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Voltammetric Determination of Sinomenine at Glassy Carbon Electrode Modified by Cysteic Acid based on Electrochemical Oxidation of *L*-cysteine

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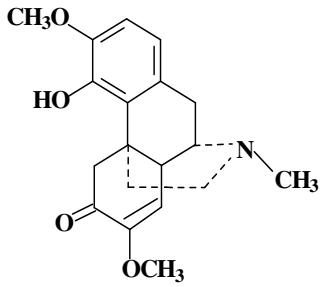
Cysteic acid based on electrochemical oxidation of *L*-cysteine (CySH) form a novel thin film material at a glassy carbon electrode (GCE) for electroanalytical determination of sinomenine. The determination of sinomenine at the modified electrode with strongly accumulation was studied by differential pulse voltammetry (DPV). The peak current obtained at + 0.650 V (vs. SCE) from DPV was linearly dependent on the sinomenine concentration in the range of 1.0×10^{-6} ~ 1.0×10^{-4} M in a B-R buffer solution (0.04 M, pH 1.81) with a correlation coefficient of 0.999. The detection limit (S/N = 3) was found to be 6.0×10^{-7} M. The low-cost modified electrode showed good sensitivity, selectivity, stability and had been applied to the determination of sinomenine in pharmaceutical formulations with satisfactory results. The electrochemical reaction mechanism of sinomenine was also discussed firstly.

Keywords: sinomenine, cysteic acid, *L*-cysteine, voltammetry, human serum

1. INTRODUCTION

The root and stem decoctions of the Chinese medicinal plant *Sinomenium acutum* Rehd. et Wils. (formerly *Sinomenium diversifolius* Diels) have been used as a folk remedy for neuralgia and rheumatoid arthritis in many areas of the Far East [1]. Chinese medical doctors have been using this plant to treat joint pain and arthritis for over 1000 years [2]. Sinomenine, (Scheme 1) [(9α, 13α, 14α)-7, 8-didehydro-4-hydroxy-3, 7-dimethoxy-17-methyl-morphina-6-one)] (C_{19} $H_{23}NO_4$) is an alkaloid isolated from the Chinese medicinal plant *Sinomenium acutum* [3]. A tablet form of the alkaloid sinomenine has also been developed in China for treatment of rheumatoid arthritis [2]. Generally, sinomenine hydrochloride is the main chemical form of sinomenine for pharmaceutical purposes [4]. It

has immunomodulating and anti-inflammatory activities [5-7]. Its anti-arthritic property is related to the anti-proliferative effects on synovial fibroblasts [6] and lymphocytes [8]. Other anti-rheumatic mechanisms are attributed to its ability to decrease PGE2 and leukotriene C4 synthesis, and inhibit NO production [9]. Although some studies on sinomenine pharmacological actions have been done, there are only a limited number of techniques described for the determination of sinomenine including non-aqueous capillary electrophoresis [10], high-performance liquid chromatography (HPLC) [11, 13], HPLC/ESI/ion trap mass spectrum [14], flow injection chemiluminescent [15], thin-layer chromatography [16-18], ultraviolet spectrophotometry [19]. However, HPLC and HPLC/ESI/ion trap mass spectrum present lower efficient and time-consuming, while thin-layer chromatography lacks quantitative precision. For these reasons, the rapid, simple and accurate method for the determination of sinomenine with high sensitivity is expected to be established.



Scheme 1. Structure of sinomenine.

Electrochemical detection of analytes is a very elegant method in analytical chemistry. The interest in developing electrochemical sensing devices for use in environmental monitoring, clinical assays or process control is growing rapidly. Electrochemical sensors satisfy many of the requirements for such tasks particularly owing to their inherent specificity, speed of response, sensitivity and simplicity of preparation. Up to date, there are only two available electrochemical methods for the determination of sinomenine in the literature including polarography with mercury electrodes [20, 21]. However, the utilization of the mercury electrodes would contaminate the environment because of their environmental toxicity if the mercury is handled with no special care, and a determination of sinomenine using a solid electrode had not been reported.

