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Glucose nanosensors based on redox polymer/glucose oxidase modified carbon fiber nanoelectrodes

Junjie Fei[a,b,1], Kangbing Wu[a], Fang Wang[a], Shengshui Hu[a,*]

[a] *Department of Chemistry, Wuhan University, Wuhan 430072, P.R. China*
[b] *College of Chemistry, Xiangtan University, Xiangtan, 411105, P.R. China*

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Abstract

This paper describes glucose nanosensors based on the co-electrodeposition of a poly(vinylimidazole) complex of $[Os(bpy)_2Cl]^{+/2+}$ and glucose oxidase (GOD) on a low-noise carbon fiber nanoelectrodes (CFNE). The SEM image shows that the osmium redox polymer/enzyme composite film is uniform. The film modified CFNE exhibits the classical features of a kinetically fast redox couple bound to the electrode surface. A strong and stable electrocatalytic current is observed in the presence of glucose. Under the optimal experimental conditions, the nanosensor offers a highly sensitive and rapid response to glucose at an operating potential of 0.22 V. A wide linear dynamic rang of 0.01–15 mM range was achieved with a detection limit of 0.004 mM. Compared with the conventional gold electrode, the nanosensor possessed higher sensitivity and longer stability. Successful attempts were made in real time monitoring rabbit blood glucose levels.

Keywords: Amperometry; Osmium redox polymer; Carbon fiber nanoelectrode; Glucose; Rabbit.

1. Introduction

The development of glucose electrodes based on immobilizing glucose oxidase (GOD) continues to receive considerable attentions in connection with the diagnosis and management of diabetes [1–3]. A wide variety of approaches have been proposed in the operation of glucose biosensors [4–8]. Wired-enzyme technology has been developed where a mediating species is chemically bound to a polymer backbone and to the enzyme in a manner that allows close contact between the FAD/$FADH_2$ centers of the enzyme, which serves to "electronically wire" the enzyme, facilitating a free flow of electrons from the enzyme to the electrode via the mediator [9,10]. A number of sensors based on this technology have been fabricated [11–13]. However, an important consideration in the future development of practical sensors is how to develop new immobilization strategies that are appropriate for the construction of miniature sensors, which requires the precise control of film deposition on a small electrode. Electrochemical immobilization provides an elegant alternative for the deposition of enzymes on very small-area electrodes of defined geometry [14]. It is reported that upon illumination, the redox polymer formed by coordinating $[Os(bpy)_2Cl^+/Os(bpy)_2Cl]^{2+}$ to poly(4-vinyl-pyridine) exchanges its inner-sphere chloride with more strongly coordinating pyridine or imidazole groups on the polymer backbones [15]. The resulting films conduct electrons when they are hydrated, and their redox segments are mobile enough to collide, even though they are tethered to the cross-linked polymer. Co-electrodeposition of redox polymer and enzymes onto macroelectrodes by applying double-step chronoamperometry has been developed [15,16]. Compared to chemical cross-linking, co-electrodeposition offer great promise to

* Corresponding author. Tel.: + 86 732 8292 205; fax: +86 732 8292 477.
E-mail addresses: sshu@whu.edu.cn (S. Hu), fei_JunJie@xtu.edu.cn (J. Fei).
[1] Tel.: +86 27 8721 8864; fax: 86 27 8764 7617.

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provide a better control to generate a coating on microelectrode, and to do so precisely in one rapid and simple step [16].

Microelectrodes and ultramicroelectrodes are considered as important analytical tools because these devices exhibited fast response time, significantly improved Faradic-to-capacitive current ratios, and substantially reduced ohmic drops [17–19]. Since Adams's group detected the amine neurotansmitters in the brain with carbon fiber microelectrodes (CFMEs) [20], CFMEs are considered as extremely useful transducers for biosensors because of their excellent properties such as high strength, low cost, and rather good biocompatibility [21]. The epoxy were involved in the process of sealing the carbon fiber in the conventional methods, which is the main factor influencing the characteristics of the electrodes [22–24]. The unavoidable bad sealing and leakage of the epoxy often resulted in the high noise, low sensitive, and short life of the electrode. Without epoxy involved, Huang developed a new method for the fabrication of low-noise carbon fiber nanoelectrodes (CFNEs) [25]. It involved flame etching of the tip to yield a smooth glassy/fiber interface, thereby increasing signal to noise in the resulting electrochemical response. Compared other methods for fabrications of CFNEs [17,26,27], the disadvantages resulting from the sealing by epoxy were thoroughly avoided and the surface of the nanoelectrodes was very smooth. Furthermore, this method is very simple, facile and low cost. Successful applications of these CFNEs have made in the detection of biological species [28–30], and however, there is no report on electrochemical immobilization of enzyme onto these CFNEs.

