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Cyclic Voltammetric Study of Hydralazine Hydrochloride, an Antihypertensive Drug

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ABSTRACT
The redox properties of hydralazine were studied by cyclic voltammetric method. The effect of scan rates on the voltammogram of hydralazine was studied and various electrochemical parameters e.g.E_{pa}, E_{pc}, I_{pa}, I_{pc}were determined. The results show a quasi-reversible behavior of hydralazine hydrochloride.

Keywords: Hydralazine, Cyclic voltammetry, Quasi-reversible, Randles-Sevcik equation

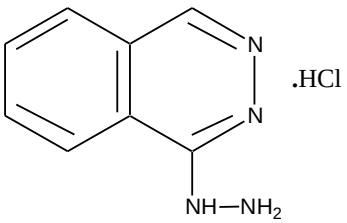
1. INTRODUCTION
Significant morbidity and mortality are consequences of heart failure. However, the therapy has been improved significantly. An estimated lifetime risk, at the age of 40, due to developing heart failure is about 40%. This situation depends upon the occurrence of unrestrained hypertension or other melancholic cardiovascular conditions [1].

There is a delicate balancing of many complex biochemical processes for normal cardiovascular performance. Disturbance of which might lead to myocardial dysfunction. It also might be a secondary cause of structural heart disease, for example, myocardial infarction (MI) or cardiomyopathic processes. Consequently, these altered signaling systems can lead to the development of myocardial dysfunction.

In the past few years several research groups have investigated the roles of ROS (reactive oxygen species) and RNS (reactive nitrogen species) in failing and normal myocardium and vasculature. Yet it remains a subject of considerable debate[2].

Signaling by RNS and modulation caused through ROS are thought to be important part of normal myocardial and vascular function. A better understanding of these mechanisms not only provides an insight into the activity of current therapies but also direct to the development of new therapeutic agents [3]. Many existing drugs for heart disease exhibit redox modulatory activity.

A combination of isosorbidedinitrate and hydralazine has recently been proved to be highly effective in treatment of heart failure [4]. Isosorbidedinitrate stimulates nitric oxide signaling. On the other hand, hydralazine is a vasodilator and antioxidant, which is responsible for inhibition of the enzymatic formation of ROS (reactive oxygen species) such as superoxide (O_2^-) by NADH and NADPH oxidases [5,6].In addition; some recent reports have shown free radical scavenging activity of hydralazine[7].The need to understand the biochemistry of superoxide and nitric oxide and their interaction has been brought to forefront by the success of this therapy[8]. Although, the mechanism of action of hydralazine is not well understood, it has been recommended for inhibition of this activity both by reducing nitrate tolerance and/or by lowering superoxide levels [6].



Scheme-1: Structure of Hydralazine Hydrochloride

Hydralazine is active in vasodilatation and reduction in total peripheral vascular resistance, which subsequently increases stroke volume, cardiac output and heart rate [9,11]. Keeping in view all these effects of hydralazine, it is useful to determine the redox properties of this drug.

Electrochemistry provides a fast, accurate and convenient method to study the equilibrium and transport properties of ionic solutes [12].In recent years, cyclic voltammetry (CV) has become a tool in medicinal chemistry. It is used to determine the lipophilicity of ionic drugs and mechanism of their transfer at ITIES (interface between two immiscible electrolyte solutions) [13,14].

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2. RESULTS

The base line of pure supporting electrolyte was found to be horizontally straight at different current sensitivities (Fig.1). Voltammogram of hydralazine hydrochloride was obtained at various scan rates i.e. at 10 mV/s, 20mV/s, 30 mV/s, 60 mV/s, 80 mV/s and 100 mV/s (Fig.2).

