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Highly sensitive and efficient voltammetric determination of ascorbic acid in food and pharmaceutical samples from aqueous solutions based on nanostructure carbon paste electrode as a sensor

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ABSTRACT

A square wave voltammetric method for the trace analysis of ascorbic acid was developed in this study. Carbon paste electrode was modified with NiO nanoparticle and 1-butyl-3-methylimidazolium tetrafluoroborate as a binder. Electro-oxidation behavior of ascorbic acid on the modified electrode was studied, which indicated that the nanostructure modified electrode could efficiently promote electrocatalytic oxidation of ascorbic acid. A fast, selective, high sensitive and simple electrochemical strategy was then developed for trace analysis of ascorbic acid using the constructed electrode. The catalytic oxidation signal exhibited a wide linear range from 0.08 to 380.0 μM toward the concentration of ascorbic acid with a sensitivity of 0.0158 μA/μM, and the limit of detection was as low as 0.04 μM. The suggested sensor was also used for quantitative determination of ascorbic acid in food and pharmaceutical samples.

1. Introduction

Ascorbic acid is a naturally organic compound and as a water-soluble vitamin has been widely applied in large quantities to food products, drinks, animal feed, pharmaceutical formulations and cosmetics due to its valuable properties such as pH regulating and antioxidant features [1,2].

Ascorbic acid participates as a key component in biological metabolism such as reducing agent in various metabolic pathways, synthesis and maintenance of collagen, blood vessels, cartilage, bones and tendons and reacts with reactive oxygen species or free radicals and probably reducing cholesterol level [3,4]. Furthermore, ascorbic acid as a vital nutrient is commonly used in therapeutical fields such as improving immunity, preventing and healing of catarrh, infertility, skin disorders, amelioration of injuries and burns, cancer, aids and clinical diagnostic applications [5,6]. Besides, in food processing industries ascorbic acid is used as an antioxidant to prevent changes in color, taste and odor of products [7].

Therefore, due to biological importance of ascorbic acid, its accurate determination in pharmaceutical, clinical and food industries samples is greatly demanded. Until now numerous techniques have been reported for quantitative determination of ascorbic acid including high performance liquid chromatography [8,9], spectrophotometry methods [10,11], fluorometry methods [12,13], solid phase analysis [14,15] and chemiluminescence methods [16]. However, all mentioned methods have major disadvantages including high cost, laborious in sample preparation and time consuming, requiring large infrastructure back up and expert knowledge, background interference for fluorometry and destroying of sample for chromatography. Recently electrochemical techniques provide accurate and rapid tools with high sensitivity for routine and reliable determination of ascorbic acid in various matrices [17–20].

Among all chemically modified electrodes, carbon paste electrodes (CPEs) received high attention due to its ease of application and regeneration, cheapness, stable response and very low ohmic resistance. Modified CPEs overcome on large over potential required for oxidation of electroactive compounds by modification of electrode surface using nanostructure materials and high conductive binder to increase the conductivity of the electrode [21]. Nanostructure materials received considerable attention due to their distinctive and unique behavior with outstanding electrical, chemical, mechanical and structural properties that make them a very attractive material for large range of applications in pharmaceutical, biological and industrial procedure [22–25]. Metal nanoparticles have been used commonly in electrochemical techniques owing to its high catalytic activity in chemical reactions and high surface area for increasing current density [26]. Since metal

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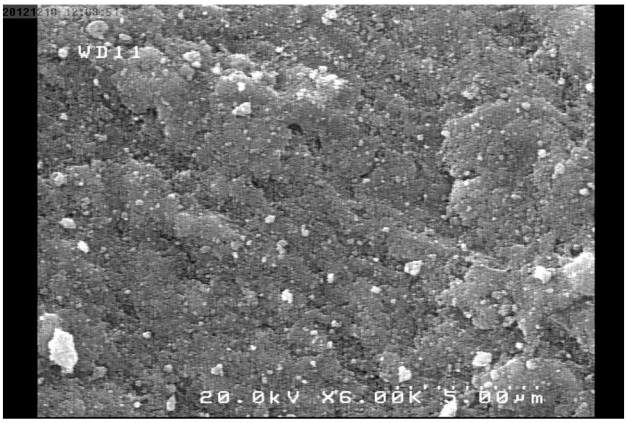


Fig. 1. SEM image of synthesized NiO nanoparticles.

nanoparticles revealed biocompatible properties, they could be applied in fabrication of electrochemical sensors to determine electroactive compound in pharmaceutical and food samples. Furthermore, using ionic liquids as a high conductive binder with specific characteristics such as good chemical and thermal stability, wide electrochemical windows and high ionic conductivity greatly benefited the fabricated electrode [27–29].

