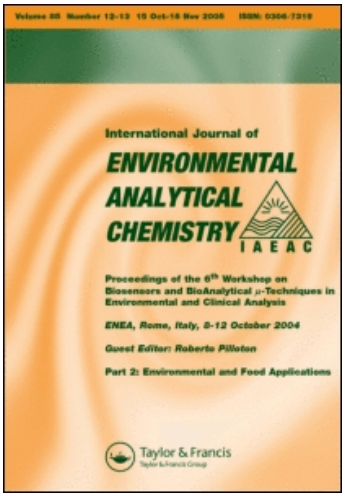


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of trace chromium(VI) by an inhibition-based enzyme biosensor incorporating an electropolymerized aniline membrane and ferrocene as electron transfer mediator

Guang-Ming Zeng[a]; Lin Tang[a]; Guo-Li Shen[b]; Guo-He Huang[a]; Cheng-Gang Niu[a]
[a] Department of Environmental Science and Engineering, Hunan University, Changsha 410082, P.R. China [b] State Key Laboratory for Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, P.R. China

To cite this Article Zeng, Guang-Ming , Tang, Lin , Shen, Guo-Li , Huang, Guo-He and Niu, Cheng-Gang(2004) 'Determination of trace chromium(VI) by an inhibition-based enzyme biosensor incorporating an electropolymerized aniline membrane and ferrocene as electron transfer mediator', International Journal of Environmental Analytical Chemistry, 84: 10, 761 — 774
To link to this Article: DOI: 10.1080/03067310410001730619
URL: http://dx.doi.org/10.1080/03067310410001730619

Intern. J. Environ. Anal. Chem.
Vol. 84, No. 10, pp. 761–774, 20 August 2004

Taylor & Francis
Taylor & Francis Group

DETERMINATION OF TRACE CHROMIUM(VI) BY AN INHIBITION-BASED ENZYME BIOSENSOR INCORPORATING AN ELECTROPOLYMERIZED ANILINE MEMBRANE AND FERROCENE AS ELECTRON TRANSFER MEDIATOR

GUANG-MING ZENG[a,*], LIN TANG[a], GUO-LI SHEN[b], GUO-HE HUANG[a] and CHENG-GANG NIU[a]

[a]*Department of Environmental Science and Engineering, Hunan University, Changsha 410082, P.R. China;* [b]*State Key Laboratory for Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, P.R. China*

(Received 23 December 2003; In final form 15 March 2004)

A novel inhibition-based glucose oxidase (GOx) biosensor for environmental chromium(VI) detection is described. An electropolymerized aniline membrane has been prepared on a platinum electrode containing ferrocene as electron transfer mediator, on which GOx is cross-linked by glutaraldehyde. The mechanism of the redox reaction on the electrode and the performance of the sensor are studied. The sensor's response to glucose decreases when it is inhibited by chromium(VI), with a lower detection limit of $0.49\,\mu g\,L^{-1}$, and the linear response range is divided into two parts, one of which is $0.49–95.73\,\mu g\,L^{-1}$ and the other is $95.73\,\mu g^{-1}$ to$8.05\,mg\,L^{-1}$. The enzyme membrane is shown to be completely reactivated after inhibition, retaining 90% activity over more than forty days. Interference to chromium(VI) determination from lead(II), copper(II), cadmium(II), chromium(III), cobalt(II), tin(II) and nickel(II) is found to be minimal, while high concentrations of mercury(II) and silver(I) may interfere with the determination of trace chromium(VI). The sensor has been used for chromium(VI) determination in soil samples with good results.

Keywords: Chromium(VI); Inhibition; Glucose oxidase; Biosensor; Polyaniline; Ferrocene

INTRODUCTION

Since the mid-1980s there has been a continuous growth in the use of biosensors for environmental analysis owing to their advantages, such as screening of various contaminants in environmental matrices, minimizing sample pretreatment, reducing the cost and time of analysis, and displaying sufficient sensitivity and selectivity [1]. Among various biosensors, recent attention has turned towards inhibition-based enzyme sensors to determine the concentrations of inhibitors in the assayed sample by measuring the inhibition degree of enzyme sensor activity, the lower detection

*Corresponding author. Fax: +86-731-8823701. E-mail: zgming@hnu.net.cn

ISSN 0306-7319 print: ISSN 1029-0397 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/03067310410001730619

limits of which can be as low as 10^{-8}–10^{-11} g L^{-1}. A number of inhibition-based enzyme sensors have been reported, such as a urease sensor for detection of mercury(II) [2], acetylcholinesterase and cholinesterase sensors for detection of organophosphate and carbamate pesticides [3,4], an oxidase sensor for detection of NO [5] and a peroxidase sensor for detection of HCN [6].

The ability to analyze trace heavy metals in the environment is now an essential requirement for safeguarding the ecosystem. Chromium(VI) (e.g., the anhydride of chromic acid, chromates, dichromates and polychromates) is a most hazardous heavy metal, discharged into the environment from industrial processes, and only a small amount can dissolve in water. It moves deeper in the soil, leaches into underground water, and remains unchanged or changes slowly in many natural waters owing to the low concentration of reductive substances. Cr(VI) has great ability to cross biological barriers. It can enter the human body through breathing, skin contact and oral ingestion with significant transfer, and can cause irritation to the nose, skin ulcers, stomach disturbances, kidney and liver damage, and even death [7]. Furthermore, the World Health Organization (WHO) has determined that Cr(VI) is a human carcinogen, which is confirmed by many reports [8]. Conventional analysis methods consist of spectrometry, flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrophotometry (GFAAS), and the recently developed atomic fluorometry spectrophotometer (AFS). These methods call for expensive equipment and complicated sample pretreatment which make them unsuitable for "on the spot" control [9]. Moreover, most methods have difficulty in analyzing only the Cr(VI) in complex samples [10].

