



Reduction of Toluidine Blue by Sulfur(IV) in Acetate Buffer Medium: Kinetics and Mechanism

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The reduction of toluidine blue (TB⁺) by sulfite [S(IV)] was studied spectrophotometrically in acetic acid-sodium acetate buffer medium in the temperature range 293-308 K. The reaction showed first order dependence on toluidine blue. The reaction was found to be first order in S(IV) at lower substrate concentration ($[S(IV)] \leq 0.07 \text{ mol dm}^{-3}$) but the reaction became second order in S(IV) at high substrate concentration ($[S(IV)] > 0.07 \text{ mol dm}^{-3}$). H⁺ ions were found to inhibit the reaction in the pH range 4.0-5.4. The rate decreased with increase in the ionic strength of the medium, but remained unchanged with decreasing dielectric constant of the medium. The oxidation reaction possibly involved a one-electron transfer step *via* a free radical mechanism. An overall enthalpy and entropy of activation were computed. A plausible mechanism and rate law corroborating the experimental results have been put forward.

Keywords: Reduction, Sulfur(IV), Toluidine blue, Kinetics and mechanism.

INTRODUCTION

Sulfite is an important substrate which is generated endogenously by the metabolism of sulfur containing amino acids such as methionine and cysteine¹. It is used as preservative in dried fruits and vegetables and in wine in millimolar concentration. It is also used as a stabilizer in many drugs. But it has been found to increase the neurotoxic effect on neuronal cells¹. As sulfite is a powerful reducing agent, a number of attempts have been made to investigate into the kinetics of its oxidation by various oxidizing agents such as chlorine dioxide², FeO₄^{2,3}, Cr(VI)⁴, Mn(III)⁵, Mn(VII)⁶, Au(III)⁷, Pt(IV)⁸ and Ir(IV)⁹ in acidic as well as in alkaline medium.

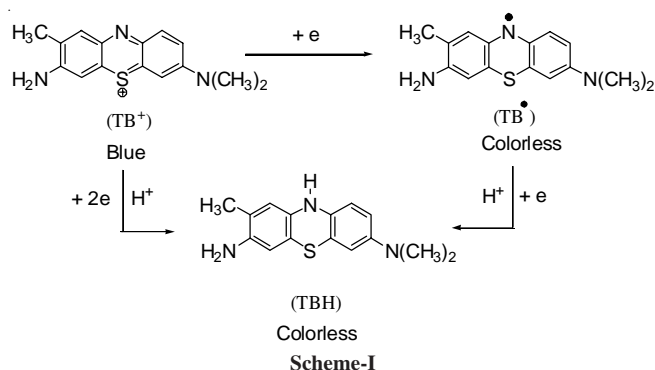
Toluidine blue (TB⁺), an acidiphilic metachromic dye, is used as a staining agent in biological systems owing to its affinity for nucleic acid and it binds to nuclear materials of tissues with a high DNA and RNA content^{10,11}. The dye has also found extensive use as a vital stain for mucosal lesions and also has been used in tissue sections to specifically stain certain components owing to its metachromic property¹². It has been used also as a vital stain to highlight potentially malignant oral lesions and may identify early lesions which could be missed out on clinical examination¹³. Toluidine blue is some time used as a polymerization inhibitor and complexing agent¹⁴. Toluidine blue is a mild oxidizing agent and may undergo either two-electron one step¹⁵ or one-electron two steps^{16,17} reduction depending on the nature of the reductant

species and toluidine blue is reduced to toluidine white (TBH). In the two steps one-electron transfer process the reduction takes place through the formation of semitoluidine blue (TB[•]) as the transient intermediate (**Scheme-I**).

A number of reactions involving toluidine blue with different reducing agents such as phenyl hydrazine¹⁵, thiourea¹⁶, dithionite¹⁷, nitrite¹⁸ and ascorbic acid¹⁹ have been reported in aqueous or weakly acidic medium. The effect of SDS micelles on reduction of toluidine blue by ascorbic acid²⁰ has been reported in aqueous acid medium. There is also a literature report²¹ involving the reduction of toluidine blue by sulfite ion in aqueous medium which attempts to elucidate the mechanism of this reaction through kinetic salt effect. However, no detailed kinetic investigation has been made to substantiate the electron transfer mechanism or to evaluate the activation and thermodynamic parameters of the reaction. It is, therefore, interesting to investigate the kinetics and mechanism of the reduction of toluidine blue by sulfite ion in acid medium in order to find out whether the reaction takes place through two one-electron transfer steps or one two-electron transfer step.

