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Research Paper

Determination of Diphacinone in Grain Samples by Using Adsorptive Stripping Voltammetry

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Abstract - *A sensitive method for the determination of the rodenticide diphacinone by differential adsorptive voltammetry (Dp-AdSV) on a hanging mercury drop electrode (HMDE) (pH 6.0) was described. The cyclic voltammograms demonstrate the adsorption of diphacinone at the mercury electrode. The optimal operational conditions of the proposed procedure were accumulated potential E_{acc} = 0.0 V, accumulation time (t_{acc}) = 60 s, scan rate = 40 mV s^{-1}, pulse amplitude = 28 mV using a universal buffer pH = 6.0 as a supporting electrolyte. The linear concentration range was found to be 2 x 10^{-10} to 3 x 10^{-7} mol/L with the lower detection limit of 1 x 10^{-10}M. The procedure was successfully applied for determination of rodenticides in formulations, food grain samples such as chick pea and maize.*

Keywords: Adsorptive stripping voltammetry, diphacinone, formulations, grain samples etc.

Introduction

Anticoagulant Rodenticides are used to control mice and rats as well as pocket gophers, squirrels and also cereal baits, mammals such as bats, moles, rabbits and hare [1]. Rodenticides act in an animal organisms as depressors of prothrombin formation and suppressors of capillary fragility leading to hemorrhages [2,3]. Diphacinone (2-diphenyl-acetyl-1) 1-H-indene-1,3 (2H)-dione is first generation indene-dione. These compounds are class-6-poisons with LD_{50} value of 2 mg/kg for rats [4] and on certain occasions cause poisoning of the household pest [5] as well as humans [6]. 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.

Several papers have been described the development of analytical procedure for quantitative estimation of diphacinone in different matrices such as spectrophotometric, flurometric and thin layer chromatographic methods [7,10], Liquid chromatographic [11,13], high performance liquid chromatographic methods employing ultraviolet detector (HPLC-UV) [14,18] and HPLC in combination with mass spectrometry (HPLC–MS)[19]. But all these methods are suffering with a lack of robustness and detection sensitivity, time-consuming, derivatization procedures and lack of sufficient selectivity and sensitivity.

Several studies have also conducted to determine rodenticides in various soil samples, formulations and animal tissues. HPLC-DAD method has been reported for the analysis of the rodenticides together with the phenylurea herbicides in soil samples [20]. Thomas *et. al.*, have studied the reversed phase in pair liquid chromatographic determination of chlorophacinone and diphacinone in steam rolled oat baits and steam rolled oat/wax baits [21]. Reversed phase ion-pair chromatography has been employed for the determination of diphacinone and indanediones in formulations [22,24]. Hunter and Sharp have modified the procedures for the determination multi-residue analysis of rodenticides in animal tissues [25]. Capillary electrophoretic separation of anti-coagulant rodenticides was described by Stathakis et al. [26] Mesmer et al. [27] proposed a simple LC-ESI-MS method for the determination of commercial rodenticides. Although these methods are successfully demonstrated the analysis of rodenticides, unfortunately, no method has been reported by electrochemical techniques for analysis of rodenticides up to now.

A survey of literature, to the best of our knowledge, reveals that no attempt has been made to study the electrochemical reduction behavior of diphacinone by employing advanced electrochemical techniques. In the present investigation, diphacinone has been selected to get more information on the reduction mechanism of carbonyl groups and electrode kinetics concerned using cyclic voltammetry (CV), differential pulse adsorptive stripping

voltammetry (Dp-AdSV). Differential pulse adsorptive stripping voltammetry has also been employed to work out analytical procedure in trace level estimation of this rodenticide in formulations and grain samples.

Material and Methods

Reagents and Solutions: Diphacionone (99%) was obtained from Labor Dr. Ehrenstorfer GmbH, Germany. The purity of the compound was confirmed based on its boiling point and TLC experiment. A stock solution ($1x10^{-5}$ M) was prepared by dissolving the appropriate amount of diphacinone in double distilled dimethylformamide. All experimental solutions were freshly prepared every time from the stock solution. Universal buffers of pH range 2.0 to 12.0 were prepared by using 0.2 M boric acid, 0.05 M citric acid and 0.1 M trisodiumorthophosphate used as a supporting electrolyte. All the chemicals used were of analytical grade (Merck).

