Int. J. Electrochem. Sci., 9 **(2014)** 6985 - 6992

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Performances of p-Aminophenol Redox Cycling by Thiols and Tris(2-carboxyethyl)phosphine on Cysteamine- and 3-Mercaptopropionic acid-Covered Gold Electrodes

Ming La, Yunxiao Feng, Chengye Yang, Changdong Chen[*]

College of Chemistry and Chemical Engineering, Pingdingshan University, Pingdingshan, Henan 467000, People's Republic of China
[*]E-mail: lmccd5631@163.com

Received: 6 August 2014 / *Accepted:* 15 September 2014 / *Published:* 29 September 2014

Amine- and carboxyl-terminal self-assembled monolayers (SAMs) have been used frequently for controlling the adsorption of biomolecules. p-Aminophenol (p-AP) redox cycling by reducing reagents have been used to improve the sensitivity for signal amplification detection. However, most of the reported reducing reagents are unsuitable for gold electrodes due to the high background current caused by the oxidation reaction of the reducing reagents on highly electrocatalytic gold electrodes. Thiols and tris(2-carboxyethyl)phosphine (TCEP) are well-known reagents for reducing quinone. In this work, we evaluated the performances of p-AP redox cycling by TCEP and thiols on cysteamine- and 3-mercaptopropionic acid (MPA)-terminal gold electrodes. The results indicated that TCEP is more suitable for p-AP redox cycling on the modified gold electrode because of its negligible background current and fast chemical reaction with the oxidized p-AP. The work will be valuable for the development of affinity biosensors with p-AP redox cycling on gold substrates.

Keywords: p-Aminophenol; alkaline phosphatase; redox cycling; tris(2-carboxyethyl)phosphine; thiols; gold electrode

1. INTRODUCTION

Electrochemical absorption biosensors are attractive for a broad range of biomedical, environmental, agricultural, and clinical analyses because they offer multiple advantages: high sensitivity, low cost, low power requirement, and high compatibility with advanced micromachining technologies. Preparation of the adsorption biosensors often relies on the controlled chemical modification of surfaces. Self-assembled monolayers (SAMs) with amine- or carboxyl-terminal on gold electrode have been commonly used for the immobilization of biomolecules, such as antigens, antibodies, and nucleic acids [1,2]. At present, sensitivity and noise reduction are very crucial for the

successful development of electrochemical absorption biosensors. Therefore, a common goal in the development of electrochemical biosensors is to improve the sensitivity. For example, many efforts have been made to reduce the detection limit by amplifying the signal using various labels, such as functionalized liposomes, enzymes, carbon nanotubes and nanoparticles [3-8].

Alkaline phosphatase (ALP) is one of the most used enzymatic labels for design of absorption biosensors [4,9]. It can remove a phosphate group from the substrate by hydrolysing phosphoric acid monoesters into a phosphate ion and a molecule with a free hydroxyl group. For example, ALP dephosphorylates p-aminophenyl phosphate (p-APP) enzymatically to produce electrochemically active species p-aminophenol (p-AP), which is detected amperometrically by substrate electrode (Fig. 1) [4]. However, ALP-based detection method suffers from drawbacks ultimately related to the limited stability of p-AP. To overcome this defect, reducing reagents can be added to the reaction mixture to prevent p-AP oxidation [10,11]. Moreover, the reducing reagents can regenerate p-AP from *p*-quinone imine (QI, the oxidation production of p-AP) in the electrochemical detection. The process of regenerating ALP-amplified electroactive species p-AP is called p-AP redox cycling, which has been employed recently in the amplified detection of proteins and DNA [11,12]. The redox cycling efficiency is related to the reducing capability and background current of the reducing reagents. The currently reported reductants such as sodium borohydride ($NaBH_4$) and hydrazine are suitable for indium-tin oxide (ITO) electrodes but not gold electrodes because of the high background currents caused by the oxidation reaction of the reductants on highly electrocatalytic metal electrodes [10,11, 13-15].

