Design, Fabrication and Validation of a Low Cost, Handheld Biochemical Analyzer

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Design, Fabrication and Validation of a Low Cost, Handheld Biochemical Analyzer

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by

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(Roll Number: 113BM0012)

based on research carried out

under the supervision of

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May 12, 2017

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Prof. Bibhukalyan Prasad Nayak
Associate Professor

May 12, 2017

Supervisors' Certificate

This is to certify that the work presented in the dissertation entitled *Design, Fabrication and Validation of a Low Cost, Handheld Biochemical Analyzer* submitted by *Punuganti Sai Akanksha*, Roll Number 113BM0012, is a record of original research carried out by her under my supervision and guidance in partial fulfillment of the requirements of the degree of *Bachelor of Technology* in *Biotechnology and Medical Engineering*. Neither this dissertation nor any part of it has been submitted earlier for any degree or diploma to any institute or university in India or abroad.

Dedication

I dedicate this thesis to my family, without whose relentless support it would not have been possible for me to come this far.

Declaration of Originality

I, *Punuganti Sai Akanksha*, Roll Number *113BM0012* hereby declare that this dissertation entitled *Design, Fabrication and Validation of a Low Cost, Handheld Biochemical Analyzer* presents my original work carried out as a bachelor student of NIT Rourkela and, to the best of my knowledge, contains no material previously published or written by another person, nor any material presented by me for the award of any degree or diploma of NIT Rourkela or any other institution. Any contribution made to this research by others, with whom I have worked at NIT Rourkela or elsewhere, is explicitly acknowledged in the dissertation. Works of other authors cited in this dissertation have been duly acknowledged under the sections “Reference” or “Bibliography”. I have also submitted my original research records to the scrutiny committee for evaluation of my dissertation.

I am fully aware that in case of any non-compliance detected in future, the Senate of NIT Rourkela may withdraw the degree awarded to me on the basis of the present dissertation.

May 11, 2017
NIT Rourkela

Punuganti Sai Akanksha

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Abstract

Biochemical Analyzer (BCA) aids in the analysis and quantification of biological fluid samples for the concentration of desired biomolecules. This measurement of substances is used in various settings – from diagnostics to prognostics and even in security checking. Current biochemical analyzers are quite expensive, need large space for installation and not suitable for on field applications due to non-portability. The current study is focused on the design and fabrication of a low cost, portable and reliable biochemical analyzer for on-site application. Briefly, the device was conceptualized comprising of an optical assembly, rotator-gear system, interfacing hardware and software components. The outer housing and the proper positioning of each component was designed on SolidWorks™ (V2014-15 SP-2, SolidWorks Corporation, USA). The same was fabricated from Acrylonitrile butadiene-styrene (ABS) by 3D printing. The optical unit incorporates inexpensive light emitting diode (LED), photo electric transducers (L14F2 photo transistor) and low cost optical interference band pass filters. The optical filter was validated by a custom made paper craft spectrometer. An Arduino UNO board was used for interfacing the detector with an LCD, which displays the readings. The measurements were based on Beer Lambert's law. Thus, a series of experiments were conducted to get optimized alignment of sensor-sample holder-light source trio. Though the device has the options to accommodate multiple optical filters, the current study was based on single bandpass interference filter with peak absorption at 505 nm (with a target to estimate blood glucose level). For an easy selection of these optical filters, a mechanical gear system was employed. As the final step, the device would be validated for its accuracy by estimating blood glucose and comparing the same against the reading by an IVD certified biochemical analyzer, Evolution 3000. Such a low cost hand held biochemical analyzer has the great potential to be used effectively in remote rural health set ups and medical camps as well.

Keywords*: *Biochemical Analyzer (BCA); LED; Arduino; Beer Lambert's Law; Phototransistor; Optical Filters.

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Chapter 1

INTRODUCTION

1.1 Background and Significance of Study

In the medical context, Biochemical analyzer is a device used to analyze the content of biological samples such as blood, urine etc. Additionally, it is used in a variety of settings ranging from nominal security checks to diagnostic purposes. For clinical applications, it is used in various types of tests for the estimation of a wide range of substances including enzyme levels (used in liver function tests, thyroid tests etc.), ion levels (e.g. magnesium and sodium) and other chemicals such as creatinine or serum albumin. The measurement of these biomolecules is necessary to diagnose various human health conditions.

These devices are based on the principle of Beer Lambert's law [1] and most generally incorporate a spectrophotometer. Existing portable Biochemical analyzers in the market utilize complex technology and numerous interfaces such as concave mirrors and other collimators in their spectrophotometers, making them expensive and difficult to carry. These incorporations make them large in size and also necessitate staff training. These large devices are useful in laboratory settings where a large number of measurements are to be made simultaneously and quickly. But these cannot be used for on-site applications, especially in case of medical camps held at rural areas. Thus, there is a lack of appropriate on-site hand held and accurate analyzers.

The proposed device incorporates these characteristics while also delivering an easy management system to the user. It is a single beam instrument based on spectrophotometric principles that have at their core, the application of the Beer-Lambert law for determining the concentration of biomolecules. Dual beam devices compare the light intensity between two light paths, one path containing a reference sample and the other the test sample. Whereas, Single beam spectrophotometers measure the relative light intensity of the beam before and after a test sample is inserted. The proposed device serves as a point of care testing instrument and will be built on spectroscopic technique that uses effective measurement of wide range of solutes by optimizing the routing channels for the transmitted light. Although the measurements from Dual-beam instruments are stable, single beam instruments ensure a dynamic range and are optically simpler and more compact [2]. Thus, this device uses a single beam principle to cater the needs of point of

care estimations and at the same time ensuring a compact design. This compact design corroborates a portable hand held device that can be carried with ease to any place, while maintaining accuracy and a user friendly interface.

The current instrument is designed to incorporate low-cost optical interference bandpass filters that allow only the required band of light to pass, eliminating the rest [3]. A light emitting diode (LED) is to be used as the light source, as it has several advantages over other light sources, including low cost, stability of light emitted and utilization of low power [4]. The system consisting of Arduino UNO board incorporated with LCD and a push button serves as the user interface [5].

1.2 Scope and Problem Statement

Existing portable Biochemical analyzers in the market utilize complex technology and numerous interfaces such as concave mirrors and other collimators in their spectrophotometers, making them expensive and difficult to carry. These incorporations make them large in size and also necessitate staff training. These large devices are useful in laboratory settings where a large number of measurements are to be made simultaneously and quickly. But these cannot be used for on-site applications, especially in case of medical camps held at rural areas. Thus, there is a lack of appropriate on-site hand held and accurate analyzers.

Measurement of a desired substance, whose concentration is useful in diagnosing a particular condition of the human health condition, proves to be difficult, especially in rural settings. It demands for the transportation of large and expensive biochemistry analyzers. Hence, there is a need for portable, low cost and user friendly biochemical analyzer. The proposed device incorporates these characteristics while also delivering an easy management system to the user.

The current study involves the design and fabrication of low cost, portable and reliable biochemical analyzer. For the prototype, a single optical filter is incorporated. But

depending upon the need and use, it can be further developed to analyze a variety of substances by incorporating appropriate number of optical filters in the filter wheel.

1.3 Objective of the Study

To design, fabricate and validate low cost, portable and reliable Biochemical Analyzer for on-site application by incorporating:

- Cost effective photoelectric transducer based detector (photodiode or phototransistor).
- Low cost optical bandpass interference filters.
- Mechanically moving user-friendly components.

1.4 Principle

Colored substances absorb a characteristic range of wavelengths. When the light from the LED falls on sample held in the cuvette, the desired analyte absorbs its characteristic peak absorption wavelength. The basic principle of the biochemistry analyzer is based on LAMBERT BEER LAW [6].

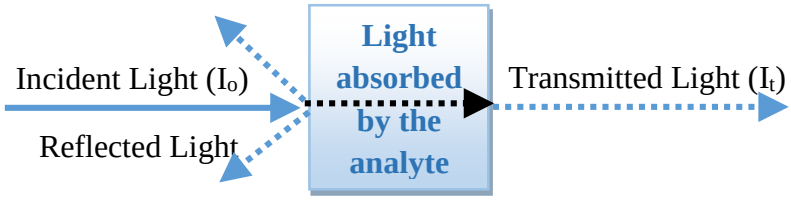


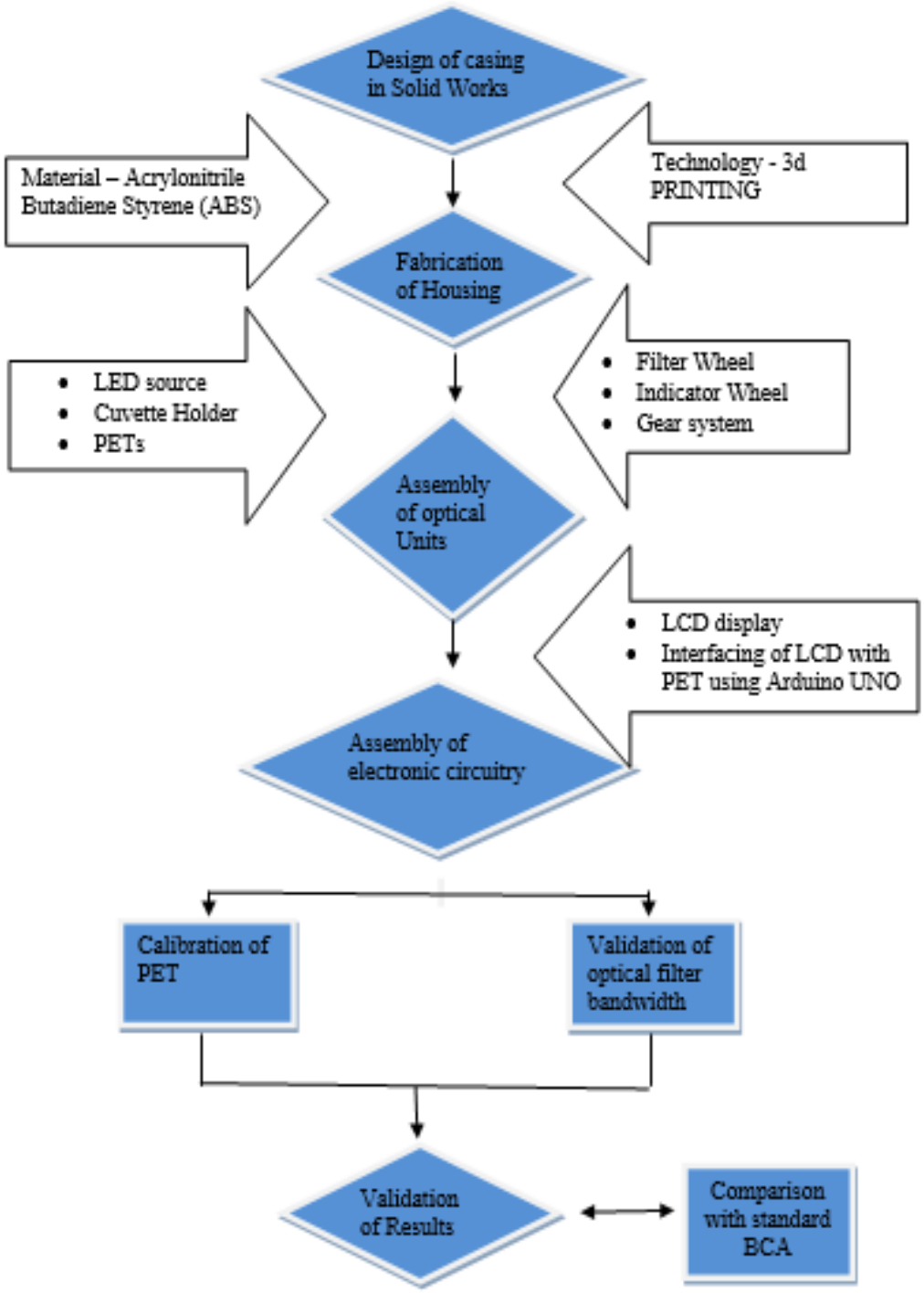
Figure 1.1: Lambert Beer Law

According to Beer Lambert's law (Figure 1.1), the amount of light absorbed is proportional to the concentration of analyte and the path length. If I_o is the intensity of light incident on the sample and I_t is the intensity of light after passing through the sample, then it is represented as -

$$I_o/I_t = e^{KLC}, \text{ where K is absorbency}$$

Thus, the concentration of the analyte can be directly measured from the transmitted light, keeping the path length constant. The intensity of transmitted light will be reflected by the voltage of the detector, as they are proportional to each other [7].

1.5 Work Plan



Chapter 2

LITERATURE REVIEW

2.1 Light Source

Colored substances absorb characteristic portion of mixed wavelengths. The remaining light will then assume the complementary color to the wavelength absorbed. The biochemical analyzer is based on the principle of Beer Lambert's law, which states a linear relationship between concentration, path length and amount of light absorbed by the analyte. Keeping the path length constant, the concentration of the analyte can be measured. Light bulbs and halogen lamps yield continuous spectrum and infrared range rays, but cannot be used in ultra violet range. Xenon discharge lamp produces both visible and ultraviolet light, but its light is composed of continuous and line spectrum with huge differences between intensities at different wavelength. It is also quite expensive and is not very stable. Light Emitting Diodes (LED) have played important role in many optical sensing devices. Due to their compactness, reliability, low power consumption, low cost, capability of automated and independent operation, and compatibility with wireless communications systems, the Light Emitting Diodes have been increasingly used in miniaturized devices [4].

2.2 Photo Detector

There are numerous photo detectors available commercially: photodiodes, phototransistors, photo darlingtons, photomultiplier tubes, photo resistors, integrated circuits, various hybrids and even thermopiles. While each one has its own advantages and disadvantages, the article by Larry Godfrey on choosing the detector, has compared and characterized the detectors based on numerous parameters. The article also provides insights on selecting the best approach for the various ultraviolet, visible and near-infrared light sensing applications. Specific application needs considered include light source, spectral characteristics, optical power, meeting electronics packaging constraints, signal-to-noise ratio, frequency bandwidth, reproducibility, cost, sensitivity, linearity and physical size [8]. Photodiodes and phototransistors prove to be better suitable for biochemical analysis purposes. Although integrated circuits and sensor electronic assemblies provide better immunity to noise compared to a photodiode, they require

expensive custom tooling and calibration processes. Photo transistors have been shown to be the best detectors for optical sensors involving low intensities of light due to their in-built amplification, higher sensitivity, faster response time and lower noise compared to the existing photodiodes and LDR (Light Dependent Resistance).

2.3 Principle of Operation

The basic principle of the biochemistry analyzer is based on LAMBERT BEER LAW. This law relates absorption of light to properties of the medium through which it travels. The amount of light penetrating into the solution which is termed as transmittance, and is expressed as the ratio of intensity of the transmitted light and intensity of the incident light beam. According to Beer Lambert's law, the amount of light absorbed is proportional to concentration of the analyte and the path length. Thus, the concentration of the analyte can be directly measured from the transmitted light, keeping the path length constant (the width of the cuvette) [9]. The intensity of transmitted light will be reflected by the voltage of the detector (photo-electric transducer), as they are proportional to each other [7].

