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# Ultratrace Kinetic Measurements of the Reduction of Methylene Blue

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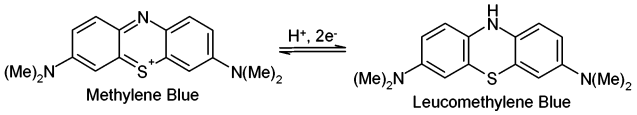
Methylene blue ($MB^+$) is a widely studied molecule currently under investigation for its properties in solar energy,[1] HIV infectivity,[2] and hydride-transfer reactions.[3] $MB^+$ is known to accept a hydride in either a concerted or stepwise manner depending on the reaction partner and conditions.[4] Previous work has shown that the kinetics for reduction in water containing ascorbic acid is first order in each reagent.[5,6] We report the second-order reduction of methylene blue to leucomethylene blue (MBH) by ascorbic acid ($H_2A$) in acetonitrile:




We carry out these measurements using cavity ring-down spectroscopy (CRDS). Previously, we have demonstrated that this technique can monitor absorbing species in a variety of organic solvents[7] and follow concentration changes on the microsecond time scale for absorbing species present in solution at the level of nanograms to picograms per milliliter. Without the use of CRDS such measurements would not have been possible. Moreover, because we employ a small, inexpensive diode laser as the light source, this type of measurement is easy to implement and should have wide applicability to the investigation of the kinetics and mechanism of organic reactions in a regime that was hitherto inaccessible.

CRDS employs highly reflective mirrors to form an optical cavity. Laser light fills the cavity with radiant energy and then is shut off. The intensity of the light is then recorded as it leaks out of the cavity through the back mirror (the cavity ring-down signal). The time constant of the exponential ring-down, $\tau$, depends on the characteristics of the cavity. The insensitivity to laser intensity combined with the multipass nature of the method provides an increase in sensitivity that is typically orders of magnitude greater than a traditional absorption measurement of the same solution sample.[8] When an absorber is present in the cavity, it contributes to the decay of the light intensity, and the value of $\tau$ is reduced. We measure the change in the decay time, $\Delta\tau$, with and without an absorber present in the cavity. Quantities such as the length of the cavity and the exact reflectivity of the mirrors then cancel out. Only knowledge of the molecular absorption coefficient $\epsilon$, which is readily obtained by a calibration curve or conventional UV−vis absorption measurements, is required to convert $\Delta\tau$ to absolute concentration.

Figure 1 shows the experimental setup. An acousto-optic modulator (AOM) chops the output of a diode laser on a time scale of Hz to MHz. A solution containing $MB^+$ from the chloride salt (1−10 nM) and $H_2A$ (1−10 $\mu$M) in acetonitrile at room temperature fills a 23-cm optical cavity that is endcapped with two mirrors whose reflectivity (99.98% at 655 nm, in air) has been checked not to change with solute or reaction product concentration. The ring-down trace is recorded by a photomultiplier and fit to an exponential function to obtain $\tau$. We utilized this method to investigate the reduction of $MB^+$ to MBH by $H_2A$ in acetonitrile with microsecond resolution.

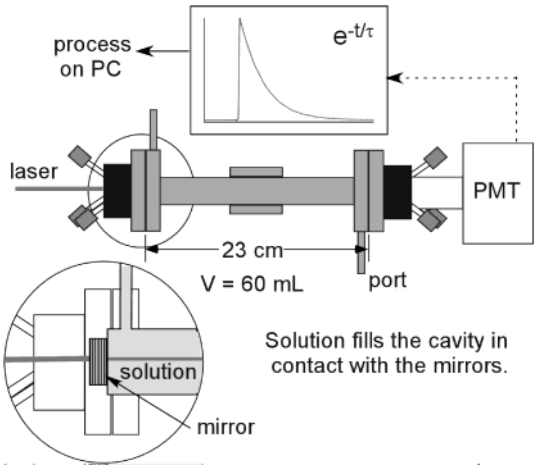



***Figure 1.*** The CRDS apparatus. A diode laser, switched by an AOM, builds up light in a cavity containing the solution to be measured. The light is then switched off, and the decay is measured by a photomultiplier and processed by oscilloscope and computer to extract the decay constant $\tau$.

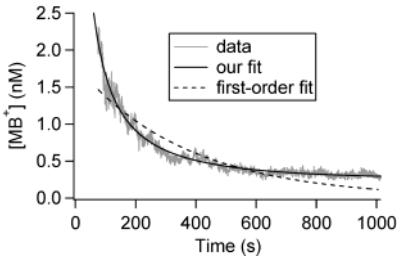



***Figure 2.*** Data from a reaction of 3.0 nM $MB^+$ with 2.5 $\mu$M ascorbic acid. The derived rate law is clearly a better description than a first-order loss. Data do not start at $t_0$ because the solutions are well mixed outside the cavity and then introduced into the chamber.

To simplify the kinetics, a several 1000-fold excess of ascorbic acid was maintained. Under these conditions, it was expected that a simple, pseudo-first-order disappearance of $MB^+$ would be observed, as had been seen for the same reaction in water.[5,6] Our data in acetonitrile clearly demonstrate more complex kinetics (Figure 2). Careful analysis of the reaction over a range of [$MB^+$] and [$H_2A$] points to a second-order reaction at early times coming to equilibrium at later times.

