



Regional Centre of Excellence in Biomedical Engineering and e-Health (CEBE)

**DESIGN OF A BIO-SENSOR-BASED PORTABLE AND RAPID POISON
DETECTION DEVICE
CASE STUDY: ORGANOPHOSPHATES POISON**

By:

ENOCK WILLIAM KAFUKUYE

Reference Number: 222000229

A Dissertation Submitted to the Regional Centre of Excellence in Biomedical Engineering and e-Health (CEBE), University of Rwanda as partial fulfilment of the requirements for the master's degree in Biomedical Engineering.

Supervised by:

Dr. Jean Felix Mukerabigwi

And

Dr. Gerard Urimubenshi


04 October 2024

DECLARATION

I, Enock William kafukuye, declare that this dissertation entitled “**DESIGN OF BIO-SENSOR BASED PORTABLE AND RAPID POISON DETECTION DEVICE. CASE STUDY: ORGANOPHOSPHATES POISON**” is my original work based on research and prototype and has not been submitted for any other degree or professional qualification.

Student Name: Enock William Kafukuye

Student Reference Number: 222000229

Student Signature: _____ 

Date: _____ **04 October 2024**



UNIVERSITY of
RWANDA

COLLEGE OF SCIENCE
AND TECHNOLOGY

Regional Centre of Excellence in Biomedical Engineering and e-Health (CEBE)

CERTIFICATE

This is to certify that the project entitled “DESIGN OF A BIO-SENSOR BASED PORTABLE AND RAPID POISON DETECTION DEVICE” is a record of original work done by Enock William Kafukuye (Reference number:222000229), a MSc. Degree student in Biomedical Engineering.

This work has been submitted under the guidance of Dr. Jean Felix Mukerabigwi and Dr. Gerard Urimubenshi

Main Supervisor:

A handwritten signature in black ink, appearing to read 'J. Mukerabigwi', written over a horizontal line.

Dr. Jean Felix Mukerabigwi

Co-Supervisor:

Dr. Gerard Urimubenshi

Biomedical Engineering Master’s Program Coordinator

Dr. Gerard Rushingabigwi

ACKNOWLEDGMENTS

I would like to thank the University of Rwanda Centre Of Excellent in Biomedical Engineering for offering me a chance to pursue this Master of Science in Biomedical Engineering. I also appreciate and thank my supervisors Dr. Jean Felix Mukerabigwi and Dr. Gerard Urimubenshi for their support and help gave me since the beginning of my Research up to the end, sincerely speaking they made a huge contribution towards the completion of my Research.

I would like also to thank the CEBE management and all lecturers who taught us this course for making sure they impart knowledge in our head which will be advantageous for our future careers as Biomedical Engineers. I worked with quite a large number of professional figures who made it easier to build my capacity in technical know-how and improve day by day my engineering skills in better practice in this field of Biomedical Engineering.

I am very grateful to the Director of CEBE, Prof Celestin Twizere, and Course Coordinator Dr. Gerard Rushingabigwi for the support and help gave me throughout the course, they worked very well, especially in coordinating our course from beginning to end.

I also thank my fellow Engineers with whom we worked together for their support, immediate availability, and advice that have contributed to completing my Research. Specifically, I would like to appreciate the contribution made by Eng. Nassor Maliki from TEMEKE Regional Referral Hospital (Biomedical Engineer), Eng. Karimu Mahmoud Khatwabi (IT specialist) from Tanzania Railway Corporation and other fellow engineers with whom we have been working together. Their distinguished support during my research is quite remarkable and indelible in my Research. I pray that their professional work and guidance attitude continue flourishing and keep up helping other young engineers.

ABSTRACT

Organophosphates are a class of chemicals that are used in a variety of products, including insecticides, herbicides, and pesticides. They are also used in some chemical warfare agents. Potential sources of organophosphate poisoning include occupational exposure such as agricultural workers, pesticide applicators, and other workers who handle organophosphates are at risk of occupational exposure.

Accidental exposure is another source of organophosphate that can be accidentally ingested, inhaled, or absorbed through the skin. Suicide or homicide are also sources of organophosphates which are used in suicide attempts or homicides.

This work describes the design of a bio-sensor-based portable and rapid poison detection device specifically organophosphate poison which is an exciting technology that has the potential to revolutionize the way we detect these dangerous chemicals.

This device is potable, lightweight, and easy to use, making it ideal for use in medical diagnostics application settings.

The methodology used to develop this device includes the proteus professional software tool which is used to interconnect circuit components and simulation of the designed device. Also, another software tool used to accomplish this project is Atmel studio version 6.2 which generates codes for the microcontroller (Arduino-uno) operations, whereby these codes make easily for Arduino-uno to receive bio-signals from bio-sensors then process them by converting them into equivalent electrical signals and finally displaced as output by using Liquid crystal display (LCD).

The key findings of this project are the rapid detection of organophosphate poison, portability sensitivity, and selectivity. The device can detect organophosphate poison within ten (10) seconds compared to other existing organophosphate poison devices which took almost an hour.

The significance of this device is that it is small, lightweight, easy to use, and rapid detection. Further research needs to be done to improve this design by integrating the device with the Internet of Things (IoT) which helps the results to be accessed by using smart devices owned by medical doctors. This technology will improve the provision of health services, especially during the interpretation of the results of poison detected.

Keywords: Organophosphates, bio-sensor, portable and detection biosensor technique

LIST OF ACRONYMS

AChE	: Acetylcholinesterase
APP	: Acute pesticide poisoning
CEBE	: Centre of Excellence in Biomedical Engineering
CRT	: Cathode Ray Tube
LCD	: Liquid Crystal Display
LED	: Light Emitting Diode
PCB	: Printed Circuit Board
OPs	: Organophosphates
PMW	: Pulse Modulated Width
WHO	: World Health Organization

LIST OF FIGURES

Figure 1: Block diagram of the proposed system.....	10
Figure 2: LCD.....	11
Figure 3: Power supply.....	11
Figure 4: Arduino Uno.....	12
Figure 5: Acetylcholinesterase (AChE) Bio-sensor	12
Figure 6: Structure of acetyl chlorine	13
Figure 7: Shows organophosphate compound react with Acetylcholinesterase enzyme	14
Figure 8: This circuit diagram shows how circuit components are interconnected.....	15
Figure 9: Circuit Simulation	16
Figure 10: Library inclusion	17
Figure 11: Sensor declaration.....	18
Figure 12: Block codes for system guidelines	19
Figure 13: Block codes for negative test.....	19
Figure 14: Simulation for negative test	20
Figure 15: Block codes for the positive test.....	21
Figure 16: Simulation for positive test result	22
Figure 17: Start -up of device	23
Figure 18: Inserting biosensor into water compound and into organophosphate compound	24
Figure 19: Waiting results for only ten(10) seconds	24
Figure 20: Negative results	25
Figure 21: Positive results	26

LIST OF TABLES

Table 1: Materials required for archiving the Research.....	9
---	---

TABLE OF CONTENTS

Declaration	ii
Certificate	iii
Acknowledgments	iv
ABSTRACT	v
LIST OF ACRONYMS	vi
LIST OF FIGURES	vii
LIST OF TABLES	viii
Table of Contents	ix
CHAPTER 1. GENERAL INTRODUCTION	1
1.1. Introduction.....	1
1.2. Problem statement.....	2
1.3. Research Questions (Hypotheses).....	2
1.4. Objectives	2
1.4.1. General Objective	2
1.4.2 Specific Objectives	2
1.5. Study scope	3
1.6. Significance of the Study.....	3
CHAPTER 2. LITERATURE REVIEW	4
2.1 Literature Review.....	4
2.1.1. Enzyme-based electrochemical biosensor for organophosphate poison detection.	4
2.1.2. Immunosensors-based biosensor for organophosphate poison detection.	4
2.2.3. DNA-based biosensors for organophosphate poison detection.	5
2.2.4. Biosensors used to detect other chemical compounds apart from organophosphate....	6
2.2.4.1. Biosensor device for cyanide detection	6
2.2.4. 2. Biosensor device for detection of botulinum neurotoxin.....	7
2.2.4.3. Biosensor device for Rapid Detection of Illicit Drugs.....	7
2.2.4.4. Biosensor device for Detection of Food Toxins.	8
CHAPTER 3. Research METHODOLOGY.	9
3.1 System Requirements.....	9
3.2. Working principle of proposed design.....	10
3.3. Block diagram of proposed portable and rapid poison detection device	10
3.4. The following are system components of the proposed design	11
3.4.1.Liquid crystal Display (LCD)	11

3.4.2. Power supply.....	11
3.4.3. Arduino Uno:	12
3.4.4. Acetylcholinesterase Bio-sensor.....	12
3.5. Basic principle of Acetylcholinesterase (AChE) biosensor.....	13
CHAPTER 4. Results.....	15
4.1.0. Circuit diagram	15
4.1. 2. Circuit Simulation.....	16
4.1.3. Library Inclusion.....	17
4.1.4. Sensor declaration.....	18
4.1.5. System guidelines	19
4.1.6. Negative test codes block.....	19
4.1.7. Negative Test	20
4.1.8. Positive test codes block	21
4.1.9. Positive test results.....	22
4.2 Summary	23
4.3. Results and discussions.....	23
4.4. Interpretation of Results.....	25
4.4.1Negative test results	25
4.4.2.Positive test results.....	26
4.4.3. Response Time.....	27
4.4.4. Selectivity	27
4.4.5. Testing.....	27
4.4.6. Comparison with Existing Methods.....	27
4.4.7.Practical Implications.....	27
4.4.8 Challenges and Future Directions	27
CHAPTER 5. CONCLUSION AND RECOMMENDATION.....	28
5.1 Conclusion	28
5.2 Recommendations.....	28
REFERENCES.....	29
APPENDICES	31
Appendix 1: Incidence rate, mortality rate, and case fatality rate for APP for children in Tanzania.....	31
Appendix 2: The utilized codes	31

CHAPTER 1. GENERAL INTRODUCTION

1.1. Introduction

Acute pesticides poisoning (APP) has been previously noted as a serious problem in Tanzania both for children and adults and in other developing and developed countries [1]. The World Health Organization (WHO) reports that over 30% of the global burden of disease in children can be attributed to environmental factors and that pesticides are a major contributor [2]. Acute pesticides poisoning (APP) in Tanzania does not yet have a surveillance system in place for cases of acute pesticide poisoning in children. Tanzanian hospitals record poisonings to the health management information system, but nothing is known about the cause.

