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Simultaneous voltammetric determination of paracetamol and ascorbic acid using a boron-doped diamond electrode modified with Nafion and lead films

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ABSTRACT

The paper describes the fabrication and application of a novel sensor (a boron-doped diamond electrode modified with Nafion and lead films) for the simultaneous determination of paracetamol and ascorbic acid by differential pulse voltammetry. The main advantage of the lead film and polymer covered boron-doped diamond electrode is that the sensitivity of the stripping responses is increased and the separation of paracetamol and ascorbic acid signals is improved due to the modification of the boron-doped diamond surface by the lead layer. Additionally, the repeatability of paracetamol and ascorbic acid signals is improved by the application of the Nafion film coating. In the presence of oxygen, linear calibration curves were obtained in a wide concentration range from 5×10^{-7} to 2×10^{-4} mol L^{-1} for paracetamol and from 1×10^{-6} to 5×10^{-4} mol L^{-1} for ascorbic acid. The analytical utility of the differential pulse voltammetric method elaborated was tested in the assay of paracetamol and ascorbic acid in commercially available pharmaceutical formulations and the method was validated by high performance liquid chromatography coupled with diode array detector.

1. Introduction

Paracetamol (N-acetyl-p-amino-phenol, acetaminophen, PA) is an effective pain killer used for the widespread relief of pains associated with many parts of the body [1]. However, the overdose of PA can lead to the accumulation of toxic metabolites which may cause hepatotoxicity and nephrotoxicity [2]. Ascorbic acid (vitamin C, 2-(1,2-dihydroxyethyl)-4,5-dihydroxyfuran-3-one, AA) plays a key role in the formation and maintenance of collagen and is a powerful antioxidant that reacts with reactive oxygen species or free radicals. It strengthens and protects the immune system, enhances iron bioavailability, and is thought to help reduce cholesterol levels [3]. AA is extensively used for the prevention and treatment of the common cold, some mental illnesses, and cancer [4,5]. In some pharmaceutical formulations, these two substances can be associated, since the presence of AA intensifies the pharmacological effect of PA, as well as promotes a protective effect with respect to PA hepatotoxicity [6]. Therefore, the simultaneous determination of PA and AA for quality control of bulk pharmaceutical and pharmaceutical formulation is very important, especially for pharmaceutical industry, which can be successfully performed by means of liquid chromatography [7–9], and spectrophotometric methods [10,11]. However, these methods are generally laborious and time-consuming.

Since PA and AA are electroactive compounds, electrochemical sensors represents an interesting alternative for their quantification. Nevertheless, as it is well known that the simultaneous determination of PA and AA by unmodified electrodes suffers from lack of selectivity due to the overlapping oxidation peaks [12]. In this sense, in recent years some approaches based mainly on electrode surface modification have been developed in order to solve this problem. According to the literature data, poly(3-methylthiophene)/palladium sub-micro-modified electrode [13], thionine immobilized multi-walled carbon nanotube-modified carbon paste electrode [14], aluminum electrode modified by thin layer of palladium [15], single-walled carbon nanotube-modified carbon-ceramic electrode [16], glassy carbon electrode modified with multi-wall carbon nanotubes dispersed in polyhistidine [17] are examples of electrode surface modification.

In 2005, the lead film electrode (PbFE) was for the first time introduced for adsorptive stripping voltammetric determinations of inorganic ions such as Co(II) and Ni(II) [18]. Till now, the electrochemistry of PbFE and its advantages compared to other bare and modified carbon electrodes have been reported for determination of several important biologically active compounds, such as caffeic acid,

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thiamine, betulinic acid etc. [19–21]. The proposed electrode exhibited interesting characteristics, such as the ability to operate in a wide range of pH media, simple preparation, good reproducibility and a simple way of electrochemical surface renewal. The lead film was usually plated in situ onto a glassy carbon surface. In this paper we first report on the utilization of a boron-doped diamond electrode (BDDE) as the support for an in situ plated lead film.

Boron-doped diamond is a modern electrode material which opens new possibilities of electrochemical investigations due to its excellent features, such as the wide potential window in aqueous solutions, low background current, long-term stability of response and low sensitivity to dissolved oxygen [22,23]. To our knowledge there are no reports on the simultaneous determination of PA and AA using a modified boron-doped diamond electrode.

Based on the above mentioned facts, the aim of the present paper was to develop a simple but sensitive electrochemical sensor for the simultaneous determination of PA and AA. In this work, we propose the use of the lead film as a sensing layer for the highly selective and sensitive quantification of PA and AA. It was shown that the current peaks for the oxidation of PA and AA can be well separated by the application of the lead film coating onto the boron-doped diamond surface. Additionally, the repeatability of paracetamol and ascorbic acid signals was improved by the application of the Nafion film coating. Based on the effective electrochemical activity of the boron-doped diamond electrode modified with Nafion and lead films toward PA and AA, a sensitive electrochemical sensor for the simultaneous determination of these species was established. Finally, the analytical performance of this sensor for the simultaneous determination of paracetamol and ascorbic acid in commercial pharmaceutical formulations without any sample pretreatment was evaluated. The results were compared to those obtained by high performance liquid chromatography coupled with diode array detector (HPLC-DAD).

