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DETERMINATION OF ASCORBIC ACID IN PHARMACEUTICAL PREPARATION AND FRUIT JUICE USING MODIFIED CARBON PASTE ELECTRODE

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ABSTRACT

Acrobic acid is key substance in the human metabolism and the rapid and accurate determination in food is of a great interest. Ascorbic acid is an electroactive compound, however poorly responded on the bare carbon paste electrodes. In this paper, brilliant cresyl blue and multi-walled carbon nanotubes were used for the modification of carbon paste electrode. Brilliant cresyl blue acts as a mediator improving the transition of electrons, whereas multiwalled carbon nanotubes increased the surface of the electrode. Both brilliant cresyl blue and multiwalled carbon nanotubes were added directly to the composite material. The electrochemical behavior of modified electode was determined in electrolyte at various pH, and the effect of the scan rate was also performed. It was shown that the electrochemical process on the surface of the modified carbon paste electrode was diffusion-controlled. The resulted modified carbon paste electrode showed a good electrocatalytic activity towards the oxidation of ascorbic acid at a reduced overpotential of +100 mV descreasing the risk of interferences. A linear response of the ascorbic acid oxidation current measured by the amperometry in the range of 0.1 – 350 μmol.L^{-1} was obtained applying the sensor for the standard solution. The limit of detection and limit of quantification was found to be 0.05 and 0.15 μmol.L^{-1}, respectively. The novel method was applied for the determination of ascorbic acid in pharmaceutical vitamin preparation and fruit juice, and the results were in good agreement with the standard HPLC method. The presented modification of carbon paste electrode is suitable for the fast, sensitive and very accurate determination of ascorbic acid in fruit juices and pharmaceutical preparation.

Keywords: ascorbic acid; carbon nanotubes; amperometry; brilliant cresyl blue

INTRODUCTION

Ascorbic acid (AA) is a significant vitamin in the diet of humans which prevents scurvy and takes part in several biological reactions **(Oguntibeju 2008)**. In recent studies, it has been found that ascorbic acid may be used as a supporting agent for the treatment of cancer **(Du et al., 2012)** and seems to be interesting in research of Alzheimer's disease **(Bowman 2012)**. 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 and vegetables. For that reason, the determination of AA is very important for biological and agro-industry. At present, vitamin C is determined using widely different techniques including colorimetric and titrimetric measurement, UV spectrophotometry, as well as fluorimetric, chromatographic and other spectroscopic methods **(Ötleş and Karaibrahimoglu, 2012)**. The most common method for analysis of vitamin C is HPLC which is more accurate, selective and sensitive than other methods mention above. However, HPLC methods require specific equipments which are very expensive, difficult in monitoring and generally time-consuming. A need has arisen for a fast, sensitive and inexpensive method for the detection of AA. Recently, a portable strip used for the rapid determination of ascorbic acid has been described **(Kudrnáčová a Kouřimská, 2015)**. The electrochemical determination is one of the approaches as was described in literature **(Skrovankova et al., 2015; Pisoschi et al., 2014)**. Research and development in amperometric sensors for the determination of AA based on the carbon paste composite material have gained increasing importance in the last few years for their advantageous properties as analytical tools, namely the easy of application, lower cost, providing direct, sensitive and fast detection of AA, in comparison with well-established, lab-based methods **(Weng et al., 2013; Li et al., 2011; Huang et al., 2014; Heli and Sattarahmedy 2015; Chang et al., 2014)**. The carbon paste electrodes have attractive advantages, such as simple preparation, low-cost implementation, renewability, low background current, and wide potential window **(Švancara et al., 2012)**. 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, anti-fouling capability and their ability to reduce over potential **(Jacobs et al., 2010; Huang et al., 2014; Bijad et al., 2014)**.

Brilliant cresyl blue (BCB) is a cationic quinine-imide dye with a planar and rigid structure which has been proven to possess promising properties as a redox catalyst. BCB can absorb strongly on the electrode surfaces and these chemically modified electrodes have been used for the determination of various organic compounds **(Lin et al., 2012; Ding et al., 2016; Shaikh et al., 2013)**. The reduction of BCB by ascorbic acid has been described **(Ulusoy et al., 2011)** therefore we chose this organic dye as a suitable redox mediator for amperometric determination of ascorbic acid.

