Cu(II)-catalysed oxidation of bioactive substrates by methylene blue in acidic medium assisted by Zn(II): Possible role of nanoparticles

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The bioactive sulphydryl substrates, cysteine hydrochloride (cys) and 2-mercaptoethylamine hydrochloride (2-ME) react with methylene blue in acidic medium in presence of Cu(II) as a catalyst. While Zn(II) has no catalytic role on its own, when it is added along with Cu(II) to the reaction system, the corresponding rate constants increase by about 25%. TEM images of the representative cys-Cu-MB system reveal the formation of the nanorods, while on adding Zn(II) to the reaction system, the morphology of the nanoparticles changes from nanorods to nanogranules. These findings are also substantiated by electron diffraction studies. It thus appears that the morphology of nanoparticles and the possible synergetic effect of Zn(II) are correlated. The synergetic effect of Zn(II) is also favoured by the presence of amino group in the substrate molecule while carboxylic group hinders it.

Keywords: Kinetics, Reaction mechanism, Nanoparticles, Synergism, Catalysis, Copper, Zinc, Metal ion catalysis, Methylene blue

In recent years, sulphur chemistry and metal ion catalysis have attracted considerable attention mainly due to the multifaceted involvement of these systems in metabolic pathways.1-5 The redox interaction between transition metals and the redox site of the ligands, besides a direct coordination is also allowed through bond or through space and perhaps this paves way for a synergetic effect. An important example of synergetic effect is the function of proteins where amino acid, H-63, simultaneously coordinates with Zn(II) and Cu(II). The Zn(II) accelerates copper-substrate interaction and contributes to proteins’ stability.7 Recently, the synergetic effect of iodide ions on the corrosion inhibition of aluminium by purine in presence of phosphoric acid has been reported.8 Largely, the metal ion activity has been attributed to the participation of nanoparticles in the homogeneous/heterogeneous domain and size, shape and surface morphology play a significant role in controlling physical, chemical, optical and electronic properties of these nanoscopic materials.