[3]. The incidence rate, mortality rate, and case fatality rate for APP for children in Tanzania are shown in Appendix 1, wherein over half of the cases, (50.9%) of the pesticide involved were unknown. Seven of the eleven cases in which specific agents could be identified involved organophosphates, making them the most frequently reported specific agent [4].

To this end, this work describes the design of a bio-sensor-based portable and rapid poison detection device specifically organophosphate poison which is an exciting technology that has the potential to revolutionize the way we detect these dangerous chemicals. Rapid and portable poison detection device is sensitive to organophosphate poison in which it takes approximately ten seconds to detect the presence of organophosphate within a saliva sample. This design technology is quick ensures safety and improves healthcare outcomes. Therefore, it is important to be able to quickly and precisely identify toxic substances in order to promptly initiate the appropriate treatments and minimize patient risk.

The methodology used to develop this device includes the proteus professional software tool which is used to interconnect circuit components and simulation of the designed device. Also, another software tool used to accomplish this project is Atmel studio version 6.2 this generates codes for the microcontroller (Arduino-uno) operations, whereby these codes make easily for Arduino-uno to receive bio-signals from bio-sensors then process them by converting them into equivalent electrical signals and finally display as output by using Liquid crystal display (LCD).

The key findings of this project are rapid detection of organophosphate poison, portability sensitivity, and selectivity. The device can detect organophosphate poison within ten (10) seconds compared to other existing organophosphate poison devices which took almost an hour.

1.2. Problem statement

The rapid deterioration of individual health, delaying treatment procedures, sample preparation method (invasive technique), device mobility challenge, and increased risk of complication are the main challenges facing many health facilities specially Temeke Regional Referral Hospital in Tanzania during Organophosphate poison detection from patients who swallowed pesticides. Designing of biosensor-based rapid and portable detection device will overcome the challenges mentioned above such as time -consuming during sample preparation, delayed treatment procedures, device mobility challenges, and sensitivity, hence reducing the number of deaths caused by swallowing organophosphate poison.

1.3. Research Questions (Hypotheses)

The following questions are the baselines that guided this study:

1. Which biomaterials and sensing elements are most suitable for the rapid and portable biosensor design?
2. How can different materials be integrated into the device to enhance its performance and durability?
3. How can the biosensor achieve rapid response times for detecting organophosphate poison?
4. How can the user interface of the biosensor be designed to be user-friendly for non-experts, such as first responders or healthcare professionals?

1.4. Objectives

1.4.1. General Objective

This project aims to design bio-sensor-based portable and rapid organophosphates poison detection device.

1.4.2 Specific Objectives

To achieve the general objective of this project, the following specific objectives are used as guiding points (realizable; most of the time, publishable):

1. To design a circuit
2. To simulate the circuit
3. To build prototype
4. To test prototype

1.5. Study scope

This is limited only to the detection of organophosphate poison from pesticide compounds using biosensors specifically acetylcholinesterase (AChE) biosensors for medical diagnostics applications. The selection of appropriate materials is based on their sensitivity, selectivity, affordability, portability, and the technology used to make them. Thereafter assembling selected components, simulate the circuit design, and finally test and validate the prototype by comparing the results with existing conventional laboratory instruments currently used in organophosphate poison in hospitals.

This research is conducted specifically at Temeke Referral Hospital in Tanzania.

1.6. Significance of the Study

The significance of this study is as follows:

- Rapid organophosphate poison detection:- Organophosphate biosensors can provide rapid and real-time detection in field settings.
- Easy to carry (portability):- The device is compact, small, lightweight, and designed for field use. They can be easy to carry and operate in field conditions.
- Easy to use (user-friendly):- The device typically involves simple procedures and a clear display (LCD) for easy interpretation. Examples include biosensors that are dipped into test tubes with chemical compounds containing organophosphate and LCD to display the results

CHAPTER 2. LITERATURE REVIEW

2.1 Literature Review

2.1.1. Enzyme-based electrochemical biosensor for organophosphate poison detection.

The enzyme-based electrochemical biosensor is desirable for organophosphate poison detection since they are easy to manufacture and deploy[5]. Furthermore, it is possible to miniaturize the required instrumentation providing compact and portable analysis devices, and they can potentially be engineered to be highly selective and sensitive. The applied electrochemical transduction method of the electrochemical biosensors is normally potentiometric or amperometric [6]. The limitations of this type of biosensor are enzyme stability, low sensitivity, time consumption during sample preparation, and complexity.

Immunosensors are biosensing devices that use immunochemical reactions in the body's serum and a variety of other media to identify specific antigens or antibodies. Bioreceptors and transducers make up most immunosensors. Utilizing a transducer, a bioreceptor transforms the producing biological signal into the desired signal by recognizing the target antigen or antibody. Because antibodies naturally bind with antigens to form an antigen-antibody complex, which is the main principle of immunosensors for detecting antibody or antigen, it is an effective method for detecting pathogens.[7].

2.1.2. Immunosensors-based biosensor for organophosphate poison detection.

Immunosensors are a type of affinity solid-state-based biosensor in which a stable complex is formed between the target analyte, antigen (Ag), and the capturing agent, antibody (Ab). The transducer creates a measurable signal as a result of this immunological reaction.[8]. The activity of immunosensors might be like immunoassays; however there is an unobtrusive contrast between them. The immunoassay test is a solid phase system in which the Ag-Ab complex is formed but where detection takes place. The Ag recognition process and Ab interaction in immunosensors take place on the same platform. [9]. Immunosensors can be divided into three main categories based on their mode of transduction: electrochemical (amperometric, potentiometric, impedimetric, and conductometric), optical, and piezoelectric. Antibody stability, cross-

reactivity, regeneration and reusability, low sensitivity, time-consumingness, and high cost are some of the limitations of this kind of biosensor.

2.2.3. DNA-based biosensors for organophosphate poison detection.

From a SELEX-built library of aptamers, the DNA aptamer was chosen because it had the highest ability to bind profenofos and other organophosphorus pesticides. [10].

On the gold nanoparticles/polyaniline composite film (AuNPs/PANI) modified graphite screen-printed electrode surface, a thiol-tethered DNA capture probe that is compatible with the chosen aptamer sequence was immobilized. The restrictions of DNA-based biosensors are less awareness, significant expense, recovery, and reusability, less particularity less strength, and obstruction from other organic parts.

Oligonucleotides aptamers, which are bioelements used for recognition, have a number of advantages over other bioelements, including their small size, which makes them easy to synthesize, their ease of modification, their low immunogenicity, and their chemical stability. They are also considered to be promising alternatives to antigen-antibody reactions.[11]. A hairpin-shaped nucleic acid motif known as Molecular Beacon (MB) has one fluorophore and one non-fluorescent quencher covalently linked to each end of its stem. This results in low fluorescence, and the loop sequence serves as a probe that is complementary to the target sequence [12]. A fluorescence signal is produced when the MB binds to a target molecule, breaking apart the stem and separating the fluorophore from the quencher.

[13] systematic Evolution of Ligands by Exponential Enrichment, or SELEX, was the method used to select T4 DNA polymerase-binding sequences from an RNA pool. In addition, the aptamer can be given additional properties through SELEX technology modification and post-SELEX optimization, such as the selection of aptamers with desired properties through the use of chemically produced or modified oligonucleotide libraries and the introduction of novel properties through chemical modification or base modification.

2.2.4. Biosensors used to detect other chemical compounds apart from organophosphate

2.2.4.1. Biosensor device for cyanide detection

Numerous microorganisms can corrupt cyanide have been accounted for. A few bacterial strains that vigorously biodegrade cyanide have been utilized to foster microbial cyanide sensors. cyanide oxidase in these microscopic organisms changes over cyanide into cyanate consuming oxygen.

Numerous microorganisms have been found to be capable of breaking down cyanide. A few bacterial strains that vigorously biodegrade cyanide have been utilized to foster microbial cyanide sensors. These bacteria's cyanide oxidase uses oxygen to convert cyanide into cyanate. The reactant action of compounds isn't, without a doubt, unmistakable for their substrates yet in addition can be delicate to hibitors. A membrane-enclosed cytochrome oxidase-based amperometric cyanide sensor has been described and characterized due to the fact that the toxicity of cyanide is caused by its binding specifically to cytochrome oxidase. Oxygen is reduced in this system by transferring electrons to cytochrome c and then cytochrome oxidase from a modified gold electrode. At encompassing oxygen fixation the current is corresponding to the cytochrome oxidase movement and hindrance of the current happens within the sight of cyanide. In the solution phase, the sensor was able to measure cyanide concentrations as low as 0.4 mol dm^3 (0.01 ppm). Five years later [14] have developed an inhibition-based sensor for cyanide using cytochrome oxidase immobilized in a carbon paste/lipid matrix with the detection limit of 0.5 M (0.013 ppm) cyanide)

The advantages of biosensors for cyanide detection are as follows, can be used to detect cyanide in a variety of samples, including blood, urine, water, and food, can be used to detect cyanide in real time and finally can be used to detect cyanide in the field.

The methodology used to develop a biosensor for cyanide detection is to identify the target molecule, Select the biological element, Design the transducer, Assemble the biosensor, and test and optimize the biosensor.

2.2.4. 2. Biosensor device for detection of botulinum neurotoxin

The biosensor used in this method contains a layer of SNAP-25 immobilized on a surface. When serum from a patient with type A botulism is applied to the biosensor, the botulinum neurotoxin in the serum will cleave the SNAP-25. The cleaved SNAP-25 can then be detected using a variety of methods, such as surface plasmon resonance or fluorescence.

