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Oxidation of Amitriptyline by Bromamine-T in Acidic Buffer Medium: A Kinetic and Mechanistic Approach

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***Abstract*—**The kinetics of the oxidation of amitriptyline (AT) by sodium N-bromotoluene sulphonamide ($C_6H_5SO_2NBrNa$) has been studied in an acidic buffer medium of pH 1.2 at 303 K. The oxidation reaction of AT was followed spectrophotometrically at maximum wavelength, 410 nm. The reaction rate shows a first order dependence each on concentration of AT and concentration of sodium N-bromotoluene sulphonamide. The reaction also shows an inverse fractional order dependence at low or high concentration of HCl. The dielectric constant of the solvent shows negative effect on the rate of reaction. The addition of halide ions and the reduction product of BAT have no significant effect on the rate. The rate is unchanged with the variation in the ionic strength ($NaClO_4$) of the medium. Addition of reaction mixtures to be aqueous acrylamide solution did not initiate polymerization, indicating the absence of free radical species. The stoichiometry of the reaction was found to be 1:1 and oxidation product of AT is identified. The Michaelis-Menton type of kinetics has been proposed. The $CH_3C_6H_5SO_2NHBr$ has been assumed to be the reactive oxidizing species. Thermodynamical parameters were computed by studying the reactions at different temperatures. A mechanism consistent with observed kinetics is presented.

***Keywords*—**Amitriptyline, bromamine-T, kinetics, oxidation.

I. Introduction

AT is a tricyclic antidepressant (TCA). It is the most widely used TCA and has the same efficacy against depression [1]. AT is used for a number of medical conditions including depressive disorders, anxiety disorders, attention deficit hyperactivity disorder, migraine prophylaxis, eating disorders, bipolar disorder, post herpetic neuralgia, and insomnia [2]. ATs in low doses relive pain. It is also used as a preventive for patients with recurring biliary dyskinesia [3]. It is also utilized in the treatment of nocturnal enuresis (bed wetting) in children. AT may be prescribed for the other conditions such as cyclic vomiting syndrome, post-traumatic stress disorder (PTSD). The symptoms and the treatment of an overdose are largely the same as for the other TCAs. The heavy dosage of AT can be dangerous. Breyer-Pfaff [4] has investigated the comparative N-glucuronidation kinetics of ketotifen and AT expressed by human UDP glucuronosyltransferases and liver microsomes. AT was reported by Breyer-Pfaff [5]. Diansong et al. [6] have studied the role of human UGT2B10 in N-glucuronidation of TCAs, AT, imipramine, clomipramine, and trimipramine. Jyothi et al. [7] have reported the ruthenium (III) catalyzed oxidative degradation of amitriptlyine-A TCA drug by permanganate in aqueous acidic medium. Ledesma et al. [8] have reported the study of the mechanisms involved in the adsorption of AT from aqueous solution onto activated carbons. Vijaya et al. [9] have investigated the design and *in vitro* evaluation of hydroxyl propyl methylcellulose based transdermal films of AT hydrochloride. Shekappa and Nandibewoor [10] have reported the oxidation of tricyclic antidepressant agent AT by permanganate in sulphuric acid medium by kinetic and mechanistic approach. AT has been proving to act as a combatant of the TrKA and TrKB receptors [11]. Now we have recently studied the kinetics of oxidation of amitriptyline by bromamine-T (BAT) in acidic buffer medium.

Aromatic N-halosulphonamides are the mild oxidants containing a strongly polarized N-linked halogen in its +1 oxidation state. The prominent member of this group chloramine-T (CAT) is a well-known analytical reagent, and the mechanical aspects of its reaction have been documented [12]-[15]. Its bromine analogue, BAT is a better oxidizing agent than CAT and chloramine-B. However, limited information on BAT reactions exists in the literature [16]-[20]. Hence, the oxidation kinetics of AT adds much to the redox chemistry. These facts prompted us to undertake the study of kinetics of oxidation of AT by BAT in acidic buffer medium with a view to elucidate the reaction mechanism.

