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Voltammetric and Spectroscopic Investigations of the Interaction between Colchicine and Bovine Serum Albumin

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Fe_3O_4 nanoparticles modified carbon paste electrode (Fe_3O_4/CPE) was constructed with the mixture of Fe_3O_4, graphite power and paraffin oil. The electrochemical behaviors of colchicine, both cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were investigated, showing an irreversible oxidation process. The oxidative peak current increased linearly with the concentration of colchicine in the range of 8.6×10^{-7} to 1.2×10^{-3} M. The detection limit was 2.6×10^{-7} M (S/N=3). The Fe_3O_4/CPE showed good selectivity, reproducibility and stability for the detection of trace colchicine. After the addition of bovine serum albumin (BSA) into colchicine solution, the oxidative peak currents decreased with the positively shift of the peak potential and no appearance of new peak. This electrochemical method was further applied to the determination of BSA in pharmaceutical formulations. The interaction between colchicine and BSA was investigated by electrochemical and spectroscopic methods. The binding constant and the number of binding sites of the interaction system were calculated by fluorescence spectroscopy.

Keywords: Colchicine, Fe_3O_4 nanoparticles, interaction, bovine serum albumin

1. INTRODUCTION

Colchicine (COLC) is a naturally occurring alkaloid used in human and veterinary medicine. It has been used as an antimitotic agent in cancer research involving cell cultures [1]. Moreover, COLC is used for alleviation of inflammatory process during podagra and for reducing pain [2]. Serum albumins are the most abundant proteins in plasma. As the major soluble protein of the circulatory system, they have many physiological functions. They can play an important role in drug disposition and efficacy [3]. Many drugs or other bioactivity small molecules bind reversibly to albumin [4-6], which implicates BSA's role as carriers. Hence, it is important to determine COLC and understand the

interactions of this drug and protein for variety of endogenous and exogenous ligands/drugs [7]. Because of its medical relevance, our work should be valuable.

So far, various methods have been reported for the determination of COLC, namely HPLC [8], spectrophotometry [9], as well as electrochemistry and voltammetric determination [10-13]. Spectroscopes including fluorescence spectroscope and UV-vis spectroscope are useful tools for the study of COLC–HSA association [6], whereas electrochemical measurement is scanty. But the investigations with spectrophotometry or HPLC require tedious pretreatment, which often leads to considerable losses of the analyte and time. In the last few years, interest has been increasing in the application of simple, sensitive, and rapid electrochemical methods in fields such as clinical, environmental and pharmaceutical analysis [14, 15]. Bodoki et al. [10] have investigated the electrochemical behaviors of COLC using graphite-based screen-printed electrodes. Zhang et al. [11] have prepared the PoPD/SWNTs composite-modified glassy carbon electrode for the determination of COLC. The mainly purpose of this study was to characterize the electrochemical behaviors of COLC and its interaction with BSA using electrochemical measurement.

In recent years, magnetic nanoparticles as special electrode immobilizing materials are becoming the focus of research [16]. Due to its special properties, such as good biocompatibility, strong superparamagnetic, low toxicity, and easy preparation process, magnetic nanoparticles modified electrodes have been used in immunology [17], cell separation processes [18] and so on. It was reported that the use of magnetic nanoparticles modified electrode could potentially result in unique properties of bioactive particles, such as increased proteins activity due to the increased surface area of nanoparticles, and good dispersion in the analyte solution leading to rapid contact between the proteins and its association and reduction of mass-transfer limitations [19]. Nanoparticles modified paste electrodes are prepared in an easy, fast and effective way by using mineral oil as binder. The resulting nanoparticles modified paste electrodes not only retains the advantages of the classical carbon paste electrode (CPE) such as the feasibility to incorporate different substances, the easy renewal and composite nature [20-21], but also keeps the ability of nanoparticles to promote electron-transfer reactions.

