Electroanalytical Determination of Oxadiazon and Characterization of Its Base-Catalyzed Ring-Opening Products

E. M. Garrido, J. L. F. C. Lima, C. Delerue-Matos, M. F. M. Borges and *A. M. Oliveira Brett*

Abstract

The electrochemical behavior of the hydrolysis products of oxadiazon was studied by cyclic and square-wave voltammetry using a glassy carbon electrode. Maximum currents were obtained at pH 12.8 in an aqueous electrolyte solution containing 30 % ethanol and the current did not decrease with time showing that there was little adsorption of the reaction products on the electrode surface. The hydrolysis products of oxadiazon were identified, after isolation and purification, as 1-trimethylacetyl-2-(2,4-dichloro-5-isopropoxyphenyl)-2-ethoxy- carbonylhydrazine (Oxa1) and 1-trimethylacetyl-2-(2,4-dichloro-5-isopropoxyphenyl) hydrazine (Oxa2) with redox potentials + 0.6 V and −0.1 V (vs. Ag/AgCl), respectively. Based on the electrochemical behavior of 1-trimethylacetyl-2-(2,4-dichloro-5-isopropoxyphenyl) hydrazine (Oxa2) a simple electroanalytical procedure was developed for the determination of oxadiazon in commercial products used in the treatment of rice crops in Portugal that contain oxadiazon as the active ingredient. The detection limit was 1×10^{-4} M, the mean content and relative standard deviation obtained for seven samples of two different commercial products by the electrochemical method were 28.4 ± 0.8 % (Ronstar) and 1.9 ± 0.2 % (Ronstar GR), and the recoveries were 100.3 ± 5.4 % and 101.1 ± 5.3 %, respectively.

Keywords: Oxadiazon, Herbicides, Square-wave voltammetry, Alkaline hydrolysis

1. Introduction

The herbicide oxadiazon [5-*tert*-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2(3*H*)-one] is a heterocyclic compound that is used to protect rice cultures, since it controls a broad spectrum of weeds and grass. It is a protoporphyrinogen oxidase inhibitor and a contact herbicide. Oxadiazon is stable in neutral or acid media but unstable in alkaline media [1], DT_{50} (half-life) 38 d (pH 9, 25 °C).

The adsorption, translocation and metabolism of oxadiazon has been studied extensively in rice plants. Oxadiazon's major degradation products in rice are carboxylic acids, alcohols, and dealkylated derivatives [2-6]. The most frequently used method for its quantification is gas chromatography [7-13]. Determina- tion by gas-chromatography is highly sensitive and selective, and sufficiently rapid for most purposes.

However, fairly high concentrations of oxadiazon in specific samples, the phytopharmaceutical products available in the Portuguese market, in which this herbicide is the only active compound and which are used to protect rice and corn crops, could be determined by using a simpler, nonpolluting, less time- consuming method provided it is sufficiently selective. It is well known that electroanalytical methods fulfil these requirements.

Oxadiazon is susceptible to base hydrolysis and the compounds, Scheme 1, formed in alkaline solution were isolated, characterized and structurally identified. Their electrochemical oxidation prop- erties have been studied as pure compounds or in a mixture.

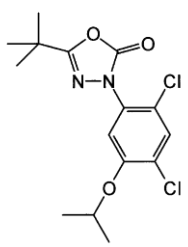
2. Experimental

2.1. Reagents and Solutions

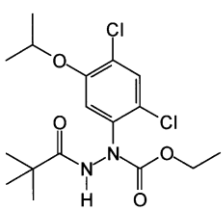
Oxadiazon was from Riedel de Haen. All reagents were analy- tical grade and aqueous solutions were prepared using purified water from a Millipore Milli-Q system (conductivity < 0.1 μS cm^{-1}). A stock solution of oxadiazon was prepared in ethanol at a concentration of 7.11×10^{-3} M. Buffer solutions used were in the pH range 1.2 to 12.8 [14, 15]. Quantitative determi- nations were carried out in a solution containing 30 % ethanol and a pH 12.8 (0.2 M KCl + 0.2 M NaOH) electrolyte solution [15].

