CATHODIC STRIPPING VOLTAMMETRY OF CLOTHIANIDIN: APPLICATION TO ENVIRONMENTAL STUDIES

Dariusz GUZIEJEWSKI[1], Sławomira SKRZYPEK[2,*], Adam ŁUCZAK[3] and Witold CIESIELSKI[4]

Lodz University, Faculty of Chemistry, Department of Instrumental Analysis, Pomorska 163, 90-236 Lodz, Poland; e-mail: [1] dguziejewski@o2.pl, [2] skrzypek@uni.lodz.pl, [3] adamluczak1@poczta.onet.pl, [4] ciesielski@uni.lodz.pl

Received September 17, 2010
Accepted January 3, 2011
Published online January 27, 2011

A new, rapid and very sensitive electrochemical method for the determination of a new pesticide clothianidin in tap and river water was developed. The electrochemical reduction and determination of clothianidin have been carried out at a hanging mercury drop electrode (HMDE) in various aqueous solutions in the pH range of 2–10 by cyclic voltammetry (CV) and cathodic stripping square wave voltammetry (SW CSV). The best results were obtained for the clothianidin determination by SW CSV method in 0.04 mol l^{-1} Britton Robinson buffer at pH 8.1. Various conditions of the procedure were checked. Elaborated electroanalytical procedure enable clothianidin determination in the concentration range of 2.0×10^{-8}–9.9×10^{-7} mol l^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) were obtained as 2.00×10^{-9} and 2.36×10^{-8} mol l^{-1}, respectively. Precision and accuracy of the developed method were checked by recovery studies in spiked tap and river water. The voltammetric determination has been validated using HPLC with UV detection.
Keywords: Clothianidin; Voltammetry; Determination; Environmental sample.

The environmental pollution by pesticides is a serious problem in last years. Many of these compounds are present in ground and surface water and their concentration level ought to be controlled, especially in drinking water. Increasing use of pesticides in agriculture and their stability is a main cause of such situation. Due to their heavy use, low level of detection of such compounds is required in analyses of many control processes, environmental and food quality[1]. That is why many techniques have been developed for quantification, such as voltammetry[2], chromatography[3–5], immunosensing[1] and biosensing[6].

Clothianidin ((*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine, cloth) is a new pesticide and belongs to the group of chloronicotinyl insecticides. Thiamethoxam, imidacloprid and thiacloprid are

Collect. Czech. Chem. Commun. 2011, Vol. 76, No. 2, pp. 131–142

doi:10.1135/cccc2010116

other examples of such compounds. They contain a nitroguanidine functional group in their structures. Insecticides like clothianidin are antagonists against nicotinic acetylcholine receptor. Due to this specific activity against the insect nervous system, these pesticides are systematic and contact compounds effective in controlling aphids, thrips, potato beetle and other various harmful pest species[7]. The important drawback of clothianidin is that it can be toxic for useful insects, i.e. bees (LD_{50} 0.0038 μg per bee)[8].

There are numerous analytical papers dealing with determination of neonicotinoid insecticides in food, agricultural and environmental samples. Most common used are GC, HPLC and mass spectrometry. There are also studies related to fluorescence[9,10], micellar electrokinetic capillary chromatography[11], as well as ELISA[12,13]. Some electrochemical methods were also developed[14–19]. To the best of our knowledge, no publications dealing with the determination of clothianidin appeared so far on hanging mercury drop electrode (HMDE)[20].

The goal of this work was to characterize this pesticide electrochemically (cyclic voltammetry, CV; cathodic stripping square wave voltammetry, SW CSV) and to develop a rapid, simple and sensitive method for the determination of this compound in various water samples.

