

## STUDY OF ELECTRODE KINETICS AND THERMODYNAMIC PARAMETERS OF ANTITUBERCULOSIS DRUG ISONIAZID AT D.M.E.

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### ABSTRACT

The polarographic study of Antituberculosis drug Isoniazid was carried out at dropping mercury electrode (DME). Study of the drug included the effect of different pH, different temperature of medium and different concentration of drug. Isoniazid showed two irreversible waves depending upon the pH. In acidic media it showed two waves while in neutral and alkaline media only one wave was observed. The diffusion current ( $i_d$ ) showed a linear dependence with the drug concentration over the range of  $2.9 \times 10^{-4}$  –  $2.9 \times 10^{-3}$ . The reduction of Isoniazid was found to be irreversible so kinetic parameters ( $K_0^{th}$ ,  $\alpha$ ) were evaluated using Meites-Israel and Gaur-Bhargava's methods. Thermodynamic parameters such as  $\Delta H_p^\ddagger$ ,  $\Delta H_v^\ddagger$ ,  $\Delta G^\ddagger$  and  $\Delta S^\ddagger$  were also evaluated.

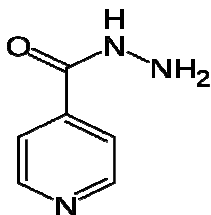
**Keywords:** Isoniazid, Direct current polarography, Kinetic parameters and thermodynamic parameters.

### INTRODUCTION

Isoniazid (pyridine-4-carboxylic acid hydrazide) also known as isonicotinylhydrazine (INH) is used as Antituberculosis - agent. Isoniazid also has an antidepressant effect, and it was one of the first antidepressants discovered. Isoniazid is a prodrug and must be activated by a bacterial catalase-peroxidase enzyme called KatG<sup>1</sup>. KatG couples the isonicotinic acyl with NADH to form isonicotinic acyl-NADH complex. This complex binds tightly to the enoyl-acyl carrier protein reductase known as InhA, thereby blocking the natural enoyl-AcpM substrate and the action of fatty acid synthesis. This process inhibits the synthesis of mycolic acid, required for the mycobacterial cell wall. A range of radicals are produced by KatG activation of Isoniazid, including nitric acid<sup>2</sup>. The study of isoniazid has been done by using different techniques like spectroscopy<sup>3</sup>, spectrophotometry<sup>4-6</sup>, EPR spectroscopy<sup>7</sup>, HPLC<sup>8</sup>, liquid chromatography<sup>9-10</sup>, differential pulse polarography<sup>11</sup>, electrochemical<sup>12-15</sup>, electroanalytical<sup>16</sup> etc. Present work is related to the electro-reduction of isoniazid at DME.

Isoniazid is odourless, and occurs as a colourless or white crystalline powder or as white crystals. It is freely soluble in water, sparingly soluble in alcohol, and slightly soluble in chloroform and in ether. Isoniazid is slowly affected by exposure to air and light.

### Structure



Iupac Name: pyridine-4-carboxylic acid hydrazide

Formula: C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O

Mol. Mass: 137.139 g/mol

[ISONIAZID] CAS No. : 54-85-3.

### MATERIALS AND METHODS

#### Apparatus

The digital D. C. Polarograph (CL-357) of Elico Limited was used to record current-voltage data. This equipment has the three electrode assembly, dropping mercury electrode as working electrode, calomel as reference electrode and platinum electrode as counter electrode.

Dropping mercury electrode had the characteristics  $m = 2.422$  mg/sec,  $t = 2.5$  sec and  $h = 60$  cm.

The Elico digital pH meter model 111E was used to measure the pH of the analytes.

#### Reagents

Isoniazid was obtained from Alpha Chemica India.

Isoniazid was dissolved in water.

All solutions were prepared freshly with doubly distilled water and analytical reagent grade chemicals (MERCCK).

#### Proposed Procedure

The general procedure used to produce DC polarograms was as follows:

An aliquot (10 ml) of experimental solution which contains B.R. Buffer, Triton-X-100 (Maxima Suppressor) and water was placed in a dry, clean polarographic cell and deoxygenated with nitrogen for 15 min. the current-voltage values were measured manually.

