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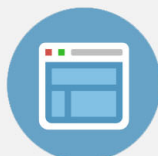
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Diffusion controlled reactions: Experimental verification of the time-dependent rate equation

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The transient effect in a diffusion limited reaction is described by a time-dependent rate coefficient: $k(t) = a + b \exp(c^2 t) \operatorname{erfc}(ct^{1/2})$, where a , b , and c are expressed in terms of the diffusion coefficient (D), the encounter distance (R), and the absolute rate coefficient (k_a); and for ionic reactions, the Onsager length (r_c). Time resolved fluorescence quenching studies on cresyl violet–potassium iodide system in water confirm the validity of the above equation. The values of D and R obtained from the fluorescence quenching studies are in good agreement with the values inferred from other sources, and the value of k_a is reasonable.

I. INTRODUCTION

The rates of rapid bimolecular chemical reactions are limited by the diffusional transport of the reactants. In such fast reactions the rate coefficient (k) is time dependent. Several theoretical models of diffusion controlled reactions have been considered in the literature¹⁻³ and these models vary in complexity. For the simplest model (Smoluchowski⁴), one gets $k(t) = \alpha + \beta t^{-1/2}$, α and β being dependent on the two parameters, mutual diffusion coefficient (D) and encounter distance (R). For a model (Collins and Kimball⁵) considering a finite value of the absolute rate coefficient (k_a), the time-dependent rate coefficient is given by

$$k(t) = a + b \exp(c^2 t) \operatorname{erfc}(ct^{1/2}), \quad (1)$$

a , b , and c are constants which depend upon the model parameters D , R , and k_a for neutral reactants,⁶ and D , R , k_a , and r_c (Onsager length) for ionic reactants.⁷ At "long" times, Eq. (1) approximates to $k(t) = \alpha' + \beta' t^{-1/2}$, a form similar to Smoluchowski's. For a typical set of values of D , R , k_a , and r_c which is relevant to our experimental system of cresyl violet, potassium iodide, and water ($D = 2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $R = 6.5 \text{ \AA}$, $k_a = 2.5 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, and $r_c = -7.2 \text{ \AA}$) the values of $k(t)$ obtained using Eq. (1) and using its long time approximation differ by $> 5\%$ and $> 50\%$ at times < 173 and < 13 ps, respectively. Experimental studies,^{6,8-12} have attempted to verify the validity of the Smoluchowski equation or the "long-time" approximation of Eq. (1) for several types of diffusion limited fluorescence quenching reactions where the quenching mechanism is of short range, such as, in electron transfer. In these attempts time-domain data were used to confirm the time dependence of the rate coefficient but quantitative agreement was poor in several cases. Recently, frequency domain fluorescence quenching studies have successfully verified Eq. (1) for several neutral or neutral/ion systems^{13,14} including complex ones involving proteins.¹⁵ However, the analysis of these systems required an assumed fixed value of k_a .

Cukier¹⁶ presented a solution for the Debye–Smoluchowski equation at low concentration in which, to the order of his calculation, the transient term is absent and the decay is therefore exponential. In subsequent work^{17,18} he used the exponential lifetime τ to discuss the analysis of steady state quenching data. In Ref. 16, Cukier comments that there may be a transient effect which arises at high concentration, but that this transient effect cannot be predicted from the solution of the Debye–Smoluchowski equation. We thus felt it was of interest to test the applicability of Eq. (1) to ionic reactants directly via time resolved measurements. A method has been recently developed¹⁹ for the analysis of quenched fluorescence time-domain data to verify the validity of Eq. (1). In this paper we use this method to analyze the quenched fluorescence decay of cresyl violet-iodide system in water. Equation (1) is found applicable for this cation/anion system and values of R , D , and k_a are obtained. The values of R and D are in good agreement with the expected values, and the value of k_a is reasonable.

II. THEORY

The time-dependent rate coefficient of a diffusion limited reaction involving ionic reactants can be obtained based on the model¹ considered by Smoluchowski (for neutral particles) and Debye (for charged particles). Flannery⁷ obtained an analytical solution of the Debye–Smoluchowski equation for the Collins and Kimball boundary condition (also referred to as radiation boundary condition or reflecting boundary condition) which gives the time-dependent concentration profile $[c(r,t)]$ of the reactant that is in excess. From the concentration profile the time-dependent rate coefficient for the reaction is obtained. The time-dependent rate coefficient is a function of four fundamental parameters of the model (reaction system), namely, the mutual diffusion coefficient (D , $\text{cm}^2 \text{ s}^{-1}$), the reaction distance (R , cm), the absolute rate coefficient (k_a , $\text{cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$), and the Onsager length (r_c , cm):

$$k(t) = a + b \exp(c^2 t) \operatorname{erfc}(ct^{1/2}), \quad (1)$$

where

$$a = \frac{\alpha_R \alpha_T}{\alpha_R + \alpha_T}, \quad (2)$$

$$b = \frac{\alpha_R^2}{\alpha_R + \alpha_T}, \quad (3)$$

$$c = \left(1 + \frac{\alpha_R}{\alpha_T}\right) \frac{D^{1/2}}{R^2} r_c \frac{\exp(r_c/R)}{\exp(r_c/R) - 1}, \quad (4)$$

$$\alpha_R = k_a \exp(-r_c/R), \quad (5)$$

$$\alpha_T = 4\pi D r_c [\exp(r_c/R) - 1]^{-1}, \quad (6)$$

and erfc refers to the complementary error function.

