DEVELOPMENT OF A LOW COST MINITAURIZED POTETNTIOSTAT FOR BIOSENSING APPLICATION USING LMP91000 AFE

A project report submitted in partial fulfillment of the requirements for the award of the degree

MASTER OF TECHNOLOGY

IN

BIOMEDICAL ENGINEERING

BY

SACHIN GANGHYAN (2K13/BME/12)

Under the supervision of

Prof. B. D. Malhotra

Department of Biotechnology

Delhi Technological University

(Formerly Delhi College of Engineering)

Delhi-110042

INDIA

DECLARATION

I, Sachin Ganghyan, hereby declares that the work entitled "Development of Low Cost, Minitaurized Potentiostat for Biosensing Application Using LMP91000 AFE" has been carried out by me under the guidance of Prof. B. D. Malhotra in Nano Bioelectronics Laboratory, Department of Biotechnology, Delhi Technological University, Delhi, India 110042

This dissertation is part of partial fulfillment of requirement for the degree of M. Tech in Biomedical Engineering. This is the original work and has not been submitted for any other degree in other university.

Sachin Ganghyan

Reg. No. - DTU/13/M.tech/396

CERTIFICATE

This is to certify that the project work entitled "**Development of Low Cost, Minitaurized Potentiostat for Biosensing Application Using LMP91000 AFE**" submitted by **Sachin Ganghyan (2K13/BME/12)** in partial fulfilment of the requirement for the award of degree of Master of Technology in Biomedical Engineering, Delhi Technological University, Delhi-110042 is an authentic record of the candidate's own work which was carried out by him under my supervision and guidance.

The information and data enclosed in this dissertation is original record of his work which has not been submitted for the award of any other diploma of degree other than the one aforementioned and has not been submitted elsewhere in India or abroad.

Date:

Prof. D. Kumar
Head, Department of Bio-Technology
Delhi Technological University
Delhi- 110042

Prof. B. D. Malhotra
Department of Bio-Technology
Delhi Technological University
Delhi- 110042

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Sachin Ganghyan

2k13/BME/12 ‘

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ABSTRACT

This project presents a novel approach of using a miniaturized potentiostat (LMP91000) and Arduino board to perform electrochemical deposition of Polyaniline (PANI) on ITO glass electrode and glucose sensing. The LMP91000 with arduino board was reconfigured to perform glucose detection. The electrode were fabricated by immobilizing *Glucose Oxidase* (GO_X) enzyme on aniline deposited electrode (GO_X/PANI/ITO). Due to its compact size and high performance, this device will effectively perform biochemical measurement on three electrode system which enables it to be used in point of care diagnosis.

1. INTRODUCTION

POC diagnostics are analytical testing platforms that can be used both in a clinical laboratory and in the vicinity of a patient using portable equipment. Therefore, the entire clinical diagnosis and treatment community is witnessing a paradigm shift from conventional diagnostic devices to the miniaturized and POC devices. These devices can fulfill the increasing demand of the medical sector for fully automated instruments that can directly use unprocessed specimens and that require minimal electronic or mechanical maintenance. POC diagnostics have the potential to provide rapid and accurate results, at reasonable costs, for the biochemical tests required by the increasing number of patients in emergency rooms (A.F.D. Cruz *et.al*. 2014). According to a recent study, approximately more than 370 million people would globally suffer from diabetes by 2030. No eventual cure for diabetes until 2030 is considered. The most permanent method of treating diabetes is a pancreas transplant which gives a patient a chance to become independent of insulin injections. In coming 10 years India would be termed as diabetes capital of the world, as per WHO. One of the greatest challenges in global healthcare is the lack of adequate diagnostic testing for early disease detection. Conventional blood glucose sensor requires expensive facilities, costly equipment and which are not practical in most of the world.

Biomedical engineering is an emerging and quickly growing field which overcomes any interruption between the microelectronics of today and the engineered science enlivened frameworks of tomorrow. It is quickly developing from material science, physics, chemistry, and information technology and electronics system. Miniaturization is the trend to manufacture ever smaller mechanical, optical and electronic products and devices. (C loncaric *et.al.* 2011) Recently much research interest has been gained towards the development of Arduino based sensing devices. Arduino is an open-source embedded platform based on flexible, easy-to-use hardware and software. The basic system consists of a microcontroller with various peripheral interfaces that is programmed by an existing software platform. The major benefits offered by Arduino include ease of setup, low cost maintenance, low cost hardware and software, open source platform, portability. Arduino based device are being designed due to its precise measurements and efficient data recording in real time. These devices are currently being used in various industries such as food, biomedical, wireless and telecommunication industries. As reported in literature, Arduino based systems are being efficiently used for specific bio sensing applications.

We present the low cost, miniaturized potentiostat for bio-sensing application using LMP91000 as a sensor AFE (analog front end), 3 electrode assembly and an open source arduino microcontroller as a controller board. It can sense the various peripheral devices by receiving input from them and can control via balanced output. This device has been used for glucose sensing application.

2-LITERATURE REVIEW

2.1 REVIEW OF LITERATURE

Diabetes mellitus is a overall general wellbeing issue. This metabolic issue results from insulin lack and hyperglycemia and is reflected by blood glucose focuses higher or lower than the typical scope of 80-120 mg/dL (4.4-6.6 mM). The illness is one of the main sources of death and inability on the planet. The confusions of engaging diabetes are various, including higher dangers of heart illness, kidney failure, or sightlessness. Such intricacies can be extraordinarily diminished through stringent individual control of blood glucose. The determination and administration of diabetes mellitus therefore obliges a tight checking of blood glucose levels. As needs be, a large number of diabetics test their blood glucose levels day by day, making glucose the most generally tried analyte. In fact, glucose biosensors represent around 85% of the whole biosensor market. (J wang *et.al*. 2007)
Such huge business sector size makes diabetes a model disease for growing new biosensing concepts. The huge economic prospects related with the administration of diabetes alongside the test of giving such compact and tight glycemic control have accordingly prompted a lot of captivating exploration what's more, inventive discovery strategies. Amperometric compound cathodes, in view of glucose oxidase (GOx), have played a driving part in the move toward simple glucose monitoring and testing and are required to assume a comparative part in the move towards ceaseless glucose observing.(J D Newman *et. al*. 2005)

The reason for design and development of 3 electrode lmp91000 using Arduino board system is to accomplish moderate distinct option for research facility quality potentiostats.

2.2 BRIEF HISTORY OF ELECTROCHEMICAL GLUCOSE BIOSENSORS:

The first glucose compound terminals started in 1962 with the improvement of the first device in Cincinnati Children's Hospital by Clark and Lyons of the. Their first glucose compound terminal depended on a paltry layer of GOx entwined more than an oxygen anode through a semipermeable dialysis film. (J. Wang *et. al*. 2008). Estimations were made in light of the observing of the oxygen devoured by the compound catalyzed response.

Platinum cathode act as negative potential which was connected to for a reductive location of the O_2 depletion. The biosensors was started its origin to this original glucose enzyme electrode. Clark's original patent in which enzymes was used for converting electro inactive substrates to electro active products. Using two electrodes, one of which was covered with the enzyme, and measuring the differential current effect of interference was corrected.

2.3. THREE ELECTRODE POTENTIOSTAT SYSTEM REQUIREMENTS:

2.3.1. Develop an inexpensive potentiostat for use in a lab setting

A commercial potentiostat can cost in the price range of $5,000 to $10,000 per channel, which is prohibitively expensive for widespread laboratory use. For example, an instructional lab environment requires a low-cost potentiostat that can be produced for individual use.

2.3.2. Document firmware for ease of use and later modification

All firmware must be documented at a level that a new user can come in and modify parameters or add new functionality without having to go through detailed circuit analysis, or programanalysis

2.3.3. Document Potentiostat so it can be produced by unskilled users

The potentiostat boards must be able to be easily assembled by unskilled users, such as first or second year electrical engineering students. This will allow for many boards to be built for laboratory use, while allowing such students to gain circuit experience.

2.3.4. Log Data for later analysis

As the potentiostat will be used in a laboratory environment, data logging is needed for later analysis and comparison.

2.3.5. Make recommendations for expanding potentiostat to three electrode version

A high level recommendation for expanding the potentiostat from a single electrode to a multi electrode model should be included for future projects.

2.4 BLOOD GLUCOSE SENSOR FOR HOME TESTING

Electrochemical biosensors are appropriate for tending to the needs of individual (home) glucose testing and have important role in the move to straightforward one-stage glucose testing. Since blood glucose home testing gadgets are utilized every day to analyze possibly life-undermining occasions they must be of greatly high caliber. The greater part of individual blood glucose screens depend on expendable screen-printed chemical cathode test strips. Such single-use test strips are mass created by the quick and thick-film (screen-printing) vapor statement process or microfabrication technique. (J.Wang *et.al*. 2007). The screen-printing innovation includes printing examples of conductors and protectors onto the surface of planar strong (plastic or clay) substrates in light of squeezing the comparing inks through a designed cover. Every strip contains the printed working and reference cathodes, with the working one covered with the essential reagents (i.e., protein, arbiter, stabilizer, surfactant, connecting, and tying specialists) what's more, layers. The reagents are regularly apportioned by an ink-plane printing innovation and stored in the dry structure. A counter cathode and an extra ('standard') working cathode might likewise be incorporated. Different layers (cross section, channel) are

frequently fused into the test strips and alongside surfactants are utilized to give a uniform specimen scope and separate the platelets. Such single-utilization gadgets take out issues of persist, cross pollution, or float. Generally speaking, in spite of their minimal effort and large scale manufacturing such sensor strips are in light of a high degree of advancement fundamental for guaranteeing high clinical precision.

Electrochemical cells typically are used to measure a wide variety of toxic and non-toxic gases such as carbon monoxide, oxygen, and hydrogen. They are based on the principals of chemical oxidation and reduction, and produce a current in proportion to the measured gas. Most cells are made up of three electrodes: working (WE), counter (CE), and reference electrodes (RE). The WE oxidizes or reduces the target gas and produces a current proportional to the gas concentration. The CE balances the generated current and the RE maintains the working electrode potential to ensure proper region of operation. Electrochemical cells are intended to interface with a potentiostat circuit. This potentiostat circuit provides current (and biasing, if required) to the CE. It maintains the WE at the same potential as the RE, and converts the output current from the WE into a voltage using a transimpedance amplifier (TIA).

Electrochemical sensors, like many sensors, have a dependence on temperature. To enable the best performance, measure the cell's temperature. Make appropriate temperature corrections based on that cell's performance vs. temperature plots, which can be found in the datasheet. The sensor, gas type, and gas concentration level dictate how much current will be output at the sensor's working electrode. To handle this variability, use a TIA with adjustable gain. Currents in the level between one to hundreds of μA are possible, so having a TIA gain in the one to hundreds of kohm range is sufficient.

Different sensors require different biasing, or for some a zero bias. Be aware of these requirements so the current produced by the sensor meets specifications. Whether the cell goes through an oxidation (CO) or reduction (NO_2) reaction to the measured gas determines if the cell produces a current into or out of the WE, respectively. The voltage at the TIA's non-inverting pin should be level-shifted appropriately to ensure maximum gain without saturating the amplifier's output in single-supply systems. For example, the TIA produces an output voltage governed by this equation: $V_{OUT} = -I_{IN} \times R_{FEEDBACK}$, where I_{IN} is current going towards the TIA across the feedback resistor. If the current into the TIA is positive (reduction reaction), V_{OUT} will be negative in reference to the noninverting pin voltage. That voltage should be raised to avoid railing the output to the negative supply. (A F D Cruz *et.al*. 2014)

Basically, it is essential that electrochemical cells have temperature correction and a potentiostat circuit that provides current sinking/sourcing, voltage biasing, current-to-voltage conversion, and level-shifting. For example, the LMP91000 a configuurable AFE potentiostat, is part of the sensor AFE family and satisfies these functions. It contains a complete potentiostat circuit with sink and source capability along with programmable TIA gain,

electrochemical cell bias, and internal zero voltage. Moreover, this sensor AFE contains an integrated temperature sensor and comes in a small 14-pin, 4-mm^2 package which allows positioning the device directly under the electrochemical cell for accurate temperature compensation and improved noise performance. (Tudos *et al*.2001; Soper *et al*. 2006; Rusling *et al*. 2010;Wang 2006)

2.5 SENSORS

A sensor can be called as analytical device which measures change in physical quantity and also converts it into a signal which can be understood by an observer or by analysis. A biological sensors consist of a transducers. A transducers is a device that can transform a signal from the interaction of an analyte with the elements which is of biological values into electrical signals. An electrical signal can be interpreted easily. Biosensors have vast characteristics which make it more valuable asset in biomedical applications. The most specific characteristics of biosensor is its selectivity. Selectivity means it will detect certain bio-chemical and does not reacts with any other contaminates. Signal stability is another important factor of a bio sensors. It performs continuous monitoring and influences the correctness of sensor. Signal stability measures the driftness while monitoring the conditions. (Choi *et al*. 2011; Justino *et al*. 2013; Kost *et. al.*1995; Kaushik *et al.* 2014)
Sensitivity of a sensors can measures the minimum change in analyte can be sensed by the sensors. Response time should be the minimum time required to analyse the change. Regenertation time is another important feature that defines the time essential to return the sensor to working state after interaction with the sample.

