

Highly Sensitive Voltammetric Sensor for Determination of Ascorbic Acid Using Graphite Screen Printed Electrode Modified with ZnO/Al₂O₃ Nanocomposite

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A sensitive and selective voltammetric sensor based on graphite screen printed electrode modified by ZnO/Al₂O₃ nanocomposite for the detection of ascorbic acid has been successfully developed. The electrochemical behavior of prepared electrode for the determination of ascorbic acid was systematically investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Under optimum conditions, the modified electrode exhibits linear responses to ascorbic acid in the ranges 1.0–100 μM, with detection limits (S/N = 3) calculated to be 0.6 μM. Finally, the developed sensor was successfully applied to the detection of ascorbic in real samples.

Keywords: Ascorbic acid; ZnO/Al₂O₃ nanocomposite; Graphite screen printed electrode; voltammetry

1. INTRODUCTION

Vitamins are organic molecules which are vital for the life. Human body provides the required vitamins from food, because it is not able to enough amounts of them or even none at all. They are divided into water-soluble and fat-soluble vitamins. Vitamins A, D, E and K are fat-soluble ones and

vitamins C and all the B vitamins are water-soluble. Water-soluble vitamins do not get stored in the body for the long time; they easily get expelled through urine. Water-soluble vitamins need to be provided more than fat-soluble ones [1].

Ascorbic acid or vitamin C plays an important role in the formation and maintenance of the collagen and also as a powerful antioxidant protecting the body against oxidative stress, and present in the human diet as a vital vitamin. Further, it is also used for the prevention and treatment of prevalent cold, mental illness, infertility, cancer and AIDS [2-4]. Also ascorbic acid is important biochemical active molecule and is widely used as food additives or antioxidants for pharmaceutical purpose. Studies show that ascorbic acid is essential for the natural synthesis of dopamine in the human body. On the other hand, large doses of ascorbic acid may reduce the risk of kidney stone formation in women. Thus, determination of this compound is very essential for pharmaceutical and biological studies [5, 6].

Numerous analytical methods for determining this vitamin has been reported, including spectrophotometry, HPLC, fluorescence, chemiluminescence and electrochemical methods, such as, potentiometry, polarography and voltammetry [7-14]. Among these methods, electrochemical methods have attracted more attention in last two decades for food and biological compounds analysis because of fast response, low detection limit, low cost, simple operation and the absence of pretreatment [15].

A screen-printed electrode (SPE) is simple, versatile, low cost, portable, easily operated, reliable, small sized, and capable of mass production. Therefore, it is applied widely in the electroanalytical measurements. In order to improve their electrochemical performance, SPEs have been modified with these nanostructures include, for example, carbon nanomaterials, Au nanoparticles, graphene, and nanostructures metal oxides. When an electrode is modified its sensitivity and selectivity is improved. Also, its detection limit compared to unmodified electrodes becomes lower and can be used as a promising device to facilitate the direct electron transfer [16-36].

Nanomaterials are used for various applications in electrochemistry [37-47]. Among metal oxides, ZnO nanostructures due to wide band gap (3.37 eV), large excitation binding energy (60 eV), non-toxicity, biocompatibility, chemical and photochemical stability, and high electron communication features is preferred for the fabrication of effective sensors. In addition, ZnO can act both as an electronic and structural promoter which exhibiting a major influence on the catalytic activity. Besides, alumina or other refractory oxides mainly increase the long-term stability as structural promoter of the catalyst system. Absence of any of these constituting components severely reduces the performance of the catalyst system. Therefore, the importance of using ZnO/Al₂O₃ nanocomposite as catalytic materials is very high [48-52].

According to the previous points, it is important to create suitable conditions for analysis of ascorbic acid in biological fluids. In this study, we describe application of novel ZnO/Al₂O₃ nanocomposite as a nanostructure sensor for voltammetric determination of ascorbic acid. The proposed sensor showed good electrocatalytic and accumulative effect on ascorbic acid. ZnO/Al₂O₃/SPE shows advantages in terms of selectivity, reproducibility, and sensitivity. Eventually, we evaluate the analytical performance of the suggestion sensor for ascorbic acid determination in real samples.

2. EXPERIMENTAL

2.1 Apparatus and reagents

The electrochemical measurements were done by an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. The screen-printed electrode (DropSens, DRP-110, Spain) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode.

All solutions were freshly prepared using double distilled water. Ascorbic acid and other reagents were of analytical grade and were provided from Merck chemical company (Darmstadt, Germany). Buffer solutions were made from orthophosphoric acid and its salts in the pH range of 2.0-9.0.

