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Conditioning of renewable silver amalgam film electrode for the characterization of clothianidin and its determination in selected samples by adsorptive square-wave voltammetry

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ABSTRACT

A new square-wave adsorptive stripping voltammetric (SWAdSV) method was developed for the determination of the neonicotinoid insecticide clothianidin (*Clo*), based on its reduction at a renewable silver amalgam film electrode (Hg(Ag)FE). The key point of the procedure is the pretreatment of the Hg(Ag)FE by applying the appropriate conditioning potential (−1.70 V vs. Ag/AgCl reference electrode). Under the optimized voltammetric conditions, such pretreatment resulted in the peak for the *Clo* reduction in Britton–Robinson buffer pH 9.0 at about −0.60 V, which was used for the analytical purpose. The developed SWAdSV procedure made it possible to determine *Clo* in the concentration range of 6.0×10^{-7}–7.0×10^{-6} mol L^{-1} (LOD=1.8×10^{-7} mol L^{-1}, LOQ=6.0×10^{-7} mol L^{-1}) and 7.0×10^{-6}–4.0×10^{-5} mol L^{-1} (LOD=1.3×10^{-6} mol L^{-1}, LOQ=4.2×10^{-6} mol L^{-1}). The repeatability, precision, and the recovery of the method were determined. The effect of common interfering pesticides was also investigated. Standard addition method was successfully applied and validated for the determination of *Clo* in spiked Warta River water, corn seeds samples, and in corn seeds samples treated with the commercial formulation PONCHO 600 FS.

1. Introduction

Nowadays, the neonicotinoid insecticides represent one of the most important and the most rapidly expanding chemical classes of insecticides introduced into the global market since the synthetic pyrethroids. The discovery of neonicotionoids can be considered as a milestone in agrochemical research over the past three decades. These pesticides are used worldwide as antagonists against nicotinic acetylcholine receptors of insect. The neonicotinoids are registered globally in more than 120 countries. They are being generally regarded as the most effective insecticides to control a broad spectrum of sucking insect pests (such as aphids, whiteflies, leaf- and plant-hoppers, trips, some micro-Lepidoptera, and a number of coleopteran pests) [1–3].

Thus, there is a growing need for the development of fast and simple methods for the characterization and determination of neonicotinoids. There are numerous analytical papers dealing with the determination of neonicotinoid insecticides in food, agricultural and environmental samples. Most commonly used are high performance liquid chromatography (HPLC) with diode array [4–6], mass-spectrometric [7], thermal lens spectroscopic [8] and amperometric [9] detection. Some electrochemical methods have also been developed [10–19]. As it is well known, modern voltammetric techniques represent a convenient alternative method in the analysis of neonicotinoids. They are fast, sensitive and inexpensive, and thus suitable for a large-scale monitoring of different electrochemically active environmental pollutants [20,21], and are often easily adaptable for in-the-field work.

Clothianidin (*Clo*, (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine, CAS: 210880-92-5) (Fig. 1) is a representative of a third generation of neonicotinoid insecticides, belonging to the nitroguanidine and thiazole subclasses. It was discovered by the former Agro Division, Takeda Chemical Industries, Co., Ltd. (currently Sumitomo Chemical Co., Ltd.) and co-developed with Bayer CropScience. At present, this compound is registered in more than 40 countries all over the world by both companies. This agent contributes to the production of crops by protecting them against damages caused by a great number of insect pests. *Clo* exhibits great biological efficacy in small amounts for a wide variety of pests such as Hemiptera, Thysanoptera,

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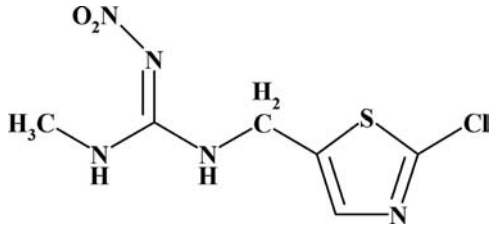

Fig. 1. Chemical structure of Clothianidin.

Coleoptera, Lepidoptera and Diptera, and a high level of operational safety has been confirmed for grains such as paddy rice, fruit trees and vegetables [22].

The methods of the determination of *Clo* are mainly based on HPLC and ultra-performance liquid chromatography (UPLC) combined with the sensitive detection by a diode-array [23], mass spectrometry [24,25]. The voltammetric methods proposed for the determination of clothianidin employed HMDE (reduction of NO_2 group, $E_p = -0.97$ V [15] and electrocatalytical effect on hydrogen evolution of guanidine group, $E_p = -1.40$ V [16]), BiF-GCE (reduction of NO_2 group, $E_p = -0.90$ V [18]), TCP-CPE (reduction of NO_2 group, $E_p = -1.25$ V [14]), or Hg(Ag)FE (reduction of NO_2 group, $E_p = -1.00$ V [19]).

One of the most commonly used working electrode materials in electrochemical methods is mercury. It is unbeatable for the voltammetric determination of electrochemically reducible compounds. However, there has been a growing concern about the use of mercury because of its toxicity. This includes the use of pure mercury as an electrode material in voltammetry. Even for laboratory use, restrictions are expected to appear in the future [20,21,26,27].

One of the promising alternatives to the family of mercury electrodes are the amalgam-based electrodes for different applications [19–25,27–47]. The wide potential window, low noise, ease of electrochemical renewal of the surface, and mechanical robustness, all this makes them applicable in flow liquid systems. Besides, simple preparation, low price, and the possibility of regeneration makes amalgam electrodes a very promising electroanalytical tool, compatible with the concepts of green analytical chemistry. To this group belongs also a relatively novel type of silver amalgam film electrode (Hg(Ag)FE) [35–39,42–45].