2.6 BIOSENSORS

A biosensor is an analytical device, used for the detection of an analyte, that combines a biological component with a physicochemical detector. Biosensors are receiving a lot of attention due to its vast characteristics like sensitivity, portability and selectivity. (P A Serra *et.al*. 2006). There are different kind of biosensors like electrochemical, optical, electronic, pyro-electric etc. In these sensors electrochemical and piezoelectric biosensors are regarded very recommendable these days. Biosensors are used to measure the concentration of biological analytes and various properties like osmolality, glucose level and oxygen level.

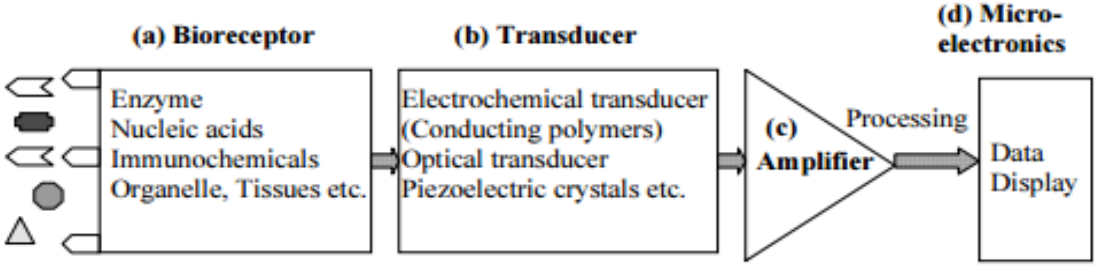


Fig: 2.1: Block Diagram of a Biosensor

Now a days, electrochemical biosensors are used for selective, specific and rapid detection of biomarkers inside biological series. The sensing routine of such electrochemical biosensors is found to be dependent on the selection of transducers and immobilizing electro-active matrix e.g. nanostructures. The introduction of nanomaterials as immobilization matrices lead to increased sensitivity, linearity, and detection at pico-molar level.

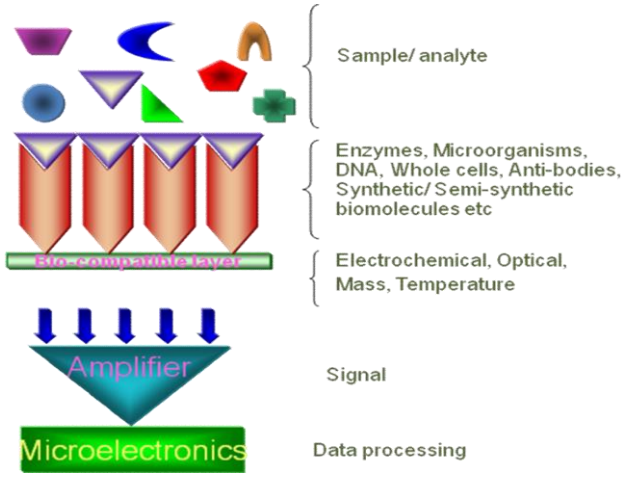


Fig. 2.2: Schematic of a Biosensor

2.6.1- Biosensor comprises of following components:

1- Biological- Enzymes, antibodies/antigens, tissues, bacteria, yeast, organelles, nucleic acids, liposomes etc.

Biological components perform following functions-

a. Specifically recognize the analyte of interest.

b. Interact with analyte to produce some physical or chemical changes that can be detected through a transducer.

2- Physical- Transducer, amplifier and microprocessor.

(a)Transducer

A transducer is a device which converts any type of signal in to an electrical signal.The signal produced as a result of interaction of bio-molecules with the analyte may be in the form of electrochemical (change in potential or current), optical (color change), calorimetric (heat measurement), piezoelectric (mass change) response and will be converted to electric signal with the choice of suitable transducer.

(b)Amplifier

The electronic signal produced by transducer is small and is amplified using an amplifier.

(c)Microprocessor

The amplified signal is fed into the microprocessor. The signal is then analyzed and interpreted and is displayed in to appropriate units

2.6.2: Functional mechanism of biosensors-

a. Analyte disperses from the solution to the electrodes/surface of biosensors.
b. Analyte responds with biological element of the biosensor which changes physicochemical assets of transducer surface.
c. The change in electronic/optical properties is dignified, converted into electrical signal which is processed, amplified and displayed on the digital system.

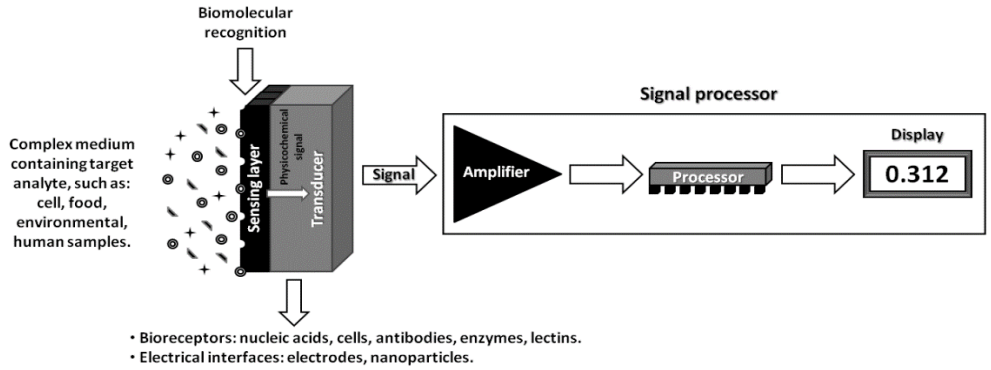


Fig. 2.3: Schematic showing main components of a Bio-receptor (a) Biocatalyst, (b) Electronic Transducer, (c) Power Amplifier, (d) Mainframe (e) Display

2.7: CHARACTERISTICS OF A BIOSENSORS

An ideal biosensor should have some important features mentioned below**:**

a. The bioreceptor must be highly **specific** for analyses, and be **stable** under normal condition.
b. The reaction should be **independent** of physical parameters such as pH, stiring and temperature as is controllable.
c. The response should be precise, accurate, reproducible and undeviating over the useful analytical range, without concentration or dillution. It should also be free from noise.
d. The probe of biosensor must be tiny and biocompatible, having no toxic or antigenic effects to be used for invasive monitoring in clinical situations,.
e. The complete biosensor should be portable, cheap, low-cost and capable of being used by normal persons.

2.8: CLASSIFICATION OF BIOSENSORS

(a) According to signal transducing element

Electrochemical biosensors: The electrochemical biosensors have been developed for detection of pathogenic bacteria in food and water. They are mainly based on the electrochemical species consumed or generated during a biological and chemical process of an analyte and substrate. The interaction produces an electrochemical signal which is measured by an electrochemical detector. Depending upon the electrochemical property measured, there are three types of electrochemical biosensor.

Potentiometric biosensors: These transducer measure potential at the working electrode with respect to reference electrode and charge generated by the selective binding at the electrode surface is detected by the biosensor. Extremely small concentration can be detected by this technique.

Amperometric biosensors: This transducer work on the priciple that a linear relationship exists between analyte concentration and current. They monitor changes in the current on the working electrode because of direct oxidation of the products. The sensor potential is set at particular value and the analyte directly or indirectly produce current at the electrode. These biosensors are highly sensitive, inexpensive and rapid.

Conductometric biosensor: This device consists of two noblemetal electrodes, which are immersed in the solution and the conductance is measured. Some enzymatic reactions convert neutral substrates into charged products, causing a change in the conductance of the medium. Although this transducer is not in widespread use, the technique is routinely used to measure salinity of marine environments.

2.8.2: The main advantages of electrochemical biosensors are:

- Faster response time
- Greater simplicity
- Low cost compared to optical, calorimetric and piezoelectric biosensors.

2.9. Application of biosensor

In recent world application of biosensors has been increasing day by day in various commercial as well as scientific research fields. The most applications fields of biosensors are explained bellow.

2.9.1: Medical applications

Among wide range of applications of biosensors, the most important application is in the field of medical diagnostics, which can be conveniently divided into vitro and in vivo diagnostics. In vitro diagnostic tests fall in three categories.

(i) Centralized tests in hospitals that include tests for glucose, lactate, uric acid, viruses, and a variety of pathogenic microbes in hospitals. DNA based biosensors are also being developed for diagnosis of hereditary diseases, viruses and cancer.

(ii) Tests in doctors clinics where analysers (in the form of portable biosensors) to be used in the nursing homes or in private clinics of practising doctors, are also being developed for testing glucose, lactate, creatinine and urea.

(iii) Tests by consumer means biosensors can be used by individuals at their residence without any help from a doctor or a nurse. In future consumer tests for pregnancy, ovulation, allergy, cancer, viruses may also become available, although medical approval for some of these consumer tests is difficult, thus delaying the long term use of such biosensors.

2.9.2: Biosensors in food industry

Biosensor can be used in food industry to measure the quality and pesticides of foods. organophosphorus and carbonate pesticides have gradually replaced the organochlorines and, although they are characterized by low environmental persistence, they generally show higher acute toxicity. The toxicity has a direct impact on human health because it causes an irreversible inhibition of acetylcholinesterase which is an enzyme involved in the nerve-impulse transmission. The measurement of the anticholinesterase activity of these pesticides can be used to as a screening test for the evaluation of the presence of these compounds in food matrices.

2.9.3: Biosensors in Agriculture

Like human beings diseases such as tuberculosis and severe acute respiratory syndrome (SARS), also occurs in the plant world, including vegetables and flowers. “Soil disease” refers to infectious disease of crop plants caused by the infection with soil micro-organisms, such as viruses, bacteria, and fungi. Therefore detection of such diseases is very important in agriculture which is possible by biosensors

2.9.4: Biosensors for environment monitoring

Biosensors can also be used for monitoring the pollution level in the environment including air, land and water. Biosensors can also be developed for monitoring the quality of water through tests for pollutants, chemical residues, pesticides, herbicides, toxins and microbes in water reservoirs. The whole cell biosensors, making use of immobilized cells of Trichosporon cutaneum (a yeast), can measure Biological oxygen Demand = BOD within 30 minutes as against 5 days needed by conventional method. Similarly, whole cell biosensors with immobilized cells of Salmonella typhimurium and Bacillus subtilis coupled with an oxygen electrode can be used for testing the Mutagenicity and carcinogenicity of various chemicals. Portable pesticide monitors for measuring the level of organophosphate and carbamate pesticides in aquatic environment are also possible by biosensor.

2.9.5: Biosensors for military and defense industry

In military and defense organizations, portable biosensors can be very useful for detection of toxic gases and the agents of chemical warfare, such as mustard and nerve gas.

2.10: CONDUCTING POLYMER

Conducting polymer or intrinsic conducting polymer ICP_s are organic polymer that conduct electricity. They can be semiconductors or may have metallic conductivity. They are organic materials like insulating polymers, they are generally not thermos-formable. They possess high electrical conductivity but mechanical properties may differ between commercially available polymers. The electrical properties can be improved by organic synthesis and by advanced dispersion techniques. (Huang, W.S *et.al*. 1986).

Conducting polymers contain (pi-electron) backbone responsible for their unusual electronic properties such as

- electrical conductivity
- low energy optical transitions
- low ionization potential
- high electron affinity

Due to this Pi-conjugated system of conducting polymers they have single & double bonds along the polymer chain. Due to the higher values of the electrical conductivity obtained in such organic polymers they are called synthetic metals. Polyaniline, Polypyrrole, Polyphenylene, polyacetylene, Polythiophene. (Jagadeesan K.K, Kumar S *et. al*. 2012)

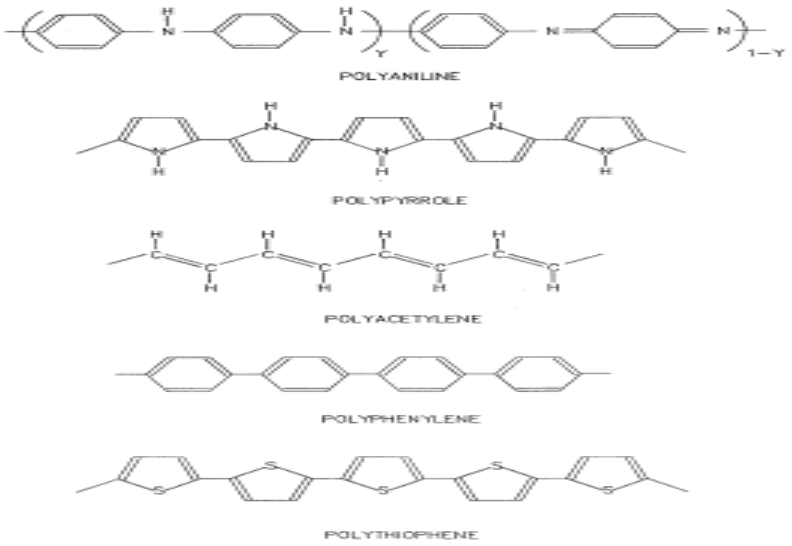


Fig.2.4: Structure of some conducting polymer commonly used in biosensors

Polyaniline (PANI) is a conducting polymer. Despite the fact that the compound itself was found more than 150 years prior, just since the mid-1980s has polyaniline caught the extreme consideration of established researchers. Due to the rediscovery of high electrical conductivity it gained intrest of scientist and researchers.