Therefore, the object of this article is to develop a novel amperometric inhibition-based GOx sensor to determine trace Cr(VI). With the addition of inhibitors to the electrolyte, the current response of the GOx sensor decreases in the presence of its substrate glucose. GOx is an ideal enzyme for studies of inhibition owing to its low cost, good stability and high specific activity [11] and it can be potentially inhibited by some heavy metals which can combine with some region in the active centre of GOx. Among the heavy metals we tested, Cr(VI) was found to be an acute inhibitor on the GOx electrode, and the inhibition was reversible, which has not previously been reported.

For inhibition studies, it is required to have a sufficient sensitivity, lifetime, and reproducibility. In this article, the immobilization of GOx is achieved by integrating traditional electrochemical polymerization with a self-assembly technique. Aniline was chosen to be electropolymerized on the electrode surface as a polymer membrane containing ferrocene as the electron transfer medium. GOx was cross-linked by glutaraldehyde on the polymer membrane. The polyaniline membrane can prevent the enzyme molecules from leaking out and hold back the penetration of some interferents in environmental samples, which adds to the sensor's stability and selectivity [12]. The cross-linking by glutaraldehyde can stabilize the steric structure of enzyme molecules and avoid enzyme denaturation under relatively severe conditions [13]. Therefore, the performance of this sensor in both glucose detection and Cr(VI) detection is shown to be better than previous reports. And the lower detection limit of 0.49 μg L^{-1} Cr(VI) is close to the detection limits of conventional methods. To analyze the Cr(VI) concentration in the environment, soil is a favorite sample. In this article, a soil extract replaced soil as a matrix to test the recovery ratios of Cr(VI), which led to good results.

With all the advantages above, this sensor holds great potential to be applied in environmental analysis for "on the spot" monitoring of trace Cr(VI).

EXPERIMENTAL

Materials

Glucose oxidase (GOx) was from Sigma, EC 1.1.3.4 from *Aspergillus niger*, with activity of 196 units mg^{-1}. β-D-glucose was from ICN Biomedicals, Aurora, OH. All other chemicals were of analytical reagent grade. Double-distilled water was used throughout. Phosphate buffer solutions of 0.0667 M KH_2PO_4 and 0.0667 M Na_2HPO_4, and acetate buffer solutions of 0.2 M HAc, 0.2 M NaAc and 1 M HAc were used in this work. A 0.5 M glucose solution was obtained by dissolving β-D-glucose in phosphate buffer (pH 7.00). The heavy metals were dissolved in 0.3% HNO_3 to prepare stock solutions of 1 g L^{-1} except Sn(II), for which $SnCl_2 \cdot 2H_2O$ was dissolved in 36% HCl and diluted with water to a concentration of 1 g L^{-1} Sn(II) and 0.3% HCl. The working standard solutions of 50 mg L^{-1} and 1 mg L^{-1} heavy metals were prepared by successive dilution of the stock solutions with acetate buffer (pH 2.55).

Apparatus

Cyclic voltammetric measurements and amperometric measurements were carried out on a PAR 273 potentiostat/galvanostat and model 270 software (EG&G Princeton Applied Research, Princeton, NJ), and Model XJP-821 polargraphic analyzer (Jiangsu Electroanalytical Instruments, Jiangsu, China). The three-electrode system used in this work consists of a Pt electrode (planar area 3.14×10^{-4} cm^2) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a Pt foil auxiliary electrode. A model CS501SP thermostat (Chongqing Wanda Experimental Equipment, Chongqing, China) was used to control the temperature. A model PHS-3C pH meter (Leici Instrument, Shanghai, China) was used to test the pH value. A centrifuge, a vacuum drying oven and a mechanical vibrator were used for sample pretreatment. During amperometric measurement, a magnetic bar was used to stir the solution.

Sample Pretreatment

The blank type of soil was collected from 100 cm underground on the unfrequented hillside of Yuelu Mountain (Changsha, China), from which large organic scraps were removed. Then the soil sample was dried (at 105°C) for 8 h, ground in a pestle and sifted through a sieve of 100 screen mesh. 40 g soil sample was placed in a 500-mL flask and 200 mL acetate buffer (pH 2.55) was added. The suspension was agitated on a mechanical vibrator at 80 r min^{-1} for 4 h, and the supernatant was centrifuged at 3500 r min^{-1} for 20 min, and then filtered through a filter paper [14]. All the work was done at room temperature unless otherwise mentioned.

GOx Sensor Fabrication

The working electrode was made by sealing a 200-μm diameter pure Pt wire in one end of a thin glass tube. The other end of the tube was sealed with ethoxyline, through

which passed a copper conductor connected to the Pt wire. The Pt electrode surface was polished with diamond paper, sonicated subsequently in 50% (volume ratio) acetate aqueous solution and water for 5 min each [15], and electrochemically pretreated by cyclic voltammetry in 0.5 M H_2SO_4 between -0.2 and $+1.2$ V at 100 mV s^{-1} until a steady state was reached.

5 mL phosphate buffer (pH 7.00) containing 0.2 M aniline and 5 mL 12 mM ferrocene (Fc) ethanolic solution were mixed together into a water/ethanol (1 : 1 volume ratio) suspension [16]. Then cyclic voltammetry between 0 and $+1.6$ V at 10 mV s^{-1} was performed on the Pt electrode vs. SCE in the mixture for 10 cycles. The resulting PA, Fc membrane was brown in color.

