Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia Vol. 10 No. 3 December 2023, 272-279
DOI: 10.20473/jfiki.v10i32023.272-279
Available online at https://e-journal.unair.ac.id/JFIKI/

Development of Colloidal Silver-based Mercury Sensors in Whitening Cream

Muhammad Hilmi Afthoni[1,2], Sherly Yunita[1], Eva Monica[1]*
[1]Department of Pharmacy, Faculty of Science and Technology, Machung University, Malang, Indonesia
[2]Department of Clinical and Community Pharmacy, Faculty of Pharmacy, University of Jember, Jember, Indonesia

*Corresponding author: eva.monica@machung.ac.id

Submitted: 26 March 2023
Revised: 16 October 2023
Accepted: 18 December 2023

Abstract

***Background**: Mercury, a hazardous heavy metal known for its toxicity to the human body, finds application in cosmetics due to its capacity to inhibit melanin formation. Traditional mercury analysis relies on resource-intensive and time-consuming instrumentation. **Objective**: This study aims to devise cost-effective and practical sensors for mercury detection. **Methods**: The sensor development process involves immobilizing the sensor onto paper, reacting it with mercury, scanning the outcome using a scanner, and subsequently quantifying RGB values using the ImageJ software. **Results**: Optimization of reagent concentrations gave a ratio of methylene blue, $AgNO_3$, gallic acid, and ascorbic acid at 0.5:7:1.5:1 generating the best results. Additionally, pH optimization within the range of 5 to 9 demonstrates stability without necessitating the inclusion of a buffer solution. Notably, the blue variant exhibits superior responsiveness during concentration optimization. Characterization of the sensor reveals a response time of 3 minutes and minimal interference of 2.145% from other substances. The sensor exhibits a linearity range of 0.5-250 ppm, regression equation $y = 8.603x + 21.124$, an R-value of 0.994, and an exceedingly low p-value of $6.9924589548512 \times 10^{-9}$. The sensor boasts a limit of detection (LOD) of 0.206 and a limit of quantification (LOQ) of 0.265, indicative of its precision. Further assessments reveal a percent relative standard deviation (% RSD) precision of 2.017% and a recovery rate of 96.14%. **Conclusion**: The sensor has exhibited stability for over one month under room temperature storage conditions. A comparison between the UV-Vis spectrophotometer and the sensor signifies no significant difference between the two methods.*

***Keywords**: $AgNO_3$, colloidal silver, mercury, methylene blue, sensor*

How to cite this article:
Afthoni, M. H., Yunita, S. & Monica, E. (2023). Development of Colloidal Silver-based Mercury Sensors in Whitening Cream. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*, 10(3), 272-279. http://doi.org/10.20473/jfiki.v10i32023.272-279

INTRODUCTION

Mercury, a chemical element, finds application in facial whitening creams owing to its capacity to impede the melanin formation process, known as melanogenesis. Melanogenesis represents a fundamental physiological pathway responsible for the generation of melanin, a pigment that absorbs light and plays a pivotal role in determining human skin colour and hair pigmentation. The melanin synthesis pathway is reliant on the enzymatic activity of tyrosinase, an enzyme with a copper-dependent mechanism responsible for converting tyrosine into melanin. Tyrosinase, being a glycoprotein, undergoes an essential N-glycosylation process to attain its active enzymatic state. Following cleavage by glucosidase, glycosylated tyrosinase assumes a properly folded conformation, rendering it amenable to active transport facilitated by Cu^{2+} within the Golgi apparatus before reaching melanosomes. Notably, inorganic forms of Mercury operate as solid inhibitors of melanin production by competing with or displacing copper ions, impairing the catalytic activity of the tyrosinase enzyme in melanin synthesis.Ultimately, this inhibition leads to the achievement of a brighter skin complexion (Haryanti et al., 2020).

