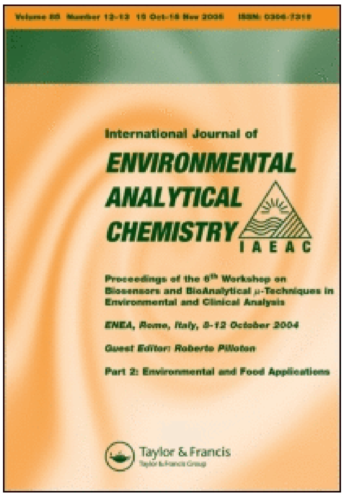

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Electrochemical Behaviour and Square Wave Voltammetry of the Rice Herbicides Molinate, Bensulfuron-Methyl, Mefenacet and Thiobencarb

E. Manuela Garrido[a][b]; Josél Costa Lima[a]; Cristina M. Delerue-matos[a]; Ana Maria Oliveira Brett[c]
[a] CEQUP/Departamento de Química-Fisica, Faculdade de Farmdcia, Universidade do Porto, Porto, Portugal [b] Institute Superior de Engenharia do Porto, Potto, Portugal [c] Departamento de Química, Faculdade de Ciěncias e Tecnologia, Universidade de Coimbra, Coimbra, Portugal

To cite this Article Garrido, E. Manuela , Lima, Josél Costa , Delerue-matos, Cristina M. and Brett, Ana Maria Oliveira(1999) 'Electrochemical Behaviour and Square Wave Voltammetry of the Rice Herbicides Molinate, Bensulfuron-Methyl, Mefenacet and Thiobencarb', International Journal of Environmental Analytical Chemistry, 75: 1, 149 — 157
To link to this Article: DOI: 10.1080/03067319908047307
URL: http://dx.doi.org/10.1080/03067319908047307

Intern. J. Environ. Anal. Chem., Vol. 75(1-2), pp. 149-157
Reprints available directly from the publisher
Photocopying permitted by license only

ELECTROCHEMICAL BEHAVIOUR AND SQUARE WAVE VOLTAMMETRY OF THE RICE HERBICIDES MOLINATE, BENSULFURON-METHYL, MEFENACET AND THIOBENCARB

E. MANUELA GARRIDO[a*], JOSÉ L. COSTA LIMA[a],
CRISTINA M. DELERUE-MATOS[a] and ANA MARIA OLIVEIRA BRETT[b†]

[a]*CEQUP/Departamento de Química-Física, Faculdade de Farmácia, Universidade do Porto, 4050 Porto, Portugal and* [b]*Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3000 Coimbra, Portugal*

(Received 26 November 1998; In final form 22 February 1999)

Cyclic, differential pulse and square wave voltammetry were applied to develop an electroanalytical procedure for the determination of a group of herbicides used to treat rice crops: molinate, bensulfuron-methyl, mefenacet and thiobencarb. The oxidation mechanism causes adsorption problems and consequent poisoning of the electrode surface by the products of the electrochemical reaction. Parameters such as pH, frequency and electrochemical electrode surface treatment were optimized. The analytical methodology developed using square wave voltammetry was applied to the determination of molinate and bensulfuron-methyl in simple or mixed commercial products.

Keywords: Molinate; bensulfuron-methyl; mefenacet; thiobencarb; herbicides; square wave voltammetry

INTRODUCTION

Molinate, bensulfuron-methyl, mefenacet and thiobencarb are herbicides used in Portugal in the treatment of rice crops. The toxicity of these compounds and the possibility of contamination of rivers and lakes or fish farms in close proximity to the rice crops explains the search for simple analytical methods for their determination.

---

[*] Permanent address: Instituto Superior de Engenharia do Porto, 4200 Porto, Portugal.

[†] Corresponding author. FAX: +351–39–835295, E-mail brett@cygnus.ci.uc.pt

Molinate [S-ethyl azepane-1-carbothioate] and thiobencarb, [S-4-chlorobenzyl diethylthiocarbamate] belong to the group of thiocarbamates herbicides. Molinate inhibits lipid metabolism whereas thiobencarb is a protein synthesis inhibitor[1]. Current methods for their analysis in aqueous or solid samples generally involve separation techniques such as gas[2–12], liquid[12–14], supercritical[15] and thin layer chromatography[16].

