Biosensors and Bioelectronics 25 (2009) 944–947

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Covalently linked silica-multiwall carbon nanotube-polyaniline network: An electroactive matrix for ultrasensitive biosensor

Shanmugasundaram Komathi [a], Anantha Iyengar Gopalan [a,b], Kwang-Pill Lee [a,b,*]

[a] Department of Chemistry Graduate School, Kyungpook National University, Daegu, South Korea
[b] Department of Nanoscience and Nanotechnology, Kyungpook National University, Daegu 702-701, South Korea

ARTICLE INFO

Article history:
Received 15 July 2009
Received in revised form 22 August 2009
Accepted 26 August 2009
Available online 1 September 2009

Keywords:
Multiwall carbon nanotube
Silica functionalization
Horseradish peroxidase
Synergistic contribution
Biosensor

ABSTRACT

A three dimensional network comprising of covalently linked multi-walled carbon nanotubes (MWNT), silica and polyaniline chains (MWNT-Silica-PANI-NW) was prepared and used as the matrix for immobilization of an enzyme (horseradish peroxidase, HRP). MWNT-Silica-PANI-NW-HRP electrode showed superior performances over the individual components and their binary combinations, in terms of enzyme stability (minimum leaching to the medium), fast electron transfer from the enzyme to the electrode (2.11 s^{-1}), high sensitivity (58.1 $\mu A\,mM^{-1}$) and low detection limit (1 pM) for the detection of hydrogen peroxide. The interconnected silica network provides open porous frame work for effective immobilization of the redox enzyme. The PANI units and MWNTs facilitate the electron transfer process and functions as molecular cable to "wire" the primary redox site of the enzyme (HRP) to the electrode. The improved performances for MWNT-Silica-PANI-NW arise from the synergistic contributions from conducting CNT, electron mediating PANI chains and porous silica network.