The oxidant, methylene blue (MB), is a cationic dye and has found enormous applications in recent past in phenomena such as memory facilitation in dementia, in photocatalytic degradation on nanocomposites and in photocatalytic reduction by TiO₂ nanotubes. The electrochemical studies on the interactions between methylene blue and surface-confined DNA as well as the kinetics of degradation of MB by alkaline hydrogen peroxide have also been reported recently. In the light of this, Cu(II)-catalyzed oxidation of bioactive sulphydryl substrates (RSH), viz., cysteine hydrochloride (cys), regarded as one of the pivots in sulphur metabolism along with glutathione and 2-mercaptoethylamine hydrochloride (2-ME, a model for coenzyme A) by methylene blue has been studied in acidic medium and the synergetic role of Zn(II) has been investigated. TEM images prove the participation of nanoparticles and a change in their morphology on adding Zn(II) along with Cu(II). In our earlier studies, we have found the inhibitory role of Zn (II) at lower concentrations (ca. 2.0×10⁻⁸ M) on the rate of Cu(II) catalysed oxidation of o-mercaptobenzoic acid and the synergetic effect of Cu(II) on Ru(III) catalyzed oxidation of cysteine by MB in acidic medium. These observations highlight the complex chemistry of these systems vis-a-vis the specificity of the substrates and metal ions acting as catalysts. This has aroused our interest in metal ion-catalysed oxidation of sulphhydril substrates by methylene blue and we have reported the kinetic studies on Ru(III) catalysed oxidation of glutathione and the oxidation of mercaptosuccinic acid involving the participation of square shaped nanocrystals. The present study underlines a correlation between the synergetic effect of Zn(II) and the morphology of the participating nanoparticles.
Materials and Methods
The solutions of L-cysteine hydrochloride (cys) and 2-mercaptimethylamine hydrochloride (2-ME) (assay 99% and 98% respectively) were prepared by dissolving the samples supplied by Riedel Dehaen AG, Germany. An exactly weighed quantity of cys and an approximately weighed quantity of 2-ME were dissolved in doubly distilled water. The latter was standardized iodosimetrically22. The solution of methylene blue was prepared by dissolving an exactly weighed quantity of the sample (Merck, Germany) in doubly distilled water and the solution was stored in dark in order to preclude the possibility of a photochemical interference. The solutions of Cu(II) and Zn(II) were prepared by dissolving exactly weighed quantities of copper sulphate (S. Merck, GR) and zinc oxide (Qualigens AR, in 5 mL of 1 M H2SO4) in doubly distilled water. The stock solution (ca. 0.1 M) of copper sulphate was standardized with a standard solution of sodium thiosulphate inter alia standard potassium iodate solution23, while the solution of Zn(II) was standardized with a standard EDTA solution using Eriochrome Black T indicator23. The solutions were subsequently used in various kinetic runs after requisite dilution. All other reagents were either BDH AnalaR, E. Merck GR or Qualigens AR grade samples. The reaction mixture, except methylene blue, was thermostatted for half an hour 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. These were thermostatted (Julabo, Germany, variation ±0.02 °C) at the desired temperature for a sufficient length of time and the aliquots were analyzed at different time intervals at 664 nm (εmax for MB = 6.76×104 M⁻¹ cm⁻¹) on an ATI-Unicam UV 2-100 spectrophotometer with the help of Beer-Lambert law plots for MB. The interference due to leuco base and the disulphide are ruled out because these species absorb strongly in the UV region of the spectrum. The oxidation product, i.e., the corresponding disulphide was prepared by oxidising the respective thiols with hydrogen peroxide24 and recrystallizing it in water methanol mixture. Dihydromethylene blue (leuco MB, referred to as H2M) was prepared by reducing methylene blue with Sn-HCl couple as described earlier25. The TEM images and surface area electron diffraction patterns were recorded on Jeol (model JEM 1230) transmission electron microscope at 117 kV for the representative reaction mixtures under the prevailing experimental conditions in presence of Cu(II) and Cu(II) and Zn(II). The reaction mixtures were allowed to evaporate at room temperature after completion of the reaction and the residue was extracted in ether. Since the reaction mixtures remain homogeneous and gave reproducible kinetic results for 90-95% completion of the reaction (Supplementary Data, Fig. S1-S4), the possibility of light scattering was ruled out. The homogeneity of the medium is again justified in the light of the fact that at low concentrations of the metal ions, the nanoparticles constitute a homogeneous lyophobic domain as reported in the case of biological lipid membranes (BLM) functioning through an interface of molecular dimensions26.

Results and Discussion
The stoichiometry of the reactions was determined analytically as well as spectrophotometrically and two moles of the substrates were found to interact with one mole of methylene blue forming the corresponding disulphide and the leuco base as shown by Eq. (1). This was verified by comparing the UV-visible and FTIR spectra of the known samples of the reaction products and those of the reaction systems (Supplementary Data, Fig. S5-S7). The characteristic frequency of the disulphide group (S-S) is in agreement with the value of 400 cm⁻¹ observed for the product isolated from the reaction mixture27.

\[ 2 \text{RSH} + \text{MB} \rightarrow \text{RSSR} + \text{H}_2\text{M} \quad \ldots (1) \]

