

Full Paper

Electrochemical Analysis of ascorbic acid in Food and Drug Samples using a Biosynthesized Ag Nanoparticle as a Mediator in Carbon Paste Matrix

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Received: 29 December 2016 / Accepted: 18 April 2017 / Published online: 15 May 2017

Abstract- In this research we describe application of *Ficus carica sub sp. rupestris* for biosynthesis of Ag nanoparticle in the first step. The synthesized nanoparticle was characterized by TEM and UV-Vis spectroscopy methods. In the continuous a carbon paste electrode modified with Ag nanoparticle (CPE/M/Ag/NPs) was fabricated as a highly sensitive sensor for ascorbic acid analysis. The CPE/M/Ag/NPs showed good catalytic activity for electrochemical analysis of ascorbic acid using voltammetric methods. The anodic peak current was linearly proportional to the concentration of ascorbic acid in the range from 0.09 to 600.0 μM with detection limit of 0.02 μM . The CPE/M/Ag/NPs was successfully applied to ascorbic acid determination in food and pharmaceuticals samples.

Keywords- *Ficus carica sub sp. rupestris*, Ascorbic acid, Modified electrode, Food and drug analysis

1. INTRODUCTION

Ascorbic acid or vitamin C is an important soluble vitamin that has important role in human body. On the other hand, it is a vital antioxidant and important reducing agent for human body. There are some minor side effects such as indigestion, particularly when taken

on an empty stomach. But it is needed to maintain the health of skin, cartilage, teeth, bone, and blood vessels. Therefore, there are many analytical methods for analysis of ascorbic acid such as HPLC [1], spectroscopy [2], chemiluminescence [3] and electrochemical sensors [4-9]. Using electrochemical sensors have more attention compare to other analytical methods due to high sensitivity and fast response [10-20].

Nano-materials with high surface area and good electrical conductivity are best choice for modification of electrochemical sensors for trace level analysis. In between, metal based nanoparticles with highly conductivity suggested for modification of many electrochemical sensors.

There are some different methods for synthesis of nanomaterials such as sol gel, hydrothermal synthesis, biosynthesis, polyol method and etc. In between, biosynthesis method has better than compare to other method due to green ability for synthesis. On the other hand, using biosynthesis method we can synthesis nano-materials with good distribution particle size [21]. There are many published papers for application of plant for biosynthesis of metal based nanoparticles and especially Ag nanoparticles [22-24].

Therefore, in this work we describe a biosynthesis method for synthesis of Ag nanoparticle as a modifier. The synthesized nanoparticle was used for modification of carbon paste electrode as a sensor in ascorbic acid determination. The CPE/M/Ag/NPs showed good ability for analysis of ascorbic acid in food and drug samples.

2. EXPERIMENTAL

2.1. Chemicals and Instrumentation

Silver nitrate, graphite powders, ascorbic acid, phosphoric acid and NaOH and other were purchased from Sigma-Aldrich. Voltammetric investigation was performed in an electro-analytical system, μ -Autolab PGSTAT 12, potentiostat/galvanostat connected to a three-electrode cell, Metrohm Model 663 VA stand linked with a computer (Pentium IV) and with Autolab software. The system was run on a PC using NOVA software.

2.2. Synthesis of Ag/NPs

A certain volume of the *Ficus carica sub sp. rupestris* extract (5 mL) was added to a 5 mL silver nitrate solution (1.0 mM) and the volume was adjusted to 40 mL with deionized water. The flask was then incubated at room temperature. The measured pH was 12.0. The suspension centrifuge and solid Ag/NPs was used for fabrication of modified electrode.

2.3. Preparation of CPE/M/Ag/NPs

The CPE/M/Ag/NPs was prepared by mixing of 0.1 g of Ag/NPs and 0.9 g of graphite powder in the presence suitable amount of paraffin oil. The obtained component was then

packed into a glass tube. Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture.

2.4. Preparation of real samples

The real samples were prepared according to our previous published paper [4]. Also, injection sample was used without any pretreatment.

3. RESULTS AND DISCUSSION

3.1. Biosynthesis of Ag/NPs

The UV-Vis spectra were suggested as a powerful technique for confirming synthesis of Ag/NPs. The UV-Vis spectrum of biosynthesis in different time is presence in Figure 1. As can be seen, in first time of synthesis (~1 h) we cannot detect any signal for formation of Ag/NPs (curve a). After moving 5 h (curve b) to 12 h (curve c) and 24 h (curve d) absorbance band increase and stable on 24 h. So, we selected this time as an optimum condition for this work.

