Biosensors and Bioelectronics 26 (2011) 2287–2291

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Reductive determination of hydrogen peroxide with MWCNTs-Pd nanoparticles on a modified glassy carbon electrode

Jung-Min You, Yu Na Jeong, Mohammad Shamsuddin Ahmed, Seul Ki Kim, Hyun Chul Choi, Seungwon Jeon*

Department of Chemistry and Institute of Basic Science, Chonnam National University, Gwangju 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 6 July 2010
Received in revised form
15 September 2010
Accepted 28 September 2010
Available online 7 October 2010

Keywords:
Hydrogen peroxide
Biosensor
Multi walled carbon nanotubes
Palladium nanoparticles
Nafion®
Electrocatalysis

ABSTRACT

This paper introduces the use of multi walled carbon nanotubes (MWCNTs) with palladium (Pd) nanoparticles in the electrocatalytic reduction of hydrogen peroxide (H_2O_2). We have developed and characterized a biosensor for H_2O_2 based on Nafion® coated MWCNTs-Pd nanoparticles on a glassy carbon electrode (GCE). The Nafion®/MWCNTs-Pd/GCE electrode was easily prepared in a rapid and simple procedure, and its application improves sensitive determination of H_2O_2. Characterization of the MWCNTs-Pd nanoparticle film was performed with transmission electron microscopy (TEM), Raman, and X-ray photoelectron spectroscopy (XPS). Cyclic voltammetry (CV) and amperometry (at an applied potential of −0.2 V) measurements were used to study and optimize performance of the resulting peroxide biosensor. The proposed H_2O_2 biosensor exhibited a wide linear range from 1.0 μM to 10 mM and a low detection limit of 0.3 μM (S/N = 3), with a fast response time within 10 s. Therefore, this biosensor could be a good candidate for H_2O_2 analysis.

1. Introduction

Hydrogen peroxide (H_2O_2) plays an important analytical role in clinics, the dye, food, and pharmaceutical industries, and the environment (Liu and Ju, 2003; Lu et al., 2008; Shu et al., 2007; Cui et al., 2005; Luo et al., 2004). Conventional H_2O_2 determination methods such as titrimetry (Hurdis and Romeyn, 1954), chemiluminescence (Hanaoka et al., 2001), fluorescence (Zhang and Wong, 1999), and spectrophotometry (Matsubara et al., 1992) are costly, complex in operation, time consuming, and involved in various sources of interference. H_2O_2 has been reduced to water *via* a two-electron transfer by effective catalysts in aqueous solution; the reduction process of H_2O_2 is related to that of O_2 in physiological systems. Electrochemical methods have the advantages of easy preparation, fast detection, low consumption, and high selectivity and sensitivity (Karyakin, 2001). But the direct electrochemical reduction of H_2O_2 at ordinary solid electrodes is a slow electrode process that requires a large overpotential, which is the major barrier for determination of H_2O_2 by electrochemical methods. Direct reduction of H_2O_2 at several metal-modified (nanostructured) electrodes, including silver (He et al., 2010), gold (Liu et al., 2010), platinum (Hrapovic et al., 2004), palladium (Huang et al., 2008), and cobalt porphyrin (Jeong et al., 2010) have been investigated. Modification of the electrode surface has been performed to enhance the rate of electron transfer and minimize overpotential. Nevertheless, it is well known that carbon nanotubes (CNTs) are suitable materials for electrode modification and support in biosensor applications because of the high accessible surface area, low electrical resistance, extremely high mechanical strength and stiffness, outstanding charge-transport characteristics, high chemical stability, and excellent biocompatibility (Guldi et al., 2005; Daniel et al., 2007; Lin et al., 2004; Callegari et al., 2004; Karyakin et al., 1999). Meanwhile, many studies have used CNTs to measure many biomolecules such as glucose and H_2O_2 (Yu et al., 2003; Wang et al., 2003, 2008; Joshi et al., 2005; Xu et al., 2007; Takamura and Matsumoto, 2008). The enhanced catalytic performances are generally attributed to metal–CNTs interaction. This interaction induces a peculiar microstructure or modification of the electron density in the metal cluster and enhances catalytic activity. The adsorption of organic species is also favored by π–π interactions between the CNTs and aromatic rings, leading to favorable reactant–product mass transportation. It is known that nanomaterials possess good conductivity that increases surface area and improved electrocatalytic performance. Recently, this lab reported the preparation of PCoTAPPNW by electrochemical polymerization; the nanowires, with SWNT, were then used for modification of GCE. The PCoTAPPNW + SWNT/GCE electrode was employed for determination of H_2O_2 by electrocatalytic reduction. The PCo-