2.2. Commercial Sample Preparation

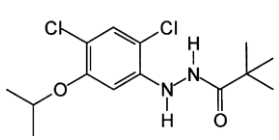
In commercial products oxadiazon is in the form of an emulsion (Ronstar) or a granulated (Ronstar GR) compound.



Oxadiazon



Oxa1



Oxa2

Scheme 1. Chemical structures of Oxadiazon, Oxa1 and Oxa2.

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A sample stock solution of the emulsion product was prepared by accurately weighing 0.5 g and dissolving it in 50.0 mL ethanol. After adding 5.0 mL of this solution to a mixture of
25.0 mL of pH 12.8, 0.2 M aqueous electrolyte buffer solution and 10.0 mL ethanol, oxadiazon was left to react by alkaline hydrolysis for 2½ hours.

A sample stock solution of the granulated product was prepared by accurately weighing 3.0 g, dissolving it in 25.0 mL ethanol and placing in an ultrasonic bath until completely dissolved. The solution was centrifuged and the supernatant solution decanted. After adding 7.0 mL of this solution to a mixture of 25.0 mL of pH 12.8, 0.2 M aqueous electrolyte buffer solution and 10.0 mL ethanol, oxadiazon was left to react by alkaline hydrolysis for 2½ hours.

After complete reaction, the sample stock solution was diluted with buffer electrolyte in order to obtain a concentration within the calibration plot range.

2.3. Apparatus

All experiments were performed using a 663 VA Metrohm system containing a glassy carbon working electrode (Metrohm 6.1204.000) (d = 3.0 mm), a glassy carbon rod counter electrode (Metrohm 6.1247.000) and a Ag/AgCl reference electrode (Metrohm 6.0728.000) attached to a Autolab PSTAT 10 poten- tiostat/galvanostat running with model GPES version 3 software, from Eco-Chemie, Netherlands. The potential range studied was from −0.5 to +1.2 V. The glassy carbon working electrode was polished every day using a polishing kit (Metrohm 6.2802.010) first with c,-Al_2O_3 (0.3 µm) and water during 60 s and after with only water during 60 s. After polishing the electrode surface was thoroughly washed with purified water.

The pH measurements were obtained with a pH-meter E 520 from Metrohm with a combined glass electrode (Metrohm

6.0202.000
).

Melting points were measured using a Kofler microscope.

Infrared spectra were recorded on an ATI Mattson Genesis Series FTIR spectrophotometer, using potassium bromide disks (Uvasol, Merck); only the most significant absorption bands are reported (vmax, cm^{-1}). ^1H and ^{13}C NMR (^1H decoupled) spectra

were acquired at room temperature on a Bruker AMX 300

instrument operating at 300.13 and 75.47 MHz, respectively. Chemical shifts are expressed as b(ppm) values relative to tetramethylsilane (TMS) as internal reference and coupling constants (J) are given in Hz; DMSO-d6 was used as sample solvent. Assignments were also made from DEPT (distortionless enhancement by polarization transfer) (underline values). Elec- tron impact mass spectra (EI-MS) were carried out on a VG AutoSpec instrument and data are reported as m/z (% of relative intensity of the most important fragments).

Thin-layer chromatography (TLC) was carried out on alumi- num sheets precoated with silica gel 60F254 with layer thickness of 0.2 mm (E. Merck). Chromatographic systems used were petroleum ether/diethyl ether (5 : 5) and chloroform/methanol (8 : 2). The spots were visualized by UV detection (254 nm) and iodine vapor. Purification of the compounds was performed by column chromatography (CC) using silica gel 60 (0.2-0.5 mm,
E. Merck) and petroleum ether/diethyl ether (7 : 3) and preparative thin-layer chromatography (PTLC) (silica gel F254, petroleum ether/diethyl ether (5 : 5)). Solvents were evaporated in a Buchi Rotavapor. Petroleum ether was of boiling range 40-60 °C.

