Development of a novel serotonin sensor based on Na-montmorillonite nanoparticles modified carbon paste electrode

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ABSTRACT

A Na-montmorillonite nanoparticles modified carbon paste electrode (CPE) denoted as serotonin sensor is described. A mixture of fine graphite powder and Na-montmorillonite nanoparticles is obtained by dispersing in paraffine, and it is applied for the preparation of serotonin sensor. The electrocatalytic oxidation of serotonin at Na-montmorillonite nanoparticles modified electrode is examined by using cyclic voltammetry (CV). The mechanism of electrochemical behavior of serotonin on Na-montmorillonite nanoparticles film electrode is investigated. The oxidation peak current linearly increased with the serotonin concentration in the range of 0.45 μM to 51 μM with a low detection limit of 57 nM. This Na-montmorillonite nanoparticles modified electrode shows high sensetivity for the determination of serotonin. It has been used to monitor serotonin in human blood serum as a sensor.   © 2009 Trade Science Inc. - INDIA

INTRODUCTION

Clay minerals have attracted the attention of electrochemists, in particular for their analytical applications[1]. The use of montmorillonite as catalyst supports to construct chemical sensors has gained much attention[2,3]. Many sensors and biosensors based on clay-modified electrodes have been developed[4-7].

Serotonin (5-hydroxytryptamine or 5-HT) is a monoamine neurotransmitter widely distributed in the brain and plays a significant role in various pharmacological, physical and biological processes including temperature regulation, muscle contraction, liver regeneration, endocrine regulation and depression[8,9]. Release or leakage of serotonin into the gut lumen has been demonstrated previously[10,11]. Serotonin has also been implicated in several gastrointestinal disorders including irritable bowel syndrome[12,13], inflammatory bowel disease[13], food hypersensitivity[14], etc. High performance liquid chromatography[15-20] and electrochemical method[21-28] have been used in the determination of 5-HT. However, it is still need to develop novel and sensitive methods for the determination of 5-HT. To our knowledge, no serotonin sensor based on the incorporation of Na-montmorillonite into carbon paste has been reported previously.

In this study, we report a carbon paste electrode incorporating Na-montmorillonite as serotonin sen-

sor for the entrapment of serotonin. This procedure constitutes an inexpensive, fast and easy method for the fabrication of serotonin sensors, by incorporating Na-montmorillonite nanoparticles into the carbon paste, followed by packing the carbon paste into the cave of a home-made electrode and smoothing it on a weighing paper. The serotonin sensor shows great electrocatalytical ability towards the oxidation of se-

rotonin and it will receive extensive application in bio-medical system. Moreover, we compared the sensor based on Na-montmorillonite -CPE with other serotonin sensors[23-27,29-31]. As shown in TABLE 1, this sensor has lower detection limit than portion of others and longer life time than others, which may make it have practical application in the determination of serotonin.

TABLE 1 : Figures of comparable methods for determination of serotonin by electrochemical sensor

Electrode	Working potential/reference electrode	detection limit	Linear range	lifetime (d)	Ref
iron(II) tetrasulfophthalocyanine/CPE	+0.33V/ (Ag|AgCl)	1μM	1~15μM	n.r.	[31]
nano-Au/overoxidized-polypyrrole/GCE	+0.37V/SCE	1nM	7nM~2.2μM	n.r.	[24]
MWNTs-DHP/GCE	+0.36/SCE	9nM	0.02~5μM	n.r.	[25]
GCRE	+0.35V/ (Ag|AgCl)	5μM	100nM~5μM	n.r.	[30]
Ru/WGE	+0.45/SCE	0.1μM	0.3~9μM	7	[26]
acetylcholine /GCE	+0.41/SCE	0.5μM	1~30μM	5	[23]
Poly(phenosafranine)/GCE	+0.28/SCE	20nM	n.r.	7	[27]
nano-Au/ITO	+0.41V/ (Ag|AgCl)	9.1nM	10nM~250μM	n.r.	[29]
Na-montmorillonite/CPE	+0.33V/SCE	57nM	0.45μM~51μM	10	our work

EXPERIMENTAL

Na-montmorillonite (SWy-2) was obtained from Source Clay Minerals Repository, University of Missouri (Columbia, MO). Graphite powder was purchased from Shanghai Reagent Factory (Shanghai, China). Paraffin oil was purchased from Wuhan Reagent Factory (Wuhan, China). Serotonin (Sigma Co.) was dissolved in redistilled water to form a stock solution of 5.7 mM and stored at 4°C. Dopamine (DA), ascorbic acid (AA), uric acid (UA) and other reagents were of analytical grade and used as received. Human blood serum obtained from the Hospital of Wuhan University was used as real biological sample in serotonin measurement. Aqueous solutions were prepared with double redistilled water.

