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Kinetics and mechanism of oxidation of Anti-tubercular drug Isoniazid by Peroxomonosulphate

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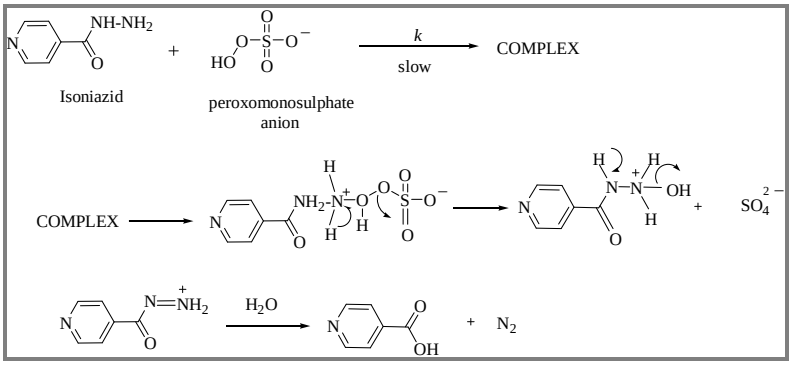
ABSTRACT

The kinetics and mechanistic aspects of oxidation of anti-tubercular drug isoniazid was studied by oxone in acidic medium. The reaction exhibits first order each in [oxone] and [isoniazid]. The reaction rate increased slightly with increase in [acid]. Variation of ionic strength had no effect on the reaction rate. The reaction is failed to induce the polymerization of acrylonitrile. The decrease in the rate of reaction with a decrease in dielectric constant of the medium was observed. The reaction was studied at six different temperatures and the thermodynamic parameters were calculated. The mechanism proposed involves the formation of isoniazid-oxone complex in the slow step, resulting in isonicotinic acid.

Graphical Abstract



Kinetic investigations in the oxidation of isoniazid.

Keywords: Isoniazid, Oxone, Kinetics, Mechanism.

INTRODUCTION

Tuberculosis is the second major cause of death from an infectious disease globally and the World Health Organization estimates that one-third of the world population is infected with Mycobacterium tuberculosis [1]. Presently the front-line treatment for infections by M. tuberculosis is Pyridine-4-carboxylic acid hydrazide commonly known as isoniazid (INH), a potent and selective agent that has been the centerpiece of tuberculosis therapy for over half- a-century, used together with rifampicin

and streptomycin. As a part of its complex mode of action, isoniazid is oxidized to produce acyl radical as an intermediate [**2**], which combines with NAD^+ to produce the actual inhibitor [**3**] of Mycobacterium. The drug has been reported to affect the metabolic activity of mycobacterial metabolism. It inhibits the activity of myeloperoxidase enzyme, which is stored in primary granules and kills the invading pathogens. This digestion and oxidative reaction of pathogens result in the production of reactive intermediates, hydrogen peroxide and superoxide. When hydrogen peroxide reacts with chloride ions results in hypochlorous acid, which damages the tissues at inflammation. Thus, inhibitors are necessary for reducing the activity of myeloperoxidase enzyme. Substituted benzoic acid hydrazides are promising inhibitors [**3**] of myeloperoxidase enzyme activity. These hydrazides react with the intermediate generated by the reaction between myeloperoxidase and hydrogen peroxide. In the absence of an inhibitor like hydrazide, such an intermediate [**4**] reacts with chloride ions to generate hypochlorous acid. Thus, isoniazid in both of its roles, a drug and an inhibitor, undergoes oxidative transformation in the presence of an enzyme as well as an oxidant.

Peroxocompounds like peroxydisulphate, perborate, peroxomonosulphate and organic peroxides are economically preferred for oxidation of organic compounds. Peroxomonosulphate (PMS), commonly known as oxone is an environmentally benign, on-chlorine oxidizing agent, used in a wide variety of industrial and consumer applications (e.g., decolorizing agent in denture cleansers, shock-oxidizer for swimming pools, repulping agent in papermaking, etc.). Lately, the use of PMS has increased rapidly in organic syntheses [**5, 6**]. The main reasons behind its popularity are favorable features such as stability, simplehandling, nontoxic nature, good solubility in water, versatility of the reagent, and low costs.