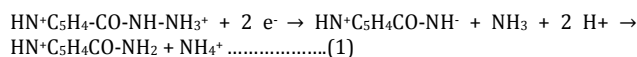
The negative potential was applied to the working electrode with 150 mV/min span rate and 100 nA/div sensitivity of current measurement. After the background polarogram had been obtained, aliquots of the required amounts of Isoniazid solution were added.

### RESULT AND DISCUSSION

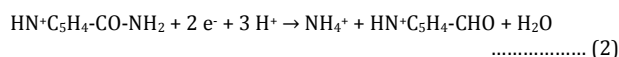
#### 1) Effect of pH of medium

The effect of pH on the current-voltage curve at a concentration  $1.458 \times 10^{-3}$  M Isoniazid was studied over the pH range 1.8 – 11.4. Isoniazid gives rise to one or two reduction peaks depending on pH. In basic medium one cathodic wave was obtained which refers to the four – electron reduction of hydrazide group to the corresponding dihydroisonicotinic amide. In acidic medium Isoniazid exhibits two cathodic waves.

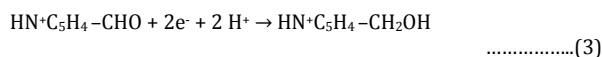
In acidic medium (pH 1.8 – 5.81) the first cathodic wave was found to be caused by a reductive splitting of the nitrogen-nitrogen bond<sup>17</sup>:



The second cathodic wave is caused by the reduction of the isonicotinic amide formed in the first reduction.



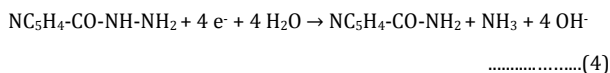
If the reduction is allowed to proceed to completion the free aldehyde is reduced to  $\gamma$ -pyridyl carbinol:



This reaction needs about 0.5 V less negative than that required for the reduction of isonicotinic acid i.e. the potential that is very close to the reduction potential of isoniazid.

Because of this reason, reaction 1 & 3 gives a single wave and reaction 2 give a separate wave.

In basic medium (pH 8.10 - 11.14) a four-electron reduction of isoniazid has been found caused by the following reaction:

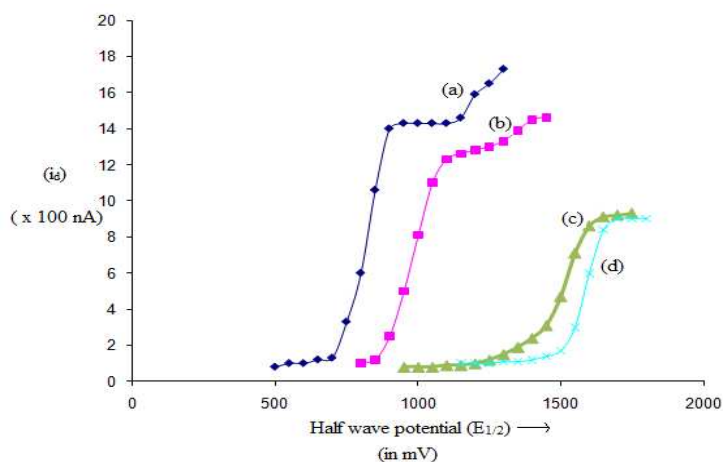


This reaction takes place in one step so that in basic medium isoniazid gives a single wave.

The diffusion current ( $i_d$ ) of first wave and half wave potential ( $E_{1/2}$ ) of first and second wave are given in table (1) and direct current polarograms (DCP) at different pH values for the reduction of Isoniazid at a concentration of  $1.458 \times 10^{-3}\text{M}$  are shown in fig (1).

**Table 1: Effect of pH on diffusion current and half wave potential of Isoniazid wave in B.R. Buffer, Isoniazid =  $1.458 \times 10^{-3}\text{M}$ , Triton-X-100 = 0.001%**

pH	$E_{1/2}$ (Volt)	$I_d \times 100 \text{ nA}$
1.8	-0.806	13.3
3.31	-0.978	11.6
4.38	1.025	6.8
5.81	-1.138	4.3
7.0	-1.474	8.4
8.1	-1.513	8.8
11.14	-1.578	8.0



