ATLANTIS PRESS

Advances in Social Science, Education and Humanities Research, volume 253

3rd Asian Education Symposium (AES 2018)

Development of Low-Cost Spectrophotometry Laboratory Practice Based on the Digital Image for Analytical Chemistry Subject

M. Lutfi Firdaus, Deni Parlindungan, Agus Sundaryono
Graduate School of Science Education
University of Bengkulu
Bengkulu, Indonesia
Email: lutfi@unib.ac.id

Muhammad Farid
Department of Physics
University of Bengkulu
Bengkulu, Indonesia
mfarid@unib.ac.id

Lena Rahmidar, Maidartati Maidartati
Department of Nursing
Universitas BSI
Bandung, Indonesia
lena@bsi.ac.id

Hermansyah Amir
Department of Chemical Education
University of Bengkulu
Bengkulu, Indonesia
hermansyah1962@gmail.com

***Abstract*—A module for laboratory practice in quantitative analytical chemistry subject has been developed. The method uses digital image colorimetry as a basis for low-cost spectrophotometry analysis. Reduction-oxidation reaction of silver nanoparticle and mercury (II) ion was used as a case study for the colorimetric experiment. The yellow color of silver nanoparticle was faded linearly to colorless with the addition of mercury ion as an analyte. The color change was then recorded with a digital camera and processed through computer software to extract the color information in the form of Red, Green and Blue. The module of laboratory practice was then applied to students in chemical education study program. The gain of the learning process was measured using pre-test and post-test. The results of the study indicate that the students were able to understand the concept of spectrophotometry by using the developed module of digital image colorimetry. Therefore, we conclude that laboratory practice of digital image colorimetry is applicable as an alternative to the traditional spectrophotometry.**

Keywords—laboratory practice; digital image; silver nanoparticle; spectrophotometry; mercury

I. INTRODUCTION

In chemistry, laboratory practice and experiment are important to achieve learning goals and to enrich student's cognitive, affective and psychomotor skills. The concepts and theories that were introduced in the classroom will be observed and verified directly by the students using an actual experiment in the laboratory. Furthermore, students are able to preserve the chemical knowledge for longer when they do and see the experiments being executed in front of their eyes. To do a laboratory practice, the presence of learning media and instruments is mandatory [1-3]. However, the cost to purchase some instruments is too high for most of the institution in developing countries. Also, for sustainability purpose, continues innovation in the chemical experiment has to be conducted in order to achieve a safer practice for environments. Chemical reagents from laboratory activities contribute to further environmental problem if not processed appropriately. The processing procedure of chemical waste is also complicated and high cost. Nowadays, the concern about the reduction of chemical substances consumption and energy saving are increasing to save our environments. Green chemistry is a term that becoming more popular because it gives us a greener alternative to the conventional laboratory practice and experiment. Some examples of green chemistry practices include prevention of chemical waste, designing safer chemicals, safer solvents, and safer energy [4–6]. Therefore, the search of an alternative low cost and environmentally benign learning media and laboratory practices without sacrificing the targetted chemical skill is crucial.

Recently, the use of digital cameras, such as the one from a smartphone, as a detector on green chemical analysis are getting popular due to its availability, portability and low cost [7–10]. Digital image-based quantitative analysis uses the color change of analyte and reagent that is linearly correlated to the concentration change of interest chemical compound. The change of the color then recorded by using the detector on the smartphone, digital camera, scanner, handycam or other colored-image capture devices that have a complementary metal-oxide-semiconductor (CMOS) or charge-coupled devices (CCD) sensors [11]. Since the method utilizes color change as an indicator for quantitative analysis, the method is often called as a digital-image based colorimetry [10]. The method employs the red–green–blue (RGB) color model that

Copyright © 2019, the Authors. Published by Atlantis Press.
This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

has values from 0 up to 255 (8 bits). Mathematical models based on Lambert-Beer law for digital images or RGB values can also be employed to construct analytical curves on absorbance versus concentration in quantitative determinations. This method has been applied successfully on determination of inorganic [10,12] and organic [13–16] samples.

