Kinetic study on the reversible hydride transfer between methylene blue and thionine

Yingjin Liu, Shunzo Yamamoto,* Yoshimichi Fujiyama and Yoshimi Sueishi

Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushima-naka,
Okayama, 700, Japan

Received 5th January 2000, Accepted 13th March 2000

The kinetics of the hydride transfer reaction between leuco methylene blue and thionine was investigated spectrophotometrically by means of the stopped-flow technique. Leuco methylene blue and leuco thionine were produced by photoreduction of methylene blue and thionine with triethylamine in ethanol in the drive syringe of the stopped-flow apparatus and their solutions were used as such in the measurement of the reaction rate. This reaction was found to be reversible, and the rate constants for forward and backward reactions were obtained. The ratio of k_f/k_b is consistent with the value of the equilibrium constant (K) which was obtained from the absorbance at 655 nm of methylene blue at equilibrium using the stoichiometric relationships and conservation conditions. The mechanism of this reversible hydride transfer reaction was discussed.

Kreevoy *et al.* found that rate constants for hydride transfer between NAD^+ analogues could be predicted by Marcus' theory of atom transfer over a wide range of structure and equilibrium constants.[1–7] They pointed out that this result indicates that the reaction involves no high-energy intermediates. For reactions in which the reactants and products have the same charge type and are structurally similar, one-step reactions of the type shown in eqn. (1) are predicted;

$$A_i^+ + A_jH \rightleftarrows A_iH + A_j^+ \qquad (1)$$

Thionine and methylene blue exist as monovalent cations in the ordinary pH region and their redox reactions are reversible. Since leuco methylene blue (MBH) and leuco thionine (TH) are very unstable and easily oxidized by coexisting oxygen in solution, few kinetic studies on the reactions of these compounds with π-acceptors have been previously reported. Recently, we have studied the kinetics of the reactions of TH and MBH with some *p*-benzoquinones[8] and ferric ion (Fe^{3+})[9] by means of a combination of the formation of MBH and TH by photoreduction of MB^+ and T^+ with triethylamine and ascorbic acid and the stopped-flow technique. By these procedures we can follow the fast reactions of MBH and TH with different oxidants.

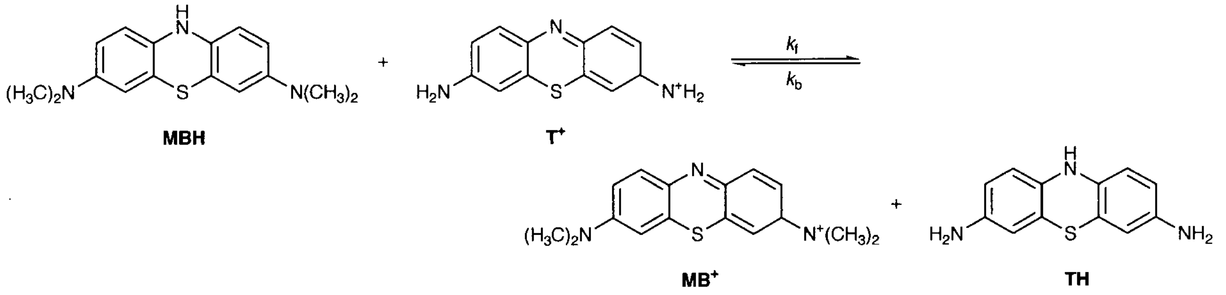
Between some cationic dyes and neutral leuco dyes a reversible one-step hydride transfer similar to that mentioned above is expected to occur because in this reaction, reactants and products have the same charge type. Olson observed color changes by mixing methylene blue and leuco safranine bluish solutions and described that this demonstration is useful for the illustration of reversible redox reactions.[10] But the detailed kinetics and mechanism for this reaction were not shown. We have checked briefly whether the hydride transfer occurs between some dyes and leuco dyes, and found that for T^+/MBH and MB^+/TH systems, the spectral changes are large and the reactions were able to be followed. This reaction was found to be reversible, as shown by eqn. (2) below.

In the present study, we followed the reactions between T^+ and MBH and MB^+ and TH by producing MBH and TH by means of the photoreduction of MB^+ and T^+ with triethylamine in ethanol in a closed system and by mixing a solution of MB^+ (T^+) with a solution of TH (MBH). This paper reports on the detailed kinetics and mechanisms of this reversible reaction.

