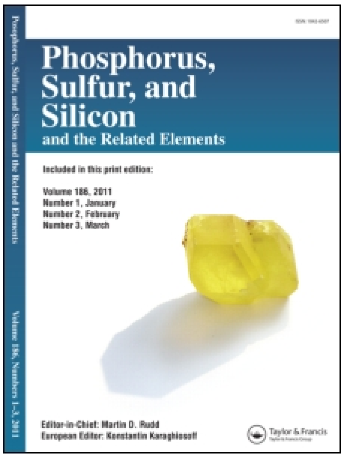

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KINETICS AND MECHANISM OF THE OXIDATION OF THIOUREA BY METHYLENE BLUE IN ACIDIC MEDIUM

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KINETICS AND MECHANISM OF THE OXIDATION OF THIOUREA BY METHYLENE BLUE IN ACIDIC MEDIUM

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The title reaction has been investigated kinetically in methanol-water medium (3% v/v) and in presence of hydrochloric acid. The reaction follows a half order kinetics in methylene blue (MB) and a first order kinetics in thiourea (TU). At higher [TU] or at lower [MB] (ca. 1.5×10^{-5} M), the order in the oxidant changes from 1/2 to zero. The rate increases linerarly on increasing [H^+]. Addition of EDTA and the variation in ionic strength has no influence on the rate except the addition of potassium chloride which may be attributed to the environmental effect. The increase in dielectric constant increases the rate. The external addition of the reaction products does not influence the rate but higher concentrations of leucomethylene blue tend to exert a slightly retarding influence. Activation parameters have been evaluated and a reaction scheme has been proposed postulating a sterically controlled nucleophilic attack of thiourea molecule on half-reduced methylene blue radical as the rate limiting step.

Key words: Thiourea; oxidation by methylene blue

Methylene blue is known to be a model electron receptor and has been extensively used for the elucidation of the mechanism of the oxidation of flavin bound enzymes.[1] The spectral properties of methylene blue have also been exploited for measuring the surface activity of synergists. Methylene blue being a cationic dye, has been used for studying the ion pair formation and recently the determination of iodide ion at ppb level has been reported.[2] The substrate also finds enormous applications as a ligand, as a metabolite in living systems and as an important ingredient in pharmaceutical formulations. Keeping this in view, the kinetics of the oxidation of thiourea has been described in this communication. It is pertinent to note that the present work constitutes a part of our investigations on the interaction of methylene blue with sulfydryl substrates[3–5] and secondly, the kinetic investigations on the oxidation of thiourea are relatively scarce.

RESULTS

Thiourea and methylene blue interact to form formamidine disulfide and the leucobase under given conditions. The formation of the disulfide was confirmed spectrophotometrically by comparing the IR spectra of the product isolated from the reaction mixture having excess thiourea with the known sample of the disulfide (Figure 1). The rate of reaction increases gradually and attains a maximum after about 3 hours when thiourea is pre-equilibrated with acid as shown in Table I. The runs were made in a nitrogen atmosphere although its absence did not make any

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* For correspondence.