So far, Hg(Ag)FE has been successfully used for the determination of several heavy metals [35,36,38,40,46–48], some popular vitamins (C1, B1 and B2) [41], and of a number of organic compounds such as moroxydine [37], blasticidin S [42], dinotefuran [43], proguanil [45], acibenzolar-S-methyl [44], nitenpyram, thiacloprid and clothianidin [19].

Relying on our previous experiences with square-wave voltammetric (SWV) determination of *Clo* [19] at the renewable Hg(Ag)FE, we continue the investigation of *Clo* by using the method of square-wave adsorptive stripping voltammetry (SWAdSV). The aim was to improve the application of Hg(Ag)FE in environmental analyses. Special attention was paid to the influence of the conditioning potential on the mechanism of the electrode reaction. A new SWAdSV signal was used for the determination of *Clo* in spiked river water, corn seeds samples, as well as in the corn samples treated with the commercial formulation PONCHO 600 FS.

2. Experimental

2.1. Apparatus

All SWV measurements were performed using an EmStat USB potentiostat with the PSTrace 2.4 software (Palm Instruments BV, The Netherlands) and an M164 electrode stand (MTM Anko Instruments, Cracow, Poland). The experiments were performed in a three-electrode system consisting of a Pt wire as the counter electrode, a silver/silver chloride electrode (Ag/AgCl, 3.00 mol L^{-1} KCl) as the reference, a renewable Hg(Ag)FE with 12 mm^2 surface (MTM Anko Instruments, Cracow, Poland) and HMDE (MTM Anko Instruments, Cracow, Poland) as the working electrodes. All the potentials were referred to the Ag/AgCl reference electrode. A pH meter (type CP-315, Elmetron, Poland) with a conjugated glass membrane electrode was also used.

2.2. Chemicals and solutions

All chemicals used were of analytical reagent grade. The analytical standard of *Clo* (purity 99.5%) was purchased from Dr. Ehrenstorfer (Germany). A fresh *Clo* stock solution (1.00×10^{-3} mol L^{-1}) was prepared weekly by dissolving 6.24 mg of the pesticide in 25.0 mL of water-acetone mixture (4:1, v/v). All further dilutions were prepared from the stock solution as required using triply distilled and deionized water. Argon (5N) was obtained from Linde Gas (Poland), and was used without further purification. Acetone (99.5%) and nitric acid (65.0%) were of analytical grade, products of POCh (Poland).

The Britton–Robinson (B–R) buffer solutions (0.04 mol L^{-1}) were used as the supporting electrolytes for the voltammetric measurements. The B–R buffers were prepared by successive addition of 0.20 mol L^{-1} sodium hydroxide (POCh) to the mixture of 0.04 mol L^{-1} phosphoric acid (85.0%, POCh), 0.04 mol L^{-1} boric acid (POCh) and 0.04 mol L^{-1} acetic acid (99.5%, POCh), to obtain the required pH value, covering the pH range of 4.0–12.0.

The river water sample was collected from the Warta River (Pławno, Poland) and stored in the fridge at 4.0 °C for one week before analysis. Corn seeds samples were obtained from the Institute of Field and Vegetable Crops (Novi Sad, Serbia).

2.3. Voltammetric procedure

The general procedure used to obtain the SWAdSV curves was as follows: 10.0 mL of the supporting electrolyte (5.0 mL of the B–R buffer mixed with 5.0 mL of the water) was transferred to the voltammetric cell, and the solution was purged with argon for 600 s with stirring. When an initial blank was recorded, the required volumes of *Clo* solution were added by means of a micropipette. If some reagents were subsequently added, the solution was purged with argon for a further 30 s.

To clean the Hg(Ag)FE surface (in the case of necessity, which is recognized as the loss of the sensor sensitivity), chemical activation in 2.0% HNO_3 was applied. Besides, the procedure of refreshing the Hg(Ag)FE surface was carried out before each measurement [47]. After the formation of a new layer, a conditioning step was performed by the application of an appropriate negative potential for a certain period of time. Next, the accumulation step at a constant potential was applied with stirring of the solution. After the resting period ($t_{eq} = 15$ s), a negative ongoing potential scan was applied. The reported signals were measured without subtracting the blank signal, and using the smoothing procedure available in PSTrace 2.4.

In the present study, the highest analytical signal was obtained in the B–R buffer pH 9.0. The measurement parameters in the SWAdSV procedure were as follows: the conditioning potential −1.70 V, conditioning time 30 s, accumulation potential −0.20 V, accumulation time 20 s, amplitude 100 mV, frequency 100 Hz, step potential 4 mV. All measurements were carried out at the ambient temperature of the laboratory (20.0 °C).

The voltammograms of *Clo* solutions were recorded at the same parameters as for pure supporting electrolyte. The recovery of the compound was calculated in sextuplicate experiments.

2.4. Determination of Clo in selected samples

2.4.1. River water samples

As first, 5.0 mL of the B–R buffer pH 9.0, 4.0 mL of the distilled water and 1.0 mL of the river water was placed in the voltammetric cell and the SWAdSV curve of the blank was recorded.

