

Uric acid detection in visible spectrum

Afiqah Yaacob¹, Nor Hafizah Ngajikin², Nurfatihah Che Abd Rashid³, Siti Hajar Aminah Ali⁴,
Maslina Yaacob⁵, Suhaila Isaak⁶ and Noran Azizan Cholan⁷

^{1,2,3,4,5,7}Faculty of Electrical and Electronic Engineering, Universiti Tun Hussein Onn Malaysia, Malaysia

⁶School of Electrical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Malaysia

Article Info

Article history:

Received Dec 19, 2019

Revised Mar 19, 2020

Accepted Mar 30, 2020

Keywords:

Beer-Lambert law

Spectrophotometer

Uric acid

ABSTRACT

The measurement of uric acid based on the optical absorption at visible light spectrum is investigated and tested. Sensing in the visible region was conducted for determination of suitable wavelength that produces high sensitivity and accuracy performance based on the Beer-Lambert law calculation. In this work, the uric acid is detected by detecting sodium urate as a product of chemical reaction between uric acid with sodium hydroxide buffer. The setup has been tested for uric acid concentration ranging from 15 mg/dL to 85 mg/dL. Three wavelengths have been analyzed which are 460 nm, 525 nm and 630 nm. Measured data at 460nm wavelength exhibits the highest sensitivity, which is 0.0012 (mg/dL)-with 86.51% accuracy. Detection of uric acid at visible light spectrum offers a low-cost sensor based on visible LEDs and photodiode is possible to be realized.

This is an open access article under the [CC BY-SA](https://creativecommons.org/licenses/by-sa/4.0/) license.



Corresponding Author:

Nor Hafizah Ngajikin,
Faculty of Electrical and Electronic Engineering,
Universiti Tun Hussein Onn Malaysia,
86400 Parit Raja, Batu Pahat, Johor, Malaysia.
Email: norhafizah@uthm.edu.my

1. INTRODUCTION

Uric acid sensor has received much attention in recent years due to the increasing number of gout patients worldwide. Gout is a disease developed from the excess amount of uric acid present in blood and urine. Uric acid dissolves in blood and travels to the kidney to be eliminated through urine. Excess amount of uric acid will not only lead to gout, but it can also lead to kidney failure, heart disease, high cholesterol, diabetes and even hypertension [1, 2]. It is important to regulate the amount uric acid in maintaining the health of the bloodstream, since low amount of uric acid may lead to atherosclerosis and stroke [2]. Uric acid can be monitored using two methods; blood test measurement [3-6] and urine test measurement [1, 7-15]. A healthy range for uric acid levels in blood serum is from 3.5 to 7.2 mg/dL in men and from 2.6 to 6 mg/dL for women [14]. Meanwhile, the healthy range of uric acid in human urine is ten times greater than the uric acid levels in blood where it is ranging from 25 to 74 mg/dL [1, 12, 14]. In recent years, there has been extensive research on uric acid analytical techniques to detect uric acid in human urine such as electro-analytical, luminescence, chromatography and spectroscopy. As for spectroscopy system [1, 7-10, 16-21], the system detects uric acid by using chemical reagent or buffer solution to the sample to be tested by analyzing light absorbance or transmittance value. Spectroscopy shows a promising linear uric acid detection in a broad range which is from 0.58 to 58.84 mg/dL [14] and 58.84 to 218.56 mg/dL [13]. Thus, make it suitable for uric acid detection in both blood and urine.

