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Colorimetric Sensor of Hg(II) Ion from AgNPs-MO Quantified Using DIC with ED Equation Approach

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Abstract
Mercury is one of the heavy metals that has high toxicity, not only for the environment but also for human health. Mercury is found in many cosmetic products; unfortunately, for people who do not understand the dangers of mercury cosmetic ingredients, their skin will become red rashes. The novelty of this article is to use colorimetric sensor for mercury combined with Digital Image Colorimetry (DIC). The image is captured with the the smartphone camera and quantified using the Euclidean Distance (ED) Equation approach. In addition, the synthesized AgNPs also use the green synthesis method with natural materials as reducing agents and stabilizers. Therefore, the purpose of this study is to determine the best synthesis conditions and determine the detection performance of mercury (II) ions. The research began with the synthesis of mercury detection substances, namely silver nanoparticles reduced and stabilized by Moringa oleifera leaf extract (AgNPs-MO). In this study, it was found that silver nanoparticles began to form at 1 hour accompanied by heating at 120°C using a hotplate, while 6 hours when without heating. The optimum pH condition is 10. This mercury detection method has good sensitivity and accuracy values, with RSD values of less than 1% for each analyte in 5 repetitions.

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INTRODUCTION

The mercury (II) ion is one type of toxic and persistent heavy metal ion that is harmful to the environment and living things. Mercury(II) ions are often used as skin whitening ingredients by several cosmetic manufacturers, even though the use of mercury(II) ions in small doses is able to have a damaging impact on the body, such as skin discoloration, dark spots, allergies, and irritation (Indriaty et al., 2018). Therefore, the Indonesian government limits the use of mercury as stipulated in the Regulation of the Head of the Food and Drug Administration of the Republic of Indonesia Number 17 of 2014 concerning requirements for microbial and heavy metal contaminants in cosmetics. The mercury concentration limit should not exceed 1 mg/kg or 1 mg/L (Safitri et al., 2020).

Despite knowing the dangers of mercury and that its use has been restricted, there are still many cosmetic manufacturers who continue to produce mercury-containing cosmetic products. BPOM banned ingredient in cosmetics most commonly found in the market is mercury, with a percentage of 43.6%. This makes people who do not understand the dangers of cosmetic ingredients victims of the products they buy (Sende et al., 2021).

Based on the explanation above, the analysis of mercury in components of life is very important. So far, mercury(II) ions have been analyzed with atomic absorption

spectrometry (AAS) and inductively coupled plasma mass spectrometer (ICP-MS) instruments (Yaniarty & Wimpy, 2024). Instrument analysis has the disadvantage of complicated operation, high cost, and must be done in the laboratory, so the portability value is low. In order to minimize the impact caused by mercury, real-time monitoring analysis is needed that is easy, economical, and fast (Bakhru Thohir & Sabila, 2021).

A widely developed method to answer the problem is the colorimetric method; this is because it is practical and cost-effective. Colorimetric detection is able to display single or multi-color changes due to the interaction of the detected target molecules (Rohsaita et al., 2024). In recent years, colorimetric detection has been massively applied to the detection of several chemical compounds, such as heavy metals (Susanto & Mudasir, 2020), harmful anions, harmful organic compounds (Nata et al., 2022), and biomolecules (Khumngern et al., 2024).

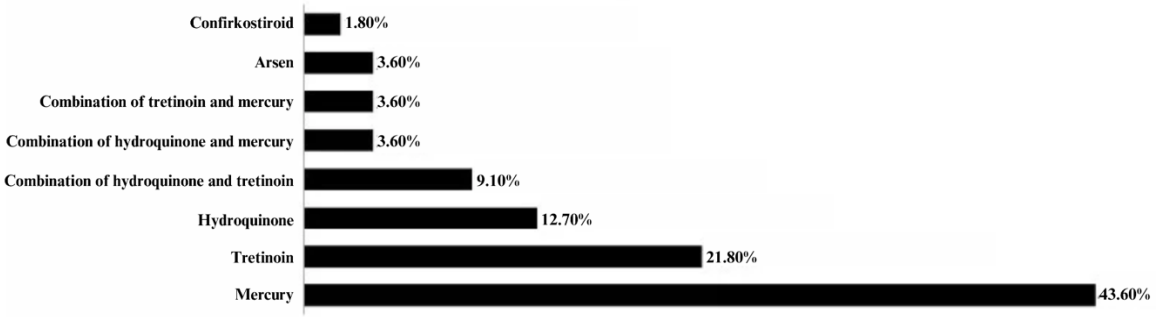

Figure 1. Percentage of banned ingredients in cosmetics (Sende et al., 2021)

