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Short communication

A biosensor prepared by co-entrapment of a glucose oxidase and a carbon nanotube within an electrochemically deposited redox polymer multilayer

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ABSTRACT

A glucose biosensor based on a nanocomposite made by layer-by-layer electrodeposition of the redox polymer into a multilayer containing glucose oxidase (GOx) and single-walled carbon nanotubes (SWCNT) on a screen-printed carbon electrode (SPCE) surface was developed. The objectives of the electrodeposition of redox polymer are to stabilize further the multilayer using a coordinative cross-linked redox polymer and to wire the GOx. The electrochemistry of the layer-by-layer assembly of the GOx/SWCNT/redox polymer nanocomposite was followed by cyclic voltammetry. The resultant biosensor provided stable and reproducible electrocatalytic responses to glucose, and the electrocatalytic current for glucose oxidation was enhanced with an increase in the number of layers. The biosensor displayed a linear range from 0.5 to 6.0 mM, a sensitivity of 16.4 μA/(mM cm^2), and a response time of about 5 s. It shows no response to 0.05 mM of ascorbic acid, 0.32 mM of uric acid and 0.20 mM of acetaminophen using a Nafion membrane covering the nanocomposite-modified electrode surface.

1. Introduction

Highly sensitive and selective enzyme-based biosensors are used in an increasing number of clinical, environmental, agricultural, and biotechnological applications [1]. Determination of glucose is one of the most popular and well-known applications of biosensors [2]. Enzyme-based electrochemical glucose biosensors can be fabricated by the incorporation of glucose oxidase (GOx) with a suitable electrochemical transducer. In amperometric biosensors, the immobilization of the enzyme and the construction of highly effective electrical communication among the redox centers of enzymes and electrodes remain challenges [3].

Due to its efficient electron shuttling properties combined with the polymeric structure and promoting a stable adsorption, the redox polymer based on osmium bipyridine (bpy)-complexed poly(4-vinylpyridine) (PVP) or poly (1-vinylimidazole) (PVI) has been extensively used for the "wiring" of redox enzymes [4] and microbial cells [5] on the electrode surface. The application of redox polymers to achieve efficient electric communication between the gram-positive organism *Bacillus subtilis* and an electrode was also studied [6]. To increase the sensitivity of biosensors, the complex of redox polymers and GOx was utilized in layer-by-layer (LBL) deposition approaches to increase loading of enzyme and mediator on the electrode [7]. Although more enzymes can be immobilized on electrodes through LBL, a thick composite film also increases electron transfer resistance and slows down substrate diffusion. The incorporation of nano-sized materials into a sensing device was extensively used to improve the conductivity of composition film [8]. For example, incorporation of carbon nanotubes (CNT) [9] and metal nanoparticles [10] greatly increases biosensor sensitivity and response speed due to their high chemical stability, high surface area, and unique electronic properties.

In this study, we describe a highly sensitive and stable biosensor for glucose sensing based on a GOx/CNT/redox polymer nanocomposite, which was made through an incorporation of electrostatic layer-by-layer deposition and electrodeposition technique. The analytical performance of the resultant glucose biosensor was evaluated in terms of sensitivity, reproducibility, and stability.

2. Material and methods

2.1. Materials

Glucose oxidase (GOx, EC 1.1.3.4 from *Aspergillus niger*) was purchased from Sigma-Aldrich. 1-Vinylimidazole, 2,2′-dipyridyl (bpy), sodium dithionite, and Nafion were purchased from Sigma-Aldrich. Ethylene glycol and K_2OsC1_6 were purchased from Acros. 2,2′-Azobis (isobutyronitrile) (Polysciences) was purified by double recrystallization from methanol and stored at −20 °C. Acetamidophenol (AP), ascorbic acid (AA), and uric acid (UA) were of analytical grades and were used as received. Os-$(bpy)_2C1_2$ was prepared using a reported method [11]. Poly(1-vinylimidazole) (PVI) and the redox polymer, designated as PVI-Os, were synthesized according to a previously published protocol [12]. Carbon ink (Electrodag 423SS) was obtained from Acheson

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Colloids (Japan). Single-walled CNTs (SWCNTs) were obtained from Shenzhen Nanotech Port Co. Ltd. (China) and the as-received MWCNT was treated using an established method [13].