As afore mentioned, interference cause by other substance in the sample in the spectroscopy system is minimize by adding a chemical reagent or buffer solution. In 2007, Yamaguchi has developed a simple and highly sensitive spectrophotometric method for the determination of uric acid based on fading of the o-hydroxyhydroquinonephthalein (QP)-palladium (II)-hexadecyltrimethylammonium complex in human urine. The absorbance measurement has been conducted using a Shimadzu spectrophotometer with deuterium and tungsten halogen lamp as the light source. This technique however has limitation in linearity range, where the linearity occurs in between 0.001 mg/dL to 0.02 mg/dL at 635 nm visible operating wavelength [18]. Later, a simple spectrophotometric method based-uricase enzyme for the detection of uric acid in normal urine and gout patient's urine samples has been developed based on the reaction of hydrogen peroxide (H₂O₂) with yellow color of 4-Aminodiphenylamine Diazonium Sulfate (variamine blue RT salt) to yield a pale yellow-green coloured solution at 269 nm wavelength im ultraviolet (UV) spectrum [8]. The calibration plot of different concentrations of uric acid was found to be linear between 9 mg/dL to 234 mg/dL with 20 minutes response time. Although broad sensing range has been achieved, the sensor requires very long response time. Besides of using chemical reagent, enzymes or buffer, uric acid also can be detected directly from the solution using spectrophotometer at 294.46 nm wavelength [19]. Although the method is simple, the effect of interefence by other substance is not studied since there is no chemical reagent was added. In this work, the uric acid detection at visible region was conducted by using NaOH buffer solution without any additional chemical reagent. Detection of the uric acid in this work is carried out by detecting sodium urate as a product of uric acid and NaOH mixing process.

2. THEORY

Analysis of sensitivity and accuracy in this work was calculated using Beer Lambert law. The Beer-Lambert law defines the attenuation of light to the properties of the sample in terms of transmittance, T and absorbance, A as stated in (1) and (2-5). In this work, the output light intensity after passing through cuvette without sample, I_{ref} , and with sample, I_o , is measured by using spectrometer. Configurations of a basic spectrophotometer system with the addition of input and output fiber is illustrated in Figure 1.

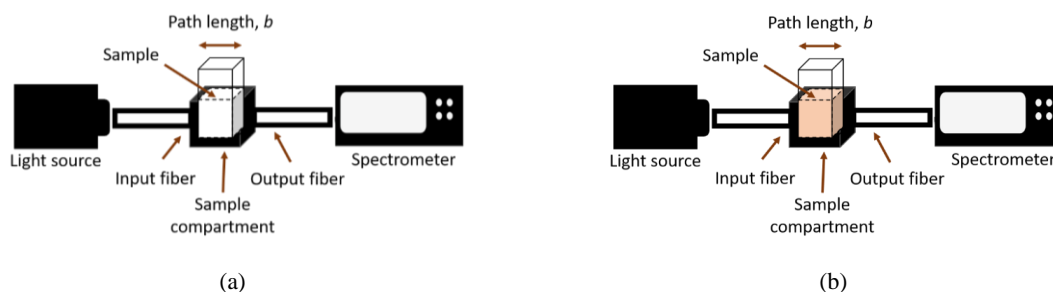


Figure 1. Spectrophotometer system (a) without sample and (b) with sample

The transmittance value based on (1) can be used to define the absorbance, A , of the sample. The relationship between transmittance, T and absorbance, A is shown in (2) [20-22].

$$T = \frac{I_o}{I_{ref}} \quad (1)$$

$$A = \log \frac{1}{T} = \epsilon bc \quad (2)$$

The (2) also relates absorbance, A , concentration of sample, c , sample path length, b , and the absorptivity of the sample, ϵ . This relationship is known as the Beer-Lambert law. Based on (2), the concentration of a substance is directly proportional to the amount of light absorbed or inversely proportional to the logarithm of the transmitted light [23, 24]. The relationship between transmittance, T and absorbance, A is illustrated in Figure 2.

Analytical analysis is done by analyzing the calibration curve which consists of a plot of absorbance versus concentration series of standard solution [20]. If the curve-fit is linear, the sensitivity of the curve fit is its slope [25]. Thus, sensitivity is given as in (3).

$$\text{Sensitivity} = \frac{\Delta A}{\Delta c} \text{ [(mg/dL)}^{-1}] \quad (3)$$

ΔA is the absorbance difference and Δc is concentration difference. The (3) shows that for a fixed concentration difference, higher absorbance will contribute to a higher sensitivity of spectrophotometer [26]. The Standard unit for sensitivity of the tested sensor is dependent on the unit used for the sample concentration. Thus, in this paper, the unit used for sensitivity is $(\text{mg/dL})^{-1}$. As for calibration accuracy, this parameter refers to how close the measured value, C_{measured} with the linear-fit real value, C_{real} [25]. The (4) shows equation used for accuracy calculation based on the data from absorbance plot in Figure 3. The following section will describe procedure for sample preparation and experiment that was conducted.

