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Kinetics of Oxidation of Cysteine by Bromamine-T in H_2SO_4 Medium

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The kinetics of oxidation of cysteine by sodium N-bromotoluene sulfonamide (bromamaine-T or BAT) in H_2SO_4 medium has been carried out at 30°C. The effect of $[H^+]$ and $[SO_4^{2-}]$, variation of ionic strength and dielectric constant to the medium on the rate of reaction has been studied. Addition of the reaction product, toluene sulfonamide had no effect on the rate of reaction. Thermodynamic parameters have been evaluated by studying the kinetics at various temperatures. Suitable mechanism has been proposed in consistency with the kinetic results.

INTRODUCTION

The chemistry of N-halo-N-metallo-aromatic sulfonamides has attracted the attention of many investigators on account of their diverse behaviour. They are the sources of halonium cations and hypohalite species[1–3]. The kinetic investigations of the oxidation of amino acids by serveral oxidants has been reported[4–15], except with bromamine-T (BAT). We report here the detailed investigation of oxidation of cysteine by bromamine-T in H_2SO_4 medium.

EXPERIMENTAL

Bromamine-T (BAT) was prepared by the reported procedure[16] and its purity was checked by iodometry and its mass spectrum, UV, IR, ^1H and ^{13}CNMR spectra. An aqueous solution of BAT was prepared, standardized and preserved in brown bottles to prevent its photochemical deterioration. AnalaR grade cysteine (J.T. Baker Chemical Co.) was used and the solution was prepared in 0.125 mol dm^{-3} H_2SO_4. All other chemicals used were of accepted grades of purity. Triply distilled water was used for preparing aqueous solutions. Ionic strength of the reaction mixture was kept at a high value with a concentrated solution of $NaClO_4$.

The reaction was carried out in a glass stoppered pyrex boiling tube. Requisite amounts of cysteine, $NaClO_4$ and H_2SO_4 were equilibrated at 30°C. To this was added a measured amount of pre-equilibrated (30°C) aqueous solution of BAT of known concentration. The progress of the reaction was monitored iodometrically for two half lives by withdrawing aliquots of the reaction mixture at regular time intervals. The pseudo first-order rate constants calculated were reproducible

within ±3%. Regression analysis of experimental data was carried out on a EC-72 statistical calculator, to get regression coefficient r.

Stoichiometry and Product Analysis

Reaction mixture containing different compositions of cysteine and BAT were equilibrated at 30°C in presence of 0.03 mol dm^{-3} H_2SO_4 for 24 h. The iodometric determination of unreacted BAT in the reaction mixture showed that 4 moles of BAT were consumed by 1 mole of cysteine according to equation (1).

$$HOOC\text{—}\underset{NH_2}{\overset{H}{C}}\text{—}CH_2SH + 4TsNBrNa + 5H_2O \rightarrow CH_3CHO + 4TsNH_2 + H_2SO_4 + NH_3 + CO_2 + 4NaBr \quad (1)$$

The presence of aldehyde which is oxidation product of cysteine in the reaction mixture was detected by preparing its 2,4-dinitrophenylhydrazone derivative and by using Tollen's and chromic acid tests[17]. The other product ammonia was quantitatively estimated by standard micro-Kjeldahl procedure, CO_2 was detected by the conventional lime-water test. The reaction product of BAT, $TsNH_2$ was also identified by paper chromatography; benzyl alcohol saturated with water was used as the solvent with 0.5% vanillin in 1% HCl in ethanol as spray reagent (R_f = 0.905)[18].

RESULTS AND DISCUSSION

With the substrate in stoichiometric excess, at constant [cyst] and [H_2SO_4], the plot of log [BAT] versus time was found to be linear (Table-1) indicating first-order dependence of rate on [BAT]. The rate of reaction increased with increase in the [cyst] and the plot of log k′ versus log [cyst] or log [S] was linear with a slope equal to unity [Fig. 1] indicating first order dependence of rate on [cyst]. The effect of [H^+] and [SO_4^{2-}] on the rate was studied. At constant [BAT], [cyst] and [Na_2SO_4], the rate of reaction decreased with increase in [H^+], and the plot of log k versus log [H^+] was found to be linear (Table-2 and Fig. 2) with fractional slope indicating inverse fractional order dependence in [H^+]. Addition of SO_4^{2-} ions in the form of Na_2SO_4 had no effect on the rate of reaction. Hence the dependence of the rate on [H_2SO_4] reflected the effect of [H^+] only on the reaction. Addition of bromide ion in the form of NaBr had no effect on the rate. Addition of the reaction product toluene sulfonamide or variation of ionic strength of the medium had no effect on the reaction. Further, the addition of acrylamide to the reaction mixture did not initiate polymerization showing the absence of free radical species. The reaction was studied at various temperatures and the kinetic and thermodynamic parameters were evaluated (Table-3).

