Kinetics and mechanism of a fast leuco-Methylene Blue oxidation by copper(II)–halide species in acidic aqueous media

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The kinetics of a fast leuco-Methylene Blue (LMB) re-oxidation to Methylene Blue (MB) by copper(II)–halide (Cl\(^-\), Br\(^-\)) complexes in acidic aqueous media has been studied spectrophotometrically using a stopped-flow technique. The reaction follows a simple first order rate expression under an excess of the copper(II) species (and H\(^+\) (aq)), and the pseudo-first order rate constant (k\(_{\text{obs}}\)) is largely independent of the atmosphere used (air, oxygen, argon). The rate law, at constant Cl\(^-\) (Br\(^-\)) anion concentration, is given by the expression: (d[MB])/dt = (k\(_{\text{obs}}\)[H\(^+\)] + k\(_{\text{fl}}\)j(1 + K[H\(^+\)]))/[Cu\(^{n}\)][LMB] = k\(_{\text{obs}}\)[LMB], where K is the protonation constant, and k\(_{\text{fl}}\) and k\(_{\text{obs}}\) are the pseudo-second order rate constants for protonated and deprotonated forms of LMB, respectively. The rate law was determined based on the observed k\(_{\text{obs}}\) vs. [Cu\(^{n}\)] and [H\(^+\)] dependences. The rate dramatically increases with [Cl\(^-\)] over the range: 0.1–1.5 M, reflecting the following reactivity order: Cu\(^{n}\) (aq) < CuCl (aq) < . . . < CuCl\(_2\) (aq). The slow re-oxidation of LMB by oxygen has also been briefly examined at different [H\(^+\)]. ESR results provide clear evidence for the formation of an intermediate radical. The mechanistic consequences of all these results are discussed.

Introduction

Methylene Blue (which we will refer to generally as MB), a member of the thiazine class of dyes, was first synthesized in 1876.\(^1\) Since that time many investigations into its physico-chemical properties have been undertaken.\(^2\)–\(^5\) Amongst the numerous uses of MB is its fairly recent application as the key component of a wide range of optical oxygen sensors used in industry to mediate the oxidation of organic compounds by oxygen, e.g. in the Wacker process.\(^18\) The efficient complexation of copper(II) by halide ligands strongly shifts the redox potential of the Cu\(^{n}\)/Cu\(^{I}\) couple more positive, \(i.e.\) oxidising; thus, E\(_{\text{ox}}\)(Cu\(^{n}\) (aq)/Cu\(^{I}\) (aq)) = 0.159 V, but is 0.566 and 0.657 V in 1 M Cl\(^-\) and Br\(^-\), respectively.\(^19\) Halide complexation also allows Cu(II) to act as a one-electron oxidant and facilitates the rapid oxidation of copper(II) by dioxygen.\(^14\) In this paper, the kinetics of re-oxidation of LMB to MB \(b\) by Cu(II)–halide complexes have been studied in acidic aqueous solutions. In addition, some study of the much slower oxidation by O\(_2\) (aq) (without any copper(II) species) has also been carried out. The results of this work are described below.

Experimental

Materials

Methylene Blue (Aldrich), CuCl\(_2\)·2H\(_2\)O, 36% HCl, NaCl, NaBr and other chemicals were analytical grade reagents and were used without further purification. Water redistilled from alkaline permanganate was used to prepare all solutions. A

Table 1 Structure and UV/Vis absorption characteristics of Methylene Blue and its common reduced forms

<table>
<thead>
<tr>
<th>Species</th>
<th>Structure</th>
<th>Abbreviation</th>
<th>pK(_{a})</th>
<th>(E^0/V) vs. NHE</th>
<th>(\lambda_{\text{max}}/\text{nm})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene Blue (unprotonated)</td>
<td><img src="image1" alt="Structure" /></td>
<td>MB(^+)</td>
<td>0</td>
<td>—</td>
<td>660; 614; 292</td>
</tr>
<tr>
<td>Semi-reduced Methylene Blue</td>
<td><img src="image2" alt="Structure" /></td>
<td>MB(^-)</td>
<td>−3; 2; 9</td>
<td>(MB/MB(^+)) = −0.23</td>
<td>420</td>
</tr>
<tr>
<td>(unprotonated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leuco-Methylene Blue</td>
<td><img src="image3" alt="Structure" /></td>
<td>LMB(^-)</td>
<td>4.5; 5.8; 1.7</td>
<td>(MB/LMB) = 0.011 (pH 7) and 0.532 (pH 0)</td>
<td>256</td>
</tr>
<tr>
<td>(unprotonated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
stock solution of 0.2 M chromium(II) chloride in 0.1 M hydrochloric acid was obtained by reducing chromium(III) chloride using zinc amalgam in a method that is described elsewhere.20 High purity gases (argon, oxygen, air) were used throughout this work.

