

## Mechanism of oxidation of isoniazid by hypohalites in alkaline medium: A kinetic study

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Kinetics of oxidation of isoniazid (INH) by sodium hypohalites NaOX (X=Cl or Br) has been investigated at 303 K in NaOH medium. Under comparable experimental conditions the oxidation behaviour is similar with both the oxidants with first order dependence of rate each on [NaOX] and [INH] and fractional order on [OH<sup>-</sup>]. Variation of ionic strength or dielectric constant of the medium does not have any effect on the reaction while the addition of halide ions slightly retards the rate. The reaction fails to induce polymerisation of acrylonitrile. The rate increases in D<sub>2</sub>O medium and the inverse solvent isotope effect  $k(\text{D}_2\text{O})/k(\text{H}_2\text{O})$  is around 1.52. Proton inventory studies have been made in H<sub>2</sub>O – D<sub>2</sub>O mixtures. Activation parameters for the reaction have been computed from the Arrhenius plots. Isonicotinic acid has been identified as the oxidation product of INH, by GC-MS. Under identical experimental conditions the rate of oxidation of INH is found to be higher with NaOBr than NaOCl by a factor of 3. The proposed reaction mechanism and the derived law are consistent with the observed kinetics.

Although aliphatic azo compounds of the type R–N=N–H appear to be unstable, derivatives of the type R–N=N–R are much more stable. Isoniazid, an azene derivative (INH, pyridine-4-carboxylic acid hydrazine) is an antitubercular drug and is now widely used along with ribampicin and streptomycin for the chemotherapy of tuberculosis. Because of its importance, several analytical procedures are reported in the literature<sup>1-3</sup> for the assay of this drug, but there

seems to be little information on the oxidation kinetics of INH or azo compounds. Liu and Margerum<sup>4</sup> reported the kinetics of oxidation of hydrazine by aqueous iodine in the pH range 0.35-8.00, using stopped flow and pulsed accelerated flow methods. A multistep mechanism is proposed. Electrochemical oxidation of niazid and isoniazid at mercury electrodes is studied by Mellado *et al.*<sup>5</sup> in the pH range 6-13. The final products were nicotinic and isonicotinic acids. In the oxidation of INH by catalase peroxides from mycobacterium tuberculosis<sup>6</sup>, the acyl dimide py-CO-N = NH and py-CO-N<sub>2</sub><sup>+</sup> were found to be the intermediates. The mechanism proposed provides a number of possibilities for the mode of action of INH. Mechanistic studies of the action of INH and ethionamide in the chemotherapy of tuberculosis has been studied by Johnsson *et al.*<sup>7</sup>. It is noted that despite the importance of the above drugs relatively little is known about their mode of action at the molecular level. In the background of the available information on the mechanism of oxidation of INH, it is of immense interest to follow the oxidation kinetics of INH with halogen oxidants. The hypohalous acids, HOCl and HOBr, play an important role in oxidation reactions while the hypohalites, OCl<sup>-</sup> and OBr<sup>-</sup>, behave as bifunctional groups<sup>8,9</sup>. The present note reports the kinetics of oxidation of INH by OCl<sup>-</sup> and OBr<sup>-</sup> ions in alkaline medium with a view to elucidate the mechanism of oxidation of the drug in solution and also to assess the relative rates of oxidation of sodium hypohalites.

## Experimental

Sodium hypohalites, NaOCl and NaOBr, were prepared by the reported procedure<sup>10</sup>. The two hypohalite stock solutions were standardized by the iodometric method. Isoniazid (BDH, Poole, UK) was used without further purification. All other chemicals were of analytical grades. Solvent isotope studies were performed with D<sub>2</sub>O (99.2%) supplied by the BARC, Mumbai. The ionic strength of the system was maintained at a constant high value (0.5 mol

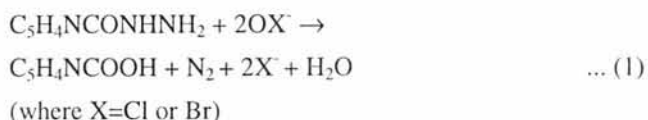
dm<sup>-3</sup>) using a concentrated solution of NaClO<sub>4</sub>. Triply distilled water used for preparing all aqueous solutions.