Thiols are well-known reagents for reducing quinone, and the direct electrochemistry of thiols at solid electrodes often needs large anodic potential to obtain the appreciable signal [16-18]. Tris(2-carboxyethyl)phosphine (TCEP) is a reducing reagent that can break disulfide bonds and reduce quinone at a fast rate [19-21]. Moreover, it is highly water soluble and stable in air. Recently, it is reported that TCEP can be used for developing electrochemical sensors with p-AP redox cycling on ITO electrodes [11,21,22]. Because gold electrode has been widely applied to create electrochemical biosensors, in this work, we demonstrated the performances of developing electrochemical sensors using p-AP redox cycling by TCEP and thiols on amine- and carboxyl-terminal gold electrodes.

2. EXPERIMENTAL

2.1 Chemicals and reagents

3-Mercaptopropionic acid (MPA), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC), , TCEP, cysteine (Cys), cysteamine hydrochloride, glutathione (GSH), 6-mercapto-1-hexanol (MCH), bovine serum albumin (BSA), KH_2PO_4 and K_2HPO_4 were obtained from Sigma-Aldrich. p-AP and Na_2SO_4 were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). The supporting electrolyte was phosphate-buffered saline solution (PBS buffer, 10 mmol L^{-1}, pH 7.4) containing 50 mmol L^{-1} Na_2SO_4. All aqueous solutions were prepared freshly with a Millipore system (Simplicity Plus, Millipore Corp.).

2.2 Evaluation the performances of p-AP redox cycling by TCEP and thiols

Gold disk electrodes with a diameter of 2 mm were polished with diamond pastes down to 3 μm and alumina pastes down to 0.3 μm, and then sonicated in ethanol and water. The cysteamine-covered electrodes were prepared by immersing the cleaned gold electrodes in an aqueous solution containing 10 mM cysteamine in the darkness for 12 hours, and the MPA-covered electrodes were prepared by immersing the electrodes in an ethanol solution containing 10 mM MPA. The performances of p-AP redox cycling by TCEP and thiols on cysteamine- and MPA-covered electrodes were compared in PBS solution with and without addition of reducing reagents. Ethanolamine was immobilized onto MPA-covered gold electrode by cross-linking ethanolamine molecules onto the MPA self-assembled monolayers (SAMs) via the EDC-mediated amine coupling reaction [1]. Specifically, the MPA-covered electrode was soaked in 40 mM EDC solution containing 0.5 mM ethanolamine for 15 min, followed by rinsing the electrode with excess water. The voltammetric determination was performed on a CHI660D electrochemical workstation (CH instruments, Shanghai, China). A platinum wire and a Ag/AgCl electrode were used as the auxiliary and the reference electrodes, respectively.

