Asian Journal of Chemistry; Vol. 23, No. 12 (2011), 5331-5334

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Preparation of Silver Doped Poly(L-Cysteine Acid) Modified Electrode and its Determination for Uric Acid

WEI MA, XUE YAO and DENGMING SUN*

School of Chemistry and Materials Science, Huaibei Normal University, Huaibei 235000, P.R. China

*Corresponding author: E-mail: sundengming@126.com

(*Received*: 6 November 2010; *Accepted*: 18 August 2011) AJC-10280

The silver doped poly(L-cysteine acid) modified electrode was prepared by cyclic voltammetric method. The voltammetric behaviour and cyclic voltammetric method determination of uric acid were studied at the modified electrode. In pH 3.0 phosphate buffer solution, the linear ranges for the determination of uric acid were 5.00×10^{-7}-1.00×10^{-4} M and 1.00×10^{-4}-5.00×10^{-4} M with the detection limit of 1.0×10^{-7} M. The method was applied to the determination of uric acid in urine with satisfactory results.

Key Words: Uric acid, Silver, L-Cysteine acid, Modified electrode, Cyclic voltammetry.

INTRODUCTION

Uric acid, a major ultimate product from purine metabolism in human body, its well-balanced level in bloods was ranged from 120-450 μmol[1,2], abnormal leveals of uric acid in human body are symptoms of several disease[3]. Recent years, the study indicated that uric acid is the risk factor that is associated with hypertension and cardiovascular[4]. It can leads to kidney damage obesity, diabetes, high cholesterol, high blood pressure and cardiovascular disease[5-7]. Otherwise, the elevated uric acid concentration in serum is often connected to gout and other conditions containing increased alcohol consumption. It is assumed that uric acid can be irreversibly oxidized in aqueous solution with the primary product of allantoin. Therefore, the detection of uric acid in human physiological fluids are essential for the diagnosis of patients suffering from a series of disorders associated with altered purine metabolism and for the development of new medicine in the clinical study.

Several methods such as enzymatic, high-performance liquid chromatography, amperometric measurement and electrochemical technique have been widely adopted to determine the concentration of uric acid[8-10]. Electrochemical techniques were generally regarded as relatively fast developed for its simplicity, ease of miniaturization, high sensitivity and relatively low cost compared with other methods[11-13].

The chemical modified electrode (CME) is widely used because it could improve the fixed functional groups' stability and electrochemical characteristics[14]. Recent years, metal doped chemical modified electrode was attracted many chemists because its obviously improved characteristics, such as sensitivity, accuracy and selectivity[15]. At present, the study focus is primary at the doping in the poly aniline[16-19], poly pyrrole[20-22] and other polyer film[23-25].

Amino acids are necessary for lives and have been of greatly used to both biologists and chemists. Many works have been done on the synthesis and determination of amino acids[26]. Poly-amino acid modified electrode have been investgated in determining some electoactive compounds. However, amino acid as single modifier to modify the electrode could not contente people's request for more accuracy, more sensitive. By metal-doped, the catalytic ability of poly-amino acid modified electrodes could be enhanced greatly. Recent years, some study of metal-doped polymer modified electrode to determine substances has been reported[25]. Nanoparticles of sliver have leaded to much more attention in electro analysis because of their particular physical and chemical properties. Nevertheless, the chemical modified electrode which is used to determine of uric acid with silver-doped in L-cysteine acid has not been reported.

In this paper, silver doped poly(L-cysteine acid) modified electrode (Ag-L-Cys/GCE) was prepared by cyclic voltammetric method, the voltammetric behaviour of uric acid and cyclic voltammetric method to determine uric acid were studied at Ag-L-Cys/GCE. The study indicated, uric acid redox rate was increased obviously at Ag-L-Cys/GCE with the silver doped with good stability, high sensitivity, wide response range and fast responding. The electrode was used to determine uric acid with satisfied result.

EXPERIMENTAL

Electrochemical measurements of cyclic voltammetry (CV) experiment were performed using a BSA100/W work station (BAS group, USA). The measurements were carried out at room temperature in a three-electrode cell. Glassy carbon electrode (bare or modified), Ag/AgCl and platinum wires were used as working, reference and counter electrodes, respectively. A digital pH/mV instrument (PHS-3C, Shanghai hongyi device corporation, shanghai, China) with an assembled electrode was utilized in pH measurements of buffer solutions used as supporting electrolytes.