In the context of the fluorescence quenching experiments, D is the sum of the diffusion coefficients of the fluorophore (D_F) and that of the quencher (D_Q), R is the unique distance at which the reaction occurs with an absolute rate coefficient k_a and the Onsager length r_c depends on the charges ($Z_1 e$ and $Z_2 e$) of the reactants, temperature (T) and dielectric constant of the solvent (ϵ):

$$r_c = \frac{Z_1 Z_2 e^2}{4\pi \epsilon_0 \epsilon k_B T}, \quad (7)$$

where k_B is the Boltzmann constant and ϵ_0 is the permittivity of free space. The Flannery's equation for $k(t)$ is applicable in the range $r \gtrsim R$ when $R \gtrsim |r_c|$, where r is the distance between the reactants. This condition imposes a certain restriction on the sizes of the molecules in relation to the charges carried by them for the Flannery equation to be valid.

As $|r_c| \rightarrow 0$, a and b in Eq. (1) are given by Eqs. (8) and (9):

$$a = \frac{k_a}{(1 + k_a/4\pi R D)}, \quad (8)$$

$$b = \frac{k_a}{(1 + 4\pi R D/k_a)}, \quad (9)$$

in which case Eq. (1) is identical to the Collins and Kimball equation for $k(t)$ derived for neutral reactants.⁶

In a fluorescence quenching experiment in which the concentration (C_Q , molecules cm^{-3}) of the quencher is far in excess of that of the fluorophore, the fluorescence decay function of an instantaneously created excited state is given by Eq. (10):

$$I(t) = A \exp\left[-\left(\tau_0^{-1} + \int_0^t k(t') C_Q dt'\right)\right]. \quad (10)$$

A in Eq. (10) is the initial intensity and τ_0 is the lifetime in the absence of the quencher. Substituting Eq. (1) for $k(t')$ in Eq. (10) and changing the variable t' to x ($= ct'^{1/2}$), one gets

$$I(t) = A \exp\left[-(\tau_0^{-1} + p)t - \frac{2q}{c^2} \int_0^{ct^{1/2}} x \exp(x^2) \operatorname{erfc}(x) dx\right], \quad (11)$$

where

$$p = a C_Q \quad (12)$$

and

$$q = b C_Q. \quad (13)$$

Experimental data of quenched fluorescence can be used to verify the validity of Eq. (11). If Eq. (11) fits the data, the values of a , b , and c obtained, are then used to calculate the fundamental parameters of the model namely, R , D , and k_a as outlined below.

For ionic reactants the value of R is obtained by solving Eq. (14) using Eq. (7). Equation (14) is obtained by using Eqs. (2)–(4) and (6):

$$\{R^4 [\exp(r_c/R) - 1]\} / [r_c \exp(2r_c/R)] = [a^2(1 + b/a)^3] / (4\pi b c^2). \quad (14)$$

With R determined, D and k_a are obtained from Eqs. (15) and (16):

$$D = \{a(a + b) [\exp(r_c/R) - 1]\} / (4\pi r_c b), \quad (15)$$

$$k_a = (a + b) \exp(r_c/R). \quad (16)$$

For neutral reactants, R , D , and k_a are obtained using Eqs. (17)–(19):

$$R^3 = [a^2(1 + b/a)^3] / (4\pi b c^2), \quad (17)$$

$$D = [a(a + b)] / (4\pi R b), \quad (18)$$

$$k_a = a + b. \quad (19)$$

III. EXPERIMENTAL

The experimental data reported in this paper were obtained using the time-correlated single photon counting (TCSPC) setup which is described elsewhere.²⁰ The entire fluorescence function including the rising edge can be well fit when a microchannel plate photomultiplier (Hamamatsu 1564 U-01) is used.²⁰ Laser grade cresyl violet was obtained from different sources. The material as perchlorate from Exciton Inc. and Aldrich Co. has a fluorescence lifetime of 2.06 ns and an absorption maximum at 585 nm in water and this material will hereafter be called CV585. The material from Lambda Physik (as chloride or perchlorate) has a fluorescence lifetime of 2.34 ns, and an absorption maximum at 590 nm in water and this material will hereafter be called CV590. Both CV585 and CV590 gave fluorescence decays that fitted well for a single exponential function. TLC purification of samples of both CV585 and CV590 did not change their fluorescence lifetimes. The emission spectra of CV585 and CV590 were also different. Apparently CV585 and CV590 are different molecules. The structure²¹ normally attributed to cresyl violet (oxazine 9) is claimed by all suppliers as indicated by the specified molecular weight. Lambda Physik has confirmed²² the structure²¹ for their sample (CV590). It is presumed that CV585 is substitutionally different from cresyl violet. Different fluorescence lifetimes have been reported^{20,23} for cresyl violet. The two values quoted are close to our values. The fluorescence of both CV585 and CV590 are quenched efficiently by iodide ion. The apparent quenching rate constants, k_q obtained by Eq. (20), for CV590 and CV585 are 1.7×10^{10} and $1.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, respectively:

$$k_q = (\tau^{-1} - \tau_0^{-1}) / C_Q. \quad (20)$$

τ is the "lifetime" of the quenched fluorescence decay fitted to a single exponential. The values of k_q for both CV590 and CV585 were larger than the conventional diffusion limit value, k_D ($= 8RT/3000\eta = 7.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ at 25°C). Values of $k_q > k_D$ are expected for diffusion limited reactions of ionic reactants of opposite charges. We have carried out experiments using both the samples. We assume that the structural differences between CV585 and CV590 are minor and hence the values of R and D calculated¹² for CV590 are also valid for CV585.

