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**3D PRINTED SPECTROPHOTOMETER FOR DRUG MONITORING IN
TUBERCULOSIS PATIENTS FOR GLOBAL HEALTH APPLICATIONS**

BY

PRAGYA HOODA

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ABSTRACT OF THESIS

3D PRINTED SPECTROPHOTOMETER FOR DRUG MONITORING IN TUBERCULOSIS PATIENTS FOR GLOBAL HEALTH APPLICATIONS

BY PRAGYA HOODA

Thesis Director:

Dr. Umer Hassan

Significant progress has been made to fight Tuberculosis (TB), and while many effective diagnoses and treatments have saved millions of lives, the infectious disease still remains to be one of the leading causes of death today. An infected patient can be prescribed with antibiotic drugs such as Rifampin for a certain period of time until treatment is complete. Drug resistant strains of TB have started to grow, creating an urgency to control the spread of this disease. Rapid diagnostic tools and different delivery methods have reduced the mortality rate from TB, however, there is still an urgent need to develop a point-of-care (POC) test that can improve the treatment process for TB patients. Here, we aim to create a POC device that can detect the amount of antibiotic in a biological sample (e.g. urine). We developed and tested a portable three-dimensional (3D) printed spectrophotometer for different biological assays and compared the results with a laboratory standard table-top instrument spectrophotometer. First, we demonstrated the accuracy of the 3D printed spectrophotometer using a Bradford Assay and found a good correlation ($R^2 = 0.96$) between our 3D printed and instrument spectrophotometer. Subsequently, assay is modified to process smaller sample volume using microcuvettes in the setup. We found a correlation coefficient of $R^2 = 0.92$ between

our 3D printed and instrument spectrophotometer. The device was then tested for a Rifampin assay and a standard curve is developed in both spiked PBS and urine samples. The correlation results from the rifampin assay will shine light on future diagnostic and drug monitoring testing for TB patients. In conclusion, our results show that our 3D printed spectrophotometer has a strong correlation compared to the real instrument spectrophotometer for absorbance-based measurements. In future, POC instruments such as our 3D printed spectrophotometer can be used by the medical staff for active TB management, and possibly to test patients response to new emerging strains of TB. Proactive diagnosis and treatment of TB patients can significantly reduce the death rate, and in turn improve the quality of human life in global health settings.

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- My parents for constantly believing in me.
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Dedications

I would like to dedicate my thesis to:

- My grandparents Maya Devi, Naurang Singh, Vimal Verma, and Jaipal Verma.

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Chapter 1: Introduction

1.1 Tuberculosis

Tuberculosis (TB) is one of the top 10 causes of death worldwide and is the leading cause of a single infectious agent ranking above HIV/AIDS. ^[1] TB is a bacterial infection that generally affects the lungs caused by *Mycobacterium Tuberculosis*. ^[1, 2] The bacterial agent can also affect other parts of the body, such as the intestines, kidneys, brain, or spine. ^[3] The dual epidemic that TB and HIV/AIDS hold on the global population causes HIV to weaken the immune system making people more prone to TB infection. ^[4]

The disease spreads when a person with TB coughs, sneezes, or spits their germs into the air. ^[1] It only takes a non-infected person to inhale a few droplets from the air to get infected with the disease. Generally, adult men account for 56% of all the TB cases in 2019, compared to 32% in adult women and 12% in children. ^[1] An infected person's common symptoms with active TB are coughing, chest pain, pain with breathing, fatigue, fever, and night sweats. ^[3] There are latent TB cases where the person will have the TB infection, but the bacteria will be in an inactive state, causing no symptoms. Latent TB makes it harder to identify the person with the disease while also delaying the road to recovery. ^[3]

1.2 Global Health

The World Health Organization (WHO) declared TB a public health issue in 1993. ^[5] The WHO and the United Nations (UN) are committed to reducing the percentage of absolute number of TB deaths to 95% by 2035. ^[2]

A person's socioeconomic status, environment, and biologics can play a factor in how vulnerable he/she can be to the infection. TB is especially relevant in low-income countries because of poor nutrition and overcrowded living or working conditions. ^[6] People may not seek treatment due to the potential fear of losing their job or not having the proper education to recognize the symptoms of TB. A lot of people live in hard to reach areas where diagnosing and treating TB is very challenging. Those who can get help face challenges of inadequate health care systems because of limited resources, low-quality drugs, and a limited number of healthcare professionals. ^[7]

1.3 Antibiotics Used for Treatment

The antibiotic treatment varies depending on the type of tuberculosis an infected person may have. TB's current recommended treatment lasts for several months and includes a combination of multiple drugs: isoniazid, rifampicin, ethambutol, and pyrazinamide. The recommended treatment for TB is to take a combination of rifampicin and isoniazid for six months and ethambutol and pyrazinamide for the first two months of the treatment period. Generally, the treatment course will depend on the health of the patient before infection. ^[8] Drug-resistant TB evolves when patients skip a dosage or stop taking the antibiotics before completing the course. Treatment for rifampin-resistant TB is more prolonged, expensive, and more toxic. ^[1, 8]

1.4 Therapeutic Drug Monitoring

Therapeutic Drug Monitoring (TDM) is a tool that clinicians can utilize to optimize the best drug dosage for patients who do not react to standard treatment regimens. ^[9]

Factors such as gender, ethnicity, weight, preexisting disorders, etc., can affect the rate at which drugs are absorbed in the body. Since drug absorption is variable among patients, it is difficult to set one ideal treatment plan for each individual. According to studies, it has been found that for patients on longer than three weeks of rifampin antibiotics, the rifampin levels are lower as compared to their initial values. ^[9] TDM can help identify patients that are slow to respond to standard treatment regimens, and clinicians can use this information to test for other strains of TB possibly.

The current approach to TDM is complex and similar applications for rich resource settings cannot be applied to low-income countries. There is an extensive process involved in collecting, processing, and shipping patient samples to laboratories. TDM can be performed to measure plasma or serum antibiotic concentrations through liquid chromatography or gas chromatography methods. Low-income countries that are hit highest with TB do not have access to similar laboratory methods. This project focuses on developing a TDM device that can test and classify the drug concentrations in a solution sample. ^[11]

1.5 Point of Care Devices

Access to laboratory testing is limited and can be time-consuming for patients to get test results on their treatment progress or symptoms. In most developing countries, centralized laboratories are not widely available due to poor infrastructure, low equipment, and medically trained personnel. ^[12] Point of Care (PoC) devices provide laboratory-quality results within minutes at the point of patient contact. The ideal qualities of the PoC device are cost-effective, user-friendly, reliable, and demonstrate

high sensitivity. ^[12] The battery-powered PoC spectrophotometer that was created for this project must work with minimal infrastructure, limited access to ground electricity and trained personnel.

1.6 Clinical Motivation

Tuberculosis holds a grave threat in every country of the world. Drug-resistant TB strains have started to grow, creating an urgency to control the spread of this disease. By testing the absorbance of antibiotics from the patient's urine, medical staff can further facilitate better diagnoses and monitoring for the patient's life. In this project, we developed and tested a point of care 3D printed spectrophotometer to measure the absorbance of different biological assays and compared the results with an instrument spectrophotometer.

Chapter 2: Point of Care 3D Device Setup

2.1 Introduction to the Point of Care 3D Device

The motivation for this project is to create a battery-powered PoC spectrophotometer. To design and develop a 3D printed spectrophotometer, the device needs to replicate the optical and electronic properties housed in an instrument spectrophotometer.

The point of care 3D printed spectrophotometer contains three main components: the optical subsystem, the 3D printed part, and the electronic subsystem. **Figure 1** exhibits the photo of the completed 3D printed device.

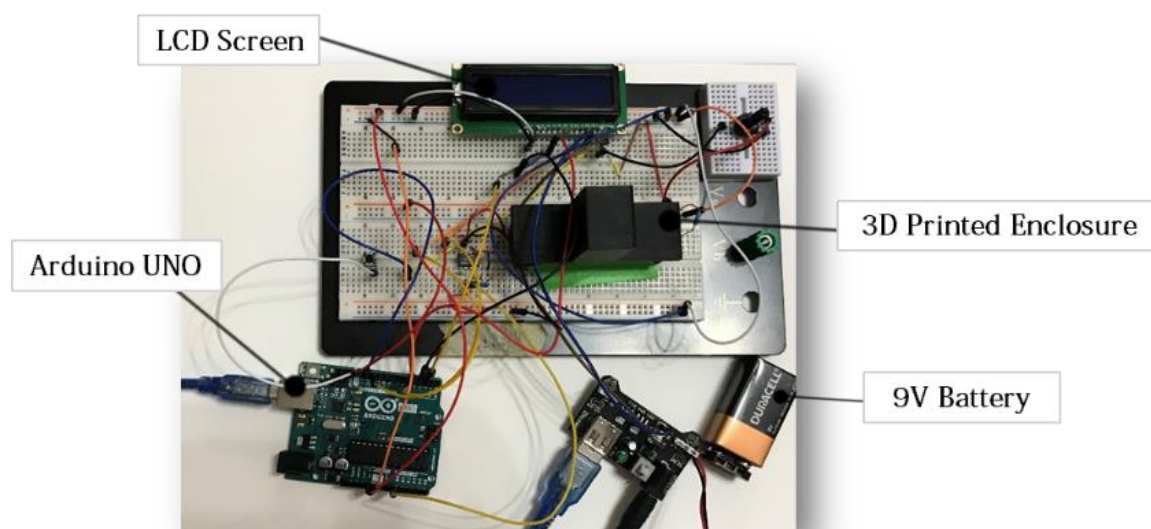


Figure 1: Photo of the 3D Printed Spectrophotometer. The main parts that are labeled are the 9V batter, Arduino UNO, Liquid Crystal Display (LCD) Screen, and the 3D printed enclosure.

The Beer-Lambert law explains that there is a linear relationship between the absorbance and concentration of a liquid sample. Beer-Lambert law is written as **Equation 1**, where A is the absorbance, ϵ is the molar absorption coefficient, c is the

molar concentration, and l is the optical path length.^[13] In our data analysis, we utilized the Beer-Lambert law to generate our standard curves by plotting the concentration values on the x-axis and the absorbance values on the y-axis.

$$A = \epsilon cl$$

Equation 1: Beer Lambert-Law equation, where A is the absorbance, ϵ is the molar absorption coefficient, c is the molar concentration, and l is the optical path length.