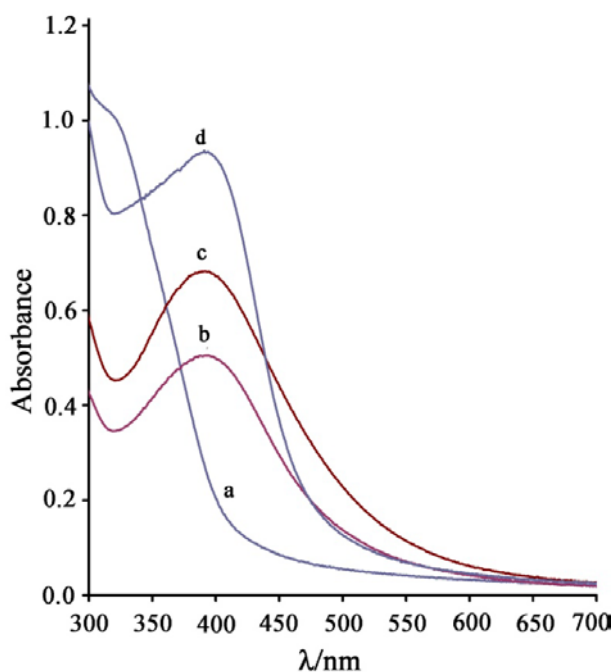


Fig. 1. Absorption spectrums of silver nanoparticles in biosynthesis process after a) 1h; b) 5h; c) 12 h and d) 24 h

In continuous, we optimized the concentration of silver nitrate in biosynthesis procedure. As can be seen the best condition was obtained in the presence of 1.0 mM silver nitrate (Fig. 2).

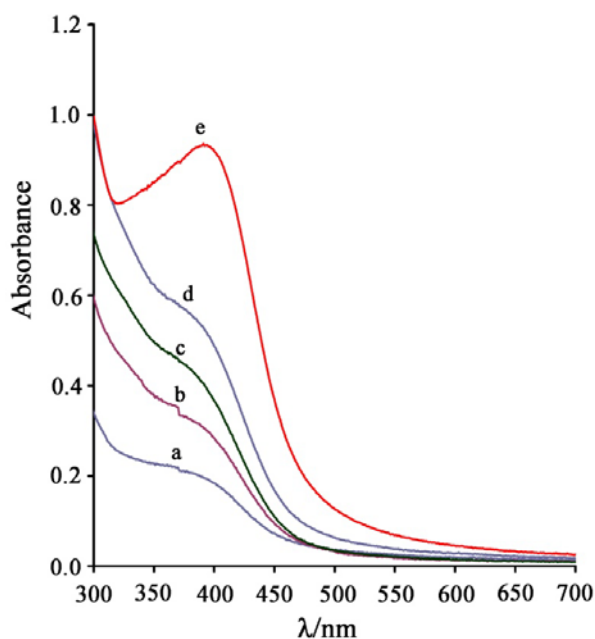


Fig. 2. Absorption spectrums of silver nanoparticles in biosynthesis process in the presence of a) 0.1 mM; b) 0.3 mM; c) 0.4 mM, d) 0.5 mM, e) 1.0 mM silver nitrate

TEM image of Ag/NPs was showed in figure 3 that confirms synthesis of Ag nanoparticle with diameter 10-20 nm.



Fig. 3. TEM image of silver nanoparticle

3.2. Electrochemical investigation

In the first step in electrochemical investigation we optimized the pH value for electrochemical determination of ascorbic acid. For this goal, we recorded square wave voltammograms of 70.0 μM ascorbic acid at a surface of CPE/M/Ag/NPs. As can be seen the best oxidation signal was obtained in pH=7.0 (Fig. 4).

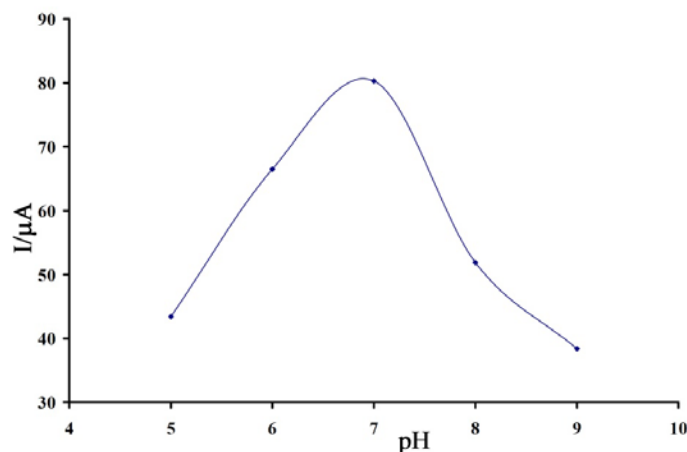


Fig. 4. Plot of oxidation current vs. pH for 70.0 μM ascorbic acid at a surface of CPE/M/Ag/NPs

Also, the relation between oxidation peak potential and pH showed in figure 5 that confirm two electrons and two protons for electro-oxidation of ascorbic acid at a surface of CPE/M/Ag/NPs. The obtained slope is very similar to Nernstian value for a two electron and two-proton.

