

Kinetic and Mechanistic Investigation on Oxidation of Levamisole hydrochloride by Bromamine-T in Hydrochloric acid Medium

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Abstract: Kinetics of oxidation of Levamisole hydrochloride (LVM) by sodium *N*-bromo-*p*-toluene sulfonamide or bromamine-T (BAT) in presence of HCl has been investigated at 303K. The Stoichiometry of the reaction was found to be 1:1 and the oxidation product of levamisole hydrochloride was identified as Levamisole *S*-oxide was characterized by GC-MS analysis. The rate of reaction shows first-order dependence on $[BAT]_0$, $[LVM]_0$ and fractional-order dependence with $[H^+]_0$. Effects of added *p*-toluene sulfonamide (PTS) and halide ions, varying ionic strength ($NaClO_4$) and dielectric constant of the medium on the rate of reaction have been investigated. Activation parameters were calculated and the reaction constants involved in the mechanisms have been computed. The proposed mechanisms and the derived rate laws are consistent with the observed kinetics.

Keywords: Kinetics, Levamisole hydrochloride, oxidation, bromamine-T, mechanism.

I. Introduction

Levamisole Hydrochloride, (6S)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-b] thiazole (LVM) was an anthelmintic and immunomodulator belonging to a class of synthetic imidazothiazole derivatives. It was discovered at Janssen Pharmaceutica (Belgium) in 1966 [1-2] and having anti cancer activities [3].

In the early stages, LVM was used for only animals like cattle, pigs and sheep. In 1971, it was found to have immunostimulatory properties then it was used for humans [4]. Currently, LVM was used in humans for diseases related to imbalances in the regulation of immune responses or deficiencies of the immune system, including autoimmune diseases, chronic and recurrent diseases, chronic infections and cancer. It had beneficial effects on host defense mechanisms and restores depressed immune responses in animals and humans.

LVM was safe effective anthelmintic to use in aquariums when administered in the proper dosage and effective against many internal parasites, especially nematodes, when used in appropriate dosages. It did not harm the bio-filter, plants, invertebrates or uninfected fish. As an added benefit, it boosts the immune competence of fish, humans, large animals, birds and some reptiles.

Use of LVM have been reported some serious side effects. It includes, allergic reactions like difficulty in breathing, closing of the throat, swelling of the lips, tongue, or face or hives, decreased bone marrow function or blood problems (fever or chills or signs of infection) nervous system problems (confusion or loss of consciousness, extreme fatigue, memory loss, muscle weakness, numbness or tingling, seizure, speech disturbances) and others.

There was no report available in the literature with regard to the kinetics of oxidation of this drug by any oxidants. The oxidation kinetic study was much benefit in understanding the mechanistic profile of LVM in acidic medium so that the study could through some light on the mechanism of metabolic conversion of LVM in biological systems.

II. Experimental

2.1 Materials

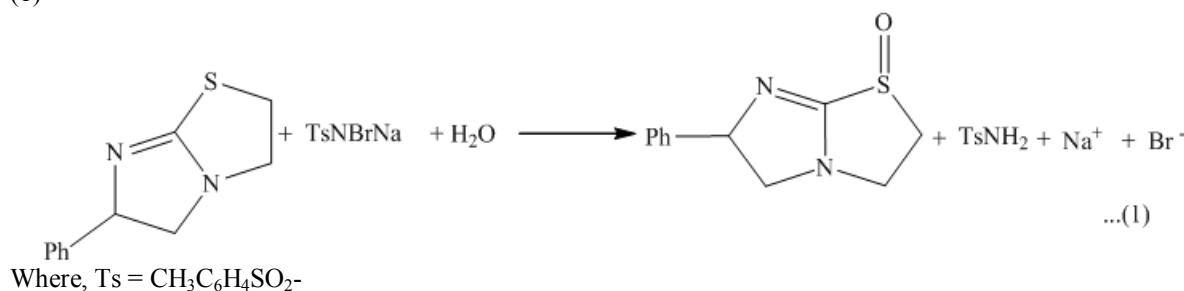
Bromamine-T was prepared [5] using chloramines-T (CAT). To a solution of CAT (20 g in 400 mL of water), 4 ml of liquid bromine was added drop wise with constant stirring at room temperature to yield dibromamine-T (DBT). The solid DBT was filtered under suction, washed thoroughly with ice cold water until all the absorbed bromine was removed and then vacuum-dried for 24h. About 20 g of DBT thus obtained was dissolved in 30 mL of 4 mol dm⁻³ NaOH with constant stirring at room temperature and the resultant aqueous solution was cooled in ice. Pale yellow crystals of BAT formed was filtered under suction, washed quickly with the minimum amount of ice cold water and dried over P₂O₅. Aqueous solution of the oxidant was standardized by iodometric procedure and preserved in brown bottles to prevent photochemical reactions.

