Simple and Rapid Device for Mercury Detection Based on The Formation of Mercury(II)-Dithizonate on Polytetrafluoroethylene (PTFE) Membrane

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ABSTRACT

A new analytical device for mercury detection has been developed by doping dithizone on to hydrophobic PTFE (polytetrafluoroethylene) membrane to form a blue dithizone membrane which instantaneously changed to orange color of mercury(II)-dithizonate complex, when this dithizone membrane was contacted to mercury(II) solution. The higher concentration of mercury showed the greater intensity of the orange colour mercury(II)-dithizone complex. The design and chemicals were optimized to obtain the best performance for mercury measurement. This method is prospective as mercury test kit for simple, low cost, and rapid semi-quantitative method for mercury(II) determination from 1-10 mg L\textsuperscript{−1} suits for on-site mercury detection and has been applied to cosmetics with satisfactory results.

Keyword: Mercury, test kit, dithizone, hydrophobic membrane, PTFE

INTRODUCTION

The hazardous mercury to human health is unquestioned. However, mercury is still widely used in some pharmaceutical products as one of active ingredients found in whitening products for lightening dark spots and stubborn pigmentation. The toxic mercury can be rapidly absorbed through the skin and affect neuro-system which leads to skin rashes, blur vision, and organ damage. While the use of mercury in cosmetic products has been banned in some countries. However, the image “white is beautiful” still remains for Asian and African woman. Therefore, skin whitening products containing mercury is still aggressively marketed in Asia and Africa [1,2]. Consequently, the development of a simple, low cost, and rapid method for on-site mercury analysis is strongly demanded for common people in developing countries to protect them from mercury poisoning.

The common standard method used for mercury analysis based on cold vapor-AAS [3] or ICP [4,5] are impractical for field analysis, expensive, and requires high analytical skills. Besides, the standard visible spectrophotometry and most spectrophotometric methods for mercury analysis involved extraction prior to detection [3], catalytic reactions [6,7], uncommon chemicals [8,9], and complicated synthesis [10]. Therefore, monitoring of mercury using those methods become ineffective and impractical for on-site determination and only can be operated by skilled people.
An effort for developing simple detection for mercury was reported by Danwiyattakul, et al. using dithizone nanofiber loaded membrane [11]. The dithizone-membrane was prepared by vacuum filtration of the dithizone nanofiber dispersion through a cellulose ester membrane. Detection of Hg(II) was done by filtering 100 mL of Hg(II) solution at pH 2-3 through the dithizone nanofiber loaded membrane with the filtration rate of 5-6 mL/min followed by colorimetric techniques using TLC scanning densitometer. This technique was reported sensitive with detection limit of 0.057 ppb and not interfered by most cations (in the presence of EDTA) and anions. Linearity of the method was not reported and the dithizone membranes using ascorbic acid was stable up to 90 days by keeping in an aluminum bag with silica gel as oxygen absorber. However, this technique is not applicable either for unskilled people or on-site mercury detection as this technique can only be operated by skilled people and requires vacuum filtration as well as TLC scanning densitometer for mercury determination. Weiwei Chen et al. [12] reporting a paper-based colorimetric device (PCD) for mercury determination, in which mercury was detected based on the suppressing oxidation capacity of PtNPs in enzymatic oxidation of 3,3,5,5-tetramethylbenzidine (TMB) loaded on PAD. The device permits determination of Hg$^{2+}$ at concentrations down to 0.01 µM within several minutes. Although this device has great potential as a new tool for field measurement of Hg$^{2+}$, however it involves uncommon reagents and complicated mechanism of detection [12]. Recently, interesting finding was reported by Longfei Cai et al. [13] involving a microfluidic paper-based analytical device (µPAD), in which mercury was determined by the length of colored precipitate of mercury(II)-dithizonate on the hydrophilic channel of the µPAD. This technique showed linear in a range of 1-30 mg L$^{-1}$ with detection limit of 0.93 mgL$^{-1}$.

Polytetrafluoroethylene (PTFE) plumber’s tape has been successfully used as hydrophobic membrane gas separator in flow based techniques which allows the aqueous solutions to come into contact without wetting the membrane and separates the gas analyte from the ionic interferences [14-16]. This PTFE plumber’s tape has not been explored elsewhere for reagent loaded membrane. The hydrophobic dithizone is expected to be well absorbed by the PTFE plumber’s tape to fabricate dithizone membrane.