On the other hand, square wave voltammogram of 70.0 μM ascorbic acid was recorded at a surface of CPE/M/Ag/NPs (Fig. 6; curve a) and bare carbon paste electrode (Fig. 6; curve b). As can be seen, the presence of Ag/NPs can be increased sensitivity of sensor for analysis of ascorbic acid and oxidation potential shifted to negative value.

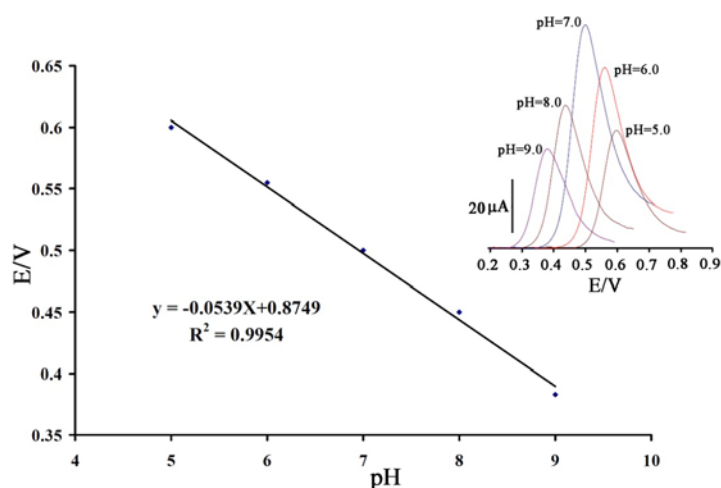


Fig. 5. Plot of potential, E, vs. pH for the electro-oxidation of 70.0 μM ascorbic acid at a surface of CPE/M/Ag/NPs. Inset: influence of pH on square wave voltammograms of ascorbic acid at a surface of the CPE/M/Ag/NPs

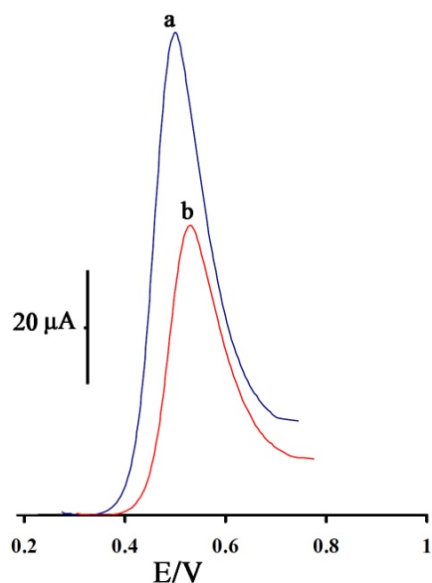


Fig. 6. Square wave voltammograms of 70.0 μM ascorbic acid at a surface of a) CPE/M/Ag/NPs and b) CPE

The Tafel plot was used for determination of α for electro-oxidation of ascorbic acid at an CPE/M/Ag/NPs (Fig. 7). Using the slope of this figure and Tafel equation, we obtained the value of α , which is equal to 0.66.

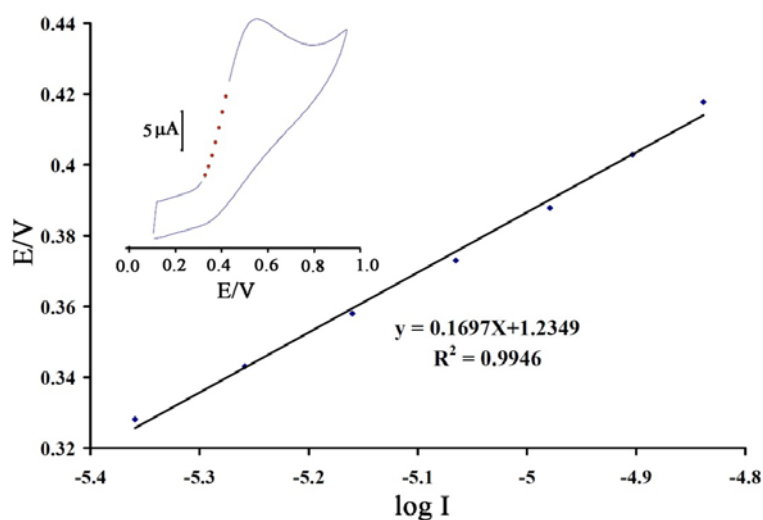


Fig. 7. Tafel Plot obtained from linear sweep voltammograms of 500 μM ascorbic acid at a surface of CPE/M/Ag/NPs

