Talanta 77 (2008) 455–459

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

A novel amperometric biosensor based on NiO hollow nanospheres for biosensing glucose

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ARTICLE INFO

Article history:
Received 29 February 2008
Received in revised form 19 June 2008
Accepted 20 June 2008
Available online 10 July 2008

Keywords:
Glucose biosensor
NiO hollow nanospheres
Glucose oxidase
Chitosan

ABSTRACT

NiO hollow nanospheres were synthesized by controlled precipitation of metal ions with urea using carbon microspheres as templates, which were for the first time adopted to construct a novel amperometric glucose biosensor. Glucose oxidase was immobilized on the surface of hollow nanospheres through chitosan-assisted cross-linking technique. Due to the high specific active sites and high electrocatalytic activity of NiO hollow nanospheres, the constructed glucose biosensors exhibited a high sensitivity of 3.43 μA/mM. The low detection limit was estimated to be 47 μM (S/N = 3), and the Michaelis–Menten constant was found to be 7.76 mM, indicating the high affinity of enzyme on NiO hollow nanospheres to glucose. These results show that the NiO hollow nanospheres are a promising material to construct enzyme biosensors.

1. Introduction

Numerous efforts have been focused on the development of biosensors in recent years because of their applications in biological and chemical analysis, clinical detection, environmental monitoring, and food processing industries [1,2]. There is always a demand for highly sensitivity, stable and disposable biosensors in determining glucose in blood and urine for the diagnosis of diabetes. Enzyme immobilization is considered as an important factor in biosensor technologies. The search for novel materials to design electrochemical biosensors is of significant at present. Recently, nanostructures such as ZnO nanowires, ZrO_2 nanoparticles, carbon nanotubes, polyaniline nanofibers, and polypyrrole nanotubes have been widely investigated as alternative matrices for immobilization of enzyme due to their exceptional electrical, optical, biocompatible properties [3–6]. Besides, their large surface area can immobilize more enzyme molecules and provide direct electron transfer between the active sites of enzyme and electrode. Among the nanomaterials, NiO nanostructures have received considerable attention in recent years due to their broad applications such as catalyst, battery cathode, gas sensors, electrochromic films, and magnetic material [7–9]. However, the application of NiO nanostructures in biosensors is rare. As NiO is a biocompatible material with a high isoelectric point (IEP) of about 10.7, which make it suitable for adsorption of proteins with low IEPs by electrostatic interaction. Moreover, NiO nanostructures with high chemical stability, high electrocatalysis, and high electron transfer capability are promising for biosensor application. In this letter, a biosensor with high sensitivity based on NiO hollow nanospheres was fabricated. NiO hollow nanospheres were synthesized by a controlled precipitation of Ni^{2+} in the presence of carbon microspheres as templates. Due to the hollow structure of NiO nanomaterials, the immobilized glucose oxidase (GO_x) showed high loading and fast electron transfer rate. The resulting biosensor exhibited high sensitivity, low detection limit to glucose. All the results provided that NiO hollow nanospheres were good matrices for protein immobilization and biosensors preparation.

2. Experimental details

2.1. Reagents

The glucose oxidase (GO_x, E.C 1.1.3.4, *from Aspergillus niger*, 200 units mg^{-1}), β-D-(+)-glucose, and chitosan (CHIT) were obtained from Sigma–Aldrich. All the other chemicals were of analytical grade supplied by Shanghai Chemical Reagent (Shanghai, China) and were used without further purification. The phosphate

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0039-9140/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.talanta.2008.06.048

