

Herbicide Clomazone Detection Using Electroanalytical Approach Using Boron Doped Diamond Electrode

Sladjana Djurdjic^{1,*}, Vesna Vukojevic¹, Sonja Jevtic², Marija V. Pergal³, Branka B. Petkovic², Dalibor M. Stankovic^{1,4,*}

¹ Innovation center of the Faculty of Chemistry, University of Belgrade, POB 51, 118, 11158 Belgrade, Serbia

² Department of Chemistry, Faculty of Natural Science and Mathematics, University of Priština, Lole Ribara 29, 38220 Kosovska Mitrovica, Serbia

³ ICTM, Center of Chemistry, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia

⁴ The Vinca Institute of Nuclear Sciences, University of Belgrade, POB 522, 11001 Belgrade, Serbia

*E-mail: sladjanadj@chem.bg.ac.rs, dalibors@chem.bg.ac.rs, daliborstankovic@vin.bg.ac.rs

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In this work, for the first time we proposed electrochemical behavior and development of an analytical procedure for quantification of pesticide clomazone. Electrochemical behavior of clomazone's at boron-doped diamond electrode is characterized with irreversible oxidation at potential of around 1.6 V, in Britton-Robinson buffer solution at pH 2. It was found that potential of this oxidation was not pH dependent excluding presence of proton in the electrochemical reaction. Square wave voltammetry method was the most appropriate for clomazone quantification and proposed procedure was successfully applied for determination of clomazone in river water samples. Obtained parameters, detection limit of 0.21 μM and working linear range from 1 to 100 μM and satisfied selectivity can indicate that presented results open new field in research of this group of pesticides and offer possible replacement to the sophisticated and expensive chromatographic methods.

Keywords: clomazone; electrochemistry; boron doped diamond electrode;

1. INTRODUCTION

Over the world several hundreds of pesticides found their application in plants treatment in order to increase agricultural production by controlling pests and plant diseases [1]. One of widely used pesticides, nowadays, is clomazone with chemical formula 2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone, extensively applied in cultivation of paddy rice, corn, tobacco [2]... This herbicide is well water-soluble compound with solubility of 1100 mg/L. Due to these facts it is now

widely investigated pesticide. Near detailed research about toxicity of clomazone and its formulation [3], there's also reported that tolerance of clomazone is linked to the state of LHC, PQ-pool and ROS detoxification in tobacco [4]. The same research group reported that pretreatment with alternation of light/dark periods improves the tolerance of tobacco to clomazone herbicide [5], while other authors reported that clomazone may be safely used in cotton to manage weeds when applied following treatments of the organophosphate insecticides phorate or disulfoton [6]. It is also recorded the clomazone's effect on hematological and some other parameters of proteins and carbohydrate metabolism [7]. Several methods were reported for the quantification of clomazone dominantly using chromatographic methods. High performance liquid chromatography with DAD detection was recorded as one of the methods for determination of clomazone [8], while this technique was also reported for the determination of clomazone in surface waters [9]. Simultaneous determination of clomazone with other pesticides were proposed by several studies using high performance liquid chromatography-electrospray ionization-tandem mass spectrometry [10], ultra-high-performance liquid chromatography-tandem mass spectrometry [11] and other techniques [1, 12, 13]. To the best of our knowledge, electrochemical behavior as well as determination of clomazone was not reported yet.

Boron doped diamond electrode, together with cathodically pretreated and anodically pretreated electrode surface, is up to date, one of the best unmodified electrode surface. Wide linear range, low background current and negligible adsorption are some of most important advantages of this electrode. Several research groups, including our, over the world widely investigate application of this electrode in electroanalytical sensing of different compounds such as drugs [14–17], vitamins [18] and different class of pesticides [19, 20].

Based on above mentioned facts, the main goal of this work was development of simple, rapid and low-cost, but at the same time, satisfactory sensitive and selective analytical method for detection of selected pesticide. Hence, electrochemical behavior of clomazone was investigated and square wave voltammetric technique was optimized and developed. Application of this approach was successfully tested in clomazone analysis in surface water samples.

