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A new approach for reduction of methylene green with ascorbic acid by de-oxygenation through carbon dioxide

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ABSTRACT

Reduction kinetics of the methylene green (MG) with ascorbic acid (AA) in acidic medium at λ_{max} 660 nm was monitored through visible spectrophotomtry in absence and presence of sodium carbonate. CO_2 release through reaction of sodium carbonate and oxalic acid, created de-oxygenated atmosphere for reduction of dye which greatly boosted the reaction rate. Initially slow reaction in presence of atmospheric oxygen proceeded rapidly when sodium carbonate was added. The reaction followed fractional order kinetics with AA and zero order kinetics with MG. The rate of reaction shows no linear dependence on [H^+] concentration as an acidic medium. The rate of reaction is directly related with the elevated concentration of salt, which suggests that the two same charged species are involved in the rate determining step. Secondary reactions at elevated temperature showed complex kinetics.

Keywords: Ascorbic Acid; Methylene Green; Sodium Carbonate; Charged Species; Secondary Reactions; Elevated Temperature

1. INTRODUCTION

Ascorbic acid (AA),a form of Vitamin C and water soluble which comes primarily from fruit and vegetables is an important micronutrient and plays many physiological roles. It occurs as l-ascorbic acid (AA) and its oxidized form, dehydro-l-ascorbic acid (DHAA), both of which are biologically active. The formula of which is $C_6H_8O_6$, behaves as a vinylogous carboxylic acid, where in the double bond ("vinyl") transmits, electron pairs between the hydroxyl and the carbonyl [1-2].

Cooper *et al.* [3] reported the photo electrochemical analysis of ascorbic acid in aqueous solution at a platinum channel electrode, using the dissolved phenothiazine dyes methylene blue and methylene green. This is achieved by measuring the current produced by immediate electro-oxidation of the reduced form of the dyes produced during the 1:1 photoreaction between the dyes and ascorbic acid induced by visible light of a wavelength of 620 - 625 nm. Strizhak [4] propose a kinetic scheme for reduction of methylene blue by ascorbic acid, characterized by the existence of two pathways: outer-sphere reduction of methylene blue according to a second-order reaction, and reduction through formation of a 1:1 complex with ascorbic acid. Strizhak [5] reported the reduction of methylene blue by ascorbic acid in the presence of copper (II) ions. The introduction of copper (II) ions increases the reaction rate, owing to the increased concentration of ascorbic acid radicals in the solution. It is shown that the inhibition of the reaction that is observed with low concentrations of copper (II) is a result of redox reactions proceeding in the system, involving oxygen dissolved in the water. Yusuf and Giirel [6] used methylene blue (MB), incorporated into titanium phosphate for determination of AA and showed that reaction can be helpful in some pharmaceutical products and are for the detection of AA as the procedure involving the reaction between triiodide and AA. AA has been determined by photo bleaching of methylene blue using continuous and FI systems, both procedures determination of AA based on its photooxidation sensitized by Toluidine Blue [7,8]. Cooper *et al*., [9] discussed the photoelectrochemical analysis of AA and decolorizing efficiency of AA to different synthetic dyes using Co/H_2O_2 system [10].

Detail literature search showed that few reports were available on the reduction of methlyene green with organic reductants but no reports were available on reduction of MG and AA in presence and absence of sodium carbonate in acidic medium. This procedure is shown to be a good alternative to routine vitamin C analysis in pharmaceutical preparations, fruit juices and soft drinks.

