1. Introduction

Pesticides represent a heterogeneous and numerous group of compounds that are used for the control of insects, fungi, bacteria, weeds, nematodes, rodents, and other pests. Phenylureas are a widely used class of herbicides in pre- and post-emergence which are highly selective and efficient for specific pests at a low dosage, and poses low toxicity to mammals. Linuron, 3-(3,4-(dichlorophenyl)-1-methoxy-1-methylurea) (Fig. 1) is one of the most important commercial urea compounds that selectively controls the germination of broadleaf weeds and grasses in crops [1]. Phenylureas are photochemically unstable, but still they can remain in soil and in the ground water [2], even in the milligram concentrations, for several weeks depending on the water temperature and pH [3]. The control of pesticides in environmental samples is of great interest due to their toxicity, carcinogenicity, and endocrine disrupting effects. Also, the detection and removal of pesticides from the industrial waste waters is important act with regard to the environmental protection.

The analysis of pesticides poses special problems since the pesticides belong to different chemical groups, have a wide range of polarity and acidic characteristics, their concentration in environmental samples is very low and the matrix sample is complex. Thus, it is of interest to improve methodologies with regard to how rapidly, accurately, and sensitively pesticides can be determined [4]. Many different analytical methods have been applied to the analysis of pesticides, mainly based on a separation by gas or liquid chromatography [5-8]. High performance liquid chromatography (HPLC) is preferably used for phenylurea analysis [9,10].
The first electrochemical analysis of linuron was performed by polarography technique [12]. More recently, linuron in water, soil and vegetable samples have been determined applying the stripping voltammetry with the carbon fiber microelectrode [13], carbon paste electrode [14], and modified carbon paste electrode with sepiolite [15] and tricresyl phosphate [16]. HPLC analysis of linuron, which involves amperometric detection using a glassy carbon electrode, was also reported [17].

Although, gas and liquid chromatography methods are sensitive and well established, they require complex and expensive instrumentation, sample preparation steps and various-step derivatization procedures. Comparing to the chromatographic methods, modern voltammetric methods are usually simple, inexpensive and rapid, as well as sufficiently sensitive to be employed for a large-scale monitoring of electrochemically active environmental pollutants [11]. Only a few electrochemical analytical studies of phenylureas pesticides have been reported in the literature [12-16]. Most of them are based on the voltammetric technique which is particularly useful in the analysis of samples that contain dispersed particles, including environmental and biological samples and industrial products. Additionally, the voltammetric methods can be applied without the sample preparation step that decreases both cost and the analysis time.

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Glassy carbon (GC) is particular useful as an electrode material because of its high chemical inertness and temperature resistance, low porosity, good electrical conductivity, reasonable mechanical and dimensional stability, high hardness and wide potential range [18]. The incorporation of heteroatom into carbon materials affects the surface and structural properties of carbon materials. Boron has been used to dope graphite, carbon-carbon composite, carbon fibers, carbon nanotubes and diamond electrodes [19-26] in order to change their electronic properties and the electrochemical behaviour. Boron doped diamond electrodes have been involved in the analysis of pesticides [26]. Although, the application of boron doped glassy carbon electrode as an indicator electrode in the potentiometric argenometric titration has been published earlier [22], to the best of our knowledge, the application of boron doped glassy carbon as an electrode for the pesticides determination has not been investigated yet.

Recently, Kalijadis et al. [27] reported the synthesis and characterisation of boron modified GC by chemically incorporation of boron into the carbon structure with a moderate temperature heat treatment. Based on X-ray diffraction pattern and Raman spectroscopy it was concluded that boron atoms are mostly substitutionally bonded in the graphene layers. Also, incorporation of boron in glassy carbon increases the number of active sites and consequently the formation of surface oxides. In this study the electrochemical determination of the linuron herbicide was performed using glassy carbon and bulk modified boron doped glassy carbon (GCB) electrodes which were synthesized in our laboratory applying the previously developed procedure [27]. The concentration of incorporated boron in glassy carbon is 2 wt.% which represents maximal substitutional solid solubility of boron into the carbon materials [28,29]. Above this concentration boron begins to enter into the interstitial position or forms a separate phase of boron carbide. The commercial GC-Metrohm electrode was used to compare the characteristics of the synthesized electrodes. Additionally, the results obtained by electrochemical determination of the linuron were compared with those obtained by the HPLC-UV procedure.

