Quantitative Kinetics of the Rapid Bromination of Isoxazole in Bromide-Free Aqueous Medium using Hydrodynamic Voltammetry

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Abstract

The kinetics of rapid bromination of isoxazole by molecular bromine at pH 4.7 have been studied quantitatively. Bromination of isoxazole is an electrophilic substitution reaction where the inductive effect and resonance effect govern reactivity and orientation respectively. Isoxazoles have a broad range of applications in pharmaceuticals and therapeutics. Hence the quantitative kinetic study will help in these applications. The bromination reactions are rapid electrophilic substitution reactions with a half-life of a few seconds, therefore the kinetic data regarding the bromination was lacking thus far. A distinct technique known as hydrodynamic voltammetry was used to perform the kinetics of the reaction. Only molecular bromine gets reduced on the working electrode during the reactions. As the solution lacked bromide ions, a decrease in the concentration of molecular Br_2 was measured with reference to diffusion current using a rotating platinum electrode (RPE). During the work, a saturated calomel electrode (SCE) was used as a reference electrode. Bromination of isoxazole followed second-order kinetics with an equal initial concentration of reactants. Thermodynamic parameters were obtained based on rate constants at five different temperatures. The obtained rate constants and activation energy were used to comment on the mechanism of the electrophilic substitution reaction. Thus, the present work quantitatively verifies the reactivity of the isoxazole in the bromination reaction.

Keywords: kinetics; hydrodynamic voltammetry; rate constants; bromination reaction; rotating platinum electrode.

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1. Introduction

Halogenation of aromatic compounds is a crucial reaction appearing in organic chemistry. Heterocyclic compounds have a cyclic structure with one or more heteroatoms in the ring[1]. The distinctive physicochemical properties and biological effects of five-membered heterocycles have placed them as key structural elements in numerous clinically important drugs[2,3]. Hence, the exploration of five-membered aromatic heterocycles remains an important research area in medicinal chemistry, intending to discover new therapeutic agents for various diseases[4-6]. Kinetics of bromination and chlorination of pyrrole and some substituted pyrrole possessing nitrogen as heteroatom has been studied previously[7]. Relative reactivity of some five-membered aromatic heterocycles in bromination reactions by molecular bromine and N-bromosuccinimide was reported in aqueous medium using kinetics as an investigational tool[8]. Structural effects on the bromination rate and selectivity of alkylbenzenes and alkoxybenzenes in aqueous solution have been studied to show aqueous free bromine species like HOBr, BrCl, Br_2, BrOCl, Br_2O and H_2OBr^+ can react with activated aromatic compounds via electrophilic aromatic substitution[9]. The quantitative kinetic data regarding the bromination reactions of five-membered aromatic heterocycles with two heteroatoms by different halogenating reagents in aqueous medium is scanty. The kinetics of the bromination of isoxazole by molecular bromine (Br_2) in aqueous medium in the absence of bromide ions was lacking in the literature, which is quantitatively assessed in the present study. The study reveals the role of reagent in the reaction mechanism. The use of aqueous medium reinforces the green chemistry principle.

Isoxazole is a five-membered heterocyclic ring having oxygen and nitrogen atoms at the 1 and 2 positions[10]. Many biologically active products contain derivatives of these heterocyclic compounds[11]. Derivatives containing isoxazole motifs possess biological activities such as anticancer, anti-inflammatory, antibacterial, antioxidant, insecticidal, antifungal, and antidiabetic[12]. Isoxazoles have unique electron-rich aromatic structures and have received much attention[13]. They make potential candidates for ring cleavage because of their weak nitrogen-oxygen bonds and aromatic character[13]. A quantitative kinetic study of the bromination of isoxazole will assist in all of these applications.