Mercury can be analyzed using several instruments, including Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Gas Chromatography Coupled to Atomic Absorption Spectrometry (GC-AAS), Cold Vapor Atomic Absorption Spectrometry (CV-AAS), Atomic Fluorescence Spectrometry (AFS), and Anodic Stripping Voltammetry (ASV) (Kristianingrum, 2009). However, analysis with this technique is costly, and the process is quite complicated. Therefore, researchers have developed colloidal silver-based chemical sensors. Colloidal silver is a nanotechnology-based product that is currently being developed and can be applied as a catalyst and optical sensor detector. The reaction mechanism in colloidal silver is that when Hg+ ions are added to the solution, an oxidation-reduction reaction will occur between Ag^0 and Hg^+ ions (Kumar *et al.*, 2017). In manufacturing colloidal silver-based sensors, methylene blue is used as a marker to detect the presence of mercury, and gallic acid is used as a capping agent so that the particles become more stable by preventing aggregation. Based on this, this study developed a colloidal silver-based chemical sensor that can detect the presence of Mercury in cosmetic products quickly, simply, and economically.

MATERIALS AND METHODS

Materials

$HgNO_3$ (Loba Chemie), methylene blue (Merck), ascorbic acid (Merck), $AgNO_3$, gallic acid (Merck), demineralized water (Hydrobatt), methanol (Merck), Whatman paper (paint No.1), phosphate buffer solution pH 5-9, dithizone (Merck), sodium hydroxide (Merck), hydrochloric acid (Merck), whitening cream samples, mica.

Tools

UV-Vis spectrophotometer (JASCO V-760), cuvette, scanner (canon LiDE 300), analytical balance (OHAUS), 50, 100, and 250 mL beakers glass (pyrex), 5, 10, 50, and 100 mL volumetric flasks (pyrex), 50 mL measuring glass (pyrex), ultrasonic (Mosinix USA), pH meter (OHAUS), drip plate, micropipette 100-1000 l and 1000-5000 l (socorex), dropper pipette, volume pipette (pyrex), ball filler, stir bar, tweezers, vial, punch hole, hairdryer, stopwatch, scissors.

Method

Sensor immobilization

The immobilization technique used is adsorption, which is done by soaking the paper in the reagent for 24 hours. The sensor paper was dried using a hairdryer. The dried paper is stored at room temperature (25℃) to maintain its stability. Paper that has been immobilized and dried is used for sensor optimization, sensor characterization, and testing on samples.

Strip test fabrication

The mercury detection strip test in cosmetic samples consists of two parts, namely the handle and the detection area as shown in Figure 1. The handle of the strip test is made of non-absorbent mica with a size of 7.5 cm x 1 cm. The detection area is made of paper glued to the bottom of the handle of the strip test.

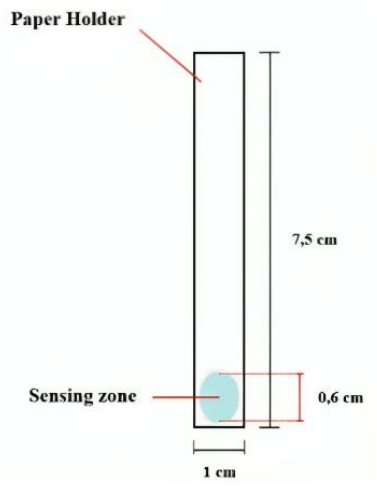

Figure 1. Strip test design

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E-ISSN: 2580-8303

Table 1. Mercury detection reagent formula

Reagent	Volume (mL) pipetted in 10 mL							
	F1	F2	F3	F4	F5	F6	F7	F8
Methylene Blue (3,127 x 10^{-3} M)	-	0.5	-	0.5	-	0.5	-	0.5
$AgNO_3$ (5,887 x 10^{-3} M)	5	5	6	6	7	7	8	8
Ascorbic Acid (0,1 M)	1	1	1	1	1	1	1	1
Gallic Acid (0,1 M)	4	3.5	3	2.5	2	1.5	1	0.5

Chemical sensor optimization

Optimization of reagent concentration

Optimization of reagent concentration was done by making reagents with variations of the formulas made in Table 1 using immobilization techniques. Each reagent that has been made was immersed in paper for 24 hours. After that, the immobilized paper was reacted with 250 ppm Mercury and then measured. All of the experiments were carried out in triplicate. Then, each formula was measured for its RGB value, and the highest RGB value was observed using the ImageJ program.

Optimization of pH

Optimization of pH was done by making a phosphate buffer solution with a pH of 5, 6, 7, 8, and 9. Furthermore, the sensor paper that had been immobilized was added with a buffer solution of various pH and drops of Mercury with a concentration of 250 ppm. Then the RGB value was measured, and the highest mean RGB value was observed using the ImageJ program (Hermanto *et al*., 2019). All of the experiments were carried out in triplicate.

