



**KINETICS AND MECHANISTIC STUDY OF CHLORAMINE-T ASSISTED OXIDATIVE
CLEAVAGE OF LABETALOL HYDROCHLORIDE DRUG IN ALKALINE MEDIUM- A
SPECTROPHOTOMETRIC STUDY**

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ABSTRACT

Chloramine-T assisted oxidation a promising method for the oxidative cleavage of labetalol hydrochloride. The kinetic of oxidation of LBT was studied spectrophotometrically by chloramines-T (CAT) as an oxidant in alkaline medium at 308 K at $\lambda_{\max}=325\text{nm}$. The experimental rate law was found to be $-d[\text{CAT}]/dt = k[\text{LBT}]^1[\text{CAT}]^1[\text{OH}^-]^{-0.61}[\text{PTS}]^{-0.63}$. The oxidation kinetics was also studied by varying the ionic strength and dielectric constant of the medium. Finally the reaction was studied at different temperatures. The products of oxidation were isolated and analyzed. Based on the experimental observations made, an appropriate rate law was derived and a possible mechanism was suggested.

KEYWORDS: Chloramine-T, labetalol hydrochloride, oxidation, spectrophotometric.

1. INTRODUCTION

Labetalol hydrochloride (LBT) is a salicylamide derivative which works as an adrenergic antagonist beta-blocker. Beta and alpha receptors increase heart rate and blood pressure. Therefore, labetalol hydrochloride (LBT) is used to lower blood pressure in myocardial infarction and unstable angina.^[1,2] Labetalol hydrochloride (LBL) is chemically designated as 2-hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl] benzamide monohydrochloride (*Figure 1*). It is a white or off-white crystalline powder, soluble in water. LBT has the empirical formula $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3 \cdot \text{HCl}$ and a molecular weight of 364.9. It has two asymmetric centers and therefore exists as a molecular complex of two diastereoisomeric pairs.

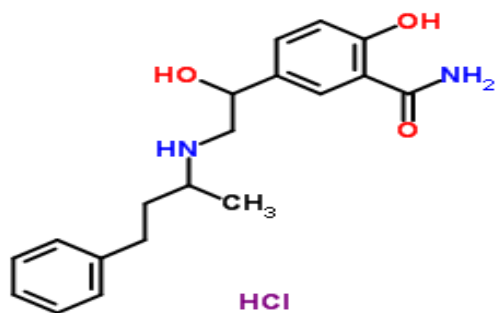


Figure 1: Chemical structure of Labetalol hydrochloride (LBT).

Chloramine-T acts as an oxidizing agent in both acidic and alkaline media.^[3-8] It undergoes a two electron change per mole to give PTS and NaCl. The redox potential of CAT-PTS^[9] is pH dependent and it decreases with the increase in the pH of the medium. Hence, the nature of the reactive oxidizing species depends upon the pH of the medium along with the observed kinetics. Several analytical methods such as TLC^[10,11], HPLC^[12-15], capillary electrophoresis^[16-18], polarography^[19] have been reported for the determination of labetalol hydrochloride in its pure and commercial dosage forms. Literature review indicated that there is no much information available on the kinetics of oxidation of LBT by any oxidant including chloramine-T. Therefore, spectrophotometric study of the kinetics and mechanism of oxidation of LBT by CAT was investigated in the present work.

2. EXPERIMENTAL METHOD AND MATERIALS

The substrate, pure labetalol hydrochloride (LBT), was purchased from YARROW CHEM PRODUCTS, Mumbai, India and was used as received. The stock solution of the LBT was prepared freshly whenever required each time. An aqueous solution of the oxidant chloramine.T (CAT) from SDFCL, Mumbai, was prepared, standardized periodically by the iodometric method and kept in brown bottles to prevent photochemical effect. Analar grade chemicals and doubly distilled water were used throughout.

