



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

Yamuna Princy S¹, Vidyavati shastry*²

¹Department of chemistry, Sea College of engineering and technology, Virgonagar, Bangalore 49, India

²Head of Department, Sea College of engineering and technology, Virgonagar, Bangalore 49, India

Article History:

Received on: 20 Jul 2020
Revised on: 20 Aug 2020
Accepted on: 27 Aug 2020

Keywords:

Drug,
Kinetic studies,
Oxidation,
Oxidation Product,
Reaction mechanism

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 [H₂SO₄]. There was no substantial effect on the rate of the reaction with KNO₃. The reaction stoichiometry shows one mole of amlodipine besylate consumes one mole of n-bromosuccinimide. The effect of temperature on the reaction rate was studied, and the activation parameters (E_a, ΔG, ΔH and ΔS) are calculated and tabulated. LC-MS technique was used to identify the oxidation product of amlodipine 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 × 10⁻⁶ in the rate equation is in good accordance with each other.



*Corresponding Author

Name: Vidyavati shastry

Phone: 7892726394

Email: vidyavati_kinetic@yahoo.co.in

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11iSPL4.4315>

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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 amlodipine 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-bromosuccinimide in 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 $\log [V_t]$ 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} mol dm⁻³, N-Bromosuccinimide from 2.0×10^{-3} to 4.0×10^{-3} mol dm⁻³ and sulphuric acid from 5.0×10^{-4} to 3.0×10^{-3} mol dm⁻³. A graph is plotted between $\log V_t$ vs. Time in seconds.

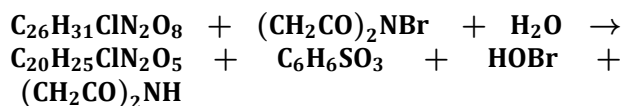
All the plots obtained in this method were as shown in (Figure 2) linear (Nadh and Sireesha, 2015) With the correlation coefficient of 0.9767 to 0.9876 as mentioned in Table 1.

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 24 hours 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.



Product analysis

The reaction mixture containing a required (amount of N-Bromosuccinimide 3×10^{-4} mol dm⁻³, 129 amlodipine besylate 1.75×10^{-3} mol dm⁻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 CCl₄. 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

Table 1: Effect of [NBS] on the rate of oxidation of Amlodipine besylate in H₂SO₄ 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
ΔH [#] (kJmol ⁻¹)	84.31
ΔS [#] (Jk ⁻¹ mol ⁻¹)	-250.6
ΔG [#] (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 H₂SO₄[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 _{cal} x 10 ⁻³ S ⁻¹
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

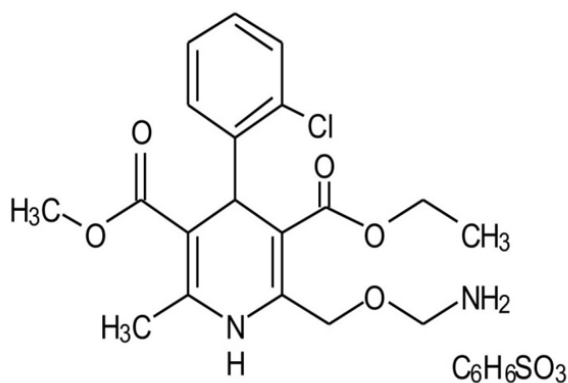


Figure 1: Chemical structure of amlodipine besylate

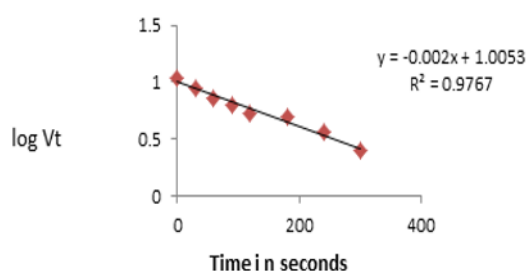


Figure 2: Plot of $\log V_t$ vs time in seconds

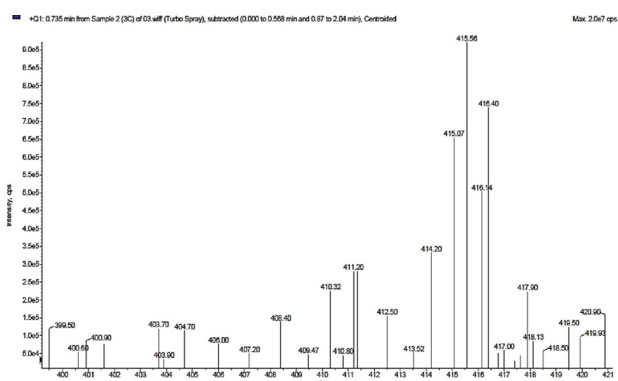


Figure 3: LCMS spectrum of the oxidation product of Amlodipine Besylate showing a molecular ion peak at 416.

