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Electrochemical Properties of Boron-Doped Diamond Electrodes and their Application in Analysis of Bioactive Compounds

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Abstract

In this Ph.D thesis, physical, chemical, and electrochemical properties of lab-made boron-doped diamond electrodes (BDDE) with different boron content (B/C ratio in gas phase during BDD film deposition) have been investigated. Scanning electron microscopy and Raman spectroscopy were used for surface characterization of the prepared BDD films. Electrochemical properties of the tested electrodes were studied by electrochemical impedance spectroscopy and cyclic voltammetry of reversible redox markers $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$. Applicability of the tested working electrodes was examined employing three various bioactive compounds – herbicide linuron, drugs mesalazine and leucovorin. Analytical methods for the determination of these analytes were developed at first using commercially available BDDE. The proposed methods were subsequently applied to the analytes determination using the investigated working electrodes. The aim was to obtain basic statistical parameters and compare them considering the different content of boron in material of the tested electrodes. It was proved that the B/C ratio significantly influences the electrochemical properties of the working BDD electrodes like a width of the potential window, size of the electrochemical active surface, rate of the charge transfer, reversibility of the observed electrode reactions, as well as the applicability in analysis of bioactive compounds.

Abstrakt

V rámci této disertační práce byly zkoumány fyzikální, chemické a elektrochemické vlastnosti laboratorně připravených borem dopovaných diamantových elektrod (BDDE) s různým obsahem boru (poměrem B/C v plynné fázi při depozici BDD filmu). Pro charakterizaci materiálu BDD filmů byla použita skenovací elektronová mikroskopie a Ramanova spektroskopie. Elektrochemické vlastnosti testovaných elektrod byly studovány s využitím elektrochemické impedanční spektroskopie a cyklické voltametrie reverzibilních redoxních systémů $[Fe(CN)_6]^{3-/4-}$ a $[Ru(NH_3)_6]^{3+/2+}$. Pro testování aplikačních možností BDDE s různým obsahem boru byl použit herbicid linuron, léčiva mesalazin a leukovorin. S využitím komerčně dostupné BDDE byly vyvinuty metody pro stanovení těchto biologicky aktivních látek, které byly poté aplikovány při jejich stanovení s využitím testovaných BDD elektrod. Cílem bylo získání základních statistických parametrů a jejich porovnání

v souvislosti s obsahem boru v materiálu elektrod. Bylo zjištěno, že poměr B/C významně ovlivňuje elektrochemické vlastnosti testovaných BDD elektrod, jako je např. šířka potenciálového okna, velikost elektrochemicky aktivního povrchu, rychlost přenosu náboje, reversibilita sledovaných elektrodových reakcí, stejně jako aplikační možnosti v oblasti analýzy bioaktivních látek.

Keywords

Voltammetry, boron-doped diamond electrode, boron content, surface characterization, electrochemical properties, linuron, mesalazine, leucovorin

Klíčová slova

Voltametrie, borem dopovaná diamantová elektroda, obsah boru, povrchová charakterizace, elektrochemické vlastnosti, linuron, mesalazin, leukovorin

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Introduction

Electrochemical methods represent instrumental analytical methods widely used in the analysis of a wide range of biologically significant substances and pollutants in the environment. Electrochemical methods have many advantages, in general the low costs of equipment, particularly fast and sensitive performance of analysis, time saving analysis, usually undemanding samples treatment, as well as easy miniaturization and automation.

Polarography has a long tradition in the Czech Republic. It was developed in 1922 by Czech chemist Jaroslav Heyrovský (Figure 1A). In 1959, he received the Nobel Prize for the invention of polarography. This method is based on measurement of current response in dependence on potential inserted on the working electrode of the electrochemical cell created by working, reference and usually an auxiliary electrode. The obtained current signal corresponds with the appropriate redox reaction of the analyte. Dropping mercury electrode (DME) is used as a working electrode in polarography. When stationary working electrodes (e.g., hanging mercury drop electrode (HMDE) or solid working electrodes) are used, the method is known as voltammetry. In Figure 1B, the first polarograph constructed by Heyrovský is shown [1-3].

Nowadays, beside mercury, carbon or metal electrodes, various new electrode materials or surface modifications of the existing electrodes are developed. The new electrode materials should meet the following requirements: low noise and residual current, a wide potential window, passivation resistance, mechanical stability, sensitivity and selectivity. Their compatibility with the principles of "green analytical chemistry" is also very important. Boron-doped diamond electrode (BDDE) belongs to the perspective electrode materials with great prospects to the future.

The present work is focused on the surface characterization and study of the electrochemical properties of BDDEs prepared under different conditions particularly with various B/C ration in the gas phase during film deposition. Moreover, their applicability as an analytical tool for determination of important bioactive compounds, concretely the herbicide linuron and drugs mesalazine and leucovorin, based on their electrochemical oxidation was investigated as well.

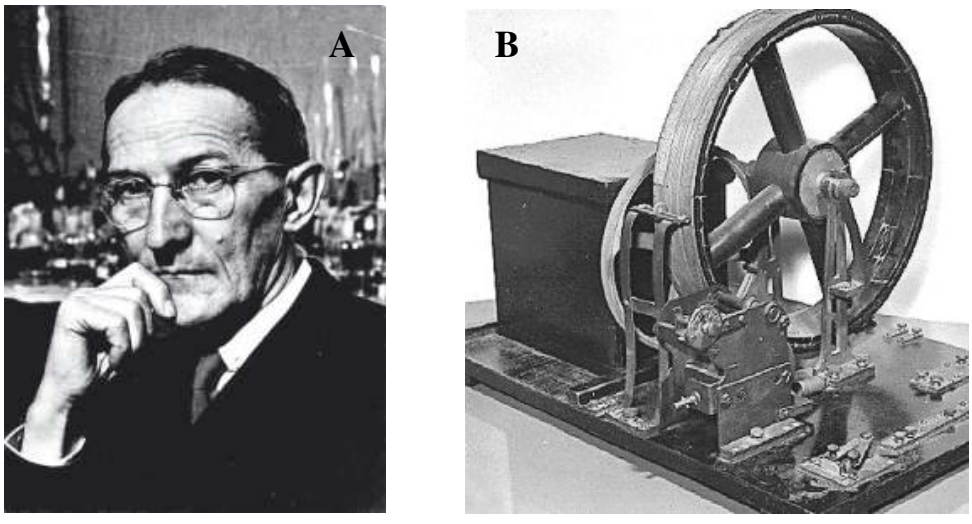

Figure 1: Prof. Jaroslav Heyrovský (A) [4] and the first polarograph (B) [5].

1. Theoretical Part

1.1 Boron-doped diamond electrode

BDDEs were introduced for electroanalytical chemistry in the early 1990s [6, 7]. The pure diamond is distinguished by outstanding mechanical and chemical stability and it is one of the best natural insulators. For its electrochemical utilization, it needs to be doped with the other elements, most often with boron [8, 9]. Diamond films are created by chemical vapor deposition (CVD) with heated filaments or microwave heating. The mixture of methane and hydrogen is usually used for diamond film deposition and diborane or trimethylboron is added to the mixture for boron doping process. Silicon served most commonly as a substrate for the diamond films. By this way, the polycrystalline boron-doped diamond films are created [8, 10, 11].

In terms of electroanalytical chemistry, the wide potential window about 3 V is the greatest advantage of BDDEs. Furthermore, these electrodes exhibit low noise. Their working surface has a paraffinic character, so low adsorption is observed during electroanalytical measurements; thereby the problems with passivation are minimized. Due to the mechanical stability, BDDEs are also suitable for measurements in flow systems [8, 9]. In general, BDDEs are most often used in the analysis of organic compounds. Various methods have been described for the determination of hydrocarbons and their derivatives [12], phenols [13], or nitrophenols [14]. Different pharmaceuticals [15, 16] or pesticides [17, 18] were also analyzed. Moreover, the surface of BDDEs can served as a suitable substrate for various modifications using e.g., metals, carbon nanomaterials, proteins, DNA, enzymes, etc. [19-21].

Due to its unique properties, BDDE can be used in a large area of analytical applications and it is very promising electrode material for the future. In the present paper, the commercially available BDDE (Figure 2A) was used to study the voltammetric behavior of the biological active compounds linuron, mesalazine, and leucovorin, and analytical methods for their determination were developed. These methods were also successfully applied in the analysis of natural and biological samples as well as of pharmaceutical preparations. The major aim of this work was to study the surface and electrochemical properties of BDDEs with various boron content (Figure 2B) in the diamond film. Also the practical applicability of these electrodes in analytical practice was investigated through the utilization of previously developed methods for mentioned analytes. The obtained statistical parameters were finally compared considering the different boron content in the electrode material.

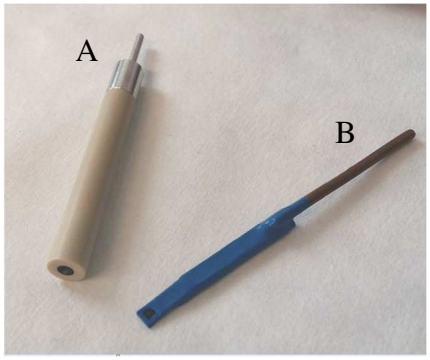

Figure 2: Commercial available (A) and lab-made (B) BDDE.

1.2 Studied substances

1.2.1 Linuron

Linuron (LIN, 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea, Figure 3) is a substituted urea herbicide used to control newly emerging and germinating grasses and broad-leafed weeds. It may be applied pre-plant, pre-emergence, post-emergence, or post-transplant using ground equipment. It is labelled for field and storehouse usage in crops such as soybean, cotton, corn, bean, potato, carrot, winter wheat, asparagus, and fruit crops. LIN acts as an herbicide through the inhibition of photosynthesis. It is a slightly toxic compound and belongs in EPA toxicity class III [22, 23].

The electrochemical determination of LIN using various types of working electrodes has been described in [24-26] and 3-electron oxidation was identified [26].

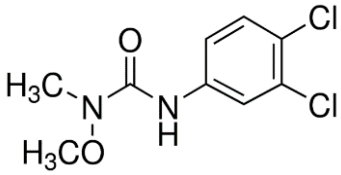

Figure 3: Structural formula of LIN [27].

