

Kinetics and Mechanism of Oxidation of L-Isoleucine and L-Ornithine Hydrochloride by Sodium N-Bromobenzenesulphonamide in Perchloric Acid Medium

Hemmige S. YATHIRAJAN*, Chapuradoddi R. RAJU, Kikkeri N. MOHANA, Sheena SHASHIKANTH and Padmarajaiah NAGARAJA

*Department of Studies in Chemistry, University of Mysore,
Manasagangotri, Mysore-570 006, INDIA
e-mail: yathirajan@yahoo.com*

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Kinetic studies of the oxidation of L-isoleucine (ISL) and L-ornithine hydrochloride (ORH) by sodium N-bromobenzenesulphonamide (bromamine-B or BAB) were studied in aqueous perchloric acid medium. The rate shows first-order dependence on both $[BAB]_0$ and $[\text{amino acid}]_0$ and inverse first-order dependence on $[H^+]$ for ISL and first-order dependence on $[H^+]$ for ORH. The rate of reaction decreased with decreases in the dielectric constant of the medium. The addition of benzenesulphonamide (BSA), which is one of the reaction products, had no significant effect on the reaction rate. The rate remained unchanged with the variation in the ionic strength of the medium for ISL, whereas the rate decreased with increases in the ionic strength of the medium for ORH. Isovaleronitrile and 3-(methylamino)propionitrile were identified as the products. Thermodynamic parameters were computed by studying the reactions at different temperatures (298-316 K). The rate laws derived are in excellent agreement with the experimental results. Plausible mechanisms are suggested.

Key Words: Bromamine-B, Oxidation, Kinetics, Amino acids

Introduction

The versatile nature of N-halogeno-N-metallo aromatic sulphonamides is due to their ability to act as sources of halonium cations, hypohalite species and nitrogen anions which act both as bases and nucleophiles. Bromamine-B is an important member of this class of reagents.

Bromamine-B (BAB; $C_6H_5SO_2NBrNa \cdot 1.5H_2O$) has received considerable attention as an oxidimetric reagent¹⁻⁵. Photolysis of aqueous BAB⁶ and conductometric study of the interaction of BAB with some metal ion solutions have been performed⁷. Crystal and molecular structure studies of bromamine-B have been reported⁸. A review of the literature shows that the kinetics of oxidation of the title compounds

*Corresponding author

using BAB have not been reported, although the oxidation of some other amino acids using BAB has been reported⁹⁻¹².

The oxidation of amino acids is of interest due to their biological importance. Amino acids play a significant role in a number of metabolic reactions. Species metabolic roles of amino acids include the biosynthesis of polypeptides and proteins and the synthesis of nucleotides. Thus the mechanism of analogous non-enzymatic chemical processes in the oxidation of amino acids is a potential area for intensive investigations. In a continuation of our work on aromatic sulphonyl haloamines^{13,14}, we report herein the kinetics of oxidation of ISL and ORH by bromamine-B in perchloric acid medium.

Experimental

Bromamine-B was prepared from Chloramine-B². Its purity was checked by iodometry and by IR, ¹H and ¹³C NMR spectroscopy. An aqueous solution of BAB was standardised iodometrically and stored in brown bottles to avoid photochemical deterioration.

Analar grade L-isoleucine and L-ornithine hydrochloride (both Sd-fine) were assayed by the standard method¹⁵. Aqueous solutions of ISL and ORH of desired strength were prepared freshly each time. All other chemicals were of analytical grade. Triple distilled water was used throughout. The constant ionic strength of the medium was maintained ($\mu = 0.5 \text{ mol dm}^{-3}$) using a concentrated solution of NaClO₄.

Kinetic measurements

The rate studies were performed under pseudo first-order conditions by keeping an excess of amino acid over BAB. Solutions of NaClO₄ and water (to keep the total volume constant for all runs) were taken in a glass-stoppered Pyrex boiling tube, and thermostated at 308 K (ISL) and 303 K (ORH). A measured amount of BAB solution, also thermostated at the same temperature, was rapidly added to the mixture in the boiling tube. The progress of the reaction was monitored by iodometric estimation of unreacted BAB in a measured aliquot of the reaction mixture at different times. The course of the reaction was studied for more than 2 half-lives. The rate constants were evaluated from the plots of log[BAB] against time. The pseudo first-order rate constants calculated were reproducible to within $\pm 3\%$.

