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Electrochemical Determination of Pindone in Agricultural Formulation and Cereal Samples by Differential Pulse Adsorptive Stripping Voltammetry

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ABSTRACT

A sensitive method for the determination of the herbicide pindone by adsorptive stripping voltammetry using a hanging mercury drop electrode at pH 4.0 was described. The cyclic voltammogram of pindone demonstrates that compound was adsorbed at the surface of mercury electrode, and the overall reduction process was under controlled diffusion. The adsorptive peak was observed at −0.3 V, and peak response was measured with respect to pH, accumulation time, potential, and scan rate. The calibration plot was found to be linear from the concentration range 3.0×10^{-9}-4.0×10^{-7} mol/L with a detection limit 2.0×10^{-9}. In addition, interference of some other pesticides on pindone determination was evaluated. Finally, the developed method was potentially applied for determination of pindone in agricultural formulation and grain samples.

Key words: *Differential pulse adsorptive stripping voltammetry, Pindone, Agricultural formulations, Grain samples.*

1. INTRODUCTION

Pindone is an anticoagulant drug for agricultural use. It is commonly used as rodenticide in the management of rats, mice, and rabbit population. It has long persistence which leads to accumulation in soil and crops that have been treated directly. Pindone 2-(2,2 dimethyl-1-oxopropyl)1-H-indene-1,3(2H)-dione is first generation indene-dione. The increased commercial availability of these compounds has resulted in an increase in accidental and intentional ingestion for both animals and human beings. Analytical methods for rapid and accurate determination of them are required both for diagnosis of the intoxication and for forensic purposes.

Survey of literature has indicated that several analytical methods have been employed for the analysis of pindone in various samples. Marek and Koskinen [1] reported multiresidue analysis of seven anticoagulant rodenticides by high-performance liquid chromatography (HPLC)-electrospray mass spectrometry. Determination of pindone through fast kinetic method was developed by Sendra *et al*. [2]. Multi residue determination of anticoagulant rodenticides in animal serum by HPLC with a diode array/flurometric detection is promising technique [3,4]. Imran *et al*. [5] reported analytical methods for determination of anticoagulant rodenticides in biological samples. Column-switching ultra HPLC-ESI-MS/MS was used for simultaneous determination of rodenticides in tissues [6]. Liquid chromatography-tandem mass spectrometry was used for comprehensive characterization of rodenticides in wastewater [7]. Beauregard *et al*. [8] and Menzie *et al*. [9] reported spectrophotometric methods for the determination of pindone in plasma. Chen *et al*. developed a method for the determination of pindone in animal liver and human plasma by ionization tandem mass spectrometry [10,11].

However, the above-mentioned methods are often complex time consuming and involve expensive apparatus. In electroanalytical chemistry, adsorptive stripping voltammetry (Adsv) is widely recognized as one of the most sensitive methods. Adsv is often an appropriate electroanalytical technique for trace determination of pesticides including biological active substances and environmental samples [12-16]. In contrast to the conventional voltammetry, the enhanced sensitive of stripping voltammetry is attributed to the accumulation of surface concentration of analyte from pre-concentration procedure.

2. MATERIALS AND METHODS

Differential pulse Adsv (Dp-Adsv) and cyclic voltammetry measurements were carried out using

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Metrohm E-506 (Herisau, Switzerland) Polarecord in combination with a Metrohm 663 VA stand and 612 VA scanner in conjunction with a hanging mercury drop electrode (HMDE). Cyclic voltammetric studies were performed with 797VA computrace. The three-electrode system was completed by means of an pt wire as a counter electrode and saturate calomel electrode as the reference electrode. All experiments were performed at room temperature. The pH measurements were made by a Metrohm 632 pH-meter.