Therefore, the nature and mechanistic aspects of the oxidation of isoniazid by oxidants like Oxone will be helpful in understanding the metabolic activity and assessment of its oxidative stress as well as its analytical assay [**7**]. In the present study we aim to investigate thoroughly the kinetics and mechanism of oxidation of isoniazid by oxone in sulphuric acid medium, to identify the active species of the substrate, oxidant and oxidation products and evaluate the related kinetic and thermodynamic parameters of the reaction.

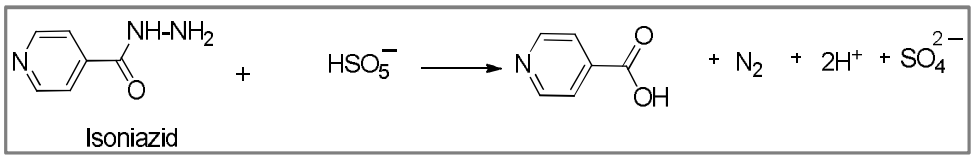
MATERIALS AND METHODS

Double distilled water was used throughout the work. All the chemicals used were of reagent grade. The stock solution of oxone (Aldrich) was prepared by dissolving in water and standardizing iodometrically. The solution of isoniazid (Aldrich) was prepared by dissolving requisite amount in water. The ionic strength was maintained using sodium sulphate, and to vary hydrogen ion concentration sulphuric acid was used. Acetic acid (BDH) was purified by refluxing with chromic acid and acetic anhydride for 6 h and then distilled and used to study the effect of solvent polarity on the reaction medium and acrylonitrile was used directly as received to study the intervention of free radical formation during the reaction. Purity of the substrates was checked by their melting points, UV, IR and NMR spectra. Separation and identification of organic intermediates in the reaction were performed using high performance liquid chromatography (HPLC). The experiments were performed with Shimadzu equipment using an ion-exchange column at 45ºC and a UV detector working at 220 nm. The intermediates and the products were identified from their retention time (t_r).

Kinetic measurements: All the kinetic measurements were carried out in black–coated vessels at constant temperature (± 0.1ºC) and performed under pseudo-first-order conditions with [Isoniazid] >> [oxone]. The reaction was initiated by the rapid addition of known amounts of oxidant to reaction mixtures containing the required amounts of substrate, sulphuric acid, and water in glass–stoppered Pyrex boiling tubes that were thermostated at the same temperature. The progress of the reaction was monitored by iodometric determination of unconsumed [oxone] in known aliquots of the reaction mixtures at different time intervals. However, before adopting iodometric method, it was ensured that the presence of isoniazid in the quenching solution of potassium iodide did not change the oxonetitre

value. Also, the presence of H_2O_2 in the oxone sample was tested. Tests with permanganate showed the absence of free hydrogen peroxide and hence this reagent was used without further purification. The course of the reaction was studied for at least two half–lives. The rate constants (*k*, s^{-1}) were determined from the pseudo-first-order plots of log [oxidant] against time. The pseudo-first-order plots were linear ($r^2 \geq 0.99$) for more than 80 % completion of the reaction and the rate constants (*k*, s^{-1}) were reproducible within ± 5 % .

Stoichiometry and product analysis: Different reaction mixtures with different sets of reactants containing various amounts of oxone and isoniazid at fixed concentration of acid, ionic strength and temperature were allowed to react for 24 h in an inert atmosphere. After completion of the reaction, the unreacted oxone was estimated iodometrically. The obtained results indicated that one mole of oxone consumed one mole of isoniazid in the predominant reaction as represented in the following equation.