**Preparation of LMB solution**

De-oxygenated, acidic, blue solutions of MB at the desired concentration (up to $5 \times 10^{-4} \text{ M}$) were titrated with Cr$^{3+} (\text{aq})$ until they became colourless. At this point, a drop of MB solution was added to obtain a pale blue colour solution of predominantly LMB. LMB was prepared fresh for each series of kinetic measurements.

**Measurements**

**ESR measurements.** Room-temperature ESR spectra of the solutions were recorded with an ESR Bruker Physik 418 S reflection type spectrometer in X-band (ca. 9.5 GHz) with a 100 kHz modulation of the steady magnetic field. The microwave frequency was monitored with a 18 GHz microwave counter 2440 (Marconi Instruments). The magnetic field was measured with an automatic MJ-110R NMR-type magnetometer (Radiopan). A flat quartz cell was used.

**Kinetic measurements: oxidation by copper(II)-halide complexes.** The rate of oxidation of LMB to MB was followed spectrophotometrically, via the appearance of the product MB, using a stopped-flow technique combined with rapid scanning on a UV/VIS spectrometer. The instrument used for this work was a HP8453 UV/VIS spectrophotometer equipped with home made stopped-flow apparatus thermostatted using an external Julabo F25 cryostat. The “dead-time” of this stopped-flow system was 0.1 s. Scans of the UV/VIS spectrum of the reaction solution were taken over the range 500–800 nm, every 0.1 s. The spectrophotometric results of a typical kinetic experiment are illustrated in Fig. 1.

The reaction was carried out at 288, 298 and 308 K under an air atmosphere and additionally at 288 K under argon and de-aerated dioxygen purged solutions at an ionic strength kept at 1.0 or 2.0 M with NaCl, NaBr and NaNO$_3$. In all this work the concentration of LMB was $1 \times 10^{-5} \text{ M}$, but the concentration of Cu(II) was varied over the range (0.2–1.5) $\times 10^{-5} \text{ M}$ and that of H$^+$ over the range 0.001–0.1 M. In all this work the time scale of the reoxidation of MB was 1 to 60 s. Each kinetic run was repeated 5 times. The pseudo-first order rate constants were calculated numerically by a nonlinear least-squares method from absorbance versus time data, recorded at 666 nm, for up to 95% of the overall absorbance change. The relative standard errors of the single $k'_{\text{obs}}$ values were 0.5–2% and for the average values of five runs were 0.5–5%.

**Kinetic measurements: oxidation by dissolved dioxygen**

As before, a rapid scanning spectrophotometer was used to monitor the oxidation of LMB, although this time the oxidant was dissolved oxygen. In a typical experiment, the reaction was started by an injection of 0.04 cm$^3$ of LMB solution into a thermostated 1 cm cell containing a solution of HCl and NaCl, in the concentrations necessary to give a final ionic strength of 1.0 M, that had been purged with air or pure dioxygen. The solution was stirred throughout the experiment. Typically, the final concentration of LMB was ca. $1 \times 10^{-7} \text{ M}$, and the concentration of H$^+$ was 1 M (HCl). Scans of the UV/VIS spectra of the reaction solution were taken every 5 s. The typical time scale of the re-oxidation process in acidic media was ca. 2000–4000 s i.e. much slower than that for any of the Cu(II)-halide oxidants. Each kinetic run was repeated three times. The kinetics of LMB reoxidation to MB by oxygen are not simple (possibly due to a gas-to-liquid mass transfer effect) and, thus, the rate of reaction was roughly evaluated by an initial rate method. In order to convert the rate data in absorbance units per second to concentration units per second, a value for $k_{\text{obs}}$ of 7.8 $\times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ was taken for MB. In this work the relative standard error for the mean rate value was 3%. Additional runs were carried out in NaHCO$_3$-buffer. The reaction was so fast that the stopped-flow technique was applied.