Apparatus: Voltammetric measurements were made using a Metrohm-E-506 (Herisan, Switzerland) Polarecord in combination with a Metrohm 663 VA stand and 612 VA Scanner. Cyclic voltammetric studies were performed with 757 VA computrace. The dropping mercury electrode had an area of 0.0223 cm^2 at a drop time of 2s. A three-electrode system consisting of a medium size hanging mercury drop electrode, a platinum wire as a counter electrode and saturated calomel electrode (SCE) as a reference electrode were used for CV and DP-AdSV (28-30). All reported potentials were referenced to the SCE electrode. Solutions were deoxygenated with high purity nitrogen gas for about 10-15 min prior to the performance of each experiment. An Elico LI-120 digital pH meter was used to measure the pH of the buffer solutions

Voltammetric measurements: In order to select suitable conditions for the determination of diphacinone using AdSV, various instrumental parameters were studied. When the pulse amplitude was varied over 20–30 mV in cyclic voltammetry, the peak current was increased linearly up to 28 mV and finally it was selected. The best scan rate was obtained with 40mVs^{-1}over the range of 35–55 mVs^{-1}. The most well defined signals with a reasonably high sensitivity were obtained with a universal buffer of pH 6.0. At this pH, appropriate concentration of diphacinone 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}) 0.0V was applied during the accumulation period (t_{acc} 60s) 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 10s), after which a potential scan was performed between 35 and 55 mVs^{-1} by using adsorptive stripping voltammetry.

Recommended Procedure for Dp-AdSV: A 10 ml of universal buffer was placed in a polarographic cell and deoxygenated by passing nitrogen 5 min. The voltammetric response was obtained using a pulse-repetition time 0.5 sec and amplitude of 28 mV with scan rate 40 mVs^{-1}. The pre-concentration potential of 0.0 V was applied for a selected period of time followed by 10 sec rest and then a differential adsorptive pulse voltammetric scan in the negative potential direction. For multi-step standard addition experiments, small increments (0.2 ml) of standard solution of diphacinone were added followed by recording the voltammogram

Analytical procedure: The required quantity of formulation corresponding to $1x10^{-5}$ M was accurately measured and transferred into a 100 ml voltammetric flask containing 50 ml of dimethylformamide. A solution of approximately $1x10^{-7}$ M was prepared by diluting this stock solution with an appropriate universal buffer.

Known amounts of diphacinone were sprayed on grain (wheat and rice) samples (25 g) and left for 1-2 h. The samples were prepared by treatment of a grounded sample with 50ml portion of acetone. After being shaken the samples for 5 min, this mixture was transferred into a centrifuge tube, and centrifuged at 2000 rpm for 5 min. After settling time of 2 min, the extracts were transferred into a 100 mL volumetric flask. The above extraction procedure was repeated twice; all the extracts were collected and allowed to dry. The residue of diphacinone was dissolved in dimethylformamide and transferred to a 100 mL volumetric flask. Then this sample solution was analyzed by the voltammetry and voltammograms are recorded in the same manner as descried earlier.

The same procedure was applied for the analysis residue of diphacinone in real samples. For this, the grains (Chickpea & Maize) were sprayed with 500 gai/ha of diphacinone formulations. The spraying was done with a foot sprayer, and nozzle was adjusted carefully to deliver uniform deposits. The samples were collected in a randomized manner from the respective fields and left for 1-2 h. The extracts were prepared by above procedure and the residues of diphacinone were determined by Dp-AdSV method. The samples of grains were drawn at 0, 4 and 7 day intervals after the application to study the carryover of the residues.

Results and Discussion

Diphacinone exhibits only one polarographic wave/peak over the pH range 2.0-6.0 with all the techniques. This wave/peak was attributed to the simultaneous reduction of three carbonyl groups of which two are at positions 1 and 3 of indene ring and the third is of the phenylacetyl ring involving six electrons. No reduction was observed in basic medium ($8 \leq pH \leq 12$) for carbonyl groups (31) due to the precipitation of electroactive species.

