The design of turbidimeter for $Mg_2P_2O_7$ in LAMP product of *Salmonella Typhi* detection

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Abstract

In this publication, we design a turbidimeter for $Mg_2P_2O_7$ sedimentation by using PID and microcontroller. The product was tested via the process of increasing the amount of *Salmonella Typhi's* DNA by *Loop Mediated Isothermal Amplification* (LAMP). The LAMP reaction works at a constant temperature in the range from 60 °C to 65 °C, and $Mg_2P_2O_7$ absorbs light at a wavelength of 650 nm. Therefore, this design adopts the principles of medical science, temperature control, physics of light, electronics and microcontrollers. The design and construction of this device consisted of four main parts: 1) the light source, which used four LEDs and voltage divider circuits, 2) the PID temperature controller, composed of temperature sensor, an optocoupler, and resistive heating element, 3) signal conditioning, consisting of four photodiodes and current to voltage converters. The sample under test was prepared in Eppendorf tube and mounted to an aluminum block. The final part, control, and signal processing was implemented using an Arduino Mega 2560, which also facilitated computer screen display. The software was designed to exhibit two modes of operation; firstly, warm-up mode, which heats up the aluminum block to the desired temperature, reaching stability within 15minutes. The second mode was turbidity measurement, which maintains temperature control and measures for turbidity of $Mg_2P_2O_7$ simultaneously. Test results showed that this device can adequately control the temperature of the sample tube at all of the values selected, which were *60*, *61*, *62*, *63*, *64* and *65* °C. Light absorbance was read every 5 seconds and used to identify Salmonella Typhi DNA from LAMP reaction by measuring the turbidity of $Mg_2P_2O_7$ from the transmitted light. Results indicate that this design is effective for this application.

Keywords**:** *$Mg_2P_2O_7$, Salmonella Typhi, Loop Mediated Isothermal Amplification (LAMP), turbidity, turbidimeter*

1. Introduction

At present, changes in the environment and various behaviors of human beings have led to many emerging diseases. Food also the same, there are many kinds of bacteria-contaminated in food. Salmonella typhi, a bacterium that causes food poisoning (Salmonellosis) by people who are infected with diarrhea, typhoid fever, nausea, and vomiting, often found in patients infected with food contamination, which is found in poultry. Therefore, it is an important infection and must be inspected in a fresh chicken factory regularly. In this study, the researchers interested in the design of the device to detect *Salmonella Typhi* (S. *Typhi*) by means of increasing the amount of DNA using a LAMP technique.

There are several methods for nucleic acid amplification. Polymerase chain reaction (PCR) is the most widely used (Saiki et al., 1988). However, the PCR method has several limitations such as it took 3-4 hours to inspect and also use specific equipment. In the detection of the infection each time, so it takes a long time and the cost is high. Later, Notomi et al. (Notomi et al., 2000) developed a new technique for nucleic acid amplification by means of Loop Mediated Isothermal Amplification (LAMP). LAMP work on isothermal condition ranging within 60-65 °C and the reaction take time to complete approximately 45 minutes to 1 hour. LAMP reaction product(Mori, Nagamine, Tomita, & Notomi, 2001) was shown as

$$DNA_{n-1} + dNTP \rightarrow DNA_n + P_2O_7^{4-} \quad [1]$$

$$P_2O_7^{4-} + 2Mg^{2+} \rightarrow Mg_2P_2O_7 \quad [2]$$

In cases diagnosis of infectious deceases, it is important to examine the presence of nucleic acid in the samples. From the LAMP product, the presence of nucleic acid was shown with turbidity of the white

specimen from magnesium pyrophosphate formation ($Mg_2P_2O_7$). The turbidimeter uses the 650 nm light to detect $Mg_2P_2O_7$ specimen (Mori, Kitao, Tomita, & Notomi, 2004).

Mori et al. (Mori et al., 2004) made an apparatus to measure the turbidity of eight samples simultaneously and maintain a constant temperature of LAMP reactions. They found that the initial time (Tt) that can detect the turbidity of the LAMP reaction solution was depended on the quantity of the initial template DNA. The relationship of the initial time (Tt) and the log of the amount of initial template DNA was linearity which useful for the quantitative analysis of amounts of initial DNA present in a sample.

Sappat, A. et al. (A. Sappat, Jaroenram, Kiatpathomchai, Lomas, & Tuantranont, 2010; Assawapong Sappat et al., 2009) designed the turbidity detection of shrimp *Taura Syndrome Virus* (TSV) by RT-LAMP reaction. The process was clarified by comparing the result with the PCR technique. The designed system was successful to detect TSV in shrimp samples for field application.

