Int. J. Electrochem. Sci., 10 **(2015)** 4770 - 4778

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

A Disposable Cholesterol Enzyme Biosensor Based on Ferrocene-Capped Gold Nanoparticle Modified Screen-Printed Carbon Electrode

Bijun Feng and You-Nian Liu[*]

College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan 410083, P. R. China
[*]E-mail: liuyounian@csu.edu.cn

Received: 5 March 2015 / *Accepted:* 2 April 2015 / *Published:* 28 April 2015

In this paper, a novel amperometric cholesterol biosensor with modified of cholesterol oxidase and cholesterol esterase at ferrocene-capped gold nanoparticle modified screen-printed carbon electrode has been developed. Firstly, the ferrocene-capped gold nanoparticle was synthesized. Then the enzyme ink containing Nafion, cholesterol oxidase, cholesterol esterase and ferrocene-capped gold nanoparticle was dropped into the surface of the screen-printed carbon electrode. The electrocatalytic behavior of cholesterol at cholesterol biosensor was investigated employed electrochemical methods. Under optimized conditions, the catalytic current at cholesterol biosensor exhibited a linear relationship with cholesterol in the range from 50 μM to 15 mM with a detected limit of 12 μM at 0.3 V vs Ag/AgCl. Furthermore, the selectivity, specificity and stability of cholesterol sensor was investigated and it was applied to measure the real samples of whole blood, the results was almost same with the data obtained from automatic biochemical analyzer.

Keywords: Cholesterol; screen printed carbon electrode; ferrocene-capped gold nanoparticle

1. INTRODUCTION

Determination of the cholesterol level in human blood is of great significance in clinical analysis[1,2]. High cholesterol accumulation in blood serum is strongly related with coronary heart disease, arteriosclerosis and myocardial infarction[3,4]. In previous studies, the detection of cholesterol is mainly based on spectrophotometry, which often involves complex operational procedure[5,6]. So it is important to develop a sensitive and selective method for cholesterol detection. Cholesterol determination is performed by enzymes, such as cholesterol oxidase and cholesterol esterase. These

together can be used to monitor both native and esterified cholesterol levels[7]. Cholesterol esterase catalyzes the hydrolysis of cholesterol ester to form cholesterol, then the cholesterol is oxidized by cholesterol oxidase and hydrogen peroxide (H_2O_2) is produced as follows[8]:

Cholesterol ester + $H_2O \rightarrow$ Cholesterol + Fatty acids (1)

Cholesterol + $O_2 \rightarrow$ H_2O_2 + Cholest-4-en-3-one (2)

It is known that the oxidized potential of H_2O_2 is high(above +0.6 V)[9], some researchers used horseradish peroxidase (HRP) or electron transfer mediators, such as ferrocyanide and prussian blue to measure H_2O_2 reduction current at lower potential to avoid the influence of reductants such as acetaminophen, ascorbic acid, cysteine, and uric acid[10,11]. Some researchers employed the nanoparticle materials such as gold nanoparticles, carbon nanotubes(CNTs) to promote electron-transfer reactions at low over-potential[12-14]. Wen and co-workers developed a disposable organophosphorus pesticides enzyme biosensor based on magnetic composite carbon nanotubes modified screen printed carbon electrode[15]. CNTs can facilitate electron transfer between the electroactive species and the electrode. Gold nanoparticles (AuNPs) are well-known low-dimensional functional materials with large surface-to-volume ratios and are biocompatible with biosystems[16,17]. It also has been widely used to low the oxidized potential in electrochemical analysis[18,19]. Another approach is employing the permselective membranes such as Nafion to block diffusion of anions interferences to the electrode surface[20,21].

In general, enzyme is often immobilized according to absorption、entrapment、cross-linking or covalent attachment[22-25]. The cross-linking or covalent attachment is more advantageous than physical adsorption which can avoid serious enzyme leaching from the surface[26]. Electrochemical sensors based on screen printed technology has the advantages of simple operation, low cost, easy operation, and is convenient for industrial production, has a great development prospect[27,28]. Thus the aim of this work is introducing of a simple manipulation and sensitive cholesterol biosensor modified of cholesterol oxidase and cholesterol esterase at ferrocene-capped gold nanoparticle modified screen-printed carbon electrode. Ferrocene as the mediator, gold nanoparticle used to facilitate electron transfer between cholesterol oxidase and electrode and nafion was employed to eliminate the anions electroactive interferences, according to investigate the electrochemical behaviors of the cholesterol at biosensor, the concentration of cholesterol can be determined.