Polyaniline has numerous appealing preparing properties amongst the cluster of directing polymers. On account of its rich properties, polyaniline is a standout amongst the most observed of the previous 50 years Polymerized from the cheap.

Poly-aniline(PANI) can be found in one of three glorified states:

1. white/clear & colorless ,Leucoemeraldine – $(C_6H_4NH)_n$

2. Green for the emeraldine salt, blue for the its base Emeraldine – $([C_6H_4NH]_2[C_6H_4N]_2)_n$

3. Blue/violet (per)nigraniline – $(C_6H_4N)_n$

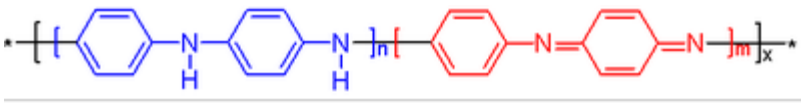


Fig.2.5: n+m = 1,main polyaniline structures, x = half degree of polymerization

The enzyme sensors developed from PANI have been intensively investigated byusing potentiometry or amperometry as the electrochemical detection technique. Most immunosensor derived from PANI are based on conductometry. The use of stable polymers with a specific receptor structure is important for their bioanalytical purpose. A relatively durable conjugated polymer such as PANI is considered as a three-dimensional network of intrinsically conducting macromolecular wires, which are able to transfer electrical signals.(S Ivanov *et. al*. 2003) The advantage of using PANI for the biosensor development lies in its capability as a biomolecule (*e.g.* enzyme) entrapment matrix. Its transducer characteristics to convert a biochemical signal into an electric signal result into signal amplification.

Further functionalization of such polymers with biospecific agents will provide more efficient biosensor membranes. PANI is a stable, electrochromic, processible conducting polymer, and it provides and will provide larger scope of usefulness in biosensors and micro-fabrications. As one of the main components in organic electronics, PANI will contribute to the development of new generation of portable and flexible analytical devices to be used in biological applications

3-MATERIALS AND METHODOLOGY

3.1: MATERIALS

The material requirements are:
The Arduino board (microcontroller) was purchased from Sumeet instruments and LMP91ooo was purchased from texas instruments. Arduino software was downloaded from http://www.arduino.cc, aniline , NHS N-hydroxysulfosuccinimide[$C_4H_5NO_3$], Sodium diphosphate dehydrate[$Na_2HPO_4.2H_2O$], Sodium Monophosphate[NaH_2PO_4], solutions were purchased from Thermo-Fisher scientific. EDC [$C_8H_{17}N_{13}$] 1-(3-dimetyamino)-propyl)-3-etylcarbodimide hydrochloride and GOx enzyme 1 mg/dl aspergillus-niger G17141-50KU of AR grade were procured from Sigma Aldrich, HCl was procured from Merc and all other chemicals were of premium grade.

3.2: HARDWARE REALISATION

Before designing hardware requirements that module must fulfill, are set:

- Miniature design with cheap and available components.
- Usage of advance integrated circuits for signal processing of electrochemical sensors
- Usage of the industrial protocols to read and control data in modules. The prospects of storing the data and setting in modules
- The prospects that the module accepts data from 3 electrode system.

3.2.1: THE PROPOSED ARCHITECHTURE

The assembly consist of a 3 Electrode system, LMP91000 sensor analog front end system, an open source arduino microcontroller board.

LMP91000 is connected to microcontroller through SCL and SDA pins which is called as a I2C communication data bus, the V_{dd} (+5V) supply which is operating voltage of this IC is given through microcontroller board, the analog output of the IC which comes at Vout pin is being fed to the analog pin A5 of the board which will display this output on the display device or a computer to which it is connected.

The 3 electrode which are working electrode (WE), Counter electrode (CE), Reference electrode (RE) is connected to Pin 12, 13, 14 respectively which are named same.

DAP is connected to ground for short circuit protection for LMP91000 IC, MENB is set to active low, DGND and Ground pin is connected to ground and Vref is set floating

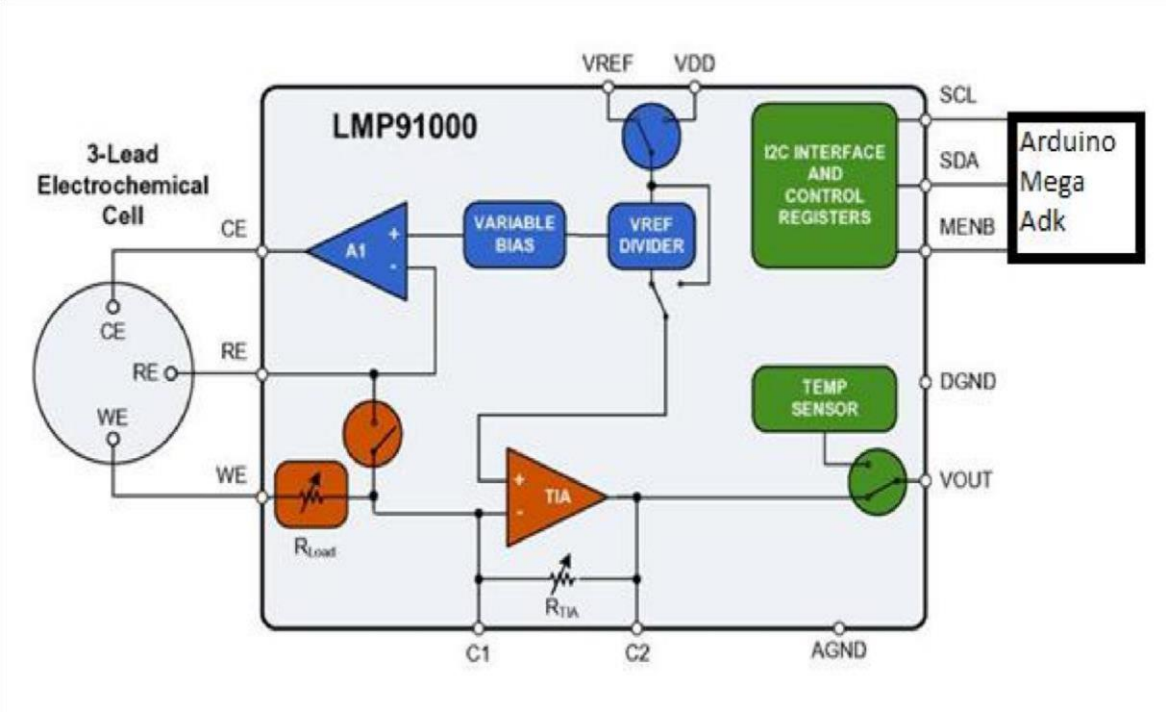


Fig. 3.1-Block diagram of a whole system with connections

3.2.2: ARDUINO ATmega2560

The proposed system is based on a low cost Arduino mega ADK board. The Arduino MEGA ADK is a microcontroller board based on the ATmega 2560. And it is precisely designed shield that implements a suitable analog front-end. It has 54 digital input/output pins. In this board, we have 54 digital input/ output pins. out of these 54 pins, 14 can be used as PWM outputs. It has 16 analog input pins and these pins can take inputs from another system in analog and this can be used for further implementation. 4 UARTs (hardware serial port). This Arduino board has 16 MHz crystal oscillator, a power jack, a USB connection an In system programming header, and a reset button.

Fig. 3.2- Photograph of Arduino Mega ADK

Table-1: Specification of Arduino Mega ADK

Microcontroller	ATmega2560
Operating Voltage	5V
Input Voltage (recommended)	7-12V
Input Voltage (limits)	6-20V
Digital I/O Pins	54 (of which 14 provide PWM output)
Analog Input Pins	16
DC Current per I/O Pin	40 mA
DC Current for 3.3V Pin	50 mA
Flash Memory	256 KB of which 8 KB used by bootloader
SRAM	8 KB
EEPROM	4 KB
Clock Speed	16 MHz

Power to the board:

The input power is given with the help of AC to DC adapter or battery to get started. By using USB cable it can be simply synchronised to a computer. The Arduino Mega can be powered with an external power supply via the USB connection. The power source is selected automatically.

The Arduino board cooperate by an external supply of 6 to 20 volts. If power supplied to the board is less than 7V, the pin 5V may supply less than 5 volts. Due to this our board become unstable. It power supplied to the board is more than 12V, the voltage regulator may get overheat and may got damaged. Therefore the operating range is 7 to 12 V. The supply pins are described as:

$V_{IN.}$ The input voltage pin to the Arduino board while using an external power source (as against to 5 volts from the USB connection or other regulated supply).

5V: The regulated power supply used to operate the microcontroller and remaining components on the board. This supply fed either from V_{IN} using on- board regulator or be supplied by USB.

3.3V: The on-board regulator generate a 3.3 volt power supply is generated by maximum current drawn from 50Ma pin.

Memory of Arduino board:
The ATmega2560 has 256KB of flash memory which is used for saving programms and out of this 8KB is used for the bootloader. 8 KB of SRAM and 4KB of EEPROM (which can be write and read with using library of EEPROM).

Input and o**utputs**

the 54 digital pins on the board can be used as an output and input pins. These pins can be used as output and input pins using digitalWrite(), pinMode(),and digitalRead() functions. These pins operates on 5V power supply and each pin receive or supply current of 40mA maximum and It has a pull up resistor of 20-50 kohms.

In addition, some pins have some specialised functions:

- Serial 1: 19 (RX) and 18 (TX); Serial: 0 (RX) and 1 (TX); Serial 2: 17 (RX) and 16 (TX); Serial 3: 15 (RX) and 14 (TX). Used to transmit (TX) and receive (RX) the transistors serial data.

  Pins 0 and 1 are also connected to the corresponding pins of the ATmega8U2 USB-toTTL Serial chip.

- External Interrupts: 3 (interrupt 1), 18 (interrupt 5), 2 (interrupt 0), 19 (interrupt 4), 20 (interrupt 3), and 21 (interrupt 2). These pins can be configured to trigger an interrupt a rising or falling edge, on an low value, or a change in value. Thoroughly discussed attachinterrupt() function for details.
- PWM: 0 to 13. Provide 8-bit PWM output with the analogWrite() function.

3.2.3: LMP91000 Sensor AFE System

The LMP91000IC is a programmable Analog Front End (AFE). It is used for micro power electrochemical sensing applications. Lmp91000 delivers a complete single solution between a microcontroller and a sensor which generates the O/P voltage proportional to the cell current. The LMP91000's reprogrammability allows it to support multiple electrochemical sensors such as 2-lead galvanic cell and 3-lead toxic gas biosensors and with a single design on the multiple discrete solutions. The final program will be uploaded on µcontroller using Arduino environment. The sample taken will generate small potential on three 3-lead electrochemical cell which is then converted into digital form. I2C interface is used to interface the arduino board with lmp91000.

Electrochemical potentiostat cells work by the impedance change of an electrolyte as it comes in contact with certain chemical or gas. These sensors have three connection for electrode assembly i.e. Working Electrode (WE), Reference Electrode (RE), Counter Electrode (CE). In operation current is driven in the CE connection and the circuitry monitor the voltage at RE points. A closed loop control circuit keeps this voltage constant, which in turn changes the return current present at WE. The resulting return current at the WE connection can be converted to a voltage via a trans-impedance amplifier (TIA).

Fig3.3: Photograph of the lmp91000 IC mounted on a PCB

Electrochemical cells typically are used to measure a wide variety of toxic and non-toxic gases such as carbon monoxide, oxygen, and hydrogen and it can also measures the change in concentration of an analyte in a solution. These cells are based on the principals of chemical oxidation and reduction. And it produces a current in proportion to the measured concentration. Most cells are made up of three electrodes: working (WE), counter (CE), and reference electrodes (RE). The WE oxidizes or reduces the target chemical changes and produces a current proportional to the concentration. The CE balances the generated current and the RE maintains the working electrode potential to ensure proper region of operation. Electrochemical cells are intended to interface with a potentiostat circuit. This potentiostat circuit provides current (and biasing, if required) to the CE. It maintains the WE at the same potential as the RE, and converts the output current from the WE into a voltage using a transimpedance amplifier (TIA).

Electrochemical sensors, like many sensors, have a dependence on temperature. To enable the best performance, measure the cell's temperature. Make appropriate temperature corrections based on that cell's performance vs. temperature plots, which can be found in the datasheet.

The sensor, gas type, and gas concentration level dictate how much current will be output at the sensor's working electrode.

To handle this variability, use a TIA with adjustable gain. Currents in the level between one to hundreds of uA are possible, so having a TIA gain in the one to hundreds of kohm range is sufficient.

Different sensors require different biasing, or for some a zero bias. Be aware of these requirements so the current produced by the sensor meets specifications. Whether the cell goes through an oxidation (CO) or reduction (NO_2) reaction to the measured gas determines if the cell produces a current into or out of the WE, respectively. The voltage at the TIA's non-inverting pin should be level-shifted appropriately to ensure maximum gain without saturating the amplifier's output in single-supply systems. For example, the TIA produces an output voltage governed by this equation:

$V_{OUT} = -I_{IN} \times R_{FEEDBACK}$, where I_{IN} is current going towards the TIA across the feedback resistor. If the current into the TIA is positive (reduction reaction), V_{OUT} will be negative in reference to the non-inverting pin voltage. That voltage should be raised to avoid railing the output to the negative supply.