Here we develop a new module for laboratory practice to be used in quantitative analytical chemistry experiment based on digital image colorimetry for undergraduate students. As a case study, we use silver nanoparticles (AgNPs) as a coloring agent for mercury (II) ion analysis. The experiment is proceeded by theoretical courses in regular class on the topics of analytical chemistry and green chemistry.

II. METHOD

A. Research Design

This research used quasi experimental with one group pre-test post-test design. The laboratory practice was conducted to 35 students of second-year undergraduate (as a research subject) with the help from four laboratory assistants. The designated time for the practice was three hours, including pre-test, main experiment activities and post-test. Data were collected by two instruments, namely questionnaires and open-ended questions. Both instruments were given at pre-test and post-test sessions.

B. Preparation of Silver Nanoparticles

As a sample, we focused on a toxic heavy metal Hg(II) analysis by using silver nanoparticles (AgNPs) as a probe and coloring agent. Silver nitrate was used as a precursor for AgNPs biosynthesis, while HgCl2 was used as the analyte of interest. Both compounds were purchased from Merck (Germany). The yellow color of AgNPs will disappear with the addition of Hg(II). AgNPs were synthesized using plant extract. The detail procedures were published elsewhere [17,18].

C. Digital Image-Based Colorimetry

The synthesized AgNPs was mixed with different concentration of Hg(II) ions to make a calibration curve. The solution in a mixture was then placed in a cuvet and ready for spectrometry analysis (Biospectrometer, Eppendorf) and smartphone camera shoot (Samsung J2). Image from a digital camera was secured in a memory card and relocated to the laptop using a card reader.

TABLE I. STANDARD CALIBRATION FOR DIGITAL IMAGE COLORIMETRY

[Hg(II)] (ppm)	Red	Green	Blue	I_R	I_G	I_B
0	121.5	120.5	76.5	0	0	0
10	119.3	124.6	88.9	-8.1	14.5	65.4
20	119.8	128.9	101.9	-6.4	291	124.4
30	123.6	134.2	110.2	7.3	46.7	158.4
40	122.7	136.1	119.8	4.2	52.8	194.6
50	131.6	145.0	130.2	3.4	80.4	231.1
60	136.5	150.7	140.9	50.6	97.1	265.3

The specific image area in a square was selected to average the Red, Green and Blue color values. The average R, G and B values were calculated using Matlab software (MathWorks).

III. RESULTS AND DISCUSSION

Table 1 shows the result of average RGB color values for designated standard concentrations of Hg(II) ion. The plot of RGB values against concentration initially produces a hyperbolic curve. To retrieve a linear line for further simple calculation, such as standard calibration curve, the initial RGB values were transformed to logarithmic ratios following the Lambert-Beer law derivation equation as follow:

$$I_R = log(\frac{R_0}{R_S})$$

$$I_G = log(\frac{G_0}{G_S})$$

$$I_B = log(\frac{B_0}{B_S})$$

I_R, I_G and I_B are the intensity for Red, Green and Blue, respectively. R_0, G_0, B_0, and R_s, G_s, B_s are the Red, Green and Blue values of blank and sample, respectively. Here, digital camera functions as a spectrophotometer analyzing the light passing through the Hg(II) solution. The word 'intensity' here is the number of RGB values of the digital image and, thus, different from that used in UV-Visible spectrometry. More detail procedure was published elsewhere [19].

The intensity of RGB color values against various Hg(II) concentrations is shown at Figure 1. The highest slope of the calibration equation for the system was Blue color which is the complementary color to the yellowish-brown of AgNPs. Therefore, all students used the Blue color for further calculation to achieve the best sensitivity. On the other hand, Green and Red color values give poor correlation and sensitivity. The log conversion of RGB value against various concentrations is shown in Figure 1 (upper panel). The accuracy of the new method was comparable to the conventional method, i.e. spectrophotometry, as shown on the Figure 1 (lower panel).

From the study, we found some of the questions that were hard to asnwer by students, as follow:

- What is the performance (accuracy and precission) of digital image-based colorimetry compared to spectrophotometry?
- Why is the maximum absorbance of yellowish-brown AgNPs appear at Blue (420 nm) wavelength?
- How can you justify that the RGB color values extracted from a smartphone can be used to measure the concentration of a sample?