2. Experimental procedures

2.1. Reagents

Phenol-formaldehyde resin (PFR) was purchased from FENOLIT (Borovnica, Slovenia). Boric acid, sulphuric acid, acetic acid, phosphoric acid, sodium hydroxide, sodium chloride, potassium chloride, calcium carbonate, sodium nitrate, sodium hydrogen carbonate, silver chloride and methanol (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea), acetamiprid (N-(6-chloro-3-pyridyl)acetyl-N,N'-cyano-N-methyl-acetamidine), tebufenozide (N-tert-butyl-N'(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide), dimethoate (O,O-dimethyl S-[2-(methyamino)-2-oxoethyl]dithiophosphate), imidacloprid (N-(6-chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl)nitramide), carbendazim (methyl 1H-benzimidazol-2-ylcarbamate) and Galolin mono® (commercial formulation which contents 500 g dm⁻³ of linuron) were obtained from Fitofarmacija a.d. (Zemun, Serbia). All pesticides are of 95% purity. Nitrogen, purity 99.995% was supplied from Messer (Messer, Tehnogas, Serbia). Deionised water and deuterium oxide (99.8% D₂O) were used for preparing aqueous solution for the voltammetric measurements.
water was supplied from Millipore purification system (Bedford, MA, USA).

The stock pesticide solution (0.8 μmol cm⁻³) was prepared in methanol and kept in the dark at -18°C. Working solutions were prepared daily by diluting the stock solution with either 0.1 mol dm⁻³ sulphuric acid or Britton-Robinson (BR) buffer (0.04 mol dm⁻³ boric acid, 0.04 mol dm⁻³ phosphoric acid and 0.04 mol dm⁻³ acetic acid, pH was adjusted with 0.2 mol dm⁻³ sodium hydroxide), covering the pH range from 0.7 to 7.0.

2.2. Instrumentation
A 797 VA Computrace analyzer (Metrohm, Switzerland) controlled by 797 VA Computrace software ver. 1.2 was applied for all voltammetric measurements. A Pt rod was the auxiliary electrode and an Ag/AgCl/KCl (3 mol dm⁻³) was the reference electrode, to which all the potentials refer. A disc glassy carbon electrode produced by Metrohm (GC-Metrohm) was applied for comparing with glassy carbon and boron doped glassy carbon electrodes prepared in our laboratory. The working area of a GC-Metrohm electrode was 0.04 cm². The working area of the home-made electrodes was 0.07 cm².

Comparative HPLC measurements were performed using Dionex-0650 liquid chromatograph (Dionex, USA), Agilent Eclipse XDB-C18 column (4.6 mm x 250 mm, 3.5 μm) and UV-detector.

2.3. Preparation of glassy carbon and boron doped glassy carbon electrodes
A commercial phenol-formaldehyde resin (PFR) was used as the starting material for synthesis of the glassy carbon. PFR was poured into a mould to obtain a polymer 0.14 cm thick. The polymerization was carried out at 90°C for 24 hours. The polymer samples were cut in plates and carbonized under a nitrogen gas stream, with the programmed heating rates: 5°C min⁻¹ to 100°C, 0.1°C min⁻¹ to 500 °C and 0.2°C min⁻¹ to 1000°C.

A boron doped glassy carbon was prepared by mixing PFR with boric acid, to obtain 2 wt.% boron in the precursor. The mixture of resin and boric acid was poured into the mould, polymerized, cut in plates and then carbonized under the same conditions as the unmodified glassy carbon samples. The electrodes were made in the form of rolls. More details about the synthesis of both glassy carbon and bulk modified boron doped glassy carbon are given in [27].

2.4. Measurement procedure
2.4.1. Voltammetry on glassy carbon electrode
Voltammetric measurements were carried out in a glass electrochemical cell at ambient temperature. 10 cm³ of the supporting electrolyte (0.1 mol dm⁻³ sulphuric acid or BR buffer) were introduced into the cell and the solution was purged with nitrogen for 5 min. Before measurement, the buffer-immersed working electrode was electrochemically activated by potential cycling in the range from -0.1 to 1.6 V, sweep rate 0.1 V s⁻¹ (50 cycles). Then, an appropriate amount of linuron solution was added and the measurement was carried out. Measurement parameters in differential pulse voltammetry (DPV) were as follows: start potential of -0.1 V, end potential of 1.6 V, pulse amplitude of 0.05 V, pulse time of 0.04 s, sweep rate of 0.1 V s⁻¹. Before adding linuron, the blank was recorded (supporting electrolyte) under the same conditions.