2.2 Kinetic procedure

Reactions were carried out under pseudo first-order conditions with a known excess of $[\text{substrate}]_0$ over $[\text{oxidant}]_0$ at constant temperature 303 K in glass stoppered Pyrex boiling tubes coated black from outside to eliminate any photochemical deterioration. A Raaga digital proportional temperature controller (CH-61) was used to maintain the desired temperature with an accuracy of $\pm 0.1^\circ\text{C}$. Requisite amounts of solutions of substrate, HCl and enough water to keep the total volume constant (50 ml) for all kinetic runs were equilibrated at 303K for about 30 min. A measured amount of BAT solution, also equilibrated at the same temperature was rapidly added to the reaction mixture which was periodically shaken for uniform concentration. The progress of the reaction was monitored by withdrawing measured aliquots (5 ml each) from the reaction mixture at regular time intervals and determined the unreacted BAT iodometrically. The course of the reaction was studied more than two half-lives. The pseudo first-order rate constants (k' s⁻¹) calculated from the linear plots of $\log [\text{BAT}]$ versus time were reproducible within $\pm 5\%$.

2.3 Stoichiometry and product analysis

Reaction mixtures containing varying proportions of BAT and LVM were equilibrated in presence of 10×10^{-3} M at 303 K. Determination of the unreacted BAT showed that one mole of LVM utilized one moles the oxidant. The observed stoichiometry has been presented by (1)



The reactions were performed under stirred conditions for 12 hours in presence of HCl at 303K. After completion of the reaction (monitored by TLC), the reaction products were neutralized with alkali and extracted with ether. The reaction products were alienated by column chromatography on silica gel (60-200 mesh) and detected by spot test [6] which revealed the formation of Levamisole S-oxide as oxidation product of LVM and the reduction product of BAT was p-toluene sulfonamide. The oxidation product was confirmed by GC-MS analysis. The GC-MS data were obtained from 17A Shimadzu gas chromatograph with a QP-5050 Shimadzu mass spectrometer. The mass spectra showed a molecular ion peaks at $m/z = 220$ [Fig.1] clearly confirming the oxidation product of LVM is levamisole S-oxide.

The reduction product of the BAT, p-toluene sulfonamide, was detected [7] by thin layer chromatography using light petroleum-chloroform-1-butanol (2: 2: 1v/v) as the solvent and iodine as the spraying agent and it was further confirmed by GC-MS analysis. The mass spectrum showed a molecular ion peak at $m/z = 171$ [Fig.2].

III. Kinetic Results And Discussions

The oxidation of LVM by BAT in the presence of HCl medium carried out in a measurable rate. The reaction orders have been determined from the slopes of $\log k'$ versus respective logarithmic concentration of LVM, HCl and MeOH except [BAT], by keeping the others concentrations constant.

3.1 Effect of Reactants on the reaction rate.

Under the conditions $[\text{LVM}]_0 \gg [\text{BAT}]_0$, plots as $\log [\text{BAT}]$ vs. time are linear representing a first-order dependence of rate on $[\text{BAT}]_0$. The pseudo first-order rate constnts (k') remained unaltered [Table. 1] by the disparity in $[\text{BAT}]_0$, corroborate the first-order dependence on $[\text{BAT}]_0$. The increase in the rate with increase of $[\text{LVM}]_0$ (5×10^{-3} to 30×10^{-3} mol dm⁻³) [Table. 1], gives a linear plot of $\log k'$ vs. $\log[\text{LVM}]$ having a unit slope demonstrating a first-order dependence of the reaction rate on $[\text{LVM}]_0$. Additional plot of k' vs. $[\text{LVM}]$ was linear passing through the origin [Fig.3] signifying the first-order dependence of rate on $[\text{LVM}]_0$. The rate of reaction augmented with increase in $[\text{HCl}]$ [Table. 1] and a plot of $\log k'$ vs. $\log [\text{HCl}]$ was linear with fractional slope (0.4) [Fig. 4] showing a fractional-order dependence of the rate on $[\text{HCl}]$

Table. 1 Effect of [BAT], [LVM] and [HCl] on the reaction rate at 303 K.