The nature of the wave was found to be diffusion controlled in the buffer systems selected. The irreversible nature of the wave was confirmed by log plot analysis of the peak (E_{dc} versus (i/i_d-i)) (32). The absence of the anodic peak in the cyclic voltammogram was also confirmed the irreversibility (Fig. 1). The wave height decreased as pH was increases and gave characteristic $E_{1/2}$ in all the buffer systems.

In the present study, 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 was carried out in pH 6.0 at applied potential of 0.0 V. The reduction product

formed after controlled potential electrolysis was identified as the corresponding hydroxy derivative by IR spectral studies. (O-H stretching frequency of broad peak appears at in between 3650cm^{-1}to 3250cm^{-1}).

The determination of number of electrons (n) involved during electrode process in diphacinone has been carried out by millicoulometry. According to this technique 'n' is found to be six for three carbonyl groups of diphacinone in acidic medium (pH ≤ 6.0).

The values obtained for diffusion coefficient (D), transfer coefficient (-E_m) and heterogeneous forward rate constant (K_f) are given in Table 1. The diffusion coefficient values evaluated from cyclic voltammetry was indicating the controlled diffusion and electrode surface adsorption. The variation of diffusion current with supporting electrolyte pH influences the diffusion coefficient values. The reason for slight variation in diffusion coefficient values with increase in pH may be attributed to the decrease in the availability of protons with increase in pH of the supporting electrolyte. The rate constant values are in general found to decrease with increase in pH indicating that the electrode reaction tends to become more and more irreversible with change in pH.

Differential pulse-adsorptive stripping voltammetric studies

Figure 2 shows differential pulse adsorptive striping voltammogram for 1.0x10^{-7} mol/ diphacinone with hanging mercury drop electrode. The systematic studies of the various experimental parameters that affect the adsorptive striping voltommograms response were carried out with hanging mercury drop electrode in order to establish the optimum conditions (accumulation Potential: 0.0V, rest time: 10s, Stirring rate: 2000 rpm; Scan rate: 40mVs^{-1}, Concentration: 1x10^{-7} M).

Effect of pH and accumulation potential

The influence of pH on the Dp-Adsv response of 1x 10^{-5} M diphacinone has been studied using hanging mercury drop electrode with the same optimized conditions (pulse amplitude: 28 mV) and the maximum peak current was obtained at pH 6.0 (Fig.3).

The influence of accumulation potential (E_{acc}), on the developed peak current was examined over the potential range of -0.4 to 0.8 V and strong adsorption at 0.0V was observed (Fig.4). A further decrease in the potential led to reduced peak current, presumably due to the detrimental effect of the irreversible reduction of carbonyl groups. Thus 0.0 V was chosen as the optimum accumulation potential for all the measurements.

Effect of accumulation time

Figure 5 showed the effect of accumulation time on the differential pulse adsorptive voltammetric peak current of the diphacinone varying with accumulation time. At first, the peak current increases with the accumulation time (t_{acc}). However, after a specific accumulation time, 60 s, the peak current tends to level off, illustrating that adsorptive equilibrium of diphacinone on the mercury electrode was achieved.

Effect of scan rate

The dependence of the Dp-Adsv developed peak current on the scan rate shows that a 40 mVs^{-1} gives the maximum response. A sharper and more sensitive peak was obtained at pulse amplitude of 28 mV. A universal buffer solution was used as the supporting electrolyte. Under these conditions, the diphacinone was pre-concentrated by accumulation on to the mercury electrode surface prior to the voltammetric measurements.