3. RESULTS AND DISCUSSION

3.1 Background current of TCEP and thiols on cysteamine- and MPA-covered gold electrodes

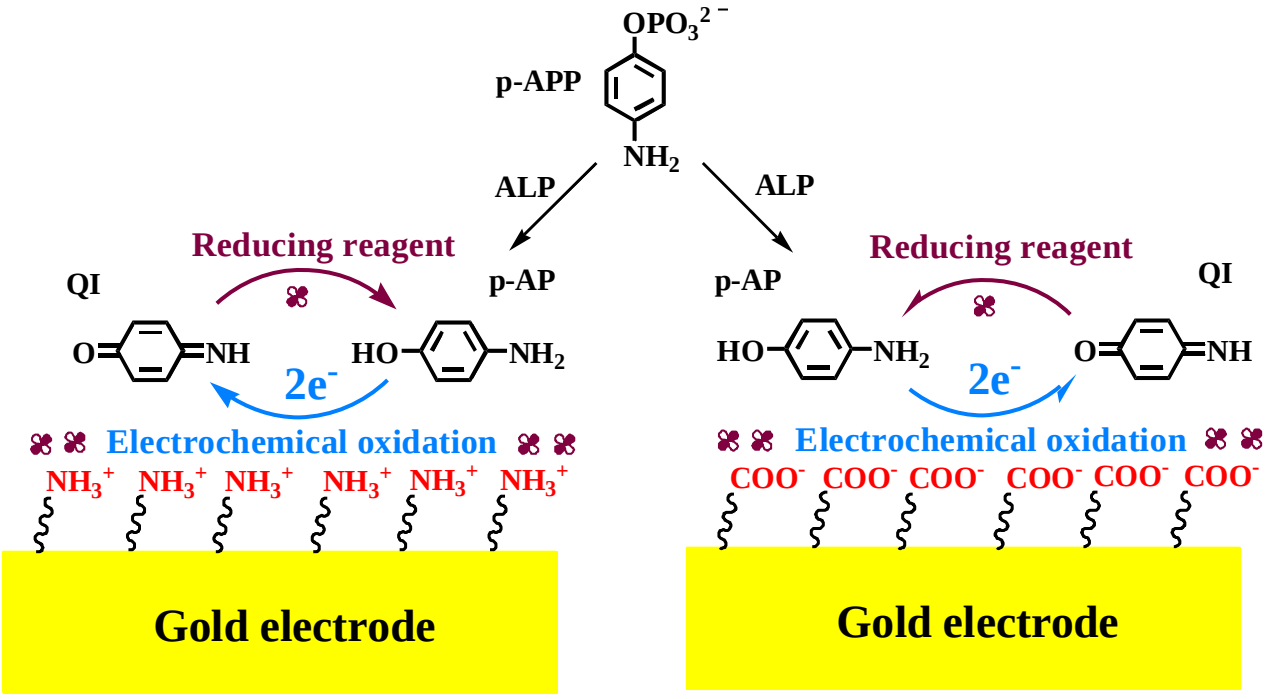

Figure 1. p-AP redox cycling by reducing reagent on the amine- and carboxyl-terminal gold electrodes.

SAMs are an inexpensive and versatile surface coating for molecular recognition for sensors, and, in particular, alkanethiol. The exposed functional terminus of SAM is utilized for the attachment

of biomolecules through covalent bond formation or non-covalent interaction. For example, SAMs with amine- and carboxyl-terminal on gold have been used frequently for controlling the adsorption of biomolecules. As mentioned in Introduction, p-AP redox cycling by chemical reductants been employed recently in the amplified detection of biomolecules. However, most of these reagents are only used on indium-tin oxide (ITO) electrodes but not gold electrodes due to the highly electro-catalysis of gold. Since cysteamine and MPA modified SAMs on gold electrodes are the most commonly employed for immobilization of biomolecules, we therefore investigated the performances of p-AP redox cycling on cysteamine- and MPA-covered gold electrodes (Fig. 1).

We first compared the background current of TCEP and several thiol-containing compounds on the modified gold electrodes. As shown in Fig. 2, the background current of TCEP (red curve) and GSH (blue curve) are compared to that of the control (black curve), while the oxidation (anodic) current of Cys (magenta curve) and cysteamine (green curve) are much higher than that of TCEP and GSH on both cysteamine- and MPA-covered gold electrodes. The low background current of GSH implied that GSH is hard to be oxidized on the modified electrode. The results also implied that TCEP and GSH are preferred for the application on the modified electrodes. Note that the background current of cysteamine was higher than that of Cys on cysteamine-covered gold electrodes, but lower than that of Cys on MPA-covered gold electrodes. A possible explanation is that the positively charged amine groups (Fig. 2A) hinder the access of the positively charged cysteamine to the electrode surface, but the negatively charged MPA SAMs facilitate the electron transfer of positively charged cysteamine (Fig. 2B).

