Determination of Rutin on the Poly(*p*-aminobenzene sulfonic acid) Modified Glassy Carbon Electrode

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The electrochemical behaviors of rutin at a poly(*p*-aminobenzene sulfonic acid) modified glassy carbon electrode (PABSA/GC) have been investigated. Rutin can generate a pair of well-defined redox peaks at the PABSA/GC located at 0.440 V (E_{pa}) and 0.396 V (E_{pc}) (*vs.* saturated calomel electrode (SCE)), respectively. The results indicate that the reaction involves two electrons transfer, accompanied by two protons and the electrochemical process is controlled by adsorption. The electrochemical parameters of rutin on the modified electrode were calculated with the results of the charge transfer coefficient (α), the number of electron transfer (n) and the electrode reaction rate constant (k_s) as 0.61, 2.08 and 2.18 s^{-1}, respectively. Under the selected conditions, the oxidation peak current was linear to the rutin concentration over the range of 2.5×10^{-7}—1.0×10^{-5} M with a detection limit of 1.0×10^{-7} M. The proposed method has been successfully applied to the determination of compound rutin tablets with no interference from the coexisting ascorbic acid.

Key words rutin; *p*-aminobenzene sulfonic acid; modified electrode

Rutin (3′,4′,5,7-tetrahydroxyflavone-3β-D-rutinoside) is one of the most bioactive flavonoids, also known as vitamin P and was thought to be an activating factor for Vitamin C.[1] Rutin has been shown to act as a scavenger of various oxidizing species, superoxide anion (O_2^-), hydroxyl radical and peroxyl radicals.[2] As a result of this, its several pharmacological activities have been widely exploited including antibacterial, antiinflammatory, antitumor, antiallergic, antiviral and antiprotozoal properties.[3,4] Hence, the determination of rutin is of considerable interest. Capillary electrophoresis,[5] HPLC,[6] flow injection analysis,[7] spectrophotometry[8] and electrochemical techniques have been used to determine rutin.

Electrochemical methods for the determination of rutin are more sensitive, less expensive and less time-consuming than the other methods. One of the major problems in the determination of rutin by electrochemical methodology is interference. As flavonoids, rutin can often be found together with ascorbic acid (AA)[9] and may hydrolyze to quercetin in pharmaceutical preparations if not well stored. Rutin has the similar oxidation potential on the bare electrodes with AA or quercetin. Electrodes modified with β-cyclodextrin incorporated carbon nanotube,[10] gilo peroxidase,[11] CeO_2 nanoparticle,[12] ionic liquid[13] have been used for the determination of rutin in the presense of AA or quercetin. However, these modified electrodes are tedious to prepare and costly or lack of stability. It is necessary to detect rutin in convenient, sensitive and high selective methods till date.

Conducting polymers have been widely used as electrode modifiers for their good stability, reproducibility, more active sites and homogeneity in the past two decades. Polymers based on aniline and aniline derivatives have been of particular interest due to their many desirable features such as facile preparation, environmental stability, high conductivity, *etc.* Recently, poly(*p*-aminobenzene sulfonic acid) modified electrodes have been reported for the electrochemical study of several compounds, including dopamine,[14] uric acid,[15] H_2O_2,[16] tyrosine,[17] trifluoperazine,[18] phenylephrine and chlorprothixene.[19] Nevertheless, there is no report about electrochemical determination of rutin by using poly(*p*-aminobenzene sulfonic acid) modified electrodes.

In this work, a poly(*p*-aminobenzene sulfonic acid) modified glassy carbon electrode (PABSA/GC) was fabricated by electropolymerization and the electrochemical behavior of rutin was investigated at it. It was found PABSA/GC showed excellent electrocatalytic activity for the oxidation of rutin. A method for the determination of rutin with simple, stable, sensitive and selective characteristics was developed. The proposed method was further used for the determination of rutin tablet samples with satisfactory results.

Experimental

Reagents Rutin ($C_{27}H_{30}O_{16}\cdot3H_2O$, M=664.56) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and its stock solution (1.0×10^{-3} M) was prepared with ethanol. *p*-Aminobenzene sulfonic acid (*p*-ABSA) was of analytical-reagent grade from Tianjin BASF Chemical Co. The supporting electrolyte was a 0.1 M phosphate buffer saline (PBS) (pH 4.0). Other reagents used were of analytical grade, and their solutions were prepared with redistilled water.