2.4 Sequence of Components in Optical Assembly

The optical bandpass interference filter passes light waves with wavelengths falling within the given range, while attenuating those outside the range through the phenomenon of interference. The optical filter in most cases is placed after the sample, to obtain accurate measurements by the elimination of scattering effects. It is placed before the sample only in case of instruments involving a monochromatic system in order to separate the resultant light rays after absorption into their respective wavelength components [3].

Chapter 3

MATERIALS AND METHODS

3.1 Components

The salient features of the current biochemical analyzer are it low cost, portability and reliability. The following components have been chosen accordingly, in order to incorporate these characteristics:

1. For the optical assembly:
    a. Optical bandpass interference filters
    b. Filter wheel
    c. Indicator wheel
    d. Axle and Gear system

2. For the detector, a photoelectric transducer (photo-transistor)

3. For the Electronic Circuitry:
    a. Arduino UNO board
    b. LCD display
    c. Push Button

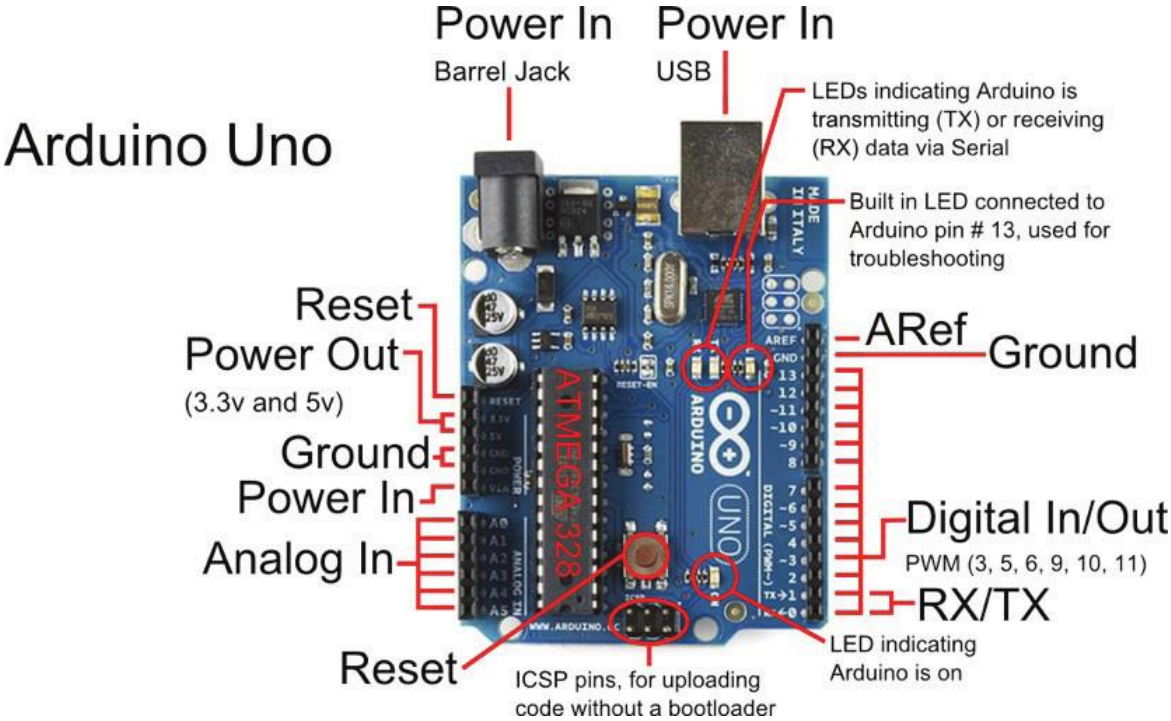


Figure 3.1: Arduino UNO Board

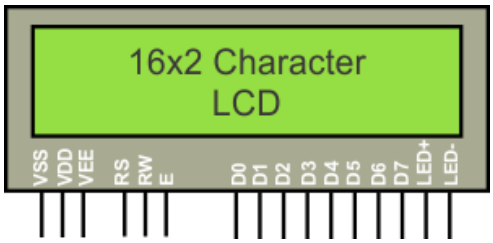


Figure 3.2: 16x2 LCD Display

Figure 3.3: Push Button (4 pin)

3.2 Design and Fabrication of Casing

The outer casing of the biochemical analyzer was designed using SolidWorks™ (V2014-15 SP-2, SolidWorks Corporation, USA) software. It was done by first determining the dimensions of respective components to be used - namely the Arduino UNO board, cuvette and LCD display. Based on these dimensions the casing of the biochemical analyzer has been designed in SolidWorks™.

The casing was divided into two parts- the upper casing (Figure 3.5) and the lower flat base (Figure 3.4). This was done to provide the ease of replacing or fixing any inner component of the biochemical analyzer. An opening was given on the upper casing through which the cuvette will be introduced. A lid (Figure 3.6) to close the opening was provided along with a small fillet based locking system. Four projections were given at the base of the upper casing which would fit into corresponding holes of the lower flat base. The resulting model is as shown in Figure 3.7.

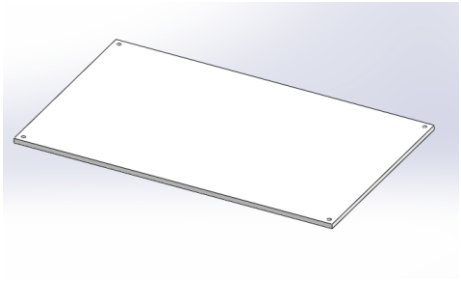


Figure 3.4: Base of the Outer Casing designed in SolidWorks™

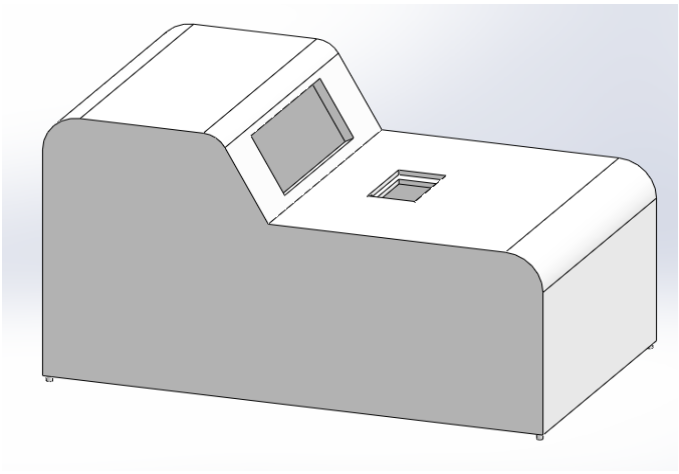


Figure 3.5: Outer Chassis (fixed on top of the base) designed in SolidWorks™

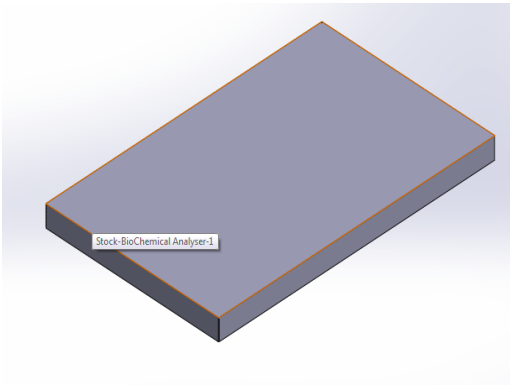


Figure 3.6: Lid for sample opening designed using SolidWorks™

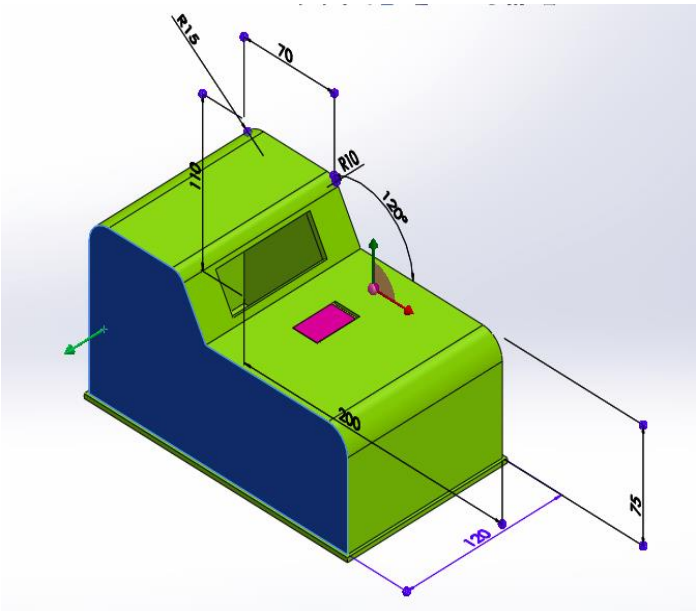


Figure 3.7: Outer Casing designed in SolidWorks™ (all measurements in mm)

3.3 Validation of L14F2 Phototransistor

3.3.1 Glucose Concentration

The L14F2 is an npn Phototransistor with a gain factor of 1075. Out of the available photodetectors, L14F2 was chosen as the best option due to its in-built amplification, higher sensitivity, faster response time and lower noise compared to the existing photodiodes and LDR (Light Dependent Resistance) [10] [11]. Due to the weak light signals involved, the amplification provided by the phototransistor is much needed, as it eliminates the use of external amplification circuitry which in turn adds noise to the readings. The output values of L14F2 were measured using Arduino UNO for varying glucose concentrations with white LED as the light source. Readings were taken in a dark room (to avoid interference from extraneous light sources) as shown in Table 4.1.

Figure 3.8: L14F2 Phototransistor

3.3.2 Optimal Distance

Experiments were conducted in order to determine the optimized distance between LED, Cuvette and PT sensor in various configuration. The readings were taken in a dark room in order to eliminate interference from extraneous light sources. Initially, the cuvette and the LED light source were kept close together and the sensor (PT) was gradually moved away from the LED at intervals of 0.5cm. The readings were taken from Arduino at each step. This procedure was repeated for both blank (i.e. distilled water) and dye (crystal violet stain) solutions. Thereafter, another configuration was adopted to confirm the conclusions obtained from this setup. This involved the crystal violet stain as the sample. Readings were taken while decreasing its concentration by adding constant volume of distilled water, while also keeping the three components in three different configurations. The setup and circuit used are as shown in Figure 3.9 and Figure 3.10. The Arduino UNO was

accordingly programmed to obtain the output voltage from the PT sensor. The output voltage was computed as the average of 250 samples (each sample taken within an interval of 1ms).

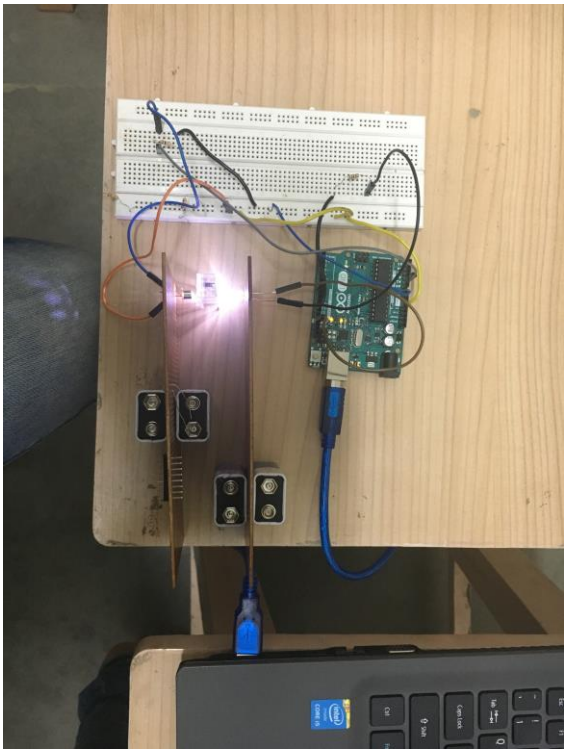


Figure 3.9: Top View of the sensor validation setup

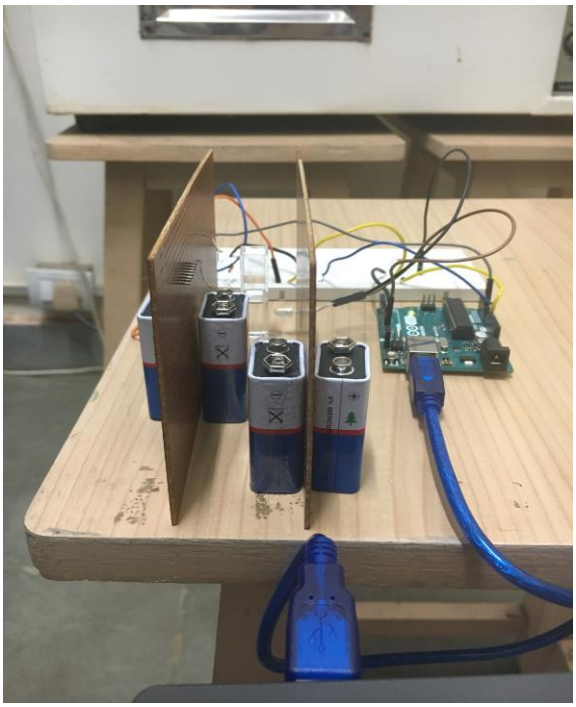


Figure 3.10: Side View of the sensor validation setup

3.4 Validation of Optical Filter

The optical bandpass interference filter was purchased from Adonis Biotech Pvt. Ltd., (India). A bandpass interference filter passes light waves with wavelengths falling within the given range, while attenuating those outside the range through the phenomenon of interference. The optical filter obtained is shown in Figure 3.11, 3.12 and 3.13.

Figure 3.11: Top View of the Optical Filter

Figure 3.12: Back View of the Optical Filter

Figure 3.13: Side View of the Optical Filter

Public Lab (www.publiclab.org) is an open source, online community that aims to solve environmental issues through science. It develops and applies the developed open source tools to environmental exploration and investigation by designing accessible DIY techniques. The Papercraft Spectrometer Kit is a DIY foldable paper spectrometer developed by the Public Lab that can be attached to either a laptop or a smartphone in order to obtain the spectrum of any desired material by isolating the incident light into its constituent wavelength components [12]. The instructions to construct the spectrometer and the final output are as shown in Figure 3.14 and Figure 3.15. The present hand-made instrument incorporates a small portion of compact disk (CD) as the diffraction grating, which thus separates the incident light.