EXPERIMENTAL

Instrumentation

All experiments were performed at μAutolab/GPES (General Purpose Electrochemical System, Version 4.9, EcoChemie) computer-controlled electrochemical system. A controlled growth mercury drop electrode (MTM Anko Instruments, Poland) was used (area 1.02×10^{-2} cm^2). All potentials were referred to the Ag|AgCl (3 M KCl) reference electrode. The counter electrode was a platinum wire. All measurements were carried out after solutions deoxygenating with argon for 10 min (and for 60 s before each consecutive measurement at ambient temperature of the laboratory (20 °C)). For the analytical application, the following parameters have been employed: pulse amplitude 100 mV, frequency 200 Hz, potential step 7 mV.

The Waters 600E liquid chromatograph included Waters 600 Controller, Waters 600 Pump, Waters 2487 Dual Absorbance Detector, Waters 717 plus Autosampler. Separation was performed on the C18 column (250 mm × 4.6 mm × 5 μm, ANALYSENTECHNIK PerfectSil Target OSD-3) kept in ambient temperature.

Materials

Clothianidin was purchased from Dr. Ehrenstorfer GmBH (Germany). A stock solution of 1.0×10^{-3} mol l^{-1} was prepared daily by dissolving an accurate mass of the pesticide in an appropriate volume of acetone and water (40% v/v) and methanol and water (30% v/v) for voltammetric and HPLC measurements, respectively. Working solutions for voltammetric

investigations were prepared by dilution of the stock solution with water. Methidathion, diazinon, acibenzolar S-methyl, demeton, cyromazin, thiophanate methyl, dodine and fenthion (stock solution of 1.0×10^{-3} mol l^{-1} each) were purchased from Sigma–Aldrich. 0.04 mol l^{-1} Britton–Robinson (BR) buffer pH 2–10 and borax buffer pH 7–9 were used as supporting electrolytes. All other chemicals were POCh S.A. (Poland) and analytical-reagent grade. All solutions were prepared with distilled and deionized water.

Water samples from Sokolowka (River water I) and Bzura (River water II) rivers were stored frozen until assay.

Calibration Procedure for Voltammetric Determination

Appropriate volumes of clothianidin working solution were transferred into the voltammetric cell to obtain related clothianidin concentrations. For optimum conditions described in the experimental section, linear calibration curves for SW CSV analysis were constructed in the clothianidin concentration ranges of 2.0×10^{-8}–2.0×10^{-7} and 2.0×10^{-7}–9.9×10^{-7} mol l^{-1} with applied accumulation time 5 and 10 s, respectively. The repeatability, accuracy and precision were checked.

Recovery Studies

To study the accuracy and repeatability of the applied methods, recovery experiments were carried out using the standard addition method. In order to know whether the constituents of biological samples show any interference with the analysis, known amounts of pure clothianidin solutions were added to the pre-analysed specimen of water and mixtures were analysed by the proposed SW CSV method. The recovery results were calculated using the related calibration equations after six repeated experiments.

Analysis Procedure of Environmental Sample

The general procedure used to obtain cathodic stripping voltammograms was as follows: 10 ml of the supporting electrolyte (5 ml of the buffer mixed with 5 ml of distilled, tap or river water) were placed in the voltammetric cell and the solution was purged with argon for 10 min with stirring. During the accumulation step, an anodic potential (–0.35 V) was applied with stirring the solution. After the accumulation period, the solution was equilibrated for 5 s. Following the equilibrium step, a negative ongoing potential scan was applied. To receive a well-shaped peak of clothianidin, the supporting electrolyte current was subtracted from the recorded clothianidin one.

Working Chromatographic Procedure

Working solution of 1×10^{-5} M clothianidin was prepared by diluting an appropriate volume of stock solution in water. Standard samples of spiked water (filtered through filtering paper) were prepared by transferring of 300 µl of clothianidin working solution to a 50-ml standard flask and made up to the mark with a river or tap water sample. The final concentration of clothianidin in each water sample was 6×10^{-8} mol l^{-1}.

To a 10-ml standard flask, 8.333 ml of a spiked water were transferred and made up to the mark with distilled water. The clothianidin concentration was determined by means of stan-

dard addition method (SAM). Concentrations of clothianidin standard additions were as follows: 5×10^{-8}, 1×10^{-7} and 1.5×10^{-7} mol l^{-1}.