Colorimetric detection is easy to observe, as it simply uses the sense of sight (Suharman & Rahayu, 2020). However, colorimetric quantification results have a high subjective value in interpreting color (Effriandi et al., 2019); therefore, in this study, the Euclidean Distance (ED) approach was used in the quantification process. ED will convert colors into RGB numeric values and convert them into numbers that can be read easily (Thohir et al., 2022).

Silver nanoparticles (AgNPs) can be used as a colorimetric detection of Hg(II) ions (Hong et al., 2022). This study uses new renewable materials as reducing agents and stabilizers in the synthesis of silver nanoparticles, namely Moringa leaves (*Moringa oleifera*). The use of natural materials has many advantages; besides being able to substitute hazardous chemicals, it has a role at the same time, so it will be more environmentally friendly and save chemicals (Thohir, 2023). Therefore in this study, AgNPs reduced by *Moringa oleifera* leaves (AgNPs-MO) will be synthesized, which will be used for the colorimetric detection of Hg(II) ions (Nowak, 2023).

The essential question of this research is how the effect of temperature and pH variations on the synthesis stage and how the detection performance. Meanwhile, the aim of this study is to determine the effect of temperature and pH on the synthesis of AgNPs-MO and determine its detection performance on mercury analytes.

METHOD

Tools and Materials

The tools used in this research include a set of glassware, hotplate, analytical balance, magnetic stirrer, and UV-Vis spectrophotometer. The materials used in this study are *Moringa oleifera* leaf powder, silver nitrate ($AgNO_3$), mercury(II) chloride ($HgCl_2$), aquabides (H_2O), and sodium hydroxide (NaOH). The research flow should be presented in this section with an image caption. The image caption is placed as part of the image *title (figure caption*) instead of part of the image. The methods used in completing the study are written in this section.

Synthesis of AgNPs-MO

The aqueous extract of *Moringa oleifera* leaves was taken as much as 15 mL and adjusted the pH to 11 using 0.6 M NaOH (Lomelí-Rosales et al., 2022). Then, it was reacted with 10 mM $AgNO_3$ as much as 1 mL by stirring at 600 rpm with various time variations. Determination of the optimum reaction time in the synthesis of AgNPs-MO was carried out at time variations of 1; 2; 4; 6; and 8 hours. In each time variation, two treatments were given, namely without heating and heating with a *hotplate* temperature of 120 °C. The AgNPs-MO colloids formed were analyzed using a UV-Vis spectrophotometer at a wavelength of 320–700 nm (Janah et al., 2022).

In addition to time and temperature variations, the optimum pH variation for *Moringa oleifera* leaf extract was carried out; the pH applied was 6; 7; 8; 9; 10; 11; and 12.

