An electrochemical biosensor based on cobalt nanoparticles synthesized in iron storage protein molecules to determine ascorbic acid

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Abstract

The electrochemical detection of ascorbic acid (AA) was investigated using a cobalt(III)–ferritin immobilized on a self-assembled monolayer modified gold electrode in phosphate buffer solution (pH 7.5). The modified electrode showed excellent electrochemical activity for oxidation of AA. The response to AA on the modified electrode was examined using cyclic and differential pulse voltammetry techniques. The resulting biosensor showed a linear response to AA in a concentration range from 6.25×10^{-6} to 2.31×10^{-5} M with sensitivity of 86,437 μAM^{-1} and detection limit of 4.65×10^{-6} M based on a signal-to-noise ratio of 3. Electrochemical parameters including the charge transfer coefficient (α) and the apparent heterogeneous electron transfer rate constant (k_s) for AA were found to be 0.52 and 1.054 Sec^{-1}, respectively. It has been shown that, using this modified electrode, AA can be determined with high sensitivity, low detection limit, and high selectivity. Volume 00, Number 0, Pages 1–6, 2015

Keywords: *cobalt nanoparticles, biosensor, ferritin, direct electron transfer, ascorbic acid*

1. Introduction

In recent years, there has been a great interest in the design, fabrication, and application of sensitive and selective electrochemical sensors. Electrochemical sensing is a convenient way to determine species in solution both quantitatively and qualitatively [1, 2]. The benefit of electroanalytical techniques over other detection methods such as luminescence, spectroscopy, and chromatography is due to their cost effectiveness, the ease of use, accuracy, and consistency [2, 3]. In these areas, the development of voltammetric sensors to determine certain neurotransmitters, such as ascorbic acid (AA) as an essential

Abbreviations: *α, charge transfer coefficient; λ, reorganization energy for electron transfer; ν, scan rate; AA, ascorbic acid; C_i, irreversible chemical reaction; CV, Cyclic voltammetric; DET, direct electron transfer; DPV, differential pulse voltammetry; DTSP, dithiobis-N-succinimidyl propionate; E_q, quasireversible electrochemical process; k°, standard heterogeneous rate constant; K_S, heterogeneous electron transfer rate constant; SAMs, self-assembled monolayers.*

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Received 20 March 2015; accepted 10 June 2015