The heavy chain acts as a vector. It binds to receptors located at nerve terminals of the neuromuscular junction to assure internalization, and upon reduction of the disulfide bond, the light chain translocates into the cytoplasm. Light chains are metalloproteases with high substrate specificity, directed against single peptide bonds in SNARE proteins. SNAREs drive synaptic vesicle fusion and exocytosis of acetylcholine, thus their proteolytic inactivation blocks neuromuscular transmission presynaptically, resulting in muscle paralysis. Eight distinct BoNT serotypes (BoNT/ A to H) are known. Human botulism is mainly due to BoNT/A, B or E. Type A botulism has the highest incidence in the United States and constitutes the severest form. In addition, BoNT/A is the most frequently used toxin in therapeutic applications to treat disorders resulting from hypercontraction or hypersecretion and in cosmetic applications[14]. A number of in vitro assays have been developed in the last decade, based mainly on immunodetection of the toxin heavy chain or on endopeptidase assays to reveal the enzymatic activity of the light chain[14]. The metalloprotease activity of BoNT/A light chain which specifically cleaves a single peptide bond (Q197–R198) in the synaptic SNARE SNAP-25 can provide a very sensitive means of detection and assays based on ELISA and mass spectrometry have been reported.

The methodology used to develop biosensors for cyanide detection is to identify the target molecule, Select the biological element, design the transducer, assemble the biosensor, and test and optimize the biosensor.

2.2.4.3. Biosensor device for Rapid Detection of Illicit Drugs.

A biosensor device for rapid detection of illicit drugs is a device that uses a biological element, such as an antibody or enzyme, to detect the presence of a specific illicit drug in a sample.[15] The biological element is typically immobilized on a transducer, which is a device that converts the binding of the illicit drug to the biological element into a measurable signal. The methodology used to develop biosensor devices for the rapid detection of illicit drugs is Immunoassay-based biosensors, immunoassay-based biosensors for rapid detection of illicit drugs typically use an

antibody that specifically binds to a specific illicit drug, and Enzyme-based biosensors for rapid detection of illicit drugs typically use an enzyme that is inhibited by a specific illicit drug.

2.2.4.4. Biosensor device for Detection of Food Toxins.

Biosensors for the detection of food toxins are devices that use a biological element, such as an antibody, aptamer, or enzyme, to detect the presence of a specific food toxin in a sample. The biological element is typically immobilized on a transducer, which is a device that converts the binding of the food toxin to the biological element into a measurable signal. Food toxins can be natural, chemicals, additives, and pesticides, presence of metals[16]. The methodology used to develop biosensors for the detection of food toxins is similar to the methodology used to develop biosensors for other applications. The general steps involved are as follows to identify the specific food toxin or toxins that the biosensor will be used to detect, select the biological element, design the transducer, assemble the biosensor, and test and optimize the biosensor. The analyte of interest is recognized by the biocomponent or recognition element, and the binding event is converted to a detectable signal by the transducer. The processor reads this signal.

CHAPTER 3. RESEARCH METHODOLOGY.

3.1 System Requirements

The research comprises the following materials as shown in **Table 1** below.

Table 1: Materials required for archiving the research.

S/N	Name of material/item	Specifications	Purchase type	Quantity
1	Microcontroller	Arduino uno Processor	local	1
2	Liquid Crystal Display (LCD)	20x4 i2c	local	1
3	Biosensor	Chemical Sensor	import	1
4	Power Supply(Adaptor)	12VDC	Local	1
5	Circuit board(PCB)	20X10	Local	1
6	Pesticide	Organophosphate(Ops) poison	local	1litre
7	White Transparent tubes	plastic	Local	2
8	Jampers	Male to Female	Local	5m
9.	Soldering wire		Local	5m
10	Waterproof body	Medium-size	Local	1

3.2. Working principle of proposed design

The input sensation from the biosensor is imported into the microcontroller to stimulate and actuate the output responses. The sensor itself senses the input solution (organophosphate) and then reads the generated bauds range which lies from 0-1023, as according to the program, the system is set to detect the positive result as bauds reach from 50% to above that is equivalent to 512 bauds. Then if it is positive results or negative results LCD will display. The Controller takes inputs the processes them to get the output which can be a positive or a negative test result.

The system is powered by 12V which is regulated to get an operation supply of 5V dc.

3.3. Block diagram of proposed portable and rapid poison detection device

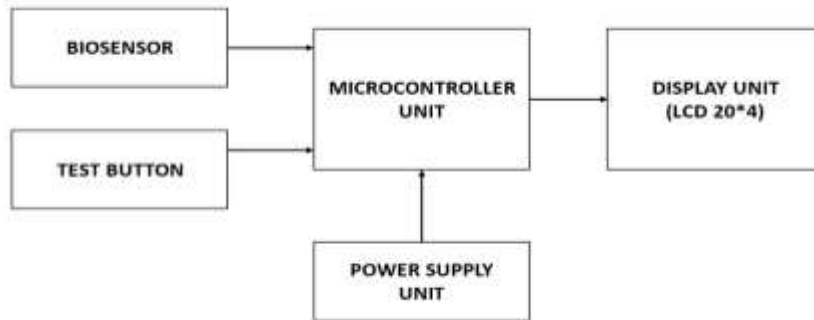


Figure 1: Block diagram of the proposed system

3.4. The following are system components of the proposed design

3.4.1. Liquid crystal Display (LCD)

Is the technology used for displays in notebooks and other smaller computers. Like light emitting diode (LED) and gas plasma technology, LCD allows displays to be much thinner than cathode ray tube (CRT) technology. The advantage of LCD is that it consumes much less power than LED and gas displays because they work on the principle of blocking light rather than emitting it [17].



Figure 2: LCD

3.4.2. Power supply

The official stance on supplying power directly to the 5V pin to the Arduino uno is thus; 5V. These pin outputs are regulated 5V from the regulator on the body. But the circuit allows a 12-volt relay to operate on a 5V or 9V supply. The maximum source voltage required by the system is about 30V DC [18].



Figure 3: Power supply

3.4.3. Arduino Uno:

Is a microcontroller board-based device. It has 14 digital input/output pins of which 6 can be used as PWM output, a 16MHz ceramic resonator, an ICS header, a USB connection, 6 analog inputs, a power jack, and a reset button. This contains all the required support needed for the microcontroller [19].



Figure 4: Arduino Uno

3.4.4. Acetylcholinesterase Bio-sensor

Biosensors is short for biological sensor. The device is made up of a transducer and biological element that may be an enzyme, an antibody, or nucleic acid. The bio-element interacts with the analysts being tested and the biological response is converted into an electrical signal by the transducer [20].



Figure 5: Acetylcholinesterase (AChE) Bio-sensor

The following below are characteristics of good biosensors;

- High sensitivity; this indicates how much the output of the device changes with unit and input.
- Linearity; the output should change linearity with the input.
- High resolution: Resolution is the smallest change in the input that the device can detect.
- Less noise and disturbance
- Less power consumption.

3.5. Basic principle of Acetylcholinesterase (AChE) biosensor.

Biosensors are widely used as devices suitable for fast analysis of toxic compounds. The enzyme Acetylcholinesterase (AChE) is a biorecognition element sensitive to inhibition by organophosphates as well as carbamate pesticides, nerve agents, several natural toxins, and some drugs.

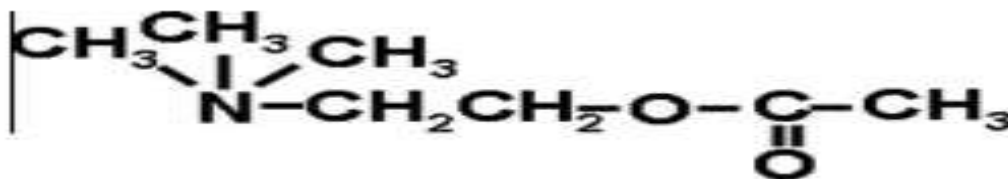


Figure 6: Structure of acetyl chlorine

Acetylcholinesterase (AChE) biosensors work on inhibitory effects. When the analyte is not present in the solution, the substrate acetylthiocholine is converted into thiocholine and acetic acid. Thiocholine is oxidized by an applied voltage. In the presence of an inhibitor, the conversion of acetylthiocholine is decreased or even null. Furthermore, the anodic oxidation current is inversely proportional to the concentration of pesticides in samples and the exposed time as well[20]. The principle of an electrochemical biosensor based on AChE and oxidation of thiocholine is shown below