Basically, it is essential that electrochemical cells have temperature correction and a potentiostat circuit that provides current sinking/sourcing, voltage biasing, current-to-voltage conversion, and level-shifting. For example, the LMP91000, a configurable AFE potentiostat, is part of the sensor AFE family and satisfies these functions. It contains a complete potentiostat circuit with sink and source capability along with programmable TIA gain, electrochemical cell bias, and internal zero voltage. Moreover, this sensor AFE contains an integrated temperature sensor and comes in a small 14-pin, 4-mm^2 package which allows positioning the device directly under the electrochemical cell for accurate temperature compensation and improved noise performance.

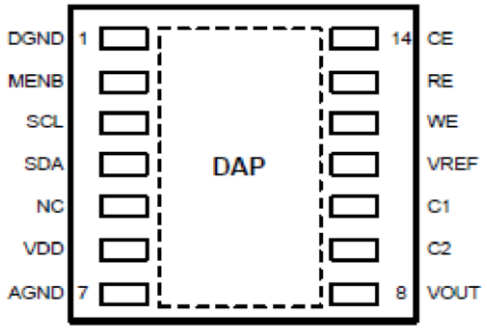


Fig.3.4- Pin diagram of LMP91000

Table-2:Pin description of LMP91000

Name	Pin	Description
DGND	1	Connect to ground
MENB	2	Module Enable, Active Low
SCL	3	Clock signal for I^2C compatible interface
SDA	4	Data for I^2C compatible interface
NC	5	Not Internally Connected
VDD	6	Supply Voltage
AGND	7	Ground
VOUT	8	Analog Output
C2	9	External filter connector (Filter between C1 and C2)
C1	10	External filter connector (Filter between C1 and C2)
VREF	11	Voltage Reference input
WE	12	Working Electrode. output to drive the Working Electrode of the chemical sensor
RE	13	Reference Electrode. Input to drive Counter Electrode of the chemical sensor
CE	14	Counter Electrode. output to drive Counter Electrode of the chemical sensor
DAP		Connect to AGND

The LMP91000 generates an output voltage proportional to the cell current. Transimpedance gain is user programmable through an I^2C compatible interface from 2.75kΩ to 35okΩ making it easy to convert current ranges from 5µA to 750µA full scale. optimized for micro-power applications, the LMP91000 AFE works over a voltage range of 2.7V to 5.25 V. The cell voltage is user selectable using the on board programmability. In addition, it is possible to connect an external transimpedance gain resistor.

A temperature sensor is embedded and it can be power cycled through the interface. The output of this temperature sensor can be read by the user through the V_{OUT} pin. It is also possible to have both temperature output and output of the TIA at the same time; the pin C2 is internally connected to the output of the transimpedance (TIA), while the temperature is available at the V_{OUT} pin.

Depending on the configuration, total current consumption for the device can be less than 1oμA. For power savings, the transimpedance amplifier can be turned off and instead a load impedance equivalent to the TIA's inputs impedance is switched in.

Table-3: Absolute maximum rating

ESD Tolerance [3]	
Human Body Model	2kV
Charge-Device Model	1kV
Machine Model	200V
Voltage between any two pins	6.0V
Current through VDD or VSS	50mA
Current sunk and sourced by CE pin	10mA
Current out of other pins[4]	5mA
Storage Temperature Range	-65°C to 150°C
Junction Temperature [5]	150°C

LMP91000 is programmed using its data sheet. In our project it is used for electrochemical sensing applications. To program this IC we have to manipulate the following four components. They are TIA Gain, V_{REF}, R_{LOAD} and BIAS VOLTAGE**.**

Control amplifier

The control amplifier has two tasks:

a) Initial charge for sensor.

b) A bias voltage for sensor.

A1 has the capability to drive up to 10mA into the sensor in order to provide a fast initial conditioning. A1 is able to source and sink current according to the gas sensor connected (oxidizing or reducing gas sensor). It can be modified to reduce system power consumption. However modifying A1 is not recommended, as it may take a long time for the sensor to recover from this situation.

Variable Bias

The voltage required by a biased gas sensor between its reference and working electrodes is provided by the Variable Bias block circuitry provides the amount of bias. The bias voltage can be programmed to be 1% to 24% (14 steps in total) of the supply, or of the external reference voltage. Using I^2C interface the 14 steps can be programmed. The polarity of the bias can be also programmed.

Internal zero

The internal Zero is the voltage at the non-inverting pin of the TIA. The internal zero can be programmed to be either 20%, 67%, 50%, of the external reference voltage or the supply. This provides both adequate headroom for the counter electrode of the sensor for swing, Best use of the ADC's full scale input range is in case of sudden changes in the gas concentration, and.

The Internal zero is maintained through an internal voltage divider. Using I2C interface this divider is programmed.

Temperature sensor

The embedded temperature sensor can be switched off during gas concentration measurement to save power.

The temperature measurement is activated through the I^2C interface. The temperature output is available at the V_{OUT} pin until the configuration bit is reset. Voltage is the output signal of the temperature sensor, with reference to ground of the LMP91000 (AGND).

LMP91000 is programmed using its data sheet. In our project it used for electrochemical sensing applications. To program this IC we have to manipulate the following four components. They are TIA Gain, V_{REF}, R_{LOAD} and BIAS VOLTAGE**.**

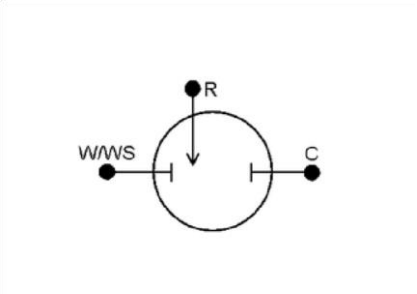
Table-4: Electrical Characteristics

Parameter		Test Conditions		Min (2)	Typ (3)	Max (2)	Units
TIA_GAIN	Transimpedance gain accuracy				5		%
	Linearity				±0.05		%
	Programmable TIA Gains	7 programmable gain resistors			2.75 3.5 7 14 35 120 350		kΩ
		Maximum external gain resistor			350		
TIA_ZV	Internal zero voltage	3 programmable percentages of VREF			20 50 67		%
		3 programmable percentages of VDD			20 50 67		
	Internal zero voltage Accuracy				±0.04		%
RL	Programmable Load	4 programmable resistive loads			10 33 50 100		Ω
	Load accuracy				5		%
PSRR		2.7 ≤VDD≤5.25V	Internal zero 20% VREF	80	110		dB
			Internal zero 5o% VREF				

	Power Supply Rejection Ratio at RE pin		Internal zero 67% VREF				
	Temperature Error	TA=-40˚C to 85˚C		**-3**		**3**	°C
	Sensitivity	TA=-40˚C to 85˚C			-8.2		mV/ °C
	Power on time					1.9	ms
EXTERNAL REFERENCE SPECIFICATION							
VREF	External Voltage reference range			1.5		VDD	V
	Input impedance				10		MΩ

3.2.4-ELECTRODE ASSEMBLY

An electrode is a conductive or semi-conductive solid that interfaces with an electrolyte solution. The 3 common electrodes are: Working, Reference



And Counter (or Auxiliary).

Working electrode is that electrode which is under observation. In our experiment working electrode is ITO glass surface.

The auxillary or counter is the electrode in the cell that finalizes the current path.

All electrochemistry experiments (with non-zero current) must have a counter-working pair. In general experiments the auxillary is simply the current sink/source and so relatively inert materials like graphite or platinum are ideal, though not necessary. In some experiments the auxillary electrode is part of observation and the material composition and setup will vary accordingly.

As their name suggests Reference electrodes are, electrodes that serve as experimental reference points. Specifically they are a reference for the potential (sense) measurements. Reference electrodes should, therefore, hold a constant potential during testing, ideally one which is known on an absolute scale. This is accomplished by first having ideally or low, no current flow through them, and second by being "well poised" which means that even if some current does flow it will not affect the potential. While many electrodes could be well poised there are several that are very commonly used and commercially available: Silver/Silver Chloride, Saturated Calomel, Mercury/Mercury (mercurous) oxide, Copper/Copper Sulphate, Mercury/Mercury Sulphate and more. There are other couples that are often referenced but are not often used today such as the Normal Hydrogen Electrode.

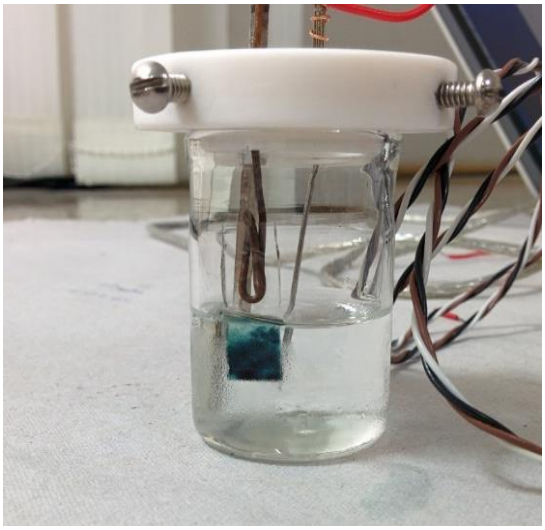


Fig.3.5: photograph of the 3 electrode cell

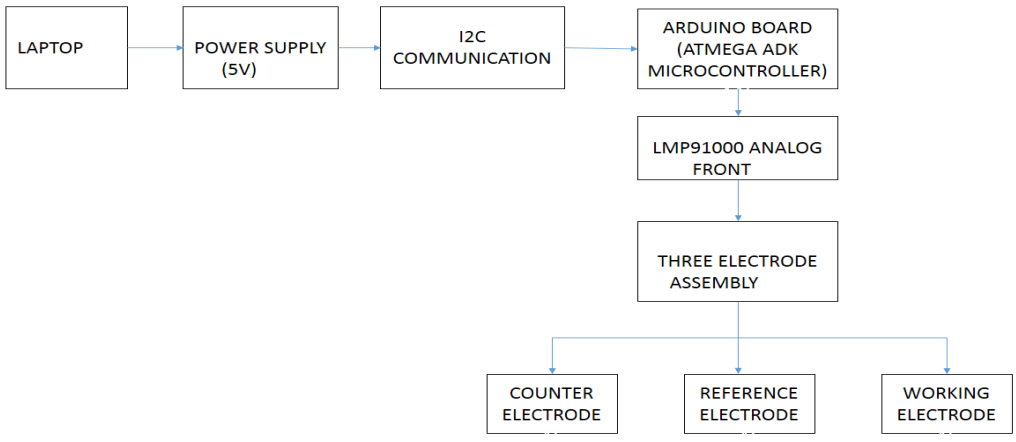
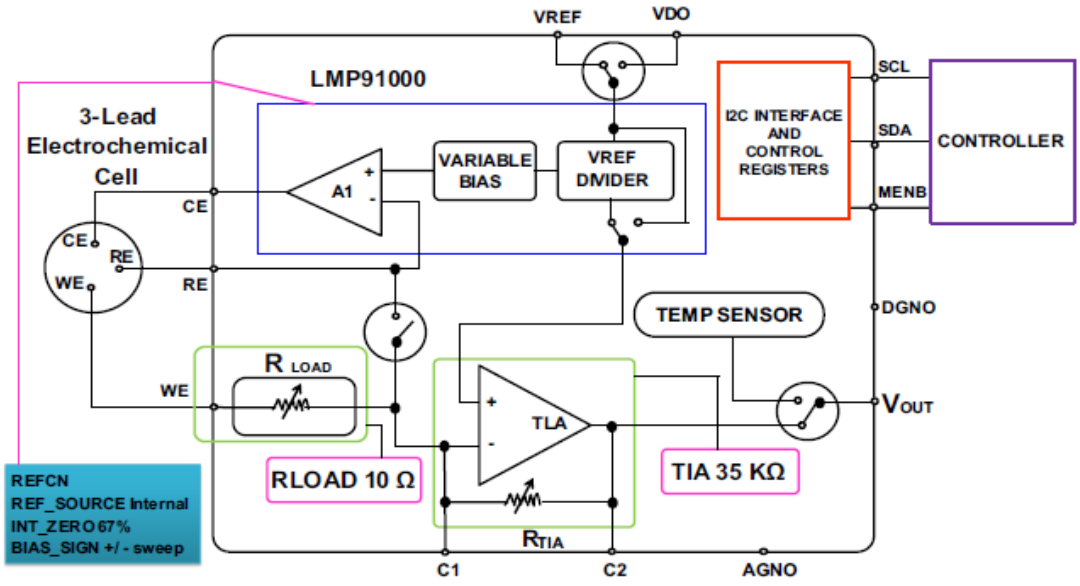


Fig. 3.6- Block diagram of the proposed architecture

3.3-METHODS

The project is divided in to three module

- Module 1- Interfacing of LMP91000 with Arduino Mega ADK
- Module 2- Electrochemical deposition of Polyaniline by aurdino based three electrode system and Immobilization of GOx enzyme on electrode
- Module 3- Glucose sensing and control experiment