Influence of the dissolved oxygen on the rate and the synergetic role of Zn(II)
In oxidation of both the substrates, viz., cysteine and 2-ME by MB, the kinetics of Cu(II)-catalyzed reaction were found to be significantly influenced by dissolved oxygen28,29. However, on adding Zn(II) to the reaction system, an identical behaviour was noticed under aerobic and anaerobic conditions (Supplementary Data, Fig. S8-S11) that indicates the kinetic significance of Zn(II) in these reaction systems. Another significant role played by Zn(II) is its synergetic effect on the catalytic activity of Cu(II) (ca. 4.0×10⁻⁶ M for cys and 2.0×10⁻⁵ M for 2-ME) in these reactions. It may be pointed out here that traces of Cu(II) (~ 10⁻⁶ M to 10⁻⁵ M) are present even in distilled water and thus the concentration of Cu(II) reported presently denotes the amount of the metal ion added externally to the reaction system. Zn(II) (ca. 5.0×10⁻⁵ M) does not exhibit any catalytic activity in the
oxidation of cysteine and 2-ME but the half order rate constants ($k_{1/2}$) increase from $3.8 \times 10^{-6}$ to $4.6 \times 10^{-6} \text{ M}^{1/2} \text{ s}^{-1}$ and from $1.6 \times 10^{-6}$ to $2.0 \times 10^{-6} \text{ M}^{1/2} \text{ s}^{-1}$ in the case of cystein-Cu-MB and 2-ME respectively on adding Zn(II) along with Cu(II) to the reaction systems. It is worthwhile to note that the reproducibility of rate constant for the kinetic runs was found to be within 5%. Hence, the variation in the magnitude of rate constants (21% and 25% in the case of cystein-Cu-MB and 2-ME-Cu-MB systems respectively) has been perceived as the influence of Zn(II) on the catalytic activity of Cu(II). It thus appears that Zn(II) has a supporting catalytic influence in these systems, probably due to some synergy and also due to its inhibitory role as reported in the ribonucleolytic systems, probably due to some synergy and also due to its role as a Lewis acid. This is, in contrast to its inhibitory role as reported in the ribonucleolytic activity of rTbMP42$^{30}$ which is attributed to the chemical modification of amino acids known to coordinate Zn(II). The reaction follows a half order kinetics in MB in the oxidation of cysteine (Fig. 1), but in the reaction with 2-ME, the order in MB varies from zero to unity (the corresponding rate constants are $k_0$ and $k_1$ respectively) on varying the concentration of 2-ME (Table 1). The order in both the substrates is unity. The rate increases on increasing [MB] and attains a limiting value at higher [MB] ($ca. > 1.5 \times 10^{-5} \text{ M}$). The half order rate constant increases from $2.1 \times 10^{-6}$ to $7.8 \times 10^{-6} \text{ M}^{1/2} \text{ s}^{-1}$ on increasing [HCl] from $2.5 \times 10^{-3}$ to $3.0 \times 10^{-2} \text{ M}$ in the oxidation of cysteine, but shows a maximum in the oxidation of 2-ME at a particular [H$_2$SO$_4$] ($ca. 2.5 \times 10^{-7} \text{ M}$). In these variations, the ionic strength of the system was maintained at a constant level by adding KCl and KNO$_3$ in the oxidation of cysteine and 2-ME, respectively.

The rate increases on adding KCl and NaCl to the reaction system, which may be attributed to the competitive formation of the ion pair of the cationic dye with chloride ion$^{17}$, while the addition of KNO$_3$ did not affect the rate of reaction. An increase in the dielectric constant of the medium increases the rate. The dielectric constant of the medium was varied by adding different volumes of ethanol ranging between 2% and 20% (v/v). In the oxidation of cysteine, the rate increased on increasing the concentration of Cu(II) showing a fractional dependence. This is in contrast to the observations made for Cu(II)-catalysed oxidation of this substrate$^{26}$ in absence of Zn(II) where the rate increased slightly and attained a limiting value at higher concentrations of Cu(II). This again highlights the specific mode of participation of metal ions acting as catalysts in these systems. In the oxidation of 2-ME, the rate showed a maximum at a particular [Cu(II)] ($ca. 3.0 \times 10^{-5} \text{ M}$) and then decreased with increasing concentration. It is found that the order in MB is zero at lower concentrations of Cu(II) ($ca. 1.0 \times 10^{-5} \text{ M}$) and at higher concentrations ($ca. > 6.0 \times 10^{-5} \text{ M}$), the order in MB is unity. In the intermediate concentration range of Cu(II), the reaction showed a half order kinetics in MB.