All electrochemical measurements were performed on a CHI 830 electrochemical analyzer (Shanghai Chenhua Co., China). The measurements were carried out in a conventional electrochemical cell with a serotonin sensor working electrode (4.5 mm in diameter), a Pt wire counter electrode and a saturated calomel reference electrode. All potentials reported were versus

the saturated calomel electrode (SCE). The serotonin sensor was prepared as follows: 100 mg graphite powder, 30 mg Na-montmorillonite and 16 μL paraffin oil were mixed by hand to produce a homogenous carbon paste. The carbon paste was packed into the cave of a home-made carbon paste electrode and then smoothed on a weighing paper. Scanning electron microscopy (SEM) was performed with a Hitachi X-650 microscope.

A volume of 10 mL 0.1 M phosphate buffer solution (PBS) (pH 7.0) was used as supporting electrolyte and a certain volume of serotonin stock solution was added into the supporting electrolyte to study the electrochemical response of serotonin at Na-montmorillonite modified carbon paste electrode.

RESULTS AND DISCUSSION

Surface morphology of different electrodes

In this work, the surface morphologies of CPE and Na-montmorillonite modified carbon paste electrode (serotonin sensor) are characterized by SEM (Figure 1). As shown in Figure 1A, the surface of CPE is very

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loose. Many nanoparticles are observed on the surface of serotonin sensor, as shown in Figure 1B. The carbon paste incorporating Na-montmorillonite nanoparticles has a surface with plenty of micropores of nanosizes that allow the free entry of serotonin to the inner of the serotonin sensor.
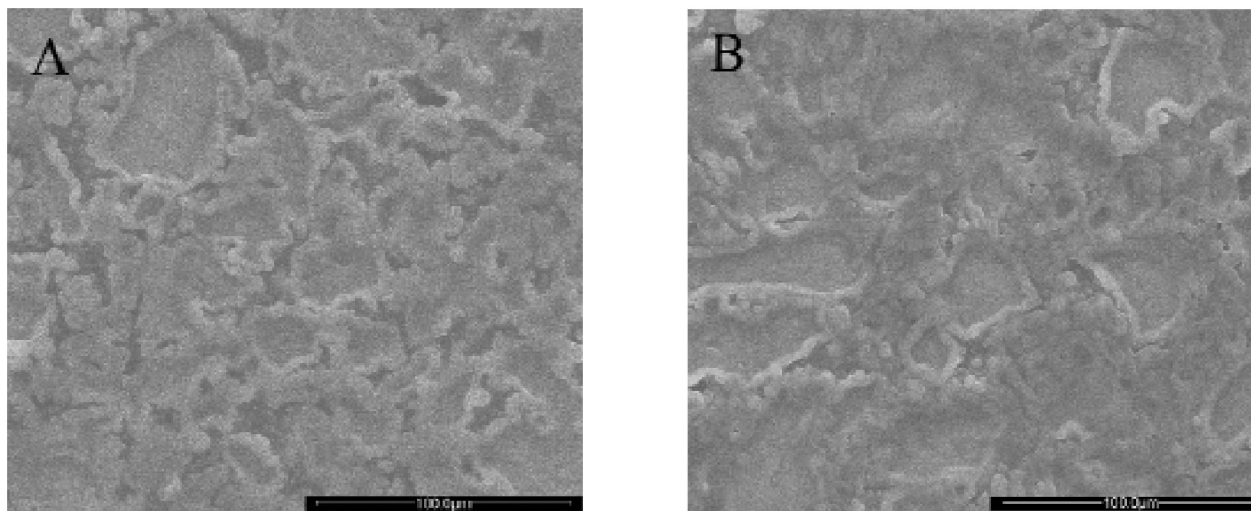

Figure 1 : SEM images of the surface of bare CPE (A) and serotonin sensor (B).