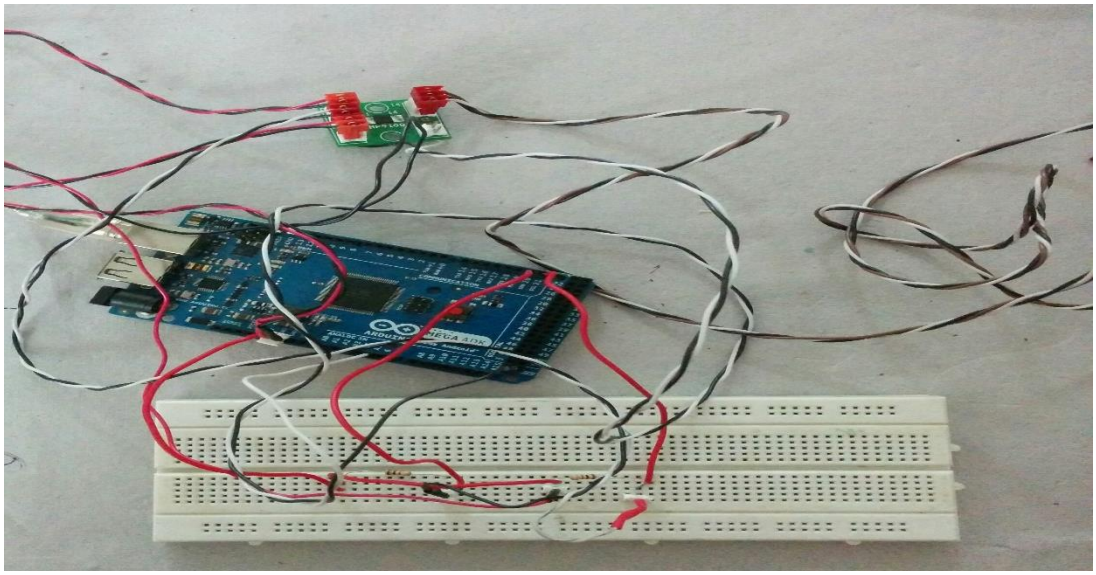


Fig: 3.7- photograph of assembled prototype device

3.3.1- INTERFACING OF LMP91000 WITH ARDUINO MEGA ADK

We interface the LMP91000 IC with arduino board via I2C interfacing using SCL (clock Pulse) and SDA (data bus). Arduino environment is downloaded and Programming is done using arduino environment in the microcontroller (ATMEGA 2560). The USB cable is used to interface the arduino board with pc and Download the arduino environment.

1. Connect the board:

The Arduino Mega automatically draw power from either the USB connection to the computer or an external power supply. The power source is selected with a jumper, a small piece of plastic that fits onto two of the three pins between the USB and power jacks. Connect the Arduino board to your computer using the USB cable. The green power LED should go on.

2. Install the drivers

After installing the drivers, upload the program on the arduino board and run the appropriate circuit. Given below is the example which was performed successfully on arduino environment. In this way the interfacing with pc was done.

I2C INTERFACE:

The I2C interface works in Standard mode (100kHz). Pull up resistors or current sources are needed on the SDA and SCL pins to drive them high when they are not in low condition. A logic zero is transmitted by driving the output low. A high logic is transmitted by discharging the yield and allowing it to be pulled-up automatically. The defined pull up resistor qualities will depends upon thetotal operating speed and total bus capacitance. The LMP91000 accompanies a 7 bit transferred to a fixed address location: 1001 000.

I2C is an acronym for "Inter-Integrated Circuit". In the late 1970s, Philips' semiconductor division (now**NXP**) saw the need for simplifying and standardizing the data lines that travel between various integrated circuits in their products. The I2C bus their solution. Which reduced the number of wires to 2 (SCL – clock and SDA – data)

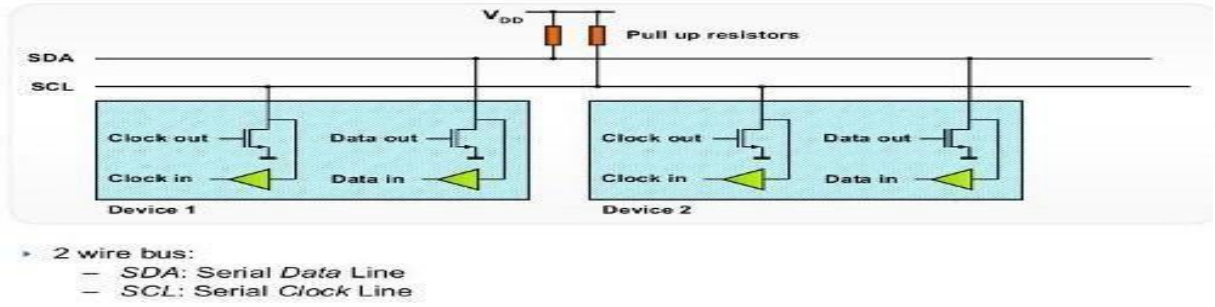


Fig. 3.8- Pull up resistors for I2C bus

WRITE AND READ OPERATION

In order to start any write and read operation with the LMP91000, MENB needs should be low during the whole communication. Then a start condition is generated by master while SCL is high driving SDA from high to low. The start condition is always followed by a Read/Write bit and a 7-bit slave address and. After these 8 bits have been transmitted by the master, SDA is free by the master and the LMP91000 either NACKs or ACKs the address. If the slave address matches, the master is LMP91000 ACKs. If the address don't match, the master is LMP91000 NACKs. For a write operation, the master follows the ACK by sending the 8-bit register address pointer. Then the LMP91000 ACKs the allocation by driving SDA low. Next, the master directs the 8-bit data to the LMP91000. Then the LMP91000 ACKs the transfer by driving SDA low. The master should generate a stop condition at this point and set the MENB in logic high optionally.

A address pointer needs to be sets first for read operation requires the LMP91000 address, also in this case the master needs to set low logic for MENB, Before reading from the desired register the master needs to write to the device and fixed the address pointer This type of read requires a start, the slave address, the address pointer, a write bit, a Repeated Start (if appropriate), , and a read bit. Following this sequence, the LMP91000 sends out the 8-bit data of the register.

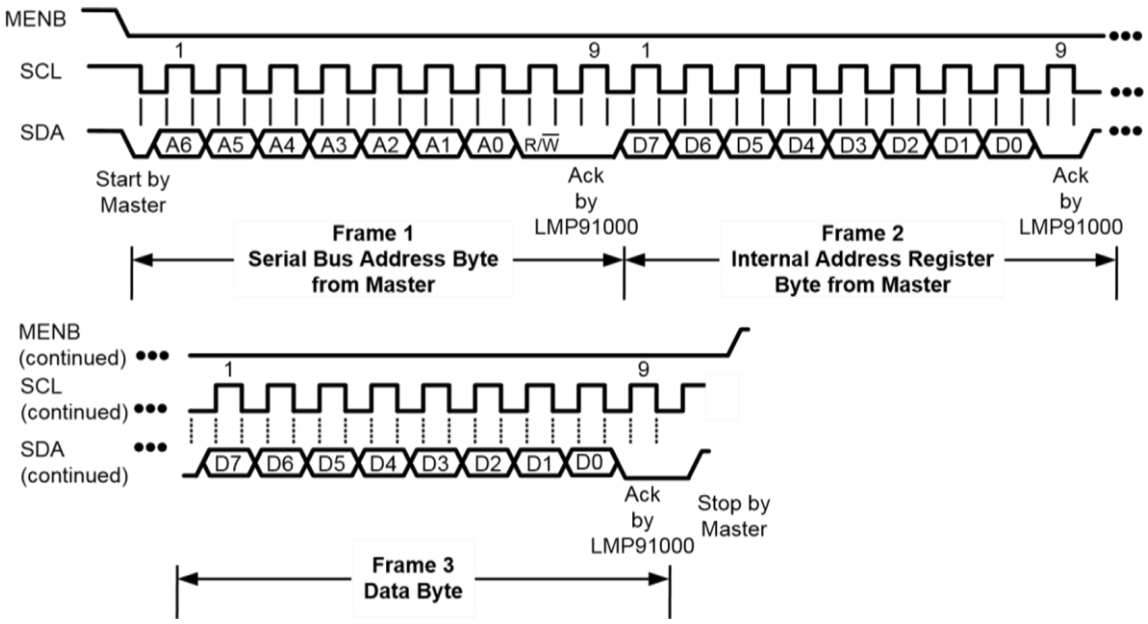


Fig. 3.9: Register write transaction

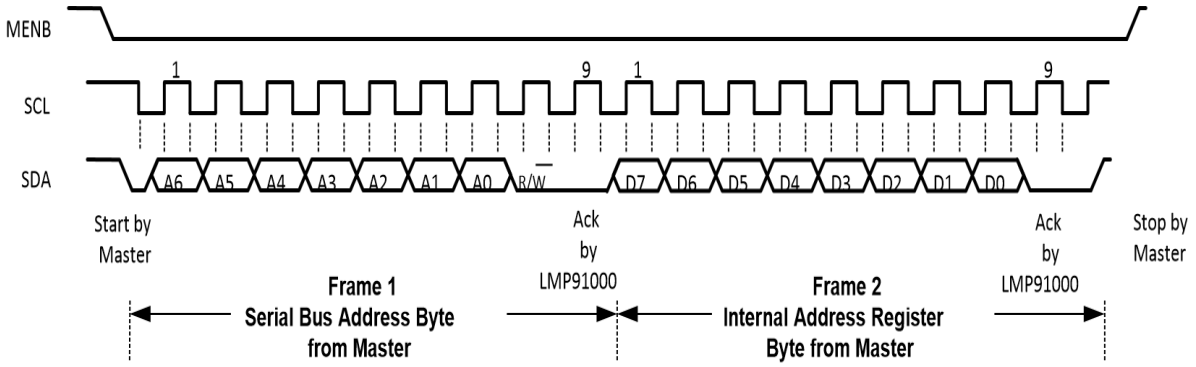


Fig. 3.10 Pointer set transaction

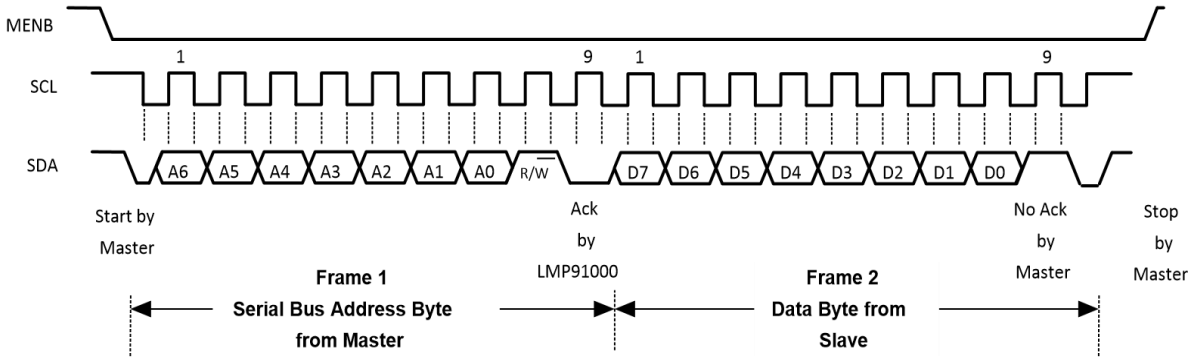
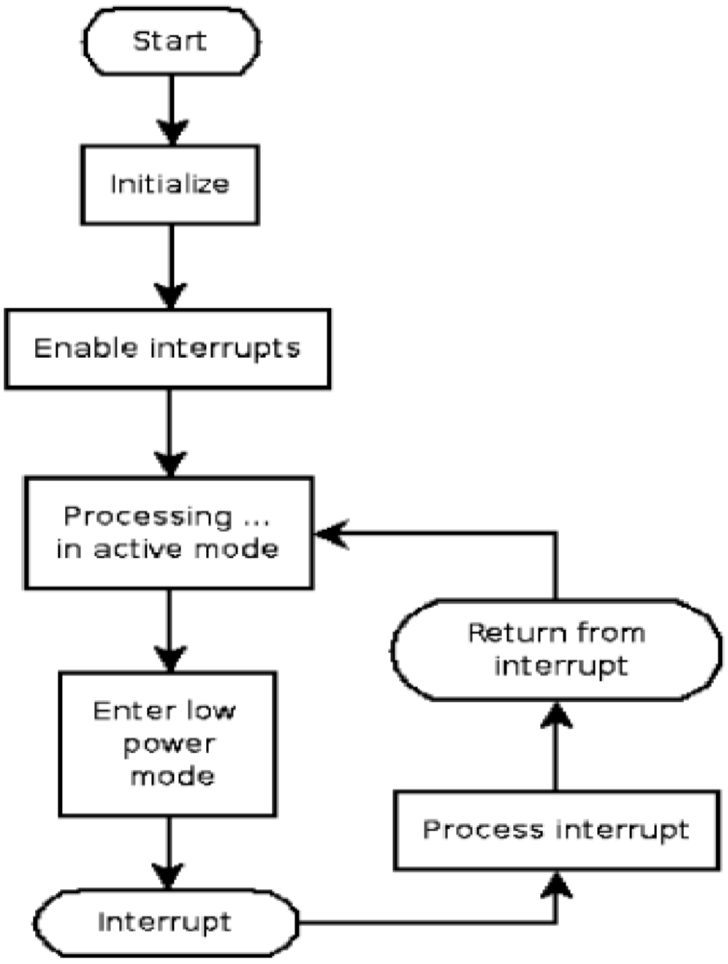