The details of the analysis of the quenched fluorescence data are described elsewhere.¹⁹ Briefly, the quenched fluorescence decay, $F(t)$ is the convolution [Eq. (21)] of the instrument response function, $R(t)$ and the decay function, $I(t)$ [Eq. (11)]. The iterative reconvolution method²⁴ with parameter adjustment by the Marquart procedure was used to fit $F(t)$ for Eq. (11). The free parameters used in the fit were A , p (ns^{-1}), q (ns^{-1}), and c ($\text{ns}^{-1/2}$). The weighted residuals were subjected to various tests of randomness²⁵⁻²⁷: Calculation of reduced chi-square, Durbin-Watson parameter (DWP), up-down (+/-) runs test parameter, and plots of weighted residuals and autocorrelation functions.

It is essential to take account of time shift (δ) between $R(t)$ and $F(t)$ in the TCSPC experiment.²⁸ The parameters q and c in the transient part of Eq. (11) are sensitive to the value of δ . Analysis of $F(t)$ was carried out for values of δ

ranging from -10 to 10 ps in 1 or 0.5 ps intervals.

Simulated data for quenched fluorescence were created as follows: Noise-free data was computed using Eq. (21):

$$F(t) = \int_0^t R(s)I(t-s)ds, \quad (21)$$

where $R(t)$ is an experimentally measured, numerically smoothed instrument response function and $I(t)$ is given by Eq. (11). The values of the parameters (A , p , q , and c) were chosen to produce a decay profile similar to an experimental one with a peak count of 2×10^4 (or 10^5). Gaussian noise is added to all the data.²⁵ The time range or data range for the analysis of the experimental or simulated data was chosen to include the rising edge and to exclude data beyond 20 counts (or 0.1% of the peak) in the decay portion of the curve.

IV. RESULTS AND DISCUSSION

The fluorescence decay profile of cresyl violet (CV585 and CV590) in water quenched by potassium iodide does not fit to a single exponential function. Shift (δ)-optimized best fits of a typical set of quenched and unquenched fluorescence decays of CV585 are shown in Fig. 1. The quenched fluorescence decay fitted to a single exponential gives a nonrandom distribution of weighted residuals [Fig. 1(E)] and autocorrelation functions. The values of the statistical test parameters indicate that the quenched fluorescence decay is ade-