**Results and discussion**

**Preliminary observations**

In order to get reproducible kinetic data, special precautions were employed when preparing the leuco-Methylene Blue stock solution. The literature methods for LMB preparation include many variants of MB photobleaching, and thermal reduction of MB by ascorbic acid, some sugars or amino acids.8,9,14,15 Most of the above methods are inconvenient for kinetic studies because an excess of the reductant is left in the solution or adsorption of the dye on the zinc granules makes it difficult to adjust the concentration of LMB to the desired level. In this work, satisfactory results were obtained by titrating MB solutions with Cr$^{3+} (\text{aq})$ until ca. 95% of the MB were converted into LMB. Although this method introduces inert chromium(III) species, mainly Cr$^{3+} (\text{aq})$ ions, into the reaction mixture at a concentration of ~ $2 \times 10^{-3} \text{ M}$, these ions are transparent within the UV/VIS absorbance range studied and do not appear to affect the kinetics of oxidation of LMB to MB by the relevant oxidants. Formation of MB$^-$ radicals via disproportionation, i.e. reaction (1), is expected to be insignificant at the low ($10^{-5} \text{ M}$) concentrations of the dye under study, given the low equilibrium constant for the process, i.e. (3–6) $\times 10^{-6};$2

$$\text{LMB} + \text{MB} \rightleftharpoons 2\text{MB}^-$$

The addition of a significant excess of copper(II) salt (typically $>10^{-4} \text{ M}$) to an acidic aqueous LMB solution ($10^{-5} \text{ M}$) containing a vast excess of Cl$^-$ and/or Br$^-$ anions causes the rapid formation of MB, whereas practically no oxidising action of Cu$^{2+} (\text{aq})$ ions is observed in nitrate media, under otherwise the same reaction conditions. Perchlorates can not be used at high concentration because of the precipitation of a Methylene Blue perchlorate salt. In all this work, the recorded absorbance versus time profiles gave good fits to simple first order kinetics. In contrast to the rapid kinetics of LMB re-oxidation by Cu(II)-halide complexes in acid, the spontaneous oxidation of LMB by dissolved oxygen is quite a slow process under acidic
conditions, but the rate increases dramatically with pH, reaching a stopped-flow time scale at pH > 6.

The reactants and the products

The protolytic forms of LMB include: H$_3$LMB$^{-}$, H$_2$LMB$^{2-}$, HLMB$^-$ and LMB$^-$. From the data in Table 1 it is possible to construct the following structure–pH diagram illustrated in Scheme 1.

From the data contained in Scheme 1, it follows that at pH < 1 the protonated cationic form, H$_3$LMB$^{2+}$, predominates; at pH > 7 LMB exists mainly as the deprotonated anion, LMB$^-$. The protolytic forms of LMB include:

- The reactants and the products
- The percentage distribution of the various copper($^{II}$) complexes in solutions, at different [Cl$^{-}$] and [Br$^{-}$] concentrations, was evaluated based on the values of these stability constants.

The measured values of the pseudo-first order rate constants ($k'_{obs}$) were found to be practically independent of the initial concentration of LMB over the range (0.5–2) × 10$^{-3}$ M as measured at the chosen wavelength of 666 nm, where the electronic transition for the monomer of MB is observed.

The measured values of $k'_{obs}$ obtained under a wide variety of different reaction conditions are given in Table 3. The measured values of $k'_{obs}$ obtained under a wide variety of different reaction conditions are given in Table 3. The measured values of $k'_{obs}$ obtained under a wide variety of different reaction conditions are given in Table 3. The measured values of $k'_{obs}$ obtained under a wide variety of different reaction conditions are given in Table 3.

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The percentage distribution of the various copper($^{II}$) and copper($^{I}$) complexes in solutions, at different [Cl$^{-}$] and [Br$^{-}$] concentrations, was evaluated based on values of the reported stability constants.

\[ \phi_i = \frac{100\%}{1 + \sum_{j=1}^{n} \beta_j [L]^j} \]  

where L = Cl$^{-}$ (Br$^{-}$) and $\phi_i$ is the percentage distribution of Cu$^{II}$ (aq), or Cu$^{I}$ (aq) and $\phi_i$ are the percentage distributions of the other ion species ($i = 1–4$) present. The results of these calculations are given in Table 2.