This paper described the fabrication and characterization of an enzyme-based nanosensor for glucose based on co-electrodeposition of redox polymer and GOD onto a low-noise CFNE. When the adsorbed redox polymer and enzyme were electrochemically reduced, the redox polymer and enzyme could be irreversible cross-linked and thereby be irreversible co-electrodeposited onto the electrode surface. Here we show that the redox polymer and GOD can be easily co-electrodeposited onto the surface of CFNEs under mild conditions by applying a steady reducing potential as reported previous [31]. Prior to redox polymer's coating, the conventional carbon fiber electrode need pretreated by exposure to 1 Torr O_2 plasma [32]. However, before the modification with redox polymer and enzyme, the freshly fabricated CFNEs did not need any pretreated. The SEM image shows that the resulting thin redox polymer/enzyme film is very uniform and contains little impurities. Such coupling of nanoscale transducers with the inherent electrocatalytic activity of the osmium redox polymer results in attractive performance characteristics for small-space biosensing of glucose, such as high sensitivity, good reproducibility, and rapid response. Compared with conventional macroelectrodes, the nanosensor possessed higher sensitivity and longer stability.

2. Experimental

2.1. Reagents and apparatus

All electrochemical measurements were carried out with a computer-controlled model CHI 660A electrochemical workstation (CH Instrument, Austin, USA) at room temperature. A small two-electrode electrochemical cell (~0.25 mL) was employed with an Ag/AgCl reference electrode and a modified CFNE working electrode for the small volume measurements. In amperometric experiments, the working electrode was poised at 0.22 V, well in the plateau region of glucose electrooxidation. All potentials in this paper were quoted with respect to the Ag/AgCl. A scanning electron microscope (SEM X650, Hitachi, Tokyo, Japan) was used for observation of the CFNEs and the redox polymer/enzyme film modified CFNEs.

The synthesis of osmium redox polymer reported here has been described elsewhere [15,16]. In this preparation, $[Os(bpy)_2Cl_2]^{+/2+}$ (bpy = 2,2′-bipyridine) was synthesized from K_2OsCl_6, and then poly(4-vinylimidazole-co-acrylamide) partially complexed with $[Os(bpy)_2Cl]^{+/2+}$. Glucose oxidase (GOD, EC 1.1.3.4, from *Aspergillus niger*, 191 units mg^{-1}) was purchase from Fluka. Glucose was purchased from Shanghai Boao Bio-scientific Co. Ltd. (Shanghai, China). The physiological buffer solution (PBS; pH 7.3), which was used as electrolyte, was prepared from phosphate buffer (0.02 M) and sodium chloride (0.15 M). The osmium redox polymer solution and GOD solution were prepared with a PBS buffer. All other chemicals were of certified analytical grade, and all solutions were prepared with doubly distilled water.

2.2. Preparation of CFNE and modified CFNE

The method to fabricate CFNE was previously described [25]. Carbon fibers (7 μm in diameter, Kureha Chemical Industry Co. Ltd, Tokyo, Japan) were sonicated for 5 min sequentially in acetone, alcohol, and doubly distilled water. The cleaned carbon fiber was then connected to the end of a 0.1 mm diameter copper wire using a high-purity silver conductive paint (SPI Supplies, West Chester, USA) and dried at room temperature. A 1.0 mm diameter glass capillary was pulled on the flame of the gas lamp to form very thin tip with an inner diameter of 15–30 μm. The carbon fiber-copper wire was inserted into the glassy capillary tip and a 1.0 cm length of the carbon fiber was exposed from the tip. The capillary tip was fused on the flame to seal the carbon fiber. The protruding carbon fiber was etched slowly on the bottom of the flame to get a desired length and a nanometer scale tip. The freshly prepared CFNE was tested by cyclic potential scans in 0.1 mM potassium ferricyanide in 0.1 M KCl at a scan rate of 25 mV s^{-1}. CFNEs that did not respond were discarded and the survival rate of the CFNEs is about 90%. The experiment results also showed that the stable currents of potassium ferricyanide at

CFNEs have a good linear relationship with the length of CFNEs.