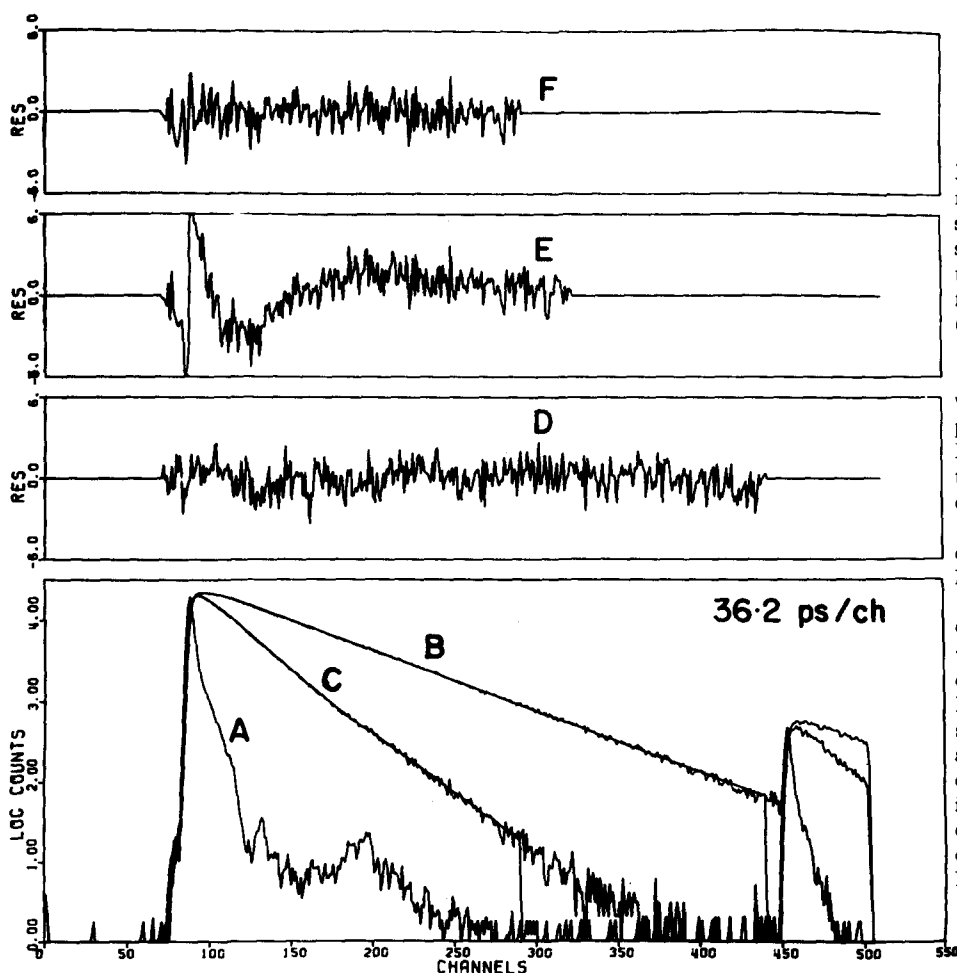


FIG. 1. Curves A, B, and C are the instrument response function [$R(t)$], fluorescence emission of CV585 in water, and fluorescence emission of CV585 quenched by KI (47 mM) in water, respectively. The response function and fluorescence emission were obtained at 590 and 610 nm, respectively. Curve B was fitted ($\delta = -7$ ps) to a single exponential function ($\tau_0 = 2.06$ ns); the random distribution of weighted residuals (curve D) and statistical test parameters (reduced chi-square = 1.13, DWP = 1.70) indicate that the single exponential fit is adequate. The shift-optimized best fit of curve C to a single exponential function ($\delta = -7.2$ ps, $\tau = 0.853$ ns) gives a nonrandom distribution of weighted residuals (curve E) and unacceptable statistical test parameters (reduced chi-square = 4.48 and DWP = 0.58). Curve C is fitted to Eq. (11) (see the text for values of parameters) for $\delta = -4$ ps; the random distribution of weighted residuals (curve F) and statistical test parameters (reduced chi-square = 1.24, DWP = 1.91, Runs test = 115, against an expected value of 116.5 ± 7.6) indicate the adequacy of the fit. Smooth curves passing through curves B and C are plots of calculated emissions for good fit which lead to distributions of residuals shown by curve D and F, respectively.

quately fitted to Eq. (11) as shown [Fig. 1(F)] by the randomness of the weighted residuals. It was found that the data can adequately fit for values of δ in the range, $-5 < \delta < -1$ ps. Although the fit is adequate for δ values in this range, the optimized values of the parameters, especially q and c , strongly depend upon the value of δ . Figure 2 shows the variation of the values of the parameters p , q , and c and the variation of the values of statistical test parameters chi-square and DWP obtained by fitting the quenched fluorescence decay to Eq. (11) for various values of δ . The chi-square was minimum (1.22), the DWP was maximum (1.93), and the Runs test parameter was optimum (119.0, against an expected value of 116.5 ± 7.6) for $\delta = -3$ ps. In this case the optimized values of the parameters p , q , and c were $0.530 \pm 0.005 \text{ ns}^{-1}$, $10.5 \pm 31.7 \text{ ns}^{-1}$, and $37.1 \pm 115 \text{ ns}^{-1/2}$, respectively. For $\delta > -3$ ps the values of parameters q and c sharply increased and had much greater uncertainties. For $\delta < -3$ ps the values and the relative uncertainties for q and c became less. The effect of variation of δ on p and A (not shown in Fig. 2) is marginal. Based on the standard criterion of satisfactory statistical tests of randomness on the weighted residuals, any choice in the range $-5 < \delta < -1$ ps is acceptable. In this range, the parameters p , q , and c are estimated to be $0.524\text{--}0.530 \text{ ns}^{-1}$, $1.96\text{--}23.7 \text{ ns}^{-1}$, and 6.7--

$79.7 \text{ ns}^{-1/2}$, respectively. With such a large range for q and c , it is clearly impossible to verify the validity of the model. This difficulty in data analysis was resolved by comparing the experimental data with a similar analysis of simulated data.

Quenched fluorescence decay data were simulated using an instrument response function with 36.2 ps/ch resolution for the following values of simulation parameters: $p = 0.57 \text{ ns}^{-1}$, $q = 2.58 \text{ ns}^{-1}$, $c = 6.34 \text{ ns}^{-1/2}$, and $\tau_0 = 2.06 \text{ ns}$. The peak count was 2×10^4 and $\delta = 0$. These values were chosen so that the simulated data resembles the fluorescence data for CV585/KI(47 mM) in water. The simulated data were fit to Eq. (11) with $\delta = 0$. The optimized values obtained for the parameters were, $p = 0.582 \pm 0.013 \text{ ns}^{-1}$, $q = 2.41 \pm 0.93 \text{ ns}^{-1}$, and $c = 6.2 \pm 2.8 \text{ ns}^{-1/2}$. The randomness of the weighted residuals were confirmed by the various statistical tests. It is noted that the input values for p , q , and c were recovered fairly accurately, but the uncertainties associated with q and c were fairly high. This uncertainty in the values of the parameters is presumably due to the reduction in the information content of the data by the addition of random noise.

The simulated data was then analyzed by shifting the instrument response function towards $F(t)$ by 1 ps ($\delta = 1$ ps) and this analysis gave the following results: $p = 0.594 \pm 0.006 \text{ ns}^{-1}$, $q = 15.9 \pm 40.3 \text{ ns}^{-1}$, $c = 43.9 \pm 113 \text{ ns}^{-1/2}$, and the distribution of weighted residuals is acceptably random. With $\delta = -1$ ps, the results of the analysis are: $p = 0.565 \pm 0.018 \text{ ns}^{-1}$, $q = 1.37 \pm 0.29 \text{ ns}^{-1}$, $c = 3.13 \pm 0.98 \text{ ns}^{-1/2}$ and the distribution of weighted residuals is acceptably random. These results (for $\delta = 1$ ps and $\delta = -1$ ps) are similar to the results of analysis of experimentally measured quenched fluorescence decay for $\delta = -3$ ps and $\delta = -5$ ps, respectively (see Fig. 2). Figure 3 shows the variation of the statistical test parameters (chi-square and DWP) and the optimized values of the parameters (p , q , and c) for the analysis of the simulated data for various values of δ . The variation of chi-square, DWP, p , q , or c with δ for the simulated data (Fig. 3) is similar to that observed for the experimental data (Fig. 2).

It is clear from the above analysis that a very precise value of δ_{opt} is required to extract meaningful parameters from fits of quenched fluorescence decays of Eq. (11). Published data for conventional²⁸ and microchannel plate²⁹ photomultipliers indicate that δ_{opt} is unlikely to be zero unless excitation and emission wavelengths are identical. Other sources, such as pathlength differences and differences in the image shape between fluorescence and instrument response measurements may also influence the value of δ . The above analysis also shows that δ_{opt} cannot be determined (for 10^4 peak counts and 36.2 ps/ch resolution) on the basis of statistical tests on the weighted residuals. However, the analysis of the simulated data suggested that the sensitivity of the parameters q and c with respect to δ can be used directly to determine δ_{opt} .