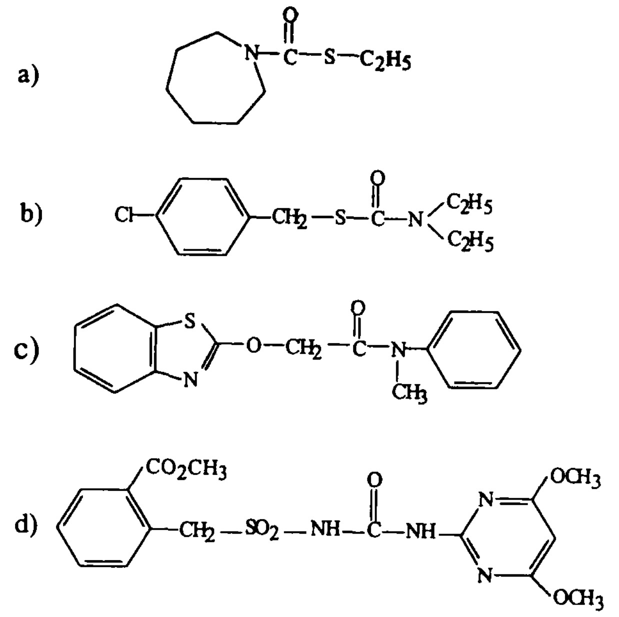

FIGURE 1 Structures of a) molinate, b) thiobencarb, c) mefenacet and d) bensulfuron-methyl

Bensulfuron-methyl, [α-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-*o*-toluic acid], belongs to the group of sulphonylureas which have an important role in agriculture and are rapidly and efficiently metabolized by plants, animals and soils. It inhibits acetolactate synthase, which is necessary for the synthesis of the amino acids valine and isoleucine required for plant growth[1]. Due to their polarity and chemical instability the analytical determination of sulphonylureas is a big challenge. Since they suffer thermal degradation at the high temperatures used in gas chromatography and mass spectrometry[17], the use of these techniques for their determination is not possible. The most common used method for the determination of bensulfuron-methyl is liquid chromatography with spectrophotometric [18; 19] or photocondutivity[20] detection.

Mefenacet, [2-(1,3-benzothiazol-2-ylowy)-N-methylacetanilide], inhibits cell division and growth[1] and can be determined in water by high performance liquid chromatography (HPLC) with spectrophotometric detection or gas chromatography with mass spectrometric detection[21].

In this work the electrochemical oxidation of molinate, thiobencarb, bensulfuron-methyl and mefenacet (Figure 1) was studied and an electroanalytical method was developed for their determination in commercial products used to protect rice and corn crops. Of the four herbicides studied another methodology (AOAC)[2] only exists for molinate for comparison of its doseage in phytopharmaceutical products; this has been used to validate the new electroanalytical methodology.

EXPERIMENTAL

Apparatus

All experiments were performed using a 663 VA Metrohm system containing a glassy carbon working electrode (Metrohm 6.1204.040) ($d = 3.0$ 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 potentionstat/galvanostat running with model GPES version 3 software, from Eco-Chemie, Netherlands. The potential range studied was from +0.0 to +1.9 V.

The glassy carbon working electrode was polished every day using a polishing kit (Metrohm 6.2802.010) first with α-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 made with a pH-meter E 520 from Metrohm using a combined glass electrode (Metrohm 6.0202.000).

Experiments for the AOAC Method[2] were done using a gas chromatograph Chrompack, model Cp 9000, with flame ionization detector and a column Chrompack 3% silicone OV-17 on Gas Chrom Q 60–80 mesh of 1.8m × ¼" × 4mm SS.

Reagents and solutions

Molinate, thiobencarb, bensulfuron-methyl and mefenacet were from Riedel de Haen. All reagents were analytical grade and aqueous solutions were prepared using purified water from a Millipore Milli-Q system (conductivity < 0.1 μS cm^{-1}). Stock solutions were prepared in acetonitrile at concentrations of 5.4×10^{-2} M for molinate, 1.2×10^{-2} M for thiobencarb, 5.7×10^{-3}M for bensulfuron-methyl and 4.8 × 10^{-3} M for mefenacet. Supporting electrolytes were 0.3M Britton-Robinson buffer solutions in the pH range 1.9–11.9[22] and 0.2 M KCl + 0.2 NaOH was used at pH 12.1[23]. The sample stock solution was diluted in the electrochemical cell with buffer electrolyte in order to obtain a concentration within the calibration curve range. Quantitative determinations were carried out at pH 1.9 for molinate, thiobencarb and mefenacet and at pH 12.1 for bensulfuron-methyl. Due to the difference in pH the maximum percentage of aqueous buffer to still ensure the solubility of the herbicides is 83% for thiobencarb and 50% for bensulfuron-methyl. All solutions were kept at the most from one day to the next in the dark at 4 °C.

