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Review

The kinetics of fast fluorescence quenching processes

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Abstract

The results of fluorescence quenching experiments which bear on the kinetics of fast bimolecular processes are reviewed and analyzed critically in light of ongoing developments of the theory of these processes. A set of recommendations for 'best' experimental procedures and data analysis methods is proposed.

Keywords: Fluorescence quenching; Fast bimolecular processes; Smoluchowski–Collins–Kimball model

1. Introduction

The kinetics of fast bimolecular reactions in solution has been a subject of interest for both theoreticians and experimentalists for a long time. Diffusion-limited processes are of particular interest because of the many physical, chemical and biological processes, including the dynamics of proteins, enzymes and membranes, the aggregation of colloidal particles, electron transfer reactions and ion association–dissociation reactions, whose kinetics are influenced by diffusion. Photophysical methods, involving the diffusion-limited quenching of the fluorescence of short-lived excited states, have proved particularly useful in these studies [1–12,13].

A particular case of importance throughout chemistry is that of diffusion-limited reactions with time-dependent reaction rate coefficients (cf. Refs. [1,14] and references therein). Fluorescence quenching is the process of choice for studying such diffusion-controlled reactions, in which the time-dependence of the quenching rate coefficient is manifest in non-exponential decay of the fluorescence of the donor in the presence of the quencher. Time-correlated single photon counting (TCSPC) and more general time-domain and frequency-domain methods are the only techniques which can provide temporal fluorescence quenching data with sufficiently high precision and sensitivity to permit various theoretical models of the nonexponential form of the decay to be tested [1,2].

Measurements of the temporal profile of the fluorescence intensity produced from electronically excited molecules in the presence of a quencher permit an investigation of kinetic processes in the first stages of the reaction, i.e., on the femtosecond to picosecond time scale. If appropriate experimental and data treatment methods are employed, one may be able to obtain some unique information about the system under study, such as the reaction distance, R, the specific rate constant, κ, the diffusion coefficient, D, the Onsager length, r_c, the lifetime of a encounter pair, $[A^* \cdots Q]$, and the activation energy for the quenching process, E_a. Using the results of such an analysis it can be possible to determine the existence of different orientations of fluorophores and quenchers, to recognize the microscopic structure of the system, and even to determine the rates of elementary processes such as energy and charge transfer.

The main objective of this paper is to present and analyze the results of fluorescence quenching experiments which bear on the kinetics of fast bimolecular processes. Comprehensive reviews of earlier work may be found in Refs. [1,2,4,5,14]. The last of these reviews was published in 1985 [1] at a time when very little experimental data from time-resolved emission experiments was available. Since then, the use of picosecond and femtosecond laser excitation sources and microchannel plate detectors in TCSPC systems have lead to a huge improvement in the resolution of time-resolved fluorescence data [15–17].

Ware and Nemzek [18] were the first to observe the phenomenon of time-dependent fluorescence quenching, often

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referred to as the transient effect, in their pioneering study of the fluorescence quenching of 1,2-benzanthracene by carbon tetrabromide in high viscosity solvents. However, even twenty-five years later some aspects of this phenomenon remain unclear. Here, in an effort to provide additional clarity, we analyze or re-analyze all of the more recent reports of time-dependent fluorescence quenching in which the Smoluchowski–Collins–Kimball (SCK) model and its extensions have been used, in whole or in part, to determine the time-dependent rate coefficients, $k(t)$. We provide explicit equations for both $k(t)$ and $I(t)$, the temporal profile of the fluorescence intensity, at various levels of approximation, and discuss how they can be used in describing the dynamics of fast bimolecular processes. Most importantly, we focus on experimental studies of the kinetics of partly diffusion-controlled fluorescence quenching in fluid solution, the data from which can be used to test the limits of validity of these theories of fast bimolecular reactions.

The simplest possible kinetics scheme describing the fluorescence quenching reaction is as follows:

excitation (1) $A \xrightarrow{h\nu} A^*$

intramolecular decay (2) $A^* \xrightarrow{1/\tau^0} A$

quenching (3) $A^* + Q \xrightarrow{k(t)} \text{Products}$,

Here A and A* represent the donor in the ground and electronically-excited states respectively, τ^0 represents the lifetime of A* in the absence of quencher, and process 3 should be understood as any chemical or physical interaction between A* and Q which leads to the quenching of A* with no significant change in the concentration of the quencher, [Q]. The central question concerns the form of the reaction rate coefficient $k(t)$, and the consequent temporal form of the fluorescence intensity decay profile. For the scheme above, the decay of the donor fluorescence intensity in the presence of a quencher as a function of time $I(t)$ may be written as follows:

$$I(t)=I(0)\exp\left[\frac{-t}{\tau^0}-[\mathrm{Q}]\int_0^t k(t')\mathrm{d}t'\right] \tag{1}$$

where $I(0)$ represents the fluorescence intensity at time, $t=0$, and [Q] represents the molar concentration of the quencher, Q.