A volume of 100.0 μL of *Clo* stock solution was transferred to the 10.0 mL calibrated flask and filled up to the mark with the river water. Next, 1.0 mL of the spiked river water was poured to the voltammetric cell containing 5.0 mL of B–R buffer and 4.0 mL of distilled water. When the SWAdS voltammogram of the spiked river water sample was recorded, the standard addition method was applied. Consecutive additions of *Clo* were added with a micropipette to the voltammetric cell. The concentrations of the standard addition in the cell were at the levels: 1.0×10^{-6} and 2.0×10^{-6} mol L^{-1}. The voltammograms were recorded after each addition, and the recovery of *Clo* was calculated for six runs.

2.4.2. Corn seeds samples

A random sample (2 kg) of the corn seeds belonging to medium-sized flat fraction of the corn hybrid NS 6010 (Institute of Field and Vegetable Crops, Novi Sad) was divided into three parts. One part of the corn seeds was left untreated and was used for checking interferences. The other two parts were prepared (cleaned and dried) for treatment, one of them was spiked with the standard solution of *Clo* and the other was treated with PONCHO 600 FS (see below).

2.4.2.1. Preparation of the untreated corn seeds samples. Four seed corn samples (30 g/each) were crushed in a mortar to small pieces. To each sample, 100.0 mL of acetone was added, the mixture carefully shaken manually, then filtered using Büchner funnel and the organic phases were collected. The solid phase was washed two times with 50.0 mL of acetone, and the organic extract was added to the liquid phase. The extract was then left to evaporate at 25.0 °C to dry material. The residue was dissolved in 5.0 mL of 0.01 mol L^{-1} HCl. The acidic solution was transferred to a 50.0 mL flask and filled up to the mark with acetone.

5.0 mL of the B–R buffer pH 9.0 and 5.0 mL of the untreated corn seeds samples were placed in the voltammetric cell, and the SWAdSV curve of the blank was recorded. Next, appropriate volume of the standard solution of *Clo* (1.0×10^{-3} mol L^{-1}) was added (from 10 μL to 80 μL) and the voltammograms were recorded after each addition.

2.4.2.2. Corn seeds samples spiked with the standard Clo solution. Four seed corn samples (30 g/each) were crushed in a mortar to small pieces. All samples were sprayed with the standard solution of *Clo* and left for several hours to dry. To each sample, 100.0 mL of acetone was added, the mixture carefully shaken manually, then filtered using Büchner funnel and the organic phases were collected. The solid phase was washed two times with 50.0 mL of acetone, and the organic extract was added to the liquid phase. The extract was then left to evaporate at 25.0 °C to dry material. The residue was dissolved in 5.0 mL of 0.01 mol L^{-1} HCl. The acidic solution was transferred to a 50.0 mL flask and filled up to the mark with acetone.

After recording the voltammogram of the blank (5.0 mL of B–R buffer pH 9.0 and 4.0 mL of distilled water), 1.0 mL of the solution of spiked corn sample solution was poured to the voltammetric cell. When the SWAdSV curve of the spiked corn sample was recorded, the standard addition method was applied. Consecutive additions of *Clo* were added with a micropipette to the voltammetric cell. The concentrations of the standard addition of *Clo* were from 1.0×10^{-6} to 2.0×10^{-6} mol L^{-1}. The voltammograms were recorded after each addition, and the recovery of *Clo* was calculated for four runs.

2.4.2.3. Corn seeds samples treated with PONCHO 600 FS. 1 kg of the untreated corn seeds was treated with the commercial formulation PONCHO 600 FS (48.96% clothianidin, Bayer CropScience) in the concentration of the manufacturer of 600 mL 100 kg^{-1} (6.00 mL kg^{-1} of seed) by the Seed Treater HEGE 11 (Hege Maschinen GmbH, Waldebnurg, Germany), and dried naturally in thin layer on blotting paper at room temperature.

Four seed corn samples (10.5 g/each) treated with the commercial formulation PONCHO 600 FS, were crushed in a mortar to small pieces. The rest of the sample preparation procedure was identical to that described in Section 2.4.2.2.

After recording the voltammogram of the blank (5.0 mL of B–R buffer pH 9.0 and 5.0 mL of distilled water), 20 μL of the treated with PONCHO 600 FS corn seeds solution was added to the voltammetric cell. When the SWAdS voltammogram of sprayed corn sample was recorded, the standard addition method was applied. Consecutive additions of *Clo* were added by means of a micropipette to the voltammetric cell. The concentrations in the cell were from 2.0×10^{-6} to 4.0×10^{-6} mol L^{-1}. The voltammograms were recorded after each addition, and the recovery of *Clo* was calculated for four runs.

3. Results and discussion

3.1. Influence of the conditioning potential and time

Based on the previous knowledge [15,16], the voltammetric response of *Clo* at the hanging mercury drop electrode (HMDE) in the B–R buffer pH 8.0 consists of three signals. Peak I (at about −1.00 V) is related to a four-electron reduction of the nitro group to hydroxylamine group, peak II (at about −1.40 V) is ascribed to the further reduction of the hydroxylamine group to the corresponding amino group, and peak III (at about −1.70 V) is due to the electrocatalytic evolution of hydrogen evolution from the guanidine group, which is also present in the structure of *Clo* (Fig. 1). An analogous pattern is also observed using Hg(Ag)FE (without applying the conditioning step) (Fig. 2).

As is the case with every solid working electrode, the Hg(Ag)FE requires also renewal and conditioning of the sensor surface. It was found that the conditioning potential (E_{cond}) had a very strong effect on the *Clo* peak current and peak potential. Therefore, the effect of the conditioning potential on the neonicotinoid SWV signals was the first critical choice. The influence of this parameter on the behavior of *Clo* (3.0×10^{-5} mol L^{-1}) was studied in the potential range from 0 V to −2.00 V (Fig. 3).

