Inhibitive Determination of Hg(II) in Aqueous Solution Using Urease Amperometric Biosensor

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Received: November 7, 2018
Accepted: December 17, 2018
DOI: 10.22146/ijc.40617

Abstract: An amperometric biosensor for the indirect determination of Hg(II) has been developed based on inhibition of urease (EC 3.5.1.5) immobilized into alginate–chitosan polyelectrolyte complexes membrane. The biosensor response was monitored by following the reduction peak of hydrolyzed urea at around -0.15 V. The amperometric biosensor has a dynamic range 40–90 ppb Hg(II) with limit of detection of 66.45 ppb toward Hg(II) ions, repeatability (CV) value of 0.86% and only Ag(I) as the main potential interference. The sensor showed a stable and reproducible response for more than 2 weeks when it stored dry at 4 °C. The analytical results of Hg(II)-spiked water sample showed a good agreement with those obtained by atomic absorption spectrometry method, suggesting that the developed method may be applied in the determination of Hg(II) in the water samples.

Keywords: amperometric biosensor; urease; alginate–chitosan; inhibition; Hg(II) ion

INTRODUCTION

Environmental pollution caused by heavy metal is becoming a great problem due to its effect on human health. Mercury, a very toxic metal that can affect the central nervous system and disturb haemin synthesis as well as cause disorder of the central nervous system [1-3]. Mercury is widely present in air, water, and soil as elemental or metallic mercury, inorganic mercury compounds, and organic mercury compounds [4]. Hence, simple and rapid detection of mercury at very low concentrations levels in environmental and biological samples is needed and very important for assurance against acute intoxications and long-time exposure that may lead to many acute diseases and even death [5].

Several analytical methods such as atomic absorption spectrometry (AAS) [6], inductively coupled plasma with mass spectrometry (ICP–MS) [7] as well as electrochemistry [8], have been developed for detecting mercury in the environment. However, these methods have limitations, such as taking a long time to carry out, using large amounts of chemical reagents and expensive equipment, and requiring qualified operators to perform the multi-step sample preparation and complex analytical procedures. These limitations make the methods unsuitable for the purpose of on-site and on time measurements [9]. On the other hand, anodic stripping voltammetry (ASV) is one of the most favorable techniques for the determination of heavy metal ions due to its low cost, high sensitivity, easy operation and ability to analyze element speciation as well as portable application [10].

Biosensor technology is a powerful alternative to conventional analytical techniques, combining the specificity and sensitivity of biological systems in small devices. Recently it is very common in the literature to use biosensors for the determination of mercury in
polluted environmental samples [11]. Most of the biosensors for detecting metal ions are designed on the inhibition of an enzyme. At a constant substrate concentration, inhibition causes a reduced enzyme activity and leads to a decrease in response signal which is proportional to the amount of heavy metal ions as an inhibitor in the sample. For example, enzymatic amperometric biosensors for the measurement of Hg$^{2+}$ based on its inhibitory action on urease activity has been developed [12] by using screen-printed carbon electrodes and screen-printed carbon electrodes modified with gold nanoparticles. The same enzyme was used in the development of a simple optical fiber biosensor for the determination of heavy metal ions such as Hg$^{2+}$, Ag$^{+}$, Cu$^{2+}$, Zn$^{2+}$, Co$^{2+}$ and Pb$^{2+}$ [13]. The metal is well known for its ability to react with sulfhydryl groups of proteins, which are frequently responsible for the enzyme’s active center [14].

Immobilization of enzyme into suitable support material is one of the most important steps in designing a biosensor since it plays an important role in the overall biosensor performance. In the last decade, various matrixes have been used as enzyme immobilization supports. For instance, polyelectrolyte complexes (PEC) used as a membrane for enzyme immobilization, and it has been reported that immobilized enzymes on PEC retain higher activities than its native enzymes and they are stable [15]. For these reasons, PEC is potentially attractive materials for applications in biotechnology, including biosensor. Alginate as polyanion and chitosan as a polycation, when dissolved in appropriate condition, can interact each other through the carboxyl group of alginate and amino group of chitosan [16] and its ionic interactions are the main interactions inside the network of PEC. PEC formed is expected to provide a better application in biosensor due to their unique structure and properties. Alginate–chitosan polyelectrolyte complexes membrane has not been previously used in enzyme inhibition biosensors for the determination of Hg$^{2+}$.

