

Determination of Ascorbic Acid Using Multi-Wall Carbon Nanotubes Immobilized on Carbon Paste Electrode

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Abstract: In this study, multi-wall carbon nanotubes (MWCNTs) immobilized on carbon paste electrode (CPE) was employed for the amperometric detection of ascorbic acid (AA). The influence of pH on the electrocatalytic effect of MWCNTs/CPE towards the oxidation of AA was studied; the effect of the potential applied and the stirring speed for amperometric detection of AA was investigated. The calibration curve was obtained for AA concentrations in a range of 1-100 μM . The detection limit for the method proposed was found to be 0.1 μM in the amperometric mode.

Keywords: Carbon paste electrode; Multi-wall carbon nanotubes; Electrocatalytic oxidation; Modified electrode; Voltammetry; Amperometry; Determination; Ascorbic acid.

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Introduction

Ascorbic acid (AA) is a significant vitamin in the diet of humans. Ascorbate prevents scurvy and takes part in several biological reactions. It is believed to be the primary antioxidant in human blood plasma. It has been used in the prevention and treatment of common cold, mental illness, infertility, cancer and AIDS [1, 2]. However, AA cannot be synthesized by humans and must be supplied from various natural and prepared foods, drugs and physiological fluids, fruit juices, soft drinks, vegetables.

As a result, the determination of AA is very important for biological and agro-industry. Many methods have been developed for this purpose. However, most of them have been based on its reducing properties. These methods lack specificity and are prone to interferences by other reducing agents in the sample [3-6].

The alternative method for measurement in complex materials such as physiological fluids or foodstuffs is chromatography. However, these methods require specific equipments which are very expensive, difficult in monitoring and generally time-consuming [7-13]. A need has arisen for a fast, sensitive and inexpensive method to detect AA and a substitution of analytical methods with a biosensor should be very significant.

Research and development in amperometric sensors for determination of AA have gained increasing importance in the last few years for their advantageous properties as analytical tools, namely the easy application, lower cost, providing direct, sensitive and fast detection of AA, in comparison with well-established, lab-based methods [14-24].

Among all the carbon electrodes, the carbon paste electrode (CPE) is an appealing and widely used electrode material in the fields of electrochemistry, electroanalysis, etc. due to its attractive advantages, such as simple preparation, low-cost implementation, renewability, low background current, and wide potential window [25]. Carbon nanotubes (CNTs) modified CPE have been applied in many studies due to the unique properties of CNTs such as large active surface area, high electronic conductivity, high mechanical resistance properties, anti-fouling capability and their ability to reduce over potential [26-28]. In this study, a simple modification procedure of MWCNTs on the surface of CPE for amperometric detection of AA was reported.

Experimental

Chemicals and Reagents

All the reagents were purchased in Sigma-Aldrich (Czech Republic). Distilled water purified by deionized water system was used in this study ($G \leq 0.055 \mu\text{S}$). The soluble oxygen was removed from all the solutions by purging with argon for 15 min (purity 99.99%, Linde Technoplyn, Prague, Czech Republic).

A solution of AA (10^{-2} M) was prepared freshly in deionized water and was kept in a dark bottom during the experiments. Briton Robinson (BR) buffer solution was used as supporting electrolyte.

The Preparation of Multi Wall Carbon Nanotubes modified Carbon Paste Electrode (MWCNTs/CPE)

The CPEs were prepared as following: 0.5 g of graphite powder 5.5-7.0 μm (CR-5, Maziva Týn n. L., s.r.o., Czech Republic) and 130 μL of mineral oil (M5904, Sigma-Aldrich, Germany) was exhaustively hand mixed together in ceramic mortar by ceramic pestle. The resulting paste was packed into the Teflon piston holder (3.0 mm inner diameter) [29,30].