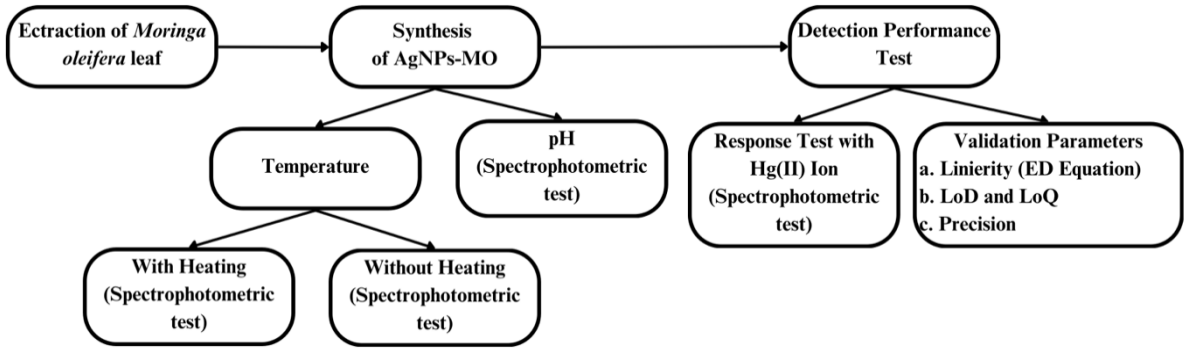

Figure 2. Research procedures

Hg(II) ion detection test and sensor detection performance

AgNPs-MO with optimum conditions was taken as much as 10 mL and put into a 50 mL volumetric flask. Then, distilled water was added until the limit mark. This dilute NP-MO solution was then put into 9 vial glasses of 2 mL each. After that, in each vial glass, 250 mL of Hg^{2+} solution was added with a concentration variation of 0.005, 0.01, 0.05, 0.1, 0.5, 1, and 5 mM. AgNPs-MO that had been contacted with Hg^{2+} solution was analyzed using UV-Vis spectrophotometry at a wavelength of 300–800 nm. In addition, the contact solution was also photographed using the device, and the RGB value was determined using the color picker and converted into an ED value (Thohir et al., 2022).

Meanwhile, the detection performance test was carried out on the detection linearity test variable using Hg(II) ion concentrations of 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, and 3 mM. LoD, LoQ, and Precision.

RESULTS AND DISCUSSION

Synthesis of AgNPs-MO

Determination of the optimum reaction time and temperature has the basic purpose of knowing information about what time and temperature AgNPs-MO could be optimally synthesized. At first, silver nanoparticles were synthesized using *Moringa oleifera* leaf extract as a reducing agent and stabilizing agent. $AgNO_3$, which acts as a precursor for Ag^+ metal ions, will be reduced to Ag^0 (Akintelu et al., 2021). The duration of the reaction between Ag^+ and plant extracts varies greatly depending on the type of plant extract used; this is because each plant has different active and reducing substances. The longer the reaction time, the more nanoparticles will be formed, which will increase the absorbance value (Velmurugan et al., 2014). $AgNO_3$ and plant extracts react to form nanoparticles in 3 hours without heating. The

research from which requires a duration of 1 hour for the formation of silver nanoparticles with heating (Rahmayani et al., 2019).

In this study, the synthesis of AgNPs-MO with heating was carried out with the help of a hotplate at 120 °C. The results of the UV-Vis spectra of colloidal AgNPs-MO are presented in Figure 4a. It can be seen that if accompanied by heating, silver nanoparticles begin to form only after 1 hour, as evidenced by the appearance of an absorption peak at a wavelength of 400 nm. Formation of silver nanoparticles occurs at wavelengths of 400 to 450 nm (Rekso & Sudradjat, 2018). The same thing also happened at reaction times of 2 hours and 4 hours. Unlike the case when the reaction time is 6 hours and 8 hours, there has been a shift in wavelength towards a larger direction (*red shift)*, which indicates an increase in size due to agglomeration in silver nanoparticles, which is likely caused by excessive heat exposure (Alauhdin et al., 2022).