In this work, a simple and fast immobilization method based on PEC is described using synthesized alginate-chitosan membrane as PEC, and their application in amperometric biosensing for determination Hg(II) in aqueous solution has been proposed for the first time. The optimized condition for urease immobilization on PEC was defined, and the effects of experimental parameters, such as pH and temperature, and the stability of the biosensor response are studied including its analytical characteristics toward Hg(II) ions.

### EXPERIMENTAL SECTION

#### Materials

The urease used for preparing the biosensor was E.C. 3.5.1.5. from jack beans (Type III, U1500) 272 u/g and it was stored at 40 °C. Sodium alginate was 300–400 cp from brown algae and chitosan was 95% deacetylated from crab shell, they were purchased from Sigma (St. Louis, USA). Hydrochloride acid (37%), glacial acetic acid (98%), sodium hydroxide (NaOH) and potassium dihydrogen phosphate (KH$_2$PO$_4$) were received from Merck (Germany). A stock solution of urea (1000 μg/mL) was prepared in aqueous solution. Mercury dinitrate (Hg(NO$_3$)$_2$), silver nitrate (AgNO$_3$), cadmium chloride (CdCl$_2$), cupric sulfate (CuSO$_4$), lead nitrate (Pb(NO$_3$)$_2$) solutions were prepared by appropriate dilution. All other reagents were analytical grade and were used without further purification. Solutions were prepared with deionized water.

#### Instrumentation

Electrochemical protocols were performed with a PalmSens portable potentiostat/galvanostat, with the PSTrace program and accessories (PalmSens Instruments BV, 3992 BZ Houten, the Netherlands). The portable potentiostat was interfaced with a computer controlled by PS Trace 4.2 software for data acquisition and experimental control. A pH meter model IM-20E (TOA Electronic Ltd.) was employed for all pH measurements.

#### Procedure

**Preparation of membrane**

The membrane was synthesized by mixing two polymer solutions consisting of chitosan hydrosol and alginate hydrosol, as described by Kulig et al. [16]. Chitosan hydrosol was prepared from 1 g of chitosan that was dispersed into 25 mL distilled water and then dissolved by adding 5 mL of glacial acetic acid with
stirring at 400 rpm using a magnetic stirrer for 12 h to form a homogeneous mixture. Alginate hydrogel was prepared by dissolving 1 g of alginate in 25 mL of distilled water, by stirring at 400 rpm and allowed to dissolve overnight (12 h). The formed mixed polymers were the hydrogel solution. Then, both polymers (2%) were homogenized by homogenizer IKA (T18 basic, Ultra Turrax, Staufen, Germany) for 90 sec. The mixtures were poured into polypropylene beakers of 60 mL to make a gel membrane and then chilled for further used. IR absorption of the prepared alginate-chitosan hydrogels was measured using the KBr pellet method at a compression pressure of 2500 lb/m^2 on an FT-IR spectrophotometer (FT-IR 1600 Perkin Elmer Co Japan). SEM (Scanning Electron Microscopy) studies were carried out on alginate-chitosan hydrogel after coating with gold-palladium on the SEM (model Joel LV 5600 USA).

**Immobilization procedure**

The mixtures of alginate-chitosan are used as the solid support for the enzyme immobilization. Before the hydrogel mixture (50 mL alginate-chitosan) was used, the mixture was added with 2 mL of 32% HCl and then adjusted with NaOH 10% (w/v) to reach pH at 5.28. The mixture was then stirred until homogenous. Afterward, 3 mL of hydrogel mixture was taken and added to 1 mL phosphate buffer (pH 6.5). The buffered mixture was added with 3 mL urease and then stirred for ± 4 h at room temperature. The mixture was transferred to the glass mold with 1 mm depth in order to produce alginate-chitosan PEC membrane with immobilized enzyme. Finally, the membrane was stored at 40 °C for 24 h for the aging process to make the dry alginate-chitosan PEC urease membrane.