Explain the mechanism take place on the Hg(II) ion determination using AgNPs as a probe.

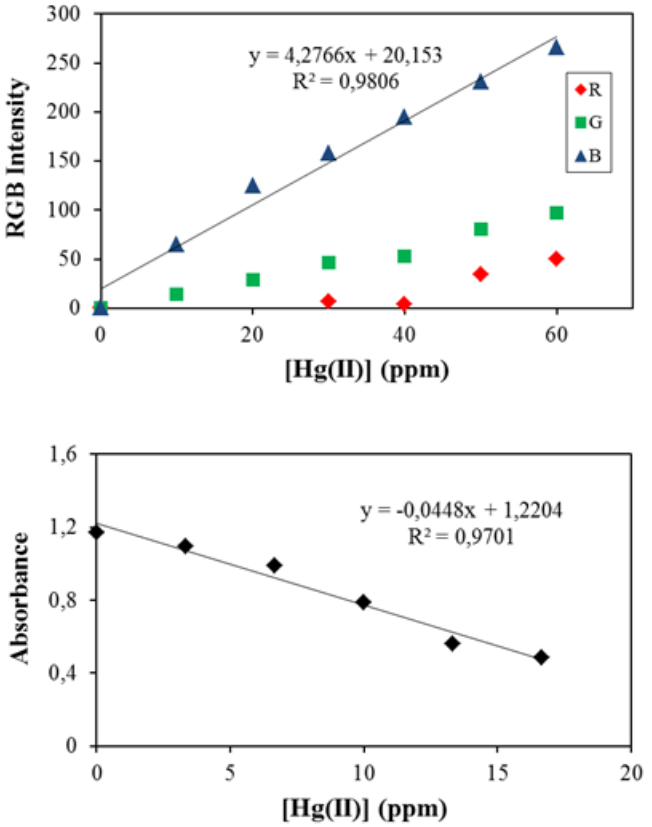

Fig. 1. The results of calculated RGB intensity using a digital camera (upper panel), and absorbance measurement using conventional visible spectrophotometer (lower panel).

TABLE II. RESULTS OF PRE- AND POST-TEST OF THE LABORATORY PRACTICE

Parameter	Pre-test	Post-test
Number of students (*n*)	35	35
Total Sum	2770	3018
Average Score	76	84
Standard Deviation	2.5	2.3
Minimum Score	75	80
Maximum Score	80	87

Questionnaires were analyzed qualitatively to obtain the description of the digital image-based colorimetry. On the other hand, Table 2 shows the results of question answer at pre-test and post-test sessions. The number of students involved were 35 students. From our study, we can see that after laboratory practice, there is a positive shift on student's knowledge. While the standard deviation were comparable, the average scores for pre-test and post-test were 76 and 84, respectively. The minimum score at pre-test (i.e. 75) were increase to 80 after the laboratory experiments. The highest score at post-test was 87, pointing out that the developed module for laboratory practice was successfully implemented to achieve the learning goals.

IV. CONCLUSION

Based on our study, we concluded that the developed module for laboratory practice on the topic of image-based colorimetry is adequately fulfilled the targeted learning outcomes to study the concept of chemistry on the subject of spectrophotometry. The ability to understand and to answer the question analyzed from questionnaire and open-ended questions at pre-test compared to post-test improved from 76 to 84 score. In addition, the new modul of laboratory practice significantly reduces the usage of chemicals, capital cost, and electric power.

REFERENCES

[1] V. Talanquer, "Chemistry Education: Ten Facets To Shape Us," J. Chem. Educ., vol. 90, no. 7, pp. 832–838, Jul. 2013.

[2] J. Henderleiter and D.L. Pringle, "Effects of Context-Based Laboratory Experiments on Attitudes of Analytical Chemistry Students," J. Chem. Educ., vol. 76, no. 1, pp. 100–106, 1999.

[3] R.C. Wedlich, A.E. Libera, A. Pires, and C. Tellarini, "Good Laboratory Practice. Part 2. Recording and Retaining Raw Data," J. Chem. Educ., vol. 90, no. 7, pp. 858–861, Jul. 2013.