Unless otherwise stated, all chemicals and reagents used are of analytical grade. Buffers were prepared using water from a Milli-Q ultrapure water system. Phosphate buffer (PB, 0.02 M) was prepared by mixing stock solutions of potassium dihydrophosphate and sodium hydroxide. The glucose solution was made from 1 M stock solution that has been left to mature overnight and diluted with a phosphate buffer to the expected concentration.

2.2. Apparatus

Amperometric and cyclic voltammetric (CV) experiments were performed with a CHI 832 (Shanghai, China). All experiments were carried out with a conventional three-electrode system with the screen-printed carbon electrode as the working electrode, a platinum foil as the counter electrode, and an Ag/AgCl (saturated potassium chloride) as the reference electrode. A thermostated bath was used to maintain the temperature at 25 ± 1 °C.

Scanning Electron Microscopy (SEM) was performed using a Quanta 200 (FEI, Philips) apparatus. The acceleration voltage was 20 kV. Samples were gold-sputtered prior to SEM measurements.

2.3. Fabrication of the biosensor

The screen-printed carbon electrode (SPCE) was prepared by a reported method [14] and geometric area of electrode is 0.05 cm^2. To facilitate the adsorption of the negatively charged GOx and SWCNT, the SPCE was functionalized with cationic PVI-Os by electrodeposition of a layer of PVI-Os onto the electrode surface. This was accomplished by applying a steady reducing potential (−1.4 V vs. Ag/AgCl) for 200 s on an SPCE in PB (pH 7.1, 20 mM) containing PVI-Os (0.7 mg/mL) [15]. The resultant electrode was then removed and washed with deionized water. For the formation of the multiple coordinate linkages between enzyme molecules and the redox polymer, we followed the experimental procedure reported in Heller's paper [15] with slight modification. Fabrication of the GOx/SWCNT/PVI-Os nanocomposite was as follows: First, the functionalized electrode was immersed in a solution of SWCNT for 20 min, followed by electrodeposition of a layer of PVI-Os. Second, the resultant electrode was immersed in a solution of GOx for 40 min, followed by electrodeposition of a layer of PVI-Os. Each assembly step was followed by washing with deionized water to remove any excess material. The GOx/SWCNT/PVI-Os nanocomposite was then formed by repetition of the two steps described above until the required number of layers was obtained. The last layer of the nanocomposite was an electrodeposited PVI-Os layer.

3. Results and discussion

3.1. Fabrication of the Biosensor based on the GOx/SWCNT/PVI-Os nanocomposite

The development of highly sensitive amperometric biosensor is dependent upon the increase in the density of sensing elements in a spatially distinct region. To achieve this, we have formed a nanocomposite consisting of enzyme, SWCNT and redox polymer through an incorporation of electrostatic layer-by-layer deposition and electrodeposition technique. The first step in the fabrication of these nanocomposite structures was functionalization of SPCE surface with the polycation. A layer of PVI-Os was electrodeposited onto the SPCE surface as an adhesion layer, which was observed in the cyclic voltammogram of the resultant electrode in blank PB (results not shown). GOx has an isoelectric point of about 3 [16]. Therefore, at pH 7.4, the GOx and SWCNTs are negatively charged, allowing adsorption onto the cationic PVI-Os layer electrodeposited on the SPCE surface. Alternating deposition of redox polymers, SWCNT and enzymes permitted the buildup, layer by layer, of a nanocomposite structure.

