Glucose Biosensor Based on a Glassy Carbon Electrode Modified with Polythionine and Multiwalled Carbon Nanotubes

Wenwei Tang¹*, Lei Li², Lujun Wu³, Jiemin Gong³, Xinping Zeng²*

¹ Department of Chemistry, Tongji University, Shanghai, China, ²School of Life Science and Technology, Tongji University, Shanghai, China

Abstract

A novel glucose biosensor was fabricated. The first layer of the biosensor was polythionine, which was formed by the electrochemical polymerization of the thionine monomer on a glassy carbon electrode. The remaining layers were coated with chitosan-MWCNTs, GOx, and the chitosan-PTFE film in sequence. The MWCNTs embedded in FAD were like “conductive wires” connecting FAD with electrode, reduced the distance between them and were propitious to fast direct electron transfer. Combining with good electrical conductivity of PTH and MWCNTs, the current response was enlarged. The sensor was a parallel multi-component reaction system (PMRS) and excellent electrocatalytic performance for glucose could be obtained without a mediator. The glucose sensor had a working voltage of −0.42 V, an optimum working temperature of 25°C, an optimum working pH of 7.0, and the best percentage of polytetrafluoroethylene emulsion (PTFE) in the outer composite film was 2%. Under the optimised conditions, the biosensor displayed a high sensitivity of 2.80 μM mM⁻¹ cm⁻² and a low detection limit of 5 μM (S/N = 3), with a response time of less than 15 s and a linear range of 0.04 mM to 2.5 mM. Furthermore, the fabricated biosensor had a good selectivity, reproducibility, and long-term stability, indicating that the novel CTS+PTFE/GOx/MWCNTs/PTH composite is a promising material for immobilization of biomolecules and fabrication of third generation biosensors.

Introduction

Glucose has always been an analyte that has received much attention because of its vital effect in clinical and environmental analysis. Since the first concept of an enzyme-based biosensor was proposed by Clark and Lyons [1], tremendous interest has been focused on the fabrication of glucose enzyme biosensors. The enzyme biosensor has experienced three stages in previous years. Due to its better selectivity and stability, the third-generation biosensor based on the direct electrochemistry of glucose oxidase has been widely developed [2–4].

However, for the flavin adenine dinucleotide (FAD) that is the active centre of glucose oxidase (GOx) and is deeply embedded in the molecular cavity [5], it is generally difficult to establish direct electron transfer (DET) between the active centre of GOx and conventional electrodes. To enhance this communication, nanomaterials have been extensively used in biosensors to achieve DET. Various nanomaterials composed of carbon [6,7] metal oxides [8,9] and conducting polymers [10,11] have demonstrated the capabilities of DET. However, significant challenges remain in the fabrication of practicable biosensors that show superior bioelectrocatalysis, precise specificity, and high stability.

Multiwalled carbon nanotubes (MWCNTs) have unique electronic, mechanical, and thermal properties, which leads to their potential application in the fabrication of biosensors. Many recent reports have demonstrated that the integration of MWCNTs and conducting polymers [12,13] or metal nanoparticles [14,15] could greatly improve the direct electrochemistry of GOx.

Recently, conducting polymers have been extensively employed for the construction of electrochemical biosensors due to their conductive nature and superior stability [16]. Thionine (TH) is a phenoxyazine dye (CH₃COOC₂H₅N₃S) with two amino groups symmetrically distributed on each side of its aromatic rings [17]. Under a suitable voltage, the aromatic rings of TH can be linked together via –NH– bridges to form polythionine (PTH) [18]. The PTH has good conductivity and exhibits a fast charge transfer capacity, showing great potential for various future applications [19,20].

