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Square wave voltammetric determination of ascorbic acid in food and pharmaceutical samples using a novel room temperature ionic liquid ZnO nanoparticles carbon paste electrode

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Abstract

In this paper we report synthesis and application of ZnO nanoparticle (ZnO/NPs) and 1, 3-dipropylimidazolium bromide as high sensitive sensors for voltammetric determination of ascorbic acid (AA) using carbon paste electrode. The ZnO/NPs was characterized with different methods such as TEM and XRD. The cyclic voltammogram showed an irreversible oxidation peak at 0.35 V (vs. Ag/AgCl_{sat}), which corresponded to the oxidation of AA. Compared to common carbon paste electrode, the electrochemical response was greatly improved. The electro-oxidation of AA occurred in a pH-dependent $2e^-$ and $2H^+$ process, and the electrode reaction followed a diffusion-controlled pathway. Under the optimum conditions, the voltammetric oxidation peak current of AA showed two linear dynamic ranges with a detection limit of 0.04 μ M for AA. The novel sensor has been found selective and successfully implemented for the determination of AA in fresh vegetable juice; fruit juices, tablet and tablet samples without previous preparation and were compared with a published electrochemical method.

Keywords: Ascorbic acid, 1, 3-dipropylimidazolium bromide, ZnO nanoparticle; Carbon paste electrode

1. Introduction

Compared to conventional electrodes, chemically modified electrodes (CMEs) offer unique well recognized advantages, especially in situations where the target analyte requires high overpotential, i.e. electrocatalysis, and also electroanalysis [1-4]. This characteristic of chemically modified electrodes arises from the advantageous combination of conventional electrochemical techniques with the chemical, structural and other specific properties of the modifying layer(s) [5-8].

A new room temperature ionic liquid has been used as a new kind of modifier for a chemically modified electrode [9-12]. This room temperature ionic liquid is composed entirely of ions and exists as a liquid at room temperature with the characteristics of negligible vapour pressure and good solubility and chemical stability. As a new green media, the room temperature

ionic liquid has many unique optical and electrochemical properties, such as higher ionic conductivity and wider electrochemical windows [13-18].

Ascorbic acid (AA), also known as vitamin C, is found in numerous natural sources. Its determination has received great attention in analytical chemistry due to the wide use in soft drinks and drugs [19]. It is also important clinically to determine its concentration in blood, urine [20] and tissues. Its quantification in foods and beverages has received increasing importance [21-23]. For the determination of ascorbic acid, several analytical methods have been proposed such as chromatography [24, 25], spectrophotometry [26, 27], mass spectrometry [28], flow injection [29, 30], chemiluminescence and electrochemical methods [31, 32] have been proposed for its determination in different matrices and at different levels.

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In this study, we describe the synthesis and application of a novel ZnO/NPs modified carbon ionic liquid paste electrode, which utilizes 1, 3-dipropylimidazolium bromide as a binder. The electrochemical behavior of AA at ZnO/NPs modified carbon ionic liquid electrode (ZnO/NPs/IL/CPE), at carbon paste electrode modified with ionic liquid (CP/ILE), at ZnO/NPs paste electrode (ZnO/NPs/CPE), and at carbon paste electrode (CPE) was investigated. The results showed the superiority of ZnO/NPs/IL/CPE to the other electrodes in terms of better reversibility and higher sensitivity. The proposed method is selective and sensitive enough for the determination of AA in real samples such as fruit juices, vegetables and tablet samples with good reproducibility.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade purchased from Merck (Darmstadt, Germany) unless otherwise stated. Doubly distilled water was used throughout. Ascorbic acid was used from Merck.

A 1.0×10^{-3} mol L⁻¹ ascorbic acid solution was prepared daily by dissolving 0.0176 g ascorbic acid (Merck) in water and the solution was diluted to 100 mL with water in a 100-mL volumetric flask. The solution was kept in a refrigerator at 4 °C in dark.

Phosphate buffer (sodium dihydrogen phosphate and disodium monohydrogen phosphate plus sodium hydroxide, 0.1 mol L⁻¹) solutions (PBS) with different pH values were used.