Electrochemical response of serotonin

Figure 2 shows the cyclic voltammograms of different electrodes in 0.1 M PBS (pH=7.0). At the bare CPE, only a weak oxidation peak at 0.33 V with a peak current of 6.97 µA is observed in the presence of 28.4 µM serotonin (curve b). There is no electrochemical signal at the serotonin sensor in the absence of 28.4 µM serotonin (curve a), whereas, a sensitive oxidation peak (I_p=17.88 µA) is observed at 0.33 V at the serotonin sensor in the presence of 28.4 µM serotonin (curve c). These

suggest that the serotonin sensor can act as a promoter to facilitate the electrochemical oxidation of serotonin. This may be contributed to the incorporated Na-montmorillonite. Because of the small dimensions and porous structure, Na-montmorillonite enlarges the surface area of the electrode (e. g. a larger electrochemical reaction interface) and improves the adsorptive property of the sensor for serotonin, which brings the great increase of the oxidation peak current of serotonin.

Optimization of experimental parameters

As the quantity of Na-montmorillonite mixed into the carbon paste can affect the electrochemical response of serotonin strongly, we optimized the quantity of Na-montmorillonite mixed into the carbon paste. The experimental results show that when the quantity of Na-montmorillonite mixed into 100 mg carbon paste is in the range of 10 mg to 35 mg, the oxidation peak current of serotonin at the serotonin sensor increases with the increase of the mixed quantity of Na-montmorillonite, gets its highest at 30 mg and then decreases. This can be explained as follows: the adsorption quantity of serotonin at the serotonin sensor increased with the increase of the mixed quantity of Na-montmorillonite and gets its biggest at 30 mg; however, with the continuous increase of the mixed quantity of Na-montmorillonite,
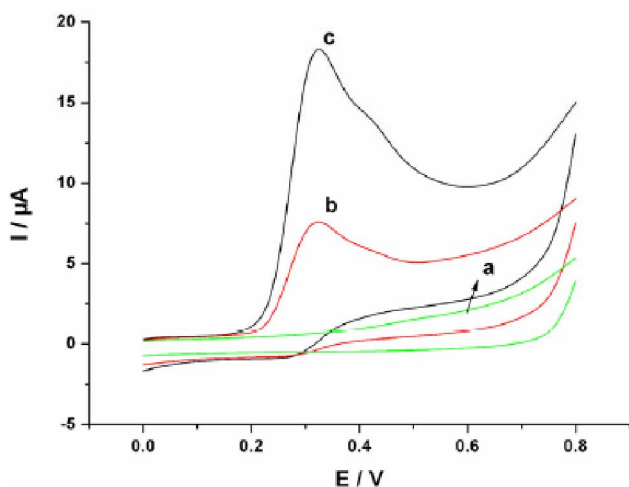

Figure 2 : Cyclic voltammograms of bare CPE (b) and serotonin sensor (a, c) with (b and c) and without (a) 28.4 µM serotonin in 0.1 M phosphate buffer solution (pH=7.0). Scan rate: 100 mV/s.

the film is so thick that it inhibits the electron transfer between serotonin and serotonin sensor.
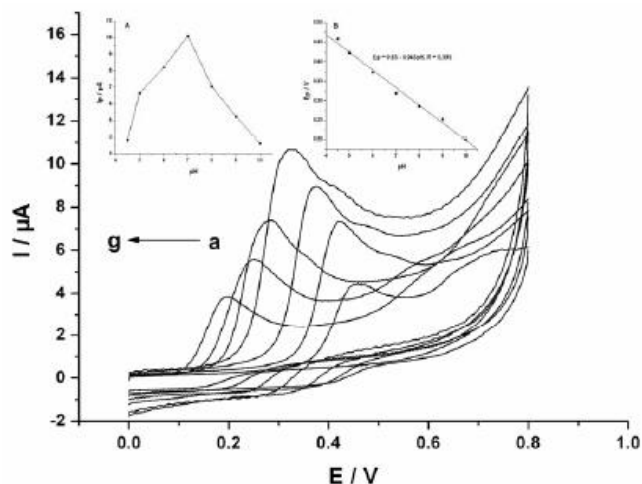

Figure 3 : Cyclic voltammograms of 28.4 µM serotonin at serotonin sensor in the phosphate buffer solution of different pH values (form a to g: 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0, respectively). Insert A: plot of the dependence of the peak current on the pH. Insert B: plot of the dependence of the peak potential on the pH.