2.2 Optical Subsystem

The 3D printed spectrophotometer was set up so that the light emitted from the light-emitting diode (LED) passed through the solution sample, and focused onto the photodiode for a voltage output. Traditional instrument spectrophotometers utilize a monochromator to change the wavelength of the light source in the machine. Since the 3D printed device does not have those capabilities, two different wavelength LEDs for the specified solution concentration were used to detect the absorbance.

A convex lens focuses parallel light onto one focal point. In the 3D device, a 10mm diameter, uncoated, plano-convex lens from Edmund optics was utilized to emit the light from the sample onto the PDB-C139 photodiode.^[14] The effective focal length of the convex lens is 10mm and can be utilized for a wavelength range of 400-2500nm.^[15] The 10mm focal length was implemented in the design process by placing the photodiode 10mm away from the lens. As the light from the LED passes through the cuvette, the convex lens takes the emitted light and focuses it onto a narrow point of the photodiode chip. **Figure 2** summarizes the optical path from the light source onto the photodiode.

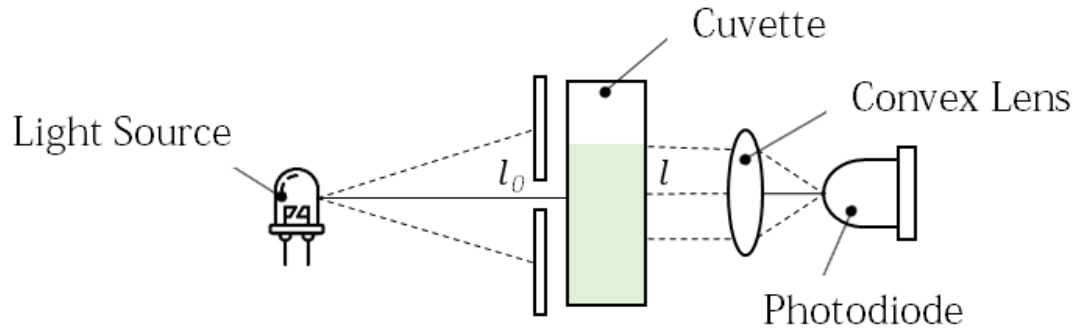


Figure 2: Schematic drawing of the optical pathway from the start of the LED light source passing through the cuvette onto the convex lens which focuses the transmitted light onto the photodiode. I_0 is the intensity of the incident light and I is the intensity of the light after it passes through the cuvette.

2.3 3D Printed Part

A spectrophotometer requires a black, light isolated environment to prevent outside ambient light from affecting the photodiode's readings. All three pieces were printed in a polylactic acid (PLA) material at the Rutgers Makerspace.

Piece 1 is the enclosure that houses the LED, cuvette, piece 3, and the photodiode.

Figure 3 shows the dimensions of piece 1. Chamber 1 is a 21.88mm long space that holds the yellow 595nm LED or blue 475nm LED depending on the experimental assay.

^[16, 17] Chamber 2 houses the 13mm x 13mm x 45mm cuvette sample. The chamber is big enough to enclose both the macro and microcuvettes. Chamber 3 occupies piece 3 that holds the uncoated 10mm diameter plano-convex lens, and chamber 4 is where the convex lens focuses the transmitted light from the sample onto the active chip region of the photodiode. Chamber 4 is kept at 10mm from the photodiode to maintain the effective focal length of the convex lens. Altogether, piece 1 enclosure is 65mm x 21mm x 20mm.

Piece 2 is the removable lid that will go over the cuvette to block the outside light from entering inside the piece 1 enclosure. The removable top lid is large enough to cover the top of the cuvette to maintain a dark environment. **Figure 4** shows the dimensions of piece 2.

Piece 3 is a separate PLA piece that holds the plano-convex lens in place. Piece 3's dimensions are 17mm x 18.5mm and is inserted in chamber 3 of the piece 1 enclosure. **Figure 5** shows the dimensions of piece 3.

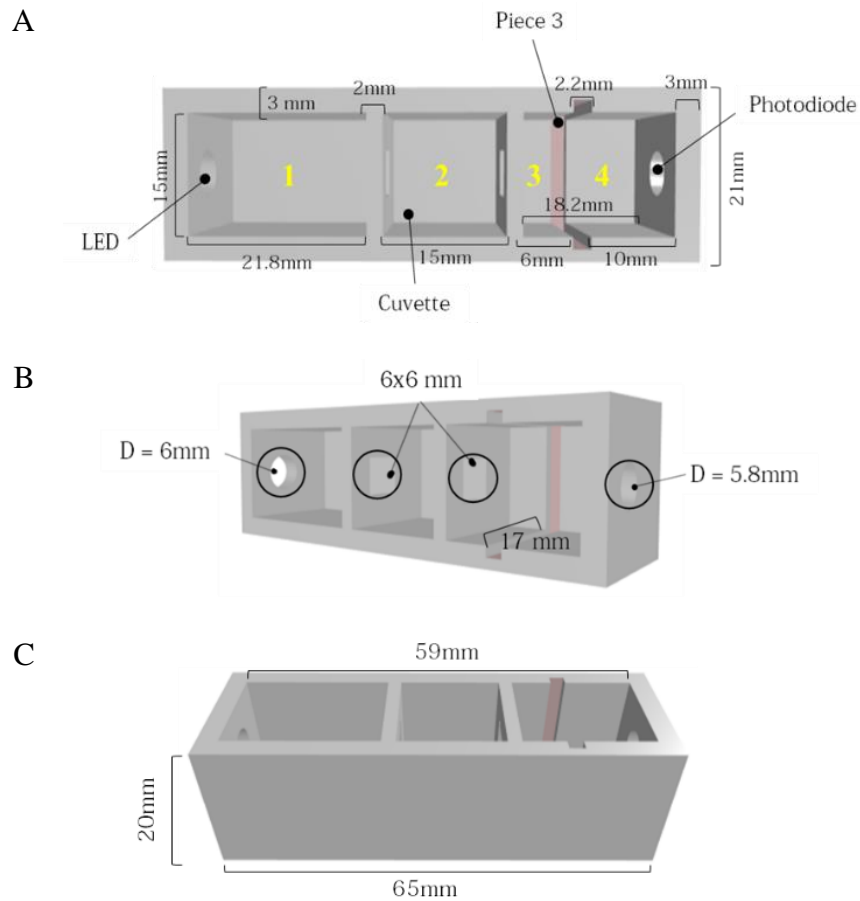


Figure 3: AutoCAD schematic of the dimensions of piece 1 where (A) is the top view of the enclosure that shows the 4 chambers, (B) the side view that shows where the LED and photodiode are housed in the enclosure, and (C) the outside dimensions of the enclosure.

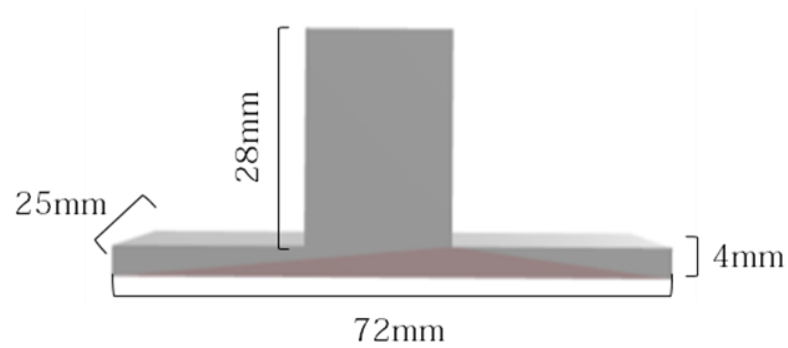


Figure 4: AutoCAD schematic of the dimensions of piece 2.

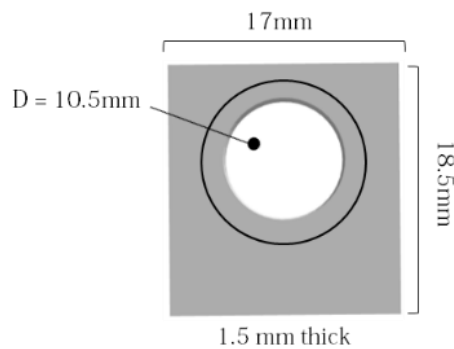


Figure 5: AutoCAD schematic of the dimensions of piece 3. The outer dimensions of piece 3 are 17 x 18.5 x 1.5, and it fits into chamber 3 of the piece 1 enclosure.

2.4 Electronic Subsystem

The purpose of the circuit board is to mimic the electronic capabilities found in an instrument spectrophotometer. **Figure 6** shows the electronic and optical path from the light source to the output voltage on the LCD screen. The following parts were used in building the circuit board:

- 9V Battery
- Power Controller
- LCD Screen
- Potentiometer

- Yellow 595nm LED & Blue 475nm LED
- PDB-C139 Silicon Photodiode
- LTC1050 Amplifier
- Resistors for the LED and Amplifier
- Capacitor for the Amplifier
- Arduino UNO

Figure 7 shows the fritzing diagram of the circuit that was built for the 3D spectrophotometer. The 9V battery powers the device and is connected to the power controller which converts the voltage from the battery into the 5V that is required for the device. The battery powers the Arduino, LCD screen, and LED. The specified LED is chosen based on the experimental assay. The yellow 595nm LED is used for the Bradford assay and switched out for the blue 475nm LED for the Rifampin Assay.

In the enclosure, the LED turns on, the light passes through the sample in the cuvette, and with the help of the convex lens, the transmitted light is focused at one focal point on the PDB-C139 photodiode chip. The LTC1050 amplifier takes in the output from the photodiode and converts it into a readable output and contains less noise. The amplifier is connected to the Arduino UNO, where the readings from the amplifier are converted into voltage output reading on the LCD screen. The potentiometer was used to lower or increase the brightness of the LCD screen. A resistor is used with the LED, so it does not receive too much voltage and break. Every amplifier needs a capacitor and resistor for its calculations.

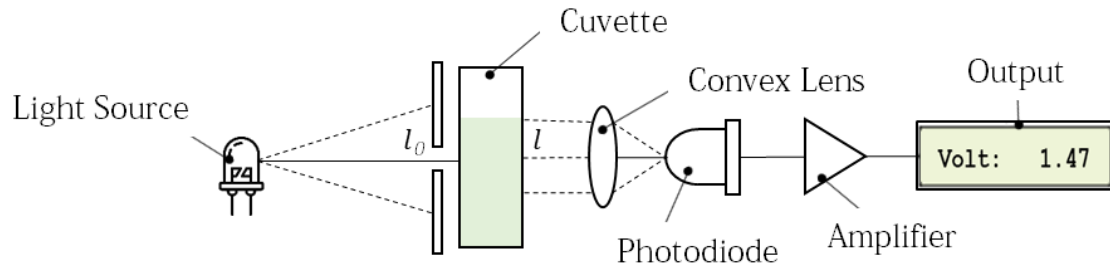


Figure 6: The light source from the LED passes through the cuvette sample onto the convex lens. The convex lens focuses the light on the photodiode, and then the amplifier outputs the voltage readings onto the LCD screen. I_0 is the intensity of the incident light and I is the intensity of the light after it passes through the cuvette.

