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Kinetics of urease inhibition-based amperometric biosensors for mercury and lead ions detection

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ABSTRACT

The sensing element of biosensor is prepared by the immobilization of urease onto nano-structured polyaniline (PANi)-Nafion® (NSPN)/Au/Al_2O_3 synthesized by the chronopotentiometric technique. The sensitivity of lead ion at an amperometric urease based biosensor is obtained to be 743.5 μA ppm^{-1} for the linear dynamic range of 0.1–1.0 ppm Pb^{2+}. The irreversible inhibiting kinetic models of the mercury and lead ions at the amperometric biosensors are proposed. The values of K_d (dissociation equilibrium constant of adsorbed type of enzyme and heavy metal ion) and k_{irr} (irreversible inhibition reaction rate constant) for mercury and lead ions are obtained to be 0.45 ppm, 1.66×10^{-3} s^{-1}, 8.67 ppm and 3.16×10^{-3} s^{-1}, respectively. Compared with lead ion detection, the higher sensitivity to mercury ion of the biosensor is mainly caused by a higher inhibition efficiency (k_{irr}/K_d).

1. Introduction

The non-biodegradable heavy metal ions existing in our environment can have major toxic and cumulative effects on living organisms and humans. Hence it is very important to monitoring the toxic heavy metal ions with a cost-effectiveness and convenient procedure [1]. Mercury ion came from coal burning, mining and industrial wastes can cause deterioration of the brain, kidneys, and developing fetus [2,3]. Lead ion caused the damage to the nervous system is mainly came from automobile exhausts, tap-water with lead pipes, old paints and incinerator and mining wastes [3]. Heavy metal ions can be detected by various techniques, including mass spectrometric, optical and electrochemical methods [1,3,4]. Electrochemical technique have attracted great interest in the detection of heavy metal ions due to the relative short analytical time, low power cost, high sensitivity and capability for in-situ measurement [1]. Furthermore, biosensors provide a rapid pathway with simple devices for measuring heavy metal ions [5,6]. Hence the inhibition-based biosensors with electrochemical transducer can provide successful combination between biotechnology and modern electronics for analysis the level of heavy metal ions [6].

Electrochemical enzyme inhibition-based biosensors have been widely used to detect heavy metal ions for food safety and environmental analysis [1,3,6–8]. Based on the glucose oxidase, HRP (horseradish peroxidase), invertase, L-lactate dehydrogenase and urease enzymatic inhibition, the concentrations of mercury ion have been detected with the electrochemical biosensors [6–9]. A conductometric biosensor with three-enzyme system (invertase, mutarotase and glucose oxidase) is developed for inhibitory determination of Hg^{2+} [10]. Mercury ion can also be detected by the potentiometric biosensors [11–13], and a good linear relationship with the logarithm of Hg^{2+} concentration in the range from 0.0004 to 0.4 ppm [11]. Based on urease inactivation by the presence of heavy metal ions (Hg^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+}), an amperometric urease-glutamic dehydrogenase coupled assay is developed for screening heavy metal ions in environmental samples [14]. Using poly(vinylferrocenium) (PVF^{+}) modified electrode, an urease-inhibition based amperometric biosensor for monitoring Hg^{2+} in battery samples gave reliable results when compared to atomic absorption spectrometric findings [15]. The optimum conditions for immobilizing urease on the screen-printed carbon electrodes (SPCEs) used to fabricate the urease-inhibition based amperometric biosensor for the detection of Hg^{2+} was evaluated by experimental design technique [16]. In our previous work, the sensitivities to monitoring Hg^{2+} are 2397.5 and 2884.0 μA ppm^{-1} cm^{-2} with linear range of 0–0.1 ppm for the urease loadings of 1.06 and 2.12 U on urease/NSPN (nano-structured polyaniline-Nafion®)/Au/Al_2O_3 sensing electrode [17].

Recent developments have shown the use of electrochemical biosensors with urease, alkaline phosphatase, glucose oxidase, nitrate reductase and L-lactate dehydrogenase as enzymes,

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respectively, for detection of lead ion [6–9,18]. The linear range for detecting the level of lead ion with conductometric sol-gel-immobilized-urease biosensor is 20.72 −207.2 ppm [19]. The concentration of lead ion can also be detected by the potentiometric urea biosensors [20,21]. The insignificant sensitivity for monitoring the level of Pb^{2+} using an amperometric biosensor based on immobilized urease and glutamic dehydrogenase is obtained [14]. Hence the amperometric urease based biosensor for detection of lead ion is seldom reported in the literature. To the best of our knowledge, the kinetics for the electrochemical urease inhibition-based biosensor for measuring the concentrations of heavy metal ions, such as mercury and lead ions, are not reported. The heavy metal ion sensing properties using an amperometric biosensor can be comprehended through the kinetic study.

The amperometric urea biosensor based on the home-made Nafion® (urease)/NSPN/Au/Al_2O_3 sensing electrode is used for inhibitive detection of lead ion in the aqueous solution. The kinetics of the amperometric urease/NSPN/Au/Al_2O_3 biosensor for detecting mercury and lead ions are also investigated in this work.