Several instrumental parameters which directly affect the voltammetric response at the HMDE, such as drop size, stirring rate, pulse amplitude, rest period and purge time were optimized. For this study each variable was changed while the others were constant. The working conditions decided up on were medium drop size, 2000 rpm, 28mV and 10s. The stripping currents were not modified when varying the rest period, since it was found that 10s was sufficient for the formation of a uniform concentration of the analyte in the mercury drop. Other experimental parameters such as temperature and ionic strength were optimized. The stripping peak currents were not modified when the temperature varied between 293-323K. Ambient conditions (298K) were selected for this study. The optimal values of these parameters were then chosen from the study of 1x 10^{-7} M diphacinone in universal buffer of pH 6.0. The peak current of diphacinone was found to increase with scan rate.

Quantitative determination of diphacinone in formulation by Dp-Adsv.

The above developed analytical procedure was successfully applied to the determination of diphacinone in formulations. The required quantity of diphacinone corresponding to a stock solution 1.0 x 10^{-5} M was accurately measured and transferred into a 100 ml voltammetric flask containing 50 ml of dimethylformamide. A standard solution of 1x10^{-7}M was prepared by diluting this stock solution with an appropriate universal buffer. The assay result for rodenticide formulations at pH 6.0 are given in Table 2.

Quantitative determination of diphacinone in grain samples

The above described method was applied to the determination of diphacinone in grain samples. Grains such as rice and wheat were chosen for the analysis of diphacinone. Recovery obtained for the determination of diphacinone (in pure and formulations) in grain samples were presented in Table 3. The proposed method was fast, simple and sensitive to analyse diphacinone in grain samples with good recoveries.

The present method was also applied to the quantitative determination of diphacinone in grain samples such as chickpea and maize. The analytical procedure of grain samples described in experimental section. The amount 500 gai/ha was added to the grain samples and samples were drawn at 0, 4 and 7 days to study the carryover of the residues. Results were presented in Table 4. With time, the residue levels of these pesticides gradually reduced. The maximum recoveries obtained for diphacinone in grains samples were 99.1% and 99.2%, respectively. The obtained results demonstrate the suitability of the developed

DP-AdSV method for the determination of diphacinone in spiked grain samples.

Conclusions

The reliability of the proposed differential pulse adsorptive stripping voltametric method for the determination of diphacinone was investigated by analyzing this rodenticide on grain samples. Recoveries of diphacinone in pure and formulation were ranging from 98.9% to 99.0% for wheat, 98.5% to 99.1% for rice and 98.0% to 99.1% for wheat, 97.6% to 98.3% for rice respectively. The percentage recoveries of diphacinone in spiked grain samples were in the range of 89.2% to 99.1% for chick pea and 88.9% to 99.2% for maize respectively. The results attained here with proposed DP-AdSV procedure allows simple, sensitive, accurate, fast response and low cost quantitative method for determination of diphacinone in different grain samples. Cyclic voltammetric experiments have shown that the electrode system in diffusion contrlloed at low concentration levels and high scan rates. AdSV provides an enhanced signal due to adsorption of the analytes and reversibility of the electron transfer reactions.

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Electrode Mechanism

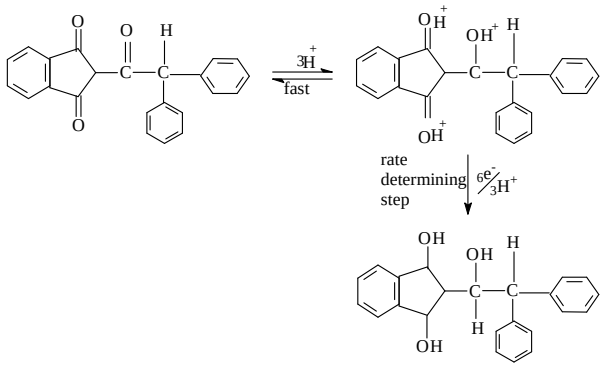

Table 1: Typical differential pulse polarography & cyclic voltammetirc data of diphacinone (Concentration: $1x10^{-5}$M; Pulse amplitude: 50 mV, Drop Time: 2 sec

pH of the Electrode	DPP			CV		
	$-E_m$ (V)	10^5 D ($Cm^2 s^{-1}$)	K_f, ($Cm\ s^{-1}$)	$-E_m$ (V)	10^5 D ($Cm^2 s^{-1}$)	K_f, ($Cm\ s^{-1}$)
2.0	0.11	2.68	$7.44x10^{-4}$	0.10	2.51	$7.83x10^{-4}$
4.0	0.24	2.46	$6.98x10^{-5}$	0.22	2.38	$7.83x10^{-4}$
6.0	0.29	2.27	$5.0x10^{-6}$	0.30	2.15	$7.83x10^{-4}$