From the results in Table 2, it appears that any copper($^{II}$) produced via reaction (2) will be almost exclusively [CuCl$^{3-}$] and [CuCl$_2$]$^{2-}$ anions under all the levels of [Cl$^{-}$] used in this work. In addition, at [Cl$^{-}$] = 1.0 M, the usual level of Cl$^{-}$ employed in this kinetic study, any Cu($^{II}$) ions will exist predominantly as [CuCl]$^{2+}$ (48%), with [CuCl$_2$] and [CuCl$_3$] in comparable amounts (24 and 18%, respectively) and the aqua and [CuCl$_2$]$^{2-}$ complexes at the low value of 5%.

In all this work the concentration of Cu($^{II}$) was in significant excess compared to that of LMB and, as a consequence, the oxidation of LMB to MB, via reaction (2), was always complete, as confirmed by the recorded final absorbance and the known molar absorption coefficient of MB.

Oxidation by copper($^{II}$)-halide complexes

All the absorbance versus time kinetic profiles for the oxidation of LMB by Cu($^{II}$)-halides gave an excellent fit to first-order rate kinetics throughout the visible range, in the presence of an excess of [Cu$^{II}$] and [H$^+$]. Thus, the measured values of the pseudo-first order rate constants ($k'_{obs}$) were found to be practically independent of the initial concentration of LMB over the range (0.5–2) × 10$^{-3}$ M as measured at the chosen wavelength of 666 nm, where the electronic transition for the monomer of MB is observed.

The measured values of $k'_{obs}$ obtained under a wide variety of different reaction conditions are given in Table 3. The measured values of $k'_{obs}$ obtained under a wide variety of different reaction conditions are given in Table 3. The measured values of $k'_{obs}$ obtained under a wide variety of different reaction conditions are given in Table 3.

A plot of the variation in the calculated value of $k'_{obs}$ for reaction (2), as a function of the concentration of the copper($^{II}$)-halide species, [Cu$^{II}$], for three different reaction temperatures is illustrated in Fig. 2. All three plots are good straight lines with an approximate zero intercept (eqn. (9)).

\[ k'_{obs} = k_{cu}[Cu^{II}] \]  

Indeed, an analysis of all the data in Table 3 based on eqn. (9) gave an excellent fit to this equation and the results of this analysis of the data in the form of values of $k_{cu}$ are contained in Table 4. Note that, the pseudo-second order rate constant, $k_{cu}$ characterises the reactivity of the Cu($^{II}$)-halide species towards LMB, for a defined H$^+$ and halide concentration.

Although most of the kinetic data reported here were obtained at constant chloride concentration, some work was also carried out using 1 M Br$^-$. 
Table 2  Percentage levels ($\phi$) of copper(II) and copper(I) complexes in Cl$^{-}$ (Br$^{-}$) media of different concentrations$^a$

<table>
<thead>
<tr>
<th>Conc. Cl$^{-}$ (Br$^{-}$) ion/M</th>
<th>Complex</th>
<th>Cu$^{II}$(aq)</th>
<th>CuL$^{m-1}$ (aq)</th>
<th>CuL$^{m-2}$ (aq)</th>
<th>CuL$^{m-3}$ (aq)</th>
<th>CuL$^{m-4}$ (aq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Cu$^{II}$</td>
<td>50 (97)</td>
<td>48 (3)</td>
<td>2 (&lt;1)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>Cu$^{I}$</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&gt;86 (&gt;99)</td>
<td>14</td>
<td>—</td>
</tr>
<tr>
<td>0.5</td>
<td>Cu$^{II}$</td>
<td>13 (87)</td>
<td>64 (12)</td>
<td>16 (1.0)</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Cu$^{I}$</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&gt;56 (&gt;99)</td>
<td>44</td>
<td>—</td>
</tr>
<tr>
<td>1.0</td>
<td>Cu$^{II}$</td>
<td>5 (75)</td>
<td>48 (21)</td>
<td>24 (4)</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Cu$^{I}$</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&gt;39 (&gt;99)</td>
<td>61</td>
<td>—</td>
</tr>
<tr>
<td>1.5</td>
<td>Cu$^{II}$</td>
<td>2 (65)</td>
<td>33 (27)</td>
<td>25 (7.3)</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Cu$^{I}$</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&gt;30 (&gt;99)</td>
<td>70</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$ Calculated based on stability constants at 298 K, $I$ = 1.0 M.$^{22,23}$