Apparatus Cyclic voltammogram (CV) and differential pulse voltammogram (DPV) experiments were performed on a CHI660C elcectrochemical workstation (Shanghai Chenhua Co., China) controlled by a microcomputer with CHI660C software. A three-electrode system was used, where a saturated calomel electrode (SCE) served as the reference electrode, a platinum wire electrode served as the auxiliary electrode and a poly(*p*-aminobenzene sulfonic acid) modified electrode (PABSA/GC) or a glassy carbon electrode (GC) served as the working electrode. All electrochemical measurements were carried out in a 10 ml cell at room temperature.

Fabrication of Poly(*p*-aminobenzene sulfonic acid) Modified Electrode Prior to use, the bare GC was polished to a mirror-like surface with 0.3 and 0.05 μm Al_2O_3 slurry on emery paper, then rinsed with redistilled water and sonicated in 1 : 1 nitric acid, acetone and redistilled water for 10 min, respectively.

The modification of the glassy carbon electrode is based on reference.[20] The pretreated electrode was placed in 2.0×10^{-3} M *p*-ABSA and 0.1 M PBS (pH=6.8), then it was conditioned by cyclic sweeping between −1.5 and +2.5 V at 100 mV/s for 10 scans. After the modification, the electrode was taken out and rinsed with distilled water. Prior to measurement, the electrode was continuously cycled from −1.0 to +1.0 V in blank PBS (pH=6.8). Finally, the resulted electrode was carefully rinsed with redistilled water, and stored in air for later use.

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Results and Discussion

Electropolymerization of *p*-ABSA at the GC Surface Voltammograms of 2.0×10^{-3} M *p*-ABSA in 0.1 M PBS (pH=6.8) at the GC are shown in Fig. 1. Anodic peak and cathodic peak were observed with peak potential value at +1.1 V (peak 1), −0.6 V (peak 2) and +0.3 V (peak 3), respectively. With a continuous scanning, the peaks were observed larger, reflecting the continuous growth of the film. After the polymerization, a homogeneous brown polymer film was formed on the electrode surface. These facts indicated that *p*-ABSA was deposited on the surface of GC by electropolymerization that is accordant with the reference.[20)]

Cyclic Voltammograms of Rutin at Bare GC and PABSA/GC Figure 2 shows electrochemical response of 5.0×10^{-6} M rutin at GC and PABSA/GC, respectively. At the bare GC, the electrochemical response of rutin was rather poor, indicating that its adsorption at the GC surface was weaker and the electrochemical reaction was quite slow. By contrast, at the PABSA/GC modified electrode, obvious oxidation and reduction peaks were observed, with the anodic peak potential (E_{pa}) at 0.440 V, the corresponding cathodic peak potential (E_{pc}) at 0.396 V. The peak current was about 10 times larger than that at the GC. This result indicates that the use of poly (*p*-aminobenzene sulfonic acid) as a modifier strengthen the adsorption of rutin on the electrode.

According to the equation,[21)] $I_p=n^2F^2A\Gamma v/4RT=nFQv/4RT$, where n is the number of electrons, v scan rate (100 mV/s), Q the charge amount (6.448 μC) and other parameters have their usual meaning (F Faraday constant, R the gas constant, T room temperature), the electron transfer number was calculated to be 2.06.

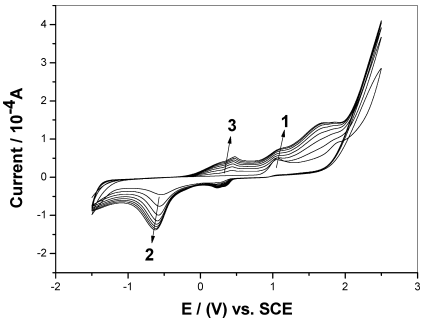

Fig. 1. Cyclic Voltammograms of the GC Modification with *p*-ABSA in 0.1 M PBS (pH 6.8) Containing 2.0×10^{-3} M *p*-ABSA

Cycles: 10, scan rate: 0.1 V/s.