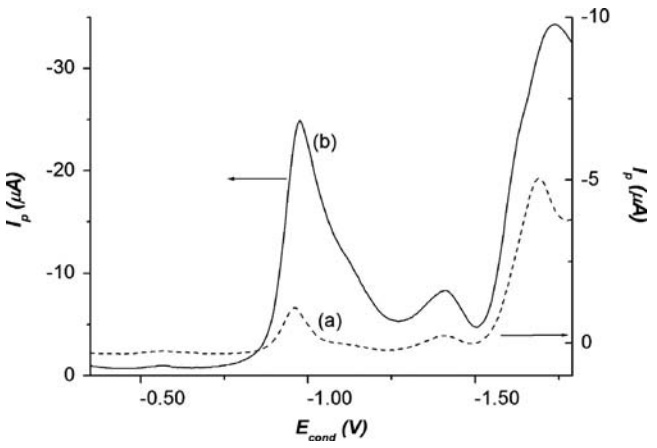

Fig. 2. SWV response of 3.0×10^{-5} mol L^{-1} *Clo* recorded in B–R buffer pH 9.0 at HMDE (a) and Hg(Ag)FE without applying the conditioning step (b). Measurement parameters: frequency f=100 Hz, amplitude E_{sw}=60 mV, and step potential ΔE=7 mV.

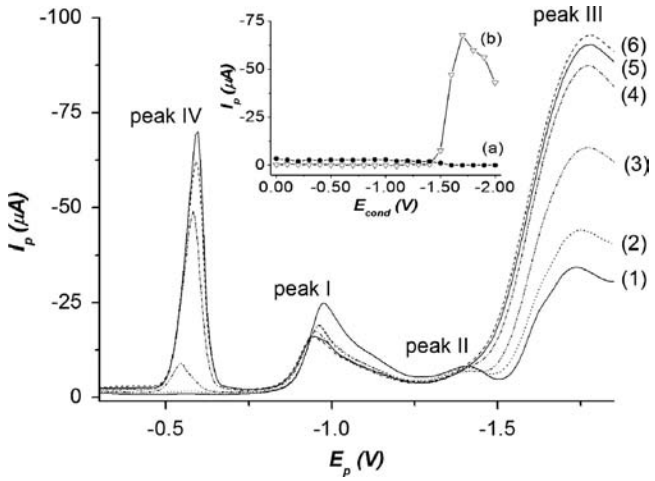

Fig. 3. Effect of the conditioning potential on the SWV signals of 3.0×10^{-5} mol L^{-1} *Clo* (1) 0 V, (2) −1.20 V, (3) −1.50 V, (4) −1.60 V, (5) −1.70 V, and (6) −1.80 V. The conditions were the same as in Fig. 2 and the conditioning time t_{cond}=15 s. The inset shows the dependence of the SWV peak current on the conditioning potential: (a) peak II (at about −1.40 V), and (b) peak IV (at about −0.60 V).

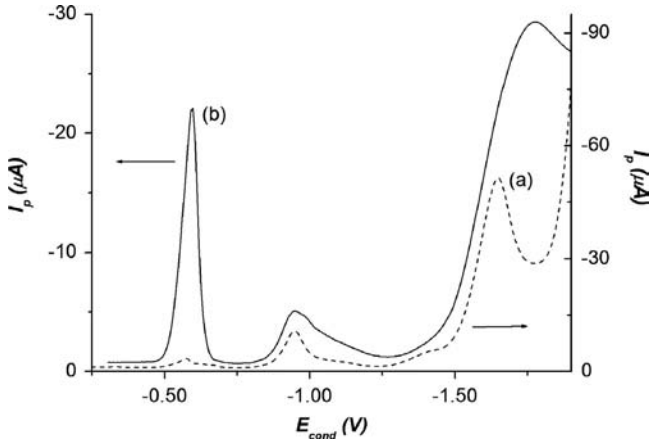

Fig. 4. SWV signals of 3.0×10^{-5} mol L^{-1} *Clo* recorded in the B–R buffer pH=9.0 at (a) HMDE and (b) Hg(Ag)FE. Measurement parameters: conditioning potential E_{cond}=−1.70 V, conditioning time t_{cond}=15 s, frequency f=100 Hz, amplitude E_{sw}=60 mV, and step potential ΔE=7 mV.

It was found that in the investigated conditioning potential range between 0 V and −1.10 V still two well separated reduction signals were observed (B–R buffer, pH 9.0). The current (I_p) of the first peak (Fig. 3, peak I) is by several times higher compared to that of the second peak (peak II). At the conditioning potential more negative than −1.20 V, the signal at −1.40 V (peak II) is going down, but a new peak at about −0.60 V appears (peak IV). The height of this peak increases till the conditioning potential of −1.70 V (I_p=68.0 μA), and then decreases as it is shown in the inset of Fig. 3. It appeared that the potential of the new signal was independent of the kind of electrode, since the peak position was the same when using Hg(Ag)FE and HMDE. However, as can be seen from Fig. 4, the Hg(Ag)FE is much more sensitive (i.e. the peak IV current is by about 7 times higher).

