Electrochemical Studies on 3-methyl-4-(4'-nitroarylhydrazono)-2-isoxazolin-5-one

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Polarographic and cyclic voltammetric reduction of 3-methyl-4-(4'-nitroarylhydrazono)-2-isoxazolin-5-one were carried out in 50% (V/V) dimethylformamide containing buffer solutions of pH 1.10 - 10.10. The compound exhibits two polarographic waves in the pH range 1.10 - 5.60 and three waves in the pH range 8.30 - 10.10. Cyclic voltammetric results are similar. 3-methyl-4-amino-2-isoxazolin-5-one was isolated as one of the products after controlled potential electrolysis of the compound at pH 4.60. Based on the results obtained, suitable mechanisms are suggested for the reduction of the title compound in various media.

Isoxazoles and isoxazolines possess antituberculous, antifungal, antibacterial and hypoglycemic properties [1-2]. These properties are generally due to their redox behaviour. Because of the close resemblence that exists between the biological and electrochemical reduction processes, the study of electrochemical reduction of the title compound is of interest. A survey of the literature [3-5] reveals that the redox behaviour has been influenced considerably by the constituents of buffer solution. Polarographic investigations [3] on some 4-arylhydrazono-2-isoxazolin-5-ones in different buffers (Clark and Lubs buffers of pH 2.0, 5.8, 7.0, Walpole buffers of pH 3.6, 4.4 and Britton-Robinson buffers of pH 9.0, 10.6) in 40% methanolic solution reveal an increase in half-wave potential in the entire pH range studied. However, earlier studies on the electrochemical behaviour of 3-methyl-4-(substituted benzeneazo)-2-isoxazolin-5-ones in Britton-Robinson buffer solutions of pH 2.1 - 10.1 in 50% (V/V) dimethylformamide [4,5] indicate an increase in the half-wave potential only upto pH 7.1 and constant thereafter. Our earlier studies showed the reduction of the hydrazone group in acidic and neutral media and azomethine anion reduction in alkaline solutions; but Goyal's results [3] show reduction of hydrazono group in the entire pH range. The medium of study probably influences redox pathway of a system. Further a possibility always exists that any given buffer may exert undesirable effects in the biochemical or biological system being studied. So, it is

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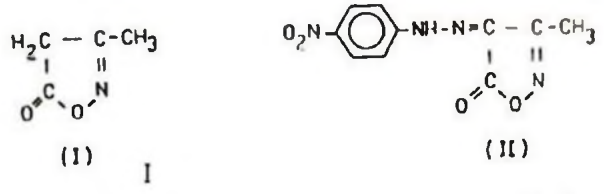
desirable to carry out replicate studies using structurally different types of buffers [6]. Therefore, a study of electrochemical behaviour of the compound under varied experimental conditions is a necessity rather than a luxury. With this in view, we are presenting the results of the polarographic and cyclic voltammetric reduction of 3-methyl-4-(4'-nitro arylhydrazono)-2-isoxazolin-5-one in Bower and Bates (HCl, KCl; pH 1.10 and 2.10), Gomori (Sodium acetate, acetic acid; pH 3.60-5.60) and Gottschalk (Ammonia, ammonium chloride; pH 8.30 - 10.10) buffer solutions.

EXPERIMENTAL

The title compound was prepared by the method given in the literature [7]. p-Nitroaniline (0.01 mol) was diazotized, the diazonium salt was filtered into a cooled solution of sodium acetate (5g) and acetoacetic ester (0.01 mol) in ethanol (25 ml). The resulting solid, 2-(4'-nitro arylhydrazono)butyrate-1,3-dione, was filtered, washed with water and dried. It was recrystallised from ethanol. A mixture of 2-(4'-nitro arylhydrazono) butyrate-1,3-dione (0.005 mole) in ethanol (20 ml) and hydroxylamine hydrochloride (0.005 mole) in water (5 ml) & sodium acetate (1.0 g) was refluxed for 4 hours. Shining orange yellow crystals separated on cooling and was recrystallised from ethanol. m.p. 212°C. Purified compound was identified by spectral studies.

IR spectra (KBr cm^{-1}) 3310 (>NH stretch), 1720 (>C=O cyclic), 1520 (-NH-N=C<), 1460 and 1560 (five membered heterocyclic ring), 750 (substituted phenyl);

pmr spectra (DMSO-d6) δ = 2.35 (3H, s, CH_3), 7.1-7.60 (4H, m, substituted phenyl), 9.5 (1H, s, -NH-N=C<).