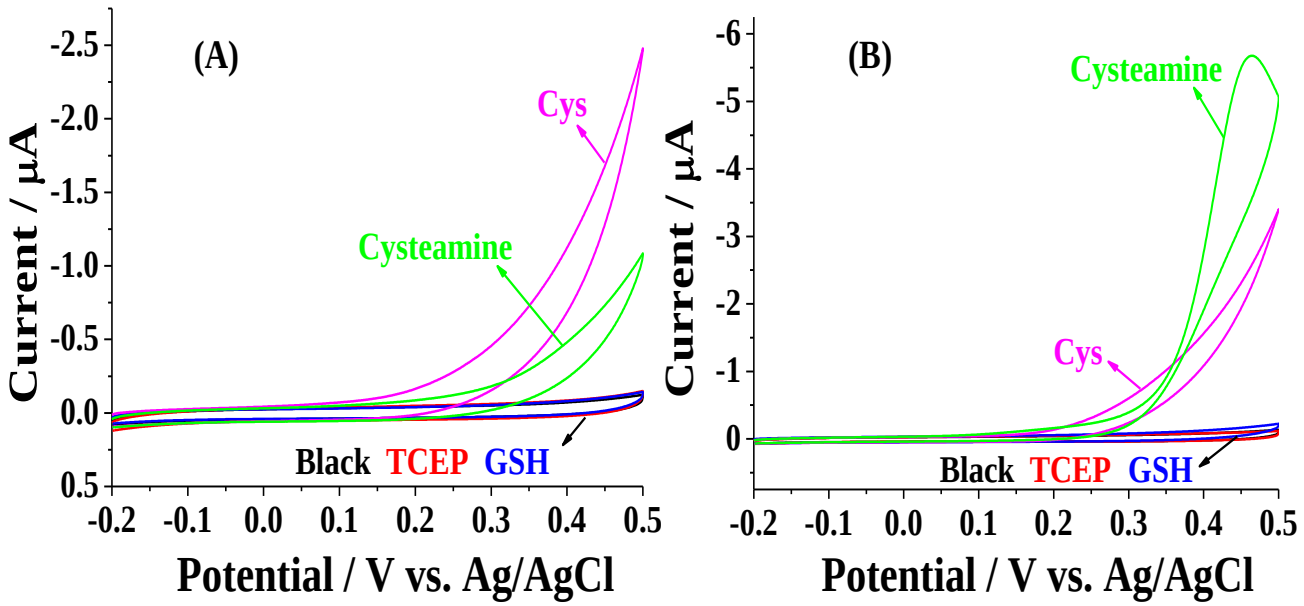

Figure 2. Cyclic voltammograms (CVs) of cysteamine- (A) and MPA- (B) covered gold electrode in the absence and presence of different reducing reagents. The concentrations of all the reducing reagents were 2 mM. The scan rate was 20 mV/s.

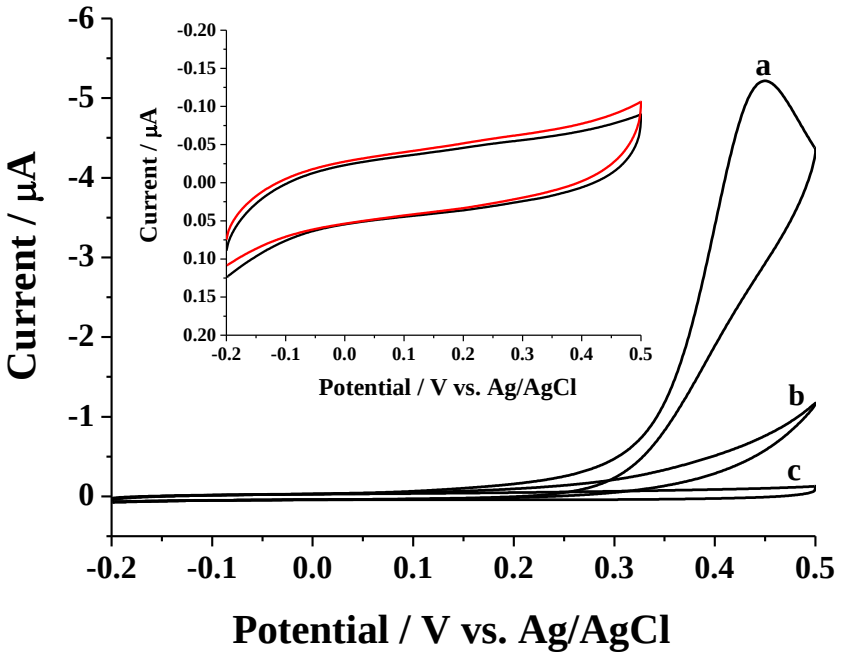

Figure 3. CVs of MPA-covered gold electrode in PBS solution with (curve a) and without (curve c) addition of cysteamine. Curve b was acquired at ethanolamine-modified MPA-covered gold electrode in cysteamine-containing solution. The inset shows that the CVs of MCH-covered electrode in the absence (black curve) and presence (red curve) of cysteamine. The concentration of cysteamine was 2 mM.