DOI: 10.1002/bab.1410

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nutritional factor, has gained a great deal of attention in recent years [4, 5]. AA, also known as vitamin C, is one of the most important vitamins widely exist in various plants and taking part in some important biological reactions such as free radical scavenging, cancer preventing, and immunity improving [6]. This compound exists widely in food, plant, and animal tissues, but cannot be synthesized by the human body. A recommended daily intake of AA is about 70–90 mg. Inadequate intake will result in the symptoms of scurvy, gingival bleeding, and so on; excess AA intake will also lead to urinary stone, diarrhea, and stomach convulsion [7]. Oxidant stress associated with insulin resistance and noninsulin-dependent diabetes mellitus contributes to poor insulin action found that increased oxidative stress has been suggested to be involved in the pathogenesis and progression of diabetic tissue damage. AA is an important antioxidant in human. Antioxidant terminates the chain of the reaction by removing free radicals and inhibits other oxidation reaction by oxidizing themselves. AA is structurally similar to glucose and can replace it in many chemical reactions, and thus is effective in prevention of nonenzymatic glycosylation of proteins. In addition, AA acts as a regulator of catabolism of cholesterol to bile acid in guinea pig and has been demonstrated to be an important factor in lipid regulation. Several studies showed decreased basal AA level in diabetic patients and also it is suggested that oxidative stress is increased in diabetes. High doses of AA (2 g/day) have been shown to improve blood glucose regulation and reduce serum cholesterol and triglyceride in type 2 diabetes patients [8, 9]. Due to the importance of AA in life cycle, its determination in aqueous solution is very important. AA can be easily oxidized electrochemically at conventional electrodes, which have been used to detect AA. However, the direct electrochemical oxidation of AA is possible, but requires high overpotentials at bare electrodes, which may lead to electrode fouling by its oxidation products with poor reproducibility, low selectivity, and sensitivity [7–12]. Therefore, several approaches have been used to overwhelm these problems, including the modification of electrode surface with polymers [13], ionic liquid [14], metal nanoparticles [15], carbon nanotubes [16], self-assembled monolayers (SAMs) terminated with suitable functionalized groups [17], and macrocyclic compounds [18]. Among the electrochemical techniques, a biosensor based on direct electron transfer (DET), that is, the third-generation biosensor, between an electrode and immobilized biomolecules such as proteins and enzymes has gained great interest among scientists due to its importance in elucidating the intrinsic thermodynamic and kinetic properties of proteins and its potential application in biosensors and biofuel cells [19–22]. It is not so convenient to process the DET between redox proteins and electrodes, since the three-dimensional structure of proteins obstructs interaction with the electrode or because of its denaturation upon adsorption onto the surface of the electrode and following passivation of the electrode surface [23]. SAMs are excellent model systems to study wetting, adhesion, lubrication, corrosion, nucleation, and protein adsorption [24]. Due to the formation of strong S–Au covalent bond, alkanethiols and disulfides are able to produce a stable SAM on the gold surface. This self-assembling method yields highly organized monomolecular films on the electrode surface and can be applied to contrive the surface energy of the electrode. These monolayers afford high flexibility to design the surfaces in some cases the oriented anchorage of biomolecules, leading to achieve a high improvement in biosensor fabrication [25–31]. Ferrtin is an iron storage protein with 24 polypeptides subunits arranged with 432 symmetry to form nearly a spherical molecule with 12 nm in overall diameter with hollow interiors of 8 nm in diameter. In the native ferritin, iron is stored within an 8 nm diameter cavity formed by the protein as a particle of ferric oxyhydroxide (which may also contain phosphate). The protein has six twofold symmetry axes, four threefold symmetry axes, and three fourfold symmetry axes. At the subunit junctions around the threefold symmetry axis, acidic amino residues accumulate and form hydrophilic channels. These channels are thought to allow metal ions passage. Around the fourfold symmetry axis, hydrophobic amino acids are gathered to form hydrophobic channels, and this channel is considered to be an outlet of proton (or entrance of electron), but it is not yet clear. The naturally occurring iron core of ferritin can be removed *in vitro* by chemical and electrochemical reduction. Ferritin is also known to form cores of metal compounds other than iron [32–38]. So, ferritin is able to be reconstituted in the presence of various electroactive materials (CdS, Co, Mn, Ni, Pd, and Pt), which can make it suitable for biosensor and biofuel cell applications [28].

Also, we have used self-assembly technology to immobilize cobalt(III)–ferritin on gold electrode for the construction of cobalt(III)–ferritin biosensor. We investigated the DET of cobalt(III)–ferritin at gold electrode and found an electrochemical response at the electrode, which is modified by dithiobis-*N*-succinimidyl propionate (DTSP). On the one hand, DTSP forms a stable SAM on a gold surface owing to the strong S–Au covalent bond, and on the other hand, it reacts with the protein. The surface electrochemical behavior of cobalt(III)–ferritin on gold electrode, modified by DTSP, has been already reported [24].

In the present study, the electrochemical activity of the modified electrode for the oxidation of AA has been investigated. To evaluate the possible analytical application of the modified electrode, it was used for the detection of AA at micromolar concentration range.

2. Experimental

2.1. Materials and reagents

Horse spleen apoferritin was purchased from Sigma and was used without further purification. All other chemicals were purchased from Merck, Germany.

2.2. Apparatus

All electrochemical experiments were performed via using an electroanalyzer (SAMA 500). A conventional three-electrode

cell was used with a saturated Ag/AgCl (3 M NaCl) electrode as a reference electrode, a platinum wire as a counter electrode, and a gold electrode as a working electrode. All experiments were carried out at ambient temperature. All pH measurements were performed using a Metrohm 632 pH-meter (Herisau, Switzerland).