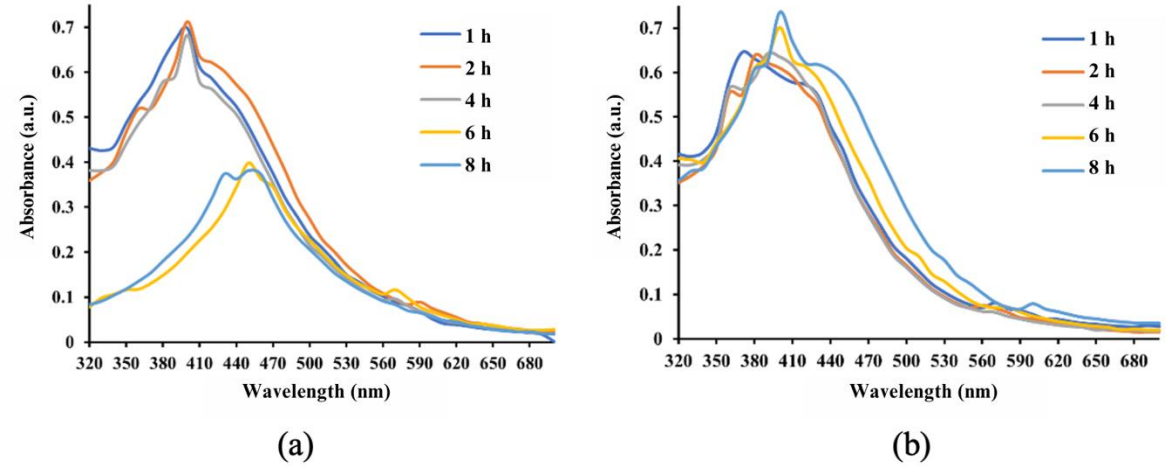

Figure 3. Visible spectrophotometric test results of AgNPs-MO: (a) with heating; (b) without heating.

Meanwhile, the UV-Vis spectra of AgNPs-MO colloids synthesized without heating can be seen in Figure 4b. In the reaction time of 1 hour to 4 hours, silver nanoparticles have not been formed because there is no absorption peak at a wavelength of 400 to 450 nm. Silver nanoparticles are only formed when the reaction time applied reaches 6 hours to 8 hours, as evidenced by the absorption peak at a wavelength of 400 nm. AgNPs has successfully synthesized silver nanoparticles at room temperature with a reaction time of 6 hours for reference (Khan et al., 2018).

Figure 4. Aqueous extract of *Moringa oleifera* leaves that have been conditioned with pH variations of 6 to 12.

Both methods proved to be successful in synthesizing AgNPs-MO; the difference is in the time applied, and for the process of shortening the reaction time, the synthesis condition that uses additional heat energy from outside is chosen. Seen in Figure 4, the silver nanoparticles formed also have spectra that have tails; this is a typical picture when using reducing agents from

natural materials, due to the large number of matrices in natural materials, so the results are not really homogeneous in size (Janah et al., 2021).

The formation of silver nanoparticles is strongly influenced by the pH conditions of the reaction medium (Chugh et al., 2021). The determination of the optimum pH conditions is to obtain the optimum reaction-reducing agent conditions for reducing Ag^{+} to Ag^{0}. pH will help the reducing agent in increasing the number of OH groups, which have a significant effect on the reduction process (Janah et al., 2021). In this study, the aqueous extract of moringa leaves was adjusted at pH ranging from 6 to 12. In this conditioning, there was a change in color, namely the intensity of the yellow color getting thicker, which can be seen in Figure 4. Then, each aqueous extract of moringa leaves that had been adjusted at pH was reacted with $AgNO_3$ to form silver nanoparticles at a reaction time of 1 hour accompanied by *hotplate* heating at 120°C. The colloidal nanoparticles formed were then analyzed using a UV-Vis spectrophotometer at a wavelength of 320 to 700 nm to determine the formation of AgNPs-MO.

