Design, Development and Testing of a Mini Potentiostat for E-scalpel Application

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Dedication

I would like to dedicate this work to many people I love. Particularly, my father, mother, sister, and husband without whose sacrifice it would not be possible.

Acknowledgements

I would like to express my heartiest gratitude to my supervisor Dr. Sharmistha Bhadra for imparting her guidance, advice, and wisdom throughout the whole time of my master's studies. I am very thankful to her for not only giving me the opportunity to study in McGill University under her kind supervision but also providing me scholarship to carry out my research. I would also like to thank Dr. Geraldine Merle for allowing me to conduct my experimental works in Montreal General Hospital.

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Abstract

With the increasing need of sensors for disease detection, electrochemical sensors have been designed, developed, and miniaturized to perform testing for medical diagnostics. Numerous experiments have been carried out to produce market competitive biosensors. This research work is focused on designing the electronic reader part of a e-scalpel for oral cancer detection. The e-scalpel will have a 3-electrode electrochemical sensor connected to a mini-potentiometer situated in the handle of the scalpel. The objective of the work is design and implement a mini-potentiostat that can send data wirelessly to a base station.

The experimental work involves designing and implementing a system on board to be used as a potentiostat for a 3-electrode system in a tris-HCl solution with Zn protein. The potentiostat is responsible to carry out Cyclic Voltammetric (CV) and Chronoamperometric (CA) analysis by providing required voltage values to a 3-electrode sensing system. The potentiostat is provided command through a computer system by means of a microcontroller and DAC. A wireless Bluetooth module does the output reading for CV and CA analysis by sending it to serial monitor of a PC.

Initially the system was developed on breadboard with through hole components to validate the performance of the designed circuit and its components. Once the functionality of breadboard of circuit conformed that desired CV and CA curves are obtained, the design is transferred into the PCB format to develop the mini-potentiostat. The mini-potentiostat is able to generate CV and CA curves required for measurements with 3-electrode electrochemical sensors. In future the battery of the system will be miniaturized and the mini-potentiostat will be integrated with an e-scalpel for oral surgeon. The integration of electronic reader and chemical sensor to provide convenient biomedical usage is achieved with this work.

Abrégé

Avec la croissance rapide des capteurs, les capteurs électrochimiques ont été conçus, improvisés et miniaturisés pour effectuer des tests de diagnostic médical. De nombreuses expérimentations ont été menées pour produire des biocapteurs compétitifs sur le marché. Ce travail de recherche est axé sur la conception de la partie lecteur électronique d'un e-scalpel pour la détection du cancer de la bouche. L'e-scalpel aura un capteur électrochimique à 3 électrodes connecté à un mini-potentiomètre situé dans le manche du scalpel. L'objectif du travail est de concevoir et de mettre en œuvre un mini-potentiostat qui peut envoyer des données sans fil à une station de base.

Le travail expérimental consiste à concevoir et mettre en œuvre un système embarqué destiné à être utilisé comme potentiostat pour un système à 3 électrodes dans une solution de tris-HCl avec protéine Zn. Le potentiostat effectue une analyse voltamétrique cyclique (CV) et chronoampérométrique (CA) en fournissant les valeurs de tension requises à un système de détection à 3 électrodes. Le potentiostat est controlé par un système informatique au moyen d'un microcontrôleur et d'un DAC. Un module Bluetooth sans fil diffuse les resultats de l'analyse CV et CA au moniteur série d'un PC

Initialement, le système a été développé sur une maquette avec des composants à trous traversants pour valider les performances du circuit conçu et de ses composants. Une fois que la fonctionnalité de la maquette du circuit est conforme aux courbes CV et CA souhaitées, le circuit electronique est transférée au format PCB pour développer le mini-potentiostat. Le mini-potentiostat est capable de produire les courbes CV et CA nécessaires aux mesures avec des capteurs électrochimiques à 3 électrodes. A l'avenir, la batterie du système sera miniaturisée et le mini-potentiostat sera intégré à un e-scalpel pour chirugie buccal. L'intégration d'un lecteur électronique et d'un capteur chimique pour fournir une utilisation biomédicale pratique est réalisée avec ce travail.

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List of Acronyms

CV	Cyclic Voltammetry
CA	Chrono Amperometry
WE	Working Electrode
RE	Reference Electrode
CE	Counter Electrode
SCE	Saturated Calomel Electrode
GCE	Glassy Carbon Electrode
PCB	Printed Circuit Board
TH	Through Hole
SMD	Surface Mount Devices
SMT	Surface Mount Technology
ADC	Analog-to-Digital Converter
DAC	Digital-to-Analog Converter
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic acid
IDE	Integrated Development Environment
DIP	Dual in-line package
SOIC	Small Outline Integrated Circuit
TQFP	Thin Quad Flat Pack
ISP	In-System-Programmer
USB	Universal Serial Bus
GUI	Graphical User Interface
EM	Electromagnetic

List of Quantities

Frequency	kHz
Current	nA
Supply Voltage	V
Length	mm
Scan Rate	mV/s
Applied Potential	V
Input Voltage	mV
Input Resistor	kΩ
Feedback Resistor	kΩ
Capacitance	µF
Supply Current	mA
Quiescent Current	µA
Output Current	A
Power Supply	mAh
Clock Speed	MHz

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CHAPTER 1: Introduction and Literature Review

1.1 Electrochemical Sensors

Electrochemical Sensors are popular for detecting and monitoring various parameters of daily life. The sensing system serves health and environmental inspection by responding to physical movement or chemical ion. The electrical response of a sensor results in a signal which is primarily monitored based on electrical potential, current and resistance [1].

Electrochemical biosensor provides information about the chemical nature of its environment which are used in various medical, biological, and biotechnological applications. Sometimes the conversion of biological information into electrical signal is challenging due to the complexity of connecting an electronic device directly to a biological environment [2]. Electrochemical biosensors efficiently analyze the content of a biological sample due to the direct conversion of a biological event to an electronic signal.

1.2 Types of Electrochemical Sensors

There are mainly three types of electrochemical sensors: potentiometric, voltammetric and amperometric sensors. The suitable sensor for an experiment can be chosen mainly depending on output range, target object, control interface and sensor size.

1.2.1 Potentiometric Sensors

In potentiometric sensors information about the composition of a sample is obtained from the potential difference between two electrodes. There are three basic types of potentiometric devices: ion- selective electrodes (ISEs), coated wire electrodes (CWEs) and field effect transistors (FETs) [25,26]. ISEs typically use an ionophore as the sensing platform to ensure selectivity toward a specific ion of interest. In the classic configuration, the electrodes are mainly membrane-based devices with ion-conducting materials that separate the sample from the inside of the electrode. One electrode is the working electrode whose potential is determined by its environment. The other electrode is a reference electrode whose potential is fixed by a solution containing the ion of interest at a constant activity as shown in Figure 1.1.

In the classical Coated Wire Electrodes (CWEs) design, a conductor is directly coated with an appropriate ion-selective polymer membrane (usually polyvinyl chloride, polyvinyl benzyl chloride) or polyacrylic acid) to form an electrode system that is sensitive to electrolyte concentrations [27]. The CWE response is similar to that of classical ISE, with regard to detectability and range of concentration. The great advantage is that the design eliminates the need for an internal reference electrode, resulting in benefits during miniaturization.

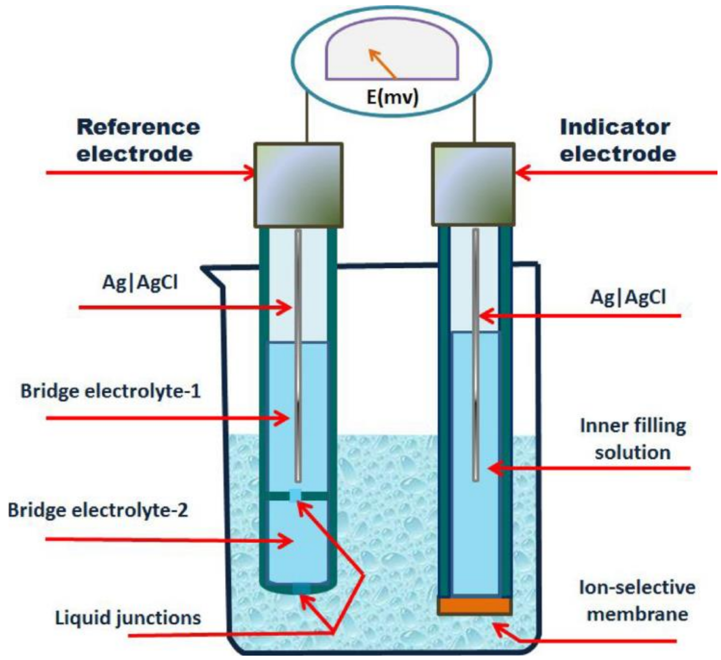

Figure 1. 1: Potentiometric cell assembly with a conventional, liquid inner contact ion selective membrane electrode as indicator electrode and a double junction reference electrode

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1.2.2 Voltammetric Sensors

Voltammetry is the study of current as a function of applied potential. The basic principle of voltammetry is to apply a known potential to an electrode and measure current generated by the redox operation of analyte. Several types of experiments may be performed to gather information from voltammetry including cyclic voltammetry, square wave voltammetry, and stripping voltammetry to name a few common techniques. Recent review articles have focused on specific applications or methods for voltammetric sensors.

Voltammetric sensors with laterally placed working electrode are an interesting alternative to classic electrodes since they offer enhanced performance in electroanalysis. Their characteristics include easier preparation for measurement, removal of interferences and gas bubbles, amplification of mass-transport, and possibility of miniaturization and automation. Porada et al. did custom designing of cyclically renewable, silver, gold, bismuth, glassy carbon, ceramic and amalgam annular band, bi-band, ring, and multidisc electrodes [28]. They represent the current trends in electroanalysis, aimed at reducing of amount of the used toxic electrode materials, like mercury and its compounds, and organic solvents used for a sample preparation.

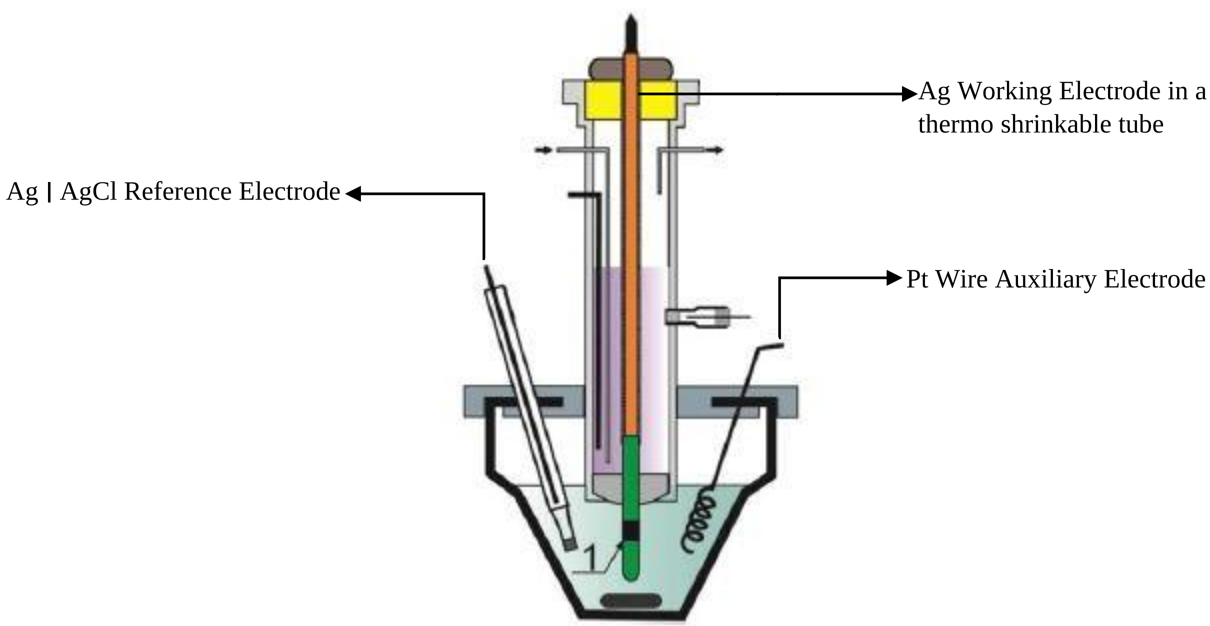

Figure 1. 2: Three electrodes in a voltammetric sensor after functioning of the renewable silver (Ag) electrode.

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Their presented mercury-based electrodes turned out to be useful in determination of variety of inorganic and organic species by means of voltammetry and tensammetry. The measurements had been conducted in a typical three-electrode cell with Pt wire as an auxiliary electrode, the Ag |

AgCl | KCl reference electrode and MF-AgSAE, AgLAF-AgSAE or RAgLAFm-E as a working electrode which was prepared in their laboratory. The influence of various inorganic cations (Cu^{2+}, Cd^{2+}, Co^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, Tl^{+}, etc), SAS, and humic and fulvic acids in great excess with respect to the analyte was examined [29]. For each sensor type, they renovated the mechanical and electrochemical surface before measurement based on the voltammetric and tensammetric curves. Particular attention was put on electrochemical characteristics of the sensors and their application for determination of trace amounts of metal ions and their complexes, organic compounds, and surface-active substances by means of tensammetry, and anodic, cathodic, adsorptive and catalytic adsorptive stripping voltammetry.

1.2.3 Amperometric Sensors

Amperometric measurements are made by recording the current flow in the cell at a single applied potential. Just like voltammetric sensors, the essential operational feature of amperometric devices is the transfer of electrons to or from the analyte. During an amperometric measurement, the working electrode, or sensor, is held at a constant potential while the current is monitored. The current is then related to the concentration of the analyte present [6,7].

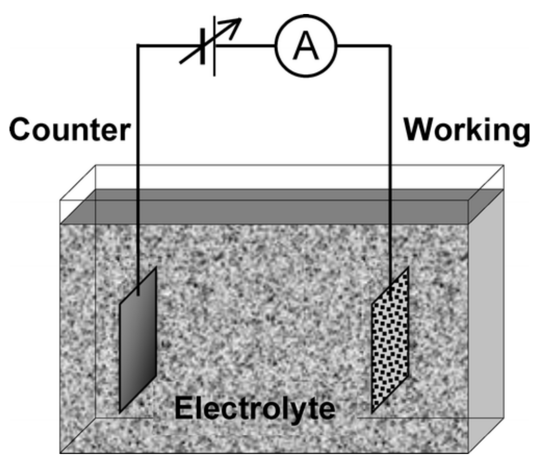

Figure 1. 3: Amperometric sensor with two-electrode configuration for liquids

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The basic instrumentation requires controlled-potential equipment, and the electrochemical cell consists of two electrodes immersed in a suitable electrolyte as shown in Figure 1.3. The performance of amperometric sensors is strongly influenced by the working electrode material. Consequently, much effort has been devoted to electrode fabrication and maintenance. Mercury was very attractive as an electrode material for many years because it has an extended cathodic potential range window, high reproducibility, and a renewable surface. The hanging mercury drop electrode or mercury film electrode was the most popular working electrode for stripping analysis [6]. Solid electrodes (carbon, platinum, gold, silver, nickel, copper, dimensionally stable anions) have been very popular as electrode materials because of their versatile potential window, low background current, low cost, chemical inertness, and suitability for various sensing and detection applications.

Fortunately, the miniaturization of the working electrode has gained much attention and microelectrodes were developed with dimensions not greater than 2 μm, increasing the possibility of in vivo and in vitro measurements with small apparatus. This resulted in a predictable boon for the development of amperometric sensors for real sample analysis. An example of this advance was the development of biosensors. They are capable of permitting a bio specific reagent, immobilized or retained at a suitable electrode, to convert the biological recognition process into a quantitative amperometric response.

1.3 Three Electrode Configuration

For electrochemical testing setup, the electrochemical sensors require three electrodes: working electrode (WE), reference electrode (RE) and counter electrode (CE) or auxiliary electrode. Each electrode has an own unique purpose to the system.

1.3.1 Working Electrode

The Working Electrode is the electrode where the potential is controlled, and current is measured. For many electrochemical experiments, the WE serves as an inert material. It is the medium of transportation for the electrons, along with which the 3-electrode system eventually generates the redox reaction inside the electrochemical cell.

High quality working electrodes like platinum and gold electrodes are perfect for use in standard cyclic voltammetry electrochemistry cells [4,5]. Due to their resistance to oxidation in both air and acids, platinum and gold disc working electrodes are widely used in standard cyclic voltammetry electrochemistry cells to investigate the oxidation and reduction potentials of organic and inorganic semiconductors. Organic semiconductors such as polymers in solution can be drop-cast onto the disc to form films, or alternatively the electrochemical cell can be set up to measure the properties of a material in solution. Both platinum and gold disc working electrodes are easy to use and clean.

On the other hand, Glassy Carbon Electrode (GCE) has a very homogeneous surface. The same electrode can be used again and again with polishing [9]. GCE is cheaper than gold electrode. The very impressive mechanical properties and chemical resistance of GCE are a major advantage for an electrode in a lab, but a major disadvantage in using it in any kind of scaled down and mass-produced electrochemical sensor. So, it is a chemically stable electrode for small size electrochemical cells despite its relatively large over-potentials of oxygen and hydrogen evolutions which creates problem in mass level operation. Figure 1.4 shows some of the working electrodes used frequently in labs.

(a)

(b)

Figure 1. 4: (a) Platinum and Gold Electrodes (b) Glassy Carbon Electrode Rod

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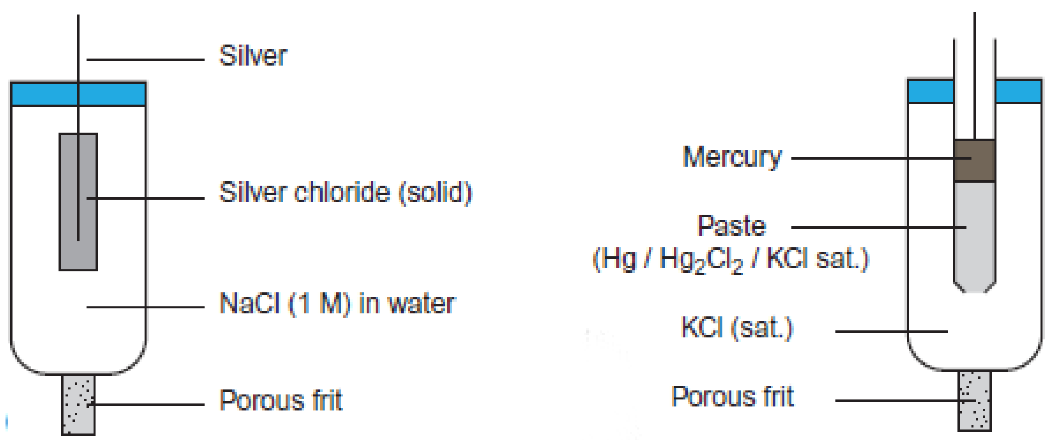
1.3.2 Reference Electrode

The Reference Electrode (RE) is used to measure the WE potential. RE should have a constant electrochemical potential as long as no current flows through it. Since the reference electrode passes negligible current, the voltage-drop between the reference and working electrode is often very small [10]. Thus, with the three-electrode system, the reference potential is much more stable, and there is compensation for voltage drop across the solution.

The most common lab Reference Electrodes are the saturated calomel electrode (SCE) and the silver/silver chloride (Ag/AgCl) electrodes as shown in Figure 1.5. SCE is based on the reaction between elemental mercury and mercury chloride, filled with saturated potassium chloride (36% w/w). On the other hand, Ag/AgCl is based on the reaction between elemental silver and silver chloride. It is filled with saturated potassium chloride (36% w/w).

Both of the chloride solutions are enclosed in electro porous KT glass frit/junction with black protective cover for glass frit. Ag/AgCl may be preferred in some cases where use of mercury, which is toxic, should be avoided. However, Ag/AgCl tends to react more with other ions. Because reference systems based on Ag/AgCl can be fabricated on a small scale, they are often used in integrated micro or miniature implantable systems.



(a) (b)

Figure 1. 5: (a) silver-silver chloride electrode and (b) saturated-calomel electrode

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1.3.3 Counter (Auxiliary) Electrode

The Counter, or Auxiliary, Electrode is a conductor that completes the cell circuit. The CE in lab cells is generally an inert conductor like platinum or graphite. In field probes, it is generally another piece of the WE material. The current that flows into the solution via the WE leaves the solution via the CE [12]. The electrodes are immersed in an electrolyte (an electrically conductive solution). The collection of the electrodes, the solution, and the container holding the solution are referred to as an electrochemical cell.

Oxidation, solvent, and acid resistant platinum wire counter electrodes are widely used in standard cyclic voltammetry electrochemistry cells. Platinum is a good choice of material for a counter electrode mostly due to its inertness. It is important for all electrochemical methods that the counter electrode allows current to flow without causing a chemical change in the solution being studied, as this would alter the results of the experiment or measurement. Figure 1.6 shows platinum wire with different structures.

Figure 1. 6: Platinum wire Counter Electrode in different forms.

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1.4 Potentiostat Fundamentals

A potentiostat is an electronic instrument that controls the voltage difference between a WE and a RE and it does not measure the current between CE and WE. All electrodes are contained in an electrochemical cell. Figure 1.7 shows a commercial potentiostat with electrodes positioned at different probes. The potentiostat implements this control by injecting current into the cell through a CE. The controlled variable in a potentiostat is the cell potential and the measured variable is the cell current.

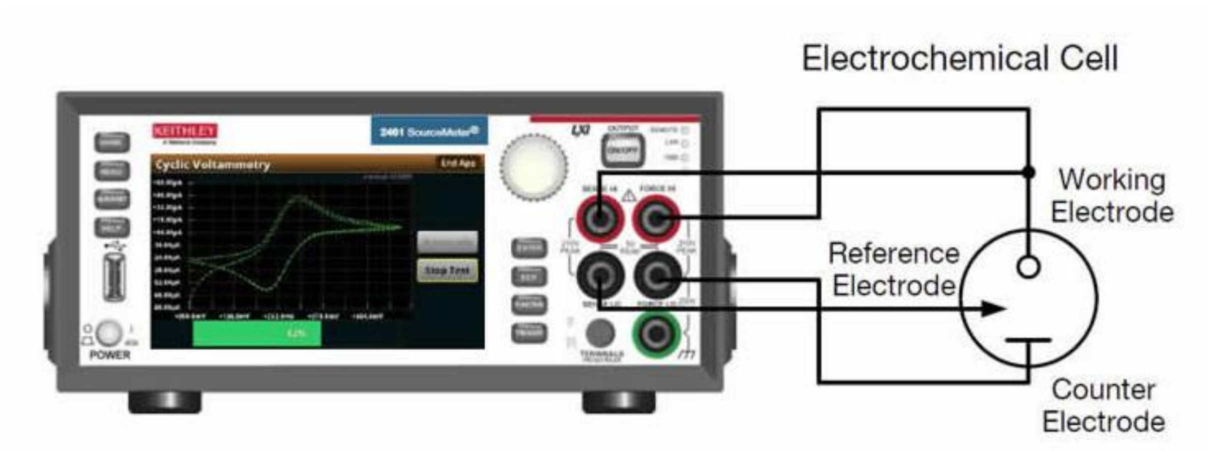

Figure 1. 7: Keithley's 2461-EC connected to 3-electrode cell.

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A basic potentiostat can be modeled as an electronic circuit consisting of four components: the electrometer, the I/E converter, the control amplifier, and the signal. The schematic representation of a potentiostat with these components is shown in Figure 1.8.

1.4.1 The Electrometer

The electrometer circuit measures the voltage difference between the reference and working electrodes. Its output has two major functions: it is the feedback signal in the potentiostat circuit, and it is the signal that is measured whenever the cell voltage is needed. An ideal electrometer has infinite impendence and zero current. In reality, the reference electrode passes a very small amount of current. Current through the reference electrode can change its potential, but this current is usually so close to zero that the change is negligible. The capacitance of the electrometer and the resistance of the reference electrode form an RC circuit. If the RC time constant is too large it can limit the effective bandwidth of the electrometer. The electrometer bandwidth must be higher than the bandwidth of all other components in the potentiostat.

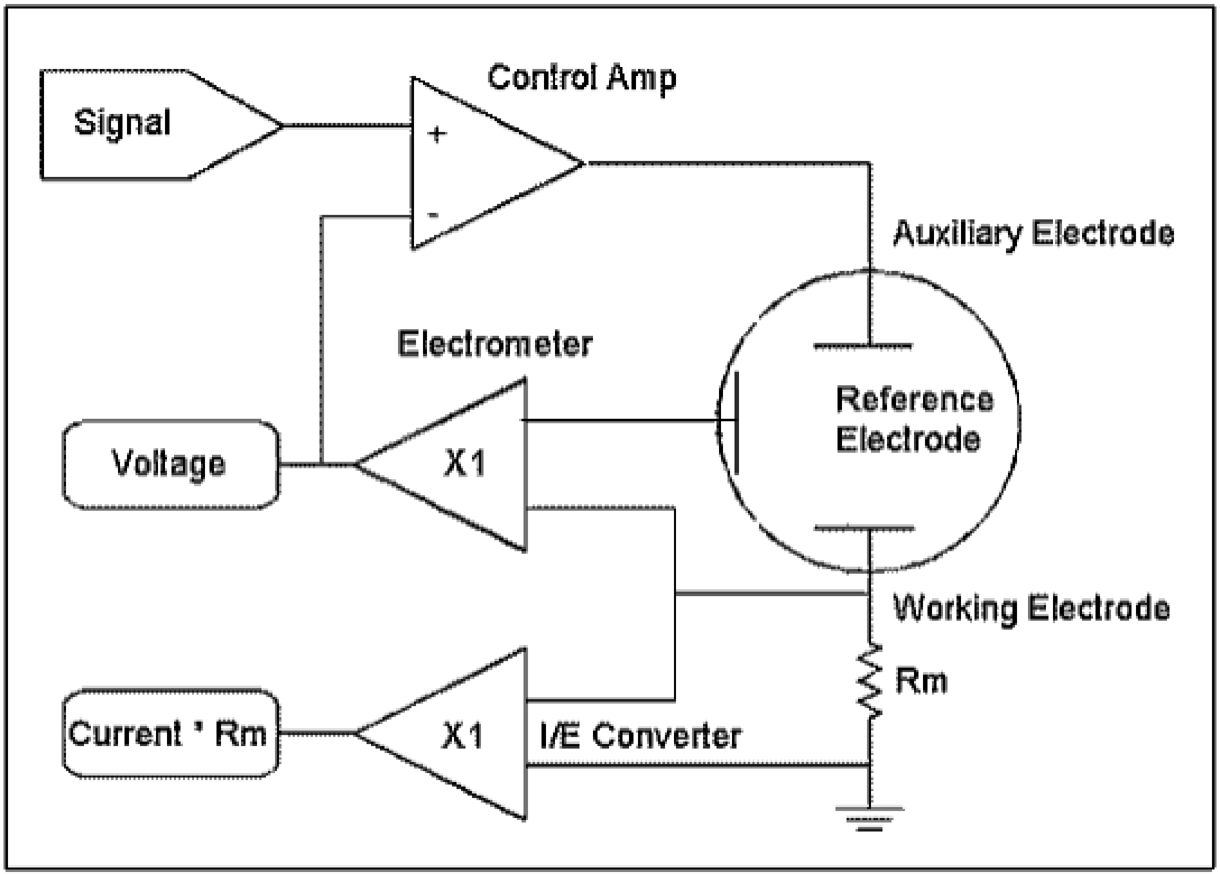

Figure 1. 8: Schematic representation of a three-electrode controlled potential apparatus. X1 on an amplifier indicates it is a unity gain amplifier.

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1.4.2 The I/E Converter

The current to voltage converter measures the cell current. The cell current is forced through a current measurement resistor, Rm. The resulting voltage across this resistor is a measure of cell current. During the course of an experiment, cell current can change by several orders of magnitude. Such a wide range of current cannot be accurately measured by a single resistor. Modern potentiostats have a number of Rm resistors and an “I/E autoranging” algorithm that selects the appropriate resistor and switches it into the I/E circuit under computer control. The bandwidth of the I/E converter depends strongly on its sensitivity. Unwanted capacitance in the I/E converter along with Rm forms an RC circuit. In order to measure small currents, Rm must be sufficiently large. This larger resistance, however, increases the RC time constant of the circuit limits the I/E bandwidth. For instance, no potentiostat can measure 10nA at 100 kHz.