**Construction of amperometric biosensor**

Construction of amperometric biosensor was used PalmSens Electrochemical. Here the alginate-chitosan PEC membrane was coated on Screen-Printed Electrode (SPE), where immobilization of urease was packed onto an electrode surface. Design of SPE with membrane sensor was carried out by coated onto an electrode surface as shown in Fig. 1. In the presence of increasing amount of Hg(II) and a constant substrate concentration, the activity of the enzyme decreases so that the signal of the sensor is measured using a PalmSens. Amperometric measurements were performed in an electrochemical cell at room temperature. The steady-state current response was recorded in a phosphate buffer (0.1 M, pH 6.0) with a urea solution (75 mM) and an operation potential of -0.15 V vs. Ag/AgCl. Signals were evaluated as the difference of the registered current to the current baseline obtained before the addition of the analyte.

**Detection of Hg(II) ions in the real sample**

To evaluate the feasibility of the amperometric biosensor for Hg(II) detection in the real water sample, the tap water obtained from our lab with no further processing was selected as the real sample. Hg(II) ion in the tap water solutions was prepared by adding HgCl_2 into the tap water at the desired concentration. Besides the tap water used as a real sample, other water samples collected from the river and sea were also used as the real samples. The sample obtained from Sekotong district – West Lombok. Here, the water samples were first filtered with a 0.22 µm syringe filters and then spiked with standard Hg(II) solutions to prepare stock solutions with various Hg(II) concentrations. The electrochemical intensity was recorded on PalmSens under the same conditions. As a comparison, the CV-AAS (GBC HG 300) was used as the reference method for the determination of Hg(II) ions in the real water samples.
RESULTS AND DISCUSSION

PEC Membrane for Enzyme Immobilization

In the preliminary work, the various mass ratio at 0.5:1; 1:1; 1:0.5 of Na-alginate and chitosan were used, and it was found to be optimum at the ratio of 1:1 to form an excellent PEC membrane. This is due to the fact that at this ratio, the ionic interaction between –NH₂⁺ group of chitosan and –COO⁻ a group of alginate form the strongest interaction compared to another ratio. In addition, the drying temperature and pH of the mixture also greatly affect these ionic interactions. The drying temperature of the alginate-chitosan PEC membrane was optimum at room temperature, even though the time needed was longer (± 72 h). Since it was treated at the room temperature, it produced a strong and well formation of the PEC membrane formed. While, at the higher drying temperature, such as at 60 °C it was produced a weak formation of the alginate-chitosan as PEC membrane. This is due to the fact that at 60 °C, the PEC membrane formed was brittle and easy to breakdown due to weak ionic bonds were formed inside the membrane. In term of pH, the formation of the alginate-chitosan PEC membrane occurred at pH of 5.28. The carboxylate groups of alginate are indicated by the presence of the carboxylate ions formed, whereas the NH₂ group of chitosan is protonated. The interaction of the two opposing charges of these functional groups causes the salt formation that promotes the well-ionic bond formation of the PEC membrane.

The IR spectrum of the alginate-chitosan membrane is shown in Fig. 2. There is absorption at the wavenumber (cm⁻¹): 3429 (–OH from alginate/–NH₂ of chitosan), 2924 (CH sp²), 1578 (–COO⁻). The reaction between the carboxylic group of alginate and amine groups of the chitosan give absorption at the wavenumber (cm⁻¹): 1740–1630 (C=O) and 1630–1510 (NC=O). However, in fact, there is no C=O and the NC=O absorption in the IR spectrum of the alginate-chitosan PEC membrane. This indicates that the formation of the alginate-chitosan membrane involves ionic interaction only. The peak intensity was observed at 1398 cm⁻¹ confirming stronger electrostatic interaction in the sample with the alginate and chitosan ratio of 1:1. The presence of the aforementioned bands in the alginate-chitosan PEC membrane mixture indicates the appearance of ionic bonds between amine groups of the chitosan and carboxyl group of the alginate.

The surface and cross-section morphology of the alginate, chitosan, and alginate-chitosan membrane were studied by Scanning Electron Microstructure (SEM) as shown in Fig. 3. The morphology of the blended alginate-chitosan membrane was less homogenous than the alginate or the chitosan only. Here, the alginate-chitosan PEC membrane showed irregular, fibrous structures of surface and rough cross-section morphology, with pores and clusters of the sodium alginate–chitosan aggregated particles. It was observed that the complex aggregates appear in Fig. 3 as the segments with round/spherical structures.
Fig 3. SEM images of surface morphology of (a) alginate; (b) chitosan; and (c) alginate–chitosan PEC membrane