Commercial sample preparation

Molinate, thiobencarb, bensulfuron-methyl and mefenacet are constituents of herbicide formulations available on the Portuguese market. Three commercial products with different relative proportions[24; 25] were analysed: (*I*) molinate; (*II*) molinate + bensulfuron-methyl [1:0.01 (w/w)], (*III*) and molinate + bensulfuron-methyl + mefenacet [1:0.02:0.3 (w/w)].

Molinate samples are granular and the sample stock solution was prepared by accurately weighing 0.91 g of the commercial sample which was first ground finely and after adding 50.00 mL acetonitrile the flask was immersed in an ultrasonic bath for 15 minutes. Different volumes from the supernatant liquid were diluted with buffer electrolyte pH = 1.9 in order to obtain a concentration within the calibration curve range.

The commercial herbicides that contained bensulfuron-methyl (*II* e *III*) were also granular, and the sample was prepared by weighing 2.90 g of the commercial sample which was ground and added to 20.00 mL acetonitrile; the flask was then immersed in an ultrasonic bath for 20 minutes at a temperature below 20°C. The solution was centrifuged and different volumes of the supernatant liquid were diluted with buffer electrolyte pH = 12.1 in order to obtain a concentration within the calibration curve range.

RESULTS AND DISCUSSION

Cyclic voltammetry

The oxidation of these herbicides was first studied by cyclic voltammetry so as to probe the oxidation mechanism, which proved to be irreversible for all of them

(Figure 2). The oxidation peak at pH = 1.9 occurred for molinate and mefenacet at +1.6V and for thiobencarb at +1.7 V. However, for bensulfuron-methyl in pH 12.1 solution, the oxidation peak was at +1.0 V. A plot of I vs. v½ is a straight line passing through the origin, indicating that the oxidation is of the soluble species for all these herbicides. Nevertheless, the products of oxidation adsorb very strongly on the electrode surface causing poisoning of the electrode and irreproducibility.

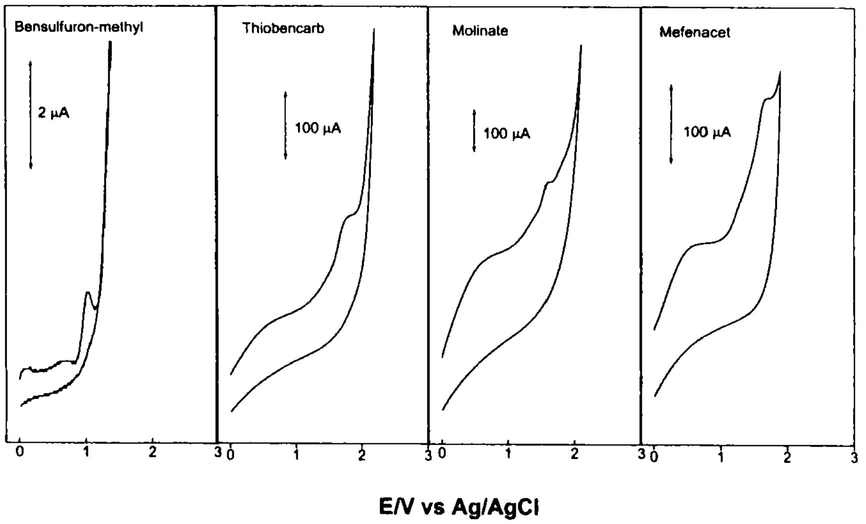

FIGURE 2 Cyclic voltammograms in pH 12.1 of 2.1×10^{-4}M bensulfuron-methyl and in pH=1.9 of 4.6×10^{-4}M thiobencarb, 1.5×10^{-3} M molinate and 6.8×10^{-5}M mefenacet. Scan rate 100 mV/s

In the potential range for the oxidation peak potentials of molinate, thiobencarb and mefenacet between +1.4 and +1.7 V vs. Ag/AgCl, the background currents are high. These were significantly reduced by electrochemically treating the electrode. Very good results concerning the reproducibility of the peak and peak definition were obtained when a potential of −1.5 V was applied to the electrode during 1 second before each scan. This procedure was used throughout all the experiments for all the herbicides.

