Anal. Chem. **2008,** *80,* 2826−2831

Electrochemiluminescent Biosensor for Hypoxanthine Based on the Electrically Heated Carbon Paste Electrode Modified with Xanthine Oxidase

Zhenyu Lin, Jianjun Sun, Jinhua Chen, Liang Guo, Yiting Chen, and Guonan Chen*

Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University) and Department of Chemistry, Fuzhou University, Fuzhou, Fujian, 350002, China

A new electrochemiluminescent (ECL) biosensor based on an electrically heated carbon paste electrode (HCPE) that was surface modified by xanthine oxidase (XOD) was designed and constructed in this work. It was found that the ECL intensity of luminol could be enhanced at the surface of XOD/HCPE by adding hypoxanthine (HX) to the solution, and there was a linear relationship between the ECL intensity and the concentration of HX. On the basis of this, an ECL enzyme biosensor can thus be developed to detect HX. However, because the activity of XOD is highly dependent on temperature, the biosensor is very sensitive to the temperature of the electrode. Also, because the temperature of the electrode may also affect the diffusion and convection of the luminescent compounds near the electrode surface, a suitable temperature for XOD/HCPE has to be controlled to achieve the best ECL signal. The key feature of the designed biosensor is that the temperature of the electrode is controllable so the most suitable temperature for the enzyme reaction can be obtained. The obtained results showed that the ECL enzyme biosensor exhibited the best sensitivity at an electrode temperature of 35 °C for the detection of HX. The detection limit was 30-fold lower than that at room temperature (25 °C).

Heated electrodes have attracted great interest from both theoretical and practical points of view in recent years.[1−12] The main feature of these electrodes is that direct electrical heating only heats the electrode but leaves the bulk solution's temperature unchanged. This technique is based on a symmetric electrode arrangement, complicated manual fabrication, and special equipment for generating alternating heating current.[12] In a recent work, Wang and coauthors successfully designed an electrically heated carbon paste electrode (HCPE) to perform adsorptive stripping measurement of trace nucleic acids.[13] The carbon paste electrodes (CPE) have several advantages, such as simplicity in fabrication and treatment and good biocompatibility, making it easily immobilized by bioactive substances. Moreover, the temperature of a CPE can also be controlled easily.[14,15]

To the best of our knowledge, heated electrodes have not been applied to study the effect of temperature on an enzyme's reaction, although it was found that an enzyme's activity was highly dependent on temperature. For example, xanthine oxidase (XOD) is most active at about 40 °C.[16] One difficulty is that the electrode temperature cannot be controlled. With the technique of HCPE, this problem now can be solved. The enzyme can be easily adsorbed on the HCPE surface and worked with at its most suitable temperature to improve the sensitivity of the sensors.

Hypoxanthine (HX) is an essential metabolite to degrade adenine nucleotide, which is mainly accumulated in biological tissues. The determination of HX is very important for the quality control of fish products in food industries.[17] Various methods have been proposed for the determination of trace amounts of HX, such as paper chromatography, ion exchange chromatography, HPLC, and capillary electrophoresis.[18−22] However, these methods require complicated and time-consuming procedures. Polarography and

* To whom correspondence should be addressed. E-mail: gnchen@fzu.edu.cn. Fax: 86-591-83713866.

