Journal of the Taiwan Institute of Chemical Engineers 42 (2011) 662–668

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice

Preparation of urease/nano-structured polyaniline-Nafion®/Au/Al_2O_3 electrode for inhibitive detection of mercury ion

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ARTICLE INFO

Article history:
Received 14 July 2010
Received in revised form 14 November 2010
Accepted 26 November 2010
Available online 26 January 2011

Keywords:
Mercury ion
Nano-structured polyaniline-Nafion®
composite film
Amperometric biosensor
Cyclic voltammetry
Urease

ABSTRACT

The level of mercury ion in the aqueous solution was detected based on the inhibition of urease/NSPN/Au/Al_2O_3 (urease/nano-structured PANi-Nafion®/Au/Al_2O_3) sensing electrode. When the NSPN composite film/Au/Al_2O_3 was prepared at 60 μA/cm^2 and used as the sensing electrode, the maximum sensitivity for monitoring the level of NH_4^+ in PBS was obtained to be 2980.3 μA/mM/cm^2 due to the dense composite film with exiguous crevices. The detection limit and the sensitivity of amperometric urea biosensor based on urease (2.12 U)/NSPN/Au/Al_2O_3 obtained at pH 7.0 and 25 °C were 0.005 mM and 4397.5 μA/mM/cm^2, respectively. The sensitivities to monitoring mercury ion were 2397.5 and 2884.0 μA/ppm/cm^2 for the Hg^{2+} concentration in the range of 0–0.1 ppm with the urease loadings of 1.06 and 2.12 U. The detection limit of the amperometric mercury ion sensor based on the urea biosensor was found to be 0.01 ppm. The sensing properties of the amperometric mercury ion biosensor were significantly affected by the concentration of urea in the solution.

1. Introduction

Heavy metal ions existing in our environment can have major toxic effects on living organisms and humans. Mercury is a known biotoxicant that can accumulate in the human body and show up in the food chain. A high level of mercury ion can cause deterioration of the brain, kidneys, and developing fetus (Chen *et al.*, 2007). Therefore the detection of mercury ion existing in the environment, drinking water and foods is a very important issue for our health. Several complex and expensive methods and techniques, such as atomic absorption spectrometry (AAS) (Hafez *et al.*, 2001; Vinas *et al.*, 2001), gas chromatography–inductively coupled plasma-mass spectrometry, atomic fluorescence spectrometry (AFS), and inductively coupled plasma-atomic emission spectrometry, and high-performance liquid chromatography (HPLC) (Chen *et al.*, 2007), have been established to detect the level of mercury ions.

Electrochemical biosensors have advantages over other existing measurement systems because they can provide rapid, simple and low-cost on-field determination of many biological active species and a number of dangerous pollutants. Based on the principles of the enzymatic inhibition, electrochemical biosensors have been widely used to detect heavy metal ions for food safety and in environmental monitoring (Amine *et al.*, 2006). Amperometric glucose biosensors have been used to measure the level of mercury ion in the solution (Alexander and Rechnitz, 2000; Malitesta and Guascito, 2005; Mohammadi *et al.*, 2002). The linear detection range of Hg^{2+} based on the amperometric glucose biosensor by immobilizing the glucose oxidase with poly-*o*-phenylenediamine obtained is 2.5–200 μmol/l (Malitesta and Guascito, 2005). Mercury(II), mercury(I), methylmercury, and mercury–glutathione complex are detected with the amperometric H_2O_2 biosensor based on horseradish peroxidase with methylene blue as the redox mediator (Han *et al.*, 2001). The detection range of mercury(II) is found by inhibiting the invertase activity in the concentration range of 1×10^{-8} to 1×10^{-6} M based on the amperometric sucrose biosensor (Mohammadi *et al.*, 2005).

Recently biosensors based on urease have been widely used to detect the level of heavy metal ions (Komaba *et al.*, 1998; Krawczyk *et al.*, 2000; Kuralay *et al.*, 2007; Rodriguez *et al.*, 2004; Yang *et al.*, 2006; Zhylyak *et al.*, 1995). The linear dynamic range of a conductometric Hg^{2+} biosensor with urease immobilized on an interdigitated gold electrode by glutaraldehyde (GA) is 1–50 μM (Zhylyak *et al.*, 1995). The linear detection range of the potentiometric Hg^{2+} biosensors based on the urease immobilized by polypyrrole (PPy) (Komaba *et al.*, 1998) and polyvinylchloride (PVC) (Krawczyk *et al.*, 2000) are reported to be 0.1–100 μM and

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doi:10.1016/j.jtice.2010.11.007

0.05–1.0 μM, respectively. The effect of the Au particle size on the detective properties of a potentiometric Hg^{2+} biosensor based on the urease immobilized on self-assembled gold nanoparticles is investigated (Yang *et al.*, 2006). The linear detection range of an amperometric Hg^{2+} biosensor based on urease immobilized in poly(vinylferrocenium) film measured at 0.7 V (vs. SCE) is 9.2–420 μM (Kuralay *et al.*, 2007).

