Kinetics of Methylene Blue Reduction by Ascorbic Acid

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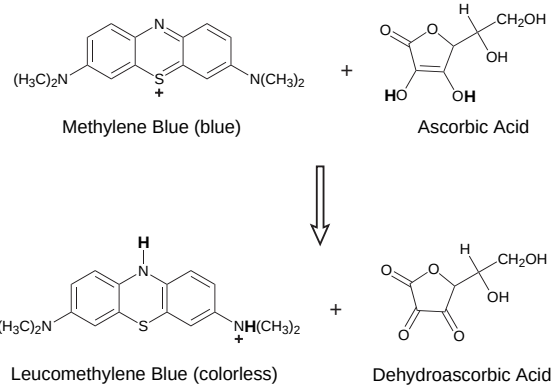
Methylene blue (MB^+) is a water-soluble cationic dye molecule that has been widely studied since its synthesis in 1876. It is easily reduced to the colorless hydrogenated molecule, leucomethylene blue, by a variety of agents (*1*). Leucomethylene blue can, in turn, be oxidized back to the blue color. Older investigations of the electrochemistry, acid–base chemistry, and dimerization of MB^+ are readily available in the literature (*2–4*). More recently, MB^+ and its one- and two-electron reduced forms have been studied as important species in the $MB^+/HS^-/O_2$ oscillating reaction system under continuous-flow stirred tank reaction conditions (*5*). The role of MB^+ excited states in fast H-atom abstraction reactions has been investigated by pulsed laser techniques (*6*). There have been two interesting recent publications on spatial pattern formation in methylene blue reaction systems under basic conditions (*7, 8*).

In a teaching context, the reaction of this dye in oxygenated basic glucose systems forms the basis of the well-known "blue bottle" experiment (*9, 10*). The range of applications in an educational setting was broadened by a recent article in this *Journal* by Snehalatha, Rajanna, and Saiprakash (*11*). This group reported that the kinetics of methylene blue reduction by ascorbic acid can be easily and quickly studied under strongly acidic conditions as a "clock reaction". The overall reaction, shown below, is drawn to emphasize the two half-reactions in the system.[1] Since the color-fading reaction typically takes only a few seconds or minutes, it is feasible to study the system under many different conditions during a laboratory or class period. Such studies should then permit quantitative evaluation of the dependence of rate on factors such as the concentrations of MB^+ and ascorbic acid, pH, temperature, solvent, and ionic strength.



We have recently used the suggestions of Snehalatha et al. in an introductory kinetics experiment in physical chemistry. Students were asked to find a suitable kinetic system to study, and this seemed to be an excellent choice. Unfortunately, however, we quickly discovered that the methylene blue–ascorbic acid reaction is not a clock reaction in the usual sense (*12, 13*). That is, there is no sudden color change, but rather a fading of the blue color, and the fading can be observed well beyond initial rate conditions. Also, the initial concentration of the species responsible for the color change, MB^+, will not always be the same in a set of runs, so that the color change may not correspond to a change in a fixed quantity of reactant. These confusions, which are discussed more fully below, can easily lead to an erroneous rate law.

In spite of the problems of a clock reaction approach, we still think that this reaction is an excellent example for introductory experimentation in kinetics. Since most laboratories have access to spectrophotometric equipment, the color-fading reaction can easily be followed by measuring the absorbance of MB^+ over time at λ_{max} = 665 nm. The absorbance decay curves can be measured under pseudo first-order conditions, and each curve can be obtained in only 1–10 min. Since MB^+ chemistry is well known in the literature, we simply illustrate this approach below for three of the more obvious questions: how does the rate law depend on MB^+ concentration, on ascorbic acid concentration, and on HCl concentration?

Experimental Procedure

Three sets of mixtures were prepared, using pipets, from three stock solutions of commercially available reagents. The stock solutions, which were stored at room temperature but not otherwise thermostated, were 3.9×10^{-4} M MB^+ (chloride salt), 0.10 M ascorbic acid (Asc), and 1.2 M HCl. In each set, one species was varied while the other two were kept at fixed initial concentrations. In all cases the MB^+ concentration was less than 1% of the H^+ and ascorbic acid molarities, thus ensuring pseudo first-order conditions. The MB^+ component was mixed quickly with the other components, and then a portion of the total volume was transferred within a few seconds to a cuvette (1.00-cm path length). Kinetic measurements were made at room temperature (24 °C) with a Hewlett-Packard 8452A diode array spectrophotometer (2-nm resolution). Although this instrument permitted acquisition of 400–700-nm spectra on each measurement cycle, the kinetic data reported here could be obtained equally well with a less expensive instrument operating at λ_{max}. Measurements were made at time intervals between 2 and 10 seconds, depending on the specific reaction rate. Data points were transferred to a computer through a GPIB interface controlled by a LabVIEW program (*14*). Absorbance values at a few selected wavelengths, including 665 nm, were stored in EXCEL format