2. EXPERIMENTAL

2.1 Materials

Carbon ink and insulation ink were acquired from JUJO (Tokyo,Janpan). The hydrophilic film and double sided adhesive tape were from 3M China Co., Ltd(Shenzhen, China) hydroxyethyl cellulose(HEC), nafion, uric acid, ascorbic acid, ferrocenemethanol, cholesterol oxidase, cholesterol esterase and triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO, USA). 11-Ferrocenyl-1-undecanethiol (Fc-C11SH) was purchased from Dojindo Laboratories (Kumamoto, Japan).Aqueous solutions were prepared using Millipore water (Simplicity Model, Billerica, MA, USA).

2.2 Electrode Preparation

We prepared the screen-printed carbon electrode contained a working electrode and a reference/counted electrode. The react area was constructed according to printed the insulation ink at working electrodes and the counter electrode strips. Then 1 mg hydroxyethyl cellulose (HEC), 1 ku cholesterol esterase, 2 ku cholesterol oxidase and 10 uL nafion were dissolved into 1 mL phosphate buffer solution (pH=7.4) containing 50 uLferrocene-capped gold nanoparticle solution and hydrated 2 h at room temperature. The ferrocene-capped gold nanoparticle prepared followed the literature[28]. Acquired 10 μL above solution dropped into the working electrode, dried at 45 ^{o}C for 0.5 h, For fabrication of the stable sample reaction cell, the hydrophilic film and double sided adhesive tape was covered the react area.

2.3 Detection of cholesterol at cholesterol biosensor

All experiments were carried out at room temperature unless otherwise stated. All electrochemical measurements were carried out with a model CHI832C Electrochemical Workstation (CH Instruments, Austin, TX, USA). The supporting electrolyte was 0.1 M NaCl. For amperometric detection of cholesterol at electrochemical cholesterol biosensor, the applied potential was set at 0.3 V. Cholesterol solution prepared followed the litetures.[29,30] The cholesterol was dissolved in mixture solution of isopropanol, triton X-100 and phosphate buffer with the ratio of 10:4:86 by weight to prepare 1.0 mM and 4.0 mM stored cholesterol solution. The value of the current was acquired at 20s at amperometric i-t curve. After amperometric measurements of cholesterol in PBS solution at intervals, the electrodes were kept dry at room temperature. All experiments were carried out at room temperature unless otherwise stated.

2.4 Real sample preparation and analysis

Aliquots (1.0 mL) of blood samples were accessed to the heparin anticoagulation tube. For measurement of the cholesterol concentration in blood sample, the results were compared to those determined with automatic biochemical analyzer(Beckman Instruments, Inc., California, USA) without any treatment.

For the stability of electrodes, we chose a bath of electrodes containing 50 strips to a sealed packaging. After each measurement, the rest of electrodes were maintained in sealed packaging at room temperature to keep clean and dry. In order to investigate the recovery of the proposed screen-printed electrode, all samples were accessed from healthy donor without any treatment, the stock solutions (5 mM and 20 mM) were added into the same volume of blood samples immediately.

3. RESULTS AND DISCUSSION

3.1 Electrochemical analysis of cholesterol biosensor

The amperometric I-t method was employed to investigate the behavior of cholesterol biosensor with modified of cholesterol oxidase and cholesterol esterase at ferrocene-capped gold nanoparticle or ferrocenemethanol（Fc-OH） modified screen-printed carbon electrode. As shown in figure 1, the current of cholesterol biosensor modified with ferrocene-capped gold nanoparticle was higher than modified with Fc-OH, and the reaction rate was also more quickly than modified with Fc-OH, which due to the large surface-to-volume ratios and biocompatible of gold nanoparticle. The results revealed that the gold nanoparticle could facilitate electron transfer between cholesterol oxidase and electrode, and the reaction time was about 20s when the potential of cholesterol biosensor was set at 0.3 V.