In the present paper, we described the use of cysteic acid based on electrochemical oxidation of *L*-cysteine (CySH) to form a novel thin film material at a glassy carbon electrode (GCE) for electroanalytical determination of sinomenine in pharmaceutical formulations. A sensitive anodic oxidative peak of sinomenine at + 0.65 V (*vs*. SCE) was used for quantitative determination by using differential pulse voltammetry. A good linear relationship was realized between the anodic peak current and sinomenine concentrations in the range of 1.0×10^{-6} ~ 1.0×10^{-4} M with the detection limit of 6.0×10^{-7} M. Compared with the amperometric method with the bare glassy carbon electrode, the detection limit of this method decreased one orders of magnitude. The electrochemical reaction mechanism of sinomenine was also discussed firstly. The modified electrode showed significantly enhanced accumulation of sinomenine compared with a bare glassy carbon electrode. This method has the advantages of rapid and simple operation, very low interference and high accuracy in

pharmaceutical formulations for the determination of sinomenine. This cysteic acid film is considered to be a promising, low-cost, steady and biocompatible material for the modification of electrodes.

2. EXPERIMENTAL PART

2.1. Apparatus

Cyclic voltammetric (CV) and differential pulse voltammetric experiments were carried out at a CHI 660B electrochemical workstation (Chenhua Instruments, China). All electrochemical experiments employed a conventional three-electrode system with a glassy carbon electrode or a cysteic acid modified glassy carbon electrode (3.0 mm in diameter) as a working electrode, a platinum wire as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. All potentials reported in this paper were referenced to the SCE. All of the electrochemical experiments were carried out at 25 ºC.

2.2. Chemicals and solutions

L-cysteine (CySH) was obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Sinomenine was purchased from Jiangxi Herbfine Hi-tech Co., Ltd (Jiangxi, China). All the other reagents used were of analytical grade. Doubly distilled water was obtained by purification through a Millipore water system and used throughout. A stock aqueous solution of 1.0×10^{-3} M sinomenine was prepared with the doubly distilled water, and kept it in the dark under refrigeration (below 4 ºC). The B-R buffer solutions (0.04 M) at various pH values were used as base solutions for the electrochemical determination of sinomenine.

2.3. Fabrication of the cysteic acid modified glassy carbon electrodes

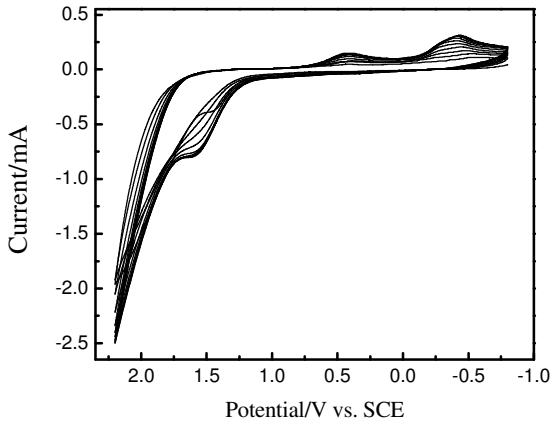


Figure 1. Cyclic voltammograms of the glassy carbon electrode modified by cysteic acid in 0.04 M HCl solution containing 2.50×10^{-3} M *L*-cysteine; scan potential, - 0.80 to 2.20 V; scan rate, 100 $mV \cdot s^{-1}$.

Before modification, the glassy carbon electrode was polished with 1, 0.3 and 0.05 μm α-alumina powder, consecutively rinsed thoroughly with doubly distilled water after each polishing step, and then sonicated in 1:1 nitric acid, acetone, and doubly distilled water in that order. The modified electrode was prepared by cycling the electrode potential between - 0.80 and + 2.20 V at the scan rate of 100 $mV \cdot s^{-1}$ in 0.04 M HCl solution containing 2.50×10^{-3} M *L*-cysteine. Figure 1 shows the cyclic voltammograms of *L*-cysteine at a bare glassy carbon electrode. After 10 consecutive cycles, the surface of the glassy carbon electrode was covered with a certain blue substance. The modified electrode was rinsed with ethanol and distilled water to remove the physically adsorbed and unreacted species from the electrode surface. We then electroactivated the modified electrode by cyclic scanning from - 0.20 V to + 0.60 V in a B-R buffer solution (0.04 M, pH 1.81), and a steady cyclic voltammogram was obtained. Finally, the electrode was dried with a stream of high purity nitrogen.

