Int. J. Electrochem. Sci., 9 **(2014)** 7672 - 7679

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Short Communication

PdS Nanoparticle Label Based DNA Biosensor for Rapid Detection of Mercury (II)

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Received: 4 September 2014 / *Accepted:* 11 October 2014 / *Published:* 28 October 2014

In the present study, we describe an electrochemical biosensor for Hg^{2+} detection by using the DNA probe. Two specific sequences for the probes, one has to ensure that the probe A hybridized with probe B modified on PdS nanoparticle to form stable DNA duplexes only in the presence of Hg^{2+} at a given operating temperature. After dissolving the PdS particles from the electrode, a mercury-film electrode is used for electrochemical detection of these Pd^{2+} ions which offer sensitively electrochemical signal transduction. The limit of detection of this assay in buffer is 16 nmol L^{-1}. The detection is also specific for Hg^{2+} without being affected by the other metal ions, such as Cd^{2+}, Li^{+}, Ba^{2+}, K^{+}, Ca^{2+}.

Keywords: Electrochemical biosensor, Mercury (II), DNA hybridization, PdS nanoparticles, T-Tmismatches

1. INTRODUCTION

The development of highly sensitive and selective methods of detecting the mercury (Hg^{2+}) contaminant in aqueous media is of great interest because of the serious threat of mercury pollution to human health and environment[1-3].

To date, several methods have been developed for detecting Hg^{2+} using probes made of molecular fluorophores[4-5], molecular chromophores[6-7], semiconductor nanocrystals[8-9], redox-active moieties[10-11], proteins[12], and oligonucleotides[13-14]. Detection and analysis methods based upon fluorescence, cyclic voltammetry, UV-Vis spectroscopy, and inductively coupled plasma mass spectrometry (ICP-MS) have been developed. These methods, however, require complicated instrumentation in certain cases, and often are limited with respect to sensitivity or selectivity.

Recently, the coordinate interaction between Hg^{2+} and bisthymine has attracted significant interest [15-16]. In detail, T-T mismatches in DNA duplexes selectively and strongly capture Hg^{2+} (binding constant higher than A-T), and the metal mediated T-Hg-T forms stable DNA duplexes. The specific interaction between thymine/mercury(II)/thymine (T-Hg^{2+}-T) has been widely used for Hg^{2+} detection[17-19]. A doubly-labeled Hg^{2+} specific probe was firstly reported to detect Hg^{2+} based on Hg^{2+} induced probe folding which brought the dye (fluorescein) and the quencher (dabcyl) into close proximity to favorintramolecular energy/electron transfer.

