Bioelectrochemistry 74 (2008) 62–65

Contents lists available at ScienceDirect

Bioelectrochemistry

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

Short communication

An electro-catalytic biosensor fabricated with Pt–Au nanoparticle-decorated titania nanotube array

Qing Kang, Lixia Yang, Qingyun Cai *

State Key Laboratory of Chemo/Biosensing and Chemometrics, Department of Chemistry, Hunan University, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 25 October 2007
Received in revised form 17 May 2008
Accepted 2 June 2008
Available online 12 June 2008

Keywords:
Catalytic
Titania
Nanotube array
Nanoparticle
Electrochemical

ABSTRACT

A Gold–Platinum nanoparticle-decorated titania nanotubular electrode is fabricated by electrochemically depositing Au and Pt nanoparticles onto a highly-oriented titania nanotube array. The prepared electrode, characterized by SEM and EDX, shows remarkably improved catalytic activities in the oxidation of hydrogen peroxide. By modifying the electrode with glucose oxidase (GOx) the resultant glucose biosensor exhibits a high sensitivity to glucose in the range of 0 to 1.8 mM with a response time of 3 s and detection limit of 0.1 mM.

1. Introduction

Electrochemical biosensors are an active research field attracting considerable attention as potential successors to a wide range of analytical techniques with high sensitivity, rapid response and relatively high selectivity [1,2]. The sensitivity can be significantly improved by introducing metal nanoparticles into the sensing interface [1,2] to facilitate the electron transfer. Noble metals are the most researched catalysts because of their high catalytic activities in many chemical reactions [1–10]. The modification of electrode surfaces with redox-active metal nanoparticles has led to the development of various electrochemical sensors [1–10]. Gold and platinum nanoparticles are one of the most intensive researched metals in design of electrodes [1,2,6–10]. Gold and platinum nanoparticles are electrodeposited onto microelectrodes to increase active centers with enhanced mass and electron transport characteristics [1,2,6–10], showing sensitive response to H_2O_2 over a wide range of concentrations [7–10]. The detection of H_2O_2 is of interest in biosensing as a usual product of oxidation reactions catalyzed by oxidoreductases in the presence of oxygen [7–10].

The catalytic efficiency (the reaction current) is always highly related to the electrode surface area. Various methods have been used to increase the electrode surface area, such as the modification of electrodes with carbon nanofibers [12,13], carbon nanotubes [14–16] and using nanoporous electrodes [17–18]. In recent years anodized titania nanotube arrays (TiO_x NT) are attracting increasing interest due to their easy preparation, high orientation, large surface area, high uniformity, and excellent biocompatibility [19–23]. A Co–Ag–Pt nanoparticle-decorated TiO_2 nanotube array shows a high catalytic activity [20]. The 10 μm long TiO_2 nanotubes prepared by anodization of titanium in dimethyl sulfoxide and HF electrolyte demonstrated significantly stronger clot formation at reduced clotting times as compared with nanocrystalline TiO_2 nanoparticles [21]. The size-adjustable property of titania nanotubes make them useful in drug delivery [22]. Titania nanotubes filled with gentamicin were explored in the application of local antibiotic therapy at the site of implantation. A biosensor for the detection of H_2O_2 was fabricated by modifying TiO_2 nanotube arrays with Horseradish Peroxidase and thionine [23]. These successful applications indicate that the highly-ordered, vertically oriented titania nanotube arrays are a promising functional material, and ideal as sensor substrates. To the authors' knowledge, however, no work has been reported on the research of the catalytic activities of Pt–Au nanoparticle-decorated titania nanotube array toward the oxidation of H_2O_2, and no work has been reported on using such a material to fabricate a biosensor.

In this work, an amperometric catalytic electrode was developed by decorating titania NT arrays with Au and Pt nanoparticles. The Electrochemical catalytic activity of the as-prepared electrode in response to H_2O_2 was investigated. An amperometric glucose oxidase (GOx) biosensor was fabricated by modifying GOx on the as-prepared electrode, and a sensitive response to glucose was achieved.

2. Experimental section

2.1. Materials

Titanium foil (99.8%, 0.127 mm thick) was purchased from Aldrich (Milwaukee, WI). Sodium fluoride, sodium chloride, sodium hydrogen

* Corresponding author.
E-mail address: qycai0001@hnu.cn (Q. Cai).