2.2 Synthesis of ZnO/Al₂O₃ nanocomposite

Aluminum hydroxide was prepared by dissolving of 3 g of Al(NO₃)₃·9H₂O in 100 ml of distilled water. The pH of solution was set to 8 by ammonia solution and it was kept at 60 °C for 18 h. The precipitate was washed by ethanol and acetone three times, respectively. The Al(OH)₃ was prepared by aging of precipitate at 75 °C for 24 h.

A solution of zinc nitrate (0.3 M) was prepared in 80 ml of distilled water. The pH of solution was set to 9.5 by ammonium solution (25%) and the 0.13 g of aluminum hydroxide was added to the solution and the solution was mixed for 2h at room temperature. The solution was aged at 90 °C for 4 h at 250 rpm stirring rate. The precipitate of ZnO-Al₂O₃ was washed by ethanol and distilled water, respectively.

2.3 Preparation of modified electrode

A bare screen-printed electrode was coated with ZnO/Al₂O₃ nanocomposite as follows. A stock solution of ZnO/Al₂O₃ in 1 mL aqueous solution was prepared by dispersing 1 mg ZnO/Al₂O₃ with ultrasonication for 1 h, and a 5 µl aliquot of the ZnO/Al₂O₃/H₂O suspension solution was casted on the carbon working electrodes, and waiting until the solvent was evaporated in room temperature.

3. RESULTS AND DISCUSSION

3.1 Electro-oxidation of ascorbic acid at a ZnO/Al₂O₃/SPE

Fig. 1 depict the cyclic voltammetric responses for the electrochemical oxidation of 100.0 µM ascorbic acid at ZnO/Al₂O₃/SPE (curve a) and bare SPE (curve b). The anodic peak potential for the oxidation of ascorbic acid at ZnO/Al₂O₃/SPE (curve a) is about 335 mV compared with 370 mV for

that on the bare SPE (curve b). Similarly, when the oxidation of ascorbic acid at the ZnO/Al₂O₃/SPE (curve a) and bare SPE (curve b) are compared, an extensive enhancement of the anodic peak current at ZnO/Al₂O₃/SPE relative to the value obtained at the bare SPE (curve b) is observed. In other words, the results indicate that the ZnO/Al₂O₃ nanocomposites improve the ascorbic acid oxidation signal.

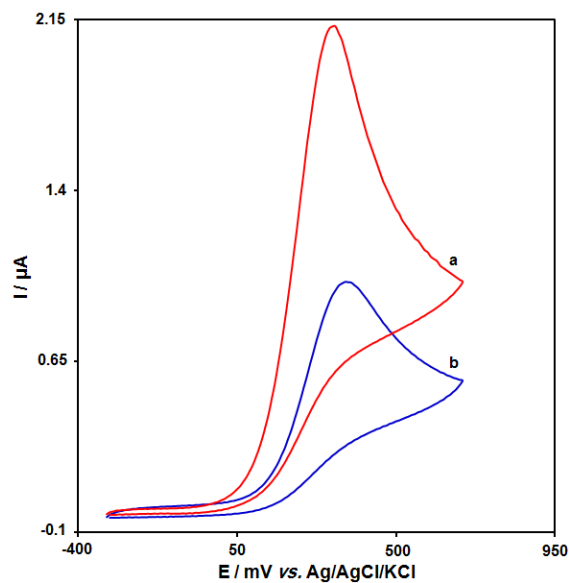


Figure 1. Cyclic voltammograms of (a) ZnO/Al₂O₃/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 100.0 μM ascorbic acid at the scan rate 50 mVs⁻¹.

