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# Electrochemical method for point-of-care determination of ciprofloxacin using boron-doped diamond electrode

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**Abstract:** This paper presents an electrochemical behavior study and quantification of fluoroquinolone antibiotic ciprofloxacin using boron-doped diamond electrode. Ciprofloxacin provides a diffusion-driven electrode reaction with an irreversible and poorly defined peak at +1.6 V *vs.* Ag/AgCl electrode in the presence of Britton-Robinson buffer solution pH 4. Applying differential pulse voltammetry (modulation amplitude of 60 mV, modulation time of 50 ms), the calibration curve with high linearity ($R^2$ = 0.997) was obtained within the concentration range of (0.74 – 20.0) × $10^{-6}$ mol $L^{-1}$ with the detection limit of 6.0 × $10^{-7}$ mol $L^{-1}$ and repeatability expressed by relative standard deviation of 2.7 % (for 20 measurements). Interference study was performed to explore the selectivity of the elaborated procedure. By analysis of pharmaceutical dosages and model human urine samples, the ciprofloxacin content with the recovery values ranging from 88.4 to 121.2 % were determined. The developed approach using point-of-care electrochemical sensor based on boron-doped diamond material could represent inexpensive analytical alternative to separation methods and could be beneficial in analysis of biological samples and in the quality control in pharmaceutical industry.

**Keywords:** ciprofloxacin, boron-doped diamond, differential pulse voltammetry, detection limit, pharmaceuticals

## Introduction

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)quinolone-3-carboxylic acid) belongs to second generation of fluoroquinolone antibiotics fluorinated at C6 position with the structure related to nalidixic acid. This broad-spectrum antibiotic is effective through suppressing the synthesis of bacterial DNA (Torriero et al., 2006). Thanks to its antimicrobial properties, ciprofloxacin is widely used in medicine for treating the infections caused by gram-positive and gram-negative bacteria such as urinary, tissue, digestive and pulmonary infections as well as in veterinary medicine (Shan et al., 2015; Zhang et al., 2013). Moreover, it is recommended for treatment of patients who have been exposed to anthrax (Ensafi et al., 2012). However, extensive consumption may result in allergic reactions and antibiotic resistance. In this sense, the development of fast and reliable analytical methods and coupled protocols for the point-of-care determination of ciprofloxacin in terms of clinical monitoring and pharmaceutical quality control is of high importance.

Up to date, the most common analytical methods employed for the quantification of ciprofloxacin traces are based on high-performance liquid chromatography (Vella et al., 2015), liquid chromatography (González et al., 2006), capillary zone electrophoresis (Xu et al., 2015), spectrophotometry (Nagaralli et al., 2002) and chemiluminiscence (Liang et al., 2004). Although these methods are sensitive and accurate, they are expensive, complicated and time consuming with the need of skilled operators. Electrochemical methods meet simple instrument with low instrumental costs and rapid response to be exhibited. In particular, they overcome the shortcomings of spectral and separation methods while providing comparable sensitivity levels.

Regarding electrochemical methods in relation to ciprofloxacin, its behaviour and quantification has been extensively studied using modified carbonaceous electrodes based on carbon paste electrode (Zhang et al., 2013) and glassy carbon electrode (Kawde et al., 2014). Modifiers such as graphene (Ensafi et al., 2012; Shan et al., 2015) and multi-walled carbon nanotubes (Fotouhi et al., 2010; Zhang et al., 2013) have been usually used in order to improve current response giving rise to higher sensitivity. In addition, an electrochemical method based on enhancement effect of cetyltrimethylammonium bromide was developed (Yi et al., 2007). The reduction behaviour of ciprofloxacin was investigated using hanging mercury drop electrode with a low detection limit of 7 × $10^{-9}$ mol $L^{-1}$ (Al-Ghamdi et al., 2014). Electrochemical biosensor based on horseradish peroxidase immobilized on a rotating disk was also employed for the determination of ciprofloxacin (Torriero et al., 2006). The lowest

detection limit of $2.6 \times 10^{-9}$ mol $L^{-1}$ was obtained in the work of Kingsley et al. (2016). It was proven that using copper zinc ferrite nanoparticle modified carbon paste electrode, the oxidation potential is shifted to lower values in comparison with a plain electrode. Developed electrochemical sensor was subsequently applied to simultaneous determination of ciprofloxacin and paracetamol. On the other hand, it is worth mentioning that utilization of electrocatalyst for an enhancement of particular electrochemical reaction is oftentimes tedious with the total cost of measurement to be significantly increased. Hence, the development of simple, rapid and cost-effective method for electroanalytical determination of ciprofloxacin is still needed.

