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Possibilities of Voltammetric Determination of Pyridine Herbicide Picloram Using Boron-Doped Diamond Electrodes

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Abstract: Voltammetric behavior of a wide-spread used pyridine herbicide picloram (PCR) was investigated using two different types of boron-doped diamond working electrodes: commercial available and self-assembled. PCR provided one irreversible oxidation peak in an acidic medium occurring at very positive potential (about +1.5 V *vs.* Ag/AgCl/ 3 mol L^{-1} KCl) in both cases. 1 mol L^{-1} sulfuric acid was selected as the most suitable supporting electrolyte for electrochemical oxidation of PCR. Differential pulse voltammetric methodology was elaborated and its operating parameters were optimized to achieve the best analytical performance for determination of PCR. Both working electrodes provided high repeatability, wide linear concentration range and low limit of detection. Applicability of proposed method was verified by analysis of PCR in model samples of tap and natural waters and human urine, respectively.

Keywords: Picloram; Boron-doped diamond electrode; Voltammetry; Determination.

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Introduction

EPA (United States Environmental Protection Agency) defines pesticide as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Generally, pesticides are a large group of various compounds and could be classified, according to the target organism, as herbicides, insecticides, fungicides and other substances like plant regulators, defoliants or desiccants [1]. Expansion of using pesticides could be observed in the last 100 years, primarily due to the high requirements on the agricultural production. Ideally, pesticides should be highly selective and destroy only the pest without harming other living organisms but most pesticides are not selective and could harm, even in a low concentration, to non-target organisms [2,3]. Some of them could persist in the environment, accumulate among a food chain and could be detected in food samples even over maximum residua limit (MRL), e.g. [4-10]. Therefore, the development of sensitive analytical methods for the determination of different pesticides, which allow to be applied rapidly, reliably and without complicated samples pretreatment, is still highly actual. Recently, various analytical methods such as gas chromatography (GC) [11], liquid chromatography (LC) [12], spectrophotometry [13] or enzyme-linked immunosorbent assays (ELISA) [14] have been described for pesticides analysis.

Electrochemical methods represent a good alternative to the above mentioned analytical approaches, especially due to the low costs of instrumentation, fast and sensitive analysis and possibility of miniaturization. Many substances applying as pesticides contain the different oxidisable and reducible function groups and thus various electrochemical methods could be employed for their analysis. Development of new electrode materials which could replace liquid mercury, due to its alleged toxicity, is one of the current trends of the electrochemistry [15]. A boron-doped diamond (BDD) is one of the novel carbon-based material, which has been studied more in detail in the last twenties years, due to its excellent electrochemical properties like wide potential range, very low and stable background current, high thermal conductivity or mechanical and chemical stability [16-20]. This electrode material has also been employed as a sensitive electrochemical tool in determination of various pesticides or their degradation products, e.g. carbamate pesticides [21,22], nitrophenols [23-28], pentachlorophenol [29], insecticides parathion [30] and methylparathion [26], herbicide atrazine [31] and fungicides bupirimate [32], dimetomorph [33] and kresoxim-methyl [34].

Picloram (PCR, Fig. 1) is the most persistent member of pyridine herbicide family, which acts as an auxin mimic substance [35]. Its half-life in soils, depending on conditions of application, type and pH of the soil and on the moisture, can vary from one month to three years. This compound is very good water soluble and thus mobile in soils, which could lead to contamination of the natural and ground waters [35-39]. The MRL of PCR for natural and tap waters, respectively, is defined by EPA as 0.5 mg L^{-1} (2 μmol L^{-1}). Analytical methods like gas or liquid chromatography (e.g. in [40-44]), spectrophotometry [45] and fluorescence [46] in various constructions have been already utilized as effective tools for determination of PCR.

Voltammetric methods, especially in combination with mercury electrodes, have also been applied for analysis and mechanistic studies of PCR. Gilbert and Mann utilized dropping mercury electrode (DME) in combination with pulse polarography [47] and subsequently Whittaker and Osteryoung used the same method for determination of PCR and other pyridine herbicide Dowco 290 [48]. Massaroppi et al. employed static mercury drop electrode (SMDE) and square wave voltammetry (SWV) for electroanalytical determination of this herbicide [49]. Sequential injection SWV with hanging mercury drop electrode (HMDE) was applied as a tool for determination of PCR as well [50]. Electrochemical reduction of PCR and clopyralid (other member of pyridine herbicide family) on a mercury pool electrode was investigated by Mellado et al. [51]. The same authors also dealt with adsorption-desorption processes on mercury [52,53] and carbon electrodes [53].

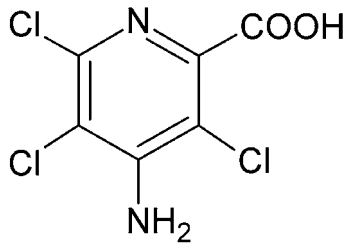

Fig. 1: *The chemical structure of picloram.*

The comparison of the voltammetric behavior of PCR on two boron-doped diamond electrodes (commercial BDDE and self-assembled (SA-BDDE)) is investigated in the present paper. Optimum working conditions for differential pulse voltammetric determination of PCR on both working electrodes were found and the proposed sensitive methods were employed for the analysis of PCR in model environmental and biological samples.

