Electrochemical Determination of Artemisinin Using a Multi-wall Carbon Nanotube Film-modified Electrode

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Artemisinin, the effective ingredient of Chinese herb *Artemisia annua L* (Qinghao in Chinese), has been proved to be effective to antimalarial. Herein, a reliable, sensitive and convenient electrochemical method was developed for the determination of artemisinin utilizing the excellent properties of multi-wall carbon nanotube (MWNT). The electrochemical behavior of artemisinin was investigated. It is found that the reduction peak current of artemisinin remarkably increases and the peak potential shifts positively by 240 mV at the MWNT film-modified electrode. These phenomena indicate that the MWNT film exhibits efficient catalytic activity to the electrochemical reduction of artemisinin. The effects of pH value, amount of MWNT, scan rate and accumulation time were examined. The limit of detection (S/N = 3) is as low as 10 μg L^{-1}. Finally, this newly developed method was used to determine the content of artemisinin in *Artemisia annua L*.

Key Words : Artemisinin (Qinghaosu), Analysis, Carbon nanotube, Electrochemistry, Film modified electrode

Introduction

Artemisinin (Fig. 1, also called Qinghaosu in Chinese), a natural product isolated from the Chinese herb *Artemisia annua L* (Qinghao in Chinese), has served as a useful prototype for the development of less neurotoxic, third generation antimalarial drugs.[1] It is proven that artemisinin works quickly, appears safe and well-tolerated, and might decrease malaria transmission by inactivating or killing gametocytes.[2] In China, *Artemisia annua L* is very popular and widely used. Therefore, it is very important and interesting to develop a simple and reliable method for the determination of artemisinin.

Until now, various methods have been reported for the determination of artemisinin such as high-performance liquid chromatography (HPLC),[3-5] reversed-phase HPLC,[6,7] thin-layer chromatography,[8,9] supercritical fluid chromatography,[10] fluorescence,[11] HPLC-mass spectrometry (HLPC-MS)[12] and flow-injection capillary electrophoresis.[13] Otherwise, the electrochemical behavior of artemisinin was examined using electrochemical and spectroelectrochemical techniques,[14-17] suggesting that artemisinin is electrochemical-active and can be reduced at negative potential due to the peroxide bond. So, a differential pulse polarographic method using dropping mercury electrode was reported for the determination of artemisinin.[18] The linear range is from 6.4 $\times 10^{-7}$ to 3.2 $\times 10^{-5}$ mol L^{-1}, and the detection limit is 58 μg L^{-1}. Additionally, a metalloporphyrin-nano Au-chitosan modified electrode was also reported for the determination of artemisinin with detection limit of 1.7 $\times 10^{-9}$ mol L^{-1}.[19] However, to the best of our knowledge, electrochemical determination of artemisinin using a carbon nanotube film-modified electrode has not been reported.

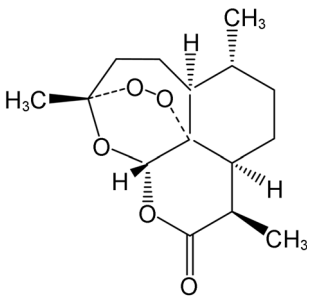

Figure 1. Molecular structure of artemisinin.

Since discovery by Iijima, carbon nanotube (CNT), including single-wall carbon nanotube (SWNT) and multi-wall carbon nanotube (MWNT), has obtained increasing attention due to its unique structure and extraordinary properties.[20,21] CNT possesses subtle electronic properties, huge surface area, efficient catalytic activity and strong adsorption ability. These excellent properties suggest that CNT is a fascinating electrode material, and now, it was widely used in electrochemistry and electroanalytical chemistry.[22-24] In this work, MWNT was successfully dispersed into water under the assistance of dihexadecyl hydrogen phosphate (DHP), giving a stable and homogeneous MWNT/DHP suspension. After evaporating water, a MWNT/DHP composite film-modified electrode was prepared. The electrochemical behavior of artemisinin was examined. Compared with the unmodified electrode, the MWNT film-modified electrode shows great difference to artemisinin. On one hand, the reduction peak current of artemisinin significantly increases, revealing that the MWNT film-modified electrode can greatly improve the determining sensitivity. On the other

hand, the reduction peak potential of artemisinin shifts positively by 240 mV, suggesting that MWNT exhibits catalytic ability to artemisinin. Based on this, an electrochemical method with high sensitivity and rapid response was developed for the determination of artemisinin.

