A Fluorimetric Approach to Studying the Effects of Ionic Strength on Reaction Rates

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An Undergraduate Steady-State Fluorescence Laboratory Experiment

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The photophysical properties of the quinine dication are well documented in the scientific literature *(1–4)*. In the educational literature, this species has been used as the fluorescent compound in an experiment designed to illustrate the principles of fluorimetry to undergraduate students *(5)*. The quenching of quinine fluorescence, particularly by halide ions, has also been widely studied *(6–11)* and has been utilized in the educational literature to illustrate the application of the Stern–Volmer equation *(5)*.

This simple, steady-state fluorescence experiment for undergraduate students involves studying the quenching of quinine fluorescence by Cl^- and Br^- in solutions of different ionic strength. The experiment enhances the student's understanding and experience in the technique of fluorimetry and illustrates how the Debye–Hückel law can be used in conjunction with the Stern–Volmer equation to study how the ionic activity of a solution influences the rate of a reaction. The experiment has particular merit as a seemingly unique situation where the Debye–Hückel law can be applied without the use of simplifying assumptions that are usually needed in the calculation of ionic activity coefficients. Such an example has not been presented in the educational literature to date. A sequence of postlaboratory tasks to facilitate a stepwise approach to the manipulation of experimental data is given in the Supplemental Material.[W]

Theory

A complete kinetic scheme for the excitation of the quinine dication followed by the excited-state quenching of its fluorescence by a halide ion is given in the Supplemental Material.[W] Students use this scheme in conjunction with the Debye–Hückel law to derive eq 1, which describes the dependence of the observed, second-order rate constant for the quenching process, k_q, on the ionic strength, I, of the solution,

$$\ln\left(k_q\right) = \ln\left(k_q^{\circ}\right) - \frac{4\,AI^{1/2}}{1 + BaI^{1/2}} \qquad (1)$$

where k_q° is the value of k_q when the ionic strength is 0 *(12)*; A and B are constants whose numerical values at 20 °C in aqueous solution are 1.1611 and 3.274×10^9, respectively *(13)*; and a is the ionic size parameter, a constant for a given system.

At a given ionic strength, experimental fluorescence quenching data can be modeled quantitatively by the Stern–Volmer equation *(14, 15)* and a value of the Stern–Volmer constant, K_{SV}, can be calculated. The constant K_{SV} is related to the lifetime of the excited state, τ_0, as follows,

$$K_{SV} = k_q\tau_0 \qquad (2)$$

where, in this case, the quinine dication *(16, 17)* is the excited state. From eq 1 it is clear that a plot of $\ln K_{SV}$ versus $I^{1/2}/(1 + BaI^{1/2})$ is linear and has a slope equal to $-4A$ (i.e., -4.644 at 20 °C) and a vertical axis intercept equal to $\ln(\tau_0 k_q^{\circ})$.

Experimental Section

Solutions

Students are provided with stock solutions of each of the halide quenchers, NaCl and NaBr, and mixed stock solutions containing QBS and HNO_3. They prepare a total of 32 test solutions: 16 solutions for each halide quencher. All test solutions contain the same concentration of quinine, 2.0×10^{-5} M. Each halide quencher is studied in solution at four different values of the nominal ionic strength, which in turn, is determined by the HNO_3 content. At each ionic strength a set of test solutions is made that comprises four solutions in which the halide concentration is zero, 1.0×10^{-3}, 3.0×10^{-3}, or 5.0×10^{-3} M. These concentrations were deliberately chosen so that the variation in quencher concentration does not significantly affect the "nominal" ionic strength. The complete details of the test solutions and the procedure for making these are given in the Supplemental Material.[W]

