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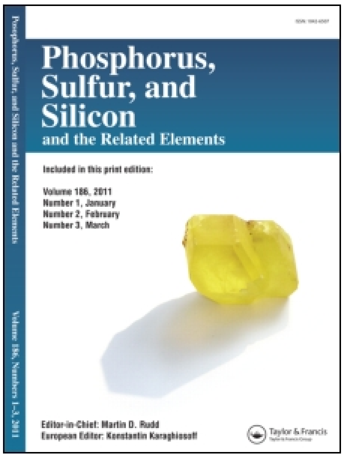

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KINETICS OF Cu(II) CATALYSED-OXIDATION OF CYSTEINE HYDROCHLORIDE BY METHYLENE BLUE IN ACIDIC MEDIUM

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The kinetics of Cu(II) catalysed-oxidation of cysteine hydrochloride under anaerobic conditions and in presence of hydrochloric acid have been described. The stoichiometry is found to be 2:1 and cystine is the oxidation product. The order is unity in cysteine as well as in methylene blue but at higher [cysteine] and low [MB], the order in MB changes to half. The rate increases on increasing [H^+] and [copper sulphate] but at higher [catalyst] the rate attains a limiting value. Similarly, the rate tends to become independent of variation in [H^+] at higher concentration. The increase in ionic strength increases the rate but it is not influenced by the addition of cystine or the leuco base. The reaction scheme postulating the interaction of cysteine with protonated MB as the rate determining step has been verified by studying the reaction under aerobic conditions.

Key words: Cysteine; oxidation by methylene blue.

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Cysteine, a metabolite and precursor in coenzyme A synthesis is widely regarded as a pivot in sulphur metabolism along with glutathione. The oxidant used presently is methylene blue which has been employed as a model electron receptor for mitochondrial electron transfer processes[1,2] and in continuation of our work,[3-6] the kinetics of Cu(II)-catalysed interaction of cysteine with this oxidant have been studied in acidic medium under anaerobic as well as aerobic conditions. It may be recalled that the oxidation of cysteine under diverse conditions has been investigated[7] and recently kinetic studies on the copper-catalysed oxidation of this substrate in alkaline medium by dioxygen[8] and on the oxidation of Cu(II) complexes of cysteine[9] have been reported. The kinetic features reported herein are markedly different from earlier studies and in spite of a scanty resemblance with those obtained for the oxidation of thiourea by this oxidant,[5] several new features of this reaction have made these reaction systems extremely interesting.

RESULTS

The order of reaction in methylene blue was determined with the help of Ostwald's isolation method. The runs having an excess concentration of cysteine over the oxidant were made in presence of hydrochloric acid (ca. 3.0×10^{-3} M). The reaction follows a first order kinetics in MB but at low concentrations of the oxidant (ca. $\leq 2.0 \times 10^{-5}$ M), the order changes from unity to half as shown by the representative runs given in Tables I and II. The rate constants obtained for all such runs are consolidated in Table III.

This conclusion was verified by graphical as well as van't Hoff's differential methods. Order in cysteine is nearly one (1.15) as confirmed by plotting log [rate]

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†For correspondance.

TABLE I

Pseudo first order kinetics in MB in its reaction with cysteine hydrochloride (excess) in acidic solution

$[Cy] = 6.0 \times 10^{-3}$

$[MB] = 2.5 \times 10^{-5}M$

$[HCl] = 3.0 \times 10^{-3}M$

$[CuSO_4] = 1.0 \times 10^{-5}M$

Temp= 35°C

$I = 3.0 \times 10^{-3}$

Time in sec	$(a-x)\times 10^5$ M	$k_1 \times 10^4 sec^{-1}$	$k_{1/2} \times 10^6$ $mol^{1/2} lit^{-1/2} sec^{-1}$
0	2.500	-	-
60	2.425	5.1*	2.7*
170	2.325	4.3	2.1
295	2.200	4.3	2.1
420	2.075	4.4	2.1
600	1.925	4.3	2.1
720	1.850	4.2	1.9
900	1.700	4.3	1.9
1020	1.625	4.2	1.9
1200	1.500	4.2	1.9
1320	1.400	4.4	1.9
1500	1.300	4.4	1.8
1620	1.200	4.5	1.9
1810	1.100	4.5	1.9
1920	1.050	4.5	1.8
2100	0.975	4.5	1.8
2400	0.875	4.4	1.7
2700	0.750	4.4	1.7
3000	0.650	4.5	1.6