Table 2: Recoveries of diphacinone in formulation by Dp-AdSV

Compound	Labeled Amount	Amount Found	Recovery %	RSD
Ditrac	2.00	1.91	98.5	0.78
	6.00	5.85	97.6	0.13
	10.00	9.90	99.0	0.10
	15.00	14.80	98.6	0.12
	20.00	19.60	98.0	0.08
Ramik	2.00	2.94	98.0	0.47
	6.00	4.86	97.2	0.08
	10.00	9.89	98.9	0.09
	15.00	14.70	98.0	0.14
	20.00	19.60	98.0	0.11

Table 3: Estimation of Diphacinone in Pure forms and in formulations

Pesticide	Additional Amount (mg)	Average Amount found		Recovery (%)	
		Wheat	Rice	Wheat	Rice
Diphacinone Pure	2.00	1.98	1.98	99.0	99.1
	4.00	3.96	3.94	98.9	98.5
	6.00	5.80	5.40	99.0	90.0
Diphacinone Formulations					
Ditrac	2.00	1.98	1.95	99.1	97.6
	4.00	3.94	3.92	98.5	98.1
	6.00	5.80	5.84	99.1	97.3
Ramik	2.00	1.96	1.96	98.0	97.8
	4.00	3.94	3.92	98.5	98.1
	6.00	5.93	5.96	98.8	98.3

Table 4: Determination of diphacinone in grain samples (Chick Pea and Maize)

Diphacinone formulation	Dosage ga.i/ha	Sampling Days	Residue level mg kg^{-1}		SD		RSD		Recovery	
			Chick pea	Maize	Chick pea	Maize	Chick pea	Maize	Chick pea	Maize
Ditrac	500	0	5.04	4.92	0.02	0.01	0.52	0.20	99.1	97.5
		4	1.02	1.06	0.01	0.01	0.98	0.98	95.2	91.2
		7	0.08	0.07	0.00	0.00	0.00	0.00	90.1	87.4
Ramik	500	0	4.97	5.05	0.03	0.05	0.72	1.02	98.7	99.2
		4	1.02	1.13	0.01	0.01	0.99	0.88	93.4	92.8
		7	0.06	1.00	0.00	0.01	0.00	1.00	89.2	88.9