Fig 4. The mechanism of Hg(II) ion sensing with the biosensor

**Biosensor Scheme**

Electrochemical biosensors have been used directly in monitoring heavy metal ions based on enzyme inhibition. This method generally requires quantitative measurement of enzyme activity or percentage inhibition as the basis of analytical methods by using appropriate transducers [18]. The equation is generally shown in the Eq. (1)

\[
E_{oks} + S \leftrightarrow [E_{oks} - S] \rightarrow E_{red} + P
\]

Fig. 4 shows the amperometric biosensor mechanism based on urease inhibition for the detection of Hg(II) ions in solution. Urease, when breaking the carbon-nitrogen bond on the amide bond of urea to CO₂, NH₃, and water, is in an oxidized enzyme state by forming the intermediate product of the enzyme-substrate oxide bond.

The product formation of urea hydrolysis is followed by the reforming of the enzyme in a reduction state. Redox enzymes are formed as a result of the reduction and oxidation reactions in enzymes as Eq. (2), where the resulting electrons can be detected using an electrochemical transducer.

\[
E_{red} \rightarrow E_{oks} + e^- \tag{2}
\]

Furthermore, inhibition measurements are made by introducing Hg(II) ions. In the biochemical mechanism, urease inhibition by Hg(II) ions is generated by the bond formation between Hg(II) ion and the sulphydryl group (-SH) as part of the urease. Inhibition of urease activity by Hg(II) ions then give rise to the slower rate of urea hydrolysis and even sometimes lead to loss of activity. Electrocatalytic changes before...
and after inhibition can provide information on the presence of a number of Hg(II) ions so that it can be used as electrochemical devices in detecting Hg(II) ions concentration in the system.

**Electrochemical Characteristics**

Monitoring of Hg(II) electrochemically could be done by enzyme inhibition. Urea hydrolysis using urease as a catalyst and NH$_4^+$ formation was determined using a redox enzyme reaction system [19]. The presence of heavy metals in sample inhibited urease activity, resulting in lower NH$_4^+$ production. Here, amperometric biosensor used screen-printed configuration of three electrodes (disposable electrode). This biosensor is based on measuring the changes in the current of the working electrode due to direct oxidation or reduction of the product of the biochemical reaction [12]. Decreasing activity due to the presence of heavy metals was proportional to the number of heavy metals in the sample.

Generally, the operating principle of biosensor could be described electrochemically, urea solution hydrolyzed to CO$_2$ and NH$_3$OH with urease as immobilized redox enzyme in the matrix. The resulting product NH$_4^+$ diffused through the membrane layer to affect the electrode with a potential of +0.1 V forming cyclic voltammetry describing the electrochemical characteristics of the biosensor, as shown in Fig. 5.

**Optimization of Experimental Parameters**

In terms of increased performance of the biosensors, there are many parameters affecting the sensor response such as response time, working potentials, pH, substrate concentration, temperature and time of Hg inhibition. The optimization of the experimental parameters is an essential step in the analytical characteristics of the mercury biosensors performance toward Hg(II) ions. Table 1 shows some investigated-experimental parameters and their optimum value.

The sensor response time is the time taken for the sensor to obtain optimum signal intensity and then variations in the signal afterward are no longer perceived. The measurement of sensor response time with scan range 0 to 20 sec. It was found that the time of 4 sec was the response time of the biosensor. Since after 4 sec the sensor signal did not rise significantly with increasing time.

The effect of working potential on the current in a steady-state was measured with a potential range from 0 to -0.5 V in urea solutions (75 mM), pH of 6 (phosphate buffer solution) using 50 ppb of Hg(II) ions. The steady-state current of the electrocatalytic reduction process of Hg(II) ions was increased rapidly from -0.5 to -0.15 V but slower as result of a larger change in the working potential of -0.15 to 0 V. Change in current increases as a result of the electrocatalytic activity due to inhibition of Hg(II) ions is used as a basis for measurements. In addition, low working potential leads to the influence of electroactive species. Hence, -0.15 V was chosen as