2. Materials and methods

2.1. Reagents

An acetate buffer, used as a supporting electrolyte for the proposed voltammetric method, was prepared from CH_3COOH and NaOH obtained from Sigma-Aldrich and Merck, respectively. Paracetamol (PA) and ascorbic acid (AA) were purchased from Sigma-Aldrich. The stock solutions of PA and AA were prepared in deionized water before starting set of experiments and stored at 4 °C in the dark until used. 0.01 mol L^{-1} solution of $Pb(NO_3)_2$ was prepared from reagent obtained from Sigma-Aldrich. Nafion (5% w/w solution, Sigma-Aldrich) was diluted with ethanol, in order to reach a 1% w/v concentration. HPLC-grade acetonitrile and trifluoroacetic acid (TFA) were from Merck. The brand name and composition of PA and AA formulations are the following: Febrisan (sample A, 750 mg PA, 60 mg AA, and excipients) and Fervex D (sample B, 500 mg PA, 200 mg AA, and excipients). All solutions were prepared using ultra-purified water (> 18 MΩ cm) supplied by a Milli-Q system (Millipore, UK).

2.2. Apparatus

The electrochemical experiments were carried out using a μAutolab analyzer equipped with USB electrochemical interface and drive GPES 4.9 software package (Eco Chemie, The Netherlands) in conjunction with a three-electrode system and a personal computer for data storage and processing. A three-electrode cell system consisted of a silver/silver chloride/potassium chloride (Ag/AgCl/KCl, 3 mol L^{-1}) reference electrode, a platinum wire as counter electrode, and a bare BDDE electrode (boron doping level of 1000 ppm, electrical resistivity of 0.075 Ω cm) purchased in an inert polytetrafluoroethylene (PTFE, Teflon) body with inner diameter of 3 mm (Windsor Scientific Ltd., United Kingdom) as the working electrode was employed for the electrochemical studies. The pH measurements were made on an Elmetron pH meter CI-316.

A high-resolution microscope FEI DualBeam™ Quanta™ 3D FEG (scanning electron microscope (SEM) with focused ion beam (FIB)) equipped with an energy dispersive X-ray spectrometer (EDS) as well as inverted metallurgical microscope Eclipse MA 200 (Nikon) were used for the electrode surface characterization.

Chromatographic measurements were performed using a high performance liquid chromatograph (VWR Hitachi Chromaster 600, Merck, Darmstadt, Germany) with pump (5160), degasser, thermostat (5310), autosampler (5260), DAD detector (5430), LiChrospher 100 (Merck, Darmstadt, Germany) C18 reversed-phase column (25 cm × 4.0 mm i.d., 5 μm particle size) and EZChrom Elite software.

2.3. Procedures

2.3.1. Procedure of Nafion film coating

For the preparation of the boron-doped diamond electrode modified with Nafion film, a 0.5 μL drop of the Nafion solution (1% w/v) was placed onto the electrode surface and left to dry in air for about 5 min in a room temperature before the lead film coating and PA and AA determination. The quality of the deposition was systematically checked with an optical microscope during the first experiments and occasionally during further experiments.

2.3.2. Procedure of lead film coating and voltammetric measurements of PA and AA

The Nafion covered boron-doped electrode was modified by the lead film using an in situ plating method. The lead film coating and voltammetric determinations of PA and AA were carried out in a solution containing 0.1 mol L^{-1} acetate buffer (pH 6.0 ± 0.1), 5×10^{-5} mol L^{-1} Pb(II) and variable concentrations of paracetamol and ascorbic acid. The potential of the electrode was changed in the following sequence: 1.0 V for 30 s and −1.45 V for 60 s. The first step was applied to clean the electrode from the lead remaining after the preceding measurement. In the next step, the lead film was plated onto the Nafion covered boron-doped diamond surface. During these steps the solution was stirred using a magnetic stirring bar. Then the stirring was stopped and after 5 s equilibration time, the anodic differential pulse voltammograms (DPVs) were recorded between −1.45 and 1.0 V with amplitude of 50 mV and scan rate 20 mV s^{-1}. The oxidation peak current of lead was much larger than the oxidation peaks currents of PA and AA, so the recorded voltammograms were cut in the potential range from −0.25 to 1.0 V. All voltammetric measurements were carried out in undeaerated solutions.

2.3.3. Chromatographic measurements

The results obtained in the course of the determination of PA and AA in pharmaceutical formulations by the proposed voltammetric method were compared to those obtained by high performance liquid chromatography coupled with diode array detector (HPLC-DAD). The chromatographic conditions, e.g., type of column and mobile phase composition for analysis of ascorbic acid and paracetamol were established on the basis of literature [24,25]. The analytes were separated on C18 reversed-phase column at flow rate of 1.0 mL min^{-1}. The gradient elution was used to enable the determination of both compounds in single chromatographic run. Mobile phase consisted of water containing 0.025% TFA (solvent A) and acetonitrile containing 0.025% TFA (solvent B). The gradient profile was as follow: 0–3.0 min, 100% A; 3–4 min, 100–85% A; 4–10 min, 85% A and 15% B. The quantification was

conducted at 240 nm for paracetamol and 245 for ascorbic acid. The temperature of autosampler and thermostat was 10 °C. The mean peak areas were taken for the construction of calibration curve. For each point, five measurements were made. The data were analyzed by linear regression least square model. Samples were analyzed by HPLC-DAD in triplicate and the concentrations of PA and AA were calculated from the calibration plot.

2.3.4. Sample preparation

The pharmaceutical was prepared by the following procedure. The content of five sachets were carefully grounded to a fine powder, and then a quantity of homogeneous powder equivalent to the average mass per sachet was dissolved in 250 mL of deionized water by sonication for 5 min. A suitable aliquot of the sample solution was analyzed using the chromatographic method (HPLC-DAD) and voltammetric method (DPV).