Experimental

Materials

Thionine (T^+Cl^-) and new methylene blue (Basic Blue 24: NMB^+Cl^-) were purchased from Tokyo Chemical Industry. Methylene blue (MB^+Cl^-), crystal violet (CV^+Cl^-), malachite green (MG^+Cl^-), leuco crystal violet (CVH) and leuco malachite green (MGH) were purchased from Wako Pure Chemical Industries. These compounds were used as received. Leuco thionine (TH), leuco methylene blue (MBH) and leuco new methylene blue (NMBH) were produced by photoreduction of these parent dyes as mentioned below. Triethylamine was used as received. Ethanol was dried over a molecular sieve 3A-1/16 and distilled. Ethanol-d_1 (99.5%) was purchased from Aldrich and used as received.



(2)

DOI: 10.1039/b000046i

Kinetic measurements

MBH (or TH, NMBH) solution was prepared by irradiating MB^+ (or T^+, NMB^+) and triethylamine solution in ethanol, deaerated by bubbling with nitrogen gas, with visible light supplied by a 650 W projector lamp. On irradiation, the solution changed from colored to colorless immediately. This indicates that the leuco dyes were formed. Ethanolic solutions of dyes and triethylamine and dyes as reaction partners for leuco dyes were separately charged in drive syringes of a stopped-flow apparatus (Otsuka Electronic stopped-flow spectrophotometer, RA-401). The two solutions were deaerated by bubbling with nitrogen gas before they were charged. The former solution was photoreduced by irradiation with visible light and a solution of leuco dyes was produced. Mixing was performed within 1 ms by means of nitrogen gas pressure. After mixing, rapid-scan spectra were taken every 2 or 10 ms. For MB^+/TH and T^+/MBH systems, after mixing, the changes in the absorbance at 655 nm were monitored. The runs were repeated several times. During the measurements, the solution of TH (or MBH) was being irradiated continuously to prevent re-oxidation of the leuco dyes by oxygen remaining in the solutions. All kinetic measurements were performed at 25.0 ± 0.1 °C.

Results

We have checked briefly whether hydride transfer occurs between some dyes and leuco dyes.† It was found that the reaction between NMBH and MB^+ is very fast and proceeds almost to completion, and that a back reaction was not observed. As mentioned above, however, it was found that for the T^+/MBH and MB^+/TH systems, the spectral changes are large and the forward and backward reactions were able to be followed.

As mentioned above, we produced TH and MBH by photoreduction of thionine and methylene blue with triethylamine. Fig. 1 shows the absorption spectra of MB^+ taken before and after photoreduction [(1) and (2)] and the spectrum of an equivolume mixture of MBH and *p*-chloranil (CA) solutions (3). It was reported that some *p*-benzoquinones oxidized MBH rapidly and quantitatively.[8] Fig. 1 can be explained by the formation of MBH by the photoreduction and re-production of MB^+ by oxidation with CA.

Fig. 2 shows the absorption spectra of MB^+ and T^+ in ethanol. Fig. 3 shows the rapid scan spectra for the reaction between MB^+ and TH and between MBH and T^+. Fig. 3 shows the rapid formation of T^+ and the simultaneous decrease in MB^+ concentration. Fig. 3 also shows that the reaction does not proceed to completion, and the reaction attains equilibrium fairly rapidly. A clean isosbestic point indicates the absence of significant concentrations of any intermediate species.

As is shown in Fig. 2, the absorption by T^+ at 655 nm (which is the wavelength of the peak of MB^+) is nearly negligible. Time-dependent curves of the absorption at 655 nm after averaging the repeated runs from both sides of the reaction are provided as supplementary data.† The absorbance at 655 nm increased rapidly and attained constant values after the mixing of an MBH solution with T^+ solutions with several concentrations. Although the T^+ concentrations are in excess of the MBH concentration, the constant values depend on the T^+ concentration. On the other hand, the absorption at 655 nm decreased rapidly and also attained almost constant values after the mixing of the MB^+ solution with TH solutions of several concentrations. These findings indicate that the reactions from both sides do not proceed to completion from either side, and these reactions are reversible.