In the current work a great attempted have been done to fabricate a novel modified carbon ionic liquid paste electrode using NiO nanoparticles and 1-butyl-3-methylimidazolium tetrafluoroborate (BMITFB) as a binder. The electrochemical behavior of ascorbic acid was investigated and the obtained results demonstrate the advantage of BMITFB/NiO/NPs/CPE in compare to the bare carbon paste electrode in terms of higher sensitivity and reliability. The well fabricated BMITFB/NiO/NPs/CPE revealed an extraordinarily low background current, an extensive operating potential window, convenient modification, reproducibility, renewability and low cost. The analytical performance of the fabricated electrochemical sensor was evaluated by determination of ascorbic acid in liposome dope with ascorbic acid and food samples.

2. Materials and methods

2.1. Chemical and reagents

Ascorbic acid, NaOH, mineral oil, methanol and graphite powder were obtained from Merck. 1-butyl-3-methylimidazolium tetrafluoroborate was purchased from Sigma-Aldrich. Phosphate buffer solution (PBS) with various pH was prepared by mixing the stock solution of 0.1 M H_3PO_4. The doubly distilled water was used in all solution preparations. All of the other chemicals were purchased in analytical grade from Merck.

2.2. Apparatus

Voltammetric measurements were performed on a Sama-500 electrochemical workstation (Isfahan, Iran). A three-electrode system was employed with a modified or unmodified carbon paste electrode as working electrode, a Pt wire as counter electrode, and a $Ag/AgCl/KCl_{sat}$ electrode as reference electrode.

Microstructure and surface morphology of nanoparticles were identified by a scanning electron microscope (SEM, Philips).

2.3. Preparation of BMITB/NiO/NPs/CPE

BMITB/NiO/NPs/CPE was prepared by mixing 0.2 g of BMITB, 0.8 g of paraffin, 0.1 g of nanoparticles and 0.9 g of graphite powder. Then the mixture was mixed well for 1 h until a uniformly wetted paste was obtained. A portion of the paste was filled firmly into a glass tube as described above to prepare BMITB/NiO/NPs/CPE. When necessary, a new surface of BMITB/NiO/NPs/CPE was obtained by pushing an excess of the paste out of the tube and polishing it on a white paper.

2.4. Real sample preparation

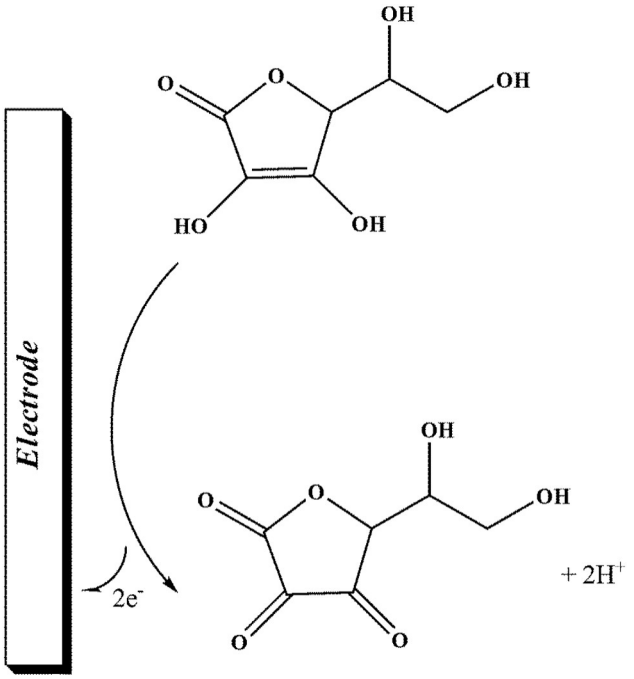
Vegetable and fruit juices were obtained using a mechanical squeezer. The juices obtained were filtered into a beaker and acidified (pH = 2) using citric acid. A 2.0-mL portion of the filtrate was added to the phosphate buffer solution pH = 7.0 in voltammetric cell. Standard addition method was used for determination of ascorbic acid in the juice samples.

Ten tablets of ascorbic acid powdered in mortar and then dissolved in 100 mL water with ultrasonication. Then, 1.0 mL of the solution plus 9.0 mL of the buffer (pH 7.0) was used for the analysis with standard addition method.