High viscosity paraffin ($d = 0.88$ kg L⁻¹) from Merck was used as the pasting liquid for the preparation of the carbon paste electrodes. Chemicals and solutions

2.2. Apparatus

Cyclic voltammetry, chronoamperometry, and differential pulse voltammetry were performed in an analytical system, μ -Autolab with (μ 3AUT 71226) PGSTAT (Eco Chemie, the Netherlands). The system was run on a PC using NOVA software. A conventional three-electrode cell assembly consisting of a platinum wire as an auxiliary electrode and an Ag/AgCl/KCl sat electrode as a reference electrode was used. The working electrode was a CPE, ZnO/NPs/CPE, CP/ILE or a ZnO/NPs/IL/CPE. X-ray powder diffraction studies were carried out using a STOE diffractometer with Cu-K α radiation ($k = 1.54$ Å). Samples for transmission electron microscopy (TEM) analysis were prepared by evaporating a hexane solution of dispersed particles on amorphous carbon coated copper grids.

2.3. Synthesis of ZnO/NPs

To prepare of ZnO/NPs, in a typical experiment, a 0.25M aqueous solution of zinc nitrate (Zn (NO₃)₂•4H₂O) and 0.5 M aqueous solution of sodium hydroxide (NaOH) were prepared in distilled

water. Then, the beaker containing NaOH solution was heated at the temperature of about 55°C. The Zn (NO₃)₂ solution was added drop wise (slowly for 1.5 h) to the above-heated solution under high-speed stirring. The beaker was sealed at this condition for 2 h. The precipitated ZnO/NPs were cleaned with deionized water and ethanol then calcined at 200 °C for 2 hours.

2.4. Preparation of the sensor

ZnO/NPs/IL/CPE was prepared by mixing of 0.2 g of 1,3-dipropylimidazolium bromide, 0.8 g of the liquid paraffin, 0.2 g of ZnO/NP, and 0.9 g of graphite powder. Then the mixture was mixed well for 50 min until a uniformly wetted paste was obtained. A portion of the paste was filled firmly into one glass tube as described above to prepare ZnO/NPs/IL/CPE. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper.

2.5. Preparation of real samples

Fresh juices were obtained using a mechanical squeezer. The juices obtained were filtered into a beaker and acidified (pH =2) using citric or sulfuric acid. A 1.0 mL portion of the filtrate was added to the supporting electrolyte solution in voltammetric cell. Vegetable juices were obtained using a grater (polymer material) and a centrifuge respectively, a 1.0 mL portion of vegetable juice was subjected for the voltammetric measurement. In all cases the amounts of vitamin C in the samples were evaluated by the standard addition method.

For the tablets, an accurately weighed portion of finely powdered sample obtained from three tablets, equivalent to about 50 mg of ascorbic acid dissolved in 100 mL water with ultrasonication. Then, 0.1 mL of the solution plus 9.9 mL of the buffer (pH 7.0) was used for the analysis with standard addition method.

2.6. Dichlorophenolindophenol (DCPIP) titration method [33]

The indophenol solution was standardized by titration with 2.0 mL of standard ascorbic acid solution and 5 mL of HPO₃+HOAc solution to the end point (a persistent rosypink color). The consumption of the blank was determined by titration indophenol solution with 7 mL of HPO₃+HOAc solution plus a given amount of water equivalent to the volume indophenol solution used in the previous standardization titration.

For sample titration, a 100 mL portion of the juice was mixed with an equal volume of HPO₃+HOAc solution before filtering. A volume of the filtrate equivalent to about 250 mg of ascorbic acid was then titrated with indophenols solution using the same procedure as described above including the titration of the blank.

3. Results and discussion

3.1. X-Ray diffraction and TEM of ZnO nanoparticles

The X-ray diffraction data were recorded by using Cu K_{α} radiation (1.5406 \AA). The intensity data were collected over a 2θ range of $20\text{--}80^{\circ}$. The average grain size of the samples was estimated with the help of Scherrer equation using the diffraction intensity of (101) peak. X-ray diffraction studies confirmed that the synthesized materials were ZnO with wurtzite phase, and all the diffraction peaks agreed with the reported JCPDS data, and no characteristic peaks were observed other than ZnO. The mean grain size ($D=17 \text{ nm}$) of the particles was determined from the XRD line broadening measurement using Scherrer equation:

$$D = K\lambda / (\beta \cos\theta) \quad (1)$$

where λ is the wavelength (Cu K_{α}), β is the full width at the half-maximum (FWHM) of the ZnO (101) line, and θ is the diffraction angle. A definite line broadening of the diffraction peaks is an indication that the synthesized materials are in nanometer range. The lattice parameters calculated were also in agreement with the reported values. The reaction temperature greatly influences the particle morphology of as prepared ZnO powders. Figure 1 show the XRD patterns of ZnO nanoparticles.