At this point it will be useful to define the terms static quenching, pseudoquenching, time-independent quenching and time-dependent quenching, and to distinguish among them. The term static quenching refers to that process by which a fluorophore is quenched at the instant of its excitation by a ground state quencher which lies sufficiently close to the donor molecule (and with the appropriate orientation) to render the fluorophore-quencher pair dark. The fraction of excited molecules which are made non-radiative by this process of static quenching will be concentration-dependent, but no diffusion is required to permit the quenching to occur. This process will be functionally time-independent at the temporal resolution of the particular measuring system employed. Pseudoquenching is the term which has been employed to describe a process in which the emission intensity of a fluorophore is altered by ground state complex formation and governed by ground state equilibrium processes [19]. There is a continuum of possible interaction energies in the ground state and so any distinction between pseudoquenching and static quenching will be somewhat arbitrary. Time-independent dynamic quenching is a process described by a pseudo-first order time-independent rate constant in which a quencher at constant bulk concentration and an excited fluorophore whose concentration decreases exponentially with time diffuse together and 'react' (interact in the general case) so as to reduce the concentration of the excited fluorophore via a true bimolecular encounter. Such a process gives rise to an exponential decay of fluorescence intensity which can be observed (in principle at least) at long times in any fluorescent system, and is embodied in the standard Stern–Volmer form of the quenching kinetics. At intermediate times, the rate coefficient describing the quenching process will be time-dependent, and it is this process which has sometimes been referred to as the transient effect. Fig. 1 illustrates the three time regimes associated with these three processes.

The quenching of an electronically excited state of a polyatomic molecule in a fluid medium has usually been modeled as a bimolecular diffusion-reaction problem. It is not our intention to describe the theory of fluorescence quenching in detail, but in order to provide sufficient background we begin by introducing the most frequently used expressions for the rate coefficients of such processes and the functions which describe the fluorescence decay of an excited donor in the presence of a quencher. For more detail, the reader is referred to a recent review which deals with the theory of such processes (Ref. [8] and references therein). The SCK treatment

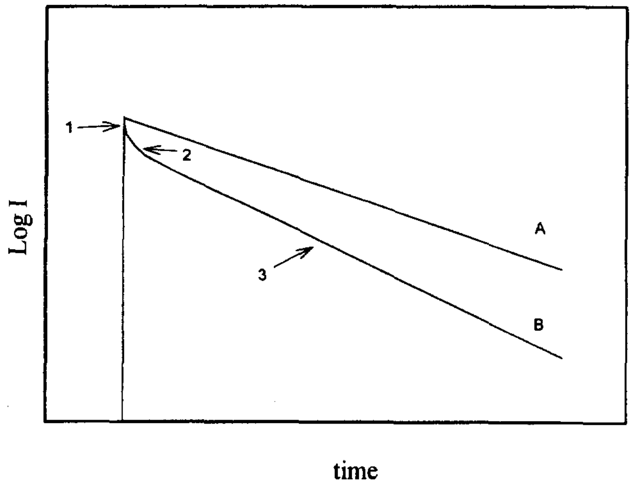


Fig. 1. Schematic diagram of logarithm of fluorescence intensity vs. time showing: (A) a single exponential decay, and (B) a nonexponential decay. Curve B has three time regions: (1) a near-delta function accounting for static or pseudoquenching, (2) the time-dependent regime, and (3) the time-independent dynamic quenching regime.

is the starting point for this discussion, and it is followed by a review of several limiting cases and simplifications which can be made under experimentally verifiable conditions. In particular, the forms of $k(t)$ which have been suggested by Nemzek and Ware [18,20], Flannery [21,23], Flannery and Mansky [22], and Periasamy et al. [24] will be introduced.

2. The Smoluchowski–Collins–Kimball model

The classical treatment of diffusion-controlled reactions began with the pioneering work of Smoluchowski [25]. In the Smoluchowski theory, an infinite reaction rate is assumed, and the reaction rate coefficient is of the form

$$k(t)=4\pi RDN'\left(1+\frac{R}{\sqrt{\pi Dt}}\right) \tag{2}$$

Here R is the sum of the donor and quencher radii, D is the sum of the diffusion coefficients of the donor and quencher, and $N'=N/1000$ where N is Avogadro's number. The decay of the donor's fluorescence in the presence of quencher resulting from this form of $k(t)$ is given by

$$I(t)=I(0)\exp\left(-\frac{t}{\tau^0}-4\pi RDN'[\mathrm{Q}]t\left[1+\frac{2R}{\sqrt{\pi DT}}\right]\right) \tag{3}$$

