Mechanism and Kinetics Studies of Oxidation of the Pharmaceutical Drug Amlodipine Besylate by N-bromosuccinimide in Aqueous Acidic Medium

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ABSTRACT

The oxidation of the pharmaceutical drug Amlodipine besylate [AML] by N-Bromosuccinimide [NBS] was investigated aqueous acidic medium under pseudo-first-order condition. The experimental results indicated that the reaction exhibits first-order concerning N-bromosuccinimide, fractional-order concerning [AML] and sulphuric acid [H₃SO₄]. There was no substantial effect on the rate of the reaction with KNO₃. The reaction stoichiometry shows one mole of amloidipine besylate consumes one mole of n-bromosuccinimide. The effect of temperature on the reaction rate was studied, and the activation parameters (Eₐ, ΔG, ΔH and ΔS) are calculated and tabulated. LC-MS technique was used to identify the oxidation product of amloidipine besylate. Based on experimental results, a mechanism is proposed, and constants K₁, K₂ and k₃ involved in the mechanism were evaluated. The observed rate constant values and the experimental value calculated by substituting the value of k₃ = 4.0 × 10⁻³ s⁻¹, K₂ = 1272 moldm⁻³s⁻¹ and K₁ = 1.96 x 10⁻⁶ in the rate equation is in good accordance with each other.

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INTRODUCTION

Chemical kinetics is concerned with understanding the rates of chemical reaction, the speed at which chemical species can transform into new substances by breaking and reforming the chemical bonds (Srgoura et al., 2011). It can reasonably guide us to control the reaction conditions and improve the reaction rate, to increase the production of chemical products, and also guide us to learn how to suppress or slow down the reaction rate of side reactions to reduce the consumption of raw materials (Zhang et al., 2020; Keith, 2012). A knowledge of reaction kinetics of pharmaceuticals under various conditions helps identify the mechanism or the sequence of steps by which a reaction occurs (Bajaj, 2012). Knowledge about the topic of chemical kinetics is vital for the pharmacists as they encounter the impact of the chemical degradation of drugs in the course of their everyday activities. Therefore the topic of chemical kinetics is helpful for pharmacists for both safety and economic reasons. A straightforward titration method has been developed to investigate the kinetics of oxidation of amloidipine besylate (AML) with N-Bromosuccinimide in acidic medium. Amlodipine is a dihydropyridine calcium antagonist (Shohin et al., 2010). It is chemically (2-[(2-amino-ethoxy)-methyl]-4-2-chlorophenyl-1, 4-dihydro-6-methyl-3, 5-pyridine dicarboxylic acid-3-ethyl-5-methyl ester (Salam, 2019). AML shown in
(Figure 1) is used for its antianginal, antiarrhythmic and antihypertensive activity. Likewise, it is also used in the treatment of variant angina (Mahmoud et al., 2012). Amlodipine is official in USP, BP, and EUP (Taylor, 1989; Zhang et al., 2000). N-Bromosuccinimide is used as an oxidant for a variety of substrate in acidic medium (Kakde and Bawane, 2009). N-Halocompounds such as n-chlorosuccinimide and n-bromosuccinimidein the field kinetics are wide as they are a source of positive halogens (Singh et al., 2000), and used as an oxidant in both acidic as well as in alkaline medium for most of the reductants. NBS is widely used as an oxidising agent for the determination of many pharmaceutically important compounds because of its sensitivity, accuracy and low cost. It is more stable in neutral, aqueous or slightly acidic medium (pH 4.5) and can, therefore, be used for oxidation at relatively lower pH (Bhandarkar et al., 2010; Hassan, 2019).

The oxidation of AML with NBS was carried out with a vision to (i) find appropriate rate law (ii) To propose scheme (iii) to identify the oxidation products of the reaction of AML with NBS.

MATERIALS AND METHODS

The chemical reagent Amlodipine Besylate used in this study was offered by CIPLA industries ltd. AR grade chemicals, AML, KI, Thiosulphate, and NBS, were used during this work. The N-bromosuccinimide solution was prepared by dissolving a measured quantity of NBS in water and stored in an amber coloured bottle, AML solution was prepared by dissolving in methanol (Kumar et al., 2018). 0.1M sulphuric acid is used during this process, and the starch indicator is freshly prepared any time before the beginning of titration. NBS solution is standardised iodometrically (Vogel et al., 1989). 10% solution of potassium iodide was used for this work potassium iodide solution is prepared by dissolving 10g of KI in 100 ml of water and stored in the amber coloured bottle to avoid photochemical deterioration.