Pindone was obtained from Sigma-Aldrich with declared purity of 99.4%. These samples are used directly without any further purification. Pindone (1×10^{-3} mol/l) stock solution was prepared by dissolving an appropriate amount of herbicide in dimethylformamide (DMF). The supporting electrolyte was Universal buffers of pH range from 2.0 to 12.0 was prepared using 0.2 mol l^{-1} boric acid, 0.05 mol l^{-1} citric acid, and 0.1 mol l^{-1} trisodium orthophosphate (Merck). All the chemicals used were of analytical grade. Twice-distilled water used to prepare the solutions. Appropriate amounts of pindone stock solution were placed in a cell containing a buffer solution. A stream of oxygen-free nitrogen gas was purged through the solution for 10 min. The voltammetric response was obtained using a pulse repetition time of 2 s, with an amplitude of 50 mV and a scan rate of 20 mV/s. For multistep standard addition experiments, small increments of the standard solution (0.2 ml) are added, and the voltammograms are recorded for each addition under similar conditions. To select a suitable medium for Dp-Adsv studies, various supporting electrolytes such as 0.1 mol.dm^{-3} HCl, 0.1 mol.dm^{-3} $HClO_4$, and universal buffer were tested. The most well-defined signal with a reasonably high sensitivity was obtain with a universal buffer of pH 4.0. The optimum conditions for the determination of pindone at pH 4.0 were found to be a pulse amplitude of 50 mV and applied potential of −0.3V versus saturated calomel electrode (SCE), respectively. The above-described procedure was successfully employed for the determination of pindone in their formulations and cereal samples.

3. RESULTS AND DISCUSSION

Pindone exhibits a single well-defined wave/peak corresponding to the simultaneous reduction of three carbonyl groups, i.e., two carbonyl groups at position 1, and 3 of indene ring and the third is at tertiary butyl. Typical cyclic voltammograms of pindone are shown in Figure 1. The reduction process is found to be irreversible as seen from the absence of anodic peak on the reverse scan in cyclic voltammetry and disobedience of Tomes' criterion confirmed the electrode process to be irreversible. Reduction wave is not obtained for pindone in basic medium due to the precipitation of electroactive species.

The reduction process of this compound was found to be diffusion controlled and adsorption on the electrode surface in the buffer systems studied as evidenced from linear plots i_p versus $v^{1/2}$ passing through origin. The shift of peak potential (E_p) toward more negative values with increase in concentration of depolarizer shows that the electrode processes is irreversible. This is further confirmed by log-plot analysis. The variation of peak potentials with scan rates and absence of anodic peak in the reverse scan in cyclic voltammetry indicates the irreversible nature of the electrode processes. The experimental constancy of $i_p/Cv^{1/2}$ with scan rate (v) has shown the electrode process to be free from any kinetic complications. The total number of electronsinvolved during the electrode reaction for pindone has been determined by millicoulometry with a platinum wire as anode and mercury pool as cathode and is found to be two. Controlled potential electrolysis experiments are conducted at −0.3 V versus SCE at pH 4.0 and corresponding decay is noted using a galvanometer. The electrolysis is allowed to proceed virtually to completion. The product formed after controlled potential electrolysis has been identified as the hydroxy derivative and it is confirmed by I.R. spectral studies where characteristic peak of the hydroxy derivative is obtained (O-H stretching frequency of broad peak appears at in between 3650 cm^{-1} and 3250 cm^{-1}). Electrode mechanism of pindone is shown in Figure 2.

3.1. Dp-Adsv Studies

In this work, the electrochemical studies with HMDE using Dp-Adsv carried out to indicate that an adsorption process occurs on the mercury electrode surface which can be used as an effective pre-concentration step before voltammetric measurement (Figure 3). An exhaustive study of dependence of adsorptive peak currents on pH, accumulation potential, accumulation time, and scan rate was performed using pindone concentration 1.0×10^5 mol/L.

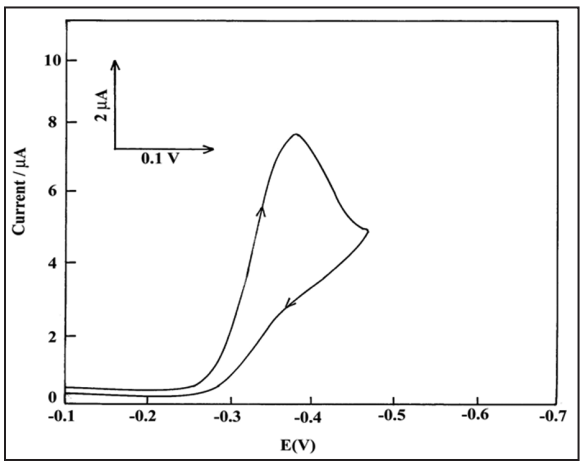

Figure 1: Cyclic voltammogram of pindone at pH 4.0 concentration: 0.25 mM, scan rate: 40 mVs^{-1}.