1.4.3 The Control Amplifier

The control amplifier compares the measured cell voltage to the desired cell voltage and drives current into the cell to force these voltages to be the same. The control amplifier works on the principle of negative feedback. The measured voltage enters the amplifier in the negative or inverting input. Therefore, a positive perturbation in the measured voltage creates a decrease in the control amplifier output, which counteracts the initial change. The control amplifier has a limited output capability, for the PARSTAT is 10 V and 200 mA.

1.4.4 The Signal

In modern potentiostats, the signal circuit is a computer-controlled voltage source. Proper choice of number sequences allows the computer to generate constant voltages, voltage ramps and sine waves at the signal current output.

1.5 Motivation and Objective

Electrochemical detection with a small system on board has become more popular in the past few years for biomedical detection and diagnosis. Ghanim and Abdullah designed to produce a low-cost Lab-on-a-chip device for electroactive diagnosis by performing standard DNA ladder results [61]. They presented a disposable microchip fabricated using commercially available PCB substrate. Jolly et al. developed a fully integrated Lab-on PCB device by utilizing peptide nucleic acid (PNA) as a probe for novel genetic analysis [62]. They addressed issues in conventional DNA systems, understanding how high-performance assays could be employed in commercially fabricated sensing microelectrodes.

The aim of this research is to design a mini-potentiostat that can be integrated into an electronic scalpel for non-invasive oral cancer detection. The electronics scalpel will have a 3-electrode electrochemical cell which will be fabricated on the blade of scalpel. The handle of the scalpel will contain the mini-potentiostat which will be connected to the 3 electrodes on the blade. Currently a team at MUHC (Spell out) is working on the 3-electrode system for oral cancer detection and have a bulky electrode system. The signal from oral saliva induces chemical reaction to 3-electrode system which is responsible for the Voltammetric or Amperometric changes. The work in this thesis uses their bulky electrode system. In future once they develop a blade with the electrode system that can be easily integrated with the mini potentiostat to complete the e-scalpel.

As high-precision instrumentation is becoming more portable and cost-effective, the healthcare industries are making more compact and small size testing devices for faster diagnosis at home. They save a lot of time and reduce damage as early detection of disease is always much more curable. The compact testing kits are built for accuracy and rapid detection of a specific condition from human sweat, saliva, or urine [15]. This research work is concentrated to build a potentiostat circuitry and then test it with the 3-electrode system. When the through-hole circuitry works properly, then it moves to the miniature PCB version.

Oral cancer occurs due to uncontrolled growth of cells in the mouth and is currently the sixth most common cancer [16]. If undetected at an early stage, this cancer metastasizes in the body leading to death. Knowledge about cancer biomarkers has increased tremendously during the last years providing great opportunities for improving the management of cancer patients by enhancing the efficiency of detection and efficacy of treatment. Cancer biomarkers could include a broad range of biochemical entities such as nucleic acids, proteins, sugars, lipids, and small metabolites, cytogenetic and cytokinetic parameters as well as whole tumor cells found in the body fluid [17]. The monitoring of the protein IL-8 and its mRNA can detect oral cancer cells. Earlier, research works were performed taking amperometric measurements for this purpose. The electrochemical transducers employed were dual screen-printed carbon electrodes (SPdCEs) composed of two elliptic carbon working electrodes (6.3mm2 each), a carbon counter electrode and an Ag pseudo-reference electrode. Furthermore, a specific cable connector (ref. DRP-BICAC, DropSens) acted as interface between the SPdCEs and the potentiostat.

With the advancement in technology, small size potentiostats named Pocket-Stat Mini-Stat are becoming more popular. The small size and low cost of these devices allow handheld, user-friendly testing, and large-scale production. Adam et al. manufactured a MiniStat that incorporates the three key components of a potentiostat: output stage, input stage, and control/communications stage, into a single 27 mm x 20 mm footprint [18]. The device was programmed using C programming language which accurately performed chronoamperometry, cyclic voltammetry, and anodic stripping square wave voltammetry. The potential between the WE and RE was controlled by maintaining an inverse potential at the CE. The highly precise current measurement of input circuit was read by an Analog to Digital Converter (ADC) where the circuit converted the small changes of current within the electrochemical cell to a voltage. The DAC was updated to output the appropriate potential difference to the cell.

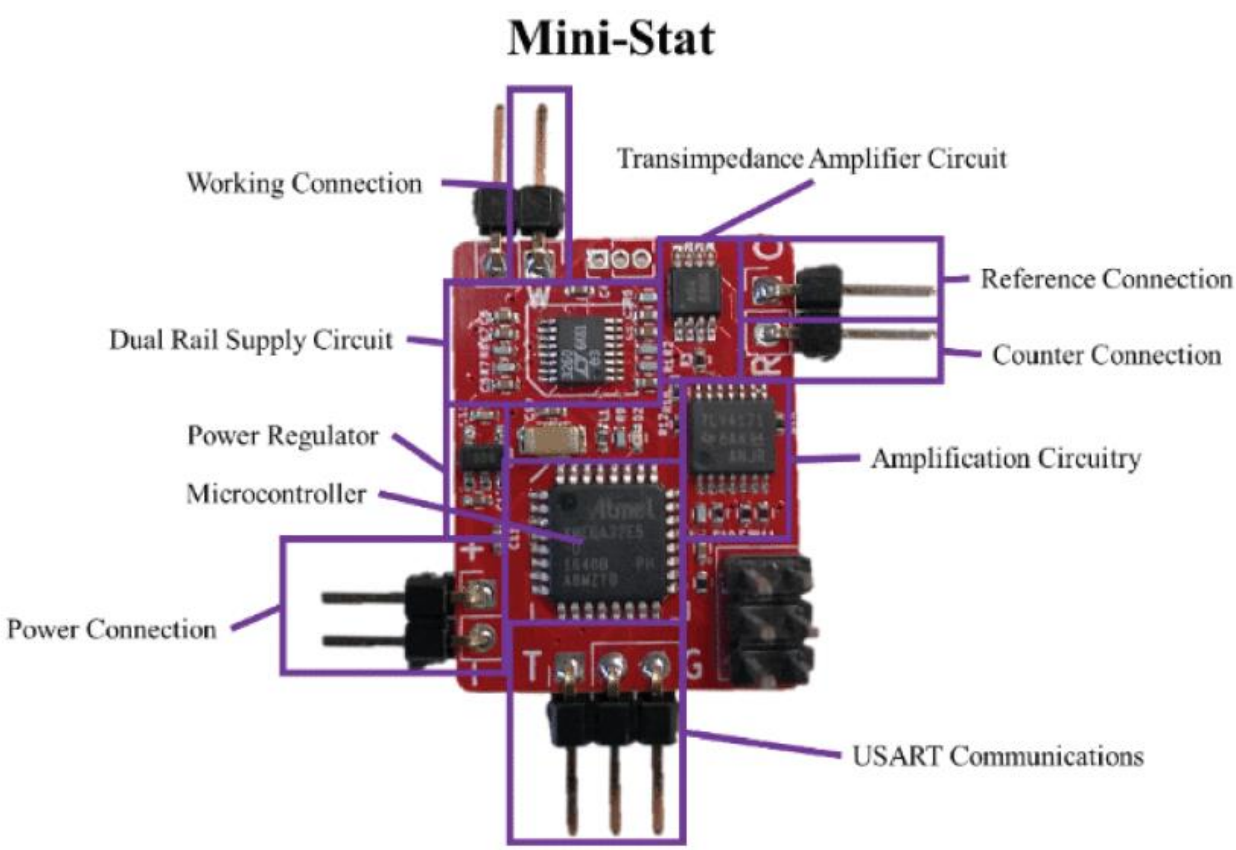

Figure 1. 9: The MiniStat device including the fabricated PCB and the populated components.

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1.6 Thesis Organization

In this chapter, different types of electrochemical sensors have been discussed. They are different by electrode and solution arrangement. So, next the 3-electrode system has been discussed broadly and how they are associated with commercial potentiostat. This chapter also explains how handheld potentiostats are used to detect oral cancer. Finally, an example of potentiostat has been shown with PCB fabrication to visualize the idea of integrated biosensor.

In Chapter 2, the main design idea for the overall system has been discussed. Here, the op-amp configurations which are responsible for constructing potentiostat circuitry are explained in detail. Then the voltage regulator diagrams are shown which will provide a stable power supply to our system. Next, the microcontroller platform, it's contribution with hardware and software parts have been discussed thoroughly with Bluetooth module.

In Chapter 3, we have implemented all the design materials of chapter 2 on a bread board system with all the Through-Hole (TH) electronic components of our biosensor system with potentiostat. At later part of this chapter, CV, CA output readings are shown with repeatability of measurements, comparison with commercial potentiostat results and reason for discrepancy with output obtained by different methods.

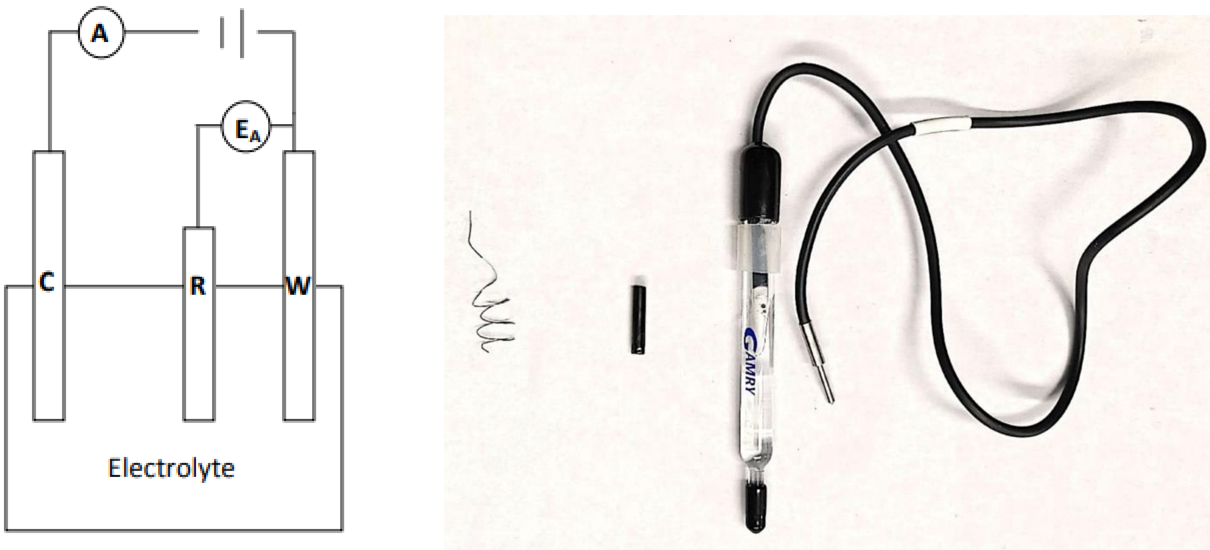
Chapter 4 explains the overall design in Altium Designer to compile the total system onto a small PCB board. The detail of soldering Surface Mount Device (SMD) components have been discussed too. Finally, Chapter 5 provides conclusion and future works.

CHAPTER 2: Design

2.1 Three Electrode Configuration

As discussed in the previous chapter, the three-electrode system consists of a working electrode, counter electrode, and reference electrode. The applied potential (E_A) is measured between the W and R and the resulting current is measured in the C lead as shown in Figure 2.1(a).

For this research work, a Saturated Calomel Electrode (SCE) has been used as RE which is a robust electrode based on the reaction between mercury and mercury chloride. It has a potential of 0.242 V in normal room temperature. Here, platinum wire and glass carbon electrode have been used as CE and WE as shown in Figure 2.1(b). Technically, they need to be manufactured from inert materials where CE size should be much larger than WE. So, it was the reason behind choosing these materials as electrodes.



(a) (b)

Figure 2. 1: (a) Simple schematic of three electrode configuration where EA is the applied voltage and C, W, and R are the counter, working and reference electrodes respectively, (b) Platinum, Glassy Carbon and SCE used as CE, WE and RE for experiment.

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2.1.1 Cyclic Voltammetry

Cyclic voltammetry (CV) is recorded by the three-electrode system where they are submerged in an analyte. In CV, a known potential is applied to a working electrode for measuring current generated by reduction or oxidation of the analyte.

In CV, the potential of the working electrode is ramped linearly versus time. The potential ramp is inverted when the working electrode's potential reaches the maximum potential programmed, forming a triangular function (Figure 2.2). In a conventional CV experiment, the system is scanned between a range of potentials in multiple in a triangular waveform manner.

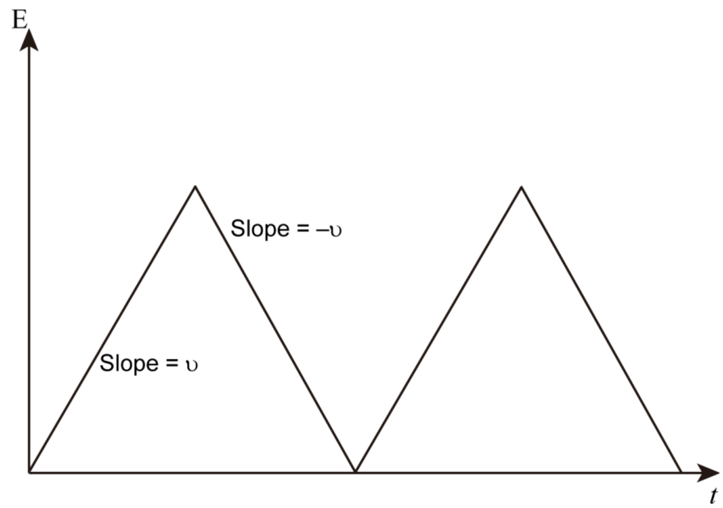

Figure 2. 2: A schematic diagram for a triangular waveform with a scan rate of υ and –υ

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The current between the working and the counter electrodes is measured and plotted vs. the applied potential between WE and RE. The current is proportional to the square root of scan rate. At a slow scan rate, the current density would result in a steady-state value. Thus, the common scan rate in a sweep voltammetric method normally ranges from 10 mV/s to 1000 mV/s.

CV measurements look at redox (reduction-oxidation) processes of an analyte in solution. Additionally, these measurements give the information if it is a reversible, quasi-reversible or irreversible process [32]. The CV curve has a duck shaped feature which can be explained by Nernst equation. Here, the reduction will not occur until the potential is sufficiently reducing. The current decreases with time as analyte near electrode is depleted.

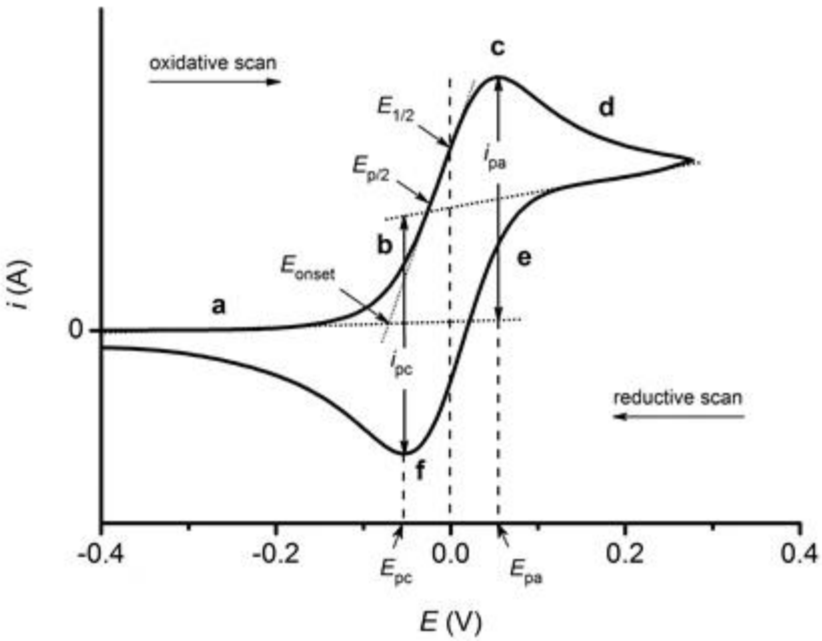

Figure 2. 3: Cyclic voltammogram for an electrochemically reversible one-electron redox process

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The 'duck-shaped' plot generated by CV is called a cyclic voltammogram. In Figure 2.3, the scan starts at -0.4V and sweeps forward to more positive, oxidative potentials. Initially the potential is not sufficient to oxidize the analyte (a). As the onset (E_{onset}) of oxidation is reached the current exponentially increases (b) as the analyte is being oxidized at the working electrode surface. Here the process is under electrochemical control with the current linearly increasing with increasing voltage with a constant concentration gradient of the analyte near the electrode surface within the diffuse double layer.

The current response decreases from linearity as the analyte is depleted and the diffuse double layer grows in size. The current reaches peak maximum at point c (anodic peak current (i_{pa}) for oxidation at the anodic peak potential (E_{pa}). The process is now under mixed control: more positive potentials cause an increase in current that is offset by a decreasing flux of analyte from further and further distance from the electrode surface.

From this point the current is limited by the mass transport of analyte from the bulk to the diffusion double layer, which is slow on the electrochemical timescale and therefore does not satisfy the

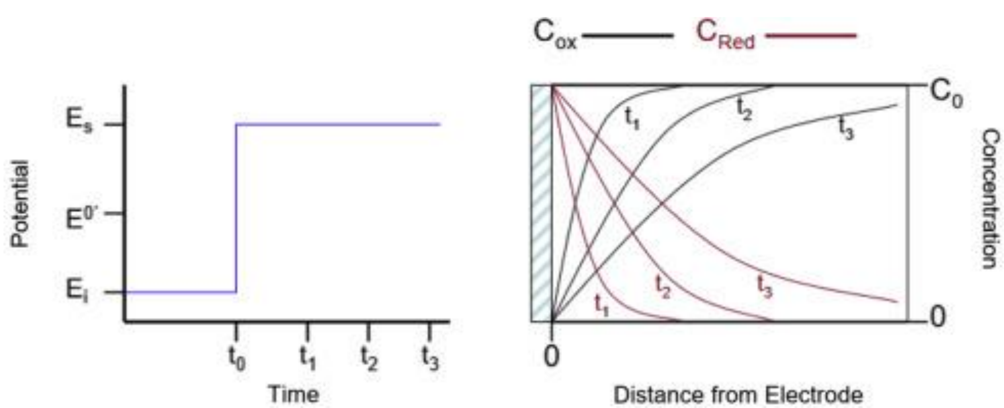
Nernst equation. This results in a decrease in current (d) as the potentials are scanned more positive until a steady state is reached where further increases in potential no longer has an effect. Scan reversal to negative potentials (reductive scan) continues to oxidize the analyte until the applied potential reaches the value where the oxidized analyte which has accumulated at the electrode surface can be re-reduced (e).

The process for reduction mirrors that for the oxidation, only with an opposite scan direction and a cathodic peak (i_{pc}) at the cathodic peak potential (E_{pc}) (f). The anodic and cathodic peak currents should be of equal magnitude but with opposite sign, provided that the process is reversible [33].

2.1.2 Chronoamperometry

Chronoamperometry (CA) is one of simplest voltammetric methods, and yet, it is one of the most frequently used voltammetric methods. CA, in its most basic form, consists of applying a single voltage step at a specific time and then measuring the current that results from the applied potential.

In CA, the intensity of the current produced after the application of a potential is measured with time. CA is a well-established amperometric technique. In this method, a pulse potential is applied to a working electrode and the current passing through the cell is determined versus time [21]. Changes in the current appear in response to rises or decreases in the diffuse layers of the analyte at the surface of the working electrode.



(a) (b)

Figure 2. 4: (a) Choosing a specific voltage for CA (b) CA curves for different time.

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Figure 2.4 (a) refers to choosing a suitable voltage for CA analysis. This figure depicts that after time t_0, the potential saturates to E_s. So, choosing a potential value close to E_s as input voltage is ideal for CA analysis. Figure 2.4 (b) shows multiple CA analysis over different period t_1, t_2 and t_3.

2.1.3 Commercial Potentiostat

To check the pattern from a commercial potentiostat, we have used VersaSTAT4 commercial potentiostat. The VersaSTAT4 can be connected with a 3-electrode system and produce Cyclic Voltammetry (CV), Chrono Amperometry (CA) curve within a specific input voltage range or time period. This 3-electrode experiment with VersaSTAT4 was done at Faculty of Medicine, McGill University in Montreal General Hospital (MGH).

(a) (b)

Figure 2. 5: (a) VersaSTAT4 setup in MGH (b) 3-electrode setup connected to VersaSTAT4.

VersaSTATs operate within the VersaStudio software package. Introduced in 2007, this software is the data acquisition program for all Princeton Applied Research potentiostats, including single channel and multichannel designs. The complete VersaStudio software provides full access to the capabilities of the VersaSTAT series and PARSTAT series of instruments [34]. With this software, flexible experiment setup can automatically sequence the potentiostatic, galvanostatic, and impedance capabilities of the VersaSTAT hardware. The results can be displayed and in a wide variety of axis formats for DC and AC experiments. Figure 2.6 shows a display window with options to start a Chronoamperometric experiment.

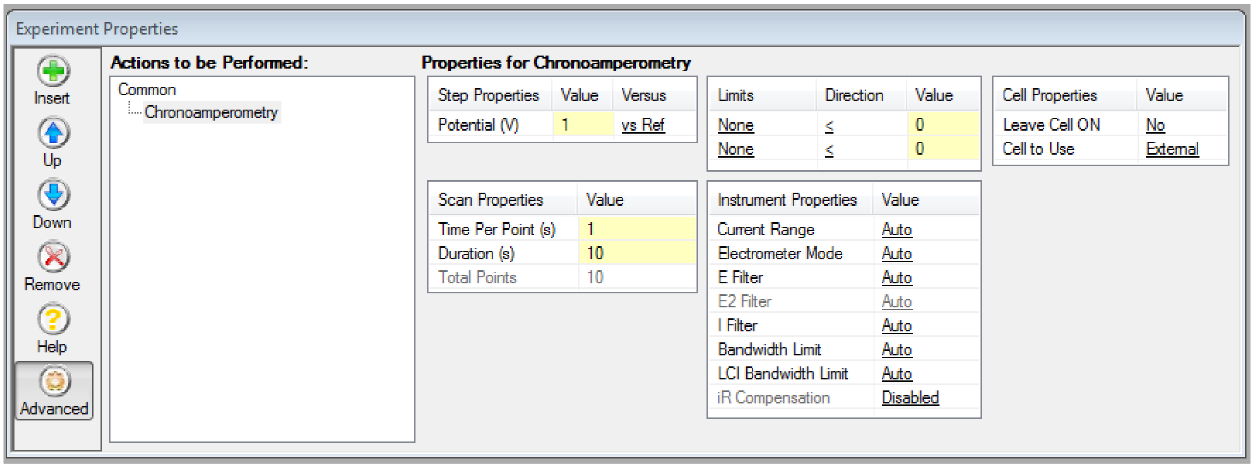


Figure 2. 6: Properties setup panel for Chronoamperometric Experiment

After setting the options for time period, scan rate, input voltage range etc. we can run the desired operation in VersaStudio. Figure 2.7 shows a CV curve within the input voltage range -1.5V to -800mV. As we mentioned from Figure 2.3, it always follows a duck shape curve which can be observed here too.

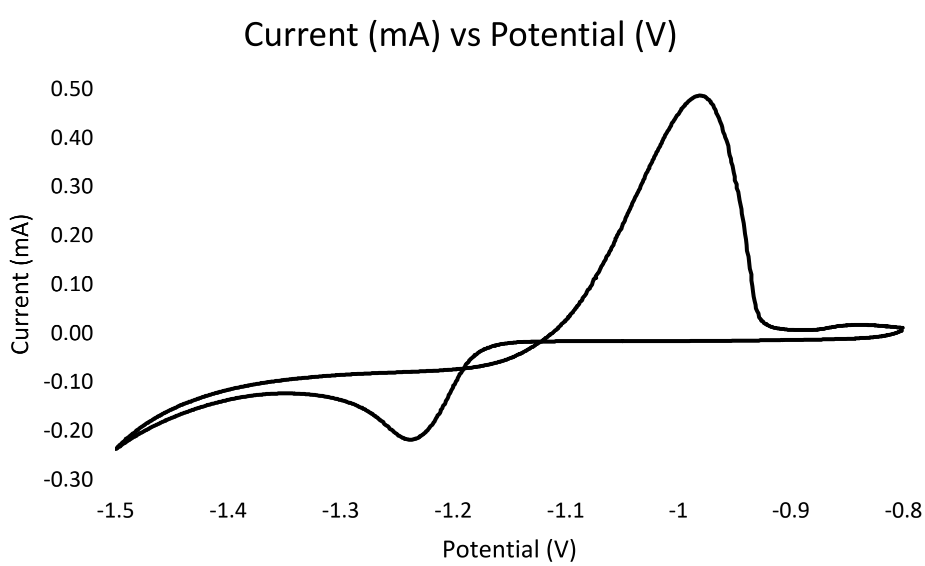
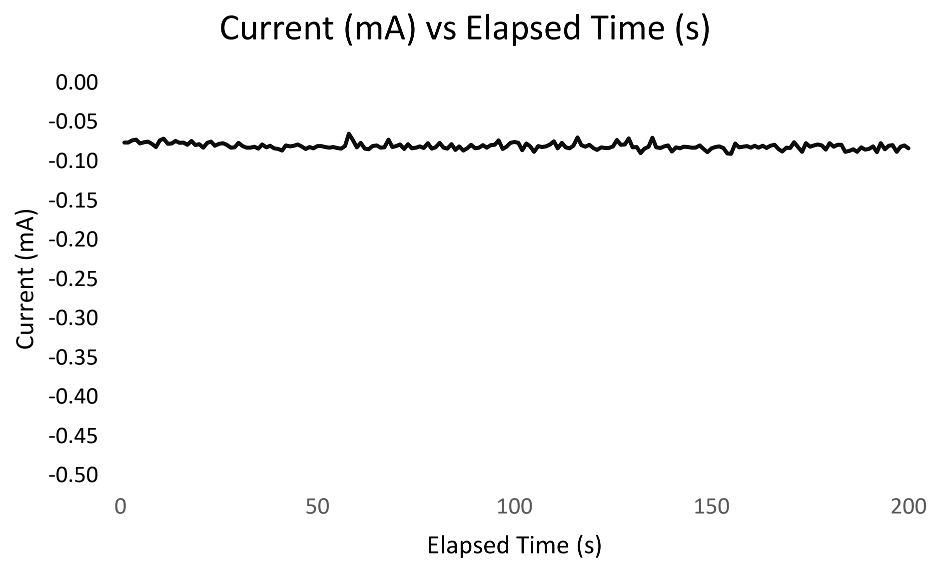
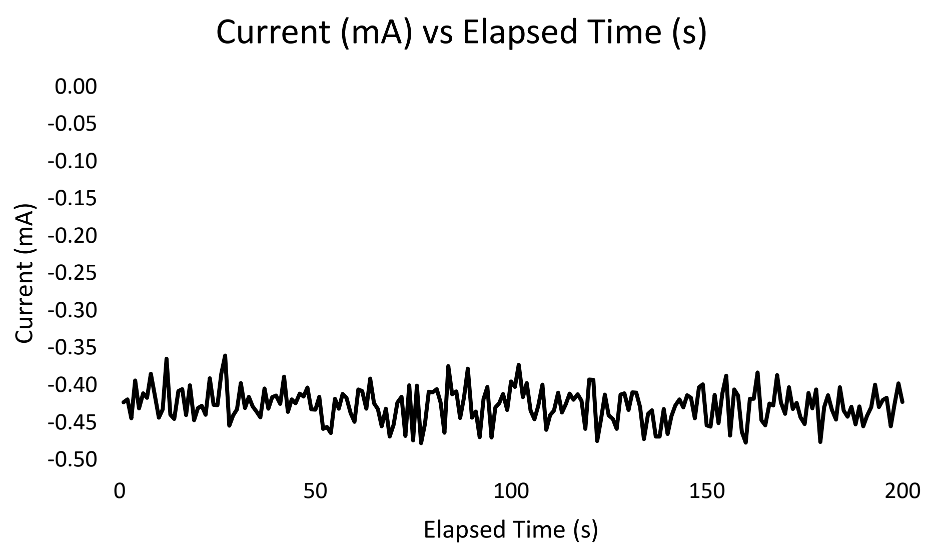


Figure 2. 7: CV curve with 3-electrodes in Tris HCl solution

Similarly Figure 2.8 shows CA curve for Tris HCl solution. For CA, the input voltage is kept constant and output current is observed within a specific time interval. After a while, the current value saturates and reaches to a constant value like the blue curve for no Zinc. The red curve with Zinc shows changes in current value due to anodic noise.