The buildup of multilayer was assessed by cyclic voltammetry. From Fig. 1, a pair of well-defined, chemically reversible CV peaks, with cathodic peak at 246 mV and anodic peak at 214 mV, was observed after the first GOx/SWCNT/PVI-Os layer was assembled. This can be ascribed to the oxidation and reduction of the redox polymer's Os(bpy) complexes at the electrode. Reduction and oxidation peak currents were increased with an increase in the number of GOx/SWCNT/PVI-Os layers, suggesting each assembling component links alternately as a submonolayer and plays a role as a platform on which layerforming counterparts are bound. The redox peaks of the multilayer electrodes were slightly more separated as the number of added layers increased (50 mV for three layer and 87 mV for five layer modified electrodes), which seems to be due to the rather slower charge transfer displayed by the PVI-Os as the successive GOx/SWCNT/PVI-Os layers deposited [11]. The most important finding, however, is that the surface concentration of PVI-Os increased as the multilayer formation proceeded, indicating that the growth of GOx/SWCNTs/PVI-Os layer on an SPCE surface and the electrical connectivity of $Os(bpy)_2$ groups in the network are maintained. Subsequently, cyclic voltammograms at a scan rate of 10 mV/s were used to characterize the number of electroactive osmium redox sites present in each layer, an important criterion for the assessment of the sensor's electrochemical function. The area under the anodic peak was integrated from the voltammogram and used to determine the surface coverage of osmium redox sites present per adsorbed layer. A linear relationship between charge density and number of layer was observed, and the charge density of the nanocomosite-modified electrode is about 3.7×10^{-9} mol cm^{-2}. This value higher than that reported for $Os(bpy)^{3/2+}$ layers without SWCNTs (about 4.1×10^{-11} mol cm^{-2}) [11].

The morphology of the nanocomposition on glass carbon electrode (GCE) was characterized by SEM. Fig. 2 displays typical SEM images of a bare GCE (A) and a GCE after modified with nanocomposition (B). As shown in Fig. 2B, the substrate was mostly covered with homogeneous, porous and three-dimensional SWCNT films after nanocomposition was formed on GCE.

The cyclic voltammograms of SPCEs modified with five layers at different scan rates ranging from 5 to 200 mV/s are shown in Fig. 3. The relationship between peak current and scan rate was linearly proportional to the square root of the scan rate ($Ip_a/\mu A = -7.82 + 4.18(v/\mathrm{mV\ s^{-1}})^{1/2}$ and $Ip_c/\mu A = 6.00 - 3.52(v/\mathrm{mV\ s^{-1}})^{1/2}$, R = 0.997); this indicates a diffusion-controlled redox process. The

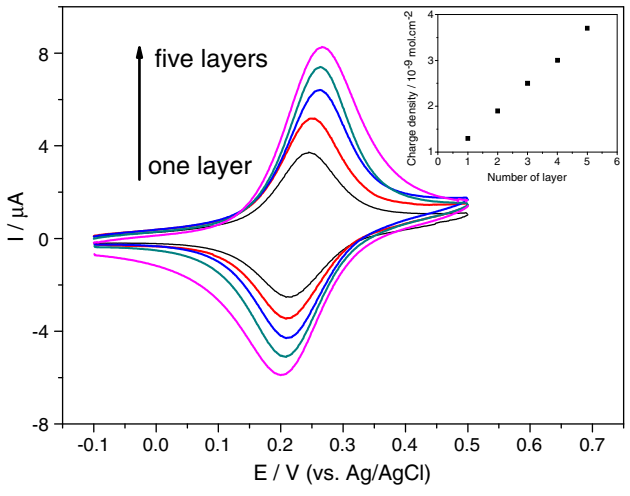

Fig. 1. Cyclic voltammograms for the nanocomposite-modified SPCEs with different numbers of GOx/SWCNT/PVI-Os layers, at a scan rate of 10 mV/s in 20 mM PB (pH 7.4) containing 0.15 M NaCl. From inner to outer: one to five layers. Inset: relationship between the charge density and the number of layers.