1.2.2 Mesalazine

Mesalazine, also known as mesalamine or 5-aminosalicylic acid (5-ASA, 5-amino-2-hydroxybenzoic acid, Figure 4), is well-established compound used in the management of inflammatory bowel disease (IBD). This drug is most useful for the treatment of mild to moderate flares of ulcerative colitis and to treat inflammation of the digestive tract (Crohn's disease). 5-ASA is an active moiety of sulfasalazine drug, which is metabolized to sulfapyridine and mesalazine. 5-ASA has the advantage of being generally well-tolerated and safe for long-term use with flexible dosing. As a derivate of salicylic acid, 5-ASA is also an antioxidant that traps free radicals, which are potentially damaging by products of metabolism [28-30]. According to the literature 5-ASA acts by blocking the production of leukotrienes and prostaglandins, inhibiting bacterial peptide-induced neutrophil chemotaxis and adenosine-induces secretion, scavenging reactive oxygen metabolites [31].

The electrochemical determination of 5-ASA in connection with various working electrodes and methods has been described in [32-34]. The oxidation process to quinone-imine form via a two-electron, two-proton oxidation steps was proposed [35].

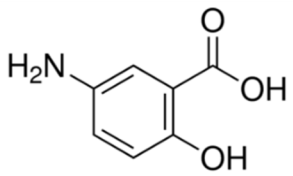

Figure 4: Structural formula of 5-ASA [36].

1.2.3 Leucovorin

Leucovorin (LV, 5-formyltetrahydrofolate monoglutamate, Figure 5) or folinic acid is a metabolite of folic acid [37]. It is its reduced form. LV is used in the treatment of cancer diseases, most often as an antidote to protect healthy cells from the toxic effects antifolates which are used in high quantities during chemotherapy. It is administrated e.g., with methotrexate to reduce its negative side effects [38] or in combination with 5-fluorouracil to enhance its effectiveness during treatment of various serious diseases [39].

Voltammetric behavior of LV has been described on mercury [40, 41] and silver solid amalgam electrodes [41]. In this paper, the determination of LV via its oxidation was firstly investigated and the applicability of commercial as well as lab-made BDDEs was studied.

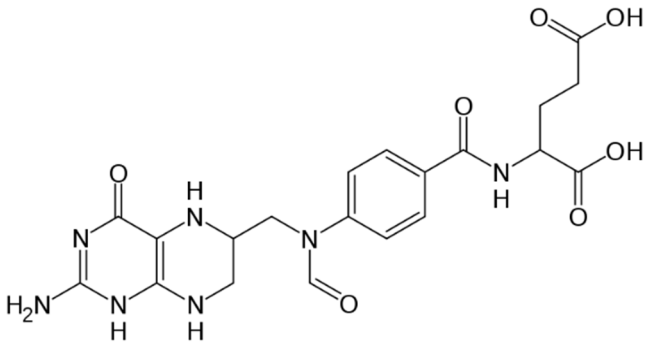

Figure 5: Structural formula of LV.

2. Experimental Part

2.1 Chemicals

All of the used chemicals were of p.a. purity and all solutions were prepared in distilled water. Britton-Robinson buffer (BRB) of pH 2-12 consisting of the mixture of acidic (0.04 mol L^{-1} H_3PO_4, H_3BO_3, and CH_3COOH, all from Ing. Petr Švec – PENTA, Czech Republic) and alkaline component (0.2 mol L^{-1} NaOH from Lachema, Czech Republic) was used as a supporting electrolyte. The electrolytes based on various concentrations of HNO_3 were prepared from 96% HNO_3 (Ing. Petr Švec – PENTA , Czech Republic). 0.1 mol L^{-1} KCl was prepared by dissolution of its powder (Ing. Petr Švec – PENTA, Czech Republic). Standard solution of 1×10^{-3} mol L^{-1} LIN was prepared by dissolution of LIN powder (99.7 % purity, (Sigma-Aldrich)) in 70% acetonitrile (Ing. Petr Švec – PENTA , Czech Republic). Standard solution of 0.01 mol L^{-1} 5-ASA was prepared by dissolution of 5-ASA powder (Sigma-Aldrich) in 0.1 mol L^{-1} HCl (Ing. Petr Švec - PENTA, Czech Republic). Standard solution of 1×10^{-3} mol L^{-1} LV (Sigma-Aldrich) was prepared by simple dissolution of its calcium salt powder in the distilled water. All these standard solutions were stored in refrigerator. 0.5 mol L^{-1} H_2SO_4 (96 %, Lach-Ner, Czech Republic) was used for the pretreatment of all BDDE. Stock solutions of $K_3[Fe(CN_6)]$ and $[Ru(NH_3)_6]Cl_3$ (2.5×10^{-3} mol L^{-1}; ≥ 99 %, Sigma-Aldrich) were prepared in 0.1 mol L^{-1} KCl.

2.2 Instrumentation

Voltammetric measurements were carried out by computer controlled analyzer Eco-Tribo Polarograph (Polaro-Sensors, Czech Republic) equipped by software POLAR.PRO version 5.1 or using an Autolab potentiostat/galvanostat model PGSTAT 12 (Metrohm Autolab B. V., Netherlands) controlled by NOVA 1.11 software. The measuring system consisted of three electrodes – BDDE as a working electrode, saturated argentchloride reference electrode (Monokrystaly, Czech Republic), and platinum wire (Monokrystaly, Czech Republic) as an auxiliary electrode. Commercially available BDDE with working surface of 7.07 mm^2 and B/C ratio in the gas phase of 1000 ppm (as declared by the producer) was supplied by Windsor Scientific (UK). Tested laboratory prepared BDDEs (Slovak University of Technology in Bratislava, Faculty of Electrical Engineering and Information Technology, Institute of Electronics and Photonics) provided working surface of 0.43 mm^2 and B/C ratio in gas phase of 1000, 2000, 4000, 8000, 10 000, and 20 000 ppm. All measurements were carried out at laboratory temperature of 23 ± 2 °C without the oxygen removing.

Electrochemical impedance spectroscopic (EIS) measurements were performed with PGSTAT302N + FRA2 module controlled by NOVA 1.10 software (Metrohm Autolab B. V., Netherlands). Raman spectra were measured at room temperature using ISA Dilor-Jobin-Yvon-Spex LabRAM confocal system with 632.8 nm He-Ne laser. SEM was carried out using JEOL JSM-7500F scanning electron microscope. Values of pH were measured by pH-meter Accumet AB150 (Fisher Scientific, Czech Republic).

All graphical dependencies were constructed using Excel software (Microsoft, USA). Parameters of calibration curves and confidence intervals were calculated using OriginPro 9 software (OriginLab Corporation, USA) on the level of significance 0.05.

Statistical parameters like limit of detection (LOD) and limit of quantification (LOQ) were calculated from the calibration dependencies as three times and ten times, respectively, of standard deviation of intercept divided by the slope.

2.3 Procedures

For the fabrication of tested lab-made BDDE the n-type Si(100) wafer with 2-µm-thick SiO_2 layer (CVD, Oxford PlasmaLab 80) was used as a substrate for BDD films deposition. First, the substrates were cleaned with isopropanol and deionized water and subsequently seeded in the ultrasonic bath using a nanodiamond powder <10 nm (CAS No. 7782-40-3, Sigma Aldrich) diluted in demineralized water. The BDD films were deposited for 4 h (resulting thickness ~ 1 µm) using double bias enhanced hot filaments reactor (HF CVD) which uses heated tungsten filaments to activate CH_4 and H_2. B/C ratio was changed from 1000 to 20 000 ppm. All of the used working BDDEs (the lab-made and the commercial one as well) were activated before starting the work. The insertion of anodic potential (+3 V, 60 s) and subsequently cathodic potential (−3 V, 300 s) in 0.5 mol L^{-1} H_2SO_4 was applied before LIN and 5-ASA analysis. The electrode thus prepared was used without further treatment and no regeneration or activation step was inserted between the particular measurements. The activation for LV analysis was carried out in 0.5 mol L^{-1} H_2SO_4 too, but the cathodic potential (−2 V, 60 s) was first inserted on the electrode and then anodic (+2 V, 60 s). Subsequently, 20 cyclic voltammograms between −1 and +2 V were recorded to ensure stabilization of the obtained signal. Besides the activation, the regeneration step consisting of the regeneration potential (+2 V, 5 s) was inserted between each scan. This procedure led to the O-terminated electrode surface.

Cyclic voltammetry (CV) was utilized for the experiments focused on electrochemical characterization of the tested BDDEs and for the study of voltammetric behavior of the analyzed compounds using commercially available BDDE as well. This electrode served also for the development of voltammetric methods for LIN, 5-ASA, and LV determination. Proposed parameters of differential pulse voltammetry (DPV) for the determination of LIN were following: initial potential (E_{in}) +400 mV, final potential (E_{fin}) +1600 mV, scan rate (v) 50 mV s^{-1}, pulse height +70 mV and pulse width +20 ms. Parameters of square wave voltammetry (SWV) for the determination of 5-ASA were following: E_{in} = 0 mV, E_{fin} = +2200 mV, potential step 7 mV, amplitude 70 mV and frequency 25 Hz. Finally, parameters of DPV for the determination of LV: E_{in} = −700 mV, E_{fin} = +1800 mV, v = 40 mV s^{-1}, pulse height +50 mV and pulse width +20 ms. These methods were then applied for measurements with tested BDDEs.

Natural water (for LIN), human urine (5-ASA), and pharmaceutical preparations (5-ASA, LV) were analyzed as practical samples during dissertation thesis. Natural water and urine samples were spiked with standard solutions of the appropriate analytes to a suitable concentration and they were only diluted by supporting electrolyte before analysis. The solutions of pharmaceutical preparations were prepared by dissolution in the appropriate solvent (0.1 mol L^{-1} HCl for 5-ASA and distilled water for LV) and mixed with the electrolyte. All quantitative analyses were performed by the standard addition method and were 5-times repeated.

3. Results and discussion

The aim of this work was to study electrochemical properties of BDDEs with various content of boron and comparison of their application options for the determination of different bioactive compounds. Firstly, analytical methods for the determination of these compounds were developed using commercially available BDDE and subsequently were applied to the tested BDD electrodes.

