CHAPTER VII

KINETICS AND MECHANISM OF OXIDATION
OF PYRAZINAMIDE BY
BROMAMINE-T IN PERCHLORIC ACID
MEDIUM
Oxidation of Pyrazinamide: A Review

Pyrazinamide (PZ) is an antitubercular drug having a wide spectrum of activity. Pyrazinamide (PZ), the pyrazine analogue of nicotinamide, is an antibacterial drug, which finds application in combating a wide range of infections. PZ is an important sterilizing drug that shortens tuberculosis (TB) therapy. PZ is indicated for the initial treatment of active tuberculosis.

Multivariate calibration analysis method has been developed for the estimation of PZ. The plasma and urine pharmacokinetic parameters of pyrazinamide and its metabolites have been studied by Lacroix, after a single oral dose of pyrazinamide 27 mg kg⁻¹ in 9 healthy subjects. In contrast, oxidation by xanthine oxidase occurred very rapidly.

Mehmedagic, Aida et al have reported the determination of pyrazinamide and its main metabolites in rat urine by high performance liquid chromatography. 5-OH-pyrazinamide and 5-OH-pyrazinoic acids standards were obtained by enzymic synthesis.

Simon, V. et al have reported the Transition Metals Effect on the Structure of Pyrazinamide Complexes. The authors study the structural changes induced in these samples as evidenced by thermal behavior and ESR of new \([\text{Cu}(\text{PZA})_2\text{Cl}_2]\) and \(\{[\text{M}(\text{PZA})][\text{Hg(}\text{SCN})_4]\}\) (M = Cu, Cd, Ni). Structural transformations are correlated with the thermal transformations described as multi-step processes consisting in crystalline phase transition, decomposition, melting and thermal oxidation.

Fanchiang, Y. T et al have reported the electron transfer on chromium (III) bound pyrazine radicals. Kinetic data support a sequence in which the green radical cation, formulated as Cr (III) pyrazine, dissociates \((k_1)\) to the parent pyrazine and \(\text{Cr}^{2+}\) which, in turn, may react with Co(III) \((k_2)\) or return to the radical cation \((k_1)\). Values of \(k_1/k_2\) obtained from measurements on different Co (III) systems are in agreement, and \(k_2\) values for the reactions of fluoro- and bromopentamminecobalt (III) complexes with \(\text{Cr}^{2+}\) are consistent with literature rates. Muralidharan, B. et al have reported cyclic voltammetric
studies on the electrochemical reduction of pyrazinamide.

A review of the literature reveals the absence of oxidation kinetics of PZ with any oxidant. In the light of the available information, the present studies reports for the first time a detailed kinetics of oxidation of PZ with BAT in Perchloric acid medium in order to (i) elucidate the reaction mechanism (ii) put forward appropriate rate law (iii) ascertain the reactive species of BAT and (iv) identify the products of the reaction.

The present investigations therefore were taken up to investigate the oxidation kinetics of this drug by BAT in acid medium at 303K to ascertain the mechanisms of metabolic conversion of PZM in biological systems and also to identify the reactive species of the oxidants in acidic medium.

Section 7.2

KINETICS OF OXIDATION OF PYRAZINAMIDE BY BROMAMINE-T IN HClO4 MEDIUM

This section reports the kinetics and mechanism of oxidation of Pyrazinamide by BAT in the presence of HClO4 medium at 303K.

The details about the preparation of reagents and the experimental procedures are similar to those given in Section 1.8 of Chapter 1.

