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Voltammetric determination of the herbicides nitralin and oryzalin in agricultural formulations, vegetables and grape juice samples

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Abstract

A sensitive method for the determination of the herbicides nitralin and oryzalin by adsorptive stripping voltammetry (AdSV) at a hanging mercury drop electrode (HMDE) (pH 6.0) was described. The cyclic voltammograms demonstrate the adsorption of these compounds at the mercury electrode. A symmetric study of the various operational parameters that affect the stripping response was carried out by differential pulse voltammetry. With an accumulation potential of −0.5 V and a 80 s accumulation time, the limit of detection was 2.47×10^{-8} mol/L and 1.5×10^{-8} mol/L, the relative standard deviation ($n = 10$), correlation coefficient values 1.14%, 0.998, 1.48%, 0.999 at concentration levels of 8.3×10^{-8} mol/L to 1.5×10^{-6} mol/L and 2×10^{-8} mol/L to 1.0×10^{-5} mol/L for both compounds. The degree of interference of some other pesticides on the differential pulse adsorptive stripping signal for nitralin and oryzalin was evaluated. Finally the proposed method was applied for determination of nitralin and oryzalin in agricultural formulations, vegetables and grape juice samples.

Keywords: DP-AdSV determination; Nitralin; Oryzalin; Formulations; Vegetables and grape juice samples

1. Introduction

Nitralin [4-methylsulfonyl-2,6-dinitro-*N*-*N*-dipropylaniline] and oryzalin [4-(dipropylamino)-3,5-dinitrobenzenesulfonamide] are dinitroaniline class of herbicides and are selective pre-emergence surface applied herbicides used for control of annual grasses and broadleaf weeds in fruit trees, nut trees, vineyards, bermudagrass turf, ornamentals and several crops. Nitralin is generally used on extensive potato cultures. Oryzalin inhibits the growth of germinating weed seeds (Meister, 1992).

Oryzalin has a low acute toxicity to mammals (US Environmental Protection Agency, 1987). The oral LD_{50} for technical oryzalin in rats and mice is >5000 mg/kg (WSSA Herbicide Handbook Committee, 1989). Microbial degradation may be responsible for the breakdown of oryzalin in soils. It is subject to photodecomposition, but not volatilisation at the soil surface (WSSA Herbicide Handbook Committee, 1989).

Anticriptosporidial activity of nitralin in an in vitro cultivation model of *Cryptosporidium parvum* is reported (Arrowood, Mead, Tixie, & You, 1996). The abiotic reduction of dinitroaniline herbicides are reported (Song & Arnold, 2003). The importance of abiotic reductive transformations as a sink for four dinitroaniline herbicides has been evaluated.

The influence of dinitroaniline herbicides on growth, sporulation and infectivity of four *Phytophthora* spp. pathogenic to deciduous fruit trees has been studied by Wilcox (1996). The pre-emergence and post-emergence herbicides are applied for large crabgrass (*Digitaria sanguinalis*) and goose grass (*Eleusine indica*) control in bermudagrass (*Cynodon dactylon*) (Johnson Jack, 1996).

The analysis of nitralin herbicide was performed by means of variety of techniques such as IR spectrometry, GLC and capillary gas chromatography (Deleu & Copin,

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1982) with N–P detector. Electrochemical properties of nitralin are evaluated (Southwick, Willis, Dasgupta, & Keszthelyi, 1976).

Liquid chromatographic technique and amperometric detection method using a hanging mercury drop electrode for the determination of nitralin in soil samples have been developed (Ruiz de Erenchun, Goicolea, Gomez de Balugera, Portela, & Barrio, 1997). RileyMellissa and Keese (1996) have compared the results of solid phase extraction techniques for herbicides.

The pharmacokinetic studies of the herbicide and antitumor compound oryzalin in mice were reported (Dvorakova, Dorr, Gallegos, McClure, & Powis, 1997). Combined oryzalin with adventitious regeneration for an efficient chromosome doubling of trihaploid kiwi fruits have been studied (Chalak & Legave, 1996).