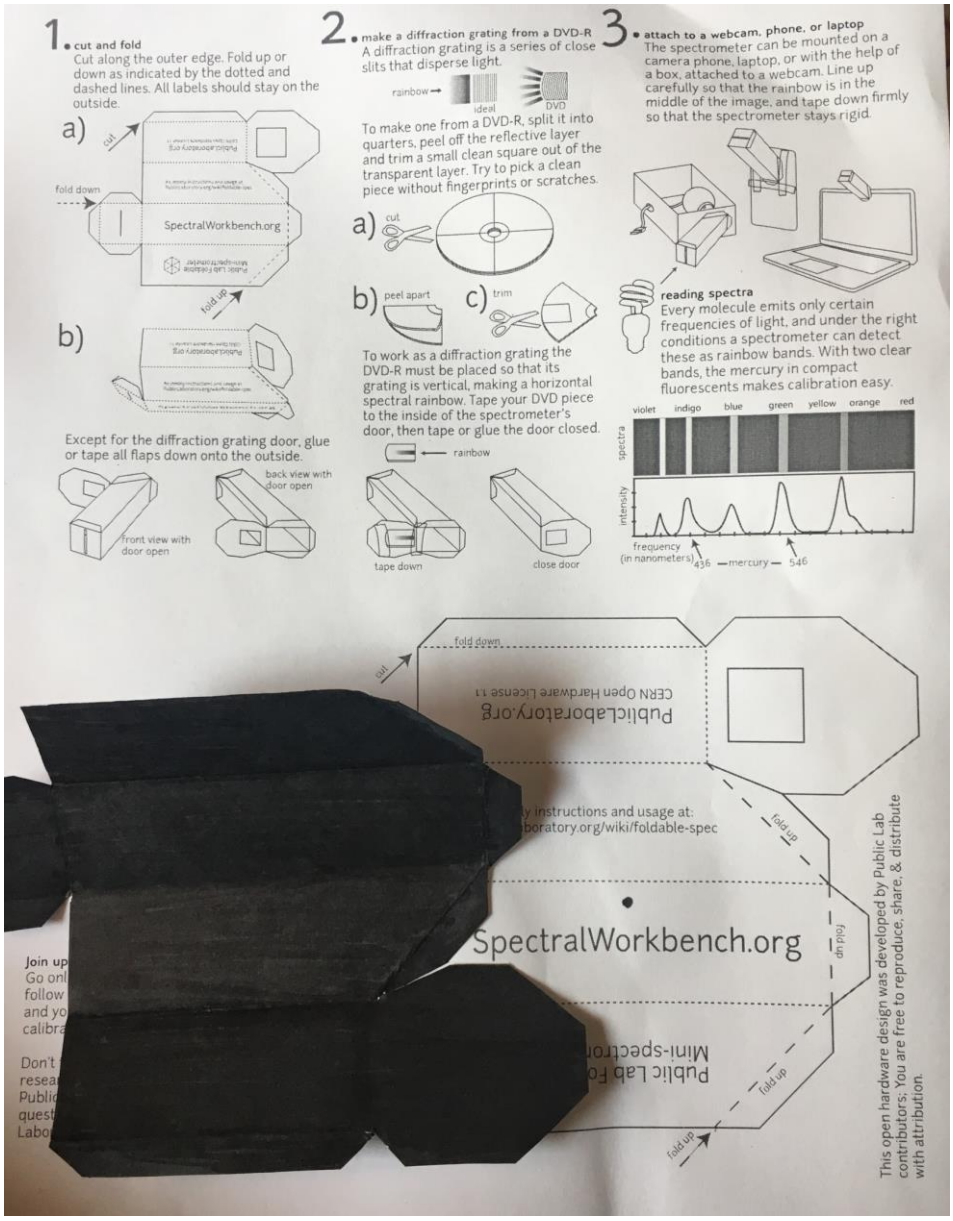


Figure 3.14: Instructions for the Papercraft Spectrometer

Figure 3.15: Front View of the Handmade Paper Spectrometer

Figure 3.16: Back View of the Handmade Paper Spectrometer

The resolution and stability of the spectrometer depends on the digital camera used to record light in the visible range, ~400-700 nanometers, which also determines the range of the device. The resolution is mainly limited by the resolution of the camera and is generally around 3 nanometers per pixel. In the clinical context, a resolution of 3nm is prescribed to be adequate. It is also affected by the narrowness of the slit.

The completed spectrometer was used to validate the current optical filter by mounting it onto a laptop (Figure 3.17). The spectral data was collected and analyzed by using a web based software, Spectral Workbench (www.spectralworkbench.org), which is an open source software developed by the Public Lab and can be used for other spectrometers as well. This software enables the attachment of the spectrometry kit,

scanning and saving of the samples, wavelength calibration of the spectrometer, plotting of the light intensity as a function of wavelength among other functions. A CFL table lamp was used as the source of light. In order to get scaled data, the spectrometer is wavelength calibrated using the spectrum of the CFL light which allows the spectrometer to display a wavelength value for any color of light incident on it [13].

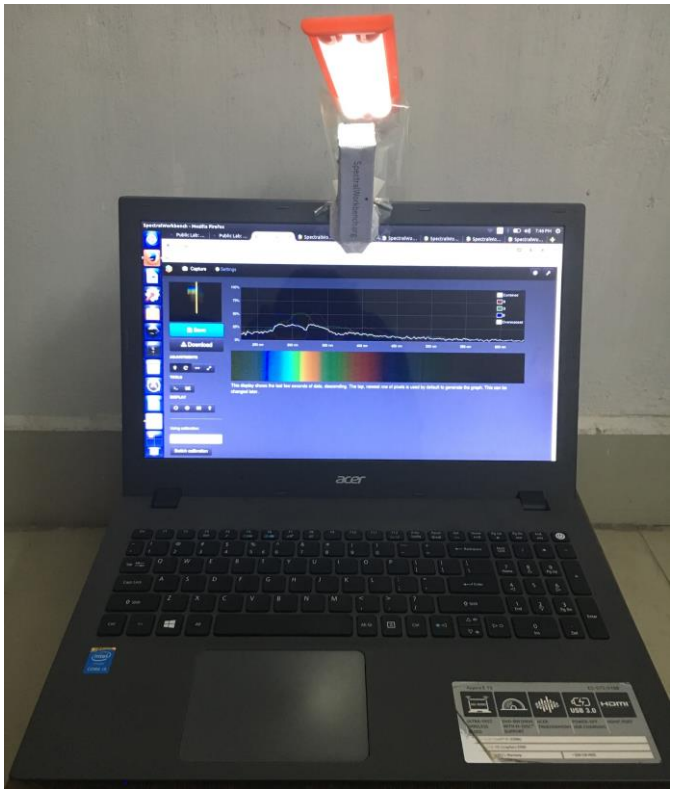


Figure 3.17: Spectrometer attached to Laptop and readings taken in Spectral Workbench

3.5 Electronic Interface

3.5.1 General Protocol

The user interface is provided by an LCD display, inter-connected with two push buttons, both of which are interfaced with the Arduino UNO board. This interface serves to prompt the user in the correct direction in a step by step process to obtain correct results. The Arduino is programmed to display the particular step in the analysis process on the LCD. The general protocol consists of four steps: adjustment of filter (based on the biomolecule to be measured), measurement of blank solution, measurement of standard solution and measurement of sample solution, at the end of which the results are displayed. After the completion of each step, the user presses 'YES/OK' button in

response and thus initiates the next step. After the button is pressed at the completion of a step, the next step is displayed on the LCD. If at any step the user wishes to terminate the process, then upon pressing the 'NO' button, the Step 1 (adjustment of filter) is thereby accessed. The flow of steps is as shown in the flow diagram (Figure 3.18). The LED and PT connected to the Arduino accordingly perform their respective functions upon receiving information from the Arduino. LED is switched on only when taking the readings, with a delay time of 3sec for stabilization. As before, an average of 250 samples is taken as the final output value.

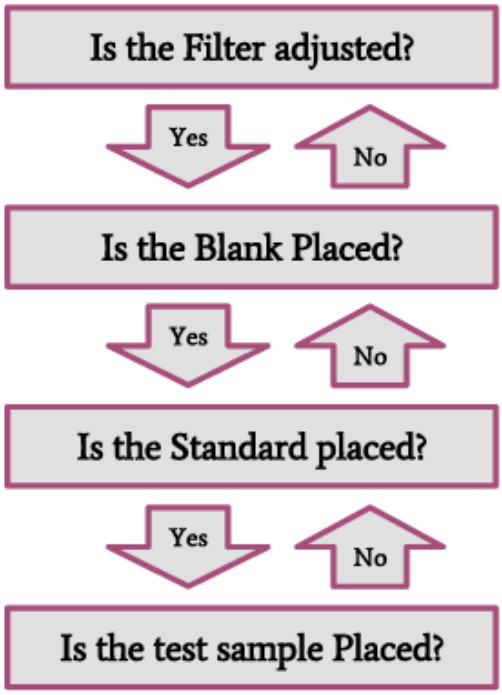


Figure 3.18: Process Flow Diagram for General Protocol

The working of the LCD and push button using Arduino platform were first tested separately. Next, the two components were interconnected and the board programmed using Arduino Software. The current device was tested with reagents from Tulip Diagnostics (P) Ltd., (India) and it is designed so that it can also be used with other reagents in order to aid the measurements. These reagents react on the analyte and bring its peak absorption to the visible range. This group of reagents use three solutions: blank, standard and test sample. The absorbance of the standard and test samples are then measured against the Blank solution. From these values, the concentration of required analyte is computed as per the protocol [14].

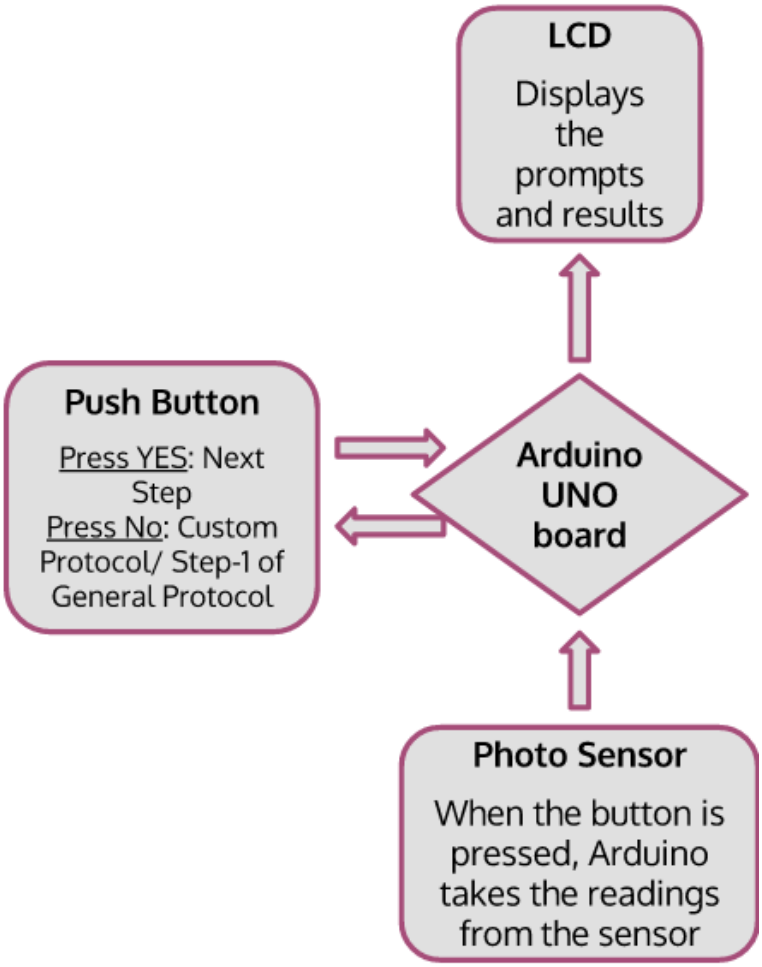


Figure 3.19: Flow Diagram describing the Electronic Interface in General Protocol

3.5.2 Custom Protocol

The custom protocol allows the user to change various parameters depending on the measurement of the desired biomolecule concentration. Pressing 'NO' at the first step (filter adjustment) of the general protocol guides the user to the custom protocol. This protocol is programmed to include the variables: Delay Time (for stabilization and taking readings), Units (mg/dl, g/dl, ug/ml etc.,), Low Limit Value, High Limit Value, Maximum Linear Range and Number of Decimal Points (in the resulting concentration). Measurement of the concentration of the biomolecule within its corresponding linear range gives an approximate linear and accurate response, thus the maximum linear range might vary depending on the molecule to be measured. If the concentration obtained is above the high limit value, then the sample is to be diluted so as to bring down its concentration into the linear limit range [14].

The above parameters are changed by pressing the 'Increment' button ('NO' button in the previous case provides this incremental function), while the 'YES/OK' button is pressed to confirm the parameter value that has been set. These set values are then incorporated into the general protocol while taking the measurements. The flowchart of the custom protocol is shown in Figure 3.20. During the general protocol, the electronic interface involves the use of only information flow between Arduino, Push Buttons and LCD and does not involve the LED and PT as the protocol does not involve taking readings.