Table 1. Some Experimental First-Order Decay Constants

Set A: total vol 22 mL [HCl] = 0.27 M [Asc] = 0.023 M		Set B: total vol 30 mL [MB^+] = 1.3×10^{-5} M [HCl] = 0.20 M		Set C: total vol 22 mL [MB^+] = 3.5×10^{-5} M [Asc] = 0.045 M	
[MB^+] / M	k_{exp} s^{-1}	[Asc] / M	k_{exp} s^{-1}	[HCl] / M	k_{exp} s^{-1}
3.5×10^{-5}	0.056	0	0	0	0.023
1.8×10^{-5}	0.062	0.0033	0.0072	0.11	0.054
1.8×10^{-5} no O_2	0.059	0.0067	0.0173	0.22	0.087
8.9×10^{-6}	0.064	0.0167	0.0365	0.44	0.136
3.5×10^{-6}	0.064	0.0333	0.0709	0.55	0.158
av k	$6.1(\pm0.4)\times10^{-2}$				

for later analysis. Full visible spectra were also displayed on the computer screen for each acquisition cycle, and this showed that no absorbing species were present other than MB^+. Since the analytical light source used by the instrument is reasonably intense, we checked for a possible photobleaching effect (*15*) by changing the time the instrument shutter was open by a factor of ten (0.1–1.0 s). Comparison of chemically identical runs under these conditions revealed no discernible difference in decay rates. Room light levels also had no observable effect.

Results

The concentrations and experimental first-order decay constants for several runs are provided in Table 1. Figure 1 shows first-order decay plots for several concentrations of MB^+ under conditions of constant initial HCl and ascorbic acid molarities (Set A). The negative slopes of the lines are the k_{exp} values, and it is evident from both this figure and the table that k_{exp} does not depend on the initial MB^+ concentration. One of the runs was repeated with deoxygenated solutions. The slope was virtually unchanged and provided no indication of an O_2 effect under these rather fast reaction conditions ($t_{1/2} \approx 11$ s). Similar plots were used to obtain k_{exp} values for all later runs. In all but one case, A_∞ was < 0.01 and had very little impact on the k_{exp} evaluations.

Figures 2 and 3 show the dependence of k_{exp} on ascorbic acid (set B) and HCl (set C). These graphs suggest a first-order dependence on ascorbic acid and on HCl concentration over a 5- to 10-fold concentration range. Even at 0 M HCl, however, MB^+ still decays in the presence of 0.045 M ascorbic acid. Taken together, the data imply an empirical rate law of the form:

$$\frac{d[MB^+]}{dt} = -\{k_0 + k_1[HCl]\}[Asc][MB^+] = -k_{exp}[MB^+] \quad (1)$$

Combining all the results, we find $k_0 = 1.0$ (±0.2) $M^{-1}\,s^{-1}$ and $k_1 = 5.3$ (±0.7) $M^{-2}\,s^{-1}$.

For most of our results, first-order decay plots were linear over four to six half-lives, and final absorbances were near zero (< 0.015). However, the 400–700-nm displays on the LabVIEW program almost always showed a noisy but discernible MB^+ spectrum at long times. We believe that this may be due to a slow oxidation of leucomethylene blue, perhaps by dissolved oxygen. In competition with the much faster acid-catalyzed MB^+ decay rates, this could lead to a low steady-state MB^+ concentration near the end of the reaction. The dissolved O_2 concentration in equilibrium with air is about 2.6×10^{-4} M (*16*), considerably greater than the MB^+ concentrations in these studies. The decay of MB^+ in 0.045 M ascorbic acid with no added HCl was much slower than the other HCl runs ($t_{1/2} \approx 30$ s) over about 7 min, and in this case the absorbance leveled off at 0.1. In a more extreme case, we studied the decay of MB^+ in 0.011 M ascorbic with no HCl. Here, the half-life was about 3 min, and the MB^+ absorbance leveled off at 0.22, as shown in Figure 4. After several additional minutes, oxygen from a compressed gas cylinder was bubbled through a frit into the solution for a few minutes, restoring much of the MB^+ intensity (0.71 at 665 nm). The color intensity grew to $A = 0.77$ during the next 3 min and then remained fairly stable for several hours. The visible spectrum was due to MB^+ during all these changes. These results clearly show that oxygen can play a significant role in the system under some conditions.[2]