Fluorescence Measurements

The fluorescence intensity of each solution at 20 °C is measured using a commercial spectrofluorimeter (Perkin-Elmer model LS-50) where the excitation wavelength is set to 350 nm (the wavelength of maximum absorbance of quinine) and the emission wavelength is set to 450 nm (the wavelength of maximum emission of the quinine species in acidic solution). It is expected that the experiment could also be performed successfully using a fluorimeter described elsewhere *(5)* that has been designed specifically for student use. Furthermore, the experiment could be shortened considerably by having the students perform fluorescence measurements on only one of the two quenching systems.
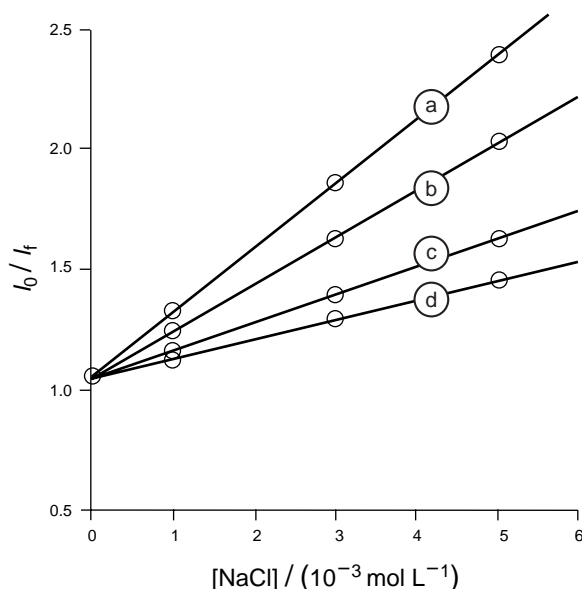
Figure 1. Stern–Volmer plots at 20 °C for the quenching of quinine (2.0 × 10⁻⁵ M) fluorescence at 450 nm by NaCl where the nominal ionic strength of the test solution is: (a) 1.26×10^{-2} M, (b) 5.26×10^{-2} M, (c) 2.026×10^{-1} M, and (d) 5.026×10^{-1} M. Excitation wavelength: 350 nm.
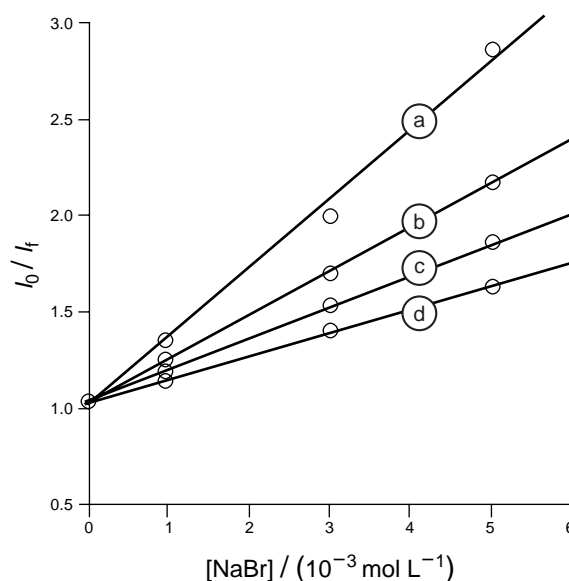

Figure 2. Stern–Volmer plots at 20 °C for the quenching of quinine (2.0 × 10⁻⁵ M) fluorescence at 450 nm by NaBr where the nominal ionic strength of the test solution is: (a) 1.26×10^{-2} M, (b) 5.26×10^{-2} M, (c) 2.026×10^{-1} M, and (d) 5.026×10^{-1} M. Excitation wavelength: 350 nm.

Hazards

The levels of NaCl, NaBr, and quinine bisulfate (QBS; Eastman Kodak) used in this experiment are considered to be safe and pose no immediate hazard. However, care should always be exercised when handling solutions containing nitric acid and safety goggles should be worn when handling such chemicals.

Results and Discussion

For each set of test solutions at a given ionic strength, a Stern–Volmer plot is constructed using the measured fluorescence intensities. The Stern–Volmer plots for the quenching of quinine by Cl⁻ and Br⁻ are shown in Figures 1 and 2, respectively. Each plot is a straight line with a correlation coefficient, r^2, in the range $.995 \leq r^2 \leq 1.000$ (99.9% confidence level, calculated using appropriate values of the Student t-statistic) and vertical axis intercept, y_0, in the range $.967 \leq y_0 \leq 1.021$ (99.9% confidence level). The linear regression parameters for these lines are given in Table 1. Figure 3 shows plots of $\ln K_{SV}$ versus $I^{1/2}/(1 + BaI^{1/2})$ for the quenching of quinine fluorescence by Cl⁻ and Br⁻. The abscissa values for each plot were calculated using the appropriate value of the ionic size parameter for the particular quencher *(18)*, $a_{Cl^-} = 4.56$ Å and $a_{Br^-} = 4.98$ Å. The data show that the value of K_{SV} decreases with increasing ionic strength *(14, 16)*. This can be attributed to the ionic effect of the solvent medium on the value of the quenching rate constant, k_q *(15)*. More importantly, it is clear that the slope of each plot compares favorably with the theoretical value of $-4A$.

