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Kinetic Study of Ascorbic Acid using Methylene Blue in the presence of Iron(II) in Acidic Medium

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ABSTRACT

The kinetics of oxidation of ascorbic acid using methylene blue were investigated in acidic medium by the spectrophotometric method within the temperature range of 30–70 °C. The kinetic measurements were conducted under pseudo–first-order conditions in which the concentration of ascorbic acid was maintained at least ten times higher than that of methylene blue. A 1:1 stoichiometric ratio was obtained for methylene blue and ascorbic acid consumed. The reaction followed first-order kinetics with respect to both the oxidant and the substrate. The results revealed that the oxidation rate increased with increasing ascorbic acid concentration, pH, temperature, acid concentration, and ionic strength, while an increase in methylene blue concentration resulted in a decrease in the rate. The Arrhenius activation energies in the absence and presence of Fe(II) ion were 38.29 kJ mol^{-1} and 19.15 kJ mol^{-1}, respectively. The reduction in activation energy confirms the catalytic role of Fe(II) ions.

Keywords: Methylene blue, Ascorbic Acid, Kinetics, Oxidation-Reduction, Qualitative analysis.

INTRODUCTION

Vitamin C (ascorbic acid) is a water-soluble antioxidant first isolated in 1928 by Szent-Györgyi. It is readily oxidized and easily degraded by alkali, oxygen, light, and heat [1]. Ascorbic acid functions widely as a reducing agent in biological and chemical environments [2]. The L - enantiomer (vitamin) undergoes oxidation to dehydroascorbic acid via two-electron processes, making it an important substrate for redox kinetic studies [3,4]. Ascorbic acid is an essential nutrient that is also found in fruits and vegetables and organ meat (e.g., liver and kidney) in variable quantities [2]. It is an organic acid with antioxidant properties in chemical and biological systems.

Understanding the kinetics of ascorbic acid oxidation is crucial for elucidating mechanistic pathways and optimizing reaction conditions [5,6]. Researchers have reported that transition metals significantly influence the oxidation pathway of antioxidants by altering electron-transfer routes. [7.8].

Methylene blue (MB^+) is a thiazine dye that undergoes reversible reduction to leucomethylene blue (LB^+) in acidic medium. Its redox behavior and electron-transfer capacity make it a useful probe in kinetic studies of reducing agents [9,10]. Existing literature has documented oxidation of ascorbic acid by inorganic oxidants, just as the redox behaviour of methylene blue with inorganic reductants. [5] Also, the influence of metal ions on dye decolorization was known [1,7,8]. However**, t**he catalytic effect of Fe(II) on the ascorbic acid–methylene blue redox system is scarcely reported. Little information also exists on how pH, ionic strength, and dye concentration affect this system.

This study aims to investigate the kinetics and mechanism of oxidation of ascorbic acid by methylene blue in an acidic medium. Specific objectives are: to determine the reaction order with respect to ascorbic acid and methylene blue; to evaluate the effects of acid concentration, ionic strength, and temperature; to determine activation and thermodynamic parameters; to assess the catalytic influence of Fe(II) ions on the reaction rate; and to confirm product formation using thin-layer chromatography.

MATERIALS AND METHODS

Materials

Ascorbic acid, Methylene blue, Iron(II) salt, HCL, UV-Visible Spectrophotometer (Model: Shimadzu UV-1800, Shimadzu Corporation, Japan).

Preparation of Solutions

Methylene blue stock: Prepared from accurately weighed dye and diluted to required concentrations.

Ascorbic acid stock: Prepared fresh before each experiment due to instability of solutions; Prepared to obtain required acid strengths (0.01 - 0.05 M); Ionic strength: Adjusted with NaCl solutions; and

Fe(II) catalyst: Prepared from $FeSO_4 \cdot 7H_2O$.