EXPERIMENTAL

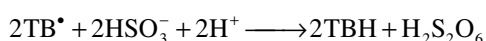
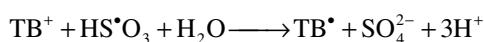
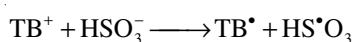
Sodium sulfite (Anhydrous Extra pure AR, SRL), toluidine blue (SRL), hydrochloric acid (GR, Merck), oxalic acid (Extra pure AR, SRL), acetic acid (AR, SRL), sodium hydroxide (GR, Merck), sodium sulfate (Anhydrous Extra pure AR, SRL) and



acrylamide (Spectrochem) were used without further purification. The stock solution of sodium sulfite (0.5 mol dm^{-3}) was prepared fresh everyday by dissolving known amount of the salt in a volumetric flask. The aqueous solution of toluidine blue ($2.03 \times 10^{-4} \text{ mol dm}^{-3}$) was prepared by direct weighing and stored in the dark. Standard sodium acetate solution was prepared by neutralizing standard acetic acid solution with standard NaOH and required acid buffer was prepared by mixing required amount of standard acetic acid and standard sodium acetate solution. All the solutions were made in doubly distilled water.

Kinetic measurements: The kinetics of the reaction was studied under pseudo-first order condition ($[\text{S(IV)}] \gg [\text{TB}^+]$). A UV-visible spectrophotometer (Shimadzu, model UV-2401PC) fitted with a thermostatted cell compartment was used to monitor the decay of toluidine blue concentration by measuring absorbance (A) of the reaction mixture at 605 nm (λ_{max} of toluidine blue) at different time intervals. The pseudo-first order rate constant (k_{obs}) was evaluated from the slope of the linear plot of $\log A$ versus time and the results were reproducible to within $\pm 5 \%$.

Stoichiometry: The stoichiometry of the reduction of toluidine blue by sulfite was studied in presence of excess sulfite compared to toluidine blue. The concentration of the reagents were $[\text{TB}^+] = 6 \times 10^{-4} \text{ mol dm}^{-3}$, $[\text{S(IV)}] = 4 \times 10^{-2} \text{ mol dm}^{-3}$ and $\text{pH} = 4.70$ at 308 K. The reaction mixture was purged with nitrogen in order to avoid aerial oxidation of sulfite and it was allowed to stand for 8 h. The excess of sulfite was then estimated iodometrically²². Blank experiment (from which toluidine blue was excluded) was also carried out. The average of different experiments indicated that the reaction stoichiometry was $[\text{TB}^+]:[\text{S(IV)}] = 2:3$. Overall stoichiometry of the reaction is in accordance with the following redox steps:



Test for free radical: Sodium sulfite solution ($8 \times 10^{-2} \text{ mol dm}^{-3}$) in acetate buffer at pH 4.7 was mixed with 15 % (w/v) acrylamide solution and purged with nitrogen followed by the addition of toluidine blue ($2.03 \times 10^{-5} \text{ mol dm}^{-3}$) and allowed to stand at room temperature. The solution became turbid within 0.5 h and on keeping a white polymeric product was obtained. Blank experiments where either toluidine blue

or sulfite were excluded showed no such polymerization. This probably indicates that free radicals intervene in the reaction and conforms with the observations made earlier in the oxidation of S(IV) by Mn(III)⁵ and Ir(IV)⁹.

RESULTS AND DISCUSSION

Absorption spectra of toluidine blue: The absorption spectra of toluidine blue solution in water have been recorded for five different concentrations of toluidine blue ($1.01, 2.03, 3.04, 4.06$ and $5.07 \times 10^{-5} \text{ mol dm}^{-3}$) between 500-700 nm (Fig. 1). The absorption maximum was found at 605 nm. The absorbance values of toluidine blue solution at the λ_{max} with different concentrations [$(1.01-5.07) \times 10^{-5} \text{ mol dm}^{-3}$] in aqueous solution were obtained from the spectra and Beer's law was found to be valid within the concentration range studied. The molar extinction co-efficient (ϵ) at 605 nm has been calculated to be $23,560 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ (reported value $24,000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ²¹).