II. Material and Methods

A. Experimental

BAT was prepared and purified by using the procedure of Nair and Indrasenan [21]. Its purity was checked by iodometric and spectroscopic data. Aqueous solutions of BAB were prepared and standardized by the iodometric method and stored in amber colored bottles. Analar grade AT was used, and aqueous solution of the substrate was prepared. Whenever required, all other chemicals used were of acceptable grades of purity. A constant ionic strength of the reaction mixture was maintained by adding a concentrated $NaClO_4$. Triply distilled water was used for preparing the aqueous solutions. A pH 1.2 buffer solution of potassium chloride and hydrochloric acid was prepared [22], and its pH value was checked with a pH meter.

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B. Kinetic Measurement

The kinetic runs were performed under pseudo-first order condition of $[AT]_0 << [BAT]_0$. Mixture containing requisite amount of solutions of the AT, $NaClO_4$, and buffer of known pH were taken in stoppard pyrex glass tube, whose outer surface was coated black to eliminate the photochemical effects. A required amount of pH 1.2 buffer solutions was added to maintain a constant volume for all runs. The tube was thermostated in a water bath set at a given temperature. To this solution, was added a measured amount of standard BAT solution, and it was also pre-equillibrated at the same temperature to initiate the reaction. The reaction mixture was shaken intermediately for uniform concentration. The progress of the reaction was monitored by withdrawing aliquots of the reaction mixture at regular time intervals and by spectrophotometrically measured the unreacted AT for two half-lives. The pseudo first-order rate constants k′ calculated were reproducible within ±3.0%. The regression analysis of the experiment was carried out on an origin 5.0 HP computer to obtain the regression coefficient r.

C. Stoichiometry

Reaction mixtures containing different composition of AT and BAT were equilibrated at 303 K in an acidic buffer (hydrochloric acid + potassium chloride) of pH 1.2 for 24 hours. The analysis showed that one mole of BAT reacted with the same mole of AT to give AT N-oxide, according to the following stoichiometry which is represented by using (1)

$$C_{20}H_{23}N + ArSO_2NBrNa + H_2O \rightarrow C_{20}H_{23}NO + ArSO_2NH_2 + NaBr \quad (1)$$

D. Product Analysis

The reaction mixture in the stoichiometric ratio in the presence of buffer medium was allowed to progress for 24h at 303 K. After the completion of the reaction (monitored by TLC), the reaction mixture was neutralized, and the products were extracted with diethyl ether. The organic products were subjected to spot tests and TLC analysis. The N-oxide product corresponding to AT N-oxide was confirmed by GC-MS analysis, and the mass spectral data for the AT N-oxide products were obtained on a17A Shimadzu gas chromatography with LCMS-2010A Shimadzu mass spectrometer. The mass spectra showed a parent ion peak at 293 amu. Fig. 1 confirms the formation of AT N-oxide in the reaction mixture of AT and BAT. The reaction product p-toulenesulphonamide ($ArSO_2NH_2$) was detected by paper chromatography [23]. Benzyl alcohol saturated with water was used as the solvent with 0.5% vanillin in 1% HCl in ethanol as the developing reagent (Rf =0.905).

III. Results

A. Effects of Varying Reactant Concentrations on the Rate

The stoichiometry of the AT-BAT reaction was found to be of 1:1 ratio, under pseudo first order conditions of [BAT] >> [AT] at constant [BAT], pH and temperature, and the plot of log (A_0/A_t) versus time was linear indicating a first order dependence of the reaction rate on [AT]. Ao and A_t are the absorbances of the reaction mixture at time intervals of zero and t, respectively. The pseudo-first order rate constants k′ obtained at 300 °C are independent of [AT], further confirming the first order dependence on [AT] Table I. At constant pH, [AT], ionic strength, and temperature, the rate increased with increasing [BAT] as seen in Table I. Further, a plot of log k′ versus log [BAT] was linear with first order dependence on [BAT] as shown in Fig. 2.