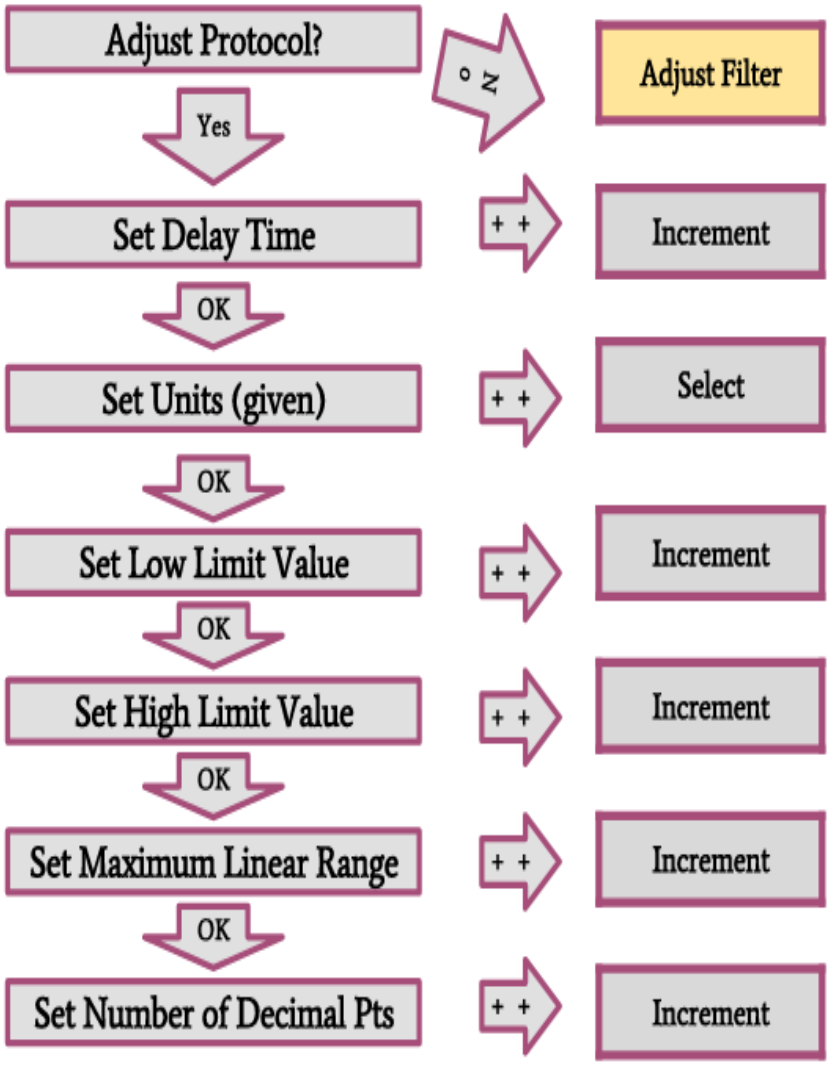


Figure 3.20: Process Flow Diagram for Custom Protocol

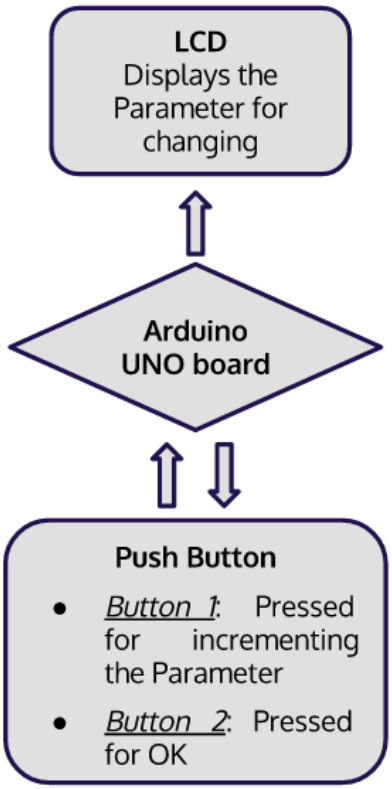


Figure 3.21: Flow Diagram describing the Electronic Interface in Custom Protocol

3.6 Embedding the Electronic Circuitry

The electronic interface comprises of total four circuits: push button circuit, LED circuit, PT sensor circuit and LCD circuit. As the first three circuits involve basic connections and are quite small, the components of the respective circuits were soldered onto Vero board. However, the LCD circuit was made onto a PCB.

In order to model the LCD circuit onto a PCB, Fritzing software was used. It is an open-source hardware initiative affiliated with the Arduino used for designing PCBs and modeling electronic circuits. PCB, an acronym for printed circuit board, allows signals and power to be routed between physical devices. Solder is the metal that makes the electrical connections between the surface of the PCB and the electronic components.

The circuit was first designed on breadboard (Figure 3.22) after which the schematic circuit was developed (Figure 3.23). The connections on the PCB board were then made accordingly, giving rise to the PCB circuit layout (Figure 3.24). The circuit layout was

then exported (Figure 3.25) and imprinted onto a copper clad PCB board using heat iron. The imprinted circuit paths were then marked with a permanent marker, in order to prevent the etching of traces. This marked board was then etched with ferric chloride solution to remove the copper layer, leaving behind the copper traces (Figure 3.26).

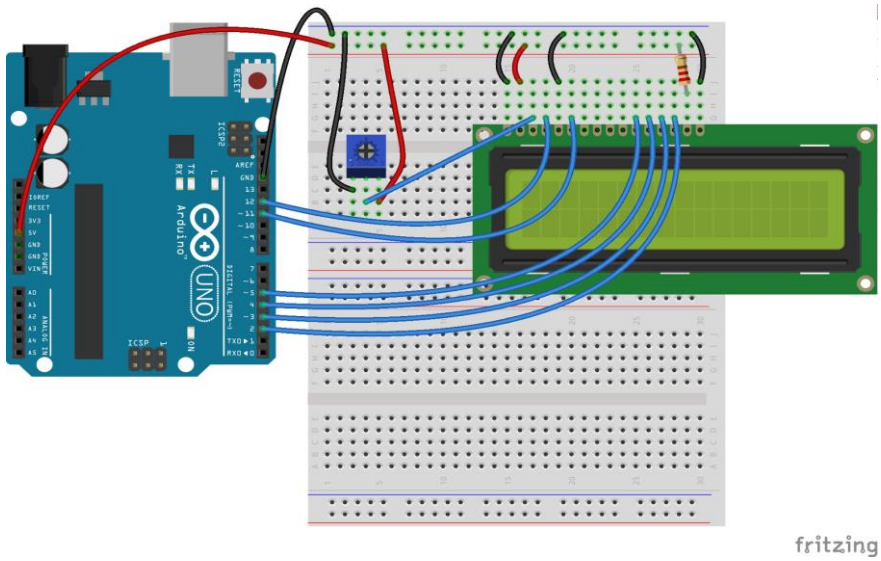


Figure 3.22: LCD circuit on breadboard

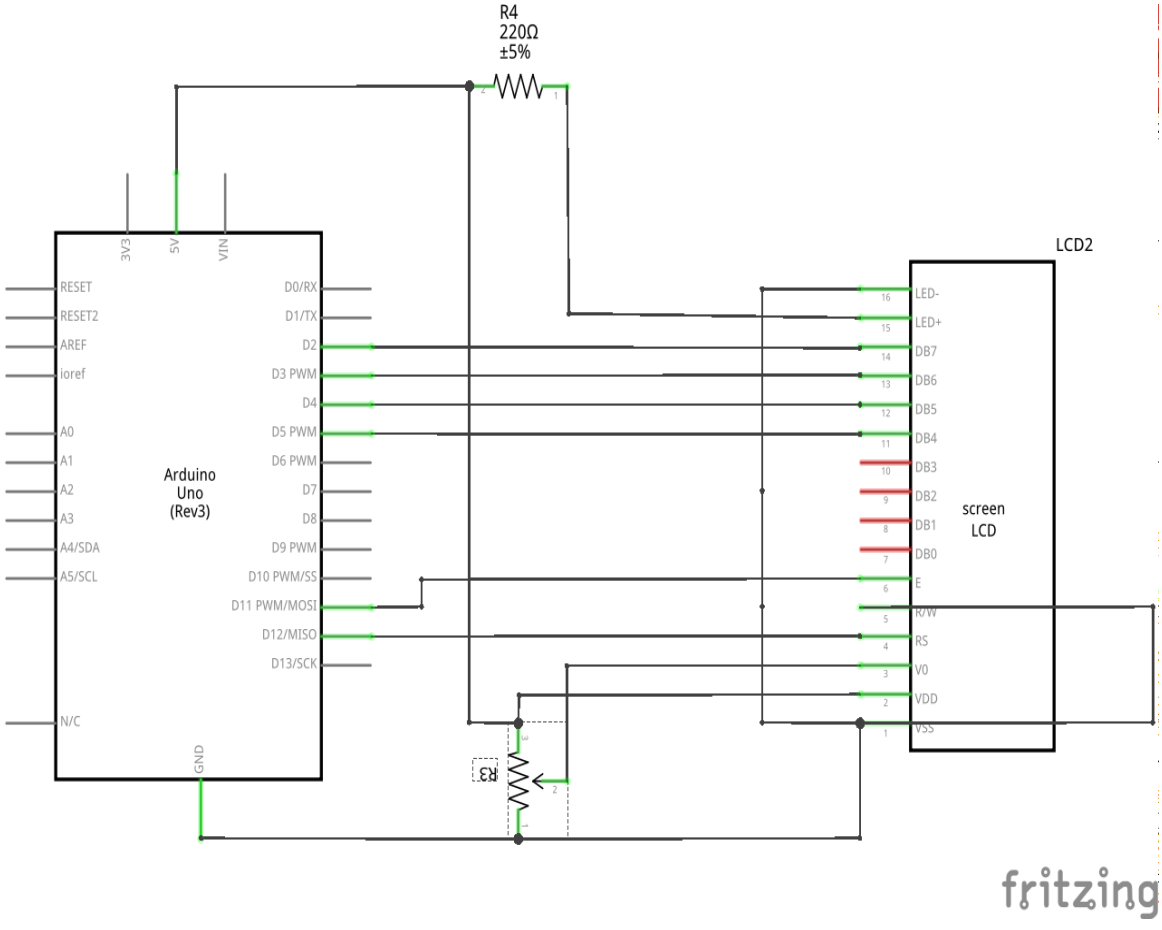


Figure 3.23: Schematic Diagram of the LCD circuit

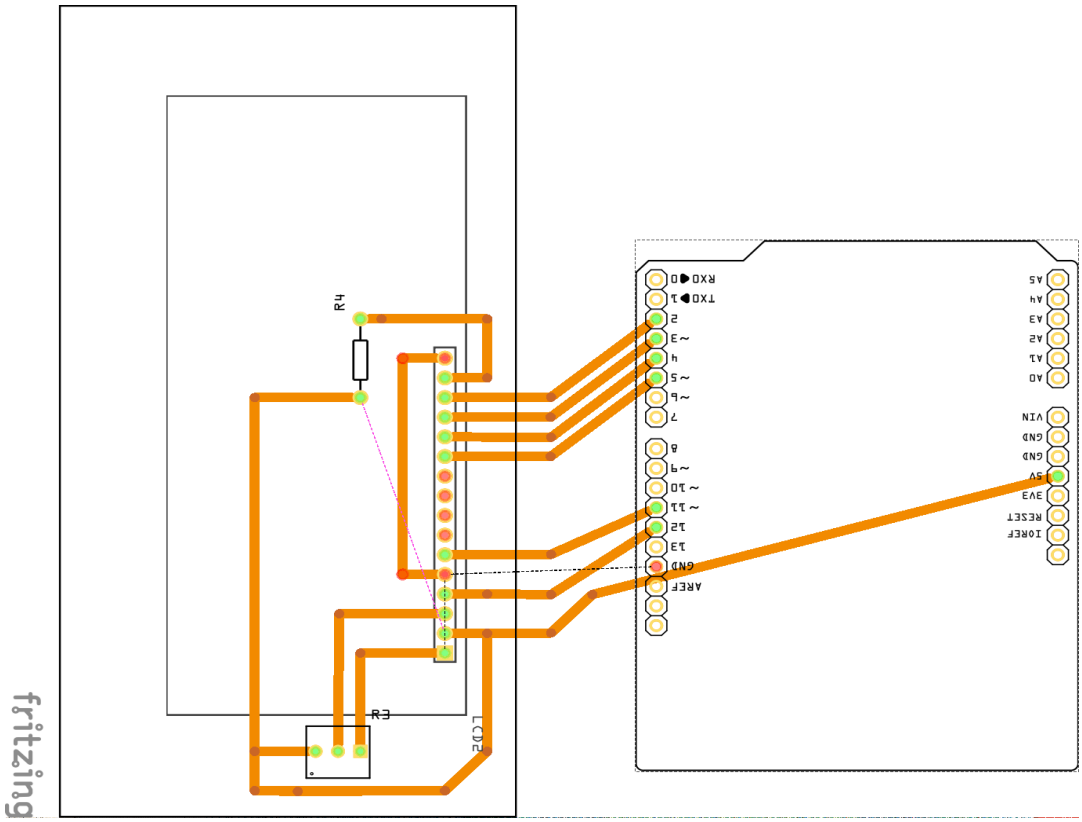


Figure 3.24: PCB design of the LCD Circuit

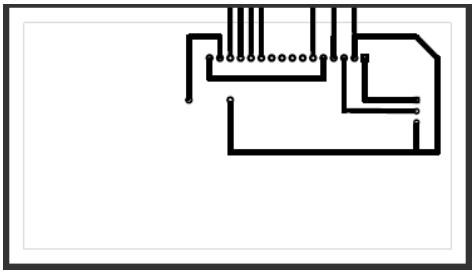


Figure 3.25: PCB layout of the LCD

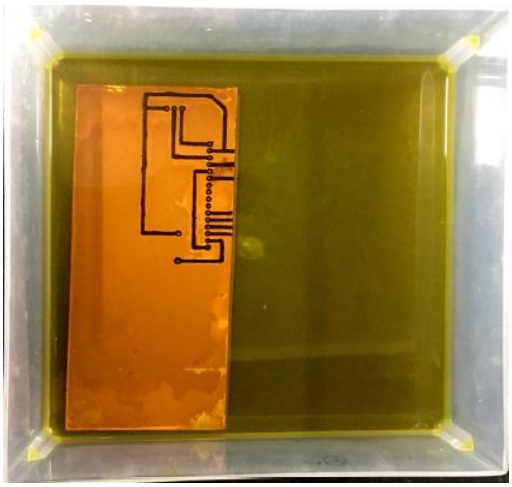


Figure 3.26: Etching of Imprinted PCB in Ferric Chloride solution

3.7 Design of Optical Assembly

The optical assembly consists of filter wheel, indicator wheel, PT holder, LED holder and cuvette holder. The assembly unit was designed first on paper (Figure 3.27 and Figure 3.28) and the dimensions of the optical assembly were selected according to the size of the components (i.e., cuvette, PT and LED) and that of the outer casing of the device. After finalization of the dimensions, the assembly components were modelled in SolidWorks™ (V2014-15 SP-2, SolidWorks Corporation, USA) software (Figures: 3.29, 3.30, 3.31, 3.32, 3.33 and 3.34). The models of the individual elements were then changed into STL format and fed into the 3D printer. The 3D printer used was Dimension SST 1200es™ (Stratasys Ltd., USA) machine, with Acrylonitrile Butadiene Styrene as the build material.

