KINETICS OF OXIDATION OF PHARMACEUTICAL DRUG
METFORMIN HYDROCHLORIDE BY BROMAMINE –T IN NaOH
MEDIUM. A MECHANISTIC STUDY

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ABSTRACT
The kinetics of oxidation of Metformin hydrochloride (MET) by bromamine-T (BAT) has been investigated in NaOH medium at 303 K. The reaction followed first-order kinetics with respect to [BAT], [MET] and fractional-order with [OH⁻]. First-order kinetics with respect to [BAT] was observed for the oxidation of metformin hydrochloride. An increase in medium concentration increased the rate of reaction. The effect of added halide ions, reduction product p-toluene sulfonamide (PTS), ionic strength (NaClO₄) and dielectric constant of the medium was studied on the rate of reaction. The main products were identified by spot test, GC–MS spectral analysis. A mechanism was proposed on the basis of experimental results. The activation parameters have been determined from the Arrhenius plots. With respect to the slow step of the mechanism were evaluated, and the thermodynamic parameters was also determined and discussed.

KEYWORDS: Kinetics, oxidation, Metformin hydrochloride , bromamine-T, mechanism.

1. INTRODUCTION
Metformin Hydrochloride (MET) [N, N–Dimethylimidodicarbonimidic diamide hydrochloride] is an anti-diabetic drug [¹]. MET is a biguanide, belong to a class of insulin sensitizers. This is widely used for the treatment of type 2 diabetes mellitus, its primary action is to inhibition of hepatic glucose production and increases the sensitivity of insulin. It
works by decreasing the amount of sugar made by the liver and decreasing the amount of sugar absorbed into the body. As a result, MET can help the body to respond better to its own insulin and decrease blood sugar levels \(^2\).

The review of available literature reveals that, there was no information available on the oxidation kinetics of MET with any oxidant. There was a need for understanding the oxidation mechanism of this compound so that the study could throw some light on the fate of the compound in biological system and also the behavior of BAT towards MET in the presence of NaOH medium. Therefore the present chapter reports the kinetics of oxidation of MET with BAT in NaOH medium at 303 K.

2. EXPERIMENTAL

2.1 Materials
Bromamine-T was prepared \(^3\) using chloramines-T (CAT). To a solution of CAT (20 g in 400 mL of water), about 4 ml of liquid bromine was added drop wise with constant stirring at room temperature to yield dibromamine-T (DBT). The solid DBT was filtered under section, washed thoroughly with ice cold water until all the absorbed bromine was removed and then vacuum-dried for 24h. About 20 g of DBT thus obtained was dissolved in 30 mL of 4 mol dm\(^{-3}\) NaOH with constant stirring at room temperature and the resultant aqueous solution was cooled in ice. Pale yellow crystals of BAT formed was filtered under suction, washed quickly with the minimum amount of ice cold water and dried over P\(_2\)O\(_5\). Aqueous solution of the oxidant was standardized by iodometric procedure and preserved in brown bottles to prevent photochemical deterioration.

Pharmaceutical grade Metformin Hydrochloride (supplied by Wanbury Limited, Bangalore, India) was used as received. All other reagents, sodium perchlorate, Methanol, CAT, and KI were of analar grade doubly distilled water was used throughout the investigations.

2.2 Kinetic procedure
Reactions were carried out under pseudo first-order conditions with a known excess of \([\text{substrate}]_0\) over \([\text{oxidant}]_0\) at constant temperature 303 K in glass stoppered Pyrex boiling tubes coated black from outside to eliminate any photochemical deterioration. A Raaga digital proportional temperature controller (CH-61) was used to maintain the desired temperature with an accuracy of ± 0.1°C. Requisite amounts of solutions of substrate, HCl
and enough water to keep the total volume constant (50 ml) for all kinetic runs were equilibrated at 303K for about 30 min. A measured amount of BAT solution, also equilibrated at the same temperature was rapidly added to the reaction mixture which was periodically shaken for uniform concentration. The progress of the reaction was monitored by withdrawing measured aliquots (5 ml each) from the reaction mixture at regular time intervals and determined the unreacted BAT eudiometrically. The course of the reaction was studied more than two half-lives. The pseudo first-order rate constants (k/s⁻¹) calculated from the linear plots of log [BAT] versus time were reproducible within ± 5%.

