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Nonlinear Fluorescence Quenching and the Origin of Positive Curvature in Stern–Volmer Plots

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Abstract: A generalization of the Smoluchowski theory of diffusion-controlled reactions is used to explain the anomalous increase in the quenching of fluorescence which appears as positive curvature in Stern–Volmer plots. The present explanation is based on the increase in nearest neighbors which occurs when either the fluorescence lifetime or bimolecular lifetime of the fluorophore is short. The calculations depend only on the fluorescence lifetime, an encounter radius, diffusion constants of the fluorophore and the quencher, and the intrinsic reactivity. Data obtained by three different experimental groups are examined, and in each case the theory and experiment are in good agreement up to quencher concentrations on the order of 0.1 M.

I. Introduction

At low concentrations the quenching of fluorescence by an acceptor molecule in solution follows the classic Stern–Volmer relationship[1]

$$I_0/I = 1 + k_Q[Q] \tag{1}$$

where k_Q is called the quenching constant, I is the steady-state intensity of fluorescence at the quencher concentration [Q], and I_0 is its value without quencher. The Stern–Volmer equation predicts a linear dependence of I_0/I on quencher concentration, at least if the quenching constant is a true constant. The existence and magnitude of the quenching constant, however, can be understood in terms of the competing processes of fluorescence and quenching, namely,

$$A \xrightarrow{h\nu} A^* \tag{2}$$

$$A^* \xrightarrow{\tau_0^{-1}} A \tag{3}$$

$$A^* + Q \xrightarrow{k^{\text{obsd}}} A + Q \tag{4}$$

where τ_0 is the fluorescence lifetime, and k^{obsd} is the rate constant for the quenching reaction. Using the fact that I is proportional to the steady-state concentration of A, gives

$$k_Q = k^{\text{obsd}}\tau_0 \tag{5}$$

Since the fluorescence lifetime is independent of the quencher concentration, Stern–Volmer plots will be linear as long as k^{obsd} is independent of quencher.

It has been known for many years that certain quenching reactions lead to curved Stern–Volmer plots.[2,3] Both positive curvature and negative curvature have been observed.[4-6] Negative curvature involves a decrease in k_Q and is associated with a change in the absorption and fluorescence spectrum of the fluorophore.[5] Deviations from the Stern–Volmer equation in such reactions have been explained by the existence of multiple fluorescing states[4] or by a compound formation.[5] On the other hand, a variety of quenching reactions have been reported which exhibit positive curvature in Stern–Volmer plots, yet which show no evidence of multiple excited states or molecular association. For example, the oxygen quenching of perylene in dodecane[7] shows a large positive curvature, even though there is no detectable change in the absorption spectrum of perylene up to oxygen concentrations of 1 M.

Several explanations of positive derivations from Stern–Volmer behavior have been advanced. The one most commonly used is that a "static" quenching mechanism is important at higher quencher concentrations. The static quenching mechanism is a slight variation on the theme of molecular association. According to Frank and Wawilow[3] it involves interactions with quenchers that are within a sphere of action of the fluorophore at the moment of excitation. However, the quenching of anthracene fluorescence by oxygen or sulfur dioxide[6] leads to a sphere of action with a radius of 30 Å, which is at odds with the fact that these quenching reactions are collisional rather than resonant. Consequently, Bowen and Metcalf[6] gave another interpretation of static quenching involving the quencher–fluorophore pairs that "are in juxtaposition at any instant". These pairs appear in the Bowen–Metcalf scheme as if they were molecularly associated with an equilibrium constant K. This type of association leads to the expression

$$I_0/I = (1 + K[Q])(1 + k^{\text{obsd}}\tau_0[Q]) \tag{6}$$

A third kind of explanation of positive curvature is due to Noyes[8-10] and, independently, Weller.[11] Their work is based on the assumption that the quenching reactions are rapid, and so are under diffusion control. Using the Smoluchowski theory they calculate a dependence of k^{obsd} on quencher concentration arising from time-dependent effects. This theory depends only on an encounter radius and a mutual diffusion constant, the same parameters used to explain the low quencher concentration experiments. Nonetheless, this theory is not in good agreement with experiment. To our mind, this is not surprising, since the experimental data in question were obtained under steady-state conditions which eliminate the transient effects assumed in the theory.

In this paper we offer an explanation of the positive curvature in Stern–Volmer plots based on a generalization of Smoluchowski's theory of diffusion effects on reactions in solution.[12,13] The calculation uses results obtained from mechanistic nonequilibrium statistical thermodynamics[14-16] and is based on the stationary nonequilibrium distribution of quencher molecules around an average fluorophore. In the standard Smoluchowski treatment,[10] the lifetime of the molecular sink is taken as infinite, which is clearly inappropriate when the lifetime of the fluorophore is short.