(a)



(b)

Figure 2. 8: CA curve for Tris HCl solution (a) No Zinc (b) Zinc

2.2 Overall System

This research work consisted of different modules like chemical solution-electrodes, electronic components, microcontroller, and Bluetooth module. They were introduced in the research experiments in a step-by-step manner when required. The following diagram summarizes the whole process:

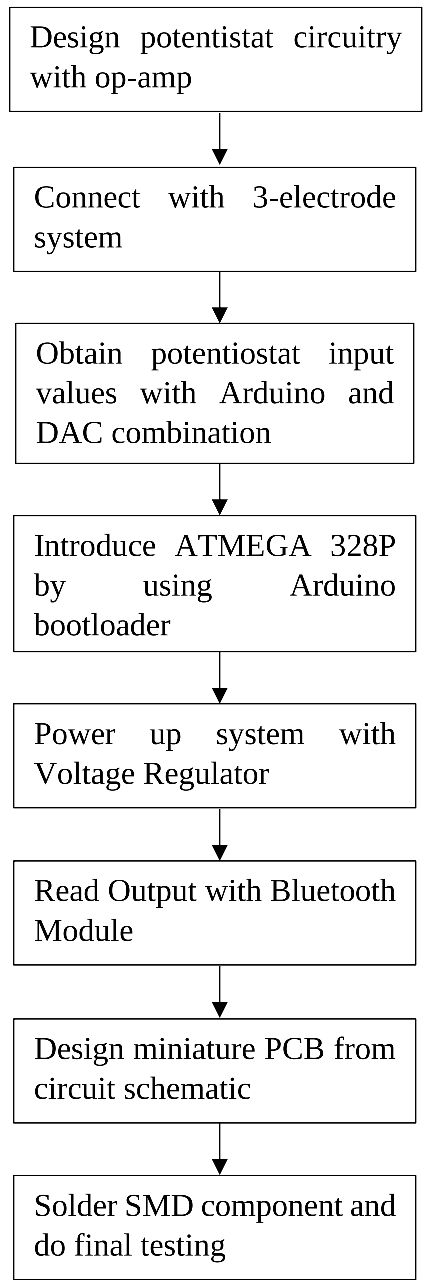


Figure 2. 9: Overall system to design biosensor.

To design our mini potentiostat, the approach first started with circuit simulation and testing on breadboard with Through-Hole (TH) electronic components. Firstly, the potentiostat circuitry was built on a breadboard with electrode connections. We got the output values by powering with a regular power supply and changing input voltage values by another power supply. After a while, we developed the design by replacing the input voltage in an integrated microcontroller environment with Arduino and DAC combination to get voltage changes at a regular interval controlled by a program from Arduino IDE. At this point, the analog readout was done with Arduino too. Also, later we did burn the bootloader and included a separate ATMEGA 328P. Next, the main power supply was replaced by a small 9V battery and voltage regulator to power up the total system. Then we introduce a pair of Bluetooth module to read the output data instead of Arduino. Afterwards, we designed this whole circuitry in Altium for the PCB prototype. Lastly, we soldered the Surface-Mount Devices (SMD) onto the PCB or FR4 board and tested output results.

2.2.1 Op-Amp Circuitry

Operational amplifiers (op amp) are used extensively in signal conditioning or filtering or to perform mathematical operations such as adding, subtracting, integration, and differentiation [35]. Op-amp is fundamentally a voltage amplifying device designed to be used with external feedback components such as resistors and capacitors between its output and input terminals. These feedback components determine the resulting operation of the amplifier. For the potentiostat circuitry, multiple op-amp with different configurations have been used. Their operation principle will be discussed in this section.

2.2.1.1 Inverting Op Amp

In the Inverting Amplifier circuit, the operational amplifier is connected with feedback to produce a closed loop operation. the output is fed back to the negative or inverting input through a resistor (R_f). The input signal is applied to this inverting pin through a resistor (R_{in}). The positive pin is connected to ground.

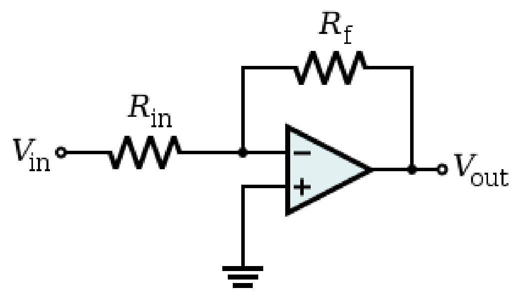


Figure 2. 10: Inverting Op-Amp

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$$V_{out} = -\frac{R_f}{R_{in}} V_{in}$$

The configuration in Figure 2.10 allows for the production of a signal that is complementary to the input. The ratio of R_f to R_{in} determines the value of V_{out} with respect to V_{in}.

2.2.1.2 Summing Op Amp

Summing amplifier can combine numbers of input signal to a single output that is the weighted sum of the applied inputs. A simple inverting amplifier can easily be modified to summing amplifier, if several input terminals are connected in parallel to the existing input terminals as shown below:

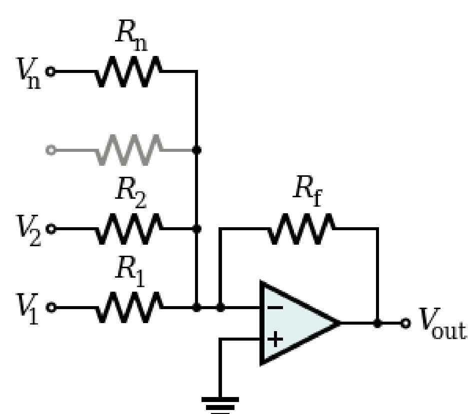

Figure 2. 11: Summing Op-Amp with n number of inputs

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$$V_{out} = -R_f(\frac{V_1}{R_1} + \frac{V_2}{R_2} + \cdots + \frac{V_n}{R_n})$$

Here, n numbers of input terminal are connected in parallel in Figure 2.11. This allows the output voltage to be easily calculated if more input resistors are connected to the amplifiers inverting input terminal.

2.2.1.3 Voltage Follower

Voltage follower is also known as unity gain buffer. From the inverting configuration, if the feedback resistor, Rf = 0 and input resistor, R2 = ∞), then the resulting circuit would have a fixed gain of “1” (unity).

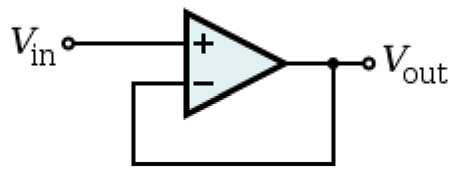

Figure 2. 12: Voltage Follower configuration with Op-Amp

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$$V_{out} = V_{in}$$

The circuit in Figure 2.12 allows for the creation of a very high impedance input and low impedance output. This is useful to interface logic levels between two components or when a power supply is based on a voltage divider.

2.2.1.4 Integrator

A particularly interesting application of op-amps is the realization of mathematical operations. Integration can be accomplished by using a typical inverting op-amp configuration as shown in Figure 2.13, but with a capacitor in the feedback path instead of a resistor. A resistor is often connected in parallel to the capacitor for saturation issues.

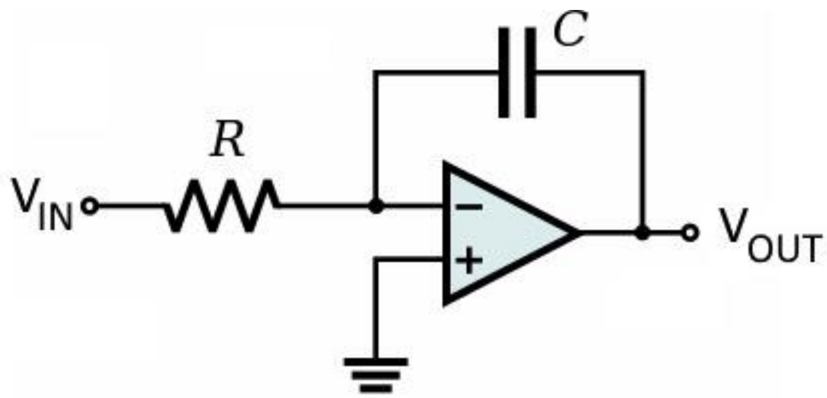

Figure 2. 13: Op-Amp as an Integrator

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$$V_{out} = -\frac{1}{RC}\int V_{in}\,dt$$

The equation refers that the output voltage of the op-amp integrator is proportional to the negative integral of the input voltage, and the constant of proportionality is the feedback capacitance multiplied by the input resistance.

2.2.2 Voltage Regulator

A voltage regulator (VR) generates a fixed output voltage of a preset magnitude that remains constant regardless of changes to its input voltage or load conditions. VRs keep the voltages from a power supply within a range that is compatible with the other electrical components. While voltage regulators are most commonly used for DC/DC power conversion, some can perform AC/AC or AC/DC power conversion as well.

There are two main types of voltage regulators: linear and switching [37]. Both types regulate a system's voltage, but linear regulators operate with low efficiency and switching regulators operate with high efficiency. In high efficiency switching regulators, most of the input power is transferred to the output without dissipation.

2.2.2.1 Linear Regulator

A linear regulator employs an active (BJT or MOSFET) pass device (series or shunt) controlled by a high gain differential amplifier. It compares the output voltage with a precise reference voltage and adjusts the pass device to maintain a constant output voltage.

Linear regulators are step-down converters, so the output voltage is always below the input voltage. They are generally easy to design, dependable, cost-efficient, and offer low noise as well as a low output voltage ripple. Linear regulators, such as the MP2018, only require an input and output capacitor to operate as shown in Figure 2.14. Their simplicity and reliability make them intuitive and simple devices for engineers and are often highly cost-effective.

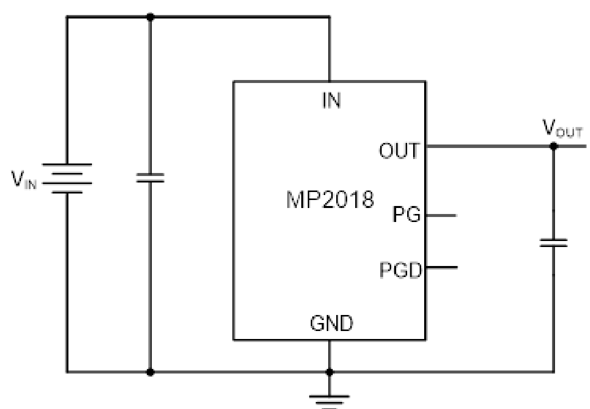

Figure 2. 14: MP2018 Linear Regulator

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2.2.2.2 Switching Regulator

A switching regulator circuit is generally more complicated to design than a linear regulator, and requires selecting external component values, tuning control loops for stability, and careful layout design. Switching regulators can be step-down converters, step-up converters, or a combination of the two, which makes them more versatile than a linear regulator. Advantages of switching regulators include that they are highly efficient, have better thermal performance, and can support higher current and wider VIN / VOUT applications. They can achieve greater than 95% efficiency depending on the application requirements. Unlike linear regulators, a switching power supply system may require additional external components, such as inductors, capacitors, FETs, or feedback resistors. The HF920 in Figure 2.15 is an example of a switching regulator.

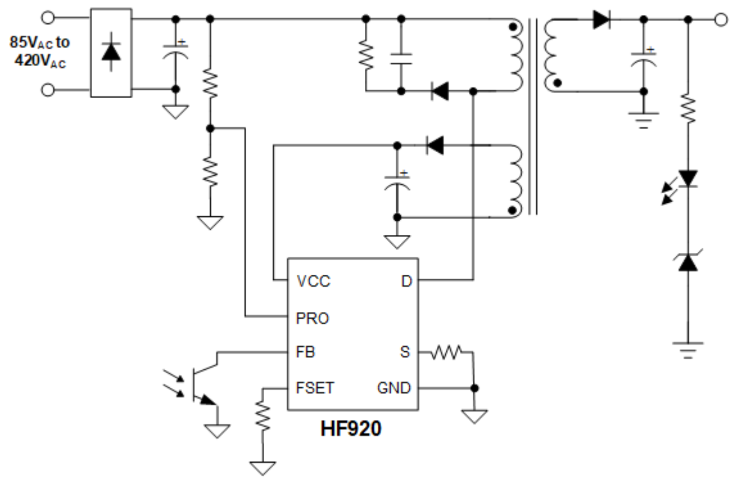

Figure 2. 15: HF920 Switching Regulator

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2.2.2.3 Positive Voltage Regulator

For this research work, step down linear regulators have been used to meet up power supply of different circuit components. So, here the output voltage is always below the input voltage. To power up Op-amp, Arduino, and DAC components a power supply of +5V is needed. We have used UA78L00 Series positive voltage linear regulators to convert 9V battery voltage into +5V.

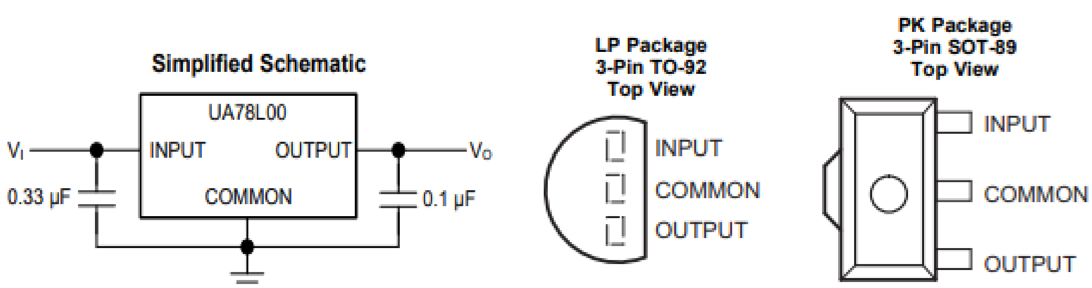

Figure 2. 16: Schematic and Package view of UA78L00 Series

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As we can see, 78L00 series voltage regulator is very easy to design and need only two simple capacitors of value 0.33 µF and 0.1 µF. One of these regulators can deliver up to 100 mA of output current. The internal limiting and thermal-shutdown features of these regulators help to protect the device from overload.

To power up our bluetooth module, a 3.3V supply is needed. The MCP1755/MCP1755S is a family of CMOS Low Dropout (LDO) voltage regulators that can deliver up to 300 mA of current while typically consuming only 68.0 µA of quiescent current. Output voltages available for the MCP1755/MCP1755S range from 1.8V to 5.5V. The LDO output is stable when using only 1 µF of output capacitance as shown in Figure 2.17.

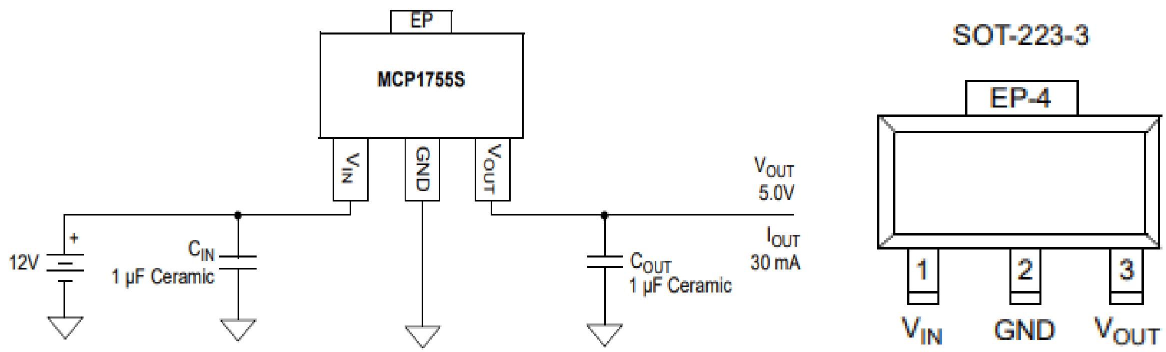

Figure 2. 17: Schematic and Package view of MCP1755/MCP1755S

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The MCP1755/MCP1755S family has a true current foldback feature. When the load impedance decreases beyond the MCP1755/MCP1755S load rating, the output current and voltage will gracefully fold back towards 30 mA at about 0V output. When the load impedance increases and returns to the rated load, the MCP1755/MCP1755S will follow the same foldback curve as the device comes out of the current foldback.

2.2.2.4 Negative Voltage regulator

The L79L series of three-terminal negative regulators employ internal current limiting and thermal shutdown, making them essentially indestructible. If adequate heat-sink is provided, they can deliver up to 100 mA output current. In our experiment, it was used to convert 9V supply to -5V for powering up op-amp and DAC. The circuitry is very simple just like UA78L00 series. It needs only two simple capacitors of value 0.33 µF and 0.1 µF for input and output respectively.

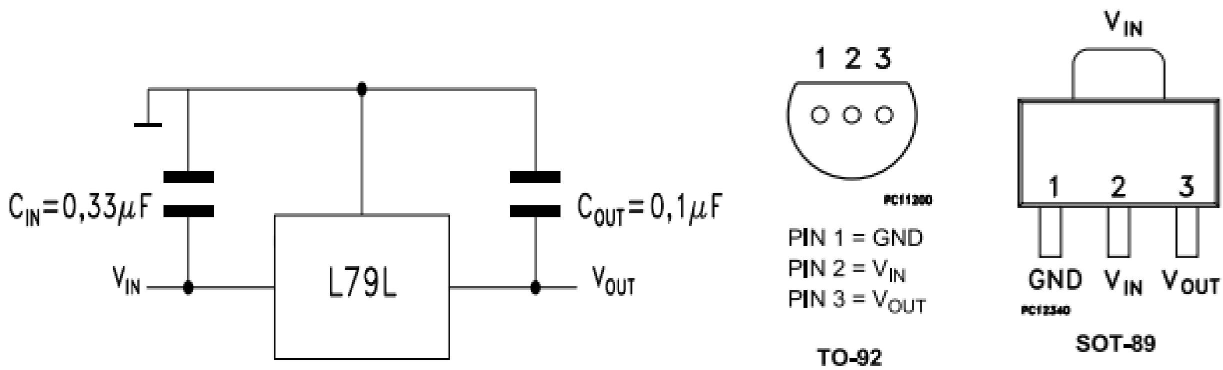

Figure 2. 18: Schematic and Package view of L79L Series

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To convert the positive battery voltage +9V into -5V, a dc-to-dc converter is needed first to get a negative voltage of -9V. Later it would be converted to -5V with L79L series regulator. The TC962 is a high voltage DC-to-DC converter. Using improved design techniques and CMOS construction, the TC962 can source as much as 80 mA. As an inverter, the TC962 can put out voltages as high as 18V and as low as 3V without the need for external diodes. Only two external capacitors of 10 µF are required for inverter applications as shown in Figure 2.19.

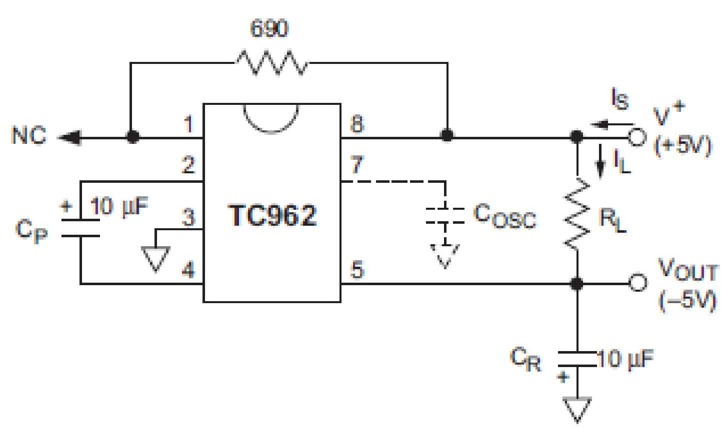

Figure 2. 19: Circuit Schematic of TC962

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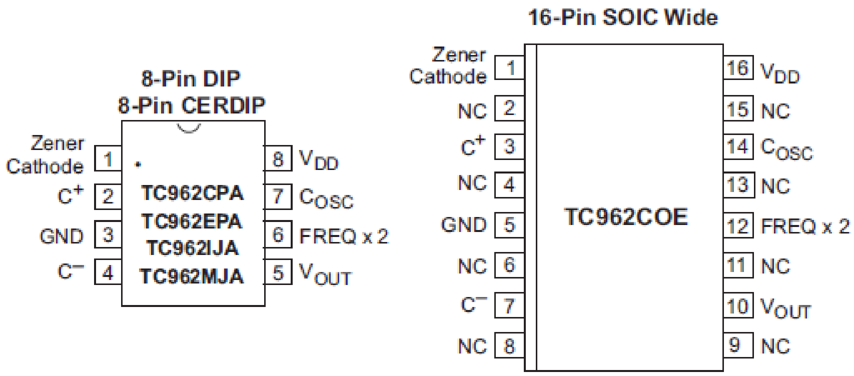

Figure 2. 20: DIP and SOIC pin diagram for TC962

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2.2.3 Battery

To power up the whole system, a battery with sufficient current supply is needed. For this research work, we are using a 9V Alkaline Energizer battery as shown in Figure 2.21(a) with a capacity of 600mAh. This battery can provide a continuous supply of 100mA current whereas our system needs 40~45 mA. To make contact between battery and our system, a battery connector like Figure 2.21(b) with 101.6mm wire has been attached.

(a) (b)

Figure 2. 21: (a) 9V Alkaline Manganese Dioxide Battery (b) Battery Connector Snap

Reprinted with permission from [42] © Energizer Battery Company for (a) and from [43] © 2017, Keystone Electronics for (b).

2.2.4 Microcontroller Platform

To run the potentiostat circuitry with continuous voltage supply at regular intervals, a microcontroller is needed. As we are using a DAC 0808 IC to supply voltage from a binary counter, the easiest way to operate this would be an Arduino. Arduino offers several models with different characteristics. The main differences between the modules are the number of inputs and outputs, the type of microcontroller and the capacity of the Flash memory [52]. In our experiment, we are using Arduino UNO as it has been the go-to board for quick prototyping, Arduino Projects, and DIY Projects. It has the following features:

- Microcontroller Unit: ATmega328
- Architecture: AVR
- Operating Voltage: 5V
- Digital I/O pins: 20 (of which 6 can produce PWM)
- Analog inputs: 6
- Flash memory: 32 KB (2 KB of this used by bootloader)
- Clock speed: 16 MHz

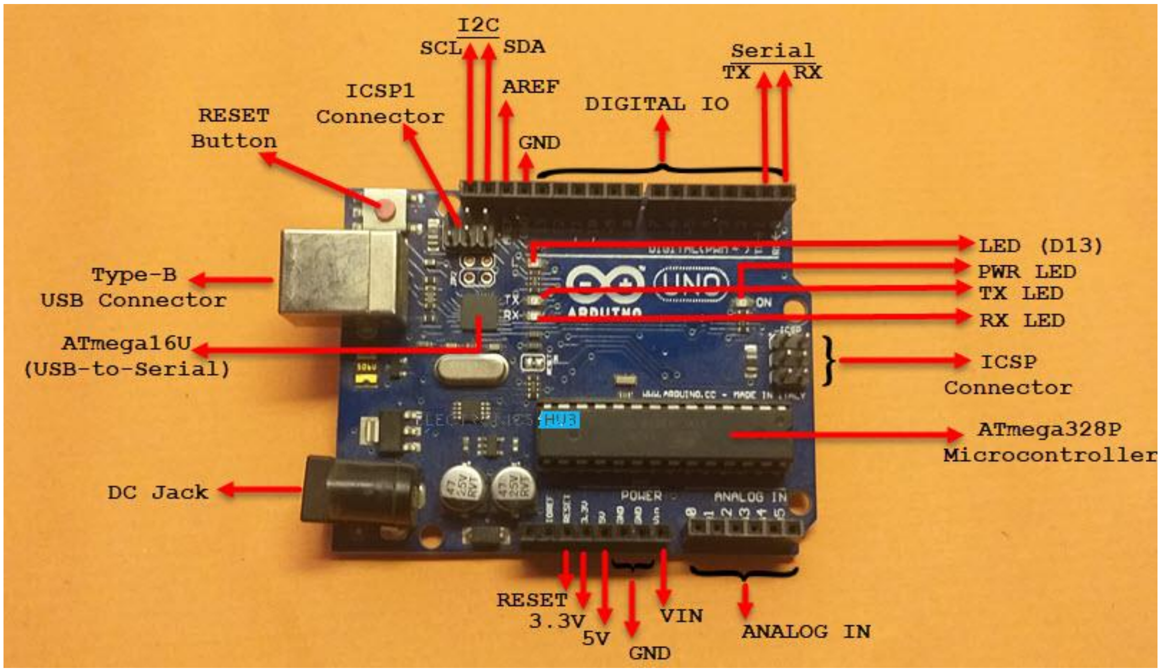

Figure 2. 22: Layout of Arduino UNO board

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Arduino UNO is based on ATmega328P Microcontroller, an 8-bit AVR Architecture based MCU from ATMEL. Arduino UNO comes in two variants: one consists of a 28-pin DIP Microcontroller while the other consists of 32 lead Quad Flat Package (QFP) Microcontroller. We are using the 28-pin DIP version for our Through-Hole (TH) system here as shown in Figure 2.22. The QFP package will be discussed later with SMD components on PCB board.

The type-B USB connector on the left short edge of the board is used for powering on the board as well as programming the Microcontroller. There is also a 2.1 mm DC jack to provide external power supply.

Of the 28 pins available on the UNO board, 20 pins are associated with input and output. In that 14 pins (D0 to D13) are true digital IO pins, which can be configured as per the application using pinMode(), digitalWrite() and digitalRead() functions. Digital IO pins 3, 5, 6, 9, 10 and 11 are capable of producing 8-bit PWM Signals by using analogWrite() function [53,54]. There are also 6 Analog Input Pins (A0 to A5). All the analog input pins provide a 10-bit resolution ADC feature, which can be read using analogRead() function. The Analog Input pins can be configured as Digital IO pins, if required.

Digital IO pins 0 and 1 are used as Serial RX and TX pins to receive and transmit serial data. These pins are connected to the serial pins of the on-board USB to Serial Converter IC. The final communication interface is the SPI. Digital IO Pins 10, 11 12 and 13 can be configured as SPI pins SS, MOSI, MISO and SCK respectively.

2.2.4.1 Arduino IDE

The Arduino Integrated Development Environment - or Arduino Software (IDE) - contains a text editor for writing code, a message area, a text console, a toolbar with buttons for common functions and a series of menus. It connects to any Arduino board to upload programs and communicate with them. As we are connecting the Arduino board with a DAC0808 IC, we need to assign values for the 8-bit binary counter. They will be later converter to analog voltage by DAC which would be the input values for our potentiostat circuitry.

The 8 digital input pins of DAC can be easily assigned values from Digital IO pins of Arduino. Here, we are considering digital pins 2 to 9 from Arduino leaving pins 0 and 1 for RX and TX. If pins 2 to 9 are configured as an OUTPUT with pinMode(), digitalWrite() will enable (HIGH) or disable (LOW) the internal pullup on the input pin. The syntax needed to be used as follows:

digitalWrite(pin, value)

where pin = 2 to 9

value = 0 or 1

This method is useful when only one input voltage is needed at a time. But as we can recall from the CV curve, a range of input values were needed at that time. So, the 8-bit counter needs to be changed maintaining a certain delay interval. In this case, running the bitRead() function within a for loop can generate continuous binary values:

bitRead(x, n)

where, x = the number from which to read.

n = which bit to read, starting at 0 for the least-significant (rightmost) bit.

