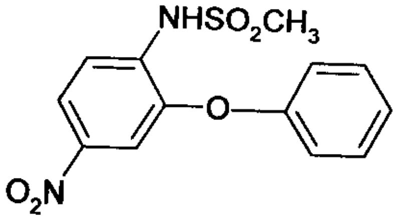
Results and Discussion

3.1 Electrochemical behavior of nimesulide in B.R buffer solutions of different pH values:-

Nimesulide is [N-(4-nitro-2-phenoxyphenyl)methane-sulfonamide] anti-inflammatory agent drug and its structural- formula has given as following :-



The aim of the present work in this part includes studying of the electrochemical behavior of nimesulide at Hg-electrode using DC-polarography and cyclic voltammetry techniques. The study also aimed to optimize the experimental and instrumental conditions for the determination of the lowest possible concentration of the investigated drugs in pure and in pharmaceutical dosage forms using cathodic adsorptive linear sweep voltammetry technique at the glassy carbon electrode.

3.1.1 DC polarography:-

3.1.1. i-Current-potential curves:-

The polarographic behavior of $1x10^{-4}$ M nimesulide was studied in Britton-Robinson buffer solutions containing 10% (v/v) ethanol of different pH values in the range from 2.0 to 12.0. The polarograms exhibited two reduction waves within the entire pH range studied corresponding to the reduction of the electro-active nitro-group ($-NO_2$), Fig. (1). The half-wave potentials ($E_{1/2}$) of the first and second reduction waves are pH dependent being shifted to more negative values on increasing the pH of the electrolysis solution.

3.1.1. ii- Effect of pressure at mercury height:-

The effect of mercury height "h" on the limiting current of the polarographic waves was examined according to the following equation[100]:

$$i_l = kh^x \text{ -- } (3.1)$$

or

$$\log i_l = \log k + x \log h \text{ ------------------------------------ } (3.2)$$

where k is constant and x is an exponent and depends on the nature of current produced during the reduction process. For diffusion-controlled current x= 0.5 and for adsorption controlled current x=1.0 while for kinetic controlled current x=0.05. However, x may have intermediate values between above mentioned ones if the electrode process is governed by the combinations of two kinds of these processes. The plots of log i_l against log h for nimesulide reduction at different pH values namely; 1.50, 7.10 and 10.50, displayed linear correlations, Fig. (2). The values of the exponent (x) for the first and second reduction waves are found to be in the range between 0.53 and 0.65 at the different pH values Table (1). This behavior indicate that the reduction process is controlled mainly by diffusion with some adsorption contribution.

3.1.1. iii- Analysis of the polarographic waves:-

Analysis of the polarographic waves is of quite importance to evaluate and confirm the degree of reversibility of the electrode reaction and to suggest the electrode reaction mechanism.

The equation of reversible polarographic waves is written in the form [101]:

$$E_{d.e} = E_{1/2} - (0.0591/n) \log [i/(i_d - i)] \text{ ------------------- } (3.3)$$

Where $E_{d.e}$ is the potential of the dropping mercury electrode i_d is the diffusion current, $E_{1/2}$ is the half–wave potential and n is the total number of electrons involved in the reversible reaction.

For irreversible electrode process the following equation of Mietes [98] is used:

$$E_{d.e} = E_{1/2} - (0.0591/\alpha n_a) \log [i/(i_d - i)] \text{ --------------------- } (3.4)$$

Where α is the transfer coefficient and n_a is the number of electrons involved in the rate determining step. The plots of log $[i/(i_d-i)]$ against $E_{d.e}$ for the first and second reduction waves of nimesulide reductions at different pH values showed linear correlations, Fig. (3 a,b). The reciprocal slopes (S_1) of the linear plots are in range from 76-138 mV and from 108-138 mV for the first and second waves; respectively, Table (2a). The values of (S_1) revealed the irreversible nature of the electrode process.

3.1.1. iv- Half- wave potential ($E_{1/2}$) –pH curves:-

On increasing the pH of the electrolysis solution the half-wave potential ($E_{1/2}$) of nimesulide reduction get shifted to more negative potentials. This behavior is further confirming the irreversible nature of the electrode reaction. The number of hydrogen ions (Z_H^+) involved in the rate determining step was determined from the rate change of $E_{1/2}$ with the pH of the electrolysis solution. The plots of $E_{1/2}$ versus pH gives straight line consisting of one segment for the first reduction wave and the second segment for second wave, Fig. (4). The slopes of $E_{1/2}$-pH curves (S_2) amount to 66 and 93, 105 mV for the first and second waves; respectively, Table (2b).

The number of hydrogen ions (Z_H^+) participating in the rate determining step is determined according to the following relationship [102]:

$$Z_H^+ = (\Delta E_{1/2}/\Delta pH)/\ (E_{d.e}/\ \log\ [i/(i_d-i)] = S_2/S_1 \text{ -------------(3.5)}$$

Inspection of the data listed in Table (2b), indicates that the number of hydrogen ions (Z_H^+) is equal to unity. This means that the rate determining step should involve one proton and two electrons.

Further confirmation of the degree of irreversibility of the electrode reaction was achieved by determining the transfer coefficient (α) values at the probable ratio of (Z_H^+/n_a) according to the following equation:

$$\alpha = (0.059/S_2)(Z_H^+/n_a) \text{ ------------------------------(3.6)}$$

The probable ratio of (Z_H^+/n_a) may have one of the following values 0.5, 1.0 or 2.0. the most probable α- values were determined and found to be suitable at the ratio of (Z_H^+/n_a) amounting to 0.5, 1.0 and 2.0, Table (2). This behavior is further confirming the irreversibility of the electrode process and the rate determining step should involve one proton and two electrons.

3.1.2. Cyclic voltammetry:-

The cyclic voltammetric behavior of 1×10^{-4} M of nimesulide was investigated in aqueous Britton-Robinson buffer solutions containing 10% (v/v) ethanol of different pH values at the glassy carbon electrode. The voltammograms displayed single irreversible cathodic peak in the cathodic scan in all media, Fig. (5). On the anodic scan no oxidation peaks were observed.

On increasing the scan rate (υ), the cathodic peak potential (E_{pc}) is shifted to more negative potentials indicating the irreversible nature of the reduction peak. Furthermore, on increasing the pH of the electrolysis solution, the peak potential (E_{pc}) is shifted to more negative values indicating the consumption of hydrogen ions in the transfer step [102]. On employing the following relationship [103].

$$E_p = -1.14\,(RT/\alpha n_aF) + (RT/\,\alpha n_aF)\ln(K_{f,h}/D^{1/2}) - (RT/2\,\alpha n_aF)\ln(\alpha n_a v) \text{---} (3.7)$$

The plots of E_{pc} versus ln v at different pH values give linear correlations Fig. (6). The slope values were used for the determination of αn_a. The values of the transfer coefficient (α) were calculated at the probable n_a values and found to be less than 0.7 at n_a=2.0, Table (3). This behavior is further confirmed the irreversible nature of electrode process.

On using Randles-Sevik equation [99] of irreversible electron transfer process:

$$I_p = 3.01\times10^5\, n(\alpha n_a)^{1/2}\, A\, D^{1/2}\, C_{ox.}\, v^{1/2} \text{ ---------------} (3.8)$$

The plots of i_p versus square root of scan rate ($v^{1/2}$) give linear relationships slightly deviating from the origin, Fig. (7). This behavior confirming that the electrode process of nimesulide is mainly controlled by diffusion with some adsorption contribution.

3.1.2.i- Kinetic parameters of the electrode reaction:-

a- Rate constant ($k^{o}_{f,h}$):

In the present work, the rate constant ($k_{f,h}$) was calculated at different pH values from the polarographic data by the method based on Koutecky equation [104]:

$$\bar{i} / i_d = 0.87 \, [(k_{f,h} \, .t^{1/2}) \, D^{1/2}] / 1 + 0.87 \, [k_{f,h} \, .t^{1/2}] \, D^{1/2} \text{---------}(3.9)$$

where $\bar{i}$ is the average current, i_d is the diffusion current, t is the drop time, D is the diffusion coefficient and $k_{f,h}$ is the rate constant at the potential corresponding to the current i.

The rate constant ($k_{f,h}$) can be related to the other kinetic parameters viz. α, the transfer coefficient, n_a the number of electrons involved in the rate-determining step, $k^{o}_{f,h}$, the rate constant at E=0 vs. NHE, and E is the electrode potential vs. NHE by the relation:

$$k_{f,h} = k^{o}_{f,h} \exp \, [-\alpha n_a \, F / RT] \, E \text{----------------------}(3.10)$$

plot of ($k_{f,h}$) against the potential (E) is a straight line with reciprocal slope proportional to αn_a which can then be evaluated. Issa et al. [45-46] gave a simplified relationship for calculating the rate constant ($k_{f,h}$) which is based on the Koutecky [104] equation :

$$(k_{f,h}) = (i / i_d - i) \, x \text{---------------------------------}(3.11)$$

where x is given by :

$$x = D^{1/2} / 0.87 \, t^{1/2}$$

and

$$\log k_{f,h} = \log \, (i / i_d - i) + \log x \text{----------------------}(3.12)$$

b- Activation Free Energy (ΔG^*):

From the absolute rate theory [105], the activation free energy can be obtained . for a totally irreversible wave, the activation energy ΔG^* can be related to the rate constant ($k_{f,h}$) at potential E according to the equation :

$\Delta G^* = (RT/0.434) \log K_1T/h + \log \delta + (\alpha n_a F/2.303) E - \log k_{f,h}$--------(3.13)

Where K_1 is the Boltzmann constant, h is the Plank's constant and δ is a factor which can be equal to 2×10^{-8} cm. By substituting the numerical values of the constants in the above equation, at 298 ° K, we obtain:

$\Delta G^* = 5778.8 [5.097 + (\alpha n_a/0.0591) E - \log k_{f,h}]$----------------(3.14)

At E=0, the equation become :

$\Delta G^* = 5778.8 (5.097 - \log k_{f,h})$--------------------------(3.15)

The rate constant $k^o_{f,h}$ of the electrode reactions of nimesulide is calculated from the data obtained by dc-polarography measurements in Britton-Robinson buffer solution of various pH values. From the data given in table (4). The value of the rate constant $k^o_{f,h}$ decreases with the increasing pH which means that the electrode reaction becomes more difficult and more irreversible. Also, the values of ΔG^* increases with increasing pH of the electrolysis medium. This may be explained on the basis that the protonation of the molecule, at lower pH, before its diffusion to the electrode surface, takes place through the contribution of the H^+ directly with the depolarizer molecule which decreases the activation energy ΔG^*. The H^+ ion activity decreases with rise of pH, hence ΔG^* increases. In alkaline media, the proton required for pre-protonation of the depolarizer is supplied from a water molecule which requires much higher energy.

The electrode reaction mechanism:-

To study the reduction mechanism of nimesulide at mercury electrode, it is necessary to calculate the number of electrons consumed in the electro-reduction process using Ilkovic equation.

The apparent diffusion coefficient (D^o) of the drug can be determined using the following equation [106] :

$$D^{o} = 3.22\times10^{-5} / (V_{m}) \quad , \mathrm{cm}^{2}\,\mathrm{s}^{-1} \text{ ---------------------------}(3.16)$$

Where V_m is the apparent molar volume of nimesulide and is equal to molecular weight/density .

The D^o values obtained in aqueous solutions .

In the present investigation, 10% (v/v) ethanol was used. Thus, the viscosity of the medium should be taken into account, the corrected values of D^o was calculated and listed in Table (4).

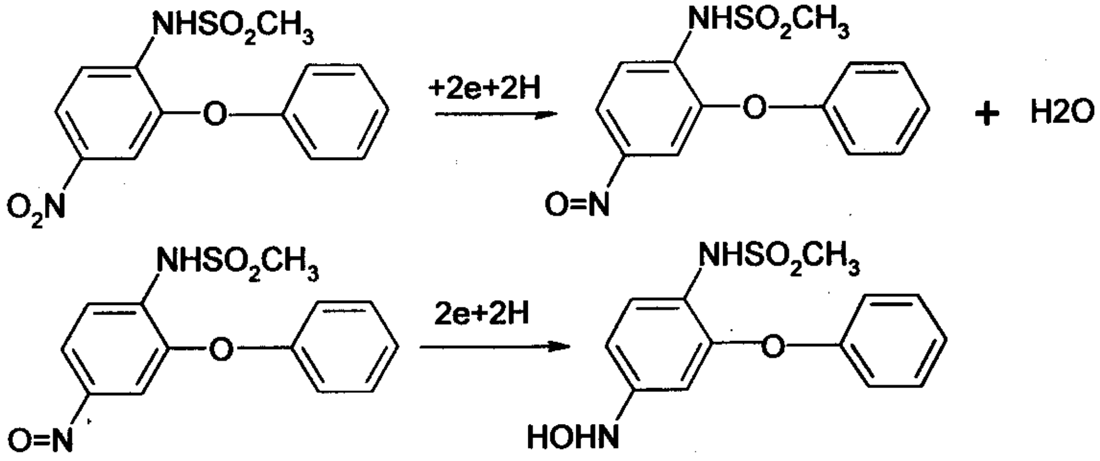
By substituting for the corrected values of (D^o), i_d, t, m and C in Ilkovic equation, it was found that four electrons are consumed in the reduction process of $-NO_2$ center of nimesulide druge in both acidic and alkaline solutions .

These results were confirmed using controlled potential coulometry at large mercury pool-cathode. The amount of charge (Q) was directly taken from a digital coulometer. The electrolysis potential was taken at the limiting current value. By applying the following equation for (Q) we can determined n :

$$Q = nFW/M \text{ ---------------------------------------}(3.17)$$

Where W is the weight of nimesulide (grams) and M its molecular weight .

The average value of n was found to be (4 ± 0.2) electrons/molecule were consumed in both the forgoing results of DC-polarography and cyclic voltammetry and in terms of nimesulide structural gensis the following electrode reaction mechanism was proposed:



3.1.3. Cathodic adsorptive stripping voltammetry (CAdSV) of nimesulide:-

In this part nimesulide is determined in pure and in pharmaceutical dosage forms using linear scan stripping voltammetry technique by optimizing both the experimental and instrumental conditions. The experimental conditions were optimized in terms of studying the effect of nature of supporting electrolytes, buffer solutions, as well as the effect of pH values. Instrumental conditions were optimized by studying the influence of deposition time (t_d), deposition potential (E_d), step height and scan rate on the stripping peak current of nimesulide. The results of both DC-polarographic and cyclic voltammetric studies at the glassy carbon electrode indicated the presence of some adsorption and association contribution of nimesulide at the electrode surface.

Based on the previous electrochemical data, cathodic adsorptive stripping voltammetry (CAdSV) is used for the quantification of nimesulide in pure and applied dosage forms.

3.1.3.i- Effect of supporting electrolyte and pH:

The adsorptive stripping current was measured as a function of pH over 2.57-8.18 range, the best signal was obtained at pH 5.30, which was selected as optimal value for subsequent studies. The variation of CAdSV peak current with pH, obtained for 5x 10^{-5} M nimesulide accumulated for 240 s. at potential of -0.4V is shown in Fig. (8).

The nature and acidity of the supporting electrolytes are some of the most important factors which strongly influence the analyte and its cathodic reduction and adsorption processes.

A number of supporting electrolytes were used, Fig. (9) they are namely KCl, $NaClO_4$ (pH=6.66), acetate buffer, phosphate buffer, and Britton-Robinson buffer. Among the various investigated supporting electrolytes, the best voltammetric signal in terms of sensitivity (peak height) and resolution (peak shape) have been secured using Britton- Robinson buffer.

3.1.3.ii- Effect of accumulation time and potential:

The deposition of the analyzed drug on the surface of the glassy carbon electrode is one of the essential conditions for highly sensitive determinations. The effect of accumulation potential on the peak height was examined over the potential range +100 to -800 mV for $5x10^{-5}$ M nimesulide at accumulation time 240 s., Fig. (10a). The relation between peak heights i_p and deposition potential E_d is shown in Fig. (10b). The peak current increased steadeily with decreasing the potential value till it reaches maximum value at E_d = -400 mV, where it decreased sharply after this inflection point. Thus, E_d = -400 mV will be adopted as optimum operational values for the following works as it ensured the highest voltammetric signal .

The effect of accumulation time of the drug was examined in the range 0-360 s. the amount of nimesulide accumulated on the electrode surface increased as the deposition time increased till 60 s, further increase of time resulted in decreasing the peak height. A time of accumulation of 60 s was chosen for analytical purposes. The voltammograms obtained for $5x10^{-5}$ M nimesulide in B. R. buffer solution of pH 5.30 and E_d = -0.4 V are shown in Fig. (11a). The dependence of peak height on accumulation time are illustrated in Fig. (11b).

3.1.3.iii- Effect of scan rate:

The effect of scan rate on the peak current and peak potential was studied. The relationship between i_p or E_p and υ at 240 s. accumulation time shows an increase in the peak current with increasing the scan rate, Fig. (12b). The peak potential was shifted to more negative values with increasing the scan rate.

The scan rate 500 mv s^{-1} was used for further voltammetric determination of the drug.

3.1.3.iv- Effect of step height:-

The effect of step height on the CAdSV peak current of nimesulide reduction was recorded at different values, Fig. (13a).

A well definit peak was observed at 5 mV step height. On increasing step height up to 150 mV the CAdSV peak current decreases till it becomes ill-defined. From the plot of peak current (i_p) versus step height it noted that i_p is inversely proportional to step height, Fig. (13b).

3.1.3.v- Calibration graph, detection limit and precision to nimesulide:-

A stock solution of nimesulide $1x10^{-3}$ M was prepared in absolute ethanol and the various concentrations in range from 10^{-4} to 10^{-7} M were obtained by accurate dilution. The relation between peak current (i_p) and concentration was represented by the straight line equation .

$$ip = aC + b \qquad \text{--------------------------}(3.18)$$

Where a and b are the slope and the intercept of straight line

The calibration graph of nimesulide was recorded at the optimum conditions from B. R. buffer solution of pH= 5.30 accumulation for 60 sec. period at a potential -0.4 V. The CAdSV peak current of nimesulide was recorded using step height 5 mV and scan rate (υ) 500 mV/sec. For accurate measurements, each concentration was recorded in four different replicate experiments.

The peak current of nimesulide increases linearly with concentration in the range $4\ x10^{-7} - 8\ x10^{-6}$ M (Fig. 14a,b).

At concentration higher than $8\ x10^{-6}$ M, a curvature of the calibration graph is observed. Further increases in concentration are accompanied with an increase in the peak current, and another straight line can be obtained in the range $1x10^{-5} - 1\ x10^{-4}$ M .

The first calibration curve is described by the following regression equation :

$$I_p\ (\mu A) = 7.735 + 2.343\ x10^{6}\ C\ (M)\ ; \qquad r = 0.9946\ , n = 8$$

where i_p is the peak current and C is the concentration.

The detection limit was calculated using the following equation [107]:

$$dl = 3\ (s.d/a)$$

s.d the standard deviation of the blank sample and a is the slope of the calibration curve so the detection limit (dl) of nimesulide at the lower concentrations is 2.8 x10^{-7}. the precision data expressed as relative standard deviation (R.S.D), was evaluated for different concentrations of nimesulide in the first calibration curve (I), Table (6).

The second calibration curve (II) can be described by the following regression equation:

$$I_p\ (\mu A) = 26.51 + 2.495 \times 10^5\ C\ (M); \qquad r = 0.9906, n = 6$$

Determination in sulide tablets dosage form:-

The porposed voltammetric method was applied to the determination of nimesulide in its tablets (sulide tablets labled to contain 100 mg nimesulide per tablet). The recoveries were calculated with reference to the calibration graph. As can be seen from the results shown in Table (7), the method gave satisfactory recovery data. The statistical calculations for the assay results show good precision of the porposed method.

The percentage recoveries based on the avarege of six separate determinations were 99.68±1.74 .

Calibration in serum:-

The voltammetric method suggested could be successfully applied using standard addition method to determination of nimesulide in spiked serum samples over the specific concentration range. The applicability of CAdSV procedure wad evaluated by estimating its recovery from spiked human serum samples. The results obtained by this recovery test for nimesulide have a mean recovery of 98.37% and 98.62% with standard deviation of ± 2.10 and ± 3.45 for 1.84 and 6.14 µg of nimesulide in 10 ml of serum samples, respectively. Accordingly, the preliminary results in spiked serum samples suggest that this methodology may have application in the assay of the drug in biological fluids such as plasma.

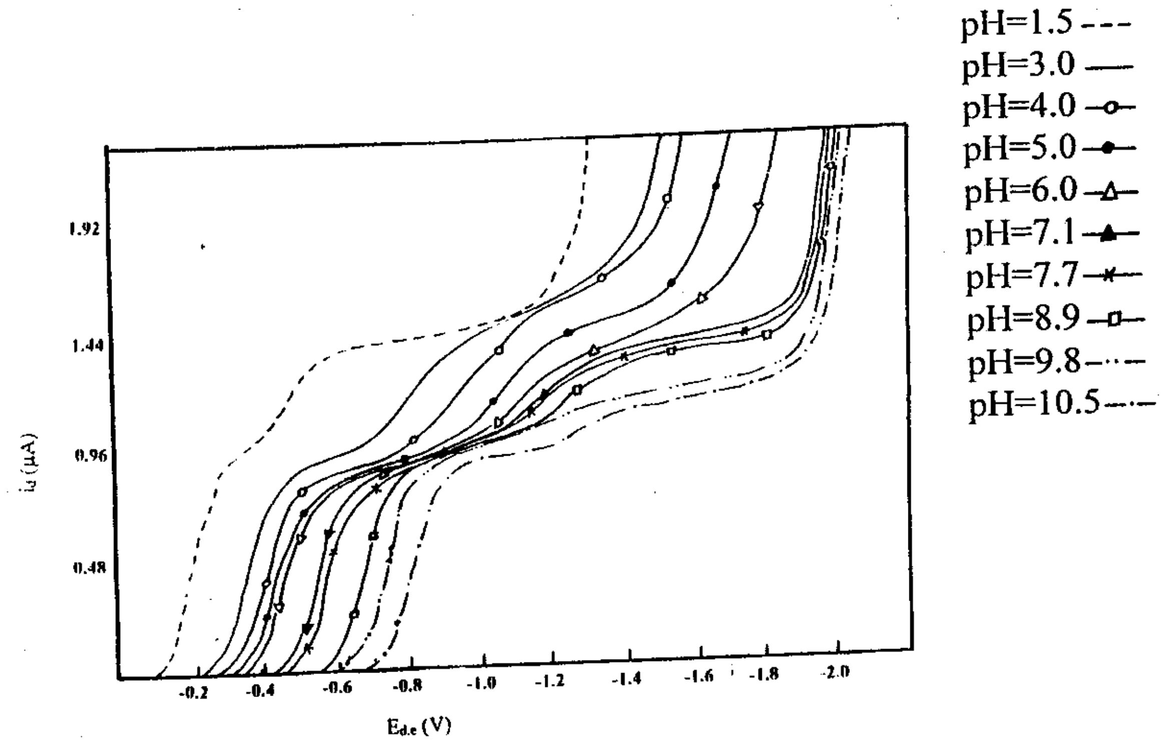


Fig. (1): DC-polarograms of $1x10^{-4}$ M of nimesulide in B.R. buffer solution of different pH values

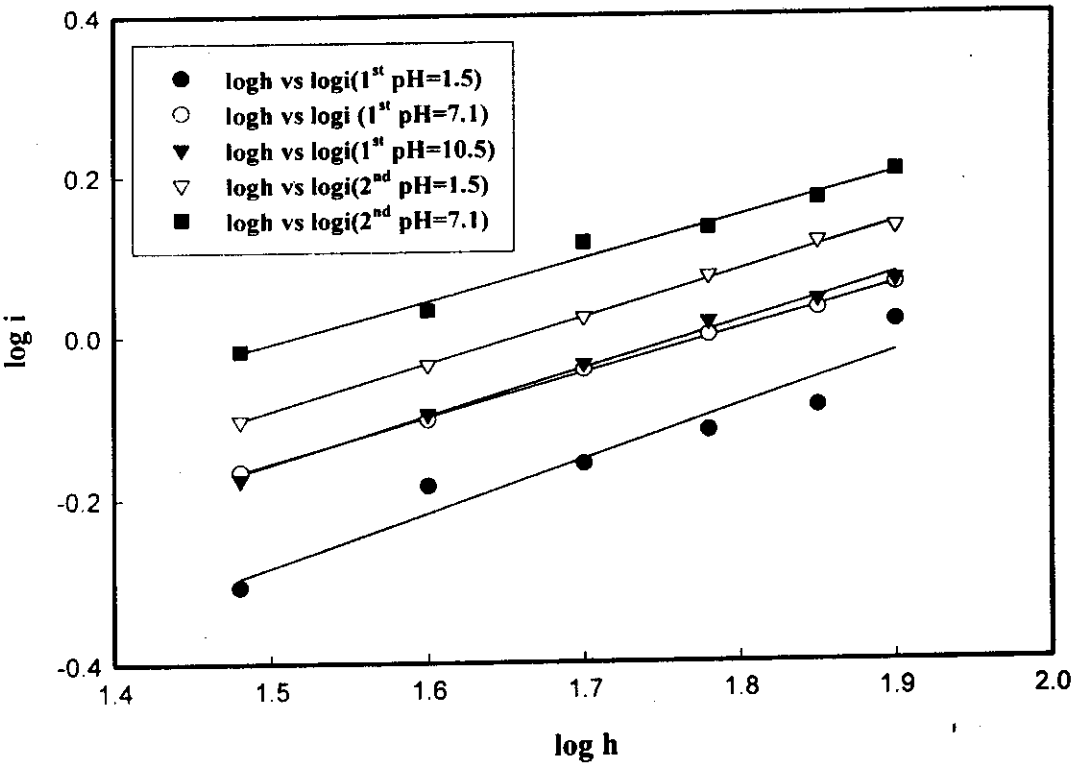


Fig. (2): logi-logh plots of Nimsulide at different pH values.

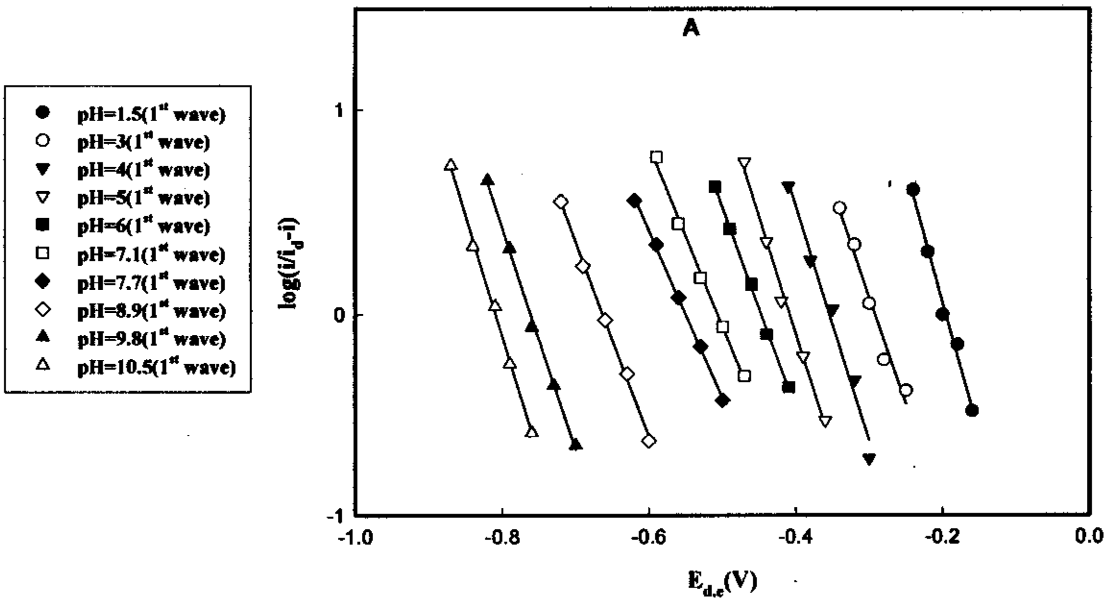


Fig. (3a): Analysis of the polarographic waves of Nimsulide at different pH values.

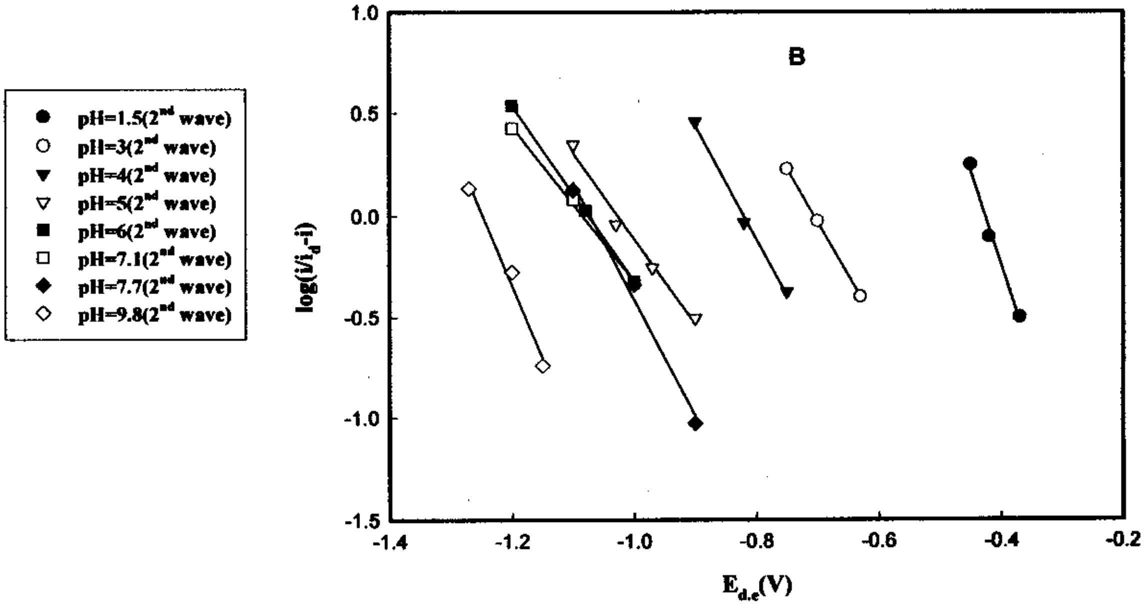


Fig. (3b): Analysis of the polarographic waves of Nimsulide

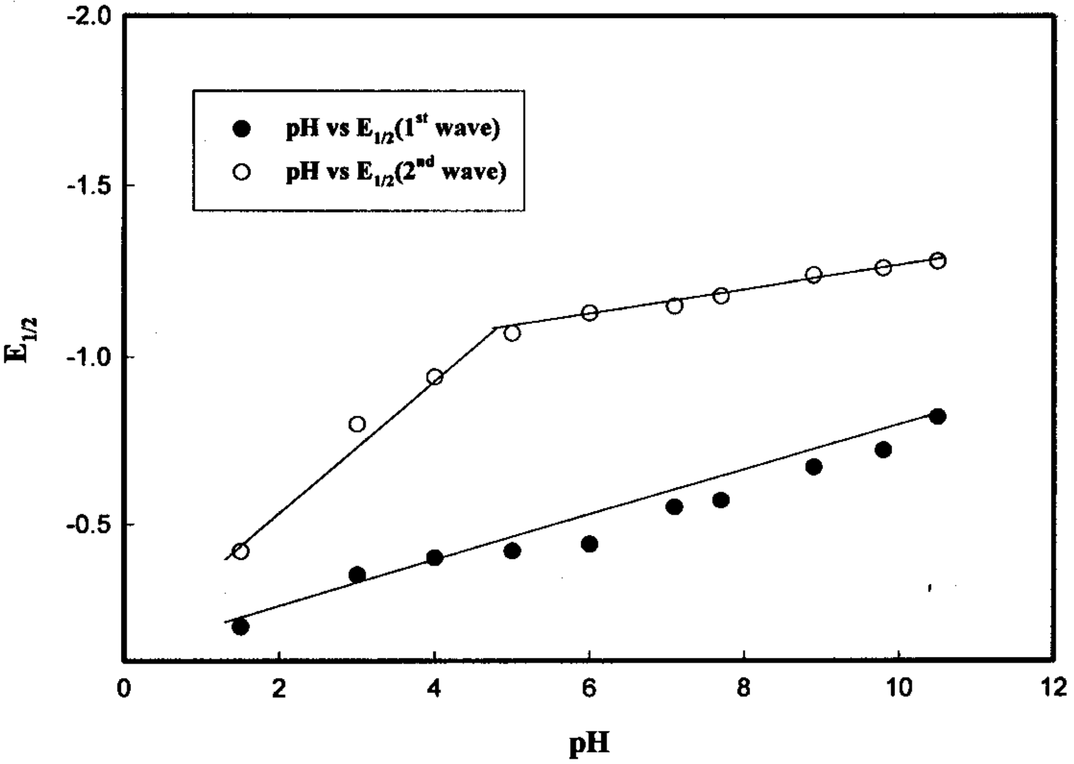
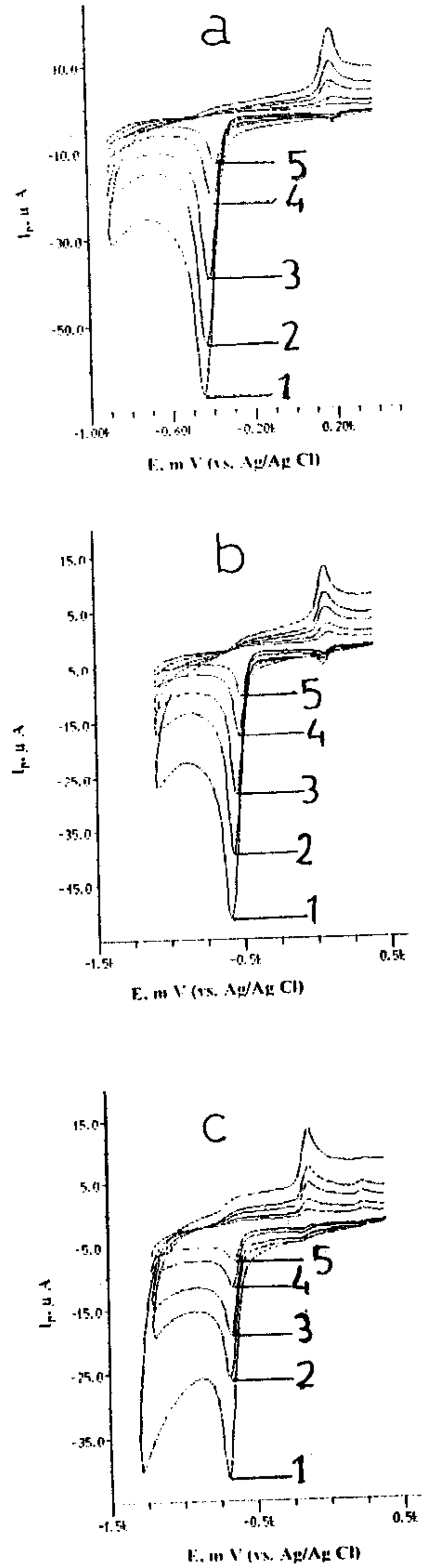


Fig. (4): $E_{1/2}$-pH plots of Nimsulide



1) scan rate=500 mV/s
2) scan rate=300 mV/s
3) scan rate=200 mV/s
4) scan rate=100 mV/s
5) scan rate=50 mV/s

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4) scan rate=100 mV/s
5) scan rate=50 mV/s

Fig. (5): Cyclic voltammograms of 1x 10-4 M of nimesulide in B.R. Buffer solution at different scan rates; (a) pH=3.10, (b) pH=4.60 (c) pH=7.34.

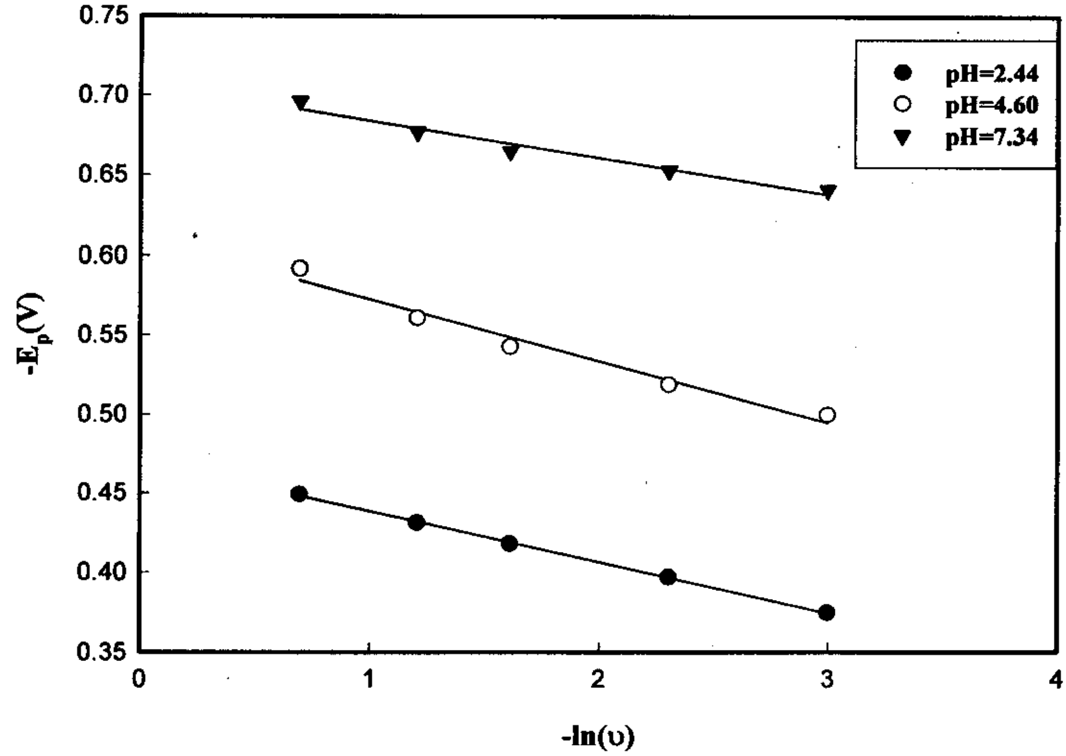


Fig. (6): E_p-ln(υ) plots of Nimesulide at different pH values.

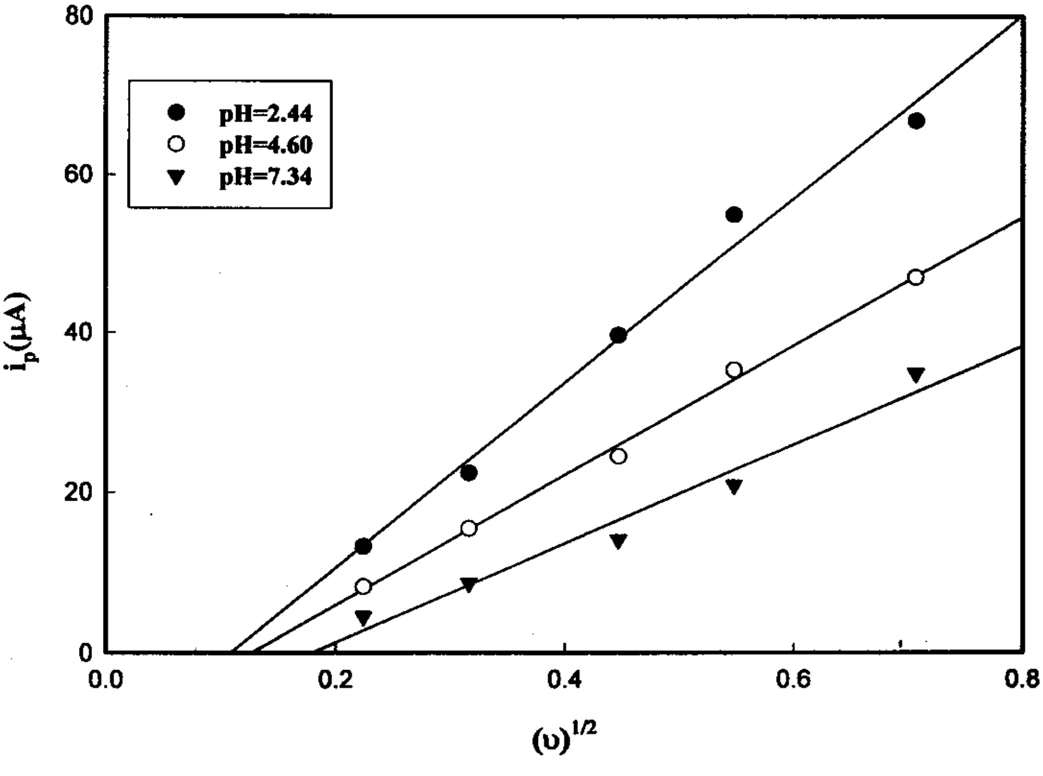


Fig. (7): i_p-$(\upsilon)^{1/2}$ plots of Nimesulide at different pH values.

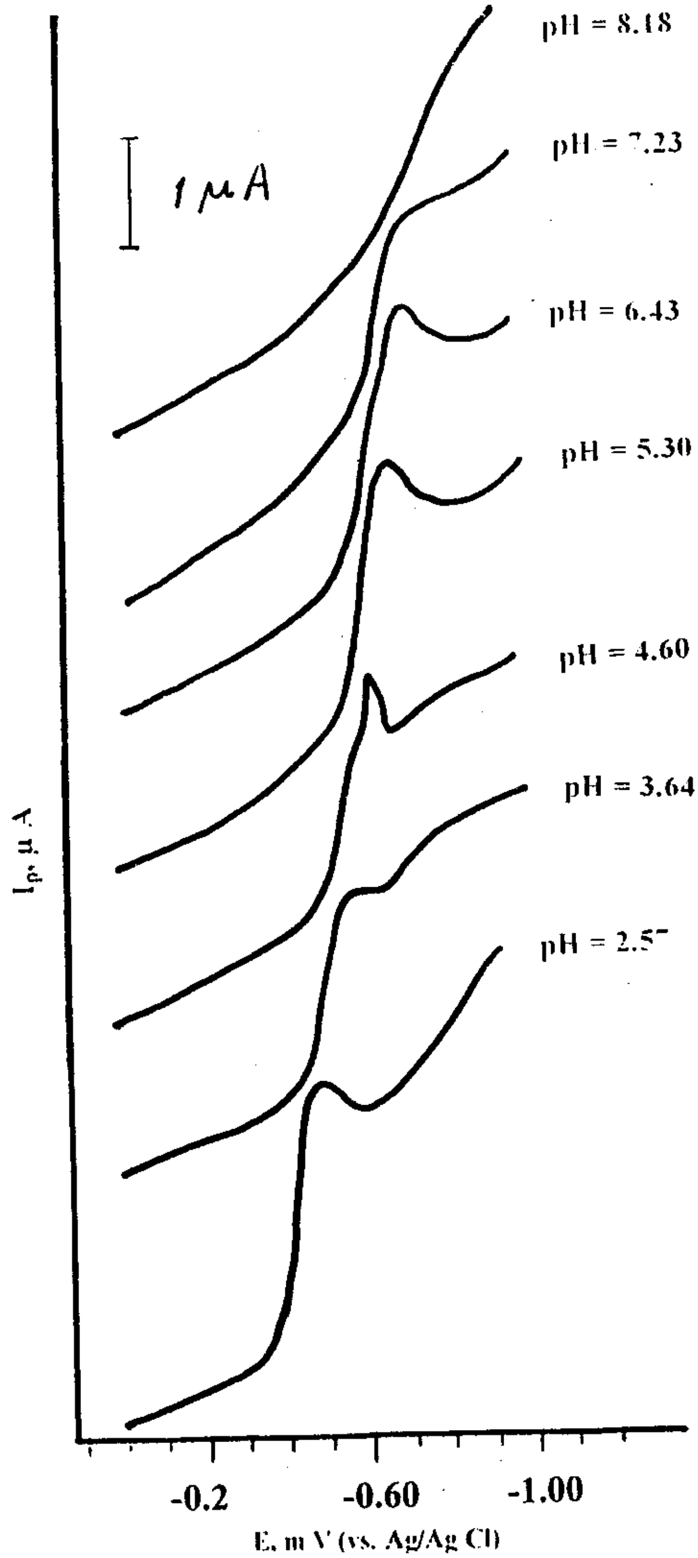


Fig. (8): Effect of pH on the CAdSV peak of $5x10^{-5}$ M of nimesulide in B.R. Buffer solution at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.

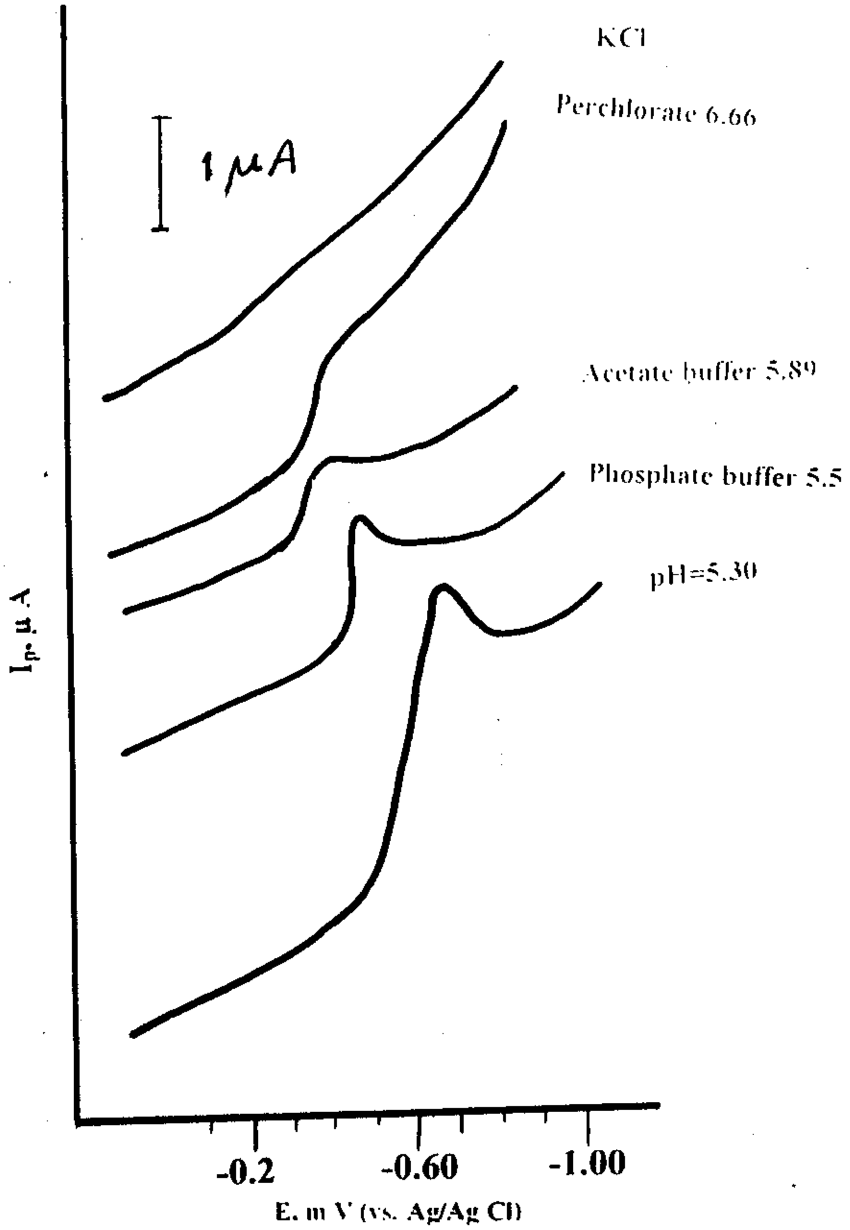


Fig. (9): Effect of different supporting electrolyte solution on the CADS peak of $5x10^{-5}$ M of nimesulide at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.

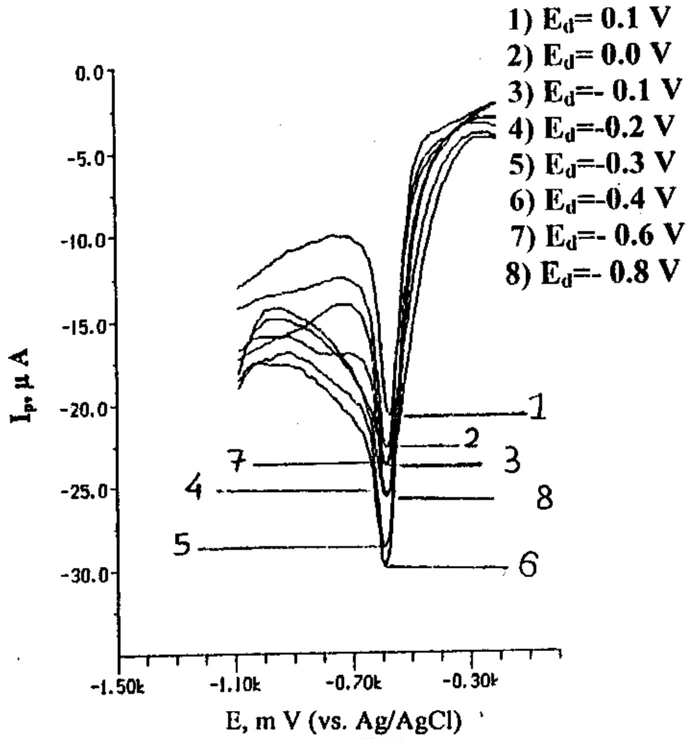


Fig. (10a): Effect of deposition potential on 5x10^{-5} M of nimsulide in B.R. buffer solution of pH=5.30 at:t$_d$=240 s, scan rate=100 mV/s,and pulse height=5 mV

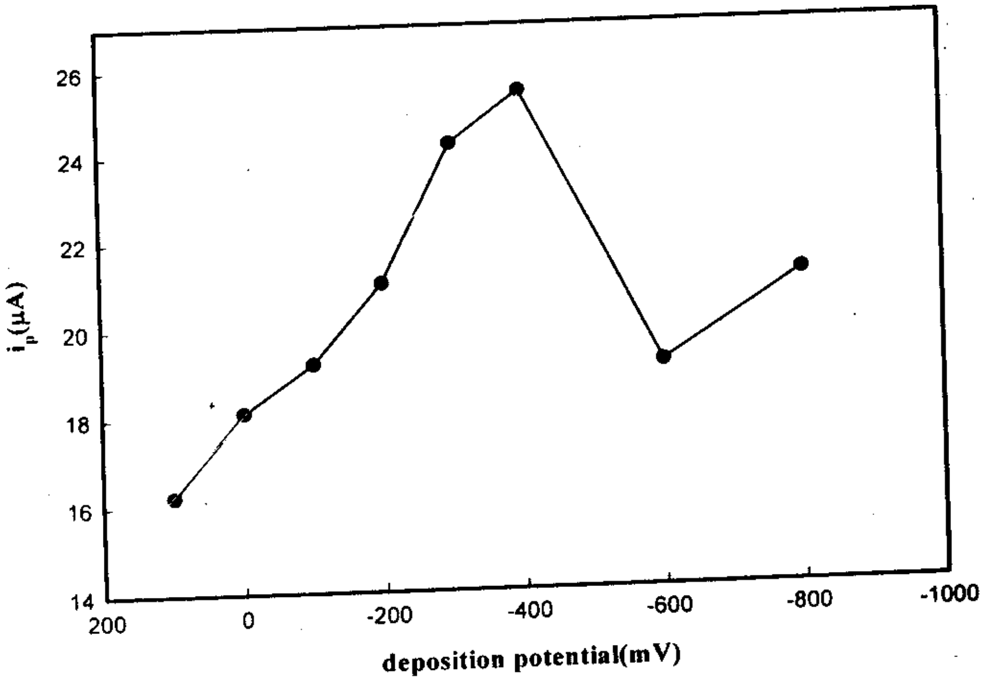


Fig. (10b): Effect of deposition potential on 5x10^{-5}M of Nimsulide in B.R. buf solution of pH=5.30 at:t$_d$=240s,scan rate=100 mV/s,and pulse height=5

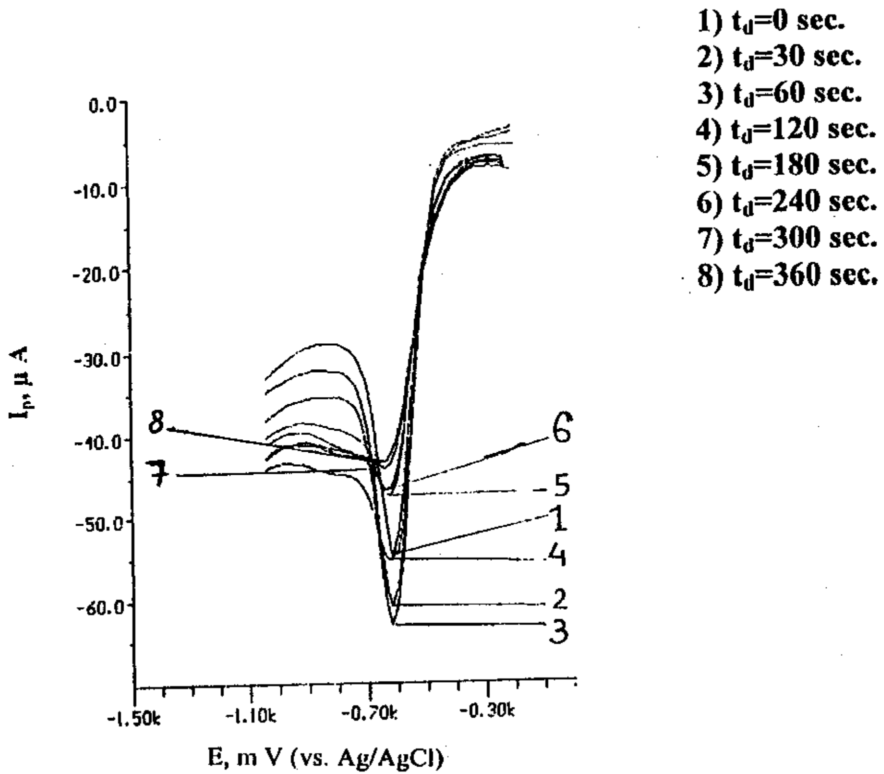


Fig. (11a): Effect of deposition time on 5x10^{-5} M of Nimsulide in B.R. buffer solution of pH=5.30 at:Ed= -0.4 V, scan rate=500mV/s, and pulse height=5 mV

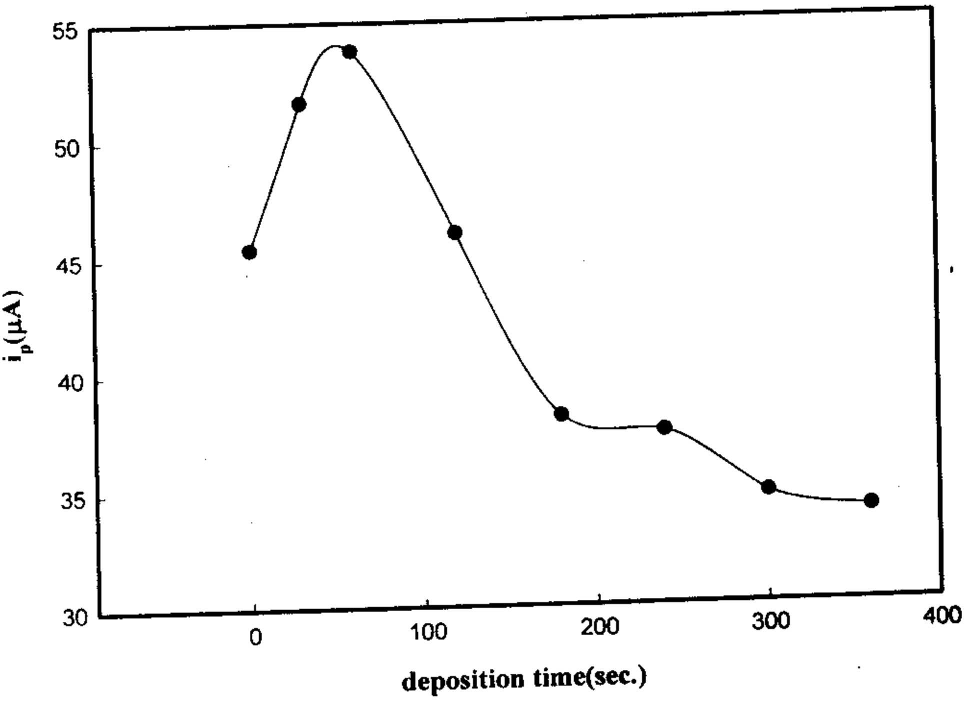


Fig. (11b): Effect of deposition time on 5x10^{-5}M of Nimsulide in B.R. buffer solution of pH=5.30 at:E_d= -0.4 V, scan rate=500 mV/s, and pulse height=5 m

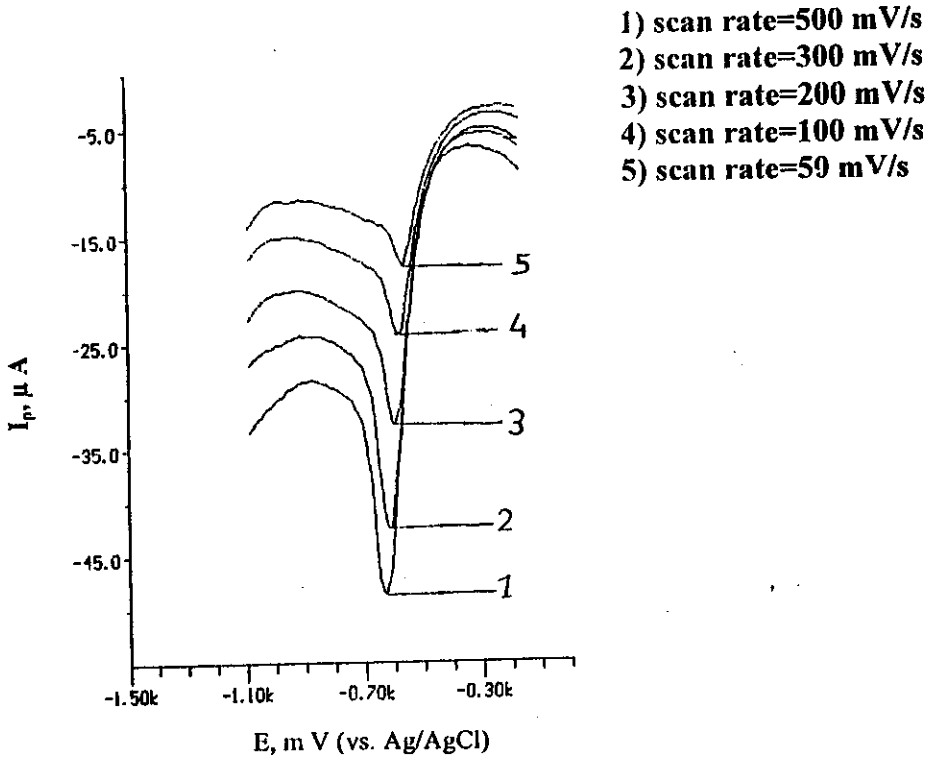


Fig. (12a): Effect of scan rate on 5x10^{-5} M of Nimsulide in B.R. buffer solution of pH=5.30 at:td=240 s,E$_d$=-0.4V, and pulse height=5 mV

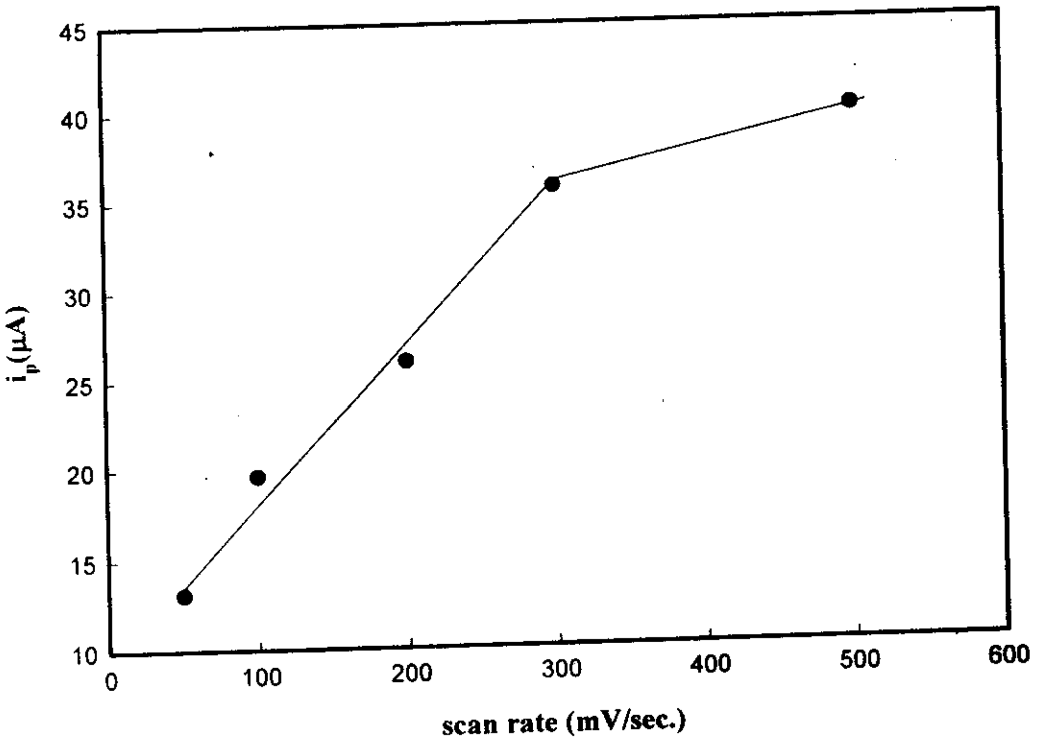


Fig. (12b): Effect of scan rate on 5x10^{-5}M of Nimsulide in B.R. buffer solution of pH=5.30 at:t$_d$=240 s,E$_d$=-0.4V, and pulse height=5 mV