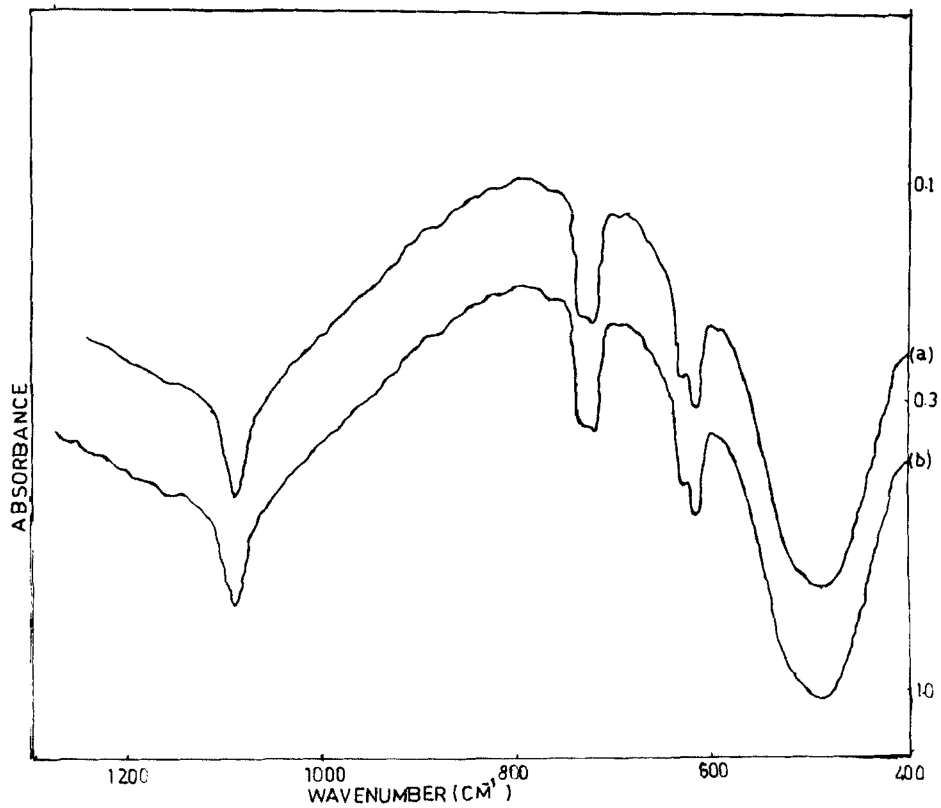


FIGURE 1 (a) IR spectrum of formamidine disulfide obtained on oxidation of TU with H_2O_2; (b) IR spectrum of the product isolated from the reaction system.

significant change in the rate of reaction as illustrated by the representative runs given in Table II.

The reaction follows a pseudo half order kinetics with respect to methylene blue, when 400 to 700 fold excess concentration of thiourea over the oxidant (ca. 3.0×10^{-5} M) is employed. The calculated pseudo half order rate constants are given

TABLE I

Rate constants for varying pre-equilibration time of thiourea

Time of equilibration with HCl (min)	$k_{1/2} \times 10^6 \text{mole}^{1/2} \text{litre}^{-1/2} \text{sec}^{-1}$
15	0.83
30	1.1
60	1.4
120	1.6
150	1.7
180	1.7

[MB] = 3.0×10^{-5} M
[TU] = 1.5×10^{-2} M
[HCl] = 12.0×10^{-2} M
[KCl] = 2.0×10^{-2} M
I = 14.0×10^{-2} M
CH_3OH = 3% (v/v)
Temp = 35°C

TABLE II

In Absence of Nitrogen			In presence of Nitrogen		
Time in Seconds	$[MB] \times 10^5$ M	$k_{1/2} \times 10^6 mole^{1/2}$ $litre^{-1/2}$ sec^{-1}	Time in Seconds	$[MB] \times 10^5$ M	$k_{1/2} \times 10^6 mole^{1/2}$ $litre^{-1/2}$ sec^{-1}
0	3.000	—	0	3.000	—
60	2.900	3.1*	60	3.000	—
300	2.725	1.7	180	2.875	1.3*
420	2.650	1.6	300	2.775	1.4
600	2.475	1.7	600	2.500	1.6
900	2.250	1.6	900	2.250	1.6
1200	2.000	1.7	1200	2.100	1.5
1500	1.800	1.6	1500	1.825	1.6
1800	1.650	1.6	1800	1.658	1.6
2100	1.425	1.6	2100	1.450	1.6
2400	1.275	1.6	2400	1.275	1.6
2700	1.100	1.6	2700	1.100	1.6
3000	0.900	1.7	3000	0.925	1.6

Average value of $k_{1/2}$ (excluding* value)
= $1.6 \times 10^6 mole^{1/2}$ $litre^{1/2}$ sec^{-1}
c.v. = 3.95%

Average value of $k_{1/2}$ (excluding* value)
= $1.6 \times 10^6 mole^{1/2}$ $litre^{-1/2}$ sec^{-1}
c.v. = 4.65%

$[MB] = 3.0 \times 10^{-5}$ M
$[TU] = 1.5 \times 10^{-2}$ M
$[HCl] = 12.0 \times 10^{-2}$ M
$[KCl] = 2.0 \times 10^{-2}$ M
$I = 14.0 \times 10^{-2}$ M
CH_3OH = 3% (v/v)
Temp = 35°C

TABLE III

Rate constants at different concentrations of TU

Run	$[TU] \times 10^2$ M	$k_{1/2} \times 10^6 mole^{1/2}$ $litre^{-1/2}$ sec^{-1}
1	1.20	1.2
2	1.34	1.3
3	1.50	1.6
4	1.64	1.7
5	1.80	1.9
6	2.10	2.1

$[MB] = 3.0 \times 10^{-5}$ M
$[HCl] = 12.0 \times 10^{-2}$ M
$[KCl] = 2.0 \times 10^{-2}$ M
$I = 14.0 \times 10^{-2}$ M
CH_3OH = 3% (v/v)
Temp = 35°C

in Table III. The results shown in Table III indicate that the rate increases linearly on increasing [TU]. A plot of log $k_{1/2}$ versus log [TU] gives a straight line with a slope of 1.1 suggesting a first order behaviour in thiourea. This was further confirmed by the initial rate measurement method.