Stoichiometry

Reaction mixtures containing varying ratios of BAT and PZ were equilibrated in presence of HClO4 (5 × 10^{-3} mol dm^{-3}) at 303 K for 24 hrs. Iodometric estimation of un-reacted BAT in the reaction mixture showed that one mole of BAT was consumed per mole of PZ, confirming the following stoichiometry:

\[
R\text{CONH}_2 + R'\text{NBrNa} + H_2O \rightarrow R\text{NH}_2 + R'\text{NH}_2 + Na^+ + Br^{-} + CO_2
\]  

(7.1)

Where \( R = C_4H_7N_2 \) and \( R' = CH_3C_6H_4SO_2 \).
**Product analysis**

The reduction product of the oxidant \textit{p}-toluenesulfonamide (PTS) was detected\textsuperscript{11} by paper chromatography. Benzyl alcohol saturated with water was used as solvent with 0.5 % vanillin in 1 % HCl solution in ethanol as spray reagent. It was further confirmed by its melting point, 138 – 139 °C (lit. m.p. 137 – 140°C) and IR spectral data (N-H stretch: 3365, 3275; Ar.C=O: 1556, 1448; SO\textsubscript{2} asymmetric stretch: 1334 SO\textsubscript{2} symmetric stretch: 1160; S-N stretch: 997 cm\textsuperscript{-1}).

The reaction mixture was extracted with ether and the oxidation product of pyrazinamide was separated and identified as aminopyrazine by its melting point 118 – 120 °C (lit.m.p.119 -120°C). It was further confirmed by elemental analysis and \textsuperscript{1}H NMR spectral studies. Elemental analysis data: N - 43.29 (44.159), C- 51.21(50.467), H - 5.39(5.257). \textsuperscript{1}H NMR (CDCl\textsubscript{3}): \delta 7.99 - 7.97 (m, 2H), 7.90 - 7.89 (m, 1H), 4.82 (s, 2H). \textsuperscript{1}H NMR spectra was recorded using λ 300 MHz NMR spectrometer. The evolved CO\textsubscript{2} gas was detected by the lime water test.

**RESULTS**

The oxidation of PZ with BAT has been kinetically investigated at different initial concentrations of the reactants in the presence of HClO\textsubscript{4} at 303 K.

**Effect of varying reactant concentrations on the rate**

With the substrate in excess, at constant [HClO\textsubscript{4}], [PZ], and temperature, the [BAT]\textsubscript{0} was varied. Plots of log [BAT] versus time were linear (r > 0.996) indicating a first-order dependence of the rate on [BAT]\textsubscript{0}. The pseudo first-order rate constants (k') calculated from the slopes are given in Table.7.1. The values of k' are unaltered with variation of [BAT]\textsubscript{0} confirming the first- order dependence of the rate on [BAT]\textsubscript{0}. Under similar experimental conditions, an increase in [PZ]\textsubscript{0} increased the k' values (Table 7.1). A plot of log k' versus log [PZ]\textsubscript{0} was linear (Fig7.1; r = 0.998) with a slope of 0.77 indicating a fractional-order dependence on [PZ]\textsubscript{0}. 

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Effect of varying [HClO₄] on the rate

The [H⁺] was varied using HClO₄. The rate increased with increase in [H⁺] (Table 7.1). A plot of log k' versus log [H⁺] was linear (Fig 7.2; r = 0.998) with a slope of 0.87 indicating a fractional-order dependence on [H⁺].

Effect of halide ions

At constant [H⁺], addition of Br⁻ in the form of NaBr, had no significant effect on the rate of reaction. Also addition of Cl⁻, in the form of NaCl did not show any effect on the reaction rate (Table 7.2).

Effect of added p-toluenesulfonamide concentration

The addition of reduced product of oxidant, p-toluenesulfonamide (PTS) had no significant effect on the rate indicating that, it is not involved in pre-equilibrium step before the rate-determining step (Table 7.2).

Effect of varying ionic strength and dielectric permittivity

The reaction rate remained unaffected by varying ionic strength of the medium through addition of NaClO₄ (0.1–0.8 mol dm⁻³) (Table 7.3). The dielectric permittivity of the medium was varied by adding different proportions (0 - 40 %, v/v) of methanol to the reaction mixture. The rate decreased with decrease in dielectric permittivity of the reaction mixture (Table 7.3). Plot of log k' versus 1/D, (Fig. 7.3; r=0.989), where D is the dielectric permittivity of the medium (D values are taken from the literature) gave a straight line with a negative slope. Blank experiments showed that methanol was not oxidized by BAT under the experimental conditions employed.