Experimental

Chemicals and Reagents

All chemicals used for the preparation of the standard solutions, supporting electrolytes and other stock solutions were of p.a. purity. Picloram (CAS No. 1918-02-1, Sigma Aldrich, Czech Republic) was used as received without any further purification. The standard solutions of PCR were prepared by dissolution of PCR powder in 50 % acetonitrile (Lach-ner, Czech Republic) and the solutions were stored in a glass flask in a refrigerator. PCR working solutions were prepared daily by dilution of the stock solution with the supporting electrolyte. Various supporting electrolytes such as nitric acid and sulfuric acid were purchased from Lachema (Brno, Czech Republic). Britton-Robinson buffer solution (BR) was prepared by mixture of the same concentrations (0.04 mol L^{-1}) of orthophosphoric acid, boric acid and acetic acid (all three purchased from Lachema, Brno, Czech Republic) in deionized water and adjusting to the desired pH value with 0.2 mol L^{-1} sodium hydroxide (Lachema, Czech Republic). All solutions were prepared in double-distilled deionized water with resistivity greater than 18 MΩ cm.

Electrochemical Apparatus and Other Instrumentation

All measurements were provided in the three-electrode set up, where the commercial BDDE (active area: 7.07 mm^2) or SA-BDDE (active area: 0.43 mm^2) served as a working electrode, Ag/AgCl/3 mol L^{-1} KCl as a reference and platinum wire as an auxiliary electrode. The commercially available BDDE (Fig. 2a) was inserted in polyether ether ketone body with inner diameter of 3 mm, resistivity of 0.075 Ω cm and boron doping level of 1000 ppm (declared by Windsor Scientific Ltd, United Kingdom as a producer). The voltammetric measurements were performed with AUTOLAB PGSTAT 302N (Metrohm Autolab B.V., The Netherlands) potentiostat/galvanostat controlled by NOVA 1.7 software. All the pH values of solutions were measured using pH meter Model 215 (Denver Instrument, USA) with a combined electrode, which was daily calibrated with standard buffer solutions. The standard solutions of PCR were prepared using ultrasonic bath Bandelin Sonorex (Schalltec GmbH, Germany). All the potentials reported in this paper were given against Ag/AgCl/3 mol L^{-1} KCl at a laboratory temperature of 25 ± 2 ºC. The Raman spectrum was measured at a room temperature on ISA Dilor-Jobin Yvon-Spex Labram confocal system with 632.8 nm He-Ne

laser. Scanning electron microscopy (SEM) was carried out on JEOL JSM-7500F scanning electron microscope.

Fabrication of the Self-Assembled Sensor Based on Boron-Doped Diamond

The n-type Si(100) wafer with 1.4 µm thick SiO_2 layer (CVD, Oxford PlasmaLab 80) was used as a substrate. The Si substrates were cleaned with isopropanol and deionized water firstly and then they were seeded in the ultrasonic bath using a nanodiamond powder <10 nm (CAS No. 7782-40-3, Sigma Aldrich). The BDD films were deposited 4 hour (~1 µm) using double bias enhanced hot filaments reactor (HF CVD) [54]. A boron-doped nanocrystaline diamond was achieved by adding trimethylboron (TMB) to the 0.5% CH_4 and H_2 gas mixture. The B/C ratio in the gas phase was 10 000 ppm. The deposition was performed in the pressure 3 000 Pa at a temperature 650 ± 20 °C. The active area (0.43 mm^2) of working electrode was created in 400 nm SiO_2 (CVD, Oxford PlasmaLab 80) by using standard optical lithography and wet etching in BOE solution (6:1 volume ratio of 40% NH_4F in water to 49% HF in water). Subsequently, the electrode chip (10×3 mm^2) was electrically connected by Ag polymer paste (CB115, DuPont) to the printed circuit board's support and completely passivated by non-conducting paste (548X, DuPont). This sensor is shown in Fig. 2b.

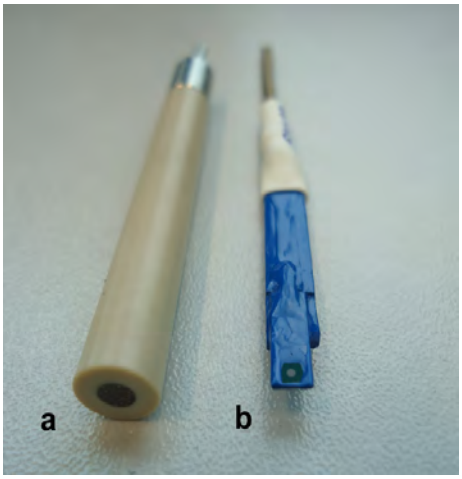

Fig. 2: *Photo of commercially available BDDE (a) and self-assembled BDDE (b).*

Voltammetric Measurements

A known volume of the PCR standard solution was pipetted into a 20 mL volumetric flask and then filled up with the supporting electrolyte. This solution was subsequently transferred quantitatively into a voltammetric cell. Cyclic voltammetry (CV) was used for the

investigation of dependence between voltammetric response of PCR and pH of the supporting electrolyte and for studying the effect of scan rate on the current response of PCR. Differential pulse voltammetry (DPV) was examined for the purpose of quantification. Five cyclic voltammograms were obtained for each measurement, and the last scan was always considered for the evaluation and making the figures reported in this paper. The DP voltammograms were recorded after optimization of instrumental parameters under followed working conditions: E_{in} = +0.6 V, E_{fin} = 2 V, modulation amplitude 75 mV, modulation time 50 ms and scan rate 20 mV s^{-1}. Prior to use at the beginning of every work way, BDD electrode surface was rinsed with deionized water and anodically pretreated by applying +2 V during 180 s in 1 M H_2SO_4 solution in order to clean the electrode surface (get rid of any impurities) followed by the cathodic pretreatment at −2 V during 180 s to attain predominance of hydrogen termination of electrode surface.