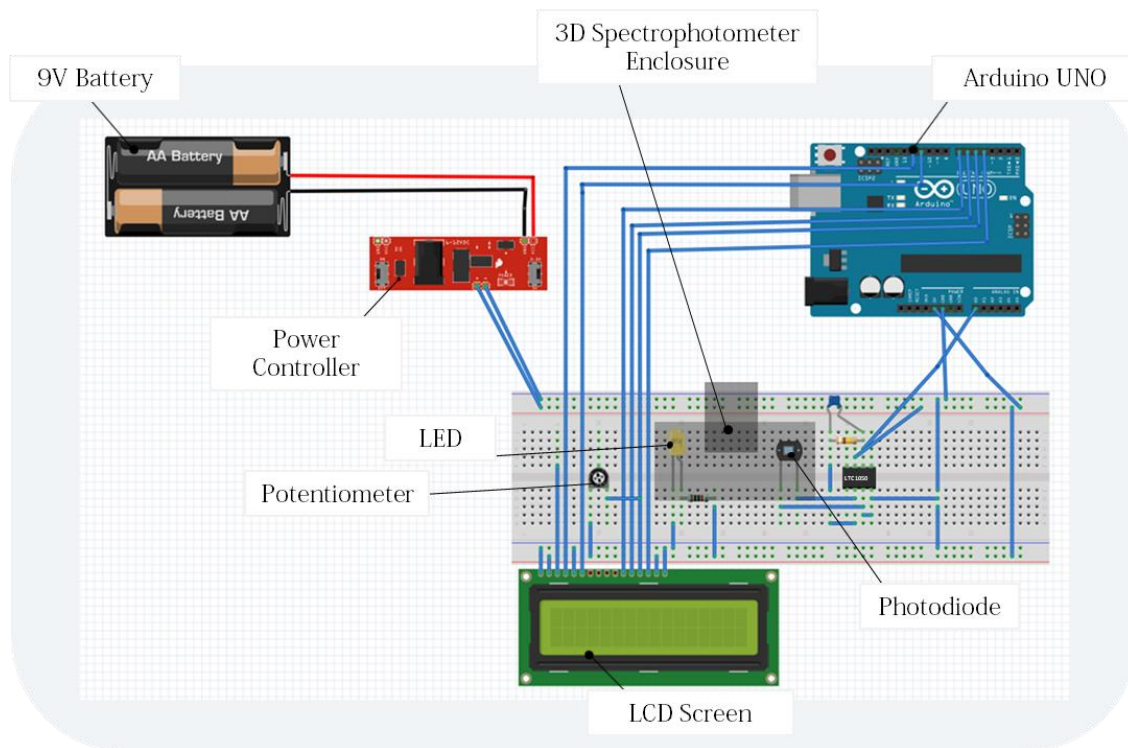


Figure 7: Fritzing diagram of the circuit board schematic.

Chapter 3: Methods

3.1 Introduction

Generating a standard absorbance vs. concentration curve requires a protein of interest and a reagent. The two experimental assays were the Bradford assay and rifampin assay. The procedures are used to validate the accuracy of the 3D device against an instrument spectrophotometer.

3.2 Bradford Assay in Macrocuvettes

For this experiment, the selected protein was Bovine Serum Albumin (BSA) with a Bradford reagent containing Coomassie Brilliant Blue. In the presence of protein, the Bradford reagent changes into a blue color.

The UV-1600PC spectrophotometer was turned on and allowed 20 minutes to warm up. The Bradford Reagent was removed from the refrigerator and gently mixed in the bottle until it was at room temperature. In the meantime, the stock solution was created by measuring 0.1 grams of BSA on a weighing scale and added into a tube that contains 10 mL of 1X PBS. The stock solution was set aside for 10-15 minutes until all BSA particles dissolved in the tube.

After the BSA dissolved, the tubes for the serial dilution were prepared. Eight (8) 1.5mL tubes were used for the serial dilution and an eighth tube for the excess dilution liquid at the end. Tubes 1-7 were filled with 500 μ L of 1X PBS. To begin the serial dilution, 500 μ L of standard BSA stock solution was added to tube 1. 500 μ L from the previous tube was transferred to the next (e.g., 500 μ L from tube 1 was added to tube 2) until the end of the dilution. Tube 7 will contain the excess solution, so 500 μ L from tube

7 was transferred into the extra tube 8. Tube 8 was not included in the standard curve measurements. The generated serial dilutions of BSA contain concentrations of (5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml, 0.15625 mg/ml, and 0.078125 mg/ml).

The maximum volume capacity in the macrocuvettes is 3.5mL. 0.05mL from each tube (tubes 1-7) was removed and added to seven cuvettes. 1.5mL room temperature Bradford Reagent was then added to each cuvette. The blank used to calibrate the instrument spectrophotometer is a 1.55mL cuvette filled with room temperature Bradford Reagent.

Each cuvette was measured three times at a wavelength of 595nm in the instrument spectrophotometer and with the yellow 595nm LED in the 3D printed spectrophotometer. The absorbance versus concentration standard curves were generated using Microsoft Excel.

3.3 Bradford Assay in Microcuvettes

The procedure for creating a standard curve in microcuvettes is similar to the one for macrocuvettes. The only difference is the added volume of Bradford Reagent and stock sample in microcuvettes.

The generated serial dilutions of BSA contain concentrations of (5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml, 0.15625 mg/ml, and 0.078125 mg/ml). 0.025mL from each tube (tubes 1-7) was removed and added to seven cuvettes. 0.75mL room temperature Bradford Reagent was added to each cuvette. The same polystyrene spectrophotometer microcuvettes were prepared for measurement for both the instrument

and 3D spectrophotometer. The blank used to calibrate the instrument spectrophotometer is a 0.775mL cuvette filled with room temperature Bradford Reagent.

Each cuvette was measured at a wavelength of 595nm in the instrument spectrophotometer and with the yellow 595nm LED in the 3D printed spectrophotometer. Due to the structure of the microcuvettes, the 3D printed spectrophotometer cannot generate valid readings if the microcuvettes are inserted right side up. To overcome this issue, microcuvette caps were used so that the tube can be flipped upside down for the light to pass through the solution and generate voltage readings. The absorbance versus concentration standard curves were generated using Microsoft Excel.

3.4 Rifampin Assay in Microcuvettes in PBS

The UV-1600PC spectrophotometer was turned on and allowed 20 minutes to warm up. The powder rifampin was removed from the freezer. The stock solution was created by measuring 10mg of powder rifampin and added in a 1.5mL tube with 1mL of 1X PBS. The stock solution was vortexed and set aside in the fridge to dissolve.

Fourteen (14) tubes were required to conduct the serial dilution for the PBS rifampin assay. However, the 14th tube is used as excess and not involved in the standard curve measurements. 1.8 mL of 1X PBS was added to tube 1, and 500 μ L of 1X PBS was added to tubes 2-13. The rifampin stock solution was removed from the fridge, and 200 μ L of the stock was added to tube 1. 1mL from the previous tube was transferred to the next (e.g., tube 1 was transferred to tube 2) until the end of the dilution. At the end of the serial dilution, tube 13 will contain the excess solution, so 1 mL from tube 13 was transferred into the extra tube 14. The generated serial dilutions of rifampin contain

concentrations of (1 mg/ml, 0.67 mg/ml, 0.44 mg/ml, 0.2963 mg/ml, 0.198 mg/ml, 0.13 mg/ml, 0.088 mg/ml, 0.059 mg/ml, 0.039 mg/ml, 0.026 mg/ml, 0.017 mg/ml, 0.012 mg/ml, 0.0077 mg/ml).

After completing the serial dilution, 800 μ L isoamyl alcohol was added to tubes 1-13. The tubes were vortexed for 20 seconds and then centrifuged at 4000RPM at room temperature for 5 minutes. After centrifugation, the upper layer (\sim 675 μ L) was pipetted out from the tubes and inserted into the microcuvettes. The blank used for the rifampin assay is the upper layer of 500 μ L of PBS and 800 μ L after centrifugation. Measurements were taken at 475nm in the instrument spectrophotometer and with a blue 475nm LED in the 3D printed spectrophotometer. Due to the structure of the microcuvettes and the design of the 3D printed spectrophotometer, microcuvette caps were used so that the cuvette can be flipped upside down for the light to pass through the solution. The absorbance versus concentration standard curves was generated using Microsoft Excel.

3.5 Rifampin Assay in Microcuvettes in Urine

The procedure for the rifampin assay in urine is the same as the PBS procedure, except that all PBS components are replaced with urine. Create the stock solution with 10mg of powder rifampin and 1mL of synthetic urine in a 1.5 mL tube.

In preparation for the serial dilution, 1.8 mL of synthetic urine was added to tube 1 and 500 μ L into tubes 2-13. The titrations were conducted the same way as the serial dilution for the procedures described in 3.3 Rifampin Assay in Microcuvettes in PBS. The generated serial dilutions of rifampin contain concentrations of (1 mg/ml, 0.67 mg/ml, 0.44 mg/ml, 0.2963 mg/ml, 0.198 mg/ml, 0.13 mg/ml, 0.088 mg/ml, 0.059

mg/ml, 0.039 mg/ml, 0.026 mg/ml, 0.017 mg/ml, 0.012 mg/ml, 0.0077 mg/ml). The blank used for the rifampin assay is the upper layer of 500 μ L of urine and 800 μ L after centrifugation. Measurements were taken at 475nm in the instrument spectrophotometer and with a blue 475nm LED in the 3D printed spectrophotometer. The microcuvette caps were used for the 3D printed spectrophotometer readings.

3.6 Data Analysis

To execute an accurate results analysis from both the instrument and 3D spectrophotometer, Microsoft Excel was used for standard curve outputs and absorbance calculations. The concentrations are plotted on the x-axis and absorbance on the y-axis.

The instrument spectrophotometer has the needs to calibrate per the blank sample. After calibrating the instrument spectrophotometer with the blank solution, absorbance readings of each cuvette were taken 3 times for error analysis and standard deviation. This procedure is the same throughout all assays in this project for the instrument spectrophotometer.