The rate increases on increasing [MB] as shown in Table IV. It is seen that at higher concentrations of methylene blue, the reaction adheres to half order kinetics in the oxidant as verified by plotting log $k_{1/2}$ against log [MB] which gives a straight line with a slope of 0.6. At lower [MB] (ca. $< 2.5 \times 10^{-5}$ M), however, the time order in methylene blue is found to be zero. In spite of this transition in time order in the oxidant, the concentration order is found to be unity as confirmed by measuring the rates after 10% reduction of the oxidant and subsequently plotting log

TABLE IV

Rate constants at different [MB]

[MB] × 10^5 M	$k_{1/2}$ × 10^6mole$^{1/2}$ litre$^{-1/2}$ sec^{-1}	k_0 × 10^9mole litre^{-1} sec^{-1}
1.25	—	3.0
1.45	—	3.6
2.00	—	4.7
2.50	1.4	—
3.00	1.6	—
3.50	1.7	—

[TU] = 1.5 × 10^{-2} M

[HCl] = 12.0 × 10^{-2} M

[KCl] = 2.0 × 10^{-2} M

I = 14.0 × 10^{-2} M

CH_3OH = 3% (v/v)

Temp = 35°C

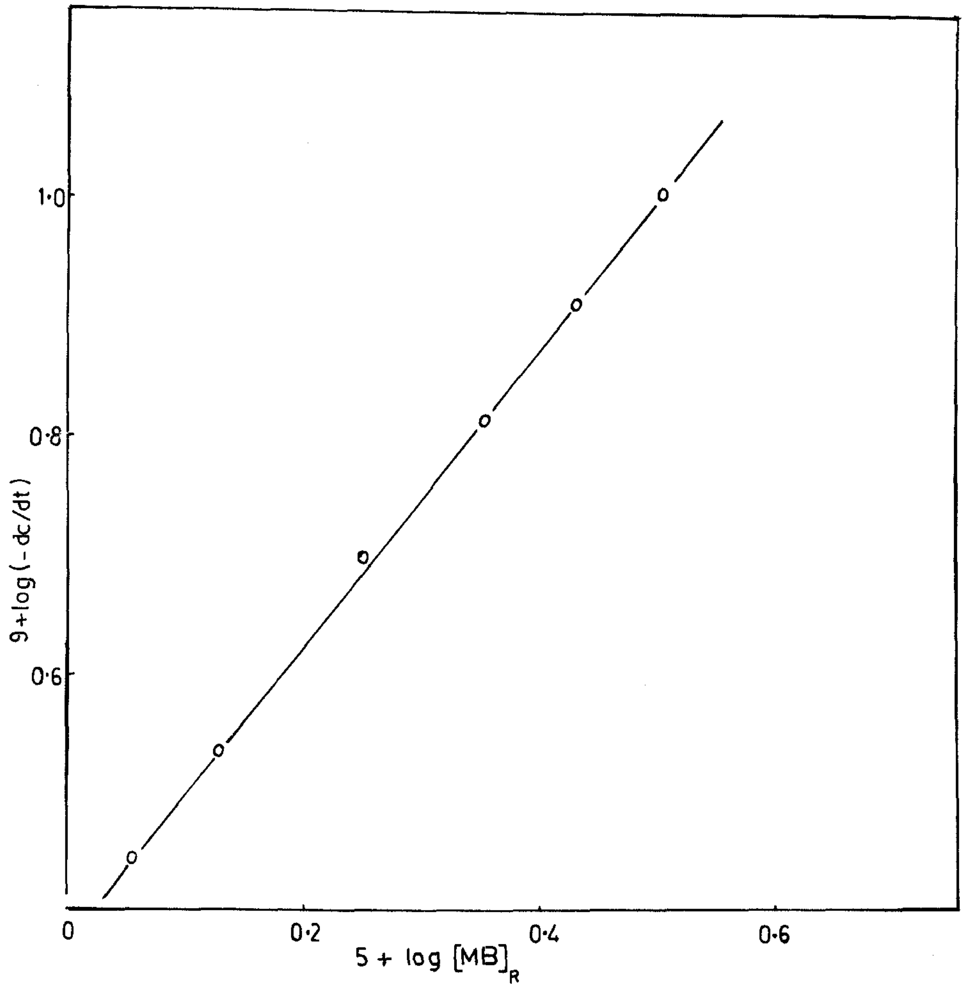


FIGURE 2 Initial concentration of MB = 1.25, 1.45, 2.00, 2.50, 3.00, 3.5 × 10^{-5} M. $[MB]_R$ = Residual concentration of MB after 10% completion of the reaction. [TU] = 1.5 × 10^{-2} M; CH_3OH = 3% (v/v); [HCl] = 12.0 × 10^{-2} M; I = 14.0 × 10^{-2} M; [KCl] = 2.0 × 10^{-2} M; Temp. = 35°C.

(−dc/dt) against log [MB] residual which yields a straight line with a slope equal to 1.2 (Figure 2).

The transition in order in methylene blue was further checked by making a number of runs having 600 to 1400 fold excess of thiourea while employing a low [MB] (ca. 1.5×10^{-5} M). It was found that at lower [TU], reaction follows a half order kinetics whereas at higher [TU], the zero order dependence in methylene blue is noticed. The rate constants obtained at different [TU] are given in Table V. This indicates that the critical concentration ratio of the principal reactants has got a very significant kinetic influence.

