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KINETICS AND MECHANISM OF OXIDATION OF AMITRIPTYLINE HYDROCHLORIDE DRUG BY CHLORAMINE-T IN ALKALINE BUFFER MEDIUM: A SPECTROPHOTOMETRIC APPROACH

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ABSTRACT

Kinetics of oxidation of amitriptyline hydrochloride drug (AMT) by chloramine-T (CAT) in alkaline buffer medium (9.3) at 306 K has been investigated spectrophotometrically at λ_{max}378 nm. The reaction showed first-order dependence on [CAT] and [AMT] $_o$ and inverse fractional-order dependence on [OH^-] and [PTS]. Stoichiometry of the reaction was found to be 1:1 with respect to the substrate and oxidant respectively. The oxidation products were identified by spectral analysis. Variation of ionic strength had no effect on the rate. Addition of p-toluene sulphonamide (PTS) retarded the rate of the reaction. Activation parameters have been computed. Probable mechanism and the relevant rate law have been deduced for the observed kinetic results.

KEYWORDS: Amitriptyline hydrochloride, CAT, oxidation-kinetics, alkaline medium.

INTRODUCTION

The chemical name of Amitriptyline hydrochloride (AMT), a tricyclic antidepressant (TCA) is 3-(10,11-dihydro-5H-dibenzol(a,d)cyclohept-5ylidene)propyldimethylamine] hydrochloride and it constitutes an important class of aerotherapeutics and belongs to the first generation of antidepressant drugs.[1] It is used to treat depression, irritable bowel syndrome, diabetic neuropathy, post-traumatic stress disorders and for migraine prophylaxis. Amitriptyline is also used as a treatment for bed wetting (nocturnal enuresis) in case of children. The common side effects of Amitriptyline drug include vomiting, constipation,

diarrohea, mouth pain, weight changes, rashes, decreased sex ability and increased urination.[2-4] Chloramine-T (CAT, p-$CH_3C_6H_4SO_2NClNa.3H_2O$) is known as a strong oxidant in both acidic and alkaline media. Also, it is a good source of electroplilic chlorine acts as an oxidizing agent both in acidic and alkaline media. It undergoes a two-electron change per mole to give PTS and NaCl in the oxidation reaction. The redox potential of CAT-PTS is pH dependent. The redox potential is found to decrease with an increase in the pH of the medium. Hence, the nature of the reactive oxidizing species taking part in the reaction can be predicted based upon the pH of the medium and the observed kinetics. Chloramine-T behaves as a strong oxidizing species in aqueous solutions.[5] The review of the available literature reveals that no information is available on the oxidation kinetics of Amitriptyline with chloramine-T. A review of previous studies reveals a few reports about oxidation and degradation reaction of amitriptyline under various conditions.[6-12] A number of methods like chromatography[13], conductometry, analytical[14] and spectrophotometry[15], have been used for the determination of amitriptyline hydrochloride. However, there is sparse comprehensive studies on the kinetics and mechanism of oxidation of amitriptyline by different oxidants. So, the investigation on the oxidation kinetics of AMT with CAT in alkaline buffer medium was taken up in the present study to explore the mechanistic aspects of the redox system. Such a study maybe beneficial to the kineticists who are working on the mechanistic chemistry of pharmaceuticals in biological systems. The chemical formula of AMT is $C_{20}H_{24}ClN$ and its structure is given in Fig.1.

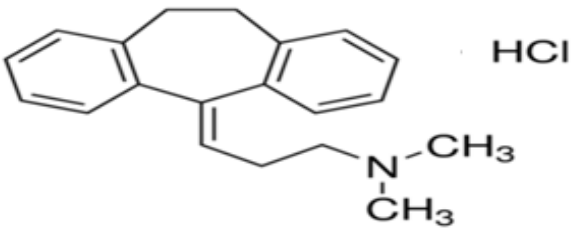


Fig. 1: Structure of amitriptyline hydrochloride.