100 mg of the untreated multi-wall carbon nanotubes (MWCNTs) was added to 50 mL of nitric acid: sulfuric acid (3:1). The mixture was sonicated for about 1 hour at 25°C and then was heated in 100°C water bath for 4 hours to obtain a relative stable suspension, then washed with distilled water by centrifugation (4000 rpm, 10 min) to neutrality and dried using infrared lamp for 4 h. The treated MWCNTs were dissolved in N, N-dimethylformamide (DMF) and were sonicated for one hour before casting 10 μL on the surface of clean CPE for preparation of MWCNTs/CPE.

Electrochemical Measurement

Three electrode system consisting of carbon paste electrode (working), Ag|AgCl|3.0 M KCl (reference) and platinum wire (counter electrode) connected to PalmSens (Ivium Technologies, Netherland) was used for electrochemical measurement. The surface of CPE was regenerated by renewing and polishing it on wet filter paper before each measurement.

All experiments were repeated at least 5 times and results represented an average with standard deviation. The statistical differences were calculated using analysis of variance at the probability factor $p = 0.05$.

Results and Discussion

Effect of the Concentration of MWCNTs

MWCNTs with various concentrations from 0.5 to 3.0 mg/mL were prepared and casted on the surface of CPE. After that, the electrode was immersed in BR buffer solution (pH 7.0) and their cyclic voltammograms were recorded in the presence of $2 \cdot 10^{-5}$ M AA in the potential range from -400 to 800 mV (scan rate 50 mV/s; potential step 25 mV).

Fig. 1 shows the influence of concentration of MWCNTs on the behavior of MWCNTs/CPE. As can be seen in this figure, the amount of MWCNTs significantly influences the oxidation peak potential of AA. At the low concentration of MWCNTs, the oxidation current of AA was significantly higher ($p < 0.05$) than those determined in higher concentration. The lowest oxidation peak potential was found at the concentration of 2.0 mg/mL MWCNTs ($p < 0.05$) and was chosen for the next experiments.

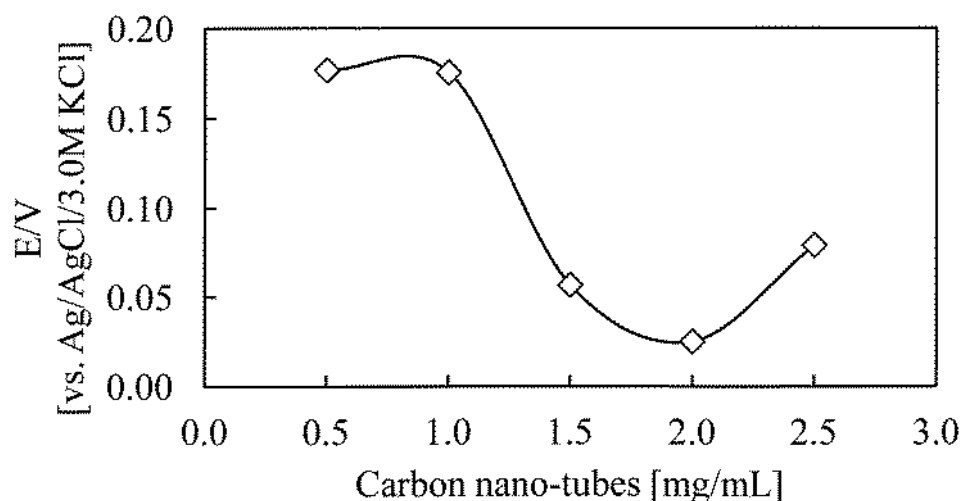


Fig. 1: The influence of the amount of carbon nanotubes on the oxidation peak potential of AA derived from cyclic voltammograms of MWCNTs/CPE in the presence of 2×10^{-5} M AA. Experimental conditions: supporting electrolyte: BR buffer pH 7.0; potential range: -400 to 800 mV; potential step: 25 mV; scan rate: 50 mV/s.

