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ORIGINAL PAPER

Electrochemical determination of ascorbic acid at the surface of a graphite electrode modified with multi-walled carbon nanotubes/tetradecyltrimethylammonium bromide

Mahdie Motahary · Sayed Mehdi Ghoreishi · Mohsen Behpour · Mahshid Golestaneh

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Abstract A multi-walled carbon nanotubes (MWCNTs)–tetradecyltrimethylammonium bromide (TTAB) film-coated graphite electrode (GE) was fabricated, and the electrochemical oxidation of ascorbic acid (AA) was studied in Britton–Robinson (B–R) buffer (pH 7.0) using cyclic, square wave, and differential pulse voltammetry (CV, SWV, and DPV). An electroanalytical study of AA and acetaminophen (ACOP) and of several mixtures of these compounds in different ratios was made. A sensitive linear voltammetric response for AA was obtained for the concentration range of 5×10^{-7} to 1.7×10^{-4} mol L^{-1}, with a correlation coefficient of 0.992, and the detection limit for AA was found to be 1.1×10^{-7} mol L^{-1} using DPV. The relative standard deviation (RSD) was 2.7%, suggesting that the film electrode has excellent day-to-day reproducibility. The proposed voltammetric approach was also applied to the determination of the AA concentration in commercial tablets.

Keywords Ascorbic acid · Graphite electrode · Tetradecyltrimethylammonium bromide · Multi-walled carbon nanotubes · Voltammetry

1 Introduction

In the last few decades, nanoparticle research has witnessed tremendous interest in nanoscience and nanotechnology. As non-metal particles, carbon nanotubes (CNTs) have been intensely studied because they are promising candidates for a wide range of applications. Since the discovery of CNTs by Iijima [1] in the early 1990s [1, 2], CNTs have attracted considerable attention due to their extraordinary structural, mechanical, electrical, and electrochemical properties as well as their promise in the field of materials science. Due to their unique structure, CNTs can behave electrically as a metal or as a semiconductor. The subtle electronic behavior of CNTs reveals that they have the ability to promote electron-transfer reactions, when they are used as an electrode material in electrochemical reactions [3]. CNTs are molecular-scale wires with high electrical conductivity, extremely high mechanical strength and modulus, and can be divided into two categories: single-wall carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). CNTs have been widely used in electroanalytical chemistry [4–7]. In many novel applications, CNTs have been studied for their use in electrochemical energy devices [8], ultra high-strength engineering fibers [9, 10], catalysts [11] and quantum wires [12]. Moreover, CNTs' chemical stability, low mass density, low resistivity, and large surface area make CNTs an ideal electrode material making CNT electrodes widely used for electrochemical reactions. In some reports, the MWCNTs have been cast on glassy carbon electrodes (GCE) to form CNT-modified electrodes. These modified electrodes have been successfully used in the oxidation of dopamine (DA) [7], electrochemical studies of proteins [13], electrocatalysis of oxygen [14], nitric oxide [15], and NADH [5]. Their performance has been found to be superior to other carbon electrodes in terms of reaction rates and reversibility. The SWCNTs have also been cast on a GCE to form a CNT film, which showed very stable electrochemical behavior and could be utilized to catalyze the electrochemical reaction of some biomolecules such as

M. Motahary · S. M. Ghoreishi (✉) · M. Behpour · M. Golestaneh
Department of Analytical Chemistry, Faculty of Chemistry, University of Kashan, Kashan, I. R. Iran
e-mail: s.m.ghoreishi@kashanu.ac.ir

DA [16], 3,4-dihydroxyphenylacetic acid, and ascorbic acid (AA) [17].

AA or vitamin C is an essential nutrient for higher primates and a small number of other species [18]. It is often added to various food products and pharmaceuticals. Importance of AA has led to a considerable effort to develop voltammetric methods for the determination of AA in biological samples. Recent clinical studies have demonstrated that the content of AA in biological fluids can be used to assess the amount of oxidation stress in human metabolism. Excessive oxidative stress has been linked to cancer, diabetes mellitus, and hepatic disease [19]. However, it is difficult to determine AA by direct oxidation at bare electrodes because of the high overpotentials that are needed, the fouling effect by its oxidation products, poor reproducibility, low selectivity, and poor sensitivity. Thus, much interest has been focused on the use of mediators and modified electrodes to catalyze the electrochemical oxidation of AA. For example, electrode surfaces modified with immobilized quinine groups [20], adsorbed TCNQ [21], deposited nickel pentacyanonitrosylferrate [22], covalently attached amino acids [23, 24], a functionalized self-assembled monolayer of 4-aminothiophenol [25], electro-polymerized films of polypyrrole [26, 27], and self-doped polyaniline [28–30] have all been employed via mediator oxidation.