1567-5394/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2008.06.004

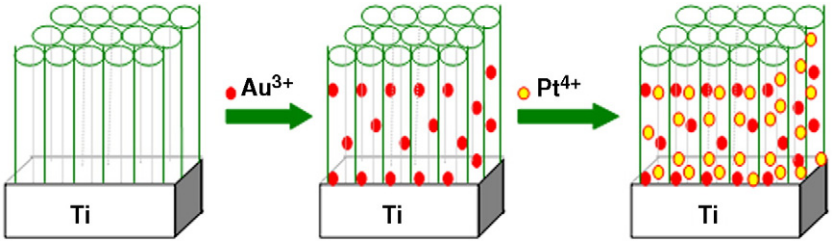

Fig. 1. Schematic deposition process of Au and Pt nanoparticles.

sulfate, chloroauric (III) acid, hexachloroplatinic (IV) acid, glucose, ascorbic acid and uric acid of analytical reagent grade were purchased from commercial sources and used as supplied. GOx (lyophilized powder, 215 U mg^{-1}, from *Aspergillus niger*) was purchased from Yakult (Japan). Double distilled water was used throughout the experiments.

2.2. Instrumentation

The catalyst topography was characterized using a field-emission scanning electron microscope (FE-SEM) operating at 5 kV (JSM 6700F; JEOL, Tokyo, Japan). An energy dispersive X-ray (EDX) spectrometer fitted to the scanning electron microscope on a nanoprobe mode was used for elemental analysis. The cyclic voltammetry (CV) and amperometric measurements were carried out by an electrochemical working station (CHI 660B; CH Instruments. Inc., Austin, TX), using a Pt wire counter electrode(Aldrich, 99.9% purity, 1 mm diameter) and a Ag/AgCl (saturated by KCl) reference electrode.

2.3. Electrode preparation

Prior to anodization, titanium foils of size 0.3×2.5 cm were ultrasonically cleaned in acetone and ethanol for each 5 min, respectively. The cleaned titanium foils were anodized in an electrolyte containing 0.1 M NaF and 0.5 M $NaHSO_4$ at room temperature for 5 h in a two-electrode configuration with a platinum cathode. The resulting nanotube arrays formed on the titanium substrate were amorphous, showing good conductivity in an external-applied electric field [24,25]. The titanium foil was only partially immersed in the electrolyte, with the upper un-anodized portion used as an electrical contact. The geometrical area of the anodized part (both sides) was 0.6 cm^2. Au, Pt nanoparticles were electrodeposited on the titania nanotubes in a plating bath containing 10 mM H_2PtCl_6 and 10 mM $HAuCl_4$ using chronopotentiometry at a current density of 50 μA s^{-1} in a standard three-electrode configuration with a titania/Ti working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl (saturated by KCl) reference electrode. The catalytic activity of the Au, Pt nanoparticle-decorated nanotubes in the oxidation of H_2O_2 was determined by cyclic voltammetry at a scan rate of 100 mV s^{-1} in a 10 mM pH 7.3 phosphate buffer solution (PBS) and 0.1 M NaCl containing 0.1 M H_2O_2, The pH was measured with Mettler–Toledo Delta 320 pH meter.

The enzyme solution was prepared by dissolving 20 mg of GOx in 1 mL of 10 mM PBS (pH 7.3) containing 0.1 M NaCl. The Pt/Au modified titania nanotublar electrode was loaded with 3-μL as-prepared enzyme solution, and dried in air. The enzyme modified biosensor was stored in 10 mM PBS (pH 7.3) containing 0.1 M NaCl at 4 °C. Amperometry was carried out in 10 mM PBS (pH 7.3) containing 0.1 M NaCl.

3. Results and discussion

3.1. Characterization of titania NT electrode decorated with the Pt–Au nanoparticles

The as-fabricated titania NT array films are amorphous and semiconductive [20,21], showing an enhanced conductivity in an externally-applied electric field in which the polarization of titania results in electron hopping between neighboring chains as described by Mataré [26]:

$$Ti^{3+}–O–Ti^{4+} \rightarrow Ti^{4+}–O–Ti^{3+}$$

The conductivity of the titania NT film facilitates the electrodeposition of metal nanoparticles, resulting in uniform deposition of the metal nanoparticles and excellent electron transfer between electrode and adsorbents, both properties of which are essential to obtaining a high catalytic activity. Fig. 1 illustrates the fabrication procedure of the Au and Pt nanoparticle-decorated titania NT electrode. The Au nanoparticles was first electrodeposited on the titania NT film and was followed by the Pt nanoparticles deposition. In relation to Au nanoparticles, Pt nanoparticles are more sensitive to detect H_2O_2. The first deposited Au would enhance the conductivity of titania and facilitate the sequent Pt electrodeposition.