In this study, we investigated the step-wise immobilizations of PTH, MWCNTs, GOx, and chitosan-PTFE composite films onto a glassy carbon electrode to build a biosensor for measuring glucose. A mediator-free GOx-based glucose biosensor was constructed though a layer-by-layer self-assembly approach. Electrochemical impedance spectroscopy was used to monitor the biosensor modification process. DET between the active site of the GOx and the electrode was achieved, and its behaviour as a biosensor and its enzyme stability were investigated by electrochemical techniques.
Experimental

Reagents

Glucose oxidase (GOx, EC 1.1.3.4, 100 U/mg, from Aspergillus niger) and bovine serum albumin (BSA) were purchased from MP Biomedicals Co., Ltd. (Shanghai). The multiwalled carbon nanotubes (MWCNTs, >95% purity) were obtained from the Chengdu Organic Chemicals Co., Ltd. of the Chinese Academy of Science (Chengdu, China). Uric acid (UA) was purchased from Sigma. D-glucose, chitosan, glacial acetic acid, glutaraldehyde, vitamin C, L-cysteine (L-cys), and the polytetrafluoroethylene emulsion (60% PTFE) were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd (SCRC, China).

Apparatus and measurements

Amperometric experiments and cyclic voltammetric experiments were performed on an Autolab AUT72230 electrochemical work station (Metrohm China Ltd.). A conventional three-electrode system was applied: the modified or unmodified GCE electrode (3 mm diameter) acted as the working electrode, and a saturated calomel electrode (SCE) and a platinum wire served as the reference and counter electrodes, respectively. All of the electrochemical experiments were carried out at room temperature.

Scanning electron microscopy (SEM) images were taken using a Tescan 5236 SEM (Tescan China Ltd.).

Electrode modification

The glassy carbon electrode (GCE, Φ = 3 mm) was polished with 1.0 and 0.05 μm α-Al2O3 slurries successively and was then rinsed with water, acetone, absolute ethanol, and doubly-distilled water. According to the method described by Wang [21], the PTH/GCE was built and covered with a golden PTH film after 50 cycles of the electrodeposition of thionine (the potential range was between +0.1 and −0.55 V at 50 mV s⁻¹).

Two mg MWCNTs was dispersed by ultrasonication in 1 mL 0.5% chitosan (CTS) solution. One mg GOx and 7.5 mg bovine serum albumin (BSA) were added to 100 μL 0.1 M PBS to prepare a 10 mg mL⁻¹ GOx aqueous solution.

Preparation of the outer chitosan-PTFE composite film: the 60% (w/w) PTFE solution was dispersed by ultrasonication in a 0.5% chitosan solution, which was finally diluted to different ratios of 1%, 2%, 5%, and 10% (w/w) chitosan/PTFE suspension (CTS+PTFE).

A CTS+PTFE/GOx/MWCNTs/PTH/GCE coating was prepared facilely by successively casting 6 μL 2 mg mL⁻¹ MWCNTs, 6 μL 10 mg mL⁻¹ GOx aqueous solution (using glutaraldehyde crosslinking as the fixation method), and 5 μL 2% (w/w) chitosan/PTFE suspension onto the pre-treated PTH/GCE. Each casting
was performed after the previous cast had been air-dried. Finally, the modified GCE was immersed in PBS with a pH = 7.0 to remove the loosely absorbed GOx and was stored at 4°C under dry conditions.

For comparison, MWCNTs/GCE, PTH/GCE, MWCNTs/PTH/GCE, and GOx/MWCNTs/GCE coatings were also prepared according to the same procedure. The assembly process of CTS+PTFE/GOx/MWCNTs/PTH is shown in Figure 1.

Results and Discussion

SEM characterisations of MWCNTs and electropolymerisation of thionine

SEM characterisation of the MWCNTs. Figure 2 shows the SEM image of the MWCNTs deposited onto the glassy carbon surface. The disordered filamentous carbon nanotubes, with tube diameters of tens of nanometres, built a porous and three-dimensional structure, which can provide a conductive, porous, and biocompatible microenvironment for enzyme immobilization.

Electropolymerisation of thionine. During the process of thionine electropolymerisation (Figure 3), the currents of a pair of reversible redox peaks increased gradually with increasing scan numbers, which can be attributed to the redox reaction of the PTH at the electrode. The anodic peak was located at ~0.22 V and the cathodic peak was at ~0.26 V after 50 scans. The results are very similar to Wang’s study [21], and the electrode is also covered by a firm golden film after electropolymerisation. In addition, a derived redox peak was located at ~0.07 V, which may be due to the differences in the raw materials.