Different analytical methods have been employed to evaluate the AA concentration in pharmaceutical formulations, foods, and biological fluids. These methods include chromatography [31, 32], electrochemistry [32], and spectrophotometry [32, 33]. Methods involving color measurement are less convenient because they are based on derivatization of the analyte to produce a colored compound, which is time-consuming. However, electrochemical methods are more promising because they possess quick response times, low cost, simplicity of instrumentation, high sensitivity, and the possibility of miniaturization.

A major drawback associated with voltammetry is the poor selectivity of measurements. This is particularly important in the case of AA because its direct oxidation on metal (or carbon) electrodes leads to poor sensitivity with large overpotentials and fouling problems caused by the adsorption of oxidation products [34–36]. Recent studies have demonstrated the advantages of using specific unmodified surfaces that accelerate electron-transfer rates such as edge plane pyrolytic graphite electrodes to perform electroanalytical determinations. Using these materials allows discrimination between concomitant analytes (for instance, DA, AA, and serotonin) [37, 38].

In this study, a MWCNTs–tetradecyltrimethylammonium bromide (TTAB)-modified graphite electrode (GE) was fabricated for the first time by us to determine AA. The electrochemical behavior of AA suggested that MWCNTs-modified GE exhibits an obvious electrocatalytic effect on the oxidation of AA, since it greatly enhances the oxidation peak current of AA as well as lowering its oxidation overpotential. After optimizing the experimental parameters, this electrode has used for the direct measurement of AA. Compared with other published methods, this new method possesses many advantages such as a very low detection limit, fast response, low cost, and simplicity.

2 Experimental

2.1 Chemicals and reagents

AA and TTAB were purchased from Merck. MWCNTs with purity >95% (40–60 nm in diameter) were obtained from the Chinese Academy of Sciences. The Britton–Robinson (B–R) buffer solution was comprised of phosphoric acid, boric acid, and glacial acetic acid, and the pH value was adjusted with NaOH. All reagents were of analytical grade. All solutions were prepared with deionized water.

2.2 Electrodes and instrumentation

All the electrochemical measurements were carried out with a M273A Electrochemical Workstation (America, EG&G Corporation). A conventional three-electrode system was employed, consisting of a MWCNTs–TTAB-modified GE as a working electrode (Metrohm), a saturated calomel reference electrode (SCE) (Metrohm) and a Pt wire counter electrode (Metrohm). Solution pH values were determined using a 691 pH meter (Metrohm Swiss made) combined with glass electrode (Metrohm). Deionized water was formed with an ultrapure water system (smart 2 pure, TKA, Germany). MWCNTs were dispersed with an ultrasonic bath (SONOREX DIGITAL, 10P, BANDELIN).

2.3 Fabrication of MWCNTs-modified GE

Following the procedure of a previous study [14], 5 mg MWCNTs and 5 mg TTAB were dispersed into 5 mL of deionized water, and then sonicated for about 15 min to give a stable and homogeneous MWCNTs–TTAB suspension. Prior to modification, the GE was mechanically polished to a mirror finish with alumina paste of different grades, rinsed and sonicated (3 min) in deionized water. Finally, the GE was coated with 5 μL of the MWCNTs–TTAB suspension and the water was allowed to evaporate under ambient conditions at room temperature. This procedure took about 1 h time. The TTAB film-coated GE was

prepared by the procedure as explained above, but without MWCNTs.

2.4 Sample preparation procedure

A 1.0×10^{-3} mol L^{-1} AA standard solution was simply prepared by dissolving AA in distilled water. A 0.2 mol L^{-1} B–R (pH 7.0) solution was prepared by mixing the equal volume of 0.2 mol L^{-1} phosphoric acid, 0.2 mol L^{-1} boric acid and 0.2 mol L^{-1} glacial acetic acid and the pH value was adjusted with 0.5 mol L^{-1} NaOH.