While running these values through DAC, we need to check the output from potentiostat to observe for specific voltages. Then we have to figure out a specific counter range within which the potentiostat is giving a complete CV cycle. For our experiment, the acceptable result was while the binary counter was operating in the range from 6-7 bit. As the first 6-bit binary number $100000_2 = 32_{10}$ and the last 7-bit binary number $1111111_2 = 127_{10}$, the uploaded sketch is as follows:

```
for (int counter = 32; counter < 127; counter++)
{
  displayBinary(counter);
  delay(delayInterval);
  }
```

After selecting specific range of input voltage for CV and setting time interval for CA, an option is needed to select the operation. A switch case within a void loop helps to complete this operation. We can select the option through a serial monitor to get CV or CA output values. If we choose other options, the monitor will show error. The uploaded sketch is as follows:

```
switch(inByte)
{
  case 'a': //Cyclic Voltammetry
  break;
  case 'b':  //Chronoamperometry
  break;
  default:
  Serial.println("error!");
  break;
 }
```

switch case controls the flow of programs by allowing programmers to specify different code that should be executed in various conditions. A switch statement compares the value of a variable to the values specified in case statements [55]. When a case statement is found whose value matches that of the variable, the code in that case statement is run. The break keyword exits the switch statement and is typically used at the end of each case. Without a break statement, the switch statement will continue executing the following expressions until a break, or the end of the switch statement is reached.

Lastly, to get the output current in CV or CA curve, there needs to be a simultaneous readout system in Arduino beside digital input to DAC. The function analogRead() reads the value from the specified analog pin [53]. Arduino boards contain a multichannel, 10-bit analog to digital converter. This means that it will map input voltages between 0 and the operating voltage 5V into integer values between 0 and 1023. On an Arduino UNO, for example, this yields a resolution between readings of 5 volts / 1024 units or, 0.0049 volts (4.9 mV) per unit. The uploaded sketch is as follows:

```
int sensorValue=analogRead(adcch1);

float voltage_mV=sensorValue*(5.0/1023.0);

Serial.print(voltage_mV, 4);  //mentions 4 points after decimal

Serial.print("\t");
```

The Analog reading channel in Arduino can only read positive values. The last stage of our potentiostat was an inverting amplifier. So, we put another inverting amplifier as mentioned in section 2.2.1.1 to get a positive readout.

2.2.4.2 Arduino as ISP

After the program code had worked well with our potentiostat circuitry, we need to migrate from an Arduino board to a standalone ATmega 328P microcontroller on a breadboard. This is needed for further consideration of PCB where we would need to print all the SMD ICs to a single FR4 board. This migration requires to burn the bootloader onto ATmega 328P using an Arduino board as an in-system program (ISP).

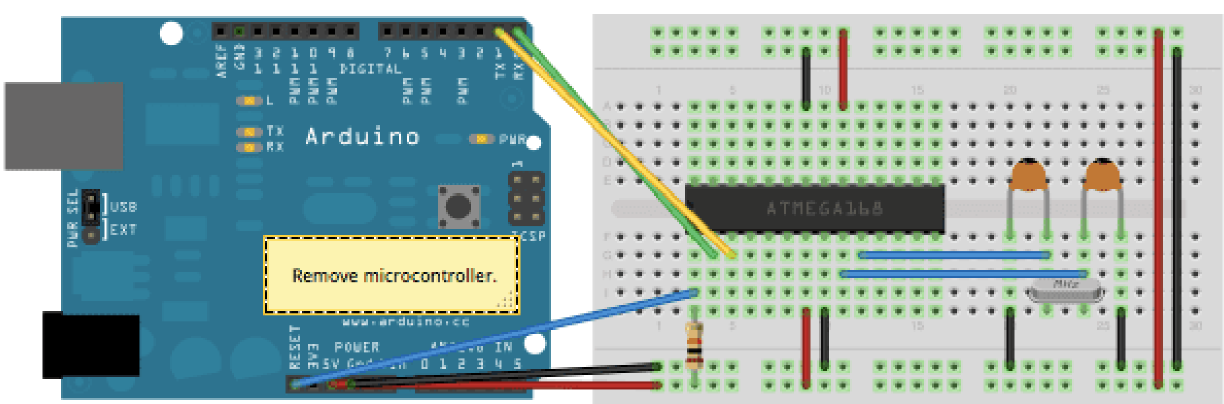

Figure 2. 23: Using Arduino board to burn the bootloader onto an Atmega328P on a breadboard.

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Firstly, we need to open the ArduinoISP sketch from the Examples section of Arduino IDE and upload it onto the Arduino board. Then we need to connect pins 10, 11, 12, and 13 of the Arduino board to 1, 17, 18 and 19 pins of ATmega 328P on breadboard keeping all other connections as Figure 2.23 (without Tx, Rx and RESET). After selecting Tools > Board menu > Arduino Duemilanove and Tools > Programmer > Arduino as ISP we can run Tools > Burn Bootloader. After burning the bootloader, we can remove the jumper wires connected to pins 10, 11, 12, and 13 of the Arduino board and connect everything just like Figure 2.23.

2.2.5 Digital-to-Analog Converter (DAC)

Digital-to-Analog converter IC and is used for converting 8-bit digital data input to analog signal output. DAC0808 IC is used in applications where a digital to analog conversion is required [56]. The chip used to be popular a decade ago and its applications used to be many but at present there are many modern IC which can perform DAC with higher resolution, speed and efficiency. So, this device is mainly used for reference and beginner applications. In this research, it is used to visualize the 8-bit counter coming out of Arduino and then get the analog output to pass through an op-amp to potentiostat.

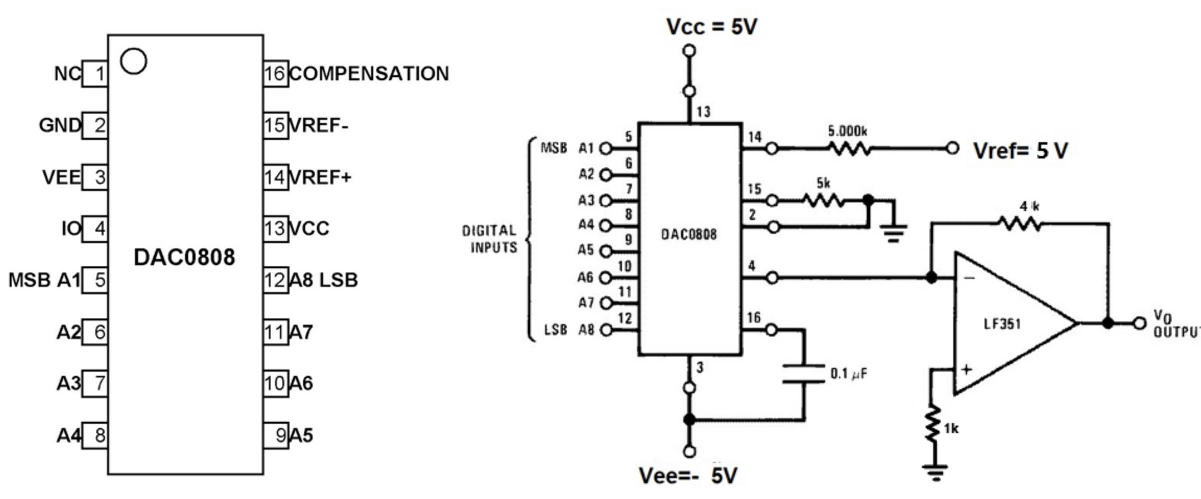


Figure 2. 24: DIP package and circuit schematic of DAC0808.

Reprinted with permission from [46] © 2011, Texas Instruments.

The device takes in parallel 8-bit data from Arduino and converts that data into analog signal at the output. The analog output from DAC is a current quantity and this needed to be converted to a voltage parameter for using in application easily. So, to convert the current parameter into voltage parameter we used op-amp circuit as shown in circuit which is called current-to-voltage converter.

Equation of Output Voltage after Conversion

$$V_o = V_{ref}\left(\frac{A_1}{2} + \frac{A_2}{4} + \frac{A_3}{8} + \frac{A_4}{16} + \frac{A_5}{32} + \frac{A_6}{64} + \frac{A_7}{128} + \frac{A_8}{256}\right)$$

Considering only the LSB as HIGH, $V_o = 5 \times \frac{1}{256} = 19.5mV$

This value refers that whenever a counter value changes, there will be a change of 19.5 mV to V_0 or to the input of potentiostat.

2.2.6 Bluetooth Module

In the previous sections we mentioned about setting up potentiostat circuitry with electrode setup, provide input voltage with Arduino and DAC, then read the output current with ADC pin in Arduino. After that, we want to send this output through a wireless module. We have considered RN4871 CLICK which is a Bluetooth® 4.2 Low Energy Module. The RF mikroBUS™ Click™ platform is convenient as it provides an evaluation expansion board which is easy to work on bread board. Figure 2.25 shows a CLICK module with pin diagram of built in RN4871 chip.

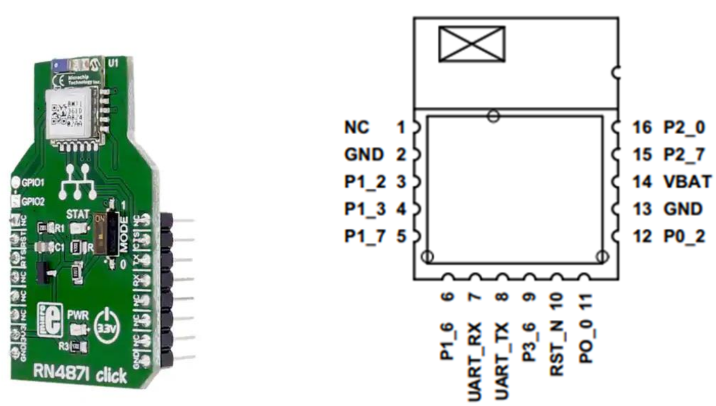


Figure 2. 25: CLICK Module and IC pin diagram of RN4871.

Reprinted with permission from [47] © 2016, Microchip Technology.

As we can see from the diagram, Pin#16 of the module should be grounded, Pin#7 and Pin#8 should be connected to R_X and T_X ports of an USB serial adapter, respectively. Pin#14 and Pin#13 are VCC (3.3V) and ground, which have to be connected for programing.

In the next steps, we need to run ISupdate programmer for providing some necessary information to the Bluetooth module. Firstly, we connect the Bluetooth module to an Arduino for power supply. Then we need to check from ISupdate if the COM port is connected. Next, firmware hex files should be loaded in the module which takes around 20 seconds.

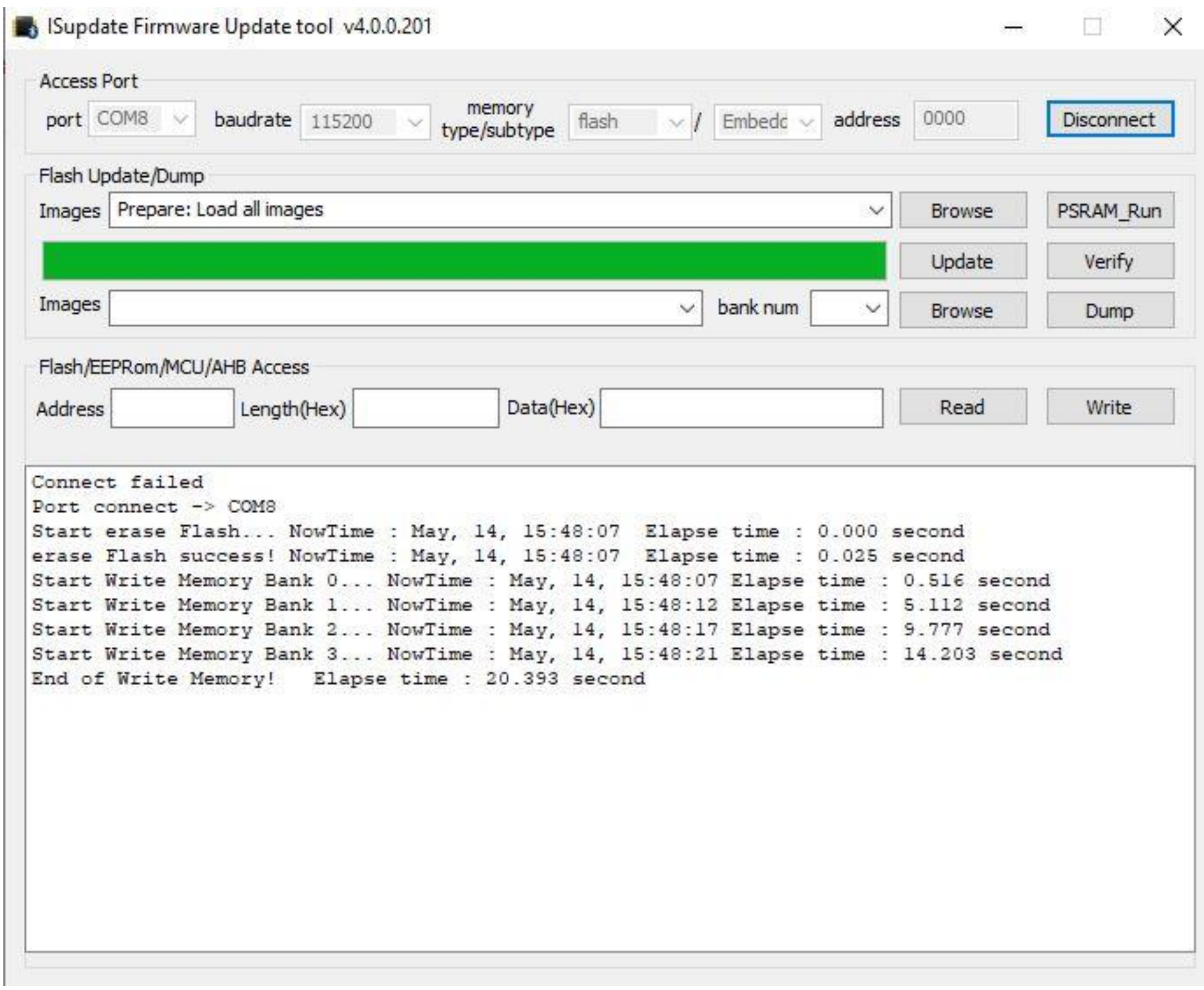


Figure 2. 26: ISupdate Firmware tool with RN4871 click module.

After that we need to download a serial monitor software CoolTerm to setup the Bluetooth module with a specific name, put instructions to discover and receive data from it. While pressing **$$$** the serial monitor shows **>CMD**. Next, we can put the commands of Figure 2.27 on Arduino Serial Monitor:

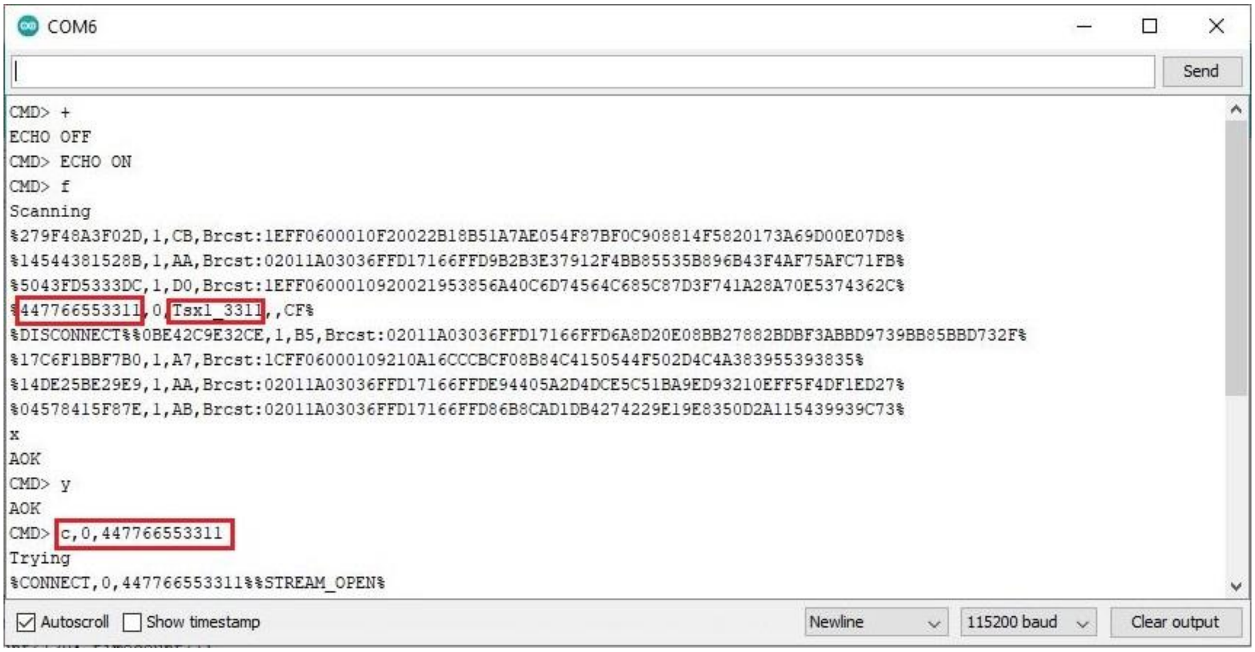


Figure 2. 27: Commands to detect and connect Bluetooth.

Entering 'f' on the monitor starts searching for Bluetooth. Our desired Bluetooth is Tsx1_3311. So, we copy the address and enter c,0,address to connect with it. Once it is connected, the monitor will show %CONNECT.

2.3 Altium Designer

In the earlier sections of this paper, different circuit diagrams have been mentioned starting from voltage regulator, potentiostat to Arduino and DAC. While working with breadboard, we can segment this circuit and test their output in a step-by-step manner to avoid complications. Circuits on breadboard take much step, require many wires, and need to have space between different ICs for placing multimeter and taking readings.

Next, when we move to the PCB layout, the strategy is opposite as we need to accommodate all the ICs within a very small area. For this purpose, we have chosen Altium designer to bring the whole schematic in one place and then move to PCB.

Schematic capture is the process of creating a logical representation of an electronic circuit. This makes it easier to put a complex circuit in one place as well as place many components and net list together for easier visual representation [57]. The circuit is typically created in sections: the processor and Bluetooth, the analog to digital processing of inputs, the potentiostat, the power supply, and so on.

2.3.1 Schematic Drawing

Altium Designer provides a powerful Manufacturer Part Search panel that can be used to search for real-world manufactured parts. The component that we ultimately solder onto the board needs to be represented, or modeled, in each design domain as a symbol on the schematic and as a footprint on the board [58]. If any component is not available in the manufacturer library, we can use Altium tools to make their symbol and footprint from the measurements given in datasheet. For example, the audio connector where the 3 electrodes are going to be connected is prepared in this customized manner.

There are two ways to connect the components in Altium Designer. We can connect them by wiring the pins together or by placing netlist identifiers to connect the pins in that net. The second idea is mostly helpful when we are using a specific voltage or net multiple times that wiring them in the schematic would create mess. Lastly, we can establish the wiring and design after the project is compiled.

2.3.2 PCB Layout

After putting together every component, connection, and notation in the schematic the circuitry is defined and ready to go to for PCB layout. Firstly, we need to check our schematic for design rule violations that keep the schematic from being synchronized with PCB layout. Once PCB layout is created, this first synchronization step will ensure that any later change in the schematic can be immediately imported into the PCB layout [59]. Next, we need to create a new PCB file in our current project, and then use the schematic capture tool to import footprints for components into your new PCB. Afterwards, we have to define a layer stack for the new PCB. Once we have completed these tasks, we can now start arranging components and routing traces between them.

It is always a good idea to define board size at the beginning and set origin accordingly. Board size can be also changed later but setting an approximation while starting helps to stick with the design plan. From **View > Board Planning Mode** and **Design > Redefine Board Shape** options we can fix the board area. For our experiment, the board size is 57.4 mm × 34.5 mm. Then we need to figure out the required layer numbers and place component accordingly. To keep it a simple design, we are using two layers. The bottom layer contains all the voltage regulators or power up ICs. So, the battery connection will be provided here. All the other ICs and related connections are on top layer. The next step is very important where we need to do routing which will provide connection between all the electronic components. In this step we need to spend good amount of time to first plan the components in a way that will result in least possible routing conflicts [60]. Routing width is another important factor to keep in consideration. Most of the routing widths used in this PCB are 0.1mm to save space, keep the PCB size small and avoid interference between adjacent connections. Only the routing width of voltage levels and grounds are kept at 0.3mm. Also, the hole size of vias needs to be selected according to company guidelines. The smallest available hole size of our manufacturer PCBWay was 0.2mm. So, we had to limit the size there though routing width was 0.1mm.

After we have carefully done all the routing, checked all the connections and matched with the schematic the next task is polygon pour. It can be done from the **Place > Polygon Pour** option. On a signal layer, we can place a solid polygon pour to define an area for carrying large power supply currents, or as a ground-connected area for providing electro-magnetic shielding. In our PCB, the largest polygon area on top layer is for +5V and on bottom it is for GND plane.

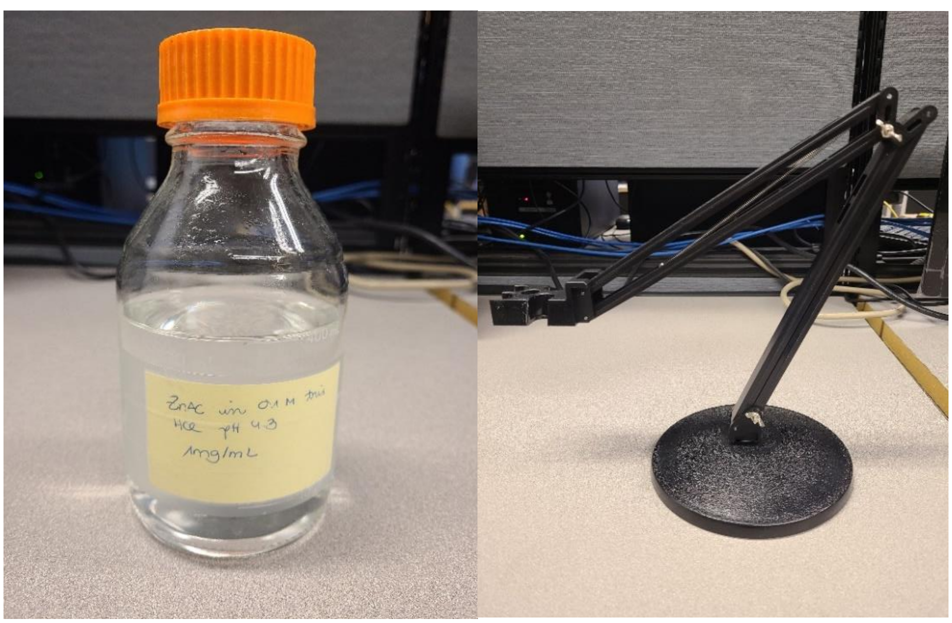
After the polygon pour, our PCB design is almost done. Next, we have to prepare the design files to be sent to the manufacturer. For that purpose, we have to create a **Keepout Layer** on the outer boundary of our board. This layer helps the manufacturer to recognize that they need to print the area inside keepout layer. The last task is to create **Gerber** and **NC Drill** files from the **File > Fabrication Output** option which will be inside Output Job folder. Finally, we need to send this folder to our manufacturer for PCB fabrication.

CHAPTER 3: Implementation on Breadboard

3.1 Chemical Setup

For the chemical setup, we need 3 electrodes submerged in a solution as mentioned in section 2.1. As we described earlier, glassy carbon and platinum wire electrodes were used as WE and CE respectively. The solution used here as electrolyte was Zinc in tris HCl with pH 4.3 as shown in Figure 3.1(a). This solution and electrodes were taken from Faculty of Medicine, McGill University in Montreal General Hospital (MGH). The SCE used as RE was ordered from Gamry Instruments. It has a phone jack at the end of reference cable which can be connected to an alligator clip and plugged directly to the desired op-amp pin of potentiostat. To connect the electrodes at specific IC pins, we used 50cm wires with double ended alligator clips.

To keep all the electrodes in place while they were submerged in solution, an electrode arm was needed. We ordered an Electrode Swing Arm from Thermo Fisher Scientific to hold electrodes and swing the arm according to solution height as well as distance among electrodes (Figure 3.1(b)). The electrode arm protects electrodes from accidental breakage where the adjustable arm allows for a wide variety of electrode positions. It also has a weighted base for stability.



(a) (b)

Figure 3. 1: (a) Zinc in Tris HCl Solution and (b) Electrode Swing Arm

3.2 Potentiostat Circuitry

As we depict from section 1.3.1, The potentiostat circuitry involves 3 op-amps to be connected with 3 electrodes. We took LMC 6484 op-amp IC as it can accommodate 4 op-amp configuration simultaneously.

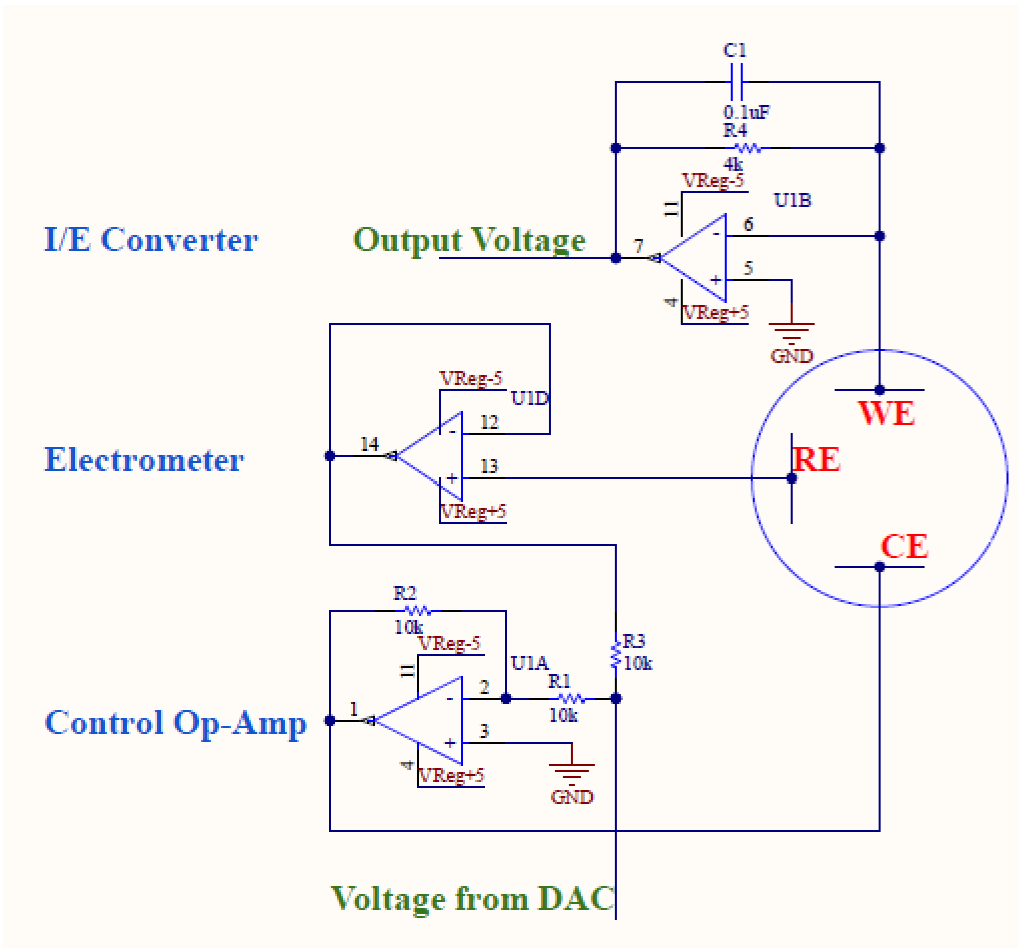


Figure 3. 2: Potentiostat circuit schematic

As shown in Figure 3.2, the microcontroller-controlled voltage from DAC gets into the inverting input pin 2 of Control Op-Amp. This op-amp works as an inverting amplifier and its output is connected to the Platinum wire CE. The Electrometer op-amp is simply built upon the principle of voltage follower. Here, the non-inverting input is connected to the Saturated Calomel RE. For the last op-amp, glassy carbon WE is connected to inverting input. This op-amp works as a current to voltage converter. As we from section 2.1.3, the output current will be in the µA range. As the 3 electrodes' work function operates in mV range, putting the resistor as 4kΩ will provide us output current in µA.