The appearance of the peak at −0.60 V at Hg(Ag)FE can be connected with the fact that the reduction of nitrocompounds involves a series of one-electrode additions, chemical steps and an important reaction intermediate is the nitrosocompound [49]. For example, nitrobenzene radical anion can be generated by electrochemical reduction in aqueous solutions at pH 13 and next the nitrobenzene radical anion turn into the nitrosobenzene form [49]. Redox equilibrium between nitrosobenzene and phenylhydroxylamine describe the following reaction: $PhNO+2e+2H^+ \leftrightarrow PhNHOH$ [50]. The procedure of the Hg(Ag)FE pretreatment by applying the appropriate conditioning potential (−1.70 V vs. SCE), maybe influences the important reactions intermediate (for example, easier forming the nitrosocompound radical anion, which is the base of next intermediate reactions leading to nitrosocompound).

The influence of the conditioning time (t_{cond}) of *Clo* was investigated in the range from 0 s to 50 s. It was found that the t_{cond} higher than 15 s did not influence significantly the peak current. Hence, the time of 30 s was chosen as the optimal one for the further studies.

3.2. Cyclic voltammetric behavior of Clo

The technique of cyclic voltammetric (CV) enables one to obtain the basic characteristics of the studied signals regarding the mechanism of the electrode reactions, their kinetic parameters and reversibility system in a relatively short period of time. The CV behavior of *Clo* was studied at a concentration level of 5.0×10^{-5} mol L^{-1} in the B–R buffer pH 9.0, after holding the potential of Hg(Ag)FE at E_{cond}=−1.70 V for 30 s. The CV curves recorded at the scan rates (ν) from 0.02 V s^{-1} to 0.40 V s^{-1} in the potential range from −0.10 V to −1.90 V exhibit also a cathodic peak at about −0.60 V (Fig. 5a). No anodic peak appeared on the reverse scan at the scan rate up to 0.30 V s^{-1}, which confirms the irreversibility of the electrode process. At the scan rates 0.35 V s^{-1} and 0.40 V s^{-1}, a small anodic signal is seen at −0.58 V, which suggests a change in the nature of the electrode process, and the cathodic peak potential shifts to more negative values. The peak current (I_p) increased linearly as the potential scan rate ν increased from 0.02 V s^{-1} to 0.40 V s^{-1} corresponding to the equation: $\log I_p(\mu A)=0.993\log\nu(V\,s^{-1})+1.859$, R^2=0.995 (Fig. 5b). The slope coefficient of the equation is close to 1.0, which was expected for the adsorptive electrode process. The character of the process confirms also the exponential dependence of $I_p=f(\sqrt{\nu})$ shown in Fig. 5b. The small cathodic–anodic signal around −0.30 V is probably related to the impurities of the supporting electrolyte.

3.3. Effect of pH

The electroanalytical behavior of neonicotinoids is significantly influenced by the pH of the solution [12,13,18,19,42,44,26,51]. The influence of the hydronium ion concentration on the reduction signal of *Clo* at about −0.60 V was studied in a wide range of pH values (4.0–12.0) using the B–R buffers as the supporting electrolyte. As it is evident from Fig. 6a, the increase in the pH resulted in the shift of the signal toward more negative potentials. Such behavior indicates the involvement of protons in the electrode reaction and that the proton-transfer reaction precedes the electrode process proper [11]. A well-shaped, most symmetrical and intense peak of *Clo* (3.0×10^{-5} mol L^{-1}) was obtained in the alkaline solutions of the pH 8.0–10.0 (Fig. 6a, curves 3–5). As can be seen from the inset of Fig. 6a, the peak height increases substantially with the increase in the pH, reaching a maximum at the pH 9.0, to decline afterwards. Hence, this pH value was chosen as the optimal for the determination of *Clo*. The E_p–pH plot (Fig. 6b) exhibits three linear parts with two breaks at approximately pH 6.0 and 8.5, with the slopes of the respective three linear segments of 37 mV pH^{-1} (R^2=0.995), 3 mV pH^{-1} (R^2=0.994), and 32 mV pH^{-1} (R^2=0.999). Such behavior is probably due to the change in the rate determining step of the reduction process [52].