The Pt/PA, Fc electrode was soaked in 2.5% (volume ratio) glutaraldehyde aqueous solution in thermostat at 37°C for 30 min, and then rinsed with water and dried in air. 1.2 mg GOx was dissolved in 20 μL phosphate buffer (pH 7.00), and a 2 μL aliquot of the GOx solution (equivalent to 23.52 unit of the enzyme) was pipetted onto the electrode surface and dried in air, then rinsed with phosphate buffer (pH 7.00) to remove non-covalent adsorptive material.

The whole process of sensor fabrication from pretreatment to GOx immobilization took only about 2.5 h. The Pt/PA, Fc/GA/GOx electrode was kept in phosphate buffer (pH 7.00) at 4°C in a refrigerator when not in use.

Measurement

The cyclic voltammetric scans were performed between 0 and $+1.1$ V at 10 mV s^{-1} vs. SCE in phosphate buffer (pH 7.00) containing glucose with different concentrations without stirring. The peak voltage was around $+0.7$ V, which was typically chosen as the optimum voltage for amperometric measurement to test the electrode characteristics and determine glucose concentration.

For inhibition studies, aliquots of standard Cr(VI) solutions were pipetted into buffer solutions containing known concentrations of glucose for amperometric measurement at $+0.7$ V, plotting the change in current as a function of Cr(VI) concentration. The optimum pH and substrate concentration for inhibition studies were found. Possible interferents such as Pb(II), Cu(II), Cd(II), Cr(III), Hg(II), Ag(I), Co(II), Sn(II) and Ni(II) were also tested under the same conditions. After each inhibition, the working electrode was reactivated by soaking in phosphate buffer (pH 7.00) for 8 min.

The soil extract was analyzed by atomic absorption spectrophotometry to confirm that the background concentrations of Cr(total), Pb(II), Cu(II), Cd(II) were 0, 20, 0 and 0 μg L^{-1} respectively according to the national standard [17]. Then a 10-mL soil extract was used as the blank solution to measure the recovery ratios of Cr(VI) concentration added.

Each calibration experiment was done three times to obtain the mean value.

RESULTS AND DISCUSSION

Growth of Polymer Membrane and Mechanism of the Biocatalytic CV Response

Electrochemical polymerization was used to deposit polyaniline on the surface of the Pt electrode. The thickness of the membrane is controlled by the number of cycles

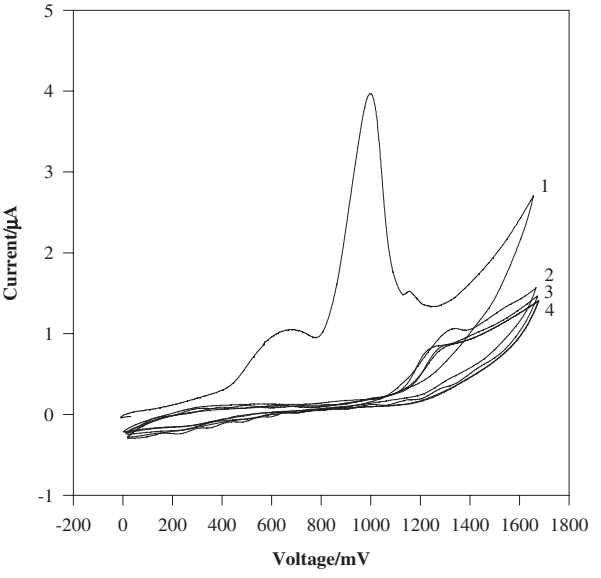


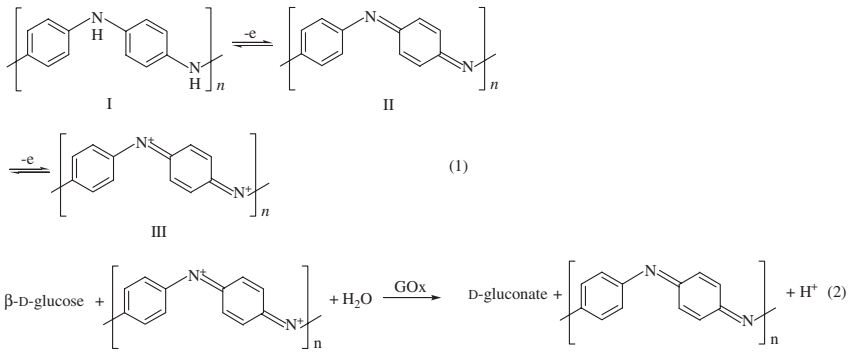
FIGURE 1 Cyclic voltammograms for electrochemical polymerization of aniline in water/ethanol mixture at 10 mV s^{-1} on Pt electrode vs. SCE. 1, 2, 3, 4 are the sequence numbers of the scanning cycles.

of the CV scan. The cyclic voltammograms are shown in Fig. 1. The highest peak indicates the polymerization of aniline. Because the electrical conductivity of the polymer membrane is much lower than that of the naked Pt electrode, the peak current in the later cycles declines drastically [15]. Ferrocene is entrapped in the polymer membrane as an electron transfer mediator to improve the electron transfer of the redox reaction. Therefore, when the pH of the buffer solution is between 4.5 and 7, the redox reactions on the electrode at +0.7 V can be represented as follows [18,19]:

$$\beta\text{-D-glucose} + Fc^+ + H_2O \xrightarrow{GOx} \text{D-gluconate} + Fc + H^+$$
$$Fc \rightarrow Fc^+ + e^-$$

The electrochemical activity of polyaniline increases swiftly as pH decreases. Scheme 1(1) shows the redox reactions of polyaniline at low pH. The anodic and cathodic peaks of reaction between I and II in the CV study are +0.21 V and +0.10 V respectively; the anodic and cathodic peak of reaction between II and III are +0.78 V and +0.68 V respectively [20]. When the pH of the acetate solution is between 2 and 4.5, with the working voltage in the amperometric study at +0.7 V, the electrochemical redox of polyaniline is activated and affects the redox reactions on the electrode as an electron mediator as shown in Scheme 1(2). The substitution of ferrocene and polyaniline for O_2 as electron transfer mediators greatly increases the speed of the redox reaction and the current response of the electrode.