The metal cations are introduced into the nanotubes by capillarity and electric field force, and electrochemically reduced to form nanoparticles. EDS spectrum (Fig. 2b) taken from the inner nanotube indicates the presence of Au and Pt, with a few nanoparticles at an average size of 20 nm existing on the NT surface as shown in Fig. 2(a). The metal loading was controlled by the duration of the deposition.

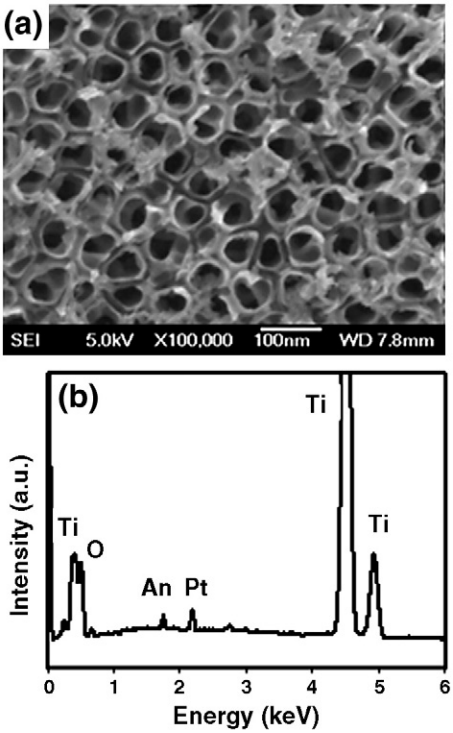

Fig. 2. SEM images of the Pt–Au nanoparticle-decorated titania NT (a) and EDS spectrum of the deposited metals (b).

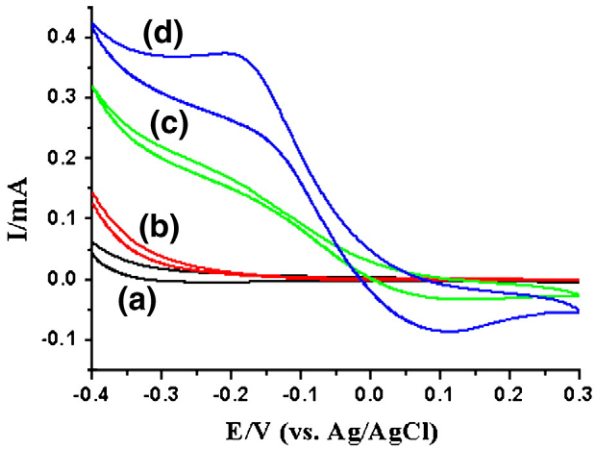

Fig. 3. Cyclic voltammograms of electrodes: (a) bare TiO_x NT, (b) Au/TiO_x NT, (c) Pt/TiO_x NT, (d) Pt–Au/TiO_x NT in 0.1 M H_2O_2 containing 10 mM PBS (pH 7.3) and 0.1 M NaCl at a scan rate of 100 mV s^{-1}.

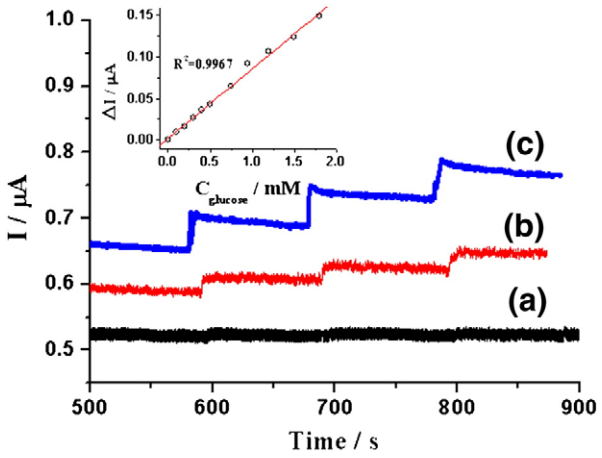

Fig. 5. Amperometric responses of the GO_x/Pt–Au/TiO_x NT sensor upon adding continuously (a) 0 mM, (b) 0.2 mM, and (c) 0.4 mM glucose in 10 mM PBS (pH 7.3) containing 0.1 M NaCl at −0.2 V versus Ag/AgCl (saturated by KCl) reference electrode. The glucose concentration is the final one. The inset shows calibration curve for glucose concentrations between 0.1 mM and 0.8 mM.