MATERIAL AND METHODS

The stock solution of chloramine-T (SDF CHEM LIMITED) was prepared in double distilled water and standardized iodometrically. Amtriptyline hydrochloride drug (AMT) (LOBA CHEMIE) was used without further purification. Aqueous solutions of desired strengths were freshly prepared prior to use. Other reagents were of analytical grade.

Kinetic Measurement

Kinetic runs were performed under pseudo first- order conditions with an excess of [CAT] over that of the substrate [Amitriptyline] at 306 K. For each run, requisite amounts of amitriptyline and the alkaline buffer (sodium tetraborate and sodium hydroxide) of known pH were mixed in a stoppered pyrex glass tube whose outer surface was coated black to avoid photochemical effects. Constant temperature was maintained by employing a thermostat controlled water bath. To this solution was added a measured amount of pre-equilibrated CAT solution. The reaction mixture was shaken for uniform concentration. The course of the reaction was monitored spectrophotometrically by measuring the absorbance at λ_{max} 378 nm at regular time intervals for three half-lives. The pseudo first -order rate constants k´(s^{-1}) were calculated.

Stoichiometry Reaction

Reaction mixtures containing different compositions of amitriptyline hydrochloride and CAT were equilibrated at 306 K in alkaline buffer of pH 9.3 for 24 hours. The iodometric determination of unreacted CAT in the reaction mixture showed that one mole of CAT was consumed by one mole of amitriptyline as shown below in Eq.1.

$$C_{20}H_{23}N + C_7H_7ClNO_2SNa + H_2O \longrightarrow C_{20}H_{23}NO + C_7H_9NO_2S + NaCl \quad (1)$$

Products Analysis

The reaction mixture under standard conditions with CAT concentration in excess of [AMT] was kept at 306 K for 48 h. TLC technique was used to monitor completion of the reaction and later the reaction products were neutralized by HCl acid and then extracted with ethylacetate. The oxidation products were characterized by LCMS spectrum. The LCMS spectrum (Fig.2) showed a (M^++1) ion peak at 293 amu indicating the presence of amitriptyline N-oxide. The other product of the reaction, the reduction product, PTS ($TsNH_2$) of CAT, was identified using paper chromatography and confirmed by LCMS spectrum (Fig.3). The first compound was confirmed as amitriptyline N-oxide also by IR spectrum (Fig.4b) which showed peaks at 1482 $cm^{-1}$for N-O stretching in addition to the C-H stretching at 2932 cm^{-1} for the oxidation product. The absence of N-O stretching peak in the IR spectrum (Fig.4a) of the parent compound of AMT further confirms that AMT has been oxidized to amitriptyline N-oxide.

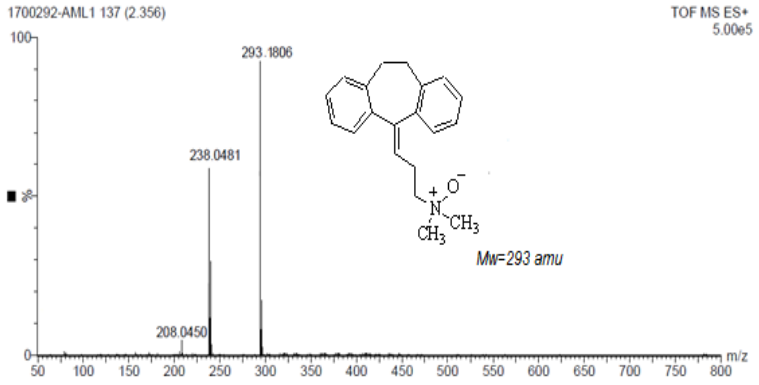


Fig. 2: LC-MS of Amitriptyline N-Oxide with peak at 293 amu.

