DIRECT DETECTION OF LOW CONCENTRATION URIC ACID IN THE VISIBLE LIGHT SPECTRUM UTILIZING SPECTROSCOPY METHOD

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To my beloved parents, families, and friends.

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ABSTRACT

A high concentration of uric acid in human blood will form a crystal that accumulates in the joints and causes inflammation and severe pain to the patient. In literature, the direct detection spectroscopy technique has been employed in uric acid detection due to its eco-friendly and rapid response features. Despite these advantages, the linearity range obtained is very limited. Therefore, to enhance the linearity range, a direct detection-based spectroscopy method in the visible light spectrum is proposed in this work. The enhancement is attributed to the relatively low molar attenuation coefficient in the visible light spectrum. This work analyzed the detection of uric acid in the visible spectrum utilizing a halogen lamp as a light source. The uric acid stock solution in this project was prepared by diluting the uric acid powder in deionized (DI) water. Then, 2, 4, 6, 8, and 10 mg/dL sample solutions were produced by diluting the stock solution based on molarity formulation. These samples were then transferred into a sample compartment for the measuring process. In measuring the samples, the output intensity spectrum was monitored as the concentration varies from 2 to 10 mg/dL. The linearity range, linearity, sensitivity, limit of detection (LoD), stability precision, and relative standard deviation (RSD) of the developed spectrophotometer were studied. The sensor performance at sample wavelengths of 600nm, 650nm, 700nm, 750nm, 800nm, 850nm, and 900nm was analyzed. The highest spectrophotometer sensitivity of 0.0515 $(mg/dL)^{-1}$ was achieved at 700nm wavelength. However, this sample wavelength has a low linearity value, which is about 91%. As for the linearity performance, the best linearity was achieved at 850nm wavelength with 98% linearity value. All the sample wavelengths exhibited more than 99% precision with less than 1% RSD for 300 seconds measurement duration, which indicates a highly stable detection and good reproducibility. The selectivity of the optimal operating wavelength offers comparable linearity and sensitivity performances of the developed spectrophotometer with rapid detection and high stability performances.

ABSTRAK

Kepekatan asid urik yang tinggi dalam darah manusia akan membentuk kristal yang terkumpul di sendi dan menyebabkan keradangan dan kesakitan yang teruk kepada pesakit. Dalam kajian terdahulu, kaedah pengesanan langsung spektroskopi telah digunakan dalam pengesanan asid urik disebabkan ciri-ciri mesra alam dan tindak balas segera. Walaubagaimanapun, julat linar yang diperolehi adalah sangat terhad. Bagi meningkatkan julat linar, pengesanan langsung spektroskopi dalam spektrum cahaya nampak telah dicadangkan dalam kerja ini. Peningkatan ini disebabkan oleh pekali pelemahan molar asid urik yang agak rendah dalam spektrum cahaya nampak. Kerja ini menganalisis pengesanan asid urik dalam spektrum berkenaan menggunakan lampu halogen sebagai sumber cahaya. Larutan stok asid urik telah disediakan dengan melarutkan serbuk asid urik dalam air ternyah-ion (DI). Kemudian, larutan sampel 2, 4, 6, 8 dan 10 mg/dL dihasilkan dengan mencairkan larutan stok berdasarkan formula molariti. Sampel-sampel ini kemudiannya dipindahkan ke dalam bekas sampel untuk proses pengukuran. Dalam pengukuran sampel, spektrum keamatan cahaya keluaran dipantau pada kepekatan berbeza dari 2 hingga 10mg/dL. Julat linar, kelinaran, sensitiviti, had pengesanan (LoD), ketepatan kestabilan dan sisihan piawai relatif (RSD) bagi pengesan yang dibangunkan telah dikaji. Prestasi spektrofotometer pada panjang gelombang 600nm, 650nm, 700nm, 750nm, 800nm, 850nm dan 900nm telah dianalisis. Sensitiviti spektrofotometer tertinggi 0.0515 $(mg/dL)^{-1}$ dicapai pada gelombang 700nm. Walau bagaimanapun, gelombang sampel ini mempunyai nilai linear yang rendah, iaitu kira-kira 91%. Bagi prestasi linear, linear terbaik dicapai pada gelombang 850nm dengan 98% nilai linear. Semua sampel gelombang mempamerkan lebih daripada 99% ketepatan dengan kurang daripada 1% RSD untuk tempoh pengukuran 300 saat, menunjukkan pengesanan yang sangat stabil dan kebolehulangan yang baik. Pemilihan gelombang operasi optimum dalam kaedah pengesanan ini dapat menawarkan nilai linear dan prestasi sensitiviti spektrofotometer yang baik dengan pengesanan pantas dan prestasi kestabilan yang tinggi.