Solutions of uric acid (Sigma, USA): 1.00×10^{-4} M. Phosphate buffer solutions (PBS) were prepared with 0.1M KH_2PO_4-K_2HPO_4 and adjusting the pH with 0.1M H_3PO_4 and 0.1M K_3PO_4 as supporting electrolyte. L-Cysteine acid and other chemicals reagents were of analytical grade unless stated otherwise. All aqueous solutions were prepared using doubly distilled deionized water.

Preparation of Ag-L-Cys/GCE: The bare glassy carbon electrode was polished successively with 0.05 μm alumina on brown textmet. Prior to modification, the electrode was rinsed with 1:1 HNO_3, ethanol and then sonicated in doubly distilled water for 5 min. Whereafter, the polymeric film was deposited by cyclic sweeping from 2.4 to -0.9 V at the scan rate of 200 mV/s for 10 cycles in the solution which contains 0.064M HNO_3, 5.0×10^{-4} M $AgNO_3$, 4.5×10^{-3} M L-cysteine acid and 2.5×10^{-2} M KNO_3. Finally, the Ag-L-Cys/GCE was rinsed by doubly distilled water.

Method: Ag-L-Cys/GCE, Ag/AgCl and platinum wires were used as working, reference and counter electrodes, respectively. Cyclic sweeping from 0.20-0.95 V at 180 mV s^{-1} in pH 3.0 phosphate buffer solution containing 5.0 mL uric acid standard solution with the quiet time of 8 s. After every sweeping, the electrode was rinsed in doubly distilled water.

RESULTS AND DISCUSSION

Preparation of the Ag-L-Cys/GCE: Fig. 1 shows the cyclic voltammograms (CVs) of the polymeric procedure of the Ag-L-Cys/GCE. In the first potential sweep, an obvious weak reductive peak at the peak potential of -0.5 V was observed. During the following 9 cycles, the peak potential was invariableness with the peak current gradually decrease. The result indicated that with the prosecution of polymerization, the polymer film at the electrode surface went to integrity and stability, at the same time, the speed of polymerization was slower.

Electrochemical behaviour of uric acid at the Ag-L-Cys/GCE: Fig. 2 shows the cyclic votammogrames of uric acid at bare GCE (1), Ag/GCE (2), L-Cys/GCE (3) and Ag-L-Cys/GCE (4). From the fig, uric acid shows an obvious oxidation peak response with the potential of 0.623 at bare GCE. But at the other three modified electrode, all voltammograms presented a pair of irreversible redox peaks with the potential value of the anodic and the cathodic peak being 0.637 and 0.573 V at Ag/GCE, 0.632 and 0.573V at L-Cys/GCE, 0.639 and 0.576V at Ag-L-Cys/GCE, respectively. It is shown that their peak potential separation (ΔE_p) was 56, 59 and 63 mV.

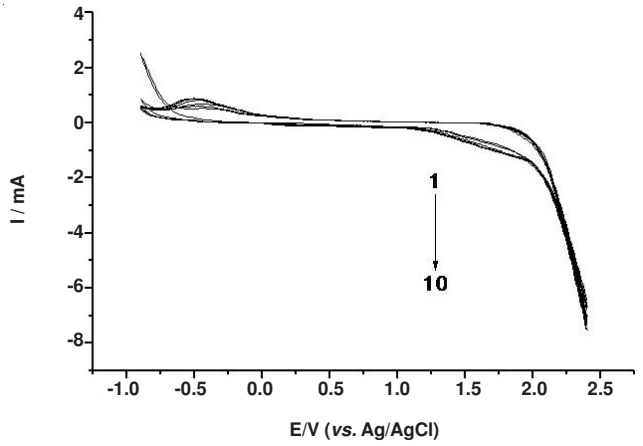


Fig. 1. Cyclic voltammetric curves of silver and L-cysteine acid in the polymerization process. Scan rate: 200 mV/s; from 1-10 indicate the total number of sweeps

The anodic peak current of Ag-L-Cys/GCE is the largest contrast against other electrode, it shows that Ag-L-Cys film have the best catalytic ability.

