Working electrode surface area, is used to ensure the problems of current overload is minimized.

Fig 3.5 Graphite Rod, as counter electrode

3.2.3 Cell and Connecting Wires

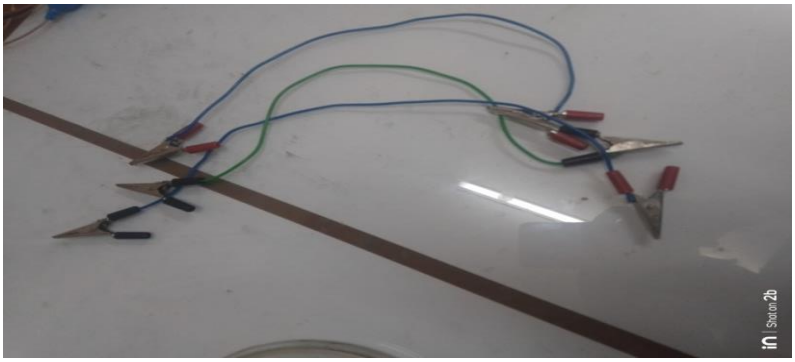


Fig 3.6 Connecting wires used in this project work

Connecting wires are used to connect the three electrodes, namely Working Electrode, Reference Electrode and Counter Electrode to the potentiostat AUTOLAB machine. Care should be taken to ensure that the leads of the electrodes are not touching each. Also the alligator clips in the connecting wires should be new, and shiny to ensure they have not been corroded.

3.2.4 PC with Nova Software

Fig 3.7 PC with Nova software, used in this project work

Nova 2.1.6 software was used to run the AUTOLAB potentiostat machine, in a PC without any internet connection, to ensure absolutely no chance of malware infections.

3.3 Material Development

Four electrocatalytic materials have been developed in this project. Their detailed synthesis or development processes are discussed in this section.

3.3.1 Ni and Ni with Nanocarbon

Stainless steel(SS) samples were polished by series of emery papers 2/0 to 3/0 and cleaned by oxalic acid, water and then dried in normal room temperature. The electrolyte was 0.65 M $NiCl_2$, 0.30 M $NiSO_4$, 0.90 M H_3BO_3 and 1.5 gm/l $CH_3(CH)_{11}OSO_3Na$ (sodium dodecyl sulfate). The sample was electrodeposited in the electrolyte at 60^oC in a galvanostatic circuit at a current density of 300 mA/cm^2. The Ni-coated sample was rinsed

in water and left in normal room temperature overnight. The coated surface was then cleaned with acetone and ethanol, respectively. For Ni-nanocarbon coating, the sample was electrocoated in the presence of suspended nanocarbon particle at 60ºC in a galvanostatic circuit at a current density of 300 mA/cm^2 with continuous stirring at 180 rpm.

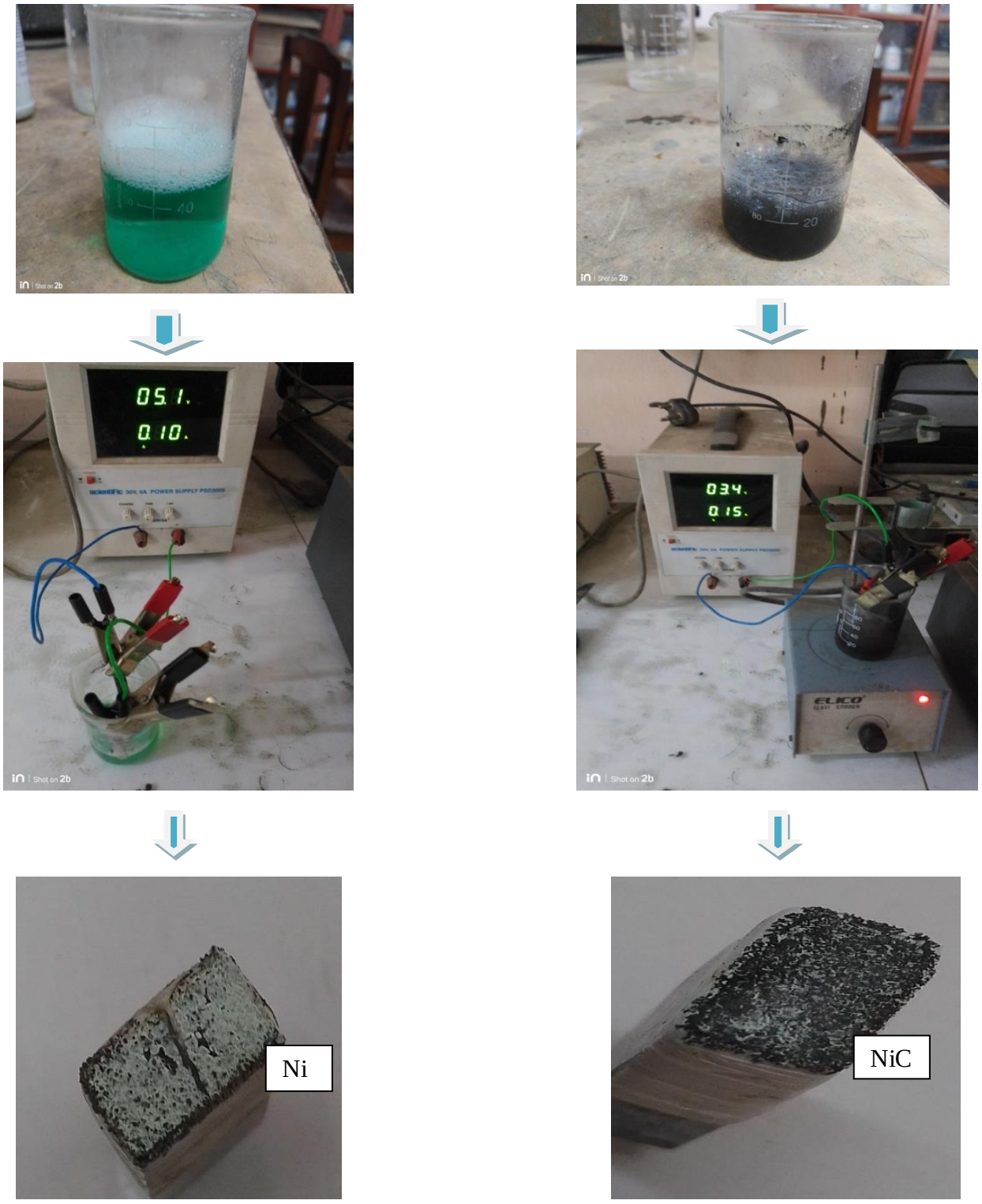


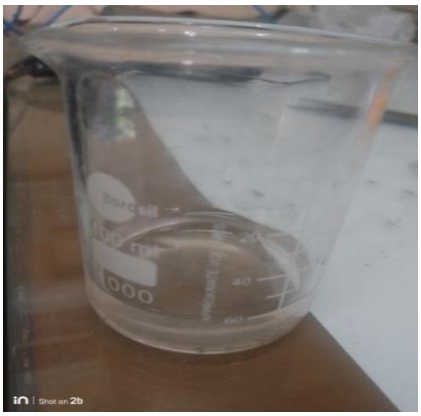
Fig 3.8 Flowchart showing the development pathway of Ni(on the left) and NiC (on the right)

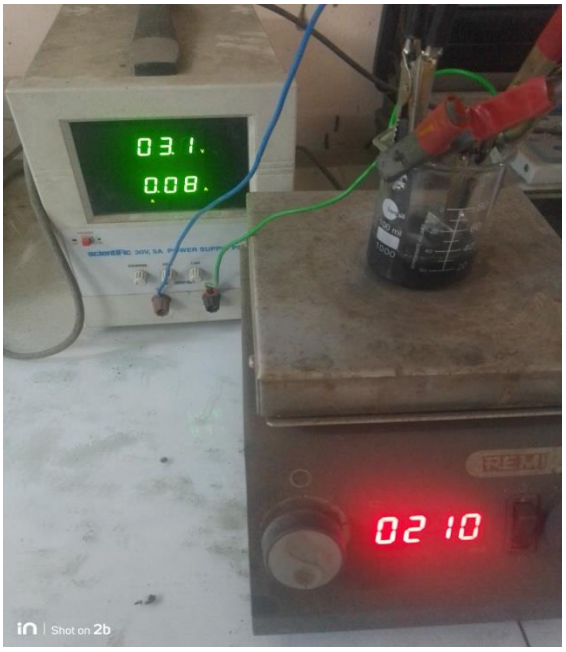
3.3.2 MnO2 and MnO2 with nanoCarbon

The coating was done on stainless steel plate by electrodeposition technique as stated below. The steel samples were polished by series of emery papers 1/0 to 3/0 and cleaned by acetone, water and dried by hot air. The sample was electrodeposited galvanostatically in a potentiostat machine with three-electrode system (working electrode 304 stainless steel, counter electrode graphite rod and calomel reference electrode) in $MnSO_4$ solution. The optimum condition of electrodeposition was found out by performing a series of experiments varying the parameters, controlling the electrodeposition process. The condition was 0.35 M $MnSO_4$ and 0.60 M H_2SO_4 at a temperature of 65^oC at a current density of 260 mA/cm^2 . The coated sample was rinsed by acetone and ethanol and dried at a temperature of 80^oC for 6 h. For MnO_2-nanocarbon coating, graphite powder prepared by ball mill grinding of graphite electrode was used. The sample was electrodeposited by a DC power supply machine in the same solution as stated above with the presence of suspended nano-graphite powder under similar condition.

Electroplating parameters	**Range**	**Optimum**
$MnSO_4$: M	0.25 – 0.4	0.34
H_2SO_4 : M	0.55 – 0.65	0.6
Carbon powder: g	-------	0.2
pH value	11-14	11.6-13.6
Bath Voltage: V/V	2-6	4-6
Applied current density : mA/cm^2	214-270	250 - 260

Table 3.3 Showing the electrodepositon parameters for development of MnO_2 and MnO_2 C electrocatalytic material





MnO_2

MnO_2C

Fig 3.9 Flowchart showing the development pathway of MnO_2(on the left) and MnO_2C (on the right)

Chapter 4
Results and Discussion

4. Results and Discussion

Having made the experimental setup as discussed in chapter 3, a series of experiments have been performed to find out whether glucose in the Simulated Body Fluid(SBF) can be electrochemically converted to energy by clean technology and thereby open up a new science & technology to reduce sugar of a diabetic patient by alternative, innovative route. Various electrochemical tests like Cyclic Voltammetry(CV), Chronoamperometry(CA), Electrochemical Impedence Spectroscopy(EIS) and Potentiodynamic Polarisation(PD) were performed with different electrocatalytic materials viz Platinum(Pt),Nickel (Ni), Nickel with nanocarbon(NiC), Manganese dioxide(MnO_2) and Manganese dioxide with nanocarbon(MnO_2 C) in artificially made Simulated Body Fluid(SBF) with different concentrations of sugar, in accordance with diabetic patients worldwide. The developed electrocatalytic materials were synthesised by electrodeposition in solution containing the required ions and at a predetermined potential, with variation of the current, to control the morphology of the structure.

The following section give result and discuss the result obtained in different experiments performed.

4.1 Cyclic Voltammetry

In this project, four different materials were developed by the process of electrodeposition. Cyclic voltammetry (CV) test was performed for all of them, to evaluate and characterise their electrochemical behaviour in SBF(PBS solution with 200mgdl glucose). The glucose is expected to be oxidised to gluconolactone on the electrocatalytic surface, giving off hydrogen ions and electrons.

Figure 4.1 depicts the CV curves of the different developed electrocatalytic materials in PBS solution containing 200mgdl glucose. The test parameters for running the CV test are as follows.

Test Parameters	Value
Start Potential	-0.3 V wrt SCE
Upper Vertex Potential	1.5 V wrt SCE
Lower VertexPotential	-0.5 V wrt SCE
Stop Potential	0.2 V wrt SCE
Number of scans	2
Scan Rate	100 mV/sec
Step	0.00244 V

Table 4.1 Test Parameters for running Cyclic Voltammetry(CV) test for the various electrocatalytic materials in SFB(Phosphate Buffer Solution with 200mgdl glucose concentration)

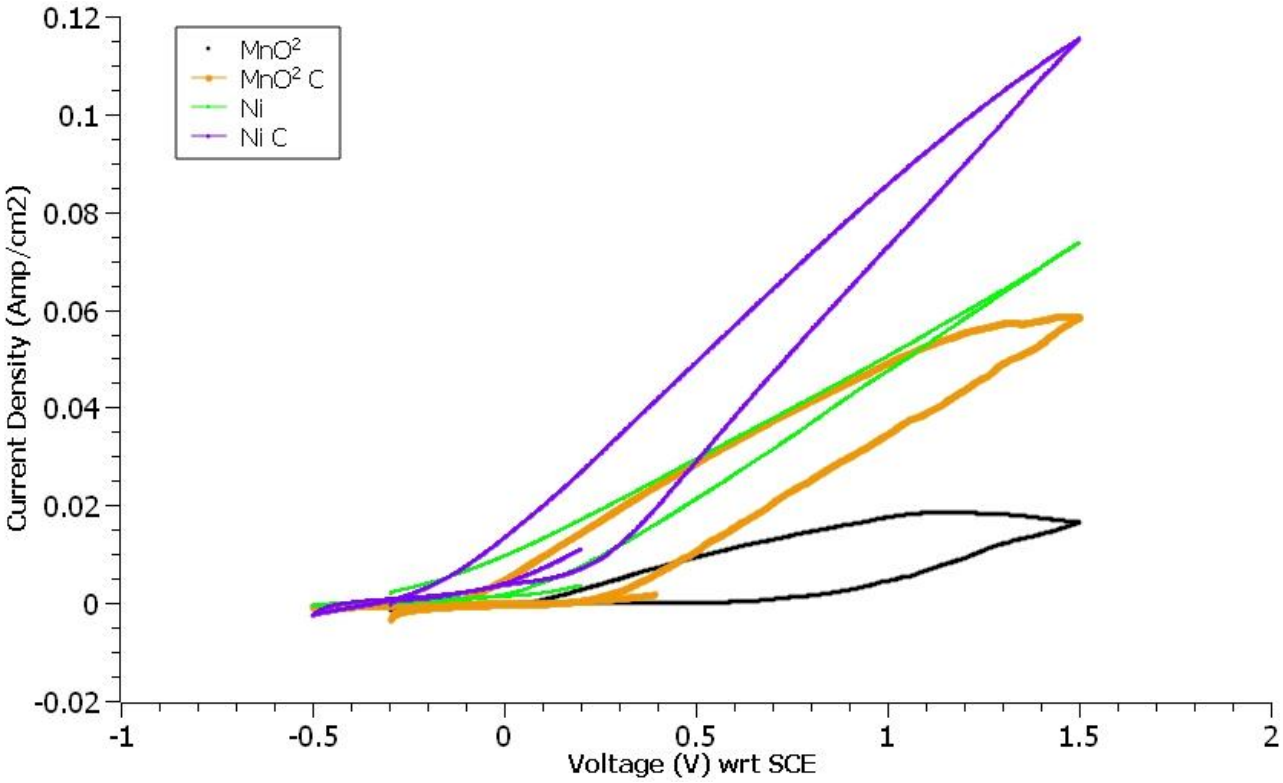


Fig 4.1 Cyclic Voltammetry(CV) curves for the various electrocatalytic materials in SFB(Phosphate Buffer Solution with 200mgdl glucose concentration)

The electrochemical oxidation of glucose over the anode surface is reflected by the current rise (y axis) at an onset potential of around + 0.05 V versus SCE. It is seen that the nature

of the curves of all the four materials is similar with a peak current density, which indicates the rate of electrochemical oxidation of the fuel glucose, on the anode surface. The enclosed area of the curve indicates the energy content from the fuel cell with electrochemical oxidation. It is noted that the electrode made with Ni-nanocarbon (Ni C) outperforms all other electrodes. The highest current density achieved is over 120 mA/cm^2 which is very encouraging, i.e., an electrode surface of 10 cm^2 will produce 1.2 A. The performance of MnO_2-nanocarbon is also good where the maximum current density is over 60 mA/cm^2. It is also to be noted, that the energy obtained is enhanced when nanocarbon is added to either Ni or MnO_2, in comparison to non nanocarbon samples.