2. Experimental

2.1. Reagents and materials

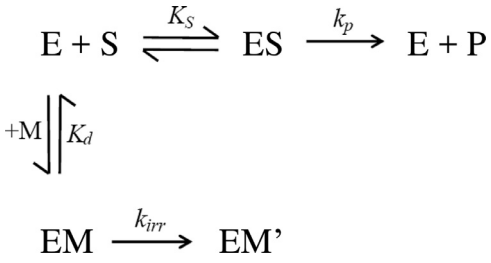
Aniline with a purity of 99.5% (Merck, GR) and 37% hydrochloric acid (Merck, GR) were used as accepted to be the monomer and the catalytic acid to prepare the PANi and its composite films. 5 wt% Nafion® solution purchased from Aldrich was used as to cast onto the electrode and synthesize PANi-Nafion® composite films. KH_2PO_4 (Showa, EP grade (99%)) and Na_2HPO_4 (Showa, EP grade (99%)) were used to prepare a buffer solution of pH 7.0. Urease (type III from Jack beans, 17,000 unit g^{-1}) was purchased from Sigma. Urea (Showa, 99%) and NH_4Cl (Showa, 99%) used in this work was not further purified before usage.

2.2. Preparation of sensing electrodes

The detail for preparing Nafion®(urease)/NSPN/Au/Al_2O_3 sensing electrode was described in our previous work [17]. Firstly, alumina plate (Al_2O_3-S-4″×4″×0.635 mm, U. E. Co., LTD) used as the substrate of sensing electrode was immersed in 3.0 M NaOH aqueous solution, washed with de-ionized (DI) water, immersed in 3.0 M HCl aqueous solution, and finally washed with DI water to remove the grease and residuals on alumina plate. The pattern of Au electrode on the alumina plate defined by a shadow mask was prepared by sputtering technique (sputtering coater JOEL JFC-1200). The procedure for cleaning the as-prepared Au/Al_2O_3 electrode was same as above. Nafion®/Au/Al_2O_3 electrode prepared by casting 8 μl 1 wt% Nafion® solution (diluting 5 wt% Nafion® solution (Aldrich) with DI water) on Au/Al_2O_3 electrode was used as working electrode to synthesize NSPN composite film in 0.1 M aniline and 1.0 M HCl aqueous solution at 5 °C by chronopotentiometric technique (electrochemical analyzer CHI 614A). Nafion®(urease)/NSPN/Au/Al_2O_3 sensing electrode was prepared by casting 4 μl urease in PBS (phosphate buffer solution, pH 7.0) and 4 μl 1 wt% Nafion® solution in series on the surface of NSPN/Au/Al_2O_3, and then drying at 4 °C for 4 h. The homemade Nafion®(urease)/NSPN/Au/Al_2O_3 electrode was stored at 4 °C pH 7.0 PBS.

2.3. Sensing properties

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 had been investigated in PBS by cyclic voltammetry (CV) technique at 25 °C [17]. By adding a suitable concentration of Hg^{2+} ($HgCl_2$, ACROS (≥99.5%)) and Pb^{2+} ($Pb(NO_3)_2$, Showa (GR, 99.5%)) into 1.0 mM urea in PBS the inhibition behavior of the urease based amperometric biosensor were also studied by CV technique. The cyclic voltammograms in the absence and presence of various concentrations of Hg^{2+} and Pb^{2+} were recorded with the scanning rate of 50 mV s^{-1} and in the potential range of −0.4–0.4 V, respectively. The sensing currents for various concentrations of heavy metal ions (Hg^{2+} and Pb^{2+}) were obtained by subtracting the peak current in the presence of heavy metal ions from that in the absence of heavy metal ions.



Scheme 1. Competitive inhibition of urease by the heavy metal ion.

3. Theoretical analysis

In general, the enzyme reactions in the presence of inhibitor are often complex. Mainly, the reversible and irreversible mechanisms of the reaction between the enzyme and inhibitor are considered [6]. As reported in our previous study [17], the initial cathodic peak current of Nafion®(2.12 U urease)/NSPN/Au/Al_2O_3 in the presence of 1.0 mM urea was obtained to be 235.7 μA, and the cathodic peak current was decreased to 138.1 μA by adding 0.1 ppm Hg^{2+}. Then the sensing electrode was washed with DI water to remove the residual Hg^{2+}. Then the sensing electrode was placed in 1.0 mM urea PBS without Hg^{2+}, the steady cathodic peak current was obtained to be 132.9 μA similar to that for the presence of 0.1 ppm Hg^{2+}. Therefore, the irreversible reaction mechanism between the enzyme and inhibitor for the detection of Hg^{2+} and Pb^{2+} at an amperometric biosensor based on Nafion® (urease)/NSPN Au/Al_2O_3 electrode was proposed and illustrated in Scheme 1. Firstly a part of enzyme (E) could reversibly adsorb heavy metal ion (inhibitor) to form EM, and the equilibrium dissociation constant for EM was obtained to be

$$K_d = [E][M]/[EM] \quad (1)$$

where [E] and [M] were the concentrations of enzyme and metal ion, respectively. Then the inactive enzyme-inhibitor complex (EM') was formed by the formation of a covalent bond between the enzyme active center and the heavy metal ion. Based on Scheme 1, the enzyme activity ([E]+[EM]=[ε]) inhibition rate could be expressed as [22],

$$-\frac{d[\varepsilon]}{dt} = -\frac{d([E]+[EM])}{dt} = k_{irr}[EM] \quad (2)$$

The initial enzyme concentration was

$$[E_0] = [E] + [EM] + [EM'] = [\varepsilon] + [EM'] \quad (3)$$

Substituting [E]=[ε] − [EM] into Eq. (1), the concentration of EM can be expressed as

$$[EM] = \frac{[\varepsilon]}{(1 + K_d/[M])} \quad (4)$$

By substituting Eq. (4) into Eq. (2) and integrating the equation with the initial condition (t = 0, [ε]=[E_0]), the relationship of