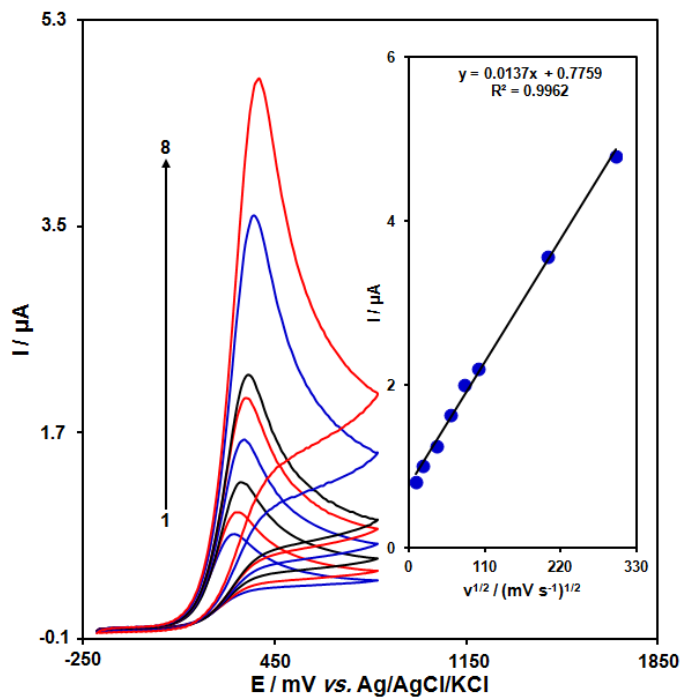


Figure 2. Cyclic voltammograms of ZnO/Al₂O₃/SPE in 0.1 M PBS (pH 7.0) containing 70.0 μM ascorbic acid at various scan rates; numbers 1-13 correspond to 10, 20, 40, 60, 80, 100, 200.0, 300.0, 400.0, 500.0, 600.0, 700.0 and 800 mV s⁻¹, respectively. Insets: (A) variation of anodic and cathodic peak current vs. $v^{1/2}$. (B) anodic peak potential vs. $\log v$

Potential scan rates on the oxidation current of ascorbic acid has been optimized (Fig. 2). According to the obtained results, increase of the scan rate caused an increase in the peak current. Also, from the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$) over a wide range from 10 to 300 mV s^{-1} , it can be concluded that the oxidation process is a type of diffusion controlled ones

Fig. 3 shows a Tafel plot that was drawn from points of the Tafel region of the LSV. Tafel slope of 0.1152 V obtained in this case which corresponds well with the involvement of one electron in the rate determining step of the electrode process, assuming a charge transfer coefficient of $\alpha=0.49$ [53].

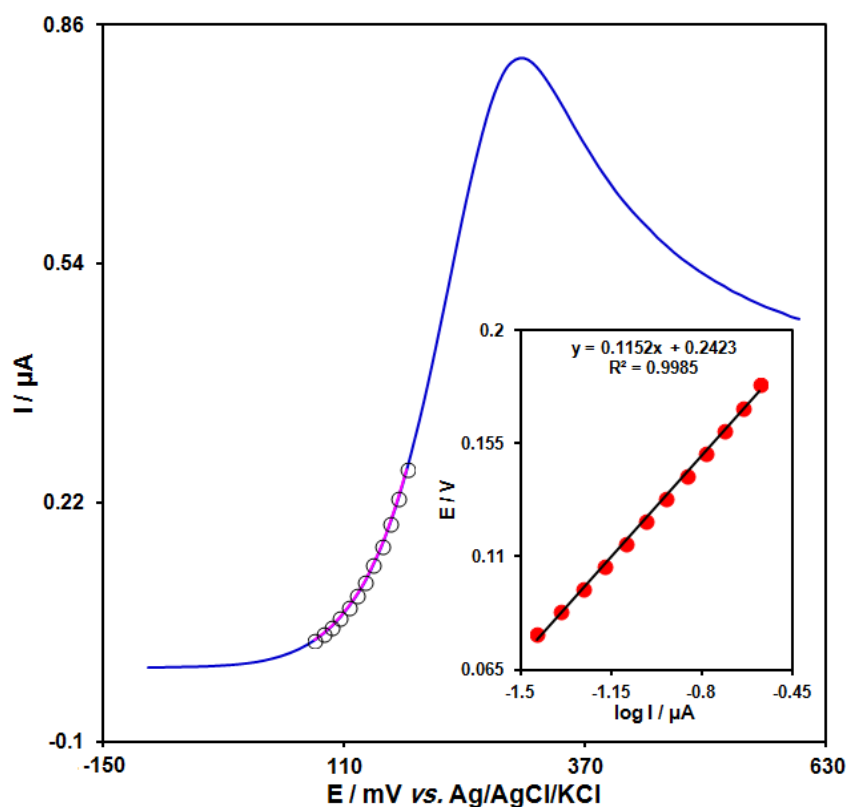


Figure 3. LSV (at 10 mV s^{-1}) of electrode in 0.1 M PBS (pH 7.0) containing $70.0 \mu\text{M}$ ascorbic acid. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV.

3.2 Chronoamperometric studies

Chronoamperometric measurements of ascorbic acid at $\text{ZnO/Al}_2\text{O}_3/\text{SPE}$ were done by set the working electrode potential at 0.35 V for the various concentrations of ascorbic acid in PBS (pH 7.0) (Fig.4). For an electroactive material (ascorbic acid in this case) with a diffusion coefficient of D , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [53].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

Where D and C_b are the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I vs. $t^{-1/2}$ were used, with the best fits for different concentrations of ascorbic acid (Fig. 4A). The slopes of the resulting straight lines were then plotted vs. ascorbic acid concentration (Fig. 4B). Based on the obtained slope and Cottrell equation the mean value of the D was found to be $5.35 \times 10^{-5} \text{ cm}^2/\text{s}$.

