Reduction of 3,7-Bis(dimethylamino) phenazothionium Chloride by Benzenethiol in Aqueous Nitric Acid Medium: A Mechanistic Approach

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Original Research Article	

ABSTRACT

The kinetics of the reduction of 3,7-bis(dimethylamino)phenazothionium chloride (MB^+) by benzenethiol in aqueous solution has been investigated in the acid range $0.3 \leq [H^+] \leq 0.9$ mole dm^{-3}, ionic strength (μ) of the reaction medium in the range: $0.4 \leq \mu \leq 1.0$ mole dm^{-3} ($NaNO_3$) and temperature, T = 29ºC. The reaction is first order in both [oxidant] and [reductant] and display an inverse order acid dependence with an overall reaction that conforms to the rate law:

$$\frac{-d}{dt}[MB^+] = (Kk_4[H^+]^{-1} + k_6)([MB^+][C_6H_5SH])$$

at $[H^+]$ = 0.3 mole dm^{-3} and μ = 1.0 mole dm^{-3} ($NaNO_3$). The rate of the reaction increased with increase in the ionic strength and decrease in dielectric constant of the reaction medium. Added $HCOO^-$ and Cl^- accelerated the rate of the reaction. Spectroscopic investigation did not reveal the


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formation of any stable reaction intermediate. Test for free radical was positive and a plausible mechanism consistent with these observations has been proposed.

1. INTRODUCTION

A recent review of methylene blue (here and thereafter represented by MB^+ for convenience, (Fig. 1) by Schirmer et al. [1], reveals an array of its many uses especially in the field of medicine. It is reported to be the first fully synthetic drug in medicine. In recent years, there was a surge of interest in MB^+ as an anti-malarial agent and as a potential treatment of neurodegenerative disorders such as alzheimer's disease, possibly through its inhibition of the aggregation of tau protein. However, in most of these applications, literature on the mechanism of its reactions is scanty. In the last two decades, it has been shown that, reduction of MB^+ can be achieved with BrO_3^- [2], SCN^- [3], H_2O_2 [4], $UV-H_2O_2$ [5,6], ascorbic acid[7-11], Ce(IV) [12], thiosulphate [13], β-mercaptoethanol [14], photo-decomposition on TiO_2 [15,16], photo-reduction by the ureas and thioureas [17]. This work reports an investigation on the dynamics of oxidation of benzenethiol by methylene blue, with the aim of gaining insight and understanding of the mechanistic pathway of methylene blue reaction with benzenethiol.
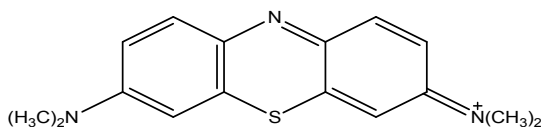
Fig. 1: Structure of 3,7-bis(dimethylamino)phenazothionium chloride (methylene blue, MB^+)

2. MATERIALS AND METHODS

All the reagents used were of analytical grade and were used as supplied. Standard solution of methylene blue was prepared by dissolving accurate weight amount of the dye in a known volume of water. The spectrum of the solution was determined over the wavelength range 400 – 700 nm. The λ_{max} which is the wavelength of maximum absorption was found to be 665 nm. This agrees with the value reported by Busari et al. [18], Snehalatha et al. [8], Ukoha [14], Sarah and Paul [7], and Mishra et al. [19]. A stock solution of benzenethiol (C_6H_5SH) was prepared by mixing a known volume of C_6H_5SH with little cm^3 of concentrated nitric acid and made up to the mark of volumetric flask with distilled water. Standard solutions of sodium formate, sodium chloride and sodium nitrate were prepared by dissolving a known weight of respective salts in known volume of distilled water.