The optimization of the pH is very important for the electrochemical determination of serotonin as the pH value of the supporting electrolyte can also affect the electrochemical response of serotonin strongly. Figure 3 shows the electrochemical behaviors of serotonin on the serotonin sensor in different
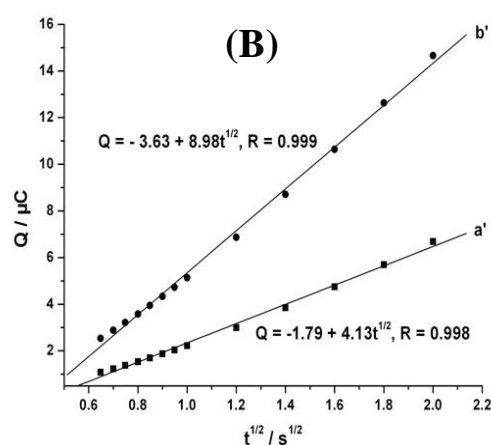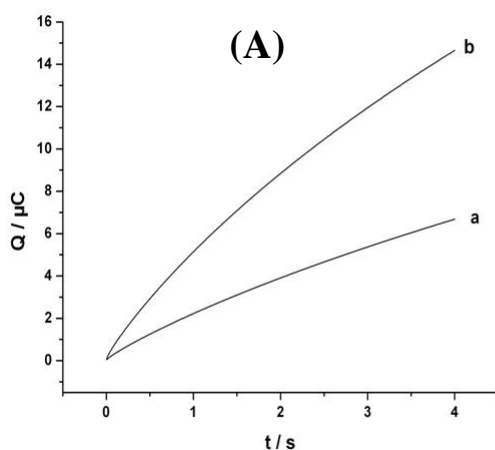
pH values. It is clear that the highest peak current is obtained in the pH = 7.0 buffer solution (Figure 3, insert A), due to the best biology activity of serotonin in this situation. The peak potential has a good linear relationship with solution pH in the range of 4.0 to 10.0 (Figure 3, insert B), indicating a proton participated electrochemical process and the participated proton number is the same as the transfer electron number.

The electrochemical oxidation mechanism of serotonin at the serotonin sensor was investigated by linear sweep voltammetry (LSV). The oxidation peak currents of serotonin increase linearly with the scan rates in the range of 50–300 mV/s, $I_p(\mu A) = 2.533 + 0.064\upsilon(mV/s)$, R=0.994. This suggests that the process of the electrode reaction is controlled by adsorption. The oxidation of serotonin at the serotonin sensor is also totally irreversible. According to the Laviron theory[32], the peak potentials and the scan rates should accord the relationship as follows:

$$Ip = nFQ\upsilon/4RT \tag{1}$$

where Ip is peak current, n is electron transfer number, F is Faraday constant, Q is charge, υ is scan rate, n is electron transfer number, T is temperature. According to equation (1), we can calculate the electron transfer number n = 1.72 ≈ 2.

Figure 4 : Chronocoulometry (A) and the linear relationships between charge and the square root of time (B) for 28.4 µM serotonin at bare CPE (a, a') and serotonin sensor sensor (b, b'). The initial potential is 0.32 V and the final potential is 0.34 V.

Enhanced adsorption of serotonin at serotonin sensor

To verify the enhanced adsorption of serotonin at the serotonin sensor, the chronocoulometry of serotonin at bare CPE (curve a) and serotonin sensor (curve

b) is performed (Figure 4A). The plots of charge against the square root of time $(Q–t^{1/2})$ show good linear relationships at bare CPE (curves a') and serotonin sensor (curves b') in the presence of 28.4 µM serotonin (Figure 4B). According to the integrated Cottrell equation[33],

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the relationship between Q and $t^{1/2}$ can be described as follows:

$$Q = 2ncFAD^{1/2}t^{1/2}\pi^{-1/2} + Q_{dl} + Q_{ads} \qquad (2)$$

$$Q_{ads} = nFA\Gamma \qquad (3)$$

where A is the electrode area, c is the concentration of serotonin, D is the diffusion coefficient, Q_{dl} is the double layer charge, Q_{ads} is the adsorption charge, Γ is the surface coverage, and other symbols have their usual significance. Considering $n = 2$, $A = 0.16$ cm^2, $c = 2.84 \times 10^{-5}$ M, $\pi = 3.14$, $F = 96500$ C/mol, according to equation (2), we can calculate that the D is 1.74×10^{-5} cm^2/s and 8.23×10^{-5} cm^2/s for CPE and serotonin sensor, respectively; according to equation (3), we can calculate that the Γ is 5.8×10^{-11} mol/cm^2 and 1.18×10^{-10} mol/cm^2 for CPE and serotonin sensor, respectively. The larger Γ of serotonin sensor may be attributed to its larger surface area which was brought by the incorporation of Na-montmorillonite. The mixed Na-montmorillonite leads to the more adsorbed amount (i.e. the surface coverage) of serotonin at serotonin sensor than that at CPE. From these results, it is clear that the enlargement of the oxidation peak current of serotonin at serotonin sensor is mainly due to the enhanced adsorption of serotonin in the Na-montmorillonite mixed carbon paste film.