In this work, the cheap and widely available PTFE plumber’s tape is explored for a simple, rapid, and applicable for onsite mercury determination by unskilled people. The dithizone membrane was simply by immersing the PTFE membrane in dithizone chloroform solution. The dithizone was instantaneously absorbed by the PTFE membrane forming blue-dithizone-membrane. When the blue-dithizone-membrane was dipped in mercury solution in aqueous solution, the membrane changes the colour from blue to orange-red of mercury(II)-dithizonate (Hg(II)-DTZ) complex due to the reaction of mercury(II) ions with dithizon on membrane. The color intensity depends on the concentration of Hg(II) solutions. The concentration of mercury(II) was determined by comparing the color intensity of sample with standard chart color of mercury. The effects of geometric membrane, stability of dithizone membrane, dithizone concentration were then studied. The detection technique based on RGB Colorimeter was also presented. The proposed mercury kit was applied to determine the concentration of Hg(II) ions in the commercial cosmetics samples. This simple, rapid, and low cost technique is expected to be very useful for preventing public from mercury poisoning.

EXPERIMENT

Chemicals and instrumentation

The tools used in this study included a set of glassware, analytical balance, RGB image photometer, and RGB Colorimeter program. The materials used in the present study were pro-
analytical grade chemicals (pa), such as dithizone (Merck, Germany), mercury(II) chloride (Merck, Germany), nitric acid (Merck, Germany), chloroform (Merck, Germany), demineralized water, commercial PTFE plumber’s tape membrane, mica, and cosmetic samples.

Instrumentation applied for analysis should be written all tools are used during research. They can contain instrumentation specification or operational conditions include brand manufacturer. For example: FTIR spectrophotometer (Shimadzu FTIR QP89500, sample was analyzed using NaCl plate or thin film).

**Procedure reaction**

**Preparation of mercury samples**

The 1000 ppm mercury stock solution was prepared by dissolving 0.1350 g standard mercury chloride, HgCl\(_2\) into 100 mL 0.1 M nitric acid solution. A lower concentration of mercury was prepared by diluting the stock solution into the corresponding volume of HNO\(_3\) 0.1 M.

**Preparation of dithizone solution**

The stock solution of 0.1% dithizone was prepared by dissolving 500 mg of dithizone into 50 mL chloroform. The lower concentration of dithizone solution was prepared by diluting the stock liquor into the corresponding volume of chloroform

**Construction of kit test design**

The design construction of mercury kit test was created in such way as a simple, low cost, and easy use for unskilled society. It was formulated using mica strip material with PTFE plumber’s tape membrane coated at the end tip by adhesive properties of the PTFE plumber's tape on mica as shown in Figure 1.

![Figure 1](image)

**Figure 1.** Construction design of PTFE membrane for mercury test kit

**Optimization of doping time for dithizone on PTFE membrane**

The optimum time for doping dithizone on the PTFE membrane was done by dipping the stick-PTFE membrane in the dithizone solution under various doping time of 1, 3, 5, 7, and 9 minutes, then the color intensity of dithizone absorbed were observed against doping time. The time required for optimum doping was indicated by the highest intensity of the blue dithizone on the PTFE membrane. For more accurate results, the color of the results obtained is photographed and read with RGB colorimeter and processed with RGB Colorimeter program.

**Optimization of membrane layers**

The PTFE membrane was layered on the mica stick with variations of 2, 4, 6, 8, and 10 layers noted in Figure 4 as I, II, III, IV, and V. Then, the sticks with various membrane layers
(I-V) were doped in 2 mL of 0.01% dithizone for 1 minute, lifted, and then immersed into the reaction tube containing 3.0 mL of 5.0 mg/L mercury solution, followed by shaking for 2 minutes.

**Influence of membrane dimension**

This experiment was carried out using the optimum membrane layer (6 layers), with membrane dimension varying from 1x1 cm², 1x0.5 cm², and 0.5x0.5 cm². These membranes were then dipped into 2 mL of 0.01% dithizone solution for 1 minute, followed by immersion in 3.0 mL of 5.0 mg/L mercury solution, and shaken for 2 minutes. The same procedure was done for mercury 10.0 mg/L.

**Optimization of dithizone concentration**

Determination of optimum dithizone solution concentration was done by preparing a series concentration of mercury(II) solution from 0-10 mg/L in 11 test tubes. Then, the 11 stick membrane from the previous experiments were dipped into 0.01% dithizone solution for 1 minute followed by immersion in the 11 test tubes containing 3.0 mL of various mercury concentration of 0-10 mg/L, and shaken for 2 minutes. The optimum dithizone concentration was determined by the dithizone solution which gives highest intensity of orange Hg(II)-DTZ complex on the membrane. For more accurate results, the results obtained were photographed and read using RGB colorimeter.