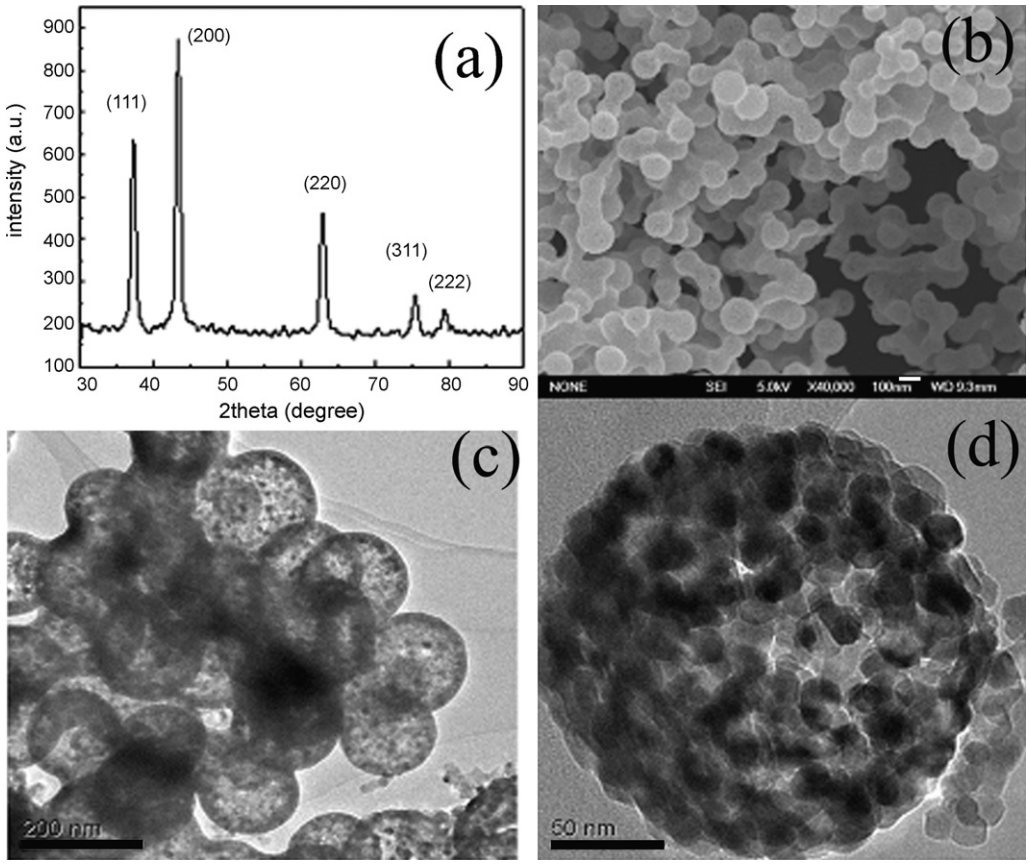

Fig. 1. (a) XRD pattern of as-grown NiO hollow nanospheres. (b) SEM image of NiO hollow nanospheres. (c) and (d) TEM images of a single hollow nanosphere.

buffer solutions (PBS, pH 6.98) were prepared from 0.04 M Na_2HPO_4 and 0.027 M KH_2PO_4. All the solutions were prepared with doubly distilled water, which was purified with a Milli-Q purification system.

2.2. Apparatus

The electrochemical experiments were carried out with a CHI 660A electrochemical workstation (Shanghai, China) with a conventional three-electrode cell. The modified or unmodified glassy carbon (GC) electrodes were used as the working electrode. The Pt wire and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively. All the solutions were purged with high purity nitrogen for at least 20 min prior to experiments and a nitrogen environment was then kept over the solution in the cell. The morphology and size of the synthesized NiO hollow nanospheres were characterized by scanning electron microscopy (SEM) [JSM-6700F] and transmission electron microscope [JEOL-3010F]. The operating voltage of SEM and TEM were 5 kV and 200 kV, respectively. The crystal structure of the sample was determined by X-ray diffraction (XRD) [D/max 2550 V, Cu Kα radiation].