3. KINETIC MEASUREMENT

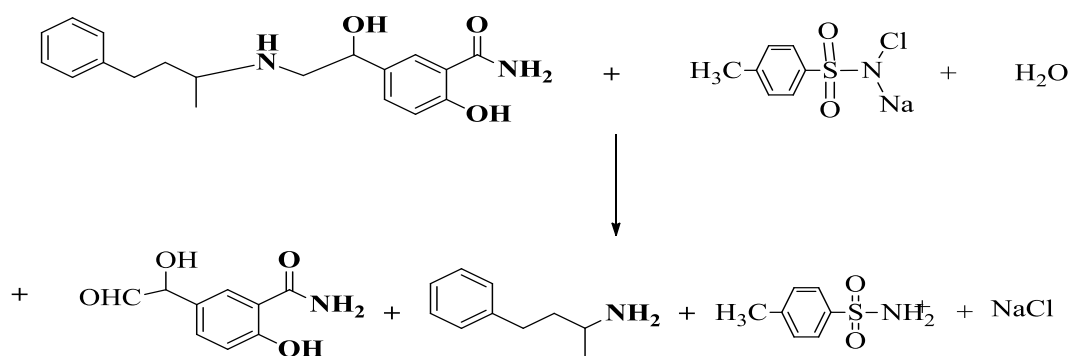
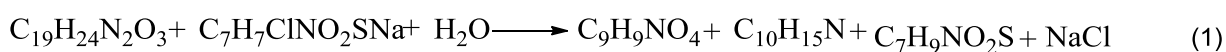
The kinetics of oxidation of labetalol hydrochloride (LBT) by chloramine-T in alkaline medium was studied spectrophotometrically at $\lambda_{\text{max}} = 325 \text{ nm}$ at 308K.

Kinetic runs were performed under pseudo first order conditions with an excess of CAT over (LBT) at 308K. For each run, requisite amounts of LBT and NaOH were mixed in a stoppered pyrex glass tube, whose outer surface was coated black. A required measure of distilled water was added to keep up a constant volume in all runs. Constant temperature was maintained employing a thermostat controlled water bath. To this solution was added a measured amount of the pre equilibrated CAT solution to give a known over all concentration. The reaction mixture was shaken for uniform concentration.

The course of the reaction was monitored spectrophotometrically using LMSP-UV1200 by measuring the absorbance at λ_{max} of 325 nm at regular intervals for two half-lives.

4. STOICHIOMETRY AND PRODUCT ANALYSIS

Varying proportions of the oxidant CAT to the reactant LBT in NaOH medium were equilibrated at 308 K for 24 h. Aliquots of the reaction mixture were idometrically titrated with standard thiosulphate solution, utilizing starch indicator, to decide the end point of unaltered CAT. The mole proportion (number of moles of CAT used per mole of LBT) was ascertained. The consequence of LBT response with CAT demonstrated a distinct stoichiometry of 1: 1 (Equation: 1 and Scheme 1).



Scheme 1: Stoichiometry of LBT and CAT.

After completion of the reaction, the reaction products were initially neutralized by the addition of HCl and were extracted with ethyl acetate, and detected by thin layer chromatography. The products were characterized by LC-MS spectra which showed peaks at m/z 195 and 150 (Figs 2 and 3), indicating the presence of oxidation products 2-hydroxy-5-(1-hydroxy-2-oxoethyl)

benzamide and 4-phenylbutan-2-amine respectively. Reduction product of the oxidant (PTS) was identified by paper chromatography using butanol-acetic acid-water (4:1:1 v/v/v) as solvent and iodine as reducing agent ($R_f = 0.90$). This was further confirmed by LC-MS (Fig 4) showing a peak at m/z 172.

