



CHEMICAL KINETIC AND MECHANISTIC STUDY OF OXIDATION OF ISONIAZID BY AMMONIUM METAVANADATE IN AN ACIDIC MEDIUM BY SPECTROPHOTOMETRY

Pore Sanjay Vishnu

Department of Chemistry, Bharati Vidyapeeth's Matoshri Bayabai Shripatrao Kadam Kanya

Mahavidyalaya Kadegaon, Sangli, Maharashtra, India

*Corresponding author: poresanjay67@gmail.com

ABSTRACT

The chemical kinetic and mechanistic study of oxidation of Isoniazid by ammonium metavanadate in an acidic medium has been studied spectrophotometrically by maintaining pseudo-first order reaction condition in which concentration of Isoniazid was in excess than oxidizing agent. The concentration dependence of Isoniazid, ammonium metavanadate, medium of the reaction, ionic strength, specific ions, temperature, dielectric constant and various solvents on the rate of oxidation reaction was observed. The determination of reactive intermediate, intervention of free radical, stoichiometry and end product analysis was carried out using standard parameters of the reaction. The order of reaction with respect to oxidant is one. The decrease in rate constant with increase in the concentration of Isoniazid is attributed to greater stability of the complex due to solvation. The specific rate of the reaction increases with increase in the concentration of acid. The oxidation rate is not influenced by increase in ionic strength and changing the various salts. The specific rate of the oxidation of Isoniazid is directly proportional to the dielectric constant of the reaction medium. The thermodynamic parameters temperature coefficient (1.97), energy of activation ($E_a=56.57 \text{ KJmol}^{-1}$), enthalpy of activation ($\Delta H^\ddagger=53.35 \text{ KJmol}^{-1}$), entropy of activation ($\Delta S^\ddagger=-135.75 \text{ J K}^{-1}\text{mol}^{-1}$) and free energy of activation ($\Delta G^\ddagger = 95.84 \text{ KJmol}^{-1}$) were obtained by carrying out the reaction at seven different temperatures. The mol ratio of Isoniazid: ammonium metavanadate is found to be 1:4 and it was independent of concentration of medium of the reaction. The mechanism in terms of the active species of the oxidant and Isoniazid is proposed.

Keywords: Isoniazid, Chemical Kinetics, Mechanistic study, Spectrophotometry, Oxidation.

1. INTRODUCTION

Chemical kinetics is a key sub discipline of chemistry that studies the reaction rate in every elemental step and corresponding catalytic mechanism. Hydrazides are obtained by preparing derivatives of Carboxylic acids and hydrazines [1]. It's multiple use in various branches of chemistry [2-6] especially pharmaceutical chemistry [7], needs to study the mechanism of their oxidation in detail. The formation of corresponding acids [6, 8-14] is observed during the most of the oxidation reactions of hydrazines. Literature survey shows that, the chemical kinetic study of oxidative degradation of Isoniazid by ammonium metavanadate is not extensively studied. The present study has been carried out with a view to postulate general mechanism for the oxidation of Isoniazid with ammonium metavanadate. Ammonium metavanadate, a versatile oxidizing agent, has also been used to oxidize many organic compounds like hydroxyl

amine hydrochloride, acetaniides, L-Cysteine, glycerol and cyclohexanol [15-19]

2. MATERIAL AND METHODS

The ammonium metavanadate (AR grade), hydrazine hydrate (BDH 99%), anhydrous sodium bicarbonate, sulphuric acid, salt, and freshly distilled ethanol was used. Ethyl ester of pyridine 4 carboxylic acid was prepared by esterification [20] and converted to Isoniazid by using the prescribed procedure [21]. An equimolar mixture of ethyl ester of Pyridine 4 Carboxylic acid and hydrazine hydrate (B.D.H. 99%) was refluxed for more than fifteen minutes. Then enough absolute ethanol was added through the condenser to get clear solution and further refluxed for more than six hours. The excess of hydrazine hydrate, solvent ethanol and other unreacted material was removed by distilling the solution under reduced

pressure. The resulting Isoniazid recrystallized from ethanol and stored in amber colored bottles and kept in dark place.

The distilled water obtained by redistillation in the presence of a few crystals of KMnO_4 and a few pellets of KOH using borosil glass distillation assembly was used throughout the experiment. The 0.01 M solution of oxidant ammonium metavanadate was prepared by dissolving accurately calculated and weighed quantity of ammonium metavanadate in hot double distilled water using Pyrex glass measuring flask. The standardization of ammonium metavanadate (v) solution was done by titrating it against standard $\text{Fe}(\text{NH}_4)_2\text{SO}_4$ solution by using diphenylamine as an indicator. Similarly, the stock solution of NaClO_4 was prepared by dissolving equivalent quantities of Na_2CO_3 and HClO_4 (70% E. Merck) in H_2O to maintain ionic strength. Standard Isoniazid solution was prepared by dissolving it in aqueous alcoholic medium.