2.4. Determination of sinomenine

The cysteic acid modified-glassy carbon electrode, the platinum wire counter electrode, and the saturated calomel reference electrode (SCE) were immersed in 20.00 mL B-R buffer solution (0.04 M, pH 1.81). A certain amount of sinomenine was added in the solution, with stirring by a magnetic stirrer. The stirring was stopped after the electrochemical accumulation for 10 s was at - 0.70 V. Then the differential pulse voltammetry (DPV) was immediately performed to scan from + 0.40 to +1.10 V after quiet time of 20 s. To establish the optimum conditions for the determination of sinomenine by means of the DPV technique, various instrumental parameter variables were studied, and the optimum conditions were as follows: scan rate, 4 $mV \cdot s^{-1}$; sampling width, 0.05 s; pulse amplitude, 50 mV; and pulse period, 0.2 s. The anodic peak current of sinomenine at + 0.65 V was recorded (Figure 2(A)). The standard addition method was applied to quantitative determination of sinomenine. After the determination, a renewal of the electrode was easily accomplished by soaking the modified electrode in the B-R buffer solution to cyclically scan between - 0.2 V and 1.2 V for about 6 cycles.

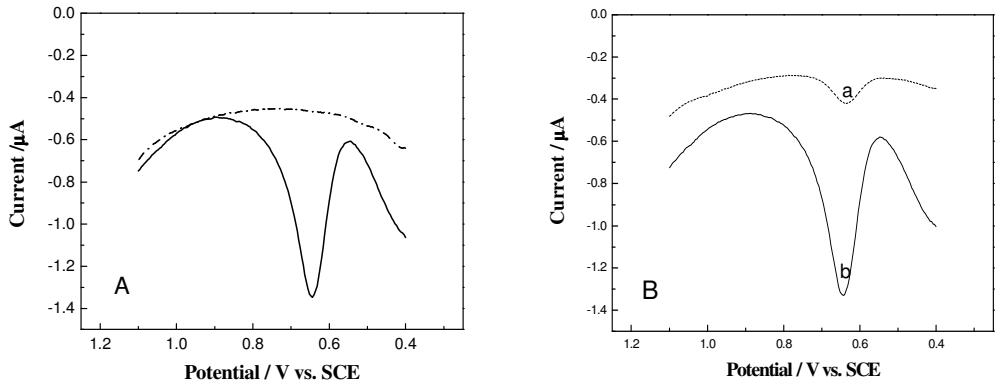


Figure 2. (A) Differential pulse voltammograms of 6.0×10^{-5} M sinomenine and blank (dash line) at the modified electrode in the B-R buffer solution (0.04 M, pH 1.81). (B) Differential pulse voltammograms of 6.0×10^{-5} M sinomenine at different electrodes in the B-R buffer solution (0.04 M, pH 1.81). (a) (Dot line): the bare GCE; (b) (Solid line): the cysteic acid/GCE. Accumulation potential

under stirring, - 0.70 V; accumulation time, 10 s; scan rate, 4 $mV \cdot s^{-1}$; sampling width, 0.05 s; pulse amplitude, 50 mV; pulse period, 0.2 s; sensitivity, 1.0×10^{-5} $A \cdot V^{-1}$.

3. RESULTS AND DISCUSSION

3.1 The role of materials modified on the glassy carbon electrode

Many electrochemical researchers have been devoted to the determination of CySH [22, 23]. And it is widely accepted that the reaction occurs by the following oxidation reaction mechanism on electrodes [23-26]:

$$CySH \leftrightarrow CyS^- + H^+ \quad (1)$$

$$CyS^- \rightarrow CyS^{\cdot}_{ads} + e^- \quad (2)$$

$$2CyS^{\cdot}_{ads} \rightarrow CySSCy \quad (3)$$

Herein, electrochemical oxidation of CySH on a glassy carbon electrode was also investigated by the cyclic voltammetry in the wide potential range of - 0.8 ~ 2.2 V at a scan rate of 100 $mV \cdot s^{-1}$ in 0.04 M HCl solution containing 2.5×10^{-3} M CySH. Figure 1 shows the cyclic voltammograms of *L*-cysteine at a glassy carbon electrode. There was an irreversible oxidation peak at ca. + 1.3 V at the first segment, which implied that CySH was oxidized to *L*-cystine (CySSCy). Another irreversible oxidation peak was observed at ca. + 1.6 V with further voltammetric cycling. In many cases, cysteic acid ($CySO_3H$) was found as an oxidation product of CySSCy [24-27]. Fei et al. determined that the oxidation product of CySH can be further oxidized to chemisorbing molecules (cysteic acid) under a high positive potential [26]. Ralph et al. demonstrated that CySH was adsorbed on the electrode by using AC voltammetry on GCE, and its further oxidation to cysteic acid was proposed [25]:

$$RSSR + 3\ H_2O \rightarrow RSO_3H + 5\ H^+ + 5\ e^- \quad (4)$$

By using cyclic voltammetric and polarization measurements, Spataru et al. confirmed that the functional group SO_3H of cysteic acid was strongly adsorbed at GCE [23]. So cysteic acid based on the electrochemical oxidation of CySH is similar to Nafion with a sulfonated group, and it can be used as a novel electrode modifier due to its attractive ion-exchange characteristics. However, Nafion is more expensive than CySH. Therefore cysteic acid film has more significant advantages and electroanalytical application than Nafion film.

Figure 2(B) shows the peak current response of sinomenine at the cysteic acid/GCE is enhanced compared with the bare GCE under identical experimental conditions. It was attributed to the electrostatic attraction of modifier layer for the protonized amine groups of sinomenine (pK_a 7.97 [28]) molecules carrying positive charges in the acid solution. The negatively-charged functional groups of the cysteic acid, the sulfonated group, can attract sinomenine onto the modifier film and improve favorable accumulation of sinomenine to result in higher sensitive current response.

3.2. Optimization of experimental conditions

Some conventional supporting electrolytes were tested, such as HCl, H_2SO_4, NaOH, phosphate buffer, and B-R buffer solution. Higher sensitive and well-defined peaks of sinomenine in voltammograms were obtained in the B-R buffer solution (0.04 M, pH 1.81). The effects of accumulation potential and accumulation time on the differential pulse voltammetric current response for sinomenine were studied. The accumulation step proceeded in constantly stirred solution and the voltage-scanning step was performed after 20 s of quiet time. The peak current of sinomenine was the highest at - 0.70 V as the accumulation potential (Figure 3(A)). It is attributed to the existence of protonized sinomenine in the cationic form at pH 1.81, and more favorable accumulation at negative potential. So an accumulation potential of - 0.70 V was chosen in all the subsequent work.

The effect of accumulation time on the peak currents was also investigated. Figure 3(B) shows the peak currents of sinomenine increased with increasing the accumulation time within 10 s, which is attributed to the adsorption of sinomenine at the modified electrode surface. Further postponement of the accumulation time did not increase the response after 10 s owing to the surface adsorption saturation. For practical purposes, a 10 s accumulation period was found to be sufficient for the determination.

The pH value of the base solution has a significant influence on the oxidation current response of sinomenine at the cysteic acid/GCE. Figure 3(C) shows the effect of the pH value of the base solution in the range of 1.81 ~ 5.00 on the peak current. The anodic peak current increased monotonically with pH value decreasing. But the interference of water electrolysis became serious when the pH value was lower than 1.81. So the B-R buffer solution (0.04 M, pH 1.81) was selected for the determination.