2.3. Synthesis of NiO hollow nanospheres

The NiO hollow nanospheres were synthesized by controlled precipitation method and the as-prepared carbon microspheres were used as templates [10,11]. Briefly, the carbon microspheres were synthesized through the polycondensation reaction of glucose under hydrothermal condition. In a typical procedure, 8 g of glucose was dissolved in 40 ml of deionized water. The solution was then transferred to a 40 ml Teflon-lined autoclave and maintained at 180 °C for 6 h. Black products were obtained after centrifugation. 5 mmol of $Ni(NO_3)_2$ was dissolved in a mixture of 10 ml of deionized water and 80 ml of ethanol to form a clear solution. 0.5 g of as-prepared carbon microspheres was dispersed in the solution above with the aid of ultrasonication to give a suspension. 3 g of urea was then added to the suspension with vigorous stirring. Finally, the mixture was transferred into a round bottom flask, and kept at 60 °C for 2 days. The obtained products were subjected to four centrifugation/water and ethanol wash/redispersion cycles and then dried at 80 °C for 12 h in an oven, followed by calcinations at 500 °C in air atmosphere. Then an ashen product was collected.

2.4. Preparation of CHIT/GO_x/NiO/GC electrode

The GC electrode was polished by 0.3 and 0.05 μm aluminum slurries, and then was cleaned by dipping into 1:1 (v/v) aqueous solution of HNO_3, deionized water and ethanol with the assistance of ultrasonication prior to the experiment. The as-prepared NiO hollow nanospheres were dispersed in ethanol by ultrasonication for 1 h to give a 3 mg/ml suspension. Firstly, 8 μl of the mixture of NiO hollow nanospheres and ethanol was firstly cast on the surface of GC electrode and dried at room temperature. Secondly, 5 μl of GO_x solution (10 mg/ml, dissolved in 0.067 M PBS, pH 6.98) was dropped onto the surface of GC electrode modified by NiO nanospheres. Finally, 5 μl of 0.5 wt.% CHIT solution dissolved in acetic acid solution was dropped onto the surface of GO_x/NiO/GC electrode to avoid the leakage of the enzyme. The device was dried at 4 °C overnight in a refrigerator, followed by washing step to remove the unimmobilized GO_x. The CHIT/GO_x/NiO/GC electrode was stored in PBS and kept at 4 °C in a refrigerator when not in use.

3. Results and discussions

3.1. Characterization of NiO hollow nanoshperes

The XRD pattern of the as-prepared product is shown in Fig. 1(a), where all the diffraction peaks could be well assigned to cubic-phase of NiO (JCPDS file No. 01-1239) with a lattice parameter of 0.4195 nm. The absence of impurity peaks suggests the high purity of NiO hollow nanospheres. The crystal size of the NiO particles is estimated by Scherrer formula ($D = 0.9\lambda/(\beta \cos\theta)$) from the (200) crystal surface to be about 12 nm [12], which is in agreement with the TEM image (Fig. 1(c)). Fig. 1(b) is a typical SEM image of the NiO hollow nanospheres with diameters of about 150 nm. And it clearly shows that the product consists of interconnecting hollow nanospheres. TEM image (Fig. 1(c) and (d)) shows that the hollow nanospheres are composed of many NiO nanocrystals, which are interconnected with each other to form the NiO hollow nanospheres. The brighter region around the nanocrystals indicates a large amount of pores exist in the NiO hollow nanospheres, which is useful for the adsorption of proteins.

3.2. Electrochemical performance of the modified electrodes

The enzyme electrode was characterized by cyclic voltammetry between the potentials of −0.3 V and 0.5 V, as shown in Fig. 2(a). Curves a and b are the cyclic voltammetric responses of CHIT/GO_x/NiO/GC electrode in stirred 0.067 M (pH 6.98) PBS in the absence and presence of 1 mM hydroquinone. No electrochemical response is observed in the absence of hydroquinone (curve a). The results indicate that hydroquinone could be used as a diffusional electron-transferring mediator. In the present of hydroquinone, a pair of redox peaks with formal potential at −0.14 V and +0.35 V versus reference electrode was clearly observed, which can be assigned to two-electron reversible redox reaction of benzoquinone/hydroquinone. With the addition of 2 mM, 3 mM, 4 mM, 5 mM glucose into the buffer solution containing hydroquinone, a sharp current increase at potential above 0.35 V was observed. The electrocatalytic peak current increases with the increasing concentration of glucose in PBS. These results indicate that the immobilized GO_x on NiO nanoparticles retains its electrocatalytic activity for the oxidation of glucose. The typical enzyme-dependent catalytic process can be described as follows:

$$QH_2 - 2e \rightarrow Q \quad (1)$$

$$GO_x(FAD) + G \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} GO_x(FAD - G) \xrightarrow{k_2} GO_x(FADH_2) + GI \quad (2)$$

$$GO_x(FADH_2) + Q \xrightarrow{k_3} GO_x(FAD) + QH_2 \quad (3)$$

where GO_x (FAD) and GO_x (FADH2) represent oxidized and reduced forms of glucose oxidase, Q and QH_2 are the benzoquinone and hydroquinone, and G and GI are β-D-(+)-glucose and gluconO-D-lactone, respectively. As the addition of glucose, the cathodic current decreased because the reduced form of the enzyme restrained the reduction of the benzoquinone at the electrode. Simultaneously, hydroquinoe was regenerated and then reoxidized at the electrode, resulting in the increase of oxidation current. In the absence of mass transport limitations, the increase of the oxidation current (i_{cat}) can be expressed as

$$\frac{1}{i_{cat}} = \frac{1}{2Fk_3S\Gamma_E^0}\frac{1}{[Q]_0} + \frac{1}{2FS\Gamma_E^0}\left(\frac{1}{k_2} + \frac{1}{k_{red}[G]}\right),$$

where F is the Faraday's constant, S the electrode area, Γ_E^0 the surface concentration of enzyme, $[Q]_0$ the mediator concentration, G the glucose concentration in PBS, $k_{red} = k_1k_2/(k_{-1} + k_2)$

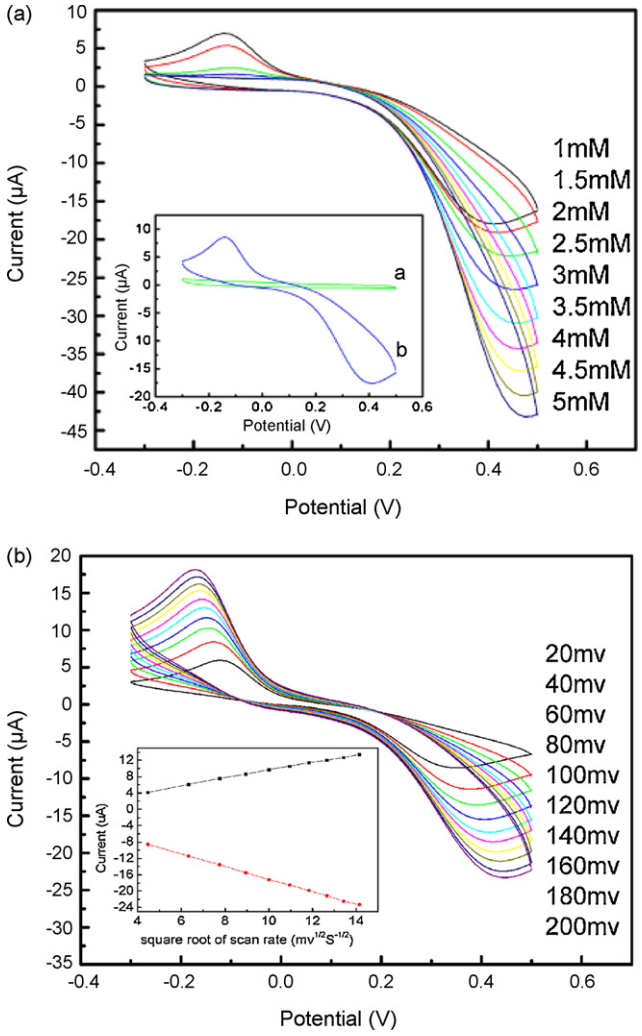

Fig. 2. (a) CVs of the electrode in the presence of glucose with different concentrations. Inset: CVs of the electrode in the presence (curve a) and absence (curve b) of hydroquinone. (b) CVs of the electrode in 0.067 M PBS (pH 6.98) containing 1 mM hydroquinone at different scan rate.