The samples prepared as previously described were incubated at 4 °C in the autosampler. A volume of 25 µl was injected in a chromatographic column. As a mobile phase, methanol and water at the ratio of 50:50 (v/v) pumped with flow rate 0.8 ml min^{-1}, were used. During the analysis, solvents were degassed by helium. UV detection was employed at 270 nm. Retention time of clothianidin was 8 min.

RESULTS AND DISCUSSION

Electrochemical Reduction of Clothianidin

In order to understand the electrochemical process of clothianidin, reduction on the HMDE, CV and SW CSV techniques were carried out. Typical square wave voltammogram of 3.0×10^{-6} M clothianidin in borax buffer (pH 8.1) is shown in Fig. 1. Clothianidin has two main cathodic peaks, the first one appeared at about –0.97 V (peak I) and the second one at –1.34 V (peak II). At very negative potential, approximately at –1.7 V, the third peak appeared (peak III).

The electrochemical behavior of clothianidin was studied over a wide pH range (2–10) at HMDE in different buffered aqueous media using SW CSV technique. The voltammetric signals were strongly pH dependent (Fig. 2).

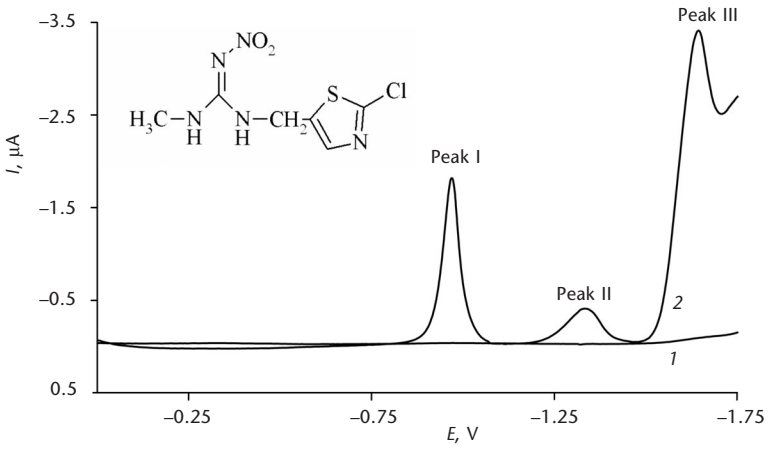


FIG. 1
SW CSV voltammogram of 3×10^{-6} M clothianidin in BR buffer pH 8.1; conditions: frequency 50 Hz, amplitude 25 mV, step potential 5 mV, t_{acc} = 30 s at 0 V. Blank (*1*), added clothianidin (*2*). Inset: clothianidin structure

The highest peak currents were received at pH 8.1. The peak potential of the peak I was shifted with increasing pH from 2.39 to 5.25 to more negative potential. Above pH 6, this signal appeared to be pH independent. Peak II depends linearly on pH in the whole investigated range. For analytical purposes, the best response (with regard to the peak current sensitivity and peak morphology, as well as buffer capacitance) was obtained for peak I in BR buffer at pH 8.1. This signal is always higher compared to peak II. The results of carried out experiments are similar to those received for thiamethoxam[17] and imidacloprid[21]. The typical DP polarogram of thiamethoxam in pH 8 presents two signals, the first peak at about –1 V and the second one at –1.32 V, the peak current of the first one is much higher than of the second one at the same time. The electrochemical study of imidacloprid[21] by DPP at different pH values demonstrates the occurrence of two different reduction processes, at pH 6.8 two peaks at –0.90 and –1.38 V were obtained. The mentioned pesticides include nitro group in their structure, which is easy reducible at HMDE. Cyclic voltammogram of clothianidin is very close to that of imidacloprid[15] and proves irreversible two step reduction.

