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Voltammetric Study of the Hydrolysis Product of Bendiocarb at the Glassy Carbon Electrode

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Abstract. Differential pulse and square wave voltammetric methods are proposed for the determination of the N-methylcarbamate insecticide Bendiocarb based on the oxidation of its hydrolysis product. A single peak ($+0.7$ V (pH 3.5)) is observed at the glassy carbon electrode. Linear relationships between the peak intensity and the concentration were obtained up to 5 μg/ml, with detection limits of 0.77 μg/ml for DPV and 0.44 μg/ml for SWV, respectively. The methods have been applied satisfactorily for the analysis of river water samples spiked with the pesticide by using the extraction with diethyl ether as preconcentration step. Concentration values as low as 0.11 μg/ml were determined with a recovery of $96 \pm 3\%$ (SWV) and $91 \pm 2\%$ (DPV).

Key words: Differential pulse voltammetry; square wave; pesticide; river water; bendiocarb.

Bendiocarb [2,3-isopropylidenodioxyphenyl N-methylcarbamate] belongs to the N-methyl carbamate pesticides. It is an insecticide acting by cholinesterase inhibition, effective as a contact and stomach poison and with some systemic activity in crop plants. In agriculture it is used for seed treatment and in granular formulations for the control of soils pests particularly in maize and sugar beet. It is also used as foliar spray on other crops [1].

Different papers appeared in the literature concerning its determination in a variety of matrices, such as: milk [2], tissues [3, 4], commercial formulations [5–10], fruits [11–13], water [14–16], grains [7], wool [17], soil [18–20]. The most frequently used methods for its determination are HPLC [2, 8, 16, 17, 21], GC [11–13, 20, 22, 23], SFC [3, 4, 24] and spectrophotometry [5, 7, 9, 10, 15]. Although there are fluorimetric [6, 14], voltammetric [18, 19] and amperometric [25] techniques too. In HPLC, fluorescence [21] and UV (at 220 nm [2] and 254 nm [8, 16]) detectors are used. The determination of Bendiocarb by spectrophotometry involves a derivatization of its alkaline hydrolysed product. In relation to voltammetric techniques, Hichman and Ramanathan [19] propose a differential pulse polarographic method for monitoring the degradation of Bendiocarb in soil from its reduction signal at a dropping mercury electrode, when measuring at a very negative potential (-0.94 V) (vs. a Ag/AgCl electrode). Hernández et al. [18] proposed a differential pulse voltammetric method based on an oxidation process by using a carbon paste electrode modified with C18 (the C18 comes from a Sep-Pack Plus from Millipore with silicon base). They carried out an accumulation for 8 minutes in Britton-Robinson buffer at pH 5 and the measurement was performed in the same buffer solution at pH 9 ($Ep = 1.17$ V). The limit of detection was 0.69 μg/ml.

On the other hand, some papers describe the determination of several N-methyl carbamate insecticides such as Fenobucarb, Carbaryl and Carbofuran by using their alkaline hydrolysis reactions and voltammetric methods [26, 27] or HPLC with electrochemical detection [28].

The aim of the present paper is to develop a voltammetric procedure for the determination of

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Bendiocarb by differential pulse and square wave voltammetry after a hydrolysis to its phenol derivative, and the application of the proposed method to the analysis of spiked river water samples.

Experimental

Apparatus

The studies were performed with an Autolab computer-controlled potentiostat (Eco Chemie, Holland) PSTAT 10 equipped with a Metrohm (Herisau, Switzerland) 663 VA stand. This system was connected with a PC 486 equipped with the General Purpose Electrochemical System (GPES 3) version 3.2 software package. The stand includes a three-electrode system, an Ag/AgCl-3M KCl reference electrode, a platinum wire auxiliary electrode and a glassy carbon electrode (Metrohm Ref. 6.1204.040) (diameter: 3 mm) as working electrode.

Reagents

Bendiocarb (99%), Fenobucarb (98%) and Pirimicarb (99%) were obtained from Riedel-de Haën (Seelze, Germany); Ethiofencarb (97.5%) was obtained from Dr. Ehrenstorfer (Augsburg, Germany); and Carbofuran (99.5%) was obtained from Krompek (Barcelona, Spain). They were used without further purification, and solutions of them in ultrapure water (40 µg/ml) were prepared and stored in the dark at 4 °C.