TABLE-1
EFFECT OF VARYING REACTANT CONCENTRATION ON THE RATE OF REACTION

$[H_2SO_4] = 3.0 \times 10^{-2}$ mol dm^{-3}, $\mu = 0.5$ mol dm^{-3}, T = 303 K.

10^3 [BAT] mol dm^{-3}	10^3 [cyst] mol dm^{-3}	10^4 k′ sec^{-1}
2.0	4.0	8.00
3.0	4.0	8.02
4.0	4.0	8.01
5.0	4.0	8.01
6.0	4.0	8.12
7.0	4.0	8.14
5.0	2.0	3.98
5.0	3.0	6.10
5.0	4.0	8.01
5.0	4.5	9.12
5.0	5.0	10.20

Plot of log k′ versus log [cyst]
r = 0.9997, order = 1.0

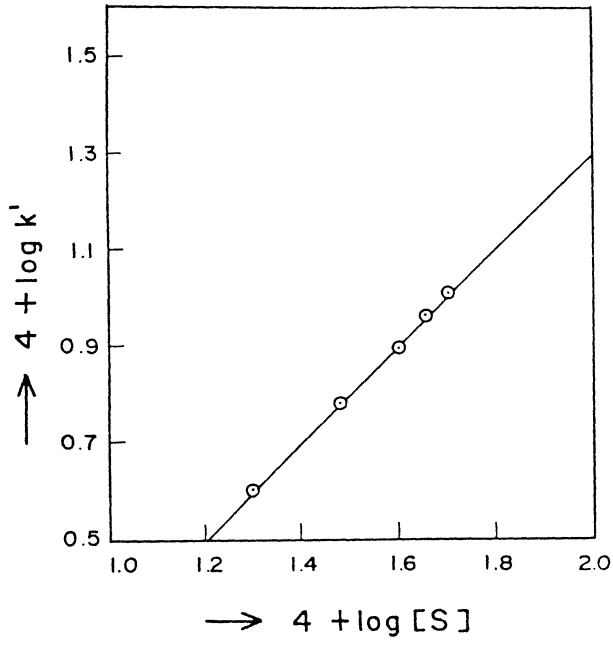


Fig. 1 [BAT] = 5.0×10^{-3} mol dm^{-3}, $[H_2SO_4] = 3.0 \times 10^{-2}$ mol dm^{-3}, $\mu = 0.5$ mol dm^{-3}, T = 303K

TABLE-2

EFFECT OF $[H^+]$ ON THE RATE OF REACTION AT CONSTANT $[Na_2SO_4]$

$[Cyst] = 4.0 \times 10^{-3}$, mol dm^{-3}, $[BAT] = 5.0 \times 10^{-3}$ mol dm^{-3}, $\mu = 0.5$ mol dm^{-3}, $[Na_2SO_4] = 0.2$ mol dm^{-3}, T = 303 K.

$[H^+]$ mol dm^{-3}	10^4 k′ sec^{-1}
0.02	13.90
0.04	10.40
0.06	8.01
0.08	6.94
0.12	5.13
0.14	4.46