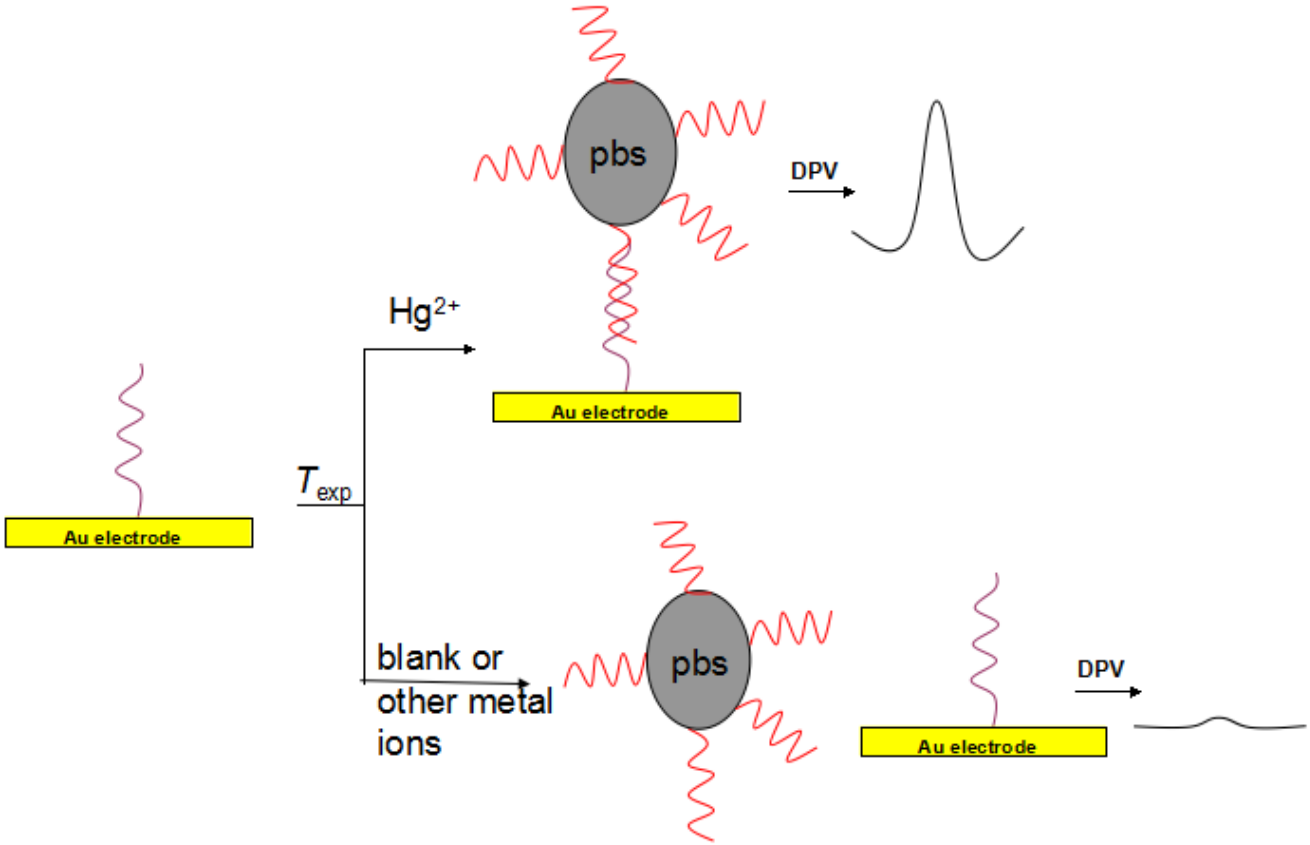

Figure 1. Schematic representation of the procedure to prepare electrochemical biosensor for the determination of mercury (II).

In the present study, we describe an electrochemical biosensor for Hg^{2+} detection by using the DNA probe (Figure 1). Two specific sequence for the probe [20], one has to ensure that the probe A and B form stable DNA duplexes only in the presence of Hg^{2+} at a given operating temperature. In the absence of Hg^{2+}, these probes do not form DNA duplexes because of a lower melting temperature (Tm) than the operating temperature due to mismatches formed in the DNA duplexes.

The probe B is labeled with MB and immoblized on Au nanoparticle to construct DNA functional nanoparticle. The probe A is immobilized on electrode surfaces to capture DNA functional nanoparticle in present of Hg^{2+} in aqueous solution, and the electrochemical signal of MB provides a readout signal for the quantitative detection of Hg^{2+}. This proposed biosensor takes the advantage of

the signal amplification effect of the labeled Au nanoparticle, resulting in high detection sensitivity. Therefore, this electrochemical biosensor is expected to have wide applications in environment monitoring.

2. EXPERIMENTAL

2.1 Reagents

The 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Shen energy Biocolor Biological Science and Technology Inc. (Shanghai, China). Bovine serum albumin (BSA) and thrombin were purchased from Dingguo Biotechnology Inc. (Shanghai, China). The nitric acid, cadmium chloride, sodium hydroxide, PBS (0.1 mol L^{-1}, PH=7.3), sodium acetate buffer (1 mol L^{-1}, pH=5.3) and other reagents were commercially available and of analytical reagent grade.

The DNA was obtained from Sangon Biotechology Inc. (Shanghai, China) with the following sequences:

Probe A: 5'-HS-C6-T_{13}

Probe B: 5'-NH_2-C6-A_6TA_6

2.2 Apparatus

Differential pulse voltammetry (DPV) measurements were performed using a CHI 660 Electrochemical Analyzer (CHI Instrument Inc., USA). The JB-1 stirring machine (Branson, Shanghai, China) and a TDL-16B centrifuge (Anting Science Instrument Inc., Shanghai, China) were used. The three-electrode electrochemical detection system consisted of a Au working electrode with sensing area of 3.14 mm^2, a Ag/AgCl reference electrode (saturated KCl) and a platinum wire counter electrode. The detection was carried out in a 5 mL electrochemical cell containing a mercury-coated glassy carbon working electrode (2 mm diameter), an Ag/AgCl reference electrode, and a platinum wire counter electrode.