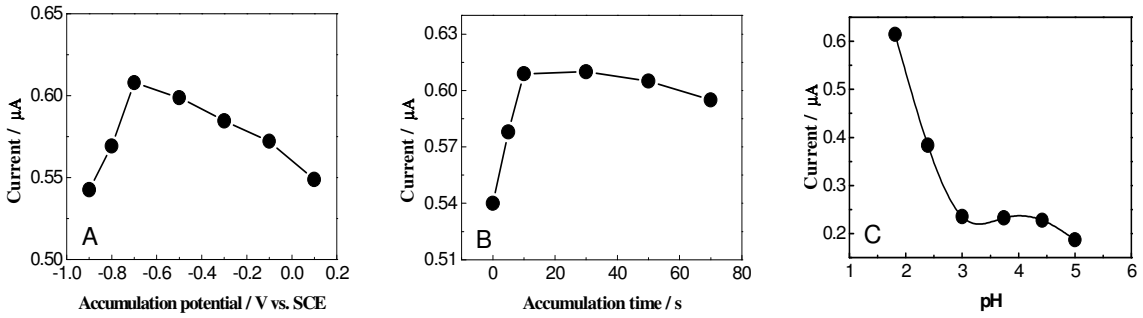
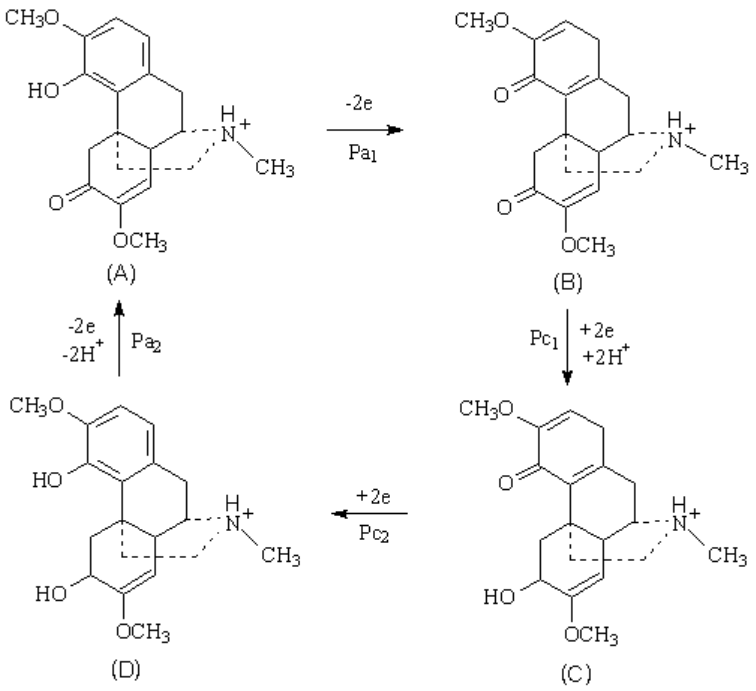


Figure 3. (A) Effect of accumulation potential on the anodic peak current of the DPV. (B) Effect of the accumulation time on the anodic peak current of the DPV. (C) Effect of pH on the anodic peak current of the DPV. The concentration of sinomenine, 5.0×10^{-5} M; scan rate, 4 $mV \cdot s^{-1}$; Quiet time, 20 s; Electrolyte, B-R buffer solution (0.04 M).

3.3. Electrochemical reaction mechanism of sinomenine at the cysteic acid/GCE

In order to study some aspects of electrochemical behaviors of sinomenine, the cyclic voltammetry was carried out in the B–R buffer solution (Figure 4(A)). The possible mechanism was

illustrated by the cycle voltammograms of sinomenine shown in Scheme 2. When the potential scanned from − 0.2 to +1.2 V, a sinomenine molecule (A) at the electrode was firstly oxidised to formation of a quinone derivative B because a hydroxy linked with a phenyl structure was easily oxidised (the peak at 0.680 V in Figure 4(A)). Following the initial oxidation, when a reverse scan was curried out, a corresponding reduction peak was observed. It was attributed to the fact that another carbonyl group of the oxidation produce B was reduced to a hydroxyl group, and this yielded the compound C. When the scan potential declined sequentially, another reduction reaction undertook, and carbonyl group inked with a phenyl structure was reduced back to previous hydroxyl to form compound D. So the subsequent reoxidation of compound D were obviously observed under subsequent scans (the reduction peak at 0.30 V and the oxidation peak at + 0.48 V in Figure 4(A)). The scan rate effect on the peak currents of 1.0×10^{-4} M sinomenine at the cysteic acid/GCE was investigated (Figure 4(B). As the scan rate increased, all the peak currents of sinomenine increased into the positive direction. Four good linearity between the peak currents (i_p) of sinomenine and the square root of scan rate ($v^{1/2}$) was obtained over the range of 10 ~ 90 mV·s^{-1}, which demonstrated that the electrode reaction of sinomenine was a diffusion-controlled process.