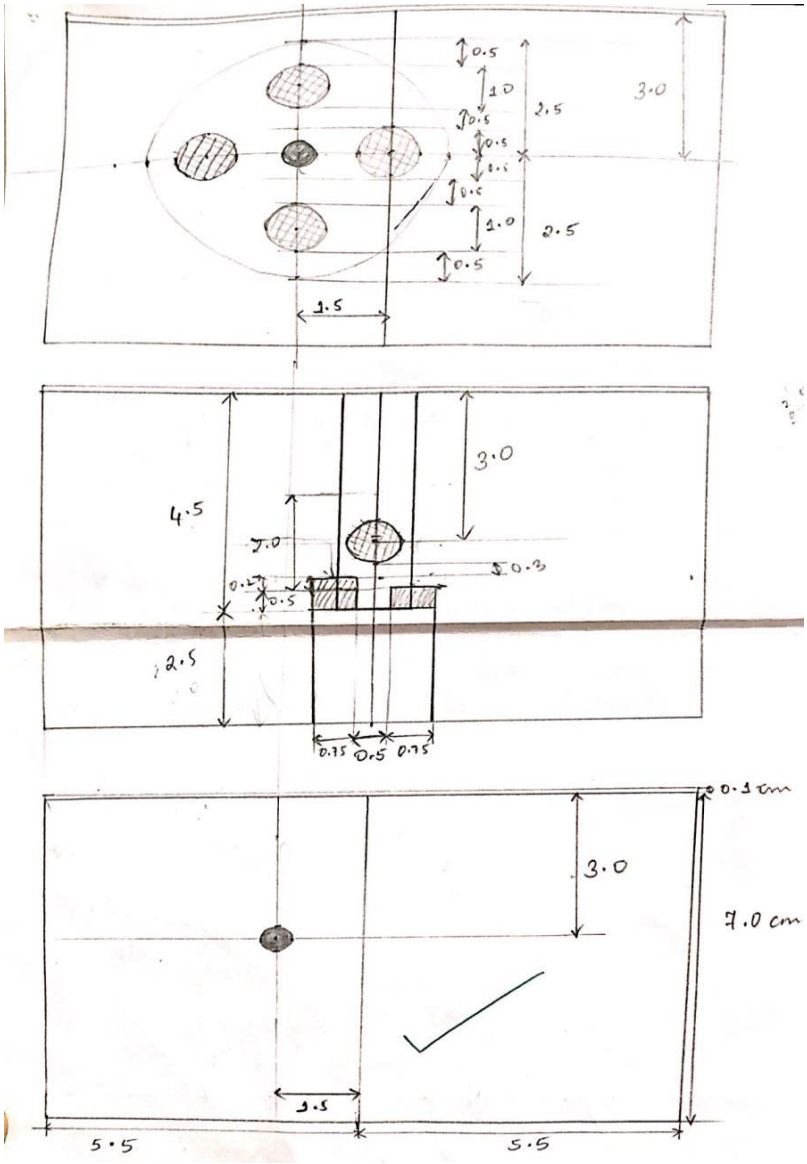


Figure 3.27: Side View of the Optical Assembly within BCA (Filter wheel, Indicator wheel, and cuvette holder)

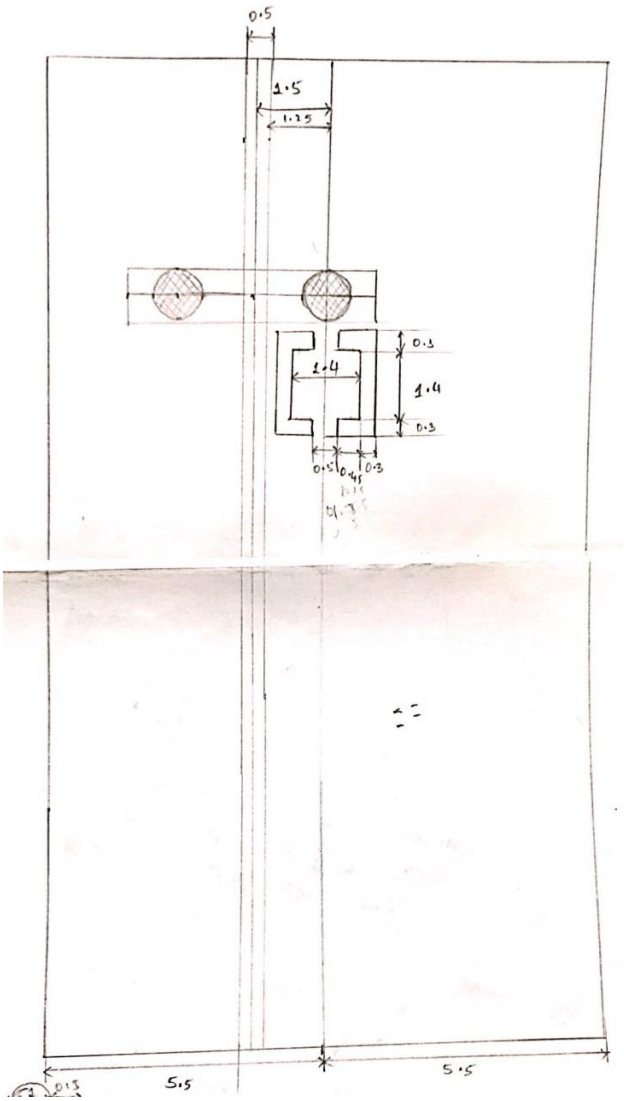


Figure 3.28: Top View of the Assembly Placement

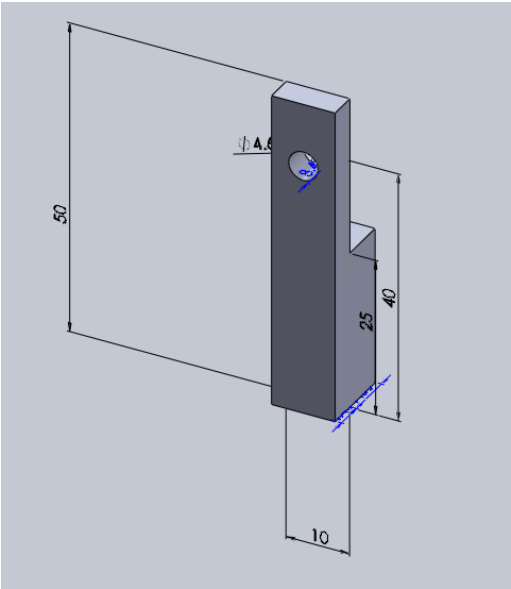


Figure 3.29: SolidWorks™ Model of PT Holder

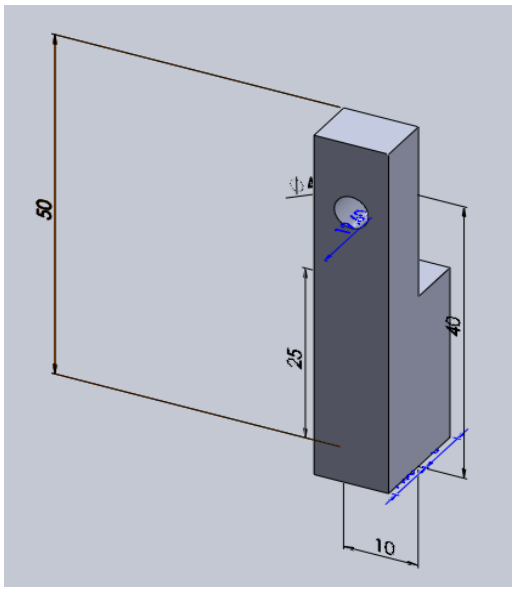


Figure 3.30: SolidWorks™ Model of LED Holder

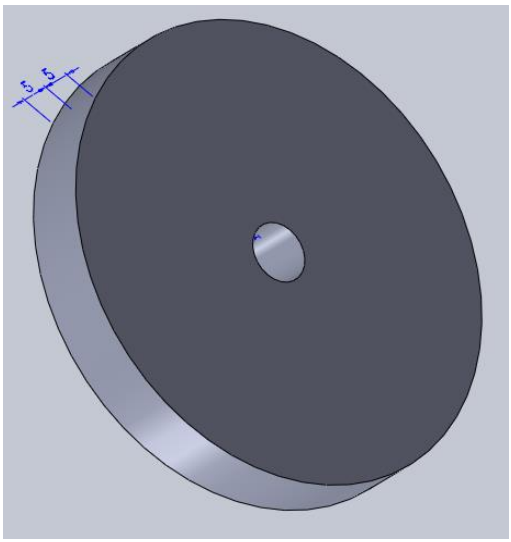


Figure 3.31: SolidWorks™ Model of Indicator Wheel

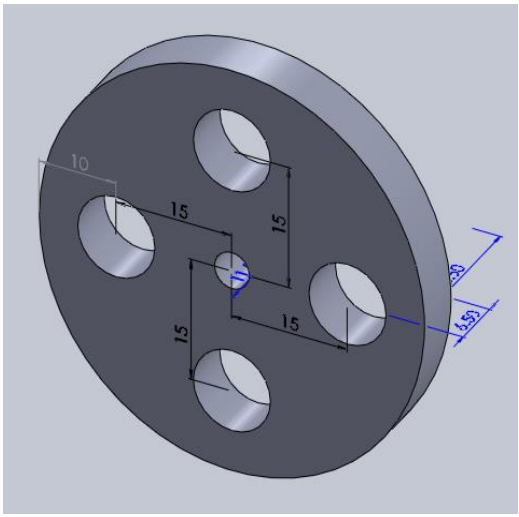


Figure 3.32: SolidWorks™ Model of Filter Wheel

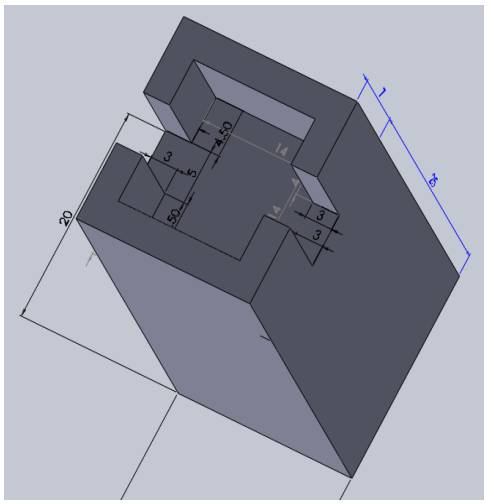


Figure 3.33: SolidWorks™ Model of Cuvette Holder

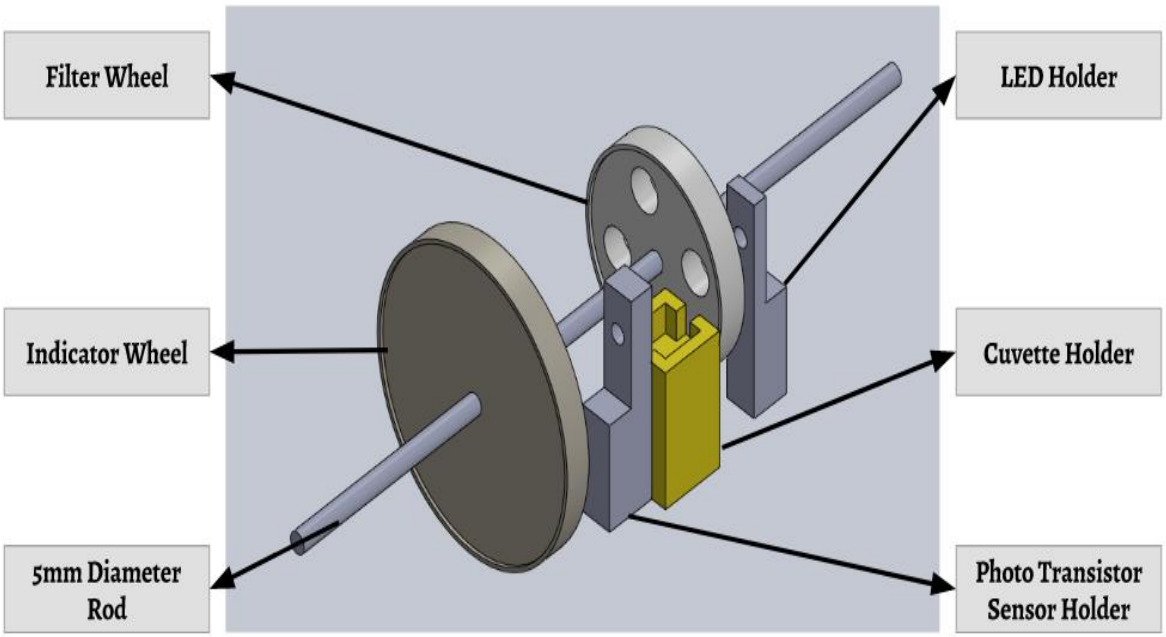


Figure 3.34: SolidWorks™ model of the complete optical assembly

3.8 Assembly of BCA

The two modules of the device - the Electronic Interface and the Optical Assembly - were then integrated onto the 3D printed outer casing of the BCA. The electronic interface consists of four separate circuit boards - that of the push buttons, LCD, LED and PT. The optical assembly consists of four components - cuvette holder, PT holder, filter wheel and indicator wheel. All these elements were arranged in their respective places onto the casing of the device.

3.8 Validation of BCA

The current device was initially validated for human blood samples by comparing with an IVD certified Biochemical Analyzer. In the next phase, it was validated with known glucose concentrations ranging from 80 mg/dL to 140 mg/dL.

3.8.1 Glucose Measurement protocol

The device was validated for glucose using the Glucose GOD/POD method. The current device was tested with reagents from Tulip Diagnostics (P) Ltd., (India). However, the in house device is compatible with reagents from other manufacturers after modifying the protocol as per the manufacturer's instructions. These reagents react on the analyte and bring its peak absorption to the visible range. This group of reagents uses three solutions: blank, standard and test sample [14].

According to the protocol, the solutions are prepared by adding 2 ml of the glucose reagent in three separate, dry test tubes. For 20 µl of distilled water, glucose standard and the sample are added to the Blank (B), Standard (S) and Test Sample (T) solutions respectively. The three solutions are then incubated for 10 minutes at 37 ^{o}C.

3.8.2 Determination of Glucose Concentration

The glucose concentration was determined as per the Glucose Kit Protocol. If the output voltages obtained for the three solutions Blank B, Standard S and Test Sample T are represented as V_B, V_S and V_T respectively, then the absorbance of the Standard (S), is given by [7] [14]:

$$\text{Abs (S)} = 2\text{-}\log_{10}(100 \times V_S / V_{B)}$$

Similarly, the absorbance of Test Sample is given by:

$$\text{Abs (T)} = 2\text{-}\log_{10}(100 \times V_T / V_{B)}$$

The total glucose concentration is then computed as:

$$\text{Concentration (mg/dL)} = (\text{Abs (S)} / \text{Abs (T)}) \times 100$$

3.8.3 Using Human Blood Samples

The current device was validated [15] initially for human blood samples. The results were compared with an IVD certified Biochemical Analyzer, Evolution 3000. The glucose concentration was measured using the serum from the blood samples. An approximate of 3 ml of blood was extracted from each of the subjects. The avoid glycolysis or other undesirable reactions which influence the glucose concentration in the blood sample, a small amount of sodium fluoride was added to the test tube before collecting [14]. The sample was then centrifuged at 1000 g for 10 minutes [16]. The serum was then collected from the resulting supernatant.

3.8.4 Using Known Glucose Concentrations

The current device validated using known glucose concentrations in distilled water in the next phase. The instrument was validated for accuracy, linearity and precision.

i. Accuracy

Accuracy is described as the assessment of the proximity of the measured value to the true value of the substance being measured. It is the process of determining whether the test system is producing valid and accurate results or not. In the present study, accuracy was determined by measuring 11 samples with known glucose concentrations. The Error Index parameter was used for this determination [17]. It is calculated by subtracting the known glucose concentration (X) from the measured value (Y) and dividing by the Total Allowable Error (TEa). The equation is given by: (Y-X)/TEa. The Error Index for each X-Y pair must fall within the range of -1 to 1 for the device to fall under the acceptability criteria for accuracy. The Total Allowable Error for glucose is given to be $\pm$ 6 mg/dL [18].

ii. Linearity

A device is said to be linear when the measured value is directly proportional to the actual concentration of the substance in the test sample. Thus, the relationship between the measured values and the actual concentration of the analyte is characterized by a straight line. Linearity verification on the current device was determined by first plotting the known values on the X-axis and the measured values on the Y- axis. The corresponding slope and the intercept of the plot are determined, using which a predicted value, P, was calculated for each of the X-

value. The predicted values were then plotted against the known values and a straight line drawn to connect the data points on the graph. The Systematic Error due to non-linearity is then computed by subtracting the measured values from the predicted values. For a device to be linear, this Systematic Error must be less than 50 % of the Total Error as well as the Total Allowable Error for each data point [19].

iii. Precision

Precision can be described as the repeatability or reproducibility of the measurements of the same sample run at different times. The coefficient of variation (CV) is used to measure precision. Precision is categorized into two types: between day and within day. Between day was tested by running two known glucose concentrations for 5 consecutive days. Within day was tested by running two known glucose concentration samples 20 times in one day. The CV is calculated for each of the concentrations in both the cases. The acceptability criteria for a device to be precise states that the % CV for each run must be equal to or less than that specified by the manufacturer [20] [21].