Collins and Kimball [26,27] modified Smoluchowski's theory by taking the finite rate of the quenching 'reaction' into account, giving

$$k(t)=a+b\exp(c^2t)\mathrm{erfc}\left(c\sqrt{t}\right) \tag{4}$$

and

$$I(t)=I(0)\exp\left(\frac{b[Q]}{c^2}\right)\exp\left[-t\left(\frac{1}{\tau^0}+a[\mathrm{Q}]\right)-\frac{b[\mathrm{Q}]}{c^2}\left(\exp(c^2t)\mathrm{erfc}\left(c\sqrt{t}\right)+\frac{2c\sqrt{t}}{\sqrt{\pi}}\right)\right] \tag{5}$$

where

$$a=k_\mathrm{r}\left[1+\frac{k_\mathrm{r}}{4\pi RDN'}\right]^{-1},\quad b=k_\mathrm{r}\left[1+\frac{4\pi RDN'}{k_\mathrm{r}}\right]^{-1},$$

$$c=\left[1+\frac{k_\mathrm{r}}{4\pi RDN'}\right]\frac{\sqrt{D}}{R},\quad \mathrm{erfc}(x)=\frac{2}{\sqrt{\pi}}\int_x^\infty e^{-z^2}\mathrm{d}z$$

Eq. (5) contains a term, $\exp(b[\mathrm{Q}]/c^2)$, with a concentration-dependent exponent which accounts for the process of static quenching.

2.1. The short time approximation

If $c\sqrt{t}\ll 1$, one can expand the product of the two functions, $\exp(x^2)$ and $\mathrm{erfc}(x)$, in three different ways to obtain three different forms of $k(t)$:

$$k(t)=a+b\exp(c^2t)-\frac{2bc}{\sqrt{\pi}}\sqrt{t}\exp(c^2t) \tag{6}$$

$$k(t)=a+b-\frac{2bc}{\sqrt{\pi}}\sqrt{t} \tag{7}$$

$$k(t)=a+b\exp(c^2t)-\frac{2bc}{\sqrt{\pi}}\sqrt{t} \tag{8}$$

No experimental data which will enable one to examine the quenching kinetics in this time region have been obtained to date.

2.2. The long-time approximation

The product of the two functions $\exp(x^2)$ and $\mathrm{erfc}(x)$ can be approximated in two ways for the case of $c\sqrt{t}\gg 1$, yielding two forms for the function which describes $I(t)$, Type A and Type B.

2.2.1. Type A

Here $k(t)$ has the same form as it has in the SCK model, and with $c\sqrt{t}\gg 1$ Eq. (5) reduces to

$$I(t)=I(0)\exp\left(\frac{b[\mathrm{Q}]}{c^2}\right)\exp\left(-t\left(\frac{1}{\tau^0}+a[\mathrm{Q}]\right)-\frac{2b[\mathrm{Q}]}{c\sqrt{\pi}}\sqrt{t}\right) \tag{9}$$

If $c\sqrt{t}>7$, then Eq. (9) gives values of $k(t)$ within 1% of those of Eq. (6). For a typical system with $D=2.84\times10^{-5}$ cm^2 s^{-1}, $R=6.74$ Å, $k_\mathrm{a}=2.4\times10^{-11}$ cm^3 s^{-1} molecule^{-1}, $[\mathrm{Q}]=0.1$ M, and $\tau^0=4.35$ ns, using $c\sqrt{t}>7$ it is apparent that the Eq. (9) is valid for times $t>1.96$ ns. However, in practice Eq. (9) will provide an adequate fit to data of practical importance (i.e., $I(0)\approx2\times10^4$) for times far shorter than such theoretical values. This apparent contradiction can be resolved by consideration of the errors in the intensities of time-domain experimental data. It has been shown that in practice for a typical system such as that described above, Eq. (9) can apply to any data corresponding to $t>190$ ps [28].

2.2.2. Type B

Once again we assume that $c\sqrt{t}\gg 1$, but now we first approximate the rate coefficient $k(t)$ by

$$k(t)=a+\frac{b}{c\sqrt{\pi t}} \tag{10}$$

so that the temporal fluorescence intensity profile has the form

$$I(t)=I(0)\exp\left(-\left(\frac{1}{\tau^0}+a[\mathrm{Q}]\right)-\frac{2b[\mathrm{Q}]}{c\sqrt{\pi}}\sqrt{t}\right) \tag{11}$$

This equation is often written in the following more familiar form

$$I(t)=I(0)\exp\left(-\frac{t}{\tau^0}-4\pi R'DN'[\mathrm{Q}]t\left[1+\frac{2R'}{\sqrt{\pi Dt}}\right]\right) \tag{12}$$

where $1/R'=1/R+D/\kappa R^2$ and κ is the specific rate constant which is associated with k_r via the formula $k_r=\kappa 4\pi R^2 N'$. Now to have agreement within 1% between the exact values of $I(t)$ given by Eq. (5) and the approximate ones of Eq. (11) or Eq. (12) requires $c\sqrt{t}>90$. Note also, in Eqs. (11) and (12), the absence of a concentration-dependent pre-exponential term.