Stoichiometry and product analysis

The stoichiometry of the oxidation was determined by thermally equilibrating varying ratios of [BAB] to [amino acid] under pseudo first-order conditions in presence of H⁺ ions (0.12-0.24 mol dm⁻³). The observed stoichiometry may be represented as



where R = C₆H₅SO₂, R' = C₂H₅CH(CH₃) for ISL and (CH₂)₃NH₂ for ORH.

The nitriles produced isovalero nitrile and 3-(methylamino)propionitrile, which were identified by their colour reactions¹⁶ with hydroxylamine and ferric chloride. Benzenesulphonamide was detected among the reaction products by thin layer chromatography¹⁷ with a mixture of petroleum ether, chloroform and n-butanol (2:2:1 v/v) as the mobile phase and iodine as the detection agent ($R_f = 0.88$).

Results and Discussion

The kinetics of oxidation of ISL and ORH by BAB in perchloric acid medium were investigated at several concentrations of reactants and H^+ . At constant $[HClO_4]$ and $[amino\ acid]_0$ ($[amino\ acid] \gg [BAB]_0$), plots of $\log[BAB]$ vs. time were linear indicating a first-order dependence of rate on $[BAB]_0$. The rate constant k was not affected by a change in $[BAB]_0$ (Table 1). The pseudo first-order rate constant increased with the increase in $[amino\ acid]$ (Table 1). Plots of $\log k$ vs. $\log[amino\ acid]$ (Fig. 1) were linear with slopes equal to 0.9907 (ISL) and 1.03 (ORH) indicating first-order dependence of the rate on concentration for both the amino acids. The rate constant decreased with an increase in $[HClO_4]$ (Table 1) for ISL, whereas the rate constant increased with an increase in $[HClO_4]$ (Table 1) for ORH. The orders with respect to $[H^+]$, as calculated from the slopes of the plots of $\log k$ against $\log[HClO_4]$ (Fig. 2) were -1.04 and 1.05, indicating inverse first-order and first-order dependence of rate on $[H^+]$ for ISL and ORH respectively.

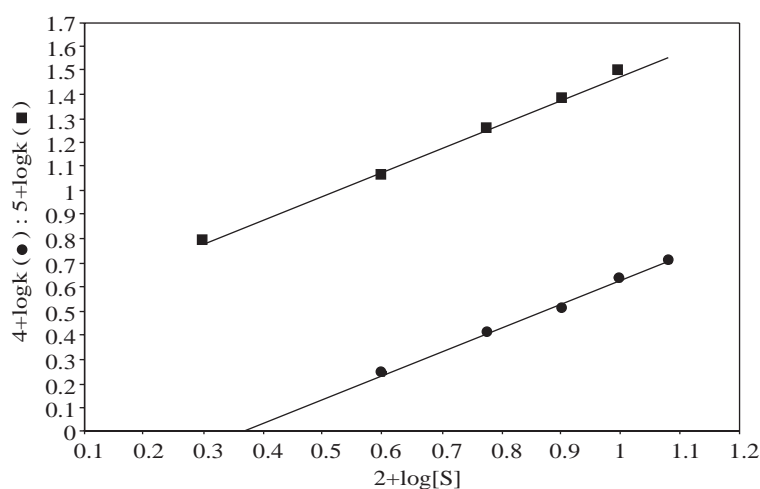


Figure 1. Plot of $4 + \log k$ (●) : $5 + \log k$ (■) \gg $1 + \log[S]$.

In the case of ISL, the rate of reaction remains unaffected when increasing the ionic strength of the medium by adding sodium perchlorate ($0.3\text{--}1.0\text{ mol dm}^{-3}$), but the rate decreased with increases in the ionic strength of the medium for ORH. The dielectric constant of the medium was varied by adding different proportions of CH_3CN (0-30% v/v) to the reaction mixture. The rate decreased with increasing proportions of CH_3CN (Table 2). Plots of $\log k$ vs. $1/D$, where D is the dielectric constant of the medium, were linear with a negative slope. Blank experiments showed that CH_3CN was not oxidised by BAB under the present experimental conditions. The addition of BSA had no effect on the reaction rate, and the addition of reaction mixture to aqueous acrylamide solution did not initiate polymerisation, showing the absence of free radical species.