3.1 Voltammetric determination of linuron, mesalazin, and leucovorin

Voltammetric behavior of LIN, 5-ASA, and LV was investigated using CV in connection with commercially available BDDE. It was found that all tested analytes provide oxidation signals suitable for analytical purposes. Because of the high sensitivity, DPV and SWV were tested for the determination of the interest substances. Final parameters of the developed voltammetric methods are summarized in experimental part. Subsequently, a number of concentration dependences were measured and the calculated statistical parameters, like linear dynamic range (LDR), LOD, and LOQ, are presented in Table 1. The examples of the obtained concentration dependences under the optimized conditions for LIN, 5-ASA, and LV are shown in Figure 6. Finally, model solutions as well as real samples of nature waters, pharmaceutical preparations and human urine were successfully analyzed. Also the repeatability of measurement and determination, respectively, was verified by calculation of the values of relative standard deviations (RSD).

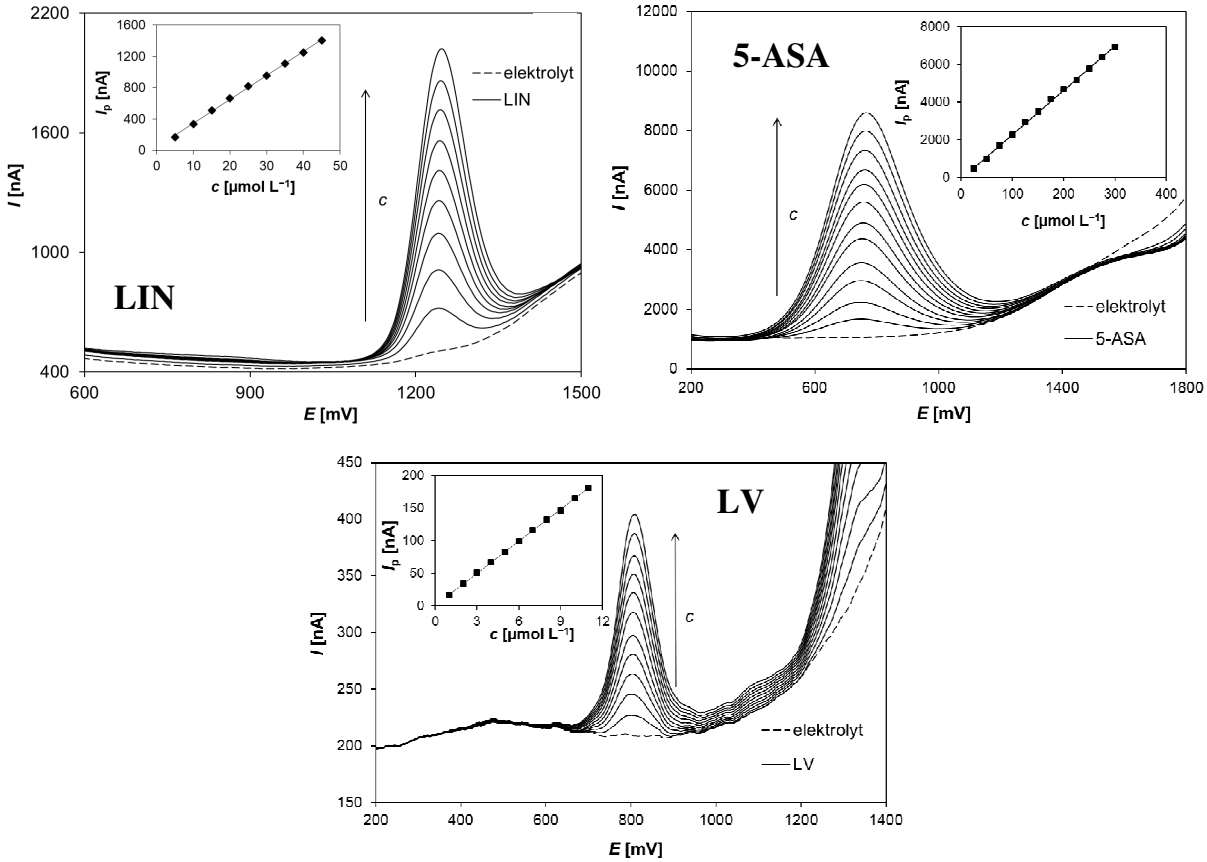

Figure 6: DP (LIN, LV) and SW (5-ASA) voltammograms of the analytes obtained under the optimized conditions in dependence on concentration with the appropriate linear dependences of I_p on c (c_{LIN} = 5.0×10^{-6}-4.5×10^{-5} mol L^{-1}, $c_{5\text{-ASA}}$ = 2.5×10^{-5}-3.0×10^{-4} mol L^{-1}, c_{LV} = 1.0×10^{-6}-1.1×10^{-5} mol L^{-1}).

Table 1: Summary of pH values and some statistical parameters for the determination of LIN, 5-ASA, and LV

	Linuron	Mesalazine	Leucovorin
Method	DPV	SWV	DPV
pH	BRB (pH 2)	BRB (pH 7)	BRB (pH 3)
LOD [mol L^{-1}]	1.4×10^{-7}	7.0×10^{-7}	1.5×10^{-8}
LOQ [mol L^{-1}]	4.7×10^{-7}	2.3×10^{-6}	5.0×10^{-8}
LDR [mol L^{-1}]	5.0×10^{-7}–1.2×10^{-4}	2.0×10^{-6}–3.0×10^{-4}	1.5×10^{-7}–2.5×10^{-5}
$RSD_S(5)$ [%]	≤1.49	≤1.50	≤2.57
$RSD_M(11)$ [%]	0.84	2.70	0.70

3.2 Surface characterization of BDDE

At first, all prepared BDDEs were examined for morphology by scanning electron microscopy. Figure 7A shows the SEM image (45° angle view) of the interface of deposited BDD film and the surface of silicon oxide layer on Si substrate. All of the tested films represent continuous coatings without cracks and voids that completely covered SiO_2 substrate. This compact structure will not allow the penetration of the supporting electrolyte during the application in electroanalytical measurements. It was found that the grain size increases with increasing B/C ratio.

The Raman spectra measured at the wavelength of 632 nm for BDD films for individual B/C ratios are presented in Figure 7B. It shows bands characteristic for BDD. One sharp peak at 1335 cm^{-1} due to sp^3 C–C bonds can be observed for the un-doped diamond film (0 ppm). It implies that a high-quality diamond was deposited on the silicon substrate. Nevertheless, this signal weakens gradually as the B/C ratio increases. Two broad bands centered at approximately 470 and 1220 cm^{-1} are associated with the actual boron incorporation in the lattice. The maximum at 470 cm^{-1} may originate from a pair of boron atoms and shifts with the increase of the boron content to lower wavenumbers. Raman spectra exhibit also significant maximum around 1580 cm^{-1} corresponding to the sp^2 graphitic carbon bonds, which indicate the presence of graphite like impurities at grain boundaries. The bands placed around 520 and 960 cm^{-1} are associated with first and second order of Si.

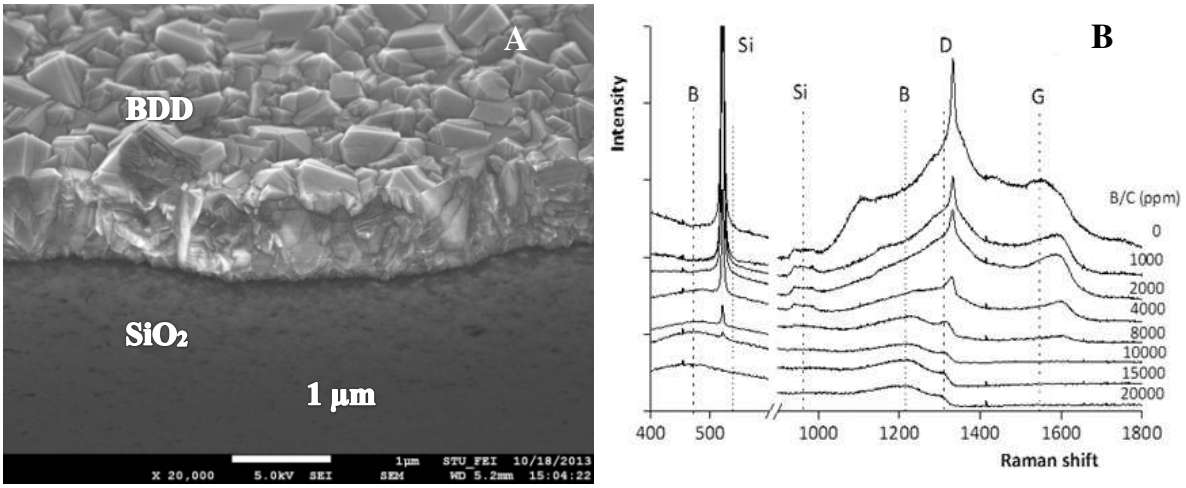

Figure 7: SEM image of the interface of the deposited BDD film and SiO_2 substrate (A) and Raman spectra of BDD films with the boron content from 0 to 20 000 ppm (B).

3.3 Electrochemical characterization of BDDE

3.3.1 Electrochemical impedance spectroscopy

The electrochemical impedance spectroscopy (EIS) is a very useful tool with the well-established theory that describes the response of a circuit to the alternating current/voltage as a function of the applied frequency. Different electric equivalent circuits (EEC) can be used for modeling and characterization of surface modified electrodes or other electrochemical systems. In our experiments EEC consisted of the serial resistance (R_1), connected to a loop with R_2 and a Warburg element, which takes into consideration the diffusion phenomena. The constant phase element (CPE), which represents contribution of capacitance, is parallel connected with R_2 and Warburg element (Z_w). The scheme of the used circuit is included in Figure 8.

The electrochemical characterization of BDD films with the range of B/C from 0 to 20 000 ppm was successfully studied over a wide frequency region in the redox system ($[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} KCl with utilization of EIS. For our purposes, the method including the measurement of real and imaginary parts of impedance concerning the physical properties was used. The Nyquist plots (Figure 8) of the electrochemical impedance spectra were obtained at the appropriate potentials determined from cyclic voltammograms. It can be described as semicircles near the origin at high frequencies. Only one semicircle was recorded for each tested electrode. This result is related to a charge transfer process at the solid/liquid interface. Also it can be related to the incorporation of substitutional boron within the diamond lattice. The EIS data correspond very well with the following results of CV, and are consistent with a model describing the charge transfer at BDD surface [44]. The relationship between impedance and rate of electron transfer were elucidated and correlate with the range of B/C from 0 to 20 000 ppm. It is obvious in Figure 8 that the impedance decreases with the increasing of boron doping level; it means the electron transfer is much more significant with the increasing of B/C ratio. The decrease of impedance of the analyzed electrochemical system corresponds to the decrease in resistivity of the BDDE with the increase of boron doping level.