(I) I (II)

3-methyl-2-isoxazolin-5-one

II

3-methyl-4-(4'-nitroarylhydrazono)-2-isoxazolin-5-one

Stock solution (1.0×10^{-2} M) of the compound was prepared in AR grade N,N'-dimethylformamide. The buffer solutions employed in the present investigations were prepared by the method given in the literature [6]. The experimental solutions for polarographic and cyclic voltammetric studies were prepared by mixing 1 ml of the stock solution of the compound, 4 ml DMF and 5 ml of buffer solution of desired pH. The pH of the solutions was checked with ELICO pH meter. The dissolved oxygen in the test solution was removed by passing pure nitrogen gas for about 15 minutes.

Polarographic current-voltage curves were recorded with ELICO CL-25 polarograph. A saturated calomel electrode and a

platinum foil were used as reference and counter electrodes respectively. The capillary characteristics: $m^{2/3}.t^{1/6}$ = 1.80 $mg^{2/3}$ $s^{1/2}$ at h = 80 cm, open circuit in water.

The cyclic voltammeter consisted of a PAR 173 potentiostat, PAR 175 universal programmer and a RE 0074 X-Y recorder. A single compartment cell (model 303 SMDE) supplied by PAR - with silver wire as reference electrode and platinum wire as counter electrode - was used. A hanging mercury drop electrode (SMDE 303) with drop area of 0.0096 cm^2 was used as the working cathode.

Controlled potential coulometry was carried out in a Lingane H-type cell [8] using ELICO potentiostat. The total number of electrons involved in the electrode process were evaluated adopting the following typical procedure. The cathodic compartment of the cell was filled with 0.01 M solution (5 ml) of the substrate, dimethylformamide (20 ml) and buffer solution of desired pH (25 ml; pH 3.60 or 8.30) and the solution was electrolysed at a constant potential (at -1.15 V in pH 3.60 and at -1.95 V in pH 8.30). The progress of the electrolysis was followed by recording the reduction currents (i) at different times (t). The number of electrons consumed per molecule was calculated from i-t curves following the procedure outlined by Lingane.

Controlled potential electrolysis of the compound was carried out on the preparative scale in the same way as above by electrolysing 50 ml of the test solution of pH = 4.60 at -1.20 V vs SCE. After the completion of electrolysis, 1 ml of the solution was withdrawn and tested qualitatively for the presence of aromatic amine. Rest of the solution was evaporated in vacuo to half its original volume, shaken with ether, the ether extract was separated, dried over anhydrous sodium sulphate and partially concentrated to afford a yellow residue. The compound was identified by elemental analysis and IR spectral data.

RESULTS AND DISCUSSION

Polarographic Studies

The compound exhibits two polarographic waves in the pH range 1.10-5.60, and three waves in the pH range 8.30 - 10.10.

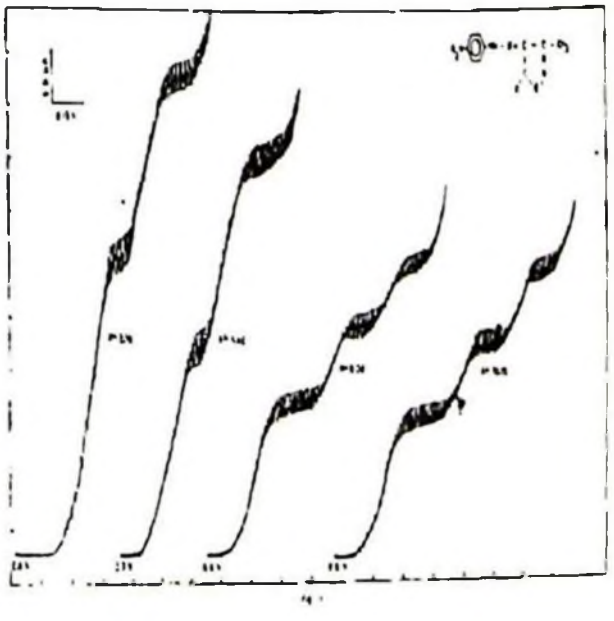


Fig. 1. Polarograms
Depolariser: 3-methyl-4-(4'-nitroarylhydrazono)-2-isoxazolin-5-one
Medium: buffers of different pH in dimethylformamide (50% V/V)