In this work, a Fe_3O_4 nanoparticles modified carbon paste electrode (Fe_3O_4/CPE) was prepared. Electrochemical behaviors and determination of COLC on the Fe_3O_4/CPE has been investigated utilizing cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The interaction of COLC and BSA was charactered by the electrochemical method, fluorescence and UV-vis spectroscope. It was found that this modified electrode showed excellent electrocatalytic activity to the oxidation of COLC. And based on COLC as the electro-active probe, an electrochemical method for the direct quantitative determination of BSA was developed.

2. EXPERIMENTAL PART

2.1. Chemicals and reagents

COLC ($C_{12}H_{25}NO_6$) and Bovine Serum Albumin (98% purity, molecular weigh 66000) were purchased from Sigma. Fe_3O_4 magnetic nanoparticles with size of about 30nm were purchased from

Anhui Maanshan Powder Engineering Co. (Anhui, China). Graphite power (spectrum grade, average particle size 4 μm) and paraffin oil were from Shanghai Chemical Reagent Co. (Shanghai, China). All other reagents were of analytical grade and were used as received without further purified. Deionized double-distilled water was used throughout the experiments.

2.2. Apparatus

A computer-controlled electrochemical workstation (CHI 660A) was carried out for electrochemical measurement. A conventional three-electrode configuration was used, with a saturated calomel electrode (SCE) as the reference electrode, a platinum wire as auxiliary electrode, and a Fe_3O_4-CPE as working electrode. Potentials are reported vs. SCE. The pH value of the solution was determined using a pHS-25 acidity meter (Weiye, Shanghai). Ultraviolet visible (UV-Vis) spectra experiments were carried out on a Lambda 17 UV-Vis spectrophotometer with wave-length range of 250-300 nm (Perking-Elmer, USA) equipped with 1.0 cm quartz cells. Fluorescence spectra were recorded on RF-540 Spectrofluorimeter (Shimadzu Corporation, Japan) equipped with 1.0 cm quartz cells and a thermostat bath.

2.3. Preparation of the paste electrodes

The conventional carbon paste electrode (CPE) was prepared by mixing 80 mg graphite power and 20 μL paraffin oil. Fe_3O_4 nanoparticles modified carbon paste electrode (Fe_3O_4-CPE) was constructed by adding 10 mg Fe_3O_4, 70 mg graphite power, and 20 μL paraffin oil. Mixing proceeded thoroughly to produce a well-proportioned paste. A portion of the paste was then packed tightly into the cavity (2 mm diameter, 2 mm depth) of a Teflon electrode holder, and a bare copper wire had been inserted through the opposite end to produce electrical contact. The composite surface was smoothed on a weighing paper and rinsed carefully with double-distilled water prior to each experiment.

2.4. Experimental method

2.4.1. Electrochemical measurement

CV had been employed for investigating the electrochemical response of COLC sweeping between 0.8 and 1.35 V at 0.1 V s^{-1} in 0.1 M H_2SO_4 solution. DPV was performed for the quantitative determination of COLC at the same potential range. Pulse amplitude was set to be 50 mV and Pulse width 50 ms.

2.4.2. UV-vis absorption spectra

The appropriate amount of 1.0 mM COLC have been added into 2.0 mL of 0.1mM BSA at [COLC]/[BSA] ratios of 0/1、0.2/1、0.4/1、0.6/1、0.8/1、1.0/1、1.2/1. The mixture was diluted to

the scale and shaked homogeneously. After reaction at room temperature for 10min, the UV spectrums of BSA were recorded. Under the same conditions, the double-distilled water was used as the blank solution.