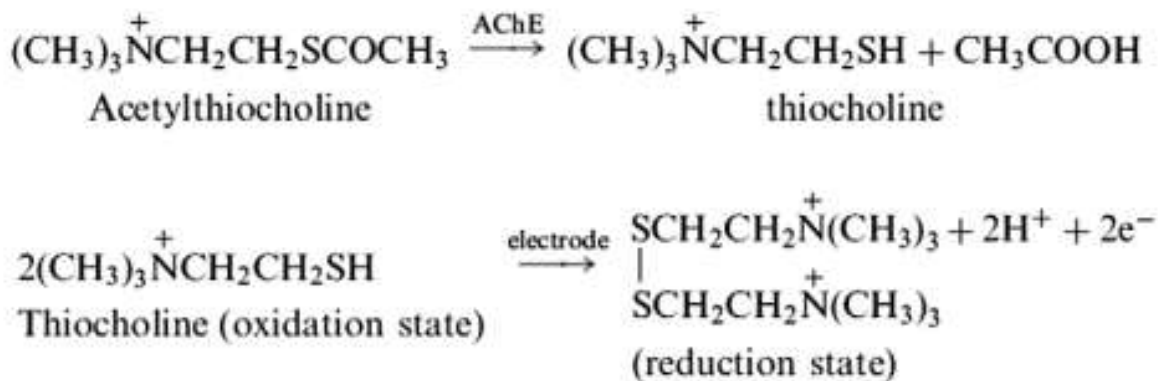


Figure 7: Shows organophosphate compound react with Acetylcholinesterase enzyme

CHAPTER 4. RESULTS

4.1.0. Circuit diagram

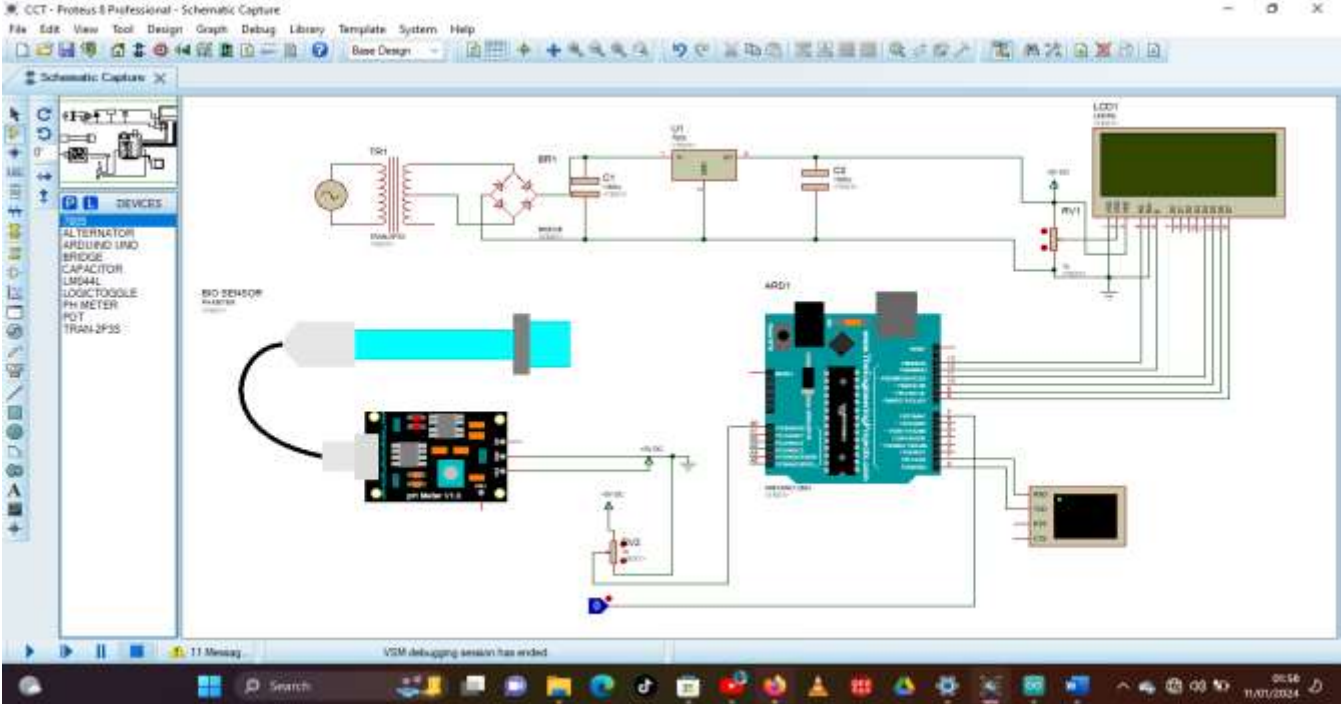


Figure 8: This circuit diagram shows how circuit components are interconnected.

4.1. 2. Circuit Simulation

The simulation is done to check whether the designed circuit will work as per design before building a prototype. This simulation is done through software called Proteus.

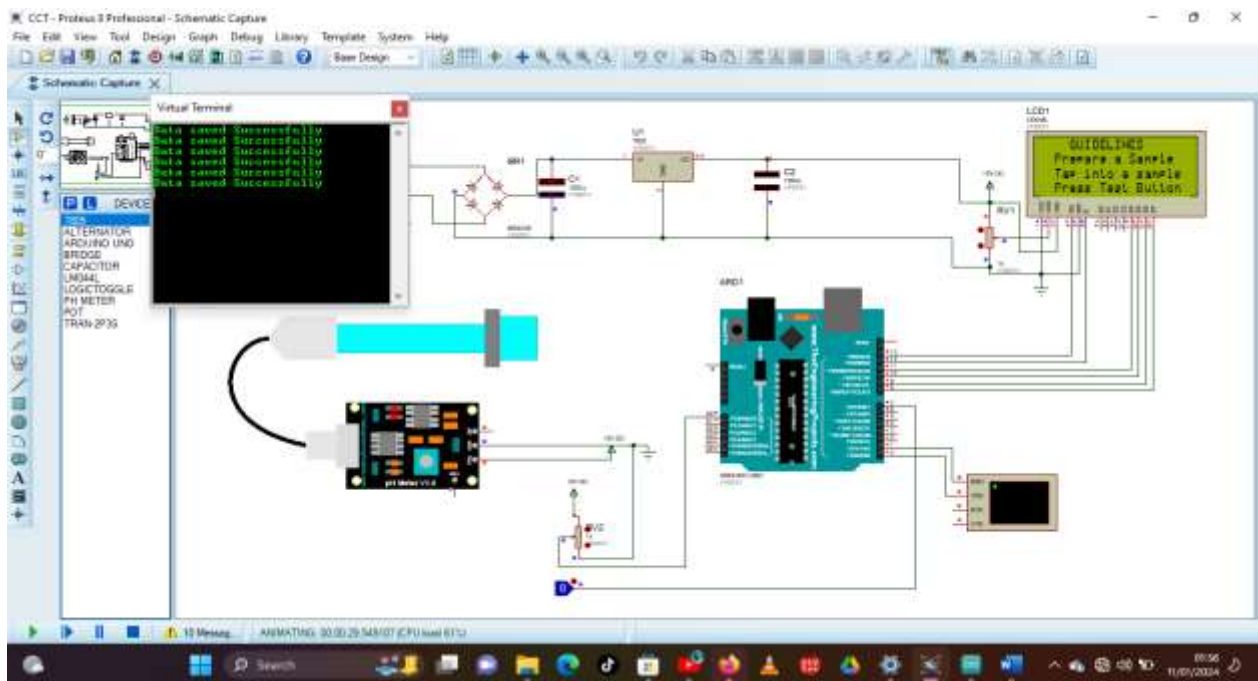


Figure 9: Circuit Simulation

4.1.3. Library Inclusion

These are libraries used to initiate programs and to interconnect with our external peripherals.

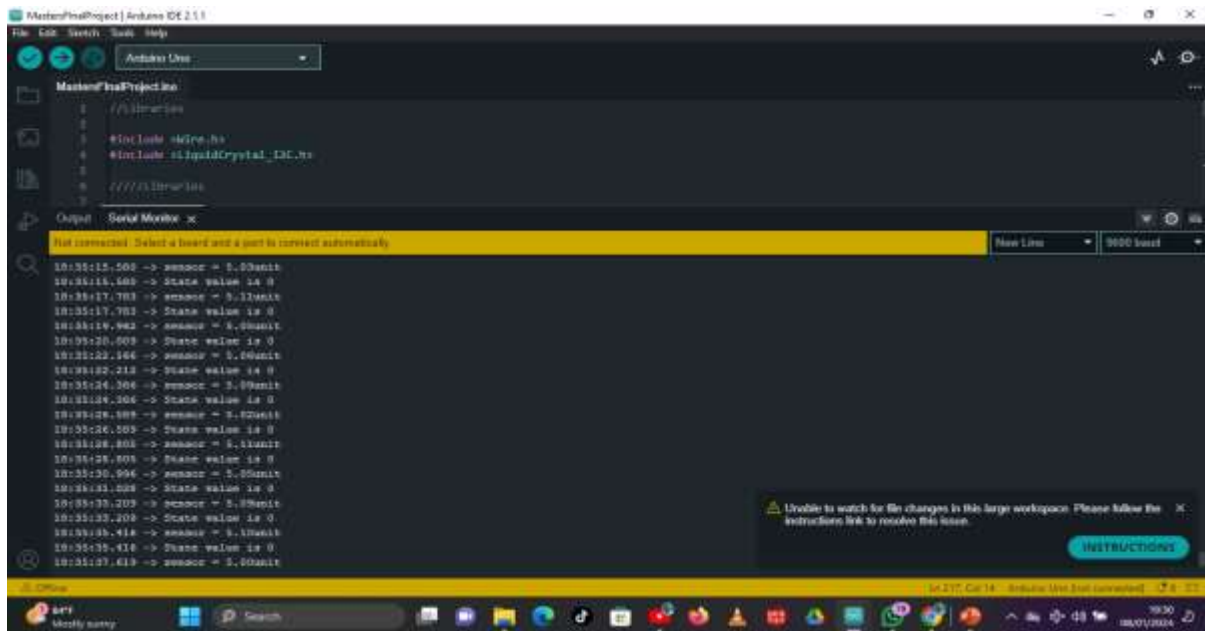


Figure 10: Library inclusion

4.1.4. Sensor declaration

This part enables a program to be aware of the sensors used on it.