The effect of pH

The electrochemical behaviour of this group of herbicides was studied over a large pH range between 1.9 and 11.9, using differential pulse voltammetry, except for bensulfuron-methyl for which it was necessary to use square wave voltammetry.

First, for molinate and mefenacet the results showed that for solutions with pH values between 1.9 and 4.0 there was no variation in the peak potential, E_p, which occurs at +1.5 V vs. Ag/AgCl. For higher pH values the peak definition was very poor. The next herbicide studied was thiobencarb and the peak potential, E_p, occurred at +1.7 V and was pH independent between 1.9 and 4.0. Finally, for bensulfuron-methyl the peak potential, E_p, varies linearly with pH, with a slope of 70 mV which suggests a one electron one proton transfer oxidation mechanism. Plots of I_p vs. pH for all the herbicides showed that higher currents are observed for pH = 1.9. This is in agreement with the described mechanisms of anodic oxidation of thiocarbamates and carbamates[26, 27] involving a one electron transfer with the formation of a cation radical intermediate which then dimerises.

The nature of the oxidation products and the number of electrons involved can be predicted by comparison with data in the literature and two references have been added and the text has been improved

Electroanalytical determination of the herbicides

The electroanalytical determination of molinate, thiobencarb, bensulfuron-methyl and mefenacet in commercial products is not easy without previous chromatographic separation since their oxidation peaks occurred for pH = 1.9 at very close values of potential. However, by careful selection of the experimental conditions, sample solubility in the supporting electrolyte, relative proportions in the commercial products and pH, it was possible to quantify molinate in samples *I* and *II* and bensulfuron-methyl in samples *II* and *III*.

In Table I are shown the results for 7 determinations of each sample of molinate and bensulfuron-methyl showing the mean (% w/w) and standard deviation and also, for molinate, the comparison with the results obtained by the AOAC method[2] and recovery data. As the oxidation products absorb strongly on the electrode surface, square wave voltammetry with electrochemical cleaning was the procedure used for the analytical determinations in the commercial products.

The sample and molinate calibration standards, between 4.96×10^{-4} and 9.02×10^{-4} M were prepared according to the description in the experimental section. Square wave voltammograms obtained for sample and standards are shown in Figure 3; the calibration plot exhibited a straight line ($r = 0.996$, n = 5). Molinate was always determined at pH = 1.9. In fact in compound *II* the % w/w of molinate is 100 times higher than that of bensulfuron-methyl. At these pH values bensulfuron-methyl has a low solubility so does not interfere in the determination.

The accuracy of these electrochemical results was assessed by comparing with those obtained with the AOAC method (Table I). This reference method uses gas chromatography for the determination of molinate in commercial products, with the internal standard cycloate. The relative errors were 1.5 %.

TABLE I Determination of molinate and bensulfuron-methyl, using the voltammetric method (VM), the reference method (RM) and recovery

Sample	*Molinate*			*Bensulfuron-methyl*
	VM (%w/w)	*RM (%w/w)*	*Recovery(%)*	*VM(%w/w)*
I[a]	7.38 ± 0.12 (1.6%)[b]	7.28 ± 0.12 (1.6%)[b]	100.2 ± 2.1 (2.1%)[b]	
II[a]	7.90 ± 0.08 (1.0%)[b]	7.78 ± 0.03 (0.3%)[b]	101.8 ± 4.2 (4.1%)[b]	0.0826 ± 0.0074 (8.9%)[b]
III[a]				0.0703 ± 0.0060 (8.6%)[b]

[a] Samples of different phitopharmaceutical preparations:
I-Molinate; *II*- Molinate + Bensulfuron-methyl; *III*- Molinate + Bensulfuron-methyl + Mefenacet.
[b] Mean, standard deviation and variation coefficient of seven determinations

The determination of bensulfuron-methyl in compounds *II* and *III* was done at pH = 12.1. At this pH neither molinate nor mefenacet showed any oxidation peaks. The sample and the calibration standard solutions, 3.22×10^{-5} and 1.57×10^{-4} M, were prepared according to the description in the experimental section. The square wave voltammograms obtained for both are shown in Figure 3. The calibration plot showed a straight line ($r = 0.996$, n=5).