**Fig. 1: Effect of pH on Isoniazid Polarogram (a) at pH 1.8, (b) at pH 3.8, (c) at pH 7.0 and (d) at pH 11.14**

When we increase the pH of the solution from 1.8 to 11.4,  $i_d$  of the first wave decreases continuously; in acidic medium  $i_d$  of the second wave also decreases but in neutral and basic medium second wave collapses with the first wave. Hence it shows Isoniazid gives two reduction waves in acidic medium and only one in basic medium. The half wave potentials ( $E_{1/2}$ ) of the polarographic waves gets shifted towards more negative potential with an increase in pH from 1.8 to 5.81 (acidic medium) these results are supported by Zuman and co-workers<sup>18-20</sup>, in basic medium  $E_{1/2}$  decreases continuously from pH 7.0 to 11.14 these results are supported by Zuman<sup>21</sup>. These results show that in strong acidic medium the reduction of hydrazide group is easier, with increase in the pH, its reduction becomes difficult.

The polarogram at pH 7.0 was much sharper and well defined so this pH was chosen for subsequent work. Further study is carried out only for the first wave.

## 2) Effect of concentration of drug

The effects of concentration of Isoniazid on polarogram are listed in table (2), the concentration of Isoniazid was varied from  $2.9 \times 10^{-4}\text{M}$  to  $2.9 \times 10^{-3}\text{M}$ . The plots of  $\log [i/(i_d-i)]$  Vs  $E_{d.e}$  were linear with slope values much higher than expected for reversible reaction which suggest that electrode reaction is irreversible. The values of  $E_{1/2}$  are almost constant and  $i_d$  increases with increasing concentration of Isoniazid as expected. Fig (2) shows a linear relationship between  $i_d$  and concentration of drug. Since the reduction of isoniazid is irreversible hence kinetic parameters, like forward rate constant ( $K_{fh}$ ) and transfer coefficient ( $\alpha n$ ) have been calculated using Meites-Israel and Gaur-Bhargava's methods.

Meites - Israel modification of Kotecky's method:-

$$E_{d.e.} = E_{1/2} - \frac{0.0542}{\alpha n} \log \frac{i}{(i_d-i)} \quad (1)$$

$$E_{1/2} = \frac{0.0591}{\alpha n} \log \frac{1.349 K_{fh}^0 t^{1/2}}{D^{1/2}} \quad (2)$$

Gaur-Bhargava modification:-

$$E_{d.e.} = E_{1/2} - \frac{0.05915}{\alpha n} \log \frac{i}{(i_d-i)} \quad (3)$$

$$E_{1/2} = \frac{0.05915}{\alpha n} \log \frac{K_{fh}^0 t^{1/2}}{(\text{antilog } C) D^{1/2}} \quad (4)$$

Where

$K_{fh}$  = formal rate constant for forward reaction

$D$  = diffusion coefficient

$\alpha n$  = transfer coefficient

$E_{d.e.}$  and  $E_{1/2}$  were determined with respect to calomel electrode. The values of  $\alpha n$  were obtained by equation-1 (Meites - Israel method) and equation-3 (Gaur-Bhargava method). The values of  $K_{fh}$  determined by equation-2 (Meites - Israel method) and equation-4 (Gaur-Bhargava method). The values of diffusion coefficient ( $D$ ) were determined by using Ilkovic equation.

$$(i_d)_{\text{max}} = 706 n D^{1/2} C m^{2/3} t^{1/6} \quad (5)$$

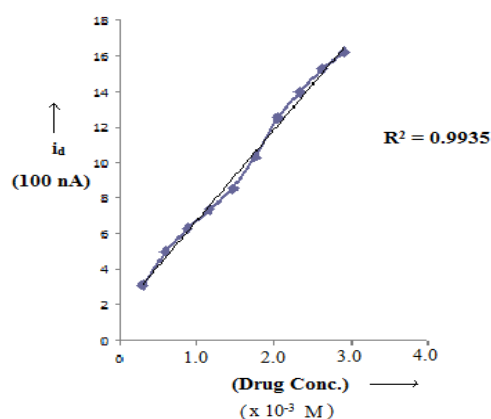
All symbols have their usual meanings.

