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Voltammetric determination of imidacloprid and thiamethoxam

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Abstract: A simple voltammetric method using a glassy carbon working electrode was developed for the determination of two members from the neonicotinoid group of insecticides: imidacloprid and thiamethoxam. The experiments showed that the voltammetric response depends on the mode of electrode surface pretreatment and the polarization mode. The response appeared to be linear in the range from 0.028 to 0.50 mg/cm^3 for both analytes. The limit of detection was 0.0077 mg/cm^3 for imidacloprid and 0.0085 mg/cm^3 for thiamethoxam, the limit of quantitation was 0.026 mg/cm^3 and 0.028 mg/cm^3, respectively. The developed method was applied for the determination of these insecticides in potato samples sprayed with Confidor 200-SL or Actara 25-WG as well as commercial formulations of imidacloprid and thiamethoxam. A recovery trial was performed to assess the accuracy of the results, the recovery values being between 95–102 % for both of neonicotinoids.

Keywords: imidacloprid, thiamethoxam, cyclic voltammetry, electrochemical detection.

INTRODUCTION

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] (Fig. 1A) and thiamethoxam [3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinane-4-ylidene(nitro)amine (Fig. 1B) belong to a relatively new class of insecticides, known as neonicotinoids. Their physical, chemical and toxicological properties have been summarized in a pesticide manual.[1] Neonicotinoids interfer with the nicotinic acetylcholine receptor and, therefore, have specific activity against the insect nervous system. This unique mode of action makes them highly desirable for controling insects that are developing resistance to conventional organophosphate, carbamate, and pyrethroid insecticides.[2] Thiamethoxam and imidacloprid also have minimal effects on beneficial insects, low toxicity to-

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ward mammals, and do not produce teratogenic or mutagenic effects. Because of this selective activity, they have been recommended for treatment of seed of several major field crops. They are systematic and contact insecticides, effective in controlling aphids (*Aphididae*), thrips (*Thrips tabaci*), potato beetle (*Leptinotarsa decemlineata*), and various other harmful pest species.[2,3]

Several analytical procedures for the determination of neonicotinoid insecticides in foods, agricultural and environmental samples have been proposed. Gas chromatography (with prior derivatization)[4,5] and high performance liquid chromatography (HPLC) with diode array,[6,7] mass-spectrometric[8–10] and amperometric (reductive pulsed mode)[11] detection have been commonly used. There are also some electrochemical methods (differential-pulse polarography[12] and square wave adsorptive stripping voltammetry on a HMDE[13]) for the determination of imidacloprid in commercial formulations or in spiked river water samples. However, to the best of knowledge, there are no electroanalytical methods for the determination of thiamethoxam.

The aim of this work was to develop a rapid, simple and sufficiently sensitive voltammetric method on glassy carbon (GC) working electrode for the determination of imidacloprid and thiamethoxam in model system and in residue-incurred potato samples.

EXPERIMENTAL

Apparatus. Voltammetric measurements were carried out on a computerized AMEL setup, Easyscan 5000, Milan, Italy. The stand includes a three-electrode system, a Radiometer saturated calomel electrode (SCE), a platinum ring auxiliary electrode and a GC (Tokai, HTT 3000 °C) working electrode.

The spectrophotometric study was carried out on an ANTHELIE Data Spectrophotometer, SECOMAM, France.

Reagents and solutions. All chemicals used were of analytical reagent grade. The reference standards were: imidacloprid Pestanal (Riedel-de-Haën, Seelze, Germany), purity 99.9 % and thiamethoxam (Syngenta, Basel, Switzerland), purity 99.5 %. The applied commercial formulations of pesticides were Conifdor SL-200 (Bayer, Germany) and Actara 25-WG (Syngenta, Basel, Switzerland), Bancol 50-WP (Pliva, Zagreb, Croatia) as synthetic products with active component: imidacloprid, thiamethoxam, bensultap [S,S'-dimethylaminotrimettylene di(benzenthiosulfonate)], respectively, and Z Stop (Bioenvironmental Center, Zrenjanin, Serbia and Montenegro) a bacteria-originated bioinsecticide. Primary stock solutions were prepared by dissolving each reference standard in double distilled water or in buffer solutions at a concentration of 0.50 mg/cm^3. Standard solutions in the range of: 0.0010–0.50 mg/cm^3 were obtained by diluting the primary stock solutions. Spectrophotometric investigation of these solutions showed that the content and propertiers did not change over a three-week period if the solutions were kept in the dark at 4 °C.