The observed linear dependence of limiting current on the square root of the height of the mercury column (i *vs* $h^{1/2}$) and the concentration of the depolariser (i *vs* C) suggests a diffusion controlled nature of the limiting currents. Semi-logarithmic analysis of the waves suggest their irreversible nature. The half wave potential values of the waves shift to more negative values with increase in pH in acidic pH's. This indicates a participation of proton in the reduction process. Since the compound 3-methyl-2-isoxazolin-5-one (I) has failed to exhibit any polarographic wave under similar experimental conditions, the observed waves of the compound II are attributed to the reduction of the nitro and hydrazono groups present in the compound. Typical plarograms are shown in Fig. 1. The polarographic data for the compound at different pH values are given in Table 1.

The electro-reduction studies on 2-(3-nitro-1-phenylhydrazono)-dimedone in buffered methanolic solutions [9] has shown that the reduction of the nitro group occurred first; this is followed by that of the hydrazone group. The first step observed in the present studies in the pH range 1.10 - 5.60 is also assigned to the reduction of the nitro group and the second to that of the hydrazone group. These results are in agreement with earlier reports given in the literature [3] for other 4-arylhydrazono-2-isoxazolin-5-ones.

The half-wave potential ($E_{1/2}$) shifts to more negative values with increase in pH

Table 1

Polarographic data of 3-methyl-4-(4'-nitro arylhydrazono)-2-isoxazolin-5-one

Conc. : 1 mM; Medium : 50% (V/V) dimethylformamide]

I Step		II Step		III Step	
$-E_{1/2}$V	I_{lim} µA	$-E_{1/2}$V	I_{lim} µA	$-E_{1/2}$V	I_{lim} µA
pH 1.10					
0.36	46.0	0.65	24.2		
pH 2.10					
0.42	45.2	0.75	25.4		
pH 3.60					
0.50	31.4	0.86	30.2		
pH 4.60					
0.57	30.5	0.93	29.1		
pH 5.60					
0.64	29.2	1.02	28.6		
pH 8.30					
0.87	22.5	1.32	11.4	1.65	9.0
pH 9.20					
0.89	23.0	1.32	10.6	1.65	9.6
pH 10.10					
0.89	23.4	1.32	10.2	1.65	10.4

in acidic media and remains constant in the alkaline medium. A plot $E_{1/2}$ *vs* pH is given in Fig.2. The following mechanism is formulated for this type of behaviour.

$AH \longrightarrow A^{\cdot} + H^{+}$ ...
(1) Characterised by pK_1

$AH + ne \longrightarrow P_1$ ... (1a) at E_1

$A^{\cdot} + ne \longrightarrow P_2$ ... (1b) at E_2

The depolarizer can exist in two forms, AH and $A^{\cdot}$, the relative concentrations being governed by equilibrium (1). Both AH and $A^{\cdot}$ are electroreducible (1a and 1b). The reduction 1a is pH dependent because H^{+} is consumed during the formation of AH. Reduction (1b) is pH independent. The slope change in the plot may be attributed to the electroactive nature of both acidic and basic forms of the depolarizer. At $pH > pK_1 + 1$ (higher pH values), pH independent half-wave potentials are observed because form $A^{\cdot}$ is predominent in the solution. Thus $E_{1/2}$ *vs* pH plot consists of two linear segments intersecting each other and the pH at the point of intersection of the two linear plots is approximately equal to pK_1 (Fig. 2).

