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Mechanism of the Reduction of Methylene Blue by Thiourea in Aqueous Acidic Medium

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Abstract: The kinetics of the reduction of methylene blue (MB^+) by thiourea (TU) in aqueous acidic medium, using spectrophotometric method, has been studied under pseudo-first order conditions of $T=25.0\pm1.0^{o}C, I=1.0$ mol dm^{-3}(NaCl), $[H^+]$ = 0.4 mol dm^{-3}(HCl) and λ_{max} = 660 nm. The reaction is second order and first order with respect to thiourea and methylene blue. The reaction shows a first order dependence on hydrogen ion concentration, while the rate of the reaction increased with increase in ionic strength but decreased with decrease in the dielectric constant of the reaction medium. The results of spectroscopic investigation and Michaelis-Mentens analysis support the formation of an intermediate complex in the course of the reaction. The reaction therefore conforms to the rate law $-\frac{d[MB^+]}{dt} = (a + b\,[H^+])[MB^+][TU]^2$.A plausible mechanism consistent with the inner-sphere mechanism has been proposed for this reaction.

Key Words: Kinetics, thiourea, reduction, stoichiometry, mechanism.

INTRODUCTION

Methylene blue (3, 7-Bis(dimethylamino) phenazo -thionium chloride, MB^+) is a water soluble polyaromatic cationic redox dye that has been widely studied since its synthesis in 1876[1]. This is largely due to its numerous uses in various field of science such as: in chemistry[2-4], biology[5-7], industries[8] and medicine[6,9], Methylene blue is easily reduced by various reducing agents to the colourless hydrogenated molecule, leucomethylene blue (LMB), which can be oxidized back to the blue colour. These processes have found application in numerous inventions like data recording holographic industries, optical data storage, food and pharmaceutical industries. Such reduction is also used in checking the purity of milk[10,11].

Thiourea, a thiocarbamide,(hereafter designated as TU), and the sulphur analogue of urea, has been known for over a century and a quarter during which time it has found a variety of uses in biological and chemical fields[12-15]. Most noted of these have been its uses as a plant growth stimulator to break bud dormancy and increase crop yield and more recently as a theurapeutic agent to threat thyroid dysfunction[16]. It belongs to a class of drugs used in the treatment of hyperthyroidism[17,18].

From the outlined uses of both methylene blue and thiourea and the kinetic investigations from literature, there is paucity of report dealing with reduction of methylene blue by thiourea. It is therefore considered worthwhile to investigate the electron transfer reaction between these organic compounds. It is hoped that the findings will find uses in understanding the electron transfer processes in the established uses of methylene blue and thiourea.

EXPERIMENTAL

Materials

All reagents and solvents were of analytical grade (BDH) and were used without further purifications. HCl was used to furnish H^+ to the reaction medium and NaCl was used to maintain a constant ionic strength for each run. Preparation of each solution was carried out using distilled water.

Methods

Spectrophotometric titration using the mole ratio method was employed in determining the stoichiometry of the reactants. The mole ratio of thiourea was varied between 1: 0.25 to 1: 2.25 (MB^+/ [TU] at $[MB^+] = 1.5\times 10^{-5}$ mol dm^{-3}; $[H^+] = 0.4$ mol dm^{-3}, $\mu = 1.0$ mol dm^{-3} (NaCl), and $T = 25.0 \pm 1.0^{o}C$. The reaction mixtures were allowed to undergo complete reaction as evident from the constancy of repeated measurements of absorbance at 660 nm. The absorbance (A_∞) was plotted against the mole ratio of the reactants and the point of sharp inflection in the plot gave the stoichiometry of the reaction.

The rate of reaction was studied under pseudo-first order condition with thiourea in at least 1000 fold excess over $[MB^+]$ at the stated conditions in Table 1 by monitoring the decrease in the absorbance of methylene blue at 660 nm using Corning Colorimeter 253. From the slopes of pseudo-first order plots of log (A_t-A_∞) versus time, the pseudo-first order rate constant (k_1) were determined. The third order rate constant (k_3) were obtained from $k_3 = k_1/[TU]^n$, where n is the order with respect to thiourea.

