Determination of Heavy Metal Ions Using Conductometric Biosensor Based on Sol-Gel-Immobilized Urease

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Heavy-metal ions are ubiquitous in nature, thus resulting in a serious environmental problem. Due to their high toxicity, there is an obvious need to determine them rapidly on site at trace levels. Although the typical detection methods such as atomic absorption spectrometry and inductively coupled plasma mass spectrometry are widely used for the determination of heavy-metal ions, both methods require very sophisticated equipment and can not be used for field monitoring. Therefore, there is a need for simple and potable detection method. In general, electrochemical methods are able to selectively detect heavy-metal ions with less complex instrumentation. The techniques developed so far include ion-selective electrodes, polarography, and other voltammetric methods. In recent years, electrochemical biosensors have received a great attention as promising alternatives for the determination of heavy-metal ions. A number of examples have been reported and most of the biosensors are based on the use of urease enzyme immobilized by glutaraldehyde crosslinking with bovine serum albumin on electrode surface. The determination of heavy-metal ions using the urease-immobilized biosensor is based on the measurement of the urease enzymatic activity which is inhibited by heavy-metal ions. It is well known that the inhibition of the urease by these ions results from the reaction with sulfhydryl groups of the active site of the enzyme. The urease converts urea into ammonium and bicarbonate ions.

\[
\text{NH}_2\text{CONH}_2 + 3 \text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_4^+ + \text{HCO}_3^- + \text{OH}^- 
\]

For monitoring the enzymatic reaction, ammonium ion-selective electrode, amperometry, and conductometry have been employed. Among the transduction methods developed, conductometric transducer is quite simple and easily fabricated because it has no reference electrode. Recently, we reported a new type of disposable conductometric biosensor based on sol-gel-immobilized-urease on a screen-printed interdigitated array (IDA) electrode for the determination of urea in human urine and serum. Therefore, durable conductometric biosensors based on the sol-gel-immobilized-urease can be advantageously used in harsh conditions such as environmental monitoring. In this article, the analytical characteristics of the present biosensor for the determination of heavy-metal ions will be described.

Experimental Section

Urease (EC 3.5.1.5, type III from Jack beans with the activity of 22,000 units/g) was obtained from Sigma (St. Louis, MO, USA). Tetramethyl orthosilicate (TMOS, 98% purity) and heavy-metal ions were purchased from Aldrich (Milwaukee, WI, USA). The heavy-metal ions used in the urease inactivation study were prepared from Hg(NO_3)_2, Cu(NO_3)_2, Cd(NO_3)_2, and Pb(NO_3)_2. All chemicals used were reagent grade and were used without further purification. Thick-film urea biosensor based on the sol-gel-immobilized urease was fabricated as described previously.

A Hewlett-Packard 4284A impedance analyzer was used to measure admittance and phase angle. A sinusoidal voltage of 10 kHz with amplitude of 10 mV peak-to-peaks was applied to the analyzer to ensure not only negligible Faradaic processes of electroactive species in the test solution but also the stability of the immobilized urease on the IDA electrode. The output signal of the impedance analyzer was fed into the computer through a general purpose interface board.

All measurements were carried out in a 10 mL glass cell filled with 5.0 mM imidazole-HCl buffer at pH 7.5 at room temperature. The sample solution was intensively stirred. In the first step of the assay the biosensor response of 1.0 mM urea solution was measured for 7.5 min. Then the biosensor was pre-incubated in a test solution containing a heavy-metal ion for 10 min. After the pre-incubation, the biosensor was intensively washed with 5.0 mM imidazole-HCl buffer. The biosensor response of 1.0 mM urea was again measured for 7.5 min. The level of inhibition due to the action of a heavy-metal ion was evaluated by comparison of the biosensor response at 7.5 min before and after the pre-incubation in the heavy-metal ion solution according to the following equation:

\[
\text{Inhibition} (\%) = \left\{ \frac{(R_0 - R)}{R_0} \right\} \times 100 \%
\]

where \(R_0\) is the admittance response of 1.0 mM urea obtain-

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ed at 7.5 min before the pre-incubation of the biosensor with the heavy-metal ions and $R$ is the admittance of 1.0 mM urea obtained at 7.5 min after the pre-incubation of the biosensor with the heavy-metal ions for 10 min.

**Results and Discussion**

By taking advantage of sol-gel method, urease has been immobilized on the screen-printed IDA electrode using tetramethyl orthosilicate (TMOS) as a sol-gel precursor. Since the performance of the resulting biosensor can be optimized through changing TMOS/water ratio in the acid-catalyzed hydrolysis of the sol-gel stock solution, we used a relatively high TMOS/water ratio of 3.0 in order to form relatively dense sol-gel matrix with small pore-size, thus resulting in a higher enzyme loading and a larger magnitude of the biosensor response.