Optimization of test concentration

Optimization of test concentration was carried out by making mercury concentrations of 0.5, 1, 5, 10, 50, 100, and 250 ppm. Tests were carried out using sensor paper that had been immobilized and then dripped with various concentrations of Mercury that had been prepared. Then, the colour intensity was measured at each concentration using the ImageJ program to determine the RGB mean value and look for the most linear colour response. Optimal optimization can be done by seeing the colour change and giving the most linear colour response value; all the experiments were carried out in triplicate (Hidayat *et al*., 2017).

Chemical sensor characterization

Response time

The timing of the sensor was done by measuring the time it takes for the reagent to react with Mercury. The response time was determined by dipping the test strip that had been immobilized. The measurements were carried out every minute for 30 minutes using a stopwatch and the RGB values were measured from the scan results using the ImageJ program and the mean RGB values were obtained. The measurement results show that the most optimal time was generated from the mean RGB; all of the experiments were carried out in triplicate (Hermanto *et al*., 2019).

Selectivity

The determination of selectivity was carried out using a mercury standard based on the results of optimization that had been added with interfering components (nipagin, nipasol, and triethanolamine). The selectivity was determined by measuring the Δ mean RGB value using the ImageJ program. Selectivity is good if it has a value of % interference < 5%.

Linearity

Determination of linearity was done by dipping the test strip into the reagent with a test concentration range of 0.5-250 ppm. After that, a linearity curve was made between concentration and Δ mean RGB. From this curve, the value of the regression equation was obtained. Furthermore, the ANOVA data processing was done using Microsoft Excel to determine the p-value (Hermanto *et al*., 2019).

Limit of detection (LOD) dan limit of quantitation (LOQ)

Determination of LOD and LOQ values was done by making several mercury standard solutions with concentrations below the smallest concentration of the linearity test range. Then, the linearity curve between concentration and Δ mean RGB was determined (Hermanto *et al*., 2019).

Precision

The precision value can be determined by calculating the relative standard deviation (RSD) of 6 measurements made using different test strips. Interday testing (repetition on various days) was performed using sample solutions of different concentrations; then, the colour change was measured using the ImageJ program so that the mean RGB value was obtained (Hermanto *et al*., 2019).

Accuracy

Determination of accuracy was carried out by the standard addition method, namely by calculating the % recovery from three times the addition of analyte of 30%, 45%, and 60% of the sample concentration from the test concentration. Next, the cream sample was

P-ISSN: 2406-9388
E-ISSN: 2580-8303

prepared, and then the test strip was dipped in the solution, and the colour change was observed. The Δ mean RGB value of the measurement results is then entered into the regression equation to obtain the mercury concentration in the sample (Hermanto *et al*., 2019).

Stability

Determination of stability was done by storing the sensor at room temperature (25℃). The sensor response was measured every day until it reached a 15% decrease from the original sensor response (Hermanto *et al*., 2019).

Mercury testing using a UV-Vis spectrophotometer

The standard solution was made by weighing 10 mg of Mercury in a 10 mL volumetric flask and then diluting it to various concentrations. Next, a sample solution was made by weighing 100 mg of the cream sample and dissolving it in 100 mL of demineralized water, which resulted in a sample solution with a concentration of 1000 ppm. The sample solution was diluted to 10 ppm by means of a 100 µL pipette dissolved in a 10 mL volumetric flask. After that, it was checked using a UV-Vis spectrophotometer in a way that each of the standard solution and sample was pipetted as much as 100 µL and added with 100 µL of 10 ppm dithizone solution (Jamaluddin & Reazul, 2003).

Sensor application

Tests using the test strip method were carried out by dipping the test strip in each sample that had been prepared by weighing 100 mg of cream and dissolving it in 100 mL of water. Then 0.1 mL of the solution was taken and dissolved in 10 mL of water. After dipping, the results of the color change can be compared with the color results of the mercury standard.