Experimental

Reagents. All reagents were of analytical grade and used directly. Artemisinin stock solution was prepared by dissolving artemisinin (Sigma, USA) into ethanol, and stored at 4 °C. Multi-wall carbon nanotube (MWNT, purity > 95%) was purchased from Shenzhen Nanotech Port Co., Ltd, China. Dihexadecyl hydrogen phosphate (DHP) was obtained from Fluka (Buchs, Switzerland).

Instruments. All the electrochemical measurements were performed using 830B Electrochemical Analyzer (CH Instruments, USA). A conventional three-electrode system, consisting of a MWNT film-modified glassy carbon working electrode (3-mm in diameter), a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode, was employed. Scanning electron microscopy (SEM) was performed with a FEI-Quanta 200 microscope.

Fabrication of MWNT film-modified GCE. The MWNT film-modified glassy carbon electrode (GCE) was fabricated as follows.[25] Firstly, a 5.0-mg MWNT and 5.0-mg DHP were dispersed into 5.0 mL re-distilled water by 30-min ultrasonic agitation to give a homogeneous MWNT suspension. Secondly, the GCE surface was mechanically polished with 0.05 μm alumina slurry to a mirror finish, rinsed and then sonicated in redistilled water. Finally, the GCE surface was coated with 10.0 μL MWNT/DHP suspension and allowed to evaporate water under an infrared lamp. The DHP film-modified GCE was prepared by the same procedure but without MWNT.

Analytical procedure. Unless otherwise stated, the pH 7.0 phosphate buffer (0.1 mol L^{-1}) that deaerated with nitrogen was used as supporting electrolyte for artemisinin analysis. The analytical procedure contains accumulation step and determining step. Firstly, artemisinin was accumulated onto the MWNT film-modified GCE surface by 3-min stirring. After that, the linear sweep voltammograms from −0.30 to −1.10 V were recorded, and the reduction peak current at −0.75 V was measured for artemisinin.

Sample preparation. An amount of 1.0 g dried leaves of *Artemisia annua L*, collected from Enshi of Hubei Province, was ground into powder and then sieved through a No. 14 mesh stainless steel sieve. The resulting powder was then extracted with 50.0 mL of ethanol for three times. After being extracted, the extract was filtered into a calibrated flask and then diluted to 250.0 mL for determination.

Results and Discussion

SEM image of MWNT-DHP film. The SEM image of MWNT-DHP composite film on the GCE surface is shown in Figure 2. It is very clear that the GCE surface is coated with a uniform and thin MWNT film.

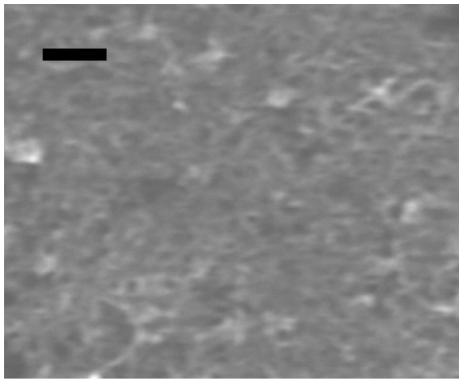

Figure 2. SEM image of MWNT film on GCE surface, scale bar = 50 nm.

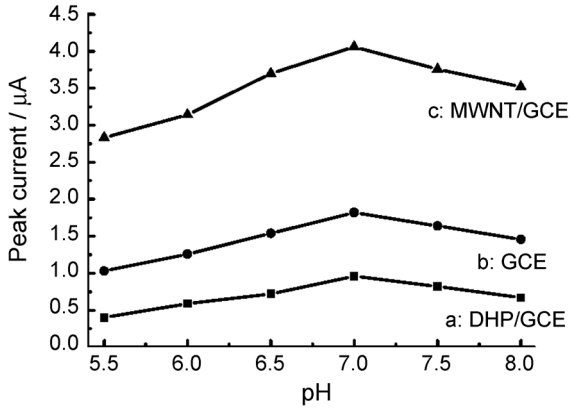

Figure 3. Dependence of reduction peak current of 30.0 mg L^{-1} artemisinin as a function of pH at DHP-modified GCE (a), GCE (b) and MWNT/DHP modified GCE (c).