There is no automatic method to calibrate the 3D printed spectrophotometer. With that in mind, voltage readings were noted for incidents of the 3D enclosure empty and with the cuvette inserted. The difference of both values were taken to find the displacement value (ΔV) from the readings (e.g. $V_{\text{EMPTY}} - V_{\text{CUVETTE}}$). The voltage readings were recorded 3 times for each cuvette for error analysis and standard deviation. The absorbance was calculated based on **Equation 2**, where I_0 is the average of all three Blank cuvette readings and I is the voltage difference of (empty – cuvette):

$$A = \text{Log}_{10}\left(\frac{l}{l_0}\right)$$

Equation 2: Absorbance Equation where l_0 is the average of all three blank cuvette readings and l is the voltage difference of (empty - cuvette).

Chapter 4: Results

4.1 Bradford Assay in Macrocuvettes

Absorbance measurements in macrocuvettes were measured for both instrument and 3D printed spectrophotometer. The cuvettes were measured at a wavelength of 595nm in the instrument spectrophotometer, and with a yellow 595nm LED in the 3D spectrophotometer.

The absorbance correlation curve exemplifies the correlation between the absorbance readings from the 3D and instrument spectrophotometer. The Bland-Altman plot displays the statistical relationship of the 3D and instrument spectrophotometer. **Figure 8** shows the standard absorbance vs. concentration curve using the instrument spectrophotometer. **Figure 9** shows the standard absorbance vs concentration curve using the 3D printed spectrophotometer. The correlation plot between both methods are shown in **Figure 10**. The Bland-Altman analysis plot is shown in **Figure 11**.

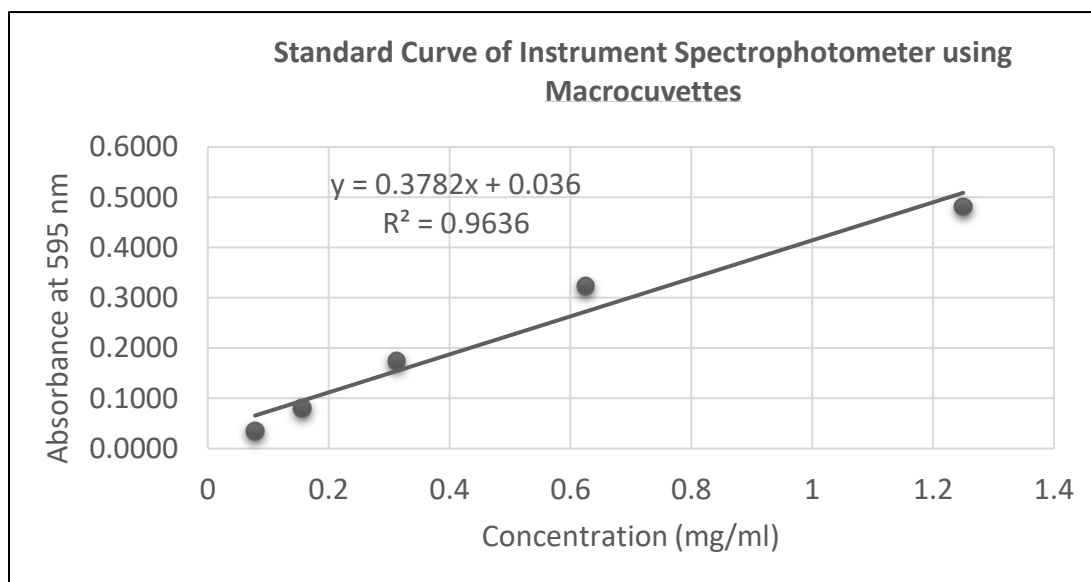


Figure 8: Standard curve of instrument spectrophotometer using macrocuvettes.

Concentration range of 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml, 0.15625 mg/ml,

0.078125 mg/ml. Correlation value = 0.9636, and equation of standard curve is $y = 0.3782x + 0.036$.

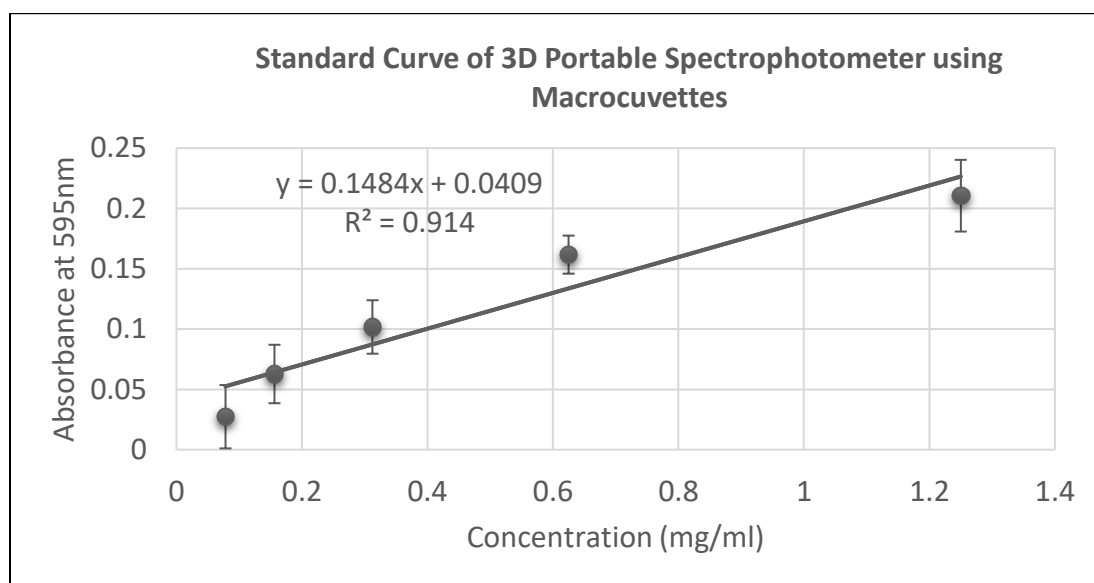


Figure 9: Standard curve of 3D printed spectrophotometer using macrocuvettes.

Concentration range of 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml, 0.15625 mg/ml, 0.078125 mg/ml. Correlation value = 0.914, and equation of standard curve is $y = 0.1484x + 0.0409$.

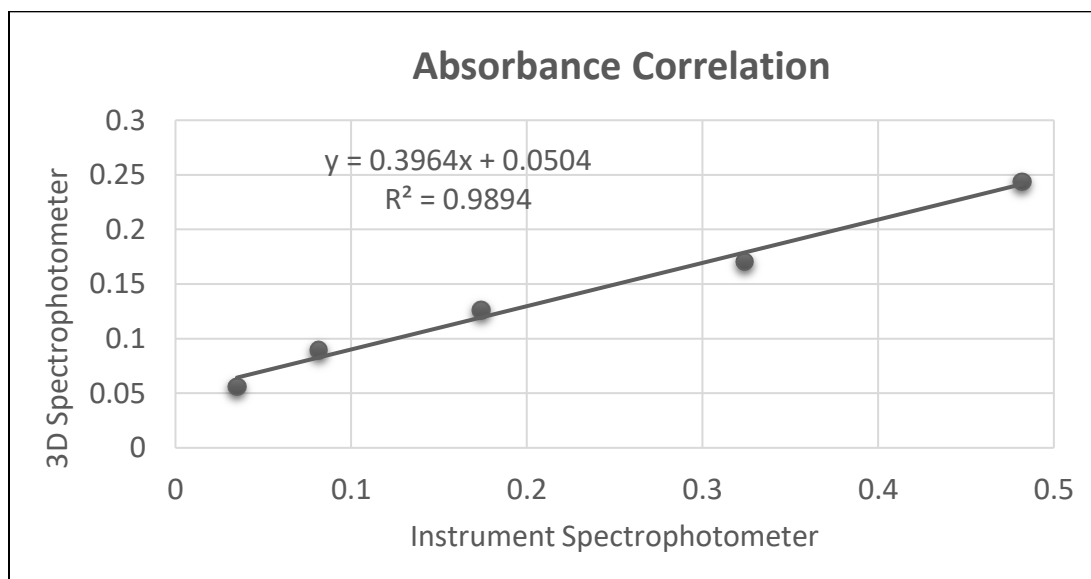


Figure 10: Standard absorbance correlation curve comparing the instrument and 3D printed spectrophotometer using macrocuvettes. Correlation value = 0.9894, and equation of standard curve is $y = 0.3964x + 0.0504$.

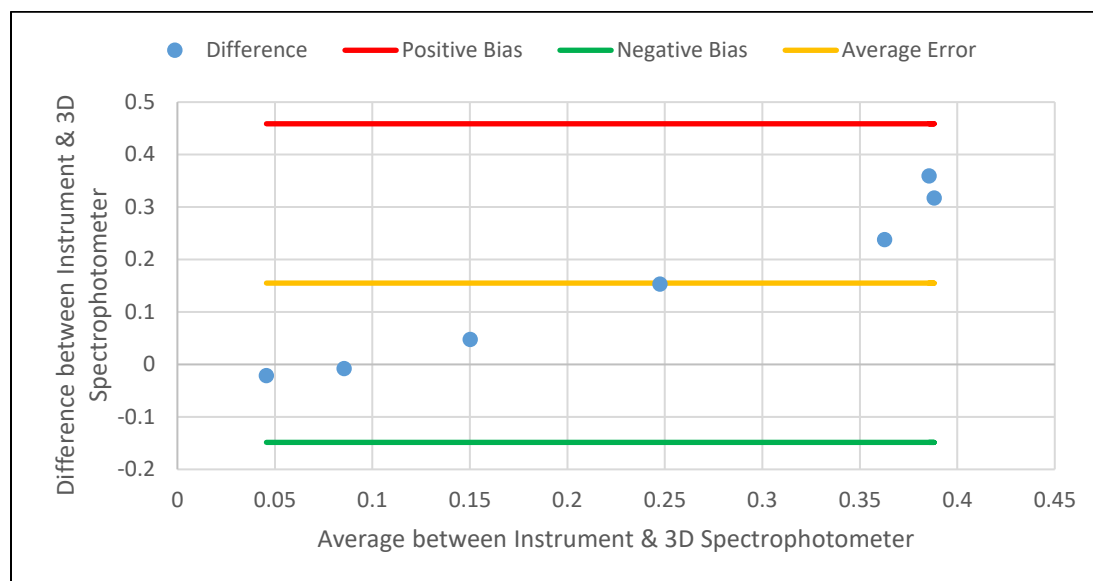


Figure 11: Bland-Altman analysis of the absorbance measurements compared between the instrument and 3D printed spectrophotometer. Exhibiting a central mean of 0.155, a lower limit of -0.149, and an upper limit of 0.459.