### Kinetic measurements

The kinetic runs were performed under pseudo-first order conditions with [substrate] >> [oxidant] in glass stoppered pyrex boiling tubes coated black on the outside to eliminate photochemical effects. The oxidant and the required amounts of INH, NaOH, NaClO<sub>4</sub> solutions and H<sub>2</sub>O (to keep the total volume constant for all runs), taken in separate boiling tubes, were thermostated for 30 min at 303 K. The reaction was initiated by the rapid addition of oxidant to the mixture and its progress was monitored by iodometric determination of the unreacted oxidant in measured aliquots (5 ml each) of the reaction mixture at regular intervals of time. The reaction was monitored for two half-lives. The pseudo-first order rate constants, *k*, calculated from the linear plots of log [oxidant] versus time were reproducible within  $\pm 3\text{--}4\%$ . Regression analysis of the experimental data was carried out on an EC-75 statistical calculator to obtain the regression coefficient, *r*.

### Stoichiometry

Varying ratios of oxidant and INH under conditions, [oxidant] >> [INH] in the presence of 0.04 mol dm<sup>-3</sup> NaOH were equilibrated at 303 K for 48 h. Determination of unreacted oxidant (OCl<sup>-</sup> or OBr<sup>-</sup>) in the reaction mixture showed that one mole of INH consumed two moles of oxidant confirming the following stoichiometry:



### Product analysis

The oxidation product INH, isonicotinic acid, in the reaction mixture was detected by the colour reaction with sodium pentacyanoamine ferroate<sup>11</sup> and also identified by its melting point of 313–314 °C (lit. m.p. 310–315 °C). Its identity was further confirmed by mass spectra analysis. The GC-MS data were obtained on a 17A Shimadzu gas chromatograph with a QP-5000 Shimadzu mass spectrometer. The mass spectrum showing and M<sup>+</sup> parent ion peak at mass / charge 122.05 clearly confirms the isonicotinic acid.

Among reaction products, nitrogen and chloride and bromide ions were identified by the conventional tests. Test for the presence of free radicals in reaction mixture with aqueous acrylamide solution was found to be negative.

### Results and discussion

The oxidation of isoniazid by sodium hypohalites (NaOCl or NaOBr) was carried out in the presence of NaOH at 303 K. The same oxidation behaviour was observed for both the oxidants (OX<sup>-</sup>).

With the substrate in excess at constant [NaOH], [INH] and temperature, plots of log [OX<sup>-</sup>] versus time were linear (*r* > 0.9960) indicating a first order dependence on [OX<sup>-</sup>]. Further, the pseudo-first order rate constant *k* was not affected with increase in [OX<sup>-</sup>] confirming the first order dependence on [OX<sup>-</sup>] (Table 1). The rate increased with increase [INH] (Table 1) and plots of log *k* versus log [INH] were linear (*r* > 0.9962) with unit slopes showing a first order dependence of rate on [INH] with both OCl<sup>-</sup> and OBr<sup>-</sup> ions. At constant [OX<sup>-</sup>], [INH] and temperature, the reaction rate increased with increase

Table 1 – Effect of varying oxidant, substrate and NaOH concentrations on the rate of reaction  
*I* = 0.5 mol dm<sup>-3</sup>, temp = 303 K