2.1 Stoichiometric Studies

The stoichiometry of the reaction of methylene blue with benzenethiol was determined at λ_{max} = 665 nm by spectrophotometric titration using the mole ratio method. The concentration of benzenethiol range was (4.68 – 93.5) x 10^{-6} mole dm^{-3} with a constant concentration of methylene blue (1.87 x 10^{-5} mole dm^{-3}), $[H^+]$ = 0.3 mole dm^{-3} and T = 29ºC. The absorbances of the solutions were measured at λ_{max}, wavelength of maximum absorption = 665 nm, after the reactions were allowed to go to completion. This was when constant absorbance values were obtained.

2.2 Determination of Order

The rate of reaction was studied by monitoring the decrease in absorbance of methylene blue at λ_{max} = 665 nm using Corning 252 Colorimeter. Kinetic measurements were carried out under pseudo-first order condition with the $[C_6H_5SH]$ in at least 100 - fold excess over $[MB^+]$ at 29^0C. Ionic strength of the reaction medium as well as hydrogen ion concentration were maintained constant for each of the runs.

3. RESULTS AND DISCUSSION

The result of the stoichiometry determination of the reaction shows that one mole of benzenethiol was consumed per mole of methylene blue according to equation 1.

$$2MB^+ + 2C_6H5SH \longrightarrow 2MB + C_6H_5SSC_6H_5 + 2H^+ \quad [1]$$

Similar stoichiometry have been reported in the reactions of methylene blue with thiosulphate ion [13], mercaptoacetic acid [14], L-ascorbic acid [20], thiourea [21], and 1-methyl-2-thiourea [22].

Pseudo-first order plots of log $(A_t - A_\infty)$ against time (where A_t and A_∞ are the absorbances at time, t and at infinity respectively) were linear to more than 90% extent of the reaction. The linearity of the plots suggests that the reaction is first order with respect to [MB$^+$]. The order of the reaction with respect to [C$_6$H$_5$SH] was determined by plotting log k_1 against log [C$_6$H$_5$SH] from Table 1. The gradient of the linear graph was about unity. The first order obtained with respect to methylene blue is in agreement with what has been reported in the literature [10,18,21-24].

Table 1: Pseudo-first order and second order rate constant for the reaction of [MB$^+$] and [C$_6$H$_5$SH] at [MB$^+$] = 1.87 x 10^{-5} mole dm^{-3}, [H$^+$] = 0.3 mole dm^{-3}, T = 29^{0}C, μ = 1.0 mole dm^{-3} and λ$_{max}$ = 665 nm

10^3[C$_6$H$_5$SH], mole dm^{-3}	$10^3 k_1$, s^{-1}	k_2, dm^3 mole^{-1} s^{-1}
1.300	0.77	5.89
1.496	0.86	5.75
1.683	0.99	5.88
1.870	1.08	5.78
2.057	1.19	5.76
2.244	1.28	5.71
2.430	1.37	5.64
2.618	1.56	5.46

Within the hydrogen ion concentration range 0.3 ≤ [H$^+$] ≤ 0.9 mole dm^{-3} at a constant ionic strength, μ = 1.0 mole dm^{-3} and T = 29°C while keeping the [oxidant] and [reductant] constant, the reaction exhibited an inverse acid dependence. The nature of such H$^+$ dependence, suggests release of proton in a pre-equilibrium step and that the deprotonated form of benzenethiol is non-reactive. This observation conforms with the finding in the reaction of this dye with ascorbic acid [8] but at variance with its reduction with thiol compounds such as mercaptoacetic acid [14], whose rate constant increases with increase in [H$^+$]. The pseudo-first order rate constants, k_1, and the acid dependence second order rate constants k_2, determined are reported in Table 2. Linear plot of k_2 versus 1/[H$^+$] produced a straight line with an intercept (Fig. 2), suggesting that one of the two rate determining steps (equation 4) is preceeded by a rapid deprotonation equilibrium [25]. Both the deprotonated and the native species are reactive. A slope of 0.495 was obtained with R=0.996, when a linear plot of logk_1 versus

log[H$^+$] was made (Fig. 3), an indication that order with respect to deprotonation of benzenethiol is probably one (equation 3). We suspect that other interactions within the reaction medium might have taken place to account for the non-integral value.