Effect of pH

In order to study the effect of pH on the electrochemical behavior of MWCNTs/CPE and bare CPE, BR buffer with a wide range of pH from 3 to 10 were used as supporting electrolyte in recording cyclic voltammograms of MWCNTs/CPE and bare CPE in the potential range -400 to 800 mV; scan rate 50 mV/s; potential step 25 mV. It was observed that pH significantly influenced electrochemical behavior of both MWCNTs/CPE and bare CPE since the oxidation peak potentials obtained in pH range from 7 to 10 were significantly lower than those obtained in acidic medium (pH 3.0, 4.0, 5.0, 6.0). At MWCNTs/CPE, although pH 8.0 gave significant lower oxidation peak potential, pH 7 was chosen for the amperometric determination of AA due to the sharper peak obtained from this supporting electrolyte.

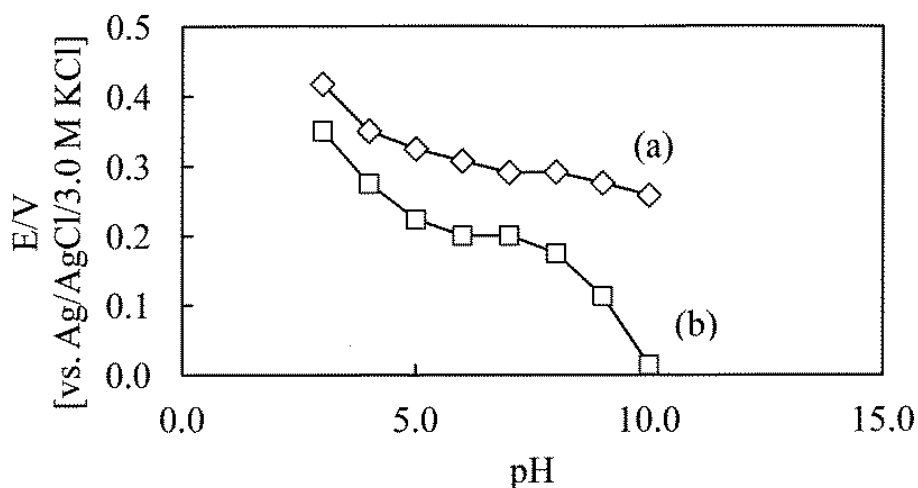


Fig. 2: The influence of pH on the oxidation peak potential of AA derived from cyclic voltammograms using: (a) bare CPE, (b) MWCNTs/CPE; in the presence of 2×10^{-5} M AA. Experimental conditions: supporting electrolyte: BR buffer pH 7.0; potential range: -400 to 800 mV; potential step: 25 mV; scan rate: 50 mV/s.

Electrocatalytic Effect of MWCNTs/CPE Towards Oxidation of AA

Fig. 3 overleaf shows the typical cyclic voltammogram of AA (2×10^{-5} M) in BR solution pH 7 derived from both MWCNTs/CPE (curve b) and bare CPE (curve c) in the potential range -400 to 800 mV; scan rate 50 mV/s; potential step 25 mV. It was observed that the peak current of AA obtained from MWCNTs/CPE was higher while the peak potential was lower than those obtained from bare CPE. The potential was shifted from to (about 150 mV). This is caused by the electrocatalytic effect of MWCNTs on the oxidation of AA [26-28].

Amperometric Detection of AA

Influence of Stirring Speed. Various value of stirring speed from 100 to 500 rpm at the fix applied potential 100 mV was employed for amperometric measurement of AA added to BR buffer pH 7.0 as supporting electrolyte. The concentration step of AA was 2×10^{-5} M for each addition. The highest measured current was obtained at applied speed of stirring 400 rpm. At the stirring speeds higher than 400 rpm, the responses of amperometric detection are not stable due to the significant influence of noise. For that reason, the stirring speed 400 rpm was chosen for the next experiments.