RESULTS AND DISCUSSION

Chemical sensor optimization

The reagent concentration optimization was carried out to obtain reagents that could detect Mercury optimally. In optimizing the reagent concentration, reagents were made with variations of 8 formulas using a combination of methylene blue, $AgNO_3$, ascorbic acid, and gallic acid. Determination of the reagent concentration was carried out on sensor paper that had been immobilized for 24 hours, which was then added with 250 ppm mercury. The results of the reagent concentration optimization experiment can be seen in Figure 2. This optimization experiment was conducted through three replication attempts. The results of reading the RGB values on ImageJ and observing color changes directly by the eye, the optimal reagent concentration is formula 6, because it has the highest Δ mean blue value.

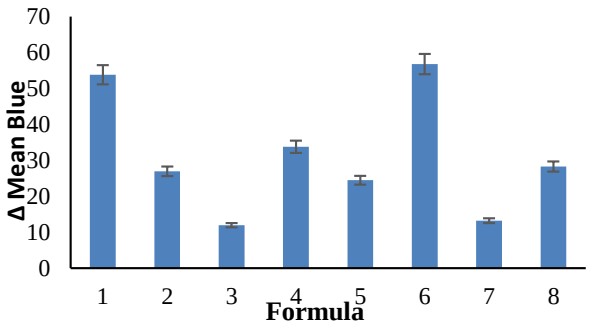

Figure 2. Comparison of reagent concentration to color change

The sensor's pH was optimized to know the optimal working pH on this sensor. The immobilized sensor paper was added with a buffer solution at pH 5, 6, 7, 8, and 9, and then reacted with 250 ppm mercury concentration. The results of reading RGB values using ImageJ and pH optimization data can be seen in Figure 3, where these results indicate that this sensor was not affected by pH because there was no significant change in the five pH values, so this sensor did not need additional buffer.

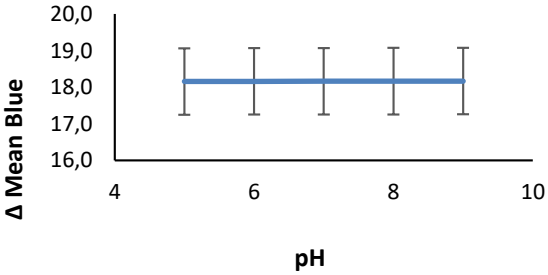

Figure 3. Sensor response at various pH to color change

The test concentration was optimized to know the most linear colour response produced by the sensor. The test concentration was optimized by making a serial solution of mercury concentration with levels of 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 50 ppm, 100 ppm, and 250 ppm. After that, the sensor paper that had been immobilized was reacted with a solution of various levels of Mercury. The results of reading RGB values using ImageJ as well as optimization test concentration data can be seen in Figure 4, where from these results, it can be seen that the blue colour response provides the most optimal response compared to other colours, with an R-value of 0.9923 so that the value of mean blue is used as a characterization reading and sensor testing.

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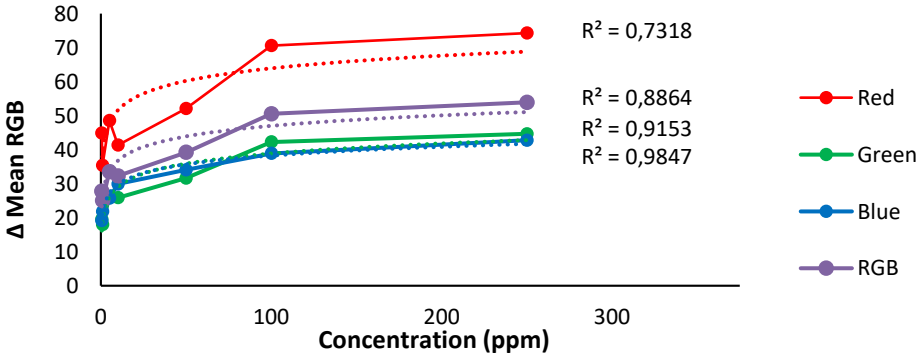