4.2 Bradford Assay in Microcuvettes

Figure 12 shows the standard absorbance vs. concentration curve using the instrument spectrophotometer. **Figure 13** shows the standard absorbance vs concentration curve using the 3D printed spectrophotometer. The correlation plot between both methods are shown in **Figure 14**. The Bland-Altman analysis plot is shown in **Figure 15**.

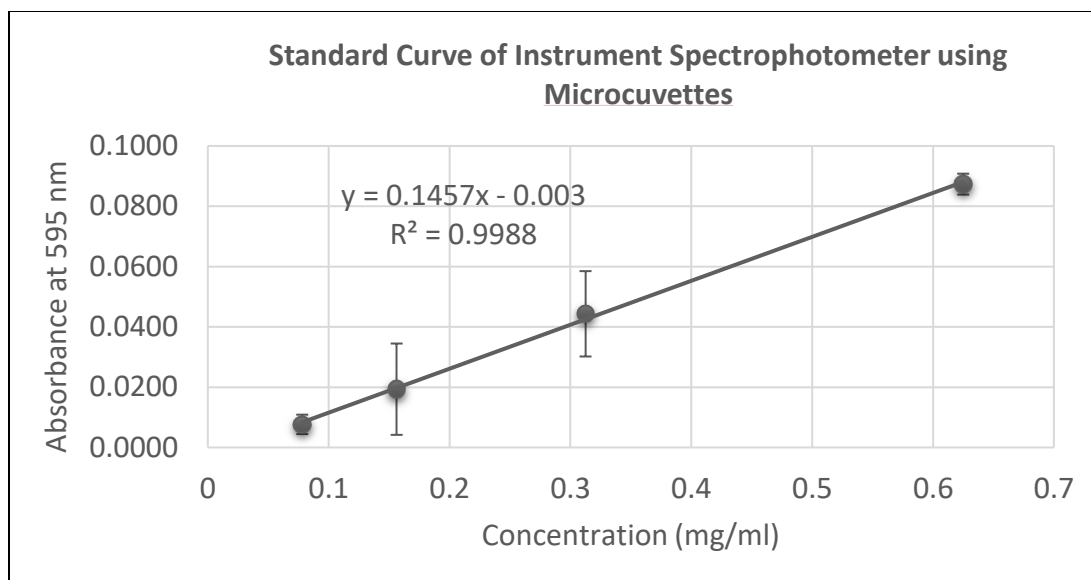


Figure 12: Standard curve of instrument spectrophotometer using microcuvettes.

Concentration range of 0.625 mg/ml, 0.3125 mg/ml, 0.15625 mg/ml, 0.078125 mg/ml.

Correlation value = 0.9988, and equation of standard curve is $y = 0.1457x - 0.003$.

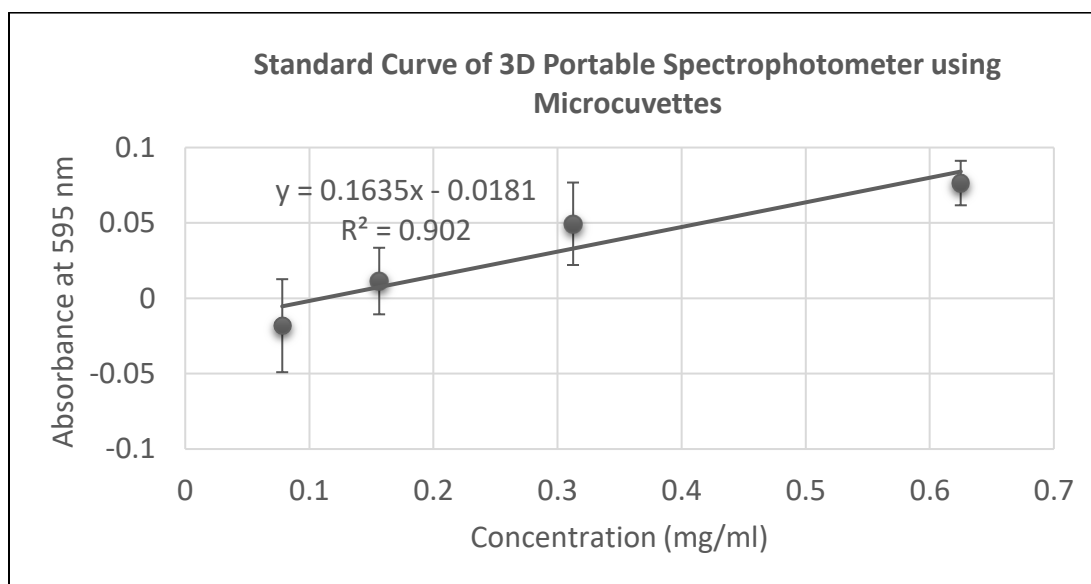


Figure 13: Standard curve of 3D printed spectrophotometer using microcuvettes.

Concentration range of 0.625 mg/ml, 0.3125 mg/ml, 0.15625 mg/ml, 0.078125 mg/ml.

Correlation value = 0.902, and equation of standard curve is $y = 0.1635x - 0.0181$.

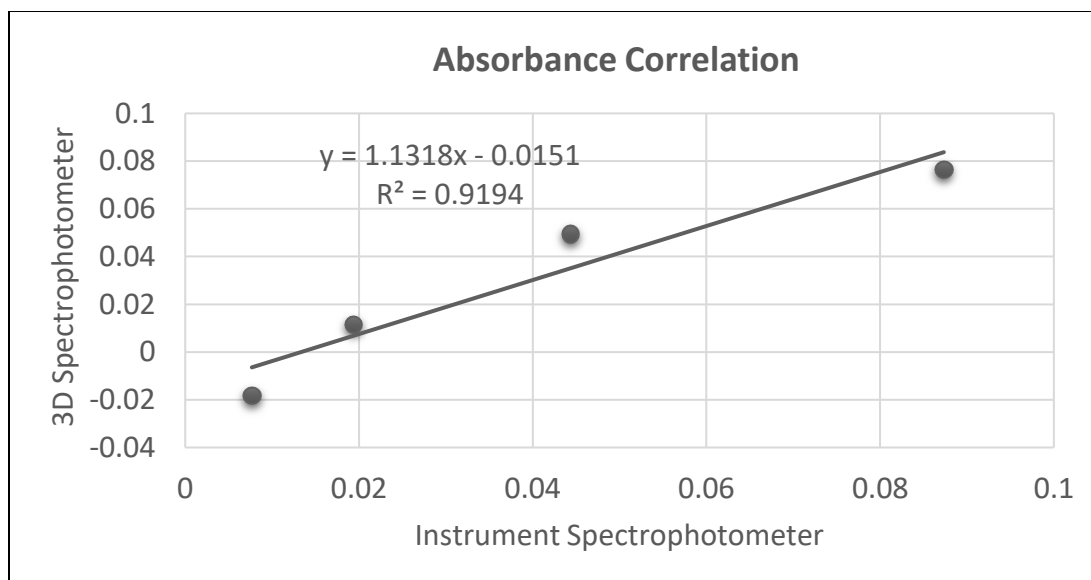


Figure 14: Standard absorbance correlation curve comparing the instrument and 3D printed spectrophotometer using microcuvettes. Correlation value = 0.9194, and equation of standard curve is $y = 0.1318x - 0.0151$.

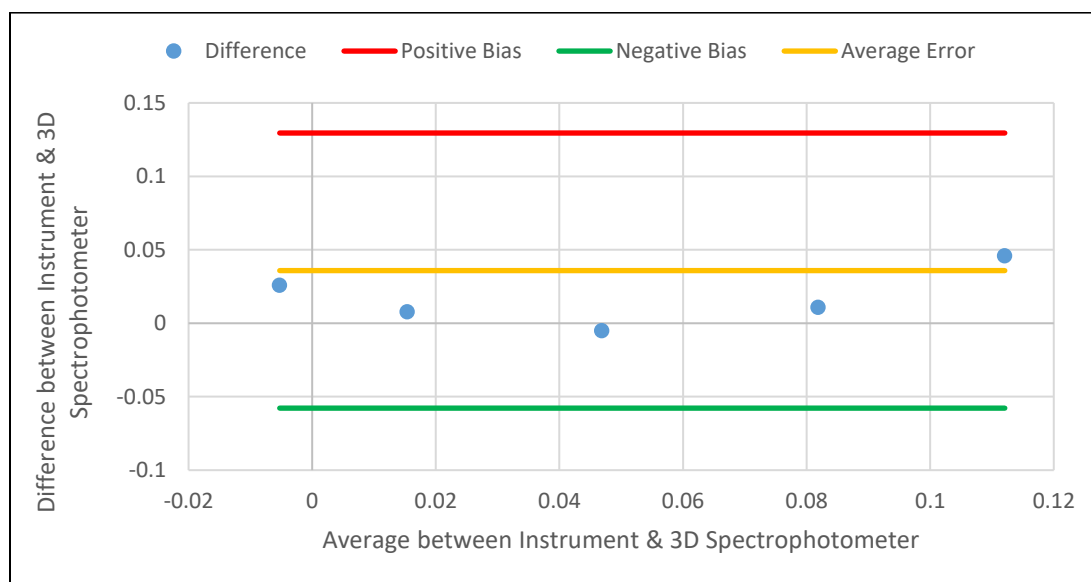


Figure 15: Bland-Altman analysis of the absorbance measurements compared between the instrument and 3D printed spectrophotometer. Exhibiting a central mean of 0.036, a lower limit of -0.058, and an upper limit of 0.129.

4.3 Rifampin Assay Results in PBS

Figure 16 shows the standard absorbance vs. concentration curve using the instrument spectrophotometer. **Figure 17** shows the standard absorbance vs concentration curve using the 3D printed spectrophotometer. The correlation plot between both methods are shown in **Figure 18**. The Bland-Altman analysis plot is shown in **Figure 19**.

