

Quinine Fluorescence Quenching at Low Ionic Strength

S. W. BIGGER,¹ P. J. WATKINS,¹ B. VERITY²

¹*School of Life Sciences and Technology F008, Victoria University of Technology, Footscray Park Campus, P.O. Box 14428, Melbourne City Mail Centre, Melbourne, 8001, Australia*

²*School of Chemical Technology, University of South Australia, Mawson Lakes, 5095, Australia*

Received 7 December 1999; accepted 9 March 2000

ABSTRACT: The effect of ionic activity on the quenching of the quinine dication fluorescence by chloride and bromide ions is examined in solutions of sufficiently low ionic strength to enable ionic activities to be taken into account by means of the Debye-Hückel equation. An equation for the dependence of the Stern-Volmer constant on the ionic strength is derived in a novel way and an iterative, linear least-squares computer program is described from which the optimized values of the ionic size parameter for chloride ($a_{\text{Cl}^-} = 4.56 \pm 0.07 \text{ \AA}$) and bromide ($a_{\text{Br}^-} = 4.98 \pm 0.23 \text{ \AA}$) quenching were obtained. The ratio of these parameters ($a_{\text{Br}^-}/a_{\text{Cl}^-} = 1.09 \pm 0.07$) is found to be similar to the ratio of the ionic radii of the two species, $r_{\text{Br}^-}/r_{\text{Cl}^-} = 1.08$. The experimental results suggest that the quenching of quinine fluorescence by bromide ions is about 28% faster than that observed for chloride ions. The results are consistent with the notion that the quenching process is a dynamic one involving the formation of an activated complex whose rate of deactivation is dependent on the ionic strength of the medium. © 2000 John Wiley & Sons, Inc. *Int J Chem Kinet* 32: 473–477, 2000

INTRODUCTION

The chemical and photophysical behaviors of the quinine dication have led to its use in studies of fluorescence [1,2] and, in particular, as a quantum yield standard [3]. However, the use of quinine as a life-time standard is only appropriate under conditions where its complex decay kinetics [4,5] can be approximated as a single exponential process [6]. Quinine has also been studied in numerous fluorescence quenching experiments, particularly ones involving halide ions [6–15]. In early work, the quenching of the quinine fluorescence by halide ions was attributed to the formation of a nonfluorescent complex between the two species [9–11].

The results of an early study [12] as well as those

of a recent study [16] show that the Stern-Volmer constant for the quenching of quinine fluorescence by halide ions is markedly affected by the ionic strength of the solution. The observed decrease in the quenching rate constant with increasing ionic strength is believed to arise from the influence of the ionic strength on the diffusion-controlled rate constant for the bimolecular quenching process [16]. This can be explained by there being either a decrease in the intrinsic rate constant for the reaction due to the “kinetic salt” effect [16], or a decrease in the effective encounter distance due to screening of the charges on the reactants [17].

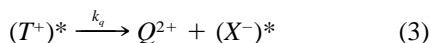
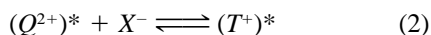
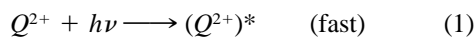
This article examines the effects of ionic activity on the quenching of the quinine dication fluorescence by both chloride and bromide ions with a view toward establishing whether the influence of the ionic activities on the transition state alone is adequate to describe the observed kinetic behavior. Unlike the systems described in a previous article [16], the solutions used in

Correspondence to: S. W. Bigger (stephen.bigger@vu.edu.au)
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the present study were of sufficiently low ionic strength to enable ionic activities to be taken into account by the application of the Debye-Hückel equation. In particular, a novel derivation of the equation for the dependence of the Stern-Volmer constant on the ionic strength is given which requires none of the usual assumptions. An iterative, linear least-squares computer program is also described which enables optimized values of the ionic size parameter to be obtained from the experimental data.