3.2. The catalytic activity toward the oxidation of H_2O_2

Fig. 3 shows the cyclic voltammograms of H_2O_2 on four different electrodes: (a) bare TiO_x NT electrode, (b) Au/TiO_x NT electrode, (c) Pt/TiO_x NT electrode, and (d) Pt–Au/TiO_x NT electrode with a Au loading of 1.30×10^{-8} mol cm^{-2} and a Pt loading of 2.60×10^{-8} mol cm^{-2} and a Au:Pt molar ratio of 1/2. The Pt–Au modified electrode (d) exhibits the highest electrochemical catalytic activity toward H_2O_2. The peak current on the Pt–Au/TiO_x NT electrode is three times that on the Pt/TiO_x NT electrode, eight times that on the Au/TiO_x NT electrode, and fifteen times that on the unmodified TiO_x NT electrode. The significantly increased catalytic activity of Pt–Au loaded electrode can be attributed to the enhanced conductivity due to the loaded Au and Pt nanoparticles and the electrochemically catalytic activity of Pt. The high surface area of the TiO_x NT offered more reactive sites as compared with a solid support [20] and a high catalytic activity was therefore achieved.

The electro-catalytic activity of the Pt–Au/TiO*x* NT electode toward H_2O_2 was assessed by quantitative analysis of the amperometric response to continuous injections of 10 μM H_2O_2 (Fig. 4). From Fig. 3 it can been seen that there is a good response from the reduction of H_2O_2 at the potential of −0.2 V. Therefore the potential of −0.2 V was used as the operational potential of the Pt–Au/TiO_x NT electrode. As shown in Fig. 4, a linear response is achieved between 10 and 80 μM H_2O_2 with a detection limit of 10 μM and a response slope of 2.92 μA mM^{-1} (R^2=0.9935).

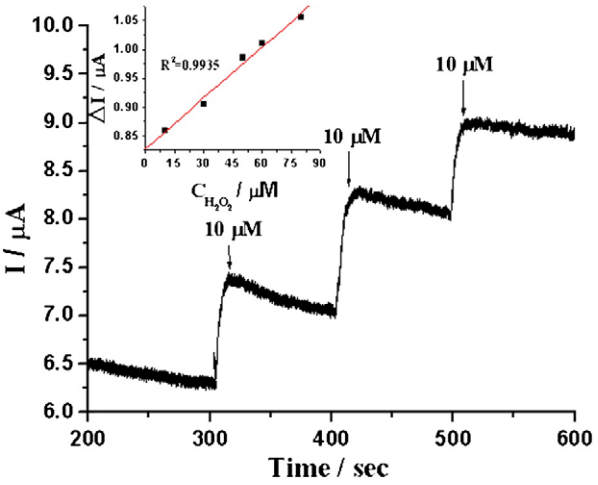

Fig. 4. Amperometric responses of the Pt–Au/TiO_x NT electrode upon adding continuously 10 μM H_2O_2 in 10 mM PBS (pH 7.3) containing 0.1 M NaCl at −0.2 V vs Ag/AgCl (saturated by KCl). 10 μM H_2O_2 is the final concentration. The inset shows the calibration curve.