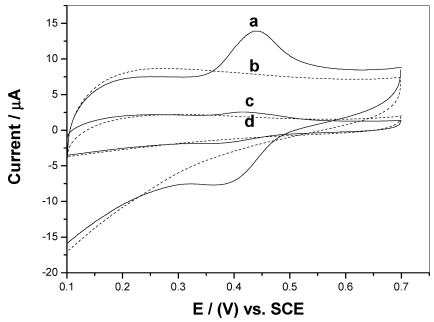

Fig. 2. CVs of 5.0×10^{-6} M Rutin (a, c) in 0.1 M PBS (pH 4.0) and Blank (b, d) at PABSA/GC (a, b) and GC (c, d)

Accumulating time: 300 s, deposition potential: 0 V, scan rate: 0.1 V/s.

Effect of pH on the Electrochemical Response The effect of solution pH values on the rutin peak potential was investigated (Fig. 3A). When the pH changed from 2.0 to 8.0, both the anodic peaks and cathodic peaks shifted to the negative direction. This indicated proton takes part in the reaction. There is a linear relationship between the anodic peak potential and the pH value, and the linear regression equation is $E_{pa}=0.671-0.058$ pH, $R=0.9994$. As the slope is 58 mV/pH, which is very close to the anticipated Nernstian value of 59 mV at 25 °C for equal number of protons and electrons transfer reaction. According to the equation, $-0.059x/n=-0.058$, where n is the electron transfer number and x is the number of hydrogen ion participating in the reaction, it can be concluded that the proton number involved is equal to the electron-transfer number in the electrochemical reaction and $x=n=2$. Therefore, the redox reaction of rutin on the PABSA/GC is a two-electron and two-proton process and the electrode reaction equation is as follows.

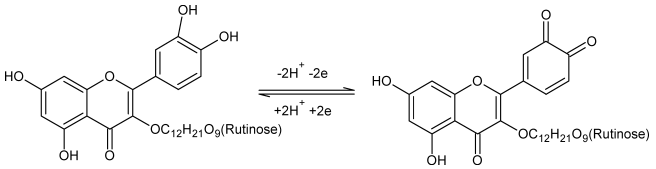

Figure 3B shows the relationship between anodic peak current in CVs and pH of the solution. The peak current increased with the pH of the solution until it reached 4.0, and then it deceased rapidly and nearly disappeared above 8.0. This is related to the proton taking part in the electrochemical reaction. In basic solutions, the electrochemical reaction becomes more difficult due to the shortage of proton. In addition, rutin turns to anions ($pK_1=7.0$) at a high pH[22)] and the terminal sulfonic acids of *p*-ABSA will be deprotonated (pK_a of *p*-ABSA is about 3.23),[23)] giving rise to the electrostatic repulsion between rutin and the modified electrode, which makes the peak current decrease greatly. Therefore, acidic solutions were suitable for the determination of rutin, pH 4.0 was chosen as the optimum pH value in this experiment.

Effect of Scan Rate on the Electrochemical Response The effect of scan rate (v) on the anodic and cathodic peak currents of 5.0×10^{-6} M rutin was studied by CV at various sweep rates. The peak currents of rutin grew with the increasing of scan rates in the range of 10—500 mV/s and there were good linear relationships between the peak currents and v (Fig. 4A). The regression equation was $I_{pa}=1.929+44.603v$ (I_{pa}: μA, v: V/s, $R=0.9970$); $I_{pc}=-1.555-37.262v$ (I_{pc}: μA, v: V/s, $R=0.9980$), indicating the redox process at the modified electrode was adsorption-controlled.

From Fig. 4B, it can be seen that the redox peak potentials were moved with the increase of the scan rate and the peak-to-peak separation increased. At higher scan rates, the peak potentials and log v showed linear relationships. A linear relationship between the E_p with the log v was established and two straight lines were got with two linear regression equations as $E_{pa}(V)=0.074\log v+0.540$ ($R=0.9960$) and $E_{pc}=-0.046\log v+0.360$ ($R=0.9940$).

According to the Laviron's equations[24)]:

$$E_{pa}=E^{\circ\prime}+\frac{2.303RT}{(1-\alpha)nF}\log v$$

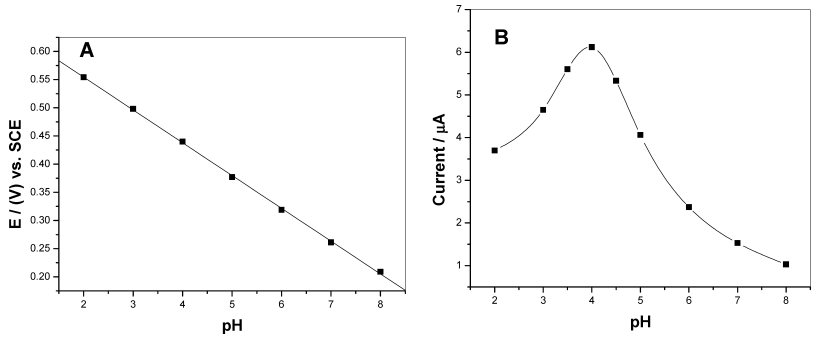

Fig. 3. The Relationship of pH of the Solution on the Peak Potential (A) and Peak Current (B) of 5.0×10^{-6} M Rutin at PABSA/GC
Other conditions are as Fig. 2.