The reactions were studied at different temperatures (298-316 K) (Table 2). From the Arrhenius plots of $\log k$ vs. $1/T$, activation energy and other thermodynamic parameters for ISL and ORH were found to be $E_a = 70.6, 107.2$; $\Delta H^\ddagger = 68.0, 104.7$; $\Delta G^\ddagger = 26.22, 24.99\text{ kJ mol}^{-1}$; $\Delta S^\ddagger = 135.1, 261.15\text{ JK}^{-1}\text{ mol}^{-1}$, and $\log A = 10.4, 16.7$ respectively.

Table 1. Effect of varying [BAB], [amino acid], [HClO₄], ionic strength on the reaction rate. $\mu = 0.5 \text{ mol dm}^{-3}$; T = 308 K (ISL) and 303 K (ORH)

| Amino acid | $10^3[\text{BAB}]$ (mol dm^{-3}) | $10^2[\text{amino acid}]$ (mol dm^{-3}) | $10[\text{HClO}_4]$ (mol dm^{-3}) | $k \times 10^4 \text{ (s}^{-1}\text{)}$ |
|------------------|------------------------------------------------|-------------------------------------------------------|-------------------------------------------------|-----------------------------------------|
| ISL | 0.3 | 8.0 | 1.6 | 3.203 |
| | 0.5 | 8.0 | 1.6 | 3.266 |
| | 0.7 | 8.0 | 1.6 | 3.345 |
| | 1.0 | 8.0 | 1.6 | 3.277 |
| | 3.0 | 8.0 | 1.6 | 3.315 |
| | 1.0 | 4.0 | 1.6 | 1.738 |
| | 1.0 | 6.0 | 1.6 | 2.572 |
| | 1.0 | 8.0 | 1.6 | 3.266 |
| | 1.0 | 10.0 | 1.6 | 4.311 |
| | 1.0 | 12.0 | 1.6 | 5.079 |
| | 1.0 | 8.0 | 1.2 | 4.350 |
| | 1.0 | 8.0 | 1.4 | 3.755 |
| | 1.0 | 8.0 | 1.6 | 3.266 |
| | 1.0 | 8.0 | 2.0 | 2.637 |
| | 1.0 | 8.0 | 2.4 | 2.168 |
| | ^a 1.0 | 8.0 | 1.6 | 3.289 |
| ^b 1.0 | 8.0 | 1.6 | 3.224 | |
| ORH | 3.0 | 6.0 | 2.0 | 1.773 |
| | 5.0 | 6.0 | 2.0 | 1.780 |
| | 7.0 | 6.0 | 2.0 | 1.762 |
| | 9.0 | 6.0 | 2.0 | 1.768 |
| | 5.0 | 2.0 | 2.0 | 0.617 |
| | 5.0 | 4.0 | 2.0 | 1.151 |
| | 5.0 | 6.0 | 2.0 | 1.773 |
| | 5.0 | 8.0 | 2.0 | 2.368 |
| | 5.0 | 10.0 | 2.0 | 3.071 |
| | 5.0 | 6.0 | 1.2 | 0.975 |
| | 5.0 | 6.0 | 1.6 | 1.245 |
| | 5.0 | 6.0 | 2.0 | 1.779 |
| | 5.0 | 6.0 | 2.4 | 2.077 |
| | 5.0 | 6.0 | 2.8 | 2.351 |
| | ^c 5.0 | 6.0 | 2.0 | 2.201 |
| | ^d 5.0 | 6.0 | 2.0 | 1.223 |
| | ^e 5.0 | 6.0 | 2.0 | 1.059 |
| | ^f 5.0 | 6.0 | 2.0 | 1.762 |