(1) Gründler, P.; Zerihun, T.; Kirbs, A.; Grabow, H. *Anal. Chim. Acta* **1995**, *305*, 232−240.
(2) Wang, J.; Gründler, P.; Flechsig, G. U.; Jasinski, M.; Lu, J. M.; Wang, J. Y.; Zhao, Z. Q.; Tian, B. M. *Anal. Chim. Acta* **1999**, *396*, 33−37.
(3) Korbut, O.; Gründler, P. J. *Electroanal. Chem*. **2001**, *506*, 143−148.
(4) Gründler, P.; Kirbs, A; Zerihum, T. *Analyst* **1996**, *121*, 1805−1810.
(5) Voss, T.; Gründler, P.; Brett, C. M. A.; Brett, O. A. M. *J. Pharm. Biomed. Anal.* **1999**, *19*, 127−133.
(6) Beckmann, A; Schneider, A.; Gründler, P. *Electrochem. Commun*. **1999**, *1*, 46−49.
(7) Jasinski, M.; Kirbs, A.; Schmehl, M.;. Gründler, P. *Electrochem. Commun*. **1999**, *1*, 26−28.
(8) Beckmann, A.; Coles, B. A.; Compton, R. G.; Gründler, P.; Marken, F.; Neudick, A. *J. Phys. Chem. B* **2000**, *104*, 764−769.
(9) Zerihun, T.; Gründler, P. J. *Electroanal. Chem*. **1996**, *404*, 243−248.
(10) Gründler, P.; Flechsig, G. U. *Electrochim. Acta* **1998**, *43*, 3451−3458.
(11) Zerihun, T.; Gründler, P. *J. Electroanal. Chem*. **1996**, *415*, 85−88.
(12) Gründler, P. *Fresenius' J. Anal. Chem*. **1998**, *362*, 180−183.
(13) Wang, J.; Gründler, P.; Flechsig, G. U.; Jasinski, M.; Rivas, G.; Sahlin, E.; Paz, J. L. L. *Anal. Chem*. **2000**, *72*, 3752−3756.
(14) Wang, J.; Musameh, M.; Mo, J. W. *Anal. Chem*. **2006**, *78*, 7044−7047.
(15) Valentini, F.; Amine, A. *Anal. Chem*. **2003**, *75*, 5413−5421.
(16) Xue, H. G.; Mu, S. L. *J. Electroanal. Chem*. **1995**, *397*, 241−247.
(17) Mulchandani, A.; Long, J. H. T.; Male, K. B. *Anal. Chim. Acta* **1989**, *221*, 223−238.
(18) Halfpenny, A. P.; Brown, P. R. *J. Chromatogr.* **1985**, 349, 275−82.
(19) Kassemsam, B. O.; Perez, B. S.; Murry, J.; Jones, N. R. *J. Food Sci.* **1963**, *28*, 28−37.
(20) Tarr, H. L. A. *J. Food Sci.* **1966**, *31*, 846−854.
(21) Jones, N. R.; Murry, J. *J. Sci. Food Agric.* **1962**, *13*, 475−480.
(22) Nguyen, A. L.; Luong, J. H. T. *Anal. Chem*. **1990**, *62*, 2490−2493.

10.1021/ac702471r CCC: $40.75
Published on Web 03/04/2008

stripping voltammetry have also been developed as alternative methods, which were based on the direct oxidation of HX or its complexes at the electrode surface.[23,24] XOD can catalyze the oxidation of HX by molecular oxygen to yield uric acid,[25−27] so the XOD-based biosensors that measure either the consumed oxygen or the produced hydrogen peroxide and/or uric acid have been demonstrated as the satisfactory sensors for HX.[28−30] Because the presence of hydrogen peroxide can enhance the electrochemiluminescence of luminol,[31−33] an electrochemiluminescent (ECL) sensor can thus be developed to determine HX.[34]

The aim of this work is to develop an ECL biosensor whose temperature can be easily controlled to get the most suitable temperature for the enzyme reaction. A HCPE modified with XOD (XOD/HCPE) has been designed and prepared. An ECL detection system equipped with XOD/HCPE was then used to determine HX concentration. The effect of the electrode temperature on the enzyme activity was investigated in detail.

EXPERIMENTAL SECTION

Chemicals. XOD, alinine, and luminol were obtained from Sigma Chemical Co. and used without further purification. The mineral oil was obtained from Aldrich. Other chemicals were analytical grade or better. Double distilled water was used throughout. The concentrations of stock solution of HX and luminol were 1.0 mmol/L, and these were stored in the refrigerator. The stock standard solution was used to prepare working standard solutions daily by suitable dilution. The ferro-/ferricyanide solution was prepared in 0.05 mol/L KCl.

Apparatus. A laboratory-built ECL detection system equipped with a heated carbon paste electrode (HCPE) was used in the experiment.[35] A function generator with a transformer was used for heating. In all heating experiments the frequency was adjusted to 100 kHz. The high frequency was used to prevent the ac voltage from interfering with the electrode process.[1] The transformer was used to filtrate the direct current generated through the function generator. By changing the output of the function generator, the temperature of the HCPE was controlled.

The ECL detection system consisted of a BPCL ultraweak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) and a CHI 660a electrochemical system (CH Instruments). A three-electrode electrochemical cell with an optically flat bottom was used. The HCPE was used as the working electrode. Platinum wire and Ag/AgCl (saturated with KCl) were used as counter and reference electrodes, respectively.