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2. EXPERIMENTAL

2.1. Materials and Methods

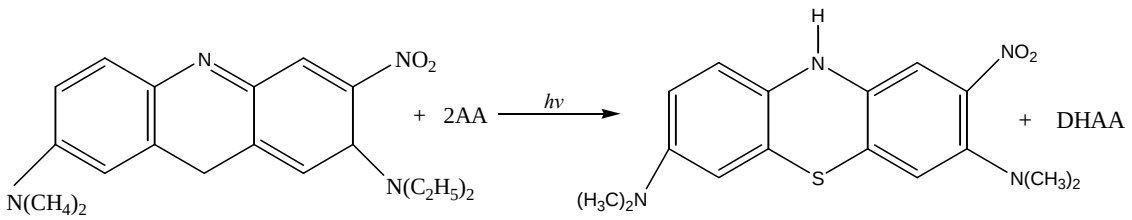
All reagents of pure quality (E-Merk) were used throughout the experiment. Dye stock solution of 3 × 10^{-5} mol·dm^{-3} was prepared in 100 ml of deionized distilled water. Solution of dye was preserved in polyethylene bottle. Stock solutions of ascorbic acid of 0.1 mol·dm^{-3}, Oxalic acid of 0.5 mol·dm^{-3}, Na_2CO_3 of 0.1 mol·dm^{-3}, KNO_3 of 2 mol·dm^{-3}, was prepared in distilled water

2.2. Kinetic Measurements

Five sets of mixture were prepared from stock solution. In each set one specie was varied while concentrations of other four were kept fixed. Each component were mixed together and portion was transferred to the cuvette (1 cm) to record a change in optical density of methylene green. Kinetic measurements were made with visible spectrometer 180 A at λ_{max} 660 nm of methylene green at room temperature in thermostatic water bath (FJPSO UK) [11,12].

2.3. Stoichiometry

The stoichiometry of the reaction mixture of MG and AA were determined by mixing the reagents in molar ratios of 2:1,1:1 and 1:2 with oxalic acid in excess. Quick de-coloration was observed in the bottle contain molar ratio in 1:2 as compared to other ratios showed that MG react with AA in 1:2 ratios in aqueous medium according to following equation [13].



methlyene green

leucomethylene green

3. RESULTS AND DISCUSSION

In the present work reduction of the methylene green with ascorbic acid were studied with variable concentrations of dye, ascorbic acid, and oxalic acid keeping one parameter varied and other constant in acidic medium in absence and presence of sodium carbonate [11,12,14]. The values of rate constant were determined through a plot of lnA vs time for each parameter concentration from the slope of line and reported in the **Tables 1**, **3-7**. A plot of log [dye] and log k were evaluated for each variable. It was observed that rate of reaction was very slow and took long time for complete de-colorization of the dye in absence of sodium carbonate according to following **Eqs.1-3**.

$$AH_2 \rightleftharpoons AH^- + H^+ \quad \text{fast} \tag{1}$$

$$MG + H^+ \longrightarrow MGH^+ \quad (\text{slow in absence of sodium carbonate}) \tag{2}$$

$$MGH^+ + e^- \xrightarrow{h\upsilon} MGH + A \tag{3}$$

The above reactions were significantly enhanced in presence of sodium carbonate with reduced de-coloration time. The total de-coloration time reduced to 5 min. while at the same pH value, but in the absence of sodium carbonate the total de-coloration was achieved in a time more than 60 min. Addition of sodium carbonate in reaction mixture results in the brisk effervescence of CO_2 (confirmed by using lime water which turn milky) with rapid de-coloration of dye indicating that CO_2 atmosphere helps in fast bleaching of the MG. The change in concentration of sodium carbonate proves that release of CO_2 in the reaction mixture affected the reduction kinetics of MG (**Table 1**) and showed that CO_2 displace the oxygen due to which equilibrium shift in forward direction with the formation of leuco dye [15]. The decline in the time of de-coloration due to the innovation of CO_2 during reaction by the addition of sodium carbonate throughout course of reaction is also supported by the early study of Gest and Stokes [16] who reported that the time required for the reduction of methylene blue was greatly increased by CO_2 removal when no substrates were added. Spectral changes for reduction of dye were recorded and presented in **Figure 1**. clearly showed that dye reduced without shift in wavelength in absence and presence of sodium carbonate.

Chemical oxygen demand (COD) decreases sharply with evolution of CO_2 which greatly influences the reduction of dye by providing the oxygen free atmosphere for reduction (**Table 2**). Reduced COD showed that CO_2 atmosphere helps in the reduction of MG through the movement of H^+ ion in the reaction mixture [16]. The

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Table 1. Effect of Sodium Carbonate (Na_2CO_3) on Rate of Reduction [MG^+] = 1 × 10^{-5} mol·dm^{-3}, [Ascorbic Acid] = 5 × 10^{-3} mol·dm^{-3}, [$H_2C_2O_4$] = 0.18 mol·dm^{-3}, [KNO_3] = 0.2 mol·dm^{-3}, [Na_2CO_3] = (1 - 5) × 10^{-2} mol·dm^{-3}, Temperature = 303 K.s.