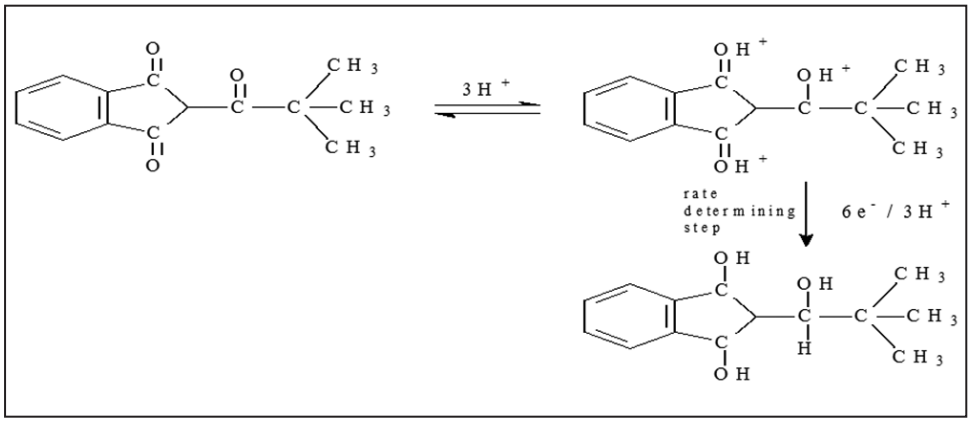

Figure 2: Electrode mechanism of pindone.

3.1.1. Influence of pH

The pH of a solution is a critical factor affecting the rate and equilibrium state of the accumulation process and rate of the electrode reaction. The influence of the pH on peak current of pindone was studied in the pH range of 2-6. Maximum peak current was obtained in the pH range 2-4 (Figure 4). In this work, a pH value of 4 was selected for further experiments because the optimum pH for production of homogeneous mercury film was found to be pH 4 and this pH was also near to the optimum pH for determination of pindone. The influence of the pH on the Dp-AdSV response was studied at HMDE at 1×10^{-5} mol/L with accumulation times 60 s.

3.2. Influence of Accumulation Potential

The accumulation potential is also a major factor affecting the sensitivity. The optimal pre-concentration potential condition is between −0.7 and −1.5 V. Figure 5 shows the effect of the accumulation potential on the magnitude of the stripping peak current. It can be seen that the largest peak current was obtained at a potential of −0.3 V for pindone. The sensitivity decreases sharply at potential <−0.3 V. Thus, an optimum pre-concentration potential −0.9 V is used in the subsequent studies for pindone.

3.3. Influence of Accumulation Time

The adsorption behavior of pindone is particular importance to be used to enhance the sensitivity of voltammetry. The effect of accumulation time on peak currents for 1×10^{-5} M pindone in universal buffer pH 4.0 was investigated. Maximum peak current was obtained at the 60 s. Therefore, accumulation time of the 60 s was chosen for further analytical studies (Figure 6).

3.4. Influence of Scan Rate

The effect of scan rate on the peak current was studied over the range of 5-80 mVs^{-1}. When scan rate was increased from 5 to 20 mVs^{-1}, the peak current is increased. However, at scan rate higher than 20 mVs^{-1}, no remarkable increase in peak current was observed.