In the present investigation, nitralin and oryzalin have been selected to get more information on the reduction mechanism of nitro groups and electrode kinetics concerned using cyclic voltammetry, differential pulse adsorptive stripping voltammetry. Differential pulse adsorptive stripping voltammetry has also been employed to work out analytical procedure in trace level estimation of these herbicides in formulations, vegetable samples and grape juice samples.

2. Materials and methods

2.1. Apparatus

Voltammetric measurements were made using Metrohm E-506 (Herisau, Switzerland) polarecord in combination with Metrohm 663 VA stand and with 612 VA scanner. Cyclic voltammetric studies were performed with 757 VA Computrace. A three-electrode system consisting of a medium size hanging mercury drop electrode, a Pt wire as a counter electrode and saturated calomel electrode is a reference electrode was used. All reported potentials were referenced to the SCE electrode. Solutions were deoxygenated with high purity nitrogen for 10–15 min prior to each experiment. An Elico LI-120 digital pH meter was used to measure the pH of the buffer solutions.

2.2. Reagents

The samples of nitralin and oryzalin were purchased from Dow Agro, USA, and Dow Elanco, Indianapolis, IN. These samples are used directly without any further purification. The purity of the sample was tested by determining their melting points and TLC experiments. Stock solution (1.0×10^{-3} mol/L) was prepared by dissolving nitralin and oryzalin in double distilled dimethylformamide. All dilute solutions were freshly prepared daily from the stock solution. Universal buffers of pH range from 2.0 to 12.0 were prepared by using 0.2 mol/L boric acid, 0.05 mol/L citric acid and 0.1 mol/L trisodium orthophosphate used as a supporting electrolyte. All the chemicals used were of analytical grade.

2.3. Voltammetric measurements

In order to select suitable conditions for the determination of nitralin and oryzalin using AdSV, various instrumental parameters were studied. Pulse amplitude of 28 mV was selected because the peak current was increased linearly up to 28 mV. When the pulse amplitude was varied over 20–30 mV. The scan rate was 35–55 mV s^{-1}. The best result was obtained with 40 mV s^{-1}. The most well defined signals with a reasonably high sensitivity were obtained with a universal buffer of pH 6.0.

2.4. Voltammetric procedure

The solution containing the universal buffer of pH 6.0, nitralin and oryzalin at appropriate concentrations was placed into the polarographic cell, through which a nitrogen stream was passed for 15 min before recording the voltammogram. The selected accumulation potential (E_{acc} –0.5V) was applied during the accumulation period (t_{acc} 80 s) while the solution was kept under stirring. After the accumulation time had elapsed, stirring was stopped and the selected accumulation potential was kept on the mercury drop for a rest time (t_r 15 s), after which a potential scan was performed between 35 and 55 mV s^{-1} by using adsorptive stripping voltammetry.

2.5. Preparation of solutions and analytical procedure

The required quantity of formulation corresponding to a 1.0×10^{-3} mol/L stock solution is accurately measured and transferred into a 50 mL volumetric flask containing acetone. Standard solution of approximately 1.0×10^{-6} mol/L is prepared by dilution of this stock solution with universal buffer.

A known amount of nitralin (planavin 75) is sprayed on potato and soybean crops and left for 1–2 h. These samples are prepared by the treatment of crushed vegetables with 100 mL of acetone. After being shaken with the use of a laboratory shaker for 5 min, this mixture was transferred into a centrifuge tube, and centrifuged at 3000 rpm for 5 min. After a settling time of 2 min, the extracts were transferred into a 50 mL flask. The above extraction procedure was repeated twice, all the extracts were collected and allowed to dry. The residue of nitralin is dissolved in dmf and transferred into a 50 mL volumetric flask. Then this sample solution was analyzed by the voltammetry and voltammograms are recorded in the same manner as described earlier.

Commercial grape juice samples used for testing were obtained from the many brands available in the local market. Samples were diluted as 1:1 ratio with double distilled water, before its filtration for the measurement. A 100 mL aliquot of juice samples were spiked with known amounts of oryzalin stock solution at different concentration levels. Then the sample was placed into a cell containing a suitable buffer.