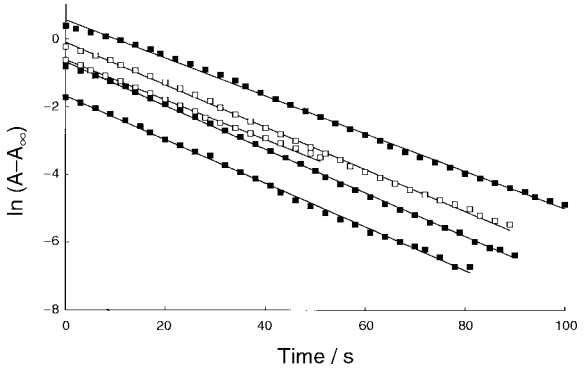


Figure 1. First-order decay plots for MB^+ absorbance at 665 nm under constant [HCl] and [Asc] conditions. The solid lines are unweighted least-squares fits to the data points. The order of initial absorption intensities corresponds to the concentration order shown in set A of Table 1. The open-square plots compare deoxygenated conditions (by N_2 bubbling) with normal air-exposed solutions for $[MB^+]_0 = 1.8\times10^{-5}$ M conditions.

Discussion

In the "clock reaction" approach of Snehalatha et al., one measures the time required for the blue color to disappear visually (*11*). For a gradually fading system, this is inherently more difficult and subjective than measuring the time to a sudden color appearance or disappearance. Furthermore, in the present cases, the time to disappearance, which we call "t_{dis}" is never short enough to be in the "initial rate" regime. To illustrate this, let us suppose that [MB^+] = x and that the decay is described by the simple first-order equation

$$\ln(x/x_0) = -kt$$

where $k = k_{exp}$, the pseudo first-order rate constant in the present studies. If the threshold concentration of MB^+ is x_{dis} (i.e., if we assume that the eye can just detect blue color at x_{dis}), then the above equation leads to a simple expression for t_{dis}.

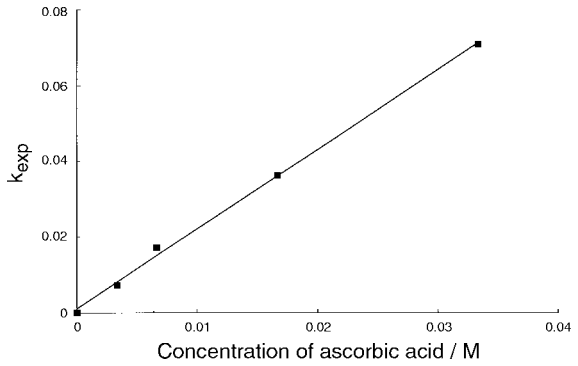


Figure 2. k_{exp} as a function of ascorbic acid molarity (set B). Slope = 2.1 M^{-1} s^{-1}.

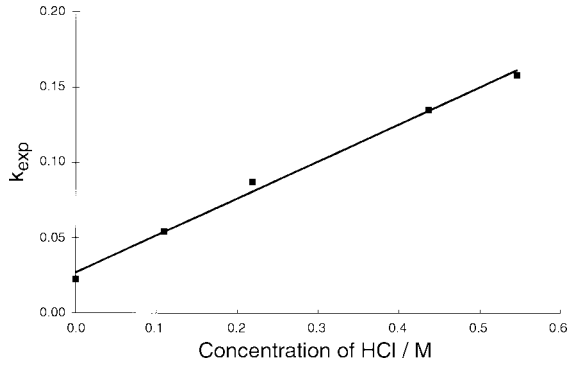


Figure 3. k_{exp} as a function of HCl molarity (set C). Slope = 0.25 M^{-1} s^{-1}, intercept = 0.027 s^{-1}.

$$t_{dis} = \frac{1}{k} \ln \frac{x_0}{x_{dis}} \quad (2)$$

and a corresponding "average rate":

$$\text{average rate} = \frac{x_0 - x_{dis}}{t_{dis}} = k \frac{x_0 - x_{dis}}{\ln\left(\frac{x_0}{x_{dis}}\right)} \quad (3)$$

