Smart box for *E. coli* detection using ABS plastic sheet under enzymatic reaction

BY

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A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF ENGINEERING IN BIOMEDICAL
KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG
ACADEMIC YEAR 2022

SCHOOL OF ENGINEERING

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

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ABSTRACT

In that project, we aimed to produce a device for detecting *Escherichia coli* (*E. coli*) in contaminated food or beverages. The *E. coli* detection device prototype was created using the Autodesk Inventor Professional 2022 software. The structure of this device consisted of 4 parts. The first part is a sample tray, which had two holes used to be put with MUG-coated ABS plastic sheets. One hole was dropped with distilled water and used as a control, and the other hole was dropped with a sample solution to check *E. coli* existence. The second part is the main body of the device, which helped obstructed the light from outside of the device to get into the device and also provided space for the UV light and electrical circuit to be set up. The third part is the back lid, which was used to install the switch of UV light and also obstructed the light from outside of the device to get into the device. The fourth part is the top lid, which also obstructed the light from outside of the device to get into the device and had a little gap for the observers to look into the device and observe the reaction under UV light. The ABS plastic sheets used in this project were formed by bringing the ABS plastic filament to dissolve with acetone and poured into petri dishes and let them dry and form plastic sheets. Two methods used to apply MUG to the fabricated plastic were investigated. The first one involved combining MUG into the ABS solution before being poured into petri dishes and left to be dry. The second one involved coating MUG onto the surface of the dried and formed ABS plastic sheets. In comparison, the coating

MUG onto ABS plastic sheet surface method was more effective than the combing MUG into ABS plastic sheet texture method. Because combining method had difficulties in being observed the blue fluorescence light due to the whiteness and turbidness of the plastic texture occurred during combining MUG into ABS plastic sheet texture process and the plastic texture did not absorb the liquid well, so it resulted in the sample solutions not being able to encounter MUG in the plastic texture. But the coating method, whiteness and turbidness of the plastic texture did not affect the observation of the blue fluorescence light that much because the reaction causing the blue fluorescence light emission occurred in the dropped sample solution and since there would be MUG leaking into the sample solution, the sample solution would encounter the MUG well. This paper also provides additional experiments conducted during the project, including thermal stability tests, applying heat during plastic sheet fabrication, and adjusting the concentration of the components used to make ABS plastic solution.

ACKNOWLEDGEMENTS

I would like to thank you, my advisors, Asst. Prof. Dr. Treesukon Treebupachatsakul and Assoc. Prof. Dr. Suejit Pechprasarn for always giving very good advices and supporting me well. Both of them always have a patience to educate me, correct me, give me chances every time I made mistakes and also provide me with a very good environment for working. Both of them are truely feasible in terms of academic knowledge and have very good ethics. I am so happy, so pround and so grateful to have both of them as my advisors.

Thank you, Asst. Prof. Dr. Kasama Srirussamee for lending me solutions and materials needed for my project and giving many very good advices.

Thank you, Assoc. Prof. Dr. Matthew Paul Gleeson for offering me to lend me solutions and materials from your laboratory and giving me advices to improve my work.

Thank you, Assoc. Prof. Dr. Chuchart Pintavirooj for helping me detect the broken part in my circuit constructed for my project and also lending me your tools and equipments

The completion of this thesis would not have been possible without their assistance and participation in every stage of the process.

Tanapath Sotaro Sagolgiet

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LIST OF SYMBOLS/ABBREVIATIONS

Symbols/Abbreviations	Terms
BME	Biomedical Engineering
SIIE	School of International Interdisciplinary Engineering Programs
SAN	Styrene-Acrylonitrile
ABS	Acrylonitrile Butadiene Styrene
ABS-PC	ABS-Polycarbonate alloys
GUD	β-D-Glucuronidase
MUG	4-Methylumbelliferyl-β-D-glucuronide
°C	Degree Celsius
wt%	Weight percentage
g/cm^3	Gram per cubic centimeter
ppm	Parts per million
UV	Ultraviolet
rt-PCR	Real-time Polymerase Chain Reaction
E. coli	*Escherichia coli*
µM	Micromolar
ml	Milliliter
g	Gram
w/v	Weight per volume
w/w	Weight per weight
PVC	Polyvinyl chloride
CAD	Computer-Aided Design
V	Voltage
W/m	Watts per meter
A	Amperes
DC	Direct Current
AC	Alternating Current

CHAPTER 1
INTRODUCTION

This chapter begins with the background and significance of the research, which introduces alternative technique for *Escherichia coli (E. coli)* detection which is the fluorogenic test for detecting β-D-glucuronidase (GUD) activity. We created a device that can detect the presence of the *E. coli*. In this device, there is a tray that is consisted of 2 holes, in the both holes, a piece of ABS plastic sheet that had been coated with MUG would be installed. For the first hole, it would be dropped with distilled water and used as the control sample. For the second hole, it would be dropped with the liquid sample that was suspected to have *E. coli.* If the *E. coli* presents, the GUD that is secreted from the *E. coli* would react with MUG from the ABS plastic sheet and produce 4MU. In this device, the UV LED is installed, once the UV LED is turned on and there is the presence of 4MU, the 4MU would emit blue fluorescence light which would show the presence of *E. coli*. Subsequently, the research objectives are described, followed by the scope of this project.

1.1 Background and significance of the study

Pathogenic bacteria contamination in victuals products is a serious problem; one of the pathogenic bacteria that commonly contaminate the victuals product is *Escherichia coli (E. coli)*. In nature, *E. coli* can be found in humans' large intestines and warm-blood animals. Hygiene of the victuals products can be indicated by *E. coli* contamination. Humans can get *E. coli* infected by consuming raw meat, milk, and vegetables. Once *E. coli* is consumed, it can cause low to high severity disease whether they are diarrhea, respiratory disease, blood stream infection and so on. There have already been many methods for detecting *E. coli* whether they are the multiple tube technique (MTT), the real-time polymerase chain reaction (real-time-PCR) and the like. Those methods are quite effective but most of them are very complicated, needed to perform in the laboratory with specific tools and very time consuming. Therefore, the significance of my study is to invent a device that can detect the presence of *E. coli* more convinient. The alternative technique that I chose to apply to my project is the fluorogenic test for detecting β-D-glucuronidase (GUD) activity. The catalytic reaction between GUD secreted by strains of *E. coli* and 4-methylumbelliferyl-β-D-glucuronide (MUG) produces 4-

methylumbelliferone (4MU) which emit blue fluorescence. *E. coli* can be easily detected by observing the blue fluorescence under the ultraviolet (UV) light and the amount of *E. coli* can be defined by the blue fluorescence intensity.

1.2 Objectives

1.2.1 To fabricate the rapid *E. coli* detection sheet under the enzymatic reaction of 4-methylumbelliferyl-β-D-glucuronide (MUG) and β-D-glucuronidase (GUD)

1.2.1.1 Combing MUG into ABS plastic sheet texture

1.2.1.2 Coating MUG onto ABS plastic sheet surface

1.2.2 To design and produce a prototype of *E. coli* detection device

1.3 Scope of the study

1.3.1 The catalytic reaction between β-D-glucuronidase (GUD) secreted by strains of *E. coli* and 4-methylumbelliferyl-β-D-glucuronide (MUG) produces 4-methylumbelliferone (4MU).

1.3.2 Acrylonitrile-butadiene-styrene (ABS) is chosen to be be polymer material.

1.3.3 Autodesk Inventor Professional 2022 is used to design the prototype of the device

CHAPTER 2
REVIEW OF THEORY RELATED

This chapter discusses about the main theory related to this project. First one is the theory about Acrylonitrile-Butadiene-Styrene (ABS) which will be used as the main material for plastic sheet fabrication. Next is gelatin and glycerol which will be the additional components added to give some desired properties. And the last one is the catalytic reaction between GUD and MUG to produce 4MU.

2.1. Acrylonitrile-Butadiene-Styrene (ABS)

As shown in Figure 1 [1], ABS is usually made by systematically polymerizing molecules such as acrylonitrile, butadiene, and styrene. As shown in Figure 2a and 2b [1], the ABS terpolymer has two phases: a continuous phase of styrene–acrylonitrile (SAN) and a scattered phase of polybutadiene. ABS that can be bought in stores has qualities that range from medium to high impact, low to high surface gloss, and high heat warping [2].

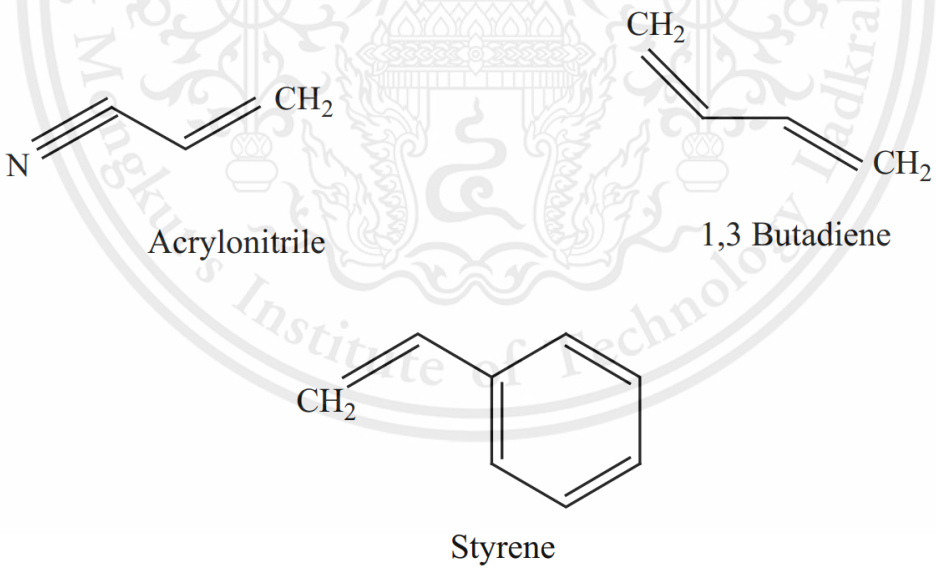


Figure 1. Monomer units of ABS. [1]

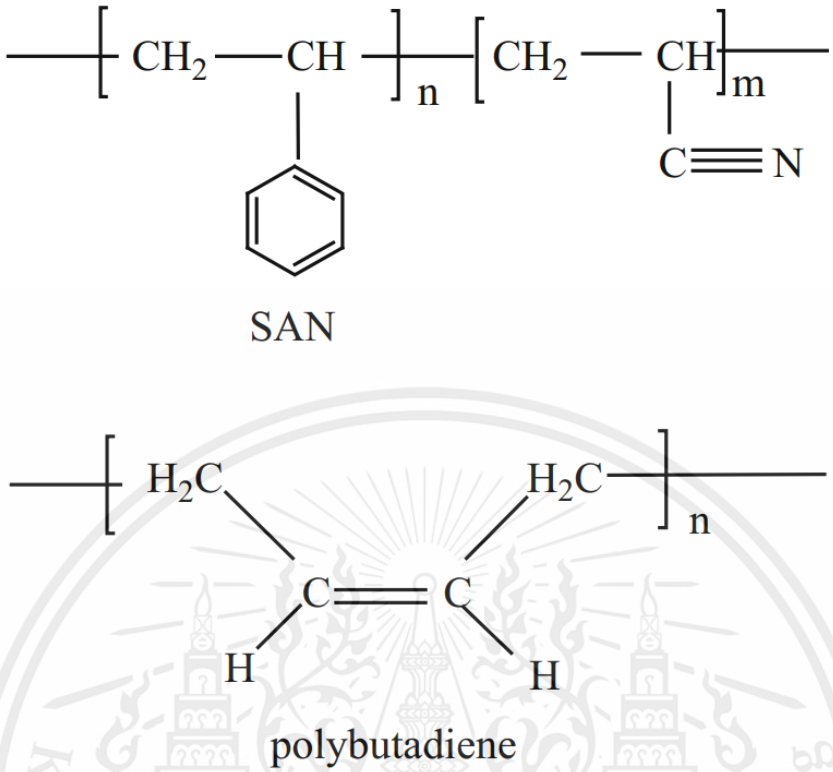


Figure 2. a). SAN phase of ABS. b). Butadiene rubber phase of ABS. [1]

ABS plastics have a high level of toughness (even when they are cold), enough stiffness, good temperature stability, and a high level of resistance to chemical attack and stress cracking from the environment. Other important things about ABS are that it is cheap, durable, and has a low rate of heat expansion. Because it is easy to shape, ABS parts can be made that stay the same size and have a better finish. No other plastics material has such a great mix of qualities that are important to technology. ABS traits are mainly determined by molecular and physical factors. The molecular mass and chemistry of the matrix, the type of rubber, the volume ratio of the rubber to the continuous phase, the size of the rubber particles, the structure of the bonded rubber, and the amount of additives all play important roles. In fact, you can control the size, spread, and texture of the rubber particles to get ABS with the best impact strength. By making the un-grafted SAN phase have more butadiene rubber and bigger molecules, it becomes tougher. Depending on the grade and the surface of the mold or sanding roll, surface gloss values of up to 95% are possible. ABS can be used to make high performance ABS-polycarbonate alloys (ABS-PC) that have a good mix of toughness and heating qualities. So, it is possible to make a wide variety of goods that meet the needs of the users.

The chemical qualities of product ABS are affected by the ongoing styrene–acrylonitrile (SAN) phase. It doesn't usually react to acids, alkalis, or salt solutions in water. But ABS doesn't like water, and the angle of touch between water and ABS is 81.0 ± 0.6. Due to the presence of leftover lubricant and the polarity of the nitrile side groups, the polymer can absorb up to 1.5% of water when stored in watery media. The paraffinic oils don't mix with the ABS plastics. But depending on the type and amount of the rubber part, some weight gain may be seen. They can also stand up to both animal and plant fats and a variety of makeup creams. The SAN phase of ABS can be dissolved in halogenated hydrocarbons, aromatics, esters, and ketones. Oxidizing agents, like chemical acids, break up the links and cause the polymer to break down. Environmental stress breaking in ABS is low, and it can be dropped even more by raising the amount of acrylonitrile in the SAN phase and its molecular weight. Because there are double bonds in the butadiene rubber phase, the surface yellows and turns gray when exposed to heat, light, and aging. The hardness of the rubber also decreases. Oxidation also makes materials weaker. Rubber phase falls apart when working temperatures are higher than 280 °C because it loses its toughness. At temperatures above 300 C, a polymer chain breaks down. When harsh processing methods are used, the rubber and matrix stages can also be destroyed [3–10]. Table 1 [1] shows the general features of ABS.