Kinetic measurements

All the kinetic measurements were carried out in an iodine flask at 299k under pseudo-first-order condition with the concentration of amlodipine besylate ten times greater than the oxidant. A known amount of AML drug solution along with the required amount of 0.1M sulphuric acid, 10% potassium iodide, and water is taken in a glass stoppered iodine flask. The reaction was started with the addition of the NBS solution. The excess of oxidant present in the reaction mixture was titrated with sodium thiosulphate solution using a freshly prepared starch indicator. The endpoint is the disappearance of blue colour. The titre values are noted. The titration is repeated with various aliquots at different time intervals from zero time until completion of reaction at least up to 80%. Generally, 8-10 titre values were obtained.

From the titre values, A plot of log [Vt] vs time was drawn, and from their slopes, the pseudo-first-order rate constants were obtained. The reaction was repeated with varying concentrations of amlodipine besylate from 1.0 × 10⁻³ to 2.5 × 10⁻³ moldm⁻³, N-Bromosuccinimide from 2.0 × 10⁻³ to 4.0 × 10⁻³mol dm⁻³ and sulphuric acid from 5.0 × 10⁻⁴ to 3.0 × 10⁻³moldm⁻³. A graph is plotted between log Vt vs. Time in seconds.

All the plots obtained in this method were as shown in (Figure 2) linear (Nadh and Sireesha, 2015) With the correlation coefficient of 0.9767 to 0.9876 as mentioned in Table 1.

RESULTS AND DISCUSSION

Stoichiometry

To propose the mechanism for AML oxidation with NBS, different proportions of AML and NBS in acidic medium was collected and equilibrated for 24hours at room temperature.

The excess oxidant present in the reaction mixture is determined by titrating against thiosulphate. Hence by plotting a graph of volume of thiosulphate vs concentration of NBS, the concentration of unreacted NBS can be estimated from the intercept. It shows that 1 mole of substrate AML utilised 1 mole of oxidant NBS confirming the stoichiometry.

\[
\begin{align*}
C_{26}H_{31}ClN_2O_8 + (CH_2CO)_2NBr + H_2O &\rightarrow C_{20}H_{25}ClN_2O_5 + C_6H_6SO_3 + HOBr + (CH_2CO)_2NH
\end{align*}
\]

Figure 1: Chemical structure of amlodipine besylate
Table 1: Effect of [NBS] on the rate of oxidation of Amlodipine besylate in H$_2$SO$_4$ medium (Representative run).

| Time in seconds | Vol of thio Vt (ml) | log Vt |
|-----------------|---------------------|--------|
| 0               | 11.0                | 1.041  |
| 30              | 9.0                 | 0.954  |
| 60              | 7.3                 | 0.863  |
| 90              | 6.1                 | 0.785  |
| 120             | 5.4                 | 0.732  |
| 180             | 4.7                 | 0.672  |
| 240             | 3.9                 | 0.591  |
| 300             | 2.5                 | 0.397  |

Table 2: The Rate constant value at different temperatures

| The temperature in (K) | K x 10^{-3}(S^{-1}) |
|-------------------------|----------------------|
| 299                     | 2.2                  |
| 303                     | 3.4                  |
| 308                     | 6.9                  |
| 313                     | 9.7                  |

Table 3: The activation parameter values calculated for the oxidation of AML with NBS in acidic medium.

| Activation parameters | Values     |
|-----------------------|------------|
| Ea (kJmol$^{-1}$)     | 82.0       |
| $\Delta$H$^\#$(kJmol$^{-1}$) | 84.31     |
| $\Delta$S$^\#$(Jk$^{-1}$mol$^{-1}$) | -250.6 |
| $\Delta$G$^\#$(kJmol$^{-1}$) | 15.8      |

Table 4: Effect of varying concentrations of oxidant [3x10$^{-4}$] moldm$^{-3}$, substrate e[1.75 x 10$^{-3}$] moldm$^{-3}$ and H$_2$SO$_4$[1.5x 10$^{-2}$] moldm$^{-3}$ on the reaction rate at 305 K

| [NBS] x10$^{-4}$ (moldm$^{-3}$) | [AMB] x10$^{-3}$ (moldm$^{-3}$) | [H$_2$SO$_4$] x 10$^{-2}$ (moldm$^{-3}$) | $K_{obs}$ x 10$^{-3}$ S$^{-1}$ | $K_{cat}$ x 10$^{-3}$ |
|--------------------------------|---------------------------------|----------------------------------------|-------------------|-----------------|
| 3.0                            | 1.0                             | 1.5                                    | 2.1               | 1.9             |
| 3.0                            | 1.5                             | 1.5                                    | 2.3               | 2.0             |
| 3.0                            | 1.75                            | 1.5                                    | 2.3               | 2.0             |
| 3.0                            | 2.0                             | 1.5                                    | 2.6               | 2.2             |
| 3.0                            | 2.25                            | 1.5                                    | 2.7               | 2.3             |
| 3.0                            | 2.5                             | 1.5                                    | 2.9               | 2.2             |
| 2.0                            | 1.75                            | 1.5                                    | 1.9               | 2.0             |
| 2.5                            | 1.75                            | 1.5                                    | 2.1               | 2.0             |
| 3.0                            | 1.75                            | 1.5                                    | 2.4               | 2.0             |
| 3.5                            | 1.75                            | 1.5                                    | 2.4               | 2.0             |
| 3.0                            | 1.75                            | 1.5                                    | 2.6               | 2.0             |
| 3.0                            | 1.75                            | 1.0                                    | 2.2               | 1.8             |
| 3.0                            | 1.75                            | 1.5                                    | 2.3               | 2.0             |
| 3.0                            | 1.75                            | 2.5                                    | 2.4               | 2.2             |
| 3.0                            | 1.75                            | 3.0                                    | 2.8               | 2.3             |
Product analysis