$$\text{Accuracy} = \left(1 - \left| \frac{C_{\text{real}} - C_{\text{measured}}}{C_{\text{real}}} \right| \right) \times 100 \text{ [\%]} \quad (4)$$

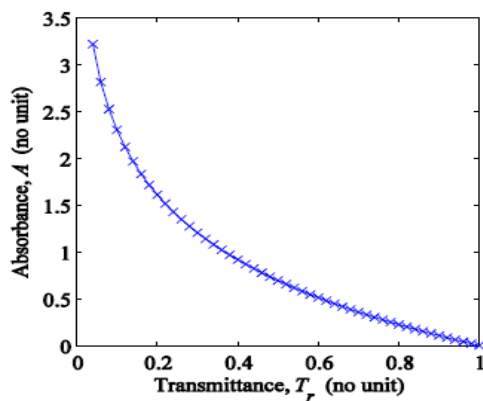


Figure 2. Graph of absorbance versus transmittance [22]

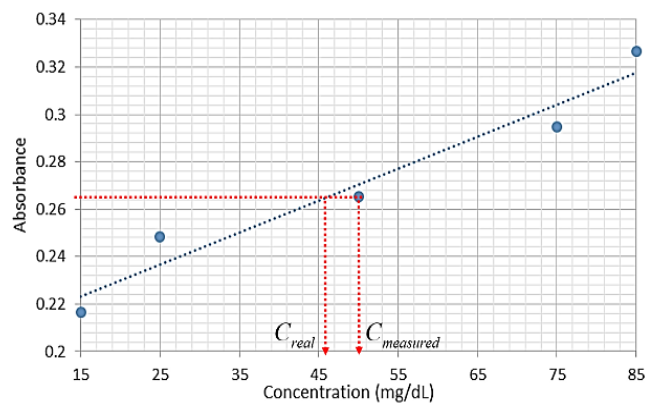


Figure 3. Accuracy measurement from absorbance plot

3. EXPERIMENT

Uric acid solution is prepared by mixing uric acid powder into sodium hydroxide (NaOH) solution [4, 7, 24]. In this research, uric acid and sodium hydroxide solution was purchased from Axon Scientific. The sample is prepared by mixing uric acid powder in 0.1 M NaOH solution as shown in Figure 4 (a) and (b). Table 1 shows the amount of uric acid powder that needs to be added to 80 mL of NaOH solution for producing uric acid sample with 15 mg/dL, 25 mg/dL, 50 mg/dL, 75 mg/dL and 85 mg/dL concentration. The sample preparation process was carried out at room temperature. Mixture of uric acid powder and NaOH solution will produce sodium urate and H₂O [24]. Higher concentration of uric acid in NaOH solution will form a solid sodium urate as shown in Figure 4 (c). Therefore, the mixture needs to be stirred for about 3 minutes before being transferred into the cuvette for fully dilution process as visualized in Figure 4 (d). In this measurement, 1.5 mL of sample were transferred into each cuvette for the characterization process as in Figure 4 (e). Figure 4 illustrates the steps taken for standard uric acid sample preparation.

Experiment was carried out by using tungsten-halogen as the light source (1) and spectrometer as optical detector. In this experiment, variable optical attenuator (2) is used to ensure intensity of light is not saturated at the spectrometer. The maximum photon count is limit to a maximum peak of 14000 photon count. Light from optical fibre is collimated into parallel beam using Ocean Optics 74-UV collimating lens (3) to interact with solution in cuvette (4) as shown in Figure 5. Then, light from cuvette is collected using the collimating lens and delivered to Ocean Optics HR4000CG-UV-NIR high resolution spectrometer (5). Ocean Optics OceanView spectrometer operating software is used to obtain and process data from spectrometer. Uric acid concentration is calculated by comparing intensity of light that passes through sample, I_o and does not pass through sample, I_{ref} . Wavelengths selected to sample uric acid are 460 nm, 525 nm and 630 nm based on available visible LED in the market.