2.4. Alkaline Hydrolysis of Oxadiazon

Oxadiazon (500 mg) was dissolved in 80 mL of ethanol and 50 mL of electrolyte (pH 12.8). The solution was stirred at room temperature in a one-neck flask equipped with a magnetic bar. After complete disappearance of oxadiazon (TLC), an aliquot of the solution was used for electrochemical studies (see Section 3). The remaining solution was neutralized by addition of HCl (15 %) and extracted with ethyl ether (3 x 100 mL). The combined organic phases were washed with H_2O and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product was prepurified by CC. After solvent evaporation the residue was purified by PTLC. 1-Trimethylace- tyl-2-(2,4-dichloro-5-isopropoxyphenyl)-2-ethoxycarbonylhydra- zine (Oxa1) and 1-trimethylacetyl-2-(2,4-dichloro-5- isopropoxyphenyl)hydrazine (Oxa2) were obtained as white crystals. Oxa1 was obtained as a diastereomeric mixture (see NMR).

2.5. Spectral Data

2.5.1. Oxadiazon

^1H NMR b: 7.85 (1H, *s*, C<u>H</u>Ar), 7.59 (1H, *s*, C<u>H</u>Ar), 4.67
(1H, *m*, OC<u>H</u>$(CH_3)_2$), 1.30 (15H, *hrs*, CH($\underline{H}_3$)$_2$, C(C$\underline{H}_3$)$_3$). ^{13}C
NMR b: 162.6, 152.4, 151.8, 131.6, <u>1</u>30.6, 124.5, 122.3, <u>1</u>15.9,
<u>7</u>2.3, 32.4, <u>2</u>6.6, <u>2</u>1.5. Other data are similar to those described in
the literature [4, 5].

2.5.2. 1-Trimethylacetyl-2-(2,4-dichloro-5-isopropoxyphe- nyl)-2-ethoxycarhonylhydrazine (Oxa1)

Mp 88-91 °C. IR vmax: 3485 (N-H), 2977, 1739 (NH-
C=O), 1691(C=O), 1560, 1479, 1398, 1375, 1329, 1277, 1238,
1188, 1138, 1105, 1066, 1018, 962, 750, 659. EI-MS m/z (%):
394 (M + 4, 7) · 393 (M + 3, 8) · 392 (M + 2, 42) ·, 391 (M + 1, 17)$^{+\cdot}$, 390 (M, $^{+\cdot}$, 358 (7), 357 (21), 356 (21), 355
(84), 349 (7), 348 (17), 347 (12), 346 (24), 322 (12), 321 (12),
320 (11), 319 (23), 318 (86), 280 (10), 279 (9), 278 (61), 277
(16), 276 (87), 237 (7), 236 (6), 235 (37), 234 (11), 233 (65), 207
(9), 206 (5), 205 (37), 204 (9), 203 (65), 195 (16), 194 (46), 193
(80), 192 (70), 191 (100), 85 (38), 57 (70). ^1H NMR b: 10.50

(1H, *s*, NH, exchanged with D_2O), 10.41 (1H, *s*, NH, exchanged with D_2O), 7.63 (2H, *s*, CHAr), 7.28, 7.24 (2H, *s*, CHAr), 4.45 (2H, *m*, $CH(CH_3)_3$), 4.11 (4H, *q*, OCH_2CH_3), 1.29, 1.27 (12H, *d*, *J* = 6, $CH(CH_3)_2$), 1.14 (24H, *hrs*, $C(CH_3)_3$, OCH_2CH_3). ^{13}C NMR b 177.4, 151.9, 139.1, 129.9, 122.6, 121.8, 114.2, 71.8, 62.2, 37.5, 26.8, 21.4, 14.3.