Britton–Robinson buffer solutions were (prepared from a 0.04 mol/dm^3 stock solution in phosphoric (Merck, Darmstadt, Germany), boric (Merck, Darmstadt, Germany), and acetic (Merck, Darmstadt, Germany), acids; and by adding 0.2 mol/dm^3 sodium hydroxide (Merck, Darmstadt, Germany) to the required pH value.

Extraction was carried out with dichloromethane (CH_2Cl_2, p.a. J. T. Baker, Phillipsburg, NJ, USA).

Potato samples. The potato samples were obtained from several sources: from local grocery stores at different geological locations, from members of Terras Eco Cooperative from Subotica

(Serbia and Montenegro), and from the city market in Novi Sad (Serbia and Montenegro). The analyzed samples were: sample **1** (non-sprayed potato), sample **2** (sprayed with Bancol 50-WP and bioinsecticide Z Stop), sample **3** (sprayed with Confidor 200-SL), sample **4** (sprayed with Actara 25-WG) and sample **5** (occasional purchase on the city market Novi Sad).

Each potato sample was washed in double distilled water, dried at room temperature, chopped into small pieces and mixed. 600 g of potato sample (**1** and **2**) was weighed in a beaker and fortified with an aliquot of the pesticide working standard mixture (0.050; 0.10; 0.20 and 0.50 mg/kg potato). The samples were allowed to stand for 24 h in the dark at 4 ºC. The control vegetable samples (**1** and **2**) and the residue-incurred samples without pesticide fortification (**3–5**) were processed by following a similar stepwise procedure.

The residue-incurred potato sample was boiled in 500 cm^3 of double distilled water for 30 min. Subsequently, the sample was colled to room temperature. The ground sample was then processed as described in the following section.

Extraction. 150 cm^3 of CH_2Cl_2 were added to the sample, the mixture was carefully shaken manually for 1 h, then wringed in Multipress, and the liquid phase was collected. The solid phase was washed with 50 cm^3 CH_2Cl_2, and the obtained organic extract was added to the liquid phase. The obtained liquid phase was quantitatively transferred to an extraction funnel. The liquid–liquid extraction was repeated three times by adding each time 20 cm^3 of CH_2Cl_2 to the aqueous phase. The organic extract was evaporated on a rotary vacuum evaporator at 25 ºC to dryness and the residue dissolved in 2.00 cm^3 of buffer solution pH 7.0 under sonication, to assist dissolution. The obtained solution was transferred to the voltammetric cell for measurement.

Voltammetric investigations. Before each measurement, the GC was polished with alumina powder (Kemika, Zagreb, Croatia) of different particle size, suspended in double distilled water, using finally the 0.3 μm grade, to attain a mirror finish. Afterwards, the GC was washed in an ultrasonic bath with double distilled water to remove any residual polish. The electrode had to be appropriately activated to attain better functioning.

The voltammetric analyses were carried out in 2.00 cm^3 of supporting electrolyte pH 7.0 after optimized electrode pretreatment with *ex situ* potential cycling (10 cycles) in the potential range from 0.4 V to – 1.9 V (*vs*. SCE) before each measurement. Bearing in mind that electrochemical pretreatment in the solutions of analytes could bring about different changes on the GC surface, the electrodes were pretreated *ex situ*. This was carried out in an aqueous solution of the supporting electrolyte pH 7.0, using an electrochemical cell identical to the one used in the voltammetric experiments. After recording the background voltammograms, an aliquot of the neonicotinoid standard was introduced and the cycle was repeated. The peak area was measured as the difference between the two voltammograms, and the determination was always carried out using the first cycle. In the case of residue-incurred samples, sample **1** was taken as the matrix of untreated potato. All the data were obtained at ambient temperature in a voltammetric cell purged with nitrogen for 10 min.

Spectrophotometric investigations. The absorption spectra of imidacloprid and thiamethoxam were recorded in the wavelength range of 200–400 nm, in buffer solutions of pH 2.0–9.0. As the best defined spectra were obtained at pH 7.0, this value was chosen for recording the spectra of solutions of different concentrations, *i.e.*, for the determination. Another reason for choosing this pH was the fact the pH of the potato matrix was about this value. However, because of the effects of the matrix, attempts to determine imidacloprid and thiamethoxam in the extracts of residue-incurred potato samples prepared by the described procedure were unsuccessful.