A preliminary study on the amperometric urea biosensor based on the PANi(urease)-Nafion®/Au composite electrode prepared by the cyclic voltammetry (CV) technique has been reported in our previous work (Luo and Do, 2004). The effect of the cycle number for preparing PANi-Nafion® composite film and the materials for immobilizing urease on the sensitivity of amperometric urea biosensor were studied (Luo and Do, 2004). In the recent year, the oriented PANi nanowires have been successfully synthesized without any template by the chronopotentimetry technique (Liang *et al.*, 2002; Liu *et al.*, 2003). Compared with the PANi-Nafion® composite film prepared by the CV technique, the sensitivity of the amperometric urea biosensor based on the nano-structured PANi-Nafion® (NSPN) composite film prepared by the chronopotentiometry technique will be promoted due to the increase in the surface area of the composite film. It is of interest to study the characteristics of amperometric urea biosensor and determination of Hg^{2+} by immobilizing the urease on the NSPN composite film.

The sensing properties of amperometric urea biosensor based on NSPN/Au/Al_2O_3 prepared by the chronopotentiometry technique were systematically investigated in this work. The amperometric urea biosensor was also used to detect the level of mercury ion in the aqueous solution.

2. Experimental

Alumina plate (Al_2O_3-S-4″ × 4″ × 0.635 mm, U. E. Co., LTD) used as the substrate of sensing electrode was first immersed in 3.0 M NaOH aqueous solution for 30 min and washed with deionized (DI) water for several times. Then the alumina plate was immersed in 3.0 M HCl aqueous solution for 30 min and washed with DI water for several times. The pattern of Au electrode on the alumina plate was defined and prepared by a shadow mask and a sputtering coater (JOEL JFC-1200). The Au/Al_2O_3 electrodes were then immersed in 3.0 M NaOH aqueous solution for 10 min, and washed with DI water for several times. By the same procedures, the Au/Al_2O_3 electrodes were immersed in 3.0 M HCl aqueous solution and washed by DI water. Nafion®/Au/Al_2O_3 electrode was prepared by casting 8 μl 1 wt% Nafion® solution (diluting 5 wt% Nafion® solution (Aldrich) with DI water) on the Au/Al_2O_3.

Using Nafion®/Au/Al_2O_3 as the working electrode, the NSPN composite film was synthesized in 0.1 M aniline and 1.0 M HCl aqueous solution at 5 °C by the chronopotentiometry technique (electrochemical analyzer CHI 614A). The urease was immobilized onto the surface of PANi-Nafion® composite film by casting 4 μl urease PBS (phosphate buffer solution, pH 7.0) and 4 μl 1 wt% Nafion® solution in series, and then drying at 4 °C for 4 h. Then the Nafion®(urease)/NSPN/Au/Al_2O_3 plate electrode was stored at 4 °C pH 7.0 PBS.

Using Pt wire and Ag/AgCl/3 M $NaCl_{(aq)}$ as the counter and reference electrodes, the sensing properties of urea biosensor based on the homemade Nafion®(urease)/NSPN/Au/Al_2O_3 plate electrode were investigated in PBS by cyclic voltammetry (CV) technique. By adding a suitable concentration of Hg^{2+} ($HgCl_2$, ACROS (≥99.5%)) into urea PBS the inhibitive behaviors of the amperometric urea biosensor were also studied by CV technique. The cyclic voltammograms of a suitable concentration of urea in the absence and presence of various concentrations of Hg^{2+} were recorded with the scanning rate and range of 50 mV/s and −0.4 to 0.4 V, respectively. The sensing currents for various concentrations of Hg^{2+} were obtained by subtracting the peak current in the presence of Hg^{2+} from that in the absence of Hg^{2+}.

3. Results and discussion

3.1. Effect of the current density for preparing NSPN/Au/Al_2O_3 electrode

3.1.1. Relationship of potential and time

Using Nafion®/Au/Al_2O_3 as the working electrode, nano-structured PANi was electropolymerized on the surface of Au to form PANi-Nafion® composite film with the constant current density method in 0.1 M aniline and 1.0 M HCl aqueous solution. The relationships between the potential and time for preparing NSPN composite films with various current densities are illustrated in Fig. 1. For the current density of 40 μA/cm^2 the potential sharply increased from 0.37 V to a maximum value of 0.84 V with the increase in the electrolysis time from 0 to 126 s, and then the potential decreased to a stable value of 0.76 V by further increasing the electrolysis time to 300 s (curve (a) of Fig. 1). The fast increase in the potential for a time less than 30 s was deduced to be the nucleation of PANi on the Au, and the slower potential increasing rate in the time range of 30–126 s might be due to the growth of the PANi nuclei. When the PANi nuclei were grown and overlaid, the potential for the electropolymerization of PANi decreased from the maximum to a stable value when the electrolysis time was greater than 126 s. A similar potential-time behavior was obtained as illustrated in the curve (b) of Fig. 1 for the current density of 60 μA/cm^2. Increasing the current density from 40 to 60 μA/cm^2 the electrolysis time to achieve the maximum potential decreased from 126 to 88 s, which was caused by increasing the PANi electropolymerization rate.