3. Results and discussion

3.1. Voltammetric behaviors of PA and AA

The oxidation mechanism of paracetamol (PA) and ascorbic acid (AA) has been extensively investigated in the literature. In the case of PA, a two-electron and two-proton transfer process occurs to produce the relatively stable ($t_{1/2}$=47 min.) oxidized product N-acetyl-p-quinoneimine [26,27]. AA undergoes a reversible two-stage redox process. The first stage is a one-electron step leading to the intermediate L-ascorbate(II) radical anion (also called monodehydroascorbic acid or semidehydroascorbic acid). A further one-electron stage follows, leading to the formation of L-dehydroascorbic acid [28,29].

The electrochemical behaviors of paracetamol and ascorbic acid on the bare BDDE, the lead film covered BDDE as well as the BDDE and GCE covered with Nafion and lead films were studied by differential pulse voltammetry. Fig. 1A shows differential pulse voltammograms obtained at unmodified and modified BDDEs in a 0.1 mol L^{-1} acetate buffer (pH 6.0 ± 0.1) in the presence of 2×10^{-5} mol L^{-1} PA, 2.5×10^{-5} mol L^{-1} AA, while scanning in the positive direction. Fig. 1B shows differential pulse voltammograms for a mixture of PA and AA recorded with the boron-doped diamond electrode and glassy carbon electrode modified with Nafion and lead films. As observed, the use of the lead film as a modifier of BDDE provided a remarkable peak separation about of 470 mV between anodic peak potentials of PA and AA with a substantial increase in the anodic peak currents. In the presence of the Nafion and lead layers onto the BDDE surface a slight increase in the anodic peak current of PA and a shifting towards less positive value of the AA oxidation peak were observed, when compared to the BDDE modified with lead film. However, the peak separation of PA and AA was still very satisfactory (430 mV). According to the literature data one of the advantages of using a Nafion coating is the improvement of mechanical stability of mercury and bismuth layers [30,31]. Thus, repeatability studies were carried out on the BDDEs modified with lead film as well as Nafion and lead films. It has to be noted that the lead film deposited onto the Nafion covered BBDE surface was stripped off electrochemically after each measurement cycle at the potential of +1.0 V for 30 s. The repeatability was determined by successive measurements (n=20) of paracetamol (2×10^{-5} mol L^{-1}) and ascorbic acid (2.5×10^{-5} mol L^{-1}) solution. The BDDE was covered by the Nafion film one time before twenty consecutive deposition of the lead film and determination of PA and AA. The relative standard deviations values were 7.5% and 1.8% (for PA), and 13.3% and 5.9% (for AA) for the BDDE modified with lead film and the BDDE modified with Nafion and lead films, respectively. These

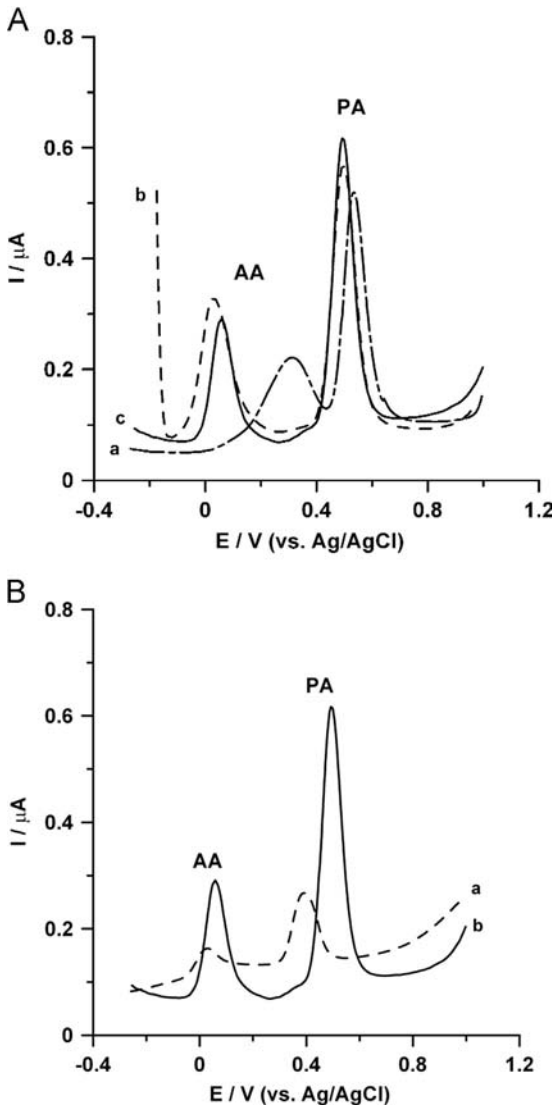

Fig. 1. (A) Differential pulse voltammograms obtained at (a) a bare BDDE, (b) the BDDE modified with lead film, (c) the BDDE modified with Nafion and lead films (solid line) in a 0.1 mol L^{-1} acetate buffer (pH 6.0 ± 0.1) in the presence of 2×10^{-5} mol L^{-1} PA, 2.5×10^{-5} mol L^{-1} AA and 0 (curve a) or 5×10^{-5} mol L^{-1} Pb(II) (curves b and c). (B) Differential pulse voltammograms obtained at (a) the GCE modified with Nafion and lead films, (b) the BDDE modified with Nafion and lead films (solid line) in solutions containing 2×10^{-5} mol L^{-1} PA, 2.5×10^{-5} mol L^{-1} AA and 5×10^{-5} mol L^{-1} Pb(II). The lead film was deposited for 60 s at −1.45 V. The Nafion film coating was carried out by applying a 0.5 μL drop of 1% (w/v) Nafion solution. DPV parameters: amplitude of 50 mV, modulation time of 40 ms, scan rate of 20 mV s^{-1}.

