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

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Received 14th June 2002, Accepted 16th October 2002
First published as an Advance Article on the web 23rd December 2002

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'_{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: $(d[MB^+])/dt = ((k_aK[H^+] + k_b)/(1 + K[H^+]))\cdot[Cu^{II}][LMB] = k'_{obs}[LMB]$, where K is the protonation constant, and k_a and k_b 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'_{obs} *vs.* $[Cu^{II}]$ and $[H^+]$ dependences. The rate dramatically increases with $[Cl^-]$ over the range: 0.1–1.5 M, reflecting the following reactivity order: $Cu^{2+}(aq) \ll CuCl^+(aq) < \ldots < CuCl_4^{2-}$. 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 physicochemical properties have been undertaken.[2–15] 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 the food industry for food packaging under a modified atmosphere of nitrogen or carbon dioxide (MAP).[10,11] The principle behind such sensors is that the reduced (leuco) form of MB, (which we shall refer to generally as LMB) is colourless in the absence of oxygen, but readily re-oxidised by O_2 to MB, *i.e.* its brightly coloured blue original form. The structures and UV/VIS characteristics of Methylene Blue and its common reduced forms are given in Table 1.

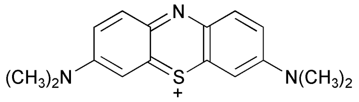
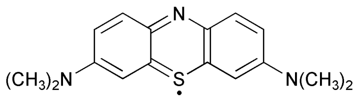
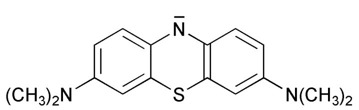
It is well known that copper(II) complexes can act as catalysts in dioxygen oxidation processes, or as direct oxidants,[16] and, for that reason, copper is important as a microelement in biological systems.[17] Copper salts are also key components of catalytical mixtures used in industry to mediate the oxidation of organic compounds by oxygen, *e.g.* in the Wacker process.[18] The efficient complexation of copper(I) by halide ligands strongly shifts the redox potential of the Cu^{II}/Cu^{I} couple more positive, *i.e.* oxidising; thus, $E^0(Cu^{2+}(aq)/Cu^+(aq)) = 0.159$ 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(I) to copper(II) by dioxygen.[18] In this paper, the kinetics of re-oxidation of LMB to MB^+ 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), $CuCl_2\cdot 2H_2O$, 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

Species	Structure	Abbreviation	pK_a	E^0/V *vs.* NHE	λ_{max}/nm
Methylene Blue (unprotonated)		MB^+	0	—	660; 614; 292
Semi-reduced Methylene Blue (unprotonated)		$MB^{\bullet}$	−3; 2; 9	$(MB/MB^{\bullet}) = -0.23$	420
Leuco-Methylene Blue (unprotonated)		LMB^-	4.5; 5.8; 1.7	(MB/LMB) = 0.011 (pH 7) and 0.532 (pH 0)	256

DOI: 10.1039/b205786g

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 to 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 × 10^{-4} M) were titrated with Cr^{2+}(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.

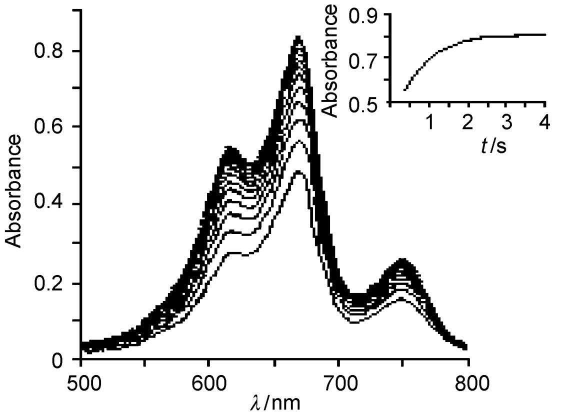


Fig. 1 Observed spectral changes during LMB oxidation by Cu^{II}–halide species in air-saturated solution $[H^+] = 0.1$ M, $T = 298$ K, $I = 1.0$ M (Na^+, H^+, Cl^-); scans were recorded every 0.2 s, $\lambda = 666$ nm. The insert illustrates the measured variation in the absorbance of the reaction solution at 666 nm as a function of reaction time. The kinetics of MB recovery give a good fit to a first-order rate equation.