B. Effects of pH and Halide Ions Concentration on the Rate

At constant $[BAT]_o$ $[AT]_o$ and temperature, the rate of the reaction increased with decrease in $[H^+]$ as it is seen in Table II. The plots of log k′ versus log $[H^+]$ were linear (r>-0.9949) with fractional slope indicating an inverse negative fractional order of approximately -0.7 in $[H^+]$. Addition of Cl^- or Br^- ions in the form of NaCl or NaBr at constant $[H^+]$ and ionic strength did not affect the rate, suggesting that chloride or bromide ions were not involved in the reaction rate.

C. Effects of [TSA] and Ionic Strength on the Rate

The addition of toulenesulfonamide [2.0×10^{-4} M to 20.0×10^{-4} M] had no effect on the rate, indicating that it is not involved in a pre-equilibrium to the rate determining step. The variation of ionic strength of the medium using $NaClO_4$ (0.02-0.020M) had no effect on the rate.

D. Effects of Dielectric Constant and Temperature on the Rate

The variation of the solvent composition using MeOH (5.0 to 20.0% v/v) did not affect the rate. The reaction was studied at varying temperatures 303-318 K. The activation parameters namely energy of activation (Ea), enthalpy of activation ($(\Delta H^{\neq})$, entropy of activation and free energy of activation ($\Delta G^{\neq}$) were obtained from the Arrhenius plots of log k′ versus 1/T. The kinetic and activation parameters obtained are presented in Table III.

E. Test for Free Radicals

The addition of the reaction mixture to the aqueous acrylamide monomer solutions in the dark did not initiate polymerization, indicating the absence of in situ formation of free-radical species in the reaction sequence.

IV. Discussion

The existance of similar equalibria in acid and alkaline solutions of N-metallo-N-haloarylsulphonamides is reported by Pryde and Soper [24], Morris et al. [22] and Bishop and Jennings [25]. BAT is analogous to CAT and behaves as a strong electrolyte in aqueous solutions forming different species as shown in (2)-(6):

$$ArSO_2NBrNa \rightleftharpoons ArSO_2NBr^- + Na^+ \quad (2)$$

$$ArSO_2NBr^- + H^+ \rightleftharpoons ArSO_2NHBr \quad (3)$$

$$2ArSO_2NHBr \rightleftharpoons ArSO_2NH_2 + ArSO_2NBr_2 \quad (4)$$

$$ArSO_2NHBr + H_2O \rightleftharpoons ArSO_2NH_2 + HOBr \quad (5)$$

$$HOBr + H^+ \rightleftharpoons H_2O^+Br \quad (6)$$

In acidic solutions, the probable oxidizing species are the free acid ($ArSO_2NHBr$), dibromamine-B ($ArSO_2NHBr_2$), HOBr and H_2O^+Br. If $ArSO_2NHBr_2$ were the reactive species, then the rate law would predict a second order dependence of rate on [BAT], which is contrary to the experimental observation. As (5) indicates a slow hydrolysis, if HOBr is the primary oxidizing species, a first order retardation of rate by the added $ArSO_2NH_2$ (toulenesulphonanide) is expected, so this is contrary to the experimental results. Hardy and Johnstan, [26] who have studied the pH dependence of relative concentrations of the species present in acidified CAT solutions of comparable molarities, have shown that $ArSO_2NHBr$ is the likely oxidizing species in acidic medium.