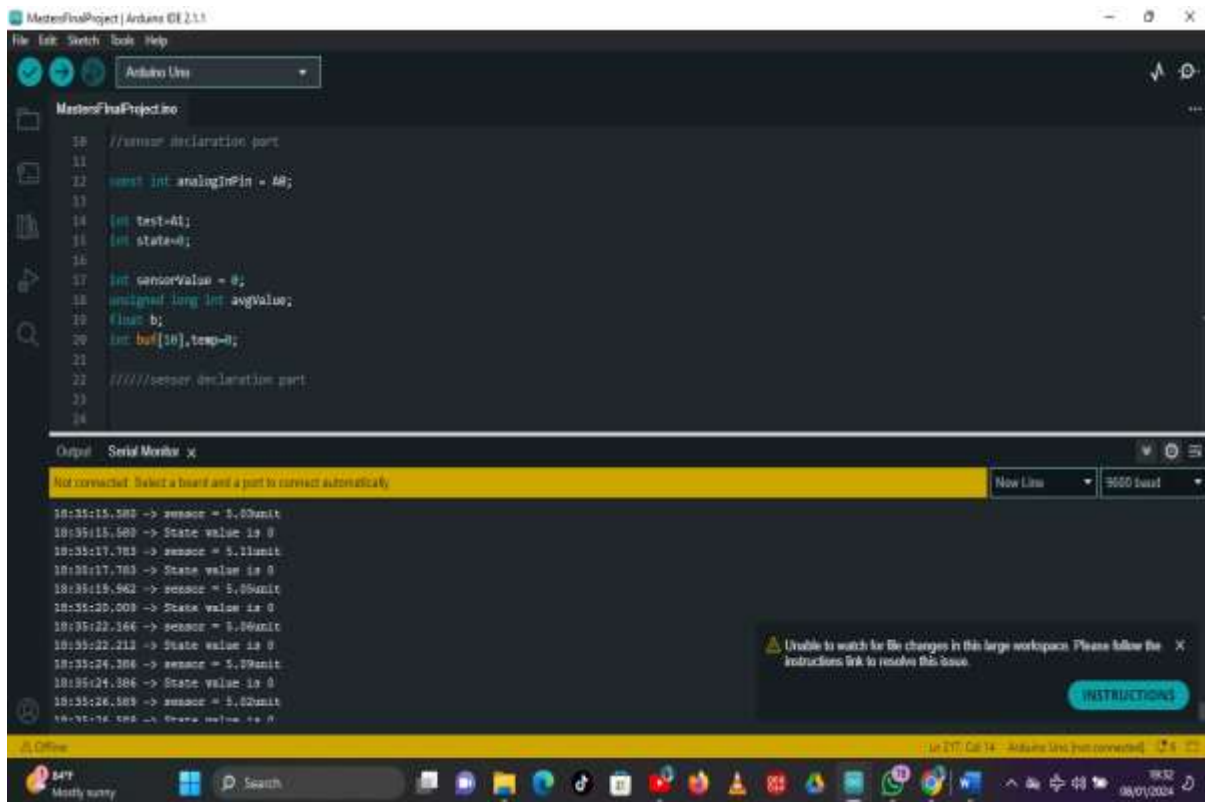
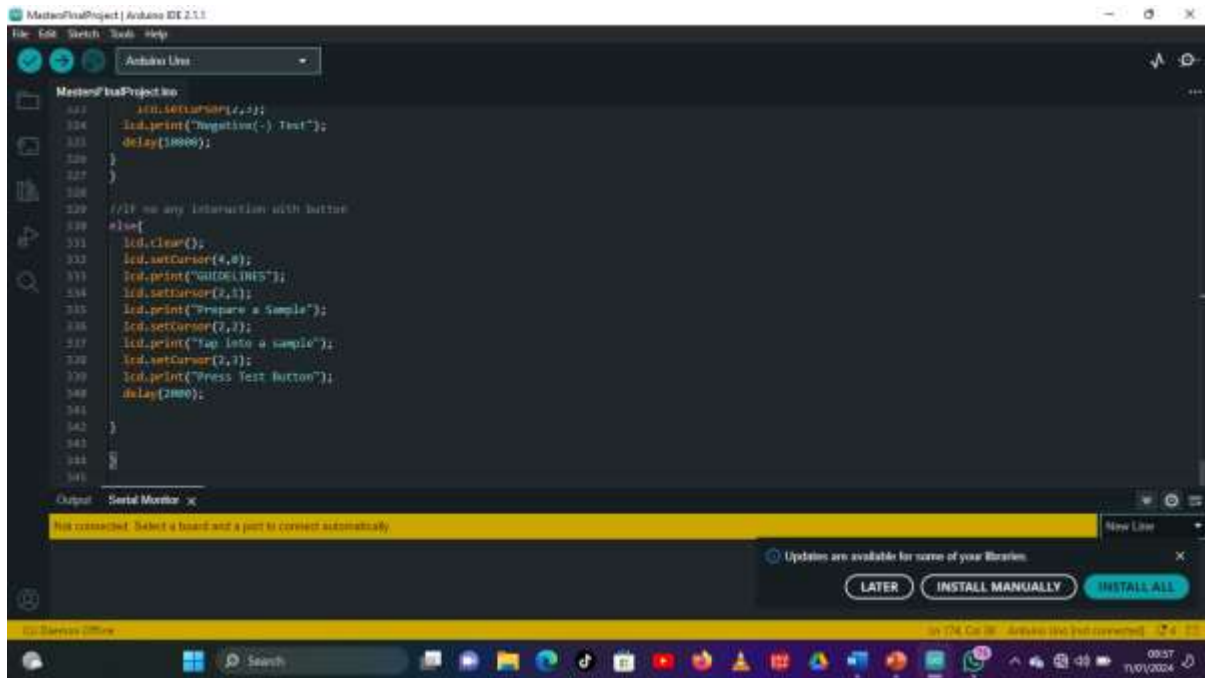


Figure 11: Sensor declaration

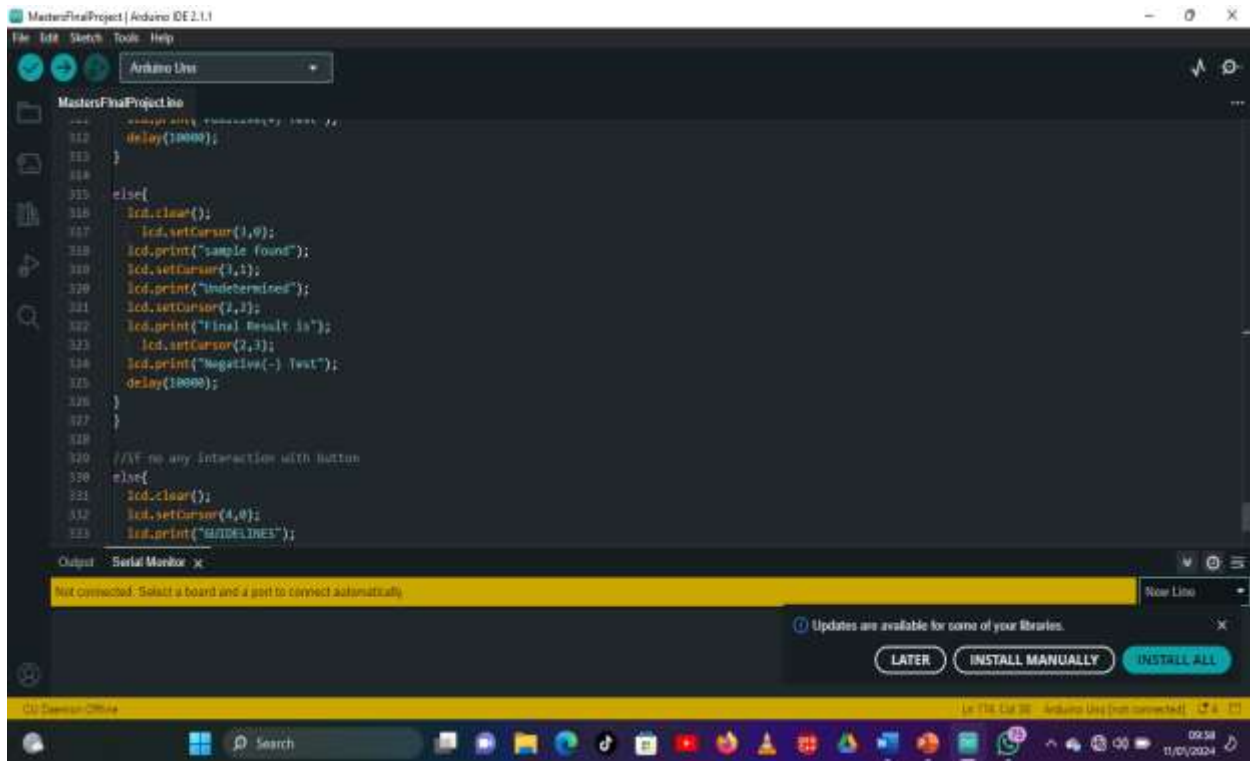
4.1.5. System guidelines



```
323 lcd.setCursor(2,2);
324 lcd.print("Negative(-) Test");
325 delay(1000);
326 }
327 }
328 }
329 //IF no any interaction with button
330 else{
331 lcd.clear();
332 lcd.setCursor(4,0);
333 lcd.print("GUIDELINES");
334 lcd.setCursor(2,1);
335 lcd.print("Prepare a Sample");
336 lcd.setCursor(2,2);
337 lcd.print("Tap into a sample");
338 lcd.setCursor(2,3);
339 lcd.print("Press Test Button");
340 delay(2000);
341 }
342 }
343 }
344 }
```

Figure 12: Block codes for system guidelines

4.1.6. Negative test codes block



```
312 delay(1000);
313 }
314 }
315 else{
316 lcd.clear();
317 lcd.setCursor(1,0);
318 lcd.print("sample found");
319 lcd.setCursor(1,1);
320 lcd.print("Undetermined");
321 lcd.setCursor(2,1);
322 lcd.print("Final Result is");
323 lcd.setCursor(2,3);
324 lcd.print("Negative(-) Test");
325 delay(1000);
326 }
327 }
328 }
329 //IF no any interaction with button
330 else{
331 lcd.clear();
332 lcd.setCursor(4,0);
333 lcd.print("GUIDELINES");
```

Figure 13: Block codes for negative test

4.1.7. Negative Test

This is a test that does not give the expected results, for instance from the figure below, the Biosensor was dipped into a pesticide compound but it found that organophosphate is absent within the pesticide chemical compound. Then microcontroller (Arduino uno) sends a command to the LCD to display the results “sample found is undetermined organophosphate not present”.