2.2.3. *Classical kinetics approximation*

If $c\sqrt{t}\gg 1$ and also $t\gg b^2/(ca)^2$, the expression for $k(t)$ may be written as follows:

$$k(t)=a=\frac{k_r k_{\mathrm{diff}}}{k_r+k_{\mathrm{diff}}}=\text{constant} \tag{13}$$

The rate coefficient is now time-independent and is correctly referred to more commonly as the rate constant. Note that if $k_r\gg 4\pi RDN'$, Eq. (13) reduces to the common expression for diffusion controlled processes, $k(t)=4\pi RDN'$, whereas if $k_r\ll 4\pi RDN'$, we arrive at the limit in which the reaction is controlled by the chemical step of the process and $k(t)=k_r$.

When these classical kinetics approximations are employed we obtain the well-known expression for the fluorescence decay of a donor in the presence of a quencher:

$$I(t)=I(0)\exp\left(-t\left(\frac{1}{\tau^0}\right)+a[\mathrm{Q}]\right) \tag{14}$$

which is often presented in the form of a single exponential intensity decay:

$$I(t)=I(0)\exp(-t/\tau) \tag{15}$$

where τ is the fluorescence lifetime of the donor in the presence of the quencher. These equations lead directly to the well-known classical Stern–Volmer equation in which the ratio of the quantum yields of fluorescence in the presence and absence of quencher is a linear function of quencher concentration, *viz.*

$$\Phi^0/\Phi=1+K_{\mathrm{SV}}[\mathrm{Q}] \tag{16}$$

where the Stern–Volmer constant, $K_{\mathrm{SV}}=k_{\mathrm{Q}}\tau^0$ and k_{Q} and τ^0 are the second order rate constant for quenching and the lifetime of the fluorescence in the absence of quencher, respectively.

3. Debye–Smoluchowski–Collins–Kimball theory

In 1942 Debye [29] extended the theory of Smoluchowski by taking into account the possible interactions between species in solution. The time-dependent rate coefficients of diffusion-limited reactions involving ionic reactants has been considered extensively by Weller [11,30–32], Hong and Noolandi [33,34], Pedersen and Sibani [35], Flannery [21,23], Flannery and Mansky [22], Rice et al. [36], Szabo [8] and Green [37]. Exact analytical equation for $k(t)$ within the framework of the Debye–Smoluchowski–Collins–Kimball (DSCK) theory have not yet been determined, and Delaire et al. [38] suggest that such solutions are not possible. The theoretical developments extending the SCK model to include the interactions among species, and the experimental attempts to verify the theoretical predictions associated with these extensions have been reviewed by Szabo [8], by Eads et al. [39] and by Periasamy et al. [24].

According to Flannery [21,23] and Flannery and Mansky [22] when $R\geq |r_c|$ (here r_c represents the Onsager length) the form of $k(t)$ which is valid for all times, t, is:

$$k(t)=\alpha\left[1+\frac{\alpha_{\mathrm{R}}}{\alpha_{\mathrm{T}}}\exp(y^2)\operatorname{erfc}(y)\right] \tag{17}$$

where

$$\alpha=\frac{\alpha_{\mathrm{R}}\alpha_{\mathrm{T}}}{\alpha_{\mathrm{R}}+\alpha_{\mathrm{T}}}=4\pi R_{\mathrm{HN}}DN'$$

$$\alpha_{\mathrm{R}}=k_r\exp(-r_c/R)$$

$$\alpha_{\mathrm{T}}=4\pi r_c DN'[\exp(r_c/R)-1]^{-1}$$

$$y=\left(1+\frac{\alpha_{\mathrm{R}}}{\alpha_{\mathrm{T}}}\right)\frac{(Dt)^{1/2}}{R^2}r_c\frac{\exp(r_c/R)}{\exp(r_c/R)-1}$$

$$R_{\mathrm{HN}}=\frac{r_c}{(1+4\pi Dr_c N'/k_r)\exp(r_c/R)-1}$$

$$r_c=\frac{Z_{\mathrm{A}}Z_{\mathrm{Q}}e^2}{4\pi\varepsilon k_{\mathrm{B}}T}$$

In the above expression for the Onsager length, $Z_{\mathrm{A}}e$ and $Z_{\mathrm{Q}}e$ represent the charges of the donor and quencher, respectively, ε is the dielectric constant of the medium, and k_{B} is the Boltzmann constant. It is interesting to note that Eq. (17) for ionic interacting molecules can be expressed in the form of Eq. (4) by reformulating the parameters *a*, *b*, and *c*, *viz.*

$$a'=\frac{\alpha_{\mathrm{R}}\alpha_{\mathrm{T}}}{\alpha_{\mathrm{R}}+\alpha_{\mathrm{T}}}$$

$$b'=\frac{\alpha_{\mathrm{R}}^2}{\alpha_{\mathrm{R}}+\alpha_{\mathrm{T}}}$$

$$c'=y=\left(1+\frac{\alpha_{\mathrm{R}}}{\alpha_{\mathrm{T}}}\right)\frac{(Dt)^{1/2}}{R^2}r_c\frac{\exp(r_c/R)}{\exp(r_c/R)-1}$$

