Synergetic influence of mercaptoacetic acid on the reaction of thiourea with methylene blue in acidic medium

K K Mishra* & Upasana Dwivedi
Department of Chemistry, Rani Durgavati University, Jabalpur 482 001, India
Email: kkmishra.chem@gmail.com

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Thiourea and methylene blue interact in a mole ratio of 2:1 in the presence of HCl forming the corresponding disulphide and leucomethylene blue. The order in thiourea is unity while that in methylene blue is zero at higher concentrations (ca. >2.5×10^{-5} M). At lower concentrations, the order in methylene blue is -1/2; this kinetic deviation depends on the nature of the thiol acid exhibiting synergetic effect. Mercaptoacetic acid does not react with methylene blue under the prevailing conditions; however it exerts a synergetic effect on the reaction. The rate increases linearly on increasing [H^+] with the reaction having a large negative entropy of activation. Electrospray induced mass spectrometry, 1H NMR and FTIR data indicate the participation of a persulphide-like species formed due to thiourea–mercaptoacetic acid interaction, which is perhaps responsible for the synergetic effect of mercaptoacetic acid.

Keywords: Kinetics, Reaction mechanism, Synergetic effect, Mercaptoacetic acid, Thiourea-methylene blue reaction

The participation of sulphhydryl substrates in regulation of biochemical processes is well known; for example, the rate of respiration of liver mitochondria is regulated by sulphhydryl substrates which is increased by thyroxine in absence of ADP. Further, thiourea inhibits thyroxine biosynthesis and thus, a mutual effect on the reactivity of thiourea and sulphhydryl substrates can be foreseen—an aspect not investigated so far. This prompted us to study the kinetics of the oxidation of thiourea and its derivatives by a model electron receptor, methylene blue in acidic medium, mediated by thiolacids. Incidentally, methylene blue has also found enormous applications such as in facilitation of memory in dementia, photocatalytic degradation on nanocomposites, photocatalytic reduction by TiO_2 nanotubes, etc. Recently, the degradation of methylene blue by DNA and kinetic studies on its degradation by alkaline H_2O_2 have been reported.

Our kinetic results indicate that thiourea is the principal substrate in its reaction with methylene blue while thiolacids, viz., mercaptosuccinic acid and mercaptoacetic acid, do not react with methylene blue at low concentrations. At these concentration levels, however, the rate of thiourea-methylene blue reaction is appreciably enhanced by the addition of thiolacids, perhaps due to their synergetic effect. A comparison of the results reported herein which describes the synergetic influence of mercaptoacetic acid on the reaction, with those reported earlier highlights the fact that kinetic features of the reaction appreciably depend on the nature of thiolacid causing synergetic effect. It may also be added that studies on synergetic effect in enzymatic systems are quite prevalent but in non-enzymatic systems, such studies are scarce and thus, present investigations may give an insight into the chemistry of the compatibility of the oxidation of thiourea with thiolacids in living systems.

Experimental
Thiourea (TU, RSH) and mercaptoacetic acid (thioglycolic acid, TGA) were obtained from Reanal Budapest, Hungary and E Merck, Germany (assay 99 % and 80 % respectively). The solutions were prepared in doubly distilled water. The thiourea solution was equilibrated with HCl (0.01 M) for about three hours so that TU attains maximum reactivity. The solution of TGA was standardized iodimetrically. The equilibrated TU solution has been used as the principal reactant. The solution of methylene blue, (MB, E. Merck, Germany) was also prepared in doubly distilled water and was kept in dark since light and temperature affect its stability. All other reagents such as hydrochloric acid, potassium chloride, etc., used in these investigations were either E. Merck GR or BDH AnaLAR grade samples. Doubly distilled water was used throughout these investigations. The runs were made in the presence of hydrochloric acid and by adding potassium chloride to maintain a fixed ionic strength (ca. 0.11 M).