2.4.2. Liquid chromatography
For the HPLC-UV analysis all samples were filtered through 0.22 μm syringe filters. The mobile phase was 70% methanol in water. The separation was performed at isocratic regime with a flow rate of 0.7 cm³ min⁻¹. Linuron was detected at wavelength of 230 nm with a retention time of 12 min.

2.4.3. Sample preparation
Linuron herbicide, technical purity (95%) was dissolved in methanol (0.8 μmol cm⁻³). After this step supporting electrolyte (0.1 mol dm⁻³ H₂SO₄) was spiked with the linuron solution to concentration inside calibration graph. The commercial formulation Galolin mono® was dissolved in methanol and analysed after a stepwise dilution with deionised water to the required linuron concentration.

3. Results and discussion

3.1. Electrochemical behaviour
The differential pulse voltammograms of 0.036 μmol cm⁻³ linuron in 0.1 mol dm⁻³ sulphuric acid, which were recorded using GC, GCB and GC-Metrohm electrodes, are presented in Fig. 2. Due to the fact that the electrodes investigated in this study (GC, GCB and GC-Metrohm) have the different working surfaces, the current density (j, expressed as the ratio of the current intensity of the oxidation peak of linuron and the surface of the working electrode) have the different working surfaces, the current density (j, expressed as the ratio of the current intensity of the oxidation peak of linuron and the surface of the working electrode) was adopted for the presentation of the experimental results. One well defined oxidation peak (not visible in the blank) with peak maxima at E_p (potential of the linuron oxidation) 1.31, 1.34 and 1.28 V was observed for GCB, GC and GC-Metrohm electrodes, respectively. The main characteristics of the peaks are given in Table 1. The current density of the linuron oxidation peak at GC, GCB and GC-Metrohm follows the trend: j(GCB) > j(GC-
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Methrom) > \(j(GC)\). This higher current density obtained for GCB is probably due to increasing number of active places at the boron modified carbon surface [27]. The linuron oxidation peak recorded by commercial carbon electrode is higher than home-made unmodified glassy carbon electrode, but lower than boron doped glassy carbon electrode.

The potential of linuron oxidation peak on the investigated glassy carbon electrodes is close to potential of linuron oxidation (1.3 V) obtained by the measurement of the linuron in the soil using a carbon fibre as the working electrode and phosphate buffer pH 2.0 as supporting electrolyte [13]. However, de Lima et al. [14] obtained two oxidation peaks of linuron at electrode potential of 0.39 and 0.56 V applying carbon paste electrode and BR buffer pH 5.5 as supporting electrolyte.

Carbon based electrode usually has to be pretreated either mechanically or electrochemically in order to improve the sensitivity and selectivity. Electrochemical pretreatments (also called electrochemical activation) are the most frequently applied methods, because they are effective, very convenient and can be performed \textit{in situ} [13,14,30-33]. The electrodes investigated in this study were electrochemically activated, by the potential cycling in the range from -0.1 V to 1.6 V, with the speed of 1 cycling at 6 seconds (50 cycles) under inert atmosphere. Fig. 3 shows the effect of the electrochemical pretreatment of GCB and GC-Metrohm electrodes on linuron voltammogram (0.036 \(\mu\text{mol cm}^{-3}\) in 0.1 mol dm\(^{-3}\) sulphuric acid as electrolyte). The potential of linuron oxidation shifted toward more negative potential after electrochemical activation, \textit{i.e.}, from 1.34 to 1.27 V and from 1.35 to 1.29 V for GC-Metrohm and GCB electrodes, respectively. It is clear from Fig. 3 that the electrochemical pretreatment led to significant improvement of the current density of linuron peak. The current density of oxidation peaks of linuron without electrochemical pretreatment are 12.8 and 8.7 \(\mu\text{A cm}^{-2}\) and after potential cycling are 28.1 and 22.5 \(\mu\text{A cm}^{-2}\) applying GCB and GC-Metrohm electrodes, respectively.

In some cases, decreasing of the initial potential or increasing the end potential in the pretreatment process can activate carbon-based electrodes and improve electrochemical signals. However, decrease of the initial potential of the cyclization (-0.7 V) for all the investigated carbon based electrodes did not improve the electrochemical response of the linuron. On the contrary, the obtained electrochemical peaks were less defined. Because of that, in almost all further presented experimental results, the investigated electrodes were activated by the potential cycling in the working potential range from -0.1 V to 1.6 V.