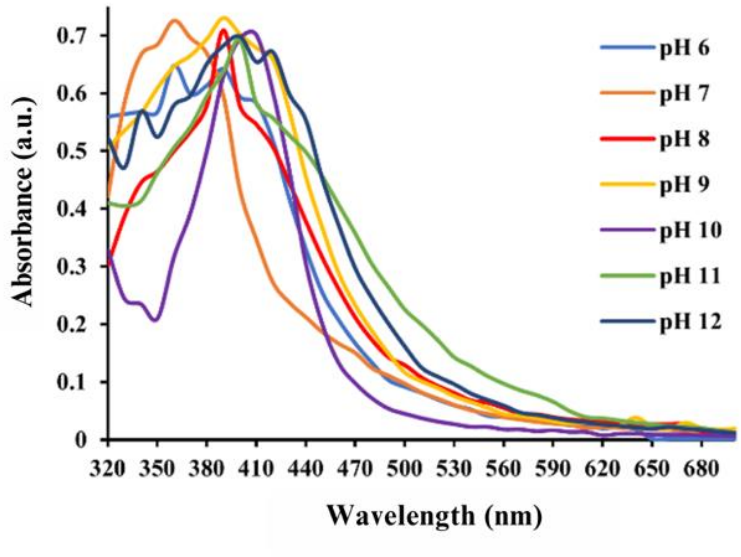

Figure 5. Silver nanoparticles synthesized at varying pH

The UV-Vis spectra of colloidal AgNOs-MO at varying pH (6 to 12) are shown in Figure 4. At pH 6 and 7, there is no indication of the formation of silver nanoparticles because the absorption peak is formed at a wavelength of 370 nm. Meanwhile, at pH 8 and 9, silver nanoparticles began to form because there was an absorption peak at a wavelength of 400 nm. At pH 10, an ideal UV-Vis spectra shape was obtained with a single absorption peak at a wavelength of 410 nm. At pH 11, there is also still an indication of silver nanoparticles because there is an absorption peak at a wavelength of 400 nm. A different thing happens at pH 12, which shows an absorption peak at a larger wavelength (*red shift),* which indicates an increase in nanoparticle size due to the formation of AgOH so that it can cause the morphology of unshaped nanoparticles (Janah et al., 2021).

In some studies, the optimum pH conditions in various plant extracts are different. Recent research concluded that silver nanoparticles synthesized from *Vernonia amygdalina* plant extract have an optimum synthesis pH at pH 12 (Tesfaye et al., 2023). Silver nanoparticles synthesized from *Aconitum volaceum* leaf extract have optimum pH conditions at pH 11 (Khan et al., 2018). In addition, synthesized silver nanoparticles using *Ocimum sanctum* leaf extract, with the optimum synthesis pH condition being pH 10 (Bere et al., 2019). However, most of the optimum pH for the synthesis of silver nanoparticles from plant extracts is in alkaline

conditions (pH > 7). This is because acidic media can slow down the rate of reduction caused by electrostatic repulsion of anions in the reaction mixture (Tesfaye et al., 2023).

Detection Performance Test

Response Test with Hg(II) Ion

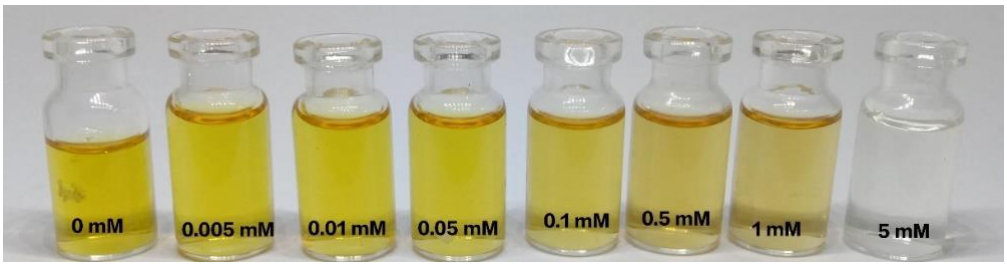

Figure 6. AgNPs-MO colloids after contact with various concentrations of mercury(II) ion standard solution.

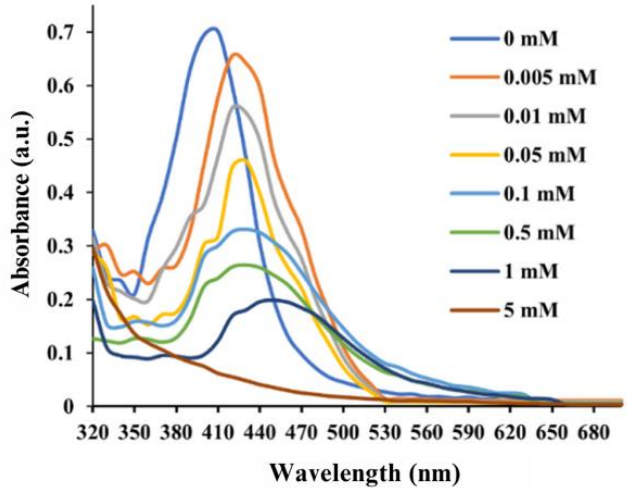

Figure 7. UV-Vis spectra of AgNPs-MO colloids that have been contacted with various concentrations of Hg(II) ion solution.