As already mentioned, addition of Zn(II) accelerated the rate in both the cases, but subsequently the rate constants remained unaffected on varying its concentration from $1.0 \times 10^{-5}$ to $12.5 \times 10^{-5} \text{ M}$. The addition of the corresponding disulphides did not produce a change in the rate. The rate of reaction again remained unaffected on adding dihydromethylene blue to the system in the oxidation of cysteine, whereas in

![Fig. 1—Half order plots for MB at varying concentrations of cysteine. (1, ■) 1.0×10^{-5} \text{ M}; (2, ▲) 2.0×10^{-5} \text{ M}; (3, ▲) 3.0×10^{-5} \text{ M}; (4, ▲) 4.0×10^{-5} \text{ M}; (5, ▲) 5.0×10^{-5} \text{ M}; (6, ▲) 6.0×10^{-5} \text{ M}; (7, ▲) 7.0×10^{-5} \text{ M}; (8, ▲) 8.0×10^{-5} \text{ M}; (9, ▲) 9.0×10^{-5} \text{ M}; (10, ▲) 1.0×10^{-4} \text{ M}; (11, ▲) 2.0×10^{-4} \text{ M}; (12, ▲) 3.0×10^{-4} \text{ M}; (13, ▲) 4.0×10^{-4} \text{ M}; (14, ▲) 5.0×10^{-4} \text{ M}; (15, ▲) 6.0×10^{-4} \text{ M}; (16, ▲) 7.0×10^{-4} \text{ M}; (17, ▲) 8.0×10^{-4} \text{ M}; (18, ▲) 9.0×10^{-4} \text{ M}; (19, ▲) 1.0×10^{-3} \text{ M}; (20, ▲) 2.0×10^{-3} \text{ M}; (21, ▲) 3.0×10^{-3} \text{ M}; (22, ▲) 4.0×10^{-3} \text{ M}; (23, ▲) 5.0×10^{-3} \text{ M}; (24, ▲) 6.0×10^{-3} \text{ M}; (25, ▲) 7.0×10^{-3} \text{ M}; (26, ▲) 8.0×10^{-3} \text{ M}; (27, ▲) 9.0×10^{-3} \text{ M}; (28, ▲) 1.0×10^{-2} \text{ M}; (29, ▲) 2.0×10^{-2} \text{ M}; (30, ▲) 3.0×10^{-2} \text{ M}; (31, ▲) 4.0×10^{-2} \text{ M}; (32, ▲) 5.0×10^{-2} \text{ M}; (33, ▲) 6.0×10^{-2} \text{ M}; (34, ▲) 7.0×10^{-2} \text{ M}; (35, ▲) 8.0×10^{-2} \text{ M}; (36, ▲) 9.0×10^{-2} \text{ M}; (37, ▲) 1.0×10^{-1} \text{ M}. Temp. = 35 °C; µ = 5.06×10^{-3} \text{ M}.]

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*Table 1—Rate constants at varying concentrations of substrates. [Cys] = 1.0×10^{-5} \text{ M}; [2-ME] = 2.0×10^{-5} \text{ M}; [HCl] = 5.0×10^{-3} \text{ M}; [Cu(II)] = 2.0×10^{-5} \text{ M}; [Zn(II)] = 1.0×10^{-5} \text{ M}; [KCl] = 2.0×10^{-5} \text{ M}; Ethanol = 4.0% (v/v); Temp. = 35 °C; µ = 5.06×10^{-3} \text{ M}.*

*Table 2—Rate constants at varying concentrations of substrates. [Cu(II)] = 2.4×10^{-5} \text{ M}; [HCl] = 5.0×10^{-3} \text{ M}; [Cu(II)] = 4.0×10^{-6} \text{ M}; [Zn(II)] = 1.0×10^{-5} \text{ M}; [KCl] = 2.0×10^{-5} \text{ M}; Ethanol = 4.0% (v/v); Temp. = 35 °C; µ = 5.06×10^{-3} \text{ M}.*
the oxidation of 2-ME, the rate of reaction slightly
decreased on increasing the concentration of leuco
base. The time of equilibration of Cu(II) with other
ingredients of the reaction system did not affect the
rate. The activation parameters of the reactions were
evaluated by making the runs at different temperatures
ranging between 25 and 45°C. \( \Delta H^* \), \( \Delta S^* \) and \( \Delta G^* \) for
the oxidation of cysteine and 2-ME were found to be
43 kJ mol\(^{-1}\), –210 J K\(^{-1}\) mol\(^{-1}\), 106 kJ mol\(^{-1}\), and, 38 kJ mol\(^{-1}\), –231 J K\(^{-1}\) mol\(^{-1}\) and 70 kJ mol\(^{-1}\) respectively.