10^4 [BAT]/M	10^3 [LVM]/M	10^3 [HCl]/M	$k(\times 10^4 \text{ s}^{-1})$
05	10	10	3.30
10	10	10	3.40
15	10	10	3.42
20	10	10	3.38
10	05	10	1.77
10	10	10	3.40
10	20	10	6.55
10	30	10	11.0
10	10	05	2.65
10	10	10	3.40
10	10	50	7.00
10	10	100	9.50
10	10	500	16.2

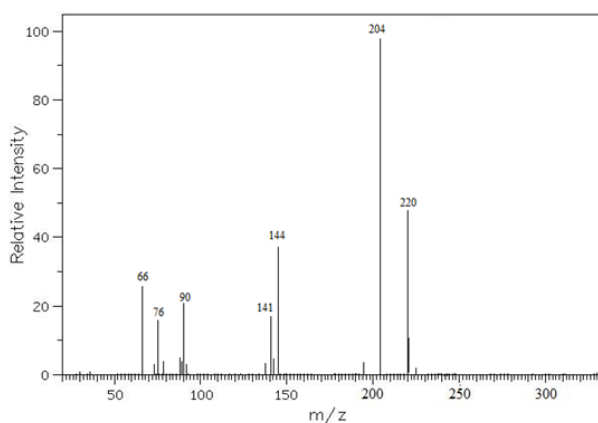


Fig. 1 GC- Mass spectrum of levamisole S-oxide with its molecular ion peak at 220 amu

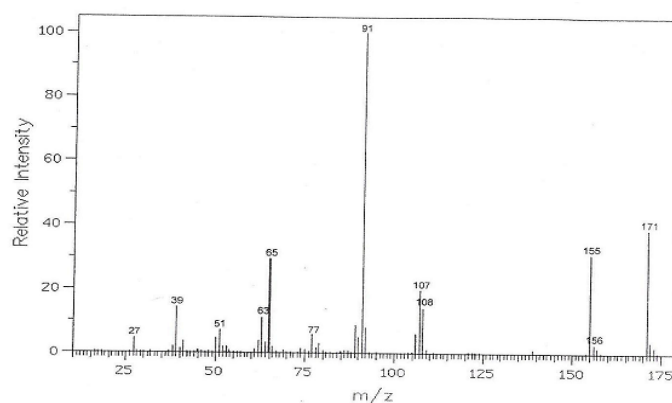


Fig. 2 GC-Mass spectrum of p-toluenesulfonamide with its molecular ion peak at 171 amu.

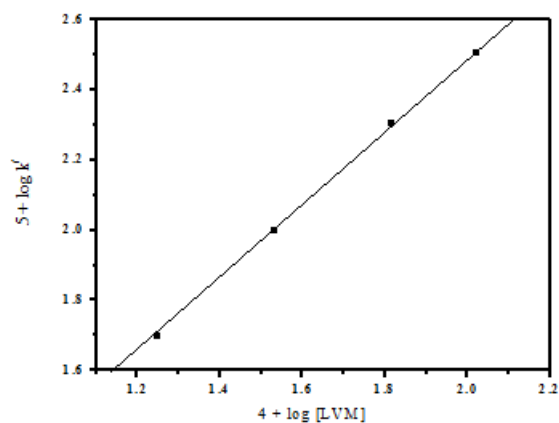


Fig. 3 Effect of variation of concentration of LVM on the oxidation of LVM by BAT in HCl at 303 K (Conditions as in Table.1)

