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Original Article

Spectroscopic Study on Oxidation of Theobromine by Potassium Permanganate in Alkaline Medium

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ARTICLE INFO	ABSTRACT

The kinetics of oxidation of *Theobromine* by alkaline permanganate at a constant ionic strength of 0.05mol/dm³was studied spectrophotometrically. The reaction between permanganate and theobromine in aqueous alkaline medium exhibits 2:1stoichiometry. The reaction is of first order in substrate concentration and fractional order with respect to concentration of both permanganate and alkali. The pH influence indicated that the oxidation is base catalyzed. The reaction rate was found to increase with increasing ionic strength and dielectric constant of the medium. The proposed reaction mechanism involves the formation of a 1:1 intermediate complex between theobromine and an alkali-permanganate species in a pre-equilibrium step, which was confirmed by both spectral and kinetic evidence. The complex decomposes slowly in a rate-determining step, resulting in the formation of free radical. The free radical generated reacts with another alkali permanganate species in a subsequent fast step to yield the final products, which were identified by spot test and spectral studies. The reaction constants involved in the different steps of the reaction mechanism were calculated. The activation parameters with respect to the slow step of the mechanism were determined.

Keywords: Theobromine, spectrophotometrically, dielectric constant, free radical.

1. INTRODUCTION

Oxidation reduction reactions are very essential reaction in environment and are regarded as key transformers in the organic synthesis. Certain alkaloids present in our food items gets oxidises that give defensive power to body.

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Theobromine, formerly known as **xantheose**, is a bitter alkaloid of the cacao plant, with the chemical formula $C_7H_8N_4O_2$. It is found in chocolate, as well as in a number of other foods, including the leaves of the tea plant and the cola nut. It is classified as a xanthine alkaloid, which also include the similar compounds theophylline and caffeine.

Despite its name, the compound contains no bromine— *theobromine* is derived from *Theobroma*, the name of the genus of the cacao tree. This finds utility on cough, a common condition that effects the vast majority of people lives and airway sensory nerve function in humans. [1]
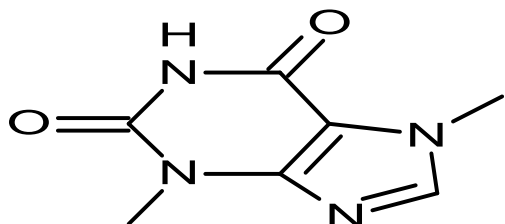
Fig 1: Chemical structure of Theobromine
MW: 180.17, Formula: $C_7H_8N_4O_2$

Theobromine has diuretic stimulant and relaxing effect. It can lower the blood pressure because it can dilate the blood vessels [2].

High blood pressure can adversely affect cardiac function, and the relaxation of smooth muscle tissue and vasodilation can help reduce the heart work-load.

Existence: Theobromine is in found in chocolate bar and molten chocolate. Chocolate is made from the cocoa bean, which is a natural source of theobromine. Cocoa powder can vary in the amount of theobromine from 2% to higher levels around 10%. It is also found in a variety of other plants like tea and guarana.

The structure Fig: 1, of theobromine is similar to that of Caffeine, a purine alkaloid is an antioxidant and radioprotector against the oxic pathway of radiation damage on catabolism generates other purine alkaloid, theobromine and finally uric acid [3].

The catabolic products of alkaloid caffeine exhibit both antioxidant and chemo preventive properties of caffeine-bearing beverages, such as tea [4].

Absorption spectral measurement is a simple but effective method in confirming the complex formation and structure [5]. The oxidant used in the present study, potassium permanganate is the most important, eco-friendly and powerful multi-electron, widely used oxidizing agent [6-17].

The medium conditions that is the pH of the medium governs the mechanisms [18].

By the literature survey we could understand that various alkaloid oxidation has been done by in acidic medium and the absence of literature for alkaline medium oxidation of alkaloid, made us curious for the present theobromine oxidation in alkaline $KMnO_4$ to happen.