Fig. 3.11: Register read transaction

SOFTWARE IMPLEMENTATION



In manual operation mode LMP91000EVM was set, which define the users to set the function parameters depending on the type of electrochemical experiments and mesurements desired. In our case, the TIA Control (TIACN) registers were set to assign a value of 35 kΩ and the R_{LOAD} register to 10 Ω. These registers are related with two different parameters, decrease the gain of the trans impedance amplifier or the TIA control increases and the R_{LOAD} register sets the value of the resistor which bridges the working electrode and the TIA amplifier. This resistor is used to control the value of the current drawn from the sensor from the TIA during the measurements. Moreover, the reference control register (REFCN) permits the configuration of the internal zero, bias source, and reference source. The reference source was fixed to be internal, so no external supply was necessary. The default value for this voltage is 2.5V

The internal zero parameter was set to be 67% of the reference voltage, which gives the output voltage of the board to be 1.67 V when there is no electrochemical activity in the sensor connected to the three-electrode system. The BIAS_SIGN register was preset to switch automatically from positive to negative and from negative to positive in order to maintain a full range CV. Finally, the BIAS register was controlled by a cycle that changed the value of the differential voltage between the reference electrode and working according to the values allocated to the REFCN parameters. By programming the LMP91000EVM with the conditions previously mentioned, It can perform CV with variable sampling rates in the potential range from 0.6 V to 0.7V. As mentioned the LMP91000EVM outputs is a voltage proportional to the cell current. The value of this current can be found by plugging the output voltage of the LMP91000 in the TIA amplifier transfer function equation, taking into consideration the parameters set for the CV measurement using the equation

$$Iout = (Vout - VINT\_Z)/TIAgain.$$

3.3.2: ELECTROCHEMICAL DEPOSITION OF POLYANILINE BY AURDINO BASED THREE ELECTRODE SYSTEM AND IMMOBILIZATION OF GOX ENZYME ON ELECTRODE

Prior to aniline deposition, ITO plates are pre-cleaned with ethanol, acetone and with abundant amounts of de-ionized water. Further, ITO plates are immersed in a solution of$H_2O_2/NH_4OH/H_2O$ (1 : 1 : 5, V/V) for about 35 min at 81°C, to obtain uniformly distributed OH groups on the ITO surface, after which these are carefully rinsed with de-ionized water and are dried. The freshly prepared Glass ITO Electrode has been placed into a solution containing 0.3 M aniline in 1 M HCl and exposed to a potential between 0V and +1.2V versus reference electrode at a scan rate of 100 mV/s using aurdino based three electrode system. The Electrochemical polymerization was continued for about 1o min until PANI gets uniformly deposited on the ITO electrode. The deposition and different phase of polyaniline has been seen in video1. The GOx emzyme is mixed with 1-(3-dimetyamino)-propyl)-3-etylcarbodimide hydrochloride (EDC,0.2M) and N-hydroxysulfosuccinimide (NHS, 0.1 M) to form covalent bond between the COOH-terminal of the enzymes and the amine group of polyaniline. The GOx/PANI//ITO bioelectrodes are preserved for about 5h in a moist chamber at room temperature (25°C) for binding of the preferred enzymes.

3.3.3- GLUCOSE SENSING AND CONTROL EXPERIMENT

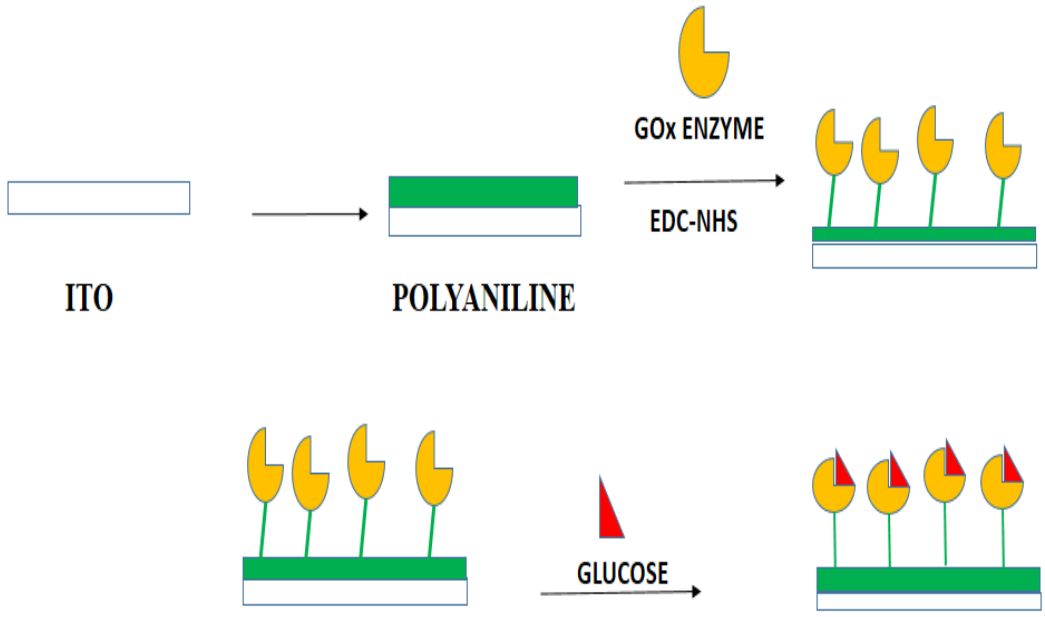


Fig. 3.11-Fabrictaion of GOx electrode and glucose detection

4. RESULTS AND DISCUSSION

MODULE-1- INTERFACING OF LMP91000 WITH ARDUINO MEGA ADK

After connecting the LMP91000 with the Arduino mega ADK controller board, the interfacing is done with the help of a programming which is done by initializing the I2C communication between the two devices by a Arduino environment software

The Arduino environment can be drawn-out using libraries, like most programming platforms. Libraries provide extra properties and functionallity for use in sketches, e.g. manipulating data and working with hardware. In our project we are using wire library. Wire library is used for Two Wire Interface (I2C/TWI) for receiving and sending data over a web of sensors or devices.

.

The following program is developed as

#define LMP91000_SLV_ADDR_WRITE 0x90

#define LMP91000_SLV_ADDR_READ 0x91

#define LMP91000_Slave_Address ox48

#define STATUS 0x00

#define MODECN 0x12

#define Fet_Short_Enabled 0x80

#define Deep_sleep 0x00

#define Two_lead_gnd 0x01

#define Standby 0x02

#define Three_Lead_Amperometric 0x03

#define Temperature_meas_TIA_Off 0x06

#define Temperature_meas_TIA_On 0x07

#define LOCKCN 0x01

#define Write_TIACN_REFCN 0x00

#define Read_TIACN_REFCN 0x01

#define TIACN 0x10

#define Gain_External_Resistance 0x00

#define Gain_2p75KOhm 0x04

#define Gain_3p5KOhm 0x08

#define Gain_7KOhm 0x0C

#define Gain_14KOhm 0x10

#define Gain_35KOhm 0x14

#define Gain_120KOhm 0x18

#define Gain_350KhOhm ox1C

#define Rload_10_Ohm 0x00

#define Rload_33_Ohm 0x01

#define Rload_50_Ohm 0x02

#define Rload_100_Ohm 0x03

#define REFCN 0x11

#define Internal_Vref 0x00

#define External_Vref 0x80

*#define Internal_Zero_20 0*00*

*#define Internal_Zero_50 0*20*

*#define Internal_Zero_67 0*40*

#define Internal_Zero_Bypassed 0x60

#define Bias_Negative 0x00

#define Bias_Positive 0x10

#define Bias_o_Percent 0x00

#define Bias_1_Percent 0x01

#define Bias_2_Percent 0x02

#define Bias_4_Percent 0x03

#define Bias_6_Percent 0x04

#define Bias_8_Percent 0x05

#define Bias_10_Percent 0x06

#define Bias_12_Percent 0x07

#define Bias_14_Percent 0x08

```
#define Bias_16_Percent 0x09
#define Bias_18_Percent 0x0A
#define Bias_20_Percent 0x0B
#define Bias_22_Percent 0x0C
#define Bias_24_Percent 0x0D
#include <Wire.h>
#define sampleDelay 100
void main()
{
Serial.begin(9600);
pinmode(7, OUTPUT);
pinmode(9, OUTPUT);
pinmode(A5,INPUT);
pinmode(A6,INPUT);
pinmode(A8,INPUT);
Wire.begin();
LMP_CFG();
}
void loop()
{
/*
digitalWrite(7,HIGH);
digitalWrite(9,LOW);
delay(sampleDelay);
Serial.print(analogRead(A5));
Serial.print(",");
delay(sampleDelay);
*/
// MOSFET control logic and test measurements
```

```
digitalWrite(9,HIGH);
digitalWrite(7,LOW);
delay(sampleDelay);
Serial.print(analogRead(A7));
Serial.print(",");
Serial.println(analogRead(A4));
Serial.println(analogRead(A4)*5.0/1024);
}
/* Configure LMP91000 for the intended operation */
void LMP_CFG(void)
{
/* MODECKN register: Configure device mode to Three Lead Amperometric*/
Wire.beginTransmission(LMP91000_Slave_Address);
Wire.write(MODECN);
//Wire.write(Three_Lead_Amperometric);
Wire.write(Three_Lead_Amperometric); // 2-wire galvanic mode
Wire.endTransmission();
/* LOCKN register: Unlock for configuring TIACN and REFCN */
Wire.beginTransmission(LMP91000_Slave_Address);
Wire.write(LOCKCN);
Wire.write(Write_TIACN_REFCN); // Unlock
Wire.endTransmission();


/* Sets the Reference Control Register to select the following parameters:
* External reference voltage | Internal zero selection = VREF*0.2 |
* Bias polarity = positive | BIAS selection = VREF*0.24 */
Wire.beginTransmission(LMP91000_Slave_Address);
Wire.write(REFCN);
```

Wire.write(Internal_Vref | Internal_Zero_50 | Bias_Negative | Bias_0_Percent);

Wire.write(Internal_Vref | Internal_Zero_20 | Bias_Positive | Bias_0_Percent);

Wire.endTransmission();

/ Sets the TIA Control Register to select 35okohm gain resistance*

** and load resistance (100 ohm)*/*

Wire.beginTransmission(LMP91000_Slave_Address);

Wire.write(TIACN);

Wire.write(Gain_350KhOhm | Rload_100_Ohm);

Wire.endTransmission();

// LOCKN register: LOCK for configuring TIACN and REFC

Wire.beginTransmission(LMP91000_Slave_Address);

Wire.write(LOCKCN);

Wire.write(Read_TIACN_REFCN); // Lock

Wire.endTransmission();

}

After interfacing we can use LMP91000 sensor AFE system with 3 electrode cell for biosensing i.e, glucose detection

MODULE 2- ELECTROCHEMICAL DEPOSITION OF POLYANILINE BY AURDINO BASED THREE ELECTRODE SYSTEM AND IMMOBILIZATION OF GOX ENZYME ON ELECTRODE

The most significant advantage of electrochemically prepared PANI(polyaniline) is that the polymer is deposited directly on the electrode surface, when its thickness and properties can be controlled via the quantity of electrical charges consumed during polymerization. Moreover, PANI provides a very suitable environment for enzyme immobilization.

After connecting the board with pc, Program was successfully uploaded on the microcontroller which is given on next page. The program was uploaded:

1. To generate the potential from 0.2V to 1.5V between working and reference electrode.

2. To generate the potential at the scan rate of 100 mV/s

The codes for following command are as follow

```
#include <Wire.h>
byte val = 0;
int ledPin = 9;    // LED connected to digital pin 9
void setup()
{
  Wire.begin(); // join i2c bus (address optional for master)
}
void loop()  {
  // fade in from min to max in increments of 5 points:
  Wire.beginTransmission(72); // transmit to device #72 (0x2c)
                              // device address is specified in datasheet
  Wire.write(byte(0x00));            // sends instruction byte
  Wire.write(val);             // sends potentiometer value byte
  Wire.endTransmission();     // stop transmitting
for(int fadeValue = 0 ; fadeValue <= 110; fadeValue +=3) {
    analogWrite(ledPin, fadeValue);
    delay(30);
  }
  // fade out from max to min in increments of 5 points:
  for(int fadeValue = 110 ; fadeValue >= 0; fadeValue -=3)
  {
     analogWrite(ledPin, fadeValue);
     delay(30);
  }
}
```

After generating these potential, the aniline starts depositing on the Glass ITO surface as aniline electro-polymerization is more feasible in acidic aqueous solution with the help of arduino programming platform, we developed a program which deposit the aniline on the ITO glass surface. The Electrochemical polymerization was continued for about 10 minutes until PANI gets deposited on the glass ITO electrode.