* Corresponding author. Tel.: +82 625303380; fax: +82 625303389.
E-mail address: swjeon3380@naver.com (S. Jeon).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.09.053

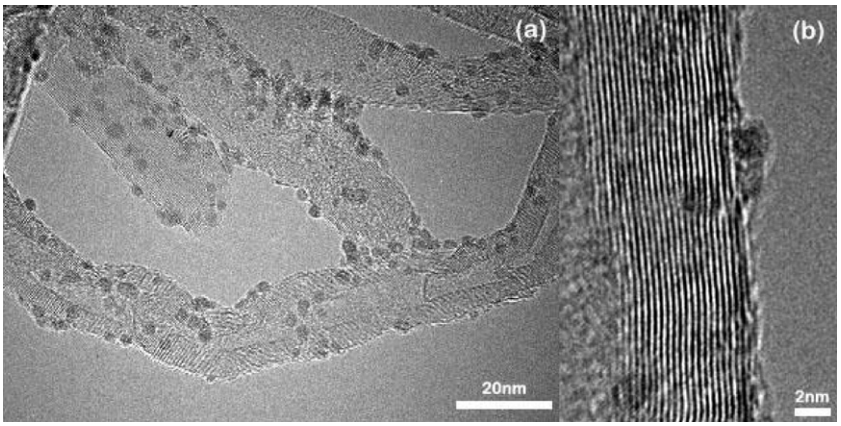

Fig. 1. TEM image of MWCNTs-Pd on a gold grid.

TAPPNW + SWNT/GCE showed a wide linear range, fast response time, and good selectivity toward the electrocatalytic reduction of H_2O_2, due to the synergistic effects between PCoTAPPNW and SWNT (Jeong et al., 2010).

In this study, we have introduced newly synthesized nanoparticles denoted as MWCNTs-Pd, and characterized them *via* TEM, Raman, and XPS. They were then used for modification of GCE, and the proposed Nafion®/MWCNTs-Pd/GCE electrode was characterized as a biosensor for H_2O_2 by means of SEM, CV, and chronoamperometry. The MWCNTs-Pd nanoparticles were applied on the GCE surface by dropping, followed by coating with Nafion® by dropping. The H_2O_2 was determined with an amperometric method at an applied potential of −0.2 V.

2. Experimental

2.1. Chemicals

All MWCNTs were parched from Carbon Nano Tech. Co., Ltd. (Pohang, South Korea). The diameter and length ranged between 20–30 nm and 1.0–2.0 μm, respectively. Nafion® was purchased from Aldrich. All other reagents used were of analytical grade and without further purification. The phosphate buffer saline (PBS) (0.1 M pH 7.4) was prepared by $NaHPO_4$ and the pH adjusted with 0.1 M NaOH. Doubly distilled water was used to prepare all aqueous electrolyte solutions.

2.2. Instruments

A three-electrode assembled cell was employed, consisting of the modified GCE (3.0 mm diameter) as the working electrode, a platinum-wire as a counter electrode, and a Ag/AgCl (3.0 M NaCl) electrode as the reference electrode. Electrochemical techniques, including CV and CA, were performed using a BAS 100B/W voltammetric analyzer (Bioanalytical Systems, West Lafayette, IN, USA) in a grounded Faraday cage. The pH measurements were performed by a pH glass electrode with a JENCO meter. The TEM observations were carried out in a JEM-2200FS microscope at 200 kV. Raman spectra were obtained at room temperature using an inVia Reflex (Renishaw 1000, New Mills, Gloucestershire, UK) micro-Raman spectrometer with 632.8 nm laser line. All XPS analyses were performed using a VG multilab 2000 spectrometer (ThermoVG Scientific, Southend-on-Sea, Essex, UK) in an ultra high vacuum. Survey scan data was collected using a pass energy of 50 eV. The content of Pd in the Pd–CNT nanocomposite was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) with an OPTIMA 4300 DV (Perkin Elmer). Prior to the measurement, the sample was treated with a mixture of HNO_3, HF and HBO_3 in order to dissolve it completely.