**Standard mercury color chart**

Standard mercury color chart was developed by dipping the stick-PTFE membrane into optimum concentration of dithizone solution under optimum dipping time, and then immersed into various concentrations (0.01 mg/L) of mercury solution. The results obtained were photographed and processed using RGB colorimeter and RGB Colorimeter program for a better performance. These colors were used as standard colors for determining mercury concentrations in the sample.

**Application of Mercury Test Kit on Cosmetics**

Cosmetics containing mercury were weighed accordingly then dissolved into 2.0 mL of 1.0 M HNO₃, and diluted to 10 mL followed by filtration. Then, the mercury test kit was immersed in mercury(II) solution for 2 minutes and the resulting color was compared to the standard color to determine its mercury concentration.

![Figure 2. The Principle reaction of mercury test kit](image-url)
RESULT AND DISCUSSION

The proposed mercury test kit was based on the formation of mercury(II)-ditizonate complexes on the PTFE hydrophobic membrane. The blue dithizone on the PTFE membrane will turn reddish orange after reacting with mercury by forming a mercury(II)-ditizonate complex (Figure 2). The dithizone doping technique on PTFE membrane is advantageous, since this method can avoid the use of carcinogenic organic solvent of chloroform.

Mercury test kit design

The mercury test kit was designed to facilitate a fast and affordable analysis. The clear mica was used as a stick to hold the membrane and ease doping process as well as detection. PTFE membrane was selected because the membrane is hydrophobic, thus it can absorb a non-polar dithizone reagent. When the membrane containing blue dithizone was immersed into mercury solution, the dithizone reacted with mercury(II) to form orange-red of Hg(II)-DTZ complex, as shown in Figure 3.

Figure 3. The principle design of mercury test kit

Figure 4. Optimization of time for doping dithizone on PTFE membrane. Conditions: dithizone 0.01% (b/v) in chloroform, 5.0 mg/L mercury(II) in 0.1 M HNO₃ (pH 1); 6 layers membrane of 1x1 cm², I-V = doping time of 1-9 minutes.

Optimization of dithizone doping time

The optimization of doping time for dithizon on to PAD-membrane was done to find out the optimal time for doping dithizone on to the entire PAD-PTFE membrane. The optimization
was observed based on the color intensity value of each condition. The experiments were carried out using 6 layer membrane with dimension of 1x1 cm², which was then dipped in 2 mL of 0.01% dithizone solution under variations of time of 1, 3, 5, 7, 9 and noted as I, II, III, IV & V. The results of the experiment can be seen in Figure 4.

It can be seen from Figure 4 that the doping of dithizone from 1-9 minutes gave the same color intensity of blue dithizone on PTFE membrane, and intensity readings obtained from RGB Imaging photometer and RGB Colorimeter program showed the same values from 1-9 minutes. Thus, 1 minute doping was selected as the optimum time for doping dithizone for the reason of time efficiency.

**Optimization of membrane layer**

The optimization of membrane layer was performed to obtain the maximum absorption capacity of dithizone on to PTFE membrane sufficiently to complex with mercury(II) in the sample; thus it is expected to produce maximum color intensity of the Hg(II)-DTZ complex. The result of the experiments can be seen in Figure 5.

![Figure 5](image-url)

*Figure 5. The effect of membrane layers. Conditions: dithizone 0.01% (b/v) in chloroform, 5.0 mg/L mercury(II) in 0.1 M HNO₃ (pH 1); membrane I-V = membrane with 2-10 layers.*

It is known from Figure 5 that increasing the number of membrane layers on mica stick for mercury test kit from I-III (2-6 membrane layers) increased the intensity of the Hg(II)-DTZ color as expected. However, further additional layers from IV-V (8-10 layers), the color of Hg(II)-DTZ remained constant (the obtained color were equal to condition of III). This is because under the small number of membrane layer (conditions I and II), the dithizone absorbed on the membrane was insufficient to complex with all mercury(II) in the sample, thus not all of mercury(II) formed Hg(II)-DTZ complex, and therefore the color intensity obtained was not maximal. The higher number of membrane layer (condition of III-V) the dithizone absorbed on the membrane showed sufficient to complex the mercury in solution presented by similar color intensity among those three conditions. This indicated that maximal absorption of dithizone required for complexing mercury in sample has been achieved. Therefore, condition III (6 layer membrane) was selected as the optimum number of layer because it gave the highest color intensity with minimal layers. Condition III also showed the uniform dispersion of mercury complexes compared to the conditions of IV and V.
Influence of membrane dimension

The dimension of the membrane needs to be optimized because the membrane dimension influences the area of contact between dithizone and mercury(II) and also influences the color dispersion; thus influence the color intensity. Theoretically, the smaller membrane dimension has smaller color dispersion, resulting in higher color intensity. However, the results showed that the larger dimension gave higher color intensity as depicted in Figure 6.