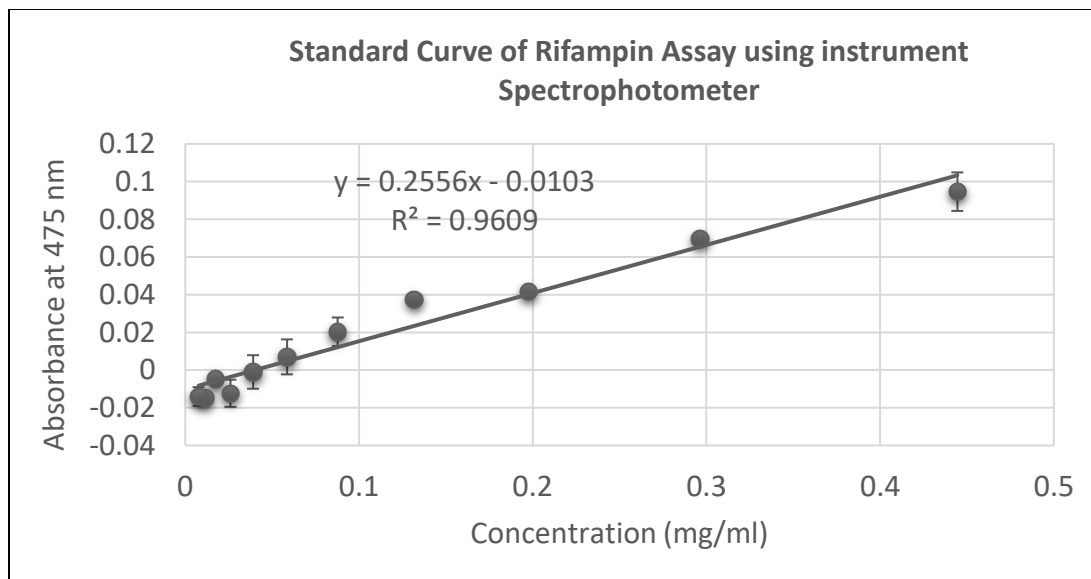


Figure 16: Standard curve of instrument spectrophotometer using microcuvettes.

Concentration range of 0.44 mg/ml, 0.2963 mg/ml, 0.198 mg/ml, 0.13 mg/ml, 0.088 mg/ml, 0.059 mg/ml, 0.039 mg/ml, 0.026 mg/ml, 0.017 mg/ml, 0.012 mg/ml, 0.0077 mg/ml. Correlation value = 0.9609, and equation of standard curve is $y = 0.2556x - 0.0103$.

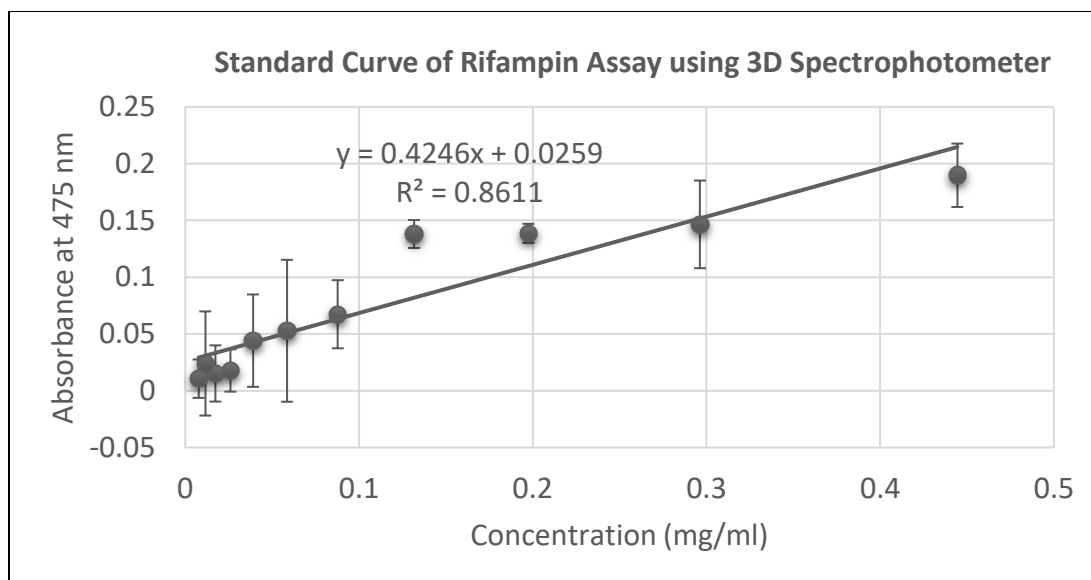


Figure 17: Standard curve of 3D printed spectrophotometer using microcuvettes.

Concentration range of 0.44 mg/ml, 0.2963 mg/ml, 0.198 mg/ml, 0.13 mg/ml, 0.088 mg/ml, 0.059 mg/ml, 0.039 mg/ml, 0.026 mg/ml, 0.017 mg/ml, 0.012 mg/ml, 0.0077 mg/ml. Correlation value = 0.8611, and equation of standard curve is $y = 0.4246x + 0.0259$.

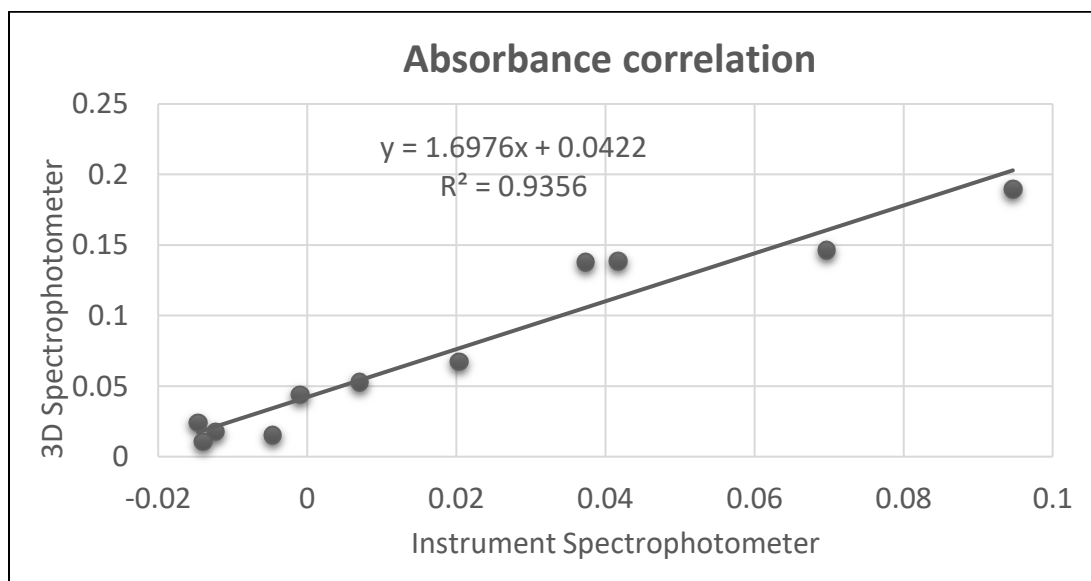


Figure 18: Standard absorbance correlation curve comparing the instrument and 3D printed spectrophotometer using microcuvettes. Correlation value = 0.9356, and equation of standard curve is $y = 1.6976x + 0.0422$.

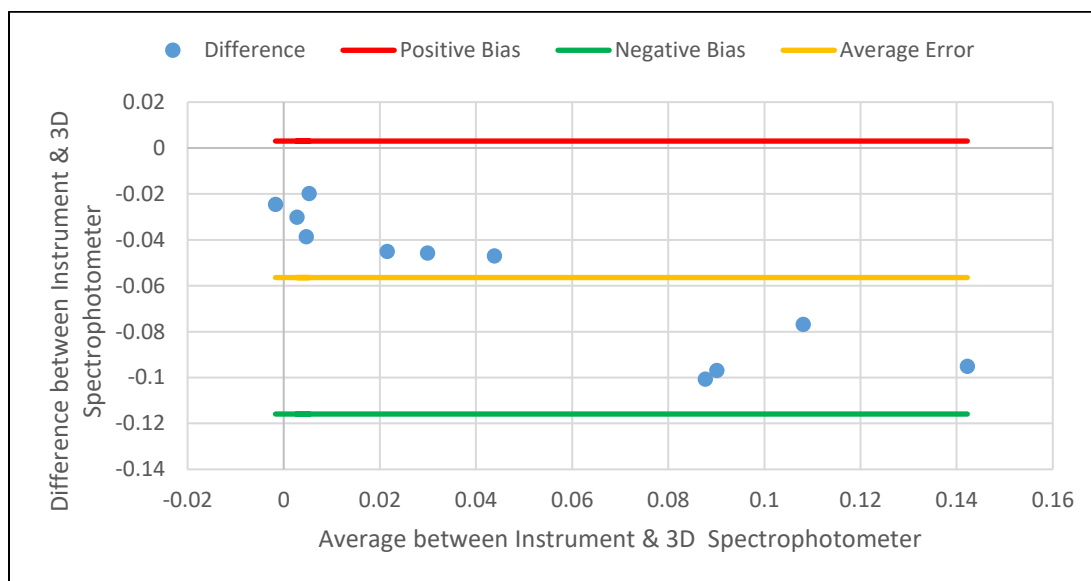


Figure 19: Bland-Altman analysis of the absorbance measurements compared between the instrument and 3D printed spectrophotometer. Exhibiting a central mean of -0.056, a lower limit of -0.116, and an upper limit of 0.003.

4.4 Rifampin Assay Results in Urine

Figure 20 shows the standard absorbance vs. concentration curve using the instrument spectrophotometer. **Figure 21** shows the standard absorbance vs concentration curve using the 3D printed spectrophotometer. The correlation plot between both methods are shown in **Figure 22**. The Bland-Altman analysis plot is shown in **Figure 23**.