The latter is equivalent to the rates measured by Snehalatha et al. This "average rate" will be proportional to the rate constant k as long as x_0 and x_{dis} are constant from run to run. This condition is met when $[MB^+]_0$ is constant, and in such a case it is legitimate, for example, to measure average rates at different temperatures and use these to determine an activation energy (*10, 11*). However, the assumption that k is proportional to "average rate" is invalid when x_0 is changed. Figure 5 shows a log/log plot based upon eq 3 for typical values of k, x_{dis}, and x_0. The slope of the plot is 0.72, comparable to the experimental slope of 0.68 reported in Figure 1, plot B of the Snehalatha paper. A slope value less than 1 has been regarded as evidence that the reaction dependence on $[MB^+]_0$ is less than first order (*11*). This is clearly incorrect; the low slopes are instead artifacts of the x_0 and x_{dis} terms in eq 3. It may be of minor interest to note that as we change the value of x_{dis} to extremely small values, with resulting large increases in t_{dis}, the plot slope approaches 1.

It has also been suggested (*11*) that the dependence on [HCl] is less than first order, again on the basis of log/log plots. Since the data in Figure 3 provide reasonable evidence that the rate is first order in [HCl], we believe that a slope <1 for log(average rate) vs log[HCl] is also an artifact, resulting from the nonzero reaction rate at 0.0 M HCl. To test this, we used eq 3, the conditions of set C in Table 1, and the k values predicted by Figure 3 to simulate log(average rate) vs log[HCl] behavior for the nonzero HCl molarities. Using $x_{dis} = 4 \times 10^{-7}$ M as in Figure 5 and $x_0 = 3.5 \times 10^{-5}$ M, we obtain a log/log plot that is reasonably linear with a slope of 0.68. Snehalatha et al. report a value of 0.76 for a similar plot. Our first-order dependence on ascorbic acid concentration does agree with the Snehalatha conclusion. Their log(average rate)/log[Asc] slope of 1 would be expected in this case, since a fixed initial MB^+ concentration was used. Even in this case, however, there is no particular advantage of a log/log plot over the more direct presentation of Figure 2. Log/log plots are often useful for data spanning several orders of magnitude, but they are unnecessarily obscure for data plots over short ranges and domains.

The reduction of MB^+ by ascorbic acid might well proceed by a complex mechanism such as the one proposed by Snehalatha et al. However, the kinetic data would also be consistent with, for example, a simple bimolecular reaction of

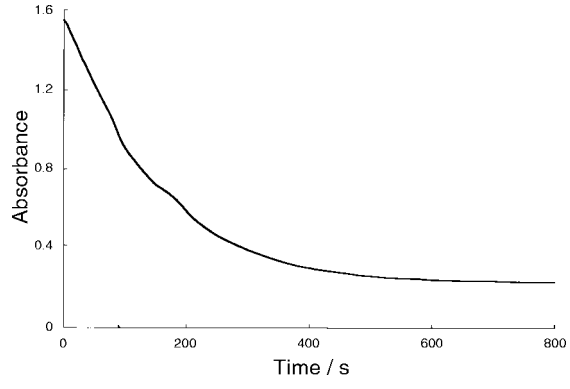


Figure 4. Absorbance decay profile for [HCl] = 0 M, [Asc] = 0.011 M, $[MB^+]_0 = 3.5 \times 10^{-5}$ M. The full 400–700 nm visible spectrum corresponds to methylene blue at all times.

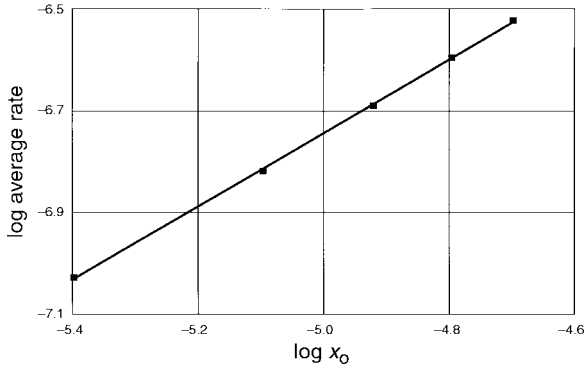


Figure 5. A log/log simulation of the "average rate" expression, eq 3, vs x_0 for reaction conditions approximating set A in the present study, and a $[MB^+]$ variation study in ref *9*. k_{exp} = 0.06 s^{-1}, the x_0 molarity range is (2–40) $\times 10^{-6}$, and x_{dis} is 4×10^{-7}M. The slope of the plot is 0.72.

ascorbic acid with MB^+. A small amount of a reactive species such as HMB^{2+} in equilibrium with H^+ and MB^+ could explain the HCl dependence. A pK_a of 0.0 is cited for HMB^{2+} in ref *6*, and the same reference summarizes reported dimer formation constants for $(MB^+)_2$, another species which might be significant in the mechanism. The reaction with ascorbic acid requires a net transfer of two H^+ and 2 e^-, but the overall reduction may well occur in sequential steps (*5*). Evidence for such possibilities would require studies beyond the scope of simple kinetic explorations. Additional kinetic characterization of the reaction also may be sought from studies of the rate dependence on temperature, solvent, and ionic strength. The first two are easily implemented, as pointed out by Snehalatha and others. Since the ionic strength contribution of HCl is already high in these studies, it is not surprising that small further increases due to additional salts produce no significant effect on MB^+ reduction rates (*11*).