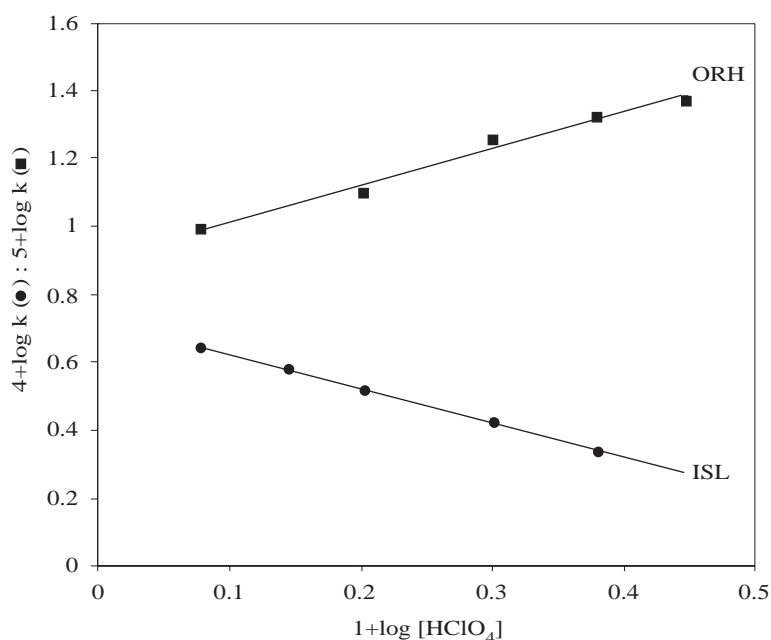
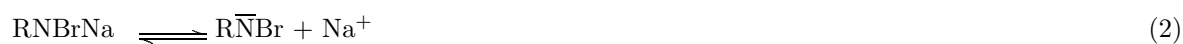
$\mu = 0.25 \text{ mol dm}^{-3}$ (b) Excess of BSA (c) $\mu = 0.3 \text{ mol dm}^{-3}$
(d) $\mu = 0.7 \text{ mol dm}^{-3}$ (e) $\mu = 1.0 \text{ mol dm}^{-3}$ (f) Excess of BSA

Bromamine-B is analogous to chloramine-T in its properties. The work of Hardy and Johnston¹ and our work on conductometric studies⁷ showed that the free acid formed can undergo disproportionation and/or hydrolysis. Therefore the possible oxidising species in acidified BAB solutions are RNHBr, RNBr₂ and HOBr.

Table 2. Effect of solvent composition and temperature on the reaction rate.

$10^3[\text{BAB}] = 1.0 \text{ mol dm}^{-3}$; $10^2 [\text{ISL}] = 8.0 \text{ mol dm}^{-3}$; $10[\text{H}^+] = 1.6 \text{ mol dm}^{-3}$; $\mu = 0.5 \text{ mol dm}^{-3}$;
 $10^3[\text{BAB}] = 5.0 \text{ mol dm}^{-3}$; $10^2 [\text{ORH}] = 6.0 \text{ mol dm}^{-3}$; $10[\text{H}^+] = 2.0 \text{ mol dm}^{-3}$; $\mu = 0.5 \text{ mol dm}^{-3}$

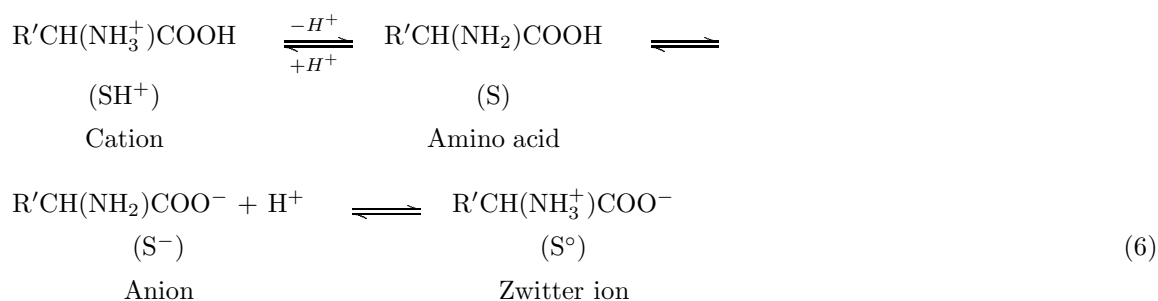
| T(K) | Acetonitrile-water (v/v %) | | $k \times 10^4 \text{ (s}^{-1}\text{)}$ | |
|------|----------------------------|-------|-----------------------------------------|-------|
| | ISL | ORH | ISL | ORH |
| 308 | 0-100 | - | 3.266 | - |
| 308 | 10-90 | - | 2.322 | - |
| 308 | 20-80 | - | 1.590 | - |
| 308 | 30-70 | - | 0.798 | - |
| 303 | - | 0-100 | - | 1.779 |
| 303 | - | 10-90 | - | 1.118 |
| 303 | - | 20-80 | - | 0.704 |
| 303 | - | 30-70 | - | 0.434 |
| 298 | - | 0-100 | - | 0.942 |
| 303 | 0-100 | 0-100 | 1.631 | 1.779 |
| 308 | 0-100 | 0-100 | 3.266 | 4.244 |
| 312 | 0-100 | - | 5.142 | - |
| 313 | - | 0-100 | - | 7.338 |
| 316 | 0-100 | - | 8.516 | - |