Figure 6. The effect of membrane dimension. Conditions: as in Figure 5, with membrane dimensions of I (1x1 cm²), II (1x0.5 cm²), and III (0.5x0.5 cm²).

Based on Figure 6, it can be seen that the color intensity of Hg(II)-DTZ increases with the dimension size of membrane (the largest membrane shows the highest color intensity). This fact is inversely proportional to the expectation. This fact is inversely proportional to the expectation. This phenomena occurred may because of the difficulty of mercury(II) to reach the reaction zone and react with dithizone on the small size membrane. The large dimension of membrane gives the higher area of contact to react with mercury in the sample solution, and thus gives higher color intensity of Hg(II)-DTZ. The dimension size of 1x1 cm² was chosen for further experiments because it gives the highest intensity and homogenous orange color of the mercury(II)-dithizonate complex.

Optimization of dithizone concentration.

Optimization of dithizone concentration was performed to determine the optimum concentration of dithizone that reacts with mercury(II) 0-10 mg/L and gives different colors for different mercury concentrations. It was expected that the higher concentration of mercury will result in higher orange color intensity of HG(II)-DTZ. This experiment was used as a reference for developing reference standard color for determining mercury(II) concentration. The experimental results of series concentration of standard mercury(II) solution from 0-10 mg/L using various dithizone concentrations from 0.01-0.05% is shown in Figure 7.

Figure 7 showed that the use 0.03% dithizone concentration give a clear, uniform (homogeneous), and significant color changes for 0-10 mg/L mercury concentrations. In order to obtain homogenous color, it is important to choose the homogenous PTFE plumber’s tape as the membrane. The concentration of 0.03% dithizone was selected as optimum, and used for the basis of mercury test kit development. The series color obtained from 0.03% dithizone was then used as the bases for developing reference standard color chart. In order to obtain a smooth
color of reference standards, the obtained colors were read their intensity using RGB colorimeter program and print out to give standard color shown in Figure 8.

**Figure 7.** Optimization of dithizone concentration. Conditions: as in Figure 5, with dithizone 0.01-0.05% and mercury(II) 0-10 mg/L.

**Figure 8.** Reference standard original color and processed by RGB Colorimeter

**Selectivity Test**

The effect of interfering ions, such as silver ion, lead ion, copper ion, cobalt ion, cadmium ion, and zinc ion were studied by involving various concentration of interfering ions, whilst the concentration of mercury was kept constant. For a more accurate calculation, the measurements were based on RGB-colorimeter readings. The results depicted in Table 1 showing that the proposed mercury test kit method can be considered as selective as the presence of silver ion
up to 12 mg/L, lead ion up to 60 mg/L, copper ion up to 18 mg/L, cobalt ion up to 18 mg/L, cadmium ion up to 60 mg/L, and zinc ion up to 18 mg/L did not significantly interfere the measurement shown by the recovery of close to 100%. However, concentration of silver, copper, cobalt, and zinc ions higher than 18 mg/L (three times of mercury) interfered the measurements, indicated the far recoveries of mercury from 100%.

Table 1. Study of interferences

<table>
<thead>
<tr>
<th>Ions added to 6.0 mg/L of mercury(II)</th>
<th>[metal ions] mg/L</th>
<th>Measured mercury(II), mg/L</th>
<th>%-Recovery*</th>
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</thead>
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<tr>
<td></td>
<td>0</td>
<td>6.08 ± 0.02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6.10 ± 0.02</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.10 ± 0.03</td>
<td>98.3</td>
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<tr>
<td>Ag⁺</td>
<td>18</td>
<td>6.76 ± 0.03</td>
<td>109.3</td>
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<td></td>
<td>60</td>
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<td>6.08 ± 0.02</td>
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<td>6</td>
<td>6.11 ± 0.03</td>
<td>98.5</td>
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<tr>
<td></td>
<td>12</td>
<td>6.11 ± 0.02</td>
<td>98.5</td>
</tr>
<tr>
<td>Pb²⁺</td>
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<td>6.13 ± 0.01</td>
<td>98.8</td>
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<tr>
<td></td>
<td>60</td>
<td>6.13 ± 0.03</td>
<td>98.8</td>
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<tr>
<td></td>
<td>0</td>
<td>6.03 ± 0.05</td>
<td>-</td>
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<tr>
<td></td>
<td>6</td>
<td>5.97 ± 0.03</td>
<td>99.5</td>
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<tr>
<td>Cu²⁺</td>
<td>12</td>
<td>5.97 ± 0.18</td>
<td>99.5</td>
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<td>18</td>
<td>5.92 ± 0.04</td>
<td>98.6</td>
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<td>60</td>
<td>4.53 ± 0.01</td>
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<tr>
<td></td>
<td>6</td>
<td>6.01 ± 0.05</td>
<td>99.9</td>
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<td>Co²⁺</td>
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<td>5.98 ± 0.06</td>
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<tr>
<td>Cd²⁺</td>
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<tr>
<td></td>
<td>60</td>
<td>7.21 ± 0.01</td>
<td>120.2</td>
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</table>