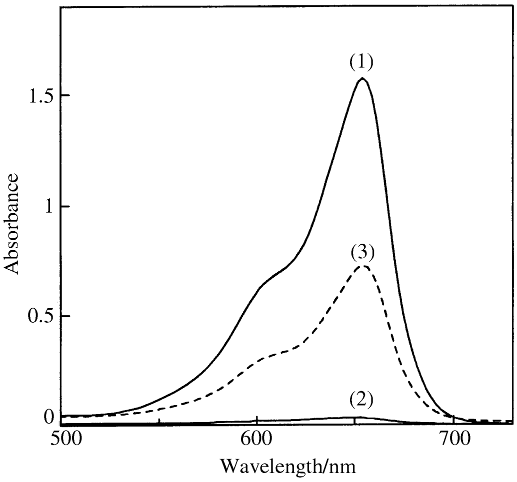


Fig. 1 Absorption spectra of MB^+ taken (1) before and (2) after photoreduction and (3) absorption spectrum of an equivolume mixture of MBH and CA.

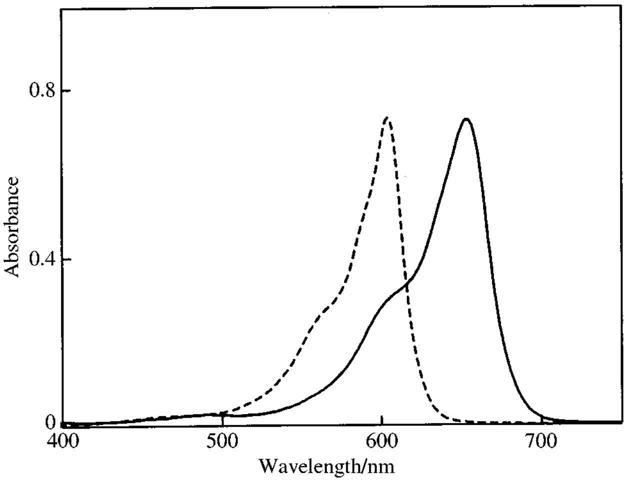


Fig. 2 Absorption spectra of MB^+ (——) and T^+ (– – –) in ethanol.

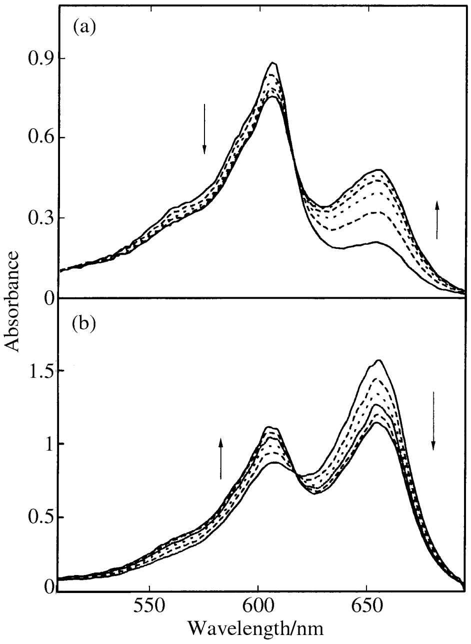


Fig. 3 Rapid-scan spectra for reactions between (a) MBH and T^+ and (b) MB^+ and TH at 25 °C in ethanol. Cycle time, (a) 20 ms and (b) 8 ms.

---

† Available as electronic supplementary information. See http://www.rsc.org/suppdata/cp/b0/b000046i/.

Kreevoy *et al.* determined the second-order rate constants for reversible reactions with the aid of three equations.[1] The first one is conventional for the description of pseudo-first-order reactions. This equation could only be used for reactions which left no significant fraction of the limiting reagent unconsumed in the end. Reactions which come to equilibrium with significant fractions of both reactants still remaining, were described by the second equation if the initial concentrations of the reactants were equal, and by the third equation if they were unequal (in this case the spectroscopically inactive reactant was used in at least 10-fold excess). In the present study, the concentrations of reactants were not equal and that of one of the reactants was not in excess. Therefore, we can not use the equations presented by Kreevoy *et al.*