In this research, we applied the Beer-Lambert law to detect $Mg_2P_2O_7$, with the transmitted light from the solution in LAMP reaction, by performing the designed apparatus to determine the increasing of $Mg_2P_2O_7$ in solutions. The device could maintain a constant temperature (60-65 ºC) of the sample solution in Eppendorf tube, 650 nm spectrum LED work as a light source, the detector was a photodiode and the current to voltage amplifier and temperature control along with measuring transmitted light with Arduino Mega 2560. The turbidity of LAMP reaction solution was studied in the dilutions of *Salmonella Typi*. The results were compared to nanodrop spectroscopy.

2. Objectives

In this publication, we design a turbidimeter for $Mg_2P_2O_7$ sedimentation by using PID and microcontroller. The product was tested via the process of increasing the amount of *Salmonella Typhi's* DNA by *Loop Mediated Isothermal Amplification* (LAMP).

3. Materials and Methods

3.1 Materials

The material on this research can be divided into 2 groups. The first material group is the designed turbidimeter which composed of 4 parts such as 1) the light source, 2) the PID temperature controller part, 3) the signal conditioning part and 4) microcontroller and display as shown in Figure 1. The second material group is used for testing the designed turbidity. We prepared the dilutions of *Salmonella Typi* with the four different concentration in 8 Eppendorf tubes, each concentration value of the solution was filled in two tubes for the test with the designed device and compare the result by using Nanodrop spectrophotometer to find the concentration of $Mg_2P_2O_7$ during the LAMP reaction.

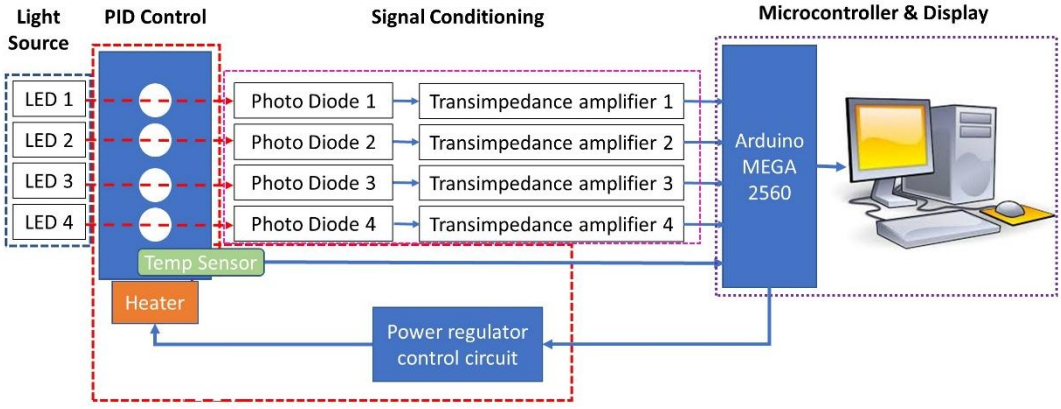


Figure 1 Block diagram of the designed turbidimeter

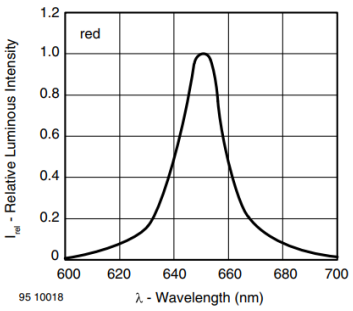
3.2 **Methods**

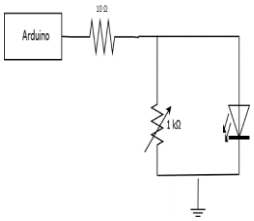
For the designed device, we give the detail for each part as:

1) The light source

We design the light source used 4 LEDs and voltage divider circuits for control each luminescent of the LED as shown in Figure 2.

