Polish J. Chem., **77**, 475–480 (2003) **COMMUNICATION**

Reactivity of Two Protolytic Forms of Methylene Blue in Reaction with Ascorbic Acid

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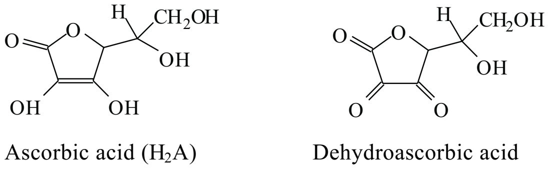
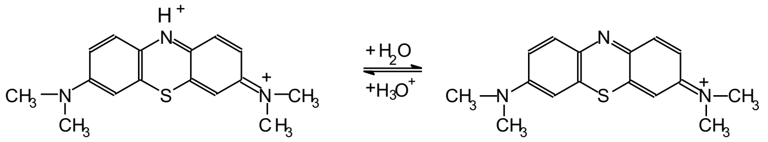
Among numerous contemporary uses of methylene blue (which we will refer to generally as MB), a thiazyne group dye, one of more recent is the application of its *leuco* form (LMB) as an optical dioxygen sensor [1,2]. Previously [3], the kinetics of LMB oxidation by Cu(II)–Cl^- complexes have been described. The reverse process, transformation of MB into LMB, has been studied in thermal and photochemical reactions, using several different reductants, including ascorbic acid (H_2A) (Scheme 1).

Scheme 1



Kinetic studies on MB reduction by H_2A have been reviewed recently [4,5]. Methylene blue exists in aqueous solutions at a very low concentration ($< 1 \cdot 10^{-5}$ M) practically exclusively in the monomeric form, which undergoes protonation near pH = 0 (Scheme 2); the acidity constant for MBH^{2+} has been roughly estimated as 1 M [6].

Scheme 2



Ascorbic acid undergoes protolytic reactions shown in (1) [7]:

$$H_2A \underset{}{\overset{pK_1 = 4.0}{\rightleftarrows}} HA^- \overset{pK_2 = 11.3}{\rightleftarrows} A^{2-} \qquad (1)$$

Further protonation, leading to the H_3A^+ cation, can take place in strongly acidic media, but no quantitative data about this reaction have been found.

It has been established [4] that the rate expression for methylene blue reduction by ascorbic acid contains first-order terms in concentrations of the oxidant and the reductant at constant $[H^+]$ for low ($<10^{-5}$ M) [MB] and moderately high (up to 0.1 M) $[H_2A]$. The first stage of the redox process – formation of $MB^\bullet$ radical – is the rate controlling step. The rate increases with the increase of $[H^+]$ within pH 1→ 0 range, but the interpretation of this effect is ambiguous and two alternative mechanisms have been proposed [4].

The aim of this work was to reexamine the rate dependence on $[H^+]$ in order to rationalize the role of the protolytic reactions in reduction of methylene blue by ascorbic acid. Two independent sets of the experiments were performed: (i) protolytic equilibrium studies for the dye and (ii) kinetic measurements. Figure 1 illustrates the electronic spectra (visible region) of methylene blue, recorded at different H^+_{aq} concentrations, using a diode-array spectrophotometer HP 8453.

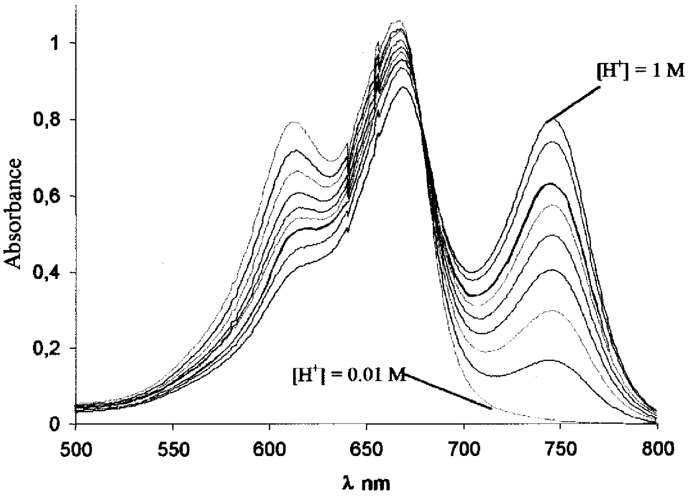


Figure 1. Electronic spectra of methylene blue at different H^+_{aq} – concentrations.