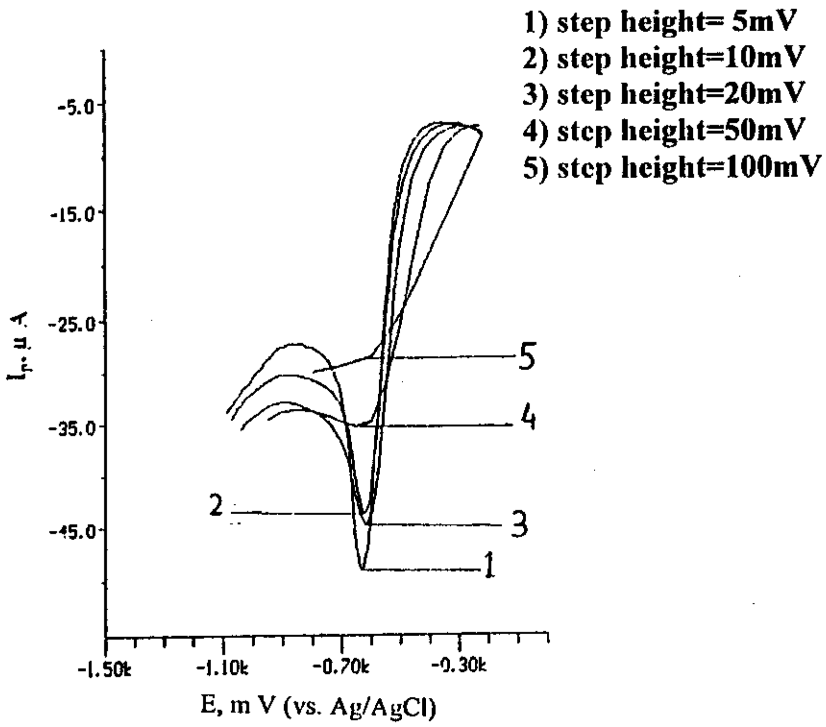


Fig. (13a): Effect of step height on 5x10-5M of Nimsulide in B.R. buffer solution of pH=5.30 at: td=240s,scan rate=500mV/s,and Ed=-0.4V

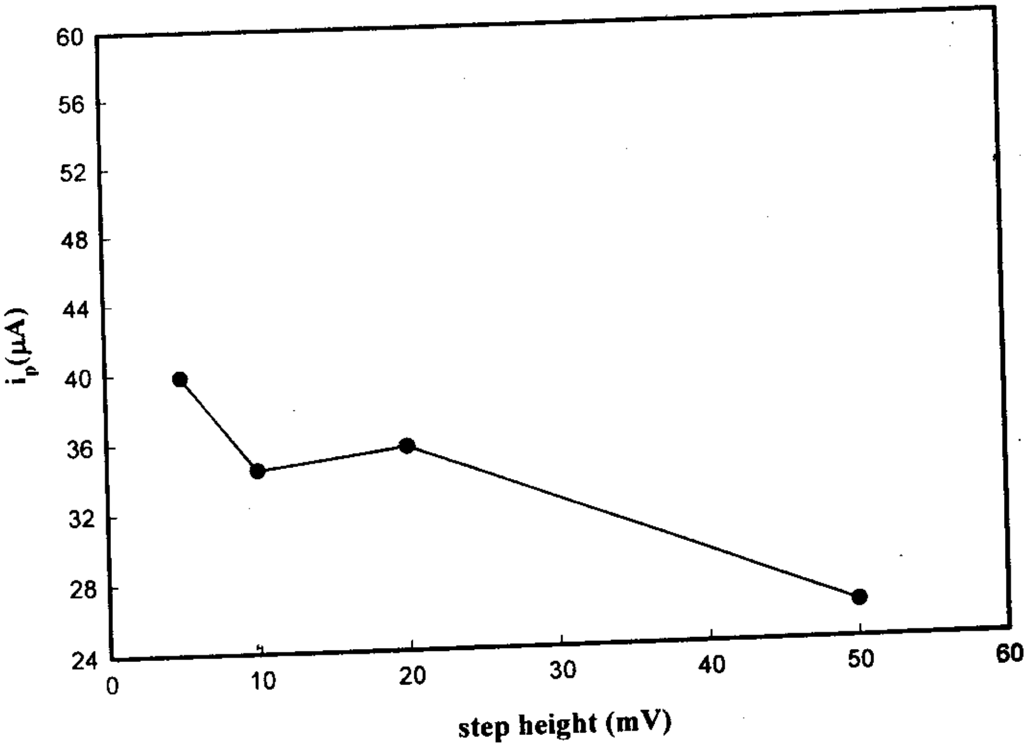


Fig. (13b): Effect of step hieght on $5x10^{-5}$M of Nimsulide in B.R. buffer solution of pH=5.30 at:t_d=240s,scan rate=500 mV/s,and E_d=-0.4V

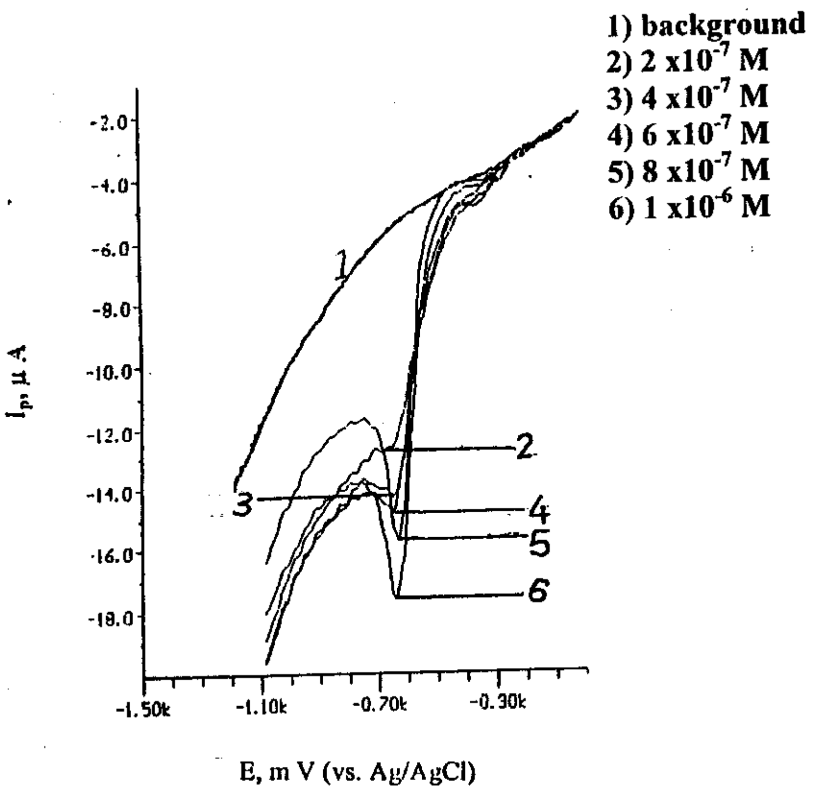


Fig. (14a): Effect of concentration of nimesulide in B.R. buffer solution of pH=5.30 at: td=60 s, Ed= -0.4 V, scan rate 500 mV/s and step height= 5 mV

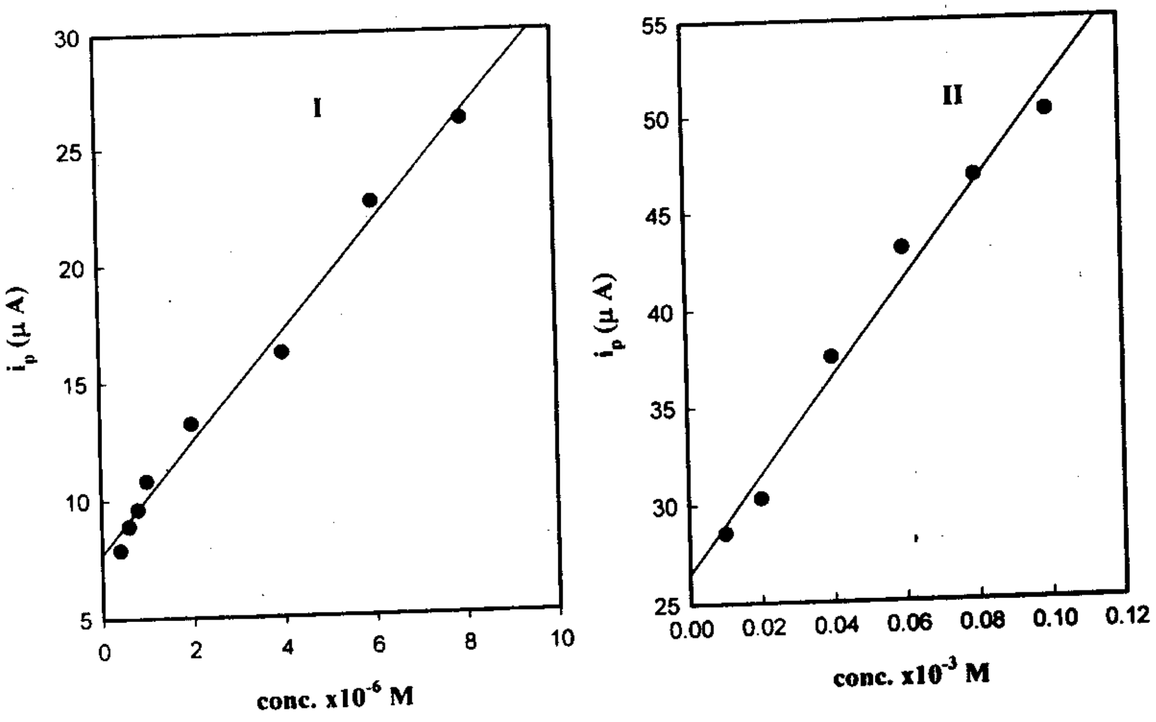


Fig. (14b): calibration curve for nimesulide

Table (1) : polarographic data for 1x10^{-4} M of nimesulide in B.R. buffer solution of different pH values at 25°C

pH	i_d(μA)		$-E_{1/2}$		Δ $\log i_l$/Δ log h	
	A	B	A	B	A	B
1.50	0.99	0.45	0.20	0.42	0.65	0.58
3.00	0.96	0.50	0.35	0.80		
4.00	0.88	0.58	0.40	0.94		
5.00	0.85	0.46	0.42	1.07		
6.00	0.83	0.40	0.44	1.13		
7.10	0.86	0.37	0.55	1.15	0.55	0.53
7.70	0.85	0.35	0.57	1.18		
8.90	0.90	0.32	0.67	1.24		
9.80	0.88	0.22	0.72	1.26		
10.50	0.90	sh.	0.82	1.28	0.59	sh.

Where A:1st wave B: 2nd wave sh: ill-defined

Table(2a) : Values of S_1, αn_a and α for $1x10^{-4}$ M of nimesulide in B.R buffer solution of different pH values, as calculated from DC-polarographic analysis.

pH	Slope(S_1) mV		αn_a		α			
					n=1	n=1	n=2	n=2
	A	B	A	B	A	B	A	B
1.50	76.75	108.34	0.77	0.55	0.77	0.55	0.39	0.28
3.00	95.51	191.20	0.62	0.31	0.62	0.31	0.31	0.16
4.00	86.88	179.86	0.68	0.33	0.68	0.33	0.34	0.17
5.00	87.87	237.53	0.67	0.25	0.67	0.25	0.34	0.13
6.00	101.83	233.64	0.58	0.25	0.58	0.25	0.29	0.13
7.10	114.16	264.55	0.52	0.22	0.52	0.22	0.26	0.11
7.70	122.70	173.61	0.49	0.34	0.49	0.34	0.24	0.17
8.90	100.50	152.20	0.57	0.39	0.57	0.39	0.29	0.20
9.80	91.91	138.89	0.64	0.43	0.64	0.43	0.32	0.22
10.50	84.75	90.100	0.70	0.66	0.70	0.66	0.35	0.33

Where A:1st wave B: 2nd wave sh: ill-defined

Table (2b): Values of (Z_H^+) and (α) as determined for different ratios (Z_H^+/n_a) at 0.5, 1.0 and 2.0. The number of protons calculated from slope (S_2) of the $E_{1/2}$-pH plots and (S_1) for nimesulide in B.R. buffer solutions of different pH values at 25°C.

pH	S_1 mV		S_2 mV		$(Z_H^+)=(S_2/S_1)$		Z_H^+/n_a		
							0.5	1.0	2.0
	A	B	A	B	A	B			
1.50	76.75	108.34	66	93	0.86	0.86	0.45	0.89	1.79
3.00	95.51	191.20	66	93	0.69	0.49	0.45	0.89	1.79
4.00	86.88	179.86	66	93	0.76	0.52	0.45	0.89	1.79
5.00	87.87	237.53	66	93	0.75	0.34	0.45	0.89	1.79
6.00	101.83	233.64	66	105	0.65	0.45	0.45	0.89	1.79
7.10	114.16	264.55	66	105	0.58	0.40	0.45	0.89	1.79
7.70	122.70	173.61	66	105	0.54	0.60	0.45	0.89	1.79
8.90	100.50	152.20	66	105	0.66	0.69	0.45	0.89	1.79
9.80	91.91	138.89	66	105	0.72	0.76	0.45	0.89	1.79
10.5	84.75	90.100	66	105	0.78	1.10	0.45	0.89	1.79

Where A:1st wave B:2nd wave

Table (3): Data obtanied for 1x10^{-4} M of nimesulide in B.R. buffer solution of different pH values using cyclic voltammetry at different scan rates, at 25°C.

pH	Scan rate (mV/s)	-Ep (V)	-dEp/d(lnv)	αna
3.10	500	0.449	0.032	0.40
	300	0.4314		
	200	0.418		
	100	0.397		
	50	0.375		
4.60	500	0.592	0.0390	0.33
	300	0.561		
	200	0.543		
	100	0.519		
	50	0.500		
7.34	500	0.696	0.0231	0.56
	300	0.677		
	200	0.665		
	100	0.653		
	50	0.641		

Table (4): Kinetic parameters obtained from D-C measurements for 1×10^{-4} M nimesulide in B. R. buffer solution of different pH's.

pH	D°(Cm²/s)	$K^{o}_{f,h}$ (Cm/s)	ΔG* (K.cal/mole)
1.50	1.90×10^{-6}	5.00×10^{-9} [a]	77.34 [a]
		2.00×10^{-9} [b]	79.41 [b]
3.00	1.90×10^{-6}	1.00×10^{-9} [a]	79.90 [a]
		1.00×10^{-9} [b]	75.64 [b]
4.10	1.90×10^{-6}	1.20×10^{-10} [a]	86.78 [a]
		7.76×10^{-10} [b]	82.12 [b]
5.00	1.40×10^{-6}	7.76×10^{-11} [a]	87.89 [a]
		7.00×10^{-10} [b]	82.36 [b]
6.00	1.40×10^{-6}	5.01×10^{-11} [a]	88.98 [a]
		3.00×10^{-10} [b]	48.48 [b]
7.10	1.40×10^{-6}	2.88×10^{-11} [a]	90.37 [a]
		1.30×10^{-10} [b]	86.58 [b]
7.70	1.30×10^{-6}	5.75×10^{-12} [a]	94.41 [a]
		1.38×10^{-11} [b]	92.21 [b]
8.90	1.30×10^{-6}	4.27×10^{-12} [a]	95.13 [a]
		4.17×10^{-13} [b]	100.98 [b]
9.80	1.10×10^{-6}	7.94×10^{-14} [a]	105.17 [a]
		3.47×10^{-14} [b]	107.22 [b]
10.5	1.10×10^{-6}	5.62×10^{-16} [a]	117.60 [a]
		2.40×10^{-20} [b]	142.82 [b]

Where a : 1st wave , b : 2nd wave .

Table (5) : Cathodic adsorptive stripping peak current (i_p) of $5x10^{-5}$ M nimesulide in B.R.buffer solution of pH=5.23 at different conditions (t_d, E_d, scan rate and pulse height).

Deposition time (t_d), sec	Deposition potential(E_d), V	Scan rate (mV/s)	Pulse height (mV)	i_p (µA)
Effect of (td):				
0	-0.4	500	5	45.40
30				51.60
60				53.80
120				46.00
180				38.10
240				37.40
300				34.70
360				34.00
Effect of (E_d):				
240	0.10	100	5	16.20
	-0.00			18.10
	-0.10			19.20
	-0.20			21.00
	-0.30			24.20
	-0.40			25.40
Effect of scan rate:				
240	-0.4	500	5	40.00
		300		35.70
		200		26.00
		100		19.60
		50		13.10
Effect of pulse height:				
240	-0.4	500	5	39.80
			10	34.40
			20	35.60
			50	26.00
			100	
			150	
			200	

Table (6): Calibration curve data of nimesulide in B.R.buffer solution of pH=5.23 using CAdSV at t_d= 60sec., E_d= -0.4 V, scan rate = 500 mV/s, and step height = 5 mV.

Conc. taken	Ip(μA)	Mean	S.D.	**R.S.D. %**
$4x10^{-7}$ M	**7.71**	**7.90**	**0.16**	**2.02**
	8.01			
	8.07			
	7.82			
$6x10^{-7}$ M	**8.71**	**8.90**	**0.18**	**2.02**
	8.84			
	8.94			
	9.14			
$8x10^{-7}$ M	**9.63**	**9.63**	**0.03**	**0.31**
	9.63			
	9.71			
	9.68			
$1x10^{-6}$ M	**10.72**	**10.80**	**0.07**	**0.69**
	10.85			
	10.88			
	10.76			
$2x10^{-6}$ M	**13.40**	**13.24**	**0.21**	**1.59**
	13.40			
	13.22			
	12.95			
$4x10^{-6}$ M	**16.32**	**16.20**	**0.05**	**0.31**
	16.38			
	16.20			
	15.90			
$6x10^{-6}$ M	**22.74**	**22.55**	**0.04**	**0.18**
	22.60			
	22.66			
	22.50			
$8x10^{-6}$ M	**25.88**	**26.11**	**0.24**	**0.91**
	26.15			
	26.18			
	26.22			

Table (7): Assay of nimesulide in dosage form in B.R.buffer solution at pH=5.23 using CAdSV at t_d= 60sec., E_d= -0.4 V, scan rate = 500mV/s, and step height= 5mV.

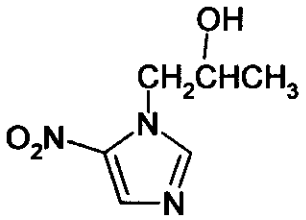
Conc.taken	Conc. calculated	Recovery %	Mean Recovery(%) ± S.D.
$2x10^{-7}$ M	$1.94x10^{-7}$M	97.00	99.75 ± 2.51
	$2.20 x10^{-7}$M	99.00	
	$2.06 x10^{-7}$M	103.0	
	$2.00x10^{-7}$M	100.0	
$4x10^{-7}$ M	$3.96x10^{-7}$M	99.50	101.18 ± 1.48
	$4.10x10^{-7}$M	102.50	
	$4.12x10^{-7}$M	103.00	
	$3.99 x10^{-7}$M	99.75	
$6x10^{-7}$ M	$5.90 x10^{-7}$M	98.33	99.72 ± 1.40
	$5.90 x10^{-7}$M	98.33	
	$6.06 x10^{-7}$M	101.0	
	$6.10 x10^{-7}$M	101.3	
$8x10^{-7}$ M	$7.78 x10^{-7}$M	97.25	98.53 ± 2.05
	$8.00x10^{-7}$M	100.0	
	$7.85x10^{-7}$M	98.13	
	$7.90 x10^{-7}$M	98.75	
$1x10^{-6}$ M	$1.00 x10^{-6}$M	100.0	99.25 ± 1.29
	$0.99 x10^{-6}$M	99.00	
	$1.00 x10^{-6}$M	100.0	
	$0.98x10^{-6}$M	98.00	

Table (8) : Assay of nimesulide in serum in B.R. buffer solution of pH=5.30 using CAdSV at td= 60 sec., Ed= -0.4 V, scan rate= 500 mV/s, and step height= 5 mV.

Conc.taken µg/10ml	Conc.found µg/10ml	Recovery %	Mean Recovery(%) ± S.D.
1.84	1.84	100.0	98.37± 2.10
	1.81	98.37	
	1.78	96.74	
	1.84	100.0	
6.14	6.05	98.50	98.13± 3.45
	6.05	98.50	
	5.83	95.00	
	6.29	102.5	

3.2. Electrochemical behavior of secnidazole in B.R buffer solutions of different pH values:-

secnidazole is [α,2-dimethyl-5-nitro-1H-imidazole 1-ethanol] and has the following structural formula :



This part includes the study of the electrochemical behavior of secnidazole at Hg-electrode using DC polarography and cyclic voltammetry techniques. The study aimed to optimize the experimental and instrumental conditions for determination of the lowest possible concentration of the investigated pharmaceutical compound in pure and dosage forms using cathodic adsorptive stripping voltammetry.

3.2.1 DC polarography:-

3.2.1. i-Current potential curves:-

The polarographic behavior of $2x10^{-4}$ M of secnidazole was studied in B.R. buffer solutions containing 10% (v/v) ethanol of different pH values in the range from 2 to 12 . The polarogrammes exhibit two reduction waves within the pH range studied corresponding to the reduction of NO_2 group (nitro-group), Fig. (15). The half-wave potential ($E_{1/2}$) of the reduction waves is pH dependent, and shifted to more negative values on increasing the pH of the electrolysis solution .

3.2.1. ii-Effect of pressure at mercury height:-

The effect of mercury height "h" was examined according to equation (3.1). The plots of log i_l against log h for secnidazole showed linear correlations at different pH values, Fig. (16). The slope values (x) are found in the range between 0.53 and 0.60 which indicate that the reduction process is controlled by diffusion and some adsorption contribution, Table (9).

3.2.1. iii-Analysis of the polarographic waves:-

Analysis of the polarographic waves is quite important to evaluate the degree of reversibility of electrode reaction and to suggest the electrode mechanism On using equation (3.4), the plots of log $[i/(i_d-i)]$ against $E_{d.e.}$ for secnidazole reduction at different pH values showed linear correlations, Fig. (17). The straight lines with reciprocal slopes (S_1) amounting in the range from (62.08 - 88.73) and (108-263) mV for the first and second waves; respectively, Table (10). This behavior revealed the irreversible nature of the electrode process.

3.2.1.iv-Half- wave potential -pH curves :-

The plots of the rate shift of $E_{1/2}$ of secnidazole versus the pH of the electrolysis solution showed linear correlations for both the first and second waves, respectively. The slope values (S_2) of the first and second waves amount to 60.56 and 140 mV, respectively, Table (10).

The number of hydrogen ions (Z_H^+) involved in the rate determining step for secnidazole reduction was calculated from both slopes of logarithmic analysis and $E_{1/2}$ plots according to equation (3.5). At all pH values Z_H^+ was found to be unity, Table (10).

The most probable values of α-parameter as shown in Table (11a), were determined at the probable ratio of (Z_H^+/n_a) using equation (3.6) and the probable (Z_H^+/n_a) ratio was found to fit with (Z_H^+/n_a) = 0.5, Table (10).

This behavior further confirms the irreversibility of electrode process and the rate determining step involves one proton and two electrons.

3.2.2 Cyclic voltammetry:-

The cyclic voltammetric behavior of $1x10^{-4}$ M of secnidazole was investigated in aqueous Britton-Robinson buffer solutions of different pH values (2-12) containing 10% (v/v) ethanol at glassy carbon electrode. The voltammograms displayed single irreversible cathodic peak in the cathodic scan in all media, where as, in the anodic scan there is no oxidation peaks were observed, Fig.(19).

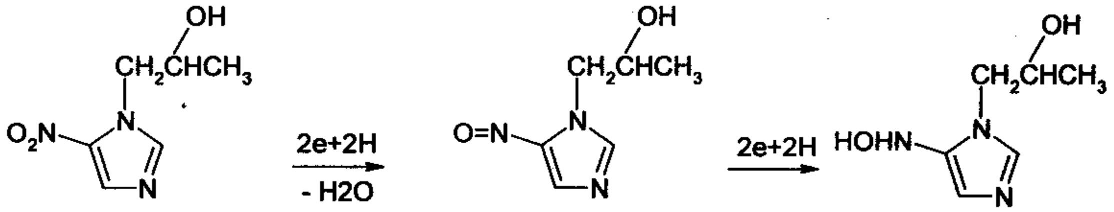
On increasing the scan rate (υ), the cathodic peak potential (E_{pc}) was shifted to more negative potentials indicating the irreversible nature of the reduction peak. Furthermore, on increasing the pH of the electrolysis solution the peak potential (E_{pc}) is shifted to more negative values indicating the consumption of hydrogen ions in the electron transfer step. On applying equation (3.7), the plots of E_p versus ln υ gives linear correlations. Fig. (20). The slope values were used for the determination of αn_a. The values of the (α) transfer coefficient were calculated at the probable n_a values and found to be less than 0.40 at n_a=2.0 Table (11b). This behavior further confirms the irreversible nature of electrode process. According to equation (3.8), the plots of i_p_vs square root of scan rate gives linear relation deviating from the origin, Fig. (21). This behavior confirms that the electrode process of secnidazole is controlled mainly by diffusion with some adsorption contribution.

3.2.2.i- Kinetic parameters of the electrode reaction:-

The rate constant $k^o_{f,h}$ of the electrode reaction of secnidazole is calculated from the data obtained by dc-polarography measurements in Britton-Robinson buffer solution of various pH values. Equation (3.12) was used in this determination and the values of the rate constant $k_{f,h}$ decrease with increasing the pH which means that the electrode reaction becomes more difficult and more irreversible, Table (12). Also, the values of ΔG* were determined using equation (3.14) and increased with increasing pH of the electrolysis medium, Table (12). This may be explained on the basis that the protonation of the molecule, at lower pH, before its diffusion to the electrode surface, takes place through the contribution of the H^+ ion directly with the depolarizer molecule which decreases the activation energy ΔG*. The H^+ ion activity decreases with rise of pH, hence ΔG* increases. In alkaline media, the proton required for pre-protonation of the depolarizer is supplied from a water molecule which requires much higher energy.

The electrode reaction mechanism:-

To study the reduction mechanism of secnidazole at mercury electrode, it is necessary to calculate the number of electrons concumed at this reduction, firstly we should calculate the (diffusion coefficient) D from equation (3.16) and the corrected values shown in Table (12). The number of electrons (n) consumed in the reduction of nitro group in acidic and basic medium were 4 electrons. The number of electrons were confirmed by controlled potential coulmetry method according to equation (3.17) the avarge value of n is (4±0.2) so the suggested mechanism as the following:



3.2.3 Cathodic adsorptive stripping voltammetry(CAdSV) of secnidazole:-

Cathodic adsorptive stripping voltammetry was used for the quantitative determination of secnidazole in pure and dosage forms. The instrumental and experimental conditions were optimized in this investigation. The instrumental conditions are deposition time (td), deposition potential (Ed), step height and scan rate. The experimental conditions includs the nature of supporting electrolyte and pH of the electrolysis solution.

3.2.3.i- Effect of pH and supporting electrolyte:

In cathodic adsorptive voltammetry, current of secnidazole is recorded as function of potential in Britton- Robinson buffer solution of pH (2.44 to 9.22) the recorded peak at various pH values due to the reduction of nitro-group at the surface of glassy carbon electrode and well developed peak is noted at pH=5.23, Fig. (22).

We determined the secnidazole in some supporting electrolyte like (phosphate, acetate, $NaClO_4$ and KCl buffers) we found that in presence of $NaClO_4$,KCl the peak disappeared, it means that the absence of this anion best of secnidazole and the pH=5.23 is most suitable for secnidazole Fig. (23)

3.2.3.ii- Effect of accumulation time and potential:

The deposition of the analyzed drug on the surface of the glassy carbon electrode is one of the essential conditions for highly sensitive determinations. The effect of accumulation potential on the peak height was examined over the potential range +100 to -800 mV for $5x10^{-5}$ M secnidazole at accumulation time 240 s., Fig. (24a). The relation between peaks height i_p and deposition potential E_d is shown in Fig. (24b). The peak current increased steadily with decreasing the potential value till it reaches maximum value at E_d = -200 mV, where it decreased sharply after this inflection point. Thus, E_d = -200 mV will be adopted as optimum operational values for the following works as it ensured the highest voltammetric signal .

The effect of accumulation time of the drug was examined in the range 0-360 s. the amount of secnidazole accumulated on the electrode surface increased as the deposition time increased till 60 s, further increase of time resulted in decreasing the peak height. A time of accumulation of 60 s was chosen for analytical purposes. The voltammograms obtained for $5x10^{-5}$ M secnidazole in B. R. buffer solution of pH 5.23 and $E_d = -0.2$ V are shown in Fig. (25a). The dependence of peak height on accumulation time are illustrated in Fig. (25b).

3.2.3.iii- Effect of scan rate:

The effect of scan rate on the peak current and peak potential was studied. The relationship between i_p or E_p and υ, at 240 s. accumulation time shows an increase in the peak current with increasing the scan rate, Fig. (26a,b). The peak potential was shifted to more negative values with increasing the scan rate.

The scan rate 500 mv s^{-1} was used for further voltammetric determination of the drug.

3.2.3.iv- Effect of step height:-

The effect of step height on the CAdSV peak current of secnidazole reduction was recorded at different values, Fig. (27a).

A well defint peak was observed at 20 mV step height. On increasing step height up to 150 mV the CAdSV peak current decreases till become ill-defined. From the plot of peak current (i_p) versus step height it noted that i_p is inversely proportional to step height, Fig. (27b).

3.2.3.v-Calibration curve, detection limit and precision:

Under the optimum conditions , a good linear correlation was obtained between the monitored voltammetric peak current and secnidazole concentration in the range6 $x10^{-6}$ to 1 $x10^{-4}$ M Fig. (28). Least-squares treatment of the calibration graph yielded the following equation:

$$ip(\mu A) = 3.35 + 90.25 \times 10^{6}\ C\ M; \quad r = 0.9958 \quad n = 8$$

the lowest detectable concentration (dl) of secnidazole was 1.7 x10^{-6} M. The reproducibility of this analytical method was evaluated for different concentrations of the drug, Table (14) .

Analysis of pharmaceutical formulation:

the proposed method was successfully applied to the assay of the studied drug in the pharmaceutical dosage forms (secnidazole tablets, labeled to contain 500 mg per tablet). The percentage recoveries for secnidazole are shown in Table (15). The method gave a good recovery. The percentage recovery based on five different determinations was 100.76 ± 1.89 .

Analysis of serum:

Serum samples were prepared as described before, and two different concentration were analysed using the proposed method (1.03 and 3.42 µg /ml). The recovery and the standard deviation were evaluated from four measurements for each sample, Table (16). The mean recovery for the two different concentrations was 102.01 ± 2.76.

This means that the proposed method can be used for determination of secnidazole in biological samples.

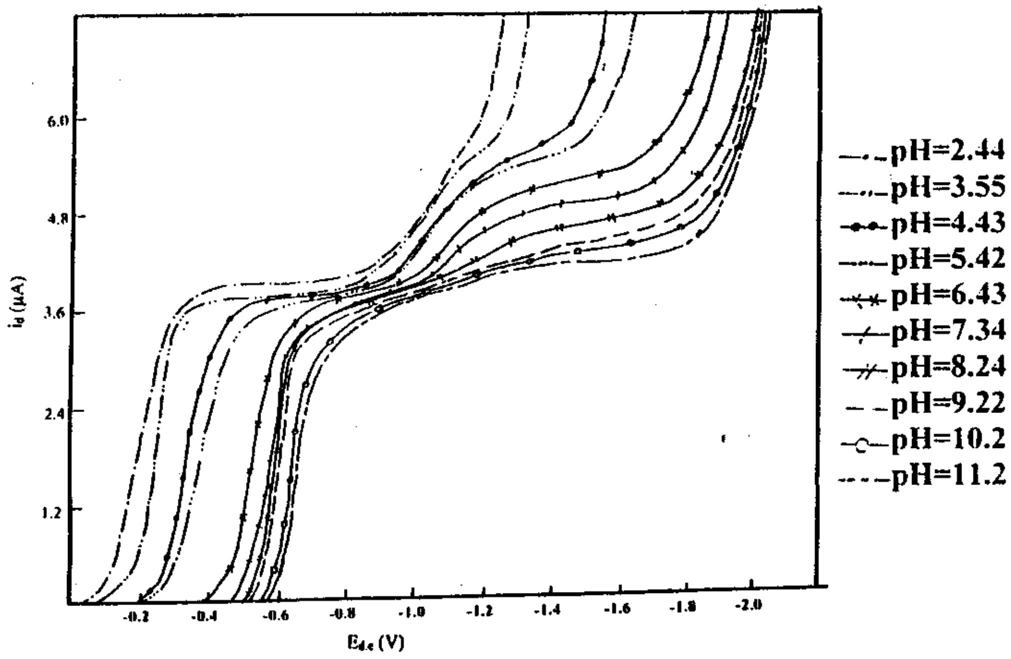


Fig. (15): DC-polarograms of $1x10^{-4}$ M of secnidazole in B.R. buffer solution of different pH values

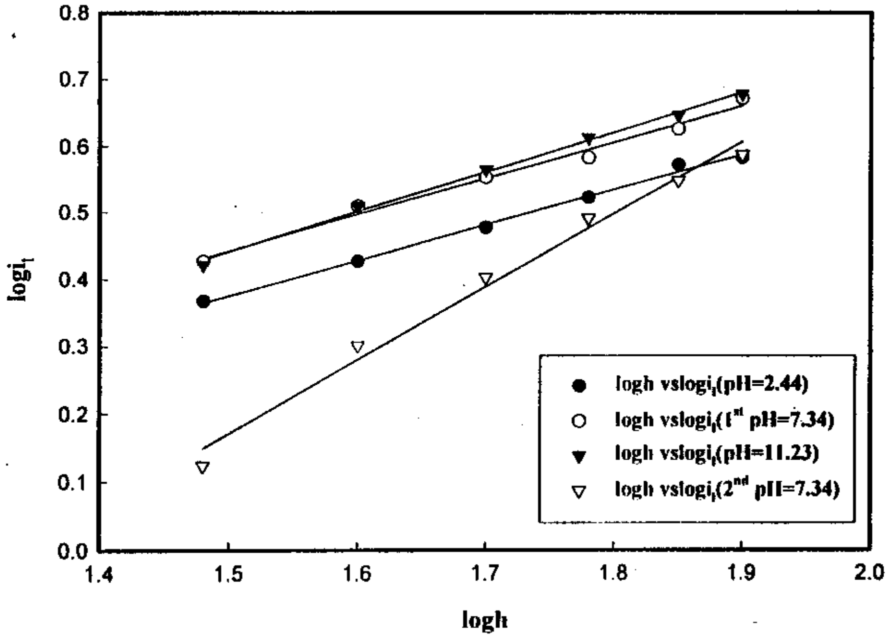


Fig. (16): log i-log h plots of secnidazole at different pH values

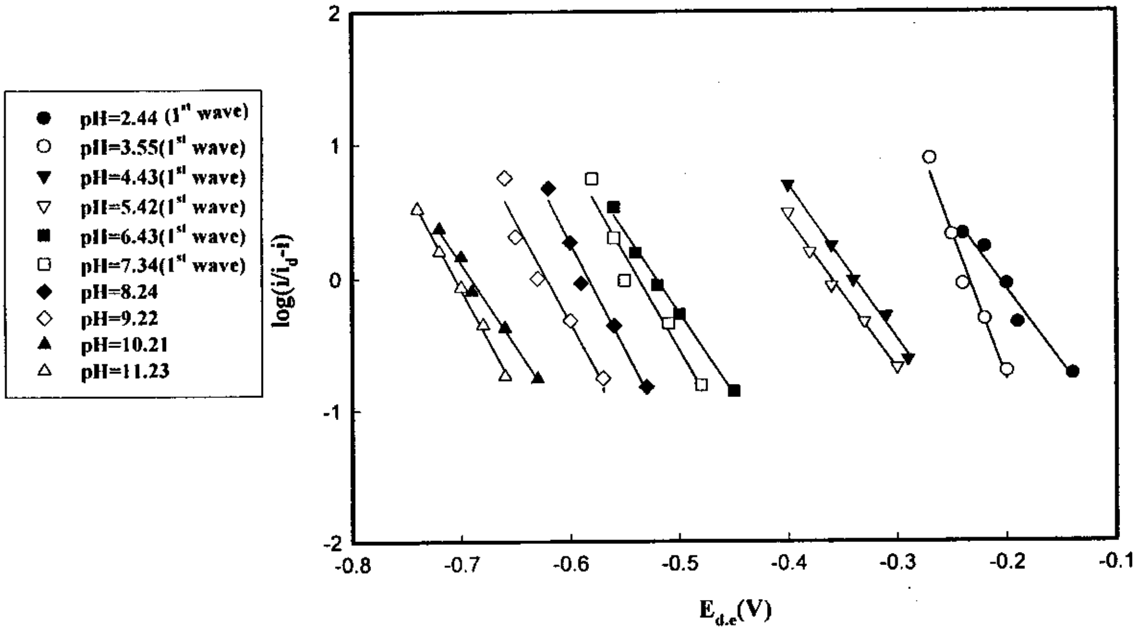


Fig. (17a): Analysis of the polarographic waves of secnidazole at different pH values

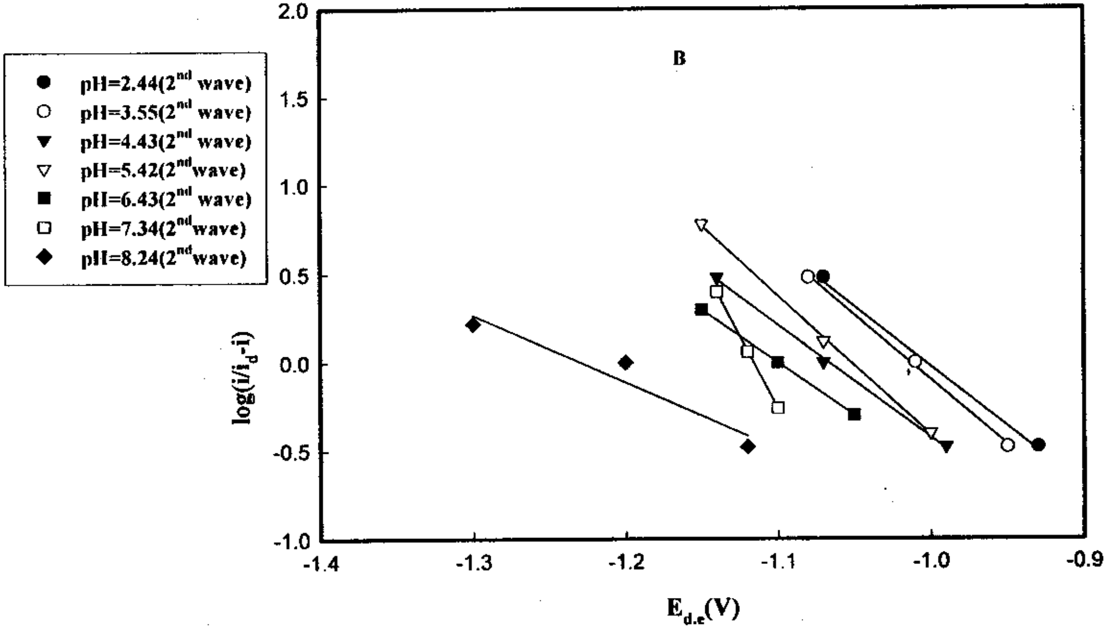


Fig. (17b): Analysis of the polarographic waves of secnidazole at different pH values

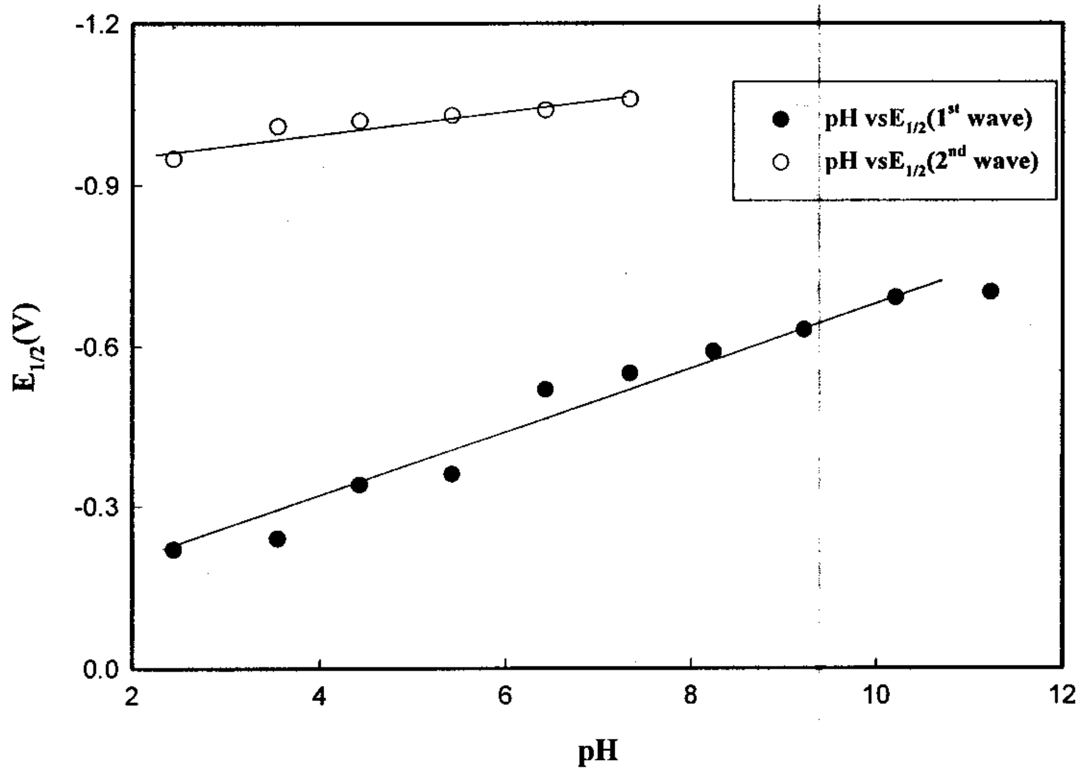


Fig. (18): $E_{1/2}$-pH plots of Secnidazole

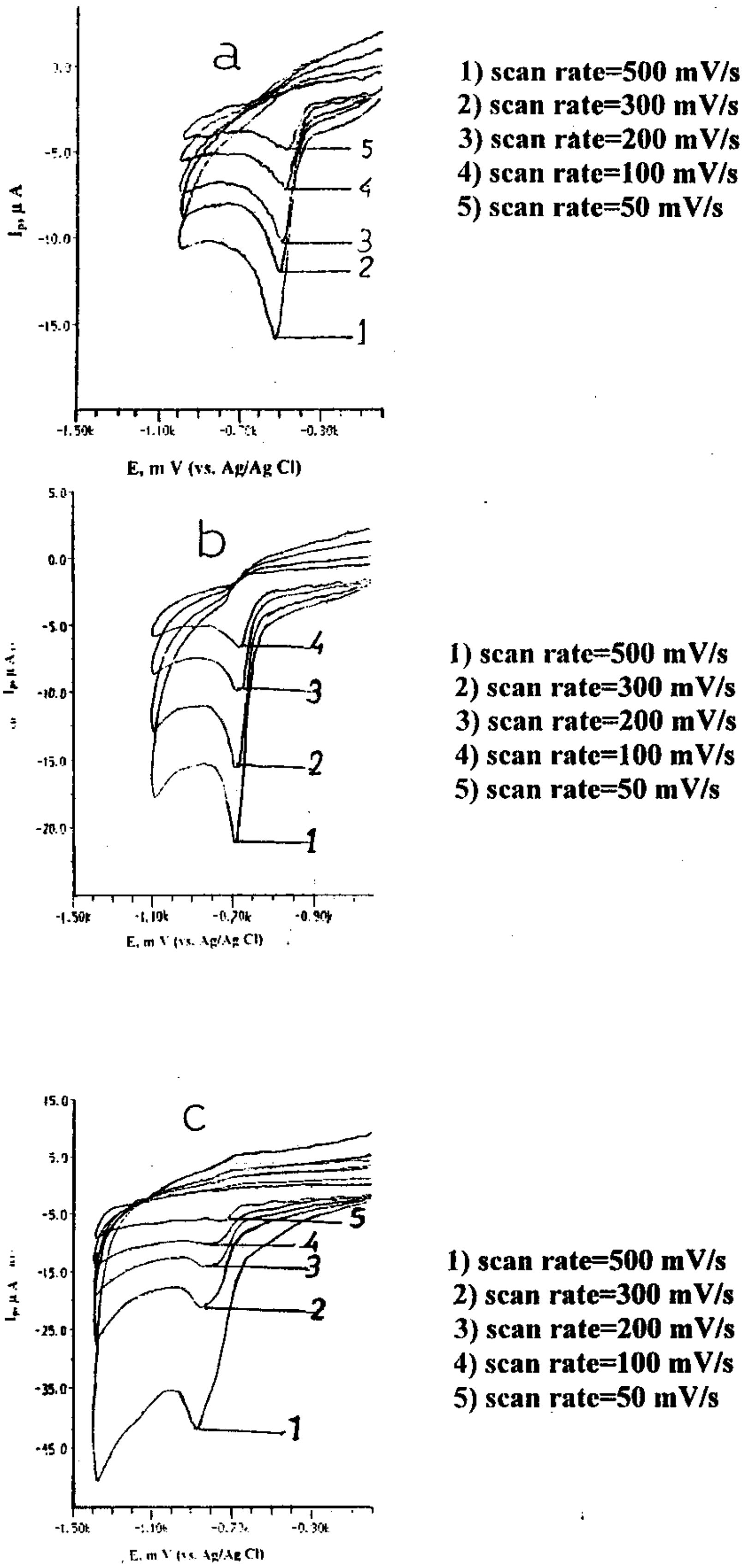


Fig. (19): Cyclic voltammograms of 1x 10^{-4} M of secnidazole in B.R. Buffer solution at different scan rates;(a) pH=3.10 , (b) pH=7.23 (c)pH=10.21 .

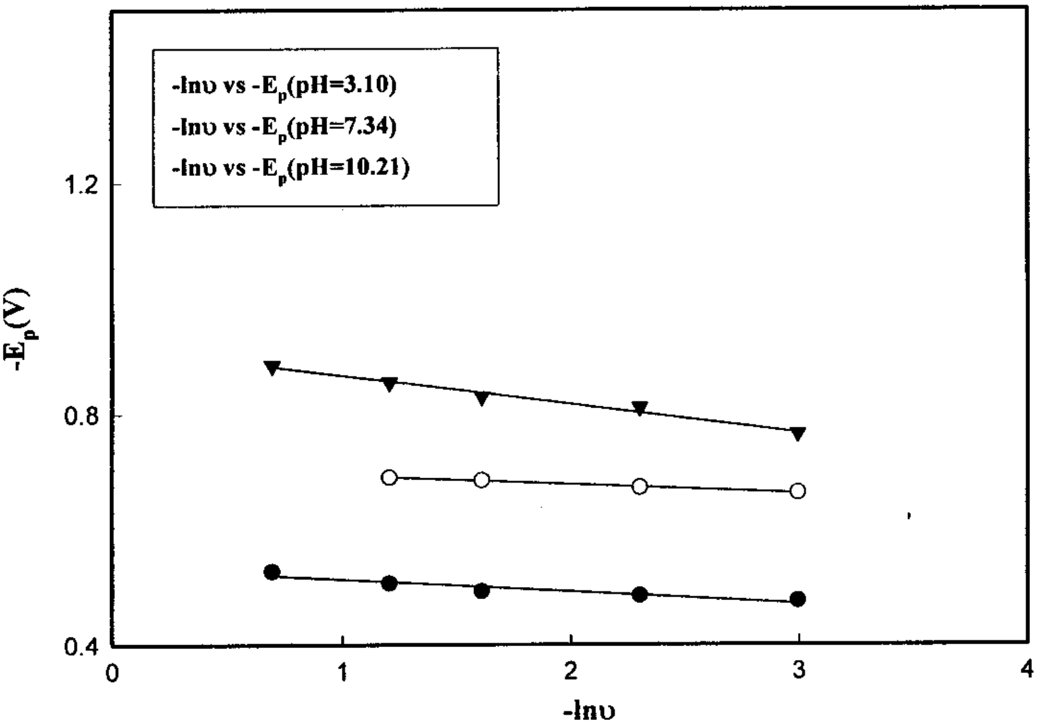


Fig. (20): E_p-ln(υ) plots of Secnidazole at different pH values.

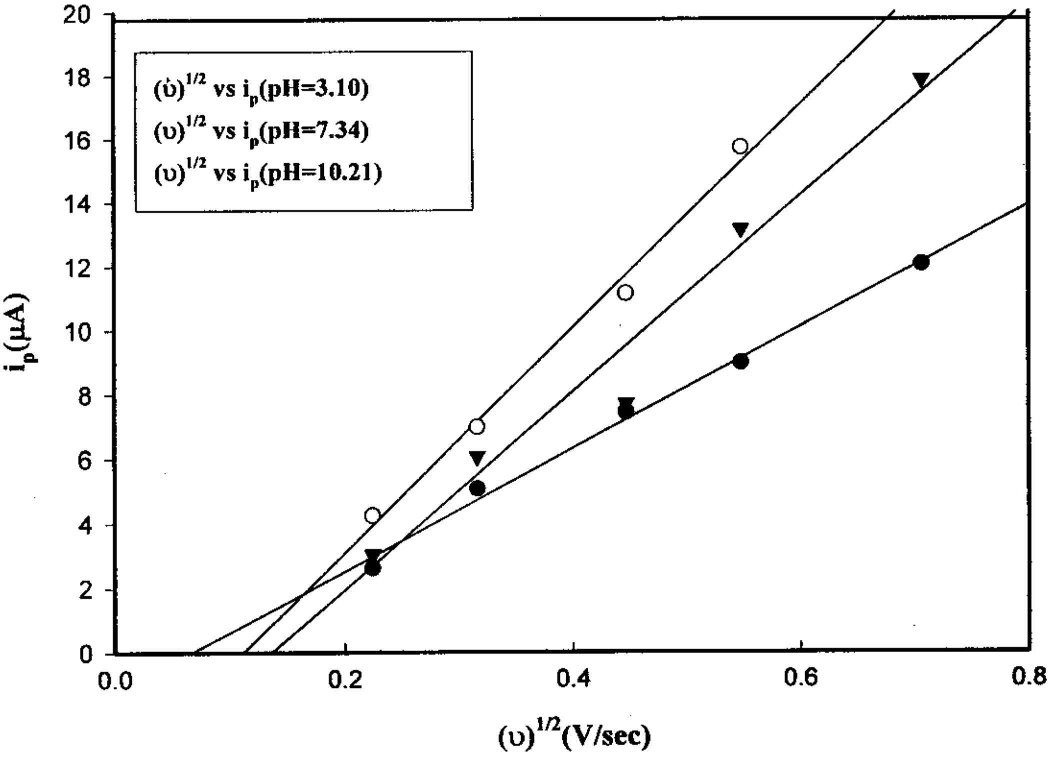


Fig. (21): i_p-$(\upsilon)^{1/2}$ plots of Secnidazole at different pH values.

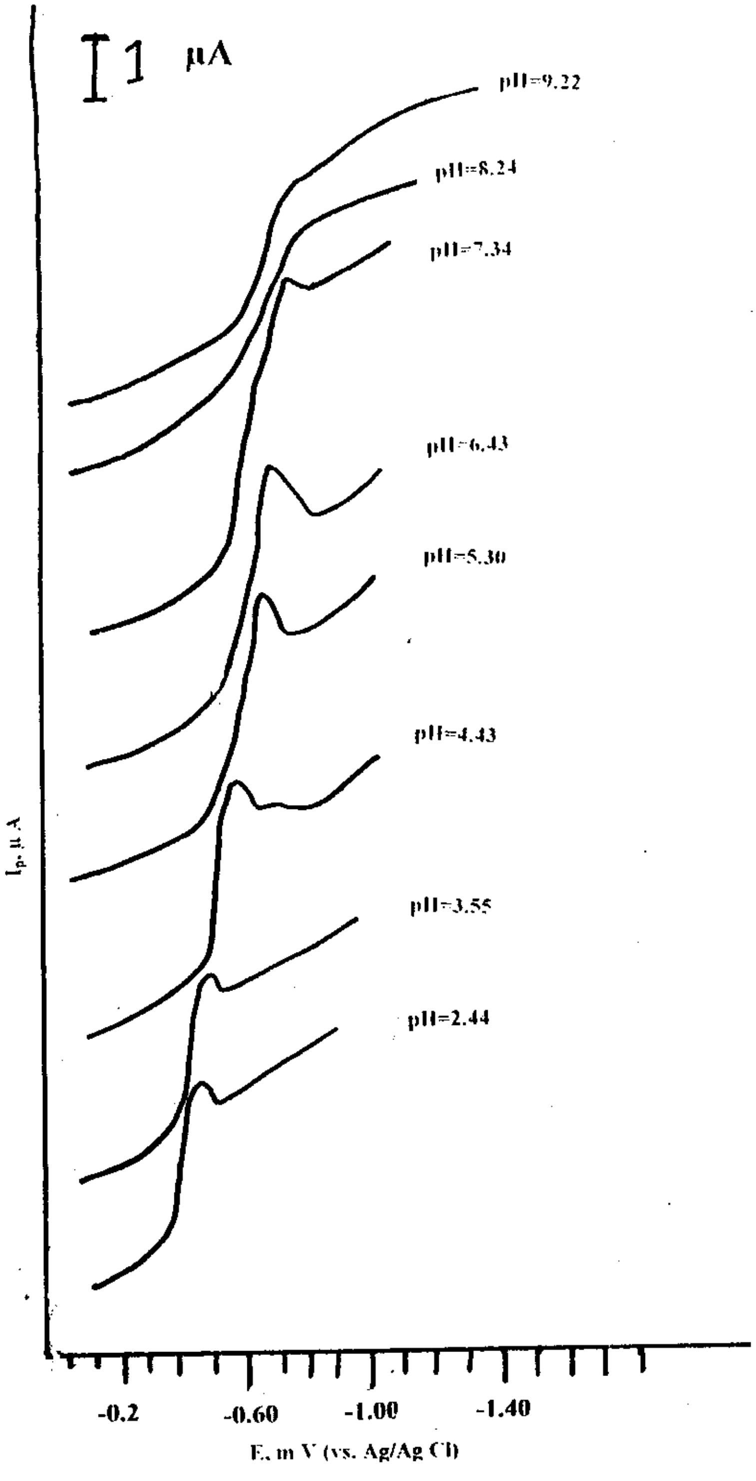


Fig. (22): **Effect of pH on the CAdS peak of $5x10^{-5}$ M of secnidazole in B.R.Buffer solution at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.**

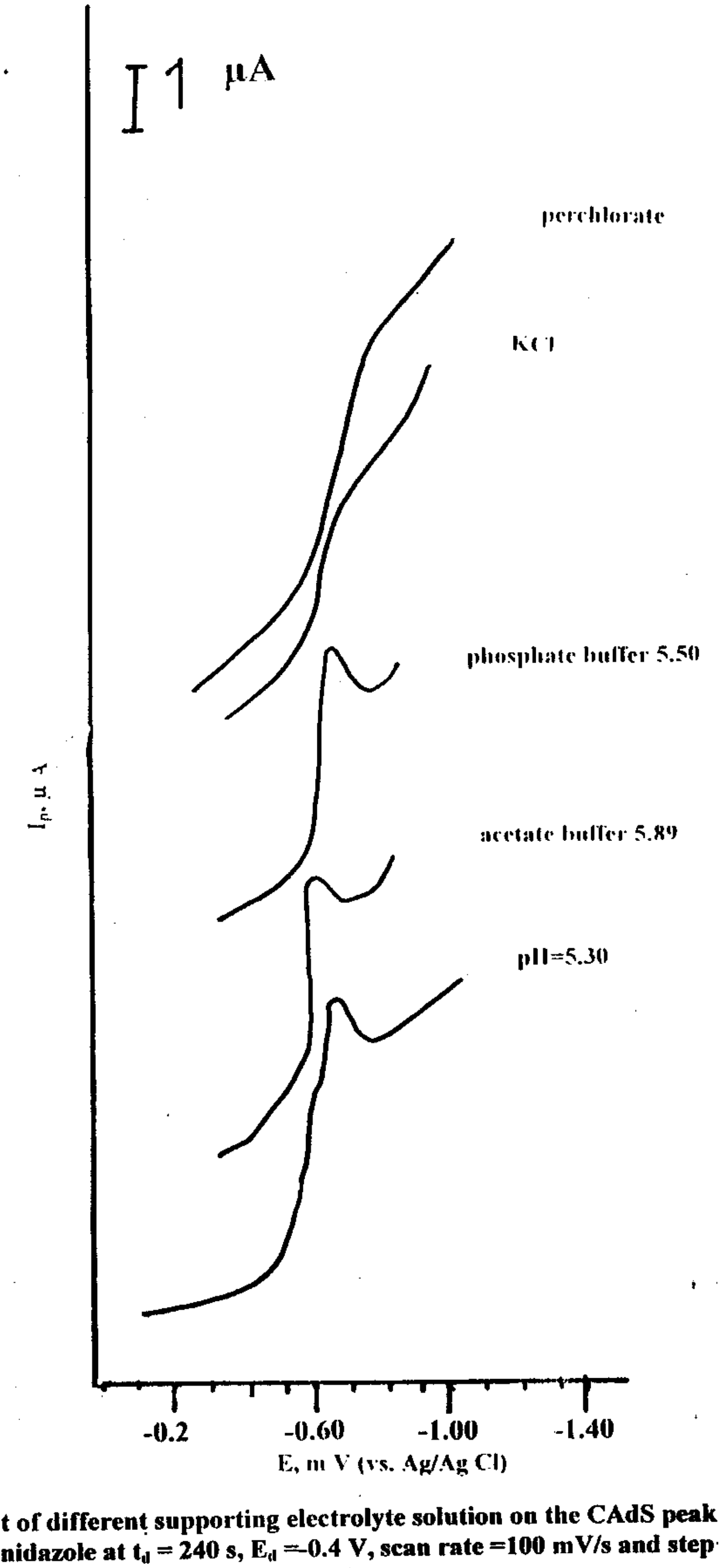


Fig. (23): Effect of different supporting electrolyte solution on the CAdS peak of $5x10^{-5}$M of secnidazole at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.