For the reversible second-order reaction between MBH and T^+, the following relation is obtained:

$$\frac{x_e}{2ab-(a+b)x_e}\ln\frac{ab(x_e+x)-(a+b)x_e x}{ab(x_e-x)}=k_f t \quad (3)$$

where a and b are the initial concentrations of MBH and T^+ and x and x_e are the concentrations of MB^+ after the time t and at equilibrium. This equation becomes identical to the second equation of Kreevoy *et al.* if $a = b$ and to the third equation if $a \ll b$. If the value of the left-hand side of eqn. (3) at time t is $Y(t)$, a linear relationship between $Y(t)$ and t is expected. However, there are some dead-times in the measurements by the stopped-flow method and the dead-times seem to be different in each run. Therefore, we rewrite eqn. (3) to the following equation

$$\Delta Y = Y(t) - Y(t_0) = k_f(t - t_0) \quad (4)$$

where t_0 is the time when the measurement was begun. From the slopes of the straight lines between ΔY and $(t - t_0)$ for reactions between MBH and T^+ and for the reverse reaction from MB^+ and TH, the values of k_f and k_b were obtained (the linear relations between ΔY and $(t - t_0)$ are shown as supplementary data†). The values of k_f and k_b are listed in Table 1. The use of eqn. (3) requires that concentrations are calculated from absorbances, and this was done using the molar absorption coefficient ($\varepsilon = 92\,000\ M^{-1}\ cm^{-1}$ for the absorption of MB^+ at 655 nm in ethanol). In the case that the concentrations are not known (molar absorption coefficients are unknown or the absorption bands of reactants and products are largely overlapped), the following equation is useful (the derivation of this equation is given in the Appendix).

$$\frac{1}{1-\alpha_{t+\Delta t}} = \exp(k_1\Delta t)\frac{1}{1-\alpha_t} + \frac{1}{r}[\exp(k_1\Delta t) - 1] \quad (5)$$

Here, $r = k_1/k_2 x_e$ and $\alpha = x/x_e$, (α_t and $\alpha_{t+\Delta t}$ are the values of α at the time t and at the time $t + \Delta t$). At a fixed time interval (Δt), a plot of $1/(1 - \alpha_{t+\Delta t})$ *vs.* $1/(1 - \alpha_t)$ should be linear with a slope of $\exp(k_1\Delta t)$ and an intercept of $(1/r)[\exp(k_1\Delta t) - 1]$. The values of α were obtained by $\alpha = (A_t - A_0)/(A_\infty - A_0)$, where A_0, A_t and A_∞ are the absorbances at 655 nm at times 0, t and ∞. From the slope and intercept, k_1 and r can be obtained (Fig. 4), and are listed in Table 2. Both rate constants (k_f and k_b) can be calculated by the procedure shown in the Appendix. These values are listed in Table 2. The results are consistent with those obtained by the first method.

Table 1 Rate constants for forward and backward reactions between MBH and T^+

$10^5[MBH]_0/M$	$10^5[T^+]_0/M$	$10^{-5}\ k_f/M^{-1}\ s^{-1}$
1.6	2.3	1.08
1.6	3.3	1.02
1.6	4.9	1.12
1.6	7.3	1.02
		Average 1.06 ± 0.05
$10^5[MB^+]_0/M$	$10^5[TH]_0/M$	$10^{-5}\ k_b/M^{-1}\ s^{-1}$
1.8	0.76	9.29
1.8	1.1	8.34
1.8	1.5	7.93
1.8	1.9	7.80
		Average 8.3 ± 0.7
		$k_f/k_b = 0.13 \pm 0.02$

The advantages of the second method can be summarized as follows: (i) we can obtain the values of k_f and k_b by a single run from the one side of the reaction. (ii) Since $\alpha = x/x_e = (A_t - A_0)/(A_\infty - A_0)$, we can apply this method by knowing the change in the absorbance, even when we do not know the concentrations of the species (we do not know the molar absorption coefficient). The value of x_e can be calculated using a, b and r. This method was proposed previously by Zeng *et al.*[11,12] for the thermokinetics of reversible reactions where the rates of heat production by the reactions were measured. This method can be applied when the linear response of the extent of reaction can be observed to equilibrium. Since both