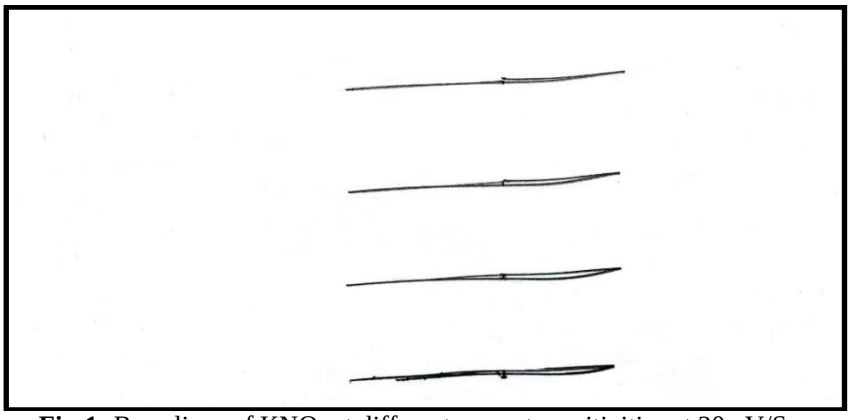

Fig-1: Base-lines of KNO_3 at different current sensitivities at 20mV/Sec
A = 300 mAmp/Volt, B = 200 mAmp/Volt, C = 100 mAmp/Volt D = 500 mAmp/Volt

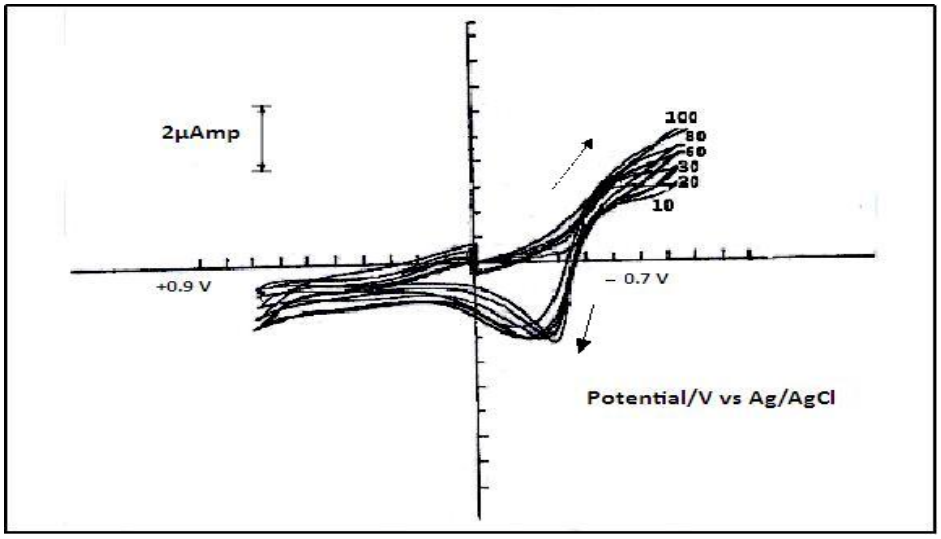

Fig-2: Overlay voltammogram of Hydralazine at different scan rates at Pt electrode Vs Ag/AgCl reference electrode.

Table-1: Different Parameters Obtained from cyclic voltammograms of Hydralazine Hydrochloride at different scan rates

Scan rate (mV/s)	**E_{pa} (mV)**	**$E_{pa/2}$ (mV)**	**E_{pa}-$E_{pa/2}$ (mV)**	**E_{pa}-E_{pc} (mV)**	**I_{pa} (µA)**	**βn_b**
10	515	400	115	180	5.00	0.417 ± 0.01
20	540	410	130	250	4.60	0.369 ± 0.01
30	565	425	140	295	6.20	0.343 ± 0.01
60	620	385	235	410	7.20	0.204 ± 0.01
80	670	415	255	460	5.80	0.188 ± 0.01
Scan rate (mV/s)	**E_{pc} (mV)**	**$E_{pc/2}$ (mV)**	**E_{pc}-$E_{pc/2}$ (mV)**	**I_{pc} (µA)**	**I_{pa}/I_{pc}**	**αn_a**
10	330	390	-60.0	10.2	0.490	0.800 ± 0.01
20	290	365	-75.0	8.60	0.535	0.640 ± 0.01
30	270	365	-95.0	7.40	0.816	0.505±0.01
60	210	340	-130	7.00	1.028	0.369 ± 0.01
80	210	320	-110	10.4	0.828	0.436 ± 0.01
100	170	300	-130	8.40	0.786	0.369 ± 0.01