Validation of the analytical methods. The linearity of the methods was determined by constructing calibration curves for both the voltammetric and the spectrophotometric method. The limit of detection (LOD) and the limit of quantitation (LOQ) (Table I) were calculated from the calibraton curves using the equations:[14] LOD = $3s/m$, and LOQ = $10s/m$, where s is the standard deviation of the intercept and m is the slope of the calibration curve. In the case of potato samples, all of the recovery experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Voltammetric behavior

As observed very early,[15] and later confirmed by many reports, including the recent authoritative finding by Chen and McCreery,[16] the electrode kinetics on GC is crucially dependent on the mode of electrode surface pretreatment. For this reason, the mechanical and electrochemical pretreatment was carried out in a wide range of pH values (2.0–9.0). Generally, it appeared that the electrochemically pretreated electrode had about 150 mV lower potential for hydrogen evolution than the polished electrode, and the residual current was significantly higher in the case of polished surface. As for the duration of potential cycling, it appeared that the voltammograms obtained after 10 cycles were identical.

As can be seen from Fig. 1A, a single cathodic peak at about –1.2 V (*vs.* SCE) with no peak in the reverse scan, indicates the irreversible nature of the electrode reaction. Continued cycling results in a shift of the peak potential toward more negative values and a large decrease in the peak current and area. The effect was also present even after stirring of the solution. This suggests potential involvement of adsorption. Similar behavior was also observed for thiamethoxam (Fig. 1B).

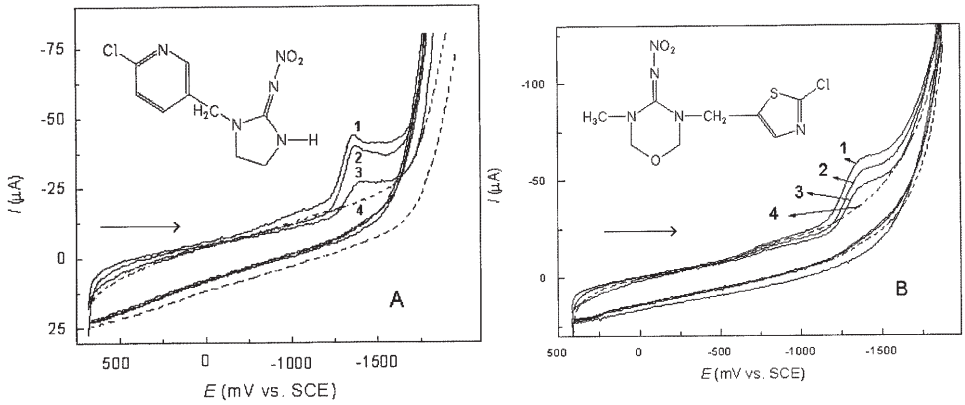

Fig. 1. Repetitive cyclic voltammograms of 0.03 mg/cm^3 solutions of imidacloprid (A) and thiamethoxam (B): 1) 1st; 2) 2nd; 3) 3rd cycles; 4) blank. GC electrode polished, pH 7.0, v = 500 mV/s.

On the basis of the comparison of the relative standard deviations (RSD), it can be concluded that the series of voltammograms recorded in neonicotinoid model solutions with an *ex situ* pretreated electrode (Table I) showed a two-times more reproducible response than a wet-polished working electrode. A dry-polished electrode gave the strongest but insufficiently reproducible signals. These observations are supported by the report of other authors[18] that the behavior of a GC electrode depends on the mode of its mechanical or electrochemical pretreatment in nitrobenzene solution, which is generally accepted as a model compound with a nitro group.

Voltammetric behavior of imidacloprid and thiamethoxam at a GC electrode was examined in supporting electrolyte of various pH values (Fig. 2). At pHs lower

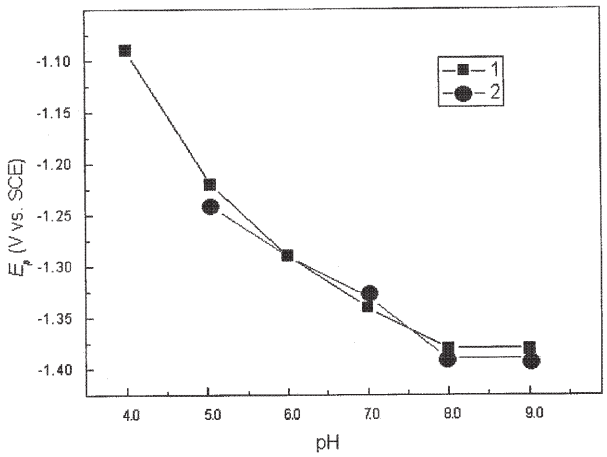

Fig. 2. Dependence of the peak potential on pH: 1) imidacloprid; 2) thiamethoxam, c = 0.03 mg/cm^3, v = 500 mV/s.