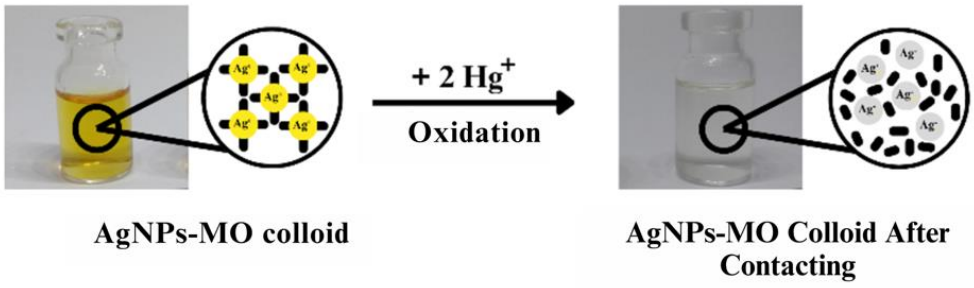

Figure 8. Simulation of the interaction mechanism of AgNPs-MO with Hg(II) ions.

AgNPs-MO colloids were tested for their response to Hg(II) ions at various concentrations. Silver nanoparticles are able to experience a decrease in color intensity when contacted with mercury (II) ions (Oluwafemi et al., 2019). This is in line with the research results that can be seen in Figure 6. When the AgNPs-MO colloid is contacted with mercury (II) ions, there is a decrease in color intensity, where the higher the concentration of mercury (II) ions added to the colloidal nanoparticles, the color will fade. Significant color changes began to occur when AgNPs-MO was contacted with a 0.1 mM concentration of Hg(II) ions.

In addition to the determination by direct eye, analysis using a UV-Vis spectrophotometer was also used to determine the response of AgNPs-MO to Hg(II) ions (Suriyakala et al., 2022). From Figure 7, when contacted with the analyte Hg(II) ions, there is a shift in the absorption peak at a larger wavelength (*red shift*). This is evidence that there is a change in the morphology of the AgNPs-MO colloid caused by the oxidation of AgNPs-MO by Hg(II) metal ions into Ag(I) ions again. The occurrence of this oxidation reaction is due to the higher reduction potential value of Hg(II) ions than Ag(I) (Sari et al., 2017). The simulation of the interaction mechanism is shown in Figure 8.

Validation Parameters

Linearity

The first validation parameter is the determination of linearity, carried out by contacting AgNPs-MO with Hg(II) ion analyte at concentrations of 0.1, 0.5, 1, 1.5, 2, 2.5, and 3 mM (Thohir et al., 2022). In Figure 9, it can be seen that there is a reduction in the intensity of the yellow color until it becomes colorless, starting in the concentration range of Hg(II) ions from 0.1 mM to 3 mM. This linearity determination aims to ensure the indication that the greater the concentration of Hg^{2+} analyte added, the Euclidean Distance value will also increase. Determination of linearity will produce a standard curve that can later be used in the quantification process of Hg(II) ions, as shown in Figure 10.

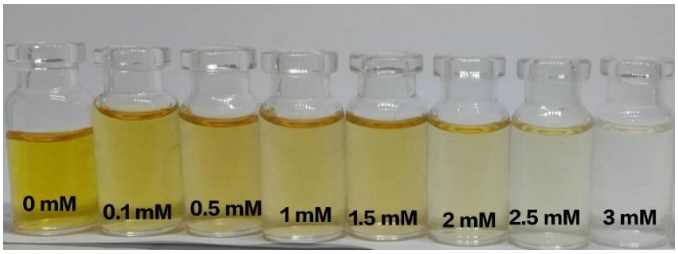

Figure 9. AgNPs-MO contacted with Hg(II) ions in the concentration range of 0.1 to 3 mM.