An alternative calibration procedure is the standard addition method. We divided the unknown sample into two portions, so that known amounts of the analyte (a spike) can be added to one portion of these two samples, the original and the original plus spike, are then analyzed. The sample with the spike will show a larger analytical response than the original sample due to the additional amount of analyte added to it. The above method was used to estimate the compound in these samples.

The difference in analytical response between the spiked and unspiked samples is due to the amount of analyte in the spike. This provides a calibration point to determine the analyte concentration in the original sample.

3. Results and discussion

3.1. Cyclic voltammetric studies

The electrochemical behavior of nitralin and oryzalin has been examined over the pH range from 2.0 to 12.0. A single well defined peak is observed throughout the pH range for these two compounds in all the techniques. This single peak is attributed to the simultaneous reduction of two nitro groups in eight electron process to the corresponding hydroxylamine groups. Typical cyclic voltammograms are shown in Figs. 1 and 2.

In cyclic voltammetric experiments, a small anodic peak (a_1) has been observed in the reverse scan at higher pH values (pH > 10.0) for these two compounds. In the second scan, another small cathodic peak (c_2) at more positive potentials than c_1 is noticed. The anodic peak (a_1) may be due to the oxidation of hydroxylamine formed at c_1 to nitroso derivative to the hydroxylamine again.

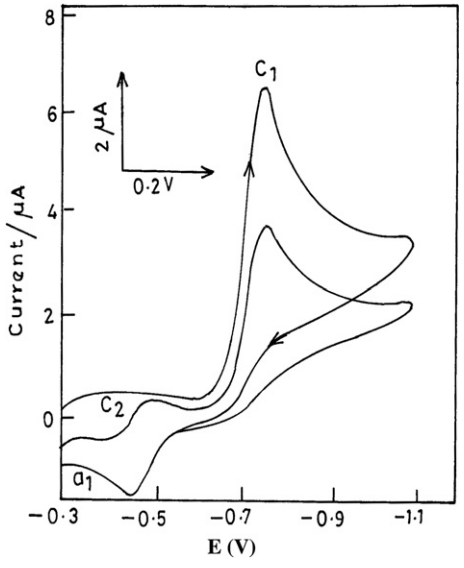

Fig. 1. Typical cyclic voltammogram of nitralin for an accumulation time of 80 s at HMDE, accumulation potential: −0.5 V; rest time: 10 s; stirring rate: 1500 rpm; scan rate: 40 mV s^{-1}; pulse amplitude: 28 mV; concentration: 1×10^{-5} mol/L; pH: 12.0.

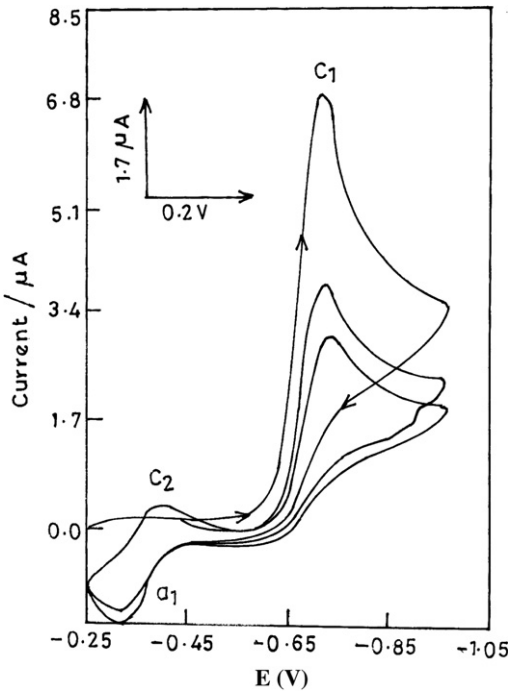

Fig. 2. Typical cyclic voltammogram of oryzalin for an accumulation time of 80 s at HMDE, accumulation potential: −0.5 V; rest time: 10 s; stirring rate: 1500 rpm; scan rate: 40 mV s^{-1}; pulse amplitude: 28 mV; concentration: 1×10^{-5} mol/L; pH: 10.0.