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 amlodipine besylate, the concentration of the reductant was varied from 1.0×10^{-3} to $2.5 \times 10^{-3} \text{ mol dm}^{-3}$, keeping the constant concentration of NBS $3.0 \times 10^{-4} \text{ mol dm}^{-3}$ and Sulphuric

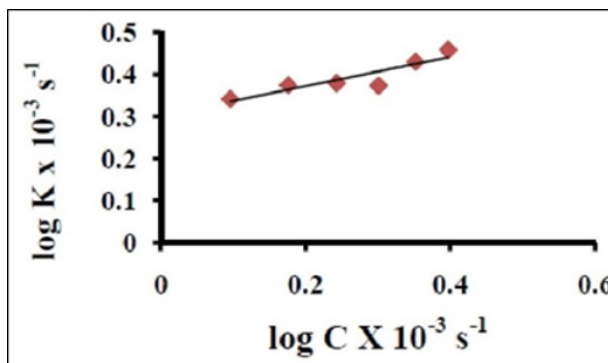


Figure 4: First order plot to study the variation of [AML] on reaction rate at a constant concentration of NBS, H_2SO_4 along with 10% KI and starch.

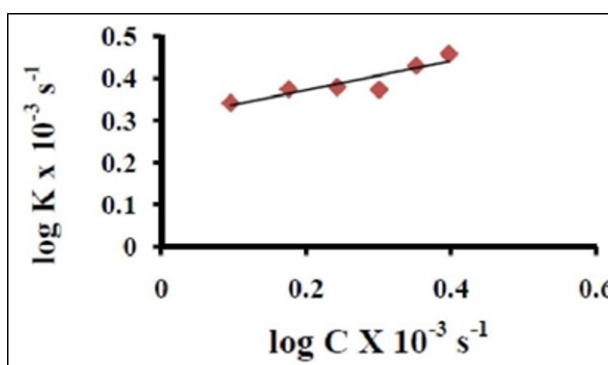


Figure 5: First order plot to study the variation of [NBS] on reaction rate at a constant concentration of AML, H_2SO_4 along with 10% KI and starch.