2.3. Preparation of biosensor

According to our previous work, apoferritin was used as a constraining reaction environment for the synthesis of cobalt(III) nanoparticles. Under a range of synthetic conditions, the protein cage remains unaltered [38]. Self-assembly technique provides a facile method to immobilize cobalt(III)–ferritin and it can be utilized to provide a matrix for the fabrication of biosensors. Therefore, the cleaning of the gold electrode is a critical important step for SAM formation, which it should be accomplished systematically. Electrode pretreatment consisted of manually polishing the electrode surface with alumina slurry of 0.5 mm, until the surface looks a mirror. Then, the electrode was sonicated in pure ethanol for 5 Min. After the mechanical cleaning, the electrodes underwent a chemical treatment by immersion in a piranha solution (1:3 mixture of 30% H_2O_2–conc. H_2SO_4) for 5 Min, and then the electrode was thoroughly washed with water. The next step was the gold electrode modification process using DTSP, which underwent dissociative chemisorption onto the gold electrode. The DTSP solution was dissolved in DMSO (5 mM). The modified electrode was prepared by spreading 5 μL of 5 mM DTSP solution onto the gold electrode with a microsyringe. Cobalt(III)–ferritin immobilization on DTSP-modified gold electrode was performed by dipping in 1 mL of cobalt(III)–ferritin for 18 H about 4 °C with applied potential of 1.0 V against Ag/AgCl, and then was rinsed with 100 mM HEPES buffer containing 60 mM of NaCl (pH 8.7). Protein was then covalently attached to the modified Au electrode through the reaction of the terminal succinimidyl groups with amino groups of cobalt(III)–ferritin molecules [24]. Then, this electrochemical biosensor was used to detect AA.

2.4. Characterization of the AA biosensor

Cyclic voltammetric (CV) and differential pulse voltammetry (DPV) were carried out in quiescent solutions with the scan rate of 100 mVs^{-1}. All potentials were reported versus the Ag/AgCl reference electrode.

3. Results and Discussion

3.1. Electrochemical behavior of biosensor toward AA

Electrochemical oxidation of AA using immobilized cobalt(III)–ferritin onto modified electrode was investigated by recording cyclic voltammograms. For bare gold electrode, no anodic response of AA oxidation was observed in the potential range from –1,000 to −400 mV (Fig. 1a). However, when cobalt(III)–ferritin/DTSP/Au electrode (biosensor) was dipped into buffer containing AA, the obvious increase in anodic peak current

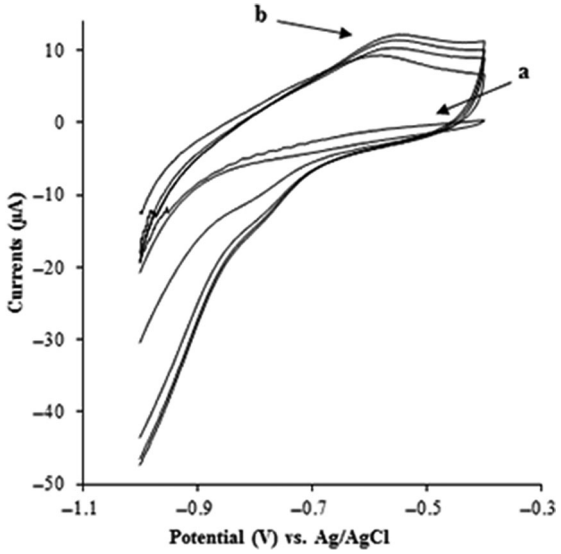


FIG. 1 *Cyclic voltammograms of 1 mM AA at a bare electrode (a) and cobalt(III)–ferritin/DTSP/Au electrode (biosensor) with different concentrations of AA from inner to outer: 0.0, 9.25, 0.126, and 0.187 μM (b) in 0.1 M PBS (pH 7.5); scan rate: 100 mV/Sec.*

was observed with the increase in the concentration of AA (Fig. 1b). It confirms that immobilized cobalt(III)–ferritin has high catalytic ability for AA oxidation. Therefore, this biosensor is very suitable for the fabrication of third generation of mediatorless biosensors to determine AA.