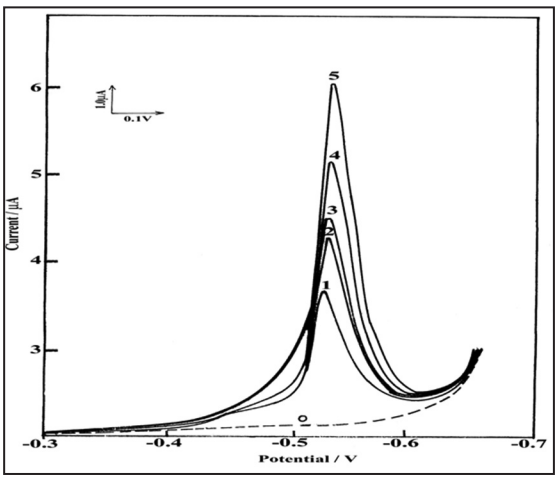

Figure 3: Typical differential pulse adsorptive stripping voltammogram of pindone at hanging mercury drop electrode. (pH 4.0) concentration: 1×10^{-5} M.

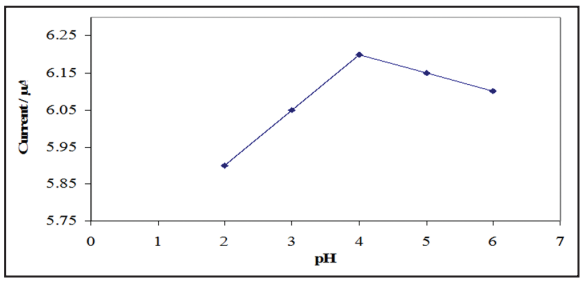

Figure 4: Effect of pH on pindone solution at hanging mercury drop electrode.

In addition, at scan rates above 25 mVs^{-1},baseline was increased. Hence, a scan rate of 20 mVs^{-1} was selected as the optimum value. The stripping currents were not modified when varying the rest period. The chosen value, 15 s, is sufficient to allow the formation of a uniform concentration of the analyte in the mercury drop and to ensure that the subsequent stripping step is performed in a quiescent solution. Other instrumental parameters, such as drop size and pulse amplitude, which directly affect the voltammetric response, were optimized. Under the optimum conditions of

pH 4, 60 accumulation time, −0.3 V accumulation potential, 20 mVs^{-1} scan rate, and 50 mV pulse amplitude, a linear relationship was obtained between concentration range 3.0×10^{-9}-4.0×10^{-7} mol/L. The calibration plot was found to be linear with correlation coefficient of 0.9995. A detection limit of 2.0×10^{-9} was estimated from quantitation of pindone under the optimum conditions.

3.5. Recommended Analytical Procedure

Analytical procedure for the voltammetric determination of pindone has been described as follows. 1 ml of standard solution of pindone (1.0×10^{-5} M) is taken in voltammetric cell and 9 ml of the supporting electrolyte

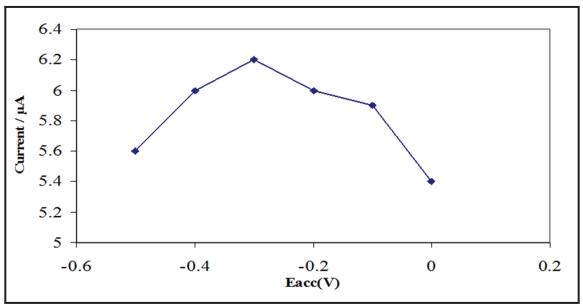

Figure 5: Effect of accumulation potential on the differential pulse adsorptive stripping voltammetry response of pindone at hanging mercury drop electrode.

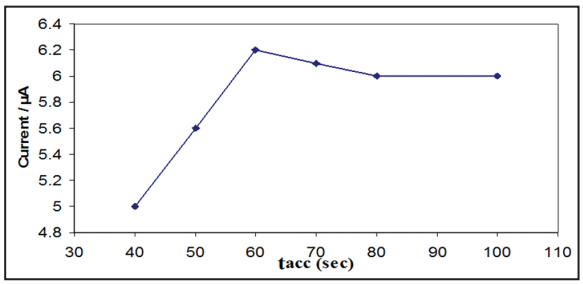

Figure 6: Effect of accumulation time on the differential pulse adsorptive stripping voltammetry response of pindone at hanging mercury drop electrode; accumulation time: 60 s.