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(1) N. J. Turro, "Modern Molecular Photochemistry", W. A. Benjamin, New York, 1978.

(2) E. R. Jette and W. West, *Proc. R. Soc. London Ser. A*, **121**, 299–312 (1928).

(3) I. M. Frank and S. I. Wawilow, *Z. Phys.*, **69**, 100–110 (1931).

(4) G. K. Rollefson and H. Boaz, *J. Phys. Colloid Chem.*, **52**, 518–527 (1948).

(5) H. Boaz and G. K. Rollefson, *J. Am. Chem. Soc.*, **72**, 3435–3443 (1950).

(6) E. J. Bowen and W. S. Metcalf, *Proc. R. Soc. London Ser. A*, **206**, 437–447 (1951).

(7) J. R. Lakowicz and G. Weber, *Biochemistry*, **12**, 4161–4170 (1973).

(8) R. M. Noyes, *J. Am. Chem. Soc.*, **79**, 551–555 (1957).

(9) R. M. Noyes, *J. Phys. Chem.* **65**, 763–765 (1961).

(10) R. M. Noyes, *Prog. React. Kinet.*, **1**, 131–160 (1961).

(11) A. Weller, *Z. Phys. Chem.*, **13**, 335–352 (1957).

(12) J. Keizer, *J. Phys. Chem.*, **85**, 940–941 (1981) [see Errata, *Ibid.*, **85**, 2862 (1981)].

(13) J. Keizer, *J. Phys. Chem.*, **86**, 5052–5067 (1982).

(14) J. Keizer, *J. Chem. Phys.*, **64**, 1679–1687 (1976).

(15) J. Keizer, *J. Chem. Phys.*, **65**, 4431–4444 (1976).

(16) J. Keizer, *J. Chem. Phys.*, **69**, 2609–2618 (1978).

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As we explain in the following sections, these lifetime effects are included in the present theory and give rise to a quantitative explanation of fluorescence quenching even at high concentration of quencher molecules.

II. Effect of Diffusion on Fluorescence

The fluorescence quenching mechanism written in eq 2–4 seems to be independent of diffusion. Nonetheless, it is well known that diffusion can limit the rate of fluorescence quenching by controlling the encounter rate of the fluorophore and the quencher. The classical explanation of this effect is due to Smoluchowski[17] and is based on a picture of quencher molecules reacting with a stationary fluorophore sink. Recently we have developed a theory of diffusion effects on solution-phase reactions[12,13,18] which uses the mechanistic theory of nonequilibrium statistical thermodynamics to calculate bimolecular rate constants. For simple reactivity models the results of that theory reduce to the Smoluchowski theory in dilute solution. At high concentrations of reactants several effects are predicted, including an increase in the rate constant, k^{obsd}, in eq 4.

To assess the effect of diffusion on reaction rates it is necessary to calculate the radial dependence of the concentration of one reactant species around a central reactant of the other species. Although the choice of the central species is immaterial, because its concentration will be small, we chose to locate the fluorophore at the center and to determine the radial distribution of quencher molecules. The concentration of quencher at a radial distance r will be

$$c(r) = [\mathrm{Q}]g_{\mathrm{A^*Q}}(r) \tag{7}$$

where $g_{\mathrm{A^*Q}}(r)$ is the radial distribution function[19] of the quencher around an average excited fluorophore molecule and [Q] is the bulk molar concentration of Q. The radial distribution function is said to measure "correlations" between A* and Q because it is related to a measure of their statistical dependence on one another. Indeed, the correlation function of their concentrations is given by[20]

$$\langle([\mathrm{A}^*](\mathbf{r}) - [\mathrm{A}^*])([\mathrm{Q}](\mathbf{r}') - [\mathrm{Q}])\rangle = [\mathrm{A}^*][\mathrm{Q}](g_{\mathrm{A^*Q}}(|\mathbf{r} - \mathbf{r}'|) - 1)$$

where the angular brackets represent an ensemble average and, for example, $[\mathrm{A}^*](\mathbf{r})$ is the actual molar concentration of A* at $\mathbf{r}$. If A* and Q are statistically independent of one another, then $g_{\mathrm{A^*Q}} = 1$, the correlation function vanishes, and A* and Q are said to be "uncorrelated". This occurs, for example, for uncharged molecules in dilute solution at equilibrium.