2.3 Preparation of nano PdS

$Pb(NO_3)_2$ and Na_2S solutions were filtered through a 22 μm microporous membrane filter prior to use. PbS nanoparticles were prepared according to the literature [21] by using mercaptoacetic acid as the stabilizer. In brief, 9.22 μL mercaptoacetic acid was added to 50 mL 0.4 mmol L^{-1} $Pb(NO_3)_2$ solution, and then the pH was adjusted to 7 with 0.5 mol L^{-1} NaOH. The solution was bubbled with nitrogen for 30 min, followed by the slow addition of 1.34 mmol L^{-1} Na_2S to the mixture solution. The molar ratio of Na_2S to $Pb(NO_3)_2$ was kept at 2.5. The reaction was carried out for 24 h under nitrogen protection and then gradually a brown colloid which is the PdS nanoparticles covered with a carboxyl group was obtained. As TEM images show, the diameter of PdS nanopatricles was about 7 nm (Figure 2).

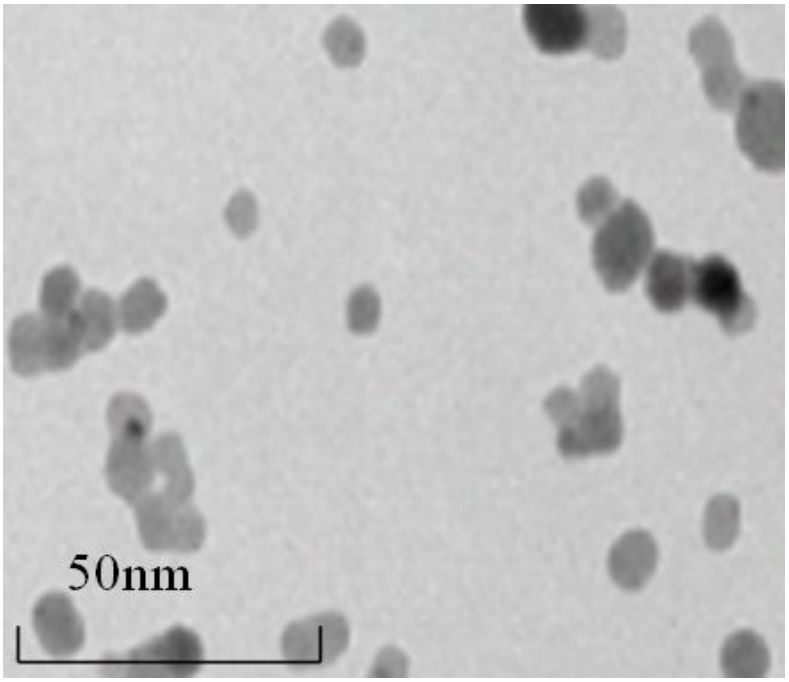

Figure 2. TEM image of the synthesized PdS nanoparticles

2.4 Preparation of DNA–PdS conjugate

200 μL of 0.1 mol L^{-1} imidazole was added to 2 OD of 5-amido-capped detection probe B. After stirred for 30 min, 100 μL of 0.1 mol L^{-1} EDC and 5 mL of PdS colloids were added to the mixture. The resulting mixture was stirred for 12 h at room temperature and then continued to centrifugate for at least 25 min at 14,000 rpm to remove the excessive DP. The yellow DNA-PdS precipitate was washed by 0.1 mol L^{-1} PBS and re-dispersed in 0.1 mol L^{-1} PBS. Then, the resulting solution was stored in the refrigerator for further use.

2.5 Immobilization of the probe A on Au electrode

The immobilization of probe A on Au electrode via Au-S binds was accomplished by first cleaning gold slides with a piranha solution (70% sulfuric acid, 30% H_2O_2) (Caution: Piranha solution reacts violently with many organic materials and should be handled with great care), followed by treatment of the electrodes with nitric acid, then with water and drying under air. 2 μL of appropriate probe A solution was dropped onto the electrode surface and the interaction was remained for 16 h. After that, the electrode was rinsed three times with phosphate buffer (0.1 mol L^{-1}, pH = 7.3) before next program.

2.6 Hg-induced DNA hybridization reaction

Fig.1 represents the procedure of preparing electrochemical biosensor for Hg^{2+} determination via DNA hybridization. The hybridization reactions were done by immersing the probe A-modified Au

electrode in the prepared solution containing appropriate Hg^{2+} and 600 μL of 10^{-9} mol L^{-1} DNA-PdS, and then incubated at 34 ^{o}C for 35 min with stirring. During this time, probe A that initially immoblized on Au electrode preferred to form DNA duplexes structure with probe B modified on PdS nanoparticle. The amount of Hg^{2+} could be indicated by the signal of DNA-PdS on the electrode which came from Hg-induced DNA hybridization.