2.3 Stoichiometry and product analysis

Reaction mixtures containing varying ratios of BAT and MET in the presence of NaOH were equilibrated at 303 K for 24 hours. The results of estimation of unreacted Bromamine-T indicated 1:1 stoichiometry that is one mole of Metformin Hydrochloide consumed one mole of Bromamine-T.

\[
\begin{align*}
\text{NH}_2 - \text{TsNHNa} + \text{H}_2\text{O} & \rightarrow \\
\text{NH}_2 + \text{TsNH}_2 + \text{Na}^+ + \text{Br}^- \\
\end{align*}
\]

(1)

After completion of the reaction (monitored by TLC), the reaction product were neutralized with HCl and extracted with ether. The reaction products were subjected to spot test [4] and chromatographic analysis (TLC technique) which revealed the formation of Metformin N-oxide as the oxidation product of metformin in acid medium. The oxidation product was separated by column chromatography and confirmed by GC-MS analysis. The GC-MS data were obtained from 17A Shimadzu gas chromatograph with a QP-5050 Shimadzu mass spectrometer. The mass spectra showed a molecular ion peaks at 145amu Fig. 1 is clearly confirming metformin N-oxide. It was also noticed that there was no further oxidation of product under present kinetic conditions.

p-Toluene sulfonamide (TsSO₂NH₂), the reduction product of the BAT, was extracted by ethyl acetate and identified [5] by TLC using petroleum ether- chloroform-1-butanol (2: 2: 1 v/v) as the solvent and iodine as the as spray reagent. Further the molecular ion peak in the mass spectrum at 171 amu Fig.2 and its MP 138 - 139°C (lit. mp 137°C– 140°C) confirms p-
toluenesulfonamide. It was also observed that there was no further oxidation of these products under the present experimental kinetic conditions.

3. KINETIC RESULTS OF OXIDATION REACTION

The oxidation of MET by BAT in the presence of Sodium hydroxide (NaOH) medium at 303 K was performed in a measurable rate. The reaction orders have been determined from the slopes of log $k'$ versus respective logarithmic concentration of MET, NaOH and MeOH except [BAT], by keeping the others concentrations constant.

3.1 Effect of Reactants

At constant $[\text{MET}]_0$, $[\text{NaOH}]$ and temperature, where $[\text{MET}] \gg [\text{BAT}]_0$, plots of log $[\text{BAT}]$ versus time were linear, indicating a first-order dependence of rate on $[\text{BAT}]_0$. The pseudo first-order rate constants $k'$, obtained are listed in [Table.1]. Further, the values of $k'$ are unaltered with variation of $[\text{BAT}]_0$, confirming first-order dependence on $[\text{BAT}]_0$.

The rate increased with increase in $[\text{MET}]_0$ range $5 \times 10^{-3}$ to $30 \times 10^{-3}$ and a plot of log $k'$ versus log $[\text{MET}]$ was linear with a slope of (+1.0), indicating a first-order dependence on $[\text{MET}]_0$. Further, a plot $k'$ versus $[\text{MET}]$ was also linear with a y-intercept, confirming the first-order dependence on $[\text{MET}]$ [Table.1 and Fig.3.

The effect of NaOH concentration was studied in the range of $10 \times 10^{-3}$ to $100 \times 10^{-3}$ mol dm$^{-3}$, keeping MET, BAT concentration and temperature at constant values. The rate constants increased with increase in the concentration of NaOH. The plots of log $k'$ vs [NaOH] were linear with positive fractional slopes (+0.73) indicates positive fractional order dependence of rate on [NaOH] Table.1 and Fig. 4.
Table 1 Effect of varying concentration of Bromamine-T, Metformin Hydrochloride and NaOH on the rate of the reaction at 303 K.

<table>
<thead>
<tr>
<th>$10^4$ [BAT]/M</th>
<th>$10^3$ [MET]/M</th>
<th>$10^3$ [NaOH]/M</th>
<th>$k'$ ($\times10^4$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>10</td>
<td>10</td>
<td>1.66</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
<td>2.54</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>10</td>
<td>2.36</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>10</td>
<td>2.91</td>
</tr>
<tr>
<td>10</td>
<td>0.05</td>
<td>10</td>
<td>1.31</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
<td>2.54</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>10</td>
<td>5.00</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>10</td>
<td>9.83</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
<td>2.54</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>20</td>
<td>5.00</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>50</td>
<td>7.77</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>100</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Fig. 1 GC-Mass spectrum of metformin-N-oxide with its molecular ion peak at 145 amu.