The data presented in Figure 3 show that Br⁻ quenches the fluorescence of quinine more efficiently than the Cl⁻ species. Indeed, the vertical axis intercepts of the two plots can be used to calculate the ratio of the quenching rate constants at zero ionic strength, $k_q^o(Br^-)/k_q^o(Cl^-) = 1.23$. Thus the quenching of quinine fluorescence by Br⁻ occurs at a rate that is about 23% faster than that obtained for Cl⁻ quenching *(18)*. Absolute values of the k_q values can be calculated from the intercepts assuming that $\tau_0 = 17.5$ ns is a good approximation of the fluorescent lifetime of quinine in a solution of zero ionic strength *(16, 17)*. The calculated k_q^o values are thus $k_q^o(Cl^-) = 2.45 \times 10^{10}$ M⁻¹ s⁻¹ and $k_q^o(Br^-) = 3.01 \times 10^{10}$ M⁻¹ s⁻¹. These values compare favorably with those reported in the literature *(18)*.

Table 1. Linear Regression Parameters Associated with the Stern–Volmer Plots Shown in Figures 1 and 2

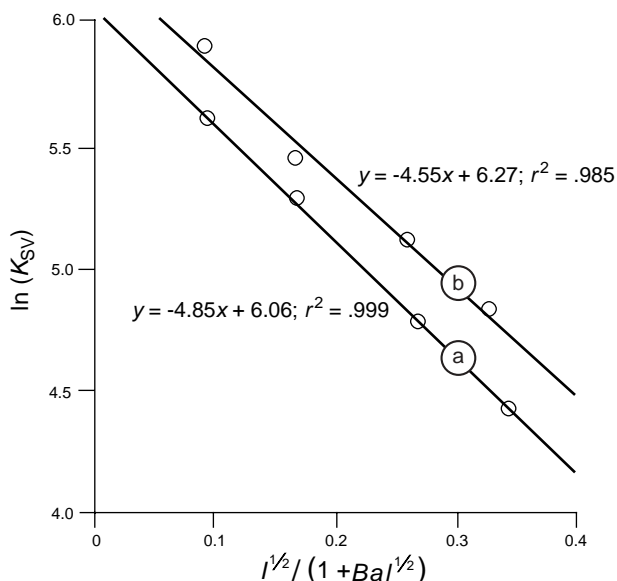| Quenching	I	K_{SV}	y_0	r^2
Cl⁻	0.0126	265.3	1.003	.9999
	0.0526	199.2	0.998	.9999
	0.2026	115.3	0.995	.9996
	0.5026	80.6	0.996	.9992
Br⁻	0.0126	361.2	0.957	.9932
	0.0526	227.8	0.996	.9999
	0.2026	161.1	1.006	.9992
	0.5026	120.9	0.999	1.0000

Figure 3. Plots of ln K_{SV} versus $I^{1/2}/(1 + BaI^{1/2})$ for the quenching of quinine fluorescence at 20 °C by: (a) Cl⁻ ions where a_{Cl^-} = 4.56 Å, and (b) Br⁻ ions where a_{Br^-} = 4.98 Å. Ionic size parameters were obtained from ref 18.

Conclusions

This simple experiment can be used to demonstrate to undergraduate students that the rate of a reaction can be controlled by the ionic strength of the medium in which it proceeds. In particular, the quenching of the quinine dication fluorescence by Cl⁻ or Br⁻ species is adequately described as a dynamic process involving the formation of an activated complex whose rate of deactivation depends upon the ionic strength of the medium. The exercise also illustrates that the observed decrease in the Stern–Volmer quenching rate constant with increasing ionic strength can be quantitatively modeled. Such quantitative modeling enables the student to determine the relative efficiencies by which the Cl⁻ and Br⁻ species quench the fluorescence of quinine.

ᵂSupplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

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