Electrochemical behaviour of CTS+PTFE/GOx/MWCNTs/PTH/GCE

EIS can give more detailed information on the impedance changes during the modification process. When presented in a Nyquist plot, the impedance spectrum includes a semi-circular portion corresponding to the electron-transfer-limited process and a linear part resulting from the diffusion process [22]. The diameter of the semicircle corresponds to the electron transfer resistance (RET) of the redox probe at the electrode interface. A smaller RET value implies that the probe has a higher interfacial electron transfer rate.

Compared with bare GCE (Figure 4), the diameter of the semicircle greatly and successively decreased after the modification with MWCNTs/GCE, PTH/GCE, and MWCNTs/PTH/GCE, which indicated that both the MWCNTs and the PTH are good conductive materials, with the latter showing better conductive performance. The combination of MWCNTs and PTH on the GCE (MWCNTs/PTH/GCE) can greatly reduce the electrode resistance and promote a higher electron transfer rate between the redox probe and the electrode surface. However, an increase in
the electron transfer resistance was observed after CTS+PTFE/GOx was immobilised on the probe, suggesting a decrease in the conductivity of CTS+PTFE/GOx/MWCNTs/PTH/GCE due to the immobilization of non-conductive PTFE and biomacromolecules. The results of the impedance change during the electrode modification can provide evidence for the successful immobilization of MWCNTs, PTH, GOx, and other materials on the electrodes.

Figure 5 (A) shows that for the GCE, there was no obvious redox peaks; two pairs of distinct redox peaks (−0.2 V and −0.07 V, respectively) were observed for PTH/GCE, which were confirmed as the characteristic peaks of PTH according to its electropolymerisation. For GOx/MWCNTs/GCE, there was a pair of standard redox peaks, whose reduction and oxidation peak potentials were −0.443 V and −0.471 V, respectively. These peak potentials were very close to the standard electrode potential of GOx, which is derived from the FAD/FADH2 GOx redox cofactor [23,24]. The results also proved that the MWCNTs can directly promote electron transfer between GOx and the electrode. At −0.45 V, −0.27 V, and −0.13 V, three pairs of well-defined and nearly symmetric redox peaks were observed in GOx/MWCNTs/PTH. This result indicated that both GOx and PTH maintained good redox activities and that the modification layers provided a proper “micro room” for GOx. Both of the redox peak potentials were negatively shifted, which may be due to the parallel multi-component reaction system (PMRS). On the one hand, the redox species on the biosensor kept their separate redox characteristics; on the other hand, they were interdependent in the modification layers as a whole. Due to the high-impedance of GOx and PTFE, the peak current value of PMRS declined.

Figure 6. CVs of the CTS+PTFE/GOx/MWCNTs/PTH/GCE electrode in 0.1 M air-saturated PBS, at scan rates of 10, 20, 50, 100, 150, 200, and 300 mV s⁻¹ (A); The value of the enzyme peak current as it changes with the scanning speed (B); The value of the PTH peak current as it changes with the scanning speed (C).

doi:10.1371/journal.pone.0095030.g006
compared with PTH/GCE. The results are consistent with the previous electrochemical impedance spectral study.

From Figure 5 (B), it can be observed that the ratio of the GOx oxidation and reduction peaks is close to 1 (ipa1/ipc1 < 1) and ΔEp1 = 30 mV; however, that ratio for PTH is slightly greater than 1 (ipa2/ipc2 > 1) and ΔEp2 = 20 mV. These results further demonstrated the good reversibility of PMRS.

Both the anodic and cathodic peak currents of GOx and PTH increased linearly with an increase in the scan rate from 10 to 300 mV s⁻¹ (Figure 6 (A), (B), and (C)), which is characteristic of a surface-controlled quasi-reversible electrochemical process [25]. These results suggested that GOx and PTH were immobilised as adsorbed reactants on the electrode surface.