Bromination of five-membered aromatic heterocycles with two heteroatoms is an electrophilic substitution reaction where the inductive effect and resonance effect play important roles in manifesting orientation and reactivity[14]. Bromination of isoxazole by molecular bromine in aqueous medium containing bromide ions (Br^-) is a slow reaction as tribromide (Br^{3-}) formed

is a weak electrophile[15]. Hence molecular bromine will be the only brominating reagent. These bromination reactions have a half-life of a few seconds which require special techniques such as competition technique, relaxation technique, pulse radiolysis, femtochemistry technique, attosecond technique[16]. The present work makes use of an electroanalytical technique known as hydrodynamic voltammetry[17-20] to study the kinetics of the bromination reactions of isoxazole considering their rapidity. The technique allows the use of low concentrations of substrate and reagent solutions i.e. up to 10^{-6} M. Diffusion current in hydrodynamic voltammetry is obtained due to the transport of reagent to the electrode by convection which helps to remove the interference from natural convection that can complicate measurements in stagnant solutions. The reactions are uncatalyzed. Bromination reactions of isoxazole at five temperatures and pH 4.7 will be carried out in aqueous medium.

The rate constants of the bromination reactions will help in determining the nucleophilicity of substrate, steric constraints and thermodynamic parameters such as the energy of activation, enthalpy of activation, entropy of activation and Arrhenius factor. The bromination reaction under study is shown in Fig.1.

2. Experimental

2.1 Chemicals

Stock solutions of isoxazole, molecular bromine (Br_2) and potassium nitrate (KNO_3) were prepared. The stock solutions were diluted as per the requirement during the experiment. All chemicals used were of analytical grade. Distilled water was used for the preparation of solutions. Using starch indicator, the Br_2 solution was standardized by iodometric titration against standard sodium thiosulphate. Molecular bromine was kept in amber-colored ground glass stoppered bottles to prevent exposure to sunlight. Sodium acetate and acetic acid buffer were used to maintain pH 4.7 throughout the kinetic study.

2.2 Instrumentation

Diffusion current was measured using a moving coil mirror galvanometer with a lamp and scale arrangement. A rotating platinum electrode (RPE) is employed as a working electrode and a saturated calomel electrode (SCE) as a reference electrode. The RPE in the glass tube has platinum wire at the tip. To bring about a convection RPE is rotated with the help of a motor having a speed of 600 rpm. The reduction potential of Br_2 is 1.09 V. A constant potential of 0.2 V is applied to the RPE through a potentiometer. At this applied voltage shift, the reduction

potential of Br_2 with the decrease in concentration is negligible. RPE used as a cathode is connected to the positive terminal of the potentiometer while SCE is to the negative terminal. A constant potential is applied during the kinetic study using a potentiometer. Br_2 is reduced at the working electrode generating the diffusion current measured in terms of lamp and scale galvanometer. A shunt maintains the diffusion current within the boundary of the galvanometer scale. The galvanometer deflection was measured on the scale, where 1cm corresponds to 1 nA. Electrochemical reactions taking place at the electrodes are:

$$Br_2 + 2e^- \longrightarrow 2Br^- \qquad \text{At RPE}$$

$$2Hg + 2Cl^- \longrightarrow Hg_2Cl_2 + 2e^- \qquad \text{At SCE}$$

The electrochemical cell was maintained at a constant temperature using a thermostat throughout the kinetic study.

2.3 Calibration of diffusion current

The diffusion current for a series of Br_2 solutions at five temperatures and pH 4.7 was calibrated. KNO_3 was used as a supporting electrolyte to eliminate the contribution of migration current to convection current, which helps in linearizing current at RPE. Both electrodes were dipped in a 50 cm^3 solution of 5×10^{-4} M Br_2. Sodium acetate and acetic acid buffer was used to maintain pH of 4.7 during the calibration. The galvanometer light spot was adjusted to zero deflection on the scale for the KNO_3 solution. Diffusion current was then measured for 5×10^{-4} M molecular bromine solution which was constituted of 5×10^{-2} M KNO_3 solution and buffer components. With the help of a shunt, the galvanometer deflection for the solution was adjusted to show the maximum value. Similarly, diffusion current was measured for remaining bromine concentrations keeping the shunt constant (Table 1). Calibration of the diffusion current at five different temperatures and pH 4.7 is shown in Fig.2.