Correlations between molecules can develop for several reasons. It is well known, for example, that charged molecules are correlated by their electrostatic interaction. In dilute solution this is described in the Debye–Hückel theory by a radial distribution function of the form[21]

$$g(r) = (B/r)\exp(-\kappa r)$$

where κ^{-1}, the Debye correlation length, measures the thickness of the ion atmosphere. Correlations can also develop at nonequilibrium stationary states between molecules that are uncorrelated at equilibrium. This phenomenon is quite general[15,16] and provides a key to understanding the effect of diffusion on rapid reactions in solution.

The radial distribution function for steady-state illumination of the quenching reactions in eq 3 and 4 has been calculated in earlier work.[12,13] For the special conditions of dilute solution of fluorophore, a great excess of quencher, and neglect of intermolecular interaction, one finds that

$$g_{\mathrm{A^*Q}}(r) = 1 - \frac{(k^{\text{obsd}}/N')}{4\pi D'r}\left[\frac{2\beta}{2\beta-\alpha}\exp(-r\beta^{1/2}) - \frac{\alpha}{2\beta-\alpha}\exp(-r(\alpha/2)^{1/2})\right] \tag{8}$$

where $\alpha = (\tau_0^{-1} + k^{\text{obsd}}[\mathrm{Q}])/D_{\mathrm{A^*}}$, $\beta = (\tau_0^{-1} + k^{\text{obsd}}[\mathrm{Q}])/D'$, $N' = 6.023 \times 10^{20}$ converts the units to moles per liter, $D' = D_{\mathrm{A^*}} + D_{\mathrm{Q}}$, and $D_{\mathrm{A^*}}$ and D_{Q} are the diffusion constants of A* and Q. There are two correlation lengths in eq 8, namely,

$$(\alpha/2)^{-1/2} = [2D_{\mathrm{A^*}}/(\tau_0^{-1} + k^{\text{obsd}}[\mathrm{Q}])]^{1/2} \tag{9}$$

and

$$\beta^{-1/2} = [D'/(\tau_0^{-1} + k^{\text{obsd}}[\mathrm{Q}])]^{1/2} \tag{10}$$

These lengths have an interesting physical interpretation. Recall first that $k^{\text{obsd}}[\mathrm{Q}]$ is the pseudo-first-order rate of quenching of A* fluorescence. Thus $\tau_0^{-1} + k^{\text{obsd}}[\mathrm{Q}]$ is the complete first-order rate constant for loss of A*. Consequently its inverse, $(\tau_0^{-1} + k^{\text{obsd}}[\mathrm{Q}])^{-1}$, in eq 9 and 10 equals the average lifetime $\tau_{\mathrm{A^*}}$ of a fluorophore molecule. Finally we recall that the root-mean-square displacement due to Brownian motion in a time $\tau_{\mathrm{A^*}}$ is $(6D\tau_{\mathrm{A^*}})^{1/2}$. Thus the correlation lengths in eq 9 and 10 measure the root-mean-square displacement of A* and the root-mean-square relative displacement of A* and Q during the lifetime of a fluorophore molecule.

Notice that if the fluorescence lifetime is infinite, the two correlation lengths increase in dilute solution like $[\mathrm{Q}]^{-1/2}$, which happens to be identical with the dependence of the Debye length on concentration.[21] Thus for an infinite lifetime the limit of dilute solution gives $\alpha = \beta = 0$, and eq 8 reduces to the usual Smoluchowski result[10]

$$g_{\mathrm{A^*Q}}(r) = 1 - \frac{(k^{\text{obsd}}/N')}{4\pi D'r} \tag{11}$$

However, fluorescence lifetimes of organic molecules are often the order of 1 to 100 n,[1] *and so eq 8 must be used even in dilute solution.*

By comparing eq 8 and 11 it is seen that the effect of the correlation lengths is to increase the magnitude of the radial distribution function with respect to that given by the Smoluchowski theory. According to eq 7 that will increase the local concentration of quencher molecules which, in turn, increases the apparent reaction rate. This effect gives rise to a quencher concentration dependence of the observed rate constant. Said in another way: as the quencher concentration is increased, the bimolecular lifetime, $1/k^{\text{obsd}}[\mathrm{Q}]$, is decreased. Consequently, there is a decrease in the number of quencher molecules that are eliminated from the immediate neighborhood of average fluorophore during its lifetime. This increases the concentration of quenchers about the average fluorophore, and so increases the observed rate constant.