Boron-doped diamond (BDD) has been recognized as one of the most promising and sensing materials owing to properties such as large working potential window in aqueous solutions, high electrochemical stability and hardness, biocompatibility, very low and stable capacitive current and resistance to fouling (Cinková et al., 2015). Recently, Garbellini et al. (2015) proposed a voltammetric method for the determination of ciprofloxacin using a cathodically pretreated BDD electrode coupled with square-wave voltammetry (SWV) and differential pulse voltammetry (DPV) with detection limits of $2.46 \times 10^{-6}$ and $4.4 \times 10^{-7}$ mol $L^{-1}$, respectively. The developed DPV method was applied to determination of ciprofloxacin in synthetic urine samples and SWV was employed for studies on the interaction of the fluoroquinolone with double-stranded DNA.

In this paper, the voltammetric behaviour of ciprofloxacin was studied on BDD electrode. The developed electroanalytical method was applied to determination of ciprofloxacin in pharmaceutical dosages and model human urine samples. It may represent rapid and cost effective alternative to separation and spectral methods.

## Materials and Methods

Cyclic voltammetric (CV) and differential pulse voltammetric (DPV) measurements were performed using an electrochemical system Autolab PGSTAT 101 (Metrohm Autolab B.V., The Netherlands), controlled by the NOVA (version 1.8) software. The electrochemical cell was equipped with a BDD working electrode (boron doping level of 1000 ppm, Windsor Scientific Ltd., United Kingdom), an Ag/AgCl/3 mol $L^{-1}$ KCl reference electrode and a Pt wire as a counter electrode. All pH values were measured using pHenomenal® pH 1100L meter (VWR, Slovakia) with a combined electrode (glass-reference electrode).

Ciprofloxacin was obtained from Sigma Aldrich (Slovakia) and used without any further purification. Its stock solution ($1.0 \times 10^{-3}$ mol $L^{-1}$) was prepared by dissolution in double-distilled water. The solutions of lower concentrations were prepared from the stock solution by appropriate dilution with supporting electrolyte. Britton-Robinson buffer solution was prepared by mixing of 0.04 mol $L^{-1}$ of the components (phosphoric acid, acetic acid and boric acid) and adjusting with sodium hydroxide (0.2 mol $L^{-1}$) to required pH value. All chemicals were of analytical grade purity. Double-distilled deionized water with resistivity no less than 18 MΩ cm was used throughout the experiments.

Prior to launching the measurement, the BDD electrode was always rinsed with deionized water and the surface was pretreated by simple cycling in 0.5 mol $L^{-1}$ sulphuric acid using potentials ranged from –2.0 to +2.0 V until a stable signal was detected (usually 10 cycles). The experimental conditions for DPV were as follows: modulation amplitude of 60 mV and modulation time of 50 ms. The linear least-squares regression (OriginPro 8.5, OriginLab Corporation, USA) was used for the assessment of calibration curve and the relevant results (slope and intercept) were reported with a confidence interval for 95 % probability. The detection limit (LOD) was calculated as three times the standard deviation of the intercept divided by the slope of calibration curve.

Commercial samples of pharmaceutical tablets Ciprinol® 500 with the declared content of ciprofloxacin (500 mg) were purchased in a local pharmacy. The stock solution of sample of the tablets was prepared as follows: ten tablets were accurately weighed (mean weight of one tablet was 777 mg) and powdered in mortar. An equivalent portion of 227 mg of homogenized powder was dissolved in 2 mol $L^{-1}$ sulphuric acid. The mixture was subsequently filtered through a filter paper, transferred into 100 mL volumetric flask and filled up with water. An appropriate aliquot (50 µL) of this solution was added to the electrochemical cell to 20 mL of supporting electrolyte. The standard addition method with respective volumes of 0.5, 1 and 1.5 mL of $1.0 \times 10^{-4}$ mol $L^{-1}$ ciprofloxacin stock standard solution was used to analyze the commercially available pharmaceutical tablets.

