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Electrochemistry and voltammetric determination of colchicine using an acetylene black-dihexadecyl hydrogen phosphate composite film modified glassy carbon electrode

Huajie Zhang *

Department of Pharmacy, Wenzhou Medical College, Wenzhou 325000, China

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Abstract

The electrochemical behavior of colchicine at an acetylene black-dihexadecyl hydrogen phosphate (denoted as AB-DHP) composite film coated glassy carbon electrode (GCE) was investigated using cyclic voltammetry (CV), linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV). Compared with the poor electrochemical signal at the unmodified GCE, the electrochemical response of colchicine at the AB-DHP film modified GCE was greatly improved, as confirmed from the significant peak current enhancement. The remarkable peak current enhancement indicates that the AB-DHP modified GCE has great potential in the sensitive determination of colchicine. Thus, all the experimental conditions, which influence the electrochemical response of colchicine, were studied and the optimum conditions were achieved. Finally, a sensitive and simple voltammetric method with a good linear relationship in the range of 1.0×10^{-7}~4.0×10^{-5} mol/L, was developed for the determination of colchicine. The detection limit of colchicine was also examined and a low value of 4.0×10^{-8} mol/L for 4-min accumulation was obtained ($S/N=3$). This electrode was successfully applied to detect colchicine in human urine samples.
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Keywords: Colchicine; Voltammetry; Acetylene black; Modified electrodes; Urine sample

1. Introduction

Colchicine is an alkaloid prepared from the dried corns and seeds of colchicum autumnale, the autumn crocus or meadow saffron. The molecular structure of colchicine is shown in Fig. 1. Colchicine is a very old drug, which is still used to treat or prevent acute gouty arthritis. In gastroenterology, it may be used to slow the formation of fibrous tissue in the liver that occurs with conditions such as cirrhosis and primary biliary cirrhosis. Recently, the side effects of colchicine also have been recognized. For example, it shows genotoxicity in vitro and in vivo systems even at low concentrations. Therefore, the development of a rapid, simple and sensitive method for colchicine determination is of great importance and interest.

So far, various methods have been reported for the determination of colchicine, namely high-performance liquid chromatography (HPLC) [1,2], reversed-phase HPLC [3,4], HPLC coupled to mass spectrometry [5,6], spectrophotometry [7], as well as immunoassay [8]. However, the report regarding the electrochemistry and voltammetric determination of colchicine is very limited [9]. In the present work, a novel chemically modified electrode: acetylene black-dihexadecyl hydrogen phosphate (AB-DHP) composite film coated glassy carbon electrode (GCE), was described for the electrochemistry and voltammetry of colchicine.

Acetylene black, a particularly pure form of graphitic, carbon black pigment, is made by the controlled combustion of acetylene in air under pressure. On account of its excellent electric conductivity, large specific surface area and strong adsorptive ability, acetylene black has been widely used in electrochemistry and electroanalysis.

* Tel.: +86 577 86689882; fax: +86 577 86689881.
E-mail address: zhang_huajie@hotmail.com.

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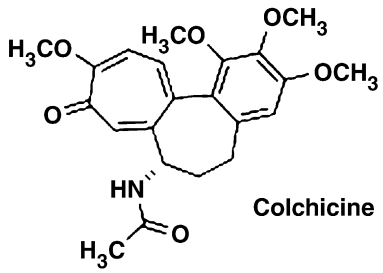

Fig. 1. Chemical structure of colchicine.

In this work, almost insoluble acetylene black (AB) was successfully dispersed into water in the presence of a special surfactant, dihexadecyl hydrogen phosphate (DHP), and the resulting AB-DHP suspension was very homogeneous and stable. After that, the AB-DHP composite film modified GCE was fabricated by coating the AB-DHP suspension on the GCE surface followed by water evaporation. The resulting composite film modified electrode has some dominant advantages including easy fabrication, low cost and low background current. Moreover, the electrochemical response of colchicine at the AB-DHP modified GCE was investigated, and the results reveal that AB-DHP cast film can remarkably enhance the electro-oxidation signal of colchicine as well as its determining sensitivity. Based on this, a simple and sensitive electrochemical method was proposed for colchicine determination.