THEORY

The observed fluorescence life-time of quinine is found to decrease as the concentration of quencher is increased [16], which supports the notion that the quenching process is a dynamic one and is not a static process involving the formation of a nonfluorescent complex between the fluorophore and quencher, which has been proposed previously [8–11]. A kinetic scheme for the excited-state quenching of the fluorescence of the quinone dication, Q^{2+} , by a halide ion, X^- , is given in reactions (1)–(3):



where $(T^+)^*$ is a transient complex that is formed presumably in a fast, reversible reaction between $(Q^{2+})^*$ and X^- , and k_q is the second-order rate constant for the quenching process. The equilibrium constant, K , for the formation of the transient complex produced in reaction (2) is given by Eq. (4):

$$K = (\gamma_+/\gamma_{2+}\gamma_-)[(T^+)^*]/[Q^{2+}][X^-] \quad (4)$$

where γ_+ , γ_{2+} , and γ_- are the activity coefficients of $(T^+)^*$, Q^{2+} and X^- , respectively.

Since the rates of reactions (1) and (2) are presumably fast, the observed rate of quenching will be determined by the rate of the process shown in reaction (3). Thus, the following equalities can be derived readily:

$$\begin{aligned} \text{Rate} &= k[(T^+)^*] = kK(\gamma_{2+}\gamma_-/\gamma_+)[Q^{2+}][X^-] \\ &= k_q[Q^{2+}][X^-] \quad (5) \end{aligned}$$

$$k_q = k_q^0(\gamma_{2+}\gamma_-/\gamma_+) \quad (6)$$

where k is a constant and k_q^0 is the value of the observed quenching rate constant, k_q , when the ionic strength is zero [18]. At zero ionic strength all ionic activity coefficients are unity and so $k_q^0 = kK$.

An equation for the dependence of k_q on the ionic strength can be derived from Eq. (6) by multiplying the numerator and denominator by γ_- , applying the definitions of mean activity coefficients, and taking the natural logarithm of both sides of the resulting equation:

$$k_q = k_q^0(\gamma_{2+}\gamma_-/\gamma_+\gamma_-) = k_q^0(\gamma_{2:1})^3/(\gamma_{1:1})^2 \quad (7)$$

$$\ln(k_q) = \ln(k_q^0) + 3 \ln(\gamma_{2:1}) - 2 \ln(\gamma_{1:1}) \quad (8)$$

Using the Debye-Hückel law for *mean* ionic activity coefficients, as it was originally intended to be used, the following expressions apply:

$$\ln(\gamma_{2:1}) = -|z_{2+}z_-|AI^{1/2}/(1 + BaI^{1/2}) \quad (9)$$

$$\ln(\gamma_{1:1}) = -|z_+z_-|AI^{1/2}/(1 + BaI^{1/2}) \quad (10)$$

where z_+ , z_{2+} , and z_- are the charges on the $(T^+)^*$, Q^{2+} , and X^- species, respectively, I is the ionic strength, a is the ionic size parameter, and A and B are constants whose numerical values at 20°C in aqueous solution are 1.1611 and 3.274×10^9 , respectively [19]. Substituting Eqs. (9) and (10) in Eq. (8) leads to the following expression:

$$\ln(k_q) = \ln(k_q^0) - 4 AI^{1/2}/(1 + BaI^{1/2}) \quad (11)$$

An expression similar to Eq. (11) has been applied in studying the effect of ionic strength on the fluorescence quenching of quinine by monovalent negative ions [12].

EXPERIMENTAL SECTION

Quinine bisulfate (6-methoxy-cinchonan-9-ol, $C_{20}H_{24}N_2O_2 \cdot H_2SO_4 \cdot 7H_2O$, 1:1 sulfate salt) from Eastman Kodak was used as received. The fluorescence characteristics of the quinine was determined and found to be in agreement with previous work [6,20]. All other chemicals used in the study were analytical grade: NaCl (BDH Chemicals Limited), NaBr (BDH Chemicals Limited), and HNO_3 (BDH Chemicals Limited). Prepared stock solutions of these reagents in distilled water were tested and found to be free of fluorescent impurities.