Human urine samples (U1 and U2) were taken from two volunteers prior to experiments. After filtration, an aliquot volume of fresh urine (0.2 mL) was placed into the electrochemical cell and 20 mL of supporting electrolyte was added. Subsequently, this solution was suitably fortified with standard solution of 0.1 mL $1.0 \times 10^{-3}$ mol $L^{-1}$ ciprofloxacin stock standard solution to achieve a required concentra-

tion. The analysis was undertaken using standard addition method with the respective volumes of 0.1, 0.2 and 0.3 mL of $1.0 \times 10^{-3}$ mol $L^{-1}$ ciprofloxacin stock standard solution.

## Results and Discussion

A redox reaction of organic compounds mainly depends on the pH of supporting electrolyte. Therefore, the influence of its pH on the current response of ciprofloxacin was examined. The electrochemical behaviour of ciprofloxacin at BDD electrode was first investigated using CV in potential range from –1.0 V to +2.0 V in $1.0 \times 10^{-3}$ mol $L^{-1}$ ciprofloxacin in Britton-Robinson buffer solution (pH 2—12). The best results were obtained in mildly acidic and neutral media (pH 4—7), while the peak current was decreasing in pH 8—12. Thus, Britton-Robinson buffer solution pH 4 was employed for further studies. Fig. 1 shows poorly defined peak corresponding to the oxidation of ciprofloxacin at +1.6 V vs. Ag/AgCl suggesting the electron transfer kinetics at BDD electrode is sluggish. Moreover, no cathodic peak appeared in the reverse scan indicating that the oxidation process was irreversible. The scan rate effect was explored recording cyclic volt-

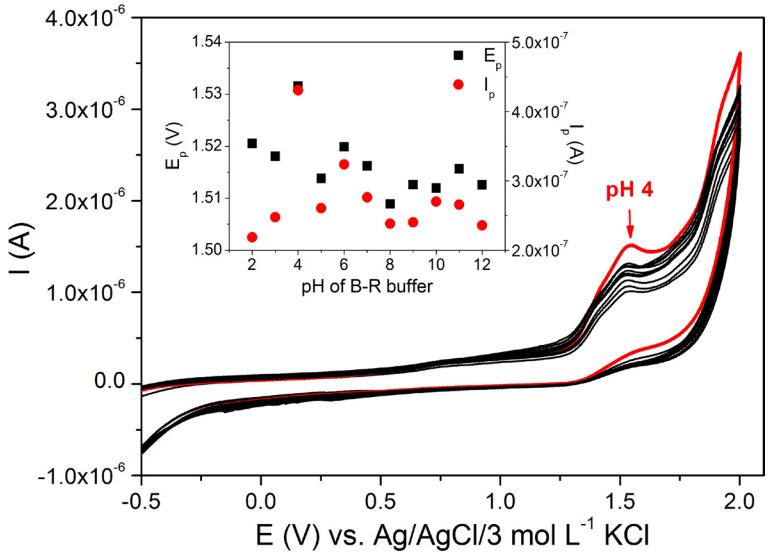


**Fig. 1.** Cyclic voltammograms of $1.0 \times 10^{-3}$ mol $L^{-1}$ ciprofloxacin in Britton-Robinson buffer solution (pH 2—12) at BDD electrode and a scan rate of 100 mV $s^{-1}$. Effect of pH on the peak potential $E_p$ and peak current $I_p$ of ciprofloxacin at BDD electrode appears in the inset.