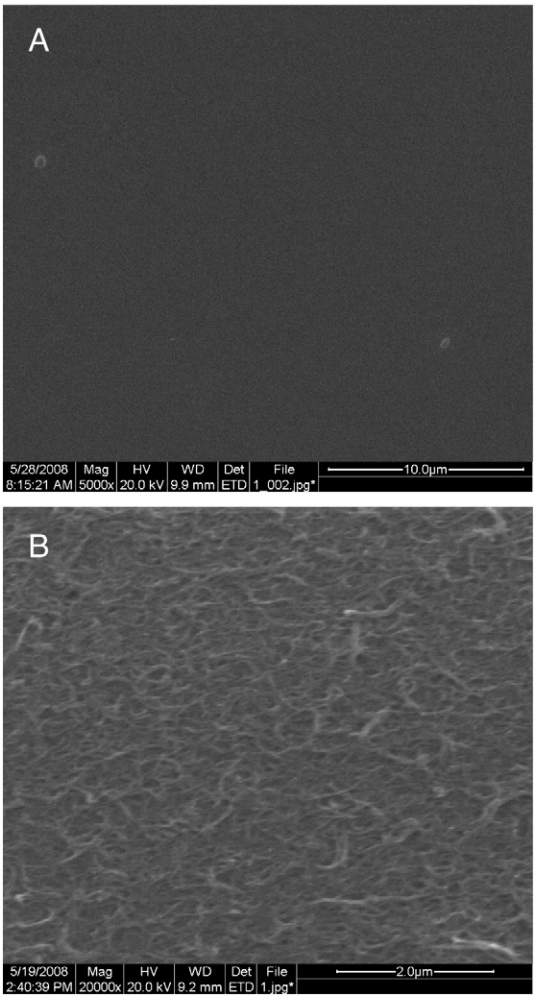

Fig. 2. Scanning electron micrographs (SEM) of the bare GCE (A) and nanocomposition modified GCE (B).

behavior may be due to that electrons are transported in the GOx-wiring gel through collisions between segmentally mobile Os^{2+} and Os^{3+} redox centers tethered to the polymer's backbone [17]. Further successive potential scanning at 100 mV/s for 1 h recorded a decrease of less than 3% in the peak current. This illustrates that the electroactive components were not free to diffuse from the electrode surface. This stability of nanocomposite seems to be due to the structural integrity of the multilayered network, which results from the multiple coordinative linkages formed between enzyme molecules and redox polymer as described by Heller et al. [15].

3.2. Electrocatalysis of nanocomposite-modified SPCEs for the oxidation of glucose

The bioelectrocatalytic signals from multilayer modified electrodes were traced by cyclic voltammetry in 10 mM glucose solution (Fig. 4). All the cyclic voltammograms registered from multilayer modified electrodes in the presence of glucose substrate were typical for the enzyme-catalyzed and -mediated voltammograms. The entire network is electrochemically connected with immobilized PVI-Os so that the enzymatic reaction can be efficiently transformed to the electrical currents. The anodic currents from multilayered electrodes were significantly enhanced with the increase of the number of deposited layers, and demonstrate that the immobilized enzyme retained its activity during the multilayer-forming and signaling processes. From one to five GOx/SWCNT/PVI-Os layers, the catalytic current of the nanocomposite-modified electrode showed an increase from 6.1 to 12.7 μA at the potential of 0.30 V (vs. Ag/AgCl). This suggests that the PVI-Os wired GOx was becoming increasingly accessible and the sensitivity improved with increasing amounts of mediator and enzyme on the electrode surface. The nanocomposite-modified electrodes containing more than five GOx/SWCNT/PVI-Os layers showed no further signal enhancement but exhibited a tendency for gradual decline in catalytic current [18]. Therefore, SPCEs modified with five layers of GOx/SWCNT/PVI-Os were used as biosensors in subsequent experiments.

To evaluate the analytical performance of the biosensors, calibration experiments were performed (Fig. 5). The amperometric signals were registered at the potential of 0.30 V (vs. Ag/AgCl). The bioelectrocatalytic signals from the biosensors reached 95% of the steady-state values within 5 s, implying a negligible diffusional restriction for the analytes, and the steady-state currents were maintained with no fluctuation. The linear regression equations was $I/\mu A = 0.82\ C/mM + 0.087$ in the range 0.5 (R.S.D. = 0.8%) to 6.0 mM (R.S.D. = 3.2%) and a correlation of 0.995. A sensitivity of 16.4 μA/(mM cm^2) was obtained. Compared to using a pure GOx/redox polymer film modified electrode [7], the sensitivity for detection of