Table 2: Acid dependence rate constants for the reaction of [MB$^+$] and [C$_6$H$_5$SH] at [MB$^+$] = 1.87 x 10^{-5} mole dm^{-3}, [C$_6$H$_5$SH] = 1.87 x 10^{-3} mole dm^{-3}, T = 29^{0}C, μ = 1.0 mole dm^{-3} and λ$_{max}$ = 665 nm

| [H$^+$], mole dm^{-3}	$10^2 k_1$, s^{-1}	k_2, dm^3 mole^{-1} s^{-1}
0.3	1.081	5.78
0.4	0.964	5.16
0.5	0.847	4.53
0.6	0.773	4.13
0.7	0.724	3.87
0.8	0.679	3.63
0.9	0.625	3.34

The observed first order and second order rate constants (k_1 and k_2 respectively) were determined as a function of ionic strength of reaction medium in the range 0.4 ≤ μ ≤ 1.0 mole dm^{-3} (NaNO$_3$) and are reported in Table 3. The plot of log k_2 against √μ (Fig. 4) indicates a positive Debye salt effect. This result suggests an interaction of species carrying same charge signs in the activated complex. According to Snehalatha et al. [8], reactions between two pairs of ions of like charges are usually accelerated by increasing ionic strength because of the increased favorable interactions of the activated complex with the denser ionic environment.

Table 3: Pseudo-first order and the ionic strength, μ dependence rate constants for the reaction of [MB$^+$] and [C$_6$H$_5$SH] at [MB$^+$] = 1.87 x 10^{-5} mole dm^{-3}, [C$_6$H$_5$SH] = 1.87 x 10^{-3} mole dm^{-3}, T = 29°C and λ$_{max}$ = 665 nm