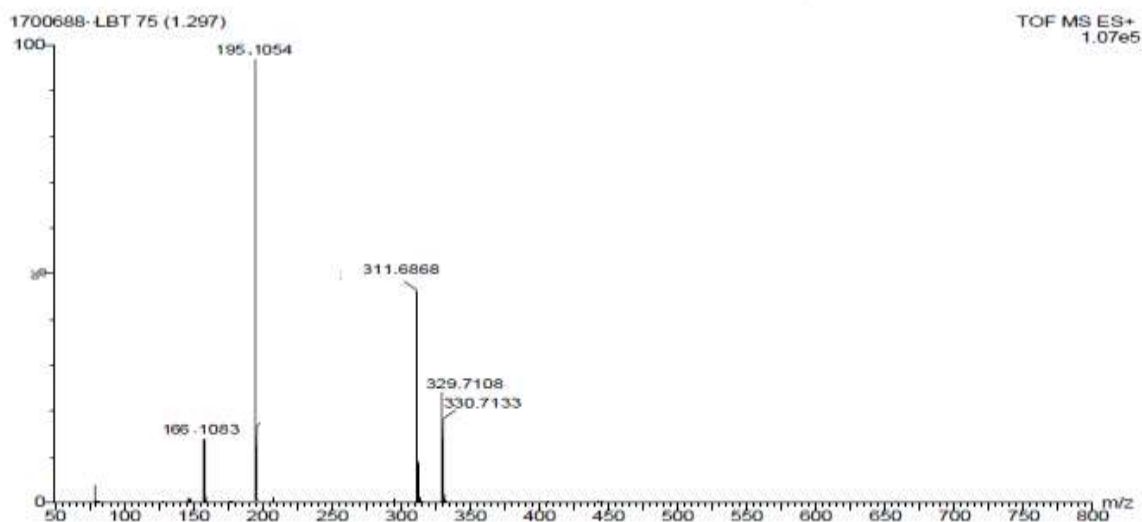


Fig 2: LC -MS of 2-hydroxy-5-(1-hydroxy-2-oxoethyl) benzamide with its $M+1$ ion peak at 195 amu.

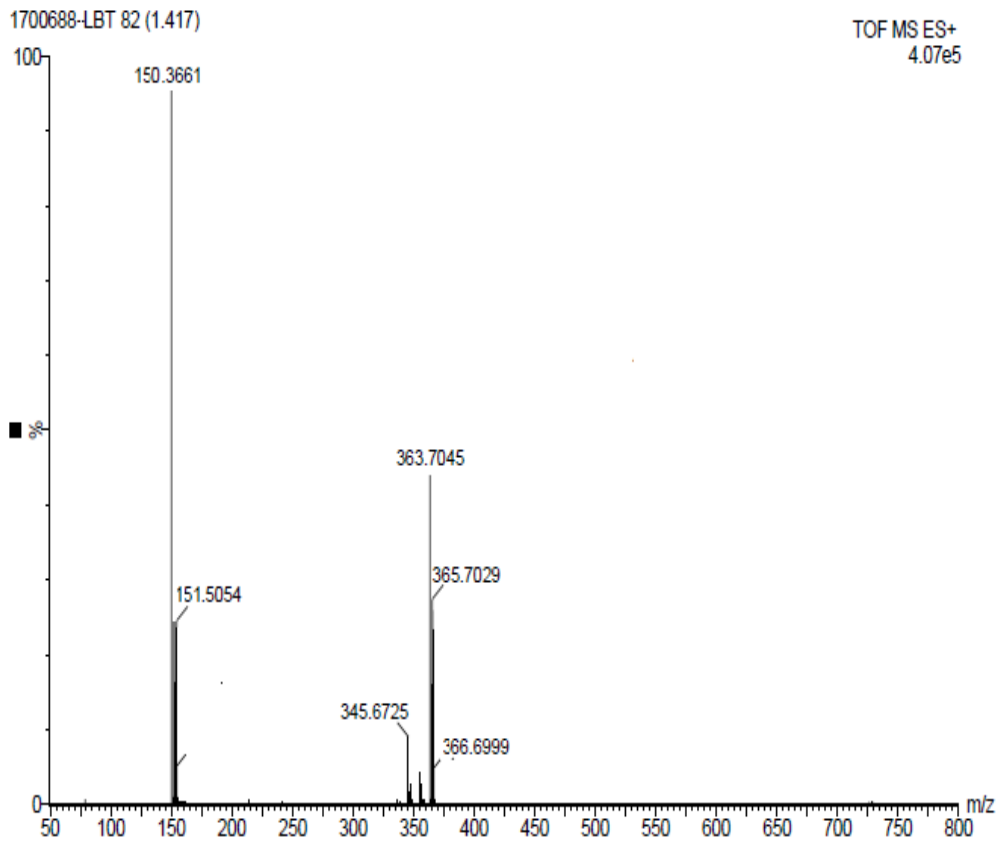


Fig.3: LC -MS of 4-phenylbutan-2-amine with its M+1 ion peak at 150 amu.