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LIST OF SYMBOLS AND ABBREVIATIONS

γ	–	Gamma
ΔI	–	Intensity difference
Δc	–	Concentration difference
ε	–	Molar absorptivity or molar attenuation coefficient
λ	–	Wavelength
μ	–	Mean
σ	–	Standard deviation
R^2	–	Correlation of determination
C_1	–	The concentration of stock solution (mg/dL)
C_2	–	The concentration of working solution (mg/dL)
CO_2	–	Carbon dioxide
H_2O_2	–	Hydrogen peroxide
$I_{average}$	–	Average value of the stable set of n measurement
I_n	–	intensity value of the nth measurement
I_o	–	Intensity of light passing through reference cell
V_1	–	The volume of stock solution needed to make the working solution
V_2	–	The finale volume working solution
V_3	–	The volume of DI water needed to make the working solution
A	–	Absorbance
a.u	–	Arbitrary unit
b	–	Sample path length
c	–	Sample concentration
c	–	Molar concentration of the uric acid measured in mol/L
c	–	Speed of light constant (3×10^8 ms^{-1})

CCD	–	Charge Coupled Device
CL	–	Chemiluminescence
DI	–	Deionized
E	–	Photon energy
f	–	Frequency
ħ	–	Planck's constant 6.63 x 10^{-34} J s
HPLC	–	High-performance liquid chromatography
Hz	–	Hertz
I	–	Intensity of light passing through sample with concentration cell
J	–	Joules
L	–	Liter
LED	–	Light emitting diode
LoD	–	Limit of Detection
m	–	Mass of the uric acid powder in gram (g)
mg/dL	–	Milligram per deciliter
mm	–	milimeter
ms	–	Millisecond
N	–	smallest scale of the sensor receiver component
NIR	–	Near infrared
nm	–	nanometer
r	–	Correlation coefficient
RSD	–	Relative Standard Deviation
R-squared	–	Coefficient of determination
S	–	Sensitivity
SMA	–	SubMiniature version A
T	–	Transmittance
TMB	–	Tetramethylbenzidine
TTL	–	Through The Lens
TV	–	television
UA	–	Uric acid
USB	–	Universal Serial Bus
UV	–	Ultraviolet

UV-VIS	–	Ultraviolet - Visible
v	–	Final volume of the solution in litre (L)
VIS	–	Visible
w	–	wavenumber
w	–	Formula molecular weight measured in g/mol

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

In 1776, the organic heterocyclic compound of uric acid was discovered by a Swedish chemist, Carl Wilhelm Scheele. Later, Alfred Garrod (1819–1917) successfully proved the relation between increased uric acid and gout. Garrod took his study even further by suggesting dietary regulation of consumption of purine-rich food as an important measure to combat gout.

Uric acid ($C_5H_4N_4O_3$) is a substance that exist in human blood due to purine's breakdown. Purine comes from consumption of protein such as meat, nut, and seafood. During the human metabolism, uric acid is produced and is dissolved in the blood before being sent to the kidney for its elimination through urine. A normal range of uric acid levels in a man's blood is from 3.5 to 7.2 mg/dL while for a woman's blood, the normal range is from 2.6 to 6 mg/dL[1]. Hyperuricemia is a condition when the human body either produces too much or is unable to excrete enough uric acid. According to the Japanese Society of Gout and Nucleic Acid Metabolism, hyperuricemia is outlined as a condition of serum uric acid with over 7.0 mg/dl, regardless of the gender and age [2]. It is crucial to monitor the uric acid level because an excess amount of uric acid will lead to gout, kidney failure, heart disease, high cholesterol, diabetes and even hypertension [3]–[6]. On the other hand, the lower amount of the substances may lead to atherosclerosis and stroke [6].

