On the Quencher Concentration Dependence of Fluorescence Quenching: The Role of Solution Dielectric Constant and Ionic Strength

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Abstract: A simple calculation of the dependence on quencher concentration of a fluorophore's lifetime τ is presented. Positive deviations from the linear Stern–Volmer relation are predicted and are shown to be due to the correlations among the quenchers when they are nondilute. When the fluorophores and quenchers are ions, the solution dielectric constant and ionic strength can strongly influence the magnitude of the deviations from linear Stern–Volmer behavior. For oppositely charged ions, a solution which is dilute with respect to quencher concentration can appear to be quite concentrated when the dielectric constant is low. When the intrinsic rate (the rate when the fluorophore and quencher are in contact) is not too much faster than the rate of diffusion, a regime can be reached where $1/\tau$ appears to return to linear Stern–Volmer behavior with slope given by the intrinsic rate.

The natural lifetime τ_0 of a fluorophore in solution can be shortened to τ by the presence of quenchers of concentration [Q] according to

$$\frac{1}{\tau} = \frac{1}{\tau_0} + k_f \qquad (1)$$

where k_f is the contribution from quenching. For diffusion-controlled quenching by *dilute* quenchers $k_f = k_D[Q]$ where $k_D = 4\pi Da$ with $D = D_A + D_Q$ the sum of the fluorophore and quencher diffusion coefficients and a is their encounter separation.[1] This expression for k_f and eq 1 lead to the linear Stern–Volmer (SV) intensity law,[2] $I_0/I = 1 + k_D[Q]\tau_0$. If the quenchers are *not* dilute, the fluorophore spatial distribution about a quencher is influenced by the presence of pairs, triplets, ..., of quenchers. Therefore, k_f cannot be obtained by multiplying k_D, the result for one fluorophore and one quencher, by [Q].

In this paper we present a simple treatment of this concentration effect. We consider the case where the fluorophore and quencher may interact by a long-range potential V appropriate to ionic reactants. Then the dielectric constant and ionic strength of the solution are important determinants of the [Q] dependence of $1/\tau$ (as is true for dilute quenchers). Furthermore, we consider diffusion *influenced* reactions where the intrinsic reaction rate k_0—the rate for quenching when the fluorophore and quencher are in contact—is not necessarily much greater than k_D, as required for diffusion control.

Three regimes are identified in this work: First, when $\nu = k_0/k_D$ is small, deviations from linear SV behavior are found to be small. Second, when ν is large (diffusion control) the quencher concentration effects can be dramatic. When the reactants are of opposite charge not only is the dilute quencher rate enhanced but so is the deviation from linear SV behavior. For like charged reactants the dilute rate is reduced and so is the deviation from linear SV behavior. Third, for $\nu \gtrsim 1$ (finite ν) and high quencher concentration, the rate constant approaches the *dilute reaction control* result $k_f = k_0[Q]$. In this case, $1/\tau$ appears to obey a linear

SV law with slope given by the intrinsic rate constant k_0. This last result offers the important possibility of measuring reaction rates which would ordinarily be dominated by diffusion.

Recent calculations[3] of k_f for the nondilute situation have shown a faster increase than linear for k_f. In the specific context of fluorescence quenching several calculations of $1/\tau$ along with comparisons to experiment have appeared.[4-6] Keizer[5] has also shown how to include long-range potential effects in the nondilute case. We recently presented a systematic calculation of the concentration effect which focuses on the long-range potential case.[7] The first correction to the dilute behavior of this calculation is exact, and we find that the simple approximate calculation presented here agrees with the systematic calculation's result.