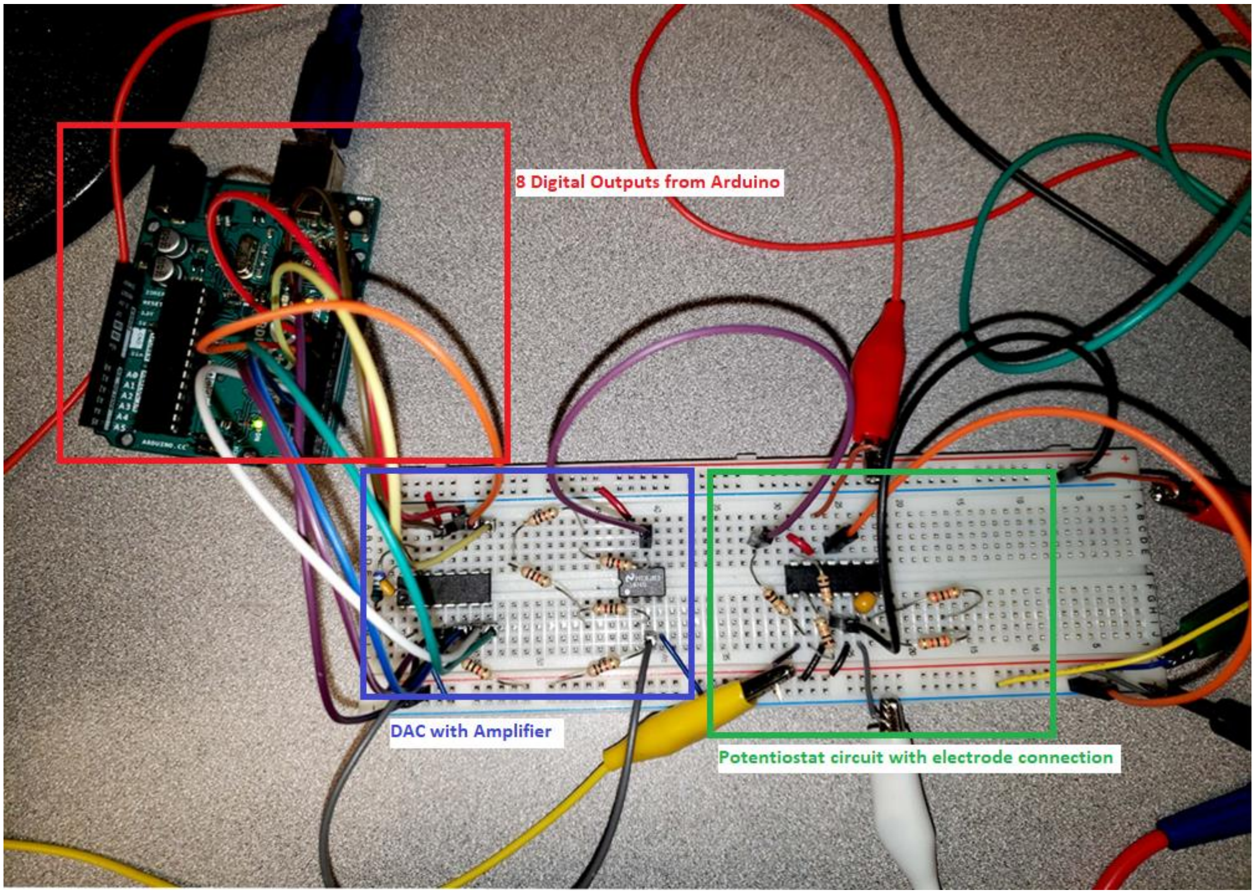


Figure 3. 3: Potentiostat circuit with DAC and Arduino on breadboard

Figure 3.3 shows a breadboard representation of the circuit in Figure 3.2. Here, LMC6484 op-amp IC has been used to accommodate all 3 op-amps mentioned previously. The DAC with Amplifier resembles the circuit on Figure 2.24. At this point we are using the pins from Arduino board to supply DAC inputs.

3.3 Load Testing

The entire system in our circuitry operates with different voltage levels. So, several voltage regulators were used here. While we checked the different desired voltage outputs with multimeter, another way to ensure is load testing. In this method we connect a potentiometer or variable resistor in parallel to the output capacitor. An ammeter is also in series connection with the potentiometer.

The goal is to change the resistor value and check how much maximum current can be provided. Figure 3.4 shows the voltage regulator diagram denoting load testing with MC79L05 negative regulator.

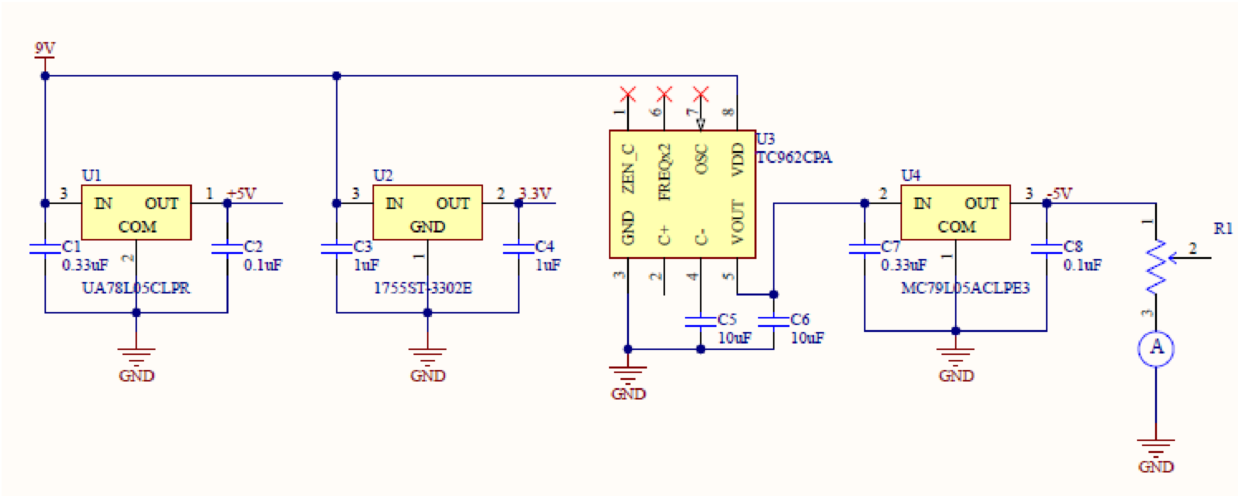


Figure 3. 4: Voltage Regulator circuit schematic with load testing

Table 1 shows a comparison between the desired and experimental maximum output current values. The desired values for different regulator ICs were collected from their datasheets.

Table 1. Maximum Current Values for Voltage Regulator ICs

Voltage Regulator IC	**Desired Maximum Current Value (mA)**	**Experimental Maximum Current Value (mA)**
UA78L05	100	81
MC79L05	100	87
TC962	80	70
MCP1755S	300	110

All the regulators cannot reach the desired value. But this is enough to run our system as it does not require more than 50mA.

Figure 3.5 provides a close observation to the bread board circuitry of voltage regulator. A 100 kOhms 0.5W through hole trimmer potentiometer has been used for load testing purpose.

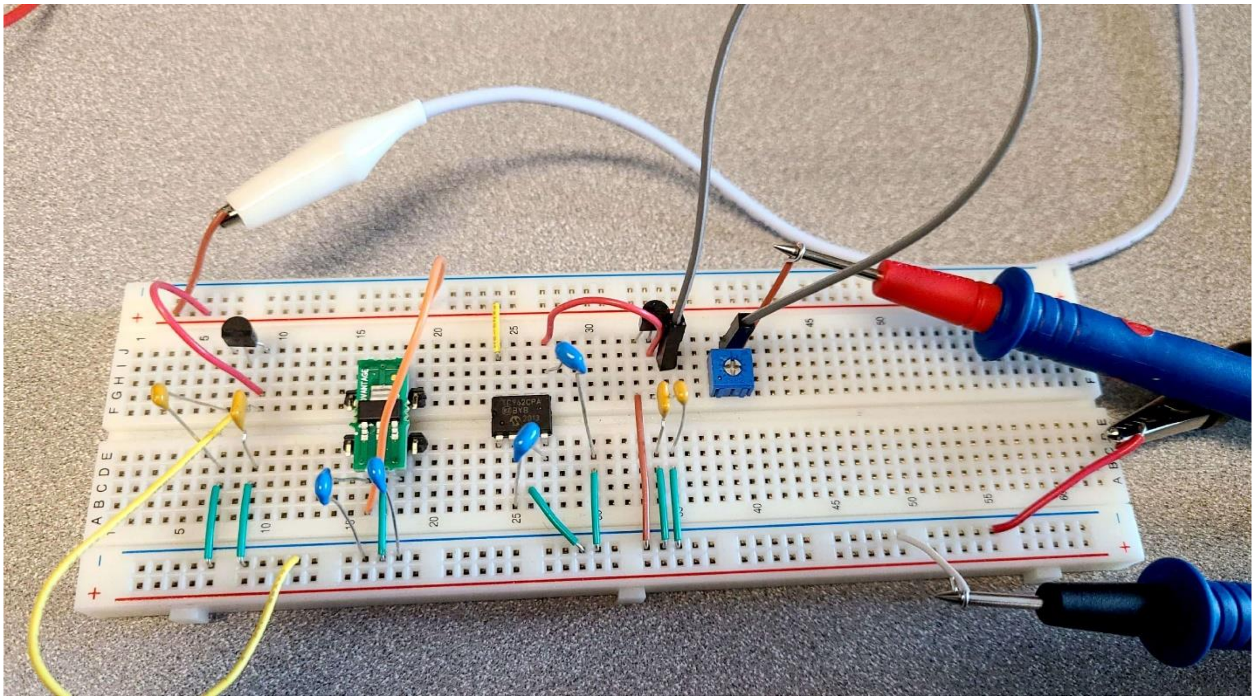


Figure 3. 5: Load Testing on bread board

3.4 Complete Circuitry

After testing our circuitry at different stages, we need to combine the whole bread board circuit for result acquisition. The complete circuit has five different stages. Each of the stage has their dedicated purpose to contribute to the system.

3.4.1 Voltage Regulator

As we mentioned in Table 1, we used 4 different ICs to meet up the different power supply requirements of our circuit. All of these are step down linear regulator who need to provide continuous supply of +5V, 3.3V and -5V. So, a 9V power supply is used as their input voltage. The only task of TC962 is to invert the voltage level to -9V which can work as the input for -5V supply voltage.

3.4.2 DAC with Amplifier

This stage consists of a DAC0808 and LM741 op-amp as an amplifier. The main function of DAC is to take digital 8-bit input from Arduino or Atmega328P and convert that into an analog output [46]. This analog value is amplified and used as input to the potentiostat circuit. As we can see from the calculation in section 2.2.5, the DAC output changes 19.5mV with each digital input bit change. Both DAC0808 and LM741 need ±5V from voltage regulators to power up.

3.4.3 Potentiostat circuit with Electrode connection

For the potentiostat circuit we need 3 op-amp configuration as shown in Figure 3.2. So, we have chosen LMC 6484 and provided connection with electrodes as per the points mentioned in Figure 3.2. We are using the fourth op-amp in LMC6484 as an inverting amplifier. The output voltage of I/E converter will be a negative value as per the previous values provided from DAC. We need to read this value with ADC in Arduino or Atmega328P. That's why we are converting it to a positive value by using fourth op-amp as inverting amplifier. Just like LM741, the op-amp IC LMC6484 needs ±5V from voltage regulators to power up.

3.4.4 Atmega328P after bootloading

Earlier we were interfacing the DAC circuitry with digital inputs from Arduino as shown in Figure 3.3. As mentioned in section 2.2.4.2, we did the operation burn bootloader to an Atmega328P IC by using Arduino as ISP code and wiring like Figure 2.23. Now, all the DAC interfacing and output reading of potentiostat will be done by Atmega328P IC instead of Arduino board [45]. Atmega328P needs +5V from voltage regulator to power up.

3.4.5 Receiver and Transmitter Bluetooth

We are using two RN4871 CLICK modules as discussed in section 2.2.6. The receiver module is connected to the Atmega328P chip and collects potentiostat output values. The transmitter Bluetooth is connected to the Arduino board. It collects data in a wireless way, passes to the ADC readout which is shown on the serial monitor screen of Arduino IDE.

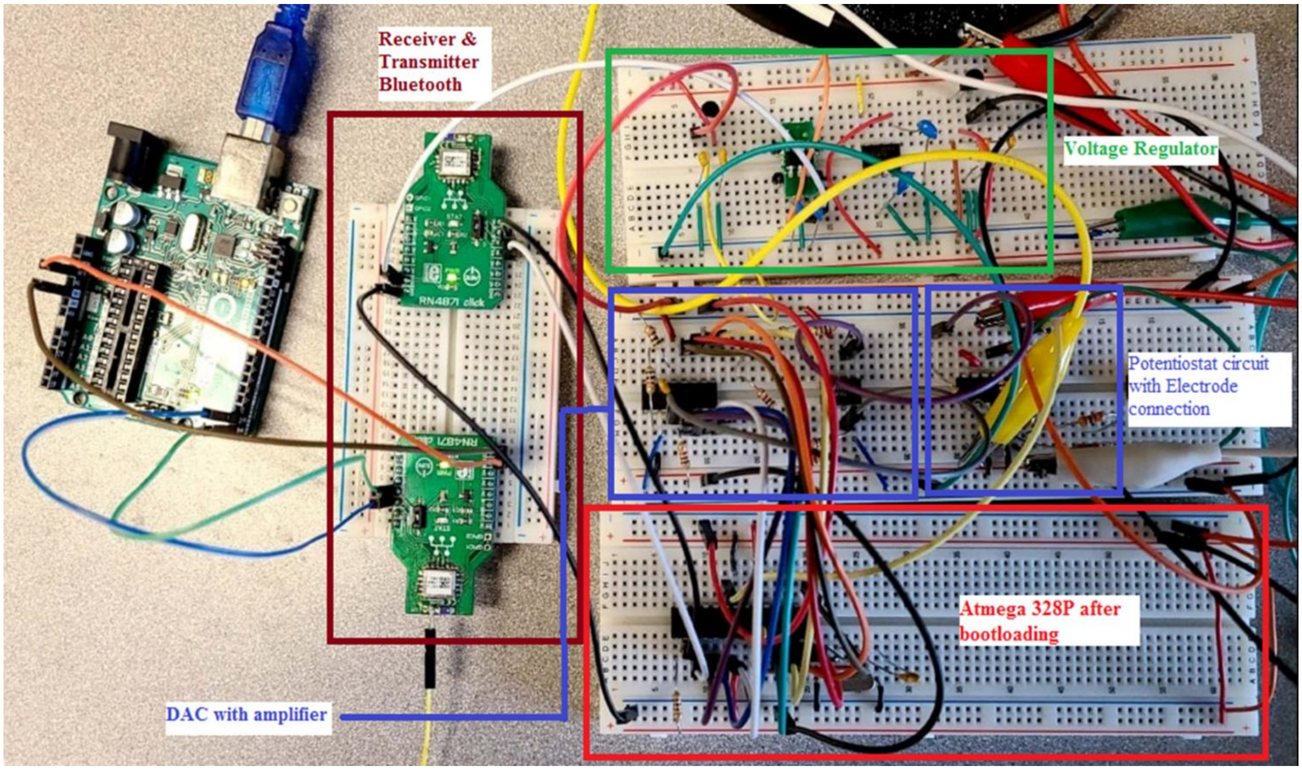


Figure 3. 6: Total bread board system for biosensor circuit

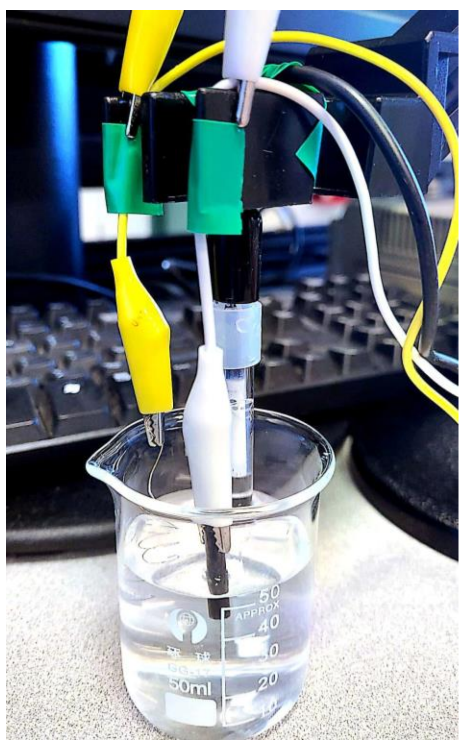


Figure 3. 7: 3-electrodes connected to potentiostat with alligator clips.

3.5 Results

In this section we will discuss about the different Cyclic Voltammetry and Chrono Amperometry outputs we got on bread board, measured with different methods.

3.5.1 Cyclic Voltammetry Multimeter Measurements

Before introducing ADC reading in Arduino IDE, we were using the microcontroller only for input purpose through binary counter. At that time, we were checking the potentiostat outputs manually with multimeter which resulted in graphical representation as Figure 3.8.

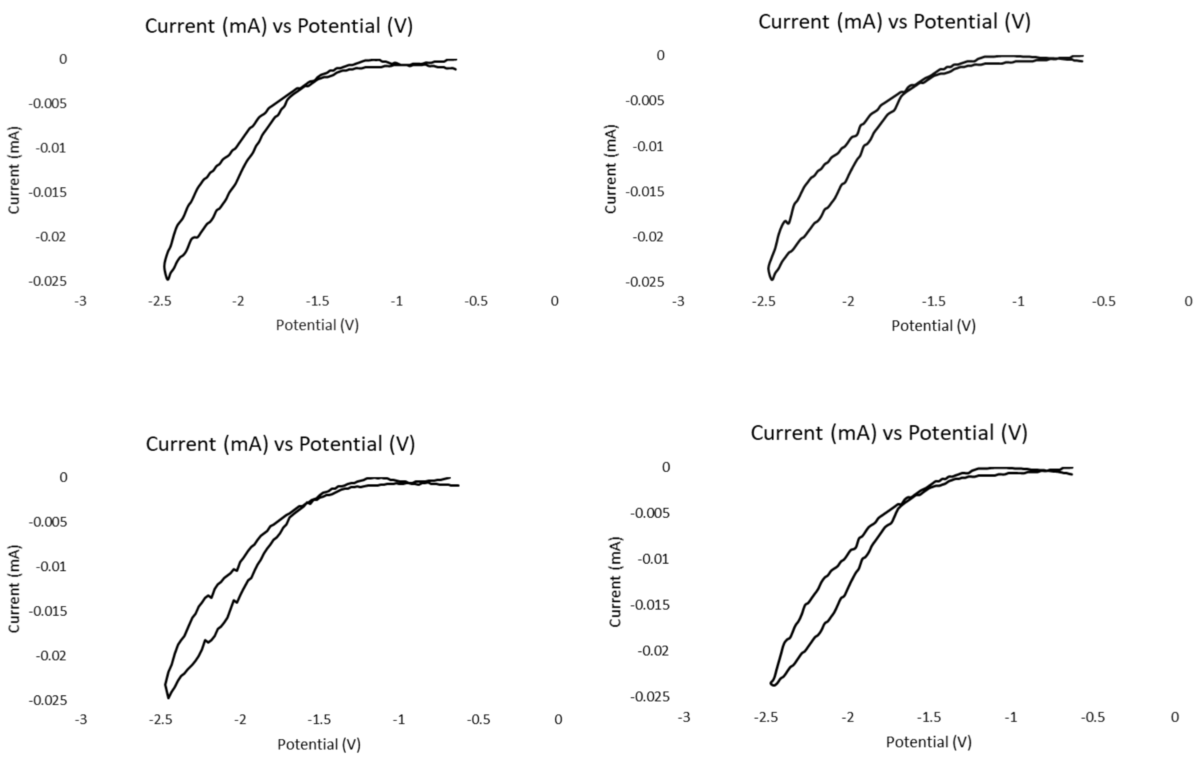


Figure 3. 8: Potentiostat cyclic voltammetry output reading with multimeter.

The commercial potentiostat could use triangular wave as input whereas we used ramp voltage as input. That’s why the output CV curves in Figure 3.8 differs from Figure 2.7. On the other hand, by repeated measurements with multimeter, we can see the consistency in their output behavior where they have maintained cyclic pattern of duck shaped curve. Table 2 shows a series of input voltage and output current values used for generating the curves.

Table 2: Output Current vs Input voltage for cyclic voltammetry curves with Multimeter Reading

V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)
-0.624	0	-1.077	-7.5E-07	-1.54	-2.8E-06	-2.02	-1.4E-05
-0.642	-2.5E-08	-1.097	-8.8E-07	-1.56	-3E-06	-2.04	-1.5E-05
-0.662	-2.5E-08	-1.117	-8.8E-07	-1.58	-3E-06	-2.06	-1.5E-05
-0.681	-5E-08	-1.137	-8.8E-07	-1.6	-3.3E-06	-2.08	-1.6E-05
-0.7	-2.5E-07	-1.157	-8.8E-07	-1.62	-3.5E-06	-2.1	-1.6E-05
-0.72	-2.5E-07	-1.177	-8.8E-07	-1.64	-3.8E-06	-2.12	-1.7E-05
-0.74	-2.5E-07	-1.197	-8.8E-07	-1.66	-4E-06	-2.14	-1.7E-05
-0.76	-2.5E-07	-1.22	-1E-06	-1.69	-4.5E-06	-2.16	-1.8E-05
-0.78	-3.8E-07	-1.24	-1E-06	-1.71	-5.3E-06	-2.18	-1.8E-05
-0.8	-3.8E-07	-1.26	-1E-06	-1.73	-5.6E-06	-2.2	-1.9E-05
-0.818	-3.8E-07	-1.28	-1.1E-06	-1.75	-6.3E-06	-2.22	-1.9E-05
-0.837	-5E-07	-1.3	-1.1E-06	-1.77	-6.5E-06	-2.24	-2E-05
-0.857	-5E-07	-1.32	-1.3E-06	-1.79	-7E-06	-2.26	-0.00002
-0.877	-5E-07	-1.34	-1.3E-06	-1.81	-7.5E-06	-2.28	-0.00002
-0.897	-5E-07	-1.36	-1.4E-06	-1.83	-8E-06	-2.3	-2E-05
-0.917	-6.3E-07	-1.38	-1.5E-06	-1.85	-8.5E-06	-2.33	-2.2E-05
-0.937	-6.3E-07	-1.4	-1.8E-06	-1.87	-9.3E-06	-2.35	-2.2E-05
-0.957	-6.3E-07	-1.42	-1.9E-06	-1.89	-9.8E-06	-2.37	-2.2E-05
-0.977	-6.3E-07	-1.44	-2E-06	-1.91	-1.1E-05	-2.39	-2.3E-05
-0.997	-6.3E-07	-1.46	-2E-06	-1.93	-1.1E-05	-2.41	-2.4E-05
-1.017	-6.3E-07	-1.48	-2.1E-06	-1.95	-1.2E-05	-2.43	-2.4E-05
-1.037	-7.5E-07	-1.5	-2.3E-06	-1.98	-1.3E-05	-2.45	-2.5E-05
-1.057	-7.5E-07	-1.52	-2.5E-06	-2	-1.3E-05	-2.47	-2.3E-05

V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)
-2.45	-2.2E-05	-1.98	-9E-06	-1.5	-2E-06	-1.03	-5E-07
-2.43	-2.1E-05	-1.95	-8.3E-06	-1.48	-1.8E-06	-1.01	-3.8E-07
-2.41	-2E-05	-1.93	-7.8E-06	-1.46	-1.6E-06	-0.99	-5E-07
-2.39	-1.9E-05	-1.91	-7.5E-06	-1.44	-1.5E-06	-0.973	-6.3E-07
-2.37	-1.8E-05	-1.89	-7E-06	-1.42	-1.3E-06	-0.956	-6.3E-07
-2.35	-1.8E-05	-1.87	-6.5E-06	-1.4	-1.3E-06	-0.935	-6.3E-07
-2.32	-1.7E-05	-1.85	-6.3E-06	-1.38	-1E-06	-0.917	-7.5E-07
-2.3	-1.6E-05	-1.83	-6E-06	-1.36	-1E-06	-0.896	-6.3E-07
-2.28	-1.5E-05	-1.81	-5.5E-06	-1.34	-8.8E-07	-0.875	-6.3E-07
-2.26	-1.5E-05	-1.79	-5.3E-06	-1.32	-7.5E-07	-0.857	-6.3E-07
-2.24	-1.4E-05	-1.77	-5E-06	-1.3	-6.3E-07	-0.839	-6.3E-07
-2.22	-1.4E-05	-1.75	-4.8E-06	-1.28	-6.3E-07	-0.818	-5.8E-07
-2.2	-1.3E-05	-1.73	-4.5E-06	-1.26	-5E-07	-0.797	-7.5E-07
-2.18	-1.3E-05	-1.71	-4.3E-06	-1.24	-3.8E-07	-0.78	-7.5E-07
-2.16	-1.3E-05	-1.69	-4E-06	-1.22	-2.5E-07	-0.763	-7.5E-07
-2.14	-1.2E-05	-1.67	-3.8E-06	-1.2	-1.3E-07	-0.74	-8.8E-07
-2.12	-1.2E-05	-1.65	-3.5E-06	-1.18	-1.3E-07	-0.717	-8.8E-07
-2.1	-1.1E-05	-1.63	-3.3E-06	-1.15	-5E-08	-0.702	-8.8E-07
-2.08	-1.1E-05	-1.61	-3.3E-06	-1.13	-7.5E-08	-0.687	-1E-06
-2.06	-1.1E-05	-1.59	-3E-06	-1.11	-5E-08	-0.672	-1E-06
-2.04	-1E-05	-1.57	-2.8E-06	-1.09	-2.5E-07	-0.657	-1E-06
-2.02	-0.00001	-1.55	-2.5E-06	-1.07	-2.5E-07	-0.642	-1E-06
-2	-9.5E-06	-1.52	-2.4E-06	-1.05	-3.8E-07	-0.627	-1.1E-06

3.5.2 Cyclic Voltammetry ADC Reading in Arduino IDE

After adding analogRead() function in the IDE code, we could put the simultaneous digital input and read analog output. The only circuit configuration we needed was an inverting op-amp to the output of potentiostat as the ADC reader could only read positive outputs. The idea was to convert the negative output to ADC readable positive value. Figure 3.9 shows the graphical representations of various cyclic voltammetry curves.

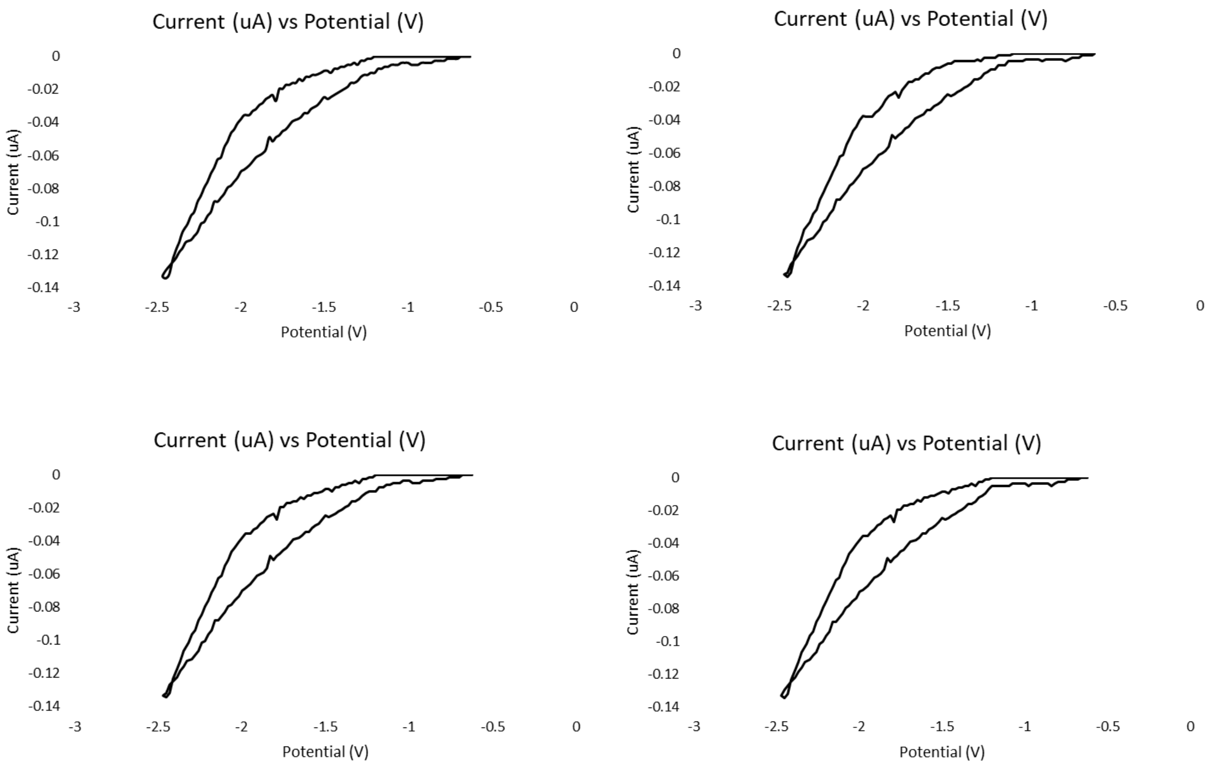


Figure 3. 9: Potentiostat cyclic voltammetry output reading with ADC.