HPLC-grade water was obtained from a Milli-Q system (Millipore, Bedford, MA., USA). Diethyl ether (free of phenolic antioxidants) was of HPLC grade. All other chemicals were of analytical reagent grade.

General Procedure

A suitable volume of the Bendiocarb solution between 0.5 and 5 µg/ml, 5 mL of 0.5 M $NaClO_4$ and 1 mL of 0.5 M NaOH were introduced in a 25 mL calibrated flask. The solution was diluted with water to near 20 mL, shaken for a few seconds and allowed to stand for 10 min. Then, 0.5 mL of glacial acetic acid was added and the solution was diluted to the mark with ultrapure water. The obtained solution (pH ≈ 3.5) was transferred to the electrochemical cell and the differential pulse voltammogram (DPV) as well as the square wave voltammograms (SWV) in the range + 0.4 to 1 V were registered in duplicate at the following instrumental conditions: DPV: scan rate 10 mV/s and pulse amplitude 30 mV; and SWV: frequency 80 Hz, step potential 5 mV and pulse amplitude 30 mV. Prior to each scan, the glassy carbon electrode was carefully smoothed with a cotton soaked in DMF during two minutes and subsequently with another cotton soaked in water for several seconds.

Procedure for the Determination in River Water Samples

In a separation funnel, 100 mL of river water sample spiked with appropriate amounts of Bendiocarb (previously filtered through a 0.45 µm nylon filter) of a pH around 4 (by adding HCl), were extracted twice with 20 mL of diethyl ether (free of phenolic antioxidants) by shaking the mixture vigorously for 5 min. The organic phase was separated and evaporated to dryness by passing a nitrogen stream. The general procedure described above was applied to the residue obtained but using in this case a 10 mL volumetric flask and adapting the procedure to this volume.

Results and Discussion

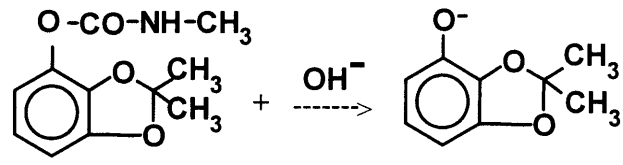
The N-methyl carbamate insecticide Bendiocarb undergoes an alkaline hydrolysis reaction giving rise to its phenolic product (2,3-isopropylidenodioxyphenol [1], Fig. 1). Bendiocarb in aqueous media is not electroactive at the glassy carbon electrode in the + 0.1 to + 1 V potential range, but the hydrolysis product is readily oxidized due to the appearance of the phenolic group.

By varying the hydrolysis time and the sodium hydroxide concentration, it was found that a 0.02 M sodium hydroxide concentration was sufficient to obtain a quasi-instantaneous hydrolysis of Bendiocarb.

With respect to the supporting electrolyte composition, it was found to be convenient to work in the presence of $NaClO_4$ 0.5 M in order to minimize surface fouling by the phenol oxidation product and to increase the reproducibility for successive scans.

Influence of pH

The influence of the pH on the oxidation signal of the hydrolysis product of Bendiocarb was studied in the range of 2.0–5.5 by adding different amounts of acetic acid to the sample after the hydrolysis, and by using DPV and SWV techniques. One peak was detected over the whole pH range investigated. The change in the peak current with the pH was not significant. On the other hand, the peak potential (Ep) shifted linearly to less positive values when increasing the pH and this with similar slopes in DPV and SWV. This suggested the participation of protons in the oxidation process (DPV: $Ep = -0.0489\ pH + 0.891$ and SWV: $Ep = -0.0454\ pH + 0.893$). From these results, we decided