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**Table 1. Optimization of experimental parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Optimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response Time (sec)</td>
<td>0–20</td>
<td>4</td>
</tr>
<tr>
<td>Working Potential (V)</td>
<td>-0.5–0</td>
<td>-0.15</td>
</tr>
<tr>
<td>pH</td>
<td>5–8</td>
<td>7</td>
</tr>
<tr>
<td>Substrat Concentration (mg/L)</td>
<td>50–100</td>
<td>75</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20–35</td>
<td>25</td>
</tr>
<tr>
<td>Inhibition Time (min)</td>
<td>4–8</td>
<td>7</td>
</tr>
</tbody>
</table>

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![Fig 5. The cyclic voltammogram showing that the increase in current is proportional to the increase of NH$_4^+$ in urea hydrolysis with a scan rate of 1.0 V/s, scan range of -0.5 to 0.5 V](image-url)
One important aspect in determining the optimum condition of the biosensor is the selection of pH. The reaction of urea hydrolysis as the basis of biosensor measurement depending on the pH value. Changes in pH are the effects of analyte interactions, which in turn changes in pH environment can be shown by sensor response sensitively. The influence of matrix and other electrocatalytic interference can be minimized by using a buffer solution. Furthermore, the stability of the sensor response with pH change can be controlled. The effect of pH on the current in steady-state with pH variations of in the pH range from 5 to 8 in a phosphate buffer solution with 75 mM urea solution are investigated. The phosphate buffer solution at pH 7 was found to be optimum, and it was used as an optimum pH in the enzymatic reaction for further measurement in this study.

An enzymatic reaction will take place effectively when the substrate concentration is proportional to the KM value of the enzyme. Therefore, it is necessary to optimize the substrate concentration in order to obtain clearly observed results. The optimum substrate concentration (urea) in this study was determined by varying urea concentrations between 50 to 100 mM with an incremental concentration of 5 mM. Here, the concentration of 75 mM was found to be optimum for the urea concentration. This concentration was used for the amperometric biosensor measurement based on the urea inhibition by Hg(II) ions.

The working temperature of the enzymatic reaction affects the changes in the blank and inhibition signal. The sensor responds to temperature change. It was seen that room temperature, 25 °C was the optimum temperature of this enzymatic reaction. The higher temperature causes enzyme activity decreased due to the denaturation of immobilized enzyme molecules. The temperature of 25 °C was then selected as the working temperature for further amperometric measurements of the Hg(II) biosensor.

Optimization of inhibition time is intended to give Hg(II) ion enough time for binding with urease, so before and after inhibition, the sensor response can be optimized. So that the measurements of intensity differences before and after inhibition can be read at the optimum signal. By introducing Hg(II) ions solution for 7 min, inhibition of Hg(II) ions has been optimized for this measurement.

Response to Biosensors

The response of amperometric biosensors has been investigated under optimum conditions. The amperometric biosensor response curve showed changes in amperometric current due to Hg(II) ions concentration. As shown in Fig. 6, the amperometric curve of the biosensor for successive addition of Hg(II) ions solution. The inset shows the plot of the calibration curve between the amperometric currents and concentration of Hg(II) ions has good linearity with the correlation coefficient, $r = 0.978$. The positive value of $r$ indicated the inhibited Hg(II) ions increased is proportional to its concentration. The resulting linear correlation occurred in the range of Hg(II) ion concentrations between 40–90 ppb. The detection limit was determined using the analyte concentration when the inhibited enzyme was 10%. This 10% inhibition was chosen because it assumes that at this value the difference of intensity before and after inhibition could be observed with 90% confidence value. Detection limit obtained was...
66.45 ppb for Hg(II) ions. Repeatability was calculated using the coefficient of variation (CV). The obtained Cv value of 0.86% for Hg(II) (n = 3) describes that the data has good precision or repeatability.

The apparent constant of Michaelis-Menten (Km(app)), where the enzyme affinity was reflected with a ratio of microscopic kinetic constants. It could be determined by the Lineweaver-Burk electrochemical equation. It was found that the K_m value was 0.115 mM without inhibitor and 0.126 mM with V_max value was 0.02 µA/sec at 50 ppb Inhibitor. The values of obtained K_m tend to increase after the addition of inhibitors; this is due to the substrates and inhibitors competed in attacking the active side of the enzyme. In this system, the substrate binding energy is equal to the binding energy of inhibitor, means that some enzymes will bind to the substrate and some will bind to the inhibitor so that the resulting inhibitory value is proportional to the relative concentration and affinity of both (substrate and inhibitor) [20]. The inhibitory value could be overcome by enhancing substrate concentration.