Figure 4. Response of color reading to test concentration

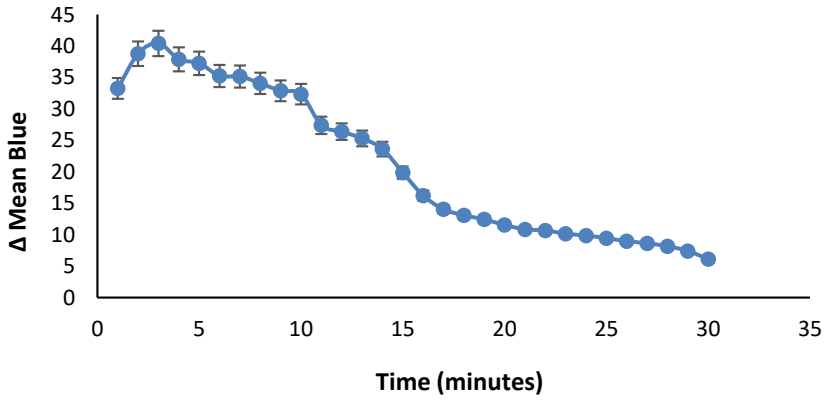

Figure 6. Sensor response time to color change (Δ mean blue)

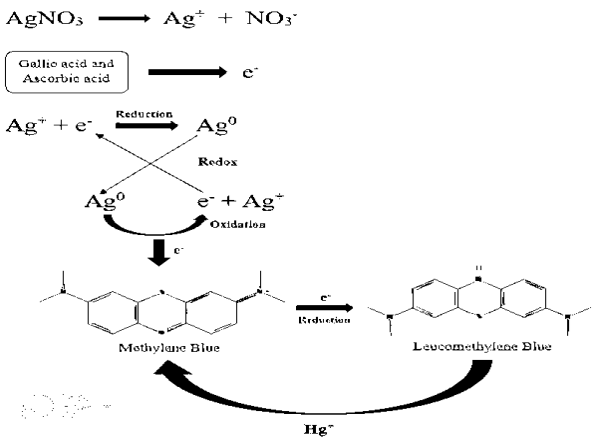

Figure 5. Reaction schematic of mercury with methylene blue, AgNO3, gallic acid, and ascorbic acid

Sensing mechanism

In developing a colloidal silver-based mercury sensor, a reaction occurs between gallic acid and ascorbic acid with $AgNO_3$, which will then reduce methylene blue. The reduction of methylene blue is influenced by colloidal silver, which is formed as a result of the reduction of $AgNO_3$. In the electron transfer process, when there is a significant difference in redox potential between the acceptor and the donor, then there is a possibility of restriction in the electron transfer (Clayden & Greeves, 2012). However, electron transfer will proceed easily if the effective catalyst has an intermediate redox potential between the acceptor and donor. In this case, colloidal silver acts as a mediator of electron transfer and contributes to methylene blue by acting as a redox catalyst. Gallic acid and ascorbic acid reduce Ag^+ from $AgNO_3$ to Ag^0, where colloidal silver acts as an electron transfer mediator and donates to methylene blue as a redox catalyst, also reducing methylene blue to leucomethylene blue (Mona *et al*., 2018). This colourless leucomethylene blue, when it reacts with Hg^+, an oxidation process will occur, which releases electrons, and methylene blue will capture electrons, thus making the blue colour of methylene blue reform, as shown in Figure 5.

Chemical sensor characterization

Response time testing was carried out to measure the time required for Mercury by optimizing the concentration to react with the reagents in the sensor to give the most optimal color response results. This measurement is carried out every minute for 30 minutes where the sensor reacts with a standard Mercury solution with a concentration of 250 ppm. The results of the response time testing can be seen in Figure 6, where the most optimal sensor response time is obtained, which is in the 3rd minute.