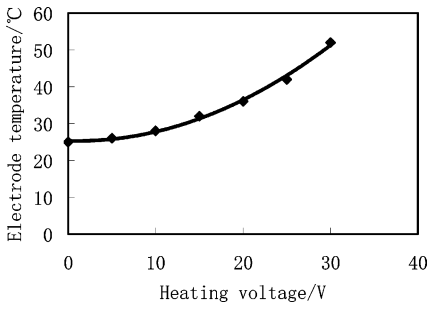

Figure 1. Relationship between the heating voltage and the temperature of the electrode surface.

Construction of the HCPE. Fabrication of this HCPE is very similar to that described in the previous report.[36] Before being modified by XOD, the working area of the carbon paste was renewed by carefully polishing with weighing paper. The temperature of the HCPE was calibrated before the experiments and is shown in Figure 1. The stationary temperature of the CPE during heating was measured according to the reported methods.[9,35] In brief, the temperature coefficient of the electrode potential for isothermal was determined first; the relationship between the electrode potential and the heating voltage (V) was determined. Then the relationship between the heating voltage and the temperature of the electrode surface (T_e) could be deduced.

Preparation of HCPE/XOD Electrode. Portions of 5.0 mg of XOD enzyme, 3.0 mg of bovine serum albumin (BSA), 15 μL of water, and 2 μL of 5% glutaraldehyde were mixed first. Then 10 μL of the mixed solution was spread on the working area of the HCPE. The electrode was then dried at room temperature until a hard yellowish gel layer was obtained. The electrode was then washed with a 0.05 mol/L phosphate buffer (pH 7.0) to remove the excess glutaraldehyde and dried again. The prepared XOD/HCPE was stored at 4 °C in phosphate buffer.

Procedure for ECL Measurement. The ECL cell was washed with 0.2 mol/L nitric acid and water, consecutively, before use. Luminol and different volumes of HX solution were added to a 10 mL volumetric flask and diluted with the buffer solution to the required volume. A volume of 5 mL of this solution was transferred to the ECL cell. Cyclic voltammetry (CV) measurements were carried out to get the ECL signal when the working electrode was heated to different temperatures.

RESULTS AND DISCUSSION

Principle of the ECL Biosensor for HX. There are many ECL biosensors based on enzyme reactions, especially the glucose ECL sensors.[37−40] The principle of the current biosensor is illustrated in Figure 2. The HX in the solution reacts with the XOD immobilized on the surface of the electrode to produce H_2O_2.[41] In an alkaline or neutral medium, luminol is electrochemi-

(23) Househam, B. C.; Vandenberg, C. M. G.; Riley, J. P. *Anal. Chim. Acta* **1987**, *200*, 291−303.
(24) Hu, S. H.; Xu, C. L.; Cui, D. F. *Chin. J. Anal. Sci.* **1999**, *15*, 468−471.
(25) Mao, L. Q.; Yamamoto, K. *Anal. Chem. Acta* **2000**, *415*, 143−150.
(26) Hu, S. H.; Xu, C. L.; Luo, J. H.; Luo, J.; Cui, D. F. *Anal. Chem. Acta* **2000**, *412*, 55−61.
(27) Xue, H. G.; Mu, S. L. *J. Electroanal. Chem.* **1995**, *397*, 241−247.
(28) Niu, J. J.; Lee, J. Y. *Sens. Actuators, B* **2000**, *62*, 190−198.
(29) Hasebe, Y.; Gokan, A.; Uchiyama, S. *Anal. Chem. Acta* **1995**, *302*, 21−27.
(30) Okuma, H.; Watanabe, E. *Biosens. Bioelectron.* **2002**, *17*, 367−372.
(31) Xu, G. B.; Zhang, J. Z.; Dong, S. J. *J. Microchem.* **1999**, *62*, 259−265.
(32) Marquette, C. A.; Blum, L. J. *Anal. Chim. Acta* **1999**, *381*, 1−10.
(33) Zhu, L. D.; Li, Y. X. *Sens. Actuators, B* **2002**, *84*, 265−270.
(34) Blum, L. J. *Bio- and Chemi-Luminescent Sensors*; World Scientific: Singapore, 1997.
(35) Lin, Z. Y.; Sun, J. J.; Guo, L.; Chen, G. N. *Anal. Chim. Acta* **2006**, *564*, 226−230.
(36) Chen, Y. T.; Lin, Z. Y.; Sun, J. J.; Chen, G. N. *Electrophoresis* **2007**, *28*, 3250−3259.
(37) Lawrence, N. S.; Deo, R. P.; Wang, J. *Anal. Chem.* **2004**, *76*, 3735−3739.
(38) Pereiar, C. M.; Oliveira, J. M.; Silva, R. M.; Silva, F. *Anal. Chem.* **2004**, *76*, 5547−5551.
(39) Liu, G.; Lin, Y.; Ostatna, V.; Wang, J. *Chem. Commun.* **2005**, *27*, 3481−3483.
(40) Eric, B.; Yu, Q. *Anal. Chem.* **2006**, *78*, 3965−3984.
(41) Dodeigne, C.; Thunus, L.; Lejeune, R. *Talanta* **2000**, *51*, 415−439.