2.5 Analytical procedure

Fifteen milliliters of 0.2 mol L^{-1} B–R buffer solution (pH 7.0) containing a specific amount of standard solution of AA was added to an electrochemical cell. Electrochemical measurements were carried out by CV and recorded in the potential range of −0.1 to 1.0 V at a scan rate of 0.1 V s^{-1} after pausing 30 s. DPV employed the following parameters: $E_{initial} = -0.8$ V, $E_{final} = 0.3$ V, scan rate = 20 mV s^{-1}. SWV was recorded from −0.5 to 0.5 V and the current peak at 0.3 V was measured. The SWV parameters were as follows: SWV frequency = 10 Hz, step height = 0.25 mV, amplitude = 10 mV.

3 Results and discussion

3.1 Electrochemical behaviors of AA

In order to illustrate the electrocatalytic effect of MWCNTs on AA, the electrochemical properties of AA on four different kinds of working electrodes were examined using SWV and the results are shown in Fig. 1. Curve (a) in Fig. 1 is related to the absence of AA in solution on a bare electrode. Under identical conditions, the oxidation peak height of AA at the TTAB-modified GE decreases by a factor of two compared to the bare GE (curve b). On the bare GE, 3×10^{-5} mol L^{-1} AA yields a very low oxidation peak in B–R buffer at pH 7.0 (curve c). TTAB forms a perfect thin film on the GE surface, inhibiting the electron transfer between AA and the GE. The peak oxidation current therefore decreases compared to the bare GE. The MWCNTs film-coated GE is shown in curve d. However, the peak oxidation current of AA at the MWCNTs–TTAB-modified GE increases significantly (curve e) in comparison with that of a bare GE. The remarkable peak current enhancement and the fall of the oxidation overpotential testify to the electrocatalytic effect of the MWCNTs-modified GE on the oxidation of AA. In conclusion, modifying a GE with MWCNTs–TTAB greatly improves the sensitivity of measuring AA because of the unusual

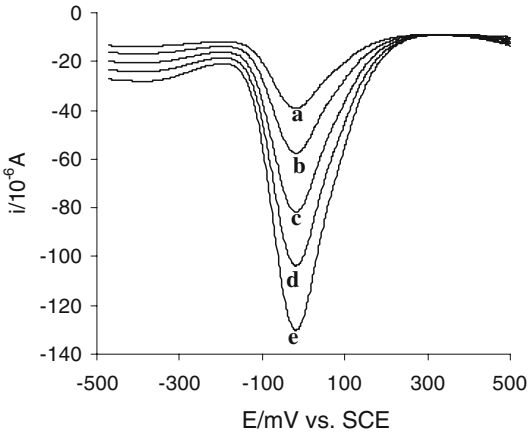


Fig. 1 Square wave voltammetric responses of 3.0×10^{-5} mol L^{-1} AA in 0.2 mol L^{-1} B–R buffer solution (pH 7.0), absence of ascorbic acid in solution on bare electrode (*a*), at TTAB film-modified GE (*b*), bare GE (*c*), MWCNTs film-coated GE (*d*), and MWCNTs–TTAB film-coated GE (*e*)

structure and properties of MWCNTs such as the very large specific area, strong adsorptive ability, and subtle electronic properties.

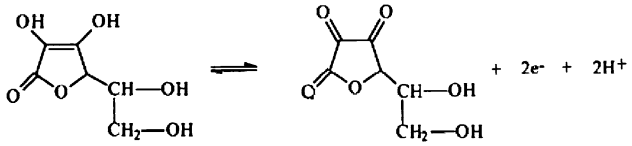
3.2 Mechanism of the oxidation of AA at MWCNTs–TTAB composite film

The pH of the supporting electrolyte has a significant influence on the electrooxidation of AA on the modified electrode. The electrooxidation of AA was studied over the pH range of 2.0–11.0 in a 0.2 mol L^{-1} B–R buffer (Fig. 2a (a–i)). The potential of the oxidation peak shifted to less positive potentials with increasing pH as depicted in Fig. 2a. The peak potential (E_{pa}) versus pH is depicted in Fig. 2b and the peak current (i_{pa}) versu pH is depicted in Fig. 2c. As can be seen the peak current in pH = 7.0 is maximum, therefore pH = 7.0 was selected as the optimum pH and this pH was used in all following experiments. The relationship of the peak potential versus pH can be expressed by following equation:

$$E_{pa}\,(\text{mV}) = 402.7 - 62.88\,\text{pH}\ (r = 0.991)$$

The slope 62.88 mV/pH was close to the theoretical 59.1 mV/pH slope predicted by the Nernst equation [39]. This result suggests that two electrons and two protons are transferred in the oxidation of AA.