With a larger dynamic range and higher time resolution it should be possible to obtain δ_{opt} directly by examining the weighted residuals. Simulated data were created using an instrument response function with a time resolution of 10.6

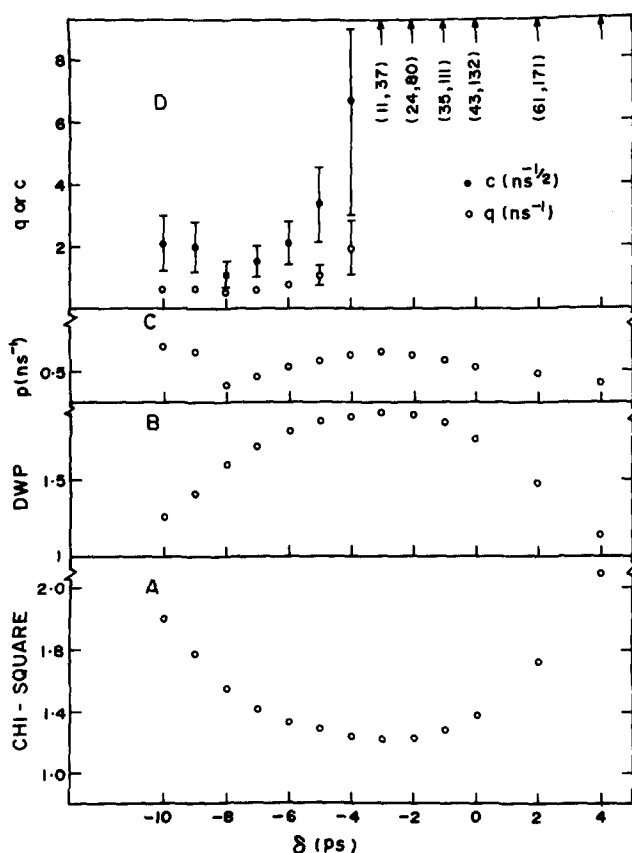


FIG. 2. Results of the fit of Eq. (11) to the quenched fluorescence decay of CV585-KI (47 mM) in water for various values of the time-shift parameter, δ . The plots show variations with δ of reduced chi-square (A), DWP (B), p (ns^{-1}) (C), and q (ns^{-1} , open circles) (D) and c ($\text{ns}^{-1/2}$, filled circles) (D) with δ . The arrows in (D) indicate that the values of q and c are outside the boundary. For these cases the values of q and c ($c > q$) are shown below the arrows.

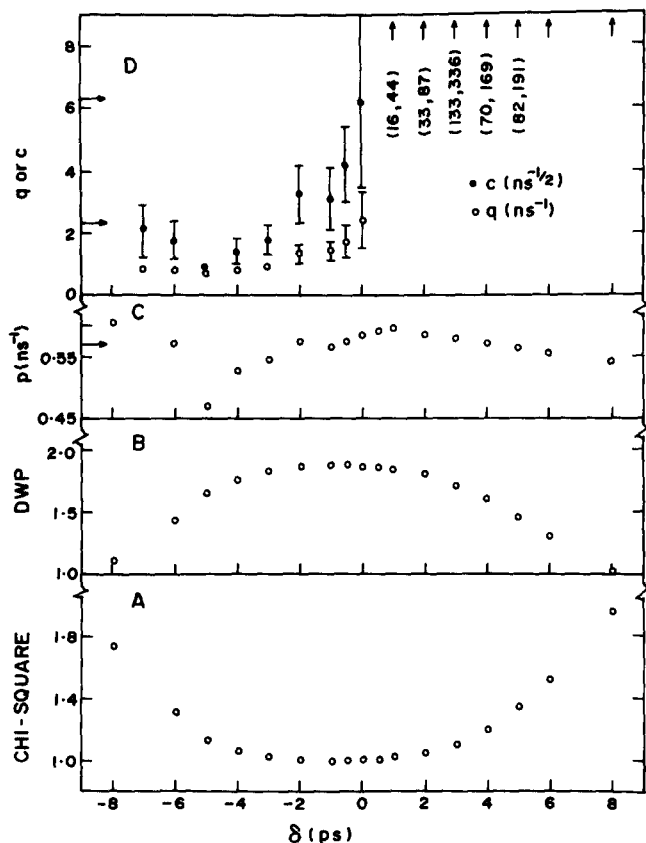


FIG. 3. Results of the fit of Eq. (11) to the simulated data (see the text for details) with a peak count of 2×10^4 and time resolution of 36.2 ps/ch. The plots show variations with δ of reduced chi-square (A), DWP (B), p (ns^{-1}) (C), q (ns^{-1} , open circles) (D), and c ($\text{ns}^{-1/2}$, filled circles) (D). The horizontal arrows in (C) and (D) indicate the input values used for simulation. The vertical arrows in (D) indicate that the values of q and c are outside the boundary. For these cases, the values of q and c ($c > q$) are given below the arrows.