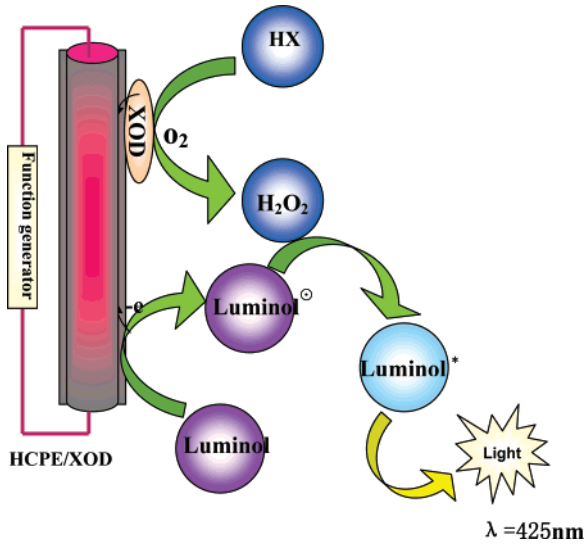

Figure 2. Principle of the ECL biosensor for HX.

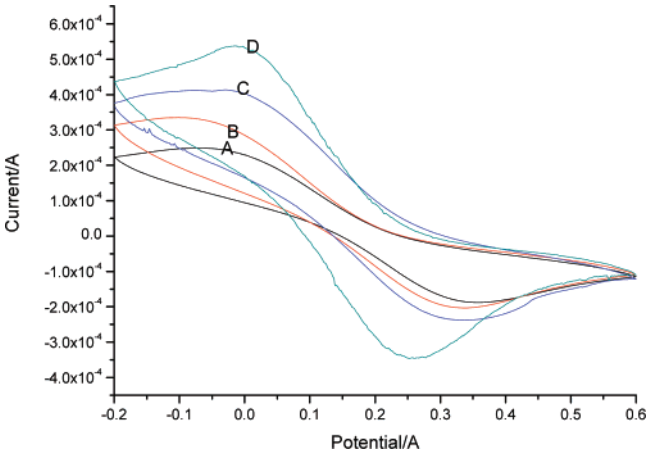

Figure 3. CV of $K_3[Fe(CN)_6]$ on HME in KCl solution at different T_e values: $K_3[Fe(CN)_6]$, 0.005 mol/L; KCl, 1 mol/L; scan rate 50 mV/s; A, 25 °C; B, 30° C; C, 35 °C; D, 45 °C.

cally oxidized to form an anion.[42−44] Further oxidizing the resulting diazo compound in the presence of hydrogen peroxide will produce the excited state of 3-aminophtalate.[32,45] The excited state of 3-aminophtalate goes back to the ground state to give light. Hydrogen peroxide participates in this ECL reaction in the form of the peroxide anion HOO• or an electrochemically formed superoxide radical $O_2^{\bullet -}$.[46,47] On the basis of the enhanced ECL of luminol, HX can be determined indirectly. It was reported that at elevated temperature, the reaction of H_2O_2 on the electrode would become quicker.[48] Our novel heated electrode would offer excellent electrocatalytic activity toward the reduction and oxidation of hydrogen peroxide that was liberated in the enzymatic reaction between XOD and HX, enabling a more sensitive determination of HX.