Average value of k_1 (excluding* value = $4.4 \times 10^{-4} sec^{-1}$
C.V. = 2.6%

Average value of $k_{1/2}$(excluding* value) = $1.9 \times 10^{-6} mol^{1/2} lit^{-1/2} sec^{-1}$
C.V. = 7.9 %

against log [MB] (Figure 1). The rates were measured by drawing tangents on the concentration-time curves for the runs described in Figure 1 at the points where 10% MB was reduced by cysteine. As usual, the mirror technique was used for this purpose. Here again, the order in MB changes from unity to half at higher concentration of cysteine (ca. 7.2×10^{-3} M).

The rate increases on increasing the ionic strength which could be varied by adding KCl, NaCl and KNO_3 to the reaction mixture. The addition of KCl and NaCl, however, results in a change in order in MB and the transition to half order kinetics in the oxidant is more significant in presence of KCl. Such a change in the kinetic feature may be attributed to the metachromatic shift in methylene blue in presence of KCl and NaCl as already reported.[10] The linear plots of log $k_{1/2}$ against $\sqrt{I}$ approximately give a slope of two. The rate increases on increasing the concentration of the catalyst but at higher $[CuSO_4]$ the rate almost levels off as revealed by the plot of log $[CuSO_4]$ against log $k_{1/2}$ (Figure 2).

The rate increases with increasing hydrogen ion concentration as its square root but at higher $[H^+]$, the rate constant tends to attain a limiting value (Table IV). The ionic strength of the system was maintained constant by adding the requisite amount of KCl.

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TABLE II

Pseudo half order kinetics in MB in its reaction with cysteine hydrochloride (excess) in acidic solution

[Cy] = 6.0×10^{-3}M

[MB] = 2.0×10^{-5}M

[HCl] = 3.0×10^{-3}M

[CuSO$_4$] = 1.0×10^{-5}M

Temp = 35°C

I = 3.0×10^{-3}M

Time in seconds	$(a-x) \times 10^5$ M	$k_{1/2} \times 10^6$ $mol^{1/2} lit^{-1/2} sec^{-1}$	$k_1 \times 10^4 sec^{-1}$
0	2.000	-	-
70	1.950	1.6	3.5
140	1.900	1.6	3.6
210	1.850	1.6	3.7
300	1.800	1.5	3.5
600	1.625	1.5	3.4
900	1.400	1.6	4.0
1200	1.225	1.6	4.1
1500	1.100	1.5	3.9
1800	0.900	1.6	4.4
2100	0.775	1.6	4.5
2400	0.625	1.6	4.8

Average value of $k_{1/2}$ = $1.6 \times 10^{-6} mol^{1/2} lit^{-1/2} sec^{-1}$

C.V. = 3.4%

Average value of k_1 = $3.9 \times 10^{-4} sec^{-1}$

C.V. = 11.9%

TABLE III

Rate constant of the reaction of cysteine hydrochloride (excess) at different [MB]

[MB] $\times 10^5$M	$k_1 \times 10^4 sec^{-1}$	$k_{1/2} \times 10^6$ $mol^{1/2} lit^{-1/2} sec^{-1}$
3.50	4.2	-
3.00	4.2	-
2.50	4.4	-
2.00	-	1.60
1.50	-	1.55
1.25	-	1.25

[Cy] = 6.0×10^{-3}M; [HCl] = 3.0×10^{-3}M; [CuSO$_4$] = 1.0×10^{-5}M;
I = 3.0×10^{-3}M; Temp = 35°C