results clearly indicated that the Nafion layer improves the repeatability of paracetamol and ascorbic acid signals. This phenomena can be connected with a better stability of the lead film deposited onto the Nafion layer. This finding confirms the advantages of using the Nafion and lead films as modifiers of the BDDE surface for the simultaneous determination of PA and AA. At the GCE and BDDE modified with Nafion and lead films, well-shaped and easy to measure oxidation peaks of PA and AA were observed (Fig. 1B). Taking into account the surface areas of GCE (1.77 mm^2) and BDDE (7.09 mm^2) it can be stated that the sensitivities for PA and AA are similar on the two electrodes. However, the background current is lower at the BDDE modified with Nafion and lead films than at the GCE modified with polymer and Pb layers. Moreover, the use of BDDE as a support for the Nafion and lead films provided a larger separation of peak potentials (430 mV) than the peak separation of PA and AA obtained at GCE (360 mV).

Therefore, the BDDE was confirmed as the best choice for further studies.

3.2. Composition of measurement solution

The influence of the following supporting electrolytes on the peak resolution and peak currents was checked: nitric acid, acetate buffer (pH 4.6, 5.7 and 6.0 ± 0.1), PIPES buffer (pH 6.9 ± 0.1), sodium hydroxide, ammonium buffer (pH $= 8.3 \pm 0.1$) at 0.1 mol L^{-1}. For stabilization of Pb(II) at mild alkaline conditions, potassium sodium tartrate (0.1 mol L^{-1}) was added to an ammonium buffer used as a supporting electrolyte. Additionally, it has to be mentioned that the lead film can be plated in situ in solution of sodium hydroxide because of Pb(II) instead of hydrolyzing, forms complexes with OH^- ($Pb(OH)_4^{2-}$). These complexes are soluble in aqueous media so can undergo electrochemical reduction on the electrode surface [21]. The measurements were carried out in solutions containing 2×10^{-5} mol L^{-1} PA, 2.5×10^{-5} mol L^{-1} AA and 5×10^{-5} mol L^{-1} Pb (II). The best peak resolution and peak currents of PA and AA were obtained in acetate buffer (pH 6.0 ± 0.1). Thus, further measurements were performed in acetate buffer medium, and its concentration was evaluated from 0.05 to 1 mol L^{-1}. Highest values of peak currents with good resolution were obtained at 0.1 mol L^{-1} concentration, thus being adopted for subsequent experiments.

The peak currents of PA and AA recorded at the BDDE modified with Nafion and lead films were investigated at increasing Pb(II) concentrations to establish a suitable 'standard' Pb(II) concentration. 2.5×10^{-5} mol L^{-1} additions of $Pb(NO_3)_2$ were made to a solution containing 0.1 mol L^{-1} acetate buffer (pH 6.0 ± 0.1) 2×10^{-5} mol L^{-1} PA and 2.5×10^{-5} mol L^{-1} AA, and deposited at -1.45 V for 60 s under stirring. The obtained results were presented in Fig. 2. From concentrations of Pb(II) of 5×10^{-5} to 1.25×10^{-4} mol L^{-1} for PA and AA maximal, stable and well reproducible peaks were observed. On the basis of these results the $Pb(NO_3)_2$ concentration of 5×10^{-5} mol L^{-1} was chosen for further study.

3.3. Optimization of electrode modification

In this work the Nafion covered boron-doped diamond electrode was prepared by the application of a drop of Nafion solution onto the electrode surface. Then, the electrode was modified by the lead film using an in situ plating method. The thickness of the

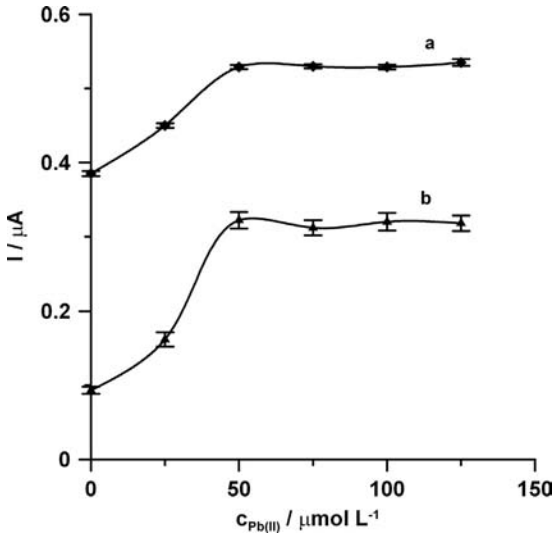

Fig. 2. Effect of the Pb(II) concentration on the peaks currents of 2×10^{-5} mol L^{-1} PA (a) and 2.5×10^{-5} mol L^{-1} AA (b). Other measurements parameters are the same as in Fig. 1. Vertical error bars represent the standard deviation ($n = 3$).

Nafion film is directly connected to the concentration of Nafion in the coating solution. Assuming a uniform distribution of the Nafion film on the electrode surface, the average thickness of the Nafion film, l_{Nafion}, can be calculated using the formula [30]:

$$l_{Nafion} = \frac{m_{Nafion}}{\pi R^2 d_{Nafion}}$$

where m_{Nafion} is the mass of the Nafion attached to the electrode surface, d_{Nafion} is the density of the Nafion film (1.58 g cm^{-3}) and R is the electrode radius (1.5 mm). By using a 0.5 μL drop of Nafion solution containing 0.5, 1, 1.5, 2 and 4% w/v Nafion, films were produced with thickness of 0.22, 0.45, 0.67, 0.9 and 1.8 μm.