Biocatalytic activity of GOx on the biosensor

The electrocatalytic activity of GOx for oxygen was convincing proof of the biocatalytic activity of the GOx immobilised on the electrode surface [26]. From Figure 7, three pairs of redox peaks were observed in both N₂-saturated and air-saturated PBS solutions. Compared to the peak currents of both GOx and PTH in the corresponding N₂-saturated solution, an increase in the cathodic peak current and decrease in anodic peak current were observed in the air-saturated solution. This response can be explained as follows:

Electrode : GOx(FADH₂) → GOx(FAD) + 2e⁻ + 2H⁺  (1)

In N₂-saturated PBS, DET occurred between the active site of the enzyme and the stable bioelectrode.

Enzyme layers : GOx(FADH₂) + O₂ → GOx(FAD) + H₂O₂  (2)

When in an air-saturated solution, the electrochemically formed GOx (FADH₂) catalysed the reduction of oxygen dissolved in the solution. The relative consumption of GOx (FADH₂) and generation of GOx (FAD) directly led to the changes in the peak currents of GOx. Because GOx and PTH were both in the PMRS of the electrode, the peak currents of PTH had the same tendency to change, which can be used as an indicator of the change in GOx in turn.

Glucose Sensing Performance of CTS+PTFE/GOx/MWCNTs/PTH/GCE

In Figure 8 (A), three pairs of redox peaks (GOx located at −0.45 V and PTH located at −0.3 V) were observed in the PBS without glucose. With increases in the glucose concentration, both the anodic and cathodic peak currents decreased, which can be explained by the following reactions:

Enzyme layers : Glucose + GOx(FAD) → gluconic acid + GOx(FADH₂)  (3)

Electrode : GOx(FAD) + 2e⁻ + 2H⁺ → GOx(FADH₂)  (4)

The equation indicates that GOx (FAD) is directly involved in the glucose oxidation reaction. As the glucose concentration
increases, GOx (FAD) is competitively employed by glucose and increasingly converted into GOx (FADH₂), leading to a continuous decrease in the cathodic and corresponding anodic peak currents of both GOx and PTH.

Figure 8 (B) depicts the plot of the cathodic peak currents of PTH vs. the glucose concentrations. The cathodic current decreased obviously with an increasing glucose concentration up to 10 mM. When the glucose concentration continued to increase, the current decreased slowly and finally flattened out. This result arose from GOx consumption and is similar to the research results of Si Peng [14], which suggested that the GOx on the surface of such an electrode is able to simultaneously demonstrate DET with the electrode and to retain its catalytic activity towards glucose. The sensing mechanism in our system is different from the first and second generation glucose sensors.

Figure 9 (A) shows that each injection of 0.25 mM glucose resulted in a decrease of 50 nA in the cathodic current for the first 10 additions, which gave rise to a sensitivity of 2.80 μA mM⁻¹ cm⁻². In addition, the current decreased steeply and then reached 95% of the steady-state current in less than 15 s. The current verses time plot showed a well-defined behaviour typical of an enzymatic reaction with a linear range up to 2.5 mM, and a current plateau was observed when the glucose concentration was higher than 2.5 mM, which suggests typical enzymatic reaction kinetics [28,29].

The calibration curve (current vs. glucose concentration) and the Lineweaver Burk plot (current⁻¹ vs. concentration⁻¹) were obtained from the amperometric response (Figure 9 (B)). The linear regression equation of \( I (\mu A) = 0.196 (\mu A \text{ mM}^{-1}) \text{C (mM)} - 0.01 (\mu A) \text{ (R = 0.997)} \) was derived from the calibration curve and revealed that the glucose sensor has a detection limit as low as 5.0 μM (S/N = 3).