A tentative mechanism for the oxidation of AA at electrode surface has been proposed as [40]



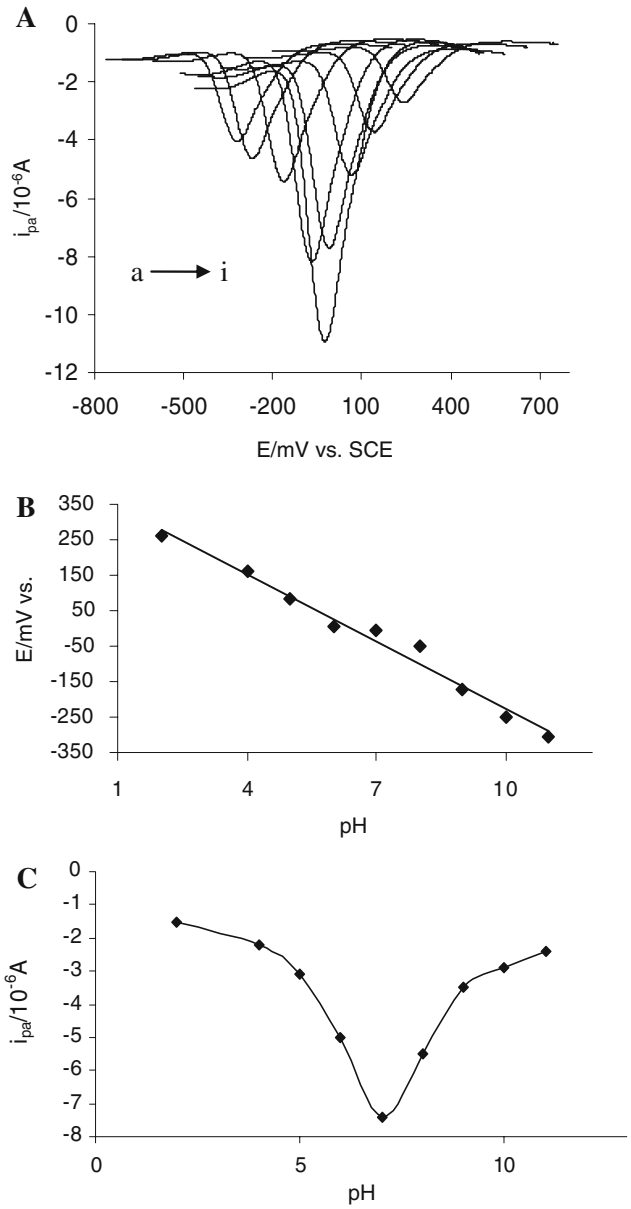


Fig. 2 **a** Differential pulse voltammograms of 4.0×10^{-6} mol L^{-1} AA in 0.2 mol L^{-1} B–R buffer solution (pH 7.0) at different pH values (*a–i*): 2.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0: (*insert*), **b** plots of E_{pa} versus pH, **c** i_{pa} versus pH

3.3 Effect of scan rate on the voltammetric response of AA

The effects of scan rate (υ) on the oxidation current of AA were also examined. The peak current increased linearly with the increase of square root of scan rate that ranged from 20.0 to 280.0 mV s^{-1}, and it can be expressed as follows:

$$i_{pa}\,(\mu A) = -0.6218\upsilon^{1/2} - 12.46\ (r = 0.9546)$$

These results show that the electrode process is controlled by the diffusion step. The relationship between