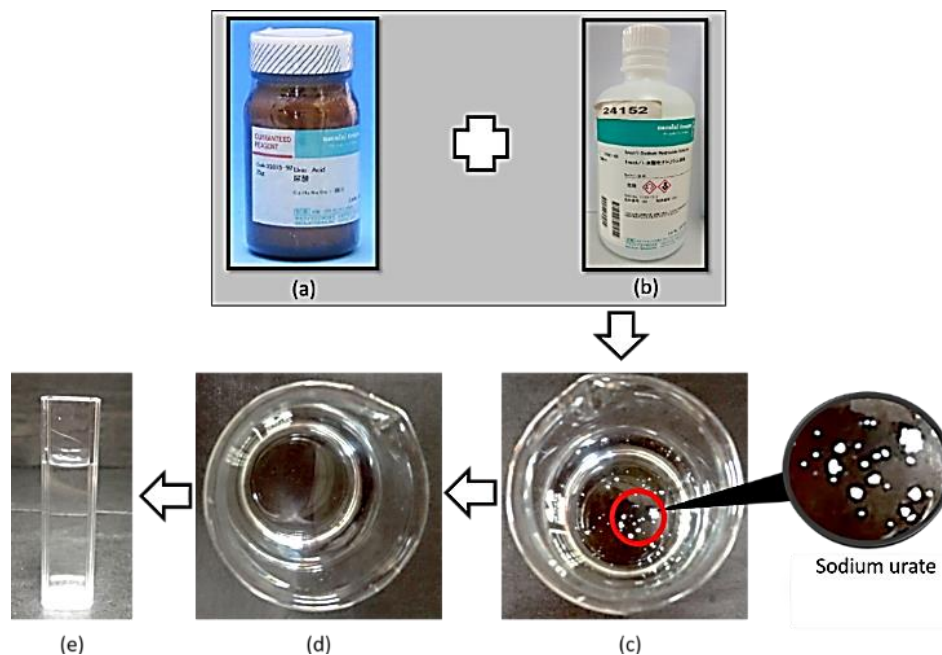


Figure 4. Preparation of standard uric acid sample; (a) uric acid powder (b) NaOH solution (c) sample after adding NaOH with uric acid producing particles of sodium urate (d) sample after stirred using glass rod (e) transfer 1.5 ml into cuvette for measurement

Table 1. Amount of uric acid powder added into 80 mL of NaOH solution

Concentration (mg/dL)	Uric Acid powder (mg)
15	12
25	20
50	40
75	60
85	68

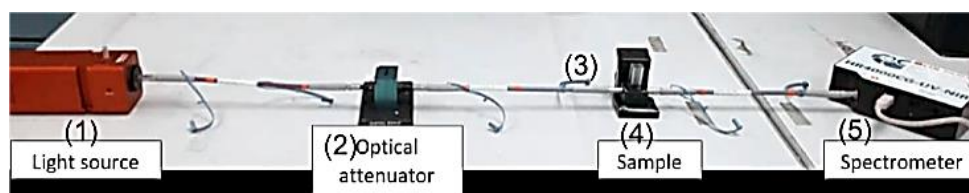


Figure 5. Experimental setup

4. RESULTS AND DISCUSSION

Figure 6 visualized the intensity spectra observed using HR4000CG-UV-VIS Ocean Optics spectrometer for SLS201 Stabilized Tungsten Halogen light operated at 400 nm to 700 nm wavelength range. This figure shows that light intensity will decrease as the concentration of uric acid increases, in which obey the Beer-Lambert law. The relationship between light intensity with absorbance is associated using (1) and (2). Calculated absorbance is plotted as in Figure 7 while calculated sensitivity and accuracy is tabulated in Table 2.

Table 2 shows that the highest sensitivity is at 460 nm wavelength which is $0.0012 \text{ (mg/dL)}^{-1}$. Although all tested wavelength demonstrate a similar pattern of absorbance due to substance in the sample, amount of the light absorb is difference, thus exhibit difference sensitivity performance. For accuracy performance, 525 nm wavelength spectrophotometer system produces accuracy above 90%. The result agrees well with what has been reported in the previous literature where less error will occur when the system has absorbance value within the recommended range, which are between 0.2 and 0.8 [22].