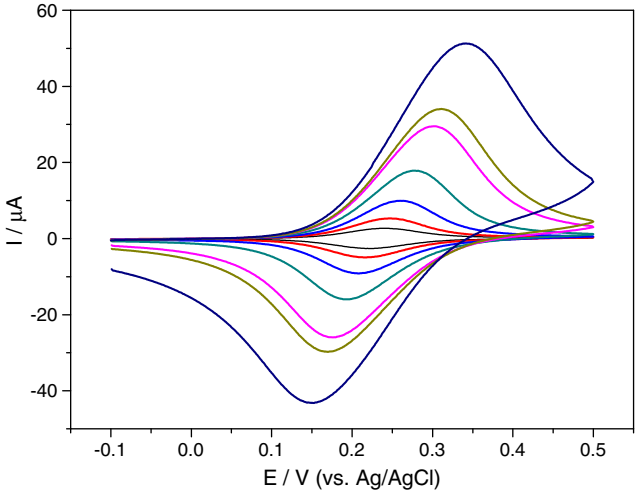

Fig. 3. Cyclic voltammograms of SPCEs modified with five GOx/SWCNT/PVI-Os layers at various potential scan rates. Potential scan rates (from inner to outer): 5, 10, 20, 40, 80, 100, and 200 mV/s.

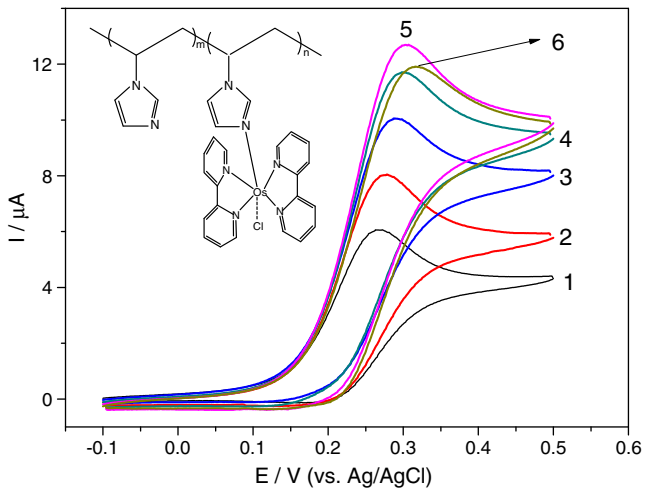

Fig. 4. Cyclic voltammograms of SPCEs modified with different numbers of GOx/SWCNT/PVI-Os layers in the presence of 10 mM glucose. The layer number is shown in the figure. The potential scan rate was 10 mV/s. Inset: structure of the redox polymer PVI-Os.

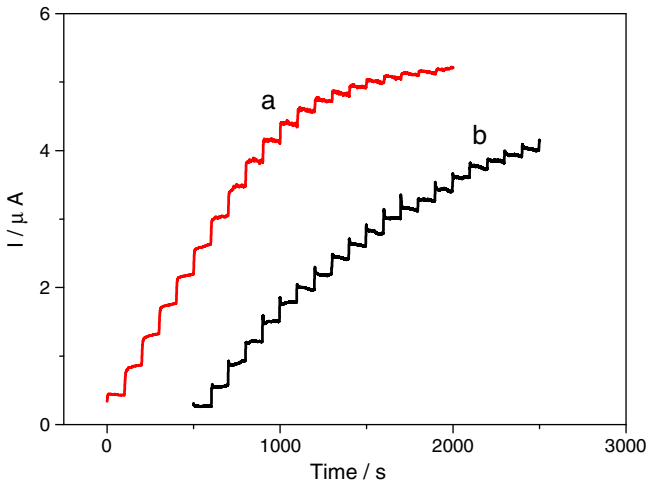

Fig. 5. Amperometric response of the nanocomposite-modified electrode to the addition of glucose aliquots into stirred PB (pH 7.4). Steps represent the response of the nanocomposite-modified electrode to additions of 0.5 mM glucose. a, nanocomposite-modified electrode without Nafion membrane; b, nanocomposite-modified electrode with Nafion membrane. E = 0.30 V (vs. Ag/AgCl).