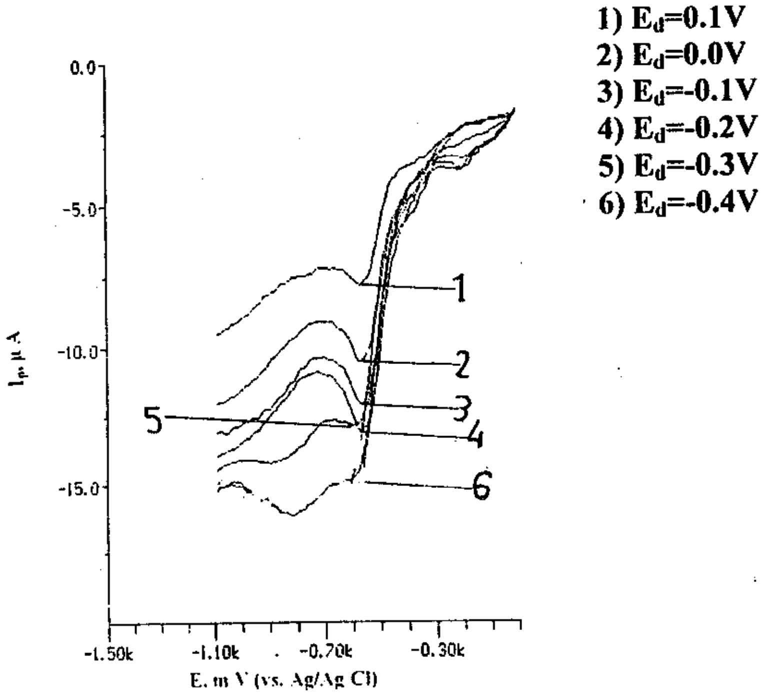


Fig. (24a): Effect of deposition potential on $5x10^{-5}$ M of secnidazole in B.R. buffer solution of pH=5.23 at: t_d=240s,scan rate=100 mV/s,and pulse height=5 mV

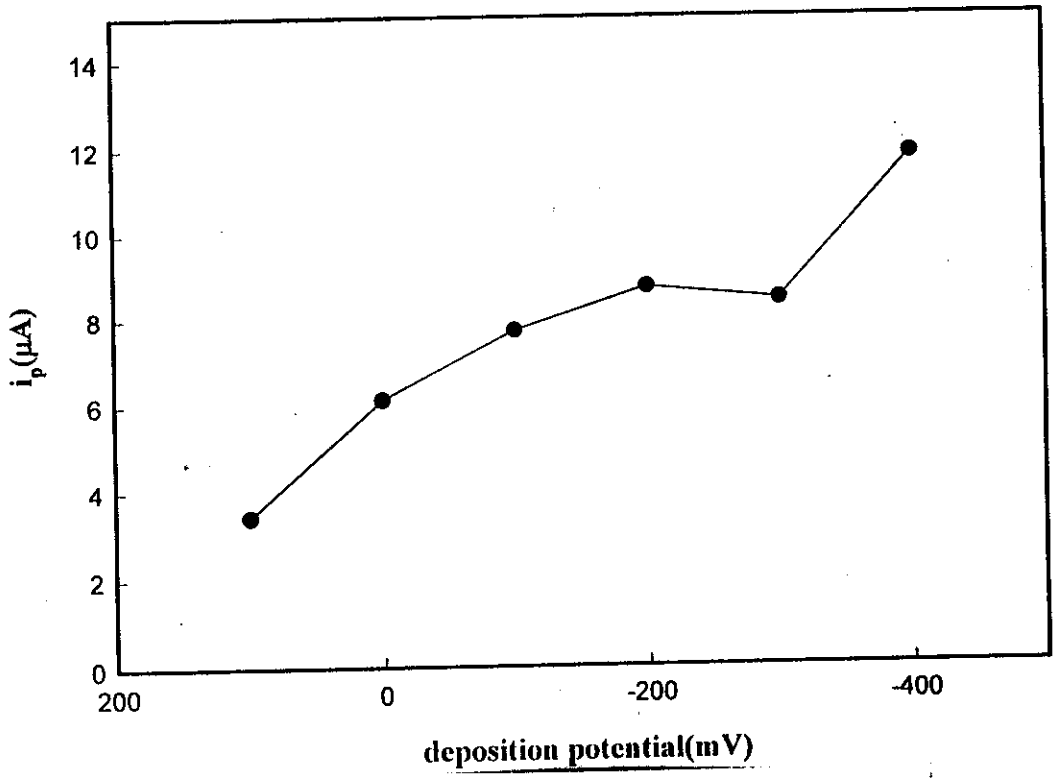


Fig. (24b): Effect of deposition potential on $5x10^{-5}$ M of secnidazole in B.R. buffer solution of pH=5.23 at: t_d=240s, scan rate=100 mV/s,and pulse height=5 mV

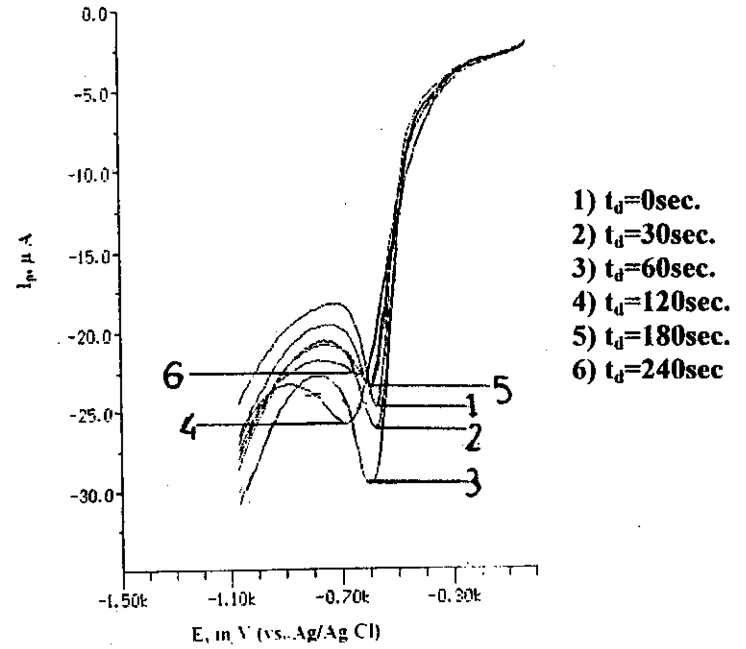


Fig. (25a): Effect of deposition time on $5x10^{-5}$ M of secnidazole in B.R. buffer Solution of pH=5.23 at scan rate=100 mV/s,E_d=-0.4V,and pulse height=20 mV

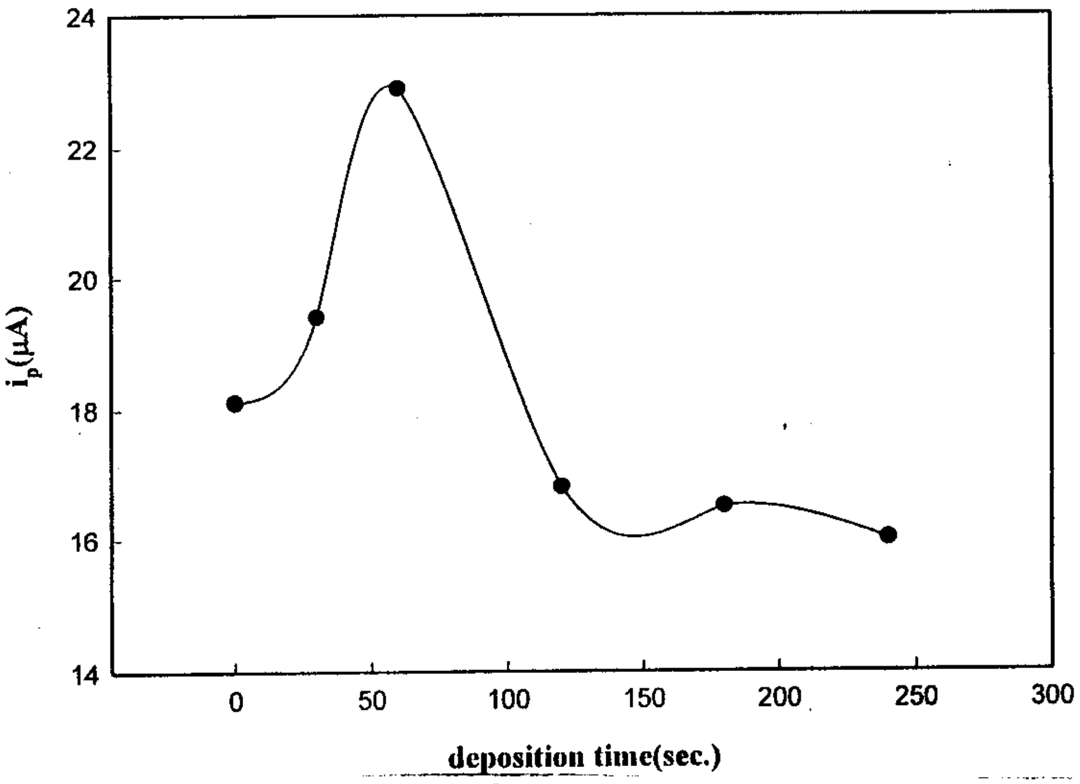


Fig. (25b): Effect of deposition time on $5x10^{-5}$ M of secnidazole in B.R. buffer solution of pH=5.23 at: scan rate=100 mV/s, E_d=-0.4V,and pulse height=20 mV

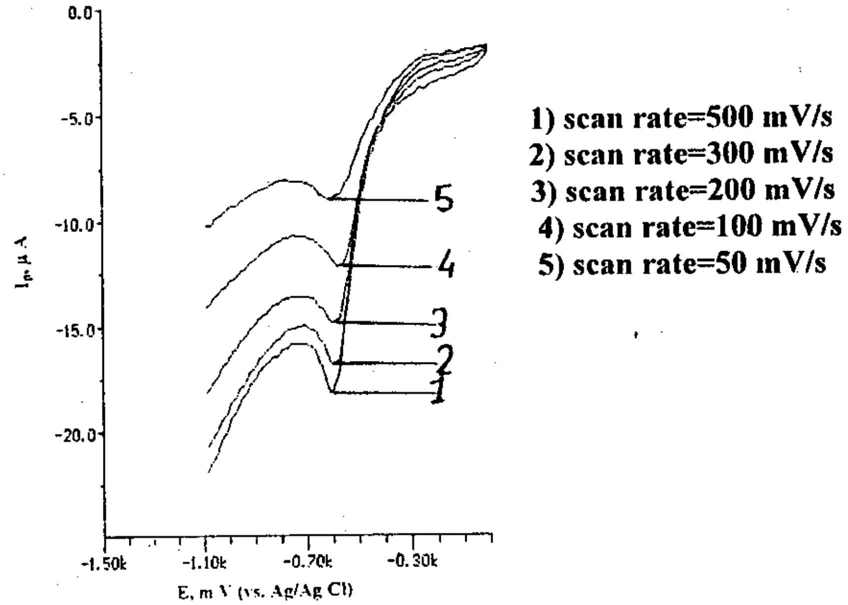


Fig. (26a): Effect of scan rate on 5x10-5M of Secnidazole in B.R. buffer solution of pH=5.23 at: t_d=240s,Ed=-0.4V,and pulse height=5mV

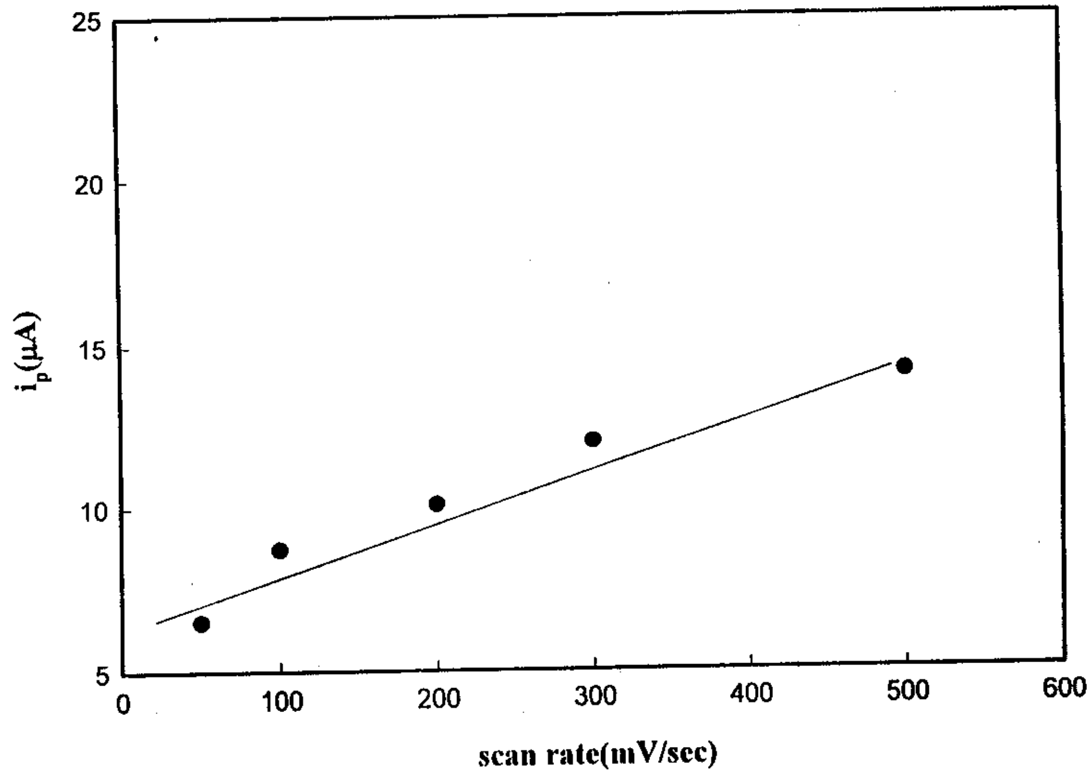


Fig. (26b): Effect of scan rate on 5x10^{-5}M of Secnidazole in B.R. buffer solution of pH=5.23 at:t_d=240s,E_d=-0.4V,and pulse height=5 mV

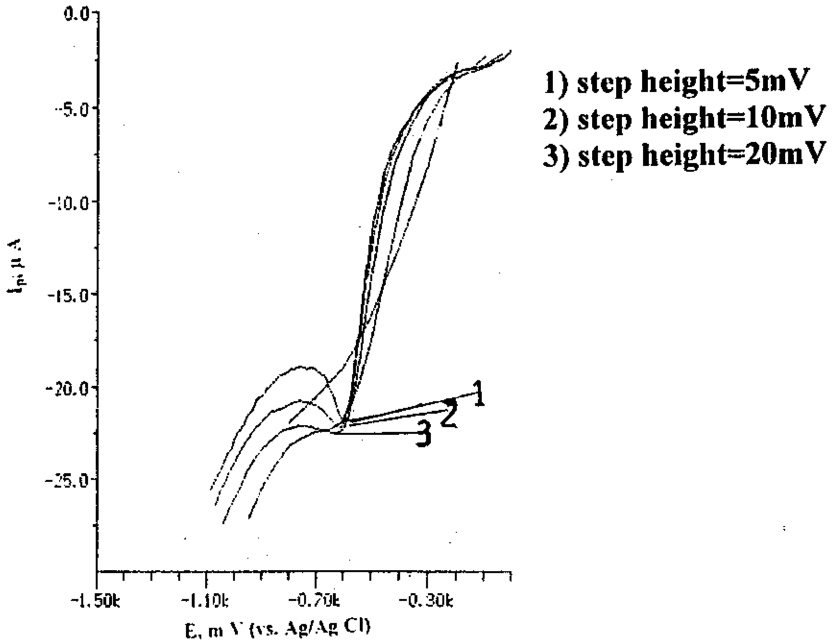


Fig. (27a):Effect of step height on 5x10-5M of Secnidazole in B.R. buffer solution of pH=5.23 at: t_d=240s,Ed=-0.4V,and scan rate=100 mV/s

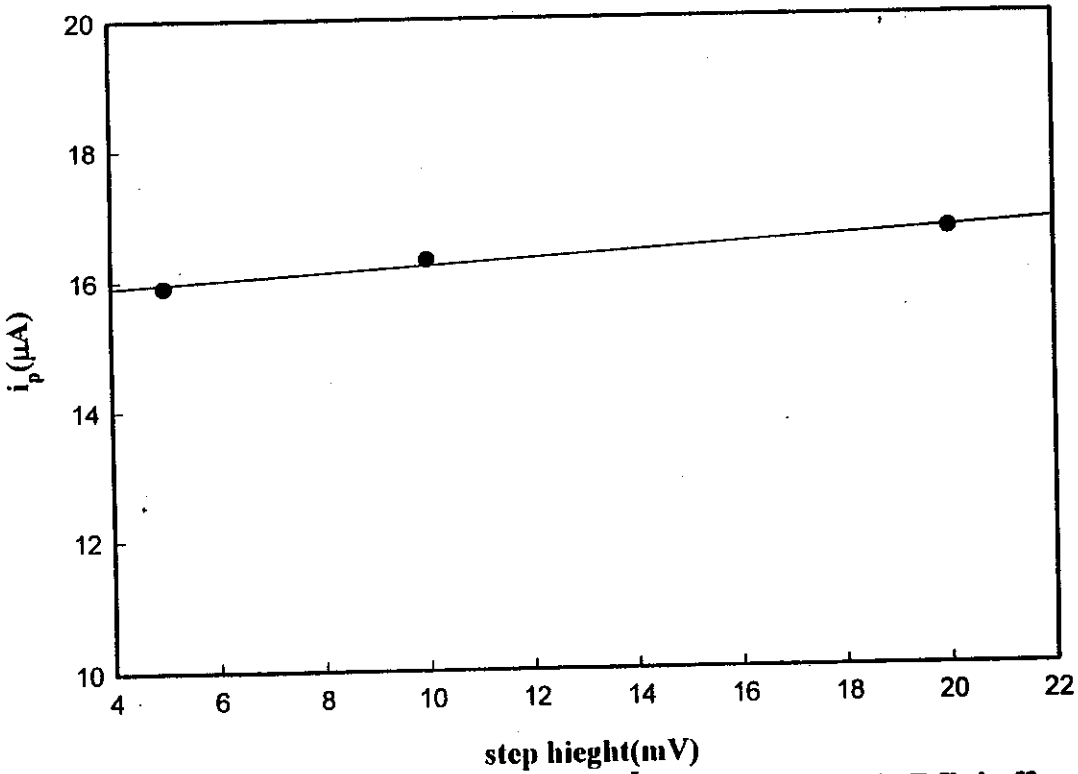


Fig. (27b): Effect of step height on $5x10^{-5}$M of Secnidazole in B.R. buffer solution of pH=5.23 at:t_d=240s,E_d=-0.4V,and scan rate=100 mV/s

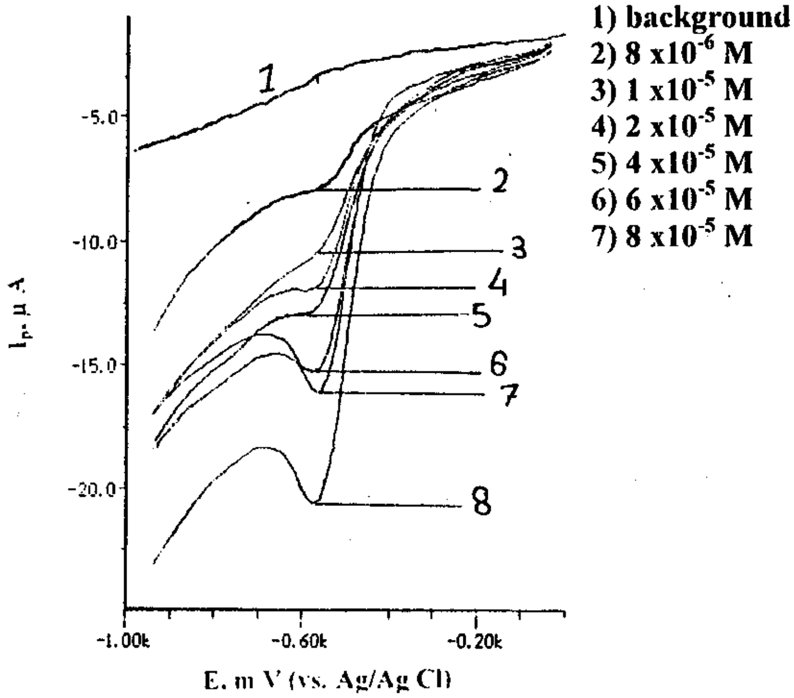


Fig.(28a): **Effect of concentration of secnidazole in B.R. buffer solution of pH=5.23 at: td=30 s, Ed= -0.2 V, scan rate 500 mV/s and step height= 20 mV**

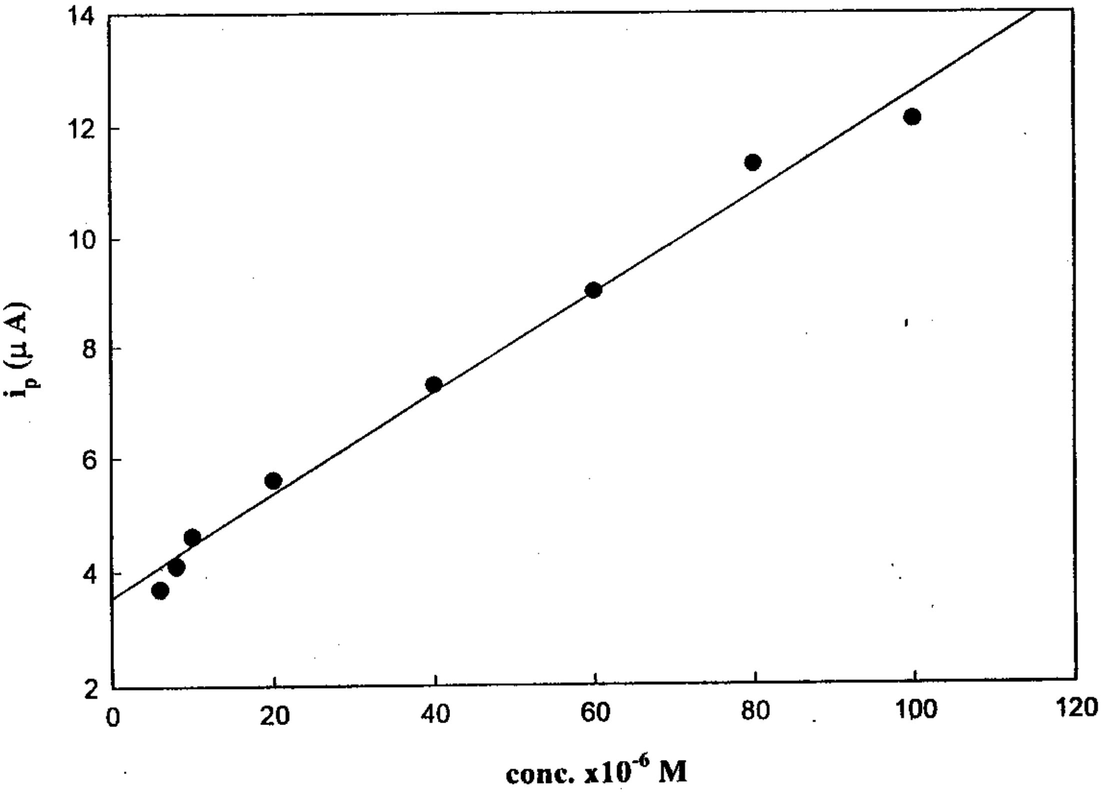


Fig. (28b): calibration curve for secnidazole

Table (9): polarographic data for 1x10^{-4}M of Secnidazole in B.R. buffer solution of different pH values at 25c°

pH	i_d(μA)		$-E_{1/2}$		Δ logi$_l$/Δ logh	
	A	B	A	B	A	B
2.44	3.92	1.80	0.22	0.95	0.53	0.57
3.55	3.80	1.68	0.24	1.01		
4.43	3.68	1.64	0.34	1.02		
5.42	3.56	1.52	0.36	1.03		
6.43	3.56	1.28	0.52	1.04		
7.34	3.48	1.20	0.55	1.06	0.55	1.09
8.24	3.44	0.72	0.59	sh.		
9.22	3.32	sh.	0.63	–		
10.2	3.40	–	0.69	–		
11.2	3.36	–	0.70	–	0.60	sh.

Where A:1st wave B: 2nd wave sh. ill-defined

Table (10): Values of (Z_H^+) and (α) as determined for different ratios (Z_H^+/n_a) at 0.5, 1.0 and 2.0. The number of protons calculated from slope(S_2)of the $E_{1/2}$-pH plots and (S_1) for secnidazole in B.R. buffer solutions of different pH values at 25°C

pH	S_1 mV		S_2 mV		$(Z_H^+)=(S_2/S_1)$		Z_H^+/n_a		
							0.5	1.0	2.0
	A	B	A	B	A	B			
2.44	88.73	147	60.56	140	0.68	0.95	0.48	0.97	1.93
3.55	84.89	135	60.56	140	0.71	1.03	0.48	0.97	1.93
4.43	86.10	156	60.56	140	0.70	0.90	0.48	0.97	1.93
5.42	88.10	127	60.56	140	0.69	1.10	0.48	0.97	1.93
6.43	80.94	108	60.56	140	0.75	1.30	0.48	0.97	1.93
7.34	69.13	154	60.56	140	0.88	0.91	0.48	0.97	1.93
8.24	62.08	263	60.56	140	0.98	0.53	0.48	0.97	1.93
9.22	65.29	sh.	60.56	140	0.92	sh.	0.48	0.97	1.93
10.21	79.56	-	60.56	140	0.76	-	0.48	0.97	1.93
11.23	64.89	-	60.56	sh.	0.93	-	0.48	0.97	1.93

Where A: 1st wave B: 2nd wave sh: ill-defined

Table (11a): Values of (αna) and (α) for $2x10^{-4}$ M of Secnidazole in B.R buffer solution of different pH values, as calculated from reciprocal slope (S_1) of the log (i/i_d-i) -$E_{d.e}$ plots.

pH	Slope(S_1) mV		αn_a		α			
					n=1	n=2	n=1	n=2
	A	B	A	B	A	A	B	B
2.44	88.73	147	0.41	0.40	0.66	0.33	0.40	0.20
3.55	84.89	135	0.70	0.43	0.70	0.35	0.43	0.22
4.43	86.10	156	0.69	0.38	0.69	0.34	0.38	0.20
5.42	88.10	127	0.67	0.46	0.67	0.33	0.46	0.23
6.43	80.94	108	0.73	0.54	0.73	0.37	0.54	0.27
7.34	69.13	154	0.86	0.97	0.86	0.43	0.97	0.49
8.24	62.10	263	0.95	0.21	0.95	0.48	0.21	0.11
9.22	65.29	sh.	0.91	sh.	0.91	0.45	sh.	sh.
10.2	79.55	–	0.74	–	0.74	0.37	–	–
11.2	64.89	–	0.91	–	0.91	0.46	–	–

Where A: 1st wave B: 2nd wave sh : ill-defined

Table (11b): Data obtanied for $1x10^{-4}$ M of Secnidazole in B.R. buffer solut of different pH values using cyclic voltammetry at different scan rates, at 25°C.

pH	Scan rate (mV/s)	-Ep (V)	-dEp/d(lnv)	αn_a
3.10	500	0.527	0.021	0.61
	300	0.506		
	200	0.492		
	100	0.484		
	50	0.475		
7.34	500		0.016	0.80
	300	0.689		
	200	0.684		
	100	0.671		
	50	0.662		
10.21	500	0.885	0.050	0.26
	300	0.855		
	200	0.830		
	100	0.811		
	50	0.765		

Table (12): Kinetic parameters obtained from D-C measurements for $2x10^{-4}$ M secnidazole in B. R. buffer solution of different pH's.

pH	D^o(Cm^2/s)	$K^o_{f,h}$ (Cm/s)	ΔG* (K.cal/mole)
2.44	5.60 x10^{-6}	3.20 x10^{-8} [a]	72.76 [a]
		3.98 x10^{-11} [b]	89.56 [b]
3.55	5.60 x10^{-6}	6.00 x10^{-9} [a]	76.97 [a]
		4.68 x10^{-13} [b]	100.71 [b]
4.43	5.60 x10^{-6}	8.32 x10^{-10} [a]	81.93 [a]
		3.89 x10^{-13} [b]	101.17 [b]
5.42	3.10 x10^{-6}	2.29 x10^{-10} [a]	85.17 [a]
		4.17 x10^{-14} [b]	106.77 [b]
6.43	3.10 x10^{-6}	2.19 x10^{-12} [a]	96.82 [a]
		4.07 x10^{-15} [b]	112.61 [b]
7.34	3.10 x10^{-6}	1.35 x10^{-14} [a]	109.64 [a]
		1.29 x10^{-17} [b]	127.05 [b]
8.24	2.20 x10^{-6}	1.38 x10^{-16} [a]	121.09 [a]
		3.02 x10^{-18} [b]	130.70 [b]
9.22	2.20 x10^{-6}	1.29 x10^{-16} [a]	121.30 [a]
10.2	1.90 x10^{-6}	5.09 x10^{-17} [a]	123.61 [a]
11.2	1.90 x10^{-6}	9.55 x10^{-18} [a]	127.83 [a]

Where a : 1st wave, b : 2nd wave .

Table (13): Cathodic adsorptive stripping peak current(i_p)of $5x10^{-5}$ M Secnidazole in B.R.buffer solution of pH=5.23 at different conditions (t_d, E_d, scan rate and pulse height).

Deposition time (t_d), s.	Deposition potential (E_d), V	Scan rate (mV/s)	Pulse hieght (mV)	i_p (µA)
Effect of (td):				
0	-0.2	500	20	18.10
30				19.40
60				22.90
120				16.80
180				16.50
240				16.00
Effect of (E_d):				
240	0.1	100	5	3.41
	-0.01			6.11
	-0.1			7.70
	-0.2			8.71
	-0.3			8.41
Effect of scan rate:				
240	-0.2	500	5	12.10
		300		11.98
		200		10.08
		100		8.730
		50		5.530
Effect of pulse height:				
240	-0.2	500	5	15.9
			10	16.3
			20	16.7
			50	
			100	
			150	
			200	

Table (14): Assay secnidazole in B.R.buffer solution of pH=5.23 using CAdSV at td= 30 sec., Ed= -0.2 V, scan rate= 500 mV/s, and step height= 20 mV.

Conc.taken	Ip(μA)	Mean	S.D	R.S.D %
$6x10^{-6}$ M	3.77 3.68 3.72 3.63	3.70	0.059	1.59
$8x10^{-6}$ M	4.10 4.08 4.08 4.13	4.09	0.024	0.58
$1x10^{-5}$ M	4.69 4.55 4.62 4.55	4.60	0.066	1.43
$2x10^{-5}$ M	5.63 5.63 5.70 5.58	5.63	0.049	0.88
$4x10^{-5}$ M	7.19 7.35 7.26 7.38	7.29	0.086	1.18
$6x10^{-5}$ M	8.82 8.94 9.12 9.08	8.99	0.137	1.52

Table (15): Assay of secnidazole in dosage form in B.R.buffer solution at pH=5.23 using CAdSV at t_d= 60sec., E_d= -0.2 V, scan rate= 500mV/s, and step height = 20 mV.

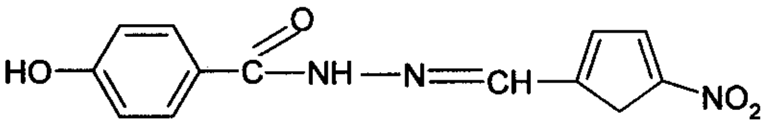
Conc.taken	Conc. calculated	**Recovery %**	**Mean** Recovery(%) ± S.D.
$1x10^{-5}$ M	**$0.99x10^{-5}$ M**	**99.00**	**100.5 ±2.44**
	$1.02\ x10^{-5}$ M	**102.0**	
	$0.98\ x10^{-5}$ M	**98.00**	
	$1.03x10^{-5}$ M	**103.0**	
$2x10^{-5}$ M	**$2.04x10^{-5}$ M**	**102.3**	**102.3 ±2.40**
	$1.96x10^{-5}$ M	**98.00**	
	$2.04x10^{-5}$ M	**102.0**	
	$2.04\ x10^{-5}$ M	**102.0**	
$4x10^{-5}$ M	**$4.10\ x10^{-5}$ M**	**102.5**	**101.6 ±2.17**
	$4.05x10^{-5}$ M	**101.3**	
	$4.10x10^{-5}$ M	**102.5**	
	$3.99\ x10^{-5}$ M	**100.0**	
$6x10^{-5}$ M	**$5.93\ x10^{-5}$ M**	**98.83**	**99.95 ±1.41**
	$6.09x10^{-5}$ M	**101.5**	
	$6.05x10^{-5}$ M	**100.8**	
	$5.92\ x10^{-5}$ M	**98.67**	
$8x10^{-5}$ M	**$8.10\ x10^{-5}$ M**	**101.3**	**100.7 ±1.06**
	$8.09\ x10^{-5}$ M	**101.3**	
	$7.99\ x10^{-5}$ M	**99.87**	
	$8.04x10^{-5}$ M	**100.5**	

Table (16): Assay of secnidazole in serum in B.R. buffer solution of pH=5.23 using CAdSV at td= 30 sec., Ed= -0.2 V, scan rate= 500mV/s, and step height= 20 mV.

Conc.taken μg/10 ml	Conc. found μg/10 ml	**Recovery** %	**Mean** Recovery(%) ± S.D.
1.03	**1.05**	**101.9**	**101.0 ±1.37**
	1.04	**101.0**	
	1.04	**101.0**	
	1.03	**100.0**	
3.42	**3.63**	**103.5**	**103.1 ±4.16**
	3.54	**103.5**	
	3.59	**105.2**	
	3.42	**100.0**	

3.3 Electrochemical behavior of nifuroxazide in B.R buffer solutions of different pH values:-

Nifuroxazide is [5-nitro-2-furaldehyde p-hydroxybenzoyl hydrazone] and has the following structural formula:



This part includes the study of electrochemical behavior of nifuroxazide at Hg-electrode using of DC polarography and cyclic voltammetry techniques The study aimed to optimize the experimental and instrumental conditions for determination of the lowest possible concentration of the investigated Pharmaceutical compound in pure and dosage forms using cathodic adsorptive stripping voltammetry .

3.3.1 DC polarography:-

3.3.1.i- Current potential curves:-

The polarographic behavior of $1x10^{-4}$ M of nifuroxazide was studied in B.R. buffer solutions containing 10% (v/v) ethanol of different pH values in the range from 2 to 12 . The polarogrames exhibit two reduction waves within the pH range studied corresponding to the reduction of NO_2 group (nitro-group), Fig. (29), the half-wave potential ($E_{1/2}$) of the reduction waves pH dependent, and being shifted to more negative values on increasing the pH of the electrolysis solution .

3.3.1.ii- Effect of pressure at mercury height:-

The effect of mercury height "h" was examined according to equation (3.1). The plots of log i_l against log h for nifuroxazide showed linear correlations at different pH values, Fig. (30). The slope values (x) are found in the range between 0.42 and 0.66 which indicate that the reduction process is controlled by diffusion and some adsorption contribution, Table (17).

3.3.1.iii- Analysis of the polarographic waves:-

Analysis of the polarographic waves is quite important to evaluate the degree of reversibility of electrode reaction and to suggest the electrode mechanism On using equation (3.4), the plots of log $[i/(i_d-i)]$ against $E_{d.e.}$ for nifuroxazide reduction at different pH values showed linear correlations, Fig. (31). The straight lines with reciprocal slopes (S_1) are in the range to (54.44 -86.66) and (63.41-152.67) mV for the first and second waves; respectively, Table (18). This behavior revealed the irreversible nature of the electrode process.

3.3.1.iv- Half- wave potential –pH curves:-

The plots of the rate shift of $E_{1/2}$ of nifuroxazide versus the pH of the electrolysis solution showed linear correlations for both the first and second waves respectively. The slope values (S_2) of the first and second waves amount to 46.8 and 41.4 mV, respectively, Table (18).

The number of hydrogen ions (Z_H^+) involved in the rate determining step for nifuroxazide reduction was calculated from both slopes of logarithmic analysis and $E_{1/2}$ plots according to equation (3.5). At all pH values Z_H^+ was found to be unity, Table (18).

The most probable values of α-parameter as shown in Table (19a), were determined at the probable ratio of (Z_H^+/n_a) using equation (3.6). the probable (Z_H^+/n_a) ratio was found to fit with (Z_H^+/n_a) = 0.5, Table (18).

This behavior further confirmed the irreversibility of electrode process and the rate determining step involves one proton and two electrons.

3.3.2Cyclic voltammetry:-

The cyclic voltammetric behavior of $1x10^{-4}$ M of nifuroxazide was investigated in aqueous Britton-Robinson buffer solutions of different pH values (2-12) containing 10% (v/v) ethanol at glassy carbon electrode. The voltammograms displayed single irreversible cathodic peak in the cathodic scan in all media, where as in the anodic scan, no oxidation peaks were observed, Fig.(33).

On increasing the scan rate (υ), the cathodic peak potential (E_{pc}) was shifted to more negative potentials indicating the irreversible nature of the reduction peak. Furthermore, on increasing the pH of the electrolysis solution the peak potential (E_{pc}) is shifted to more negative values indicating the consumption of hydrogen ions in the electron transfer step. On employing equation (3.7), the plots of E_p versus ln υ gives linear correlations Fig.(34) . The slope values were used for the determination of αn_a. The values of the (α) transfer coefficient were calculated at the probable n_a values and found to be less than 0.28 at n_a=2.0, Table(19b). This behavior is further confirmed the irreversible nature of electrode process. According to equation(3.8), the plots of i_p versus square root of scan rate ($\upsilon^{1/2}$) gives linear relation slightly deviating from the origin, Fig.(35). This behavior confirming that the electrode process of nifuroxazide is controlled mainly by diffusion with some adsorption contribution.

3.3.2.i- Kinetic parameters of the electrode reaction:-

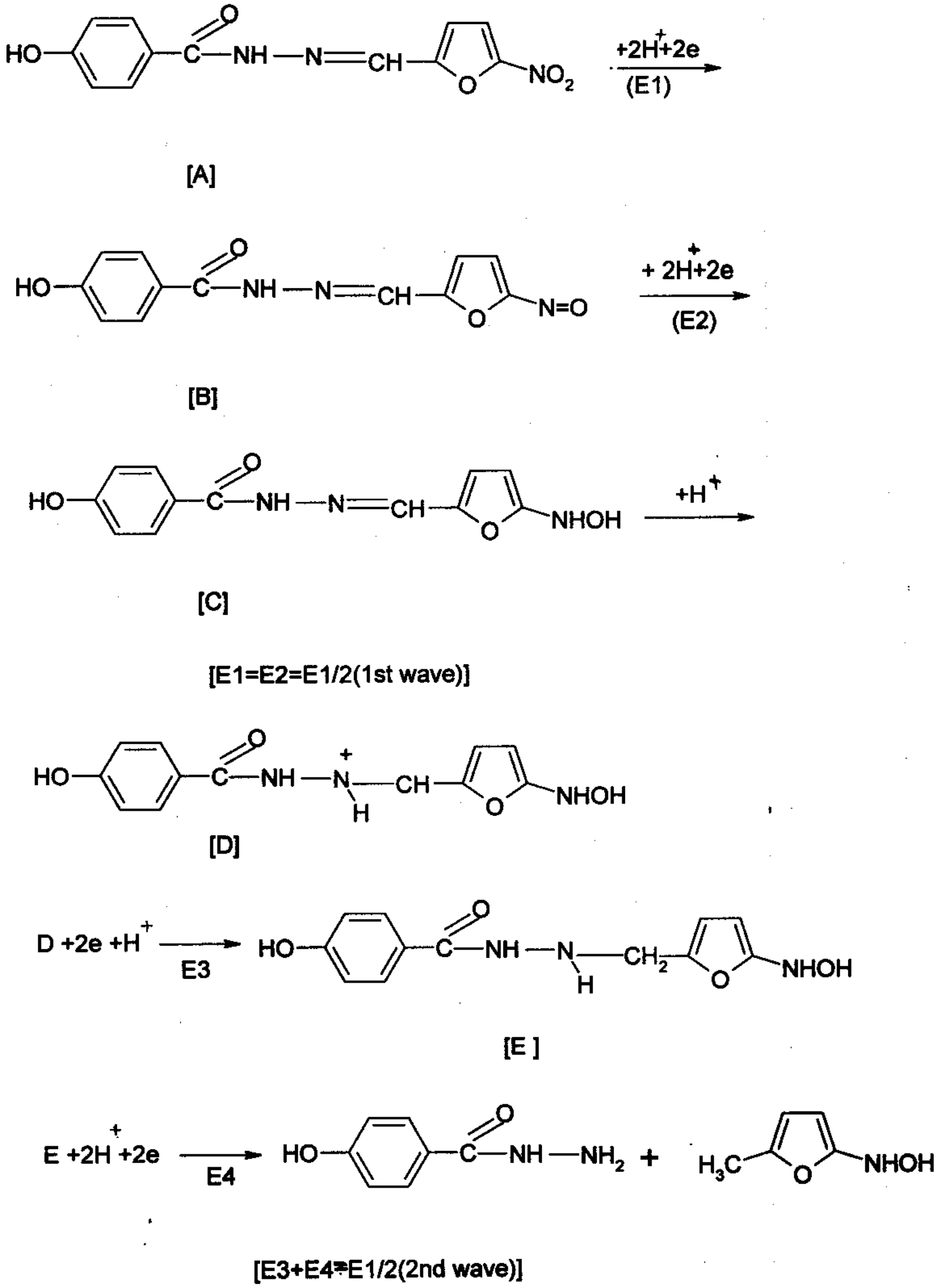
The rate constant $k^{o}_{f,h}$ of the electrode reaction of nifuroxazide is calculated from the data obtained by dc- polarography measurements in Britton-Robinson buffer solution of various pH values. Equation (3.12) was used in this determination and the values of the rate constant $k_{f,h}$ were found to be decreases with increasing the pH which means that the electrode reaction becomes more difficult and more irreversible, Table (20). Also, the values of ΔG^* were determined using equation (3.14) and found to be increased with increasing pH of the electrolysis medium, Table (20) . This may be explained on the basis that the protonation of the molecule, at lower pH, before its diffusion to the electrode surface, takes place through the contribution of the

H^+ ion directly with the depolarizer molecule which decreases the activation energy ΔG* . The H^+ ion activity decreases with rise of pH, hence ΔG* increases. In alkaline media, the proton required for pre-protonation of the depolarizer is supplied from a water molecule which requires much higher energy.

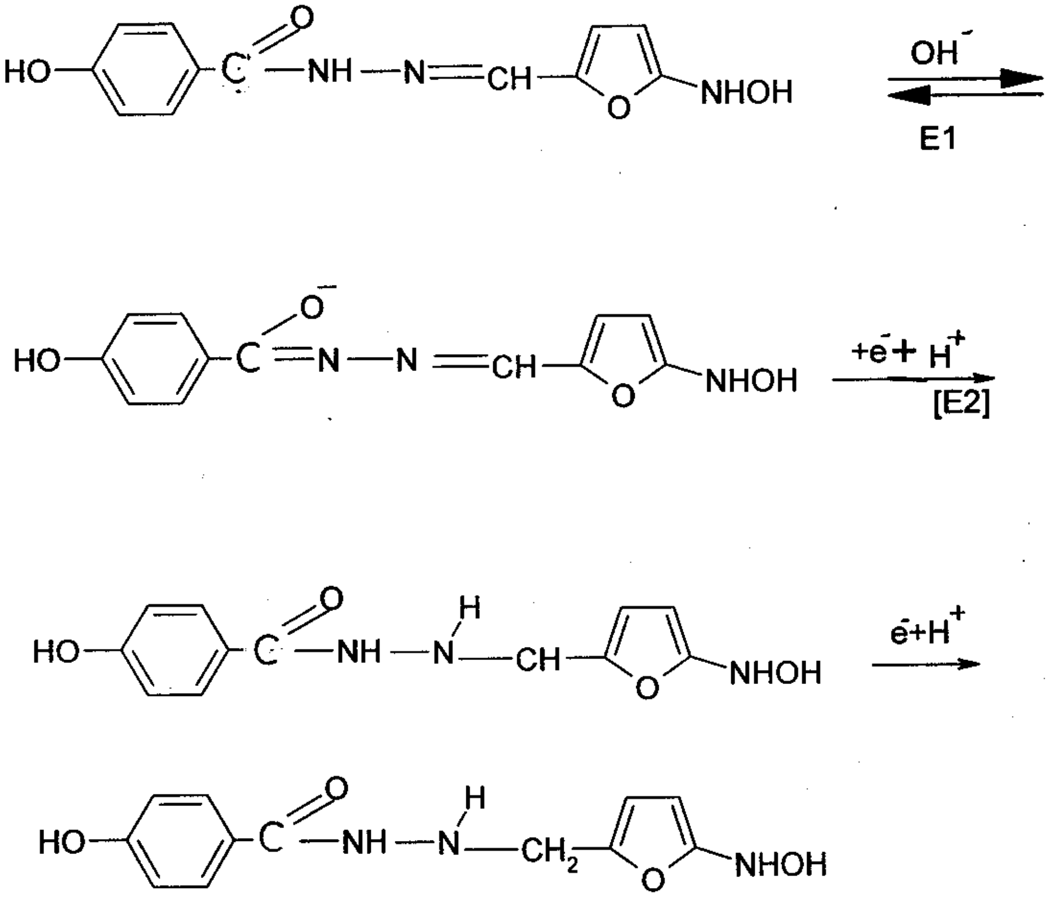
The electrode reaction mechanism:-

To study the reduction mechanism of nifuroxazide at mercury electrode, it is necessary to calculate the number of electrons concumed at this reduction, firstly we should calculate the (diffusion coefficient) D from equation (3.16) and the corrected values shown in Table (20). The number of electron found that 8 electrons are consumed in the reduction of oxime and nitro groups (N=C), (NO_2) groups in acidic and 6 electrons at basic medium for two groups, and the number of electrons were confirmed by controlled potential coulmetry method according to equation (3.17) the avarge value of n is (8±0.2) so the suggested mechanism as the following:

In acidic medium :-


HO
C
NH
N
CH
O
NO2
+2H+ +2e
(E1)
[A]
N=O
+ 2H+ +2e
(E2)
[B]
NHOH
+H+
[C]
[E1=E2=E1/2(1st wave)]
[D]
D +2e +H+
E3
CH2
[E]
E +2H+ +2e
E4
NH2
H3C
[E3+E4=E1/2(2nd wave)]

In alkaline medium :-



3.3.3 Cathodic adsorptive stripping voltammetry(CAdSV) of nifuroxazide:-

Cathodic adsorptive voltammetry was used for the quantitative determination of nifuroxazide in pure and dosage forms . the instrumental and experimental conditions were optimized in this investigation. The instrumental conditions are deposition time (td), deposition potential (Ed), step height and scan rate. While the experimental conditions are including the nature of supporting electrolyte and pH of the electrolysis solution.

3.3.3.i- Effect of pH and supporting electrolyte:

In cathodic adsorptive voltammetry current of nifruxazide is recorded as function of potential in Britton- Robinson buffer solution of pH(2.71 to 11.00) the recorded peak at various pH values due to the reduction of nitro-group at the surface of glassy carbon electrode and well developed peak is noted at pH=8.18 Fig.(36).

We determined the nifruxazide in some supporting electrolyte like (phosphate, acetate, $NaClO_4$ and KCl buffers) we fined thate in presence of $NaClO_4$,KCl the peak disappeared it means that the absence of this anion best of nifruxazide and the pH=8.18 is most suitable for nifruxazide Fig.(37)

3.3.3.ii- Effect of accumulation time and potential:

The deposition of the analyzed drug on the surface of the glassy carbon electrode is one of the essential conditions for highly sensitive determinations. The effect of accumulation potential on the peak height was examined over the potential range +100 to -800 mV for $5x10^{-5}$ M nifuroxazide at accumulation time 240 s., Fig. (38a). the relation between peaks height i_p and deposition potential E_d is shown in Fig. (38b). The peak current increased steadily with decreasing the potential value till it reaches maximum value at E_d = -400 mV, where it decreased sharply after this inflection point. Thus, E_d = -400 mV will be adopted as optimum operational values for the following works as it ensured the highest voltammetric signal .

The effect of accumulation time of the drug was examined in the range 0-360s. the amount of nifuroxazide accumulated on the electrode surface increased as the deposition time increased till 60 s, further increase of time resulted in decreasing the peak height . A time of accumulation of 60 s was chosen for analytical purposes. The voltammograms obtained for $5x10^{-5}$ M nifuroxazide in B. R. buffer solution of pH 8.18 and E_d = -0.4 V are shown in Fig. (39a). The dependence of peak height on accumulation time are illustrated in Fig. (39b).

3.3.3.iii- Effect of scan rate:

The effect of scan rate on the peak current and peak potential was studied. The relationship between i_p or E_p and υ at 240 s., accumulation time shows an increase in the peak current with increasing the scan rate, Fig. (40a,b). The peak potential was shifted to more negative values with increasing the scan rate.The scan rate 500 mv s^{-1} was used for further voltammetric determination of the drug.

3.3.3.iv- Effect of step height:-

The effect of step height on the CAdSV peak current of nifuroxazide reduction was recorded at different values, Fig. (41a).

A well defint peak was observed at 5 mV step height. On increasing step height up to 150 mV the CAdSV peak current decreases till become ill-defined. From the plot of peak current (i_p) versus step height it noted that i_p is inversely proportional to step height, Fig. (41b).a\ and all data shown in Table (22).

3.3.3.v- Calibration curve, detection limit and precision to nifuroxazide:-

A calibration plot was obtained from known concentrations of nifuroxazide using the peak current in μA as analytical response Fig.. (42). Series of standard solution (four replicates) of the drug were prepared by following the procedure described. The calibration graph was found to be linear over the range 4 x10^{-7} to 2 x10^{-6} M. The calibration graph yielded the following regression equation :

$$Ip\ (\mu A) = 0.70 + 5.265 \times 10^{6}\ C\ M; \qquad r = 0.9979 \quad n = 6$$

The detection limit obtained for the developed voltammetric method was 6.5 x10^{-8} M. In order to assess the precision as the standard deviation for concentration with the relativestandard deviation were evaluated, Table (22).

Analysis of pharmaceutical formulation:

the proposed method was successfully applied to the assay of the studied drug in the pharmaceutical dosage forms (antinal, drotazide and diax tablets, labeled to contain 200 mg per tablet for three dosage forms) . The percentage recoveries for nifuroxazide are shown in Table (23, 24, 25) respectively for three dasage forms. The method gave a good recovery. The percentage recovery based on five, three and five different determinations respectively to three drugs were (99.54 ± 1.72), (100.93 ±2.74) and (100.54 ±1.66) respectively.

Analysis of serum:

serum samples were prepared as described before, and two different concentration were analysed using the proposed method (1.63 and 5.44 µg /ml). The recovery and the standard deviasion were evaluated from four measurements for each sample, Table (26). The mean recovery for the two concentration was 100.06 ± 2.94.

this means that the proposed method can be used for determination of nifuroxazide in biological samples.

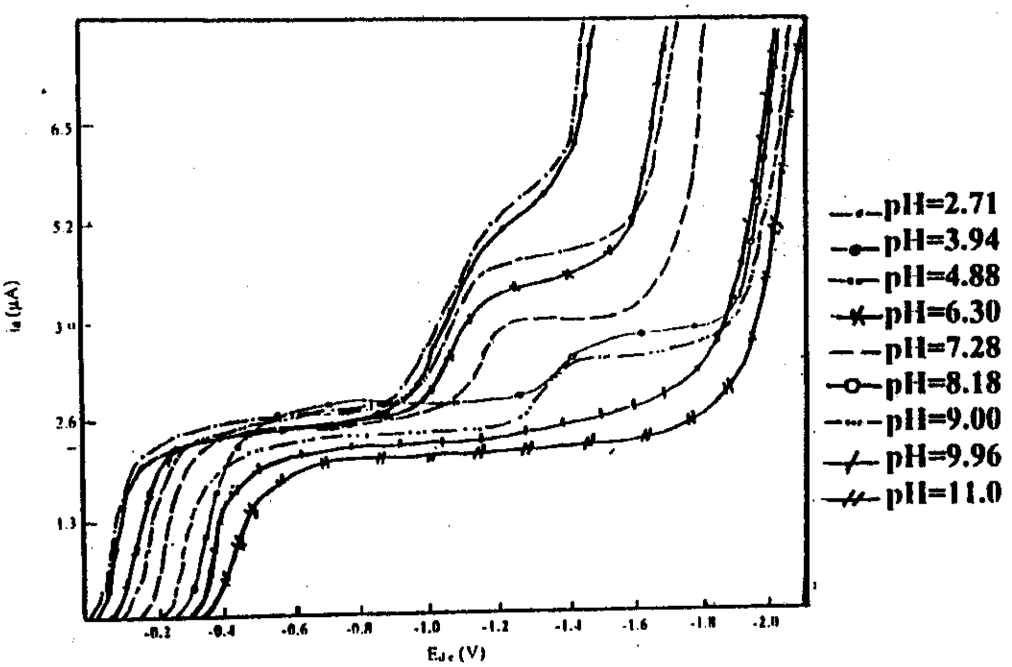


Fig.(29): DC-polarograms of 1x10^{-4} M of nifuroxazide in B.R. buffer solution of different pH values

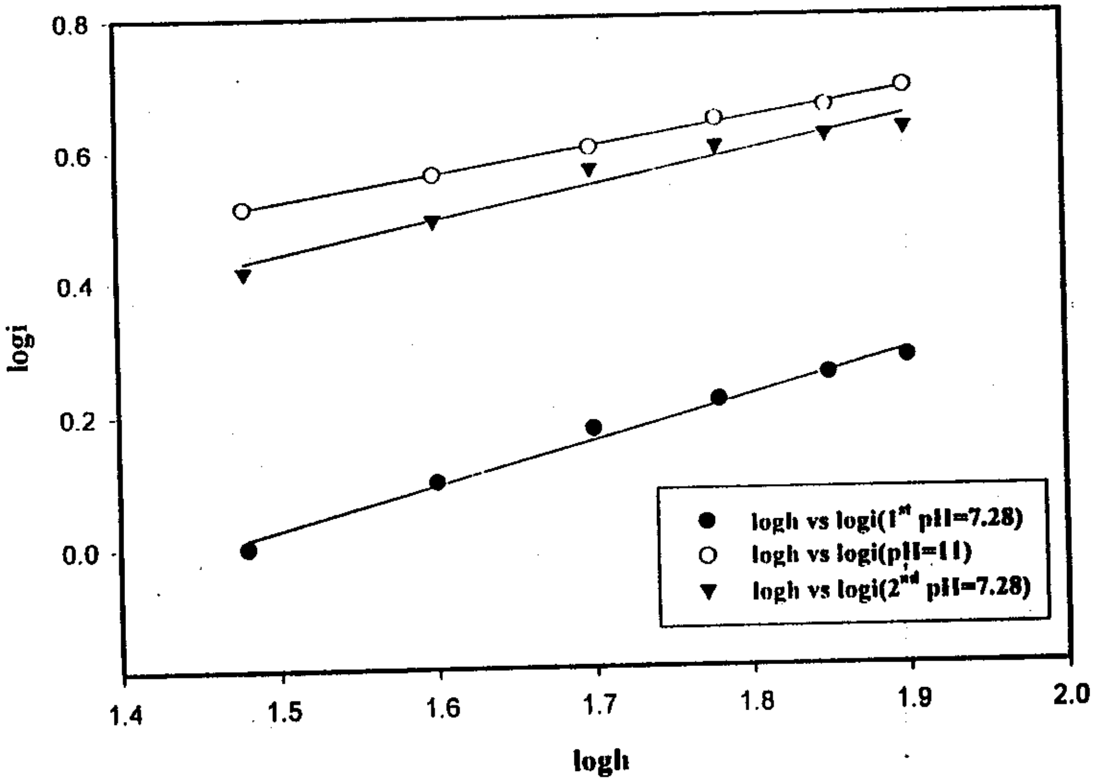


Fig. (30): logi-logh plots of Nifruxazide

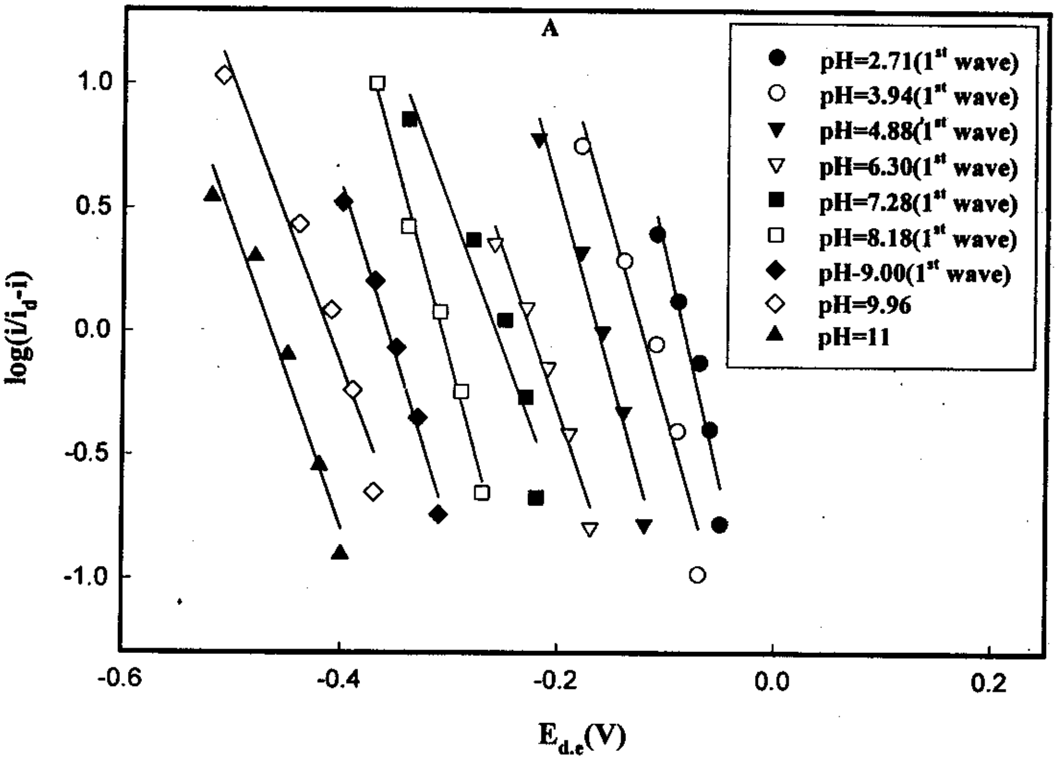


Fig. (31a): Analysis of the polarographic waves of nifruxazide at different pH values

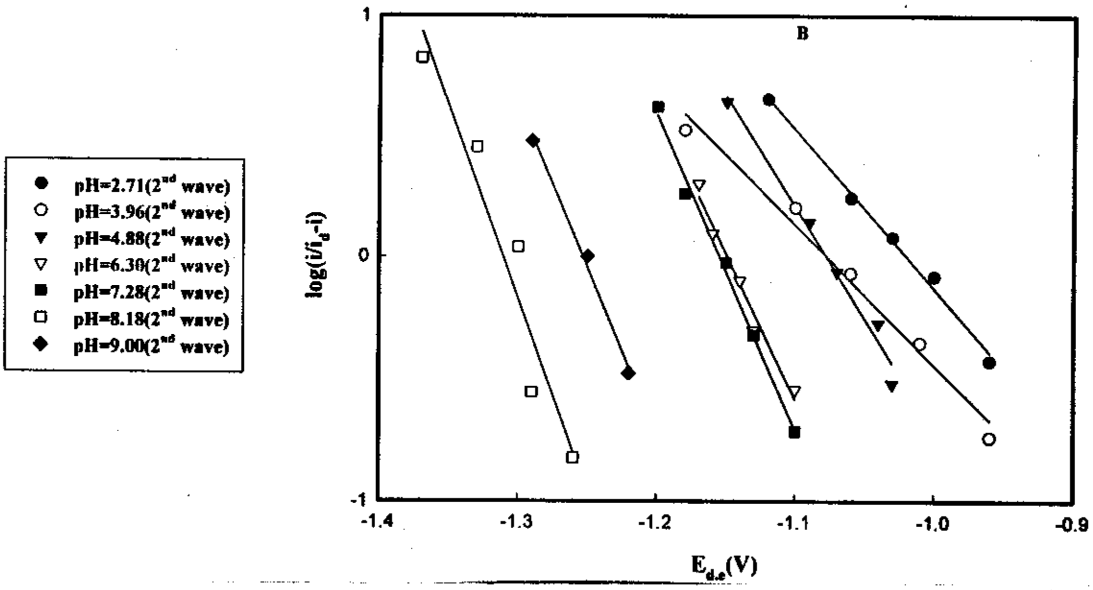


Fig. (31b): Analysis of the polarographic waves of nifruxazide at different pH values

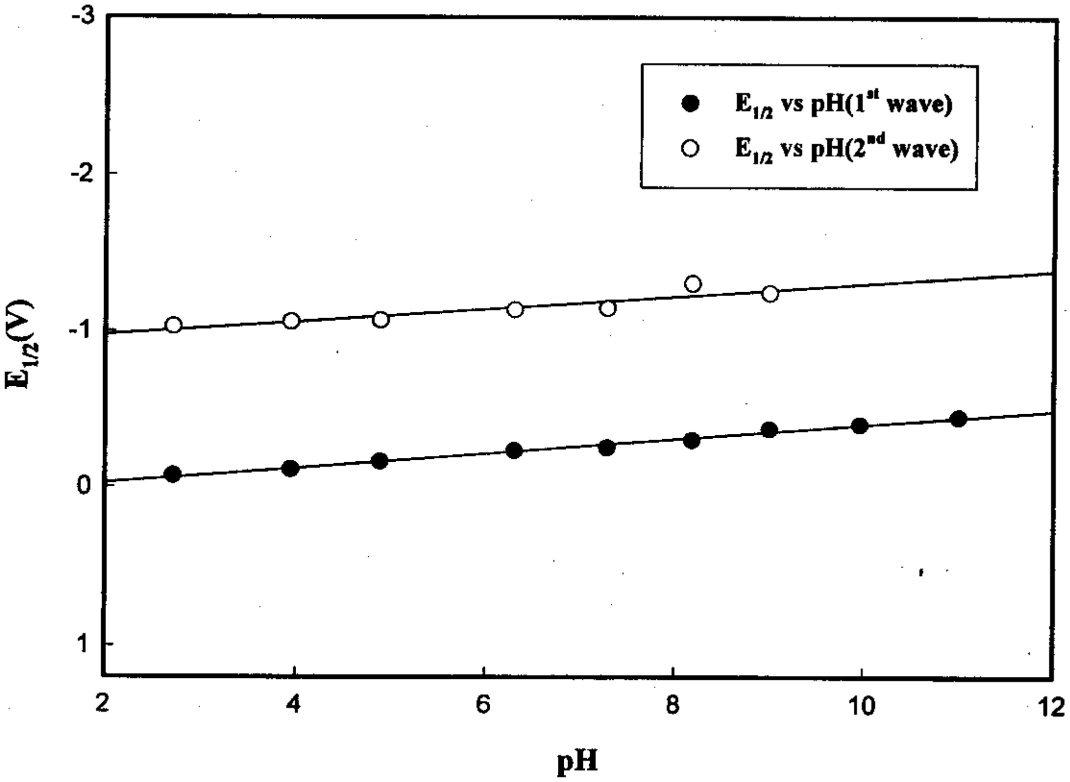


Fig. (32): $E_{1/2}$-pH plots of Nifruxazide

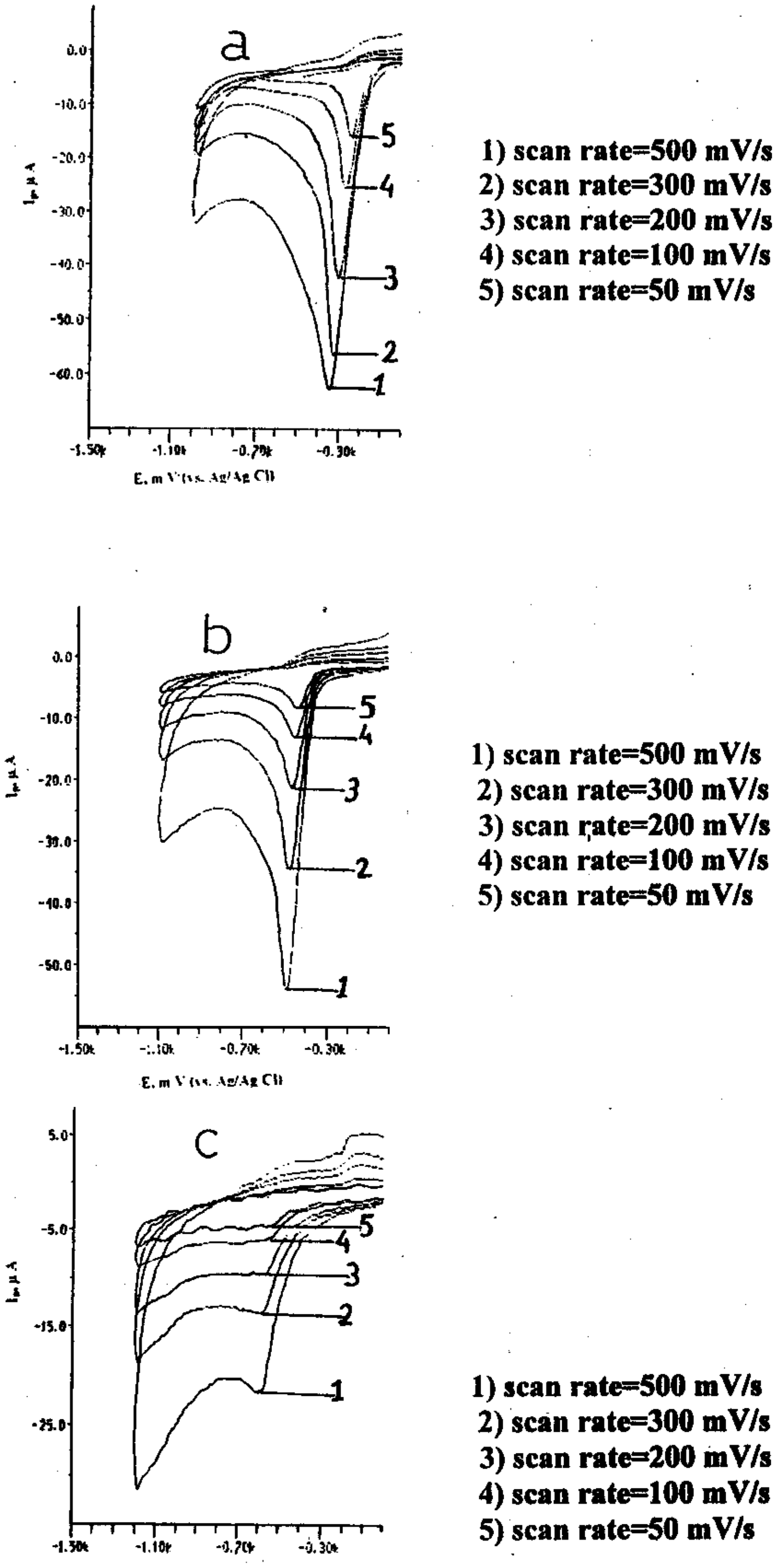


Fig. (33): Cyclic voltammograms of 1x 10^{-4} M of nifuroxazide in B.R. Buffer solution at different scan rates;(a) pH=2.71 , (b) pH=7.28 (c)pH=11.00 .