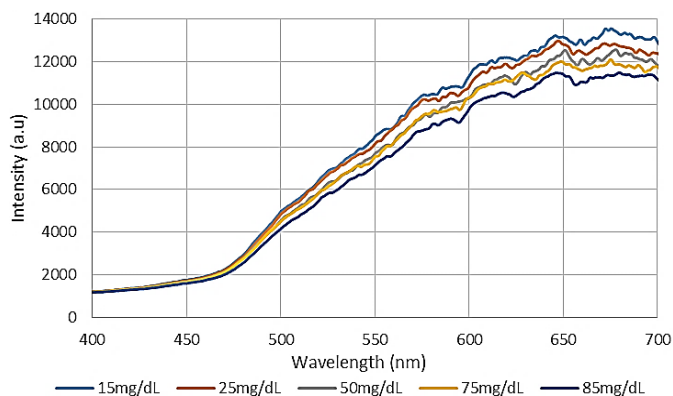


Figure 6. Intensity spectra of different uric acid concentration

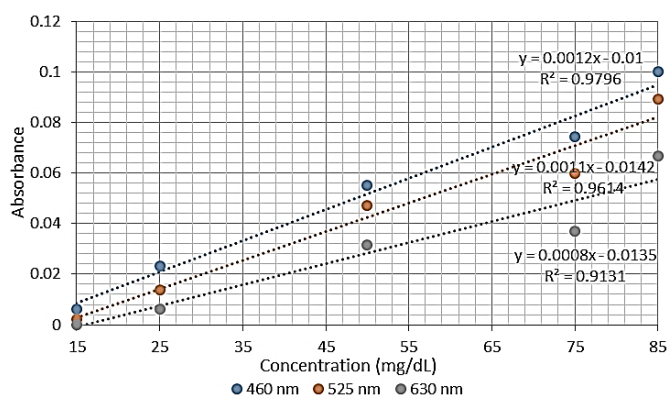


Figure 7. Absorbance analytical curve

Table 2. Spectrophotometer performance for selected tested wavelength

Wavelength (nm)	Sensitivity (mg/dL) ⁻¹	Accuracy (%)
460	0.0012	86.51
525	0.0011	91.17
630	0.0008	84.27

5. CONCLUSION

The research study was carried out to analyze sensitivity and accuracy performance of spectrophotometer system at different sampled wavelength using absorbance analytical technique. The absorption was calculated using Beer's Lambert law formula. Through the analysis, the spectrometer was able to observe the current concentration of uric acid and its absorption wavelength at visible light region. In this work, spectrophotometer system operated at 460nm wavelength has the highest sensitivity while only system at 525 nm wavelength has accuracy higher than 90%. As discussed, the accuracy is influenced by limitation of Beer-Lambert law where absorbance value beyond 0.2 and 0.8 will have large error. The spectrophotometer system with visible light source will offer a simple and economical uric acid detection system and can be applied in biomedical application.

ACKNOWLEDGEMENTS

The authors are grateful to Universiti Tun Hussein Onn Malaysia (UTHM) for supporting this research work under Postgraduate Research Grant (GPPS), grant no: H408 and Research University Grant (RUG) TIER 1 Scheme, grant no: H162.

REFERENCES

- [1] Azmi N. E., et al., "A simple and sensitive fluorescence based biosensor for the determination of uric acid using H₂O₂-sensitive quantum dots/dual enzymes," *Biosensors and Bioelectronics*, vol. 67, pp. 129-133, 2015.
- [2] Pormsila W., S. Krähenbühl, P. C. Hauser, "Capillary electrophoresis with contactless conductivity detection for uric acid determination in biological fluids," *Analytica Chimica Acta*, vol. 636, no. 2, pp. 224-228, 2009.