4.2 Potentiodynamic Polarisation

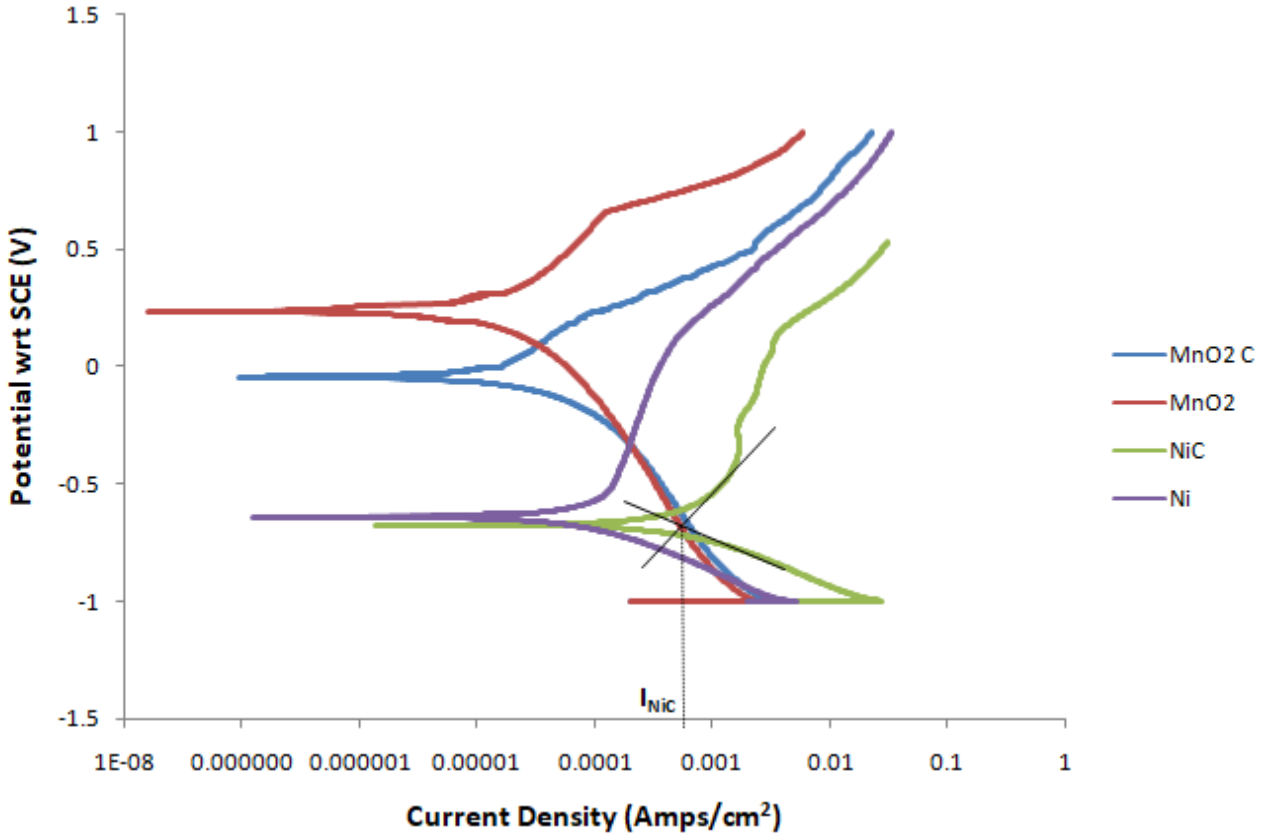


Fig 4.2 Tafel Plot for the various electrocatalytic materials in SFB(Phosphate Buffer Solution with 200mgdl glucose concentration)

The fig no. 4.2 depicts branch of potentiodynamic polarisation curves for different electrocatalytic materials, with and without nanocarbon. The curve which shifts to the right

most, produce max cell current density. It is seen, that the electrocatalytic material, Nickel with nanocarbon (NiC) produce max delivery current. The magnitude of the current can be found out by superimposing anodic and cathodic Tafel lines, as shown in the figure. It is seen that for NiC electrode, the current is coming around 1 mA/cm^2, that is, an electrode of size of 1 inch by 1 inch, in a battery of 10 electrodes, can produce over 129 mA, which is enough current to run small electronic gadgets.

4.3 Chronoamperometry

Having obtained a highly energetic electrode with a very good maximum current density and energy content, it is interesting to find out how the current decays with time by performing a CA test. Just like the CV test, here also, all the four developed materials have been tested by Chronoamperometry (CA). Any stable electrocatalytic surface produces a steady current for a certain period of time before its surface is contaminated and becomes electrochemically less active. Figure 4.3 shows the chronoamperometry (CA) graphs of all the four developed materials, in the PBS solution containing 200mgdl concentration. The results are similar to those obtained in the CV study in Figure 4.1. The effect of graphene is seen to have increased the steady-state current density and has made the material more energetic. The steady-state current with graphene–nickel–electrode is found around 70 mA/cm^2 . That is, an electrode with a surface area of 100 cm^2 in a glucose fuel cell can produce up to 7A of current, which is very good for running motors or other useful devices without the generation of pollutants.

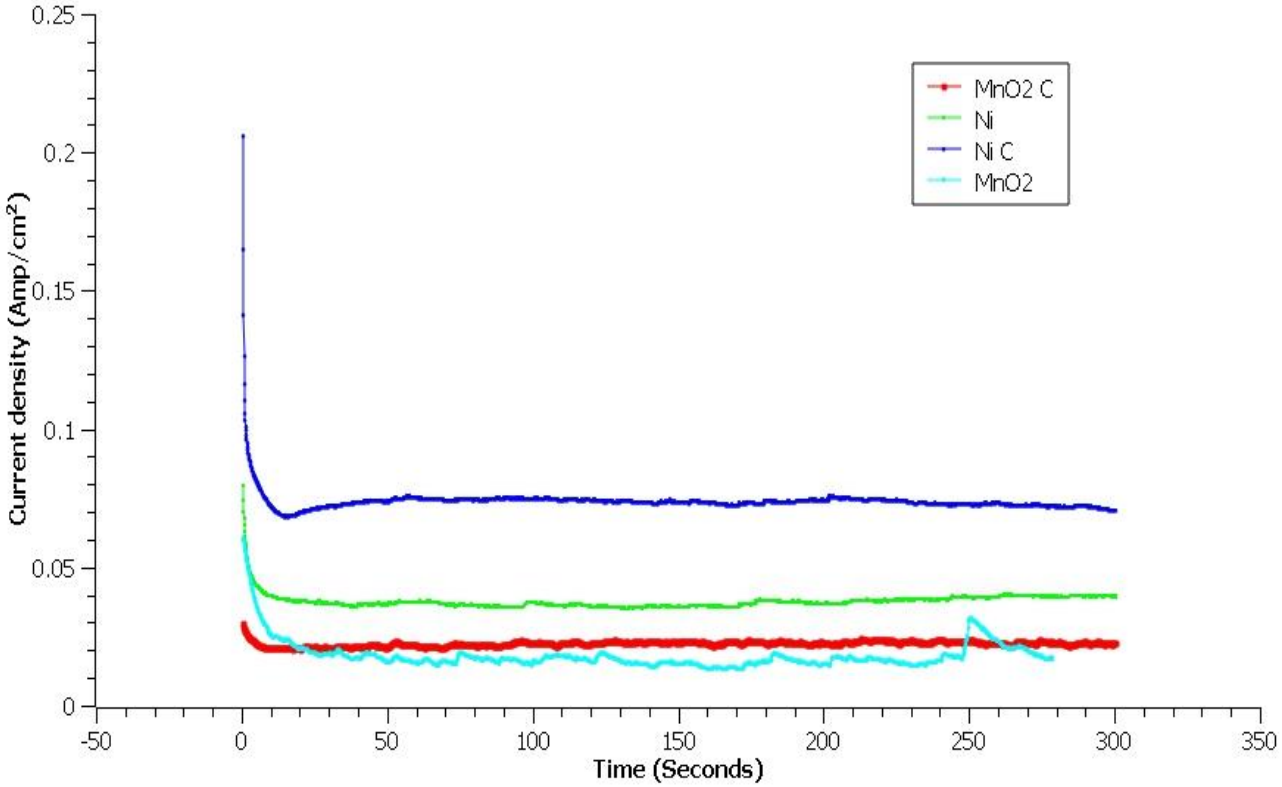


Fig 4.3 Chronoamperometry (CA) curves for the various electrocatalytic materials in SFB(Phosphate Buffer Solution with 200mgdl glucose concentration)

4.4 Electrochemical Impedence Spectroscopy(EIS)

The electrochemical phenomena occurring at the metal– electrolyte interface can be represented by R-L-C circuit and are studied by EIS for better understanding of the fundamental aspects of oxidation of the fuel on the electrolytic substrate. In general, the interface consists of layer of (+ ve) charge and a layer of (- ve) charge which is called electrical double layer, which produces a capacitance or pseudo-capacitance. In addition, there are resistance loads like polarization resistance (R_p) and solution resistance (R_s). There may also be induction or more capacitance due to coating. The phenomenon can be interpreted by Nyquist and Bode plots, which are depicted and discussed in the following section for various electrocoated electrocatalysts.

Fig 4.4 shows the Nyquist plots for different electrocatalytic materials. The diameter of the semicircle represents the polarisation resistance(R_p) . It is seen that NiC electrocatalytic material shows minimum diameter of the semicircle, which indicates it is a high

electrocatalytic material, as far as electrochemical conversion of sugar is concerned. The polarisation resistance(R_p) value of NiC in this solution, is around 24 ohms.

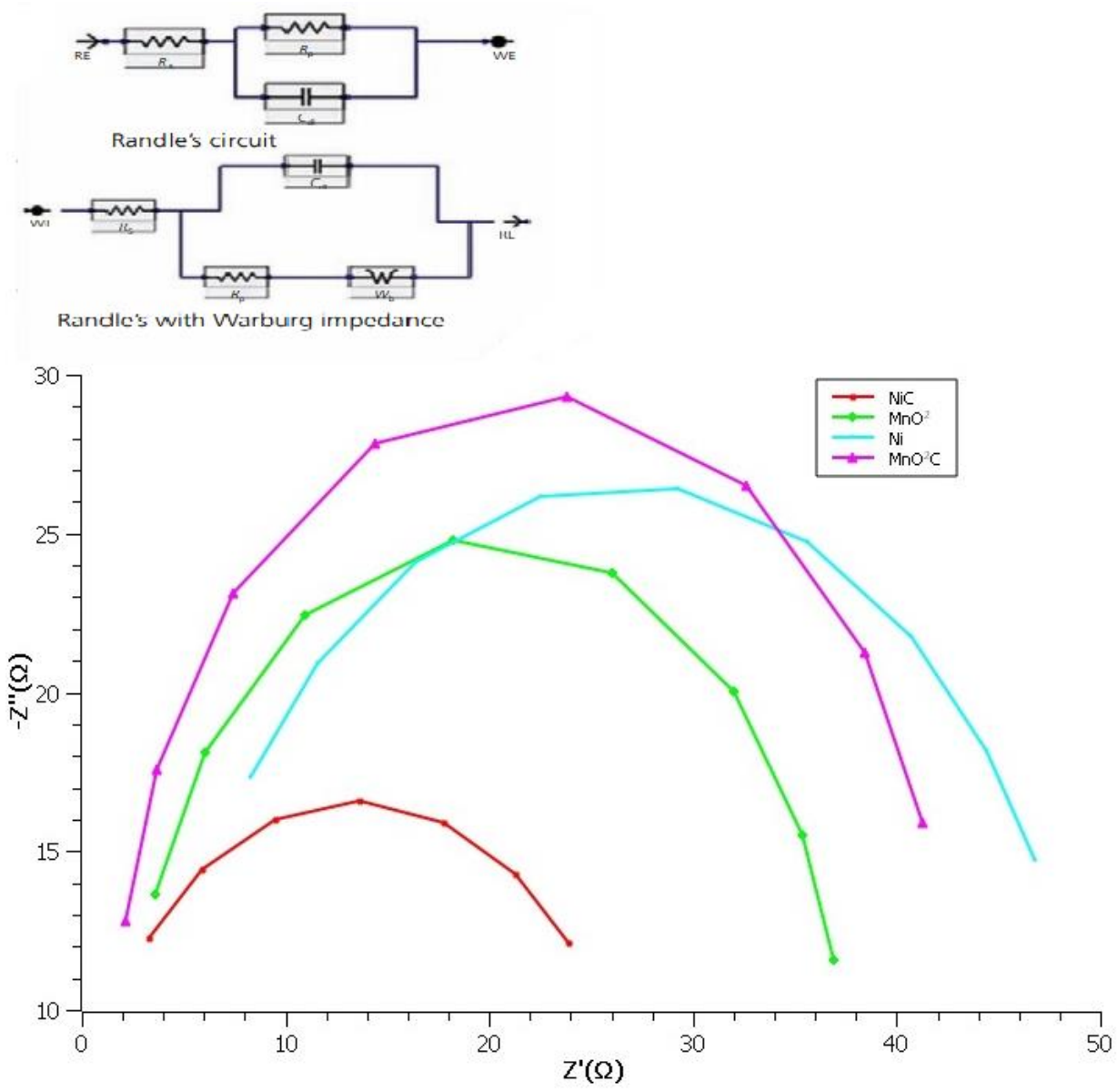


Fig 4.4 Nyquist Plot for the various electrocatalytic materials in SFB(Phosphate Buffer Solution with 200mgdl glucose concentration)

After NiC material, MnO_2 shows the second lowest diameter, indicating the second lower polarisation resistance(R_p) of around 36 ohms. After that MnO_2 C shows the polarisation resistance(R_p) value of around 42 ohms, and lastly Ni material shows an R_p value of 46 ohms.