Physical properties	Metric	English	Comments
Density	1.04 g/cc	0.0376 lb/in^3	Grade count = 3
Melt flow	18–23 g/10 min	18–23 g/10 min	Average = 21.3 g/10 min; grade count = 3
Mechanical properties			
Hardness, rockwell *R*	103–112	103–112	Average = 110; grade count = 3
Tensile strength, yield	42.5–44.8 MPa	6160–6500 psi	Average = 44 MPa; grade count = 3
Elongation at break	23–25 %	23–25 %	Average = 24.3 %; grade count = 3
Flexural modulus	2.25–2.28 GPa	326–331 ksi	Average = 2.3 GPa; grade count = 3
Flexural yield strength	60.6–73.1 MPa	8790–10,600 psi	Average = 68.9 MPa; grade count = 3
Izod impact, notched	2.46–2.94 J/cm	4.61–5.51 ft-lb/in	Average = 2.8 J/cm; grade count = 3
Electrical properties			
Arc resistance	120 s	120 s	Grade count = 1
Comparative tracking index	600 V	600 V	Grade count = 1
Hot wire ignition, HWI	15 s	15 s	Grade count = 1
High amp arc ignition, HAI	120 arcs	120 arcs	Grade count = 1
High voltage arc-tracking rate, HVTR	25 mm/min	0.984 in/min	Grade count = 1
Thermal properties			
Maximum service temperature, air	88–89 °C	190–192 °F	Average = 88.7 °C; grade count = 3
Deflection temperature at 1.8 MPa (264 psi)	88–89 °C	190–192 °F	Average = 88.7 °C; grade count = 3
Vicat softening point	100 °C	212 °F	Grade count = 1
Flammability, UL94	HB	HB	

Table 1. General properties of ABS. [1]

2.2. Gelatin

Glycine, proline, and hydroxyproline make up around 57% of the 18 different complex amino acids that make up gelatin chemically, while other notable amino acid families including glutamic acid, alanine, arginine, and aspartic acid make up the remaining 43% [11]. Gelatin includes a combination of single and double unfolded chains with hydrophilic nature, and it contains 25.2% oxygen, 6.8% hydrogen, 50.5% carbon, and 17% nitrogen [12]. A-chains (one polymer/single chain), b-chains (two a-chains covalently crosslinked), and c-chains (three covalently crosslinked a-chains) make up the chemical structure of gelatin and have molar masses of approximately 90 × 103, 180 × 103, and 300 × 103 g/mol, respectively [13]. The structure will disintegrate into colloids during processing, however at temperatures below 35 to 40 °C, the state will change to gelatinous [14]. Long-term boiling of the gelatin aqueous solution, however, will modify its characteristics owing to breakdown and prevent it from reforming after chilling. Additionally, the viscosity and gel strength of gelatin fluctuate with the relative molecular mass distribution, while pH, temperature, and electrolyte condition may all have an impact. The manufacturer has established a crucial standard for evaluating the gel strength of gelatin [15]. Depending on the grade and concentration of the gelatin, the gel melting point is the top limit, and the lower limit, which is ice crystallized, has happened at the freezing point [16]. Gelatin gel will be present at a low temperature range. Gelatin should be maintained below 4 °C in order to enhance its viscosity, which rises with concentration. Mammalian gelatin melts into a solution reversibly at 37 °C because the triple helix shape changes back to the coiled state. Hydrogen linked connection zones often hold the physical gelatin network together [17]. However, owing to thermal reversibility, physical gelatin gels are unstable at physiological temperature and above [18]. It will restrict utilization in applications like tissue engineering and medication administration where gels must be stable for a certain amount of time before disintegrating [19]. Therefore, to address this issue and stabilize the gelatin gels, chemical or enzymatic crosslinking is preferred [20]. A recognizable sequence in gelatin is the amino acid. The largest source of amino acid synthesis is collagen hydrolysis from animal tissues such bone, tendon, and skin [21]. Collagen is composed of three polypeptide chains. A triple helix that is interwoven and stable in structure forms the inter-chain hydrogen bond. In order to generate sufficient swelling and collagen solubilization for gelatin extraction, an appropriate chemical pre-treatment will break noncovalent linkages. The triple-helix shape often breaks down, the chains unwind, and the molecules separate into

smaller pieces as a result of the breakdown of hydrogen and hydrophobic connections [22]. Therefore, by cleaving the hydrogen and covalent bonds that preserve the triple-helix shape and turn soluble gelatin into a coiled form, it is conceivable to convert collagen to soluble gelatin [23]. Affinity, high dispersibility, low viscosity characteristics, dispersion stability, and water retention are only a few of the excellent physical qualities that gelatin has overall. Gelatin is a significant food ingredient because of its coating, hardness, and reversibility properties, according to Ramos et al. [24]. Gelatin may be used extensively as an emulsifier, dispersion, and clarifier since it is both a thickening and foaming agent [25].

2.3. Glycerol

Pure glycerol is colorless and odorless and tastes pleasant. Viscous at room temperature. Glycerol boils at 290 °C at 101.3 kPa. Table 2 [26] shows reduced-pressure values. Glycerol produces azeotropes with biphenyl and nonazeotropes with water. Figure 3 [27,28] shows glycerol-water solution vapor pressure curves at different concentrations. Experimentally determined liquid–vapor equilibria of glycerol–water solutions are crucial for distillation and fractionation [29–31]. Figure 4 [26] shows 13.3 and 1.33 kPa boiling and condensing curves. Calculation techniques are also theoretical [32].

Temperature, °C	Pressure, kPa
290	101.3
266	53.3
222	13.3
204	6.67
175	2.00
152	0.67
130	0.18
100	0.03
20	<0.0001

Table 2. Vapor pressure vs. temperature of glycerol. [26]

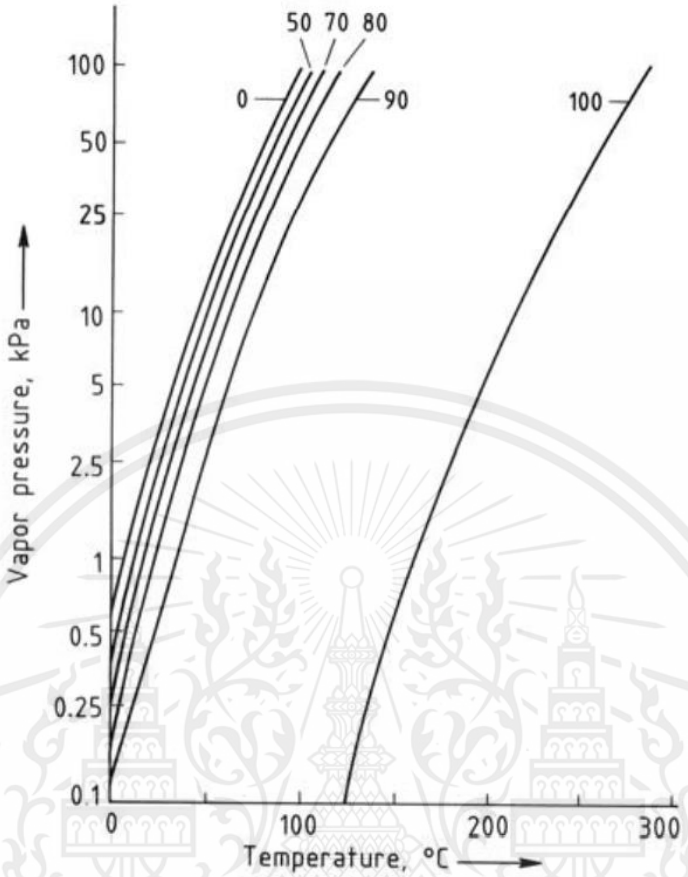


Figure 3. Vapor pressure of glycerol – water solutions (wt % glycerol). [27,28]

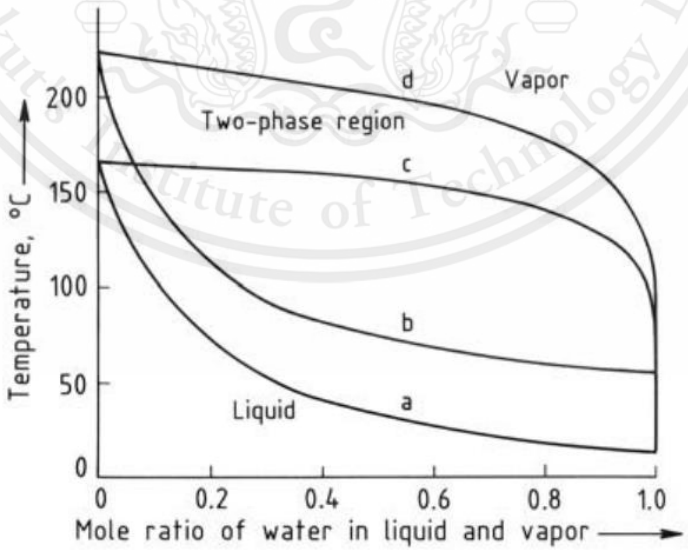


Figure 4. Liquid–vapor equilibria for glycerol–water solutions a) Boiling curve, 1.33 kPa; b) Boiling curve, 13.3 kPa; c) Condensing curve, 1.33 kPa; d) Condensing curve, 13.3 kPa. [26]

Pure glycerol freezes about 18°C. Due to supercooling, crystalline states are rare. 70–110 °C produces a solid, glassy condition. Glycerol crystals seed crystallization at 0 °C. Glycerol–water solutions freeze at Figure 5 [26]. An eutectic mixture forms at 66.7 wt% glycerol, fp 46.5 °C.

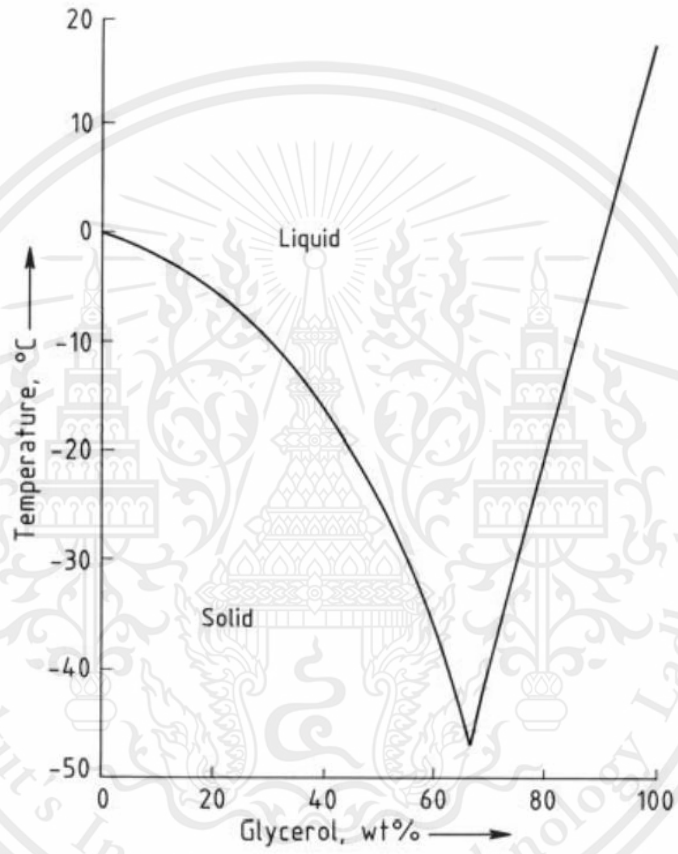


Figure 5. Freezing point of glycerol–water solutions. [26]

Glycerol is 1.261 g/cm^3 (20 °C). [27] provides glycerol-water density tables. From 0 to 200 °C, a mean density change of 0.0007 g/cm^3 per unit temperature change may be utilized for preliminary estimates. Glycerol compresses half as much as water tabulates the refractive index of aqueous glycerol from 0 to 100% in 1% increments. Glycerol-water solutions (0–100 wt %) from 40 to 100 °C have been measured for viscosity [27]. Figure 6 [26] shows curves. Aqueous glycerol solutions at any concentration give moisture until equilibrium with atmospheric water vapor [27]. Figure 7 [26] shows glycerol solution relative humidity.