2.7 Electrochemical detection

After a thorough washing procedure, the PdS nanoparticles on the gold substrate were dissolved by adding 200 μL of 0.10 mol L^{-1} HNO_3. Then 1.8 mL acetate buffer (0.1 mol L^{-1}, pH=5.3) was added into 200 μL of HNO_3 solution (containing dissolved Pd^{2+}). Electrochemical detection of the dissolved Pd^{2+} were performed at a mercury-film electrode using a 5 min deposition at -1.0 V in an acetate buffer solution (0.1 mol L^{-1}, pH=5.3). After the electrochemical deoxidation, DPV was immediately performed with the scan range from -0.8 to -0.2 V (Incr E 0.004 V, amplitude 0.05 V, pulse width 0.05 s, pulse period 0.2 s), resulting in an analytical signal due to the oxidation of Pd, which relates to the amount of the PdS nanoparticles for the hybridization format. The DPV peak height at a potential of -0.50 V of the oxidation of Pd was used in all of the measurements. The mercury-film electrode was prepared on a polished glassy carbon electrode by applying a potential of -1.10 V for 10 min in a 0.1 mol L^{-1} HCl solution containing 100 mg L^{-1} Hg^{2+}.

3. RESULTS AND DISCUSSION

3.1 Principle of detection of Mercury (II) based on Hg^{2+}-induced hybirdization

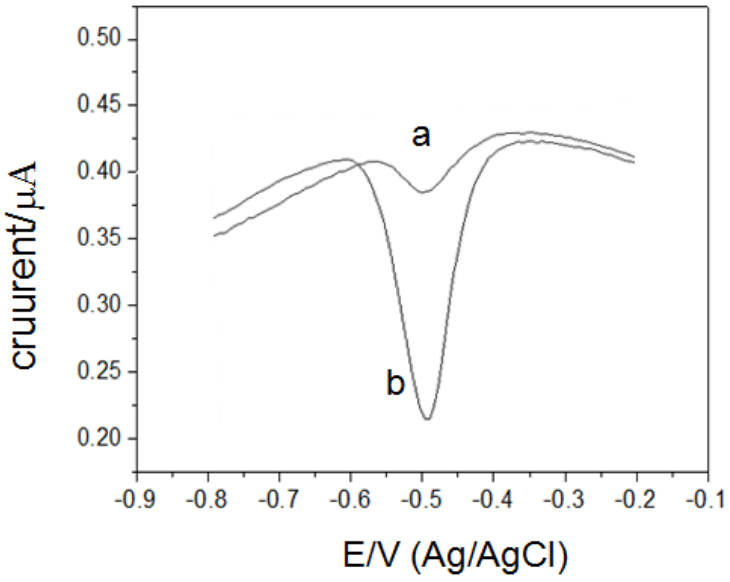

Figure 3. DPV response of the biosensor in the prepared solution containing 1 nmol L^{-1} DNA-PbS (a) without Hg^{2+} (b) in the presence of Hg^{2+} (c =1280 nmol L^{-1}).

The detection of mercury (II) was performed under the optimum stringency conditions determined above (0.10 mol L^{-1} $NaNO_3$, 34°C). Aliquots of various concentrations of Hg^{2+} were prepared from one concentrated Hg^{2+} stock solution (1 mmol L^{-1}). Firstly, the Probe A/AuE immersed into the prepared solution containing 1 nmol L^{-1} DNA-PbS, and then incubating it at 34°C for 30 minutes, only a relatively small DPV current signal was obtained as shown in Figure 3, curve a. Contrarily, a marked electrochemistry signal was obtained (Figure 3, curve b) after the Probe A/AuE was immersed in the solution containing the DNA-PbS and Hg^{2+}.