Scheme 2. The electrochemical reaction mechanism of sinomenine at the cysteic acid/GCE.

3.4. Calibration curve and detection limit

Under preceding optimum detection conditions, anodic peak currents were proportional to the sinomenine concentrations in the range of 1.0×10^{-6} ~ 1.0×10^{-4}. The linear equation is i_{pa} (μA) =

0.0010 + 0.01219*C* (μM) with a correlation coefficient, r = 0.999 (Figure 5). The detection limit of sinomenine was 6.0×10^{-7} M in terms of the role of a signal to noise ratio of 3:1 (S/N = 3).

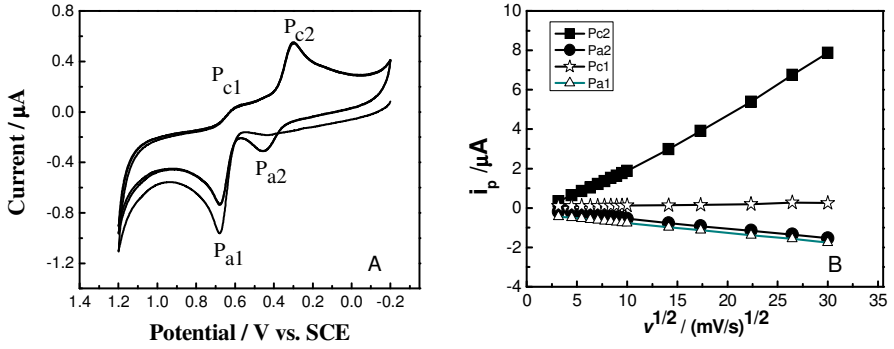


Figure 4. (A) Cyclic voltammograms of 1.0×10^{-4} M sinomenine at the cysteic acid/GCE in the B-R buffer solution (0.04 M, pH 1.81). Scan rate: 10 mV·s^{-1}. (B) Plots of the peak currents of 1.0×10^{-4} M sinomenine at the cysteic acid/GCE at different scan rates in the B-R buffer solution (0.04 M, pH 1.81).

When sinomenine concentration was more than 1.0×10^{-4} M, the current response decreased gradually and its plateau was observed, this is attributed to the saturation of sinomenine on the modified electrode.

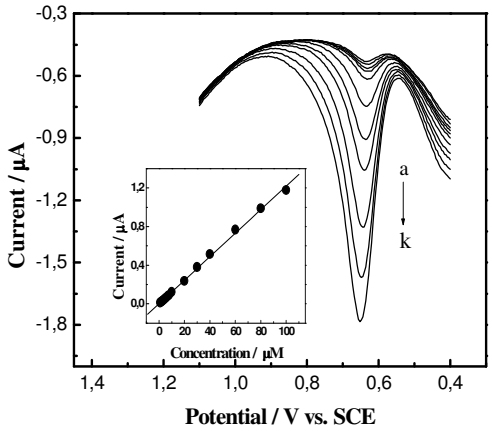


Figure 5. Differential pulse voltammograms of sinomenine at the cysteic acid/GCE in the B-R buffer solution (0.04 M, pH 1.81). The concentrations of sinomenine: (a) 5.0×10^{-6} M ; (b) 6.0×10^{-6} M ; (c) 7.0×10^{-6} M ; (d) 8.0×10^{-6} M ; (e) 1.0×10^{-5} M ; (f) 2.0×10^{-5} M; (g) 3.0×10^{-5} M ; (h) 4.0×10^{-5} M; (i) 6.0×10^{-5} M; (j) 8.0×10^{-5} M ; (k) 1.0×10^{-4} M. Inset: Plot of the anodic peak current of sinomenine versus the concentration of sinomenine. The experimental parameters are similar to Figure 2.