Figure 2a shows the CVs of 1.0 mM AA in 0.1 M PBS at different scan rates. The peak currents were observed to increase with increasing the scan rates. Moreover, the oxidation peak increased with increasing the scan rates and a linear relationship is found between the peak current and square root of scan rate, with a correlation coefficient of 0.98 (Fig. 2b). This behavior indicates a diffusion-controlled process at the electrode surface [11, 12]. From Fig. 2a, it was possible to gather the information shown in Table 1 regarding the influence of the scan rate (ν) on the anodic and cathodic peak potentials of this system. The ΔE_p values were much higher than that of expected, 59 mV for $n = 1$ electron or for a reversible system. From the variation of ΔE_p values with the potential scan rate, shown in Table 1, one may conclude that the AA electrochemical oxidation is a quasi-reversible mass transfer-controlled process. Typical curves for this case, at small values of λ (reorganization energy for electron transfer), higher scan rates, and essentially reversible behavior, are found. For large values of λ (lower scan rates), no current is observed on scan reversal and the shape of the curve is similar to a totally irreversible charge transfer (Fig. 2a). When the rate of the charge-transfer reaction is sufficiently slow, the observed behavior depends on the standard heterogeneous rate constant (k°) and transfer coefficient (α) as well as the

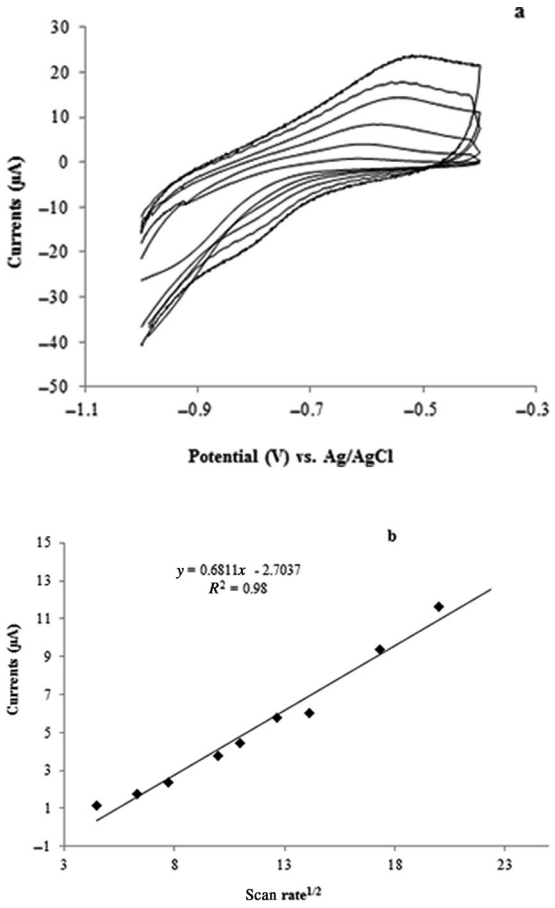


FIG. 2 *(a) CVs of the biosensor when exposed to 1.0 mM AA in a 0.1 M PBS (pH 7.5) at different scan rates (from inner to outer): 20, 40, 60, 100, 120, and 160 mV/Sec. (b) Plot of anodic peak currents versus the root of scan rate.*

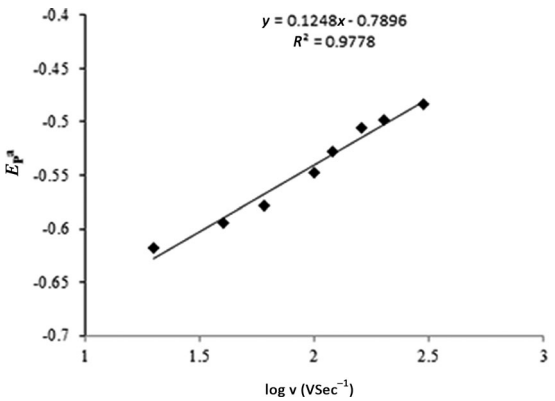


FIG. 3 *Relationship between the anodic peak potential E_P^a and logarithm of scan rate (log ν), obtained from CVs in Fig. 2a.*

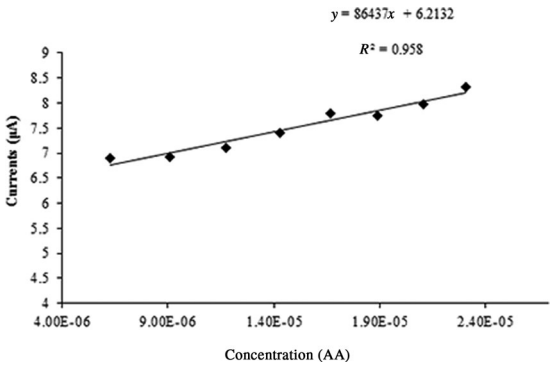


FIG. 4 *Calibration curve demonstrating AA detection at modified electrode.*

TABLE 1 ***Variation of the voltammetric parameters as a function of the potential scan rate (v) corresponding to the CVs as shown in Fig. 2a***