2. Experimental

2.1. Reagents

1.0×10^{-3} mol/L colchicine stock solution was prepared by dissolving colchicine (Sigma) into re-distilled water, and stored at 4 °C in the dark. Dihexadecyl hydrogen phosphate (DHP) was purchased from Fluka Chemical Reagent Corporation. Acetylene black (AB: particle size=200–300 nm) was purchased from Shanghai Chemical Reagent Co. Ltd., Shanghai, China. Other chemicals were of analytical grade quality. All the chemicals were used as received. Re-distilled water was used for all solutions.

2.2. Apparatus

Electrochemical experiments were performed with a computer controlled model CHI-830A Electrochemical Analyzer (CHI, Shanghai Chenhua Apparatus Corporation, China). A conventional three-electrode system with an AB-DHP-modified GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode was employed. Unless specified otherwise, all potentials reported here are referred to SCE.

2.3. Surface preparation and modification

Prior to modification, the GCE (3 mm in diameter) was polished with 0.3 and 0.05 μm aluminum slurry sequentially and sonicated in re-distilled water for 3 min between each polishing step. The modification of AB-DHP composite film onto the GCE surface was as follows [10]: 5 mg AB and 5 mg DHP were added into 5 mL water and sonicated in water bath for 10 h, resulting in a black and homogeneous suspension. Then 10 μL of 1 mg/mL AB-DHP suspension was cast on the clean GCE surface and dried under an IR lamp. The DHP-modified GCE was prepared by the same procedure as explained above, but without AB.

2.4. Procedure

Unless otherwise stated, 0.1 mol/L $HClO_4$ was used as the supporting electrolyte for colchicine determination. The accumulation step was carried out under open-circuit while stirring the solution for 4 min, then the differential pulse voltammograms from 0.80 to 1.20 V were recorded after 15 s quiet time, and finally the peak current at 1.08 V was measured. Prior to and after every measurement, the AB-DHP film coated GCE was activated by five successive cyclic voltammetric sweeps between 0.60 V to 1.25 V at 50 mV/s in 0.1 mol/L $HClO_4$ supporting electrolyte to give a fresh electrode surface.

3. Results and discussion

3.1. Electrochemical behavior of colchicine

Cyclic voltammetry (CV) is a widely-used and effective tool to investigate the electrochemical property of electrochemical active species. Here, the electrochemical property of colchicine on the AB-DHP film coated GCE was examined by CV, and the results shown in Fig. 2. The

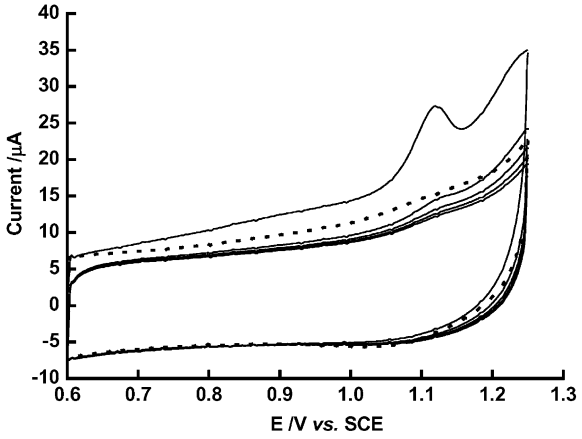

Fig. 2. Cyclic voltammograms of an AB-DHP composite film modified GCE in 0.1 mol/L HClO4 at 100 mV/s (dotted line). Solid line: dotted line+2.0×10^{-5} mol/L colchicine.