The calibration curve was constructed from the average of five replicate measurements for each PCR calibration solution. The peak currents (I_p) recorded using CV and DPV were evaluated from the straight lines connecting the minima before and after the peak maximum without any background correction. The current densities (j) of measured currents were calculated due to the different active areas of the working electrodes. The linear least-square regression in OriginPro 7.5 (OriginLab Corporation, USA) was used for the evaluation of calibration curve and the relevant results (slope and intercept) were reported with confidence interval for 95% probability. The limit of detection (LD) was calculated as three times the standard deviation for the blank solution (supporting electrolyte) divided by the slope of the calibration curve.

Preparation of the Model Samples

Commercially Available BDDE. One sample of a tap water, three samples of natural waters and sample of human urine were analyzed using BDDE. The tap water was sampled from the water supply in Bratislava (Slovak Republic), samples of natural waters were collected from the rivers Elbe (Pardubice, Czech Republic) and Danube (Bratislava, Slovak Republic) and from the nameless brook which is closed to the agricultural area (Kameničany, Northwest part of the Slovak Republic). The water samples were analyzed with no further pretreatment or purification. None of the water samples contained measurable amount of PCR. Therefore, all water samples were spiked with stock solution of PCR (200 µL of 1 mmol L^{-1} in 100 mL of water sample) to concentration level of 2 µmol L^{-1} (0.5 mg L^{-1}), which is declared by EPA as

a maximum contaminant level for PCR. 10 mL of spiked water was diluted with the supporting electrolyte (1 mol L^{-1} H_2SO_4) to 20 mL. This solution was subsequently transferred quantitatively into the voltammetric cell and analyzed.

The sample of human urine was obtained from non-smoker female volunteer of the age of 28. The sample was stored in the refrigerator after sampling and it was analyzed without any further pretreatment about 15 hours after sampling. 1 mL of the urine was pipetted into a volumetric flask, then spiked with stock solution of PCR (50 µL of 1 mmol L^{-1}) a filled up with the supporting electrolyte (1 mol L^{-1} H_2SO_4) to 20 mL. The prepared solution was quantitatively transferred into the voltammetric cell and analyzed.

Self-Assembled BDDE. One sample of a tap water and one sample of natural water were analyzed using SA-BDDE. The tap water was sampled from the water supply in Bratislava (Slovak Republic). Sample of natural water was collected from the river Danube (Bratislava, Slovak Republic) and analyzed with no further pretreatment or purification. None of the water samples contained measurable amount of PCR. Therefore, the water samples were spiked with stock solution of PCR to concentration level of 2 µmol L^{-1} (tap water) and 10 µmol L^{-1} (river water), respectively. 10 mL of spiked water was diluted with the supporting electrolyte (1 mol L^{-1} H_2SO_4) to 20 mL. This solution was subsequently transferred quantitatively into a voltammetric cell and analyzed.

Determination of PCR. The content of PCR in all samples was determined by standard addition method, when at least two standard additions were performed. Each determination was repeated five times and relative standard deviation of 5 repeated determinations ($RSD_D(5)$) was calculated [55].

Results and Discussion

Diamond Film Properties of SA-BDDE

Fig. 3 shows SEM image of detail of the edge BDD/SiO_2 after BOE etching and Raman spectrum of the BDD sensor. The film morphology consists of the diamond crystals size from 200 to 300 nm and completely overcoated with SiO_2, which constitutes a perfectly defined surface of the working electrode (Fig. 3a). The Raman spectrum reveals a characteristic

spectrum for boron-doped polycrystalline diamond films (Fig. 3b). One sharp peak centered at 1 325 cm^{-1} (called as "D" disordered graphite, represent sp^3 carbon bonding), the broad band centered at 1 580 cm^{-1} (called "G" graphitic sp^2 carbon phases) and two broad bands of approximately 500 and 1 220 cm^{-1} for a boron peak originates from local vibrational modes of boron pairs [56]. The bands centered at 520 cm^{-1} and 960 cm^{-1} are assigned to first and second-order of Si [57].

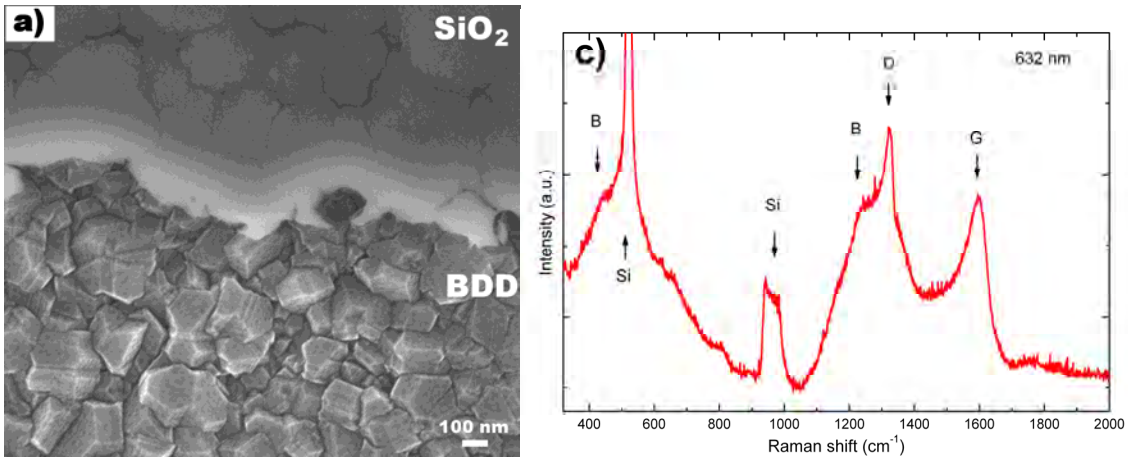

Fig. 3: *SEM image of detail of the edge BDD/SiO_2 after BOE etching (a) and Raman spectrum of BDD (b).* Legend: *B* – boron; *Si* – silicon; *D* – disordered graphite (sp^3); *G* – graphitic carbon (sp^2).