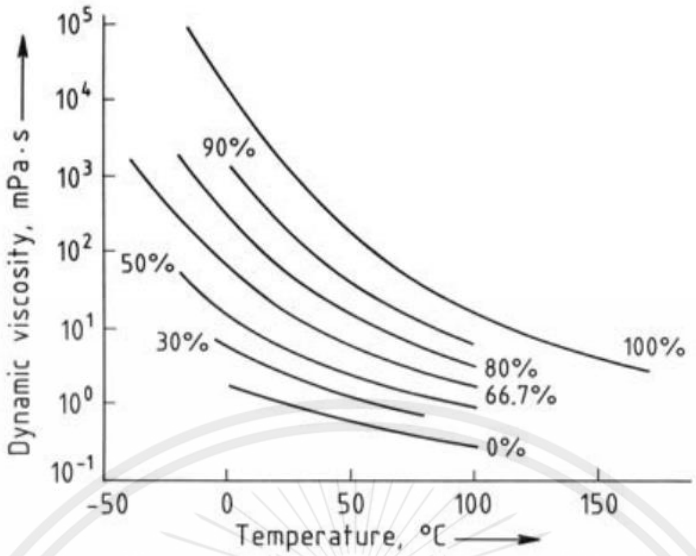


Figure 6. Dynamic viscosity of glycerol – water solutions. [26]

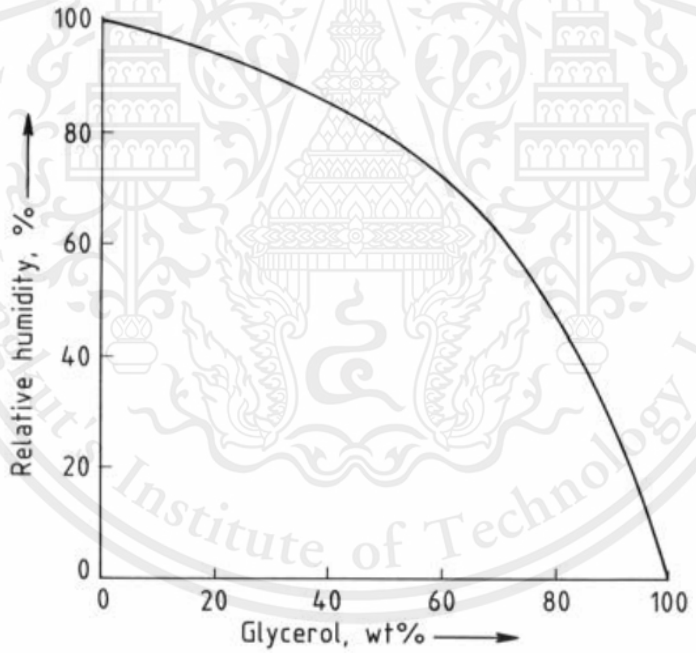


Figure 7. Relative humidity over aqueous glycerol (20 – 100 C). [26]

Due to its three hydroxyl groups, glycerol is a solvent like water and simple aliphatic alcohols. It mixes well with water, methanol, ethanol, and the isomers of propanol, butanol, and pentanol. It mixes well with phenol, glycol, propanediols, amines, and heterocyclic compounds having nitrogen atoms in the rings (pyridine, quinoline). Acetone, diethyl ether, and dioxane reduce its solubility. Hydrocarbons, long-chain aliphatic alcohols, fatty oils, and

halogenated solvents like chloroform hardly dissolve glycerol. Ternary systems like glycerol–water–phenol and glycerol–ethanol–benzene have large temperature-dependent miscibility gaps. Glycerol creates azeotropes with other chemicals [33]. Glycerol dissolves numerous organic and inorganic substances (see Table 2 [26]), making it helpful for pharmaceutical manufacture. Glycerol, like other liquids, has temperature- and pressure-dependent gas solubility [34]. Table 3 [26] shows glycerol's physical characteristics [28].

M_r	92.09
mp	18.0 °C
bp (101.3 kPa)	290.0 °C
Density (20 °C)	1.261 g/cm^3
Refractive index n_D^{20}	1.4740
Dynamic viscosity (20 °C)	1.410 Pa · s
Compressibility (28.5 °C)	2.1×10^{-4} MPa^{-1}
Gravity coefficient of thermal expansion (15 – 20 °C)	0.000615 K^{-1}
Surface tension (20 °C)	63.4 mN/m
Heat of formation	−669 kJ/mol
Heat of combustion	−1662 kJ/mol
Heat of vaporization (55 °C)	88.2 kJ/mol
(195 °C)	76.1 kJ/mol
Heat of fusion (18 °C)	18.3 kJ/mol
Heat of solution (infinite dilution)	− 5.8 kJ/mol
Heat capacity (298 K < T < 383 K)	$C_p(T) = 987.98 + 4.7074\times T$ in J kg^{-1} K^{-1}
(− 80 °C)	1.91 kJ kg^{-1} K^{-1}
(− 108 °C)	0.91 kJ kg^{-1} K^{-1}
Thermal conductivity (0 °C)	0.29 W m^{-1} K^{-1}
Diffusion constant of water into glycerol (20 °C)	1.33×10^{-11} m^2/s
Specific electrical conductivity (20 °C)	0.1 µS/cm
Relative dielectric constant (25 °C)	42.48
Flash point	177 °C
Fire point	204 °C
Autoignition temperature on glass	429 °C
Calorific value	18 kJ/g
Solubility of KCl (99.5 % glycerol) (25 °C)	6.01 g/100 g
(90 °C)	8.78 g/100 g
Solubility of NaCl (99.5 % glycerol) (25 °C)	7.22 g/100 g
(90 °C)	7.31 g/100 g
Solubility of Na_2SO_4 (99.5 % glycerol) (25 °C)	0.20 g/100 g
(90 °C)	0.63 g/100 g
Solubility of N_2 (99.25 % glycerol) (15 °C)	0.553 vol %
Solubility of CO_2 (99.26 % glycerol) (15 °C)	43.8 vol %

Table 3. Physical properties of glycerol. [26]

Glycerol reacts like other alcohols (Alcohols, Aliphatic; Alcohols, Polyhydric). The interior secondary hydroxyl group is less reactive than the terminal primary hydroxyl groups. At 160 °C, modest concentrations of strong mineral acid generate acrolein smell (odor

threshold 0.2–0.4 ppm). Acrolein forms rapidly at 200 °C and above. Acrolein is hardly detectable when heated under neutral or alkaline conditions. Alkaline glycerol dehydrates at 180 °C, creating ether-linked polyglycerols. Glycerol absorbs water quickly and is destroyed by bacteria at room temperature. Glycerol readily oxidizes to aldehyde, carboxyl, or carbonyl groups [35, 36]. Glycerol may be biochemically oxidized [37]. Catalytic hydrolysis [38] or microbiological conversion [39] of glycerol to acrolein and 1,3-propanediol were also studied. Glycerol reaction products include:

1. Mono-, di-, and triesters of inorganic and organic acids;
2. Mono- and diglycerides of fatty acids made by transesterification of triglycerides (from fats);
3. Aliphatic esters and aromatic esters, which are made by reacting with alkylating or arylating agents [40, 41];
4. Polyglycerols, which are made when glycerol and epichlorohydrin react with alkaline hydrolysis or when water is taken out between molecules with an alkaline catalyst;
5. Cyclic 1,2- or 1,3-acetals or ketals that are made when aldehydes or ketones react with them;
6. Mono- or diglycerates are made when alkali or metal alcoholates react with them.
7. Glycerol polyoxyalkylenes are made when glycerol is alkoxylated with ethylene oxide or propylene oxide in an alkaline environment.

2.4. The catalytic reaction between β-D-glucuronidase (GUD) secreted by strains of *E. coli* and 4-methylumbelliferyl-β-D-glucuronide (MUG) produces 4-methylumbelliferone (4MU).

The development of effective techniques for the detection of GUD has recently received a lot of attention. Numerous techniques, including chemosensors and colorimetric techniques, have been developed [42-47]. In terms of high sensitivity and selectivity, a fluorescent probe-based approach, particularly an organic fluorescent probe, stands out among the others. However, difficult synthesis, inadequate photobleaching resistance, and excessive cytotoxicity are inevitable drawbacks of organic fluorescent probes [48-52]. In contrast, inorganic nanoprobes have evolved into a potent analytical technique with impressive benefits, including a straightforward production procedure, great biocompatibility, a high capacity for

photobleaching resistance, and adequate dispersion in water [44,45,53,54]. Additionally, the current techniques for detecting GUD based on inorganic probes are still not sensitive or selective enough to monitor GUD, which significantly restricted their usefulness in illness diagnosis. Therefore, it is currently difficult to create techniques for GUD detection that have superior biocompatibility, easy operation, high sensitivity, and selectivity.

Alternately, numerous detection techniques that are based on the enzymatic activities of *E. coli* are frequently employed in the microbiological analysis. One example of this is the fluorogenic test, which is used to detect the activity of -D glucuronidase (GUD). The 4-methylumbelliferyl--D-glucuronide (MUG) substrate is the target of a particular binding by the GUD enzyme, which results in the formation of the fluorogenic radical product 4-methyl umbelliferone (4MU) [55]. Furthermore, 94–96% of the *E. coli* strains produce the GUD enzyme [56, 57]. As a consequence, the GUD enzyme may be used as a fluorescence biomarker to identify the *E. coli* strains [58]. When the MUG compound is added to conventional bacterial media, *E. coli* may be easily recognized by viewing the blue fluorescence in the medium when illuminated by ultraviolet (UV) light [59, 60]. This identification method is facilitated by the presence of the MUG component. The quantity of *E. coli* that is present may be 14 measured, just as with the rt-PCR method, by the intensity of the blue fluorescence [61].

CHAPTER 3
METHODOLOGY

3.1 ABS plastic sheet fabrication

The suitable combination of ABS plastic that is used to make the device for detecting *E. coli* in food and beverage products is needed to be created. The device was designed in two types. First one is combining MUG into the texture of ABS plastic, this plastic is fabricated in the form of a sheet, the desired properties of this type must be flexible, tough and liquid-permeable but it must not dissolve in water or other liquid from the food and beverage product. It must let the GUD enzyme from *E. coli* to react with MUG substrate inside the package material but it must not let MUG substrate to leak out of the device material. The second one is coating MUG onto the surface of ABS plastic, this plastic is fabricated in the form of a sheet, the desired properties of this type must be able to release MUG from the surface into the liquid sample that is suspected that it contains *E. coli* and the texture of the plastic is flexible and tough.

The overall process in my method for fabricating the plastic film and defining the suitable combination was dissolving the ABS plastic in acetone and adding other components to adjust the properties of the plastic texture for it to have proper properties. The experiment was set as shown in Figure 8 to fabricate ABS plastic sheet.

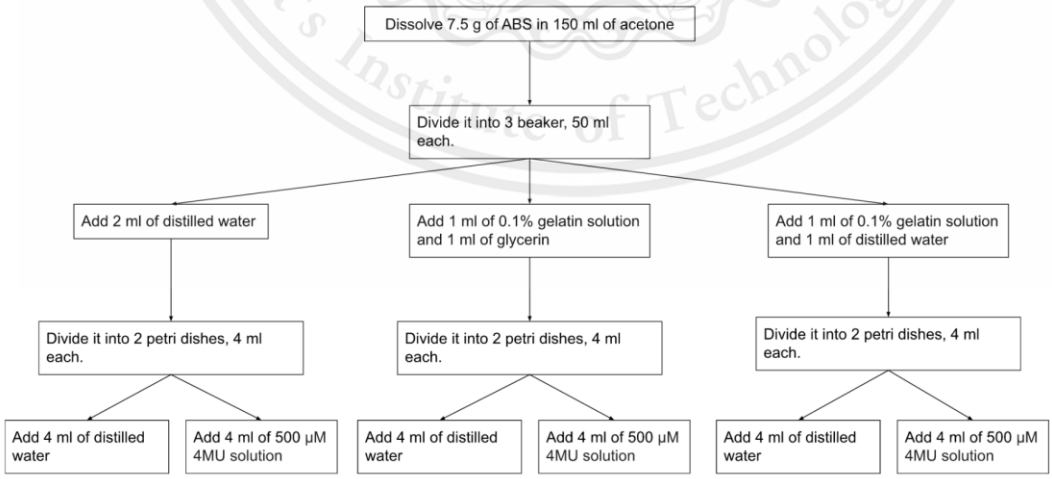


Figure 8. ABS fabrication procedure.

In the experiment in Figure 8, 7.5 g of ABS in 150 ml of acetone was dissolved and divided into 3 beakers, 50 ml each. Then, one beaker that contained 50 ml of ABS solution was added with 2 ml of distilled water and divided into 2 beakers, 4 ml each. Then, 4 ml of distilled water was added into one 4 ml ABS solution beaker and 4 ml of 4MU solution was added into another 4 ml ABS solution beaker and mixed all of them well. Then, they were poured into two petri dishes and glass slides were also dipped into each petri dish and let the mixed ABS solution coat the glass slides and left all of them to dry overnight. After leaving all of them to dry, They were put under the UV light and the result was collected. Then these procedures were repeated but in 50 ml ABS beaker were added with gelatin and glycerin instead and these procedures were repeated another time but in 50 ml ABS beaker were added with gelatin and distilled water instead.

3.2 Thermal Stability

In this experiment, the heat tolerance of 4MU and MUG is tested in order to find the range of the temperature that 4MU and MUG are be able to operate.

4MU was tested in different temperatures to be observed if it could still emit blue fluorescent light. 4MU solution which had concentration of 500 μM was devided into 6 microcentrifuge tubes, 1 ml each. First tube, it was not heated and it was left at room temperature to be set as the controlled sample. Other 4 tubes were heated in a test tube heater with different temperatures. The second tube was heated to 60 °C, third tube was heated to 70 °C, fourth tube was heated to 80 °C, fifth tube was heated to 90 °C and sixth tube was heated to 100 °C, then fluorescence emission was observed under UV light.

Next, the temperature tolerance of MUG was tested to be observed if it could still react with GUD and produced 4MU. The 170 units MUG solution was devided into 18 microcentrifuge tubes, 250 ml each and 500 ml of distilled water was dropped into a microcentrifuge tube to set it as the controlled sample. MUG solution microcentrifuge tubes were heated in a water bath with different temperatures. The first 3 tubes were heated to 25 °C, second 3 tubes were heated to 60 °C, third 3 tubes were heated to 70 °C, fourth 3 tubes were heated to 80 °C, fifth 3 tubes were heated to 90 °C and sixth 3 tubes were heated to 100 °C for 15 minutes each, all of the microcentrifuge tubes were left to cool down to room temperature, then 500 μM GUD was dropped into 3 microcentrifuge tubes in 25 °C set, 250 ml each, by 500 μM GUD being dropped in one tube and waiting 30 seconds then being dropped in another

tube and waiting another 30 seconds then being dropped in the last tube, then waiting for controlling 15 minutes of reaction time before collecting result by observing fluorescence emission under UV light. Then I repeated these steps for microcentrifuge tubes in the 60 °C, 70 °C set, 80 °C set, 90 °C set and 100 °C set.