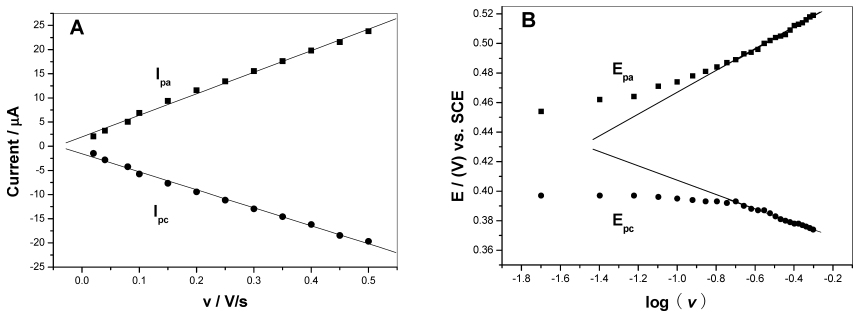

Fig. 4. The Relationship of Scan Rate on the Peak Current (A) and Peak Potential (B) of 5.0×10^{-6} M Rutin at PABSA/GCE
Other conditions are as Fig. 2.

$$E_{pc} = E^{\circ\prime} - \frac{2.303RT}{\alpha nF}\log v$$

$$\log k_s = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nFv} - \frac{(1-\alpha)\alpha nF\Delta E_p}{2.3RT}$$

The values of α and n were calculated to be 0.61 and 2.08, respectively. The value of k_s was further calculated to be $2.18\ s^{-1}$.

Variation of Peak Current with Accumulating Time and Potential For consideration of the adsorption of rutin at the modified electrode, the effect of accumulating time and accumulating potential was investigated. The peak current increased with an increase in accumulating time. When accumulation time was above 5 min, it achieved a maximum value for 5.0×10^{-6} M rutin. So 5 min was generally chosen as the accumulation time. The accumulation potential had little effect on the peak current of rutin in the range from -0.4 to $+0.4$ V and the potential around 0 V was obviously favorable for obtaining the maximal peak currents, so 0 V was selected as the accumulation potential.

The Response Mechanism of Rutin at the Modified Electrode Polyaniline is known to have the ability to interact with many components through hydrogen bonding and through π–π stacking.[25] Poly(*p*-aminobenzene sulfonic acid) film is consisted of many high conjugated dimer molecules and has the similar property with polyaniline.[26] Rutin, which has three aromatic rings, can be adsorbed to the polymer surface through π–π stacking between the aromatic rings and the dimers. On the other hand, the stacking of conjugated aromatic rings might provide a perfect pathway for charge transport from the rutin molecules to the GC which facilitates the electron transfer processes. Therefore, the redox signal of rutin is notably enhanced at PABSA/GC compared with GC.

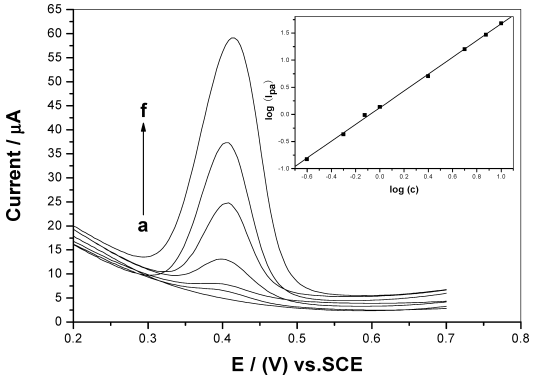

Fig. 5. The DPV Curves Containing Different Concentrations of Rutin (a→f: μM): 0, 0.75, 1.0, 2.5, 5, 7.5, 10 in 0.1 M PBS (pH 4.0) at PABSA/GC
Amplitude: 40 mV; pulse width: 50 ms; pulse period: 200 ms; sensitivity: 1.0×10^{-5} A/V; other conditions are as Fig. 2.