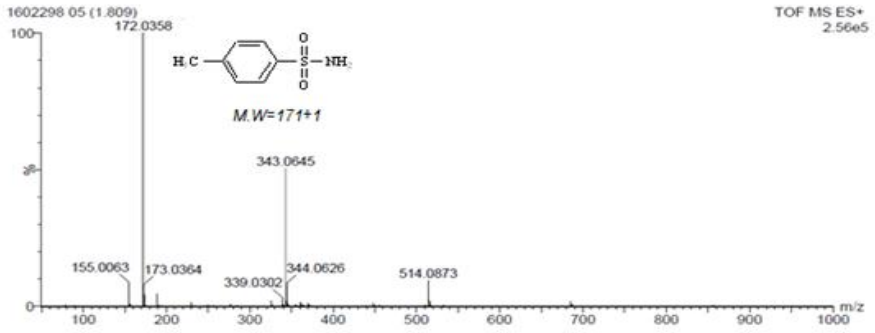


Fig. 3: LC-MS of PTS (reduction product of CAT).

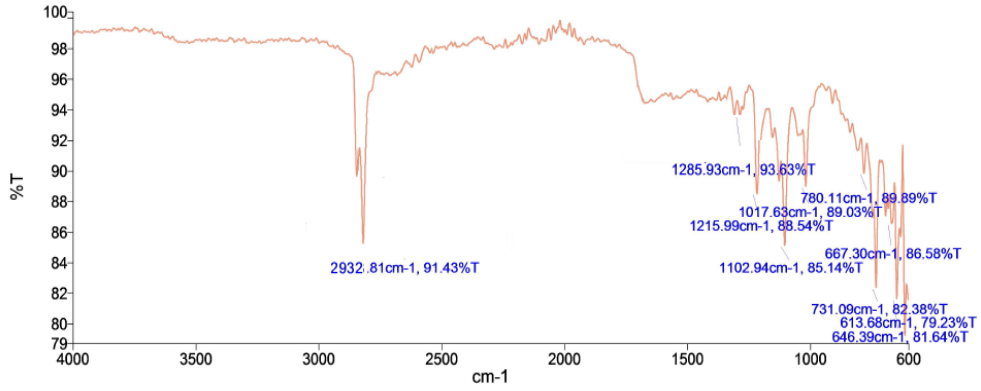


Fig. 4a: IR-Spectrum of pure Amitriptyline.

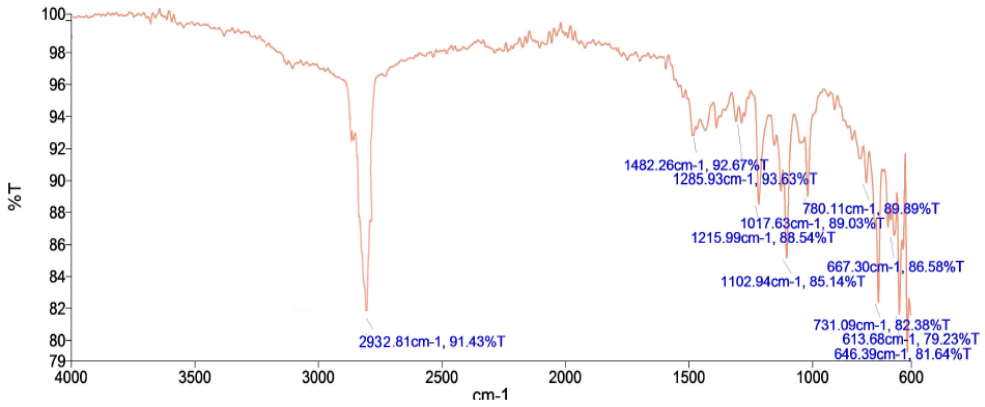


Fig. 4b: IR-Spectrum of Amitriptyline-N-Oxide.

KINETIC RESULTS

Effect of Reactant Concentration on the Oxidation rate of AMT

With the concentration of CAT in excess, at constant $[AMT]_o$, pH and temperature, for the standard run, [AMT] = 3.0×10^{-4} moldm^{-3}, $[CAT]_o = 3.0 \times 10^{-3}$ mol dm^{-3} and pH of 9.3. at 306 K. The rate constant k' was constant with increase in [AMT] supporting first-order dependence on [AMT] (Table 1). With $[CAT]_o$ increasing, value of k' increased. The order with respect to CAT was obtained by plotting log k' vs log [CAT]. The plot was linear with a slope value of 1.14 confirming the first order dependence of rate on [CAT].(Fig.5).