Prior to their modification, all CFNEs used in the experiment were freshly made with no chemical or electrochemical pretreated. The modification of CFNE was performed in an electrodeposition PBS solution containing the osmium redox polymer (0.75 mg mL^{-1}) and GOD (0.5 mg mL^{-1}) by applying 2 min a steady reducing potential of −1.4 V. The resulting modified CFNEs were thoroughly washed with water. In order to eliminate the ascorbic acid interference, 0.5% Nafion solution was dropped on the tip of the modified CFNE then left to dry at room temperature.

2.3. Experiment in real time monitoring rabbit blood glucose level

Male New Zealand white rabbits, weighing ∼2.0 kg, were anesthetized with ketamine 50 mg kg^{-1} and xylazine 10 mg kg^{-1} and then secured on a blanket. A catheter was indwelled into the left ear arteries for the blood sampling and each time 0.15 mL blood sample was withdrawn. Then an aliquot of 0.05 mL blood sample was diluted to 0.25 mL with PBS buffer. The redox polymer/enzyme-modified CFNE was immersed in this solution and amperometric response was recorded. Before sample withdrawing, a dose of 50 unit kg^{-1} of body weight heparin solution was administrated as anticoagulant. In each experiment, a 50% glucose solution (300 mg kg^{-1}) was administered into the right ear vein to induce a rapid rise in glucose concentration. A rapid decline in glucose concentration was then induced by an insulin injection (0.5 unit kg^{-1}). Once an in vivo monitoring was finished, the catheter was explanted from animal.

3. Results and discussion

3.1. SEM characterization

The SEM images of CFNE and redox polymer/enzyme-modified CFNE are shown in Fig. 1 General view of the CFNE showed that a smooth needle-shaped carbon fiber is tightly sealed in capillary (Fig. 1(a)). Fig. 1(b) displays the tip of the CFNE and it shows that the diameter of the tip is ∼100 nm, and the surface is smooth. Fig. 1(c) further demonstrates the smooth surface of CFNE before modification. Fig. 1(d) shows that the electrodeposition method can form a uniform redox polymer/enzyme composite film on the surface of CFNE and the composite film contain little impurities.