The above stoichiometric equation is consistent with the results of product analyses. The oxidation product of isoniazid was identified as the corresponding isonicotinic acid by both spectral and chemical analyses. The yield was about 89%. The melting point of the recrystallized isonicotinic acid was found to be 309°C (lit. m.p = 310°C). The formation of isonicotinic acid was confirmed by IR Spectral (KBr) data (i) a band at (*ν*)1690 cm^{-1} due to –C=O stretching of acid and (ii) a band at (*ν*) 2840 cm^{-1} due to -OH stretching. Isonicotinic acid was further confirmed from ^{1}H-NMR spectrum (DMSO);*δ* 8.7 ppm (s, Ar-2H), 7.52 ppm (s, Ar-2H) and 10.6 ppm (s, 1H–carboxylic acid OH) respectively. Similar oxidation product of isoniazid with different experimental condition was reported earlier [**8-10**].

RESULTS AND DISCUSSION

Effect of Concentration and Kinetic runs: At fixed concentration of other reactants and when [isoniazid] is in 10–fold excess over [oxone], the disappearance rate of [oxone] followed first-order rate law as was observed from the log initial rate (with respect to concentration/time) versus log [oxone] ($r > 0.998$) for more than three half-lives of the reaction. Further, the pseudo–first-order rate constant (*k*, s^{-1}), evaluated from the slopes of such plots remained unchanged Table 1 with the variation of [oxone], confirming the first order dependence of the rate on [oxone].Under the same experimental conditions, the rate of the reaction increased linearly with increase in [isoniazid] (Table 1) and the plot of log k_{obs} against log [isoniazid] was linear with unit slope ($r \geq 0.99$) indicating first order dependence of the rate on [isoniazid]. Further, the double reciprocal plot of k_{obs} versus [isoniazid] was also linear ($r>0.99$) passing through the origin **(**Figure 1), inferring the formation of an intermediate between oxone and isoniazid in the slow step.

The effect of $[H^+]$ on the reaction rate was studied in order to establish the active species of reactants present in the solution. At fixed concentrations of substrate (isoniazid), oxone, and other conditions remaining constant, the reaction rate increased linearly with increase in $[H_2SO_4]$ shown in table 1. The effect of ionic strength of the medium on the reaction rate was studied using Na_2SO_4, with other experimental conditions held constant. There was no significant effect of ionic strength on the reaction rate. The dielectric constant (D) of the medium (Table 2) was varied using different proportions of acetic acid from 30-60%. The D values were calculated from the equation $D = D_W V_W + D_A V_A$, where D_W and D_A are the dielectric constants of pure water and acetic acid respectively, and V_W and V_A are the volume fractions of components water and acetic acid respectively in the total

mixture. The reaction rate decreased with a decrease in dielectric constant of the medium. Plot of log *k* versus 1/D (Figure 2) was found to be linear with negative slope. Blank experiments performed showed that acetic acid was not oxidized significantly by oxone under prevailing conditions.

Table 1. Dependence of rate on the factors influencing the oxidation of Isoniazid by oxone in acidic medium at 300 K

[Oxone] × 10^{-4} (mol. dm^{-3})	[Isoniazid] × 10^{-3} (mol. dm^{-3})	[H_2SO_4] (mol. dm^{-3})	k× 10^3 (s^{-1})
2.50	2.00	0.05	4.20
4.00	2.00	0.05	4.18
5.00	2.00	0.05	4.22
7.50	2.00	0.05	4.21
10.00	2.00	0.05	4.19
15.00	2.00	0.05	4.21
5.00	**0.50**	0.05	1.22
5.00	**1.00**	0.05	2.18
5.00	**2.00**	0.05	4.22
5.00	**4.00**	0.05	7.17
5.00	**5.00**	0.05	9.59
5.00	**10.00**	0.05	18.42
5.00	2.00	**0.02**	2.90
5.00	2.00	**0.03**	3.45
5.00	2.00	**0.04**	3.83
5.00	2.00	**0.05**	4.22
5.00	2.00	**0.06**	4.71
5.00	2.00	**0.08**	5.20