Effect of varying temperature

The reaction was studied at different temperatures (293 – 313 K) keeping other experimental conditions constant (Table 7.4). From the linear Arrhenius plot of log k' versus 1/T (Fig.7.4; r = 0.998), activation energy and other thermodynamic parameters for the composite reaction were computed. These are compiled in Table 7.4.
Test for free radicals

The addition of aqueous acryl amide monomer solution to the reaction mixture in an inert atmosphere did not initiate polymerization indicating the absence of free radical species in the reaction sequence.
### Table 7.1
Effect of varying concentrations of reactants and HClO₄ on rate

<table>
<thead>
<tr>
<th>$10^3 [\text{BAT}]_0$ (mol dm$^{-3}$)</th>
<th>$10^3 [\text{PZ}]$ (mol dm$^{-3}$)</th>
<th>$10^3 [\text{HClO}_4]$ (mol dm$^{-3}$)</th>
<th>$10^4 k'$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>10.0</td>
<td>5.0</td>
<td>5.21</td>
</tr>
<tr>
<td>2.5</td>
<td>10.0</td>
<td>5.0</td>
<td>5.28</td>
</tr>
<tr>
<td>5.0</td>
<td>10.0</td>
<td>5.0</td>
<td>5.26</td>
</tr>
<tr>
<td>7.0</td>
<td>10.0</td>
<td>5.0</td>
<td>5.30</td>
</tr>
<tr>
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<td>1.0</td>
<td>5.0</td>
<td>0.84</td>
</tr>
<tr>
<td>1.0</td>
<td>2.5</td>
<td>5.0</td>
<td>1.80</td>
</tr>
<tr>
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<td>5.0</td>
<td>5.0</td>
<td>2.80</td>
</tr>
<tr>
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<td>20.0</td>
<td>5.0</td>
<td>8.21</td>
</tr>
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<td>1.0</td>
<td>50.0</td>
<td>5.0</td>
<td>15.43</td>
</tr>
<tr>
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<td>10.0</td>
<td>1.0</td>
<td>1.22</td>
</tr>
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<td>1.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.31</td>
</tr>
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<td>40.0</td>
<td>32.13</td>
</tr>
<tr>
<td>1.0</td>
<td>10.0</td>
<td>50.0</td>
<td>40.02</td>
</tr>
</tbody>
</table>

$\mu = 0.5$ mol dm$^{-3}$, $T = 303K$

### Table 7.2
Effect of Variations of $p$-toluenesulfonamide, NaBr, NaCl on the rate

<table>
<thead>
<tr>
<th>$[\text{PTS}] \times 10^3$ mol dm$^{-3}$</th>
<th>$10^4 k$ (s$^{-1}$)</th>
<th>$[\text{NaBr}] \times 10^4$ mol dm$^{-3}$</th>
<th>$10^4 k$ (s$^{-1}$)</th>
<th>$[\text{NaCl}] \times 10^3$ mol dm$^{-3}$</th>
<th>$10^4 k$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>5.21</td>
<td>0.0</td>
<td>5.21</td>
<td>0.0</td>
<td>5.21</td>
</tr>
<tr>
<td>1.0</td>
<td>5.25</td>
<td>1.0</td>
<td>5.12</td>
<td>1.0</td>
<td>4.92</td>
</tr>
<tr>
<td>5.0</td>
<td>5.12</td>
<td>2.0</td>
<td>5.30</td>
<td>2.0</td>
<td>5.10</td>
</tr>
<tr>
<td>10.0</td>
<td>5.30</td>
<td>5.0</td>
<td>5.28</td>
<td>5.0</td>
<td>5.34</td>
</tr>
<tr>
<td>20.0</td>
<td>5.34</td>
<td>8.0</td>
<td>5.25</td>
<td>8.0</td>
<td>4.98</td>
</tr>
</tbody>
</table>

$[\text{BAT}]_0 = 1 \times 10^3$ mol dm$^{-3}$; $[\text{PZ}]_0 = 10 \times 10^3$ mol dm$^{-3}$; $[\text{HClO}_4] = 5 \times 10^3$ mol dm$^{-3}$; $\mu = 0.5$ mol dm$^{-3}$; $T=303K$. 