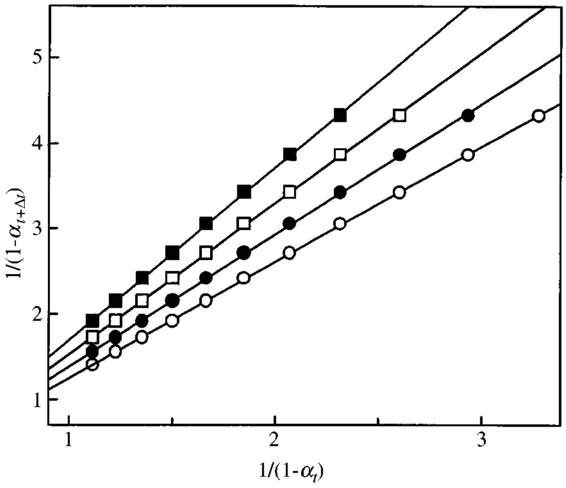


Fig. 4 Plots of $1/\alpha_{t+\Delta t}$ *vs.* $1/\alpha_t$ for the reaction between MBH and T^+ in ethanol. Initial concentrations; $[MBH]_0 = 1.6 \times 10^{-5}$ M, $[T^+]_0 = 3.3 \times 10^{-5}$ M, Δt = 21 (○), 30 (●), 39 (□) and 48 ms (■).

Table 2 Values of parameters involved in eqn. (5) and rate constants for forward and backward reactions between MBH and T^+

$10^5[MBH]_0/M$	$10^5[T^+]_0/M$	Δt/ms	k_1/s^{-1}	r	$10^5 x_e/M$	$10^5 k_2/M^{-1}\ s^{-1}$	$10^{-5}\ k_f/M^{-1}\ s^{-1}$	$10^{-5}\ k_b/M^{-1}\ s^{-1}$
1.6	2.3	27	12.6	−3.49	0.57	−6.33	1.35	7.62
1.6	3.3	21	14.6	−3.22	0.60	−7.56	1.12	8.68
1.6	3.3	30	14.5	−3.37	0.63	−6.83	1.20	8.03
1.6	3.3	39	14.6	−3.19	0.59	−7.76	1.10	8.86
1.6	3.3	48	14.7	−3.13	0.58	−8.10	1.08	9.17
1.6	4.9	18	18.2	−3.29	0.69	−8.02	1.08	9.09
1.6	7.3	12	20.8	−3.73	0.83	−6.72	1.08	7.80
							Average 1.14(0.10)	8.5(0.6)
							$k_f/k_b = 0.13 \pm 0.02$	

Table 3 The values of absorbance at 655 nm at equilibrium and equilibrium constant between MBH and T^+ (MB^+ and TH)

$10^5[MBH]_0/M$	$10^5[T^+]_0/M$	Abs^∞ at 655 nm	K
1.6	2.3	0.47	0.13
1.6	3.3	0.55	0.12
1.6	4.9	0.66	0.14
1.6	7.3	0.77	0.14
$10^5[MB^+]_0/M$	$10^5[TH]_0/M$	Abs^∞ at 655 nm	K
1.8	0.76	0.98	—
1.8	1.1	0.76	0.10
1.8	1.5	0.63	0.18
1.8	1.9	0.46	0.16
			Average = 0.14 ± 0.03

methods can be applied to the present case, the usefulness of the second method was confirmed for determining the rate constants for forward and backward reactions by a single run followed spectrophotometrically.

Equilibrium was established starting with known concentrations of MB^+ and TH or T^+ and MBH. The concentration of MB^+ at equilibrium was determined from the absorbance of MB^+ at 655 nm. The concentrations of the other three species could then be evaluated by using the stoichiometric relationships and conservation conditions. The equilibrium constant, K, was evaluated from its defining equation;

$$K = \frac{[TH]_e[MB^+]_e}{[T^+]_e[MBH]_e} \tag{6}$$

The value of K is shown in Table 3.

Discussion

As is shown in Tables 1 and 2 the values of k_f and k_b, evaluated by the two methods, are in good agreement with each other. Further, the value of K directly obtained (shown in Table 3) is in good agreement with the ratios of k_f/k_b. These consistent results show the validity of the evaluation of the rate constants with the second method.