The reaction mixture containing a required amount of N-Bromosuccinimide \(3 \times 10^{-4} \text{moldm}^{-3}\) and 129 amloidipine besylate \(1.75 \times 10^{-3} \text{moldm}^{-3}\) along with other reagents after titration with sodium thiosulphate was kept for 24 hours at room temperature. During this process, the substrate AML was converted entirely into a product. Using a separating funnel, the product in the reaction mixture was extracted by washing it several times using CCl\(_4\). The extracted product is separated from the aqueous phase. The separated organic phase is sent to LC-MS for product analysis. From the LC-MS spectra obtained, the oxidative product is identified (Asha et al., 2018). The LC-MS shows a molecular ion peak at 407, as shown in (Figure 3) confirming the oxidative product (Argekar and Powar, 2000; Bahrami and Mirzaeei, 2004).

Effect of Amlodipine Besylate

The oxidation of AML with NBS was kinetically investigated at several initial concentrations of the reactants in Sulphuric acid medium at 299k. To determine the concentration concerning amloidip-
ine besylate, the concentration of the reductant was varied from $1.0 \times 10^{-3}$ to $2.5 \times 10^{-3} \text{moldm}^{-3}$, keeping the constant concentration of NBS $3.0 \times 10^{-4} \text{moldm}^{-3}$ and Sulphuric acid $1.5 \times 10^{-3} \text{moldm}^{-3}$ the value of $k_{obs}$ increased with increase in AML concentration, and a plot of log $K$ vs log [AML] was linear with $R^2 = 0.81$, indicating a fractional order dependence concerning to substrate amloidine besylate as shown in (Figure 4).

**Effect of N-bromosuccinimide**

To determine the order concerning oxidant, N-bromosuccinimide study was conducted by varying the concentration of [NBS] $2.0 \times 10^{-3}$ moldm$^{-3}$ to $4.0 \times 10^{-3} \text{moldm}^{-3}$ at 299K at a constant concentration of [AML] $1.75 \times 10^{-3} \text{moldm}^{-3}$ and [Sulphuric acid] $1.5 \times 10^{-3} \text{moldm}^{-3}$. A plot of log $K$ vs log C shows first-order dependence concerning NBS as shown in (Figure 5).

**Effect of ionic strength on the reaction rate**

The effect of ionic strength on the rate of the reaction is investigated by increasing concentration of $\text{KNO}_3$ from 0.1 to 0.3 moldm$^{-3}$ by keeping the constant concentration of [AML] $1.75 \times 10^{-3} \text{moldm}^{-3}$ and NBS $3.0 \times 10^{-4} \text{moldm}^{-3}$. It has been observed that the reaction remains constant with an increase in the concentration of Potassium nitrate. Variation of ionic strength of the medium showed no significant effect on the reaction rate.

**Effect of sulphuric acid**

The rate of the reaction was carried out by keeping the constant concentration of [AML] $1.75 \times 10^{-3} \text{moldm}^{-3}$ and [NBS] $3.0 \times 10^{-4} \text{moldm}^{-3}$ varying concentration of sulphuric acid from $5 \times 10^{-4}$ to $3 \times 10^{-3} \text{moldm}^{-3}$. The rate of the reaction increases with an increase in the volume of sulphuric acid. A plot of log k’ vs log C shows fractional-order dependence.

**Effect of temperature**

The kinetic reaction pathway is influenced much by temperature variation; oxidation of AML with NBS was studied at a different temperature ranging from 299 k to 313k. When the reaction was conducted, the other experimental conditions and reaction concentration of [AML] $1.75 \times 10^{-3} \text{moldm}^{-3}$ [NBS] $3.0 \times 10^{-4} \text{moldm}^{-3}$ and [Sulphuric acid] 0.5 moldm$^{-3}$ it has been observed that the reaction remains constant with an increase in the concentration of Potassium nitrate. Variation of ionic strength of the medium showed no significant effect on the reaction rate.