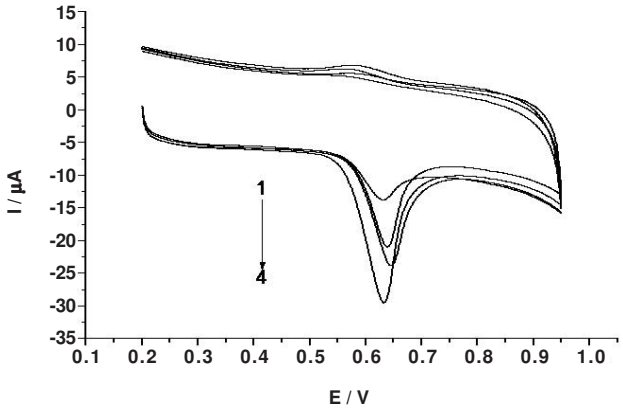
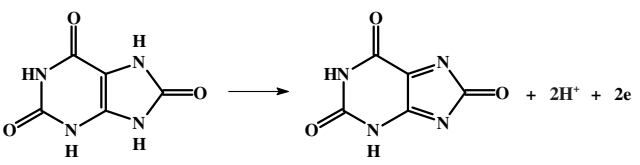


Fig. 2. Cyclic voltammetric curves of 1.00×10^{-4} $M_{uric\ acid}$ at GCE (1), Ag/GCE (2), L-Cys/GCE (3) and Ag-L-Cys/GCE (4). PBS: 3.0 scan rate: 180 mV/s

To investigate the pH dependence on uric acid response at the modified electrode, cyclic voltammograms of uric acid at the Ag-L-Cys/GCE were recorded from pH 2.5-11.0. As shown in Fig. 3, the oxidation peak potential (E_{pa}) and re-reduction peaks potential (E_{pc}) were all shifted to negative direction with the relationship of E_{pa}-pH was E_{pa} (V) = 0.8107-0.06243 pH, R = 0.9910. So the slope were 62.4 mV/pH (close to the theoretical value of 59 mV/pH), showing that the proportion of the electron and proton reactions is 1:1.

Thus, the electrode reaction mechanism could be described[3] as:



when pH < 3.0, with the accretion of pH, oxidation peak's current increased, but when pH reached 3.0, peak current took on a depressed trend with the accretion of pH. As well, the

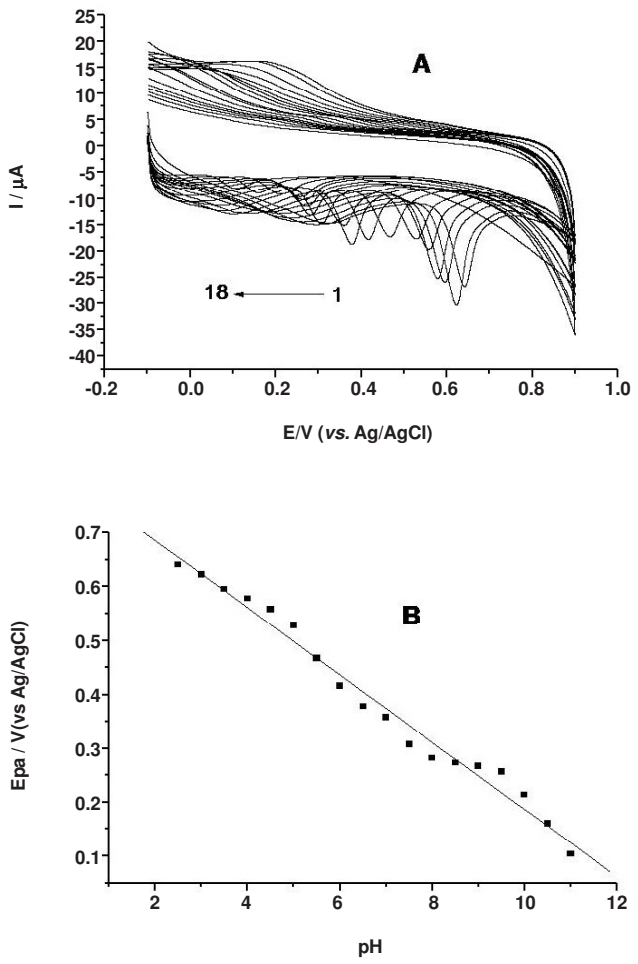


Fig. 3. Effects of solution pH at Ag-L-Cys/GCE on the cyclic voltammetric response of 1.0×10^{-4} $M_{uric\ acid}$(A) and the relationship curve between the peak potential and pH(B). Scan rate: 50 mV/s; pH from 1-18: 2.5, 3.0,3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0

cathodic peak current exhibited a similar current when pH < 3.5, but when pH > 3.5, the peak current rapidly felled, it would disappeared when pH > 6.0. The anodic and the cathodic peak current were turned to tiptop at pH of 3.0 and 3.5, respectively. Considering the anodic peak's response was good and steady, phosphate buffer solution of pH 3.0 was chosen in this paper.