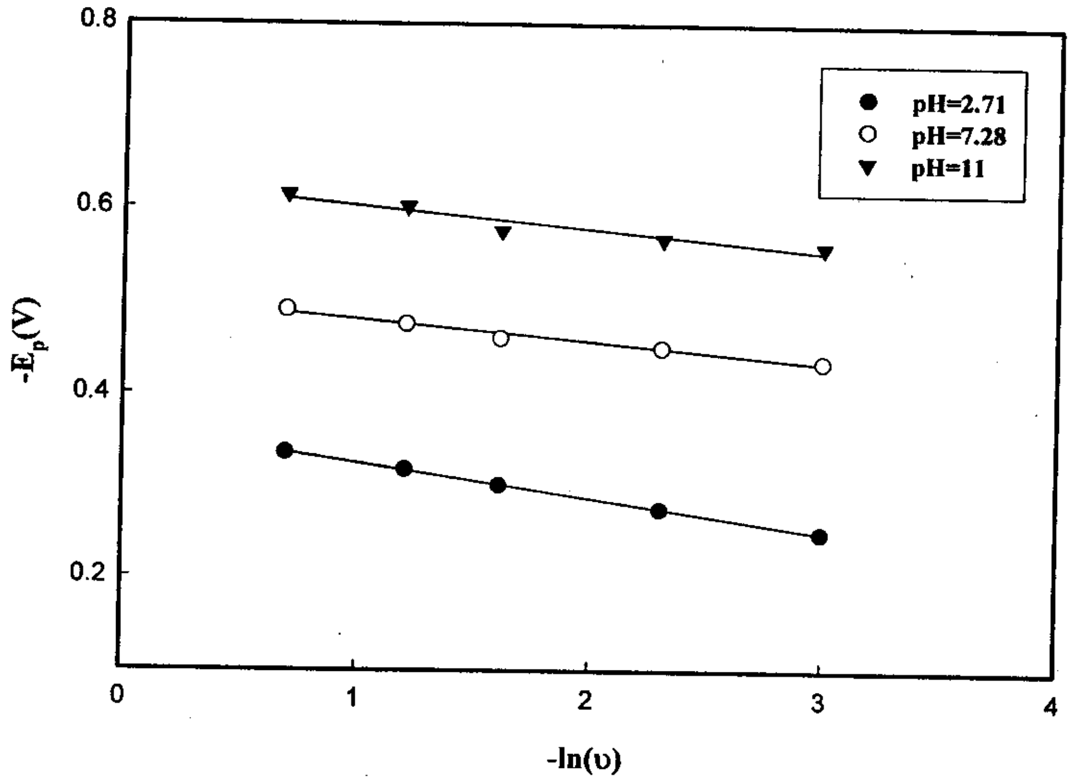


Fig. (34): E_p-ln(υ) plots of Nifruxazide at different pH values.

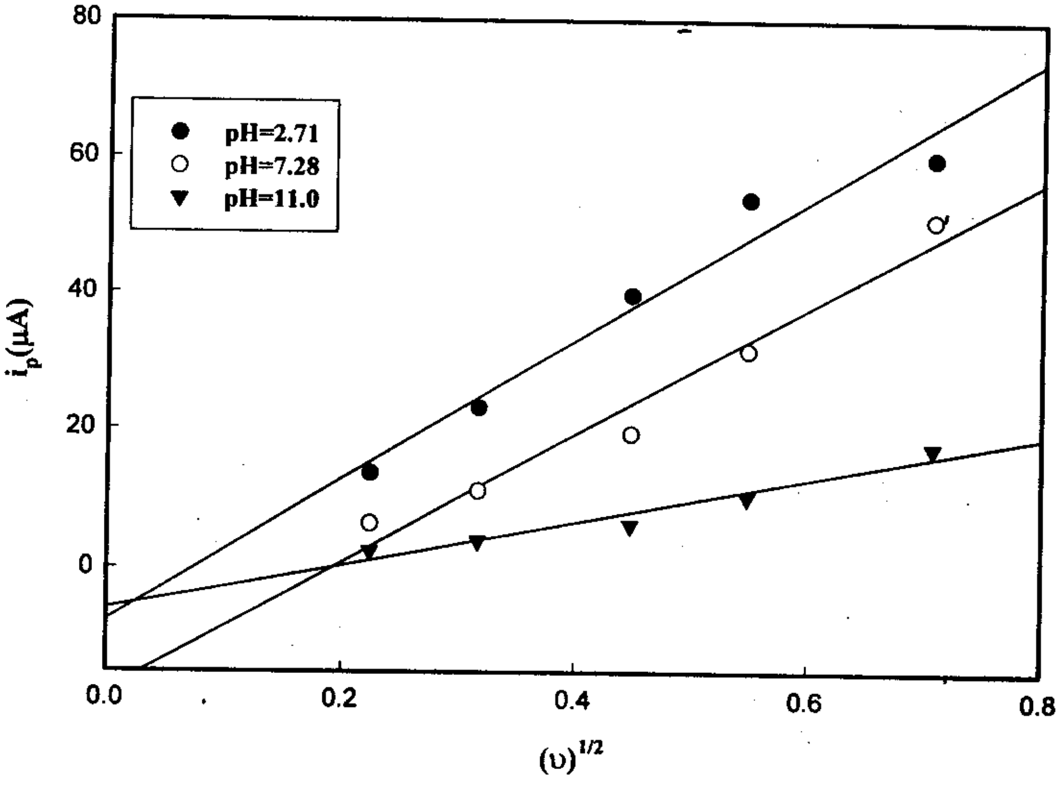


Fig. (35): i_p-$(\upsilon)^{1/2}$ plots of Nifruxazide at different pH values.

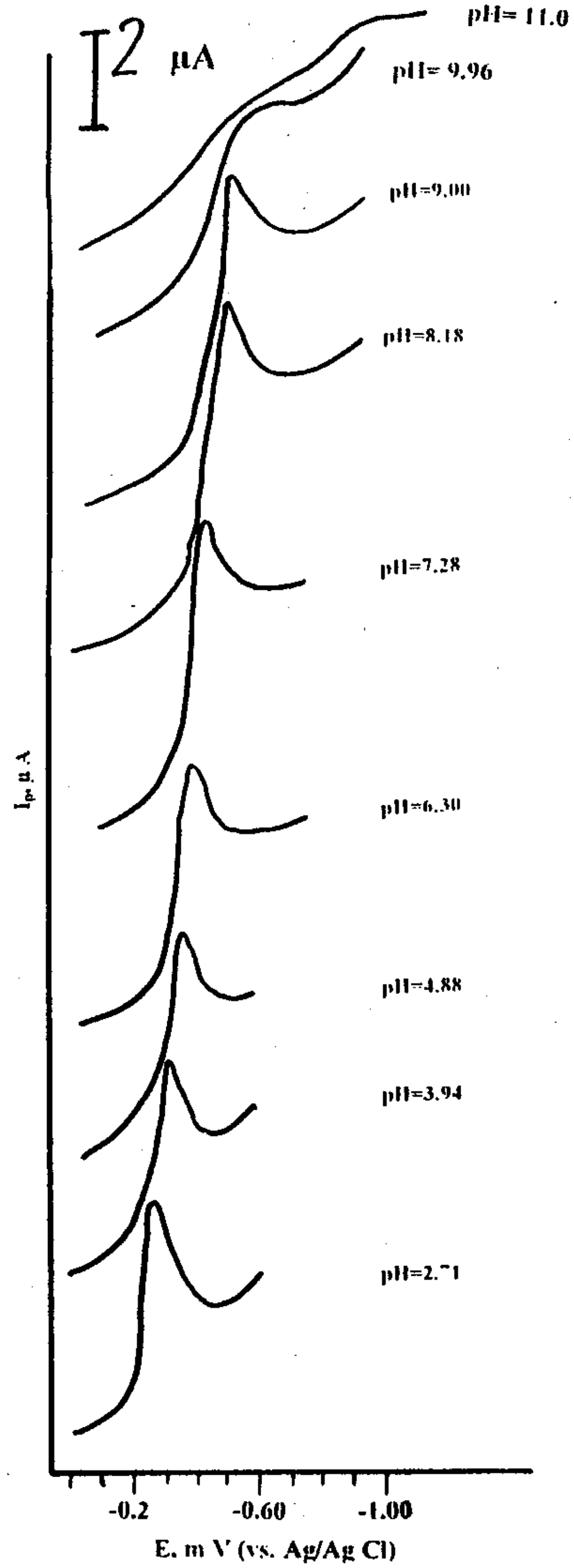


Fig. (36): Effect of pH on the CADS peak of 5x10^{-5} M of nifuroxazide in B.R. Buffer solution at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.

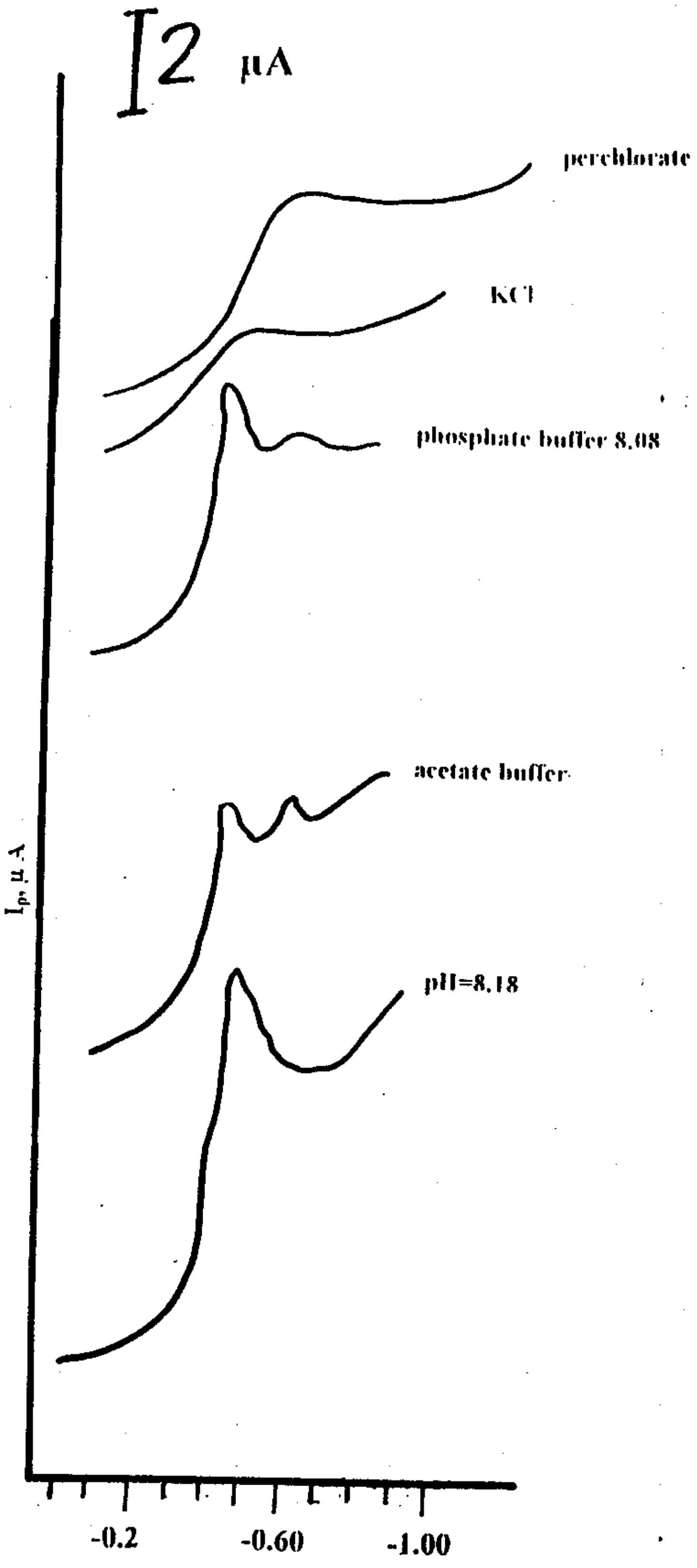


Fig. (37): **Effect of different supporting electrolyte solution on the CADS peak of 5×10^{-5} M of nifuroxazide at $t_a = 240$ s, $E_d = -0.4$ V, scan rate =100 mV/s and step height = 5 mV.**

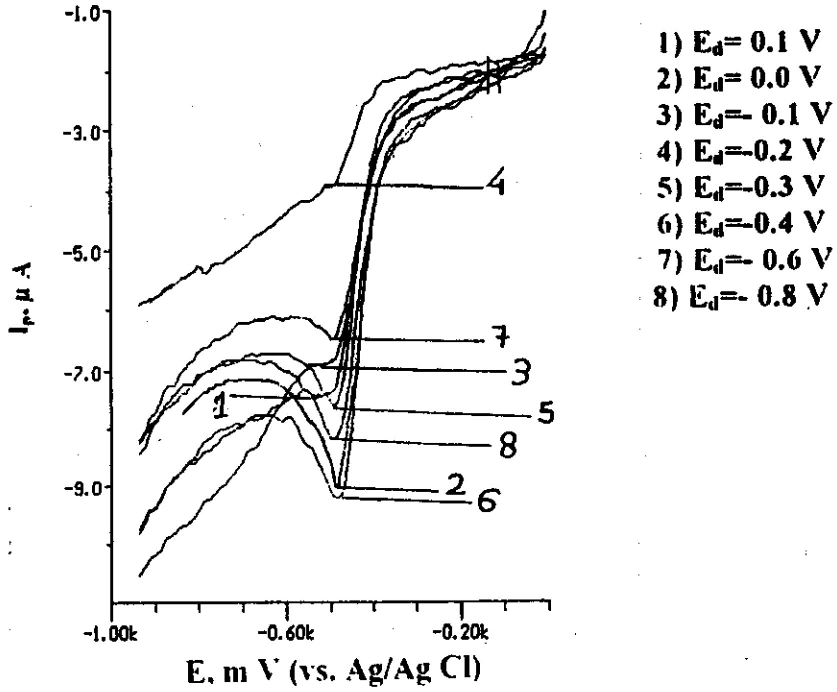


Fig. (38a): **Effect of deposition potential on $5x10^{-5}$ M of nifruxazide in B.R. buffer Solution of pH=8.18 at scan rate=100 mV/s, t_d=240 s, and pulse height=5 mV.**

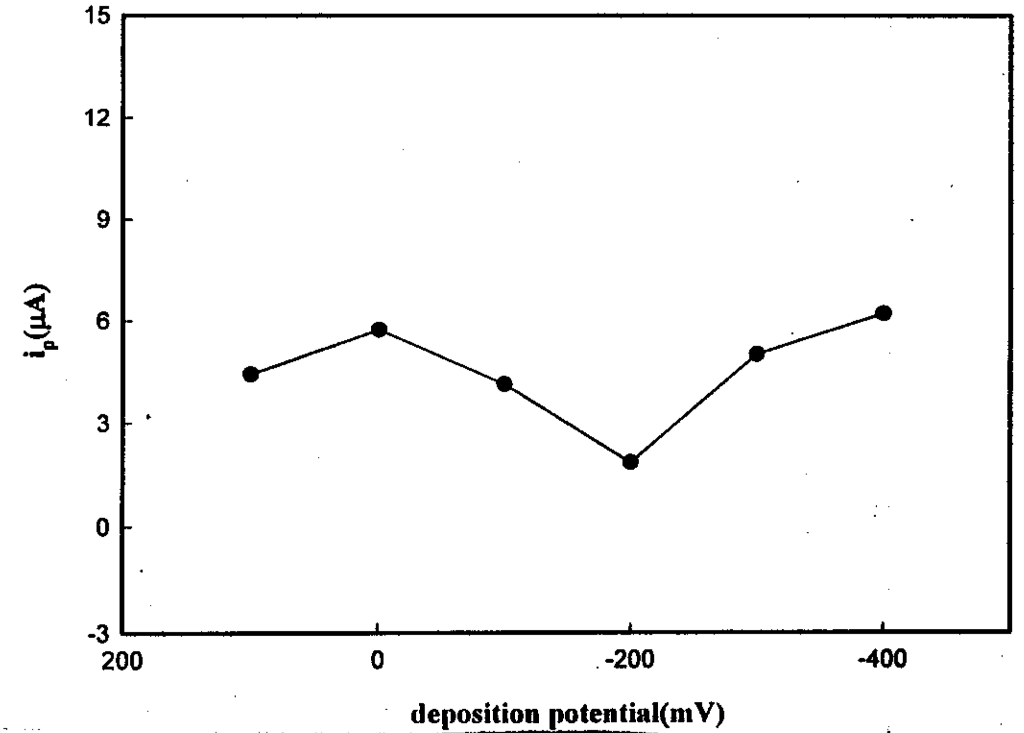


Fig. (38b): Effect of deposition potential on $5x10^{-5}$ M of nifruxazide in B.R. buffer solution of pH=8.18 at scan rate=100 mV/s, t_d=240 s, and pulse height=5 mV.

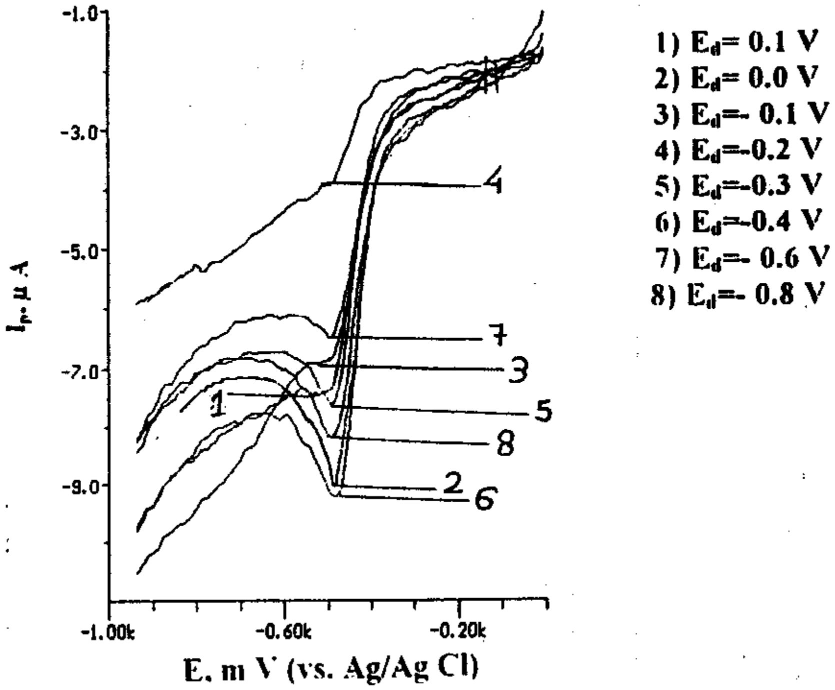


Fig. (38a): Effect of deposition potential on $5x10^{-5}$ M of nifruxazide in B.R. buffer Solution of pH=8.18 at scan rate=100 mV/s, t_d=240 s, and pulse height=5 mV.

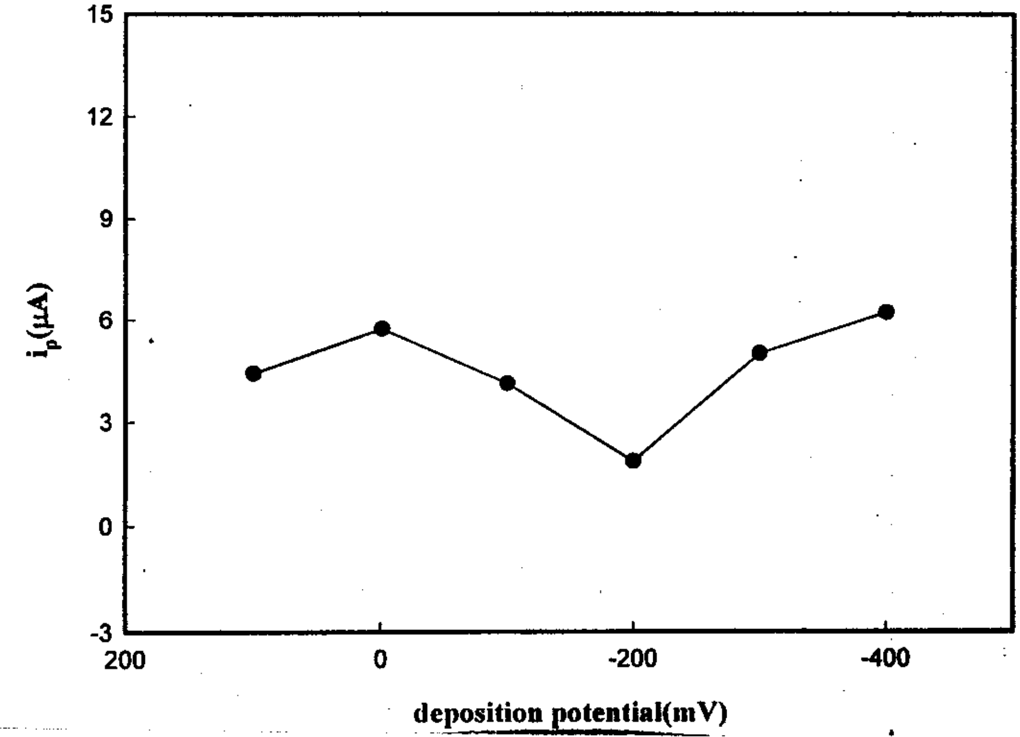


Fig. (38b): Effect of deposition potential on $5x10^{-5}$ M of nifruxazide in B.R. buffer solution of pH=8.18 at scan rate=100 mV/s, t_d=240 s, and pulse height=5 mV.

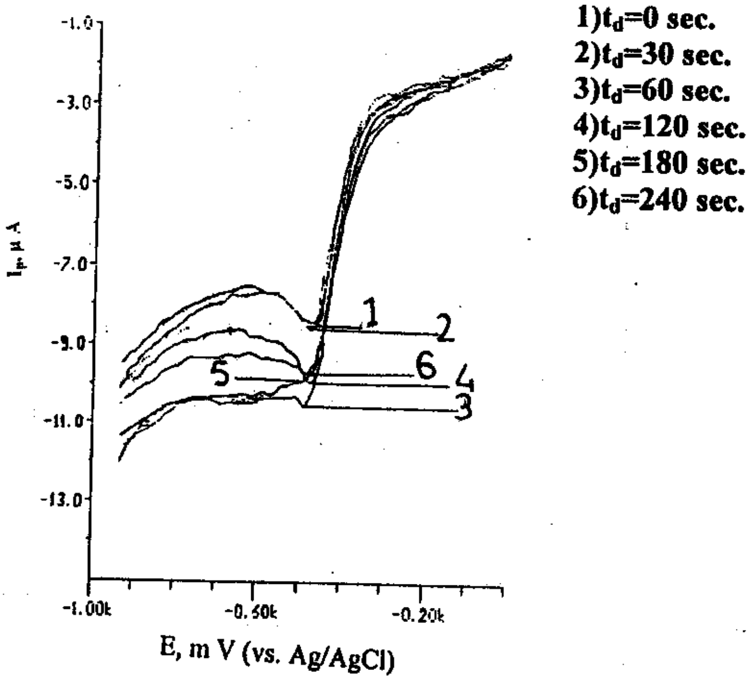


Fig. (39a): Effect of deposition time on 5x10^{-5} M of nifruxazide in B.R. buffer Solution of pH=8.18 at scan rate=500 mV/s s, E_d=-0.4V,and pulse height=5 mV.

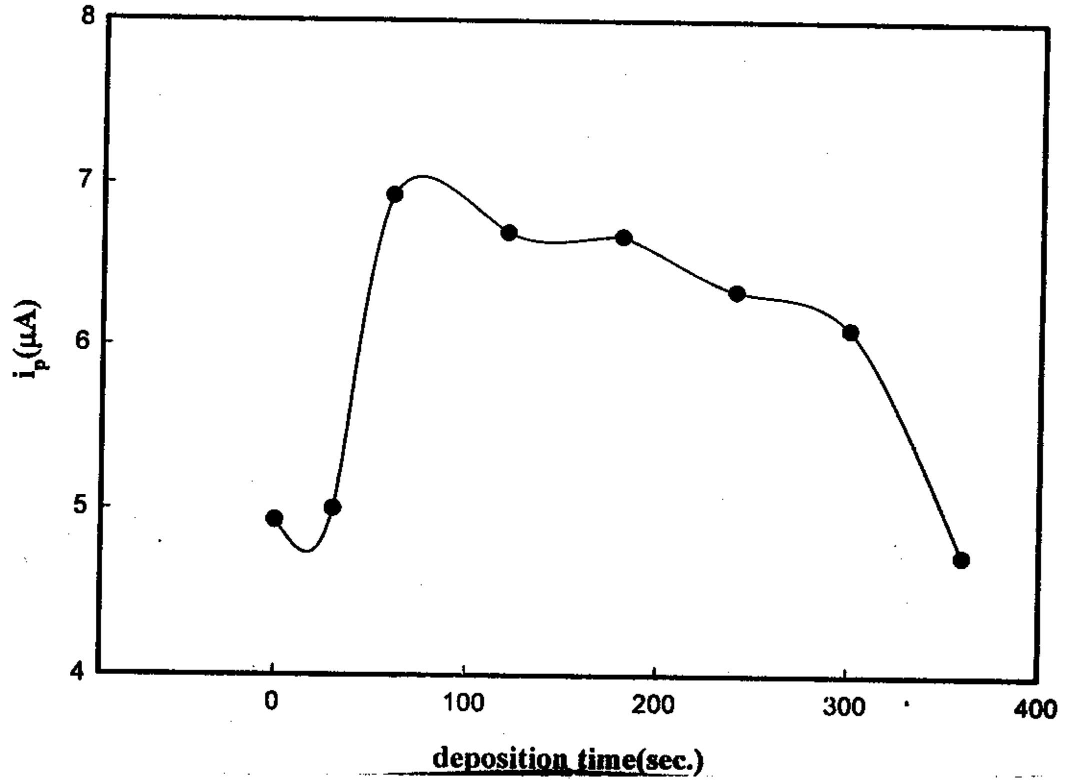


Fig. (39b): Effect of deposition time on 5x10^{-5} M of nifruxazide in B.R. buffer solution of pH=8.18 at scan rate=500mV/s s, E_d=-0.4V,and pulse height=5 mV.

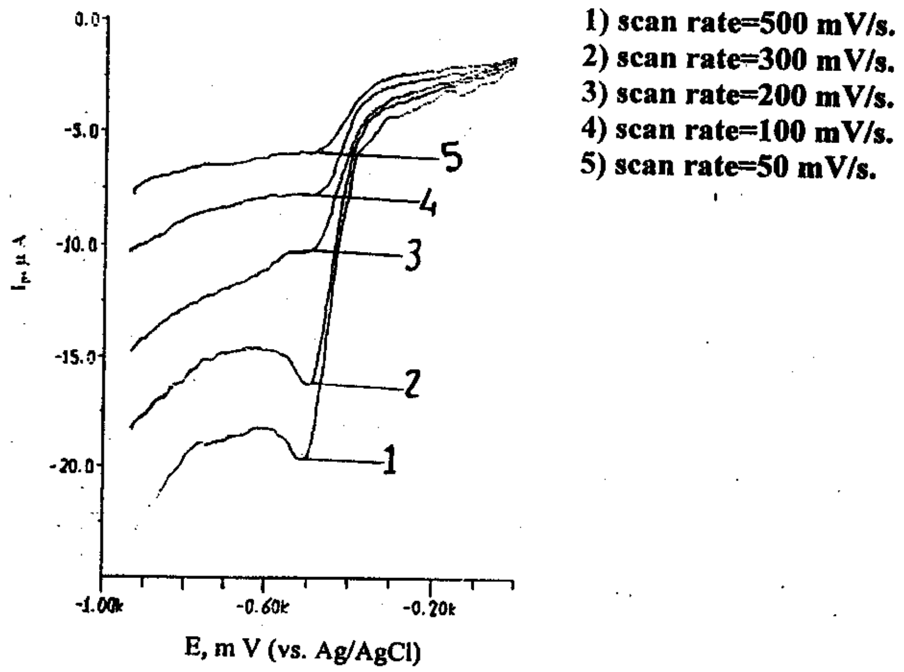


Fig. (40a):.Effect of scan rate on $5x10^{-5}$M of Nifruxazide in B.R.buffer solution of pH=8.18 at t_d=240 s,E_d=-0.4V,and pulse height=5 mV

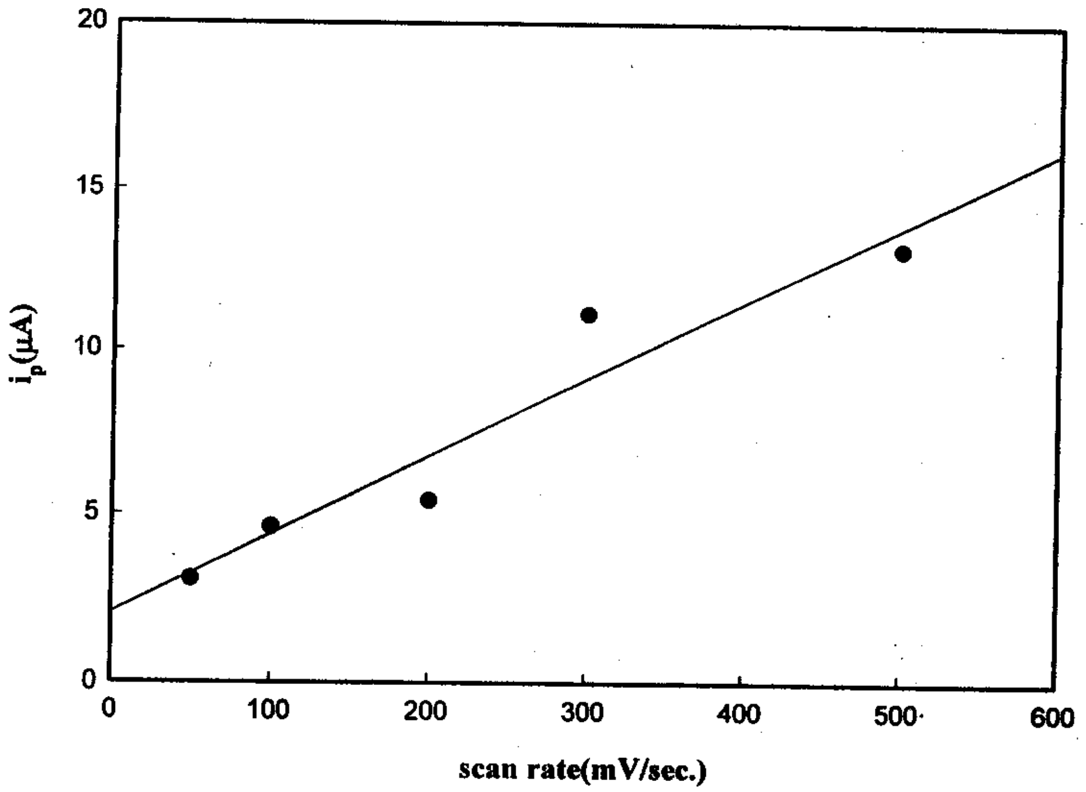


Fig. (40b): Effect of scan rate on $5x10^{-5}$M of Nifruxazide in B.R.buffer solution of pH=8.18 at t_d=240 s,E_d=-0.4V,and pulse height=5 mV.

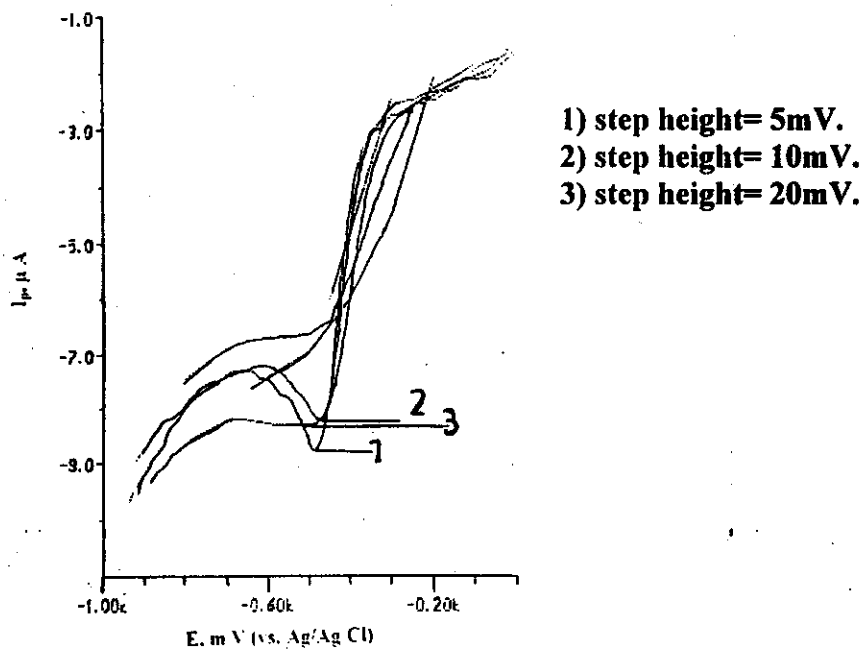


Fig. (41a): Effect of pulse height on 5x10-5M of Nifruxazide in B.R.buffer solution of pH=8.18 at t_d=240 s, E_d=-0.4V,and scan rate=100 mV/s.

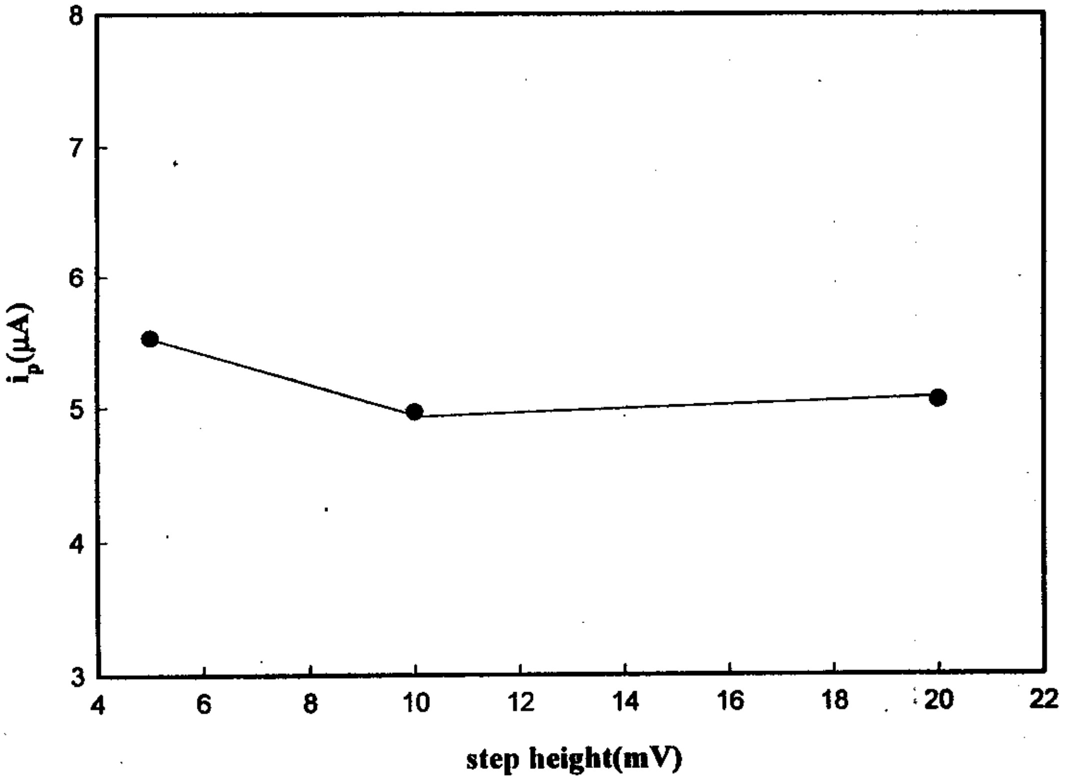


Fig. (41b): Effect of pulse height on $5x10^{-5}$M of Nifruxazide in B.R.buffer solution of pH=8.18 at t_d=240 s,E_d=-0.4V,and scan rate=100 mV/s.

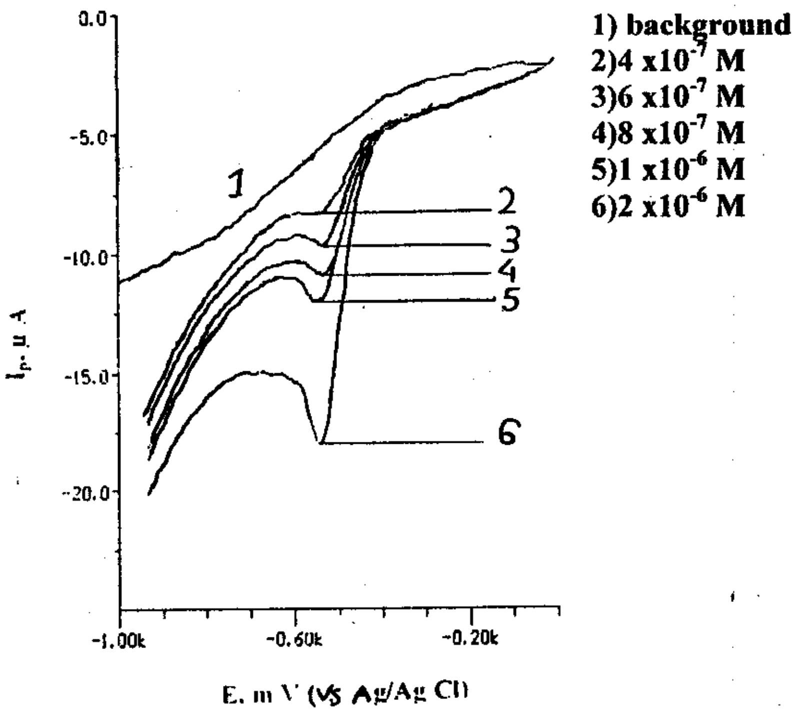


Fig. (42a): Effect of concentration of nifuroxazide in B.R. buffer solution of pH=8.18 at: t_d=60 s, E_d= -0.4 V, scan rate 500 mV/s and step height= 5 mV

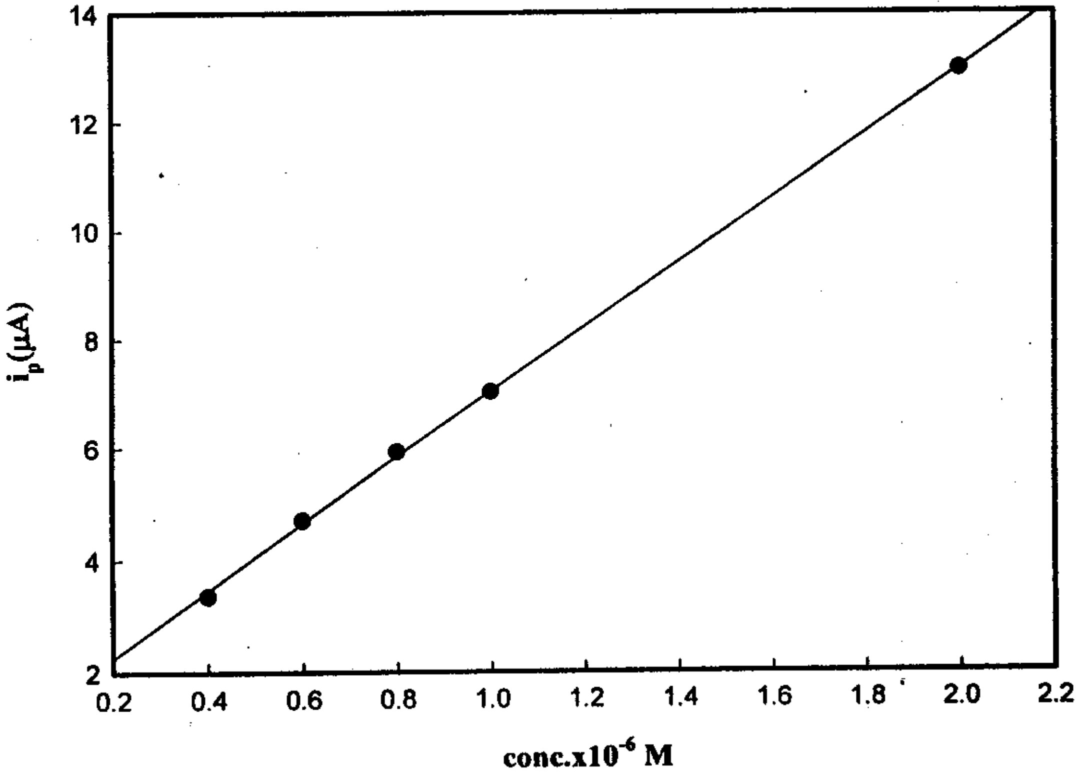


Fig. (42b): calibration curve for nifuroxzide.

Table(17) : Polarographic data for $1x10^{-4}$M of nifuroxazide in B.R. buffer solution of different pH values at 25c°

pH	i_d(μA)		$-E_{1/2}$		Δ logi_l/Δ logh	
	A	B	A	B	A	B
2.71	2.40	2.16	0.07	1.03	0.56	0.48
3.94	2.28	2.12	0.11	1.06		
4.88	2.16	2.08	0.16	1.07		
6.30	2.12	1.88	0.23	1.14		
7.28	2.08	1.44	0.25	1.15	0.66	0.42
8.18	2.00	0.88	0.30	1.31		
9.00	1.92	0.76	0.37	1.25		
9.96	1.80	sh.	0.40	sh.		
11.0	1.72	–	0.45	–	0.52	sh.

Where A:1st wave B: 2nd wave sh: ill-defined

Table(18): Values of (Z_H^+) and (α) as determined for different ratios (Z_H^+/n_a) at 0.5, 1.0 and 2.0 of the number of protons calculated from slope(S_2)of the $E_{1/2}$-pH plots and (S_1) for nifuroxazide in B.R. buffer solutions of different pH values at 25C°

pH	S_1 mV		S_2 mV		(Z_H^+)=(S_2/S_1)		Z_H^+/n_a		
							0.5	1.0	2.0
	A	B	A	B	A	B			
2.71	54.44	152.67	46.8	41.4	0.86	0.27	0.63	0.78	2.50
3.94	66.98	174.22	46.8	41.4	0.70	0.28	0.63	0.78	2.50
4.88	65.19	109.29	46.8	41.4	0.72	0.38	0.63	0.78	2.50
6.30	79.24	83.96	46.8	41.4	0.59	0.49	0.63	0.78	2.50
7.28	85.62	77.16	46.8	41.4	0.55	0.54	0.63	0.78	2.50
8.18	63.13	63.41	46.8	41.4	0.74	0.65	0.63	0.78	2.50
9.00	72.05	73.86	46.8	41.4	0.65	0.56	0.63	0.78	2.50
9.96	86.66	sh.	46.8	sh.	0.54	sh.	0.63	0.78	2.50
11.0	81.90	—	46.8	—	0.57	—	0.63	0.78	2.50

Where A:1st wave B:2nd wave sh: ill-defined

Table(19a): Values of (αna) and (α) for $1x10^{-4}$ M of nifuroxazide in B.R buffer solution of different pH values, as calculated from reciprocal slope (S_1) of the log (i/i_d-i) -$E_{d.e}$ plots.

pH	Slop(S_1) mV		αn_a		α			
					n=1	n=2	n=1	n=2
	A	B	A	B	A	A	B	B
2.71	54.44	152.62	1.09	0.39	1.09	0.55	0.39	0.20
3.96	66.98	174.22	0.88	0.34	0.88	0.44	0.34	0.17
4.88	65.19	109.29	0.91	0.54	0.91	0.45	0.54	0.27
6.30	79.24	83.96	0.75	0.70	0.75	0.38	0.70	0.35
7.28	85.62	77.16	0.69	0.77	0.69	0.35	0.77	0.39
8.18	63.13	63.41	0.94	0.93	0.94	0.47	0.93	0.47
9.00	72.05	73.86	0.82	0.80	0.82	0.41	0.80	0.40
9.96	86.66	sh.	0.68	sh.	0.68	0.34	sh.	sh.
11.0	81.90	–	0.72	–	0.72	0.36	–	–

Where A: 1st wave B: 2nd wave sh: ill-defined

Table (20): Kinetic parameters obtained from D-C measurements for 1×10^{-4} M nifuroxazide in B. R. buffer solution of different pH's.

pH	D^{o}(Cm^{2}/s)	$K^{o}_{f,h}$ (Cm/s)	ΔG^{*} (K.cal/mole)
2.71	5.60×10^{-6}	9.00×10^{-9} ^{a}	75.82 ^{a}
		2.24×10^{-11} ^{b}	90.98
3.94	5.60×10^{-6}	2.70×10^{-10} ^{a}	84.75 ^{a}
		1.58×10^{-12} ^{b}	97.65
4.88	5.60×10^{-6}	5.00×10^{-11} ^{a}	88.98 ^{a}
		4.68×10^{-15} ^{b}	112.28 ^{b}
6.30	4.30×10^{-6}	3.00×10^{-11} ^{a}	90.26 ^{a}
		1.55×10^{-19} ^{b}	138.17 ^{b}
7.28	4.30×10^{-6}	6.00×10^{-12} ^{a}	94.30 ^{a}
		3.55×10^{-21} ^{b}	147.61 ^{b}
8.18	4.30×10^{-6}	1.12×10^{-13} ^{a}	104.29 ^{a}
		2.57×10^{-23} ^{b}	160.00 ^{b}
9.00	3.40×10^{-6}	1.26×10^{-14} ^{a}	109.78 ^{a}
		1.45×10^{-27} ^{b}	184.75 ^{b}
9.96	3.40×10^{-6}	1.02×10^{-14} ^{a}	110.31 ^{a}
11.0	3.40×10^{-6}	8.71×10^{-15} ^{a}	110.70 ^{a}

Where a : 1st wave , b : 2nd wave .

Table(19b) : Data obtained for $1x10^{-4}M$ of nifuroxazide in B.R. buffer solution of different pH values using cyclic voltammetry at different scan rates, 25°C.

pH	Scan rate (mV/s)	-Ep (V)	-dEp/d(lnv)	αn_a
2.71	500	0.34	0.038	0.34
	300	0.32		
	200	0.30		
	100	0.28		
	50	0.25		
7.28	500	0.49	0.023	0.56
	300	0.48		
	200	0.46		
	100	0.45		
	50	0.44		
11.0	500	0.62	0.025	0.51
	300	0.60		
	200	0.58		
	100	0.57		
	50	0.56		

Table(21) :Cathodic adsorptive stripping peak current(i_p)of $5x10^{-5}M$ nifuroxazide in B.R.buffer solution of pH=8.18 at different conditions (t_d, E_d, scan rate and pulse height).

Deposition time (t_d), sec	Deposition potential(E_d), V	Scan rate (mV/s)	Pulse hieght (mV)	i_p (μA)
Effect of (td):				
0	-0.4	500	5	4.93
30				5.00
60				6.93
120				6.70
180				6.68
240				6.34
300				6.11
360				4.73
Effect of (E_d):				
240	0.1	100	5	4.43
	-0.01			5.73
	-0.1			4.13
	-0.2			1.86
	-0.3			5.01
	-0.4			6.21
Effect of scan rate:				
240	-0.4	500	5	13.18
		300		11.11
		200		5.42
		100		4.62
		50		3.06
Effect of pulse height:				
240	-0.4	500	5	5.53
			10	4.97
			20	5.06
			50	
			100	
			150	
			200	

Table (22): Calibration curve data of nifuroxazide in B.R.buffer solution of pH=8.18 using CAdSV at t_d= 60 sec., Ed= -0.4 V, scan rate = 500mV/s, and step height= 5 mV.

Conc.taken	Ip(µA)	**Mean**	**S.D**	**R.S.D %**
$4x10^{-7}$ M	**2.48**	**2.50**	**0.070**	**2.80**
	2.41			
	2.55			
	2.58			
$6x10^{-7}$ M	**3.87**	**3.90**	**0.096**	**2.48**
	3.83			
	3.94			
	3.98			
$8x10^{-7}$ M	**5.16**	**5.09**	**0.123**	**1.24**
	4.98			
	4.98			
	5.22			
$1x10^{-6}$ M	**6.34**	**6.18**	**0.093**	**1.50**
	6.20			
	6.08			
	6.08			
$1.5x10^{-6}$ M	**8.52**	**8.60**	**0.175**	**2.03**
	8.74			
	8.52			
	8.65			
$2x10^{-6}$ M	**10.98**	**11.1**	**0.254**	**2.28**
	10.80			
	11.34			
	11.28			

Table (23): Assay of nifuroxazide in dosage form (Antinal) at pH=8.18 using CAdSV at t_d= 60sec., E_d= -0.2 V, scan rate= 500mV/s, and step height= 5 mV.

Conc.taken	Conc. calculated	Recovery %	Mean Recovery(%) ± S.D.
$4x10^{-7}$ M	$4.01 x10^{-7}$ M	100.3	99.37 ±1.96
	$3.93 x10^{-7}$ M	98.25	
	$4.06 x10^{-7}$ M	101.5	
	$3.90 x10^{-7}$ M	97.50	
$6x10^{-7}$ M	$5.90 x10^{-7}$ M	98.33	99.74 ±1.16
	$6.01 x10^{-7}$ M	100.2	
	$5.97 x10^{-7}$ M	99.50	
	$6.06 x10^{-7}$ M	101.0	
$8x10^{-7}$ M	$8.04 x10^{-7}$ M	100.5	99.34 ±2.30
	$7.80 x10^{-7}$ M	97.50	
	$7.80 x10^{-7}$ M	97.50	
	$8.15 x10^{-7}$ M	101.9	
$1x10^{-6}$ M	$1.01 x10^{-6}$ M	101.0	100.3 ±1.00
	$1.00 x10^{-6}$ M	100.0	
	$0.99 x10^{-6}$ M	99.00	
	$1.01 x10^{-6}$ M	101.0	
$2x10^{-6}$ M	$1.96 x10^{-6}$ M	98.00	99.00 ±2.16
	$1.94 x10^{-6}$ M	97.00	
	$2.02 x10^{-6}$ M	101.0	
	$2.00 x10^{-6}$ M	100.0	

Table (24): Assay of nifuroxazide in dosage form (Drotozide) at pH=8.18 using CAdSV at t_d= 60sec., E_d= -0.2 V, scan rate= 500mV/s, and step height= 5 mV.

Conc.taken	Conc. calculated	Recovery %	Mean Recovery(%) ± S.D
$8x10^{-7}$ M	**$8.06\ x10^{-7}$ M**	**100.8**	**100.06 ±1.95**
	$8.20\ x10^{-7}$ M	**102.5**	
	$7.90\ x10^{-7}$ M	**98.75**	
	$7.86\ x10^{-7}$ M	**98.25**	
$1x10^{-6}$ M	**$1.06\ x10^{-6}$ M**	**106.0**	**104.3 ±5.06**
	$0.99\ x10^{-6}$ M	**99.00**	
	$1.02\ x10^{-6}$ M	**102.0**	
	$1.06\ x10^{-6}$ M	**106.0**	
$2x10^{-6}$ M	**$1.96\ x10^{-6}$ M**	**98.00**	**99.50 ±1.22**
	$2.01\ x10^{-6}$ M	**100.5**	
	$1.99\ x10^{-6}$ M	**99.50**	
	$2.00\ x10^{-6}$ M	**100.0**	

Table (25) : Assay of nifuroxazide in dosage form (Diax) at pH=8.18 using CAdSV at t_d= 60sec., E_d= -0.2 V, scan rate= 500mV/s, and step height= 5 mV.

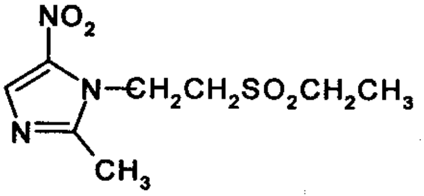
Conc.taken	Conc. calculated	**Recovery** %	**Mean** Recovery(%) ± S.D
$2x10^{-7}$ M	**$2.06\ x10^{-7}$ M**	**103.0**	**101.0 ±1.82**
	$2.00\ x10^{-7}$ M	**100.0**	
	$2.00\ x10^{-7}$ M	**100.0**	
	$2.02\ x10^{-7}$ M	**101.0**	
$4x10^{-7}$ M	**$3.99\ x10^{-7}$ M**	**99.75**	**99.56 ±1.19**
	$4.04\ x10^{-7}$ M	**101.0**	
	$3.96\ x10^{-7}$ M	**99.00**	
	$3.94x10^{-7}$ M	**98.50**	
$6x10^{-7}$ M	**$6.12\ x10^{-7}$ M**	**102.0**	**101.1 ±1.73**
	$6.00\ x10^{-7}$ M	**100.0**	
	$6.09\ x10^{-7}$ M	**101.5**	
	$6.05\ x10^{-7}$ M	**100.8**	
$8x10^{-7}$ M	**$8.18\ x10^{-7}$ M**	**102.3**	**100.8 ±1.47**
	$8.04\ x10^{-7}$ M	**100.5**	
	$7.96\ x10^{-7}$ M	**99.50**	
	$8.08\ x10^{-7}$ M	**101.0**	
$1x10^{-6}$ M	**$0.98\ x10^{-6}$ M**	**98.00**	**100.3 ±2.08**
	$1.00\ x10^{-6}$ M	**100.0**	
	$1.00\ x10^{-6}$ M	**100.0**	
	$1.03\ x10^{-6}$ M	**103.0**	

Table (26): Assay of nifuroxazide in serum in B.R.buffer solution of pH=8.18 using CAdSV at td= 60 sec., Ed= -0.4 V, scan rate= 500mV/s, and step height= 5mV.

Conc.taken µg/10 ml	Conc. Calculated µg/10 ml	**Recovery** %	**Mean** Recovery(%) ± S.D
1.63	**1.61**	**98.77**	**100.8 ±2.69**
	1.66	**101.8**	
	1.66	**101.8**	
	1.64	**100.6**	
5.44	**5.38**	**98.89**	**99.35 ± 3.19**
	5.57	**102.4**	
	5.18	**95.22**	
	5.49	**100.9**	

3.4 Electrochemical behavior of tinidazole in B.R buffer solutions of different pH values:-

Tinidazole is -[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitro-1H-imidazole], and has the following structural formula :



This part includes study of e electrochemical behavior of tinidazole at Hg-electrode using of DC polarography and cyclic voltammetry techniques. The study aimed o to optimize the experimental and instrumental conditions for determination of the lowest possible concentration of the investigated pharmaceutical compound in pure and dosage forms using cathodic adsorptive stripping voltammetry.

3.4.1 DC polarography:-

3.4.1.i Current potential curves:-

The polarographic behavior of 1x10^{-4} M of tinidazole was studied in B.R. buffer solutions containing 10% (v/v) ethanol of different pH values in the range from 2 to 12 . The polarogrammes exhibit two reduction waves within the pH range studied corresponding to the reduction NO_2 group (nitro-group), Fig.(43) the half-wave potential $E_{1/2}$ of the reduction waves are pH dependent, and being shifted to more negative values on increasing the pH of the electrolysis solution .

3.4.1.ii Effect of pressure at mercury height:-

The effect of mercury height "h" was examined according to equation (3.1). The plots of log i_l against log h for tinidazole showed linear correlations at different pH values, Fig.(44). The slope values (x) are found in the range between 0.44 and 0.82 which indicate that the reduction process is controlled by diffusion and some adsorption contribution, Table (27) .

3.4.1. iii-Analysis of the polarographic waves:-

Analysis of the polarographic waves is quite important to evaluate the degree of reversibility of electrode reaction and to suggest the electrode mechanism On using equation (3.4), the plots of $\log[i/(i_d-i)]$ against $E_{d.e.}$ for tinidazole reduction at different pH values showed linear correlations, Fig.(45). The straight lines with reciprocal slopes (S_1) amounting in the range to (54.05 - 106.61) and (161-256) mV for the first and second waves; respectively, Table (28). This behavior revealed the irreversible nature of the electrode process.

3.4.1.iv-Half- wave potential –pH curves :-

The plots of the rate shift of $E_{1/2}$ of tinidazole versus the pH of the electrolysis solution showed linear correlations for both the first and second waves respectively . The slope values (S_2) of the first and second waves is amounting to 54.05 and 150 mV, respectively ,Table (28).

The number of hydrogen ions (Z_H^+) involved in the rate determining step for tinidazole reduction was calculated from both slopes of logarithmic analysis and $E_{1/2}$ plots according to equation (3.5). At all pH values Z_H^+ was found to be unity, Table (280).

The most probable values of α- parameter as shown in Table (29a). were determined at the probable ratio of (Z_H^+/n_a) using equation (3.6). the probable (Z_H^+/n_a) ratio was found to fit with (Z_H^+/n_a) = 0.5 at Table (28).

This behavior is further confirmed the irreversibility of electrode process and the rate determining step involves one proton and two electrons.

3.4.2 Cyclic voltammetry :-

The cyclic voltammetric behavior of 1×10^{-4} M of tinidazole was investigated in aqueous Britton-Robinson buffer solutions of different pH values (2-12) containing 10% (v/v) ethanol at glassy carbon electrode. The voltammogram displayed single irreversible cathodic peak in the cathodic scan in all media, where as, in the anodic scan there is no oxidation peaks were observed, Fig.(47).

On increasing the scan rate (υ), the cathodic peak potential (E_{pc}) was shifted to more negative potentials indicating the irreversible nature of the reduction peak. Furthermore, on increasing the pH of the electrolysis solution the peak potential (E_{pc}) is shifted to more negative values indicating the consumption of hydrogen ions in the electron transfer step. On employing equation (3.7), the plots of E_p versus ln υ gives linear correlations, Fig.(48). The slope values were used for the determination of αn_a The values of the (α) transfer coefficient were calculated at the probable n_a values and found to be less than 0.4 at na=2.0, Table(29b). This behavior is further confirmed the irreversible nature of electrode process. According to equation(3.8), the plots of i_p versus square root of scan rate ($\upsilon^{1/2}$) gives linear relation slightly deviating from the origin, Fig.(49). This behavior confirming that the electrode process of tinidazole is controlled mainly by diffusion with some adsorption contribution.

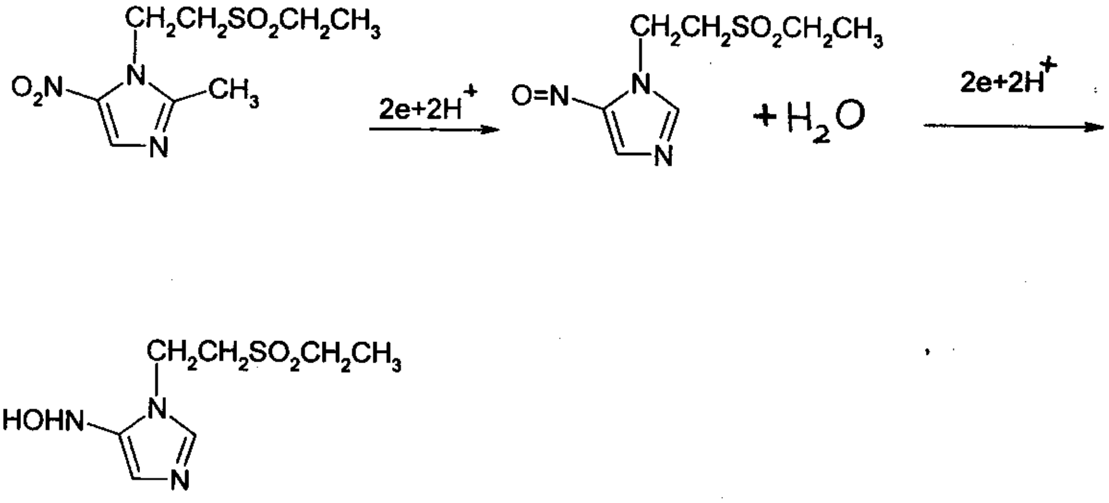
3.4.2.i-Kinetic parameters of the electrode reaction :-

The rate constant $k^o_{f,h}$ of the electrode reaction of tinidazole is calculated from the data obtained by dc- polarography measurements in Britton-Robinson buffer solution of various pH values. Equation (3.12) was used in this determination and the values of the rate constant $k_{f,h}$ were found to be decreased with increasing the pH which means that the electrode reaction becomes more difficult and more irreversible, Table (30) . Also, the values of ΔG^* were determined using equation (3.14) and found to be increased with increasing pH of the electrolysis medium, Table (30) . This may be explained on the basis that the protonation of the molecule, at lower pH, before its diffusion to the electrode surface, takes place through the contribution of the H^+ ion directly with the depolarizer molecule which decreases the activation energy ΔG^* . The H^+ ion activity decreases with rise of pH, hence ΔG^*

increases. In alkaline media, the proton required for pre-protonation of the depolarizer is supplied from a water molecule which requires much higher energy.

The electrode reaction mechanism :-

To study the reduction mechanism of tinidazole at mercury electrode, it is necessary to calculate the number of electrons concumed at this reduction, firstly we should calculate the (diffusion coefficient) D from equation (3.16) and the corrected values shown in Table (30). The number of electron (n) found that 4 electron are consumed in the reduction of nitro group at acidic and basic medium. The number of electrons were confirmed by controlled potential coulmetry method according to equation (3.17) the avarge value of n is (4±0.2) so the suggested mechanism as the following:



3.4.3 Cathodic adsorptive stripping voltammetry(CAdSV) of tinidazole :-

Cathodic adsorptive voltammetry was used for the quantitative determination of tinidazole in pure and dosage forms . the instrumental and experimental conditions were optimized in this investigation. The instrumental conditions are deposition time (td), deposition potential (Ed), step height and scan rate. While the experimental conditions are including the nature of supporting electrolyte and pH of the electrolysis solution.