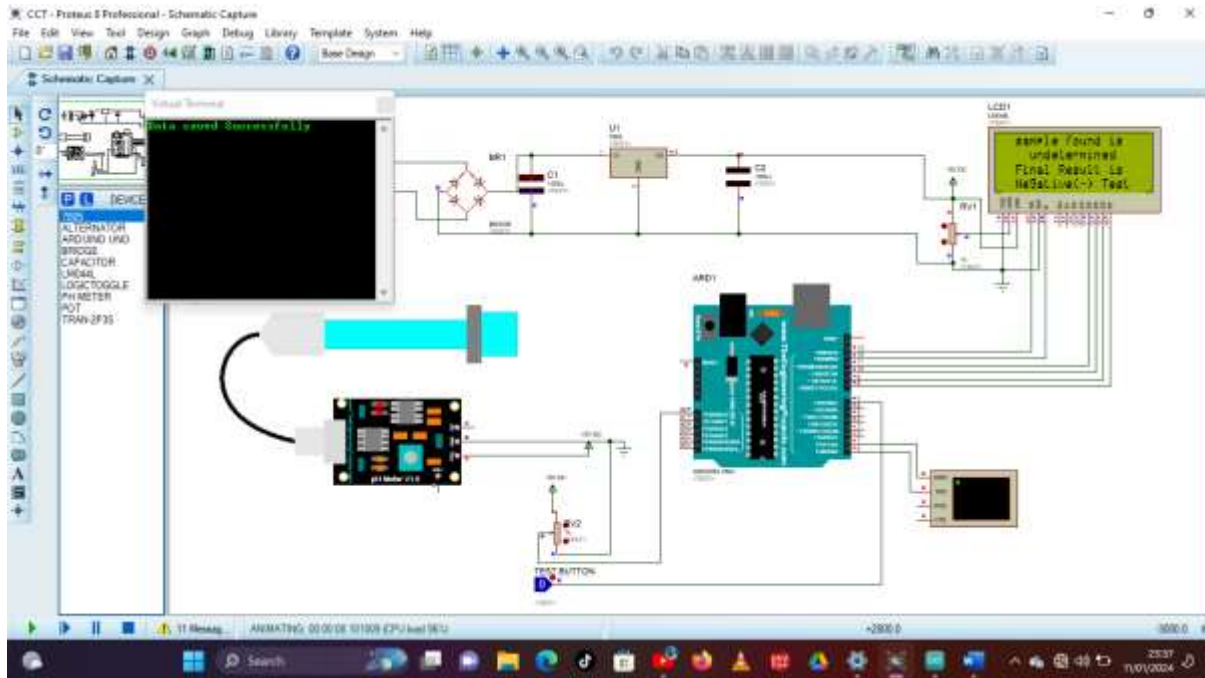
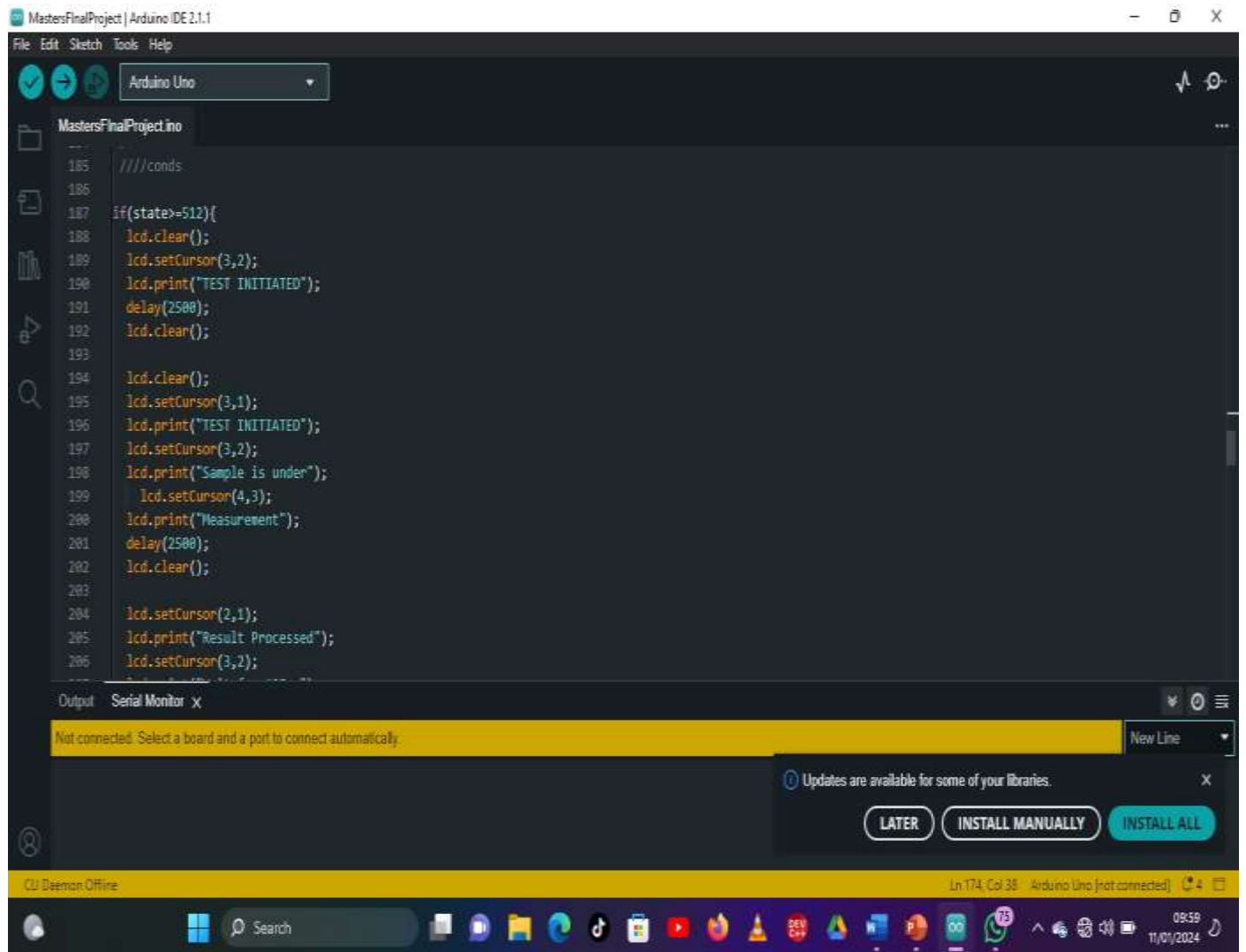


Figure 14: Simulation for negative test

4.1.8. Positive test codes block



```
MastersFinalProject | Arduino IDE 2.1.1
File Edit Sketch Tools Help
Arduino Uno
MastersFinalProject.ino
185  ///conds
186
187  if(state>=512){
188    lcd.clear();
189    lcd.setCursor(3,2);
190    lcd.print("TEST INITIATED");
191    delay(2500);
192    lcd.clear();
193
194    lcd.clear();
195    lcd.setCursor(3,1);
196    lcd.print("TEST INITIATED");
197    lcd.setCursor(3,2);
198    lcd.print("Sample is under");
199    lcd.setCursor(4,3);
200    lcd.print("Measurement");
201    delay(2500);
202    lcd.clear();
203
204    lcd.setCursor(2,1);
205    lcd.print("Result Processed");
206    lcd.setCursor(3,2);
```

Output Serial Monitor x
Not connected. Select a board and a port to connect automatically. New Line

Updates are available for some of your libraries. LATER INSTALL MANUALLY INSTALL ALL

CU Daemon Offline Ln 174, Col 38 Arduino Uno [not connected] 09:59 11/01/2024

Figure 15: Block codes for the positive test

4.1.9. Positive test results

This is a test that gives expected results, for instance from the figure below, the Biosensor was dipped into pesticide compound and it found that organophosphate is present within the pesticide chemical compound. Then microcontroller(Arduino uno) commands LCD to display the results “ sample found is determined organophosphate presence”.

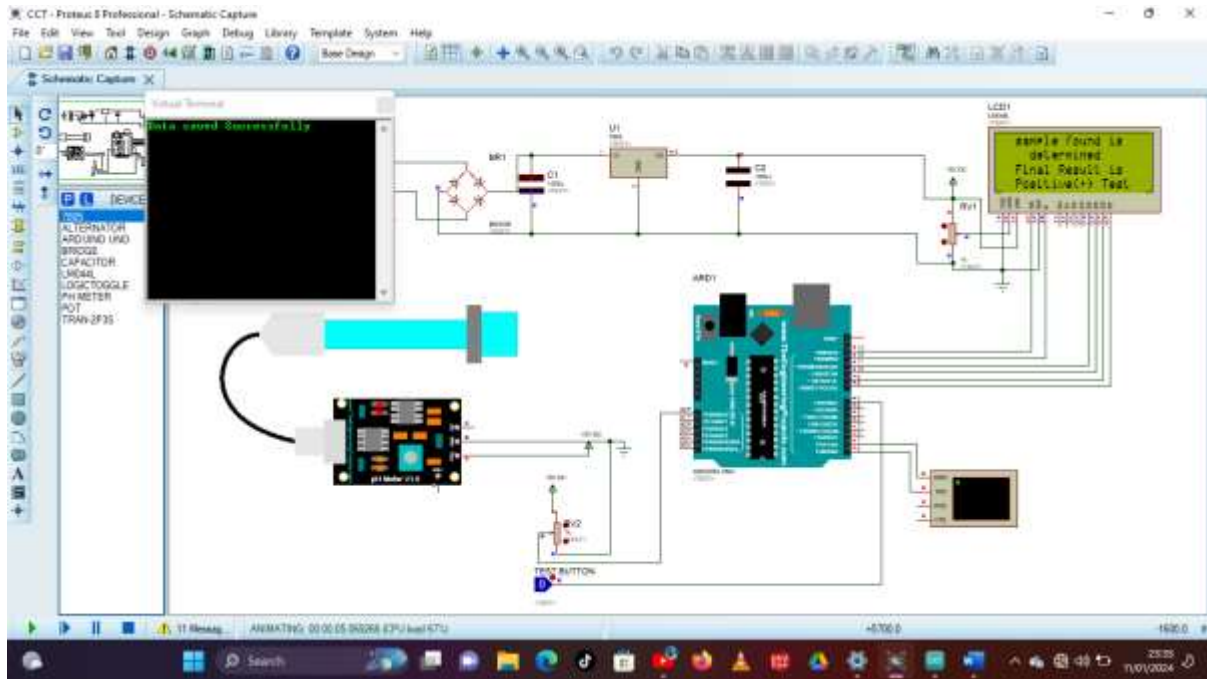


Figure 16: Simulation for positive test result

4.2 Summary

From the experiment made on both negative and positive tests, shows that the biosensor used which is the Acetylcholinesterase biosensor is sensitive to organophosphate-only detection. The device is very quick to almost within one minute in responding and gives results. For the negative test, we saw that biosensor didn't recognize the presence of organophosphate poison. But for a positive test, the biosensor recognizes the presence of organophosphate poison within a pesticide.