In the long-time limit $k(t)$ is given by

$$k(t)=4\pi R_{\mathrm{HN}}DN'\left(1+\frac{R_{\mathrm{HN}}}{\sqrt{\pi Dt}}\right) \tag{18}$$

which is of the same form as that previously derived by Hong and Noolandi [33,34]. It has been shown that in the limit $r_c \to 0$ (Eq. (17)) for $k(t)$ is identical to the Smoluchowski–Collins–Kimball equation derived for non-interacting species. In this same limit Eq. (18) also is of the form of the Smoluchowski equation for non-interacting species.

Periasamy et al. [24] have derived expressions for the rate coefficient and temporal fluorescence intensity profile in different time domains using the DSCK theory, and these are given below.

3.1. Short-time approximation

For the case of $y \ll 1$,

$$k(t)=m-n\sqrt{t}, \qquad (y\ll 1), \tag{19}$$

and

$$I(t)=I(0)\exp\left[-\left(\frac{1}{\tau^0}+m[\mathrm{Q}]\right)t+\frac{2}{3}n[\mathrm{Q}]t^{3/2}\right] \tag{20}$$

where

$$m=4\pi R_{\mathrm{HN}}DN'(1+\alpha_{\mathrm{R}}/\alpha_{\mathrm{T}})$$

$$n=m\left(\frac{\alpha_{\mathrm{R}}}{\alpha_{\mathrm{T}}}\right)^2\left(\frac{D}{\pi}\right)^{1/2}\frac{r_c}{R^2[1-\exp(-r_c/R)]}$$

3.2. SCK approximation

No exact analytical equation for $k(t)$ which is valid for all times is known in the framework of the DSCK theory. However, according to Refs. [21–23] when $R \geq |r_c|$ the expression for $k(t)$ valid for all times t (Eq. (17)) is of the form

$$k(t)=a'+b'\exp(c'^2t)\mathrm{erfc}\left(c'\sqrt{t}\right) \tag{21}$$

so that the temporal fluorescence decay profile is given by

$$I(t)=I(0)\exp\left(\frac{b'[\mathrm{Q}]}{c'^2}\right)\exp\left[-t\left(\frac{1}{\tau^0}+a'[\mathrm{Q}]\right)\right.$$
$$\left.-\frac{b'[\mathrm{Q}]}{c'^2}\left(\exp(c'^2t)\mathrm{erfc}(c'\sqrt{t})+\frac{2c'\sqrt{t}}{\sqrt{\pi}}\right)\right] \tag{22}$$

3.3. Long-time approximation

If $y \gg 1$

$$k(t)=p+q/\sqrt{t} \tag{23}$$

where $p=4\pi R_{\mathrm{HN}}DN'$ and $q=(16\pi D)^{1/2}R_{\mathrm{HN}}{}^2N'$. The intensity profile is given by

$$I(t)=I(0)\exp[-(ut+v\sqrt{t})] \tag{24}$$

where $u=1/\tau^0+4\pi R_{\mathrm{HN}}DN'[\mathrm{Q}]$ and $v=8\sqrt{\pi D}R_{\mathrm{HN}}^2N'[\mathrm{Q}]$.

3.4. Classical kinetics approximation

If $y \gg 1$ and $t \gg q^2/p^2$,

$$k(t)=p \tag{25}$$

and

$$I(t)=I(0)\exp[-(1/\tau^0+p[\mathrm{Q}])t] \tag{26}$$

This is the well-known single-exponential decay of the fluorescence of the donor in the presence of the quencher.

The equations for the rate coefficient and temporal profile of the fluorescence intensity in the presence of quencher for non-interacting molecules are similar to those given above for interacting molecules, with the exception of the definitions of parameters. In the case of interacting molecules we have at least one extra parameter, the Onsager length.