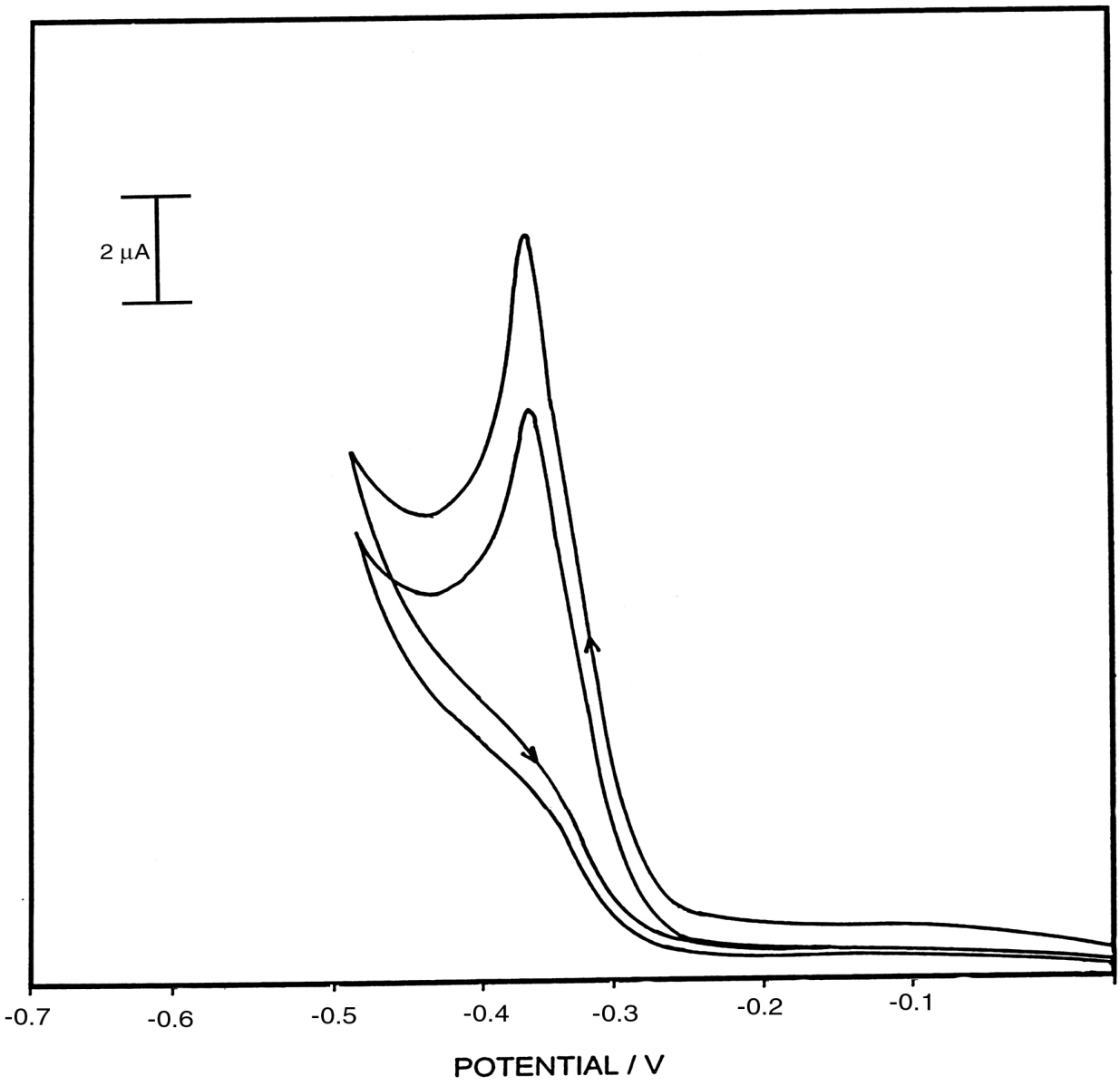

Figure 1: Typical cyclic voltammograms of diphacinone (Scan Rate: 40 mVs^{-1}, Concentration: 1 x 10^{-5}M)