Plot of log k′ versus log $[H^+]$
r = 0.9998, order = –0.58

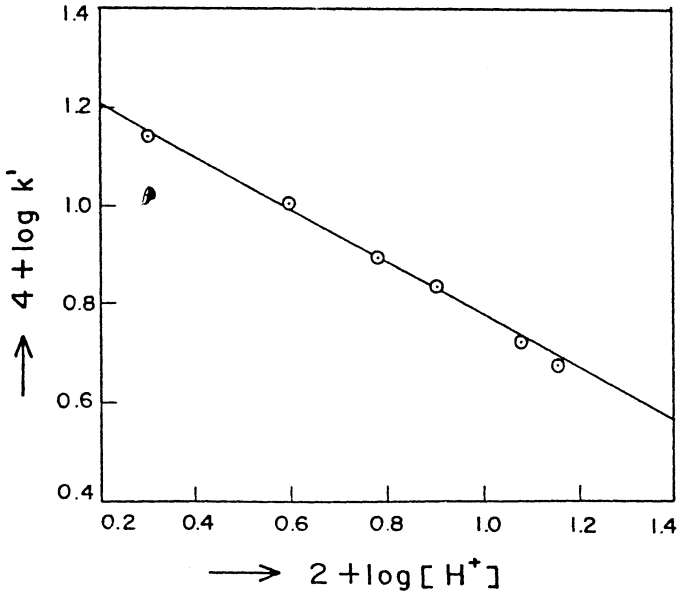


Fig. 2 [cyst] = 4.0×10^{-3} mol dm^{-3}, [BAT] = 5.0×10^{-3} mol dm^{-3}, $\mu = 0.5$ mol dm^{-3}, $[Na_2SO_4] = 0.2$ mol dm^{-3}, T = 303K

TABLE-3
KINETIC AND THERMODYNAMIC PARAMETERS FOR THE OXIDATION OF CYSTEINE BY BROMAMINE-T

E_a kJ mol^{-1}	$\Delta H^{\neq}$ kJ mol^{-1}	$\Delta S^{\neq}$ JK mol^{-1}	$\Delta G^{\neq}$ kJ mol^{-1}
47.12	44.58	−157.20	92.51

Pryde and Copper[19], Morries *et al*[20], Bishop and Jennings[21] have shown the existence of similar equilibria in acid and alkaline solutions of N-metallo-N-haloarylsulfonamides, bromamine-T (TsNBrNa or p-$CH_3C_6H_4SO_2NBrNa$), like its chlorine analog chloramine-T, behaves as a strong electrolyte in aqueous solutions forming different species as shown in equations (2–6),

$$TsNBrNa \rightleftharpoons TsNBr^- + Na^+ \quad (2)$$

$$TsNBr^- + H^+ \rightleftharpoons TsNHBr \quad (3)$$

$$TsNHBr + H_2O \rightleftharpoons TsNH_2 + HOBr \quad (4)$$

$$2TsNHBr \rightleftharpoons TsNH_2 + TsNBr_2 \quad (5)$$

$$HOBr + H^+ \rightleftharpoons H_2OBr^+ \quad (6)$$

In acid medium, the probable oxidizing species are the free acid (TsNHBr), dibromamine-T ($TsNBr_2$), HOBr and H_2OBr^+. The involvement of $TsNBr_2$ in the mechanism leads to a second order rate law according to equation (5) which is contrary to experimental observations. As equation (4) indicates, a slow hydrolysis, if HOBr were the primary oxidizing species, a first order retardation of the rate by the added $TsNH_2$ would be expected contrary to the experimental result. Hardy and Johnston[17] who have studied the pH dependent relative concentrations of the species present in acidified haloamines solution of comparable molarities have shown that TsNHBr is the likely oxidizing species in acid medium. Narayanan *et al.*[22] and Subhashini *et al.*[23] have reported that monohaloamines can be further protonated at pH < 2 as shown in the following equations (7) and (8) for chloramine-T and chloramine-B respectively.

$$CH_3C_6H_4SO_2NHCl + H^+ \rightleftharpoons CH_3C_6H_4SO_2\overset{+}{N}H_2Cl \quad (7)$$

$$C_6H_5SO_2NHCl + H^+ \rightleftharpoons C_6H_5SO_2\overset{+}{N}H_2Cl \quad (8)$$

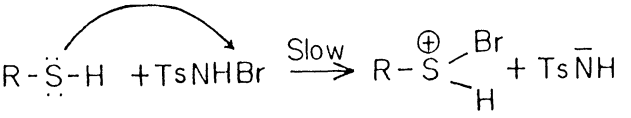
The second protonation constants for chloramine-T and chloramine-B are 102 and 61 ± 5 repectively at 25°C. Gupta[24] believes that the values could be lower than those reported by the above workers[22, 23]. In the present case the inverse fractional order in $[H^+]$ suggests that the deprotonation of $TsNH_2Br^+$ results in the regeneration of TsNHBr which is likely to be the active oxidizing species involved in the mechanism of the cysteine oxidation. Based on the preceding discussion a mechanism (Scheme-I) is proposed for the reaction