2. EXPERIMENTAL

All reagents used during this work were of analytical grade. Britton-Robinson buffer solution (BRBS) was used as supporting electrolyte, prepared by adding of 0.04 M of each, sulfuric acid, phosphoric acid and boric acid. pH of buffer was adjusted with 0.2 M sodium hydroxide. For experiments at pH 1, 0.1 M hydrochloric acid was used.

Electrochemical experiments were done in conventional three electrode cell (total volume of 10 mL), equipped with boron doped diamond electrode (BDD, 3 mm diameter, 1000 ppm of boron doping level, Windsor Scientific, UK) 3M KCl Ag/AgCl electrode and platinum wire of large surface as working, reference and counter electrode, respectively. CHI 760b (USA) potentiostat/galvanostat was used for electrochemical measurements. For pH measurements pH meter model Orion 1230 equipped with combined glass electrode model Orion 9165BNWP (USA).

Electrochemical behavior of clomazone was tested by cyclic voltammetry. First, the clomazone response was tested in potential range of -1.5 to 2 V at BDD electrode in BRBS at pH 2, with scan rate 100 mV/s. Second, under the same conditions, clomazone in the anodic range of potentials was also examined. In addition, using a cyclic voltammetry was tested a possible oxidation mechanism of clomazone at BDD electrode by 0.1 mM of clomazone at different pH values of supporting electrolyte under the afore mentioned conditions.

For the detection of clomazone were used two methods. Differential pulse voltammetry (DPV) was applied in the potential range of 0 to 2 V with amplitude of 10 mV. Square wave voltammetry (SWV) was applied the same potential range. For this method were optimized parameters such as amplitude (40 mV) and frequency (20 Hz), because this method gave a better electrochemical response of clomazone than DPV.

Limit of detection and limit of quantification were calculated as 3 and 10 times the ratio of signal to baseline (noise), respectively.

Surface water samples were taken from Belgrade rivers Danube and Sava. Collected samples were stored at 4°C and, on the measuring day were filtered throughout 45 µM syringe filter. Samples were diluted with supporting electrolyte in ratio 1:2 and directly analyzed, under previously optimized experimental conditions. Recovery experiments were done, in order to investigate matrix effect. For this purpose standard addition method was used. All experiments were done in triplicate.

3. RESULTS AND DISCUSSION

3.1. Electrochemistry of clomazone

Electrochemical behavior of clomazone was tested using cyclic voltammetry. In anodic potential region, well-defined and sharp oxidation peak was observed at potential of 1.6 V in BRBS at pH 2 (Figure 1). In the reversed scan there was not noticed cathodic electroactivity, indicating irreversible oxidation of clomazone in selected supporting electrolyte using BDD electrode. Inset of Figure 1 shows electrochemical behavior of clomazone in positive potential region in BRBS at pH 2. Subsequently, pH influence on the peak potential was tested in the range of 1 to 12. Cyclic voltammograms obtained during this study are shown in Figure 2. It was found that best oxidation response was achieved in BRBS at pH 2, taking into account peak shape and peak current. Negligible or no peak potential shift was noticed with change of pH of electrolyte. This can be attributed to the no proton involved in the electrochemical reaction on the electrode surface. Similar phenomenon was observed in the literature [21]. Based on this study electrochemical clomazone oxidation mechanism can be suggested as two electron processes divided in two steps where the first step is corresponding radical formation and loss of one electron and the second step is one electron formation of radical anion rearomatized clomazone. However, for confirmation of these statements and detailed mechanism, additional studies are required. For development of analytical methodology for clomazone detection, based on these measurements, BRBS at pH 2 was selected and all further experiments were done in this supporting electrolyte.