2.4 Kinetic measurements

A quantitative kinetic assessment of the bromination of isoxazole by molecular bromine was carried out. Isoxazole solution and molecular bromine solution of the same concentrations were taken in separate beakers and kept in a thermostat. Supporting electrolyte and buffer components were also kept in the thermostat. All components were mixed in a reaction vessel after attaining a constant temperature. A stopwatch was started when the molecular bromine solution was mixed with the substrate solution. The concentration of substrate and reagent was 5×10^{-4} M in the 50 cm^3 mixture in the reaction vessel. The diffusion current was recorded every 20 seconds. The measurements were taken thrice for the precise results and the standard deviation for the measurements was determined. Corresponding concentrations of molecular

Br_2 were evaluated from the calibration curve. A similar procedure is repeated for five different temperatures maintaining a constant pH of 4.7 (Table 2-6).

3. Results and Discussion

The calibration of the diffusion current for the reagent was linear (Fig.2). A straight-line graph of $1/[Br_2]$ vs time was obtained for the uncatalyzed bromination reactions of isoxazole by Br_2 (Fig.3). The nature of the graph confirms that the reaction follows second-order kinetics in the aqueous medium. The corresponding rate equation can be written as

$$\text{Rate} = k\,[\text{Isoxazole}]\,[Br_2] \quad (1)$$

The rate constants for the bromination of isoxazole at five temperatures are compiled in Table 7.

According to the thermodynamical formulation of rate constants, the equilibrium between reactants and activated complex may be manifested in terms of thermodynamical functions[21]. Thermodynamic parameters are determined from the Arrhenius plot (Fig. 4) using rate constants (Table 8).

The energy of activation is calculated using

$$Ea = -2.303 \times R \times \text{slope of Arrhenius plot} \quad (2)$$

Pre-exponential Factor (A) is calculated using

$$k = A.\, e^{Ea/RT} \quad (3)$$

The entropy of activation (ΔS^*) was calculated using

$$k = e^2(k_BT/h)(e^{\Delta S^*/R})(e^{-Eexp/RT}) \quad (4)$$

Where,

k = Rate constant for the reaction

T = Temperature

k_B = Boltzmann's constant,

h = Planck's constant,

E_{exp} = Experimental energy of activation

R = Gas constant.

The negative value of entropy of activation obtained for the bromination of isoxazole indicates that the reaction follows an associate mechanism in which a single activated complex is formed. The enthalpy of activation was calculated using

$$E_{exp} = \Delta H^* + 2RT$$

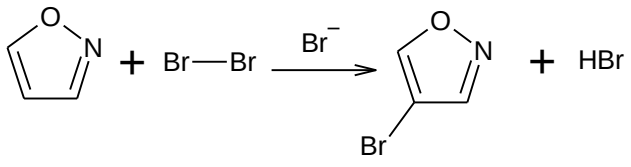
Molecular Br_2 undergoes polarization in the aqueous medium and bond dipole $Br^{\delta+}$- $Br^{\delta-}$ is formed. This dipole consists of one bromine atom with a formal positive charge ($Br^{\delta+}$) and

another bromine atom with a formal negative charge ($Br^{\delta-}$). The delocalized π-electron cloud of the isoxazole attacks the positive end of the dipole ($Br^{\delta+}$) to form a resonance-stabilized carbocation which is a slow step. The negative end of the reagent ($Br^{\delta-}$) abstracts the proton and the substrate molecule regains aromaticity in the second step. The second step is faster as compared to the first step. This is the arenium ion mechanism[22-24]. Fig.4 shows the mechanism of bromination of isoxazole by molecular bromine. The deactivating effect of the annular nitrogen decreases the reactivity of isoxazole than furan and pyridine in electrophilic substitution[25]. Electrophilic substitution of bromine takes place at the C_4 position on isoxazole leading to the formation of 4-bromoisoxzole[26,27]. The formation of the monosubstituted product was confirmed by thin-layer chromatography where solvents used were ethanol and n-hexane in 4:6 ratio.