CV and ECL of Luminol at HCPE. The ferrocyanide redox system was used to examine the voltammetric behavior of the CPE (see Figure 3). The results showed that at elevated electrode surface temperature, the peaks became high and sharp and the separation of the anodic and cathodic peak potentials became small, indicating that the electrode reversibility had been improved at higher electrode surface temperature. These results are very consistent with that reported in the literature.[13] However, if the temperature was too high, the carbon particles inside the paste tended to lose contact in some extent, which caused higher resistance and increased the separation of the anodic and cathodic peak potentials. So the most suitable electrode surface temperature should be sought to improve the reversibility of the electrochemical reaction. In our experiments, we found that if the temperature was higher than 60 °C, the separation of the anodic and cathodic peaks potential would become larger.

Figure 4A shows the CV curves of luminol at different electrode surface temperatures. It is seen that the current is increased at elevated electrode surface temperature. The improvement of the current rate can be attributed to the enhanced convective transport. When the electrode was heated, the temperature of the solution near the electrode surface was increased. A temperature gradient layer near the electrode surface was formed, which speeded up the diffusion and convection.[9,36] Because of this greatly improved mass transfer at elevated temperature, a greater current flow was observed.

ECL usually occurs at the surface of electrode or in the solution adjacent to the electrode. Therefore, adjusting the position of the electrode or changing the material of the electrode can always improve the behavior of ECL. The ECL occurring at the surface of the electrode is not only influenced by the diffusion of the luminescent compound at the electrode but also influenced by the convection of the luminescent compounds near the electrode surface. Among them, the convection can be greatly affected by temperature. As reported previously, the ECL intensities of $Ru(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$/oxalate were greatly increased at elevated platinum electrode temperature.[36] Our results showed that the ECL of luminol was also obviously affected by the electrode temperature.

The ECL behaviors of luminol at HCPE under CV scanning are shown in Figure 4B. As can be seen, the ECL intensity increases with increase of the electrode surface temperature. The ECL intensity at an electrode surface temperature of 48 °C is improved about 70% in comparison to that at 25 °C. This significant increase of the ECL intensitycan mainly be attributed to improved mass transfer to the electrode surface at elevated temperature.

In order to determine how diffusion and convection is affected by the temperature, the Stoke−Einstein equation can be applied to calculate the diffusion coefficient[49]

$$D = \frac{kT}{6\pi r_i \eta}$$

where D is the diffusion coefficient, k is Boltzmann's constant, T is the absolute temperature, r_i is the radius of the diffusing species, and η is the viscosity of the solution.

(42) Sakura, S. *Anal. Chim. Acta* **1992**, *262*, 49−57.
(43) Cui, H.; Xu, Y.; Zhang, Z. F. *Anal. Chem.* **2004**, *76*, 4002−4010.
(44) Cui, H.; Zou, G. Z.; Lin, X. Q. *Anal. Chem.* **2003**, *75*, 324−331.
(45) Richter, M. M. *Chem. Rev.* **2004**, *104*, 3003−3036.
(46) Ghosh, S.; Sarker, D.; Misra, T. N. *Sens. Actuators, B* **1998**, *53*, 58−62.
(47) Fähnrich, K. A.; Pravda, M.; Guilbault, G. G. *Talanta* **2001**, *54*, 531−559.
(48) Lau, C.; Flechsig, G. W.; Gründler, P.; Wang, J. *Anal. Chem. Acta* **2005**, *554*, 74−78.
(49) Baransik, A. S. *Anal. Chem.* **2002**, *74*, 1294−1301.