In the present case, the inverse fractional order in $[H^+]$ suggests that the protonation of $ArSO_2NHBr$ results in the generation of $ArSO_2N^+H_2Br$, which is likely to be the active oxidizing species involved in the mechanism of AT oxidation. Based on the preceding discussion, the following mechanism is proposed for the scheme of reactions.

$$ArSO_2N^+H_2Br \overset{K_1}{\rightleftharpoons} ArSO_2NHBr + H^+ \quad fast$$

$$ArSO_2NHBr + S \overset{k_2}{\rightleftharpoons} X \quad slow$$

$$X + H_2O \xrightarrow{k_3} Product$$

$$rate = k_2[ArSO_2NHBr][S] \quad (7)$$

The total effective concentration of $[BAT]_t$ from the above reactions is given by:

$$[BAT]_t = [ArSO_2N^+H_2Br] + [ArSO_2NHBr] \quad (8)$$

From step (1)

$$K_1 = \frac{[ArSO_2NHBr][H^+]}{[ArSO_2N^+H_2Br]} \quad (9)$$

$$\therefore [ArSO_2N^+H_2Br] = \frac{[ArSO_2NHBr][H^+]}{K_1} \quad (10)$$

Substitute (10) in (9)

$$[BAT]_t = \frac{[ArSO_2NHBr][H^+]}{K_1} + [ArSO_2NHBr]$$

$$\therefore [BAT]_t = [ArSO_2NHBr]\left\{\frac{[H^+]+1}{K_1}\right\}$$

$$[ArSO_2NHBr] = \frac{K_1[BAT]_t}{[H^+]+K_1} \quad (11)$$

Since

$$rate = k_2[ArSO_2NHBr][S]$$

This equation leads to the following rate law

$$rate = \frac{k_2K_1[BAT]_t[S]}{[H^+]+K_1} \quad (12)$$

This is in good agreement with the experimental data, including a first order in substrate, oxidant, and inverse fractional order in $[H^+]$

Since, rate = $k_{obs}[BAT]_t$, (12) can be transformed into (13)

$$k_{obs} = \frac{k_2K_1[S]}{[H^+]+K_1} \quad (13)$$

$$\frac{1}{k_{obs}} = \frac{[H^+]+K_1}{k_2K_1[S]} \quad (14)$$

or

$$\frac{1}{k_{obs}} = \frac{[H^+]}{k_2K_1[S]} + \frac{1}{k_2[S]} \quad (15)$$

A plot of $1/k'$ versus $[H^+]$ at constant [substrate] [BAT] and temperature from (15) was found to be linear with

$$slope = \frac{1}{k_2K_1[S]} \text{ and } Intercept = \frac{1}{k_2[S]} \quad (16)$$

The value of K_1 deprotonation constant (0.13) and k_2 (0.901) were calculated from the slope and intercept of the plots. Furthermore, the protonation constant $K_P = 1/K_1$ (7.21) of $ArSO_2NHBr$ was evaluated. The values of protonation constant K_P are similar to the value reported in previous publication [19]. Therefore, constancy of K_P values forms a strong indirect evidence for the existence of the reacting species $ArSO_2N^+H_2Br$. The rate constants and thermodynamic parameters show that the reaction is faster in bromamine-B than in BAT. The moderate value of enthalpy of activation ($\Delta H^{\neq}$) is supportive for the proposed mechanism in schemes, and the highly negative entropy of activation ($\Delta S^{\neq}$) indicates the formation of rigid transition state by an associative process in both oxidants BAB and BAT.

TABLE I
EFFECT OF VARYING REACTANT CONCENTRATION ON THE RATE BUFFER PH = 1.2; μ = 0.1 MOL DM^{-3}; Λ_{MAX}= 410 NM. T = 303K

[BAT] x 10^3 mol dm^{-3}	10^4[AT] mol dm^{-3}	10^5k′ (s^{-1})
1.0	1.0	4.46
2.0	1.0	10.0
3.0	1.0	19.0
4.0	1.0	22.0
5.0	1.0	28.0
6.0	1.0	34.6
3.0	0.6	19.1
3.0	0.8	19.3
3.0	1.0	19.0
3.0	1.2	20.1
3.0	1.5	18.9