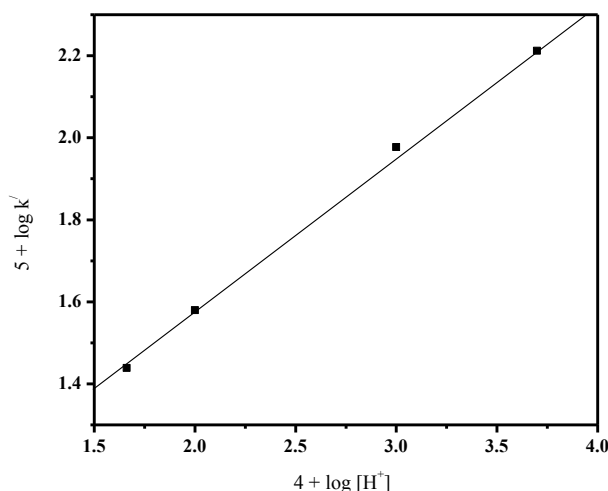


Fig. 4 Effect of medium (HCl) on the oxidation of LVM by BAT in HCl at 303 K (Conditions as in Table .1)

3.2 Effect of Halide ions, PTS and Ionic strength of the medium on the reaction rate

At constant $[H^+]$ ($10 \times 10^{-3} \text{ mol dm}^{-3}$) maintained with HCl, the addition of NaCl (1.0×10^{-2} to $50.0 \times 10^{-2} \text{ mol dm}^{-3}$) did not affect the rate of reaction [Table. 2]. Consequently, the dependence of rate on $[HCl]$ substantiated the effect of $[H^+]$ only. Correspondingly, addition of NaBr (1.0×10^{-2} to $50.0 \times 10^{-2} \text{ mol dm}^{-3}$) showing the no effect on the rate. These results specify that there is no role for halide ions in the reaction.

Addition of p-toluene sulfonamide (1.0×10^{-4} to $50.0 \times 10^{-4} \text{ mol dm}^{-3}$) to the reaction blend did not alter the rate considerably [Table. 2]. This signifies that PTS is not involved in any step preceding to the rate determining step in the scheme proposed.

The effect of ionic strength (I) of the medium on the rate was studied in a range of 1.0×10^{-3} to $50.0 \times 10^{-3} \text{ mol dm}^{-3}$ using NaClO_4 solution keeping other experimental conditions constant [Table. 2]. The rate was not altered significantly suggesting that nonionic species are involved in the rate determining step.

Table. 2 Effect of varying $[\text{NaCl}]$, $[\text{NaBr}]$, $[\text{PTS}]$ and NaClO_4 on the reaction rate.

$10^2[\text{NaCl}] / [\text{NaBr}] \text{ M}$	$k' (\times 10^4 \text{ s}^{-1})$
1	3.30 (5.74)
5	3.47 (5.95)
10	3.40 (5.99)
20	3.38 (5.77)
50	3.42 (5.80)
$10^4 [\text{PTS}]/\text{M}$	
1	2.49
5	2.44
10	2.64
20	2.48
50	2.50
$10^3 [\text{NaClO}_4]/\text{M}$	
1	2.66
5	2.55
10	2.65
20	2.52
50	2.42

Values in parenthesis refers to the rate constants with NaBr
 $[\text{BAT}] = 10 \times 10^{-4} \text{ mol dm}^{-3}$; $[\text{LVM}] = 10 \times 10^{-3} \text{ mol dm}^{-3}$; $[\text{HCl}] = 10 \times 10^{-3} \text{ mol dm}^{-3}$; $T = 303 \text{ K}$

3.3 Effect of Dielectric constant of medium on the rate of reaction

The dielectric constant (D) of the medium was varied using different amounts of methanol and H_2O (0-30% v/v). The reaction rate decreases with an increase in methanol content [Table.3]. Plots of $\log k'$ vs. $1/D$ [Fig.5] were found to be linear with negative slopes (0.25).

Table. 3 Effect of varying dielectric constant of medium on the reaction rate.