3.3. The sensor response to glucose

A glucose biosensor was fabricated by modifying the Pt–Au/TiO_x NT electrode with glucose oxidase (GOx). Generally, biosensors enzymes are immobilized to the sensor surface by either cross–linking with, e.g. glutaraldehyde [7,10] or being protected with a thin layer of, e.g. Nafion [8,9] to avoid the loss of enzymes. In this work the excellent biocompatibility and the nanotubular structure of the Pt–Au/TiO_x NTs provide a hole-rich electrode structure for the direct immobilization of GOx. The reproducibility experiments showed that GOx was firmly adsorbed on the sensor without significant loss. Fig. 5 displays the amperometric response of the biosensor to different concentrations of glucose: (a) 0 mM, (b) 0.2 mM, (c) 0.4 mM at −0.2 V versus Ag/AgCl (saturated by KCl) reference electrode in 10 mM PBS (pH 7.3) containing 0.1 M NaCl. The biosensor exhibits a linear response to glucose in the concentration ranging from 0 mM to 1.8 mM, with a sensitivity of 0.08366 μA mM^{-1}, a figure of merit of 109.5 mM^{-1} and a response time of 3 s. Defining the detection limit as the concentration that can be detected at 3 times the noise level, the detection limit is 0.1 mM glucose, which is much lower than the serum glucose concentration of diabetic patients. Under normal physiological conditions, serum glucose concentration is 3.8–6.1 mM. Diabetic urine appears when blood glucose is generally higher than 9 mM, the renal glucose threshold. The achieved detection limit is lower than these achieved on an amperometric glucose biosensor based on electrodeposition of platinum nanoparticles onto covalently immobilized carbon nanotube electrode (0.4 mM) [8] and a wireless, remote query glucose biosensor based on a pH-Sensitive Polymer (0.6 mM) [11].

The effect of electroactive interferents such as ascorbic acid and uric acid, which are commonly present in physiological samples of glucose, were investigated. The responses of the enzyme electrode to 0.1 mM ascorbic acid and 0.5 mM uric acid are 2.15 nA and 14.82 nA, respectively, which are negligible to the electrode response to the physiological normal level of glucose (5.6 mM).

The proposed biosensor showed a good repeatability with a relative standard deviation of 3.6% (n=6) in response to 1 mM glucose, and relative stability. The sensor response to 1 mM glucose was still retained at 72.58% value of the initial response after 25 days.

4. Conclusions

A Pt–Au nanoparticle-modified electrode was fabricated with highly-ordered titania nanotube array as substrate. The as-prepared electrode exhibited a high catalytic efficiency to the oxidation of H_2O_2 due to the high surface area of the nanotubular structure, the excellent biocompatibility of TiO_2, and the high catalytic activity of the loaded Pt nanoparticles. By modifying the electrode with glucose oxidase (GOx) the resultant glucose biosensor exhibits a linear response to glucose in the range of 0 to 1.8 mM with a response time of 3 s and detection limit of 0.1 mM.

Acknowledgments

We are grateful for the financial support from the National Science Foundation of China under the grant 20475016 and 20775024, and the Specialized Research Fund for the Doctoral Program of Higher Education under grant 20050532024.