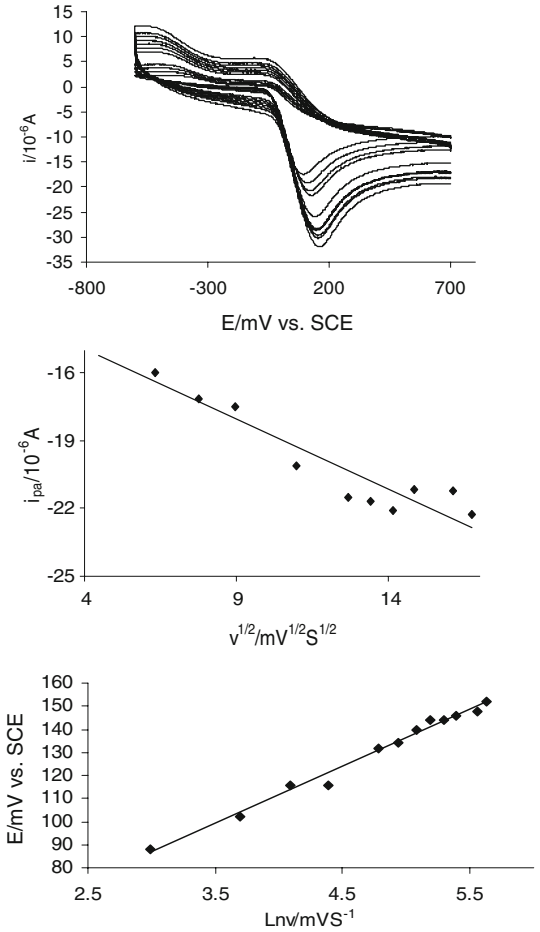


Fig. 3 **a** Cyclic voltammograms of 2.0×10^{-4} mol L^{-1} AA at the modified electrode at different scan rates from 0.02 to 0.28 Vs^{-1} in 0.2 mol L^{-1} B–R buffer solution (pH 7.0). **b** A linear relationship between the peak current and the square root of scan rate. **c** A linear relationship between the peak potential and the logarithmic scan rate

the oxidation peak potential and scan rate is described by the following equation:

$$E_{pa}\,(mV) = 24.787\ \ln\upsilon + 12.335\ (r = 0.994)$$

These results are described in Fig. 3. The slope indicates that the value of αn_α is 0.52, where α is the transfer coefficient and n_α is the number of electrons transferred in the rate-determining step. On the basis of above results, with a n_α value of 2, the value of α is calculated to be 0.26, which is reasonable for a irreversible electrode processes. Based on the above discussion, the oxidation process of AA is controlled by the diffusion step and two electrons are involved in the reaction.

The relation between the anodic peak oxidation current, i_{pa} (mA), diffusion coefficient of the electroactive species, D_o (cm^2 s^{-1}), and scan rate, υ (V s^{-1}), is given by [41]:

$$i_{pa} = \left(2.99 \times 10^5\right) n\alpha^{1/2} A C_o^{*} D_o^{1/2} \upsilon^{1/2},$$

where n is the number of electrons exchanged in oxidation, α is the transfer coefficient, A is the apparent surface area of the electrode (cm^2), and C_o* is the concentration of the electroactive species (mmol L^{-1}). Using the above equation, the diffusion coefficient D_o of AA was determined 4.68×10^{-7} cm^2s^{-1}.

3.4 Calibration curve

The calibration curve for AA in pH 7.0 was measured by DPV and SWV. For DPV the best parameters on the MWCNTs–TTAB film-coated GE were found to be: pulse amplitude = 20 mV; scan rate = 20 mV s^{-1}; and pulse width = 100 ms. For the MWCNTs–TTAB-modified GE, the linear segment increases from 5×10^{-7} to 1.7×10^{-4} mol L^{-1} with a regression equation of $i_{pa} = -372110C - 8.7$ ($r = 0.992$, C in mol L^{-1}, i_{pa} in μA) (Fig. 4).

It was found that this method can detect 5.0×10^{-7} mol L^{-1} AA. RSD of 2.7% for 6×10^{-7} mol L^{-1} AA ($n = 6$) indicates the excellent reproducibility of the test. The long-term stability of the MWCNTs–TTAB-modified GE was evaluated by measuring the current responses at a fixed AA concentration of 6×10^{-7} mol L^{-1} over a period of 3 weeks. The MWCNTs–TTAB-modified GE was used daily and stored in air. The current response deviated only 3.1% over the 3-week period, revealing that the MWCNTs-modified GE fabricated by this method possesses long-term stability.

However, for SWV, the linear range was from 7.0×10^{-7} to 9.5×10^{-5} mol L^{-1} ($r = 0.986$), and the detection limit of AA was 4.6×10^{-7} mol L^{-1} (Fig. 5).