The Influence of Supporting Electrolyte on the Voltammetric Response of Picloram

The choice of supporting electrolyte is essential for voltammetric analyses because it plays an important role in an electrode reaction of studied analyte. In our case, the effect was examined using CV from −1.5 V to 2 V (vs. Ag/AgCl 3 mol L^{-1} KCl) with 0.9 mmol L^{-1} PCR working solution. It was found, that PCR provided only one oxidation (anodic) peak in acidic media on both working electrodes as evidenced from Fig. 4. Black curves illustrate records before and after addition of PCR on SA-BDDE and grey curves belong to measurements on commercial BDDE. The arrows indicate direction of the scan. Various electrolytes like nitric acid, Britton-Robinson buffer and sulfuric acid, respectively, were tested as a suitable medium for recording of PCR oxidation and the highest current response was recorded in sulfuric acid. Therefore, the influence of sulfuric acid concentration on the PCR response was studied more in detail and obtained results are shown in Fig. 5, where the dependence between peak current density and peak potential, respectively, and concentration of sulfuric acid is shown. It is obvious, from Fig. 5a, that the highest current response density could be recorded in the more

concentrated sulfuric acid and thus 1 mol L^{-1} H_2SO_4 was selected as a supporting electrolyte for all subsequent analysis. On the other hand, the position of the recorded anodic signal was independent on the concentration of sulfuric acid and the signal did not move significantly, which is clear from Fig. 5b.

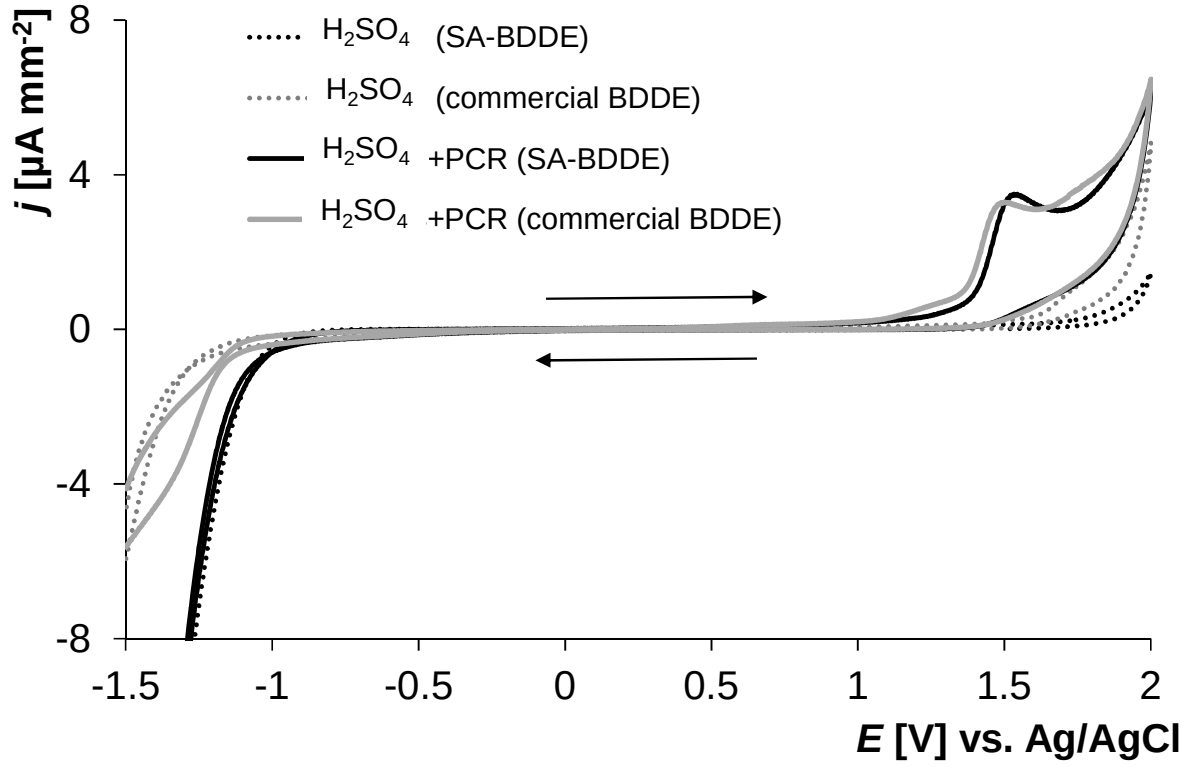

Fig. 4: *Cyclic voltammograms recorded on SA-BDDE and commercial BDDE, respectively, in 1 mol L^{-1} H_2SO_4 in absence (dotted lines) and presence (continuous lines) of 0.9 mmol L^{-1} PCR.* Experimental conditions: CV, E_{in} = −1.5 V, E_{fin} = 2 V, v = 100 mV s^{-1}.