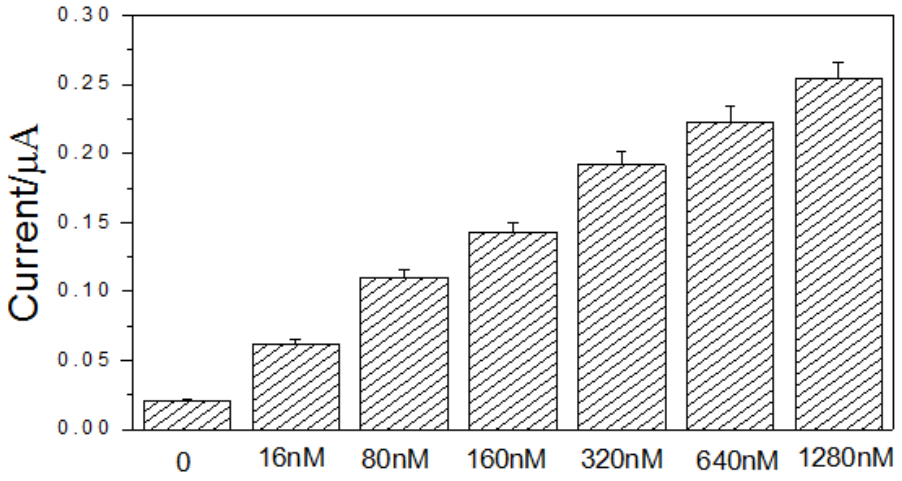

Figure 4. Graph of average DPV signal intensity as a function of the Hg^{2+}concentration in buffer.

It demonstrated that Probe A hybridization with Probe B only in present of Hg^{2+}, and then produced a significant electrochemistry signal. Without Hg^{2+}, there is little of adsorption of DNA-PbS onto the electrode and resulting in a small current signal. The probe A reaction with 1 nmol L^{-1} DNA-PdS probes in present of various concentrations of Hg^{2+}. Significantly, signal intensity proportionally correlates with Hg^{2+} concentration (Figure 4) and the detection limit of Hg^{2+} in buffer is 16 nmol L^{-1}.

3.2 Detecting specificity

In order to investigate the selectivity of the assay, other metal ions (Cr^{3+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Li^{+}, Ba^{2+}, K^{+}, Ca^{2+}, Mg^{2+}, Pb^{2+}) at 1 μmol L^{-1} were tested in a similar way. As shown in Figure 5, under stringency conditions, only the Hg^{2+} showed a significant response. These results showed that an extraordinary specificity of the detection system to the Hg^{2+} could be obtained. The results above fully indicated that the biosensor was sensitive and selective for Hg^{2+} assay. Compared to other electrochemical biosensor [22,23], the present biosensor possessed lower limit of detection, better selectivity. It indicated that this electrochemical biosensor could be applied to portable detection of Hg^{2+} in the emergency pollutant accident.

3.3 Detection of Hg^{2+} in Natural Media.

Standard solutions of varying Hg^{2+} concentration (0, 10, 100, 200, 400, 600, 800, and 1000 nmol L^{-1}) were prepared from a concentrated stock solution of $[Hg(ClO_4)_2]$ in the collected lake. The DNA-PbS, the buffer, the surfactant, and the salt solutions were also prepared in the lake water to evaluate the robustness of the assay under natural conditions. The detection of mercury (II) (Hg^{2+}) in natural media was performed in a manner similar to that used for the buffer samples and the detection limit of Hg^{2+} in lake water is 16 nmol L^{-1}. In conclusion, the electrochemical biosensor could be used for detecting mercury (II) in real water, effectively.