The magnitudes of k_f and k_b reflect the differences in the reactivities of MBH and TH and in those of T^+ and MB^+. From the data of the rate constants for oxidations of MBH and TH by *p*-benzoquinones (BQ's) and Fe^{3+} presented in the previous papers, the reactivity of MBH is lower than that of TH (the ratio of rate constants for MBH and TH, k(MBH)/k(TH) = 0.29 (2-methoxy-*p*-BQ), 0.29 (2,5-dimethyl-*p*-BQ), 0.36 (2,6-dimethyl-*p*-BQ), 0.39 (2,5-di-*tert*-butyl-*p*-BQ) and 0.35 (Fe^{3+})). Similar values were obtained for different oxidants. This shows that the value of about 0.3 is a measure of the difference in the reactivity of MBH and TH for oxidation. On the other hand, the reactivity of T^+ and MB^+ as electron or hydride acceptors depends on the redox potential $E^0(A/A^-)$. From the values of $E^0(A/A^-)$ for T^+ and MB^+ (−0.11 for T^+ and −0.05 for MB^+),[13] it is clear that the reactivity of MB^+ is higher than that of T^+. Since the reactivities of TH and MB^+ are higher than those of MBH and T^+, respectively, the value of k_f obtained was smaller than that of k_b, and an equilibrium constant less than unity was obtained.

For the hydride transfer between NAD^+ analogues, Kreevoy *et al.*[1] postulated the direct hydride transfer mechanism and Ohno *et al.*[14] suggested the stepwise electron–proton–electron transfer (or electron–hydrogen transfer) mechanism. For the nearly symmetrical reversible nature of the present reaction, the potential energy diagram of the reaction must be nearly symmetrical, and therefore in the stepwise mechanism the rate-determining step must be the step of proton-transfer located at the centre and the asymmetrical electron-hydrogen transfer mechanism is eliminated.

Since a hydride or a proton is transferred in a rate-determining step in the above mechanism, a kinetic isotope effect is expected. We tried to produce leuco methylene blue-d (MBD) by using ethanol-d_1 instead of ethanol as solvent and measured the rate of the reaction between MBD and T^+. The values of k_f and K obtained for MBD were 0.71×10^5 M^{-1} s^{-1} and 0.13. The ratio of k_f^H/k_f^D was about 1.55, while K^H/K^D is nearly unity. The isotope effect observed for k_f is not high but is not negligible. It is higher than would be expected for a secondary or a solvent isotope effect. This suggests that the hydride (or proton) transfer is the rate-determining step.

A high isotope effect will be expected for the near symmetrical transition state such as in the present reaction. Smaller isotope effects may show that the hydrogen atom which is involved in the leuco dye comes partly from the O–H (O–D) bond of ethanol, but partly from C–H bonds. The possibility of the latter is supported by the fact that one of the products observed is acetaldehyde in the photoreduction of methylene blue in ethanol.[15] If the content of MBD is γ in leuco methylene blue formed in ethanol-d_1, k_f^D obtained in ethanol-d_1 is expressed by

$$k_f^D = \gamma k_D + (1-\gamma)k_f^H \tag{6}$$

where k_D is the value of k_f for pure MBD. Therefore, the ratio of k_f^D/k_f^H is given as follows;

$$\frac{k_f^D}{k_f^H} = \gamma\frac{k_D}{k_f^H} + (1-\gamma) \tag{7}$$

If $\gamma \leqq 0.5$ (it is assumed that only less than half of the hydrogen atom involved in leuco dye comes from the O–D bond in ethanol-d_1), the isotope effect for k_f larger than 3.4 was obtained using the value obtained for k_f^H/k_f^D (1.55).

As mentioned above, a clean isosbestic point was obtained and this shows the absence of significant concentrations of any intermediate species. Although we can not distinguish the direct hydride transfer mechanism from the stepwise electron–proton–electron transfer mechanism, we tentatively propose the following simple mechanism:

$$MBH + T^+ \rightleftarrows (MBG\cdots T^+) \quad \text{(fast)}$$

$$(MBH\cdots T^+) \rightleftarrows (MB^+\cdots TH) \quad \text{(slow)}$$

$$(MB^+\cdots TH) \rightleftarrows (MB^+ + TH \quad \text{(fast)}$$

Here, $(MBH\cdots T^+)$ and $(MB^+\cdots TH)$ are complexes involving π–π interaction *via* the aromatic rings. This mechanism is the same as that proposed by Kreevoy *et al.* for hydride transfer between NAD^+ analogues.[1]