To confirm this hypothesis, we investigated the background current of cysteamine on the neutral SAMs, prepared by cross-linking ethanolamine molecules onto the MPA SAMs via the EDC-mediated amine coupling reaction. As a result, we found that the background current decreases apparently after the modification with ethanolamine (*cf.* curves a and b in Fig. 3), demonstrating that the neutral SAMs hinder the access of cysteamine to the electrode surface. The current is still higher than that of the control (curve c), indicating that not all the carboxyl groups on electrode were activated by EDC for coupling with ethanolamine, since low background current was observed on neutral MCH-covered surface (see the inset in Fig. 3).

3.2 p-AP redox cycling by TCEP and GSH

In view of the low background of TCEP and GSH, we tested their electrocatalytic activities in p-AP redox cycling on MPA-covered electrode. As shown in Fig. 4A, a reversible anodic peak of p-AP was observed on MPA-covered electrode in the absence of TCEP and GSH. However, upon the addition of TCEP to p-AP solution, an increase in the oxidation current and a decrease in the reduction current was observed, implying that QI resulting from the electrochemical oxidation of p-AP was reduced immediately by TCEP. However, only a slight increase in the oxidation current was observed in the presence of GSH. The results indicated that the chemical reaction of QI to TCEP is faster than to GSH. Therefore, TCEP is more suitable p-AP redox cycling on MPA-covered gold electrode in view of its fast reaction with the oxidized p-AP.

In the ALP-based electrochemical sensors, the major drawback is the instability of p-AP resulting from the dephosphorylation of p-aminophenyl phosphate by ALP. Reducing reagents are commonly added to the reaction mixture to protect p-AP from oxidation. We also investigated the stability of p-AP in the absence and presence of TCEP. As show in Fig. 4B, the electrochemical signal of p-AP decreased after 60 min, while no apparent difference in the CVs was observed after incubation for 1 and 60 min in the presence of TCEP. The result demonstrated that TCEP prevented the aerial oxidation of p-AP in this process. Note that TCEP did not inhibit the enzymatic activity of ALP. Therefore, TCEP is suitable for p-AP redox cycling.