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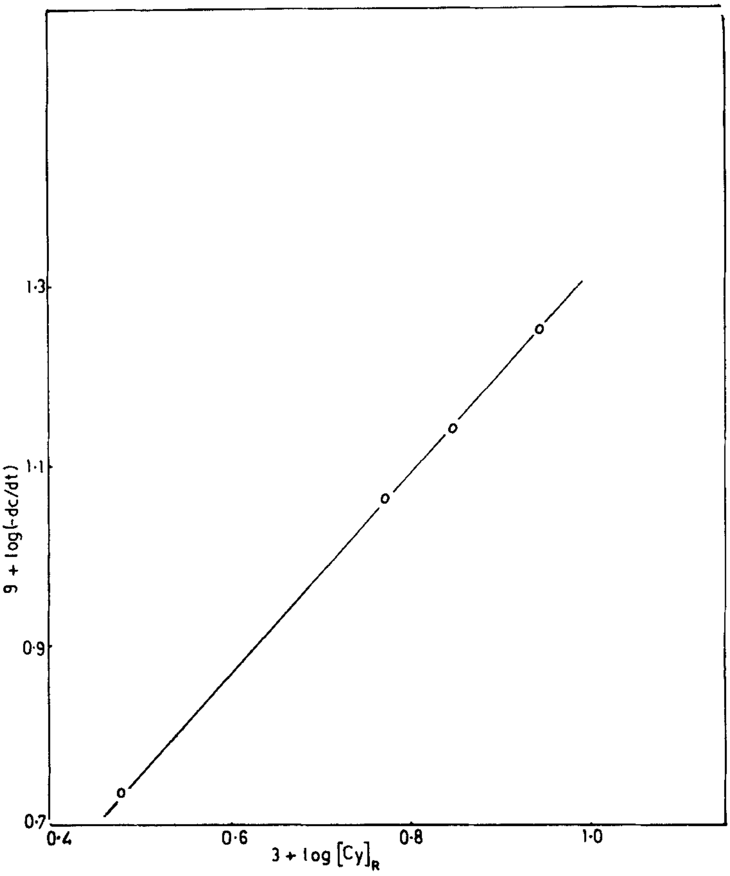

FIGURE 1 Effect of variation of [Cy] on the kinetics of Cu(II) catalysed interaction of cysteine with methylene blue in acidic solution [MB] = 3.0 × 10^{-5} M; [HCl] = 3.0 × 10^{-3} M; [$CuSO_4$] = 1.0 × 10^{-5} M; I = 3.0 × 10^{-3} M; Temp = 35°C; [Cy] × 10^3 M = 3.0, 6.0, 7.2, 9.0.

The rate constant decreases on decreasing the dielecric constant of the medium. Cystine and leuco base when added externally did not influence the rate of reaction.

The pseudo first order rate constants were determined at different temperatures ([Cy] = 6.0 × 10^{-3} M; [MB] = 3.0 × 10^{-5} M) keeping all other conditions unchanged and ΔH^*, ΔS^* and ΔG^* were found to be 40.2 kJ mol^{-1}, -174.3 kJ^{-1} mol^{-1} and 95.0 kJ mol^{-1}, respectively.

The kinetic features of the reaction were also studied in presence of externally added KCl. The order in cysteine is again found to be unity while a half order kinetics in MB was observed under these conditions.

DISCUSSION

Methylene blue exists as protonated species in acidic medium[11-15] while cysteine is known to form complexes with metal ions.[16] Further, cupric ions have been frequently used as catalyst for the oxidation of sulphydryl compounds[17] and here