Appendix

The bimolecular reversible reaction between MBH + T^+ has the rate law

$$\frac{d[MB^+]}{dt} = k_f[MBH][T^+] - k_b[MB^+][TH] \tag{A1}$$

where, k_f and k_b are the rate constants for the forward and backward reactions. If the initial concentrations of MBH and T^+ are a and b and those of MB^+ and TH are 0, and the concentrations of MB^+ after time t is x, then the concentrations of reactants and products at time t are given as follows;

$$[MBH] = (a-x),\ [T^+] = (b-x),$$

$$[MB^+] = x \text{ and } [TH] = x.$$

The rate of production of MB^+ is then given by

$$\frac{dx}{dt} = k_f(a-x)(b-x) - k_b x^2 \tag{A2}$$

If x_e is the concentration of MB^+ at equilibrium, when the net rate of the reaction is zero,

$$k_f(a - x_e)(b - x_e) - k_b x_e^2 = 0 \tag{A3}$$

From eqn. (A2) and (A3) we can prove that

$$\frac{dx}{dt} = k_1(x_e - x) + k_2(x_e - x)^2 \tag{A4}$$

where $k_1 = k_f(a + b - 2x_e) + 2k_b x_e$ and $k_2 = k_f - k_b$. Integrating eqn. (A4), we obtain

$$\ln \frac{[k_1 + k_2(x_e - x)]k_e}{(k_1 + k_2 x_e)(x_e - x)} = k_1 t \tag{A5}$$

Let $r = k_1/k_2 x_e$ and $\alpha = x/x_e$, and considering the experimental values α_t (at time t) and $\alpha_{t+\Delta t}$ (at time $t + \Delta t$), we have

$$\frac{1}{1 - \alpha_{t+\Delta t}} = \exp(k_1 \Delta t) \frac{1}{1 - \alpha_t} + \frac{1}{r} [\exp(k_1 \Delta t) - 1] \tag{A6}$$

Using k_1, k_2 and r, x_e, k_f and k_b are expressed as follows:

$$x_e = \frac{ab}{a + b} \frac{r + 2}{r + 1} \tag{A7}$$

$$k_f = \frac{k_1 + 2k_2 x_e}{a + b} \tag{A8}$$

$$k_b = \frac{k_1 - (a + b - 2x_e)k_2}{a + b} \tag{A9}$$

From Table 2 and eqn. (A7), we can calculate the final reaction extent x_e and the value of k_2 using $k_2 = k_1/rx_e$. Finally, both rate constants (k_f and k_b) can be calculated from eqn. (A8) and (A9).

References

1 R. M. G. Roberts, D. Ostovic and M. M. Kreevoy, *Faraday Discuss. Chem. Soc.*, 1982, **74**, 257.
2 D. Ostovic, R. M. G. Roberts and M. M. Kreevoy, *J. Am. Chem. Soc.*, 1983, **105**, 7629.
3 M. M. Kreevoy and I. H. Lee, *J. Am. Chem. Soc.*, 1984, **106**, 2550.
4 D. Ostovic, I. H. Lee, R. M. G. Roberts and M. M. Kreevoy, *J. Org. Chem.*, 1985, **50**, 4206.
5 M. M. Kreevoy and A. T. Kotcherar, *J. Am. Chem. Soc.*, 1990, **112**, 3579.
6 Y. Kim, D. G. Truhlar and M. M. Kreevoy, *J. Am. Chem. Soc.*, 1991, **113**, 7837.
7 I. H. Lee, E. H. Jeovrg and M. M. Kreevoy, *J. Am. Chem. Soc.*, 1997, **119**, 2722.
8 S. Yamamoto, Y. Fujiyama, M. Shiozaki, Y. Sueishi and N. Nishimura, *J. Phys. Org. Chem.*, 1995, **8**, 805.
9 Y. J. Liu, S. Yamamoto and Y. Sueishi, *J. Phys. Org. Chem.*, 1999, **12**, 194.
10 E. S. Olson, *J. Chem. Educ.*, 1977, **54**, 366.
11 J. S. Liu, X. C. Zeng, A. M. Tian and Y. Deng, *Thermochim. Acta*, 1993, **36**, 43.
12 Y. J. Liu, S. Yamamoto, S. Q. Cheng, Y. Q. Zhang and X. C. Zeng, *J. Sichuan Univ.*, 1999, **36**, 287.
13 H.-J. Timpe and S. Neuenfeld, *J. Chem. Soc., Faraday Trans.*, 1992, **88**, 2329.
14 A. Ohno, T. Shio, H. Yamamoto and S. Oka, *J. Am. Chem. Soc.*, 1981, **103**, 2045.
15 Y. Usui, *Bull. Chem. Soc. Jpn.*, 1965, **38**, 206.