2.4.3. Fluorescence measurement

4.0 mL of 0.1mM BSA, and an appropriate amount of 1.0 mM COLC were sequentially added into a 10-ml calibrated tube. The mixture was diluted to the scale with double-distilled water and shaked homogeneously. After reaction at room temperature for 10min, Fluorescence spectrums of BSA were recorded. An excitation wavelength of 296 nm and emission wavelength of 350 nm were used throughout. The widths of slit were set to be 10 nm.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behaviors of COLC

CVs of 0.1 mM COLC at CPE and Fe_3O_4/CPE were shown in Fig. 1. In 0.1 M H_2SO_4 solution, no redox peaks were observed at 0.8～1.35 V potential range at CPE (Fig.1a) and Fe_3O_4/CPE (Fig.1b). The background response of Fe_3O_4/CPE was larger than that of CPE, which indicated that the surface area of Fe_3O_4/CPE was larger. An anodic peak corresponding to the oxidation of COLC at 1.26 V was visible at CPE (Fig. 1c). After the electrode modified by Fe_3O_4, a peak current enhancement was observed (Fig. 1d). The reason for the better performance of the Fe_3O_4/CPE may be due to the large effective surface area of the Fe_3O_4 nanoparticles, which results in a higher rate of electronic transport to the electrode surface. No corresponding reduction peak was seen on the reverse sweep, indicating that the electrode reaction of COLC was an irreversible process.

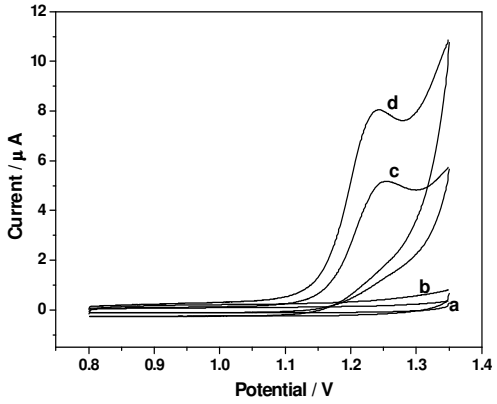

Figure 1. CVs of (a, c) CPE and (b, d) Fe_3O_4/CPE in 0.1 M H_2SO_4 solution without (a, b) and with (c, d) 0.1mM COLC. Scan rate: 0.1 V s^{-1}.

The influence of scan rate on the response of COLC at the Fe_3O_4/CPE was studied by CV. The peak currents were proportional to the square root of scan rates (as shown in Fig. 2 and the inset plot). The linear regression equation is expressed as i_{pa} (A) = 2.218×10^{-5} $v^{1/2}$ (V s^{-1}) $^{1/2}$ + 6.045×10^{-7} (r = 0.999), which indicates that the electrochemical response of COLC on Fe_3O_4/CPE is a diffuse controlled process.

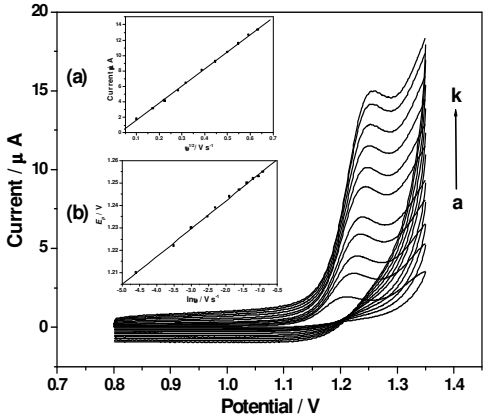

Figure 2. CVs of 0.1 mM COLC in 0.1 M H_2SO_4 at Fe_3O_4/CPE over a range of scan rates (a to k): 0.01, 0.03, 0.05, 0.08, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 V s^{-1}. Inset: Plots of anodic peak current (a) and potential (b) *vs* the square root of scan rates.