Two stages of the potential increase were found by preparing PANi at the current densities of 80 and 100 μA/cm^2 (curves (c) and (d) of Fig. 1). The increase in potential in the first stage was mainly caused by the nucleation of PANi on the electrode surface similar to that for the current densities of 40 and 60 μA/cm^2. However the potential in the second stage different from those with the current

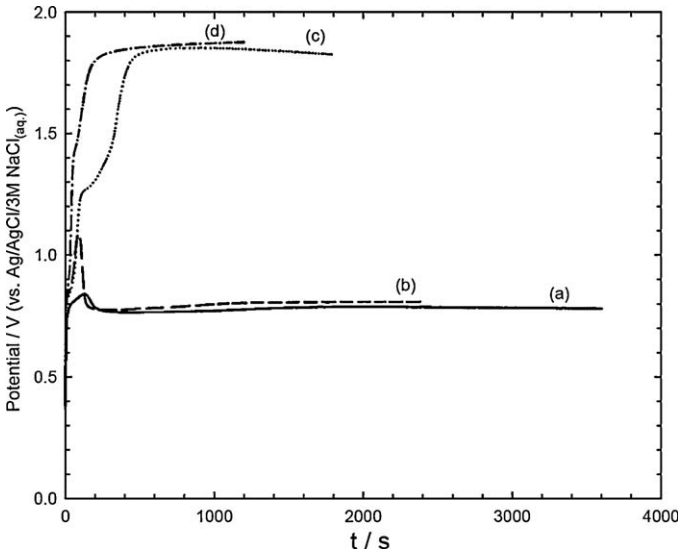

Fig. 1. Effect of electrolysis time on the potential for preparing NSPN composite film. Working electrode: Nafion®/Au/Al_2O_3 (8 μl 1.0 wt% Nafion® solution cast on Au (0.4 cm × 0.5 cm)), counter electrode: Pt wire, reference: Ag/AgCl/3 M $NaCl_{(aq)}$, [aniline] = 0.1 M, [HCl] = 1.0 M, T = 5 °C. Current density for preparing PANI-Naion®: (a) 40, (b) 60, (c) 80 and (d) 100 mA/cm^2.

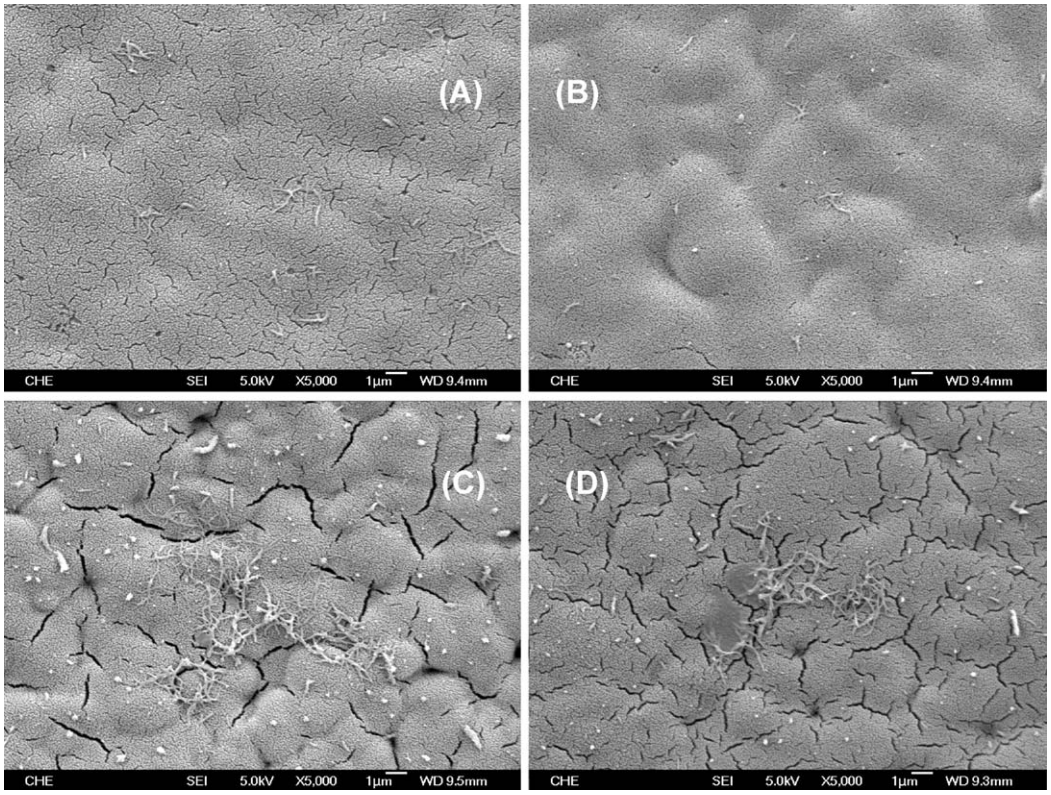

Fig. 2. SEM micrographs of NSPN composite film prepared at current density of (A) 40, (B) 60, (C) 80, and (D) 100 μA/cm^2.

density less than 60 μA/cm^2 was increased to a stable value of 1.85 V for current density of 80 μA/cm^2. When the current density for preparing PANi was equal or less than 60 μA/cm^2, the electropolymerization of PANi was inferred to be controlled by the reaction kinetics on the electrode surface, and hence a relatively lower stable potential was obtained in the final stage. On the other hand, for the current density greater than 60 μA/cm^2, the electropolymerization of PANi was controlled by the diffusion of aniline (monomer) from the bulk solution through the Nafion® film to the electrode surface. When the electrolysis time increased, the potential should be increased to reduce the concentration of aniline on the electrode surface, and compensate the increase in the diffusion layer thickness. Finally the constant concentration gradient was obtained at a relative higher potential to satisfy the constant polymerization rate due to the constant current density applied.