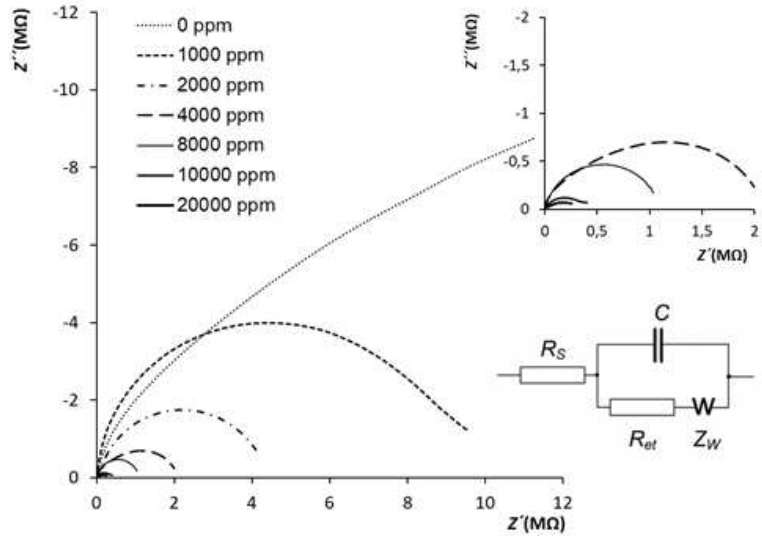

Figure 8: Electrochemical impedance spectrograms for 2.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} KCl recorded on BDDEs with the range of B/C from 0 to 20 000 ppm (inserted the detail of this graph); frequency range from 0.1 Hz to 100 kHz, signal amplitude of 10 mV.

3.3.2 Cyclic voltammetry of redox systems $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$

For electrochemical characterization of BDD films cyclic voltammetry (CV) of redox systems of $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$ was measured under the following parameters: v = 100 mV s^{-1}, potential range from −1600 mV to +1800 mV. CV of the above mentioned redox systems was used also for the measurements of dependencies on v (10, 20, 50, 100, 200, 300, 400, and 500 mV s^{-1}). $[Ru(NH_3)_6]^{3+/2+}$ is typical representative of redox systems provided *outer sphere* reactions and $[Fe(CN)_6]^{3-/4-}$ *inner sphere* reaction on BDD electrodes. From CV of these redox systems various parameters like width of the potential window, reversibility of the electrode reaction, the electrochemically active surface, and the apparent electron transfer rate constant (k^0_{app}) were calculated and compared.

The first step of the characterization of the tested BDDE was recording of the width of the potential window. 0.1 mol L^{-1} KCl was used as a supporting electrolyte. The border potential on the anodic and cathodic side as well was the value, when the current reached ±1500 nA. The increasing B/C ratio in the gas phase caused narrowing of the potential window (Table 2). Moreover, the narrowing was more significant in the cathodic region. Particularly, the width decreased of about 900 mV comparing BDDE 1000 ppm and 20 000 ppm and the widest potential window, namely 3660 mV, was observed for BDDE with B/C 1000 ppm.

Table 2: Potential values corresponding to the anodic (E_a) and cathodic (E_c) border of potential window and the calculated total usable potential range for each particular electrode (E_{tot})

B/C [ppm]	E_a [mV]	E_c [mV]	E_{tot} [mV]
1000	+1960	−1700	3660
2000	+1760	−1510	3270
4000	+1660	−1430	3090
8000	+1590	−1390	2980
10000	+1610	−1200	2810
20000	+1550	−1180	2730

Reversibility of the electrode reactions for the mentioned redox markers was examined next. The example of the obtained cyclic voltammograms of $[Fe(CN)_6]^{3-/4-}$ are depicted in Figure 9 and for $[Ru(NH_3)_6]^{3+/2+}$ in Figure 10. Considering reversibility, it is apparent that this parameter grown with increasing boron content, which is obvious from the increasing of the particular signals and decreasing of their potential differences. Particular values of the anodic and cathodic peak heights (I_{pa} and I_{pc}), their ratio (I_{pa}/I_{pc}), and values of the anodic and cathodic peak potential (E_{pa} and E_{pc}) and their difference (ΔE_p) are summarized in Table 3. The parameter I_{pa}/I_{pc} ranged from 0.86 to 0.98 for both redox markers. The highest values limiting to the theoretical value 1 confirming reversibility of the electrode reaction were found for BDDE with the highest B/C (10 000 ppm for both redox systems and 20 000 ppm for $[Fe(CN)_6]^{3-/4-}$ and surprisingly for BDDE with B/C 1000 ppm. On the other hand, the potential difference, which is the most important criteria for assessment of the reversibility, was especially for the system $Fe(CN)_6]^{3-/4-}$ enormous (ranged from 1406 to 491 mV) and did not limit to the theoretical value of 59 mV. Considering a decreasing trend of the potential difference, it was again confirmed, that the reversibility increased with the boron content and the best result, namely the lowest

value of ΔE_p, was obtained for BDDE with B/C 10 000 ppm. Moreover, ΔE_p defined for this electrode and $[Ru(NH_3)_6]^{3+/2+}$ limiting to the theoretical value 59 mV, which is typical for the one-electron reversible electrode reaction. The slight increase of ΔE_p for electrode with B/C 20 000 ppm may be explained by a higher content of sp^2 hybridized carbon in these BDD films. The significant difference in values of ΔE_p between $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$ may be probably due to the *outer* and *inner sphere* nature of the used redox markers.

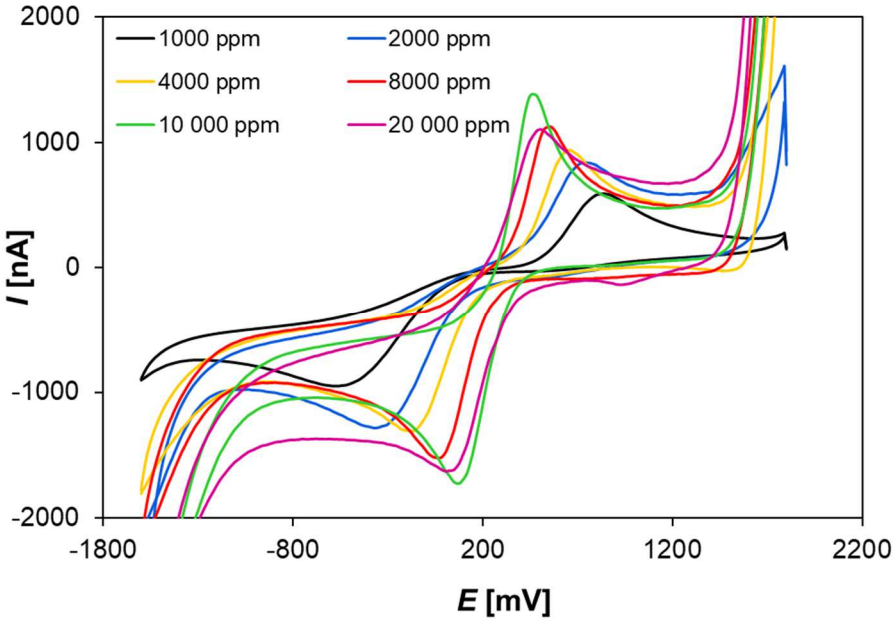

Figure 9: Cyclic voltammograms of $[Fe(CN)_6]^{3-/4-}$ recorded on the lab-made BDDE. Method – CV, E_{in}=E_{fin} = −1600 mV, E_{switch} = +1800 mV, v = 100 mV s^{-1}; supporting electrolyte – 0.1 mol L^{-1} KCl; $c([Fe(CN)_6]^{3-/4-})$ = 2.5×10^{-3} mol L^{-1}.

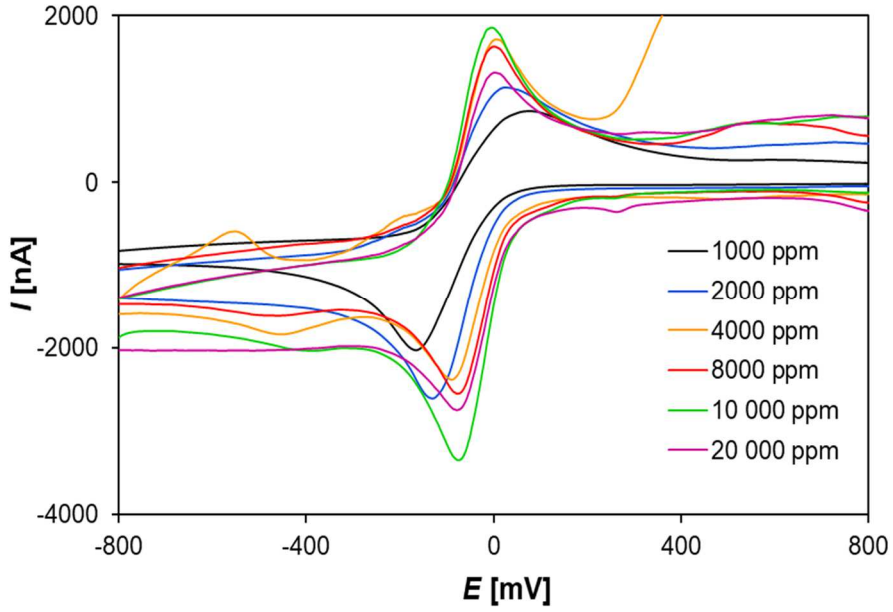

Figure 10: Cyclic voltammograms of $[Ru(NH_3)_6]^{3+/2+}$ recorded on the lab-made BDDE. Method – CV, E_{in}=E_{fin} = −1600 mV, E_{switch} = +1800 mV, v = 100 mV s^{-1}; supporting electrolyte – 0.1 mol L^{-1} KCl; $c([Ru(NH_3)_6]^{3+/2+})$ = 2.5×10^{-3} mol L^{-1}.