2.3. Formulation of MWCNTs-Pd nanoparticles

The as-received MWCNTs were first oxidized in a hot acid solution of HNO_3 and H_2SO_4 (1:3 by volume) at 90 °C for 3 h to remove impurities and generate surface functional groups. The acidified MWCNTs were dispersed in THF and NaSH aqueous solution then added for thiolation. In order to disperse Pd nanoparticles into the carbon template, we have followed a procedure using a Pd colloidal solution. Sodium tetrachloropalladate (II) (Na_2PdCl_4, 249.83 mg) was dissolved in deionized water (30 mL), followed by addition of 10 mL of 4-dimethylaminopyridine (DMAP, 119.9 mg). After that, thiolated MWCNTs (223.8 mg) in 100 mL of deionized water was added to this solution. The $NaBH_4$ solution was slowly dropped into the mixture and vigorously stirred for 30 min until the color changed from pale yellow to black. The resulting slurry was filtered, washed thoroughly with deionized water, and dried in a vacuum oven to give the MWCNTs-Pd nanoparticles.

2.4. Electrode modification

The GCE surface was highly polished with alumina paste, washed by distilled water, and finally rinsed with methanol. Then GCE surface was coated with 5.0 μL black MWCNTs-Pd suspension. After drying at room temperature, 5.0 μL of a 1% Nafion® solution was further dropped onto the electrode surface. The Nafion®/MWCNTs-Pd/GCE electrode was washed with distilled water before and after each experiment. All experiments were carried out in an argon atmosphere at room temperature.

3. Results and discussion

3.1. Characterization of MWCNTs-Pd modifier

The detailed morphology and structure of the MWCNTs-Pd are shown in Fig. 1. The TEM images show that nano-sized particles were highly dispersed with the MWCNTs (Fig. 1(a)). Smaller and highly dispersed nanoparticles were much more abundant than larger aggregates and revealed the nanoparticles to be anchored on the surface of MWCNTs. The average particle size was estimated to be ~2.0 nm. The high-resolution TEM (HRTEM) image shows that the graphitic sheets of MWCNTs-Pd maintained a highly ordered crystalline structure (Fig. 1(b)). However, the lattice fringes of the anchored nanoparticles were not obtained regarding the parameters of lattice, ordering, and orientation.

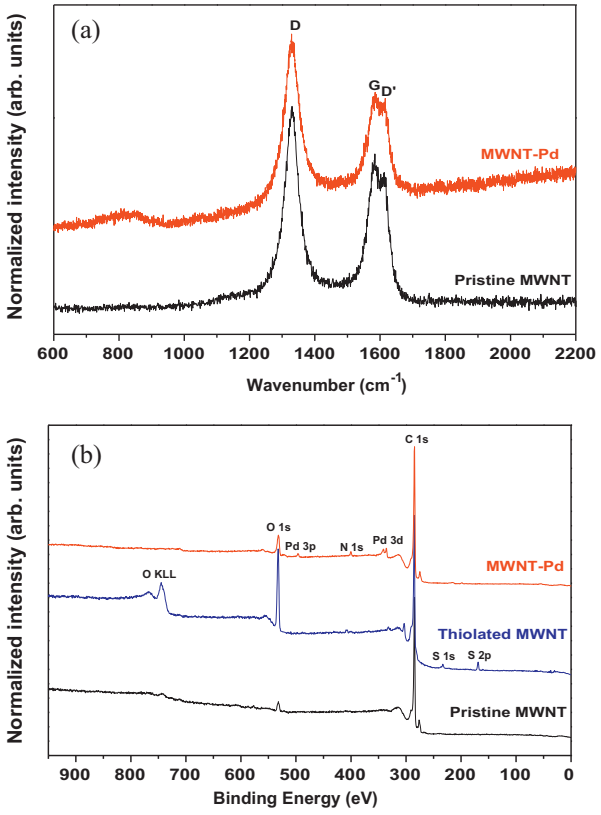

Fig. 2. (a) A Raman spectra of MWCNTs-Pd and MWCNTs. (b) XPS survey spectra of pristine MWCNTs, thiolated MWCNTs, and MWCNTs-Pd.