Concentration of Na_2CO_3 × 10^2 mol·dm^{-3}	Velocity v × 10^5	Specific rate constant (k_{obs}) × 10^3 s^{-1}	1/(k_{obs})	% decoloration
1.0	–10 .00	1.0	1000	55.0
2.0	–3.00	1.2	833.33	60.48
3.0	–3.00	1.2	833.33	73.134
4.0	–3.00	3.2	312.5	81.223
5.0	–3.00	2.9	344.828	90.415

Table 2. Chemical Oxygen Demand in presence of sodium carbonate. [MG^+] = 1 – 5 × 10^{-5} M Mixture, [MG^+] = 1 × 10^{-5} M, [Ascorbic Acid] = 6 – 9 × 10^{-3} M, [$H_2C_2O_4$] = 0.06 – 0.3 M, [KNO_3] = 0.2 M, [Na_2CO_3] = 1 × 10^{-2} M, Temperature = 303 K.

[MG] × 10^5 mol·dm^{-3}	COD	Ascorbic Acid × 10^3 mol·dm^{-3}	COD
1.0	–307.2	5.0	585.6
2.0	–208	6.0	572.8
3.0	–195.2	7.0	572.8
4.0	–188.8	8.0	566.4
5.0	–256	9.0	579.2

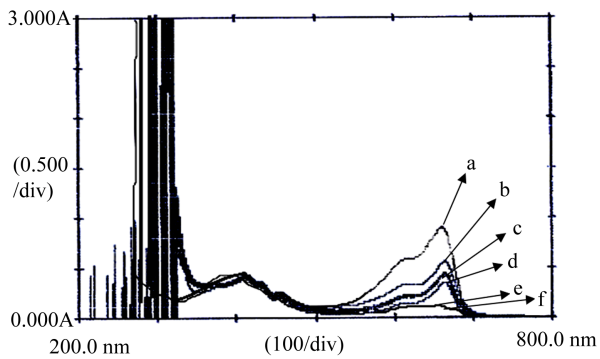

Figure 1. Spectral scan of MG with ascorbic acid in presence and absence of sodium carbonate. Where a = 5 × 10^{-3} mol·dm^{-3}, b = 6 × 10^{-3} mol·dm^{-3}, c = 7 × 10^{-3} mol·dm^{-3}, d = 8 × 10^{-3} mol·dm^{-3}, e = 9 × 10^{-3} mol·dm^{-3}.

effect of change in concentration of oxalic acid on rate of reaction showed no a linear dependence on [H^+]. This may be attributed with that the varied concentration of oxalic acid as a medium, showed no effect on rate of reduction (**Table 3**). This indicated that oxalic acid is involved in the release of CO_2 with sodium carbonate as varied concentration of sodium carbonate decrease the reduction time according to following equations no. (4 - 7).

$$H_2C_2O_4 + Na_2CO_3 \longrightarrow Na_2C_2O_4 + CO_2 + H_2O \quad (4)$$

$$MG + AH_2 \xrightarrow[H_2/2e^-]{} MGH^+ + AH$$
$$\text{fast } (CO_2 \text{ atmosphere}) \quad (5)$$

$$MG + AH \longrightarrow MGH^+ + A^- \quad \text{fast} \quad (6)$$

$$MGH^+ + e^- \xrightarrow{h\upsilon} MGH + A \quad \text{fast} \quad (7)$$

The dye and ascorbic acid concentration were varied to analyze the effect of reduction of MG on rate of reduction. Results showed that the reaction followed fractional order kinetics with ascorbic acid and zero order kinetics with methylene green (**Tables 4** and **5**). COD increases with the increase in concentration of dye (**Table 2**). The values of percent de-coloration decreases with the increase in concentration of dye in reaction solution (**Table 4**) [17-19]. Unexpectedly it was observed that the rate constant of MG reduction appears to be the same in concentration range of 2 × 10^{-5} to 5 × 10^{-5} mol·dm^{-3} also, when concentration of ascorbic acid was varied in solution containing a fixed amount of the dye, the de--coloration percentage increases with the increase in the concentration of dye. This reflects that the reaction depends upon the concentration of ascorbic acid (**Figure 2**). However, beyond certain limits the value of rate constant decreases (**Table 5**). The hydrogen ion abstraction and electron donation by ascorbic acid take place through following reaction which leads to the formation of leuco dye with dehydroxy –1- ascorbic acid (DHAA) (**Eq.8**) [1-3,19]..