Without the presence of ferrocene, the anodic current at the Pt/PA/GOx electrode shows little response to the addition of glucose to the phosphate buffer (pH 7.00) in cyclic voltammetric scans. Figure 2 presents the cyclic voltammograms on a Pt/PA/GOx electrode and on a Pt/PA, Fc/GOx electrode in the absence and presence



SCHEME 1 Schematic diagram of the redox reactions on the working electrode.

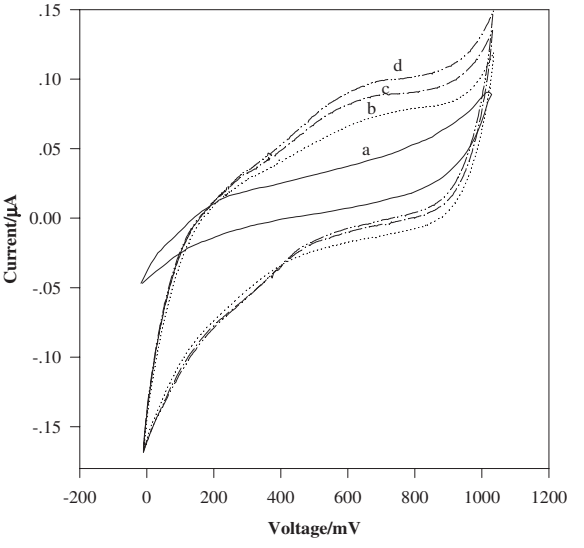


FIGURE 2 Cyclic voltammograms obtained in blank phosphate buffer (pH 7.00) at 10 m Vs^{-1}, on Pt/PA/GOx electrode without the presence of ferrocene (a), and on Pt/PA,Fc/GOx electrode containing glucose of the following concentrations: 0 (b), 5 mM (c) and 10 mM (d).

of glucose. The figure shows that the presence of ferrocene greatly increases the current on the electrode in phosphate buffer (pH 7.00), and the anodic current evidently rises at around +0.7 V with increasing glucose concentration, although the oxidation peak is not sharp because the surface area of the sensor is quite small. The sensitivity of the sensor to glucose is also shown to be highest at +0.7 V in the subsequent amperometric measurement.

Electrode Characteristics

pH Effect

The pH value of the working solution is usually regarded as having a very important effect on the performance of the enzyme electrode and its sensitivity towards the substrate and inhibitor. The pH range of an enzyme electrode often shifts from the pH

limits of the dissolved enzyme's activity for many reasons, such as the immobilization and the buffer solution [9]. As previously reported, the pH range of a dissolved GOx from *Aspergillus niger* is 3.4–7.5, while the pH range of GOx immobilized on activated carbon is 2.5–9 [21]. In this work, the solid immobilization of GOx and stabilization of its molecular structure may improve its pH adaptability, and make it still active even at low pH. And probably because of the difference of the redox mechanism and buffer solution at high and low pH values, as mentioned earlier, the anodic current change towards 1 mM glucose in acetate buffer (pH 2.10–3.50) is larger than in phosphate buffer (pH 4.39–6.98) as shown in Fig. 3. But when the pH is lower than 2.55, the current drops owing to the loss of GOx activity. Moreover, the sensor's response to glucose is proved to be more sensitive in acetate buffer (pH 2.55) than in phosphate buffer (pH 6.24) in the experiment.

Glucose Calibration by Amperometric Measurement

Because the working pH of most GOx electrodes in the literature is around 6, pH 6.24 is chosen as the calibration pH for glucose in this article to be comparable with others. Figure 4 shows the anodic current change with the addition of glucose to phosphate buffer (pH 6.24) measured by amperometry at +0.7 V. The change in current reaches a steady state in approximately 60 s owing to the catalytic reaction. The plot of the current change vs. glucose concentration in Fig. 4(A) indicates that the lower detection limit is 0.1 mM and the linear range is 1–17 mM. Five replicate measurement of a 7 mM glucose solution gives an RSD of 2.87% for a mean current change value of 3.768 nA. The current response to a specific glucose concentration can remain constant for up to 30 min, which is an advantage for the inhibition test.

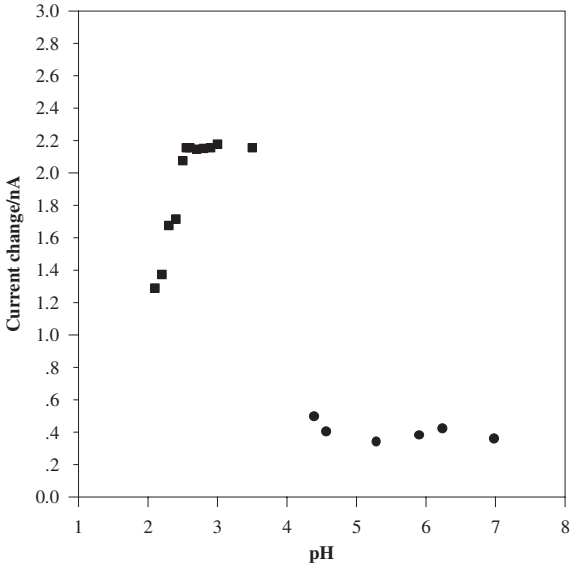


FIGURE 3 The amperometric current change towards 1 mM glucose vs. pH value in phosphate buffer (•) and acetate buffer (■) at +0.7 V.