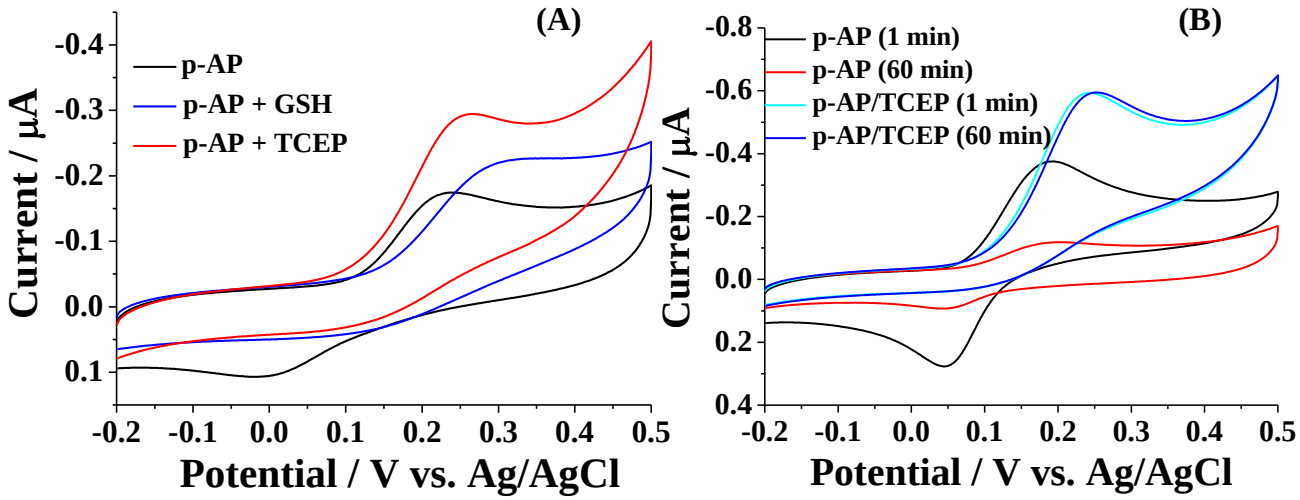

Figure 4. (A) CVs of MPA-covered gold electrode in 20 μM p-AP solutions with or without GSH and TCEP. (B) CVs at the modified electrode in 50 μM p-AP solutions in the absence and presence of TCEP. p-AP solutions were incubated with and without TCEP for 1 min and 60 min at room temperature, respectively. The other experimental conditions are the same as those in Figure 2.

3.3 Effect of BSA on the p-AP redox reaction

The real samples contain approximately 150–400 mg L^{-1} plasma proteins [23]. To prevent the non-specific absorption, serum proteins were commonly used to block the unreacted gold surface in the elaboration of affinity biosensors. For example, BSA has been applied successfully in the biochemical fields including enzyme-linked immunosorbent assays (ELISAs), immunoblots, and immunohistochemistry, because of its stability to increase signal in assays, its lack of effect in many biochemical reactions, and its low cost. Here, we also investigated the effect of BSA on the redox reaction of p-AP. As shown in Fig. 5, no apparent difference was observed in the voltammograms of MPA-covered electrode before and after coating with BSA. However, we found that the oxidation current of p-AP on BSA-coated MPA-covered electrode is higher than that on the MPA-covered electrode, while the reduction current in the former is lower than that in the latter. A reasonable explanation is that BSA catalyzed the p-AP redox reaction. The result is acceptable since BSA contains cysteine residues that could reduce the oxidized p-AP. Thus, BSA not only avoids the non-specific absorption but also prevent the auto-oxidation of p-AP to some extent.

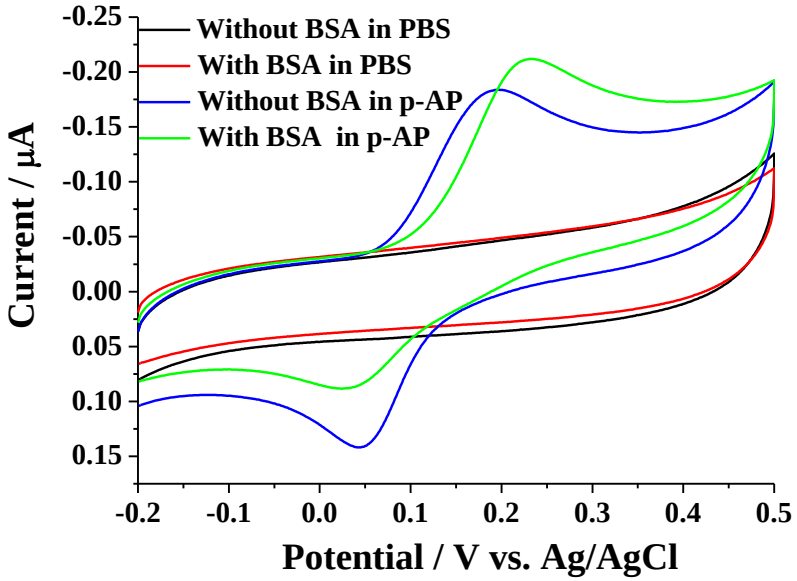

Figure 5. CVs of MPA-covered gold electrode before and after coating with BSA in the absence and presence of 20 μM p-AP. The other experimental conditions are the same as those in Figure 2.