Table 3. Effect of Oxalic Acid ($H_2C_2O_4$) on Rate of Reduction of MG With Ascorbic Acid. [MG^+] = 1 × 10^{-5} mol·dm^{-3}, [Ascorbic Acid] = 6 × 10^{-3} mol·dm^{-3}, [$H_2C_2O_4$] = (0.06 – 0.3) mol·dm^{-3}, [KNO_3] = 0.2 mol·dm^{-3}, [Na_2CO_3] = 1 × 10^{-2} mol·dm^{-3}, Temperature = 303 K.

Concentration of $H_2C_2O_4$ mol·dm^{-3}	Velocity v × 10^5	Specific rate constant (k_{obs}) × 10^3 s^{-1}	1/(k_{obs})	% decoloration
0.06	–3.0	1.3	767.23	77.241
0.12	–3.0	1.3	767.23	77.241
0.18	–3.0	1.3	767.23	77.241
0.24	–2.0	1.2	500.00	71.312
0.3	–3.0	1.3	767.23	77.241

Table 4. Effect of Methylene Green [MG^+] on Rate of Reduction. [MG^+] = (1 – 5) × 10^{-5} mol·dm^{-3}, [Ascorbic Acid] = 6 × 10^{-3} mol·dm^{-3}, [$H_2C_2O_4$] = 0.18 mol·dm^{-3}, [KNO_3] = 0.2 mol·dm^{-3}, [Na_2CO_3] = 1 × 10^{-2} mol·dm^{-3}, Temperature = 303 K.

Concentration of [MG^+] × 10^5 mol·dm^{-3}	Velocity v × 10^5	Specific rate constant (k_{obs}) × 10^3 s^{-1}	1/(k_{obs})	% decoloration
1.0	–4.0	1.8	555.56	71.111
2.0	–4.0	1.0	1000	81.361
3.0	–5.0	1.0	1000	89.194
4.0	–5.0	1.0	1000	93.657
5.0	–7.0	1.0	1000	82.282

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Table 5. Effect of Ascorbic Acid on Rate of Reduction of MG. $[MG^+] = 1 \times 10^{-5}$ mol·dm^{-3}, [Ascorbic Acid] = (6 – 9) × 10^{-3} mol·dm^{-3}, $[H_2C_2O_4] = 0.18$ mol·dm^{-3}, $[KNO_3] = 0.2$ mol·dm^{-3}, $[Na_2CO_3] = 1 \times 10^{-2}$ mol·dm^{-3}, Temperature = 303 K.

Concentration of Ascorbic acid × 10^3 mol·dm^{-3}	Velocity $\upsilon \times 10^5$	Specific rate constant (k_{obs}) × 10^3 s^{-1}	$1/(k_{obs})$	% decoloration
5.0	–10.00	2.7	370.37	81.223
6.0	–4.00	1.8	555.56	71.111
7.0	–10.00	5.5	181.82	57.667
8.0	–20.00	14.3	69.93	67.025
9.0	–9.00	121.0	8.265	49.738