The slope and intercept of $E = f(\mathrm{pH})$ (peak potential–pH dependences) for both signals of the neonicotinoid pesticides clothianidin, imidacloprid and thiamethoxam are also very similar. For the clothianidin peak I, the slope and intercept are equal to 0.112 V pH^{-1} and 0.267 V, respectively. In the

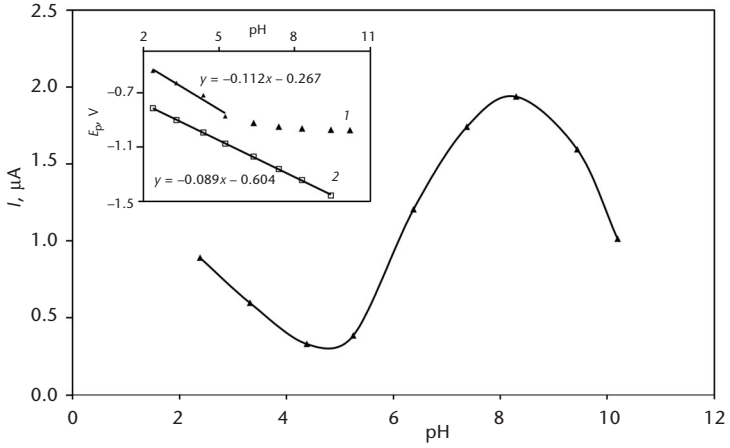
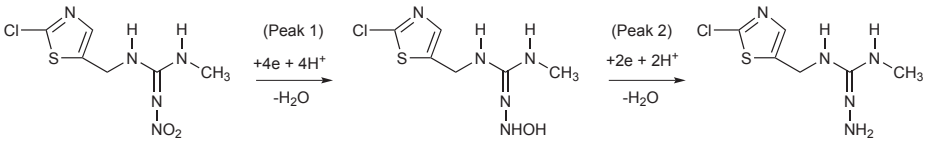


FIG. 2
Dependence of peak I current on pH, 3×10^{-5} M clothianidin; conditions as in Fig. 1. Inset: dependence of peak I (*1*) and peak II (*2*) potential on pH

case of imidacloprid[14] and thiamethoxam[17], the slope of the same dependence is equal to 0.110 and 0.082 V pH^{-1}, respectively. In literature, the intercept of peak I potential versus pH is given only in the case of imidacloprid and reaches 0.278 V. Comparing these results, one can confirm the same type of electrode mechanism. Minor difference in the slope in the case of thiamethoxam can be explained due to the used technique as both clothianidin and imidacloprid were investigated using square wave voltammetry and the first pesticide with differential pulse voltammetry. The same comparison can be done in the case of peak II. The slope of the same dependence for clothianidin, imidacloprid and thiamethoxam reaches 0.089, 0.072 and 0.071 V pH^{-1}, respectively. The intercept is equal to 0.604 and 0.847 V for clothianidin and imidacloprid, respectively. Hence, based on the comparison, it could be assumed that the numbers of electrons and the mechanism involved in the reduction of the compounds are identical. Such a mechanism is described in literature for imidacloprid and thiamethoxam as follows: peak I relates to 4-electron reduction of NO_2 group to NHOH one, peak II corresponds to further reduction of hydroxylamine group to an appropriate amino group (Scheme 1). Similar deduction was used for the polarographic characterization of thiamethoxam[17]. Such a mechanism is well described in literature in connection with electrode reactions of nitro compounds[22]. We believe that peak III can be ascribed to electrocatalytical effect on hydrogen evolution of guanidine group[23] also present in the structure of clothianidin.

All experiments were conducted in the constant room temperature of 20 °C. The effect of the potential scan rate between 50 and 1000 mV s^{-1} on the clothianidin peak I current was evaluated. Scan rate studies were than performed to assess whether the processes on the electrode were under diffusion or adsorption control. The relationships between current and scan rate were linear according to the equation $I_p = Av^x$, indicating diffusion control. The x values 1.0 and 0.5 are expected for adsorption- and diffusion-controlled reactions[24], respectively. The regression of the loga-



SCHEME 1
Proposed mechanism of clothianidin reduction

rithm of the peak current vs the logarithm of the scan rate gave a slope value of 0.81 (the correlation coefficient of the straight line 0.999) indicating that the electrode process is controlled by both diffusion and adsorption[25].