Detection of serotonin at serotonin sensor

Cyclic voltammetry measurements were carried out in a stirred deaerated 0.1 M PBS (pH 7.0). The accumulation was carried out at open circuit with stirring the solution for 170 s, and then kept quiet for 10 s. The voltammograms in the potential range of -0.4 V ~ 0.8 V were recorded. The current response exhibits an excellent linear relationship with the concentration of serotonin in the range of 0.45 μM to 51 μM, Ip (μA) = 7.38 + 1.96c (μM), R = 0.9996. The sensitivity of the serotonin sensor is 1.96 μA/(μM). The detection limit was detected for eight times and estimated to be 57 nM (signal-to-noise ratio = 3.0).

Stability and reproducibility study

Stability and reproducibility are key elements of the electrode performance. It was observed that the serotonin sensor remains 93% of its initial response after it was kept in air for ten days, which indicates

that the modified electrode has good stability. The linear sweep voltammetric responses of 28.4 μM serotonin at the serotonin sensor were almost the same with a relative standard deviation (RSD) of 3.36% (n = 10), which suggests that this serotonin sensor have a good reproducibility.

Selectivity study

TABLE 2 : The influences of some ions and organic compounds on the peak current of 28.4 μM serotonin at serotonin sensor.

Interferant	Concentration(mM)	Signal change (%)
DA	0.5	16.23
AA	0.5	3.5
UA	0.5	2.3
Glucose	5	0.26
K^+	0.5	0.12
Na^+	0.5	0.16
Ca^{2+}	0.5	0.22
Mg^{2+}	0.5	0.27
Fe^{3+}	0.5	2.9

TABLE 2 shows the anti-interference ability of the serotonin sensor. The experimental results indicate that 0.5 mM Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, 5 mM glucose did not interfere and 0.5 mM DA interfere the determination of 28.4 μM serotonin. Here, if the addition of a given substance causes a current change of 5% or more, we consider it has interference.

Real samples analysis

TABLE 3 : Results of the recovery experiment.

concentration of serotonin from sample (μM)	concentration of serotonin from stock solution (μM)	theoretic concentration of serotonin (μM)	practical concentration of serotonin (μM)	recovery (%)
0.103	0.5	0.603	0.592	98.17
0.103	0.5	0.603	0.608	100.83
0.103	0.5	0.603	0.612	101.49
0.103	0.7	0.803	0.792	98.63
0.103	0.7	0.803	0.809	100.75
0.103	0.7	0.803	0.812	101.12

We tested the practical application ability of the sensor towards serotonin by using linear sweep voltammetry. 10 mL 0.1 M PBS (pH 7.0) was used as the supporting electrolyte in a conventional electrochemical cell, a certain volume of human blood serum was added into the cell to make up serotonin solution and then a certain volume of serotonin stock

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solution was added into the cell to examine the recovery result. The experimental results in TABLE 3 show that the recovery is 100.17% in average (n = 6) and this result is satisfied, it suggests that the sensor have practical application in the determination of serotonin.

CONCLUSION

In summary, in this paper, the serotonin sensor was prepared on the basis of incorporating Na-montmorillonite into carbon paste. The incorporated Na-montmorillonite enlarges the surface area of the serotonin sensor and improves its adsorptive property for serotonin, due to its small dimensions and porous structure, this result in improving the electrochemical response of serotonin. The experimental parameters, such as incorporated Na-montmorillonite quantity and pH values were optimized; moreover, the mechanism of the oxidation of serotonin on the serotonin sensor was discussed. This serotonin sensor has good properties for the determination of serotonin. The oxidation peak currents of serotonin have a good linear relationship with the serotonin concentrations in the range of 0.45 μM to 51 μM and the detection limit is low to 57 nM. The serotonin sensor was used for the measurement of serotonin in human blood serum and the result showed that the serotonin sensor has a high sensitivity and may have practical applications in serotonin monitoring system.

ACKNOWLEDGEMENTS

REFERENCES

[1] Z.Navra'tilova', P.Kula; Electroanalysis., **15**, 837 **(2003)**.

[2] P.V.Kamat; J.Electroanal.Chem., **163**, 389 **(1984)**.

[3] T.J.Pinnavaia; Science, **220**, 365 **(1983)**.