The effect of concentration of ascorbic acid on CPE/M/Ag/NPs has been studied in 0.1 M PBS (pH 7.0) as can be seen in figure 8. Where anodic peak current increased with increasing

in concentration range 0.09 to 600.0 μM with limit of detection 0.02 μM for ascorbic acid analysis.

One of the most important advantages of CPE/M/Ag/NPs is having good selectivity. To test the selectivity of CPE/M/Ag/NPs upon the determination of ascorbic acid using square wave voltammetry, some interferents were investigated by the addition of various interference species to 0.1 M PBS in the presence of 50.0 μM of ascorbic acid. The results indicate in table 1. As can be seen, there are not any important interference for ascorbic acid analysis at a surface of CPE/M/Ag/NPs.

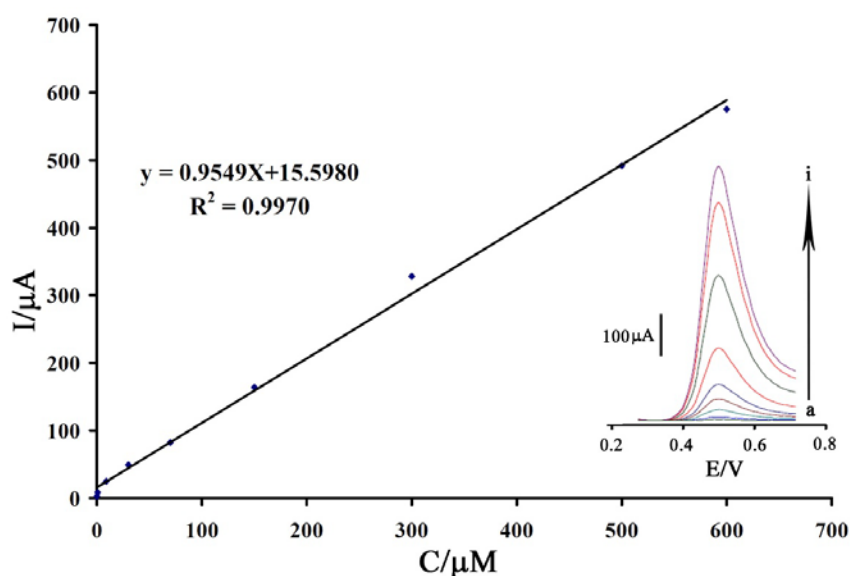


Fig. 8. The plots of the I_{pa} as a function of ascorbic acid concentration. Inset shows the SWVs of CPE/M/Ag/NPs (pH 7.0) containing different concentrations of ascorbic acid

Table 1. Interference of some foreign substances for 50.0 μM of ascorbic acid

Species	Tolerante limits ($W_{\text{Substance}}/W_{\text{Analytes}}$)
Glucose, Li^+ , F^- , K^+ , Na^+ , Br^- , Alanine, Tryptophan, methionine	1000
Starch	Saturation

Finally the ability of CPE/M/Ag/NPs was check for analysis of ascorbic acid in injection and food samples. The obtained data are presence in table 2 that confirm ability of proposed sensor for ascorbic acid analysis.

Table 2. Determination of ascorbic acid in practical samples (n=3)

Sample	Ascorbic acid	Found (Ascorbic acid)	Found (Ascorbic acid)
	Added (μM)	Proposed method (μM)	HPLC method (μM)
Injection	5.00	4.95 \pm 0.78	5.44 \pm 0.75
Energy drink	-	< LOD	< LOD
Pimento Juices	-	55.77 \pm 0.87	56.01 \pm 1.2
Orange Juices	-	185.50 \pm 2.33	187.33 \pm 5.44

4. CONCLUSION

In this paper, a carbon paste electrode modified with Ag/NPs has been successfully developed to investigate ascorbic acid at pH 7.0. The anodic peak current was linearly proportional to the concentration of ascorbic acid in the range from 0.09 to 600.0 μM with detection limit of 0.02 μM . The CPE/M/Ag/NPs was successfully applied to ascorbic acid determination in food and pharmaceuticals samples.

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