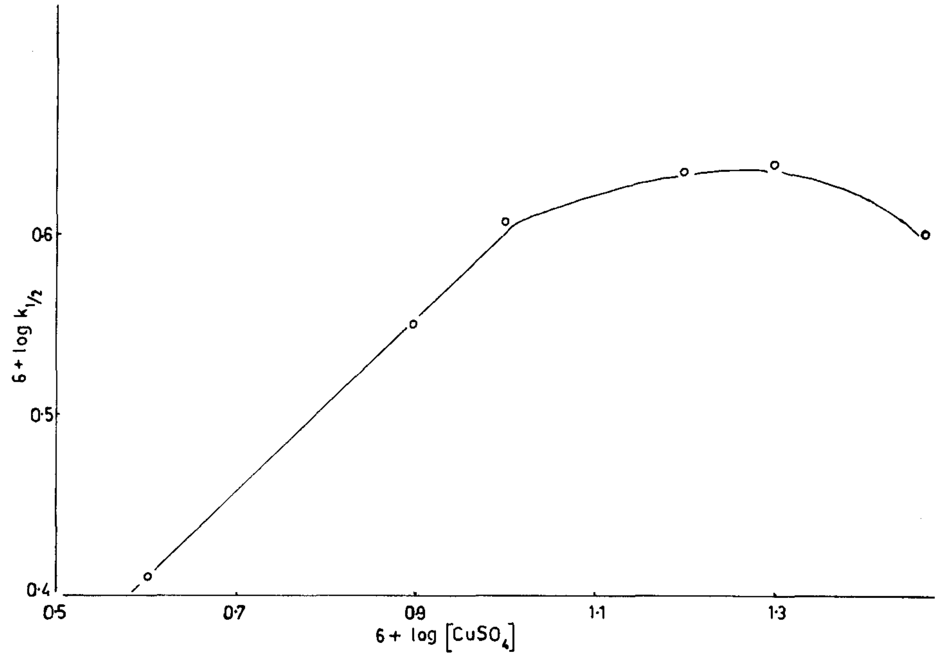

FIGURE 2 Effect of variation of [Cu(II)] on the kinetics of Cu(II) catalysed interaction of cysteine with methylene blue in acidic solution. [Cy] = 6.0×10^{-3} M; [MB] = 3.0×10^{-5} M; [HCl] = 3.0×10^{-3} M; I = 3.9×10^{-2} M; Temp = 35°C; $[CuSO_4] \times 10^6$ M = 4.0, 8.0, 10.0, 16.0, 20.0, 30.0.

TABLE IV

Rate constant of the reaction of cysteine hydrochloride (excess) with MB at different $[H^+]$

$[HCl] \times 10^3 M$	$k_{1/2} \times 10^6 mol^{1/2} lit^{-1/2} sec^{-1}$
2.0	3.4
3.0	4.2
6.0	5.7
8.0	6.6
10.0	6.6

[Cy] = 6.0×10^{-3}M; [MB] = 3.0×10^{-5}M; $[CuSO_4]$ = 1.0×10^{-5}M;
I = 3.9×10^{-2}M; Temp = 35°C

too, a complex designated as *C* is presumed to be formed with Cu(II) in acidic medium as revealed by IR spectra of cysteine on interaction with Cu(II) (Figure 3). It is evident from the spectrum that such an interaction mainly involves the bending vibrational modes of SH group resulting in the distortion of normal square planar Cu-cysteine complex and this may account for the specific catalytic influence exhibited, in situ, by cupric ions. An interpretation of kinetic data may be given in terms of the participation of Cu(I) obtained on reduction of Cu^{2+} ion by cysteine but such a possibility is ruled out in the light of the fact that cysteine is easily

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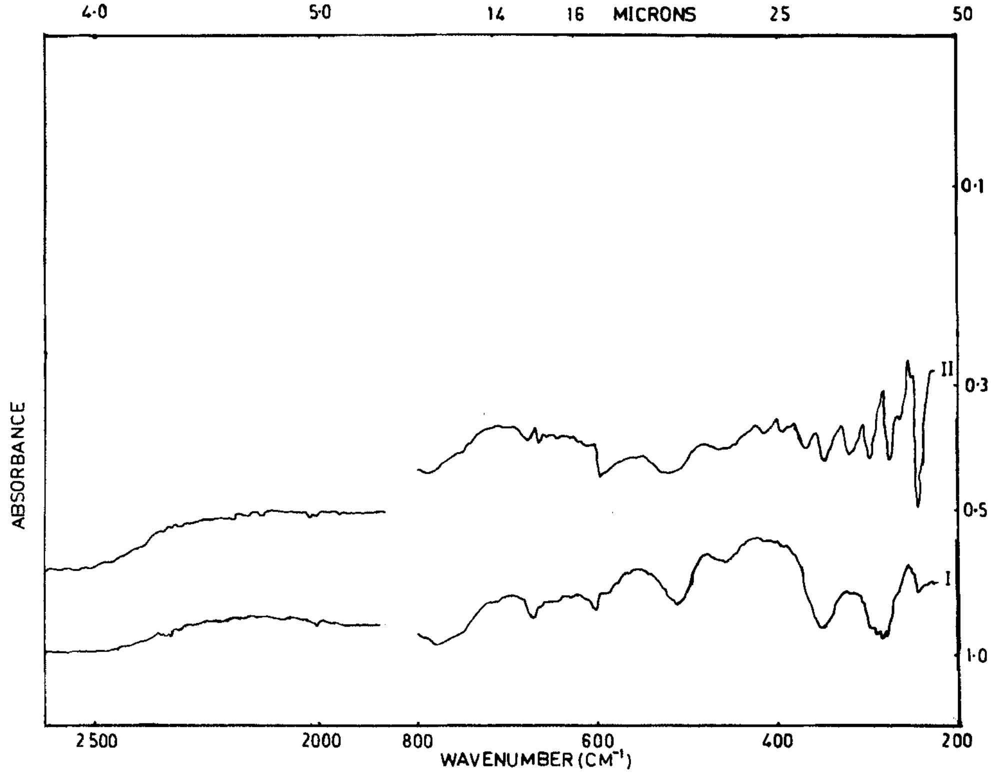