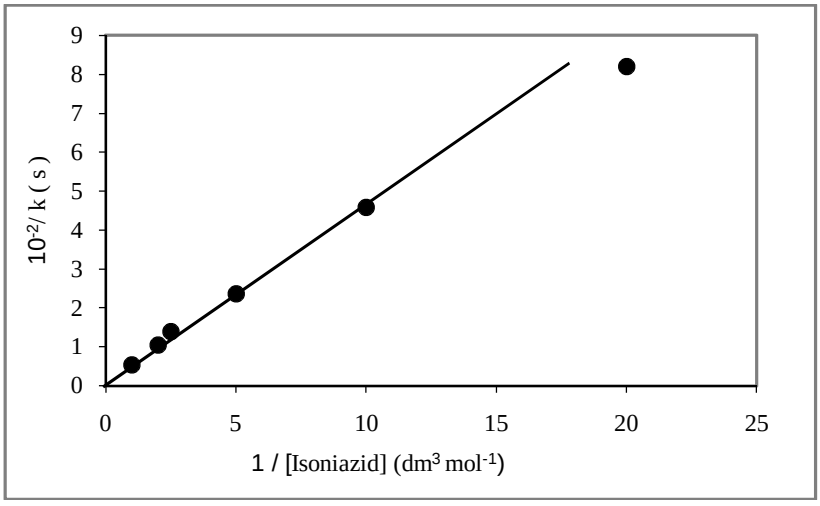


Figure 1. Plot of 1/*k* against 1/[Isoniaizid]

Table 2. Effect of dielectric constant on the reaction rate at 300 K

AcOH : H_2O	D[a]	k× 10^3 (s^{-1})
30 : 70	53.19	4.95
40 : 60	46.49	4.42
50 : 50	39.79	3.91
60 : 40	33.09	3.14

Experimental conditions: [Isoniazid] =2.0 × 10^{-3} mol·dm^{-3}, [Oxone] = 5.0 × 10^{-4} mol·dm^{-3}, [H_2SO_4] = 0.05 mol·dm^{-3};
D[a]: Values are calculated from the values of pure solvent.

Test for Free Radicals: The reactions were studied in the presence of added acrylonitrile to

understand the intervention of free radicals. There was no effect of added acrylonitrile (0.1–1.0 mold m^{-3}) on the reaction rate, and also no precipitate due to polymerization of acrylonitrile was observed, suggesting the absence of any free radical formation in the reaction. To further confirm the absence of free radicals in the reaction pathway, the reaction was carried out in the presence of 0.05 mold m^{-3} of 2, 6-di-*t*-butyl-4-methylphenol (butylated hydroxyl toluene or BHT). It was observed that the BHT was recovered unchanged, almost quantitatively.

Effect of Temperature: The oxidation of isoniazid was studied in the temperature range (Table 3) of 293–313 K and the activation parameters were evaluated from the slope of Arrhenius plot (Figure 3) of log *k* versus 1/T are: *E*a = 40.78± 1.5 kJ $mole^{-1}$, $\Delta H^{\neq}$ = 398.29± 1.5 kJ $mole^{-1}$, $\Delta S^{\neq}$ = –151.64 J K^{-1} $mole^{-1}$ and $\Delta G^{\neq}$ = 83.78± 1.2 k J $mole^{-1}$ at 300 K at concentrations of oxone = 5.00×10^{-4}moldm^{-3}, Isoniazid = 2.00×10^{-3} mold m^{-3}; H_2SO_4 = 0.05 moldm^{-3}. Large negative value of entropy indicates that the complex is more ordered than the reactants.

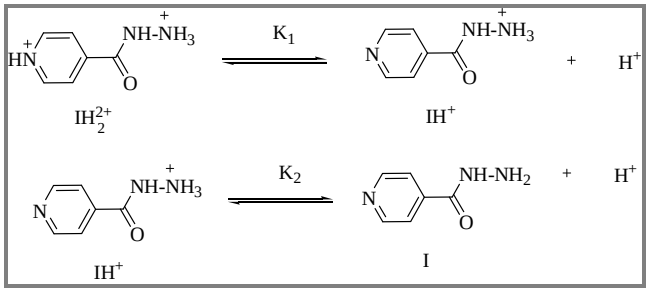
Table 3. Influence of temperature on the rate of the reaction

Temp (K)	293	298	300	303	308	313
$k\times 10^3$ (s^{-1})	2.68	3.45	4.22	4.60	5.75	7.57