- [3] Zhuang Q. Q., et al., "Peroxidase-like activity of nanocrystalline cobalt selenide and its application for uric acid detection," *International Journal of Nanomedicine*, vol. 12, pp. 3295-3302, 2017.
- [4] Boroumand S., M. A. Chamjangali, G. Bagherian, "Double injection/single detection asymmetric flow injection manifold for spectrophotometric determination of ascorbic acid and uric acid: Selection the optimal conditions by MCDM approach based on different criteria weighting methods," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 174, pp. 203-213, 2017.
- [5] Liao L. T., et al., "Evaluation of an electrochemical biosensor for uric acid measurement in human whole blood samples," *Clin Chim Acta*, vol. 436, pp. 72-77, 2014.
- [6] Pleskacova A., et al., "Simultaneous Determination of Uric Acid, Xanthine and Hypoxanthine in Human Plasma and Serum by HPLC-UV: Uric Acid Metabolism Tracking," *Chromatographia*, vol. 80, no. 4, pp. 529-536, 2017.
- [7] Rocha D. L., Rocha F. R., "A flow-based procedure with solenoid micro-pumps for the spectrophotometric determination of uric acid in urine," *Microchemical Journal*, vol. 94, no. 1, pp. 53-59, 2010.
- [8] Hamzah H. H., et al., "Spectrophotometric Determination of Uric Acid in Urine Based-Enzymatic Method Uricase with 4-Aminodiphenylamine Diazonium Sulfate (Variamine Blue RT Salt)," *Journal of Analytical & Bioanalytical Techniques*, vol. S7, no. 011, 2013.
- [9] Villa J. E., Poppi R. J., "A portable SERS method for the determination of uric acid using a paper-based substrate and multivariate curve resolution," *Analyst*, vol. 141, no. 6, pp. 1966-1972, 2016.
- [10] Jin D., et al., "Quantitative determination of uric acid using CdTe nanoparticles as fluorescence probes," *Biosensors and Bioelectronics*, vol. 77, pp. 359-365, 2016.
- [11] Yu J., et al., "A novel chemiluminescence paper microfluidic biosensor based on enzymatic reaction for uric acid determination," *Biosensors and Bioelectronics*, vol. 26, no. 7, pp. 3284-3289, 2011.
- [12] Ballesta-Claver J., et al., "Disposable biosensor based on cathodic electrochemiluminescence of tris (2,2-bipyridine) ruthenium (II) for uric acid determination," *Analytica Chimica Acta*, vol. 770, pp. 153-160, 2013.
- [13] Pavliček V., et al., "Very fast electrophoretic determination of creatinine and uric acid in human urine using a combination of two capillaries with different internal diameters," *Electrophoresis*, vol. 35, no. 7, pp. 956-961, 2014.
- [14] Fanjul-Bolado P., et al., "Uric Acid Determination by Adsorptive Stripping Voltammetry on Multiwall Carbon Nanotubes Based Screen-Printed Electrodes," *Electroanalysis*, vol. 27, no. 5, pp. 1276-1281, 2015.
- [15] Langsi V. K., et al., "Synthesis and characterisation of non-bonded 1.7 μm thin-shell (TS1. 7-100nm) silica particles for the rapid separation and analysis of uric acid and creatinine in human urine by hydrophilic interaction chromatography," *Journal of Chromatography A*, vol. 1506, pp. 37-44, 2017.
- [16] Khajehsharif H., et al., "The comparison of partial least squares and principal component regression in simultaneous spectrophotometric determination of ascorbic acid, dopamine and uric acid in real samples," *Arabian Journal of Chemistry*, vol. 4, no. 2, pp. S3451-S3458, 2014.
- [17] Martinez-Pérez D., et al., "A reagent less fluorescent sol-gel biosensor for uric acid detection in biological fluids," *Analytical Biochemistry*, vol. 322, no. 2, pp. 238-242, 2003.
- [18] Yamaguchi T., et al., "Spectrophotometric determination of uric acid based on fading of o-hydroxyhydroquinonephthalen-palladium (II)-hexadecyltrimethyl-ammonium complex," *Analytical Sciences*, vol. 23, no. 2, pp. 223-226, 2007.
- [19] Norazmi N., et al., "Uric acid detection using uv-vis spectrometer," *IOP Conf. Series: Materials Science and Engineering*, 2017.
- [20] Hardesty J. H., B. Attili, "Spectrophotometry and the Beer-Lambert Law: An Important Analytical Technique in Chemistry," Collin, 2010.
- [21] Rashid N. C. A., et al., "Spectrophotometer with Enhanced Sensitivity for Uric Acid Detection," *Chinese Optics Letter*, vol. 17, no. 8, pp. 1-5, 2019.
- [22] Marcus T. C., et al., "Alternative wavelength for linearity preservation of Beer-Lambert Law in ozone concentration measurement," *Microwave and Optical Technology Letters*, vol. 57, no. 4, pp. 1013-1016, 2015.
- [23] Turgeon M. L., "Linne & Ringsrud's Clinical Laboratory Science - E-Book: The Basics and Routine Techniques," *Elsevier Health Sciences*, 2015.
- [24] Teque N., "Uric Acid," 2018, [Online]. Available: <https://www.nacalai.co.jp/ss/ec/ECsrchdetl.cfm?Dum=1&syohin=3591592&syubetsu=3>
- [25] Dunn P. F., "Measurement and Data Analysis for Engineering and Science," Third Edition, *CRC Press*, 2014.
- [26] Welz B., et al., "High-Resolution Continuum Source AAS: The Better Way to Do Atomic Absorption Spectrometry," Wiley, 2006.