ps/ch (instead of 36.2 ps/ch) for the same simulation parameters: $p = 0.57 \text{ ns}^{-1}$, $q = 2.58 \text{ ns}^{-1}$, and $c = 6.34 \text{ ns}^{-1/2}$. The noise-free peak count was 2×10^4 . The simulated data was analyzed for $\delta = 0$ which gave $p = 0.568 \pm 0.008 \text{ ns}^{-1}$, $q = 2.44 \pm 0.50 \text{ ns}^{-1}$, and $c = 5.89 \pm 1.44 \text{ ns}^{-1/2}$ and the weighted residuals were random. This simulated data was analyzed for various values of δ and the results are plotted in Fig. 4. It is to be noted that the chi-square and DWP variation with δ is steeper compared to that observed for the simulated data with 36.2 ps/ch resolution. Figure 5 shows the analysis of data when the peak count is increased from 2×10^4 to 1×10^5 and the time resolution is kept the same (10.6 ps/ch). The chi-square and DWP values vary steeply with respect to δ . It appears possible, therefore, using experimental data comparable to simulated data to optimize δ_{opt} with an accuracy of less than 0.5 ps solely based on the randomness criterion of weighted residuals. The uncertainties associated with the optimized values of parameters at $\delta = \delta_{\text{opt}}$ are also considerably less. The simulated data (Fig. 5) for $\delta = 0$ gave the following results: $p = 0.57 \text{ ns}^{-1}$, $q = 2.25 \pm 0.1 \text{ ns}^{-1}$, and $c = 6.1 \pm 0.75 \text{ ns}^{-1/2}$. However, the sharp variation of the parameters, especially q and c even in the region $\delta_{\text{opt}} \pm 0.5 \text{ ps}$ could still pose a problem in

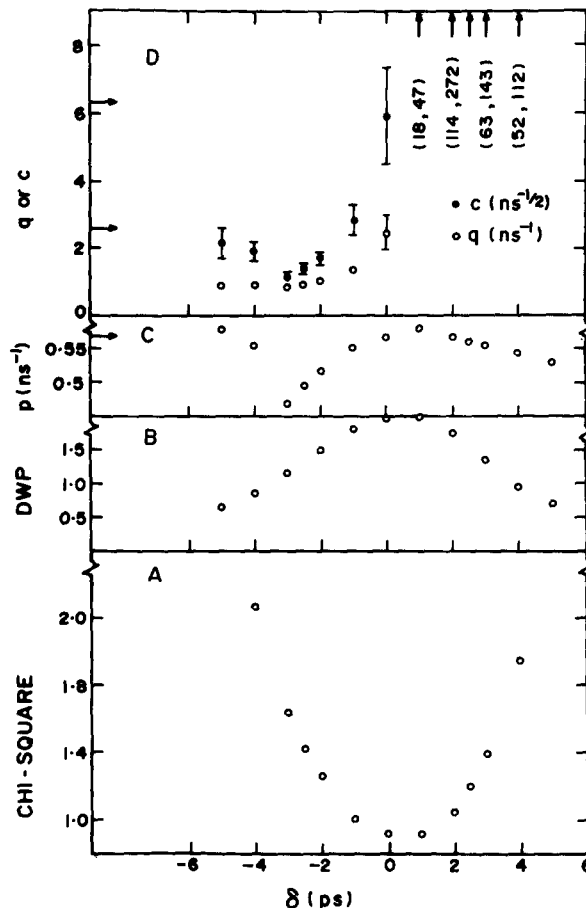


FIG. 4. Results of the fit of Eq. (11) to the simulated data (see the text for details) with a peak count of $\sim 2 \times 10^4$ and time resolution of 10.6 ps/ch. See Fig. 3 for explanations.

choosing δ_{opt} to determine the correct values of the parameters.

We adopt the following procedure for determining δ_{opt} : (i) As a first condition the quenched fluorescence decay fitted to Eq. (11) must generate results (p, q, c , chi-square and DWP) which when plotted as a function of δ exhibit patterns similar to those obtained for simulated data, as in Fig. 3. (ii) δ_{opt} is then chosen as that value of δ for which the uncertainty in the value of c is approximately 50% of c and for $\delta = \delta_{\text{opt}} + 1 \text{ ps}$, the uncertainty in c exceeds the value of c . It is to be noted that the guidelines are set by the results of the simulated data of comparable peak counts and time per channel. Based on these criteria the value of δ_{opt} is found to be -4 ps for the quenched fluorescence data (Fig. 2).

The quenched fluorescence decay of CV585 at other concentrations of KI (12.5, 26.3, and 82.6 mM) were fit to Eq. (11) and the results for each decay followed the patterns similar to those shown in Fig. 2. Values of δ_{opt} were determined as outlined above and the values of parameters p , q , and c were determined for each concentration. The value of δ_{opt} for these concentrations were -1.5 (12.5 mM), -3.0 (26.3 mM), and -3.5 ps (82.6 mM).

The parameters p , q , and c obtained from the analysis of quenched fluorescence decays were plotted against concentration of the quencher (Fig. 6). It is seen that the plots of p vs C_Q and q vs C_Q satisfy the linear relationship predicted by