The apparent Michaelis-Menten constant \( K_M \) was calculated using the electrochemical version of the Lineweaver Burk
The percentage of PTFE in the outer composite film was 2%. In addition, the best voltage of 0.42 V, an optimum working temperature of 25°C, and an optimum working pH of 7.0. In Figure 9 (B), which is much lower than the values of 8.5 mM for GOx immobilised on literature angle nanotubes [30], 13.9 mM for GOx immobilised on silica/multi-walled carbon nanotube/polyacrylonitrile nanocomposite layer redox polymers [31], and 14.6 mM for GOx immobilised on gold nanoparticles [32]. The smaller KM indicates that this biosensor has a superior enzymatic activity and a higher affinity for the glucose substrate [33].

Optimization conditions for the glucose detection system

As shown in Figure 10, the sequence of the absolute differences between the starting and ending current values was $I_{\text{start}}-I_{\text{end}}<I_{\text{start}}-I_{\text{bstart}}=I_{\text{bend}},$ which was attributed to the combination of differently modified materials. For the glucose detection range, the order size was Step C>Step B>Step A, which showed that the biosensor using the CTS+PTFE/GOx/MWCNTs/PTH/GCE composite had a better amperometric response. The reason was that the CTS+PTFE film had good permeability and could make glucose molecules diffuse into the electrode surface smoothly and orderly.

We also did a series of research experiments to optimise the working conditions of the glucose sensor (the related figures and data are not shown). The biosensor has an optimum working voltage of $-0.42 \text{ V}$, an optimum working temperature of 25°C, and an optimum working pH of 7.0. In addition, the best percentage of PTFE in the outer composite film was 2%.

Selectivity, reproducibility, and stability of the glucose biosensor

To evaluate the selectivity of the biosensor, many interfering biomolecules, including uric acid (UA), ascorbic acid (AA), and L-cysteine (L-cys), that normally co-exist with glucose in real samples (human blood) were investigated (Figure 11). The current generated from the interfering species, such as 1.0 mM of UA, AA, and L-cysteine, was negligible, indicating that the biosensor had a strong anti-interference ability. This strong anti-interference ability can mainly be attributed to the low working voltage and reduction method we adopted in this study, which can effectively avoid the oxidation of the interfering substances at high voltages.

We put three sensors in PBS solution, which had a glucose concentration of 1.0 mM, to perform current tests. Then, the sensors were saved in the refrigerator at 4°C to perform stability tests. As Figure 12 shows, the current response had no obvious changes at the beginning after 2 days. After 3 weeks, the current responses decreased to 89.6%, 86.7%, and 91.7% of the initial response. In Figure 12, the relative standard deviation (RSD) was small and the reproducibility was good. The biosensor displayed an acceptable stability, which can be attributed to the following two properties: the immobilization of the MWCNTs and the thionine electropolymerisation on the electrode surface were very stable, which can provide a friendly environment for GOx and maintain its bioactivity; and the CTS+PTFE composite can effectively prevent enzyme leakage and prolong the life of the biosensor.

The mechanism of DET of the sensor

In Figure 13, the active center of GOx (FAD) is buried deeply, only a few GOx can occur DET (DET-GOx), and the other part is just loaded up without DET-GOx (WDET-GOx). Both of them have biological catalysis activity, and can catch glucose and oxide them.

The current response of CTS+PTFE/GOx/MWCNTs/PTH is from the competitive reaction of glucose. Glucose competively spreads to DET-GOx and reacts with it, thereby the DET-GOx is reduced, and thus the response current is decreased. DET-GOx influences detection performance. The MWCNTs embedded in FAD are like “conductive wires” connecting FAD with electrode, reduce the distance between them and are propitious to DET. Combining with good electrical conductivity of PTH and MWCNTs, the current response is enlarged. According to response current, glucose concentration can be detected.