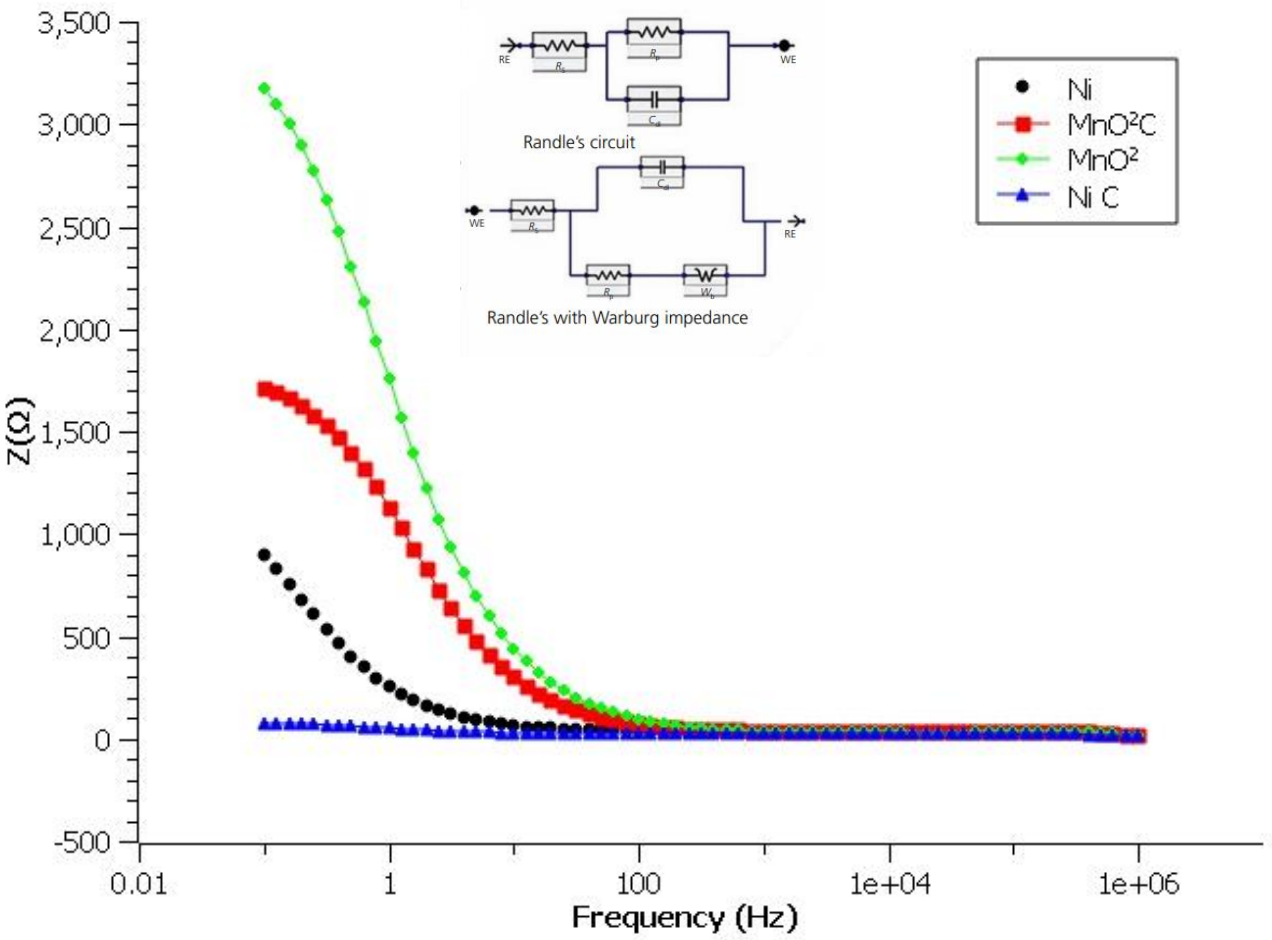


Fig 4.5 Bode Plot (impedence) for the various electrocatalytic materials in SFB(Phosphate Buffer Solution with 200mgdl glucose concentration)

The Bode Plot in the fig 4.5 shows the impedence values for the various electrocatalytic materials. The impedance is indicated by the difference of the top and bottom y coordinate (Z) of any curve. The Bode Curve also agree with the result obtained in the Nyquist Plot. It is seen that the y value or the impedence value of Nickel with nanocarbon(NiC) is minimum(100 ohms), indicating high energetic material. After it, Nickel(Ni) is showing less impedence value, of around 800 ohms . Next comes MnO_2 C with 1600 ohms. The worst performing material is MnO_2 with 3100 ohms, showing least energetic properties among the materials.

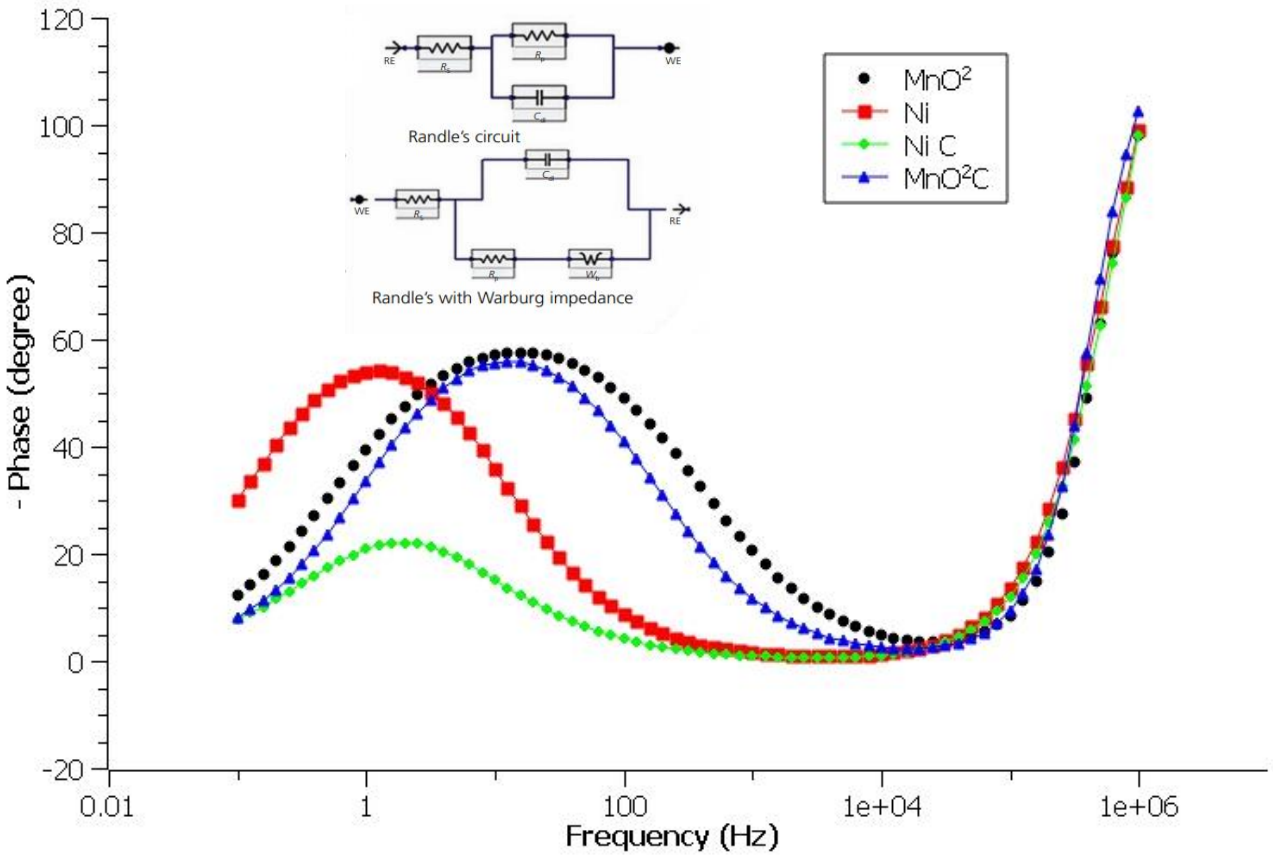


Fig 4.6 Bode Plot (phase angle) for the various electrocatalytic materials in SFB(Phosphate Buffer Solution Solution with 200mgdl glucose concertration)

The presence of the capacitive load produces an angle shift towards −90°. If the phase angle shift is less than 90°, then it is a pseudocapacitance. It behaves like a capacitive load, but it is not a pure capacitance. It can be seen (Figure 4.6) that the phase angle is shifted towards −60° for all the electrodes at a particular frequency, indicating an R–L–C circuit with a capacitive load; however, it is not a pure capacitance but a pseudocapacitance.

4.5 Material Characterisation

After successful electrochemical testing of the developed electrocatalytic materials, material characterisation was done, to find out the constituents of the developed materials as well as their morphology. Two material characterisation tests were done, namely X Ray Diffraction(XRD) and Scanning Electron Miscroscopy(SEM).

4.5.1 X Ray Diffraction(XRD)

Fig 4.7A shows the XRD pattern of electrodeposited Nickel nanocarbon (Ni C) material, on SS substrate. It shows the peak intensity at different 2 theta values. The bigger peaks are from Ni and nanocarbon phases.

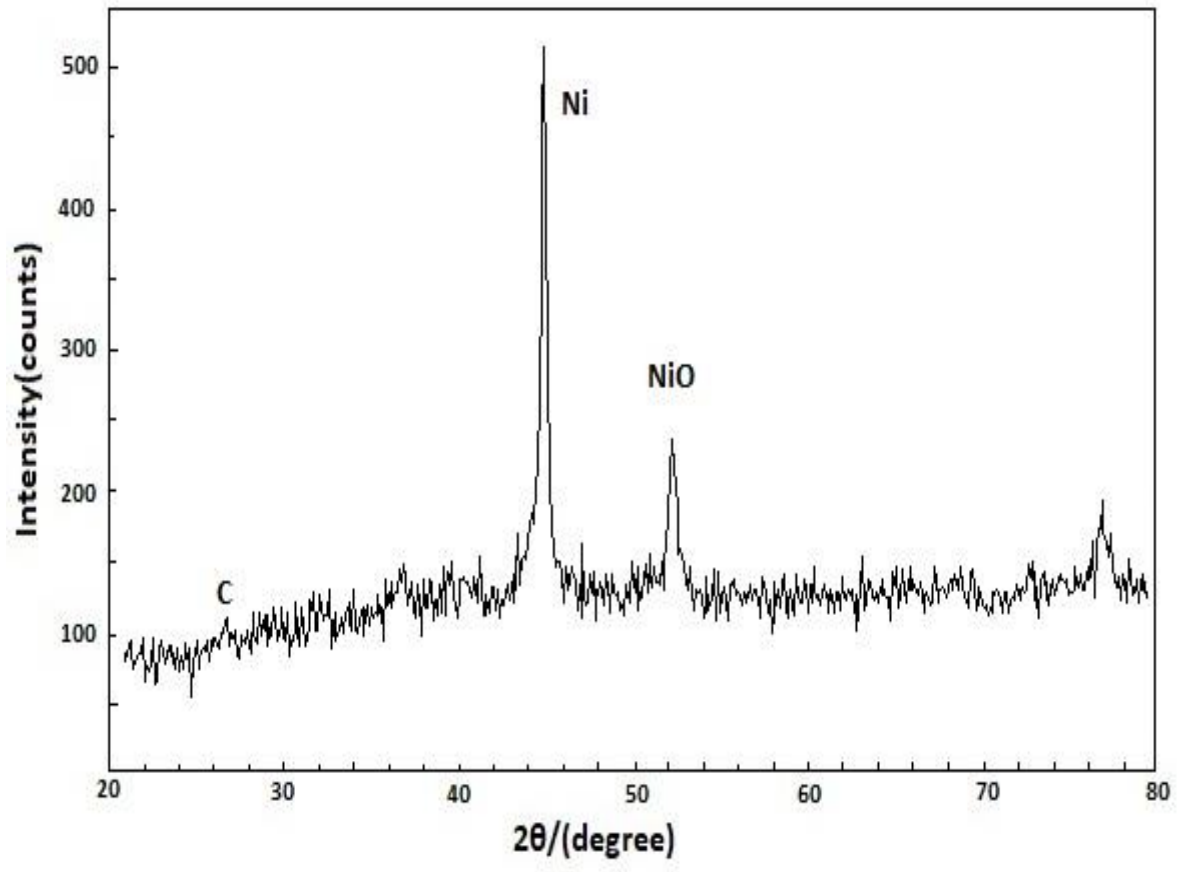


Fig. 4.7A XRD image of NiC

Similarly, fig 4.7B shows the XRD pattern of electrodeposited MnO_2 with nanocarbon, on SS substrate. The smaller peaks are from the constituents of the SS substrate (being not of interest in the present investivation), were not considered in the figure. This confirms electrodeposition of Ni with nanocarbon, and MnO_2 with nanocarbon, on SS substrate.