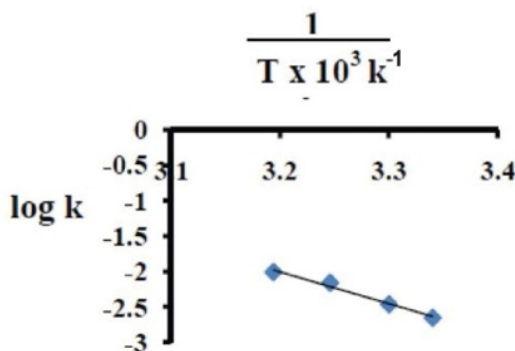
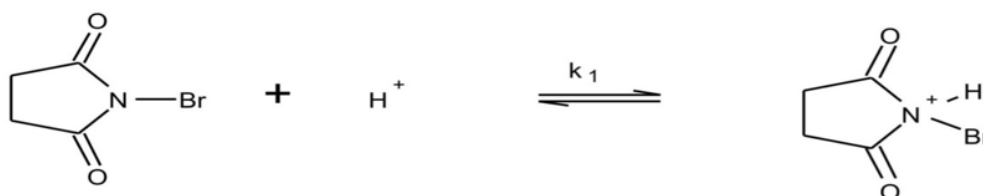
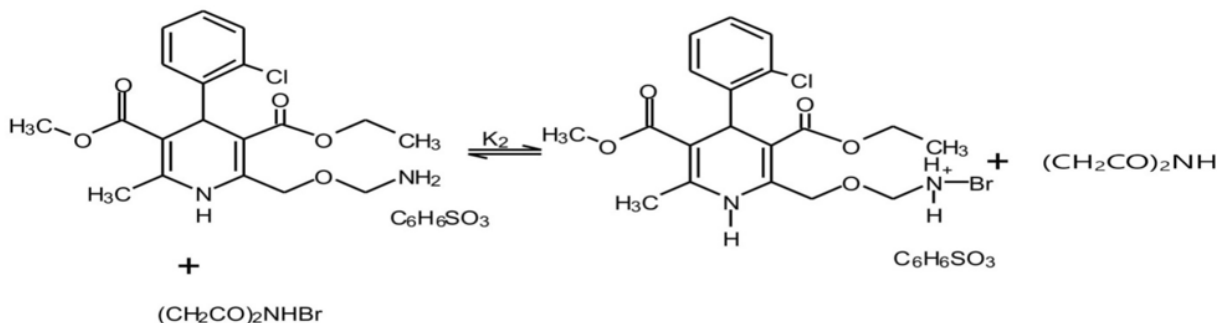
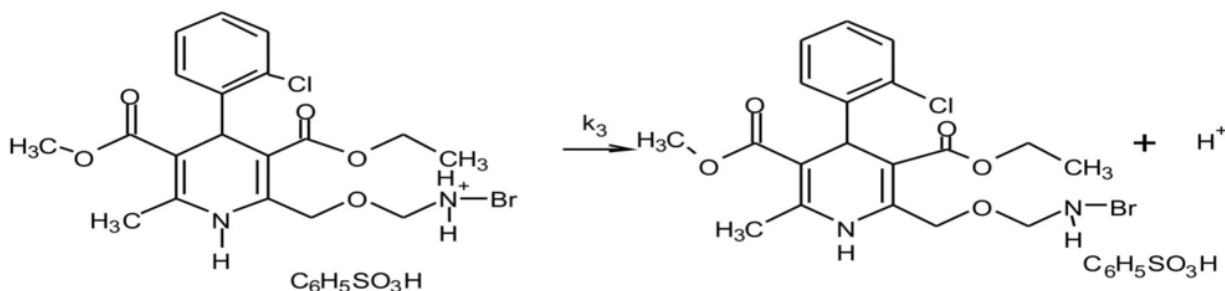
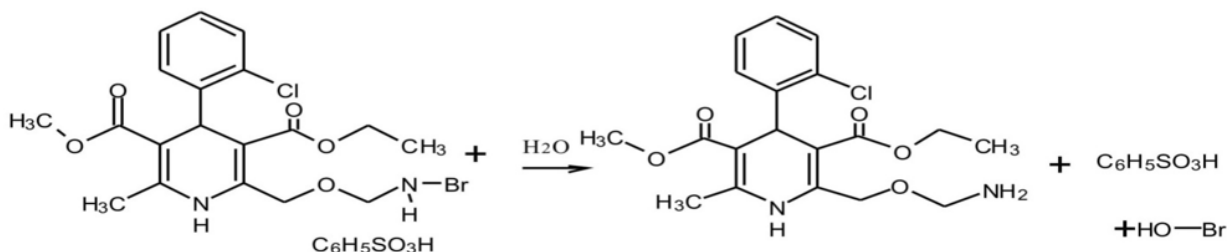
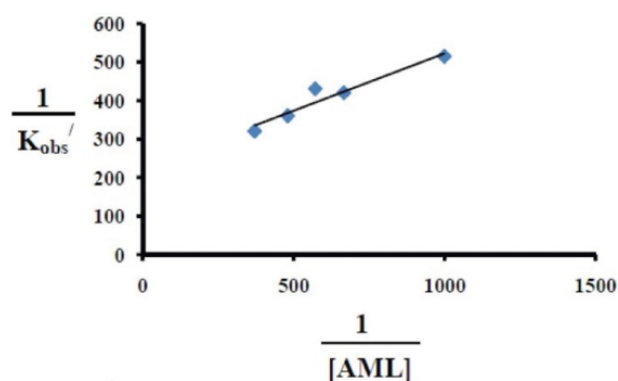


Figure 6: A Plot of $\log K$ VS $1/T$ at different temperatures.

acid. $1.5 \times 10^{-3} \text{ mol dm}^{-3}$ the value of k_{obs} increased with increase in AML concentration, and a plot of $\log K$ vs $\log [\text{AML}]$ was linear with $R^2 = 0.81$, indicating a fractional order dependence concerning to substrate amlodipine besylate as shown in (Figure 4).

Effect of N-bromosuccinimide

To determine the order concerning oxidant, N-

step : 1**Step -2****Step -3****Step :4****Figure 8: A detailed Mechanistic interpretation for the oxidation of AML with NBS.****Figure 7: A Plot of 1/k_{obs} vs 1/ [AML]**

bromosuccinimide study was conducted by varying the concentration of [NBS] from $2.0 \times 10^{-3} \text{ mol dm}^{-3}$ to $4.0 \times 10^{-3} \text{ mol dm}^{-3}$ at 299K at a constant concentration of [AML] $1.75 \times 10^{-3} \text{ mol dm}^{-3}$ and [Sulphuric acid] $1.5 \times 10^{-3} \text{ mol dm}^{-3}$. 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 studied by increasing concentration of $[\text{KNO}_3]$ from 0.1 to 0.3 mol dm^{-3} by keeping the constant concentration of [AML] $1.75 \times 10^{-3} \text{ mol dm}^{-3}$ [NBS] $3.0 \times 10^{-4} \text{ mol dm}^{-3}$ and sulphuric acid 0.5