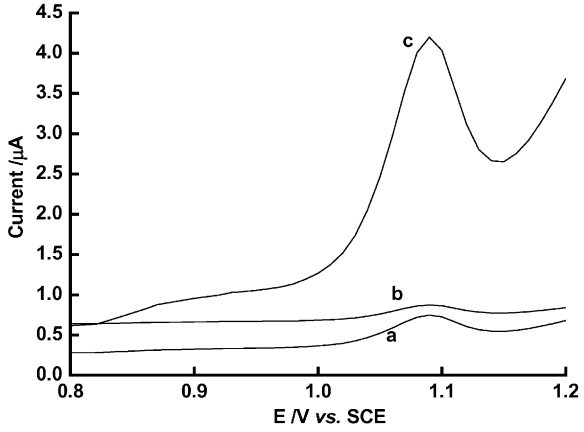

Fig. 3. Differential pulse voltammograms (DPV) of 1×10^{-6} mol/L colchicine in 0.1 mol/L $HClO_4$ at unmodified GCE (a), DHP film modified GCE (b), and AB-DHP composite film modified GCE (c) after 4-min open-circuit accumulation. The best DPV parameters are pulse amplitude=50 mV, scan rate=20 mV/s, pulse width=50 ms.

dotted line depicts the cyclic voltammograms of the AB-DHP film coated GCE in 0.1 mol/L $HClO_4$ free of colchicine at 100 mV/s. During the potential sweep from 0.60 to 1.25 V, no redox peak was observed. However, a well-defined oxidation peak with high peak current appears at 1.12 V upon addition of 2×10^{-5} mol/L colchicine (solid line). Nevertheless, the oxidation peak current of colchicine shows a remarkable decrease during the successive cyclic voltammetric sweeps. After the second sweep, the peak current decreases slightly and finally remains unchanged. The decrease in peak current may be caused by the fact that the adsorption of colchicine or its oxidative product occurs at the electrode surface and therefore, passivates the electrode surface. Thus, the oxidation peak current in the first anodic sweep was recorded for colchicine analysis in the following studies. Otherwise, the experiment results also show that the used electrode surface can be renewed easily by five successive cyclic voltammetric sweeps between 0.60 to 1.25 V at 50 mV/s in 0.1 mol/L $HClO_4$, suggesting that the adsorption at the electrode surface is weak. Fig. 2 also tells that the electrode process of colchicine on the AB-DHP modified GCE is totally irreversible since there is no corresponding reduction peak during the reverse potential scan from 1.25 to 0.60 V.

Otherwise, the electrochemical behaviors of colchicine at three different working electrodes (i.e., bare GCE, DHP-modified GCE and AB-DHP film coated) were compared by differential pulse voltammetry (DPV), and the results shown in Fig. 3. The best DPV parameters in this work are pulse amplitude=50 mV, scan rate=20 mV/s, pulse width=50 ms, accumulation time=4 min. At a bare GCE, a poorly-defined oxidation peak with very low peak current was observed at 1.08 V for 1×10^{-6} mol/L colchicine after 4-min open-circuit accumulation (curve a). Under identical conditions, the oxidation peak of colchicine almost decreases by 50% at a DHP-modified GCE (curve b). Dihexadecyl hydrogen phosphate (DHP) can form a perfect thin film on GCE surface, and thus blocks the mass transportation and electron transfer of colchicine, so the peak current conversely decreases compared with that at a bare GCE. However, the oxidation peak current of colchicine (E_p=1.08 V) is significantly improved at the AB-DHP composite film modified GCE (curve c), compared with those at bare GCE and DHP modified GCE. The remarkable peak current enhancement is undoubtedly attributed to the unique characteristics of AB such as high surface area and strong adsorptive abilities. In conclusion, the AB-DHP film coated GCE greatly improves the sensitivity of d colchicine determining.

3.2. Influence of supporting electrolyte on colchicine oxidation

The electrochemical responses of colchicine at AB-DHP-modified GCE have been examined in several electrolytes, including H_2SO_4, KCl, HAc, H_3PO_4, NaOH, $HClO_4$, Britton–Robison buffer of pH 2.0~12.0, acetate buffer of pH 1.0~5.5 and phosphate buffer of pH 5.0~8.0 (each 0.1 mol/L). The experiment results show that the highest oxidation peak current was obtained in 0.1 mol/L $HClO_4$ for colchicine, otherwise, the background current is very low and the oxidation peak is well-defined. Therefore, 0.1 mol/L $HClO_4$ was used for determining colchicine.