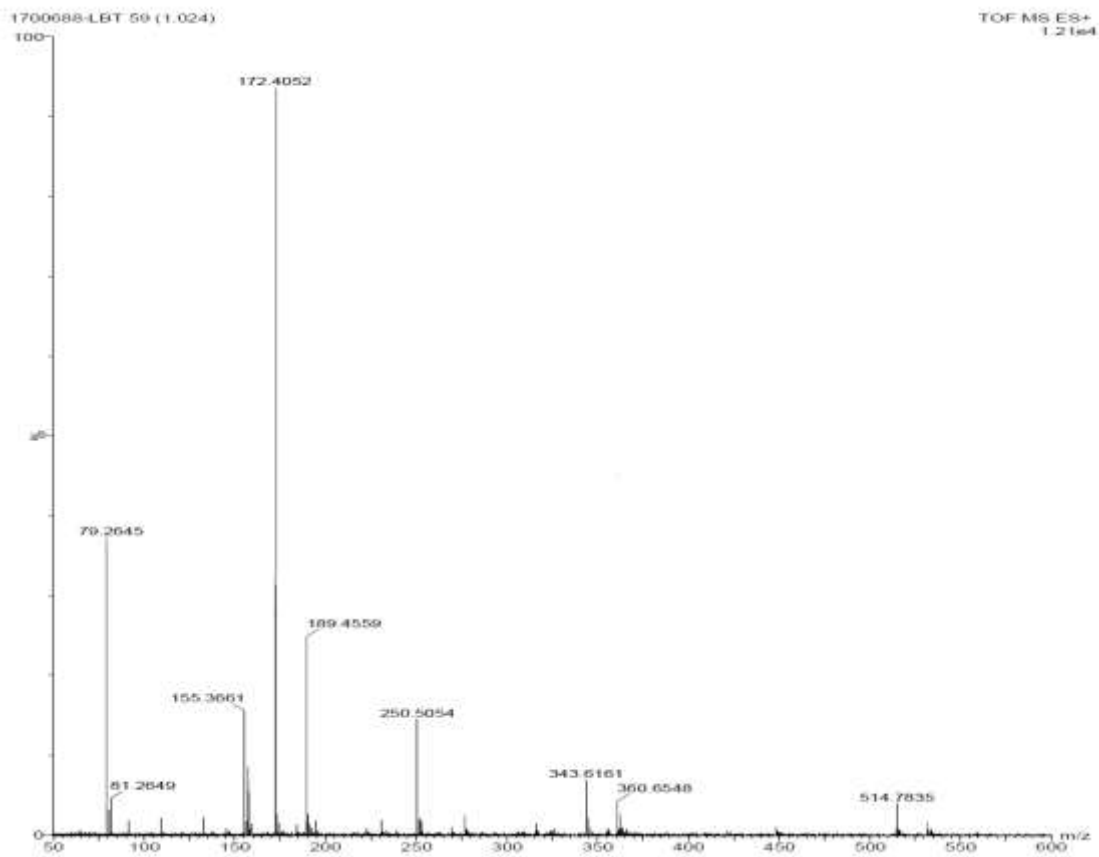


Fig.4: LC -MS of PTS reduction product of oxidation of LBT by CAT.

5. RESULTS

5.1 Effect of concentrations of LBT and CAT on the reaction rate

With the concentration of CAT in excess, at constant $[CAT]_0$, $[NaOH]_0$ and temperature, plot of $\log(\text{abs})$ vs time was found to be linear with an intercept on y-axis which indicates a first order dependence of the rate on $[LBT]_0$ ($R^2 = 0.999$). An increase in $[CAT]_0$ resulted in the increase in the value of k' for the oxidation reaction. A graph of $\log k'$ vs $[CAT]_0$ was linear with slope value equal to 1.00 (Table 1, Fig 5). This clearly indicated the first order dependence of rate on the concentration of CAT. The values of pseudo first order rate constants (k') are tabled in Table 1.

5.2 Effect of concentration of NaOH on the rate

The rate of the reaction was studied at different $[NaOH]_0$ by keeping other conditions constant. It was found that the rate of oxidation decreased with an increase in the concentration of OH^- (Table 1). The order with respect to $[NaOH]$ was found to be -0.61 ($R^2 = 0.999$, Fig 6). This clearly indicated an inverse fractional order dependence of rate on $[OH^-]$.

5.3 Effect of reduction product (PTS) on the rate

The rate of the reaction decreased with the addition of *p*-toluene sulfonamide (reduction product of CAT) which indicated the formation of reduction product in a fast pre-equilibrium to the rate limiting step. Further, the plot of $\log k'$ vs $\log [PTS]$ gave a straight line (Table 2, Fig. 7, $R^2 = 0.983$) with a slope of -0.63 .

5.4 Effect of dielectric constant of the medium on the rate

Methanol of different compositions (0-30% v/v) was used in order to study the effect of varying the dielectric constant of the medium. It was noticed that the rate decreased with the increase in the methanol content for the oxidation reaction of LBT with CAT. The plot of $\log k'$ vs $1/D$ was linear ($R^2 = 0.997$) with a negative slope.