Table 3: Basic parameters of cyclic voltammograms measured on all tested BDDE for redox systems $Fe(CN)_6^{3-/4-}$ and $Ru(NH_3)_6^{2+/3+}$ in 0.1 mol L^{-1} KCl

B/C [ppm]	I_{pa} [nA]	I_{pc} [nA]	I_{pa}/I_{pc}	E_{pa} [mV]	E_{pc} [mV]	ΔE_p [mV]
$[Fe(CN)_6]^{3-/4-}$						
1000	500	−507	0.98	829	−577	1406
2000	510	−576	0.88	720	−355	1075
4000	672	−767	0.88	659	−163	822
8000	849	−947	0.89	550	−24	574
10 000	1039	−1053	0.98	545	72	473
20 000	694	−710	0.98	503	12	491
$[Ru(NH_3)_6]^{3+/2+}$						
1000	1161	−1268	0.92	71	−167	238
2000	1226	−1389	0.88	26	−132	158
4000	1283	−1407	0.91	26	−91	117
8000	1400	−1530	0.92	4	−71	75
10 000	1560	−1612	0.97	−10	−71	61
20 000	972	−1128	0.86	8	−77	85

Electroactive area of the working electrodes (A) was calculated from the CV voltammogram of $[Fe(CN)_6]^{3-/4-}$ utilizing Randles-Ševčík equation [42] for the v of 100 mV s^{-1}. The results are summarized in Table 4. It is evident that the active surface increased with the boron content and the largest area was calculated for BDDE with B/C 10 000 ppm. It could be assumed, that the higher active surface is related with the smaller formed crystals. The significant reduction was observed for films with higher B/C ratio, which could be again caused by the worsen quality of the film.

Table 4: Calculated values of the electrochemical active surfaces and the apparent heterogeneous electron-transfer rate constants for all tested BDDE.

B/C [ppm]	A $[Fe(CN)_6]^{3-/4-}$ [mm^2]	k^0_{app} $[Fe(CN)_6]^{3-/4-}$ [cm s^{-1}]	k^0_{app} $[Ru(NH_3)_6]^{3+/2+}$ [cm s^{-1}]
1000	0.25	1.8×10^{-9}	5.2×10^{-4}
2000	0.26	1.2×10^{-8}	2.7×10^{-3}
4000	0.30	7.0×10^{-7}	5.5×10^{-3}
8000	0.36	9.0×10^{-6}	1.3×10^{-2}
10 000	0.60	1.1×10^{-4}	9.7×10^{-2}
20 000	0.30	5.0×10^{-5}	7.7×10^{-3}

The influence of the v was examined next. This parameter varied from 10 to 500 mV s^{-1}. Anodic and cathodic signal increased with the scan rate but the dependence was non-linear. On the other hand, the linearity was observed for the dependence between peak height and the square root of the scan rate (Figure 11). This result is typical for the diffusion controlled electrode reaction, which is common for BDDE due to their low ability to adsorb analytes on the working surface. Very similar outcomes were obtained for both redox systems.

Finally, the attention in the electrochemical characterization was focused on the influence of scan rate and square root of the scan rate, respectively, on the value of

ΔE_p and calculation of the apparent heterogeneous electron transfer rate constants (k^0_{app}) employing the Nicholson approach [43]. It was proved, that the most differentiated signals were recorded on BDDE with B/C 1000 ppm and the difference decreased with increasing B/C. The lowest values were determined for BDDE with the highest B/C. Values of k^0_{app} were calculated for v = 100 mV s^{-1}. It is obvious (Table 4), that k^0_{app} increased with growing B/C in the gas phase. It can be concluded, that higher k^0_{app} indicated faster heterogeneous electron transfer and confirmed favorable electrochemical properties of the working electrodes. Thus, the electrodes of the best electrochemical features can be assumed for BDDE with higher B/C in the gas phase, namely 10 000 ppm.

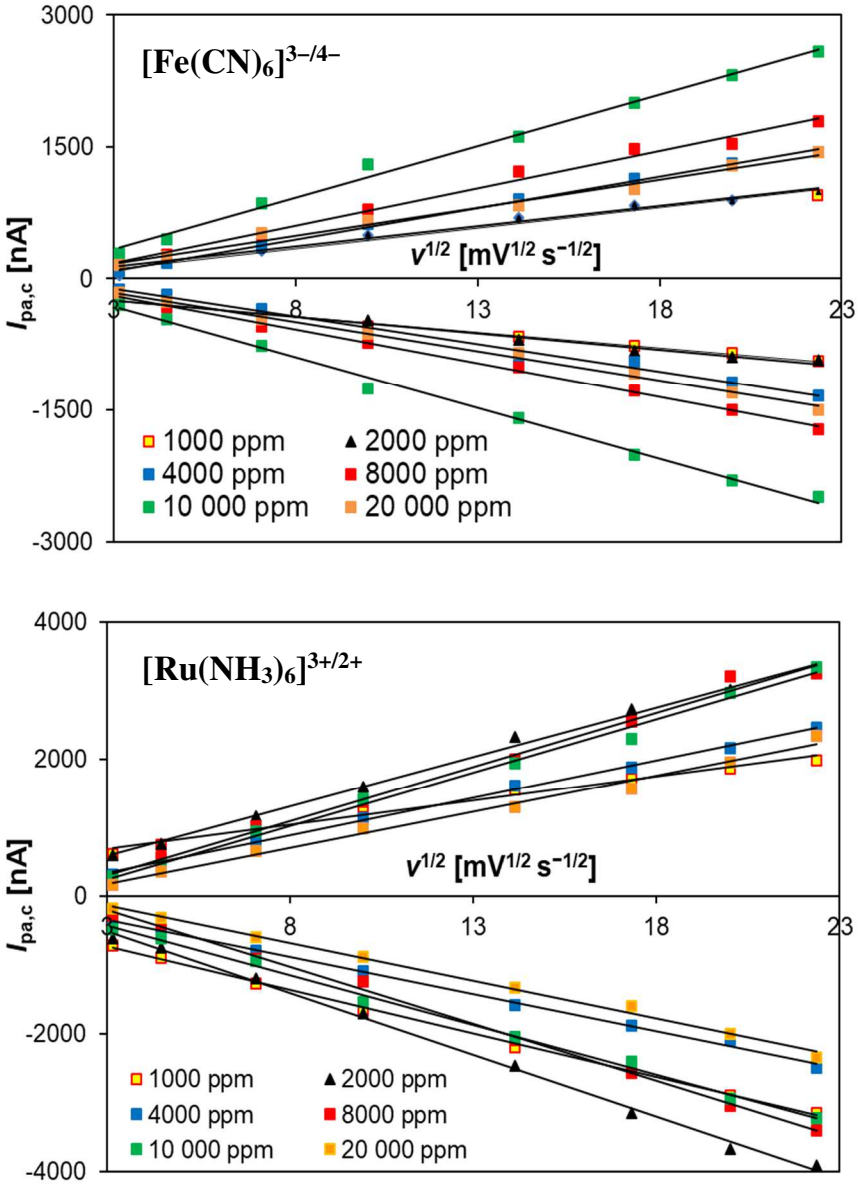

Figure 11: Dependences of $I_{pa,c}$ on $v^{1/2}$ for 2,5×10^{-3} mol L^{-1} $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$ obtained with tested BDDEs. Method – CV, electrolyte – KCl (0,1 mol L^{-1}), E_{in} = −1600 mV, E_{switch} = +1800 mV, v = 10-500 mV s^{-1}.

3.4 Applicability of BDDE with various B/C ratio in the analysis of bioactive compounds

The target of this work, beside the electrochemical characterization of the lab-made BDDE, was examination and assessment of their analytical utilization for LIN, 5-ASA, and LV determination. DP voltammograms in presence of 5×10^{-6} mol L^{-1} LIN and 5×10^{-6} mol L^{-1} LV, and SW voltammogram in presence of 5×10^{-5} mol L^{-1} 5-ASA were recorded using all tested electrodes and the values of I_p were evaluated. The obtained dependencies of I_p on B/C are shown in Figure 12. It is evident, that the I_p increased with increasing B/C up to 10 000 ppm for all tested substances. For 5-ASA and LV an I_p increases also between 10 000 and 20 000 ppm, but the growth in the current response does not match to the increase of boron content in the BDD film. In case of LIN, I_p significantly decreases between 10 000 and 20 000 ppm. It could be concluded, that electrode with B/C 10 000 ppm provided comparable or even better electrochemical properties than BDDE with B/C 20 000 ppm and increasing of the B/C did not lead to improving of the electrode's features.