3.4.3.i- Effect of pH and supporting electrolyte:

In cathodic adsorptive voltammetry current of tinidazole is recorded as function of potential in Britton- Robinson buffer solution of pH(2.03 to 10.55) the recorded peak at various pH values due to the reduction of nitro-group at the surface of glassy carbon electrode and well developed peak is noted at pH=6.13 Fig.(50).

We determined the tinidazole in some supporting electrolyte like (phosphate, acetate, $NaClO_4$ and KCl buffers) we fined thate in presence of $NaClO_4$,KCl the peak disappeared it means that the absence of this anion best of tinidazole and the pH=6.13 is most suitable for tinidazole Fig.(51)

3.4.3.ii- Effect of accumulation time and potential :

The deposition of the analyzed drug on the surface of the glassy carbon electrode is one of the essential conditions for highly sensitive determinations. The effect of accumulation potential on the peak height was examined over the potential range +100 to -800 mV for $5x10^{-5}$ M tinidazole at accumulation time 240 s., Fig. (52a). the relation between peaks height i_p and deposition potential E_d is shown in Fig. (52b). The peak current increased steadily with decreasing the potential value till it reaches maximum value at E_d = -400 mV, where it decreased sharply after this inflection point. Thus, E_d = -400 mV will be adopted as optimum operational values for the following works as it ensured the highest voltammetric signal.

The effect of accumulation time of the drug was examined in the range 0-360s. the amount of tinidazole accumulated on the electrode surface increased as the deposition time increased till 30 s, further increase of time resulted in decreasing the peak height . A time of accumulation of 30 s was chosen for analytical purposes. The voltammograms obtained for $5x10^{-5}$ M tinidazole in B. R. buffer solution of pH 6.13 and E_d = -0.4 V are shown in Fig. (53a). The dependence of peak height on accumulation time are illustrated in Fig. (53b).

3.4.3.iii- Effect of scan rate :

The effect of scan rate on the peak current and peak potential was studied. The relationship between i_p or E_p and υ at 240 s., accumulation time shows an increase in the peak current with increasing the scan rate, Fig. (54a,b). The peak potential was shifted to more negative values with increasing the scan rate.The scan rate 500 mv s^{-1} was used for further voltammetric determination of the drug.

3.4.3.iv- Effect of step height :-

The effect of step height on the CAdSV peak current of tinidazole reduction was recorded at different values, Fig. (55a).

A well defint peak was observed at 10 mV step height. On increasing step height up to 150 mV the CAdSV peak current decreases till become ill-defined. From the plot of peak current (i_p) versus step height it noted that i_p is inversely proportional to step height, Fig. (55b). And all data shown in Table (31).

3.4.3.v-Calibration curve, detection limit and precision :

under the optimum condition , a good linear correlation was obtained between the monitored voltammetric peak current and tinidazole.concentration in the range 1 $x10^{-6}$ to 1 $x10^{-5}$ M Fig.(56). Least-square treatment of the calibration graph of the first curve yielded the following equation :

$$i_p(\mu A) = 2.89 + 0.91 \times 10^6 \, C \, M; \quad r = 0.9956 \quad n = 6$$

the lowest detectable concentration (dl) of tinidazole was 2.6 x10^{-7} M. The reproducibility of thus analytical method was evaluated for different concentrations of the drug, Table (32) .

the second calibration curve can be described by the follwing regression equation :

$$i_p(\mu A) = 11.31 + 0.91 \times 10^6 \, C \, M; \quad r = 0.9995 \quad n = 8$$

Analysis of pharmaceutical formulation :

the proposed method was successfully applied to the assay of the studied drug in the pharmaceutical dosage forms (protozole tablets, labeled to contain 500 mg per tablet). The percentage recoveries for tinidazole are shown in Table (33). The method gave a good recovery. The percentage recovery based on six different determinations were 100.49 ± 1.45.

Analysis of serum :

serum samples were prepared as described before, and two different concentration were analysed using the proposed method (0.148 and 4.92 μg /ml). The recovery and the standard deviasion were evaluated from four measurements for each sample, Table (34). The mean recovery for the two concentration was 99.57 ± 2.25.

this means that the proposed method can be used for determination of tinidazole in biological samples.

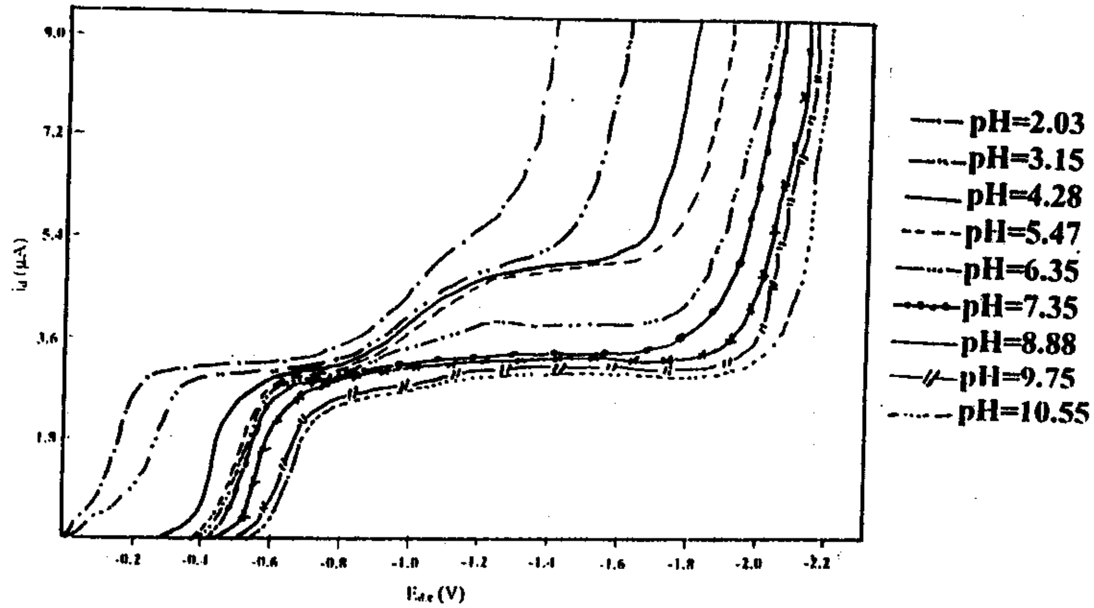


Fig. (43): DC-polarograms of $1x10^{-4}$ M of tinidazole in B.R. buffer solutions of different pH values

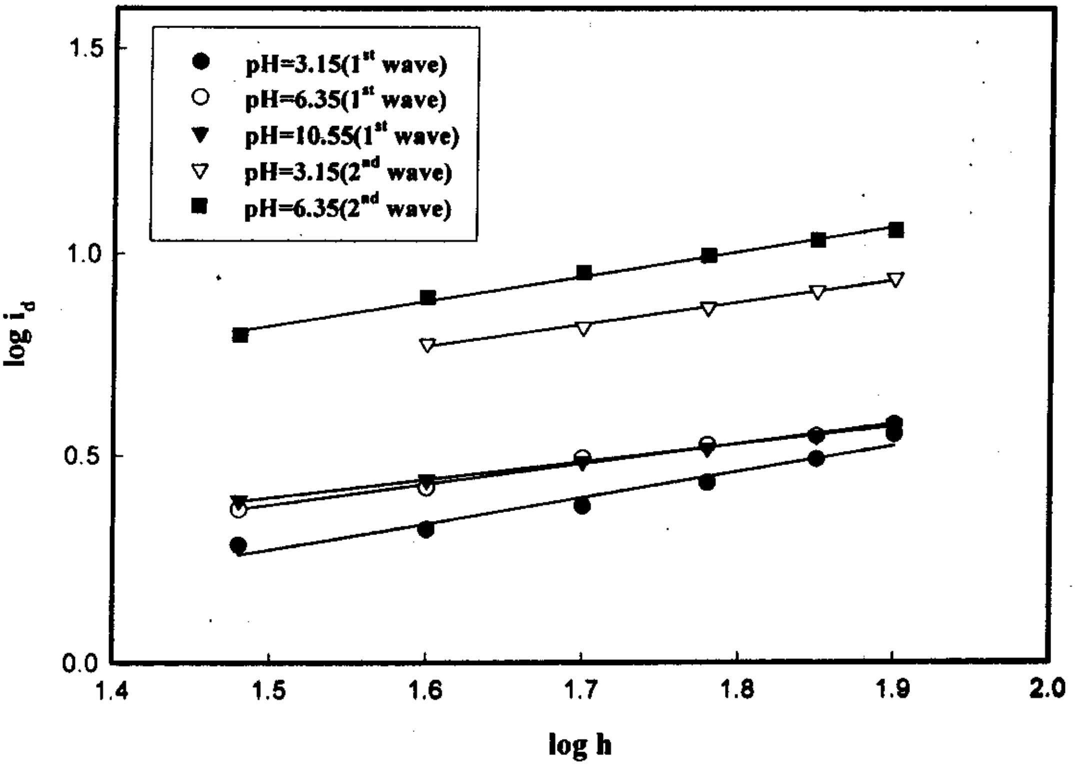


Fig. (44): log i_d-log h plots of tinidazole at different pH

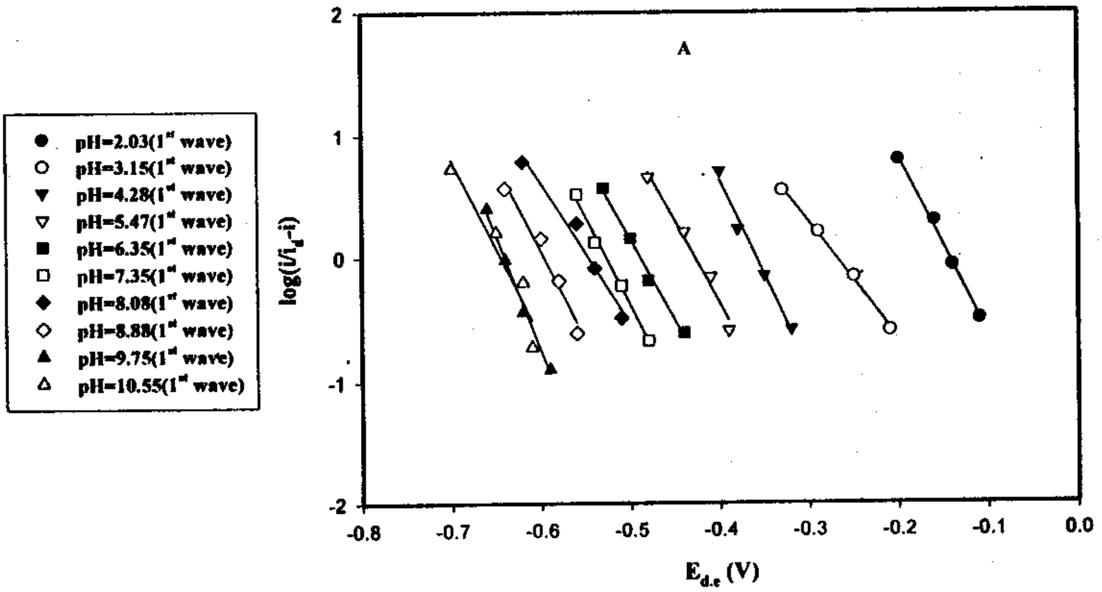


Fig. (45a): $E_{d.c.}$ & $\log(i/i_d\text{-}i)$ for tinidazole to 1st wave.

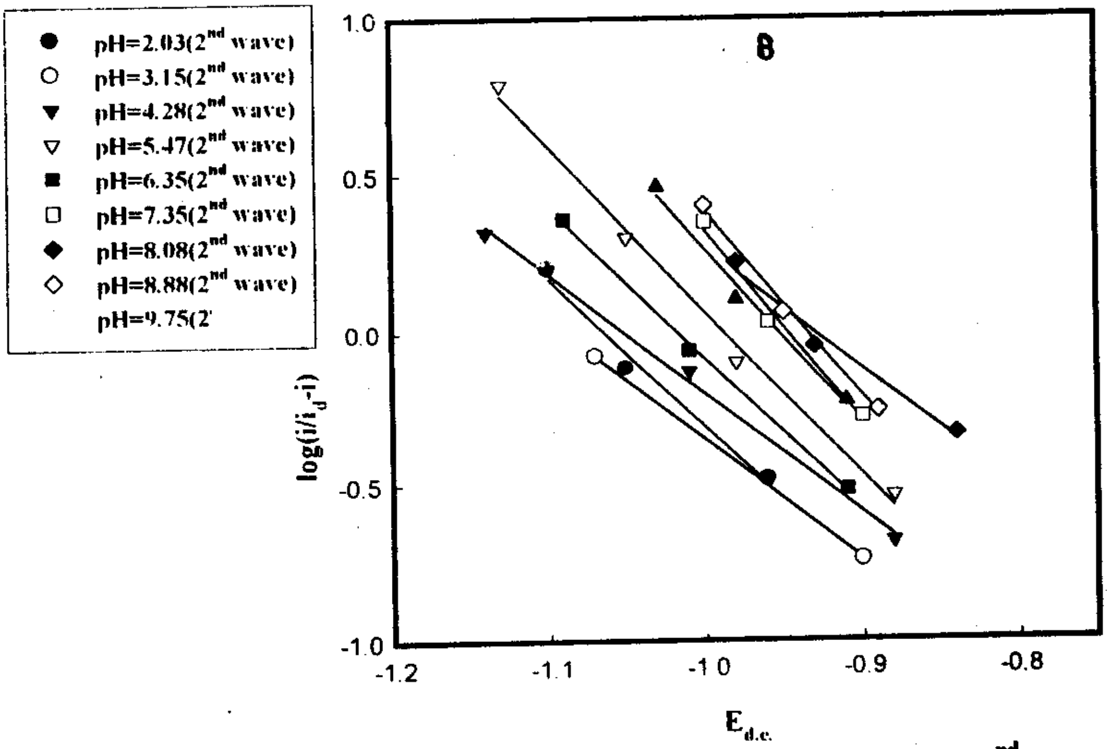


Fig.(45b): $E_{d.c.}$ & $\log(i/i_d\text{-}i)$ for tinidazole to 2nd wave.

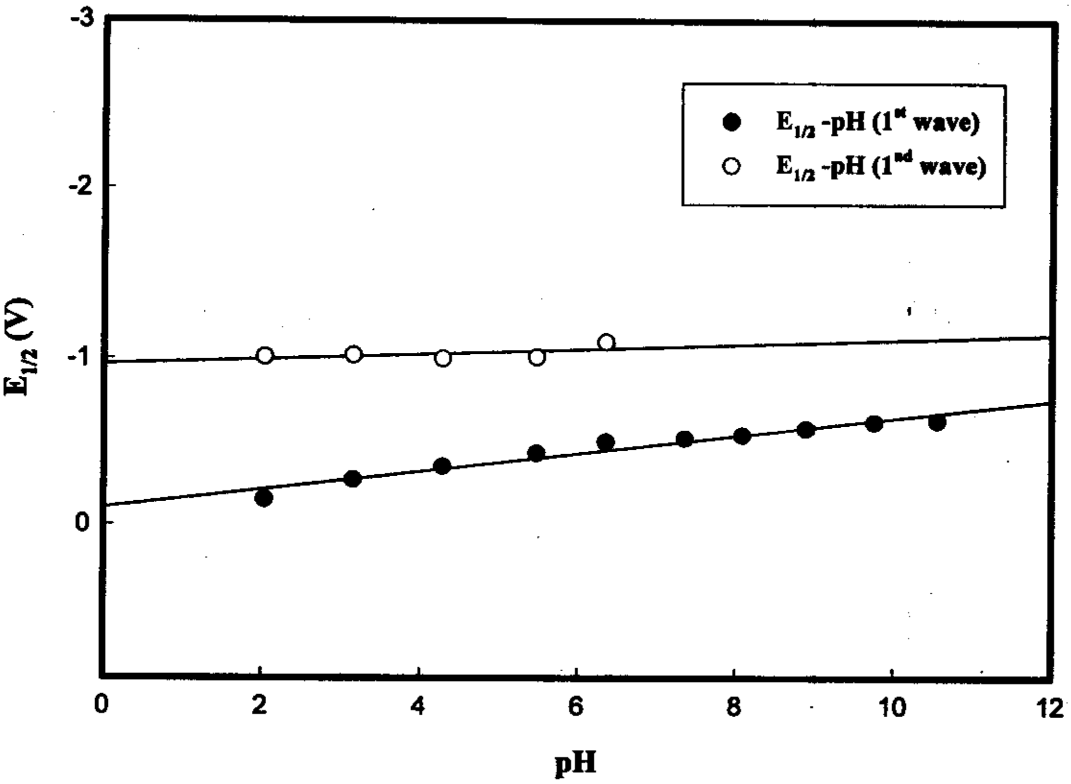


Fig. (46): $E_{1/2}$- pH plots of tinidazole.

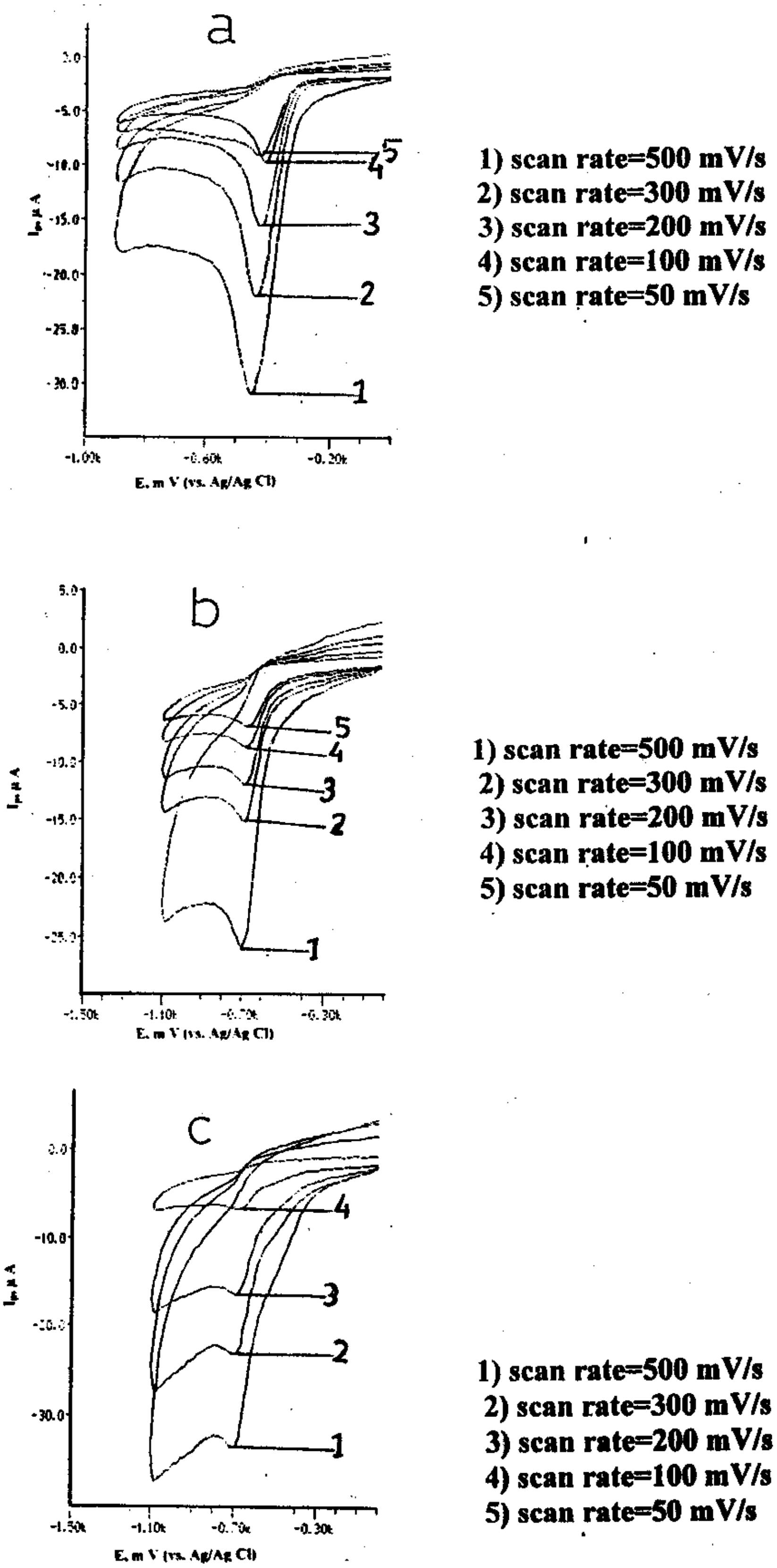


Fig. (47): Cyclic voltammograms of 1x 10^{-4} M of tinidazole inB.R. Buffer solution at different scan rates;(a) pH=3.20 , (b) pH=7.26 (c)pH=8.87 (d) pH=9.78

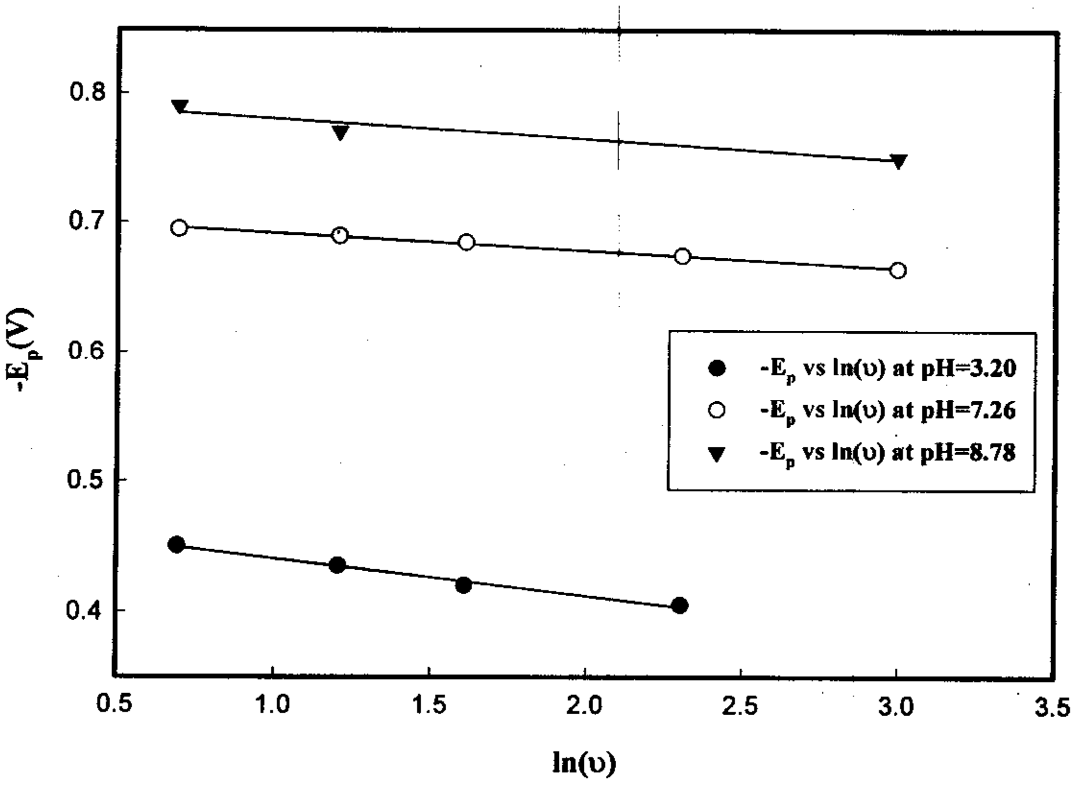


Fig. (48): E_p-ln(υ) plots of tinidazole at different pH values.

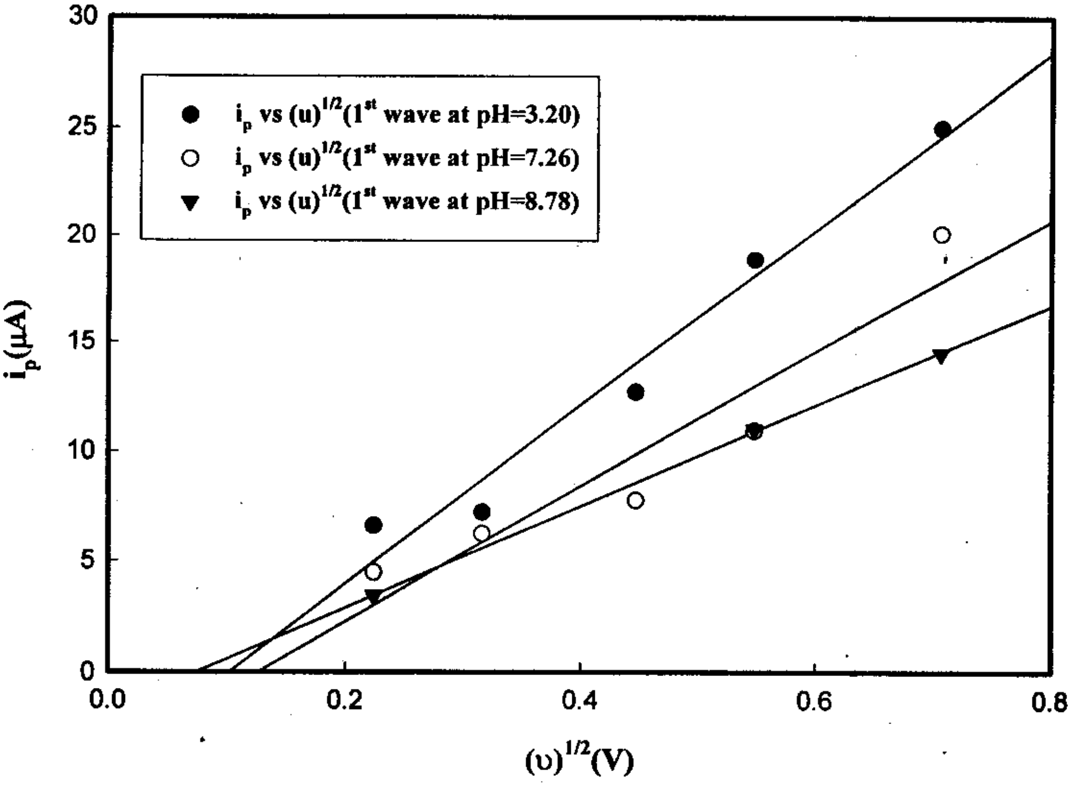


Fig. (49): i_p-$(\upsilon)^{1/2}$ plots of tinidazole at different pH values.

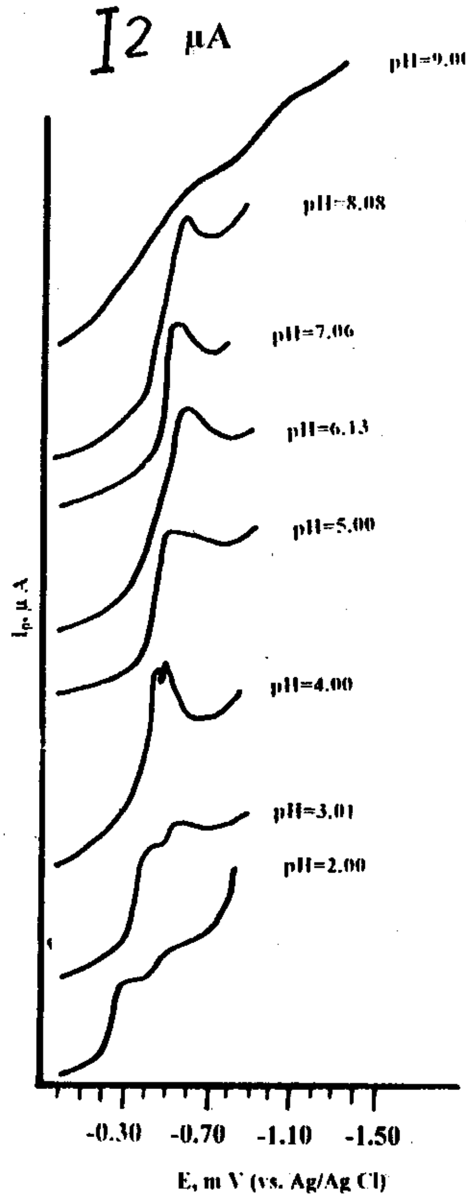


Fig. (50): Effect of pH on the CAdS peak of $5x10^{-5}$ M of tinidazole in B.R. Buffer solutions at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.

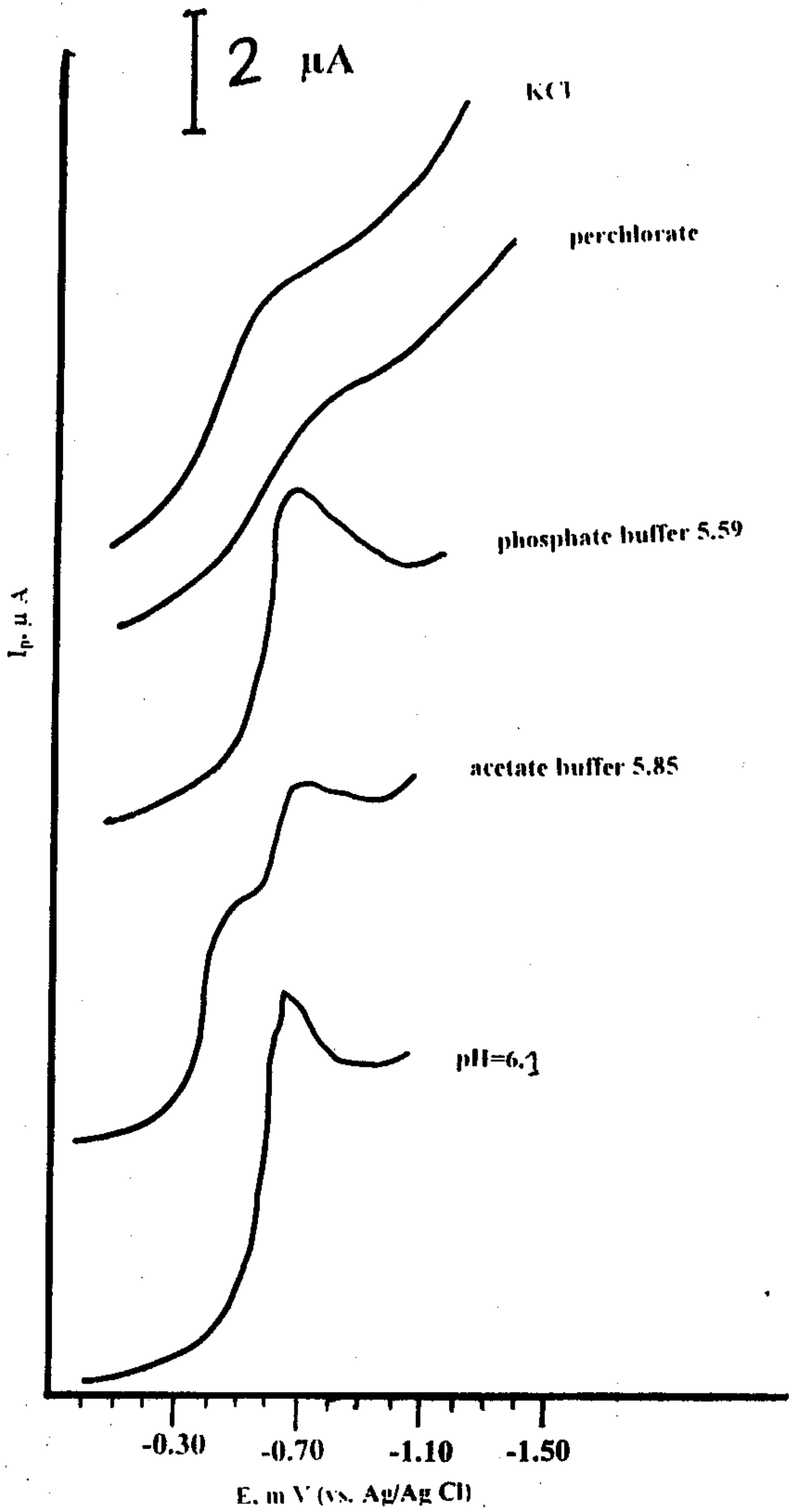


Fig. (51): Effect of different supporting electrolyte solution on the CAdS peak of $5x10^{-5}$ M of tinidazole at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.

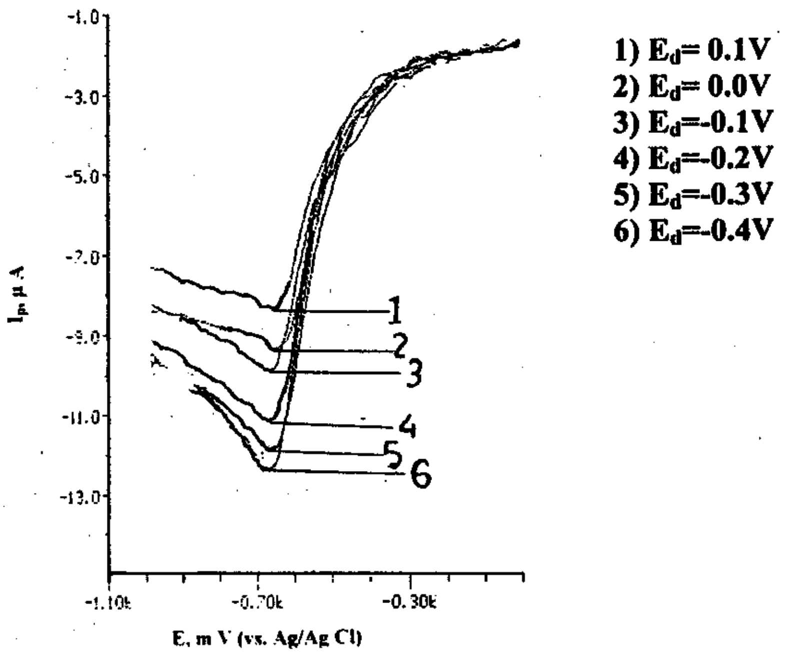


Fig. (52a): Effect of deposition potential on 5x10^{-5}M of tinidazole in B.R. buffer Solution of pH=6.13 at scan rate=100mV/s s, t_d=240 s and pulse height=5 mV.

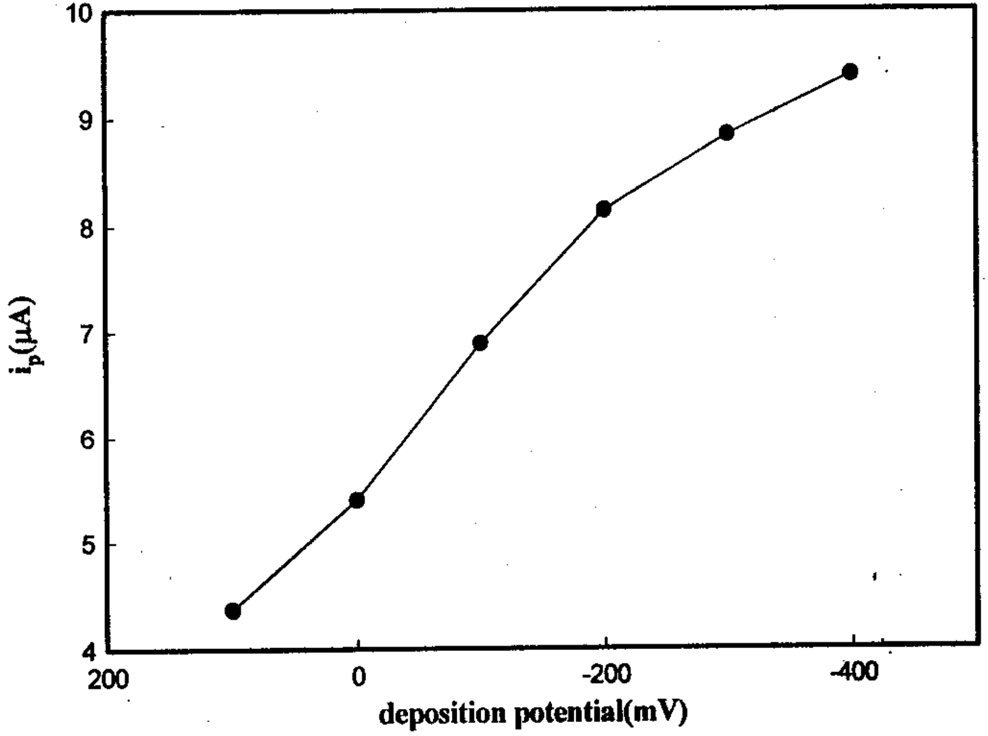


Fig. (52b): Effect of deposition potential on 5x10^{-5}M of tinidazole in B.R.buffer Solution of pH=6.13 at scan rate=100mV/s s,t_d=240 s,and pulse height=5 mV.

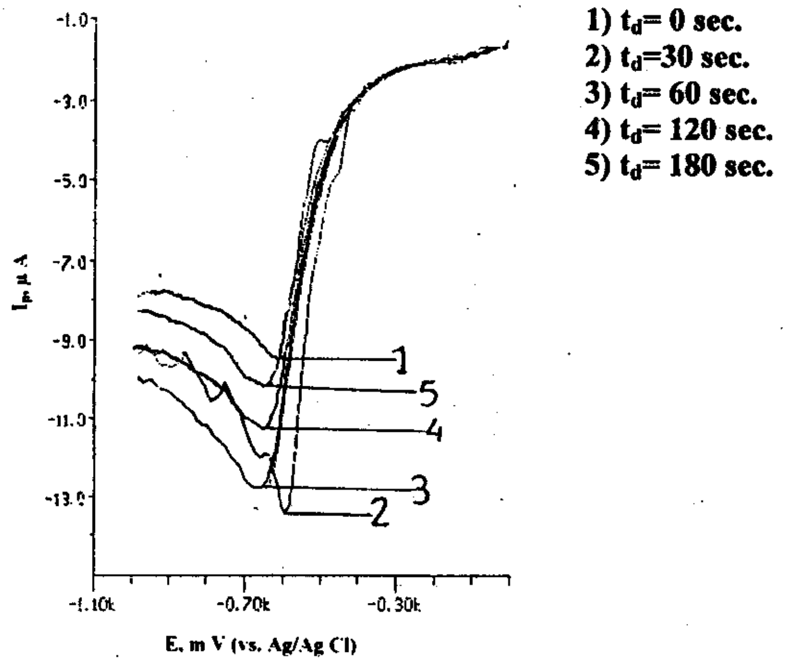


Fig. (53a): Effect of deposition time on $5x10^{-5}$M of tinidazole in B.R. buffer Solution of pH=6.13 at scan rate=500mV/s s, E_d=-0.4V,and pulse height=10 mV.

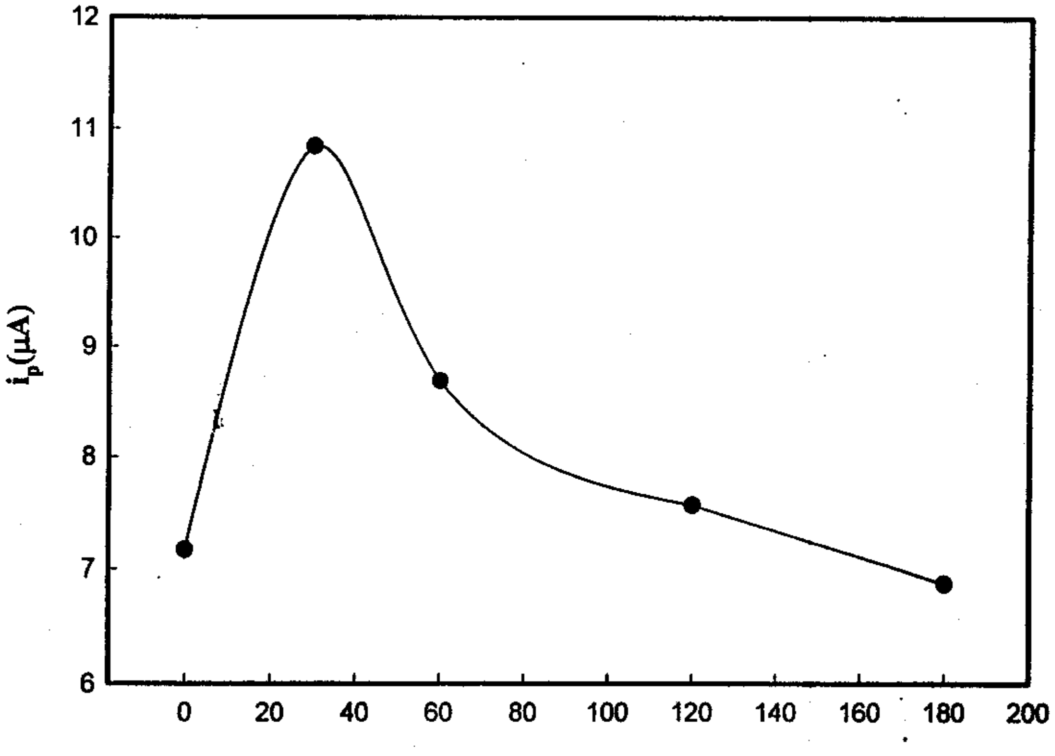


Fig. (53b): Effect of deposition time on $5x10^{-5}$M of tinidazole in B.R.buffer Solution of pH=6.13 at scan rate=500mV/s s,E_d=-0.4V,and pulse height=10 mV.

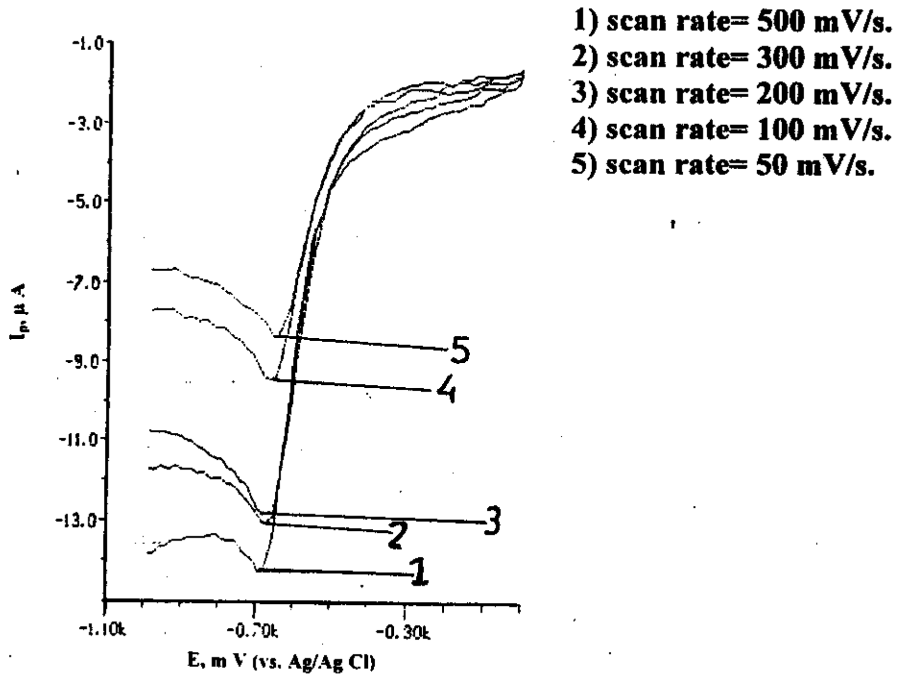


Fig. (54a): Effect of scan rate on 5×10^{-5}M of tinidazole in B.R. buffer solution of pH=6.13 at E_d= -0.4V s, t_d=240 s and pulse height=5 mV.

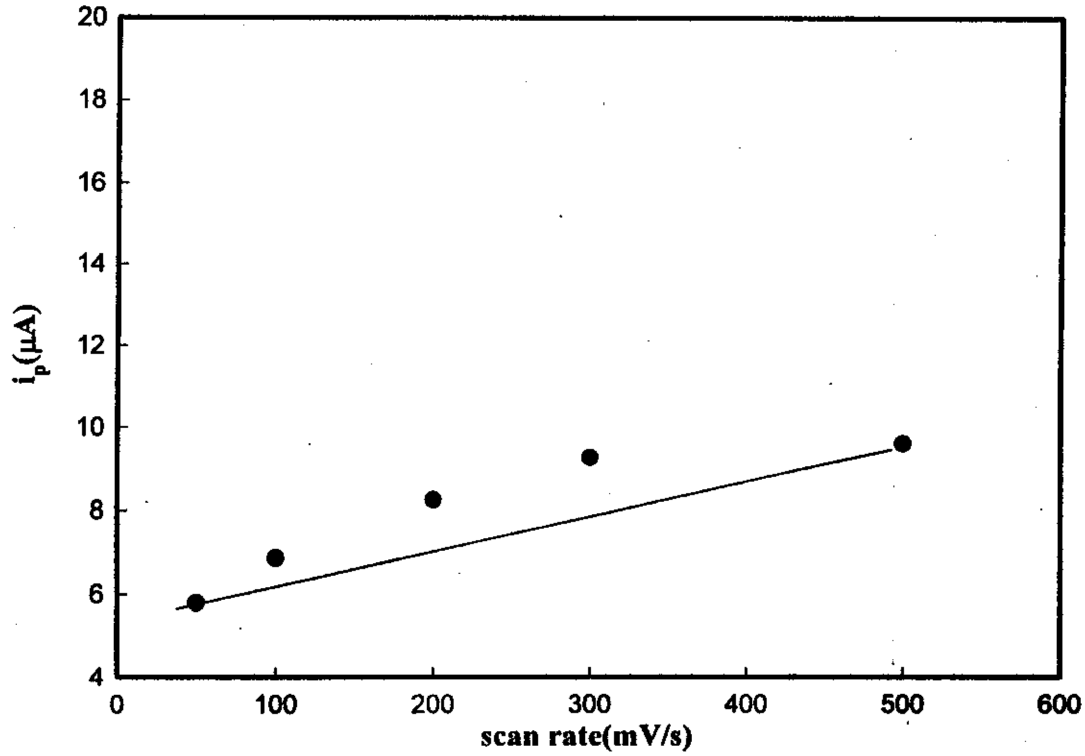


Fig. (54b): Effect of scan rate on 5×10^{-5}M of tinidazole in B.R.buffer solution of pH=6.13 at Ed= -0.4V s,td=240 s,and pulse height=5 mV.

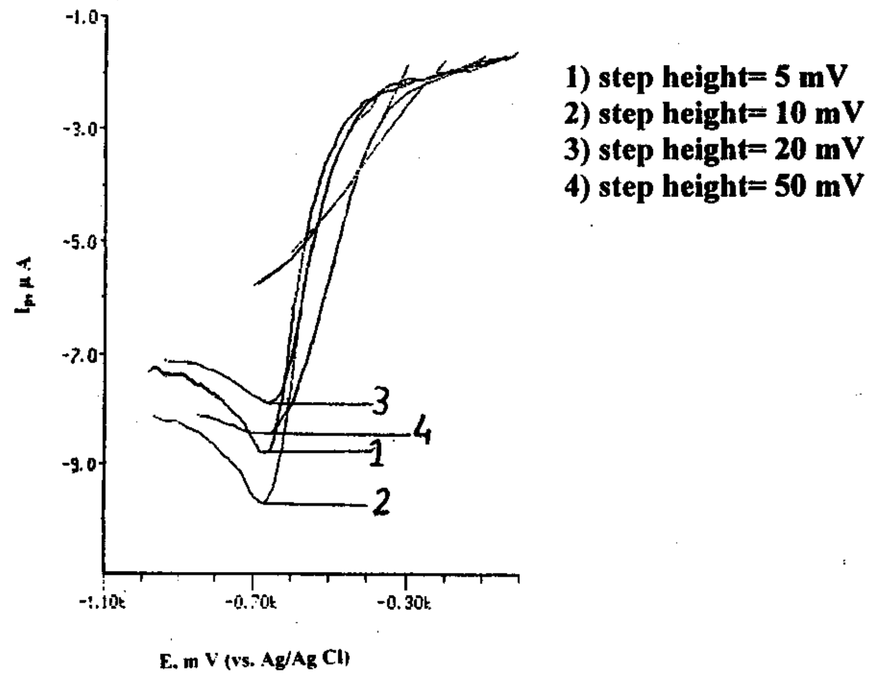


Fig. (55a): Effect of step height on $5x10^{-5}M$ of tinidazole in B.R.buffer solution of pH=6.13 at E_d= -0.4V s, t_d=240 s and scan rate =500 mV/s.

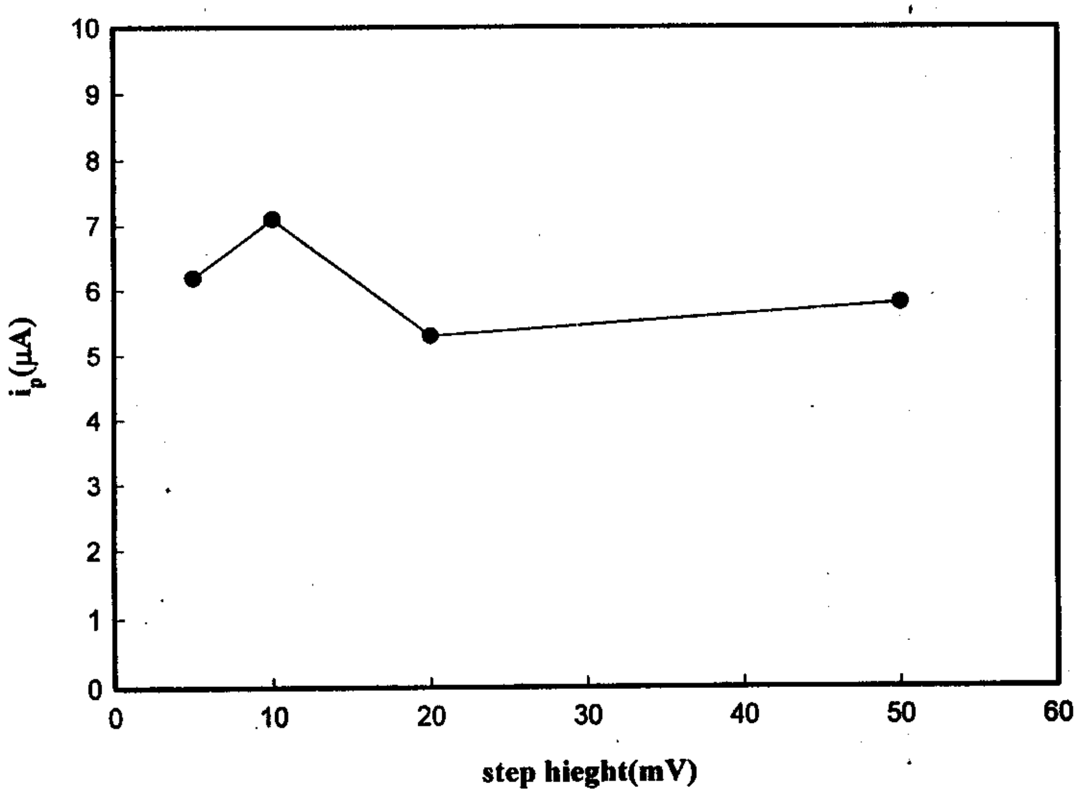


Fig. (55b): Effect of step height on $5x10^{-5}M$ of tinidazole in B.R.buffer solution of pH=6.13 at Ed= -0.4V s, td=240 s and scan rate =500 mV/s.

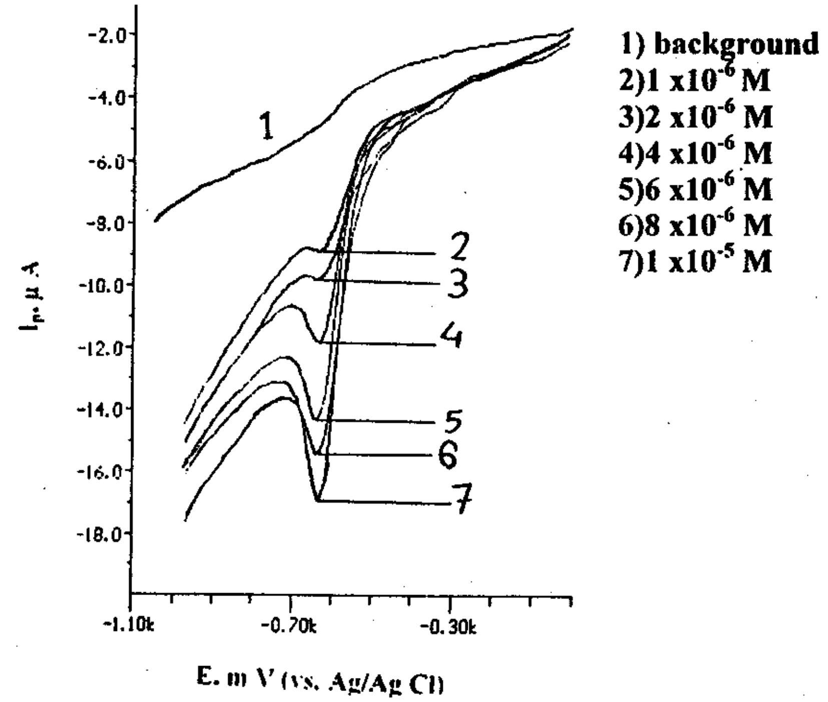


Fig.(56a): Effect of concentration of tinidazole in B.R. buffer solution of pH=6.13 at: t_d=30 s, E_d= -0.4 V, scan rate 500 mV/s and step height= 10 mV

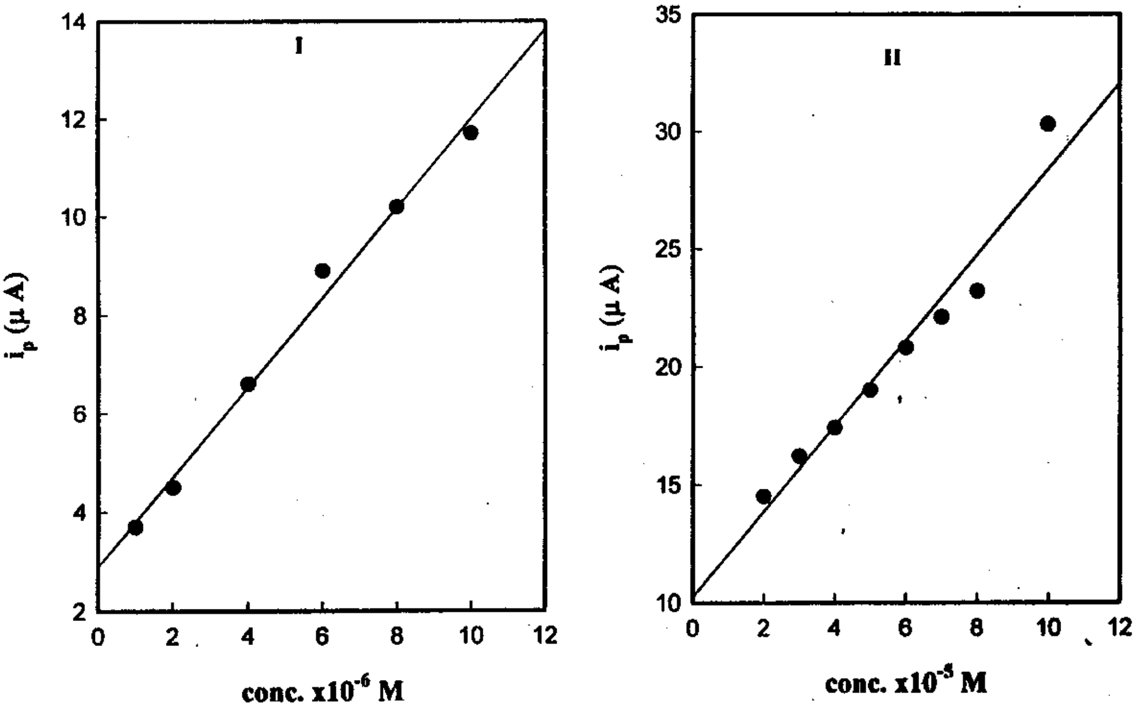


Fig. (56b): calibration curve for tinidazole

Table(27): polarographic data for 1×10^{-4}M of tinidazole in B.R. buffer solution of different pH values at 25c°

pH	i_d(μA)		$-E_{1/2}$		Δ logi_l/Δ logh	
	A	B	A	B	A	B
2.03	3.12	2.10	0.15	1.00		
3.15	3.06	1.68	0.27	1.01	0.63	0.54
4.28	3.00	1.56	0.35	1.02		
5.47	2.94	1.44	0.43	1.03		
6.35	2.94	0.78	0.50	1.10	0.50	0.61
7.35	2.82	sh.	0.52	sh.		
8.08	2.80	—	0.54	—		
8.88	2.76	—	0.58	—		
9.75	2.64	—	0.62	—		
10.55	2.58	—	0.63	—	0.44	sh.

Where A:1st wave B: 2nd wave sh: ill-defined

Table(28) : Values of (Z_H^+) and (α) as determined for different ratios (Z_H^+/n_a) at 0.5, 1.0 and 2.0 of the number of protons calculated from slope(S_2)of the $E_{1/2}$-pH plots and (S_1) for tinidazole in B.R. buffer solutions of different pH values at 25C°

pH	S_1 mV		S_2 mV		(Z_H^+)=(S_2/S_1)		Z_H^+/n_a		
							0.5	1.0	2.0
	A	B	A	B	A	B			
2.03	69.16	208	54.05	150	0.78	0.72	0.55	1.09	2.19
3.15	106.61	256	54.05	150	0.51	0.60	0.55	1.09	2.19
4.28	64.89	256	54.05	150	0.83	0.60	0.55	1.09	2.19
5.47	75.02	204	54.05	150	0.72	0.74	0.55	1.09	2.19
6.35	76.22	161	54.05	150	0.71	0.93	0.55	1.09	2.19
7.35	69.88	256	54.05	150	0.77	0.59	0.55	1.09	2.19
8.08	87.18	167	54.05	150	0.62	0.90	0.55	1.09	2.19
8.88	69.78	175	54.05	150	0.77	0.86	0.55	1.09	2.19
9.75	54.05	sh.	54.05	150	1.00	sh.	0.55	1.09	2.19
10.6	69.20	—	54.05	150	0.78	—	0.55	1.09	2.19

Where A:1st wave B:2nd wave sh: ill-defined

Table(29a):Values of (αna) and (α) for $2x10^{-4}$ M of tinidazole in B.R buffer solution of different pH values, as calculated from reciprocal slope (S_1) of the log $(i/i_d\text{-}i)$ -$E_{d.e}$ plots.

pH	Slop(S_1) mV		αna		α			
					n=1	n=2	n=1	n=2
	A	B	A	B	A	A	B	B
2.03	69.16	208	0.85	0.28	0.85	0.43	0.28	0.14
3.15	106.6	256	0.55	0.23	0.55	0.28	0.23	0.12
4.28	64.89	256	0.91	0.23	0.91	0.45	0.23	0.12
5.47	75.02	204	0.79	0.29	0.79	0.39	0.29	0.15
6.35	76.22	161	0.77	0.37	0.77	0.39	0.37	0.19
7.35	69.88	256	0.84	0.23	0.84	0.42	0.23	0.12
8.08	87.18	167	0.68	0.35	0.68	0.34	0.35	0.18
8.88	69.78	175	0.85	0.34	0.85	0.42	0.34	0.17
9.75	54.05	sh.	1.09	sh.	1.09	0.55	sh.	sh.
10.6	69.20	–	0.85	–	0.85	0.43	–	–

Where A: 1st wave B: 2nd wave sh: ill-defined

Table(29b) : Data obtanied for 1x10^{-4}M of tinidazole in B.R. buffer solution of different pH values using cyclic voltammetry at different scan rates, at 25°

pH	Scan rate (mV/s)	-Ep (V)	-dEp/d(lnv)	α
				n=1
3.20	500	0.45		
	300	0.44		
	200	0.42	0.028	0.45
	100	0.41		
	50	0.25		
7.26	500	0.70		
	300	0.69		
	200	0.68	0.013	0.98
	100	0.67		
	50	0.66		
8.78	500	0.79		
	300	0.77	0.016	0.80
	50	0.75		

Table (30): Kinetic parameters obtained from D-C measurements for $1\text{x}10^{-4}$ M tinidazole in B. R. buffer solutions of different pH's.

pH	D°(Cm^2/s)	K°$_{f,h}$ (Cm/s)	ΔG* (K.cal/mole)
2.03	2.40 x10^{-5}	4.00 x10^{-7} [a]	66.42 [a]
		1.23 x10^{-8} [b]	75.19 [b]
3.15	2.40 x10^{-5}	3.00 x10^{-7} [a]	67.18 [a]
		1.20 x10^{-9} [b]	81.01 [b]
4.28	2.40 x10^{-5}	6.00 x10^{-9} [a]	76.66 [a]
		1.15 x10^{-9} [b]	81.11 [b]
5.47	2.40 x10^{-5}	7.90 x10^{-10} [a]	82.02 [a]
		5.50 x10^{-10} [b]	82.96 [b]
6.35	1.50 x10^{-5}	4.37 x10^{-11} [a]	89.31 [a]
		3.80 x10^{-11} [b]	89.68 [b]
7.35	1.50 x10^{-5}	2.51 x10^{-13} [a]	102.30 [a]
		6.02 x10^{-13} [b]	100.07 [b]
8.08	1.50 x10^{-5}	4.90 x10^{-16} [a]	117.90 [a]
		5.80 x10^{-13} [b]	100.17 [b]
8.88	7.20 x10^{-6}	4.57 x10^{-17} [a]	123.90 [a]
		5.80 x10^{-13} [b]	100.17 [b]
9.75	7.20 x10^{-6}	1.23 x10^{-17} [a]	127.17 [a]
10.55	7.20 x10^{-6}	2.40 x10^{-18} [a]	131.30 [a]

Where a : 1st wave , b : 2nd wave .

Table(31):Cathodic adsorptive stripping peak current(i_p)of $5x10^{-5}$M Tinidazole in B.R.buffer solution of pH=6.13 at different conditions (t_d, E_d, scan rate and pulse height).