Chapter 4

RESULTS AND DISCUSSION

4.1 Outer Casing

The designed casing was 3D printed using Dimension SST 1200es™ (Stratasys Ltd., USA) 3D printer (Figure 4.2) with Acrylonitrile Butadiene Styrene (ABS) as the base material. The upper part of the casing had to be divided so that the 3D printed model was produced without any damage. The produced casing is as shown in the Figure 4.1. ABS is a lightweight, thermoplastic polymer with robust mechanical properties. The most important properties of ABS are its resistance to heat and toughness. However, it does allow light to pass through it to a certain extent. In order to maintain the casing completely immune to light rays, an additional layer of Polyurethane Lacquer (PU Lacquer) has to be coated.

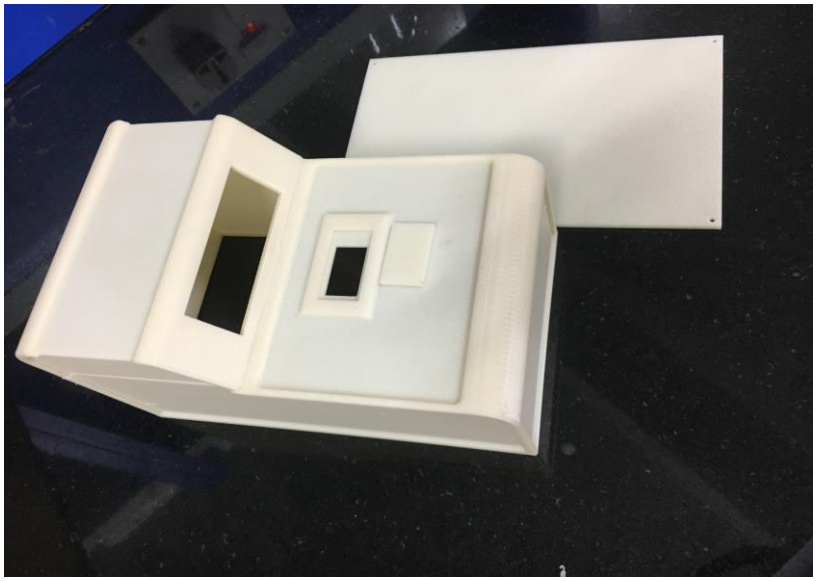


Figure 4.1: 3D printed Biochemical Analyzer outer casing

Figure 4.2 Dimension SST 1200es™ 3D Printer

4.2 Validation of L14F2 Phototransistor

4.2.1 Glucose concentration

The output voltage from the L14F2 is seen to increase with increasing glucose concentrations. This can be either attributed to the increased scattering of higher amount of particles or the increasing nature of the phototransistor with increasing light intensity. The former maybe due to the fact that the amount of glucose taken was in the order of g/ml whereas in human blood samples, the glucose levels are in the range of mg/dl. The results obtained can be made more accurate and sensitive by the addition of chromophores (reagents).

Table 4.1: Output voltage of L14F2 for various glucose concentrations

S.No	Weight of Glucose Used (gm)	Concentration (M)	Output Voltage (V)
1.	0	- (blank)	4.907
2.	0.09	0.05	4.907
3.	0.18	0.1	4.917
4.	0.36	0.2	4.917
5.	0.54	0.3	4.922

4.2.2 Optimal Distance

The output obtained for the first alignment - cuvette and LED placed together while the PT is gradually moved away from the LED - is shown in Figure 4.3 and Figure 4.4. According to the graphs obtained in both the cases - for blank and for colored solution (crystal violet stain/dye) - the amplification is highest when the PT is placed in close proximity to the LED and cuvette. This alignment could thus aid in detecting the lowest possible concentration. Thus, the amplification associated with this alignment was further confirmed by the output obtained from decreasing concentration of dye (addition of equal amounts of distilled water). As seen from Figure 4.5, when the PT is placed far away (8.2cm) from the LED, the output obtained is randomized, erroneous and incongruous with that of the concentration of the dye used. However, when the PT is brought within

0.5cm distance to that of cuvette (cuvette is also placed at 0.5cm from LED), the output obtained (Figure 4.6) shows an increased sensitivity and was in accordance with that of the decreasing concentration of the dye. When all the three elements are further brought in close proximity (i.e., LED, Cuvette and PT are all juxtaposed next to each other), the output (Figure 4.7) demonstrates the highest sensitivity with decreasing concentration of the dye. Hence it was concluded that the optimal distance between the PT, LED and cuvette was when all the three are placed as close as possible to each other. This alignment was then incorporated into the optical assembly and accordingly designed and fabricated.

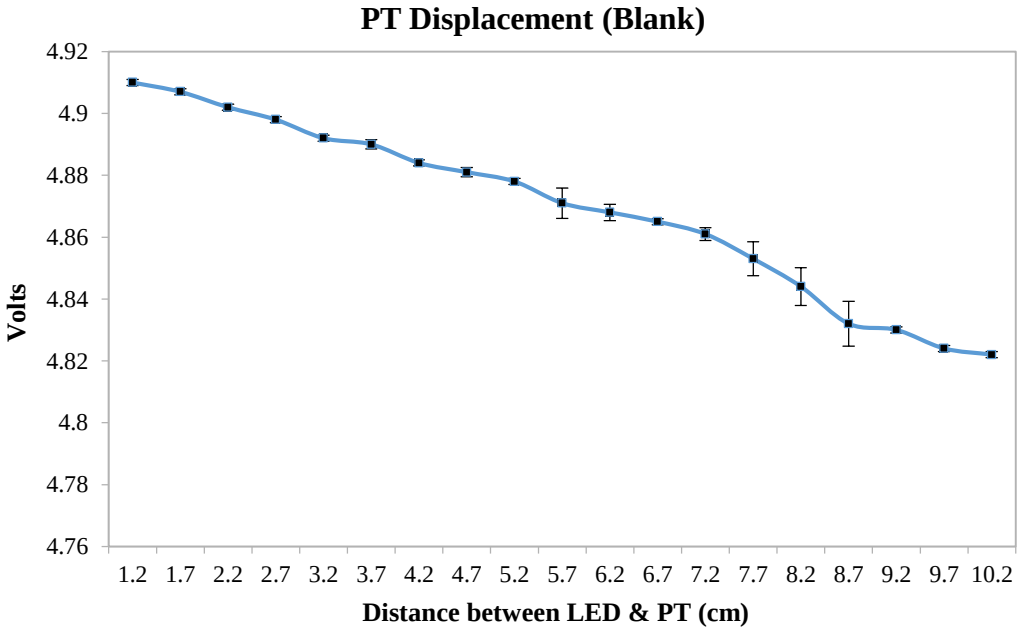


Figure 4.3: Output Voltage obtained with PT displaced away from the LED with Distilled water as the sample (blank) (Cuvette and LED placed together)

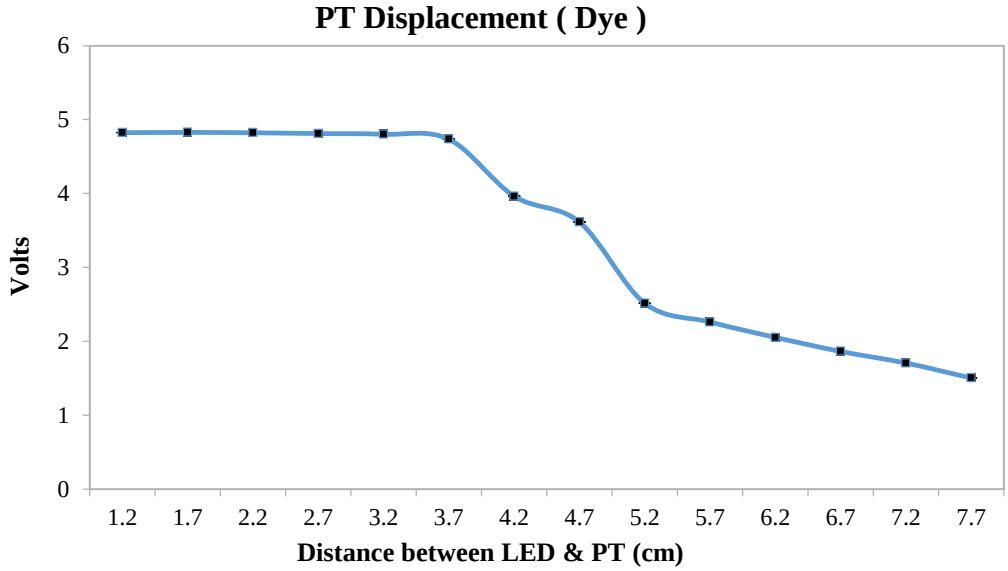


Figure 4.4: Output Voltage obtained with PT displaced away from the LED with Crystal Violet Stain (colored solution) as the sample (dye) (Cuvette and LED placed together)

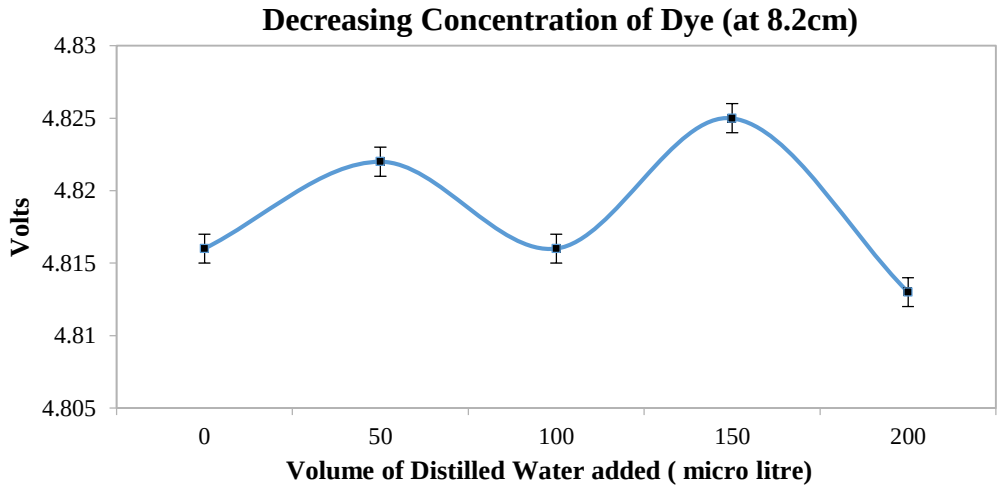


Figure 4.5: Output Voltage obtained with decreasing concentration of the dye (Cuvette placed at 0.5cm from LED; PT placed at 8.2cm from LED)

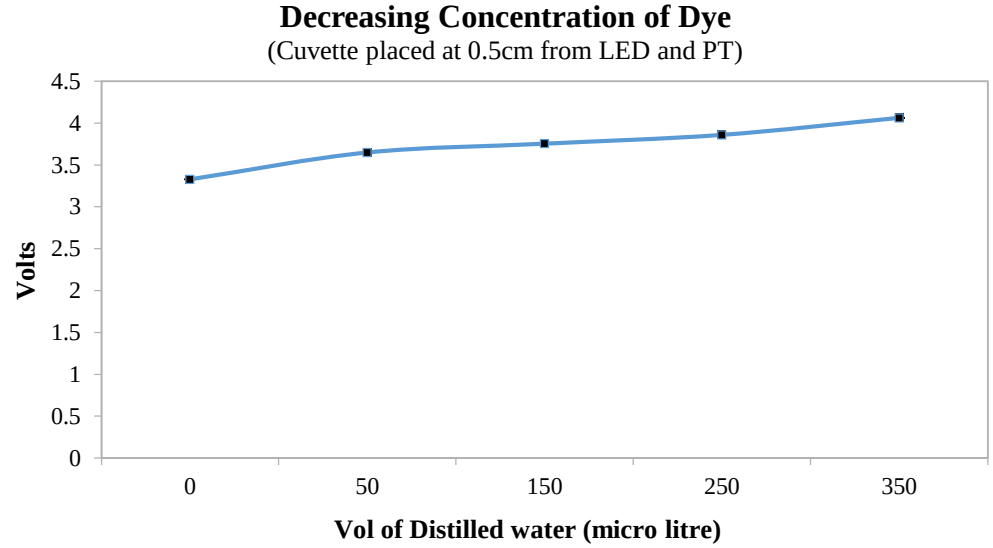


Figure 4.6: Output Voltage obtained with decreasing concentration of the dye (Cuvette placed at equal distance of 0.5cm from both LED and PT)

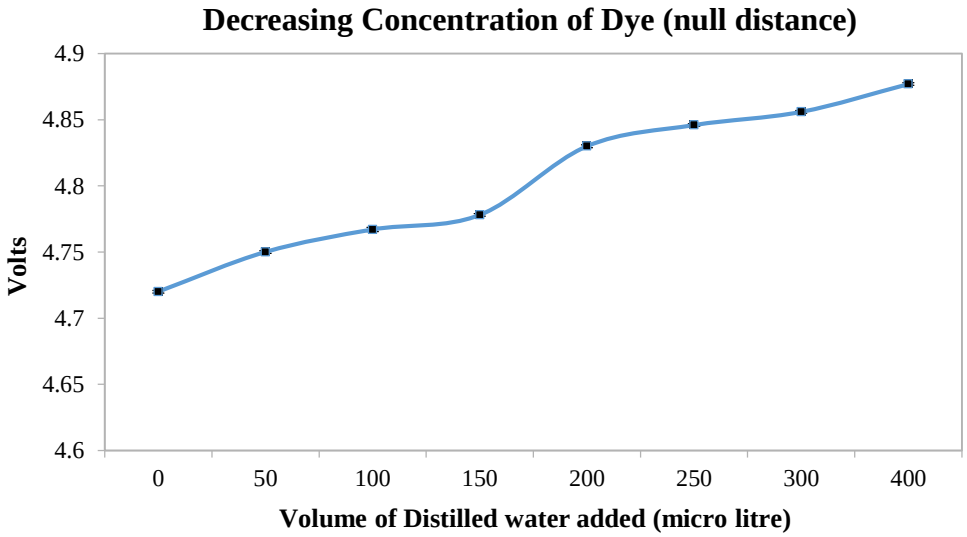


Figure 4.7: Output Voltage obtained with decreasing concentration of the dye (Cuvette placed in between LED and PT; all three placed next to each other in close proximity)

4.3 Validation of Optical Filter

The spectrum of the CFL tube obtained from the Spectral workbench is shown in Figure 4.8 (its zoomed version with enunciated plot shown in Figure 4.9). This spectrum was used for calibrating the spectrometer which was then used to obtain the spectrum of the optical filter (Figure 4.10).