mol dm^{-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 reaction was carried out by keeping the constant concentration of [AML] $1.75 \times 10^{-3} \text{ mol dm}^{-3}$ and [NBS] $3.0 \times 10^{-4} \text{ mol dm}^{-3}$ varying concentration of sulphuric acid from 5×10^{-4} to $3 \times 10^{-3} \text{ mol dm}^{-3}$. The rate of the reaction increases with an increase in the volume of sulphuric acid. A plot of $\log k'$ vs \log Sulphuric acid 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 313 K. When the reaction was conducted, the other experimental conditions and reaction concentration of [AML] $1.75 \times 10^{-3} \text{ mol dm}^{-3}$ [NBS] $3.0 \times 10^{-4} \text{ mol dm}^{-3}$ and [sulphuric acid] 0.5 mol dm^{-3} were kept constant. The rate constant of the reaction shows a proportional increase with an increase in temperature, as mentioned in Table 2. A Plot of $\log k$ vs $1/T$ as shown in (Figure 6) gave them activation parameters to justify the oxidation AML vs NBS in an acidic medium. Arrhenius equation expresses the variation of the reaction rate with temperature, its integral form is

$$k = A e^{-E_a/RT}$$

The integrated form of the Arrhenius equation is given by

$$\ln k = \ln A - E_a/RT$$

$$\log k = \log A - E_a/2.303RT$$

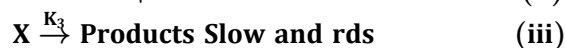
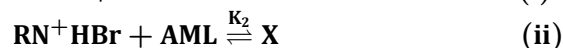
The above equation indicates the fact that a straight line is obtained when a graph is plotted between $\log k$ vs $1/T$. The slope is $-E_a/2.303RT$ and E_a activation energy is calculated easily. The activation parameters can be brought out using Arrhenius equation. The activation parameters are calculated and listed in Table 3.

$$\Delta H = E_a - RT; k = (k_B T/h) e^{\Delta S^\ddagger} e^{-\Delta H^\ddagger/RT}; \Delta G = \Delta H^\ddagger - T\Delta S^\ddagger$$

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 H_2SO_4 hence the protonated species of NBS, RN^+HBr is the most likely oxidising species as shown in step (i) of the reaction scheme. The

protonated species RN^+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).



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

$$\text{Rate} = -d\text{RNBr}/dt = K_3[\text{X}]$$

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

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

3. From step 1 of scheme

$$\text{RNBr} = [\text{RN}^+\text{HBr}] / K_1[\text{H}^+]$$

4. From step 2 of scheme

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

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

$$[\text{RNBr}]_t = [\text{RN}^+\text{HBr}]/K_1[\text{H}^+] + [\text{X}]/K_2[\text{AML}] + [\text{X}]$$

6. On rearranging equation 5

$$\text{X} = K_1 K_2 [\text{RNBr}]_t [\text{AML}][\text{H}^+] / 1 + K_1[\text{H}^+] + K_1 K_2 [\text{AML}][\text{H}^+]$$

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

$$\text{Rate} = K_1 K_2 k_3 [\text{RNBr}]_t [\text{AML}][\text{H}^+] / 1 + K_1[\text{H}^+] + K_1 K_2 [\text{AML}]$$

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

Since $\text{Rate} = k/[[\text{RNBr}]_t]$, under pseudo-first order conditions of $[\text{NBS}]_o \ll [\text{AML}]_o$, equation 7 can be transformed as eq8, eq9 and eq10 (Sukhdev and Puttaswamy, 2013).

$$8. \quad K_{obs} = K_1 K_2 k_3 [\text{AML}] [\text{H}^+] / 1 + K_1 [\text{H}^+] + K_1 K_2 [\text{AML}]$$

$$9. \frac{1}{K_{obs}} = \frac{1}{K_1 K_2 k_3 [AML][H^+]} + \frac{1}{K_2 k_3 [AML]} + \frac{1}{k_3}$$

$$10. \frac{1}{K'} = \frac{1}{K_2 k_3 [AML][H^+]} \left\{ \frac{1}{K_1 [H^+]} + 1 \right\} + \frac{1}{k_3}$$

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_2 was found to be $4.0 \times 10^{-3} s^{-1}$ and $0.1272 \text{ moldm}^{-3} s^{-1}$. The value of K_1 was found to be 1.96×10^{-6} . The experimental value tabulated in (Table 4) is calculated by substituting the value of k_3 , K_2 , K_1 in the rate equation. $K_{obs} = \frac{K_1 K_2 k_3 [AML][H^+]}{1 + K_1 [H^+] + K_2 [AML]}$. The k 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: $\text{rate} = k / [NBS]_0 [\text{Substrate}]^x [H^+]^y$. 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 between oxidant and substrate was obtained by the plot of $1/K_{obs}$ vs $1/[AML]$. LC-MS found the product of the reaction.

ACKNOWLEDGEMENT

The authors kindly acknowledge the support and encouragement of principal and management of SEA College of Engineering and technology, and also the generosity of CIPLA Industries Ltd for offering their API'S.

Funding report

The authors declare that they have no funding support for this study.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

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