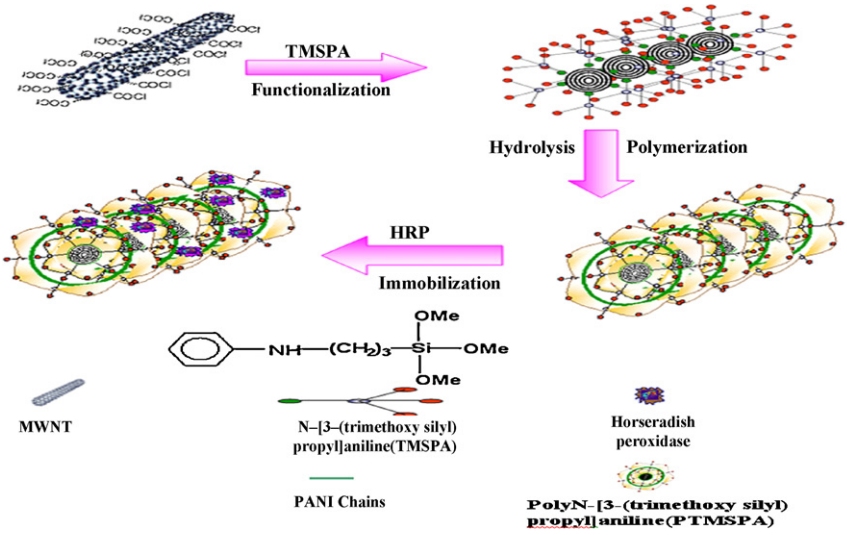
1. Introduction

Carbon nanotubes (CNTs) possess the ability to promote electron transfer reactions involving enzymes and they have raised great expectations in electrochemical biosensing, biofuel cells and biochip fabrication ((Lin et al., 2004) and (Kim et al., 2007)). However, the hydrophobicity of CNTs poses a few problems such as stability of the adsorbed or immobilized enzyme and approach of the analyte to active enzyme sites. These problems can cause detrimental effect on the performances of biodevices and thus need to be solved. Surface functionalization of CNTs, through which the prerequisites for applications could be embedded, is receiving recent attention especially towards biosensor fabrication and detection of biomolecules/drugs ((Hu et al., 2004), (Singh et al., 2005) and (Pantarotto et al., 2003)). Sol–gel derived inorganic materials such as silicas, are particularly attractive for enzyme immobilization because they exhibit tunable porosity, chemical inertness, and negligible swelling in aqueous solution ((Luckarift et al., 2004) and (Xia et al., 2006)). Silica imparts biocompatibility and hydrophilicity for the immobilization of enzymes as well as prevents enzyme leakage. CNTs-silica composites have been developed as new materials for nanodevices ((Mulvaney et al., 2000) and (Caruso, 2001)). However, CNTs-silica composites have not yet been used for electrochemical biodevices. Though silica is ideally suited for immobilization of enzyme, it is necessary to have a conducting ramified network of molecular wires close to the enzyme for allowing electron transfer (ET) between enzyme and the electrode. Polyaniline (PANI) is extensively used as an electron transducer to augment the signal produced by enzymes and also to eliminate electrode fouling. It is explicit from literature that CNTs, silica and PANI are individually having a few beneficial characteristics for enzyme based biodevices but as well have undesirable effects on electrochemical device performances, when they are used alone ((Ivnitski et al., 2008) and (Ma et al., 2006)). We intend to integrate CNT, silica and PANI in a single matrix to form "electron wire" for the immobilized enzyme. We aimed to achieve improved performances for biodevices through synergistic advantageous contributions from each component.

Here, we present the first report on the utility of interconnected structure of multiwall carbon nanotube (MWNTs), silica network, and PANI chains for immobilization of a redox enzyme (horseradish peroxidase, HRP) and fabrication of a biosensor. MWNT-silica-PANI-NW was expected to have superior performances in terms of stable enzyme immobilization (minimum tendency to leach into medium) and biocompatibility (performance in physiological conditions) due to the presence of silica network and electron transfer capability from enzyme to electrode through MWNT-PANI molecular wire. Experiments were designed to demonstrate the advantages of MWNT-silica-PANI-NW over the individual components and their binary combinations for enzyme immobilization and biosensor performance towards detection of hydrogen peroxide.

* Corresponding author. Tel.: +82 53 950 5901; fax: +82 53 952 8104.
E-mail addresses: algopal_99@yahoo.com (A.I. Gopalan), kplee@knu.ac.kr (K.-P. Lee).

0956-5663/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2009.08.041



Scheme 1. Preparation of MWNT-silica-PANI-NW and immobilization with HRP.

The key aspect of the present investigation is the preparation of 3D NW through covalent linking of MWNT, silica and PANI chains (Scheme 1). Typically, preparation of MWNT-silica-PANI-NW consists of two major steps: (i) functionalization of MWNT with *N*-[3-(trimethoxy silyl) propyl]aniline (TMSPA) (MWNT-f-TMSPA) to obtain MWNT-f-TMSPA and (ii) subsequent polymerization of MWNT-f-TMPSA with TMPSA. The amine units in TMSPA as well as in MWNT-f-TMPSA undergo simultaneous oxidation leading to grafting of poly(*N*-[3-(trimethoxy silyl) propyl]aniline (PTMSPA) onto the surface of MWNTs. The mild acidic condition causes hydrolysis and condensation of methoxy groups in silyl part to generate PTMSPA 3D NW, which comprises of silica frame interconnected with PANI chains (Scheme 1), (Herein referred as MWNT-silica-PANI-NW).

2. Experimental

2.1. Chemicals and reagents

N-[3-(trimethoxy silyl) propyl]aniline (TMSPA) (99%), hydrogen peroxide (30%) and ammonium persulphate were purchased from Sigma–Aldrich, Inc., USA. Multi-walled carbon nanotubes (MWNTs) (10–50 nm in diameter) were obtained from CNT Co. Ltd., Incheon, Korea. Aqueous solutions of H_2O_2 were prepared in phosphate buffer (pH 7) afresh at the time of carrying out the experiments. For electrochemical studies, indium tin oxide (ITO) coated glass plate (specific surface resistance of about (10 Ω) was used as the working electrode. ITO electrode was degreased with acetone and rinsed with distilled water prior to use. A constant surface of 1 cm^2 was maintained by masking the other part of the working electrode with lacquer.