3.5 Simultaneous determination of AA and ACOP

The mixtures of AA and ACOP were made as follows: 1:1, 1:2, 1:4, 2:1. A blurry oxidation peak by SWV was observed in the potential range from −0.7 to 0.7 V for the mixture of ACOP and AA in B–R (pH 7.0) (Fig. 6), illustrating that the oxidation peaks of ACOP and AA can be separated on the MWCNTs/TTAB/GE. In Fig. 6, the anodic peak potentials of ACOP and AA oxidations on the MWCNTs/TTAB/GE were at approximately 290, −100 mV, respectively. This tests shows that the two compounds can be easily separated from each other.

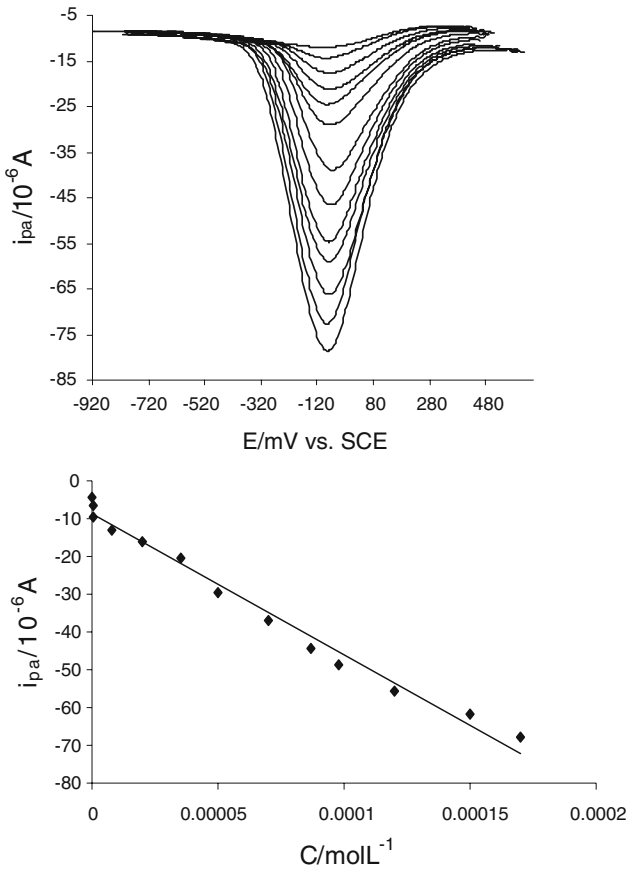


Fig. 4 DPV of AA on TTAB–MWCNTs-GE at different AA concentrations from 5.0×10^{-7} to 1.7×10^{-4} mol L^{-1}: (*insert*) linear relationship between i_{pa} and the concentration of AA

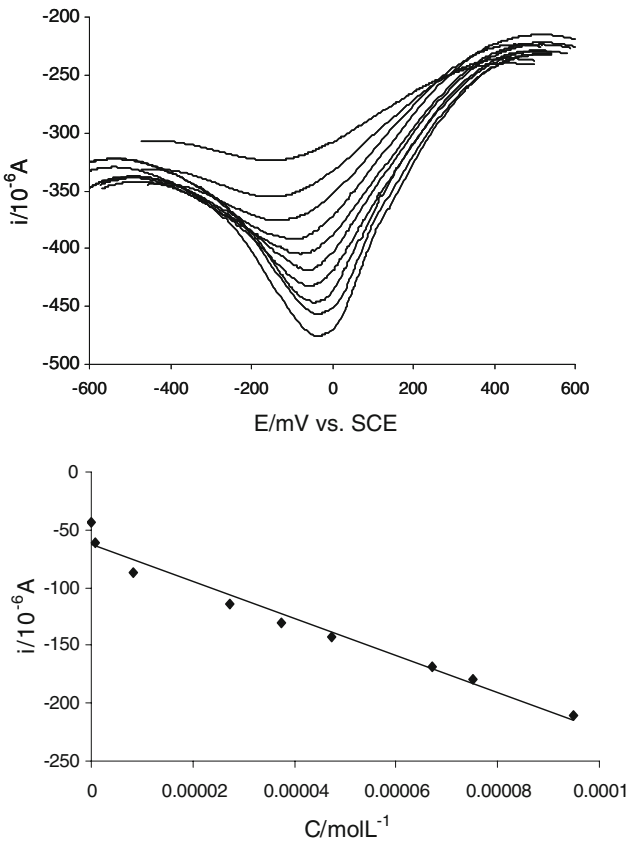


Fig. 5 SWV of AA on TTAB–MWCNTs-GE at different AA concentrations from 7.0×10^{-7} to 9.5×10^{-5} mol L^{-1}: (*insert*) linear relationship between i and the concentration of AA