Voltage 2.2 V
Wavelength 650 nm
Working Temperature – 40 to 100 °C



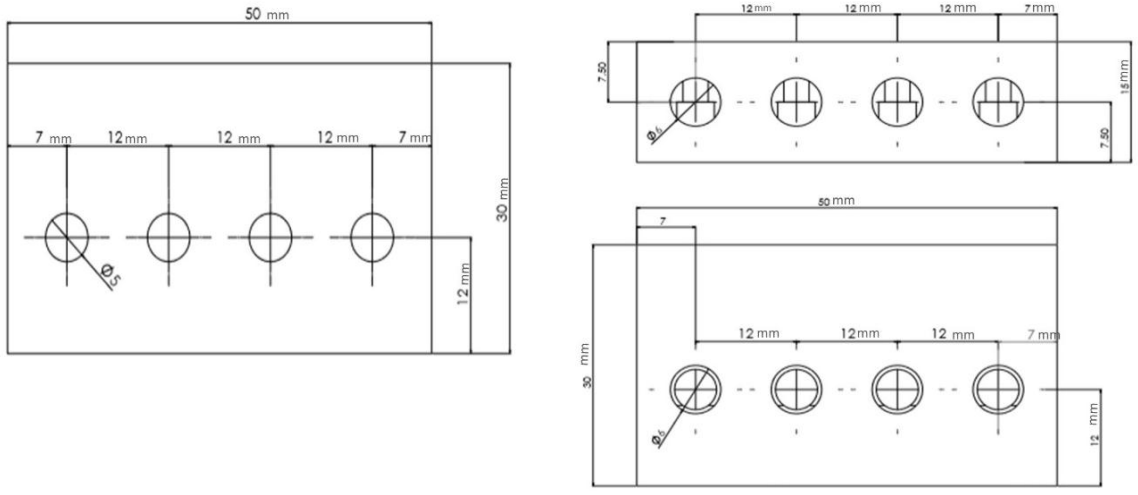


(a) (b) (c)

Figure 2 (a) LED specification (b) Characteristic graph of relative intensity vs. wavelength of LED (c) voltage divider circuit for control lamination of LED.

2) The PID temperature controller

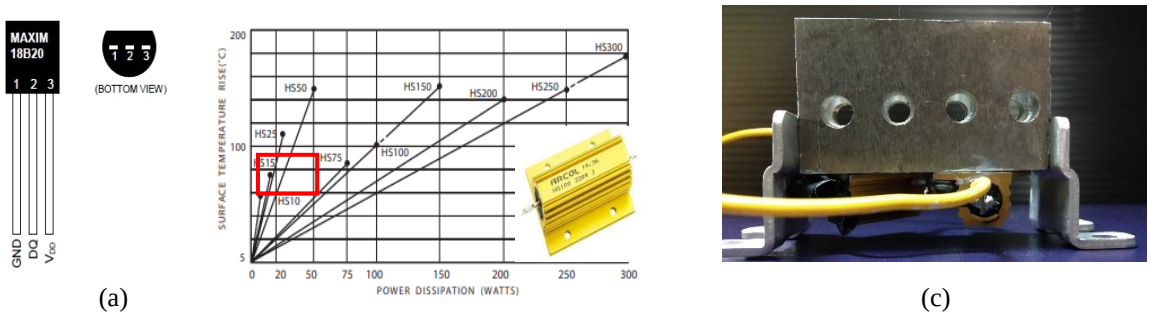
On this section, we used the aluminum block to be the medium for control the constant temperature of the solution in Eppendorf tubes. The aluminum block was designed as shown in Figure 3. The PID control was composed of a temperature sensor, DS18B20, for feedback control and DC resistor heater as shown in Figure 4. The two-aluminum housed axial wire wound panel mount resistors, 22Ω ±5% 15W, were paralleled connection and placed at the bottom of the aluminum block, while the temperature sensor was placed at the center inside the aluminum block.



(a) (b)

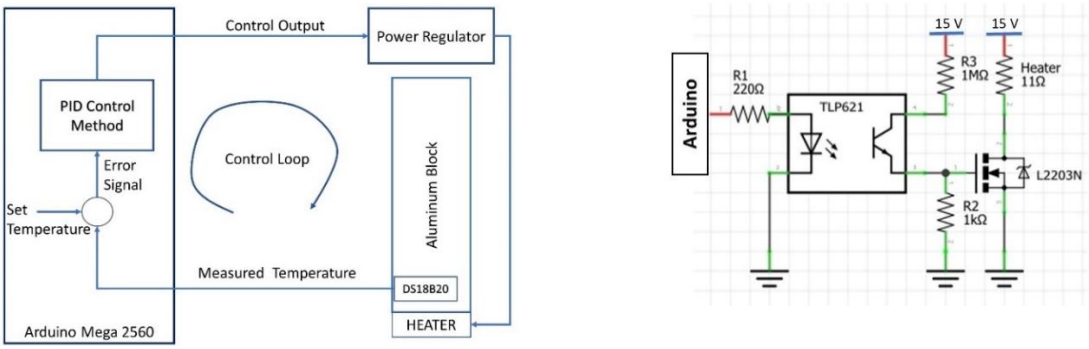
Figure 3 (a) Right side view (b) Top view and Left side view

For the power regulator control circuit, we use optoisolator TLP621 to prevented and separated the microcontroller from the 15 V source. The 15 V source was turned on or turned off according to FETL2203N and PID parameter in the programming. The circuit as shown in Figure 5.