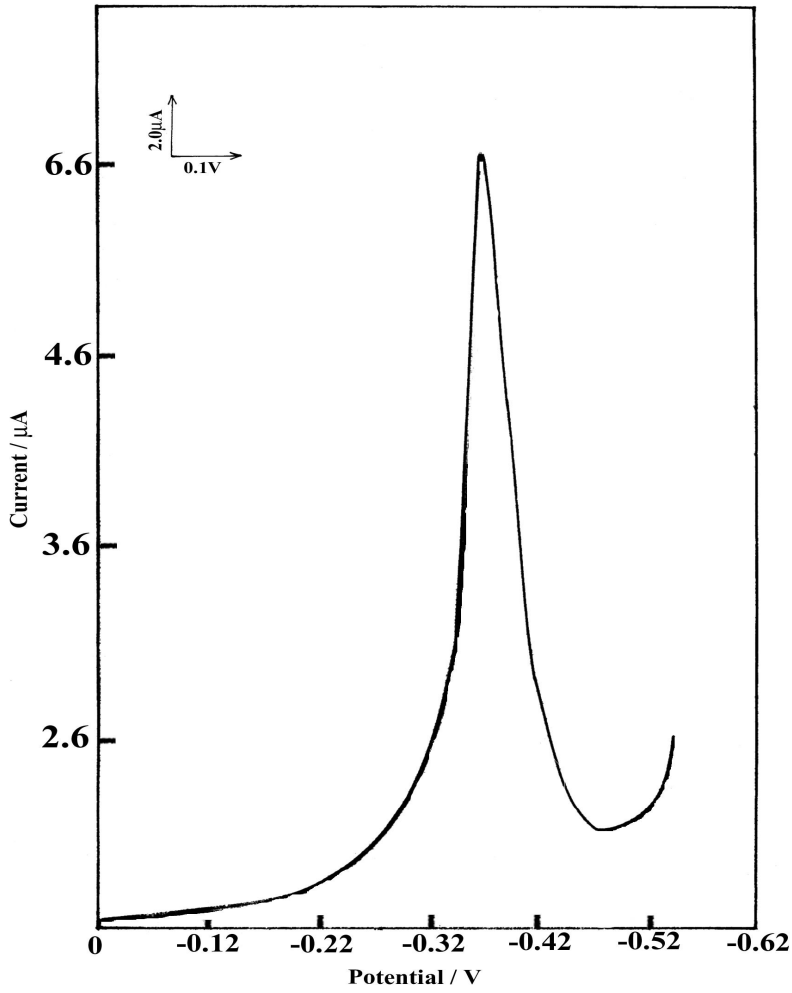

Figure 2: Typical differential pulse adsorptive stripping voltammograms of Diphacinone at HMDE. Accumulation Potential: 0.0V, rest time: 10s, Stirring rate: 2000 rpm, Scan rate: 40mVs^{-1}, Concentration: 1x10^{-7} M

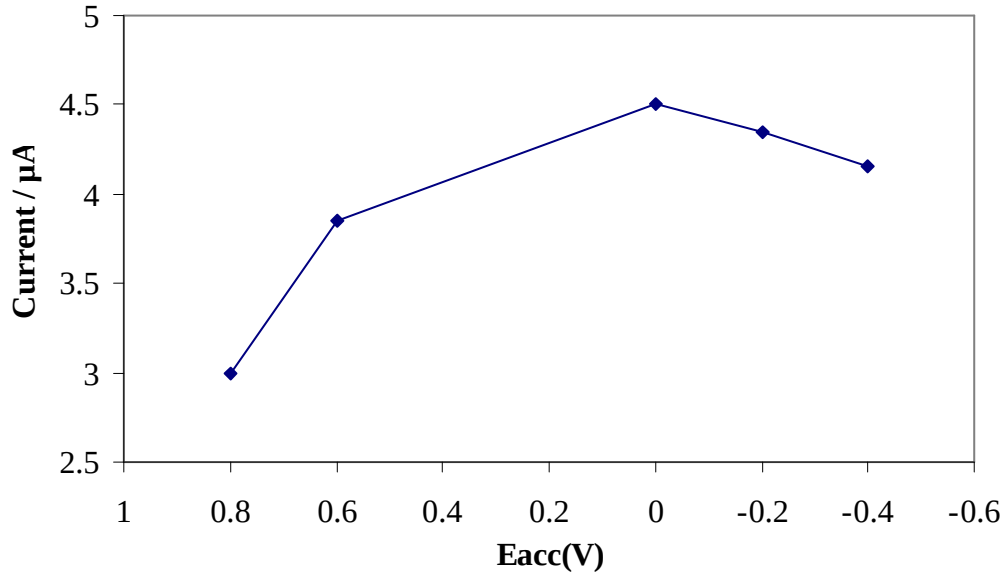

Figure 3: Effect of accumulation potential on the DP-AdSV response of diphacinone at HMDE, accumulation time: 60 sec., rest time: 10 sec., stirring rate: 2000 rpm, scan rate: 40 mVs^{-1}; pulse amplitude: 28 mV