Deposition time (t_d), sec	Deposition potential(E_d), V	Scan rate (mV/s)	Pulse hieght (mV)	i_p (μA)
Effect of (td):				
0	-0.4	500	10	7.17
30				10.84
60				8.69
120				7.57
180				6.88
Effect of (E_d):				
240	0.1	100	5	6.37
	-0.01			5.40
	-0.1			6.89
	-0.2			8.14
	-0.3			8.84
	-0.4			9.39
Effect of scan rate:				
240	-0.4	500	5	9.61
		300		9.46
		200		9.25
		100		6.86
		50		5.8
Effect of pulse height:				
240	-0.4	500	5	6.19
			10	7.1
			20	5.3
			50	5.8
			100	
			150	
			200	

Table (32): Calibration curve data of tinidazole in B.R.buffer solution of pH=6.13 using CAdSV at t_d= 30sec., E_d= -0.4 V, scan rate= 500 mV/s, and step height= 10 mV.

Conc.taken	Ip(µA)	Mean	S.D.	R.S.D. %
$1x10^{-6}$ M	**3.66**	**3.56**	**0.030**	**0.84**
	3.60			
	3.68			
	3.68			
$2x10^{-6}$ M	**4.46**	**4.56**	**0.091**	**1.97**
	4.64			
	4.64			
	4.52			
$4x10^{-6}$ M	**6.53**	**6.54**	**0.050**	**0.76**
	6.53			
	6.62			
	6.51			
$6x10^{-6}$ M	**8.99**	**8.91**	**0.073**	**0.82**
	8.96			
	8.86			
	8.84			
$8x10^{-6}$ M	**10.15**	**10.21**	**0.064**	**0.62**
	10.25			
	10.16			
	10.28			
$1x10^{-5}$ M	**11.54**	**11.68**	**0.091**	**0.78**
	11.58			
	11.74			
	11.78			

Table (33): Assay of tinidazole in dosage form (protozole) in B.R. buffer solution at pH=6.13 using CAdSV at t_d= 30sec., E_d= -0.4 V, scan rate = 500 mV/s, and step height= 10 mV.

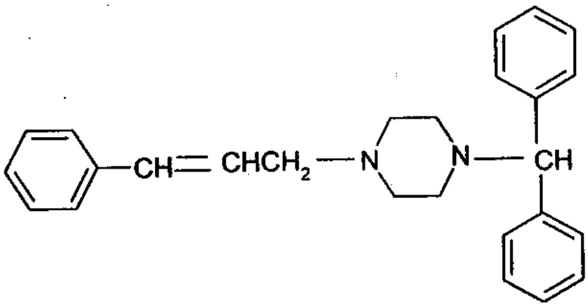
Conc.taken	Conc. calculated	Recovery %	Mean Recovery(%) ± S.D.
$1x10^{-6}$ M	$0.98 x10^{-6}$ M	98.00	101.0 ±2.70
	$1.03 x10^{-6}$ M	103.0	
	$1.03 x10^{-6}$ M	103.0	
	$1.00 x10^{-6}$ M	100.0	
$2x10^{-6}$ M	$1.98 x10^{-6}$ M	99.00	100.5 ±1.41
	$2.04 x10^{-6}$ M	102.0	
	$2.00 x10^{-6}$ M	100.0	
	$2.02 x10^{-6}$ M	101.0	
$4x10^{-6}$ M	$3.97 x10^{-6}$ M	99.25	100.4 ±1.29
	$4.07 x10^{-6}$ M	101.8	
	$4.04 x10^{-6}$ M	101.0	
	$3.98 x10^{-6}$ M	99.50	
$6x10^{-6}$ M	$6.20 x10^{-7}$ M	103.3	101.2 ±2.01
	$6.00 x10^{-7}$ M	100.0	
	$6.06 x10^{-7}$ M	101.0	
	$6.03 x10^{-7}$ M	100.5	
$8x10^{-6}$ M	$7.98 x10^{-6}$ M	99.88	99.87 ±0.48
	$8.03 x10^{-6}$ M	100.4	
	$7.94 x10^{-6}$ M	99.25	
	$8.00 x10^{-6}$ M	100.0	
$1x10^{-5}$ M	$1.01 x10^{-5}$ M	101.0	100.0 ±0.81
	$1.00 x10^{-5}$ M	100.0	
	$1.00 x10^{-5}$ M	100.0	
	$0.99 x10^{-5}$ M	99.00	

Table(34):Assay of tinidazole in serum in B.R.buffer solution of pH=6.13 using CAdSV at td= 30 sec., Ed= -0.4 V, scan rate= 500mV/s, and step height= 10 mV.

Conc. taken μg/10ml	Conc. Calculated μg/10ml	Recovery %	Mean Recovery(%)± S.D
0.148	**0.147**	**99.32**	**99.49 ±1.38**
	0.146	**98.65**	
	0.150	**101.4**	
	0.146	**98.65**	
4.94	**4.88**	**98.78**	**99.64 ±3.12**
	5.10	**103.2**	
	4.98	**100.8**	
	4.73	**95.75**	

3.5. Electrochemical behavior of cinnarizine in B.R buffer solutions of different pH values:-

cinnarizine is [1-trans-cinnamyl-4-diphenylmethylpiperazine] and has the following structural formula :



This part includes the study of electrochemical behavior of cinnarizine at Hg-electrode using of DC polarography and cyclic voltammetry techniques. The study aimed to optimize the experimental and instrumental conditions for determination of the lowest possible concentration of the investigated pharmaceutical compound in pure and dosage forms using cathodic adsorptive stripping voltammetry .

3.5.1 DC polarography:-

3.5.1. i-Current potential curves:-

The polarographic behavior of $1x10^{-4}$ M of cinnarizine was studied in B.R. buffer solutions containing 10% (v/v) ethanol of different pH values in the range from 2 to 12 . The polarogrammes exhibit two reduction waves within the pH range studied corresponding to the reduction CH=CH group Fig.(57) the half-wave potential $E_{1/2}$ of the reduction waves are pH dependent, and being shifted to more negative values on increasing the pH of the electrolysis solution .

3.5.1. ii-Effect of pressure at mercury height :-

The effect of mercury height "h" was examined according to equation (3.1). The plots of log i_l against log h for cinnarizine showed linear correlations at different pH values, Fig.(58). The slope values (x) are found in the range between 0.43 and 0.82 which indicate that the reduction process is controlled by diffusion and some adsorption contribution, Table (35) .

3.5.1. iii-Analysis of the polarographic waves:-

Analysis of the polarographic waves is quite important to evaluate the degree of reversibility of electrode reaction and to suggest the electrode mechanism On using equation (3.4), the plots of $\log[i/(i_d-i)]$ against $E_{d.e.}$ for cinnarizine reduction at different pH values showed linear correlations, Fig.(59). The straight lines with reciprocal slopes (S_1) amounting in the range to (55.39 -96) and (162.8-221.7) mV for the first and second waves; respectively, Table (36). This behavior revealed the irreversible nature of the electrode process.

3.5.1.iv-Half- wave potential –pH curves :-

The plots of the rate shift of $E_{1/2}$ of cinnarizine versus the pH of the electrolysis solution showed linear correlations for both the first and second waves respectively . The slope values (S_2) of the first and second waves is amounting to 76.2 and 198 mV, respectively ,Table (36).

The number of hydrogen ions (Z_H^+) involved in the rate determining step for cinnarizine reduction was calculated from both slopes of logarithmic analysis and $E_{1/2}$ plots according to equation (3.5). At all pH values Z_H^+ was found to be unity, Table (36).

The most probable values of α- parameter as shown in Table (37a). were determined at the probable ratio of (Z_H^+/n_a) using equation (3.6). the probable (Z_H^+/n_a) ratio was found to fit with (Z_H^+/n_a) = 0.5 at Table (36).

This behavior is further confirmed the irreversibility of electrode process and the rate determining step involves one proton and two electrons.

3.5.2 Cyclic voltammetry :-

The cyclic voltammetric behavior of 1×10^{-4} M of cinnarizine was investigated in aqueous Britton-Robinson buffer solutions of different pH values (2-12) containing 10% (v/v) ethanol at glassy carbon electrode. The voltammogram displayed single irreversible cathodic peak in the cathodic scan in all media, where as in the anodic scan there is no oxidation peaks were observed, Fig.(61).

On increasing the scan rate (υ), the cathodic peak potential (E_{pc}) was shifted to more negative potentials indicating the irreversible nature of the reduction peak. Furthermore, on increasing the pH of the electrolysis solution the peak potential (E_{pc}) is shifted to more negative values indicating the consumption of hydrogen ions in the electron transfer step. On employing equation (3.7), the plots of E_p versus ln υ gives linear correlations Fig.(62). The slope values were used for the determination of αn_a The values of the (α) transfer coefficient were calculated at the probable n_a values and found to be less than 1.0 at n_a=2.0, Table(37b). This behavior is further confirmed the irreversible nature of electrode process. According to equation(3.8), the plots of i_p versus square root of scan rate($\upsilon^{1/2}$) gives linear relation slightly deviating from the origin, Fig.(63). This behvior confirming that the electrode process of cinnarizine is controlled mainly by diffusion with some adsorption contribution.

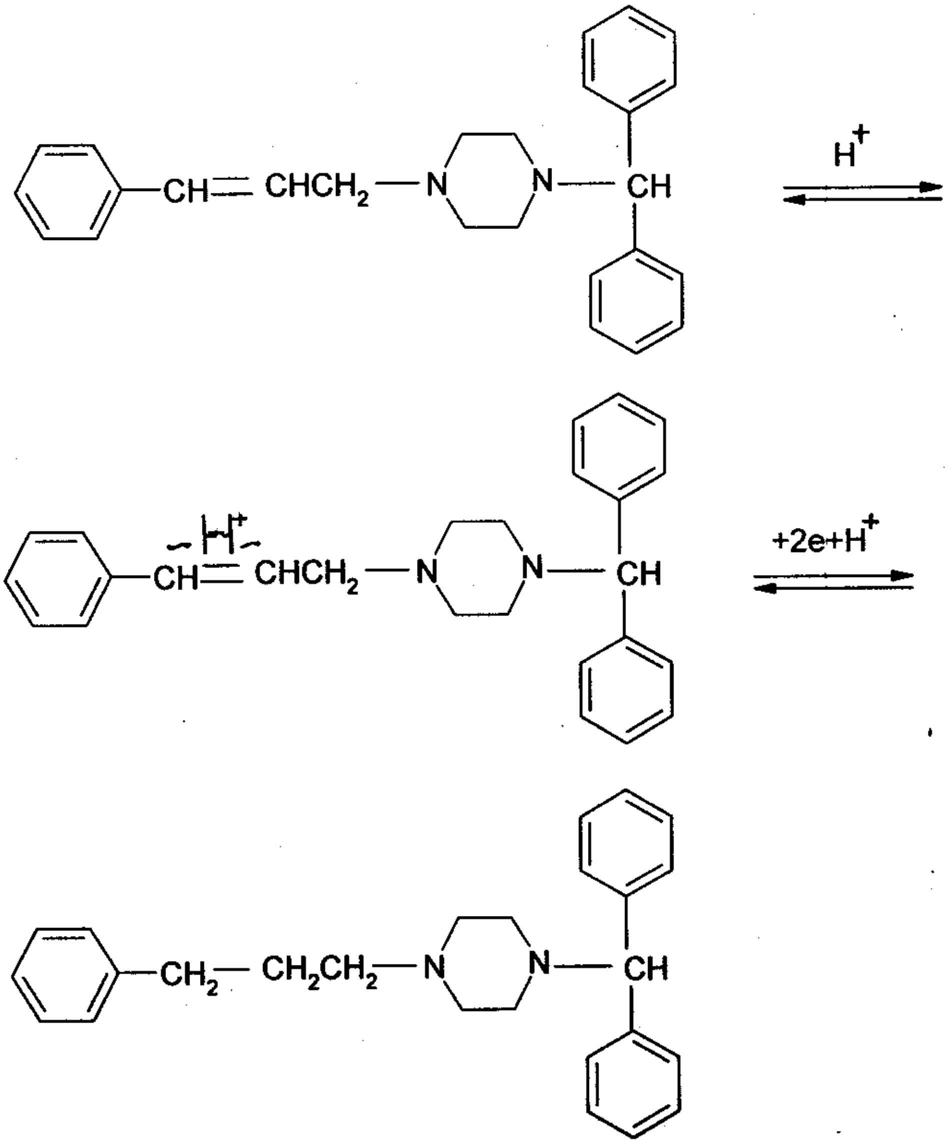
3.5.2.i-Kinetic parameters of the electrode reaction :-

The rate constant $k^o_{f,h}$ of the electrode reaction of cinnarizine is calculated from the data obtained by dc- polarography measurements in Britton-Robinson buffer solution of various pH values. Equation (3.12) was used in this determination and the values of the rate constant $k_{f,h}$ were found to be decreased with increasing the pH which means that the electrode reaction becomes more difficult and more irreversible, Table (38) . Also, the values of ΔG^* were determined using equation (3.14) and found to be increased with increasing pH of the electrolysis medium, Table (38). This may be explained on the basis that the protonation of the molecule, at lower pH, before its diffusion to the electrode surface, takes place through the contribution of the H^+ ion directly with the depolarizer molecule which decreases the activation energy ΔG^* . The H^+ ion activity decreases with rise of pH, hence ΔG^*

increases. In alkaline media, the proton required for pre-protonation of the depolarizer is supplied from a water molecule which requires much higher energy.

The electrode reaction mechanism :-

To study the reduction mechanism of cinnarizine at mercury electrode, it is necessary to calculate the number of electrons concumed at this reduction, firstly we should calculate the (diffusion coefficient) D from equation (3.16) and the corrected values shown in Table (38). The number of electron (n) found that 2 electrons are consumedin the reduction of (C=C) group at acidic and basic medium,and the number of electrons confirmed by controlled potential coulmetry method according to equation (3.17) the avarge value of n is (2±0.2) so the suggested mechanism as the following:



3.5.3 Cathodic adsorptive stripping voltammetry(CAdSV) of cinnarizine:-

Cathodic adsorptive voltammetry was used for the quantitative determination of cinnarizine in pure and dosage forms . the instrumental and experimental conditions were optimized in this investigation. The instrumental conditions are deposition time (td), deposition potential (Ed), step height and scan rate. While the experimental conditions are including the nature of supporting electrolyte and pH of the electrolysis solution.

3.5.3.i- Effect of pH and supporting electrolyte:

In cathodic adsorptive voltammetry current of cinnarizine is recorded as function of potential in Britton- Robinson buffer solution of pH(2.54 to 8.69) the recorded peak at various pH values due to the reduction of nitro-group at the surface of glassy carbon electrode and well developed peak is noted at pH=2.54 Fig.(64).

We determined the cinnarizine in some supporting electrolyte like (phosphate, acetate, $NaClO_4$ and KCl buffers) we fined that in presence of $NaClO_4$,KCl the peak disappeared it means that the absence of this anion best of cinnarizine and the pH=2.54 is most suitable for cinnarizine Fig.(65)

3.5.3.ii- Effect of accumulation time and potential :

The deposition of the analyzed drug on the surface of the glassy carbon electrode is one of the essential conditions for highly sensitive determinations. The effect of accumulation potential on the peak height was examined over the potential range +100 to -800 mV for $5x10^{-5}$ M cinnarizine at accumulation time 240 s., Fig. (66a). the relation between peaks height i_p and deposition potential E_d is shown in Fig. (66b). The peak current increased steadily with decreasing the potential value till it reaches maximum value at E_d = 0.0 mV, where it decreased sharply after this inflection point. Thus, E_d = 0.0 mV will be adopted as optimum operational values for the following works as it ensured the highest voltammetric signal .

The effect of accumulation time of the drug was examined in the range 0-360s. the amount of cinnarizine accumulated on the electrode surface increased as the deposition time increased till 60 s, further increase of time resulted in decreasing the peak height . A time of accumulation of 60 s was chosen for analytical purposes. The voltammograms obtained for $5x10^{-5}$ M cinnarizine in B. R. buffer solution of pH 5.23 and E_d = 0.0 V are shown in Fig. (67a). The dependence of peak height on accumulation time are illustrated in Fig. (67b).

3.5.3.iii- Effect of scan rate :

The effect of scan rate on the peak current and peak potential was studied. The relationship between i_p or E_p and υ at 240 s., accumulation time shows an increase in the peak current with increasing the scan rate, Fig. (68 a,b). The peak potential was shifted to more negative values with increasing the scan rate. The scan rate 500 mv s^{-1} was used for further voltammetric determination of the drug.

3.5.3.iv- Effect of step height :-

The effect of step height on the CAdSV peak current of cinnarizine reduction was recorded at different values, Fig. (69a).

A well defint peak was observed at 10 mV step height. On increasing step height up to 150 mV the CAdSV peak current decreases till become ill-defined. From the plot of peak current (i_p) versus step height it noted that i_p is inversely proportional to step height, Fig. (69b). and all data shown in Table (39).

3.5.3.v-Calibration curve, detection limit and precision :

A calibration plot was obtained from known concentrations of cinnarizine using the peak current in μA as analytical response Fig. (70). Series of standard solution (four replicates) of the drug were prepared by following the procedure described. The first calibration graph was found to be linear over the range 2 x10^{-7} to 1 x10^{-6} M. The calibration graph yielded the following regression equation :

$$Ip\ (\mu A) = 0.94 + 16.2 \times 10^{6}\ C\ M; \qquad r = 0.9988 \quad n = 5$$

The detection limit obtained for the developed voltammetric method was 9 x10^{-9} M. In order to assess the precision as the standard deviation for concentration with the relative standard deviation were evaluated, Table (40).

The regression equation to second calibration curve is :

$$Ip\ (\mu A) = 27.75 + 0.19 \times 10^{6}\ C\ M; \qquad r = 0.9882 \quad n = 8$$

Analysis of pharmaceutical formulation:

The proposed method was successfully applied to the assay of the studied drug in the pharmaceutical dosage forms (cinnarizine and stegrun tablets, labeled to contain 75 and 25 mg per tablet for two dosage forms respectively). The percentage recoveries for cinnarizine are shown in Table (41 and 42) respectively for two dasage forms. The method gave a good recovery.. The percentage recovery based on five different determinations respectively to two drugs were (100.33 ± 1.79) and (100.01 ±1.33) respectively .

Analysis of serum:

Serum samples were prepared as described before, and two different concentration were analysed using the proposed method (2.20 and 7.34 µg /ml). The recovery and the standard deviasion were evaluated from four measurements for each sample, Table (43). The mean recovery for the two concentration was 99.72 ± 1.09.

this means that the proposed method can be used for determination of cinnarizine in biological samples.

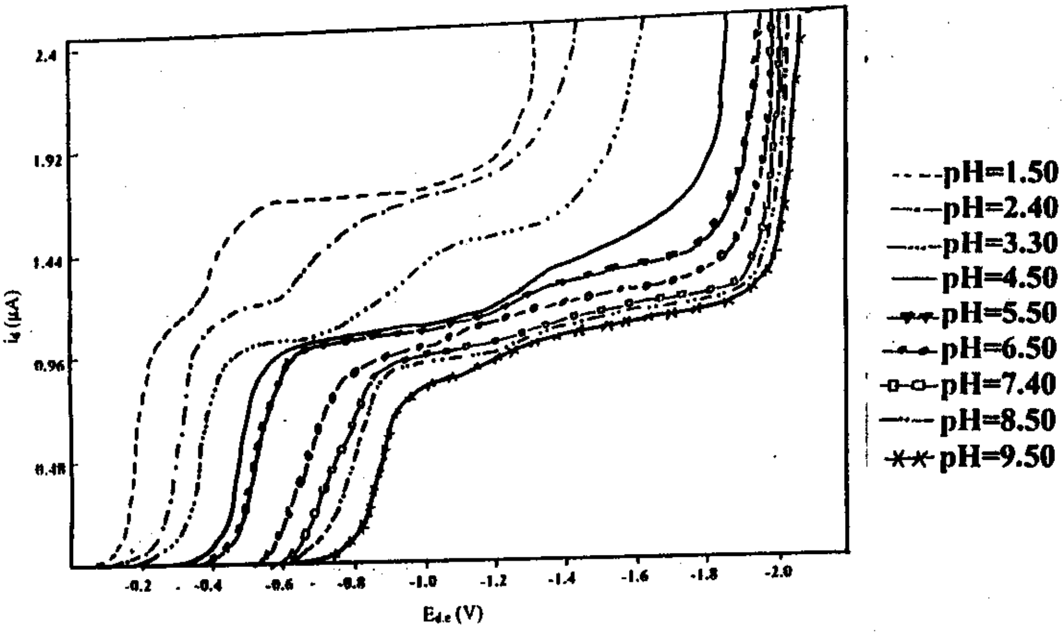


Fig. (57): DC-polarograms of $1x10^{-4}$ M of cinnarizine in B.R. buffer solution of different pH values

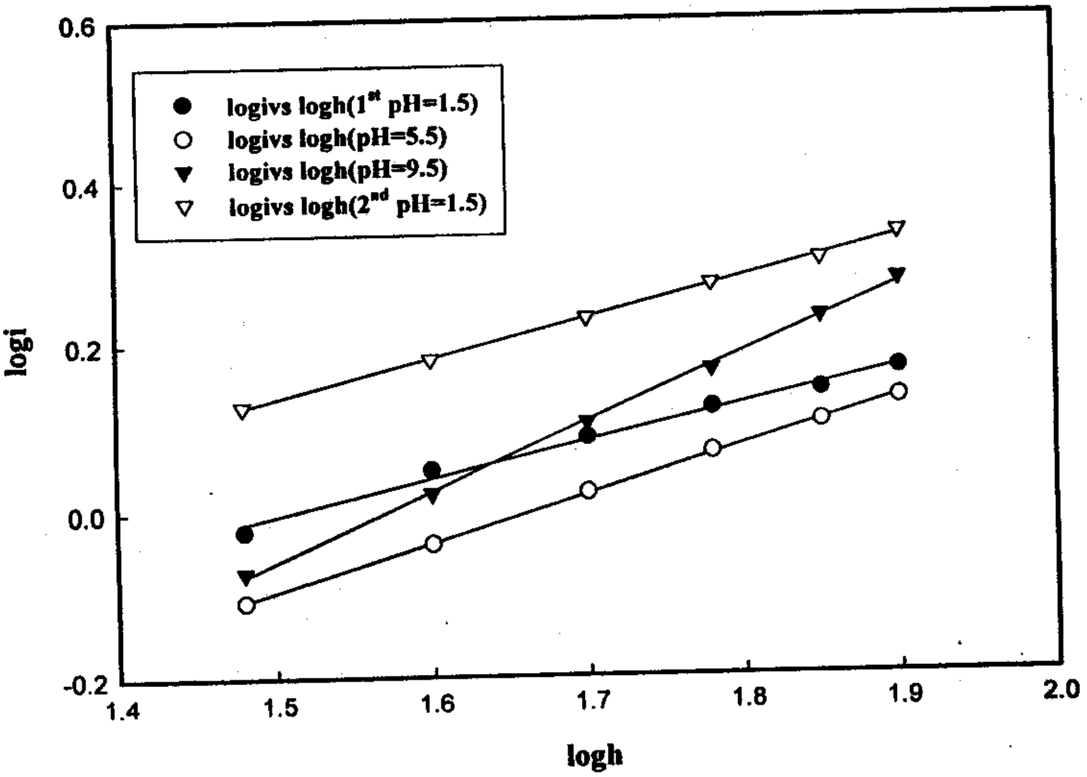


Fig. (58): logi-logh plots of Cinnarzine

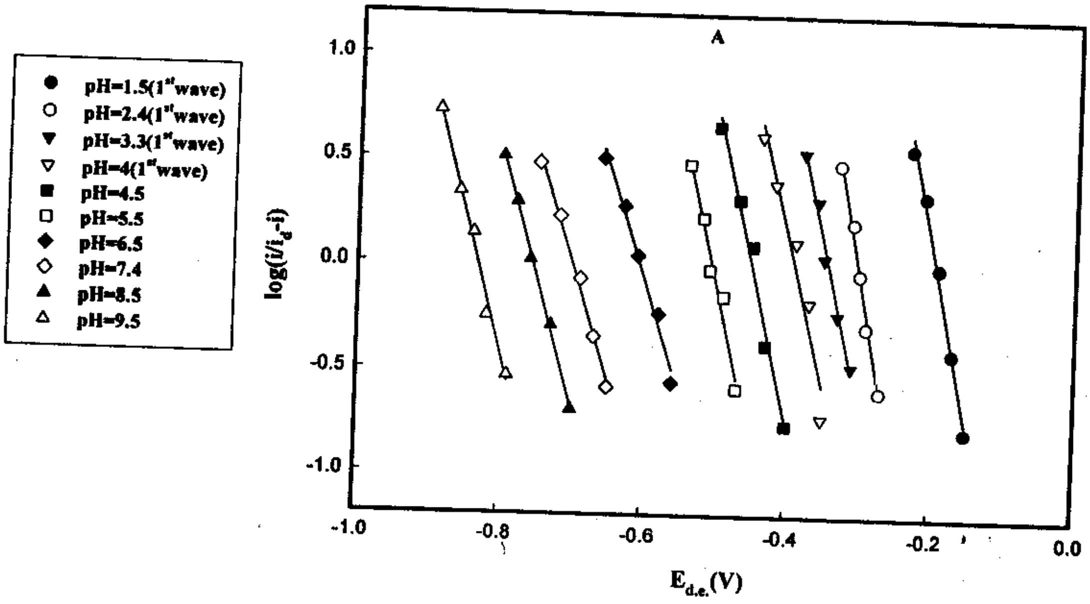


Fig. (59a): Analysis of the polarographic waves of Cinnarzine at different pHvalues

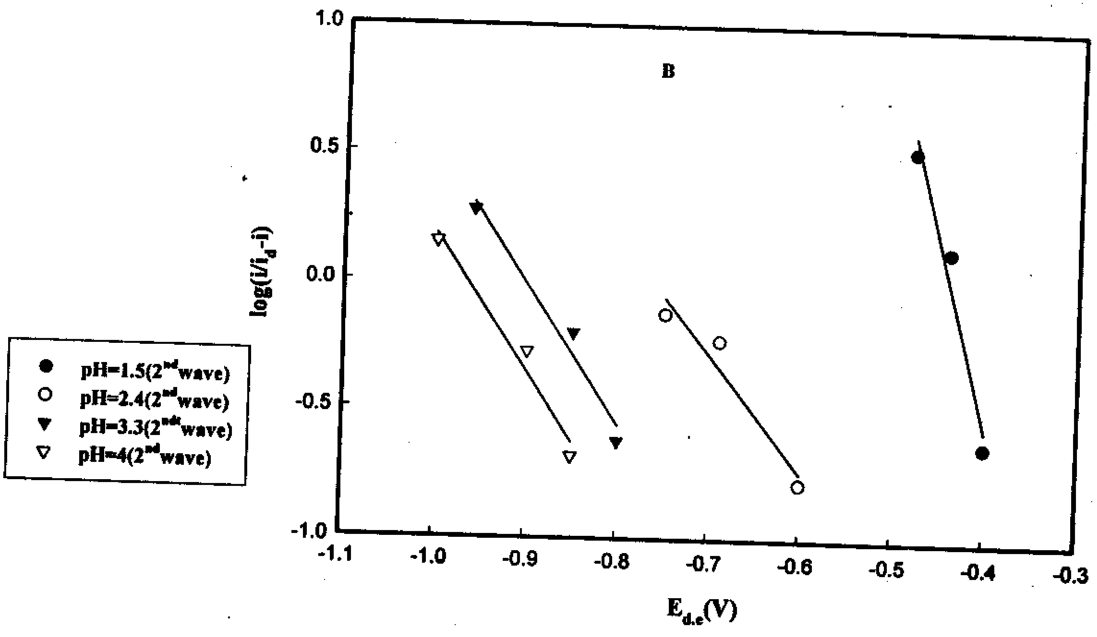


Fig. (59b): Analysis of the polarographic waves of Cinnarzine at different pHvalues

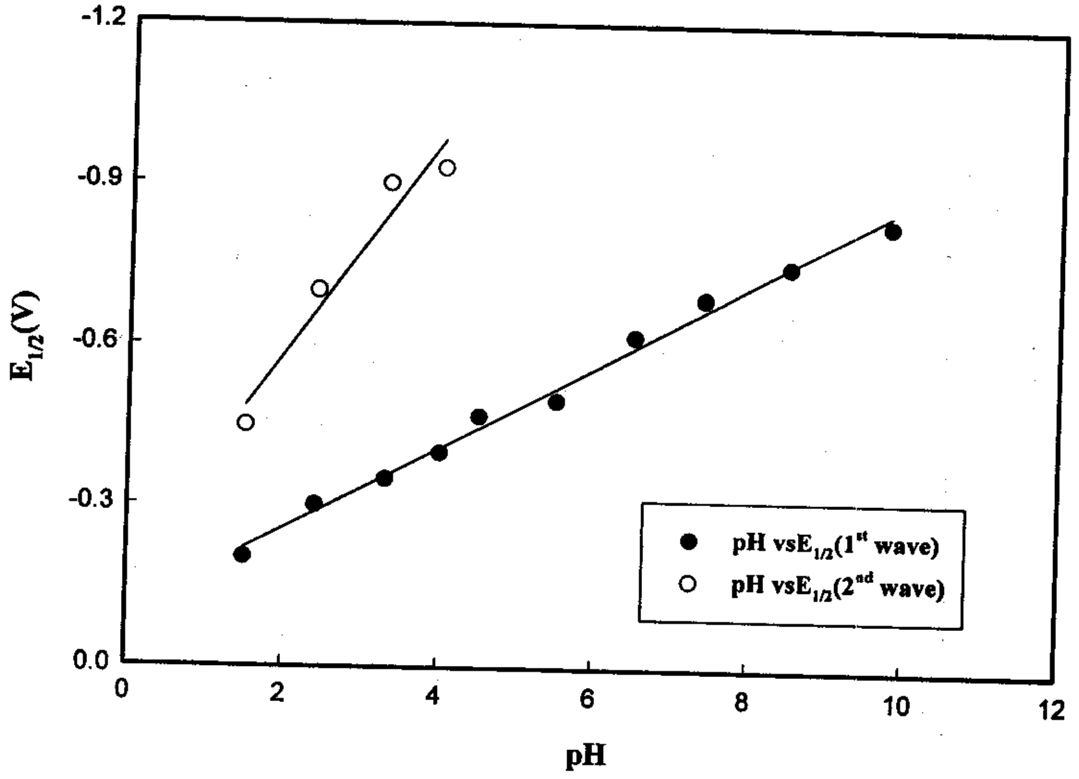
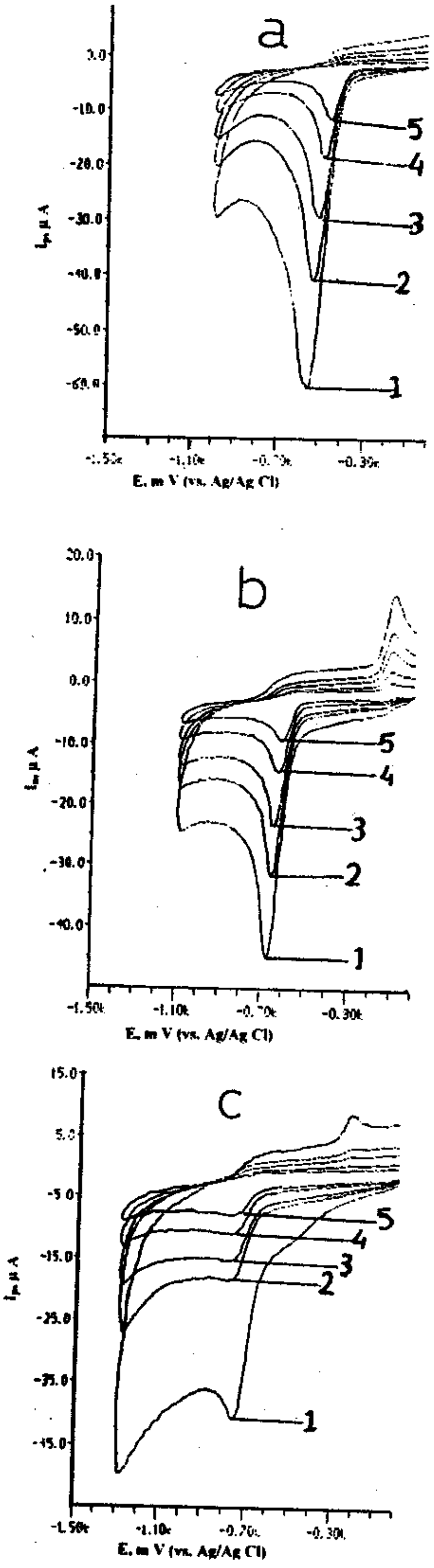


Fig. (60): $E_{1/2}$-pH plots of Cinnarzine



1) scan rate=500 mV/s
2) scan rate=300 mV/s
3) scan rate=200 mV/s
4) scan rate=100 mV/s
5) scan rate=50 mV/s

1) scan rate=500 mV/s
2) scan rate=300 mV/s
3) scan rate=200 mV/s
4) scan rate=100 mV/s
5) scan rate=50 mV/s

1) scan rate=500 mV/s
2) scan rate=300 mV/s
3) scan rate=200 mV/s
4) scan rate=100 mV/s
5) scan rate=50 mV/s

Fig. (61): Cyclic voltammograms of 1x 10^{-4} M of cinnarizine in B.R. Buffer solutions at different scan rates;(a) pH=3.67 , (b) pH=7.26 (c)pH=8.80.

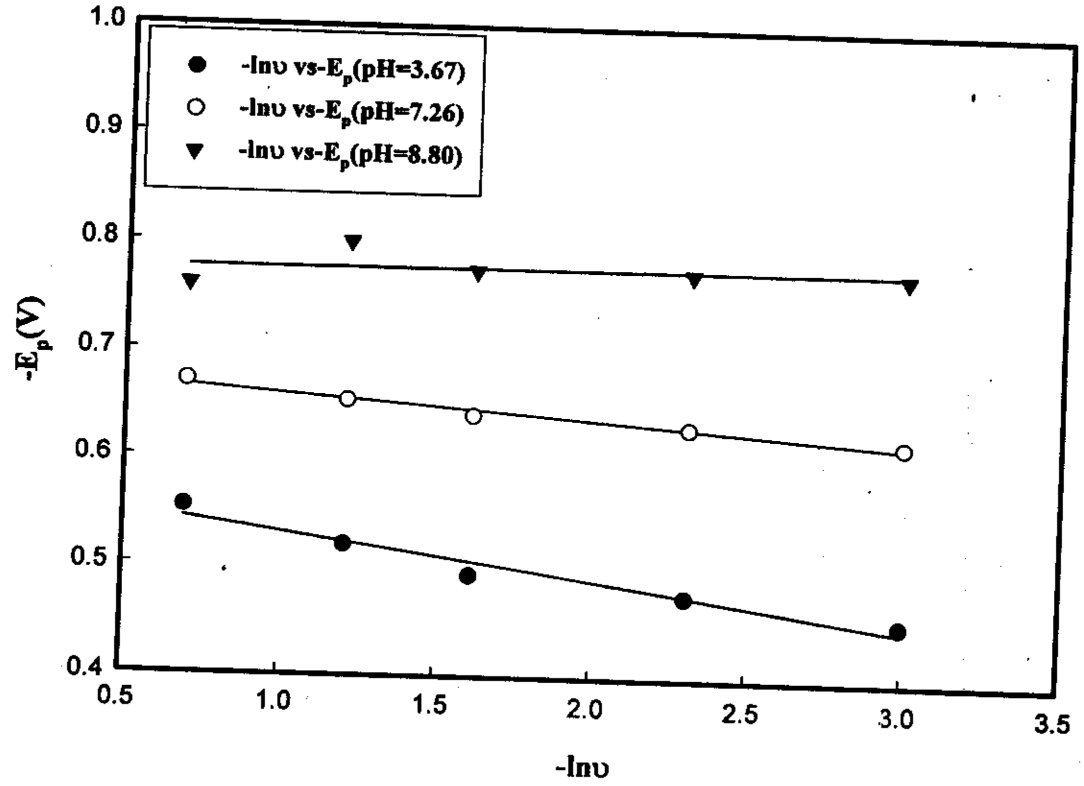


Fig. (62): E_p-ln(υ) plots of Cinnarzine at different pH values.

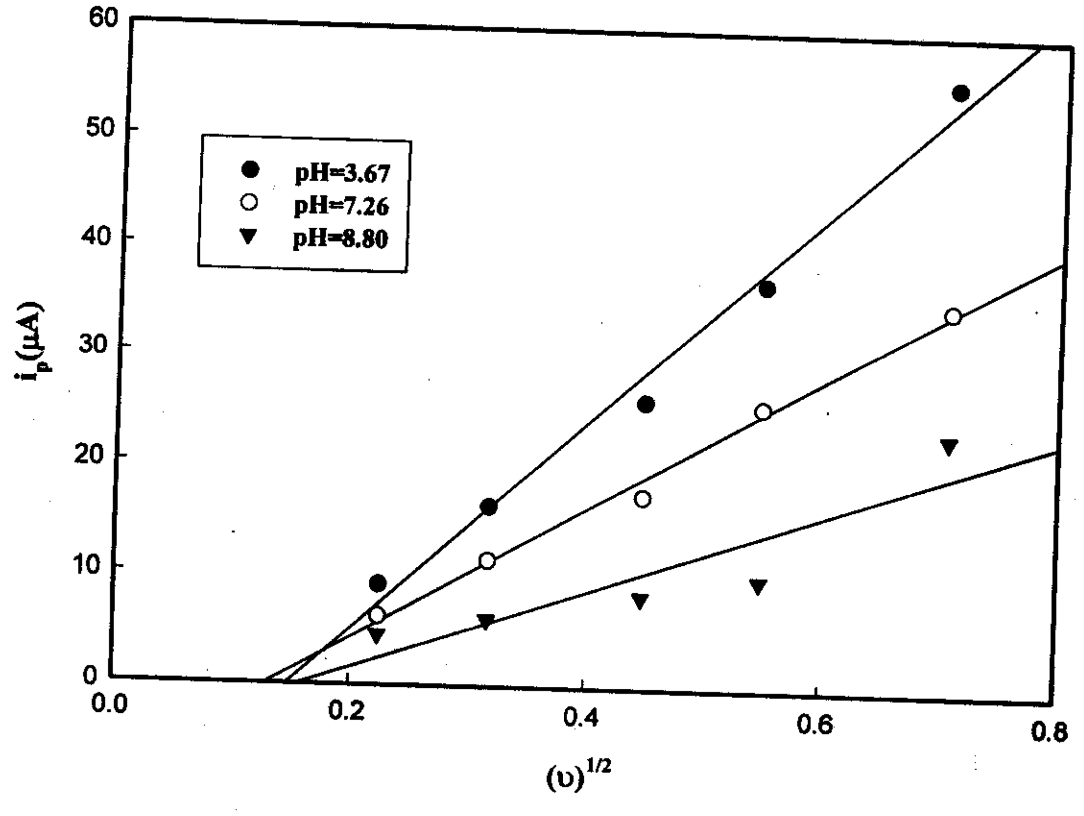


Fig. (63): i_p-$(\upsilon)^{1/2}$ plots of Cinnarzine at different pH values.

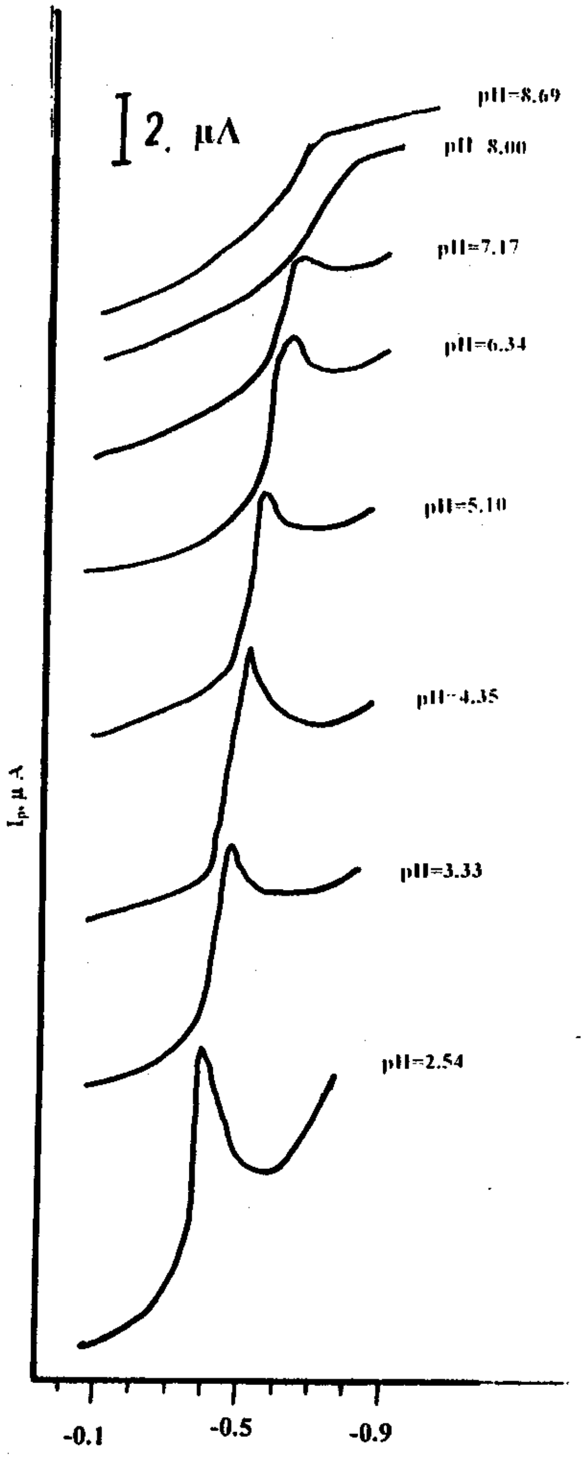


Fig. (64): **Effect of pH on the CAdS peak of 5×10^{-5} M of cinnarizine in B.R. Buffer solution at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.**

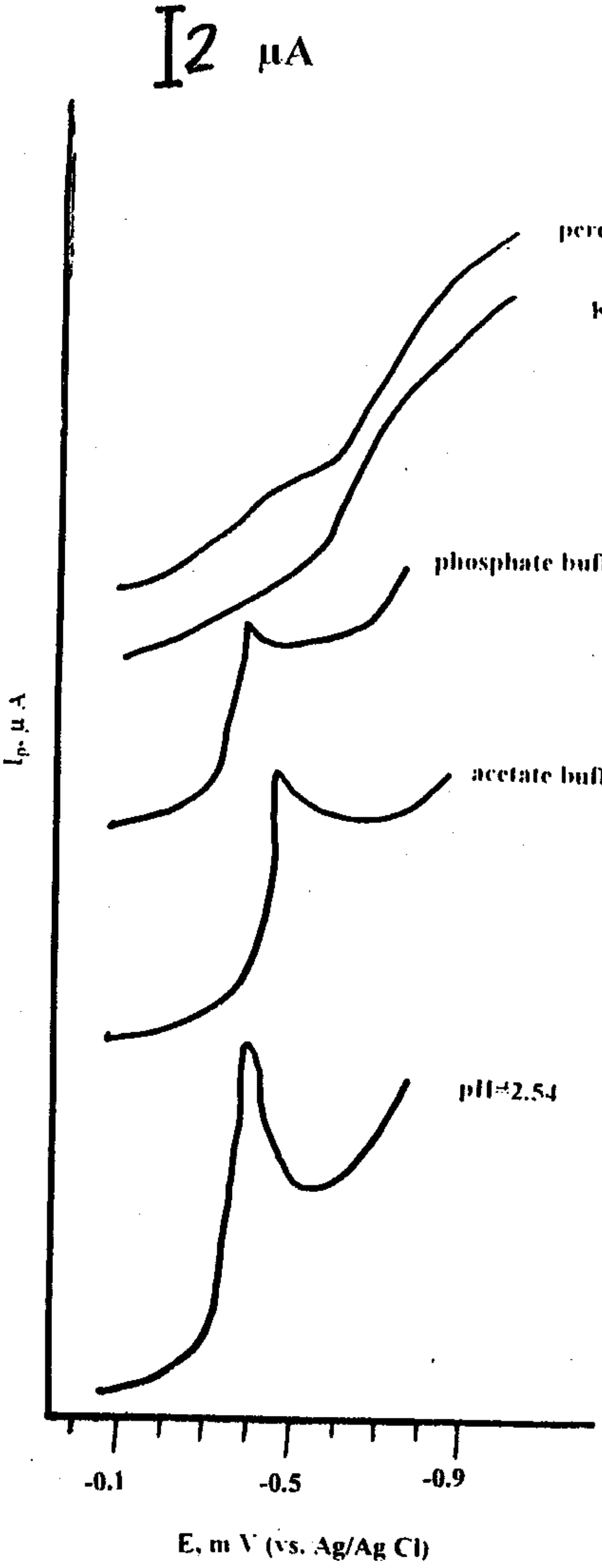


Fig. (65): **Effect of different supporting electrolyte solution on the CAdS peak of $5x10^{-5}$ M of cinnarizine at t_a = 240 s, E_a =-0.4 V, scan rate =100 mV/s and step height = 5 mV.**

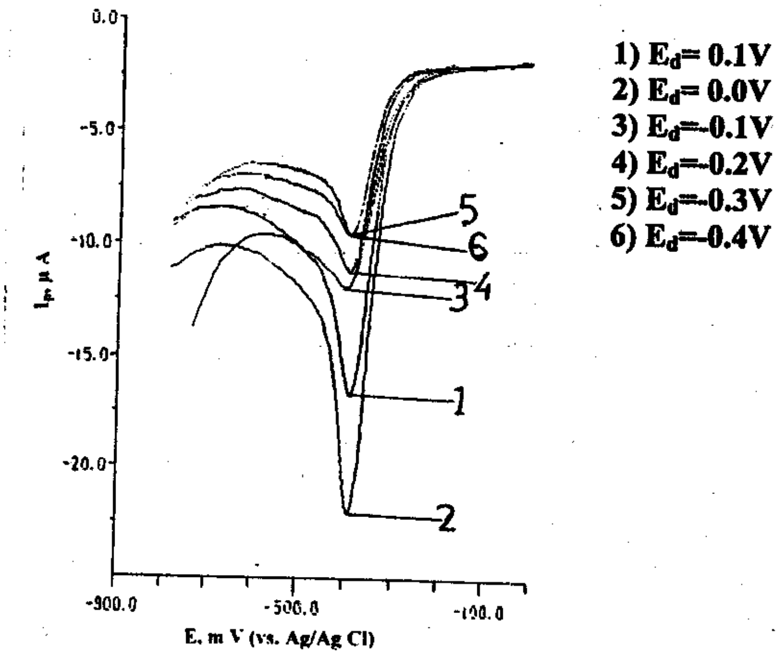


Fig. (66a): **Effect of deposition potential on 5x10-5M of Cinnarzine in B.R.buffer solution of pH=2.54 at td=240 s,scan rate=100mV/s,and pulse height=5 mV.**

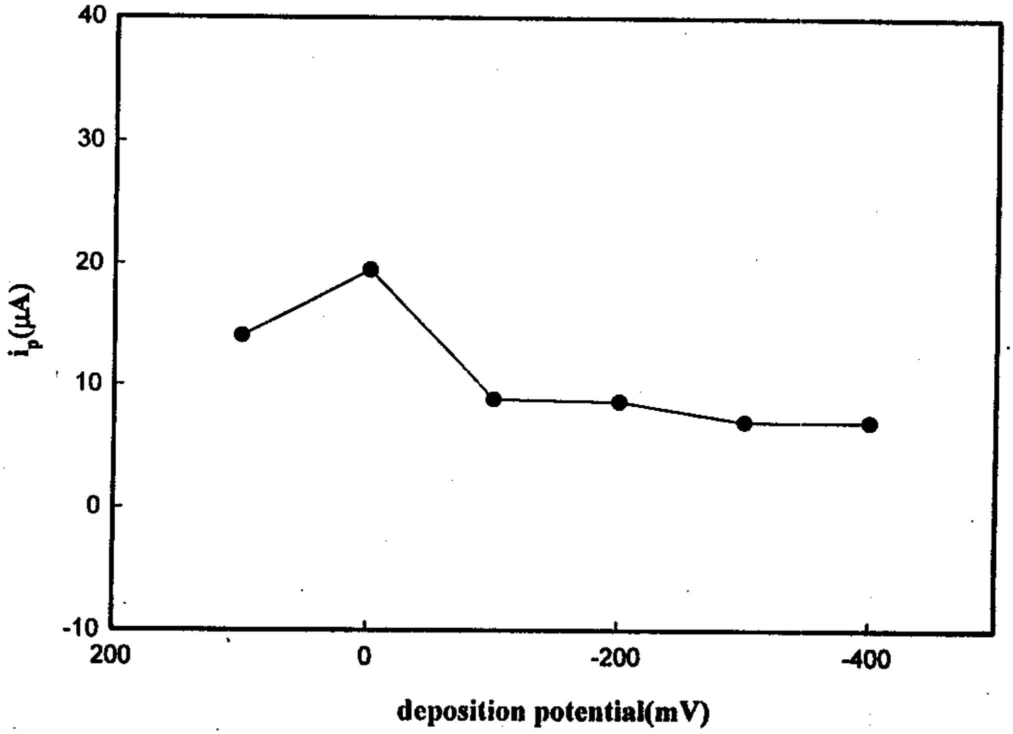


Fig. (66b): **Effect of deposition potential on $5x10^{-5}$M of Cinnarzine in B.R.buffer solution of pH=2.54 at td=240 s,scan rate=100mV/s,and pulse height=5 mV.**

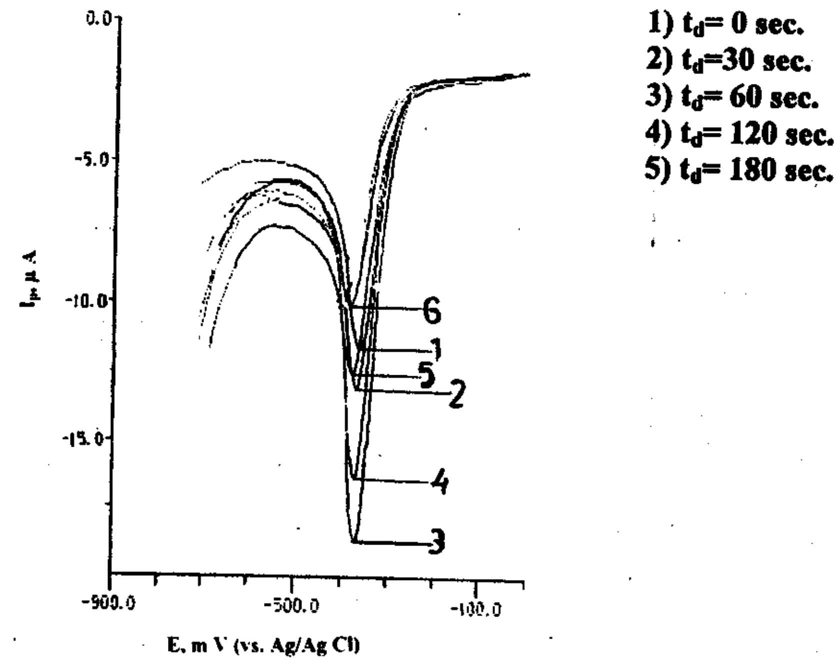


Fig. (67a): Effect of deposition time on $5x10^{-5}M$ of cinnarizine in B.R.buffer Solution of pH=2.54 at scan rate=500mV/s s, E_d=0.0V,and pulse height=10 mV.

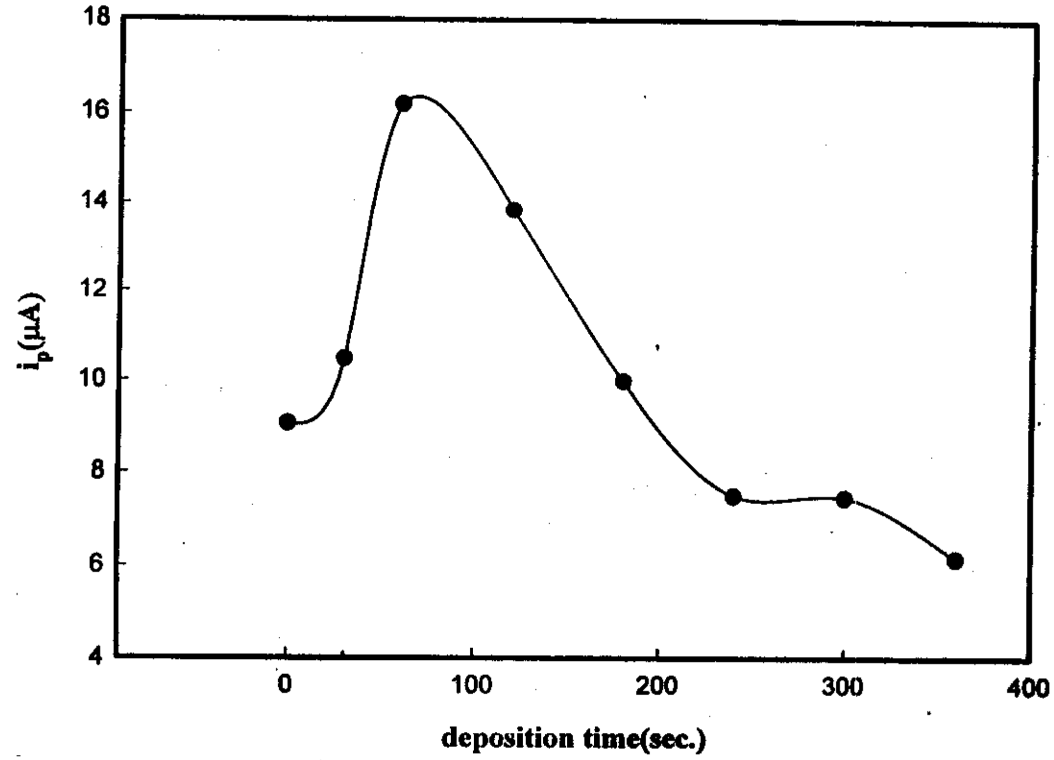


Fig. (67b): Effect of deposition time on $5x10^{-5}M$ of Cinnarzine in B.R.buffer solution of pH=2.54 at scan rate= 500mV/ s,E_d=0.0V,and pulse height=10 mV.

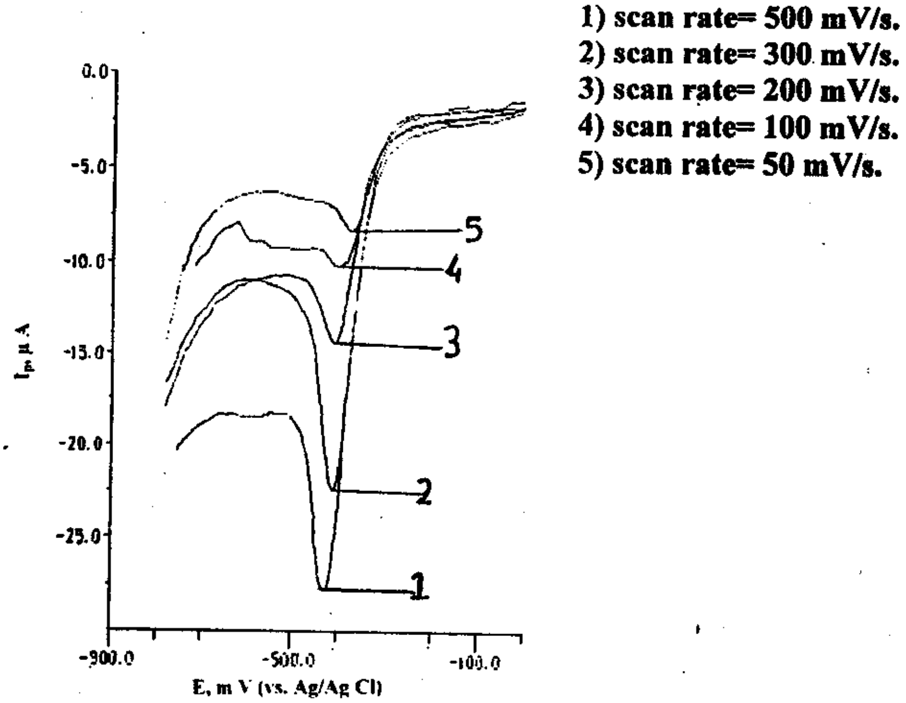


Fig. (68a): Effect of scan rate on 5x10^{-5}M of cinnarizine in B.R.buffer solution of pH=2.54 at E_d= -0.4V s, t_d=240 s,and pulse height=5mV.

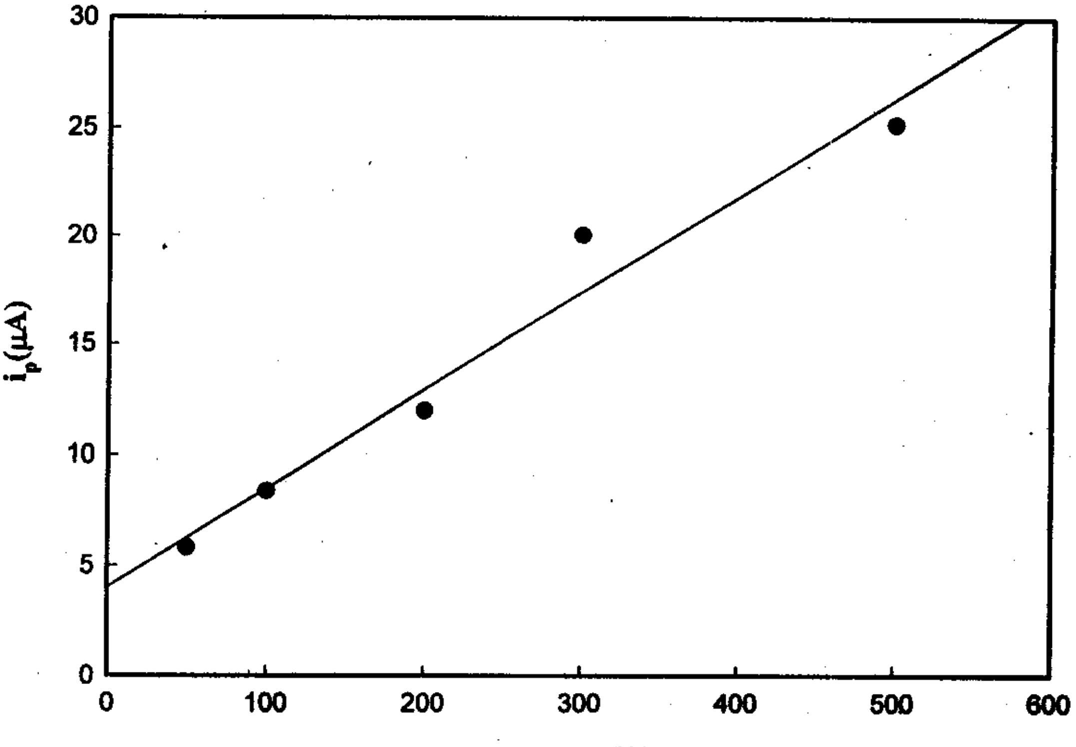


Fig. (68b): Effect of scan rate on 5x10^{-5}M of Cinnarzine in B.R.buffer solution of pH=2.54 at t_d=240 s,E_d=-0.4V,and pulse height=5mV.

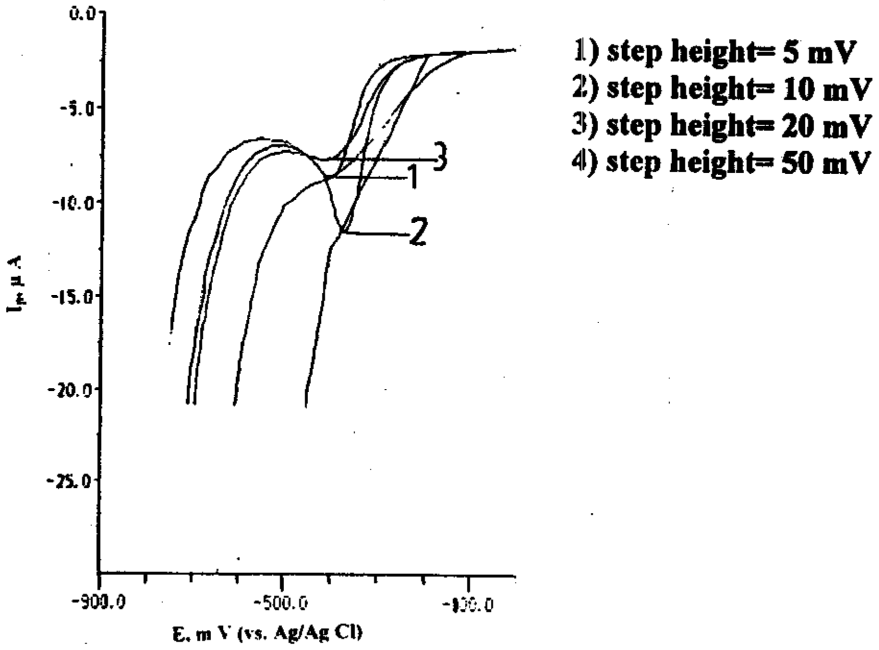


Fig. (69a): Effect of step height on $5x10^{-5}$M of cinnarizine in B.R.buffer solution of pH=2.54 at E_d= -0.4V s, t_d=240 s and scan rate =500 mV/s.