[4] P. Anastas and N. Eghbali, "Green Chemistry: Principles and Practice," Chem. Soc. Rev., vol. 39, no. 1, pp. 301–312, 2010.

[5] H.M. Mohamed, "Trends in Analytical Chemistry Green , environment-friendly , analytical tools give insights in pharmaceuticals and cosmetics analysis," Trends Anal. Chem., vol. 66, pp. 176–192, 2015.

[6] R.A. Sheldon, "Fundamentals of green chemistry: efficiency in reaction design," Chem. Soc. Rev., vol. 41, no. 4, p. 1437, 2012.

[7] N. Moonrungsee, S. Pencharee, and N. Peamaroon, "Determination of Iron in Zeolite Catalysts by a Smartphone Camera-Based Colorimetric Analyzer," Instrum. Sci. Technol., vol. 9149, no. January, p. 10739149.2015.1137587, 2016.

[8] V. Oncescu, D. O'Dell, and D. Erickson, "Smartphone based health accessory for colorimetric detection of biomarkers in sweat and saliva.," Lab Chip, vol. 13, no. 16, pp. 3232–8, Aug. 2013.

[9] P. Jarujamrus et al., "Use of a Smartphone as a Colorimetric Analyzer in Paper-based Devices for Sensitive and Selective Determination of Mercury in Water Samples," Anal. Sci., vol. 34, no. 1, pp. 75–81, 2018.

[10] M.L. Firdaus, W. Alwi, F. Trinoveldi, I. Rahayu, L. Rahmidar, and K. Warsito, "Determination of Chromium and Iron Using Digital Image-based Colorimetry," Procedia Environ. Sci., vol. 20, pp. 298–304, 2014.

[11] R.J. Meier, L.H. Fischer, O.S. Wolfbeis, and M. Schäferling, "Referenced luminescent sensing and imaging with digital color cameras: A comparative study," Sensors Actuators B Chem., vol. 177, pp. 500–506, 2013.

[12] H. El Kaoutit, P. Estévez, F.C. García, F. Serna, and J. M. García, "Sub-ppm quantification of Hg(II) in aqueous media using both the naked eye and digital information from pictures of a colorimetric sensory polymer membrane taken with the digital camera of a conventional mobile phone," Anal. Methods, vol. 5, no. 1, pp. 54–58, 2013.

[13] R.C. de Sena, M. Soares, M.L.O. Pereira, R.C.D. da Silva, F F. do Rosário, and J.F.C. da Silva, "A simple method based on the application of a CCD camera as a sensor to detect low concentrations of barium sulfate in suspension.," Sensors, vol. 11, no. 1, pp. 864–75, 2011.

[14] Journal ., "Digital Image Processing of Palm Oil Fruits International Journal of Food Digital Image Processing of Palm Oil Fruits," no. September 2015, 2006.

[15] P. Macedo dos Santos and E. R. Pereira-Filho, "Digital image analysis – an alternative tool for monitoring milk authenticity," Anal. Methods, vol. 5, no. 15, p. 3669, 2013.

[16] P. Masawat, A. Harfield, and A. Namwong, "An iPhone-based digital image colorimeter for detecting tetracycline in milk," Food Chem., vol. 184, pp. 23–29, 2015.

[17] M.L. Firdaus, I. Fitriani, S. Wyantuti, Y.W. Hartati, R. Khaydarov, J.A. McAlister, and T. Gamo, Colorimetric detection of mercury (II) ion in aqueous solution using silver nanoparticles. Analytical Sciences, vol. 33, no. 7, pp. 831-837, 2017.

[18] M. Firdaus, S. Andriana, W. Alwi, E. Swistoro, A. Ruyani, and A. Sundaryono, Green synthesis of silver nanoparticles using Carica Papaya fruit extract under sunlight irradiation and their colorimetric detection of mercury ions. In Journal of Physics: Conference Series Vol. 817, No. 1, p. 012029), 2017

[19] M.L. Firdaus, W. Alwi, F. Trinoveldi, I. Rahayu, L. Rahmidar, and K. Warsito, "Determination of Chromium and Iron Using Digital Image-based Colorimetry," Procedia Environ. Sci., vol. 20, pp. 298–304, 2014.