4. Conclusions

The bromination of isoxazole by molecular bromine in aqueous medium is of second order reaction. Rate constants and thermodynamic parameters for the rapid bromination of isoxazole by molecular bromine are determined at five different temperatures and pH 4.7. Hydrodynamic voltammetry was successfully optimized for the study of rapid kinetics of the halogenation reaction. The kinetic observations follow the stated mechanism. Green chemistry principles are followed during the study since very dilute solutions and lower concentrations of reagents are used.

Figures:



Isoxazole **4- bromoisoxazole**

Fig. 1: Bromination of Isoxazole

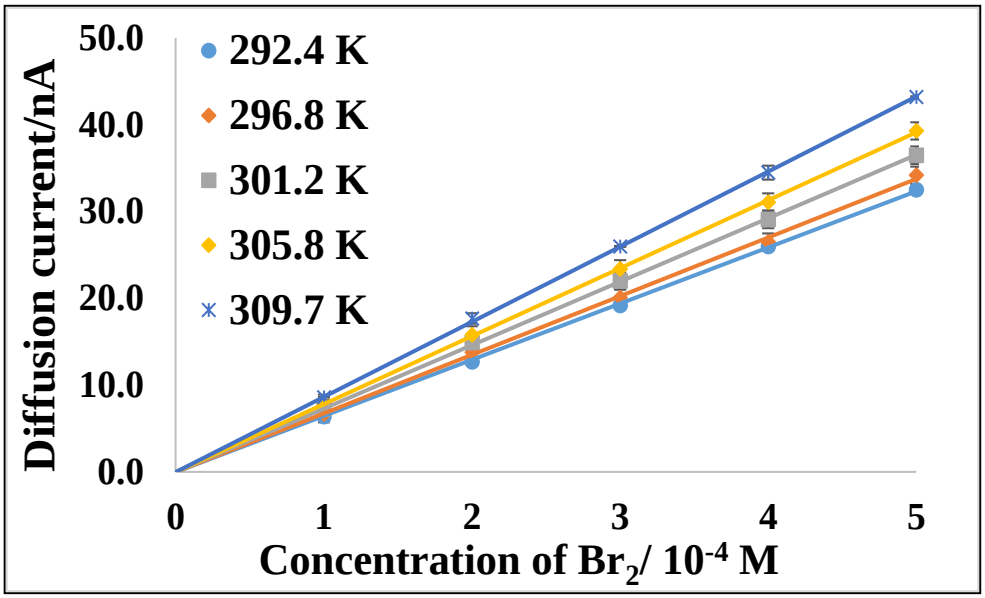


Fig. 2: Calibration of diffusion current for Br_2 at five temperatures and pH 4.7

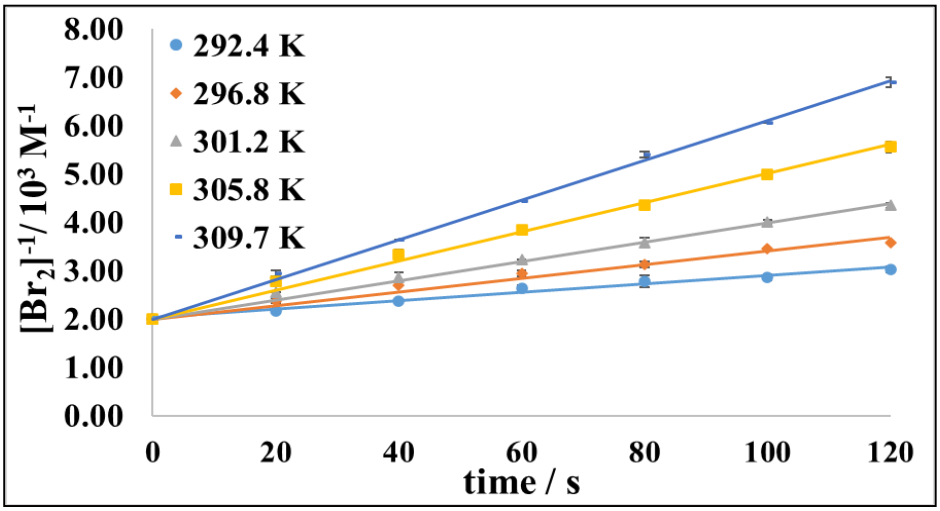


Fig. 3: Kinetics of the bromination of isoxazole by molecular Br_2 at five **temperatures and pH 4.7**