This effect can be quantitatively determined by introducing the intrinsic bimolecular reactivity,[12] $k^\circ(r)$. This function gives the dependence of the reaction rate constant on the distance separating the reactants.[13] Using it the observed bimolecular rate constant is given by

$$k^{\text{obsd}} = N'\int k^\circ(r)g_{\mathrm{A^*Q}}(r)\,\mathrm{d}\mathbf{r} \tag{12}$$

The simplest reactivity function corresponds to reaction with a rate constant k° at an encounter radius R. It has the form[12,13]

$$k^\circ(r) = k^\circ\delta(r - R)/4\pi r^2 \tag{13}$$

If the Smoluchowski radial distribution of eq 11 is used in conjunction with this reactivity, eq 12 yields the expression

$$k^{\text{obsd}} = \frac{4\pi D'Rk^\circ N'}{4\pi D'R + k^\circ} \tag{14}$$

This is the familiar result obtained from Smoluchowski's theory

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[17] M. V. Smoluchowski, *Z. Phys. Chem.*, **92**, 129–168 (1917).

[18] U. R. Steiger and J. Keizer, *J. Chem. Phys.*, **77**, 777–788 (1982).

[19] T. L. Hill, "Statistical Mechanics", McGraw-Hill, New York, 1956, Chapter 6.

[20] D. McQuarrie, "Statistical Mechanics", Harper and Row, New York, 1976, Chapter 13.

[21] W. J. Moore, "Physical Chemistry", 4th ed., Prentice-Hall, New York, 1972, pp 452.

using the so-called radiation boundary condition.[10]

To analyze data on fluorescence quenching we have chosen, for simplicity, the reactivity function in eq 13. Combining this with the radial distribution function eq 8 and rearranging, then gives

$$k^{\text{obsd}} = \frac{4\pi D'Rk^{\circ}N'C(R)}{4\pi D'RC(R) + k^{\circ}} \tag{15}$$

where

$$C(R) = \left[\frac{2D_{\text{A}^*}}{D_{\text{A}^*} - D_{\text{Q}}}\exp(-R\beta^{1/2}) - \frac{D'}{D_{\text{A}^*} - D_{\text{Q}}}\exp(-R(\alpha/2)^{1/2})\right]^{-1} \tag{16}$$

The function $C(R)$ corrects the Smoluchowski theory for fluorescence and bimolecular lifetime effects. Because α and β depend of the value of k^{obsd}, eq 16 is an implicit equation for the bimolecular quenching constant. Nonetheless, it is easily solved by iteration on a hand calculator.

In the special case that the intrinsic bimolecular reactivity k° is much greater than $4\pi D'R$, eq 15 reduces to

$$k^{\text{obsd}} = 4\pi D'RN'C(R) \tag{17}$$

This is the limit of diffusion-controlled quenching and, again, involves lifetime corrections to the usual Smoluchowski result.

Finally, we notice that the diffusion constants of A* and Q appear separately in eq 15–17. This shows that for fluorescence quenching the absolute, as well as the relative, Brownian motion of A* and Q is important. As Noyes has pointed out,[10] this effect is missing in the Smoluchowski theory which includes correlated motions that are not actually present. The appearance of the absolute diffusion constants in the present theory eliminates these correlations and, thus, increases the observed rate constant over the Smoluchowski value.

III. Effect of Quencher Concentration on Fluorescence

Based on the analysis of the previous sections, it is expected that the quenching constant will depend on quencher concentration through k^{obsd}. As we shall see, for an appreciable effect k^{obsd} must be close to its diffusion-controlled value given in eq 17. Furthermore, unless the correlation lengths are within a factor of 10 of the encounter distance, R, the correction factor $C(R)$ is essentially 1. It is possible to estimate the quencher concentration, $[\text{Q}]_{\text{min}}$, for which quencher effects on the correlation lengths first become appreciable. Using the approximate expression $k^{\text{obsd}} \approx 4\pi D'R$, together with the criterion $\beta_{\text{min}}^{-1/2} \approx 10R$ and eq 10, gives

$$[\text{Q}]_{\text{min}} \approx 1/400\pi R^3\text{N}' \tag{18}$$

For an encounter radius of $R = 5$ Å, eq 18 gives $[\text{Q}]_{\text{min}} \approx 0.01$ M. This is typical of concentrations for which positive deviations from Stern-Volmer plots first appear.[3-7]

To compare more carefully with experiment we have calculated k^{obsd}, and thence the quenching constant k_{Q} in eq 5, or the relative fluorescence intensity in eq 1, for five quenching reactions. These reactions were chosen because independent data (or at least estimates) of diffusion constants, lifetimes, and encounter radii were available. With the exception of the quenching of tryptophan fluorescence by oxygen in aqueous solution, the Stern–Volmer plots of all five reactions are described rather well by eq 17, and thus are diffusion controlled.