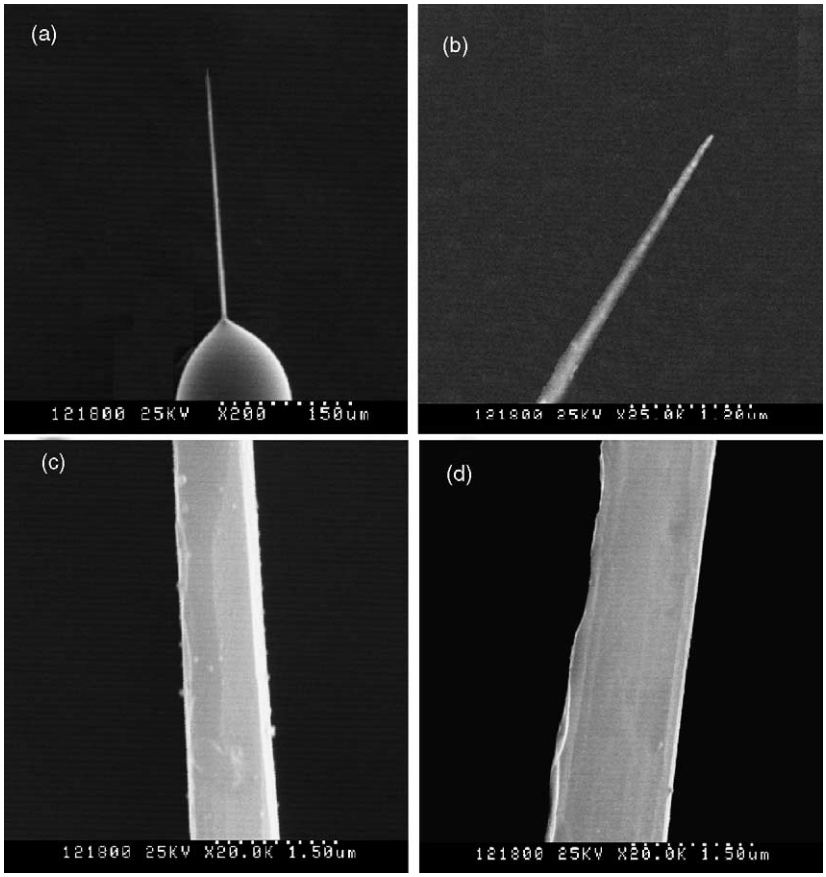

Fig. 1. SEM images of the CFNE: (a) the general view of a CFNE ×200, (b) the tip of a CFNE ×25,000, (c) The surface of a CFNE ×20,000, (d) the surface of a redox polymer/enzyme-modified CFNE ×20,000.

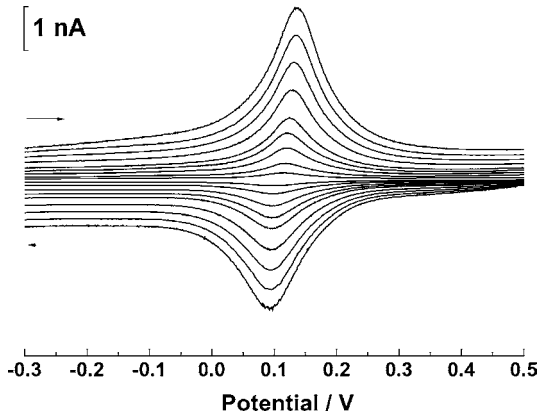

Fig. 2. Cyclic voltammograms of a redox polymer/enzyme-modified CFNE in a pH 7.3, PBS buffer. The scan rates from the innermost to the outmost waves were, 10, 25, 50, 75, 100, 150, 200, 250, 300 mV s^{-1}.

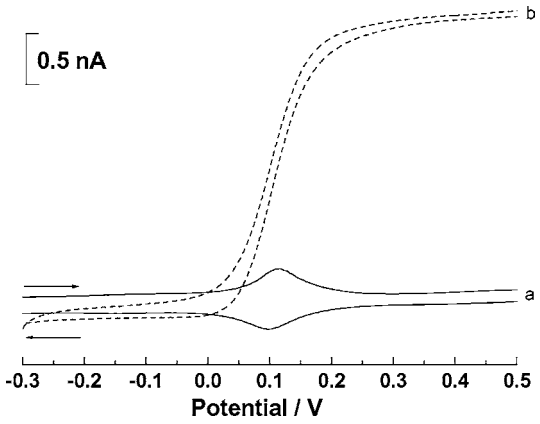

Fig. 3. Cyclic voltammograms of a redox polymer/enzyme-modified CFNE in a pH 7.3, PBS buffer in the absence of (a) and in the presence of (b) glucose (5 m mol L^{-1}). Scan rate: 25 mV s^{-1}.

3.2. Voltammetric characteristics of the nanosensor

The cyclic voltammograms of a redox polymer/enzyme-modified CFNE (Fig. 2) exhibit well-defined oxidation and reduction peaks corresponding to the oxidation and reduction of the Os^{2+}/Os^{3+} redox couple and yielding an formal potential, taken by average value of the anodic and cathodic peak potential, of 105 mV. Successive cyclic potential scans of the redox polymer/enzyme-modified CFNE displayed the nearly same voltammograms (not shown), suggesting the redox polymer/enzyme film was stable. As can be seen from Fig. 2, both anodic and cathodic peak currents increased linearly and the separation between the anodic and cathodic peak potentials ((E_p) increased only marginally (less than 30 mV), as the scan rate increased from 10 to 300 mV s^{-1}, which indicates that the redox polymer/enzyme film modified CFNE exhibits a reversible surface-bound redox process and the charge transfer from the film to the electrode is rapid. With increasing scan rate, the peak-to-peak potential separation increased and the scan rate dependent of the peak current became more complicated, indicative of a mixed linear and cylindrical semi-infinite transport within the redox polymer/enzyme film [33].

Fig. 3 shows cyclic voltammograms of a redox polymer/enzyme film modified CFNE in the PBS solution in the absence of glucose (a) and in the presence of 5 mM glucose (b). A typical catalytic electrooxidation wave is observed and the reduction peak of the redox polymer is eliminated, indicative of an efficient catalytic property for glucose. The absence of a reduction current peak shows that the film was homogeneously maintained in the reduced state by the transfer of electrons from GOD–$FADH_2$ to the Os^{3+} sites [16].