Procedure

Twenty reaction mixtures were prepared by mixing appropriate volumes of ascorbic acid, hydrochloric acid, and sodium chloride in a 1 cm path-length cuvette. The concentration of methylene blue was kept below 1% of the molar concentrations of both ascorbic acid and hydrogen ions to maintain pseudo-first-order conditions.

Methylene blue was added last to initiate the reaction. The decrease in absorbance was monitored at 665 nm, the λmax of methylene blue (molar absorptivity = 2000 $dm^3\ mol^{-1}\ cm^{-1}$). Each experimental parameter—ascorbic acid concentration, methylene blue concentration, acid concentration, ionic strength, temperature, and Fe(II) ion—was varied independently. Temperatures studied were 303, 313, 323, 333, and 343 K using a thermostated water bath.

Reaction Order and Pseudo-First-Order Behavior

Linear plots of ln(A) versus time confirmed pseudo-first-order behavior with respect to methylene blue, consistent with earlier work. [9,10] The rate law reduces to:

$$R = -k_2[\mathrm{MB}^+]$$

Thin Layer Chromatography Analysis

TLC was carried out on silica gel plates using ethanol–water (70:30) as mobile phase. Spots were visualized under UV light (254 nm). Rf values were compared with literature standards for ascorbic acid and dehydroascorbic acid.

RESULTS AND DISCUSSION

Table 1 presents the experimental concentrations used for varying ascorbic acid, methylene blue, hydrochloric acid, and ionic strength. The corresponding pseudo-first-order rate constants show: An increase in kobs with increasing ascorbic acid concentration (Set A); A slight decrease in kobs with increasing methylene blue concentration (Set B) increase in kobs with increasing acid concentration (Set C); and Minimal variation of kobs with ionic strength (Set D).

Table 1: Sample preparation and kinetic measurement condition for the analysis of methylene blue reduction

A_1	2.5×10^{-2}	Methylene Blue (M)	HCl (M)		$k_{observed}$ ($dm^3mol^{-1}s^{-1}$)
Set A: varying Ascorbic acid concentration [H_2A]					
		3×10^{-5}	1×10^{-1}	1×10^{-1}	-2.4×10^{-2}
A_2	4.5×10^{-2}	3×10^{-5}	1×10^{-1}	1×10^{-1}	-2.5×10^{-2}
A_3	6.5×10^{-2}	3×10^{-5}	1×10^{-1}	1×10^{-1}	-3.1×10^{-2}
A_4	8.5×10^{-2}	3×10^{-5}	1×10^{-1}	1×10^{-1}	-3.4×10^{-2}
A_5	10.5×10^{-2}	3×10^{-5}	1×10^{-1}	1×10^{-1}	-3.4×10^{-2}
Set B: varying Methylene concentration [MB^+]					
B_1	2.5×10^{-2}	1.0×10^{-5}	1×10^{-1}	1×10^{-1}	-1.8×10^{-2}
B_2	2.5×10^{-2}	2.0×10^{-5}	1×10^{-1}	1×10^{-1}	-1.8×10^{-2}
B_3	2.5×10^{-2}	3.0×10^{-5}	1×10^{-1}	1×10^{-1}	-1.9×10^{-2}
B_4	2.5×10^{-2}	4.0×10^{-5}	1×10^{-1}	1×10^{-1}	-1.9×10^{-2}
B_5	2.5×10^{-2}	5.0×10^{-5}	1×10^{-1}	1×10^{-1}	-1.9×10^{-2}
Set C: varying HCl concentration [H^+]					
C_1	4.0×10^{-2}	5.0×10^{-5}	1.0×10^{-2}	1×10^{-1}	-1.9×10^{-2}
C_2	4.0×10^{-2}	5.0×10^{-5}	2.0×10^{-2}	1×10^{-1}	-2.6×10^{-2}
C_3	4.0×10^{-2}	5.0×10^{-5}	3.0×10^{-2}	1×10^{-1}	-2.4×10^{-2}
C_4	4.0×10^{-2}	5.0×10^{-5}	4.0×10^{-2}	1×10^{-1}	-2.2×10^{-2}
C_5	4.0×10^{-2}	5.0×10^{-5}	5.0×10^{-2}	1×10^{-1}	-2.3×10^{-2}
Set D : varying NaCl concentration[Cl^-]					
D_1	2.5×10^{-2}	5.0×10^{-5}	1.0×10^{-2}	1.0×10^{-2}	-1.5×10^{-2}
D_2	2.5×10^{-2}	5.0×10^{-5}	1.0×10^{-2}	2.0×10^{-2}	-2.0×10^{-2}
D_3	2.5×10^{-2}	5.0×10^{-5}	1.0×10^{-2}	3.0×10^{-2}	-1.5×10^{-2}
D_4	2.5×10^{-2}	5.0×10^{-5}	1.0×10^{-2}	4.0×10^{-2}	-1.6×10^{-2}