TABLE II
EFFECT OF VARYING PH ON THE RATE OF REACTION [AT]0 = 1.0 × 10^{-4} MOL DM^{-3}; [BAT] = 3.0 × 10^{-3} MOL DM^{-3}; μ = 0.1 MOL DM^{-3}; Λ_{MAX}= 410 NM. T = 303K.

pH	[H^+]	k x 10^5(sec^{-1})
1.0	0.100	13.0
1.1	0.079	15.0
1.2	0.060	19.0
1.3	0.050	22.0
1.4	0.039	26.0
1.5	0.031	30.0

TABLE III
ACTIVATION PARAMETERS FOR THE OXIDATION OF AMITRIPYLINE BY BAT IN BUFFER MEDIUM

Ea (kJ mol^{-1})	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (JK^{-1} mol^{-1})	$\Delta G^{\neq}$ (JK^{-1} mol^{-1})
54.82	52.28	- 143.98	97.31

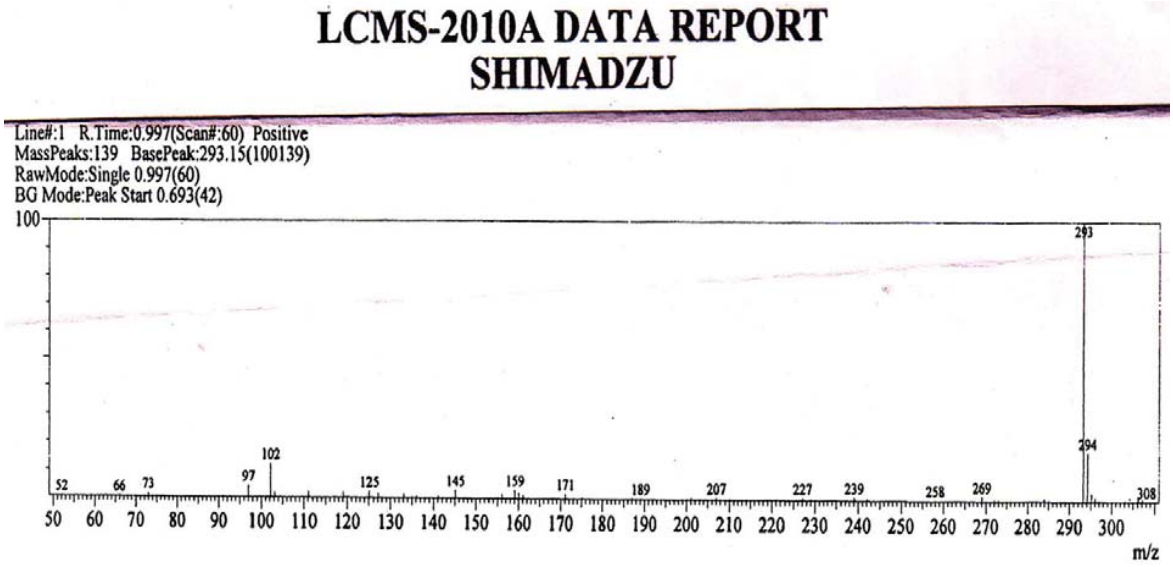

Fig. 1 Gas Chromatography Mass Spectra of AT N-oxide with Molecular Peak is obtained at 293

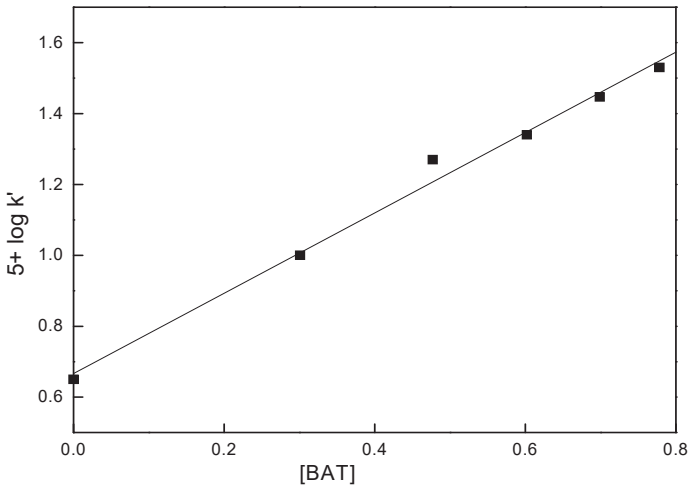

Fig. 2 Plot of log k' versus [BAT]