The effect of changes in $[H^+]$ on the reaction rate was investigated by keeping $[MB^+]$ and [TU] constant while varying $[H^+]$ between $(1.0 - 8.0) \times 10^{-1}$ mol dm^{-3} (HCl). Ionic strength, μ, was maintained at 1.0 mol dm^{-3} (NaCl) and reaction was carried out at $25.0 \pm 1.0^{0}C$. The order of reaction with respect to $[H^+]$ was obtained as the slope of the plot of log k_1 against log $[H^+]$. Variation of acid dependent rate constant with $[H^+]$ was obtained by plotting k_3 against $[H^+]$.

The ionic strength of the reaction mixture was varied between 0.50 and 2.00 mol dm^{-3} (NaCl) and maintaining $[MB^+]$, [TU] and $[H^+]$ constant at $25.0 \pm 1.0^{0}C$. Relationship of the reaction rate with changes in the ionic strength was determined by plotting log k_1 against $\sqrt{\mu}$.

The effect of medium dielectric constant, D, on the rate of reaction was investigated by using a binary solvent mixture of water and acetone. A plot of log k_1 against $\frac{1}{D}$ gave the relationship between the third order rate constant and the dielectric constant of the reaction medium.

At constant concentration of all other reactants, the effect of added ions (Mg^{2+}, Ca^{2+} , NO_3^- or SO_4^{2-}) on the rates of the reactions was investigated in the range $(3.0 - 16.0) \times 10^{-2}$ mol dm^{-3} (Table 2).

About 2g of acrylamide was added to a partially oxidized reaction mixtures followed by addition of a large excess of methanol. Control experiment was carried out by adding acrylamide to solutions of MB^+ and TU separately at the stated conditions of $[H^+]$, $[\mu]$ and temperature in Table 1. Gel formation indicates presence of free radicals.

At the completion of the reaction, the reaction mixtures were analyzed to determine the products formed. Likely products tested for were leucomethylene blue, urea and SO_4^{2-}**.** Mixtures of glucose/NaOH solution were added to the product in order to oxidize the colourless solution to $[MB^+]$. Dilute acidified solution of $BaCl_2$ was added to the product to test for the presence of SO_4^{2-}. Urea was tested for by the addition of few drops of dilute alkaline $CuSO_4$ solution.

RESULTS AND DISCUSSION

Stoichiometry

Stoichiometric result shows that one mole of MB^+ is consumed by one mole of TU as represented in the equation below,

$$MB^+ + SC(NH_2)_2 + 5H_2O \longrightarrow$$

$$SO_4^{2-} + OC(NH_2)_2 + 3H^+ + MBH \quad \text{................1}$$

A similar stoichiometry has been reported for the reactions of methylene blue with mercaptoacetic acid, L-ascorbic acid[19] and thiosulphate ion[20].

Order of Reaction

The pseudo-first plots of log (A_t-A_∞) versus time were linear for more than 70% of the reaction(where A_t and A_∞ are the absorbance at time t and at the end of the reaction respectively) .This implies a first order dependence on $[MB^+]$. A typical plot is presented in Figure 1. Pseudo-first order rate constants, k_1, determined as the slope of above are presented in Table 1.

The plot of log k_1 versus log [TU] was linear with a slope of 1.95 indicating second order dependence of the rate of reaction on [TU] (Figure 2). The third order rate constant, k_3, was obtained from $k_3 = \frac{k_1}{[TU]^2}$ and were found to be fairly constant (Table1).

Hence, the rate equation can be represented by equation 2,

$$-\frac{d[MB^+]}{dt} = k_3[MB^+][TU]^2 \quad \text{.....2}$$

Khan and Sarwar[21], Sarah and Paul[22], also reported a similar order in kinetics of methylene blue reduction by ascorbic acid.