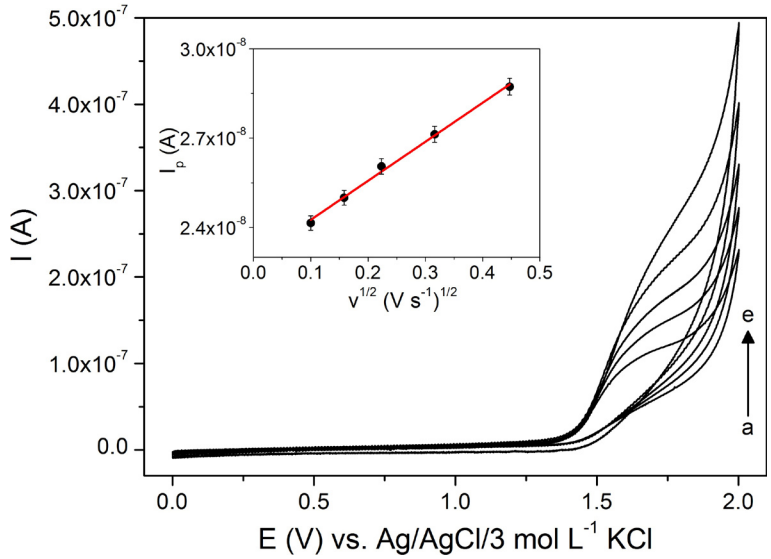


**Fig. 2.** Cyclic voltammograms of $1.0 \times 10^{-4}$ mol $L^{-1}$ ciprofloxacin for scan rates ($v$): (a) 0.01, (b) 0.025, (c) 0.05, (d) 0.1 and (e) 0.2 V $s^{-1}$ in Britton-Robinson buffer solution pH 4 at BDD electrode. The peak current $I_p$ as a function of square root of the scan rate $v^{1/2}$ appears in the inset.

ammograms in $1.0 \times 10^{-4}$ mol $L^{-1}$ ciprofloxacin in Britton-Robinson buffer solution pH 4 at scan rates in the range of 0.01—0.2 V $s^{-1}$ (Fig. 2). The peak current of ciprofloxacin increased linearly with square root of scan rate ($v^{1/2}$) and shifted slightly to positive values. This shift is typical for diffusion-limited electrode processes (Švorc et al., 2015). The dependence may be expressed by the following equation (1):

$$I_p(\mathrm{A}) = (2.30 \pm 0.01) \times 10^{-8} + (1.31 \pm 0.05) \times \times 10^{-8} \times v^{1/2}\,(\mathrm{V\ s^{-1}}),\ R^2 = 0.996 \quad (1)$$

In order to optimize the experimental set-up for ciprofloxacin determination, different DPV parameters influencing the current response of analyte were analyzed. The ranges of studied parameters were 10—150 ms and 25—150 mV for modulation time and modulation amplitude, respectively. The measurement was performed in $1.0 \times 10^{-4}$ mol $L^{-1}$ ciprofloxacin in Britton-Robinson buffer solution pH 4 and the optimized experimental parameters were as follows: modulation time of 50 ms and modulation amplitude of 60 mV.

Based on the optimized experimental conditions described above, the calibration curve was constructed. The peak current $I_p$ was plotted against the concentration of ciprofloxacin in the range of $(0.74—20.0) \times 10^{-6}$ mol $L^{-1}$. The DPV results show oval peak at the potential of +1.35 V vs Ag/AgCl (Fig. 3). The analytical parameters are summarized in Table 1. The LOD was found to be $6.0 \times 10^{-7}$ mol $L^{-1}$ and is slightly higher when compared to that reported by Garbellini et al. (2015) reached at cathodically pretreated BDD electrode. The repeatability was examined by measuring 20 successive DPV responses at the ciprofloxacin concentration level of $4.8 \times 10^{-6}$ mol $L^{-1}$. The relative standard deviation (RSD) was found to be 2.7 % showing the notable stability of BDD electrode. Overall, wide linear concentration range, sufficient sensitivity and excellent repeatability obtained with BDD as a modification-free electrode surface make the proposed method a suitable electrochemical platform for ciprofloxacin sensing in pharmaceuticals and biological samples.

**Tab. 1.** Analytical parameters for the determination of ciprofloxacin by DPV in Britton-Robinson buffer solution pH 4 at the BDD electrode ($n = 3$).

| Analytical parameter | Value |
|---|---|
| Intercept (A) | $(2.19 \pm 0.26) \times 10^{-9}$ |
| Slope (A L $mol^{-1}$) | $(1.35 \pm 0.03) \times 10^{-3}$ |
| Linear concentration range (mol $L^{-1}$) | $(0.74 - 20.0) \times 10^{-6}$ |
| Coefficient of determination | 0.997 |
| Detection limit (mol $L^{-1}$) | $6.0 \times 10^{-7}$ |
| Repeatability (%)* | 2.7 |

*RSD calculated for 20 replicate of measurements using $4.8 \times 10^{-6}$ mol $L^{-1}$ ciprofloxacin.