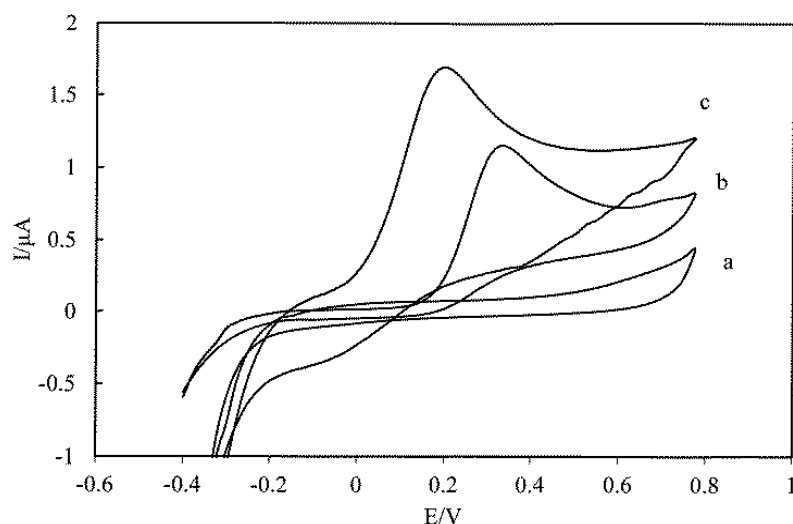


Fig. 3: Cyclic voltammograms of MWCNTs/CPE a) in the absence of AA; b) in the presence of 2×10^{-5} M AA; c) bare CPE in the presence of 2×10^{-5} M AA. Experimental conditions: supporting electrolyte: BR buffer pH 7.0; potential range: -400 to 800 mV; potential step: 25 mV; scan rate: 50 mV/s.

Influence of the Potential Applied. Various potentials from 0 to +500 mV at the stirring speed 400 rpm were applied for amperometric detection of AA, with the concentration of 2×10^{-5} M AA for each addition to BR buffer solution (pH 7.0); see the following Fig. 4.

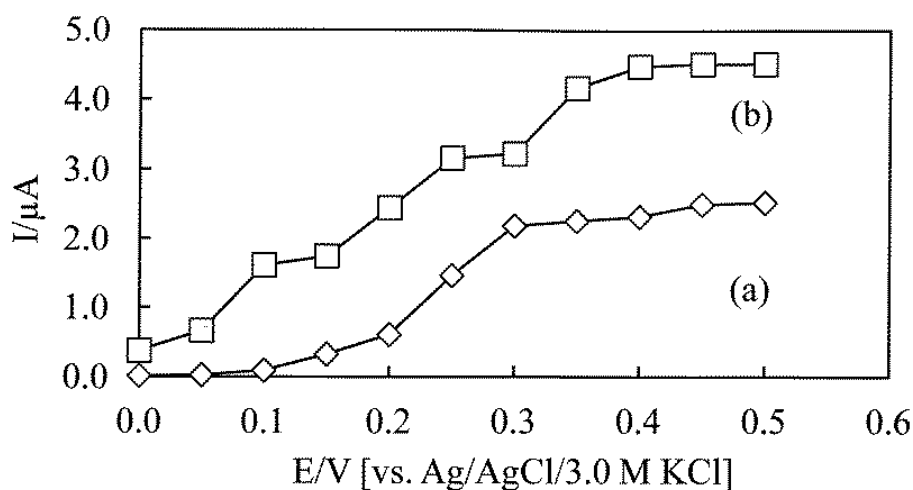


Fig. 4: The influence of working potential on oxidation current of 2×10^{-5} M AA by hydrodynamic amperometry using: (a) bare CPE, (b) MWCNTs/CPE. Experimental conditions: supporting electrolyte: BR buffer pH 7.0; speed of stirring: 400 rpm.

As can be seen in Figure, the higher potential applied, the higher peak current obtained for both MWCNTs/CPE and bare CPE, while the peak currents obtained from MWCNTs/CPE were significant higher than those obtained from bare CPE. The potential +100 mV was chosen for MWCNTs/CPE to avoid the oxidation from some interference compounds. The currents obtained at this applied potential from MWCNTs/CPE were about 18 times higher than those obtained from bare CPE.