FIGURE 3 I, IR Spectrum of cysteine in KBr. II, IR Spectrum of cysteine with Cu(II) in KBr.

oxidised in alkaline but not in the acidic medium.[18,19] Incidentally, Taylor *et al.*[19] have used Fe^{3+} ion as catalyst (ca. 10^{-4} M) which has a relatively higher oxidation potential than Cu^{2+} ion. Moreover, it has also been recorded in developing analytical procedures for quantitative determination of sulphydryl compounds with Cu(II) that the metal ion oxidises thiols quantitatively at higher concentrations and at low concentrations, mainly mercaptides are formed.[20-22]

It may also be stated here that even in alkaline medium, Ehrenberg and coworkers[7] have used Cu^{2+} ions as catalyst (ca. 10^{-5}–10^{-7} M) in the oxidation of cysteine by molecular oxygen and these authors have also explained the reaction mechanism by postulating the participation of Cu(II)-cysteine-O_2 complex and from EPR spectroscopic data, they have shown that 100 ± (2%) of Cu is present as Cu(II). This obviously rules out the possibility of Cu(I) acting as catalyst in the reaction system. It may also be mentioned that copper-cysteine complex prepared externally when added to the system, did not catalyse the reaction. Incidentally, the existence of reactive species eluding an easy stereochemical interpretation has been pointed out by Taylor and coworkers in these reaction systems and such species have been termed as activated intermediates.[20] Thus,

$$MB + H^+ \overset{K_1}{\rightleftharpoons} MBH^+ \tag{1}$$

$$RSH + H^+ \overset{K_2}{\rightleftharpoons} RSH_2^+ \tag{2}$$

$$\mathrm{RSH_2^+} + \mathrm{Cu^{II}} \xrightleftharpoons{K_3} \mathrm{C} \tag{3}$$

The protonated species MBH$^+$ is presumed to react with cysteine molecule to form thiyl and semireduced methylene blue (HM$^\bullet$) radicals.

$$\mathrm{RSH} + \mathrm{MBH^+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{RS^\bullet} + \mathrm{HM^\bullet} + \mathrm{H^+} \tag{4}$$

It may be mentioned here that the oxidation of thiols to disulphide has been widely reported to involve RS$^\bullet$ radical[23] while the formation of semireduced methylene blue radical (analogous to semiquinone radicals[24]) by the action of reducing substrates such as ascorbic acid on MB has been reported in literature.[25] The participation of free radicals in this reaction system has been qualitatively confirmed by the positive polymerisation test with acrylonitrile.[26]

The semireduced methylene blue radical interacts with the copper-cysteine complex to give the leuco base and the dimerisation of RS$^\bullet$ radicals may give the disulphide.

$$\mathrm{HM^\bullet} + \mathrm{C} \xrightarrow{k_2} \mathrm{RS^\bullet} + \mathrm{H_2M} + \mathrm{Cu^{II}} + \mathrm{H^+} \tag{5}$$

$$2\mathrm{RS^\bullet} \xrightarrow{k_3} \mathrm{RSSR} \tag{6}$$

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

$$-\frac{d[\mathrm{MB}]}{dt} = k_1[\mathrm{RSH}][\mathrm{MBH^+}] - k_{-1}[\mathrm{RS^\bullet}][\mathrm{HM^\bullet}][\mathrm{H^+}] \tag{7}$$