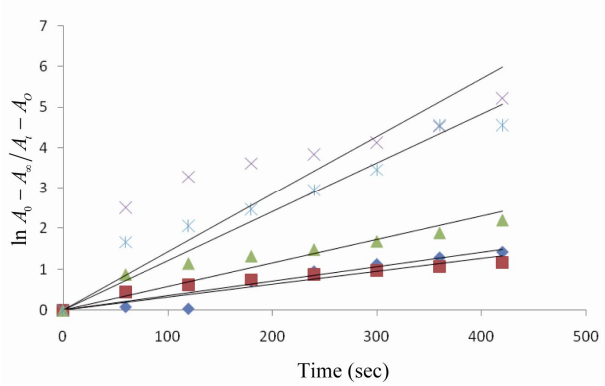
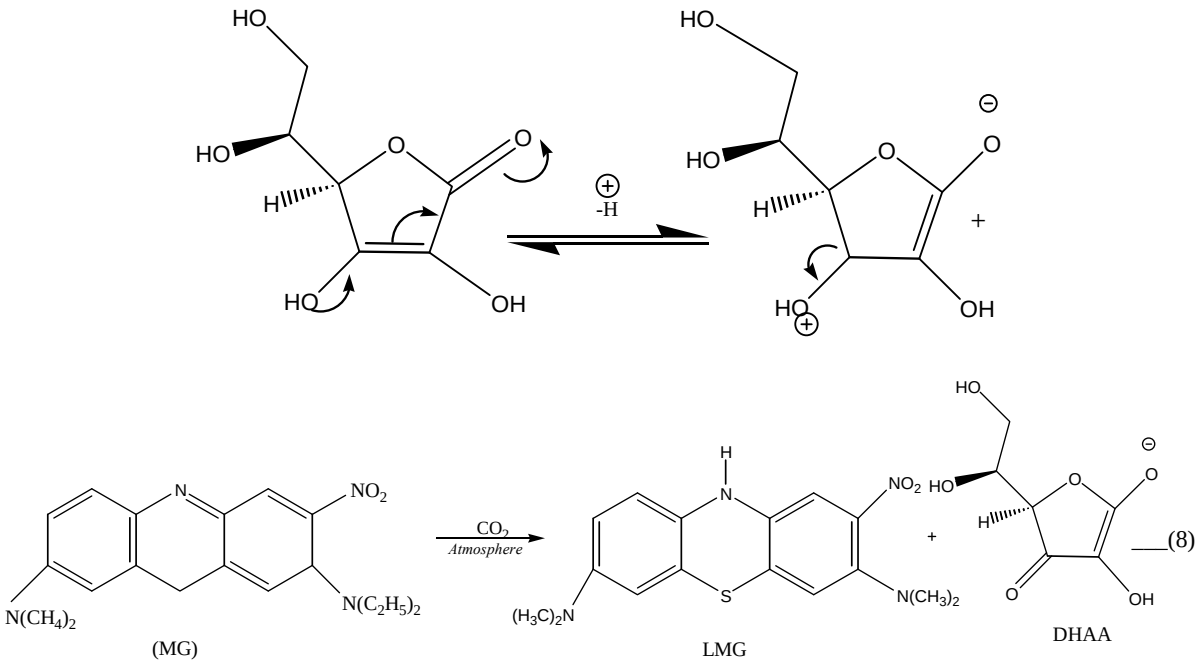


Figure 2. A plot of $\ln A_0 - A_\infty / A_t - A_\infty$ vs Time for reduction of MG with variable concentration of AA. $[MG^+] = 1 \times 10^{-5}$ M, [Ascorbic Acid] = 5 – 9 × 10^{-3} M, $[H_2C_2O_4] = 0.18$ M, $[KNO_3] = 0.2$ M, $[Na_2CO_3] = 1 \times 10^{-2}$ M, Temperature = 303 K.



The general effect of an added electrolyte on the observed rate constant of a reaction in solution is determined only by the ionic strength which showed that the rate of reaction is directly related with the elevated concentration of salt, suggested that the two same charged species are involved in the rate determining step [11,14]. The kinetic salt effect of added salt electrolyte was determined through Debye-Huckel limiting law which gives relationship between ionic strength and rate constant. The equation may be given as

$$\log k = \log k_0 + 1.02\, Z_A\, Z_B \sqrt{\mu} \qquad (9)$$

for the determination of reactive specie in the rate determining step. This equation shows that a plot of log k vs $\sqrt{\mu}$ should be linear, with the slope and intercept equal to 1.02 $Z_A Z_B$ and log k_0 respectively. The slope represents the product of charges on the species involved in the rate timing stop. If the rate limiting step is between the species of like charges, a positive slope is expected. When the reaction is in between opposite charges, it results in a negative slope. In present work effect of salt was studied at constant concentration of dye, reductant and acid by varying concentration of salt which showed that the rate constant increases with increase in an ionic strength of the reaction (**Table 6**) indicating that the reaction is in between ions of like charges in rate determining step. The plot of log k vs $\sqrt{\mu}$ is linear with