Liposomes containing ascorbic acid were prepared using film hydration method. Lecithine and cholesterol were dissolved in chloroform solvent with three different compositions of 70:30, 60:40 and 50:50, respectively. Rotary evaporator was used to evaporate the solvent, until an oily concentrated mixture was obtained and then known amount of ascorbic acid which is dissolved in di-water, was added to the mixture and for 30 min rotary evaporating process was continued until milky color suspensions containing liposomes were formed. To prevent the oxidation of Lecithine the procedure was carried out under inert atmosphere. To determine the amount of ascorbic acid loading in all formulations, the non-entrapped compounds were separated from encapsulated ones by centrifuge. The supernatant phase which contains the non-trapped ascorbic acid was separated. Standard addition method was used to determine the percentage of non-trapped ascorbic acid using proposed sensor.

2.5. Synthesis of NiO nanoparticles

To prepare the nanoparticles (NiO), 0.25 M aqueous solution of $NiO(NO_3)_2 \cdot 6H_2O$ and a 0. 5 M aqueous solution of NaOH was prepared in distilled water. The $NiO(NO_3)_2 \cdot 6H_2O$ solutions were added drop wise (slowly for 3.0 h) to the above solution under high-speed stirring. The beaker was sealed at this condition for 3 h. The precipitated $Ni(OH)_2$



Scheme 1. Eelectrochemical mechanism for electro-oxidation of ascorbic acid.

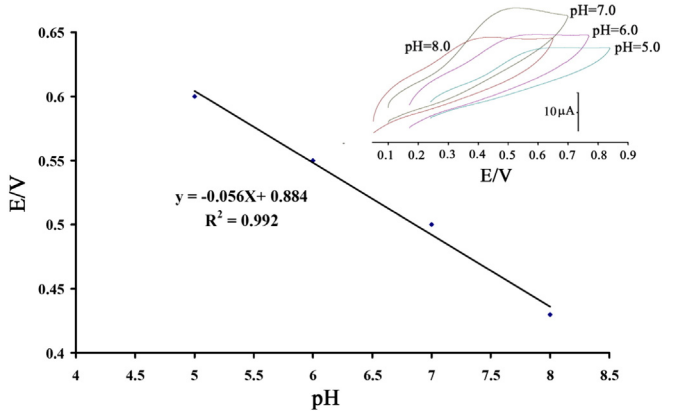


Fig. 2. Plot of potential, E, vs. pH for the electro-oxidation of 500 μM ascorbic acid at a surface of BMITFB/NiO/NPs/CPE. Inset: influence of pH on cyclic voltammograms of ascorbic acid at a surface of the modified electrode (pH 5–8, respectively).

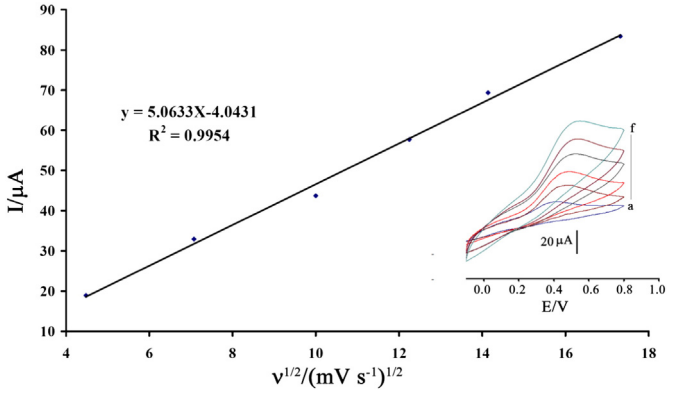


Fig. 4. Plot of I_{pa} versus $\nu^{1/2}$ for the oxidation of ascorbic acid BMITFB/NiO/NPs/CPE. Inset shows cyclic voltammograms of ascorbic acid at BMITFB/NiO/NPs/CPE at different scan rates of (a) 20, (b) 50, (c) 100, (d) 150, (e) 200 and (f) 300 mV s^{-1} in 0.1 M phosphate buffer, pH 7.0.

was cleaned with deionized water and ethanol then calcined at 400 °C for 1.5 h for synthesis of NiO/NPs.

3. Results and discussion

3.1. Morphological investigation by SEM

Morphology of NiO nanoparticles was characterized by SEM (Fig. 1). The dark spots correspond to NiO/NPs, which were only synthesized in our synthesis condition. Nanoparticles with nearspherical shapes were synthesized.