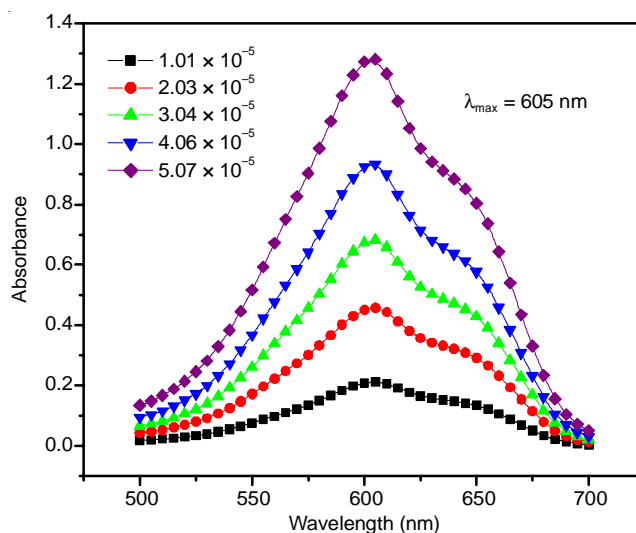


Fig. 1. Absorption spectra of toluidine blue at different concentrations in aqueous medium

Effect of variation of $[\text{TB}^+]$: The rate of the reaction was studied at various initial concentration of toluidine blue ($(1.02-4.08) \times 10^{-5} \text{ mol dm}^{-3}$) keeping buffer pH, $[\text{S(IV)}]$ and temperature constant at 4.7, $8.0 \times 10^{-2} \text{ mol dm}^{-3}$ and 298 K, respectively. The pseudo-first order rate constant was found to remain unchanged at about $(12.8 \pm 0.5) \times 10^{-4} \text{ s}^{-1}$ (Table-1). The result indicates that the reaction is first order with respect to toluidine blue.

Effect of variation of $[\text{S(IV)}]$: The effect of changing the substrate concentration was studied at four different temperatures (293, 298, 303 and 308 K) keeping pH of buffer and $[\text{TB}^+]$ constant at 4.7 and $2.03 \times 10^{-5} \text{ mol dm}^{-3}$, respectively. At a constant temperature an increase in sodium sulfite concentration was found to enhance the rate of oxidation (Table-1). The plot of k_{obs} versus $[\text{S(IV)}]$ at each temperature was found to be two inter-secting straight lines, one passing through origin (Fig. 2) indicating that the reaction is first order in S(IV) at lower substrate concentration ($[\text{S(IV)}] \leq 0.07 \text{ mol dm}^{-3}$), but the order increases at high substrate concentration ($[\text{S(IV)}] > 0.07 \text{ mol dm}^{-3}$).

TABLE-1
EFFECT OF $[TB^+]$, $[S(IV)]$ AND $[H^+]$ ON THE RATE OF
OXIDATION OF SULPHITE BY TOLUIDINE BLUE AT 298 K

$10^5 [TB^+]$ (mol dm ⁻³)	$10^2 [S(IV)]$ (mol dm ⁻³)	$10^5 [H^+]$ (mol dm ⁻³)	$10^4 k_{obs}$ (s ⁻¹)
1.02	8.0	1.99	12.8
2.04	8.0	1.99	12.5
3.06	8.0	1.99	13.3
4.08	8.0	1.99	12.7
2.04	2.0	1.99	1.08
2.04	6.0	1.99	6.04
2.04	10.0	1.99	31.4
2.04	14.0	1.99	54.2
2.04	17.5	1.99	90.0
2.04	8.0	0.40	39.91
2.04	8.0	1.26	14.56
2.04	8.0	1.99	12.44
2.04	8.0	5.01	3.91
2.04	8.0	10.0	2.46