Working electrode = Pt
Reference electrode = Ag|AgCl
Temperature = 25 ± 1°C
Current sensitivity = 20µA/V
Supporting electrolyte (KNO3) = 0.1M

The values of $(E°)_c$ and $(E°)_a$ were also determined for hydralazine by calculating Ip85% at all scan rates (Table 2)[17]. Electrochemical parameters like E_{pa}, E_{pc}, I_{pa}, I_{pc} were determined from these voltammograms (Table 1). The value of αn_a (where n_a is the number of electron involved in the rate determining step and α is the transfer coefficient) was calculated, for the quasi-reversible reaction of hydralazine according to the following equation;
$\alpha n_a = 0.048/ (E_p-E_{p/2})$[15,16]. The calculated values of αna at the Pt electrode at various potential scan rates are given in Table.1.

Table-2: Redox Potential obtained from cyclic voltammogram of Hydralazine Hydrochloride at different scan rates.

ν (V/s)	$\nu^{½}$ (V/s)	E_{pc} (V)	$E_{pc/2}$ (V)	$(E°)_c$ (V)	E_{pa} (V)	$E_{pa/2}$ (V)	$(E°)_a$ (V)
0.010	0.100	0.330	0.390	0.250	0.515	0.400	0.440
0.020	0.141	0.290	0.365	0.170	0.540	0.410	0.450
0.030	0.173	0.270	0.365	0.160	0.565	0.425	0.480
0.060	0.245	0.210	0.340	0.090	0.620	0.350	0.510
0.080	0.283	0.210	0.320	0.040	0.670	0.370	0.520
0.100	0.316	0.170	0.300	0.020	0.720	------	0.540

Working electrode = Pt
Reference electrode = Ag|AgCl
Temperature = 25 ± 1°C
Current sensitivity = 20 µA/V
Supporting electrolyte (KNO3) = 0.1M
Concentration of Hydralazine Hydrochloride = 0.01 M

Table-3: Diffusion coefficient of Hydralazine Hydrochloride at different scan rates.

ν (mV/s)	ν (V/s)	$\nu^{1/2}$	I_{pa} (µA)	I_{pa} (A)	$D^{1/2}$	D ($cm^2 s^{-1}$)
010	0.01	0.100	5.00	5.00 E-06	1.64 E-03	2.71 E-06
020	0.02	0.141	4.60	4.60 E-06	1.07 E-03	1.15 E-06
030	0.03	0.173	6.20	6.20 E-06	1.18 E-03	1.39 E-06
060	0.06	0.245	7.20	7.20 E-06	9.67 E-04	9.35 E-07
080	0.08	0.283	5.80	5.80 E-06	6.75 E-04	4.55 E-07
100	0.10	0.316	6.60	6.60 E-06	6.87 E-04	4.71 E-07
ν (mV/s)	**ν (V/s)**	**$\nu^{1/2}$**	**I_{pc} (µ A)**	**I_{pc} (A)**	**$D^{1/2}$**	**D ($cm^2 s^{-1}$)**
010	0.01	0.100	10.2	1.02 E-05	3.36 E-03	1.13 E-05
020	0.02	0.141	8.60	8.60 E-06	2.00 E-03	4.00 E-06
030	0.03	0.173	7.40	7.40 E-06	1.41 E-03	1.98 E-06
060	0.06	0.245	7.00	7.00 E-06	9.40 E-04	8.84 E-07
080	0.08	0.283	10.4	1.04 E-05	1.21 E-03	1.46 E-06
100	0.10	0.316	8.40	8.40 E-06	8.74 E-03	7.64 E-05

Working electrode = Pt
Reference electrode = Ag|AgCl
Temperature = 25 ± 1°C
Current sensitivity = 20 µA/V
Supporting electrolyte (KNO3) = 0.1M
Concentration of Hydralazine Hydrochloride= 1x10-5 mol cm-3
n = number of electron transfer = 1
A= Area of the electrode = 0.0113 cm2
D1/2 = Ip / (2.69x105) (n)3/2 A C (v)1/2

The value of diffusion coefficient, 'D', was calculated using Randles-Sevcik equation, which is as follows[12]:

$$\mathbf{I_p = 2.69 \times 10^5 \, n^{3/2} A \, D^{1/2} C \, v^{1/2}}$$

Where,

I_p = Peak current (Amp)
n = Number of electron transfer
A = Electro-active area of the electrode (cm^2)
D = Diffusion coefficient (cm^2 Sec^{-1})
C = Concentration (mole cm^{-3})
v = Scan rate (Volts/Sec)

The values of diffusion coefficient determined at different scan rates are given in Table.3.