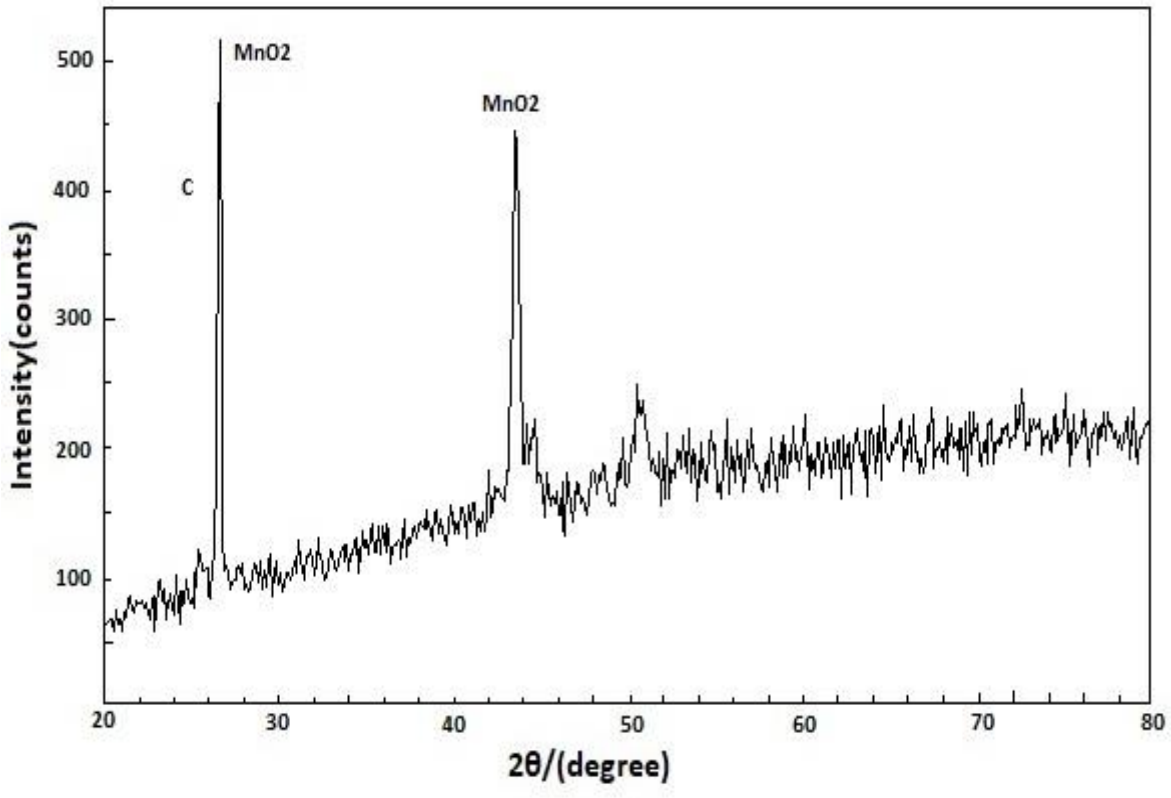


Fig. 4.7B XRD image of MnO_2C

4.5.2 Scanning Electron Microscopy (SEM)

The morphologies of the developed electrocatalytic materials, Nickel with nanocarbon(NiC) and Manganese dioxide with nanocarbon (MnO_2C) were examined under SEM. SEM images morphology is shown in Fig 4.8. The NiC material shows a 3D microscopic structure with a high surface to volume ratio. The images show a much higher 3D electrocatalytic surface area available for the electrochemical oxidation of the fuel, which produces high current, which was found in the electrochemical characterisation. It is

interesting to find that the nano Carbon particles are randomly distributed within the Nickel matrix.

The morphology of the MnO_2 C material exhibits an effective more 3D space on which electrochemical oxidation of glucose has taken place. This increase in effective surface area accounts for the higher current, being delivered from the oxidation of glucose.

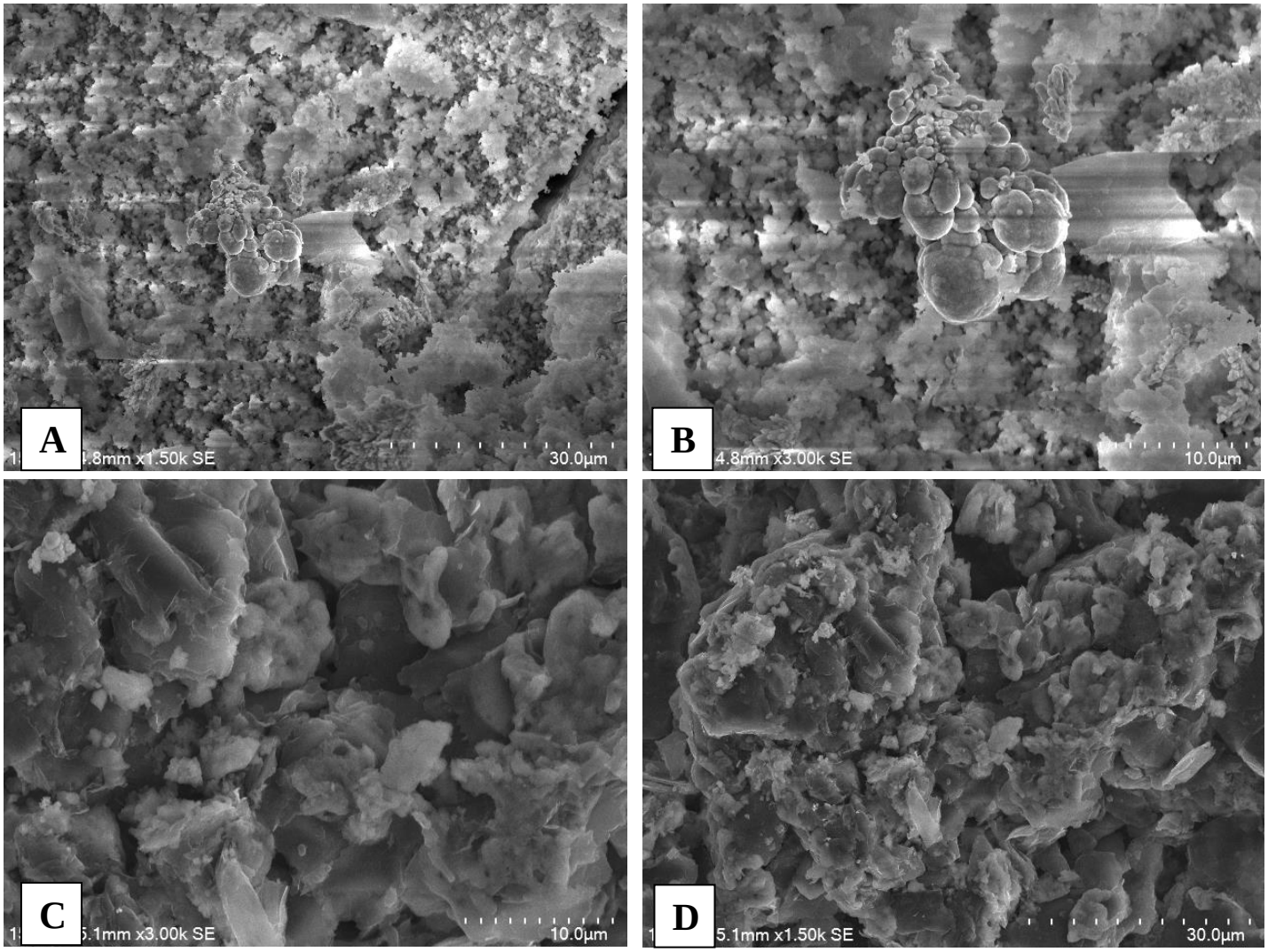


Fig 4.8 SEM image of NiC and MnO_2C. A) NiC at x1500 magnification B) NiC at x3000 magnification. C) MnO_2C at x3000 magnification D) MnO_2C at x1500 magnification

4.6 Electroxidation of glucose in SBF solution

Having obtained very successful results in the foregoing discussion of CV, CA, PD and EIS, now it is inquired whether glucose in the body fluid decreases or not.

The concentration of the glucose in the solution is proportional to current density. So, if the change in current density can be properly determined, it will be easy to find out, how much glucose concentration has been decreased.

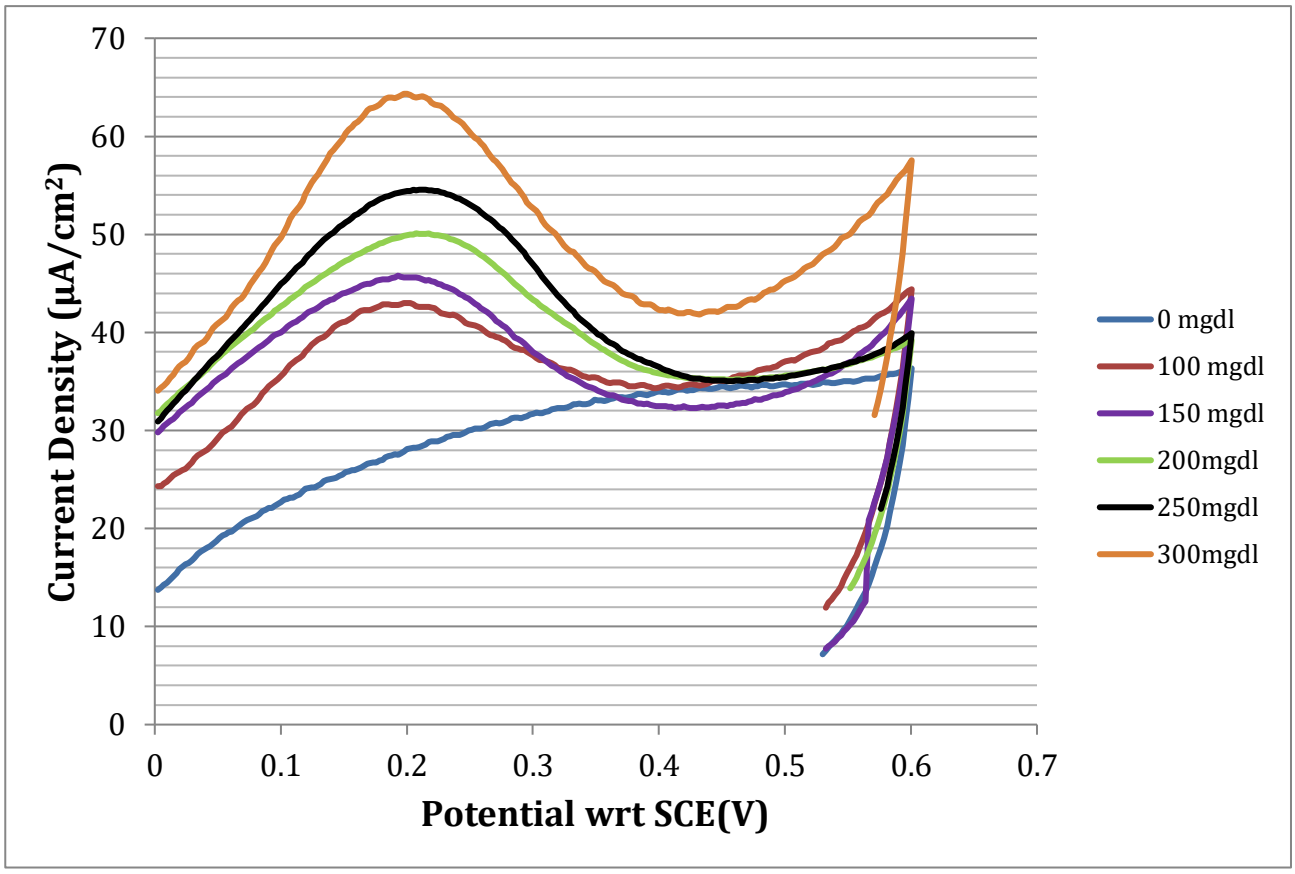


Fig 4.9 Calibration Curves showing different peak current density values for different glucose concentrations for detection of glucose concentration in SBF(Phosphate Buffer Solution with 0.01M NaOH)

For this measurement, some known solution of different sets of glucose concentration were experimented with CV, and by mathematical modelling, the equation relating glucose concentration with the current density, was found out. Fig no, shows the calibration curve. It shows the different concentration of glucose in the body fluid, that gives various different peak current density. The ordinate(y axis) is the glucose concentration in mg/dl and abscissa(x axis) is the current density in µA/cm^2. The equation developed from mathematical modelling is given below. It is seen it is a linear equation, with over 95% matching. Using the developed equation, the glucose concentration is found out in the tabulated format.

The calibration curve has shown a good linear regression results, with the R-Squared (R^2 or the coefficient of determination) value of 0.974. The results of the correlation is presented by the following equation.

Glucose(mgdl) = 8.821*Current(μA/cm^2) – 253.1 **(1)**

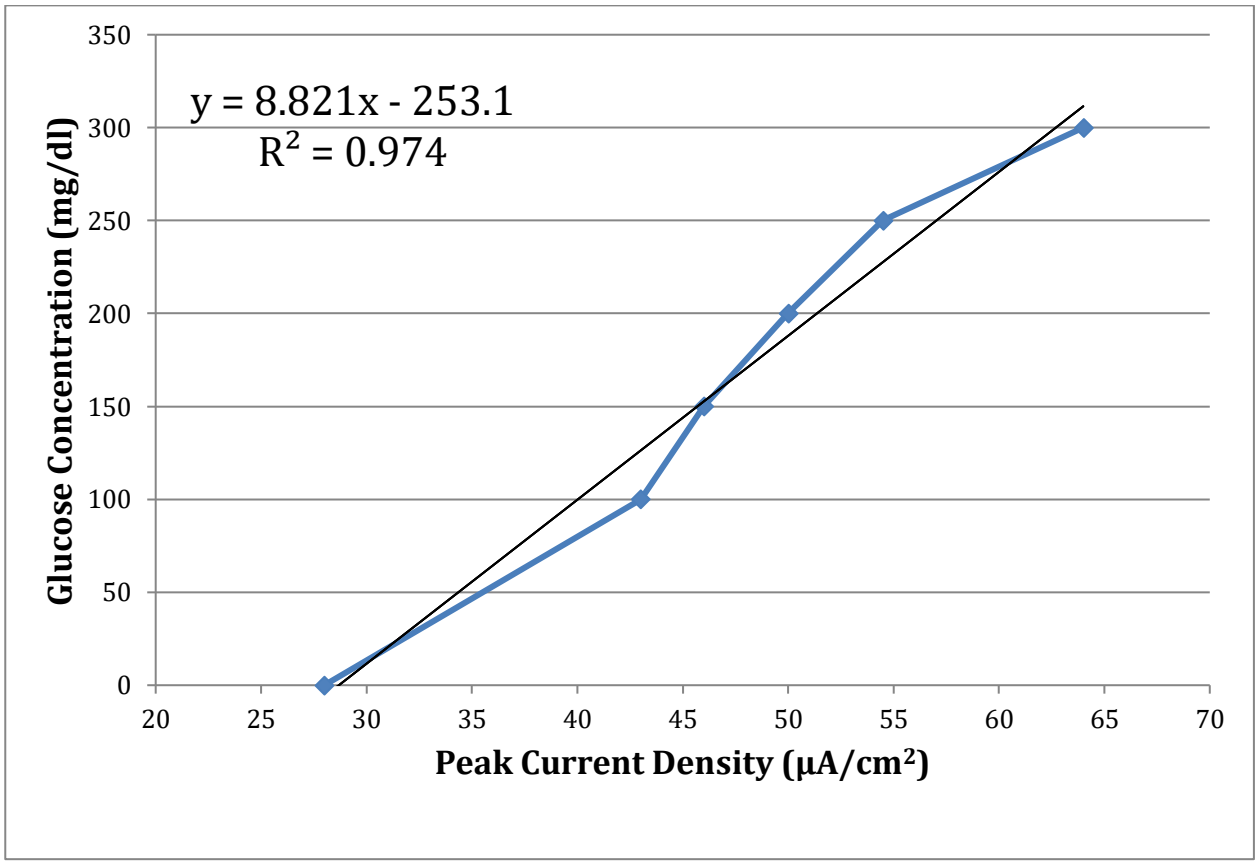


Fig 4.10 Linear Regression Plot showing linear relationship of glucose concentration with peak current densities, with the linear regression equation.