**Reaction Scheme**

In most of the investigations, the molecular NBS acts only through its positive polar end. The rate of the reaction increase by an increase in the concentration of the $\text{H}_2\text{SO}_4$ hence the protonated species of NBS, $\text{RN}^+\text{HBr}$ is the most likely oxidising species as shown in step (i) of the reaction scheme. The protonated species $\text{RN}^+\text{HBr}$ reacts with AML to form an intermediate complex, which further undergoes hydrolysis to form products. Considering the above facts and all experimental data obtained, the following mechanism may be suggested for the oxidation of AML with NBS in an acid medium, as shown in (Figure 8).

\[
\begin{align*}
\text{RNBr} + \text{H}^+ & \rightleftharpoons \text{RN}^+\text{HBr} & (i) \\
\text{RN}^+\text{HBr} + \text{AML} & \rightleftharpoons \text{X} & (ii) \\
\text{X} & \rightarrow \text{Products Slow and rds} & (iii)
\end{align*}
\]

In the above scheme X is the intermediate complex species whose possible structure is shown in Scheme 8 in which a detailed mechanistic interpretation of AML and NBS reaction in acid medium is presented.

**Kinetic Rate law**

1. Step 3 in scheme determines the overall rate

\[
\text{Rate} = -\frac{d[RNBr]}{dt} = K_t[X]
\]

2. If the total effective concentration of NBS in solution is represented by [RNBr]$_t$, then

\[
[RNBr]_t = [RNBr] + [RN^+\text{HBr}] + [X]
\]

3. From step 1 of scheme

\[
[RNBr] = \frac{[RN^+\text{HBr}]}{K_t[H^+]} 
\]

4. From step 2 of scheme
Figure 8: A detailed Mechanistic interpretation for the oxidation of AML with NBS.

\[ \text{RN}^+\text{HBr} = \frac{[X]}{K_2}\text{[AML]} \]

5. Substituting equation 3 and 4 in equation 2 we have

\[ [\text{RNBr}]_t = \frac{[\text{RN}^+\text{HBr}]/[\text{H}^+]}{K_2} + \frac{[X]}{K_2}\text{[AML]} + [X] \]

6. On rearranging equation 5

\[ X = K_1K_2[\text{RNBr}]_t [\text{AML}][\text{H}^+] / 1 + K_1[\text{H}^+] + K_1K_2[\text{AML}][\text{H}^+] \]

7. Substituting for [X] from equation 6 in equation 1 we have.

\[ \text{Rate} = \frac{K_1K_2k_3[\text{RNBr}]_t[\text{AML}][\text{H}^+] / 1 + K_1[\text{H}^+]}{1+K_2[\text{AML}]} \]

The Rate law as shown in equation (7) is in good agreement with the experimental results, wherein the first-order dependence of rate on [NBS], fractional-order dependence each on [AML] and [H+]

Since Rate = k/ [RNBr]t, under pseudo-first order conditions of [NBS] << [AML], equation 7 can be transformed as eq8, eq9 and eq10 (Sukhdev and Puttaswamy, 2013).

8. \[ K_{obs} = \frac{K_1K_2k_3 [\text{AML}][\text{H}^+] / 1 + K_1[\text{H}^+]}{1+K_2[\text{AML}]} \]

9. \[ 1/K_{obs} = 1/K_1K_2k_3[\text{AML}][\text{H}^+] + 1/K_1k_3[\text{AML}] + 1/k_3 \]
10. $1/K' = 1/K_s k_3 [AML][H^+]$ (1/ $K_s [H^+] + 1$) + $1/k_3$

Based on the above equation (10), the rate law was verified by plotting a graph of $1/k_{obs}$ vs $1/[AML]$ as shown in (Figure 7) at constant $[H^+]$. From the slope and intercept of the above plot, the value of $k_3$ and $K_s$ was found to be $4.0 \times 10^{-5}$ s$^{-1}$ and 0.1272 moldm$^{-3}$s$^{-1}$. The value of $K_s$ was found to be $1.96 \times 10^{-6}$. The experimental value tabulated in (Table 4) is calculated by substituting the value of $k_3$, $K_s$ in the rate equation. $k_{obs} = K_1 K_2 K_3 [AML][H^+] / 1 + K_1 [H^+] + K_2 [AML]$. The experimental values are in good agreement with k calculated values, as shown in (Table 4).

CONCLUSIONS

The following interpretations are drawn, based on the outcomes obtained from research work done. The reaction obeys the experimental rate law: rate = $k_1 / [NBS]_0 \times [Substrate] \times [H^+]^n$. The function of hydrogen ion is vital to this reaction, as the rate of reaction increases with an increase in the volume of sulphuric acid. The observed negative value of entropy indicates the formation of the activated complex. The evidence for complex formation was verified by plotting a graph of $1/k_{obs}$ vs $1/[AML]$. LC-MS found the product of the reaction.

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Conflict of interest

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