μ, mole dm^{-3}	$10^3 k_1$, s^{-1}	k_2, dm^3 mole^{-1} s^{-1}
0.4	4.47	2.39
0.5	5.39	2.88
0.6	6.34	3.39
0.7	7.44	3.98
0.8	8.36	4.47
0.9	9.16	4.90
1.0	10.81	5.78
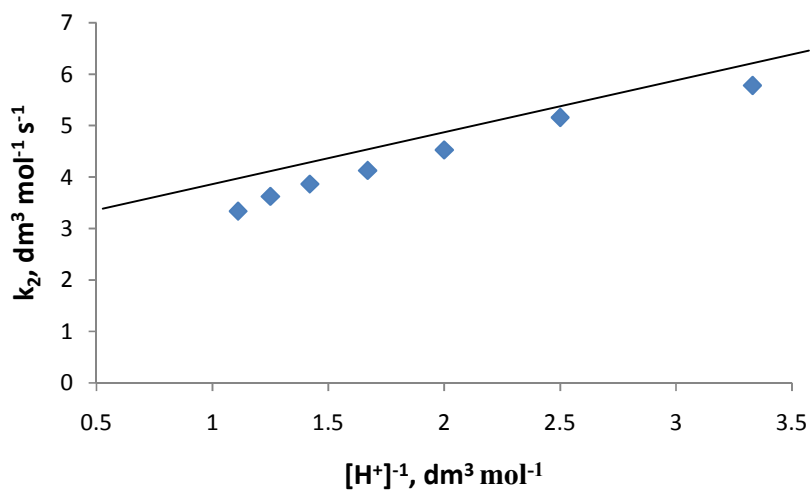
Fig. 2: Plot of the dependence of hydrogen ion concentration on second order rate constant of MB$^+$- C_6H_5SH reaction
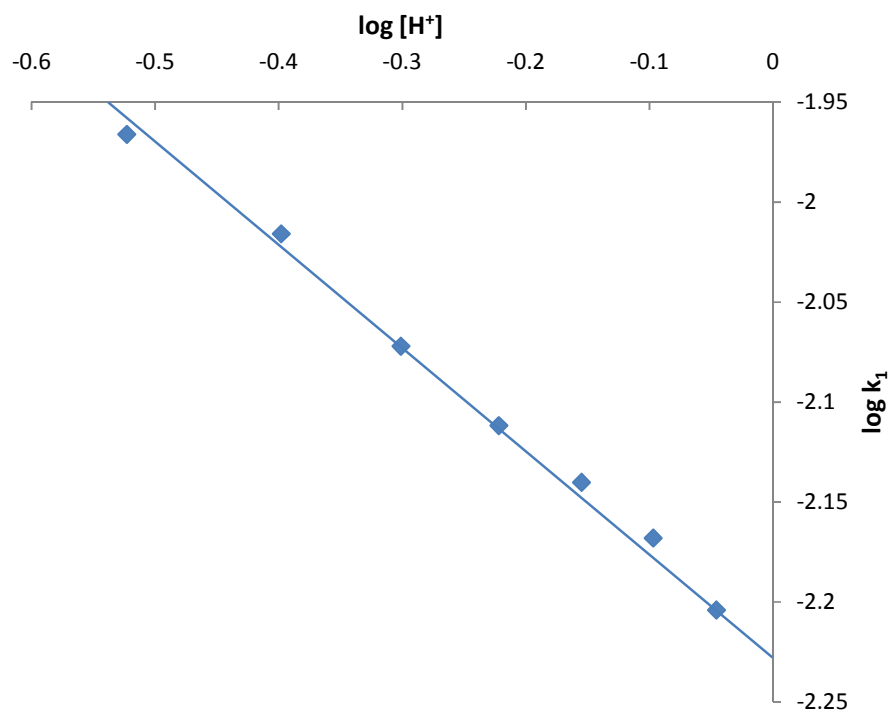
Fig. 3: Plot of log k_1 versus log [H$^+$] of MB$^+$- C_6H_5SH reaction
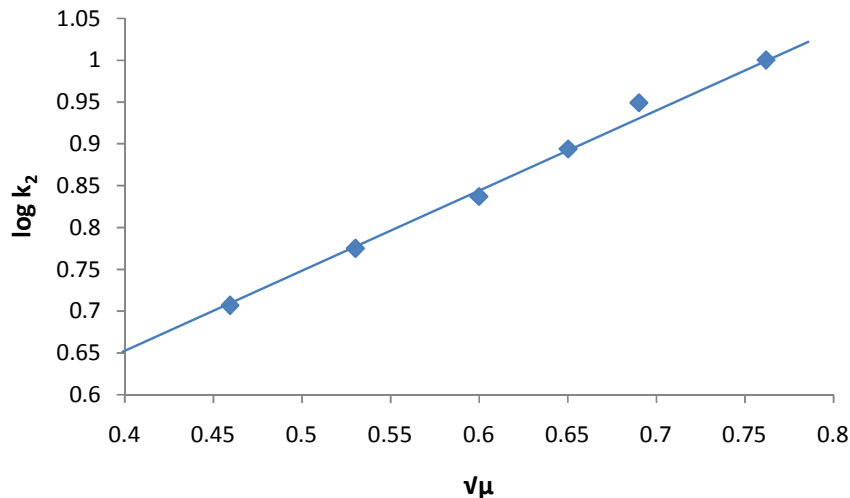
Fig. 4: Effect of ionic strength on MB$^+$- C$_6$H$_5$SH reaction rate

3.1 Effect of Added Anions on Reaction Rate

Table 4 shows increase in reaction rate with increase in concentration of the anions (HCOO$^-$ and Cl$^-$). Plots of k_2 against [anions] for these reactions were found to be linear (Figs. 5 & 6). Ion catalysis of reaction rate has been reported as being characteristic of the outersphere reaction mechanism because of the possibility of the intervention of these ions in the activated complex [26]. In this investigation, the anions HCOO$^-$ and Cl$^-$ were found to catalyzed the reaction. This suggests that the coordination integrity of the reactants are intact prior to and during the electron transfer process implying the possibility of an outersphere reaction mechanism.