As it is seen, a new band at λ_{max} = 747 nm is characteristic for the protonated form of methylene blue. Isosbestic point at λ_{max} = 676 nm is consistent with the single protolytic reaction:

$$HMB^{2+} + H_2O \xrightleftharpoons{K_a} MB^+ + H_3O^+ \tag{2}$$

The acid dissociation constants at three temperatures at constant ionic strength $I = 1.0$ M (H^+, Na^+, Cl^-) were determined from absorbance $[H^+]$ data, using a nonlinear least-squares method:

$$\frac{1}{[H^+]_i} = \frac{A_b - A_i}{A_i - A_a} \cdot \frac{1}{K_a} \tag{3}$$

where A_a and A_b are the absorbances of the conjugate acid and base, respectively.

Table 1 presents the acidity constants and standard enthalpy and entropy for reaction (2).

Table 1. Acidity constants and standard enthalpy and entropy for reaction (2).

Temperature	K_a, M	ΔH^0	-4.6 ± 0.6 kJ mol^{-1}
298.3 K	0.748 ± 0.04	ΔS^0	-18 ± 2 J mol^{-1} K^{-1}
312.6 K	0.675 ± 0.02	ΔG^0	0.7 kJ mol^{-1}
327.5 K	0.635 ± 0.03	$K^{298}_{a(calc)}$	0.745 M

The reaction (2) is weakly exothermic and slightly increases the ordering of the solvent (Table 1). From the acidity constant it is obvious, that methylene blue exists practically exclusively in its conjugate base form at pH > 2, whereas the conjugate acid predominates substantially only below pH = 0. Kinetics of methylene blue reduction by ascorbic acid have been studied under conditions, which allowed for essential simplification of the examined system: (i) concentrations of MB were very low ($3–9 \cdot 10^{-6}$ M), what practically eliminates MB dimerization, (ii) using argon atmosphere in order to minimalize LMB reoxidation by dissolved dioxygen, (iii) applying $[H^+]$ range 0.05–0.9 M, what practically eliminates the deprotonated forms of ascorbic acid and keeps both protolytic forms of methylene blue in comparable amounts. Reduction of the dye causes its bleaching, as it is shown in spectral scans taken during the MB → LMB transformation (Fig. 2).

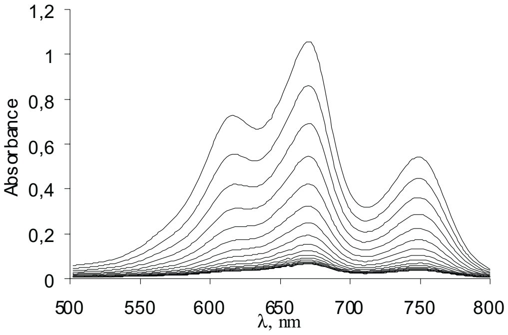


Figure 2. Changes in electronic spectra of methylene blue during reaction with ascorbic acid.

The redox process was followed spectrophotometrically, using a stopped-flow Spektrakinetic Workstation 1990, Applied Photophysics. The pseudo-first order rate constants (k_{obs}) were calculated numerically by a nonlinear least-squares method from absorbance-time data taken at isosbestic point for HMB^{2+} and MB^+ forms. Each kinetic run was repeated 3–5 times. The standard errors of the mean k_{obs} were below 2%. The obtained k_{obs} are independent of [MB] for initial concentrations 3, 6 and $9 \cdot 10^{-6}$ M. The rate increases linearly with the increase of [ascorbic acid] (Fig. 3).

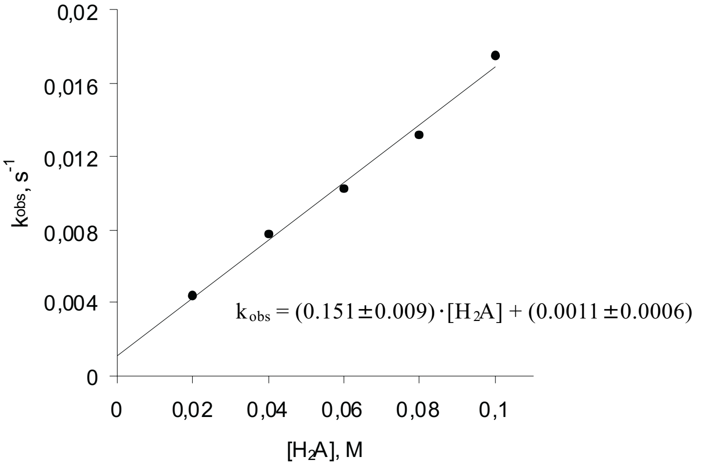


Figure 3. Dependence of the pseudo-first order rate constant on ascorbic acid concentrations, $[H^+] = 0.05$ M, $I = 1.0$ M (H^+, Na^+, Cl^-), T = 278 K, argon atmosphere.

