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Sensitive and Selective Detection of Mercury Ions by Potentiometric Biosensor Based on Urease Immobilized in Chitosan–Poly(Vinyl Alcohol) Hydrogel Film

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In this paper, a potentiometric biosensor was fabricated by immobilizing urease into chitosan–poly(vinyl alcohol) hydrogel film at the surface of a pH electrode. Based on the inhibition effect of Hg^{2+} to the activity of immobilized urease, the proposed biosensor was used for detection of Hg^{2+}. The results showed that the inhibition rate exhibited a good linear relationship with the logarithm of Hg^{2+} concentration in the range from 0.002 μM to 2μM. Defined as 10% inhibition rate, the detection limit was found to be 0.001 μM. The concentration for 50% inhibition rate was calculated to be 0.03μM. Furthermore, the proposed biosensor exhibited excellent selectivity to Hg^{2+}. Other heavy metal ions, such as Ag^{+}, Cu^{2+}, Pb^{2+}, Cd^{2+} have littler influence on Hg^{2+} determination. The biosensor was successfully applied for determination of Hg^{2+} in industrial wastewater samples. The results obtained by the biosensor were consistent with those measured by inductively coupled plasma mass spectrometry (ICP-MS) method.

Keywords: Hg^{2+}; Potentiometric biosensor; Urease; Chitosan; Poly(vinyl alcohol)

1. INTRODUCTION

As a liquid metal at room temperature, mercury owns many interesting properties, such as excellent electrical conductivity and unusual capacity to form amalgams with other metals. These characteristics make mercury be used widely in industrial manufacture and mineral extraction [1, 2]. During the application, mercury is released into the environment continually. But mercury is high toxicity and bioaccumulation [3]. Even if people are exposed very low doses of mercury, important problems may be caused. Among all kinds of mercury species, Hg^{2+} is highly reactive and most toxic [4]. It can be methylated by aquatic organisms. Then methylmercury is biomagnified through the food

chain [5]. For this reason, sensitive and selective detection of Hg^{2+} has received much attention and becomes an important research area [6].

The typical analytical methods used for the determination of Hg^{2+} include atomic fluorescence spectroscopy (AFS) [7], atomic absorption spectroscopy (AAS) [8], inductively coupled plasma mass spectrometry (ICP-MS) [9] and inductively coupled plasma-optical emission spectrometry (ICP-OES) [10]. Although these methods are well established, they rely on expensive and sophisticated instruments and are time consuming. On the other hand, special person are required to perform these methods. For these reasons, simple and fast procedures which are sensitive enough to replace the traditional methods are especially valuable.

Hg^{2+}, such as other heavy metal ions, is well known as an enzyme inhibitor. Applying this phenomenon, enzyme based biosensors for inhibitive determination of Hg^{2+} could be developed. These methods have received widespread attention in recent years for their economical, simple and easy-to-use properties. Determination of Hg^{2+} by enzyme based biosensors can be very sensitive, since the reduction of enzyme activity by single inhibitor molecule can be noticeable due to amplification effect. Several enzymes have been used for determination of Hg^{2+} such as glucose oxidase [11, 12], peroxidase [13, 14], acetylcholinesterase [15], invertase [16, 17], and urease which is the most frequently applied as it is relatively cheap and easily available [18-21]. But the inhibition of enzymatic reaction by Hg^{2+} is not specific, which leads to poor selectivity. Many other heavy metal ions such an Ag^{+}, Cu^{2+} can also actuate as an enzyme inhibitors. Different heavy metal ions may cause cross-sensitivity [22] or some synergistic phenomena [23]. How to improve the selectivity becomes the important and difficult aspect of enzyme based biosensors for Hg^{2+} determination. In the investigation of Volotovsky et al., adding small amount of NaI into the sample could suppress sensitivity to Ag^{+} and the interference by Cu^{2+} could be restrained by rewash the sensor in EDTA solution [21]. But this method is inconvenient. On the other hand adding NaI and rewashing by EDTA may cause decrease in sensitivity for Hg^{2+} determination, as Hg^{2+} can combine with I^{-} or EDTA.