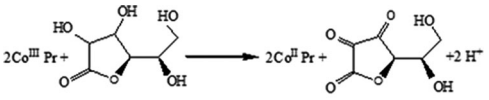
ν (mV Sec^{-1})	E_P^a (mV)	E_P^c (mV)	(E_P^a – E_P^c) (mV)
20	–618	–	–
40	–595	–	–
60	–578	–	–
100	–547	–782	235
120	–528	–795	267
160	–506	–812	306
200	–498	–820	322
300	–483	–830	347

kinetic parameter λ for the following reaction. The irreversible following reactions cause the voltammetric wave to shift toward positive values, and this shift away from $E^{0'}$ causes a decrease in the rate of the charge-transfer reaction. All of these electrochemical results suggest a typical E_qC_i mechanism, which includes a quasireversible electrochemical process (E_q) followed by an irreversible chemical reaction (C_i) [39, 40]. A study of iron binding to ferritin that can catalyze ascorbate oxidation was done that Fe^{3+} within the ferritin shell can be directly reduced by AA and Fe(II) ions can be released from ferritin by AA [41, 42]. It looks the same reaction may occur here. The AA oxidation process on the surface of the biosensor is as follows: AA diffuses from the bulk solution to the biosensor surface [7, 43], and then complex reactions take place between the central metal ion and AA, and Co(II) ions can be released from ferritin by AA. On the biosensor surface, the oxidation of AA in a 0.1 M PB (pH 7.5) occurs as a two-step

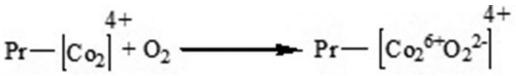
TABLE 2 *Performances of the cobalt(III)–ferritin/DTSP/Au electrode for AA sensing compared to previously reported works*

AA biosensor	*Sensitivity*	*Detection limit (μM)*	*Linear range*	*Reference*
Cobalt(III)–ferritin/DTSP/Au	86,437 μAM^{-1}	4.65	6.25–2.31 μM	Present work
CoPc–MWNTs/GC	–	1	2.6–10 μM	Zuo et al. [7]
Pd-CNF/CPE	–	15	0.05–4 mM	Huang et al. [45]
Ppy-FCNM/CPE	–	33.8	0.10–1.00 mM	Raoof et al. [46]
		13.4	0.10–0.95 Mm	
Poly(caffeic acid)/ GC	–	7	20 μM–1 mM	Ren et al. [47]

quasireversible electrochemical process (E_q) followed by an irreversible chemical reaction (C_i) (Eqs. (1) and (2)):



Eq. 1



Eq. 2

Figure 3 shows the relationship between the peak potential ($E_P{}^a$) and the logarithm of scan rate (log ν) for biosensor in 0.1 M BPS (pH 7.5) containing 1 mM of AA. The peak potential ($E_P{}^a$) changed linearly versus log ν with a linear regression equation of $E_p{}^a = 0.1248 \log \nu - 0.7896$; $R^2 = 0.9778$ (ν mV Sec^{-1}) in the range from 40 to 300 mV Sec^{-1}. For a redox monolayer modified electrode, the peak potentials can be represented by Laviron:

$$E_{pa} = E^{0'} - \frac{2.3RT}{(1-\alpha)nF} \log \frac{(1-\alpha)Fn\nu}{RTK}$$

$$\log K_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(RT/nF\nu) - \frac{\alpha(1-\alpha)nF\Delta E_p}{2.3RT}$$

where α is the electron transfer coefficient; n is the number of electrons; R, T, F, and K are gas, temperature, Faraday constant, heterogeneous electron transfer rate constant, respectively. According to the slope of anodic process, it was calculated that $(1-\alpha)\,n = 0.476$. Given $0.3 < \alpha < 0.7$ in general [44, 45], it could be concluded that $n = 1$ and $\alpha = 0.52$. So, the redox reaction between the cobalt(III)–ferritin prosthetic group and AA is a single electron transfer process. Also, the heterogeneous electron transfer rate constant could be estimated to be 1.054 Sec^{-1}, which was higher than that of previously reported for other protein immobilization studies [46], indicating that this biosensor provided a suitable environment to increase the electron transfer rate of cobalt(III)–ferritin.