The influence of some possible organic biomolecules was investigated by adding the particular compounds to $1.0 \times 10^{-5}$ mol $L^{-1}$ ciprofloxacin in Britton-Robinson buffer pH 4 under optimized

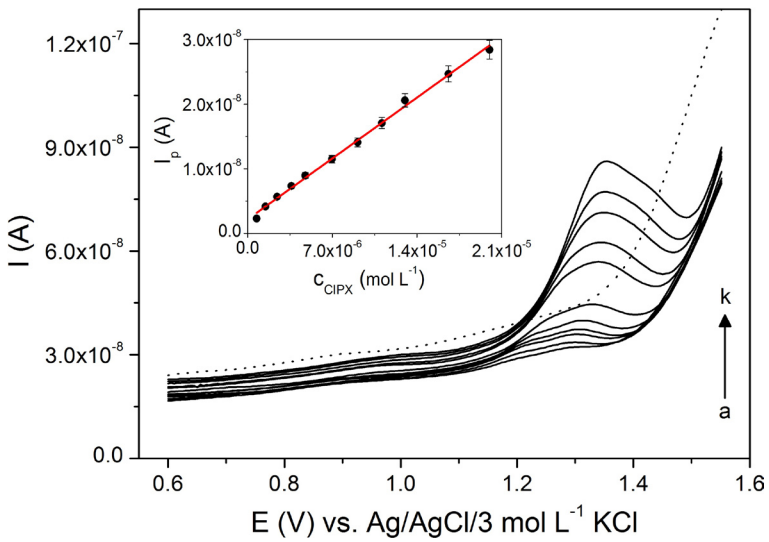


**Fig. 3.** DP voltammograms for various concentrations of ciprofloxacin: (a) $7.4 \times 10^{-7}$; (b) $1.5 \times 10^{-6}$; (c) $2.4 \times 10^{-6}$; (d) $3.6 \times 10^{-6}$; (e) $4.8 \times 10^{-6}$; (f) $7.0 \times 10^{-6}$; (g) $9.1 \times 10^{-6}$; (h) $1.1 \times 10^{-5}$; (i) $1.3 \times 10^{-5}$; (j) $1.7 \times 10^{-5}$ and (k) $2.0 \times 10^{-5}$ mol $L^{-1}$ in Britton-Robinson buffer solution pH 4 (dotted line) at BDD electrode. The optimized DPV parameters: modulation amplitude of 60 mV, modulation time of 50 ms. The dependence between peak current and ciprofloxacin concentrations appears in the inset.

DPV operating conditions. Caffeine, dopamine, folic acid, penicillin V and amlodipine are known to be oxidized at the potential close to the oxidation potential of ciprofloxacin. Therefore, these compounds may influence the ciprofloxacin voltammetric response. The effects were evaluated in the concentration ratios of ciprofloxacin to interfering agent as follows: 1:1, 1:2, 1:5, 1:10, 1:50 and 1:100. It was found that the signal of ciprofloxacin decreases at 10-fold excess of caffeine and 5-fold excess of dopamine. The oxidation peak of ciprofloxacin became enlarged after minor additions of folic acid. However, in its 50-fold excess a decrease of ciprofloxacin response was recorded accompanied by increasing background current. Furthermore, the signal of ciprofloxacin decreased in the presence of both penicillin V and amlodipine. To sum up, the significant influence was noticed even in the minor excess of tested biomolecules making the usage of this method slightly restricted in analysis of biological samples with the particular amount of these substances.

Finally, the application of the proposed method was performed by determining the concentrations of ciprofloxacin in commercially available pharmaceutical tablets and model human urine samples by means of the standard addition method. An aliquot of the tablet solution was added into the Britton-Robinson buffer solution pH 4 followed by additions of ciprofloxacin. DP voltammograms of ciprofloxacin in pharmaceuticals are displayed in Fig. 4. The peak attributed to the oxidation of ciprofloxacin was slightly moving from +1.3 to +1.4 V vs. Ag/AgCl with each standard addition. The declared of the compound amount was 500 mg per tablet and the value found was (442 ± 37) mg per tablet. Subsequently, the proposed method was used to determine ciprofloxacin in model human urine samples (indicated as U1 and U2). The results are listed in Table 2. The calculated recovery values (88.4 % for tablets and 90.7 and 121.2 % for urine samples) indicate that there is mild matrix effect in the analyzed samples. However, despite this fact, the proposed method could be considered as sufficiently accurate for quantification of ciprofloxacin in pharmaceutical and biological samples. Additionally, to enhance the analytical performance and to provide complete validation of the developed method, the