Distilled water was used in preparing "sets" of aqueous solutions all containing quinine at a constant

level of 2.0×10^{-5} M and with each set having a different ionic strength. Each set comprised six solutions where the halide (NaCl or NaBr) concentration was: zero, 1.0×10^{-3} , 2.0×10^{-3} , 3.0×10^{-3} , 4.0×10^{-3} or 5.0×10^{-3} M. These halide concentrations were deliberately chosen so that the variation in quencher concentration does not affect significantly the "nominal" ionic strength of each set of solutions. Indeed, in calculating the latter, the contribution made by the quencher was assumed to be equal to the "average" quencher concentration of 2.5×10^{-3} M.

In a previous study, it was shown that the effect of electrolytes on the rate of quenching is related to the ionic strength and not simply to the acidity of the solution [11]. Nonetheless, the "kinetic salt" effect can be produced successfully by varying the acidity of the test solution. Thus, the ionic strength of each set of solutions was fixed by the addition of HNO₃ to produce a final HNO₃ concentration in the range 2.5×10^{-3} to 2.0 M in the case of solutions containing NaCl, and 5.0×10^{-3} to 5.0×10^{-1} M in the case of the NaBr solutions.

Fluorescence intensities at 450 nm (the wavelength of maximum emission of the quinine species in acidic solution) were measured at 20°C using an excitation wavelength of 350 nm (the wavelength of maximum absorbance of the quinine dication) on a Perkin-Elmer model LS-50 spectrofluorimeter.

RESULTS AND DISCUSSION

At a given ionic strength of solution, the observed quenching of fluorescence can be modeled quantitatively by the following form of the Stern-Volmer equation [11,21]:

$$I_f^0/I_f \approx 1 + k_q\tau_0[Q] \quad (12)$$

where I_f is the fluorescence intensity, I_f^0 is the fluorescence intensity when $[Q] = 0$, and τ_0 is the fluorescence lifetime when $[Q] = 0$. Thus, a plot of I_f^0/I_f against $[Q]$ is a straight line that has a slope equal to the Stern-Volmer constant, K_{SV} where $K_{SV} = k_q\tau_0$.

For each set of solutions of a given ionic strength, a Stern-Volmer plot was constructed using the fluorescence intensities measured at 450 nm, the wavelength of maximum emission. Figure 1 shows selected Stern-Volmer plots that are typical of the results obtained for the quenching of quinine by chloride ions. Similar behavior was also observed for bromide ion quenching. In all cases in this study, the Stern-Volmer plots were straight lines each with a correlation coefficient, r^2 , in the range $0.994 \leq r^2 \leq 1.000$ (99.9%

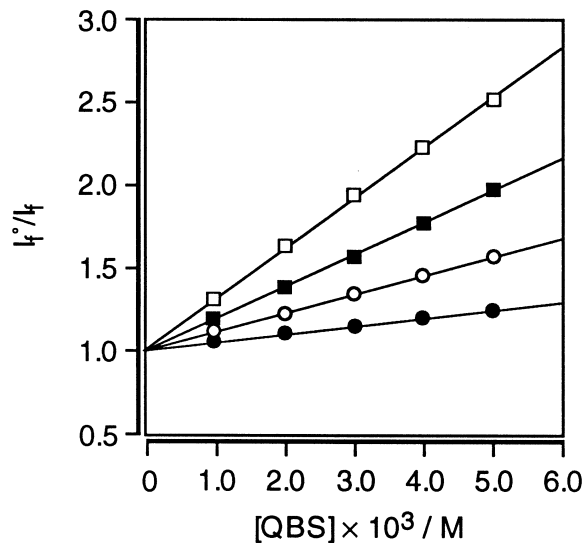


Figure 1 Typical Stern-Volmer plots at 20°C for the quenching of quinine (2.0×10^{-5} M) fluorescence at 450 nm by NaCl where the nominal ionic strength of the test solution is: (a) \square , 5.6×10^{-3} M; (b) \blacksquare , 5.26×10^{-2} M; (c) \circ , 2.53×10^{-1} M; and (d) \bullet , 2.00 M. Excitation wavelength: 350 nm.

confidence level, calculated using an appropriate value of the Student t -statistic) and vertical axis intercept, y_0 , in the range $0.981 \leq y_0 \leq 1.011$ (99.9% confidence level). The linearity of these plots confirms that the variation in the quencher concentration within a given set of solutions does not affect significantly the nominal ionic strength.