3.5. Repeatability and Recovery

We examined the lifetime of cysteic acid/GCE, and demonstrated that the cysteic acid film retained 98.7 % of its initial response after 1 month of storage in B-R buffer solution (0.04 M, pH 1.81). Such stability seems to be acceptable for most practical applications. Because the modified

electrode can adsorb the cation sinomenine, it was necessary to renew the electrode surface. We found that the renewal of the electrode surface is easily accomplished by soaking the modified electrode in phosphate buffer solution and cycling its potential between - 0.2 V and 1.2 V about 6 cycles. Sinomenine of 1.0×10^{-5} M was determined repeatedly with the same cysteic acid modified glassy carbon electrode for 9 times. The average current was 0.6 μA with a relative standard deviation (RSD) of 2.6 % (n = 9), which indicates that the modified electrode possessed a good reproducibility. After detecting sinomenine in every five human serum samples, the modified electrode was washed in the B-R buffer solution under stirring, and then transferred into a standard solution of 5.0×10^{-5} M sinomenine to record its oxidation peak current. Its average current was 0.61 μA with the relative standard deviation (RSD) of 2.1 % (n = 5). Such stability seemed to be acceptable for most practical applications.

The recovery tests of sinomenine in the range from 1.0×10^{-5} to 4.0×10^{-5} M were performed. The results are listed in Table 1. The recoveries varied in the range from 94.6 % to 103.8 %.

Table 1. Recovery of sinomenine.

Added / μM	Found / μM	Recovery (%)
10.0	9.46	94.6
20.0	19.80	99.0
30.0	30.48	101.6
40.0	41.52	103.8

Table 2. Determination results of sinomenine in solid tablets at the cysteic acid/GCE.

Batch No.	HPLC Method (n =5) (mg/tablet)	Present method (n=5) (mg/tablet)	Present method R.S.D* (%)
Lijun® No. 409360	19.32	19.54	3.2
Zhengqing Fengtongning No. 0511108	19.41	19.73	3.9

* R.S.D is relative standard deviation.

3.6. Interference

For the following solid pharmaceutical formulations analysis, tablet excipient, such as starch, was added in the system of the determination. It was not found that the results were changed in the presence of the excipient. When the concentration of sinomenine was 2.0×10^{-5} M, no interferences were observed in the presence of 4.0×10^{-3} M of KCl, NaCl, NH_4Cl, lactose, glucose, *D*-fructose, citric acid, Vitamin B_1, Vitamin B_2, Vitamin B_6, respectively.

3.7. Determination of the pharmaceutical preparation samples

The developed DPV method for the sinomenine determination was applied to a commercial preparation (sinomenine tablets: Xian Lijun Pharmaceutical Co. LTD., China, Lijun®, batch No. 0409360, labeled amount of 20 mg per tablet; Hunan Zhengqing Pharmaceutical Co. LTD., China, , batch No. 0511108, labeled amount of 20 mg per tablet). Five random tablets were ground to fine powder, and then were transferred into a 100.0 mL calibrated flask. It was dissolved for 5 min with water using supersonic vibrator. 100 μL sample solution was added in 20.00 mL B−R buffer solution (0.04 M, pH 1.81), with stirring by a magnetic stirrer. Then the differential pulse voltammetry was immediately performed to scan under preceding optimum conditions for the determination of sinomenine. Table 2 gives the results of DPV analysis of the commercial preparation. The HPLC was employed to compare the validity of the developed method [11]. There was no significant difference between two methods, and a good agreement was achieved. The results show that the proposed methods could be recommended for the determination of sinomenine in solid tablets. The developed method could easily be used in quality control laboratory for the analysis of sinomenine in solid pharmaceutical formulations.

4. CONCLUSIONS

A glassy carbon electrode modified by cysteic acid based on electrochemical oxidation of *L*-cysteine was fabricated. Sinomenine could be sensitively determined by voltammetry because cysteic acid with cation exchange property drastically increased the current response of sinomenine. In 0.04 M B-R buffer solution (pH 1.81). The peak current obtained from the DPV was linearly dependent on sinomenine concentration in the range of 1.0×10^{-6} ~ 1.0×10^{-4} M in 0.04 M B-R buffer solution (pH 1.81), with a correlation coefficient of 0.999 and a detection limit of 6.0×10^{-7} M. The modified electrode was also showed to be applicable in the detection of sinomenine in solid pharmaceutical formulations with excellent sensitivity. This thin film is considered to be a promising, low-cost, and steady material in the modification of electrodes. It is hope that the cysteic acid-modified electrode will be a good application for further sensor development.