The reaction mixture, except methylene blue (MB), was thermostatted for half an hour (Julabo, Germany; variation ± 0.02 °C) at the desired temperature and the solution of MB was subsequently added to the reaction system. Reaction vessels (Pyrex, England),...
coated black from outside with Black Japan were used. Aliquots were withdrawn at different time intervals and the concentration of methylene blue was determined at 664 nm by using Thermospectronic Unicam UV-530 spectrophotometer, with the help of Beer-Lambert law plots for MB.

The interference due to leuco base and the disulphide was ruled out because these species absorb strongly in the UV region of the spectrum. The oxidation products, viz., the corresponding disulphides were prepared by oxidizing the respective substrates with hydrogen peroxide and recrystallizing in ether. Dihydromethylene blue (leuco base) was prepared by reducing MB with Sn-HCl couple and the excess of hydrogen gas was boiled off. The solution was subsequently stored under nitrogen atmosphere. All the solutions were prepared afresh for each run. Kinetic runs made under aerobic and anaerobic conditions were compared and due to their similar kinetic behavior, detailed investigations were made only under aerobic conditions.

Results and discussion

The stoichiometry of the reaction was determined spectrophotometrically and two moles of thiourea are found to react with one mole of MB. The corresponding disulphide and dihydromethylene blue have been identified as the end products by comparing UV-visible and FTIR spectra of the known samples of these compounds and those of the corresponding reaction mixture. It has already been reported that the reactivity of thiourea appreciably depends on the time of protonation and thus, TU is shown to be protonated to give RSH$_2^+$ (equilibrium constant $K_1$). TU and TGA react very insignificantly with MB in presence of HCl (ca. 0.10 M; extent of reaction is 15 % and 3.8 % respectively in 70 min.), but on adding TGA to TU–MB reaction system, the rate is considerably enhanced and 87 % reaction is completed in 47 min. This indicates that TGA exerts a synergetic influence on the reactivity of TU towards MB in acidic medium. It is likely that the thiolic acid dissociates to produce the thiolate ion (R/S$^-$, equilibrium constant $K_2$).

Further, the rate of reaction is not affected by the addition of acetic acid; thus it is evident that the presence of sulphonyl group in the inductor is essential for the synergetic effect. An attempt was made to understand the mode of interaction between TU and TGA and for this purpose, ESI-MS and $^1$H NMR spectroscopy were used. ESI-MS data reveal the transfer of sulphur atom from dissociated TGA to thiourea molecule producing a reactive intermediate which may be an onium ion (OI), which has perhaps a persulphide like structure (Eq. 1)

$$\text{RSH}_2^+ + \text{S. CH}_2\text{COOH} \rightarrow \text{R-S}^- + \text{H}_2\text{O} + \text{CO}_2$$

The formation of a persulphide like species is indicated by the gain and simultaneous loss of 32 mass units by the TU fragment 85 and TGA fragment 148.8 respectively to give the fragment with $m/z$ equal to 116.8 in TU-TGA mixture (Supplementary Data, Figs S1-S3). This contention is similar to the findings of Fontecave and coworkers who have reported the stimulatory effect of operon SufS on the activity of operon SufE in *E. coli* due to the transfer of sulphur atom between cysteine moieties. It may, however, be added that the phenomenon is quite complex vis-à-vis the species involved in the transfer process and eludes an easy interpretation. The interaction postulated in Eq. (1) seems plausible because $^1$H NMR spectra of TU-TGA mixture show the presence of R-S$'$ group ($\delta$ 3.2 ppm), carboxylate ion ($\delta$ 7.2 ppm), -OH and $-\text{NH}_2$ groups ($\delta$ 5.2 ppm and $\delta$ 4.0 ppm) respectively (Supplementary Data, Fig. S4). Similarly, FTIR spectra indicate changes in stretching and bending vibrational modes of TU and TGA molecules when they are taken together. FTIR spectra of protonated TU, TGA and TU-TGA mixture indicates that the interaction between TU and TGA involves the stretching modes of C-N, C-H, S-H, C-S and N-H groups (1097, 3169, 2686, 731 and 3440 cm$^{-1}$ respectively) and the bending modes of S-H (504, 621 cm$^{-1}$) and N-H groups (1599 cm$^{-1}$). Further, the formation of the intermediate OI also involves the participation of $-\text{COOH}$ and N-C=S groups (Supplementary Data, Figs S5-S8).

