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New applications of boron-doped diamond electrode for voltammetric determination of ascorbic acid

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An unmodified boron-doped diamond electrode was used for the voltammetric determination of ascorbic acid (AA). The measurements were carried out in an acetate buffer solution of pH = 4.6 by differential pulse voltammetry (DPV). Under the optimized experimental conditions, AA gaves linear response in a broad concentration range from $5 \cdot 10^{-7}$ to $2 \cdot 10^{-3}$ mol/dm^3. The detection limit was $1.63 \cdot 10^{-7}$ mol/dm^3. The proposed procedure was applied for simple and fast voltammetric determination of ascorbic acid in human urine samples and commercially available dietary supplements.

1. INTRODUCTION

Ascorbic acid (AA, vitamin C, or 2-(1,2-dihydroxyethyl)-4,5-dihydroxyfuran-3-one) is a water-soluble organic compound that plays an important role in many living processes [1]. Vitamin C is easly oxidized to L-dehydroascorbic acid [2]. It is metabolized to inactive sulphide and oxalic acid and excreted in urine [3]. Due to its antioxidant (it reacts with free radicals and reactive oxygen species) and pH regulator properties, ascorbic acid is broadly present in food, drinks and pharmaceuticals [4, 5]. It plays an important role in collagen biosynthesis, strengthens and protects the immune system, improves iron bioavailability and probably

reduces cholesterol level [6]. Furthermore, vitamin C is widely found alongside other biologically and pharmaceutically active substances such as acetaminophen in different pharmaceutical formulations and biological fluids [7]. The use of the complementary presence of ascorbic acid strengthens the positive effect of acetaminophen and diminishes its toxicity towards the liver [8, 9]. AA is mainly used in prevention and treatment of common cold [10]. Vitamin C can be also applied in treatment of diseases caused by radicals such as cancer or Parkinson's disease [11]. Thus, the development of a sensitive, selective and simple method for the determination of ascorbic acid is of the great importance.

Various methods were employed for the determination of vitamin C including: titration [12], fluorimetry [13, 14], spectrophotometry [15, 16], chemiluminescence [17], chromatography [18–20], mass spectrometry [21] and electrochemical techniques such as voltammetry using various working electrodes and supporting electrolytes. Among voltammetric techniques for determination of vitamin C, many of them involve the use of modified electrodes [22–25]. The main disadvantage of this type of electrode is its time-consuming preparation [26].

In this paper, we used a bare boron-doped diamond (BDD) electrode for voltammetric detection of ascorbic acid. We demonstrated that sometimes it not necessary to apply chemical modification and/or electrochemical pre-treatment of electrode surface in order to improve sensitivity or selectivity of the analysis.

To date, few methods using unmodified boron-doped diamond electrode have been proposed for the analysis of vitamin C in pharmaceutical formulations [3, 4, 26]. In this study, we present new applications of bare BDD electrode for simple and fast determination of ascorbic acid by differential pulse voltammetry (DPV) in human urine sample and commercially available supplements.

2. EXPERIMENTAL

2.1. Apparatus

All measurements were performed using the µAutolab analyser with a USB electrochemiacal interface, driven by a GPES 4.9 software package produced by Eco Chemie, the Netherlands. A conventional three-electrode quartz cell with the volume of 10 cm^3 was used with an Ag/AgCl as a reference electrode, a platinum wire – as a counter electrode and a bare BDD electrode (boron doping level of 1000 ppm,

electrical resistivity of 0.075 Ωm) purchased in an inert polytetrafluoroethylene (PTFE, Teflon) body with inner diameter of 3 mm (Windsor Scientific Ltd., United Kingdom) – as a working electrode. The BDD electrode was polished daily using 0.3 μm alumina slurry on a Buehler polishing pad.