On presuming steady state for HM$^\bullet$ and RS$^\bullet$

$$[\mathrm{HM^\bullet}] = \frac{k_1[\mathrm{RSH}][\mathrm{MBH^+}]}{k_{-1}[\mathrm{RS^\bullet}][\mathrm{H^+}] + k_2[\mathrm{C}]} \tag{8}$$

On substituting [HM$^\bullet$] in Equation (7)

$$-\frac{d[\mathrm{MB}]}{dt} = k_1[\mathrm{RSH}][\mathrm{MBH^+}]\left[\frac{k_2[\mathrm{C}]}{k_{-1}[\mathrm{RS^\bullet}][\mathrm{H^+}] + k_2[\mathrm{C}]}\right] \tag{9}$$

For evaluating [RS$^\bullet$], the cubic equation

$$k_{-1}k_3[\mathrm{RS^\bullet}]^3[\mathrm{H^+}] + k_2k_3[\mathrm{C}][\mathrm{RS^\bullet}]^2 - 2k_1k_2[\mathrm{C}][\mathrm{RSH}][\mathrm{MBH^+}] = 0 \tag{10}$$

was transformed to the standard form

$$P_3(x) = a_3x^3 + a_2x^2 + a_1x + a_0 = 0 \tag{11}$$

The real root of x represented by x_1 is given by[5,27]

$$x_1 = -\frac{a_2}{3a_3} + (\{\sqrt{P^3 + Q^2}\} - Q^{1/3}) - (\{\sqrt{P^3 + Q^2}\} + Q^{1/3}) \tag{12}$$

where

$$P = \frac{a_1}{3a_3} - \left(\frac{a_2}{a_3}\right)^2 \tag{13}$$

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and

$$Q = \frac{1}{2a_3}\left(a_0 - \frac{a_2^3}{27a_3^2} - a_2 p\right) \tag{14}$$

On substituting $a_1 = 0$ and presuming $P^3 \rightarrow 0$ under the conditions $k_{-1}[H^+] \gg k_2[C]$;

$$x_1 = -\frac{a_2}{3a_3} + (-2Q)^{1/3} \tag{15}$$

and

$$Q = \frac{1}{2a_3}\left(a_0 - \frac{a_2^3}{27a_3^2}\right) \tag{16}$$

On substituting the values of a_3, a_2 and a_0 from Equation (10);

$$Q = -\frac{1}{2k_{-1}k_3[H^+]}\left[2k_1k_2[C][RSH][MBH^+] + \frac{(k_2k_3[C])^3}{27k_{-1}k_3[H^+]^2}\right] \tag{17}$$

or,

$$Q = -\left[\frac{a[C][RSH][MBH^+][H^+] + (k_2k_3[C])^3}{2 \times 27(k_{-1}k_3[H^+])^3}\right] \tag{18}$$

Where

$$a = 27 \times 2k_1k_2k_{-1}^2k_3^2$$

Or,

$$[RS^\bullet] = \frac{a[C][RSH][MBH^+][H^+]^2 + (k_2k_3[C])^3}{27(k_{-1}k_3[H^+])^3} \tag{19}$$

(Under the conditions $k_{-1}[H^+] \gg k_2[C]$).

Thus, the rate of reaction is given by

$$-\frac{d[MB]}{dt} = \frac{3k_1k_2k_3[RSH][MBH^+][C]}{[a[C][RSH][MBH^+][H^+]^2 + (k_2k_3[C])^3]^{1/3}} \tag{20}$$

on substituting $[MBH^+] = K_1[MB][H^+]$ and $[C] = K_2K_3[RSH][Cu^{II}][H^+]$ the rate expression is given by

$$-\frac{d[MB]}{dt} = \frac{b[RSH]^2[MB][H^+]^2[Cu^{II}]}{[a'[RSH]^2[MB][Cu^{II}][H^+]^4 + (b'[RSH][Cu^{II}][H^+])^3]^{1/3}} \tag{21}$$