Electrochemical behavior of artemisinin. The electrochemical response of artemisinin in pH 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 phosphate buffer was individually examined using cyclic voltammetry. At the unmodified, DHP film-modified and MWNT-DHP film-modified GCEs, only a reduction peak is observed for artemisinin. Figure 3 shows the reduction peak current of 30.0 mg L^{-1} artemisinin as a function of pH value. As pH value gradually increasing from 5.5 to 7.0, the reduction peak current of artemisinin also gradually increases. When the pH value further increases from 7.0 to 8.0, the reduction peak current contrarily shows gradual decline. So, the optimal supporting electrolyte is selected as pH 7.0 phosphate buffer because the peak current is highest.

In pH 7.0 phosphate buffer, the electrochemical reduction of 30.0 mg L^{-1} artemisinin was investigated using liner sweep voltammetry (LSV). At the unmodified GCE (Fig. 4a), a reduction peak is observed at −0.99 V for artemisinin, and the peak height is very low. However, the reduction peak current obviously increases at the unmodified GCE after 3-min accumulation (Fig. 4b), suggesting that accumulation can improve the determining sensitivity of artemisinin. Under the identical conditions, it is found that the reduction peak current of artemisinin notably decreases at the DHP film-modified GCE (Fig. 4c). The DHP film on GCE surface

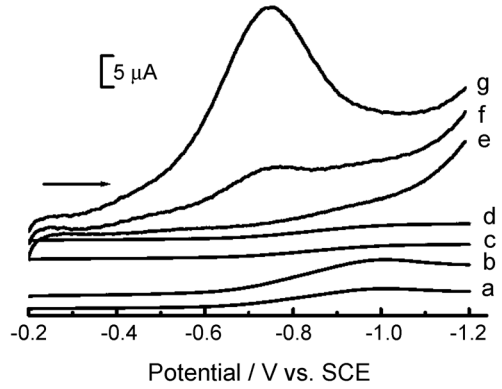

Figure 4. LS voltammograms of 30.0 mg L^{-1} artemisinin in pH 7.0 phosphate buffer at GCE (a,b); DHP-modified GCE (c,d) and MWNT/DHP modified GCE (f,g). (b,d,g): after 3-min accumulation; (e): blank voltammograms of MWNT/DHP film. Scan rate: 100 mV s^{-1}.

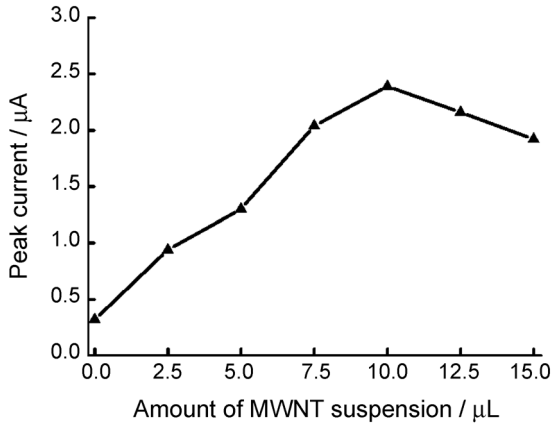

Figure 5. Effect of amount of MWNT/DHP suspension on the reduction peak current of 3.0 mg L^{-1} artemisinin. Accumulation time: 3 min.

is perfect and has poor electric conductivity. So, the mass transport and electron transfer of artemisinin at the DHP film-modified GCE becomes difficult, resulting in the great peak current decline. After 3-min accumulation, the reduction peak current of artemisinin almost keeps unchanged at the DHP film-modified GCE (Fig. 4d).