3.2 Effect of Change in Dielectric Constant on Reaction Rate

The effect of change in dielectric constant (D) on the rate of the MB$^+$ - [C$_6$H$_5$SH] reaction was investigated at constant [oxidant], [reductant], μ and temperature. This was done by varying the % of acetone in acetone-water mixture as shown by equation 2. The decrease in rate constants with increase in D of reaction medium is presented in (Table 5). Observation of this nature is consistent with interaction of species of unlike charges or charged – neutral molecule in the rate determining step. This assertion conforms with equations 4 and 6. However, this is in contrast to the increase in reaction rate obtained upon increase in ionic strength of reaction medium.

Therefore, we suspect that other interaction could be taking place in the reaction medium to account for the latter observation.

Table 4: The effect of added HCOO$^-$ and Cl$^-$ on the rate of the reaction of methylene blue and benzenethiol at [MB$^+$] = 1.8 x I0^{-5} mole drn^{-3}, [C$_6$H$_5$SH]= 1.87 x 10^{-3} mole drn^{-3}, μ = 1.0 mole drn^{-3}, [H$^+$] = 0.3 mole drn^{-3}, T = 29^{0}C and λ_{max} = 665 nm

[HCOO$^-$]

10^3[HCOO$^-$], mole dm^{-3}	$10^2 k_1$, s^{-1}	k_2, dm^3 mole^{-1} s^{-1}
20	1.38	7.40
40	1.43	7.65
80	1.60	8.54
120	1.68	8.99
180	1.81	9.67
200	1.92	10.27
300	2.10	11.23

[Cl$^-$]

| 10^3[Cl$^-$], mol dm^{-3}	$10^2 k_1$, s^{-1}	k_2, dm^3 mol^{-1} s^{-1}
20	1.30	6.97
40	1.37	7.31
80	1.46	7.80
120	1.57	8.39
180	1.69	9.06
200	1.75	9.36
300	2.98	10.59

Table 5: The effect of variation of dielectric constant on the rate constants of MB$^+$ - C$_6$H$_5$SH Reaction at [MB$^+$] = 1.8 x 10^{-5} mole drn^{-3}, [C$_6$H$_5$SH] = 1.87 x 10^{-3} mole drn^{-3}, μ = 1.0 mole drn^{-3}, [H$^+$] = 0.3 mole drn^{-3}, T = 29°C and λ$_{max}$ = 665 nm

| D	% acetone	$10^2 k_1$, s^{-1}	k_2, dm^3 mole^{-1} s^{-1}
81.0	0	1.197	5.79
80.4	1	1.244	6.65
79.8	2	1.403	7.54
79.2	3	1.533	8.20
78.6	4	1.621	8.67
78.0	5	1.733	9.27
77.4	6	1.907	10.20
76.8	7	2.019	10.80
76.2	8	2.132	11.40
75.6	9	2.289	12.20

$$D_{reaction\ medium} = \frac{D_{H_2O} \times V_{H_2O} + D_{acetone} \times V_{acetone}}{total\ volume\ of\ solution}\quad [2]$$

where D_{H_2O}, V_{H_2O}, $D_{acetone}$ and $V_{acetone}$ are dielectric constant of water, volume of water, dielectric constant of acetone and volume of acetone respectively.