The influence of the Nafion concentration in the range from 0.5 to 4% w/v on the voltammetric responses of 2×10^{-5} mol L^{-1} PA and 2.5×10^{-5} mol L^{-1} AA at the boron-doped diamond electrode modified with Nafion and lead layers was studied. The lead film was deposited from the solution containing 5×10^{-5} mol L^{-1} Pb(II) for 60 s at -1.45 V. The obtained results indicate that the Nafion films produced by 0.5% and higher than 1% w/v Nafion solution was too thin or thick for PA determination. In the case of AA decline in the sensitivity was observed for a higher than 1.5% w/v concentration of Nafion solution. If the Nafion layer is too thin, some parts of the electrode can be not covered and the efficiency lower. In the case of too thick Nafion film, an effective mass transport can be hindered. For further experiments a 0.5 μL drop of Nafion solution at concentration of 1% w/v was used for the preparation of the BDDE modified with Nafion and lead films.

The thickness of the lead film could be controlled by varying the Pb(II) concentration in the sample solution as well as the potential and the time of the lead film deposition. The influence of the Pb(II) concentration on the voltammetric responses of PA and AA was investigated in Section 3.2. Next, the potential of the lead film deposition onto the Nafion covered boron-doped diamond electrode surface was changed in the range from -1.3 to -1.5 V and its influence on the peak currents of 2×10^{-5} mol L^{-1} PA and 2.5×10^{-5} mol L^{-1} AA was studied. The ascorbic acid peak attained maximal value as the deposition potential of the lead film was -1.45 V. In the case of paracetamol determination the peak currents were constant in the range tested of the lead film deposition. For further study the potential of -1.45 V was chosen. Next, the time of the lead film deposition onto the Nafion covered BDDE surface was studied. The deposition time was changed in the range from 0 to 120 s and its influence on the oxidation peaks of PA and AA was studied. It was observed that the oxidation peak currents of 2×10^{-5} mol L^{-1} PA and 2.5×10^{-5} mol L^{-1} AA attained maximal and stable values as the lead film was deposited in the time range from 50 to 120 s, so for further measurements the lead film deposition time of 60 s was chosen.

The surface of the electrode was characterized by optical microscopy and scanning electron microscopy (Fig. 3). Optical microscopy images show the surface of Nafion covered BDDE (Fig. 3a) and Nafion covered BDDE plated with lead (Fig. 3b). The lead deposit on the Nafion covered boron doped diamond electrode is clearly visible as a black deposit covering a large proportion of the electrode surface (Fig. 3b). The SEM image of the surface of the BDDE modified with Nafion and lead films as well as mapping EDS of the region presented in SEM image are showed in Fig. 3c and d, respectively. Brighter areas (gray and white) in the EDS map indicate an abundance of Pb. The EDS map of the lead coating on the Nafion covered electrode (Fig. 3d) revealed that the accumulated lead film was relatively, but not entirely, uniform in term of thickness and distribution over the surface. The presence of locations with higher thickness of the lead film appeared as the brightest patches (white) can be connected with higher thickness of the Nafion film in these places (Fig. 3d). This can be attributed to more efficient replanting of Pb at the end of the forward pulse as

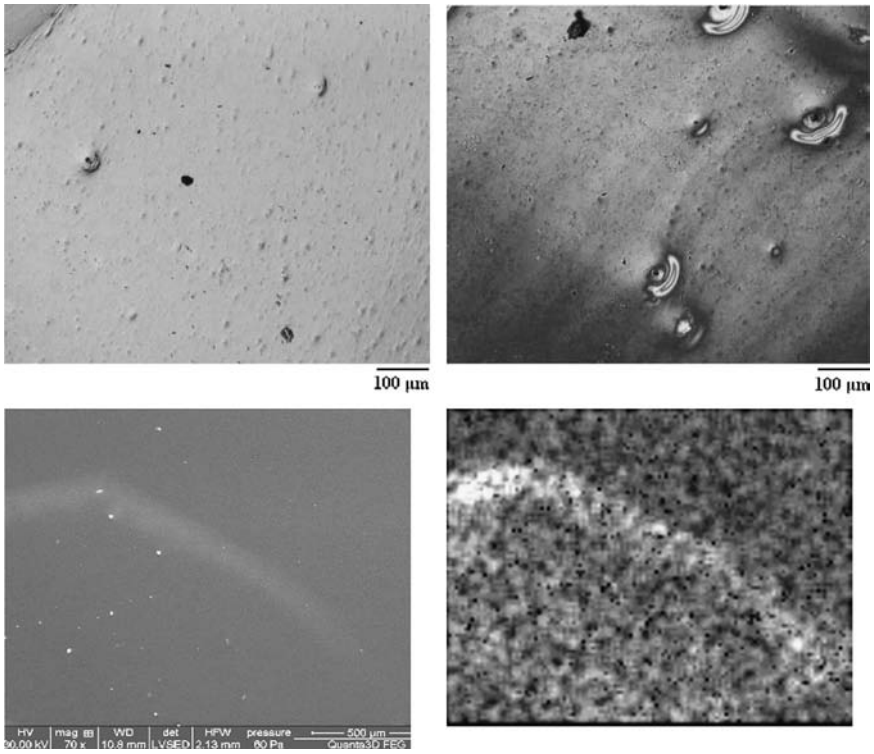

Fig. 3. Optical microscopy images of the surface of: (a) the Nafion covered BDDE, (b) the Nafion covered BDDE plated with lead. SEM image (c) of the surface of the BDDE modified with Nafion and lead films as well as mapping EDS (d) of the region presented in SEM image. Brighter areas (gray and white) in the EDS map indicate an abundance of Pb. The lead film was deposited for 60 s at -1.45 V from solution containing 0.1 mol L^{-1} acetate buffer (pH 6.0 $\pm$ 0.1) and concentration of 5×10^{-5} mol L^{-1} Pb(II). The Nafion film coating was carried out by applying a 0.5 μL drop of 1% (w/v) Nafion solution.