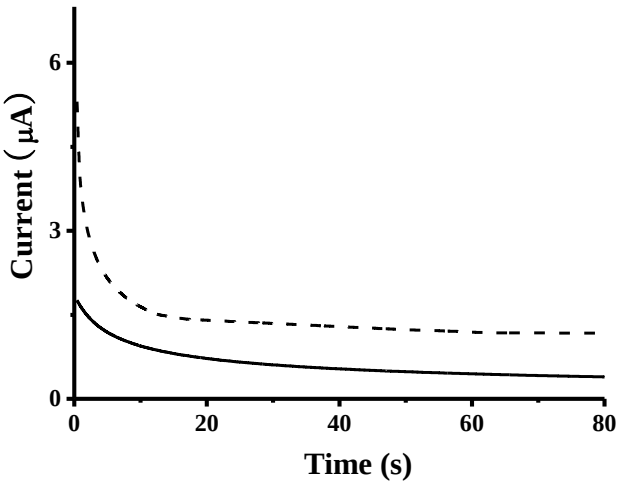

Figure 1. The I-t curve of 5 mM cholesterol at cholesterol biosensor modified ferrocene-capped gold nanoparticle (dashed line) or ferrocene (solid line) as mediate with the potential of 0.3 V.

3.2 Detection of cholesterol at cholesterol biosensor

Cholesterol ester was hydrolyzed by cholesterol esterase to form cholesterol, and the cholesterol was oxidized by cholesterol oxidase, in this process, hydrogen peroxide (H_2O_2) was produced. When in the addition of ferrocene-capped gold nanoparticle as the mediator, the H_2O_2 was prevented formation[32] and the reaction can be described as followed:

Cholesterol ester + $H_2O \rightarrow$ Cholesterol + Fatty acids (1)

Cholesterol + $Fc^+ \rightarrow$ Fc + Cholest-4-en-3-one (2)

$Fc + e^+ \rightarrow Fc^+$ (3)

According to the above reactions, the cholesterol concentration can be acquired by the electrochemical oxidation of ferrocene-capped gold nanoparticle AT cholesterol biosensor. As shown in figure 2, the oxidized current increased with addition of cholesterol and can be detected using

electrochemical method. Figure 3 depicts the plot of the electrocatalytic current at the enzyme screen-printed electrodes with cholesterol concentration in the range from 50 uM to 15 mM. The value of the current was acquired at 20s at amperometric I-t curve when the potential was set at 0.3V. The oxidized peak current at the cholesterol biosensor exhibits a linear relationship with the cholesterol concentration, and the linear equation is I(uA) =0.18[cholesterol](mM) + 0.46×10^{-6}. The limits of detection were calculated on the basis of three times of the background noise and the value was found to be 12 uM.

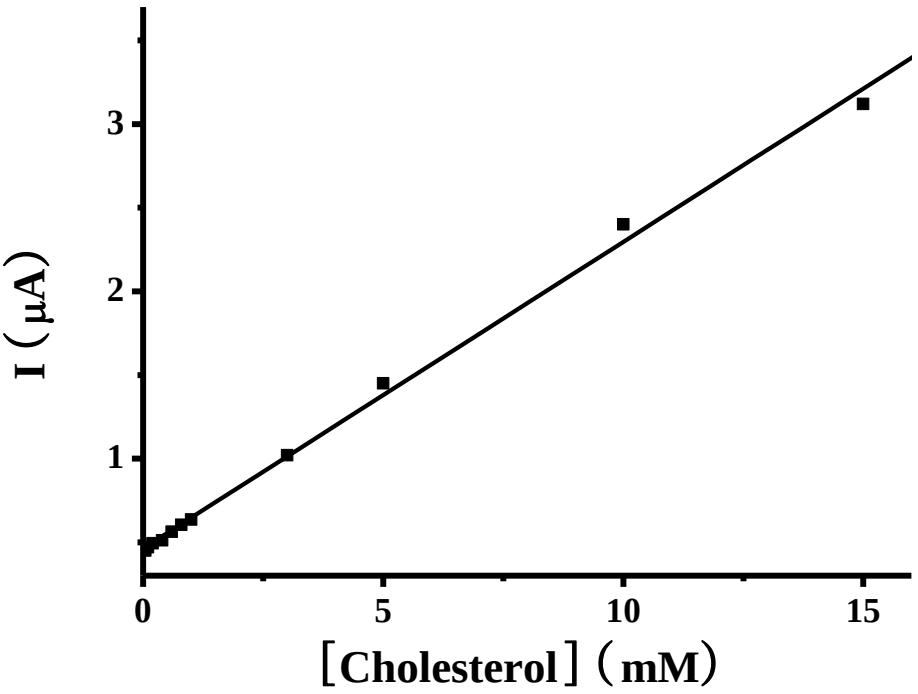

Figure 2. The calibration curve of cholesterol concentration from 0.05-15 mM with with the potential of 0.3 V.