A linear plot of $1/k_1$ against $l/[C_6H_5SH]$, also called Michaelis- Menten plot (Fig. 7) which passed through the origin, shows that there was no binuclear complex formation of significant stability during the course of the reaction, affirming that an outersphere mechanism for this reaction is probable.
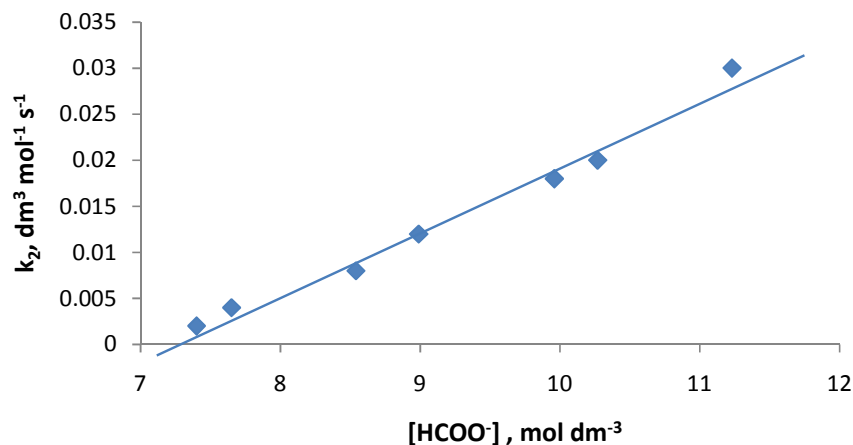
Fig. 5. Plot of [HCOO$^-$] dependence on second order rate constants of MB$^+$ - C$_6$H$_5$SH reaction
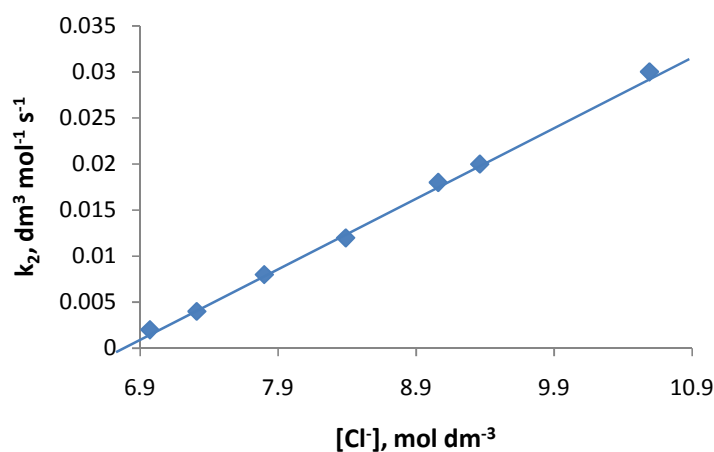
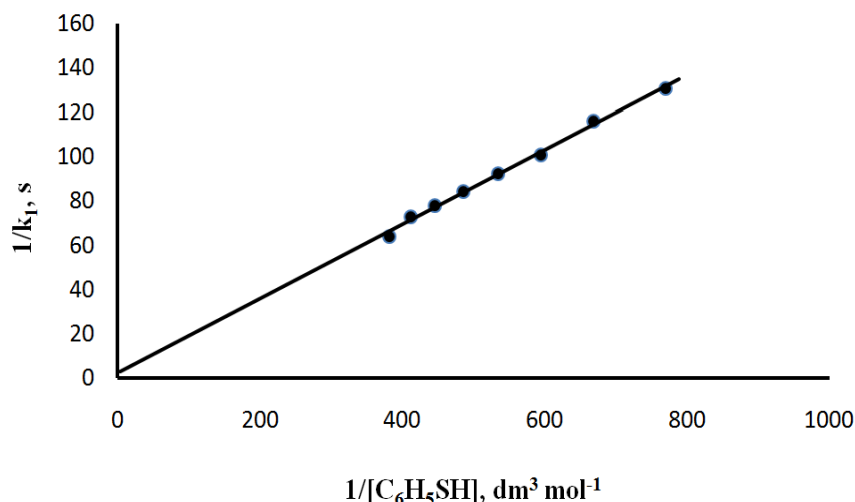**Fig. 6: Plot of [Cl$^-$] dependence on second order rate constants of MB$^+$ - C$_6$H$_5$SH reaction**
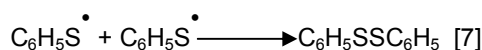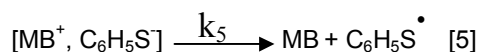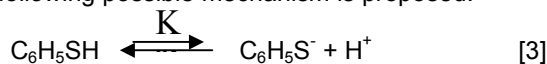
Fig. 7: Plot of $1/k_1$ versus $1/C_6H_5SH$ of MB^+- C_6H_5SH reaction