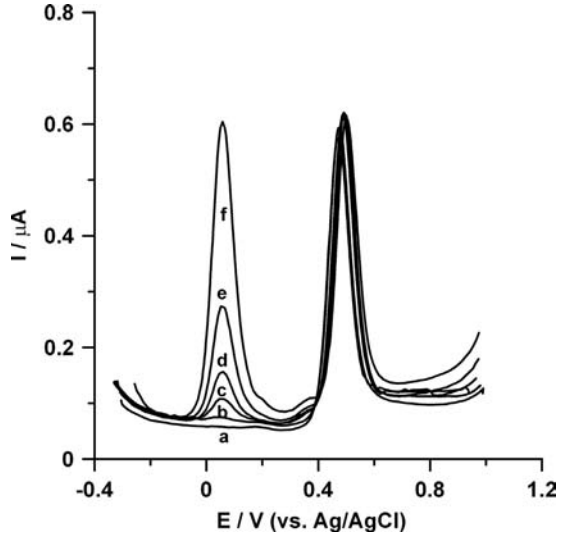

Fig. 4. Differential pulse voltammograms obtained in 0.1 mol L^{-1} acetate buffer (pH 6.0 $\pm$ 0.1) at the BDDE modified with Nafion and lead films in the course of determination of low concentrations of AA in the presence of PA at concentration of 2×10^{-5} mol L^{-1}: (a) 0, (b) 1×10^{-6}, (c) 5×10^{-6}, (d) 1×10^{-5}, (e) 2×10^{-5}, and (f) 5×10^{-5} mol L^{-1} AA. The Nafion film coating was carried out by applying a 0.5 μL drop of 1% (w/v) Nafion solution. DPV parameters: amplitude of 50 mV, modulation time of 40 ms, scan rate of 20 mV s^{-1}.

the Nafion film helped confine the cationic oxidized species close to the electrode surface.

3.4. Optimization of instrumental parameters of DPV

In the present studies differential pulse scan mode for the voltammetric determinations of paracetamol and ascorbic acid was used. The influence of different levels of the instrumental parameters of DPV on the peaks height for 5×10^{-5} mol L^{-1} PA and AA was investigated. It was found that modulation amplitude of 50 mV, modulation time of 40 ms and scan rate of 20 mV s^{-1} appeared to be the optimum values of differential pulse scan mode parameters, so were used for calibration graphs constructions.

3.5. Calibration graphs

In order to evaluate the electrochemical response of different concentration of PA and AA when both substances are present in the sample solution, the described below studies under the optimal analytical conditions were carried out. For this, PA concentration was changed in the range from 5×10^{-7} to 1×10^{-3} mol L^{-1}, while the AA concentration was equal to 2.5×10^{-5} mol L^{-1}. Analogously, the influence of the AA concentration was checked by increasing the AA concentration from 1×10^{-6} to 1×10^{-3} mol L^{-1}, while setting the PA concentration at 2×10^{-5} mol L^{-1}. An examination of the obtained results allowed concluding that the peaks oxidation of PA and AA are fairly constant, while the peak currents of AA and PA increase as their concentrations are increased, respectively.

The obtained results also allowed to the construction of the calibration graphs of PA and AA when both substances are presented in the sample solution. The calibration graph for PA on the BDDE modified with Nafion and lead film in the presence of 2.5×10^{-5} mol L^{-1} AA was linear from 5×10^{-7} to 2×10^{-4} mol L^{-1} and obeyed the equation $y = 0.016x + 0.025$, where y is the peak current (μA) and x is the paracetamol concentration (μmol L^{-1}). The correlation coefficients (r^2) was 0.9983. The calibration graph for AA in the presence of 2×10^{-5} mol L^{-1} PA was linear from 1×10^{-6} to 5×10^{-4} mol L^{-1} and obeyed the equation $y = 0.010x + 0.039$, where y is the peak current (μA) and x is the ascorbic acid concentration (μmol L^{-1}). The correlation coefficients (r^2) was 0.9987. The detection limits of PA and AA

Table 1
Comparison of various electroanalytical method proposed for the simultaneous determination of PA and AA.

Electrode	Linear range [μmol L^{-1}]	Detection limits [μmol L^{-1}]	Peak resolution	Reference
BDDE	PA: 10–100 AA: 10–100	0.85 0.77	220 mV	[32]
BDDE	PA: 10–70 AA: 10–100	0.97 0.80	220 mV	[33]
MWCNPE	PA: 39.4–146.3 AA: 100–700	2.1 7.1	350 mV	[34]
Pt/PMT/Pd	PA: – AA: –	– –	400 mV	[13]
CNT-TCP	PA: 0.1–100 AA: 1–100	0.05 0.3	303 mV	[14]
Pd/Al	PA: 100–3000 AA: 100–3000	50 50	400 mV	[15]
SWCNT/CCE	PA: 0.2–150 AA: 5–700	0.12 3	320 mV	[16]
GCE/MWCN-Polyhis	PA: 0.25–0.1 AA: 25–2500	0.032 0.76	373 mV	[17]
BDDE modified with Nafion and lead films	PA: 1–200 AA: 1–500	0.17 0.52	430 mV	This work

MWCNPE: multiwalled carbon nanotube paste electrode, Pt/PMT/Pd: poly(3-methylthiophene)/palladium sub-micro-modified Pt electrode, CNT-TCP: thionine immobilized multi-walled carbon nanotube modified carbon paste electrode, Pd/Al: aluminum electrode modified by thin layer of palladium, SWCNT/CCE: single-walled carbon nanotube-modified carbon-ceramic electrode, and GCE/MWCN-Polyhis: glassy carbon electrode modified with multi-wall carbon nanotubes dispersed in polyhistidine.