(a) (c)

Figure 4 (a) Temperature sensor (DS18B20) (b) Resistor Heater and the characteristic graph of power dissipation and temperature (c) the set of heaters were placed at the bottom of the aluminum block.



(a) (b)

Figure 5 (a) PID Control Diagram (b) Power regulator control circuit

3) The signal conditioning

This part composes of four groups of the photodiode and the current to voltage converter circuit. The photodiode (BPX65) had characteristics that good response to both working temperature range and the good response for the 650 nm wavelength light. Since the current of the photodiode that response to the intensity of incident light in microampere then we need to amplify the current change by using the current to voltage converter circuit as shown in Figure 6.

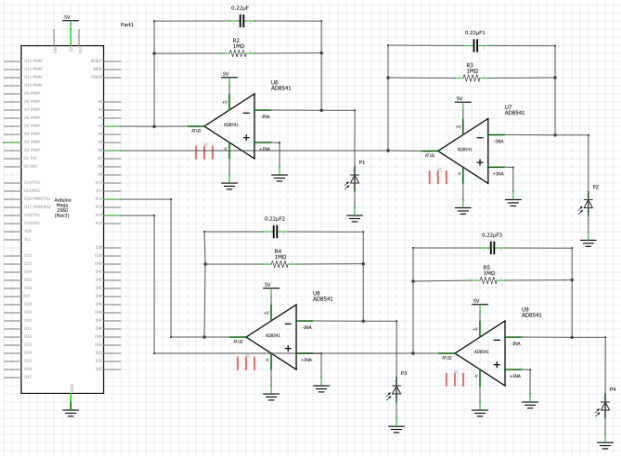


Figure 6 The signal conditioning part

4) Microcontroller and display

Arduino Mega 2560 were used as the microcontroller to control both temperature and measured the turbidity of four Eppendorf tubes. The turbidity measurements were calculated according to Beer-Lambert's law as the equation (1) and the measurement repeated once every 5 s for 60 minutes.

$$Turbidity = -\ln\left(\frac{I_{OUT}}{I_{IN}}\right) \quad (1)$$

When I_{OUT} is the intensity of the transmitted light from each Eppendorf tube.

I_{IN} is the average intensity of the transmitted light from each Eppendorf tube within the first period (10 minutes) by the assumption that there are no $Mg_2P_2O_7$.

The designed program was divided into three modes, which is the temperature selection mode, warm up aluminum block mode, and turbidity measurands mode, the flow chart as shown in Figure 7.

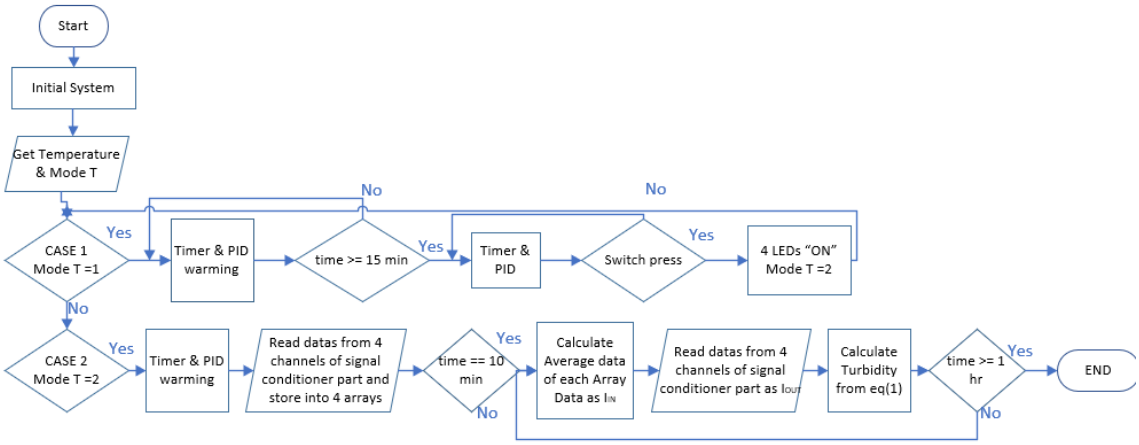


Figure 7 The flow chart of programming

4. Results and Discussion

The designed device was shown in Figure 8. We separated the aluminum heat block, light source, and photodiode out of the control circuit and microcontroller since the working temperature was higher than 60 °C and some electronic device do not work well.