4. Statistical thermodynamics of non-equilibrium processes

Keizer [9,10,40–42] and Weller [11] have been responsible for important adaptations of the theory of the statistical thermodynamics of nonequilibrium processes to the problem of fast kinetics, and was the first to take the problem of nonequilibrium into account. The advantage of this theory is that it makes it possible to find analytical equations for $k(t)$ and also for the Stern–Volmer function, Φ^0/Φ. Applying Keizer and Weller's theory to the processes of fluorescence quenching results in the following modified Stern–Volmer type relationship:

$$\frac{\Phi^0}{\Phi}=1+\frac{4\pi RDN'k_r C(R)\tau^0[\mathrm{Q}]}{k_r+4\pi RDN'C(R)\tau^0} \tag{27}$$

where:

$$C(R)=\frac{2D_{\mathrm{Q}}}{[(D_{\mathrm{Q}}-D_{\mathrm{A}})\exp(-R/L_{\mathrm{AQ}})+(D_{\mathrm{Q}}+D_{\mathrm{A}})\exp(-R/L_{\mathrm{A}})]}$$

$$L_{\mathrm{AQ}}=\sqrt{\frac{(D_{\mathrm{Q}}+D_{\mathrm{A}})\tau^0}{1+k_q\tau^0[Q]}}$$

$$L_{\mathrm{A}}=\sqrt{\frac{D_{\mathrm{A}}\tau^0}{1+k_q\tau^0[\mathrm{Q}]}}$$

Here D_{A} represents the diffusion coefficient of the donor, and D_{Q} is the diffusion coefficient of the quencher. Eq. (27) has been successfully applied to a number of diverse excited donor, quencher, solvent systems [9,10,40–42] and has always produced a good fit with the experimental results.

Nonequilibrium statistical thermodynamics is also very helpful in resolving the apparent discrepancies among the results of steady state, time-resolved time-domain and time-resolved frequency-domain experiments on the same systems [43–45]. Molski and Keizer were the first to find the relationships among $k^0(z)$, the Laplace transform of the quenching rate coefficient in the time domain, $k(t)$, the rate constant measured under steady-state conditions, k^{ss}, and the mean field rate coefficient in the frequency domain, $k^{mf}(\omega)$. These relationships can be expressed as follows [45]:

$$k^{mf}(\omega)=(i\omega+1/\tau^0+[Q]k^{ss})k^0(i\omega+1/\tau^0+[Q]k^{ss}) \tag{28}$$

and

$$k^{ss}=(1/\tau^0+[Q]k^{ss})k^0(1/\tau^0+[Q]k^{ss}) \tag{29}$$

where ω is the angular frequency.

Although discrepancies between time-domain and steady-state experiments have often been reported, the analysis of Molski and Keizer appears to provide a reasonable explanation for them. Eqs. (28) and (29) are the outcomes of the rigorous application of the theory of non-equilibrium statistical thermodynamics to the problem of fast fluorescence quenching, and clearly represent a better approach than the phenomenological one used by Andre et al. [46,47]. Szabo [8] and Zhou and Szabo [48] have also contributed to this subject. More experimental work in the area would clearly be desirable.

5. Analyses of experimental results

5.1. Early work

Among the earlier experimental work in the area, the quenching of the fluorescence of anthracene and its derivatives deserves particular attention. Anthracene quenching in solvents of different viscosity was first studied by Bowen and Metcalf [49] in 1951. From measurements of the fluorescence intensity with and without quencher, I and I_0, respectively, they concluded that a complex was formed between ground state anthracene and carbon tetrabromide, proposed that the presence of such a ground state complex was responsible for the large observed values of the rate constant of fluorescence quenching in this system, and determined what they believed was the equilibrium constant for its formation. An alternative interpretation of these results was provided by Noyes [50] in 1957, who showed that the magnitude of the supposed equilibrium constant increased with temperature, contrary to expectation for a ground state complex [51]. Ware and Novros [52] measured both Φ^0/Φ and the ratio of the fluorescence lifetime with and without quencher, τ^0/τ, for the same system, and with these data in hand also reinterpreted the results of Bowen and Metcalf. Ware and Novros [52] showed that very good agreement between their experimental results and the predictions of the Smoluchowski theory could be obtained by including a term describing non-stationary diffusion and also taking the effect of complex formation between anthracene and carbon tetrabromide (so-called pseudoquenching [19]) into account. Ware and Novros [52] and others showed that when non-stationary diffusion was accounted for, the Stern–Volmer equation can be expressed as follows in the low quencher concentration regime:

$$\frac{\Phi^0}{\Phi}=1+4\pi R'DN'\left(1+\frac{R'}{\sqrt{D\tau^0}}\right)\tau^0[Q] \tag{30}$$

The data on anthracene fluorescence quenching by carbon tetrabromide were also analyzed by Stevens [53] who employed the nearest-neighbor quenching model and obtained the following equation:

$$\frac{\Phi^0}{\Phi}=1+\frac{4\pi RDN'k_r[Q]}{4\pi RDN'+k_r[1-(2\pi N'[Q])^{1/3}R]} \tag{31}$$

where k_r is the internal rate constant of the reaction describing the quenching within A* − Q encounter pairs. The values of D and R obtained by Stevens are in very good agreement with the values given in Ref. [52]. Eq. (31) has subsequently been used successfully in the analysis of a rather large number of systems involving an excited donor, quencher and fluid viscous solvent [53–56].