3.3 Heating during ABS plastic sheet fabrication

The experiment conducted is shown in Figure 9. The purposes of this experiment were to fabricate plastic film using ABS plastic which could be composed of other components whether they were MUG, 4MU and so on by trying to heat during the fabrication process to make the ABS plastic after combining with other components be homogeneous and form a sheet of plastic and to make MUG in the plastic texture can react with GUD solution and produce 4MU. The experiment was started from the left procedure branch in Figure 9, 4MU solution was used because it needed to be observed how 4MU would glow in this transparent ABS plastic. 50 ml ABS solution was poured into a separated beaker and 2 ml of water was added and this 50 ml ABS solution that had been added with 2 ml of water was devided into 2 beakers, 4 ml each. Next, 4 ml of distilled water was put into one beaker and 4MU solution was put into another beaker. hot plates were used to heat 2 ABS solution beakers, a distilled water beaker and a 4MU solution beaker at 70°C. Once all of these 4 beakers were heated, one ABS solution was poured into a distilled water beaker and another ABS solution was poured into a 4MU solution beaker. This procedure was repeated but the temperature was changed from 70°C to 80°C. This procedure was stopped being done because all of the ABS solutions instantly became solid after being added into distilled water or added into 4MU solution.

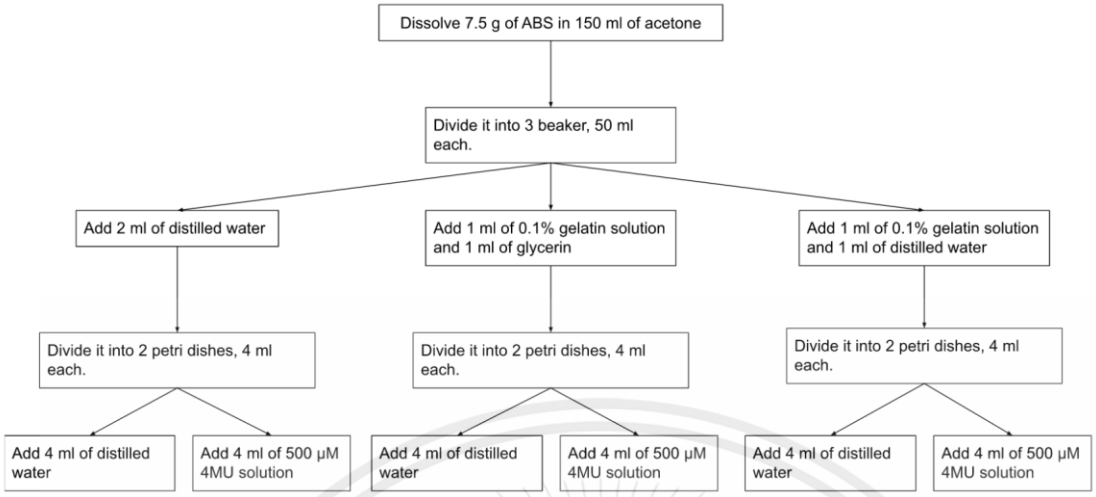


Figure 9. ABS fabrication procedure.

The method of heating was changed from using hot plates to using a bathtub instead. 50 ml ABS solution was poured into a separated beaker and 2 ml of water was added and this 50 ml ABS solution that had been added with 2 ml of water was devided into 2 test tubes, 4 ml each. Next, 4 ml of distilled water was put into one test tube and 4MU solution was put into another test tube. All of these test tubes were boiled in a bathtub at 80℃. Once all of these 4 beakers were heated, One ABS solution was pored into a distilled water test tube and another ABS solution was poured into a 4MU solution test tube. Then, this procedure was repeated 2 times but the temperature was changed to 90℃ for the first time and the temperature was changed to 100℃ for the second time and for both 2 times, heated ABS solution was only combined with heated distilled water and heated ABS solution was not combined with heated 4MU solution because it was needed to be observed if the solution still instantly became solid after the combining and 4MU solution was not wanted to be wasted.

3.4 Adding more acetone in ABS plastic sheet fabrication process

In this experiment, the ABS plastic solution was needed to be diluted in order to be a ble to be combined with other components. A new method was conducted by attempting to dilute the concentration of the ABS solution by adding 10 ml of acetone solution more into each beaker at number 1 in Figure 10 before adding 4 ml of distilled water into one beaker and adding 4 ml of 4MU solution into another beaker. The result shows that the major parts of the solution did not become solid and there were only a tiny part of the solution that became solid. After one ABS solution was completely mixed with distilled water and another ABS solution

with 4MU solution, they were separately poured into two petri dishes and glass slides were dipped into each petri dish and the mixed ABS solution was let to coat the glass slides and all of them were left to dry overnight. After leaving all of them to dry, they were put under the UV light.

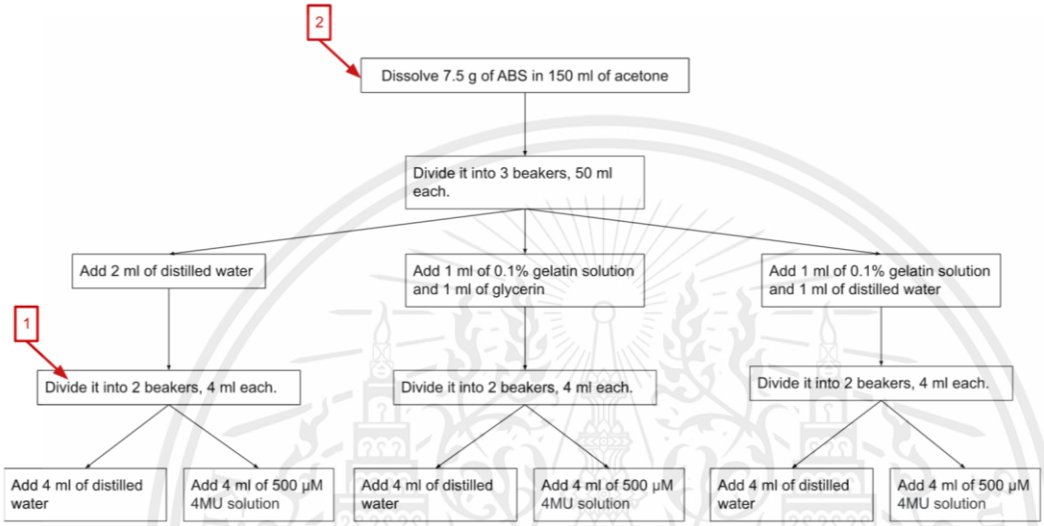


Figure 10. ABS fabrication procedure.

3.5 Increasing ABS plastic concentration in ABS plastic sheet fabrication process

Normally, the ABS concentration used to form a plastic sheet was 5% w/v. In this experiment, the amount of the ABS plastic was increased to be 10% w/v. The procedures of this current experiment are shown in Figure 11.

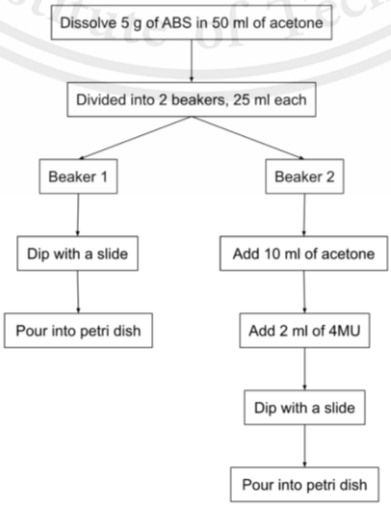


Figure 11. ABS plastic fabrication procedure adjusted ABS concentration.

The 5 g of ABS was dissolved in 50 ml of acetone, then it was divided into 2 beakers. In beaker 1, a glass slide was dipped and the plastic solution was let to coat the surface of a glass slide, then the rest of the solution was poured in a petri dish. In beaker 2, 10 ml acetone was added and 2 ml of 4MU was came behide, then a glass slide was dipped and the plastic solution was let to coat the surface of a glass slide, then the rest of the solution was poured in a petri dish. All of the solutions in the petri dishes and glass slides were left to be dry.

3.6 Coating MUG onto ABS plastic sheet surface

In this experiment, the ABS plastic sheet was coated with a different method and different substance. Normally after the ABS sheet was formed on a petri dish, it would be peeled off and proceed to further process, but for this new coating method, the plastic sheet wasn't peeled off from the petri dish. Two ABS plastic sheets was prepared in two petri dishes, then acetone was poured on to the surface of the ABS plastic sheet in both petri dishes to slightly dissolve their surface and it was left for around 30 seconds then the excess acetone was poured off. Once their surfaces were dissolved and became a glue-like texture, MUG solution was immediately poured onto one petri dish and gelatin combined with MUG was also poured onto the another petri dish. For the petri dish that was poured with MUG solution, it was left for several hours for MUG to bind to the ABS plastic sheet then the excess MUG solution was poured off, then GUD solution was poured into this petri dish and it was left for 15 minutes for the reaction to occur. For the petri dish that was poured with gelatin, it was left for the gelatin to dry and become solid and stick onto the surface of the ABS plastic sheet, then GUD solution was poured into this petri dish and it was left for 1 hour for the GUD solution to be absorbed into gelatin and reacted with MUG.

3.6.1 Effects of acetone on efficiency of MUG

The purpose of this experiment was to find out if acetone affected the efficiency of fluorescence production from the catalytic reaction between MUG and GUD. Therefore, 1 ml of MUG was combined with 1 ml of acetone in a test tube and it was left for 15 minutes then 1 ml of GUD was added and it was left for 15 minutes for the reaction to occur. In another test tube 1 ml of MUG was combined with 1 ml of GUD and it was left for 15 minutes for the reaction to occur and then 1 ml of distilled water

was added to adjust the volume of the solution to be equal to the previous solution which was 3 ml. For the third test tube, 3 ml of distilled water was added to make it as a controlled sample.

3.6.2 Leakage of MUG in to GUD

There were two experiment conducted in this topic. The purpose of the first experiment was to find out how many times MUG could leak into the GUD solution and blue fluorescence occured. The excess GUD solution that had been dropped onto the surface of the ABS plastic that had been coated with MUG was collected. The GUD solution had been dropped and left for 15 minutes for the reaction to occur, I repeated this procedure for 4 times.

The purpose of the second experiment was to ensure that there was actually MUG leaking into the GUD solution, so 4 solutions were prepared for being inspected in the spectrophotometer which were MUG, GUD, 4MU and GUD solution that had been dropped onto ABS plastic sheet coated with MUG.

3.7 Designing and producing the prototype of *E. coli* detection the device

The prototype of the *Escherichia coli* detection device was designed using Autodesk Inventor Professional 2022 program. The structure of this device consists of 4 parts as shown in Figure 3. First part is a sample tray, this part has two holes used to be put with MUG coated ABS plastic sheets, one hole would be dropped with distilled water and used as a control and the another hole would be dropped with solution that was expected to contain *E. coli* or GUD. The second part is the main body of this device. This part helps obstruct the light from outside the device to get into the device and also provides space for the UV light and electrical circuit to be set up. The third part is the back lid. This part is used to install the switch of UV light and also obstruct the light from outside the device to get into the device. The fourth part is the top lid. This part also obstructs the light from outside the device to get into the device and has little gap for the observers to look into the device and observe the reaction under UV light. Next, the electrical circuit used for this device was also designed and constructed. The components of this circuit are rocker switch, 10A fuse, 5V 5A switching power supply and 5V 15W/m UV light emitting 395 - 405 nm UV light.

CHAPTER 4
EXPERIMENTAL RESULT AND DISCUSSION

4.1 ABS plastic sheet fabrication

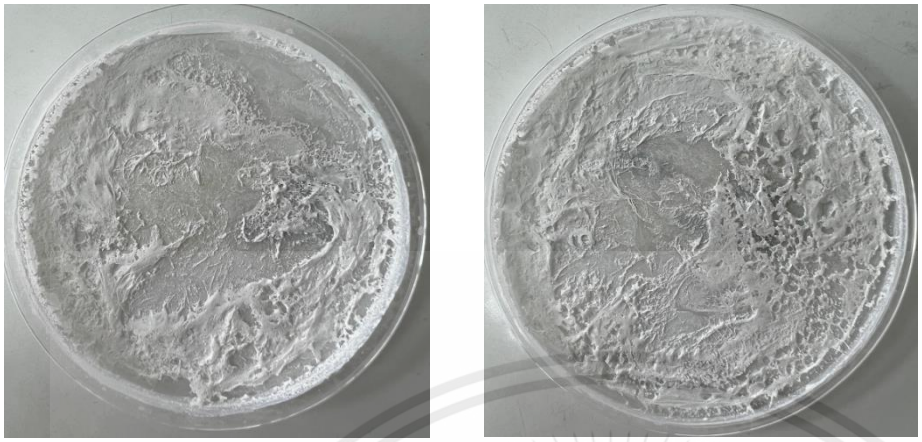
From the results in Figure 12, the texture of the plastic both the control dish and added 4MU dish and when they are put under the UV light, it could not be distinguished the different between the controlled dish and the added 4MU dish. Because the plastic used was white, therefore it reflexed the color of UV light also. The results from dipping method in Figure 12, the texture of the film formed was not flexible and was very fragile.

From the results in Figure 13 , the texture of the plastic both the control dish and added 4MU dish and when they were put under the UV light, it could not be distinguished the different between the controlled dish and the added 4MU dish. Because the plastic used was white, therefore it reflexed the color of UV light also. The results from dipping method in Figure 13, the texture of the film formed was not flexible and was very fragile but it became more film-like than the film formed in Figure 12.