The irreversibility of the electrode process is confirmed by log plot analysis of the peak. The variability of the peak potential with scan rate also indicates the irreversible nature of the electrode process. The E_p values of nitralin and oryzalin are found to be dependent on pH and shift towards more negative values with the increase in pH of the buffer solutions, indicating proton involvement in the electrode process. The number of protons involved in the rate determining step is found to be two.

Millicoulometry is employed to find out the number of electrons involved in the electrode process. It is found to be eight (for each nitro group four electrons) for the reduction of two nitro groups simultaneously in nitralin and oryzalin in both acidic and basic medium.

Controlled potential electrolysis (cpe) has been carried out in a modified cell with mercury pool cathode, saturated calomel electrode and platinum wire as anode. This experiment is carried out in pH 4.0 at applied potential of −0.5 V. After electrolysis, the reduced products are extracted with ether. The ethereal layer is evaporated on water bath and the products are identified as the corresponding hydroxylamine. The isolation product is confirmed as hydroxylamine by IR spectral data (N–H stretch: 3420 cm^{-1}, O–H stretch: 3060–3000 cm^{-1}, N–H bend: 1500 cm^{-1}).

3.2. Differential pulse AdSV studies

The electrochemical studies with hanging mercury drop electrode, using differential pulse adsorptive stripping

voltammetry carried out for indicate that an adsorption process occur on the mercury electrode surface which can be used as an effective pre-concentration step prior to voltammetric measurement (Figs. 3 and 4). An exhaustive study of the dependence of adsorptive peak currents on pH, accumulation potential, accumulation time and scan rate was performed using 10^{-5} mol/L nitralin and oryzalin solutions.

3.3. Effect of pH

Voltammograms were recorded at different pH values and a maximum intensity for pH 6.0 was obtained. At this pH nitralin and oryzalin yields a single well defined peaks.

3.4. Effect of accumulation potential

The influence of the accumulation potential on the peak height of nitralin and oryzalin were studied from −0.2 V to −1.0 V and a strong adsorption at −0.5 V was observed. Therefore, this potential was used as the optimum accumulation potential for all the measurements.

3.5. Effect of accumulation time

At first, i_p increased linearly with t_{acc}, indicating that before adsorptive equilibrium is reached, the longer the pre-concentration time, the more nitralin and oryzalin were adsorbed, and the larger the peak current. However, after a specific accumulation time, the peak current tended to level off, illustrating that adsorptive equilibrium of nitralin and oryzalin on the mercury electrode surface was achieved.

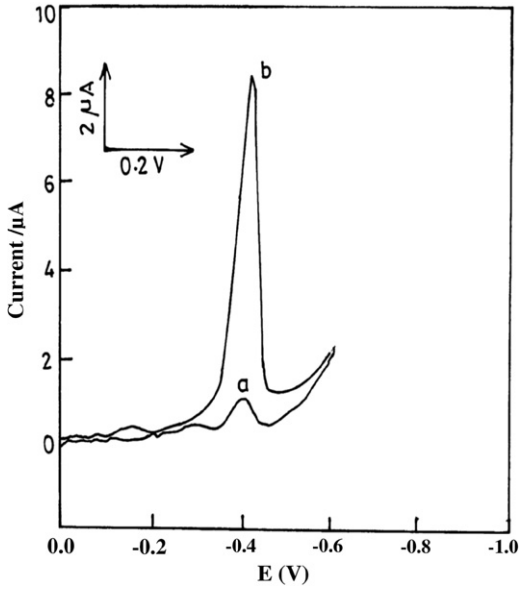

Fig. 3. Typical differential pulse adsorptive stripping voltammogram of nitralin for an accumulation time of 80 s at HMDE (pH 6.0) (b); blank solution (a); accumulation potential: −0.5 V; rest time : 10 s; stirring rate: 1500 rpm; scan rate: 40 mV s^{-1}; pulse amplitude: 28 mV; concentration: 1×10^{-5} mol/L.