The order in MB was determined employing Ostwald isolation method by making runs with 600 fold excess of TU over [MB] (ca. 2.0 $\times 10^{-5}$ M)
and it was found to be -1/2 (Fig. 1). The order in MB remains -1/2 at its lower concentrations (ca. ≤ 2.5 × 10⁻⁵ M, Table 1); however at higher concentrations of MB (ca. ≥ 3.0 × 10⁻⁵ M) the order shows a transition from -1/2 to zero (Fig. 2). It may be pointed out here that MB obeys Beer-Lambert law up to a concentration of 3.5 × 10⁻⁵ M and thus, variations in [MB] could not be made beyond that concentration level. The rate constant for -1/2 order reaction (k₋₁/₂) gradually increases from 1.1 × 10⁻¹¹ M⁵/₂ s⁻¹ to 3.3 × 10⁻¹¹ M⁵/₂ s⁻¹ on increasing [MB] from 1.0 × 10⁻⁵ M to 2.5 × 10⁻⁵ M, but the zero order rate constant (k₀) remains unchanged under these conditions. The rate constant k₋₁/₂ was calculated by using the expression for nth order rate constant on substituting n = -1/2 in Eq. (2),

$$k = \frac{1}{n(n-1)} \left[ \frac{1}{(a_0 - x)^{n-1}} - \frac{1}{a_0^{n-1}} \right] \quad \ldots (2)$$

Table 1: Rate constants at varying concentrations of methylene blue. ([TU] = 6.0 × 10⁻³ M; [TGA] = 6.0 × 10⁻⁴ M; [HCl] = 0.1 M; [KCl] = 0.01 M; µ = 0.11 M; Temp. = 35 °C)

<table>
<thead>
<tr>
<th>[MB] × 10⁻⁵ (M)</th>
<th>k₋₁/₂ × 10⁻¹¹ (M⁵/₂ s⁻¹)</th>
<th>k₀ × 10⁹ (M s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>1.5</td>
<td>1.7</td>
<td>-</td>
</tr>
<tr>
<td>2.0</td>
<td>2.1</td>
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<tr>
<td>2.5</td>
<td>3.3</td>
<td>-</td>
</tr>
<tr>
<td>3.0</td>
<td>-</td>
<td>9.3</td>
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<tr>
<td>3.4</td>
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where a₀ and t denote the initial concentration of methylene blue and time respectively.

The value of k₋₁/₂ increases from 1.5 × 10⁻¹¹ to 5.0 × 10⁻¹¹ M³/₂ s⁻¹ on increasing [TU] from 4.0 × 10⁻³ to 12.0 × 10⁻³ M and the plot of log k₋₁/₂ and log [TU] is linear with a slope of 1.1. This shows that the order in thiourea is unity. TGA exerts a synergetic effect on the reactivity of TU but the rate increases very slightly on increasing [TGA]. The plot of log k₋₁/₂ against log [TGA] is linear with a slope of 0.2, which further suggests a complex dependence of rate on [TGA]. The kinetic features of the reaction of TU with methylene blue in acidic medium in absence of thiolacids indicate that TU is the principal reactant. When the concentrations of TU and TGA were simultaneously varied while keeping a constant ratio, the rate shows a direct proportionality with the concentration of total sulphydryl group.