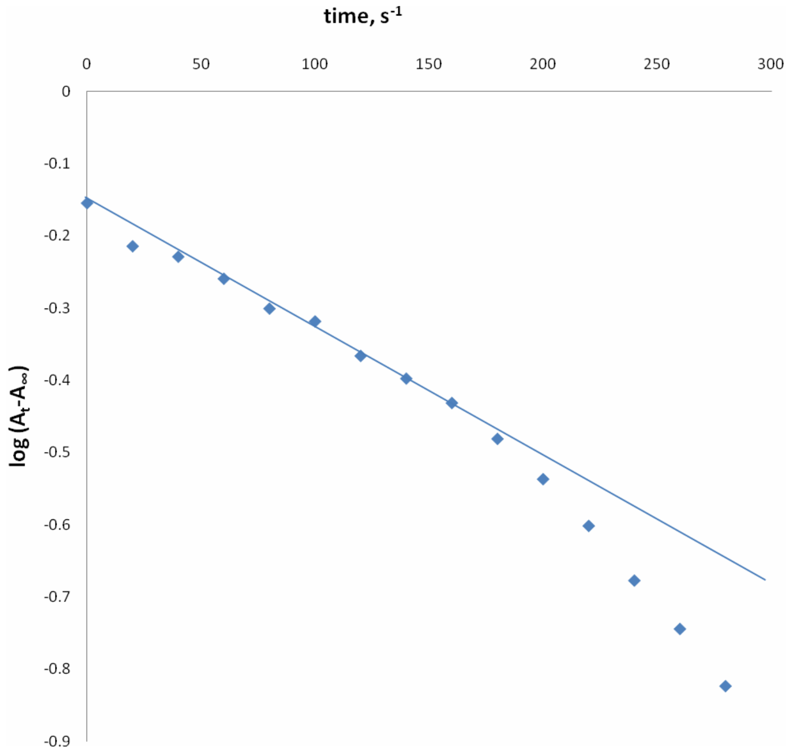

Figure 1: Typical Pseudo-first order plot for the redox reaction of MB^+ with TU
$[MB^+] = 1.5 \times 10^{-5}$ mol dm^{-3}; $[TU] = (3.0 – 13.5) \times 10^{-2}$ mol dm^{-3}
$[H^+] = 0.4$ mol dm^{-3}, $\mu = 1.0$ mol dm^{-3} (NaCl), T = 25.0 ± 1.0°C and $\lambda_{max} = 660$ nm

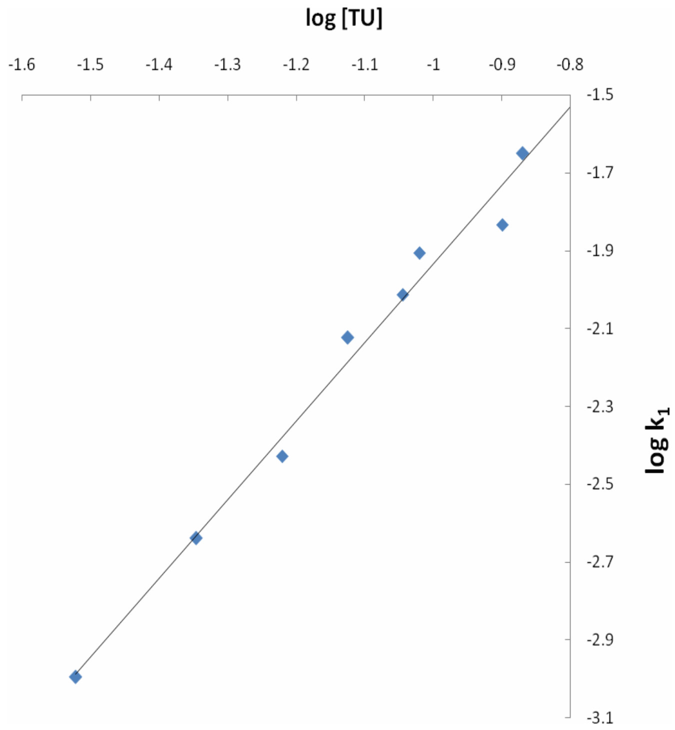

Figure 2: Plot of Log k_1 versus log [TU] for the redox reaction of MB^+ with TU
$[MB^+] = 1.5 \times 10^{-5}$ mol dm^{-3}; $[TU] = (3.0 – 13.5) \times 10^{-2}$ mol dm^{-3}
$[H^+] = 0.4$ mol dm^{-3}, $\mu = 1.0$ mol dm^{-3} (NaCl), T = 25.0 ± 1.0°C and $\lambda_{max} = 660$ nm

Table 1: Pseudo-first order and third order rate constants for the reaction of MB^+ with TU. $[MB^+] = 1.5 \times 10^{-5}$mol dm^{-3}, $\lambda_{max} = 660$ nm and T = 25.0 ± 1.0°C