BIOGRAPHIES OF AUTHORS



Afiqah Yaacob received her Bachelor degree from Universiti Teknologi Mara (UiTM). Currently, she is pursuing her Masters degree in Faculty of Electrical and Electronic Engineering, Universiti Tun Hussein Onn Malaysia (UTHM) in the field of spectrophotometer application.



Nor Hafizah Ngajikin received her B.E and M.E in Electronic Engineering from Universiti Teknologi Malaysia (UTM) in 2001 and 2003 respectively. She was awarded a Ph.D from UTM for her work on MEMS Fabry-Perot optical tunable filter. From 2004-2017, she served as a senior lecturer and researcher at Lightwave Communication Research Group (LCRG), UTM. She is currently a lecturer at Universiti Tun Hussein Onn Malaysia. Her research interest includes an optical devices and sensors for biomedical applications.



Nurfatihah Che Abd Rashid received her B.E and M.E in Electrical Engineering from Universiti Teknologi Malaysia (UTM) in 2015 and 2019 respectively. She has involved in research on spectrophotometric detection for uric acid and soil nutrient applications. She is currently pursuing a PhD degree in Faculty of Electrical and Electronic Engineering, Universiti Tun Hussein Onn Malaysia (UTHM).



Siti Hajar Aminah Ali is a lecturer at Universiti Tun Hussein Onn Malaysia (UTHM). Her work focuses on image processing and neural network. She received her Bachelor of Electrical-Telecommunications Engineering and Master of Electrical-Electronics and Telecommunications from Universiti Teknologi Malaysia (UTM), Skudai, Malaysia. Her highest education is Doctor of Philosophy in Engineering from Kobe University, Japan.



Maslina Yaacob was awarded a Ph.D from Universiti Teknologi Malaysia (UTM) for her work on Wide Range Analysis of Ozone Gas Concentration in Ultraviolet Region. Her B.E and M.E in Electrical Engineering were also awarded from UTM in 2007 and 2010, respectively. Her research interest includes an optical devices and optical sensors for gas sensing applications. She is currently a lecturer and researcher at Universiti Tun Hussein Onn Malaysia.



Suhaila Isaak received her Bachelor Degree in Electrical and Electronic Engineering from Universiti Teknologi Malaysia in 1998. In 2001, she completed her Master in Science (Electronic) from Universiti Putra Malaysia. She was awarded the Ph.D degree in Electrical and Electronics Engineering from University of Nottingham, England in 2011. Her major research interests are high-speed Geiger mode avalanche photodiode, analog integrated CMOS design, VLSI circuit, high speed photon counting on FPGA, and photonic experimental analysis on biological prototype system in visible light range. Currently, she has involved on integrated linear array photon camera as spectroscopy system for macronutrient soil analysis.



Noran Azizan Cholan was born on 31st August 1979 in Segamat, Johor, Malaysia. He received his bachelor degree in Electronics Engineering from Universiti Tenaga Nasional Malaysia in 2002. Afterwards in 2004, he obtained his master degree in Electronics-Telecommunications Engineering from Universiti Teknologi Malaysia. In 2010, he enrolled as a PhD student in Universiti Putra Malaysia. During his PhD study in 2012, he went to Swansea University, UK and The Hong Kong Polytechnic University, Hong Kong for research attachment. He completed his PhD in 2014 before serving as a senior lecturer in Universiti Tun Hussein Onn Malaysia. As of now, he has been authors/co-authors for 25 journal and 16 conference proceeding papers. His research interests include lasers, amplifiers, optics modeling, microwave optics and optical sensors.