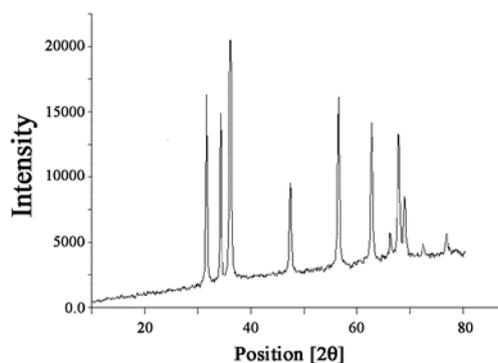


Figure 1. XRD patterns of ZnO nanoparticles.

The morphology of the as-grown nanostructures was characterized by TEM technique. Fig. 2 presents a typical TEM image of ZnO/NPs nanoparticle. It is clear that in this case, a ZnO nanoparticle was successfully prepared.

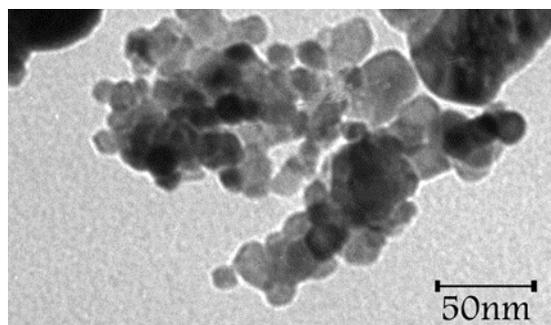


Figure 2. TEM image of ZnO nanoparticles.

3.2. Electrochemical investigation

AA can be oxidized at positive potential depends on the electrode type and solution pH [11]. We anticipated that the oxidation of AA would be pH dependent. In order to ascertain this, the voltammetric

response of AA at a surface of ZnO/NPs/IL/CPE was obtained in solutions with varying pH. Result shows, the peak potential of the redox couple was pH dependent with a slope of -52.0 mV/pH unit at 25°C which was equal to the anticipated Nernstian value for a one-electron, one-proton electrochemical reaction. It can be seen that the maximum value of the peak current was appeared at pH 7.0 (Fig. 3), so this value was selected throughout the experiments.

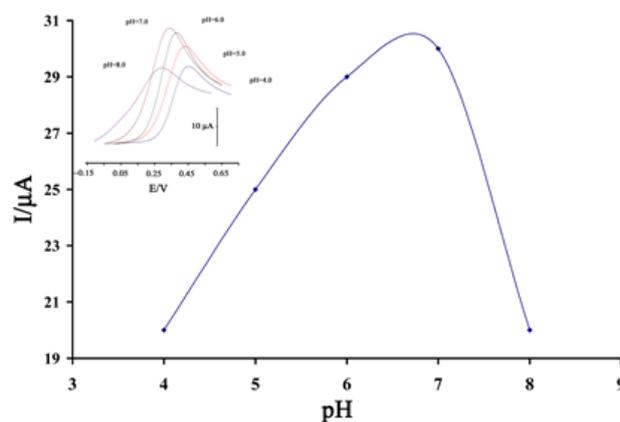


Figure 3. Current-pH curve for electrooxidation of $50.0 \mu\text{M}$ AA at ZnO/NP/IL/CPE with a scan rate of 50 mV s^{-1} . Inset) influence of pH on cyclic voltammograms of AA at a surface of the modified electrode, (pH 4, 5, 6, 7, and 8, respectively).

Figure 4 (inset) shows the current density derived from the cyclic voltammograms responses of $50 \mu\text{M}$ AA (pH 7.0) at the surface of different electrodes with a scan rate of 100 mV s^{-1} . The results show that the presence of ZnO/NPs and IL together cause increasing the active surface of the electrode. The direct electrochemistry of AA on the modified electrode was investigated by linear sweep voltammetry. Figure 4 showed the typical linear voltammogram responses of $50 \mu\text{M}$ AA at pH 7.0 at the surface of different electrodes with a scan rate of 100 mV s^{-1} . ZnO/NPs/IL/CPE exhibited significant oxidation peak current around 370 mV with the peak current of $33.4 \mu\text{A}$ (Fig. 4, curve a). In contrast, low redox activity peak was observed at ZnO/NP/CPE (Fig. 4, curve c) and at unmodified CPE (Fig. 4 curve d) over the same potential range. The AA oxidation peaks potential at ZnO/NP/CPE and at CPE observed around 450 and 460 mV vs. the Ag/AgCl/KCl sat reference electrode with the oxidation peaks current of 11.5 and $4.5 \mu\text{A}$, respectively. In addition, at the surface of bare IL/CPE, the oxidation peak appeared at 370 mV with the peak current was $19.0 \mu\text{A}$ (Fig. 4, curve b), which indicated the presence of ILs in CPE could enhance the peak currents and decrease the oxidation potential (decreasing the overpotential). A substantial negative shift of the currents starting from oxidation potential for AA and dramatic increase of current of AA oxidation. The results indicated that the presence of ZnO/NPs on ZnO/NPs/IL/CPE surface had great improvement with the electrochemical response, which was partly due to excellent characteristics of ZnO/NPs