In addition, the effect of the scan rate on the peak current of uric acid was investigated in pH 3.0 phosphate buffer solution, Fig. 4. The oxidation peak potential of uric acid was positively removed and the oxidation peak current went to increase with the accretion of scan rate. In the range from 40-600 mV/s, the logarithm of oxidation peak current and the scan rate were found to be proportional: $\log (I_{pa}) = -5.492 + 0.6061 \log \nu$, r = 0.9968. Furthermore, the oxidation peak current was discovered to be proportional to the square root of the scan rate with the range from 20-600 mV/s: $I_{pa} = -1.639 \times 10^{-5} + 6.909 \times 10^{-6}\nu^{1/2}$ (V/s), r = 0.9986. It is reasonable to ensure that a diffusion-controlled irreversible oxidation process of uric acid at the surface of modified electrode. Based on the above, we can affirmed that the reaction is controlled primary by the diffusion of uric acid. When the scan rate was over 180 mV/s, the peak figure became ungainly. So in this paper, the scan rate was employed for 180 mV/s.

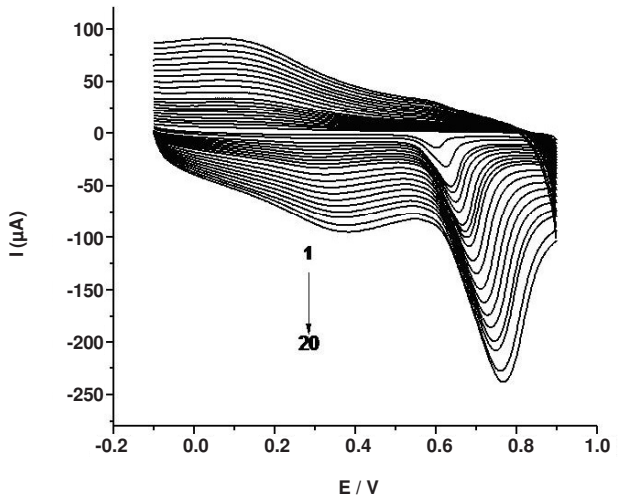


Fig. 4. Cyclic voltammograms of 1.0×10^{-4} M uric acid in pH 3.0 PBS. Scan rate from 1-20: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 240,280, 320, 360, 400, 440, 480, 520, 560, 600 mV/s

The quiet time was changed every time to do a group of experiment. The result indicated that peak current went to tiptop when the quiet time is 8 s. Thus, the optimal quiet time was chosed as 8 s.

Concentration determination of uric acid: As shown in Fig. 5, to determine uric acid at the modified electrode by cyclic voltammetry. At the optimal condition, the oxidation peak current was taken on two liner relations, they were proportional to the concentration of uric acid from 5.00×10^{-7}-1.00×10^{-4} M and 1.00×10^{-4}-5.00×10^{-4} M, the liner regression equation were $I_{pa} = 1.067 + 2.709 \times 10^{5}C_{uric\ acid}$ (M) and $I_{pa} = 24.98 + 3.128 \times 10^{4}C_{uric\ acid}$ (M), with the correlation coefficient of R = 0.9950 and R = 0.9980, respectively. The detection limit was 1.0×10^{-7} M.

Electrode reproducibility and stability: The reproducibility and stability of the modified electrode were determined in 50 parallel experiments. The relative standard was 3.0 %. Put the modified electrode in air at room temperature for 30 days, the same shape and current of the voltammetric curves of uric acid showed a better stability and reproducibility of Ag-L-Cys/GCE.

Interferences: The effect of other foreign compounds such as amino acids and commonly iron in pharmaceutical preparations were studied by preparing solutions containing 5.00×10^{-5} $M_{uric\ acid}$ by addition of an appropriate amount to give an error of ± 5.0 %.