3.2. Electrochemical investigation

Scheme 1 shows electro-oxidation mechanism for ascorbic acid in aqueous buffer solution. According to Scheme 1, we found that electro-oxidation of ascorbic acid is relative to pH value in buffer solution. So, this parameter optimized in the first step of ascorbic acid analysis. The influence of the buffer solution pH on electrochemical oxidation of ascorbic acid for the BMITB/NiO/NPs/CPE was investigated at various pH values ranging from 5.0 to 8.0 (Fig. 2 insert). As can be seen the oxidation peak potentials shifted toward negative potential with the increase in pH values that confirm proposed mechanism in Scheme 1. On the other hand, the maximum catalytic peak current was obtained at pH 7.0 for ascorbic acid analysis. According to obtained data in this investigation, we selected pH = 7.0 as the optimum condition for the ascorbic acid determination.

Cyclic voltammetry was used for investigation of electrochemical oxidation signal of ascorbic acid on the different modified electrode (Fig. 3). BMITB/NiO/NPs/CPE exhibited large oxidation current with the oxidation peak current of 163.5 μA (curve a). On the other hand, weak electrochemical oxidation activity peak was observed at CPE (curve d) over the same condition. The ascorbic acid oxidation peak current at NiO/NPs/CPE and at CPE was observed around 960 and 1000 mV with the oxidation current of 58.5 and 21.5 μA, respectively. Also, at the surface of BMITB/CPE, the oxidation peak current was 101.5 μA (curve b), which indicated that the presence of BMITB in CPE could enhance ascorbic acid oxidation peak currents. The results indicated that the presence of synthesized NiO nanoparticles and BMITB on BMITB/NiO/NPs/CPE surface had great improvement with the electrochemical oxidation response of ascorbic acid.

Investigation of scan rate can be useful for study of electrochemical mechanisms and kinetic characteristics of ascorbic acid. Fig. 4 insets showed the cyclic voltammograms of ascorbic acid on BMITB/NiO/NPs/CPE between −0.1 and 0.8 V at various scan rates. As can be seen in Fig. 4, the oxidation peak currents (Ip) increased linearly vs. $\nu^{1/2}$ from 20 to 300 mV s^{-1} indicating that the oxidation of ascorbic acid on BMITB/NiO/NPs/CPE was a diffusion controlled process.

To obtain further information on the rate determining step, a Tafel plot was developed for the ascorbic acid at a surface of BMITB/NiO/NPs/CPE using the data derived from the raising part of the current–

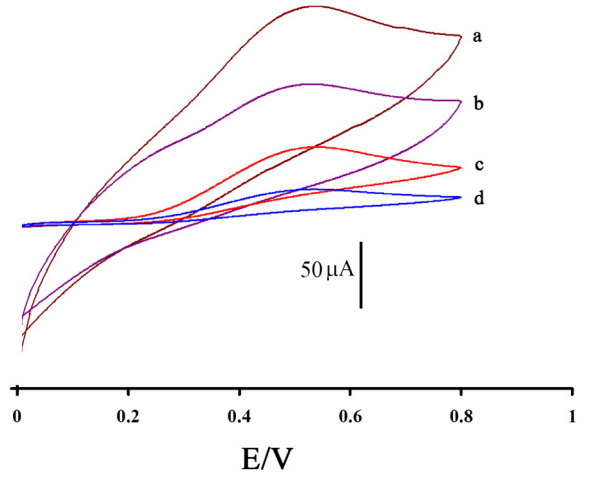


Fig. 3. Cyclic voltammograms of (a) BMITFB/NiO/NPs/CPE, (b) BMITFB/CPE, (c) NiO/NPs/CPE and (d) CPE in the presence of 500 μM ascorbic acid pH 7.0, respectively.

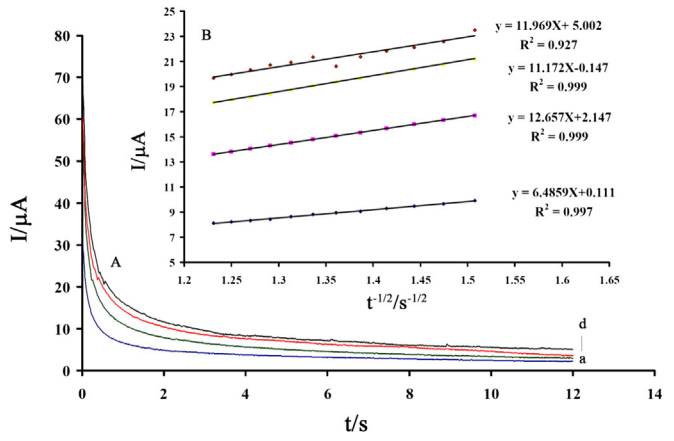


Fig. 5. (A) Chronoamperograms obtained at BMITFB/NiO/NPs/CPE in the presence of (a) 100; (b) 200; (c) 300 and (d) 400 μM ascorbic acid in the buffer solution (pH 7.0). (B) Cottrell's plot for the data from the chronoamperograms.