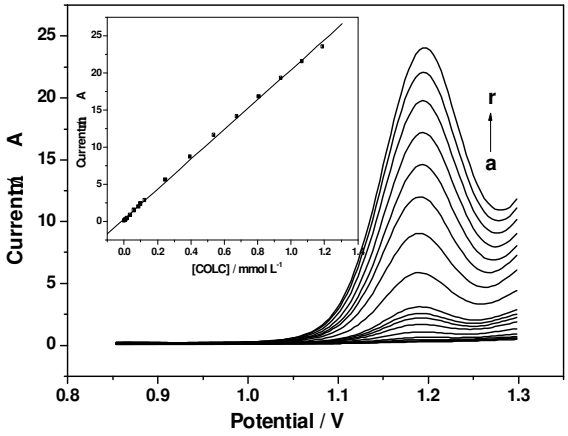

Figure 3. DPV of COLC in 0.1 M H_2SO_4 at Fe_3O_4/CPE at different concentrations (a to r): 8.56×10^{-7}, 3.13×10^{-6}, 5.68×10^{-6}, 8.49×10^{-6}, 1.68×10^{-5}, 3.58×10^{-5}, 6.16×10^{-5}, 8.61×10^{-5}, 9.79×10^{-5}, 1.23×10^{-4}, 2.49×10^{-4}, 3.95×10^{-4}, 5.37×10^{-4}, 6.75×10^{-4}, 8.09×10^{-4}, 9.39×10^{-4}, 1.07×10^{-3}, 1.19×10^{-3}M. Inset: plots of the oxidative current and concentration of COLC.

DPV was adopted for the determination of COLC. The anodic peak current was linearly related to the concentration of COLC in the range of 8.6×10^{-7} to 1.2×10^{-3} M (Fig.3), with the linear regression equation: i_{pa} (A) = 3.23×10^{-7} +0.020C (i_{pa}: A, C: M). The linear range was wider than most of the reported valves [10-13]. The detection limit was 2.6×10-7 M (S/N=3), which was lower than 2 μM at

glassy carbon electrode [13]. The reproducibility of Fe_3O_4/CPE for the determination of COLC was investigated. The relative standard deviation (RSD) was 1.9% for 8 successive determinations of 0.1 mM COLC with a single electrode. The results suggested that Fe_3O_4/CPE posed a good repeatability for the determination of COLC.

To demonstrate the application of this electrochemical sensor for the determination of COLC in pharmaceutical formulations, the recovery test was carried out by adding known amounts of COLC standard solution to injection. As shown in Table 1, the recoveries were from 97.2% to 103.1%. The excellent recoveries suggest that the method is reliable for the quantitative determination of COLC in pharmaceutical preparations.

Table 1. Recovery test of COLC

Samples ($c / 10^{-5}$ mol L^{-1})	Added ($c / 10^{-5}$ mol L^{-1})	Found ($c / 10^{-5}$ mol L^{-1})	Recovery (%)
2.00	1.96	4.02	103.1
2.00	3.84	5.78	98.4
2.00	5.66	7.50	97.2
5.00	4.30	9.40	102.3
5.00	6.10	11.2	101.6
5.00	7.83	12.7	98.3

Possible interference for the detection of COLC at Fe_3O_4/CPE was investigated by adding various ions to 0.1 M H_2SO_4 solution in the presence of 50 μM COLC. From the experiments, no interference could be observed for the following organic compounds: dopamine (50), ascorbic acid (50), uric acid (50), oxalic acid (100), tartaric acid (100), and citric acid (100). And the inorganic species, such as 300- Mg^{2+}, Al^{3+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, K^+, Na^+, Cl^-, Ac^- and SO_4^{2-} did not interfere. The results indicate the present method is adequate for the determination of COLC in real samples.

3.2. Studies of the interaction between COLC and BSA

3.2.1. Electrochemical behavior of COLC in the presence of BSA

DPVs of COLC in the absence and presence of BSA were recorded in Fig.4. An anodic peak at Fe_3O_4/CPE could be observed in H_2SO_4 solution containing 0.1 mM COLC. After the addition of BSA, the oxidation peak current of COLC decreased obviously with the positive shift of the peak potential and no new peaks appeared in the same scan potential range. The results show that there are interactions between COLC and BSA, which result in the formation of electro-inactive complexes. It was difficult for COLC in the complex to make contact with the electrode surface and subsequently be

oxidized at that surface. Furthermore, the concentration of free COLC on the electrode surface decreased, thus the oxidation peak current of COLC reduced.