Calibration Curve. After the experiments above, the optimum conditions for preparation of MWCNTs/CPE and application of this electrode for amperometric detection of AA was employed: speed of stirring: 400 rpm; applied potential: +100 mV. The calibration curve was obtained for AA concentrations in a range of 1-100 μM , as can be seen in Fig. 5 (inset). The precision of the method was investigated by repeated injections ($n = 10$, 100 μL) of the $5.0 \times 10^{-5} \text{ M}$ AA and the relative standard deviation (RSD) was found to be 6.4 %, while detection limit was calculated to be $1.0 \times 10^{-7} \text{ M}$ for amperometric determination of AA.

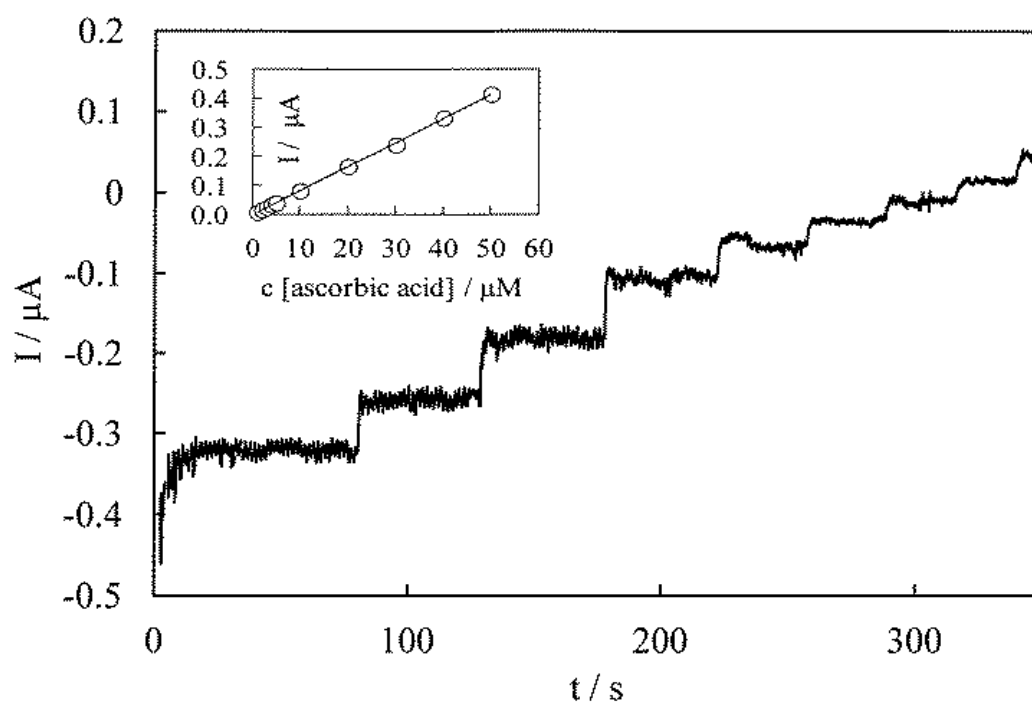


Fig. 5: Typical hydrodynamic amperogram of $1.0 \times 10^{-5} \text{ M}$ ascorbic acid obtained at the MWCNTs/CPE. Experimental conditions: supporting electrolyte: BR buffer pH 7.0; speed of stirring: 400 rpm; applied potential: +100 mV. Inset: calibration curve obtained from the amperometric measurements.

Conclusions

This study has demonstrated that MWCNTs/CPE showed significant electrocatalytic activity towards the AA oxidation. It was observed that the oxidation peak of AA shifted from +350 mV at CPE to +150 mV at the MWCNTs/CPE. Besides, an enhancement in the oxidation peak of AA was also observed. The amperometric detection of AA with MWCNTs/CPE was successfully tested; the detection limit of AA being estimated as 0.1 μM in this study.

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