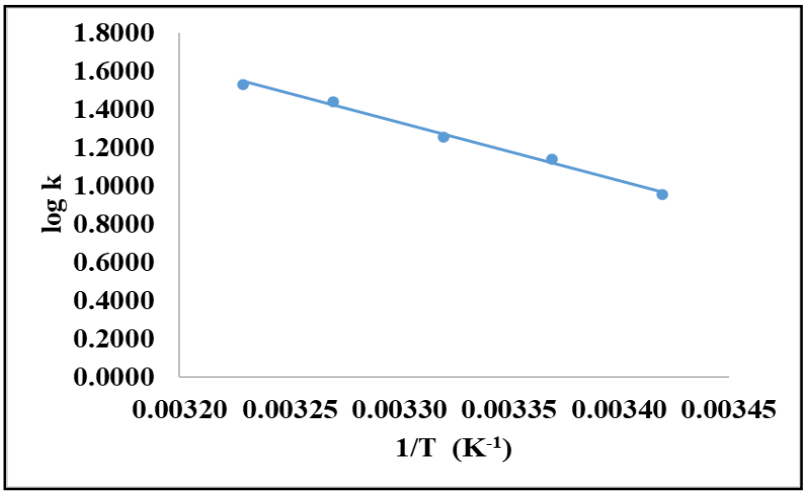


Fig. 4: Arrhenius plot for the iodination of isoxazole by Br_2

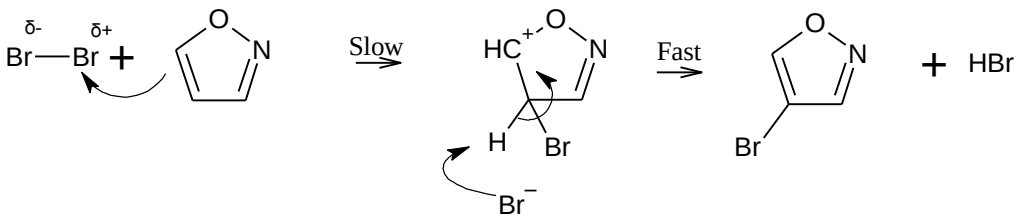


Fig. 5: Mechanism for the bromination of isoxazole by molecular Br_2

Tables:

Table 1: Calibration of diffusion current for Br_2 at five temperatures and pH 4.7

[Br_2]/ 10-4 M	**Mean diffusion current at various temperatures/ nA**				
	292.4 K	296.8 K	301.2 K	305.8 K	309.7 K
1	6.4	6.7	7.3	7.9	8.6
2	12.7	13.8	14.9	15.8	17.7
3	19.2	20.1	22.0	23.4	26
4	26.0	26.5	29.1	31.1	34.5
5	32.5	34.2	36.5	39.3	43.2

Table 2: Kinetics of bromination of isoxazole by Br_2 at 292.4 K and pH 4.7

Time /s	Diffusion current/nA				Standard deviation	Standard error	$[Br_2]/10^{-4}$ M	$[Br_2]^{-1}/10^3$ M^{-1}
	I	II	III	mean				
0	32.5	32.5	32.5	32.5	0.00	0.00	5.00	2.00
20	29.6	29.6	29.6	29.6	0.00	0.00	4.50	2.22
40	27.2	27.3	27.2	27.2	0.06	0.03	4.20	2.38
60	25.7	25.8	25.9	25.8	0.10	0.06	3.90	2.56
80	23.2	23.4	23.2	23.3	0.12	0.07	3.60	2.78
100	21.6	21.6	21.6	21.6	0.00	0.00	3.40	2.94
120	19.7	19.6	19.6	19.6	0.06	0.03	3.20	3.13