2.2. Instrumentation

The morphology of MWNT-silica-PANI was investigated by a Field emission scanning electron microscope (FESEM) Hitchachi S-4300 with a field emission gun operated at 200 kV. UV–visible spectra were recorded using Shimadzu UV-2101 spectrophotometer in the range of 300–800 nm. Fourier transform infrared spectra were recorded by Perkin-Elmer Lamda 9N-1062 spectrometer. Cyclic voltammetry and amperometric measurements were performed in Iviumstat and Compactstat (The Netherland). The modified working electrodes were prepared by drop casting 20 μL of the suspension, prepared by dispersing 10 mg of MWNT-silica-PANI-NW or MWNT-f-TMSPA or MWNT in 1 mL of *N*,*N* dimethyl formamide (DMF) onto ITO surface and dried at 40 °C in vacuum oven. Electrochemical experiments were performed using a three electrode electrochemical cell set up, that contained HRP/MWNT-silica-PANI-NW or HRP/MWNT-f-TMSPA or HRP/MWNT as working electrode, an Ag/AgCl (reference) electrode, and a platinum wire (auxiliary) electrode. The electroactivity of MWNT-silica-PANI-NW electrode was evaluated by recording cyclic voltammogram in 0.1 M HCl in the potential range 0–0.8 V. The biocatalytic activity of MWNT-silica-PANI-NW electrode was evaluated by cyclic voltammetry for a solution of 1 mM H_2O_2 in 0.1 M phosphate buffer solution (PBS). The amperometric responses of HRP/MWNT, HRP/MWNT-f-TMSPA, HRP/MWNT-silica-PANI-NW electrodes towards H_2O_2 were recorded under steady state conditions in PBS (pH 7) by applying a constant potential of +0.1 V at the working electrode. When the background current became stable, the response signal were measured for the successive addition of H_2O_2 (10 μL) into 0.1 M PBS.

2.3. Preparation of MWNT-silica-PANI-NW composite

2.3.1. Preparation of MWNT functionalized N-[3-(trimethoxy silyl) propyl]aniline (MWNT-f-TMSPA)

Initially, MWNTs were carboxylated by adopting the procedure in the earlier literature (Yu et al., 2008; Santhosh et al., 2006). 50 mg of MWNT-COCl was refluxed with 30 mM *N*-[3-(trimethoxy silyl) propyl]aniline using THF at 60 °C for 24 h. The TMSPA functionalized MWNTs (MWNT-f-TMSPA) were filtered, washed and dried in vacuum.

2.3.2. Preparation of MWNT-silica-PANI-NW

10 mg of MWNT-f-TMSPA was dispersed in 20 mL of 0.1 M HCl and sonicated for 5 min. To the MWNT-f-TMSPA dispersed solution, 5 mL of ammonium persulphate (30 mM) was added drop wise at 5 °C. The dark green precipitate, MWNT-silica-PANI-NW was filtered washed with water and dried in vacuum oven at 35 °C.

2.3.3. Preparation of HRP/MWNT-silica-PANI-NW electrode

HRP/MWNT-silica-PANI-NW electrode was prepared by drop casting 20 μL of the suspension, prepared by dispersing 10 mg of MWNT-silica-PANI-NW in 1 mL of *N*,*N* dimethyl formamide (DMF) onto ITO surface and dried at 40 °C in vacuum oven. 10 μL of HRP (50 mg/mL) was dropped onto the surface of MWNT-silica-PANI-NW electrode through a micro pipette and allowed to dry at room

temperature for 30 min. In the similar way, HRP/MWNT-f-TMSPA and HRP/MWNT electrodes were fabricated.

3. Results and discussion

3.1. Morphology

FESEM images of MWNT-f-TMSPA (Supporting information: S1) and MWNT-silica-PANI-NW (Supporting information: S1) are presented. The interconnected spherical particles in Fig. 1 are probably threaded through MWNTs. The spheres are sol–gel formed silica particles with sizes in the range of 1–2 μm. High volt transmission electron microscope (HVTEM) image of MWNT-silica-PANI-NW (Fig. 1, inset) indicates, CNTs are embedded in silica framework. The silica layer (Supporting information: S1) and CNTs (Supporting information: S1) are clearly differentiated under higher magnifications. The silica-PANI network is evident from the mesh like morphology with nanocavities (dark regions in Supporting information: S1). The interconnected spheres indicate that MWNTs are the bridging units to connect silica-PANI-NW. The existence of PANI units in MWNT-silica-PANI-NW is also evident from FTIR spectroscopy and UV–visible spectroscopy (Supporting information: S2). The presence of a strong peak around 1130 cm^{-1} confirms the presence of Si–O–Si linkages. The prominent peaks around 1510 cm^{-1} and 1606 cm^{-1} are assigned for aromatic amine and imine units, respectively as reported for pristine PANI. UV–visible spectrum of MWNT-silica-PANI-NW shows the presence of polaronic and bipolaronic transitions of PANI chains.