Comparing Figure 3.9 to Figure 3.8, we got an observation of ADC quantization error that occurs due to the difference between the analog signal and the closest available digital value at each sampling instant from the ADC. So, the curve shape varies from manual multimeter reading. The y-axis or current values change in these two observations due to change in output gain at inverting amplifier. The output gain was changed by changing resistor value to 5.1kΩ to minimize quantization error for better output curves for the ADC reader case. Table 3 shows a series of input voltage and output current values used for generating the curves.

Table 3: Output Current vs Input voltage for cyclic voltammetry curves with ADC Reading

V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)
-0.624	0	-1.077	-4.9E-09	-1.54	-2.9E-08	-2.02	-7.3E-08
-0.642	-1.2E-09	-1.097	-4.9E-09	-1.56	-3.1E-08	-2.04	-7.6E-08
-0.662	-1.2E-09	-1.117	-4.9E-09	-1.58	-3.2E-08	-2.06	-7.8E-08
-0.681	-1.2E-09	-1.137	-4.9E-09	-1.6	-3.4E-08	-2.08	-7.9E-08
-0.7	-1.2E-09	-1.157	-7.3E-09	-1.62	-3.4E-08	-2.1	-8.3E-08
-0.72	-2.5E-09	-1.177	-7.3E-09	-1.64	-3.7E-08	-2.12	-8.6E-08
-0.74	-2.5E-09	-1.197	-7.3E-09	-1.66	-3.8E-08	-2.14	-8.8E-08
-0.76	-2.5E-09	-1.22	-9.8E-09	-1.69	-3.9E-08	-2.16	-8.8E-08
-0.78	-3.7E-09	-1.24	-9.8E-09	-1.71	-4.2E-08	-2.18	-9.4E-08
-0.8	-4.9E-09	-1.26	-1.1E-08	-1.73	-4.4E-08	-2.2	-9.7E-08
-0.818	-3.7E-09	-1.28	-1.2E-08	-1.75	-4.5E-08	-2.22	-1E-07
-0.837	-3.7E-09	-1.3	-1.5E-08	-1.77	-4.8E-08	-2.24	-1E-07
-0.857	-3.7E-09	-1.32	-1.6E-08	-1.79	-4.9E-08	-2.26	-1.1E-07
-0.877	-3.7E-09	-1.34	-1.6E-08	-1.81	-5.1E-08	-2.28	-1.1E-07
-0.897	-3.7E-09	-1.36	-1.8E-08	-1.83	-4.9E-08	-2.3	-1.1E-07
-0.917	-3.7E-09	-1.38	-2E-08	-1.85	-5.6E-08	-2.33	-1.1E-07
-0.937	-4.9E-09	-1.4	-2.1E-08	-1.87	-5.9E-08	-2.35	-1.2E-07
-0.957	-3.7E-09	-1.42	-2.2E-08	-1.89	-6E-08	-2.37	-1.2E-07
-0.977	-3.7E-09	-1.44	-2.3E-08	-1.91	-6.1E-08	-2.39	-1.2E-07
-0.997	-3.7E-09	-1.46	-2.4E-08	-1.93	-6.4E-08	-2.41	-1.2E-07
-1.017	-3.7E-09	-1.48	-2.6E-08	-1.95	-6.6E-08	-2.43	-1.3E-07
-1.037	-3.7E-09	-1.5	-2.4E-08	-1.98	-6.8E-08	-2.45	-1.3E-07
-1.057	-4.9E-09	-1.52	-2.7E-08	-2	-7E-08	-2.47	-1.3E-07

V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)
-2.45	-1.3E-07	-1.98	-3.8E-08	-1.5	-6.1E-09	-1.03	0
-2.43	-1.3E-07	-1.95	-3.8E-08	-1.48	-6.1E-09	-1.01	0
-2.41	-1.2E-07	-1.93	-3.6E-08	-1.46	-4.8E-09	-0.99	0
-2.39	-1.2E-07	-1.91	-3.4E-08	-1.44	-4.8E-09	-0.973	0
-2.37	-1.1E-07	-1.89	-3.2E-08	-1.42	-4.8E-09	-0.956	0
-2.35	-1.1E-07	-1.87	-2.8E-08	-1.4	-4.8E-09	-0.935	0
-2.32	-1E-07	-1.85	-2.6E-08	-1.38	-4.9E-09	-0.917	0
-2.3	-9.7E-08	-1.83	-2.4E-08	-1.36	-4.9E-09	-0.896	0
-2.28	-9.4E-08	-1.81	-2.3E-08	-1.34	-4.9E-09	-0.875	0
-2.26	-8.8E-08	-1.79	-2.7E-08	-1.32	-3.7E-09	-0.857	0
-2.24	-8.4E-08	-1.77	-2.2E-08	-1.3	-4.9E-09	-0.839	0
-2.22	-7.9E-08	-1.75	-2E-08	-1.28	-2.5E-09	-0.818	0
-2.2	-7.6E-08	-1.73	-1.7E-08	-1.26	-2.5E-09	-0.797	0
-2.18	-7.1E-08	-1.71	-1.7E-08	-1.24	-2.5E-09	-0.78	0
-2.16	-6.7E-08	-1.69	-1.6E-08	-1.22	-2.5E-09	-0.763	0
-2.14	-6.2E-08	-1.67	-1.6E-08	-1.2	-1.2E-09	-0.74	0
-2.12	-6.1E-08	-1.65	-1.3E-08	-1.18	-1.2E-09	-0.717	0
-2.1	-5.5E-08	-1.63	-1.2E-08	-1.15	-1.2E-09	-0.702	0
-2.08	-5.1E-08	-1.61	-1.2E-08	-1.13	-1.2E-09	-0.687	0
-2.06	-4.6E-08	-1.59	-9.7E-09	-1.11	0	-0.672	0
-2.04	-4.4E-08	-1.57	-8.5E-09	-1.09	0	-0.657	0
-2.02	-4E-08	-1.55	-8.5E-09	-1.07	0	-0.642	0
-2	-3.8E-08	-1.52	-7.3E-09	-1.05	0	-0.627	0

3.5.3 Cyclic Voltammetry Output with Bluetooth Reading

After getting the results with ADC, we did bootloading as discussed in section 2.2.4.2 and separated the Atmega328P chip. The main goal was to read output with Bluetooth instead of Arduino. We did the similar work for PCB part in section 4.6. As mentioned in section 3.4.5, we could connect 2 Bluetooth modules accordingly and read the output values on serial monitor. Figure 3.10 shows the graphical representations of the cyclic voltammetry curves obtained by this procedure.

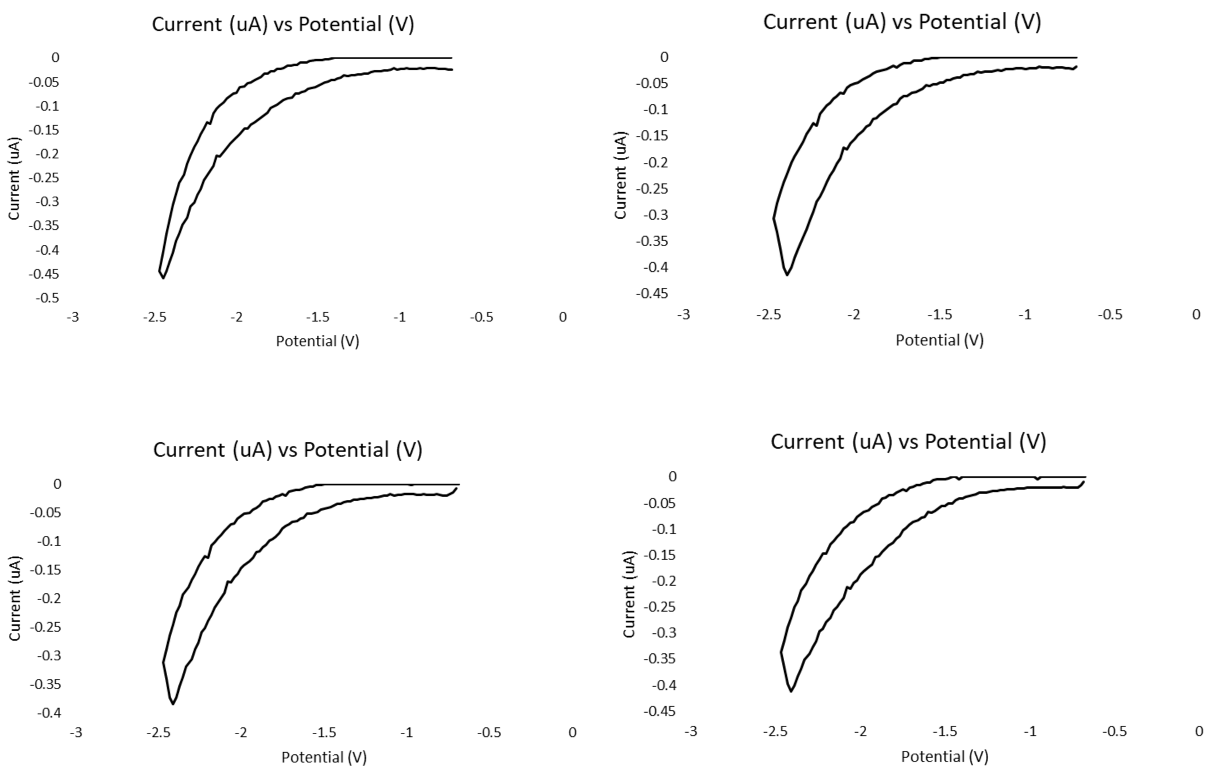


Figure 3. 10: Potentiostat cyclic voltammetry output reading with Bluetooth.

In this experiment, RN4871 CLICK Module was used as Bluetooth. During our experiments, there was a time delay in between compiling the code and finding and connecting to the Bluetooth. That's why sometimes the beginning and end points of CV curves of Figure 3.10 could not merge at a point near X-axis as the Bluetooth missed some initial values. Also, to minimize the quantization error due combined Atmega328P ADC and Bluetooth reading, the output gain was changed by changing resistor values to 20kΩ which fitted better at this stage of experiment. Table 4 shows a series of input voltage and output current values used for generating the curves.

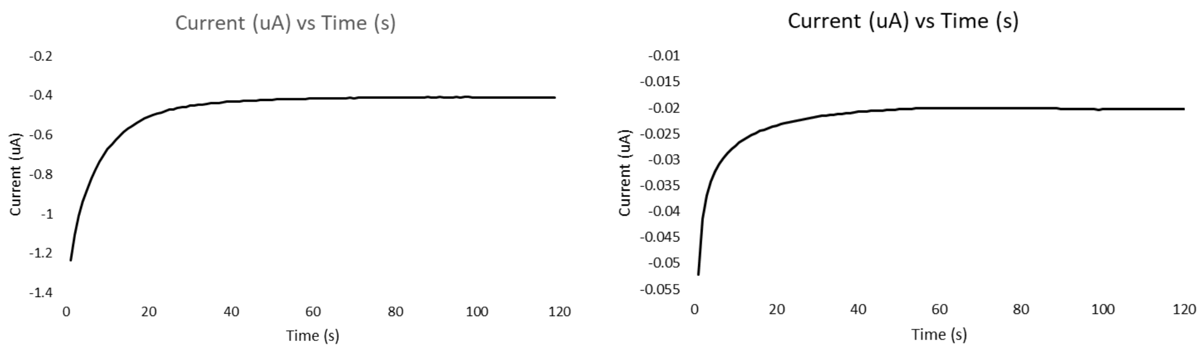
Table 4: Output Current vs Input voltage for cyclic voltammetry curves with Bluetooth Reading

V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)
-0.7	-1.59E-08	-1.157	-2.57E-08	-1.62	-7.58E-08	-2.1	-2.32E-07
-0.72	-1.96E-08	-1.177	-2.45E-08	-1.64	-7.82E-08	-2.12	-2.4E-07
-0.74	-1.96E-08	-1.197	-2.69E-08	-1.66	-8.31E-08	-2.14	-2.49E-07
-0.76	-1.96E-08	-1.22	-2.69E-08	-1.69	-8.68E-08	-2.16	-2.57E-07
-0.78	-1.96E-08	-1.24	-2.81E-08	-1.71	-9.17E-08	-2.18	-2.7E-07
-0.8	-1.83E-08	-1.26	-2.93E-08	-1.73	-9.65E-08	-2.2	-2.79E-07
-0.818	-1.96E-08	-1.28	-3.06E-08	-1.75	-1.01E-07	-2.22	-2.91E-07
-0.837	-1.96E-08	-1.3	-2.93E-08	-1.77	-1.14E-07	-2.24	-2.97E-07
-0.857	-1.96E-08	-1.32	-3.3E-08	-1.79	-1.19E-07	-2.26	-3.15E-07
-0.877	-1.96E-08	-1.34	-3.54E-08	-1.81	-1.26E-07	-2.28	-3.27E-07
-0.897	-2.08E-08	-1.36	-3.67E-08	-1.83	-1.31E-07	-2.3	-3.4E-07
-0.917	-2.08E-08	-1.38	-3.79E-08	-1.85	-1.38E-07	-2.33	-3.51E-07
-0.937	-2.08E-08	-1.4	-4.03E-08	-1.87	-1.44E-07	-2.35	-3.69E-07
-0.957	-2.08E-08	-1.42	-4.16E-08	-1.89	-1.52E-07	-2.37	-3.84E-07
-0.977	-2.08E-08	-1.44	-4.4E-08	-1.91	-1.54E-07	-2.39	-4.01E-07
-0.997	-2.08E-08	-1.46	-5.01E-08	-1.93	-1.67E-07	-2.41	-4.12E-07
-1.017	-2.08E-08	-1.48	-5.13E-08	-1.95	-1.74E-07	-2.43	-3.97E-07
-1.037	-2.2E-08	-1.5	-5.5E-08	-1.98	-1.81E-07	-2.45	-3.67E-07
-1.057	-2.2E-08	-1.52	-5.62E-08	-2	-1.87E-07	-2.47	-3.37E-07
-1.077	-2.2E-08	-1.54	-6.11E-08	-2.02	-1.98E-07	-2.45	-3.14E-07
-1.097	-2.32E-08	-1.56	-6.48E-08	-2.04	-2.04E-07	-2.43	-2.88E-07
-1.117	-2.32E-08	-1.58	-6.84E-08	-2.06	-2.14E-07	-2.41	-2.7E-07
-1.137	-2.32E-08	-1.6	-6.72E-08	-2.08	-2.11E-07	-2.39	-2.51E-07

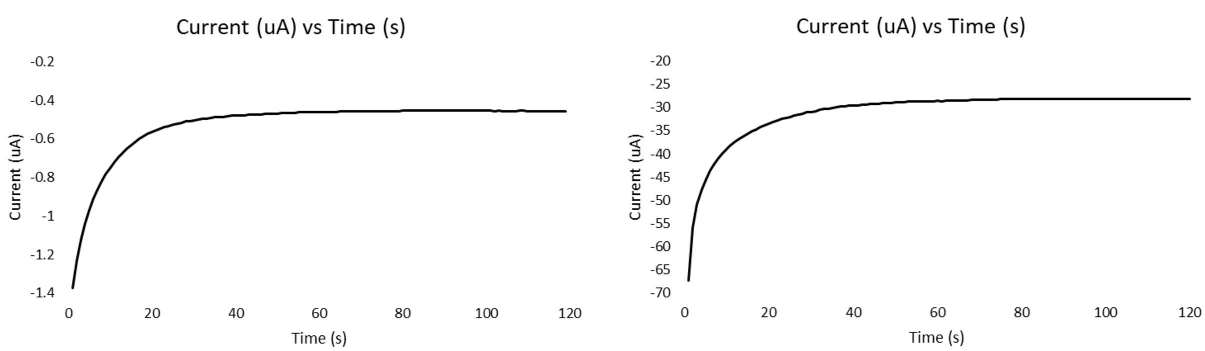
V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)
-2.37	-2.38E-07	-1.93	-5.74E-08	-1.5	-4.9E-09	-1.07	0
-2.35	-2.18E-07	-1.91	-5.38E-08	-1.48	-2.45E-09	-1.05	0
-2.32	-2.05E-07	-1.89	-5.01E-08	-1.46	0	-1.03	0
-2.3	-1.91E-07	-1.87	-4.16E-08	-1.44	0	-1.01	0
-2.28	-1.81E-07	-1.85	-3.91E-08	-1.42	-4.9E-09	-0.99	0
-2.26	-1.67E-07	-1.83	-3.54E-08	-1.4	0	-0.973	0
-2.24	-1.58E-07	-1.81	-3.42E-08	-1.38	0	-0.956	-3.68E-09
-2.22	-1.48E-07	-1.79	-2.93E-08	-1.36	0	-0.935	0
-2.2	-1.48E-07	-1.77	-2.69E-08	-1.34	0	-0.917	0
-2.18	-1.3E-07	-1.75	-2.32E-08	-1.32	0	-0.896	0
-2.16	-1.23E-07	-1.73	-2.69E-08	-1.3	0	-0.875	0
-2.14	-1.15E-07	-1.71	-1.96E-08	-1.28	0	-0.857	0
-2.12	-1.09E-07	-1.69	-1.83E-08	-1.26	0	-0.839	0
-2.1	-9.9E-08	-1.67	-1.47E-08	-1.24	0	-0.818	0
-2.08	-9.41E-08	-1.65	-1.47E-08	-1.22	0	-0.797	0
-2.06	-8.8E-08	-1.63	-1.22E-08	-1.2	0	-0.78	0
-2.04	-8.68E-08	-1.61	-1.1E-08	-1.18	0	-0.763	0
-2.02	-7.7E-08	-1.59	-9.78E-09	-1.15	0	-0.74	0
-2	-7.21E-08	-1.57	-6.1E-09	-1.13	0	-0.717	0
-1.98	-6.6E-08	-1.55	-4.9E-09	-1.11	0	-0.702	0
-1.95	-6.36E-08	-1.52	-3.68E-09	-1.09	0	-0.687	0

3.5.4 Chronoamperometry Output Measurements

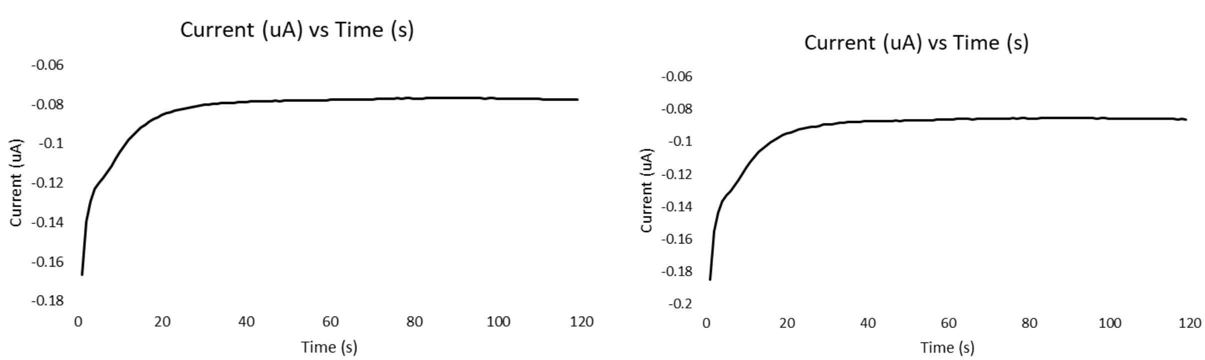
We took the chronoamperometry output current values using all three methods like cyclic voltammetry. Here, the outputs were more stable as they got saturated after a certain time. The output current was measured until 2 minutes as in Figure 3.11 to show the consistency of values. Table 5 shows a series of time and output current values used for generating the curves.



(a)



(b)



(c)

Figure 3. 11: Chronoamperometry output reading with (a) Multimeter (b) ADC and (c) Bluetooth

Table 5: Output Current vs Time for Chronoamperometry curves with Bluetooth Reading

Time (s)	I_{out} (A)	Time (s)	I_{out} (A)	Time (s)	I_{out} (A)	Time (s)	I_{out} (A)
1	-7.5E-05	31	-3.4E-05	61	-3.2E-05	91	-3.1E-05
2	-6.2E-05	32	-3.4E-05	62	-3.2E-05	92	-3.1E-05
3	-5.7E-05	33	-3.4E-05	63	-3.2E-05	93	-3.1E-05
4	-5.3E-05	34	-3.4E-05	64	-3.2E-05	94	-3.1E-05
5	-5.1E-05	35	-3.3E-05	65	-3.2E-05	95	-3.1E-05
6	-4.9E-05	36	-3.3E-05	66	-3.2E-05	96	-3.1E-05
7	-4.7E-05	37	-3.3E-05	67	-3.2E-05	97	-3.1E-05
8	-4.5E-05	38	-3.3E-05	68	-3.1E-05	98	-3.1E-05
9	-4.4E-05	39	-3.3E-05	69	-3.1E-05	99	-3.1E-05
10	-4.3E-05	40	-3.3E-05	70	-3.1E-05	100	-3.1E-05
11	-4.2E-05	41	-3.3E-05	71	-3.1E-05	101	-3.1E-05
12	-4.2E-05	42	-3.3E-05	72	-3.1E-05	102	-3.1E-05
13	-4.1E-05	43	-3.3E-05	73	-3.1E-05	103	-3.1E-05
14	-4E-05	44	-3.2E-05	74	-3.1E-05	104	-3.1E-05
15	-4E-05	45	-3.2E-05	75	-3.1E-05	105	-3.1E-05
16	-3.9E-05	46	-3.2E-05	76	-3.1E-05	106	-3.1E-05
17	-3.9E-05	47	-3.2E-05	77	-3.1E-05	107	-3.1E-05
18	-3.8E-05	48	-3.2E-05	78	-3.1E-05	108	-3.1E-05
19	-3.8E-05	49	-3.2E-05	79	-3.1E-05	109	-3.1E-05
20	-3.7E-05	50	-3.2E-05	80	-3.1E-05	110	-3.1E-05
21	-3.7E-05	51	-3.2E-05	81	-3.1E-05	111	-3.1E-05
22	-3.7E-05	52	-3.2E-05	82	-3.1E-05	112	-3.1E-05
23	-3.6E-05	53	-3.2E-05	83	-3.1E-05	113	-3.1E-05
24	-3.6E-05	54	-3.2E-05	84	-3.1E-05	114	-3.1E-05
25	-3.6E-05	55	-3.2E-05	85	-3.1E-05	115	-3.1E-05
26	-3.5E-05	56	-3.2E-05	86	-3.1E-05	116	-3.1E-05
27	-3.5E-05	57	-3.2E-05	87	-3.1E-05	117	-3.1E-05
28	-3.5E-05	58	-3.2E-05	88	-3.1E-05	118	-3.1E-05
29	-3.4E-05	59	-3.2E-05	89	-3.1E-05	119	-3.1E-05
30	-3.4E-05	60	-3.2E-05	90	-3.1E-05	120	-3.1E-05

CHAPTER 4: Implementation on PCB

4.1 Altium Design Files

After working with the bread board circuitry, the next step is to design PCB prototype and work with SMD components. For this purpose, we have firstly sorted out the equivalent SMD components of our bread board components. Next, as we discussed in section 2.3, we drew the circuit schematic in Altium designer. Here, we had to keep in mind if the footprint of all our required SMD components were available in Altium Manufacturer Library. Figure 4.1 shows the schematic diagram used to design the PCB prototype.

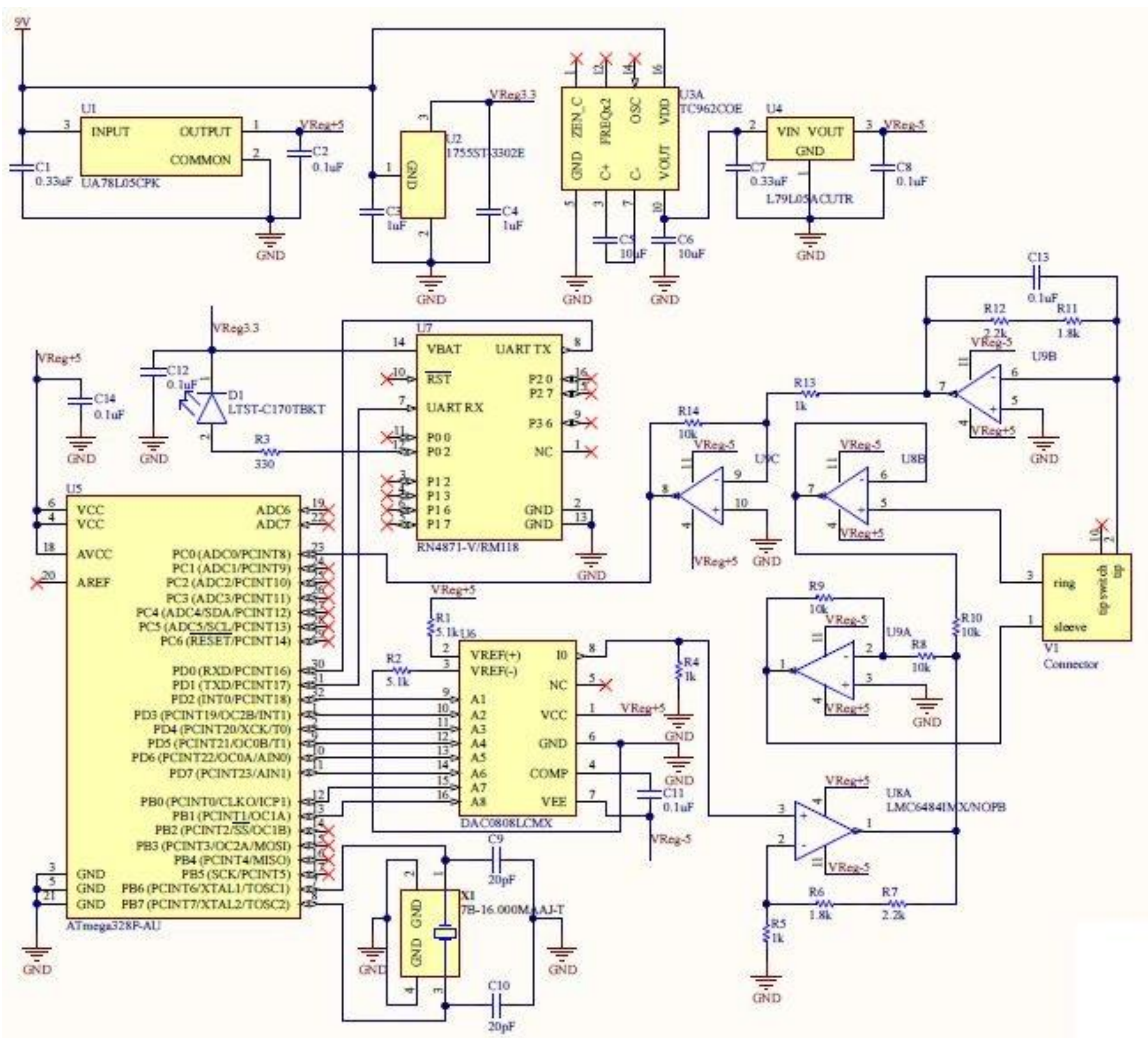
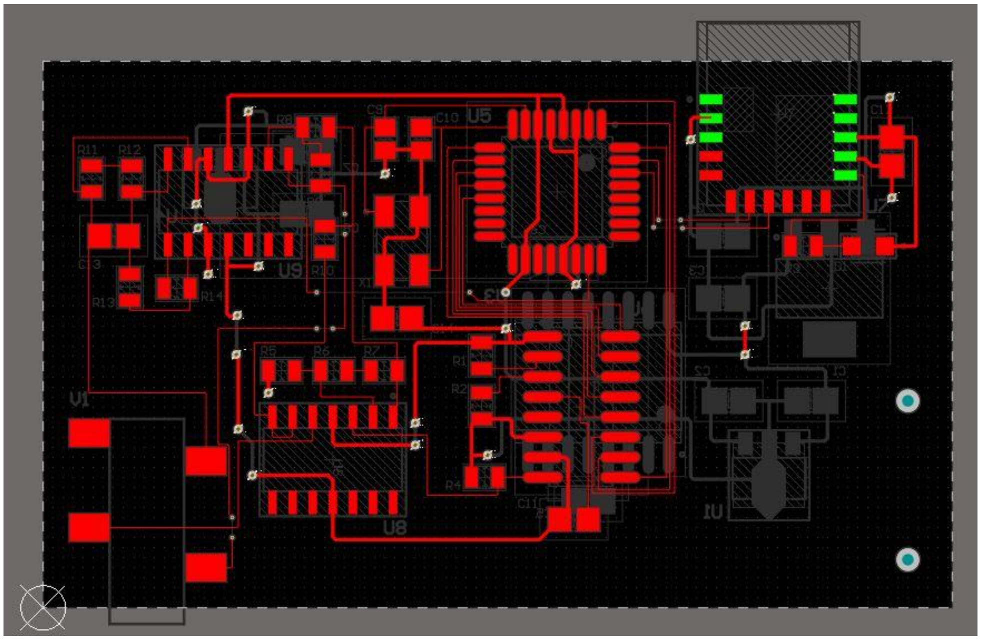
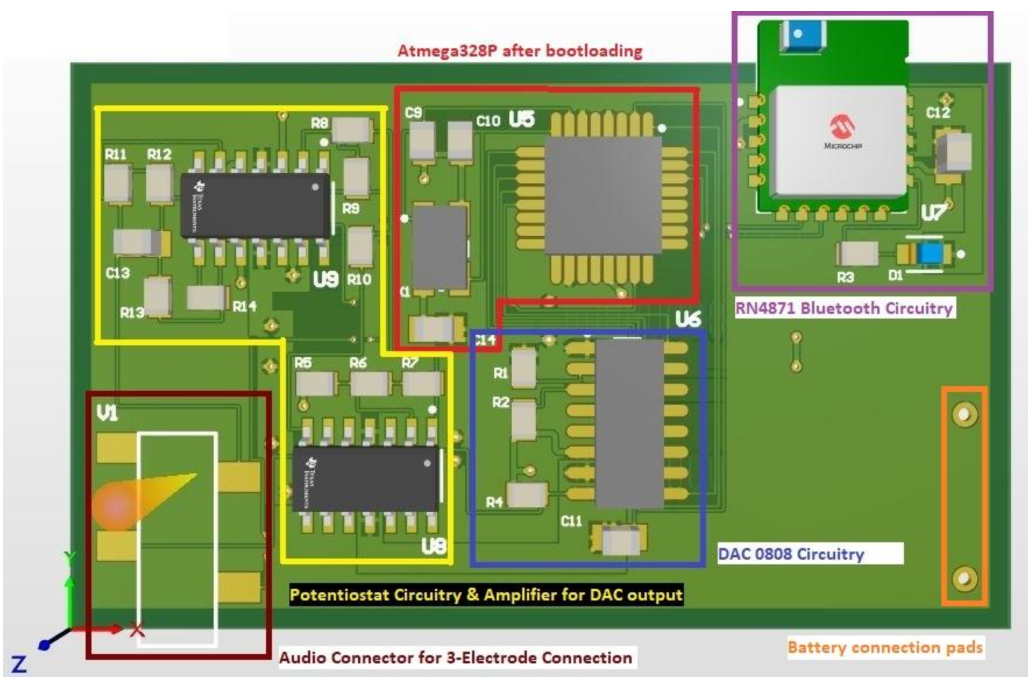


Figure 4. 1: Schematic diagram in Altium designer

When our schematic is ready, we proceed to generate the PCB layout. For the ease of the experiment, we have considered only two principal layers: top and bottom layer.