The rate increases linearly on increasing [H⁺] (Table 2), which can be attributed to the protonation

Table 2: Effect of variation of [H⁺] on the rate. ([TU] = 6.0 × 10⁻³ M; [TGA] = 6.0 × 10⁻⁴ M; [MB] = 2.0 × 10⁻⁵ M; [HCl] = 0.1 M; [KCl] = 0.01 M; µ = 0.11 M; Temp. = 35 °C)

<table>
<thead>
<tr>
<th>[HCl] (M)</th>
<th>k₀ × 10⁹ (M s⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>0.05</td>
<td>4.7</td>
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<tr>
<td>0.10</td>
<td>6.2</td>
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<tr>
<td>0.20</td>
<td>9.6</td>
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<td>0.25</td>
<td>10.0</td>
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<td>0.30</td>
<td>11.0</td>
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<td>0.35</td>
<td>13.0</td>
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of methylene blue\textsuperscript{18} to produce the species MBH\(^+\) (equilibrium constant \(K_4\)). In these variations, the ionic strength of the system was kept constant \((\text{ca. } 0.35 \text{ M})\) by adding the requisite amount of KCl. It was observed that the runs are kinetically smooth at a relatively lower ionic strength while at higher ionic strength \((\text{ca. } \geq 0.40 \text{ M})\), the runs tend to become erratic. Due to this, all the kinetic runs except those made with varying [H\(^+\)], were made at an ionic strength of 0.11 M. The order in MB shows a transition from -1/2 to zero under these conditions and the zero order rate constant increases from \(4.7 \times 10^{-9}\) to \(13.0 \times 10^{-9}\) \(M\ s^{-1}\) on increasing [H\(^+\)] from 0.05 to 0.35 M. The plot of \(\log k_o\) and \(\log [H^+]\) gives a straight line with a slope of 0.8, which indicates a near linear dependence of rate on [H\(^+\)].

The rate of reaction is not affected on varying the ionic strength and dielectric constant of the reaction system. The addition of the corresponding disulphides and dihydroxy methylene blue to the system externally does not affect the rate of reaction. The influence of addition of leuco base was ascertained by making blank runs with equivalent amount of Sn dissolved in HCl, since Sn-HCl couple was used to reduce MB\(^+\). It may be pointed out here that 0.01 M HCl was used along with Sn metal to reduce methylene blue to give the leuco base. In the investigations on the effect of external addition of leuco base on the rate of reaction, an aliquot of MB ranging from 0.5 mL to 2.0 mL was added to the reaction system containing a maximum of \(2.0 \times 10^{-5}\) M HCl, while 0.10 M HCl was already present in the system. Blank runs were however made to probe the effect of addition of the equivalent amounts of Sn and HCl on the rate of reaction.

The activation parameters, viz., \(\Delta H^*\), \(\Delta S^*\) and \(\Delta G^*\), were determined by making the runs at different temperatures and were found to be 26.8 kJ mol\(^{-1}\), -366.4 J K\(^{-1}\) mol\(^{-1}\) and 139.6 kJ mol\(^{-1}\) respectively. It is thus evident that the entropy change accompanying the rate determining step is largely negative and its correlation with \(\Delta H^*\) suggests that thermodynamic control is more prominent than kinetic control in this chemical reaction.

The kinetic parameters of the reaction closely resemble those obtained for the oxidation of TU in presence of thioamic acid (mercaptosuccinic acid, TMA) although there are a few deviations. The reaction adheres to -1/2 order kinetics in MB at lower concentrations as reported earlier by Mishra & coworkers\textsuperscript{7,19} in the oxidation of thiourea in presence of TMA as well as in the oxidation of thiosulphate ion by methylene blue and by Laidler & Hoare\textsuperscript{19} in the urease catalyzed hydrolysis of urea. It may, however, be pointed out that the transition in order in MB from zero to -1/2 in the present case occurs at relatively higher concentrations of methylene blue \((\text{ca. } 2.0 \times 10^{-5} \text{ M})\) as against \(1.0 \times 10^{-5}\) M in the case of TU-TMA-MB reaction\textsuperscript{7}).