3.2. Cyclic voltammetry

Interestingly, MWNT-silica-PANI-NW shows electroactivity due to the presence of PANI units and MWNTs (Supporting information: S3). A series of control experiments revealed the superior performances of MWNT-Silica-PANI-NW, in terms of electroactivity and fast ET from the immobilized enzyme to the electrode as compared to MWNT, MWNT-f-TMSPA and MWNT-PANI electrodes.

First, electroactivities of the electrodes were compared using $Fe(CN)_6^{3-/4-}$ as a redox marker (Supporting information: S4). Cyclic voltammogram of HRP/MWNT, HRP/MWNT-f-TMSPA, HRP/MWNT-silica-PANI-NW electrode was recorded in the potential range from −0.2–0.8 V (vs. Ag/AgCl) in the presence of $Fe(CN)_6^{3-/4-}$ (10 mM) at scan rates in the range 10–1000 mV s^{-1}. Voltammograms shows a well defined redox couple with a peak potential separation (ΔE_p) of 136 mV, 189.8 mV, 79 mV at the scan rate of 10 mV s^{-1} in the cases of HRP/MWNT, HRP/MWNT-f-TMSPA, HRP/MWNT-silica-PANI-NW electrodes, respectively. HRP/MWNT-silica-PANI-NW electrode shows higher peak current (5.1 μA) with a minimum peak potential separation (ΔE_p = 79 mV) at the scan rate of 10 mV s^{-1} in comparison to other electrodes.

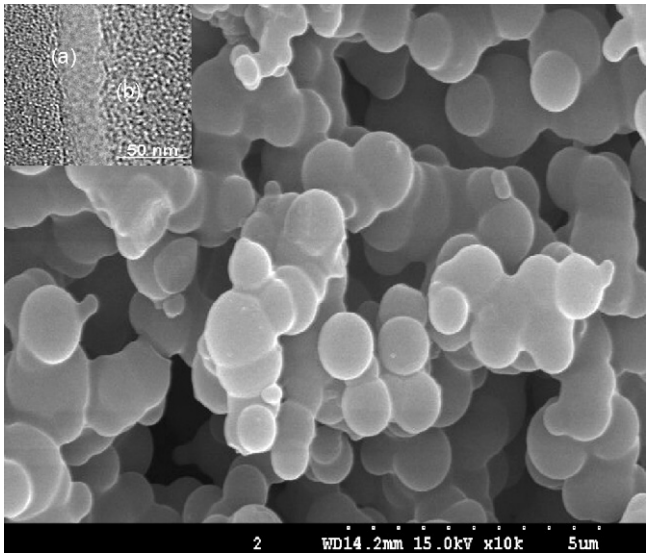


Fig. 1. FESEM image of MWNT-silica-PANI-NW. Inset: High magnification HVTEM image of MWNT-silica-PANI-NW. Further magnified images of (a) and (b) are presented in (Supporting information: S1(B)(a) and (B)(b)).

3.3. pH studies

Second, control experiments were performed to test the electron transfer ability from the enzyme (HRP) to electrode by cyclic voltammetry at different pH conditions (Supporting information: S5). HRP/MWNT-silica-PANI-NW electrode shows a higher peak current with minimum peak potential separation (79 mV) at pH 7, hence pH 7 is chosen for further for electrochemical detection of H_2O_2 studies. Also, the electron transfer ability was also verified at HRP/MWNT and HRP/MWNT-f-TMSPA electrodes (Supporting information: S6 inset). The quasi reversible redox peaks seen around 87 mV (anodic) and 11 mV (cathodic) correspond to oxidation and reduction of electroactive centers in HRP (vs. Ag/AgCl). Importantly, electron transfer was not prominent at HRP/MWNT and HRP/MWNT-f-TMSPA electrodes (Supporting information: S6 inset). The formal potential of ET process is more positive than reported for HRP-chitosan (−0.0091 V vs. NHE) (Huang et al., 2002) and HRP-gluton (−0.091 V Vs. NHE) (Liu and Hu, 2003). The smaller peak separation between anodic and cathodic processes (ΔE_p = 76 mV vs. Ag/AgCl) signifies that HRP molecules have preferred orientation in the MWNT-silica-PANI-NW matrix to impart effective ET from the enzyme to the electrode ((Long et al., 2008) and (Wu et al., 2008)). We presume that interconnected silica network may provide open porous frame work for effective immobilization of the redox enzyme. The PANI units and MWNTs facilitate the ET process and function as molecular cable to "wire" the primary redox site of the enzyme (HRP) to the electrode. Also, the electron transfer reaction at HRP/MWNT-silica-PANI-NW is a surface confined one with a linear dependence of scan rate on peak current (Supporting information: S7). The rate constant (k_s 2.11 s^{-1}) of ET reaction, calculated through Laviron model, is much higher than for HRP immobilized on pyrolitic graphite (Tatsuma et al., 1998).