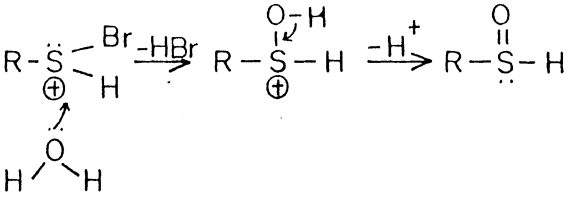
$$TsNH_2Br \xrightarrow{K_1} TsNHBr + H^+ \quad \text{(i) fast}$$

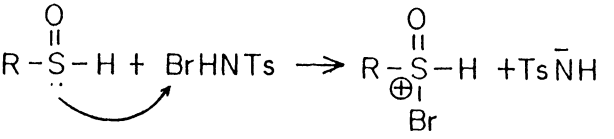
$$TsNHBr + S \xrightarrow{k_2} X \quad \text{(ii) slow}$$

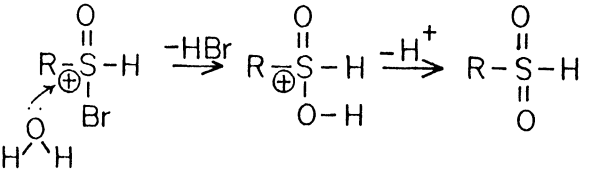
$$X \xrightarrow{k_3} \text{products}$$

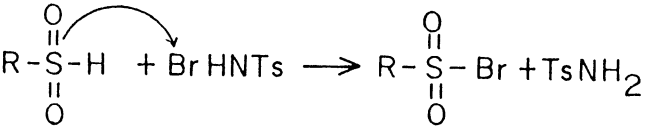
Scheme-I

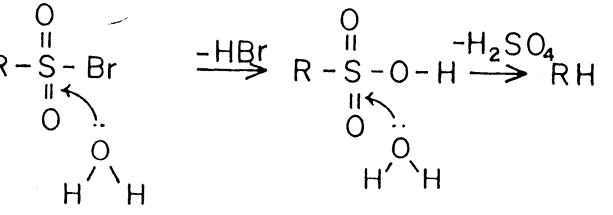
I 

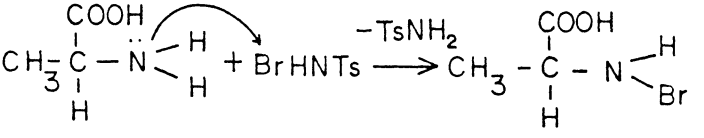
II 

III 

IV 

V 

VI 

VII 

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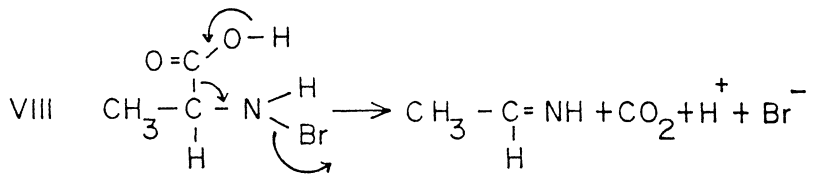
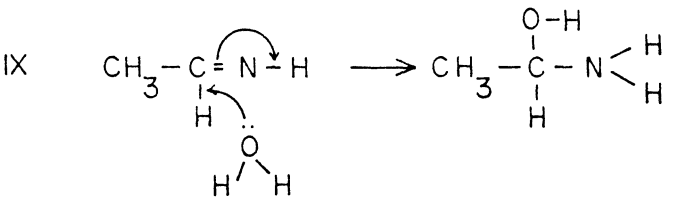
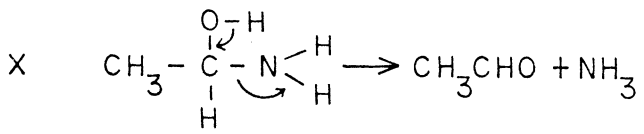
Scheme-II







Here R = $HOOC-\underset{\underset{NH_2}{|}}{CH}-CH_2$

Ts = $P-CH_3C_6H_4SO_2$

Scheme-II

In Scheme-I, S represents the cysteine substrate while X represents the complex intermediate species A detailed mechanistic interpretation of cysteine-BAT reaction in acid medium is represented in scheme-II.