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**Figure 2.** DP voltammograms of linuron (0.036 \(\mu\text{mol cm}^{-3}\)) in 0.1 mol dm\(^{-3}\) sulphuric acid applying studied electrodes (A) GCB, (B) GC and (C) GC-Metrohm, and corresponding baselines signed as dot lines. Experimental conditions: voltage step 0.006 V, sweep rate 0.05 V s\(^{-1}\). The electrodes were electrochemically activated before measurement.

**Table 1.** The main characteristics of the oxidation peak of linuron (0.036 \(\mu\text{mol cm}^{-3}\)) in 0.1 mol dm\(^{-3}\) H\(_2\)SO\(_4\) observed at the investigated glassy carbon based electrodes.

| Electrode     | Peak position (V) | Peak area (\(\mu\text{A V cm}^{-2}\)) |
|---------------|-------------------|----------------------------------------|
| GC            | 1.34              | 12.8                                   |
| GCB           | 1.31              | 27.9                                   |
| GC-Metrohm    | 1.28              | 22.5                                   |

The main characteristics of the oxidation peak of linuron (0.036 \(\mu\text{mol cm}^{-3}\)) in 0.1 mol dm\(^{-3}\) H\(_2\)SO\(_4\) observed at the investigated glassy carbon based electrodes.
3.2. Influence of pH on measurements

The effect of pH on the electrochemical behaviour of linuron was investigated using sulphuric acid (0.1 mol dm\(^{-3}\) pH 0.7) and Britton-Robinson buffer (0.12 mol dm\(^{-3}\), pH 4 and 7). Concentration of linuron in examined solutions were 0.034 μmol cm\(^{-3}\) and the voltammograms were recorded from -0.1 V to 1.6 V, at the sweep rate of 0.05 V s\(^{-1}\), applying GCB electrode (Fig. 4i), and GC-Metrohm electrode (Fig. 4ii).

As clearly visible from Fig. 4, both potential and current density of oxidation peaks depend on the pH of supporting electrolyte. Decreasing of electrolyte pH from pH 7.0 to 0.7, \(E_p\) is moved toward more positive potential for 0.19 V. The potential of the linuron oxidation (\(E_p\)) was found to shift linearly towards more negative values with increasing electrolyte pH (for both investigated electrodes), and these dependences fit the equations: \(E_p = 1.366 - 0.0286\) pH \((r = 0.997)\) and \(E_p = 1.366 - 0.029\) pH \((r = 0.997)\) for GCB and GC-Metrohm electrode, respectively. These results indicate the involvement of protons in the electrochemical reaction of the linuron at unmodified and bulk modified boron doped glassy carbon electrodes [14]. Based on these results and according to literature [14,34], it can be concluded that the peak of linuron oxidation at 1.3 V was obtained by elimination of the methoxy group (replaced by a hydrogen atom) through formation of 3-(3,4-dichlorophenyl)-1-methylurea and formic acid.

Additionally, the current intensity of linuron peak strongly depends on pH of the supporting electrolyte, decreasing of pH lead to increasing current density of the linuron peak. The sharpest and most intensive linuron peak was obtained in strongly acidic solutions at pH 0.7.

3.3. Direct DPV determination of linuron at the glassy carbon based electrodes in the selected samples

The electrochemical response of the investigated electrodes (GC, GCB and GC-Metrohm) to linuron was investigated in the wide range of linuron concentration (0.0024 to 0.13 μmol cm\(^{-3}\)) by applying DPV method in 0.1 mol dm\(^{-3}\) H\(_2\)SO\(_4\) as the supporting electrolyte. All measurements were done in triplicate and the mean values are shown in Fig. 5, while the error bars represent the standard deviations. The relative standard deviation was 8-10%, for the lowest determined concentration, and less than 5%, for the concentration higher than 0.008 μmol cm\(^{-3}\). The quantitative DPV determination of linuron applying the studied glassy carbon electrodes is based on the linear relationship between the linuron concentration and the area of the linuron oxidation peak. The analytical parameters of the developed DPV method and the comparative HPLC/UV measurements are shown in Table 2. The linear dependence of the linuron peak area and linuron concentration was obtained in the concentration ranges: 0.005 - 0.1 μmol cm\(^{-3}\) and 0.005 - 0.07 μmol cm\(^{-3}\), for GCB and GC electrodes, respectively. It should be noted here that applying GC-Metrohm electrode, differential pulse voltammetry exhibits two linear concentration ranges: 0.002 - 0.028 μmol cm\(^{-3}\) and 0.028 - 0.13 μmol cm\(^{-3}\). The highest correlation coefficient \((r = 0.998)\) was obtained for the determination of linuron using GCB electrode. Also, it can be seen from Table 2 that the linearity range is broadest for the boron doped electrode. The limit of detection (LOD) was determined using the graphical method, as the lowest linuron concentration that produce the oxidation peak of the linuron, for the
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signal to noise ratio equals to 3. The LOD of linuron applying bulk modified boron doped glassy carbon electrode and commercial glassy carbon electrode was found to be 0.006 μmol cm⁻³. While, LOD of linuron applying unmodified glassy carbon electrodes is higher (0.01 μmol cm⁻³) comparing to other two investigated electrodes. The obtained results were compared to the HPLC-UV determination of linuron which is the most frequently used method for linuron determination (the main parameters of the calibration curve are given in Table 2) [35].