3.4. Amperometry

The HRP/MWNT-silica-PANI-NW electrode shows superior biocatalytic electrochemical detection of H_2O_2 in 0.1 M PBS as compared to MWNT and MWNT-f-TMSPA electrodes (Supporting information: S8). The response current values to H_2O_2 increase linearly in the concentration range of H_2O_2 from 1 pM to 1 mM (Fig. 2). The HRP/MWNT-silica-PANI-NW electrode shows higher sensitivity of (58.1 μA mM^{-1}) (Supporting information: S8(B)(a)) than HRP/MWNT (0.71 nA/mM^{-1}) and HRP/MWNT-f-TMSPA (0.05 μA/mM^{-1}) electrodes (Supporting information: S8(A)). The HRP/MWNT-silica-PANI-NW electrode exhibits much lower detection limit (1 pM) as compared to HRP/MWNT (2.1 μM) and HRP/MWNT-f-TMSPA (65 nM) electrodes. To ascertain the lower detection limit of HRP/MWNT-silica-PANI-NW, typical amperogram was recorded in the range 1–12 pM (Fig. 2, inset). The linearity between current response and concentration (in the range 1–12 pM) confirms the lower detection limit. The linear range of HRP/MWNT-silica-PANI-NW is much wider (Supporting information: S8(B)) than at HRP/MWNT, HRP/MWNT-f-TMSPA electrodes, PANI modified screen printed electrode (Luo et al., 2007a), and PANI/SiO_2/GC electrode (Luo et al., 2007b). Thus, the

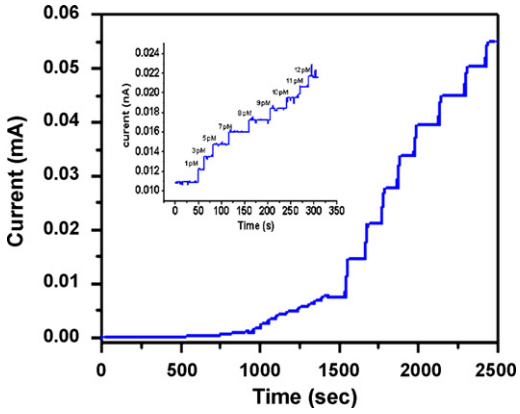


Fig. 2. Amperometric response of HRP/MWNT-silica-PANI-NW modified electrode at +0.1 V upon successive addition of 10 μL H_2O_2 in 0.1 M PBS (pH 7.0). Inset amperogram of HRP/MWNT-silica-PANI-NW modified electrode in the concentration range of H_2O_2 from 1 to 12 pM.

existence of MWNT, silica and PANI as interconnected network provides improved performances for the biosensor.

3.5. Stability of HRP/MWNT-silica-PANI-NW electrode

The stability of HRP/MWNT-silica-PANI-NW and other electrodes were monitored through enzyme leaching test (Supporting information: S9). Typically, HRP/MWNT-silica-PANI-NW showed a minimum leaching (1.9%) after three weeks as compared to 88.6%, 9.8% for HRP/MWNT and HRP/MWNT-f-TMSPA matrices, respectively. Importantly, HRP/MWNT-silica-PANI-NW based biosensor electrode shows 98% of its current response (for 1 mM of H_2O_2) after three weeks). Thus, HRP/MWNT-silica-PANI-NW matrix not only retains the enzyme for a longer period but also sustains its biocatalytic property.