Effect of pH on the Oxidation Reaction Rate of AMT

The pH of the oxidation reaction solution was varied to study the effect of pH variation on the rate of reaction keeping other conditions unchanged. The values of rate constant of the oxidation reaction decreased with an increase in pH of the medium (Table 1). The plot of log k' versus log [OH^-] was used to obtain the order with respect to alkaline buffer. The plot was linear with slope equal to -0.70 (Fig.6, R^2=0.991). This clearly indicated an inverse fractional-order dependence of rate on [OH^-].

Effect of $NaClO_4$ Concentration on the Oxidation Reaction Rate of AMT

$NaClO_4$ solution was added in successive runs in the range of 0.1 to 0.4 mol dm^{-3} to follow the effect of ionic-strength of the reaction medium on the rate maintaining other experimental conditions constant. No significant effect on the rate of oxidation of Amitriptyline was found with increase in [$NaClO_4$]. This predicted the involvement of at least one non-ionic species in the rds.

Effect of [Cl^-] and [Br^-] Ions on the Oxidation Reaction Rate of AMT

NaCl and NaBr solutions were added in the range (1.0-4.0 x 10^{-2}) mol dm^3 to the reaction mixture to ascertain the effect of Cl^- and Br^- ions on the reaction rate. The rate of oxidation of Amitriptyline remained unaffected indicating that the halide ions played no role in the reaction sequence.

Effect of p-Toluenesulfonamide (PTS) on the Oxidation Reaction Rate of AMT

The effect of [PTS] on the rate was determined. It was found that variation in [PTS] retarded the rate of the oxidation reaction. The values of the rate constant (k) decreased with increasing [PTS] (Table2). Further, the plot of log k' vs log [PTS] was linear (Fig.7, R^2 = 0.993) with a negative slope of -0.36 indicating an inverse fractional-order with [PTS] and its involvement prior to the rds in the course of reaction.

Effect of Dielectric Constant of the Medium on the Oxidation Reaction rate of AMT

The dielectric constant of the medium was varied by changing the composition of the reaction medium using methanol (0-30% v/v) to investigate the effect of dielectric constant of the medium on the reaction rate. It was observed that the rate decreased with the increase in the methanol content in the oxidation reaction of Amitriptyline with CAT. The plot of log k' vs 1/D (Table 3, Fig.8) was linear with a negative slope. The values of dielectric constant for various methanol-water mixtures employed for calculation were taken from literature.[16]

Effect of Temperature on the Oxidation Reaction Rate of AMT

The effect of temperature on the rate was studied by carrying out the kinetic measurements at different temperatures in the range (298-314 K) keeping all other experimental conditions constant. The Arrhenius plot of log k' vs 1/T was linear (Table 4, Fig.9, R^2 = 0.995). The thermodynamic activation parameters were obtained with the help of the Arrhenius plot. The oxidation reaction does not involve free radicals as no polymerization of acrylamide occurred when its aqueous solution was added to the reaction mixture.

DISCUSSION

Reactive Species of CAT

Chloramine-T acts as an oxidizing agent both in acidic and alkaline media. It undergoes a two-electron change per mole to give PTS and NaCl. The redox potential of CAT-PTS is pH dependent. The redox potential is found to decrease with an increase in the pH of the

medium. Hence, the nature of the reactive oxidizing species taking part in the reaction can be predicted based upon the pH of the medium and the observed kinetics. Chloramine-T behaves as a strong oxidizing species in aqueous solutions.[17] Depending on the pH of the medium; CAT furnishes[18-21] several types of reactive species in solutions.