2.2. Reagents

An acetate buffer (0.05 mol/dm^3), used as a supporting electrolyte, was prepared from CH_3COOH obtained from Sigma-Aldrich and NaOH purchased from Merck. The stock standard solution of ascorbic acid (AA) (10^{-2} mol/dm^3) was prepared by dissolving the reagent obtained from Sigma-Aldrich in deionized water before starting a sequence of experiments and stored at 4°C in the dark until used. 0.01 mol/dm^3 solution of $Pb(NO_3)_2$ was prepared from reagent obtained from Sigma-Aldrich. The reference material of (lyophilized) human urine was purchased from Medichem. The standard solutions of Ca(II), Mg(II), Se(VI), Fe(III), Mn(II) used to study the interference effect were obtained from Merck and Fluka for Cu(II). Stock standard solution contaning $1 \cdot 10^{-2}$ mol/dm^3 of rutin was prepared by dissolving the reagent obtained from Sigma in methanol. Stock standard solution of pantothenic acid (vitamin B_5) (from Sigma) at concentration of $1 \cdot 10^{-2}$ mol/dm^3 was prepared by dissolving in 0.02 mol/dm^3 NaOH purchased from Sigma-Aldrich. Stock standard solutions of thiamine (vitamin B_1), nicotinic acid (vitamin B_2) (obtained from Fluka) and pyridoxine (vitamin B_6) at concentration of $1 \cdot 10^{-2}$ mol/dm^3 were prepared in deionized water. The standard solutions to study the interference effect were stored in the dark at 4°C until used. All solutions were prepared using ultra-purified water (> 18 MΩ/cm) supplied by the Milli-Q system (Millipore, United Kingdom).

2.3. Sample preparation

The dietary supplements analysed were Gold Vita-Min anti-OX Super Sport capsules containing 240 mg of ascorbic acid in one capsule and Vita-Min Multiple Sport capsules containing 290 mg of AA in one capsule produced by Olimp Sport Nutrition, Poland. The dietary supplements were prepared by the following procedure. The content of three capsules were carefully mixed and then a quantity of homogeneous powder equivalent to the average mass per capsule was transferred into a 10 cm^3 calibrated flask, filled to the mark with deionized water and

shaken for 10 min. in microshaker. An aliquot of such prepared samples was filtered using 0.45 μm Millipore membrane filter to remove insoluble substances. A suitable volume of so prepared samples was added to the supporting electrolyte in the voltammetric cell. The determination of AA was carried out under optimal conditions.

In order to determine AA in human urine, 0.1 cm^3 of human urine sample was added to the supporting electrolyte (1% v/v) and the measurements were carried out under the optimal conditions.

2.4. Standard procedure of measurements

The electrochemical experiments were carried out in a 0.05 mol/dm^3 acetate buffer solution (pH = 4.6). Differental pulse voltammetry (DPV) parameters were the following: step potential of 2 mV, modulation amplitude of 50 mV, scan rate of 20 mV/s. The potential was scanned from –1.45 to 1.0 V. The measurements were carried out from undeaerated solutions.

3. RESULTS AND DISCUSSION

3.1. Voltammetric behaviors of AA

Ascorbic acid (AA) can be easly oxidated to produce dehydroascorbic acid. The process involves the release of two electrons and two protons [5] (Figure 1). For this irreversible redox couple (ascorbic acid/dehydroascorbic acid) the anodic peak height depends on the analyte concentration [26–27].

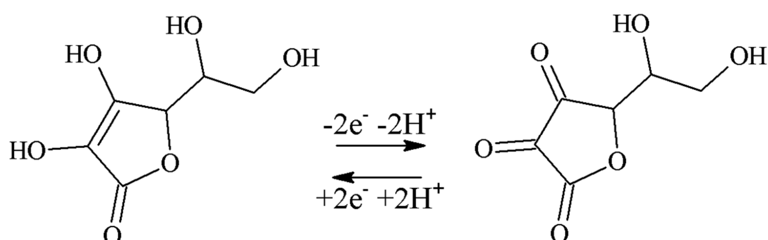

Fig. 1. Oxidation mechanism of ascorbic acid.

In order to evaluate the advantages of a bare boron-doped diamond (BDD) electrode with regard to bare glassy carbon (GC) electrode and BDD electrode modified with lead film, differential pulse

voltammograms were recordered from the solution containing 0.1 mol/dm^3 acetate buffer solution (pH = 6.0 ± 0.1) and $2.5 \cdot 10^{-5}$ mol/dm^3 AA. In the case of lead film electrode, $5 \cdot 10^{-5}$ mol/dm^3 $Pb(NO_3)_2$ was added to the supporting electrolyte and the lead film was plated in situ onto the BDD support at potential of –1.45 V for 60 s. As it can be seen in Figure 2, analytical signals of AA obtained at GC and BDD electrodes have similar values. However, the use of BDDE makes it possible to reduce background current and to obtain better formed peak of AA. In the presence of lead film on the BDD surface no increase in the anodic peak current of AA was observed, which is in accordance with the literature [28]. Therefore, the BDDE was confirmed as the best choice of further study.

