Ultratrace Kinetics by Cavity Ring-Down Spectroscopy

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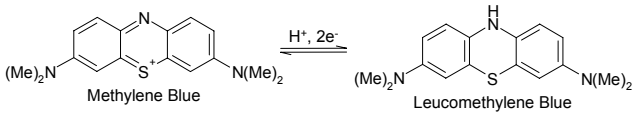
We report the ability to follow concentration changes on the microsecond timescale for absorbing species present in solution at the level of parts per billion to parts per trillion. This measurement permits the investigation of the kinetics and mechanism of organic reactions in a regime that was hitherto inaccessible. We illustrate this work by examining the reduction of methylene blue (MB^+) to leucomethylene blue (MBH) by ascorbic acid (H_2A) in acetonitrile. We carry out these



measurements using cavity ring-down spectroscopy (CRDS), which we have demonstrated can monitor absorbing species in a variety of organic solvents.[1] 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.

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, τ, depends on the characteristics of the cavity. The insensitivity to laser intensity coupled with the multipass nature of the method provides an increase in sensitivity that is typically orders of magnitude greater than a traditional absorption measurement in the same solution sample.[2] When an absorber is present in the cavity, it contributes to the decay of the light intensity, and the value of τ is reduced. We measure the change in the decay time, $\Delta\tau$, with and without an absorber present in the cavity. Quantities such as refractive index of the solution, length of the cavity, and exact reflectivity of the mirrors then cancel out. Only knowledge of the molecular absorption coefficient ε is required to convert $\Delta\tau$ to absolute concentration. This information can be readily obtained by means of conventional UV-VIS absorption spectroscopy.

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 μM) in acetonitrile at room temperature fills a 23-cm optical cavity that is endcapped with two mirrors (99.98% reflectivity at 655 nm). The ring-down trace is recorded by a photomultiplier and fit to an exponential function to obtain τ. We illustrate this method by investigating the reduction of MB^+ to MBH by H_2A. Net hydride transfers often proceed by complex mechanisms; MB^+ is known to accept a hydride in either a concerted or stepwise manner depending on the reaction partner and conditions.[3] At 655 nm, only the MB^+ shows appreciable absorption, Consequently, it is possible to watch its disappearance over time with microsecond resolution.

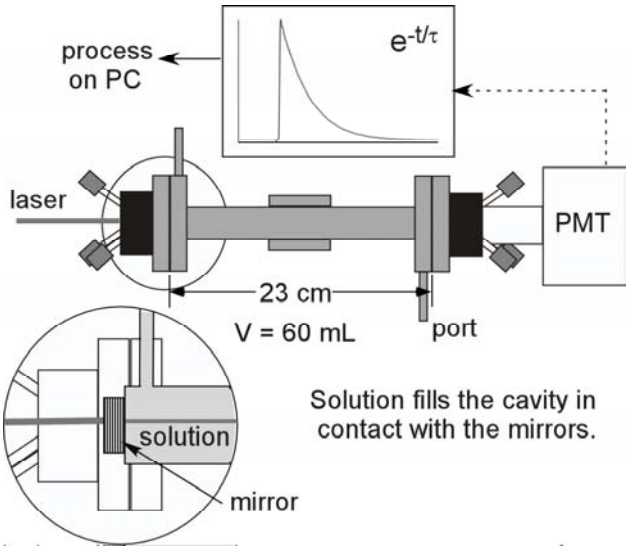


Figure 1: The CRDS apparatus. A diode laser, switched by an AOM, builds up light in a cavity containing the solution to be measured. The light is then switched off, and the decay is measured by a photomultiplier and processed by oscilloscope and computer to extract the decay constant τ.

To simplify the kinetics, a several thousand-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.[4,5] 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.[5] With initial $[MB^+]$ in the 1-10 nM range, we expect ample amounts of dissolved O_2 to drive the reverse reaction. The forward reaction is second order with respect to $[MB^+]$ and the reverse reaction is first order with respect to [MBH]. The reaction is reversed by dissolved oxygen. Thus, we write

$$\frac{d[MB^+]}{dt} = -k_f[H_2A]^n[MB^+]^2 + k_r[O_2]^n[MBH] \qquad (1)$$

Flooding the reaction with excess acid and assuming the amount of dissolved oxygen to be in excess, the kinetics reduce to

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

The only source of MBH is reacted MB^+, so we write that

$$\frac{d[MB^+]}{dt} = -k'_f[MB^+]^2 + k'_r([MB^+]_0 - [MB^+]) \quad (3)$$

This expression can be integrated with the condition

$$[MB^+]_{t=0} = [MB^+]_0 \quad (4)$$

and the assertion that rate constants must be real and positive:

$$[MB^+]_t = \frac{a - k'_r}{2k'_f} + \frac{2ak'_f[MB^+]_0^2}{b\exp(at) - 2k'_f{}^2[MB^+]_0^2} \quad (5)$$

Here

$$a = \sqrt{k'_r(k'_r + 4k'_f[MB^+]_0)} \quad (6)$$

and

$$b = a^2 + 2k'_f{}^2[MB^+]_0^2 + a(k'_r + 2k'_f[MB^+]_0) \quad (7)$$

Figure 2 shows how well this model fits our data.