The Raman spectrum consists of three bands at ~1330 (D band), 1570 (G band), and ~1610 cm^{-1} (D' band) (Fig. 2(a)). The D band was generally associated with defects located along the nanotube sidewalls. The G band corresponded to the tangential vibration modes and can be found in other sp^2 carbon materials such as graphite. The D′ band, which was a shoulder of the G band at a higher frequency, corresponded to second-order Raman scattering from variations in the D-band. The value of I_D/I_G was approximately 1.38 for the pristine MWCNTs and nearly 1.78 for the MWCNTs-Pd. These results suggest that the functionalization of the MWCNTs resulted in a decrease in crystallinity.

Fig. 2(b) shows a series of XPS survey spectra from the pristine MWCNTs, thiolated MWCNTs, and MWCNTs-Pd. For the pristine MWCNTs, the XPS data shows distinct C and O 1s peaks; no other elements were detected. However, after thiolation, the presence of the S element was detected from the thiolated MWCNTs. The relative surface atomic ratio was estimated from the corresponding peak areas and corrected with the tabulated sensitivity factors. The estimated value of the S content was near 3.0 at%. For the MWCNTs-Pd, the XPS data confirmed the presence of Pd in the nanostructures. The estimated value of the Pd content was approximately 2.7 at%. According to ICP experiments, the amount of Pd contained in the nanocomposite was 3.35 wt.%, consistent with that of the XPS results.

3.2. Electrochemical behaviour of the modified electrode

The voltammetric response of H_2O_2 on the modified electrode was recorded *via* CV. Fig. 3 demonstrates a typical CV of 1.0 mM H_2O_2 in 0.1 M PBS at pH 7.4, recorded at the (a) MWCNTs, (b) MWCNTs-SH and (c) Nafion®/MWCNTs-Pd/GCE electrode. When GCE was modified by only MWCNTs, the peak current had almost disappeared (Fig. 3(a)). In the case of MWCNTs-SH, we do not find

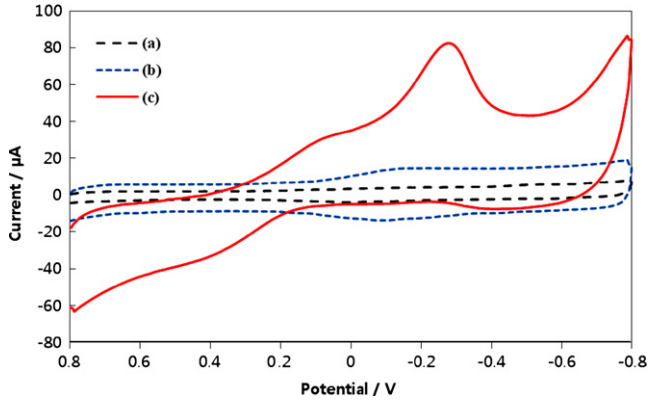

Fig. 3. Cyclic voltamograms of (a) MWCNTs, (b) MWCNTs-SH, and Nafion®/MWCNTs-Pd/GCE in 0.1 M PBS in 1.0 mM H_2O_2 at pH 7.4. Scan rate of 100 mV/s.

any peak and current response also was extremely low (Fig. 3(b)). A remarkably catalytic sharp peak was observed around −0.28 V with a sufficiently high current response (Fig. 3(c)). From the above discussion, it is very easily understandable that the Pd nanoparticles are giving extra catalytic effect with MWCNTs. Hence, the MWCNTs-Pd could easily transfer the electron which is involved with the catalytic reaction, indicating that in the modified GCE can perform sensitively *via* CV the presence of H_2O_2.