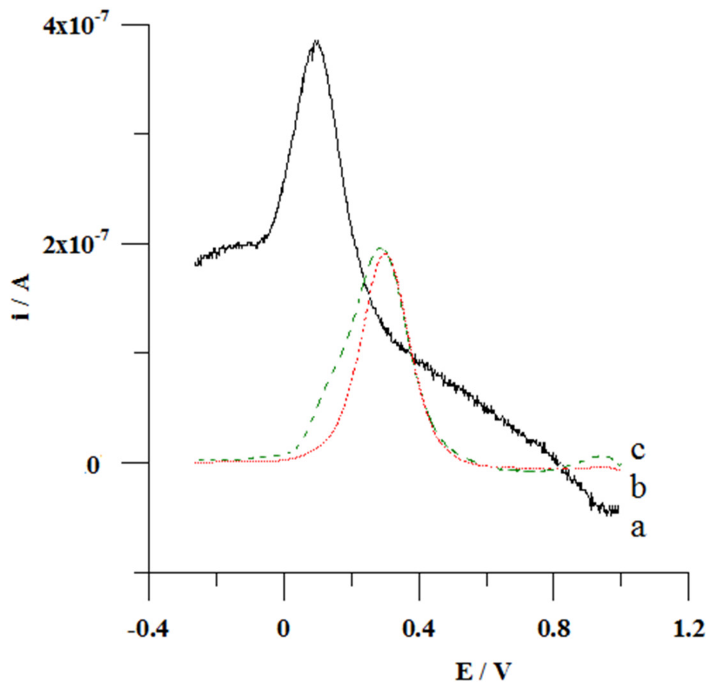

Fig. 2. Differential pulse voltammograms obtained at: a bare glassy carbon electrode (GCE) (black solid line), a bare boron-doped diamond electrode (BDDE) (red dotted line), boron-doped diamond electrode modified with lead film (green dashed line). Ascorbic acid at concentration of $2.5 \cdot 10^{-5}$ mol/dm^3 was determined in 0.1 mol/dm^3 acetate buffer solution (pH = 6.0±0.1). The lead film was deposited at –1.45 V for 30 s from solution containing $5 \cdot 10^{-5}$ mol/dm^3 $Pb(NO_3)_2$. DPV parameters: step potential of 2 mV,modulation amplitude of 50 mV, scan rate of 20 mV/s.

3.2. Composition of measurement solution

In all experiments described in this section, the potential of the electrode was changed in the following sequence: 1.0 V for 30 s and –1.45 V for 60 s. In the first step, the electrode surface was cleaned after the preceding measurement. The next potential was applied for deposition of AA onto the working electrode surface. During these steps the solution was stirred using a magnetic stirring bar.

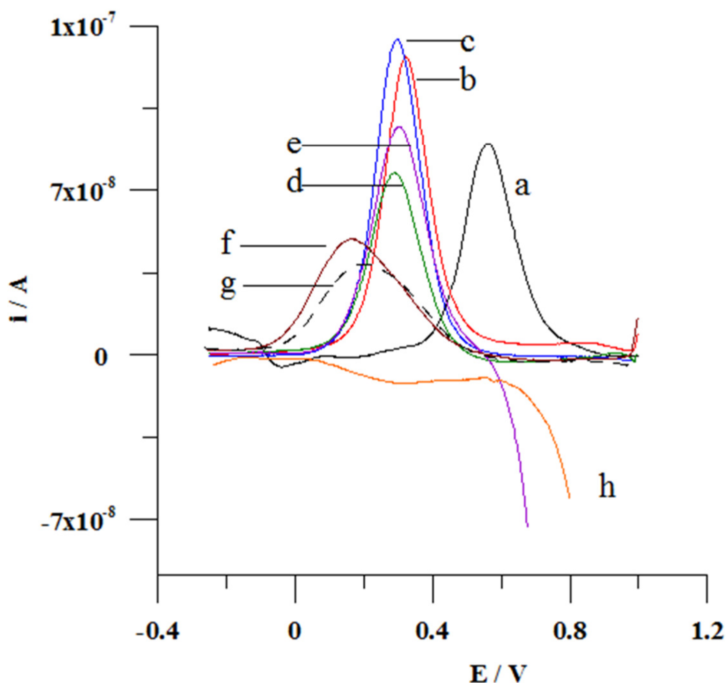

Fig. 3. Influence of the supporting electrolyte type at concentration of 0.1 mol/dm^3 on the AA peak current: a) H_2SO_4 (black line), b) acetate buffer of pH = 3.5 (red line), c) acetate buffer of pH = 4.6 (blue line), d) acetate, buffer of pH = 6.0 ± 0.1 (line green), e) PIPES buffer of pH = 6.9 ±0.1 (violet line), f) ammonium buffer of pH = 8.3 ± 0.1 (brown line), g) ammonium buffer of pH = 9.0, h) NaOH (orange line). Concentration of AA: $1 \cdot 10^{-5}$ mol/dm^3. The AA was ccumulated at –1.45 V for 60 s. Other measurement parameters are the same as in Fig. 2.