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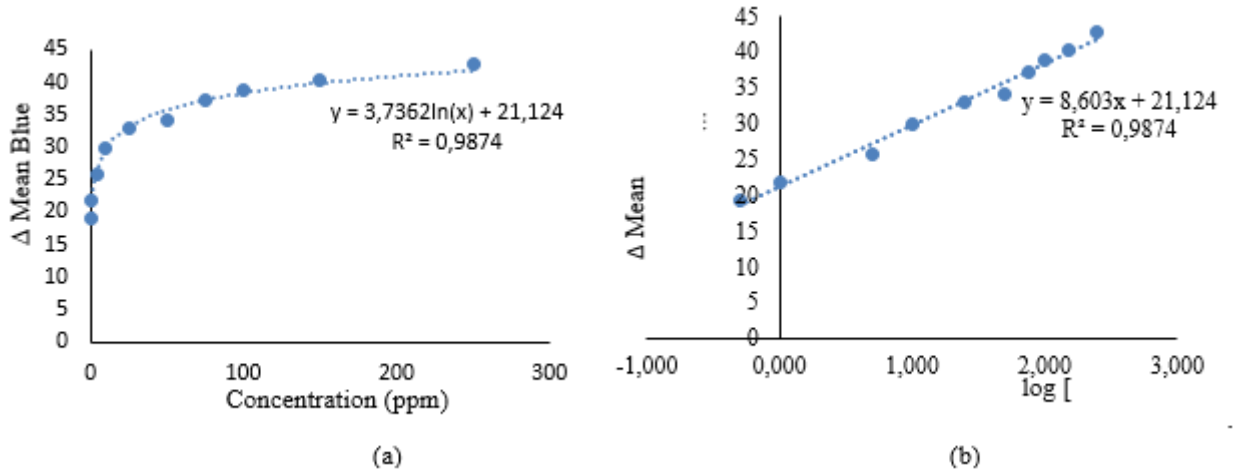

Figure 8. (a) Linearity test results in logarithmic equations; (b) linearity test results in linear equations

The selectivity test was carried out to determine the confounding component's effect. The nuisance components used in this test are usually found in cosmetics, such as methyl paraben, propylparaben, and triethanolamine (TEA). The preparation was done by mixing Mercury with the interfering component, in which 1:1, 1:10, and 1:100 ratios were used. The results of the selectivity test obtained from the presence of 3 interfering components can be seen in Table 2 and Figure 7. The % interference value is 2.145%, where this result is by the requirements of the selectivity test, which is <5%, so it can be said that the method used is not affected by the presence of a nuisance component (Nethercote & Ermer, 2012).

Table 2. Results of comparison of interference values

Comparison	Nipagin	Nipasol	TEA
1 : 1	3,639	0,706	4,193
1 : 10	2,899	1,087	3,176
1 : 100	1,200	1,384	1,017

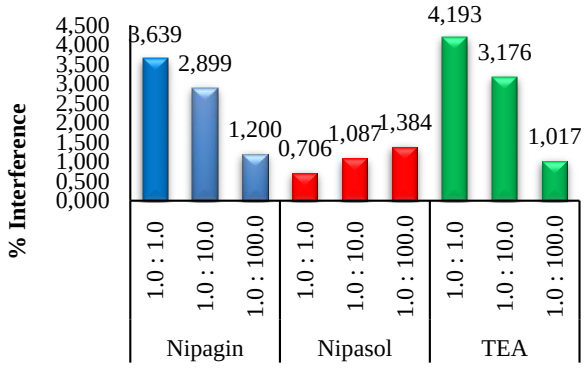

Figure 7. Results % interference selectivity test

The linearity test was carried out to determine the relationship between the detector response and changes in concentration. The concentration in the linearity test ranges from 0.5-250 ppm with 10 test points. Tested using the scanometry method, then measured RGB values in the ImageJ program. From these results, the regression equation y = 3.7362ln(x) + 21,124 is obtained for the logarithmic curve, as shown in Figure 8(a). The regression equation formed becomes y = 8.603x + 21,124, and the R-value is 0.994, as shown in Figure 8(b). After that, the ANOVA test was carried out, which obtained a p-value of 6.9924589548512 x 10-9. The results of the linearity test can be seen in Appendix F, where these results have met the requirements of good linearity, namely the correlation coefficient (r) 0.999, and the p-value of the ANOVA test is less than = 0.01(Nethercote & Ermer, 2012).

LOD is determined to know the smallest amount of analyte or sample in the sample that still gives a significant response to the sensor method. In contrast, the determination of LOQ aims to determine the most minor level or the smallest concentration of Mercury that can still be quantified for precision and accuracy determination. The results obtained from the determination of LOD and LOQ values were 0.206 ppm and 0.625 ppm, respectively.

The precision test was carried out to know the closeness of a series of measurements obtained from the sample. Test precision was determined by calculating the relative standard deviation (RSD). This test was carried out on 3 consecutive days using the same concentration (interday), each with six replications. % RSD resulting from the average precision test was 2.017%, so it can be concluded that this test has met the

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E-ISSN: 2580-8303

requirement since the % RSD was less than 7.3 for a concentration of 10 ppm sample (Huber, 2007).