The simplest model we could devise that fits our observations was a second-order loss of $MB^+$ coupled to a first-order regeneration of $MB^+$ from the product MBH. MBH is known to return to $MB^+$ in the presence of dissolved oxygen.[9] With initial [$MB^+$] in the 1−10 nM range, the concentration of $H_2A$ is much in excess as is the amount of dissolved $O_2$. Thus, we write

$$\frac{d[MB^+]}{dt} = -k'_f[MB^+]^2 + k'_r[MBH] \qquad (1)$$



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The only source of MBH comes from $MB^+$, so we write that

$$\frac{d[MB^+]}{dt} = -k'_f[MB^+]^2 + k'_r([MB^+]_o - [MB^+]) \quad (2)$$

This expression can be integrated under the constraint that the rate constants must be real and positive:

$$[MB^+]_t = \frac{a - k'_r}{2k'_f} + \frac{2ak'_f[MB^+]_o^2}{b\exp(at) - 2k'^2_f[MB^+]_o^2} \quad (3)$$

Here

$$a = \sqrt{k'_r(k'_r + 4k'_f[MB^+]_o)} \quad (4)$$

and

$$b = a^2 + 2k'^2_f[MB^+]_o^2 + a(k'_r + 2k'_f[MB^+]_o) \quad (5)$$

Figure 2 shows the fit of this model to our data.

As a check, a log−log plot of $k'_f$ vs $[H_2A]$ gives a line of slope of 0.96, essentially unity. This result confirms the pseudo-first-order dependence imposed by the excess of ascorbic acid. It follows that the forward rate constant is $k_f = k'_f/[H_2A] = (8.3 \pm 1.6) \times 10^3$ $M^{-1}$ $s^{-1}$ and the reverse is $k'_r = 86 \pm 69$ $s^{-1}$ where the errors represent one standard deviation. The large uncertainty in the reverse rate likely arises from a combination of factors. The $O_2$ concentration varies from solution to solution as we did not attempt to control it. Moreover, the portion of the curve that most affects $k'_r$ is late in the reaction when the signal is much closer to the background noise. Consequently, we believe that we have only determined a range for $k'_r$ and that it is possible the dependence is more complex than first order in [MBH]. The forward rate constant, however, is well defined. As a test of our model, a reaction was run in a solution that was well sonicated to remove as much of the dissolved oxygen as possible. The resulting reaction should show a simple second-order disappearance of $MB^+$ because the reverse reaction should be negligible. Figure 3 shows a plot of $[MB^+]^{-1}$ vs time that should be linear in the case of second-order kinetics. Clearly, removal of oxygen leads to the behavior predicted by our model.

We propose the following mechanism for the second-order formation of MBH, which is consistent with our kinetic data and previously reported results.[5,6]

In water:

$$MB^+ + H_2A \xrightarrow{k_1} MB^\bullet + H_2A^{+\bullet} \quad (6)$$

$$MB^\bullet + H_2A^{+\bullet} \xrightarrow{k_2} MBH^{+\bullet} + HA^\bullet \quad (7)$$

$$MB^\bullet + H_3O^+ \xrightarrow{k'_2} MBH^{+\bullet} + H_2O \quad (8)$$

$$MBH^{+\bullet} + HA^\bullet \xrightarrow{k_3} MBH + HA^+ \quad (9)$$

and the corresponding rate expression for product formation is:

$$\frac{d[MBH]}{dt} = k_1k'_2k_3[MB^+] = k[MB^+] \quad (10)$$

In acetonitrile, proton transfer from the solvent is not available, so the proton-transfer step occurs much more slowly. The lifetime of the $MB^\bullet$ intermediate significantly lengthens and the reaction

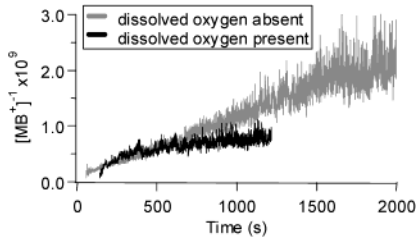


***Figure 3.*** Comparison of the reaction of 1 nM $MB^+$ with 3.1 $\mu$M ascorbic acid in the presence and absence of dissolved oxygen. A plot of conc$^{-1}$ vs time is linear for a second-order reaction. At early times, both cases are similar, but in the presence of oxygen the reaction bends toward equilibrium. Noise increases with time in an inverse concentration plot.

$$MB^\bullet + MBH^{+\bullet} \xrightarrow{k_4} MBH + MB^+ \quad (11)$$

generates MBH product. Thus, the rate expression for formation of product, still without taking the back reaction into account, becomes:

$$\frac{d[MBH]}{dt} = k_4(k_1[MB^+])(k_1k_2[MB^+]) = k'[MB^+]^2 \quad (12)$$

which is second order in $[MB^+]$. As a test of our model, a reaction was run in a solution where the solvent was 1% water in acetonitrile. The native absorption of water at 650 nm prevents us from using higher concentrations. Nevertheless, sufficient water is present to act as the proton source for the proton-transfer reaction. First-order kinetics were observed under these conditions, as expected.

We have demonstrated the use of cavity ring-down spectroscopy to follow the kinetics of organic reactions whose species are present in only nanomolar concentrations. This technique may be used to overcome common difficulties such as extremely poor solubility or limited quantity of a molecule under study. The straightforward laser system and experimental setup appear to make this type of analysis suitable for many applications. Our investigation of methylene blue reduction kinetics differs from past experiments in two important respects. First, our increased sensitivity has allowed us to use much smaller dye concentrations so the relative concentration of dissolved oxygen is significant. With greater dye concentration it was necessary to bubble oxygen through solutions to see the reverse reaction.[9] Second, the aprotic solvent acetonitrile profoundly alters the reaction mechanism, making it second-order rather than first order in methylene blue.

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