such as good electrical conductivity, high chemical stability, and high surface area. The suitable electronic properties of ZnO/NPs together with the ionic liquid gave the ability to promote charge transfer reactions, good anti-fouling properties, especially when mixed with a higher conductive compound such as ILs when used as an electrode.

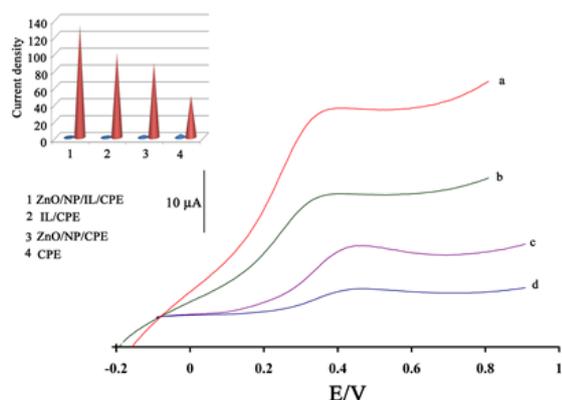


Figure 4. Cyclic voltammograms of a) at ZnO/NP/IL/CPE, b) IL/CPE, c) at ZnO/NP/CPE and d) CPE in presence of 100 μM AA at a pH 7.0, respectively. Inset: the current density derived from cyclic voltammograms responses of 100 μM AA at pH 7.0 at the surface of different electrodes with a scan rate of 50 mV s^{-1} .

The influence of potential scan rate (ν) on I_{pa} of 50 μM AA at the ZnO/NP/IL/CPE was studied by linear sweep voltammetry at various sweep rates (Fig. 5 inset). As shown in Fig. 5, the peak currents of AA grow with the increasing of scan rates and there are good linear relationships between the peak currents and $\nu^{1/2}$ (Fig. 5). The regression equation is $I_{\text{pa}} = 5.9717 - 25.0670 \nu^{1/2}$ (I_{pa} : μA , ν : mVs^{-1} , $R^2 = 0.9911$), indicating the redox process of 50 μM AA at the ZnO/NP/IL/CPE was diffusion-controlled.

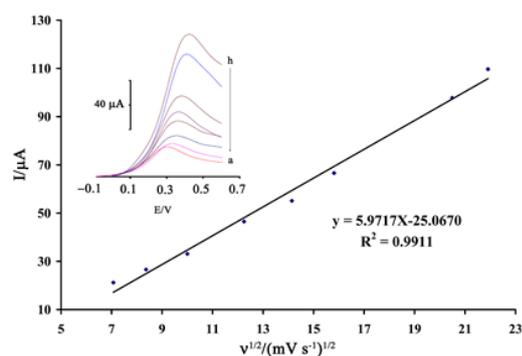


Figure 5. Plot of I_{pa} versus $\nu^{1/2}$ for the oxidation of AA at ZnO/NP/IL/CPE. Inset shows cyclic voltammograms of AA at ZnO/NP/IL/CPE at different scan rates of 50, 70, 100, 150, 200, 250, 420 and 480 mV s^{-1} in 0.1 M phosphate buffer, pH 7.0.

To obtain further information on the rate determining step, a Tafel plot was developed for the AA at a surface of ZnO/NP/IL/CPE using the data derived from the raising part of the current–voltage curve (Fig. 6). The slope of the Tafel plot is equal to

$n(1-\alpha)F/2.3RT$ which comes up to 0.1908 V decade^{-1} . We obtained α as 0.8.