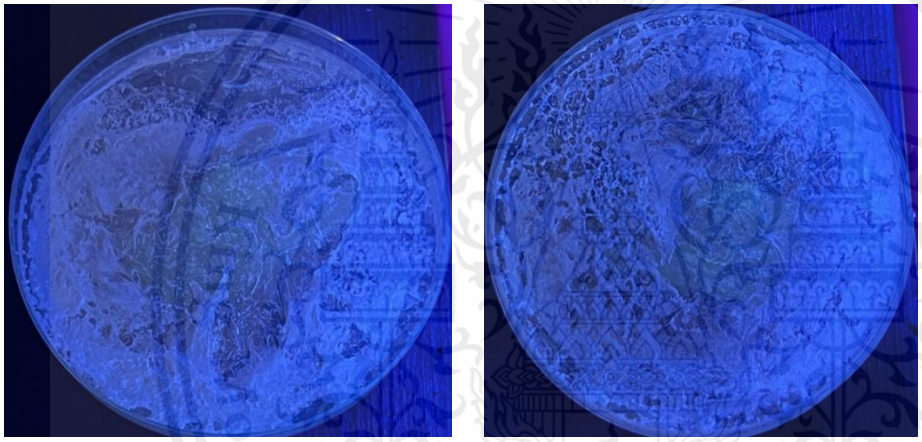
From the results in Figure 14 , the texture of the plastic both the control dish and added 4MU dish and when they are put under the UV light, it could not be distinguished the different between the controlled dish and the added 4MU dish. Because the plastic used was white, therefore it reflexed the color of UV light also. The results from dipping method in Figure 13, the texture of the film formed was not flexible and was very fragile but it became more film-like than the film formed in Figure 12 and Figure 13.

During the process of mixing ABS plastic solution with other components, there were some problems occurred. The ABS instantly became solid after being mixed with other components which made the mixing process be difficult and inefficient.

a). b)



c). d).



e). f).

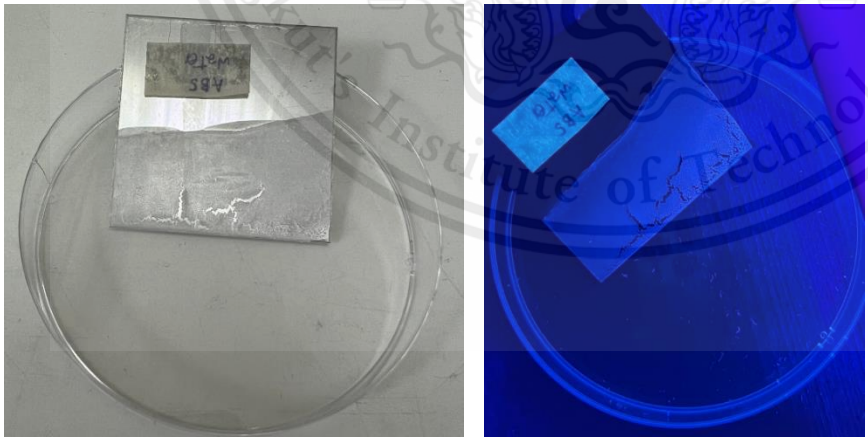
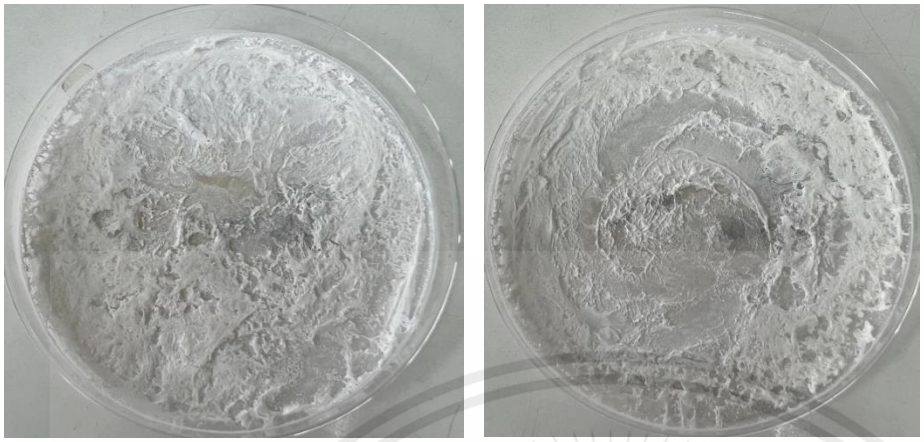


Figure 12. Results in distilled water added branch. a). Controlled dished. b). 4MU added dish. c). Controlled dish under UV light. d). 4MU added dish under UV light. e). Controlled slide. f). Controlled slide under UV light.

a). b).

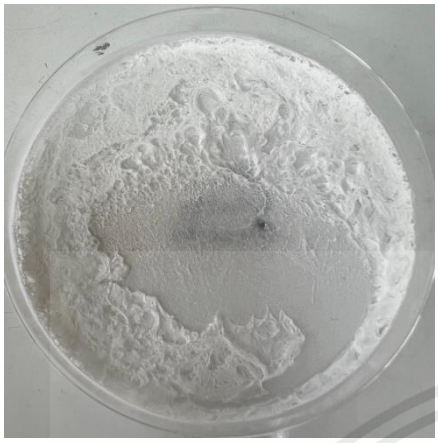


c). d).

e). f).

Figure 13. Results gelatin added branch. a). Controlled dish. b). 4MU added dish c). Controlled dish under UV light. d). 4MU added dish under UV light. e). Controlled slide. f). Controlled slide under UV light.

a).



b).

c).

d).

e).

f).

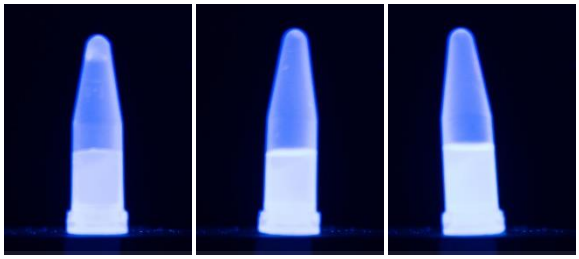
g). h).

Figure 14. Results Gelatin and glycerin added branch. a). Controlled dish. b). 4MU added dish c). Controlled dish under UV light. d). 4MU added dish under UV light. e). Controlled slide. f). 4MU added slide. g). Controlled slide under UV light h). 4MU added slide under UV light.

4.2 Thermal Stability

Due to the previous experiments that there were the attempts to add other components into melted plastic solution but the solution instantly became solid after dropping other components in because the temperature of the solution was too low, the plastic solution was needed to be heated to around the range of 60 °C to 100 °C during adding other components. To do that, it was needed to be ensured that 4MU and MUG could still operate in this temperature range.

a). b). c).



d). e). f).

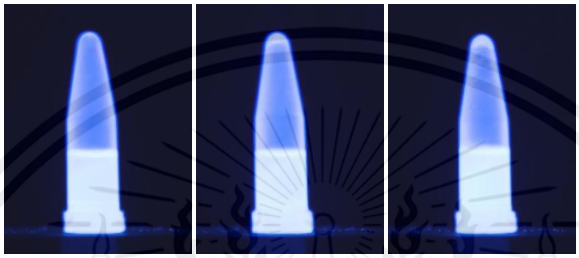
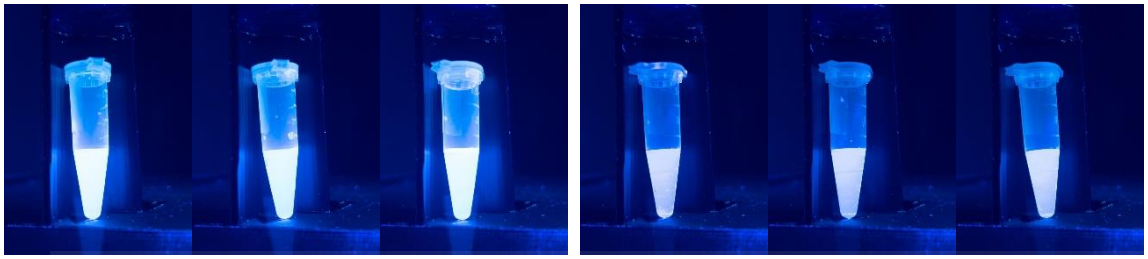


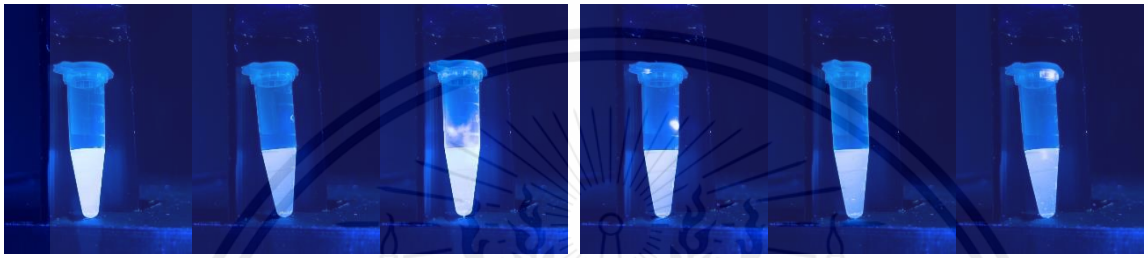
Figure 15. Glowing of 4MU fluorescence at different temperatures from 25 °C to 100 °C. a). 4MU at 25 °C b). 4MU heated at 60 °C c). 4MU heated at 70 °C d). 4MU heated at 80 °C e). 4MU heated at 90 °C f). 4MU heated at 100 °C.

From Figure 15, the fluorescence emission was observed under UV light. 4MU at every temperature from 25 °C to 100 °C could still emit blue fluorescent light, the intensity of 4MU at each temperature was slightly different.

a). b).



c). d).



e). f).

f).

Figure 16. a). Glowing of 4MU fluorescence produced by reaction between unheated MUG and GUD b). Glowing of 4MU fluorescence produced by reaction between heated MUG at 60 °C and GUD c). Glowing of 4MU fluorescence produced by reaction between heated MUG at 70 °C and GUD. d). Glowing of 4MU fluorescence produced by reaction between heated MUG at 80 °C and GUD. e). Glowing of 4MU fluorescence produced by reaction between heated MUG at 90 °C and GUD. f). Glowing of 4MU fluorescence produced by reaction between heated MUG at 100 °C and GUD. g). Distilled water (control sample) .

As the glowing of 4MU fluorescence observed in Figure 16, the brightness of the fluorescence light gets dimmer when the MUG is heated at higher temperature. Therefore, MUG can tolerate heat at a temperature range between 60 °C to 100 °C. However, the thermal stability of MUG reduced with increasing temperature resulted in reducing the binding property with GUD. Constantly, the amount of 4MU fluorescence product decreases as the results shown in Figure 16. For heating the melted plastic solution while adding other components to prevent the solution from instantly becoming solid, I should heat the melted plastic solution at around 60 °C to 70 °C because if I heat the solution at higher temperature, MUG molecules will lose their ability to bind with GUD or the chemical structure is altered, which affects the amount of observable 4MU product.

4.3 Heating during ABS plastic sheet fabrication

The result shows that all of the ABS solutions instantly became solid after being added into distilled water at 80℃, 90℃, and 100℃ and instantly became solid after being added into 4MU solution at 80℃. These results are shown in Figure 17. From the result of this experiment, it shows that heating during combining ABS solution with other components did not prevent the ABS solution from instantly becoming solid.

a). b).

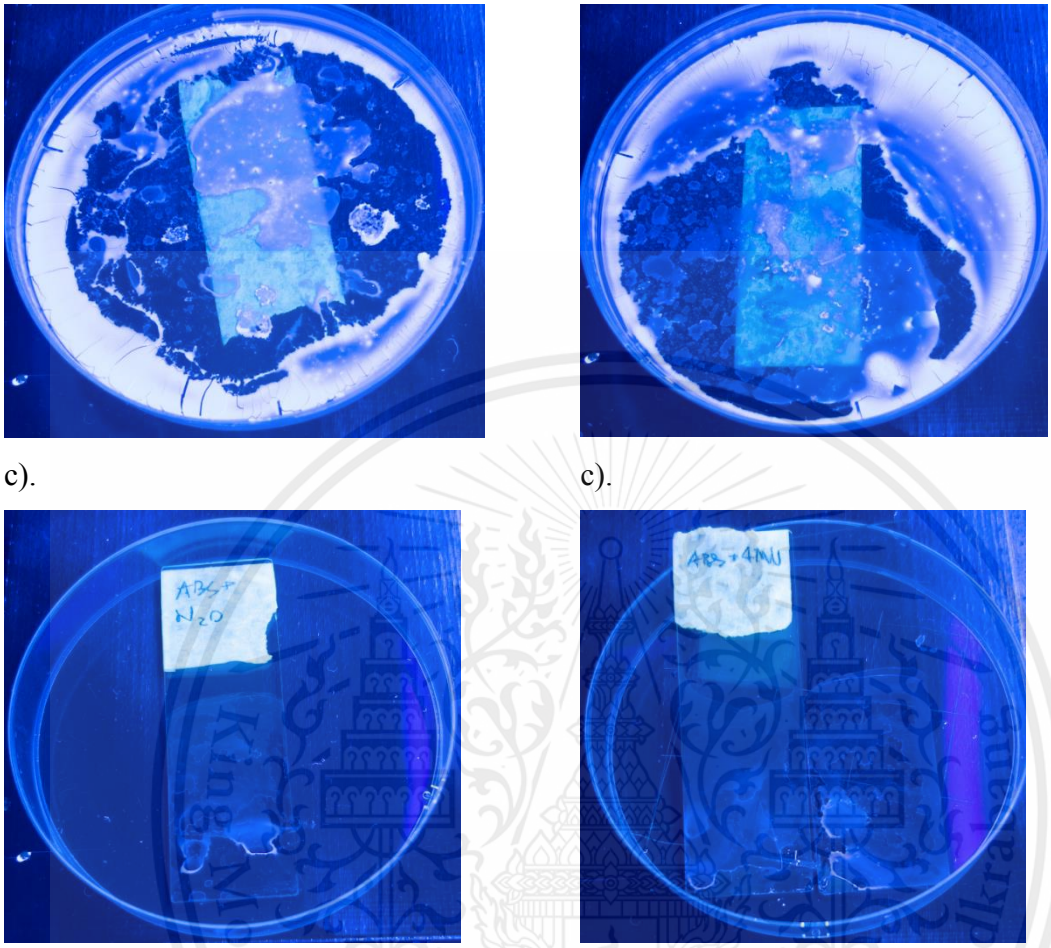
c). d).

Figure 17. a). Combination of ABS and 4MU solution at 80°C. b). Combination of ABS solution and distilled water at 80°C. c). Combination of ABS solution and distilled water at 90°C. d). Combination of ABS solution and distilled water at 100°C.