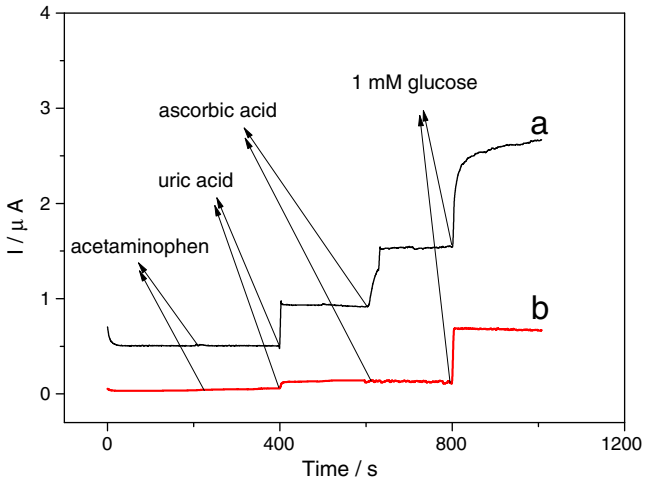

Fig. 6. Amperometric response recorded by the nanocomposite-modified electrode in a stirred solution to which 0.20 mM acetamidophenol, 0.32 mM uric acid, and 0.05 mM ascorbic acid aliquots were added, respectively. a, nanocomposite-modified electrode without Nafion membrane; b, nanocomposite-modified electrode with Nafion membrane. E = 0.30 V (vs. Ag/AgCl).

glucose was improved by using the nanocomposite modified electrode because much more glucose-accessible redox polymer-wired GOx was loaded onto the electrode surface. This increase may be attributed to the three-dimensional distribution of the redox polymer-wired GOx, because the SWCNTs in the nanocomposite enable the formation of porous nanostructures with high surface areas. In addition, the carbon nanotubes also act as a backbone in multilayer film and facilitate electron transfer across the multilayer film. As shown in Table 1, the sensitivity for glucose detection was also improved compared with similar type of sensors. A detection limit of 0.1 mM was estimated based on the glucose concentration that is required to produce a signal greater than three times the noise level.

The selectivity is an important feature to be considered for enzymatic glucose sensors. To evaluate selectivity, the amperometric current responses to UA (0.32 mM), AA (0.05 mM), and AP (0.20 mM) are examined together with glucose at 0.3 V (vs. Ag/AgCl) of operational voltage. AP exhibited no response. In contrast, large current responses were observed for AA and UA, as shown in Fig. 6a. Nafion is a negatively charged polyelectrolyte matrix that produces a repulsive force against negatively charged molecules. The use of a thin Nafion membrane over the electrode surface may reduce the interference caused by anions present in biological media [25]. Thus, Nafion was added on the surface of the nanocomposite-modified electrode and the resultant nanocomposite was dried in air for 15 min. Negligible current was observed in the presence of UA and AA for the glucose biosensor with Nafion membrane (Fig. 6b). The linear regression equation for the nanocomposite-modified electrode with Nafion membrane was I/μA = 0.46 C/mM + 0.48 in the range of 0.5 to 6.0 mM and a correlation of 0.982. The blood glucose level is the amount of glucose present in the blood of a human. Normally in mammals, the body maintains the blood glucose level at a reference range between about 3.6 and 5.8 mM. For normal people, the sensitivity range of the biosensor can meet the requirement. But for people with diabetes, dilution of the blood is needed. Even though the Nafion membrane reduces the intensity of the biosensor response (Fig. 5b), it eliminates the interference caused by AA and UA.

The long-term stability of the biosensor was evaluated by measuring its performance every 5 days for 30 days. The sensing elements were stable for at least one month and maintained 90% activity during the entire period, with storage at 4 °C between measurements.

4. Conclusion

A highly sensitive glucose biosensor based on the GOx/SWCNT/PVI-Os nanocomposite was developed. Incorporation of SWCNT into the nanocomposite significantly improved the sensitivity of the biosensor. The nanocomposite-modified electrodes demonstrated an increase in catalytic current with every deposited layer of the GOx/SWCNT/PVI-Os. The resultant biosensors showed sensitivity of 16.4 μA/(mM cm^2), a linear range of up to 6 mM and a detection limit of 0.1 mM glucose (S/N = 3).