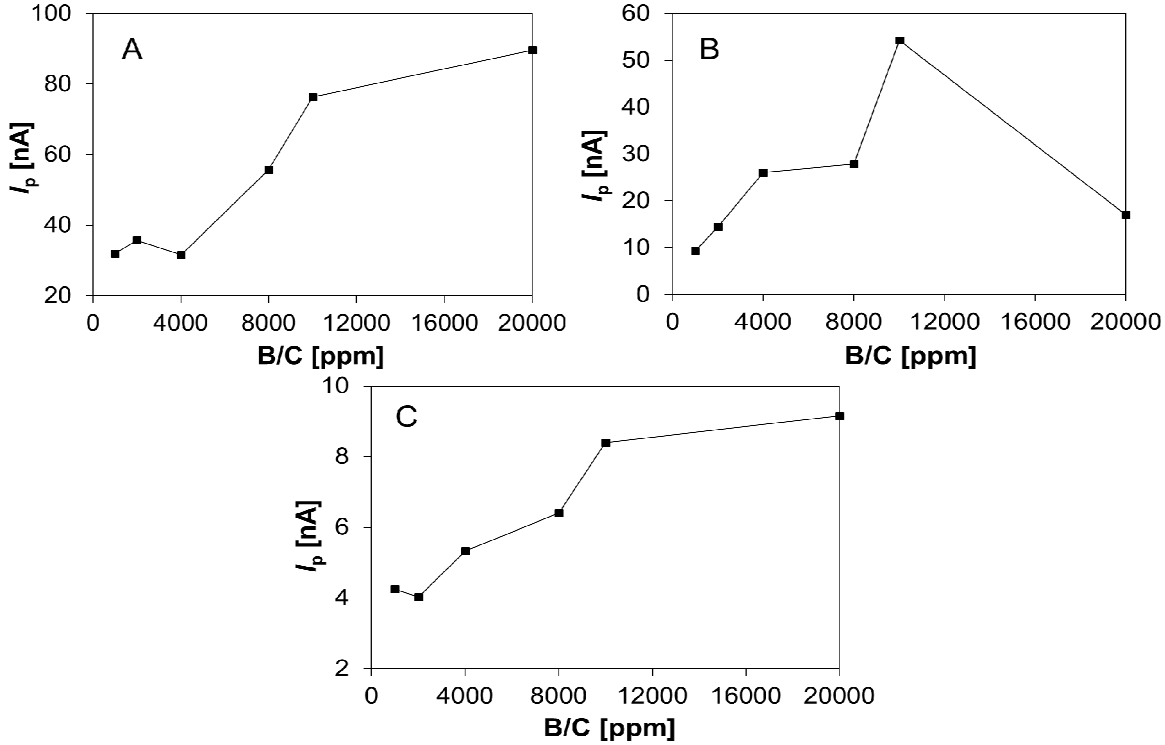

Figure 12: The dependencies of analytes I_p on the B/C ratio obtained using tested BDDEs (5×10^{-5} mol L^{-1} 5-ASA (A), 5×10^{-6} mol L^{-1} LIN (B), 5×10^{-6} mol L^{-1} LV (C)). Method: DPV, BRB (pH 2), E_{in} = +400 mV, E_{fin} = +2000 mV, v = 50 mV s^{-1}, pulse height = 70 mV, pulse width = 20 ms (LIN); SWV, BRB (pH 7), E_{in} = 0 mV, E_{fin} = +1800 mV, A = 70 mV, f = 25 Hz (5-ASA); DPV, BRB (pH 3), E_{in} = −700 mV, E_{fin} = +1800 mV, v = 40 mV s^{-1}, pulse height = 50 mV, pulse width = 20 ms (LV).

After that, series of concentration dependencies were measured for all tested analytes on all of the tested electrodes to obtain statistical parameters like LDR, LOD, LOQ, or RSD of repeated measurements (n = 11)). Examples of the obtained concentration dependences for LIN in the range from $2{,}50\times10^{-6}$ to $2{,}25\times10^{-5}$ mol L^{-1} , for 5-ASA from $5{,}0\times10^{-5}$ to $4{,}5\times10^{-4}$ mol L^{-1}, and for LV from $1{,}0\times10^{-6}$ to $1{,}8\times10^{-5}$ mol L^{-1} are illustrated in Figures 13-15. In the inset of these Figures,

voltammetric curves obtained using BDDE (10 000 ppm) corresponding with the appropriate dependence are placed. It is evident from figures that the peak heights increased linearly with LIN, 5-ASA, and LV concentration. The value of slope increases for all analytes with increasing B/C ration, but in case of LIN and LV the slope decrease for BDDE (20 000 ppm). Calculated statistical parameters are summarized for LIN in Table 5, 5-ASA in Table 6, and LV in Table 7. Very good results were, as it was expected, obtained for electrodes with higher content of boron (8000 and 10 000 ppm) and surprisingly also for electrode with B/C 1000 ppm. In the case of BDDE with B/C 20 000 ppm, the deterioration of monitoring parameters was observed.

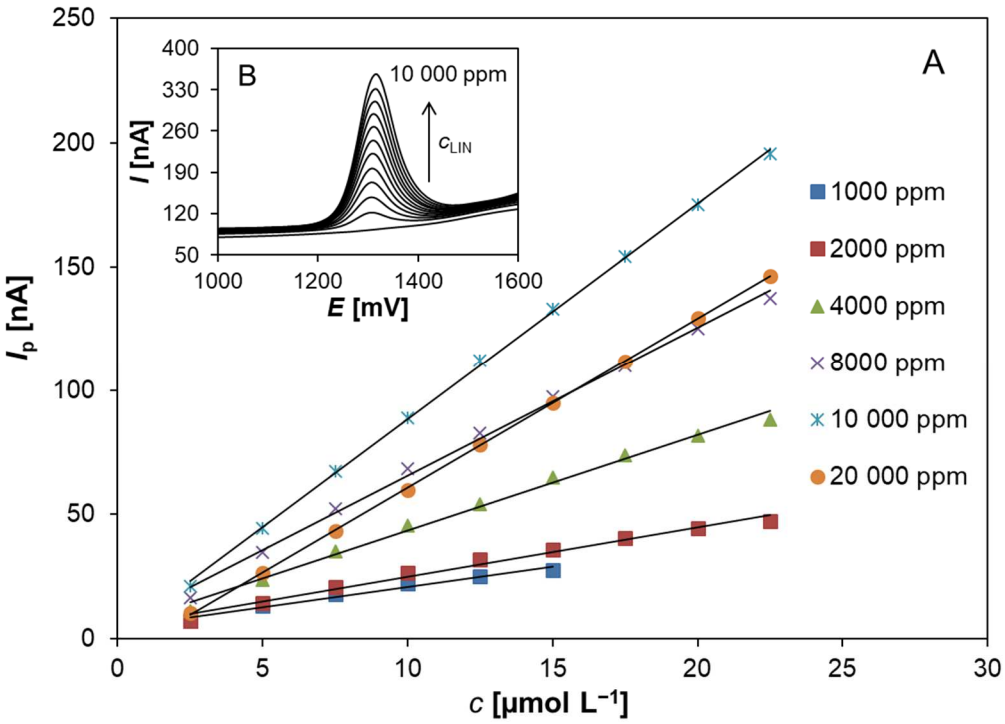

Figure 13: Linear concentration dependencies of LIN in range 2.50×10^{-6}–2.25×10^{-5} mol L^{-1} obtained on the lab-made BDDEs with B/C 1000–20 000 ppm (A), example of voltammetric curves of LIN recorded on BDDE with B/C 10 000 ppm (B). Method – DPV, BRB (pH 2), E_{in} = +400 mV, E_{fin} = +2000 mV, v = 50 mV s^{-1}, pulse height = 70 mV, pulse width = 20 ms.

Table 5: Statistical parameters of LIN determination obtained with all particular BDDEs, DPV, BRB (pH 2), E_{in} = +400 mV, E_{fin} = +2000 mV, v =50 mV s^{-1}, pulse height = 70 mV, pulse width = 20 ms.

B/C [ppm]	LDR [mol L^{-1}]	LOD [mol L^{-1}]	LOQ [mol L^{-1}]	RSD_M(11) [%]
1000	2.5×10^{-7}-2.5×10^{-5}	1.1×10^{-7}	3.5×10^{-7}	1.2
2000	1.0×10^{-6}-2.5×10^{-5}	4.0×10^{-7}	1.3×10^{-6}	2.9
4000	1.0×10^{-6}-3.0×10^{-5}	3.1×10^{-7}	1.0×10^{-6}	1.6
8000	5.0×10^{-7}-7.0×10^{-5}	6.2×10^{-8}	2.1×10^{-7}	0.4
10 000	5.0×10^{-7}-9.0×10^{-5}	1.8×10^{-7}	6.1×10^{-7}	0.3
20 000	1.5×10^{-6}-8.0×10^{-5}	2.9×10^{-7}	9.5×10^{-7}	3.8

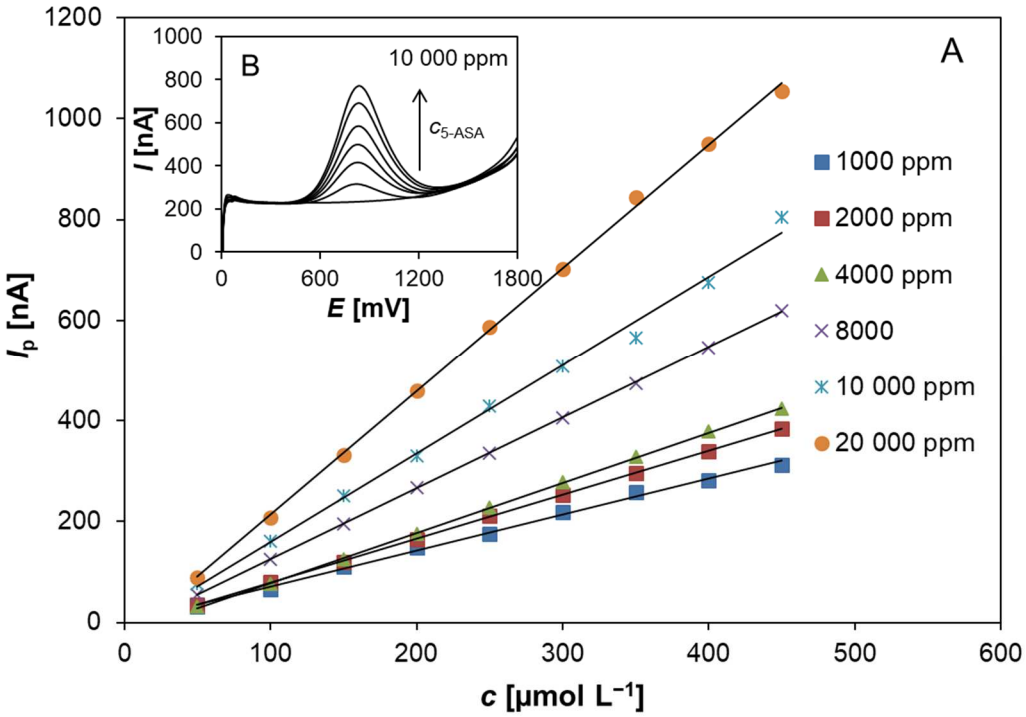

Figure 14: Linear concentration dependencies of 5-ASA in range 5.0×10^{-5}–4.5×10^{-4} mol L^{-1} obtained on the lab-made BDDEs with B/C 1000–20 000 ppm (A), example of voltammetric curves of 5-ASA recorded on BDDE with B/C 10 000 ppm (B). Method – SWV, BRB (pH 7), E_{in} = 0 mV, E_{fin} = +1800 mV, A =70 mV, f = 25 Hz.