Quenching Calculation

We introduce the calculation with the dilute, neutral (V short-ranged) case. The fluorophore number density $n(r)$ around a quencher located at the coordinate origin is, at steady state

$$n(r) = \left[1 - \frac{k_0}{k_0 + k_D} \frac{a}{r} \right] n_0 \qquad (2)$$

This result is obtained by solving the diffusion equation with the radiation boundary condition[8]

$$4\pi a^2 D \left. \frac{\partial n}{\partial r} \right|_{r=a} = k_0 n(a) \qquad (3)$$

and the boundary condition $n(r \to \infty) = n_0$, the bulk concentration. The radiation boundary condition states that the diffusion flux of fluorophore into the quencher equals the rate constant for

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reaction when the fluorophore–quencher pair is in contact times the fluorophore concentration at contact. Stated another way, k_0 is the initial (in time) rate constant, before diffusion can occur.

The rate constant k_f is obtained by dividing the fluorophore flux into the quencher $(4\pi Da^2(\partial n/\partial r)_{r=a})$ by n_0 and multiplying by the quencher number density c. (We use c rather than [Q], the molarity, as the concentration unit.) With eq 2 and 3 we obtain the standard result[8] $k_f \equiv k_f^{(0)} = k_0 k_D c/(k_0 + k_D) = k_0 c/(1 + \nu)$ where we define $\nu = k_0/k_D$ and $k_f^{(0)}$ indicates the dilute quencher limit. For $\nu \gg 1$, $k_f \to k_D c$, the diffusion control limit; for $\nu \ll 1$, $k_f \to k_0 c$, the reaction control limit. These limits reflect the rate-limiting step nature of consecutive reactions.

Debye[1,9] showed how to generalize this calculation (for diffusion control $\nu \to \infty$) to any reactant potential V; we use a related procedure which can also be used for nondilute quenchers. It involves transformation to a new space variable[10]

$$\bar{r}(r) = \left[\int_r^\infty \frac{e^{\beta V(x)}}{x^2} \, dx \right]^{-1} \quad (4)$$

and defining $\bar{n} = n \exp(\beta V)$ with $\beta = 1/k_B T$. When this transformation is carried out, the resulting equation has the form of the usual diffusion equation, but with a space-dependent diffusion "constant" $D(r)$. This transformation was used by Flannery to study the time-dependent diffusion equation; we find it of use here. If we assume that $D(r)$ is a constant then the transformed diffusion equation has the solution

$$\bar{n} = \left(1 - \frac{k_0}{k_0 + k_D} \frac{\bar{a}}{\bar{r}} \right) n_0 \quad (5)$$

where $\bar{a} = \bar{r}(r = a)$. The rate coefficient is given by

$$k_f^{(0)} = [4\pi D \bar{a}^2 c \, (\partial \bar{n}/\partial \bar{r})|_{\bar{r}=\bar{a}}]/n_0 = k_0 c/(1 + \nu) \quad (6)$$

where now $\nu = k_0/k_D$ with $k_D = 4\pi D \bar{a}$. In the diffusion-control limit $k_f^{(0)} \to 4\pi D \bar{a} c$ which is Debye's result.[9] The form of $k_f^{(0)}$ is the same as for the short-range V, only the length $a \to \bar{a}$ in the diffusion-step part, k_D.

For nondilute quenchers we hypothesize that $\bar{n}$ satisfies the equation

$$D \bar{\nabla}^2 \delta \bar{n} = k_f \delta \bar{n} \quad (7)$$

where $\delta \bar{n} = \bar{n} - n_0$. When solved with the radiation boundary condition of eq 3 this yields

$$\bar{n}(\bar{r}) = \left[1 - \frac{\nu}{1 + \nu(1 + \lambda \bar{a})} \frac{\bar{a}}{\bar{r}} e^{-\lambda(\bar{r}-\bar{a})} \right] n_0 \quad (8)$$

where $\lambda = (k_f/D)^{1/2}$. Both eq 7 and its solution eq 8 introduce the idea that there is a finite chance that quenching will take place before a specific fluorophore–quencher pair can diffuse together, as becomes more likely for increasing [Q]. Note that $1/\lambda$ is a length that characterizes the quenching power of the medium, relative to the rate of diffusion, that intervenes between the specific fluorophore–quencher pair.[11]