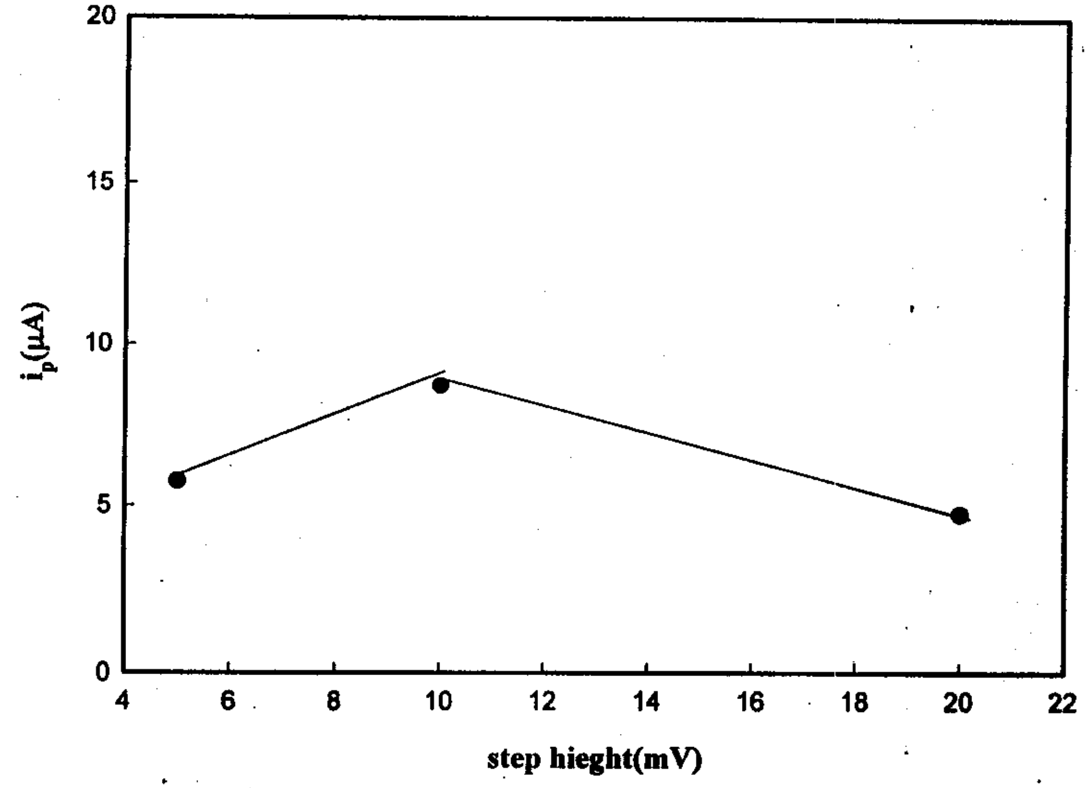


Fig. (69b): Effect of step height on $5x10^{-5}$M of Cinnarzine in B.R.buffer solution of pH=2.54 at t_d=240 s,E_d=-0.4V,and scan rate=500 mV/s.

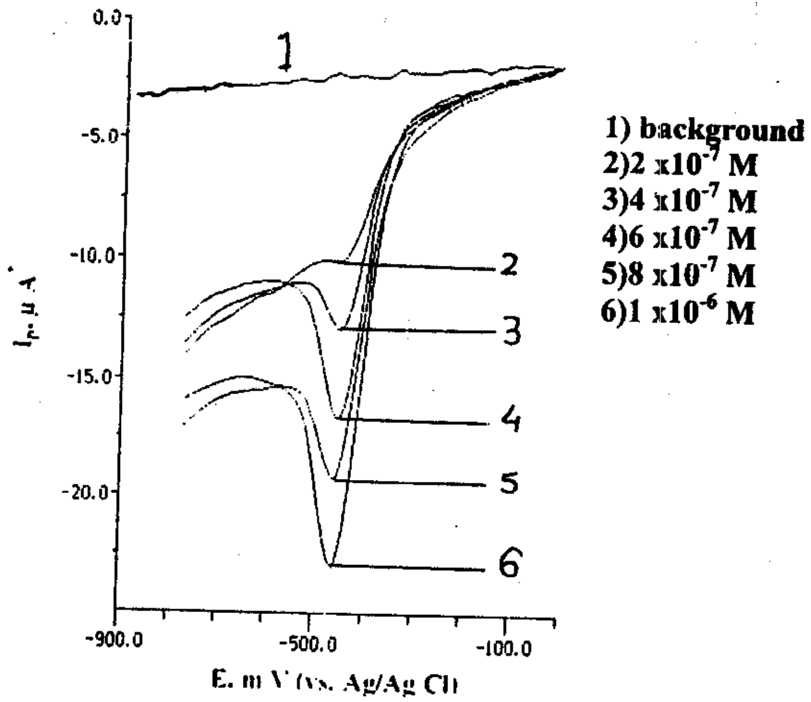


Fig. (70a) : **Effect of concentration of cinnarizine in B.R. buffer solution of pH=2.54 at: t_d=60 s, E_d= 0.0 V, scan rate 500 mV/s and step height= 10 mV**

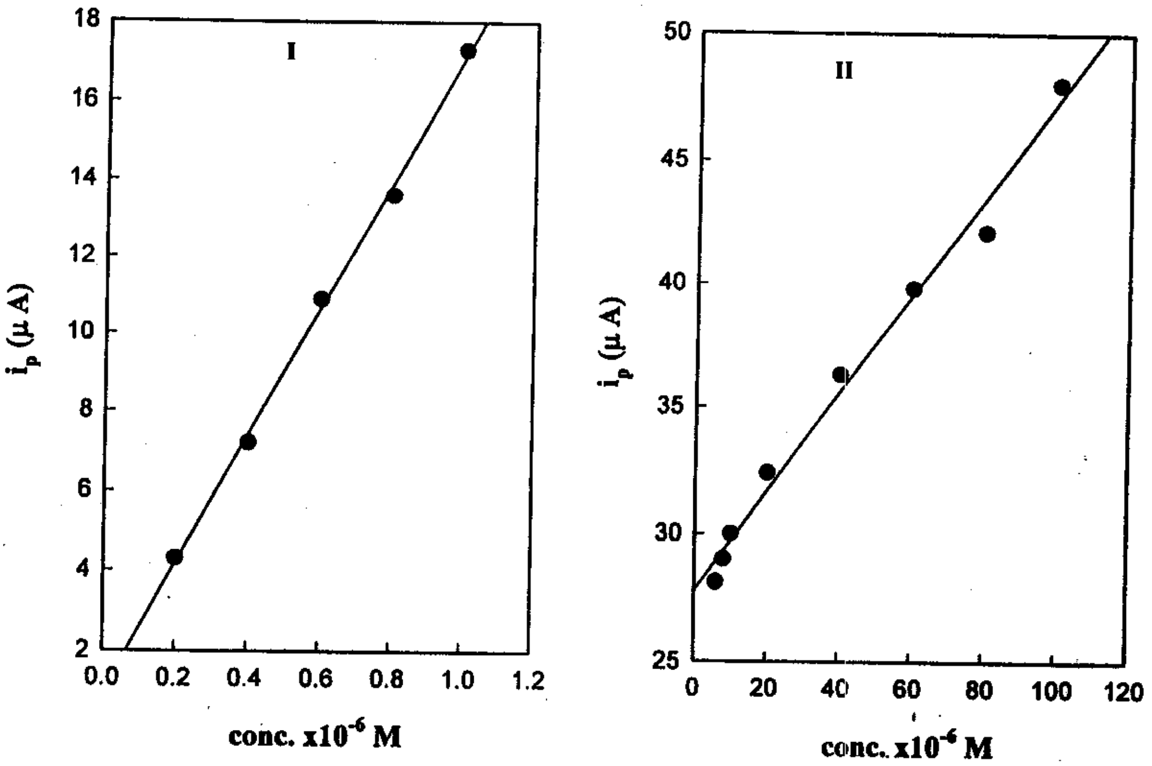


Fig. (70b): Calibration curve for cinnarizine.

Table(35): Polarographic data for 1×10^{-4}M of Cinnarizine in B.R. buffer solution of different pH values at 25 °C.

pH	i_d(μA)		$-E_{1/2}$		$\Delta \log i_l/\Delta \log h$	
	A	B	A	B	A	B
1.5	1.12	0.58	0.20	0.45	0.43	0.49
2.4	1.12	0.48	0.30	0.70		
3.3	1.02	0.46	0.35	0.90		
4.0	1.03	0.44	0.40	0.93		
4.5	1.04	sh.	0.47	sh.	0.56	sh.
5.5	1.02	–	0.50	–		
6.5	0.93	–	0.62	–		
7.4	0.91	–	0.69	–		
8.5	0.90	–	0.75	–		
9.5	0.83	–	0.83	–	0.82	–

Where A:1st wave B: 2nd wave sh: ill-defined

Table(36) : Values of (Z_H^+) and (α) as determined for different ratios (Z_H^+/n_a) at 0.5, 1.0 and 2.0 of the number of protons calculated from slope(S_2)of the $E_{1/2}$-pH plots and (S_1) for cinnarizine in B.R. buffer solutions of different pH values at 25C°

pH	S_1 mV		S_2 mV		(Z_H^+)=(S_2/S_1)		Z_H^+/n_a		
							0.5	1.0	2.0
	A	B	A	B	A	B			
1.5	58.49	169.80	76.2	198	1.30	0.85	0.39	0.78	1.6
2.4	55.39	221.70	76.2	198	1.38	0.89	0.39	0.78	1.6
3.3	67.43	184.84	76.2	198	1.13	1.07	0.39	0.78	1.6
4.0	72.21	185.98	76.2	198	1.06	1.07	0.39	0.78	1.6
4.5	68.69	sh.	76.2	sh.	1.11	sh.	0.39	0.78	1.6
5.5	68.80	—	76.2	—	1.11	—	0.39	0.78	1.6
6.5	96.00	—	76.2	—	0.79	—	0.39	0.78	1.6
7.4	94.22	—	76.2	—	0.81	—	0.39	0.78	1.6
8.5	84.08	—	76.2	—	0.91	—	0.39	0.78	1.6
9.5	77.92	—	76.2	—	0.98	—	0.39	0.78	1.6

Where **A:1st wave** **B:2nd wave** sh: ill-defined

Table(37a):Values of (αna) and (α) for 1x10^{-4} M of Cinnarizine in B.R buffer solution of different pH values, as calculated from reciprocal slope (S_1) of the log (i/i_d-i) -$E_{d.e}$ plots.

pH	Slope(S_1) mV		αna		α			
					n=1	n=2	n=1	n=2
	A	B	A	B	A	A	B	B
1.5	58.49	169.80	1.01	0.85	1.01	0.50	0.85	0.42
2.4	55.39	221.80	1.07	0.27	1.07	0.53	0.27	0.13
3.3	67.43	184.84	0.88	0.32	0.88	0.44	0.32	0.16
4	72.21	185.98	0.82	0.32	0.82	0.41	0.32	0.16
4.5	68.69	sh.	0.86	sh.	0.86	0.43	sh.	sh.
5.5	68.80	–	0.86	–	0.86	0.43	–	–
6.5	96.00	–	0.62	–	0.62	0.32	–	–
7.4	94.22	–	0.63	–	0.63	0.32	–	–
8.5	84.08	–	0.70	–	0.70	0.35	–	–
9.5	77.9	–	0.76	–	0.76	0.38	–	–

Where A: 1st wave B: 2nd wave sh: ill-defined

Table(37b): Data obtanied for $1x10^{-4}$M of Cinnarizine in B.R. buffer solution of different pH values using cyclic voltammetry at different scan rates, at 25°

pH	Scan rate (mV/s)	-Ep (V)	-dEp/d(lnv)	αna
3.67	500	0.55	0.0418	0.31
	300	0.52		
	200	0.49		
	100	0.48		
	50	0.45		
7.26	500	0.62	0.0217	0.73
	300	0.65		
	200	0.64		
	100	0.63		
	50	0.62		
8.80	500	0.76	0. 006	2.1
	300	0.80		
	200	0.78		
	100	0.78		
	50	0.78		

Table (38): Kinetic parameters obtained from D-C measurements for $1x10^{-4}$ M cinnarizine in B. R. buffer solution of different pH's.

pH	$D^o(Cm^2/s)$	$K^o_{f,h}$ (Cm/s)	ΔG* (k.cal/mole)
1.50	$2.80 x10^{-6}$	$1.05 x10^{-10}$ [a]	71.26 [a]
		$6.17 x10^{-16}$ [b]	117.3 [b]
2.40	$2.80 x10^{-6}$	$3.39 x10^{-11}$ [a]	89.96 [a]
		$3.00 x10^{-17}$ [b]	124.94 [b]
3.30	$2.80 x10^{-6}$	$5.50 x10^{-12}$ [a]	94.51 [a]
		$2.00 x10^{-19}$ [b]	137.5 [b]
4.00	$1.20 x10^{-6}$	$4.07 x10^{-12}$ [a]	95.26 [a]
		$1.00 x10^{-19}$ [b]	139.25 [b]
4.50	$1.20 x10^{-6}$	$1.00 x10^{-13}$ [a]	104.60 [a]
5.50	$1.20 x10^{-6}$	$4.57 x10^{-14}$ [a]	107.10 [a]
6.50	$9.60 x10^{-7}$	$2.23 x10^{-14}$ [a]	108.34 [a]
7.40	$9.60 x10^{-7}$	$5.13 x10^{-15}$ [a]	112.00 [a]
8.50	$9.60 x10^{-7}$	$6.31 x10^{-16}$ [a]	117.29 [a]
9.50	$9.60 x10^{-7}$	$6.31 x10^{-17}$ [a]	123.10 [a]

Where a : 1st wave , b : 2nd wave .

Table(39):Cathodic adsorptive stripping peak current(i_p)of $5x10^{-5}$M Cinnarizine in B.R.buffer solution of pH=2.54at different conditions (t_d, E_d, scan rate and pulse height).

Deposition time (t_d), sec	Deposition potential(E_d), V	Scan rate (mV/s)	Pulse hieght (mV)	i_p (μA)
Effect of (td):				
0	0.00	500	10	9.06
30				14.49
60				16.19
120				13.84
180				9.99
240				7.51
300				7.48
360				6.18
Effect of (E_d):				
240	0.1	100	5	14.07
	-0.01			19.52
	-0.1			8.85
	-0.2			8.67
	-0.3			7.00
	-0.4			7.00
Effect of scan rate:				
240	0.00	500	5	25.21
		300		20.07
		200		11.98
		100		8.37
		50		5.80
Effect of pulse height:				
240	0.00	500	5	5.73
			10	8.70
			20	4.78
			50	
			100	
			150	
			200	

Table(40) : Calibration curve data of cinnarizine in B.R.buffer solution of pH=2.54 using CAdSV at t_d= 60sec., E_d= 0.0 V, scan rate= 500mV/s, and step height= 10 mV.

Conc. taken	Ip(μA)	Mean	S.D.	**R.S.D.** %
$2x10^{-7}$M	**4.34** **4.17** **4.23** **4.30**	**4.26**	**0.082**	**1.87**
$4x10^{-7}$M	**7.16** **7.23** **7.40** **7.16**	**7.23**	**0.13**	**1.56**
$6x10^{-7}$M	**10.98** **10.69** **10.78** **10.95**	**10.85**	**0.138**	**1.27**
$8x10^{-7}$M	**13.45** **13.78** **13.62** **13.65**	**13.62**	**0.136**	**0.99**
$1x10^{-6}$M	**17.26** **17.36** **17.26** **17.42**	**17.33**	**0.079**	**0.45**

Table(41) : Assay of cinnarizine in dosage form (cinnarizine 75 mg) in B.R. buffer solution at pH=2.54 using CAdSV at t_d= 60 sec., E_d= 0.0 V, scan rate= 500mV/s, and step height= 10 mV.

Conc. taken	Conc. calculated	Recovery %	Mean Recovery(%) ± S.D
$2x10^{-7}M$	**$2.07\ x10^{-7}M$**	**103.5**	**102.1 ±2.98**
	$2.03\ x10^{-7}M$	**101.5**	
	$2.00\ x10^{-7}M$	**100.0**	
	$2.07\ x10^{-7}M$	**103.5**	
$4x10^{-7}M$	**$3.90\ x10^{-7}M$**	**97.50**	**98.87 ±1.67**
	$4.00\ x10^{-7}M$	**100.0**	
	$3.97\ x10^{-7}M$	**99.25**	
	$3.95\ x10^{-7}M$	**98.75**	
$6x10^{-7}M$	**$6.14\ x10^{-7}M$**	**102.3**	**100.9 ±1.49**
	$5.98\ x10^{-7}M$	**99.66**	
	$6.06\ x10^{-7}M$	**101.0**	
	$6.04\ x10^{-7}M$	**100.7**	
$8x10^{-7}M$	**$7.78\ x10^{-7}M$**	**97.87**	**98.99 ±1.53**
	$7.93\ x10^{-7}M$	**99.12**	
	$8.02\ x10^{-7}M$	**100.2**	
	$7.90\ x10^{-7}M$	**98.8**	
$1x10^{-6}M$	**$1.02\ x10^{-6}M$**	**102.0**	**100.8 ±1.29**
	$1.01\ x10^{-6}M$	**101.0**	
	$1.00\ x10^{-6}M$	**100.0**	
	$1.00\ x10^{-6}M$	**100.0**	

Table(42) : Assay of cinnarizine in dosage form (cinnarizine 25 mg) in B.R.buffer solution at pH=2.54 using CAdSV at t_d= 60sec., E_d= 0.0 V, scan rate= 500mV/s, and step height= 10 mV.

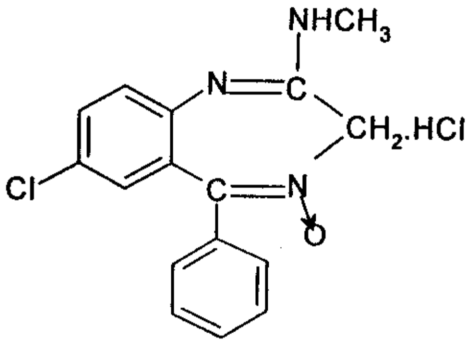
Conc. taken	Conc. calculated	Recovery %	Mean Recovery(%) ± S.D
2×10^{-7}M	2.03×10^{-7}M	101.5	100.0 ±2.12
	1.94×10^{-7}M	97.00	
	2.03×10^{-7}M	101.5	
	2.00×10^{-7}M	100.0	
4×10^{-7}M	4.03×10^{-7}M	100.8	100.0 ±1.36
	3.98×10^{-7}M	99.50	
	4.02×10^{-7}M	100.5	
	3.97×10^{-7}M	99.25	
6×10^{-7}M	6.05×10^{-7}M	100.8	100.6 ±0.87
	6.03×10^{-7}M	100.5	
	6.07×10^{-7}M	101.2	
	6.00×10^{-7}M	100.0	
8×10^{-7}M	8.10×10^{-7}M	101.3	100.4 ±0.87
	7.97×10^{-7}M	99.62	
	8.01×10^{-7}M	100.1	
	8.06×10^{-7}M	100.8	
1×10^{-6}M	0.98×10^{-6}M	98.00	99.00 ±1.41
	1.00×10^{-6}M	100.0	
	0.99×10^{-6}M	99.00	
	0.99×10^{-6}M	99.00	

Table(43):Assay of cinnarizine in serum in B.R.buffer solution of pH=2.54 using CAdSV at td= 60 sec., Ed= 0.0 V, scan rate= 500mV/s, and step height= 10 mV.

Conc. taken (µg/10 ml)	Conc. Calculated (µg/10 ml)	Recovery %	Mean Recovery(%) ± S.D
2.20	2.15	97.73	99.54 ±1.60
	2.25	102.3	
	2.19	99.54	
	2.17	98.64	
7.34	7.36	100.3	99.89 ±0.57
	7.32	99.72	
	7.37	100.4	
	7.28	99.18	

3.6. Electrochemical behavior of chlorodiazepoxide in B.R buffer solutions of different pH values:-

Chlorodiazepoxide is[7-chloro-2-(methylmino)-5-phenyl-3H-1,4-benzodiazepine-4-oxide hydrochloride] and has the following strucyure :



This part includes the study of electrochemical behavior of chlorodiazepoxide at Hg-electrode using of DC polarography and cyclic voltammetry techniques The study aimed to optimize the experimental and instrumental conditions for determination of the lowest possible concentration of the investigated pharmaceutical compound in pure and dosage forms using cathodic adsorptive stripping voltammetry .

3.6.1 DC polarography:-

3.6.1. i-Current potential curves:-

The polarographic behavior of $1x10^{-4}$ M of chlorodiazepoxide was studied in B.R. buffer solutions containing 10% (v/v) ethanol of different pH values in the range from 2 to 12 . The polarogrammes exhibit three reduction waves within the pH range studied corresponding to the reduction of C=N and oxime (NO) groups Fig.(71) the half-wave potential $E_{1/2}$ of the reduction waves are pH dependent, and being shifted to more negative values on increasing the pH of the electrolysis solution .

3.6.1. ii-Effect of pressure at mercury height :-

The effect of mercury height "h" was examined according to equation (3.1). The plots of log i_l against log h for chlorodiazepoxide showed linear correlations at different pH values, Fig.(72). The slope values (x) are found in the range between 0.31 and 0.67 which indicate that the reduction process is controlled by diffusion and some adsorption contribution, Table (44).

3.6.1. iii-Analysis of the polarographic waves:-

Analysis of the polarographic waves is quite important to evaluate the degree of reversibility of electrode reaction and to suggest the electrode mechanism On using equation (3.4), the plots of log[i/(i_d-i)] against $E_{d.e.}$ for chlorodiazepoxide reduction at different pH values showed linear correlations, Fig.(73). The straight lines with reciprocal slopes (S_1) amounting in the range to (60.94 -146.41), (49.21-91.49) and (69.83-169.49) mV for the first, second and third waves; respectively, Table (45). This behavior revealed the irreversible nature of the electrode process.

3.6.1.iv-Half- wave potential -pH curves :-

The plots of the rate shift of $E_{1/2}$ of chlorodiazepoxide versus the pH of the electrolysis solution showed linear correlations for both the first, second and third waves respectively . The slope values (S_2) of the first, second and third waves is amounting to 81.75, 54.82 and 70.17 mV, respectively ,Table (45a).

The number of hydrogen ions (Z_H^+) involved in the rate determining step for secnidazole reduction was calculated from both slopes of logarithmic analysis and $E_{1/2}$ plots according to equation (3.5). At all pH values Z_H^+ was found to be unity, Table (45a).

The most probable values of α- parameter as shown in Table (45b). were determined at the probable ratio of (Z_H^+/n_a) using equation (3.6). the probable (Z_H^+/n_a) ratio was found to fit with (Z_H^+/n_a) = 0.5 at where the values of (Z_H^+/n_a) at 0.5, 1and 2 were 0.36, 0.73 and 1.5 respectively .

This behavior is further confirmed the irreversibility of electrode process and the rate determining step involves one proton and two electrons.

3.6.2 Cyclic voltammetry :-

The cyclic voltammetric behavior of $1x10^{-4}$ M of chlorodiazepoxide was investigated in aqueous Britton-Robinson buffer solutions of different pH values (2-12) containing 10% (v/v) ethanol at glassy carbon electrode. The voltammogram displayed double irreversible cathodic peaks in the cathodic scan in all media, where as in the anodic scan there is no oxidation peaks were observed, Fig.(75).

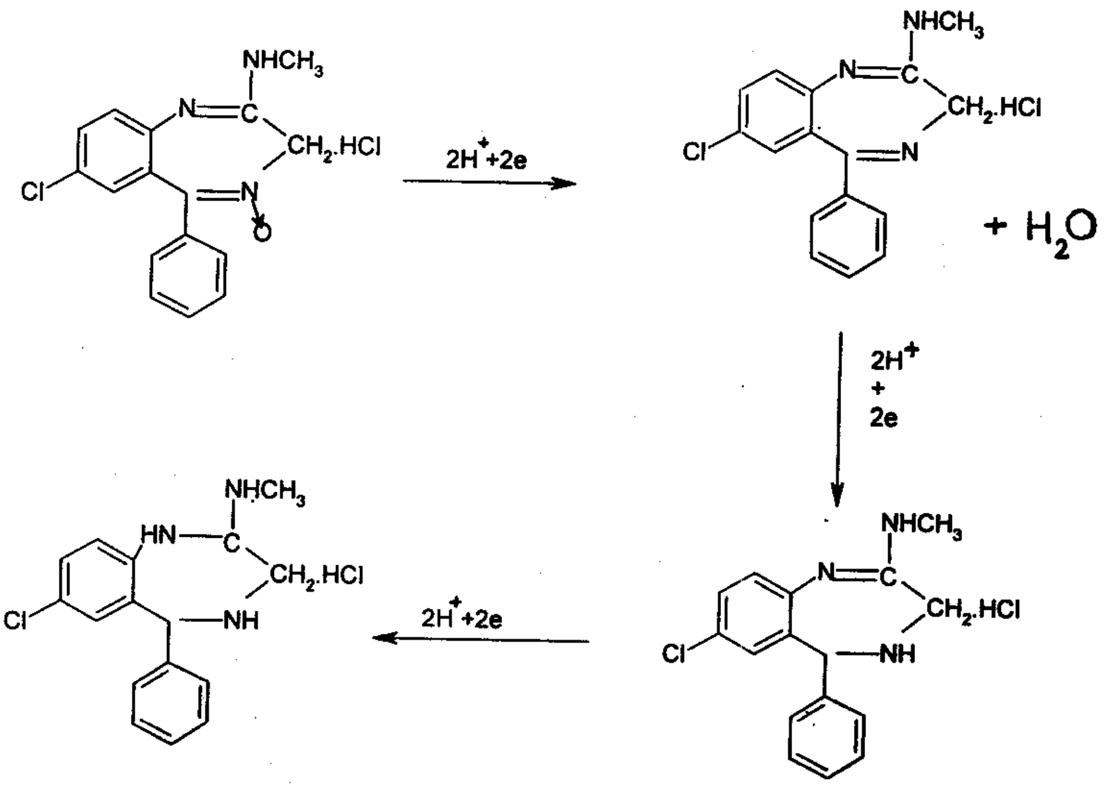
On increasing the scan rate (υ), the cathodic peak potential (E_{pc}) was shifted to more negative potentials indicating the irreversible nature of the reduction peak. Furthermore, on increasing the pH of the electrolysis solution the peak potential (E_{pc}) is shifted to more negative values indicating the consumption of hydrogen ions in the electron transfer step. On employing equation (3.7), the plots of E_p versus ln υ gives linear correlations Fig.(76) . The slope values were used for the determination of αn_a. The values of the (α) transfer coefficient were calculated at the probable n_a values and found to be less than 0.6 at n_a=2.0, Table(46). This behavior is further confirmed the irreversible nature of electrode process. According to equation(3.8), the plots of i_p versus square root of scan rate ($\upsilon^{1/2}$) gives linear relation slightly deviating from the origin, Fig.(77). This behavior is confirming that the electrode process of chlorodiazepoxide is controlled mainly by diffusion with some adsorption contribution.

3.6.2.i-Kinetic parameters of the electrode reaction :-

The rate constant $k^o_{f,h}$ of the electrode reaction of chlorodiazepoxide is calculated from the data obtained by dc- polarography measurements in Britton-Robinson buffer solution of various pH values. Equation (3.12) was used in this determination and the values of the rate constant $k_{f,h}$ were found to be decreased with increasing the pH which means that the electrode reaction becomes more difficult and more irreversible Table(47). Also, the values of ΔG* were determined using equation (3.14) and found to be increased with increasing pH of the electrolysis medium, Table(47). This may be explained on the basis that the protonation of the molecule, at lower pH, before its diffusion to the electrode surface, takes place through the contribution of the H^+ ion directly with the depolarizer molecule which decreases the activation energy ΔG^* . The H^+ ion activity decreases with rise of pH, hence ΔG^* increases. In alkaline media, the proton required for pre-protonation of the depolarizer is supplied from a water molecule which requires much higher energy.

The electrode reaction mechanism :-

To study the reduction mechanism of chlorodiazepoxide at mercury electrode, it is necessary to calculate the number of electrons concumed at this reduction, firstly we should calculate the (diffusion coefficient) D from equation (3.16) and the corrected values shown in Table (47), the number of electron (n) found that 6 electrons are consumed in the reduction of oxime group at acidic and basic medium,and the number of electrons were confirmed by controlled potential coulmetry method according to equation (3.17) the avarge value of n is (4±0.2) so the suggested mechanism as the following:



3.6.3 Cathodic adsorptive stripping voltammetry(CAdSV) of chlorodiazepoxide

Cathodic adsorptive voltammetry was used for the quantitative determination of chlorodiazepoxide in pure and dosage forms . the instrumental and experimental conditions were optimized in this investigation. The instrumental conditions are deposition time (td), deposition potential (Ed), step height and scan rate. While the experimental conditions are including the nature of supporting electrolyte and pH of the electrolysis solution.

3.6.3.i- Effect of pH and supporting electrolyte:

In cathodic adsorptive voltammetry current of chlorodiazepoxide is recorded as function of potential in Britton- Robinson buffer solution of pH(2.15 to 11.12) the recorded peaks at various pH values due to the reduction of C=N and oxime group at the surface of glassy carbon electrode and well developed peak is noted at pH=4.05 and 7.33 Fig.(78).

We determined the chlorodiazepoxide in some supporting electrolyte like (phosphate, acetate, $NaClO_4$ and KCl buffers) we fined that in presence of $NaClO_4$,KCl the peak disappeared it means that the absence of this anion best of chlorodiazepoxide and the pH=4.05 and pH=7.33 is most suitable for chlorodiazepoxide Fig.(79)

3.6.3.ii- Effect of accumulation time and potential :

The deposition of the analyzed drug on the surface of the glassy carbon electrode is one of the essential conditions for highly sensitive determinations. The effect of accumulation potential on the peak height was examined over the potential range +100 to -800 mV for $5x10^{-5}$ M chlorodiazepoxide at accumulation time 240 s., Fig. (80a, 81a). the relation between peaks height i_p and deposition potential E_d is shown in Fig. (80b, 81b). The peak current increased steadily with decreasing the potential value till it reaches maximum value at E_d = -400 mV, where it decreased sharply after this inflection point. Thus, E_d = -400 mV will be adopted as optimum operational values for the following works as it ensured the highest voltammetric signal.

The effect of accumulation time of the drug was examined in the range 0-360s. the amount of chlorodiazepoxide accumulated on the electrode surface increased as the deposition time increased till 30 s, further increase of time resulted in decreasing the peak height. A time of accumulation of 30 s was chosen for analytical purposes. The voltammograms obtained for $5x10^{-5}$ M chlorodiazepoxide in B. R. buffer solution of pH 4.04 and pH 7.33 respectivily and E_d = -0.4 V are shown in Fig. (82a, 83a). The dependence of peak height on accumulation time are illustrated in Fig. (82b, 83b).

3.6.3.iii- Effect of scan rate :

The effect of scan rate on the peak current and peak potential was studied. The relationship between i_p or E_p and υ at 240 s., accumulation time shows an increase in the peak current with increasing the scan rate, Fig. (84a,b) at pH 4.04 and Fig. (85 a,b) at pH 7.33 . The peak potential was shifted to more negative values with increasing the scan rate. The scan rate 500 mv s^{-1} was used for further voltammetric determination of the drug.

3.6.3.iv- Effect of step height :-

The effect of step height on the CAdSV peak current of chlorodiazepoxide reduction was recorded at different values, Fig. (86a, 87a).

A well defint peak was observed at 10mV step height for pH 4.04 and 7.33 On increasing step height up to 150 mV the CAdSV peak current decreases till become ill-defined. From the plot of peak current (i_p) versus step height it noted that i_p is inversely proportional to step height, Fig. (86b, 87b). and all data shown in Table (48, 49) for pH 4.04 and 7.33.

3.6.3.v-Calibration curve, detection limit and precision :

A calibration plot was obtained from known concentrations of chlorodiazepoxide using the peak current in µA as analytical response Fig. (88 and 89) at pH =4.04 and 7.33 respectively. Series of standard solution (four replicates) of the drug were prepared by following the procedure described.

The calibration graph was found to be linear over the range 2×10^{-7} to 5×10^{-6} M at pH =4.04 and range 1×10^{-6} to 1×10^{-4} at pH =7.33.

The calibration graph yielded the following regression equations:

I_p (μA) = 1.57 + 1.46 x 10^6 C M; r = 0.9982 n = 9 at pH =4.04

I_p (μA) = 5.85 + 0.16 x 10^6 C M; r = 0.9959 n = 11 at pH =7.33

The detection limit obtained for the developed voltammetric method was 5×10^{-8} M at pH =4.04 and dl at pH =7.33 is 2.5×10^{-7} M.

In order to assess the precision as the standard deviation for concentration with the relative standard deviation were evaluated, Table (50, 51).

Analysis of pharmaceutical formulation:

The proposed method was successfully applied to the assay of the studied drug in the pharmaceutical dosage forms (cloxid capsules, labeled to contain 5 and 25 mg per tablet). The percentage recoveries for chlorodiazepoxide are shown in Table (52 and 53) respectively for pH 4.04 and 7.33. The method gave a good recovery. The percentage recovery based on six different determinations respectively to two pHs were (100.92 ± 1.82) and (101.15 ±1.75) respectively.

Analysis of serum:

The results of precision in spiked serum samples, Table (54), suggest that this method can't be used for the determination of chlorodiazepoxide in human serum.

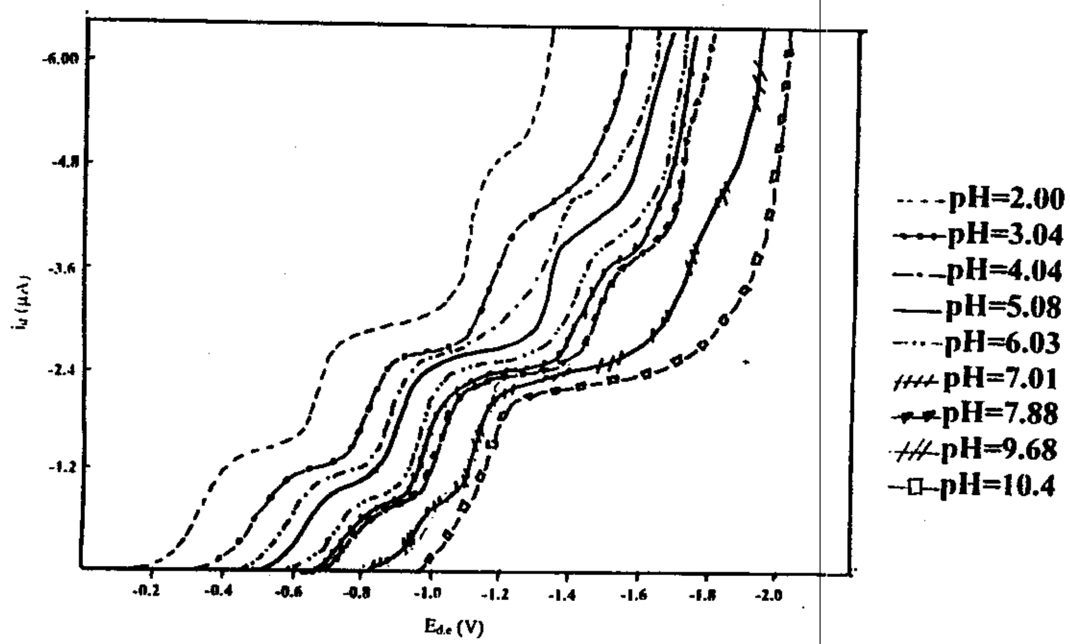


Fig. (71): DC-polarograms of $1x10^{-4}$ M of chlorodiazepoxide in B.R. buffer solution of different pH values

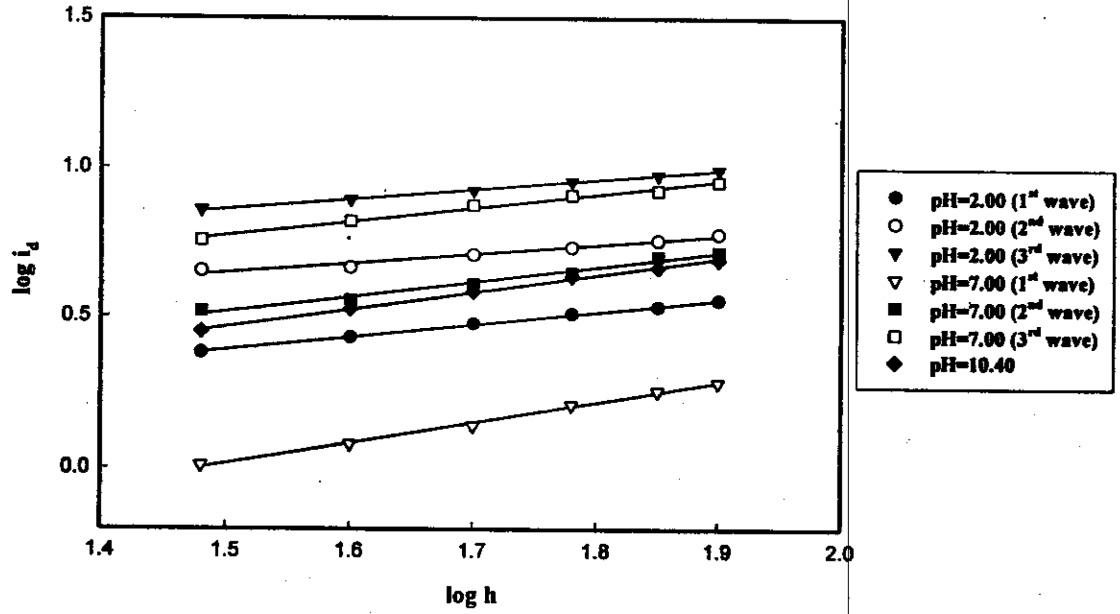


Fig. (72): log i_d-log h plots of chlorodiazepoxide

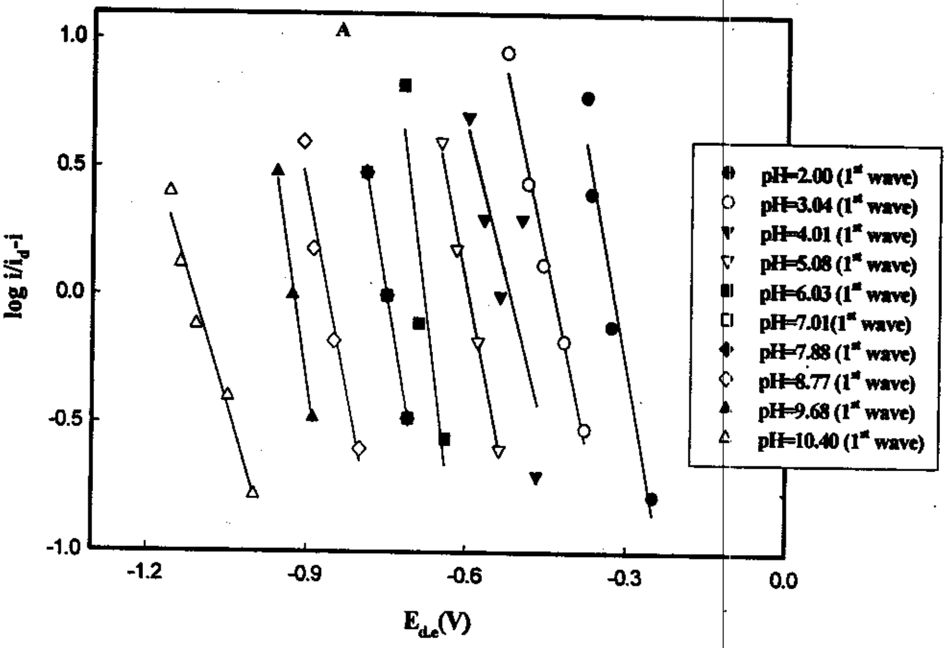


Fig. (73a): analysis of polarographic waves of chlorodiazepoxide at diffrent pH values (1st wave)

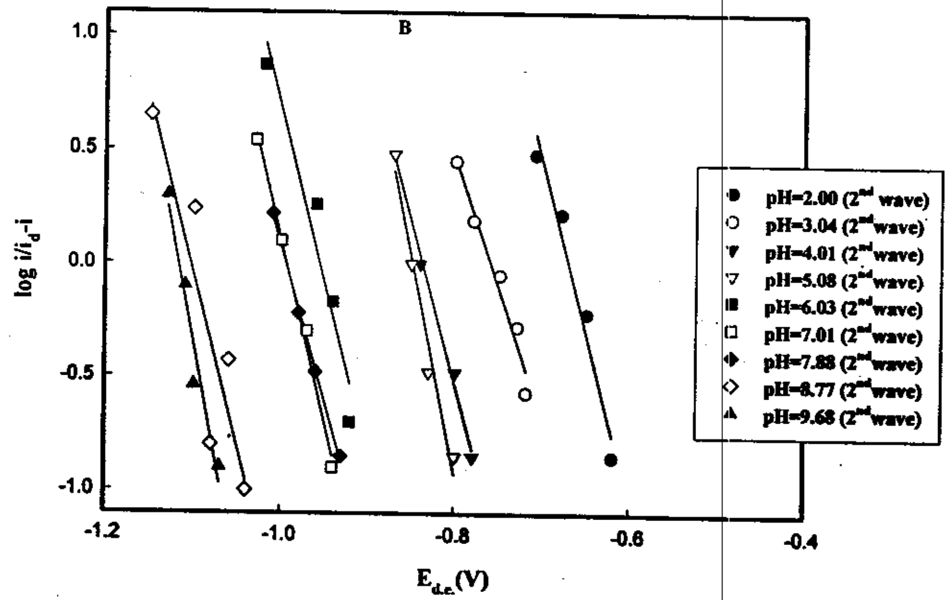


Fig. (73b): analysis of polarographic waves of chlorodiazepoxide at diffrent pH values (2nd wave)

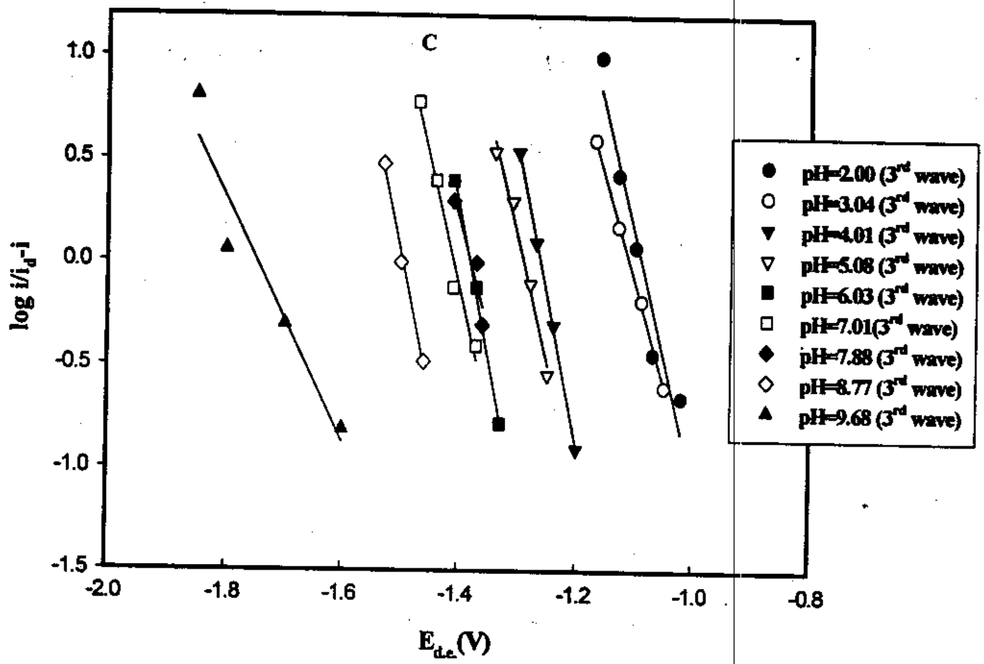


Fig. (73c): analysis of polarographic waves of chlorodiazepoxide at diffrent pH values (3rd wave)

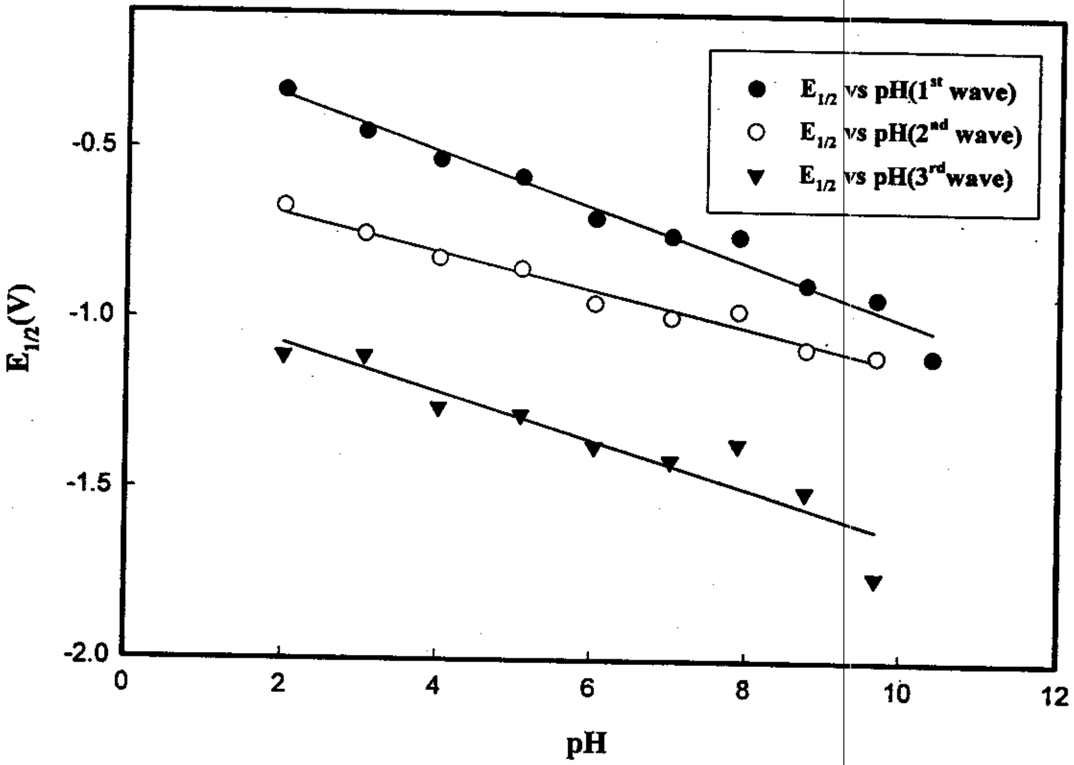


Fig. (74): $E_{1/2}$-pH plots of chlorodiazepoxide

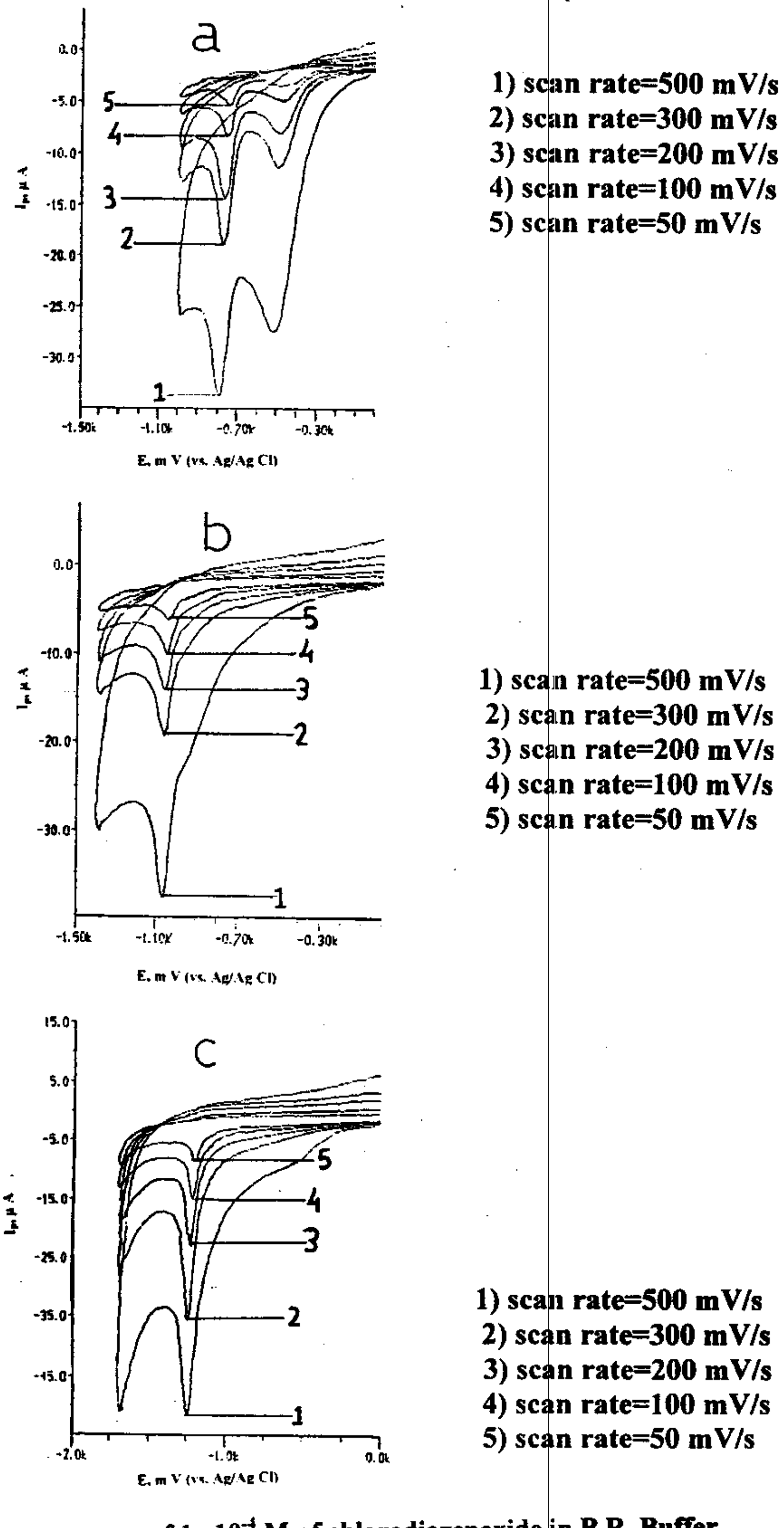


Fig. (75):Cyclic voltammograms of 1x 10^{-4} M of chlorodiazepoxide in B.R. Buffer solutions at different scan rates;(a) pH=2.15 , (b) pH=7.33 (c)pH=10.45.

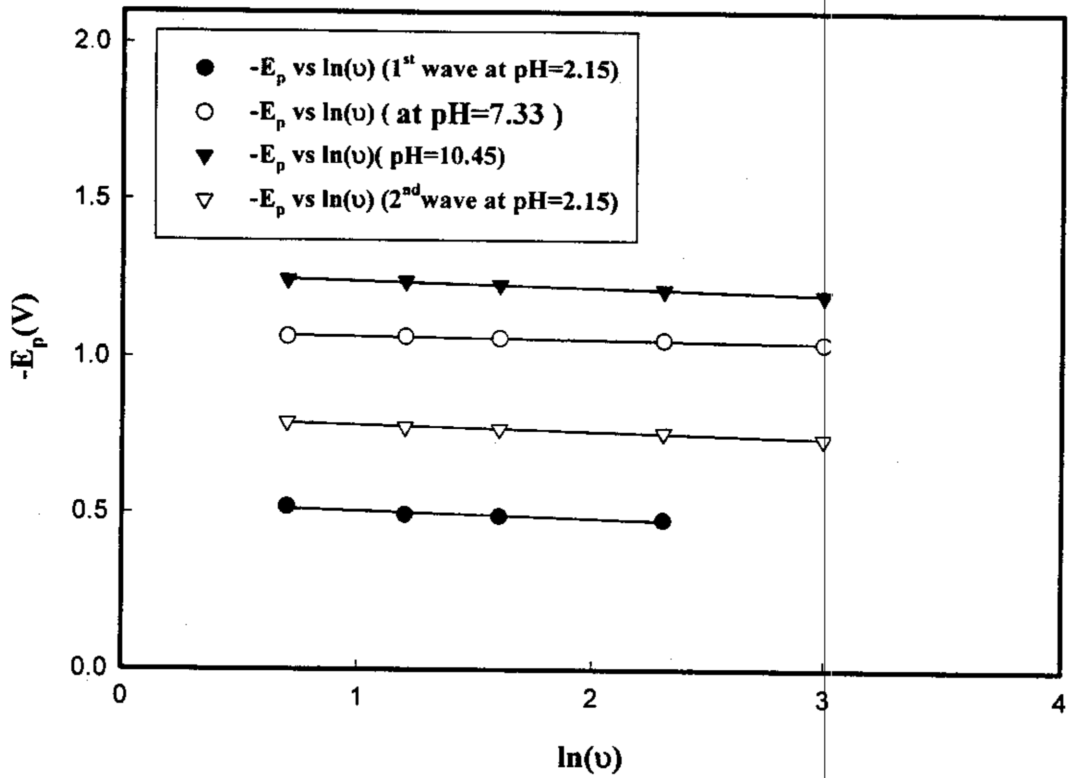


Fig. (76): E$_p$-ln(υ) plots of chlorodiazepoxide at different pH values.

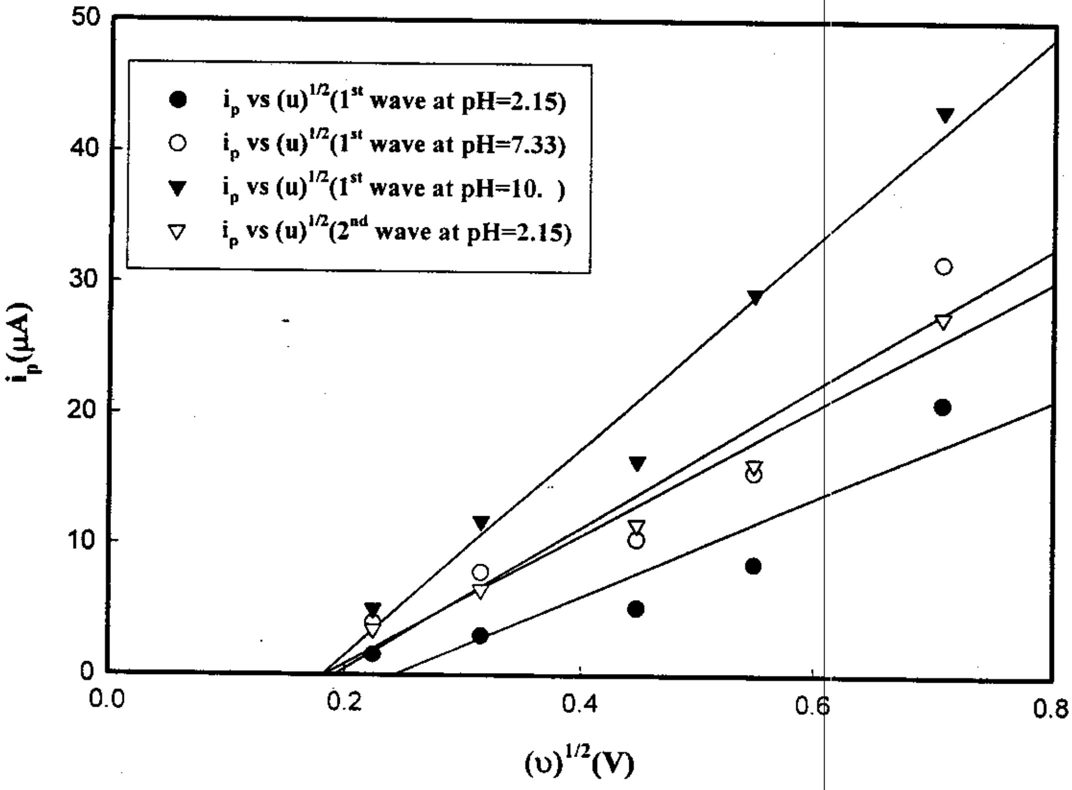


Fig. (77): i$_p$-(υ)$^{1/2}$ plots of chlorodiazepoxide at different pH values.

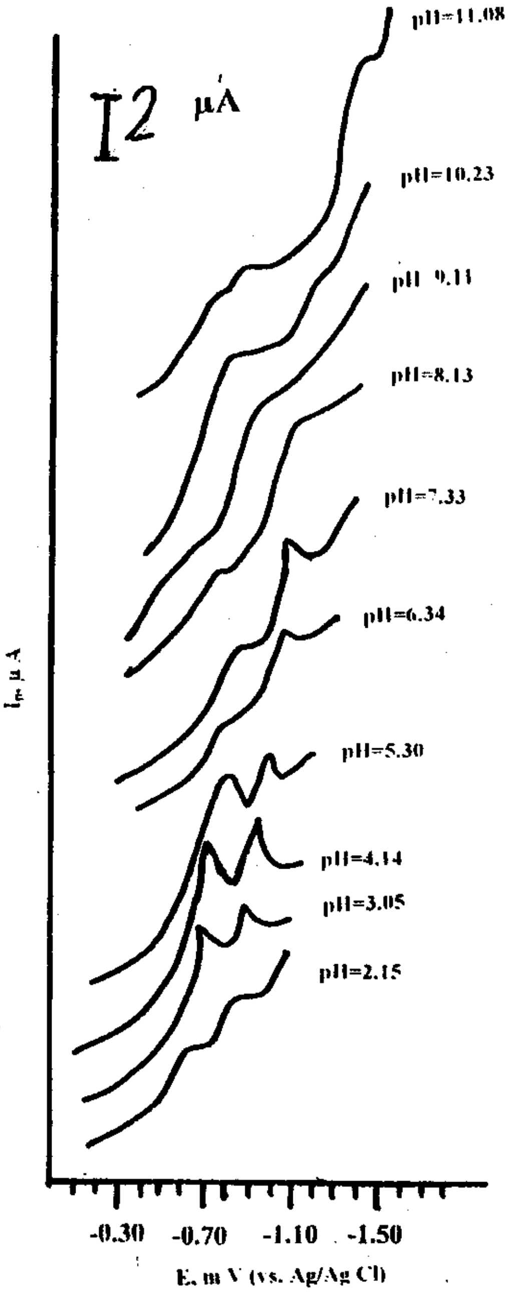


Fig. (78): **Effect of pH on the CAdS peak of 5×10^{-5} M of chlorodiazepoxide in B.R. Buffer solution at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.**

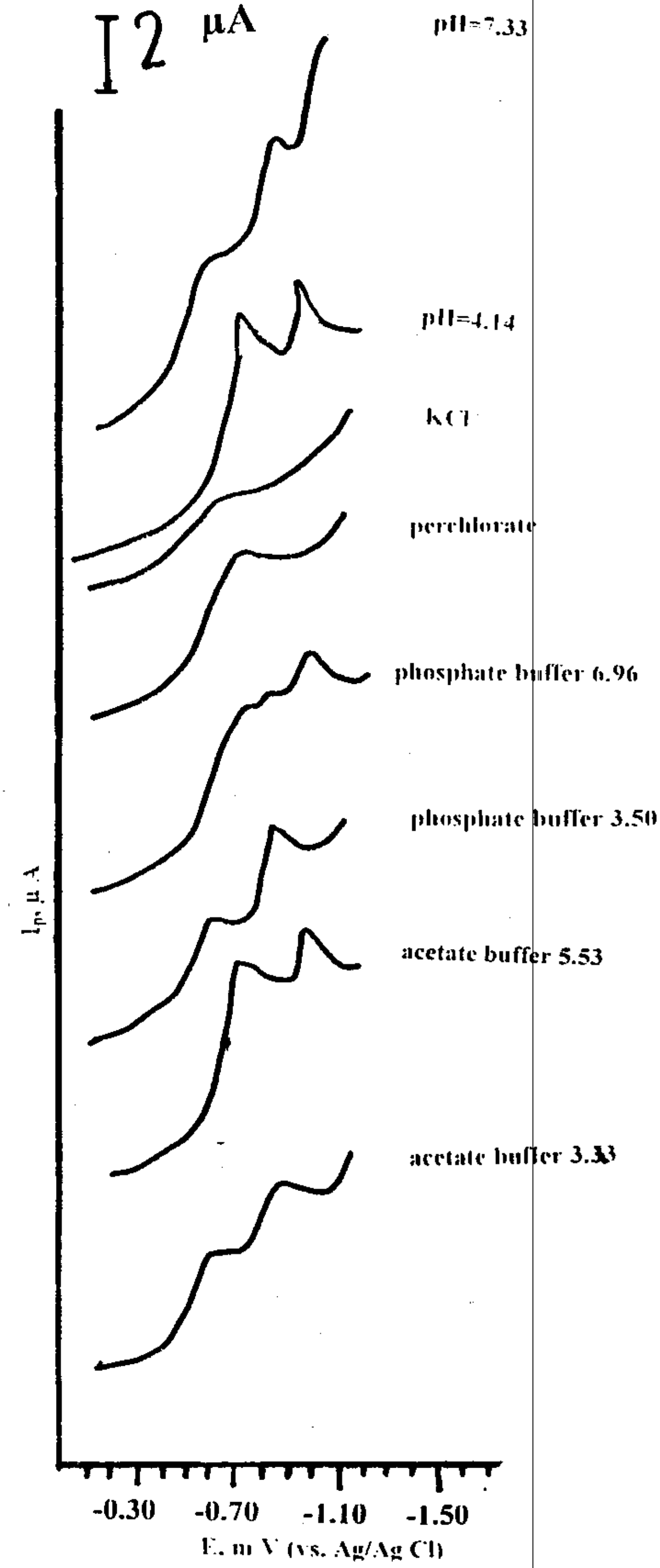


Fig. (79): Effect of different supporting electrolyte solution on the CAdS peak of $5x10^{-5}$ M of chlorodiazepoxide at t_d = 240 s, E_d =-0.4 V, scan rate =100 mV/s and step height = 5 mV.

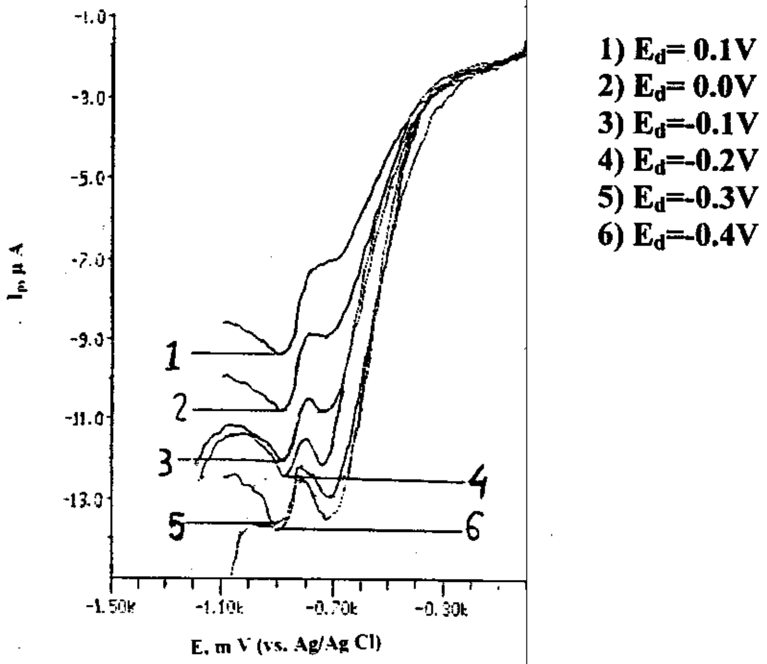


Fig. (80a):Effect of deposition potential on 5x10-5M of chlorodiazepoxide in B.R.buffer solution of pH=2.54 at td=240 s, scan rate=100mV/s,and pulse height=5mV.