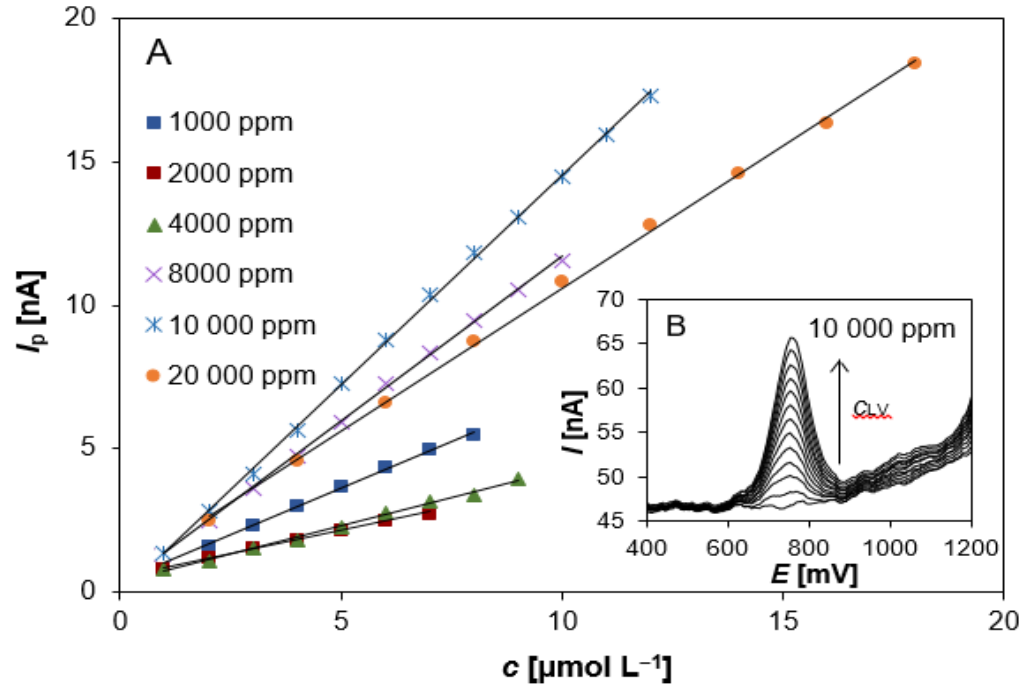

Figure 15: Linear concentration dependencies of LV in range 1.0×10^{-6}–1.8×10^{-5} mol L^{-1} obtained on the lab-made BDDEs with B/C 1000–20 000 ppm (A), example of voltammetric curves of LV recorded on BDDE with B/C 10 000 ppm (B). Method – DPV, BRB (pH 3), E_{in} = –700 mV, E_{fin} = +1800 mV, v = 40 mV s^{-1}, pulse height = 50 mV, pulse width = 20 ms.

Table 6: Statistical parameters of 5-ASA determination obtained with all particular BDDEs, SWV, BRB (pH 7), E_{in} = 0 mV, E_{fin} = +1800 mV, A =70 mV, f = 25 Hz.

B/C [ppm]	LDR [mol L^{-1}]	LOD [mol L^{-1}]	LOQ [mol L^{-1}]	$RSD_M(11)$ [%]
1000	2.0×10^{-6}-7.0×10^{-4}	6.3×10^{-7}	2.1×10^{-6}	1.7
2000	1.0×10^{-5}-9.0×10^{-4}	3.8×10^{-6}	1.3×10^{-5}	0.8
4000	2.5×10^{-5}-7.0×10^{-4}	3.7×10^{-6}	1.2×10^{-5}	1.2
8000	5.0×10^{-6}-9.0×10^{-4}	1.8×10^{-6}	5.6×10^{-6}	1.1
10 000	2.5×10^{-6}-1.0×10^{-3}	1.1×10^{-6}	3.7×10^{-6}	1.3
20 000	8.0×10^{-5}-1.0×10^{-3}	3.1×10^{-6}	1.1×10^{-5}	4.3

Table 7: Statistical parameters of LV determination obtained with all particular BDDEs, DPV, BRB (pH 3), E_{in} = −700 mV, E_{fin} = +1800 mV, v = 40 mV s^{-1}, pulse height = 50 mV, pulse width = 20 ms.

B/C [ppm]	LDR [mol L^{-1}]	LOD [mol L^{-1}]	LOQ [mol L^{-1}]	$RSD_M(11)$ [%]
1000	2.5×10^{-7}-2.5×10^{-5}	6.7×10^{-8}	2.2×10^{-7}	2.0
2000	1.0×10^{-6}-3.0×10^{-5}	3.6×10^{-7}	1.2×10^{-6}	1.7
4000	5.0×10^{-7}-3.0×10^{-5}	1.2×10^{-7}	3.7×10^{-7}	1.9
8000	5.0×10^{-7}-3.5×10^{-5}	9.0×10^{-8}	3.0×10^{-7}	3.4
10 000	5.0×10^{-7}-5.0×10^{-5}	1.0×10^{-7}	3.4×10^{-7}	1.1
20 000	2.0×10^{-6}-4.0×10^{-5}	4.2×10^{-7}	1.4×10^{-6}	0.8

Finally, repeatability of the LIN, 5-ASA, and LV determination were studied using always two model solutions for all of the examined electrodes. The standard addition method was employed for this experiment and 2 standard additions were added at least. Every determination was 5 times repeated and RSD of 5 repeated determinations (n = 5) were calculated. It was found that all of the obtained results were correct and repeatable. $RSD_D(5)$ did not exceed 4 % for LIN, 5-ASA, and LV and for all tested lab-made BDDE. Only the analyte concentrations for some of the tested electrodes should be increased due to worse obtained statistical parameters, namely higher LOD and LOQ, respectively.

Conclusion

The presented work summarizes the results of the extensive study focused on physical, chemical, and electrochemical properties of BDDE prepared by CVD with heated filaments in dependence on the boron doping level. SEM and Raman spectroscopy were used for the surface characterization, subsequently EIS and CV of redox systems $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$ were applied for the investigation of the electrochemical properties of the tested electrodes. Finally, the voltammetric behavior of the herbicide linuron and drugs mesalazine and leucovorin on commercially supplied and lab-made BDDEs was studied based on their electrochemical oxidation.

It was found that boron doping level strongly influenced the features of the working electrodes, namely the width of the potential window, reversibility of the electrode reaction, height of the background, and the ratio of signal to noise. The best results were obtained for electrodes with higher (not the highest) B/C in the gas phase, specifically 10 000 ppm. Surprisingly very good results in analytical application were obtained also for BDDE with B/C 1000 ppm despite poor results dealing with its electrochemical properties. It can be given by low background and high ration of signal to noise in case of this electrode, and especially by very good linearity of concentration dependencies.

List of References

1. HEYROVSKY, Jaroslav. Elektrolysa se rtuťovou kapkovou elektrodou. *Chemické Listy*, 1922, v. 16, p. 256-264.
2. KLOUDA, Pavel.: *Moderní analytické metody*. 2., edited and completed issue Ostrava, 132 p., 2003, ISBN 8086369072.
3. KOTRLÝ, Stanislav, CHURÁČEK, Jaroslav. *Analytická chemie III – elektroanalytické metody*. Issue 1. Pardubice: University of Chemical Technology, 82 p., 1984.
4. J. Heyrovsky Institute of Physical Chemistry. www.jh-inst.cas.cz [online]. [cit. 2018-07-07]. Available at: http://www.jh-inst.cas.cz/www/detail.php?p=2
5. Příběh kapky, Jaroslav Heyrovský, the first Czech Nobelist. www.jh-inst.cas.cz [online]. [cit. 2018-07-07]. Available at: https://www.jh-inst.cas.cz/heyrovsky/
6. PATEL, Kanaksundar., et al. Application of boron-doped CVD-diamond film to photoelectrode. *Denki Kagaku*, 1992, v. 60, i. 7, p. 659-661.
7. SWAIN, Greg. M., RAMESHAM, Rajeshuni. The electrochemical activity of boron-doped polycrystalline thin-film electrodes. *Analytical Chemistry*, 1993, v. 65, i. 4, p. 345-351.
8. PECKOVÁ, Karolína, et al. Boron-Doped Diamond Film Electrodes – New Tool for Voltammetric Determination of Organic Substances. *Critical Reviews in Analytical Chemistry*, 2009, v. 39, i. 3, p. 148-172.
9. EINAGA, Y., FUJISHIMA, A. In: BRILLAS, E, MARTINEZ-HUITLE, CA (eds) *Synthetic diamond films: Preparation, Electrochemistry, Characterization, and Applications*. Wiley, New York , 2011. 590 p. ISBN 9780470487587.
10. XU, Jishou, et. al. Boron-doped diamond thin-film electrodes. *Analytical Chemistry*, 1997, v. 69, i. 19, p. 591-597.
11. SWAIN, Greg M., et al. Application of diamond thin films in electrochemistry. *MRS Bulletin*, 1998, v. 23, i. 9 p. 56-60.
12. PECKOVÁ-SCHWARZOVÁ, K., ZIMA, J., BAREK, J. In MORETTO, LM, KALCHER, K (eds) *Environmental analysis by electrochemical sensors and biosensors*. Springer Verlag, New York, 2014, 713 p., ISBN 978-1-4939-0676-5.
13. TERASHIMA, Chiaki., et al.: Electrochemical Oxidation of Chlorophenols at a Boron-Doped Diamond Electrode and Their Determination by High-Performance Liquid Chromatography with Amperometric Detection. *Analytical Chemistry*, 2002, v. 74, i. 4, p. 895-902.
14. PEDROSA, Valber de A., et al.: Electroanalytical determination of 4-nitrophenol by square wave voltammetry on diamond electrodes. *Journal of the Brazilian Chemical Society*, 2003, v. 14, i. 6, p. 530-535.
15. WANGFUENGKANAGUL, Nattakarn, CHAILAPAKUL, Orawon. Electrochemical analysis of acetaminophen using a boron-doped diamond thin film electrode applied to flow injection system. *Journal of Pharmaceutical and Biomedical Analysis*, 2002, v. 28, i. 5 p. 841-847.