3.2. Linear range and limit of detection

Immobilization of cobalt(III)–ferritin on DTSP-modified gold electrode is a very suitable means to fabricate the third generation of mediatorless biosensors for determination of AA. Since DPV is much more current sensitive in comparison with linear sweep voltammetry, this method was employed in order to estimate the low detection limit. Figure 4 shows the calibration curve of the biosensor for successive addition of different concentrations of AA. There is a linear relation between response current and AA concentration in the range 6.25×10^{-6}–2.31×10^{-5} M. The detection limit (signal-to-noise ratio of 3) and sensitivity were 4.65×10^{-6} M and 86,437 μAM^{-1}, respectively. The detection limit, linear calibration range, and sensitivity for AA determination of mentioned biosensor are comparable and even better than those obtained using other modified electrodes [11]. Table 2 indicates the detection limit, linear calibration range, and sensitivity of this biosensor for AA determination. As it is shown, these factors are comparable and even better than those have been reported for other modified electrodes.

4. Conclusions

In this article, an AA biosensor has been designed by immobilization of cobalt(III)–ferritin molecules onto modified Au electrode using succinimidyl alkanedisulfide compounds. The electrooxidation of micromolar AA solution was performed using modified gold electrode. The prepared third-generation biosensor displayed good sensitivity and reproducibility, wide linear range, low detection limit, fast response, and good long-term stability for the detection of AA. This biosensor can provide a good electrochemical sensing platform for biosensor and biocatalysis applications.

5. References

[1] Peng, Y., Liu, F., and Ye, J. (2006) J. Chromatogr. B 830, 224–230.

[2] Shokuhi Rad, A., Mirabi, A., Binaian, E., and Tayebi, H. (2011) Int. J. Electrochem. Sci 6, 3671–3683.

[3] Power, A. C., and Morrin, A. (2013) Electroanalytical Sensor Technology. Dublin City University, Ireland.