Wang et al. developed a novel chitosan–poly(vinyl alcohol) (CTS–PVA) hydrogel with three-dimensional network structure, which showed superior selective adsorption capacity for Hg^{2+} [24]. This investigation may be helpful to fabricating biosensor for selective detection of Hg^{2+}. In this paper, a potentiometric biosensor was fabricated by immobilizing urease into CTS–PVA hydrogel film. The sensitivity and selectivity of the fabricated biosensor for Hg^{2+} determination were research in detail.

2. EXPERIMENTAL

2.1. Reagents

Chitosan (CTS) from shrimp shells(≥75% deacetylated), Urease (EC 3.5.1.5.) from Jack beans (50～100 unit/mg activity), poly(vinyl alcohol) (PVA, Mw 89,000～98,000, 99+% hydrolyzed) and glutaraldehyde solution (25% in H_2O) were purchased from Sigma-Aldrich. Sodium ethylenediaminetetraacetate (EDTA) and thioacetamide (TAA) of analytical grade were from Shanghai Aladdin Reagent Company, China. All standard solutions of heavy metals (1000 μg/mL) were

purchased from the National Institute of Metrology, China. 2% (w/v) chitosan solution was obtained by dissolution of quantified chitosan into 1% (v/v) acetic acid. The solution was stirred for about 4 h until the solid dissolved entirely.

5 mM urea solution prepared by dissolving a certain weight urea in 0.1 M Phosphate buffer solution (PBS, pH 7.4) was used to test the potential response of the fabricated urease biosensor

0.1 M citrate buffer solution (CBS) was prepared by dissolving 192.14g citric acid in 1000 mL of ultrapure water and the pH value was adjusted by NaOH, which was used to prepare the inhibitive solution of Hg^{2+}.

Regeneration solution (0.1 M Tris–HCl buffer, 10 mM EDTA 10 mM TAA) was obtained by dissolution of quantified all components in ultrapure water. Then the pH of the regeneration solution was adjusted to 7.0 with hydrochloric acid [25].

2.2. Apparatus

A flat combined pH electrode (E-901, Shanghai Ruosull Technology Co., Ltd, Chian) was used to prepare the biosensor. Potential was measured with a PXJ-1B ion meter (Jiangsu Jiangfen Electroanalytical Instrument Co., Ltd, China).

A Thermo X-7 ICP-MS (Thermo Fisher Scientific, USA) was used to determine Hg^{2+} for comparing with the results obtained by the biosensor.

2.3. Preparation of the biosensor

The enzyme based biosensor with urease immobilised in CTS–PVA hydrogel at pH electrode surface was prepared as published procedure [24] with modification: 4.9 mL 2% CTS solution was mixed mechanically with 0.42 mL 10% PVA aqueous solution and 0.5mL urease solution (50mg/mL) at room temperature for 2 h to obtain a homogeneous solution, then 0.4 mL of 25% glutaraldehyde aqueous solution were added dropwise in and the mixture was continuously stirred at room temperature for another 0.5h. 20 μL of the mixed solution was cast onto the flat combined pH electrode surface by using a microsyringe. The modified electrode was dried in air overnight. In order to obtain a uniform film, a breaker was covered over the electrode for the slow evaporation of water. Before measurement, the prepared electrode were immersed into PBS for 1 h, and then washed with ultrapure water to allow swelling and remove the excess acetic acid.

2.4. Analytical procedures

After being immersed into 5 mM urea solution, the potential response of the prepared biosensor was recorded as ΔE_0. Then the biosensor was immersed into CBS containing known concentration of Hg^{2+} for a fixed time. After washing in PBS, the biosensor was immersed into 5 mM urea solution, and the potential response was recorded as ΔE_{inh}. And the inhibition rate (I) of Hg^{2+} can be expressed as:

$$I(\%) = \frac{\Delta E_0 - \Delta E_{inh}}{\Delta E_0} \times 100 \quad (1)$$

After used, the biosensor was immersed in the regeneration solution for 10mins to restore the activity of enzyme inhibited by Hg^{2+}.