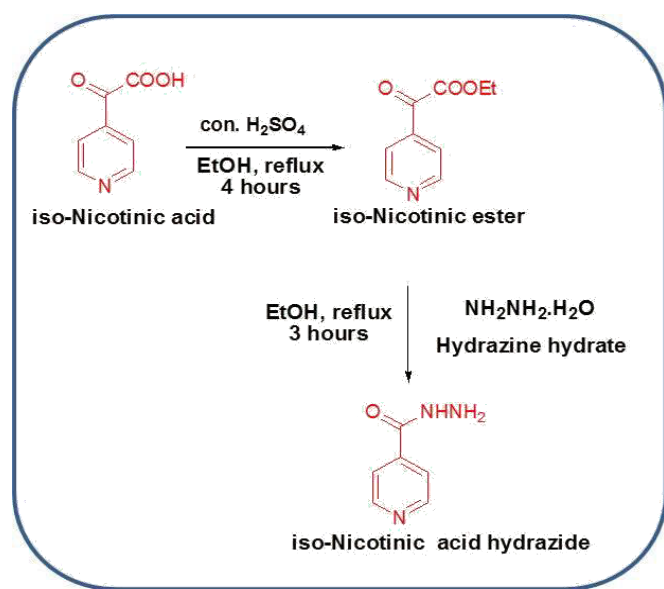


Fig. 1: Reaction Scheme

2.1. Determination of absorption maxima (λ_{max}) of the reaction

The absorption maxima *i.e.* λ_{max} of the reaction was determined by varying wavelength. It was observed that maximum absorbance for both ammonium metavanadate and a mixture of Isoniazid and ammonium metavanadate was obtained at $390 \text{ nm } \lambda_{\text{max}}$.

2.2. Method of following the kinetic reaction

The oxidant and Isoniazid were taken in separate conical flasks along with required quantities of H_2SO_4 and

NaClO_4 and were kept in a constant temperature bath at $35 \pm 0.1^\circ \text{C}$ for more than half an hour. The kinetic study was followed by mixing thermally equilibrated solution of reactants and transferring the reaction mixture to 1 cm^3 cuvette. The progress of reaction was checked by measuring absorbance of the reaction mixture at 390 nm using UV-Vis. spectrophotometer in H_2SO_4 medium using H_2O as a reference solvent. The reaction was studied under pseudo-first order condition in which, concentration of Isoniazid was in excess as compared to that of ammonium metavanadate. The reaction is found to proceed through formation of complex between ammonium metavanadate and Isoniazid. The pseudo-first order rate constant K was obtained by plotting the log of absorbance at 390 nm against time. The rate constant K for Isoniazid was found to be fairly constant at different concentrations of ammonium metavanadate.

2.3. Reaction intermediates: intervention of free radical

The induced polymerization of acrylonitrile [22] and spontaneous reduction of mercuric chloride [23] in reaction mixture itself indicates that, the present reaction involves the formation of free radical.

2.4. Effect of sulphuric acid on substrate (hydrazide)

A standard kinetic experiment was repeated by only changing the concentration of H_2SO_4 from 0.0025 to 0.1 M containing equivalent quantities of Isoniazid, oxidant, salt and temperature. The mixtures in each flask were made alkaline and tested for detection of hydrazine after three hours. It was seen that, the resultant solution in various flasks didn't give any response to ammonical silver nitrate and Fehling's solution, indicating the absence of hydrazine. Hence it can be concluded that, there is no significant hydrolysis of Isoniazid in the presence of H_2SO_4 up to 0.1 M.

2.5. Confirmation of status of acid content and to check hydrolysis of Isoniazid under experimental conditions

The confirmation of hydrolysis of substrate under experimental conditions was done by conducting similar set of experiments. It was subjected to alkalimetric estimation of acid content in the beginning and at the end. When it was found that there was no perceptible change in acid content of any mixture after a period of minimum three hours. This invariance of acid concentration can be attributed to the fact that, there

was no hydrolysis of Isoniazid under experimental conditions was observed. Similar observations are already reported [24].