10^2[TU] (mol dm^{-3})	10[H^+] (mol dm^{-3})	μ (mol dm^{-3})	10^3k_1 (s^{-1})	k_3 (dm^{6}mol^{-2} s^{-1})
3.0	4.0	1.00	1.01	1.12
4.5	4.0	1.00	2.03	1.13
6.0	4.0	1.00	3.73	1.04
7.5	4.0	1.00	7.53	1.33
9.0	4.0	1.00	9.17	1.13
10.5	4.0	1.00	12.40	1.12
12.0	4.0	1.00	14.70	1.02
13.5	4.0	1.00	22.40	1.23
9.0	1.0	1.00	3.30	0.41
9.0	2.0	1.00	6.14	0.76
9.0	3.0	1.00	7.53	0.93
9.0	4.0	1.00	9.60	1.18
9.0	5.0	1.00	13.75	1.69
9.0	6.0	1.00	14.50	1.79
9.0	7.0	1.00	16.20	2.00
9.0	8.0	1.00	17.19	2.12
9.0	4.0	0.50	8.45	1.04
9.0	4.0	0.75	9.20	1.13
9.0	4.0	1.00	10.74	1.32
9.0	4.0	1.25	12.10	1.49
9.0	4.0	1.50	14.50	1.79
9.0	4.0	1.75	17.35	2.14
9.0	4.0	2.00	18.60	2.29

Effect of Hydrogen ion Concentration

The effect of the [H^+] on the rate of reaction is reported in **Table 1.** The rates were found to be first order acid dependent on [H^+]. The plot of k_3 versus [H^+] was linear with a positive intercept. The acid dependent rate equations for the reactions can be represented by equation 3,

$$-\frac{d[MB^+]}{dt} = (a + b\,[H^+])[MB^+][TU]^2 \quad \text{------3}$$

where 'a' = 2.53 dm^3 mol^{-1} s^{-1} and 'b' = 0.221 dm^6 mol^{-2} s^{-1}

In the range $0.1 \leq [H^+] \leq 0.8$ moldm^{-3}, the rate of the reaction increases in the presence of hydrogen ions concentration (Table 1). The above equation 3 suggests that the reaction occurs via acid-dependent and acid-independent pathways. Similar report for the reduction of methylene blue have been observed by Iyun and Asala[23]; Bugaje[20] and Ukoha[24].

Effect of changes in ionic strength and total dielectric constant of the reaction medium

The relationship of reaction rate with changes in the ionic strength was determined by plotting log k_1 against $\sqrt{\mu}$ with a positive slope of 0.60 (Figure 3). The rate of reaction was found to increase with increase in ionic strength of the reaction medium (Table 1). . This positive salt effect suggests an interaction of species carrying same charge signs in the activated complex. This fact is also buttressed by the observed effect of varying the dielectric constant, D, of the reaction medium (Table 3) on the rate of reaction Thus, these results indicate a reaction pathway, involving ion-ion species[25]. Since MB^+ is positively charged, the predominantly reductant species is likely to be the protonated TU species

Effect of added ions

The rate constant was found to be unaffected by the presence of either NO_3^- or SO_4^{2-} as shown in Table 2. The presence of Ca^{2+} decreases the rate while Mg^{2+} did not affect the rate constant. The lack of catalysis as observed with the added anions and cations is in line with the formation of ion-pairs prior to electron transfer. Since the ion-pair complex does not possess a formal charge, interaction with added ions will not be very plausible hence the reaction is likely to follow inner-sphere pathway[26].