DPV Determination of Rutin Figure 5 presents DPV curves of different concentrations of rutin at PABSA/GC under the optimum conditions. A linear relationship could be established between the logarithm of peak current [Log(I_{pa})] and the logarithm of the concentration of rutin [Log(c)] in the range of 2.5×10^{-7}—1.0×10^{-5} M (the inset). The linear regression equation was Log(I_{pa}) (μA) = 1.547 Log(c) (μM) + 0.125, R = 0.9995, the detection limit of rutin was found to be 1.0×10^{-7} M (S/N = 3). In addition, the relative standard deviation (RSD) of the eight times repeated determination of 5.0×10^{-6} M rutin was 1.6%, indicating that the modified electrode showed good reproducibility. After the electrode was stored for 4 weeks, no apparent decrease of the electrochemical response to rutin was observed, which indicated the

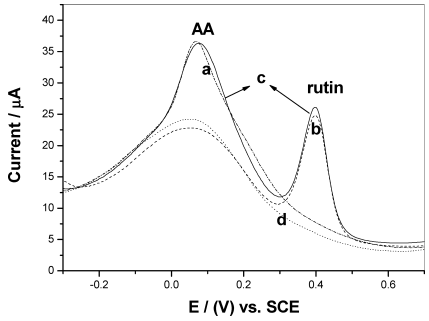

Fig. 6. DPVs of 1.0×10^{-3} M AA (a), 5.0×10^{-6} M rutin (b), 1.0×10^{-3} M AA+5.0×10^{-6} M Rutin (c) and Blank (d) at PABSA/GC

Amplitude: 40 mV; pulse width: 50 ms; pulse period: 200 ms; sensitivity: 1.0×10^{-5} A/V; other conditions are as Fig. 2.

Table 1. Determination of Rutin in Tablets[a] and the Recovery (n=5)

Sample	Detected (mg/l)	RSD (%)	Added (mg/l)	Found (mg/l)	Content[b] (mg)	Recovery (%)
1	1.568	2.1	1.595	3.185	19.60	101.4
2	1.583	2.3	1.595	3.159	19.79	98.8
3	1.726	1.8	1.595	3.310	21.58	99.3
4	1.650	1.4	1.595	3.215	20.63	98.1
5	1.571	2.7	1.595	3.209	19.46	102.7

a) Label amount: 20 mg rutin/tablet. *b*) Content of rutin was obtained by multiplying the detected value and the dilution factor.

good stability of the modified electrode.

Interferences Study AA is the main coexisting substance in compound rutin tablet samples, so the electrochemical response of rutin in the presence of AA at the modified electrode was studied. It can be seen from Fig. 6, the oxidation peaks of AA (curve a) and rutin (curve b) are located at different potential while its peaks are overlapped seriously at the bare electrode. Curve c is the differential pulse voltammogram of mixture solution (AA+rutin) with a peak to peak separation of 320 mV which is large enough for simultaneous determination in the mixed solution. As quercetin may be present as a hydrolytic product of rutin in pharmaceuticals when the rutin tablets are not well stored. The interference of quercetin was also studied, which had a peak separation of 128 mV with rutin. The modified electrode is not influenced by 25-fold quercetin less than ±5.0% relative error.

The effects of other inorganic and organic compounds on the determination of 5.0×10^{-6} M rutin were studied. The determination of rutin is not influenced by a 250-fold excess of ascorbic acid, 100-fold excess of Cu^{2+}, Ca^{2+}, Fe^{3+}, Zn^{2+}, Na^{+}, K^{+}, glucose, vitamin B1, lactic acid, folic acid, adenine, 50-fold excess of dopamine and epinephrine.

Determination of Rutin in Rutin Tablets Ten rutin tablets were powdered and mixed adequately. Twenty milligrams (rutin content) were weighed and dissolved in 100 ml ethanol with the aid of ultrasonication. Certain volume of the above solution was diluted with buffer solution by 125-fold (initial solution). At first, the concentration of rutin in initial solution was detected (see Detected) and the content in rutin tablet was obtained by multiplying the detected value and the dilution factor (see Content). Then the rutin standard solution (see Added) was added to the initial solution and the final solution was found (see Found). Finally, the recovery was calculated. Five parallel samples were analyzed with the proposed method and the results are shown in Table 1. It can be seen the current method is in good agreement with label amount and with a good recovery in the range of 98.1—102.7%.