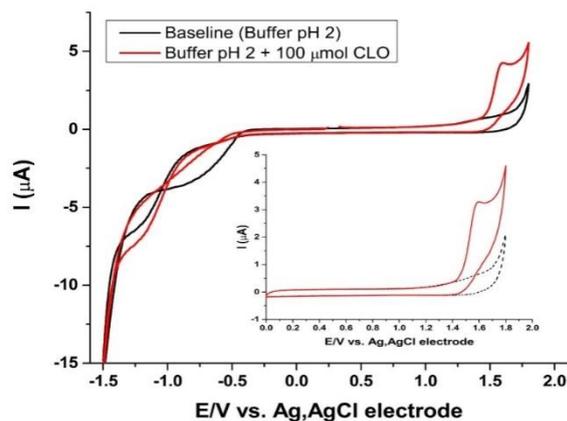


Figure 1. Cyclic voltammogram of 0.1 mM of clomazone in BRBS at pH 2 with BDD as working electrode and Ag/AgCl electrode as reference electrode. Scan rate 100 mV/s. Insert figure shown electrochemical response of 0.1 mM clomazone in anodic range potential.

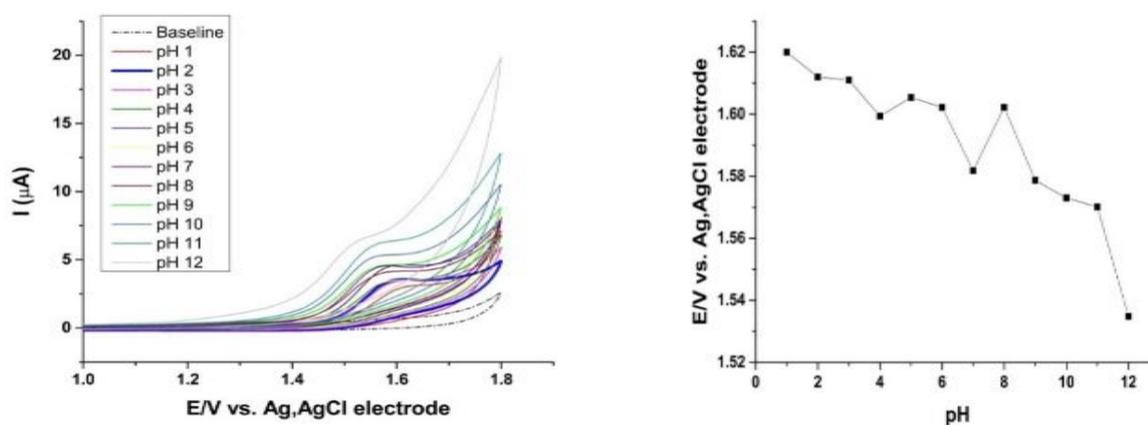


Figure 2. Cyclic voltammograms of 0.1 mM of clomazone at BDD (working) electrode and Ag/AgCl (reference) electrode in various pHs of supporting electrolytes. Scan rate 100 mV/s. Dependence of the peak potential against pH values.

In order to investigate nature of the electrochemical oxidation of clomazone at BDD electrode surface, cyclic voltammograms of 0.1 mM clomazone in BRBS at pH 2, using various scan rates, were recorder (Figure 3). It was found that increase of the scan rate is followed with oxidation peak current increase. This can be expressed with corresponding equation $I (\mu A) = 0.31657 v^{1/2} (mV/s)^{1/2} - 0.01568$ where regression coefficient was $R = 0.9996$. This function shows linear dependence over the studied range of the scan rates (10-150 mV/s). According obtained results electrochemical reaction which occurs at the electrode surface can be explained as diffusion controlled process with negligible adsorption phenomenon.