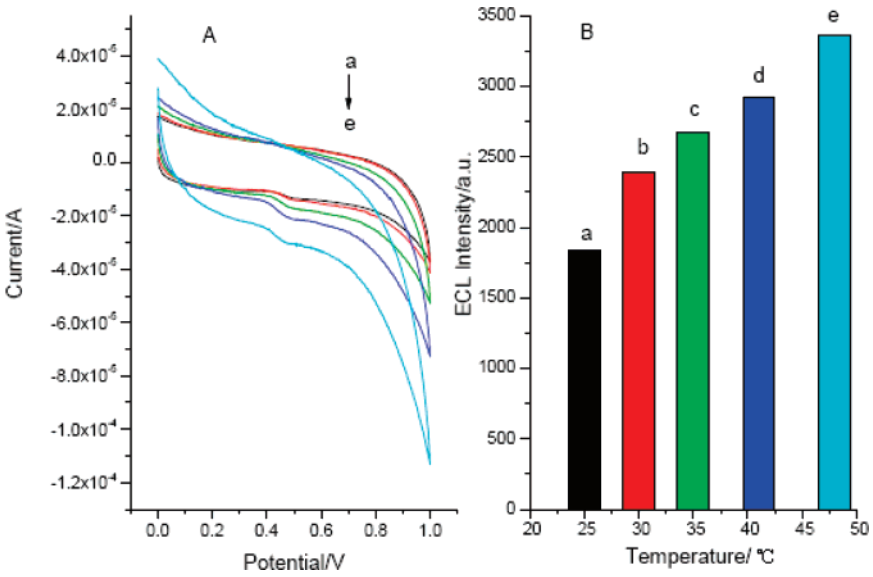

Figure 4. (A) CV curves and (B) ECL intensities for luminol in phosphate buffer solution at different electrode temperatures. Conditions: luminol, 2.0×10^{-5} mol/L; PBS solution, 0.2 mol/L (pH = 7.0); a, 25 °C; b, 30 °C; c, 35 °C; d, 41 °C; e, 48 °C; CV was performed in the range of 0−1.0 V; scan rate, 0.05 V/s.

The viscosity of the solution near the electrode surface decrease with elevation of the electrode surface temperature. In addition, the solution temperature at the electrode surface is nearly the same as that of the electrode. With a change in solution temperature from 25 to 35 °C, the viscosity of water decreases 20%; consequently, the diffusion coefficient will increase about 30%. From Figure 4A we know that the current increases about 60% and the ECL intensity increases about 54% when the electrode surface temperature changes from 25 to 35 °C. So the convection caused by the elevated electrode temperature may contribute to the other 30% increase. These indicate that the enhancement of diffusion rate and convection caused by elevating the temperature contributes 50% of the ECL intensity enhancement.

The most useful application of the heated electrode is that it can enhance the analytical signal of the analytes; however, this signal enhancement is only beneficial when the noise level is not increased at the same time. A CV scan was performed on the HCPE in the range of 0−0.2 V and measured the CL intensity simultaneously (which could be seen as the background signal of chemiluminescence (CL), since the luminol system gave nearly no ECL signal in this range); we found that no significant increase of noise could be observed when the electrode was heated. The background CL intensity of the system was also not affected by the electrode temperature. We also studied the temperature effect of the bulk solution on the background CL intensity. At elevated solution temperature, the background CL intensity and the noise of CL increase greatly, the background CL at 35 °C is about 40 times than that at 25 °C, and the noise also increased about 10 times; these will give great interference to the ECL detection. So bulk solution heating is not fit for this kind of system, though it also can increase the analytical signal of the analytes.

ECL Behaviors of Luminol−HX System at XOD/HCPE. The ECL intensity of luminol at the XOD/HCPE would increase with the addition of HX. Meanwhile, the increased intensity was found to be linear in relationship with the change in concentration of HX.

It is well-known that the medium plays an important role in the ECL reaction. We found experimentally that a phosphate buffer solution (PBS) was more favorable to the luminol−HX ECL system than an acetate buffer or borate buffer solution at the same pH (pH 7.0) value. In PBS solution, the ECL signals were more stable and symmetrical, which was more beneficial for quantitative analysis. So phosphate buffer solution was selected in subsequent experiments.

The pH value not only affected the ECL intensity of luminol but also the activity of XOD, which subsequently affected the performance of XOD/HCPE and the response of the biosensor. When the pH value was higher than 9.0, the XOD lost its activity and the background CL was very high. However, if the pH value was lower than 6.8, the ECL intensity decreased greatly. So the effect of pH on the ECL response of the sensor was investigated in the range of 6.8−9.0. It was found that the maximum enhanced ECL intensity with good stability and peak shape was obtained at pH 7.0. Therefore, PBS with pH 7.0 was chosen as the buffer in the subsequent experiments.

The uric acid generated from the enzyme reaction of HX may lead to a local pH decrease, so the buffer concentration is also an important parameter to deal with the local pH variations. In the solution of 1×10^{-5} mol/L HX, it was found that the enhanced ECL intensity (ΔI) increased with the increase of the buffer concentration and reached a constant value when the concentration reached the value of 150 mmol/L. So in this study, a buffer solution of 200 mmol/L was chosen.