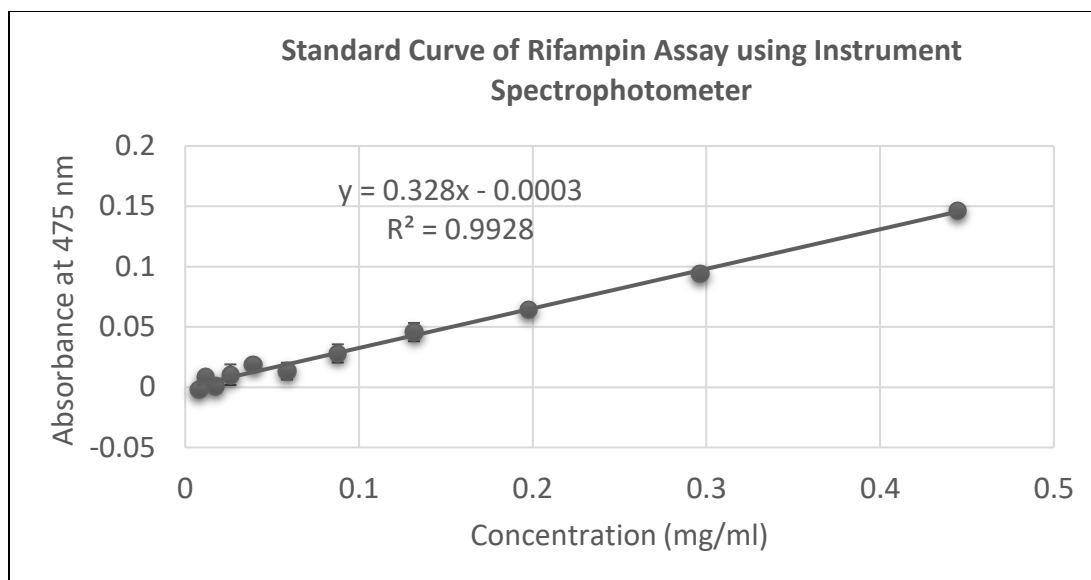


Figure 20: Standard curve of instrument spectrophotometer using microcuvettes.

Concentration range of 0.44 mg/ml, 0.2963 mg/ml, 0.198 mg/ml, 0.13 mg/ml, 0.088 mg/ml, 0.059 mg/ml, 0.039 mg/ml, 0.026 mg/ml, 0.017 mg/ml, 0.012 mg/ml, 0.0077 mg/ml. Correlation value = 0.9928, and equation of standard curve is $y = 0.328x - 0.0003$.

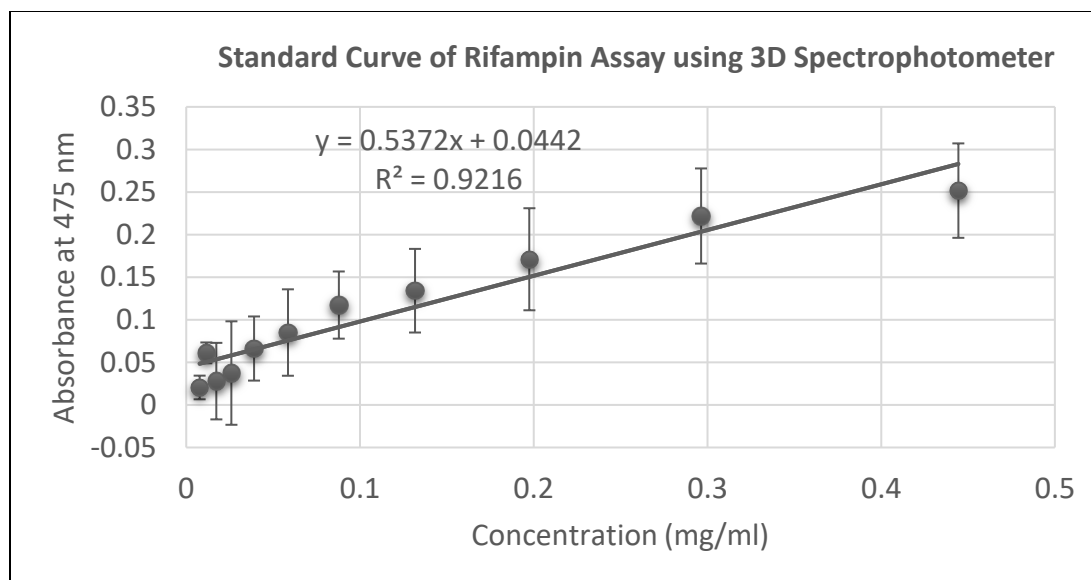


Figure 21: Standard curve of 3D printed spectrophotometer using microcuvettes.

Concentration range of 0.44 mg/ml, 0.2963 mg/ml, 0.198 mg/ml, 0.13 mg/ml, 0.088 mg/ml, 0.059 mg/ml, 0.039 mg/ml, 0.026 mg/ml, 0.017 mg/ml, 0.012 mg/ml, 0.0077 mg/ml. Correlation value = 0.9216, and equation of standard curve is $y = 0.5372x + 0.0442$.

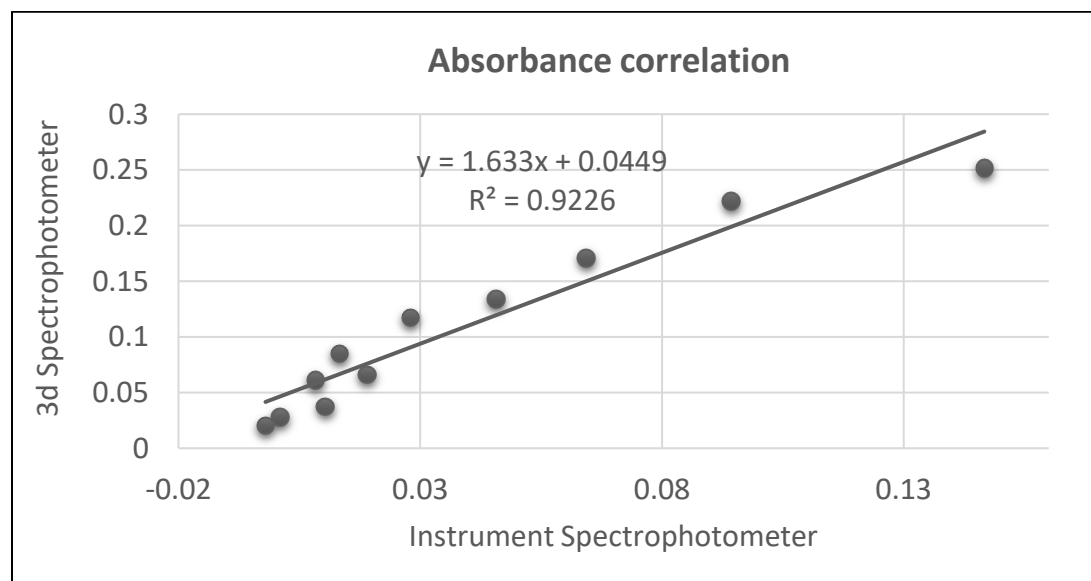


Figure 22: Standard absorbance correlation curve comparing the instrument and 3D printed spectrophotometer using microcuvettes. Correlation value = 0.9226, and equation of standard curve is $y = 1.633x + 0.0449$.

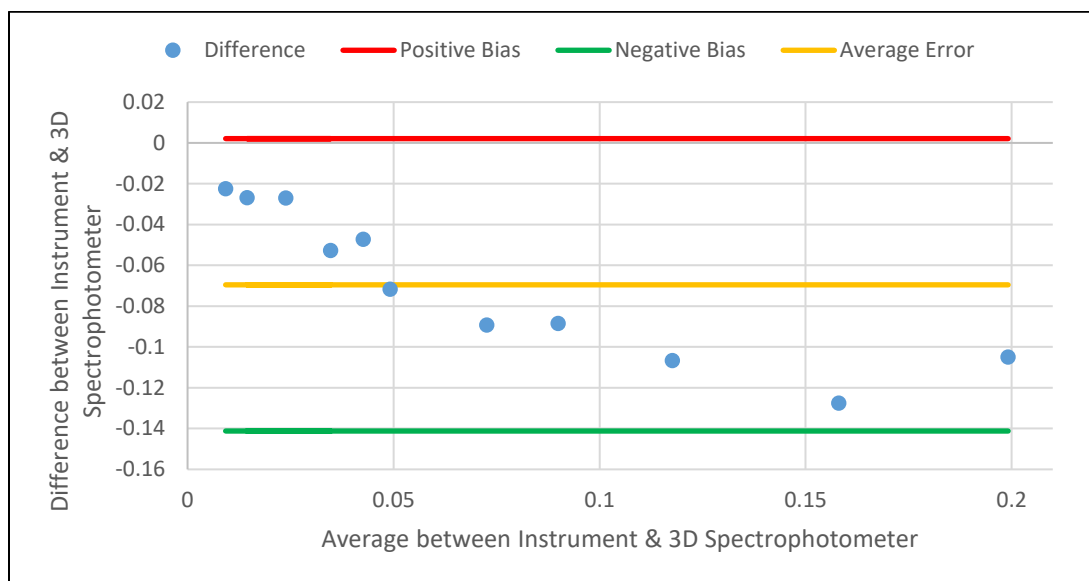


Figure 23: Bland-Altman analysis of the absorbance measurements compared between the instrument and 3D printed spectrophotometer. Exhibiting a central mean of -0.0696, a lower limit of -0.141, and an upper limit of 0.0021.

Chapter 5: Future Work

5.1 Future Work

Phase 1 of this project involved developing a 3D printed spectrophotometer that could measure the absorbance readings of any biological sample. The goal of phase 1 was to create a 3D printed spectrophotometer that incorporated an optimal optic set up with a circuit design for data acquisition and processing. A shortcoming of phase 1 is that the experimental assays require multiple manual steps, such as centrifugation in the rifampin assay. In a real point of care scenario, it would take time to take the urine samples to the lab and centrifuge them and then pipette out the upper layer to measure the absorbance.

To overcome these limitations, we developed a phase 2 device that integrates a microfluidic chip and on-chip centrifuge.

5.2 Phase 2

The purpose of phase 2 is to reduce the amount of manual steps involved in prepping the urine-rifampin samples to target a point of care device that is more compact and delivers results within minutes. Phase 2 is still in its development and the device encompasses a 3D printed centrifuge that contains a Polydimethylsiloxane (PDMS) channel, printed circuit board (PCB), and a motor.

The PDMS channel will house the rifampin-urine and isoamyl alcohol solution. After centrifugation, the aqueous layer will congregate into the center of the chip, leaving enough room to house an LED above and photodiode below to detect the absorbance from the chip. The chip can hold a volume capacity of 650 μL . **Figure 24A** shows the AutoCAD schematic of the PDMS channel and the printed channel. (**Figure 24B**)

A centrifuge is a laboratory machine that spins at a high speed to see separation in liquid solutions. ^[18] We developed an on-chip centrifuge that will spin for 5 minutes at 14000RPM with the assistance of a 1.5V motor. The motor is housed in a 3D printed enclosure of the 3D printed base. **Figure 25** shows the motor that will spin the PDMS channel in phase 2.

Additionally, we developed a PCB design to consolidate all the wiring of the phase 1 breadboard to reduce excessive wiring exposure. **Figure 26** shows the PCB schematic of all the electronic components. **Figure 27A** shows a diagram of the device before and after the channel is placed into the 3D components. **Figure 27B** exhibits the photo of the setup of the 3D centrifuge device. In the future, we hope to test the device and separation in the PDMS channel.