The rate increases on increasing $[H^+]$ (Table VI). The ionic strength of the system was kept constant (ca. 24.0×10^{-2} M) in these variations by adding the requisite amount of potassium chloride. A plot of log $k_{1/2}$ versus log $[H^+]$ gives a straight line with a slope of 1.2 suggesting a linear dependence of rate on $[H^+]$.

The rate remains practically unaffected on varying ionic strength of the system. However, the addition of potassium chloride slightly accelerates the rate which may be attributed to the environmental effect. The rate increases on increasing the dielectric constant of the medium and a plot of log $k_{1/2}$ and 1/D gives a straight line with a negative slope which indicates the possibility of the participation of similarly charged species in the rate determining step.

TABLE V

Rate constants at different [TU] at lower [MB]

[TU] $\times 10^{-2}$ M	$k_{1/2} \times 10^6$ mole$^{1/2}$ litre$^{-1/2}$ sec^{-1}	$k_0 \times 10^9$ mole litre^{-1} sec^{-1}
0.9	0.7	—
1.2	1.0	—
1.5	—	4.1
1.8	—	5.1
2.1	—	5.6

[MB] = 1.5×10^{-5} M
[HCl] = 12.0×10^{-2} M
[KCl] = 2.0×10^{-2} M
I = 14.0×10^{-2} M
CH_3OH = 3% (v/v)
Temp = 35°C

TABLE VI

Rate constants at different $[H^+]$

[HCl] $\times 10^2$ M	$k_{1/2} \times 10^6$ mole$^{1/2}$ litre$^{-1/2}$ sec^{-1}
4.0	0.6
6.0	0.8
8.0	1.1
10.0	1.5
12.0	1.8
13.5	2.2
15.0	2.6

[MB] = 3.0×10^{-5} M
[TU] = 1.5×10^{-2} M
CH_3OH = 3% (v/v)
I = 24.0×10^{-2} M
Temp = 35°C

TABLE VII

Rate constants at different $[H_2MB]$

$[H_2MB] \times 10^5$ M	$k_{1/2} \times 10^6 \text{mole}^{1/2} \text{litre}^{-1/2} \text{sec}^{-1}$
0.0	1.6
0.4	1.6
1.2	1.5
2.0	1.4
3.0	1.2

$[MB] = 3.0 \times 10^{-5}$ M

$[TU] = 1.5 \times 10^{-2}$ M

$[HCl] = 12.0 \times 10^{-2}$ M

$[KCl] = 2.0 \times 10^{-2}$ M

$I = 14.0 \times 10^{-2}$ M

CH_3OH = 3% (v/v)

Temp = 35°C

The external addition of the disulfide and the leuco base does not influence the rate but it is slightly retarded by the addition of higher concentrations of leuco methylene blue (Table VII).