Computing the rate k_f as before, but using eq 8, yields

$$k_f = \frac{k_0 k_D (1 + \lambda \bar{a}) c}{k_0 + k_D (1 + \lambda \bar{a})} \quad (9)$$

With the definitions $\lambda = (k_f/D)^{1/2}$, $\nu = k_0/k_D$, and $\tilde{\phi} = 4\pi \bar{a}^3 c/3$, eq 8 can be written as

$$(\lambda \bar{a})^2 = \frac{3\tilde{\phi}\nu(1 + \lambda \bar{a})}{\nu + 1 + \lambda \bar{a}} \quad (10)$$

The quantity $\tilde{\phi}$ is an *effective* volume fraction and can be large ($\tilde{\phi} \gg 1$) (see the discussion), in contrast to the material volume fraction $\phi = 4\pi a^3 c/3$. Solving eq 10 by iteration for λ yields k_f

from eq 9; the first iteration regains $k_f^{(0)}$ given in eq 6. Using this result in the right hand side of eq 9 yields a k_f which, combined with eq 1, predicts

$$\frac{1}{\tau} = \frac{1}{\tau_0} + k_f^{(0)} \left[1 + \left(\frac{\nu}{1 + \nu} \right)^{3/2} (3\tilde{\phi})^{1/2} \right] \quad (11)$$

This is a positive deviation from linear SV behavior which depends on the effective volume fraction $\tilde{\phi}$.

The iteration of eq 10 can be continued, but of greater interest is the general $\tilde{\phi}$ behavior for different values of ν. First consider $\nu \ll 1$ and $\tilde{\phi}$ such that $\nu\tilde{\phi}$ is also small. Then, from eq 10, λ must be small and k_f remains close to $k_f^{(0)}$, the dilute limit. (This and all our other conclusions are verified by numerical solution of eq 10). In this regime of small ν the fluorophore concentration $\bar{n}$ about a given quencher is close to the bulk concentration (cf. eq 8). Thus, the modification of $\bar{n}$ about a given quencher due to the presence of other quenchers is reduced, each quencher appears to act independently, and $k_f \to k_f^{(0)}$. Baird et al.[4] and we[12] have found this reduction in the concentration effect for the short-range potential case.

A second regime is $\nu \to \infty$, the diffusion-control limit. For large $\tilde{\phi}$, eq 10 predicts $\lambda \bar{a} = 3\tilde{\phi}$ or $k_f = k_D c(3\tilde{\phi})$, a quadratic dependence on quencher concentration. Here, k_f increases monotonously with $\tilde{\phi}$ and the greatest deviations from linear behavior occur. Diffusion control, where $\bar{n}(\bar{r} = \bar{a}) = 0$, maximizes the effect of the deviation of $\bar{n}(r)$ from the bulk value, so we expect the largest deviations from linear behavior.

The third regime is large but finite ν (numerically $\nu > 1$). The large $\tilde{\phi}$ behavior is $k_f/k_f^{(0)} = 1 + \nu$ which, with eq 6, predicts $k_f = k_0 c$. At large $\tilde{\phi}$ we find a return to the reaction-control, dilute result. Here, $\bar{n}$ is once again close to the bulk concentration n_0 and therefore the influence of many quenchers is reduced. A semiquantitative estimate of the relation of $\tilde{\phi}$ to ν in this regime can be obtained as follows. The large $\tilde{\phi}$ prediction from eq 10 is $(\lambda \bar{a})^2 = 3\tilde{\phi}\nu$. Using this in eq 8 evaluated at $\bar{r} = \bar{a}$ yields $\bar{n}(\bar{a}) = [1 - \nu/(1 + \nu + (3\tilde{\phi}\nu)^{1/2})]n_0$. For $(3\tilde{\phi}\nu)^{1/2} \gg \nu \gg 1$, $\bar{n}(\bar{a}) \sim n_0$, the bulk value. The numerical solution of eq 10 bears this out. For $(3\tilde{\phi}\nu)^{1/2} \gg \nu \gtrsim 1$ we find the "saturation" behavior just described. On the other hand, when ν is so large that $(3\tilde{\phi}\nu)^{1/2} \ll \nu$ the diffusion-control regime of k_f increasing with $\tilde{\phi}$ without limit is regained.