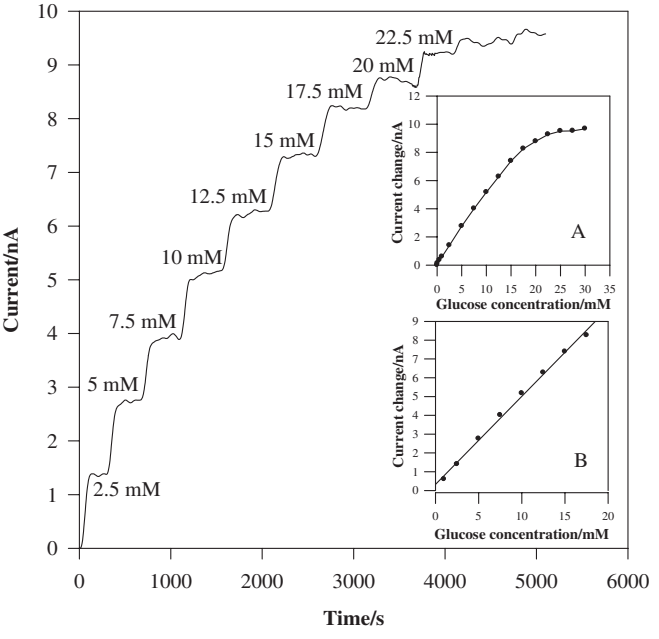


FIGURE 4 The current change vs. the addition of glucose to phosphate buffer (pH 6.24) measured by amperometry at +0.7 V. Inset: plot of current change vs. glucose concentration (A) and the regression line (B).

The linear regression of current change as a function of glucose concentration is illustrated in Fig. 4(B). The expression is

$$\Delta I = 0.3238 + 0.4687 \times C,$$

where ΔI is current change (nA), C is glucose concentration (mM), and the regression coefficient is 0.9964.

Inhibition Study

A typical inhibition curve and a dynamic recovery curve are illustrated in Fig. 5. A known concentration of Cr(VI) is directly added to the working solution containing glucose in an amperometric experiment at +0.7 V. The current change reaches a steady state in less than 80 s, so no incubation is required in this work. Then after soaking the electrode in phosphate buffer (pH 7.00) for 8 min, the anodic current on the electrode increases again in electrolyte containing the same glucose concentration.

The Effect of pH and Substrate Concentration

When Cr(VI) was added to phosphate buffer (pH 4.39–6.98), little inhibitory effect could be detected in the inhibition study. Only in acetate buffer at low pH did Cr(VI) have an evident effect on the enzyme activity. Similar results are given in the tests of many other heavy metals. Figure 6 shows the relationships between pH, inhibition degree by Cr(VI) and current change for known glucose concentrations.

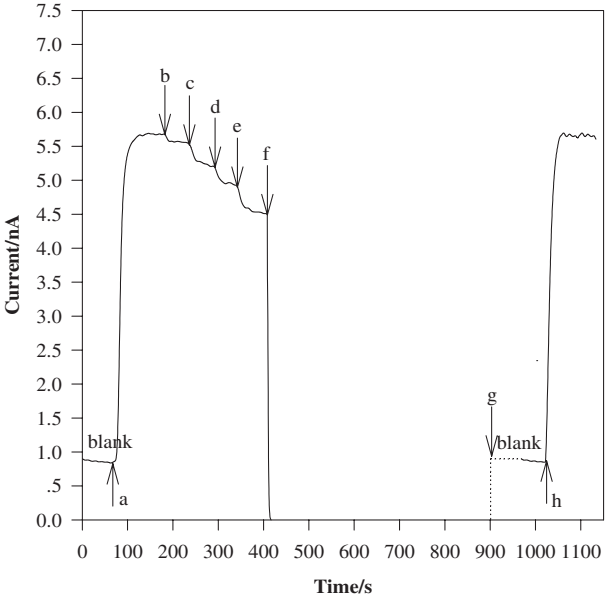


FIGURE 5 Amperometric measurement in acetate buffer (pH 2.55) at +0.7 V after the stepwise changes: additions of 2.25 mM glucose (a) and 0.5 μg L^{-1} (b), 15 μg L^{-1} (c), 100 μg L^{-1} (d) and another 100 μg L^{-1} (e) of Cr(VI) into the electrolyte, switching off the electric current and immersing the electrodes in phosphate buffer (pH 7.00) (f), switching on the electric current in acetate buffer (pH 2.55) (g) and addition of 2.25 mM glucose (h).