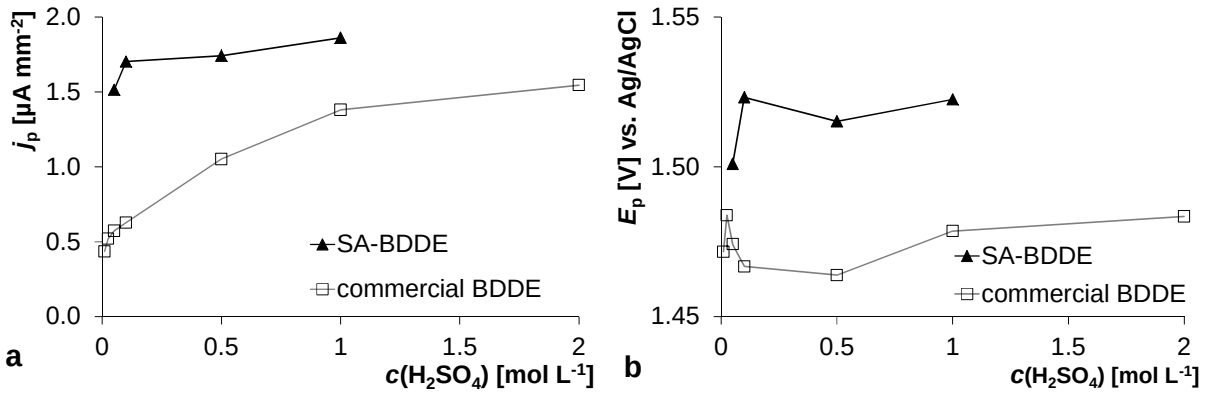

Fig. 5: *Effect of H_2SO_4 concentration on the current response density (j_p) (a) and peak position (E_p) (b), respectively, recorded on SA-BDDE (▲) and commercial BDDE (□).* Experimental conditions are the same as in Fig. 4.

The Effect of Scan Rate on the Voltammetric Response of Picloram

The effect of scan rate was examined using CV and 0.9 mmol L^{-1} PCR solution. Following scan rates were applied: 10, 25, 50, 75, 100, 150, 200 and 250 mV s^{-1} for this purpose on both working electrodes. It was ascertained, that followed signal increased linearly with the square root of the scan rate and obtained linear dependences could be described by Equation (1) for SA-BDDE and Equation (2) for commercial BDDE, respectively.

$$j_p\left[\mu A\, mm^{-2}\right] = (0.0244 \pm 0.0005)v^{1/2}\left[\left(mV\, s^{-1}\right)^{1/2}\right] + (0.0195 \pm 0.0065);\ R^2 = 0.9960 \qquad (1)$$

$$j_p\left[\mu A\, mm^{-2}\right] = (3.7031 \pm 0.0502)v^{1/2}\left[\left(mV\, s^{-1}\right)^{1/2}\right] + (0.0276 \pm 0.0016);\ R^2 = 0.9960 \qquad (2)$$

On the basis of obtained results, a diffusion-limited electrode reaction as a controlling process could be supposed. This result is oftentimes typical for this type of electrode material in determination of organic compounds due to low adsorption properties on the electrode surface. Moreover, the peak potential of the registered current response slightly shifts to the more positive potential values with the increasing scan rate thus confirming an irreversible character of the electrode reaction of PCR on the BDD surface.

Development of the Method

Two sensitive voltammetric methods DPV and SWV were tested as a suitable for voltammetric analysis of PCR. It was observed, that signals recorded using DPV were more stable and intensive and thus this technique was selected for further examination. Therefore, operating parameters of this method such as modulation amplitude and modulation time were investigated in order to optimize the experimental set-up for determination of PCR. The influence of the studied parameters was investigated using 50 μmol L^{-1} solution of PCR in case of SA-BDDE and 10 μmol L^{-1} solution of PCR for measurements on commercial BDDE. 1 mol L^{-1} H_2SO_4 was used as a supporting electrolyte for all experiments.

Modulation amplitudes from 5 to 150 mV were applied, when the fixed modulation time 50 ms was used and almost the same dependences were recorded on both BDDE. The signal of PCR increased with the raising modulation amplitude but it also expanded and shifted to the less positive potentials and thus the modulation amplitude of 75 mV was selected for all the subsequent analysis due to the convenient peak shape and sufficient

current response. On the other hand, the followed signal decreased with increasing modulation time, when modulation times varied from 10 to 100 ms, and the most stable peak was observed when 25 ms was applied as a modulation time.

Proposed method and repeatability of analysis was examined by 11 repeated measurements and calculation of relative standard deviation for 11 repeated measurements ($RSD_M(11)$). High repeatability was proved with achieved low value of $RSD_M(11)$ (0.91 % for SA-BDDE and 2.6 % for commercial BDDE, respectively). It can be concluded, that the minimal adsorption of PCR oxidation product on both BDD electrodes surface without need of any regeneration of the surface was confirmed and used working electrodes and proposed procedure proved to be suitable for precise detection and quantification of PCR.

Determination of PCR

Calibration curves were constructed by plotting of the peak current density against PCR concentration. The anodic signal increased linearly in the range from 2.5 to 90.9 μmol L^{-1} (SA-BDDE) and from 0.5 to 48.07 μmol L^{-1} (commercial BDDE), respectively, and obtained voltammetric curves are shown in Fig. 6 (SA-BDDE) and Fig. 7 (commercial BDDE), respectively. The insets in Fig. 6 and 7, respectively, illustrated the linear increases of current response density measured in dependence with concentration of PCR in the analyzed solution. These dependences could be described by Equation (3) belonging to the measurements recorded with SA-BDDE and Equation (4), which relates to the analysis on commercial electrode.

$$j_p\left[\mu A\, mm^{-2}\right] = (0.0062 \pm 0.000084)c\left[\mu mol\ L^{-1}\right] + 0.0282 \pm 0.0034\,;\ R^2 = 0.9978 \qquad (3)$$

$$j_p\left[\mu A\, mm^{-2}\right] = (0.0138 \pm 0.000056)c\left[\mu mol\ L^{-1}\right] + 0.0052 \pm 0.0003\,;\ R^2 = 0.9994 \qquad (4)$$

The detection limits were calculated from the obtained linear dependences and it was achieved relatively low values: LD(SA-BDDE) = 1.7 μmol L^{-1} (1700 nmol L^{-1}) and LD(commercial BDDE) = 70 nmol L^{-1}, respectively. The lower LD attained with commercial working electrode is caused by larger working surface and thus possibility of detection of lower concentrations of PCR, which is obvious from comparison of obtained LD (LD of commercial BDDE is about 21times higher than LD of SA-BDDE) and active surfaces of used electrodes (commercial electrode has about 16times larger active surface than

SA-BDDE). These values, especially that obtained with commercial electrode, are fully comparable with LD obtained with analysis on mercury electrodes (e.g. 83 nmol L^{-1} [47], 60 nmol L^{-1} [48], 44-156 nmol L^{-1} [49] and 149 nmol L^{-1} [50], respectively).