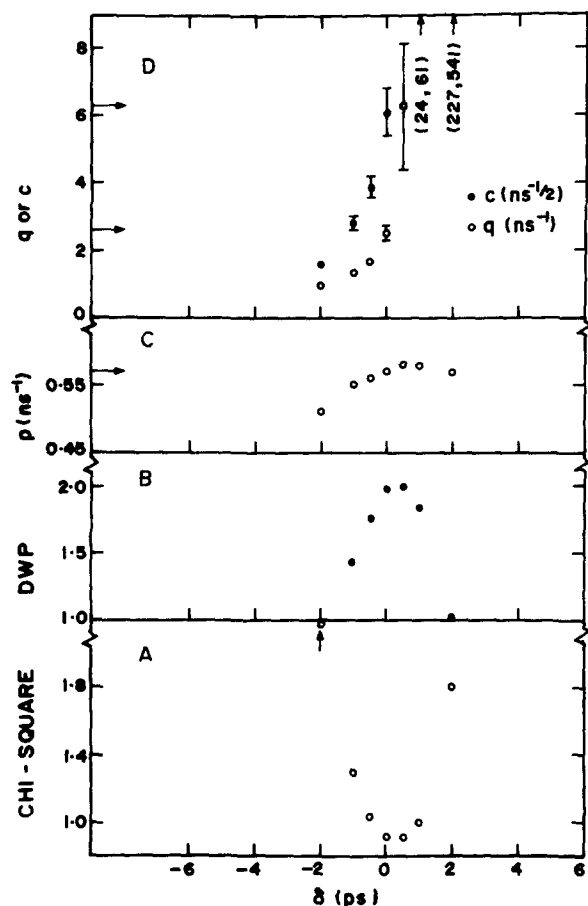


FIG. 5. Results of the fit of Eq. (11) to the simulated data (see the text for details) with a peak count of $\sim 1 \times 10^5$ and time resolution of 10.6 ps/ch. See Fig. 3 for explanations.

Eqs. (12) and (13). The value of c , within the error limits, may be considered concentration independent as expected from the theory. The slope of the p vs C_Q plot gives a value of $a = 1.87 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$. The slope of the q vs C_Q plot gives the value of $b = 5.16 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$. The value of c is in the range of $4\text{--}7 \text{ ns}^{-1/2}$ or $(1.26\text{--}2.2) \times 10^5 \text{ s}^{-1/2}$. Next we consider whether these values of a , b , and c lead to physically realistic values of D , R , and k_a .

R is obtained by solving Eq. (14). The value of r_c is -7.2 \AA for this system in water at 23°C . A graphical solution of Eq. (14) for $c = 4 \text{ ns}^{-1/2}$ (with $R > 0$) gives $R = 2.4 \text{ \AA}$ and $R = 7.35 \text{ \AA}$, of which the lower value was discarded as physically unreasonable. For other values of c , the solutions of Eq. (14) gave the following results: $R = 2.7 \text{ \AA}$ and $R = 6.15 \text{ \AA}$ for $c = 4.5 \text{ ns}^{-1/2}$; $R = 3.6 \text{ \AA}$ and $R = 4.3 \text{ \AA}$ for $c = 5.1 \text{ ns}^{-1/2}$. For $c > 5.2 \text{ ns}^{-1/2}$, Eq. (14) does not have a $R > 0$ solution. Thus, a physically meaningful range, $4.3\text{--}7.4 \text{ \AA}$, is obtained for R . Using Eqs. (16) and (17) the calculated ranges for the values of D and k_a are $1.75 \times 10^{-5} < D < 2.28 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $1.32 \times 10^{-11} < k_a < 2.66 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$, respectively.

The radii of the dye and the quencher (iodide) can be calculated using empirical³⁰ or experimental data.³¹ The radii for cresyl violet cation and iodide ion are calculated to be 3.7^{12} and 2.8 \AA ,³¹ respectively. It is gratifying to note that

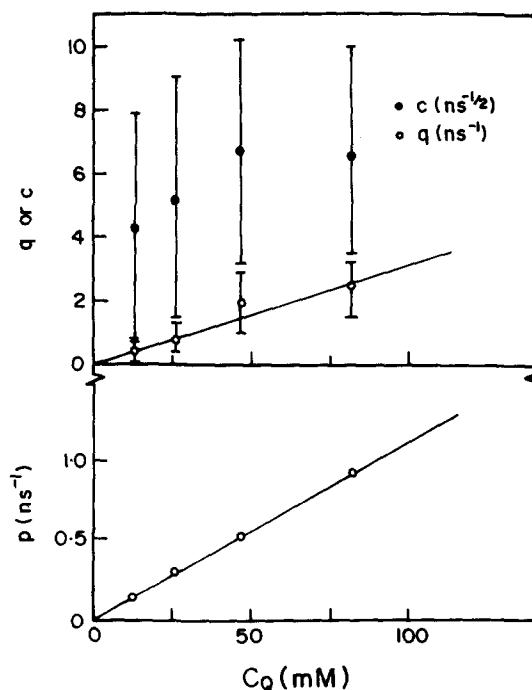


FIG. 6. Plots of the optimized values of $p(\text{ns}^{-1})$, $q(\text{ns}^{-1})$, and $c(\text{ns}^{-1/2})$ against the quencher concentration, C_Q for the CV585-KI system in water.

the range of the value of R ($4.3 < R < 7.4 \text{ \AA}$) obtained above is consistent with the sum of the radii, 6.5 \AA . If we exclude values of $R < 6.5 \text{ \AA}$ as physically unrealistic, the range of values of R becomes $6.5 < R < 7.4 \text{ \AA}$ and the range of values for D and k_a are narrowed down to $1.74 \times 10^{-5} < D < 1.88 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $2.32 \times 10^{-11} < k_a < 2.66 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$, respectively.

The value of D is the sum of the diffusion coefficients of the dye and iodide ion. The following values of diffusion coefficients are based either on an experiment (for iodide) or empirical theory (for cresyl violet): $1.865 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (KI at 25°C),³² $1.909 \pm 0.020 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (KI at 25°C),³³ $1.59 \pm 0.02 \times 10^{-5}$ (I^- at 25°C),³⁴ and $0.65 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (cresyl violet).¹² Using Edward's theory and radius of iodide ion (2.8 \AA) one obtains a value of $1.0 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for the diffusion coefficient of iodide. These values suggest that the sum of the diffusion coefficients of cresyl violet and iodide is in the range of $(1.65\text{--}2.56) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. The value of D obtained using Eq. (15) is in reasonable agreement with the above value, although the experimental values for I^- lead to values of D somewhat larger than obtained from our analysis. The agreement for all values is quite satisfactory considering that modelling the diffusion of cresyl violet by an isotropic constant is unlikely to be very accurate.