3.3. Effect of the amount of AB-DHP loaded onto the GCE surface

The relationship between the amount of AB-DHP loaded onto the GCE surface and the oxidation peak current of colchicine has been examined. Fig. 4 demonstrates that the oxidation peak current of colchicine gradually increases with improving the amount of the AB-DHP suspension from 0 to 10 μL. If the amount of AB-DHP suspension further increases to 15 μL, the oxidation peak current of colchicine changes slightly. However, the oxidation peak current conversely decreases when the AB-DHP suspension

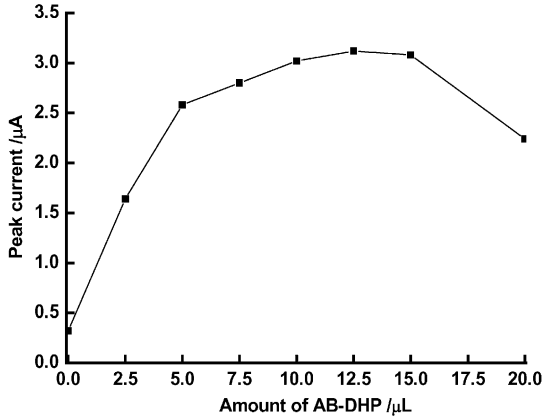

Fig. 4. Effect of the amount of the AB-DHP suspension on the oxidation current of 1.0×10^{-6} mol/L colchicine.

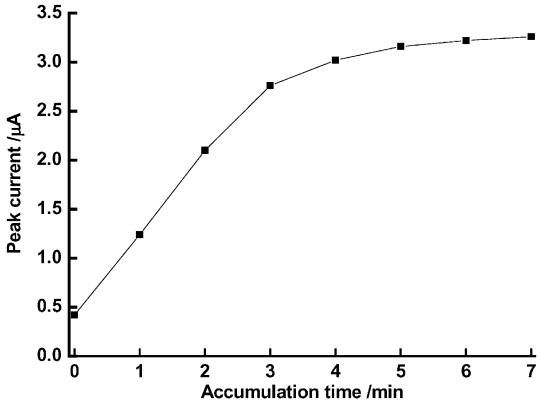

Fig. 5. Influence of accumulation time on the oxidation current of 1.0×10^{-6} mol/L colchicine at AB-DHP composite film modified GCE.

exceeds 15 μL since too much DHP retards the electron transfer and mass transportation of colchicine. Moreover, the charging current increases with enhancing the amount of the AB-DHP on the GCE surface, preventing us from determining colchicine at low concentrations level. For above-mentioned reasons, the amount of AB-DHP suspension on the GCE surface was kept at 10 μL in the present study.

3.4. Influence of scan rate

The oxidation peak currents of 1×10^{-5} mol/L colchicine at different scan rates from 25 to 300 mV/s without accumulation were measured by linear sweep voltammetry (LSV). It is found that the oxidation peak current is proportional to the scan rate, indicating that the oxidation of colchicine at the AB-DHP film-coated GCE is adsorption-controlled.

3.5. Accumulation conditions

Generally speaking, the accumulation step, including two main parameters (i.e., accumulation potential and time), is a simple and effective way to enhance the determining sensitivity. Thus, the influences of accumulation potential and time on the oxidation peak current of colchicine were examined. The oxidation peak current of 1×10^{-6} mol/L colchicine was measured after 4-min accumulation at different potentials and under open circuit by DPV. The peak currents almost keep unchanged, revealing that the accumulation potential has no influence on the oxidation peak current of colchicine at the AB-DHP film modified GCE. Thus, the accumulation step was performed under open circuit.

Fig. 5 shows the dependence of the oxidation peak current on the accumulation time. When the accumulation time is below 4 min, the oxidation peak current almost exhibits a linear relationship with the accumulation time. However, with further increasing accumulation time the plots become curved. The curvature presumably indicating the limiting value of the amount of colchicine on the AB-DHP composite film has been achieved. Considering both the sensitivity and the working efficiency, an accumulation time of 4 min was chosen as the optimal accumulation time.