The quenching of quinine fluorescence by iodide in aqueous solution is shown in Figure 1. The line represents the results of eq 17 and the circles are from the early experimental work of Jette and West[2], published in 1928. For the lifetime of quinine we have used the modern value,[22] $\tau_0 = 1.9 \times 10^{-8}$ s rather than the larger value measured by Perrin.[3] The diffusion constants are based on data given by Frank and Wawilow[3] for the Nernst–Einstein formula and are $D' = 1.6 \times 10^{-5}$ cm^2 s^{-1} and $D_{\text{I}^-} = 1.2 \times 10^{-5}$ cm^2 s^{-1}. The value used for the encounter radius is $R = 6.6$ Å, which is somewhat smaller than the sum of the hydrodynamic radii, but quite plausible. At the highest concentrations of iodide in Figure 1, the correlation lengths are calculated to be $(\alpha/2)^{-1/2} = 7.5$ Å and $\beta^{-1/2} = 10.7$ Å. At zero concentration of quencher they are $(\alpha/2)^{-1/2} = 39.0$ Å and $\beta^{-1/2} = 55.1$ Å. These short correlation lengths at low concentration are due to the fluorescence lifetime of quinine and yield a value for k^{obsd} of 1.0×10^{10} M^{-1} s^{-1}. This should be compared with the value of $k^{\text{obsd}} = 0.80 \times 10^{10}$ M^{-1} s^{-1} given by the Smoluchowski expression. Thus even in dilute solution, the lifetime correction to the Smoluchowski theory is 25% for this reaction. At 0.05 M one finds $k^{\text{obsd}} = 2.7 \times 10^9$ M^{-1} s^{-1}, so that the Smoluchowski theory is in error by a factor 3.4. The Smoluchowski prediction for I_0/I is shown by the dashed line in Figure 1.

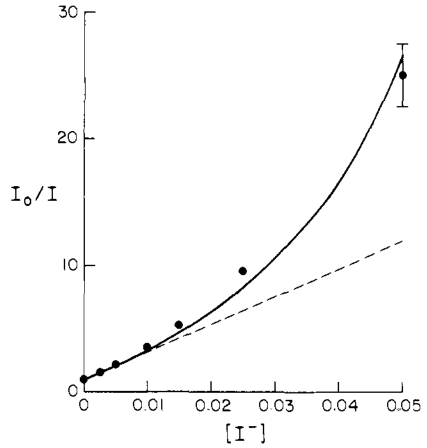

Figure 1. Stern–Volmer plot for the quenching of quinine sulfate by iodide. The circles represent experimental data from ref 2 and the full line is calculated from eq 17 using parameter values given in the text. The experimental value at 0.05 M has an estimated error of ±10%, as shown by the error bar. The dashed line gives the prediction based on the Smoluchowski equation, eq 14.

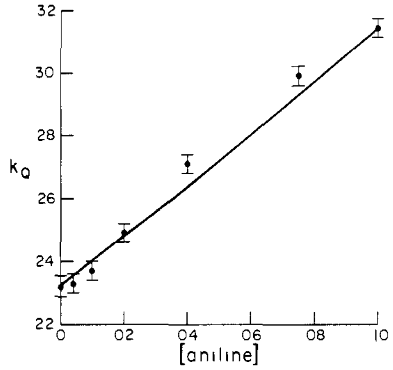

Figure 2. The quenching constant for aniline quenching of uranin fluorescence. The circles represent experimental data from ref 23, and the line is calculated from eq 17 using parameter values given in the text.

In Figure 2 we compare eq 17 with the data of Williamson and La Mer on the quenching of uranin by aniline in aqueous solution.[23] The line is based on eq 17 using $\tau_0 = 4.5 \times 10^{-9}$ s, $D' = 1.34 \times 10^{-5}$ cm^2 s^{-1}, $D_{\text{uranin}} = 3.7 \times 10^{-6}$ cm^2 s^{-1}, and $R = 3.84$ Å. The values of τ_0 and the diffusion constants are those reported experimentally,[23,24] and R was determined so that the calculated value of k_{Q} agreed with the experimental value in the limit of low aniline concentration. As the error in the experimental values is at least ±0.3, the agreement between the calculation and the experiment seems quite good.

It is worth emphasizing that the calculations in Figures 1 and 2 depend on only one empirical parameter, the encounter radius

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[22] R. F. Chen, *Anal. Biochem.*, **57**, 593–604 (1974).

[23] B. Williamson and V. La Mer, *J. Am. Chem. Soc.*, **70**, 717–721 (1948).

[24] K. C. Hodges and V. La Mer, *J. Am. Chem. Soc.*, **70**, 722–726 (1948).