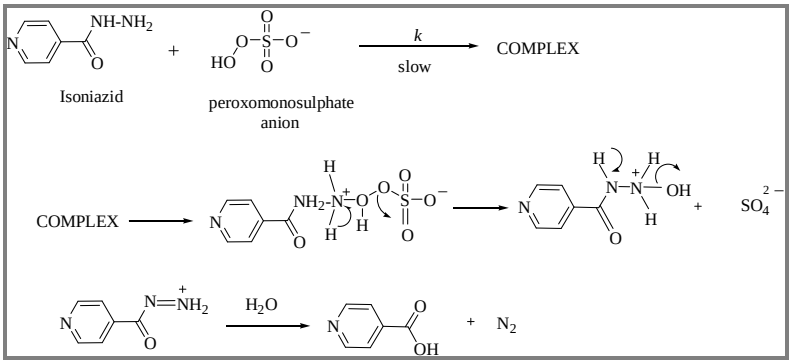
Experimental conditions: [Isoniazid] =2.0×10^{-3} mol·dm^{-3}, [Oxone] = 5.0×10^{-4} mol·dm^{-3}, [H_2SO_4] = 0.05 mol·dm^{-3}

Active Species of the Reactants: The structure of Oxone or Peroxomono sulphuric acid (PMS), used in the present study contains a sulphur atom surrounded tetrahedrally by perhydroxyl group and hydroxyl group. Peroxomonosulphate can be considered as a monosubstituted hydrogen peroxide in which one of the hydrogens is replaced by the SO_3 group, the other hydrogen comes from the acid group. Peroxide act as an oxygen donor to the organic substrate. In fact, it is the peroxide bond in these peracids [**11**] thatis mainly responsible for its reactions. The proton of the hydroxyl group is equivalent to that of sulphuric acid proton and is highly ionized while that of perhydroxyl group is weakly ionized. The *p*K value of the perhydroxyl proton is [**12**] to be 9.4, indicating that in strongly acidic *p*H, the peroxomonosulphate exists mainly in the form of HSO_5^- ion and is an effective nucleophile [**13-15**]. Since the present reaction is studied in the *p*H range of 1 to 2, the oxidant is in the form of peroxomonosulphate anion, HSO_5^-[**16**].

There are two possible protonation sites in isoniazid [**9**], the pyridine nitrogen and the $–NH_2$ group of isoniazid. The *p*K of pyridine nitrogen is found to be [**17**] 1.8 and that of the $-NH_2$ group of isoniazid is 3.5. Since the reaction was carried out in acidic medium the hydrazide moiety will be completely in its protonated form due to its very low *p*K values [**18**]. Therefore, within the range of [H^+] studied the isoniazid is completely transformed into the diprotonated form IH_2^{2+} [**9**]. Thus, the active species of isoniazid in acidic medium is IH_2^{2+}, which contains protonated pyridine nitrogen and a protonated $–NH_2$ group of hydrazide moiety. As the protonation of isoniazid occurs, the rate constant values increase slightly by varying the concentration of acid. In case of absence of protonation of oxidant and substrate the rate remains constant [**18**] with a change in [H^+]. The complexation between the protonated $–NH_2$ group and active species of the oxidant leads to further reaction while that protonated pyridine nitrogen does not undergo any further reaction.



Mechanism: Based on the aforementioned reasons and in accordance with experimental results, the mechanism as shown in scheme 1 is proposed for the isoniazid oxidation by oxone.



Scheme 1. Kinetic investigations in the oxidation of isoniazid.

The present reaction between isoniazid and oxone in acid medium has a stoichiometry of 1:1 with a first order dependence on [isoniazid] and [oxone]. The linear plot of 1/*k* against 1/[isoniazid], passing from the origin (Figure 1) infers and is evident for the complex formation between the isoniazid and oxone in the slow step. The decrease of reaction rate by increasing the composition of acetic acid in the reaction mixture (Table 2), while keeping the oxidant and substrate concentrations unchanged suggests that the dielectric constant of the medium play an important role. The linear plot of log *k* versus 1/D with a negative slope (Figure 2) suggests that there is a charge development in the transition state involving a more polar activated complex than the reactants [**19-22**], a neutral molecule and mononegative ion (HSO_5^-), suggesting a polar ionic mechanism. The Arrhenius plot of log *k* versus 1/T was a straight line. From the slope value of the straight line, the thermodynamic parameters of the reaction were calculated. The positive values of free energy of activation $\Delta G^{\#}$ and enthalpy of activation, $\Delta H^{\#}$ in the present study indicated that transition state was highly solvated while large negative value of entropy of activation, $\Delta S^{\#}$ suggested the formation of a rigid transition state with reduction of degree of freedom of molecules.