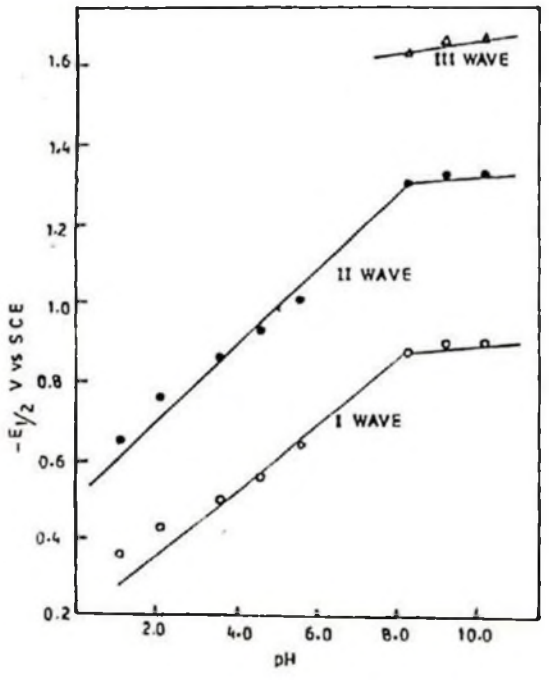


Fig. 2. Plot of $-E_{1/2}$ vs pH
Depolariser: 3-methyl-4-(4'-nitroarylhydrazono)-2-isoxazolin-5-one.
Medium: buffers of different pH in 50% V / V DMF

The $E_{1/2}$ *vs* pH plot in low pH region is linear and is governed by the following equation.

$$E_{1/2} = C - \frac{RT}{\alpha nF} \ln K_1 + \frac{RT}{\alpha nF} \ln [H^{+}]$$

Data in Table 1 shows that the height of the first wave in solution of pH values 1.10 and 2.10 is 1.5 times that of the first wave at pH values 3.60 and 5.60. This clearly shows that the reduction of nitro group at pH values 1.1 and 2.1 is directly to the amine stage but at pH values 3.6 to 5.6, the reduction stops at the hydroxylamine stage. This may be attributed to the insufficient protonation of the hydroxylamine in these pH values. It may be worth noting that the half-wave potentials in solutions of pH 1.10 and 2.10 deviate from the $E_{1/2}$ - pH plot of the first wave (Fig. 2). This deviation is because of the difference in the reaction mechanism.

The second wave represents the reduction of hydrazono group. The height of this step in media of pH 3.6 nearly corresponds to the 4 electron step observed for the first wave (cf. Table 1). Eventhough same number of electrons (4) are involved in the reduction in first and second steps in media at pH 3.6 to 5.6.

the heights of the two steps are not the same. The height of the second is found to be less and decreases further with increase of pH. This is attributed to the decrease in protonation which is a prerequisite for the reduction of hydrozono group. The conversion of azo-methine to anionic form [11] (-N⁻-N=C<) makes it susceptible for chemical cleavage in presence of hydroxyl ions to the corresponding carbonyl compound [12].

However it is surprising that the height of the second step is lower at lower pH also (pH 1.10 to 2.10). This may be attributed to the loss of one of the intermediate species in the highly acidic medium. This behaviour is similar to that reported for compounds containing hydrozono group [10].

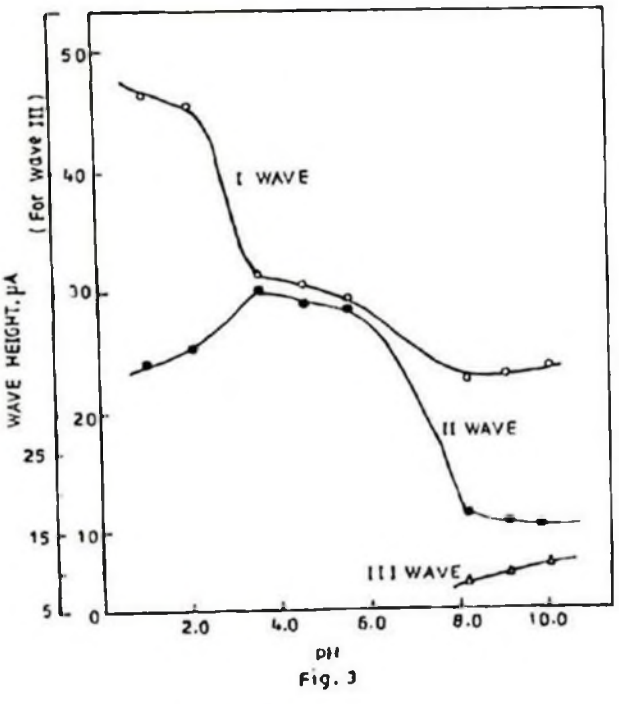


Fig. 3. Plot of Wave Height *vs* pH
Depolariser: 3-methyl-4-(4'-nitroarylhydrazono)-2-isoxazolin-5-one
Medium: buffers of different pH in 50% V / V DMF