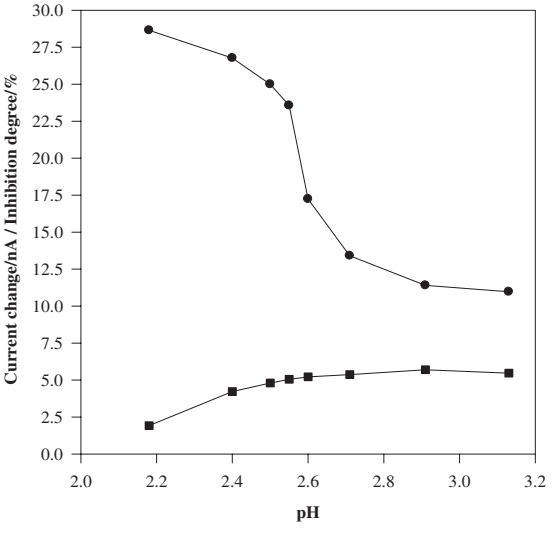


FIGURE 6 Plots of current change towards 2.25 mM glucose (●) and inhibition degree by 200 μg L^{-1} Cr(VI) (■) vs. pH value in acetate buffer at +0.7 V by amperometric measurement.

It is found that as pH rises, the degree of inhibition of the GOx electrode drops, while the current change rises. The strong pH dependence of Cr(VI) inhibition is consistent with the pH-dependent chromate/dichromate equilibrium [22,23]. Accordingly, dichromate concentration rises as the pH value of the solution drops, and the growing

inhibitory effect is mainly caused by the increasing dichromate concentration in the solution [22]. Similarly, other heavy metal ions in aqueous solution can be hydrolyzed when the pH value rises, and their inhibitory effect is found to become weaker, for those hydrolysates are less likely to inhibit GOx. On the other hand, it is believed that the relative error of calibration on electrochemical instruments decreases when the current change increases, so pH 2.55 is chosen as the optimum pH for an inhibition study. Also, we chose 2.25 mM glucose as an appropriate value because the inhibition degree drops as glucose concentration increases, as shown in Fig. 7.

Cr(VI) Calibration by Amperometric Measurement

In this article, the inhibition degree of the GOx electrode has a linear relationship with the natural logarithm of the Cr(VI) concentration. The lower detection limit should correspond to the inhibitor concentration which results in at least 4–6% of inhibition [9]. Here 4% is chosen as the inhibition degree for the lower detection limit, that is 0.49 $\mu g\,L^{-1}$. The linear range can be divided into two parts as shown in Fig. 8, one of which covers the range 0.49–95.73 $\mu g\,L^{-1}$, and the regression equation is

$$I\% = 4.8663 + 2.2535 \times C,$$

where $I\%$ s the inhibition degree, C is the natural logarithm of Cr(VI) concentration ($\mu g\,L^{-1}$), and the regression coefficient is 0.9796.

The other is 95.73 $\mu g\,L^{-1}$ to 8.05 $mg\,L^{-1}$, and the regression equation is

$$I\% = -72.8524 + 18.1656 \times C,$$

where the regression coefficient is 0.9918.

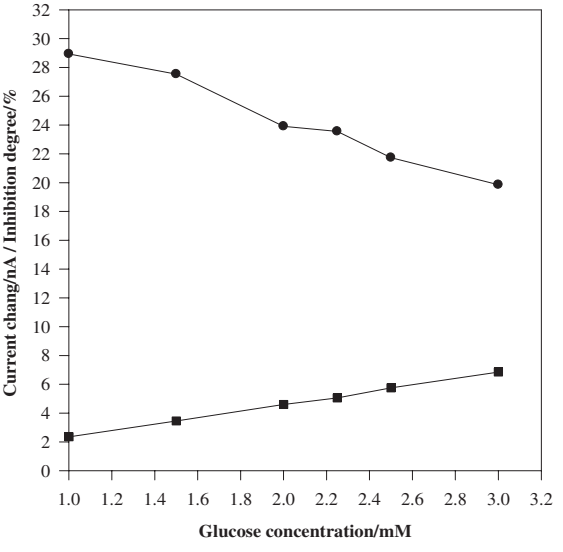


FIGURE 7 Plots of current change towards glucose (•) and inhibition degree by 200 $\mu g\,L^{-1}$ Cr(VI) (■) vs. glucose concentration in acetate buffer (pH 2.55) at +0.7 V by amperometric measurement.

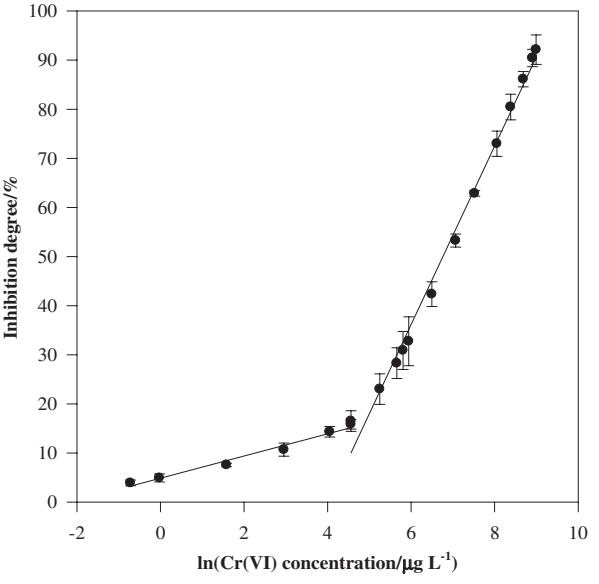


FIGURE 8 The linear regression of inhibition degree vs. the natural logarithm of Cr(VI) concentration of 0.49–95.73 μg L^{-1} and 95.73 μg L^{-1} to 8.05 mg L^{-1} in acetate buffer (pH 2.55). The vertical bars designate the standard deviation for the mean of three replicated tests.

Since each of the calibrations is done three times, and the standard deviations of inhibition degree are not more than 1.4%, the stability and reproducibility of the inhibition-based sensor are guaranteed.