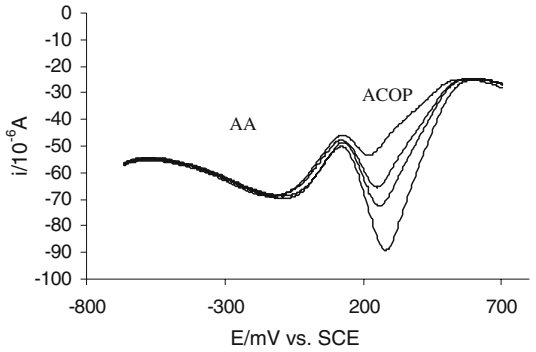


Fig. 6 SWV of mixtures of AA and ACOP were made as follows: 1:1, 1:2, 1:4, 2:1

3.6 Applications

This method was applied to the determination of AA in drug tablets. In this experiment, the concentration of AA was calculated using the standard addition method in the range of the calibration plot. Differential pulse voltammograms were then recorded under the exactly identical conditions as were employed for the calibration plot. When keeping the dilution factor in consideration, it was found that the AA concentration determined using this method was in good agreement with the reported value. The experimentally determined and reported AA amounts in tablets are listed in Table 1.

3.7 Recovery test

Recovery tests of ACOP were carried out in the concentration range of 4.0×10^{-6}–2.4×10^{-5} mol L^{-1}. The obtained results are listed in Table 2. Recoveries were found to lie in the range of 98.0–104%.

3.8 Effect of interferences

The selectivity of the MWCNTs–TTAB-modified GE was evaluated in the presence of different interfering molecules. The voltammetric responses of AA were examined in the presence of several possible interfering substances like penicillin, cloxacillin, aspirin, amoxicillin, and bromohexin. These substances are present in biological fluids and may interfere in the determination of AA with conventional methods. Differential pulse voltammograms were taken for the oxidation of AA (3×10^{-6} mol L^{-1}) after the addition of 7.5×10^{-5} mol L^{-1} of each interferent. The peak current in the absence of any interferent was 7.86×10^{-6} A. The results are given in Table 3. Thus, the response of AA at the MWCNTs–TTAB-modified GE was not affected by the examined interferents below 25-fold concentrations.

Table 2 Recovery data observed for AA at different concentrations

Added (mol L^{-1})	Detected (mol L^{-1})	Recovery (%)
4.0×10^{-6}	4.05×10^{-6}	101
1.5×10^{-5}	1.47×10^{-5}	98
2.4×10^{-5}	2.5×10^{-5}	104

Analysis carried out by DPV

Table 3 Impact of foreign species on the oxidation peak

Interference	Concentration (mol L^{-1})	DPV signal change (%)
Penicillin	7.5×10^{-5}	0.25
Cloxacillin	7.5×10^{-5}	4.2
Aspirin	7.5×10^{-5}	8.1
Amoxicillin	7.5×10^{-5}	−3.7
Bromohexzin	7.5×10^{-5}	−4.8

4 Conclusions

In this study, a MWCNTs–TTAB-modified GE was easily fabricated for electrochemical and voltammetric determination of AA. The CV, DPV, and SWV investigations showed effective electrocatalytic activity in lowering the anodic overpotentials and complete resolution of the anodic waves of AA from ACOP. High sensitivity, selectivity, and reproducibility of the voltammetric response make the proposed modified electrode very useful for accurate determination of AA in pharmaceutical and clinical preparations.

Table 1 A comparison between the observed and reported AA concentration in tablets

Tablet name (company name)	Reported concentration (mol L^{-1})	Observed concentration (mol L^{-1})	Error (%)
Vitamin C Chewable tablet 250 mg (Daru pakhsh Pharmaceutical Co. Iran)	7.0×10^{-5}	6.9×10^{-5}	−1.4
Vitamin C 1000 mg (Osve Pharmaceutical Co. Iran)	3.5×10^{-5}	3.6×10^{-5}	+2.8

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