The peak absorption of the optical filter can be seen to occur vividly at 505 nm, which is in fact the specified absorption maxima of the filter. It can also be seen from the zoomed in version (Figure 4.11) of the optical filter spectrum that the bandwidth is approximately 20nm, varying from 495 nm to 515 nm), which was the specified bandwidth in the quotation of the optical filter during its procurement. Thus the optical filter was successfully validated using the handmade spectrometer and Spectral Workbench software.

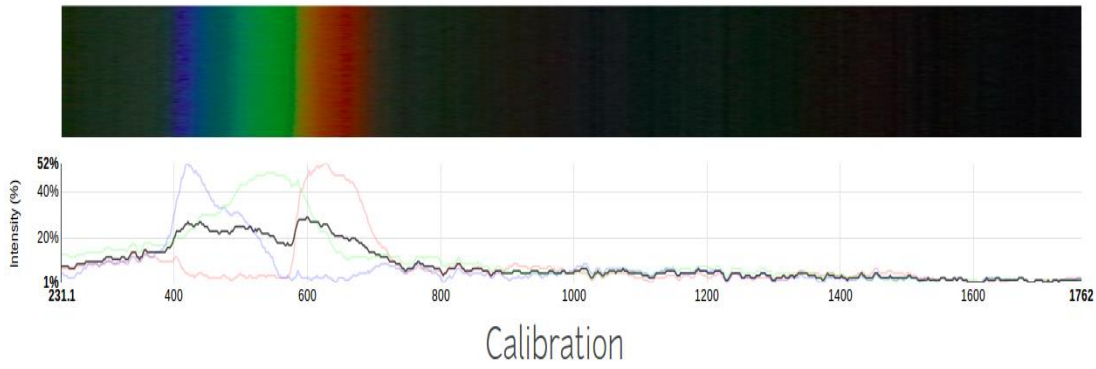


Figure 4.8: Spectrum of CFL light source used for the wavelength calibration of the optical filter

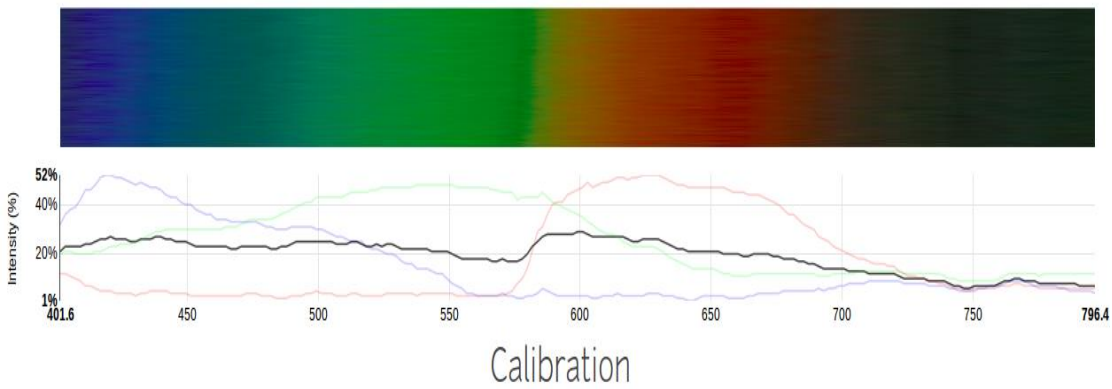


Figure 4.9: Zoomed in Spectrum of CFL light source

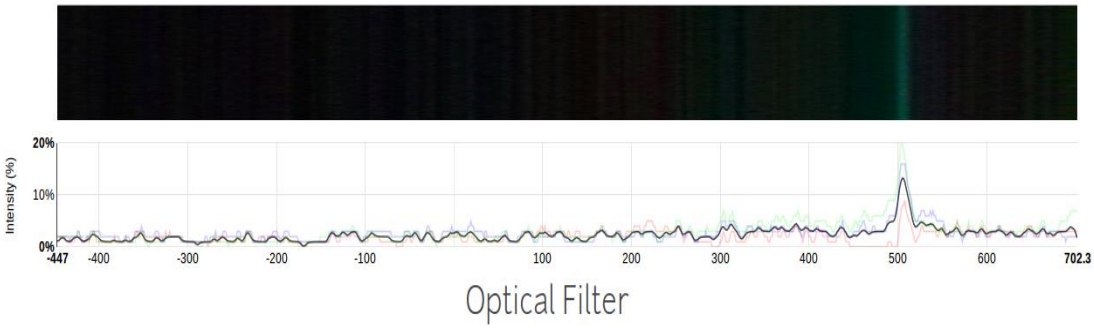


Figure 4.10: Spectrum of the Optical Filter (peak absorption at 505 nm)

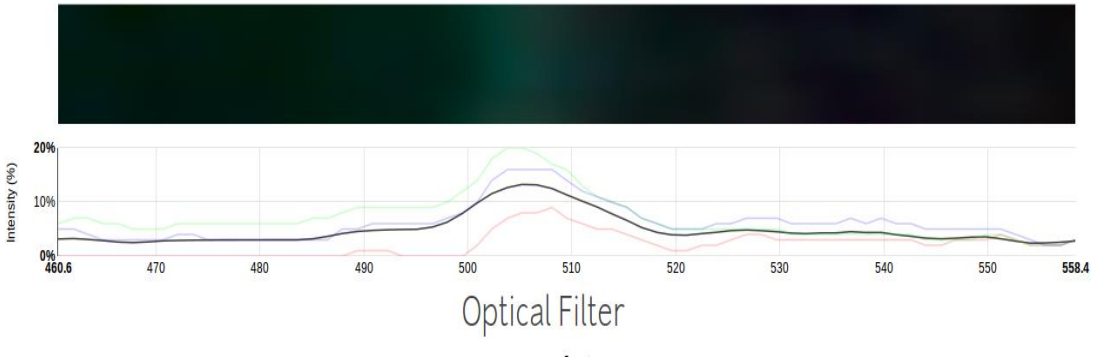


Figure 4.11: Zoomed in version of the spectrum of the optical filter (bandwidth of 20 nm from 495 nm to 515 nm; peak absorption at 505 nm)

4.4 Electronic Interface

The electronic interface was accordingly programmed in order to provide the user with prompts. This minimizes the chances of human error in carrying out the steps. The developed interface ensures an easy to use device, without any complications, thus avoiding the requirement of device operation training by the user.

The user interface comprises of an LCD display, inter-connected with two push button, all of which are interfaced with Arduino UNO board. This interface serves to prompt the user in the correct direction in a step by step process to obtain correct results. The Arduino is programmed to display the particular step in the analysis process on the LCD. Only after pressing the button, the next step is initiated which is then displayed on the LCD. The user can also change the protocol when desired.

The interface consists of the four circuits - push button circuit (Figure 4.12 (a), soldered onto Vero board), LED circuit (Figure 4.12 (b), soldered onto Vero board), PT

circuit (Figure 4.12 (c), soldered onto Vero board), and LCD circuit (Figure 4.13 and Figure 4.14, soldered onto its PCB circuit layout). The PT circuit was embedded onto the Vero board such that the resistor can be changed during the validation process of the biochemical analyzer.

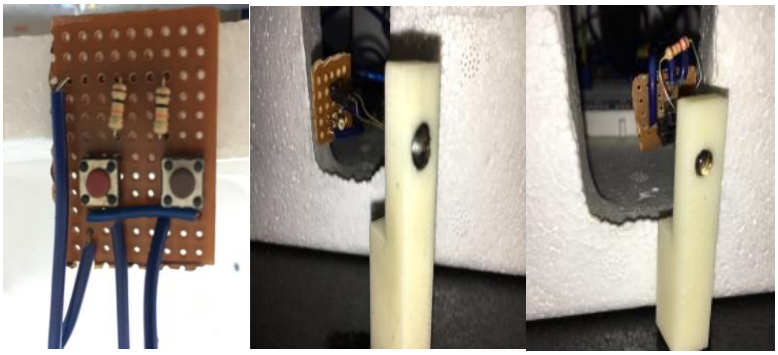


Figure 4.12: (a) Push Button Circuit (b) LED Circuit (c) PT Circuit

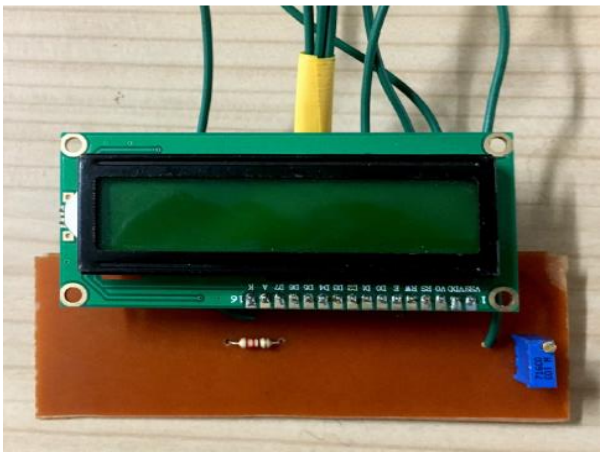


Figure 4.13: Front View of the PCB of LCD Circuit

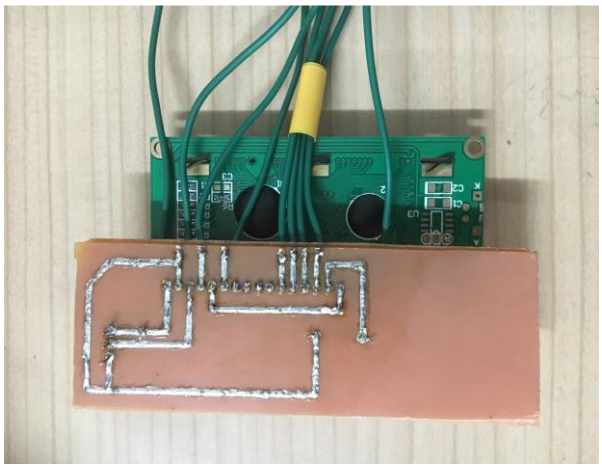


Figure 4.14: Back View of the PCB of LCD Circuit (soldered components)

4.5 Assembly of BCA

All the components of both the electronic interference and the optical assembly were assembled and fixed onto the 3D printed outer casing of the Biochemical Analyzer in their respective places, as shown in Figures 4.15 – 4.18.

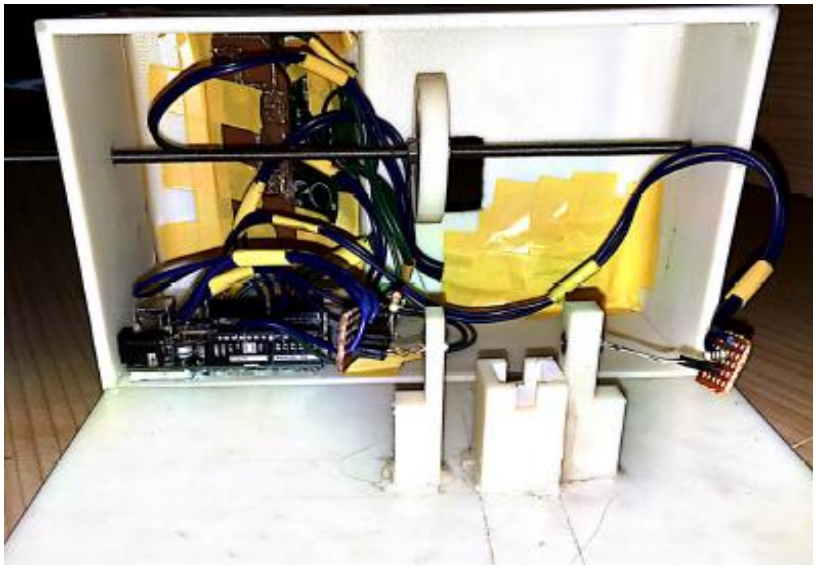


Figure 4.15: Side View of the assembly (inner)

Figure 4.16: Top View of the assembly (inner)

Figure 4.17: Top View of the BCA (outer)

Figure 4.18: Back View of the BCA (outer) (port for powering the Arduino)

4.6 Validation of BCA

4.6.1 Using Human Blood Samples

The current device was validated initially using human blood samples. The results were compared with an IVD certified Biochemical Analyzer, Evolution 3000. The glucose concentration was measured using the serum from the blood samples. The results of the validation are given in Tables 4.2, 4.3, and 4.4. The device was found to be 90.464 % accurate for human blood samples.

Table 4.2: Validation of BCA with Serum Glucose Concentration of Subject 1

S. No	Concentration obtained with Evolution 3000 (mg/dL)	Concentration obtained with BCA (mg/dL)
1	112	104.923
2	116	100.671
3	112	99.070
4	111	98.195
5	110	100.148
Mean (mg/dL)	112.2	100.601
Mean Error (%)		10.338

Table 4.3: Validation of BCA with Serum Glucose Concentration of Subject 2

S. No	Concentration obtained with Evolution 3000 (mg/dL)	Concentration obtained with BCA (mg/dL)
1	125	110.448
2	122	109.286
3	117	106.168
4	116	108.594
5	121	109.024
Mean (mg/dL)	120.2	108.704
Mean Error (%)		9.564

Table 4.4: Validation of BCA with Serum Glucose Concentration of Subject 3

S. No	Concentration obtained with Evolution 3000 (mg/dL)	Concentration obtained with BCA (mg/dL)
1	84	77.730
2	88	77.730
3	90	80.728
4	81	77.730
5	86	77.730
Mean (mg/dL)	85.8	78.330
Mean Error (%)		8.707

4.6.2 Using Known Glucose Concentrations

The current device validated using known glucose concentrations in distilled water. The instrument was validated for accuracy, linearity and precision.

i. Accuracy

The Error Index for each X-Y pair falls within -1 and 1 except two samples as shown in Table 4.16, which signifies that the current device is accurate within the acceptability range. The verification was done using 11 known glucose concentrations in distilled water: 80 mg/dL (Table 4.5), 90 mg/dL (Table 4.6), 100 mg/dL (Table 4.7), 108 mg/dL (Table 4.8), 112 mg/dL (Table 4.9), 116 mg/dL (Table 4.10), 120 mg/dL (Table 4.11), 124 mg/dL (Table 4.12), 128 mg/dL (Table 4.13), 130 mg/dL (Table 4.14) and 140 mg/dL (Table 4.15).