$$TsNClNa \rightleftharpoons Ts\bar{N}Cl + Na^+ \quad (2)$$

$$Ts\bar{N}Cl + H^+ \rightleftharpoons TsNHCl \quad (3)$$

$$2\ TsNHCl \rightleftharpoons TsNH_2 + TsNCl_2 \quad (4)$$

$$TsNHCl + H_2O \rightleftharpoons TsNH_2 + HOCl \quad (5)$$

$$TsNCl_2 + H_2O \rightleftharpoons TsNHCl + HOCl \quad (6)$$

$$HOCl \rightleftharpoons H^+ + \bar{O}Cl \quad (7)$$

$$HOCl + H^+ \rightleftharpoons H_2\overset{+}{O}Cl \quad (8)$$

In alkaline solutions of CAT, $TsNCl_2$ does not exist and the possible oxidizing species are TsNHCl, $TsNCl^-$, HOCl and OCl^-. Amongst these reactive oxidizing species , one of them acts as the oxidant species in the reaction which can be deduced from the observed kinetic data. In the present study, since the rate shows an inverse fractional- order dependence on $[OH^-]$ with increase in pH, one can conclude that HOCl, which is formed from the hydrolysis of monochloramine-T, the reactive species. This hykaswEER ASWEis further confirmed by the retarding effect of [PTS] on the rate. It can be shown that TsNHCl (free acid) undergoes hydrolysis to give PTS (reduced product of CAT) along with the formation of HOCl as indicated by Eq. 4 Hence, from the observed kinetic data, it is deduced that HOCl is the reactive species of CAT taking part in the oxidation of AMT by CAT at pH 9.3.Scheme 1 is proposed to repreaesent the reaction sequence of the oxidation reaction.

Reaction Scheme

$$Ts\bar{N}Cl + H_2O \underset{fast}{\overset{K_1}{\rightleftharpoons}} TsNHCl + O\bar{H} \quad (i)$$

$$TsNHCl + H_2O \underset{fast}{\overset{K_2}{\rightleftharpoons}} TsNH_2 + HOCl \quad (ii)$$

$$HOCl + AMT \xrightarrow[r.d.s]{k_3} X \quad (iii)$$

$$X + H_2O \longrightarrow products \quad (iv)$$

Scheme 1: **Scheme for the oxidation of AMT by CAT in alkaline buffer medium.**

In the above scheme, X represents the intermediate compound. The rate law is derived for the proposed scheme as shown below:

From steps (i) and (ii) :

$$K_1 = \frac{[TsNHCl]\,[\bar{O}H]}{[Ts\bar{N}Cl]\,[H_2O]} \quad \text{or} \quad [Ts\bar{N}Cl] = \frac{[TsNHCl][OH^-]}{K_1\,[H_2O]} \qquad (9)$$

$$K_2 = \frac{[TsNH_2]\,[HOCl]}{[TsNHCl][H_2O]} \quad \text{or} \quad [TsNHCl] = \frac{[TsNH_2]\,[HOCl]}{K_2\,[H_2O]} \qquad (10)$$

Substituting for TsNHCl from Eq.10 into Eq.9:

$$[Ts\bar{N}Cl] = \frac{[TsNH_2][HOCl][OH^-]}{K_1\,K_2[H_2O]^2} \qquad (11)$$

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

$$[CAT]_t = [TsNCl^-] + [TsNHCl] + [HOCl] \qquad (12)$$

Substituting Eqs.10 and 11 into Eq. 12 and solving for [HOCl] :

$$[HOCl] = \frac{K_1K_2[CAT]_t[H_2O]^2}{K_2[TsNH_2][\bar{O}H] + K_2[TsNH_2][H_2O] + K_1K_2[H_2O]^2} \qquad (13)$$