3.3 Effects of pH to the cholesterol biosensor

We investigated the response current of cholesterol biosensor at various pH values from 5.5 to 9.5. As shown in figure 3, the current changed value (ΔI) at cholesterol biosensor was increased with the pH increased. However, ΔI was decreased when the pH was high than 8.0, which was mainly due to the optimally catalytic efficient of cholesterol oxidase at pH=7.0[33], when the pH was too high, the catalytic efficient mainly was affected by the electron transfer, and the high pH value also affected the stability of the gold nanoparticle, Thus the best pH value at cholesterol biosensor was at 7.0-8.0, we chose pH at 7.4 in this research. Also the result was similar with conclusions acquired from Ahmet and coworkers[15].

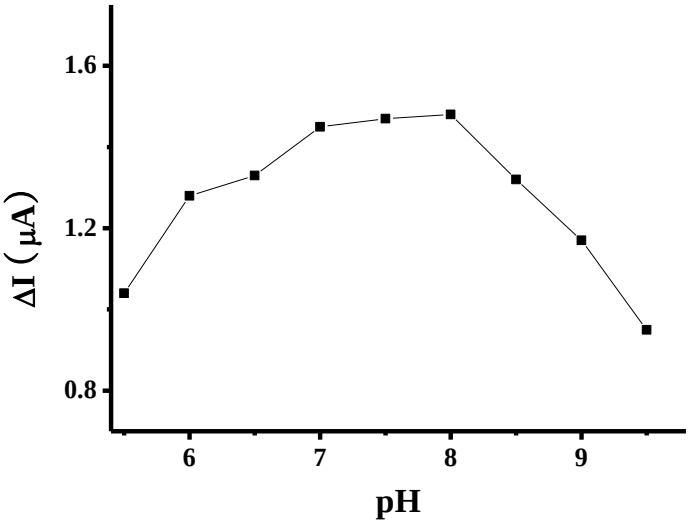

Figure 3. The current changed value with the various of pH from 5.5 to 9.5. The concentration of cholesterol was 5 mM and the potential was set at 0.3 V.

3.4 Selectivity and specificity of the cholesterol biosensor

It is necessary to study the selectivity and specificity of the cholesterol biosensor. As shown in figure 4, the electroactive species such as uric acid(UA), ascorbic acid(AA) and acetaminophen(ACP) did not change the cholesterol response in the presence of these interference species at cholesterol biosensor, which was mainly due to the nafion film to block diffusion of anions interferences to the electrode surface. The results demonstrated the cholesterol biosensor has good selectivity and specificity. So the cholesterol biosensor in our method can be used in real sample measurement.

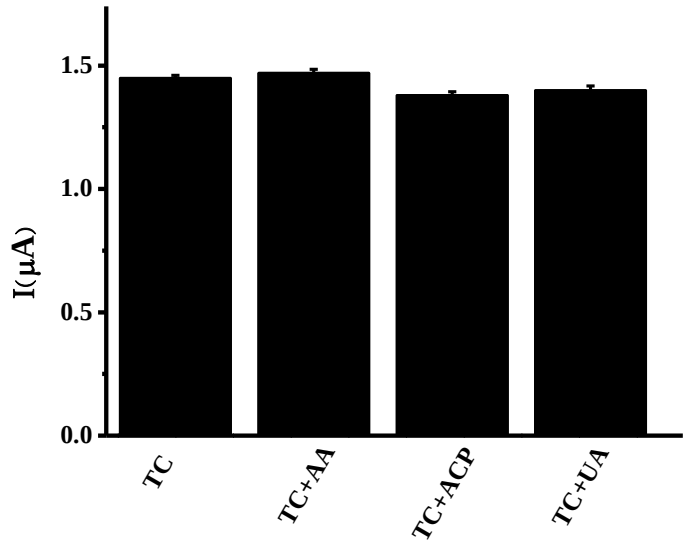

Figure 4. Selectivity of the cholesterol biosensor for detection of cholesterol (TC) in the absence or presence of redox-active species (AA, ACP and UA). Error bars represent the RSD values (n = 3).