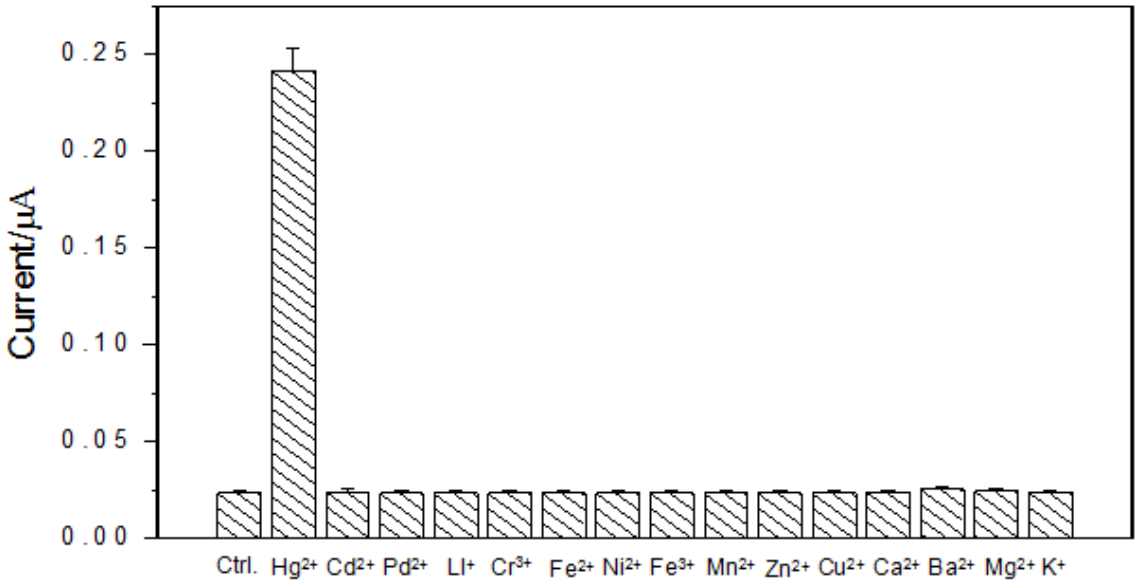

Figure 5. DPV response of the assay in the presence of various metal ions ([M^{x+}]) 1 μmol L^{-1}).

4. CONCLUSION

The present study has introduced a new mercury (II) electrochemical biosensor based on Hg^{2+}-induced hybirdiziation. As low as 16nmol L^{-1} Hg^{2+} has been specifically recognized, and other metal ion such as Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Li^{+} didn't affect the target detection. Therefore, this electrochemical biosensor is expected to have wide applications in environment monitoring.

ACKNOWLEDGEMENT
This work was financially supported by the National Natural Science Foundation of China (Grant No. 21265007, 21307161), the Special Scientific Research Funds for Central Non-profit Institutes, Chinese Academy of Fishery Sciences (No 2013C006), and JiangXi Science and Technology Committee (Grant No. 20122BAB215024, 20132BAB215032).

References

1. Z. Torres, M. Mora, R. Taylor, D. Alvarez-Bernal, H. Buelna, A. Hyodo, *Environ. Sci. Technol.*, 48(2014)6359.
2. J. Perrault, D. Miller, J. Garner, J. Wyneken, *Sci. Total Environ*., 463 (2013)61.
3. N. Zheng, Q. Wang, X. Zhang, D. Zheng, Z. Zhang, S. Zhang, *Sci. Total Environ*., 387(2007)96.
4. C. Chiang, C.C. Huang, C.W. Liu, H.T. Chang, *Anal. Chem*., 80(2008)3716.
5. L. Guo, H. Hu, R. Sun, G. Chen, *Talanta*, 79(2009)775.
6. Y. Wang, F. Yang, X. Yang, *Biosens. Bioelectron*., 25(2010)1994.
7. G. Mor-Piperberg, R. Tel-Vered, J. Elbaz, I. Willner, *J. Am. Chem. Soc*., 132(2010)6878.
8. B. Chen, Y. Yu, Z. Zhou, P. Zhong, *Chem. Lett*., 33(2004)1608.
9. X. Yan, Z. Cao, C. Lau, J. Lu, *Analyst*, 135(2010)2400.
10. J. Wang, B. Liu, *Chem. Commun*., 39(2008)4759–4761.
11. X. Zhou, D. Kong, H. Shen, *Anal. Chim. Acta*, 678(2010)124.
12. S. Jia, X. Liu, P. Li, D. Kong, H. Shen, *Biosens. Bioelectron*. 27(2011)148.
13. Liu X., Tang Y., Wang L., Zhang, J., Song S., Fan C., Wang S., *Adv. Mater*., 19(2007)1471.
14. D. Li, A. Wieckowska, I. Willner, *Angew. Chem. Int. Ed*. 120(2008)3991.
15. G. Clever, C. Kaul, T. Carell, *Angew. Chem., Int. Ed*., 46(2007)6226.
16. Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, *J. Am. Chem. Soc*., 128(2006)2172.
17. X. Xue, F. Wang, X. Liu, *J. Am. Chem. Soc*., 130(2008)3244.
18. J. Liu, Y. Lu, *Angew. Chem. Int. Ed*., 46(2007)7587.
19. D. Li, A. Wieckowska, *Angew. Chem. Int. Ed*., 47(2008)3927.
20. L. Jae-Seung, A. Chad, *Anal. Chem*., 80(2008)6805.
21. A. Radi, L. Sanchez, E. Baldrich, C. Sullivan, *Anal. Chem*., 77(2005)6320.
22. T. Li, S. Dong, E. Wang, *Anal. Chem*., 81(2009) 2144.
23. Y. Lai, Ma Y. Y., Sun L. P., *Electrochim Acta*, 56(2011) 3153.