3.6. Calibration graph

The dependence of the oxidation peak current on colchicine concentration was investigated in 0.1 mol/L $HClO_4$ by DPV. The experiment results showed that the linear concentration range was found to occur from 1×10^{-7} to 4×10^{-5} mol/L, with a regression equation of $i_p=0.14+2.98\times10^{6}\ C$ ($r=0.9986$, C in mol/L, i_p in μA). For 4-min accumulation under open circuit, the detection limit was 4.0×10^{-8} mol/L. The reproducibility of this electrochemical system was also examined by repeating the measurement of 1×10^{-6} mol/L colchicine at the same AB-DHP composite film modified GCE. After each measurement, the electrode was thoroughly rinsed with water, transferred to the blank electrolyte and scanned in the range of 0.60 to 1.25 V for five cycles to remove any adsorbate. The relative standard deviation of 5.3% for 1.0×10^{-6} mol/L colchicine ($n=10$) indicates excellent reproducibility.

The long-term stability of the AB-DHP film coated GCE was estimated by measuring the current response at a fixed colchicine concentration of 1×10^{-6} mol/L over a period of 3 weeks. The AB-DHP film coated GCE was used daily and stored in air. The experimental results show that the current response only deviates 5.7%, suggesting that the AB-DHP film coated GCE reported in this work possesses long-term stability.

3.7. Interferences

Table 1 lists the interferences of some foreign species on the determination of 1×10^{-6} mol/L colchicine. The results show that at least 100-fold concentrations of some metal ions, such as Ca^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, 20-fold concentrations of vitamin B_6, ascorbic acid (AA), uric acid (UA), xanthine (XA), dopamine (DA), vitamin A, and 50-fold concentrations of vitamin E, vitamin B_1, progesterone and caffeine, did not interfere with the oxidation signal of colchicine (signal change below 5%), indicating the

Table 1
Interferences of some foreign species on the determination of 1×10^{-6} mol/L colchicine

Foreign species	Tolerance level (mol/L)*
Ca^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}	1×10^{-4}
Vitamin B_6, ascorbic acid (AA), uric acid (UA), xanthine (XA), dopamine (DA), vitamin A	2×10^{-5}
Vitamin E, vitamin B_1, progesterone and caffeine	5×10^{-5}

*For 5% oxidation signal change.

Table 2
Determination of colchicine in urine samples

Sample	Original (mol/L)	Spiked (mol/L)	Found (mol/L)	Recovery (%)
A	0.00	8.00×10^{-6}	7.92×10^{-6}	99.0
B	0.00	5.00×10^{-6}	5.08×10^{-6}	101.6
C	0.00	2.00×10^{-6}	2.08×10^{-6}	104.0
D	0.00	8.00×10^{-7}	7.82×10^{-7}	97.8
E	0.00	5.00×10^{-7}	5.16×10^{-7}	103.2

present method was adequate for the determination of colchicine in real samples.

3.8. Analysis of colchicine in urine samples

The proposed method has been applied for the direct determination of colchicine in human urine, but no signal of colchicine was detected. Thus, this method was applied to detect colchicine in urine samples spiked with colchicine at a certain concentration. Urine samples containing colchicine were prepared by spiking urine obtained from healthy volunteers with appropriate volume of colchicine stock solution to give final colchicine urine concentrations between 5×10^{-7} to 1×10^{-5} mol/L. No sample treatment was performed, except for a urine dilution (1:2) with 0.1 mol/L $HClO_4$. The colchicine concentration was determined by the standard addition method; the results are shown in Table 2. The recoveries indicate that the accuracy and repeatability of this voltammetric method are very good. From the results listed in Table 2, it is very clear that the novel AB-DHP film coated electrode has a great potential for colchicine determination in practical samples.

4. Conclusion

The AB-DHP composite film modified GCE described in this paper is very cheap and easy to prepare, and can greatly enhance the sensitivity of determining colchicine. Compared with the unmodified GCE, the AB-DHP composite film coated GCE facilitates the oxidation of colchicine and then significantly enhances its oxidation peak current, owing to the strong adsorptive activity and huge surface area of AB film. Subsequently, a simple and sensitive electrochemical procedure was developed for the determination colchicine in real human urine samples.

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