[MeOH] % v/v	D	10 ² /D	k'($\times 10^4$ s ⁻¹)
0	76.73	1.3032	3.4
10	72.37	1.3817	3.0
20	67.48	1.4819	2.5
30	62.71	1.5946	2.0

[BAT] = 10×10^{-4} mol dm⁻³ ; [LVM] = 10×10^{-3} mol dm⁻³ ; [HCl] = 10×10^{-3} mol dm⁻³ ; T=303K

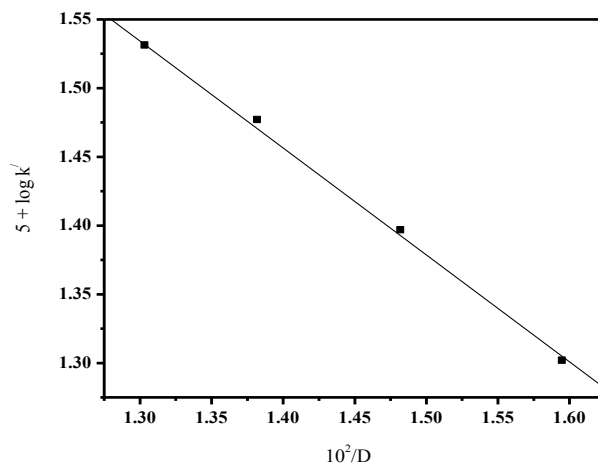


Fig. 5 Effect of Dielectric constant (MeOH) on the oxidation of LVM by BAT in HCl at 303 K (Conditions as in Table .3)

3.4 Effect of temperature

The rate constants for the oxidation of LVM with BAT at different temperature ranges from 293 to 313 K was measured by keeping the other experimental conditions constant. The temperature effect was studied for three concentration of LVM. It was observed that the rate constant of the reaction increases with increase in temperature for given concentration of LVM [Table. 6 and Fig. 6]. From the linear plot of log k' vs. 1/T, [Fig. 7] thermodynamic parameters were evaluated [Table .7].

Table .6 Effect of varying Levamisole concentrations on the reaction rate at different temperatures.

10 ³ [LVM]/M	k'($\times 10^4$ s ⁻¹)		
	293K	303K	313K
05	1.32	2.0	3.3
10	2.38	3.8	5.91
20	2.80	4.49	7.01
50	5.60	11.5	14.4

[BAT] = 10×10^{-4} mol dm⁻³ ; [HCl] = 10×10^{-3} mol dm⁻³

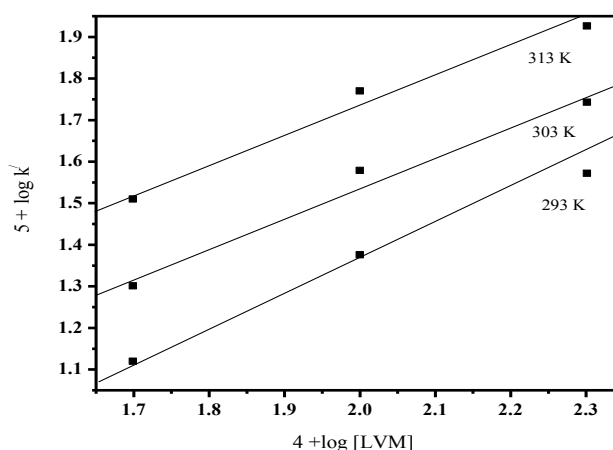


Fig. 6 Effect of variation of concentration of the LVM at different temperature on the oxidation

of LVM by BAT in HCl. (Conditions as in Table. 6)

Table .7 Effect of varying temperature and the values of activation parameters for the composite reaction between BAT and LVM

Temperature (K)	$k(\times 10^4 \text{ s}^{-1})$	Activation parameter
293	2.38	$E_a = 34.018 \text{ kJmol}^{-1}$
303	3.80	$\Delta H^\ddagger = 33.76 \text{ kJmol}^{-1}$
313	5.91	$\Delta G^\ddagger = 57.79 \text{ kJmol}^{-1}$
323	8.70	$\Delta S^\ddagger = -200.13 \text{ JK}^{-1}\text{mol}^{-1}$
		$\log A = 2.422$

$$[\text{BAT}] = 10 \times 10^{-4} \text{ mol dm}^{-3}; [\text{LVM}] = 10 \times 10^{-3} \text{ mol dm}^{-3}; [\text{HCl}] = 10 \times 10^{-3} \text{ mol dm}^{-3}$$