The device is portable, user user-friendly since even procedures for operation are very simple and short. This reduces the processing time of samples for people with pesticide (organophosphate) contamination. Acetylcholinesterase is a biomaterial and sensing element most suitable for rapid and portable biosensor design.

4.3. RESULTS AND DISCUSSIONS

When device is connected to power supply LCD display name of designed device as shown in figure below.



Figure 17: Start -up of device

Inserting Acetylcholinesterase Bio-sensor into water and into organophosphate compound respectively then , then wait results for ten(10seconds).



Figure 18: Inserting Acetylcholinesterase bio-sensor into water compound and into organophosphate compound.



Figure 19: Waiting results for only ten(10) seconds

4.4. Interpretation of Results

4.4.1 Negative test results

This indicates that the sample does not contain detectable levels of organophosphates. This suggests that the individual or environment being tested is not currently exposed to harmful levels of these chemicals.

Acetylcholinesterase Bio-sensor for detecting organophosphates often use enzymes such as acetylcholinesterase (AChE), which organophosphates inhibit. When organophosphates are present, they bind to and inhibit AChE, reducing its activity.



Figure 20: Negative results

4.4.2. Positive test results

This indicates that organophosphates are present in the sample. This is shown by the reduced activity of AChE due to its inhibition by organophosphates. A positive result suggests that there has been exposure to these chemicals, which could lead to poisoning and associated health risks.

Acetylcholinesterase Bio-sensor designed to detect organophosphates typically rely on enzymes such as acetylcholinesterase (AChE). Organophosphates inhibit the activity of AChE by binding to it, preventing the breakdown of the neurotransmitter acetylcholine.



Figure 21: Positive results

4.4.3. Response Time

The biosensor's one-minute response time demonstrated its capacity for quick detection.

4.4.4. Selectivity

Excellent selectivity against OP toxins was shown by the biosensor against other interfering molecules, including pesticides and ordinary home chemicals.

4.4.5. Testing

The biosensor was tested in real-world scenarios, including hospital settings, especially Temeke Referral Hospital. Field testing verified the biosensor's dependability and precision in identifying OP toxins, yielding outcomes in line with laboratory analytical findings.

4.4.6. Comparison with Existing Methods

Compared to traditional laboratory-based methods, such as chromatography and spectrophotometry, the biosensor offers several advantages, including portability, rapid analysis, and ease of use. Existing portable detection devices for OP poisons often suffer from limitations in sensitivity, specificity, or response time, highlighting the significance of our biosensor's performance.

4.4.7. Practical Implications

The development of a rapid and portable biosensor for OP poison detection has significant practical implications for various stakeholders, including emergency responders, healthcare professionals, environmental agencies, and law enforcement personnel.

The availability of such a device could streamline poison detection efforts, leading to improved patient outcomes, reduced environmental contamination, and enhanced public safety.

4.4.8 Challenges and Future Directions

Despite the promising results, challenges such as IoT integration need to be addressed in future research. In conclusion, the design of a rapid and portable biosensor for the detection of organophosphate poisons represents a significant advancement in poison detection technology. The results of this study demonstrate the feasibility and effectiveness of the biosensor in detecting OP poisons with high sensitivity, specificity, and rapid response time. Further research and development efforts are warranted to address remaining challenges and realize the full potential of this technology for improving public health and safety.

CHAPTER 5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Rapid poison detection devices are crucial in emergency situations, enabling swift responses to poison exposures. This technology can assist first responders, emergency medical personnel, and law enforcement in making quick decisions to mitigate the impact of poison incidents. In healthcare settings, biosensors for poison detection can aid medical professionals in diagnosing cases of poisoning promptly. This can lead to faster and more targeted treatments, improving patient outcomes and reducing the severity of poisoning incidents.

5.2 Recommendations

To invest in ongoing research to improve sensor technologies, with a focus on increasing sensitivity, reducing response times, and enhancing the overall accuracy of biosensors. This can involve exploring new materials, fabrication techniques, and signal-processing methods.

The researchers continue to investigate the use of multiple bioreceptors in biosensor designs to enhance specificity and broaden the range of detectable poisons. This can involve combinations of enzymes, antibodies, and aptamers to improve the device's versatility.

Increase public awareness of the benefits of biosensor-based poison detection devices. Educational campaigns can help promote understanding among healthcare professionals, emergency responders, and the general public about the capabilities and proper use of these technologies.

Focus on cost-effective manufacturing processes and materials to make biosensor devices more manufacturing industries should be built in East African countries, this will help to lower the cost for raw materials used to design biosensors and reduce shipment cost.

REFERENCES

- [1] E. Lekei, A. V Ngowi, and L. London, “Acute Pesticide Poisoning in Children : Hospital Review in Selected Hospitals of Tanzania,” vol. 2017, 2017.
- [2] M. Vaismoradi, S. Tella, P. A. Logan, J. Khakurel, and F. Vizcaya-Moreno, “Nurses’ adherence to patient safety principles: A systematic review,” *Int. J. Environ. Res. Public Health*, vol. 17, no. 6, pp. 1–15, 2020, doi: 10.3390/ijerph17062028.
- [3] K. Jaga and C. Dharmani, “Sources of exposure to and public health implications of organophosphate pesticides,” vol. 14, no. 3, pp. 171–185, 2003.
- [4] A. Amir, A. Raza, T. Qureshi, G. B. Mahesar, S. Jafferri, and F. Haleem, “Organophosphate Poisoning : Demographics , Severity Scores and Outcomes From National Poisoning Control Centre , Karachi,” vol. 12, no. 5, pp. 1–7, 2020, doi: 10.7759/cureus.8371.
- [5] M. Campàs, B. Prieto-simón, and J. Marty, “Seminars in Cell & Developmental Biology A review of the use of genetically engineered enzymes in electrochemical biosensors,” vol. 20, pp. 3–9, 2009, doi: 10.1016/j.semcdb.2009.01.009.
- [6] M. Z. H. Khan, M. R. Hasan, S. I. Hossain, M. S. Ahommed, and M. Daizy, “Ultrasensitive detection of pathogenic viruses with electrochemical biosensor: State of the art,” *Biosens. Bioelectron.*, vol. 166, no. July, p. 112431, 2020, doi: 10.1016/j.bios.2020.112431.
- [7] D. Lu *et al.*, “FULL PAPER A Novel Nanoparticle-Based Disposable Electrochemical Immunosensor for Diagnosis of Exposure to Toxic Organophosphorus Agents,” pp. 4371–4378, 2011, doi: 10.1002/adfm.201100616.
- [8] G. S. Id, B. Ioana, O. Hosu, and G. Marrazza, “DNA-Based Sensor for the Detection of an Organophosphorus Pesticide : Profenofos,” pp. 1–12, doi: 10.3390/s18072035.
- [9] C. Zhang *et al.*, “Organophosphorus pesticides detection using broad-specific single-stranded DNA based fluorescence polarization aptamer assay,” *Biosens. Bioelectron.*, vol. 55, pp. 216–219, 2014, doi: 10.1016/j.bios.2013.12.020.
- [10] R. Yamamoto and P. K. R. Kumar, “Molecular beacon aptamer fluoresces in the presence of Tat protein of HIV-1,” *Genes to Cells*, vol. 5, no. 5, pp. 389–396, 2000, doi: 10.1046/j.1365-2443.2000.00331.x.
- [11] C. Tuerk and L. Gold, “Systematic Evolution of Ligands by Exponential Enrichment:

- RNA Ligands to Bacteriophage T4 DNA Polymerase,” *Science* (80-.), vol. 249, no. 4968, pp. 505–510, 1990, doi: 10.1126/science.2200121.
- [12] M. Darmostuk, S. Rimpelova, H. Gbelcova, and T. Ruml, “Current approaches in SELEX: An update to aptamer selection technology,” *Biotechnol. Adv.*, vol. 33, no. 6, pp. 1141–1161, 2014, doi: 10.1016/j.biotechadv.2015.02.008.
- [13] K. K. W. Mak, H. Yanase, and R. Renneberg, “Cyanide fishing and cyanide detection in coral reef fish using chemical tests and biosensors,” vol. 20, pp. 2581–2593, 2005, doi: 10.1016/j.bios.2004.09.015.
- [14] W. John Albery, A. E. G. Cass, and Z. X. Shu, “Inhibited enzyme electrodes. Part 2: The kinetics of the cytochrome oxidase system,” *Biosens. Bioelectron.*, vol. 5, no. 5, pp. 379–395, 1990, doi: 10.1016/0956-5663(90)80017-8.
- [15] M. I. Majeed, H. Nawaz, and F. N. Arshad, “Detecting the Presence of Illicit Drugs Using Biosensors,” 2020.
- [16] A. Girigoswami, M. M. Ghosh, P. Pallavi, and S. Ramesh, “Nanotechnology in Detection of Food Toxins – Focus on the Dairy Products,” vol. 11, no. 6, pp. 14155–14172, 2021.
- [17] P. Type and S. B. Y. Customer, “LCM System,” pp. 1–21.
- [18] T. H. E. D. C. P. Supply, “The d.c. power supply,” pp. 1–6.
- [19] P. R. Manual, “Arduino UNO R3 Features,” pp. 1–13, 2022.
- [20] T. Vo-Dinh, M. J. Sepaniak, G. D. Griffin, and J. P. Alarie, “Immunosensors: Principles and Applications,” *Immunomethods*, vol. 3, no. 2, pp. 85–92, 1993, doi: 10.1006/immu.1993.1042.