2. EXPERIMENTAL

2.1 Materials and reagents

A stock solution of theobromine was prepared afresh by dissolving an appropriate amount of recrystallized sample in the required volume sodium hydroxide solution. Solutions of potassium permanganate were prepared and standardized [Vogel, 1967]

All other reagents were of analytical grade and their solutions were prepared by dissolving requisite amounts of the samples in double-distilled water. Sodium hydroxide solution was used to provide the required alkalinity. The ionic strength was maintained constant ($0.05mol/dm^3$) using KCl as an inert electrolyte.

Instrument used:

A systronics double beam UV-VIS spectrophotometer with compatibility was used to study the absorbance study, processing and data interpretation. Thermostat was used to maintain the temperature to an accuracy of $\pm0.5^oC$.

2.2 Kinetic measurements

Kinetic measurements were performed by monitoring absorbance changes using a spectrophotometer withfully thermostatted cell compartment. All kinetic measurements were performed in glass stoppered tubes coated black to eliminate photochemical effects. The reactions were carried out under pseudo first order conditions by taking a known excess of theobromine over permanganate concentration at 298K.

Appropriate amounts of theobromine, NaOH, KCl and water to keep the total volume constant were equilibrated at constant temperature.

A measured amount of permanganate solution also pre-equilibrated at thesame temperature was rapidly added to the mixture. The progress of reaction was followed by measuring the absorbance of unreacted permanganate in the reaction mixture present in 1 cm cell in a thermostatted compartment of a Systronics UV-Vis spectrophotometer at its maximum absorbance of 510nm as a function of time. The application of Beer's law to permanganate at 510nm is found out, giving =$2083\pm50dm^3mol^{-1}cm^{-1}$(literature =$2200dm^3mol^{-1}cm^{-1}$). It was verified that other constituents of the reaction mixture do not absorb significantly at thiswavelength. The pseudo-first-order rate constants, k_{obs} calculated from the linear plots of log(absorbance)*versus*time were reproducible within $\pm4\%$ and are average of at least three independent kinetic runs.

3. RESULTS AND DISCUSSION

3.1 Stoichiometry and product analysis

Different sets of concentrations of theobromine permanganate in 0.5 $moldm^{-3}$NaOH and at constant ionic strength, I = 0.05$moldm^{-3}$ were kept in a closed container under nitrogen atmosphere at 25^oC. After 24hour permanganate concentration was assayed by measuring the absorbance at510 nm. The results indicate that two moles of

permanganate reacts with one mole of theobromine as shown in equation (1). The main reaction products were identified as uric acid and Mn(VI) .The formation of Mn(VI) is identified by visible spectrum and the uric acid is identified by LC-MS Analysis. Fig: 2 (LC MS ANALYSIS)

$$+2MnO_4^- + 2OH^- \longrightarrow +2MnO_4^{2-} + 2H_2O ------1$$
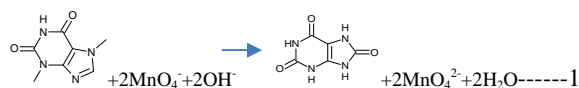
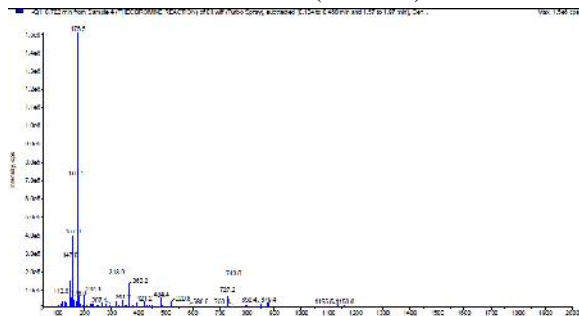THEOBROMINE PRODUCT(Uric acid)

Fig 2: LC-MS analysis of the product obtained for oxidation of theobromine with potassium permanganate

Reaction order

The slopes of $\log k_{obs}$ versus log (absorbance) graph when plotted by varying the concentration of theobromine, permanganate and alkali and in turn while keeping all other concentrations and conditions constants, the reaction orders can be determined.