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 × 10^{-5} M, but the concentration of Cu(II) was varied over the range (0.2–1.5) × 10^{-3} 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'_{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 × 10^{-5} 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 ε_{666} of 7.8 × 10^4 M^{-1} cm^{-1} was taken for MB.[2] 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 zinc amalgam.[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^{2+}(aq) until *ca.* 95% of the MB were converted into LMB. Although this method introduces inert chromium(III) species, mainly Cr^{3+}(aq) ions, into the reaction mixture at a concentration of ~ 2 × 10^{-5} 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 $MB^\bullet$ radicals *via* synproportionation, *i.e.* reaction (1), is expected to be insignificant at the low (10^{-5} M) concentrations of the dye under study, given the low equilibrium constant for the process, *i.e.* (3–6) × 10^{-6}:[2]

$$LMB + MB \rightleftharpoons 2MB^\bullet \qquad (1)$$

The addition of a significant excess of copper(II) salt (typically > 10^{-4} M) to an acidic aqueous LMB solution (10^{-5} 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+}(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:[7,9] H_3LMB^{2+}, H_2LMB^+, $HLMB^0$ and LMB^- (Table 1). 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_3LMB^{2+}, predominates; at pH > 7 LMB exists mainly as the deprotonated anion, LMB^-. Within the pH range 1–7, all the protonated and deprotonated LMB forms can act as the electron donors. According to the literature data,[21] the product of LMB oxidation exists mainly (>90%) in the monomeric form at the concentration of the dye chosen for this work, namely *ca.* 10^{-5} M. In addition, its two protolytic forms: HMB^{2+} and MB^+ are present in equal amounts at pH ≈ 0. Thus, spectra of the product of oxidation of LMB to MB at pH > 1 are attributed almost exclusively to the deprotonated form of MB, *i.e.* MB^+.

A simplified stoichiometry of the studied reaction with copper(II) complexes in acidic aqueous media (at pH = 1) is given by eqn. (2):

$$2Cu^{II} + H_3LMB^{2+} + 3H_2O \longrightarrow 2Cu^{I} + MB^{+} + 3H_3O^{+} \quad (2)$$

and, in the case of an oxygen or air atmosphere, re-oxidation of copper(I) by dioxygen takes place (eqn. (3)):

$$4Cu^{I} + O_2 + 4H_3O^{+} \longrightarrow 4Cu^{II} + 6H_2O \quad (3)$$

thus the net overall process is given by eqn. (4):

$$2H_3LMB^{2+} + O_2(aq) \longrightarrow 2MB^{+} + 2H_3O^{+} \quad (4)$$

In the presence of chloride (or bromide) anions copper(II) and copper(I) exist as aqua ions and as a series of the chloro-aqua complexes (eqns. (5) and (6)):

copper(II):

$$Cu(H_2O)_6^{2+} + nCl^- \rightleftharpoons [Cu(Cl)_n(H_2O)_{6-n}]^{(2-n)+} \quad (n = 1\text{–}4) \quad (5)$$

copper(I):

$$Cu^+(aq) + nCl^- \rightleftharpoons [Cu(Cl)_n(aq)]^{(1-n)+} \quad (n = 1\text{–}3) \quad (6)$$

The percentage distribution of the various copper(II) and the copper(I) complexes in solutions, at different $[Cl^-]$, were evaluated based on values of the reported stability constants[22,23] and eqns. (7) and (8):

$$\phi_0 = \frac{100\%}{1 + \sum_{i=1}^{4} \beta_i [L]^i} \quad (7)$$

$$\phi_{i(i=1-4)} = \phi_0 \beta_i [L]^i \quad (8)$$

where L = Cl^- (Br^-) and ϕ_0 is the percentage distribution of $Cu^{2+}(aq)$, or $Cu^+(aq)$ and ϕ_i are the percentage distributions of the other ionic 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(I) produced *via* reaction (2) will be almost exclusively $[CuCl_2]^-$ and $[CuCl_3]^{2-}$ 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 $[CuCl]^+$ (48%), with $[CuCl_2]$ and $[CuCl_3]^-$ in comparable amounts (24 and 18%, respectively) and the aqua and $[CuCl_4]^{2-}$ 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.[2]

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^{-5} M as measured at the chosen wavelength of 666 nm, where the electronic transition for the monomer of MB is observed.