The calculation and visualization of electronic
structure of methylene blue carried out by applying
the GaussianO\(_3\) package as reported by Katafias and
coworkers\(^{17}\) is shown in Fig. 2. It is evident that
electron density at carbon atom 3 and at nitrogen atom
in \( \text{para} \) position in MB molecule is relatively less, and
thus, these positions will undergo electrophilic attack.
Methylene blue is reported to be protonated\(^{20}\) in acidic
medium to give the species MBH\(^+\) (equilibrium
constant \( K \)). Cysteine and 2-ME may coordinate with
metal ions Cu(II) and Zn(II) as frequently reported in
literature; 2-mercaptoethylamine (2-ME) is known to
coordinate with metal ions to give the complex having
the structure\(^{27}\) (I).

\[
\begin{align*}
\text{S} & \quad \text{M} \\
\text{N} & \quad \text{H}_2 \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{RSH} & \quad \text{Cu(II)} & \quad \text{R'}
\end{align*}
\]

(\( \text{I} \))

It appears that the molecule RSH coordinates with
Cu(II) to form a reactive complex \( C_1 \),

\[
\text{RSH} + \text{Cu(II)} \quad \overset{k_1}{\rightleftharpoons} \quad \overset{k_{-1}}{\text{RSH}^+ + \text{Cu}^{2+}}
\]

where \( R' \) represents the remaining part of the substrate
molecules.

TEM (transmission electron microscopy) images,
using cysteine hydrochloride as the representative
compound, reveal the formation of Cu-cysteine
nanorods (size 59 nm, Fig. 3a) and perhaps their
participation during the course of reaction accounts
for the catalytic activity of the metal ion. In proteins,
Zn(II) is reported to be coordinated with four or three
amino acid residues to stabilize the protein structure
or to form a catalytic active centre\(^{7}\). Thus, the
complex \( C_1 \) may subsequently coordinate, \( \text{in situ} \),
with Zn(II) to produce another transient complex \( C_2 \)
(Eq. 3).

\[
C_1 + \text{Zn(II)} \quad \overset{k_2}{\rightleftharpoons} \quad \overset{k_{-2}}{\text{CuZnR'}}
\]

(\( \text{C}_2 \))... (3)
Zn-cysteine interaction also gives indications of the formation of nanoparticles, which are low melting and thus, could not be characterized. In the Cu(II)-Zn(II)-cysteine system, the morphology of nanoparticles changes and nanogranules are formed (size 26 nm, Fig. 3b). It may be mentioned here that morphology-dependent nanocatalysis involving some precious metal particles and transition metal particles Co using and Ni have been reported recently by Li and co-workers\(^ {31}\). Selected area electron diffraction studies (SAED) reveal that the diameter of the rings decreases when Zn(II) is added along with Cu(II) to the reaction system (Fig. 4a and 4b). Since the lighter portion in between the concentric rings increases on adding Zn(II), it indicates that the extent of scattering decreases on adding Zn(II) i.e., the dimension of the lattice increases under these conditions\(^ {32}\). The FTIR studies indicate the shifting of band at 3400 cm\(^{-1}\) in cysteine molecule to 3200 cm\(^{-1}\) on interaction with Cu(II) suggesting the participation of carbonyl group in the ligation. In the case of Zn(II) the coordination occurs through -NH stretch with characteristic frequency at about 2700 cm\(^{-1}\) (ref. 33). In the cyst-Cu-Zn system, new bands appear at 2330, 2996 and 3950 cm\(^{-1}\), which suggest the participation of C-O, N-H and O-H stretching vibrational modes under these conditions\(^ {33}\). Further, the participation of bending vibrational modes of -SH group in the ligation of cysteine with Zn(II) (species C\(_2\)) is also revealed by FTIR spectra (Supplementary Data, Figs S12–S15). It seems that in presence of Cu(II) and Zn(II), the formation of an outer sphere complex is facilitated which may result in enhancement of rate of electron transfer reaction as has been noticed presently\(^ {34}\). Since the morphology of the nanoparticles changes and their size decreases on adding Zn(II) to the system, it appears that the lattice can accommodate a larger number of nanoparticles in complex C\(_2\) which causes a synergetic effect of Zn(II) on the catalytic activity of Cu(II). This observation is analogous to the results reported by Yang and co-workers\(^ {35}\) on photoluminescence studies on mercaptocarboxylic acid stabilized CdTe nanoparticles where broad diffuse rings in SAED pattern were obtained due to the small particle size.