The influence of the following supporting electrolytes on the anodic peak current of AA was checked: sulphuric acid(VI), acetate buffer (pH: 3.5, 4.5 and 6.0 ±0.1), PIPES buffer (pH = 6.9 ± 0.1), ammonium buffer (pH 8.3 ± 0.1 and 9.0) and sodium hydroxide. The measurements were

carried out in solutions containing $1 \cdot 10^{-5}$ mol/dm^3 AA. The results obtained are presented in Figure 3. The highest and well-formed signal was observed in the acetate buffer solution (pH = 4.6), so this electrolyte was chosen for further measurements.

Next, the concentration of the acetate buffer was changed in the range from 0.01 to 0.5 mol/dm^3 and its influence on the AA peak current was studied. It was found that the current of the AA peak increases as the concentrate of acetate buffer rises to 0.05 mol/dm^3 and then slowly decreases (Figure 4). On the basis of these results, the concentration of the acetate buffer solution (pH = 4.6) 0.05 mol/dm^3 was chosen.

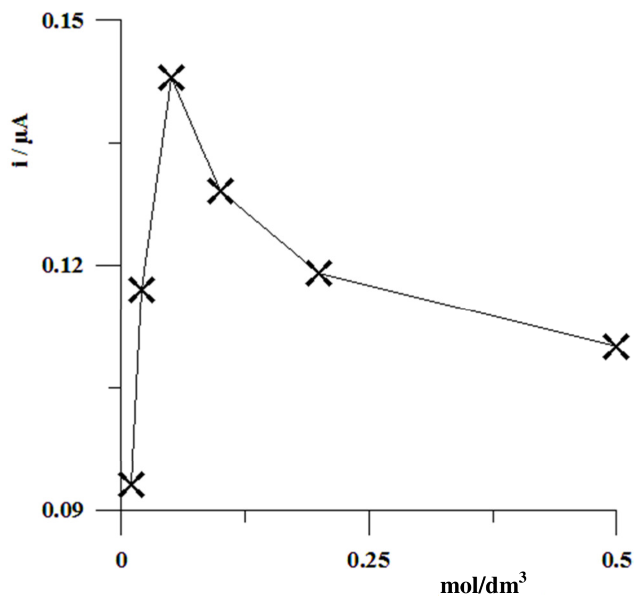

Fig. 4. Influence of the concentration of acetate buffer solution (pH = 4.6) on the AA peak current of $1 \cdot 10^{-5}$ mol/dm^3. The AA was accumulated at –1.45 V for 60 s.

3.3. Optimization of the AA determination procedure

The influence of accumulation potential on the AA peak current was studied for AA concentration of $1 \cdot 10^{-5}$ mol/dm^3. The potential was changed in the range from +0.15 to –1.5 V. The obtained results indicate that the accumulation potential has no effect on the AA signal (Figure 5A).