Now that the Calibration curves for Glucose detection have been discussed, one can now proceed towards attempting to reduce the excess glucose that is present in a diabetic patient's blood. As obtained from literature [5][6], the fasting healthy blood glucose level (BGL) is 72 to 108 mgdl.

As shown in the literature review section, a number of electrocatalytic materials were found to be very efficient in electroxidising glucose in PBS solution. Among them, Platinum is the most popular and widely recognised. A number of Chronoamperometry tests were run with Platinum, with different glucose concentrations, and for different time durations,

to observe the performance of Platinum electrode material, in electroxidising the glucose molecules in the PBS solution and thereby, reducing the glucose level. All these tests are elaborated as follows.

1) A solution of known concentration of 250 mgdl glucose was taken in the electrochemical cell, and Chronoamperometry (CA) procedure was run in it. Potential applied was 0.25V wrt SCE. And the time duration was 60 secs.

   This particular potential was arrived at, from the Calibration Curves. As it can be seen in the fig, the peak current density, for all the solutions with different glucose concentration, was obtained at a certain fixed potential value. That potential value is 0.2 V wrt SCE. It means, around this particular potential the electroxidation of glucose molecules is happening most efficiently, and as a result of it, there is a sudden increase in the flow of electrons through the working electrode, which is constituting the peak in the Cyclic voltamogram.

   But due to certain practical issues like IR drop, the exact potential for most efficient electroxidation, varies a little bit from the potential (0.2V) obtained in the CV test. After doing many hit and trial tests, that exact potential was found to be 0.25 V wrt SCE.

   After the Chronoamperometry(CA) procedure is done, now it is needed to know whether the initial glucose level (of known concentration) has been reduced or not. For that, a Cyclic Voltammetry (CV) test needs to run, to obtain the peak current density value from it, and to put the value in the linear regression equation(1) that have been obtained from the Calibration Curves. The CV test was run, with all the test parameters like Start potential, Stop potential, Upper Vertex Potential, Lower

Vertex Potential, Scan Rate, etc exactly the same as the CV procedures that were run for obtaining the Calibration Curves.

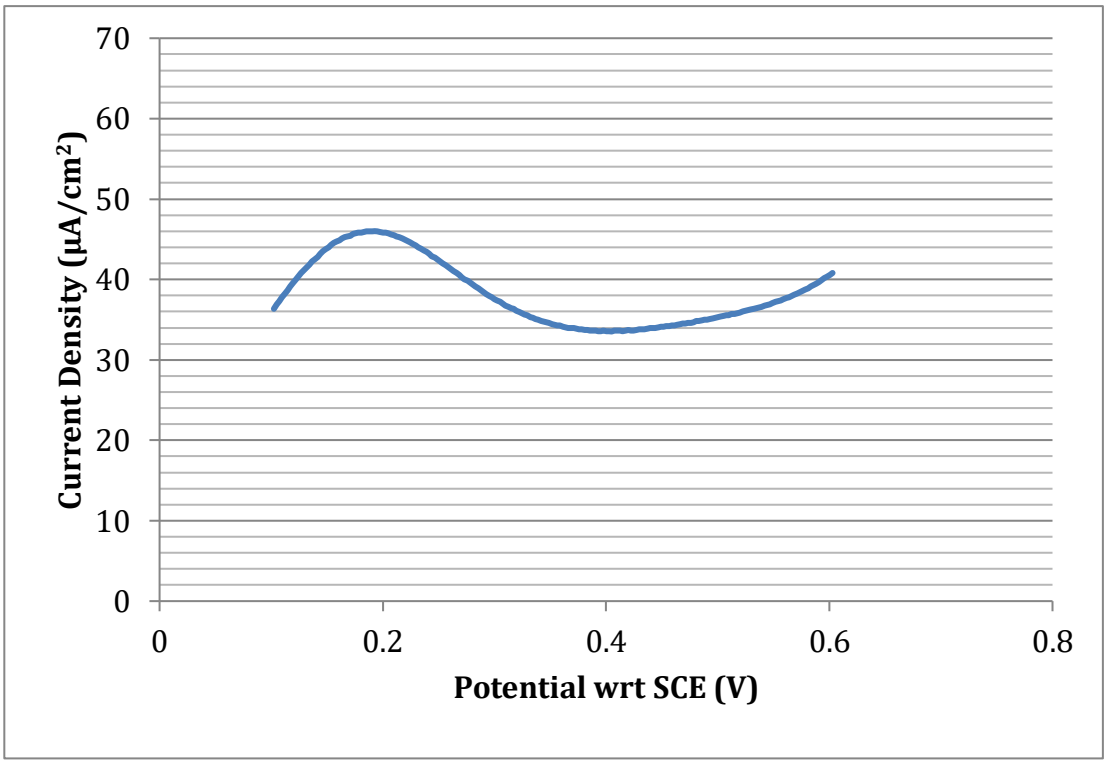


Fig 4.11 CV curve showing peak current density after running CA in 250mg/dl glucose in SBF solution (PBS solution with 0.01M NaOH)

The Cyclic Voltamogram obtained from the CV test, is shown in the above fig 4.11. As can seen from the voltamogram, the peak current density obtained is **46 µA/cm²**. Now we will put this value in the linear regression equation (1), to obtain the corresponding glucose concentration.

Glucose(mgdl) = 8.821*Current(µA/cm²) – 253.1 **(1)**

Putting the current value(46) in this equation, gives the glucose concentration of **152.67 mgdl**.

Thus, it can be clearly seen that with Platinum as working electrode, the known concentration **(250 mgdl)** of glucose in PBS solution has been successfully lowered or reduced to **152.67 mgdl**, by electroxidation via Chronoamperometry (CA)

procedure. It should be noted that the time duration of CA procedure for this test was 60 secs.

2) In the next test, a solution of known concentration of **200 mgdl** glucose was taken in the electrochemical cell, and Chronoamperometry (CA) procedure was run in it. All the rest of the test parameters and procedures are exactly the same as the previous test. That is, the applied potential was 0.25 V wrt SCE, for 60 secs.

After the CA procedure, the CV test was done to find out the concentration of glucose in the solution. The peak current from the Cyclic voltamogram was **43.5 µA/cm^2**. Putting this value in the linear regression equation (1), we get the glucose concentration to be **130.61 mgdl**.

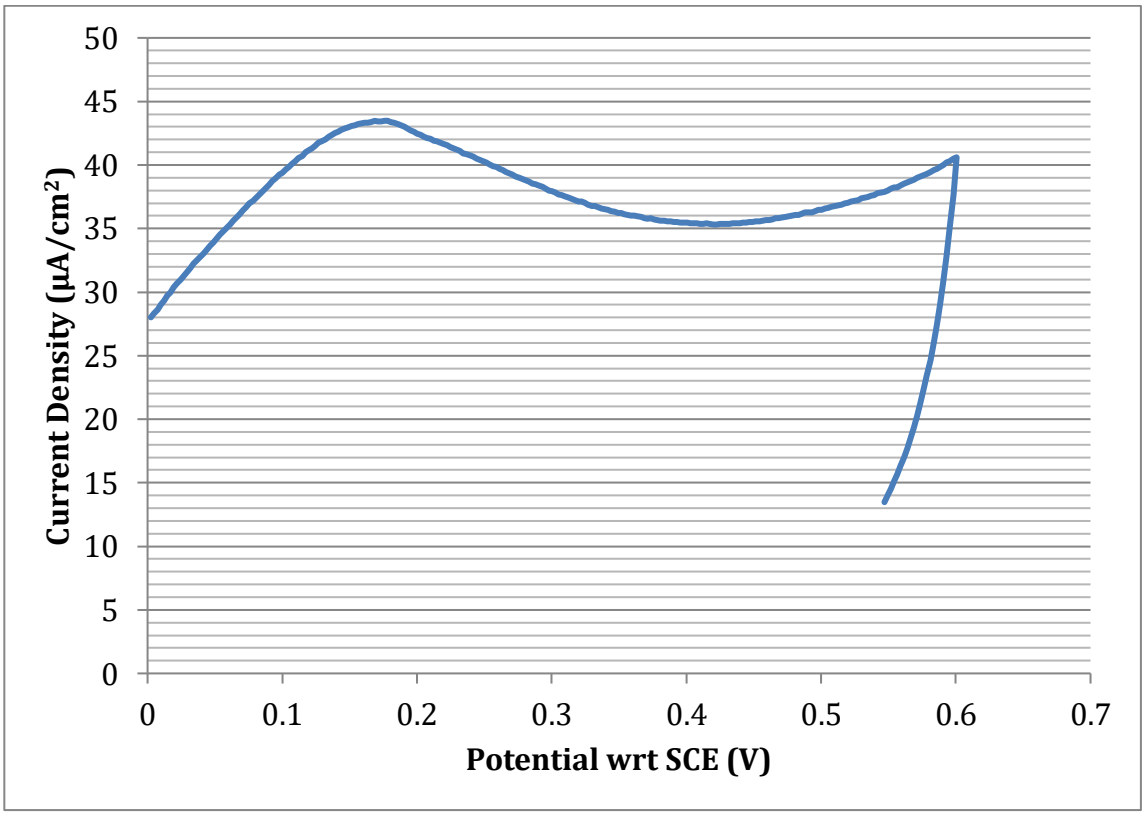


Fig 4.12 CV curve showing peak current density after running CA in 200mg/dl glucose in SBF solution (PBS solution with 0.01M NaOH)

Thus, again it was demonstrated that with Platinum as working electrode, the known concentration **(200 mgdl)** of glucose in PBS solution has been successfully

lowered or reduced to **130.61 mgdl**, by Electroxidation via Chronoamperometry (CA) procedure.

3) In the next test, a solution of known concentration of **150 mgdl** glucose was taken in the electrochemical cell, and Chronoamperometry (CA) procedure was run in it. All the rest of the test parameters and procedures are exactly the same as the previous test except the time duration of 30 secs. That is, the applied potential was 0.25 V wrt SCE, for 30 secs.

   After the CA procedure, the CV test was done to find out the concentration of glucose in the solution. The peak current from the Cyclic voltamogram was **41.5 µA/cm^2**. Putting this value in the linear regression equation (1), we get the glucose concentration to be **112.97 mgdl**.

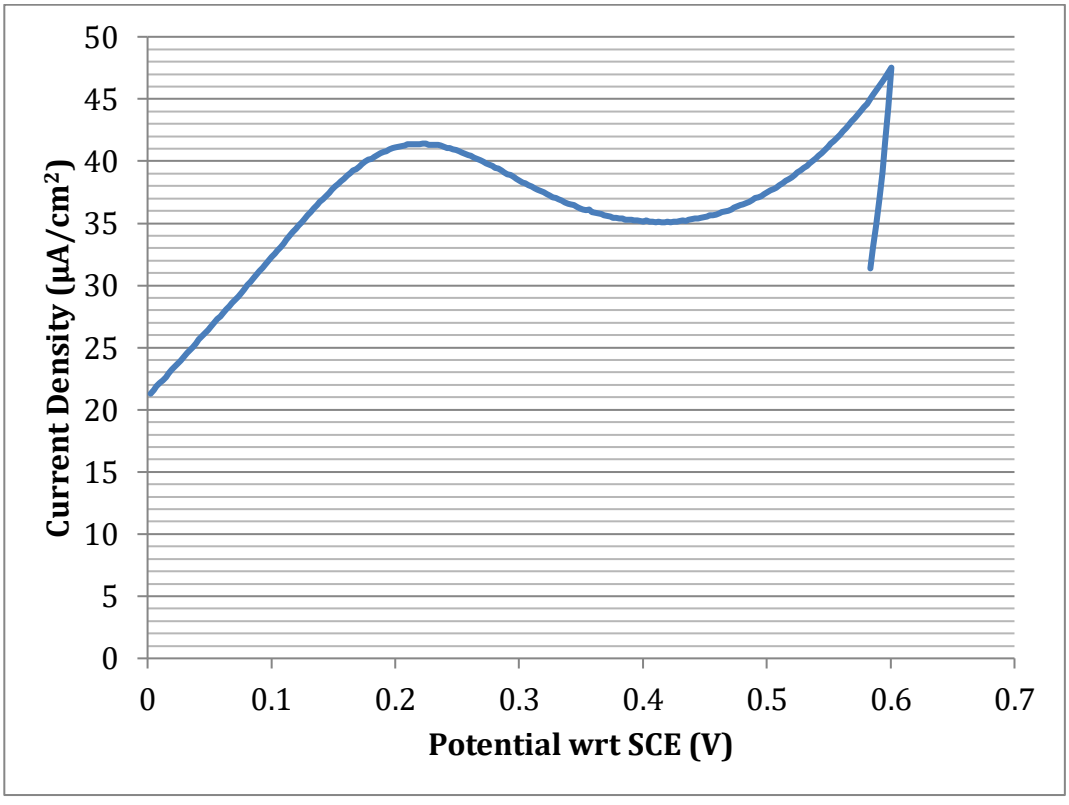


Fig 4.13 CV curve showing peak current density after running CA in 150mg/dl glucose in SBF solution (PBS solution with 0.01M NaOH)

Thus, again it was demonstrated that that with Platinum as working electrode, the known concentration **(150 mgdl)** of glucose in PBS solution has been successfully

lowered or reduced to **112.97 mgdl**, by Electroxidation via Chronoamperometry (CA) procedure.

Glucose Level(mg/dl)	Glucose Level after CA(mg/dl)	Peak Current Density ($\mu A/cm^2$)	Peak Current Density after CA($\mu A/cm^2$)	Energy released ($\mu Watt/cm^2$)
250	152.67	57.04	46	2.21
200	130.61	51.37	43.5	1.57
150	112.97	45.70	41.5	0.84

Table 4.2 Data regarding lowering of glucose level by Pt electrode in SBF(PBS solution with 0.01M NaOH) and corresponding energy released

4) Till now, we have used Platinum as the working electrode. Now we will use the materials developed by electrodeposition, to see how they perform, for electroxidation of glucose molecules in PBS solution, and thereby lowering or reducing the glucose level in the solution.

   The first material is Manganese dioxide with Nanocarbon (MnO_2C). So, just like before, a solution of known glucose concentration(200 mgdl) was taken in the electrochemical cell. Unlike in case of Platinum working electrode, no definite peak current potential can be identified for the electrodeposited MnO_2 C electrode in the Cyclic Voltammogram obtained during material characterisation. Therefore one cannot rely on Chronoamperometry procedure for the electroxidation of glucose, where a certain fixed potential is applied to the working electrode. Thus, one have to resort to Cyclic Voltammetry(CV) procedure, for the electroxidation of glucose by MnO_2 C. The following are the test parameters for the CV procedure.