(pH 4.0) is added. This is purged with O_2-free N_2 gas for 10 min before each run. The optimum conditions for the analytical determination at pH 4.0 are found to be a drop time of 2 s, a pulse amplitude of 50 mV, and an applied potential of −0.30 V. The correlation coefficient and relative standard deviation obtained using the procedure are found to be 0.99 and 1.28%, respectively, for 10 replicants.

The above-developed procedure is successfully utilized for the determination of pindone is agricultural formulations. In the present work, formulations of pindone, namely, Pival® and Pivalyn® are used for analysis. 1 ml of formulation solution corresponding to a standard solution of concentration 1.0×10^{-5} M is diluted with 9 ml of supporting electrolyte (pH 4.0) and voltammogram is recorded after deaeration. After taking the voltammogram, standard solution of pindone (0.2 ml) is added to it, deaerated for 2 min, and the voltammogram is again recorded under identical conditions. Assay results for pindone in agricultural formulations are shown in Table 1. The results presented in this study showed that the applied DP-AdSV method is to be a valuable procedure for the analysis of agricultural formulations before pesticide residue determination.

The same procedure has been applied for the determination of pindone is cereal grains such as maize and barley. Grain (maize and barley) samples (50 g) are sprayed with known amount of pindone and left for 2-3 h. Then, the samples are crushed into a fine powder. Then, the extracts are prepared by extracting a crushed sample with acetonitrile. The organic solvent is evaporated to dryness. The residue of pindone is dissolved in DMF and transferred into a 50 ml volumetric flask. The results obtained for the estimation of pindone in cereal grain by DP-AdSV are incorporated in Table 2. The data incorporated in Tables 1 and 2 show that the ingredients present in formulations in addition to pindone, and the other constituents present in cereal grains do not interfere in the proposed method. All these results

Table 1: Assay results of pindone in formulation by DP-AdSV.

Name of the formulation	Labeled amount (mg)	Amount found (mg)	Recovery (%)	Standard deviation
Pival®	5.0	4.90	98.00	0.020
	10.0	9.93	99.30	0.012
	15.0	14.94	99.60	0.016
	20.0	19.94	99.90	0.080
Pivalyn®	5.0	4.92	98.40	0.009
	10.0	9.92	99.20	0.010
	15.0	14.91	99.40	0.080
	20.0	19.92	99.60	0.090

Pulse amplitude: 50 mV, Drop time: 2 s, DP-AdSV: Differential pulse adsorptive stripping voltammetry

Table 2: Analysis of fortified cereal grains for pindone by DP-AdSV.

Name of the formulation	Labeled amount (mg)	Amount found (mg)	Recovery	Standard deviation
Maize	5.0	4.97	99.40	0.08
	10.0	9.89	98.90	0.12
	25.0	24.95	99.80	0.04
Barley	5.0	4.89	97.80	0.07
	10.0	9.96	99.60	0.05
	25.0	24.93	99.72	0.06

Pulse amplitude: 50 mV, Drop time: 2 s, DP-AdSV: Differential pulse adsorptive stripping voltammetry

demonstrate the validity of the voltammetric methodology to carry out the determination of this rodenticide at low concentration levels, which may be used in environmental monitoring and food stuff quality control after their suitable extraction into the appropriate organic solvent.

The proposed method is free from interference due to ingredients present in pindone and also other constituents present in cereal samples. The proposed method is simple, inexpensive, rapid, reliable, and sensitive and which does not involve any elaborate clean-up procedures compared to other methods.

4. CONCLUSION

This study showed the efficacy of an HMDE for the analysis of pindone in cereal samples by cyclic voltammetry and DP-AdSV voltammetry with sensitivities in the order of 10^{-9} M, respectively. Pindone could be determined with negligible interferences. With the help of cyclic voltammetry, we proposed reduction mechanisms in both acidic media. Cyclic voltammetric experiments have shown that the electrode system is diffusion-controlled. The proposed procedure is convenient to apply for the determination of pindone in cereal samples and also anticoagulant rodenticides compounds.

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****Bibliographical Sketch***

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