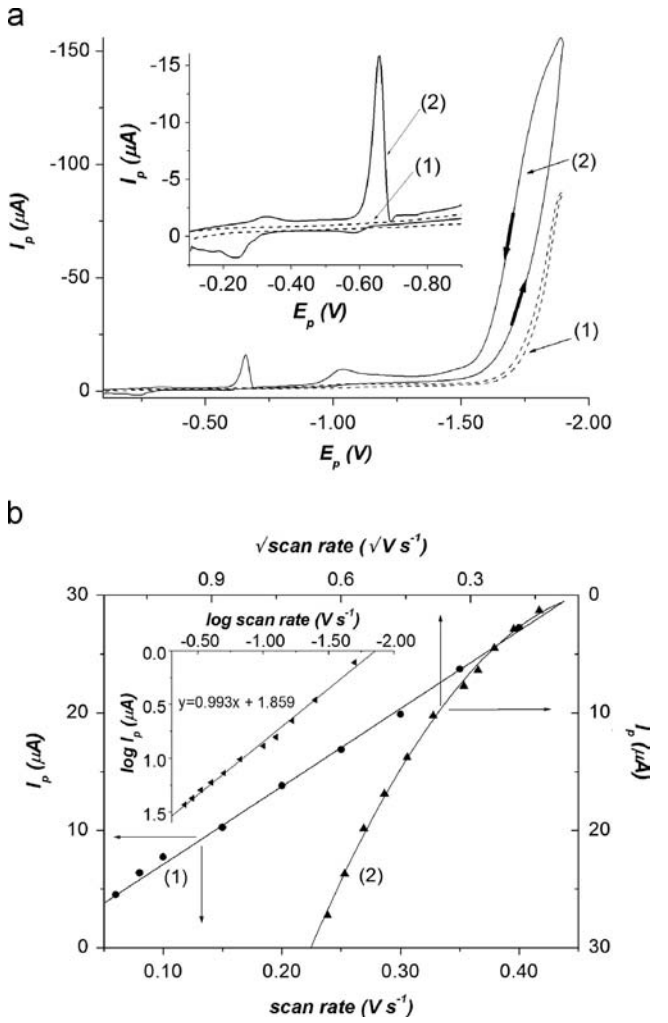

Fig. 5. (a) Cyclic voltammograms of 5.0×10^{-5} mol L^{-1} *Clo* (2) recorded in B–R buffer pH 9.0 (1). Measurement parameters: scan rate $\nu = 0.30$ mV s^{-1}, conditioning potential $E_{cond} = -1.70$ V, conditioning time $t_{cond} = 30$ s. The inset shows the same figure in a narrowed scale. (b) The linear plot of the peak current (I_p) versus the potential scan rate (ν) (1) from 0.02 V s^{-1} to 0.40 V s^{-1}; (2) the exponential dependence of $I_p = f(\sqrt{\nu})$. The inset shows the dependence of log I_p on log ν over the potential range from −0.10 V to −1.90 V.

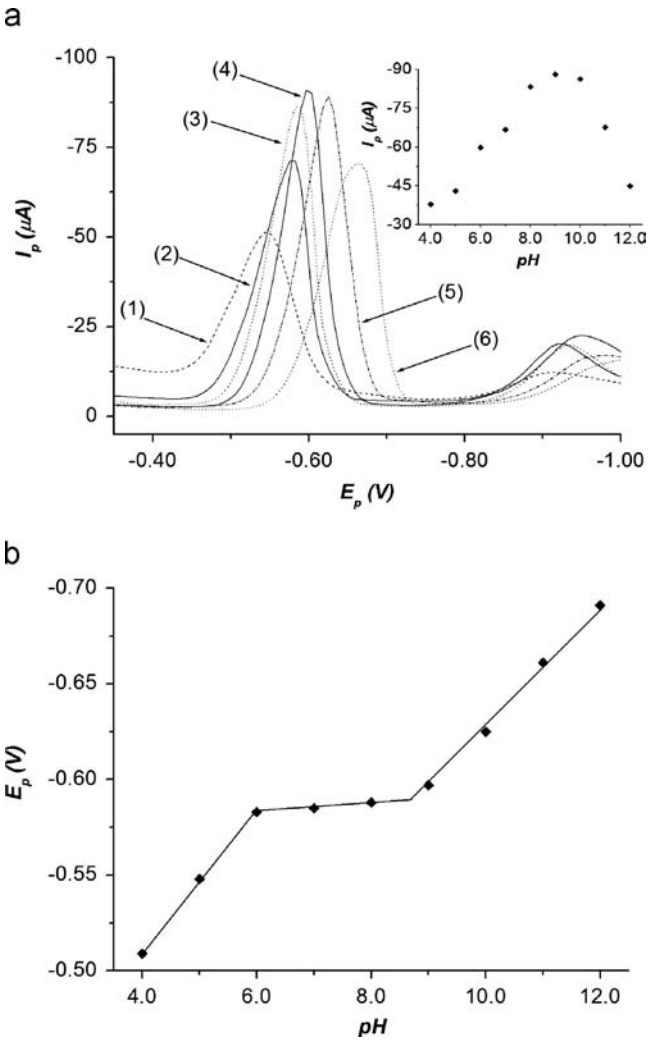

Fig. 6. (a) Effect of the pH on the SWV signal ($C_{Clo} = 3.0 \times 10^{-5}$ mol L^{-1}): (1) pH 5.0, (2) pH 7.0, (3) pH 8.0, (4) pH 9.0, (5) pH 10.0, and (6) pH 11.0. The inset shows the I_p–pH dependence for peak IV. (b) Dependence of the SWV peak potential ($C_{Clo} = 3.0 \times 10^{-5}$ mol L^{-1}) on the pH. Measurement parameters: conditioning potential $E_{cond} = -1.70$ V, conditioning time $t_{cond} = 30$ s, frequency $f = 100$ Hz, amplitude $E_{sw} = 60$ mV, and step potential $\Delta E = 7$ mV.

3.4. Influence of the SWV parameters

Taking the analytical application of the measured at −0.60 V signal of *Clo* into consideration the influence of the potential modulation parameters, such as the frequency (f), amplitude (E_{SW}), and the step potential (ΔE) was studied [53].

At the beginning, the influence of E_{SW} was investigated in the range from 10 mV to 100 mV. For the amplitudes in the range of 10–60 mV, the peak current increased linearly, while the amplitudes higher than 60 mV caused a non-linear growth of the peak (plateau). The best result according to the sensitivity and shape signal was achieved at $E_{SW} = 100$ mV.

The influence of the potential scan rate in the SWV technique was studied by varying the step height and frequency. The study of the influence of f in the range from 8 to 110 Hz showed that up to 63 Hz an ill-defined *Clo* signals were observed. The highest peak current and the best-defined shape were obtained for 100 Hz, and this value was used in the further analytical studies. In the whole range of f, the peak potential of the reduction of *Clo* shifted toward more negative values (from −0.50 V to −0.60 V) with the increase in f. The influence of ΔE was investigated in the range of 1–20 mV. The increase in this parameter in this range caused an increase in the response, while the higher ΔE values caused a distortion of the peak shape. The best-shaped response was obtained with the step potential of 4 mV, and this value was chosen as the optimal in the subsequent studies.