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1.5012 slope [20]. The positive sign of the slope

Table 6. Effect of Ionic Strength on Rate of Reduction of MG. $[MG^+] = 1 \times 10^{-5}$ mol·dm^{-3}, [Ascorbic Acid] = 6×10^{-3} mol·dm^{-3}, $[H_2C_2O_4] = 0.18$ mol·dm^{-3}, $[KNO_3] = (0.2 – 1)$ mol·dm^{-3}, $[Na_2CO_3] = 1 \times 10^{-2}$ mol·dm^{-3}, Temperature = 303 K.

$\sqrt{\mu}$ mol·dm^{-3}	Concentration of KNO_3 mol·dm^{-3}	Velocity $v \times 10^5$	Specific rate constant $(k_{obs}) \times 10^3$ s^{-1}	$1/(k_{obs})$	% decoloration
0.8809	0.2	–4.0	1.8	555.56	71.111
0.9879	0.4	–3.0	1.1	909.091	77.931
1.0844	0.6	–3.0	1.3	767.23	83.448
1.1730	0.8	–10.0	3.4	294.118	87.805
1.2554	1.0	–20.0	5.5	181.818	81.034

indicate that the species involve in the rate determining step has like charges. If Z_A and Z_B have the same sign, then Z_A Z_B is positive and the rate constant increases with ionic strength as observed in the present investigation. The value of Z_A and Z_B was obtained from the slope of the plot of logk vs $\sqrt{\mu}$ which is 1.50. The pseudo first-order rate constant (k') with respect to dye concentration was found to non significantly correlated (R^2 = 0.5717) as a function of ionic strength. From the data in Table [4] it can be observed that higher rate constants are attained at higher ionic strengths. This can be related to alterations of ionic atmosphere and changes in the charge densities around anions involved in the rate-determining step. The formation of a single highly charged ionic complex from two less highly charged ions is favored by a high ionic strength because the new ion has a denser ionic atmosphere [20].Using rate constant data at different ionic strengths, a graph was obtained employing the Brønsted–Bjerrum equation, based on the limiting law of Debye–Hückel (**Figure 3**).The rate constant at infinite dilution (k_0) is 4.2 M [the product of charges Z_A Z_B is 1.5. Considering the extended law of Debye–Hückel (**Figure 3**), k_0 is 4.2 mol·dm^{-3} Z_AZ_B is 1.50. This value is close to the expected value of 2 considering the proposed mechanism, which involves a collision between a monovalent and a biivalent cation in the rate-determining step [20].

The effect of temperature on rate of reaction indicated that elevation in temperature results in the complex kinetics which may be due to the some secondary reactions operating in the reaction mixture at a given temperature [19, 21-29]. It was observed from the **Table 7** that there is no linear relation in between the temperature elevation and rate constant which showed that temperature elevation cause secondary reactions in which dye molecule or reductant may be break down due to which black particles were appear in the reaction mixture. The values of activation parameters like entropy of activation, enthalpy of activation and energy of activation support secondary reaction predominantly degradation of dye molecule instead of reduction [14,23].

4. REACTION MECHANISM

Reaction mechanism of the reduction of MG proposed in which role of CO_2 was considered to displace the oxygen and provide deoxygenated atmosphere to increase the rate of reduction. Ascorbic acid existed in reaction mixture as follows (equation) and reduction take place by abstraction of H from AA (**Eqs.10** and **11**).

$$AH_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AH^- + H^+ \qquad (10)$$

$$MG^+ + H^+ \underset{}{\overset{k_2}{\rightleftharpoons}} MGH^+ \qquad (11)$$

$$MGH^+ + 2AH^- \xrightarrow{k_3} MGH + A$$

Rate law for abstraction of H to convert dye into colorless form is as follows

$$\frac{-d[MG]}{dt} = [MG][AH_2^+] \qquad (12)$$

$$\frac{-d[MG]}{dt} = k_2[MG][H^+] \qquad (13)$$

Rate of formation of active species of reductant according to **Eq. 2**.