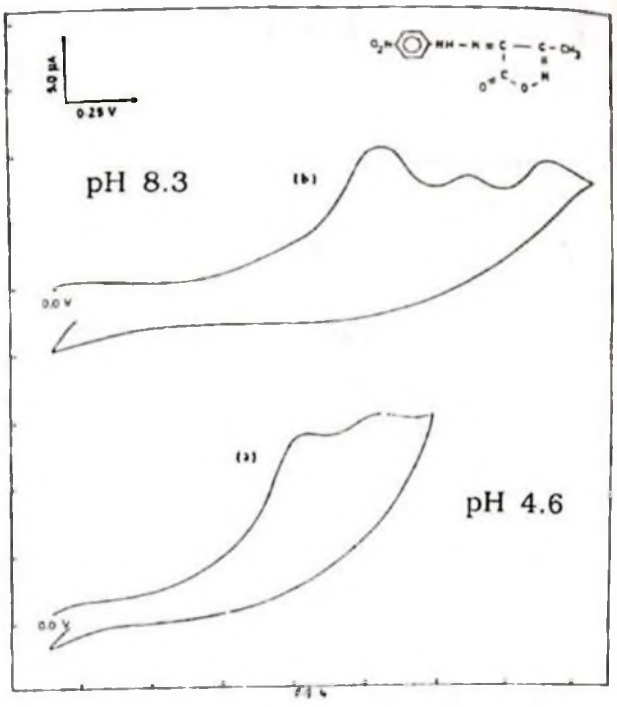


Fig. 4. Cyclic Voltammograms
Depolariser: 3-methyl-4-(4'-nitroarylhydrazono)-2-isoxazolin-5-one
Medium: buffers of different pH in 50% V / V DMF

Three reduction steps are observed in alkaline media. The first step could be a four electron reduction of nitro group to hydroxylamine group. From the position of the second wave on the potential axis, it is assumed that this is due to a 4 electron reductive cleavage of the anion [- N⁻ - N = C<]. The third wave is ascribed to the reduction of the carbonyl compound since reduction of carbonyl group occurs at much higher negative potentials. The potential at which third wave is observed corresponds to the potential at which reduction of ketonic carbonyl group is observed [13]. A plot of pH *vs* wave height is shown in Fig. 3. The increase in the height of the third wave with pH further suppports this fact since the formation of anion and its cleavage to carbonyl compound is favoured under strong alkaline conditions.

Cyclic voltammetric studies

The peaks observed correspond to the observed waves in polarography. Typical cyclic voltammograms are presented in Fig. 4 and the typical cyclic voltammetric data is presented in Table 2.

The linear plot of i_p *vs* $v^{1/2}$ passing through the origin suggest the diffusion controlled nature of the electrode process. The absence of the anodic peak indicates the irreversible nature of the reduction. This is further established by the negative shift in the peak potentials with increase in scan rate. The cyclic voltammetric studies in general substantiate the result obtained by polarography. The two peaks observed in acidic solutions are as usual attributed to the reduction of nitro and hydrazone groups. The three peaks observed in alkaline solutions are due to the reduction of nitro, azomethine anion and the carbonyl compound.

Controlled Potential Coulometry

The coulometric studies indicate the participation of 8 electrons in the

Table 2

CyclicVoltammetric Data of 3-methyl-4-(4'-nitro arylhydrazono)-2-isoxazolin-5-one

[Conc. 1mM; Medium 50% (V/V) dimethylformamide

Scan rate	$-E_{pc}$ V			i_{pc} μA			$i_{pc}/v^{1/2}C$		
VS^{-1}	I	II	III	I	II	III	I	II	III
pH = 2.10									
0.10	0.70	0.92	-	14.05	14.00	-			
pH = 4.60									
0.01	0.80	1.06	-	3.83	4.67	-	38.3	46.7	-
0.02	0.82	1.08	-	5.33	6.17	-	37.7	43.6	-
0.05	0.85	1.10	-	9.30	10.67	-	41.6	47.7	-
0.10	0.87	1.12	-	13.00	14.67	-	41.1	46.4	-
0.20	0.90	1.14	-	16.33	19.33	-	36.5	43.2	-
0.30	0.95	1.16	-	20.00	23.67	-	36.5	43.2	-
0.50	1.00	1.20	-	27.33	31.67	-	38.7	44.7	-
pH = 8.30									
0.01	1.06	1.42	1.70	3.57	2.60	3.10	35.7	26.0	31.0
0.02	1.08	1.43	1.72	5.04	3.71	4.40	35.6	26.2	31.1
0.05	1.12	1.45	1.74	8.00	5.65	6.85	35.8	25.3	30.6
0.10	1.14	1.47	1.76	10.50	8.00	9.40	33.2	25.3	29.7
0.20	1.15	1.50	1.78	14.34	11.35	13.52	32.1	25.4	30.2
0.30	1.16	1.52	1.80	20.30	13.94	16.67	37.1	25.6	30.4
0.50	1.18	1.55	1.84	26.37	18.13	21.74	37.3	25.6	30.7
pH = 10.10									
0.10	1.15	1.48	1.76	10.50	7.95	9.45			