3.3. Determination of H_2O_2

The performance of Nafion®/MWCNTs-Pd/GCE towards the determination of H_2O_2 was evaluated by an amperometric technique. The effect of applied potentials on the reduction of H_2O_2 at Nafion®/MWCNTs-Pd/GCE electrode was studied and the results are shown (Supplemental S1). In this work, the applied potential of −0.2 V was chosen. Fig. 4 illustrates the chronoamperometric response of the modified electrode to subsequent additions of H_2O_2 in PBS at an applied potential of −0.2 V. The inset of Fig. 4 represents the plot of the response currents *versus* H_2O_2 concentration. The linearity of the plot current was very reasonable a 1.0 mM concentration. The current density of the H_2O_2 reduction obtained at the Nafion®/MWCNTs-Pd/GCE electrode, with good sensitivity (change of current density per unit concentration of H_2O_2), near about 23 μA/mM. The electrode responses achieved a steady-state signal within 10 s. The linear regression equation is: $i(\mu A) = 23.25C\,(mM) - 0.68$, with a 0.9999 correlation coefficient.

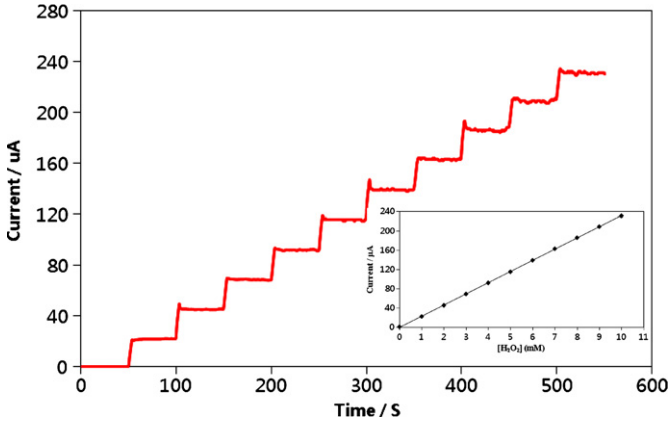

Fig. 4. The amperometric response of 1.0–10 mM H_2O_2 at Nafion®/MWCNTs-Pd/GCE. Inset: plot of response current *versus* H_2O_2 concentration. Applied potential of −0.2 V.

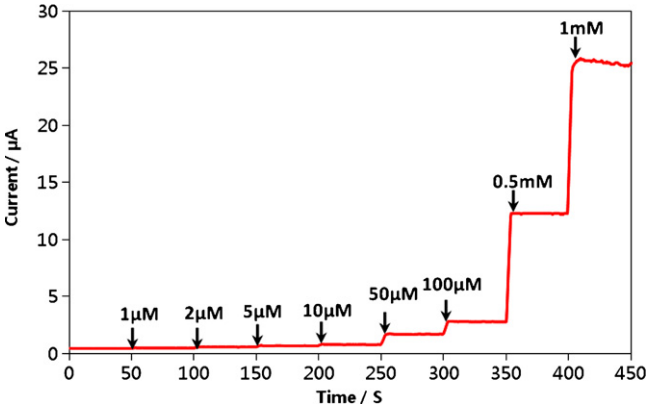

Fig. 5. The amperometric response of Nafion®/MWCNTs-Pd/GCE modified electrode to successive additions of 1 μM to 0.5 mM H_2O_2 into 0.1 M PBS solution. Applied potential of −0.2 V.

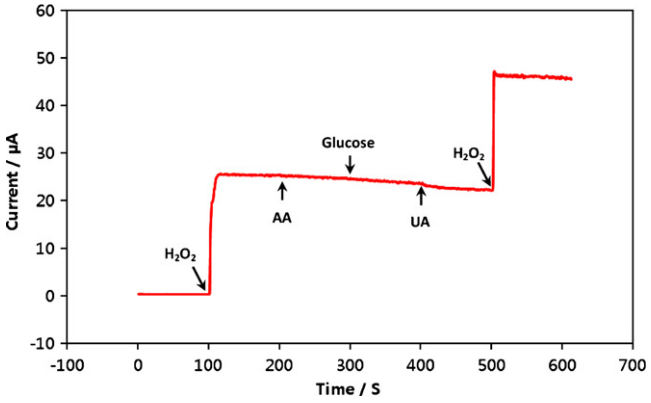

Fig. 6. Amperometric response of the hydrogen peroxide biosensor upon addition of 1.0 mM H_2O_2, 0.15 mM ascorbic acid (AA), 5.0 mM glucose, 0.5 mM uric acid (UA), and 1.0 mM H_2O_2. Applied potential of −0.2 V.