3.5 Stability of the cholesterol biosensor

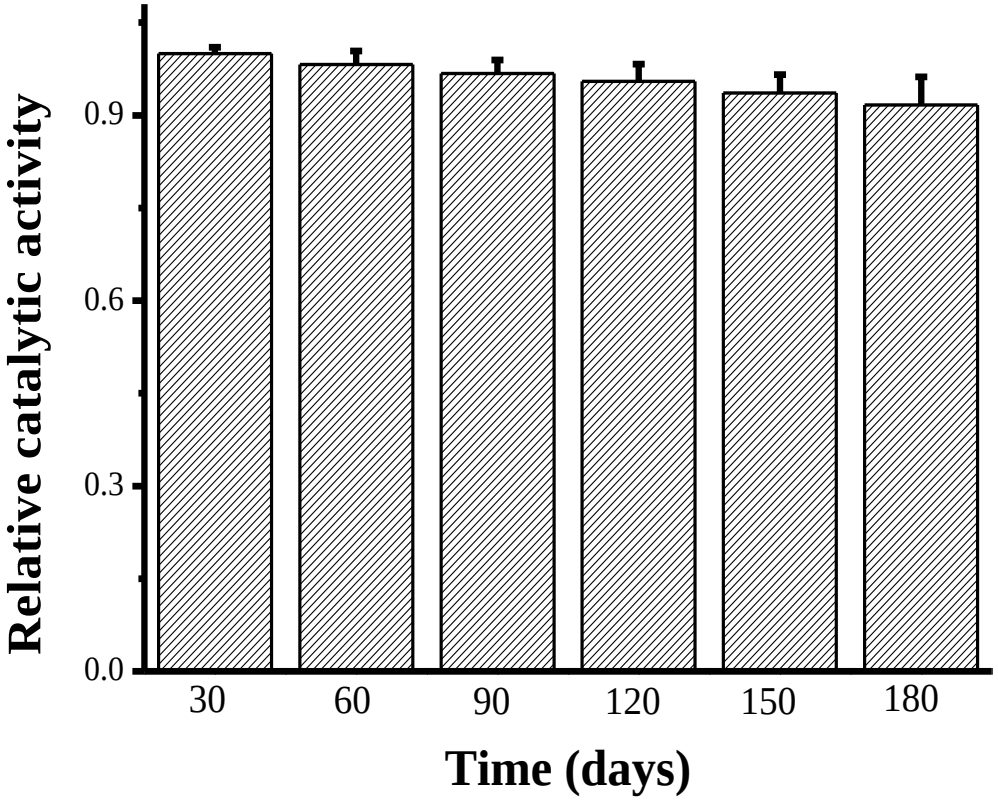

Figure 5. Stability of the cholesterol biosensor. Error bars represent the RSD values (n = 3).

The stability of the cholesterol biosensor was investigated by determined the response current of 5 mM cholesterol. As shown in figure 5, the catalytic current at screen-printed electrode kept stable after six months, the electrode still retained 91.7% of the initial current, which demonstrates that the proposed electrode can be used as the commercial electrode in real application. The stability was due to the good biocompatible of gold nanoparticles.

3.6 Real sample measurement

At least three replicate measurements were conducted for these blood samples (Some partial results were listed in Table 1) and the results are also compared to those acquired with automatic biochemical analyzer (Beckman Instruments, Inc., California, USA). As shown in Table 1, the results based on our method are in excellent agreement with those obtained with the commercial automatic biochemical analyzer. Compared to the commercial automatic biochemical analyzer, there is no significant difference of the accuracy, so the amperometric cholesterol biosensor with modified of cholesterol oxidase and cholesterol esterase at ferrocene-capped gold nanoparticle modified screen-printed carbon electrode could be used in cholesterol clinical diagnosis.

Table 1. Cholesterol concentrations determined from healthy and patient donors. (The hospital cholesterol results were acquired from automatic biochemical analyzer determined by spectrophotometry method)

Samples	The results of hospital (mmol/L)	This method (mmol/L)
1	4.2	4.6
2	5.5	5.6
3	5.2	5.0
4	5.6	5.8
5	4.1	4.2
6	2.6	2.5
7	6.6	6.4
8	3.8	4.0

4. CONCLUSIONS

In this paper, we designed a new method of amperometric cholesterol biosensor with modified of cholesterol oxidase and cholesterol esterase at ferrocene-capped gold nanoparticle modified screen-printed carbon electrode successfully. There is a good relationship between the electrocatalytic current and the cholesterol concentration. Furthermore, the electrochemical strips exhibit the good selectivity and stability. In addition, this method was also successfully employed for cholesterol detection in blood samples. We therefore conclude that the rapid and sensitive cholesterol integrated to portable devices can apply in the field of clinical analysis.