Polymerization of polyaniline is done in the existence of 1M Hydrochloric acid, i.e. at greater acidity, its deposition takes place faster and produces a product of improved conductivity. A immobile higher concentration of HCl greater than 2M concentration leads to the reduction of conductivity. Distilled water was castoff because the potential existence of iron (III) ions in tap water may quicken the aniline oxidation. These aniline deposited ITO surface is used for further sensing application.

Due to this following changes takes place on ITO surface Polymerized from the inexpensive aniline monomer, polyaniline can be found in one of three idealized oxidation states

- leucoemeraldine – white/clear & colorless $(C_6H_4NH)_n$
- emeraldine –green for the emeraldine salt, blue for the emeraldine base$([C_6H_4NH]_2[C_6H_4N]_2)_n$
- (per)nigraniline – blue/violet $(C_6H_4N)_n$

Fig.4.1: Photograph of the poly aniline deposition green in colour

Fig. 4.2: Photograph of the poly aniline deposition dark green in colour

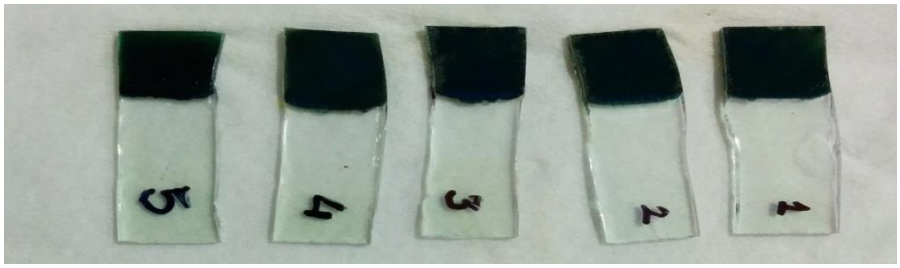


Fig. 4.3: Photograph of the several poly aniline deposited electrode

CHARACTERIZATION OF POLYANILINE:

Characterization of polyanilne can be done Scanning electron microscope (SEM) and X ray diffraction Method (XRD).

Scanning electron microscope (SEM):

SEM is a type of electron microscope that develops images of a sample while scanning a sample with a focused beam of electrons. Various signals can be produced when an electron interact with an atom of the sample. These signals contains information about the sample's surface topography and composition. Generally raster scan pattern is used by the electron beam and an image is produced when the beam's position is combined with the detected signal. SEM can achieve resolution better than 1 nanometer. Specimens can be observed in high vacuum, in low vacuum, in wet conditions (in environmental SEM), and at a wide range of cryogenic or elevated temperatures. The Hitachi-S3700 model is used to study the surface morphology of the PANI depostited on ITo glass electrode, in SEM images it can be clearly seen the thread like structure of aniline

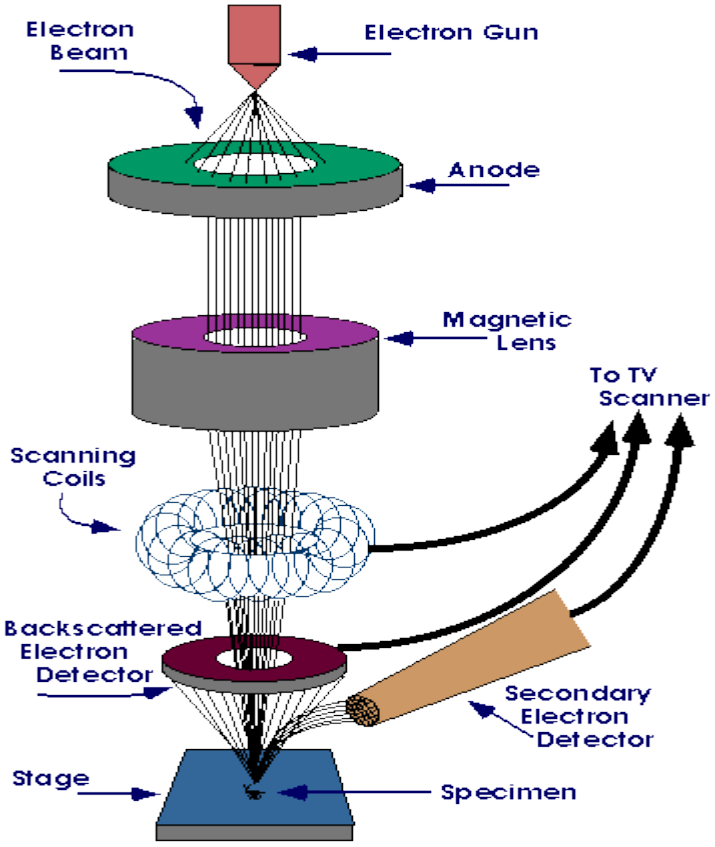


Fig. 4.4: Scanning electron microscope (SEM) Schematic

The SEM images of polyaniline Deposited on ITO glass electrode.

The thread like structure shown in fig shown below and inset clearly reveals the deposition of nanostructured PANI on the surface of glass ITO surface. The images shown below are on three scales i.e. 500µm, 1µm, 500mm.

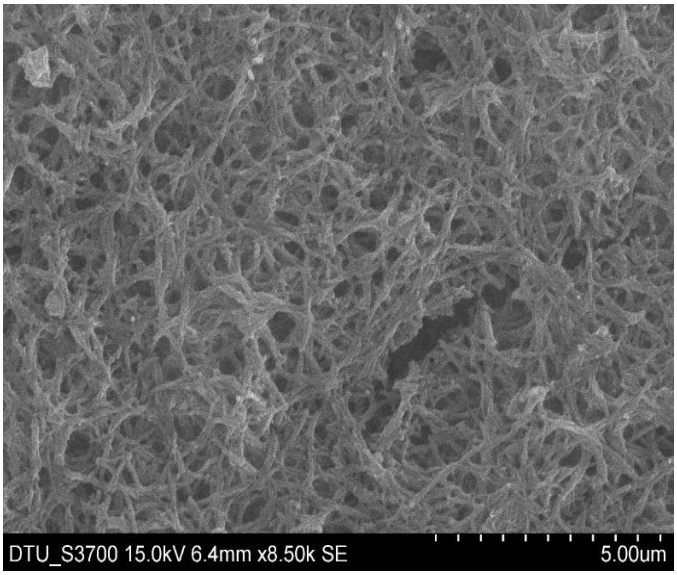


Fig. 4.5: SEM image of polyaniline(5µm)

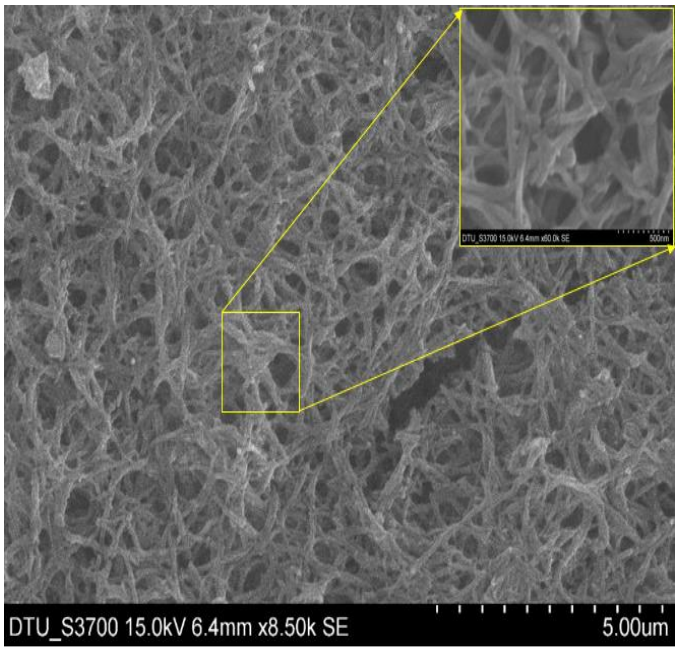


Fig. 4.6: SEM image of polyaniline(5 um) inset 500nm

XRD Study:

The atomic planes of a crystal cause an incident beam of X-rays to interfere with one another as they leave the crystal. The phenomenon is called X-ray diffraction. XRD indicates the crystallinity of the product. High crystalline products could possess metallic behavior and they are more useful than amorphous products. Generally, the ratio of half-width to height (HW/H) of an X-ray diffraction peak reflects the order of the polymer crystallinity

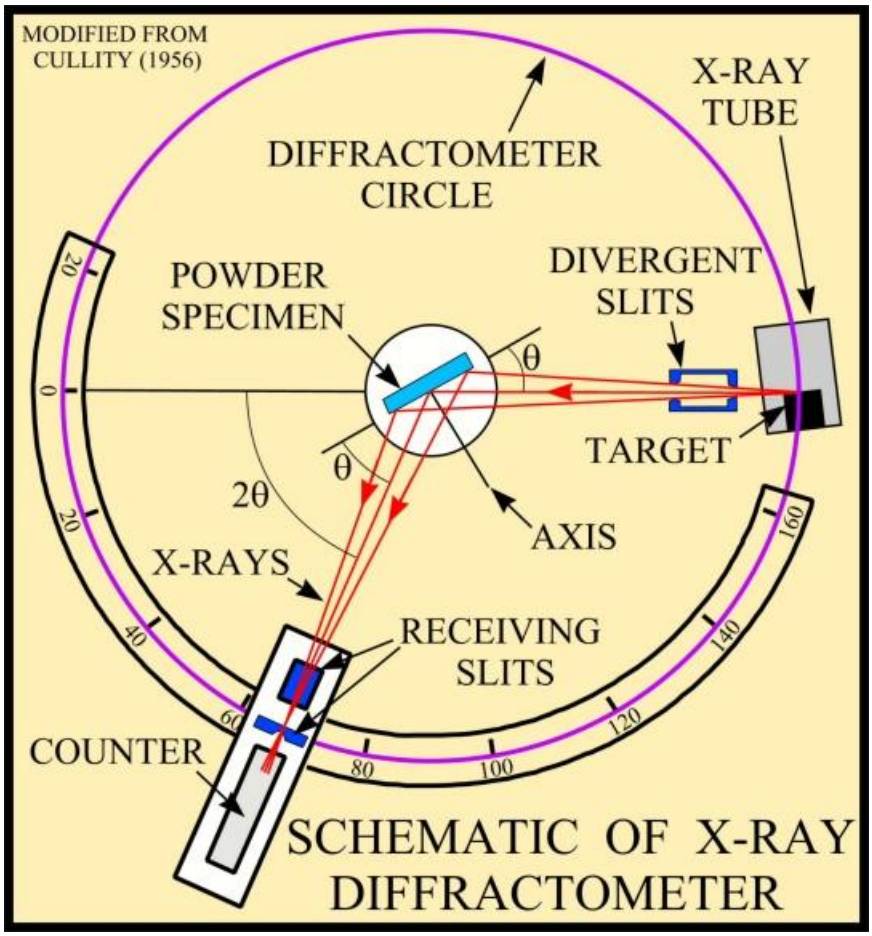


Fig. 4.7: XRD schematic

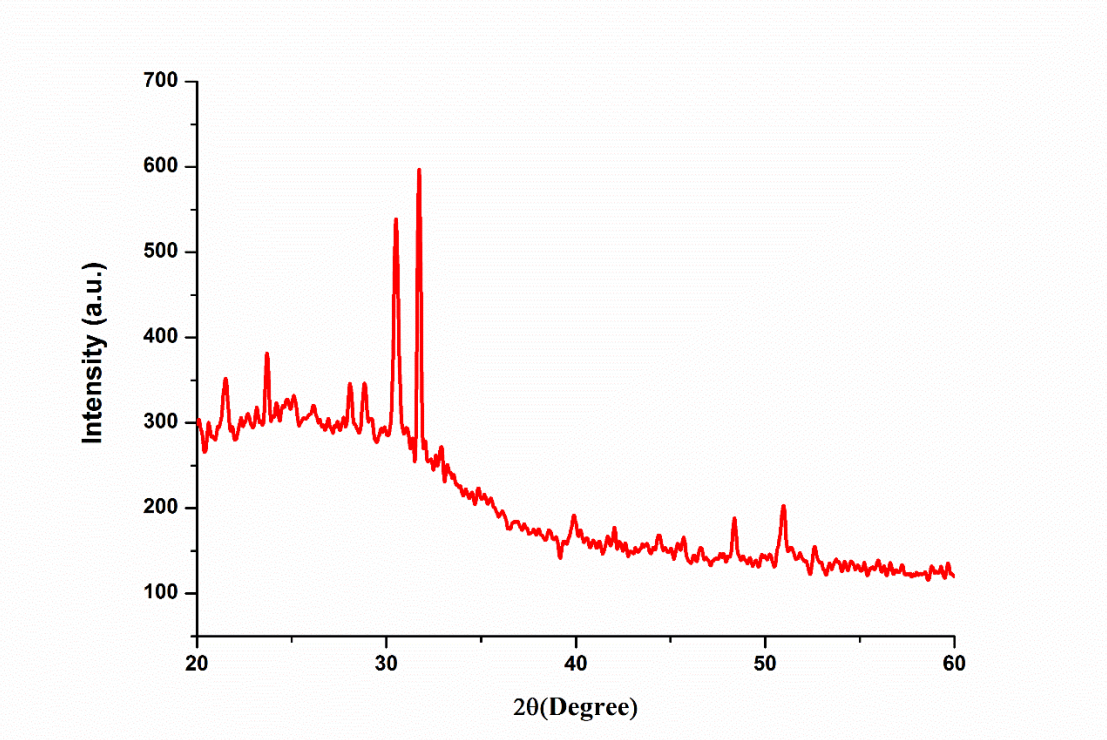


Fig. 4.8: XRD analysis of polyaniline

XRD peak confirm the crystalline properties of deposited polyaniline. Crystalline polyaniline has an X-ray diffraction pattern that shows three peaks match with crystalline polyaniline (23.6, 31.8° and 48.3°). The characteristic peaks appeared corresponding to (112), (031) and (025) crystal planes 0f PANI.