The values of dielectric constant for various methanol-water mixtures were taken from literature.^[21-22]

5.5 Effect of varying ionic strength and halide ions concentration on the rate

The ionic strength of the reaction medium was varied in the range $0.1-0.4 \text{ mol dm}^{-3}$ by the addition of $NaClO_4$ solution keeping other experimental conditions constant. It was found that there was no significant effect on the rate of oxidation of LBT. Further, the addition of halide ions in the form of $NaCl$ and $NaBr$ had no pronounced effect on the rate of oxidation of labelalol hydrochloride.

5.6 Effect of temperature and calculation of activation parameters

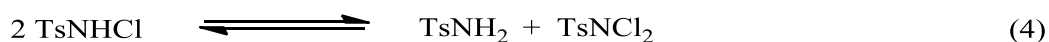
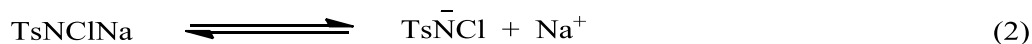
The effect of temperature on the rate was studied at different temperatures (298-318). The Arrhenius plot of $\log k'$ vs $1/T$ was found to be linear (Table 3, Fig 8, $R^2 = 0.991$). The activation parameters energy of activation (E_a), enthalpy of activation (ΔH^\ddagger), entropy of activation (ΔS^\ddagger), free energy of activation (ΔG^\ddagger) and frequency factor (A) were obtained with the help of Arrhenius plot.

5.7 Test for Free radicals

Addition of reaction mixture to an aqueous solution of acrylamide did not initiate polymerization, which indicates that no free radical is involved or generated in the reaction.

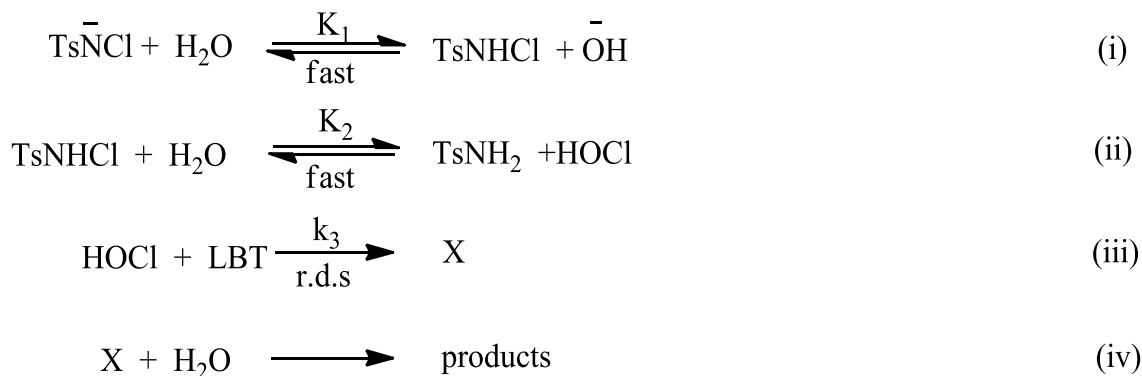
6. DISCUSSION

Chloramine-T is known to act as an oxidizing agent in both acidic and basic media. This oxidant generally undergoes two electron change in its reactions. The solution of CAT acts as a strong electrolyte and it generates different types of reactive species, based on the pH of the solution.^[23] From the investigations of Pryde and Soper^[24], Bishop and Jennings^[25], and Hardy and Johnston^[26], organic N-haloamines have shown to have similar equilibria in both acidic and in basic solutions. The possible equilibria that exist in aqueous solutions of CAT are as shown below.



Therefore, in alkaline solutions of CAT, the possible oxidizing species are TsNHCl, TsNCl⁻, HOCl and OCl⁻. Amongst these reactive oxidizing species one among them is selected from the observed kinetic data. Since there is retardation in the rate of LBT oxidation by CAT due to the addition of PTS, it can be concluded that

HOCl is the probable active species for the oxidation of LBT by CAT in NaOH medium. Based on the above discussion Scheme-2 is proposed to explain the reaction sequence for the oxidation of LBT by CAT in NaOH medium.



Scheme. 2: A general reaction scheme for the oxidation of LBT by CAT in alkaline medium.