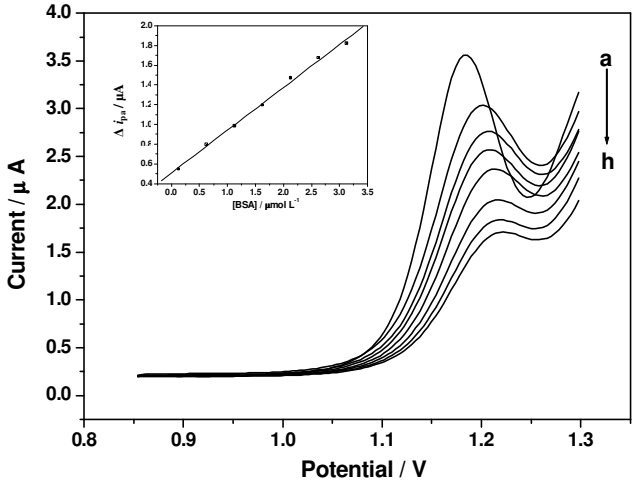

Figure 4. DPV curves of COLC after addition of BSA at different concentrations (a to h): 0, 1.25×10^{-7}, 6.25×10^{-7}, 1.13×10^{-6}, 1.63×10^{-6}, 2.13×10^{-5}, 2.63×10^{-5}, 3.13×10^{-5} mol/L. Insert: linear relationship between Δi_{pa} and c_{BSA}.

Under the optimal conditions, a linear relationship was obtained between the differences of current height of COLC in the absence and presence of BSA (Δi_{pa}) and the concentration of BSA. With a fixed COLC concentration of 0.1 mM, the linear dynamic range was within the BSA concentration from 0.12 to 3.13 μM (the inset of Fig. 4). The linear regression equation was Δi_{pa} (A) = 5.095×10^{-7}+ $0.433C_{BSA}$ (M), with the detection limit of 37 nM. Thus, it was reliable for the quantitative determination of electro-inactive BSA if COLC was used as the electro-active probe.

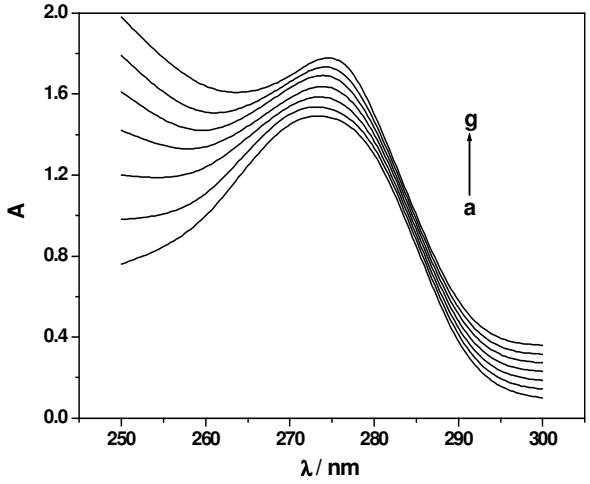

Figure 5. The UV absorption spectra of BSA after treatment with different concentration COLC. [COLC]: [BSA] from a to g: 0/1, 0.2/1, 0.4/1, 0.6/1, 0.8/1, 1.0/1 and 1.2/1.

3.2.2. UV–vis absorption spectra

Fig. 5 shows the UV–vis absorption spectra of BSA and its mixture with COLC in 0.1 M H_2SO_4 solution, which is obtained by keeping the BSA concentration constant and changing the COLC concentration. In the wavelength range from 250 to 300 nm, the maximum absorption of BSA was at 275 nm (curve a). The spectrum of proteins in the 275 nm region primarily reflects the rotatory strength of the π-π^* transitions of the peptide bond, which is relationship with the large amount of a-helix of protein **(3, 4)**. With the addition of COLC, the absorption at 275 nm increased and the protein spectrum underwent a red shift. The changes of absorption spectra indicates that there is a binding interaction between COLC and BSA, which induces the conformational change of BSA.