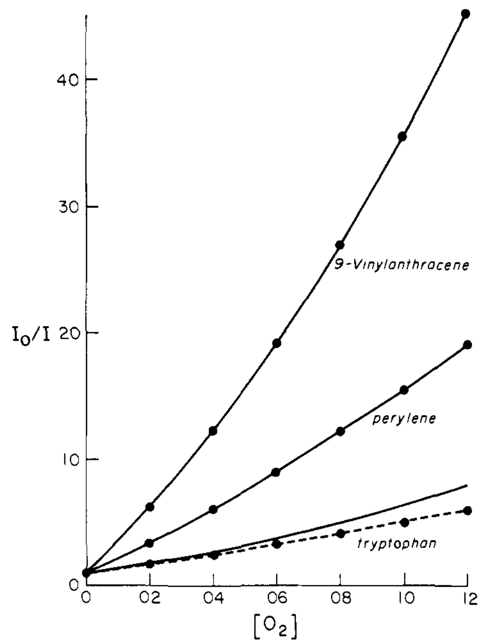

Figure 3. Stern–Volmer plots for oxygen quenching of the indicated organic compounds. The circles represent experimental results from ref 7, Table III; the full lines are calculated using eq 17; and the dashed line for tryptophan is calculated using eq 15. Parameters used for the calculations are given in the text.

R. Moreover, we have used the limiting value of the quenching rate at low concentration to fix the value of this parameter. The values of the quenching rate at higher concentration of quencher are then *uniquely* determined using eq 17. Thus there are no adjustable parameters in the high concentration calculations.

We have also compared eq 17 to the results of Lakowicz and Weber[7] on the quenching of the fluorescence of organic molecules by oxygen. Results for the fluorophores 9-vinylanthracene in dodecane and perylene in dodecane are shown in Figure 3. The experimental values of the fluorescence lifetimes of the substances used in eq 17 to calculate the full lines were $\tau_0 = 11 \times 10^{-9}$ s (9-vinylanthracene), and 5.4×10^{-9} s (perylene). Since values of the diffusion constants in dodecane could not be found, we used Ware's data[25] to estimate that D(9-vinylanthracene) = 2×10^{-5} cm^2 s^{-1}, D(perylene) = 2.2×10^{-5} cm^2 s^{-1}, and $D(O_2) = 5.6 \times 10^{-5}$ cm^2 s^{-1}. The encounter values used were R = 3.6 Å (9-vinylanthracene) and R v 3.2 Å (perylene). These encounter radii correspond to the zero oxygen concentration value of $k^{obsd} = 2.1 \times 10^{10}$ M^{-1} s^{-1} that was measured experimentally for both these reactions. The agreement between the experiments and the calculations based on eq 17 is evident in Figure 3.

Also shown in Figure 3 is the oxygen quenching of tryptophan in 0.025 M aqueous Tris buffer. The calculation using eq 17 was based on the experimentally determined values[7] $\tau_0 = 2.7 \times 10^{-9}$ s, $D(O_2) = 2.6 \times 10^{-5}$ cm^2 s^{-1}, and D(tryptophan) = 0.7×10^{-5} cm^2 s^{-1}. A value of R = 4 Å is then compatible with the experimental observation that $k^{obsd} = 1.3 \times 10^{10}$ M^{-1} s^{-1} at zero oxygen concentration.[26] The results based on eq 17 and these parameter values are plotted as the full line in Figure 3. The calculation shows significant curvature not seen in the experimental data.

There are several possible explanations for the lack of agreement between theory and experiment for the tryptophan data. First, the reactivity function in eq 13 is based on the presumption that only encounters at the radius R are effective in quenching. This is a highly simplified picture of the quenching reaction and may be seriously mistaken for the oxygen quenching of tryptophan. Second, intermolecular forces[13] have been neglected in obtaining the radial distribution function in eq 8. Third, tryptophan is not spherical, so rotational diffusion effects may be important.[18] While all these criticisms of the calculation are plausible, they should be equally applicable to the four other molecules for which the calculation is quite successful. Furthermore, a variety of organic substances quenched by oxygen in aqueous solution show no appreciable curvature of their Stern–Volmer plots.[7] Thus we were led to seek a more general explanation of the lack of curvature for oxygen quenching in aqueous solutions.

Table I. Values of the Encounter Radius R for Anthracene Quenched by Oxygen in Various Solvents[a]

solvent	$k^{obsd} = k_{exp}$, M^{-1} s^{-1}	R, Å	D', cm^2 s^{-1}
n-octyl alcohol	1.7×10^{10}	7.3	2.0×10^{-5}
isobutyl alcohol	1.8×10^{10}	5.8	3.1×10^{-5}
isopropyl alcohol	2.2×10^{10}	5.6	4.1×10^{-5}
ethyl alcohol	2.5×10^{10}	5.3	5.7×10^{-5}
benzene	3.1×10^{10}	4.5	7.9×10^{-5}
n-heptane	3.5×10^{10}	4.8	8.7×10^{-5}
acetone	3.9×10^{10}	3.8	12.7×10^{-5}

[a] R is determined as that value for which k^{obsd} calculated from eq 17 agrees with experiment; k_{exp} and D' are taken from ref 25.