Concerning thiobencarb and mefenacet, that occur always together with molinate in the commercial products, they have very similar oxidation peak potentials so they are electrochemical interferents. They were studied separately and the voltammetric conditions were similar to those of molinate. In Figure 3 are shown the square wave voltammograms obtained for mefenacet between 1.13×10^{-5} M and 6.17×10^{-5} M, and for thiobencarb between 3.70×10^{-5} and 4.41×10^{-4} M. Calibration plots showed straight lines ($r = 0.995$, n = 5) for mefenacet and (r= 0.996 , n=5) for thiobencarb.

CONCLUSIONS

Electrochemical methods can be successfully used to quantify the herbicides molinate, bensulfuron-methyl, mefenacet and thiobencarb. The method devel-

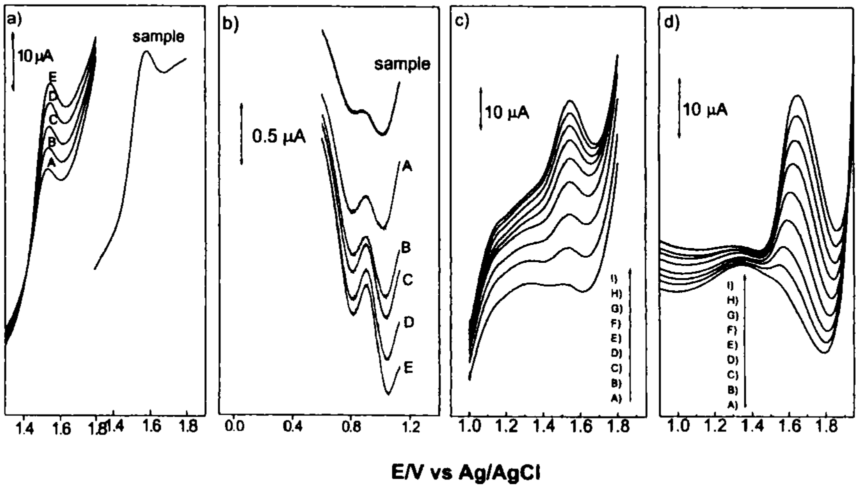

FIGURE 3 Square wave voltammograms, frequency 50 Hz, pulse amplitude 50 mV of **a)** molinate in pH=1.9 of standards of molinate (A-4.96 × 10^{-4}, B-5.98 × 10^{-4}, C-7.00 × 10^{-4}, D-8.01 × 10^{-4}, E-9.02 × 10^{-4} M) and a commercial sample; **b)** bensulfuron-methyl in pH=12.1 successive addition of standard solutions to the sample for constructing the calibration plot (A-3.22 × 10^{-5}, B-6.41 × 10^{-5}, C-9.56 × 10^{-5}, D-1.27 × 10^{-4}, E-1.57 × 10^{-4} M); **c)** of mefenacet in pH=1.9 (A-1.51 × 10^{-6}, B-3.49 × 10^{-6}, C-5.41 × 10^{-6}, D-6.86 × 10^{-6}, E-8.14 × 10^{-6}, F-9.46 × 10^{-6}, G-1.06 × 10^{-5}, H-1.14 × 10^{-5}, 1.28 × 10^{-5}M); **d)** thiobencarb (A-3.70 × 10^{-5}, B-7.38 × 10^{-5}, C-1.10 × 10^{-4}, D-1.71 × 10^{-4}, E-2.31 × 10^{-4}, F-2.90 × 10^{-4}, G-3.48 × 10^{-4}, H-4.07 × 10^{-4}, I-4.41 × 10^{-4} M). Frequency 50 Hz, pulse amplitude 50 mV

oped is simple and also permits the study of the oxidation mechanism which leads to strongly adsorption of oxidation products on the electrode surface. A comparative evaluation of the data for molinate showed that the results obtained electroanalytically were in good agreement with those obtained by the AOAC chromatographic method. It was possible to determine molinate and bensulfuron-methyl in the same commercial product without separation techniques by optimizing the pH and solubility conditions.