Bendiocarb **2,3-isopropyliden dioxyphenol**

Fig. 1. Hydrolysis of Bendiocarb

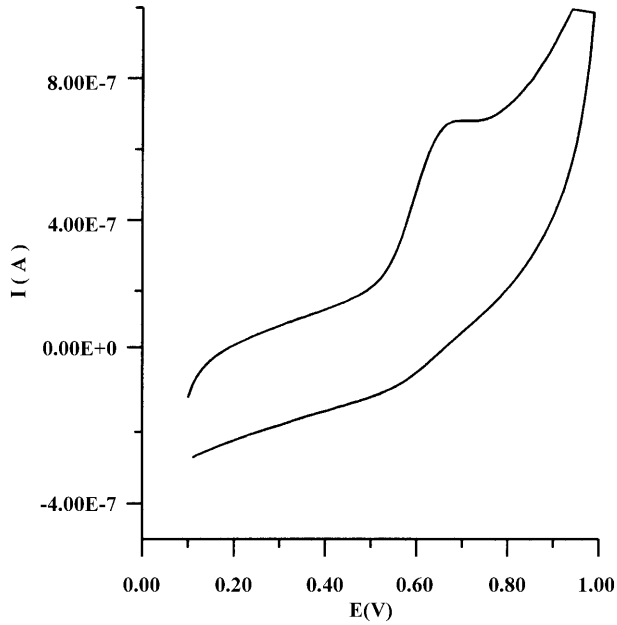


Fig. 2. Cyclic voltammogram of a 2 μg/ml hydrolysed solution of Bendiocarb. Scan rate: 50 mV/s

to select a pH value of 3.5 (by the addition of 0.5 mL of glacial acetic acid) to carry out the subsequent experiments.

Influence of Instrumental Parameters

The influence of the instrumental parameters has been studied in DPV and SWV techniques. In DPV, in the voltage range studied (10–100 mV) a linear increase of peak current with the pulse amplitude (ΔE) was found up to a value of 60 mV. In SWV techniques the influence of some parameters was examined, by varying one of them and holding the others constant. The step potential (ΔEs) was studied in the range 2–10 mV and it was found that the peak current increased linearly up to a value of 10 mV. The frequency (f) was varied between 20 and 100 Hz and the peak current was found to rise linearly up to 100 Hz and in the case of pulse amplitudes (ΔE) between 10 to 100 mV, a linear response was obtained up to 40 mV. From these results and for rapid and sensitive assays, a pulse amplitude of 30 mV in DPV and SWV, a step potential of 8 mV and a frequency of 80 Hz in SWV have been selected.

Nature of the Process

Cyclic voltammetric (CV) studies of the electrode process showed that the phenomenon was irreversible (Fig. 2). From the influence of the scan rate

Table 1. Statistical parameters for the determination of Bendiocarb

	Techniques	
	DPV	SWV
Equation	$Ip(A) = -1.49 \times 10^{-8} + 6.50 \times 10^{-8}\,C$ (μg/ml)	$Ip(A) = -2.32 \times 10^{-8} + 4.74 \times 10^{-7}\,C$ (μg/ml)
$\sigma_{intercept}$	8.87×10^{-9}	3.72×10^{-8}
σ_{slope}	2.92×10^{-9}	1.23×10^{-8}
R^2	0.987	0.995
Detection limits	0.77*	0.44*
	0.41**	0.26**

* Calculated as described by Clayton et al. [29] selecting false positive and false negative probabilities of 0.05.
** Determined as described by Winefordner and Long [30].

Table 2. Study of interferences in the determination of Bendiocarb

$C_{interferent}/C_{bendiocarb}$	Error (%)				
	Ethiofencarb*		Carbofuran*	Fenobucarb**	Pirimicarb
0.25	30[a]	3[b]	9	<2	<1
0.5	46[a]	1[b]	13	12	3
0.75	55[a]	5[b]	18	21	<1
1	64[a]	2[b]	23	22	1
1.25	68[a]	5[b]	25	22	<3

* Negative interference, **positive interference.
[a] Ip measured from valley to valley. [b] values obtained by measuring Ip from base line to Ep values.

(20–600 mV/s) a linearity between peak current and square root of scan rate was found to occur, from which it was deduced that this oxidation process is diffusion controlled.

Voltammetric Determination of Bendiocarb and Analytical Characteristics of the Method

Using the selected conditions already mentioned, a linear relationship was found between Ip and the concentration in the range studied (0.5 to 24 µg/ml). However, taking into account the concentration level of this pesticide that can be found in river water, the calibration graph and the statistical parameters have been calculated for the working range 0.5–5 µg/ml (Table 1). Five different standards solutions were prepared, each one of them in triplicate.

The relative standard deviations (RSD) of the analytical signals were calculated by using the calibration data, being 3% and 2% for 4 µg/ml (for six identical samples) in DPV and SWV, respectively. The detection limits calculated as done by Clayton et al. [29], who select a false positive and a false negative probability of 0.05, and the ones calculated as proposed by Winefordner and Long [30] are also given in Table 1.