Shown in Figure 2 is a "pseudo-code" representation [22,23] of a computer program that was used to perform iterative linear least-squares regression analyses on data extracted from the Stern-Volmer plots in order to determine an optimum value of the ionic size parameter.¹ Since $K_{SV} = k_q\tau_0$, then Eq. (11) can be used to derive:

$$\ln(K_{SV}) = \ln(k_q^0\tau_0) - 4AI^{1/2}/(1 + BaI^{1/2}) \quad (13)$$

Thus a plot of $\ln(K_{SV})$ vs. $I^{1/2}/(1 + BaI^{1/2})$ will be linear with a gradient equal to $-4A$ and a vertical axis intercept equal to $\ln(k_q^0\tau_0)$. The computer program reads corresponding pairs of K_{SV} and ionic strength values and, in an iterative loop, calculates the data pairs $\ln(K_{SV})$ and $I^{1/2}/(1 + BaI^{1/2})$, where the latter is calculated using a value of the product $B \times a$, which is set in the loop. A linear regression is then performed

¹ A listing of the computer program code, which is written in the BASIC language, is available from the authors on request.

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Optimization of "Ba" Product: Ionic Strength Effect
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set lower and upper limits of product Ba: BaMin, BaMax
set increment for Ba: deltaBa

read n values of the ionic strength, I(i), and the associated
quenching rate constant, K(i), from the data file

repeat with Ba = BaMin to BaMax step deltaBa
  repeat with i = 1 to n
    x(i) = SQR(I(i))/(1 + Ba*SQR(I(i)))
    y(i) = log(K(i))
  end repeat
  perform linear least squares regression on {x(i), y(i)}
  print Ba, gradient, intercept and regression coefficient
end repeat
end

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Figure 2 A "pseudo-code" listing of an iterative, linear least-squares regression program for determining an optimum value of the ionic size parameter from steady-state fluorescence quenching data.

on the calculated data pairs, and the gradient, intercept, and regression coefficient, r^2 , are displayed before the value of the product $B \times a$ is incremented and the process repeated. An examination of the output produced by the program in successive trials enables the value of the product $B \times a$ to be optimized in such a way that the observed gradient of the plot is equal to the theoretical value, $-4 A$. Since the value of the constant B is known at the temperature of the experiment, then the value of the ionic size parameter can be subsequently calculated from the optimized value of the product $B \times a$.

Figure 3 shows plots of $\ln(K_{SV})$ vs. $I^{1/2}/(1 + BaI^{1/2})$ for the quenching of quinine fluorescence by the halide ions Cl^- and Br^- . Each plot was produced

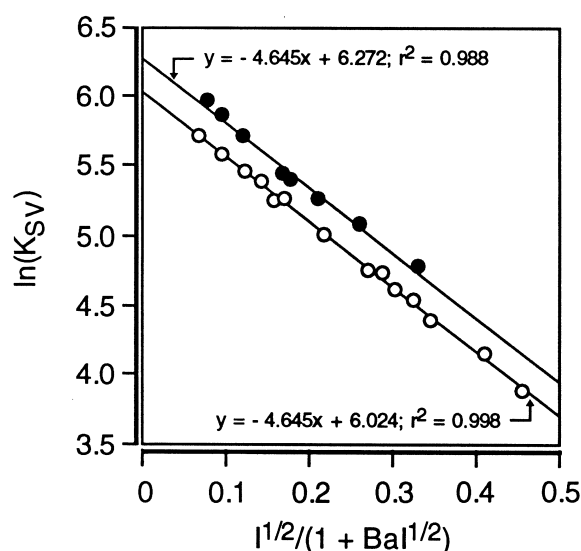


Figure 3 Plots of $\ln(K_{SV})$ vs. $I^{1/2}/(1 + BaI^{1/2})$ for the quenching of quinine fluorescence at 20°C by: (a) Cl^- ions (\circ) where $B \times a_{\text{Cl}^-} = 1.491$, and (b) Br^- ions (\bullet) where $B \times a_{\text{Br}^-} = 1.630$.