MATERIAL AND METHODOLOGY

Reagents and equipment

All the reagents were purchased from Sigma-Aldrich (Czech Republic). Deionized water was used in this study (G ≤0.055 µS). Dissolved oxygen was removed from all the solutions by purging with argon for 5 min (purity 99.99%, Linde Technoplyn, Prague, Czech Republic).

A solution of ascorbic acid (10^{-2} mol.L^{-1}) was daily prepared in deionized water and was kept in a dark bottle during the experiments. Briton Robinson (0.04 M, B-R) buffer solution containing 0.1 M KCl was used as a supporting electrolyte.

A three electrode system consisting of CPEs (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 surfaces of CPE were regenerated by renewing and polishing them on wet filter paper before each measurement.

Preparation of CPE

Both bare and modified electrodes were prepared by mixing of 0.5 g of graphite powder 5.5 – 7.0 µm (CR-5, Maziva Týn n. L., s.r.o., Czech Republic) with 130 µL of mineral oil (M5904, Sigma-Aldrich, Germany). The modified CPE electrode contained 1.0% of multiwalled carbon nanotubes (40 – 60 nm, Shenzhen NanoTech Port Co., China) (MWCNTs), and 3.0% of brilliant cresyl blue powder (BCB). The resulting paste was packed into the Teflon piston holder (3.0 mm inner diameter) (**Švancara and Metelka 2000**). The resistance of the composite material was always ≤15.0 Ω.

Electrochemical procedure

The effect of modifiers in carbon paste was investigated using cyclic voltammetry in 0.04 M Britton-Robinson buffer solution (B-R) at the pH 5.0 containing 0.1 M KCl at a scan rate of 50 mV.s^{-1} in the range of potentials from -400 mV to +1000 mV. The influence of pH between 3.0 and 9.0 was also investigated at the scan rate of 50 mV.s^{-1} in the same potential window. In order to study the effect of the scan rate on the peak potentials and peak currents, the cyclic voltammograms of the BCB-MWCNTs/CPE were recorded at different scan rates in the potentials ranged from -300 to 800 mV in B-R buffer solution at the pH 5.0 containing 0.1 M KCl.

The amperometric detection of ascorbic acid based on its electrocatalytic oxidation was studied by using BCB-MWCNTs/CPE. Aliquots of a stock solution of AA were added to the supporting electrolyte solution (B-R buffer solution at the pH 5.0 plus 0.1M KCl) after the background current reached a steady state value at an applied potential +100 mV.

Sample preparation

The proposed method was applied for the determination of AA in pharmaceutical sample (tablet, Celaskon 250 mg, Zentiva, Czech Republic) and fruit juice sample (Toma Juice Multivitamin, Pepsico CZ s.r.o., Czech Republic). The tablet (15 mg) was dissolved in deionized water, transferred to a 250 mL volumetric flask, and then was diluted by deionized water until the mark. Juice sample was used directly without any treatment. The amount of ascorbic acid was also determined using HPLC/UV (LC-10AD, Shimadzu Co., Japan) equipped with column LiChrospher RP-18e (250 × 4 mm, 5 µm). A mixture of 250 µL of dilute sample and 50 µL of internal standard (isoascorbic acid in metaphosphoric acid) was vigorously shaken following by centrifugation (13.000 rpm, 5 min). The supernatant (5 µL) was injected into the mobile phase (sodium phosphate/phosphoric acid, pH 2.0, flow rate 1 mL.min^{-1}) with detection wavelength at 263 nm.

Student´s t-test was used for determination of statistical differences between results at the probability level $p = 0.05$ (Origin Pro v. 9, OriginLab Corp., USA).

RESULTS AND DISCUSSION

Electrochemical behavior of BCB-MWCNTs/CPE

As can be seen from Figure 1, an anodic and cathodic peak was observed at +125 mV and +75 mV, respectively. The formal potential of the redox process was +100 mV, and a peak-to-peak separation was +50 mV. The reverse-to-forward current peak ration was approximately unity, which reflects the reversible electrochemical behavior of BCB.