Effect of Methylene Blue Concentration

Increasing methylene blue concentration produced a slight decrease in kobs (Fig. 1). This behaviour is consistent with substrate-limited redox reactions where excess oxidant stabilizes the oxidized dye species. [7,11].

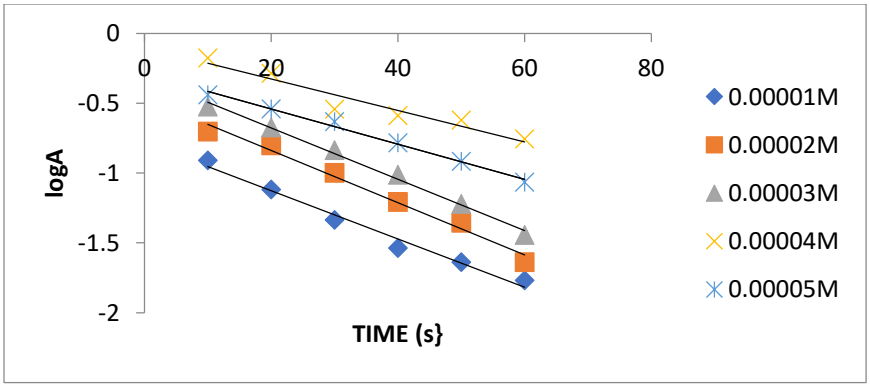

Fig 1: Plot of log A against time for the effect of varying methylene blue concentration in the absence of a catalyst

Effect of Ascorbic Acid Concentration

Increasing [H_2A] increased the pseudo-first-order rate constants (Fig. 2), confirming first-order dependence on ascorbic acid. This agrees with the reported behaviour of biological antioxidants. [4,12]

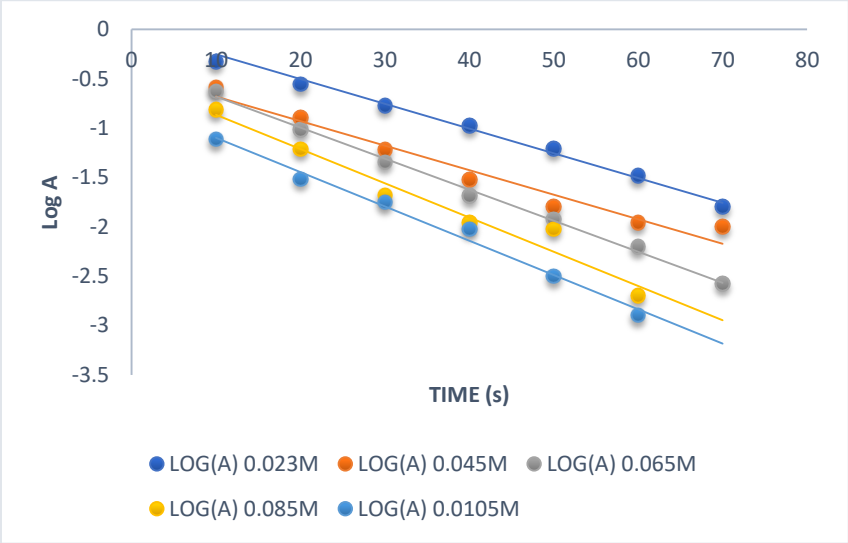

Fig. 2: Plot of log A against time for the effect of varying ascorbic acid concentration in the absence of iron (II)

