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DEVELOPMENT OF A NITROCELLULOSE MEMBRANE-BASED TEST STRIP FOR DETECTING MERCURY (II)

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Abstract

Mercury (Hg), a hazardous heavy metal, has become a significant environmental and public health concern. Practical tools for detecting mercury in water and cosmetic samples remain limited. This study aimed to develop a nitrocellulose membrane–based test strip capable of detecting mercury through its reaction with diphenylcarbazide. The development process involved the formation of a purple-colored complex resulting from the interaction between mercury ions and diphenylcarbazide, which served as the visual indicator of mercury presence. The selectivity of the test strip was evaluated against non-mercury ions, and its accuracy (%) was compared to the atomic absorption spectroscopy (AAS) method. The results revealed that the nitrocellulose membrane–based test strip was selective toward mercury ions, exhibited a clear color response with increasing mercury concentrations, and demonstrated a relatively high accuracy of 83.33%. These findings suggest that the developed test strip has strong potential as a semi-quantitative and portable tool for mercury detection.

Keywords: *mercury, test strip, diphenylcarbazide, selectivity, accuracy*

Introduction

Mercury (Hg) is one of the most hazardous environmental elements due to its persistence, bioaccumulation, and lack of biological function. Even at low doses, mercury poses serious risks to the environment and human health (Vieira, 2021). Exposure to mercury, even in small amounts, can result in significant health problems. Previous tests have shown high mercury levels in the blood, urine, and hair of local residents. Pregnant women and infants are particularly vulnerable to mercury exposure, which has been linked to neurological effects (Mallongi, 2023). Human exposure often occurs through the consumption of contaminated fish and seafood. When individuals ingest mercury-containing fish, the metal enters the body and can travel to various organs, including the brain, where it may cause adverse health effects (Wu, 2024). Mercury is also found in skin-lightening cosmetics, and the risks associated with continuous use of these products are increasingly evident. Organic mercury compounds such as ethylmercury, methylmercury, and phenylmercuric salts may be used as preservatives in certain cosmetic formulations. Mercury poisoning can occur through dermal absorption when using skin-lightening products. Inorganic mercury exposure has been associated with skin rashes, neurological disturbances, and kidney toxicity (Bastiansz, 2022).

To achieve ecologically sustainable mercury determination across diverse matrices and concentration levels, accurate and traceable measurement instruments are essential. Numerous approaches have been proposed for developing disposable optical or visual sensors for mercury ion detection (Rotake, 2020). Highly sensitive analytical techniques, such as capillary electrophoresis, chromatographic methods (gas chromatography, liquid chromatography), and spectroscopic methods (atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, and cold vapor atomic absorption), are widely used to detect mercury (Migašová, 2020). Although these laboratory-based techniques offer high sensitivity and selectivity, their application in environmental monitoring is limited by expensive instrumentation and time-consuming sample preparation. Therefore, simpler and more affordable Hg^{2+} detection methods are being explored alongside conventional techniques (Komova, 2022).

Among the most widely used approaches are spectrometric detection methods. Dithizone has been employed as a recognition reagent to selectively detect Hg^{2+} under highly acidic conditions; however, previous studies indicate that this kit is not sufficiently sensitive for mercury detection in drinking water samples (Wang, 2018). To address this limitation, Chen (2016) developed a paper-based colorimetric device (PCD) capable of real-time Hg^{2+} detection through a visible color shift. Using a fiber-optic instrument, Hg^{2+} concentrations as low as 0.01 μM could be successfully monitored, with the digital readout indicating the degree of blue color change (Chen, 2016). Sulistyarti (2019) later developed an analytical tool by doping dithizone onto a hydrophobic PTFE membrane, producing a blue dithizone membrane that instantly turned orange upon contact with mercury(II). The intensity of the orange mercury(II)–dithizonate complex increased with higher mercury concentrations, although the use of a six-layer membrane reduced efficiency (Sulistyarti, 2019).