Conclusions

In this work, we successfully introduced a promising glucose biosensor based on the immobilization of GOx onto a MWCNTs/PTH nanocomposite film. The evaluation of the CTS+PTFE/GOx/MWCNTs/PTH/GCE demonstrated that DET of GOx...
was achieved on this nanocomposite film. Cyclic voltammetry showed three pairs of well-defined redox peaks corresponding to DET of GOx (FAD/FADH₂) and PTH. The parallel multicomponent reaction system (PMRS) of the electrode played an important role in facilitating the electron transfer between GOx and the electrode surface. To the best of our knowledge, this is the first report about the direct electrochemistry of GOx based on PMRS. Furthermore, the potential use of the resulting electrode as a third generation glucose biosensor was confirmed by the amperometric response for successive additions of glucose. The biosensor showed a high sensitivity, fast amperometric response and low detection limit. Possible interfering species in blood, such as uric acid, ascorbic acid, and L-cysteine, did not influence the current response. These results demonstrate that the CTS+PTFE/GOx/MWCNTs/PTH is an attractive material for the fabrication of efficient amperometric biosensors. The method presented can be used for the immobilization and evaluation of DET of other enzymes or proteins.

Acknowledgments

We are grateful to Xingzhi Su, Min Zhang for technical advice. We thank YanFei Li for his valuable comments on the manuscript. We acknowledge Central Laboratory of Department of Chemistry at Tongji University for organizing test works.

Author Contributions

Conceived and designed the experiments: WWT LL XPZ. Performed the experiments: WWT LL XPZ. Analyzed the data: WWT LL JMW XPZ. Contributed reagents/materials/analysis tools: WWT XPZ. Wrote the paper: XPZ LL JMW.