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References

1. S. W. Chen, X. J. Mi, R. Wang, W. J. Wang, W. X. Kong, Y. J. Zhang and Y. L. Li, *Life Sci.*, 78 (2005) 232.
2. X. J. Li, Patrick Y. K. Yue, et al *Life Sci.*, 79 (2006) 665.
3. Y. W. Chen, J. Y. Li, J. B. Zhang, T. T. Zhao, et al., *Int. Immunopharmacol.*, 5 (2005) 1446.
4. J. Sun, J. M. Shi, T. H. Zhang, K. Gao, J. J. Mao, B. Li, Y. H. Sun, Zh. G. He, *World J. Gastroenterol.*, 11 (2005) 4547.

5. L. Liu, et al., *Arzneimittel-forsc.*, 44 (1994) 1223.
6. L. Liu, et al., *Int. Immunopharmacol.*, 16 (1994) 685.
7. D. Candinas, et al., *Transplantation*, 62 (1996) 1855.
8. L. Liu, et al., *Int. Immunopharmacol.*, 18 (1996) 529.
9. B. Vieregge, et al., *Planta. Med.*, 65 (1999) 80.
10. Y. Q. Li, S. Y. Ciu, Y. Q. Cheng, X. G. Chen, Z. D. Hu, *Anal. Chim. Acta*, 508 (2004) 17.
11. Pharmacopoeia Committee of the Ministry of Public Health, Chinese Pharmacopoeia (Vol.1): in sinomenine, Chinese Chemical Industry Press, Beijing, 2005, pp 135.
12. M. Moriyasu, M. Ichimaru, Y. Nishiyama, Kato, *Bunseki. Kagaku.*, 42 (1993) 659.
13. X. H. Yan, H. D. Li, W. X. Peng, F. Q. Lin, Y. Shao, Y. Q. He, *Yao Xue Xue Bao*, 32 (1997) 620.
14. Y. M. Yao, Z. R. Tan, Z. Y. Hu, X. Guo, Z. N. Cheng, L. S. Wang, H. H. Zhou, *Clin. Chim. Acta*, 356 (2005) 212.
15. J. G. Li, J. R. Lu, *Fenxi Shiyanshi*, 16 (1997) 41.
16. Z. G. Pang, B. Q. Wang, C. Fan *Fenxi Huaxue*, 23 (1995) 539.
17. N. Lin,. *Sepu*, 7 (1989) 54.
18. L. He, *Yaoxue Tongbao*, 17 (1982) 308.
19. H. Y. Deng, *Guangdong Yao Xue*, 1 (1995) 17.
20. W. Guo, J. F. Song, Z. Q. Zhang, W. B. Bi, L. J. Sun, *Fenxi Huaxue*, 23 (1997) 1232.
21. H. Zou, Z. B. Yuan, *Fenxi Huaxue*, , 25 (1997) 551.
22. H. Li, T. Li, E. Wang, *Talanta*, 42 (1995) 885.
23. N. Spataru, B. V. Sarada, E. Popa, D. A. Tryk, A. Fujishima, *Anal. Chem.*, 73 (2001) 514.
24. J. Zagal, C. Fierro, R. Rozas, *J. Electroanal. Chem.,* 119 (1981) 403.
25. T. R. Ralph, M. L. Hitchman, J. P. Millington, F. C. Walsh, *J. Electroanal. Chem.*, 375 (1994) 1.
26. S. D. Fei, J. H. Cheng, S. Z. Yao, G. H. Deng, D. L. He, Y. F. Kuang, *Anal. Biochem.*, 339(2005)29.
27. D. G. Davis, E. Bianco, *J. Electroanal. Chem.*, 12 (1966) 254.
28. Y. Ch. Liu, B. Zhao, G. Q. Wang, *J. Shenyang Pharma. University*, 16 (1999) 217.