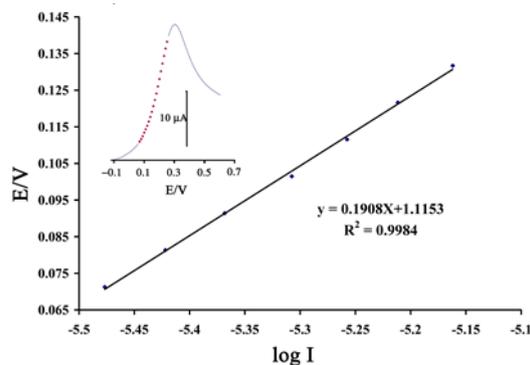


Figure 6. Tafel plot for ZnO/NP/IL/CPE in 0.1 M PBS (pH 7.0) with a scan rate of 50 mV s^{-1} in the presence of 50 μM AA.

Chronoamperometric measurements of AA at ZnO/NP/IL/CPE were carried out by setting the working electrode potential at 400 mV vs. Ag/AgCl/KCl sat for the various concentration of AA in buffered aqueous solutions (pH 7.0) (Fig. 7A). For an electroactive material (AA 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. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of AA (Fig. 7B). The slopes of the resulting straight lines were then plotted vs. AA concentration. From the resulting slope and Cottrell equation the mean value of the D was found to be $2.3 \times 10^{-5} \text{ cm}^2/\text{s}$.

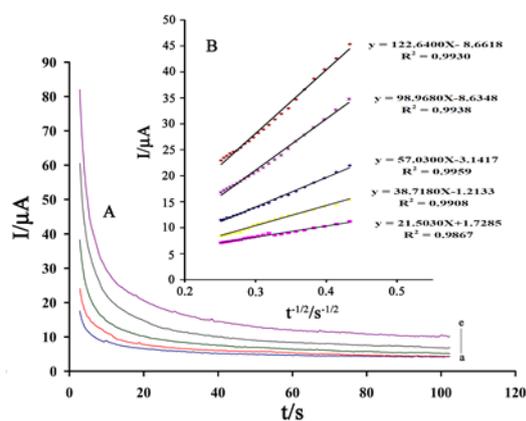


Figure 7. A) Chronoamperograms obtained at ZnO/NP/IL/CPE in the presence of a) 300; b) 400; c) 500; d) 600 and e) 700 μM AA in the buffer solution (pH 7.0). B) Cottrell's plot for the data from the chronoamperograms.

3.3. Linear dynamic range and limit of detection

Square wave voltammetry (SWV) was used to determine AA concentrations. The SW voltammograms clearly show that the plot of peak current vs. AA concentration is linear for 0.08–350 $\mu\text{mol L}^{-1}$ of AA, the regression equation being $I_{\text{p}}(\mu\text{A})$

= $(0.6449 \pm 0.0120)C_{AA} + (18.1350 \pm 0.8932)$ ($r^2 = 0.9902$, $n = 10$), where C is μM concentration of AA and I_p is the peak current. The detection limit was $0.04 \mu\text{M}$ AA according to the definition of $Y_{LOD} = Y_B + 3\sigma$.

3.4. Stability and reproducibility

The repeatability and stability of ZnO/NP/IL/CPE was investigated by square wave voltammetric measurements of $10.0 \mu\text{M}$ AA. The relative standard deviation (RSD%) for ten successive assays was 0.94% . When using four different electrodes, the RSD% for seven measurements was 1.5% . When the electrode stored in the laboratory, the modified electrode retains 97% of its initial response after a week and 94% after 45 days. These results indicate that ZnO/NP/IL/CPE has good stability and reproducibility, and could be used for AA.

3.5. Interference study

In order to evaluate the selectivity of the proposed method in the determination of AA the influence of various foreign species on the determination of $10.0 \mu\text{M}$ AA was investigated. Tolerance limit was taken as the maximum concentration of foreign substances that caused an approximate relative error of $\pm 5\%$. The results given in Table 1 show that the peak current of AA is not affected by all conventional cations, anions, and organic substances.

3.6. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of AA in tablet, fruit juice and vegetable juice samples. Based on the repeated differential pulse voltammetric responses ($n=3$) of the diluted analyte and the samples that were spiked with specified concentration of AA, measurements were made for determination of AA concentrations in food samples. The results are listed in Table 2.

In addition, a published electrochemical method [20] was used for the analysis to confirm the accuracy of the proposed method with a standard procedure. The results presented in Table 2 indicate that the modified electrode retained its efficiency for the determination of AA in real samples with satisfactory results.

Table 2. Determination of AA in real samples ($n=3$).