3.3 Reaction Mechanism

Taking recourse to the above findings, the following possible mechanism is proposed:

$$C_6H_5SH \xrightleftharpoons{K} C_6H_5S^- + H^+ \qquad [3]$$

$$MB^+ + C_6H_5S^- \xrightarrow{k_4} [MB^+, C_6H_5S^-] \quad slow \quad [4]$$

$$[MB^+, C_6H_5S^-] \xrightarrow{k_5} MB + C_6H_5S^\bullet \quad [5]$$

$$MB^+ + C_6H_5SH \xrightarrow{k_6} MB + C_6H_5S^\bullet + H^+ \quad slow \quad [6]$$

$$C_6H_5S^\bullet + C_6H_5S^\bullet \longrightarrow C_6H_5SSC_6H_5 \quad [7]$$

From equations 4 and 6 which are the rate determining steps, the rate law can be written as

$$\frac{-d}{dt}[MB^+] = k_4[MB^+][C_6H_5S^-] + k_6[MB^+][C_6H_5SH] \qquad [8]$$

and from equation 3

$$[C_6H_5S^-] = \frac{K[C_6H_5SH]}{[H^+]} \qquad [9]$$

Making the substitution of equation 9 into equation 8, the rate of the MB^+ - C_6H_5SH reaction can be represented by the expression

$$\frac{-d}{dt}[MB^+] = \frac{k_4K[MB^+][C_6H_5SH]}{[H^+]} + k_6[MB^+][C_6H_5SH] \qquad [10]$$

$$\frac{-d}{dt}[MB^+] = k_4K[MB^+][C_6H_5SH][H^+]^{-1} + k_6[MB^+][C_6H_5SH] \qquad [11]$$

Therefore, $\frac{-d}{dt}[MB^+] = (Kk_4[H^+]^{-1} + k_6)[MB^+][C_6H_5SH]$ $\qquad [12]$

Investigation of intermediate complex formation gave a negative result, suggesting that such species is not important in the reaction. Free radicals test that was carried out by adding about 1g of acrylamide to a partially oxidized mixture in excess methanol formed some suspension, an indication of generation of some free radicals in the reaction medium. This is consistent with the proposed mechanism (equations 5 and 6). It is worth noting, however, that the proposed formation of $[MB^+, C_6H_5S^-]$, an ion-pair in one of the pre-electron transfer steps did not lead to a shift in the λ_{max} of 665 nm for methylene blue. This suggests that MB^+ and this ion-pair absorb at the same λ_{max}. It is therefore proposed, based on the above results that, the reaction of methylene blue with benzenethiol occurs by the outersphere mechanism.

4. CONCLUSION

The kinetics and the mechanism of electron transfer reaction of methylene blue with the benzenethiol have been investigated in nitric acid medium. The reaction displayed a stoichiometry of 1: 1. It shows inverse first order hydrogen ion dependence and is catalyzed by added anions. The rate of the reaction increased with increasing

319

ionic strength and decreasing dielectric constant of the reaction media respectively. The test for radicals was positive. The reaction is therefore believed to occur by the outersphere mechanism and plausible mechanism consistent with these observations has been proposed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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