PPENDICES

Appendix 1: Incidence rate, mortality rate, and case fatality rate for APP for children in Tanzania

Product	Chemical group	WHO Class	Frequency	Percentage by category	Percentage of all agents
<i>(1) Known products</i>					
Zinc Phosphide	IN	1b	2	18.2%	3.7%
OP	OP	II	7	63.6%	13.2%
Sulphur	IN	IV	1	9.1%	1.9%
Endosulfanbn	OC	II	1	9.1%	1.9%
<i>S total 1</i>			<i>11</i>	<i>100.0%</i>	<i>20.7%</i>
<i>(2) Unspecific products</i>					
Livestock dip	UN	UN	1	6.67%	1.9%
Food poisoning	UN	UN	11	73.33%	20.7%
Rat poison	UN	UN	3	20.00%	5.6%
<i>S total 2</i>			<i>15</i>	<i>100.00%</i>	<i>28.2%</i>
<i>(3) Unknown</i>					
UN			27	100.00%	50.9%
<i>S total 3</i>			<i>27</i>	<i>100.00%</i>	

IN: inorganic; OP: organophosphate; OC: organochlorine; UN: unknown.

Appendix 2: The utilized codes

```
//Libraries

#include <Wire.h>
#include <LiquidCrystal_I2C.h>

////Libraries

LiquidCrystal_I2C lcd(0x27, 20, 4);

//sensor declaration part

const int analogInPin = A0;

int test=A1;
int state=0;
```

```

int sensorValue = 0;
unsigned long int avgValue;
float b;
int buf[10],temp=0;

/////sensor declaration part

//setup the device
void setup()
{
  pinMode(test,INPUT);

  Serial.begin(9600);
  lcd.init();
  lcd.begin(20, 4);
  lcd.backlight();
  lcd.setCursor(2,0);
  lcd.print("RAPID BIOSENSOR");
  lcd.setCursor(6,1);
  lcd.print("POISON");
  lcd.setCursor(2,2);
  lcd.print("DETECTION DEVICE");
  lcd.setCursor(0,3);
  lcd.print("_____");
  delay(7000);
  lcd.clear();

  lcd.setCursor(1,0);
  lcd.print("Device Initialize");
  lcd.setCursor(4,1);
  lcd.print("Data Reading");
  lcd.setCursor(2,2);

```

```
lcd.print("Data Score 0%");
lcd.setCursor(5,3);
lcd.print("Please Wait");
delay(500);
lcd.clear();

lcd.setCursor(1,0);
lcd.print("Device Initialize");
lcd.setCursor(4,1);
lcd.print("Data Reading");
lcd.setCursor(2,2);
lcd.print("Data Score 15%");
lcd.setCursor(5,3);
lcd.print("Please Wait");
delay(500);
lcd.clear();

lcd.setCursor(1,0);
lcd.print("Device Initialize");
lcd.setCursor(4,1);
lcd.print("Data Reading");
lcd.setCursor(2,2);
lcd.print("Data Score 40%");
lcd.setCursor(5,3);
lcd.print("Please Wait");
delay(1500);
lcd.clear();

lcd.setCursor(1,0);
lcd.print("Device Initialize");
lcd.setCursor(4,1);
lcd.print("Data Reading");
lcd.setCursor(2,2);
lcd.print("Data Score 57%");
```

```
lcd.setCursor(5,3);
lcd.print("Please Wait");
delay(500);
lcd.clear();

lcd.setCursor(1,0);
lcd.print("Device Initialize");
lcd.setCursor(4,1);
lcd.print("Data Reading");
lcd.setCursor(2,2);
lcd.print("Data Score 69%");
lcd.setCursor(5,3);
lcd.print("Please Wait");
delay(500);
lcd.clear();

lcd.setCursor(1,0);
lcd.print("Device Initialize");
lcd.setCursor(4,1);
lcd.print("Data Reading");
lcd.setCursor(2,2);
lcd.print("Data Score 86%");
lcd.setCursor(5,3);
lcd.print("Please Wait");
delay(1500);
lcd.clear();

lcd.setCursor(1,0);
lcd.print("Device Initialize");
lcd.setCursor(4,1);
lcd.print("Data Reading");
lcd.setCursor(2,2);
lcd.print("Data Score 97%");
lcd.setCursor(5,3);
```

```

lcd.print("Please Wait");
delay(1500);
lcd.clear();

lcd.setCursor(1,0);
lcd.print("Device Initialize");
lcd.setCursor(4,1);
lcd.print("Data Reading");
lcd.setCursor(2,2);
lcd.print("Data Score 100%");
lcd.setCursor(5,3);
lcd.print("Please Wait");
delay(1500);
lcd.clear();

lcd.setCursor(1,0);
lcd.print("Device Initialize");
lcd.setCursor(4,1);
lcd.print("Data Reading");
lcd.setCursor(0,2);
lcd.print("Data Score uploaded");
lcd.setCursor(3,3);
lcd.print("Successfully");
delay(3500);
lcd.clear();
}

//looping or overall operation of the program
void loop()
{
state=analogRead(test);

for(int i=0;i<10;i++)
{

```

```

buf[i]=analogRead(analogInPin);
delay(10);
}
for(int i=0;i<9;i++)
{
for(int j=i+1;j<10;j++)
{
if(buf[i]>buf[j])
{
temp=buf[i];
buf[i]=buf[j];
buf[j]=temp;
}
}
}
avgValue=0;
for(int i=2;i<8;i++)
avgValue+=buf[i];

float pHVol=(float)avgValue*5.0/1024/4.3;
float phValue = -5.70 * pHVol +29.5 ;
phValue=14.2-phValue;
//float phValue = -3.0 * pHVol+17.5;
Serial.print("sensor = ");
Serial.println(phValue*0.72 + String("unit")); //calibration point
Serial.println("State value is "+ String(state));
/* lcd.clear();
lcd.setCursor(0,0);
lcd.print("  pH Value");
lcd.setCursor(6,1);
lcd.print(phValue);
delay(500);
*/
////conds

```

```
if(state>=512){
  lcd.clear();
  lcd.setCursor(3,2);
  lcd.print("TEST INITIATED");
  delay(2500);
  lcd.clear();

  lcd.clear();
  lcd.setCursor(3,1);
  lcd.print("TEST INITIATED");
  lcd.setCursor(3,2);
  lcd.print("Sample is under");
  lcd.setCursor(4,3);
  lcd.print("Measurement");
  delay(2500);
  lcd.clear();

  lcd.setCursor(2,1);
  lcd.print("Result Processed");
  lcd.setCursor(3,2);
  lcd.print("Wait for 10Sec");
  delay(1500);
  lcd.clear();

  lcd.setCursor(2,1);
  lcd.print("Result Processed");
  lcd.setCursor(3,2);
  lcd.print("wait a moment");
  lcd.setCursor(9,3);
  lcd.print("10");
  delay(800);
  lcd.clear();
```

```
    lcd.setCursor(2,1);  
    lcd.print("Result Processed");  
    lcd.setCursor(3,2);  
    lcd.print("wait a moment");  
    lcd.setCursor(9,3);  
    lcd.print("9");  
    delay(800);  
    lcd.clear();
```

```
    lcd.setCursor(2,1);  
    lcd.print("Result Processed");  
    lcd.setCursor(3,2);  
    lcd.print("wait a moment");  
    lcd.setCursor(9,3);  
    lcd.print("8");  
    delay(800);  
    lcd.clear();
```

```
    lcd.setCursor(2,1);  
    lcd.print("Result Processed");  
    lcd.setCursor(3,2);  
    lcd.print("wait a moment");  
    lcd.setCursor(9,3);  
    lcd.print("7");  
    delay(800);  
    lcd.clear();
```

```
    lcd.setCursor(2,1);  
    lcd.print("Result Processed");  
    lcd.setCursor(3,2);  
    lcd.print("wait a moment");  
    lcd.setCursor(9,3);  
    lcd.print("6");  
    delay(800);
```

```
lcd.clear();

    lcd.setCursor(2,1);
lcd.print("Result Processed");
lcd.setCursor(3,2);
lcd.print("wait a moment");
lcd.setCursor(9,3);
lcd.print("5");
delay(800);
lcd.clear();
```

```
    lcd.setCursor(2,1);
lcd.print("Result Processed");
lcd.setCursor(3,2);
lcd.print("wait a moment");
lcd.setCursor(9,3);
lcd.print("4");
delay(800);
lcd.clear();
```

```
    lcd.setCursor(2,1);
lcd.print("Result Processed");
lcd.setCursor(3,2);
lcd.print("wait a moment");
lcd.setCursor(9,3);
lcd.print("3");
delay(800);
lcd.clear();
```

```
    lcd.setCursor(2,1);
lcd.print("Result Processed");
lcd.setCursor(3,2);
lcd.print("wait a moment");
lcd.setCursor(9,3);
```

```

lcd.print("2");
delay(800);
lcd.clear();

    lcd.setCursor(2,1);
lcd.print("Result Processed");
lcd.setCursor(2,2);
lcd.print("wait a moment");
lcd.setCursor(9,3);
lcd.print("1");
delay(2500);
lcd.clear();

if (phValue>=9.2)
{
    lcd.clear();
    lcd.setCursor(3,0);
    lcd.print("sample found");
    lcd.setCursor(2,1);
    lcd.print("Organophosphate");
    lcd.setCursor(2,2);
    lcd.print("Final Result is");
    lcd.setCursor(1,3);
    lcd.print("Positive(+) Test");
    delay(10000);
}

else{
    lcd.clear();
    lcd.setCursor(3,0);
    lcd.print("sample found");
    lcd.setCursor(3,1);
    lcd.print("Undetermined");
    lcd.setCursor(2,2);

```

```
lcd.print("Final Result is");
  lcd.setCursor(2,3);
lcd.print("Negative(-) Test");
delay(10000);
}
}

//if no any interaction with button
else{
  lcd.clear();
  lcd.setCursor(4,0);
  lcd.print("GUIDELINES");
  lcd.setCursor(2,1);
  lcd.print("Prepare a Sample");
  lcd.setCursor(2,2);
  lcd.print("Tap into a sample");
  lcd.setCursor(2,3);
  lcd.print("Press Test Button");
  delay(2000);
}
}
```