The presence of electron donating –NH\(_2\) group in complex C\(_2\) may enhances electron density on S atom and thus, the species C\(_2\) may interact with MBH\(^{+}\) to produce a transient species C\(^{*}\) as shown in Eq. (4).

\[
\begin{align*}
\text{C}_2 + \text{MBH}^{+} & \rightarrow \text{C}^{*} \\
\text{C}^{*} & \rightarrow \text{RS}^{•} + \text{HM}^{•} \\
\text{RS}^{•} + \text{HM}^{•} & \rightarrow \text{end products}
\end{align*}
\]

(Here R\(^{"}\) represents the remaining part of the transient complex C\(_2\)).

The transient species C\(^{*}\) may subsequently dissociate to give radicals RS\(^{•}\) and HM\(^{•}\) (half reduced methylene blue radical), which in turn may interact with the substrate molecule to give the end products. The participation of radicals such as RS\(^{•}\) and HM\(^{•}\) in these reaction systems has been frequently reported in the literature\(^ {24,26}\) and such a participation during the course of reaction was verified by the positive polymerization test with acrylonitrile\(^ {36}\). Hence,
Schematically, the referred reactions taking place in presence of Cu(II) and in presence of Cu(II), Zn(II) both can be represented as shown in Scheme 1.

\[
\begin{align*}
\text{C}^* & \xrightarrow{k_2} \text{H} \\
\text{R}^\cdot \text{S}^\cdot & + \text{(CH}_3\text{)}_2\text{N}^\cdot \xrightarrow{\text{fast}} \text{H}_2\text{M} \\
2\text{R}^\cdot \text{S}^\cdot & \xrightarrow{\text{fast}} \text{RSSR}
\end{align*}
\]

\[\text{Scheme 1}\]

On presuming step (5) as the rate determining step, the rate of reaction is given as

\[
-\frac{d[\text{MB}]}{dt} = k_3 [\text{C}_2][\text{MBH}^+] - k_3' [\text{C}^*][\text{Cu(II)}][\text{Zn(II)}][\text{H}^+]
\]

On applying steady state treatment for \(\text{C}_1\), \(\text{C}_2\) and \(\text{C}^*\) and under the conditions that [Cu(II)]×[Zn(II)] is very small, the rate expression is given as Eq. (9).

\[
\text{Since } [\text{MBH}^+] = K [\text{MB}][\text{H}^+], \text{ the rate expression is}
\]

\[
-\frac{d[\text{MB}]}{dt} = \frac{k_1' k_2' k_3' k_4'}{k_{-1}[\text{Zn(II)}][\text{MBH}^+]} [\text{RSH}][\text{Cu(II)}]
\]

\[
-\frac{d[\text{MB}]}{dt} = \frac{k_1' k_2' k_3' k_4'}{k_{-1}[\text{Zn(II)}][\text{MBH}^+]} [\text{RSH}][\text{Cu(II)}]
\]

\[
-\frac{d[\text{MB}]}{dt} = \frac{k_1' k_2' k_3' k_4'}{k_{-1}[\text{Zn(II)}][\text{MBH}^+]} [\text{RSH}][\text{Cu(II)}]
\]

\[
-\frac{d[\text{MB}]}{dt} = \frac{k_1' k_2' k_3' k_4'}{k_{-1}[\text{Zn(II)}][\text{MBH}^+]} [\text{RSH}][\text{Cu(II)}]
\]

... (9)