Quantitative Studies

In order to develop a voltammetric methodology for determination of the pesticide, we have selected SW mode, since the peaks were sharply and well defined at low concentrations of the clothianidin. The optimum instrumental conditions were chosen from the studies of the variation of the peak current on step potential, amplitude and frequency as well as quiescent time, concentration of the BR buffer, accumulation time and potential. There was no significance in equilibrium time and concentration level of the buffer within the range of 0.04–0.004 mol l^{-1}. For the analytical purposes, the best signal was obtained with step potential 7 mV, amplitude 100 mV and frequency 200 Hz. Some interesting results were obtained for investigation of the influence of accumulation time (Fig. 3) and accumulation potential (Fig. 4) on recorded signals.

For high concentration of clothianidin (3×10^{-5} mol l^{-1}) there is a significant decrease in signal current with increase of accumulation time. This can be attributed to saturation of mercury drop with the pesticide and/or inter-

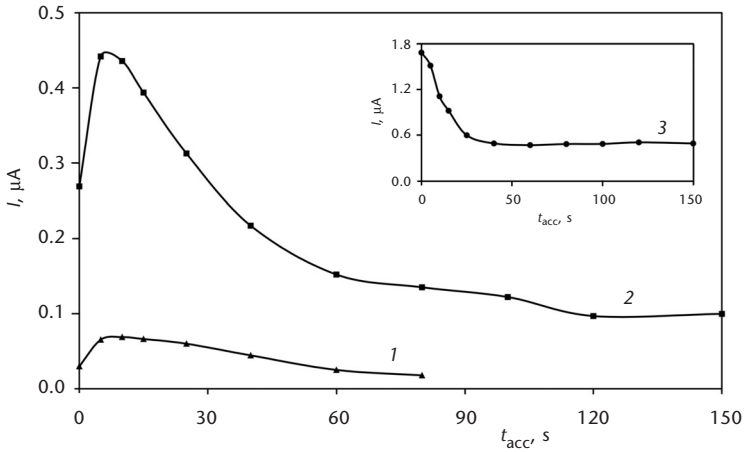


FIG. 3
Dependence of peak I signal on accumulation time for three chosen clothianidin concentrations: 3×10^{-7} (*1*), 3×10^{-6} (*2*) and 3×10^{-5} mol l^{-1} ((*3*) inset) in BR buffer pH 8.1; conditions: E_{acc} = –0.3 V, other parameters as in Fig. 1

molecular interaction hindering electrode reaction. We have confirmed the result repeating the same experiment for a one fold and two fold lower concentration of pesticide. Additional plots (Fig. 4) suggest that clothianidin is likely to adsorb on the surface of the electrode and is very sensitive to accumulation time. This can be an advantage for analytical purposes what will be described further. Accumulation potential is also of large significance and optimal value –0.35 V was chosen. Such potential additionally certifies that clothianidin does not create covalent bond with oxidized form of electrode material.

Quantitative evaluation is based on the linear correlation between the mean peak current and concentration of the pesticide. Because of high sensitivity to accumulation time, two ranges were selected: from 2.0×10^{-8} to 2.0×10^{-7} (Range 1) and from 2.0×10^{-7} to 9.9×10^{-7} (Range 2) with different accumulation times 10 and 5 s, respectively. The equations of the calibration plots are $I_p(A) = 3.06C + 3.12 \times 10^{-9}$ with linear correlation coefficient $r = 0.9998$, and $I_p(A) = 2.21C + 8.10 \times 10^{-8}$ with $r = 0.9990$, respectively (C in mol l^{-1} stands for concentration). All statistic data were obtained from six measurements. Standard deviations for intercept and slope of the calibration curves are 2.04×10^{-9} A and 1.69×10^{-2} A l mol^{-1}, respectively for Range 1, and 1.74×10^{-8} A and 2.68×10^{-2} A l mol^{-1}, respectively for Range 2.