Diphenylcarbazide (DPC) is considered a highly effective chelating agent due to its strong complexation affinity for metal ions (Mumtaz, 2021). Diphenylcarbazone solution rapidly reacts with mercury salts in an alcohol–water mixture (20:80, v/v), producing a blue or purple color indicative of mercury complex formation (Dayem, 2021). Commercial mercury detection kits remain limited; therefore, based on the considerations above, this study developed a nitrocellulose membrane–based test strip for mercury detection using diphenylcarbazide as the reactive agent. This research also examined the test strip's selectivity against other metal ions and evaluated its accuracy compared with the *atomic absorption spectroscopy* (AAS) method.

Experimental Section

Materials

The materials used in this study included 96% alcohol, 1,5-Diphenylcarbazide (Loba Chemie), 68% nitric acid, hand gloves, mercury standards, lead standards, nitrocellulose membranes, Whatman filter paper No. 42, commercial mercury test kits, and distilled water.

Procedure

Sample Preparation

Mercury samples with various concentrations were prepared by diluting mercury standards to obtain the desired concentrations: 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, and 35 ppm.

Preparation and Optimization of Test Strips

This detection technique was based on the selective chromogenic ability of Diphenylcarbazide compounds on paper, which enabled mercury ion identification. The filter paper was cut into rectangular pieces measuring 4 cm × 8 cm.

Diphenylcarbazide Attachment Process

The paper was then soaked in the Diphenylcarbazide reagent until fully saturated. Drying was carried out at room temperature for 24 hours.

Hg and Pb Ion Selectivity Test

The Pb ion selectivity test for the performance of the test kit was conducted by adding 1.5 mL of a 5 ppm mercury solution into a test tube, followed by 0.5 mL of 2 M nitric acid and 8 mL of a 1% Diphenylcarbazide solution. The mixture was reacted by shaking for 8 minutes. Absorbance was measured using a UV–Vis spectrophotometer at a maximum wavelength of 493.5 nm (Putra, 2014). All tests were performed in triplicate.

Diphenylcarbazide Strip Testing Preparation

The prepared test strips were dripped with mercury samples at concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, and 35 ppm. Each test included a control solution without a paper strip, tested in duplicate for each concentration. Color changes on the strips were observed visually and documented using a camera. The type of color produced was recorded. A positive reaction was indicated by a color change to purple (violet). The color intensity was assessed based on differences in the concentrations of the heavy metal solutions.

Accuracy Test Using Atomic Absorption Spectrophotometry

The procedure began with the preparation of a standard Hg solution. A 100 ppm mercury standard solution was prepared by transferring 10 mL of Hg stock solution into a 100 mL volumetric flask, diluting to the mark with 20% HNO_3, and homogenizing. A 10 ppm mercury standard solution was then prepared by transferring 10 mL of the 100 ppm solution into a 100 mL volumetric flask, diluting with 20% HNO_3, and homogenizing. Standard Hg solutions with concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ppm were then prepared. Each solution was measured using AAS at 253.7 nm, and the results were plotted to form a calibration curve. Sample determination was carried out by placing a mercury sample of a given concentration into a microwave digestion tube, adding 10 mL of concentrated HNO_3, and heating it in a microwave at 80°C for approximately 3 hours. After digestion, the sample was removed and mixed with 10 mL of heavy-metal-free distilled water, then transferred to a Nessler tube. The mixture was diluted to the 50 mL mark using heavy-metal-free distilled water. The sample was measured using AAS at 253.7 nm (Wulandari, 2018).

Results and Discussion

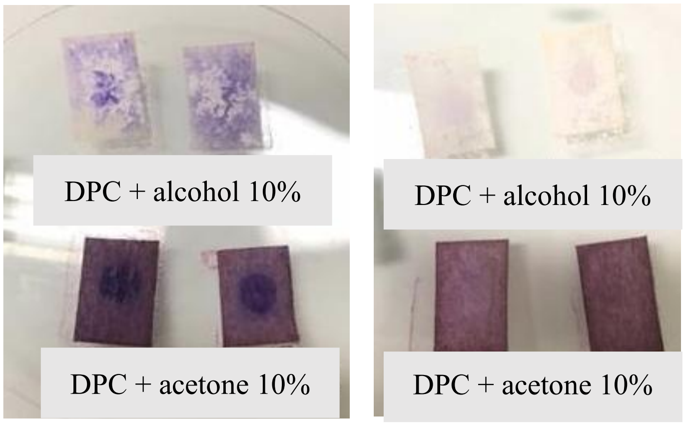
Differential Reaction of Diphenylcarbazide with Mercury (Hg) and Lead (Pb)