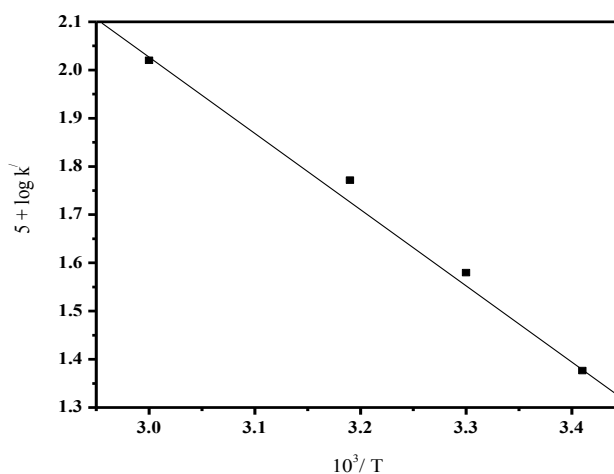


Fig. 7 Effect of Temperature on the oxidation of LVM by BAT in HCl. (Conditions as in Table 7)

3.5 Test for free radicals

The addition of small amount of the oxidation reaction mixture to the aqueous acryl amide solution did not initiate polymerization, showing the absence of free radicals species (non ionic species) during the reaction sequences.

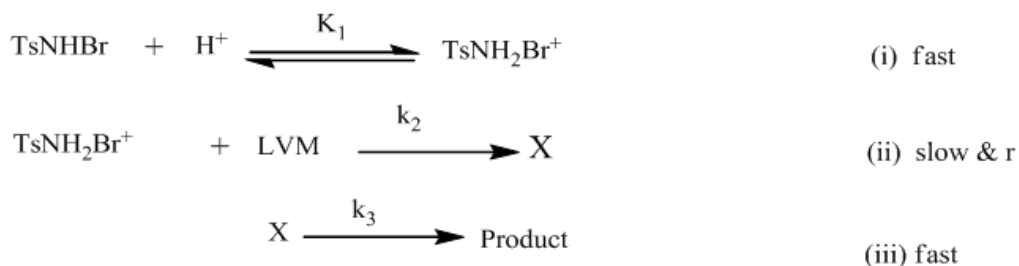
IV. Discussion

Bromamine-T (BAT) act as an oxidizing agent both in acidic and alkaline media [8–13]. The being of similar equilibrium in acid and alkaline solutions of BAT has been reported by Morris et. al. [8], Ruff and Kucsman [9], Bishop and Jennings [10], Hardy and Johnston [11], Pryde and Soper [12] and Higuchi et al. [13]. Aqueous solution of BAT (TsNBrNa) behave as a strong electrolyte [10] and depending on the pH, it furnishes different types of reactive species. The probable oxidizing species in acidified BAT solutions are the conjugate free acid (TsNHBr), dibromamine-T (TsNBr₂), hypobromous acid (HOBr) and possibly H₂OBr⁺.

If TsNBr₂ and HOBr were to perform as reactive species, then the rate law would have followed a second-order dependence on [BAT]₀ and a first-order retardation of the rate by the addition of PTS (TsNH₂) would be expected. Since, no such effect was noticed, the species HOBr was also ruled out. Therefore TsNHBr can be assumed as the reactive oxidizing species in the oxidation of LVM by BAT in acid medium.

The dependence of rate on [H⁺] indicates protonation of the oxidant. The formation of the diprotonated species with a protonation constant of 1.02×10^2 at 298 K mol dm⁻³ at 25 °C has been reported for CAT (2) in acid solution [14–15].

In the present investigations, the fractional-order with respect to [HCl] indicates that the protonation of TsNHBr results in the formation of TsNH₂Br⁺ which is likely be the active oxidizing species involved in the mechanism of levamisole oxidation.



Scheme 1

If $[\text{BAT}]_t$ is the total concentration of BAT then From Scheme 1, we get
 $[\text{BAT}]_t = [\text{TsNHBr}] + [\text{TsNH}_2\text{Br}^+]$... (3)

$$K_1 = \frac{[\text{TsNH}_2\text{Br}^+]}{[\text{TsNHBr}][\text{H}^+]}$$

or

$$[\text{TsNHBr}] = \frac{[\text{TsNH}_2\text{Br}^+]}{K_1[\text{H}^+]} \quad \dots (4)$$