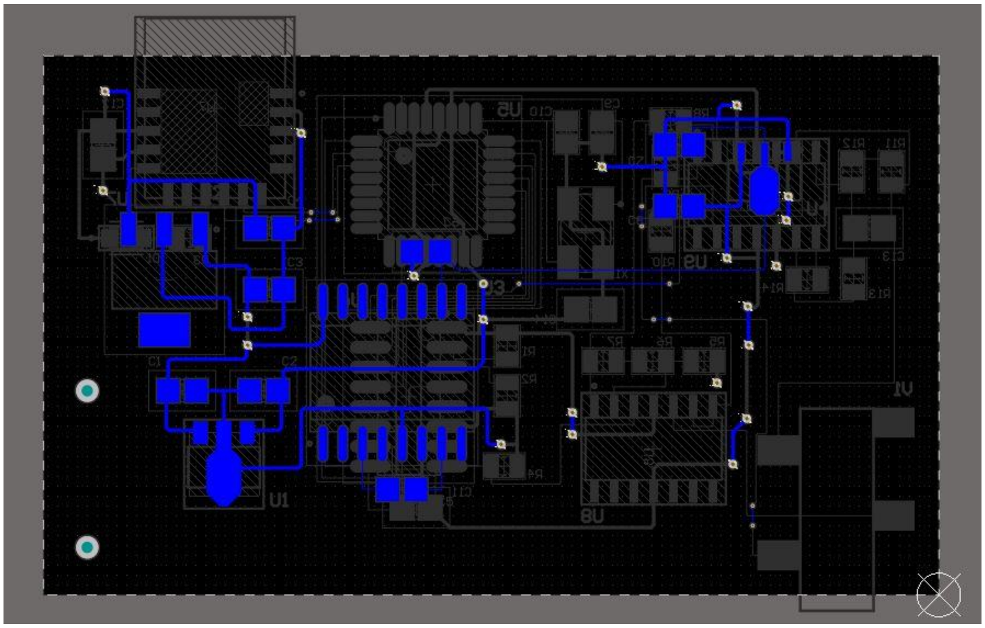
(a)



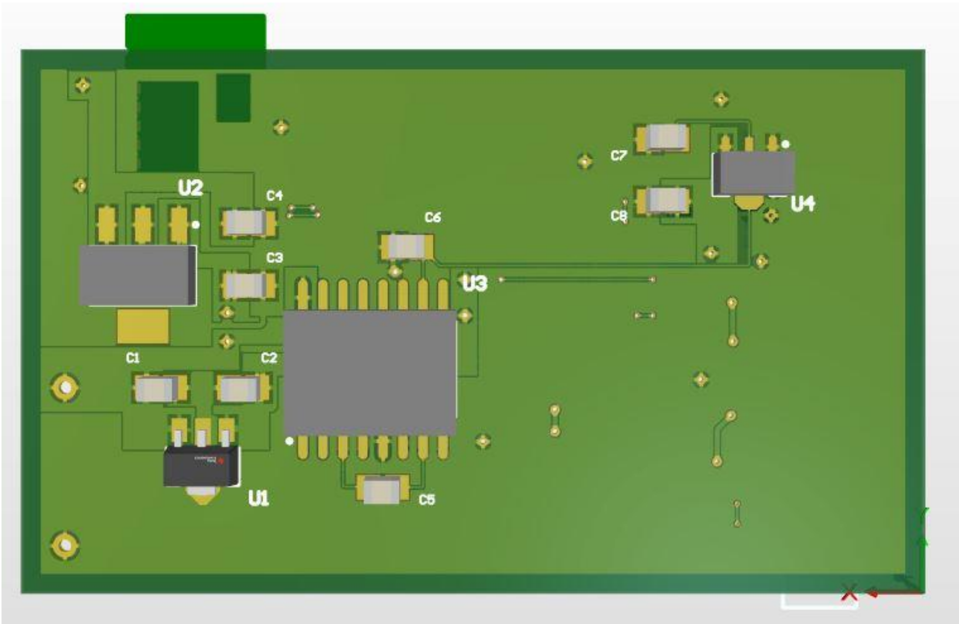
(b)

Figure 4. 2: (a) 2D and (b) 3D Layout Mode of Top Layer

The bottom layer contains all the voltage regulators which are responsible to power up the ICs on top layer. On the other hand, top layer consists of microcontroller, DAC, Bluetooth, potentiostat circuitry and audio connector which is responsible for 3-electrode connection.



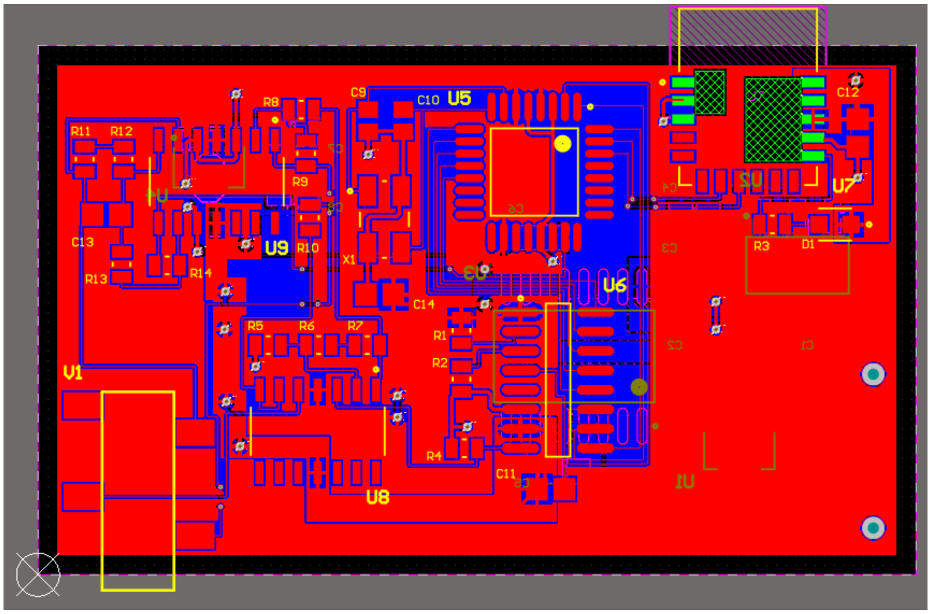
(a)



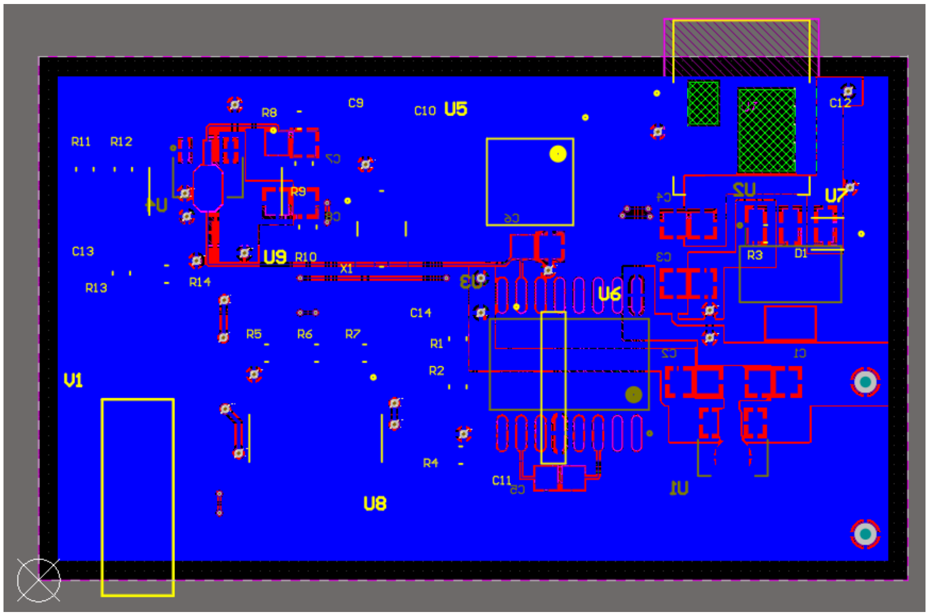
(b)

Figure 4. 3: (a) 2D and (b) 3D Layout Mode of Bottom Layer

Figure 4.2 shows 2D and 3D view of top layer. In the 2D view, all the polygon pours have been shelved for ease of visualization. The 3D view points out to all the separate ICs ad their purpose. On the right bottom side of top layer, two pads have been introduced as 9V and GND where we can connect a battery as the main power supply.



(a)



(b)

Figure 4. 4: Complete (a) Top and (b) Bottom view of PCB in 2D layout mode

Similarly, Figure 4.3 shows 2D and 3D view of bottom layer. For both Figure 4.2 (a) and 4.3 (a), the red and blue colors refer to the PCB footprint. This footprint allocates places for metal components to be soldered. Figure 4.2 (b) and 4.3 (b) show the 3D views after component placement. They are shown in our completed PCB module in Figure 4.9 (a) and 4.9 (b).

Next, we need to place Keepout Layer around our board and generate Gerber and NC Drill files for placing the order to manufacturer as mentioned in section 2.3.2. With all the layers and polygon pours the complete PCB design 2D view seems like Figure 4.4.

4.2 Working with SMD Components

While working with the SMD components, we need a dedicated workbench to pick and place them carefully. SMD components are very miniature in size which requires extra attention and practice to locate them successfully on PCB boards.

The first and foremost component needed is a soldering station which comes with a soldering iron and hot air gun. It is a multipurpose power soldering device designed for electronic components soldering. We can either solder metals with soldering iron or take small amount solder paste, place ICs correctly and used hot air gun. Proper temperature control and correct time duration is very important while using the hot air gun. Figure 4.5 (a) shows the soldering station setup.

The consistency of solder paste can be maintained by adding flux to it. As SMD components are very small, we only need a very little amount to mount them. So, we use a toothpick to put solder paste on PCB board surface. The flux has another important use. When we need to de-solder a component from the board, we can used sufficient amount of flux around it. Then we can blow the hot air gun which helps to pullout the component. Figure 4.5 (b) shows solder paste and flux inside a syringe to put a precise amount of it.

Another important component to maintain SMD components is tweezer. As they are very small in size, we need to pick them with one tweezer, place on PCB board and gently push with another tweezer while blowing hot air gun or soldering with metal. For our experiments we were using different tweezers depending on our need. They are shown in Figure 4.5 (c).

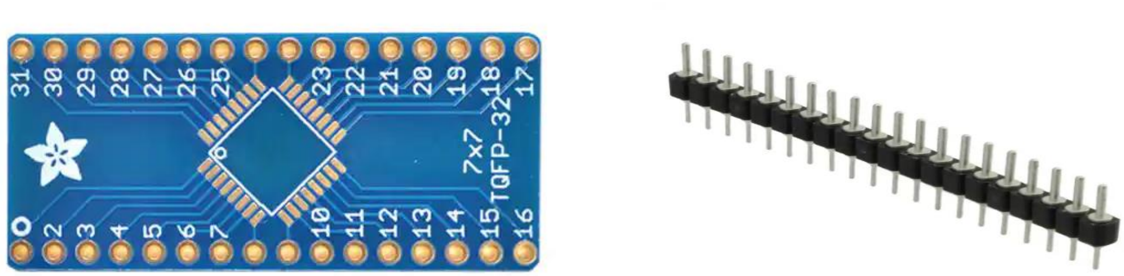
(a)

(b) (c)

Figure 4. 5:(a) Soldering station with hot air gun and iron (b) Solder Paste and Flux (c) Tweezers

4.3 Bootloading Atmega328P SMD Package

As described in section 2.2.4.2, we can use Arduino as ISP which works as a bootloader. It was the procedure for Atmega328P which is a 28 pin DIP package. While using a SMD package, we have a 32 pin Atmega328P TQFP (Thin Quad Flat Pack). Firstly, we need an adapter like Figure 4.6 (a) where we can place the IC. This adapter help to do the bootloading of an SMD package in a bread board with the help of Header connector Male pin like Figure 4.6 (b). Here we soldered the Male pins by adding metals with heated soldering iron. Then it was ready for using on breadboard.



(a) (b)

Figure 4. 6: (a) SMT Adapter 32 TQFP (b) Header connector Male pin

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Next, we soldered the Atmega328P IC in the middle of SMT adapter. So, now it just works like a DIP package and we did the bootloading in a similar manner. Figure 4.7 shows the required connections. After burning bootlader, we uploaded the code for our experiments, desoldered and picked the IC from adapter to be used on our PCB board.

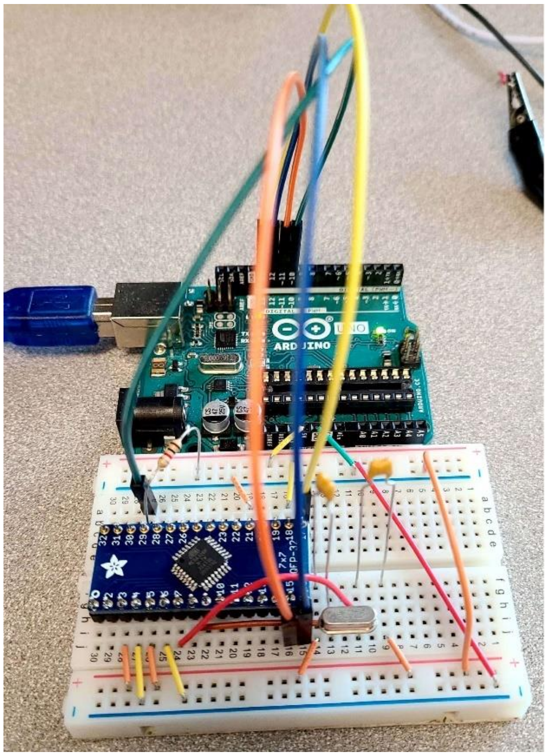

Figure 4. 7: Burning bootloader on Atmega328P TQFP.

4.4 Chemical Setup with Audio Connector

The chemical setup for bread board connection was shown in Figure 3.7. The electrodes submerged in solution were connected to their respective potentiostat location (or op-amp IC points) with the help of alligator clips and wires. While working on PCB, we chose an Audio Cable like Figure 4.8(b) as it has 3 different wires associated with it. We can connect the alligator clips from 3 electrodes to these 3 wires. Next, we have chosen a compatible Audio Jack like Figure 4.8(a). This Audio Jack will be mounted on the PCB and potentiostat circuit will get connected to electrodes by the Audio Connector.

(a) (b)

Figure 4. 8: (a) 3.50 mm Audio Jack for 3 conductors (b) 3.50 mm Audio Cable with 3′ length.

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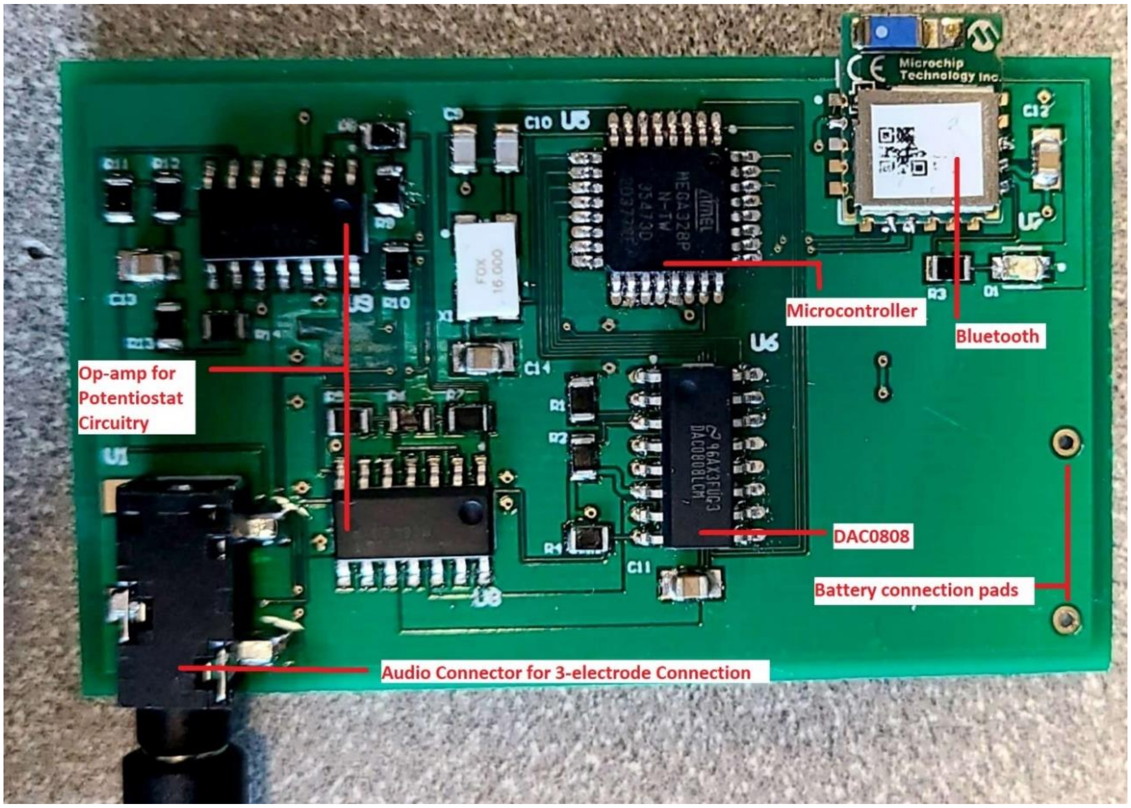
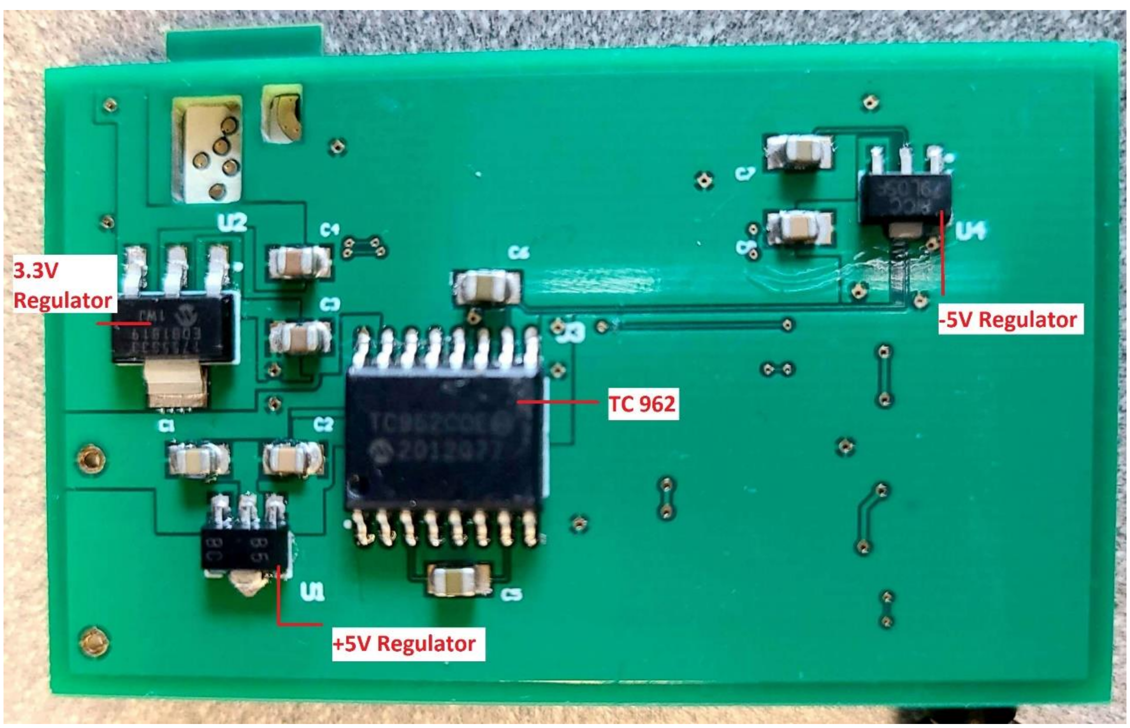
4.5 Complete Circuitry

After the successful placement of all required SMD components on our PCB prototype, our PCB board looks like Figure 4.9. It is an FR4 board ordered from PCBWay, China with a dimension of 57.4 mm x 34.5mm. We can compare them with Figure 4.2 and Figure 4.3. After preparing the PCB board, we put all other connection to power up our circuitry, receive output values by Bluetooth and display them on serial monitor. The total system is shown in Figure 4.10.



(a)



(b)

Figure 4. 9: (a) Top View and (b) Bottom View of complete PCB board

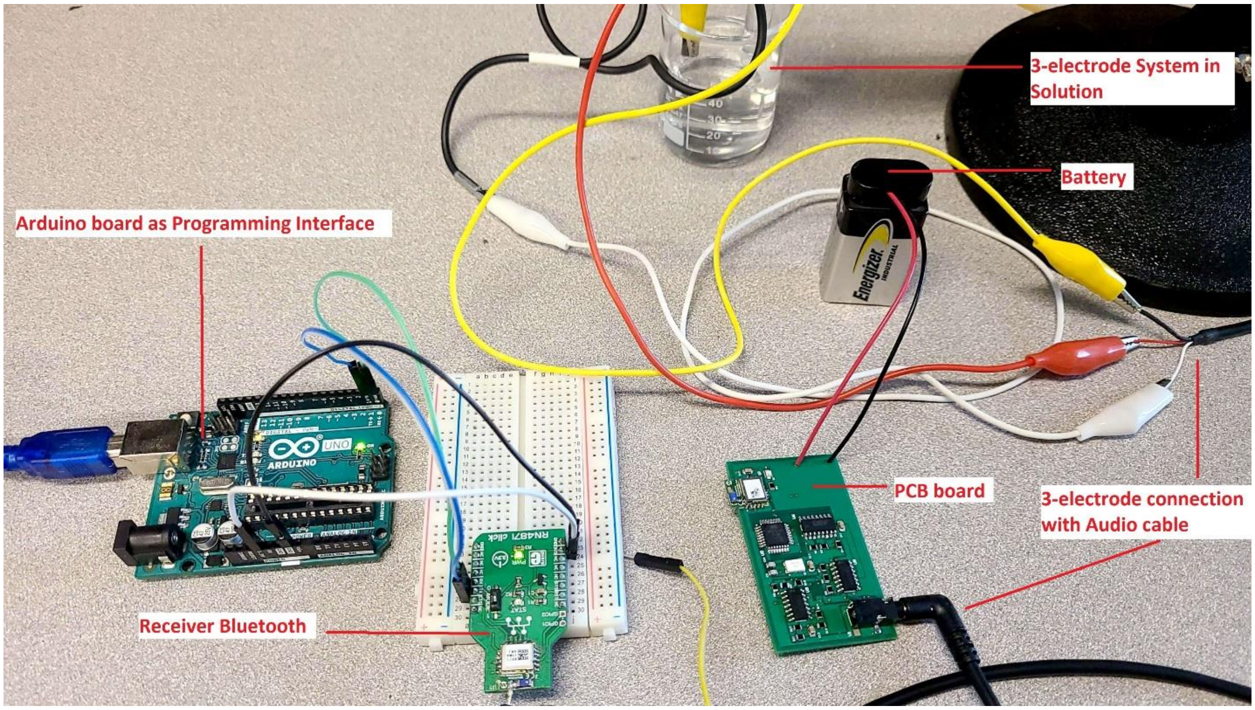


Figure 4. 10: Total system with PCB board for biosensor circuit

4.6 Results

In this section we will discuss about the Cyclic Voltammetry and Chrono Amperometry outputs we got on PCB board, measured with Bluetooth receiver.

After soldering all SMD components on PCB, we checked all the pinpoints with multimeter connectivity test. Then we powered up the PCB with 9V battery which also turned on the transmitter Bluetooth of PCB. Connecting the whole system like Figure 4.10 started giving us output values received by the RN4871 CLICK Module like section 3.4.5. Figure 4.11 shows the graphical representations of the CV curves obtained by this procedure.

As mentioned in section 3.5.3, there was a time delay in between compiling the code and finding and connecting to the Bluetooth CLICK module. This time we could reduce that delay as one of the CLICK modules got replaced by SMD Bluetooth part. So, CV curves of Figure 4.11 could merge nearly to X-axis. Next, the outputs were more stable like section 3.5.4. We measured the output current until 2 minutes as in Figure 4.12 to show the consistency of values. We had the transition from CV to CA by selecting from a switch option in Arduino IDE.