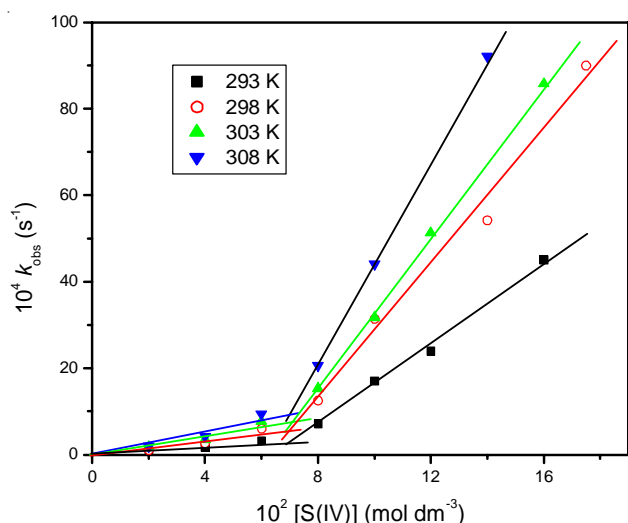


Fig. 2. Variation of pseudo-first order rate constant with change in sodium sulfite concentration. Plot of k_{obs} versus $[S(IV)]$ at four different temperatures. $[TB^+] = 2.03 \times 10^{-5}$ mol dm⁻³, pH = 4.70

Effect of variation of ionic strength: The oxidation was studied at various initial concentrations of Na₂SO₄ (0.30-0.72 mol dm⁻³) keeping pH, $[TB^+]$, $[S(IV)]$ and temperature constant at 4.7, 2.03×10^{-5} mol dm⁻³, 8×10^{-2} mol dm⁻³ and 298 K, respectively. The pseudo-first order rate constant (k_{obs}) was found to decrease with increase of ionic strength although the effect was small (Table-2).

TABLE-2
VARIATION OF PSEUDO-FIRST ORDER RATE CONSTANT
WITH CHANGE IN IONIC STRNGTH. $[TB^+] = 2.03 \times 10^{-5}$ mol
dm⁻³, $[S(IV)] = 8 \times 10^{-2}$ mol dm⁻³, pH= 4.70 AND
TEMPERATURE = 298 K

$[Na_2SO_4]$ (mol dm ⁻³)	$10^3 k_{obs}$ (s ⁻¹)
0.00	2.37
0.15	2.15
0.30	2.03
0.48	1.92
0.60	1.81
0.72	1.72

Effect of variation of $[H^+]$: The effect of variation of $[H^+]$ on the reaction rate was studied at 298 K keeping $[S(IV)]$

and $[TB^+]$ constant at 0.08 mol dm⁻³ and 2.03×10^{-5} mol dm⁻³, respectively while the pH was varied from 4 to 5.4. The reaction rate was found to decrease with an increase in $[H^+]$ (Table-1). A plot of k_{obs}^{-1} versus $[H^+]$ was a straight line having a +ve slope and +ve intercept (Fig. 3).

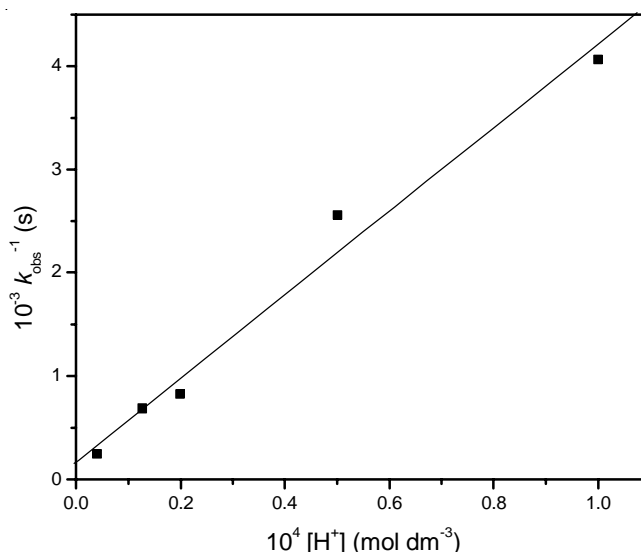


Fig. 3. Variation of pseudo-first order rate constant with change in pH of the medium. $[TB^+] = 2.03 \times 10^{-5}$ mol dm⁻³, $[S(IV)] = 8 \times 10^{-2}$ mol dm⁻³ and temperature = 298 K

Effect of variation of dielectric constant: Effect of changing solvent composition on the reaction rate was studied by the addition of 1,4-dioxane (5-30 % v/v) to the reaction mixture as mentioned in Table-3. The rate was found to remain almost unchanged with increasing the proportion of 1,4-dioxane.