This aligns with reported behavior of biological antioxidants. [13,14]

Effect of Acid Concentration (H^+/pH)

Increasing acid concentration decreased the rate (Fig. 3), indicating acid inhibition. Similar observations were reported for ascorbate oxidation by permanganate [12,13]

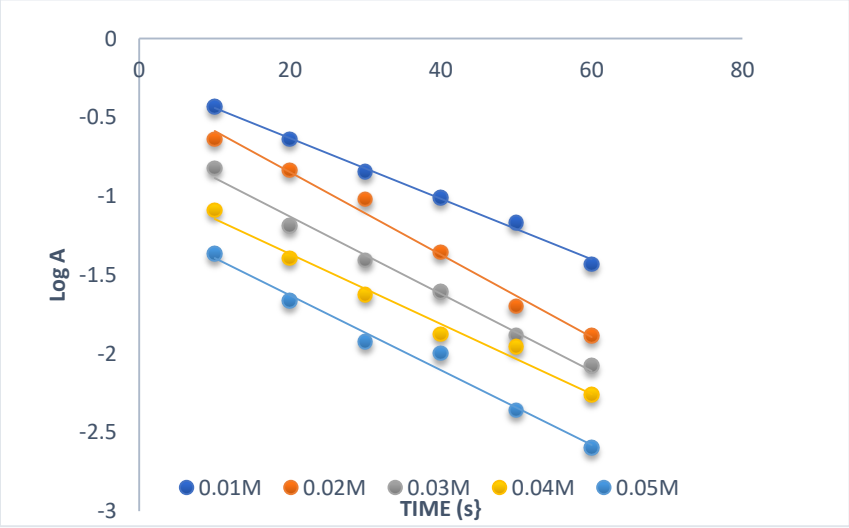

Fig. 3: A plot of log A against time for the Effect of varying [H^+] of the medium

Effect of Ionic Strength [Cl^-]

NaCl caused negligible changes in kobs (Fig. 4), showing that the rate-determining step is not significantly affected by ionic interactions, consistent with outer-sphere electron transfer. [15,16]

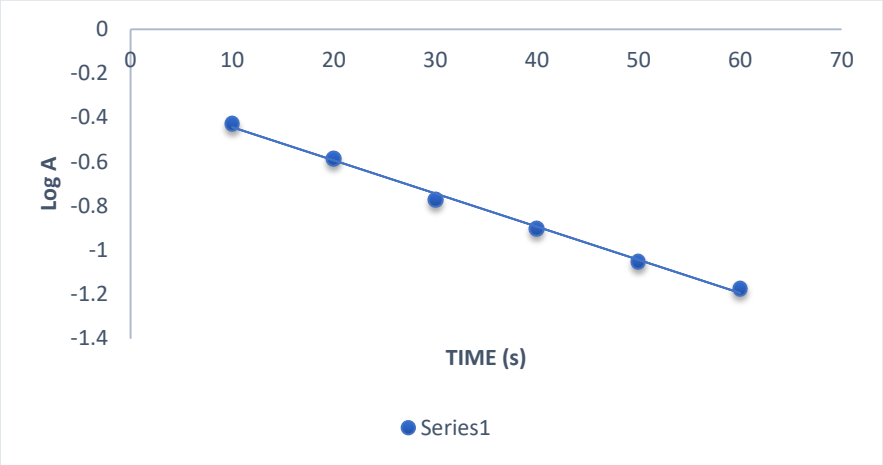

Fig. 4: A plot of log A against time for the effect of varying ionic strength concentration