References

1. Clark LC, Lyons C (2006) Electrode systems for continuous monitoring in cardiovascular surgery. Ann N Y Acad Sci 102: 28–45.
2. Wu X, Zhao F, Varcoe JR, Thummer AE, Avignone-Rossa C, et al. (2009) Direct electron transfer of glucose oxidase immobilized in an ion liquid reconstituted cellulose-carbon nanotube matrix. Bioelectrochemistry 77: 64–68.
3. Guo CX, Li CM (2010) Direct Electron Transfer of Glucose Oxidase and Biosensing of Glucose on Hollow Sponge-Nanostructured Conducting Polymer/Metal Oxide Composite. Phys Chem Chem Phys 12: 12153–12159.
4. Wang YL, Liu L, Li MG, Gao F (2011) Multifunctional carbon nanotubes for direct electrochemistry of glucose oxidase and glucose bioassay. Biosens Bioelectron 30: 107–111.
5. Wilson R, Turner APF (1992) Glucose-oxidase—an ideal enzyme. Biosens Bioelectron 7: 163–165.
6. Wu S, Ju BX, Liu Y (2007) Conductive Mesocellular Silica Carbon Nanocomposite Foams for Immobilization, Direct Electrochemistry, and Biosensing of Proteins. Adv Funct Mater 17: 565–592.
7. Gooding JJ, Wikelwo R, Liu JQ, Yang WR, Loos D, et al. (2003) Protein Electrochemistry Using Aligned Carbon Nanotube Arrays. J Am Chem Soc 125: 9006–9007.
8. Peng HF, Liang RP, Qiu JD (2011) Facile Synthesis of Fe₃O₄@Al₂O₃ Core-Shell Nanoparticles and their application to the highly specific capture of heme proteins for Direct Electrochemistry. Biosens Bioelectron 26: 3005–3011.
9. Zhu ZH, Qu LN, Niu QJ, Zeng Y, Sun W, et al. (2011) Urichinlike MnO₂ Nanoparticles for the Direct Electrochemistry of Hemoglobin with Carbon Ionic Liquid Electrolyte. Biosens Bioelectron 26: 2119–2124.
10. Wang ZY, Liu SN, Wu P, Cai CX (2009) Detection of Glucose Based on Direct Electron Transfer of Glucose Oxidase Immobilized on Highly Ordered Polyacrylamide Nanotubes. Anal Chem 81: 1638–1645.
11. Guo CX, Li CM (2010) Direct Electron Transfer of Glucose Oxidase and Biosensing of Glucose on Hollow Sponge-Nanostructured Conducting Polymer/Metal Oxide Composite. Phys Chem Chem Phys 12: 12153–12159.
12. Wu NE, Zhao F, Varcoe JR, Thummer AE, Avignone-Rossa C, et al. (2009) Direct electron transfer of glucose oxidase immobilized in an ion liquid. Bioelectrochemistry 77: 64–68.
13. Deng CY, Chen JH, Nie Z, Si SH (2010) A sensitive and stable biosensor based on the direct electrochemistry of glucose oxidase assembled layer-by-layer at the multilayer carbon nanotube-modified electrode. Biosensors and Bioelectronics 26: 2119–2124.
14. Si P, Ding SJ, Yuan J, Lou XW, Kim DH (2011) Hierarchically structured one-dimensional TiO₂ for protein immobilization, direct electrochemistry, and mediator-free glucose sensing. ACS Nano 5: 7617–7628.
15. Wang Y, Yuan R, Chai QY, Li WJ, Zhao Y, et al. (2011) Direct electron transfer: Electrochemical glucose biosensor based on hollow Pt nanosphere functionalized multilayer carbon nanotubes. Journal of Molecular Catalysis B: Enzymatic 71: 146–151.
16. Shirat MD, Tso CO, Wallace GG (2008) Amperometric glucose biosensor on layer by layer assembled carbon nanotube and polypyrrole multilayer film. Electroanalysis 20: 150–156.
17. Li QW, Zhang J, Yan H, He MS, Liu ZF (2004) Thiourea-mediated chemistry of carbon nanotubes. Carbon 42: 287–291.
18. Baothrae JM, Archer MD (1983) Dye-modified electrodes for photogalvanic cells. Electrochem Acta 20: 1513–1522.
19. Zhang KY, Zhang L, Xu JR, Wang C, Geng T, et al. (2010) A sensitive amperometric hydrogen peroxide sensor based on thionin/EDTA/carbon nanotubes—chitosan composite film modified electrode. Microchem Acta 171: 139–145.
20. Liu Y, Zhang HL, Lai GS, Yu AM, Huang YM, et al. (2010) Amperometric NADH biosensor based on magnetic chitosan microspheres/poly(thionine) modified glassy carbon electrode. Electroanalysis 22: 1725–1732.
21. Wang QX, Zhang HL, Wu YW, Yu AM (2012) Amperometric hydrogen peroxide biosensor based on a glassy carbon electrode modified with polythionine and gold nanoparticles. Microchem Acta. 176: 279–285.
22. Bard AJ, Faulkner LR (2001) Electrochemical methods: Fundamentals and applications, 2nd edn. John Wiley&Sons, New York.
23. Liu G, Paddon MN, Row JJ (2007) A molecular wire modified glassy carbon electrode for achieving direct electron transfer to native glucose oxidase. Electrochem Commun 9: 2218–2223.
24. You CP, Li X, Zhang S, Kong JH, Zhao DY, et al. (2009) Electrochemistry and biosensing of glucose oxidase immobilized on PED-accessed mesoporous carbon. Microchem Acta 167: 109–116.
25. Guo RF, Zheng JB (2009) Amine-terminated ionic liquid functionalized carbon nanotube-gold nanoparticles for investigating the direct electron transfer of glucose oxidase. Electrochim Commun 11: 608–611.
26. Zhang HF, Meng ZC, Wang Q, Zheng JB (2011) A novel glucose biosensor based on direct electrochemistry of glucose oxidase incorporated in bionanodized gold nanoparticles-carboxylate composite film. Sensor Actuat B-Chem 156: 23–27.
27. Murthy ANN, Sharma J (1998) Glucose oxidase bound to self-assembled monolayers of bis(4-pyridyl) disulfide at a gold electrode: Amperometric determination of glucose. Anal Chem Acta 363: 215–220.
28. Kamin RA, Wilson GS (1980) Rotating ring-disk enzyme electrode for biocatalysis kinetic studies and characterization of the immobilized enzyme layer. Anal Chem 52: 1198–1205.
29. Manesh KM, Kim HT, Santhosh P, Gopalani AL, Lee KP (2008) A novel glucose biosensor based on immobilization of glucose oxidase into multilayer carbon nanotubes-polylelectrolyte-brush magnetohydrodynamic membrane. Biosens Bioelectron 23: 771–779.