4. Conclusions

In, summary, the interconnected 3D NW matrix, MWNT-silica-PANI exhibits better electroactivity and electron transfer capability from enzyme to the electrode. Also, HRP/MWNT-silica-PANI-NW biosensor shows lower detection limit (picomolar), wide linear range (1 pM to 1 mM) and high sensitivity (58.1 μA/mM) for the detection of H_2O_2 at neutral pHs due to synergistic contributions from conducting CNT, electron mediating PANI chains and porous silica network. HRP/MWNT-silica-PANI-NW has adequate characteristics for application such as biochip fabrication and biofuel cells.

Supporting information

FESEM and HVTEM images (S1); UV–vis and FTIR spectroscopy (S2); Electroactivity (S3); Electroactivity of modified electrodes in the presence of $Fe(CN)_6^{3-/4-}$ (S4); Cyclic voltammogram of HRP/MWNT-Silica-PANI-NW electrode at different pH conditions (S5); Cyclic voltammograms of modified electrodes in 0.1 M PBS (pH 7.0) (S6); DET behavior (S7); Current–time curve of modified electrodes (A) calibration plot of HRP/MWNT-silica-PANI-NW electrode at different concentration range of H_2O_2 (S8); Leaching test of HRP (S9).

Acknowledgements

This research was supported by Kyungpook National University Research Fund, 2008, KOSEF-2008-05994 and KRF-2006-C00001. The authors acknowledge the Korea Basic Science Institute(Deajon)

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.08.041.

References

Caruso, F., 2001. Adv. Mater. 13, 11–22.
Huang, H., Hu, N., Zeng, Y., Zhou, G., 2002. Anal. Biochem. 308, 141–151.
Hu, H., Ni, Y., Montana, V., Haddon, R.C., Parpura, V., 2004. Nano Lett. 4, 507–511.
Ivnitski, D., Artyushkova, K., Rincón, R.A., Atanassov, P., Luckarift, H.R., Johnson, G.R., 2008. Small 4, 357–364.
Kim, S.N., Rusling, J.F., Papadimitrakopoulos, F., 2007. Adv. Mater. 19, 3214–3228.
Lin, Y., Lu, F., Tu, Y., Ren, Z., 2004. Nano Lett. 4, 191–195.
Liu, H., Hu, N., 2003. Anal. Chim. Acta 481, 91–99.
Luo, X., Vidal, G.D., Killard, A.J., Morrin, A., Smyth, M.R., 2007a. Electroanalysis 19, 876–883.
Luo, X., Killard, A.J., Morrin, A., Smyth, M.R., 2007b. Electrochim. Acta 52, 1865–1870.
Long, J.S., Silvester, D.S., Wildgoose, G.G., Surkus, A.E., Flechsig, G.U., Compton, R.G., 2008. Bioelectron. Chem. 74, 183–187.
Luckarift, H.R., Spain, J.C., Naik, R.R., Stone, M.O., 2004. Nat. Biotechnol. 22, 211–213.
Ma, Y., Ali, S.R., Dodoo, A.S., He, H., 2006. J. Phys. Chem. B 110, 16359–16365.
Mulvaney, P., Liz-Marzan, L.M., Giersig, M., Ung, T., 2000. J. Mater. Chem. 10, 1259–1270.
Pantarotto, D., Partidos, C.D., Graff, R., Hoebeke, J., Briand, J.P., Prato, M., Bianco, A., 2003. J. Am. Chem. Soc. 125, 6160–6164.
Santhosh, P., Manesh, K.M., Gopalan, A., Lee, K.P., 2006. Anal. Chim. Acta 575, 32–38.
Singh, R., Pantarotto, D., McCarthy, D., Chaloin, O., Hoebeke, J., Partidos, C.D., Briand, J.P., Prato, M., Bianco, A., Kostarelos, K., 2005. J. Am. Chem. Soc. 127, 4388–4396.
Tatsuma, T., Ariyama, K., Oyama, N., 1998. J. Electroanal. Chem. 446, 205–209.
Wu, F., Hu, Z., Xu, J., Tian, Y., Wang, L., Xian, Y., Jin, L., 2008. Electrochim. Acta 53, 8238–8244.
Xia, Y., Yang, Z., Mokaya, R., 2006. Chem. Mater. 18, 140–148.
Yu, H., Jin, Y., Peng, F., Wang, H., Yang, J., 2008. J. Phys. Chem. C 112, 6758–6763.