A nonlinear dependence of the k_{obs} *versus* $[H^+]$ shows Figure 4 and Table 2.

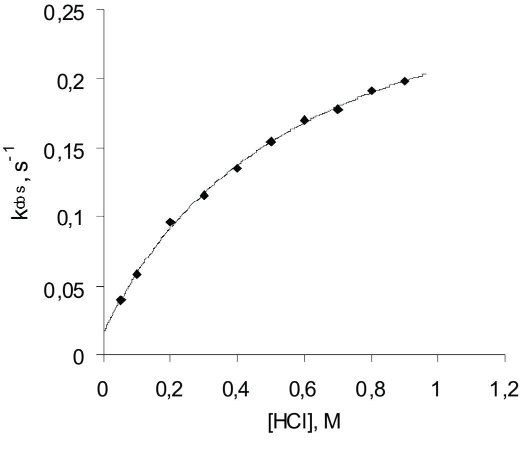


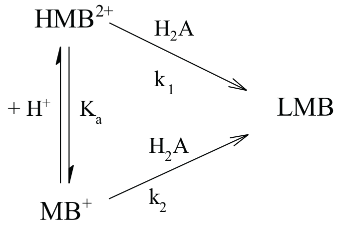
Figure 4. Dependence of the pseudo-first order rate constant on H^+_{aq} – concentration at $[H_2A] = 0.05$ M, $I = 1.0$ M (H^+, Na^+, Cl^-), T = 298 K, argon atmosphere.

Table 2. Dependence of the pseudo-first order rate constant on H^+_{aq} – concentration at $[H_2A] = 0.05$ M, $I = 1.0$ M (H^+, Na^+, Cl^-), T = 298 K, argon atmosphere.

[HCl], M	k_{obs}, s^{-1}	[HCl], M	k_{obs}, s^{-1}
0.05	0.04	0.5	0.155
0.1	0.058	0.6	0.170
0.2	0.096	0.7	0.178
0.3	0.116	0.8	0.192
0.4	0.135	0.9	0.198

The rate increase found with the increase of $[H^+]$ can be rationalized assuming the following reaction mechanism (Scheme 3):

Scheme 3



The essential feature of the presented model is the assumption that the parallel reduction of the two protolytic forms of methylene blue takes place, and that the reactivity of the conjugate acid is higher than that of the conjugate base. It has also been assumed that the only reactive form of the reductant is H_2A, because formation of the less reactive H_3A^+ cation in small amount is without kinetic importance. The reaction mechanism proposed operates with three parameters: the acidity constant (K_a) and two rate constants, which characterize the reactivity of the conjugate acid (k_1) and the conjugate base (k_2). The rate law, derived from Scheme 2 and consistent with the data presented in Figures 3 and 4, is

$$-\frac{d[\mathrm{MB}]}{dt} = \frac{k_1 + \frac{k_2}{K_a}[H^+]}{1 + \frac{[H^+]}{K_a}}[H_2A][\mathrm{MB}] \tag{4}$$

where $[\mathrm{MB}] = [\mathrm{HMB}^{2+}] + [\mathrm{MB}^+]$, thus

$$k_{obs} = \frac{k_1 + \frac{k_2}{K_a}[H^+]}{1 + \frac{[H^+]}{K_a}} \tag{5}$$

Validity of the reaction mechanism proposed can be verified basing on comparison of the kinetic parameter K_a (Table 3) with independently determined value of the acidity constant.

Table 3. Results of a least-squares fitting of $k_{obs} - [H^+]$ data to (5), T = 298.3 K, *I* = 1.0 M (H^+, Na^+, Cl^-).

Parameter	Value
k_1	$(1.66 \pm 0.32) \cdot 10^{-2}\ s^{-1}$
k_2	$(3.22 \pm 0.13) \cdot 10^{-1}\ s^{-1}$
K_a	$(6.1 \pm 0.61) \cdot 10^{-1}$ M

These values at 298 K equal to 0.61 (Table 3) and 0.745 M (Table 1), respectively, are almost the same within the error limit. Looking at the k_1 and k_2 values it is seen that the oxidant in the form of the conjugate acid reacts about 20 times faster than that in the form of the conjugate base (at 298 K).

Summing up, our results strongly support the mechanism, which attributes the observed rate increase with the increase of the $[H^+]$ to protonation of the oxidant, what produces the more reactive HMB^{2+} form.

Acknowledgments

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