The results of the comparative reaction test between diphenylcarbazide and the metals mercury (Hg) and lead (Pb) showed clear differences in color formation. A faint pink color appeared in the reaction with Pb, whereas a strong purple color was produced in the reaction with Hg, even when using the same concentration for both metals.

The solvent optimization results for diphenylcarbazide indicate that alcohol is more effective than acetone. This is due to differences in solvent properties. Alcohol, being a polar solvent, dissolves polar compounds such as diphenylcarbazide more efficiently (Henkel, 2017). The color formation in the reaction between Hg and diphenylcarbazide occurs because of the formation of a complex compound through the interaction between metal ions and ligands, which are molecules or ions that donate electron pairs to metal ions.

In this reaction, mercury ions (Hg^{2+}) function as the metal ions, while diphenylcarbazide acts as the ligand that forms a complex with them. This complex has a specific chemical structure that gives it unique properties, including a distinctive purple color. The greater the concentration of Hg ions, the more intense the resulting color, as shown in Figure 2 (Shallal, 2023).

In contrast, the reaction between diphenylcarbazide and Pb produces a faint color because Pb reacts with organic compounds such as diphenylcarbazide to form white or colorless complexes. These results demonstrate that the reaction between Hg and DPC produces a specific and recognizable color response.



(a) (b)

Figure 1. Color reaction of diphenylcarbazide (DPC) dissolved in alcohol and acetone with (a) Hg and (b) Pb

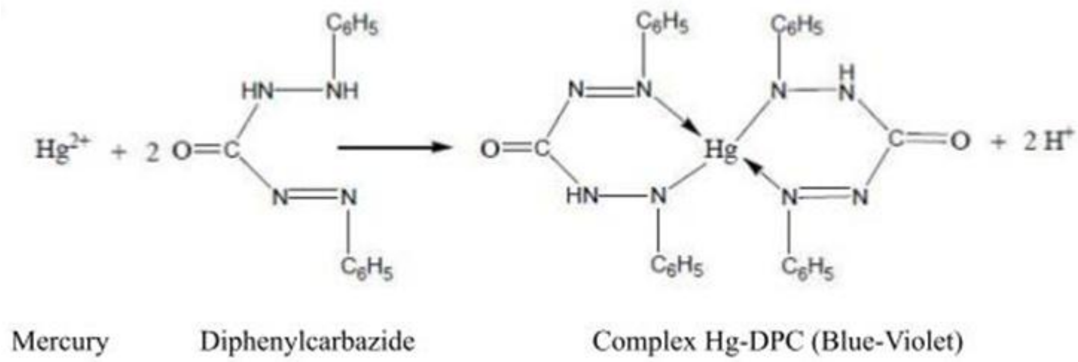


Figure 2. Reaction between Hg and DPC

Selectivity Test of the Developed Strip Test Toward Other Ions

A selectivity test was conducted to evaluate the performance of the test kit by observing the absorbance of the Hg(II)–diphenylcarbazide complex in the presence of Pb^{2+} ions. The measurement results are presented in the figure below. Selectivity refers to a method's ability to accurately and exclusively measure specific analytes in the presence of other components in the sample matrix without affecting the analytical results (Preechaburana, 2023). This study investigated the effect of Pb^{2+} ions on the performance of the mercury test kit. The results showed that Pb^{2+} ions exerted only a limited and stable effect, with an average absorbance decrease of 1.9% across all tested mercury concentrations (5–35 ppm). For instance, at a mercury concentration of 10 ppm, the absorbance of the Hg(II)–DPC complex before Pb^{2+} addition was 3.419 ± 0.215. After adding Pb^{2+} ions from a 10 ppm $PbCl_2$ solution, the absorbance changed only slightly to 3.354 ± 0.287. Statistical analysis (see Table 1) also shows that there was no significant difference between the measured concentrations of Hg(II) ions with and without the presence of Pb(II).