Conclusion

A poly(*p*-aminobenzene sulfonic acid) modified glassy carbon electrode was fabricated. The electrochemical behaviors of rutin at the modified electrode were investigated in pH 4.0 PBS. Compared with its response at GC, the electrochemical signal of rutin was improved dramatically at the proposed electrode. Under the optimized conditions, a sensitive and selective method was established for the determination of rutin and it can be applied to rutin tablet samples with good recovery. In addition, the modified electrode showed a distinct advantage of simple preparation, high selectivity, good reproducibility and good stability.

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References

1) Ding H. Y., Chao J. B., Zhang G. M., Shuang S. M., Pan J. H., *Spectrochimi. Acta, Part A*, **59**, 3421—3429 (2003).
2) Robak J., Gryglewski R. J., *Biochem. Pharmacol.*, **37**, 837—841 (1988).
3) Gene R. M., Cartana C., Adzet T., Adzet T., Marin E., Parella T., Canigueral S., *Planta Med.*, **62**, 232—235 (1996).
4) Ramanathan R., Das W. P., Tan C. H., *Int. J. Oncol.*, **3**, 115—119 (1993).
5) Chen G., Zhang H. W., Ye J. N., *Anal. Chim. Acta*, **423**, 69—76 (2000).
6) Zu Y. G., Li C. Y., Fu Y. J., Zhao C. J., *J. Pharm. Biomed. Anal.*, **2006**, 714—719 (2006).
7) Volikakis G. J., Efstathiou C. E., *Talanta*, **51**, 775—785 (2000).
8) Hassan H. N. A., Barsoum B. N., Habib I. H. I., *J. Pharm. Biomed. Anal.*, **20**, 315—320 (1999).
9) Pejic N., Kuntic V., Vujic Z., Micic S., *IL Farmaco*, **59**, 21—24 (2004).
10) He J. L., Yang Y., Yang X., Liu Y. L., Liu Z. H., Shen G. L., Yu R. Q., *Sens. Actuators, B*, **114**, 94—100 (2006).
11) Oliveira I. R. Z., Fernandes S. C., Vieira I. C., *J. Pharm. Biomed. Anal.*, **41**, 366—372 (2006).
12) Wei Y., Wang G. F., Li M. G., Wang C., Fang B., *Microchim. Acta*, **158**, 269—274 (2007).
13) Zhang Y., Zheng J. B., *Talanta*, **77**, 325—330 (2008).
14) Xu F., Gao M. N., Wang L., Shi G. Y., Zhang W., Jin L. T., Jin J. Y., *Talanta*, **55**, 329—332 (2001).
15) Tang H., Hu G. Z., Jiang S. X., Liu X., *J. Appl. Electrochem.*, **39**, 2323—2328 (2009).
16) Kumar S. A., Chen S. M., *J. Mol. Catal. A, Chem.*, **278**, 244—250 (2007).
17) Huang K. J., Luo D. F., Xie W. Z., Yu Y. S., *Colloids Surf. B*, **61**, 176—181 (2008).
18) Jin G. Y., Huang F., Li W., Yu S. N., Zhang S., Kong J. L., *Talanta*, **74**, 815—820 (2008).
19) Huang F., Jin G. Y., Liu Y., Kong J. L., *Talanta*, **74**, 1435—1441 (2008).
20) Jin G. Y., Zhang Y. Z., Cheng W. X., *Sens. Actuators B*, **107**, 528—534 (2005).
21) Bard A. J., Faulkner L. R., "Electrochemical Methods Fundamentals and Applications," Wiley, New York, 1980.
22) Mielczarek C., *Eur. J. Pharm. Sci.*, **25**, 273—279 (2005).
23) Wu Y., Li N. M., Luo H. Q., *Sens. Actuators B*, **133**, 677—681 (2008).
24) Laviron E., *J. Electroanal. Chem.*, **101**, 19—28 (1979).
25) Ikkala O. T., Pietila L. O., Passiniemi P., Vikkia T., Osterholmb H., Ahjopaloc L., Osterholmb J. E., *Synth. Mat.*, **84**, 55—58 (1997).
26) Lin X. Q., Kang G. F., Lu L. P., *Bioelectrochemistry*, **70**, 235—244 (2007).