Figure 8 The designed device

The functional testing of this research has divided into two parts: the PID control temperature part and the turbidity measurement part. For the PID control temperature part, we measure the temperature of an aluminum block by using sensor DS18B20 for each selected temperature together with measure the temperature of the solution inside the Eppendorf tube measure with the Fluke 289 true RMS multimeter.

The temperature of the aluminum block measured from the designed device for each selected temperature versus time was shown in Figure 9. We found that the temperature of the aluminum block was acceptably close to the selected temperature. The temperature of the aluminum block was increase and constant within 15 minutes.

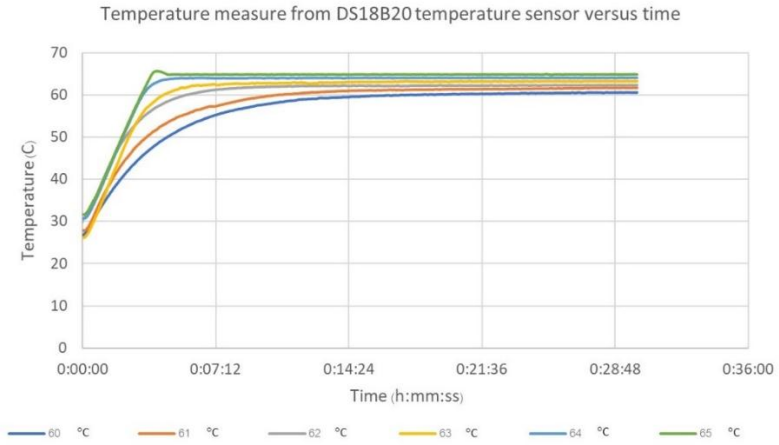


Figure 9 The selected measured temperature from DS18B20 versus time

The result of the average temperature of aluminum block measured from sensor DS18B20 and the average temperature of the solution inside the Eppendorf tube measured with the Fluke 289 true RMS multimeter after 15 minutes from start time were compared and shown in Table 1. The highest error between the temperature of the aluminum block and the solution in the Eppendorf tube is 0.79% or 0.5°C at 61°C that means the temperature of the solution inside the Eppendorf tube was acceptably close to the temperature of the aluminum block.

Table 1 Comparison result of the average temperature of the designed device and the substance's average temperature within the Eppendorf tube measure with Fluke 289 true RMS multimeter

The setting Temperature (°C)	the avg temperature of the designed device (°C)	the avg temperature of the standard device (°C)	% Error
60	60.14	59.8	0.57%
61	61.48	61.0	0.79%
62	62.12	61.9	0.36%
63	63.01	62.6	0.66%
64	64.02	64.0	0.28%
65	64.80	64.8	0%

The second part is the turbidity measurement. After 15 minutes, the designed device has a constant temperature (65 °C). *Salmonella Typi* DNA of LAMP substances were prepared in 8 Eppendorf tubes and divided into two sets of the *Salmonella Typi* DNA with the same concentration. We prepared 5-folds dilution by starting from 50 ng/µl then the concentration of *Salmonella Typi* DNA equal to 50, 10, 2 and 0.4 ng/µl for the first tube to the fourth tube respectively. We avoid disturbing the light detection of our apparatus by building 2 sets of apparatus. The first set of *Salmonella Typi* was used with the first designed

device, another set was used with the second designed device. The first device was used to control temperature and measure the transmitted light as described above; the second device was used to only control temperature to maintaining the LAMP reaction. We pipet 2 µl of each tube in the second device by start pipet at 10 minutes and repeat every 5 minutes to check the concentration of $Mg_2P_2O_7$ from light 650 nm wavelength of Nanodrop Thermo Scientific.

The result of the turbidity from the first device as shown in Figure 10. We measure the turbidity for two hours (longer than the second device for one hour) and found that the turbidity of 4 tubes was increased and decrease after that especially the first tube that has the highest concentration of *Salmonella Typhi*. From LAMP reaction [1] and [2], the higher concentration DNA got a faster reaction and reaction stop faster. The presence of $Mg_2P_2O_7$ was depending on the concentration of the initial DNA. It is possible that the experiment of Figure 10 was tested with LED light only, we didn't disturb the solution inside the Eppendorf tube. The sediment of $Mg_2P_2O_7$ may sedimentation at the bottom of the tube affect the transmitted light to a photodiode.