Study of Interferences

The influence of the presence of other carbamate insecticides (Fenobucarb, Carbofuran, Ethiofencarb and Pirimicarb) on the peak current (Ip) of the Bendiocarb oxidation peak was investigated. The binary mixtures were prepared by keeping the Bendiocarb concentration constant (1.8 µg/ml) while varying the concentration of the others within the range 0.4–2.4 µg/ml.

In Table 2 the error percentages in the determination of Bendiocarb in the presence of the other pesticides are shown. Under the conditions adjusted for the Bendiocarb determination, only Pirimicarb did not exhibit a signal from 0.4 to 1 V and no interference has been observed.

The others pesticides investigated interfere even at ratios of concentrations less than 1:1 (Table 2). In Fig. 3 (a, b, c) the voltammograms of bendiocarb alone and the voltammograms of each binary mixture (bendiocarb + interferent compound) are shown. They can explain the results of the measurements and the sign of the interferences, according to the relative position of the peaks. It is necessary to highlight the

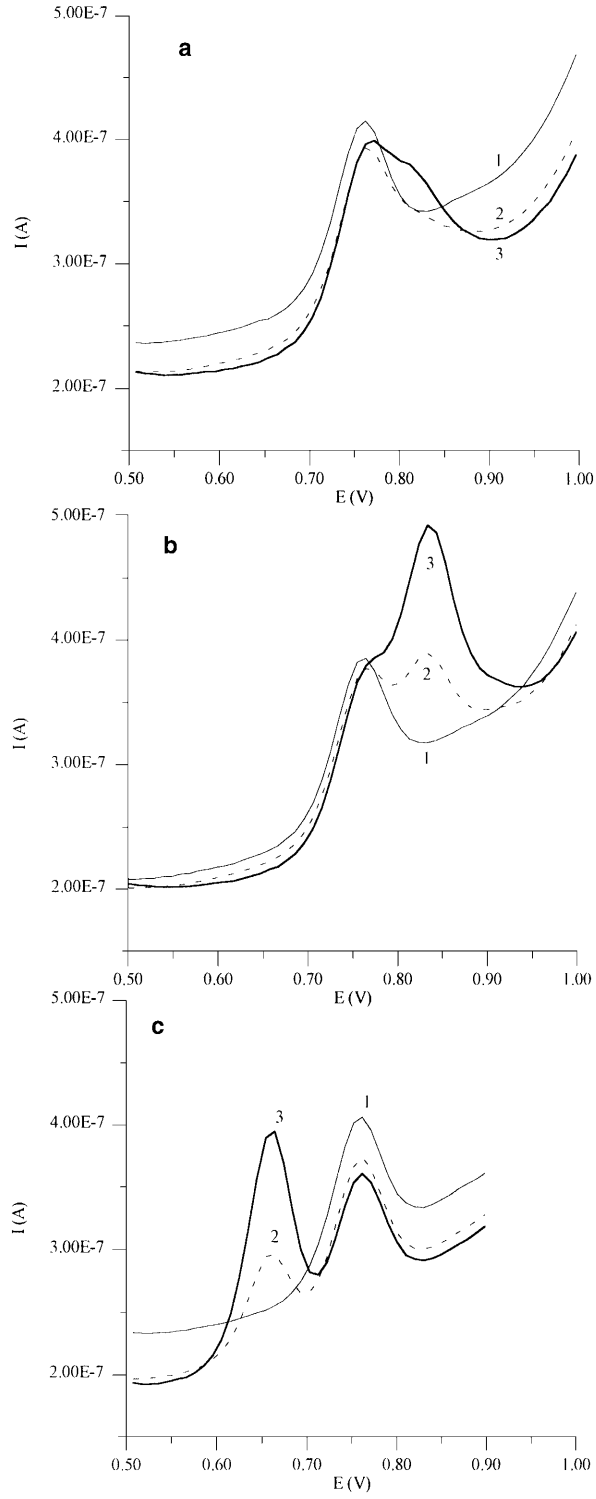


Fig. 3. Voltammograms (DPV). (a) *1* Bendiocarb 1.80 µg/ml; *2* Fenobucarb 0.83 µg/ml + 1.80 µg/ml of Bendiocarb; *3* Fenobucarb 2.2 µg/ml + Bendiocarb 1.80 µg/ml. (b) *1* Bendiocarb 1.80 µg/ml; *2* Ethiofencarb 0.90 µg/ml + bendiocarb 1.80 µg/ml; *3* Ethiofencarb 2.25 µg/ml + Bendiocarb 1.80 µg/ml. (c) *1* Bendiocarb 1.80 µg/ml; *2* Carbofuran 0.88 µg/ml + 1.80 µg/ml of Bendiocarb; *3* Carbofuran 2.21 µg/ml + Bendiocarb 1.80 µg/ml. (scan rate: 10 mV/s; pulse amplitude 30 mV)