Table 3: Kinetics of bromination of isoxazole by Br_2 at 296.8 K and pH 4.7

Time /s	Diffusion current/nA				Standard deviation	Standard error	$[Br_2]/10^{-4}$ M	$[Br_2]^{-1}/10^3$ M^{-1}
	I	II	III	mean				
0	34.2	34.2	34.2	34.2	0.00	0.00	5.00	2.00
20	28.1	28.1	28.2	28.1	0.06	0.03	4.20	2.38
40	25.2	25.2	25.2	25.2	0.00	0.00	3.80	2.63
60	23.9	23.9	23.8	23.9	0.06	0.03	3.40	2.94
80	21.1	21.3	21.3	21.2	0.12	0.07	3.10	3.23
100	19.1	19.1	19.1	19.1	0.00	0.00	2.90	3.45
120	18.3	18.3	18.4	18.3	0.06	0.03	2.70	3.70

Table 4: Kinetics of bromination of isoxazole by Br_2 at 301.2 K and pH 4.7

Time /s	Diffusion current/nA				Standard deviation	Standard error	$[Br_2]/10^{-4}$ M	$[Br_2]^{-1}/10^3$ M^{-1}
	I	II	III	mean				
0	36.5	36.5	36.5	36.5	0.00	0.00	5.00	2.00
20	28.2	28.2	28.2	28.2	0.00	0.00	3.90	2.56
40	26.9	26.8	26.7	26.8	0.10	0.06	3.50	2.86
60	23.5	23.5	23.5	23.8	0.58	0.33	3.10	3.23
80	21.7	21.8	21.9	21.8	0.10	0.06	2.80	3.57
100	18.2	18.3	18.3	18.3	0.06	0.03	2.50	4.00
120	17.3	17.4	17.4	17.4	0.06	0.03	2.30	4.35

Table 5: Kinetics of bromination of isoxazole by Br_2 at 305.8 K and pH 4.7

Time /s	Diffusion current/nA				Standard deviation	Standard error	$[Br_2]/10^{-4}$ M	$[Br_2]^{-1}/10^3$ M^{-1}
	I	II	III	mean				
0	39.3	39.3	39.3	39.3	0.00	0.00	5.00	2.00
20	28.2	28.2	28.3	28.2	0.06	0.03	3.60	2.78
40	23.6	23.8	23.7	23.7	0.10	0.06	3.00	3.33
60	20.4	20.5	20.5	20.5	0.06	0.03	2.60	3.85
80	19.0	19.0	19.0	19.0	0.00	0.00	2.30	4.35
100	15.3	15.3	15.3	15.3	0.00	0.00	2.00	5.00
120	14.2	14.0	14.0	14.1	0.12	0.07	1.80	5.56

Table 6: Kinetics of bromination of isoxazole by Br_2 at 309.7 K and pH 4.7

Time /s	Diffusion current/nA				Standard deviation	Standard error	$[Br_2]/10^{-3}$ M	$[Br_2]^{-1}/10^3$ M^{-1}
	I	II	III	mean				
0	43.3	43.3	43.3	43.3	0.00	0.00	5.00	2.00
20	32.1	32.1	32.2	32.1	0.06	0.03	3.40	2.94
40	25.3	25.3	25.3	25.3	0.00	0.00	2.75	3.64
60	20.5	20.5	20.5	20.5	0.00	0.00	2.25	4.44
80	17.2	17.3	17.2	17.2	0.06	0.03	1.85	5.41
100	14.8	14.8	14.8	14.8	0.00	0.00	1.65	6.06
120	12.8	12.7	12.9	12.8	0.10	0.06	1.45	6.90

Table 7: Rate constants for the bromination of isoxazole by molecular Br_2 at five different temperatures and pH 4.7

Temperature / K	292.4	296.8	301.2	305.8	309.7
Rate constants / M^{-1} s^{-1}	8.97	14.55	18.10	27.67	34.10

Table 8: Thermodynamic parameters for the bromination reaction

Energy of activation (Ea)	50.97 kJ/mol
Pre-exponential factor (A)	1.3742×10^{10} $M^{-1}s^{-1}$
Entropy of activation (ΔS^*)	-66.89 $Jmol^{-1}K^{-1}$
Enthalpy of activation (ΔH^*)	43.89 kJ/mol

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