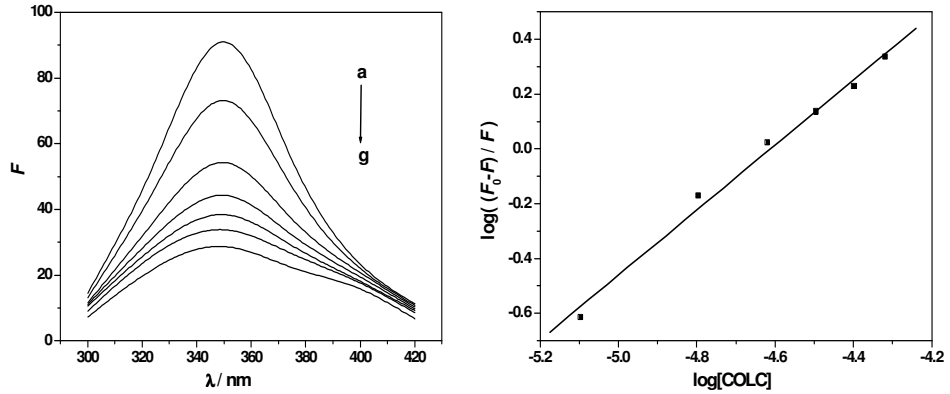

Figure 6. (A) The fluorescence spectra of BSA after treatment with COLC. $\lambda_{ex}/\lambda_{em}$=296 nm/350 nm; [BSA]=$4.0\times10^{-5}$ mol L^{-1}; [COLC] from a to g: 0, 8.0×10^{-6}, 1.6×10^{-5}, 2.4×10^{-5}, 3.2×10^{-5}, 4.0×10^{-5} and 4.8×10^{-5} mol L^{-1}. (B) Linear relationship between log ((F_0-F)/F) and log [COLC].

3.2.3. Fluorescence spectroscopy

Fluorescence spectroscopy is a powerful tool for the study of the reactivity of chemical and biological systems since it allows non-intrusive measurements of substances in low concentration under physiological conditions [22]. The interaction between COLC and BSA was also investigated by fluorescence spectroscopy. In order to discuss the results within the linear concentration range, we carried out the experiment within the linear part of log ((F_0 - F) / F) against log K_0, and stabilized the concentration of BSA at 40 μM. Concentration of COLC varied from 0 to 48μM at increments of 8μM. As can be seen from Fig. 6, the fluorescence intensity reduced obviously with the increasing concentrations of COLC, accompanied by a decrease of wavelength emission maximum (λmax). This suggests an increased hydrophobicity of the region surrounding the tryptophan site [23].

In order to investigate the quenching mechanism, the fluorescence quenching data K_0 and n were analyzed with the well-known equation:

$$\log ((F_0 - F) / F) = \log K_0 + n\log[M]$$

where F_0 and F denotes the steady-state fluorescence intensities in the absence and in the presence of quencher (COLC), respectively, K_0 is binding constant of BSA with COLC, [M] is the equilibrium concentration of the quencher, and n is the number of binding sites. By plotting log ((F_0 - F) / F) against log[M], the linear regression equation is log ((F_0 - F) / F) = 5.480+1.188 log[M] (R^2=0. 990). K_0 and n were calculated to be 3.02×10^5 L mol^{-1} and 1, respectively, suggesting the formation of COLCs–BSA complex.