This finding confirms that the presence of Pb^{2+} ions does not significantly affect the measurement of mercury, thereby maintaining the reliability of the test kit even when Pb^{2+} interference is present in the sample (Putra, 2014). In addition to demonstrating good selectivity and detection limits, many existing analytical methods can measure several heavy metal ions simultaneously. However, these conventional techniques are often difficult to operate and require skilled personnel, as well as expensive and bulky analytical instruments. As a result, rapid screening and on-site detection of trace heavy metal ions in food, water, or cosmetics remain impractical using these approaches (Si, 2024). The strip test developed in this study has the potential to serve as a portable, selective, and user-friendly tool for mercury detection in everyday applications.

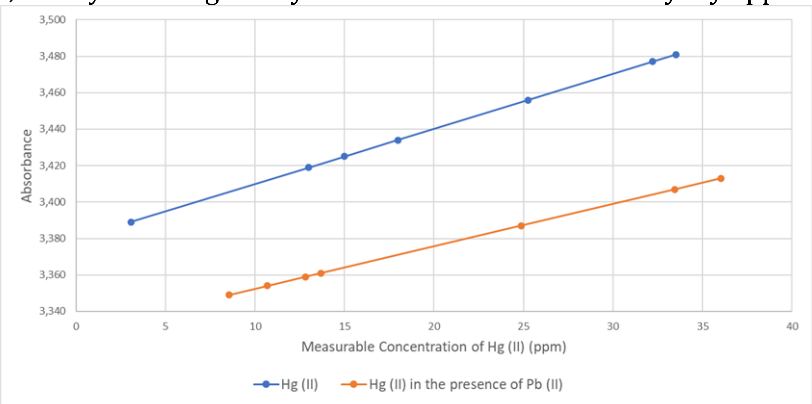


Figure 3. Selectivity Test of Hg(II) in the Presence of Pb(II)

Table 1. Selectivity Test of Hg(II) in the Presence of Pb(II)

Measurable Parameter	Concentration (ppm)	Mean Abs Hg(II) + DPC	Sd	Mean Abs Hg(II) + Pb(II) + DPC	Sd	Measurable Concentration Hg(II) + DPC (ppm)	Measurable Concentration Hg(II) + Pb(II) + DPC (ppm)	Paired t-Test
Hg(II)	5	3.389	0.315	3.349	0.234	3.100	8.546	p > 0.05 (no significant difference)
	10	3.419	0.215	3.354	0.287	13.100	10.693	
	15	3.425	0.276	3.359	0.316	15.100	12.840	
	20	3.434	0.154	3.361	0.289	18.100	13.699	
	25	3.456	0.219	3.387	0.184	25.433	24.865	
	30	3.477	0.261	3.407	0.239	32.433	33.454	
	35	3.481	0.267	3.413	0.321	33.767	36.030	

Comparison Test With Commercial Kit

The comparison test was conducted using a commercial kit, beginning with the preparation of mercury standard solutions at concentrations of 30 ppm and 700 ppm. This step is essential for ensuring the accuracy and reliability of the test. After the standards were prepared, two drops of each mercury solution were added to the commercial kit. The results showed that, in the commercial kit (see Figure 4), the standard Hg^{2+} solution at 30 ppm could not be detected. In contrast, in the developed strip test, it was clearly detected, indicated by a violet color change. However, at the 700 ppm concentration, both the commercial kit and the developed strip test produced the same purple color.

The Earth's crust contains large amounts of heavy metals, commonly defined as those with a density greater than 5 g/cm^3, such as Hg. However, living organisms contain these metals only in trace amounts, and even small quantities in the atmosphere, soil, or water can negatively affect health. Heavy metals primarily affect human health through occupational exposure, environmental contamination, or food-chain accumulation (Nowicki, 2021). Therefore, this strip test shows potential for detecting mercury at lower concentrations than commercial kits.