Catalytic Effect of Fe(II)

Fe(II) markedly increased the reaction rate (Fig. 5). Activation energy decreased from 38.29 kJ mol^{-1} to 19.15 kJ mol^{-1} in its presence, strongly supporting catalytic action. [7,11]

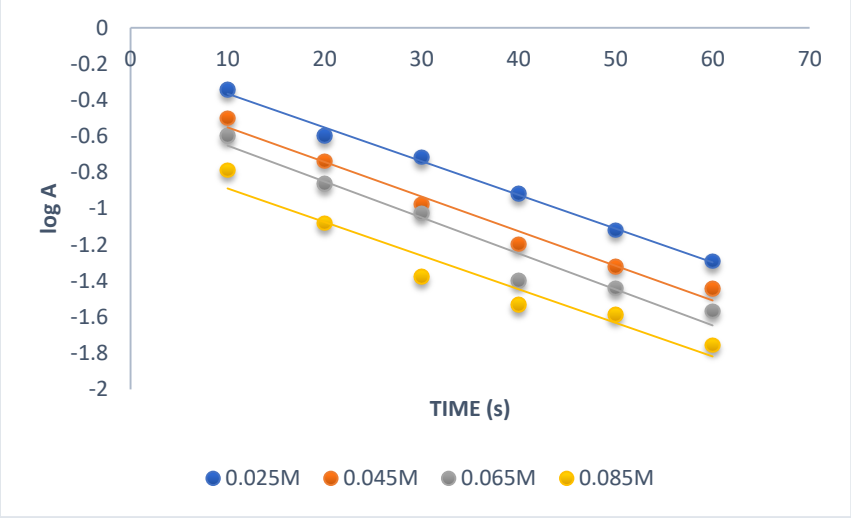

Fig 5: A plot of log A against time for the effect of iron (II) on the variation of Ascorbic Acid concentration

Effect of Temperature and Activation Parameters

The results in Figs. 6 and 7 show that increasing temperature increased rate constants, consistent with Arrhenius behavior.

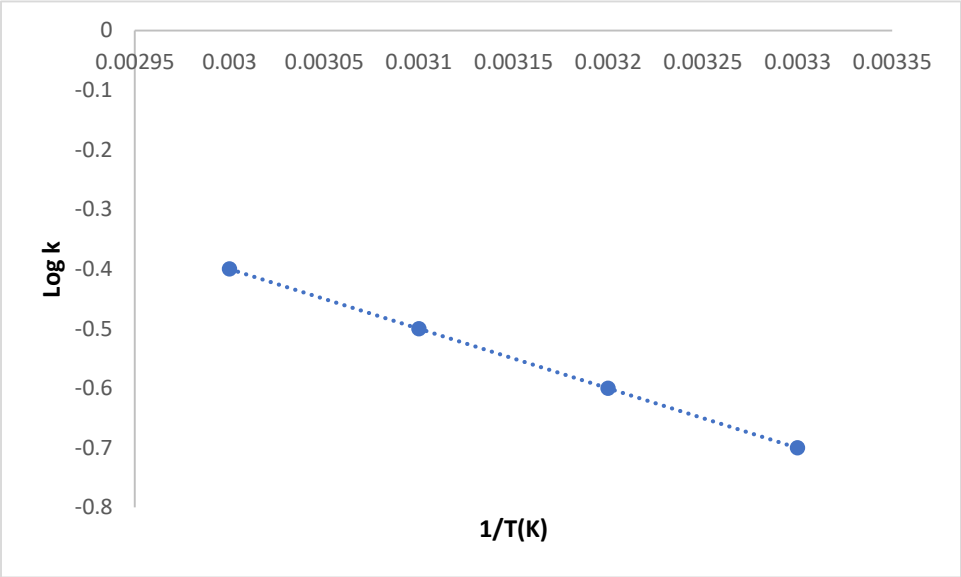

Fig 6: A plot of log k_1 against 1/T (K) for the effect of varying temperature in the absence of iron (II).