3.2.1 Dependence of $KMnO_4$

The influence of permanganate concentration on the rate of reaction were studied by differing the concentration of MnO_4^- in the range of 0.5×10^{-4}-7.5×10^{-4} mol/dm^3 at fixed concentration of other reactants theobromine (1×10^{-2} moldm^{-3}), alkali(5×10^{-1} moldm^{-3})and constant ionic strength (5×10^{-2} moldm^{-3})and temperature 25°C. The plot of graph between log Absorbance versus time was found to be linear and the order with respect to [MnO$^-_4$] was found to be fractional order.

3.2.2 Dependence of theobromine

The concentration of theobromine was varied from 0.25×10^{-2} to 3×10^{-2} moldm^{-3} at fixed concentration of MnO_4 (0.25×10^{-3} moldm^{-3}),KCl (5×10^{-2} moldm^{-3}) and OH$^-$ (5×10^{-1} moldm^{-3}). The values of k_{obs} presented table clearly shows that the reaction is fractional order with respect to theobromine.

3.2.3 Dependence of NaOH

The reaction was carried out by varying the concentration of [OH$^-$] from 1×10^{-1}-10×10^{-1} moldm^{-3} at fixed concentration of KMnO$_4$ (2.5×10^{-4} moldm^{-3}), theobromine(1×10^{-2} moldm^{-3}) and KCl (5×10^{-2} moldm^{-3}). The rate constants of the reaction were found to increase with increase in [OH$^-$] [Table1]. A plot of log kobs versus log [OH$^-$] was linear with a slope of 0.7, suggesting that reaction to be fractional order with respect to [OH-] [Fig 3].
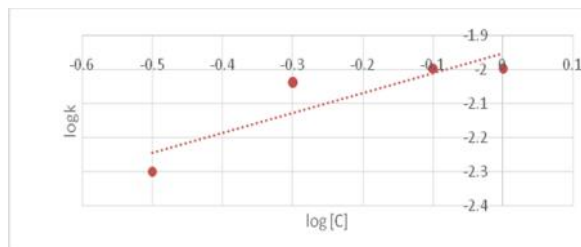

Fig 3: Effect of [OH$^-$] on the reaction between theobromine and permanganate

3.2.4 KCl dependence:

Ionic strength of reaction was studied by varying concentration of KCl from 1×10^{-2} – 10×10^{-2} moldm^{-3} at fixed concentration of KMnO$_4$ (2.5×10^{-4} moldm^{-3}), theobromine (1×10^{-2} moldm^{-3}) and OH$^-$ (0.01 moldm^{-3}) From rate constant values [Table 1] which clearly indicates, concentration of KCl has no significant effect on reaction rate.

3.3 Test for free radical (polymerisation study):

Involvement of free radical during oxidation by MnO$_4$ was studied by adding acrylonitrile [15, 16] followed by dilution with methanol resulted in perceptible precipitation, which clearly indicates reaction is intervention of free radical.

3.4 Effect of temperature:

Effect of temperature on reaction rate was studied at different temperatures (299-318K) at constant reaction condition and observed that the rate of reaction increased with increase in temperature [Table II]. The Arrhenius plot of log k versus 1/T gives straight line, from the slope and intercept, the experimental energy of activation E$_a$ and the frequency factor $\log_{10}A$ were calculated. The Eyrings parameters H$^\#$, S$^\#$ and G$^\#$ were calculated and tabulated [Table:III]

3.5 Effect of ionic strength

The effect of ionic strength and dielectric constant of the medium on the rate explains quantitatively the reaction between molecule and ion, with all these evidences, following reaction mechanism may be suggested which involve reaction of the active species of permanganate,[MnO$_4$.OH]$^{2-}$, with theobromine leading to the formation of a complex (C) in a prior equilibrium step.

[Theobromine] = 1×10^{-2} mol/dm^{-3}, [KMnO4] = 2.5×10^{-4} mol/dm^{-3}, [OH-] = 0.5mol/dm^{-3}, [KCl] = 0.05mol/dm^{-3}