3. RESULTS AND DISCUSSION

3.1. Dynamics of the enzymatic reaction

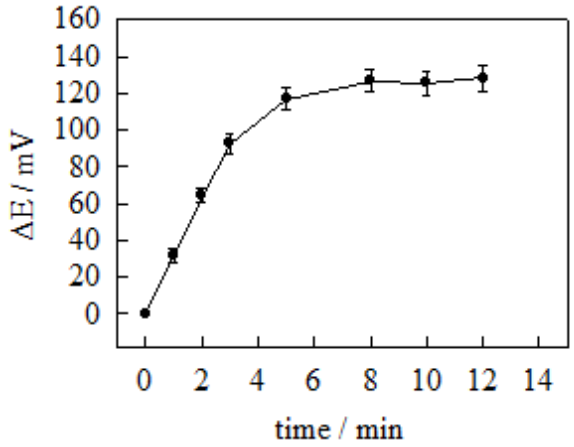

Figure 1. Typical potential response of the biosensor to urea. The concentration of urea was 5 mM.

In order to fix the response time of the biosensor, the dynamics of the enzymatic reaction was investigated. The potential response during the enzymatic reaction is shown in Figure 1.As shown, the potential response increased evidently as the enzymatic reaction time increasing from0 to 5 min, and then began to level off. The reason for this phenomenon is that enzymatically catalyzed hydrolysis of urea caused the potential response increases. After 5 min, the enzymatic reaction tends to equilibrium and the potential gradually reached a constant. Thus, a optimum response time of 5 min was chosen for the biosensor.

3.2. Influence of inhibition time and the pH of Hg^{2+} solution on biosensor sensitivity

Both the inhibition time and the pH of Hg^{2+} solution have significant effect on the inhibition efficiency of Hg^{2+} to urease. Thus, the sensitivity of the biosensor would be affected. The dependence of inhibition rate on inhibition time is shown in Figure 2.

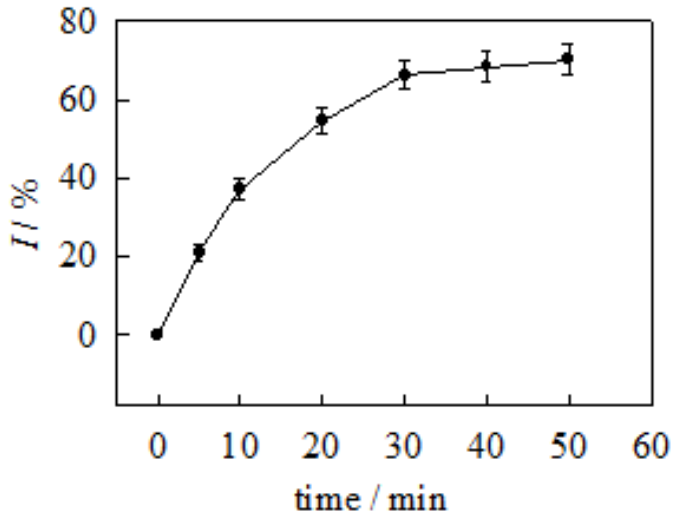

Figure 2. Dependence of inhibition rate (*I*) on inhibition time for 0.1μM concentration of Hg^{2+} (pH 5.5). The concentration of urea was 5 mM.

As can be seen, the inhibition rate increased evidently as the inhibition time increased from 5 min to 30min. When the inhibition time was longer than 30min, no essential increase of inhibition rate was observed. The influence of pH of Hg^{2+} solution on inhibition rate was tested and the result was presented in Figure 3. It was clearly shown that the inhibition rate increased with increasing pH from 2.00 to 5.50. Thus, the inhibition time and the pH of Hg^{2+} solution were chosen as 30 min and 5.50, respectively. Under these conditions, the highest sensitivity of the biosensor could be obtained.