Where $a' = aK_1K_2K_3$, $b = 3k_1k_2k_3K_1K_2K_3$; and $b' = k_2k_3K_2K_3$ or

$$-\frac{d[MB]}{dt} = \frac{b[RSH]^{1.3}[MB][Cu^{II}][H^+]}{[a'[MB][Cu^{II}][H^+] + [RSH](b'[Cu^{II}])^3]^{1.3}} \tag{23}$$

At relatively smaller concentrations of cysteine, $[RS^\bullet]$ is expected to be smaller in Equation (9) and this explains a first order kinetics in cysteine and MB under

TABLE V

Rate constant of the reaction of cysteine hydrochloride (excess) at different [MB]

$[MB] \times 10^5 M$	$k_1 \times 10^4, sec^{-1}$
3.50	2.9
3.00	3.1
2.50	3.0
2.00	2.9
1.50	3.2
1.25	3.2

$[Cy] = 6.0 \times 10^{-3} M$; $[HCl] = 3.0 \times 10^{-3} M$; $[CuSO_4] = 1.0 \times 10^{-5} M$; $I = 3.0 \times 10^{-3} M$; Temp = 35°C.

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these conditions. When [RS$^\cdot$] becomes appreciably larger then Equation (22) becomes tenable and the reaction exhibits a near first order kinetics in cysteine, a fractional order in MB, as well as with respect to hydrogen and cupric ions. It is also seen from Equation (9) that at low [MB], there will be a greater probability of recombination of RS$^\cdot$ and HM$^\cdot$ in step (4) which will result in a larger magnitude of k_{-1}. This will tend to decrease $k_{1/2}$ with decreasing [MB] as has been noticed (Table II). In fact, the transition in order in MB from unity to half at larger [Cy] or at low [MB] may be attributed to the larger value of k_{-1} under these conditions but complex nature of the chemical reaction does not permit an easy interpretation for this phenomenon. The proposed reaction scheme also explains the levelling of rate at higher [Cu^{2+}] and [H^+]. The proposed reaction scheme suggests that the equilibrium (3) will lie for to the left under aerobic conditions due to prevalence of Cu(II) and this will hamper the reaction given in step (5). This will lead to a first order kinetics even at low concentrations of MB and this postulate has been corroborated by studying the effect of variation of [MB] as shown in Table V. This evidently justifies the proposed theoretical treatment of the rate data.

EXPERIMENTAL

The samples of L-cysteine hydrochloride (abbreviated as Cy or RSH) and methylene blue (MB) were obtained from John Baker, Inc., Colorado U.S.A. and Farbwerke Hoechst, Germany, respectively. The solutions were prepared afresh in double distilled water by exact weighing. The leuco base was prepared by passing pure SO_2 gas in a solution of methylene blue as described earlier[1] while the sample of cystine was obtained from M/S Sigma, Inc. U.S.A. All other reagents were either BDH, AnalaR, E. Merck, G.R. or Sigma's extra pure quality samples. Double distilled water was used throughout these investigations. The reaction of cysteine with MB is extremely slow in absence of Cu(II) (approximately 10% reduction of MB in 1 hour) and the rate is not affected by the addition of EDTA (disodium salt, ca. $\sim 10^{-6}$ M). This obviously indicates that the trace metal ion impurities, if any, have no kinetic influence. The possibility of the participation of molecular oxygen[8] was ruled out on the grounds that the reaction is not quantitative in absence of Cu(II) under aerobic conditions (about 25% in 1 hour) even at larger [cysteine] (ca. 3.0×10^{-2} M) which indicates that the kinetics of the reaction highly depends on Cu-cysteine interaction.

The progress of the reaction was followed on a Klett-Summerson photoelectric colorimeter by measuring the depletion in absorbance of methylene blue ($\varepsilon_{max} = 4.5 \times 10^4$ lit mol^{-1} cm^{-1} at 660 nm) by using an orange filter (spectral range 590–670 nm). The runs were made in nitrogen atmosphere. The ingredients of the reaction mixture were thermostated (variation ± 0.1°C) in reaction vessels made of Pyrex glass coated with Black Japan. The aliquots were withdrawn at different time intervals and the concentration of methylene blue was calculated from the Beer's law plots.

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