2.5.3. 1-Trimethylacetyl-2-(2,4-dichloro-5-isopropoxyphenyl)hydrazine (Oxa2)

Mp 98-103ºC. IR vmax: 3336, 3226 (N-H), 2974, 1664 (C=O), 1593, 1535, 1481 (C-CAr), 1394, 1275, 1180, 1180, 1107, 856, 744, 704. EI-MS *m/z* (%): 322 $(M + 4, 8)^{+\cdot}$, 321 $(M + 3, 7)^{+\cdot}$, 320 $(M + 2, 49)^{+\cdot}$, 319 $(M + 1, 14)^{+\cdot}$, 318 (M, $64)^{+\cdot}$, 280 (5), 279 (4), 278 (21), 277 (6), 276 (31), 196 (7), 195 (6), 194 (39), 193 (17), 192 (56), 191 (19), 149 (12), 85 (21), 57 (100). ^1H NMR b: 9.69 (1H, *d*, *J* = 3, NH, exchanged with D_2O), 7.47 (1H, *d*, *J* = 3, NH, exchanged with D_2O), 7.35 (1H, *s*, C*H*Ar), 6.34 (1H, *s*, CHAr), 4.40 (1H, *m*, $CH(CH_3)_2$), 1.27 (6H, *d*, *J* = 6, $CH(CH_3)_2$), 1.19 (9H, *hrs*, $C(CH_3)_3$). ^{13}C NMR b: 176.9, 152.4, 145.1, 129.4, 111.4, 108.6, 99.7, 71.3, 37.5, 27.2, 21.6.

3. Results and Discussion

3.1. Electrochemical Study of Oxadiazon in Solution

Although no electroactive behavior was seen for oxadiazon, two oxidation peaks appeared after 2½ hours in pH 12.8, 0.2 M aqueous electrolyte buffer solution with 30 % ethanol, corre- sponding to its alkaline hydrolysis products. They were studied by cyclic and square-wave voltammetry using a glassy carbon electrode. Maximum currents were obtained at this pH. The currents did not decrease with time so there was little adsorption of the reaction products on the electrode surface.

Since there was clear evidence of a homogeneous reaction taking place in solution it was decided to investigate the chemical stability of oxadiazon which is described below. In fact, oxa- diazon in solution suffers alkaline hydrolysis and leads to two compounds which were isolated, purified and structurally char- acterized as Oxa1 and Oxa2 (Scheme 1). Their electrochemical oxidation was studied by square-wave voltammetry (Figure 1) and occurs at + 0.6 V and —0.1 V for Oxa1 and Oxa2, respectively. Cyclic voltammetry showed an irreversible oxida- tion reaction for both (Figure 2) with linear plots of peak current against square root of scan rate, showing that the reaction is of solution-soluble species.

The electrochemical oxidation of Oxa1 in solution presented one oxidation peak at + 0.6 V immediately after solution preparation (Figure 3). However, the oxidation peak of oxa- diazon decreased with time and after one day two peaks with similar intensity appeared at —0.1 and + 0.6 V, respectively Oxa2 and Oxa1 (Figure 3). Consequently it was obvious that the homogeneous reaction of alkaline hydrolysis of the ester compound Oxa1 (see below) was taking place in solution and could be followed by electron transfer reaction of the products.

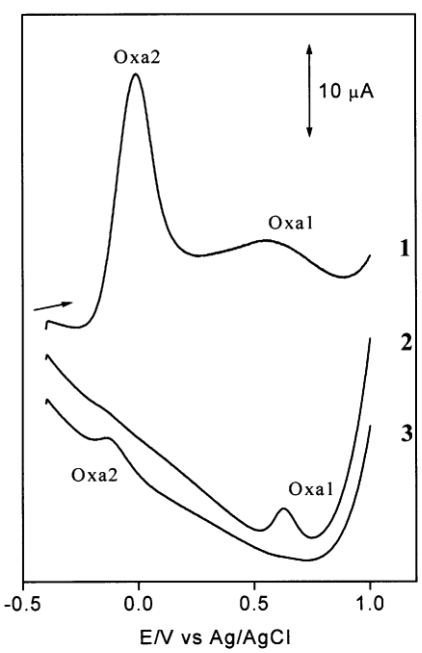

Fig. 1. Square-wave voltammograms in a solution of 30 % ethanol and pH 12.8, 0.2 M aqueous electrolyte buffer. Frequency 50 Hz, scan increment 1 mV and pulse height 50 mV: 1) 9.05×10^{-4} M oxadiazon after 2½ hours in solution; 2) 4.54×10^{-4} M Oxa1; 3) 1.25×10^{-4} M Oxa2.