Theobromine x10^{-2}mol/dm^3	[MnO$_4^-$]x10^{-4}mol/dm^3	[OH-]x10^{-1}mol/dm^3	KClx10^{-2}mol/dm^3	kobsx10^{-2}	k$_{cal}$x10^{-2}
0.25	2.5	5	5	0.66	0.66
0.5	2.5	5	5	0.71	0.7
1	2.5	5	5	.95	1.0
2	2.5	5	5	1.17	1.17
3	2.5	5	5	1.28	1.28
1	0.5	5	5	0.99	0.99
1	1.5	5	5	0.98	1.0
1	2.5	5	5	.95	0.95
1	5	5	5	0.95	0.95
1	7.5	5	5	0.94	0.94
1	2.5	1	5	0.51	0.51
1	2.5	3	5	0.82	0.82
1	2.5	5	5	0.95	0.5
1	2.5	7	5	1.0	1
1	2.5	10	5	1.5	1
1	2.5	5	1	0.51	0.5

| 1	2.5	5	2.5	0.7	0.51
1	2.5	5	5	0.95	.7
1	2.5	5	7.5	1	1
1	2.5	5	10	0.95	0.95

Table 2: Effect of variation of temperatures on reaction rate
[Theobromine] = 1×10^{-2} mol/dm^{-3}, [KMnO4] = 2.5×10^{-4} mol/dm^{-3}, [OH-] = 0.5 mol/dm^{-3}, [KCl] = 0.05 mol/dm^{-3}

Temperature in (K)	298	308	313	318
$k \times 10^{-2}$ (S^{-1})	0.9	1.75	2.5	3.35

Table 3: The activation parameters calculated for oxidation of theobromine with potassium permanganate in alkaline medium.

Activation parameters	Values
E_a (kJ/mol)	2.05
$H^{\#}$ (kJmol^{-1})	-3.8
$S^{\#}$ (J K^{-1}mol^{-1})	-303.5
$G^{\#}$ (kJmol^{-1})	76.11

It was reported [19,20] for permanganate ion in aqueous alkaline medium that the alkali combines with permanganate ion to form an alkali-permanganate species $[MnO_4.OH]^{2-}$ in a pre-equilibrium step as given by the following equilibrium

$$MnO_4^- + OH^- \xrightarrow{K1} [MnO_4.OH]^{2-} \qquad ----------2$$
Intermediate complex
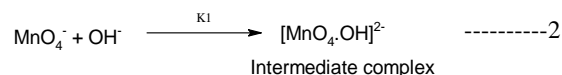
Where, K1 is the equilibrium constant for formation of alkaline species of the oxidant. This may be observed from the fractional order in OH$^-$. The formation of $[MnO_4.OH]^{2-}$ species in alkaline medium also supported by the Michaelis-Menten plot (1/kobs*versus* 1/[OH$^-$]), which is linear with a positive intercept (Fig:3).

Many studies [21-22] suggested that most of the oxidation reactions by permanganate ion in neutral and alkaline media proceed through intermediate complex formation between oxidant and substrate. The spectral evidence for the complex formation between the oxidant and substrate obtained from UV-Visspectra, as well as obeying the [sub] dependence of the rate constant to the Michaelis-Menten kinetics for the formation of intermediate complex(1/kobs*vs*.1/[sub] plot) as shown in Figure , suggest the formation of an intermediate complex. The effect of ionic strength and dielectric constant of the medium on the rate also explains quantitatively the reaction between molecule and ion, with these observations, reaction mechanism may be suggested which follows reaction of the active species of permanganate,$[MnO_4.OH]^{2-}$,with theobromine leading to the formation of a complex (C) in a prior equilibrium step.

$$C_7H_8N_4O_2 + [MnO_4.OH]^{2-} \xrightarrow{K2} [C_7H_8N_4O_2-MnO_4.OH]^{2-}$$
(C) $\qquad ---------------3$
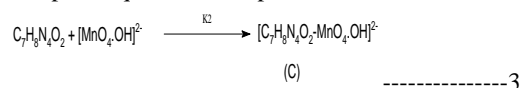
Where, K2 is the formation constant of the complex.
In the above complex, one electron is transferred from theobromine to permanganate ion. The decomoposition of

this complex in slow step forms of a free radical intermediate of theobromine and Mn(VI)

$$[C_7H_8N_4O_2-MnO_4.OH]^{2-} \xrightarrow{k_{slow}} C_6H_7N_4O_6 + MnO_4^{2-} + H_2O \qquad ----------4$$
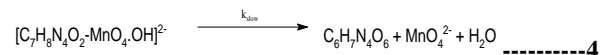
Thus intermediate free radical formed is rapidly attacked by the one more active species of the oxidant to form the final oxidation products