4.4 Adding more acetone in ABS plastic sheet fabrication process

The results from section 4.3 shows that all of the combinations at every temperature instantly became solid after mixing solutions to each other. Therefore, there was a hypothesis that the cause of the ABS solution instantly becoming solid after being combined with other components was from the excessive concentration of ABS solution.

a). b).



c). c).

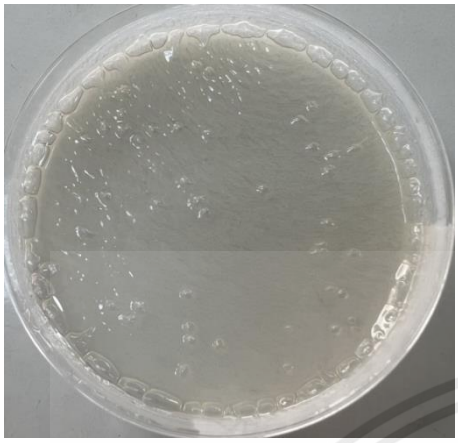
Figure 18. a). Dried ABS solution combined with distilled water in a petri dish under UV light. b). Dried ABS solution combined with 4MU in a petri dish under UV light. c). Dried ABS solution combined with distilled water on a glass slide under UV light. d). Dried ABS solution combined with 4MU in a glass slide under UV light.

From the result in Figure 18, the color of the dried mixed ABS solution was white instead of being transparent and that made it difficult to observe the emission of blue fluorescence light from the 4MU and the difference between the one that was added with 4MU and the one that was not added with 4MU could not be distinguished. The texture of the plastic was not whole, even thin film, it only formed unevenly thin layers around the circumference of the petri dishes in a and b in Figure 18. On the glass slide in c and d in Figure 18, the plastic barely formed the layer and there was only some dried plastic solution being left on it. The cause of the texture of the plastic not being whole, even thin film both in petri dishes and on glass slides was because the ABS solution concentration at number 2 in Figure 10 was too low.

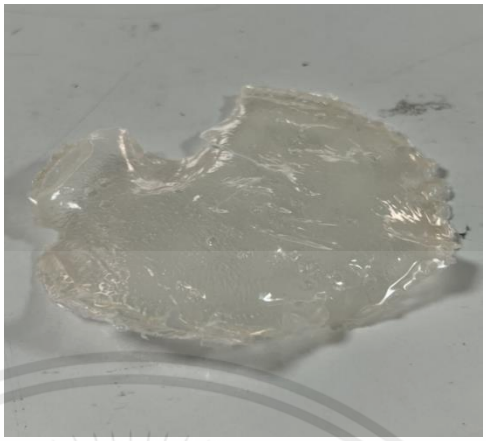
4.5 Increasing ABS plastic concentration in ABS plastic sheet fabrication process

As the results shown in Figure 19, The ABS in a petri dish formed a clear plastic sheet with some bubbles occurring on it. This plastic sheet could be peeled off the petri dish, the texture was quite tough, flexible but could not be stretched. The ABS on the glass slide didn't form well, the ABS was barely left on the glass slide. The ABS combined with 4MU in the petri dish formed a partially turbid and clear plastic sheet with quite rough texture and it could not be peeled off the petri dish and after putting under the UV light, because of the turbidness, 4MU fluorescence could not be observed. The ABS combined with 4MU on a glass slide formed a thin layer of plastic and after putting under the UV light, no fluorescence emission could be observed. From the result, increasing the concentration of ABS plastic improved the plastic formation. And this result shows that the turbidness in dried ABS plastic was caused by additional components that were combined into the ABS solution.

a).

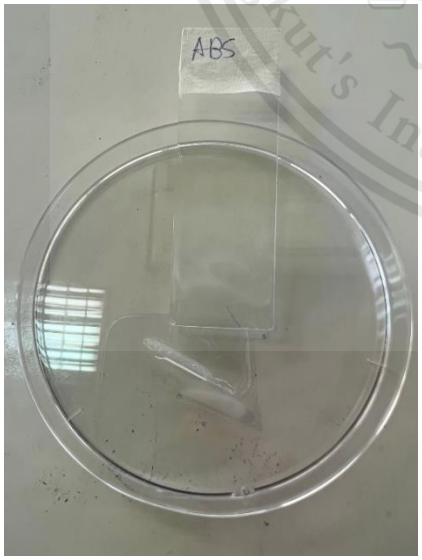


b).



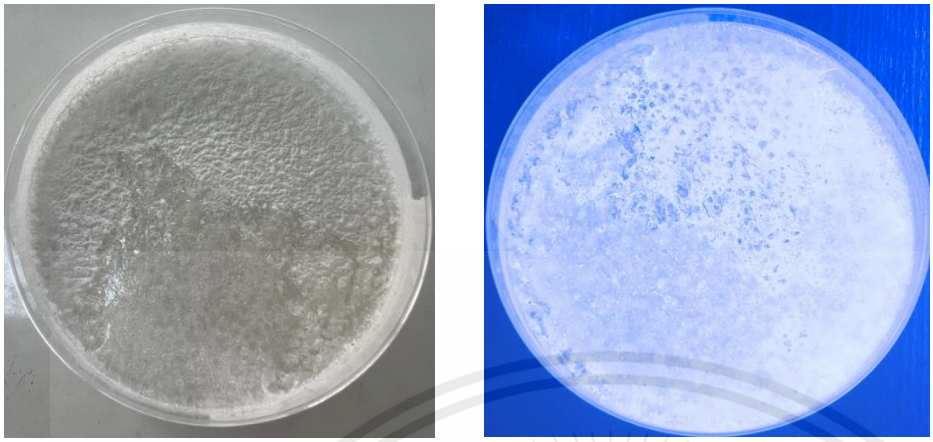
c).

d).



e).

f). g).



h). i).

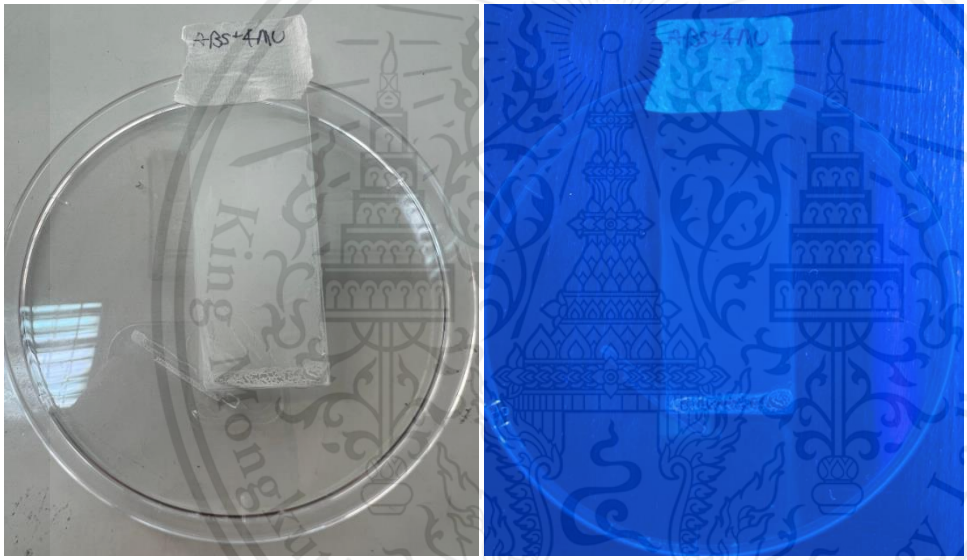


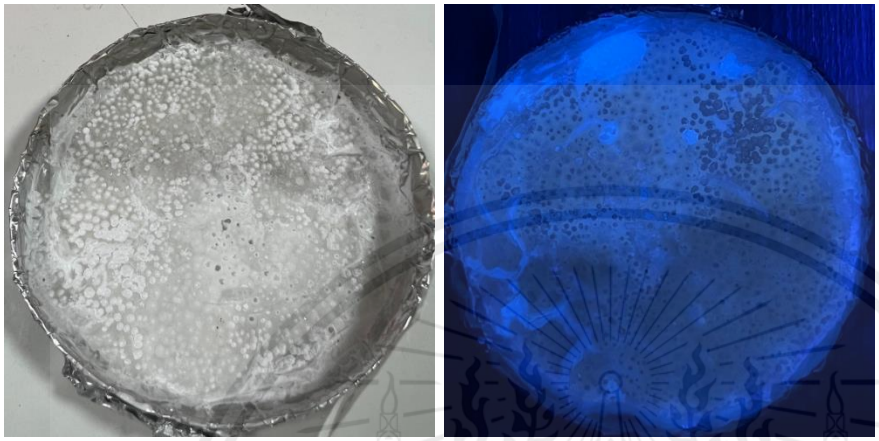
Figure 19. a). Dried ABS in a petri dish. b). Dried ABS in a petri dish under UV light. c). Peeled dried ABS from a petri dish in a. d). Dried ABS on a glass slide. e). Dried ABS on a glass slide under UV light. f). Dried ABS combined with 4MU in a petri dish. g). Dried ABS combined with 4MU in a petri dish under UV light. h). Dried ABS combined with 4MU on a glass slide. i). Dried ABS combined with 4MU on a glass slide under UV light.

4.6 Coating MUG onto ABS plastic sheet surface

From the results, sample a in Figure 20, blue fluorescence could be observed only in the area in which excess GUD solution was left but for other areas which were dry, blue fluorescence could not be observed. It shows that MUG did not properly bind to the ABS plastic surface and also leaked into the GUD solution. For the sample d in Figure 1, it could not be distinguished from the control sample (sample e in Figure 20). It shows that there was no blue

fluorescence occurred, the reasons were the GUD solution could not be absorbed into the gelatin layer or it was needed more time to be absorbed and reacted.

a). b).



c). d).

e).

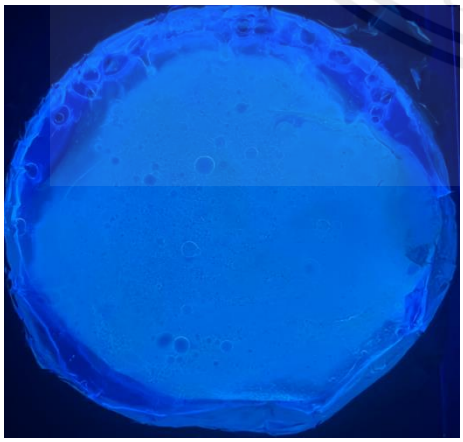


Figure 20. a). ABS plastic coated with MUG under normal room light. b). ABS plastic coated with MUG and poured with GUD solution under UV light. c). ABS plastic coated with

gelatin combined with MUG under normal room light. d). ABS plastic coated with gelatin combined with MUG and poured with GUD solution under UV light. e). ABS plastic coated with gelatin combined with MUG under UV light (control sample).

4.6.1 Effects of acetone on efficiency of MUG

From the results shown in Figure 21, The light intensity of fluorescence from MUG combined with acetone was less than the light intensity of fluorescence from MUG that had not been being combined with acetone. It shows that acetone inhibits MUG in the reaction or GUD in the reaction or both.

a). b).

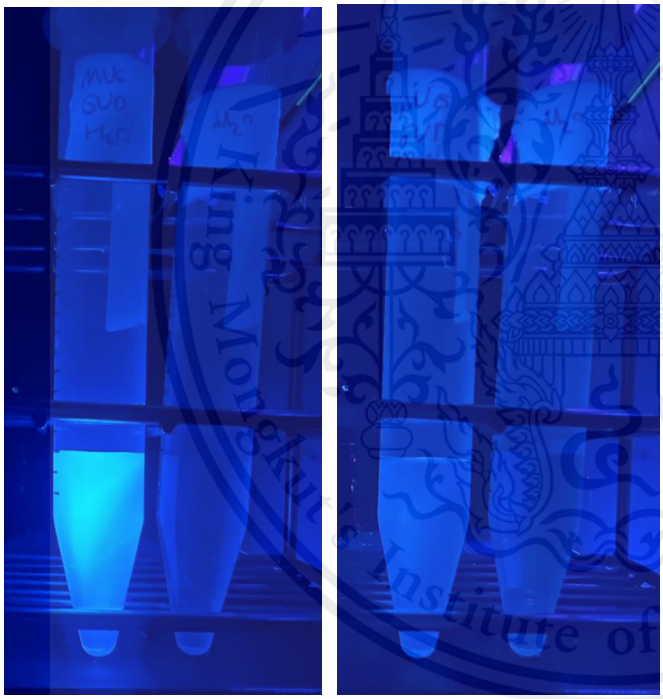


Figure 21. a). MUG combined with distilled water reacting with GUD (left) compared to distilled water (right). b). MUG combined with acetone reacting with GUD (left) compared to distilled water (right).

4.6.2 Leakage of MUG

In this experiment number of times that MUG could leak into solution was needed to be noted. The results from the Figure 22 show that there might be some MUG leak into the GUD solution because blue fluorescence light could be observed from the

solution under UV light. And every time the GUD was dropped onto the ABS plastic sheet, the intensity of blue fluorescence light got dimmer, so it shows that the amount of MUG leaking into the GUD solution was decreased once the times the GUD was dropped onto the ABS plastic sheet was increased.

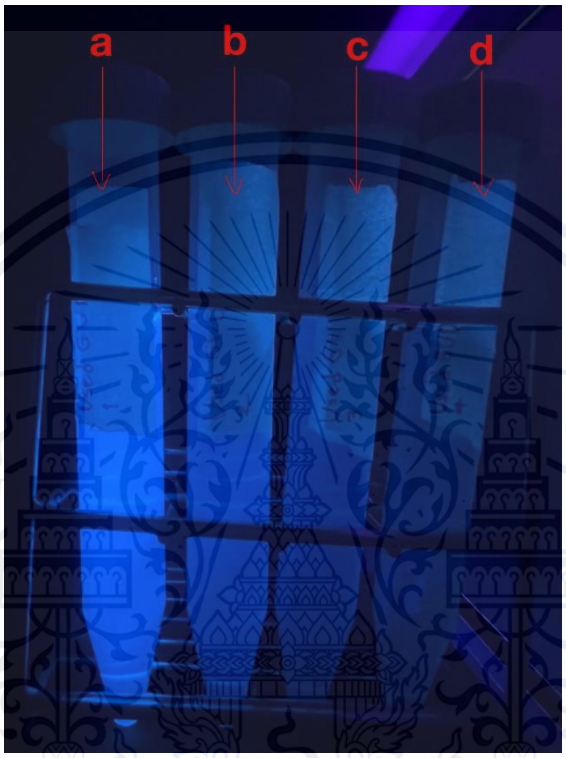


Figure 22. a). Excess GUD collected for the first time b). Excess GUD collected for the second time c). Excess GUD collected for the third time d). Excess GUD collected for the fourth time.