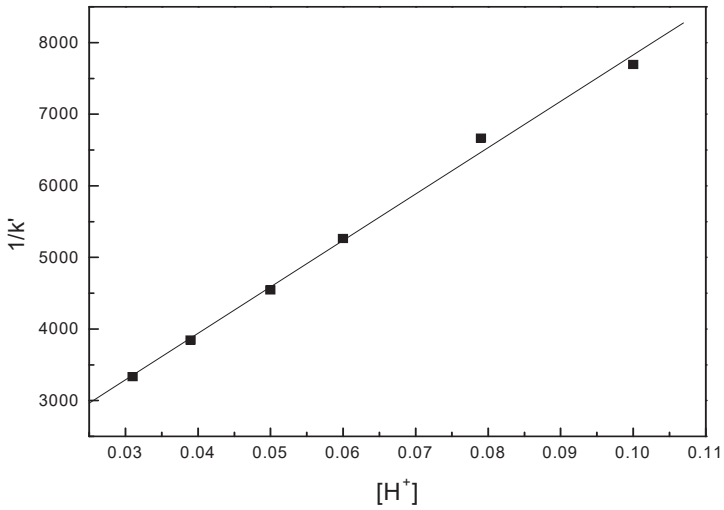

Fig. 3 Plot of1/k'versus [H^+]

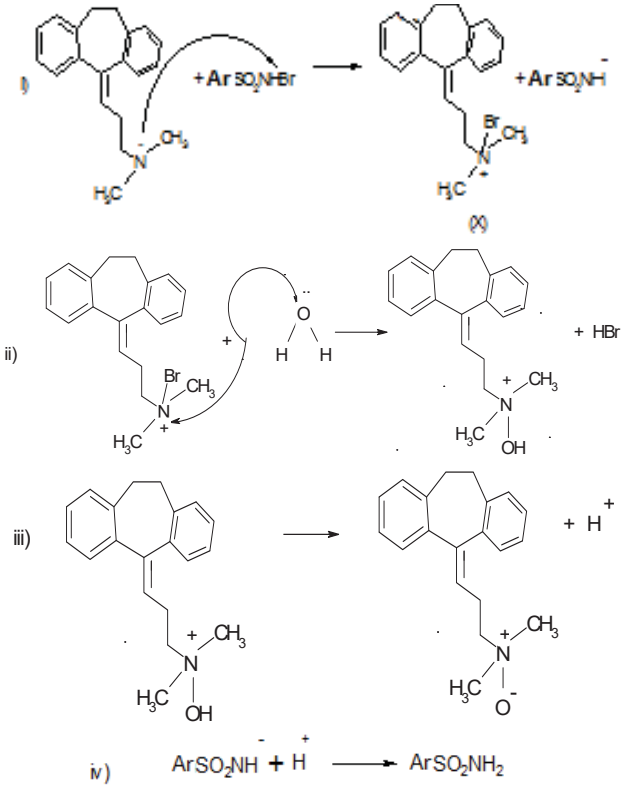

Fig. 4 Represents the Scheme of reactions of AT by BAT in buffer medium of different steps

V. Conclusion

Based on the present research work, the following conclusive remarks are drawn:

- The oxidation reaction follows similar kinetic patterns for both substrate and oxidant.
- The reaction obeys the experimental rate law.
- The thermodynamic parameters and reaction constants were evaluated.
- Reaction mechanism and rigorous kinetic modeling fit well with the experimental data.
- In the course of research work, oxidation product of AT N-oxide was identified.

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