by calculating abscissa values using the appropriate optimized value of the product $B \times a$, which, in turn, were determined using the computer program described by Figure 2. The experimental data confirm that the value of K_{SV} decreases with increased ionic strength, a trend that has been observed elsewhere for the quenching of quinine fluorescence by chloride ions [11,16] and which has been attributed to the ionic strength effect of the solvent medium on the value of the quenching rate constant, k_q [16].

In the present study, the optimized value of the product $B \times a$ for chloride ion quenching was found to be $B \times a_{\text{Cl}^-} = 1.491$. This is comparable to the value obtained by Harty and Rolefson [12], who studied a similar system; for bromide quenching, $B \times a_{\text{Br}^-} = 1.630$ was found in the present study. From these products, the ionic size parameters for the quenchers are calculated to be $a_{\text{Cl}^-} = 4.56 \pm 0.07 \text{ \AA}$ and $a_{\text{Br}^-} = 4.98 \pm 0.23 \text{ \AA}$. It is interesting to note that the ratio of the experimental ionic size parameters reflects almost exactly, and within experimental error, the ratio of the ionic radii of the two species. In particular, $a_{\text{Br}^-}/a_{\text{Cl}^-} = 1.09 \pm 0.07$ and $r_{\text{Br}^-}/r_{\text{Cl}^-} = 1.08$, the latter calculated using tabulated values of r , the ionic radius [24].

It is clear from the data presented in Figure 3 that the bromide ion quenches the fluorescence of quinine more efficiently than the chloride ion. The vertical axis intercepts of the two plots can be used to determine the ratio of the quenching rate constants at zero ionic strength, $k_q^{\circ}(\text{Br}^-)/k_q^{\circ}(\text{Cl}^-) = 1.28 \pm 0.08$, which suggests that the quenching of quinine fluorescence by the bromide ion occurs at a rate that is about 28% faster than that observed for the chloride ion. Absolute values of k_q° can be calculated from the intercepts by using an appropriate value of τ_0 .

Fluorescence life-time experiments of quinine in solutions containing different concentrations of H_2SO_4 have shown that there is a small, but significant, decrease in the fluorescence life-time from 18.9 to 17.5 ns when the acid concentration is decreased from 1.0 to 0.1 M. Such a difference can be attributed to the suppression of a nonradiative deactivation pathway by the acid [16,25]. If the value of $\tau_0 = 17.5$ ns is taken as a good approximation of the fluorescent life-time of quinine in a solution of zero ionic strength, then the calculated k_q° values are $k_q^{\circ}(\text{Cl}^-) = (2.36 \pm 0.04) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ and $k_q^{\circ}(\text{Br}^-) = (3.03 \pm 0.13) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$.

CONCLUSIONS

The experimental results conform to the derived theoretical model and, thus, support the notion that the

quenching of quinine fluorescence by halide ions can be described adequately as a dynamic process involving the formation of an activated complex whose rate of deactivation is dependent on the ionic strength of the medium. The functional dependence of the observed decrease in the Stern-Volmer quenching rate constant with increasing ionic strength can be readily derived from consideration of the mean ionic activity coefficients associated with the species comprising the activated complex and the application of the Debye-Hückel equation. By fitting the experimental data to the derived function it was found that, within experimental error, the ratio of the ionic size parameters of the two quenchers investigated is almost equal to the ratio of their ionic radii and that the quenching of quinine fluorescence by the bromide ion is about 28% faster than that observed for the chloride ion. It is anticipated that the experimental protocol described herein can be successfully applied to the future systematic study of other quenching systems.

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