The errors were 11 determined by comparison with the peak heights given by a solution of analyte containing no foreign substances. The result indicated L-leucine, L-serine, L-lysine, L-aspartic acid, L-isoleucine, carbamide (5.00×10^{-3} M), Ba^{2+}, Cu^{2+}, Cd^{2+}, Ag^{+}, Bi^{3+}, Cl^{-}, Co^{2+}, Cr^{3+}, Mg^{2+}, La^{3+}, Zn^{2+}, Sr^{2+}, Ca^{2+}, Nd^{3+}, Zr^{4+}, Mn^{2+}, Pb^{2+}, K^{+}, Na^{+}, Al^{3+}, AA (> 2.0 mg), I^{-} (1.9 mg), V^{5+}, Fe^{3+} (1.0 mg), Cr^{5+} (0.1 mg), had no interference with uric acid detection. As could be seen, most of these substances did not interfere with the determination of uric acid. Therefore, it's possible to determine uric acid which came from human body urine directly at Ag-L-Cys/GCE.

Samples analysis: Take some amount of human urine diluted to 100 mL. Then take out 5 mL to an electrochemical

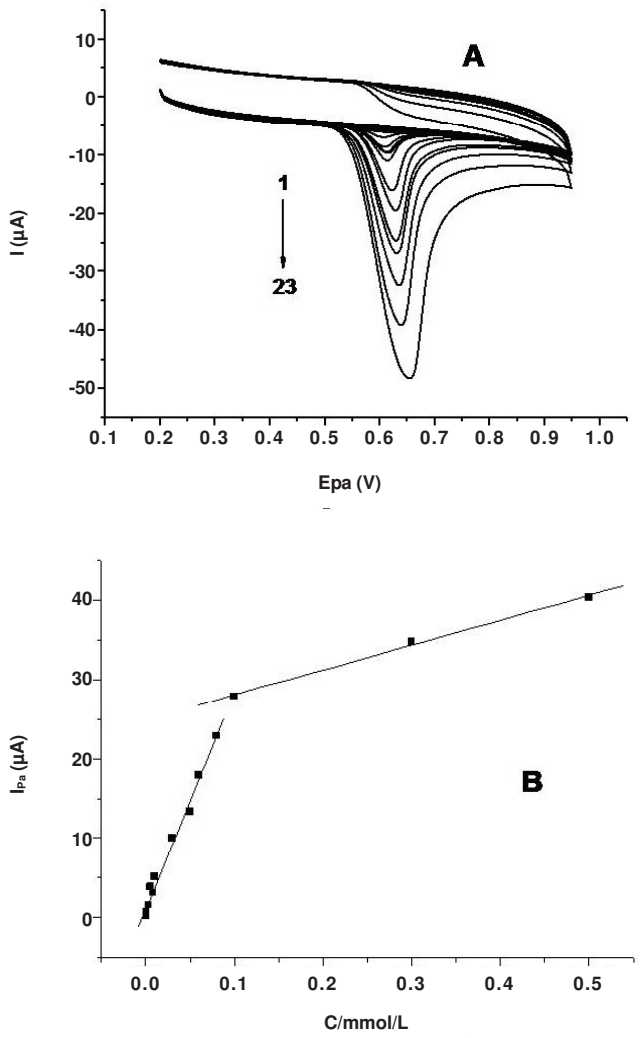


Fig. 5. (A) Cyclic voltammogams for uric acid in 0.1M phosphate buffer solution (pH 3.0) at Ag-L-Cys/GCE modified electrode at different $C_{uric\ acid}$ (from 1-23): 8.00×10^{-9}, 1.00×10^{-8}, 3.00×10^{-8}, 5.00×10^{-8}, 8.00×10^{-8}, 1.00×10^{-7}, 3.00×10^{-7}, 5.00×10^{-7}, 6.00×10^{-7}, 8.00×10^{-7}, 1.00×10^{-6}, 3.00×10^{-6}, 5.00×10^{-6}, 6.00×10^{-6}, 8.00×10^{-6}, 1.00×10^{-5}, 3.00×10^{-5}, 5.00×10^{-5}, 6.00×10^{-5}, 8.00×10^{-5}, 1.00×10^{-4}, 3.00×10^{-5}, 5.00×10^{-4} M (B) the relationship between the oxidation peak current and the concentration for uric acid

cell and added 5 mL pH 3.0 PBS. Then the test solution was determined by cyclic voltammetric measurement and the results were satisfactory. The results are listed in Table-1.