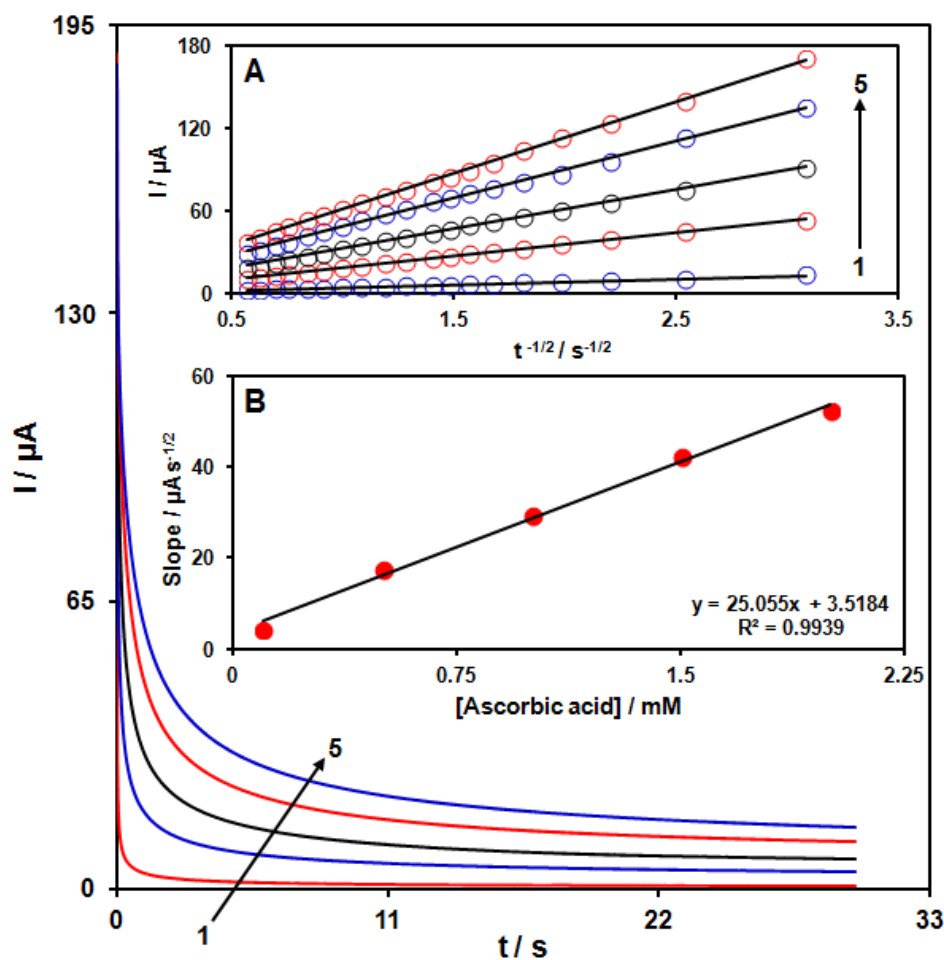


Figure 4. Chronoamperograms obtained at $\text{ZnO}/\text{Al}_2\text{O}_3/\text{SPE}$ in 0.1 M PBS (pH 7.0) for different concentration of ascorbic acid. The numbers 1–5 correspond to 0.1, 0.5, 1.0, 1.5 and 2.0 mM of ascorbic acid. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1–5. (B) Plot of the slope of the straight lines against ascorbic acid concentration.

3.3 Calibration plot and limit of detection

The peak current of ascorbic acid oxidation at the surface of the modified electrode can be used for the determination of ascorbic acid in the solution (Fig. 5). Hence, differential pulse voltammetry (DPV) experiments were carried out for different concentrations of ascorbic acid. The oxidation peak currents of ascorbic acid at the electrode surface were proportional to the concentration of the ascorbic

acid in the range of 1.0 to 100.0 μM . The detection limit (3σ) of ascorbic acid was found to be 0.6×10^{-7} M. These values are comparable with values reported by other research groups for electrocatalytic oxidation of ascorbic acid at the surface of chemically modified electrodes (see Table 1).