TABLE-3
VARIATION OF PSEUDO-FIRST ORDER RATE CONSTANT
WITH CHANGE IN DIELECTRIC CONSTANT OF THE
MEDIUM. $[TB^+] = 2.03 \times 10^{-5}$ mol dm⁻³, $[S(IV)] = 8 \times 10^{-2}$
mol dm⁻³, pH= 4.70 AND TEMPERATURE = 298 K

% (v/v) Dioxane	ϵ	$10^4 k_{obs}$ (s ⁻¹)
5	74.2	8.23
10	69.7	8.34
15	65.3	7.86
20	60.8	7.98
25	56.4	8.12
30	51.9	7.87

Effect of temperature: The pseudo-first order rate constant was found to increase with increase in temperature (Fig. 2). Although it was not possible to find out the activation parameters for the rate determining step, an attempt was made to evaluate the overall enthalpy of activation (ΔH^\ddagger) and entropy of activation (ΔS^\ddagger) [for the first part of the reaction when it is first order with respect to S(IV)] from the linear plot of $\log(k_2/T)$ versus $1/T$ using the relationship:

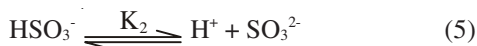
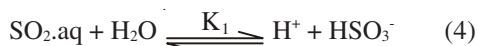
$$\log(k_2/T) = [\log(k_B/h) + \Delta S^\ddagger/2.303R] - \Delta H^\ddagger/2.303 RT \quad (2)$$

k_B and h are Boltzmann constant and Plank's constant respectively and k_2 is second order rate constant. The ΔH^\ddagger and ΔS^\ddagger values were found to be 38.9 kJ mol⁻¹ and -155.4 JK⁻¹ mol⁻¹, respectively.

Toluidine blue may undergo protonation in acidic solution.



As the reaction is retarded by H^+ ions, it is suggested that the oxidant reacts in the non-protonated form TB^+ . Again, in a solution of sodium sulfite the following equilibria exist:

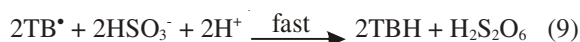
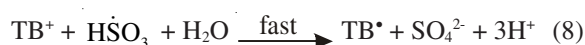
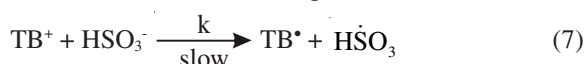


The values of the dissociation constants K_1 and K_2 are 1.6×10^{-2} and 1.0×10^{-7} , respectively at 298 K²³. Thus three reducing species, *viz.*, $\text{SO}_2(\text{aq})$, HSO_3^- and SO_3^{2-} are present in a solution of sodium sulfite. However, it has been found that at ordinary temperature in the pH range 3-6, HSO_3^- is the predominating species. In such a solution concentration of HSO_3^- is given by

$$[\text{HSO}_3^-] = \frac{[\text{S(IV)}]}{1 + \{[\text{H}^+]/K_1\} + \{K_2/[\text{H}^+]\}} \quad (6)$$

The reaction has been studied in the pH range 4.0 to 5.4 and from the known value of K_1 and K_2 it is found that within this pH range, $[\text{HSO}_3^-] \approx [\text{S(IV)}]$. Haight and coworkers⁴ have shown that in the oxidation of sulfite by Cr(VI) in the pH range 4.18-5.05, HSO_3^- ion is the active reductant species.

The reaction induces the polymerization of acrylamide solution indicating a one-electron transfer step. Hence it is suggested that HSO_3^- mainly reacts with toluidine blue in a one-electron transfer rate determining step to produce intermediate semitoluidine blue (TB^*) and radical intermediate HS^*O_3 (eqn. 7). The radical intermediate HS^*O_3 reacts with another molecule of toluidine blue to produce sulfate ion along with TB^* . The reactive semitoluidine blue (TB^*), combines with HSO_3^- in a fast step and may also produce dithionic acid in addition to sulfate as the oxidation product.



The presence of sulfate in the reaction product was verified by the addition of barium chloride in the reaction mixture. Had there been only sulfate ion as the oxidation product of sulfite, the reaction stoichiometry would have been 1:1. However, the stoichiometric results in the present study showed that two molecules of toluidine blue react with three molecules of sulfite. This type of stoichiometry is possible if dithionate is also produced in addition to sulfate. Similar observation has been reported in the oxidation of sulfite by gold(III) in acid medium⁷.