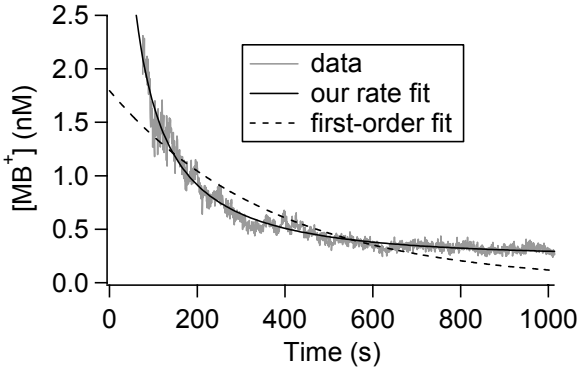


Figure 2: Data from a reaction of 3.0 nM MB^+ with 2.5 μM ascorbic acid. The derived rate law is clearly a better description than a first-order loss. Data does not start at t_0 because the solutions are well mixed outside the cavity and then introduced into the chamber.

As a check, a plot of $\log(k_f)$ vs. $\log([H_2A])$ gives a slope of 0.96, essentially unity. This result confirms the pseudo-first-order dependence imposed by the excess of ascorbic acid. The rate constants are: $k_f = 8.3 \pm 1.6 \times 10^3$ $M^{-1}s^{-1}$ and $k_r = 86 \pm 69$ s^{-1} where the error represents 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. Moreover, the portion of the curves 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. The forward rate constant, however, is well defined. As a test of our model, a reaction was run in 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 stopped or at least severely slowed. 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.

Our investigation 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 this effect.[3] Second, acetonitrile alters the reaction mechanism.

Despite these differences, some aspects of the reaction remain unchanged. Wopschall and Shain[7] showed the reduction of MB^+

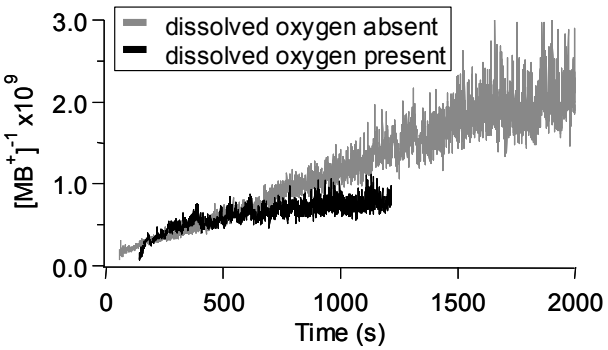


Figure 3: Comparison of the reaction of 1 nM MB^+ with 3.1 μM ascorbic acid in the presence and absence of dissolved oxygen. A plot of $conc^{-1}$ vs. time is linear for a second-order reaction. At early times, both cases are similar, but in the presence of oxygen the reaction bends toward equilibrium.

is a stepwise electron-proton-electron transfer, rather than a concerted one. Oxygen in solution also affects the formation of the radical intermediate.[8] The rate-limiting step is the proton transfer, which seems to be affected by dimerization of the dye. Dye molecules often form dimers or higher aggregates in solution. MB^+ is known to form dimers in aqueous solution, but only at concentrations above 4×10^{-5} M.[9] It might be expected to dimerize more strongly in acetonitrile than in water, possibly owing to its large dipole moment interacting with a solvent of lower dielectric constant (water = 80, acetonitrile = 37). Ionic strength of the solution from the MB^+ counter-ion does not affect the dimerization constant until it is far greater than our concentration.[10] Aggregation or the lack thereof could explain the second-order kinetics observed in the forward reaction in acetonitrile and the first-order kinetics in water. Clearly in this reaction, the solvent has a profound effect on the reaction kinetics. The back reaction seems to remain first-order irrespective of solvent.

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.

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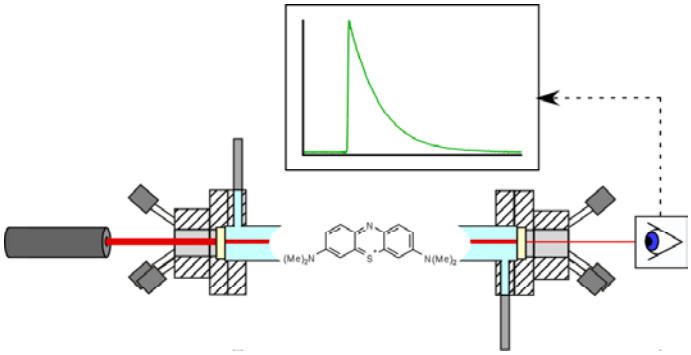

Cavity ring-down spectroscopy was used to investigate the kinetics of methylene blue reduction by ascorbic acid in acetonitrile. Because of our high sensitivity we were able to use very low concentrations (1-10 nM) of the dye. Under these conditions, we observed a second-order loss of dye as well as a competing back reaction with dissolved oxygen. The use of an inexpensive diode laser and a relatively simple setup should make ultratrace kinetic studies more accessible.