4. CONCLUSION

In this work, the performances of p-AP redox cycling by TCEP and thiols on amine- and carboxyl-terminal gold electrodes were evaluated. The background current of these reducing reagents decreases in the order of Cys > cysteamine > TCEP, GSH on amine-terminal gold electrode and decreases in the order of cysteamine > Cys > GSH, TCEP on carboxyl-terminal gold electrode. Among these reagents, TCEP is more suitable for p-AP redox cycling on the modified gold electrodes because of its negligible background current and fast chemical reaction with the oxidized p-AP. The amine- and carboxyl-terminal SAMs on gold surface have widely used for the elaboration of affinity biosensors; thus, we believe that our work will be valuable for the development of affinity biosensors using p-AP redox cycling.

ACKOWLEDGMENTS
This work was support by the Fund of Department of Science and Technology Department in Henan (KJT142102310462), the Youth Research Foundation in Pingdingshan University (20120015) and the High-Level Personnel Fund in Pingdingshan University (1011014/G).

References

1. L. Liu, D. Deng, Y. Xing, S. Li, B. Yuan, J. Chen, N. Xia. *Electrochim. Acta* 89 (2013) 616.
2. J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo, G. M. Whitesides. *Chem. Rev.* 105 (2005) 1103.
3. H. Karimi-Maleh, M. Keyvanfard, K. Alizad, V. Khosravi, M. Asnaashariisfahani. *Int. J. Electrochem. Sci.* 7 (2012) 6816.
4. E. Kim, K. Kim, H. Yang, Y. T. Kim, J. Kwak. *Anal. Chem.* 75 (2003) 5665.

5. L. Liu, J. Du, S. Li, B. Yuan, H. Han, M. Jing, N. Xia. *Biosens. Bioelectron.* 41 (2013) 730.
6. J. T. Mason, L. Xu, Z.-M. Sheng, T. J. O'Leary. *Nat. Biotechnol.* 24 (2006) 555.
7. N. Xia, D. Deng, L. Zhang, B. Yuan, M. Jing, J. Du, L. Liu. *Biosens. Bioelectron.* 43 (2013) 155.
8. N. Xia, L. Zhang, G. Wang, Q. Feng, L. Liu. *Biosens. Bioelectron.* 47 (2013) 461.
9. A. Samphao, P. Suebsanoh, Y. Wongsa, B. Pekec, J. Jitchareon, K. Kalcher. *Int. J. Electrochem. Sci.* 8 (2013) 3254.
10. J. Das, K. Jo, J. W. Lee, H. Yang. *Anal. Chem.* 79 (2007) 2790.
11. M. R. Akanda, Y.-L. Choe, H. Yang. *Anal. Chem.* 84 (2012) 1049.
12. A. Walter, J. Wu, G.-U. Flechsig, D. A. Haake, J. Wang. *Anal. Chim. Acta* 689 (2011) 29.
13. J. Das, M. A. Aziz, H. Yang. *J. Am. Chem. Soc.* 128 (2006) 16022.
14. S. J. Kwon, E. Kim, H. Yang, J. Kwak. *Analyst* 131 (2006) 402.
15. S. J. Kwon, H. Yang, K. Job, J. Kwak. *Analyst* 133 (2008) 1599.
16. T. Inoue, J. R. Kirchhoff. *Anal. Chem.* 74 (2002) 1349.
17. T. Inoue, J. R. Kirchhoff. *Anal. Chem.* 72 (2000) 5755.
18. J. C. Harfield, C. Batchelor-McAuley, R. G. Compton. *Analyst* 137 (2012) 2285.
19. R. E. Hansen, J. R. Winther. *Anal. Biochem.* 394 (2009) 147.
20. M. P. Bova, M. N. Mattson, S. Vasile, D. Tam, L. Holsinger, M. Bremer, T. Hui, G. McMohan, A. Rice, J. M. Fukuto. *Arch. Biochem. Biophys.* 429 (2004) 30.
21. M. R. Akanda, M. A. Aziz, K. Jo, V. Tamilavan, M. H. Hyun, S. Kim, H. Yang. *Anal. Chem.* 83 (2011) 3926.
22. M. R. Akanda, V. Tamilavan, S. Park, K. Jo, M. H. Hyun, H. Yang. *Anal. Chem.* 85 (2013) 1631.
23. L. Liu, S. Li, L. Liu, D. Deng, N. Xia. *Analyst* 137 (2012) 3794.