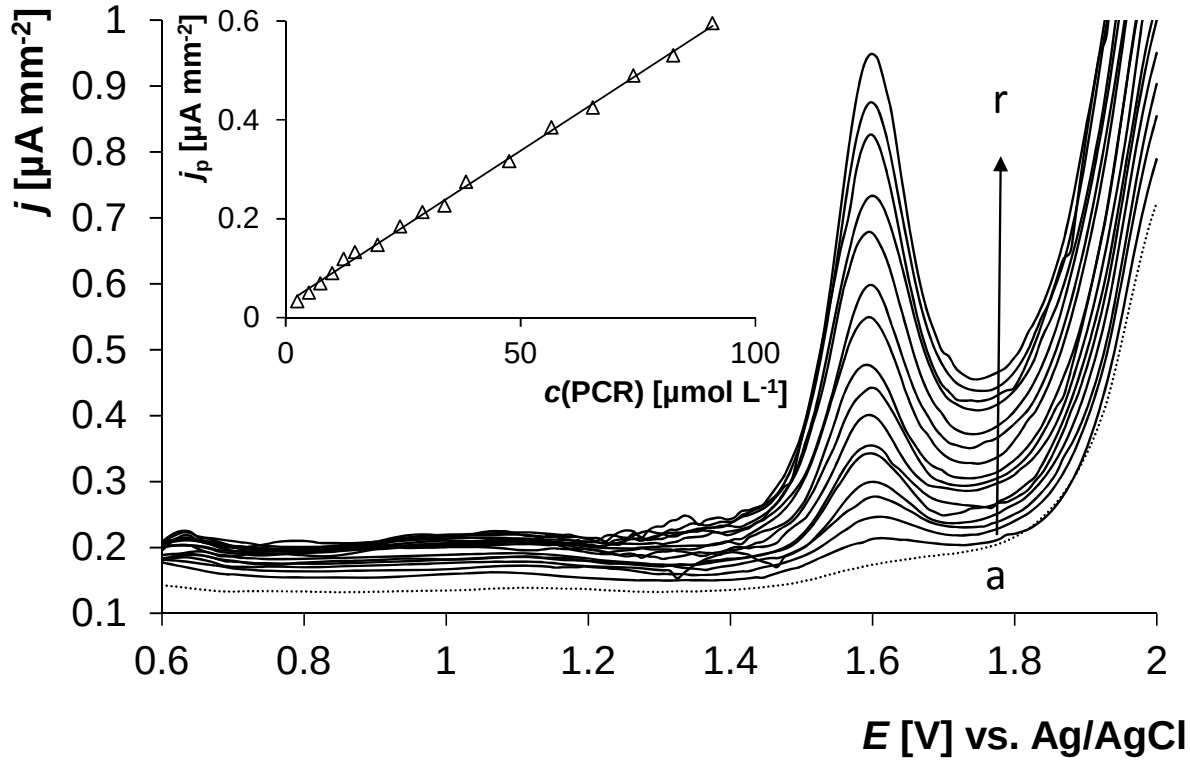

Fig. 6: *DP voltammograms of various PCR concentrations recorded on self-assembled BDD electrode*. Legend: a) 0, b) 2.50, c) 4.98, d) 7.44, e) 9.90, f) 12.35, g) 14.78, h) 19.61, i) 24.39, j) 29.13, k) 33.82, l) 38.46, m) 47.62, n) 56.60, o) 65.42, p) 74.07, q) 82.57 and r) 90.91 µmol L^{-1} PCR. Experimental conditions: supporting electrolyte: 1 mol L^{-1} H_2SO_4; DPV, E_{in} = 0.6 V, E_{fin} = 2 V, v = 20 mV s^{-1}, modulation amplitude: 75 mV, modulation time: 25 ms. Inset: Dependence of current response density (j_p) on concentration of PCR in the analyzed solution (c(PCR)).

Interference Study

The very positive position of the followed peak could be convenient for the selectivity of proposed method because many of compounds, which could be present in real samples (especially water, soils or urine), provided none signal or they can be oxidized at lower potentials. This assumption was confirmed by an interference study, which was carried out on commercial electrode, but the same results should be supposed also for the self-assembled sensor.