After a day the peak currents were unchanged and a plot of I_{pOxa1}/I_{pOxa2} with time showed that there was no further reac- tion in solution.

3.2. Study of Chemical Stability of Oxadiazon in Solution

In order to understand the electrochemistry of oxadiazon's alkaline hydrolysis products, the reaction was carried out at room temperature under the same experimental conditions as for electrochemical studies. The products were identified by other techniques. Studies were first performed with an ethanolic aqueous buffer solution of oxadiazon, 9.05×10^{-4} M (pH 12.8), which was stirred at room temperature. During the reaction the presence of the starting material ($R_f = 0.96$) was observed by TLC as well as the formation of two compounds, which were named as Oxa1 ($R_f = 0.57$) and Oxa2 ($R_f = 0.47$). After 2½ hours complete disappearance of oxadiazon was observed.

To characterize the products the reaction was scaled up (see Section 2). When the reaction was complete an aliquot (25.0 mL) was withdrawn and studied by square-wave voltammetry. The same electrochemical behavior was observed as in the preli- minary electrochemical studies (Figure 1) which led to the conclusion that the two oxidation peaks could be explained by the compounds formed in the solution.

After isolation, purification and structural characterization of the compounds formed in solution, they were identified as 1-trimethylacetyl-2-(2,4-dichloro-5-isopropoxyphenyl)-2-ethoxy- carbonylhydrazine (Oxa1) and 1-trimethylacetyl-2-(2,4-dichloro- 5-isopropoxyphenyl)-hydrazine (Oxa2) (Scheme 1).

It was concluded that oxadiazon is a heterocyclic compound which is susceptible to base hydrolysis with ring-opening and the formation of hydrazine compounds. The mechanism that is most consistent with the data obtained is shown in Scheme 2, which involves nucleophilic attack of hydroxide ion on the carbonyl group, with the formation of a tetrahedral intermediate and in situ formation of a carboxylic anion, which after ethylation gives

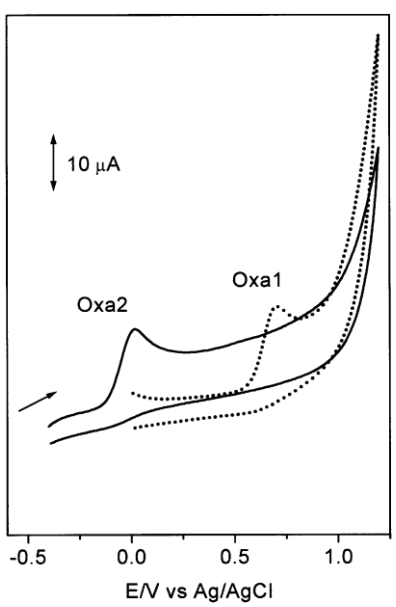

Fig. 2. Cyclic voltammograms of 1.0×10^{-3} M Oxa1 and Oxa2, in a solution of 30 % ethanol and pH 12.8, 0.2 M aqueous electrolyte buffer. Scan rate 100 mV s^{-1}.