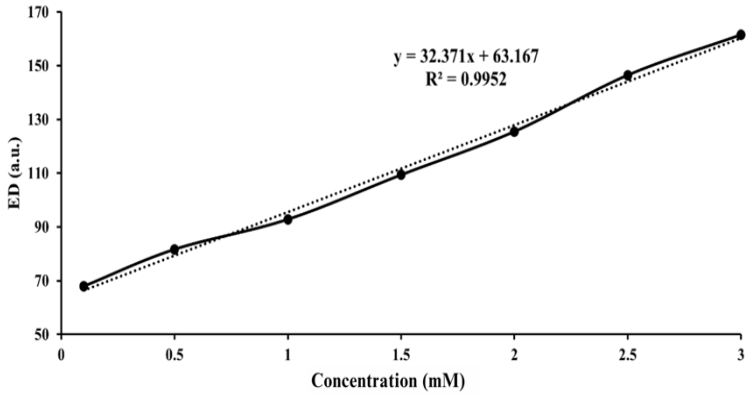

Figure 10. Standard curve of Hg(II) ions in the concentration range of 0.1 to 3 mM for AgNPs-MO by Euclidean Distance.

The main purpose of the standard curve in Figure 10 is to produce a linear regression equation so that it can be used to convert the *Euclidean distance* value obtained when measuring the test solution into the concentration of the target analyte in a sample (Nuradi & Jangga, 2020). The linear regression equation used in determining this concentration, namely, where y is the absorbance and x is the concentration of the target analyte, The linear regression equation obtained in determining linearity, namely. The R^2 value obtained is 0.9952. This value has been considered sufficient from the requirements of a data set called linear, which is R^2 more than 0.995 (Sayakti et al., 2023).

LoD and LoQ

The limits of detection (LoD) and quantification (LoQ) were calculated based on the standard deviation of 10 blanks and the slope of the standard curve using the following equation.

$$\text{LoD} = \frac{3\times\text{Sb}}{\text{m}}, \text{LoQ} = \frac{10\times\text{Sb}}{\text{m}}$$

The results obtained for the limit of detection were 0.0263 mM and for the limit of quantification 0.0877 mM. The smaller detection limit indicates the sensitivity value of the method. Meanwhile, the limit of quantification shows the smallest quantity that is able to give accurate results (Thohir et al., 2022).

Precision

Precision is one of the most important method validation parameters. Precision determination is carried out to determine the closeness of the results between one data point and other data points based on the %RSD value obtained. In this study, precision testing was carried out with *repeatability* parameters, which were carried out with the same five samples and analyzed by *Digital Image Colorimetry* through the conversion of RGB values into *Euclidean Distance* values. The results obtained are expressed as RSD values presented in Table 1.

RSD ≤ 1% means very thorough, if 1% < RSD ≤ 2% means thorough, if 2% < RSD ≤ 5% means moderate accuracy, and if RSD > 5% means not thorough. In this study, the RSD value was less than 1%, which means that this method is very thorough (Sulistyani et al., 2021).

Table 1. Data from repeatability precision analysis

Hg^{2+} concentration	**Mean ED**	**SD**	**%RSD**
0.5 mM	65.329	0.319	0.488
1.5 mM	78.387	0.248	0.316
2.5 mM	137.355	0.519	0.377

CONCLUSION

Novelty silver nanoparticles from moringa leaf extract (AgNPs-MO) have been successfully synthesized using an $AgNO_3$ precursor, which can be used as a colorimetric detection agent for Hg(II) ions and performs just as well as the classic, environmentally unfriendly methods. The synthesis was carried out in 1 hour with 120°C hotplate heating and 6 hours without heating. While the optimum pH condition in the synthesis of silver nanoparticles from moringa leaves (AgNPs-MO) for the detection of mercury(II) ions is pH 10.

AgNPs-MO when in contact with mercury(II) ions is able to produce changes in the form of reducing the intensity of yellow color to colorless. In the linearity test, the linear area results were obtained in the range of 0.1 mM to 3 mM with an R2 value of 0.9952. The LoD of this method is 0.0263 mM, while the LoQ is 0.0877 mM. This method is also considered precise because it has an RSD value of less than 1%.

RECOMMENDATIONS

This research needs to be developed by doing a better extraction process, because using natural ingredients requires an optimum formulation process. In addition, characterization also needs to be done with a variety of better instrumentation, such as PSA or TEM, in order to get a better picture of AgNPs.

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