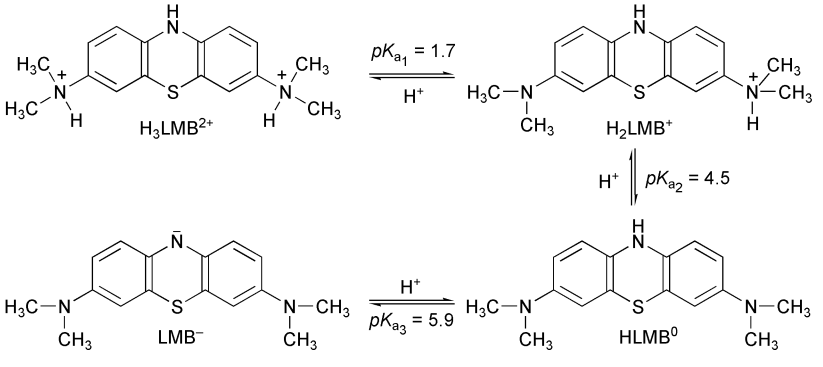
The measured values of k'_{obs} obtained under a wide variety of different reaction conditions are given in Table 3. Experiments conducted in the presence and absence of oxygen showed that the rate of LMB oxidation by the Cu(II)–halide species is independent of oxygen concentration. Thus, copper(II) species play a role of direct LMB oxidants.

A plot of the variation in the calculated value of k'_{obs} for reaction (2), as a function of the concentration of the Cu(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)).

$$k'_{obs} = k_{Cu}[Cu^{II}] \quad (9)$$

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^-.



Scheme 1

Table 2 Percentage levels (φ_i) of copper(II) and copper(I) complexes in Cl^- (Br^-) media of different concentrations[a]

Conc. Cl^- (Br^-) ion/M		Complex Cu^{n+}(aq)	$CuL^{(n-1)+}$(aq)	$CuL_2^{(n-2)+}$(aq)	$CuL_3^{(n-3)+}$(aq)	$CuL_4^{(n-4)+}$(aq)
0.1	Cu^{II}	50 (97)	48 (3)	2 ($\leqslant$1)	$\leqslant$1	$\leqslant$1
	Cu^{I}	$\leqslant$1	$\leqslant$1	>86 ($\geqslant$99)	14	—
0.5	Cu^{II}	13 (87)	64 (12)	16 (1.0)	6	1.0
	Cu^{I}	$\leqslant$1	$\leqslant$1	$\geqslant$56 ($\geqslant$99)	44	—
1.0	Cu^{II}	5 (75)	48 (21)	24 (4)	18	5
	Cu^{I}	$\leqslant$1	$\leqslant$1	$\geqslant$39 ($\geqslant$99)	61	—
1.5	Cu^{II}	2 (65)	33 (27)	25 (7.3)	28	12
	Cu^{I}	$\leqslant$1	$\leqslant$1	$\geqslant$30 ($\geqslant$99)	70	—

[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]

$[H^+]/10^{-2}$ M	$[Cu^{2+}]/10^{-4}$ M	$k'_{obs}/10^{-2}$ s^{-1}	$[H^+]/10^{-2}$ M	$[Cu^{2+}]/10^{-4}$ M	$k'_{obs}/10^{-2}$ s^{-1}
T = 288 K					
0.1	2	69.0 ± 0.7	4	2	10.1 ± 0.1
	5	179 ± 1.5		5	25.2 ± 0.2
	10	348 ± 2.1		10	48.0 ± 1.3
				15	69.8 ± 2.0
0.3	2	37.8 ± 0.2	6	2	8.60 ± 0.2
	5	94.5 ± 0.5		5	21.1 ± 0.4
	10	189 ± 1.4		10	43.8 ± 0.7
	15	283 ± 2.2		15	65.7 ± 0.8
0.6	2	24.4 ± 0.2	8	2	8.30 ± 0.1 (56.9 ± 0.4)
	5	61.0 ± 0.6		5	20.7 ± 0.3 (185 ± 2.7)
	10	119 ± 1.1		5	20.5 ± 0.1[b]
	15	183 ± 2.0		5	21.0 ± 0.4[c]
				10	42.7 ± 0.6 (443 ± 21)
				15	64.1 ± 0.8 (681 ± 34)
1	2	14.4 ± 0.1	10	2	7.70 ± 0.4 (59.9 ± 2.3)
	5	42.6 ± 0.4		5	18.9 ± 0.6 (182 ± 2.9)
	10	86.5 ± 0.9		10	39.5 ± 0.8 (384 ± 27)
	15	132 ± 1.5		15	59.3 ± 1.1 (587 ± 29)
2	2	12.5 ± 0.2			
	5	32.6 ± 0.2			
	10	63.4 ± 2.8			
	15	94.9 ± 1.3			
T = 298 K			T = 308 K		
10	2	19.9 ± 0.5	10	2	41.2 ± 0.5
	5	51.1 ± 1.5		5	124 ± 2.4
	10	96.2 ± 0.8		10	276 ± 5.6
	15	140 ± 1.0		15	422 ± 9.8