To ensure that there was actually MUG leaking into the GUD solution, spectrophotometer was used to find the presence of each components in the solution. From the results from Figure 23, the MUG leak solution graph have a trend and peak that are the combination of trend and peak of MUG, GUD and 4MU, so it is concluded that there was combination of MUG, GUD and 4MU in this GUD solution that had been dropped onto ABS plastic sheet coated with MUG.

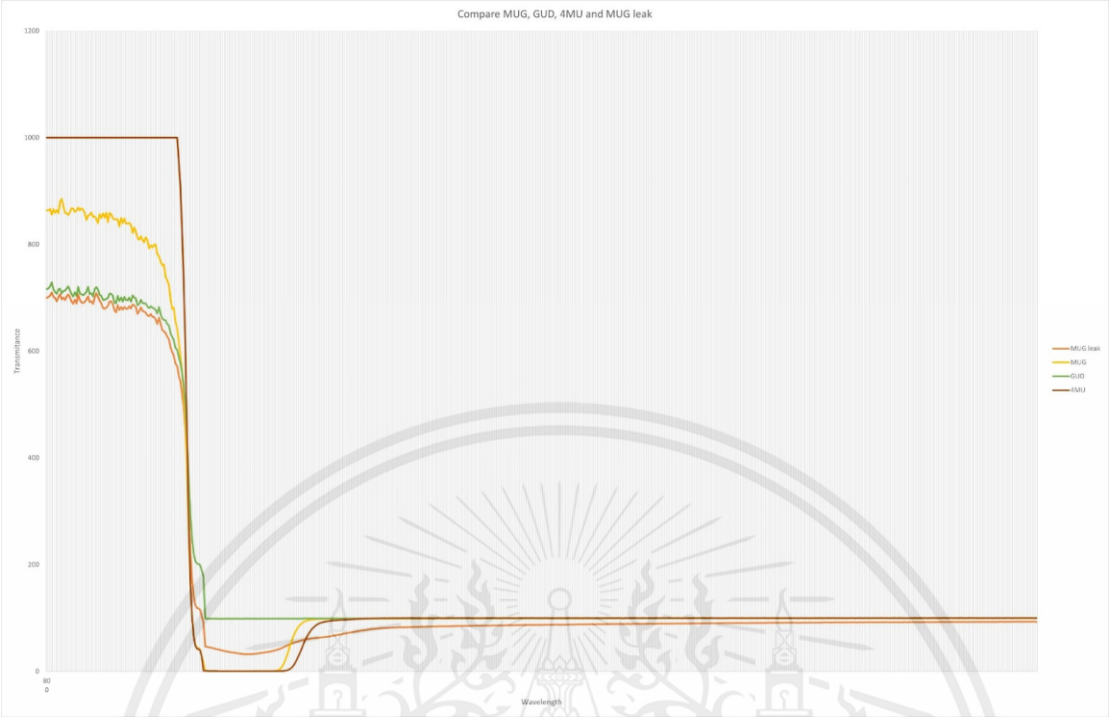
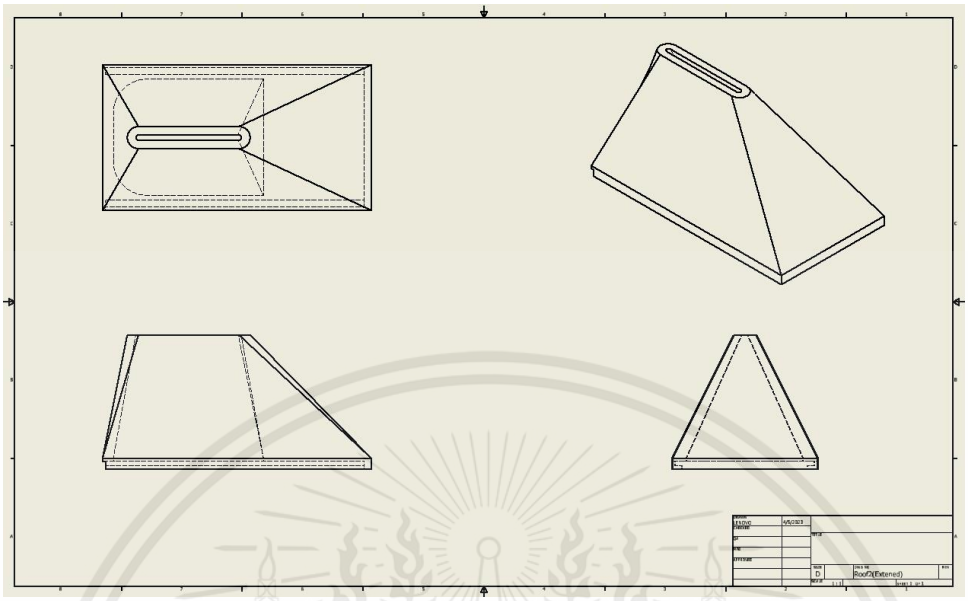


Figure 23. Graph showing the relationship between wavelength and transmittance of MUG, GUD, 4MU and GUD solution that had been dropped onto ABS plastic sheet coated with MUG (MUG leak).

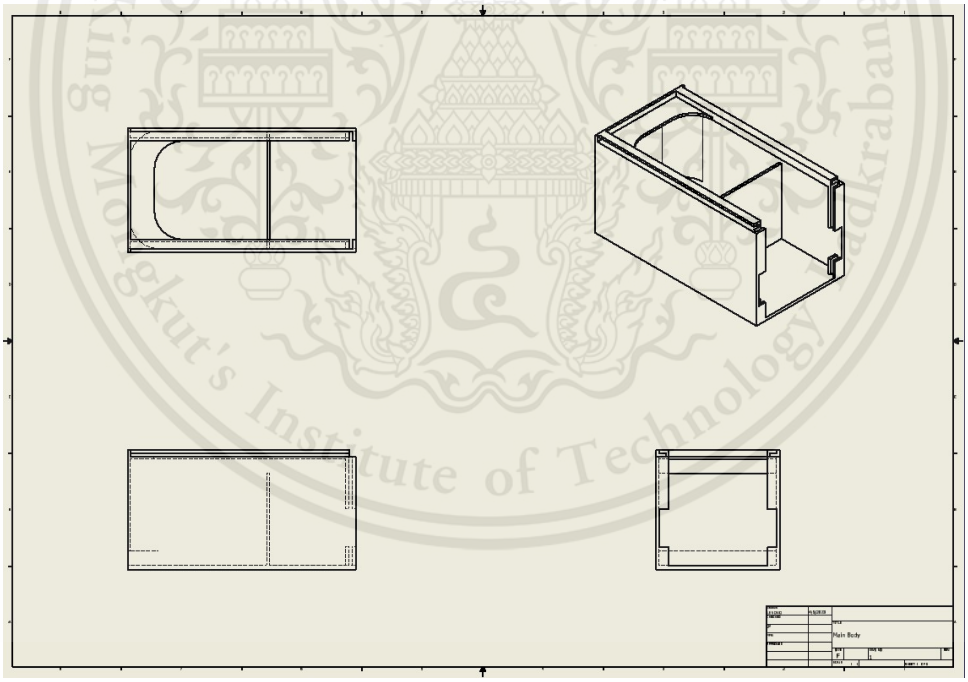
4.7 Designing and producing the prototype of *E. coli* detection the device

The 3D structure of this prototype was designed as shown in Figure 25 and it's 2D drawings of each components are shown in Figure 24. The electrical circuit components used for this prototype are shown in Figure 27 and they were construct by following the diagram shown in Figure 26. The actual 3D structure was printed as shown in Figure 28 and the actual circuit was constructed and installed in the structure of the prototype as shown in Figure 29. The opearation of this device was tested (Figure 30), once the left hole was dropped with GUD for 15 minutes, the reaction between MUG and GUD occurred which resulted in producing 4MU which emitted blue fluorescence light under UV light in the chamber of this prototype. Therefore, this prototype could operate properly as shown in Figure 30.

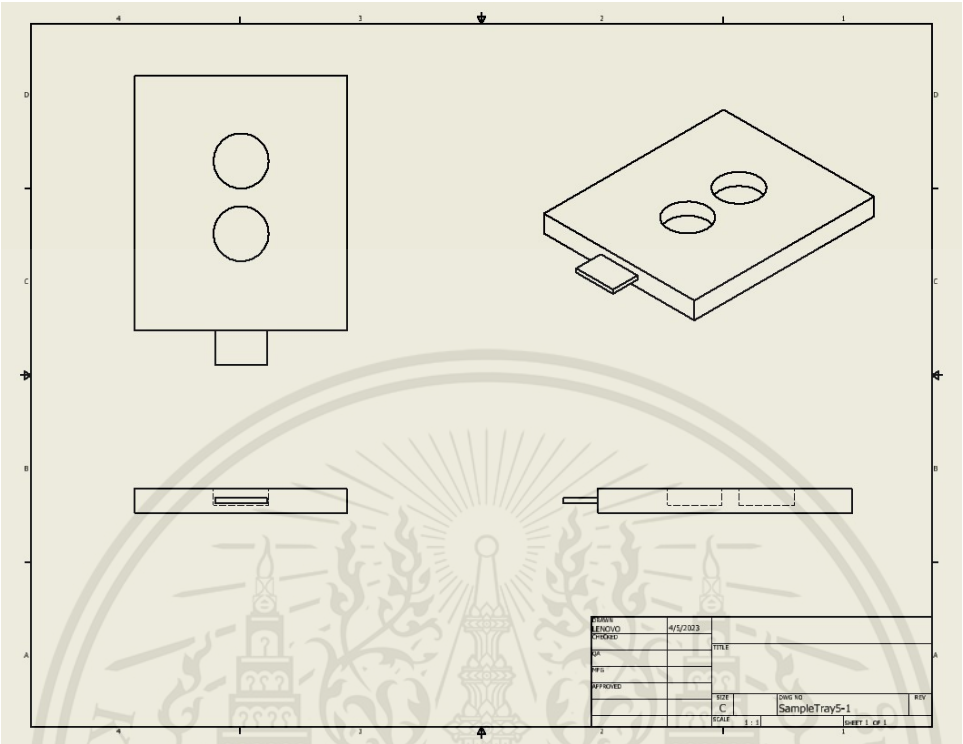
a).



b).



c).



d).

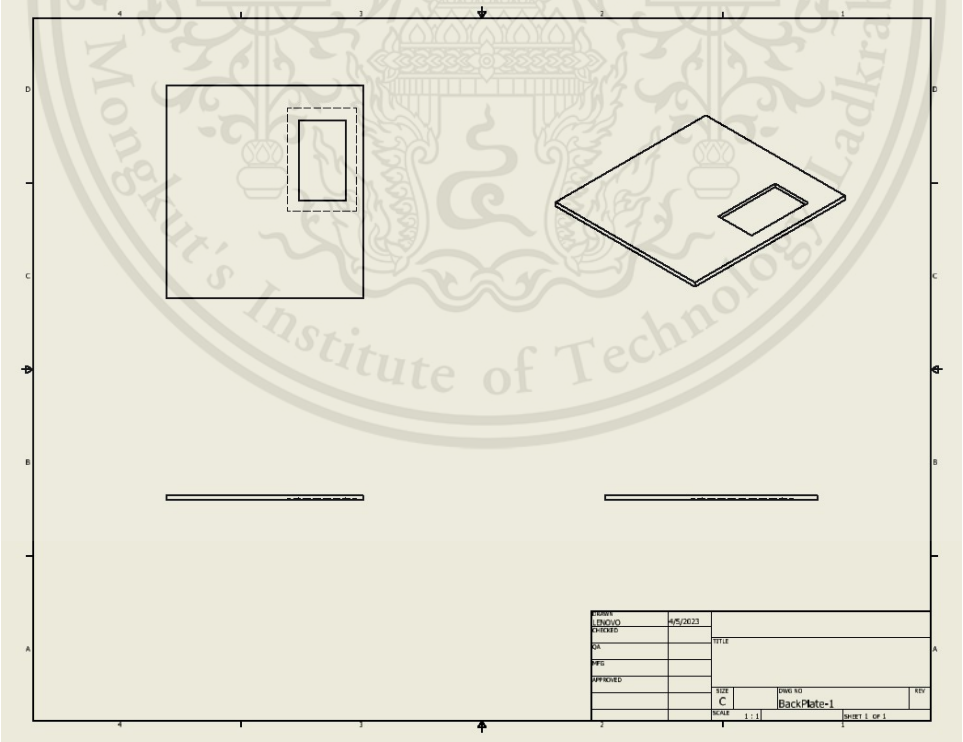


Figure 24. 2D drawing of each component of the *Escherichia coli* detection device Autodesk Inventor Professional 2022. a). Sample tray. b). Main body. c). Back lid. d). Top lid.