Figure 2. Plot of $4+\log k (\bullet) : 5+\log k (\blacksquare) >< 2+\log [\text{HClO}_4]$.


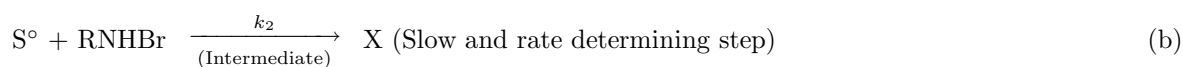


If RNHBr_2 were to be the oxidising species, then the rate law predicts a second-order dependence of rate on $[\text{BAB}]$, which is contrary to experimental observations. If HOBr is the active oxidant species, a first-order retardation of the rate by the added benzenesulphonamide is expected. However, no such effect is observed. The calculations of Hardy and Johnston on aqueous BAB and the observations of Bishop and Jennings¹⁸ and Morris et al.¹⁹ on aqueous chloramine-T solutions have clearly shown that the conjugate acid is a predominant species in acid medium that reacts with the substrate.

An amino acid is known to exist in the following equilibria in aqueous solution:



Under the present experimental conditions, the concentration of anion form will be very low and hence the possible species may be either the cation form of amino acid or zwitter ion (S°). With cation as the active species, the rate law predicts a second-order dependence of the rate on $[\text{H}^+]$, which is contrary to experimental observations. Protonated amino acid is not involved in the reaction sequence and the zwitter ion is the active species in this reaction. Scheme 1 is proposed to interpret the experimental observations in the oxidation of ISL .



Scheme 1. Reaction sequence for the oxidation of ISL by BAB .

Based on Scheme 1, the rate of reaction is given by

$$\text{Rate} = k_2[\text{S}^\circ][\text{RNHBr}] \quad (7)$$

Assuming steady state for the species S° , we have

$$\frac{d[\text{S}^\circ]}{dt} = k_1[\text{SH}^+] - k_{-1}[\text{S}^\circ][\text{H}^+] - k_2[\text{S}^\circ][\text{RNHBr}] = 0$$

$$[\text{S}^\circ]\{k_{-1}[\text{H}^+] + k_2[\text{RNHBr}]\} = k_1[\text{SH}^+]$$

$$\therefore [\text{S}^\circ] = \frac{k_1[\text{SH}^+]}{k_{-1}[\text{H}^+] + k_2[\text{RNHBr}]} \quad (8)$$

Assuming $k_{-1}[\text{H}^+] \gg k_2[\text{RNHBr}]$, equation (8) becomes

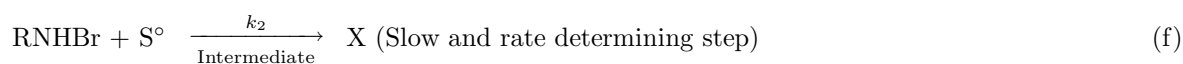
$$[\text{S}^\circ] = \frac{k_1[\text{SH}^+]}{k_{-1}[\text{H}^+]} \quad (9)$$

Substituting in (7), it is seen that

$$\text{Rate} = \frac{k_1 k_2 [\text{SH}^+][\text{RNHBr}]}{k_{-1}[\text{H}^+]} \quad (10)$$

$$\text{or } - = \frac{d[\text{BAB}]}{dt} = \frac{k_1 k_2}{k_{-1}} \frac{[\text{BAB}][\text{SH}^+]}{[\text{H}^+]} \quad (11)$$

In the case of ORH, Scheme 2 can be proposed to account for the observed kinetics.