Fig. 2 GC-Mass spectrum of p-toluenesulfonamide with its molecular ion peak at 171 amu.
Fig. 3 Effect of variation of concentration of MET on the oxidation of MET by BAT in NaOH at 303 K (Conditions as in Table .1)

Fig. 4 Effect of medium [NaOH] on the oxidation of MET by BAT in NaOH at 303 K (Conditions as in Table .1)

3.2 Effect of PTS, Halide ions and Ionic strength
During the oxidation reaction, oxidizing agent BAT gets reduced. The reduction product of BAT is p-Toluene sulphonamide (PTS). In order to study the effect of reduction product during the oxidation reaction of MET by BAT, the reduction product of BAT was added to the reaction mixture in the range of $5 \times 10^{-4}$ to $20 \times 10^{-4}$ mol dm$^{-3}$. The rate constants for
different PTS concentrations are shown in Table. 2. The data of Table. 2 showed that the rate constant remains same in the range of studied PTS concentration. It indicated that the rate constants on the oxidation of MET by BAT does not depend on PTS concentration, and hence PTS has no effect on the rate of the reaction.

In order to see the effect of halide ions such as chloride and bromide on the reaction rate, the experiments were performed taking halide salts at concentration in the range $1 \times 10^{-2}$ to $10 \times 10^{-2}$ mol dm$^{-3}$. The NaOH concentration was kept at $10 \times 10^{-3}$ mol dm$^{-3}$. Table. 2. The data of Table. 2 showed that the rate constant remains same in the range of studied halide concentration. It infers that the rate constants of the oxidation of MET by BAT does not depend on halide ions concentration, hence halide ions show no effect on the rate of the reaction.

The know the effect of ionic strength on the oxidation reaction of MET by BAT, ionic strength of the medium was varied in the reaction mixture by the addition of the solution of NaClO$_4$ in the range $5 \times 10^{-3}$ to $20 \times 10^{-3}$ mol dm$^{-3}$. The rate constants for different NaClO$_4$ concentrations are shown in Table. 2. The data in the Table. 2 shows that the rate constant remains same in the range of studied NaClO$_4$ concentrations. Hence the rate constant is independent of NaClO$_4$ concentrations. The ionic strength of the medium does not influences the present oxidation reaction.

Table. 2 Effect of varying [NaCl], [NaBr], [PTS] and NaClO$_4$ on the reaction rate.

<table>
<thead>
<tr>
<th>$10^2$[NaCl] / [NaBr] / M</th>
<th>k$'$ (×10$^4$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>2.70 (2.43)</td>
</tr>
<tr>
<td>05</td>
<td>2.63 (2.50)</td>
</tr>
<tr>
<td>10</td>
<td>2.56 (2.56)</td>
</tr>
<tr>
<td>$10^3$[PTS]/M</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>2.56</td>
</tr>
<tr>
<td>10</td>
<td>2.50</td>
</tr>
<tr>
<td>20</td>
<td>2.63</td>
</tr>
<tr>
<td>$10^3$ [NaClO$_4$]/M</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>2.63</td>
</tr>
<tr>
<td>10</td>
<td>2.56</td>
</tr>
<tr>
<td>20</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Values in parenthesis refers to the rate constants with NaBr

[BAT] = $10 \times 10^{-4}$ mol dm$^{-3}$; [MET] = $10 \times 10^{-3}$ mol dm$^{-3}$; [NaOH] = $10 \times 10^{-3}$ mol dm$^{-3}$; $T = 303$ K
3.3 Effect of Dielectric constant of Medium

The dielectric constant (D) of the medium was studied by the addition of methanol to the reaction medium. The values of the dielectric constant [D] of H₂O and methanol system of different compositions are reported in the literature. The results obtained indicated that the increase in the concentration of methanol lowers the dielectric constant of the medium, resulting in the decrease of rate of the reaction. Plots of dielectric constant [D] vs. log k' are shown in Table. 3 Fig. 5. The slope of the plot is negative with a negative value of (−0.7).