case of the ethiofencarb that would not interfere until a concentration ratio of 1:1 if the Ip of bendiocarb is determined for the Ep while considering the baseline level. For further concentration ratios the peaks are overlapped and they cannot be measured separately. On the contrary if the Ip is measured from valley to valley it would interfere at lower ratios.

Determination of Bendiocarb in River Water Samples

The method developed for the determination of Bendiocarb has been applied to the analysis of different samples of river water which were previously spiked with Bendiocarb at different concentrations.

For high sensitivity, a preconcentration step was carried out. The samples were treated and analysed as described under Experimental. Four different samples of spiked river water have been analysed in triplicate by DPV and SWV. For the case of a calibration with external standard samples the results were good with regard both to recovery and repeatability, as shown in Table 3 and in Figure 4. The results obtained have been compared with those of HPLC [16] with UV detection where the same preconcentration step was used and with a mobile phase composition of 40% of acetonitrile-water and detection at 276 nm. We can see that in general also here a good agreement is found (Table 3).

Table 3. Determination of Bendiocarb in spiked river water samples

Bendiocarb added	Bendiocarb found* (recovery ± RSD %)		
(μg/ml)	DPV	SWV	HPLC using UV-detection
0.44	91 ± 2	92 ± 1	98 ± 2
0.34	92 ± 4	89 ± 3	95 ± 1
0.26	88 ± 5	87 ± 4	95 ± 3
0.11	91 ± 2	86 ± 8	96 ± 3

* Each value is the mean of three determinations.

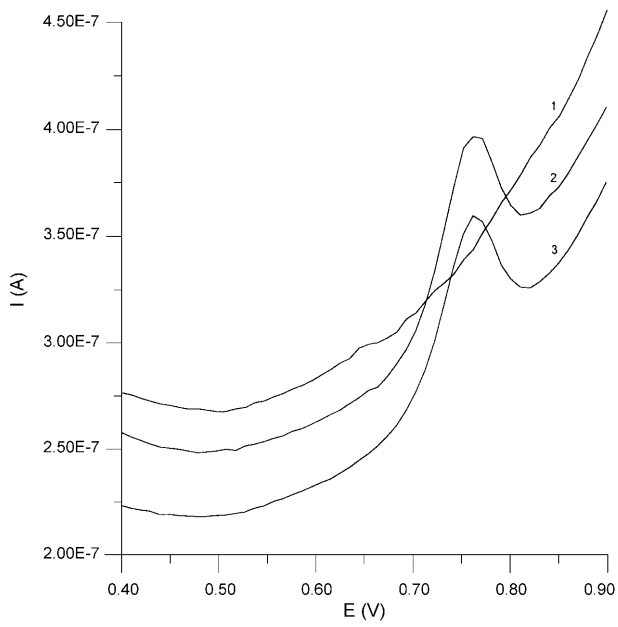


Fig. 4. Differential pulse voltammograms of: *1* Unspiked river water, extracted into diethyl ether according to the proposed procedure. *2* River water spiked with 0.11 μg/ml of Bendiocarb, treated in the same way (final concentration of 1.1 μg/ml). *3* Standard solution of Bendiocarb (1.1 μg/ml). Scan rate: 10 mV/s; pulse amplitude: 30 mV. In all cases the samples are hydrolysed following the procedure mentioned in the text

Conclusions

Two voltammetric procedures have been optimized for the determination of the N-methylcarbamate insecticide Bendiocarb, subsequent to its hydrolysis reaction. For the oxidation of the hydrolysis product one obtains a peak by using DPV and SWV with a peak potential near to + 0.7 V. The electrode process is irreversible and involves the appearance of hydrogen ions. The proposed methods have been applied to determine Bendiocarb in spiked river water samples with acceptable results.

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