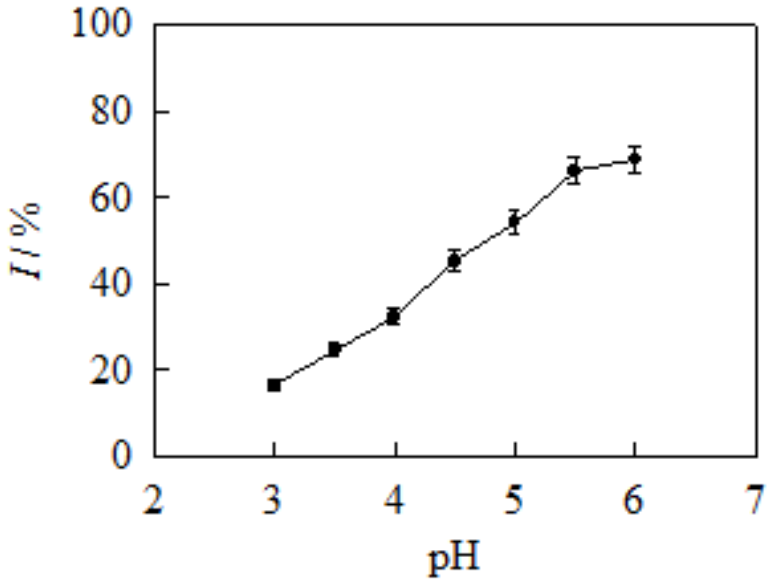

Figure 3. Dependence of inhibition rate (*I*) on the pH of Hg^{2+} solution for 0.1μM concentration of Hg^{2+}. The concentration of urea was 5 mM and the inhibition time was 30 min.

3.3. Response characteristics of the biosensor

3.3.1 Linear response range and the detection limit

Under the optimal measurement conditions, the potential response values of the biosensor to different concentrations of Hg^{2+} were tested and the results were show in Figure 4.

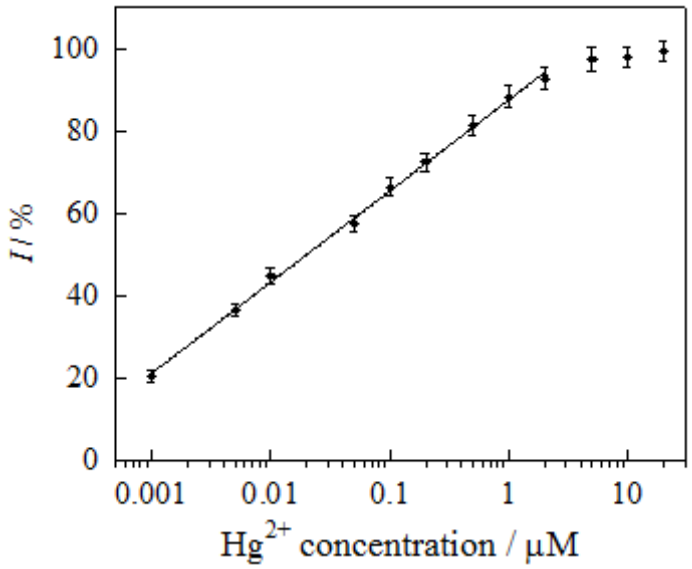

Figure 4. Calibration curve for Hg^{2+} determination by the proposed biosensor. The concentration of urea was 5 mM and the inhibition time was 30 min.

As can be seen, the inhibition rate increased evidently as the concentration of Hg^{2+} increased. The inhibition rate was linearised on semilogarithmic co-ordinates with concentrations of Hg^{2+} from 0.002 μM to 2μM. The detection limit was found to be 0.001 μM, which was defined as 10% inhibition rate. When the concentrations of Hg^{2+} was high then 3μM, the activity of urease was almost completely inhibited (the inhibition rate >95%). The concentration for 50% inhibition rate was calculated to be 0.03μM.