Table 3  Pseudo-first order rate constants for LMB oxidation by Cu$^{II}$-chloride (bromide) species; $I$ = 1.0 M (Na$^+$, H$^+$, Cl$^-$, (Br$^-$))$^a$

<table>
<thead>
<tr>
<th>[H$^+$]$^1/10^{-2}$ M</th>
<th>[Cu$^{II}$]$^1/10^{-4}$ M</th>
<th>$k_{obs}^{Cu}/10^{-2}$ s$^{-1}$</th>
<th>[H$^+$]$^1/10^{-2}$ M</th>
<th>[Cu$^{II}$]$^1/10^{-4}$ M</th>
<th>$k_{obs}^{Cu}/10^{-2}$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$T = 288$ K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>69.0 ± 0.7</td>
<td>4</td>
<td>2</td>
<td>10.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>179 ± 1.5</td>
<td>5</td>
<td>5</td>
<td>25.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>348 ± 2.1</td>
<td>10</td>
<td>10</td>
<td>48.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>2</td>
<td>37.8 ± 0.2</td>
<td>6</td>
<td>8.60 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>94.5 ± 0.5</td>
<td>5</td>
<td>5</td>
<td>21.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>189 ± 1.4</td>
<td>10</td>
<td>10</td>
<td>43.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>283 ± 2.2</td>
<td></td>
<td>15</td>
<td>69.8 ± 2.0</td>
</tr>
<tr>
<td>0.6</td>
<td>2</td>
<td>24.4 ± 0.2</td>
<td>8</td>
<td>2</td>
<td>8.30 ± 0.1 (56.9 ± 0.4)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>61.0 ± 0.6</td>
<td>5</td>
<td>5</td>
<td>20.7 ± 0.3 (185 ± 2.7)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>119 ± 1.1</td>
<td>5</td>
<td>5</td>
<td>20.5 ± 0.1$^b$</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>183 ± 2.0</td>
<td>5</td>
<td>5</td>
<td>21.0 ± 0.4$^b$</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>14.4 ± 0.1</td>
<td>10</td>
<td>2</td>
<td>7.70 ± 0.4 (59.9 ± 2.3)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>42.6 ± 0.4</td>
<td>5</td>
<td>5</td>
<td>18.9 ± 0.6 (182 ± 2.9)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>36.5 ± 0.9</td>
<td>10</td>
<td>10</td>
<td>39.5 ± 0.8 (384 ± 27)</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>132 ± 1.5</td>
<td>15</td>
<td>15</td>
<td>59.3 ± 1.1 (587 ± 29)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>12.5 ± 0.2</td>
<td></td>
<td>2</td>
<td>41.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>32.6 ± 0.2</td>
<td>5</td>
<td>5</td>
<td>124 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>63.4 ± 2.8</td>
<td>10</td>
<td>10</td>
<td>276 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>94.9 ± 1.3</td>
<td>15</td>
<td>15</td>
<td>422 ± 9.8</td>
</tr>
</tbody>
</table>

$^a$ The data in parentheses refer to $I$ = 1.0 M (Na$^+$, H$^+$, Br$^{-}$). $^b$ In argon atmosphere. $^c$ In oxygen atmosphere.

Fig. 2  Dependence of the pseudo-first order rate constant ($k_{obs}^{Cu}$) on [Cu$^{II}$] for LMB oxidation by copper(II)-Cl$^{-}$ species; [H$^+$]$^1/10^{-3}$ M, $I$ = 1.0 M (Na$^+$, H$^+$, Cl$^{-}$).