By substituting $[\text{TsNHBr}]$ from (4) into (3)

$$[\text{BAT}]_t = [\text{TsNH}_2\text{Br}^+] + \frac{[\text{TsNH}_2\text{Br}^+]}{K_1[\text{H}^+]} \quad \dots (5)$$

$$[\text{BAT}]_t = \frac{[\text{TsNH}_2\text{Br}^+]\{K_1[\text{H}^+] + 1\}}{K_1[\text{H}^+]} \quad \dots (6)$$

$$[\text{TsNH}_2\text{Br}^+] = \frac{K_1[\text{BAT}]_t[\text{H}^+]}{K_1[\text{H}^+] + 1} \quad \dots (7)$$

From slow and rate determining step

$$\text{Rate} = \frac{d[\text{BAT}]_t}{dt} = k_2[\text{TsNH}_2\text{Br}^+][\text{LVM}] \quad \dots (8)$$

By substituting $[\text{TsNH}_2\text{Br}^+]$ from (7) into (8)

$$\text{Rate} = \frac{K_1 k_2 [\text{BAT}]_t [\text{LVM}] [\text{H}^+]}{K_1 [\text{H}^+] + 1} \quad \dots (9)$$

Since $\text{Rate} = k'[\text{BAT}]_t$, then (9) can transferred as

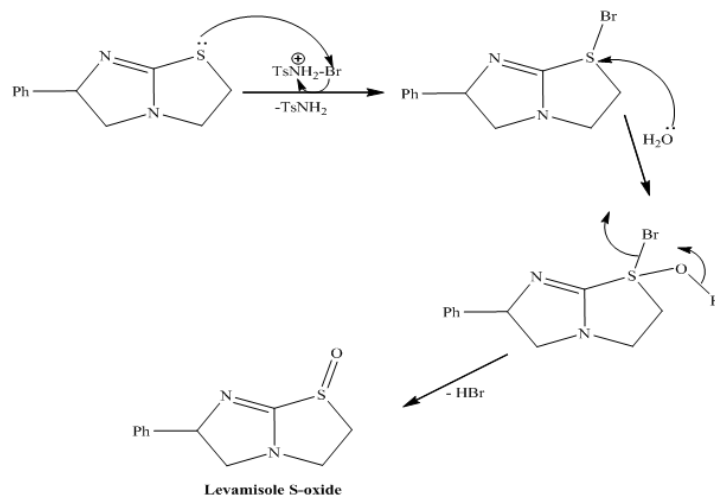
$$k' = \frac{K_1 k_2 [\text{LVM}] [\text{H}^+]}{K_1 [\text{H}^+] + 1} \quad \dots (10)$$

$$\frac{1}{k'} = \frac{1}{k_2 [\text{LVM}]} + \frac{1}{K_1 k_2 [\text{LVM}] [\text{H}^+]} \quad \dots (11)$$

$$\text{Slope} = \frac{1}{K_1 k_2 [\text{LVM}]} \quad \text{Intercept} = \frac{1}{k_2 [\text{LVM}]}$$

From the double reciprocal plot of $1/k'$ Verses $1/[\text{H}^+]$. The values of protonation constant K_1 and decomposition constant K_2 were calculated.

The plots of $1/k'$ vs. $1/[LVM]$ and $1/k'$ vs. $1/[H^+]$ at constant temperature and other reaction conditions were linear, from the slopes and intercepts of these plots, values of formation constants K_1 and k_2 and decomposition constants k_3 were calculated. The near constancy of these values supports the proposed mechanism for the oxidation of LVM by BAT. Since the rate was unity in $[LVM]$, Michaelis-Menten types of kinetics were adopted. The effects of $[LVM]$ in the rate of different temperatures (298–323 K) were examined. From the linear plots of $1/k'$ vs. $1/[LVM]$ at different temperatures, values of k_3 were computed. Using the values of k_3 activation parameters for the rate limiting step were evaluated using Arrhenius plots of $\log k_3$ vs. $1/[H^+]$.



Scheme -2

V. Conclusion

Oxidation of Levamisole Hydrochloride (LVM), by bromamine-T (BAT) in HCl medium. The rate of oxidation of LVM by BAT was found to be first-order with respect to $[LVM]$, $[BAT]$ and $[HCl]$. The stoichiometry of oxidation reaction is 1:1. The oxidation product identified was Levamisole S-oxide. The active oxidizing species involved in acid medium is $TsNHBr^+$. Activation parameters calculated from Arrhenius plot and the observed results supported the proposed mechanism and rate law.

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