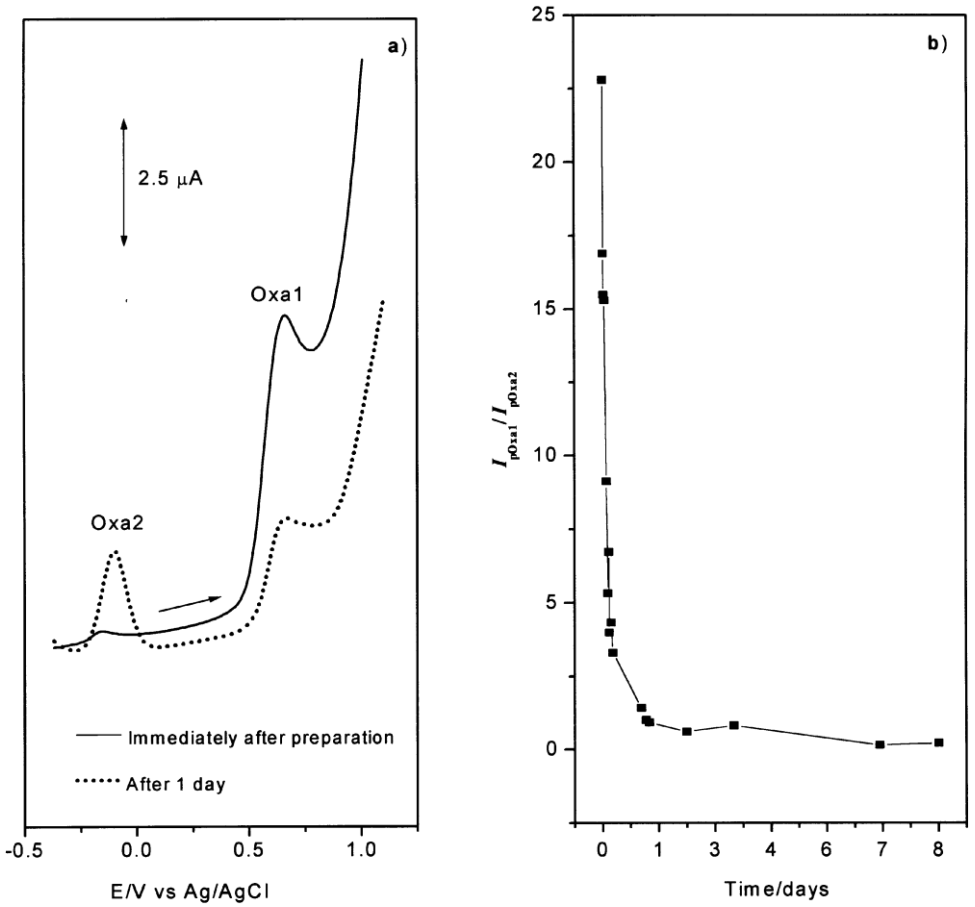

Fig. 3. Square-wave voltammograms of 2.4×10^{-3} M Oxa1 in a solution of 30% ethanol and pH 12.8 0.2 M aqueous electrolyte buffer. Frequency 50 Hz, scan increment 1 mV and pulse height 50 mV: a) (--) immediately after preparation and (· · · · ·) after one day; b) Plot of I_{pOxa1}/I_{pOxa2} vs. time.

Oxa1 and after decarboxylation leads to Oxa2. Decarboxylation of this type of intermediates is a rapid process, as proposed in the literature for similar compounds, which occurs with the displa- cement of the carboxyl group as carbon dioxide [16-18].

Although the identified compounds have not been previously described as by-products of alkaline hydrolysis of oxadiazon, Oxa2 and an analogue of Oxa1 (methyl ester derivative) were referred to in the literature as metabolites of oxadiazon in rice plants [2-4].

3.3. Electroanalytical Determination of Oxadiazon in a Commercial Product

The oxidation of Oxa2 occurs at −0.1 V and this compound, unlike Oxa1, was found to be very stable in solution. For this reason it was chosen for the indirect electroanalytical quantifi- cation of oxadiazon in commercial products that contain only oxadiazon as the active ingredient. Square-wave voltammo- grams obtained for Oxa2 in sample and standards are shown in Figure 4.

The sample and calibration standards, between 1.3×10^{-4} e 2.7×10^{-4} M were prepared according to the description in Section 2. The calibration plot gave a straight line ($r = 0.997$, $n = 5$). The detection limit was 1×10^{-4} M (3 × background noise amplitude). The values of the mean content and relative standard deviation obtained for seven samples of the two commercial products by the electrochemical method were 28.4±0.8% (Ronstar) and 1.9±0.2% (Ronstar GR), and the recoveries were 100.3±5.4% and 101.1±5.3%, respectively.