Figure 4e depicts the linear sweep voltammograms of MWNT-DHP film-modified GCE in pH 7.0 phosphate buffer without artemisinin. No redox peak is observed. After addition of 30.0 mg L^{-1} artemisinin, a sensitive reduction peak is observed at −0.75 V (Fig. 4f). Compared with curve (a), it is very clear that the reduction peak current of artemisinin significantly increases at the MWNT/DHP film-modified GCE. Moreover, the reduction peak potential shifts positively from −0.99 V to −0.75 V. The positive shift of 240 mV and remarkable peak current enhancement strongly reveal that the MWNT film exhibits highly-efficient catalytic activity to artemisinin. As expected, the reduction peak current of artemisinin greatly increases after 3-min accumulation at the MWNT-DHP film-modified GCE, which shown in Figure 4g. MWNT possesses large surface area, more active sites and strong adsorption ability, so, the MWNT film-modified GCE is more active and exhibits higher accumulation efficiency to artemisinin. Without a doubt, the electrochemical reduction signal of artemisinin greatly enhances at the MWNT film-modified GCE.

Effect of scan rate. In pH 7.0 phosphate buffer, the reduction response of artemisinin under different scan rates was investigated at the MWNT film-modified GCE. As scan rate increasing from 25 to 300 mV s^{-1}, the reduction peak current linearly increases, revealing that the reduction of artemisinin is controlled by adsorption.

The effect of scan rate (v) on the reduction peak potential (E_{pc}) was also studied. As increasing v, the E_{pc} shifts positively. According to Laviron's theory,[26] the number of electrons involved in the reduction of artemisinin can be easily achieved from the slope of E_{pc} versus log(v). In this work, the an_a is calculated to be 1.03 from the slope. Generally, α is assumed to be 0.5 for a totally irreversible electrode process. Hence two electrons are involved in the electrochemical reduction of artemisinin, which is in good agreement with published work.[14-17]

Influence of the amount of MWNT-DHP suspension. Dihexadecyl hydrogen phosphate (DHP) is a hydrophobic surfactant with poor conductivity, and can form a perfect thin film on GCE surface. So, too much DHP is unhelpful to the electron transfer and mass transportation of artemisinin. Figure 5 demonstrates the relationship between the reduction peak current of artemisinin and the amount of MWNT-DHP suspension. When the volume of MWNT-DHP suspension increases from 0.0 to 10.0 μL, the reduction peak current sharply increases. As improving the amount of MWNT-DHP suspension, the amount of MWNT at GCE surface also increases, showing higher accumulation efficiency to artemisinin. Therefore, the peak current remarkably increases. As further improving the amount from 10.0 to 15.0 μL, the peak current conversely decreases and the charging current begins to obviously increase, keeping us from determining trace level of artemisinin. In addition, the time for evaporating water also increases. For above-mentioned reasons, 10.0 μL of MWNT-DHP suspension was used to modify GCE surface.

Accumulation time. The influence of accumulation time on the reduction peak current of 3.0 mg L^{-1} artemisinin was studied, which shown in Figure 6. When the accumulation time improves from 0.0 to 3.0 min, the reduction peak current of artemisinin linearly increases. However, the reduction peak current is almost independent of accumulation time when the time exceeds 3.0 min, suggesting that the amount of artemisinin at the MWNT film tends to a limiting value. So, 3-min accumulation was employed to achieve higher sensitivity and better working efficiency.

Precision. The intra-assay precision was evaluated by assaying one modified electrode for five replicate determinations. After each measurement, the MWNT film-modified GCE was thoroughly rinsed with water, transferred into the blank electrolyte and scanned for 5 cycles. The relative standard deviation (RSD) is 5.6% for 3.0 mg L^{-1} artemisinin, suggesting that this newly developed method possesses good

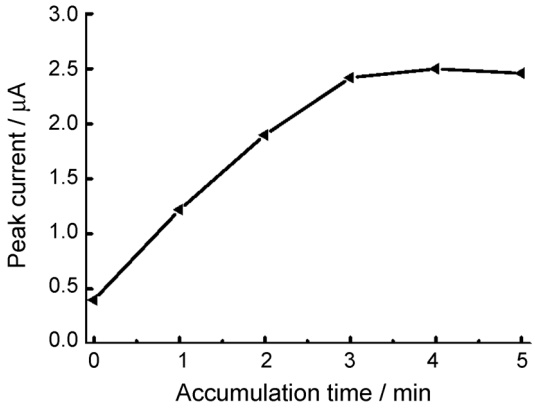

Figure 6. Influence of accumulation time on the reduction peak current of 3.0 mg L^{-1} artemisinin.

precision.