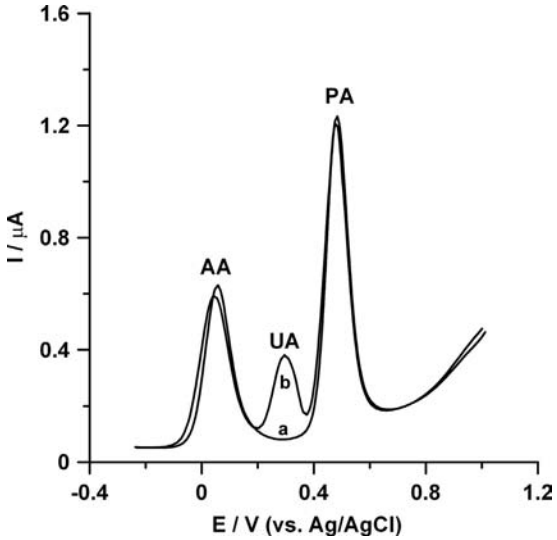

Fig. 5. Differential pulse voltammograms obtained at the BDDE modified with Nafion and lead films in the course of determination of: (a) 5×10^{-5} mol L^{-1} AA and 5×10^{-5} mol L^{-1} PA and (b) as (a)+5×10^{-4} mol L^{-1} uric acid (UA). The lead film was deposited for 60 s at −1.45 V. The Nafion film coating was carried out by applying a 0.5 μL drop of 1% (w/v) Nafion solution. DPV parameters: amplitude of 50 mV, modulation time of 40 ms, scan rate of 20 mV s^{-1}.

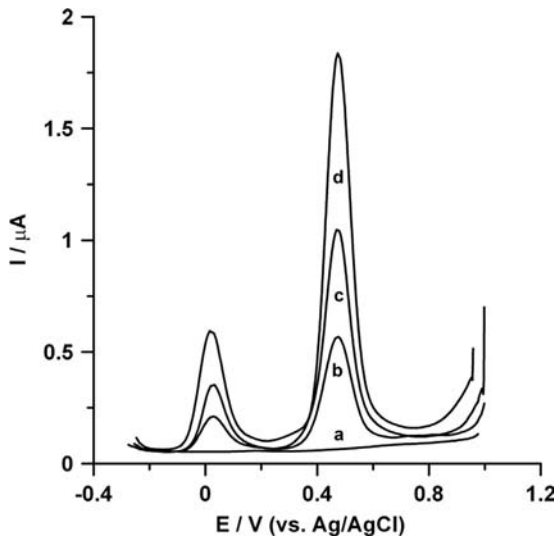

Fig. 6. Differential pulse voltammograms obtained in 0.1 mol L^{-1} acetate buffer (pH 6.0 ± 0.1) at the BDDE modified with Nafion and lead films in the course of determination of PA and AA in commercially available pharmaceutical formulation (sample B): (a) background, (b) sample, (c) as (b)+1×10^{-5} mol L^{-1} AA and 3×10^{-5} mol L^{-1} PA, and (d) as (b)+2.5×10^{-5} mol L^{-1} AA and 6.5×10^{-5} mol L^{-1} PA. Other measurements parameters are the same as in Fig. 5.

Table 2
Results obtained for the simultaneous determination of paracetamol and ascorbic acid in pharmaceutical formulations using DPV (proposed) and HPLC-DAD methods.

Sample	Compound	Label value (mg)	HPLC-DAD value[a] (mg) ± RSD (%)	DPV value[a] (mg) ± RSD (%)	E_1[b] (%)	E_2[c] (%)
A	PA	750	772.5 ± 0.5	767.0 ± 0.6	2.27	−0.71
	AA	60	61 ± 4	60 ± 3	0	−1.64
B	PA	500	497.9 ± 0.5	489.0 ± 0.4	−2.20	−1.79
	AA	200	182 ± 2	190.9 ± 0.8	−4.55	4.89

[a] Average of 3 measurements.
[b] Relative error 1 (%) = 100 × (voltammetric value − label value)/label value.
[c] Relative error 2 (%) = 100 × (voltammetric value − HPLC value)/HPLC value.

estimated from 3 times the standard deviation (n=5) for the lowest determined concentration of paracetamol and ascorbic acid divided by the slope of the linear regression equation are equal to 1.7×10^{-7} and 5.2×10^{-7} mol L^{-1}, respectively. The voltammograms obtained in the course of the determination of low concentrations of AA in the presence constant concentration of PP are presented in Fig. 4.