| 10 <sup>4</sup> [oxidant] <sub>0</sub><br>(mol dm <sup>-3</sup> ) | 10 <sup>2</sup> [INH] <sub>0</sub><br>(mol dm <sup>-3</sup> ) | 10 <sup>2</sup> [NaOH]<br>(mol dm <sup>-3</sup> ) | 10 <sup>3</sup> <i>k</i> (s <sup>-1</sup> ) |       |
|-------------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------|---------------------------------------------|-------|
|                                                                   |                                                               |                                                   | NaOCl                                       | NaOBr |
| 2.0                                                               | 6.0                                                           | 4.0                                               | 1.68                                        | 4.88  |
| 6.0                                                               | 6.0                                                           | 4.0                                               | 1.70                                        | 4.80  |
| 8.0                                                               | 6.0                                                           | 4.0                                               | 1.60                                        | 4.85  |
| 10.0                                                              | 6.0                                                           | 4.0                                               | 1.55                                        | 4.92  |
| 12.0                                                              | 6.0                                                           | 4.0                                               | 1.63                                        | 4.82  |
| 8.0                                                               | 1.0                                                           | 4.0                                               | 0.26                                        | 0.84  |
| 8.0                                                               | 3.0                                                           | 4.0                                               | 0.77                                        | 2.50  |
| 8.0                                                               | 6.0                                                           | 4.0                                               | 1.60                                        | 4.85  |
| 8.0                                                               | 10.0                                                          | 4.0                                               | 2.66                                        | 7.30  |
| 8.0                                                               | 15.0                                                          | 4.0                                               | 4.22                                        | 12.0  |
| 8.0                                                               | 6.0                                                           | 1.0                                               | 0.75                                        | 2.05  |
| 8.0                                                               | 6.0                                                           | 2.0                                               | 1.05                                        | 3.20  |
| 8.0                                                               | 6.0                                                           | 4.0                                               | 1.60                                        | 4.85  |
| 8.0                                                               | 6.0                                                           | 8.0                                               | 2.32                                        | 7.00  |
| 8.0                                                               | 6.0                                                           | 16.0                                              | 3.30                                        | 11.2  |

Table 2 – Proton inventory studies for the oxidation of isoniazid by hypohalites in H<sub>2</sub>O – D<sub>2</sub>O mixtures

[oxidant]<sub>0</sub> = 8.0 × 10<sup>-4</sup> mol dm<sup>-3</sup>, [INH]<sub>0</sub> = 6.0 × 10<sup>-2</sup> mol dm<sup>-3</sup>;  
[NaOH] = 4.0 × 10<sup>-2</sup> mol dm<sup>-3</sup>, I = 0.5 mol dm<sup>-3</sup>; temp = 303 K

| Atom fraction of deuterium<br>(n) | 10 <sup>3</sup> k (s <sup>-1</sup> ) |       |
|-----------------------------------|--------------------------------------|-------|
|                                   | NaOCl                                | NaOBr |
| 0.000                             | 1.60                                 | 4.85  |
| 0.248                             | 1.78                                 | 5.30  |
| 0.497                             | 1.95                                 | 5.93  |
| 0.745                             | 2.18                                 | 6.65  |
| 0.947                             | 2.42                                 | 7.36  |

in [NaOH] (Table 1). Plots of log *k* versus log [NaOH] were linear (*r* > 0.9978) with fractional slopes (0.57 and 0.60 with OCl<sup>-</sup> and OBr<sup>-</sup> respectively) showing a fractional order dependence of rate on [OH<sup>-</sup>]. An increase in [NaX] (NaCl for OCl<sup>-</sup> and NaBr for OBr<sup>-</sup>; 5 × 10<sup>-4</sup> – 40 × 10<sup>-4</sup> mol dm<sup>-3</sup>) in the reaction mixture slightly retarded the rate (-0.12 and -0.14 with OCl<sup>-</sup> and OBr<sup>-</sup> respectively). Variation of ionic strength (*I* = 0.1–0.8 mol dm<sup>-3</sup>) of the medium by adding NaClO<sub>4</sub> had no effect on the rate. Decrease in permittivity of the medium, by adding methanol (0–40% v/v) to the reaction mixture did not alter the rate significantly.