According to the suggested mechanism, the rate law is given by

$$V = -\frac{d[\text{ox}]}{dt} = k[\text{TB}^+][\text{HSO}_3^-] = k \frac{[\text{ox}]}{1 + K[\text{H}^+]} [\text{S(IV)}] \quad (10)$$

where, $[\text{ox}]$ stands for total toluidine blue concentration.

Thus, the pseudo-first order rate constant,

$$k_{\text{obs}} = -\frac{1}{[\text{ox}]} \frac{d[\text{ox}]}{dt} = \frac{k}{1 + K[\text{H}^+]} [\text{S(IV)}] \quad (11)$$

Or, the second order rate constant,

$$k_2 = k_{\text{obs}}/[\text{S(IV)}] = \frac{k}{1 + K[\text{H}^+]} \quad (12)$$

Eqn. 11 may also be rearranged as

$$\frac{1}{k_{\text{obs}}} = \frac{1}{k[\text{S(IV)}]} + \frac{K}{k[\text{S(IV)}]} [\text{H}^+] \quad (13)$$

Eqn. 11 shows that the reaction is first order with respect to S(IV). Plot of k_{obs} versus $[\text{S(IV)}]$ should be a straight line passing through the origin as found from experiment at lower substrate concentration. Eqn. 13 predicts that a plot of k_{obs}^{-1} versus $[\text{H}^+]$ should be a straight line with a +ve slope and +ve intercept as found in Fig. 3. From the intercept and slope of this plot the values of k (rate constant for the rate determining step) and K (protonation equilibrium constant for toluidine blue) have been evaluated to be $6.25 \times 10^{-2} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ and $1.9 \times 10^5 \text{ dm}^3 \text{ mol}^{-1}$, respectively at 298 K.

Rate determining step involved two oppositely charged ions and the rate should be retarded with increase of ionic strength as has been found in the salt effect studies (Table-2). At higher substrate concentration there may be a parallel path where two HSO_3^- ions react with one toluidine blue in the rate determining step.



And the rate law becomes

$$V = k[\text{TB}^+][\text{HSO}_3^-] + k'[\text{TB}^+][\text{HSO}_3^-]^2 \quad (14)$$

or,

$$k_{\text{obs}} = \frac{\{k[\text{S(IV)}] + k'[\text{S(IV)}]^2\}}{1 + K[\text{H}^+]} \quad (15)$$

If the second path predominates at higher substrate concentration the order with respect to $[\text{S(IV)}]$ becomes two. At this higher substrate concentration, the concentration of the free radical (HS^*O_3) is expected to be higher and thus there is a greater possibility of combination of these radicals to produce dithionic acid ($\text{H}_2\text{S}_2\text{O}_6$).

According to the mechanism suggested the reaction rate should increase with decrease in the dielectric constant of the medium. That is, k_{obs} should increase with increase in the % of 1,4-dioxane in H_2O -dioxane mixture. But the rate has been found to remain more or less unchanged with increase in the proportion of dioxane in the reaction mixture. Such an observation may be accounted for by the fact that with decrease in the dielectric constant of the medium, the combination of two oppositely charged ions TB^+ and HSO_3^- is favoured and there should be an enhancement of rate. But with decrease in dielectric constant, the combination of two oppositely charged ions H^+ and HSO_3^- should also be favoured, thereby decreasing the effective concentration of HSO_3^- and thus decreasing the rate. These two opposing effects possibly neutralize each other and the reaction rate remains unchanged. Thus all these effects of pH, ionic strength, solvent composition *etc.* support the proposed mechanism and rate law.

Conclusion

Variable reaction order with respect to S(IV) was observed in the oxidation of sulfite by toluidine blue (TB⁺) (first order at [S(IV)] ≤ 0.07 mol dm⁻³ and second order at [S(IV)] > 0.07 mol dm⁻³). H⁺ ion shows a retarding effect on the reaction rate. The oxidation takes place through a one-electron transfer step via the formation of semitoluidine blue and radical intermediate of HSO₃⁻ which ultimately produced sulfate and dithionate.

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