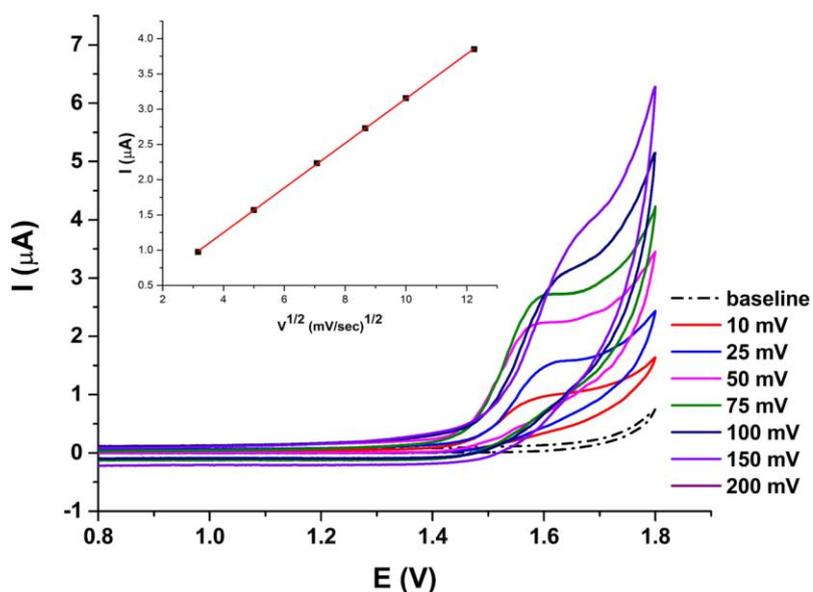


Figure 3. Cyclic voltammograms of 0.1 mM of clomazone at BDD electrode (working electrode) and Ag/AgCl electrode (reference electrode) in BRBS at pH 2 at various scan rates from 10 to 200 mV/s. Inset figure present dependence of the peak current from the square root of the scan rate.

These statements confirm advantages of selected electrode material and approves idea of this work.

3.2 Selection and optimization of quantification procedure

Electroanalytical response of 0.1 mM of clomazone in previously selected supporting electrolyte was tested using two voltammetric techniques, square wave voltammetry (SWV) and differential pulse voltammetry. It was found that better characteristics were obtained using SWV voltammetry and in further steps this technique was optimized in order to further increase SWV performances for determination of clomazone. This was done by varying pulse amplitude and frequency, in ranges 10-90 mV and 10-90 Hz, respectively. Figure 4. shows influence of different frequencies on the oxidation signal of clomazone. Increase of the pulse amplitude from 10 mV, at constant frequency of 15 Hz, cause increase of the peak current up to the value of 40 mV. Further increase does not influence on the peak characteristics or causes its decrease and due to that pulse amplitude of 40 mV was selected for further experiments. Similar experiments were done for frequency optimization and best analytical response was observed at the frequency value of 20 Hz, while pulse amplitude was at previously optimized 40 mV. Influence of the different frequency values are depicted in Figure 4. Based on these experiments, these selected parameters were used for all further experiments.

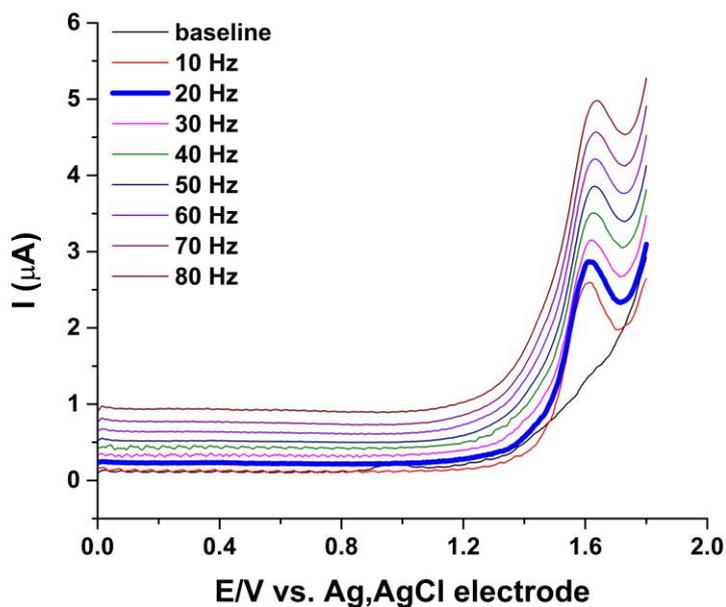


Figure 4. Optimization of the SWV frequency in the range from 10 to 80 Hz of 0.1 mM of clomazone at BDD electrode as working electrode and Ag/AgCl electrode as reference electrode in BRBS at pH 2. Pulse amplitude 40 mV.