The reaction rates were determined at different temperatures (298–313 K). Based on the linear Arrhenius plots of log *k* versus 1/*T* (*r* > 0.9895), the values of *E*<sub>a</sub>, Δ*H*<sup>‡</sup>, Δ*G*<sup>‡</sup> and Δ*S*<sup>‡</sup> were evaluated for each oxidant and were found to be: 52.5, 50.0, 90.5 k cal mol<sup>-1</sup> and -134 JK<sup>-1</sup> mol<sup>-1</sup> for NaOCl and 43.4, 40.9, 87.7 k cal mol<sup>-1</sup> and -155 JK<sup>-1</sup> mol<sup>-1</sup> for NaOBr, respectively. Rate studies in D<sub>2</sub>O medium showed that the reaction is faster in heavy water. Studies of the rate in D<sub>2</sub>O medium for NaOCl and NaOBr revealed that while *k*(H<sub>2</sub>O) is 1.60 × 10<sup>-3</sup> s<sup>-1</sup> and 4.85 × 10<sup>-3</sup> s<sup>-1</sup>, *k*(D<sub>2</sub>O) is 2.42 × 10<sup>-3</sup> s<sup>-1</sup> and 7.36 × 10<sup>-3</sup> s<sup>-1</sup>, respectively. The solvent isotope effect *k*(H<sub>2</sub>O)/*k*(D<sub>2</sub>O) = 0.66 and 0.65 for the two oxidants. Proton inventory studies were made in H<sub>2</sub>O – D<sub>2</sub>O mixtures for both the oxidants and the results are

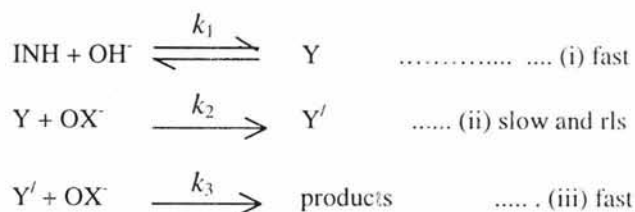
shown in Table 2. The corresponding proton inventory plots for the rate constant *k*<sup>n</sup> in a solvent mixture of deuterium atom fraction *n* are given in Fig. 1.

Reaction kinetics in basic medium showed first order dependence in both [oxidant] and [INH] at a constant [NaOH]. The reaction also showed a fractional order in [OH<sup>-</sup>] and an accelerating effect with a trend: OBr<sup>-</sup> > OCl<sup>-</sup>. The experimental rate law that represents the kinetic data is given as

$$-d[\text{OX}^-]/dt = -d[\text{INH}]/dt = k[\text{OX}^-][\text{INH}][\text{OH}^-]^a \dots (2)$$

where *a* < 1

In aqueous basic media, NaOX dissociates to form the active hypohalite ions, OX<sup>-</sup>. The first order dependence on [NaOX] suggests that OX<sup>-</sup> is the active oxidizing species. If free molecular halogen, X<sub>2</sub>, formed by the hydrolysis of NaOX is the active species involved, then the reaction rate would have had a second order dependence on [NaOX], which is contrary to the experimental observations. Absence of ionic strength effect on the rate rules out a direct interaction between OX<sup>-</sup> and OH<sup>-</sup>. On the basis of foregoing discussion coupled with the absence of free radicals in reaction mixture, a mechanism for INH oxidation by the hypohalites in basic medium is proposed as in Scheme 1:



Scheme 1

Here Y and Y' are complex intermediate species. The first step involves the basic hydrolysis of the amide<sup>12</sup> giving Y which is subjected to an electrophilic attack by the oxidant (OX<sup>-</sup>) in a rate-limiting step. The intermediate Y' formed reacts with a second molecule of the oxidant in a fast step to form the products. This is shown in Scheme 2.

Scheme 1 leads to rate law (3):

$$\text{Rate} = \frac{-d[\text{OX}^-]}{dt} = \frac{K_1 k_2 [\text{INH}] [\text{OX}^-] [\text{OH}^-]}{1 + K_1 [\text{OH}^-]} \quad \dots (3)$$

Above rate law supports the experimental results, namely, the first order dependence of  $[\text{INH}]$  and  $[\text{OX}^-]$ , and fractional order in  $[\text{OH}^-]$ , observed in the oxidation of INH by the hypohalites.