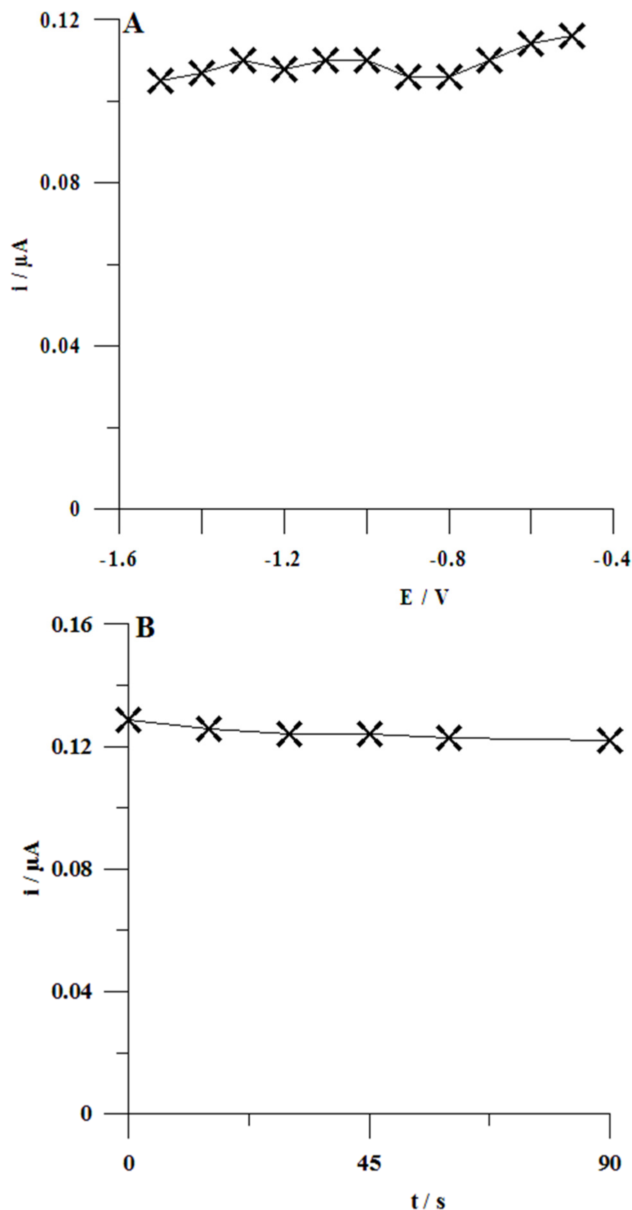

Fig. 5. Effect of accumulation potential (A) and time (B) on the peak current of $1 \cdot 10^{-5}$ mol/dm^3 AA. In the case of (A) the accumulation time of AA was 60 s. In the case of (B) the accumulation potential of AA was –1.45 V.

This shows that AA does not accumulate on the electrode surface. To confirm this fact, the effect of an accumulation time of $1 \cdot 10^{-5}$ mol/dm^3 AA was studied. The accumulation time was changed in the range from 0

to 300 s. It was observed that the extension of the accumulation time does not affect to AA peak current (Figure 5B). Therefore, in the following measurements we used differential pulse voltammetry.

Next, the need of cleaning BDD electrode surface after each measurement was checked. In this cause, 10 successive measurements of $1 \cdot 10^{-5}$ mol/dm^3 AA analytical signal using purification at 1.0 V per 30 s and without purification were performed. The relative standard deviation of the peak current of 3.8 % and 2.8 % with and without purification of the electrode surface was obtained, respectively. On the basic of these results, futher studies were performed without using a cleaning step.

3.4. Calibration graph

Under the optimal analytical conditions, determination of ascorbic acid with increasing concentration was performed. The voltammograms obtained in the course of the determination of low concentrations of AA are presented in Figure 6.

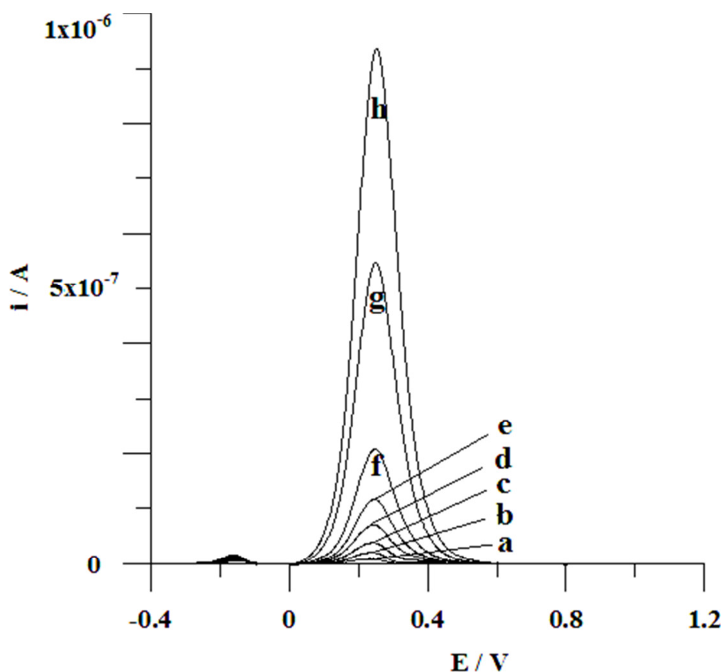

Fig. 6. Differential pulse voltammograms obtained at the BDD electrode from a solution containing 0.05 mol/dm^3 acetate buffer (pH = 4.6) and increasing AA concentration: a) $5 \cdot 10^{-7}$, b) $1 \cdot 10^{-6}$, c) $2 \cdot 10^{-6}$, d) $5 \cdot 10^{-6}$, e) $1 \cdot 10^{-5}$, f) $2 \cdot 10^{-5}$, g) $5 \cdot 10^{-5}$, h) $1 \cdot 10^{-4}$ mol/dm^3. DPV parameters: step potential of 2 mV, modulation amplitude of 50 mV, scan rate of 20 mV/s.