A comparison of the different reactivities of the two different complexes, given by the different values of $k_{obs}$ in Table 4, show that the Cu(II)-bromide complexes are about one order of magnitude higher in reactivity than these for the chloride complexes, under otherwise the same reaction conditions. The likely cause for this marked difference in reactivity is a higher copper(II) redox potential in the Br$^-$ medium, as would be consistent with an outer sphere electron transfer mechanism. $^{24}$

From the results in Table 4 it is clear that the kinetics of reaction (2) are very pH-dependent, as illustrated by a plot of $k_{obs}$ vs. [H$^+$] for LMB oxidation by copper(II)-Cl$^{-}$ species in Fig. 3 which show that the value of $k_{obs}$ decreases substantially with increasing [H$^+$]. This observed rate dependence can be most readily rationalized by assuming that the rate determining step involves the oxidation of LMB to the radical MB$^+$ and that the state of protonation of LMB is critical in determining this rate.
A rate law for the observed dependence of $k\_{obs}$ on [H$^+$] within pH range 3–1 can be derived assuming the reaction model presented below in Scheme 2 applies. In Scheme 2 (i) the rate controlling step is formation of the MB$^+$ radical; and (ii) the parallel oxidation of the protonated and the deprotonated forms takes place with rate constants $k_i$ and $k_a$, respectively.

The proposed reaction sequence (Scheme 2) leads to the following rate law, assuming constant Cl$^-$ (Br$^-$) concentrations:

$$
\frac{d[M^+]}{dt} = \frac{(k_i[H^+] + k_a)[Cu^2+] \cdot [LMB]}{(1 + k_i[H^+])}
$$

where, at constant [H$^+$]

$$
k_{obs} = \frac{(k_i[H^+] + k_a)}{(1 + k_i[H^+])}
$$

The rate constants $k_a$ and $k_i$ characterise the reactivity of the deprotonated and the protonated forms of LMB, respectively, and $K$ is the reciprocal of the acid dissociation constant of H$_2$LMB$^{2+}$. The results of a least-squares fitting of the $k_{obs}$ vs [H$^+$] data given in Table 4, and illustrated in Fig. 3, to eqn. (10) gives $k_a = (7.0 \pm 0.1) \times 10^3$ M$^{-1}$ s$^{-1}$ and $k_i = 0.3 \times 10^2$ M$^{-1}$ s$^{-1}$ and $K = (1.1 \pm 0.03) \times 10^3$ M$^{-1}$, thus $K_a = 9.1 \times 10^4$ M.

The $pK_a$ for the H$_2$LMB$^{2+}$/H$_2$LMB$^+$ couple derived from the kinetic data and the proposed kinetic scheme, i.e. $pK_a = 3.1$, is different from that reported elsewhere ($pK_a = 1.7$). However, the latter was recorded under low ionic strength conditions, whereas the high ionic strength conditions used in this work will cause a general increase in all the values of the $pK_a$'s associated with LMB. Additionally the temperature used before was 10 K higher. The limited solubility of LMB at I = 1.0 M and 288 K makes impossible a pH-metric determination of the acidity constant under the mentioned conditions. Further work is required in order to ascertain the actual $pK_a$ values of the various forms of LMB under high ionic strength conditions.

From the results of the analysis of the data illustrated in Fig. 3, using eqn. (10), it appears that the deprotonated form of LMB is ca. 20 more reactive than its protonated form.

The kinetics of reaction (2) were also studied as a function of [Cl$^-$] and the results of this work are given in Table 5.

From the results in Table 5 it appears that $k'$ $\times$ obs increases markedly, about 140 times, with a 15 fold increase of [Cl$^-$]. The limited solubility of LMB at I = 1.0 M and 288 K makes impossible a pH-metric determination of the acidity constant under the mentioned conditions. Further work is required in order to ascertain the actual $pK_a$ values of the various forms of LMB under high ionic strength conditions.

Oxidation of LMB by dioxygen

The pseudo-first order rate constants for the oxidation of LMB by oxygen (eqn. (12)):

$$
2H_2LMB^{2+} + O_2 \rightarrow 2MB^- + 2H_2O^+ \quad (12)
$$

are presented in Table 6. In all this work the concentration of oxygen, typically $> 10^{-4}$ M, is much higher than that of [LMB], which is ca. $1 \times 10^{-5}$ M. Comparison of the measured values recorded in air and oxygen saturated solution (Table 6) shows that the rate depends on concentration of dissolved O$_2$ but that the values of $k_{obs}$ are not proportional to [O$_2$]. Rather, a saturation effect is observed at high levels of [O$_2$]. Further work is required in this area to ascertain the cause of this saturation effect. A rough measure of the reactivity of LMB towards oxygen, i.e. $k_{obs}$, can be gained by assuming that at low levels of [O$_2$], i.e. air saturated conditions, $k_{obs}$ is proportional to [O$_2$] and, therefore, the pseudo-second order rate constant is given by eqn. (13):