Until the early 1970s, the experimentally observed decays of donor fluorescence in the presence of a quencher, $I(t)$ (including those of Ware and Novros [52]), were measured using equipment which had insufficient time resolution to permit reliable observation of the expected effects of non-stationary diffusion. Consequently, these decays were described exclusively by single-exponential decay functions and were interpreted using the classical or modified Stern–Volmer equations in which Φ^0/Φ is expressed as a function of both the quencher concentration and the exponential lifetime [30–32,57–61].

5.2. Experiments of Ware, Andre and collaborators

In a long series of papers starting in 1973, Ware and Nemzek [18,20], Andre et al. [46,64,65,67,68,70,71,75–77,90], Kruger [62], Peak et al. [63], Viriot et al. [66], Donner et al. [69], Bouchy et al. [72], Rima et al. [73], Baros and Andre [74], Broke et al. [78], Baros et al. [79,81,82], Dong et al. [80,83], Lewis and Ware [84–86], Ware et al. [87,88], Hui and Ware [89] proposed that a time-dependent reaction rate coefficient should be used in modeling the kinetics of fluorescence quenching, and showed that such a model can be used successfully in describing many, often complex, photophysical and photochemical systems, including: (i) simple fluorescence decay [18,20,52]; (ii) the kinetics of reversible reactions [84,89]; (iii) fluorescence quenching when the quencher affects more than one excited state [67]; (iv) the kinetics of the formation and decay excimers [77,84]; (v) the kinetics of the formation and decay of exciplexes [84,86–89]; (vi) the kinetics of fluorescence quenching taking into account static quenching; (vii) the solvent cage effect on the photolysis of benzyl chloride [71]; (viii) an explanation of the discrepancies often observed between the results of Stern–Volmer analyses obtained under conditions of pulsed compared with continuous excitation

[46,47]; and (ix) fluorescence quenching in membranes [68,74,81].

In 1973 Ware and Nemzek [18] were the first to report the direct observation of a fluorescence decay exhibiting other than single-exponential character, for the 1,2-benzanthracene/carbon tetrabromide quencher/propane-1,2-diol solvent system, as shown in Fig. 2. This was followed in 1975 by a more detailed report in which they also described the quenching of naphthalene fluorescence and reported both steady state and time-resolved emission measurements.

As expected the plots of the steady state emission intensity ratios I_0/I vs. [Q] were nonlinear, exhibiting positive deviations from linearity at higher quencher concentrations. To rationalize these results Ware and Nemzek employed the following general expression for the steady state I_0/I ratio:

$$\frac{I_0}{I}=\frac{\int\limits_0^\infty I_0(t)\mathrm{d}t}{\int\limits_0^\infty I(t)\mathrm{d}t} \tag{32}$$

where $I_0(t)=I(0)\exp(-t/\tau^0)$ is the time-resolved single exponential decay of the donor's fluorescence in the absence of a quencher, and $I(t)$ has the form of Eq. (12). Inserting these expressions into Eq. (32) gives the following modified form of the Stern–Volmer equation:

$$\frac{I_0}{I}=\frac{1+4\pi R'DN'[\mathrm{Q}]\tau^0}{1-\frac{Y}{\sqrt{X}}\sqrt{\pi}\exp\left(\frac{Y^2}{X}\right)\mathrm{erfc}\left(\frac{Y}{\sqrt{X}}\right)} \tag{33}$$

where:

$$X=1/\tau^0+4\pi R'DN'[\mathrm{Q}]$$

$$Y=4(R')^2\sqrt{\pi D}N'[\mathrm{Q}]$$

$$\mathrm{erfc}(x)=\frac{2}{\sqrt{\pi}}\int\limits_x^\infty \mathrm{e}^{-z^2}\mathrm{d}z$$

If a 1:1 complex is formed between the ground state donor and the quencher, the dependence of the steady-state emission intensity ratio can be expressed [20] as follows:

$$\left(\frac{I_0}{I}\right)_{\mathrm{ss}}=(1+K_l'[\mathrm{Q}])\frac{1+4\pi' DN'[\mathrm{Q}]\tau^0}{1-\frac{Y}{\sqrt{X}}\sqrt{\pi}\exp\left(\frac{Y^2}{X}\right)\mathrm{erfc}\left(\frac{Y}{\sqrt{X}}\right)} \tag{34}$$

where:

$$K_l'=\frac{\varepsilon_{\mathrm{AQ}}}{\varepsilon_{\mathrm{A}}}K_1,$$

$$K_1=\frac{[\mathrm{AQ}]}{[\mathrm{A}][\mathrm{Q}]}$$

Here $\varepsilon_{\mathrm{AQ}}$ is the molar extinction coefficient of the AQ complex and ε_{A} is the molar extinction coefficient of the donor. Ware and Nemzek obtained a better fit between the experimental values of I_0/I and Eq. (34), and this prompted them to recommend taking non-stationary diffusion and ground state donor–quencher complexation into account in many excited donor–quencher–solvent systems. The results of the steady-state measurements reported in Refs. [18,20] were subsequently reanalyzed by many authors who determined, for example, if the results of 1,2-benzanthracene fluorescence quenching could described by other formulations of the Stern–Volmer equation [46,47,53,62,63,91–93].