The equilibration time (t_{eq}) was checked in the range from 0 s to 30 s. It appeared that this parameter did not influence significantly the peak current. The best result was obtained for the equilibration time of 15 s.

To improve the sensitivity and detection limit of the method, the influence of the accumulation potential (E_{acc}) and accumulation time (t_{acc}) was also studied. The observed *Clo* response was highly sensitive to the accumulation factor. The dependence of the SWAdSV peak current on the E_{acc} was studied at the t_{acc} of 10 s, in the potential range from −0.05 V to −0.50 V for the *Clo* concentration of 3.0×10^{-6} mol L^{-1}. With the shift to more negative E_{acc}, an increase in the *Clo* signal was observed; the maximum attained at −0.20 V was followed by a significant decrease. Therefore, −0.20 V was chosen as the optimal deposition potential for further analytical measurements.

The dependence of the peak current on the accumulation time was investigated in the range between 0 and 50 s at the *Clo* concentration level of 3.0×10^{-6} mol L^{-1}. It was found that a maximal peak height

was achieved for the t_{acc} of 20 s, and this value was used in all further analytical studies. It should be noticed that the prolonged times resulted in a decrease of the voltammetric response. Such behavior suggests high affinity of *Clo* molecules to the Hg(Ag)FE electrode surface [16].

3.5. Analytical features of the SWAdSV method

The developed SWAdSV method using Hg(Ag)FE was tested for the determination of *Clo* under the optimal conditions described in the previous section. A good correlation between the peak current and the *Clo* concentration was obtained in the concentration ranges of 6.0×10^{-7}–7.0×10^{-6} mol L^{-1} (range 1) and 7.0×10^{-6}–4.0×10^{-5} mol L^{-1} (range 2) (Fig. 7). The characteristics of the corresponding calibration curves are summarized in Table 1.

The limits of detection (LOD) and the limits of quantification (LOQ) of *Clo* in the range 1 and 2 (1.8×10^{-7}, 6.0×10^{-7} and 1.3×10^{-6}, 4.2×10^{-6} mol L^{-1}, respectively) were calculated based on the following equations: LOD/LOQ=kSD/b (k=3 for LOD, k=10 for LOQ). The standard deviation (SD) was calculated for the intercept (six runs) and b stands for the slope of the calibration graph [54]. The

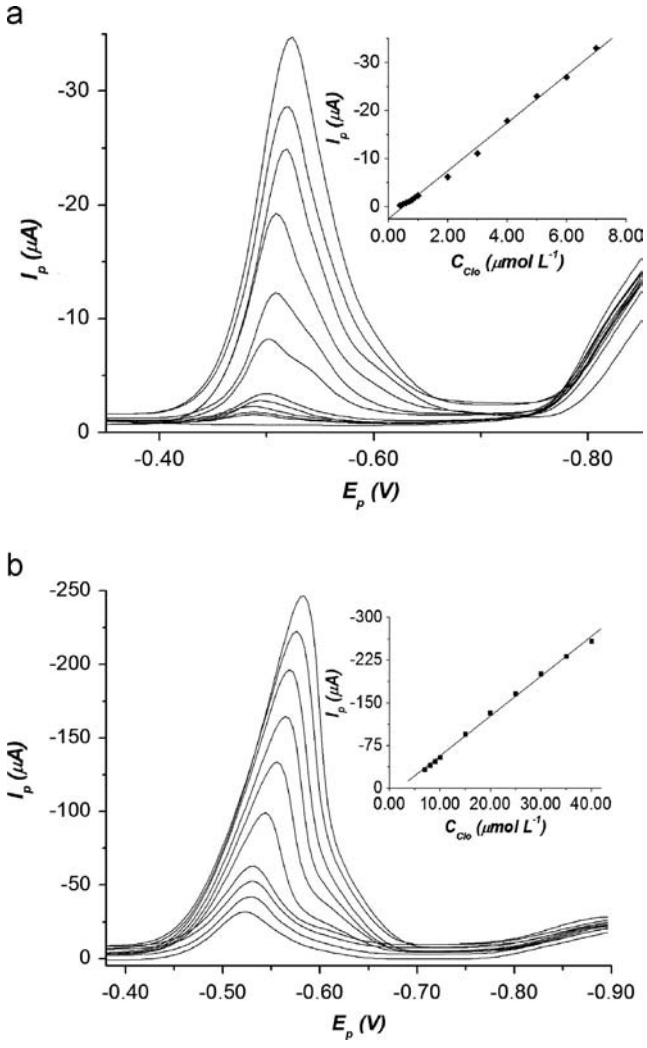

Fig. 7. SWAdSV responses of *Clo*, recorded in B–R buffer pH 9.0. Increasing concentration from the bottom: (a) range 1, $c(Clo\times10^{-6})$: 0, 0.60, 0.70, 0.80, 0.90, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, and 7.00 μmol L^{-1}; (b) range 2, $c(Clo\times10^{-6})$: 7.00, 8.00, 9.00, 10.00, 15.00, 20.00, 25.00, 30.00, 35.00 and 40.00 μmol L^{-1}. The insets show the corresponding calibration graphs of *Clo*. The conditions were the same as in Fig. 6.

Table 1
Regression data of the calibration graphs for the quantitative determination of *Clo*, B–R buffer, pH 9.0 by SWAdSV using Hg(Ag)FE.