References

[1] M. Pumera, S. Sánchez, I. Ichinose, J. Tang, Electrochemical nanobiosensors, Sens. Actuators, B, Chem. 123 (2007) 1195–1205.
[2] S.J. Guo, E. Wang, Synthesis and electrochemical applications of gold nanoparticles, Anal. Chim. Acta 598 (2007) 181–192.
[3] W.M. Wang, D. Zheng, C. Du, Z.Q. Zou, X.G. Zhang, B.J. Xia, H. Yang, D.L. Akins, Carbon-supported Pd–Co bimetallic nanoparticles as electrocatalysts for the oxygen reduction reaction, J. Power Sources 167 (2007) 243–249.
[4] S.F. Wang, F. Xie, R.F. Hu, Carbon-coated nickel magnetic nanoparticles modified electrodes as a sensor for determination of acetaminophen, Sens. Actuators, B, Chem. 123 (2007) 495–500.
[5] S. Zhao, K. Zhang, Y.Y. Sun, C.Q. Sun, Hemoglobin/colloidal silver nanoparticles immobilized in titania sol–gel film on glassy carbon electrode: Direct electrochemistry and electrocatalysis, Bioelectrochemistry 69 (2006) 10–15.
[6] R. Kumara, A.N. Maitraa, P.K. Patanjalib, P. Sharma, Hollow gold nanoparticles encapsulating horseradish peroxidase, Biomaterials 26 (2005) 6743–6753.
[7] T.Y. You, O. Niwa, M. Tomita, S. Hirono, Characterization of platinum nanoparticle-embedded carbon film electrode and its detection of hydrogen peroxide, Anal. Chem. 75 (2003) 2080–2085.
[8] X. Chu, D.X. Duan, G.L. Shen, R.Q. Yu, Amperometric glucose biosensor based on electrodeposition of platinum nanoparticles onto covalently immobilized carbon nanotube electrode, Talanta 71 (2007) 2040–2047.
[9] H. Tang, J.H. Chen, S.Z. Yao, L.H. Nie, G.H. Deng, Y.F. Kuang, Amperometric glucose biosensor based on adsorption of glucose oxidase at platinum nanoparticle-modified carbon nanotube electrode, Anal. Biochem. 331 (2004) 89–97.
[10] X.H. Kang, Z.B. Mai, X.Y. Zou, P.X. Cai, J.Y. Mo, A novel glucose biosensor based on immobilization of glucose oxidase in chitosan on a glassy carbon electrode modified with gold–platinum alloy nanoparticles/multiwall carbon nanotubes, Anal. Biochem. 369 (2007) 71–79.
[11] Q.Y. Cai, K.F. Zeng, C.M. Ruan, T.A. Desai, C.A. Grimes, A wireless, remote query glucose biosensor based on a pH-sensitive polymer, Anal. Chem. 76 (2004) 4038–4043.
[12] S.E. Baker, K.Y. Tse, C.S. Lee, R.J. Hamers, Fabrication and characterization of vertically aligned carbon nanofiber electrodes for biosensing applications, Diam. Relat. Mater. 15 (2006) 433–439.
[13] J. Niedziolka, M.A. Murphy, F. Marken, M. Opallo, Characterisation of hydrophobic carbon nanofiber–silica composite film electrodes for redox liquid immobilisation, Electrochim. Acta 51 (2006) 5897–5903.
[14] S.F. Wang, Q. Xu, Electrochemical parameters of ethamsylate at multi-walled carbon nanotube modified glassy carbon electrodes, Bioelectrochemistry 70 (2007) 296–300.
[15] S.Y. Ly, Detection of dopamine in the pharmacy with a carbon nanotube paste electrode using voltammetry, Bioelectrochemistry 68 (2006) 227–231.
[16] K.B. Wu, H. Wang, F. Chen, S.S. Hu, Electrochemistry and voltammetry of procaine using a carbon nanotube film coated electrode, Bioelectrochemistry 68 (2006) 144–149.
[17] G. Zhao, J.J. Xu, H.Y. Chen, Interfacing myoglobin to graphite electrode with an electrodeposited nanoporous ZnO film, Anal. Biochem. 350 (2006) 145–150.
[18] A. Jänes, H. Kurig, E. Lust, Characterisation of activated nanoporous carbon for supercapacitor electrode materials, Carbon 45 (2007) 1226–1233.
[19] K. Shankar, G.K. Mor, H.E. Prakasam, S. Yoriya, M. Paulose, O.K. Varghese, C.A. Grimes, Highly-ordered TiO_2 nanotube arrays up to 220 μm in length: use in water photoelectrolysis and dye-sensitized solar cells, Nanotechnology 18 (2007) 1–11.
[20] L.X. Yang, D.M. He, Q.Y. Cai, Fabrication and catalytic performances of TiO_2 nanotube array-supported Co–Ag–Pt nanoparticles, J. Phys. Chem. C 111 (2007) 8214–8217.
[21] S.C. Roy, M. Paulose, C.A. Grimes, The effect of TiO_2 nanotubes in the enhancement of blood clotting for the control of hemorrhage, Biomaterials 28 (2007) 4667–4672.
[22] K.C. Popat, M. Eltgroth, T.J. LaTempa, C.A. Grimes, T.A. Desai, Decreased Staphylococcus epidermis adhesion and increased osteoblast functionality on antibiotic-loaded titania nanotubes, Biomaterials 28 (2007) 4880–4888.
[23] S.Q. Liu, A.C. Chen, Coadsorption of Horseradish peroxidase with thionine on TiO_2 nanotubes for biosensing, Langmuir 21 (2005) 8409–8413.
[24] O.K. Varghese, M. Paulose, D. Gong, C.A. Grimes, E.C. Dickey, Crystallization and high-temperature structural stability of titanium oxide nanotube arrays, J. Mater. Res. 18 (2003) 156–165.
[25] O.K. Varghese, G.K. Mor, C.A. Grimes, M. Paulose, N. Mukherjee, A titania nanotube-array room-temperature sensor for selective detection of hydrogen at low concentrations, J. Nanosci. Nanotechnol. 4 (2004) 733–737.
[26] H.F. Mataré, Defect Electrons in Semiconductors, Wiley-Interscience, New York, 1971.