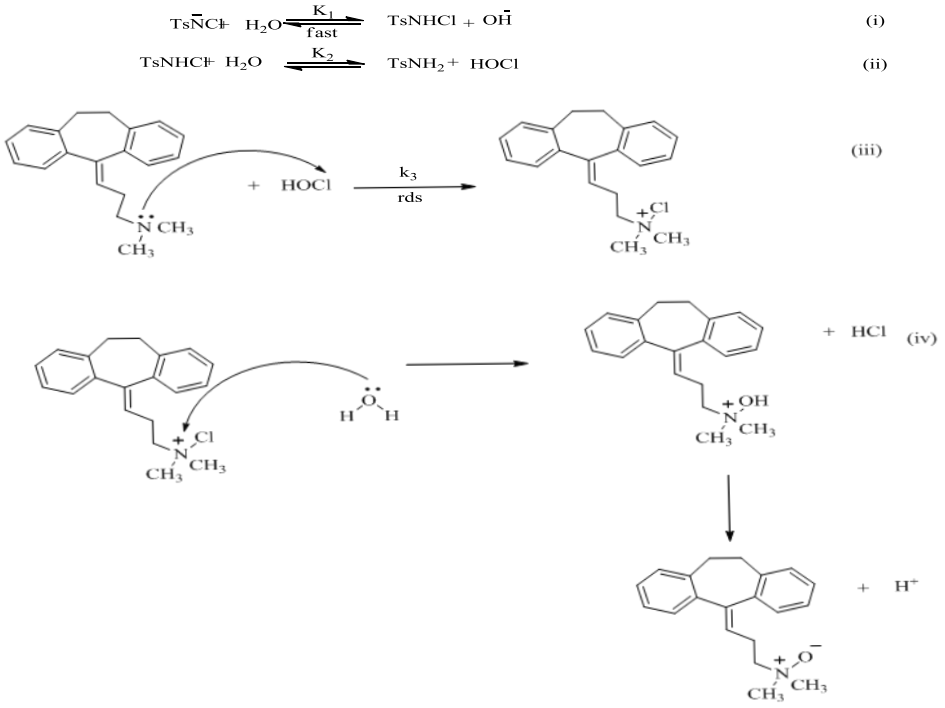
From the slow rds step (iii) in Scheme .1 :

$$\text{Rate} = k_3[HOCl]\,[AMT] \qquad (14)$$

Substituting for HOCl from Eq.13 into Eq.14,

$$\text{Rate} = \frac{K_1K_2k_3[CAT]_t[H_2O]^2\,[AMT]}{K_1[TsNH_2][\bar{O}H] + K_1[TsNH_2][H_2O] + K_1K_2[H_2O]^2} \qquad (15)$$

The above rate law (Eq.15) agrees well with the observed kinetic data.



Scheme.2: A detailed reaction scheme of the oxidation of AMT by CAT.

In Scheme.1, the first step involves the hydrolysis of the acid anion which results in the formation of conjugate acid TsNHCl with the elimination of OH^-. The TsNHCl formed undergoes hydrolysis to give the reduction product of CAT ($TsNH_2$) and HOCl which is the active oxidizing species oxidizing AMT in the present system. In the next step (rate limiting step), the oxidizing species (HOCl) interacts with the drug to give complex (X) which undergoes a series of changes to give the final oxidation products as shown in reaction mechanism Scheme.2. The mechanism is also supported by the study of the effect of the dielectric constant of the medium on the reaction to get an idea about the nature of the reaction in the rate determining step. According to Amis[22], for an interaction between two dipoles or an ion - dipole system, a plot of log k′ vs 1/D gives a straight line, with a negative slope for a reaction between a negative-ion and a dipole or between two dipoles, while a positive slope is obtained for a positive ion-dipole interaction. The former concept agrees in the present system, i.e. a dipole-dipole interaction involvement in the rate determining step. The constancy of the rate constant on addition of chloride ions also supports the proposed mechanism and 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 reaction is seen to be enthalpy controlled. The negative value of $\Delta S^{\#}$ 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 the activated complex.