It is necessarily to detect and quantify uric acid in normal range because monitoring the uric acid can be a powerful indicator for initial warning signs of diseases [3]. In 2021, a study discovered that there was a significant increase in fetal and maternal incidents in the hyperuricemia group compared with the normal uric acid

group. This study revealed that serum uric acid remained an important predictor for low birth weight and premature birth in preeclampsia patients [7]. A record from 1999 until 2009 revealed that gout admissions rose at 5.5% per year in New Zealand and at 7.2% per year in England [8]. Based on Sarawak General Hospital year 2011 and 2012 data, majority of gout patients were male with the mean age of 60.0±14.2 years. They have a mean duration of illness of 2.5±8.7 years. In addition, about 28.6% of the patients had a family history of gout in their first-degree relatives [9].

Health monitoring has received tremendous attention by the society in recent years. In general, health monitoring is carried out by measuring an amount of substance in human body that causes diseases. Detection of the substance can be done by using invasive method such as blood extraction. However, this invasive method that requires blood extraction will normally cause pain, calluses and scar to the patient especially for hemophiliacs, neonates, elderly and disable people [10]. Therefore, a non-invasive method has become an alternative to overcome the stated issues. Aside from blood, human body also excretes fluids that are categorized as human waste which can be extracted for the non-invasive health monitoring methods.

In recent years, there has been extensive research to detect uric acid using non-invasive methods such as electro-analytical [1], [6], [11]–[16], chemiluminescence [17]–[19], chromatography [20], [21] and spectroscopy [3], [4], [22]–[27]. These non-invasive methods sense the substance either from human blood or urine sample. Electro-analytical method such as voltammetry [1], capillary electrophoresis [6], electrochemical [11], and potentiometry [12] offer a simple detection method using electrode. However, the need for complex sample pretreatment and the high cost of some reagents limit their applications [28]. Meanwhile, chemiluminescence and chromatography have the capability to detect uric acid concentration in either blood or urine. However, they require involvement of complex chemical preparation process, narrow linear range, and slow response time. The spectroscopy method shows a promising linear uric acid detection in a broad range which is from 0.58 to 234 mg/dL [1], [3], [29]. In addition, the spectroscopy method is very effective as optical sensing system due to its simplicity, cost effectiveness and fast time response [22]. Moreover, the direct detection of the spectroscopy method is also eco-friendly to the environment because it does not dispose of any medical waste.

1.2 Problem Statement

High level of uric acid will lead to gout, kidney failure and even hypertension. The main indicator of gout is the level of uric acid present in blood and human urine. Therefore, it is important to monitor the concentration of uric acid for early-stage warning of this condition and diagnosis of patients.

Various types of uric acid detection methods illustrate the demand to develop a uric acid sensor with a wide range concentration measurement and fast response time. The uric concentration measurement based on spectroscopy method exhibits response time of millisecond range, which makes the sensor well-suited compared to other methods. In addition, spectroscopy shows a promising linear uric acid detection in a broad range which is from 0.58 to 234 mg/dL [1], [3], [29]. Thus, making it suitable for uric acid detection in both blood and urine.

Previous studies have reported efforts to improve linearity range of uric acid concentration measurement. Based on the spectroscopy method, the detection can be divided into indirect and direct detection of uric acid. The indirect detection requires chemical reagent, enzyme, catalyst, or buffer in its procedure, to be able to detect the product of uric acid such as hydrogen peroxide (H_2O_2). Although the indirect detection is highly sensitive, it produces chemical wastes, and its preparation is complex, costly, and time consuming. On the other hand, the direct detection of uric acid is fast, instantaneous, and eco-friendly.

In previous work, the direct uric acid measurement was done in ultraviolet region with the chosen absorption wavelength of 294.46nm [30] and 293.99nm [22]. In near infrared region, the direct detection was conducted in between 1400 nm and 1700 nm [23]. The work by Norazmi *et al.*[22] demonstrated direct detection of uric acid in the range of 40 to 100 mg/dL at an ultraviolet wavelength with a fast response of 3 seconds. Besides that, previous work by Jae Kim[23] showed that direct detection of uric acid in near-infrared utilized a cost-effective, quick, and non-invasive. However, the sensor only detected the uric acid concentration from 3 to 7 mg/dL.