3.1.2. Surface morphologies of NSPN composite film

Some small crevices were found on the NSPN composite films prepared at the current densities of 40 and 60 μA/cm^2 with the passing charge of 0.0288 C as illustrated in Fig. 2(A) and (B). On the other hand, for the same charges passed, relatively greater crevices were found on the surface of composite films when the higher current densities (80 and 100 μA/cm^2) were applied (Fig. 2(C) and (D)). A small part of the PANi fiber was beetled from the composite films and the size of fiber found from the SEM images was 150 nm when the current densities for preparing PANi were 40 and 60 μA/cm^2, respectively. Increasing the current density to 80 and 100 μA/cm^2 the amount of beetling PANi fibers increased and the feature size of PANi fibers increased to 250 nm. The Nafion® film was extruded by the greater amount and feature size of beetling PANi fibers for the preparing current density greater than 80 μA/cm^2, and resulted in the increase in the amount of crevices on the surface of composite films.

3.2. Effect of the current density for preparing NSPN composite film on the sensing NH_4^+

The ions in the bulk solution were doped/undoped into the NSPN composite film to compensate the change in charge of PANi for the redox of composite film. However the doping/undoping ion was restricted to be NH_4^+ due to the existence of the cation exchange membrane (Nafion® film) as indicated in the following equation (Luo and Do, 2004),

$$PANi^+RSO_3^- + NH_4^+ + e^- \rightleftharpoons PANiNH_4^+RSO_3^- \quad (1)$$

where RSO_3^- was represented the Nafion® of the composite film.

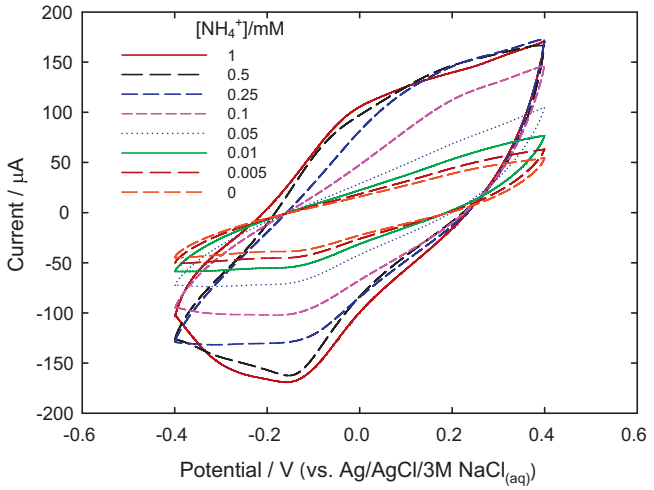

Fig. 3. Cyclic voltammograms of various concentrations of NH_4^+ on NSPN/Au/Al_2O_3 prepared at 60 μA/cm^2. Counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, T = 25 °C, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution), scan rate = 50 mV/s.

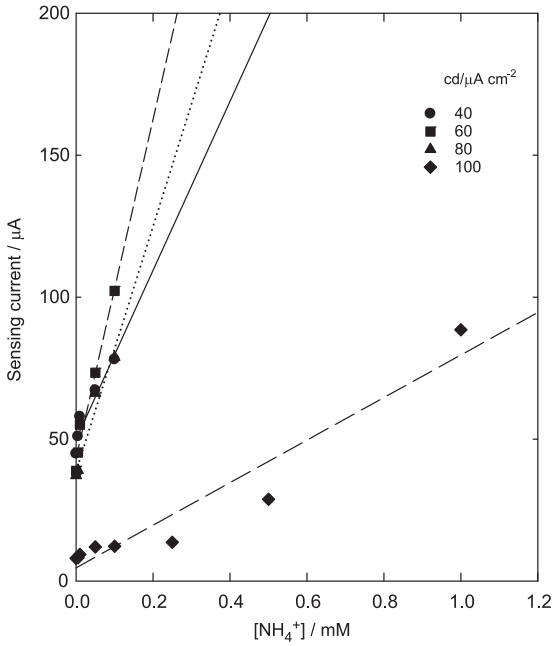

Fig. 4. Effect of the concentration of NH_4^+ on the sensing current for sensing electrodes prepared at various current densities. Sensing electrode: NSPN/Au/Al_2O_3, counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, T = 25 °C, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution).