3.3. Co-electrodeposition of redox polymer and GOD on CFNEs

Since the electrodeposition process is restricted to the electrode surface, it is effective both for the selective deposition of redox polymers and incorporated biomaterials at micro and ultramicro devices. In this work, co-electrodeposition was carried out by applying a steady reducing potential [31]. The experiment results showed that the concentration of the osmium redox polymer of 0.75 mg mL^{-1} was best for our purpose and the optimal concentration of GOD was 0.5 mg mL^{-1}. The reducing potential was examined in the range from 0 V to −1.6 V and the rate of electrodeposition increased with lowering the reduction potential, and reached a plateau at −1.4 V. A slight decrease in the total amount of material deposited was observed when the potential became more negative than −1.4 V. The dependence of the peak current of redox polymer/enzyme film modified CFNEs on the co-electrodeposited time was studied (Fig. 4). It can be seen from Fig. 4, the peak current increases linearly with the co-electrodeposited time over the range of 10–120 s, and then tends to level off due to the redox polymer/enzyme film surface coverage reached its maximum value and no longer increased with the co-electrodeposited time.

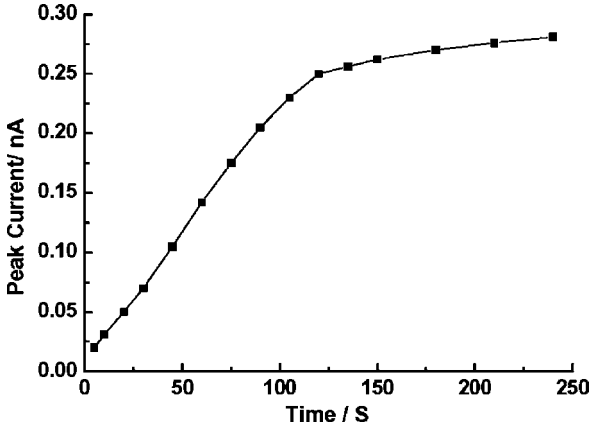

Fig. 4. The dependence of peak current of redox polymer/enzyme-modified CFNE on the co-electrodeposited time. Scan rate: 25 mV s^{-1}.

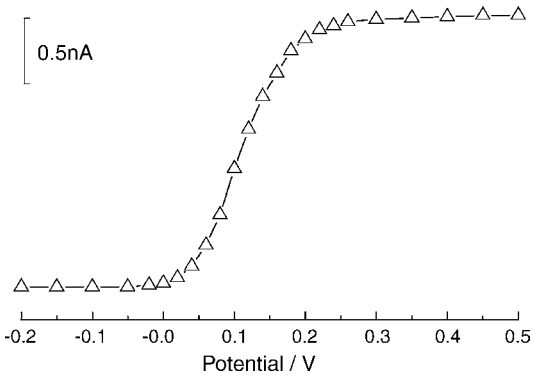

Fig. 5. Dynamic potential voltammogram of 5 mM glucose at a redox polymer/enzyme-modified CFNE.

3.4. Amperometric response of the nanosensor to glucose

Fig. 5 shows the potential dependence of 5 mM glucose response (dynamic potential voltammogram) at a redox polymer/enzyme-modified CFNE. The curve obtained has a sigmoidal shape with a reflection point coinciding with the formal potential of the osmium redox couple. The oxidation currents increase with the increasing potentials, and it finally reach a steady response at about 0.22 V. Thus the redox polymer/enzyme-modified CFNE could be used as an effective amperometric sensor for the determination of glucose. In order to reach high electrooxidation current and at the same time minimize the electrooxidation of interferants, the applied potential of the nanosensor was kept at 0.22 V in all experiments.