For comparison, the analytical performances such as the linear range and the limit of detection of the proposed biosensor and other enzyme based biosensors reported in the literatures were all summarized in Table 2. As can be seen, the linear range of the proposed biosensor was located in a relative lower range of Hg^{2+} concentrations. And the limit of detection for Hg^{2+} obtained by the proposed biosensor was much lower than by the previous reported biosensors. These results indicated that the proposed biosensor was an excellent platform for sensitive detection of Hg^{2+}.

3.3.2. Selectivity, accuracy and precision

The selectivity is obviously one of the important characteristics of the biosensors, determining whether reliable measurement of the target in sample is possible. To investigate the selectivity and

accuracy of the proposed biosensor, determination of Hg^{2+} in the presence of various heavy metal ions with 10 times concentration was carried out. The results were summarized in Table 2. As shown, the recovery of mercury ions was about 100% in the case either Hg^{2+} existed alone or coexisted with other heavy metal ions. The result of precision was also shown in Table 1. In all the cases, the relative standard deviation (RSD) was range from 2.8% to 4.2%. These results indicated that the selectivity, accuracy and precision of the proposed biosensor were satisfactory.

Table 1. Comparison of the performances for Hg^{2+} detection by enzyme based biosensors

Enzyme	Immobilization matrix	Transducers	Linear range	Limit of detection	References
Urease	Chitosan–poly(vinyl alcohol) hydrogel	Potentiometric	0.002μM-2μM	0.001μM	This work
Urease	Poly(vinyl chloride)	Potentiometric	0.05μM-1.0μM	0.02μM	19
Urease	Gold nanoparticles	Potentiometric	0.09 μM-1.99μM	0.05μM	20
Urease	Nafion	Potentiometric	Not mentioned	1μM	21
Urease	Nano-structured polyaniline-Nafion	Amperometric	0.010–0.100 ppm (0.05μM-0.5μM)	0.01 ppm (0.05μM)	26
Glucose Oxidase	Nation-MnO2 modified carbon paste	Amperometric	2.0–32.5 mg/L (10μM-162.5μM)	0.5 mg/L (2.5μM)	27
Invertase, mutarotase, glucose oxidase	Glutaraldehyde cross-linked bovine serum albumin	Conductometric	Not mentioned	0.025μM	28

Table 2. Determination of Hg^{2+} in binary mixtures

Hg^{2+} (μM)	Added cations	Added cations concentration(μM)	Determination of Hg^{2+}(μM)	Recovery(%)	RSD[a]
0.1	-	-	0.098	98	2.8
0.1	Ag^{+}	1.0	0.103	104	3.4
0.1	Cu^{2+}	1.0	0.101	101	4.2
0.1	Cd^{2+}	1.0	0.096	96	2.9
0.1	Pb^{2+}	1.0	0.097	97	3.1
0.1	Cr^{3+}	1.0	0.098	98	3.0
0.1	Ni^{2+}	1.0	0.096	96	3.6

[a] Relative standard deviation

3.3.3. Real sample analysis

To demonstrate the analytical applicability of the proposed biosensor, the concentrations of Hg^{2+} in two industrial wastewater samples were tested. Each sample was analyzed six times using the proposed biosensor, and the results were compared with those obtained by ICP-MS. The results were given in Table 3. As shown, the amount of Hg^{2+} obtained by the biosensor were in good agreement with those measured by ICP-Ms method, reflecting the utility of the proposed biosensor.

Table 3. Hg^{2+} concentrations in the wastewater samples determined by the proposed biosensor and ICP-MS method

Sample	Result of the biosensor(μM)[a]	Result of ICP-MS(μM)[a]
Industrial wastewater sample A	0.258±0.011	0.245±0.010
Industrial wastewater sample B	0.104±0.004	0.098±0.003

[a] Average of six determinations ± standard deviation

4. CONCLUSION

A potential biosensor with urease immobilized into chitosan–poly(vinyl alcohol) hydrogel film at the surface of pH electrode was used for determination of Hg^{2+}. This biosensor exhibited excellent sensitivity and selectivity. It also presented satisfactory precision. Furthermore, this biosensor was successfully used for accurate determination of Hg^{2+} in wastewater samples.

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