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

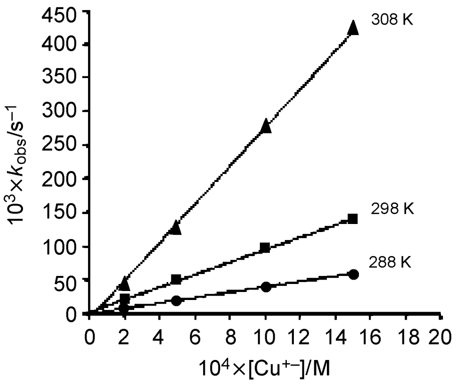


Fig. 2 Dependence of the pseudo-first order rate constant (k'_{obs}) on $[Cu^{II}]$ for LMB oxidation by copper(II)–Cl^- species; $[H^+]$ = 0.1 M, I = 1.0 M (Na^+, H^+, Cl^-).

A comparison of the different reactivities of the two different complexes, given by the different values of k_{Cu} 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^-, than Cl^- 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_{Cu} *vs.* $[H^+]$ for LMB oxidation by copper(II)–Cl^- species in Fig. 3 which show that the value of k_{Cu} 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.

Table 4 k_{Cu} values as a function of acid concentration[a]

T/K	$[H^+]/10^{-2}$ M	$k_{Cu}/10^2$ M^{-1} s^{-1}
288	0.1	34.98 ± 0.29
	0.3	18.88 ± 0.01
	0.6	12.11 ± 0.08
	1	8.72 ± 0.11
	2	6.34 ± 0.03
	4	4.73 ± 0.07
	6	4.37 ± 0.02
	8	4.26 ± 0.02
	8[b]	44.3 ± 1.0
	10	3.94 ± 0.03
	10[b]	38.6 ± 0.7
298	10	9.48 ± 0.14
308	10	27.7 ± 0.65

[a] Determined from plots of the pseudo-first order rate constants (k'_{obs}) as a function of $[Cu^{II}]$ for various fixed values of $[H^+]$; $I = 1.0$ M (Na^+, H^+, Cl^-). [b] Data for $I = 1.0$ M (Na^+, H^+, Br^-).

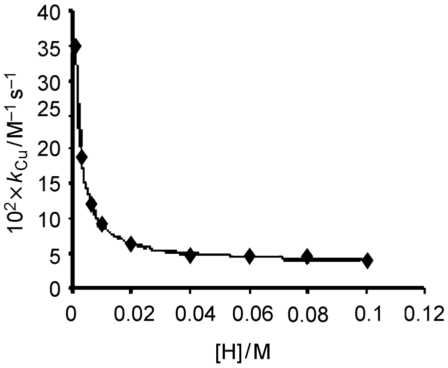
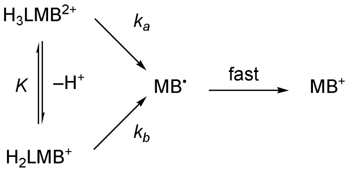


Fig. 3 Dependence of the pseudo-second order rate constants (k_{Cu}) for LMB oxidation by copper(II)–Cl^- species as a function of $[H^+]$; $T = 288$ K, $I = 1.0$ M (Na^+, H^+, Cl^-).

A rate law for the observed dependence of k_{Cu} 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 $MB^{\bullet}$ radical; and (ii) the parallel oxidation of the protonated and the deprotonated forms takes place with rate constants k_a and k_b, respectively.



Scheme 2

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

$$\frac{d[MB^+]}{dt} = \frac{(k_a K[H^+] + k_b)}{(1 + K[H^+])}[Cu^{II}][LMB] \qquad (10)$$

where, at constant $[H^+]$

$$k_{Cu} = \frac{(k_a K[H^+] + k_b)}{(1 + K[H^+])} \qquad (11)$$

The rate constants k_b and k_a 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_3LMB^{2+}. The results of a least-squares fitting of the k_{Cu} *vs.* $[H^+]$ data given in Table 4, and illustrated in Fig. 3, to eqn. (10) gives: $k_b = (7.0 \pm 0.1) \times 10^3$ M^{-1} s^{-1}, $k_a = 0.3 \times 10^3$ 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.