Test Parameters	Value
Start Potential	0 V wrt SCE
Upper Vertex Potential	1 V wrt SCE

Lower VertexPotential	0 V wrt SCE
Stop Potential	0.5 V wrt SCE
Number of scans	5
Scan Rate	100 mV/sec
Step	0.00244 V

Table 4.3 Test Parameters for CV test, to electro-oxidise 200 mg/dl glucose in SBF(PBS solution with 0.01M NaOH) by various electrocatalytic electrode materials

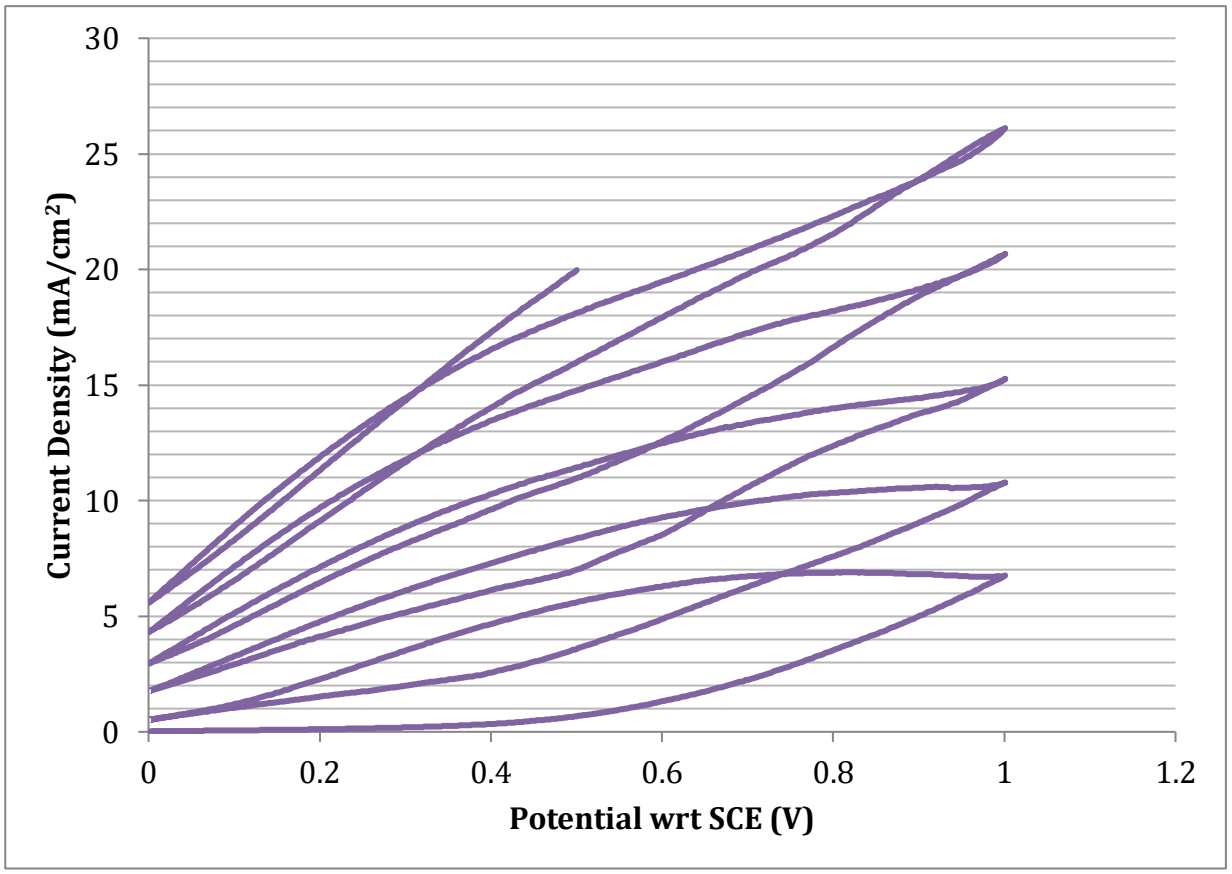


Fig 4.14 CV run, to electro-oxidise 200 mg/dl glucose in SBF(PBS solution with 0.01M NaOH) by MnO_2 C electrode

After the CV procedure is done, now it is needed to know whether the initial glucose level (of known concentration) has been reduced or not. For that, another Cyclic Voltammetry (CV) test needs to run, just like in previous cases. The CV test was run, with all the test parameters like Start potential, Stop potential, Upper Vertex Potential, Lower Vertex Potential, Scan Rate, etc exactly the same as the CV procedures that were run for obtaining the Calibration Curves.

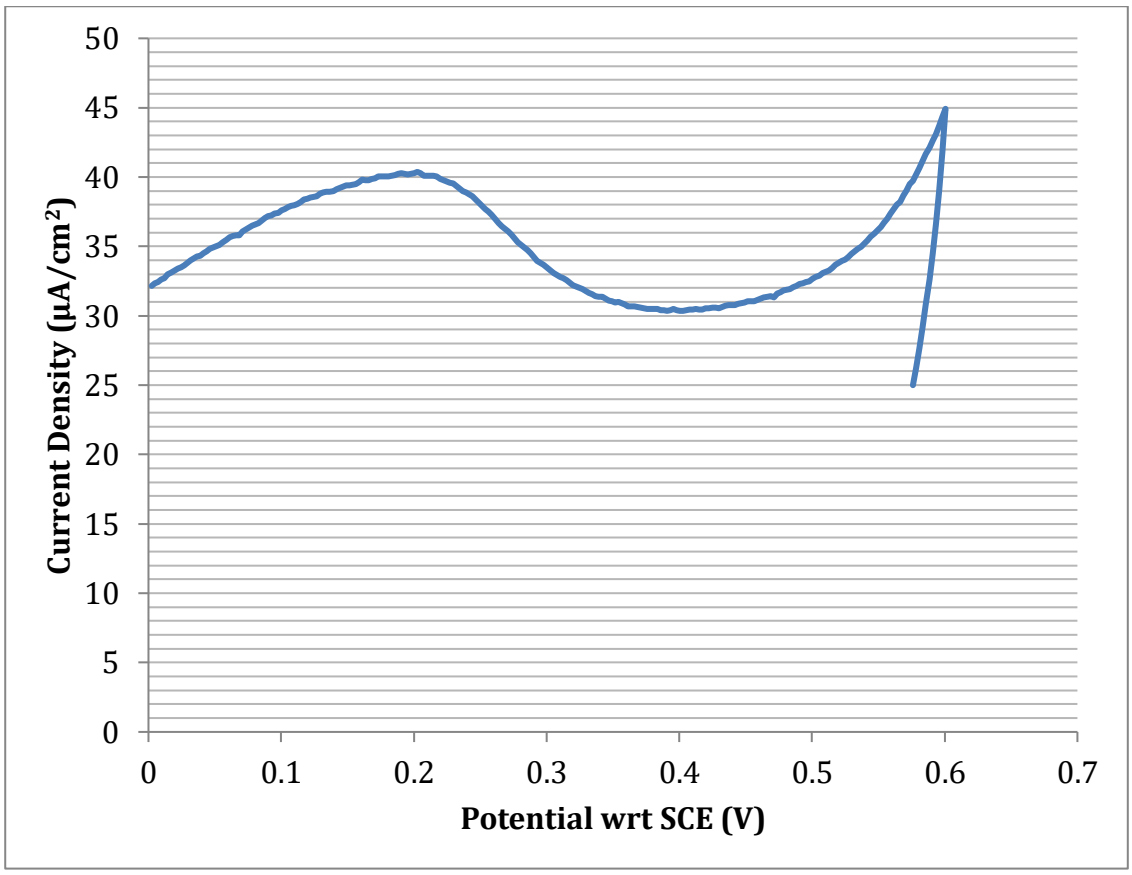


Fig 4.15 CV curve showing peak current density after running CV with MnO_2 C electrode in 200mg/dl glucose in SBF solution (PBS solution with 0.01M NaOH)

The peak current from the Cyclic voltamogram was **40 µA/cm²**. Putting this value in the linear regression equation (1), we get the glucose concentration to be **99.74 mgdl.** Thus, it was demonstrated that with electrodeposited MnO_2 C as working electrode, the known concentration **(200 mgdl)** of glucose in PBS solution has been successfully lowered or reduced to **99.74 mgdl**, by Electroxidation via Cyclic Voltammetry(CV) procedure.

5) The next material is Nickel with Nanocarbon (Ni C). A solution of known glucose concentration(200 mgdl) was taken in the electrochemical cell. Just like in case of MnO_2 C, no definite peak current potential can be identified for the electrodeposited Nickel with Nanocarbon (Ni C) electrode in the Cyclic Voltammogram obtained during material characterisation. Thus in this case as well, we will rely on CV

procedure for electroxidation of Glucose. The CV test parameters are same as the previous test.

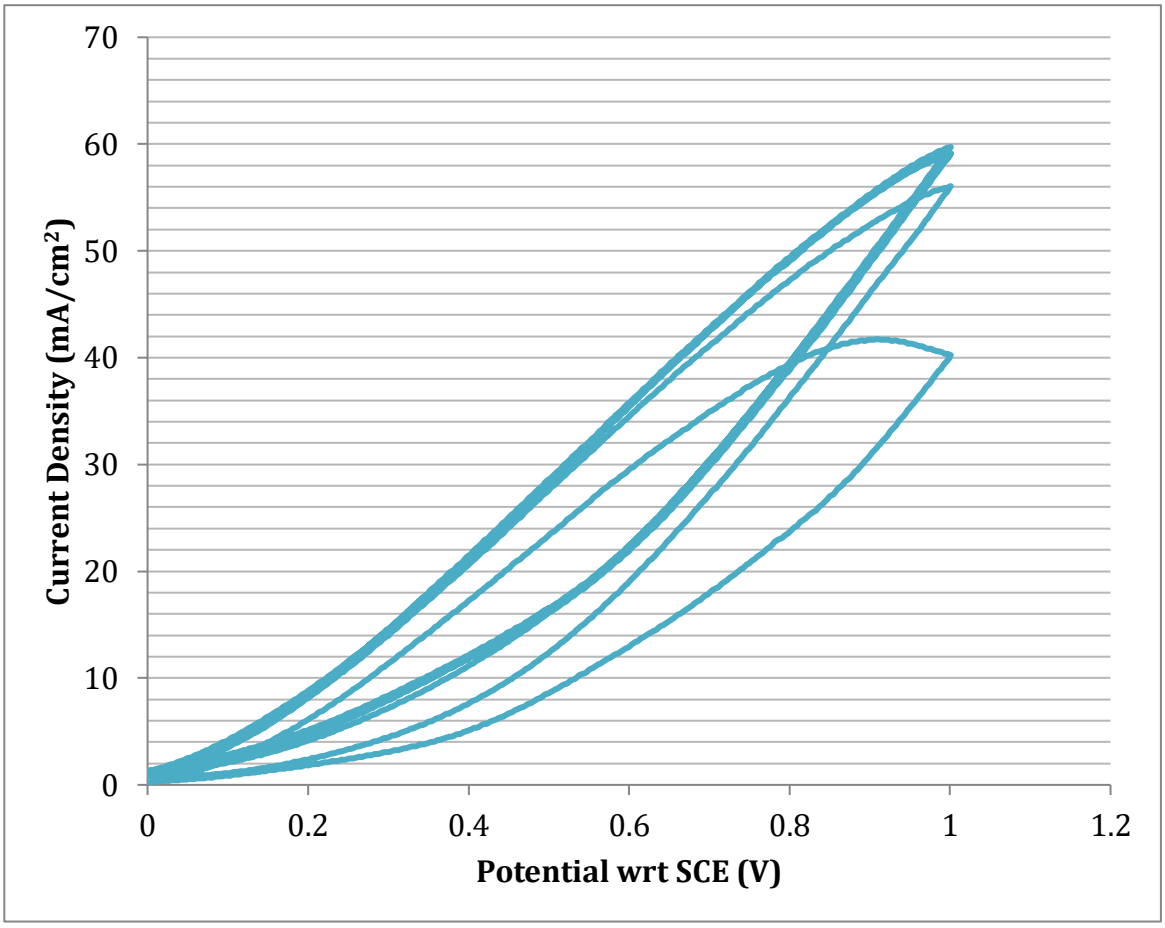


Fig 4.16 CV run, to electro-oxidise 200 mg/dl glucose in SBF(PBS solution with 0.01M NaOH) by NiC electrode

After the CV procedure is done, now we need to know whether the initial glucose level (of known concentration) has been reduced or not. For that, we need to run another Cyclic Voltammetry (CV) test, just like in previous cases. The CV test was run, with all the test parameters like Start potential, Stop potential, Upper Vertex Potential, Lower Vertex Potential, Scan Rate, etc exactly the same as the CV procedures that were run for obtaining the Calibration Curves.

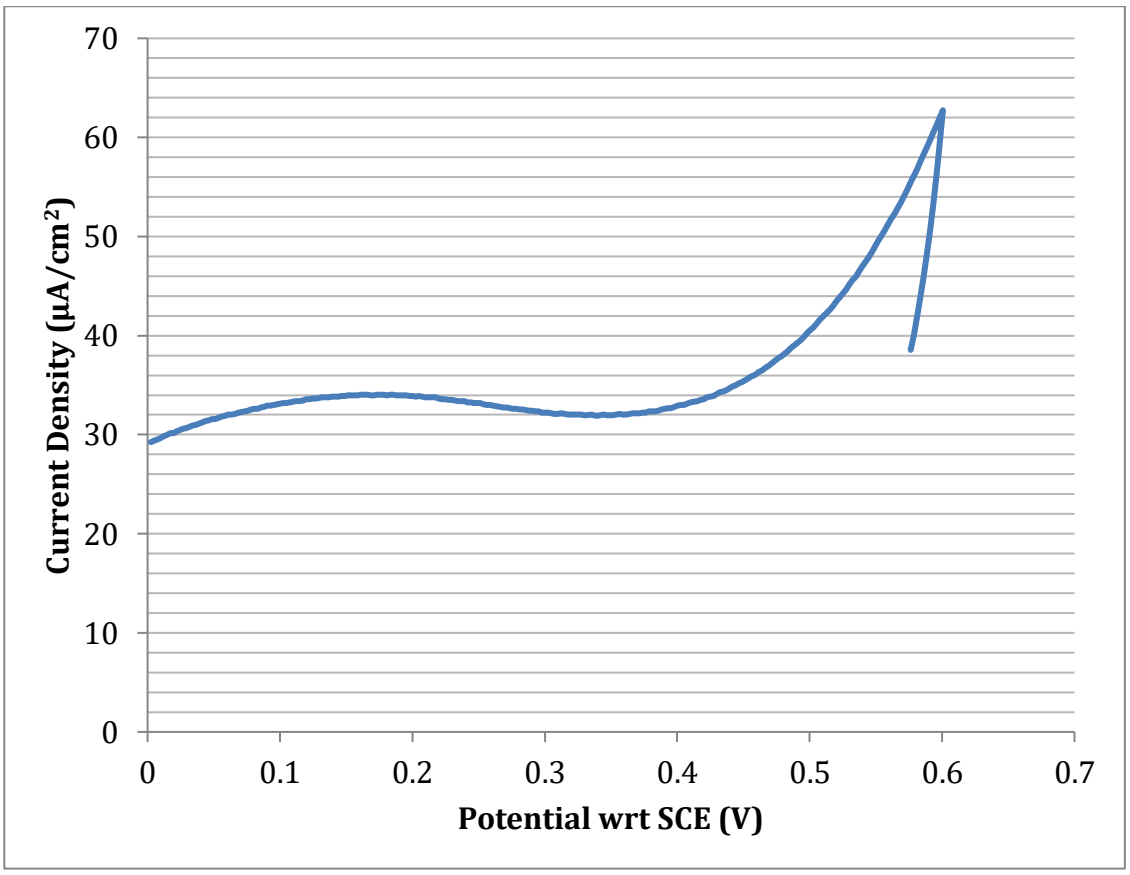


Fig 4.17 CV curve showing peak current density after running CV with NiC electrode in 200mg/dl glucose in SBF solution (PBS solution with 0.01M NaOH)

The peak current from the Cyclic voltamogram was **34.5 µA/cm²**. Putting this value in the linear regression equation (1), we get the glucose concentration to be **51.22 mgdl.** Thus, it was demonstrated that with electrodeposited Nickel Nickel Oxide with Nanocarbon (Ni C) as working electrode, the known concentration **(200 mgdl)** of glucose in PBS solution has been successfully lowered or reduced to **51.22 mgdl**, by Electroxidation via Cyclic Voltammetry(CV) procedure.