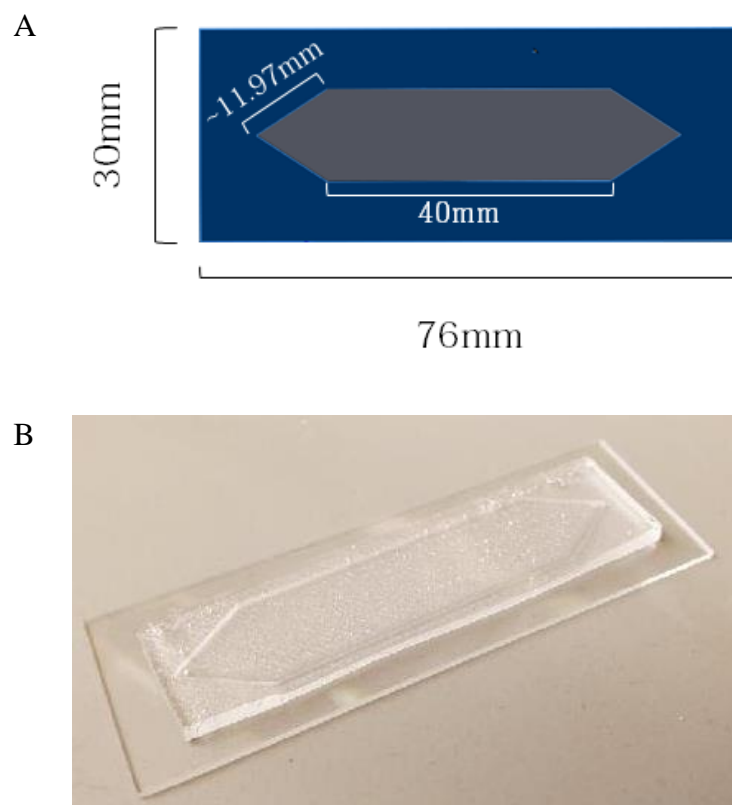


Figure 24: PDMS channel used in phase 2 of the project. (A) AutoCAD schematic of the channel for printed and (B) the printed channel in PDMS material.



Figure 25: The 1.5V motor purchased from Amazon by the seller Topoox that will spin the channel for centrifugation.

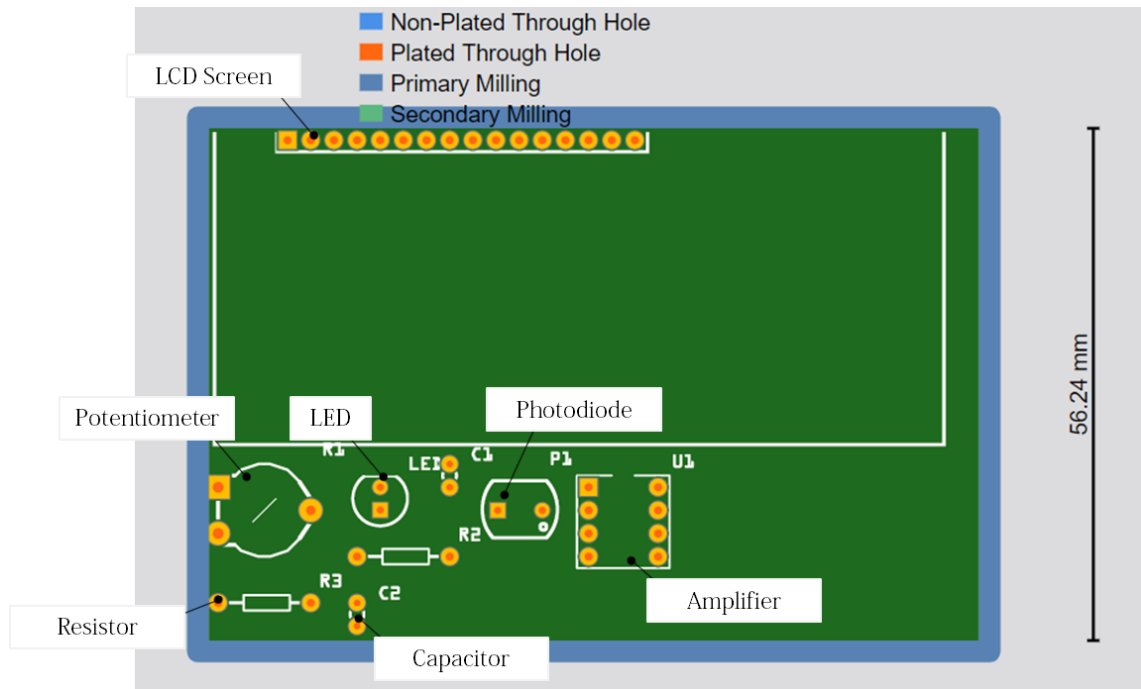


Figure 26: PCB design of the consolidated electronic components.

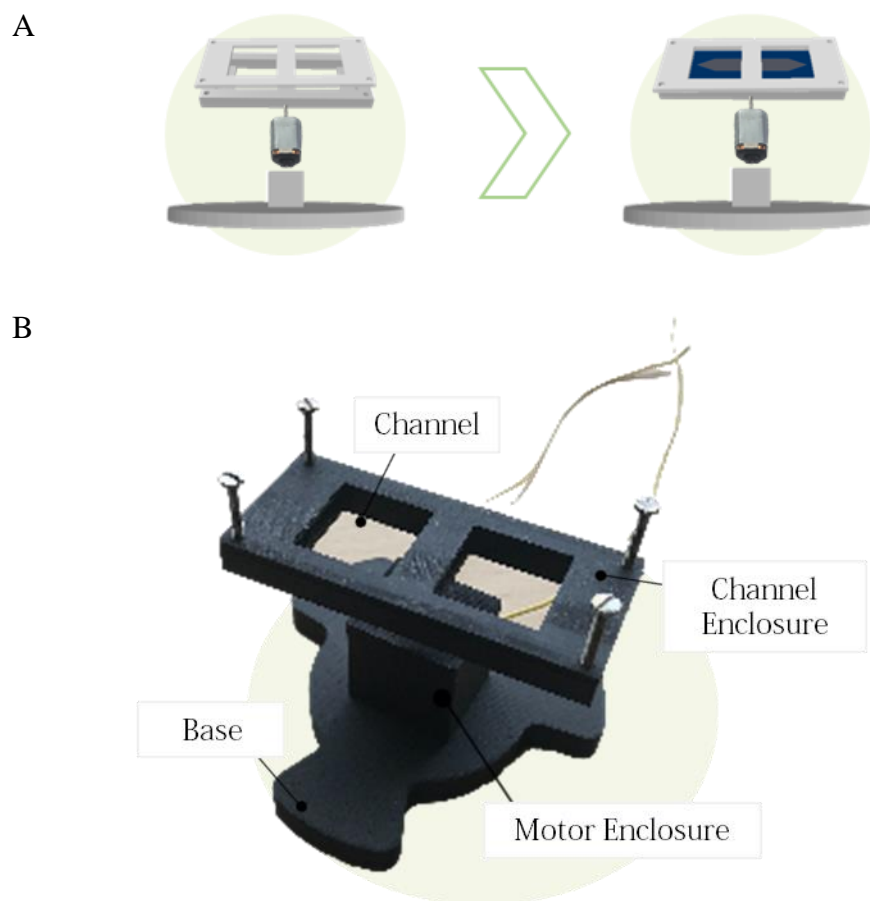


Figure 27: A design set up of the phase 2 3D printed centrifuge. (A) A diagram of the device before and after the channel is placed into the 3D components. (B) Exhibits the photo of the setup of the 3D centrifuge device.

5.3 Conclusion

We developed and tested a portable 3D printed spectrophotometer for different biological assays and compared the results with a laboratory standard table-top instrument spectrophotometer. The two assays tested at varying volumes were the Bradford and Rifampin Assay to generate a standard absorbance vs. concentration curve.

Based on the results' outcome, there is a strong correlation between the instrument and the 3D printed spectrophotometer.

There were challenges faced while testing the 3D printed device. When the cuvettes were measured in the 3D device, there were many fluctuations in the voltage output. This can be due to the battery usage and light sensitivity of the photodiode. For our experiment, the device on for 3-4 hours until all samples were measured. However, in a real-life point of care scenario, a medical clinician would only need to turn the device on for a few minutes. We can test the device with a more stable and consistent power source by connecting the device to a voltage box to resolve this issue. To overcome the light sensitivity issue, we can test the device in a dark environment by either inserting it into a box or turning off the environment's lights. As discussed before, phase 1 requires multiple manual sample processing, which we resolved by developing the phase 2 application.

When comparing the absorbance results between an instrument and 3D printed spectrophotometer, the absorbance range varies. For instance, the standard curve of the rifampin assay in synthetic urine of the instrument spectrophotometer has absorbance ranges within -0.05 to 0.2. **(Figure 20)** However, the absorbance ranges for the 3D printed spectrophotometer are within the -0.05 to 0.35 range. **(Figure 21)** The photodiode in the 3D spectrophotometer is absorbing more light than the output of the LED. There are multiple solutions to testing the effectiveness of the photodiode. One being that with the use of a stray light and a constant input voltage, we can check the voltage coming in and intensity coming out. Another test would be to see the saturation / absorbance region of the photodiode for the range at which the diode will give its effective reading.

Future modifications to phase 1 can be applied to the electronic components. The photodiode in our current device is very sensitive to light, making it difficult to test samples in sunlight. To enhance the use of the photodiode, we can use a pulse amplitude modulation to filter out noise from ambient light.^[19] This will provide more accurate readings from the cuvettes without the interference of environment light. Another electronic modification can be to use an OPT101 which is a monolithic photodiode with on-chip trans impedance amplifier.^[20] This part will replace the individual photodiode and amplifier pieces to overcome problems such as noise and current error. Utilizing the OPT101 can eliminate that additional noise that may be causing the varying difference in absorbance range in the 3D printed spectrophotometer.

Utilizing the 3D printed spectrophotometer can assist medical professionals to monitor the drug absorbance of a TB patient. TDM can help classify whether the patient has adequate or inadequate drug exposure. The printed device can provide a non-invasive approach to testing TB patients. Future strategies of this project would be to resolve the voltage readings' fluctuations and test the 3D device on known human urine concentrations and compare the absorbance results between an instrument and a 3D spectrophotometer.

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