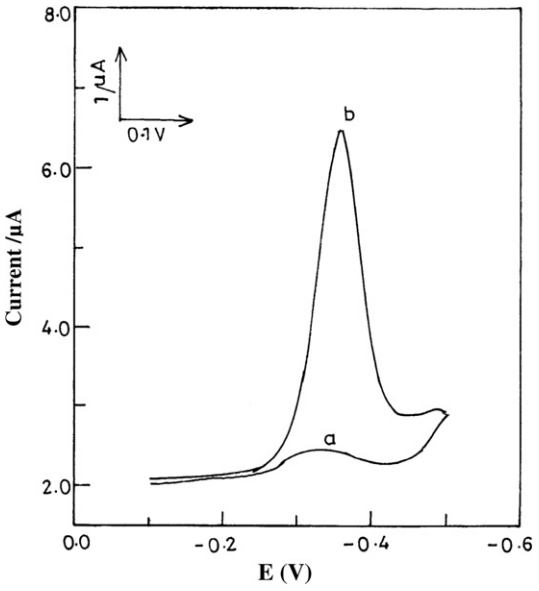

Fig. 4. Typical differential pulse adsorptive stripping voltammogram of oryzalin for an accumulation time of 80 s at HMDE (pH 6.0) (b); blank solution (a); accumulation potential: −0.5 V; rest time: 10 s; stirring rate: 1500 rpm; scan rate: 40 mV s^{-1}; pulse amplitude: 28 mV; concentration: 1×10^{-5} mol/L.

3.6. Effect of scan rate

The effect of scan rate (v) on the peak currents was evaluated for the adsorbed nitralin and oryzalin. The plot of log i_p versus log v shows a linear relationship with a correlation coefficients of 0.998 and 0.999 for two compounds.

A linear relationship of the adsorption holds between the peak current and the concentrations of nitralin and oryzalin in the ranges from 8.3×10^{-8} mol/L to 1.5×10^{-6} mol/L and 2×10^{-8} mol/L to 1.0×10^{-5} mol/L.

The optimal values of these parameters were then chosen from the study of the variation of the peak current (i_p) of 1.0×10^{-5} mol/L nitralin and oryzalin in universal buffer of pH 6.0. The peak current of nitralin and oryzalin was found to increase linearly on increase with scan increment.

The influence of several instrumental parameters known to affect the differential pulse adsorptive stripping current response at the HMDE, such as mercury drop size, stirring rate, pulse amplitude, rest period and purge time were optimized. For this study, each variable was changed while the others were kept constant. The working conditions decided upon were medium drop size, 1500 rpm, 28 mV, 10 s and 10 min. The stripping currents were not modified when varying the rest period, since it was found that 10 s was sufficient to allow for the formation of a uniform concentration of the analyte in the mercury drop.

3.7. Interference study

There is no effect due to ingredients present in formulations of ethalfluralin, nitralin and oryzalin. In case of

general nitralin formulations (Linuron and napropamide), the presence of napropamide causes no significant changes in the peak potential of the voltammetric reduction peaks obtained for nitralin, due to napropamide gives a reduction peak at −1.38 V, shifted to more negative potentials than those of the nitralin. There is no reduction behaviour of linuron is observed. Therefore, the proposed method does not involve the elaborate clean up procedures with the other methods.

3.8. Quantitative determination of nitralin and oryzalin in agricultural formulations

The above developed analytical procedure is then applied to the determination of nitralin in formulations planavin 75, nitraline and oryzalin in formulations ryzelan, El-119 and dirimal as wettable powders. Assay results for nitralin and oryzalin in formulations are given in Table 1.