The calibration graph was linear from $5 \cdot 10^{-7}$ to $2 \cdot 10^{-3}$ mol/dm^3, and was in accordance with the equation y = 7.412x + 0.148, where y is the peak current (μA) and x is AA concentration (mmol/dm^3). The correlation coefficient (R^2) was 0.9984. The detection limit estimated from 3-times the standard deviation (n = 3) for the lowest determined AA concentration was $1.63 \cdot 10^{-7}$ mol/dm^3.

The repeatability was determined by 3 successive measurements of $5 \cdot 10^{-6}$ mol/dm^3 AA solution at the same electrode. The relative standard deviation of the peak current of 2.86 % was obtained, revealing good repeatability of proposed method.

3.5. Interferences

Various ions and organic substances were examined regarding their interference with the determination of ascorbic acid. The obtained results are shown in Tables 1 and 2.

Table. 1. Influence of various ions on the $1 \cdot 10^{-5}$ mol/dm^3 AA peak current.

Ion	Concentration [mol/dm^3]	Relative signal [%]
Mg(II)	$5 \cdot 10^{-6}$	98.7
	$1 \cdot 10^{-5}$	97.0
Ca(II)	$5 \cdot 10^{-6}$	101.0
	$1 \cdot 10^{-5}$	99.2
Zn(II)	$5 \cdot 10^{-6}$	95.4
	$1 \cdot 10^{-5}$	88.6
Cu(II)	$5 \cdot 10^{-6}$	97.2
	$1 \cdot 10^{-5}$	60.2
Se(VI)	$5 \cdot 10^{-6}$	99.7
	$1 \cdot 10^{-5}$	96.2
Mn(II)	$5 \cdot 10^{-6}$	99.8
	$1 \cdot 10^{-5}$	93.9

It was found that the voltammetric signal of $1 \cdot 10^{-5}$ mol/dm^3 AA is not influenced by equal concentration of Mg(II), Ca(II), Se(VI), Mn(II), pantothenic acid (vitamin B_5), thiamine (vitamin B_1), pyridoxine (vitamin B_6) and uric acid. The addition of Zn(II), Cu(II), rutin and nicotinic acid

(vitamin B_3) at concentration of $1 \cdot 10^{-5}$ mol/dm^3 causes a decrease of the ascorbic acid signal to 88.6, 60.2, 65.3 and 88.8 % of its original value, respectively. It should be added that, in each case, the analytical signal of ascorbic acid was still well-formed and easy to measure.

Table 2. Influence of various organic substances on the $1 \cdot 10^{-5}$ mol/dm^3 AA peak current.

Organic substance	Concentration [mol/dm^3]	Relative signal [%]
Rutin	$5 \cdot 10^{-6}$	104.8
	$1 \cdot 10^{-5}$	65.3
Thiamine (vitamin B_1)	$5 \cdot 10^{-6}$	97.5
	$1 \cdot 10^{-5}$	91.6
Nicotinic acid (vitamin B_3)	$5 \cdot 10^{-6}$	98.1
	$1 \cdot 10^{-5}$	88.8
Pantothenic acid (vitamin B_5)	$5 \cdot 10^{-6}$	98.7
	$1 \cdot 10^{-5}$	96.4
Pyridoxine (vitamin B_6)	$5 \cdot 10^{-6}$	101.5
	$1 \cdot 10^{-5}$	97.1
Uric acid	$5 \cdot 10^{-6}$	98.4
	$1 \cdot 10^{-5}$	92.0

Additionally, the effect of the percentage of human urine on the $1 \cdot 10^{-5}$ mol/dm^3 AA peak current was studied. For this purpose, the human urine at concentration from 0.01 to 10 % was added to the supporting electrolyte. As can be seen in Figure 7, the AA signal was stable upon raising concentration of human urine in voltammetric cell to 1% and than started decrease at heigher concentations. The AA peak was completely suppressed where the addition of human urine was 10 %.