Based on Beer-Lambert law, the lower molar attenuation coefficient will give a higher concentration value which improves the linearity range. Each of the wavelengths has its own molar absorptivity's value. Thus, this research is carried out to investigate the wavelength other than the ultraviolet and near-infrared spectrum which could improve the linearity range of uric acid concentration in blood.

1.3 Significant of Research

An enhancement of linearity range in direct detection of low concentration uric acid in the visible light spectrum is reported in this work. The linearity range in this work is found superior than that reported in the previous direct detection-based uric acid sensor. This enhancement is attributed to the relatively low molar attenuation coefficient in the visible light spectrum. Due to the limitation of equipment, range of the visible light spectrum was set from 600 to 900nm wavelength and the sample was prepared for concentration range in between 2 to 20mg/dL. Besides the enhanced linearity range, the proposed sensor has the advantage of fast response time. This results from the avoidance of any enzyme or reagent in the sensing operation. An experimental study has been carried out to analyze the spectrophotometer performance in the aspect of linearity range, linearity coefficient, sensitivity, resolution, limit of detection (LoD) and stability precision performance. This work shows the potential of direct detection-based spectrophotometer system that operates in the visible light spectrum in sensing a wider range of low concentration uric acid.

1.4 Research Objectives

This research work embarks on the following objectives:

a) To determine optimal linearity performance for low concentration uric acid detection within the medium and high range of the visible light spectrum.

b) To characterize the developed spectrophotometer in terms of its linearity range, linearity, sensitivity, limit of detection (LOD), stability precision and relative standard deviation (RSD) performance.

1.5 Scopes of Study

The following scope of work has been identified to achieve the objectives:

a) The spectrophotometer's concept and working principle, as well as its components, were studied.

b) The effects of spectrophotometer light source intensity was measured and analyzed using a uric acid sample of concentration from 2 mg/dL to 20 mg/dL, which represent the low, medium and high uric acid concentration in human blood.

c) The normalized intensity value was determined for the analysis of the sensitivity, linearity, precision, and stability of the established portable spectrophotometer in detecting uric acid.

d) Selection of equipment to be used in the experimental setup for the optical sensor characterization based on absorption spectroscopy. The sample path length was fixed at 10 mm.

e) Selection of the wavelength range in this work is restricted by two parameters, which are cuvette material and spectrum of the light source. The plastic cuvette used in this work has a wavelength range at the visible and near-infrared (NIR), which is from 350 to 900nm wavelength. However, the halogen light source has a good transmittance in the wavelength range from 600 to 900nm. Therefore analysis in this work was carried out in between 600 to 900nm wavelength range.

1.6 Outline of the thesis

This thesis is arranged into five chapters. The first chapter introduces the thesis and describes the research work briefly.

Chapter 2 gives a brief description of the classification of the uric acid detection method. Followed by a review on spectrophotometer components; light source, detector, and sample compartment. Studies on uric acid detection based on the spectroscopy method from previous work are discussed in terms of sensor

performance. Lastly, the performance parameters such as linearity, sensitivity, limit of detection, stability precision and relative standard deviation are explained.

Chapter 3 focuses on spectrophotometer design, construction, and characterization methods. At the beginning of this chapter, the procedure of uric acid sample preparation is described. Next, the halogen light source characterization and method of detection uric acid is described.

Chapter 4 illustrates the output of the research and analysis of the results obtained from the spectrophotometer. The analysis includes system sensitivity, linearity, resolution, LoD, stability, and relative standard deviation. Comparison of the developed spectrophotometer performance in detecting uric acid using halogen light source with previous research is included to verify the significant contribution obtained from this work.

Finally, Chapter 5 states the overall conclusions and contribution of this research and discuss the possibilities for further development of this work.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter provides the background theory of spectroscopy which includes the spectrophotometer instrumentation, derivation of Beer-Lambert Law and limitation of Beer-Lambert Law. Previous studies on the detection of uric acid in blood using the spectroscopy method are also discussed. Lastly, the performance parameters such as linearity, linearity range, sensitivity, resolution, limit of detection (LoD), stability precision and relative standard deviation are explained.