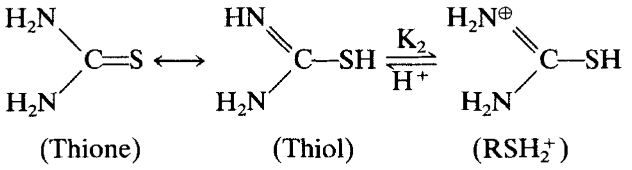
Activation parameters were determined by studying the reaction at different temperatures (30° to 50°C) and the applicability of Arrhenius equation was tested. The enthalpy, entropy and the free energy of activation were found to be 28.7 kJ mole^{-1}, -266.1 $\text{JK}^{-1}\text{mole}^{-1}$ and 110.6 kJ mole^{-1} respectively.

DISCUSSION

Methylene blue is known to be protonated in acidic medium[6–9] as shown below.

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

The kinetic results indicate that the equilibration with acid increases the reactivity of thiourea which may perhaps be attributed to the protonation of its thiol form.[10]



(2)

The protonated species may undergo a nucleophilic attack by a thiourea molecule forming thiyl radical and semi-reduced methylene blue radical (HM˙).

$$RSH + MBH^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RS^{\cdot} + HM^{\cdot} + H^+ \qquad (3)$$

It may be added here that the participation of thiyl radicals in these systems is quite prevalent in literature[11] while the formation of semireduced methylene blue radical by the action of reducing substrates such as ascorbic acid has also been reported by the earlier workers.[12] The participation of free radicals during the course of reaction has been qualitatively demonstrated by the positive polymerization test with acrylonitrile.[13]

The semireduced methylene blue radical (HM˙) is expected to have a distorted symmetry of anthracene ring as suggested in the photochemical dimerisation of anthracence[14] due to occupation of antibonding π orbital by the odd electron. This may considerably hamper the rate of nucleophilic attack by a sluggish thiourea molecule on this species. Largely due to this, this interaction has been presumed to be the rate determining step. Thus,

$$HM^{\cdot} + RSH_2^+ \xrightarrow{k_2} RS^{\cdot} + H_2M + H^+ \text{ (rate determining step)} \tag{4}$$

$$2RS^{\cdot} \xrightarrow[\text{fast}]{k_3} RSSR \tag{5}$$

The rate of reaction for the above reaction scheme would be given by the rate expression

$$-\frac{d[MB]}{dt} = k_2[HM^{\cdot}][RSH_2^+] \tag{6}$$

On presuming steady state for HM˙ and RS˙

$$[HM^{\cdot}] = \frac{k_1[RSH][MBH^+]}{k_{-1}[RS^{\cdot}][H^+] + k_2[RSH_2^+]} \tag{7}$$

and

$$k_{-1}[RS^{\cdot}]^3[H^+] + k_2k_3[RSH_2^+][RS^{\cdot}]^2 - 2k_1k_2[RSH_2^+][RSH][MBH^+] = 0 \tag{8}$$

On substituting the value of [HM˙] in Equation (6), the rate is given by

$$-\frac{d[MB]}{dt} = \frac{k_1k_2[RSH_2^+][RSH][MBH^+]}{k_{-1}[RS^{\cdot}][H^+] + k_2[RSH_2^+]} \tag{9}$$

The cubic Equation (8) was transformed in to the standard form[15]

$$p_3(x) \equiv a_3\,x^3 + a_2\,x^2 + a_1\,x + a_0 = 0 \tag{10}$$

The real root of x (x_1) is given by

$$x_1 = -\frac{a_2}{3a_3} + (\{\sqrt{p^3 + Q^2}\} - Q)^{1/3} - (\{\sqrt{p^3 + Q^2}\} + Q)^{1/3} \tag{11}$$

Where

$$P = \frac{a_1}{3a_3} - \left(\frac{a_2}{a_3}\right)^2 \tag{12}$$

and

$$Q = \frac{1}{2a_3}\left(a_0 - \frac{a_2^3}{27\,a_3^2} - a_2\,P\right) \tag{13}$$

On substituting $a_1 = 0$ and presuming $P \longrightarrow 0$ under the conditions $k_{-1}[H^+] >> k_2\,[RSH_2^+]$,

$$Q = -\left[\frac{a[RSH_2^+][RSH][MBH^+][H^+]^2 + (k_2k_3[RSH_2^+])^2}{27 \times 2 \times (k_{-1}[H^+])^3}\right] \qquad (14)$$

Where

$$a = 27 \times 2 \times k_1k_2k_{-1}^2 \qquad (15)$$

Thus,

$$x_1 = -\frac{a_2}{3a_3} + -[2\,Q]^{1/3} \qquad (16)$$

$$[RS^\cdot] = -\frac{k_2k_3[RSH_2^+]}{3\,k_{-1}[H^+]} + \left[\frac{a[RSH_2^+][RSH][MBH^+][H^+]^2 + (k_2k_3[RSH_2^+])^3}{27\,(k_{-1}[H^+])^3}\right]^{1/3} \qquad (17)$$