reduction process at pH 3.60 - 4 electrons for the conversion of $-NO_2$ to -NHOH and 4 more electrons for the cleavage of azo-methine. In the reduction process at pH 8.30, ten electrons are found to be consumed. Out of these, four electrons are consumed for reduction of $-NO_2$ to -NHOH, four for the cleavage of azo-methine anion and two electrons for the reduction of carbonyl compound. The coulometric data has been presented in Table 3.

Table 3

Coulometric data of 3-methyl-4-(4-nitro arylhydrazono)-2-isoxazolin-5-one

[Concentration 1 mM; Medium 50% (V/V) dimethylformamide]

Current μA	Time sec	n-value
pH = 3.60		
61.6	0	-
46.5	3600	8.2
39.6	5400	7.8
pH = 8.30		
42.9	0	-
36.4	3600	9.7
33.6	5400	9.8

Products from Controlled Potential Electrolysis

After carrying out the electrolysis, presence of aromatic amine in the solution was established by the standard spot test [14]. The yellow crystalline solid obtained was identified as 3-methyl-4-amino-2-isoxazolin-5-one ($C_4H_6N_2O_2$) [Found : C = 41.2%; H = 4.52%; N= 23.1%: above formula requires C = 42.1%; H = 5.26%; N = 24.56%]. IR spectra (KBr, cm^{-1}) 1587 (>C=N-), 1670 (>C=O cyclic), 3260 (>N-H stretch), 3020 (>C-CH_3), 1450, 1470 (heterocyclic ring).

Kinetic Parameters

The values of heterogeneous forward rate constant ($k^\circ_{f,h}$) have been evaluated by Meites-Israel [15] method. The values of $-E_{1/2}$ and αn_a are obtained from the intercept and slope of $E_{d.e}$ *vs* log $[i/(i_d-i)]$ plot. The values of forward rate constant in cyclic voltammetric studies have been evaluated by the following equation given in the literature [16].

$$E_{pc} = \frac{-1.14\,RT}{\alpha n_a F} + \frac{RT}{\alpha n_a F} \ln\left(\frac{k^\circ_{f,h}}{D^{1/2}}\right) - \frac{RT}{2\,\alpha n_a F} \ln(\alpha n_a v)$$

The heterogeneous forward rate constant ($k^\circ_{f,h}$) calculated at different pH values are presented in Table 4. The rate constant values in general, are observed to be high in acidic media. The rate decreases with increase in pH.

The activation free energy change, ΔG^* is obtained from the following relation given in the literature [17].

$$k^\circ_{f,h} = \frac{kT}{h} r_o \exp\left(\frac{-\Delta G^*}{RT}\right)$$

where k = Boltzman constant (erg.deg^{-1}.mol^{-1})
h = Plank's constant (erg.sec)
r_o = Mean distance between two depolarised ions in bulk solution (cm),
ΔG^* = Activation free energy change
R = Gas constant (Cal.)
T = Absolute temperature

In general the value of r_o is taken as 2 x 10^{-8} cm [17]. The values of activation free energy change increase with the rise of pH. This trend confirms an increase in the irreversibility of the electrode process with increase in pH.

A Contrast Between Present and Previous Results

In our earlier investigations on the electrochemical behaviour of the title compound in BR buffers, two waves were observed in the entire pH range (pH 2.1 to 10.1) which correspond to the reduction of nitro and hydrazono groups to hydroxylamine and heterocyclic amine along with aromatic amine respectively. But in the present investigations, it is observed that the same compound exhibit two waves at pH 1.10 and 2.10 which in contrast to our earlier findings are found to be due to the reduction of nitro to amino group and the reductive cleavage of hydrazone group. But the height of the wave in the present studies is found to be less than that expected which has been due to the hydrolysis of protonated imine; no such hydrolysis has been observed in Britton-Robinson buffer solutions at pH 2.10.