Table 1
Comparison of performances of electrochemical glucose biosensor based on different GOx/CNT or GOx/redox polymer nanocomposites.

Nanocomposite	Potential (vs. Ag/AgCl)	linear Rang (mM)	Limit of detection	Sensitivity μA mM^{-1}cm^{-2}	Ref
GOx–SWNT–PVI-Os/SPCE	0.30	0.2–7.5	0.1 mM	14.6	This work
GOx–PVI-Os/SPCE	0.20	0.3–10	0.1 mM	0.14 μA mM^{-1}	7
GOx–PVP-Os–AA/Au	0.30	2–20	N	0.015	11
GOx/CNT/PAP/Au	0.75	Up to 5	0.01 mM	11.4	[19]
GOx–MWNT–PEI/GC	−0.50	Up to 0.3	0.05 mM	106.57	[20]
GOx–FTMA–SWNT/SPCE	−0.60	0.04–0.38	N	1.73 μA mM^{-1}	[21]
GOx–biotin–Py–SWNT/Pt	0.6	0.005–13	N	37	[22]
GOx–gelatin–MWNT/GC	−0.40	6.30–20.1	N	2.47	[23]
GOx–MWNT–Sol–gel/bppg	0.30	0.2–20	0.05 μM	0.196	[24]

Note: GC glass carbon electrode; CNT carbon nanotube; PVP-Os poly[(vinylpyridine) Os(bipyridyl)$_2$Cl]; PVP-Os-AA poly[vinylpyridine Os(bisbipyridine)$_2$Cl]-*co*-allylamine; PAP Poly (o-aminophenol); PEI poly(ethylenimine); FTMA 11-(ferrocenyl)-undecyltrimethylammonium bromide; Py pyrene; bppg basal plane pyrolytic graphite; N not reported.

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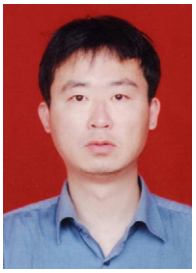
References