In the proposed scheme, in step (i). The anion TsNCl⁻ undergoes hydrolysis to give the free acid monochloramine-T, which on further hydrolysis

produces HOCl. which interacts with the LBT in the slow step to give complex (x), which finally undergoes hydrolysis to give the oxidation products.

From steps (i) and (ii),

$$K_1 = \frac{[\text{TsNHCl}][\text{OH}^-]}{[\text{TsNCl}^-][\text{H}_2\text{O}]} \quad (9.)$$

$$K_2 = \frac{[\text{TsNH}_2][\text{HOCl}]}{[\text{TsNHCl}][\text{H}_2\text{O}]} \quad (10)$$

Rearranging equation (9) and (10),

$$[\text{TsNCl}^-] = \frac{[\text{TsNH}_2][\text{OH}^-][\text{HOCl}]}{K_1 K_2 [\text{H}_2\text{O}]^2} \quad (11)$$

$$[\text{TsNHCl}] = \frac{[\text{TsNH}_2][\text{HOCl}]}{K_2 [\text{H}_2\text{O}]} \quad (12)$$

If [CAT]_t is the total effective concentration of CAT, then

$$[\text{CAT}]_t = [\text{TsNCl}^-] + [\text{TsNHCl}] + [\text{HOCl}] \quad (13)$$

Substituting equations (11) and (12) in (13), and solving for [HOCl]

$$[\text{HOCl}] = \frac{K_1 K_2 [\text{CAT}]_t [\text{H}_2\text{O}]^2}{[\text{TsNH}_2][\text{OH}^-] + K_1 [\text{TsNH}_2][\text{H}_2\text{O}] + K_1 K_2 [\text{H}_2\text{O}]^2} \quad (14)$$

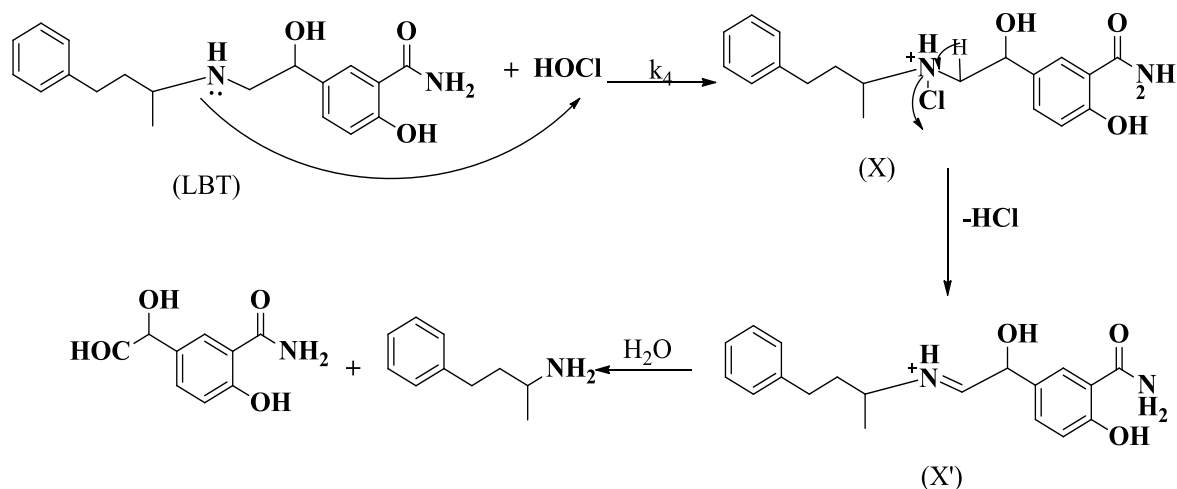
Also from step (iii)

$$\text{Rate} = k_3 [\text{HOCl}] [\text{LBT}] \quad (15)$$

Finally Substituting equations (9.14) in (9.15),

$$\text{Rate} = \frac{K_1 K_2 k_3 [\text{CAT}]_t [\text{LBT}] [\text{H}_2\text{O}]^2}{[\text{TsNH}_2][\text{OH}^-] + K_1 [\text{TsNH}_2][\text{H}_2\text{O}] + K_1 K_2 [\text{H}_2\text{O}]^2} \quad (16)$$

The above rate law (16) agrees well with the observed kinetic data, i. e a first order dependence of rate on [LBT] and [CAT] and inverse fractional order on [OH⁻] and [PTS].