Since  $\frac{-d[\text{OX}^-]}{dt} = k[\text{OX}^-]$ , Eq. (3) can be

transformed into Eqs (4) and (5)

$$k = \frac{K_1 k_2 [\text{INH}] [\text{OH}^-]}{1 + K_1 [\text{OH}^-]} \quad \dots (4)$$

$$\frac{1}{k} = \frac{1}{K_1 k_2 [\text{INH}] [\text{OH}^-]} + \frac{1}{k_2 [\text{INH}]} \quad \dots (5)$$

From the slope and intercept of double reciprocal plot of  $1/k$  versus  $1/[\text{OH}^-]$  at constant  $[\text{INH}]$  ( $r > 0.9870$ ), values of  $K_1$  and  $k_2$  for NaOCl and NaOBr were found as 25.0 and 21.1  $\text{dm}^3 \text{mol}^{-1}$  and 0.06 and 0.18  $\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$  respectively.

The proposed mechanism is supported by the increase in rate in  $\text{D}_2\text{O}$  medium since  $\text{DO}^-$  is a stronger base than  $\text{HO}^-$  and signifies fast pre-equilibrium ion transfer<sup>13</sup>. Proton inventory plots (Fig. 1) could throw some light on the nature of the transition state. The dependence of the rate constant,  $k^n$ , on the deuterium atom fraction  $n$  in the solvent mixture of  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  is given by the following form of Gross-Butler equation<sup>14,15</sup>:

$$\frac{k^0/k^n}{\text{TS}} = \frac{\pi (1 - n + n \phi_i)}{\text{RS} \pi (1 - n + n \phi_j)} \quad \dots (6)$$

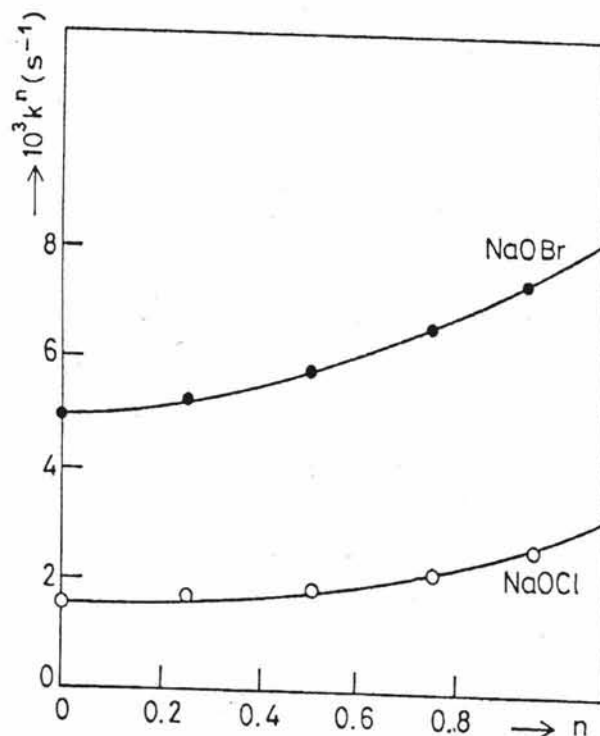


Figure 1 — Proton inventory plots of  $k^n$  versus  $n$  for the oxidation of isoniazid by NaOCl and NaOBr in  $\text{H}_2\text{O} - \text{D}_2\text{O}$  mixtures. Experimental conditions are as in Table 2.

where  $\phi_i$  and  $\phi_j$  are isotope fractionation factors for isotopically exchangeable proton sites in the transition state (TS) and in the reactant site (RS), respectively. Equation (6) allows the calculation of  $\phi_i$  if the values of  $\phi_j$  are known. However, the curvature of proton inventory plots could reflect the number of exchangeable protons in the reaction<sup>16</sup>. Plots of  $k^n$  versus  $n$  (Fig. 1) are curves in the present case and these in comparison with the standard curves indicate the involvement of a single proton or H-D exchange in the reaction sequence from the hydroxide ion. This proton exchange can be shown by comparison with standard proton inventory plots reported in the literature<sup>16</sup>. Hence the participation of hydroxide ion in the formation of transition state is inferred.