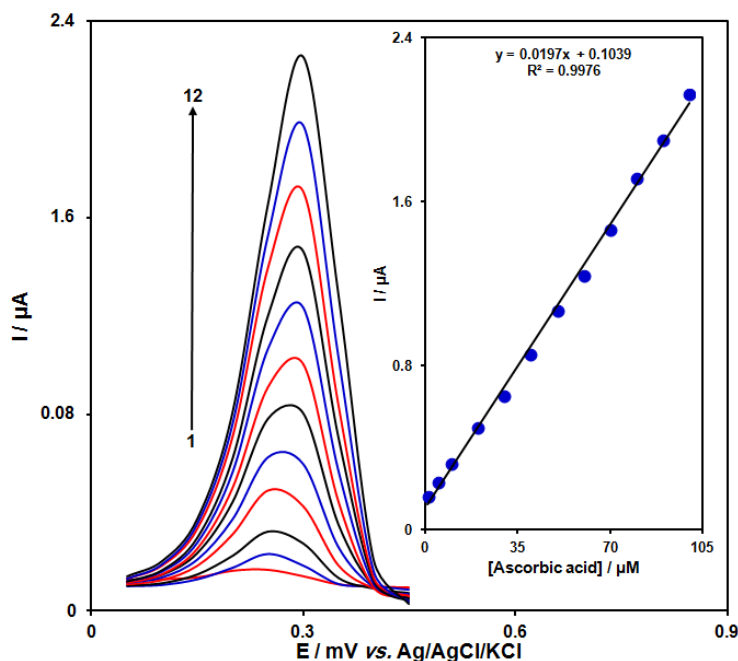


Figure 5. DPVs of ZnO/Al₂O₃/SPE in 0.1 M (pH 7.0) containing different concentrations of ascorbic acid. Numbers 1–12 correspond to 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0 and 100.0 μM of ascorbic acid. Insets: (A) a plots of the electrocatalytic peak current as a function of ascorbic acid concentration in the range of 1.0-100.0 μM .

Table 1. Comparison of the efficiency of some electrodes used in detection of ascorbic acid.

Electrode	Modifier	LOD	LDR	Ref.
Glassy carbon electrode	Cu ₂ O/CuO/reduced graphene oxide	0.31 μM	100.0-1000.0 μM	54
Pencil graphite electrode	O-phenylenediamine	0.26 μM	1.0-1000.0 μM	55
Glassy carbon electrode	Reduced graphene oxide-CO ₃ O ₄ nanocomposite	48.7 nM	1.0-51.0 μM	56
Gold interdigitated microelectrode	Graphene oxide and gold nanoparticles	0.62 μM	2.0-1050.0 μM	57
Glassy carbon electrode	Metal-organic frameworks	4.6 μM	14.1-5500.0 μM	58
Glassy carbon electrode	Silver nanoparticles	0.1 μM	0.4-450.0 μM	59
Glassy carbon electrode	Magnetite nanoparticle	0.042 μM	1.0-12.0 μM	60
Glassy carbon electrode	Sulfonazo III	0.17 nM	0.5-1300.0 μM	61
Graphite screen printed electrode	ZnO/Al ₂ O ₃ nanocomposite	0.06 μM	1.0-100.0 μM	This work

3.4 Real sample analysis

To study the analytical applicability of the proposed electrode, it was applied to the determination of ascorbic acid in Vitamin C ampoule, Vitamin C tablet and vitamin C effervescent tablets. The results for determination of ascorbic acid in real samples are given in Table 2. Acceptable recovery of the results was obtained. The reproducibility of the method was calculated by the mean relative standard deviation (R.S.D.).

Table 2. The application of ZnO/Al₂O₃/SPE for determination of ascorbic acid in vitamin C ampoule, vitamin C tablet and vitamin C effervescent tablets (n=5). All concentrations are in μM .

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Vitamin C ampoule	0	15.0	-	3.4
	2.5	17.6	100.6	1.9
	5.0	19.4	97.0	2.2
Vitamin C tablet	0	20.0	-	2.1
	2.0	21.8	99.1	3.1
	4.0	24.6	102.5	2.8
Vitamin C effervescent tablets	0	17.5	-	2.9
	3.0	21.2	103.4	1.8
	6.0	23.1	98.3	2.7

4. CONCLUSIONS

In summary, an ultrasensitive and selective ZnO/Al₂O₃/SPE nanosensor was developed for individual determination of ascorbic acid. Under the optimal operating conditions, the ZnO/Al₂O₃/SPE nanosensor showed high sensitivity and selectivity for determination of ascorbic acid with wide linear ranges (1.0-100.0 μM) and limit of detection as low as 0.6 μM . Moreover, its further application for the detections of ascorbic acid in real samples was also demonstrated with good accuracy.

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