Scheme 2. Reaction sequence for the oxidation of ORH by BAB.

$$\therefore \text{The rate of reaction} = k_2[\text{RNHBr}][\text{S}^\circ] \quad (12)$$

Assuming steady state conditions for RNHBr, we have

$$\frac{d[\text{RNHBr}]}{dt} = k_1[\text{BAB}][\text{H}^+] - k_{-1}[\text{RNHBr}] - k_2[\text{RNHBr}][\text{S}^\circ] - k_3[\text{X}][\text{RNHBr}] = 0 \quad (13)$$

Neglecting $k_3[\text{X}][\text{RNHBr}]$, equation (13) leads to

$$[\text{RNHBr}] = \frac{k_1[\text{BAB}][\text{H}^+]}{k_{-1} + k_2[\text{S}^\circ]} \quad (14)$$

Substituting (14) in (12) we get

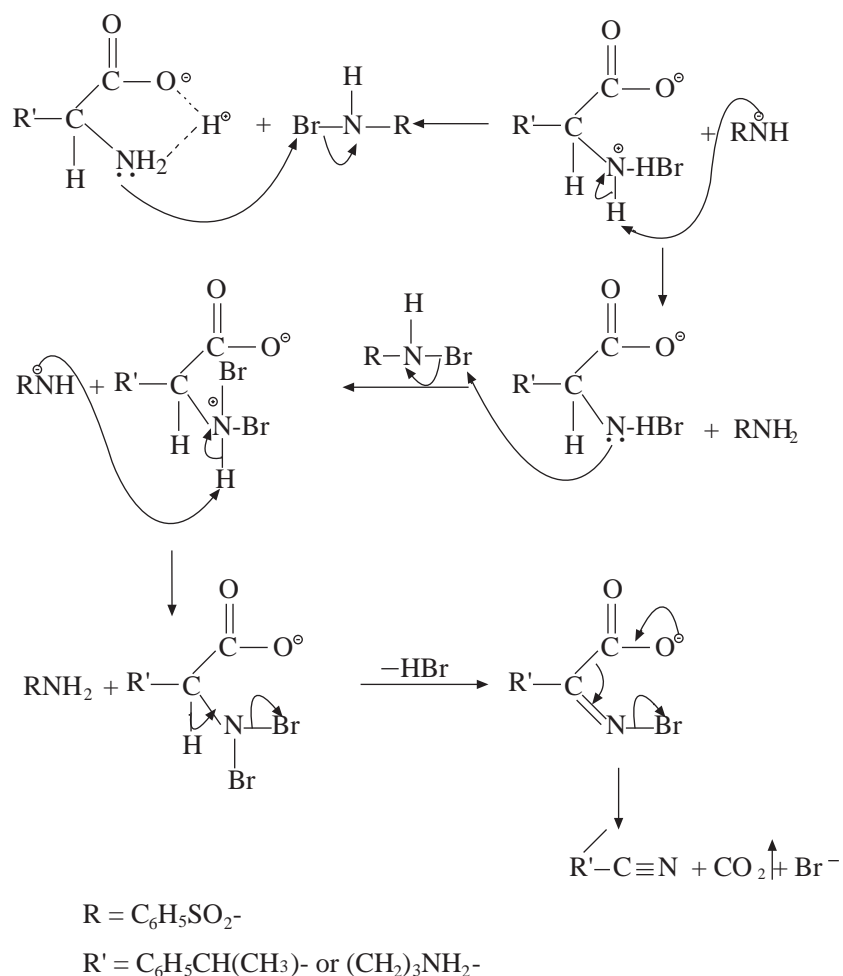
$$\text{Rate} = k_2[\text{S}^\circ] \frac{k_1[\text{BAB}][\text{H}^+]}{k_{-1} + k_2[\text{S}^\circ]} \quad (15)$$

Assuming $k_{-1} \gg k_2[\text{S}^\circ]$, equation (15) becomes

$$- = \frac{d[\text{BAB}]}{dt} = \frac{k_1 k_2}{k_{-1}} [\text{BAB}][\text{S}^\circ][\text{H}^+] \quad (16)$$

The rate laws given by equations (11) and (16) are in agreement with the experimental results.