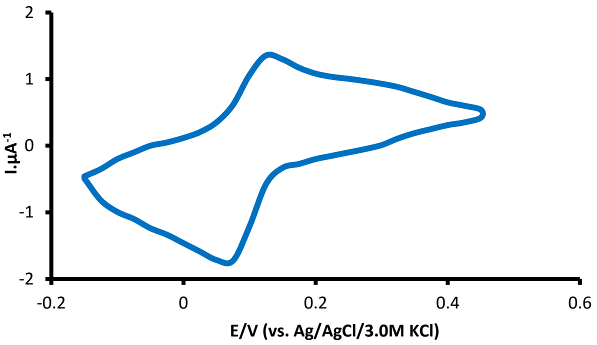

Figure 1 Cyclic voltammograms of BCB-MWCNTs/CPE in the absence of ascorbic acid in B-R buffer solution (pH 5.0) containing 0.1M KCl at the scan rate 50 mV.s^{-1}. Potential range from -150 to +450 mV, potential step 25 mV.

The electrochemical behavior of BCB-MWCNTs/CPE was investigated by recording of cyclic voltammograms in a B-R buffer solution at the pH 3.0 containing 0.1M KCl at various scan rates. The anodic (I_{pa}) and cathodic (I_{pc}) peak currents were proportional to the scan rates (v) in the range from 10 to 100 mV.s^{-1}. The equations and the regression coefficients were found to be: $I_{pa} = 0.11v^{1/2} + 0.02$ ($R^2 = 0.998$) and $I_{pc} = -0.11v^{1/2} + 0.04$ ($R^2 = 0.994$), respectively.

These results indicate that the electrochemical process on the BCB-MWCNTs/CPE is diffusion-controlled. The shifting of the potentials was not observed between 10 and 100 mV.s^{-1}.

In order to study the effect of pH on the electrochemical behavior of BCB-MWCNTs/CPE, B-R buffer with the pH ranged from 3.0 to 9.0 as a supporting electrolyte was used

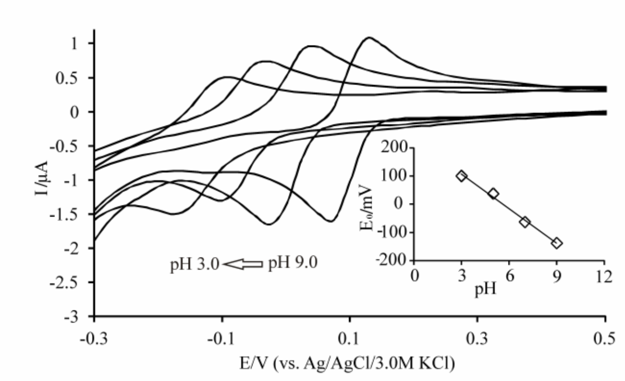

Figure 2 Cyclic voltammograms of BCB-MWCNTs/CPE in B-R buffer solutions (pH 3.0, 5.0, 7.0 and 9.0) containing 0.1M KCl, scan rate 50 mV.s^{-1}, potential range from -500 to +900 mV. Inset: Effect of formal potential (E_0) of BCB-MWCNTs/CPE on the pH.

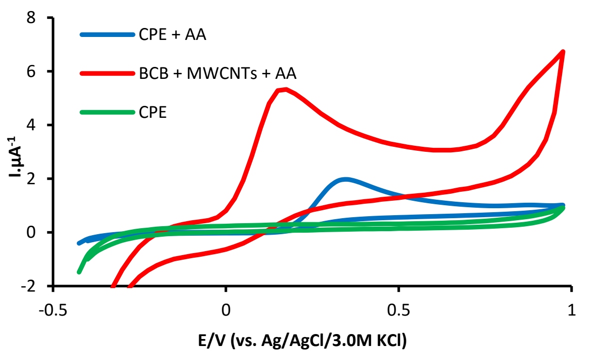

Figure 3 Cyclic voltammograms of BCB-MWCNTs/CPE and CPE in the presence of 250 μmol.L^{-1} of ascorbic acid in B-R buffer (pH 5.0) containing 0.1M KCl, scan rate 50 mV.s^{-1}, potential step 25 mV. Bare CPE (green) in buffer solution served as control.

for determination of cyclic voltammograms of BCB-MWCNTs/CPE at the scan rate of 50 mV.s^{-1}. It was seen that the pH significantly influenced the behavior of BCB-MWCNTs/CPE, since the shape and the position of the peak for redox pair was better in acidic conditions (3.0 and 5.0) in comparison with those obtained in the pH 7.0 and 9.0 (Figure 2).