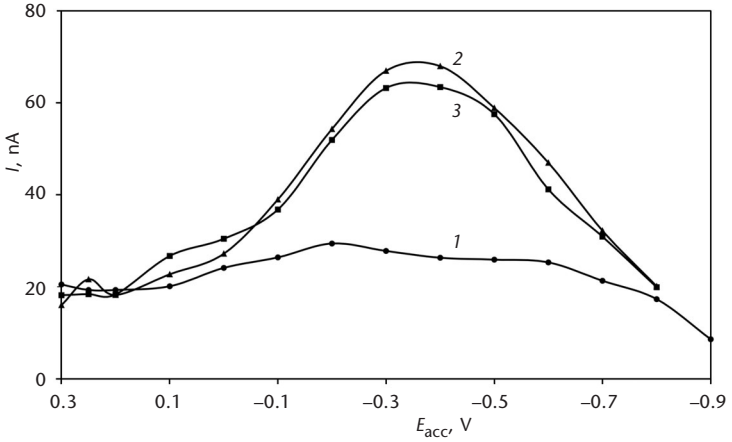


FIG. 4
Dependence of peak I current on accumulation potential for three chosen accumulation times: 0 (*1*), 10 (*2*) and 15 s (*3*) in BR buffer pH 8.1, 3×10^{-7} M clothianidin; parameters of excitation as in Fig. 1

Validation of the procedure for the quantitative determination of the clothianidin was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), repeatability, recovery, accuracy and precision. Detection and quantification limits of the procedure were calculated from the calibration curves as kSD/b, where k = 3 for LOD and 10 for LOQ, SD is standard deviation of the intercept and b is the slope of calibration line[26]. LOD and LOQ were obtained as 2.00×10^{-9} and 2.36×10^{-8} mol l^{-1}, respectively for Range 1, and 6.65×10^{-9} and 7.86×10^{-8} mol l^{-1}, respectively for Range 2. The repeatability of the measurement was calculated from six independent runs of different clothianidin concentrations. The relative standard deviations varied from 1.7 to 6.3% for lower concentration range and from 2.2 to 3.8% for higher one.

The effect of common pesticides presence was tested for possible interferences in the assay of clothianidin. It was observed that the demeton, cyromazin, thiophanate methyl and dodine did not interfere up to 50-fold in the determination. The presence of 20-fold methidathion changed the clothianidin signal for about 20%. The presence of 10-fold fenthion decreased peak current for about 50%. Acibenzolar S-methyl disturbed at concentrations equal and higher than clothianidin concentration. The presence of diazinon always hindered clothianidin peak.

Analysis of Clothianidin in Spiked Environmental Samples

The developed procedure was applied for the analyses of tap and two different samples of river (Bzura and Sokolowka) water. There was no need of any precipitation, evaporation or extraction step prior to pesticide assay. The measurements of clothianidin in spiked water were performed as described in Experimental. The applicability of proposed SW CSV technique for determination of the pesticide was tested successfully by standard addition method (Fig. 5). The concentrations of the standard addition in the cell were at the levels 2×10^{-8} and 4×10^{-8} mol l^{-1}. The clothianidin content of spiked samples was recovered in the range of 98.57–101.49% with less than 3.5% RSD (Table I). The obtained recovery results were calculated from the linear regression equations $I_p(A) = 3.06C + 3.12 \times 10^{-9}$. The good results of recovery confirm not only the accuracy of the applied technique but lack of interferences from matrix as well. The HPLC method[27] was chosen as the analytical reference method and the received results compare well with SW CSV analysis data (Table I). The same diluted river samples were studied in both methods.