TABLES AND PLOTS

Table 1: Effect of variation of reactants on the oxidation reaction rate

[AMT] = 3.0 ×10^{-4} moldm^{-3}, $[CAT]_o$= 3.0 × 10^{-3} mol dm^{-3},

pH =9.3 ,T=306 K

[AMT] ×10^3 moldm^{-3}	[CAT]×10^4 moldm^{-3}	*pH*	k×10^4 s^{-1}
2.0	3.0	9.3	1.54
3.0	3.0	9.3	**1.53**
4.0	3.0	9.3	1.55
6.0	3.0	9.3	1.56
8.0	3.0	9.3	1.52
3.0	1.0	9.3	0.75
3.0	2..0	9.3	1.05
3.0	3.0	9.3	**1.53**
3.0	4.0	9.3	2.26
3.0	5.0	9.3	3.07
3.0	3.0	9.0	3.58
3.0	3.0	9.2	2.20
3.0	3.0	9.3	**1.53**
3.0	3.0	9.4	1.23
3.0	3.0	9.6	1.02

Table 2: Effect of variation of PTS concentration on the reaction rate.

$[AMT]_o$ = 3.0× 10^{-4}mol dm^{-3}, $[CAT]_o$ = 3.0× 10^{-3}mol dm^{-3}, pH = 9.3, T=306 K

10^3[PTS] mol dm^{-3}	3+log [PTS]	10^4 k′ (s^{-1})	4+logk'
0.5	0.699	1.49	1.173
0.75	0.875	1.28	1.107
1.0	1.000	1.17	1.068
1.5	1.176	1.02	1.012
2.0	1.301	0.89	0.949

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

$[AMT]_o$ = 3.0× 10^{-4}mol dm^{-3}, $[CAT]_o$ = 3.0× 10^{-3}mol dm^{-3}, pH= 9.3, T=306 K

% MeOH (v/v)	D	1/D	10^4 k′ (s^{-1})	4+logk'
0	76.73	0.0130	1.53	1.186
10	72.37	0.0138	1.17	1.070
20	67.48	0.0148	0.86	0.938
30	62.71	0.0159	0.65	0.817

Table.4: Effect of variation of temperature and Activation parameters of the oxidation of AMT by CAT.

$[AMT]_o = 3.0\times 10^{-4}$mol dm^{-3}, $[CAT]_o = 3.0\times 10^{-3}$mol dm^{-3}, pH = 9.3, T=306 K

Temperature (K)	10^3(1/T) (K^{-1})	10^4 k′ (s^{-1})	4+logk'
298	3.355	0.79	0.898
302	3.311	1.19	1.077
306	3.267	1.53	1.186
310	3.225	2.16	1.335
314 Ea (kJ mol^{-1}) 51.12	3.184 $\Delta H^{\neq}$ (kJ mol^{-1}) 49.59	3.04 $\Delta G^{\neq}$ (kJ mol^{-1}) 93.66	1.484 $\Delta S^{\neq}$(JK^{-1} mo^{1}) -124.80

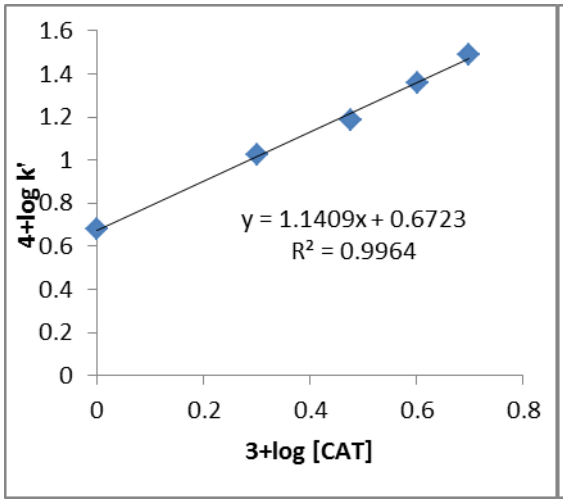


Fig. 5: A Plot of log k′ versus log [CAT]