TABLE-1
ANALYTICAL RESULTS OF SAMPLES (n = 6)

Sample	Found (μM)	RSD (%)	Added (μM)	Recovery (%)
1	138.4	2.1	20	95.20
2	76.3	3.5	20	105.0
3	108.8	2.4	20	101.8

Conclusion

A simple, quick and sensitive electrochemical technique has been developed for the voltammetric measurement of uric acid, based on a Ag-L-Cys/GCE. This technique has been successfully applied for simultaneous measurement of uric acid in human urine with satisfactory results.

ACKNOWLEDGEMENTS

This work was financially supported by the Science and Technology Foundation of Anhui province (No. 2007020203011), the Natural Science Foundation of Anhui Province Ministry of Education (No. KJ2008A122), the Foundation of Anhui Key Laboratory of Energetic Materials (KLEM2009008).

REFERENCES

1. X. Cai, K. Kalcher, C. Neuhold and B. Ogorevc, *Talanta*, **41**, 407 (1994).
2. J.M. Zen and J.S. Tang, *Anal. Chem*., **67**, 1892 (1995).
3. J.B. He, G.P. Jin, Q.Z. Chen and Y. Wang, *Anal. Chim. Acta*, **585**, 337 (2007).
4. F. Bandukwala, M. Huang and G.V.R. Prasad, *Transpl. Proc*., **41**, 1634 (2009).
5. R.J. Johnson, D.H. Kang, D. Feig, S. Kivlighn, J. Kanellis, S. Watanabe, K.R. Tuttle, R.I. Bernardo, H.A. Jaime and M. Mazzali, *Hypertension*, **41**, 1183 (2003).
6. M. Mazzali, J. Hughes, Y.G. Kim, J.A. Jefferson, D.H. Kang, K.L. Gordon, H.Y. Lan, S. Kivlighn and R.J. Johnson, *Am. J. Hypertens.*, **38**, 1101 (2001).
7. M. Reimann, A.E. Schutte, L. Malan, H.W. Huisman and N.T. Malan, *Atherosclerosis*, **197**, 638 (2008).
8. R. Dobay, G. Harsanyi and C. Visy, *Anal. Chim. Acta*, **385**, 187 (1999).
9. K. Inoue, T. Namiki, Y. Iwasaki, Y. Yoshimura and H. Nakazawa, *J. Chromatogr. B*, **785**, 57 (2003).
10. E. Popa, Y. Kubota, D.A. Tryk and A. Fujishima, *Anal. Chem*., **72**, 1724 (2000).
11. M.A. Mohammad, H. Beitollahi, B. Ganjipour, H. Naeimi and M. Nejati, *Bioelectrochemistry*, **75**, 1 (2009).
12. J.M. Zen, A.S. Kumar and D.M. Tsai, *Electroanalysis*, **15**, 1073 (2003).
13. J. Argüello, V.L. Leidens, H.A. Magosso, R.R. Ramos and Y. Gushikem, *Electrochim. Acta*, **54**, 560 (2008).
14. D.M. Sun and W.N. Hu, *Chin. J. Anal. Chem*., **25**, 913 (2008).
15. W. Ma and D.M. Sun, *Russ. J. Electrochem*., **43**, 1382 (2007).
16. K.S. Ryu and K. Kim, *J. Power Sources*, **165**, 420 (2007).
17. L. Zhang, C.H. Zhang and J.Y. Lian, *Biosens. Bioelectron*., **24**, 690 (2008).
18. P.R. Roy, T. Okajima and T. Ohsaka, *J. Electroanal. Chem*., **561**, 75 (2004).
19. P. Kalimuthu and S.A. John, *Anal. Chim. Acta*, **647**, 97 (2009).
20. J. Li and X.Q. Lin, *Sens. Actuators B*, **124**, 486 (2007).
21. K. Yamada, K. Seya and G. Kimura, *Synth. Met.*, **159**, 188 (2009).
22. S. Shahrokhian and E. Asadian, *J. Electroanal. Chem*., **636**, 40 (2009).
23. G.Z. Zhou, T. Tian and Y.X. Sun, *Anal. Test. Technol. Instrum*., **1**, 13 (2005).
24. M.E. Ghica and C.M.A. Brett, *Anal. Lett*., **39**, 1527 (2006).
25. W.N. Hu, D.M. Sun and W. Ma, *Chem. Anal. (Warsaw)*, **53**, 703 (2008).
26. A.C. Lin, D.C. Chen, C.C. Lin, H.H. Chou and C.H. Chen, *Anal. Chem*., **71**, 1549 (1999).