In contrast, CA was applied to determine the detection limit, with sampling from 1.0 to 500 μM (Fig. 5). The detection limit based on the signal-to-noise ratio (S/N = 3) was estimated to be 0.3 μM, suggesting that the H_2O_2 concentration could be easily detected at a very low concentration using the chronoamperometric method.

3.4. Necessity of Nafion®

It is well known that Nafion® is highly conductive towards cations, resists chemical attack, and is highly permeable to water. These points prompted this lab to use it in the modification of GCE the response was most impressive. Nafion® films have been used extensively for the modification of electrode surfaces and for the construction of amperometric biosensors (Wang et al., 2003). In addition, Nafion® films protect the electrode from the external stimulus. Also Nafion® coating is able to protect the electrode surface. In order to enhance the stability and reproducibility, therefore we modified the MWCNTs-pd/GCE with 1.0% Nafion® in this study. After the modifications were compared with each other (MWCNTs-Pd/GCE and Nafion®/MWCNTs-Pd/GCE) by the subsequent application of 3.0 mM H_2O_2 in PBS, 5 times responses were recorded. Between the first and fifth responses, the current response decreased approximately 18% due to the absence of Nafion® (Supplemental S2(a)). However the reproducibility of the Nafion®/MWCNTs-Pd/GCE was investigated by successively detecting 3.0 mM H_2O_2 for 5 time measurements (time interval: 2 h) and the relative standard deviation (RSD) was 1.5%, indicating a good reproducibility (Supplemental S2(b)). The stability of the Nafion®/MWCNTs-Pd/GCE for detection of 1.0 mM H_2O_2 was also investigated by amperometric responses for a long period about 20 min (stirred solution) (Supplemental S3). After 1000 s the response was still retained 96.9% value of the initial response.

3.5. Interference study

An interference study is an important part in the determination of any species *via* the electrochemical method. The anti-interference ability of the biosensor was investigated by the CA method. The effect of common interfering electroactive substances such as ascorbic acid (AA), glucose and uric acid (UA) were assessed and presented in Fig. 6. Fig. 6 shows the amperometric response for the biosensor of subsequent injection of 1.0 mM H_2O_2, 0.15 mM AA, 5.0 mM glucose, 0.5 mM UA and 1.0 mM H_2O_2 at −0.2 V. This concentrations were selected because the level of the endogenous AA, glucose, and UA is respectively about 0.125 mM, 4.4–6.6 mM, and 0.33 mM in blood sample (Hrapovic et al., 2004; Wang, 2008). As can be seen the current responses of AA, glucose and UA were observed much smaller than those of H_2O_2. The effect of possible interfering species on hydrogen peroxide detection was examined using 0.15 mM AA, 5.0 mM glucose and 0.5 mM UA which caused a decrease of 1.6%, 3.6% and 6.0% in the reduction current of 1.0 mM H_2O_2. It is well known that Nafion® is a negatively charged polyelectrolyte matrix and it reduces the permeability of negatively charged substrates (Shankaran et al., 2003; Lukachova et al., 1998). It was evident that the influence of the interfering species tested on the H_2O_2 response was negligible, indicating a high selectivity of the proposed biosensor.

4. Conclusions

We have developed and characterized a biosensor Nafion®/MWCNTs-Pd/GCE electrode, easily prepared in a rapid and simple procedure, that improved the sensitive and selective determination of H_2O_2. The attractive electrochemical and structural properties of the MWCNTs-Pd suggested a potential application towards electrocatalysis and as a biosensor. Overall, this is a simple approach to fabricate a GCE-based amperometric H_2O_2 biosensor at a lower potential (−0.2 V). Nafion® has been successfully applied on MWCNTs-Pd-modified GCE. The developed H_2O_2 biosensor Nafion®/MWCNTs-Pd/GCE possessed high sensitivity, a quick response time, a low detection limit, and good selectivity towards H_2O_2. Therefore, the Nafion®/MWCNTs-Pd/GCE electrode could be a good candidate for monitoring H_2O_2 concentration.