A typical amperometric experiment of glucose was carried out in a PBS solution without any convective stirring. Amperometric tests showed that the nanosensor exhibits stable electrochemical response for various concentrations of glucose and the oxidation current increases and reached steady values within a short time period (Fig. 6). Carbon-fiber microelectrodes of similar dimensions were found to have poor sensitivity to homogeneous catalytic reactions, even if the enzyme is well bound to the electrode surface [34]. However, since the osmium redox couple in this work is a non-diffusing collector of the enzyme-generated current, the high current–noise ratio can be attributed to the increased flux of the electrons via radial diffusion through the thin redox polymer/enzyme film to the electrode surface from enzyme molecules immobilized closed to the electrode surface [35]. The current responses were found to be linear to the glucose concentration over the range of 0.01–15 mM, and a sensitivity of 0.47 nA mM^{-1} and an intercept of 0.0064 nA with a correlation coefficient, r, of 0.9987 were obtained. The limit of detection (LOD) was 0.004 mM (S/N = 3), while the macroelectrodes of the similar film show a LOD of 0.03 mM [16], which indicated that miniaturization does result in an increase in sensitivity and improves signal to noise ratios. The precision was also estimated from two series of 15 repetitive measurements of 0.1 and 1 mM glucose solution, and the relative standard deviations (RSD) were 5.3% and 4.7%, respectively. The inter-electrode reproducibility was also investigated. The freshly etched CFNEs with good voltammetric responses in 0.1 mM potassium ferricyanide were measured by electronic microscope (×750) and the CFNEs with length of ~100 μm were selected for further studied. After the modifications, the reproducibility of the naonosensors was examined by measurement of the amperometric currents of redox polymer/enzyme film modified CFNEs in 1 mM glucose solution and a relative standard deviations of 8.5% was obtained for n = 10. These results suggest that the fabricated nanosensor has a good accuracy for the determination of glucose.

When the concentration of glucose higher than 15 mM, with the increase of glucose concentration the current did not increased linearly and a Michaelis–Menten type response was observed (insert of Fig. 6). The Michaelis–Menten constant can be obtained using Eadie–Hofstee transformations of the Michaelis–Menten equation [36] (Eq. (1))

$$i_k = i_m - K'_M \left(\frac{i_k}{\text{glucose}} \right) \quad (1)$$

where i_k is the steady-state current, i_m is the maximum steady-state current under saturating substrate conditions, K'_M is the apparent Michaelis–Menten constant. As shown in Fig. 7, an Eadie–Hoastee type plot gives a straight line. From the slope and intercept of the line, K'_M of 8.33 mM and i_m of 8.26 nA are evaluated. The apparent Michaelis constant (K'_M) characterizes the enzyme nanosensors, not the enzyme, itself, and provides a measure of the substrate concentration rang over which the nanosensors response is approximately linear. In this case, the calibration curve was linear up to 15 mM glucose, which is consistent with the obtained value of K'_M.

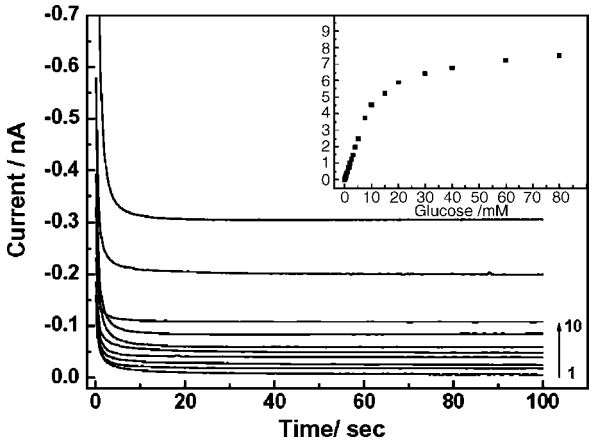

Fig. 6. Amperometric i–t curves at a redox polymer/enzyme-modified CFNE in PBS containing 0 (1), 0.02 (2), 0.04 (3), 0.06 (4), 0.08 (5), 0.1 (6), 0.15 (7), 0.2 (8), 0.4 (9) and 0.6 mM (10) of glucose with the potential hold at 0.22 V. Insert: Calibration curve of glucose at the nanosensors.