Reversibility and Regeneration

In this study, inhibition of the GOx electrode requires no incubation, and after each inhibition by Cr(VI) and other interferents [Pb(II), Cu(II), Cd(II), Cr(III), Hg(II), Ag(I), Co(II), Sn(II) and Ni(II)], it does not need any antidote such as EDTA or dimercaptopropanol, but only an 8-min soaking in phosphate buffer (pH 7.00) for its complete reactivation. Moreover, Curve 1 in Fig. 7 shows that the inhibition degree of the GOx electrode clearly decreases as the glucose concentration increases, which implies that Cr(VI) inhibition is alleviated by addition of substrate.

Considering the aforementioned stabilization of the enzyme steric structure, we presume that the inhibition action by these heavy metals is not based on their irreversible reactions with some amino acid residues such as sulfur groups in the enzyme so as to break its steric structure [22,24,25], but based on their reversible absorption on some positive or negative regions in the redox center of GOx [11,26]. From these facts, it can be concluded that the inhibition assay in this study is reversible and competitive.

On the other hand, it was previously reported that the activity of immobilized GOx could only be inhibited by heavy metals for concentrations above 10^{-5} M [27,28], since metal ions are inclined to be hydrolyzed in the pH range of those GOx sensors and less likely to cause the inhibitory effect as mentioned above. Moreover, the chromate/dichromate equilibrium of a Cr(VI) solution also depends on the pH value. So the sensor's sensitivity is strongly pH dependent. In this study, unlike most assays reported which are operated at pH 5–7, a much lower pH of 2.55 is used for Cr(VI) measurement

TABLE I The interference results of other heavy metals under the same conditions as the calibration of Cr(VI)

Interferent	*Concentration* ($\mu g\ L^{-1}$)	*Inhibition degree* (%)	*Cr(VI) concentration giving the same inhibition degree* ($\mu g\ L^{-1}$)
Cr(III)	500	9.53	7.92
Pb(II)	500	4.97	1.05
Cu(II)	500	1.56	0.23
Cd(II)	500	6.17	1.78
Sn(II)	500	Not detectable	0
Ni(II)	500	4.44	0.83
Co(II)	500	9.12	6.60
Ag(I)	275	8.91	6.02
	500	13.86	54.11
Hg(II)	275	9.40	7.48
	500	14.12	60.73

with the detection limit of $0.49\ \mu g\ L^{-1}$, which can be compared to conventional methods such as atomic absorption spectrophotometry.

Selectivity and Stability

Selectivity is an important factor in the performance of an inhibition-based enzyme sensor. Although an inhibition-based enzyme electrode can give a greater or lesser response to a few substances, it can be used for the calibration of only one acute toxicant. Nine heavy metals as interferents were measured under the same conditions as the calibration of Cr(VI). From the results shown in Table I, the interference to Cr(VI) detection from Pb(II), Cu(II), Cd(II), Cr(III), Co(II), Sn(II) and Ni(II) is found to be minimal. However, Hg(II) and Ag(I) at $500\ \mu g\ L^{-1}$ lead to 14.12% and 13.86% inhibition of the enzyme electrode, respectively, which may interfere with the determination of trace Cr(VI) in environmental samples; hence a pretreatment to remove these two metal ions is needed.

The stability of the sensor was also investigated. Figure 9 compares the change of its response to 2.25 mM glucose in acetate buffer (pH 2.55) and 5 mM glucose in phosphate buffer (pH 6.24) in 54 days, which shows that the activity of the enzyme sensor is more stable in the acetate buffer than in the phosphate buffer, and remains more than 90% at pH 2.55 forty days after being made, which confirms the reusability of the enzyme sensor over such a long period.

Application

To apply this enzyme-inhibition sensor to test Cr(VI) in environmental samples, known concentrations of Cr(VI) were added to a soil extract, representing soil, and tested by this sensor. The results are shown in Table II, with the average recovery ratio of 97.07%.

CONCLUSIONS

An inhibition-based enzyme sensor for the detection of Cr(VI) has been developed based on the inhibition of GOx. The fabrication of this sensor is low-cost, simple and

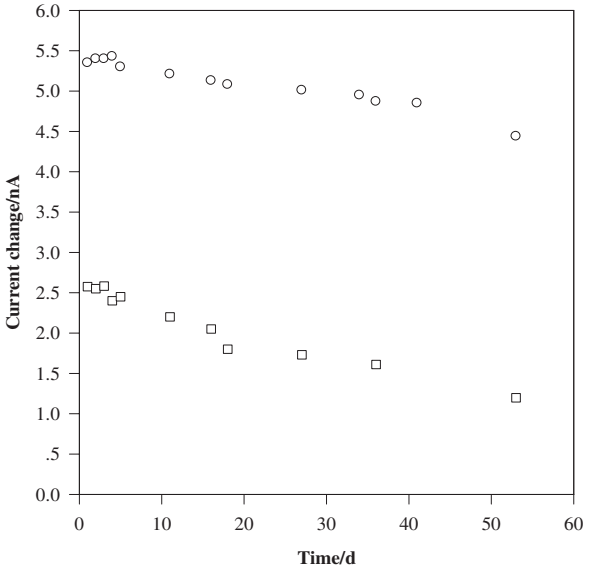


FIGURE 9 Plot of current change of the GOx sensor towards 2.25 mM glucose in acetate buffer (pH 2.55) (○) and 5 mM glucose in phosphate buffer (pH 6.24) (□) vs. time by amperometry at +0.7 V.