In our earlier studies three peaks were observed in cyclic voltammetric studies in acidic media. The first peak was attributed to the reduction of nitro group and the second and third peaks to the two step reductive cleavage of hydrazone group. But in the present studies only two peaks are observed which correspond to the reduction of nitro and hydrazone groups. The hydrazone group in the present studies undergoes reduction in a single step. Further an inverted peak which was observed (in acidic solutions)

Table 4

Kinetic Parameters

$k^{o}_{f,h}$ cms^{-1} ; ΔG° k.cal/mol

Step	Polarography $k^{o}_{f,h}$	ΔG°	CV at v 0.10V.S^{-1} $k^{o}_{f,h}$	ΔG°
pH 1.10				
I	4.14×10^{-4}	11.8		
II	2.53×10^{-6}	14.9		
pH 2.10				
I	1.48×10^{-4}	12.5	2.53×10^{-3}	10.7
II	4.98×10^{-7}	15.9	3.77×10^{-3}	10.5
pH 3.60				
I	4.22×10^{-6}	14.6		
II	6.99×10^{-9}	18.5		
pH 4.60				
I	2.03×10^{-6}	15.1	3.51×10^{-3}	10.5
II	5.61×10^{-9}	18.6	3.96×10^{-3}	10.5
pH 5.60				
I	4.11×10^{-7}	16.0		
II	9.91×10^{-10}	19.7		
pH 8.30				
I	1.92×10^{-9}	19.3	2.83×10^{-3}	10.7
II	2.91×10^{-16}	28.8	2.16×10^{-3}	10.8
III	5.79×10^{-24}	39.5	1.87×10^{-3}	10.9
pH 9.20				
I	1.26×10^{-9}	19.5		
II	2.71×10^{-16}	28.8		
III	4.43×10^{-24}	39.7		
pH 10.10				
I	1.28×10^{-9}	19.5	2.83×10^{-3}	10.7
II	2.62×10^{-16}	28.9	2.16×10^{-3}	10.8
III	4.79×10^{-24}	39.7	1.86×10^{-3}	10.9

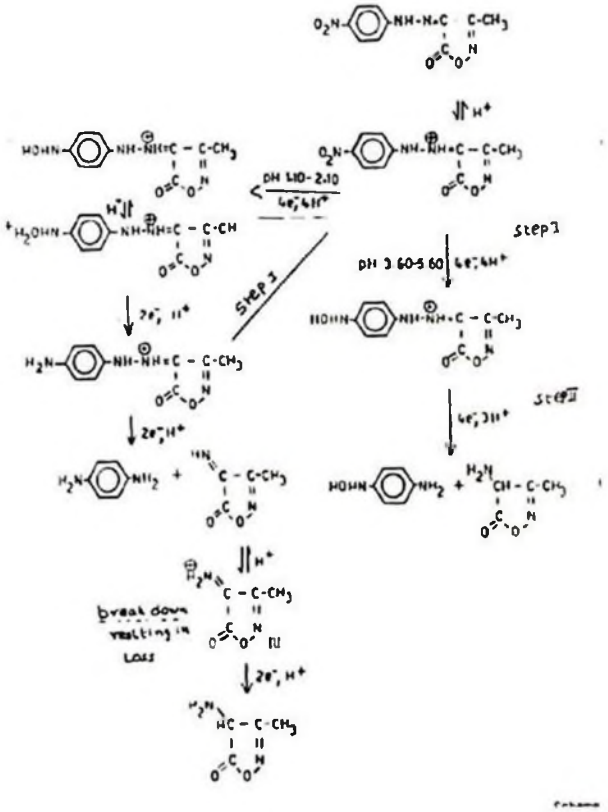
in BR buffers has been not observed in the present studies.

In alkaline solutions two waves / peaks were observed in BR buffers which correspond to the reduction of nitro and azomethine anion. But in the present studies three waves / peaks are observed which correspond to the reduction of nitro, azomethine anion and carbonyl groups. The reduction peak for the reduction of carbonyl compound which has not been observed in BR buffers has appeared clearly in the present investigations.