Additionally, paracetamol and ascorbic acid were determined by simultaneously changing their equal concentrations. The calibration graphs for PA and AA present a good linear response in the concentration range 5×10^{-7}–2×10^{-4} mol L^{-1} and 1×10^{-6}–5×10^{-4} mol L^{-1}, respectively. The corresponding calibration equations are $y = 0.015x + 0.032$ for PA ($r^2 = 0.9976$) and $y = 0.007x + 0.026$ for AA ($r^2 = 0.9985$), where y is the peak current (μA) and x is the paracetamol or ascorbic acid concentration (μmol L^{-1}). The

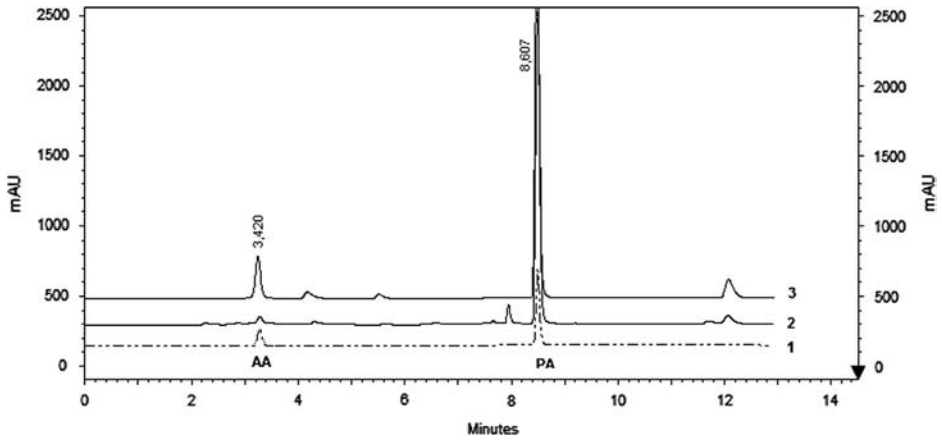

Fig. 7. Chromatogram obtained with use mixture of 0.025% TFA in water (solvent A) and acetonitrile containing 0.025% TFA (solvent B) according to gradient elution program: 0–3.0 min, 100% A; 3–4 min, 100–85% A; 4–10 min, 85% A and 15% B. Line 1 – standards, line 2 – sample A, and line 3 – sample B.

detection limits of PA and AA calculated as above are 1.75×10^{-7} and 5.5×10^{-7} mol L^{-1}, respectively.

Table 1 shows the comparison of the analytical parameters obtained by the proposed method with those obtained by other electrochemical methods proposed in the literature for the simultaneous determination of PA and AA. The comparison to other electrochemical sensors for the simultaneous determination of PA and AA shows that the proposed method using the BDDE modified with Nafion and lead films provides remarkable peaks resolution, high linear range and simplicity (when compared to other modified electrodes). The limits of detection of PA and AA are lower, comparable or in some cases a little bit higher than those obtained using other electrochemical sensors. It has to be noted that the proposed procedure of the sensor preparation is much simpler than procedures described in the literature with a lower detection limit [14,17].

3.6. Potential interfering species

Under optimal condition of DPV, potential interfering species including uric acid, caffeine, glucose, dopamine and 4-aminophenol were evaluated individually on the sensor responses for simultaneous PA and AA determination at 5×10^{-5} mol L^{-1} concentration. The effect of each for foreign species was evaluated up to proportion (analyte:interferent) 1:10 mol/mol, being observed no significant changes on peaks resolution and peak currents of PA and AA. The voltammograms obtained in the course of simultaneous determination of 5×10^{-5} mol L^{-1} PA and AA in the absence and presence of 5×10^{-4} mol L^{-1} uric acid are presented in Fig. 5. It can be seen, that the BDDE modified with Nafion and lead films can be applied for simultaneous determination of paracetamol, uric acid and ascorbic acid.

3.7. Real samples analysis

Commercial pharmaceutical formulations containing paracetamol and ascorbic acid were analyzed to simultaneously determine both substances in order to evaluate the validity of the herein proposed method. Recovery experiments carried out to evaluate matrix effects after standard-solution additions yielded a good average recovery for both substances (100.04% for PA and 97.73% for AA), indicated that there were no important matrix interferences for the sample analyzed by the proposed DPV method.

Table 2 presents the values of the amounts of PA and AA simultaneously determined in the pharmaceutical formulations employing the proposed DPV method and the HPLC-DAD method for comparison. As it can be seen in this table, no significant differences were observed between the values found with the DPV and HPLC techniques for the amounts of PA and AA in the pharmaceutical formulations. Applying the paired *t*-test to the results obtained using both methods, the calculated *t* values (1.77 for paracetamol and 1.96 for ascorbic acid) were smaller than the critical value (3.18, α=0.05). These results indicate that there are no important differences between the obtained results at the 95% confidence level. The voltammograms obtained in the course of PA and AA determination in pharmaceutical formulation (sample B) at the boron-doped diamond electrode modified with the Nafion and lead films are presented in Fig. 6. The chromatogram of standards and analyzed samples obtained with use HPLC-DAD method is shown in Fig. 7.

4. Conclusions

The feasibility of the boron-doped diamond electrode modified with Nafion and lead films for the simultaneous PA and AA determination by DPV was confirmed. The proposed method using this new sensor provided a remarkable peak separation of PA and AA of 430 mV and low limits of detection of 1.7×10^{-7} and 5.2×10^{-7} mol L^{-1}, respectively. No drawbacks related with fouling of the electrode surface and interferences were verified, making the method very suitable to obtain precise and accurate results for PA and AA determination in commercial pharmaceutical formulations. The method is also characterized by simplicity, low cost and does not require complicated sample treatments before analysis. The proposed method with the use of the boron-doped diamond electrode modified with Nafion and lead films was successfully applied for the simultaneous determination of paracetamol and ascorbic acid in the commercially available pharmaceutical formulations, with results similar to those obtained by high performance liquid chromatography coupled with diode array detector.

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