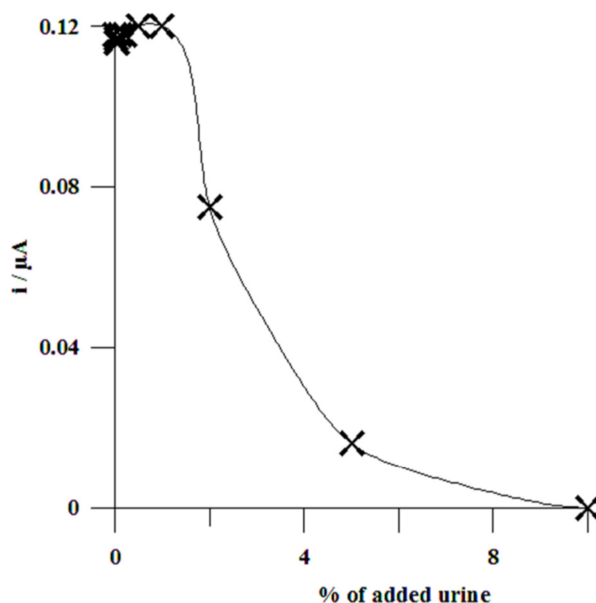

Fig. 7. Effect of percentage of added human urine on the $1 \cdot 10^{-5}$ mol/dm^3 AA peak current.

4. ANALYTICAL APPLICATIONS

Commercially available dietary supplements containing ascorbic acid were analyzed in order to evaluate the accuracy of the herein proposed method. The standard additions method was used for samples analysis spiked with aliquots in amounts of AA standards. As can be seen in Table 3, the quantitative results obtained for the capsules were in accordance with the data supplied by the manufacturer.

Table 3. The results of AA determination in dietary supplements (Gold Vita-Min anti-OX Super Sport, Vita-Min Multiple Sport) obtained by the proposed differential pulse voltammetric procedure.

Dietary supplement	Claimed [mg/capsule]	Found [mg/capsule]	Recovery ± RDS [%]
Gold Vita-Min anti-OX Super Sport	240	241.80	100.75 ± 3.11
Vita-Min Multiple Sport	290	300.50	103.62 ± 3.68

The relative standard deviations (RSD) are given for n = 3.

The recoveries are in the range from 100.75 to 103.62% and shows good accuracy of the proposed method. The voltammograms obtained in the course of AA determination in supplements (Gold Vita-Min anti-OX Super Sport) are presented in Figure 8.

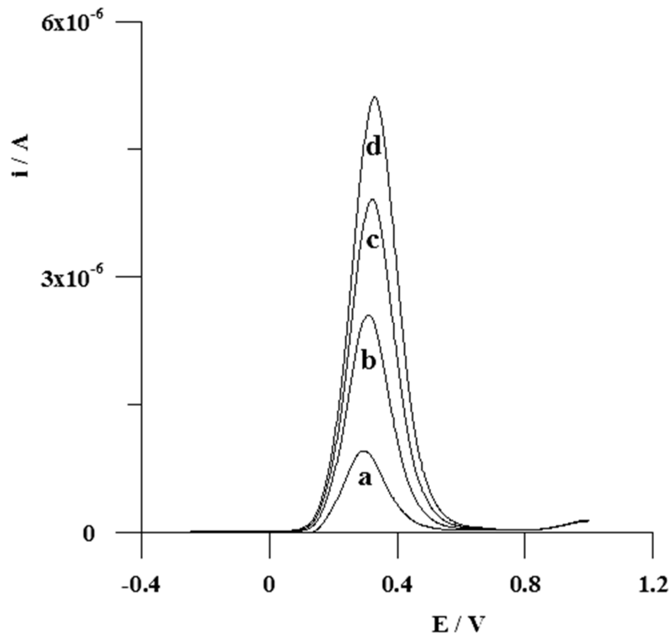

Fig. 8. Differential pulse voltammograms obtained in the course of AA determination in dietary supplement (Gold Vita-Min anti-OX Super Sport) at boron-doped diamond electrode: a) sample (10 mm^3); b) as (a) + $2 \cdot 10^{-4}$ mol/dm^3 AA; c) as (a) + $4 \cdot 10^{-4}$ mol/dm^3 AA; d) as (a) + $6 \cdot 10^{-4}$ mol/dm^3 AA.