If $k_{-1}[H^+]$ is larger;

$$[RS^\cdot] = \left[\frac{a[RSH_2^+][RSH][MBH^+][H^+]^2 + (k_2k_3[RSH_2^+])^3}{27\,(k_{-1}[H^+])^3}\right]^{1/3} \qquad (18)$$

Thus, the rate of reaction is given by

$$-\frac{d[MB]}{dt} = \frac{3k_1k_2[RSH_2^+][RSH][MBH^+]}{[a[RSH_2^+][RSH][MBH^+][H^+]^2 + (k_2k_3[RSH_2^+])^3]^{1/3}} \qquad (19)$$

or,

$$-\frac{d[MB]}{dt} = \frac{3k_1k_2K_1K_2[RSH]^2[MB][H^+]}{[a\,K_1K_2[RSH]^2[MB][H^+] + (k_2k_3K_2[RSH][H^+])^3]^{1/3}} \qquad (20)$$

$$-\frac{d[MB]}{dt} = \frac{3k_1k_2K_1K_2[RSH]^{1\cdot 3}[MB][H^+]}{[a\,K_1K_2[MB][H^+] + (k_2k_3K_2)^3[RSH]]^{1/3}} \qquad (21)$$

Equation (21) explains a near first order dependence of rate on [TU], a fractional order in MB and a complex relationship between the rate and $[H^+]$. Initially, $[RS^\cdot]$ is expected to be much smaller and it is evident from Equation (9) that the reaction will adhere to a first order kinetics in MB as has been confirmed from the initial rate measurement method. It also appears likely that the radical reaction becomes more prominent at higher [TU] or at low [MB] and thus, the recombination of radicals becomes very significant under these conditions specifically involving the disproportionation of semireduced methylene blue radical to form the parent dye as reported in case of semiquinone radicals.[16] This equilibrium would result in a limiting concentration of $HM^\cdot$ radical to give a zero order kinetics in [MB]. Incidentally, a similar transition to zero order kinetics in hexacyanoferrate (III) ion has been noticed by Mishra and Kalla in the oxidation of 1-butanethiol and 2-butanethiol in presence of externally added hexacyanoferrate (II) ions.[17]

The present investigations have unequivocally highlighted the complexities of these reaction systems and have prompted us to extend the work to probe the kinetics of the oxidation of biologically significant sulfhydryl substrates and their induced effect on the reactivity of thiourea.

EXPERIMENTAL

A standard solution of methylene blue was prepared in double distilled water by an exact weighing of the sample supplied by M/S Farbwerke Hoechst, Germany ($E_{max} = 4.5 \times 10^4$ lit. mole^{-1}cm^{-1}). The sample of thiourea obtained from Reanal (Hungary), (assay 99.2%) was used to prepare the substrate solution by dissolving an exactly weighed quantity in double distilled water.

Thiourea solution was thermostated for 3 hours at 35°C in order to facilitate its conversion into a relatively more reactive form which might probably involve the thiol-thione tautomerism. The runs were made in methanol water medium (3% v/v) and in presence of hydrochloric acid. The solutions of the oxidant and the substrate were stored in a dark place to avoid the possibility of photochemical reactions. All other reagents used in these investigations were either of BDH AnalaR or S. Merck's GR grade.

The runs were made in presence of EDTA (disodium salt) in order to check the interference of metal ion impurities present, if any, in thiourea sample. It was found that the rate remains unaffected on adding EDTA (ca. 1.0×10^{-2} M). The reaction mixtures were thermostated (variation ± 0.1°C) in reaction vessels made of Pyrex glass coated with Black Japan. The disulfide (the oxidation product) was prepared by oxidising thiourea with equivalent amount of hydrogen peroxide[18] and extracted with methanol and ether. The leucomethylene blue was obtained by bleaching a known solution of the oxidant by passing hydrogen gas liberated from Sn-HCl couple. The solutions were always stored in a nitrogen atmosphere. The kinetics of the reaction was followed colorimetrically on a Klett-Summerson photoelectric colorimeter fitted with an orange light filter, since methylene blue shows λ max at 660 mm and the spectral interference in this region due to the leucobase, thiourea and the disulfide is ruled out. The concentration of methylene blue was determined from the Beer's law plots because the law is found to be valid in the entire concentration range employed presently.

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