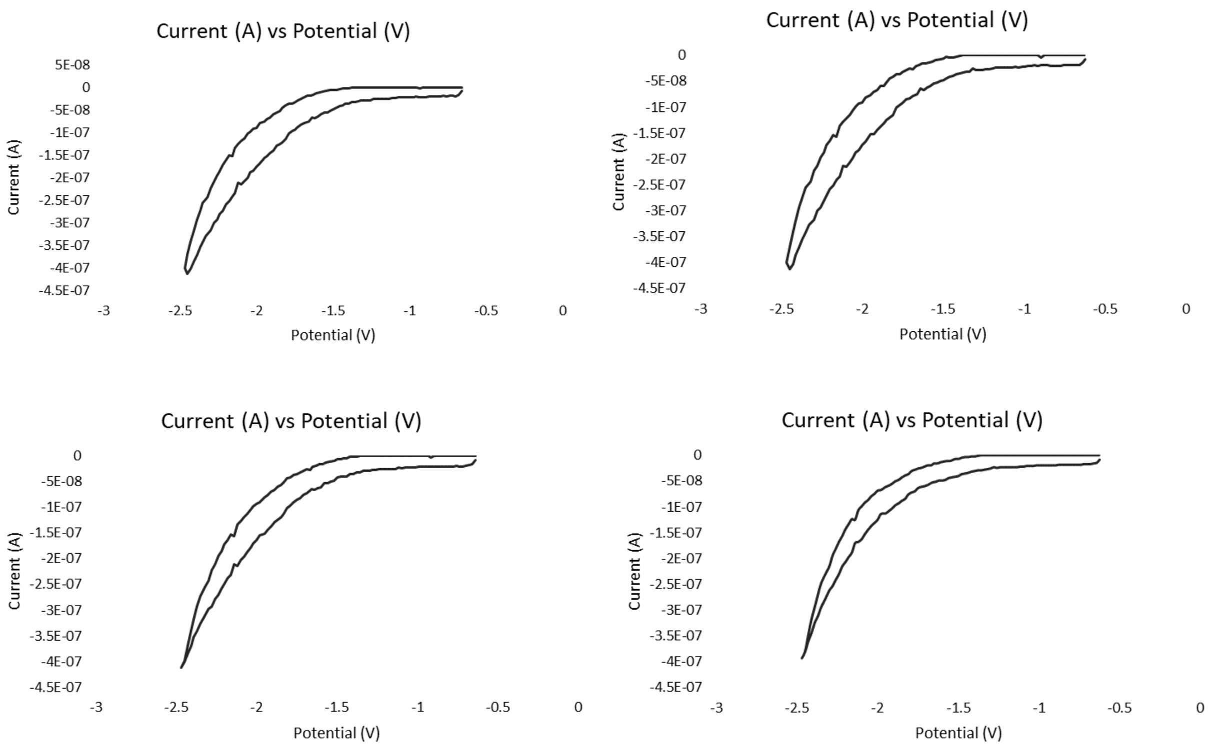


Figure 4. 11: Cyclic Voltammetry output reading with PCB board

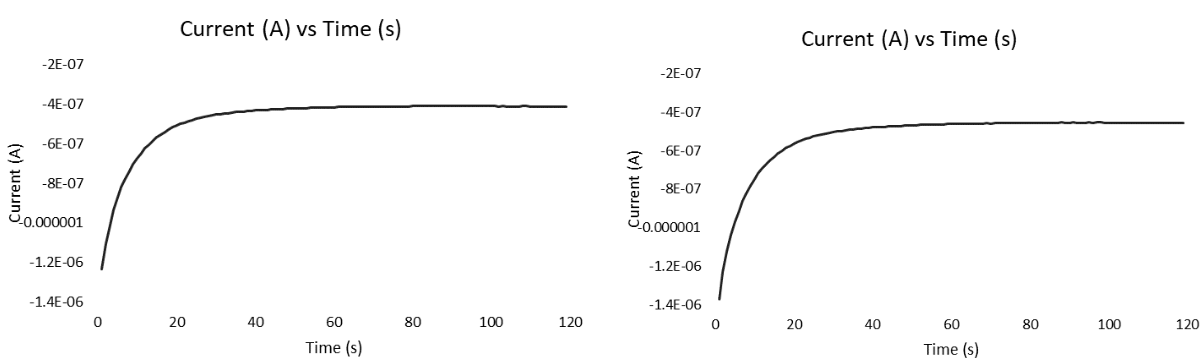


Figure 4. 12: Chronoamperometry output reading with PCB board

Table 6 and 7 show a series of data used for CV and CA experiments respectively with integrated PCB system.

Table 6: Output Current vs Input voltage for cyclic voltammetry curves with PCB system

V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)
-0.624	-7.3E-09	-1.077	-2.3E-08	-1.54	-5.3E-08	-2.02	-1.8E-07
-0.642	-1.5E-08	-1.097	-2.4E-08	-1.56	-5.4E-08	-2.04	-1.9E-07
-0.662	-1.8E-08	-1.117	-2.4E-08	-1.58	-6E-08	-2.06	-2E-07
-0.681	-1.8E-08	-1.137	-2.4E-08	-1.6	-6.2E-08	-2.08	-2.1E-07
-0.7	-2E-08	-1.157	-2.4E-08	-1.62	-6.7E-08	-2.1	-2.2E-07
-0.72	-2E-08	-1.177	-2.4E-08	-1.64	-6.5E-08	-2.12	-2.1E-07
-0.74	-2E-08	-1.197	-2.6E-08	-1.66	-7.5E-08	-2.14	-2.3E-07
-0.76	-2E-08	-1.22	-2.7E-08	-1.69	-7.8E-08	-2.16	-2.4E-07
-0.78	-2.1E-08	-1.24	-2.7E-08	-1.71	-8.3E-08	-2.18	-2.5E-07
-0.8	-2.1E-08	-1.26	-2.8E-08	-1.73	-8.6E-08	-2.2	-2.6E-07
-0.818	-2.1E-08	-1.28	-2.8E-08	-1.75	-9.2E-08	-2.22	-2.7E-07
-0.837	-2.1E-08	-1.3	-2.9E-08	-1.77	-9.5E-08	-2.24	-2.8E-07
-0.857	-2E-08	-1.32	-2.6E-08	-1.79	-1E-07	-2.26	-2.9E-07
-0.877	-2E-08	-1.34	-3.2E-08	-1.81	-1.1E-07	-2.28	-3E-07
-0.897	-2E-08	-1.36	-3.2E-08	-1.83	-1.2E-07	-2.3	-3.2E-07
-0.917	-2.1E-08	-1.38	-3.4E-08	-1.85	-1.2E-07	-2.33	-3.3E-07
-0.937	-2E-08	-1.4	-3.5E-08	-1.87	-1.3E-07	-2.35	-3.4E-07
-0.957	-2.1E-08	-1.42	-3.7E-08	-1.89	-1.4E-07	-2.37	-3.5E-07
-0.977	-2.1E-08	-1.44	-3.9E-08	-1.91	-1.4E-07	-2.39	-3.7E-07
-0.997	-2.2E-08	-1.46	-4.2E-08	-1.93	-1.5E-07	-2.41	-3.8E-07
-1.017	-2.2E-08	-1.48	-4.4E-08	-1.95	-1.5E-07	-2.43	-4E-07
-1.037	-2.3E-08	-1.5	-4.8E-08	-1.98	-1.7E-07	-2.45	-4.1E-07
-1.057	-2.2E-08	-1.52	-5E-08	-2	-1.7E-07	-2.47	-4E-07

V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)	V_{in} (V)	I_{out} (A)
-2.45	-3.7E-07	-1.98	-8.2E-08	-1.5	-6.1E-09	-1.03	0
-2.43	-3.4E-07	-1.95	-7.7E-08	-1.48	-3.7E-09	-1.01	0
-2.41	-3.2E-07	-1.93	-7.1E-08	-1.46	-4.9E-09	-0.99	0
-2.39	-2.9E-07	-1.91	-6.7E-08	-1.44	-4.9E-09	-0.973	0
-2.37	-2.7E-07	-1.89	-6E-08	-1.42	-2.5E-09	-0.956	0
-2.35	-2.6E-07	-1.87	-5.9E-08	-1.4	-1.2E-09	-0.935	0
-2.32	-2.4E-07	-1.85	-5.3E-08	-1.38	0	-0.917	0
-2.3	-2.2E-07	-1.83	-4.4E-08	-1.36	0	-0.896	0
-2.28	-2.1E-07	-1.81	-4.2E-08	-1.34	0	-0.875	0
-2.26	-2E-07	-1.79	-3.7E-08	-1.32	0	-0.857	0
-2.24	-1.9E-07	-1.77	-3.7E-08	-1.3	0	-0.839	0
-2.22	-1.7E-07	-1.75	-3.2E-08	-1.28	0	-0.818	0
-2.2	-1.6E-07	-1.73	-2.9E-08	-1.26	0	-0.797	0
-2.18	-1.5E-07	-1.71	-2.6E-08	-1.24	0	-0.78	0
-2.16	-1.6E-07	-1.69	-2.7E-08	-1.22	0	-0.763	0
-2.14	-1.4E-07	-1.67	-2.1E-08	-1.2	0	-0.74	0
-2.12	-1.3E-07	-1.65	-2E-08	-1.18	0	-0.717	0
-2.1	-1.2E-07	-1.63	-1.6E-08	-1.15	0	-0.702	0
-2.08	-1.1E-07	-1.61	-1.6E-08	-1.13	0	-0.687	0
-2.06	-1.1E-07	-1.59	-1.3E-08	-1.11	0	-0.672	0
-2.04	-9.9E-08	-1.57	-1.2E-08	-1.09	0	-0.657	0
-2.02	-9.3E-08	-1.55	-9.8E-09	-1.07	0	-0.642	0
-2	-9.2E-08	-1.52	-7.3E-09	-1.05	0	-0.627	0

Table 7: Output Current vs Time for chronoamperometry curves with PCB system

Time (s)	I_{out} (A)	Time (s)	I_{out} (A)	Time (s)	I_{out} (A)	Time (s)	I_{out} (A)
1	-1.9E-07	31	-8.9E-08	61	-8.6E-08	91	-8.5E-08
2	-1.6E-07	32	-8.9E-08	62	-8.6E-08	92	-8.5E-08
3	-1.4E-07	33	-8.8E-08	63	-8.6E-08	93	-8.5E-08
4	-1.4E-07	34	-8.8E-08	64	-8.6E-08	94	-8.5E-08
5	-1.3E-07	35	-8.8E-08	65	-8.6E-08	95	-8.5E-08
6	-1.3E-07	36	-8.8E-08	66	-8.6E-08	96	-8.5E-08
7	-1.3E-07	37	-8.8E-08	67	-8.6E-08	97	-8.6E-08
8	-1.2E-07	38	-8.8E-08	68	-8.6E-08	98	-8.5E-08
9	-1.2E-07	39	-8.8E-08	69	-8.6E-08	99	-8.5E-08
10	-1.2E-07	40	-8.8E-08	70	-8.6E-08	100	-8.6E-08
11	-1.1E-07	41	-8.7E-08	71	-8.6E-08	101	-8.6E-08
12	-1.1E-07	42	-8.7E-08	72	-8.6E-08	102	-8.6E-08
13	-1.1E-07	43	-8.7E-08	73	-8.6E-08	103	-8.6E-08
14	-1E-07	44	-8.7E-08	74	-8.6E-08	104	-8.6E-08
15	-1E-07	45	-8.7E-08	75	-8.6E-08	105	-8.6E-08
16	-1E-07	46	-8.7E-08	76	-8.5E-08	106	-8.6E-08
17	-9.9E-08	47	-8.7E-08	77	-8.6E-08	107	-8.6E-08
18	-9.7E-08	48	-8.7E-08	78	-8.5E-08	108	-8.6E-08
19	-9.6E-08	49	-8.7E-08	79	-8.5E-08	109	-8.6E-08
20	-9.5E-08	50	-8.7E-08	80	-8.6E-08	110	-8.6E-08
21	-9.4E-08	51	-8.7E-08	81	-8.6E-08	111	-8.6E-08
22	-9.3E-08	52	-8.7E-08	82	-8.6E-08	112	-8.6E-08
23	-9.3E-08	53	-8.7E-08	83	-8.5E-08	113	-8.6E-08
24	-9.2E-08	54	-8.7E-08	84	-8.5E-08	114	-8.6E-08
25	-9.1E-08	55	-8.7E-08	85	-8.5E-08	115	-8.6E-08
26	-9.1E-08	56	-8.7E-08	86	-8.5E-08	116	-8.6E-08
27	-9.1E-08	57	-8.6E-08	87	-8.5E-08	117	-8.6E-08
28	-9E-08	58	-8.6E-08	88	-8.5E-08	118	-8.6E-08
29	-8.9E-08	59	-8.6E-08	89	-8.5E-08	119	-8.6E-08
30	-8.9E-08	60	-8.6E-08	90	-8.5E-08	120	-8.6E-08

CHAPTER 5: Conclusion and Future Work

This research work involves designing a system on board for mini potentiostat that is operated by a microcontroller and can transmit data wirelessly to a base station.

- By replacing the battery with a Li-Polymer thin battery and placing the circuit board on the top of the battery, the mini potentiostat can be easily integrated within the handle of the e-scalpel.
- This mini potentiostat when connected to the miniaturized electrochemical cell on a scalpel blade would allow the oral surgeon to wirelessly transmit data indicating the status of the tissue, he/she is removing and see the status in a monitor in front of him/her. This would give them real-time feedback instead of waiting for lab result on excised tissue after surgery. This would in-turn reduce the waiting time and cost of health care system.
- The bulky wired potentiostat available in market is not good for practical use as it would involve long wires to surgeon's scalpel and would create hindrance in their movement. In short, the mini-potentiostat has potentials to integrate with e-scalpel for a hassle-free examination with easy mobility for surgeon.
- The mini potentiostat is placed on scalpel handle where the blade end works as biomarker. The biomarker can be inserted inside oral area, data can be taken and further diagnosed for cancer detection purpose. Depending on application type and potentiostat size, blade length and handle perimeter can be changed respectively to manufacture a compatible detection tool.
- As mentioned earlier, we used RN 4871 CLICK module to transmit data wirelessly. While it worked for our PCB system, there was a proposal to test it with other available devices at hospital to check for any occurring interference resulting from EM wave or noise. But due to COVID-19, the hospitals provided limited emergency access. So, it was not possible to do any extended research work in collaboration with them.

In future, there can be certain changes included to potentially enhance this design for oral cancer detection.

- The PCB design could be improvised by adding more pads for specific testing pins. These pads will further help to check the result outputs in a step-by-step manner with different ways like bread board circuit.
- The RN 4871 CLICK module can be replaced with a better one to provide more accurate graphical structure with less attempts.
- The Arduino ‘Switch’ operation can be done in a more automated way by using GUI in MATLAB coding. The GUI will provide an easier way to choose between CV and CA by a press button with mobile application like icon rather than finding alphabetical keys.
- A thin Li-Polymer battery can be used as a substitute for Alkaline battery. The reduced battery size would help to place the whole circuit arrangement on the e-scalpel.

References

[1] F.J. Holler, D.A. Skoog, S.R. Crouch, *Principles of Instrumental Analysis*, sixth edition, Thomson Brooks/ Cole, 2007.

[2] Lowe, C. R. *Biosensors. Trends in Biotechnology* 1984, 2(3), 59–65.

[3] Ma'mun A, Abd El-Rahman MK, Abd El-Kawy M. Real-time potentiometric sensor; an innovative tool for monitoring hydrolysis of chemo/bio-degradable drugs in pharmaceutical sciences. *Journal of pharmaceutical and biomedical analysis*. 2018 May 30; 154:166-73.

[4] Ambrosi, A.; Pumera, M. *Phys. Chem. Chem. Phys.* 2010, 12, 8943–8947.

[5] Chang, J.-L.; Chang, K.-H.; Hu, C.-C.; Cheng, W.-L.; Zen, J.-M. *Electrochem. Commun.* 2010, 12, 596–599.

[6] Wang, J. *Analytical Electrochemistry*, 3rd ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2006.

[7] Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2001.

[8] Stetter JR, Li J. Amperometric gas sensors a review. *Chemical reviews*. 2008 Feb 13;108(2):352-66.

[9] Chen L, Tang Y, Wang K, Liu C, Luo S. Direct electrodeposition of reduced graphene oxide on glassy carbon electrode and its electrochemical application. *Electrochemistry communications.* 2011 Feb 1;13(2):133-7.

[10] Adler SB. Reference electrode placement in thin solid electrolytes. *Journal of the electrochemical society*. 2002 Apr 2;149(5): E166.

[11] Eggins BR. Chemical sensors and biosensors. John Wiley & Sons; 2002 Apr 26.

[12] Zhang DW, Li XD, Li HB, Chen S, Sun Z, Yin XJ, Huang SM. Graphene-based counter electrode for dye-sensitized solar cells. *Carbon.* 2011 Dec 1;49(15):5382-8.

[13] testequipmentdepot, Ptentiostats, "Keithley", (Graphical Potentiostat, 2460-EC) https://www.testequipmentdepot.com/keithley/potentiostats/graphical-potentiostat-2460ec.htm

[14] Gamry Instruments. Application Notes: Potentiostat Primer. http://www.gamry.com/App_Notes/Potentiostat_Primer.htm.

[15] A. S. Paterson *et al.*, “A low-cost smartphone-based platform for highly sensitive point-of-care testing with persistent luminescent phosphors," *Lab Chip*, vol. 17, no. 6, pp. 1051_1059, 2017.

[16] O. Kujan, A.M. Glenny, R. Oliver, N. Thakker, P. Sloan, Screening programmesfor the early detection and prevention of oral cancer, *Cochrane Libr*. (2006).

[17] Bhatt, A.N., Mathur, R., Farooque, A., Verma, A., Dwarakanath, B.S., 2010. *Indian J. Med.* Res.132,129–149.

[18] Adams SD, Doeven EH, Quayle K, Kouzani AZ. MiniStat: Development and evaluation of a mini-potentiostat for electrochemical measurements. *IEEE access*. 2019 Mar 4; 7:31903-12.

[19] Elgrishi N, Rountree KJ, McCarthy BD, Rountree ES, Eisenhart TT, Dempsey JL. A practical beginner’s guide to cyclic voltammetry. Journal of chemical education. 2018 Feb 13;95(2):197-206.

[20] Rezaei B, Irannejad N. Electrochemical detection techniques in biosensor applications. InElectrochemical Biosensors 2019 Jan 1 (pp. 11-43). Elsevier.

[21] Kamat A, Huth A, Klein O, Scholl S. Chronoamperometric investigations of the electrode–electrolyte interface of a commercial high temperature PEM fuel cell. Fuel Cells. 2010 Dec;10(6):983-92.

[22] Lefrou, C., Pierre Fabry, J.-C.P., 2012. Electrochemistry: The Basics, with Examples.

[23] ossila, Products, “Working Electrode”, https://www.ossila.com/products/working-electrode

[24] basinc, Products, “Auxiliary Electrode”, https://www.basinc.com/products/ec/auxele

[25] Bobacka J, Ivaska A, Lewenstam A. Potentiometric ion sensors. Chemical reviews. 2008 Feb 13;108(2):329-51.

[26] Bakker E, Pretsch E. Nanoscale potentiometry. TrAC Trends in Analytical Chemistry. 2008 Jul 1;27(7):612-8.

[27] Lindner E, Gyurcsányi RE. Quality control criteria for solid-contact, solvent polymeric membrane ion-selective electrodes. Journal of Solid-State Electrochemistry. 2009 Jan 1;13(1):51-68.

[28] Porada R, Jedlińska K, Lipińska J, Baś B. Voltammetric Sensors with Laterally Placed Working Electrodes: A Review. Journal of The Electrochemical Society. 2020 Jan 13;167(3):037536.

[29] Lovander MD, Lyon JD, Parr IV DL, Wang J, Parke B, Leddy J. Critical Review—Electrochemical properties of 13 vitamins: a critical review and assessment. Journal of the Electrochemical Society. 2018 Jan 27;165(2):G18.

[30] Heineman, William R. and Kissinger, Peter T. "Laboratory Techniques in Electroanalytical Chemistry." Dekker, New York, 1996.

[31] Bogdan M, Brugger D, Rosenstiel W, Speiser B. Estimation of diffusion coefficients from voltammetric signals by support vector and gaussian process regression. Journal of cheminformatics. 2014 Dec;6(1):1-3.

[32] Uchida, Y.; Kätelhön, E.; Compton, R. G., Cyclic voltammetry with non-triangular waveforms: Electrochemically irreversible and quasi-reversible systems. J. Electroanal. Chem. 2018, 810, 135-144.

[33] Elgrishi, N.; Rountree, K. J.; McCarthy, B. D.; Rountree, E. S.; Eisenhart, T. T.; Dempsey, J. L., A Practical Beginner's Guide to Cyclic Voltammetry. J. Chem. Educ. 2017, 95 (2), 197-206.

[34] Maiolo L, Notargiacomo A, Marrani M, Minotti A, Maita F, Pecora A. Ultra-flexible microelectrode array nanostructured by FIB: A possible route to lower the device impedance. Microelectronic engineering. 2014 Jun 1; 121:10-4.

[35] Gayakwad RA. Op-amps and linear integrated circuit technology. Prentice Hall; 1983.

[36] Fiore JM. Operational amplifiers and linear integrated circuits: Theory and application. West Publishing Company; 1992 Jan.

[37] Monolithic Power: Voltage Regulator types: https://www.monolithicpower.com/en/voltage-regulator-types

[38] Texas Instruments (2016) UA78L00 Series Positive-Voltage Linear Regulators https://www.ti.com/lit/ds/symlink/ua78l.pdf

[39] Microchip Technology (2012) MCP1755/MCP1755S High-Performance LDO https://ww1.microchip.com/downloads/en/DeviceDoc/MCP1755-MCP1755S.pdf

[40] Texas Instruments (2003) MC7900 Series Negative-Voltage Regulators https://www.ti.com/lit/ds/symlink/mc79l.pdf

[41] Microchip Technology (2001-2012) TC962 High Current Charge Pump DC-to-DC Converter https://ww1.microchip.com/downloads/en/DeviceDoc/21484D.pdf

[42] Energizer Battery Company 9V Alkaline Zinc-Manganese Dioxide Battery https://data.energizer.com/pdfs/EN22.pdf

[43] Keystone Electronics (2017) Shielded 9V Battery Straps https://www.keyelco.com/userAssets/file/M65p30.pdf

[44] Electronics Hub (2021) Arduino Uno Board Layout https://www.electronicshub.org/arduino-uno-pinout/

[45] arduino.cc (2018) Built in Examples: Arduino to Breadboard https://www.arduino.cc/en/Tutorial/BuiltInExamples/ArduinoToBreadboard

[46] Texas Instruments (2011) DAC0808 8-Bit D/A Converter https://www.ti.com/lit/ds/symlink/dac0808.pdf

[47] Microchip Technology (2016) RN4870/71 Bluetooth® 4.2 Low Energy Module https://download.mikroe.com/documents/datasheets/rn4870.pdf

[48] Adafruit Industries LLC (2014) SMT Aadpters 3 Pack 32 QFN / TQFP https://www.adafruit.com/product/1163

[49] Harwin Inc. (2014) 20 Position SIP, SIL - Header Connector Male Pin Tin https://cdn.harwin.com/pdfs/C004XX_Round_Pin_IC_Sockets.pdf

[50] CUI Devices (2019) 3.50mm Headphone Phone Jack Stereo 3 Conductor Connector Solder https://www.cuidevices.com/product/resource/sj2-3581x-smt.pdf

[51] Tensility International Corp (2017) 3 Conductor Cable Stereo Phone Plug, 3.0' (91.44cm) https://tensility.s3.amazonaws.com/uploads/pdffiles/10-02143.pdf

[52] Badamasi YA. The working principle of an Arduino. In 2014 11th international conference on electronics, computer and computation (ICECCO) 2014 Sep 29 (pp. 1-4). IEEE.

[53] Galadima AA. Arduino as a learning tool. In2014 11th International Conference on Electronics, Computer and Computation (ICECCO) 2014 Sep 29 (pp. 1-4). IEEE.

[54] M. Banzi, Getting Started with arduino. O'Reilly Media, Inc., 2009.

[55] Nwoye CD, Usikalu MR, Babarimisa IO, Achuka JA, Ayara WA. Construction of an automatic power switch using infrared motion sensor. Journal of Informatics and Mathematical Sciences. 2017;9(2):331-7.

[56] Ashram FA. DAC Interfacing with 8051.

[57] Beltran Jr AA, Hiwatig CD, Laguna–Agustin NJ, Villanueva MB. Teaching Electronic Circuits Using Altium Designer. International Journal of Scientific Engineering and Technology. 2014;3(10):1239-43.

[58] Zhang HZ, Zhang P. Integrated design of electronic product based on altium designer [j]. Radio Commun. Technol. 2008; 6:19.

[59] Jordan B. Designer adaptation in the changing PCB industry. EE-Evaluation Engineering. 2017 Nov 1;56(11):20-3.

[60] Zlatanov N. PCB Design Process and Fabrication Challenges. InPCB West Conference 2012 Sep.

[61] Ghanim MH, Abdullah MZ. Design of disposable DNA biosensor microchip with amperometric detection featuring PCB substrate. BioChip Journal. 2013 Mar 1;7(1):51-6.

[62] Jolly P, Rainbow J, Regoutz A, Estrela P, Moschou D. A PNA-based Lab-on-PCB diagnostic platform for rapid and high sensitivity DNA quantification. Biosensors and Bioelectronics. 2019 Jan 1; 123:244-50.

Appendix

Arduino code to choose between CV and CA operation

```
int ledPin[] = {9,8,7,6,5,4,3,2}; // array of pins used in the program
int maxCount = 128;               // used for looping through each number
int delayInterval = 1000;         // delay between each light change
const int adcch1 = A0;            // ADC channel output

void setup()
{
  Serial.begin(115200);
  // Set up each pin as an output pin
  for (int i=0; i<8; i++)
  {
    pinMode(ledPin[i], OUTPUT);
  }
}

void loop()
{
  if (Serial.available()>0)
  {
    int inByte=Serial.read();

    switch(inByte)
    {
      case 'a': //Cyclic Voltammetry
          // LOOP through each number (32 to 127)
          for (int counter = 32; counter < maxCount; counter++)
          {
            // pass the count number to display Binary function
            displayBinary(counter);
            readAnalogChannel();
            delay(delayInterval);
          }
          for (int counter = maxCount-1; counter >= 32; counter--)
          {
            // pass the count number to display Binary function
            displayBinary(counter);
            readAnalogChannel();
            delay(delayInterval);
          }
          delay(3000);
          for(;;);
          break;

      case 'b':  //Chronoamperometry
            digitalWrite(2, HIGH); // sets the digital pin 2 off
            digitalWrite(3, HIGH); // sets the digital pin 3 off
            digitalWrite(4, HIGH); // sets the digital pin 4 on
            digitalWrite(5, HIGH); // sets the digital pin 5 off
            digitalWrite(6, HIGH); // sets the digital pin 6 on
            digitalWrite(7, HIGH); // sets the digital pin 7 off
```

```
            digitalWrite(8, LOW); // sets the digital pin 8 off
            digitalWrite(9, LOW); // sets the digital pin 9 off
            for (int timecount = 0; timecount<120; timecount++)
            {
            readAnalogChannel();
            delay(delayInterval);
            }
            break;

      default:
          Serial.println("error!");
          break;
     }
   }
}

void displayBinary(byte numToShow)
{
  for (int i=0; i<8; i++)
  {
    // Use the bitRead function and cycle through each bit of the binary
number
    // to see if it is a one or zero. If it is a one then turn the LED on.
    // If it is a zero turn the LED off
    if (bitRead(numToShow, i)==1)
    {
      digitalWrite(ledPin[i], HIGH);
    }
    else
    {
      digitalWrite(ledPin[i], LOW);
    }
  }
}

void readAnalogChannel()
{
   int sensorValue=analogRead(adcch1);
   float voltage_mV=sensorValue*(5.0/1023.0);
   Serial.print(voltage_mV, 4);
  // print a tab between values:
  Serial.print("\t");
  // print a new value in a new line:
  Serial.println();
  // delay before next reading:
}
```