$$
k_{obs} = \frac{k_{obs}}{[O_2]_{air}} \quad (13)
$$
The calculated values for \( k_{oa} \) are reported in Table 6 and show that the rate of reaction (2) decreases substantially when the pH of the solution is decreased from pH \( \sim 8 \) to 0. Protonated forms of LMB are expected to be less reactive than their deprotonated counterparts (see the redox potential data in Table 1). Thus, for the oxidation of LMB by oxygen, in \( \text{HCO}_3^- \) buffer solution, LMB exists practically exclusively in the anionic LMB form which is far more reactive than the protonated forms of LMB and, consequently, the rate constant for this oxidation reaction is about 8 times higher in \( \text{HCO}_3^- \) buffer solution than in very acidic solution.

Room-temperature ESR spectra of LMB solution oxidized by oxygen were measured as well. The spectra exhibit single isotropic line with \( g = 2.003 \) and peak-to-peak width, \( \Delta B_{pp} \approx 2 \text{ mT} \). The ESR parameters given above are very close to those of MB\(^+\) formed electrochemically.\(^3\) The ESR spectrum of a water–ethanol (1:9) solution of LMB is shown as example in Fig. 4.

![Fig. 4](image)

**Fig. 4** Exponential smoothing ESR spectrum of ca. \( 1 \times 10^{-3} \text{ M} \) LMB water–ethanol (1:9) solution exposed to air diffusion. Other conditions: time of reaction ca. 45 min; microwave frequency 9.4975 GHz; room temperature.

was observed that the intensity of the ESR signal was not so high and slowly increased and then decreased during the course of reaction. Finally, the ESR signal disappeared for full oxidation of LMB to MB. The ESR signal detected had no hyperfine splitting probably due to the high modulation amplitude used, thus the line width is also perturbated. On the other hand, such experimental conditions were needed to observe signal at all. Nevertheless, there is no doubt that a relatively stable, but at very low concentration, free radical is formed as one of the oxidation steps.

**Conclusion**

In conclusion, the rate of oxidation of LMB by Cu(II)–chloro and –bromo complexes is high in acid solution but decreases with increasing acidity, and the kinetics appear simple first order with respect to the concentration of LMB. In the presence of Cu(II) ions, the rate of LMB oxidation increases with increasing chloride concentration, reflecting its depend-

Table 6  Pseudo-first order rate constants, \( k_{oa} \), and \( k_{oa} \) for LMB oxidation by \( \text{O}_2 \) at 298 K; \( f = 1.0 \text{ M} \) (Na\(^+\), H\(^+\), Cl\(^-\)).

<table>
<thead>
<tr>
<th>( [\text{H}^+] / \text{M} )</th>
<th>Oxygen</th>
<th>Air</th>
<th>Oxygen</th>
<th>Air</th>
<th>( k_{oa} / \text{M}^- \text{s}^-1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.3 ( \times 10^{-3} )</td>
<td>62</td>
<td>17</td>
<td>9.1</td>
<td>1.9</td>
<td>89</td>
</tr>
<tr>
<td>1.0</td>
<td>3.3</td>
<td>2.8</td>
<td>11.7</td>
<td>2.4</td>
<td>11.7</td>
</tr>
</tbody>
</table>

\( k_{oa} = k_{oa}/[\text{O}_2\text{H}^-\text{HCO}_3^-] \) buffer solution.

ence on the concentration of the Cu\(^{2+}\) ions complexed by Cl\(^-\) ions, especially the anionic complexes. The pseudo-second order rate constants, \( k_{oa} \), increase ca. 2.7 times per 10 K. In contrast, oxidation of LMB by dissolved oxygen is very slow in acidic solution but much more rapid in neutral solution. However, the kinetics of this latter process are complex and require further study. Comparison of the reactivity of different oxidants using values of the pseudo-second order rate constants shows that reactivity of Fe(III)–Cl species\(^2\) is two orders of magnitude higher (at [Cl\(^-\)] = 0.4 M, pH = 2, \( T = 298 \text{ K} \)) than that of Cu(II)–Cl complexes and the reactivity of [Fe(CN)\(_6\)]\(^{3-}\)–oxidant\(^4\) is even higher than that of Fe(III)–Cl species.

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**References**