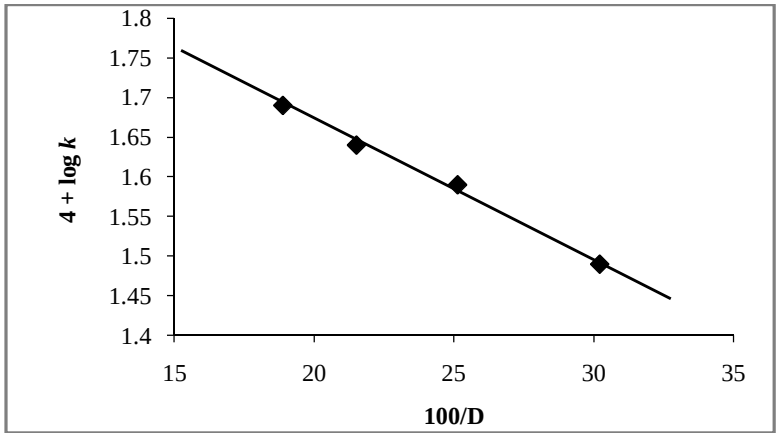


Figure 2. Plot of log *k* against 1/D.

The rate equation in consonance with the mechanism proposed is:

$$\text{Rate} = \frac{-\text{d[PMS]}}{\text{dt}} = k\,[Isoniazid][HSO_5^-] \qquad \ldots (1)$$

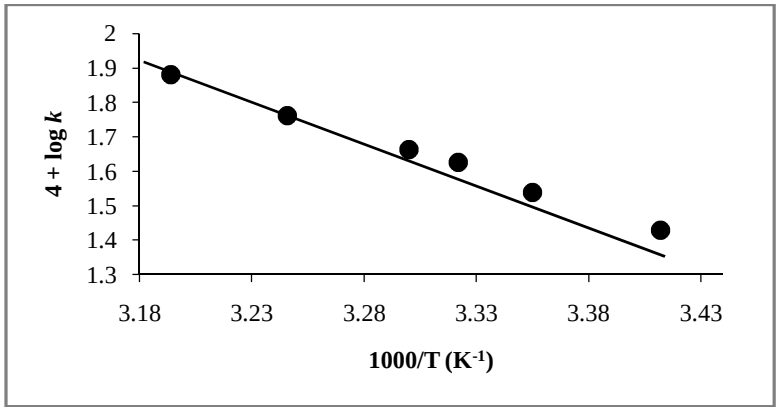


Figure 3. Arrhenius plots of log *k* versus 1/T.

The rate law (Eq. 1) is in accordance with the observed experimental results, expressing the first-order dependence on [oxone], and [isoniazid].The larger negative value of entropy of activation in conjunction with other experimental data supports the proposed mechanism outlined in the scheme 1.

APPLICATION

Inspite of many applications [23] of oxone, its use in the kinetic investigations of oxidation of anti-tubercular [24] drug isoniazid helps in improving the understanding of oxidation process of isoniazid in acidic medium. Oxone provides a green and efficient method of analysis of isoniazid, as oxone is easily available, simple in handling and less hazardous in nature. This study focuses on the development of facile and reliable method to understand the oxidation of anti-tubercular prodrug Isoniazid.

CONCLUSION

The kinetics of oxidation of isoniazid by oxone has been investigated in sulphuric acid medium. The oxidation product was identified as isonicotinic acid by spectral and chemical analyses. The reaction was carried out at six different temperatures and the activation and thermodynamic parameters were evaluated. A plausible mechanism has been proposed to explain the experimental observations.

ACKNOWLEDGEMENTS

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