**Table 2: Effect of concentration of Isoniazid on wave and hence on kinetic parameters in B.R. Buffer at pH = 7.0, Triton-X-100 = 0.001%**

Conc. (M)	E <sub>1/2</sub> (Volt)	I <sub>a</sub> × 100 nA	D (cm <sup>2</sup> sec <sup>-1</sup> )	Slope (mV)	α <sub>n</sub> (M.I.) (V)	α <sub>n</sub> (G.B.) (V)	K <sup>0</sup> <sub>fh</sub> (M.I.) (cm sec <sup>-1</sup> )	K <sup>0</sup> <sub>fh</sub> (G.B.) (cm sec <sup>-1</sup> )
0.292×10 <sup>-3</sup>	-1.527	3.1	3.2104	0.1032	0.5251	0.5731	2.32×10 <sup>-14</sup>	1.81×10 <sup>-15</sup>
0.583×10 <sup>-3</sup>	-1.526	5	2.0879	0.1028	0.5272	0.5753	1.69×10 <sup>-14</sup>	1.31×10 <sup>-15</sup>
0.875×10 <sup>-3</sup>	-1.521	6.3	1.4732	0.1015	0.5339	0.5827	1.06×10 <sup>-14</sup>	7.96×10 <sup>-16</sup>
1.166×10 <sup>-3</sup>	-1.511	7.4	1.1433	0.1006	0.5387	0.5879	8.6×10 <sup>-15</sup>	6.48×10 <sup>-16</sup>
1.458×10 <sup>-3</sup>	-1.51	8.5	0.9654	0.0972	0.5573	0.6082	2.73×10 <sup>-15</sup>	1.85×10 <sup>-16</sup>
1.75×10 <sup>-3</sup>	-1.514	10.3	0.9844	0.0914	0.5926	0.6468	3.14×10 <sup>-16</sup>	1.75×10 <sup>-17</sup>
2.041×10 <sup>-3</sup>	-1.524	12.5	1.0652	0.0852	0.6360	0.6940	1.99×10 <sup>-17</sup>	8.59×10 <sup>-19</sup>
2.333×10 <sup>-3</sup>	-1.527	14	1.0230	0.0804	0.6736	0.7351	1.93×10 <sup>-18</sup>	6.76×10 <sup>-20</sup>
2.624×10 <sup>-3</sup>	-1.525	15.3	0.9654	0.0800	0.6772	0.7390	1.59×10 <sup>-18</sup>	5.51×10 <sup>-20</sup>
2.916×10 <sup>-3</sup>	-1.525	16.2	0.8767	0.0794	0.6819	0.7442	1.15×10 <sup>-18</sup>	3.88×10 <sup>-20</sup>

Where,

K<sup>0</sup><sub>fh</sub> (M.I.) = Formal Rate Constant obtained from Meites & Israel's method K<sup>0</sup><sub>fh</sub> (M.I.) = Formal Rate Constant obtained from Gaur & Bhargava's method

**Fig. 2: Effect of drug concentration on diffusion current**

### 3) Effect of temperature of medium

The effects of temperature on polarograms are listed in table (3), temperature has a very little effect on E<sub>1/2</sub> but i<sub>a</sub> increases with the temperature. The value of α<sub>n</sub> increases with temperature. A decrease in value of α<sub>n</sub> implies that transfer of electrons becomes difficult as temperature was elevated<sup>22</sup>, in present case

transfer of electron becomes easier. Further the values of K<sup>0</sup><sub>fh</sub> decreases with increase in temperature which suggests that irreversibility increases with increase in temperature; this implies that reduction products of isoniazid are stable at higher temperature.

At 30°C well defined and well shaped wave was observed.

**Table 3: Effect of Temperature on Isoniazid wave and hence on kinetic parameters in B.R. Buffer at pH = 7.0, Isoniazid = 1.458×10<sup>-3</sup> M, Triton-X-100 = 0.001%**

Temp. (°C)	E <sub>1/2</sub> (Volt)	i <sub>a</sub> × 100 nA	D (cm <sup>2</sup> sec <sup>-1</sup> )	Slope (mV)	α <sub>n</sub> (M.I.) (V)	α <sub>n</sub> (G.B.) (V)	K <sup>0</sup> <sub>fh</sub> (M.I.) (cm sec <sup>-1</sup> )	K <sup>0</sup> <sub>fh</sub> (G.B.) (cm sec <sup>-1</sup> )
20	-1.502	9.4	1.1809	0.1393	0.3891	0.4246	6.71×10 <sup>-11</sup>	1.14×10 <sup>-11</sup>
25	-1.502	9.6	1.2317	0.1363	0.3976	0.4339	4.16×10 <sup>-11</sup>	6.73×10 <sup>-12</sup>
30	-1.504	11.4	1.7369	0.1306	0.4150	0.4529	1.73×10 <sup>-11</sup>	2.55×10 <sup>-12</sup>
35	-1.506	11.9	1.8926	0.1258	0.4308	0.4702	6.92×10 <sup>-12</sup>	9.33×10 <sup>-13</sup>
40	-1.505	12.4	2.0549	0.1219	0.4446	0.4852	3.27×10 <sup>-12</sup>	4.1×10 <sup>-13</sup>