Effect of the Temperature of XOD/HCPE on Luminol−HX ECL System. In fact, for an ECL enzyme biosensor, besides the diffusion and convection of luminescent compounds near the electrode surface being affected by the temperature of the electrode surface, the enzyme activity would also be affected. The effect of the XOD/HCPE temperature on the ECL intensity of the luminol−HX system has been investigated. Figure 5 shows the relationship between the electrode temperature and the ECL intensity. At the beginning, the ECL intensity increased with

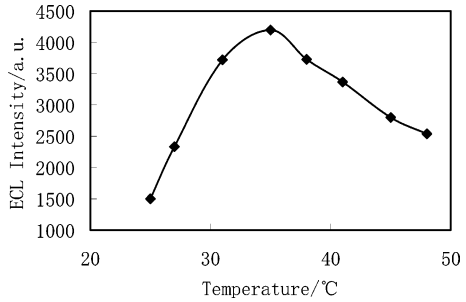

Figure 5. ECL intensities of luminol−HX system on XOD/HCPE at different electrode temperatures. Conditions: luminol, 2.0 × 10^{-5} mol/L; HX, 1.0 × 10^{-5} mol/L.

increasing XOD/HCPE temperature. At further increase of the temperature to higher than 35 °C, the ECL intensity started to decrease. This phenomenon was different from that of the luminol ECL at HCPE, as we mentioned in the above section. At 35 °C, the ECL intensity is about 3 times that at 25 °C, and the increase partly comes from the increase of mass transfer to electrode surface. We have mentioned in the above section that this type of increase is about 54% in magnitude. So most of the increase might come from the increase of the enzyme activity, and more H_2O_2 will be produced at the elevated electrode temperature.

It has been proven that temperature may affect the activity of XOD. In a certain temperature window, the enzyme's activity will increase at higher electrode temperature; more H_2O_2 will be produced, which will further enhance the ECL intensity of the present system. However, too high an electrode temperature might have decreased the enzyme's activity, so less H_2O_2 would have been generated, which subsequently would have lowered the ECL intensity of this system. Therefore, a suitable electrode temperature should be selected for an ECL enzyme biosensing system. In this system, a temperature of 35 °C was chosen for the subsequent experiments.

Figures 6A and7A show the ECL−potential curves of the luminol−HX system on XOD/HCPE with different concentrations of XOD at the electrode temperatures of 25 and 35 °C, respectively. At both temperatures, the ECL intensity increased with increasing HX concentration. In addition, at 35 °C the ECL intensity was much higher than that at 25 °C at the same HX concentration; for example, when the concentration of HX was 100 mmol/L, the ECL intensity increased about 3.3 times. It can be seen from Figure 6B that when the temperature of the XOD/HCPE system was 25 °C, the linear response with a correlation coefficient of 0.9950 was obtained over the range of 8−300 μM of HX. The detection limit for HX was 3 μM (signal-to-noise ratio = 3). However, the linear response range for HX at a temperature of 35 °C was 0.6−200 μM with a correlation coefficient of 0.9960 (see Figure 7B). The detection limit was as low as 0.1 μM, which was 30-fold lower than that obtained at 25 °C.

Stability and Reproducibility. The stability of the sensor was determined by measuring its response at regular intervals after storage at 4 °C. The response test was carried out at least 10 times with 10 min intervals using the same sampling solution (1.0 × 10^{-5} mol/L) each day. At both 25 and 35 °C, the response retained an almost constant value for the first 5 days and then decreased gradually to 70% of its initial value after 10 days. However, if the temperature of the electrode was 45 °C, the response was