Using NSPN/Au/Al_2O_3 prepared at 60 μA/cm^2 as the sensing electrode, the steady cyclic voltammograms for various concentrations of NH_4^+ in pH 7.0 PBS were shown in Fig. 3. Increasing the concentration of NH_4^+ from 0.005 to 0.5 mM the steady cathodic peak current increased from 45.3 to 161.0 μA. Further increasing the concentration of NH_4^+ to 1.0 mM the steady cathodic peak was slightly increased to 168.6 μA.

When the PANi-Nafion® composite films prepared at the current densities of 40, 80 and 100 μA/cm^2 were used as the sensing electrodes, the cyclic voltammograms for sensing NH_4^+ in PBS at the potential range of −0.4 to 0.4 V were similar to that of the sensing composite film prepared at 60 μA/cm^2. The relationships of the sensing currents (cathodic peak currents) and the concentrations of NH_4^+ illustrated in Fig. 4 revealed that the linear ranges for sensing NH_4^+ based on PANi-Nafion® composite films prepared at 40–80 μA/cm^2 were obtained as 0.005–0.1 mM (Fig. 4 and Table 1). The linear range for sensing NH_4^+ was extended to 0.005–1.0 mM for the composite film prepared at 100 μA/cm^2. The sensitivities of the amperometric NH_4^+ sensor could be evaluated from the slopes of the linear relationships between the sensing currents and the concentrations of NH_4^+.

Increasing the current density for preparing the composite films from 40 to 60 μA/cm^2 the sensitivity of the amperometric NH_4^+ sensor increased from 1487.1 to a maximum value of 2980.3 μA/mM/cm^2 (Table 1). Further increasing the preparing current density to 100 μA/cm^2 the sensitivity of the NH_4^+ sensor sharply decreased to 374.5 μA/mM/cm^2. Nafion® in the composite film played an ion selective role for sensing NH_4^+ (Luo and Do, 2004). Increasing the crevices of the composite film the selectivity for sensing NH_4^+ decreased because the doping/undoping ions (such as anions in the solution) other than NH_4^+ through the crevices were increased. Therefore the increase in amounts of crevices and beetling PANi fibers on the composite film surface would dramatically decrease the sensitivity of the amperometric NH_4^+ sensors. As shown in Fig. 2, the dense composite film with exiguous crevices and small amount of PANi fibers protruded on the composite film prepared at a current density of 60 μA/cm^2 resulted in the maximum sensitivity for sensing NH_4^+. On the other hand, a relatively large number and feature size of crevices and PANi fibers protruding from the composite surface were obtained at the current density of 100 μA/cm^2 (Fig. 2), and hence resulted in a lower sensitivity (Table 1).

Table 1
Effect of current density for preparing NSPN composite film on the sensing properties of the amperometric NH_4^+ sensor.

cd[a] (μA/cm^2)	Sensitivity (μA/mM/cm^2)	Linear range of [NH_4^+] (mM)
40	1487.1	0.005–0.1
60	2980.3	0.005–0.1
80	2157.8	0.005–0.1
100	374.5	0.005–1.0

Sensing electrode: NSPN/Au/Al_2O_3, counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, T = 25 °C, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution). Conditions for preparing NSPN composite film: [aniline] = 0.1 M, [HCl] = 1.0 M, T = 5 °C, 8 μl 1.0 wt% Nafion® solution cast on Au (0.4 cm × 0.5 cm)/Al_2O_3.

[a] Current density for preparing nano-structured PANi-Naion® composite film.

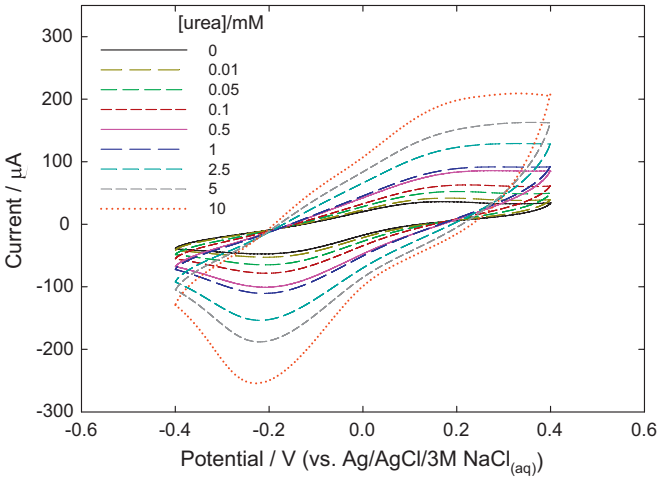

Fig. 5. Cyclic voltammograms of various concentrations of urea on urease (0.53 U)/NSPN/Au/Al_2O_3 electrode. Counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, T = 25 °C, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution), scan rate = 50 mV/s.