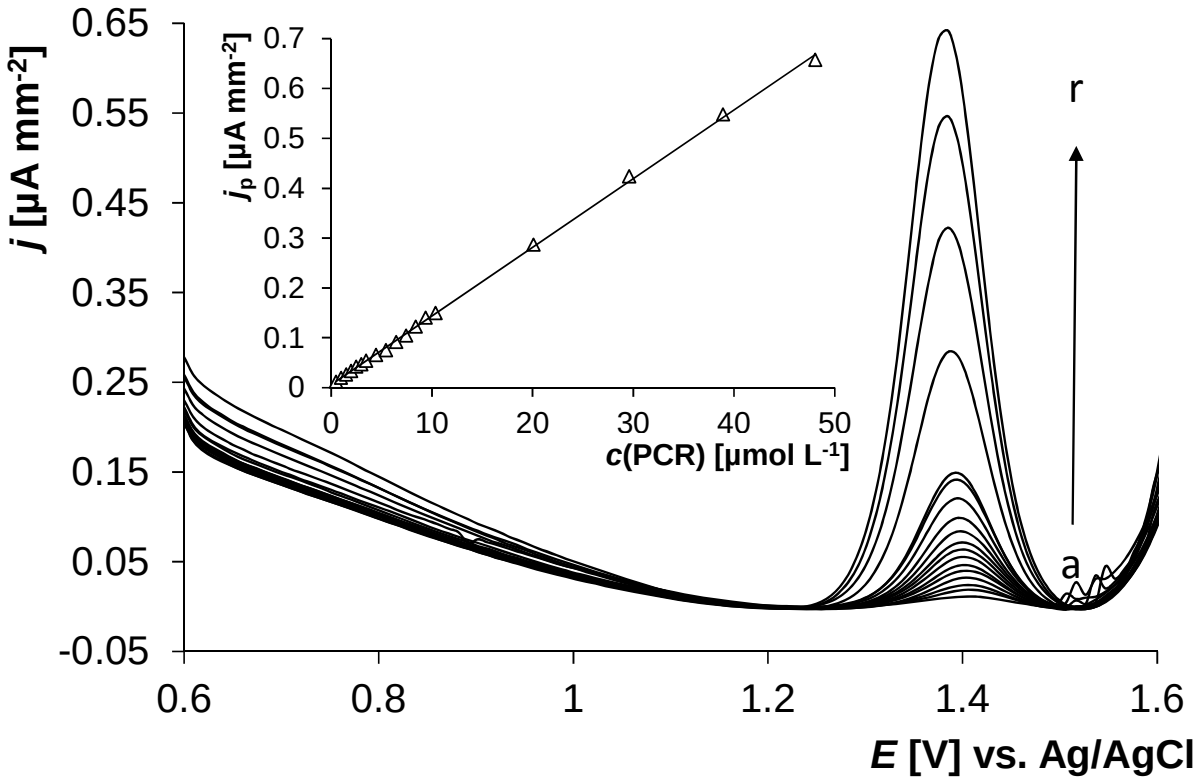

Fig. 7: *DP voltammograms of various PCR concentrations recorded on commercial BDD electrode*. Legend: a) 0.50, b) 1.00, c) 1.50, d) 2.00, e) 2.49, f) 2.99, g) 3.49, h) 4.48, i) 5.47, j) 6.46, k) 7.44, l) 8.43, m) 9.41, n) 10.39, o) 20.09, p) 29.60, q) 38.92 and r) 48,07 µmol L^{-1} PCR. Experimental conditions: supporting electrolyte: 1 mol L^{-1} H_2SO_4; DPV, E_{in} = 0.6 V, E_{fin} = 2 V, v = 20 mV s^{-1}, modulation amplitude: 75 mV, modulation time: 25 ms. Curves are after baseline correction. Inset: Dependence of current response density (j_p) on concentration of PCR in the analyzed solution (c(PCR)).

The effect of seven biomolecules (uric acid – UA, barbituric acid – BA, ascorbic acid – AA, folic acid – FA, sucrose – S, creatinine – C, and urea – U), which could be presented in human urine samples, was examined in three concentration levels (in ratios of PCR:biomolecule = 1:1, 1:10, 1:100). The influence of some wide-spread used herbicides (glyphosate – GLY and triasulfuron - TS) and other members of pyridine herbicide family (clopyralid – CLP and triclopyr – TCP) was also investigated in 2 concentration ratios (PCR:herbicide = 1:1 and 1:10). All measurements were carried out at concentration level of 5 µmol L^{-1} PCR. Obtained results are summarized in Table I. The substance was considered to interfere seriously when it gave a PCR signal change more than 5 %. It is obvious, that only FA from the group of biomolecules and TCP from herbicides caused significant decrease of the PCR signal even in concentration ratio of 1:10 (FA) and 1:1 (TCP), respectively. UA and AA also caused reduction of PCR peak but at 100times excess of this biomolecule. Other

tested molecules did not interfere in analysis of PCR significantly and it can be concluded, that BDDE could be applied, due to the good selectivity, as a working electrode for analysis of real samples.

Table I: *Influence of potential interfering agents (IA) on the voltammetric response of 5 μmol L^{-1} PCR measured on commercial BDDE.*

Interfering agent	Signal change of PCR in presence of interference agent (%)		
	1:1	1:10	1:100
UA	+1	−1.8	-9
BA	+1.3	+2.1	+4.2
AA	<0.5	−2.1	−17
FA	−3.2	−65	-
S	<0.5	−2.2	−2.1
C	−1.7	−1.6	−1.8
U	<0.5	<0.5	<0.5
CLP	+1.1	+2.8	-
TCP	−54.5	-	-
TS	−0.9	+1.8	-
GLY	<0.5	−1.9	-

Legend: UA – uric acid, BA – barbituric acid, AA – ascorbic acid, FA – folic acid, S – sucrose, C – creatinine, U – urea, CLP – clopyralid, TCP – triclopyr, TS – triasulfuron, GLY – glyphosate.

Analysis of Model Samples

As it was described above, proposed methods are sensitive and sufficiently selective and thus they could be applied for analysis of real samples. The samples were not pretreated by difficult way and only dilution by the supporting electrolyte was used prior to an analysis. Any of the real samples did not contain measurable amount of PCR and therefore, they were spiked with PCR. Added amount of PCR was determined using standard addition method, all determinations were 5times repeated and particular relative standard deviations of 5 repeated determinations ($RSD_D(5)$) were calculated. All samples (tap water, brook water and both samples of river waters) analyzed with commercial BDDE and sample of tap water for analysis on SA-BDDE were spiked with PCR to a concentration level of 2 μmol L^{-1} (0.5 mg L^{-1}). The river from Danube analyzed on SA-BDDE was spiked with higher concentration of PCR (10 μmol L^{-1}) because of the complicated matrix and lower sensitivity

of SA-BDDE in this medium. Obtained results are summarized in Table II. It is obvious, that added amounts of PCR were precisely and accurately determined and obtained results expressed as a confidence interval for 95% probability.