Parameter	Range 1	Range 2
Concentration range (mol L^{-1})	6.0×10^{-7}–7.0×10^{-6}	7.0×10^{-6}–4.0×10^{-5}
Slope (A L mol^{-1})	−5.0	−7.0
Intercept (A)	2.5×10^{-6}	1.3×10^{-5}
Correlation coefficient	0.998	0.999
Number of measurements	6	6
LOD (mol L^{-1})[a]	1.8×10^{-7}	1.3×10^{-6}
LOQ (mol L^{-1})[b]	6.0×10^{-7}	4.2×10^{-6}

[a] LOD=kSD/b (k=3 for LOD).
[b] LOQ k=kSD/b (k=10 for LOQ).

appearance the two ranges of linear relationship is probably due to the working electrode passivation or multi-layer adsorption.

The repeatability (one day) of the voltammetric procedure was estimated on the basis of six repetitive measurements at each studied *Clo* concentration, and the *RSD* did not exceed 5.0%. In order to check the correctness of the method, the *RSD* and recovery were also calculated for the different concentrations in the linear range. The *RSD* did not exceed 6.0%.

3.6. Analytical applications

3.6.1. Spiked river water

The developed procedure was successfully applied for the determination of *Clo* in the spiked samples of Warta River water, prepared as described in Section 2.4.1. For each sample, six experiments were performed. No interferences were observed from the river water components. The recovery results for *Clo* in spiked Warta River water are given in Table 2.

3.6.2. The untreated corn seeds samples

The untreated corn seeds solution was studied under the optimized voltammetric parameters to check interferences derived from corn seeds. Calibration curves received in this medium (as described in Section 2.4.2) for *Clo* shown the linear dependence between peak current and concentration in the range of 1.0×10^{-6}–8.0×10^{-6} mol L^{-1}. No interferences were observed from the corn seeds components. Any noise peaks were not found from the biological material in the studied potential range.

3.6.3. Corn seeds samples spiked with standard the Clo solution

The optimized SWAdSV procedure was also successfully applied for the determination of *Clo* in the spiked corn seeds samples. The applicability of the procedure was tested by the standard addition method. All the four replicates were performed as described in Section 2.4.2. The recovery and precision results of *Clo* in spiked corn seeds samples are given in Table 2. The method is sufficiently accurate and precise to be applied for the determination of *Clo* in corn seeds samples.

3.6.4. Corn seeds samples treated with PONCHO 600 FS

The developed voltammetric procedure was used for the determination of *Clo* in the corn seeds samples treated with the commercial formulation PONCHO 600 FS. All the experiments were performed as described in Section 2.4.2. No interferences were noticed from the components present in the corn seeds samples. The applicability of the procedure was tested by the standard addition method, by running four replicates. The recovery results given in Table 2 show that the method is suitable for

Table 2
Results of the *Clo* determination by SWAdSV technique.

Sample	Added [10^{-6} mol L^{-1}]	Found [10^{-6} mol L^{-1}]	Precision *RSD* [%]	Recovery [%][d]
Spiked river water	1.00	0.99 ± 0.04[a]	3.9	99.3
Spiked corn seeds	2.00	2.02 ± 0.12[b]	3.7	101.1
Corn seeds treated with PONCHO 600 FS	6.05	6.01 ± 0.13[b]	1.4	99.5

[a] $t(S/n^{1/2})=2.57$, $p=95\%$, $n=6$.
[b] $t(S/n^{1/2})=3.18$, $p=95\%$, $n=4$.

the determination of *Clo* in the corn seeds samples treated with PONCHO 600 FS.

3.7. Effect of interferences

Finally, we checked whether the commonly used pesticides like acibenzolar-S-methyl, fenoxanil, metam-sodium trihydrate, cyazofamid and nitrothal-isopropyl would interfere with the determination of *Clo* by the developed method under the optimized experimental conditions. The *Clo* concentration in the voltammetric cell was 1.0×10^{-6} mol L^{-1}, whereas the other pesticides were present at the levels ranging from 1.0×10^{-7} mol L^{-1} to 1.0×10^{-5} mol L^{-1}, so that the corresponding ratios to *Clo* concentration were 0.1, 0.5, 1.0, 5.0 and 10. The presence of metam-sodium trihydrate exhibited a major effect on the recorded peak current, while nitrothal-isopropyl caused a minor decrease of the measured *Clo* signal. Fenoxanil caused a pronounced decrease of the measuring signal only at a 10-fold higher concentration. On the other hand, the presence of cyazofamid had no effect on the *Clo* peak current, whereas acibenzolar-S-methyl at the ratio 1:1 and higher caused distortion of the analytical signal.

4. Conclusions

The application of an appropriate conditioning potential of Hg(Ag)FE resulted in a new SWAdSV signal of *Clo* at -0.60 V in the B–R buffer at pH 9.0. Cyclic voltammetric experiments showed the electrode mechanism is an irreversible reduction controlled by the adsorption of *Clo*. An analytical method was developed for the determination of *Clo* in the model solution under the optimized experimental conditions. The developed SWAdSV procedure was also found to be effective for the determination of *Clo* in selected samples such as spiked river water, corn seeds samples and corn seeds samples treated with the commercial formulation PONCHO 600 FS. The influence of other commonly used pesticides like acibenzolar-S-methyl, fenoxanil, metam-sodium trihydrate, cyazofamid and nitrothal-isopropyl was also studied.

The developed procedure is characterized by major advantages such as short determination time, low cost, environmental friendliness, low LOQ, and all this without pre-treatment and time consuming extraction steps. It could also be adopted for the use in quality control laboratories. Moreover, the proposed method can serve as a good alternative to HPLC, GC, LC–MS, and other analytical techniques used for pesticides analysis.

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