These kinetic results suggest that the onium ion, after rearrangement, may facilitate the formation of the more reactive thiourea analogue X along with the liberation of TGA molecule. This contention is similar to that postulated by Martin and Spence\textsuperscript{20, 21} in the oxidation of glutathione (GSH) by Mo(VI) where the formation of a reactive cyclic species of glutathione (GSH) was presumed to explain the kinetic results. The analogue X may react with the protonated methylene blue to produce the radicals RS' and HM' (half reduced methylene blue).

The transient species HM' may subsequently react with the substrate to give the leuco base and RS'.
radical which may dimerize to produce the disulphide as shown in the proposed mechanism (Scheme 1). The participation of HM’ and RS’ radicals in these reaction systems has been frequently reported in literature and in the present case, it has been verified by the positive polymerization test with acrylamide. The semireduced radicals (HD•) formed in situ in two electron reduction of some dyes (D), disproportionate to give the leuco base and the parent dye.

The equilibrium constant for the reversible combination of H2M and MB is reported to be \(3.6 \times 10^{-6}\) and thus, disproportionation reaction (Eq. 6) has been shown as a direct step. It has already been mentioned that at higher concentrations of MB, the order in MB is zero which can be explained by presuming Eq. (4) as the rate determining step. On applying steady state treatment for X and substituting \([O\text{I}], [MB\text{H}^+]\) and \([RSH_2]\) from the corresponding equilibria the rate expression is

\[
\frac{d[MB]}{dt} = \frac{k_1 k_2 K_1 K_2 K_3 K_4 [RSH][TGA][MB][H^+]}{k_{-1}[TGA] + 2 K_4 k_2 [MB][H^+]}
\]

... (7)

The order in MB is found to be \(-1/2\) at lower concentrations and it appears that the concentration of radical HM’ becomes smaller under these conditions which may pave way for Eq. (5) to become the rate determining step. Thus on applying steady state treatment for HM’ and X and substituting again \([MB\text{H}^+], [O\text{I}]\) and \([RSH]\) we get Eq. (8).

\[
\frac{d[MB]}{dt} = \frac{k_1 k_2 K_1 K_3 K_4 [RSH][TGA]}{k_{-1}[TGA] + 2 K_4 k_2 [MB][H^+]}
\]

\[
\times \left[ \frac{k_4 k_1 K_3 K_4 [RSH][TGA][MB][H^+]}{k_4 k_{-1}[TGA] + 2 K_4 K_2 [MB][H^+]} \right]^{1/2}
\]

... (8)

The rate expression (Eq. 8) predicts a near first order dependence on \(TU\) and \(H^+\) ion and also explains a near zero order kinetics in TGA. It also explains a negative order in MB but admittedly, no quantitative correlation of [MB] and negative order could be obtained for the reaction system. In spite of this, the proposed reaction scheme is in consonance with the experimental evidence.

The above study shows that the reactivity of thiourea towards methylene blue (a model electron acceptor) in acidic medium is considerably enhanced on adding low concentrations of mercaptoacetic acid due to synergetic effect. It appears that sulphhydryl substrates assist the biosynthesis of thyroxine by favouring the oxidation of thiourea. The order in MB is found to be negative (-1/2) at low concentrations of MB (ca. \(2.5 \times 10^{-5}\text{ M}\)) which is attributed to the interaction of half-reduced methylene blue radical (HM’) with a reactive analogue of thiourea produced in situ due to the participation of a persulphide-like species as revealed by ESI-MS and ¹H-NMR spectra of TU-TGA mixture.

Supplementary data

Supplementary Data associated with the article, viz., ESI-MS, FTIR and ¹H-NMR spectra (Figs S1-S8) and complete derivation of rate expressions (Eqs 7 and 8), are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA 51A(03) 458-463_Suppl Data.pdf.

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