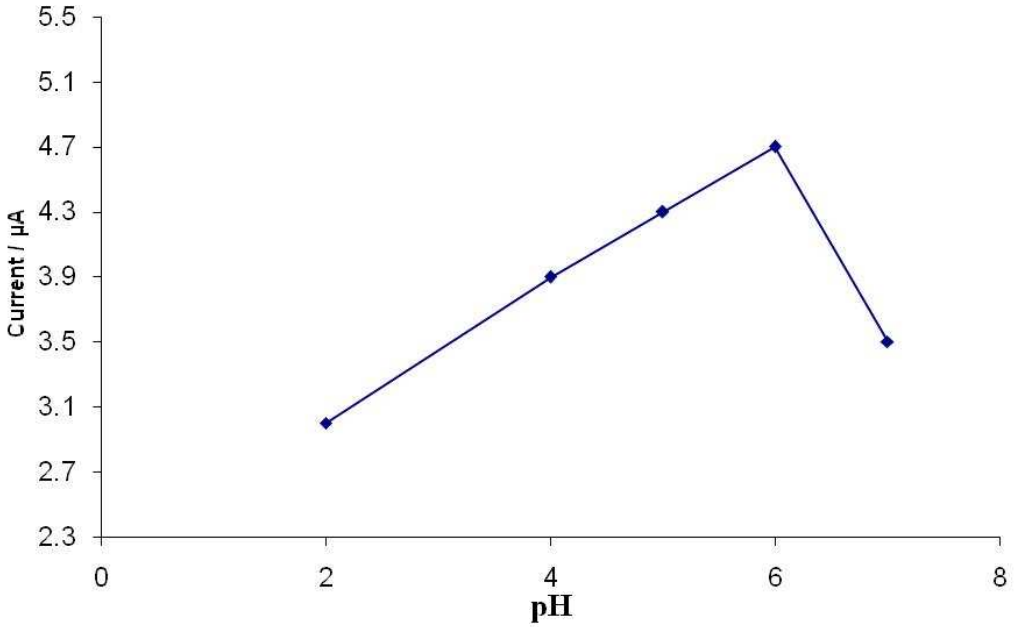

Figure 4: Effect of pH on diphacinone solution at HMDE; accumulation time: 60 sec., accumulation potential: 0.0V, rest time: 10 sec., stirring rate: 2000 rpm, scan rate: 40 mVs^{-1}, pulse amplitude: 28 mV

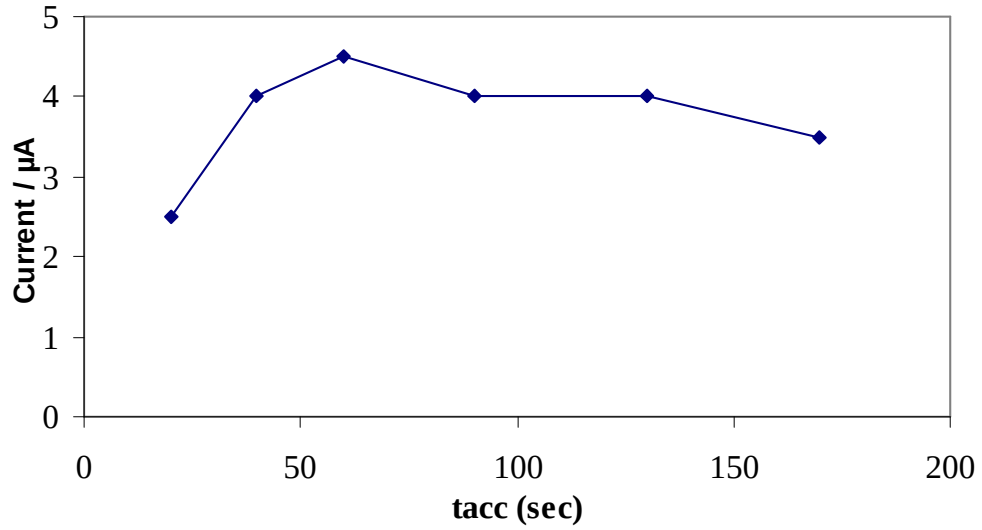

Figure 5: Effect of accumulation time on the DP-AdSV response of diphacinone at HMDE, accumulation potential: 0.0V, rest time: 10 sec., stirring rate: 2000 rpm, scan rate: 40 mVs^{-1}, pulse amplitude: 28 mV