One possibility is that *oxygen quenching of tryptophan fluorescence, while intrinsically fast, is not actually diffusion controlled.* If this were the case, then eq 15, rather than eq 17, should be used to analyze the experiments. Certainly if $k°/4\pi D'R$ is not large, then the lack of quencher dependence in $k°$ will tend to wash out the dependence of the correlation lengths on quencher concentration. Indeed, by using the previously quoted values of τ_0, $D(O_2)$, and D(tryptophan) and setting R = 9 Å and $k°/4\pi D'R = 0.8$, we find the dashed curve in Figure 3. The agreement for these parameter values is quite good, although it is worth noting that approximately the same fit is obtained using R = 8 Å and $k°/4\pi D'R = 1$.

While these values of the encounter radius are larger than one normally expects, it can be shown for nonaqueous solvents that the encounter radius for oxygen quenching is solvent dependent. Using eq 17 and the data of Ware,[27] we have calculated the encounter radius of anthracene and oxygen required to fit the experimental value of k^{obsd} at low oxygen concentration. This is shown in Table I. The trend is clearly that the slower the reaction, the larger the value of the encounter radius. For the solvent n-octyl alcohol, the predicted value of R is 7.3 Å and k^{obsd} is somewhat larger than that for tryptophan. Had we not included the fluorescence lifetime correction of eq 17, but used instead the Smoluchowski result $k^{obsd} = 4\pi D'R$, a value of 11.3 Å would have been obtained. These calculations make a value of 8–9 Å for the encounter radius of oxygen and tryptophan somewhat more plausible, although they certainly do not explain such a value.

This calculation suggests that the oxygen quenching of tryptophan is almost, but not quite, diffusion controlled in aqueous solution. Since this means that $k°/4\pi D'R$ cannot be neglected, we have had to introduce the intrinsic reactivity parameter $k°$ into the calculation. This explanation can be tested further experimentally. By carrying out the quenching experiments in aqueous mixtures at high weight percent of glycerol, it is possible to decrease diffusion constants by several orders of magnitude.[24] If our explanation of the lack of curvature for tryptophan is correct, then the ratio of $k°/4\pi D'R$ would be increased in glycerol solutions from 0.8 to a value of 100 or so. This would reduce the effect of $k°$ to the 1% level and, thus, make the reaction diffusion controlled. According to this explanation, one would expect a marked increase in the curvature of the Stern–Volmer plots for oxygen quenching of tryptophan at high weight percents of glycerol.

IV. Conclusions

The calculations in the previous section imply two general features of positive curvature for Stern–Volmer plots. First, quenching reactions which are fast enough to be diffusion con-

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(25) W. Ware, *J. Phys. Chem.*, **66**, 455–458 (1961).

(26) From Table III, ref 7.

(27) Reference 25, Tables I and II.

trolled should exhibit curvature at concentrations above about 0.01 M. The curvature is caused by the bimolecular lifetime effect which leads to a quencher dependent correlation length. The extent of curvature, however, depends strongly on the unimolecular lifetime and the diffusion constant of the fluorophore. This is readily apparent in Figure 3. While both perylene and 9-vinylanthracene have the same value of k^{obsd} at low oxygen concentration, the curvature in the Stern–Volmer plot is significantly greater for the longer lived 9-vinylanthracene.

The second general feature demonstrated by the calculations is the diminution of curvature for reactions which are not diffusion controlled. Slower reactions reduce the effect of the bimolecular lifetime on k^{obsd} with a subsequent loss of sensitivity to increased quencher concentration. While we have speculated that this is the cause of the negligible curvature for oxygen quenching of tryptophan in water, the effect itself is quite general.

The quality of agreement between experiments and the calculations based on eq 15 or 17 is good. Further tests of the theory, however, suggest themselves. For example, it would be useful to have data on quenching rates and diffusion constants in a variable solvent system like glycerol–water to test the effect of changing viscosity on the low quencher concentration value of k^{obsd}. The only data we have seen of this sort are two data points for the aniline quenching of uranin.[24] Although a calculation based on eq 17 is in agreement with these data, it would be nice to have a full range of weight percent mixtures with which to compare the theory. We are hopeful that such experiments will be forthcoming. While charge effects have not been included in the present calculations, they can in principle be added. Experimental data involving the effect of ionic strength would be particularly useful for charged systems.