Scheme 3: A detailed reaction mechanism for the oxidation of LBT by CAT in alkaline medium.

The reaction scheme is supported by many parameters like dielectric constant effect, effect of ionic strength and the effect of PTS. According to Amis^[27], for the interaction between two dipoles or an ion - dipole system, a plot of $\log k$ v/s $1/D$ gives a straight line with a negative slope for a reaction between a negative ion and a dipole or between dipole and dipole and a positive slope for a positive ion-dipole interaction. The present observation agrees with this concept in that, i.e. a dipole and a dipole interaction is involved in the rate determining step. Also, the rate of the ionic reactions is influenced by the charges carried by the ions and the ionic strength of the medium. However, in the present case, the rate constant is independent of the ionic strength. The constancy of rate constant on addition of Chloride ions also supports the proposed mechanism and the derived rate law. The proposed mechanism is also supported by observed activation parameters. The activation parameters like energy of activation and other thermodynamic parameters are moderate. The energy of activation is less for the fast reaction and vice-versa, which indicates that the oxidation reaction of LBT by CAT is enthalpy controlled. The negative value of ΔS^\ddagger indicates that the transition state is highly ordered compared to that of initial ground state which is due to greater degree of solvation during the formation of activated complex. Also, the addition of acrylamide to the reaction mixture had no effect on the reaction rate. This shows the absence of free radicals in the reacting system during oxidation of LBT with CAT.

Table 1: Effect of varying concentration of reactants on rate of oxidation.

[LBT] $\times 10^4$ M	[CAT] $\times 10^3$ M	[NaOH] $\times 10^3$ M	$k \times 10^4$ s^{-1}
1.0	2.0	2.0	3.43
2.0	2.0	2.0	3.39
3.0	2.0	2.0	3.45
4.0	2.0	2.0	3.47
6.0	2.0	2.0	3.44
3.0	1.2	2.0	2.21
3.0	1.6	2.0	2.86
3.0	2.0	2.0	3.45
3.0	2.5	2.0	4.51
3.0	3.0	2.0	5.51
3.0	2.0	1.0	4.98
3.0	2.0	1.5	3.87
3.0	2.0	2.0	3.45
3.0	2.0	2.5	2.93
3.0	2.0	3.0	2.47

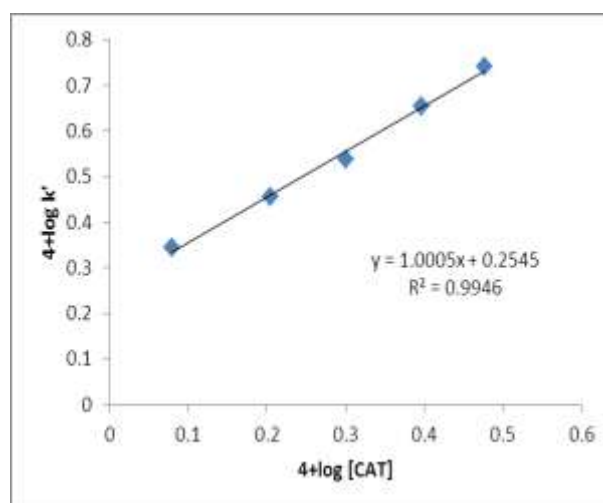


Fig 5: Plot of $\log k'$ vs [CAT].

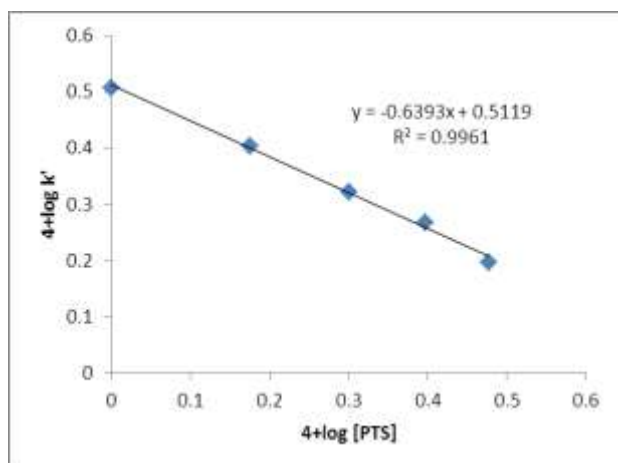


Fig 6: Plot of log k' vs[NaOH].