Halide ions slightly retard the rate which can be attributed to the removal of the active hypohalite by the reaction:



It is seen that variation of dielectric constant ( $D$ ) of the medium does not affect the rate. An

explanation can be offered in terms of the Born treatment applied by Laidler<sup>17</sup> for a dipole-dipole interaction, as shown in Eq. (7):

$$\ln k = \ln k_0 + \frac{3}{8kT} (2/D-1) \left( \frac{\mu_A^2}{r_A^3} + \frac{\mu_B^2}{r_B^3} - \frac{\mu_{\ddagger}^2}{r_{\ddagger}^3} \right) \dots (7)$$

Here  $k_0$  is the rate constant in a medium of infinite  $D$ , and  $\mu$  and  $r$  refer to the dipole moments and radii of reactants A, B and the activated complex. It is seen from Eq. (7) that the rates should be higher in a medium of lower  $D$  when  $r_{\ddagger}^3 > r_A^3 + r_B^3$ . This indicates that the extent of charge dispersal in the transition state is higher since  $r_{\ddagger}^3 > r_A^3 + r_B^3$ . On the other hand,  $r_{\ddagger}^3 \approx r_A^3 + r_B^3$  implies the absence of dielectric effect of solvent on rate, as was observed in the present investigations.

The proposed mechanism is also supported by the observed activation parameters. The moderate values of  $\Delta H^\ddagger$  and fairly large negative  $\Delta S^\ddagger$  values point towards a rigid associative transition state. The near constancy of  $\Delta G^\ddagger$  values indicate that a similar mechanism is operative in the oxidation of INH by both NaOCl and NaOBr.

A comparison of the rates of reaction of NaOCl and NaOBr shows that the rates are three times more with the bromine analogue, which could possibly be attributed to the difference in electrophilicities of the halogen species responsible for the oxidation of isoniazid.

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## References

- 1 Sarwar M, Malick A & Khan U A, *Anal Lett*, 22 (1989) 853.
- 2 Mahfouz N M A & Emara K M, *Talanta*, 40 (1993) 1023.
- 3 Nagaraj P, Murthy K C S & Yathirajan H S, *Talanta*, 43 (1996) 1075.
- 4 Liu R M & Margerum D W, *Inorg Chem*, 37 (1998) 2531.
- 5 Mellado J M R, Angulo M & Gavin R M, *J electroanal Chem*, 252 (1993) 253.
- 6 Johnsson K & Schultz P G, *J Am chem Soc*, 116 (1994) 7425.
- 7 Johnsson K, King D S & Schultz P G, *J Am chem Soc*, 117 (1995) 5009.
- 8 Swain C G & Brown J F, *J Am chem Soc*, 74 (1952) 2538.
- 9 Epstein J, Bauer V E, Saxe M & Demek M M, *J Am chem Soc*, 78 (1956) 4068.
- 10 Brauer G, *Handbook of preparative inorganic chemistry*, (Academic Press, New York) 2<sup>nd</sup> ed, vol.I (1963) 309.
- 11 Feigl F, *Spot tests in organic analysis*, (Elsevier, Amsterdam), (1956) 156.
- 12 Roberts J D & Caseiro M C, *Basic principles of organic chemistry* (W A Benjamin Inc, New York), 1965, 681.
- 13 Collins C J & Bowman N S, *Isotope effect in chemical reactions*, (Van Nostrand – Reinhold, New York), 1970, 267.
- 14 Albery W J & Davies M H, *J chem Soc, Faraday Trans*, 68 (1972) 167.
- 15 Gopalakrishnan G & Hogg J L, *J org Chem*, 50 (1985) 1206.
- 16 Isaccs N S, *Physical organic chemistry*, (Wiley, New York), 1987, 275.
- 17 Laidler K J, *Chemical kinetics*, (Tata-McGraw Hill, Mumbai), 1965, 474.