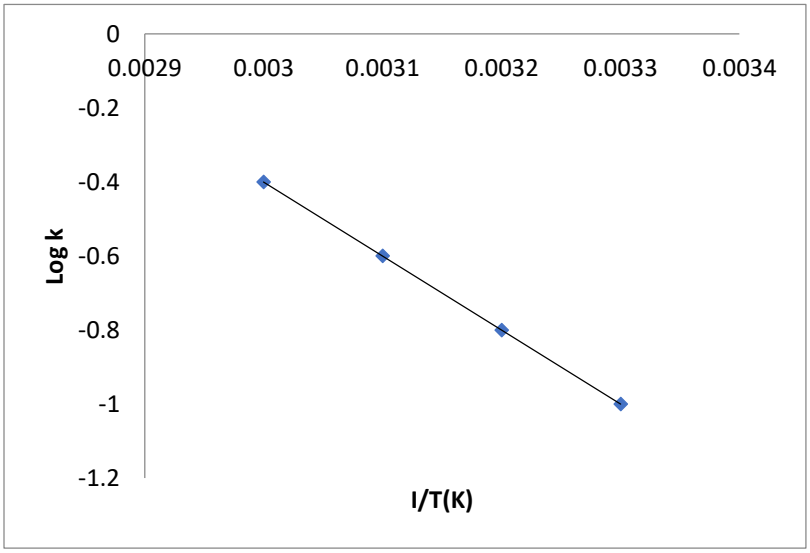

Fig 7: A plot of log k_2 against 1/T (K) for the effect of varying temperature in the presence of iron(II).

The values of the activation parameter are recorded in tables 3 and 4 for both reactions in the presence and absence of Fe(II). The thermodynamic activation parameters show negative entropy of activation (ΔS‡), indicating an ordered transition state typical of electron-transfer reactions. [4,15].

Table 2: Effects of temperature on the pseudo-first order rate constants for oxidation of ascorbic acid using Methylene Blue.

Temperature (K)	A ($dm^3mol^{-1}s^{-1}$)	B ($dm^3mol^{-1}s^{-1}$)
303	-0.7	-0.4
313	-0.6	-0.3
323	-0.5	-0.2
333	-0.4	-0.1
343	-0.3	0

Table 3: The Arrhenius and thermodynamic activation parameters for the oxidation of ascorbic acid in the presence of Fe (II) ions.

Ea. ($KJmol^{-1}$)	$\Delta H^{\#}$($KJmol^{-1}$)	$\Delta S^{\#}$ ($Jmol^{-1}K^{-1)}$
38.29	2.48	-137.8

Table 4: The Arrhenius and thermodynamic activation parameters for the oxidation of ascorbic acid in the presence of Fe (II) ions

Ea. ($KJmol^{-1}$)	$\Delta H^{\#}$($KJmol^{-1}$)	$\Delta S^{\#}$ ($Jmol^{-1}K^{-1}$)
19.15	2.5	-195.3

Thin Layer Chromatography Analysis

TLC revealed two main spots: unreacted ascorbic acid and dehydroascorbic acid. Rf values (0.73 - 0.84) align with literature data. Spot intensities suggested approximately 70% conversion to product**.** [16]

CONCLUSION

The oxidation of ascorbic acid by methylene blue proceeds via a pseudo-first-order pathway under acidic conditions. The rate is influenced by reactant concentration, pH, ionic strength, and Fe(II) catalysis. Activation energies and thermodynamic parameters support an electron-transfer mechanism with catalytic enhancement by Fe(II). TLC confirms formation of dehydroascorbic acid, with 70% of the reaction forming product. The rate of reaction tends to change while varying the concentrations of components in the mixture. The linear inverted plots further validate the pseudo-first-order nature of the reaction.

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