The effects discussed in this paper have been confined to fluorophores which do not engage in molecular association with quencher molecules. If molecular complexes are formed which do not fluoresce, then the Bowen–Metcalfe result in eq 6 combined with the definition of the lifetime of A*, i.e., $\tau = (\tau_0^{-1} + k^{\mathrm{obsd}}\cdot[\mathrm{Q}])^{-1}$, shows that

$$I_0/I = (1 + K[\mathrm{Q}])\tau_0/\tau \tag{19}$$

Thus in the absence of this type of molecular association

$$I_0/I = \tau_0/\tau \tag{20}$$

which is one of the criteria for the absence of quenching via molecular association and has been verified, for example, for the oxygen quenching of tryptophan.[7] As we have shown, the purely dynamic effects described in this paper lead to positive curvature in Stern–Volmer plots even when molecular association is absent. If static quenching via molecular association also occurs, then eq 19 shows that I_0/I will be greater than τ_0/τ. Furthermore, the molecular association constant can be obtained as the slope of a plot of $I_0\tau/I\tau_0$ vs. [Q]. An interesting limiting case occurs when the lifetime τ_0 is short enough or the quenching rate constant is small enough that $k^{\mathrm{obsd}}[\mathrm{Q}]\tau_0 << 1$. This implies that dynamic quenching is negligible and that $\tau/\tau_0 \approx 1$. In this case only static quenching will be observed and

$$I_0/I \approx (1 + K[\mathrm{Q}])$$

Quenching through this sort of purely static mechanism has been observed for several flavin compounds in aqueous solution.[28]

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Registry No. Anthracene, 120-12-7; quinine sulfate, 804-63-7; uranin, 518-47-8; 9-vinylanthracene, 2444-68-0; perylene, 198-55-0; tryptophan, 73-22-3.

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(28) G. Weber in "Flavins and Flavoproteins", E. C. Slater, Ed., Elsevier, Amsterdam, 1966, pp 15–21, Table II.

Spin Trapping of Peroxy Radicals by Phenyl-*N*-(*tert*-butyl)nitrone and Methyl-*N*-durylnitrone

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Abstract: The spin trapping of *tert*-butylperoxy and tetralylperoxy radicals by phenyl-*N*-(*tert*-butyl)nitrone (PBN) and methyl-*N*-durylnitrone (MDN) has been investigated. The peroxy radicals were generated by the alkoxy radical induced decomposition of hydroperoxides, hydrogen atom abstraction from hydrocarbon in the presence of oxygen, and decomposition of hydroperoxides by cobaltous ion and lead tetraacetate. The spin adducts of peroxy radicals were observed by ESR and their hyperfine splitting constants were determined. The spin adduct of peroxy radicals by MDN could be clearly distinguished from that of alkoxy radical. The nitrone spin adducts of oxygen radicals were found to be reasonably stable at room temperature in the dark, but they decayed readily in the ordinary laboratory light.

A process commonly referred to as "spin trapping" has been applied in the studies of free-radical chemistry and biology.[1-4] The transient radicals are scavenged by spin traps and converted to longer lived spin adducts, whose ESR spectra provide valuable information concerning the structure of the intermediate radicals and the mechanisms of the reactions in which they are involved. At the same time, however, it has been pointed out that cautions have to be observed especially in the stability of the spin adducts and interpretation of ESR spectra.[2,5,6] The trapping of oxygen radicals has received considerable attention.[2,3,6,7] We have previously reported[8] the spin trapping of peroxy radicals by

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(1) Janzen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31–40.

(2) Janzen, E. G. In "Free Radicals in Biology"; Pryor, W. A., Ed.; Academic Press: New York, 1980; Vol. 4, pp 116–154.

(3) Perkins, M. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 1–64, and references cited therein.

(4) Janzen, E. G.; Evans, C. A.; Davis, E. R. In "Organic Free Radicals"; Pryor, W. A., Ed.; American Chemical Society: Washington, D.C., 1978; pp 433–446; *ACS Symp. Ser.* No. 69.

(5) Coxon, J. M.; Gilbert, B. C.; Norman, R. O. C. *J. Chem. Soc., Perkin Trans. 2* **1981**, 379–381.

(6) Rosen, G. M.; Rauckman, E. J. In "Oxygen and Oxy-Radicals in Chemistry and Biology"; Rodgers, M. A. J., Powers, E. L., Eds.; Academic Press: New York, 1981; pp 97–108.

(7) Harbour, J. R.; Bolton, J. R. *Photochem. Photobiol.* **1978**, *28*, 231–238.

0002-7863/83/1505-1498$01.50/0