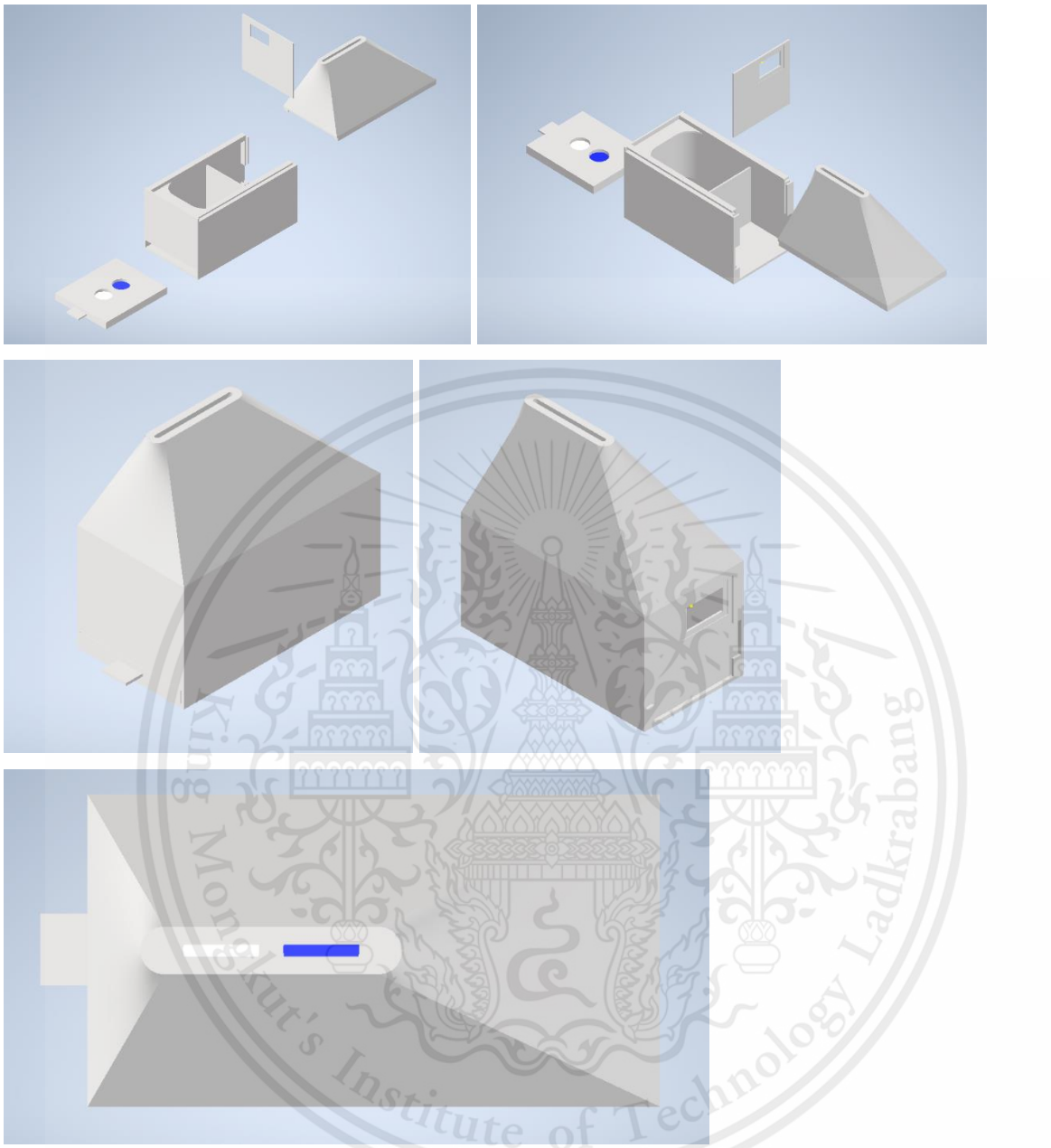


Figure 25. 3D assembly design of the *Escherichia coli* detection device using Autodesk Inventor Professional 2022.

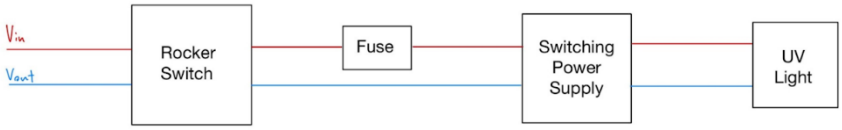
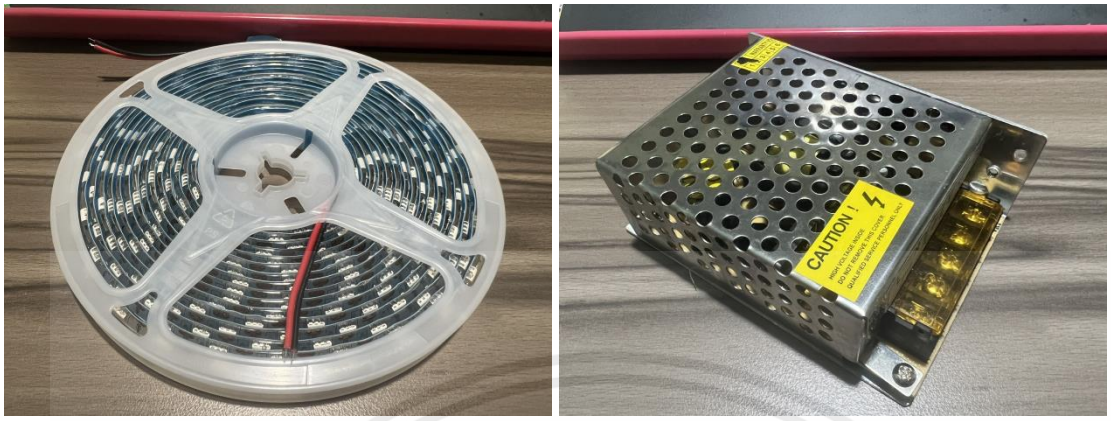


Figure 26. Electrical circuit diagram.

a). b).



c). d).

Figure 27. Electrical components used in the circuit of the device a). 5V 15W/m UV light emitting 395 - 405 nm wavelength. b). 5V 5A DC switching power supply (Input 220V AC). c). Rocker switch d). 10A fuse.

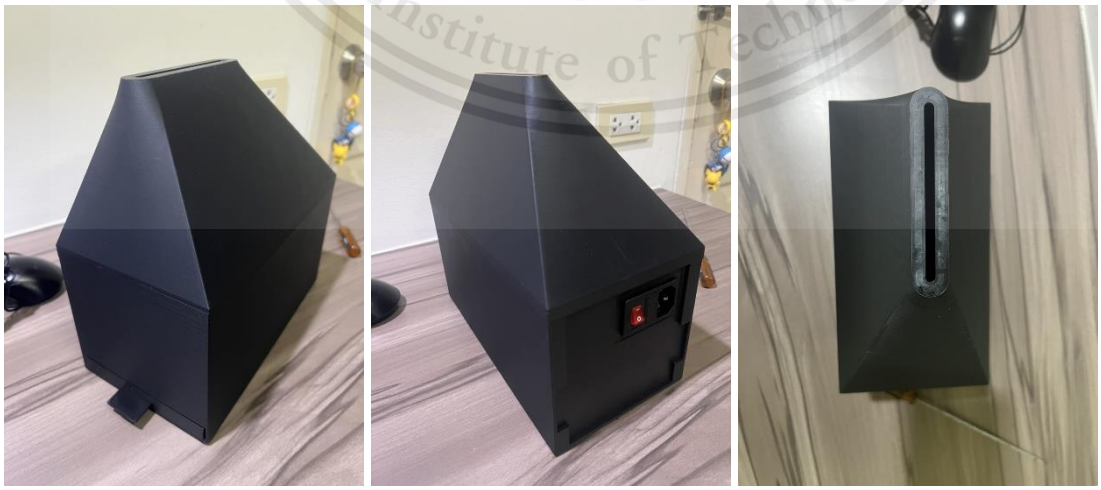


Figure 28. The actual structure of the prototype.

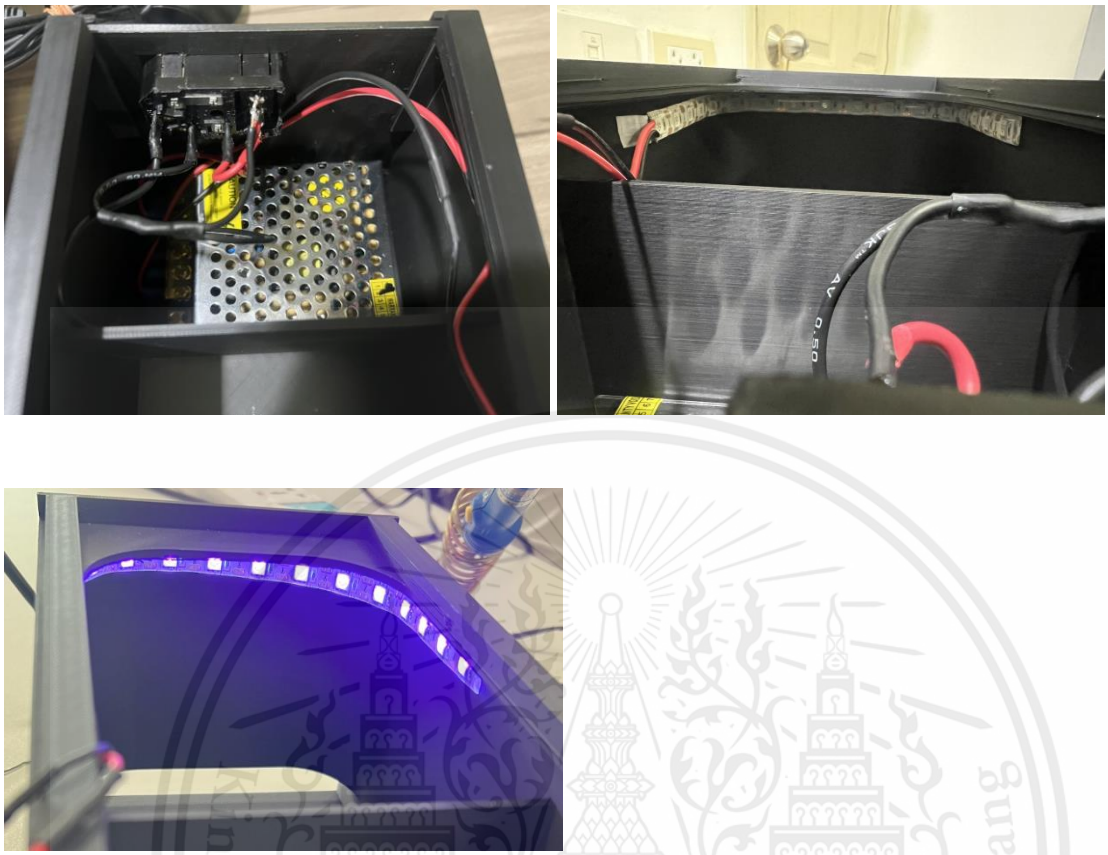


Figure 29. The actual circuit constructed inside of the prototype.

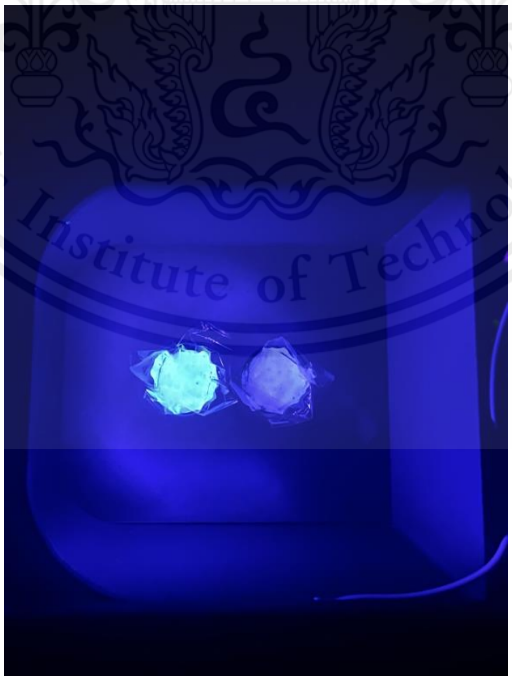


Figure 30. The operation of the prototype.

CHAPTER 5
CONCLUSION

5.1 Conclusion

The most suitable tenique for fabricating a plastic sheet that contains MUG and this MUG can react with GUD and produces 4MU and the fluorescence emitted from this 4MU is observable is coating MUG onto ABS plastic sheet surface technique. Because in comparison between coating MUG onto ABS plastic sheet surface and combing MUG into ABS plastic sheet texture, for combining method, GUD that encounter the MUG in plastic texture cannot react with MUG which results in no 4MU producing or in another case, if there are some GUD can react with MUG in the plastic texture, the 4MU fluorescence cannot be observed be cause the amount of GUD that is absorbed by the plastic sheet is too less and also the whiteness and turbidness of the plastic texture make it very difficult to observe the 4MU fluorescence. Therefore, the coating method is more efficient because the whiteness and turbidness of the plastic sheet will have less effects on the 4MU fluorescence observation because the 4MU fluorescence emission is occurred in the GUD solution due to the leakage of MUG from the plastic surface into GUD solution. For the coating method, the plastic sheet does not need to have absorbency property

The prototype of this *E. coli* detection device is very well designed and produced. The structure of this prototype is strong and practical. The UV LED in the chamber of the structure can be turned on properly and can excite the 4MU to emit blue fluorescence light. The chamber of the device is dark enough for the blue fluorescence light can be observed and the thin slit on the top lid is practical and wide enough for the observer to look into the chamber. The ABS plastic sheet coated with MUG can properly release MUG into GUD solution in the hole of the tray of this prototype, the reaction between MUG and GUD occurred which resulted in producing 4MU which emitted blue fluorescence light under UV light in the chamber of this prototype.

5.2 Suggestion

The UV source used to excite the 4MU to emit blue fluorescence should have the wavelength of 355 nm to properly excite the 4MU or the UVA which has the wavelength between 315 – 400 can excite 4MU properly as well.

There are some differences between the behavior of the 4MU produced from the 4MU powder and the 4MU produced from the reaction between GUD and MUG. The blue fluorescence emitted from this two different type of 4MU at has different intensity.

The blue fluorescence in this device can be properly observed is because the concentration of GUD solution used is very high. But in reality the sample taken from food or beverage may not be included with this high concentration of GUD in case that this food or beverage is contaminated with *E. coli*. Therefore, in practical, the blue fluorescence from the reaction between GUD and MUG may not be produced enough to be able to be observed.

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