[1] G.S. Wilson, Y. Hu, Enzyme-based biosensors for in vivo measurements, Chem. Rev. 100 (2000) 2693–2704.
[2] J. Wang, Electrochemical glucose biosensors, Chem. Rev. 108 (2008) 814–825.
[3] U. Bilitewski, Protein-sensing assay formats and devices, Anal. Chim. Acta 568 (2006) 232–247.
[4] A. Heller, B. Feldman, Electrochemistry in diabetes management, Acc. Chem. Res. 43 (2010) 963–973.
[5] S. Timur, B. Haghighi, J. Tkac, N. Pazarlioglu, A. Telefoncu, L. Gorton, Bioelectrochemistry 71 (2007) 38–45.
[6] V. Coman, T. Gustavsson, A. Finkelsteinas, C.V. Wachenfeldt, C. Hagerhall, L. Gorton, Electrical wiring of live, metabolically enhanced *Bacillus subtilis* cells with flexible osmium-redox polymers, J. Am. Chem. Soc. 131 (2009) 16171–16176.
[7] Q. Gao, X. Yang, Layer-by-layer electrodeposition of redox polymers and enzymes on screen-printed carbon electrodes for the preparation of reagentless biosensors, Chem. Commun. 1 (2004) 30–31.
[8] A. Merkoci, M. Pumera, X. Llopis, B. Perez, M. del Valle, S. Alegret, New materials for electrochemical sensing VI: carbon nanotubes, Trends Anal. Chem. 24 (2005) 826–838.
[9] H. Cui, J. Ye, W. Zhang, F. Sheu, Modification of carbon nanotubes with redox hydrogel: improvement of amperometric sensing sensitivity for redox enzymes, Biosens. Bioelectron. 24 (2009) 1723–1729.
[10] P. Santhosh, K.M. Manesh, S. Uthayakumar, S. Komathi, A.I. Gopalan, K.P. Lee, Fabrication of enzymatic glucose biosensor based on palladium nanoparticles dispersed onto poly(3,4-ethylenedioxythiophene) nanofibers, Bioelectrochemistry 75 (2009) 61–66.
[11] K. Sirkar, A. Revzin, M.V. Pishko, Glucose and lactate biosensors based on redox polymer/oxidoreductase nanocomposite thin films, Anal. Chem. 72 (2000) 2930–2936.
[12] T.J. Ohara, R. Rajagopalan, A. Heller, Glucose electrodes based on cross-linked [Os $(bpy)_2Cl]^{+/2+}$complexed poly(1-vinylimidazole) films, Anal. Chem. 65 (1993) 3512–3517.
[13] P. Asuri, S.S. Bale, R.C. Pangule, D.A. Shah, R.S. Kane, J.S. Dordick, Structure, function, and stability of enzymes covalently attached to single-walled carbon nanotubes, Langmuir 23 (2007) 12318–12321.
[14] Q. Gao, X. Cui, F. Yang, Y. Ma, X. Yang, Preparation of poly(thionine) modified screen-printed carbon electrode and its application to determine NADH in flow injection analysis system, Biosens. Bioelectron. 19 (2003) 277–282.
[15] Z. Gao, G. Binyamin, H.H. Kim, S.C. Barton, Y. Zhang, A. Heller, Electrodeposition of redox polymers and co-electrodeposition of enzymes by coordinative cross-linking, Angew. Chem. Int. Ed. 41 (2002) 810–813.
[16] W. Schumann, T.J. Ohara, H.L. Schmidt, A. Heller, Electron transfer between glucose oxidase and electrodes via redox mediators bound with flexible chains to the enzyme surface, J. Am. Chem. Soc. 113 (1991) 1394–1397.
[17] A. Aoki, A. Heller, Electron diffusion coefficients in hydrogels formed of cross-linked redox polymers, J. Phys. Chem. 97 (1993) 11014–11019.
[18] T.W. Tsai, G. Heckert, L.F. Neves, Y. Tan, D.Y. Kao, R.G. Harrison, D.E. Resasco, D.W. Schmidtke, Adsorption of glucose oxidase onto single-walled carbon nanotubes and its application in layer-by-layer biosensors, Anal. Chem. 81 (2009) 7917–7925.
[19] D. Pan, J. Chen, S. Yao, W. Tao, L. Nie, An amperometric glucose biosensor based on glucose oxidase immobilized in electro polymerized poly (o-aminophenol) and CNT composite film on a gold electrode, Anal. Sci. 21 (2005) 367–371.
[20] C. Deng, J. Chen, Z. Nie, S. Si, A sensitive and stable biosensor based on the direct electrochemistry of glucose oxidase assembled layer-by-layer at the multiwall carbon nanotube-modified electrode, Biosens. Bioelectron. 26 (2010) 213–219.
[21] N. Sato, H. Okuma, Development of single-wall carbon nanotubes modified screen-printed electrode using a ferrocene-modified cationic surfactant for amperometric glucose biosensor applications, Sens. Actuators B 129 (2008) 188–194.
[22] R. Haddad, M. Holzinger, A. Maaref, S. Cosnier, Pyrene functionalized single-walled carbon nanotubes as precursors for high performance biosensors, Electrochimica Acta 55 (2010) 7800–7803.
[23] A.P. Periasamy, Y.J. Chang, S.M. Chen, Amperometric glucose sensor based on glucose oxidase immobilized on gelatin-multiwalled carbon nanotube modified glassy carbon electrode, Bioelectrochemistry 80 (2011) 114–120.
[24] A. Salimi, R.G. Compton, R. Hallaj, Glucose biosensor prepared by glucose oxidase encapsulated sol–gel and carbon-nanotube-modified basal plane pyrolytic graphite electrode, Anal. Biochem. 333 (2004) 49–56.
[25] A.A. Karyakin, E.A. Kotelnikov, L.V. Lukachov, E.E. Karyakin, J. Wang, Optimal environment for glucose oxidase in perfluorosulfonated ionomer membranes: improvement of first-generation biosensors, Anal. Chem. 74 (2002) 1597–1603.



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