Figure 4. Comparison of mercury ion detection between the developed strip test and the commercial kit

Mercury Test Kit Accuracy Test Using Atomic Absorption Spectrophotometer

The accuracy test of the mercury test kit was performed using mercury samples at specific concentrations, and the results were compared with the standard AAS method. The color comparator of the mercury test kit (5–1000 ppm) is shown in Figure 5.

The color produced by the mercury samples matched the test kit comparator in the 500–1000 ppm range, and thus a mercury concentration of 600 ppm was selected for the accuracy test. The accuracy of the test kit was 83.33%, calculated using the formula %R = (Yf / Yc) × 100, where Yc is the true concentration and Yf is the measured concentration (Indrayanto, 2022).

High-sensitivity spectroscopy methods such as flame atomic absorption spectroscopy (FAAS), graphite furnace atomic absorption spectroscopy (GFAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) are widely used to determine heavy-metal concentrations in environmental samples (Shahbazi, 2019). In both tests, mercury at a concentration of 600 ppm produced a strip-test color corresponding to 500 ppm (see Figure 5), while the AAS results indicated a concentration of 592.9 ppm. The accuracy values obtained were 83.3% for the developed strip test and 98.83% for the AAS method. Despite the differences from the standard AAS method, the developed kit demonstrated an acceptable level of accuracy according to the Food and Drug Administration Office of Regulatory Affairs Laboratory Manual. The general acceptance criteria for accuracy in human drug analysis methods are typically 80%–120% for analysis and 70%–130% for content uniformity (Indrayanto, 2022).

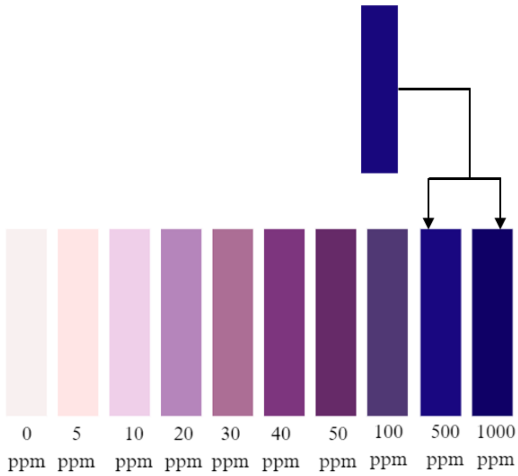


Figure 5. Visualization of the kit at various mercury sample concentrations

According to the SANTE 12682/2019 guidelines, specificity, accuracy/ repeatability, precision/reproducibility, limit of quantification (LOQ), limit of detection (LOD), sensitivity/linearity, and selectivity are the primary parameters referenced in method validation. Additional validation recommendations are provided in the Eurachem handbook (Eurachem, 1998). Method validation is essential for both new and optimized methods to ensure linearity, accuracy, and repeatability, particularly for analyses intended for use in commercial laboratories (Fitriadi, 2021).

Conclusion

This study successfully developed a test strip for mercury detection based on its reaction with diphenylcarbazide. The color formation in the reaction between mercury (Hg) and diphenylcarbazide occurs due to the formation of a complex compound through the interaction between mercury ions (Hg^{2+}) and diphenylcarbazide ligands. This complex possesses a distinct chemical structure that produces a characteristic violet color. Increasing the concentration of mercury ions enhances the intensity of the resulting color. The reliability of the test kit is maintained even in the presence of Pb^{2+} ion interference, as the findings indicate that Pb^{2+} ions do not have a significant effect on mercury measurement results. The comparison test with commercial kits further shows that the developed strip test can detect mercury at lower concentrations than those detected by commercial kits. The developed kit also demonstrated an acceptable accuracy of 83.33%, meeting the general acceptance criteria for accuracy in human drug analysis methods outlined in the Food and Drug Administration Office of Regulatory Affairs Laboratory Manual II. These criteria typically require a minimum accuracy of 80–120% for analysis and 70–130% for broader evaluations. This strip test is currently limited to detecting mercury in water and cosmetic samples; therefore, further testing is required to evaluate its applicability to other sample types, such as food and clinical specimens.

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