The applicability of the voltammetric procedure was tested by the determination of the linuron in the spiked water samples and the commercial formulation Galolin mono®, using DPV with GCB electrode (DPV-GCB) and HPLC-UV measurements (Table 3). The spiked water sample was obtained by adding of linuron solution (0.8 μmol cm⁻³ of technical purity linuron in methanol) in the supporting electrolyte to the two different concentrations (0.009 and 0.036 μmol cm⁻³). Calibration curve method was applied for the determination of the target compound. All measurements were done in triplicate. Similarly to the spike water sample, the content of the active compound in the analyzed commercial formulation was determined by calibration curve method. Good correlation between the amounts of added and found linuron in the spiked water samples (recovery was 92% and 102% for two analyzed samples) reflects the high accuracy and precision of the proposed DPV-GCB method. The satisfactory precision (RSD 2.4%) was obtained for analysis of spiked linuron samples of higher concentration (0.036 μmol cm⁻³).

Figure 4. The effect of pH on the DP voltammograms of linuron (0.034 μmol cm⁻³) recording by (i) boron doped glassy carbon (A) and (ii) GC-Metrohm (C) electrodes. Legend: 1 - pH = 0.7; 2 - pH = 4.0 and 3 - pH = 7.0. The experimental conditions: voltage step 0.006 V; sweep rate 0.05 V s⁻¹ (no electrochemical pretreatment).
Somewhat lower RSD of 8% was observed for the spiked water sample with lower linuron concentration (0.009 μmol cm⁻³). The amount of linuron in commercial formulation Galolin mono® was found to be 457 g dm⁻³ (1.83×10³ μmol cm⁻³) and it was lower than prescribed 500±25 g dm⁻³ (2×10³ μmol cm⁻³) probably due to the sampling process of viscous and sticky liquid sample. In addition, the results obtained by the DPV-GCB method are in good accordance with the results obtained with the HPLC-UV method (Table 3).

### 3.4. Interferences study

The interference of some common ions (Na⁺, K⁺, Ca²⁺, Ag⁺, Cl⁻, CO₃⁻, HCO₃⁻, NO₃⁻) and pesticides (acetamiprid, dimethoate, imidaclopride, carbendazim and tebufenozide) on the determination of linuron was tested in this study. First, DPV of linuron (0.05 μmol cm⁻³) was recorded using GCB electrode, then either selected ion was added to the final concentration that is 100 times more than linuron or selected pesticide was added to the same final concentration as linuron. The solutions were analysed by the proposed method. The results indicated that only addition of NaHCO₃ increased the peak current of linuron, all other investigated ions and pesticides neither influence on peak position nor current density of linuron oxidation peak.

Herein, it can be stated that the continuing experiments are needed to achieve lower detection limits.
limit necessary for the application of the developed method for determination of linuron pesticides in the environmental samples, to deeply investigate influence of NaHCO₃, and for better understanding of oxidation process of linuron.

4. Conclusion

The results of this study demonstrate for the first time the application of the bulk modified boron doped glassy carbon electrode synthesized in our laboratory for the voltammetric determination of the linuron herbicide. DPV signal of linuron was studied applying investigated glassy carbon electrodes at different pH. The obtained results showed that the sharpest, most symmetrical and intense peak was obtained in strongly acidic solution suggesting protons involvement in the electrochemical reaction of the linuron. Differential pulse voltammetry with boron doped glassy carbon electrode was found to have the best applicability for the linuron determination because of the broader linearity range and very good value of LOD. These results indicate that the incorporation of boron in glassy carbon directly affects the electrochemical behaviour of this material and made it significantly sensitive for the oxidation processes. Although the HPLC-UV analysis provides lower LOD of the linuron, differential pulse voltammetry with bulked modified boron doped glassy carbon electrode can represent an alternative, rapid, cheap and environmentally friendly procedure for determination of the herbicide in the commercial formulations and water samples.

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