Discussion

The deviation from linear SV behavior obtained above depends on the effective volume fraction $\tilde{\phi}$. Since $\tilde{\phi} = (\bar{a}/a)^3 \phi$, even a small material volume fraction ϕ can correspond to a large value of $\tilde{\phi}$ when $\bar{a}/a$ is large. For a Coulomb potential $\beta V = z_A z_Q r_c/r$ ($r_c = \beta e^2/4\pi \epsilon_0 \epsilon_r = 5.606 \times 10^2 \, \text{Å}/\epsilon_r$ with ϵ_r the dielectric constant)

$$\bar{a}/a = -z_A z_Q (r_c/a)/[1 - e^{z_A z_Q r_c/a}] \quad (12)$$

For univalent oppositely charged ions in water ($\epsilon_r = 78.5$) $\bar{a} = 7.13$ Å and in cyclohexane ($\epsilon_r = 2$) $\bar{a} = 280$ Å, so that choosing an encounter distance $a = 7$ Å we have a range from $\bar{a}/a \sim 1$ to ~ 40. With reference to eq 11 for $\nu \to \infty$, a magnification factor of 250 $[(3\tilde{\phi})^{1/2} = (\bar{a}/a)^{3/2} (3\phi)^{1/2} \sim (40)^{3/2} (3\phi)^{1/2}]$ is possible. Thus, a concentration effect leading to nonlinear Stern–Volmer behavior can occur at small quencher concentration. On the other hand, for like charges eq 12 gives $\bar{a}/a \sim (r_c/a) \exp(-r_c/a)$ when $r_c/a \gg 1$; the correction to dilute behavior is exponentially small, and it will appear that there is no concentration effect. Both enhancement and suppression are well-known for the dilute case,[1] and it is not surprising that such effects also arise for the nondilute quencher case.

In evaluating $\bar{a}$ for charged reactants we must also consider the solution's ionic strength, I. Control (or at least an accounting) of I as [Q] is varied to obtain a Stern–Volmer plot is recognized[13] to be important to the correct interpretation of quenching experiments for dilute quenchers—the same care must be exercised

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for nondilute quenchers. Using the Debye–Hückel potential $\beta V_{DH} = z_A z_Q (r_c/r) e^{-\kappa r}$ ($\kappa^2 = (2e^2 N_0 d\beta/1000\epsilon_0\epsilon_r) I$, with N_0 Avagadro's number and d the solution density) in eq 4 leads to $\tilde{a}_{DH}/a = (r_c/a) \exp[-(r_c/a)(1 - \kappa a)]$ for like charged univalent ions with $r_c/a \gg 1$; thus, the concentration effect is enhanced by increasing ionic strength. For oppositely charged univalent ions, $\tilde{a}_{DH}/a = (r_c/a) \exp(-\kappa r_c)$ and the concentration effect will be reduced relative to the zero ion strength result.[14] Both effects are present in the dilute case too, of course, and are due to the weakening of charge interactions by the Debye–Hückel screening. In room temperature water, for $I = 0.01$ m $\kappa = 3.3 \times 10^6$ cm^{-1} and $\kappa r_c = 0.233$; for a lower dielectric constant solvent (or more highly charged ions) κr_c can be larger with a correspondingly greater effect.

In summary, $1/\tau$ depends on $\tilde{\phi}$ and $\tilde{\phi}$ depends on ϵ_r and I; they must be properly accounted for when interpreting fluorescence quenching experiments.