2.2 Review Method of Uric Acid Detection

Uric acid (UA) is the end product of purine metabolism when one consumes protein such as soy legumes, meat and seafood [31]. It is absorbed into the blood circulatory system, filtered by the kidneys, and excreted through the urine. Therefore, the concentration of uric acid in physiological fluids such as plasma, serum and urine serves as a valuable indicator for a particular clinical condition. Increased uric acid in urine or serum can affect kidney function and blood pressure, leading to diseases such as gout, cardiovascular, hypertension and kidney disease while low uric acid levels may lead to molybdenum deficiency, copper toxicity and worsening of multiple sclerosis [32]. As such, detecting soluble uric acid levels in human bodily fluids is critical for determining the diagnosis of patients with the above conditions.

Different types of UA detection methods have been developed to measure the level of UA in human serum and urine sample. The materials used in most of the detection methods tend to be diversified and contain either enzymatic or nonenzymatic

approaches. The enzymatic reactions, caused by uricases that catalyze the oxidation of UA, mostly happen in birds, reptiles, and primates but not in humans. The uricases will convert UA into simpler molecule such as allantoin, carbon dioxide (CO_2) and hydrogen peroxide (H_2O_2). Since humans lack the enzyme due to a defective gene which could not be transcribed, the generation and excretion of UA maintain a relatively constant level [21]. In 2013, Erden *et al* [33] explained various detection methods which also included the enzymatic and spectrophotometry. These detection methods are divided into chemiluminescence, fluorescence, spectrophotometry, HPLC-mass spectrometry, ion chromatography, high performance liquid chromatography (HPLC)/isotope dilution mass spectrometry (IDMS), capillary electrophoresis-amperometry, capillary electrophoresis with chemiluminescence detection, colorimetry and enzymatic. However, such a category is very comprehensive and having a general knowledge of the current state of UA identification is important [34]. In 2017, Dong *et al* [32] mentioned a few categories of UA detection methods and the spectrophotometry was not specified in any category because it was also used together with the HPLC, CE and other methods. While Wang *et al.*[34] discussed various methods of UA detection which were classified based on the signal record technology. However, because of the cross-use of different approaches and detection methods in modern science, it often created conceptual misunderstanding. Such enzyme-based UV, fluorescent, and electrochemical methods may still exist, while others may not.

In this research, the UA detection method was reviewed based on four basic categories [35] which included spectroscopy, electro-analytical, chromatography, luminescence, and as shown in Figure 2.1. The spectroscopy method can be further classified into the use of chemicals or without chemicals on the uric acid sample.