[4] D.Barancˇok, J.Cira´k, P.Tomcˇý´k, K.Gmucova´; Bioelectrochem., **55**, 153 **(2002)**.

[5] L.Coche-Gue'rente, V.Deprez, P.Labbe'; J.Electroanal.Chem., **458**, 73 **(1998)**.

[6] S.Hu, C.Xu, G.Wang, D.Cui; Talanta, **54**, 115 **(2001)**.

[7] S.J.Yao, S.Yuan, J.H.Xu; Appl.Clay.Sci., **33**, 35 **(2006)**.

[8] N.J.M.Van, W.J.Janssens, P.M.Vanhoutte; Pharm.Res.Commun., **17**, 585 **(1985)**.

[9] T.N.Wise, L.M.Arnold, V.Maletic; CNS Spectrums, **10**, 1 **(2005)**.

[10] M.D.Coates, C.R.Mahoney, D.R.Linden, J.E.Sampson, J.Chen, H.Blaszyk, M.D.Crowell, K.A.Sharkey, M.D.Gershon, G.M.Mawe, P.L.Moses; Gastroenterology, **126**, 1657 **(2004)**.

[11] R.J.Gilkin; Clin.Ther., **27**, 1696 **(2005)**.

[12] R.J.Dearman, R.A.Skinner, N.Deakin, D.Shaw, I.Kimber; Toxicology, **206**, 195 **(2005)**.

[13] O.Nylander, L.Pihl; Acta Physiol., **186**, 45 **(2006)**.

[14] D.Grundy; J.Physiology, **575**, 1 **(2006)**.

[15] D.Bose, A.Durgbanshi, M.E.Capella-Peir'o, M.Gil-Agust', J.Esteve-Romero, S.Carda-Broch; J.Pharm.Biomed.Anal., **36**, 357 **(2004)**.

[16] R.D.Johnson, R.J.Lewis, D.V.Canfield, C.L.Blank; J.Chromatogr.B., **805**, 223 **(2004)**.

[17] P.Pastore, G.Favaro, D.Badocco, A.Tapparo, S.Cavalli, G.Saccani; J.Chromatogr.A., **1098**, 111 **(2005)**.

[18] K.Gregersen, L.Friyland, A.Berstad, P.Araujo; Talanta, **75**, 466 **(2008)**.

[19] S.Parrot, L.Lamb'as-Senas, S.Sentenac, L.Denoroy, B.Renaud; J.Chromatogr.B., **850**, 303 **(2007)**.

[20] P.C.Gunaratna, K.Karen, C.Candice, B.Kissinger; J.Neurosci.Meth., **155**, 143 **(2006)**.

[21] X.Jiang, X.Lin; Anal.Chim.Acta, **537**, 145 **(2005)**.

[22] M.James, M.J.Penningtona, P.Clinton, L.Jonesa, C.Owessona, P.Danie, M.Laughlina, A.S.Jonathan; J.Neurosci.Meth., **140**, 5 **(2004)**.

[23] G.P.Jin, X.Q.Lin, J.M.Gong; J.Electroanal.Chem., **569**, 135 **(2004)**.

[24] J.Li, X.Lin; Sensor.Actuat.B., **124**, 486 **(2007)**.

[25] K.B.Wu, J.J.Fei, S.S.Hu; Anal.Biochem., **318**, 100 **(2003)**.

[26] G.P.Jin, Q.Z.Chen, Y.F.Ding, J.B.He; Electrochim.Acta., **52**, 2535 **(2007)**.

[27] T.Selvaraju, R.Ramaraj; Electrochem.Commun., **5**, 667 **(2003)**.

[28] Z.H.Wang, Q.L.Liang, Y.M.Wang, G.A.Luo; J.Electroanal.Chem., **540**, 129 **(2003)**.

Full Paper

[29] R.N.Goyal, K.G.Vinod, M.Oyama, N.Bachheti; Talanta, **72**, 976 **(2007)**.

[30] K.Miyazaki, G.Matsumoto, M.Yamada, S.Yasui, H.Kaneko; Electrochim.Acta., **44**, 3809 **(1999)**.

[31] J.Oni, T.Nyokong; Anal.Chim.Acta., **434**, 9 **(2001)**.

[32] E.Laviron; J.Electroanal.Chem., **101**, 19 **(1979)**.

[33] A.J.Bard, L.R.Faulkner; 'Electrochemical Methods: Fundamentals and Applications'. Wiley, New York, **(2001)**.