3.3. Analytical performances of the proposed method

With plotting oxidation peak current against clomazone concentration using SWV method under optimized parameters, working linear range of the developed procedure was estimated. Voltammograms and corresponding calibration curve are presented in Figure 5. It was found that oxidation current shows linear dependence with increase of clomazone concentration in the range of 1-100 μM and this can be expressed with following equation $I (\mu\text{A}) = 0.02762 c (\mu\text{M}) - 0.01577$. Calculated limit of detection was found to be 0.21 μM and limit of quantification of 0.72 μM . By comparing our results with those reported for developed chromatographic methods [8, 10, 12, 13] it is obvious that these methods offers better characteristics, but, also, all of them required pre-concentration step before pesticides analysis, as expected content in surface water and vegetable samples is relatively low. Comparing instrumentation costs and special training for chromatographic techniques, developed electroanalytical procedure can serve as potential replacement for determination of clomazone residue in surface water. Relative standard deviation was calculated from 5 consecutive measurements of three different concentrations from calibration curve, 5, 15 and 25 μM , and was found as 2.1 %, 1.8 % and 1.7 %, respectively, indicating remarkable reproducibility of developed method.

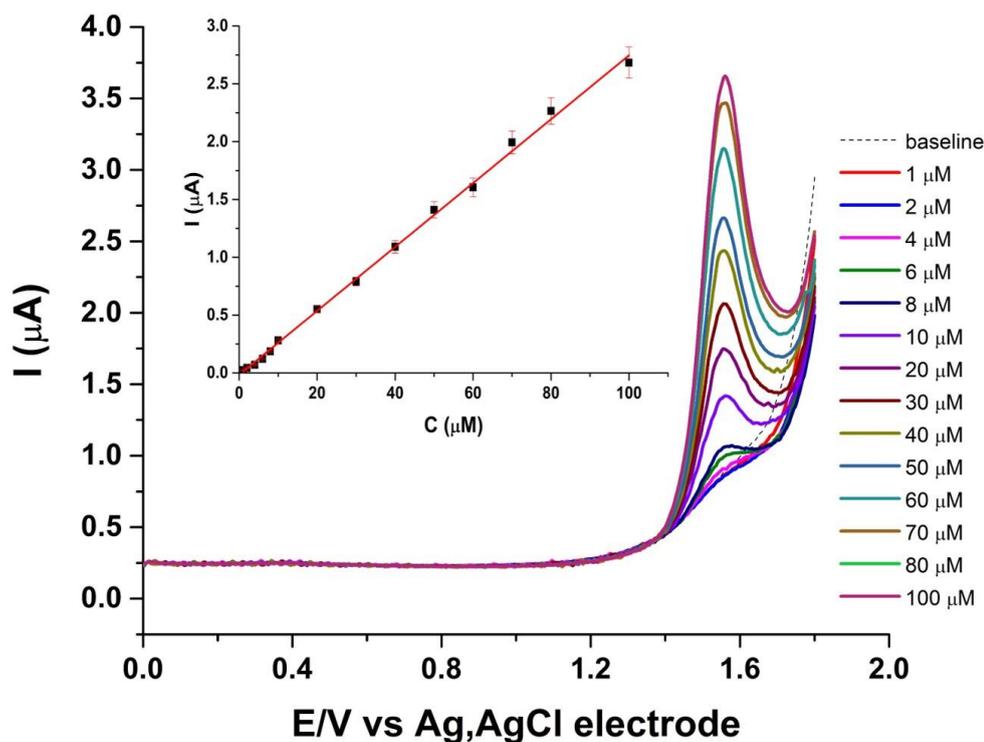


Figure 5. Voltammograms obtained for different concentrations of clomazone in positive potential range. SWV technique, working electrode BDD, reference electrode Ag/AgCl electrode, supporting electrolyte BRBS (pH 2). Pulse amplitude 40 mV, frequency 20 Hz. Insert figure shown corresponding calibration curve.