Acknowledgement

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0007864).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.09.053.

References

Callegari, A., Cosnier, S., Marcaccio, M., Paolucci, D., Paolucci, F., Georgakilas, V., Tagmatarchis, N., Va˘ızquez, E., Prato, M., 2004. J. Mater. Chem. 14, 807–810.
Cui, X., Liu, G., Lin, Y., 2005. J. Biomed. Nanotechnol. 1, 320–327.
Daniel, S., Rao, T.P., Rao, K.S., Rani, S.U., Naidu, G.R.K., Lee, H.Y., Kawai, T., 2007. Sens. Actuators B 122, 672–682.
Guldi, D.M., Rahman, G.M.A., Zerbetto, F., Prato, M., 2005. Acc. Chem. Res. 38, 871–878.
Hanaoka, S., Lin, J.M., Yamada, M., 2001. Anal. Chim. Acta 426, 57–64.

He, X., Hu, C., Liu, H., Du, G., Xi, Y., Jiang, Y., 2010. Sens. Actuators B Chem. 144, 289–294.
Huang, J., Wang, D., Hou, H., You, T., 2008. Adv. Funct. Mater. 18, 441–448.
Hurdis, E.C., Romeyn, J.H., 1954. Anal. Chem. 26, 320–325.
Hrapovic, S., Liu, Y., Male, K.B., Luong, J.H.T., 2004. Anal. Chem. 76, 1083–1088.
Jeong, H., Ahmed, M.S., Jeon, S., 2010. J. Nanosci. Nanotechnol. 10, 1–7.
Joshi, P.P., Merchant, S.A., Wang, Y., Schmidtke, D.W., 2005. Anal. Chem. 77, 3183–3188.
Karyakin, A.A., Karyakina, E.E., Schmidt, H.L., 1999. Electroanalysis 11, 149–155.
Karyakin, A.A., 2001. Electroanalysis 13, 813–819.
Liu, A., Dong, W., Liu, E., Tang, W., Zhu, J., Han, J., 2010. Electrochim. Acta 55, 1971–1977.
Lin, Y., Taylor, S., Li, H.P., Fernando, K.A.S., Qu, L.W., Wang, W., Gu, L.R., Zhou, B., Sun, Y.P., 2004. J. Mater. Chem. 14, 527–541.
Liu, S.Q., Ju, H.X., 2003. Biosens. Bioelectron. 19, 177–183.
Lu, X.B., Jhou, J.H., Lu, W., Liu, Q., Li, J.H., 2008. Biosens. Bioelectron. 23, 1236–1243.
Lukachova, L.V., Karyakin, A.A., Ivanova, Y.N., Karyakina, E.E., Varfolomeyev, E.D., 1998. Analyst 123, 1981–1985.
Luo, X.L., Xu, J.J., Zhou, W., Chen, H.Y., 2004. Biosens. Bioelectron. 19, 1295–1300.
Matsubara, C., Kawamoto, N., Takamura, K., 1992. Analyst 117, 1781–1784.
Shankaran, D.R., Uehara, N., 2003. Kato. Biosens. Bioelectron. 18, 721–728.
Shu, X.H., Chen, Y., Yuan, H.Y., Gao, S.F., Xiao, D., 2007. Anal. Chem. 79, 3695–3702.
Takamura, K., Matsumoto, T., 2008. Anal. Bioanal. Chem. 391, 951–961.
Wang, J., 2008. Chem. Rev. 108, 814–825.
Wang, J., Musameh, M., Lin, Y., 2003. J. Am. Chem. Soc. 125, 2408–2409.
Wang, Y., Wei, W., Zeng, J., Liu, X., Zeng, X., 2008. Microchim. Acta 160, 253–260.
Xu, Y., Pehrsson, P.E., Chen, L., Zhang, R., Zhao, W., 2007. J. Phys. Chem. C 111, 8638–8643.
Yu, X., Chattopadhyay, D., Galeska, I., Papadimitrakopoulos, F., Rusling, J.F., 2003. Electrochem. Commun. 5, 408–411.
Zhang, L.S., Wong, G.T.F., 1999. Talanta 48, 1031–1038.