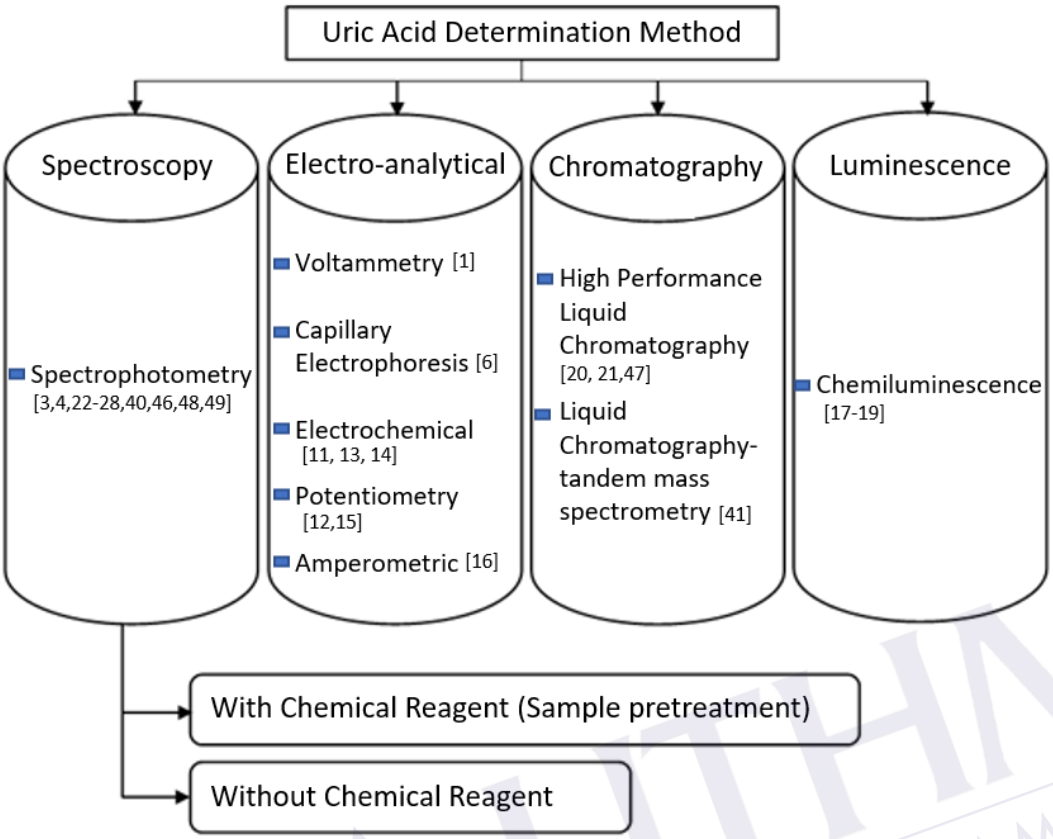

Figure 2.1 Uric acid sensing method

Electro-analytical is a technique that measures the voltage and/or current in an electrochemical cell containing the substance or chemical constituent of interest [36], [37]. Electro-analytical method can be divided into many categories such as voltammetry [1], capillary electrophoresis [6], electrochemical [11], [13], [14], potentiometry [12], [15], and amperometric [16]. Electro-analytical method for UA detection has drawn a lot of interests due to its advantages in term of fast response, easy testing procedures, low cost, and high selectivity and sensitivity [38]. This method is also used in clinical analysis because it provides high sensitivity and selectivity in detecting a variety of analytes [39]. However, most of the electro-analytical methods utilize the uricase enzyme to detect uric acid, in which has disadvantages in enzymes mobilization, reproducibility and require specific control of the working conditions [14]. Another disadvantage is that purine derivatives like caffeine appear to bind to the electrode surface, necessitating the regular removal of the detection flow cell for electrode activation or replacement [40].

The chromatography methods include High-Performance Liquid Chromatography (HPLC) using a separation technique to select the components of a

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APPENDIX A

LIST OF PUBLICATIONS

Journal

1. Afiqah Yaacob, Nor Hafizah Ngajikin, Nurfatihah Che Abd Rashid, Siti Hajar Aminah Ali, Maslina Yaacob, Noran Azizan Cholan , "Uric acid detection in visible spectrum," *Telkomnika*, vol. 18, no. 4, pp. 2035–2041, 2020.
2. Afiqah Yaacob, Nor Hafizah Ngajikin, Nurfatihah Che Abd Rashid, Maslina Yaacob, Siti Hajar Aminah Ali, Nur Ellina Azmi, Noran Azizan Cholan "Linearity range enhancement in direct detection of low concentration uric acid," *Optik*, vol. 249, p. 168243, 2022

Poster Conference

1. "Determination of uric acid using spectrophotometer", FKEE Postgraduate Poster Day Conference 2019, 24 November 2019
2. "Uric Acid Determination in Visible Spectrum", The 2nd FAST Postgraduate Virtual Symposium 2020, 21 December 2020, Silver Award

APPENDIX I

VITA

The author was born on June 7, 1987 in Kuala Lumpur, Malaysia. She was raised in Perak and went to Maktab Rendah Sains MARA Lenggong, Perak for her secondary school. She pursued her first degree studies for four years at Universiti Teknologi Mara (UiTM) Shah Alam, Selangor in Electrical Engineering. Then, in 2012 until 2019, she joined in various work field such as administration, logistic, education, telecommunication, and construction. Currently, she is pursuing her studies in Master's degree of Electrical Engineering in Universiti Tun Hussein Onn Malaysia (UTHM). Her research interests include uric acid detection and development of optical sensors. She also has been an active member of various student club such as Graduate Student Association (GSA), Electrical Engineering Postgraduate Student (EECLIPS) and IEEE UTHM Student Branch since 2019 until 2022. She has been entrusted with the position of Treasurer in GSA, Secretary I in EECLIPS and Committee Member in IEEE UTHM Student Branch. She has experience managing CSR projects around Batu Pahat, Johor, which promote ethical and sustainable activities, as well as generate goodwill and a positive reputation between UTHM students and community.