Food Anal. Methods (2016) 9:500–504
DOI 10.1007/s12161-015-0221-8

Ascorbic Acid Detection with MnO_2-Modified GCPE

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Received: 17 April 2015 / Accepted: 3 June 2015 / Published online: 12 June 2015

Abstract In this study, manganese(IV) oxide (MnO_2) nanoparticle (np)-modified glassy carbon paste electrode is used for ascorbic acid (AA) detection in fruit juice samples. The experimental parameters like MnO_2 np amount and pH were optimized by using modified full factorial design model. By means of this model, the number of experiments has been reduced. Under optimal conditions, the linear range for AA was obtained between 2.64×10^{-6} and -1.5×10^{-3} M. Limit of detection (LOD) (3 s/m) and relative standard deviation (RSD) were calculated as 8×10^{-7} M and 4.56 %, respectively. Developed sensor was applied to AA detection in fruit juice samples.

Keywords MnO_2 nanoparticles · Glassy carbon paste electrode · Ascorbic acid · Fruit juice

Introduction

The use of metal/metal oxide nanoparticle superstructures for the organization of electrochemical sensing devices is an extremely promising prospect. In electroanalytical applications, metal nanoparticles provide some important functions including the roughening of the conductive sensing interface and some catalytic properties that result in amplification of electrochemical signal (Wang 2005). Chemical properties of some nanoparticles could be different from those of the bulk materials since nanostructures have higher surface energy than bulk counterparts. Due to this property, usually nanoparticles are chemically more active than the related bulk materials.

Manganese(IV) oxides (MnO_2) are a kind of inorganic materials that show catalytic activity towards some reagents. Also, it is known that bulk MnO_2 catalyzes the decomposition of H_2O_2 while MnO_2 nanoparticles (MnO_2 np) react with H_2O_2 directly (Luo et al. 2004a, b). By using this advantage, various biosensors based on H_2O_2 monitoring have been constructed (Yao et al. 2006; Hocevar et al. 2004; Lin et al. 2005). It has also been demonstrated that by using MnO_2 np, more sensitive results were obtained both for lactate (Xu et al. 2005) and ascorbic acid (AA) (Luo et al. 2004a, b).

AA is a water-soluble vitamin which exists in biological fluids, fruit juice, and vegetables. Pharmaceutical, chemical, cosmetic, and food sectors benefit from it as an antioxidant (Prasad et al. 2011). It is widely used to prevent and treat some diseases such as common cold, mental illness, infertility, and in some manifestations of HIV infection. On the other hand, diseases such as cancer, diabetes mellitus, and hepatic disorders are related to AA concentration in the body fluids (Mbouguen et al. 2011; Mallesha et al. 2011).

Although electrochemical methods are majorly used techniques for AA detection, methods like fluorescence, chemiluminescence, high-performance liquid chromatography, capillary zone electrophoresis, flow-injection spectrophotometry, and colorimetric determination have also been utilized for this purpose (Liu et al. 2015). On the other hand, in electrochemical analyses, traditional electrodes have some disadvantages like low selectivity and sensitivity, poor reproducibility, and electrode fouling.

In present work, MnO_2 np-modified glassy carbon paste electrode (GCPE) was firstly used for detection of AA. The experimental parameters like MnO_2 np amount and pH were optimized by using modified full factorial design model. Under optimal conditions, the linear range for AA was obtained

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between $2.64x10^{-6}$ and $-1.5x10^{-3}$ M. Then, analytical characteristics were explored, and developed electrode was applied for AA detection in fruit juice samples.

Experimental

Reagents

Glassy carbon spherical powder (20–50 μM) was purchased from Alfa Aesar (www.merck.de). AA, mineral oil, manganese acetate, and potassium permanganate were purchased from Sigma-Aldrich (www.sigmaaldrich.com). All solutions were prepared by double distilled water.

Apparatus

Differential pulse (DP) measurements were carried out a μ-AUTOLAB type III electrochemical measurement system from ECO CHEMIE Instruments B.V. (Netherlands), driven by GPES software. GCPE and MnO_2 np-modified GCPE were used as working electrodes. Ag|AgCl electrode and Pt electrode were used as reference and auxiliary electrode, respectively. The electrodes were inserted into a conventional electrochemical cell. The size of MnO_2 np was measured using JEOL-JEM 2100 transmission electron microscopy (TEM). Also during the preparation of MnO_2 np, Sigma 3-16 PK-type centrifuge was used.

Preparation of MnO_2 np

MnO_2 np (20 nm in size) was prepared according to the literature (Luo et al. 2004a, b; Bai et al. 2007). Briefly, 0.10 M potassium permanganate solution (40 mL) was added to 4 mL of 1.5 M manganese acetate solution under ultrasonication at 25 °C. After reaction, the brown solution was centrifuged (about 10,000 rpm). The deposition (MnO_2 np) was washed with water repeatedly until the washing solution was neutral. Obtained MnO_2 nps were found to be in the size of 20 nm from TEM measurements (Fig. 1). The nanoparticles were kept at 4 °C in distilled water when not used.