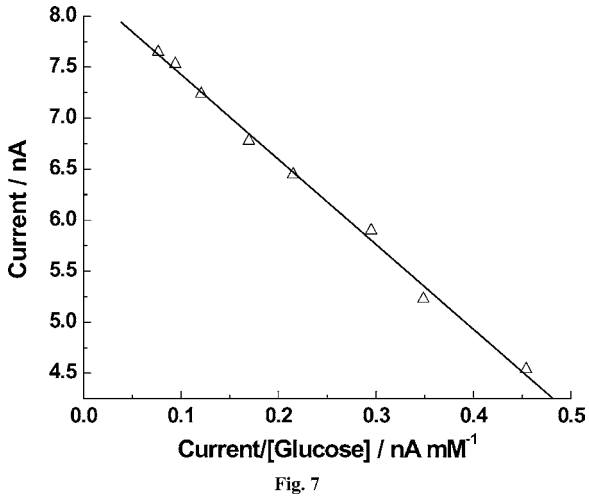

Fig. 7. Typical Eadie–Hofstee plot of relationship between the steady current and glucose concentration at a redox polymer/enzyme-modified CFNE at 0.22 V (vs. SCE).

3.5. Elimination of the interferences of AA

AA is electroactive, which often co-exists with glucose in biological systems, and can be oxidized at a redox polymer/enzyme film, resulting in an overlapping amperometric response. A coating of Nafion has been successfully used to eliminate the interference of AA in the determination of glucose [37]. After a redox polymer/enzyme film was further coated with Nafion film, cyclic voltammograms of Nafion-coated redox polymer/enzyme CFNEs were similar to that of the redox polymer/enzyme CFNEs. The amperometric response of glucose at the Nafion-coated redox polymer/enzyme film allowed little changes, indicating that the existence of Nafion film had little effect on the electrochemical characteristics of the redox polymer/enzyme film. At a Nafion-coated redox polymer/enzyme film modified CFNEs, the addition of 0.1 mM AA did not affect the amperometric response of 1 mM glucose, which showed that coating of the Nafion membrane could efficiently eliminate AA interference.

3.6. Stability and sample analysis

Stability tests were carried out at room temperature; between measurements, the nanosensor was keep at 4 °C. It was shown that the modified CFNEs maintain 90% of its initial sensitivity after 72 h. While at the similar film modified gold macroelectrode, the enzyme electrode lost 10% of its initial sensitivity after 48 h, and about 50% of its initial sensitivity after 64 h. The improvement of stability at the CFNEs may be duo to the good biocompatibility of carbon fiber.

Fig. 8 shows the variation in the nanosensor-measured glycemia of rabbit after glucose and insulin were sequentially administrated intravenous. In this experiment, the blood glucose concentration of rabbit was rapidly rise from 4.5 mM to 9.0 mM by an intravenous injection of glucose solution and

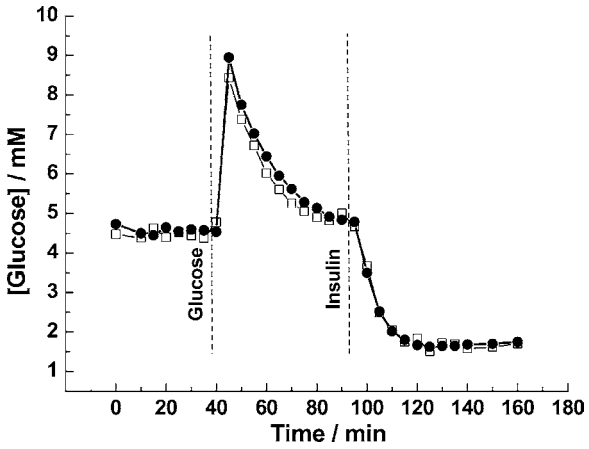

Fig. 8. Real-time monitored blood glucose concentration of rabbit with a nanosensor (□) and an YSI Model 2300 glucosememter (●).

then decreased behind the peak. After 50 min, the glucose level recovered to nearly normal glucose concentration and then a rapid decline in the glucose concentration is obtained by an intravenous insulin injection. The declined blood glucose concentration will maintain for a relative long time and then increased slowly. As also can be seen from Fig. 8, the results obtained with nanosensor are in good agreement with reference values obtained with a yellow springs blood sugar analyzer (YSI Model 2300).

4. Conclusion

We have demonstrated herewith that CFNEs modified with thin films of osmium redox polymer and GOD can be useful for the real time monitoring the blood glucose concentration of rabbits. Using electrochemical method, osmium redox polymer and GOD can be simultaneously deposited onto the low-noise CFNEs to form uniform composite films under mild conditions. The osmium complex in the resulting redox polymer/enzyme composite films effectively mediates the electron transfer from the enzyme to the substrate CFNEs and the resulting nanosensors exhibit excellent properties for glucose response.

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