Module 3- Glucose sensing

The glucose oxidase (GOx) enzyme 1mg/dl is freshly prepared in phosphate buffer (50mM, pH 7.0) prior to use and stored at 4°C. The fresh stock solution of glucose was prepred in phosphate buffer (5oo mg/dl) which is further used to make the different working solution using PBS and stored at 4°C.

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{EOD}} O_2 + 2H^+ + 2e^-$$

In this Glucose biosensor, PBS containing 5mM $[Fe(CN)_6]^{3-/4-}$ has been used as artificial mediator that carry electron between the FAD center of glucose oxidase and electrode surface by the following scheme.

$$\text{glucose} + GOx_{(ox)} \longrightarrow \text{gluconic acid} + GOx_{(red)}$$

$$GOx_{(red)} + 2[Fe(CN)_6]_{(ox)} \longrightarrow GOx_{(ox)} + 2e^-$$

$$2[Fe(CN)6]_{(red)} \longrightarrow 2[Fe(CN)6]_{(ox)} + 2e-$$

where 2[Fe(CN)6] (ox) and 2[Fe(CN)6] (red) are the oxidized and reduced forms of the mediator. The reduced form is reoxidized at the electrode, giving a current signal (proportional to the glucose concentration) while regenerating the oxidized form of the mediator. Such mediation cycle is displayed in Figure

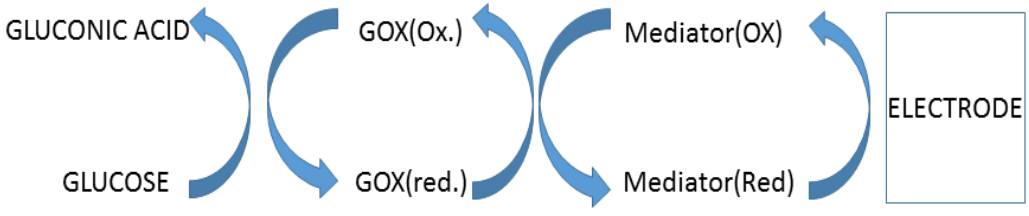


Fig. 4.9: Sequence of events that occur in glucose biosensor system

SENSING EXPERIMENT

VOLTAGE RESPONSE OF GOD ELECTRODES ON ADDING GLUCOSE SOLUTION OF DIFFERENT CONCENTRATION ON IT

After adding glucose solution to the change in response voltage of the active device glucose oxidase is the parameter of interest for sensor applications. The response potential of the device depends on several factors:

1-The contact resistance between the ITO GLASS electrode and the polymer film.
2-The geometric factor of the film and 3-the film conductivity.

The film conductivity is depends on several factors, such as analyte pH, temperature, polymer film potential, substrate concentration and enzyme loading potential-time

Change in output voltage w.r.t. to different glucose concentration will stable after 5-10 minutes

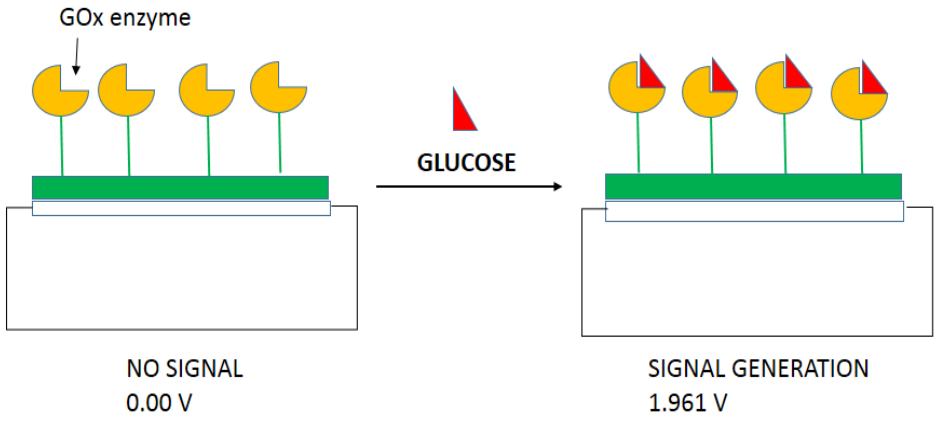


Fig. 4.10: Schematic of change in voltage due to binding of glucose with GOx enzyme on working electrode

The change in output due to adding glucose of different concentration in to the 3 electrode cell in presence of PBS buffer (pH-7) with using GOD electrode and unimmobilized electrode is given in table below. The results shows that amperometric current increases after addition of Glucose

Voltage at WE-2.323V, CE-2.395, CE-2.231V

Glucose cocentrtaion	Vout (using unimmoblized electrode)	Vout (using GOD electrode)	$Iout = \frac{Vout - Vintz}{TIAgain}$
0 mg/dl	2.4mV	1.97V	3.83 µA
100mg/dl	2.4mV	1.76V	4.40 µA
200mg/dl	2.4mV	1.49V	5.12 µA
300mg/dl	2.4mV	1.05V	6.42 µA
400mg/dl	2.4mV	0.57V	7.81 µA
500mg/dl	2.4mV	0.33V	8.48 µA

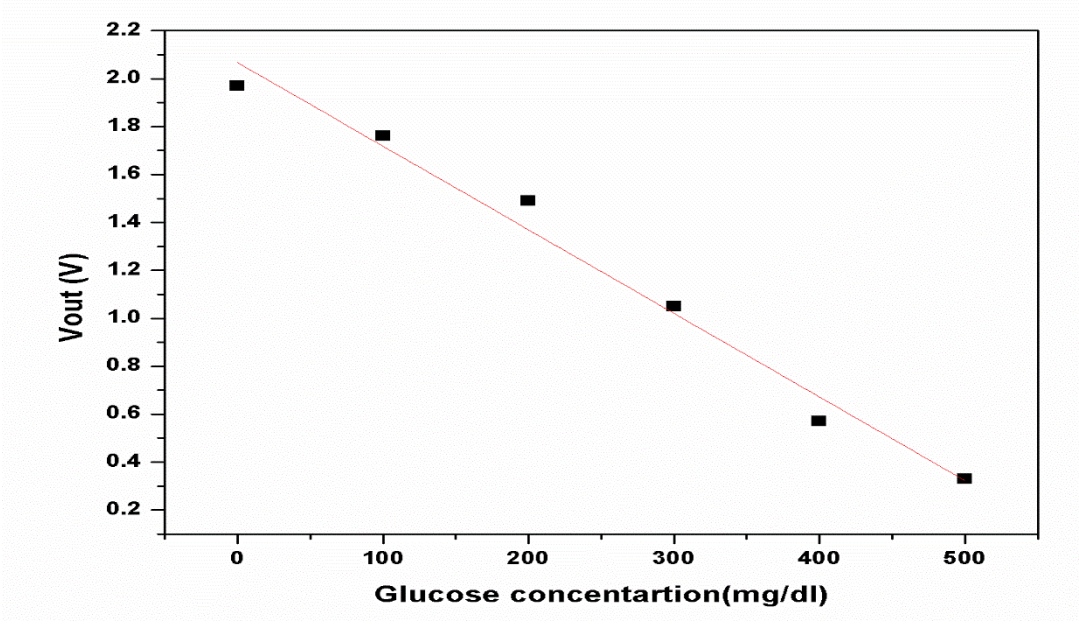


Fig. 4.11: Calibration plot between the glucose concentration & magnitude of voltage (**R_2 =0.978**)

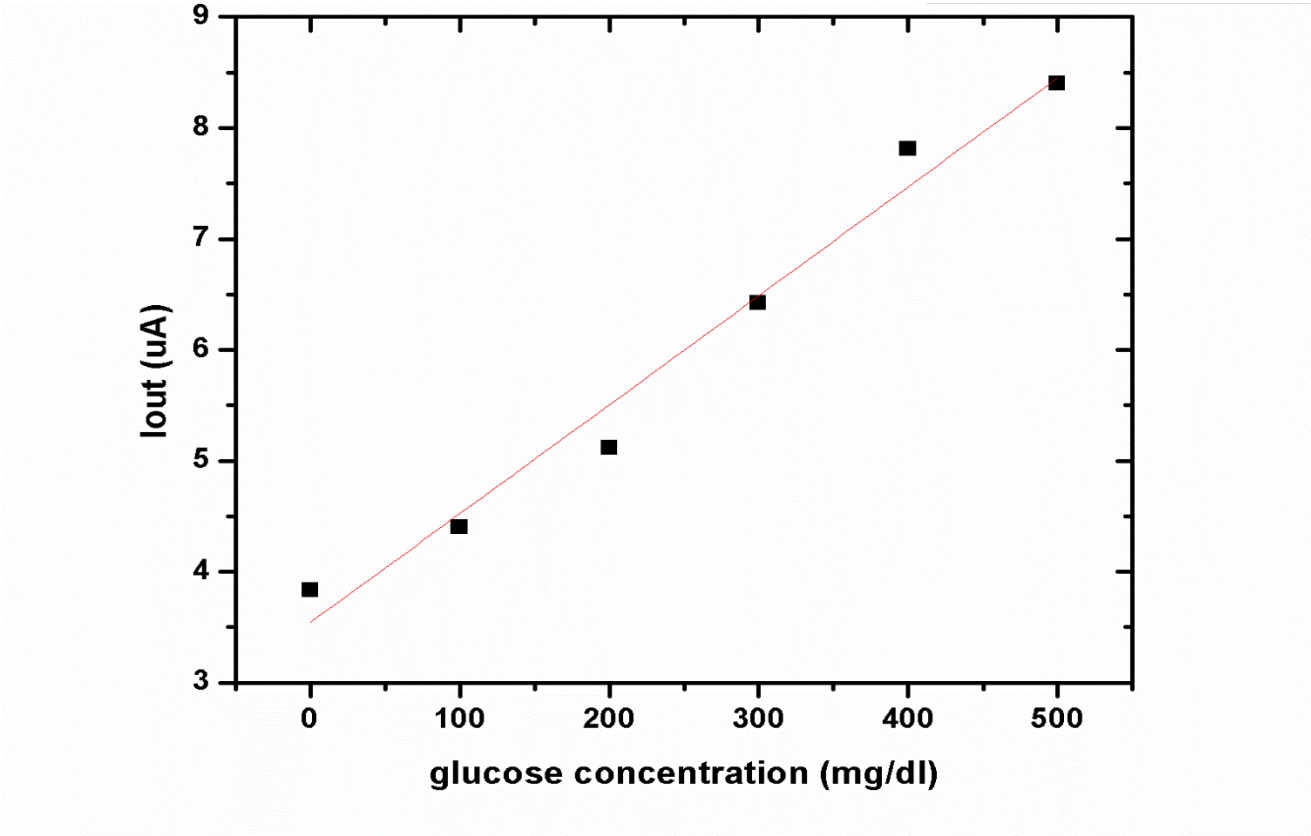


Fig. 4.12: Calibration plot between the glucose concentration & output current(**R_2 =0.978**)

CONCLUSION

We have customized a miniaturized electrcochemical system consisting of LMP91000 potentiostat as a transducer and Arduino mega ADK to develop a portable biosensing device .The Arduino environment has been used for deposition of PANI and glucose measurements using GOx immobilized glass ITO electrode. Due to its small size and fast performance, this device gives enormous potential as a widely available, point of-care diagnostic platform, especially in rural and remote areas. In addition to its influence on global healthcare, this technology is pertinent to other important applications including environmental monitoring, food safety and bio-security.

FUTURE PERSPECTIVE

Smart research on new sensing ideas has opened the door to a prevalent clinical applications of electrochemical devices. Such devices are extremely beneficial for delivering the diagnostic info in a simple, fast, and low budget fashion, and are thus uniquely qualified for meeting the demands of point-of-care transmission. The attractive properties of electrochemical devices are thus extremely auspicious for improving the efficiency of therapy monitoring and diagnostic testing, and for point-of-care testing, in general. The main defy is to convey electrochemical techniques to the patient's side for use by non-laboratory personnel without compromising reliability and accuracy. The recognition of decentralized electronic testing of diseases would thus need additional all-encompassing developmental work. It is expected that the imagination of biomedical engineers, material scientists and electrochemists and, coupled with proper resources, will revolutionize disease diagnostics such as cholesterol, hemoglobin, cancer screening etc.

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