The systematic calculation that we presented before[7] is restricted to the diffusion-controlled regime. We found that the result given

in eq 11 (for $\nu \to \infty$) is the first correction to the dilute behavior; thus the approximate method given here yields the exact first correction. At higher concentration our previous calculation is also approximate. It yields the same general behavior as found here, in particular, both calculations predict $k_f \sim (k_D c) \tilde{\phi}$ for large $\tilde{\phi}$, but have different coefficients of proportionality.

We expect that the conclusions reached here for high $\tilde{\phi}$ values are qualitatively correct, but they should not be viewed as quantitative predictions. The possibility of a return to reaction control by increasing $\tilde{\phi}$ is especially intriguing. If this regime is accessible (oppositely charged ions in low ϵ_r and I solutions) then the loss of chemical information that is a result of the diffusion-control limit can be bypassed. For example, electron-transfer reactions are difficult to study due to their rapidity (k_0, the actual transfer step is often large compared with k_D, the rate for diffusion). These reactions often are between inorganic ions of opposite charge and may become accessible by going to high quencher concentration.

(14) To obtain these simple connections between $\tilde{a}$ for Coulomb and Debye–Hückel potentials κr_c must be small. See, for example, ref 1, p 167.

Electronic Energy Levels in Long Polyenes: S₂ → S₀ Emission in *all-trans*-1,3,5,7,9,11,13-Tetradecaheptaene

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Abstract: Absorption, fluorescence, and fluorescence excitation spectra of *all-trans*-1,3,5,7,9,11,13-tetradecaheptaene have been obtained in room-temperature solutions and 77 K glasses. The heptaene, unlike shorter fluorescent polyenes, does not exhibit the characteristic gap between the origins of absorption ($1^1A_g^- \to 1^1B_u^+$) and emission. Excitation spectra and solvent shift studies lead to the assignment of two distinct emissions, $S_2 \to S_0$ ($1^1B_u^+ \to 1^1A_g^-$) and $S_1 \to S_0$ ($2^1A_g^- \to 1^1A_g^-$), with the ratio of S_2 to S_1 emission increasing with the S_2–S_1 energy gap. Extrapolation of room-temperature solution data gives a gas-phase S_2–S_1 difference of 8400 cm^{-1}. Tetradecaheptaene's "anomalous" $S_2 \to S_0$ emission, the first observed for a linearly conjugated molecule, is compared to similar violations of Kasha's rule by azulene and other polyarenes. The spectroscopic and photochemical implications of these findings for other long polyenes also are discussed.

I. Introduction

Recent research on linear polyenes ranges from theoretical investigations of the electronic states of model compounds to experiments which probe the initial photochemical events in vision and photosynthesis.[1,2] The optical spectroscopy of simple, unsubstituted polyene hydrocarbons has provided a fertile starting point for these other studies.[3] The model polyenes, both in 4.2 K mixed crystals and 77 K glasses, exhibit well-resolved (and thus informative) vibronic spectra. These spectra have established excited-state symmetries and have furnished some details of excited-state structures.

Previous work on unsubstituted all-trans polyenes

for *n* = 2-4
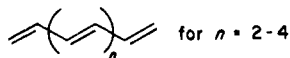
has shown that the lowest excited singlet state ($2^1A_g^-$) diverges

from the second excited state ($1^1B_u^+$) with increasing polyene length.[4] This behavior is not accounted for by current theories and raises the question of $2^1A_g^-$'s position in the long (i.e., "infinite") polyene limit. Does $2^1A_g^-$ (like $1^1B_u^+$) converge to a finite energy gap with respect to the electronic ground state ($1^1A_g^-$)? The experimental answer to this question poses an interesting challenge to theory and is closely related to such issues as the extent of bond alternation and the electronic properties of long polyene chains.[5] Extrapolation of our previous work to longer polyenes also could lead to the location and assignment of the electronic states of polyene natural products such as β-carotene ($n = 9$). The position of β-carotene's low-energy electronic states is critical to an understanding of energy-transfer processes in photosynthesis.[6] Previous reports[7] of a low-lying $2^1A_g^-$ state in

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