The accuracy test is carried out to determine the measurement method's accuracy. The accuracy test was carried out using the standard addition method with three replications. The value of % recovery obtained from the average accuracy test is 96.14%, so it can be said that this test meets the requirements % recovery is in the range of 80-110% for a concentration of 10 ppm sample (Huber, 2007).

The stability test was carried out to know the time at which the sensor gave the same and stable reaction to an analyte at the same concentration until the response time of the sensor to the analyte decreased drastically (usually more than 15% of the initial sensor response) (Kuswandi, 2008). The stability test results, which lasted for one month, showed a % decrease in the range of 0.005-0.061, indicating that the sensor is stable because it shows the % decrease, which is still far from 15%.

Comparison of mercury testing using a UV-vis spectrophotometer and sensor

A comparison of mercury testing using the UV-Vis spectrophotometer and the sensor methods was carried out to know if there were significant differences between the two methods. The results of testing the two methods were analyzed using a T-test Two-Sample Assuming Unequal Variances in Microsoft Excel. If the value of < 0.05, then H0 is rejected, meaning there is a significant effect between one independent variable and the dependent variable. Meanwhile, if the value is> 0.05, then H0 is accepted, meaning there is no significant effect between one independent variable and the dependent variable (Miller & Miller, 2010). The results obtained from the five samples can be seen in Table 3. The results of the p-value between the two methods are more than = 0.05, indicating no significant difference between the UV-Vis spectrophotometer method and the sensor method, so this sensor can be used to detect the presence of Mercury.

Sensor application

The sensor was applied to the sample directly to determine the sensor's direct response in detecting the presence or absence of mercury in the sample. The results of the application of the sensor on the sample can be seen in Figure 9, where from these results, it can be seen that the sensor has been optimum in detecting the presence of Mercury due to a change in colour according to the mercury standard.

Table 3. Comparative test results of uv-vis spectrophotometer with sensor

Sample	UV-Vis Spectrophotometer (ppm)	Sensor (ppm)	P (T<=t) *two tail*
1	15,476	15,066	0,692
	15,830	15,596	
	15,013	14,689	
2	13,419	13,092	0,787
	13,275	13,002	
	13,166	13,677	
3	12,507	12,618	0,940
	12,450	12,560	
	14,052	13,772	
4	13,664	12,882	0,112
	13,886	13,243	
	13,865	12,972	
5	15,598	15,453	0,463
	15,821	15,101	
	15,895	15,812	

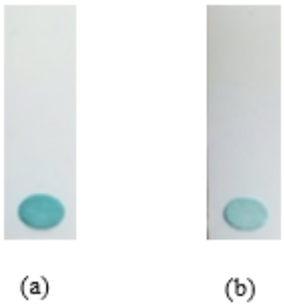

Figure 9. (a) Sensor before reacting; (b) sensor after being reacted with the sample

CONCLUSION

The optimal condition of the sensor is formula 6 with a ratio of methylene blue: AgNO3 : gallic acid: ascorbic acid = 0.5 : 7 : 1.5 : 1. The addition of buffer in this sensor is not required, and blue is the most optimum colour in generating sensor responses based on test concentrations. The sensor has a response time characterization in the 3rd minute, linear in the range of 0.5-250 ppm, LOD of 0.206 ppm and LOQ of 0.265 ppm. This sensor provides selective, precise, and accurate results. The sensor is stable for more than one month on storage at room temperature (25℃). The sensor includes measurement results that are not statistically significant when compared to the UV-Vis spectrophotometer method.

ACKNOWLEDGMENT

The author would like to thank the Pharmacy Laboratory of Ma Chung University which has supported this research by providing good facilities.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

AUTHOR CONTRIBUTIONS

Conceptualization, M. H. A.; Methodology, M. H. A.; Software, M. H. A., S. Y.; Validation, M. H. A., E. M.; Formal Analysis, S. Y.; Investigation, S. Y.; Resources, M. H. A., E. M.; Data Curation, M. H. A.; Writing - Original Draft, S. Y.; Writing - Review & Editing, E. M.; Visualization, S. Y.; Supervision, M. H. A.; Project Administration, E. M.; Funding Acquisition, M. H. A.

FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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P-ISSN: 2406-9388
E-ISSN: 2580-8303