From Scheme-I,

$$\text{rate} = \frac{-d[BAT]}{dt} = k_2[S]\,[TsNHBr] \tag{9}$$

If the total effective concentration of BAT, from Scheme-I is given by equation (10),

$$[BAT]_t = \overset{+}{TsNH_2}Br + TsNHBr \tag{10}$$

Then

$$K_1 = \frac{[TsNHBr][H^+]}{\overset{+}{TsNH_2}Br}$$

$$\therefore \quad [\overset{+}{TsNH_2}Br] = \frac{[TsNHBr][H^+]}{K_1} \tag{11}$$

Substituting (11) in equation (10),

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

$$= [TsNHBr]\left\{\frac{[H^+]+1}{K_1}\right\}$$

Then

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

$$\therefore \qquad [TsNHBr] = \frac{K_1[BAT]_t}{[H^+]+K_1} \qquad (12)$$

Since rate $\qquad k_2 = [TsNHBr][S], \qquad (13)$

Substituting (12) in (13)

$$\text{Rate} = \frac{k_2K_1[BAT]_t[S]}{[H^+]+K_1} \qquad (14)$$

Which is in agreement with the experimental data including a first order in [BAT] and [cysteine] and inverse fractional order in $[H^+]$.

Since rate = k′ [BAT] under pseudo first order condition, the rate equation can be transformed into equation (15).

$$k' = \frac{k_2K_1[S]}{[H^+]+K_1} \qquad (15)$$

$$\frac{1}{k'} = \frac{[H^+]+K_1}{k_2K_1[S]} \qquad (16)$$

$$\frac{1}{k'} = \frac{[H^+]}{k_2K_1[S]} + \frac{1}{k_2[S]} \qquad (17)$$

Based on equation (17) the plot of 1/k′ versus $[H^+]$ (Fig. 3) at constant [BAT], [substrate] and temperature was found to be linear. The values of K_1 and k_2 were calculated from the slope and intercept of the plot ($k_2 = 53.57 \times 10^{-2}$ and $K_1 = 3.72 \times 10^{-2}$). The value of deprotonation constant ($K_1 = 3.72 \times 10^{-2}$) of step (i) of Scheme-I is calculated from equation (17). Therefore the value of protonation constant (K_p) is obtained by $K_p = 1/K_1$. Further the value of K_1 26.88 is equal to that of the values obtained in the oxidation of primary amines by bromamine-T in HCl medium and in presence of Ru(III) catalyst[25]. Therefore the constancy of K_p or K_1 values forms a strong indirect evidence for the existence of the reacting species $TsNH_2Br^+$ of the oxidant, supporting the proposed mechanism of oxidation of cysteine by BAT (Scheme-I).

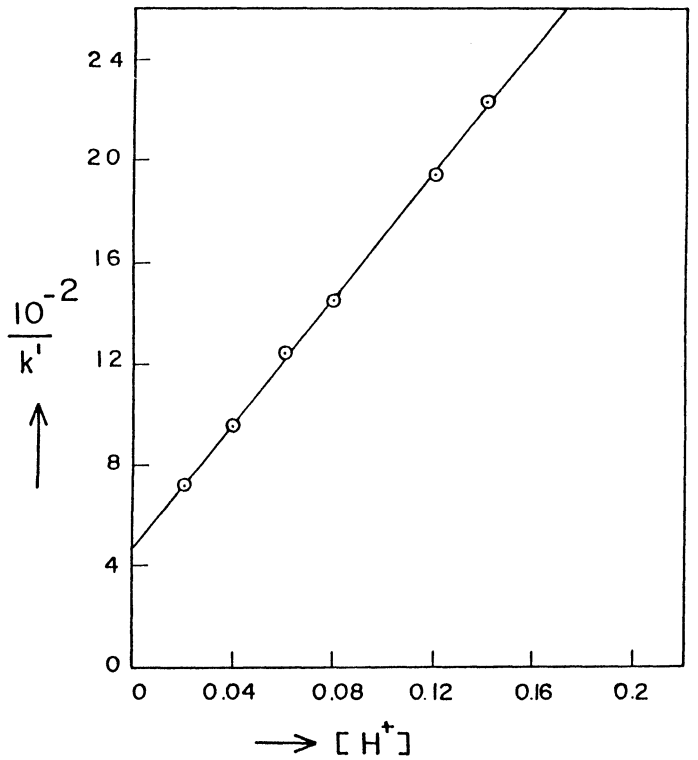


Fig. 3 $[Cyst] = 4.0 \times 10^{-3}$ mol dm^{-3}, $[BAT] = 5.0 \times 10^{-3}$ mol dm^{-3}, $[H_2SO_4] = 3.0 \times 10^{-2}$ mol dm^{-3}, $\mu = 0.5$ mol dm^{-3}, T = 303 K

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