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### Table 7.3

Effect of Variations of NaClO₄ and CH₃OH on the reaction rate

<table>
<thead>
<tr>
<th>[NaClO₄] mol dm⁻³</th>
<th>10⁴ k (s⁻¹)</th>
<th>CH₃OH (%) v/v</th>
<th>10⁴ k (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>5.30</td>
<td>0.0</td>
<td>5.21</td>
</tr>
<tr>
<td>0.2</td>
<td>5.32</td>
<td>10.0</td>
<td>4.80</td>
</tr>
<tr>
<td>0.4</td>
<td>5.24</td>
<td>20.0</td>
<td>4.22</td>
</tr>
<tr>
<td>0.6</td>
<td>5.20</td>
<td>30.0</td>
<td>3.50</td>
</tr>
<tr>
<td>0.8</td>
<td>5.40</td>
<td>40.0</td>
<td>2.70</td>
</tr>
</tbody>
</table>

[BAT]₀ = 1 × 10⁻³ mol dm⁻³; [PZ]₀ = 10 × 10⁻³ mol dm⁻³;

[HCIO₄] = 5 × 10⁻³ mol dm⁻³; μ = 0.5 mol dm⁻³; T=303K.

### Table 7.4

Effect of Temperature on the reaction rate and Activation Parameters

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>10⁴ k (s⁻¹)</th>
<th>Activation Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>2.38</td>
<td>Eₐ (kJ mol⁻¹) 62.3</td>
</tr>
<tr>
<td>298</td>
<td>3.55</td>
<td>ΔH° (kJ mol⁻¹) 59.77</td>
</tr>
<tr>
<td>303</td>
<td>5.21</td>
<td>ΔS° (JK⁻¹ mol⁻¹) -111.3</td>
</tr>
<tr>
<td>308</td>
<td>7.91</td>
<td>ΔG°# (kJ mol⁻¹) 93.49</td>
</tr>
<tr>
<td>313</td>
<td>12.52</td>
<td>-</td>
</tr>
</tbody>
</table>

[BAT]₀ = 1 × 10⁻³ mol dm⁻³; [PZ]₀ = 10 × 10⁻³ mol dm⁻³;

[HCIO₄] = 5 × 10⁻³ mol dm⁻³; μ = 0.5 mol dm⁻³.
Fig. 7.1 Plot of log$k'$ versus log$[PZ]$  
$4+\log[PZ]$

Fig. 7.2 Plot of log$k'$ versus log$[H^+]$  
$4+\log[H^+]$
Fig. 7.3 Plot of logk' versus 1/D

Fig. 7.4 Plot of logk' versus 1/T
Fig. 7.5 Plot of $1/k'$ versus $1/[PZ]$ and $1/[H^+]$
Pryde and Soper\textsuperscript{13}, Morris \textit{et al}\textsuperscript{14} and Bishop and Jennings\textsuperscript{15} have shown the existence of similar equilibrium in alkali and acidic solutions of \textit{N}-metalloy-\textit{N}-haloarylsulfonamides. Bromamine-T similar to chloramine-T, behaves as a strong electrolyte in aqueous solution. To confirm this hypothesis, conductometric and pH-titrations between aqueous solutions of BAT and HCl were performed. The conductometric behavior of BAT is identical with that of CAT\textsuperscript{13-18} while the pH titration curves observed are similar to those noted by Morris \textit{et al}\textsuperscript{14}. The possible equilibria in acidified BAT solutions are,