Table 1
Determination of ascorbic acid in real samples (n = 3).

Sample	Ascorbic acid added (μM)	Found (ascorbic acid) proposed method (μM)	Found (ascorbic acid) published method (μM) [30]	F_{ex}	F_{tab}	t_{ex}	$t_{tab(95\%)}$
Tablet	10.0	9.86 ± 0.56	10.45 ± 0.74	8.9	19.0	2.2	3.8
	20.0	20.68 ± 0.75	20.83 ± 0.95	–	–	–	–
Fruit Juices							
Orange	–	201.65 ± 1.51	200.98 ± 1.65	11.8	19.0	3.1	3.8
Lemon	–	187.55 ± 1.48	188.01 ± 1.42	10.7	19.0	3.0	3.8
Apple	–	20.65 ± 0.85	19.99 ± 0.79	9.1	19.0	2.4	3.8

±Shows the standard deviation.
F_{ex} is calculated F-value; F_{tab} is the F-value obtained from one-tailed table of F-test; t_{ex} is calculated value of t-student test; t_{tab} is the t-value obtained from the table of student t-test.

voltage curve (not shown). The slope of the Tafel plot is equal to $n(1-\alpha)F/2.3RT$ which comes up to 0.3006 V decade^{-1}. We obtained α as 0.9.

Chronoamperometric measurements of ascorbic acid at BMITB/NiO/NPs/CPE were carried out by setting the working electrode potential at 750.0 mV for the various concentrations of ascorbic acid in PBS (Fig. 5A). Using the obtained slopes and Cottrell equation (Fig. 5B) the mean value of the D was found to be 8.56×10^{-5} cm^2 s^{-1}.

3.3. Calibration plot and limit of detection

Since square wave voltammetry (SWV) was used for determination of ascorbic acid in this work. The SW voltammograms clearly show that the plot of oxidation peak current versus ascorbic acid concentration is linear for 0.08–380 μM of ascorbic acid. The detection limit was determined at 0.04 μM ascorbic acid according to the definition of $Y_{LOD} = Y_B + 3\sigma$.

3.4. Effect of interference

Interference of various inorganic and organic compounds in the determination of ascorbic acid was investigated. 150 fold concentration of glucose, fructose, lactose, sucrose, urea, thiourea, phenol, 100 fold concentration of Na^+, SO_4^{2-}, Cl^-, Mg^{2+}, Ca^{2+}, Pb^{2+}, Zn^{2+}, NO_3^-, and Ni^{+2} and 75 fold concentration of histidine, methionine, cysteine, lysine, phenyl alanine, glycine had almost no influence at the peak current of 50 μM ascorbic acid with a change of less than 5%, which suggested good selectivity of the method.

3.5. Stability and reproducibility

The repeatability and stability of BMITB/NiO/NPs/CPE were investigated by SWV measurements of 50.0 μM ascorbic acid. The relative standard deviation (RSD%) for fifteen successive assays was 2.1%. When using four different electrodes, the RSD% for fifteen measurements was 1.9%. When the electrode stored in the laboratory, the modified electrode retains 97% of its initial response after 10 days and 92% after 65 days. These results indicate that BMITB/NiO/NPs/CPE has good stability and reproducibility, and could be used for ascorbic acid.

3.6. Real sample analysis

To evaluate the applicability of the BMITB/NiO/NPs/CPE for the food and tablet sample applications, the BMITB/NiO/NPs/CPE was employed for the analysis of ascorbic acid in food juice and tablet samples. Standard addition method was used for measuring ascorbic acid concentration in the real samples. The results are given in Table 1, confirm that the modified electrode retained its efficiency for the determination of ascorbic acid in real samples.

Moreover, the encapsulation efficiency of formed liposome containing ascorbic acid was calculated by determination of the non-entrapped ascorbic acid which is remaining in supernatant phase by proposed fabricated sensor. Using standard addition method revealed 83.5% efficiency of encapsulation in composition of 70:30 (Lecithine:cholesterol) which is the best compositions in compare to the others.

4. Conclusion

The novel voltammetric sensor was developed for the fast and rapid determination of ascorbic acid. The BMITB/NiO/NPs/CPE showed great improvement to the electrode process of ascorbic acid compare to the carbon paste electrode. Under the optimum conditions, the oxidation peak current was proportional to the ascorbic acid concentration in the range of 0.08–380 μM with the detection limit of 0.04 μM. Finally, the BMITB/NiO/NPs/CPE was successfully used for the determination of ascorbic acid in real samples.

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