6) The next material is Nickel (Ni). A solution of known glucose concentration(200 mgdl) was taken in the electrochemical cell. Just like in the previous case, no definite peak current potential can be identified for the electrodeposited Nickel Nickel(Ni) electrode in the Cyclic Voltammogram obtained during material characterisation. Thus in this case as well, CV procedure will be relied on, for electroxidation of Glucose. The CV test parameters are same as the previous test.

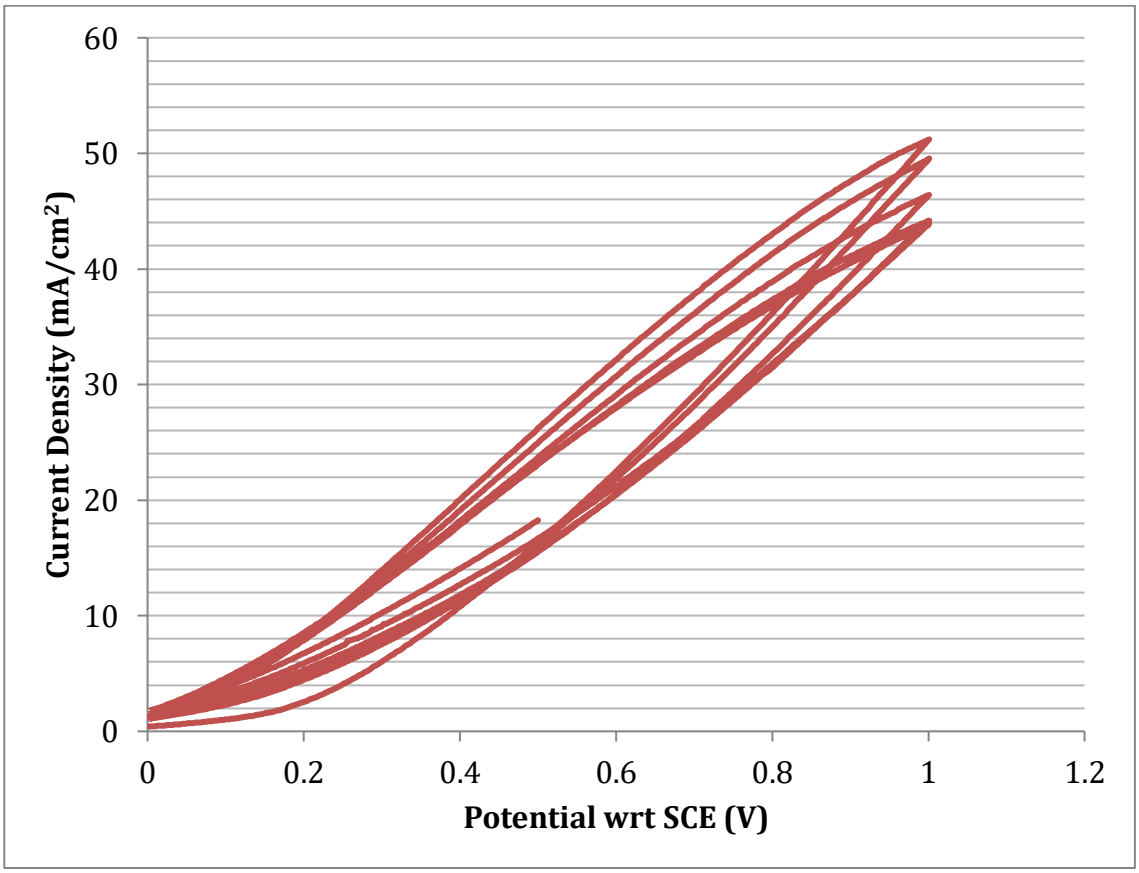


Fig 4.18 CV run, to electro-oxidise 200 mg/dl glucose in SBF(PBS solution with 0.01M NaOH) by Ni electrode

After the CV procedure is done, now we need to know whether the initial glucose level (of known concentration) has been reduced or not. For that, we need to run another Cyclic Voltammetry (CV) test, just like in previous cases. The CV test was run, with all the test parameters like Start potential, Stop potential, Upper Vertex Potential, Lower Vertex Potential, Scan Rate, etc exactly the same as the CV procedures that were run for obtaining the Calibration Curves.

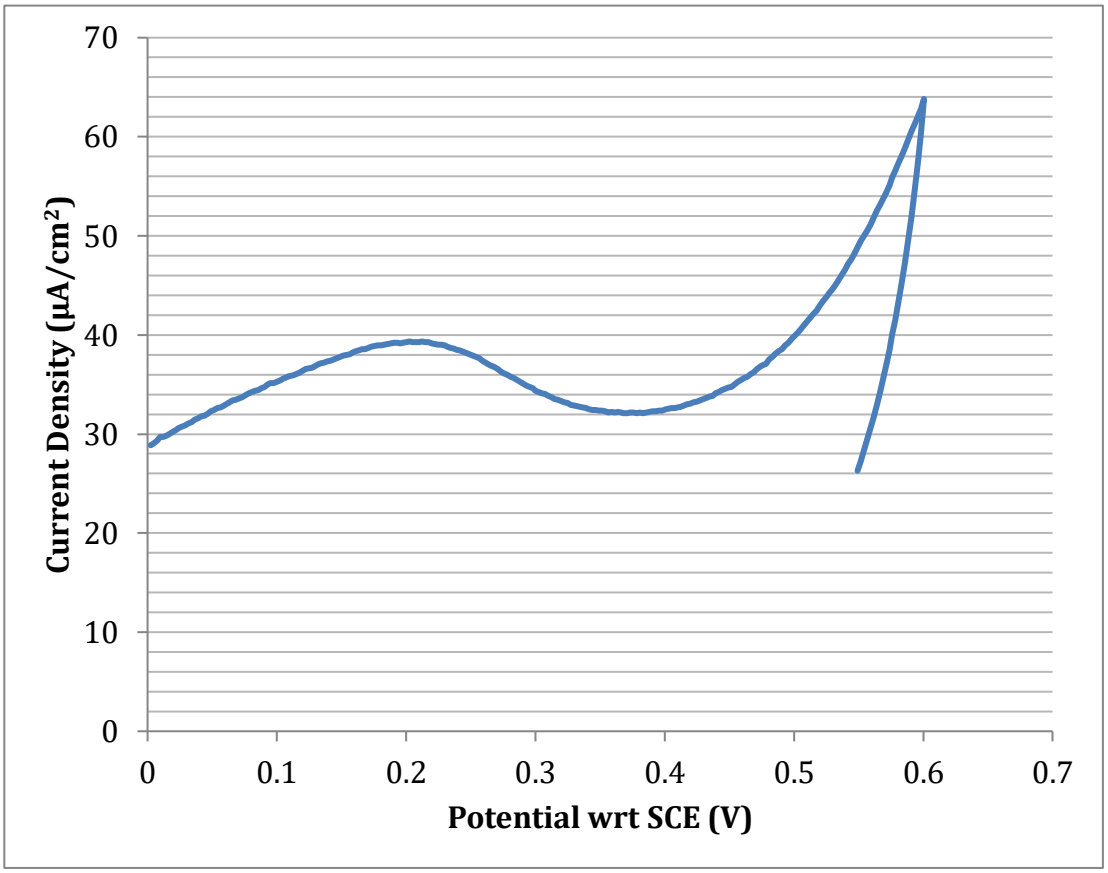


Fig 4.19 CV curve showing peak current density after running CV with Ni electrode in 200mg/dl glucose in SBF solution (PBS solution with 0.01M NaOH)

The peak current from the Cyclic voltamogram was **39 µA/cm²**. Putting this value in the linear regression equation (1), we get the glucose concentration to be **90.92 mgdl.** Thus, it was demonstrated that with electrodeposited Ni as working electrode, the known concentration **(200 mgdl)** of glucose in PBS solution has been successfully lowered or reduced to **90.92 mgdl**, by Electroxidation via Cyclic Voltammetry(CV) procedure.

7) The next material is Manganese dioxide (MnO_2). We will take a solution of known glucose concentration(200 mgdl) in the electrochemical cell. Just like in the previous case, no definite peak current potential can be identified for the electrodeposited Manganese dioxide (MnO_2) electrode in the Cyclic Voltammogram obtained during material characterisation. Thus in this case as well, we will rely on CV procedure for electroxidation of Glucose. The CV test parameters are same as the previous test.

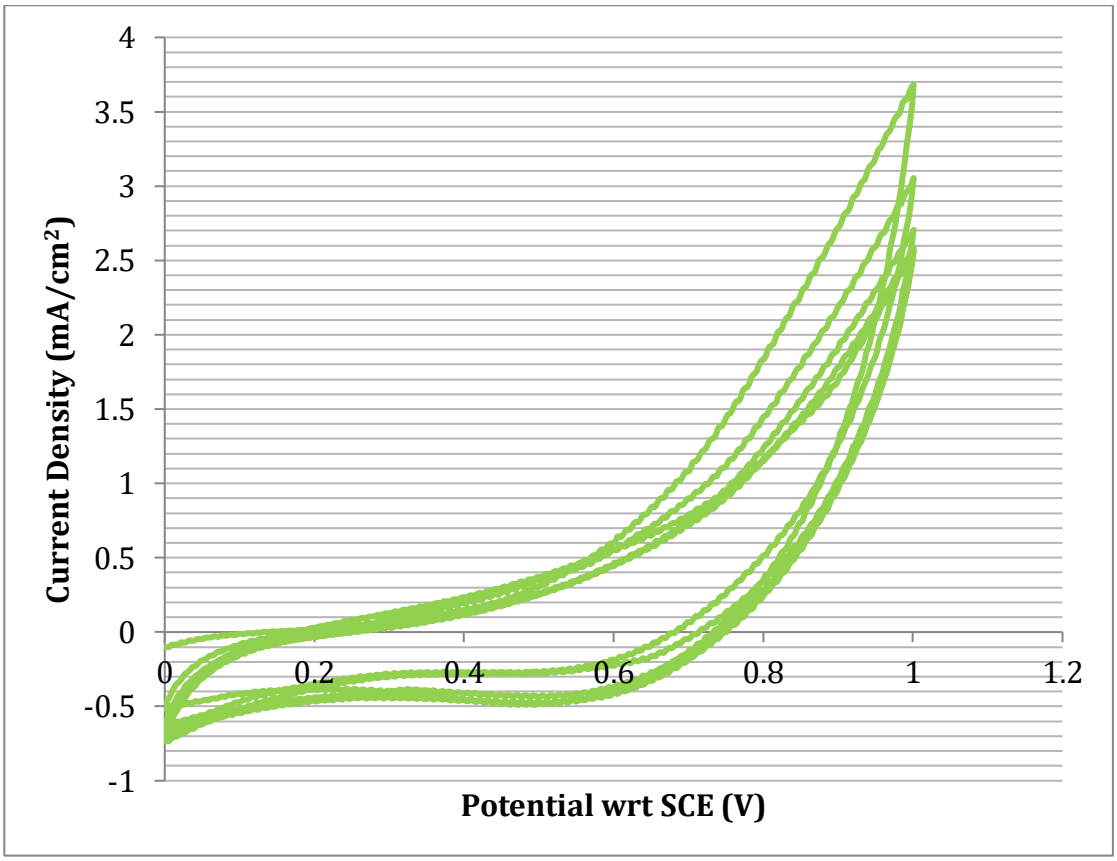


Fig 4.20 CV run, to electro-oxidise 200 mg/dl glucose in SBF(PBS solution with 0.01M NaOH) by MnO_2 electrode

After the CV procedure is done, now we need to know whether the initial glucose level (of known concentration) has been reduced or not. For that, we need to run another Cyclic Voltammetry (CV) test, just like in previous cases. The CV test was run, with all the test parameters like Start potential, Stop potential, Upper Vertex Potential, Lower Vertex Potential, Scan Rate, etc exactly the same as the CV procedures that were run for obtaining the Calibration Curves.