[13,14]. According to the equation, the surface concentration of GO_x immobilized on the electrode surface was estimated to 1.7×10^{-13} mol/cm^2, which is due to the large surface-to-volume ratio and high IEP of NiO hollow nanospheres.

Fig. 2(b) shows the cyclic voltammograms (CVs) of CHIT/GO_x/NiO/GC electrode in PBS (pH 6.98) at different scan rates. Peak-to-peak separation increases with increasing scan rate, suggesting a quasireversible behavior. Both the cathodic and anodic peaks currents are linearly proportional to the square root of scan rate in the range from 20 to 200 mV/s (Linear regression equations: Pa: $y = 0.09717 + 0.94924x$, $r = 0.999$; Pc: $y = -1.68145 - 1.53808x$, $r = -0.999$), which shows a typical diffusion-controlled electrochemical behavior.

Fig. 3(a) is a typical amperometric response of the glucose biosensor after addition of successive aliquots of 0.3 mM glucose in PBS (pH 6.98) at an applied potential of 0.35 V. It is found that the biosensor shows a rapid and sensitive response to the change of glucose concentration, which is ascribed to the good electrocatalytic property of CHIT/GO_x/NiO/GC electrode. Besides, the response time is around 8 s for the electrode to reach 95% steady-state current, indicating a fast electron exchange between GO_x and NiO. The corresponding calibration curve is shown in Fig. 3(b). The

Table 1
Glucose content determination in serum sample

Samples	Content determined by local hospital ($mmol^{-1}$)	Content determined by current method[a] ($mmol^{-1}$)	R.S.D. (%)	Relative error (%)
1	5.51	5.35 ± 0.06	2.3	−2.90
2	4.72	4.90 ± 0.05	2.8	+3.81
3	4.85	5.05 ± 0.1	3.5	+4.12
4	4.90	4.74 ± 0.06	2.5	−3.26
5	5.24	5.38 ± 0.08	3.2	+2.67

[a] Average of the three measurements.

linear detection range of NiO hollow nanospheres-based glucose biosensor is 1.5–7 mM. This kind of biosensor has a low detection limit of 47 μM (be estimated based on the signal-to-noise characteristics of these data (S/N = 3)) and a high sensitivity of 4.3 μA/mM. The high sensitivity of the enzyme electrode can be attributed to the excellent adsorption ability, high electrocatalytic activity and good biocompatibility of the NiO hollow nanospheres. The Michaelis–Menten constant of the system (K_M^{app}) in this work can be calculated from the well-known Lineweaver–Burk equation and is found to be 7.76 mM, which gives an indication of the enzyme–substrate kinetics for the glucose biosensor. The value of K_M^{app} for GO_x in this work is much smaller than GO_x immobilized by sol–gel organic–inorganic hybrid material, 22 mM, GO_x immobilized by chitosan and gold nanoparticles, 10.5 mM, GO_x entrapped in polypyrrole films, 19 mM, and glucose oxidase in Nafion film, 14.91 mM [15–18]. The results implied that the GC electrode modified by NiO hollow nanospheres exhibits a higher affinity to glucose.