16. SARTORI, Elen Romão. et al. Square-wave voltammetric determination of propranolol and atenolol in pharmaceuticals using a boron-doped diamond electrode. *Talanta*, 2010, v. 81, i. 4-5, p. 1418-1424.
17. ŠVORC, Ľubomír., et al. Green electrochemical sensor for environmental monitoring of pesticides: Determination of atrazine in river waters using a boron-doped diamond electrode. *Sens Actuators B*, 2013, v. 181, p. 294-300.
18. JANÍKOVÁ-BANDŽUCHOVÁ, Lenka, et al. Sensitive voltammetric method for rapid determination of pyridine herbicide triclopyr on bare boron-doped diamond electrode. *Electrochimica Acta*, 2015, v. 154, p. 421-429.
19. HUANG, L. C. Lora, CHANG, Huan-Cheng. Adsorption and immobilization of cytochrome c on nanodiamonds. *Langmuir*, 2004, v. 20, i. 14, p. 5879-5884.
20. YANG, Wensha, et al. DNA-modified nanocrystalline diamond thin-film as stable, biologically active substrates. *Nature Materials*, 2002, v. 1, p. 253-257.
21. TROUPE, Clare E., et al. Diamond-based glucose sensors. *Diamond and Related Materials*, 1998, v. 7, i. 2-5, p. 575-580.
22. EPA: R.E.D Facts. [cit. 2017-08-23] [online]. Available at: https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-035506_1-Mar-95.pdf
23. EXTOXNET: [cit. 2017-08-23] [online]. Available at: http://extoxnet.orst.edu/pips/linuron.htm
24. DE LIMA, F., et al.: Determination of Linuron in Water and Vegetables Samples Using Stripping Voltammetry with a Carbon Paste Electrode. *Talanta*, 2011, v. 83, i. 5, p. 1763-1768.
25. ĐORĐEVIĆ, Jelena, et al. Voltammetric Determination of the Herbicide Linuron Using a Tricresyl Phosphate-Based Carbon Paste Electrode. *Sensors*, 2012, v. 12, i. 1, p. 148-161.
26. FIGUIREDO-FILHO, Luiz C. S., et al. Electroanalytical Determination of the Linuron Herbicide Using a Cathodically Pretreated Boron Doped Diamond Electrode: Comparison with a Boron-Doped Diamond Electrode Modified with Platinum Nanoparticles. *Analytical Methods*, 2015, v. 7, i. 2, p. 643-649.
27. SIGMA ALDRICH: Safety Data Sheet. Http://www.sigmaaldrich.com/ [online]. [cit. 2017-08-23]. Available at: http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=CZ&language=cs&productNumber=36141&brand=SIAL&PageToGoToURL=http%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Dlinuron%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DCZ%26focus%3Dproduct
28. IACUCCI, Marietta, et al. Mesalazine in inflammatory bowel disease: a trendy topic once again? *Canadian Journal of Gastroenterology,* 2010, v. 24, i. 2, p. 127-133.
29. JOSHI, Ravi, et al. Free radical scavenging reactions of sulfasalazine, 5-aminosalicylic acid and sulfapyridine: mechanistic aspects and antioxidant activity. *Free Radical Research,* 2005, v. 39, i. 11, p. 1163-1172.
30. BODEGRAVEN, Ad A., MULDER, Chris J.J. Indications for 5-aminosalicylate in inflammatory bowel disease: is the body of evidence

complete? *World Journal of Gastroenterology,* 2006, v. 12, i. 38, p. 6115-6123.
31. AHNFELT-RONNE, Ian, et al. Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. *Gastroenterology,* 1990, v. 98, i. 5, p. 1162-1169.
32. SHAHROKHIAN, Saeed, et al. Investigation of the electrochemical behavior of mesalazine on the surface of a glassy carbon electrode modified with CNT/PPY doped by 1,5-naphthalenedisulfonic acid. *Electroanalysis,* 2013, v. 25, i. 11, p. 2481-2491.
33. NIGOVIĆ, Biljana, ŠIMUNIĆ, Branimir. Determination of 5-aminosalicylic acid in pharmaceutical formulation by differential pulse voltammetry. *Journal of Pharmaceutical an Biomedical Analysis,* 2003, v. 31. i. 1, p. 169-174.
34. BECKETT, Emma L., et al. Sonoelectrochemically enhanced determination of 5-aminosalicylic acid. *Talanta,* 2001, v. 54, i. 5, p. 871-877.
35. PALSMEIER, Rita K., et al. Investigation of the degradation mechanism of 5-aminosalicylic acid in aqueous solution. *Pharmaceutical Research*, 1992, v. 9, i. 7, p. 933-938.
36. SIGMA ALDRICH: Safety Data Sheet. Http://www.sigmaaldrich.com/ [online]. [cit. 2017-08-23]. Available at: http://www.sigmaaldrich.com/catalog/product/sial/y0000297?lang=en&region=CZ
37. JOLIVET, Jacques. Role of Leucovorin Dosing and Administration Schedule. *European Journal of Cancer,* 1995, v. 31, i. 7-8, p. 1311-1315.
38. BLEYER, W. Archie. The clinical Pharmacology of Methotrexate. *Cancer,* 1978, v. 41. i. 1, p. 36-51.
39. MINI, Enrico, et al. Enhancement of the antitumor effects of 5-fluorouracil by folinic acid. *Pharmacology and Therapeutics,* 1990, v. 47, i. 1, p. 1-19.
40. ALLEN, William, et al. Polarographic determination and evidence for the structure of leucovorin. *Journal of the American Chemical Society,* 1952, v. 74, i. 13, p. 3264-3269.
41. ŠELEŠOVSKÁ, Renáta, et al. Voltammetric determination of leucovorin using silver solid amalgam electrode. *Electrochimica Acta,* 2012, v. 60, p. 375-383.
42. RANDLES, J. E. B. A cathode ray polarograph. Part II.—the current-voltage curves. *Transactions of the Faraday Society,* 1948, v. 44, p. 327-338.
43. NICHOLSON, Richard S. Theory and Application of Cyclic Voltammetry for Measurement of Electrode Reaction Kinetics. *Analytical Chemistry,* 1965, v. 37, i. 11, p. 1351-1355.
44. OLIVEIRA, S. Carlos. B., OLIVEIRA-BRETT, Ana Maria. Voltammetric and electrochemical impedance spectroscopy characterization of a cathodic and anodic pre-treated boron doped diamond electrode. *Electrochimica Acta,* 2010, v. 55, i. 15, p. 4599-4605.

List of Students' Published Works

Articles dealing with the topic of Ph.D. thesis published in journals with IF

1. ŠELEŠOVSKÁ, Renáta, ŠTĚPÁNKOVÁ, Michaela, JANÍKOVÁ, Lenka, NOVÁKOVÁ, Kateřina, VOJS, Marian, MARTON, Marián, BEHÚL, Miroslav. Surface and electrochemical characterization of boron-doped diamond electrodes prepared under different conditions. *Monatshefte für Chemie – Chemical Monthly*, 2016, 147, 1353-1364.
2. ŠTĚPÁNKOVÁ, Michaela, ŠELEŠOVSKÁ, Renáta, JANÍKOVÁ, Lenka, CHÝLKOVÁ, Jaromíra. Voltammetric determination of mesalazine in pharmaceutical preparations and biological samples using boron-doped diamond electrode. *Chemical Papers*, 2017, 71, 1419-1427.
3. ŠTĚPÁNKOVÁ, Michaela, ŠELEŠOVSKÁ, Renáta, JANÍKOVÁ, Lenka, MARTINKOVÁ, Pavlína, MARTON, Marián, MICHNIAK, Pavol, CHÝLKOVÁ, Jaromíra. Porovnání borem dopovaných diamantových elektrod s různým obsahem boru při stanovení herbicidu linuronu a léčiva mesalazinu. *Chemické Listy*, 2018, 112, 389-395.
4. ŠELEŠOVSKÁ, Renáta, KRÄNKOVÁ, Barbora, ŠTĚPÁNKOVÁ, Michaela, MARTINKOVÁ, Pavlína, JANÍKOVÁ, Lenka, CHÝLKOVÁ, Jaromíra, VOJS, Marian. Influence of boron content on electrochemical properties of boron-doped diamond electrodes and their utilization for leucovorin determination. *Journal of Electroanalytical Chemistry*, 2018, 821, 2-9.
5. ŠELEŠOVSKÁ, Renáta, KRÄNKOVÁ, Barbora, ŠTĚPÁNKOVÁ, Michaela, MARTINKOVÁ, Pavlína, JANÍKOVÁ, Lenka, CHÝLKOVÁ, Jaromíra, NAVRÁTIL, Tomáš. Voltammetric determination of leucovorin in pharmaceutical preparations using a boron-doped diamond electrode. *Monatshefte für Chemie – Chemical Monthly*, in press (2018).

Other articles of the author published in journals with IF

1. ŠTĚPÁNKOVÁ, Michaela, ŠELEŠOVSKÁ, Renáta, JANÍKOVÁ-BANDŽUCHOVÁ, Lenka, CHÝLKOVÁ, Jaromíra. Voltametrické stanovení insekticidu imidaclopridu s využitím stříbrné pevné amalgámové elektrody. *Chemické listy*, 2015, 109, 527-534.
2. ŠELEŠOVSKÁ, Renáta, JANÍKOVÁ, Lenka, ŠTĚPÁNKOVÁ, Michaela, CHÝLKOVÁ, Jaromíra. Copper solid amalgam electrode as a simple and sensitive tool for voltammetric determination of the antineoplastic drug 5-fluorouracil in pharmaceuticals. *Chemical Papers*, 2017, 71, 679-688.
3. ŠTĚPÁNKOVÁ, Michaela, ŠELEŠOVSKÁ, Renáta, JANÍKOVÁ, Lenka, CHÝLKOVÁ, Jaromíra, ŠVANCARA, Ivan. Sensitive electrochemical sensor for the determination of folic acid based on a bismuth-film electrode. *Monatshefte für Chemie – Chemical Monthly*, 2017, 147, 423-433.

4. ŠELEŠOVSKÁ, Renáta, MARTINKOVÁ, Pavlína, ŠTĚPÁNKOVÁ, Michaela, NAVRÁTIL, Tomáš, CHÝLKOVÁ, Jaromíra. Comparison Study of Voltammetric Behavior of Muscle Relaxant Dantrolene Sodium on Silver Solid Amalgam and Bismuth Film Electrodes. *Journal of analytical methods in chemistry*, 2017, 2017, 12 pages.