TABLE II The results of the recovery test in soil extract as matrix

Concentration added (μg L^{-1})	*Average concentration recovered* (μg L^{-1})	*Recovery* (%)
4.31×10	$(3.98 \pm 1.20) \times 10$	92.29
1.91×10^2	$(1.86 \pm 0.37) \times 10^2$	97.52
2.86×10^2	$(2.58 \pm 0.45) \times 10^2$	90.08
1.85×10^3	$(1.81 \pm 0.01) \times 10^3$	98.09
3.15×10^3	$(3.38 \pm 0.25) \times 10^3$	107.37

fast. The adaptability and stability of the sensor at low pH in glucose determination is an advantage over other enzyme sensors for heavy metal inhibition studies. Good sensitivity, stability and reusability were all obtained. Interference from Pb(II), Cu(II), Cd(II), Cr(III), Co(II), Sn(II) and Ni(II) was found to be minimal, while high concentrations of Hg(II) and Ag(I) may interfere with the determination of trace Cr(VI); hence a pretreatment to remove these two metal ions is needed. The determination of Cr(VI) in a real sample was in good agreement with the declared concentration. All of these clearly illustrate that this GOx sensor has excellent characteristics, which deserves further study of the application in real-time environmental monitoring for trace Cr(VI).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (70171055, 50179011), the Natural Science Foundation of China for Excellent Youth (50225926), the Doctoral Foundation of the Ministry of Education of China (20020532017), the Teaching and Research Award Programme for Outstanding Young

Teachers in Higher Education Institutions of MOE, P.R.C. (TRAPOYT) in 2000 and the National 863 High Technologies Research Foundation of China (2001AA644020, 2003AA644010).

References

[1] J. Wang, *J. Pharm. Biomed. Anal.*, **19**, 47–53 (1999).
[2] T.K.V. Krawczyk, T. Moszczynska and M. Trojanowicz, *Biosens. Bioelectron.*, **15**, 681–691 (2000).
[3] S. Andreescu, A. Avramescu, C. Bala, V. Magearu and J.L. Marty, *Anal. Bioanal. Chem.*, **374**, 39–45 (2002).
[4] H.S. Lee, Y.A. Kim, D.H. Chung and Y.T. Lee, *Int. J. Food Sci. Technol.*, **36**, 263–269 (2001).
[5] E. Kilinc, M. Ozsoz and O.A. Sadik, *Electroanal.*, **12**, 1467–1471 (2000).
[6] V. Volotovsky and N. Kim, *Biosens. Bioelectron.*, **13**, 1029–1033 (1998).
[7] Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological Profile for Chromium*, *Report TP-92/08*. (US Department of Health and Human Services, Atlanta, GA, 1993).
[8] T.F. Mancuso, *Am. J. Ind. Med.*, **31**, 129–139 (1997).
[9] G.A. Evtugyn, H.C. Budnikov and E.B. Nikolskaya, *Talanta*, **46**, 465–484 (1998).
[10] World Health Organization (WHO), *Guidelines for Drinking-Water Quality*, Vol. 2, (WHO, Geneva, 1996) 2nd ed.
[11] P.W. Alexander and G.A. Rechniz, *Electroanal.*, **12**, 343–350 (2000).
[12] R. Garjonyte and A. Malinauskas, *Sens. Actuators B*, **56**, 85–92 (1999).
[13] C.L. Zou, *Acta Biochim. Biophys. Sinica* (Chinese), **24**, 393–398 (1992).
[14] I.A. Veselova and T.N. Shekhovtsova, *Anal. Chim. Acta*, **392**, 151–158 (1999).
[15] X.Q. Wu, D.J. Cao and H.W. Jiang, *J. Shanghai Teachers Univ. (Natural Sciences)* (Chinese), **23**, 159–166 (1994).
[16] P.A. Fiorito, I. Susana and C. Torresi, *J. Brazil. Chem. Soc.*, **12**, 729–733 (2001).
[17] State Environmental Protection Administration of China, GB/T17138–1997, GB/T17140–1997, GB/T17137–1997 (Chinese), Beijing, China (1997).
[18] Y. Nakabayashi, M. Wakuda and H. Imai, *Anal. Sci.*, **14**, 1069–1076 (1998).
[19] A.A. Shul'ga, M. Koudelka-Hep and N.F. Roolj, *Anal. Chem.*, **66**, 205–210 (1994).
[20] W.J. Cho and H.J. Huang, *Anal. Chem.*, **70**, 3946–3951 (1998).
[21] S.Y.S. Yeung, Y.K. Cho and J.E. Bailey, *Biotechnol. Bioeng.*, **20**, 1249–1265 (1978).
[22] J.G. Zhao, R.W. Henkens and A.L. Crumbliss, *Biotechnol. Prog.*, **12**, 703–708 (1996).
[23] F.A. Cotton, G. Wilkinson, C.A. Murillo and M. Bochmann, *Advanced Inorganic Chemistry*. (John Wiley & Sons, New York, 1999), 6th ed., p. 613.
[24] T. Danzer and G. Schwedt, *Anal. Chim. Acta*, **318**, 275–286 (1996).
[25] H.H. Wu, Z.M. Zheng and S.M. Zhou, *Acta Chim. Sinica* (Chinese), **49**, 689–693 (1991).
[26] H.J. Bright and D.J.T. Porter, In: *The Enzymes*, Vol. 12. (P.D. Boyer, Ed. Academic Press, New York, 1975), 3rd ed., pp. 421–505.
[27] A.M. Donlan, G.J. Moody and J.D.R. Thomas, *Anal. Proc.*, **26**, 369–371 (1989).
[28] A.L. Kukla, N.I. Kanjuk, N.F. Starodub and Y.M. Shirshow, *Sens. Actuators B*, **57**, 213–218 (1999).