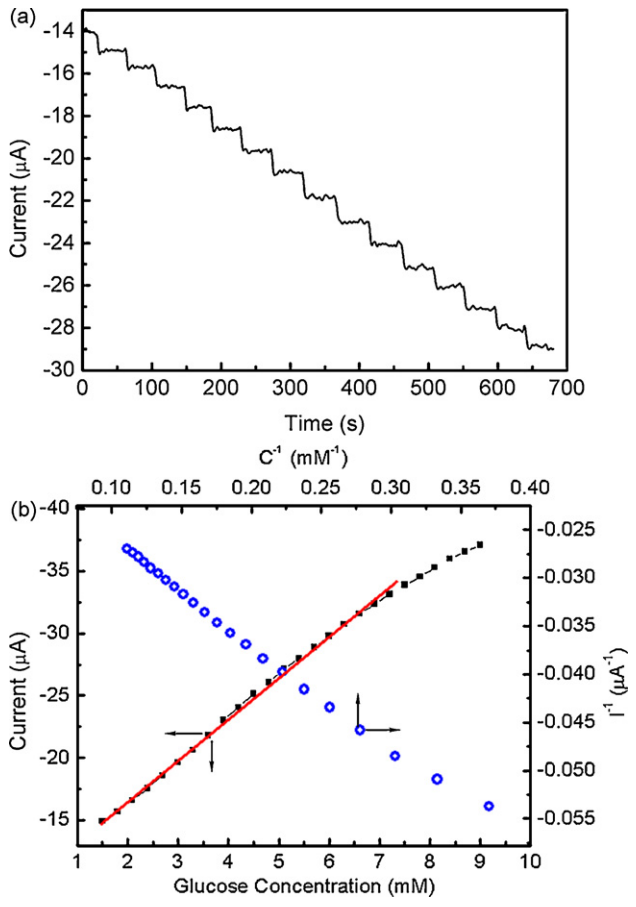

Fig. 3. (a) Amperometric responses of CHIT/GO_x/NiO/GC electrode with the successive addition of 0.3 mM glucose to the 0.067 M PBS (PH 6.98) under stirring. (b) The calibration curve of NiO/GO_x biosensor and the Lineweaver–Burk plot. The straight line is the linear fit to the calibration curve. A potential of +0.35 V (vs SCE) was applied on the working electrode in the measurement.

3.3. Repeatability and reproducibility

The reproducibility of response current of the GO_x enzyme electrode was investigated by comparing the response currents of 8 enzyme electrodes. The relative standard deviation (R.S.D.) was 5.3% at a glucose concentration of 0.2 mM. In order to evaluate the repeatability, an enzyme electrode was used to determine 1 mM glucose for 10 times continuously, and the R.S.D. of the response current was 3.5%.

3.4. Stability of the enzyme electrode

The CHIT/GO_x/NiO/GC electrode was stored in PBS (pH 6.98) at 4 °C when not in use. The stability of the biosensor was investigated using the same PBS containing 1 mM glucose. Every few days the cyclic voltammogram was recorded and using the same electrode. The peak current decreased with the increase of storage time. However, the electrode can still retain about 85% of the initial response after 20 days storage. The above results indicate that the CHIT/GO_x/NiO/GC electrode has a good stability.

3.5. Interference and real sample analysis

We also examined the interference of some electroactive compounds to the glucose response. The deviation of response was calculated according to $(I_i - I_g)/I_g$, where I_i and I_g were the steady-state current recorded for solutions containing glucose and interferent or glucose alone. The interference was investigated in the presence of physiological normal level with a glucose concentration of 5.6 mM. At CHIT/GO_x/NiO/GC electrode, the deviations caused by ascorbic acid (0.1 mM) and uric acid (0.5 mM) were 1.9% and 3.2%, respectively. This indicates that the biosensor suffers some influence from the coexistents in serum samples. However, the influence of ascorbic acid and uric acid on the glucose response was weak under the testing conditions. In order to validate the reliability of the biosensor, the determination of glucose in human serum samples was performed with the CHIT/GO_x/NiO/GC electrode. Fresh serum samples were first analyzed in the local hospital with ASCA AG-II Chemistry System (Landmark, USA). The samples were then reassayed with the CHIT/GO_x/NiO/GC electrode. For current method, a serum sample was added into the stirred 5 ml PBS (pH 6.98), and amperometric response was recorded at 0.35 V. The results were shown in Table 1. The glucose levels determined in this work were close to the values declared by local hospital, indicating that the fabricated glucose biosensor has practical potential.