*Average from triplicates with RSD < 5 %
Application and Validation of Mercury Test Kit

The validity of the mercury test kit was tested by applying the mercury test kit to determine mercury content in cosmetics. Ten cosmetics samples were prepared by acid extraction to release mercury(II) into solution. Then, the dissolved mercury content (mercury extract) was reacted with the dithizone on the membrane to form a reddish orange color. The concentration of mercury in the sample can be determined by comparing the color intensity of the samples to the reference standard color. Ten types of cosmetic brands were weighed accordingly and dissolved in 1 M nitric acid followed by dilution to meet the concentration of mercury in the range of 0-10 mg/L. Then, the PTFE membrane was dipped into 0.03% dithizone for 1 minute, followed by immersion in 3 mL of cosmetic sample extract and shaken for 2 minutes. The colors obtained were compared to the reference standard color (Figure 9) to determine the concentration of mercury(II) in cosmetics. The colors of sample also measured their intensities using RGB/RGB Colorimeter and compared the intensity values to those of reference intensities.

![Figure 9. Determination of mercury concentration in cosmetics](image)

Table 2. Validation test using cosmetic samples

<table>
<thead>
<tr>
<th>Cosmetic samples</th>
<th>Mercury Test Kit (mg/L)</th>
<th>VIS spectrophotometry (mg/L)</th>
<th>AAS (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.17 ± 0.29</td>
<td>3.17 ± 0.01</td>
<td>3.10 ± 0.12</td>
</tr>
<tr>
<td>B</td>
<td>9.33 ± 0.29</td>
<td>9.45 ± 0.01</td>
<td>9.34 ± 0.29</td>
</tr>
<tr>
<td>C</td>
<td>7.33 ± 0.23</td>
<td>7.47 ± 0.04</td>
<td>7.43 ± 0.29</td>
</tr>
</tbody>
</table>

*Average from triplicates with RSD < 5 %

The results presented in Figure 9 shows that the color from cosmetic sample II corresponds to the standard color of 1 mg/L mercury, the color from cosmetics IV corresponds to the standard color of 2.0 mg/L mercury, and cosmetic V corresponds to the standard color of 4.0 mg/L mercury. This determination was supported by the readings obtained from RGB Colorimeter, in which the intensities values of the cosmetics samples were equal with the corresponding standard colors. The proposed technique showed prospective as an alternative
early detection for mercury(II) and can be brought to analytical technique for quantitative mercury(II) analysis by further investigation for selectivity and validity.

Validity of the method was done by applying the proposed mercury test kit, followed by RGB Colorimeter readings for determining mercury in cosmetic samples, than comparing the results to those obtained from the standard visible and atomic absorption spectrophotometry methods. The validation test using cosmetic samples is depicted in Table 2, in which the data showed comparable results to all samples between the proposed mercury test kit and spectrophotometric standard methods supported by the value of t-test calculated < t-test tabulated (at α of 0.05, n = 3).

CONCLUSION

The mercury test kit has been successfully developed for a fast and simple mercury determination based on the reaction of mercury(II) and dithizonate on the PTFE membrane under optimum conditions of 6 membrane layers, membrane dimension of 1x1 cm², dithizonate doping time of 1 minute, and concentration dithizonate of 0.03%. The proposed mercury test kit has several advantages: (1) easy to make and very low cost; the PTFE plumber’s tape membrane can be found cheaply and easily elsewhere (2) very simple technique; only by dipping membrane into dithizonate solution followed by dipping the dithizonate membrane to mercury solution, whereas the previous techniques involved more complicated steps for detection, (3) fast analysis (2-3 minutes), whilst the previous methods take much longer time, (4) suits for unskilled user and on-site mercury analysis. The developed mercury test kit showed prospective for early mercury detection and has also been applied for mercury(II) analysis in cosmetics with satisfactory results.

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