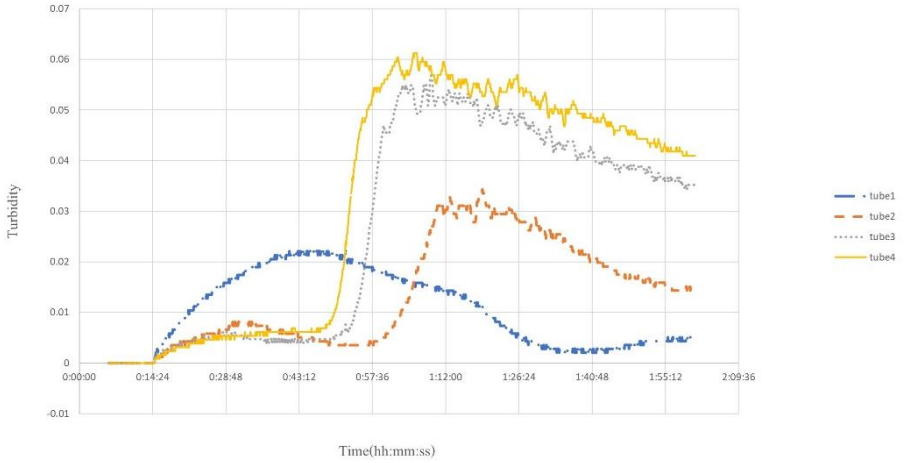


Figure 10 Turbidity measure from the designed device

The concentration of sediment of $Mg_2P_2O_7$ from the second device measure with the 650 nm light wavelength of Nanodrop Thermo Scientific was shown in Figure 11. We pipet the solution inside the Eppendorf tube at 10 minutes after start measure turbidity and pipet every 5 minutes after that. From Figure 11, we found that the concentration of $Mg_2P_2O_7$ was increased according to the concentration of *Salmonella Typhi* in each tube. After 45 minutes, The $Mg_2P_2O_7$ concentration in every tube was stable according to the LAMP reaction [1], [2] because of the limited amount of the initial substance.

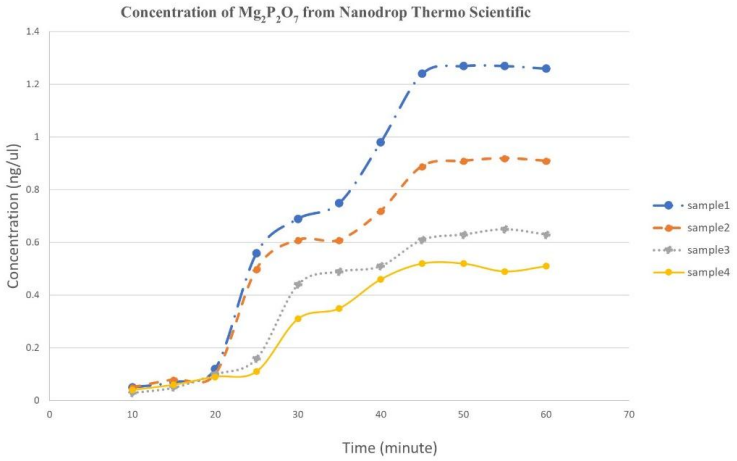


Figure 11 Concentration of $Mg_2P_2O_7$ analysis with Nanodrop Thermo Scientific

5. Conclusion

We design the turbidimeter capable to measure four samples every 5 seconds to determine the presence of $Mg_2P_2O_7$ of the *Salmonella Typhi* DNA on the LAMP Reaction. The results demonstrated that the designed turbidimeter for $Mg_2P_2O_7$ in LAMP product of *Salmonella Typhi* detection can make the isothermal condition and can also detect the presence of $Mg_2P_2O_7$ in LAMP product of Salmonella Typhi DNA as shown in this paper. The initial detection time was depending on the concentration of initial Salmonella Typhi concentration. For future work, we plan to find the equation to predict the concentration of *Salmonella Typhi* according to Mori (Mori et al., 2004) by pay attention in the first 10 minutes and make more measurand channels for the test with the more variety concentration of DNA. The testing may not only the *Salmonella Typhi* DNA from commercial but also include the sample which contaminates of *Salmonella Typhi*.

6. Acknowledgments

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