than 3.0, no imidacloprid reduction peak was noticeable. On the other hand, the reduction peak for thiamethoxam appeared only at pH 4.0 and above. The shift in the peak potential as a function of pH was linear for both insecticides, and the peak current and area increased to pH 7.0. Namely, the potentials of hydrogen evolution at pH 4.0–6.0 in case of imidacloprid and pH 5.0 or 6.0 in the case of thiamethoxam are quite close to the reduction peak of imidacloprid and thiamethoxam, but the peak current intensities and areas can be determined in both cases. At pH 7.0–9.0, the potential window is of a satisfactory width, but in alkaline medium the nitroguanidine functional group of imidacloprid undergoes alkaline hydrolysis.[19] Therefore, the optimum pH adopted for further studies was 7.0. Similar E_p–pH plots for both neonicotinoids suggests the involvement of a similar reduction mechanism at pH values above 4.0.

The log I_p–log v graph was linear over the scan rate range of 25–200 mV/s at pH 7.0 with a slope of 0.98 for imidacloprid and of 0.97 for thiamethoxam. The peak potential shifted in the cathodic direction when the scan rate was increased according to the following equations: E_p (V) = 1.2 + 0.002 v (mV/s), r = 1.00 for imidacloprid and E_p (V) = 1.1 + 0.01 v (mV/s), r = 0.98 for thiamethoxam, which confirms the irreversibility of the electrode process. At higher scan rates, the current intensity and peak area increased rapidly. The scan rate of 500 mV/s, giving well-defined and reproductable peaks, was selected as optimum for the determination.

Analytical characteristics

Quantitative evaluation was based on the linear dependence of the peak area obtained exclusively in the first cycle on concentration (Table I and Fig. 3). In the optimized procedure at pH 7.0 and 500 mV/s, the response appeared to be linear in the range from 0.028 to 0.50 mg/cm^3 for both neonicotinoids. A precision assay was carried out at two levels (0.060 and 0.50 mg/cm^3). The maximal values of the RSD were 2.1 and 2.5 % for imidacloprid and thiamethoxam, respectively.

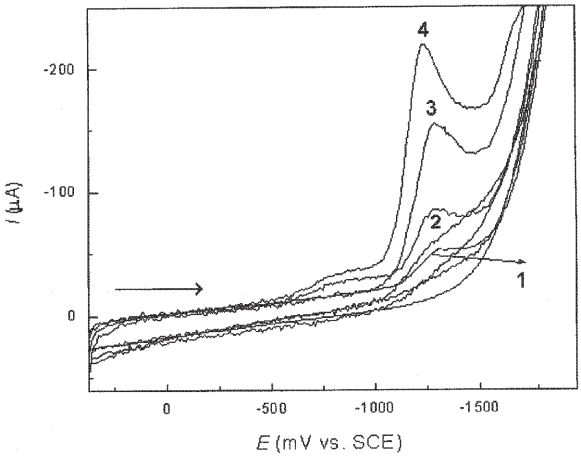

Fig. 3. Voltammograms recorded for different concentrations of imidacloprid (mg/cm^3): 1) 0.050; 2) 0.11; 3) 0.22 and 4) 0.40. GC electrode pretreated *ex situ,* pH 7.0, v = 500 mV/s.

The results of the voltammetric measurements of the standard solutions were compared with those obtained by the UV-spectrophotometric method (Table I). This method is based on the strong absorption of imidacloprid and thiamethoxam in the buffer pH 7.0 at 270 and 253 nm, respectively, due to the π-π^* transition of the nitroguanidine chromophores. It is important to notice, that in buffer solutions of pH 2.3–3.8, no absorption maxima were observed. The LOD of the spectrophotometric method was about 100 times lower than that of the voltammetry method (Table I).