4. Conclusions

An indirect electroanalytical method can be successfully used to quantify the herbicide oxadiazon in a commercial product. The electrochemically developed method is simple, enables faster determinations and also permits the study of the homogeneous alkaline hydrolysis mechanism of oxadiazon which involves the formation of two electroactive compounds.

5. Acknowledgements

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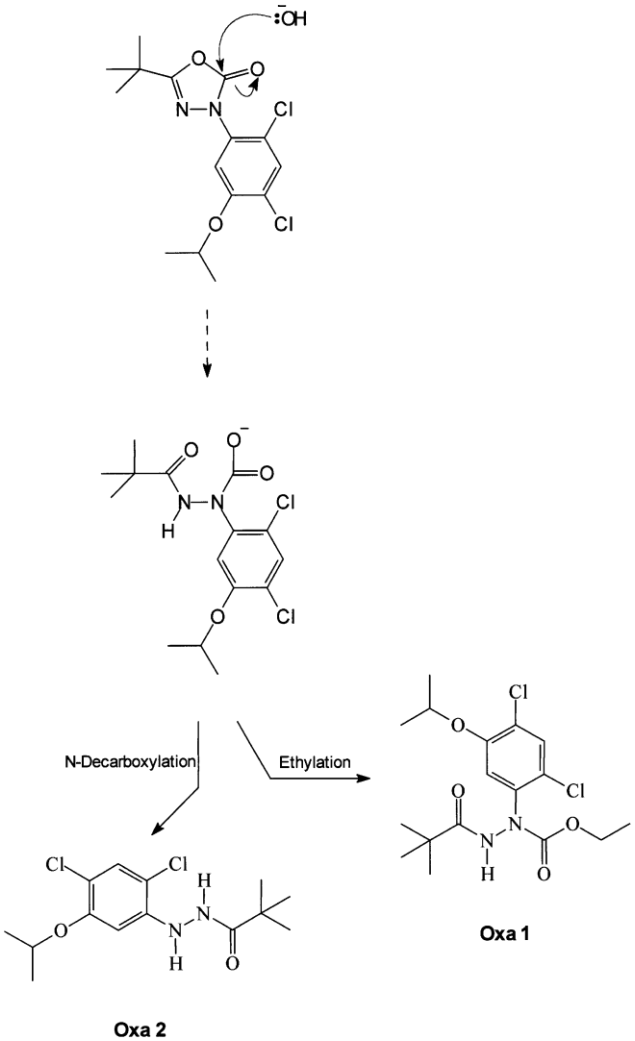

Scheme 2. Proposed mechanism for the base catalyzed hydrolysis of oxadiazon.

6. References

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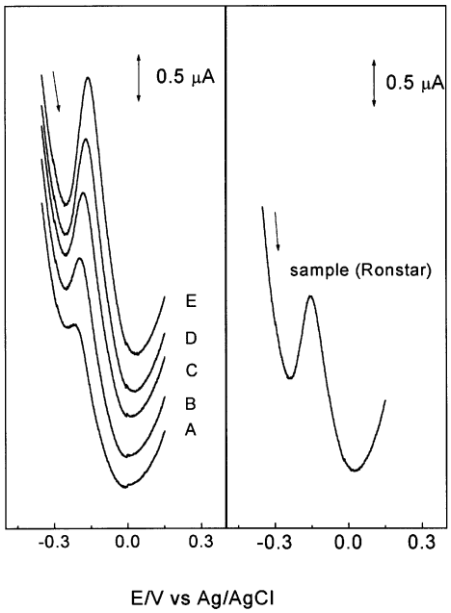

Fig. 4. Square-wave voltammograms of oxadiazon in a solution of 30 % ethanol and pH 12.8 0.2 M aqueous electrolyte buffer. Frequency 50 Hz, scan increment 1 mV and pulse height 50 mV. a) Successive additions of standard solutions for constructing the calibration plot; A: 1.30×10^{-4}, B: 1.702×10^{-4}, C: 2.098×10^{-4}, D: 2.434×10^{-4}, E: 2.720×10^{-4} M.
b) Commercial sample solution.

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