3. DISCUSSION

At first glance the cyclic voltammograms appear to show the presence of a quasi-reversible electron transfer (Figure 2). A quasi-reversible process, which is reversible at low sweep rates, becomes irreversible at higher ones, after having passed through a region known as quasi-reversible at intermediate values [18]. This transition from reversibility occurs when the relative rate of the electron transfer with respect to that of mass transport is insufficient to maintain Nernstian equilibrium at the electrode surface. In the quasi-reversible region both forward and back reactions make a contribution to the observed current [19]. This change from reversible, to quasi reversible and finally irreversible behavior can readily be seen from a plot of peak current density, J_p (Ipa or Ipc) as a function of square root of scan rate ($v^{1/2}$) (Fig.3).

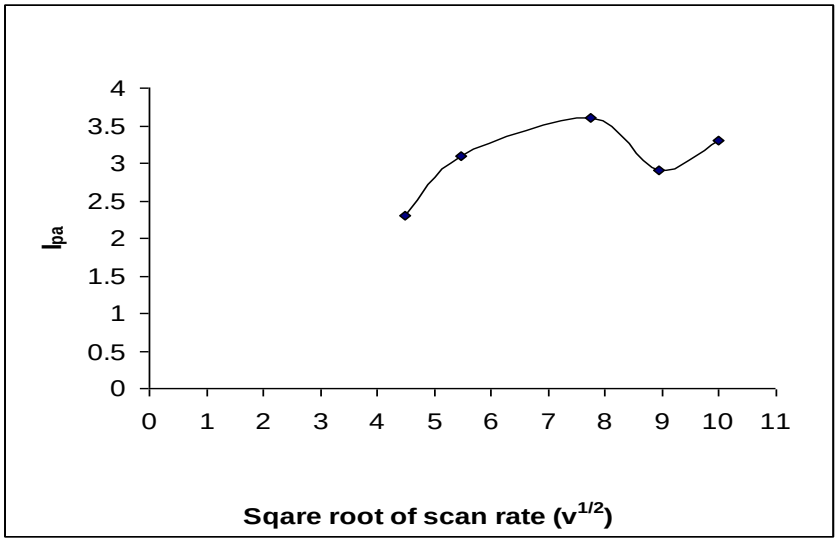

Fig-3: Variations of anodic peak current density with square root of sweep rate from the cyclic voltammograms of Hydralazine in KNO_3 (0.1M).

Other diagnostic tests for a quasi reversible system and their comparison with the results obtained from the voltammogram of hydralazine are shown in table 4[20].

The first point of table 4 for quasi-reversible behavior has been discussed in the above paragraph in which anodic (I_{pa}) peak current density was plotted against square root of scan rate ($v^{1/2}$). The plot shows that the current density increased with increasing $v^{1/2}$ but it is not proportional to it (Fig.3).

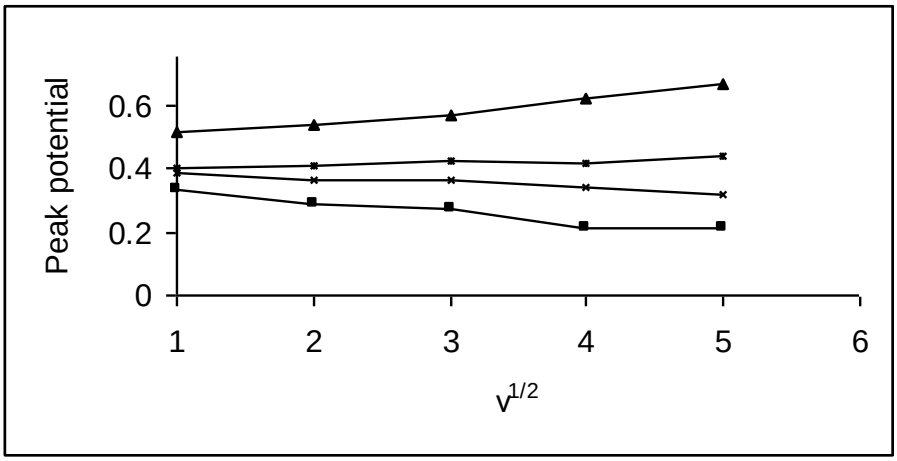

Fig-4: Variations of anodic and cathodic peak potential (Ep and Ep/2) with square root of sweep rate from the cyclic voltammograms of Hydralazine in KNO_3