Further, thermodynamic parameter (ΔH<sub>p</sub><sup>#</sup>, ΔH<sub>v</sub><sup>#</sup>, ΔG<sup>#</sup>, ΔS<sup>#</sup>) have been reported in Table (4). The enthalpy of activation at constant pressure (ΔH<sub>p</sub><sup>#</sup>) has been calculated by substituting the value of slope of the plot (log K<sup>0</sup><sub>fh</sub> v/s 1/T) in the Vant Hoff equation.

$$\Delta H_p^{\#} = 2.303R \times \text{Slope}$$

Where R = Gas constant.

The value of slope comes out to be 6.233 × 10<sup>3</sup>.

$$\Delta H_p^{\#} = \Delta H_v^{\#} + RT$$

From this relation ΔH<sub>v</sub><sup>#</sup> (enthalpy change of activation at constant volume) was evaluated, the activation free energy change (ΔG<sup>#</sup>) was determined the relationship.

$$K^0_{fh} = (KT/h)r_0 \exp(-\Delta G^{\#}/RT)$$

Where K = Boltzmann constant,

h = Plank's constant,

r<sub>0</sub> = mean distance between depolarized ions in the bulk solution,

R = Gas constant,

T = absolute temperature.

In general value of r<sub>0</sub> is taken as 2 × 10<sup>-8</sup> cm<sup>23</sup>. The entropy of activation (ΔS<sup>#</sup>) was calculated using following equation;

$$\Delta S^{\#} = (\Delta H_v^{\#} - \Delta G^{\#})/T$$

The plot of log K<sup>0</sup><sub>fh</sub> vs. 1/T is found to be linear from the slope of which the values of ΔH<sub>p</sub><sup>#</sup>, ΔH<sub>v</sub><sup>#</sup>, ΔG<sup>#</sup> and ΔS<sup>#</sup> have been evaluated and presented in Table (4).

Table 4: Thermodynamic parameters at different temperatures

Temperature(°C)	$\Delta H_p^\ddagger$ (J/Mole)	$\Delta H_v^\ddagger$ (J/Mole)	$\Delta G^\ddagger$ (J/Mole)	$\Delta S^\ddagger$ (J/Kelvin)
20	$11.93 \times 10^4$	$11.69 \times 10^4$	$74.38 \times 10^3$	$21.26 \times 10^2$
25	$11.93 \times 10^4$	$11.68 \times 10^4$	$76.88 \times 10^3$	$15.99 \times 10^2$
30	$11.93 \times 10^4$	$11.68 \times 10^4$	$80.42 \times 10^3$	$12.13 \times 10^2$
35	$11.93 \times 10^4$	$11.67 \times 10^4$	$84.14 \times 10^3$	$9.32 \times 10^2$
40	$11.93 \times 10^4$	$11.67 \times 10^4$	$87.50 \times 10^3$	$7.30 \times 10^2$

$\Delta H_p^\ddagger$  = Enthalpy change for activation process at constant pressure

$\Delta H_v^\ddagger$  = Enthalpy change for activation process at constant volume

$\Delta G^\ddagger$  = Gibbs free energy change for activation process

$\Delta S^\ddagger$  = Entropy change for activation process

A persual of the values of various quantities presents in table (4) show that activation free energy change ( $\Delta G^\ddagger$ ) is positive at all the temperatures suggesting the non spontaneous nature of electrode process. Positive value of  $\Delta S^\ddagger$  suggests that formation of activated state is accompanied by increase of entropy. From table (4) it can be concluded that as we increase the temperature values of the  $\Delta G^\ddagger$  increases and that of the  $\Delta S^\ddagger$  decreases continuously, it shows that the non spontaneity of electrode process increases with temperature.

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