TABLE I. Analytical parameters for the voltammetric and spectrophotometric methods

Parameter	Compound/Method			
	Imidacloprid		Thiamethoxam	
	Voltammetry	UV Spectrophotometry	Voltammetry	UV Spectrophotometry
Concentration range (mg/cm^3)	0.028–0.50	0.0020–0.060	0.028–0.50	0.0020–0.060
Intercept	0.018	0.0035	0.058	0.0030
Slope/(cm^3/mg)	15.62	77.76	12.75	57.74
Correlation coefficient (r)	0.999	0.999	0.999	0.999
LOD/(mg/cm^3)	0.0077	5.3×10^{-5}	0.0085	7.2×10^{-5}
LOQ/(mg/cm^3)	0.026	1.8×10^{-4}	0.028	2.4×10^{-4}
Precision (RSD/%)[a]	2.1	0.56	2.5	0.80

[a]Mean (n = 6)

Determination of imidacloprid and thiamethoxam in potato samples

The applicability of the voltammetric procedure was tested by determining two insecticides in several residue-incurred potato samples. Recovery trial for

imidacloprid and thiamethoxam extraction from fortified samples at two different concentrations (0.05 mg/kg and 0.1 mg/kg) was performed to assess the accuracy of the results; recoveries ranged from 95 to 102 %, showing the good accuracy of the procedure.

As can be seen from Fig. 4, potato-matrix extracted from the non-sprayed potato sample (curve 5) did not block the electrode surface and did not contain other voltammetrically active components, which are favorable facts for the determination. The calibration graph is linear for the same concentration interval as with the model systems.

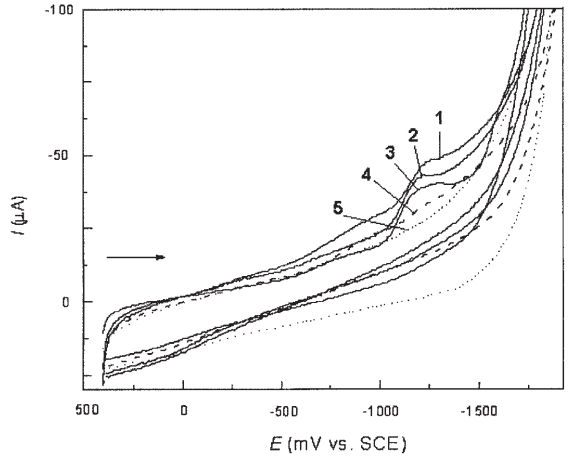

Fig. 4. Voltammograms for the sample **4** (sprayed with Actara 25-WG) (1), sample **5** (ocassional purchase on the city market Novi Sad), (2), sample **3** (sprayed with Confidor 200-SL) (3), sample **2** (sprayed with Bancol 50-WP and bioinsecticide Z Stop), (4), and sample **1** (non-sprayed potato) (5), pH 7.0, ν=500 mV/s.

Based on the result of voltammetric determination, the potatos treated with pesticide formulations according to the field needs contained about twice the amount of neonicotinoids (Fig. 4, curves 1 and 3) than is the maximum allowed residue limit[17] of 0.05 mg/kg. The potato from the city market contained about 0.09 mg imidacloprid/kg potato (Fig. 4, curve 2).

A systematic quantitative study was undertaken to verify the effect of the presence of other insecticides (Z Stop, Bancol 50-WP), the commercial forms of which are available in Serbia and Montenegro. No significant interferences were observed, which can be expected because of the absence of active components having a nitro functional group (Fig. 4, curve 4).

No signal from the reduction of nitro-groups could be observed for the cooked potato, which was to be expected in view of the thermal instability of these insecticides.

Good recoveries and low relative standard deviation reflect the high accuracy and precision of the proposed method. Moreover, the method is simple, easy and inexpensive, thus making of an excellent tool for the determination of imidacloprid and thiamethoxam in potato samples, with an accuracy which is not possible to achieve by the spectrophotometric method, without additional cleanup of the potato extract.

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ИЗВОД

ВОЛТАМЕТРИЈСКО ОДРЕЂИВАЊЕ ИМИДОКЛОПРИДА И ТИАМЕТОКСАМА

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Развијена је једноставна волтаметријска метода помоћу електроде од стакластог угљеника као радне електроде за одређивање два представника групе неоникотиноидних инсектицида: имидаклоприда и тиаметоксама. Експерименти показују да волтаметријски сигнали зависе од претретмана електродне површине и начина поларизације. Волтаметријски одзив је линеаран у опсегу концентрација 0,028–0,50 mg/cm^3 за оба аналита. Граница детекције износи 0,0077 mg/cm^3 за имидаклоприд и 0,0085 mg/cm^3 за тиаметоксам, а граница одређивања 0,026 mg/cm^3, односно 0,028 mg/cm^3. Развијена метода је примењена за одређивање ових инсектицида у узорцима кромпира третираних комерцијалним формулацијама имидаклоприда и тиаметоксама, Confidor 200 SL-200, односно Actara 25-WG. Тестови приноса, извршени ради процене тачности резултата, су били у опсегу 95–102 % за оба неоникотиноида.

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