TABLE I

Results of the clothianidin quantitative determination in spiked water and mean recovery values by SW CSV and HPLC techniques, $t(k,\alpha) = 2.57$, $p = 0.95$, $n = 6$, $k = n - 1$; concentration of clothianidin added 5×10^{-8} mol l^{-1}

Parameter	Tap water	River water I	River water II
		SW CSV	
$\overline{C} + SDt(k,\alpha)$, mol l^{-1}	$(5.07 + 0.18) \times 10^{-8}$	$(5.05 + 0.16) \times 10^{-8}$	$(4.93 + 0.10) \times 10^{-8}$
RSD	0.035	0.032	0.021
Mean recovery, %	101.49	100.92	98.57
		HPLC	
$\overline{C} + SDt(k,\alpha)$, mol l^{-1}	$(4.99 + 0.06) \times 10^{-8}$	$(5.06 + 0.09) \times 10^{-8}$	$(5.00 + 0.09) \times 10^{-8}$
RSD	0.012	0.016	0.018
Mean recovery, %	99.85	101.16	100.03

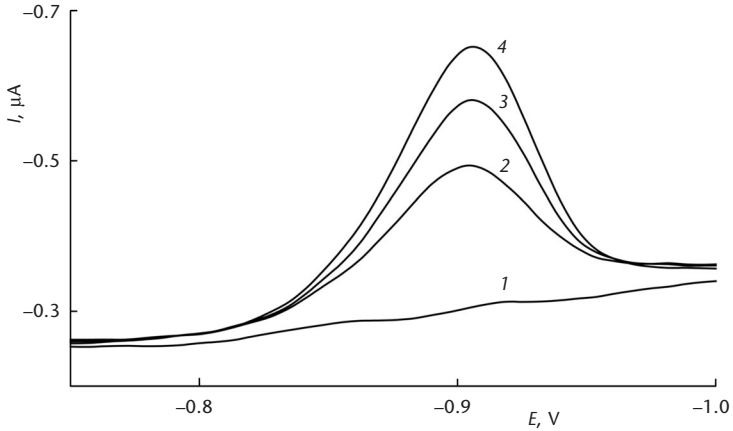


FIG. 5

SW voltammograms of spiked tap water along with standard addition method in BR buffer pH 8.1; conditions: t_{acc} = 10 s at –0.35 V, frequency 200 Hz, amplitude 100 mV, step potential 7 mV. Blank (*1*), spiked tap water (*2*), and concentrations of clothianidin standard additions 2×10^{-8} (*3*) and 4×10^{-8} mol l^{-1} (*4*)

CONCLUSIONS

In summary, the electrochemical reduction of clothianidin on hanging mercury drop electrode was established and studied for the first time. We have stated that the reduction mechanism is connected with irreversible two-step reduction of nitro group. A new square wave cathodic stripping voltammetric method of clothianidin determination in the range of 2.0×10^{-8}–9.9×10^{-7} mol l^{-1} based on recorded signal of nitro group reduction was developed. The method was also applied for the determination of clothianidin in spiked real water samples. The analysis was performed with good recoveries without any interference from the sample. Voltammetric results were validated by HPLC method and were satisfying. The quantitative assay of real samples by proposed techniques involved only dilution of environmental samples and was not time-consuming because no preliminary steps were required.

Electrochemical methods, such as differential pulse voltammetry or square wave voltammetry, belong to the most sensitive voltammetric methods. The sensitivity increases when the accumulation step is employed, so LOD and LOQ of the presented method of clothianidin determination are one of the lowest values described in literature. The proposed voltammetric method is a good alternative to the HPLC, GC, LC-MS and other pesticide analysis techniques.

This work was supported by Grant No. 505/708 from the University of Lodz, Poland.