Table. 3 Effect of varying dielectric constant of medium on the reaction rate.

<table>
<thead>
<tr>
<th>[MeOH] % v/v</th>
<th>D</th>
<th>10²/D</th>
<th>K'(×10⁴ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>76.73</td>
<td>1.3032</td>
<td>2.54</td>
</tr>
<tr>
<td>10</td>
<td>72.37</td>
<td>1.3817</td>
<td>1.94</td>
</tr>
<tr>
<td>20</td>
<td>67.48</td>
<td>1.4819</td>
<td>1.8</td>
</tr>
<tr>
<td>30</td>
<td>62.71</td>
<td>1.5946</td>
<td>1.73</td>
</tr>
</tbody>
</table>

[BAT] = 10 × 10⁻⁴ mol dm⁻³; [MET] =10 × 10⁻³ mol dm⁻³;
[NaOH] = 10 × 10⁻³ mol dm⁻³; T = 303 K

Fig. 5 Effect of Dielectric constant (MeOH) on the oxidation of Metformin (MET) by Bromamine-T (BAT) in NaOH at 303 K (Conditions as in Table. 3)

3.4 Effect of temperature

The rate constants for the oxidation of MET with BAT at different temperature ranges from 293 to 313 K was measured by keeping the other experimental conditions constant. The
temperature effect was studied for three concentration of MET. It was observed that the rate constant of the reaction increases with increase in temperature for given concentration of MET. Further the rate constant increases with increasing the concentration of MET for a given temperature Table. 4 and Fig. 6.

Also, the Arrhenius plot of log $k'$ vs.1/T, which is linear as in Table. 5 and Fig. 7, was used to calculate the thermodynamic parameters.

**Table .4 Effect of varying MET on the reaction rate at different temperatures.**

<table>
<thead>
<tr>
<th>$10^3$[MET]/M</th>
<th>k'($\times 10^4$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>293K</td>
</tr>
<tr>
<td>0.05</td>
<td>0.625</td>
</tr>
<tr>
<td>0.10</td>
<td>1.31</td>
</tr>
<tr>
<td>0.20</td>
<td>2.70</td>
</tr>
</tbody>
</table>

$[BAT] = 10 \times 10^{-4}$ mol dm$^{-3}$; $I = 5 \times 10^{-3}$ mol dm$^{-3}$; $[NaOH] = 10 \times 10^{-3}$ mol dm$^{-3}$

![Graph showing the Arrhenius plot of log $k'$ vs.1/T for varying concentrations of MET.](image)

**Fig. 6 Effect of variation [MET] at different temperature on the rate of the reaction (Conditions as in Table. 4).**
Table. 5 Effect of varying temperature and the values of activation parameters for the composite reaction between BAT and MET

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>k'(×10^4 s(^{-1}))</th>
<th>Activation parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>1.31</td>
<td>Ea = 53.80 kJmol(^{-1})</td>
</tr>
<tr>
<td>303</td>
<td>2.54</td>
<td>∆H(^#) = 51.28 kJmol(^{-1})</td>
</tr>
<tr>
<td>313</td>
<td>5.41</td>
<td>∆G(^#) = 95.35 kJmol(^{-1})</td>
</tr>
</tbody>
</table>

\[ \log A = 5.667 \]

\([\text{BAT}] = 10 \times 10^{-4} \text{ mol dm}^{-3}; [\text{MET}] = 10 \times 10^{-3} \text{ mol dm}^{-3}; [\text{NaOH}] = 10 \times 10^{-3} \text{ mol dm}^{-3} \]

Fig. 7 Effect of Temperature on the oxidation of MET by BAT in NaOH. (Conditions as in Table.5)

3.5 Test for free radicals

The addition of small amount of the oxidation reaction mixture to the aqueous acryl amide solution did not initiate polymerization, showing the absence of free radicals species (non ionic species) during the reaction sequences.
4. DISCUSSION

Investigations by Pryde and Soper \[6\] Morris et.al \[7\] and Bishop and Jennings \[8\] on sodium-N-haloarenesulfonamides have shown the similar equilibria and they behave as strong electrolyte in aqueous solutions following are the different species for BAT.