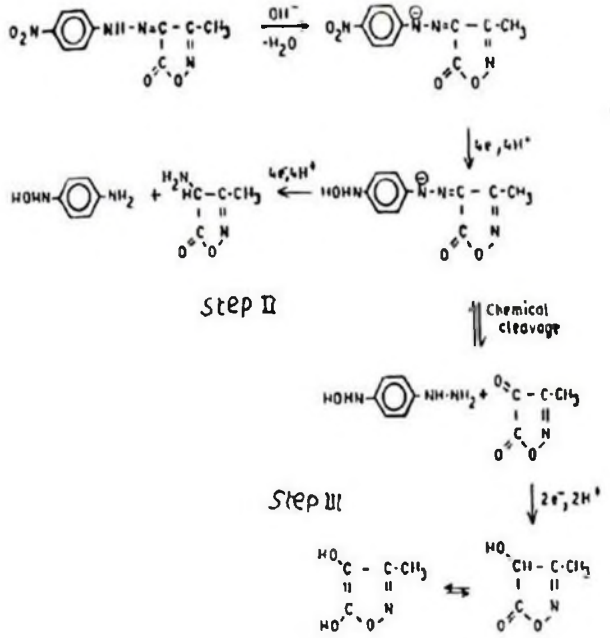
MECHANISM

Based on the observations detailed in the foregoing discussion, the mechanism shown in Scheme I and II is proposed for the reduction process in acidic and alkaline media respectively. The mechanism given in Scheme I shows four steps to account for the two waves observed in the pH range 1.20-2.10. The first wave observed is due to the 6 electron reduction of $-NO_2$ to $-NH_2$. The reason for showing it in two steps involving, 4 electrons and 2 electrons is to show that it is protonated hydroxylamine that undergoes reduction at pH 1.10 and 2.10 which is not possible in the remaining pH range. The protonated hydroxylamine reduction step occurs more easily than nitro reduction, so the two steps manifest as a single 6 electron step. Similarly steps 3 & 4 together account for the second wave. The reason for showing it in 2 two electron steps instead of a single 4 electron step is to show the formation of protonated imine (species III, cf. Scheme I that undergoes hydrolysis under strong acidic conditions. The hydrolysis process is responsible for the observed lower height of second wave at pH 1.10 & 2.10 as compared to height of the second wave observed at pH 3.60. Again these two steps occur at almost same potential and hence a single wave. The first wave observed in the pH range 3.60 - 5.60 is attributed to the 4 electron reduction of $-NO_2$ to -NHOH and the second to a 4 electron reductive cleavage of azo-methine. The coulometric data also indicate the participation of 8 electron in the electrode reaction at pH 3.60.

Reduction pattern in acidic media



Reduction pattern in alkaline media



Scheme II

Scheme II shows 3 steps to account for three waves observed in alkaline media. Step I corresponds to 4 electron reduction of $-NO_2$ to -NHOH. The half-wave potential data of the II wave in alkaline media fit well in the $E_{1/2}$ - pH plot for the second wave in the entire pH range of study. Step II is attributed to a four electron reductive cleavage of azo-methine anion. The combined height of II and III waves is almost equal to the height of the I wave in alkaline media. But step II and III together can not be attributed to a four electron reduction of azo-methine anion in two steps involving two electrons each. The position of the second wave on the potential axis suggests that this wave is due to a four electron reduction of azo-methine anion. The potential at which the third wave is observed correspond to the potential of ketonic carbonyl reduction [13]. Two electron reduction is due to the carbonyl compound, formed as a result of the chemical cleavage of azo-methine anion. The coulometric studies at pH 8.30 also indicate the participation of ten electrons in the reduction process, four electrons for the reduction of $-NO_2$ to -NHOH, four electrons for the reductive cleavage of azo-methine anion and two electrons for the reduction of carbonyl compound.

CONCLUSIONS

The results obtained in the present studies show that the nature of reduction of a given compound is largely influenced by the medium in which it is studied. The intermediates/products giving a wave in one medium may not appear in the other media and the reduction which has been found to stop at certain stage in one medium has been carried forward in the other. The reduction wave of some of the products of reduction could merge with the decomposition of some buffer media but clearly appear in the other. Thus the medium of study exert a considerable effect on the reduction pathway and the present findings envisages the need to carry out the investigations in buffer media of varied composition.

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