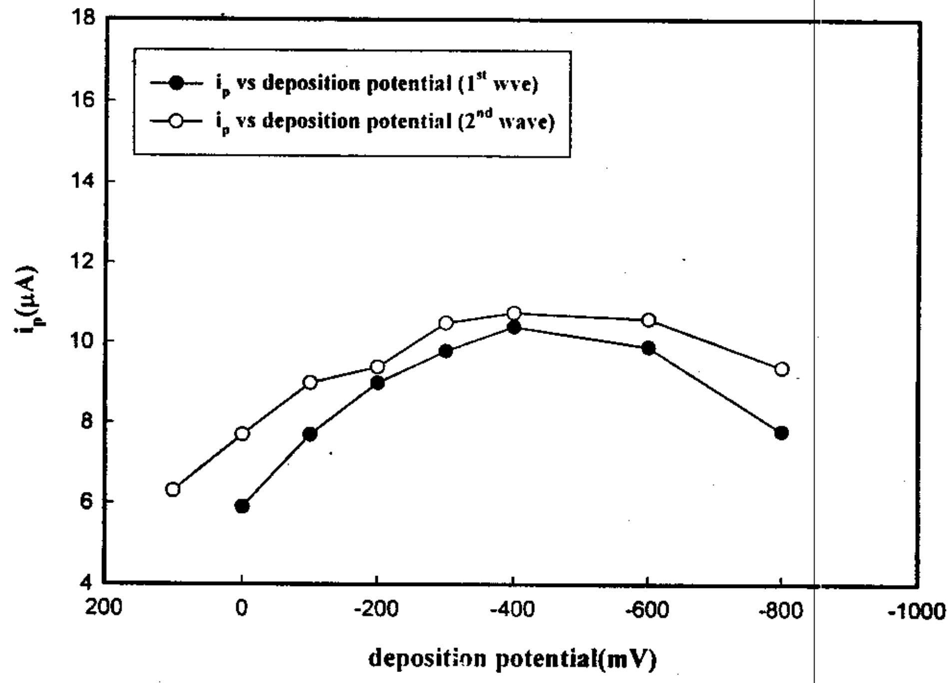


Fig.(80b): Effect of deposition potential of 5x10^{-5} M chlorodiazepoxide at pH=4.14 at: scan rate=100mV/s,t$_d$=240 sec.and step height=10 mV.

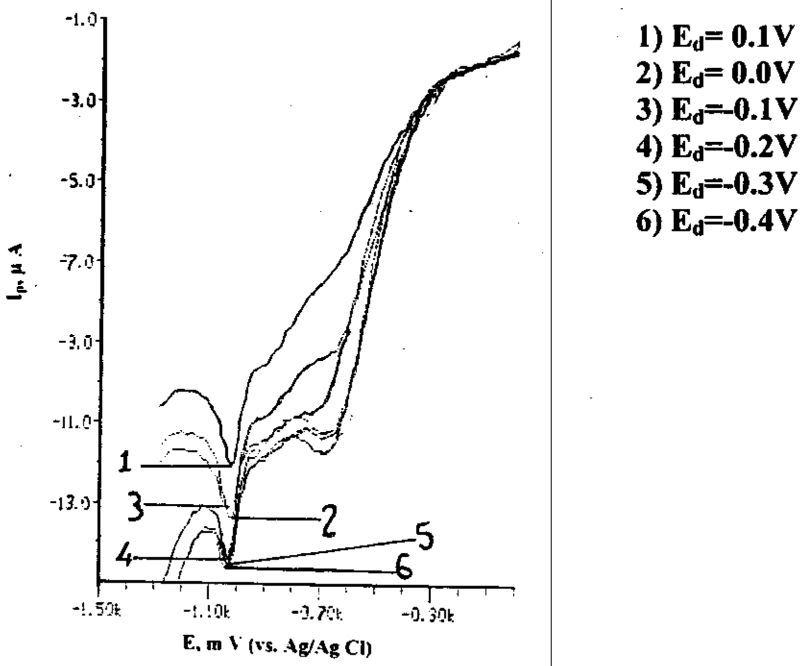


Fig. (81a): Effect of deposition potential on 5x10-5M of chlorodiazepoxide in B.R.buffer solution of pH=7.33 at td=240 s, scan rate=100mV/s,and pulse height=20mV.

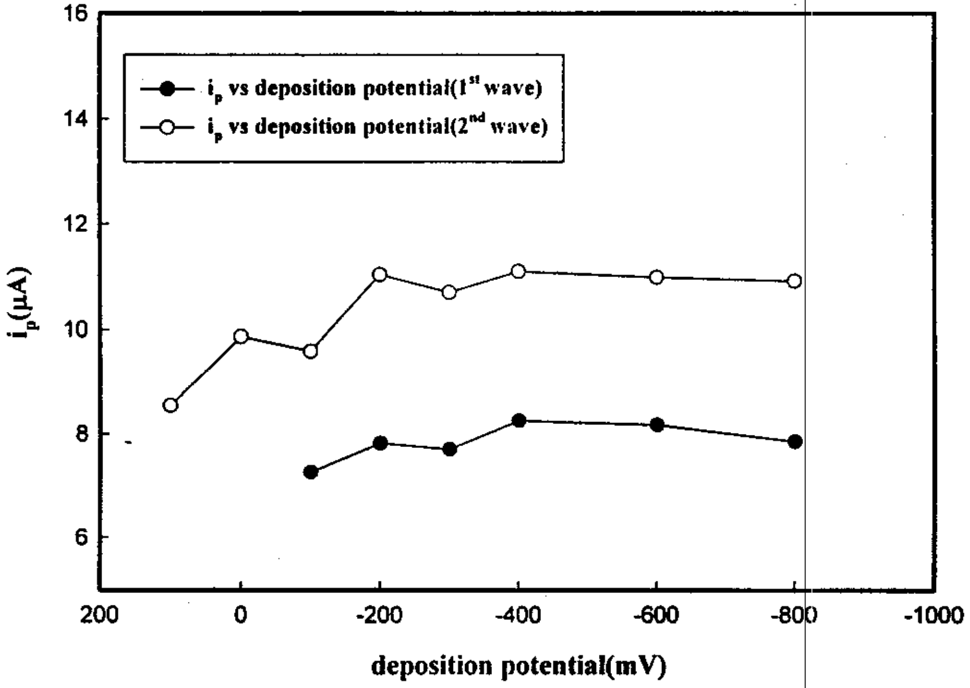


Fig. (81b): Effect of depositio potential of 5x10^{-5} M chlorodiazepoxide at pH=7.33 at: setp height=20mV,t$_d$=240 sec.nd scan rate=100 mV/s.

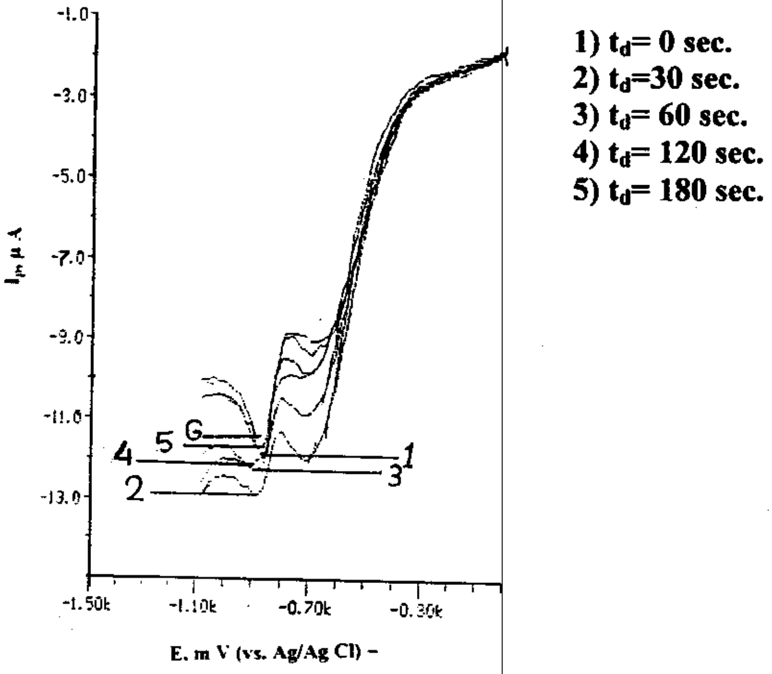


Fig. (82a): **Effect of deposition time on 5x10^{-5}M of chlorodiazepoxide in B.R.buffer Solution of pH=4.14 at scan rate=500mV/s s, E_d=0.0V,and pulse height=10 mV.**

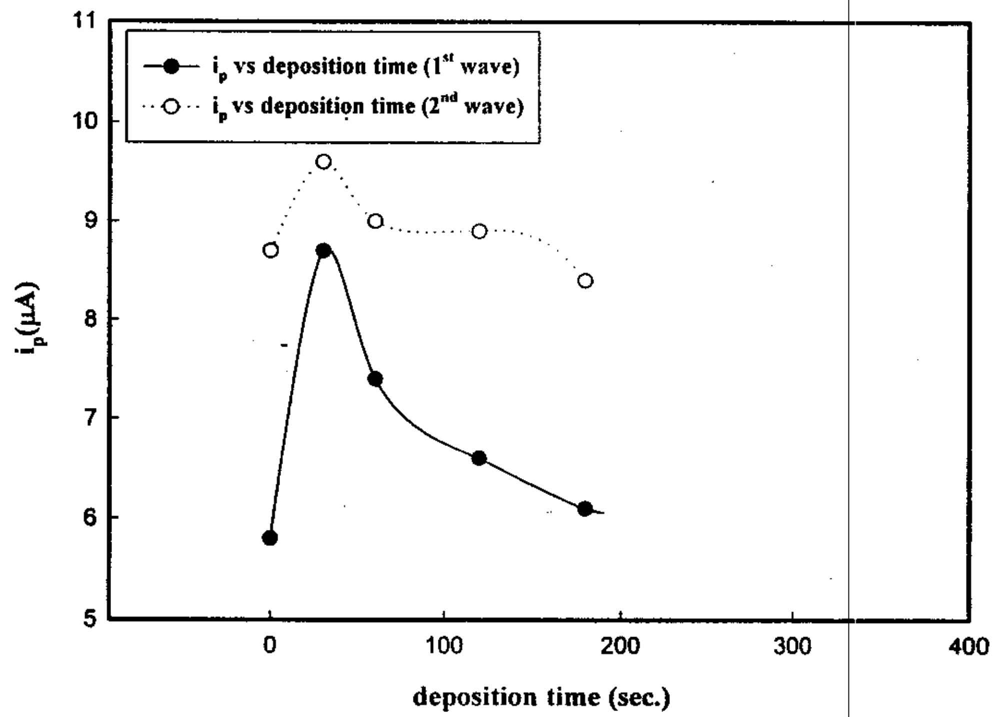
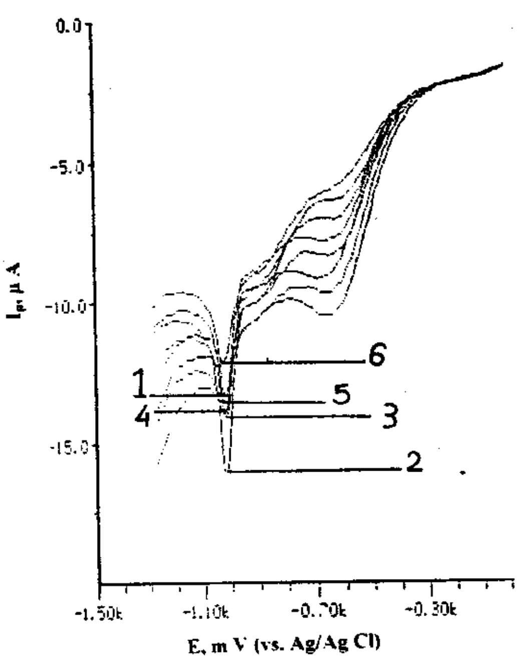


Fig. (82b): **Effect of deposition time of 5x10^{-5} M chlorodiazepoxide at pH=4.14 at: E_d= -400mV,scan rate=500 mV/s and step height=10 mV.**



1) t_d= 0 sec.
2) t_d=30 sec.
3) t_d= 60 sec.
4) t_d= 120 sec.
5) t_d= 180 sec.

Fig. (83a): Effect of deposition time on $5x10^{-5}$M of chlorodiazepoxide in B.R.buffer Solution of pH=7.33 at scan rate=500mV/s s, E_d=0.0V, and pulse height=10mV.

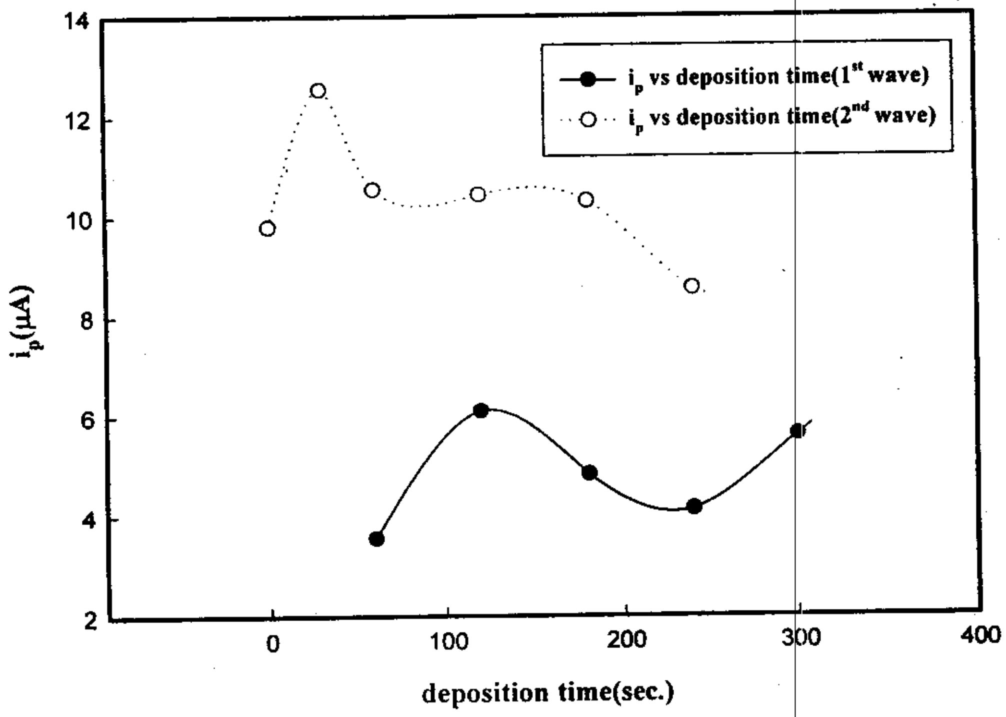


Fig.(83b):Effect of deposition time of $5x10^{-5}$ M chlorodiazepoxide at pH=7.33 at: setp height=20mV,E_d=-400 mV.and scan rate=500 mV/s.

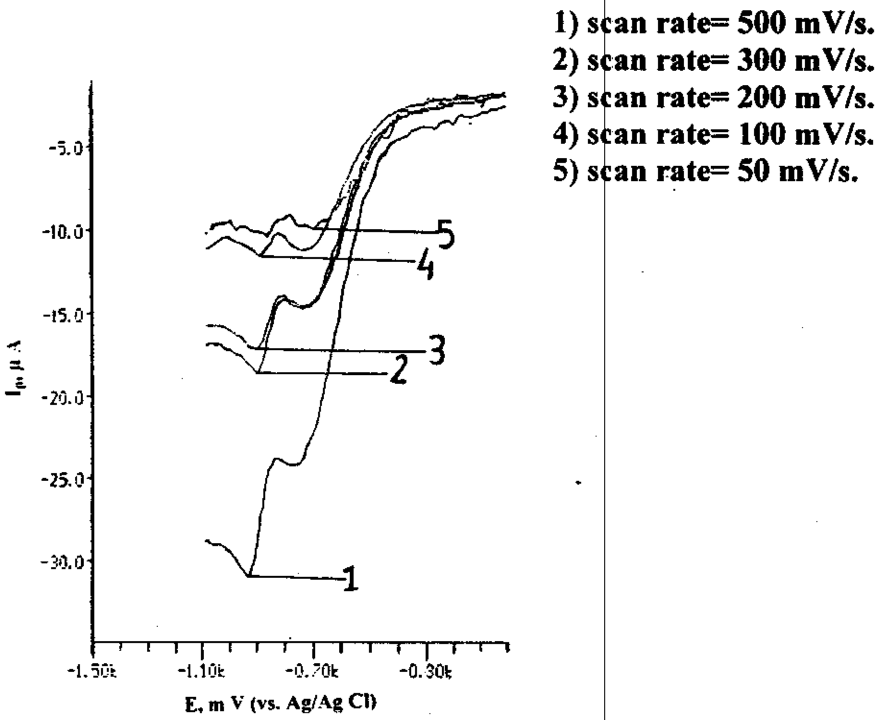


Fig. (84a): Effect of scan rate on 5x10^{-5}M of chlorodiazepoxide in B.R.buffer solution of pH=4.14 at E_d= -0.4V s, t_d=240 s,and pulse height=10 mV.

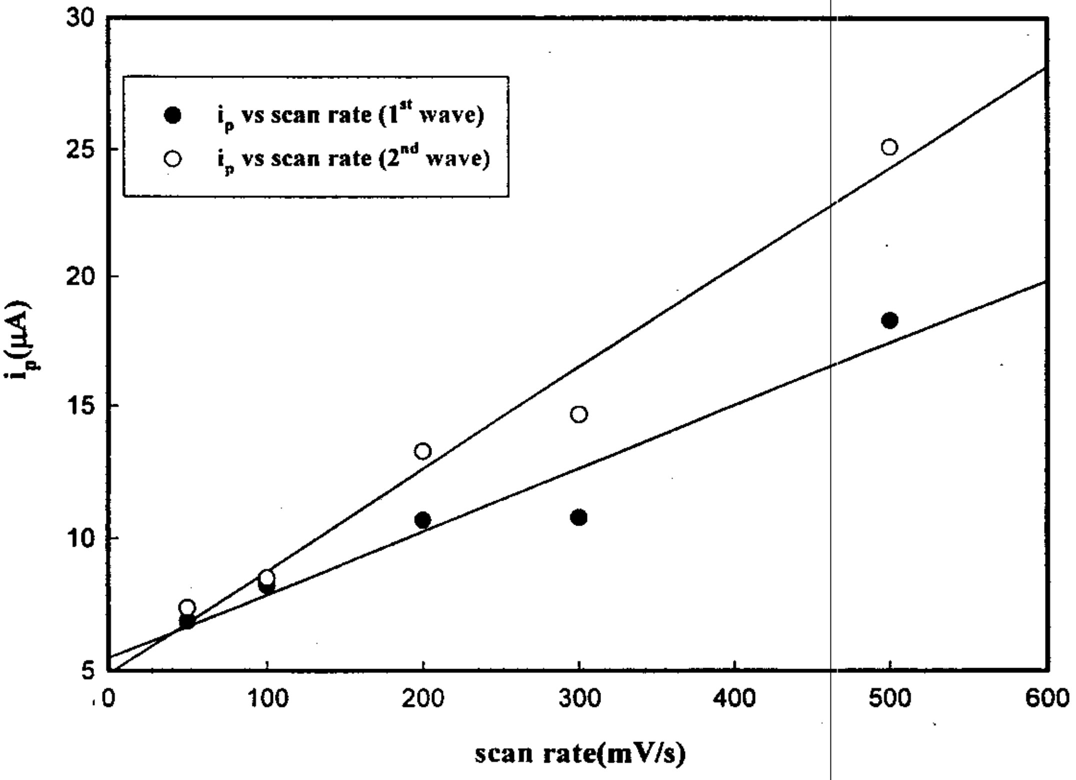


Fig. (84b): Effect of scan rate of 5x10^{-5} M chlorodiazepoxide at pH=4.14 at: E_d=-400mV,t_d=240 sec.nd step height=10 mV.

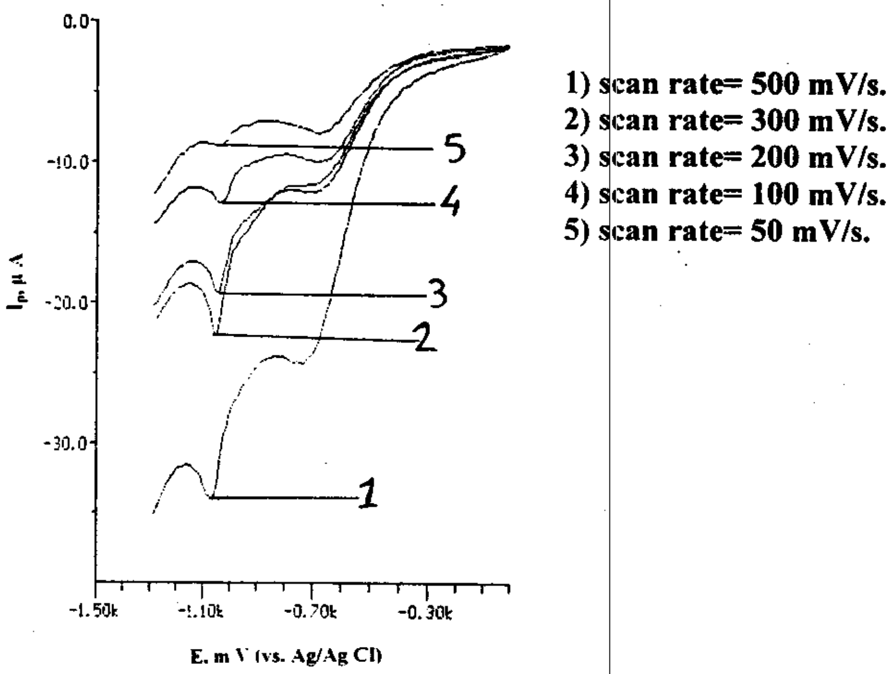


Fig. (85a): Effect of scan rate on $5x10^{-5}M$ of chlorodiazepoxide in B.R.buffer solution of pH=4.14 at E_d= -0.4V s, t_d=240 s,and pulse height=10 mV.

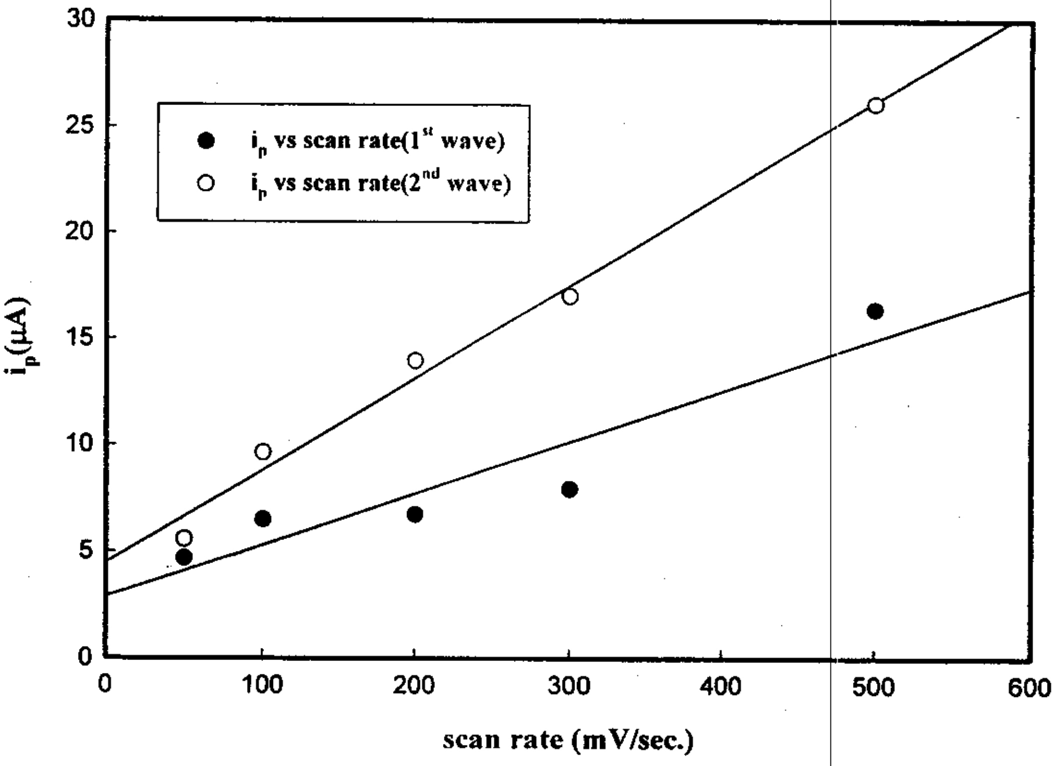


Fig. (85b): Effect of scan rate of $5x10^{-5}$ M chlorodiazepoxide at pH=7.33 at: E_d=-400mV,t_d=240 sec.nd step height=10 mV.

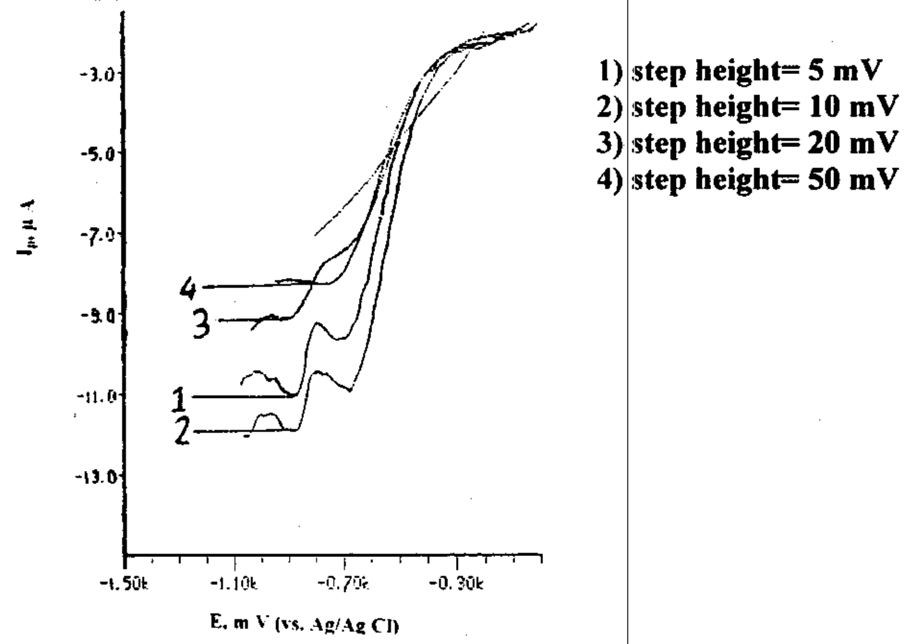


Fig. (86a): Effect of step height on 5x10^{-5}M of chlorodiazepoxide in B.R.buffer solution of pH=4.14 at E$_d$= -0.4V s, t$_d$=240 s and scan rate =100 mV/s.

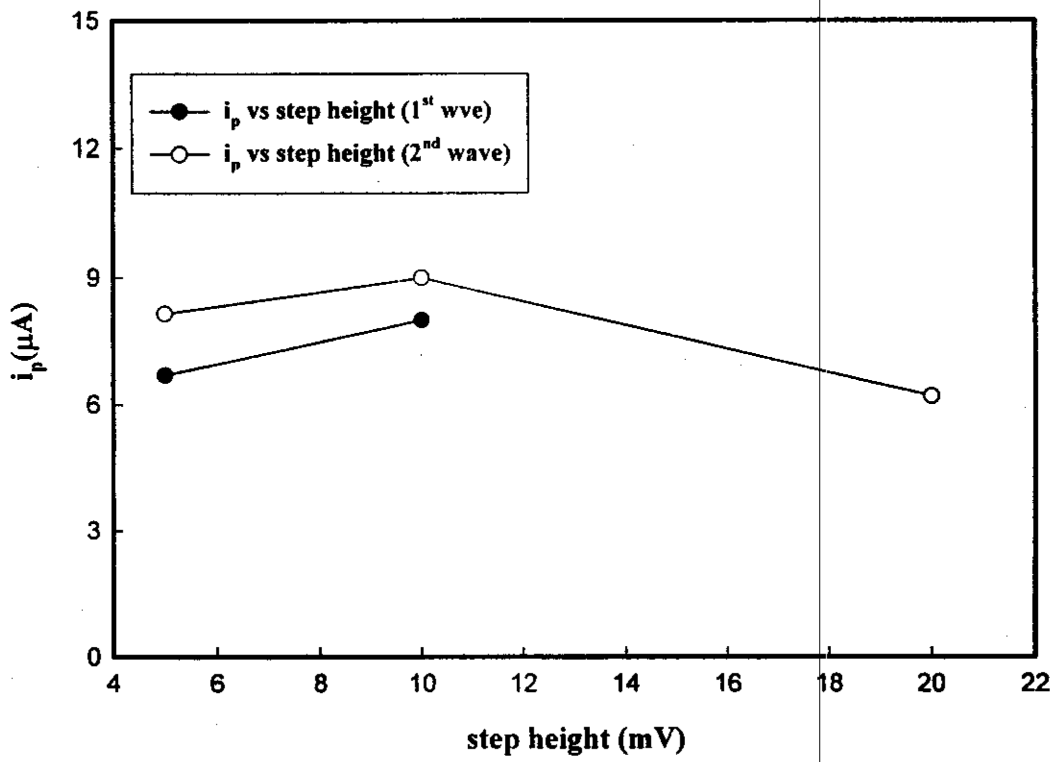


Fig.(86b):Effect of step height of 5x10^{-5} M chlorodiazepoxide at pH=4.14 at: E$_d$=-400mV,t$_d$=240 sec.) and scan rate =100 mV/s.

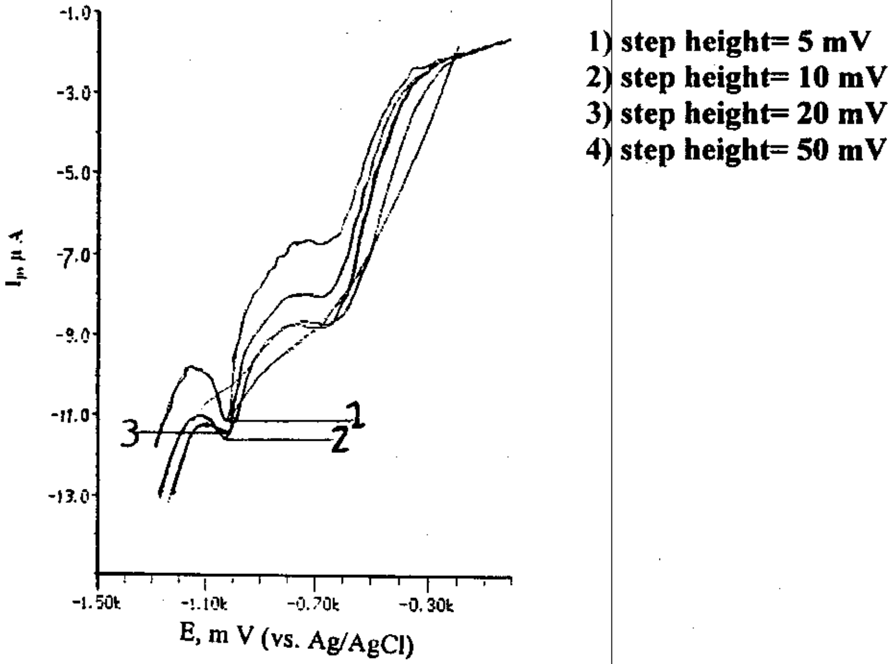


Fig. (87a): Effect of step height on $5x10^{-5}$M of chlorodiazepoxide in B.R.buffer solution of pH=7.33 at E_d= -0.4V s, t_d=240 s and scan rate =100 mV/s.

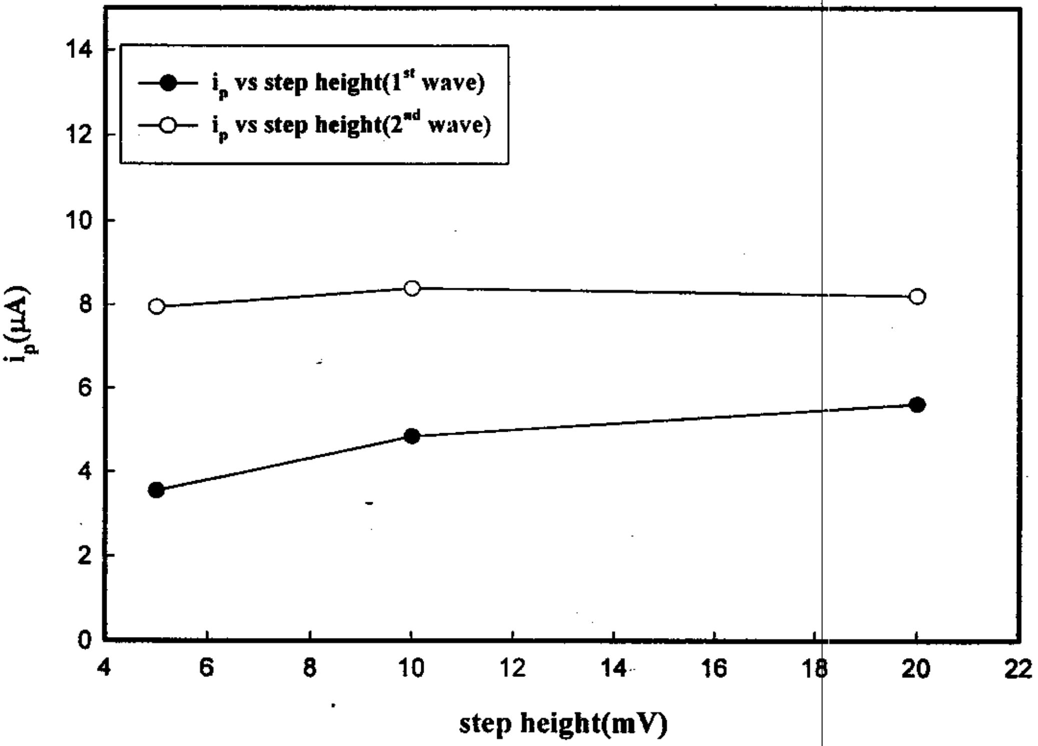


Fig. (87b): Effect of setp height of $5x10^{-5}$ M chlorodiazepoxide at pH=7.33 at: E_d=-400mV,t_d=240 sec.nd scan rate=100 mV/s.

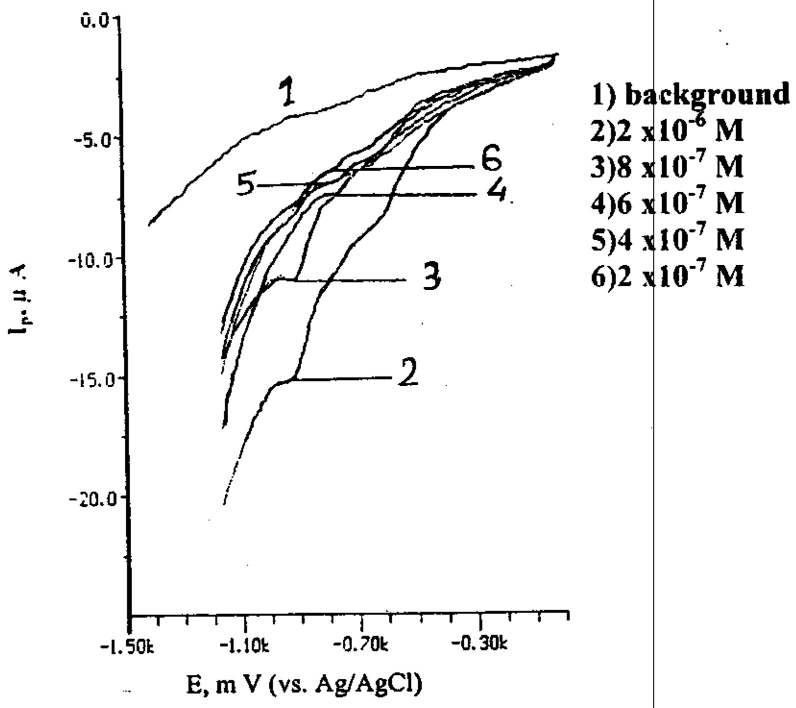


Fig. (88a): Effect of concentration of chlorodiazepoxide in B.R. buffer solution of pH=4.14 at: t_d=30 s, E_d= -0.4 V, scan rate 500 mV/s and step height=10 mV

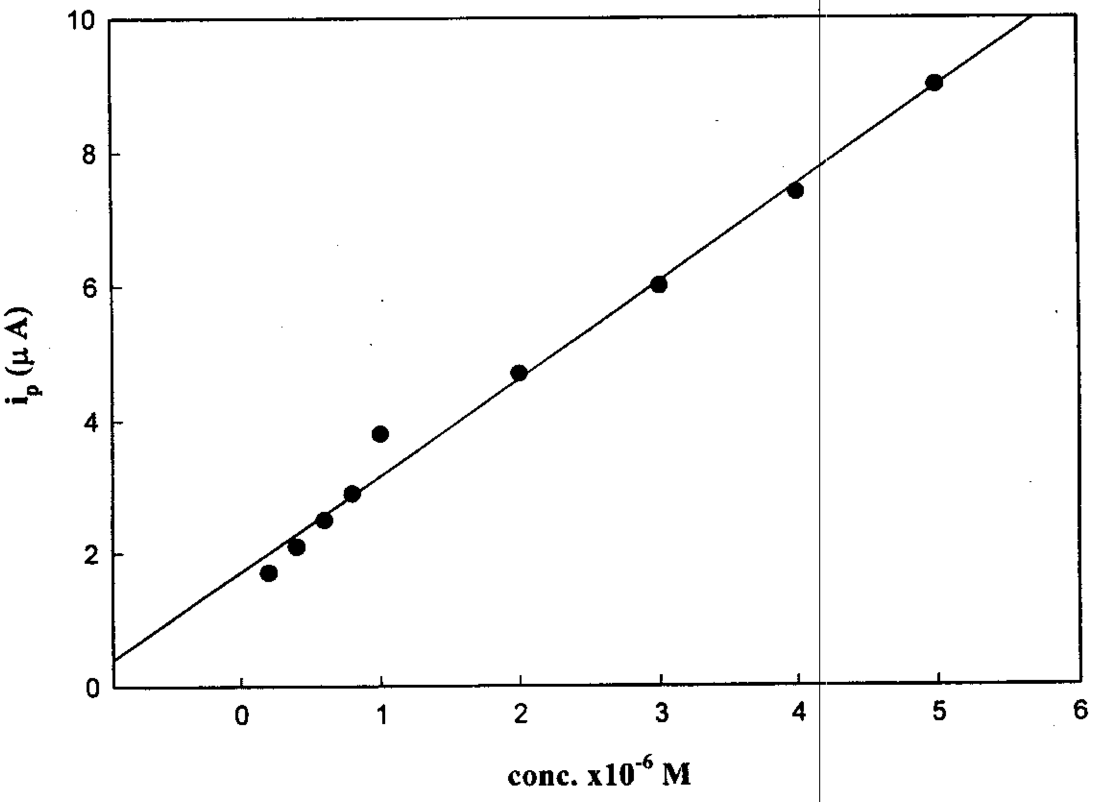


Fig. (88b): Calibration curve for chlorodiazepoxide (pH 4.14).

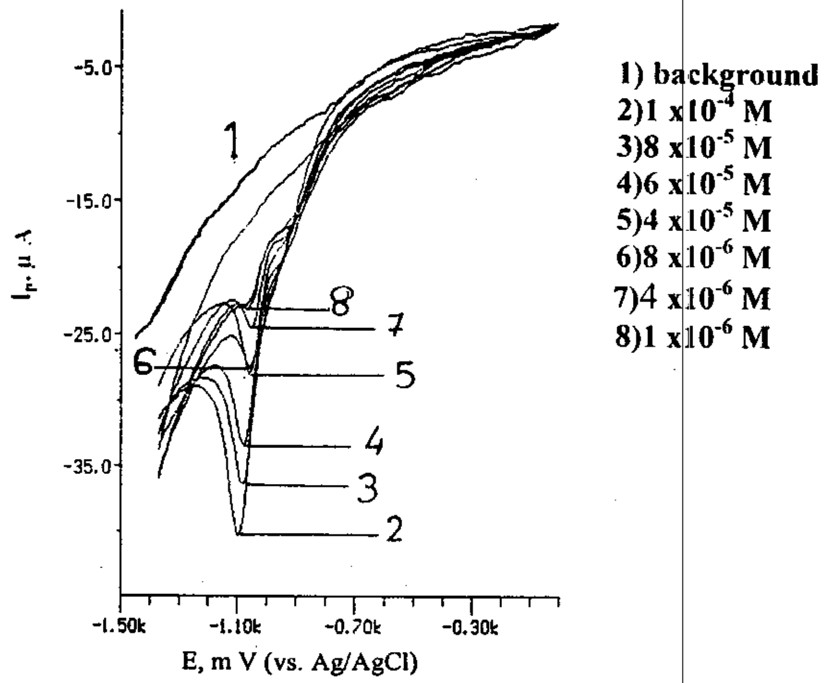


Fig. (89a): Effect of concentration of chlorodiazepoxide in B.R. buffer solution of pH=4.14 at: t_d=30 s, E_d= -0.4 V, scan rate 500 mV/s and step height=10 mV

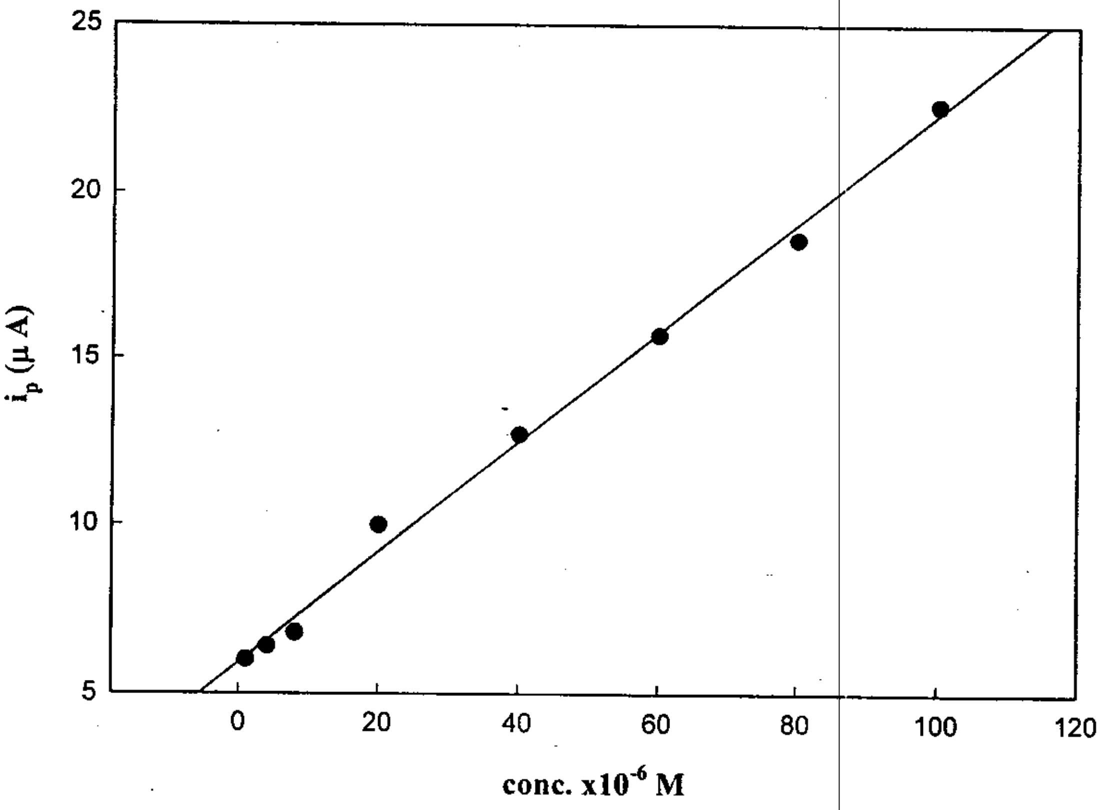


Fig. (89b): Calibration curve for chlorodiazepoxide (pH 7.18).

Table(44): Polarographic data for 1x10^{-4}M of chlorodiazepoxide inB. R. buffer solution of different pH values at 25 °C.

pH	i_d(μA)			-$E_{1/2}$			Δ logi_l/Δ logh		
	A	B	C	A	B		A	B	C
2.00	1.44	1.60	2.20	0.33	0.67	1.11	0.42	0.31	0.33
3.04	1.28	1.52	1.80	0.45	0.75	1.11			
4.01	1.20	1.48	1.76	0.53	0.82	1.26			
5.08	1.04	1.48	1.60	0.58	0.85	1. 28			
6.03	0.88	1.50	1.48	0.70	0.95	1.37			
7.01	0.76	1.60	1.36	0.75	0.99	1.41	0.67	0.50	0.46
7.88	0.72	1.56	1.44	0.75	0.99	1.47			
8.77	0.68	1.52	1.40	0.89	1.08	1.50			
9.68	0.64	1.52	sh.	0.93	1.10	1.75			
10.4	2.40	sh.	—	1.11	sh.	sh.	0.58	sh.	sh.

Where A:1st wave B: 2nd wave C: 3rd wave sh: ill-defined

Table(45a) :Values of (Z_H^+)the number of protons calculated from slpoe(S_2)of the $E_{1/2}$-pH plots and (S_1) for chlorodiazepoxide in B.R. buffer solutions of Different pH values at 25c^0

pH	S_1 mV			S_2 mV			(Z_H^+)=(S_2/S_1)		
	A	B	C	A	B	C	A	B	C
2.00	89.77	67.70	83.82	81.75	54.82	70.17	0.91	0.81	0.84
3.04	104.07	86.13	101.01	81.75	54.82	70.17	0.78	0.64	0.69
4.01	120.77	69.93	69.83	81.75	54.82	70.17	0.67	0.79	1.00
5.08	93.90	52.38	82.64	81.75	54.82	70.17	0.86	1.05	0.85
6.03	60.94	91.49	67.80	81.75	54.82	70.17	1.30	0.60	1.03
7.01	83.33	63.57	82.24	81.75	54.82	70.17	0.97	0.87	0.85
7.88	83.33	74.91	93.37	81.75	54.82	70.17	0.97	0.73	0.75
8.77	96.62	66.58	73.42	81.75	54.82	70.17	0.84	0.83	0.95
9.68	73.42	49.21	169.49	81.75	54.82	70.17	1.10	1.11	0.41
10.40	146.41	sh.	sh.	81.75	sh.	sh.	0.55	sh.	sh.

Where A:1st wave B:2nd wave C: 3rd wave sh: ill-defined

Table(45b):Values of (αna) and (α) for $1x10^{-4}$ M of chlorodiazepoxide in B.R buffer solution of different pH values, as calculated from reciprocal slope (S_1) of the log (i/i_d-i) -$E_{d.e}$ plots.

pH	Slope(S_1) mV			αna			α					
							n=1			n=2		
	A	B	C	A	B	C	A	B	C	A	B	C
2.00	89.77	67.70	83.82	0.66	0.87	0.70	0.66	0.87	0.70	0.33	0.44	0.35
3.04	104.1	86.13	101.0	0.57	0.68	0.58	0.57	0.68	0.58	0.29	0.34	0.29
4.01	120.8	69.93	69.83	0.49	0.84	0.84	0.49	0.84	0.84	0.25	0.42	0.42
5.08	93.90	52.38	82.64	0.63	1.10	0.72	0.63	1.10	0.72	0.32	0.55	0.36
6.03	60.94	91.49	67.80	0.97	0.88	0.87	0.97	0.88	0.87	0.49	0.44	0.44
7.01	83.33	63.57	82.24	0.71	0.92	0.72	0.71	0.92	0.72	0.36	0.46	0.36
7.88	83.33	74.91	93.37	0.71	0.79	0.63	0.71	0.79	0.63	0.36	0.40	0.32
8.77	96.62	66.58	73.42	0.61	0.89	0.80	0.61	0.89	0.80	0.31	0.45	0.40
9.68	73.42	49.21	169.5	0.80	1.19	0.35	0.80	1.19	0.35	0.40	0.60	0.18
10.40	146.4	sh.	sh.	0.40	sh.	sh.	0.40	sh.	sh.	0.20	sh.	sh.

Where A: 1st wave B: 2nd wave C: 3rd wave sh: ill-defined

Table(46):Data obtained for $1x10^{-4}M$ of chlorodiazepoxide in B.R. buffer Solution of different pH values using cyclic voltammetry at different Scan rates, at 25°

pH	Scan rate (mV/s)	-Ep (V)		-dEp/d(lnv)		αn_a	
		A	B	A	B	A	B
2.15	500	0.52	0.79	0.02	0.02	0.56	0.64
	300	0.50	0.78				
	200	0.49	0.77				
	100	0.48	0.76				
	50	0.47	0.74				
7.33	500	1.07		0.01		1.28	
	300	1.07					
	200	1.06					
	100	1.05					
	50	1.04					
10.45	500	1.25		0.02		0.64	
	300	1.24					
	200	1.23					
	100	1.22					
	50	1.20					

Where A: 1st peak B: 2nd peak

Table (47): Kinetic parameters obtained from D-C measurements for $1\text{x}10^{-4}$ M chlorodiazepoxide in B. R. buffer solution of different pH's.

pH	D^{o}(Cm^{2}/s)	$K^{o}_{f,h}$ (Cm/s)	ΔG* (K.cal/mole)
2.00	$1.30\ \text{x}10^{-5}$	$3.00\ \text{x}10^{-9}$ [a]	78.31 [a]
		$2.04\ \text{x}10^{-14}$ [b]	108.6 [b]
		$7.76\ \text{x}10^{-15}$ [c]	111.0 [c]
3.04	$1.30\ \text{x}10^{-5}$	$1.00\ \text{x}10^{-9}$ [a]	80.45 [a]
		$2.60\ \text{x}10^{-16}$ [b]	119.5 [b]
		$2.60\ \text{x}10^{-19}$ [c]	136.9 [c]
4.01	$1.30\ \text{x}10^{-5}$	$2.00\ \text{x}10^{-10}$ [a]	85.50 [a]
		$4.27\ \text{x}10^{-18}$ [b]	129.8 [b]
		$3.07\ \text{x}10^{-21}$ [c]	148.0 [c]
5.08	$7.20\ \text{x}10^{-6}$	$1.05\ \text{x}10^{-11}$ [a]	92.89 [a]
		$8.90\ \text{x}10^{-19}$ [b]	133.8 [b]
		$2.01\ \text{x}10^{-21}$ [c]	149.1 [c]
6.03	$7.20\ \text{x}10^{-6}$	$2.04\ \text{x}10^{-14}$ [a]	108.6 [a]
		$9.33\ \text{x}10^{-21}$ [b]	145.2 [b]
		$6.06\ \text{x}10^{-23}$ [c]	157.8 [c]
7.01	$1.50\ \text{x}10^{-6}$	$1.51\ \text{x}10^{-14}$ [a]	109.3 [a]
		$3.47\ \text{x}10^{-22}$ [b]	153.5 [b]
		$2.00\ \text{x}10^{-24}$ [c]	166.4 [c]
7.88	$1.50\ \text{x}10^{-6}$	$1.00\ \text{x}10^{-16}$ [a]	121.9 [a]
		$3.00\ \text{x}10^{-23}$ [b]	159.6 [b]
		$1.38\ \text{x}10^{-24}$ [c]	167.3 [c]
8.77	$1.40\ \text{x}10^{-6}$	$1.00\ \text{x}10^{-18}$ [a]	133.5 [a]
		$1.12\ \text{x}10^{-24}$ [b]	167.9 [b]
		$1.05\ \text{x}10^{-26}$ [c]	179.6 [c]
9.68	$1.40\ \text{x}10^{-6}$	$5.00\ \text{x}10^{-19}$ [a]	135.2 [a]
		$2.\ 05\ \text{x}10^{-30}$ [b]	200.5 [b]
		$9.05\ \text{x}10^{-27}$ [c]	180.0 [c]
10.40	$1.40\ \text{x}10^{-6}$	$2.82\ \text{x}10^{-21}$	691.9 [a]

Where a : 1st wave , b : 2nd wave , c: 3rd wave.

Table(48) : Cathodic adsorptive stripping peak current(i_p)of $5x10^{-5}$M chlorodizepoxide in B.R.buffer solution of pH=4.05at different conditions (t_d, E_d, scan rate and pulse height).

Deposition time (t_d), sec	Deposition potential(E_d), V	Scan rate (mV/s)	Pulse hieght (mV)	i_p (µA)	
Effect of (td):				A	B
0	-0.4	500	10	5.80	8.70
30				8.70	9.60
60				7.40	9.00
120				6.60	8.90
180				6.10	8.40
240				6.50	7.10
300				6.10	6.90
360				5.5	6.7
Effect of (E_d):					
240	0.1	100	5		6.30
	-0.01			5.90	7.70
	-0.1			7.70	9.00
	-0.2			9.00	9.40
	-0.3			9.80	10.5
	-0.4			10.4	10.7
Effect of scan rate:					
240	-0.4	500	5	18.3	25.1
		300		10.8	14.7
		200		10.7	13.3
		100		8.23	8.52
		50		6.90	7.40
Effect of pulse height:					
240	-0.4	500	5	6.70	8.15
			10	8.00	9.00
			20		6.20
			50		
			100		
			150		
			200		

Table(49) : Cathodic adsorptive stripping peak current(i_p)of $5x10^{-5}$M chlorodiazepoxide in B.R.buffer solution of pH=7.33 at different conditions (t_d, E_d, scan rate and pulse height).

Deposition time (t_d), sec	Deposition potential(E_d), V	Scan rate (mV/s)	Pulse hieght (mV)	i_p (μA)	
Effect of (td):				A	B
0	-0.4	500	10		9.80
30					12.6
60				3.55	10.6
120				6.10	10.4
180				4.83	10.3
240				4.13	8.56
300				3.62	6.71
360				2.94	5.99
Effect of (E_d):					
240	0.1	100	5		8.55
	-0.01				9.87
	-0.1			7.26	9.59
	-0.2			7.82	11.0
	-0.3			7.70	10.7
	-0.4			8.25	11.1
Effect of scan rate:					
240	-0.4	500	5	16.4	26.1
		300		6.49	17.0
		200		6.94	14.0
		100		6.73	9.64
		50		4.68	5.57
Effect of pulse height:					
240	-0.4	500	5	3.55	7.94
			10	4.84	8.39
			20	5.62	8.21
			50		
			100		
			150		
			200		

Table(50) : Calibration curve data of chlorodiazepoxide in B.R.buffer solution of pH=4.04 using CAdSV at t_d= 30sec., E_d= -0.4 V, scan rate = 500mV/s, and step height= 10 mV.

Conc. taken	Ip(μA)	Mean	S.D.	R.S.D. %
$2x10^{-7}$M	1.73 1.69 1.71 1.71	1.71	0.016	0.95
$4x10^{-7}$M	2.08 2.10 2.08 2.14	2.10	0.028	1.33
$6x10^{-7}$M	2.53 2.48 2.43 2.51	2.49	0.029	1.16
$8x10^{-7}$M	2.93 2.88 2.95 2.84	2.90	0.037	1.29
$1x10^{-6}$M	3.79 3.84 3.72 3.97	3.78	0.059	1.57
$2x10^{-6}$M	4.63 4.69 4.74 4.72	4.70	0.048	1.03
$3x10^{-6}$M	6.08 6.08 5.93 5.91	6.00	0.093	1.54
$4x10^{-6}$M	7.52 7.46 7.38 7.36	7.43	0.074	0.99
$5x10^{-6}$M	9.10 9.04 8.95 8.92	9.00	0.082	0.92

Table(51) : Calibration curve data of chlorodiazepoxide in B.R.buffer solution of pH=7.33 using CAdSV at t_d= 30sec., E_d= -0.4 V, scan rate= 500mV/s, and step height= 10 mV.

Conc. taken	**Ip(μA)**	**Mean**	**S.D.**	**R.S.D. %**
1x10⁻⁶M	**6.08** **6.01** **5.94** **5.94**	**5.99**	**0.067**	**1.12**
2x10⁻⁶M	**6.17** **6.10** **6.26** **6.22**	**6.19**	**0.069**	**1.11**
4x10⁻⁶M	**6.42** **6.51** **6.32** **6.36**	**6.40**	**0.082**	**1.28**
6x10⁻⁶M	**6.67** **6.63** **6.52** **6.56**	**6.60**	**0.068**	**1.03**
8x10⁻⁶M	**6.90** **6.83** **6.76** **6.72**	**6.80**	**0.079**	**1.16**
1x10⁻⁵M	**6.93** **6.98** **6.98** **7.14**	**7.01**	**0.091**	**1.29**
2x10⁻⁵M	**10.12** **9.93** **9.97** **10.08**	**10.02**	**0.090**	**0.89**

Table(51) : Continued.

Conc. taken	Ip(μA)	Mean	S.D.	R.S.D. %
$4x10^{-5}$M	**12.64** **12.76** **12.72** **12.68**	**12.70**	**0.052**	**0.41**
$6x10^{-5}$M	**15.77** **15.72** **15.70** **15.61**	**15.70**	**0.067**	**0.43**
$8x10^{-5}$M	**18.58** **18.71** **18.62** **18.54**	**18.61**	**0.073**	**0.39**
$1x10^{-4}$M	**22.50** **22.56** **22.69** **22.65**	**22.60**	**0.086**	**0.38**

Table(52) : Assay of chlorodiazepoxide in dosage form (cloxide) in B.R. buffer solution at pH=4.04 using CAdSV at t_d= 30sec., E_d= -0.4 V, scan rate= 500mV/s, and step height= 10 mV.

Conc. taken	Conc. calculated	Recovery %	Mean Recovery(%) ± S.D
1x10^{-5}M	1.01 x10^{-5}M	101.0	101.5 ±2.58
	0.99 x10^{-5}M	99.00	
	1.03 x10^{-5}M	103.0	
	1.03 x10^{-5}M	105.0	
2x10^{-5}M	2.05 x10^{-5}M	102.0	101.4 ±2.29
	2.06 x10^{-5}M	103.0	
	1.99 x10^{-5}M	99.50	
	2.01 x10^{-5}M	100.5	
4x10^{-5}M	4.09 x10^{-5}M	102.3	101.3 ±1.72
	4.05 x10^{-5}M	101.3	
	4.06 x10^{-5}M	101.5	
	4.00 x10^{-5}M	100.0	
6x10^{-5}M	6.03 x10^{-5}M	100.5	100.3 ±0.65
	6.00 x10^{-5}M	100.0	
	6.06 x10^{-5}M	101.0	
	5.99 x10^{-5}M	99.83	
8x10^{-5}M	8.22 x10^{-5}M	102.8	101.8 ±2.38
	8.22 x10^{-5}M	102.8	
	8.10 x10^{-5}M	101.3	
	8.05 x10^{-5}M	100.6	
1x10^{-4}M	0.98 x10^{-5}M	98.00	99.25 ±1.29
	0.99 x10^{-5}M	99.00	
	1.00 x10^{-5}M	100.0	
	1.00 x10^{-5}M	100.0	

Table(53) : Assay of chlorodiazepoxide in dosage form (cloxide) in B.R. buffer solution at pH=7.33 using CAdSV at t_d= 30sec., E_d= -0.4 V, scan rate= 500mV/s, and step height= 10 mV.

Conc. taken	Conc. calculated	Recovery %	Mean Recovery(%) ± S.D
$1x10^{-5}M$	$1.03\ x10^{-5}M$	103.0	102.0 ±2.70
	$1.03\ x10^{-5}M$	103.0	
	$1.02\ x10^{-5}M$	102.0	
	$1.00\ x10^{-5}M$	100.0	
$2x10^{-5}M$	$2.06\ x10^{-5}M$	103.5	101.08 ±2.38
	$2.04\ x10^{-5}M$	102.0	
	$2.00\ x10^{-5}M$	100.0	
	$2.04\ x10^{-5}M$	102.0	
$4x10^{-5}M$	$4.10\ x10^{-5}M$	102.5	101.2 ±1.52
	$3.99\ x10^{-5}M$	99.75	
	$4.01\ x10^{-5}M$	100.3	
	$4.09\ x10^{-5}M$	102.3	
$6x10^{-5}M$	$6.20\ x10^{-5}M$	103.3	101.5 ±2.20
	$6.10\ x10^{-5}M$	101.7	
	$6.00\ x10^{-5}M$	100.0	
	$6.05\ x10^{-5}M$	100.8	
$8x10^{-5}M$	$8.00\ x10^{-5}M$	100.0	99.99 ±0.90
	$7.90\ x10^{-5}M$	98.75	
	$8.03\ x10^{-5}M$	100.4	
	$8.07\ x10^{-5}M$	100.9	
$1x10^{-4}M$	$1.01\ x10^{-5}M$	101.0	100.5 ±0.81
	$1.00\ x10^{-5}M$	100.0	
	$1.01\ x10^{-5}M$	101.0	
	$1.00\ x10^{-5}M$	100.0	

Table(54): Assay of chlorodiazepoxide in serum in B.R.buffer solution of pH=4.14 using CAdSV at td= 30 sec., Ed= -0.4 V, scan rate=500mV/s, and step height= 10 mV.

Conc. taken (µg/10 ml)	**Conc. Calculated (µg/10 ml)**	**Recovery %**	**Mean Recovery(%) ± S.D**
6.72	**5.86**	**87.25**	**100.1 ±12.76**
	7.90	**117.0**	
	6.44	**95.92**	
	6.69	**99.54**	
20.2	**24.87**	**123.4**	**100.0 ±13.82**
	19.62	**97.32**	
	20.04	**99.43**	
	16.11	**79.95**	