\begin{align}
\text{RNBrNa} &\rightleftharpoons \text{RNBr}^- + \text{Na}^+ \\
\text{RNBr}^- + \text{H}^+ &\rightleftharpoons \text{RNHBr} \\
\text{RNHBr} + \text{H}_2\text{O} &\rightleftharpoons \text{RNH}_2 + \text{HOBr} \\
2\text{RNHBr} &\rightleftharpoons \text{RNH}_2 + \text{RNBr}_2 \\
\text{HOBr} + \text{H}^+ &\rightleftharpoons \text{H}_2\text{OBr}^+ 
\end{align}

Where \( R = \text{CH}_3\text{C}_6\text{H}_5\text{SO}_2 \).

Therefore, the possible oxidizing species in acidic solutions are free acid RNHBr, RNBr\textsubscript{2}, HOBr and H\textsubscript{2}OBr\textsuperscript{+}. The oxidation of haloamine-sulfonamide system is pH dependent and decrease with increasing in pH of the medium\textsuperscript{19}. In the present investigations, oxidation of pyrazinamide with BAT shows a first-order dependence on BAT and fractional-orders in acid and substrate. The involvement of RNBr\textsubscript{2} in the mechanism leads to a second-order rate law and negative effect of RNH\textsubscript{2} according to equation (7.5), which is contrary to the experimental observations. If HOBr were the primary oxidizing species, a first-order retardation of the rate by the added RNH\textsubscript{2} would be expected. However no such effect is noticed. Hardy and Johnston, who studied the pH dependent relative concentrations of the species present in acidified chloramine-T solutions of comparable molar concentrations, have shown that CH\textsubscript{3}C\textsubscript{6}H\textsubscript{4}SO\textsubscript{2}NHCl is the probable oxidizing species in acid medium. Narayanan and Rao\textsuperscript{20}, and Subhashini \textit{et al}\textsuperscript{21} have reported that, monohaloamines can be further protonated at pH 2, as shown in the following equations (7.7) and (7.8) for monochloramine-T and monochloramine-B respectively.
\[
\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{NHCl} + \text{H}^+ \rightleftharpoons \text{CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{N}^+\text{H}_2\text{Cl} \quad (7.7)
\]

\[
\text{C}_6\text{H}_5\text{SO}_2\text{NHCl} + \text{H}^+ \rightleftharpoons \text{C}_6\text{H}_5\text{SO}_2\text{N}^+\text{H}_2\text{Cl} \quad (7.8)
\]

In the present case, the fractional-order in \([\text{H}^+]\) indicates that the protonation of \(\text{RNHBr}\) results in the formation of \(\text{RN}^+\text{H}_2\text{Br}\) which is likely to be the active oxidizing species involved in the mechanism. Based on the preceding discussion, the following mechanism (Scheme 1) is proposed for the reaction in acid medium,

\[
\text{RNHBr} + \text{H}^+ \underset{k_1}{\overset{k_2}{\rightleftharpoons}} \text{RN}^+\text{H}_2\text{Br} \quad \text{fast (i)}
\]

\[
\text{RN}^+\text{H}_2\text{Br} + \text{S} \underset{k_3}{\rightarrow} \text{X} \quad \text{fast (ii)}
\]

\[
\text{X} \rightarrow \text{products} \quad \text{slow and r. d. s (iii)}
\]

**Scheme 7.1**

In Scheme 7.1, \(\text{S}\) and \(\text{X}\) represent the substrate and complex intermediate species, whose structures are shown in Scheme 7.2 where a detailed mechanism of oxidation of PZ with BAT in HClO\(_4\) medium is illustrated. An initial equilibrium involves protonation of \(\text{RNHBr}\) forming an active oxidizing species of BAT (\(\text{RN}^+\text{H}_2\text{Br}\)). In the next step, the electrophilic attack by \(\text{RN}^+\text{H}_2\text{Br}\) at the amide nitrogen of PZ results in the formation of \(\text{N}\)-bromamide intermediate (\(\text{X}\)) through fast intermolecular rearrangement, with the elimination of \(\text{RNH}_2\). This \(\text{N}\)-bromamide intermediate (\(\text{X}\)) undergoes Hofmann rearrangement followed by hydrolysis to give the products.