4. Conclusions

We have fabricated an amperometric glucose biosensor by immobilizing glucose oxidase on the surface of NiO hollow nanospheres, which were synthesized by controlled precipitation

of metal ions with urea. The fabricated CHIT/GO_x/NiO/GC electrode exhibits high sensitivity and linear response in the range of 1.5–7 mM glucose concentration due to the large specific surface area and high electrocatalytic activity of NiO hollow nanospheres. The low value of Michaelis–Menten constant of 7.76 mM indicated high enzyme affinity of glucose oxidase. The results clearly suggest that NiO hollow nanospheres provide an attractive matrix for effective immobilization of biomolecules.

Acknowledgements

This work was partly supported from "973" National Key Basic Research Program of China (Grant No. 2007CB310500), Chinese Ministry of Education (Grant No. 705040), and National Natural Science Foundation of China (Grant No. 90606009).

References

[1] N.A. Rakow, K.S. Suslick, Nature (London) 406 (2000) 710.
[2] R.W. Keay, C.J. McNeil, Biosens. Bioelectron. 13 (1998) 963.
[3] A. Wei, X.W. Sun, J.X. Wang, Y. Lei, X.P. Cai, C.M. Li, Z.L. Dong, W. Huang, Appl. Phys. Lett. 89 (2006) 123902.
[4] Y. Liu, M. Wang, F. Zhao, Z. Xu, S. Dong, Biosens. Bioelectron. 21 (2005) 984.
[5] Y. Xian, Y. Hu, F. Liu, Y. Xian, H. Wang, L. Jin, Biosens. Bioelectron. 21 (2006) 1996.
[6] E.M.I. Mala Ekanayake, D.M.G. Preethichandra, Keiichi Kaneto, Biosens. Bioelectron. 23 (2007) 107.
[7] C.L. Carnes, K.J. Klabunde, J. Mol. Catal. A, Chem. 194 (2003) 227.
[8] V. Biju, M. Abdul Khadar, Mater. Sci. Eng.: A 304–306 (2001) 814.
[9] Y. Ichiyanagi, N. Wakabayashi, J. Yamazaki, S. Yamada, Y. Kimishima, E. Komatsu, H. Tajima, Physica B 329–333 (2003) 862.
[10] X.M. Sun, Y.D. Li, Angew. Chem. Int. Ed. 43 (2004) 597.
[11] H.S. Qian, G.F. Lin, Y.X. Zhang, Poernomo Gunawan, R. Xu, Nanotechnology 18 (2007) 355602.
[12] Hyo-Jin Ahn, Hyun-Chul Choi, Kyung-Won Park, Seung-Bin Kim, Yung-Eun Sung, J. Phys. Chem. B 108 (2004) 9815.
[13] C. Bourdillon, C. Demaille, J. Gueris, J. Moiroux, J.M. Saveant, J. Am. Chem. Soc. 115 (1993) 12264.
[14] S. Zhang, Y. Niu, C. Sun, Electrochim. Acta 49 (2004) 4777.
[15] B. Wang, B. Li, Q. Deng, S. Dong, Anal. Chem. 70 (1998) 3170.
[16] D.W.M. Arrigant, P.N. Bartlett, Biosens. Bioelectron. 13 (1998) 293.
[17] B.Y. Wu, S.H. Hou, F. Yin, J. Li, Z.X. Zhao, J.D. Huang, Q. Chen, Biosens. Bioelectron. 22 (2007) 838.
[18] K.I. Ozoemena, T. Nyokong, Electrochim. Acta 51 (2006) 5131.