Table 1
Determination of nitralin and oryzalin in formulations by DP-AdSV

Name of the formulation	Labeled amount (mg)	Amount found (mg)	Recovery[a] (%)	Standard deviation
Nitralin formulations				
Planavin 75	10	9.99	99.8	0.012
	20	19.97	99.8	0.021
	30	29.9	99.6	0.014
Nitraline	10	9.96	99.6	0.018
	20	19.9	99.4	0.02
	30	29.9	99.6	0.02
Oryzalin formulations				
Ryzelan	3.0	2.94	98.0	0.026
	5.0	4.92	98.4	0.031
El-119	3.0	2.91	97.0	0.017
	5.0	4.93	98.6	0.025
Dirmial	3.0	2.9	96.3	0.021
	5.0	4.90	98.0	0.03

[a] Each value is an average of three determinations.

Table 2
Recovery studies for nitralin in spiked vegetable samples

Name of the formulation	Labeled amount (mg)	Amount found (mg)		Average recovery[a] (%)	
		Potatoes	Soybeans	Potatoes	Soybeans
Planavin 75	10	9.13	9.07	91.3	90.7
	15	13.68	13.40	91.2	89.3
	20	18.31	18.17	91.5	90.8
	25	22.76	22.66	91.0	90.6

[a] Each value is an average of three determinations.

3.9. Quantitative determination of nitralin in vegetable samples

DP-AdSV under the experimental conditions described above is used to determine nitralin in vegetable samples. Vegetables such as potato and soybeans have been chosen for the analysis of nitralin. The results obtained using the DP-AdSV method are shown in Table 2.

3.10. Quantitative determination of oryzalin in grape juice samples

The present developed method was applied to the quantitative determination of oryzalin in grape juice samples. The analytical procedure of grape juice samples described in experimental section. The amount 12.0, 1.2 μl^{-1} of oryzalin is added to the grape juice samples and the average amount found 11.73 ± 0.20 for 12.0, 1.18 ± 0.10 for 1.2 μl^{-1}. The average recoveries obtained for oryzalin in juice samples are 97.7% and 98.3%, respectively.

4. Conclusion

Recoveries of nitralin formulations (planavin 75, nitraline) ranging from 99.63% to 99.85% and 99.45% to 99.60%, respectively. The recoveries of planavin 75 are more than nitraline. The recoveries of nitralin (planavin 75) in vegetable samples such as potato and soybean are ranging from 91.04% to 91.56% and 89.34% to 90.89%.

Table 3
The comparison of the present method with other reported analytical methods

	Method 1*	Method 2*	Present method
Technique	Reductive amperometric and LC	DPP	DP-AdSV, CV, CPE and MC
Electrode	HMDE	–	HMDE
pH	2.5	7.4	6.0
Accumulation potential (V)	−1.35	−1.60, −3.30, −5.40 and −6.50	−0.5
Accumulation time (s)	9 min	–	80
Limit of detection	6.9 ng g^{-1}	–	2.47×10^{-8} mol L^{-1}
Relative standard deviation	<4%	–	1.14% ($n = 10$)
Correlation coefficients	–	–	0.998
Concentration levels	–	–	8.3×10^{-8} mol L^{-1} to 1.5×10^{-6} mol L^{-1}
Samples	Soil samples	–	Vegetables and grape juice samples

*Method 1: Ruiz de Erenchun et al., 1997; *Method 2; Purnendu K. Dasgupta et al.
Ads-SWV: adsorptive stripping voltammetry-square wave ; DP-AdSV: differential pulse adsorptive stripping voltammetry; LC: liquid chromatography; CV: cyclic voltammetry; CPE: control potential electrolysis; MC: millicoulometry.

The percentage of recoveries of nitralin comparatively high in potato samples.

The percentage of recoveries obtained for oryzalin formulations (ryzelan, El-119 and dirimal) are in the range 97.00–98.60%. From the results the recoveries of El-119 are high compared to remaining formulations of oryzalin. Recoveries of oryzalin in spiked grape juice samples in the range of 97.75–98.33% which indicates the high accuracy and reproducibility of the proposed differential pulse adsorptive stripping voltammetric method.

This work presents that highly sensitive method for the determination of nitralin and oryzalin in agricultural formulations and foodstuffs compared than the conventional methods (Table 3).

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