Table 4.5: Validation of BCA with Glucose Concentration of 80 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.860	4.833	4.837	85.150	6.437
4.860	4.833	4.837	85.150	6.437
4.861	4.833	4.838	82.100	2.626
4.860	4.832	4.836	85.679	7.099
4.860	4.833	4.837	85.150	6.437
Mean			84.646	5.807

Table 4.6: Validation of BCA with Glucose Concentration of 90 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.861	4.833	4.835	92.838	3.153
4.861	4.833	4.834	96.419	7.132
4.861	4.832	4.834	93.084	3.427
4.86	4.832	4.833	96.419	7.132
4.861	4.832	4.834	93.084	3.427
Mean			94.369	4.854

Table 4.7: Validation of BCA with Glucose Concentration of 100 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.86	4.832	4.831	103.582	3.582
4.861	4.833	4.83	110.749	10.749
4.86	4.833	4.832	103.714	3.714
4.86	4.833	4.831	107.430	7.430
4.861	4.832	4.831	103.459	3.459
Mean			105.787	5.787

Table 4.8: Validation of BCA with Glucose Concentration of 108 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.854	4.826	4.822	114.333	5.864
4.854	4.826	4.823	110.749	2.545
4.854	4.827	4.823	114.862	6.354
4.854	4.827	4.823	114.333	5.864
4.854	4.826	4.822	113.840	5.407
Mean			113.623	5.207

Table 4.9: Validation of BCA with Glucose Concentration of 112 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.855	4.825	4.819	120.075	7.209
4.856	4.825	4.819	119.429	6.633
4.857	4.825	4.82	115.685	3.290
4.857	4.825	4.819	118.824	6.093
4.857	4.826	4.82	119.429	6.633
Mean			118.688	5.972

Table 4.10: Validation of BCA with Glucose Concentration of 116 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.856	4.826	4.82	120.075	3.513
4.856	4.825	4.818	122.670	5.750
4.857	4.825	4.818	121.963	5.141
4.857	4.828	4.822	120.765	4.107
4.856	4.827	4.821	120.765	4.107
Mean			121.247	4.524

Table 4.11: Validation of BCA with Glucose Concentration of 120 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.861	4.836	4.829	128.093	6.774
4.86	4.836	4.829	129.26	7.717
4.86	4.835	4.829	124.077	3.398
4.861	4.835	4.829	123.153	2.628
4.861	4.835	4.829	123.153	2.628
Mean			125.5472	4.623

Table 4.12: Validation of BCA with Glucose Concentration of 124 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.858	4.829	4.821	127.692	2.977
4.857	4.829	4.821	128.678	3.773
4.857	4.828	4.82	127.692	2.977
4.857	4.828	4.82	127.692	2.977
4.857	4.829	4.821	128.678	3.773
Mean			128.086	3.295

Table 4.13: Validation of BCA with Glucose Concentration of 128 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.855	4.829	4.82	134.741	5.266
4.855	4.83	4.821	136.127	6.349
4.855	4.829	4.821	130.878	2.248
4.855	4.829	4.82	134.741	5.266
4.856	4.83	4.822	130.878	2.248
Mean			133.473	4.276

Table 4.14: Validation of BCA with Glucose Concentration of 130 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.86	4.834	4.825	134.741	3.647
4.861	4.835	4.825	138.605	6.619
4.86	4.833	4.824	133.458	2.660
4.86	4.834	4.824	138.605	6.619
4.861	4.834	4.825	133.457	2.660
Mean			135.773	4.441

Table 4.15: Validation of BCA with Glucose Concentration of 140 mg/dl

Output Voltage (V)			Concentration (mg/dL)	% Error
B	S	T		
4.855	4.83	4.818	148.184	5.846
4.856	4.83	4.818	146.336	4.525
4.856	4.83	4.818	146.336	4.525
4.855	4.83	4.818	148.184	5.846
4.856	4.83	4.818	146.336	4.525
Mean			147.075	5.054

Table 4.16: Verification of Accuracy

S.No	Actual Value (mg/dL) (X)	Measured Value (mg/dL) (Y)	Error Index (Y-X)/TEa
1	80	84.646	0.774
2	90	95.801	0.967
3	100	105.787	0.964
4	108	113.623	0.937
5	112	118.688	1.115
6	116	121.504	0.917
7	120	125.547	0.925
8	124	128.086	0.681
9	128	133.473	0.912
10	130	135.773	0.962
11	140	146.786	1.131

ii. Linearity

From the plot of Measured Value Vs Actual Value shown in Figure 4.19, the slope and intercept were determined which were then used for computing the predicted values (P). From Figure 4.20, it can be seen that the line connecting the data points of the predicted values is a straight line. The Coefficient of Correlation is 0.999, which greater satisfies the acceptability criteria: R > 0.975. The Systematic Error computed from the data, shown in Table 4.16 satisfies both the conditions i.e., it is less than 50 % of Total Error and the Total Allowable Error for each measurement of the concentration.

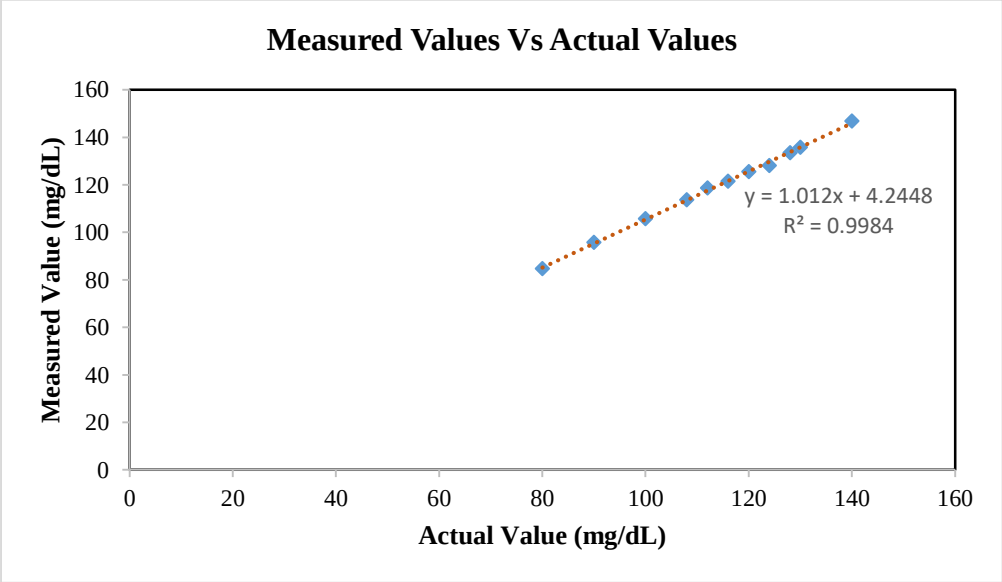


Figure 4.19: Linearity Verification by plotting of Measured Values against Actual Values

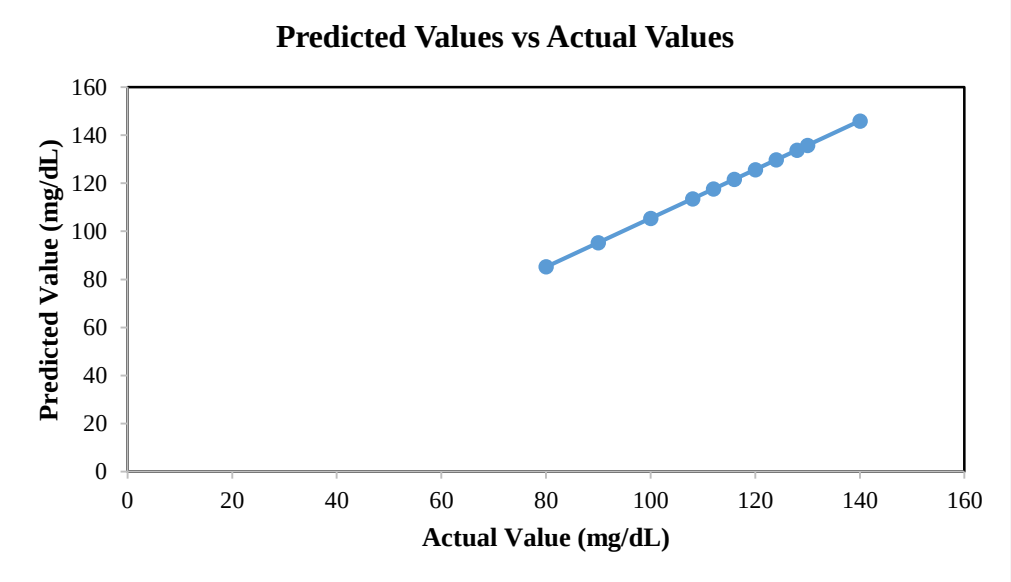


Figure 4.20: Linearity Verification by plotting of Predicted Values against Actual Values

Table 4.17: Verification of Linearity

S.No	Actual Value (mg/dL) (X)	Measured Value (mg/dL) (Y)	Predicted Value P	Systematic Error (P-Y)	50 % of Total Error 0.5*(Y-X)
1	80	84.646	85.2048	0.559	2.323
2	90	95.801	95.3248	-0.476	2.901
3	100	105.787	105.4448	-0.342	2.893
4	108	113.623	113.5408	-0.083	2.812
5	112	118.688	117.5888	-1.099	3.344
6	116	121.504	121.6368	0.133	2.752
7	120	125.547	125.6848	0.138	2.774
8	124	128.086	129.7328	1.646	2.043
9	128	133.473	133.7808	0.308	2.736
10	130	135.773	135.8048	0.032	2.887
11	140	146.786	145.9248	-0.861	3.393

iii. Precision

For between day precision verification, the % CV obtained was 0.541 in case of 116 mg/dL and 1.049 in case of 120 mg/dL glucose concentration (Table , both of which are less than 1.40, which is the manufacturer specified % CV for between day precision measurement. Similarly, the % CV for within day was obtained as 2.39 for 112 mg/dL (Table 4.17) and 2.32 for 120 mg/dL (Table 4.18) glucose concentrations. The % CV specified by the manufacturer is 2.79 which is greater than the measured % CV. Thus, the device falls within the acceptable range for precision.

Table 4.18: Between day precision verification using two known glucose concentrations

Day	Measured Values for 120 mg/dL	Measured Values for 116 mg/dL
1	123.967	122.791
2	123.997	121.504
3	125.547	122.518
4	125.961	122.232
5	127.012	121.247
Average	125.297	122.058
SD	1.314	0.660
% CV	1.049	0.541

Table 4.19: Within day precision verification using 112 mg/dL glucose concentration

S.No	112 mg/dL				
	Output Voltage (V)			Concentration (mg/dL)	% Error
	B	S	T		
1	4.854	4.827	4.822	118.580	5.875
2	4.854	4.827	4.822	118.580	5.875
3	4.854	4.827	4.822	118.580	5.875
4	4.854	4.826	4.822	114.333	2.083
5	4.853	4.826	4.822	114.862	2.556
6	4.857	4.828	4.822	120.765	7.826
7	4.857	4.828	4.822	120.765	7.826
8	4.856	4.827	4.822	117.302	4.734
9	4.856	4.827	4.823	113.840	1.643
10	4.856	4.828	4.823	117.918	5.284
11	4.856	4.827	4.821	120.765	7.826
12	4.856	4.827	4.822	117.302	4.734
13	4.854	4.826	4.822	114.333	2.083
14	4.854	4.827	4.822	118.580	5.875
15	4.854	4.826	4.822	114.333	2.083
16	4.854	4.826	4.822	114.333	2.083
17	4.854	4.826	4.823	110.749	1.117
18	4.854	4.827	4.822	118.580	5.875
19	4.854	4.827	4.823	114.862	2.556
20	4.854	4.826	4.822	114.333	2.083
Mean (mg/dL)				116.685	4.295

Table 4.20: Within day precision verification using 120 mg/dL glucose concentration

S.No	120 mg/dL				
	Output Voltage (V)			Concentration (mg/dL)	% Error
	B	S	T		
1	4.858	4.829	4.821	127.692	6.410
2	4.858	4.828	4.821	123.423	2.852
3	4.858	4.829	4.821	127.692	6.410
4	4.857	4.829	4.821	128.678	7.232
5	4.858	4.828	4.82	126.772	5.643
6	4.857	4.828	4.82	127.692	6.410
7	4.857	4.828	4.821	124.228	3.523
8	4.858	4.827	4.821	119.429	0.476
9	4.857	4.827	4.821	120.075	0.062
10	4.857	4.828	4.82	127.692	6.410

11	4.857	4.828	4.82	127.692	6.410
12	4.857	4.827	4.82	123.423	2.852
13	4.857	4.827	4.82	123.423	2.852
14	4.857	4.827	4.82	123.423	2.852
15	4.856	4.827	4.82	124.228	3.523
16	4.857	4.828	4.821	124.228	3.523
17	4.857	4.827	4.821	120.075	0.062
18	4.856	4.827	4.82	124.228	3.523
19	4.857	4.827	4.82	123.423	2.852
20	4.856	4.827	4.821	120.765	0.637
Mean (mg/dL)				124.414	3.726

Table 4.21: Comparison of within day and between day precision verification

Within Day			Between Day		
Concentration (mg/dL)	Measured % CV	Manufacturer Specified % CV	Concentration (mg/dL)	Measured % CV	Manufacturer Specified % CV
112	2.39	2.79	116	0.541	1.4
120	2.32		120	1.049	

Chapter 5

CONCLUSION

5.1 Summary

The results of the current study can be summarized as:

- A low cost, handheld Biochemical Analyzer was successfully designed and fabricated using SolidWorks^{TM} software by 3D printing technology.
- The essential components of the device i.e., optical filter, Arduino UNO, LED and L14F2 Phototransistor were embedded into the device casing.
- The optical filter was validated using in-house designed Papercraft Spectrometer.
- The proper alignment of LED, Cuvette, and PT for highest possible amplification and sensitivity was confirmed.
- Finally, the results for glucose estimation in human blood samples as well as known glucose concentrations in distilled water were successfully validated using Evolution 3000. The device was found to be 90.464 % accurate for human blood samples. Accuracy, linearity and precision were successfully verified.

5.2 Conclusion

A low cost, handheld biochemical analyzer was successfully designed and fabricated using minimal and low cost components. In the initial phase of validation, the device was validated using known concentrations of glucose as well as human blood samples for glucose against that of Evolution 3000. In the next phase, the device would be validated for other blood biomarkers since we propose that with the use of four appropriate optical filters combined with requisite programing for individual protocols, the device can be used for estimating more than 100 blood biomarkers. In addition, the device would be characterized for its static parameters (i.e. accuracy, stability, tolerance, sensitivity etc.,) so that a specification chart can be produced for the end users.

5.3 Scope for Further Research

Using the current optical filter with peak absorption maxima at 505nm, a total of approximately 21 clinical tests can be performed. Hence, the device can be further validated for these corresponding biomolecules. Additionally, a temperature lookup table can be created to aid in the sample preparation steps, depicting the corresponding temperature - sample incubation time.

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