REFERENCES

1. Bhand S., Surugiu I., Dzgoev A., Ramanathan K., Sundaram P. V., Danielsson B.: *Talanta* **2005**, *65*, 331.
2. Mirceski V., Guziejewski D., Skrzypek S., Ciesielski W.: *Croat. Chem. Acta* **2010**, *83*, 121.
3. Katsumata H., Kaneco S., Suzuki T., Ohta K.: *Anal. Chim. Acta* **2006**, *577*, 214.
4. Lambropoulou D., Sakellarides T., Albanis T.: *Fresenius J. Anal. Chem.* **2000**, *368*, 616.
5. Quintana J., Mart'l I., Ventura F.: *J. Chromatogr., A* **2001**, *938*, 3.
6. Touloupakis E., Giannoudi L., Piletsky S. A., Guzzella L., Pozzoni F., Giardi M. T.: *Biosens. Bioelectron.* **2005**, *20*, 1984.
7. Kim H. J., Liu S., Keum Y. S., Li Q. X.: *J. Agric. Food Chem.* **2003**, *51*, 1823.
8. Schmuck R., Keppler J.: *Pflanzenschutz-Nachrichten Bayer* **2003**, *56*, 26.
9. Vilchez J. L., El-Khattabi R., Blanc R., Navalon A.: *Anal. Chim. Acta* **1998**, *371*, 247.
10. Vilchez J. L., Valencia M. C., Navalon A., Molinero-Morales B., Capitain-Vallvey L. F.: *Anal. Chim. Acta* **2001**, *439*, 299.
11. Watanabe E., Eun H., Baba K., Arao T., Ishii Y., Endo S., Ueji M.: *J. Agric. Food Chem.* **2004**, *52*, 2756.

12. Carretero A. S., Cruces-Blanco C., Duran S. P., Gutierez A. F.: *J. Chromatogr., A* **2003**, *1003*, 189.
13. Kim H. J., Shelver W. L., Li Q. X.: *Anal. Chim. Acta* **2004**, *509*, 111.
14. Navalon A., El-Khattabi R., Gonzalez-Casado A., Vilchez J. L.: *Microchim. Acta* **1999**, *130*, 261.
15. Guiberteau A., Galeano T., Mora N., Parilla P., Salinas F.: *Talanta* **2001**, *53*, 943.
16. Guzsvany V., Gaal F., Bjelica L., Okresz S.: *J. Serb. Chem. Soc.* **2005**, *70*, 735.
17. Guzsvany V., Kadar M., Gaal F., Toth K., Bjelica L.: *Microchim. Acta* **2006**, *154*, 321.
18. Guzsvany V., Kadar M., Gaal F., Bjelica L., Toth K.: *Electroanalysis* **2006**, *18*, 1363.
19. Guzsvany V., Kadar M., Papp Z., Bjelica L., Gaal F., Toth K.: *Electroanalysis* **2008**, *20*, 291.
20. Papp Z., Svancara I., Guzsvany V., Vytřas K., Gaal F., Bjelica L., Abramovic B.: in: *Sensing in Electroanalysis* (K. Vytřas, K. Kalcher and I. Svancara, Eds), Vol. 4, p. 47–58. University of Pardubice, Pardubice 2009.
21. Cacho J., Fierro I., Deban L., Vega M., Pardo R.: *Pestic. Sci.* **1999**, *55*, 949.
22. Squella J. A., Bollo S., Nunez-Vergara L. J.: *Curr. Org. Chem.* **2005**, *9*, 565.
23. a) Mirceski V., Skrzypek S., Ciesielski W., Sokołowski A.: *J. Electroanal. Chem.* **2005**, *585*, 97; b) Skrzypek S., Mirceski V., Ciesielski W., Sokołowski A., Zakrzewski R.: *J. Pharm. Biomed. Anal.* **2007**, *45*, 275; c) Skrzypek S., Ciesielski W., Yilmaz S.: *Chem. Anal. (Warsaw)* **2007**, *52*, 1071.
24. Erk N.: *Anal. Biochem.* **2003**, *323*, 48.
25. Kul D., Gumustas M., Uslu B., Ozkan S. A.: *Talanta* **2010**, *82*, 286.
26. dos Santos L. B. O., Abate G., Masini J. C.: *Talanta* **2004**, *62*, 667.
27. Watanabe E., Baba K., Eun H.: *J. Agric. Food Chem.* **2007**, *55*, 3798.