$$C_6H_7N_4O_6 + [MnO_4.OH]^{2-} \longrightarrow C_7H_8N_4O_6 + MnO_4^{2-} + H_2O \qquad -------------5$$
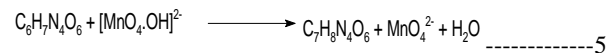
With reference to the above explained mechanism, the rate of disappearance of permanganate ion or formation of the intermediate complex can be expressed by the following rate law:

$$Rate = -d[MnO_4^-]/dt = k[C] \qquad ------------------------6$$

The rate of change complex formation with the change in substrate, and hydroxyl ion and oxidant concentrations can be deduced to give the following equation

$$Rate = \frac{kK1K2[sub][MnO_4^-][OH^-]}{1 + K1[OH^-] + K1K2[sub][OH^-]} \qquad ------------------------7$$

Under pseudo-first order conditions, large excess concentrations of substrate over [MnO$_4^-$], leads to the rate law given by

$$Rate = -d[MnO_4^-]/dt = k_{obs}[MnO_4^-] \qquad ----------------------8$$

After comparing and re-arranging Eqs. 7 and 8, we can write

$$\frac{1}{k_{obs}} = \frac{1+K1[OH-]}{kK1K2[OH-][Sub]} + \frac{1}{k} \qquad ----------------------9$$

To verify the rate law the equation can be rearranged to

$$\frac{1}{k_{obs}} = \frac{1}{kK1K2[OH-][Sub]} + \frac{1}{kK2[Sub]} + \frac{1}{k} \qquad ---------------- 10$$

According to Eq. (10), other conditions being constant, plots of 1/kobs*versus* 1/[OH-] and 1/kobs*versus* 1/[Sub] are linear and (Fig:3). The slopes and intercepts of these plots lead to the values of K1, K2, and k . The seconstants were used to calculate the rate constants over different experimental conditions .The k_{exp} & k_{obs} values are in good agreement as shown in table 1.
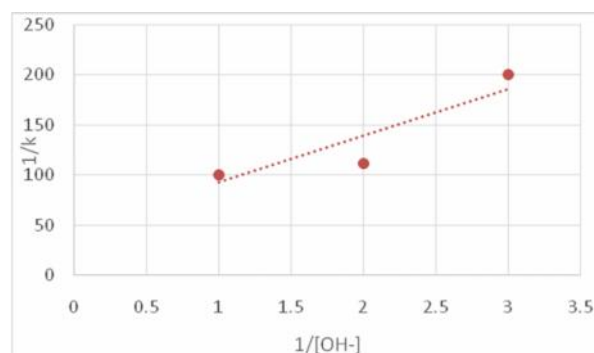
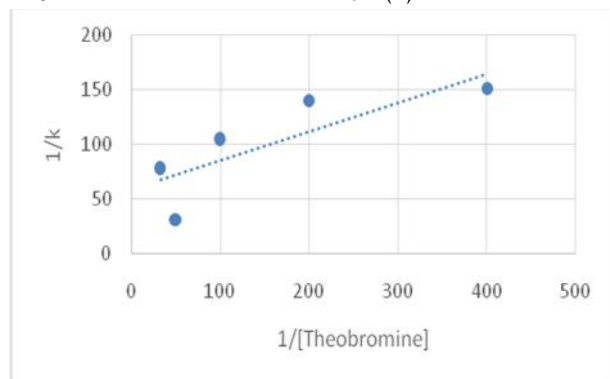
Fig 3: Michaelis-Menten plot1/kobs*versus* 1/[OH-]

Fig 4: 1/k$_{obs}$ vs 1/[Theobromine]

4. CONCLUSIONS

The reaction is studied in alkaline medium. The rate of reaction increases with increase in alkali concentration. The $[MnO_4.OH]^-$is active species for the reaction. Rate constants k, K1 &K2 is calculated. The rate constants at different temperatures are calculated, activation parameters are listed in table .The large negative value of entropy indicates the complex formation. A mechanism for the reaction is proposed & it is verified.

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