The effect of H-bonding ensures considerable electron density at the amine nitrogen through the release of its non-bonding electrons. The enhanced nucleophilicity at the N-atom facilitates an electrophilic attack by the positive bromine of RNHBr. This is more or less concerted with the release of H^+ ion and subsequent reactions take place through a fast step. The detailed mechanistic interpretation of Schemes 1 and 2 is shown in general in Scheme 3.

**Scheme 3.** Mechanism of oxidation of ISL/ORH by bromamine-B.

It is noted that a change in solvent composition by adding CH_3CN to the reaction mixture affects the rate. Many theories have been put forward to give a quantitative explanation²⁰⁻²² for the effect of the dielectric constant (D) of the medium on the kinetics of liquid phase reactions. For the limiting case of a zero angle of approach between 2 dipoles or ion-dipole system, Amis²³ has shown that in a straight line plot of $\log k'$ vs. $1/D$ a positive slope indicates a positive ion-dipole reaction, while a negative slope indicates the involvement of 2 dipoles or a negative ion-dipole reaction. In the present investigations a plot of $\log k$ vs. $1/D$ gives a straight line with a negative slope, clearly supporting the involvement of 2 dipoles in the rate determining step for both ISL and ORH. All these observations confirm the proposed mechanism.

Puttaswamy and Vaz¹² have studied the oxidation of glutamic and aspartic acids with BAB in aqueous perchloric acid medium at 30 °C. The rate shows first order dependence on both [BAB] and [amino acid] and inverse first-order dependence on $[H^+]$. At $[H^+] > 0.6 \text{ mol dm}^{-3}$, the rate levelled off, indicating zero-order dependence on $[H^+]$ and under these conditions, the rate has fractional-order dependence on [amino acid]. Succinic and malonic acids have been identified as the reaction products, with 1 mole of the amino acid consuming 2 moles of BAB. In the present investigations, however, a first-order dependence on both [BAB] and [amino acid] was noted. A first-order dependence of rate on $[H^+]$ for ORH and an inverse first-order dependence on $[H^+]$ with respect to ISL was observed. Nitriles as the products of oxidation were

noted in the present work ($[S] \gg [Ox]$) but acids or aldehydes were not. Nitriles were detected by their colour reaction. The presence of nitrile in the products was confirmed by FT-IR analysis in both cases as follows. The products were dissolved in ether in a separatory funnel, washed with dilute solution of NaOH and shaken well. The ether layer was concentrated by slow evaporation on a water bath. A small drop of the liquid remaining was taken for FT-IR analysis, which revealed a sharp peak around 2250 cm^{-1} for nitrile. Ample evidence for the formation of nitriles in these reactions has been reported earlier from our laboratories.^{3,14,24,25} The results obtained are comparable with those from kinetic studies on the oxidation of α -amino acids²⁴ and glutamic acid²⁵ by chloramine-T in acid medium.

In the case of ORH, the excess acid protonates the amino group in the side chain, which facilitates the increase in the solubility of the substrate. The reaction product BSA does not influence the rate, showing that it is not involved in pre-equilibrium. In the case of ISL, the variation of the ionic strength of the medium does not alter the rate, indicating that non-ionic species are involved in the rate limiting step, whereas with ORH the observed decrease in the rate with the increasing ionic strength of the medium is consistent with the Quinlan-Amis equation. The fairly high value of the energy of activation observed during the reaction is comparable with those observed during the oxidation of α -amino acids by CAT²⁴, where the formation of more compact chlorinated amino acid intermediate is assumed. The high entropy of activation observed during these reactions supports the predicted mechanisms. The positive value of the free energy of activation indicates that the intermediate X is strongly solvated. The mechanism is further supported by the stoichiometry of the reaction.

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