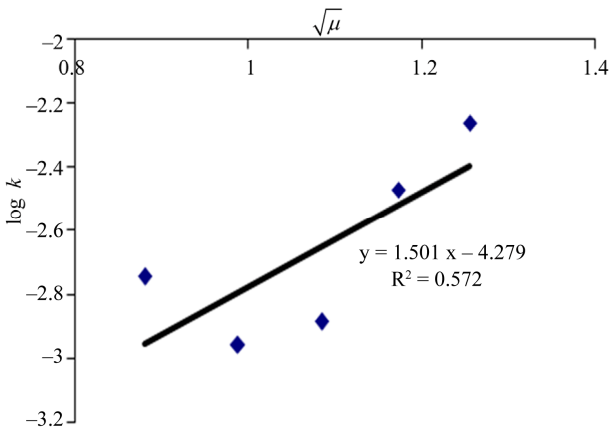

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Figure 3. A plot of logk vs $\sqrt{\mu}$. [MG^+] = 1 × 10^{-5} M, [Ascorbic Acid] = 6 × 10^{-3} M, [$H_2C_2O_4$] = 0.18 M, [KNO_3] = (0.2 – 1) M, [Na_2CO_3] = 1 × 10^{-2} M, Temperature = 303 K.

Table 7. Effect of Temperature on Rate of Reduction. [MG^+] = 1 × 10^{-5} M, [Ascorbic Acid] = 5 × 10^{-3} M, [$H_2C_2O_4$] = 0.18 M, [KNO_3] = 0.2 M, [Na_2CO_3] = 1 × 10^{-2} M, Temperature = 303 – 323 K.

Temperature (K)	Velocity $v \times 10^{-5}$	Specific rate constant (k_{obs}) × 10^3 s^{-1}	$1/(k_{obs})$	% decoloration
303	–3.00	1.6	625	65.87
308	–3.00	1.5	666.667	66.25
313	–8.00	3.3	303.03	88.22
318	–4.00	3.2	312.5	
323	–5.00	2.5	400	
$\sqrt{\mu}$ mol·dm^{-3}	$\Delta E^{\neq}$ kj·mol^{-1}	$\Delta H^{\neq}$ kj·mol^{-1}	$\Delta S^{\neq}$ kj·mol^{-1}	$\Delta G^{\neq}$ kj·mol^{-1}
0.8809	28.337	258.765	380.392	143.506

$$\frac{-d[AH_2]}{dt} = k_1[AH_2] \tag{14}$$

At equilibrium rate of formation of H = rate of reduction of dye

Hence

$$k_1[AH_2] = k_2[MG][H^+] \tag{15}$$

$$\frac{k_1}{k_2} = \frac{[MG][H^+]}{[AH_2]} \tag{16}$$

Taking $k_1/k_2 = K$

$$K = \frac{[MG][H^+]}{[AH_2]} \tag{17}$$

$$K = \frac{[H^+]}{[AH_2]} \tag{18}$$

According to **Eqs.1** and **2** reaction is in depended upon H ion concentration

$$K = \frac{\frac{-d[MG]}{dt}}{k_2[AH_2]}$$

$$[MG] = \frac{\frac{-d[MG]}{dt}}{k_2[H^+]} \tag{19}$$

$$K = \frac{\frac{-d[MG]}{dt}}{k_2[AH_2]} \tag{20}$$

$$k_2 = \frac{\frac{-d[MG]}{dt}}{k[AH_2]} \tag{21}$$

The above **Eq.21** corresponds to our results that reduction is in depended upon concentration of dye and fractional order with respect to ascorbic acid **Tables 4** and **5**.

5. CONCLUSIONS

It was concluded from above investigation that dehydrogenation of ascorbic acid with methylene green in the presence of sodium carbonate decolorizes the dye more rapidly in acidic medium which could be more economical technique in future and has a significant advantage over other studies reported in the chemical literature for the dehydrogenation of ascorbic acid or in other words it may be used for the detection of AA in different fruit samples in very diminutive quantities.

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