The inter-assay precision was estimated by determining the response of 3.0 mg L^{-1} honokiol at 10 different MWNT film-modified GCEs. The RSD is calculated to be 6.3%, indicative of acceptable fabrication reproducibility.

Linear range and limit of detection. The variation of reduction peak current with concentration was studied using linear sweep voltammetry after 3-min accumulation. When the concentration (C) gradually increases from 0.4 mg L^{-1} to 40 mg L^{-1}, the reduction peak current (i_{pc}) linearly increases, shown in Figure 7. The linear regression equation is: i_{pc} = 0.1492 + 0.7615 C (i_{pc} in μA, C in mg L^{-1}, r = 0.9986). Additionally, the limit of detection after 3-min accumulation was evaluated to be 0.1 mg L^{-1} (3.5×10^{-7} mol L^{-1}) based on 3 signal/noise ratio. If exceeding the accumulation time, the sensitivity will be further improved. For 6-min accumulation and 10-min accumulation, the limit of detection is 40 μg L^{-1} and 10 μg L^{-1}, respectively.

Interference. The possible interferences of other species on the determination of artemisinin were examined. It is found that 1000-fold concentrations of Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, 200-fold concentrations of ascorbic acid, vitamin E, vitamin B6, lysine, vitamin A, uric acid, 100-fold concentrations of tryptophane, cysteine, indole-3-acetic acid, almost do not interfere with the reduction signal of artemisinin at the MWNT film-modified GCE (error < 5%).

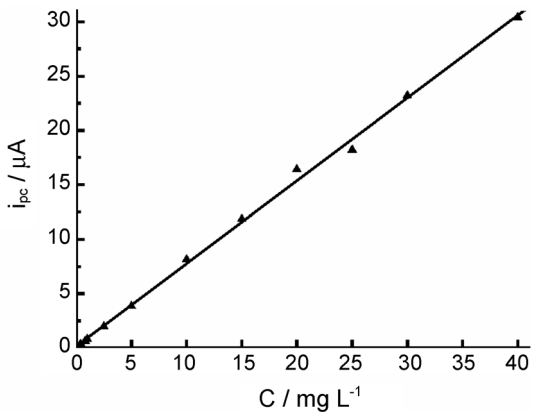

Figure 7. Calibration curve for artemisinin. Accumulation time: 3 min.

Table 1. Determination of artemisinin in *Artemisia annua L*

Sample	By fluorescence /mg g^{-1}	By this method /mg g^{-1}	RSD	Recovery
A	4.26	4.12	4.1%	100.9%
B	3.80	3.92	4.6%	97.4%
C	3.14	3.30	4.7%	96.8%
D	5.64	5.72	3.8%	99.4%
E	4.43	4.26	4.4%	99.2%

Analytical application. The newly proposed method was used to determine artemisinin in *Artemisia annua L*, which collected from Enshi of Hubei Province, China. The content of artemisinin was determined by the standard addition method, and the results are listed in Table 1. Each sample was determined in triplication, and the RSD is below 5.0%. In order to testify the accuracy of this method, the cytochrome C-catalyzed fluorescence determination[11] was also employed to determine the content of artemisinin. It is found that the results obtained by these two methods are in good agreement, indicative of good accuracy of this new method. In addition, the recovery for artemisinin standard solution was tested, and in the range from 96% to 101%, also suggesting that this new method has good accuracy.

Conclusion

MWNT possesses huge surface area, strong adsorptive ability, numerous active sites and subtle electronic properties. Therefore, the MWNT film-modified electrode catalyzes the electrochemical reduction of arternisinin and remarkably enhances the reduction signal of arternisinin. Based on this, a convenient, sensitive, precise and time-efficient method was proposed for the quantitative analysis of arternisinin.

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