Under the optimum conditions, steady-state admittance responses of the biosensor as a function of urea concentration were examined in 5.0 mM imidazole-HCl buffer at pH 7.5. As shown in Figure 1, the biosensor exhibited good responses to urea solution in the concentration range 0.05-10 mM. In particular, the urea biosensor linearly responded to the urea over the 0.05-2.5 mM concentration range and reached a plateau at around 10 mM urea. Although the time required reaching the steady-state response of the biosensor was dependent upon the urea concentration, the steady-state responses were reached in about 15 min after the injection of the urea solution. As far as the inhibition test is concerned, it is better to use the urea concentration which gives the maximum biosensor response in the linear response region as shown in Figure 1. In view of the response time and signal magnitude, the urea concentration used in the inhibition test of the biosensor to heavy-metal ions was chosen to be 1.0 mM.

The rate of enzyme inhibition by an irreversible inhibitor (heavy-metal ion in this study) is rather slow. Therefore, the biosensor to be tested should be pre-incubated for a certain period of time in the test solution containing an inhibitor in order to obtain the measurable inhibition. In order to optimize the assay time and sensitivity, the effect of pre-incubation time of the biosensor in 0.1 mM Hg$^{2+}$ solution upon the biosensor response was examined. The admittance responses of the biosensor before and after pre-incubation in the 0.1 mM Hg$^{2+}$ solution were monitored over the time course. As shown in Figure 2, the level of inhibition non-linearly depends on the incubation time. The original biosensor response (a) rapidly decreased to 59% in 5 min incubation (b) and then further decreased gradually to 47% in 20 min incubation (d) (shown in Figure 2A). These results were re-plotted in Figure 2B. From these results, it is obvious that a measurable inhibition of the urease activity by the Hg$^{2+}$ ion can be obtained within 5-10 min pre-incubation period although it is clearly dependent upon the specific metal ion and its concentration. In view of the assay time and sensitivity, an incubation time of 10 min was chosen for further experiments.

Under the predetermined optimum conditions, the biosensor has been applied to the determination of various heavy-metal ions. The calibration plots of inhibition (%) vs...
such as Nafion to produce silicate/Nafion composite, we use sol-gel silicate is combined with cation-exchange polymer, the increase of the pre-incubation time. In addition, if the range for a particular heavy-metal ion can be obtained with improved lower detection limit and an extended dynamic range for the heavy-metal ions were summarized in Table 1. It could be possible that an (S/N=3) and dynamic ranges for the heavy-metal ions were tested. The Hg$^{2+}$ ion was the most toxic component to the inhibition to the urease activity was Hg$^{2+}$ > Cu$^{2+}$ > Cd$^{2+}$ > Pb$^{2+}$, which was in good agreement with the results observed in the previous reports. The detection limits (S/N=3) and dynamic ranges for the heavy-metal ions were summarized in Table 1. It could be possible that an improved lower detection limit and an extended dynamic range for a particular heavy-metal ion can be obtained with the increase of the pre-incubation time. In addition, if the sol-gel silicate is combined with cation-exchange polymer, such as Nafion to produce silicate/Nafion composite, we would get the improved accumulation efficiency of positively charged heavy-metal ions into the enzyme layer so that the shorter incubation time and lower detection limit could be realized. Therefore, the urease biosensor immobilized in the silicate/Nafion composite film is under investigation for the measurement of heavy-metal ions.

Conclusions

The present study indicates that the sol-gel-immobilized-urease biosensor on a thick-film IDA electrode can be used as a reliable tool for the determination of heavy-metal ions in liquid samples. In comparison to the other reported urea sensors for the determination of heavy-metal ions, the pre-sent biosensor exhibits several advantages; easy production of the sensor, low cost, good sensor-to-sensor reproducibility, no chemical modification of the substrate or enzyme for the enzyme immobilization process, and further possibility to control of the biosensor performance by changing the alkoxide/water ratio in the stock sol-gel solution in the construction of the biosensor. As far as we know, this is the first report on the use of a disposable sol-gel-derived conductometric biosensor for trace heavy-metal ion measurements.

It is clear that due to the nonspecific nature of the inhibition effect, this type of biosensor based on the enzyme inhibition assay can not be used for the specific determination of a particular heavy-metal ion. Therefore, further study is under way to achieve selective detection of heavy-metal ions.

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Table 1. Analytical characteristics of the urea biosensor for the determination of heavy-metal ions. Incubation time was 10 min.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Detection limit (mM)</th>
<th>Dynamic range (mM)</th>
</tr>
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<tbody>
<tr>
<td>Hg$^{2+}$</td>
<td>0.005</td>
<td>0.005-0.5</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>0.02</td>
<td>0.02-1.0</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>0.1</td>
<td>0.1-10</td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>0.9</td>
<td>0.9-10</td>
</tr>
</tbody>
</table>

References