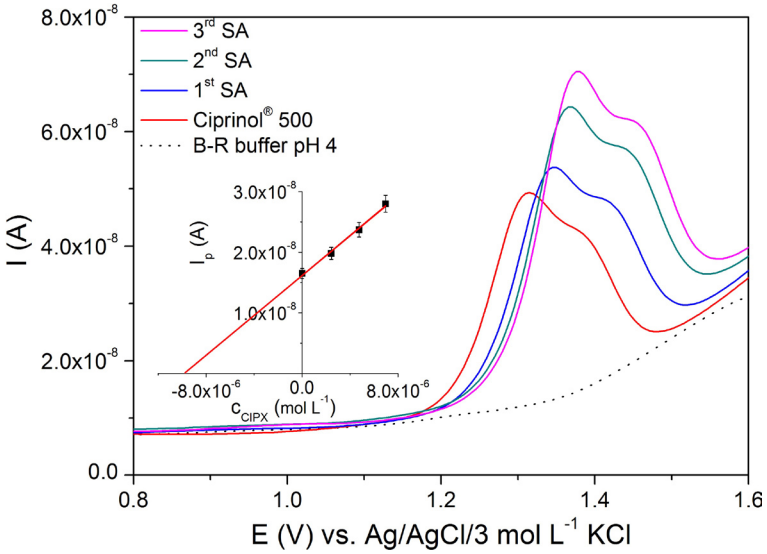


**Fig. 4.** DP voltammograms for the analysis of Ciprinol® 500 with the declared content of 500 mg ciprofloxacin using standard addition (SA) method in Britton-Robinson buffer solution pH 4 at BDD electrode. The optimized DPV parameters: modulation amplitude of 60 mV, modulation time of 50 ms. The analysis by standard addition method is depicted in the inset.

**Tab. 2.** Analysis of model human urine samples using the proposed method ($n = 3$).

| Sample | Ciprofloxacin content (mol $L^{-1}$) | | Recovery (%) |
|---|---|---|---|
| | Added | Found* | |
| U1 | $2.45 \times 10^{-6}$ | $(2.97 \pm 0.94) \times 10^{-6}$ | 121.2 |
| U2 | $4.93 \times 10^{-6}$ | $(4.47 \pm 0.48) \times 10^{-6}$ | 90.7 |

*Coverage interval calculated according ( $\bar{x} \pm t_{n-1,\alpha} \cdot \frac{SD}{\sqrt{n}}$ ); $t_{2;\,0.05} = 2.92$.

analysis of a larger amount of pharmaceutical and biological samples should be executed.

## Conclusions

In present paper, voltammetric behavior of irreversibly oxidized ciprofloxacin using BDD electrode was investigated applying CV technique. It was found that oxidation of analyte is a diffusion-controlled process with the peak potential at +1.6 V vs. Ag/AgCl. Linear concentration range from 0.74 to 20.0 × $10^{-6}$ mol $L^{-1}$, the detection limit of 6.0 × $10^{-7}$ mol $L^{-1}$ and the relative standard deviation of 2.7 % ($n$ = 20, 4.8 × $10^{-6}$ mol $L^{-1}$ ciprofloxacin) was achieved under optimal DPV parameters. Effect of possible interfering compounds such as caffeine, penicillin V, amlodipine and folic acid was evaluated with the significant impact on the analyte current response to be observed. Achieved recovery values (88.4 % for pharmaceutical, 90.7 and 121.2 % for human urine samples) confirmed satisfactory accuracy of elaborated protocol. The developed method using BDD electrode as an electrochemical sensor presents an effective, simple, fast and cost-effective alternative to separation and spectral methods and to chemically modified electrodes with the detection limit sufficient for the pharmaceutical quality control and point-of-care application to analysis of biological samples. Moreover, the benefits of using BDD electrode as a "green" electrochemical sensor for organic molecules are clearly demonstrated.

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