3.3. Sensing characteristics of amperometric urea biosensor

The level of urea in pH 7.0 PBS was measured by the CV technique based on the sensing electrode by immobilizing 0.53 U urease onto the NSPN/Au/Al_2O_3 electrode prepared at 60 μA/cm^2. As shown in Fig. 5, the cathodic and anodic peaks found at the potentials of −0.23 and 0.20 V for various concentrations of urea were due to the doping and undoping of NH_4^+ produced by the enzymatic reaction of urea in the aqueous phase (Eq. (2)) to compensate the charge changed in the redox of polyaniline (Eq. (1))

$$CO(NH_2)_2 + 3H_2O \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^- + OH^- \quad (2)$$

Compared with the results for sensing NH_4^+ (Fig. 3), a greater cathodic and anodic peak potential difference found in Fig. 5 for sensing urea might be due to the higher electric resistance, which was caused by the urease immobilized layer on the sensing electrode. Two linear ranges were found by plotting the cathodic peak current (I_{cp}^0) against the concentration of urea as illustrated in Fig. 6. The sensitivities of the amperometric urea biosensor found from the slopes of the linear relationships were 1494.2 and

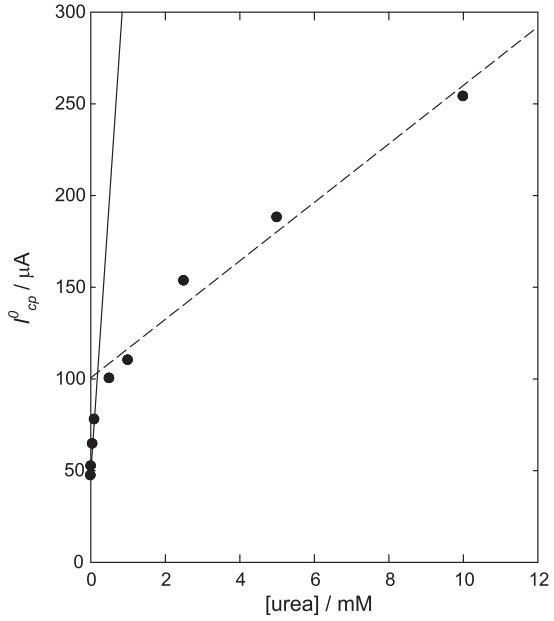

Fig. 6. Effect of the concentration of urea on the sensing current of amperometric urea biosensor. Sensing electrode: urease (0.53 U)/NSPN/Au/Al_2O_3, counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, T = 25 °C, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution).

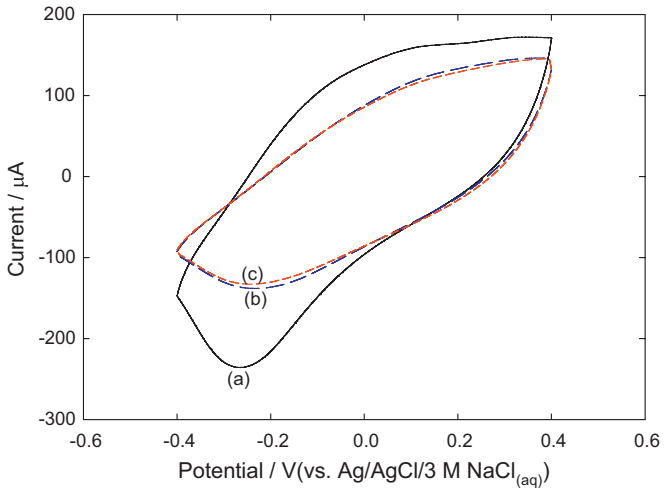

Fig. 7. Cyclic voltammograms on urease (2.12 U)/NSPN/Au/Al_2O_3. Counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, T = 25 °C, scan rate = 50 mV/s, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution). (a) 1.0 mM urea PBS, (b) 0.1 ppm Hg^{2+} in 1.0 mM urea PBS, and (c) 1.0 mM urea PBS.

79.7 μA/mM/cm^2 in the ranges 0.01–0.1 mM and 0.5–10 mM urea, respectively.

3.3.1. Effect of the loading of urease

When the amount of urease immobilized on the electrode (loading of urease) increased, the increase in the enzymatic urea reaction rate resulted in an increase in the concentration of NH_4^+ (product of the enzymatic reaction) nearby the electrode surface. Hence the sensitivity of the urea biosensor would be increased with the loading of urease on the sensing electrode. The experimental results in Table 2 indicated that the sensitivity of the urea biosensor was significantly increased from 595.9 to 3913.5 μA/mM/cm^2 by increasing the urease loading from 0.265 to 1.06 U. The sensitivity of the amperometric urea biosensor slightly increased to 4397.5 μA/mM/cm^2 by further increasing the loading of urease to 2.12 U. Furthermore the detection limit was decreased from 0.05 to 0.005 mM by increasing the urease loading from 0.265 to 1.06 and 2.12 U (Table 2).