The difference E_{pa}-E_{pc} was found to be greater than 59/n mV and increases with potential scan rate (Table.1). The last point of the table is that when scan rate increases, E_{pc}under goes a negative shift, this behavior also confirms quasi-reversible electron transfer process (Table.1).

A plot of peak potential (i.e. E_{pa} and E_{pc}) vs. log of scan rate indicates that peak potential increases with scan rate. The same results were obtained when Epa/2 and Epc/2 were plotted against log of scan rate (Fig.4).

Hydralazine hydrochloride fulfills the diagnostic tests for quasi-reversibility and does not stand the diagnostic tests for totally reversible and irreversible processes in the presence of KNO_3 (Table.4).

Table-4: Diagnostic criteria for a quasi-reversible system at 25 ± 1°C.

S. No.	Criteria for quasi-reversible system	Results obtained for Hydralazine Hydrochloride
1.	$\lvert I_p \rvert$ is not proportional to $\nu^{1/2}$ but increases as with increase in $\nu^{1/2}$	$\lvert I_p \rvert$ is not proportional to $\nu^{1/2}$but increases with $\nu^{1/2}$
2.	$\lvert I_{pa} / I_{pc} \rvert = 1$ provided $\alpha_c = \alpha_a = 0.5$	$\lvert I_{pa} / I_{pc} \rvert \approx 1$
3.	$E_{pa} - E_{pc}$ is greater than 59/n mV and increases as ν increases	$E_{pa} - E_{pc} > 59$ mV and increases as ν increases
4.	E_{pc} shifts negatively on increasing ν	On increasing ν the E_{pc} shifts negatively

4. CONCLUSION

Cyclic voltammetric study of hydralazine suggests that;

- Hydralazine shows quasi-reversible behavior.
- The diffusion coefficient value shows that it can pass through cell membrane.

Cyclic voltammetric data also shows that hydralazine can reduce different metals at different potentials due to its quasi-reversible behavior.

5. MATERIALS AND METHOD

Analytical grade chemicals were used in all the experiments, and employed without further purification. Special care was taken to wash the glassware before use. For this study CO_2 free water was used which was prepared by boiling distilled deionized water for about 10 minutes and then cooling in an airtight bottle [21].

Cyclic Voltammetric studies were carried out onCV-1B Cyclic Voltammetry controller (EF1011-00) unit, Bioanalytical System Inc. USA. Hydralazine hydrochloride was prepared in 0.1 M solution of potassium nitrate (KNO_3), the supporting electrolyte. The calibration of the instrument was accomplished following the procedure given in the installation/operation manual of the instrument. Calibration was also done by using 0.001M solution of Ferrocene in Acetonitrile containing 0.1M tetraethylamonium phosphate (TEAP) for which the ferrocenium/ferrocene reduction potential was 400mV and ΔE= 72 mV at scan rate of 100 mV/s[22].

The base-line of pure supporting electrolyte was recorded at Pt electrode vs. Ag|AgCl reference electrode, at 25 ± 1°C and at 20 mV/s. 15 mL of Hydralazine hydrochloride solution was transferred to the cell. Repeated insertion of electrode assembly in the analyte solution ensured the removal of air bubbles from the surface of the Pt electrode. The cell top was fixed firmly at the cell, and nitrogen gas was flushed for five minutes. The solution in the cell was stirred for three minutes. Adjustments related to current sensitivity, initial and final switching potential, voltage scan rate, and chart recorder sensitivity were made during nitrogen purging prior to the initiation of scan. The cyclic voltammetry was performed at various scan rates ranging from 10-100mV/s.

6. REFERENCES

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