Sample	AA Added (μM)	Found (AA) Proposed method (μM)	Found (AA) Official method (μM)	F_{ex}^a	F_{tab}	t_{ex}^b	$t_{tab(95\%)}^b$	
Tablet	5.0	5.35 ± 0.45^c	4.22 ± 0.45	5.4	19.0	1.2	3.8	
	10.0	9.74 ± 0.55	10.65 ± 0.72	-	-	-	-	
	20.0	19.84 ± 0.33	20.45 ± 0.55	-	-	-	-	
Fruit Juices	-	-	-	-	-	-	-	
	Orange "	150.66 ± 1.22	151.11 ± 1.47	11.9	19.0	3.5	3.8	
	Kiwi "	80.22 ± 0.85	79.88 ± 1.33	10.5	19.0	3.2	3.8	
	Apple "	25.35 ± 0.65	24.95 ± 0.95	9.2	19.0	3.0	3.8	
	Lemon	55.23 ± 0.45	54.95 ± 0.83	8.2	19.0	2.7	3.8	
	Vegetable juices	-	-	-	-	-	-	-
	Pimento	-	45.33 ± 0.75	45.22 ± 0.85	10.0	19.0	3.1	3.8
Dill	-	90.32 ± 0.25	90.25 ± 0.32	4.9	19.0	1.1	3.8	

^a F_{ex} Calculated F-value; Reported F value from F-test table with 95% confidence level and 2/2 degree of freedom;

^b t_{ex} Calculated t; t_{tab} (95%) Reported t value from t-student test table with 95% confidence level.

^c \pm Shows the standard deviation.

Table 1. Interference study for the determination of $10.0 \mu\text{M}$ AA under the optimized conditions.

Species	Tolerant limits ($W_{Substance}/W_{AA}$)
Glucose, Sacarose, Lactose, Ferocetose	900
Li^+ , Br^- , Al^{3+} , K^+ , ClO_4^- , Na^+ , Cl^- , CO_3^{2-} , Ca^{2+} , Mg^{2+} , SO_4^{2-}	750
Thiourea, Glutamic acid, Tryptophan, Lucine, L-Threonine, L-Phenylalanine, Glycine, Methionine, Alanine, Valine, Histidine	600
Cysteine	400
Starch	Saturation

4. Conclusion

In this work, we describe synthesis of ZnO/NPs as a high sensitive sensor for voltammetric determination of AA using carbon paste electrode. The synthesized nanoparticle was characterized with different methods such as TEM and XRD. The direct electrochemistry of AA at a surface of ZnO/NP/IL/CPE was assessed by cyclic voltammetry, chronoamperometry and square wave voltammetry methods. The presence 1,3-dipropylimidazolium bromide and ZNO nanoparticles helped AA to have a favored orientation and reduce the effective electron transfer distance. Finally, the propose sensor was successfully used for the determination of AA in food samples.

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اندازه گیری ولتامتری موج مربعی اسکوربیک اسید در نمونه های غذایی و دارویی با بکارگیری یک الکتروود خمیر کربن اصلاح شده با مایع یونی و نانوذره اکسید روی

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چکیده:

در این کار سنتز و کاربرد نانو ذرات روی اکسید و مایع یونی ۳و۱ دی پروپیل ایمیدازولینیوم بر مایه به عنوان سنسور حساس برای اندازه گیری اسکوربیک اسید با استفاده از الکتروود خمیر کربن استفاده شد. ولتاموگرام چرخه ای نشان می دهد که پیک اکسیداسیون برگشت ناپذیر در پتانسیل ۳۵۰ میلی ولت که مربوط به اکسیداسیون اسکوربیک اسید است. در مقایسه با الکتروود خمیر کربن پاسخ الکتروشیمیایی افزایش می یابد. الکترواکسیداسیون اسکوربیک اسید وابسته با pH بوده و دو الکترون و دو پروتون را در واکنش که تحت کنترل نفوذ است از خود نشان می دهد. تحت شرایط بهینه، جریان اکسیداسیون اسکوربیک اسید دو رنج خطی با حد تشخیص ۰/۰۰۴ میکرومولار از خود نشان می دهد. سنسور جدید با موفقیت و حساسیت برای اندازه گیری اسکوربیک اسید در نمونه های آب سبزیجات و میوه و نمونه قرص بکار گرفته شده است و داده ها با یک روش دیگر مقایسه شده است.

کلمات کلیدی: اسکوربیک اسید، ۳و۱ دی پروپیل ایمیدازولینیوم بر مایه، نانو ذره روی اکسید، الکتروود خمیر کربن

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