Acknowledgements

Junta Nacional de Investigação Cientifica e Tecnológica (JNICT) is thanked for financial support through project PEAM/SEL/516/95, Fundo de Apoio à Investigação do Instituto Superior de Engenharia do Porto for financial support through project P-10/98, and PRODEP for a research grant (E. Manuela Garrido).

References

[1] British Crop Protection Council, *The Pesticide Manual* (ed CDS Tomlin, 1997), 11th ed.
[2] AOAC *Association of Official Analytical Chemists* (1997), capter 7, 16th edition, 31 pp.
[3] D.G. Crosby and J.B. Bowers, *J. Agric. Food. Chem.*, **16**, 839–843 (1968)

[4] Q.X. Li, S.J. Gee, M.M. Mcchesney, B.D. Hammock and J.N. Seiber, *Anal. Chem.*, **61,** 819–823 (1989).

[5] N.L. Olson, R. Cornell, R.Cummings, R. Riech and S. Reimer, *J. Assoc. Off. Anal. Chem,* **78,** 1464–1473 (1995).

[6] C. Aguilar, F.Borrull and R.M. Marcé, *J. Chromatogr. A,* **771,** 221–231 (1997).

[7] A.A. Boyd-Boland and J.B. Pawliszyn, *J. Chromatogr. A,* **704,** 163–172 (1995).

[8] M.J. Redondo, M.J. Ruiz, R. Boluda and G. Font, *J. Chromatogr. A,* **719,** 69–76 (1996).

[9] M.J. Redondo, M.J. Ruiz, R. Boluda and G. Font, *J. Chromatogr. A,* **678,** 375-379 (1994).

[10] H. Kobayashi, K. Ohyama, N.Tomiyama, Y. Jimbo, O. Matano and S. Goto, *J. Chromatogr.,* **643,** 197-202 (1993).

[11] W. Liao, T. Joe and W.G. Cusick, *J. Assoc. Off. Anal. Chem,* **74,** 554–565 (1991).

[12] D. Puig and D. Barceló, *J. Chromatogr. A,* **673,** 55–64 (1994).

[13] F.C. Ferradal, J.R. Procopio and L.H. Hernandez, *Fresenius J. Anal. Chem.,* **343,** 382–385 (1992).

[14] C.J. Miles and H.A. Moye, *Anal. Chem.,* **60,** 220–226 (1988).

[15] T. Yarita, A. Nomura, Y. Horimoto and S. Gonda, *J. Chromatogr. A,* **750,** 175–181 (1996).

[16] S. Butz and H.J. Stan, *Anal. Chem.,* **67,** 620–630 (1995).

[17] F. Garcia and J. Henion, *J. Chromatogr.,* **606,** 237–247 (1992).

[18] S. Lacorte and D. Barceló, *Anal. Chim. Acta,* **296,** 223–236 (1994).

[19] M. Zhou, F.Q. Bramble, T.J. Devire and G.I. Norwood, *J. Assoc. Off. Anal. Chem.,* **79,** 791–796 (1996).

[20] R.V. Slates, *J. Agric. Food. Chem.,* **36,** 1207–1211 (1988).

[21] S. Kodama, A. Yamamoto, Y. Saito, N. Tokayanagi and A.Matsunaga, *Buseki-Kagaku,* **45,** 259–263 (1996).

[22] C. M. Fernández and V.C. Martin, *Talanta,* **24,** 747–748 (1997).

[23] A.M.O Brett, M.M.M Grazina, T.R.A. Macedo, C. Oliveira and D. Raimundo, *J. Pharm. Biomed. Anal.,* **11,** 203-206 (1993).

[24] Ministério da Agricultura, Desenvolvimento Rural e Pescas, Direcção-Geral de Protecção das Culturas, *Guia dos Produtos Fitofarmacêuticos,* Oeiras (1997).

[25] Ministério da Agricultura, Instituto Nacional de Investigação Agrária, Centro de Protecção da Produção Agricola, *Herbicidas para a Cultura de Arroz,* Oeiras (1992).

[26] M.H. Baizer and H. Lund, *Organic Electrochemistry,* 2nd Edition, Marcel Dekker, Inc, New York, 1983.

[27] M. Sugawara, K. Mori and J.-I. Yoshida, *Electrochim Acta,* **42,** 995–2003 (1997).