The inset in Fig. 2 shows the formal potential (E^0) of the BCB-MWCNTs/CPE plotted against pH in the range from 3.0 to 9.0. It shows a slope -40.63 mV.pH^{-1}, which is close to that given by the Nernstian equation for equal number of electrons and protons transfer process. Moreover, the both oxidation and reduction currents proportionally decreased with increasing pH. The electrochemical behavior has been documented in electrodeposited BSB film on the surface of multi-walled carbon nanotubes modified glassy carbon electrode **(Lin et al., 2012)**.

Ascorbic acid oxidation at BCB-MWCNTs/CPE

Cyclic voltammograms of BCB-MWCNTs/CPE were recorded both in the absence and presence of ascorbic acid in order to study the electrocatalytic activity of modify electrode towards AA oxidation. As shown in Figure 3, anodic and cathodic peaks were obtained at +125 and 75 mV, respectively. Oxidation of AA in the bare CPE and BCB-MWCNTs/CPE gave peak potentials at about 350 and 150 mV, respectively. The overpotential for AA oxidation was found to shift by about 200 mV.

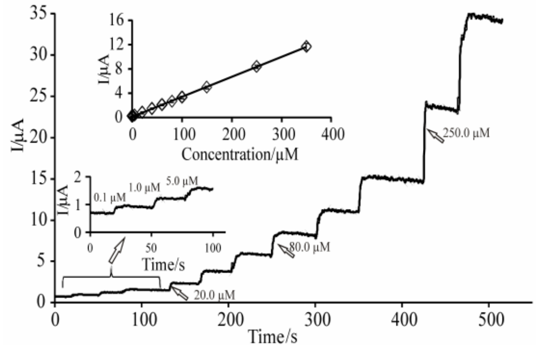

Figure 4 Amperometric current-time curves of AA with various concentrations (μM) using BCB-MWCNTs/CPE. Inlet: the plot of maximum oxidation current vs. concentration of AA. B-R buffer solution at pH 5.0, constant potential +100 mV, stirring speed 400 rpm. The vertical bars represent standard deviation (*n* = *6*).

The modification of CPE by each modifier separately was also investigated. The oxidation peak potential of AA using CPE with 1.0% of MWCNTs shifted to more negative potentials by about +50 mV with increasing ($p < 0.05$) of oxidation current of AA to 3.06 ±0.02 μA in comparison with that obtained in bare CPE (2.13 ±0.02 μA). On the other hand, the separate addition of 3.0% of BCB to CPE resulted in similar oxidation current of AA (2.91 ±0.05 μA) but more progressive shift of the peak potential to +175 mV was observed. It confirms that MWCNTs just increased the electroactive surface area of the CPE electrode (with slight increase of overpotential) whereas BCB acts as a redox mediator. This result clearly indicates that BCB-MWCNTs/CPE in B-R buffer solution (pH 5.0) containing 0.1M KCl exhibited a significant supporting electrocatalytic activity towards AA oxidation. In the study of **Zhang et al., (2013)**, the film of poly(bromcresol purple) at glassy carbon electrode also showed good electrocatalytic effect towards AA oxidation with reduced the oxidation overpotential for about 240 mV with increasing current. In view of oxidation current and the targeted analyte (AA), pH 5.0 was chosen as the best buffer since it offered a relatively high oxidation current and a low oxidation potential.

Various values of applied potential from -50 to +400 mV were used for the amperometric detection of AA. The oxidation current of AA exhibited steep increase from -50 to 100 mV. An applied potential is considered suitable when it offers relatively high oxidation current and low oxidation peak potential. The applied potential +100 mV was chosen for the amperometric determination of AA at BCB-MWCNTs/CPE since it gave the highest current (4.86 ±0.01 μA) compared with that obtained from the lower applied potentials. Besides, using low applied potential can avoid the interferences of some compounds from the matrices. Figure 4 shows the current-time curves for the amperometric responses at various concentration of AA.