Table 5 Dependence of the pseudo-first order rate constants (k'_{obs}) on $[Cl^-]$

$[Cl^-]$/M	$k'_{obs}/10^{-2}$ s^{-1}
0.1	2.38 ± 0.1
0.5	34.4 ± 0.8
1.0	125 ± 1.4
1.5	344 ± 9.2

$[H^+] = 0.1$ M and $T = 298$ K and $I = 2.0$ M (Na^+, H^+, Cl^-, NO_3^-).

The pK_a for the H_3LMB^{2+}/H_2LMB^+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).[9] 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'_{obs} increases markedly, about 140 times, with a 15 fold increase of $[Cl^-]$ (from 0.1 to 1.5 M at $I = 2.0$ M and this correlates well with an increase in the concentrations of the anionic copper(II)–chloride complexes (see Table 2). Thus, it appears that the Cu–chloride anionic species are more reactive than Cu^{2+} ions and the cationic and the neutral Cu–chloride species. The observed correlation between contents of the anionic complexes and the rate constant is consistent with higher reactivity of the negatively charged species. The reaction proceeds between unlike charged reagents thus the higher negative charge of the oxidant stimulates the electron transfer process. Interestingly an approximate ten-fold increase in k'_{obs} with increasing $[Cl^-]$ is accompanied by *ca* ten fold increase of the $[CuCl_4]^{2-}$ anion content. As a consequence it appears likely that the $[CuCl_4]^{2-}$ complex is the most reactive oxidant in reaction (2).

Oxidation of LMB by dioxygen

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

$$2H_3LMB^{2+} + O_{2(aq)} \longrightarrow 2MB^+ + 2H_3O^+ \qquad (12)$$

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.* 1×10^{-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_{O_2}, 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 constants is given by eqn. (13):

$$k_{O_2} = \frac{k_{obs}}{[O_2]_{air}} \qquad (13)$$

Table 6 Pseudo-first order rate constants, k_{obs} and k_{O_2}, for LMB oxidation by $O_{2(aq)}$ at 298 K; $I = 1.0$ M (Na^+, H^+, Cl^-)

	$k_{obs}/10^{-3}$ s^{-1}		Solubility of O_2[25]/ 10^{-4} M		
$[H^+]$/M	Oxygen	Air	Oxygen	Air	$k_{O_2}{}^a$/M^{-1} s^{-1}
$6.3 \pm 10^{-9\,b}$	62	17	9.1	1.9	89
1.0	3.3	2.8	11.7	2.4	11.7

[a] $k_{O_2} = k_{obs}/[O_2]_{air}$. [b] HCO_3^- buffer solution.

The calculated values for k_{obs} 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 ~ 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 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 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, $\Delta B_{pp} \sim 2$ mT. The ESR parameters given above are very close to those of $MB^{\bullet}$ formed electrochemically.[3] The ESR spectrum of a water–ethanol (1:9) solution of LMB is shown as example in Fig. 4. It 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.

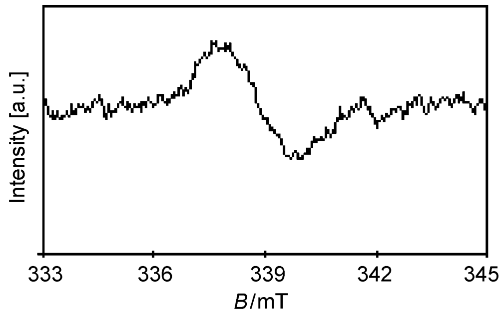


Fig. 4 Exponential smoothing ESR spectrum of *ca.* 1×10^{-3} M LMB water–ethanol (1:9) solution exposed to air diffusion. Other conditions: time of reaction *ca.* 45 min; microwave frequency 9.4975 GHz; room temperature.

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 dependence on the concentration of the Cu^{2+} ions complexed by Cl^- ions, especially the anionic complexes. The pseudo-second order rate constants, k_{Cu} 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, $T = 298$ 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.

Acknowledgements

A. P.-G. would like to express her gratitude for a TEMPUS grant sponsoring her stay at Prof. A. Mills laboratory at University of Wales, Swansea, UK. The authors wish to express theirs gratitude to Prof. F. Rozploch (Institute of Physics, Nicholas Copernicus University) for his kind help in carrying out the ESR measurements. This work was financially supported by the Polish State Committee for Scientific Research through grant no. 1631/TO9/2000/19.

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