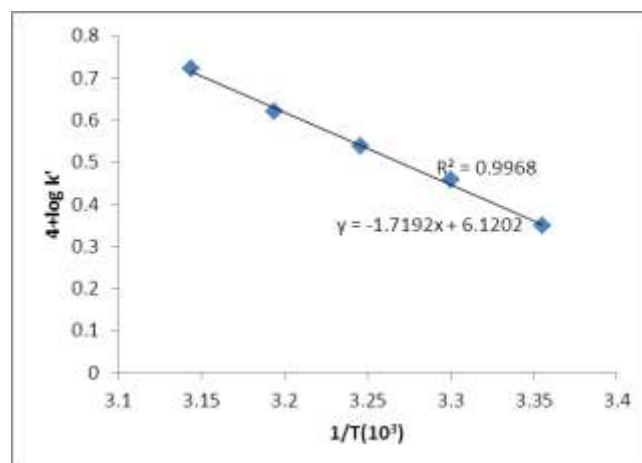


Fig 8: Plot of log k' vs1/T.

Table2: Effect of varying [PTS] on rate of oxidation.

10^4 [PTS] mol dm ⁻³	5+log [PTS]	10^4 k' (s ⁻¹)	4+logk'
1.0	0.000	3.12	0.507
1.5	0.176	2.54	0.404
2.0	0.301	2.09	0.321
2.5	0.397	1.89	0.267
3.0	0.477	1.13	0.197

$[LBT]_0 = 3.0 \times 10^{-4}$ mol dm⁻³, $[CAT]_0 = 2.0 \times 10^{-3}$ mol dm⁻³, $[NaOH]_0 = 2.0 \times 10^{-3}$ mol dm⁻³, T= 308 K

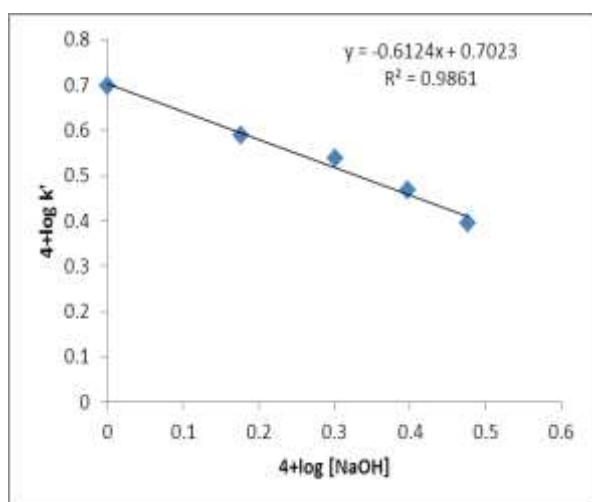


Fig 7: Plot of log k' vs[PTS].

Table 3: Temperature effect on the reaction rate and activation parameters.

Temperature K	k x 10 ⁴ s ⁻¹	Activation Parameters
298	2.23	Ea : 58.22 kJmol ⁻¹
303	2.87	ΔH^\ddagger : 63.59 kJmol ⁻¹
308	3.45	ΔS^\ddagger : -121.80 JK ⁻¹ mol ⁻¹
313	4.16	ΔG^\ddagger : 97.66 kJmol ⁻¹
318	5.27	Log A : 6.52

$[LBT]_0 = 3.0 \times 10^{-4}$ mol dm⁻³, $[CAT]_0 = 2.0 \times 10^{-3}$ mol dm⁻³, $[NaOH]_0 = 2.0 \times 10^{-3}$ mol dm⁻³, T= 308 K.

7-CONCLUSION

The kinetics of oxidation of LBT was studied by CAT in alkali medium at 308 K. The experimental rate law was $-d [CAT]/dt = k [LBT]^1 [CAT]^1 [NaOH]^{-0.61} [PTS]^{-0.63}$. The negative dielectric effect showed an dipole-dipole interaction in the rate limiting step. Also change in ionic strength of the medium had negligible effect showing that the molecule involved in the rate limiting step was neutral. Since there was no halide effect the dependence of the rate on [NaOH] reflected the effect of [OH⁻] only. The activation parameters showed that the activated complex was less strongly solvated compared to the reactant molecule and suggested the formation of a rigid activated complex. Finally an appropriate rate law was deduced with a suitable mechanism.

8-ACKNOWLEDGEMENT

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