3.4. Detection of Hg^{2+} based on the amperometric urea biosensor

3.4.1. Recovery of the sensing electrode

Using urease (2.12 U)/NSPN/Au/Al_2O_3 as the sensing electrode, the cathodic peak current was obtained to be 235.7 μA in the presence of 1.0 mM urea as illustrated in curve (a) of Fig. 7. By adding 0.1 ppm Hg^{2+} into the 1.0 mM urea PBS, the steady cathodic peak current inhibited by Hg^{2+} was obtained to be 138.1 μA (curve (b) of Fig. 7). The sensing electrode was washed with DI water for several times to remove the residual Hg^{2+}, and then placed in 1.0 mM urea PBS without Hg^{2+}, the steady cathodic peak current obtained from the cyclic voltammogram in Fig. 7 (curve c) was 132.9 μA, which was similar to the case for presence of 0.1 ppm Hg^{2+}. The experimental results indicated that the active site of urease on the sensing electrode was irreversibly combined with Hg^{2+}, and the cathodic peak current could not be recovered by removing Hg^{2+}. The similar results were obtained when the concentration of Hg^{2+} was changed to 0.5 and 1.0 ppm, respectively. Hence the sensing electrode used to monitoring the level of Hg^{2+} was renovated each time.

Table 2
Effect of urease loading on the sensing properties of amperometric urea biosensor.

Loading of urease (U)	Linear range of [urea] (mM)	Sensitivity (μA/mM/cm^2)	Detection limit (mM)
0.265	0.05–0.5	595.9	0.05
0.53	0.01–0.1	2233.0	0.01
1.06	0.005–0.1	3913.5	0.005
2.12	0.005–0.1	4397.5	0.005

Sensing electrode: urease/NSPN/Au/Al_2O_3, counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution), T = 25 °C.

3.4.2. Effect of urease loading

When the concentration of urea in PBS was fixed at 1.0 mM, the sensing currents of amperometric urea biosensor were measured in the presence of the various concentrations of Hg^{2+} at 25 °C with the urease loadings of 2.12 and 1.06 U, respectively. The activity of urease on the sensing electrode was inhibited due to the adsorption and combination with Hg^{2+} to the active center of urease, and the rate of enzymatic reaction and the sensing current would then be decreased. The sensing current (I_s) of the amperometric Hg^{2+} biosensor was defined as

$$I_s = I^0_{cp} - I_{cp} \quad (3)$$

where I^0_{cp} and I_{cp} were the cathodic peak currents of the urea biosensor in the absence and presence of Hg^{2+} in PBS. As indicated in Fig. 8, the linear relationship between the sensing current and the concentration of Hg^{2+} was found for the concentration of Hg^{2+} from 0 to 0.1 ppm. Increasing the concentration of Hg^{2+} from 0 to 0.1 ppm the sensing currents of urea biosensor immobilized with 2.12 and 1.06 U of urease increased from 0 to 57.9 and 53.9 μA. The similar sensitivities of the amperometric Hg^{2+} biosensor immobilized with 2.12 and 1.06 U urease were experimentally obtained to be 2884.0 and 2397.5 μA/ppm/cm^2, respectively. The results correlated well with the amperometric urea biosensor in the absence of Hg^{2+} illustrated in Table 2. The detection limit of the amperometric Hg^{2+} biosensor was found to be 0.01 ppm Hg^{2+}.

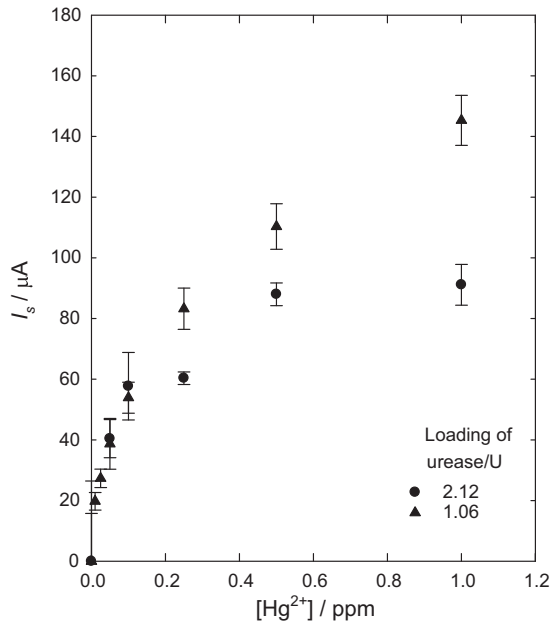

Fig. 8. Effect of concentration of Hg^{2+} on the sensing current of amperometric urea biosensor in 1.0 mM urea. Sensing electrode: urease/NSPN/Au/Al_2O_3, counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution), T = 25 °C.