The value of k_a cannot be compared with any external data. However, we observe that the value $2.32 \times 10^{-11} < k_a < 2.66 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$ ($1.4 \times 10^{10} < k_a < 1.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$) is internally consistent with the theory of diffusion limited reactions. In a diffusion limited reaction, it is expected that k_a is larger than or comparable to the rate of transport of reactants given by Eq.

(6). This condition is satisfied since the value of α_T is $2.55 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$ ($1.54 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$). The value of k_a is also larger than the steady state (time-independent) rate coefficient, a ($1.87 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$ or $1.13 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$). The quenching process is almost certainly an electron transfer reaction.¹² It would be interesting to see whether the dependence of k_a on solvent and temperature is in accord with Marcus theory.³⁵

Fluorescence quenching experiments were also carried out using CV590. Quenched fluorescence decay data for four quencher (potassium iodide) concentrations were fitted to Eq. (11) and the optimized values of the parameters p , q , and c were obtained by the method described previously. Figure 7 shows the plots of p , q , and c against C_Q . The parameters p , q , and c satisfy Eqs. (12), (13), and (4), respectively. The values obtained for a , b , and c were $1.9 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$, $4.75 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$, and $3.5\text{--}5.0 \text{ ns}^{-1/2}$, respectively. Using the values of a , b , and c , R is calculated to be $4.6 < R < 8.2 \text{ \AA}$. Using a physically realistic limit on R ($6.5 < R < 8.2 \text{ \AA}$), D and k_a are calculated to be $1.72 \times 10^{-5} < D < 1.97 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $2.2 \times 10^{-11} < k_a < 2.76 \times 10^{-11} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$. The values of D and k_a for CV590-iodide system are close to those for the CV585-iodide system.

The values we obtain for R , D , and k_a for the CV585-iodide and CV590-iodide systems in water are realistic. We assume at present, that the structure of CV585 is similar to that of CV590. As noted above, these oxazine dyes cannot be considered to be spherical in shape, as the theory assumes. The magnitude of the error contributed by the nonspherical shape of cresyl violet cannot be easily estimated. However, the rotational dynamics of cresyl violet in pure solvents is adequately described by assuming a spherical shape.³⁶ Hence, we conclude that these two systems may be considered to be reasonable examples of diffusion controlled reac-

tions that are described by the Debye–Smoluchowski model.⁷

V. CONCLUSIONS

Recently, Lakowicz and co-workers^{13–15} have successfully fitted frequency domain data of quenched fluorescence for a number of systems, including proteins, and confirmed the validity of Eq. (1) in all of them. We have demonstrated in this paper that TCSPC experimental data of moderate time resolution (36.2 ps/ch) and peak counts (2×10^4) are adequate to verify the validity of Eq. (1) for ionic reactants. Two systems of ionic reactants (CV585-iodide and CV590-iodide) were studied and for both of them the values of D , R , and k_a obtained from the fluorescence data analysis were reasonable. This is not necessarily expected since dielectric friction³⁷ and dielectric saturation³⁸ in the neighborhood of the ions may significantly alter the diffusion coefficient and the effective radius (via the Onsager length). In contrast to the frequency domain data analysis, assumption of a limiting value for the absolute rate coefficient $k_a = [4\pi R^2 \kappa$, where κ (cm s^{-1}) is the velocity of reaction] is not necessary to obtain D and R in the time-domain data analysis.

The time-domain data are very sensitive to the instrumental timeshift (δ) between instrument response function and fluorescence emission function. The estimate of an accurate value for δ appears to be crucial in the analysis of the quenched fluorescence data. This conclusion is not surprising since the transient term of the rate coefficient will be significant only at early times and any distortion of the data in that time domain, if uncorrected, may lead to erroneous results.

Our finding of nonexponential behavior of quenched fluorescence decay and a good fit to Eq. (11) indicates that the analysis of Cukier^{16–18} does not apply to our experimental system.

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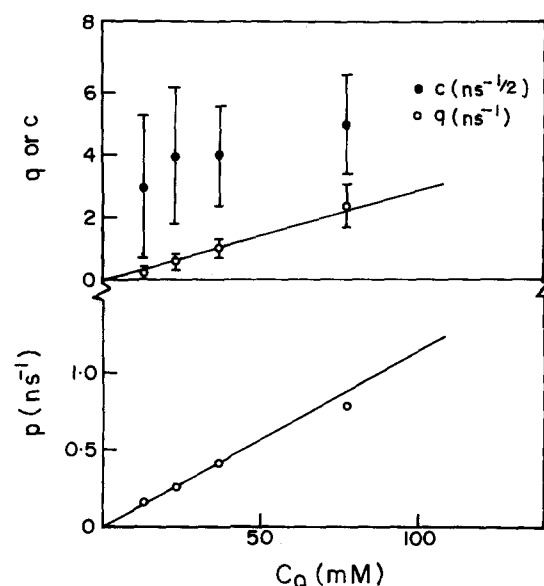


FIG. 7. Plots of the optimized values of p (ns^{-1}), q (ns^{-1}), and c ($\text{ns}^{-1/2}$) against the quencher concentration, C_Q for the CV590-KI system in water.

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