References

1. Y. C. T. Siao, Y. Chen and C. A. Lee, *Sens. Actuat. B: Chem.*, 135 (2008) 96.
2. C. Fang, J. He and Z. Chen, *Sens. Actuat. B: Chem.*, 155 (2011) 545.
3. A. Uittenbogaard, Y. S. Ying and E. J. Smart, J. Biol. Chem., 288 (2013) 6587.
4. C. Belcher, J. J. Heatley, C. Petzinger, S. Hoppes, C. D. Larner, S. J. Sheather and R. D. Macfarlane, *J. Exotic Pet Med.*, 23 (2014) 71.
5. R. A. Silva, A. M. Carmona-Ribeiro and D. F. Petri, *Colloids Surf. B: Biointerfaces*, 110 (2013) 347.
6. L. M. Estronca, H. A. Filipe, A. Salvador, M. J. Moreno and W. L. Vaz, (2014). *J. Lipid Res.*, 55 (2014) 1033.
7. A. Ahmadalinezhad and A. Chen, *Biosens. Bioelectron.*, 26 (2011) 4508.
8. R. Coulombe, K. Q. Yue, S. Ghisla and A. Vrielink, *J. Biol. Chem.*, 276 (2001) 30435.
9. A. A. Karyakin, *Electroanalysis*, 13 (2001) 813
10. S. M. Borisov and O. S. Wolfbeis, *Chem. Rev.*, 108 (2008) 423.
11. J. C. Vidal, J. Espuelas, E. Garcia and J. R. Castillo, *Talanta*, 64 (2004) 655.
12. V. Mani, B. Devadas and S. M. Chen, *Biosens. Bioelectron.*, 41 (2013) 309.
13. L. Bai, R. Yuan, Y. Chai , Y. Zhuo, Y. Yuan and Y. Wang, *Biomaterials*, 33 (2012) 1090.
14. R. Akter, M. A. Rahman and C. K. Rhee, *Anal. Chem.*, 84 (2012) 6407.
15. N. Gan, X. Yang, D. Xie, Y. Wu and W. Wen, *Sensor*, 10 (2010) 625.
16. L. Lin, J. Zhou, Y. Zhang and Lin, Z, *Electroanalysis*, 20 (2008) 1798.
17. Y. Z. Song, Y. Song and H. Zhong, *Gold Bull*, 44 (2011) 107.

18. F. F. Liang, C.Y. Liu, J. Jiao, S.Q. Li, J. Xia and J.B. Hu, *Microchim. Acta*, 177 (2012)389.
19. J. Du, X.P. Yu and J.W. Di, *Biosens. Bioelectron.*, 37 (2012) 88.
20. G. Piechotta, J. Albers and R. Hintsche, *Biosens. Bioelectron.*, 21 (2005) 802.
21. Y. Peng, C. -W. Wei, Y. -N. Liu and J. Li, *Analyst*, 136 (2011) 4003.
22. J. Wang, *Chem. Rev.*, 108 (2008) 814.
23. M. Ben-Moshe, V. L. Alexeev, and S. A. Asher, Anal. *Chem.*, 78 (2006) 5149.
24. K. Y. Chumbimuni-Torres, P. Calvo-Marzal, J. Wang, and E. Bakker, *Anal. Chem.*, 80 (2008) 6114
25. Y. Liu, M. K. Wang, F. Zhao, Z. A. Xu and S. J. Dong, *Biosens. Bioelectron.*, 21 (2005) 984.
26. Y. Peng, D. Jiang, L. Su, L. Zhang, M. Yan, J. Du, Y., Y.-N. Liu and F. Zhou., *Anal. Chem.*, 81 (2009) 9985.
27. S. Timur, L. Della Seta, N. Pazarlioglu, R. Pilloton and A. Telefoncu, *Process Biochem.*, 39 (2004) 1325.
28. T. Garcla, M. Revenga-Parra, L. Anorga, S. Arana, F. Pariente and E. Lorenzo, *Sens. Actuators B: Chem.*, 161 (2012) 1030.
29. J. Wang, J. Li, A. J. Baca, J. Hu, F. Zhou,W. Yan, and D.-W. Pang, *Anal. Chem.*, 75 (2003) 3941.
30. E. Yapar, S. K. Kayahan, A. Bozkurt and L. Toppare, *Carbohydr. Polym.*, 76 (2009) 430.
31. Y. Fadime, A. Fatma, C. Servet and Y. Ahmet, *Sensors*, 9 (2009) 6435.
32. P. C. Nien, P. Y. Chen and K. C. Ho, *Sensors*, 9 (2009) 1794.
33. J. C. Vidal, E. Garcia-Ruiz and J. R. Castillo, J. *Pharm. Biomed. Anal.*, 24 (2000) 51.