4. CONCLUSIONS

In this paper, the electrochemical behaviors of COLC and its interaction with BSA were characterized by electrochemical and spectroscopic methods. The results suggested that the response of COLC at Fe_3O_4/CPE was a diffuse controlled irreversible process. The modified electrode could be applied for the determination of COLC with wide linear range and low detection limit. When BSA was added into the COLC solution, the oxidative peak current of COLC decreased and the peak potential shifted positively. The binding constant and the number of binding sites, analyzed by fluorescence spectroscopy, were obtained. The results demonstrated that BSA interacted with COLC to form an electrochemical inactive 1:1 complex. Based on the decrease of oxidative peak current of COLC with the presence of BSA, the interaction of BSA with COLC can be further applied to the determination of micro amount of BSA.

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References

1. A. Farce, C. Loge, S. Gallet, N. Lebegue, P. Carato, P. Chavatte, P. Berthelot, D. Lesieur, *J. Enzym. Inhib. Med. Chem.* 19 (2004) 541.
2. G.C. Borstad, L.R. Bryant, M.P. Abel, D.A. Scroggie, M.D. Harris, J.A. Alloway, *J. Rheumatol.* 31 (2004) 2429.
3. R.E. Olson, D.D. Christ, *Ann. Rep. Med. Chem.* 31 (1996) 327.
4. Y.J. Hu, Y. Liu, X.S. Shen, X.Y. Fang, S.S. Qu, *J. Mol. Struct.* 738 (2005) 143.
5. J. Guharay, B. Sengupta, P.K. Sengupta, *Proteins* 43 (2001) 75.
6. Y.J Hu, Y. Liu, L.X Zhang, R.M Zhao, S.S Qu, *J. Mol. Struct.* 750 (2005) 174.
7. Y. J. Hu, Y. Liu, R. M. Zhao, S. S. Qu, *Int. J. Biol. Macromol.* 37 (2005) 122.
8. G. Aroud, M. Parkinson, *J. Pharm. Pharmacol.* 55 (2003) 10.
9. D.K. Singh, B. Srivastava, A. Sahu, *J. Indian Chem. Soc.* 81 (2004) 171.
10. E. Bodoki, S. Laschi, I. Palchetti, R. Săndulescu, M. Mascini, *Talanta* 76 (2008) 288.
11. X. H. Zhang, S. M. Wang, L. Jia, Z. X. Xu, Y. Zeng, *Sensor Actuat. B-Chem.* 134 (2008) 477.
12. H. Zhang, *Bioelectrochemistry* 68 (2006) 197.
13. E. Bodoki, R. Sândulescu, L. Roman, *Cent Eur J Chem.* 5 (2007) 766.
14. W. Sun, J. Han, K. Jiao, L. Lu, *Bioelectrochemistry* 68 (2006) 60.
15. L. Fotouhi, S. Banafsheh, M. M. Heravi, *Bioelectrochemistry* 77 (2009) 26.
16. K. Ding, *Int. J. Electrochem. Sci.*, 4 (2009) 943.

17. L. Ao, F. Gao, B. Pan, R. He, D. Cui, *Anal. Chem.* 78(2006)1104.
18. S.V. Sonti, A. Bose, *J. Colloid Interf. Sci.* 170(1995) 575.
19. L.M. Rossi, A.D. Quach, Z. Rosenzweig, *Anal. Bioanal. Chem.* 380 (2004) 606.
20. M. D. Rubianes, G. A.Rivas, *Electrochem. Commun.* 5(2003)689-694.
21. R.Y.A. Hassan , I.H.I. Habib, H. N.A Hassan, *Int. J. Electrochem. Sci.*, 3 (2008) 935.
22. Y.J. Hu., Y. Liu, R.M. Zhao, S.S. Qu, *Int. J. Biol. Macromol.* 37 (2005) 122.
23. Y.V. Il'ichev, J.L. Perry, J. D. Simon, *J. Phys. Chem. B* 106 (2002) 452; U. Kragh-Hansen, F. Hellec, B. de Foresta, M. le Maire, J.V. Møller, *Biophys. J.* 80 (2001) 2898.