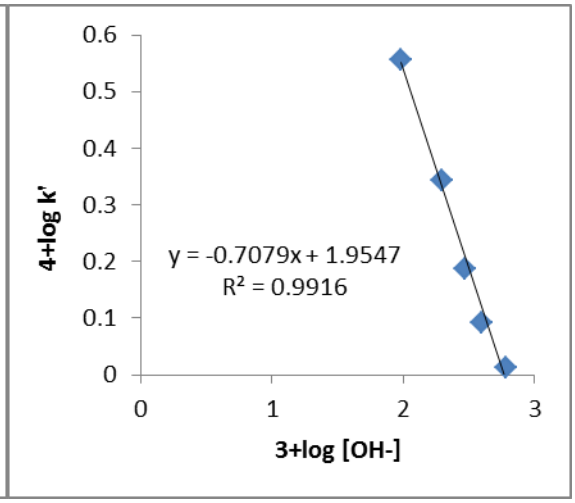


Fig. 6: A Plot of log k′ versus [OH$^-$]

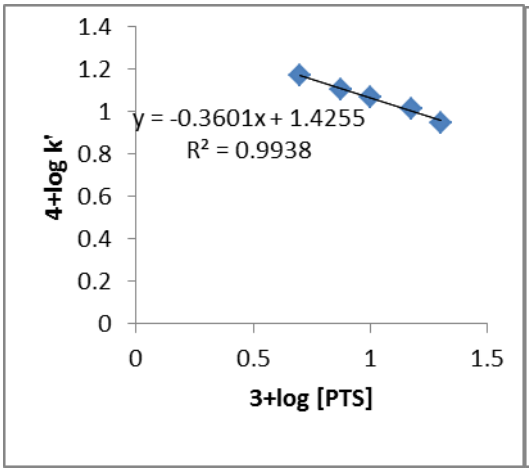


Fig. 7: A Plot of log k′versus log [PTS]

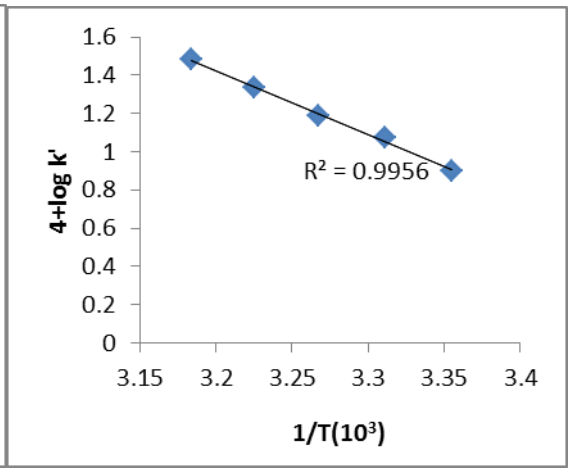


Fig. 8: A Plot of log k′ versus 1/T (10^3)

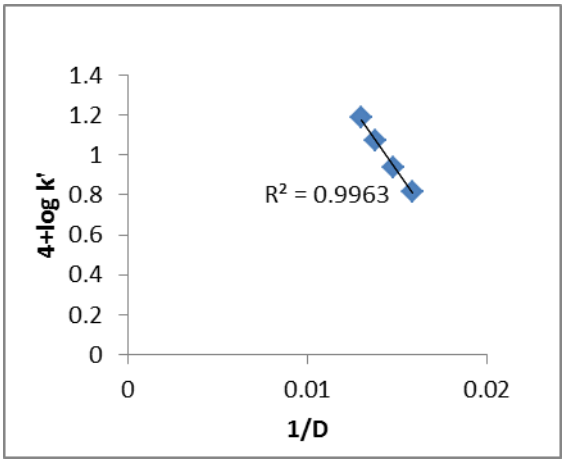


Fig.9: A Plot of log k′ versus 1/D.

CONCLUSION

- In this investigation,the kinetics of oxidation of AMT was studied by CAT in alkaline buffer medium of pH 9.3 at 306 K spectrophotometrically.
- The derived rate law was $-d[CAT]/dt = k[AMT]^{1}[CAT]^{1}[OH^{-}]^{-0.70}[PTS]^{-0.36}$.
- The activation parameters were computed and an appropriate rate law was worked out with a suitable mechanism.

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