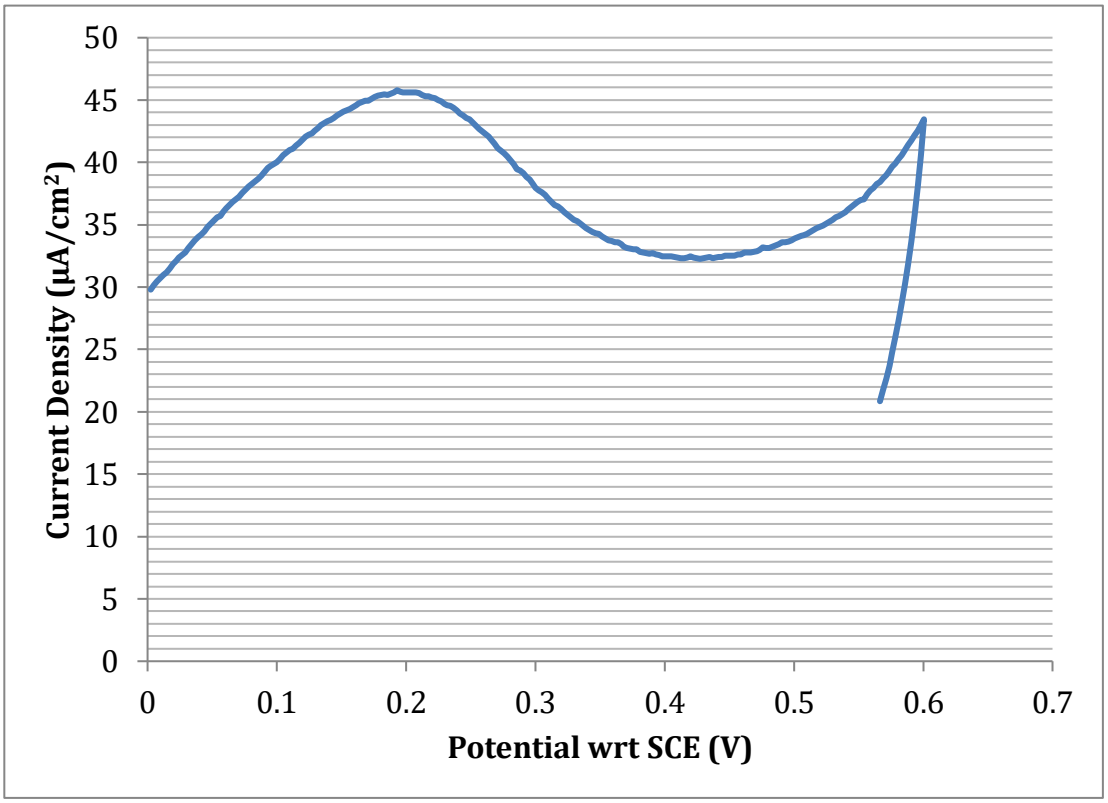


Fig 4.21 CV curve showing peak current density after running CV with MnO_2 electrode in 200mg/dl glucose in SBF solution (PBS solution with 0.01M NaOH)

The peak current from the Cyclic voltamogram was **46 µA/cm²**. Putting this value in the linear regression equation (1), we get the glucose concentration to be **152.67 mgdl.** Thus, it was demonstrated that with electrodeposited MnO_2 as working electrode, the known concentration **(200 mgdl)** of glucose in PBS solution has been successfully lowered or reduced to **152.67 mgdl**, by Electroxidation via Cyclic Voltammetry(CV) procedure.

Electrode (initially 200mg/dl glucose)	**Glucose Level after CV(mg/dl)**	**Peak Current Density ($\mu A/cm^2$)**	**Peak Current Density after CV($\mu A/cm^2$)**	**Energy released ($\mu Watt/cm^2$)**
NiC	51.22	51.37	34.5	3.374
Ni	90.92	51.37	39	2.474
MnO_2C	99.74	51.37	40	2.274
MnO_2	152.67	51.37	46	1.074

Table 4.4 Data regarding lowering of glucose level by different electrodes in SBF(200 mg/dl glucose in PBS solution with 0.01M NaOH) and corresponding energy released

The above table summaries the data regarding lowering of glucose level by different electrodes in SBF(200 mg/dl glucose in PBS solution with 0.01M NaOH) and corresponding energy released. It can be easily seen from the table that all the four electrocatalytic materials have been successful to lowering the glucose level. The energy released values are also good, in comparison to Platinum, whose data has been shown in table no.

4.7 Robotic system for glucose level control

Having succeeded to bring down the sugar level in the body fluid through electrochemical conversion, it is now thought what happens if the patient's sugar level falls below the minimum requirement (hypoglycaemia) that may lead to serious sickness of the patient, or even death. Thus it is necessary to have an automatic control, to decrease the sugar level in the diabetic patient, to the predetermined minimum value, where the patient will have normal blood sugar.

A robotic controlled circuit has been added as an electrochemical gadget to control decrease of the sugar level by cyclic voltammetry. The circuit diagram is shown in fig.4.23. In this system, an alert system have been developed with a buzzer tone and Red LED bulb, which will play low and disconnect the circuit automatically, the moment the sugar level starts dropping down below the minimum level.

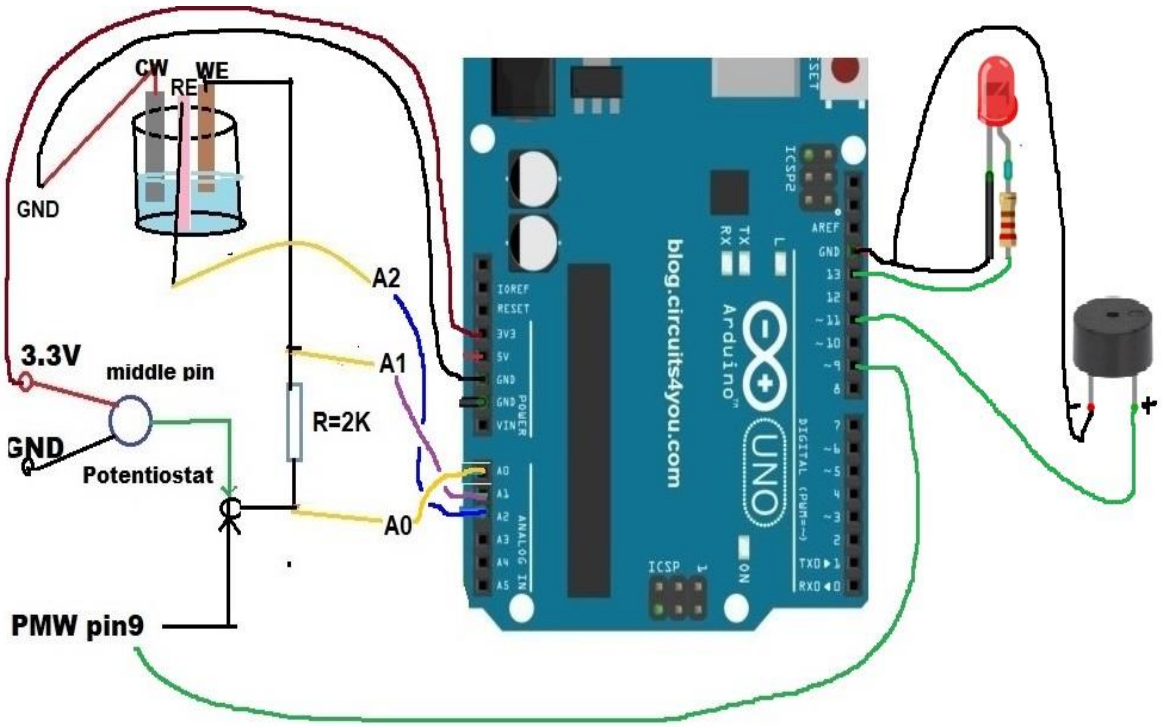


Fig 4.23 Circuit diagram of the robotic system with Arduino UNO board and alert system

The circuit consists of an Arduino board UNO, with a few analog pins and digital pins. The potential is now supplied from the digital pins of the Arduino board and not from the DC electrochemical potentiostat. The working electrode is connected to pin 9, the counter electrode is connected to the ground and the reference electrode is connected to the analog pin A2. Using a proper program code, the potential applied can be varied at a fixed scanrate, to electrochemically oxidise sugar in the body fluid solution, similar to normal

potentiostat but with more accuracy. Red LED bulb is connected to pin 13 and the other leg is connected to the ground. A buzzer is connected. The program is given below.

The program name AD_Electrochemicalsugar.ino has been illustrated below.

----------*Start of Program Code*----------

```
const int appliedvoltage = A0;//for measuring current
const int we_voltage = A1;// for measuring current
const int re_voltage = A2;// for measuring potential
int a=9;    // input to WE
int b=13;   // input to Red LED
int c=8;    // input to buzzer

float Cell_Potential = 0.0;
float R = 2000.0; // resistance of R(2K)
float we_value = 0;
float re_value = 0;
float wee_value = 0;
float ap_value = 0;
float I=0;
float Imin = 41.163   // current value in microAmp, corresponding to 110 mg/dl sugar, from  linear regression equation
```

```
void setup()
{
   pinMode(appliedvoltage, INPUT);
    pinMode(we_voltage, OUTPUT);
    pinMode(re_voltage, OUTPUT);
    pinMode(a, OUTPUT);
     pinMode(b, OUTPUT);
     pinMode(c, OUTPUT);
Serial.begin(9600);
 Serial.println("CLEARDATA");
   Serial.println("LABEL,CLOCK,CURRENT,CELLVOLTAGE");
}
void loop()
{
///////////////// forward scan
  for (int i=1; i<=205; i++)
{
     analogWrite(a,i);
  //potential
  float SamplesVa = 0;
  float SamplesVb = 0;
 we_value = analogRead(we_voltage);
 re_value = analogRead(re_voltage);
 //find potential  average of we_value and re_value
  for (int x = 0; x < 50; x++)
{ //Get 50 samples
     we_value = analogRead(we_voltage);
```

```
    re_value = analogRead(re_voltage);
  SamplesVa = SamplesVa + we_value;  //Add we_value samples together
  SamplesVb = SamplesVb + re_value;  //Add re_value samples together
  delay (3); // let ADC settle before next sample 3ms
 }
 float potavalue = SamplesVa/50;//taking average of we_values
 float potbvalue = SamplesVb/50;//taking average of re_values
 Cell_Potential = (potavalue - potbvalue)(5.0/1024)*1000; //will give cell potential in millivolt



  // Current
  float SamplesVc = 0;
  float SamplesVd = 0;
  wee_value = analogRead(we_voltage);
  ap_value = analogRead(appliedvoltage);

  for (int x = 0; x < 50; x++)
{ //Get 50 samples
   wee_value = analogRead(we_voltage);
   ap_value = analogRead(appliedvoltage);
  SamplesVc = SamplesVc + wee_value;  //Add wee_value samples together
  SamplesVd = SamplesVd + ap_value;  //Add ap_value samples together

  delay (3); // let ADC settle before next sample 3ms
 }
 float potcvalue = SamplesVc/50;//taking average of wee_values
 float potdvalue = SamplesVd/50;//taking average of ap_values
```

```
 I = (potdvalue - potcvalue)*4880/R; // current will come in microamps
                if ( I<=Imin) {


              digitalWrite(c, LOW);  // for buzzer to go low
              digitalWrite(b, LOW);  // for red LED to go low
                delay (2000);
                Serial.println("Lowest glucose level reached! The cell will shut immediately");
                break;
                   }


Serial.print("DATA,TIME,");
Serial.print(I);
Serial.print(",");
Serial.print(Cell_Potential);
 delay(2000);
              }
}
```

----------End of Program Code----------

The program output has been shown underneath.

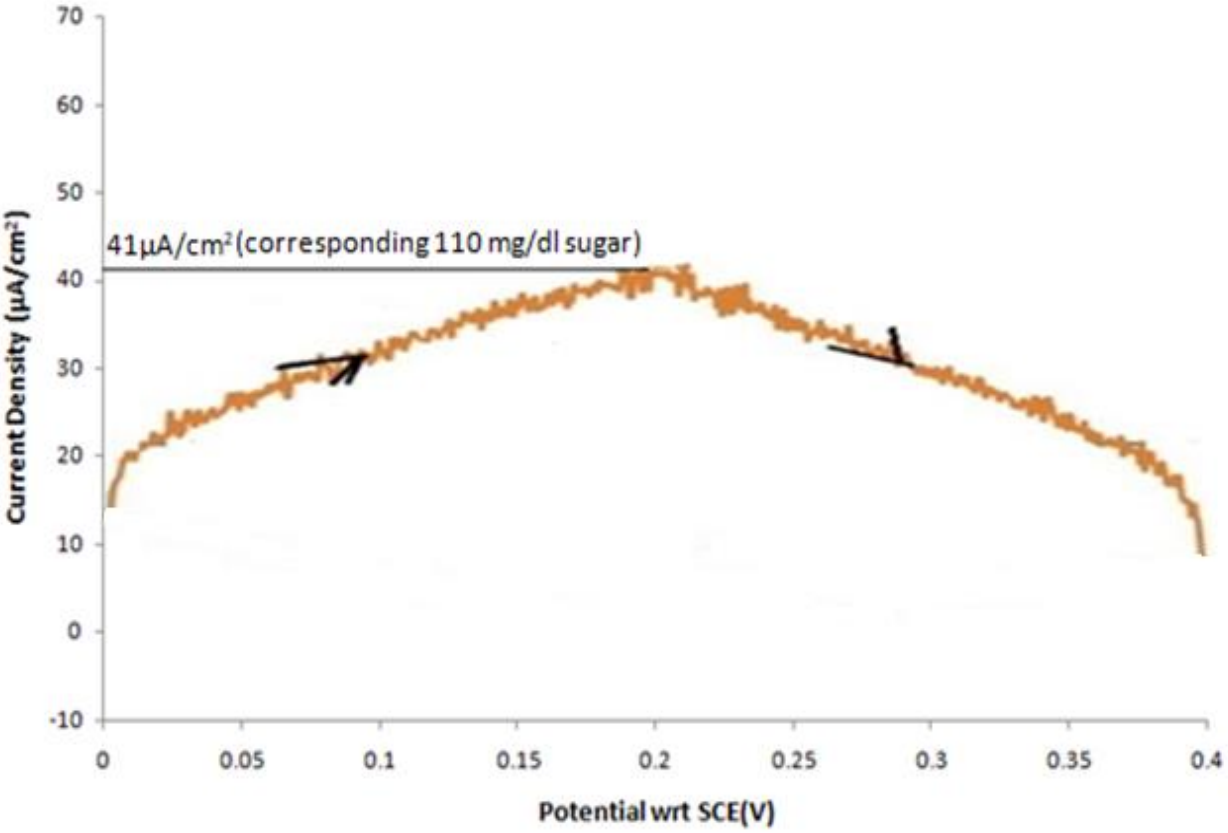


Fig 4.23 Showing the program output of the Cyclic Voltammetry

It is found that the program gives accurate alert system, when the sugar level in the simulated body fluid falls below 110 mgdl.

Chapter 5
Conclusion

5. Conclusion

This project work has attempted to find and demonstrate a novel way which can cause a potential breakthrough in the treatment of diabetes. Direct linear relationship between glucose concentration and peak current density was established, with over 97% matching. By electro-oxidising glucose in Simulated Body Fluid (SBF), the glucose concentration has been shown to decrease. Glucose, which is essentially a fuel, can be oxidised to convert itself into energy. Different working electrodes have been used in this project, including Platinum. And all of the electrodes were successful in lowering the glucose level, by electro-oxidising. Through this project work, it has been conclusively demonstrated that reduction of glucose levels by electro-oxidation is a genuine phenomena, and it holds the potential to absolutely revolutionise the diabetic treatment regime throughout the world.

Chapter 6
Reference

6. Reference

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