It should be emphasized that our results are typical of an undergraduate lab experiment and should not be regarded as "research-quality" results for several reasons. All our measurements are at a room temperature of 24 °C, and careful thermostating was not used. More importantly, we have not thoroughly explored the role of dissolved oxygen. Contrary to the suggestion of Snehalatha et al., the "blue bottle" effect does play a role in this reaction system, and the conversion of leucomethylene blue back to MB^+ can result in a persistent faint blue color under slow reaction conditions. In addition, under slow conditions we have noted that the linearity of our first-order plots is not always as good as that shown in Figure 1. Studies with deoxygenated solutions and a more careful investigation of the role of dissolved oxygen would be interesting avenues for further student exploration. Finally, it should be noted that commercial methylene blue samples can have significant impurities (*17*), and careful purification is needed prior to research-level studies of the system.[3]

Summary

Our principal conclusion is that a kinetic study of the MB^+ reaction with ascorbic acid is a good experiment in introductory kinetics. For fairly fast reaction conditions, the complications due to leucomethylene blue oxidation by dissolved O_2 are minimal. Since absorbance decay curves can be obtained in a short time, it is feasible to study a sufficient range of conditions to establish a reasonable rate law. For slower reaction conditions, where the kinetics are more complex than simple first-order decay of MB^+ to zero concentration, there are more challenging kinetic problems, suitable for investigation by more advanced students. We do not recommend the "clock reaction" approach of Snehalatha et al. for quantitative work because of the flaws outlined above. In the setting of a classroom demonstration, however, their approach is suitable for showing the qualitative effect of initial ascorbic acid molarity, HCl molarity, and temperature on the reaction rate. It is noteworthy that the educational value of MB^+ redox chemistry in introducing concepts of kinetics, mechanisms, catalysis, and steady-state conditions was espoused many years ago in this *Journal* by J. A. Campbell (*10*). In this sense, the study of MB^+ reduction with reagents such as ascorbic acid is simply extending the range of possibilities for using this molecule in the teaching of fundamental aspects of reaction rates.

Acknowledgments

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Notes

1. The MB^+ representation is somewhat generic, being similar to that of several literature presentations. A few sources locate the positive charge on one of the amine nitrogens, with appropriate double bond shifts. The S^+ structure shown is consistent with the predictions of CAChe MOPAC (version 94) PM3 calculations. Under acidic conditions additional protons will be present. The representation includes neither dimerization nor the intermediate radical resulting from a 1-electron reduction, $MB\cdot$ (*5*).

2. The underlying rate law for our plots is simple first-order: $dx/dt = -kx$, where $x = [MB^+]$. Our original reason for including an A_∞ term in the first-order plots was to correct for small drifts in lamp intensity in the diode array instrument. However, if there is significant competition between MB^+ decay and MB^+ formation due to oxidation of leucomethylene blue by excess O_2, a better rate law is $dx/dt = -kx + k_2(x_o - x)$, where $x_o - x$ represents the concentration of leucomethylene blue. This rate law integrates to $\ln(x - x_\infty) = -(k_1 + k_2)t + c$. Thus, plots of $\ln(A - A_\infty) = -(k_1 + k_2)t + c'$ permit the evaluation of $(k_1 + k_2)$ when permanent long-term absorption is due to residual MB^+. Since $dx/dt = 0$ at long times, the relationship $k_2/k_1 = x_\infty/(x_o - x_\infty)$ can be readily obtained from the differential rate law. For our conditions, the highest value of k_2 was $k_2 = 0.06k_1$; for most of the runs k_2 was less than 1% of k_1. We have assumed that k_2 is negligible in obtaining the results reported in Table 1 and in the figures.

3. Some indication of the purity problem is provided by a comparison of our MB^+ molar absorptivity of 54,000 $M^{-1}cm^{-1}$ at λ_{max} to literature values of 64,000 (*5*) and 82,000 (*6*).

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