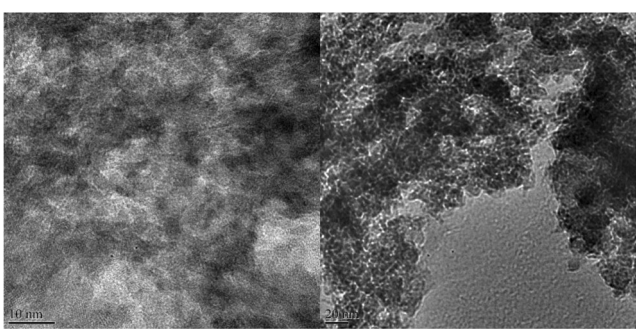

Fig. 1 TEM images of MnO2 np

Preparation of Electrodes

MnO_2 np modified-GCPE was prepared by hand mixing 80:20 (% *w/w*) glassy carbon spherical powder/mineral oil and 2 μL MnO_2. A portion of the resulting paste was then packed firmly into the electrode cavity (3.0 mm in diameter and 5.0 mm in depth) of a PTFE sieve where electrical contact was established via a copper wire.

Sample Application

The contents of AA in orange juice were determined. For this purpose, the sample solutions were stirred for a while and used as stock substrate solutions without any dilution. Then, known amount of sample solution was added to the reaction cell containing working buffer. The signals obtained from the samples were recorded, and AA concentration was calculated by using a calibration graph.

Results and Discussion

Effect of MnO_2 np

It has been known that nanoparticles provide fast mass transport, sensitivity, and electrocatalytic activity when used in electrodes (Cevik and Anik 2010). To compare the performances and observe the difference, electrochemical behavior of plain GCPE and MnO_2 np-modified GCPE were compared for the oxidation of AA. As can be seen clearly from Fig. 2, the best current value was obtained with MnO_2 np-modified GCPE. This result proves that the usage of MnO_2 np provides better sensitivity compared to bare GCPE. Considering the oxidation reaction of AA to dehydroascorbic acid, it can be stated that MnO_2 np could be fastened by this reaction (Luo et al. 2004a, b).

Optimization of pH and Electrode Structure

The experimental parameters like MnO_2 np amount and pH of acetate buffer were optimized by using a full factorial experimental design modified added overall central point.

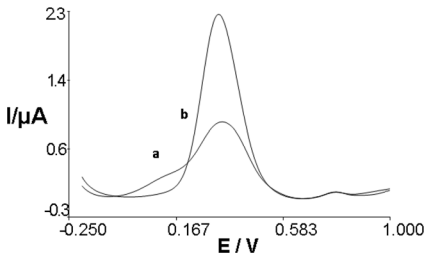

Fig. 2 Obtained DP for 0.25 mM AA voltammograms with GCPE (*a*) and MnO2 np-modified GCPE (*b*)

Table 1 The limits of experimental parameters for modified full factorial experimental design

Factor 1 (*x1*) GCPE + MnO2 np (%)		Factor 2 (*x2*) pH (−)		Response (*y*) current (mA)
Coded levels	Measured values	Coded levels	Measured values	Measured values
0	1,2	0	4,5	2,6633
1	1,6	−1	4	1,2727
−1	0,8	1	5	2,0167
1	0,8	1	5	2,16667
−1	1,6	−1	4,5	1,1313

Experimental running ranges and measured current values as response were given in Table 1. Analysis of the experimental design was conducted by Design-Expert software. Quadratic response surface function chosen for optimization was given as follows:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}{x_1}^2 + b_{22}{x_2}^2$$

Response surface function for optimized processes parameters was plotted on Fig. 3, and the optimization zone on the figure could be seen in detail.

Analytical Characteristics

The linear range for MnO_2 np-modified GCPE was found between 2.64×10^{-6} and -1.5×10^{-3} M AA with the equation of y=0.0062x+0.3676 and R^2 of 0.991 (Fig. 4). This range is better than hydroquinone-modified chitosan/carbon film electrode (Jirimali et al. 2013), polyaniline-modified screen-printed carbon electrode (Kit-Anan et al. 2012), poly(bromocresol purple)-modified glassy carbon electrode (Zhang et al. 2013), and gold nanoparticles-TiO_2 nanotubes-Ti electrode (Hosseini et al. 2011). At higher concentrations, a standard curve showed a deviation from linearity. LOD was calculated 8×10^{-7} M. The repeatability of the biosensor was tested for 25 μM of AA (n=4), and the relative standard deviation (RSD) was calculated as 4.6 %.

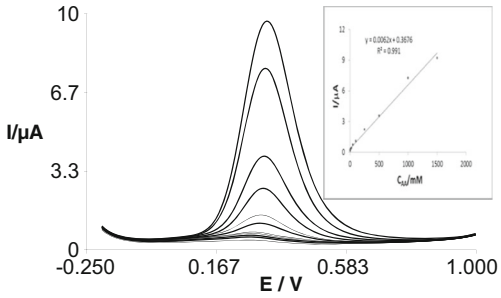

Fig. 4 DP voltammograms of the MnO2 np-modified GCPE electrode in 50 mM acetate buffer (pH 4.5) with increasing concentrations of AA. Calibration graph for AA (inside)

The obtained linear range for AA was compared with other reported literature methods for AA determination (Table 2). As can be seen from Table 2, MnO2 np-modified GCPE exhibits lower linear range.