2.6. Stoichiometry of the reaction

The number of moles of ammonium metavanadate consumed by one mole of Isoniazid in reaction under investigation means the stoichiometry of the reaction was investigated by spectrophotometric method. A set of five reaction mixtures containing a known excess of NaClO_4 over Isoniazid in the presence of $1.0 \times 10^{-2} \text{ M}$ H_2SO_4 and $5.0 \times 10^{-2} \text{ M}$ NaClO_4 was kept in a constant temperature bath at 35°C for more than 48 hours. A blank experiment without Isoniazid was carried out concurrently using identical quantities of ammonium metavanadate, H_2SO_4 and NaClO_4 after volume correction with water for Isoniazid solution.

After completion of the reaction, concentration of ammonium metavanadate (IV) was determined at 765nm ($\epsilon = 17.77$) [25] from its absorbance and molar extinction coefficient (ϵ) by using the expression $C = \text{Abs.}/\epsilon$. Finally, the moles of ammonium metavanadate(IV) formed in oxidation of one mole of Isoniazid were calculated in each case by using the relation.

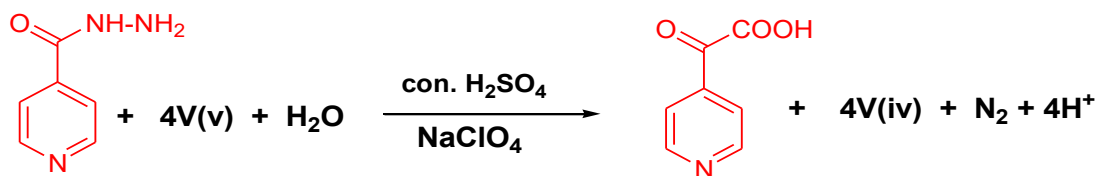
$$\text{Mole ratio} = -[\text{V (IV)}] / [\text{Isoniazid}]_0$$

The value of $[\text{V (IV)}]/[\text{Isoniazid}]_0$ for different experiments was found to be nearly equal to 4. Therefore it is concluded that 4 moles of ammonium metavanadate were required for oxidation of one mole of Isoniazid.

2.7. Identification of oxidation products

For the identification of oxidation products, the reaction was studied by using Isoniazid and ammonium meta-

vanadate in stoichiometric proportions in presence of $1.0 \times 10^{-2} \text{ M}$ H_2SO_4 and $1.0 \times 10^{-1} \text{ M}$ sodium perchlorate. The flask containing reaction mixture was kept in a thermostated water bath maintained at 35°C for 26 hours to complete the reaction. After the completion of the reaction, the reaction mixture was subjected to ether extraction and acid was separated. The presence of carboxylic acid group was detected by testing with 5 % bicarbonate solution. The amide derivative of the corresponding aromatic carboxylic acid was prepared [26]. The observed physical constant of amide derivatives was found to be in good agreement with those of benzamide. The formation of corresponding carboxylic acids in oxidation of Isoniazid was accompanied by evolution of nitrogen gas. Nitrogen was detected by lime test [27]. A mixture of lime and MnO_2 in 10:1 proportion was ignited in a small hard glass tube. A test portion of concentrated reaction mixture was rendered neutral with NaOH solution and it was added to the ignited mixture. The tube was heated slowly and the liberated gas was tested with filter paper moistened with MnNO_3 and AgNO_3 solutions. This indicator paper held at the mouth of the tube shows grey fleck, which turns blue immediately on treatment with a drop of benzidine solution which indicates the formation of nitrogen gas during the oxidation. Finally considering the products of oxidation, observed mole ratio and material balance, the oxidation of Isoniazid can be represented by the stoichiometric equation. The above equation not only consistent with stoichiometry and products of reaction, but also explains the observed gradual decrease in k values with increase in time, which can be attributed to increasing proportions of H^+ ions with the progress of the reaction.



3. RESULTS AND DISCUSSION

It was found that, the reaction proceeds with a measurable velocity using $1.5 \times 10^{-3} \text{ M}$ ammonium metavanadate, $1.5 \times 10^{-2} \text{ M}$ Isoniazid, $1.1 \times 10^{-1} \text{ M}$ NaClO_4 , $1.5 \times 10^{-2} \text{ M}$ H_2SO_4 and 390 nm λ_{max} at 35°C . The observed rate constant (k) of the reaction goes on slightly decreasing with time, the order of reaction with respect to ammonium metavanadate(v) is one.

3.1. Mechanism of the reaction

The detailed mechanism of the reaction in terms of the active species of the HVO_3 and substrate protonated Isoniazid is shown in the Fig.2.