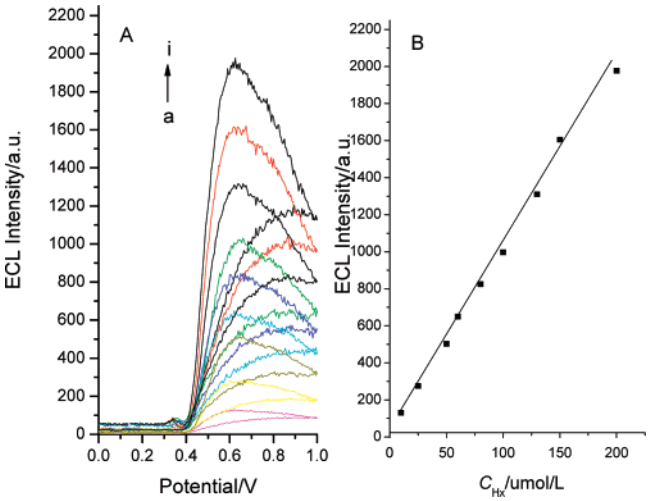

Figure 6. (A) ECL−potential curves of luminol−HX system on XOD/HCPE with different HX concentrations at electrode temperature of 25 °C; (B) calibration curve for HX. Key to labels and conditions: HX, a = 8, b = 25, c = 50, d = 60, e = 80, f = 100, g = 130, h = 150, and i = 200 μmol/L; luminol, 2.0 × 10^{-5} mol/L; PBS solution, 0.2 mol/L (pH = 7.0).

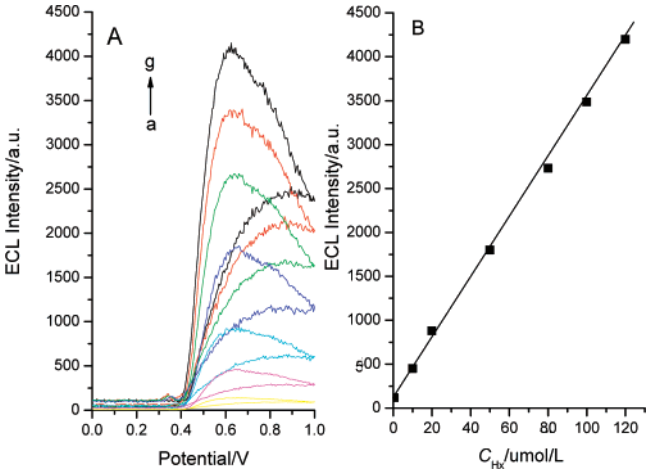

Figure 7. (A) ECL−potential curves of luminol−HX system on XOD/HCPE with different HX concentration at an electrode temperature of 35 °C; (B) calibration curve for HX. Key to labels and conditions: HX, a = 0.6, b = 10, c = 20, d = 50, e = 80, f = 100, and g = 120 μmol/L; luminol, 2.0 × 10^{-5} mol/L; PBS solution, 0.2 mol/L (pH = 7.0).

decreased after each test, which again indicated that the enzyme might have lost its activity at this high temperature and could not be recovered.

The sample solution containing 1.0 × 10^{-5} mol/L HX had been applied to study the reproducibility of the ECL sensor. The results showed that the relative standard deviation values of the ECL responses were 7.5 and 6.8% (n = 6), respectively, when the electrode temperatures were 25 or 35 °C. These mean that the reproducibility of the sensor data is well-suited to give precise measurements.

CONCLUSIONS

A new electrically heating controlled CPE modified with xanthine oxidase (HCPE/XOD) was designed and fabricated in this work, based on work in which an ECL biosensor had been developed for the detection of hypoxanthine (HX). The temper-

ature of HCPE/XOD was found not only to affect the diffusion and convection of luminescent compounds near the electrode surface but also to affect the enzyme activity; therefore, a suitable temperature of the electrode should be controlled for this ECL enzyme biosensor to achieve the best detection of HX. The present study shows that the HCPE is a useful tool to provide the suitable temperature for an ECL enzyme reaction. This heating-controlled ECL enzyme biosensor has some obvious advantages: (1) The surface of electrode easily be cleaned up and renewed. (2) Suitable control of the electrode temperature is favorable to enhance the detection sensitivity; by using the heating controlled enzyme biosensor, some enzyme systems which have no activity or lower activity at room temperature but have high activity at higher temperature can easily be investigated. In this case, the detection limit for HX atr 35 °C is found to be 30-fold lower than that at room temperature (25 °C). (3) The proposed HCPE is well-suited for combining with advanced separation techniques, such as capillary eletrochromatography and capillary electrophoresis. Some works related to this area are in process in our lab.

ACKNOWLEDGMENT

This project was financially supported by the National Nature Science Foundation of China (20735002, 20575011).

Received for review December 4, 2007. Accepted January 27, 2008.

AC702471R