Analytical Characteristics

The biosensor based on enzyme immobilization could be reused by reactivation, by introducing a 1 mM EDTA solution. The lifetime of this biosensor was determined by a decrease in enzyme activity due to inhibition for one week. Besides being caused by metal inhibition, the activity of immobilized enzyme also decreases during storage. Hence, the immobilized urease was stored at 4 °C when it was not in use, or it could lose its activity by storing at room temperature. Fig. 7 shows the cycle use of urease biosensors for the heavy metal ions detection was 5 times after reactivation because after that the enzyme activity decreased >10% due to inhibition.

Method selectivity was determined by adding a different amount of the potentially interfering substances containing another heavy metal ion that generally find in aquatic environments such as Pb(II), Cu(II), Cd(II) and Ag(I). Table 2 shows the activity of the biosensor on several heavy metal ion interferents. The analysis was conducted with the concentration ratio of Hg(II) with the interference ion was 1:10 (10 ppb Hg(II), 100 ppb interference). The inhibition relative value was very small (< 5%), indicating that Pb(II), Cu(II), Cd(II) and Ag(I) ions have no significant effect at this ratio. However, Ag(I) has a relatively larger value compared to other interference ions; this is because Ag(I) at this concentrations could affect the inhibition activity of metal ions on enzymes [21].

Detection of Hg(II) Ions in the Real Water Sample

To evaluate the ability of the biosensor prepared to be applied in the analysis of aquatic samples. The standard addition method was used, and samples spiked with various amount of Hg(II) were analyzed, as described above. The results of the determination are summarized in Table 3. The results showed that the developed biosensor has an excellent performance for the determination of Hg(II) ions in an aquatic system. This can clearly be seen by examining the analytical results presented in Table 3 which are in good agreement with those obtained from the reference method using atomic absorption spectrometry (AAS). In general, the

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**Table 2. Determination results of possible interferences tested with the biosensor**

<table>
<thead>
<tr>
<th>Interference</th>
<th>Relative Inhibition (%)</th>
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<tbody>
<tr>
<td>Pb(II)</td>
<td>+ 0.79</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>+ 0.68</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>+ 1.10</td>
</tr>
<tr>
<td>Ag(I)</td>
<td>+ 1.66</td>
</tr>
</tbody>
</table>
Table 3. Determination of Hg(II) in aquatic samples

<table>
<thead>
<tr>
<th></th>
<th>Various Water Samples</th>
<th>Biosensor (ppb)</th>
<th>AAS (std Hg(II)) (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffer</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>Tap water</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>River water</td>
<td>0.00±0.02</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>4</td>
<td>Seawater</td>
<td>39.40±0.02</td>
<td>40.2±0.02</td>
</tr>
</tbody>
</table>

*average of triplicate measurements

concentration results of analytes are smaller than that of the detection limit; thus these results are possibly somewhat biased. This indicates that the content of the analytes in the sample is in fact very small and therefore it is relatively difficult to quantify accurately by using this proposed method. However, if we compare the results with those obtained by AAS, it is still in a good agreement. This at least indicates the superior of the method even when the level of the analyte is below the limit of detection.

**CONCLUSION**

This study has shown that a simple and rapid amperometric biosensor for Hg(II) determination can be developed based on the inhibition of urease activity. The optimum conditions for biosensor were found as follows: operating potential -0.15 V vs. Ag/AgCl, supporting electrolyte: 75 mM urea solution in 0.1 M phosphate buffer (pH 6). The developed amperometric biosensor performance has a linear concentration range of 40–90 ppb Hg(II) ions, with a limit of detection of 66.45 ppb, and repeatability as represented by its CV of 0.86%. The developed biosensor is quite stable and gives reproducible response for more than 2 weeks. Ag(I) has been found to be the only potential interference with a relatively larger inhibition activity on enzymes compared to other interference ions. The results of biosensor measurement show a good agreement with those analyzed by the atomic absorption spectrometry, indicating that the biosensor system can be applied accurately for the determination of Hg(II) ions in the real water samples.

**ACKNOWLEDGMENTS**

The authors are grateful to the Minister of Research Technology and higher education of the Republic of Indonesia (Kemenristekdikti) for the research funding through doctoral dissertation research grant contract number 65F/SPP-PDDI/UN18.12/PL/2016.

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