Table 1 Comparison of carbon paste electrode (CPE) based electrochemical sensors for amperometric determination of ascorbic acid.

Electrode modification	Applied potential (mV)	LOD ($\mu mol.L^{-1}$)	Dynamic range ($\mu mol.L^{-1}$)	Reference
AF-MWCNT/EPI[1]	+420	4.1	NA	**Huang et al., 2014**
Gold decorated SiO_2@PANI[2] core-shell microsphere	+400	3.8	150.0-8000.0	**Weng et al., 2013**
Graphene doped CPE	+310	0.07	0.1-106.0	**Li et al., 2011**
Graphene oxide CoHCF[3] nanocomposite	+430	0.29	2.5-62.5	**Heli and Sattarahmady 2015**
trans-PEPACC[4]	+450	2.27	0-550	**Chang et al., 2014**
BCB/MWCNT[5]	+100	0.05	0.1-350.0	This work

[1] amino-functionalized multi-walled carbon nanotube electroactive polyamide, [2] polyaniline, [3] cobalt hexycyanoferrate. [4] photoactive and electroactive azo-based polyimide/amino-functionalized multiwalled carbon nanotubes, [5] briliant cresyl blue/muti-walled carbon nanotube.

Table 2 Ascorbic acid content ($mg.L^{-1}$) in real sample using BCB-MWCNTs/CPE and standard HPLC method ($n = 20$).

Sample	Amperometric method	HPLC method
Juice	120.1 ±5.2*	120.5 ±4.7
Tablet	25.8 ±0.5	25.2 ±0.4

* standard deviation

A linear relationship between the AA concentration and the peak oxidation current was obtained over the concentration range $1 \times 10^{-7} – 3.5 \times 10^{-4}$ $mol.L^{-1}$ (Figure 4, inlet) with equation:

$$I_{pa}\ (\mu A) = 0.033 \times c\ (\mu mol.L^{-1}) + 0.166\ (R^2 = 0.993)$$

The repeatability of the method was investigated by amperometric measurements of 5 $\mu mol.L^{-1}$ and 300 $\mu mol.L^{-1}$ of AA ($n = 20$) and the relative standard deviation (RSD) was found to be 4.5% and 5.2%, respectively. The limit of detection (LOD) using the equation LOD = $3s_b/m$, where s_b is the standard deviation of the blank response and m is the slope of the calibration plot, was found to be 5.0×10^{-8} $mol.L^{-1}$. The limit of quantification (LOQ) using the equation LOQ = $10s_b/m$ was found to be 1.5×10^{-7} $mol.L^{-1}$ (signal/noise = 10). Various modified carbon paste electrodes used for ascorbic acid determination were compared (Table 1). Among amperometric sensors based on CPE, our modification allowed the detection of AA at lower applied potential avoiding the interference species. BCB-MWCNTs/CPE electrochemical sensor is proven to be extremely sensitive, simple renewable and easy in preparation and storage.
After 45 days of storage at room temperature, the amperometric responses of AA at the concentration of 5.0 $\mu mol.L^{-1}$ was not significantly ($p > 0.05$) different in comparison with those obtained using the freshly prepared electrode. The low detection limit is an advantageous character of BCB-MWCNTs/CPE compared with the most CPE modified electrodes mentioned in Table 1. BCB-MWCNTs/CPE is also applicable for the analysis of AA in fruit juices and pharmaceutical preparations. As described in Table 2, the contents of AA in real samples did not differ from those determined by HPLC method.

CONCLUSION

This study demonstrated that BCB-MWCNTs/CPE showed significant electrocatalytic activity towards the oxidation of AA. It was observed that the oxidation peak potential of AA shifted from +375 mV at CPE to +100 mV at BCB-MWCNTs/CPE together with increasing of the oxidation current. The electrocatalytic activity of BCB-MWCNTs/CPE was investigated to detect AA by amperometry with a good linearity and sensitivity. We may conclude that BCB-MWCNTs/CPE represents a steady electrode material for electrocatalytic oxidation of AA.

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