According to the above scheme the rate of the reaction is given by $\text{Rate} = k_c [\text{Complex}]$

Substituting the value of $[\text{Complex}]$ from the equilibrium,

$$\text{Rate} = k_c K_c [\text{HVO}_3] [\text{Ar-CONH-NH}_3^+]$$

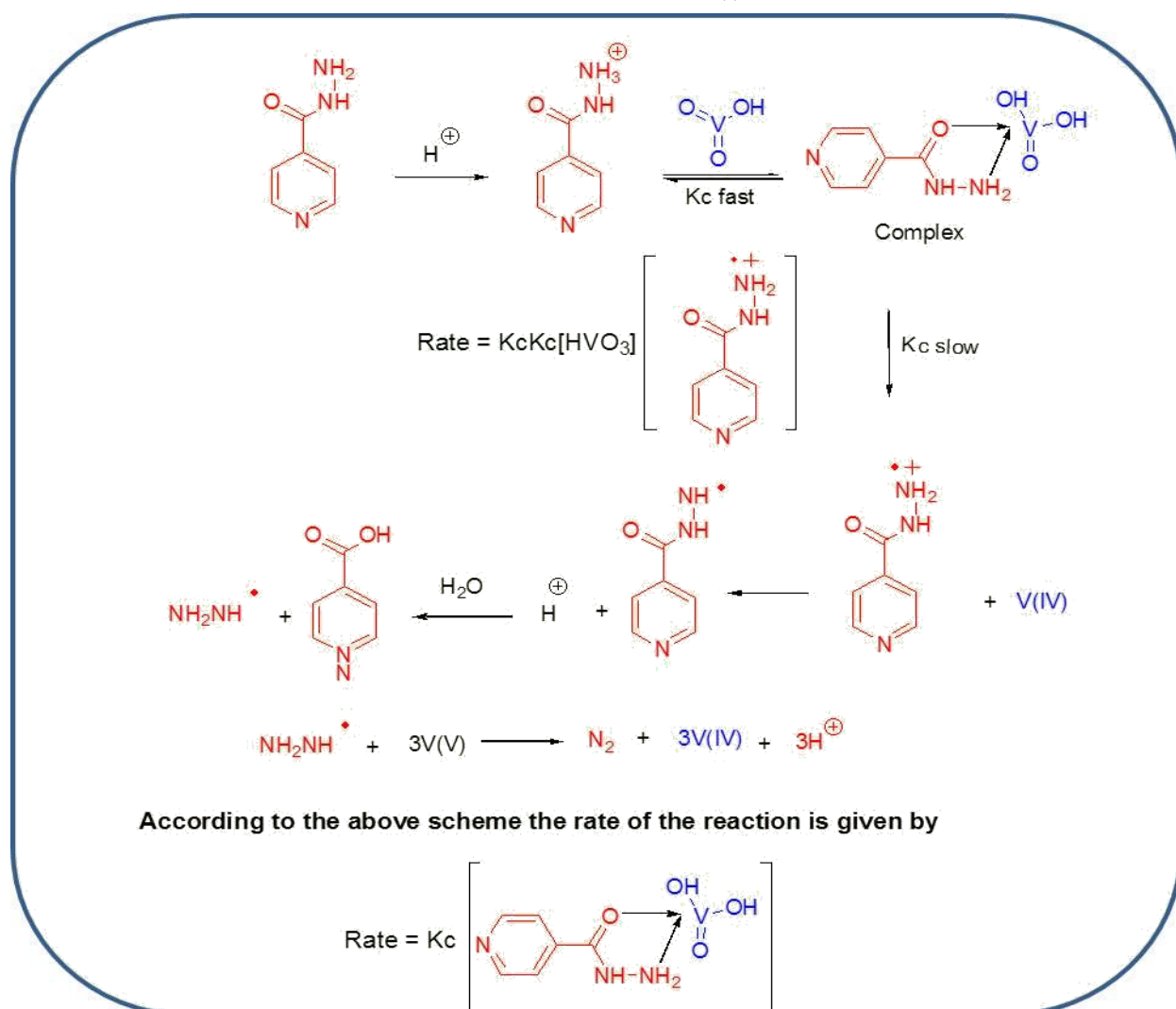


Fig. 2: Mechanism of the reaction

Table 1: Specific reaction rate constants determined by graphical method

Effect of	Unit Concentration	Multiples of Unit Concentrations and respective rate Constants						
[Isoniazid]	$[NI] \times 10^3 M$	0.3	0.6	1.2	1.5	1.8	2.4	3.0
	$k \times 10^4 sec^{-1}$	3.79	3.69	3.60	3.51	3.08	2.68	2.39
[AMV]	$[AMV] \times 10^3 M$	0.3	0.6	1.2	1.5	1.8	2.4	3.0
	$k \times 10^4 sec^{-1}$	3.66	3.67	3.62	3.51	3.58	3.41	3.50
[H ₂ SO ₄]	$[H_2SO_4] \times 10^2 M$	0.5	1.0	1.5	2.0	3.0	4.0	5.0
	$k \times 10^4 sec^{-1}$	0.961	2.06	3.51	3.71	3.93	4.10	4.31
[NaClO ₄]	$[NaClO_4] \times 10^1 M$	0.3	0.6	1.0	1.5	2.0	2.5	3.0
	$k \times 10^4 sec^{-1}$	3.52	3.44	3.51	3.41	3.54	3.44	3.50
Various Salts	$[SALT]_0 = 1.0 \times 10^{-1} M$	LiCl	NaCl	KCl	MnCl ₂	LiClO ₄	NaClO ₄	KClO ₄
	$k \times 10^4 sec^{-1}$	3.45	3.42	3.49	3.57	3.53	3.51	3.38
Dielectric Constant	Ethanol %	40	50	60	70	80	--	--
	Water %	60	50	40	30	20	--	--
	Dielectric constant	69.23	65.18	61.29	55.74	48.48	--	--
Temperature	$k \times 10^4 sec^{-1}$	4.12	3.87	3.51	2.35	1.64	--	--
	Temperature in °C	25	30	35	40	45	50	55
	$k \times 10^4 sec^{-1}$	1.76	2.65	3.51	5.17	6.84	10.12	13.41

Table 2: Various energy parameters calculated

Sr	Energy parameters	Value	Sr	Energy parameters	Value
1	Temperature Coefficient	1.97	4	Entropy of activation ΔS^\ddagger (J K ⁻¹ mol ⁻¹)	-135.75
2	Energy of activation Ea (KJ mol ⁻¹)	54.89	5	Free energy of activation ΔG^\ddagger (KJ mol ⁻¹)	95.84
3	Enthalpy of activation ΔH^\ddagger (KJ mol ⁻¹)	53.35			

4. CONCLUSION

The investigation of the effect of Isoniazid concentration on the reaction rate shows that, the pseudo-first order rate constant decreases with increase in Isoniazid concentrations. It can be attributed to greater stability of the complex in alcoholic medium probably due to solvation. The effect of oxidant concentration on the reaction shows that, the rates of oxidation of Isoniazid are almost constant with increase in concentration of ammonium metavanadate and the order of the reaction with respect to ammonium metavanadate remains one throughout the used concentrations of ammonium metavanadate. In other words, Oxidation follows the first order kinetics because the log (Abs) versus time plot in each case is linear with positive slope and intercept on log (Abs) axis. The constancy of k values at different initial [ammonium metavanadate] indicates the pseudo-first order kinetic behavior of reaction.