[4] Li, F., Li, J., Feng, Y., Yang, L., and Du, Z. (2011) Sens. Actuat. B 157, 110–114.
[5] Zhang, J., Zhu, Z., Zhu, J., Li, K., and Hua, S. (2014) Int. J. Electrochem. Sci. 9, 1264–1272.
[6] Christen, W. G., Gaziano, J. M., and Hennekens, C. H. (2000) Ann. Epidemiol. 10, 125–134.
[7] Zuo, X., Zhang, H., and Li, N. (2012) Sens. Actuators B. 161, 1074–1079.
[8] Afkhami-Ardekani, M., and Shojaoddiny-Ardekani, A. (2007) Indian J. Med. Res. 126, 471.
[9] Eze, E. D., Dawud, F. A., Zainab, A. A., Jimoh, A., Malgwi, I. S., and Isa, A. S. (2012) Asian J. Med. Sci. 4, 17.
[10] Li, F., Tang, C., Liu, S., and Ma, G. (2010) Electrochim. Acta 55, 838.
[11] Nassef, H. M., and Radi, A. E. (2007) Anal. Chim. Acta 583, 182.
[12] Mbouguen, J. C. K., Kenfack, I. T., Walcarius, A., and Ngameni, E. (2011) Talanta 85, 754.
[13] Kumar, S. A., Lo, P. H., and Chen, S. M. (2008) Biosens. Bioelectron. 24, 518.
[14] Safavi, A., Maleki, N., Moradlou, O., and Tajabadi, F. (2006) Anal. Biochem. 359, 224–229.
[15] Luo, X., Morrin, A., Killard, A. J., and Smyth, M. R. (2006) Electroanalysis 18, 319.
[16] Zhang, P., Wu, F. H., Zhao, G. C., and Wei, X. W. (2005) Biochemistry 67, 109.
[17] Shervedani, R. K., Bagherzadeh, M., and Mozaffari, S. A. (2006) Sens. Actuat. B. 115, 614.
[18] Wang, Z., Wang, Y., and Luo, G. (2002) Analyst 127, 1353.
[19] Salimi, A., Sharifi, E., Noorbakhsh, A., and Soltanian, S. (2006) Electrochem. Commun. 8, 1499.
[20] Wu, Y., and Hu, S. (2007) Microchim. Acta 159, 1.
[21] Ivnitski, D., Branch, B., Atanssov, P., and Apblett, C. (2006) Electrochem. Commun. 8, 1204.
[22] Kamitaka, Y., Tsujimura, S., Setoyama, N., Kajino, T., and Kano, K. (2007) Phys. Chem. Chem. Phys. 9, 1793.
[23] Sheng, Q., Luo, K., Li, L., and Zheng, J. (2009) Bioelectrochemistry 74, 246.
[24] Kashanian, S., Rafipour, R., Taright, F. A., and Ravan, H. (2012) IET Nanobiotechnol. 6, 102.
[25] Akram, M., Stuart, M. C., and Wong, D. K. Y. (2004) Anal. Chim. Acta 504, 243.
[26] Raj, C. R., and Behera, S. (2005) J. Electroanal. Chem. 581, 61.
[27] Orozco, J., Jorquera, C. J., and Sanchez, C. F. (2009) Bioelectrochemistry 75, 176.
[28] Won, K., Park, M. J., Yoon, H. H., and Kim, J. H. (2008) Ultramicroscopy 108, 1342.
[29] Tominaga, M., Ohira, A., Yamaguchi, Y., and Kunitake, M. (2004) J. Electroanal. Chem. 566, 323.
[30] Kim, J. W., Choi, S. H., Lillehei, P. T., Chu, S. H., King, G. C., and Watt, G. D. (2007) J. Electroanal. Chem. 601, 8.
[31] Martin, T. D., Moheit, S. A., Niichel, R. J., Peterson, S. C., Campbell, C. H., and Zapien, D. C. (1997) J. Electroanal. Chem. 420, 279.
[32] Douglas, T., and Stark, V. T. (2000) Inorg. Chem. 39, 1828.
[33] Polanams, J., Ray, A. D., and Watt, R. K. (2005) Inorg. Chem. 44, 3203.
[34] Klem, M. T., Mosolf, J., Young, M., and Douglas, T. (2008) Inorg. Chem. 47, 2237.
[35] Yoshimura, H. (2006) Colloids Surf. A: Physicochem. Eng. Aspects 282–283, 464–470.
[36] Tsukamoto, R., Iwahori, K., Muraoko, M., and Yamashito, I. (2005) Bull. Chem. Soc. Jpn. 78, 2075.
[37] Kim, I., Hosein, H. A., Strongin, D. R., and Douglas, T. (2002) Chem Mater. 14, 4874.
[38] Kashanian, S., Tarighat, F. A., Rafipour, R., and Tarighat, M. A. (2012) Mol. Biol. Rep. 39, 8793.
[39] Bard, A. J., and Faulkner, L. R. (2001) Electrochemical Methods Fundamentals and Applications, 2nd ed., John Wiley & Sons, Inc., USA.
[40] Avendano, S. C., Angeles, G. A., Silva, M. T. R., Pina, G. R., Romo, M. R., and Pardave, M. P. (2007) J. Electroanal. Chem. 609, 17.
[41] Huang, H. Q., Lin, Q. M., and Wang, T. L. (2002) Biophys. Chem. 97, 17.
[42] Liu, Y., and Hu, N. (2009) Biosens. Bioelectron. 25, 185.
[43] Wu, Y., and Hu, S. (2004) Anal. Chim. Acta 527, 37.
[44] Moghaddam, A. B., Esmaieli, M., khodadadi, A. A., Ganjkhanlou, Y., and Asheghali, D. (2011) Microchim. Acta 173, 317.
[45] Liu, P., Zhang, X., Feng L., Xiong H., and Wang S. (2011) Am. J. Biomed. Sci. 3, 69.
[46] Topoglidis, E., Astuti, Y., Duriaux, F., Grätzel, M., and Durrant, J. R. (2003) Langmuir 19, 6894.
[47] Huang, J., Liu, Y., Hou, H., and You, T. (2008) Biosens. Bioelectron. 24, 632.