\[
\begin{align*}
TsNB\text{Na} & \rightleftharpoons TsNBr^- + Na^+ \quad \ldots (2) \\
TsNBr^- + H^+ & \rightleftharpoons TsNHBr \quad \ldots (3) \\
2 TsNHBr & \rightleftharpoons TsNH_2 + TsNBr_2 \quad \ldots (4) \\
TsNHBr + H_2O & \rightleftharpoons TsNH_2 + HOBr \quad \ldots (5) \\
TsNBr_2 + H_2O & \rightleftharpoons TsNHBr + HOBr \quad \ldots (6) \\
HOBr & \rightleftharpoons H^+ + O Br^- \quad \ldots (7)
\end{align*}
\]

(Here Ts=p-CH_3C_6H_4SO_2^-)

Therefore, the possible oxidizing species in basic medium are TsNHBr, TsNBr^- and HOBr. Hardy and Johnston \[9\] have also reported the following equilibrium in alkaline solutions of BAT.

\[
\begin{align*}
TsNBr^- + H_2O & \rightleftharpoons TsNHBr + OH^- \quad \ldots (8) \\
\end{align*}
\]

For the alkali accelerating step Eq(8) can be written as

\[
\begin{align*}
TsNHBr + OH^- & \rightleftharpoons TsNBr^- + H_2O \quad \ldots (9) \\
\end{align*}
\]

In present case, the rate of the reaction is accelerated with respect to [OH^-], indicates that the anion TsNBr^- is the most likely oxidizing species.

\[
\begin{align*}
K & \quad \text{(i) fast} \\
k & \quad \text{(ii) slow & rds} \\
\end{align*}
\]

Scheme 1
The total concentration of BAT is \([\text{BAT}]_t\), then
\[
[\text{BAT}]_t = [\text{TsNHBr}] + [\text{TsNBr}^-]
\]  \(\text{(10)}\)

By substituting for \([\text{TsNHBr}]\) from equilibrium (1) of Scheme 1 in Eq. (1) and solving for \([\text{TsNBr}^-]\), one obtains.
\[
[\text{TsNBr}^-] = \frac{K [\text{BAT}]_t [\text{OH}^-]}{[\text{H}_2\text{O}] + K [\text{OH}^-]}
\]  \(\text{(11)}\)

From the slow step (ii) of Scheme 1,
\[
\text{Rate} = k [\text{TsNBr}^-] [\text{MET}] \quad \text{... (12)}
\]

By substituting for \([\text{TsNBr}^-]\) from Eq. (11) in to Eq. (12), the following rate law is obtained
\[
\text{Rate} = \frac{K k [\text{BAT}]_t [\text{MET}] [\text{OH}^-]}{[\text{H}_2\text{O}] + K [\text{OH}^-]} \quad \text{... (13)}
\]

The rate law (13) is in agreement with the experimental data, including a first order dependance of rate each on \([\text{BAT}]_t\) and \([\text{MET}]\) and a fractional order dependance on \([\text{OH}^-]\).

The effect of \([\text{MET}]\) on the rate at different temperatures (293-313K) was studied. Addition of halide ions had no effect on the rate indicating that no inter halogen compound or free bromine was formed. The reduction product PTS had no influence on the rate showing that it was not involved in pre-equilibrium. The change in the ionic strength of the medium did not alter the rate indicating that non-ionic species were involved in the rate determining step. Amis\(^{10}\) has shown that a plot of \(\log k'\) versus \(1/D\) is linear. It gives a negative slope for a reaction between the cations and dipole. The negative dielectric effect, in the present studies, supports the interaction of dipolar species in the rate limiting step.

The proposed mechanism is also supported by the moderate value of energy of activation. The fairly high positive value of free energy of activation and enthalpy of activation indicate that the transition state is highly solvated, while the negative entropy of activation suggests the formation of the compact activated complex.
5. CONCLUSION

The kinetics of oxidation of Metformin hydrochloride by Bromamine-T in NaOH medium has been studied at 303 K. Stoichiometry of the reaction was found to be 1:1. The oxidation product identified was Metformin N-oxide at is confirmed by GC-MS analysis. The active oxidizing species involved in NaOH medium is TsNHBr. Activation parameters are calculated. The scheme proposed and derived rate law has been correlated with the observed kinetics.

ACKNOWLEDGMENTS

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