Equation (10) explains a first order kinetics in the substrates, a fractional order in MB and a first order and near zero order kinetics in Cu(II) and Zn(II) respectively. The rate expression also predicts a complex dependence of rate on [H\(^+\)] as has been frequently noticed in the oxidation of functional –SH group\(^{37}\). It has already been shown that in the oxidation of 2-ME, the order in MB is zero at lower concentrations of 2-ME and Cu(II) \((ca. < 1.50 \times 10^{-2} \text{ M}; 1.0 \times 10^{-5} \text{ M} \text{ respectively})\), while it is unity at higher concentrations of the substrate and the catalyst \((ca. \geq 6.0 \times 10^{-5} \text{ M})\). In the intermediate concentration range of 2-ME \((ranging \text{ between } 1.50 \times 10^{-2} \text{ M} \text{ to } 1.80 \times 10^{-2} \text{ M})\) and Cu(II) \((1.67 \times 10^{-5} \text{ M} \text{ to } 4.00 \times 10^{-5} \text{ M})\), the order in MB is 1/2. It appears that at lower concentrations of these reacting species, the size of nanoparticles may be relatively small due to the nucleation of the particles\(^{38}\) and thus, the lattice can accommodate a larger number of nanoparticles which will facilitate the reaction between the complex \(\text{C}_2\) and \(\text{MBH}^+\) (Eq. 4). This will result in larger values of rate constants \(k_3'\) and \(k_4'\) so that \(k_3' k_4' K[\text{MB}][\text{H}^+] > k_1' k_2'\) and under the conditions that [Zn(II)] is comparatively smaller, Eq.10 will lead to a zero order kinetics in MB. At higher concentrations of 2-ME and Cu(II), agglomeration of nanoparticles may take place decreasing their reactivity. Thus, \(k_3'\) may become smaller in Eq. 4 which will lead to a smaller value of \(k_3' k_4' K[\text{MB}][\text{H}^+]\) exhibiting first order kinetics in MB as has been observed in the oxidation of cysteine. In the intermediate concentration ranges of 2-ME and catalyst, Eq. 10 may be applicable leading to a
fractional order in MB. A comparison of the kinetic results obtained for Cu(II)-catalysed oxidation of cysteine, 2-mercaptoethylamine and α-mercaptobenzoic acid (thiosalicylic acid, TSA) by methylene blue in acidic medium reveals that the addition of Zn(II) in the case of cys and 2-ME accelerates the rate of reaction while it wields a retarding influence on the reaction of TSA \(^{13}\). It was noticed that in the oxidation of TSA, the rate constant \((k_{3/2})\) decreases from 3.6×10\(^{-1}\) to 2.4×10\(^{-1}\) \(M^{1/2}\) s\(^{-1}\) on adding Zn(II) (ca. 6.0×10\(^{-7}\) M) to the reaction system, which suggests that the presence of amino group in the thiol molecule favours the synergetic effect of Zn(II) in these reaction systems. It may be pointed out here that the possibility of the formation of nanoparticles on interaction of TSA with Cu(II) could not be ascertained by recording TEM images, perhaps due to their low melting nature. Further, a larger value of \(\Delta G^*\) obtained for oxidation of cysteine (160 kJ mol\(^{-1}\) as compared to 70 kJ mol\(^{-1}\) and 49.5±2 kJ mol\(^{-1}\) obtained for the oxidation of 2-ME and TSA respectively) by MB suggests that this reaction is relatively less favoured as compared to that of 2-ME, which may be attributed to the presence of carboxylic group in cysteine molecule. This is corroborated by the observations made on the oxidation of TSA and it appears that the reaction is hampered by the presence of carboxylic group which may be attributed to the protonation of the nanoparticles by carboxylic group\(^8\). It is, thus, apparent that metal ion catalysis in these reaction systems are mediated by nanoparticles and the rate of reaction is intricately correlated with the intramolecular effect exerted by the substrate as well as on the nature of metal ions acting as catalysts in the studied systems.

### Supplementary Data

Supplementary data associated with this article i.e., Figs S1-S15, are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_52A(06)724-731SupplData.pdf.

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