The effect of concentration of H₂SO₄ on the rates of oxidation of Isoniazid shows that, the specific reaction rate increases as the concentration of H₂SO₄ increases. The reaction rates are not influenced by increase in ionic strength similarly the reactions rates are not influenced by using various salts, under the experimental conditions.

The specific rate of reactions is decreasing with decrease in dielectric constant of the reaction medium. The study of effect of temperature on oxidation of Isoniazid clearly shows that, the rate of reaction depends on temperature and it increases with increase in temperature. The values of observed rate constants were used to determine various thermodynamic parameters like temperature coefficient (1.97), energy of activation (Ea) 54.89 KJ mol⁻¹, enthalpy of activation (ΔH^\ddagger) 53.35 KJ mol⁻¹, entropy of activation (ΔS^\ddagger)-135.75 J K⁻¹ mol⁻¹ and free energy of activation (ΔG^\ddagger) 95.84 KJ mol⁻¹. The log (Abs) against time plots at different temperatures are linear which reveals that, the pseudo-first order kinetic behavior of the reaction is not affected by change in temperature. The formation of free radicals or radical ions during the course of reaction was confirmed from induced polymerization of acrylonitrile. The formation of carboxylic acids and N₂ in the oxidation of aliphatic as

well as aromatic acid hydrazides is well documented in literature. The mole ratio of Isoniazid:ammonium metavanadate is found to be 1:4 and it is independent of concentration of H₂SO₄.

The integer value of observed mole ratio, its independence on H₂SO₄ concentration and formation of only carboxylic acid along with nitrogen gas as oxidation products leads to deduce that, the two rate determining steps occurring simultaneously results in the formation of one and the same intermediate. Although the observed mole ratio (Isoniazid: ammonium metavanadate) of the reaction is 1:4, as is pointed out earlier, the order of reaction with respect to ammonium metavanadate is one.

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Conflict of interest

The author declares no conflict of interest.

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