Commercial working electrode was also investigated as a tool for determination of PCR in model sample of human urine. Urine was spiked with the herbicide to the final concentration of 50 μmol L^{-1}. An example of the determination is depicted in Fig. 8, where the curve of spiked urine with PCR is highlighted. Determined amount was consistent with added amount of PCR and results are summarized in Table II.

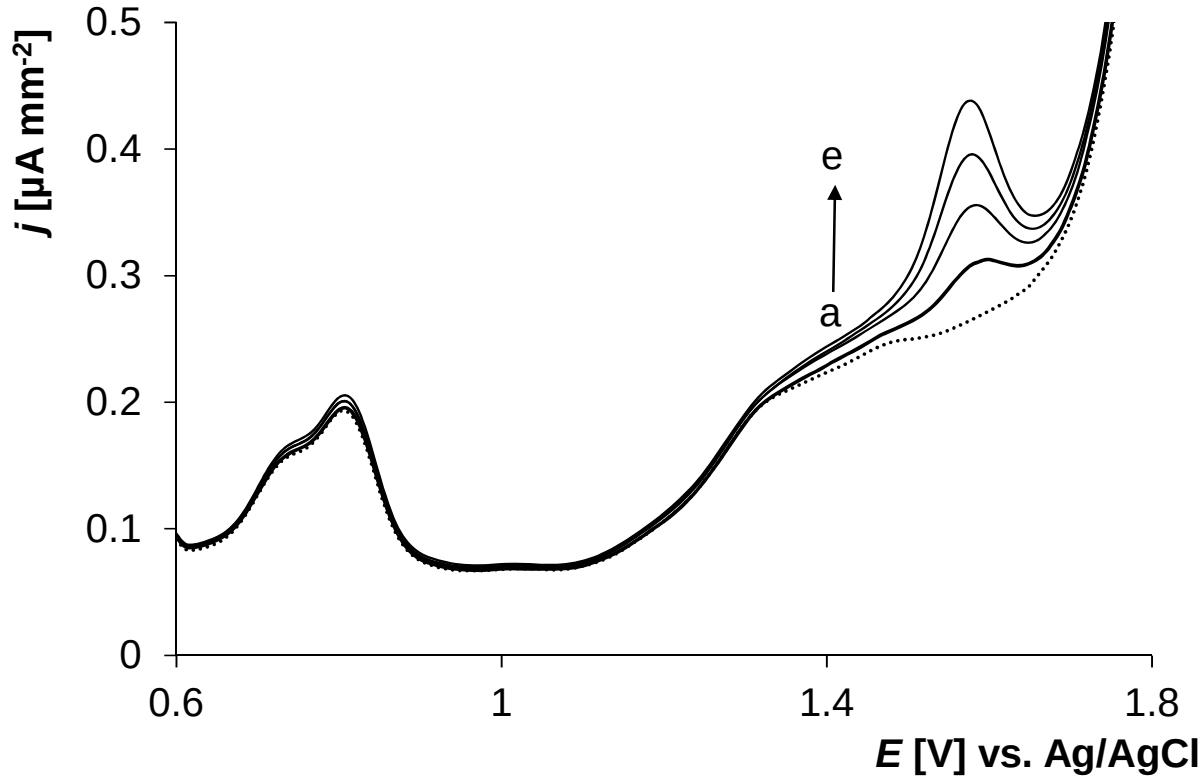

Fig. 8*: Determination of PCR in human urine using commercial BDDE.* Legend: **a**) diluted urine with supporting electrolyte, **b**) diluted urine with PCR, **c**) - **e**) additions of standard solution of PCR. Experimental conditions: supporting electrolyte: 1 mol L^{-1} H_2SO_4; DPV, E_{in} = 0.6 V, E_{fin} = 2 V, v = 20 mV s^{-1}, modulation amplitude: 75 mV, modulation time: 25 ms.

Table II: *Statistical parameters of PCR determination in the model samples of natural waters and human urine.*

Sample		SA-BDDE		Commercial BDDE	
	Added [μmol L^{-1}]	Found* [μmol L^{-1}]	RSD_D [%]	Found* [μmol L^{-1}]	RSD_D [%]
Tap water	2	1.98 ± 0.05	3.8	1.97 ± 0.06	4.4
Danube	2	-	-	2.03 ± 0.04	2.5
Danube	10	9.88 ± 0.17	3.0	-	-
Elbe	2	-	-	1.98 ± 0.04	3.1
Brook	2	-	-	1.97 ± 0.06	4.4
Urine	50	-	-	49.04 ± 1.33	4.1

* Average from 5 repeated determinations.

Conclusions

Boron-doped diamond as a perspective electrode material was tested for determination of pyridine herbicide picloram. It was found, that PCR provided one oxidation peak at very positive potential (about +1.5 V vs. Ag/AgCl/3 mol L^{-1} KCl) in strongly acidic medium. Two different constructions of BDDE, commercially available and self-assembled, in combination with DPV were tested and both provided wide linear dynamic range, low LD and good repeatibility. Moreover, achieved LD for PCR determination using the commercial electrode was fully comparable with those obtained on mercury electrodes. The aplicability of proposed methods were verified by analysis of PCR in model real samples with good accuracy. It can be concluded that the developed voltammetric methods can undoubtedly be considered as an effective, sensitive and green (environmentally acceptable) tool in analysis of PCR and other herbicides as well as may represent the electrochemical alternative to mercury electrodes.

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