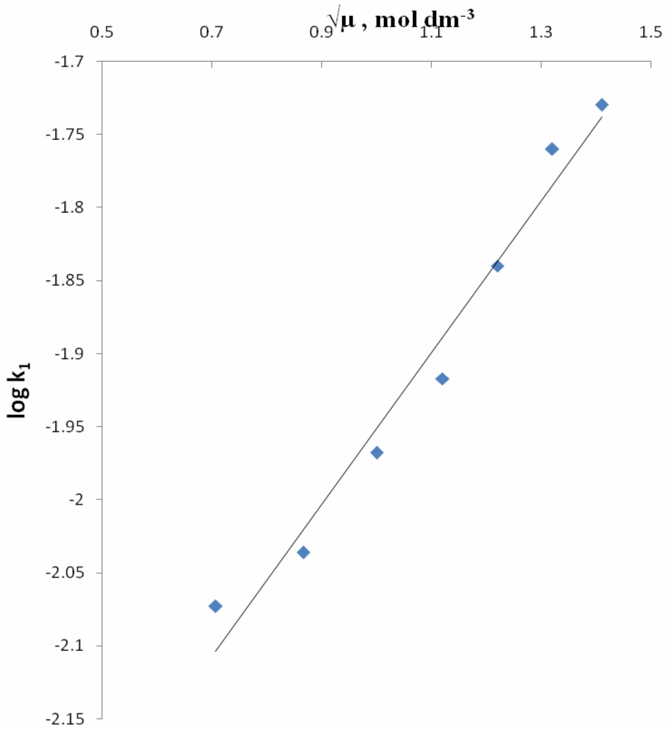

Figure 3: Log k_1 versus √μ for the redox reaction of MB^+ and TU
$[MB^+]$ = 1.5x 10^{-5} mol dm^{-3}; [TU] = 9.0 x 10^{-2} mol dm^{-3}, $[H^+]$ = 0.4 mol dm^{-3}, μ = 0.50 - 2.00 mol dm^{-3} (NaCl), T = 25.0 ± 1.0°C and λ_{max}= 660 nm

Table 2: Rate data for the effect of cations and anions on the third order rate constant for MB^+ - TU reaction. $[MB^+]$ = 1.5 x 10^{-5}mol dm^{-3}, [TU] = 9.0 x 10^{-2} mol dm^{-3}, λ_{max} = 660 nm and T = 25.0 ± 1.0°C

X	10^2[X] mol dm^{-3}	$10^2k_1(s^{-1})$	$k_3(dm^6mol^{-2}\ s^{-1})$
Mg^{2+}	0.00	1.11	1.37
	3.00	1.09	1.34
	8.00	1.05	1.29
	12.00	1.13	1.39
	14.00	0.99	1.22
	16.00	1.11	1.37
Ca^{2+}	0.00	1.11	1.37
	3.00	0.66	0.81
	8.00	0.51	0.62
	12.00	0.76	0.44
	14.00	0.22	0.27
	16.00	0.15	0.18
NO_3^-	0.00	1.11	1.37
	3.00	1.18	1.45
	8.00	1.14	1.40
	12.00	1.32	1.62
	14.00	1.29	1.59
	16.00	1.30	1.60
SO_4^{2-}	0.00	1.11	1.37
	3.00	1.19	1.46
	8.00	0.93	1.14
	12.00	1.00	1.23
	14.00	1.08	1.33
	16.00	1.03	1.27

Table 3: Effect of changes in total dielectric constant
$[MB^+] = 1.5 \times 10^{-5}$ mol dm^{-3}, $[TU] = 9.0 \times 10^{-2}$ mol dm$^{-3,}$ λ_{max} = 660 nm, T=25.0 ± 1.0°C

D	10^2 1/D	$10^3 k_1 (s^{-1})$	k_3 (dm^{6}mol^{-2}s^{-1})
81.00	1.23	11.10	1.37
79.80	1.25	11.40	1.41
78.59	1.27	8.45	1.04
77.36	1.29	6.94	0.86
76.20	1.31	5.50	0.68

Michaelis-Menten Plot

The Michaelis – Menten plot gave a negative intercept (Figure 4) which suggest the probable presence of preassociation step or presence of intermediates complex formation with appreciable equilibrium constant. This is also supported by a shift in λ_{max} from 660 nm to 670 nm when reactants were mixed together and the electronic spectra recorded after one minute of mixing. Similar negative intercept has been reported by Ukoha[19] while studying the reaction between MB^+ and mercaptoacetic acid.

Free Radical Test

Addition of acrylamide to partially oxidized reaction mixture followed by excess methanol showed no gel formation. This is suggestive of the probable absence of free radical formation during the electron transfer. On the other hand, free radical could have been formed but reacts so quickly that this method cannot detect it.