The proposed voltammetric procedure was also applied for determination of AA in human urine without any extraction step. For this purpose, 100 mm^3 human urine sample was added to the supporting electrolyte. The obtained calibration graph for the determination of AA in human urine sample was linear in the range from $1 \cdot 10^{-6}$ to $5 \cdot 10^{-5}$ mol/dm^3 (Figure 9). The linear regression equation was y = 7.041x + 63.426, where y is the peak current (μA) and x is an ascorbic acid concentration ($\mu mol/dm^3$). The correlation coefficient (R^2) was 0.9933. The detection limits estimated from 3-times the standard deviation (n = 5) for the lowest determined concentration of AA in human urine samples diluted 100 times was $3.44 \cdot 10^{-7}$ mol/dm^3. The relative standard deviation from three determinations of AA at concentration of $1 \cdot 10^{-7}$ mol/dm^3 was 1.39 %.

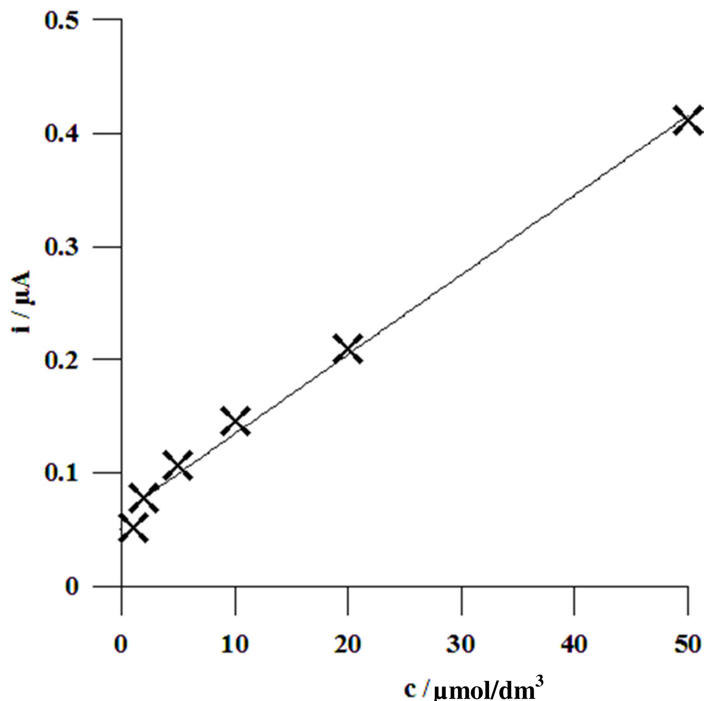

Fig. 9. Linear calibration plots of anodic current peaks versus concentration of AA in the range from $1 \cdot 10^{-6}$ to $5 \cdot 10^{-5}$ mol/dm^3 obtained at BDD electrode in human urine samples diluted 100 times.

The results inditate the potential applicability of unmodified boron-doped diamond electrode for the determination of ascorbic acid in real samples (dietary supplements and human urine) without any interferences from samples matrix.

5. CONCLUSION

The approach taken in this work describes a new application of boron-doped diamond electrode for voltammetric determination of ascorbic acid. The proposed method provides a broad linear range and low detection limit ($1.63 \cdot 10^{-7}$ mol/dm^3) without using any electrochemical pre-treatments and/or chemical modification of the BDD electrode surface. The developed procedure was successfully applied for the determination of AA in commercially available dietary supplements and human urine witout any interferences from samples matrix.

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CURRICULA VITAE

Ilona Sadok is a graduate of Maria Curie-Skłodowska University (UMCS) in Lublin, where in 2013 she obtained a master's degree in analytical chemistry. She is currently working toward her Ph.D. at UMCS in electroanalytical chemistry on designing the voltammetric sensors.

Katarzyna Tyszczuk-Rotko is now an assistant professor at the Department of Analytical Chemistry and Instrumental Analysis (Maria Curie-Skłodowska University, Lublin, Poland). She received her M.Sc. and Ph.D. degrees in Chemistry from Maria Curie-Skłodowska University in 2004 and 2006, respectively. In 2013 she achieved postdoctoral degree. Her present area of interes is stripping analysis, mainly the voltammetric determination of biologically active compound and metal ions using electrodes modified with metal films.

Agnieszka Szwagierek is a graduate of Maria Curie-Skłodowska University (UMCS) in Lublin, where she graduated first degree in biology and chemistry in 2012 and 2013, respectively. She is currently working toward her M.Sc. at UMCS in chemistry, specialization of material chemistry.