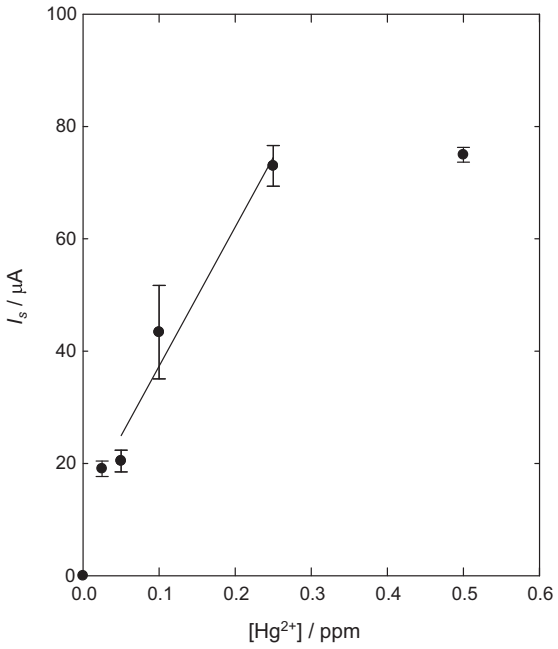

Fig. 9. Effect of concentration of Hg^{2+} on the sensing current of amperometric urea biosensor in 0.1 mM urea. Sensing electrode: urease (1.06 U)/NSPN/Au/Al_2O_3, counter electrode: Pt wire, reference electrode: Ag/AgCl/3 M $NaCl_{(aq)}$, pH 7.0 PBS (0.025 M K_2HPO_4 and 0.025 M KH_2PO_4 aqueous solution), T = 25 °C.

Table 3
Comparison of the monitoring Hg^{2+} based on urea biosensors.

Immobilization matrix	Transducers	Linear range/LOD[a]	References
Embedded by Nafion® film	Amperometric	0.010–0.100 ppm, LOD = 0.010 ppm	This work
Adsorbed on PVF[b]	Amperometric	2.5–115 ppm, LOD = 2.0 ppm	Kuralay *et al.* (2007)
Cross-linkage by GA on Au nanoparticles	Potentiometric pH-sensitive	0.027–0.541 ppm, LOD = 0.013 ppm	Yang *et al.* (2006)
Cross-linkage by GA	Conductometric	0.27–13.59 ppm	Zhylyak *et al.* (1995)
Entrapped in PVC[c]	Potentiometric	0.014–0.270 ppm	Krawczyk *et al.* (2000)
Entrapment in alginate gel	Amperometric	10–100 ppm, LOD = 2.9 ppm	Rodriguez *et al.* (2004)
Embedded by Nafion® film	ISFET[d]	C_{50}[e] = 0.41 ppm	Volotovsky *et al.* (1997)

[a] Limit of detection.
[b] Poly(vinylferrocenium).
[c] Poly(vinyl chloride).
[d] Ion selective field effect transistor.
[e] The concentration of Hg^{2+} for 50% urease inactivation.

3.4.3. *Effect of the concentration of urea*

Changing the concentration of urea in PBS from 1.0 to 0.1 mM, the urea biosensor loaded with 1.06 U urease was used to monitoring the levels of Hg^{2+}. Comparing with the amperometric Hg^{2+} biosensor in the presence of 1.0 mM urea in PBS (Fig. 8), the linear range of amperometric Hg^{2+} biosensor in the presence of 0.1 mM urea was shifted a relative higher range of Hg^{2+} concentration (0.05–0.25 ppm) as shown in Fig. 9. The sensitivity of amperometric Hg^{2+} biosensor in 0.1 mM urea PBS found to be 1247.0 μA/ppm/cm^2 was significantly less than that in 1.0 mM urea PBS with sensitivity of 2397.5 μA/ppm/cm^2. At a higher concentration of urea in PBS (1.0 mM), the amperometric Hg^{2+} biosensor had a higher background signal (I^0_{cp}), the sensing current (I_s) and the sensitivity of the amperometric Hg^{2+} biosensor hence increased.

The Hg^{2+} biosensors based on the inactivation of urease in the literature were concluded and compared in Table 3. The various transducers resulted in the various definitions of the sensitivity for sensing the concentration of Hg^{2+}. The linear Hg^{2+} concentration of this work was located in a relative lower range (0.010–0.100 ppm). The minimum limit of detection (LOD) for monitoring the concentration of Hg^{2+} was obtained to be 0.01 ppm in our results.

4. Conclusions

The NSPN composite film was prepared on Au/Al_2O_3 electrode by the chronoamperometric technique. When the NSPN composite film/Au/Al_2O_3 was prepared at 60 μA/cm^2 and used as the sensing electrode, the maximum sensitivity for monitoring the level of NH_4^+ in PBS was obtained to be 2980.3 μA/mM/cm^2 due to the dense composite film with exiguous crevices. Using urease (2.12 U)/NSPN/Au/Al_2O_3 as sensing electrode, the maximum sensitivity and the detection limit of the amperometric urea biosensor were obtained to be 4397.5 μA/mM/cm^2 and 0.005 mM in pH 7.0 PBS. Based on the amperometric urea biosensor the level of Hg^{2+} was measured by irreversibly inhibitive the activation of urease. The sensitivity and the detection limit of the amperometric mercury ion biosensor loaded with 2.12 U urease were experimentally obtained to be 2884.0 μA/ppm/cm^2 and 0.01 ppm Hg^{2+}, respectively. The sensitivity of the amperometric mercury ion biosensor loaded with 1.06 U urease was significantly increased from 1247.0 to 2397.5 μA/ppm/cm^2 by increasing the concentration of urea in PBS from 0.1 to 1.0 mM. The detection limit of the mercury ion biosensor was obtained to be 0.01 ppm.

Acknowledgments

The financial support of the Energy and Environment Research Laboratories of Industrial Technology Research Institute (ITRI), Tunghai University and Chin-Yi University of Technology is acknowledged.

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