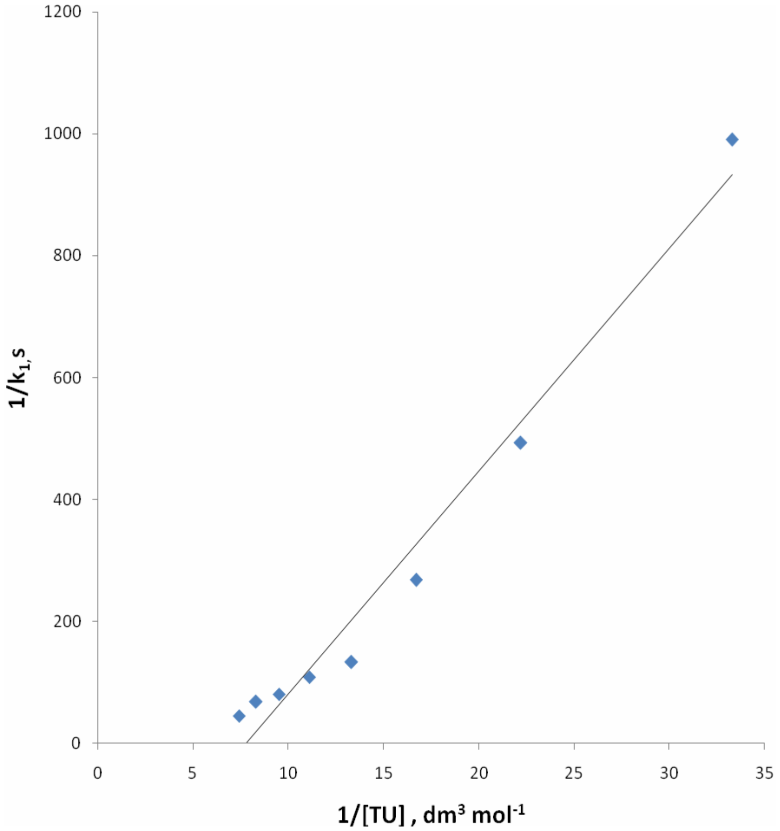

Figure 4: Michealis-Menten plot of $1/k_1$ versus 1/[TU] for the redox reaction of MB^+ and TU
$[MB^+] = 1.5 \times 10^{-5}$ mol dm^{-3}; $[TU] = (3.0 – 13.5) \times 10^{-6}$ mol dm^{-3}
$[H^+] = 0.4$ mol dm^{-3}, $\mu = 1.0$ mol dm^{-3} (NaCl), T = 25 ± 1°C and λ_{max} = 660 nm

Product Analysis
The blue colour of MB^+ was regenerated on the addition of NaOH/glucose mixture, showing the presence of leucomethylene blue. Addition of acidified $BaCl_2$ solution gave a white precipitate; an indication that SO_4^{2-} ion was one of the reaction products.

A pinkish-violet colour which gradually turned bluish was obtained with the addition of dilute alkaline $CuSO_4$ solution. This shows the presence of urea[27].
On the basis of the above results, the following mechanism has been proposed.

$$MB^+ + H^+ \overset{K_1}{\rightleftharpoons} MBH^{2+} \qquad ---(1)$$

$$MBH^{2+} + [TU] \overset{K_2}{\rightleftharpoons} [MBH^{2+}, TU] \qquad ---(2)$$

$$[MBH^{2+}, TU] + TU \xrightarrow{k_3} products \qquad ---(3)$$

$$TU + MB^+ \overset{K_4}{\rightleftharpoons} [TU, MB^+] \qquad ---(4)$$

$$[TU, MB^+] + TU \xrightarrow{k_5} products \qquad ---(5)$$

Rate = $k_3[TU.MBH^{2+}][TU] + k_5[TU.MB^+][TU]$ $\qquad ---(6)$

From equation (1) and (2)

Rate = $k_3K_1K_2[TU]^2[H^+][MB^+] + k_5[TU.MB^+][TU]$ $\qquad ---(7)$

But from equation (4)

Rate = $k_3K_1K_2[TU]^2[H^+][MB^+] + k_5K_4[MB^+][TU]^2$ $\qquad ---(8)$

Let $k_3K_1K_2$ = k and k_5K_4 = $\mathbf{k}'$

Rate = $k[TU]^2[H^+][MB^+] + \mathbf{k}'[MB^+][TU]^2$ $\qquad ---(9)$

Rate = $[(\mathbf{k}]' + \mathbf{k}[H^+])[MB^+][TU]^2$ $\qquad ---(10)$

Equation 10 is similar to equation 3.

where: k' = a = 2.53 $dm^3\,mol^{-1}s^{-1}$ and k = b = 0.221 $dm^6\,mol^{-2}\,s^{-1}$ confirming the plausibily of the proposed mechanism.

Based on the above results, the inner-sphere mechanism is proposed for the reaction.

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