From the slow step of Scheme 1,

\[
\text{rate} = k_3[X] \quad (7.9)
\]

If \([\text{BAT}]_t\) represents the total effective concentration of BAT, then

\[
[\text{BAT}]_t = [\text{RNHBr}] + [\text{RN}^+\text{H}_2\text{Br}] + [X] \quad (7.10)
\]

By substituting for \([\text{RNHBr}]\) and \([\text{RN}^+\text{H}_2\text{Br}]\) from equilibrium steps (i) and (ii) in equation (7.10) one obtains,
By substituting $[X]$ from equation (7.12) into equation (7.9), the following rate law (equation 7.13) is obtained:

$$\text{rate} = \frac{-d[BAT]}{dt} = \frac{K_1 K_2 [BAT][H^+][S]}{1 + K_1[H^+] + K_1K_2[H^+][S]}$$

The rate law (7.13) is in good agreement with the experimental results. Since rate = $k'[BAT]_i$, equation (7.13) can be transformed into equations (7.14) to (7.16),

$$k' = \frac{K_1 K_2 k_3 [H^+][S]}{1 + K_1[H^+] + K_1K_2[H^+][S]}$$

$$\frac{1}{k'} = \frac{1}{K_1 K_2 k_3 [H^+][S]} + \frac{1}{K_2 k_3[S]} + \frac{1}{k_3}$$

$$\frac{1}{k'} = \frac{1}{K_2 k_3[S]} \left( \frac{1}{K_1[H^+]} + 1 \right) + \frac{1}{k_3}$$

Based on equation (7.15) and (7.16), a plot of $1/k'$ versus $1/[S]$ and $1/k'$ versus $1/[H^+]$ at standard conditions has been found to be linear (Fig.7.5) the values of protonation constant $K_1$, formation constant and decomposition constant $k_3$, were calculated. These are found to be $K_1 = 17.4 \times 10^{-2}$ dm$^3$ mol$^{-1}$, $K_2 = 6.1 \times 10^4$ mol dm$^{-3}$ and $k_3 = 14.28 \times 10^{-4}$ s$^{-1}$ from the slope and intercept of the plot.

The effect of varying dielectric permittivity ($D$) of the medium on the rate has been described in several studies. A decrease in the rate with decreasing dielectric permittivity of the medium supports the proposed mechanism. Amis and Jaffe$^{22}$ has shown that a plot of log $k'$ versus $1/D$ gives a straight line with a negative slope for a reaction between an anion and a dipole or between two dipoles and a positive slope for a reaction between a cation and
dipole. In the present study, plot of log $k'$ versus $1/D$ is linear with a negative slope supports the suggested mechanism. The reduction product of the oxidant $p$-toluenesulfonamide did not influence the rate showing that it is not involved in any pre-equilibrium. The suggested mechanism is also supported by the values of energy of activation and other activation parameters. The high values of Gibbs free energy of activation and enthalpy of activation indicate that the transition state is solvated, while high negative value of entropy of activation accounts for the formation of compact transition state.
Scheme 7.2

RNHBr + H⁺ $\rightleftharpoons$ RNH₂Br

(i)

(ii)

(iii)

(iv)

Hofmann
REFERENCES

7 Mehmedagic, Aida; Verite, Philippe; Menager, Sabine; Tharassee, Christine; Chabenat, Christiane; Andre, Dominique; Lafont, Olivier. *Journal of Chromatography, B: Biomedical Sciences and Applications* (1997), 695(2), 365-372.