3.4. Selectivity of the proposed method

Selectivity of the developed method was tested in presence of some common species present in the surface water. Effect of nitrates, sulfates, phosphates, iron, calcium, magnesium, sodium and potassium. Additionally, presence of some other pesticide can strongly influence toward detection of clomazone and for that purpose effect of mezoctriene, sulcotriene and pethoxamid was investigated (Figure 6). It was found that firstly (water presented ions) tested compounds do not interfere with clomazone detection up to the 500-fold excess, and that these species can be considered as non-effecting. When 40 μM of mezoctriene is presented in the solution decrease of the clomazone peak current was observed. This is followed with loses of method characteristics, calibration curve can be constructed with starting point of 5 μM . This can be attributed to the obtained oxidation response of mezoctriene under selected working conditions. Similar behavior was noticed in presence of pethoxamid. On the other hand, presence of sulcotriene at same concentration level do not cause any significant changes in the oxidation peak current from clomazone and due to that can be concluded that this compound does not have influence on clomazone determination. Based on reported results, it can be considered that our developed methodology is satisfactory selective and can be applied for application in real samples.

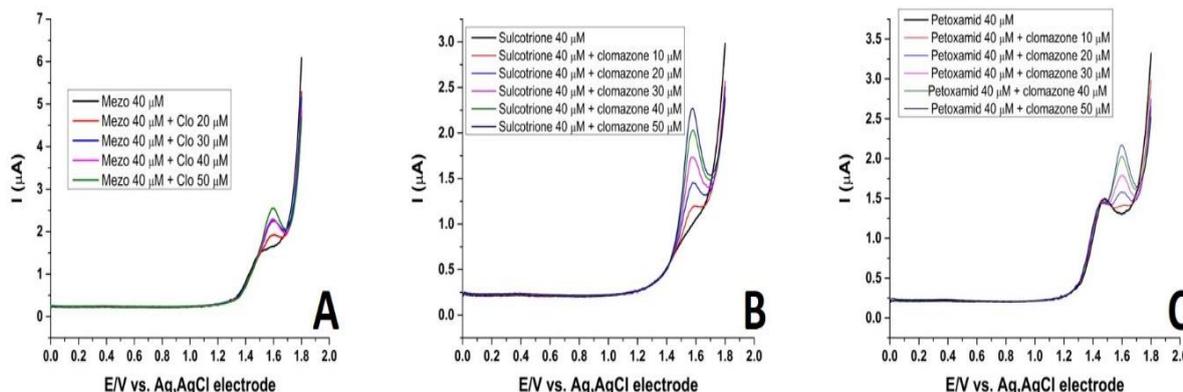


Figure 6. Electrochemical response of clomazone in the presence of some tested interferences by SWV method: A) mezotrione; B) sulcotrione; C) pethoxamid. BDD as working electrode, Ag/AgCl electrode as reference electrode, supporting electrolyte BRBS at pH 2. Pulse amplitude 40 mV, frequency 20 Hz.

3.5. Analytical application

Table 1. Results obtained for clomazone quantification using proposed analytical procedure

Sample	Found (µM)	Added (µM)	Found (µM)	Recovery (%)	Added (µM)	Found (µM)	Recovery (%)
Danube	0.00	2.00	2.11	106	5.00	7.35	103
Sava	0.00	2.00	2.04	102	5.00	7.19	102

Applicability of developed experimental procedure for detection of clomazone was investigated for analysis of surface waters (Table 1). This was done according to the procedure mentioned in Experimental section. It was found that good agreement was obtained for comparison of expected results with artificially spiked samples. Recovery tests confirmed good selectivity and negligible matrix effect for the quantification of clomazone using our technique.

4. CONCLUSIONS

In summary, novel analytical method was developed for the detection of herbicide clomazone. This work presented for the first-time application of this approach for monitoring clomazone concentration in surface water. It was shown that after procedure optimization satisfactory results were obtained. Wide concentration range and low detection limit are given, with negligible effect of interfering species. Developed procedure was successfully applied for determination of clomazone in

artificially prepared surface waters samples, indication that proposed approach can open new field in detection of herbicides.

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