Interference Study

Determination of AA in the presence of uric acid (UA) was also studied using MnO_2 np-modified glassy carbon paste electrode. For this purpose, interference studies were

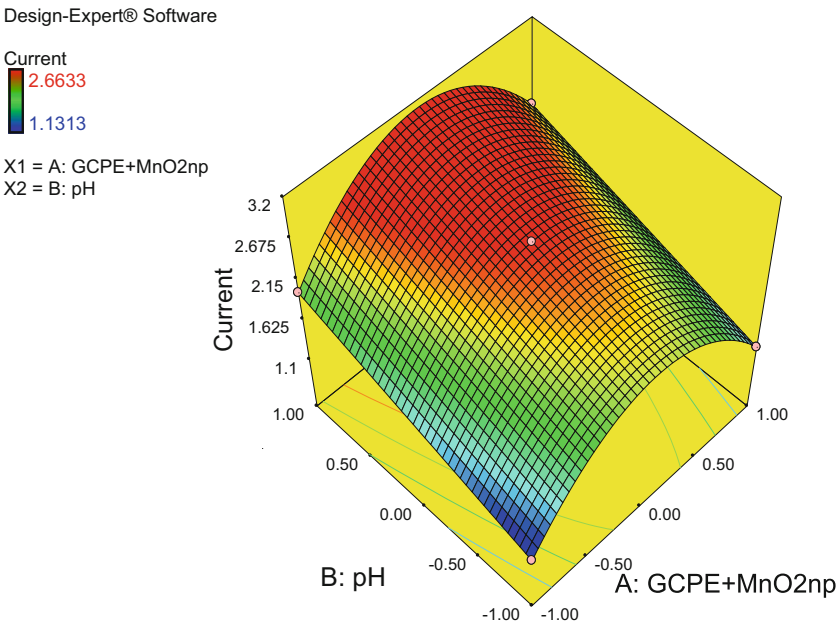

Fig. 3 Response surface function for optimization of process parameters

Table 2 Comparison of the proposed method with the literature methods for AA determination

Electrode	Linear range (μM)	pH	Interferents	References
Tosyl-CNP/GCE	10–3000	2.0	Uric acid	Amiri et al. 2015
Fe_2O_3/RG	570–3970	6.98	Uric acid	Yu et al. 2015
SWCNT/CCE	5.0–700	7.0	Acetaminophen	Habibi et al. 2011
TMP-Ba/FCs/GCE	70–1100	7.0	Uric acid	Mbouguen et al. 2011
GNP/LC/GCE	8.0–5500	7.0	Uric acid	Hu et al. 2008
MnO_2-modified GCPE	2.64–1500	4.5	Uric acid	Present work

Tosyl-CNP/GCE tosyl surface carbon nanoparticles/glassy carbon electrode
Fe2O3/RG Fe_2O_3/graphene nanocomposite
SWCNT/CCE single-walled carbon nanotube-modified carbon–ceramic electrode
TMP-Ba/FCs/GCE TMP-Ba/ferrocene/FCs
GNP/LC/GCE gold nanoparticles/L-cysteine/glassy carbon electrode

performed in acetate buffer containing 25 μM AA-25 μM UA, 25 μM AA-50 μM UA, and 25 μM AA-100 μM UA. Obtained current values were compared to 25 μM AA results. Current values showed a difference lower than 1 %. As a result, it can be concluded that UA did not show interference for detection of AA.

AA Determination in Real Samples

The sample solutions were prepared as described in section 2.5. Two different AA concentrations, 25 and 50 μM, were tried, and experiments were repeated for three times. All the results are given in Table 3. Obtained results from the biosensor were compared to the AA content of fruit juice that was calculated from product label written by the manufacturer. As can be seen from Table 3, very promising recovery values were obtained demonstrating that designed system is suitable for the detection of AA in real samples.

Conclusion

MnO2 np-modified GCPE was prepared, and its performances were examined for AA detection. The experimental parameters like MnO_2 np amount and pH were optimized by using a modified full factorial design model. Under optimum conditions, the current values display a linear relationship with respect to the concentrations of AA in the range from 2.64×10^{-6} to -1.5×10^{-3}. Meanwhile, the detection limit was 8×10^{-7} M. Developed sensor was applied to AA detection in fruit juice samples, and good recovery values were obtained.

Table 3 Measured and calculated concentrations (*n*:3)

Sample	Given concentration of AA (μM)	Found AA concentration (μM)	Recovery (%)
Fruit juice	25	26.1±0.20	104.4±0.20
	50	50.5±0.23	101±0.23

Compliance with Ethical Standards

Conflict of Interest Serdar Çevik declares that he has no conflict of interest. Ülkü Anık declares that she has no conflict of interest. Oğuz Akpolat declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Informed consent was not applicable.

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