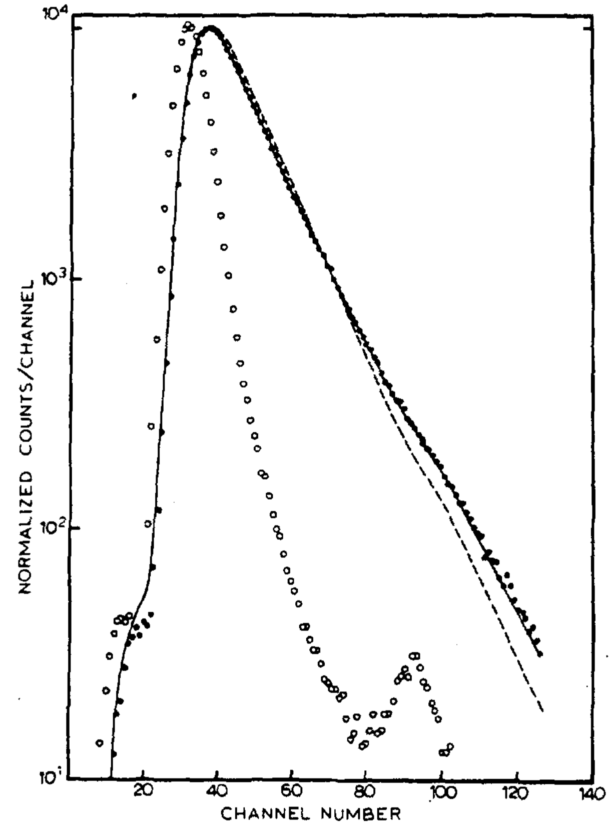


Fig. 2. Fluorescence decay of 1,2-benzanthracene in propane-1,2-diol quenched by $\mathrm{CBr_4}$. The concentration of $\mathrm{CBr_4}$ is 0.29 M and the temperature is 30°C. The dashed line represents the best fit to a single-exponential function, and the solid line represents the best fit using the Smoluchowski model. Solid points are experimental data, and open points are the instrument response function (from Ref. [18]).

The kinetics of the reversible process of exciplex formation and decay can be modeled on the following scheme:

$$\mathrm{A}\xrightarrow{h\nu}\mathrm{A}^*$$

$$\mathrm{A}^*\xrightarrow{1/\tau^0}\mathrm{A}$$

$$\mathrm{A}^*+\mathrm{Q}\xrightarrow{k(t)}\text{Exciplex},$$

$$\text{Exciplex}\xrightarrow{k_{\mathrm{b}}}\mathrm{A}^*+\mathrm{Q},$$

$$\text{Exciplex}\xrightarrow{k_{\mathrm{p}}}\mathrm{A}+\mathrm{Q}\text{ or Products}$$

where k_{b} and k_{p} are the rate constants of exciplex decay. Based on this kinetic scheme, Hui and Ware [89] derived the following approximate expression for the Stern–Volmer constant K_{SV}:

$$\underset{[Q]\to 0}{K_{SV}} = 4\pi R'DN'\tau^0\left(\frac{k_p}{k_b+k_p}\right)\left(1+\frac{R'}{\sqrt{\tau^0 D}}\right) \tag{35}$$

Independent measurements of 1,2-benzanthracene fluorescence quenching by carbon tetrabromide in propane-1,2-diol were performed in 1980 by Solie et al. [94] who, like Ware and Nemzek [20], analyzed the fluorescence decays using Eq. (13). However, the results reported in Ref. [94] are appreciably different from those given in Ref. [20]. For instance for the same quencher concentration (0.1 mol dm^{-3}), the effective radius R' determined from the data of Ref. [94] is 1 Å, while the value calculated from the data from Ref. [20] is 8.5 Å. Similarly, in 1987, Lakowicz et al. [95] also studied the kinetics of 1,2-benzanthracene fluorescence quenching by carbon tetrabromide in propane-1,2-diol, and analyzed the data using the Eq. (3). Their results at 288 K (for $[CBr_4]=0.1$ mol dm^{-3} and $T=288$ K) are very different from those given both in Refs. [20,94], e.g., $R=$ 147 Å. In our view, the differences in the results reported in Refs. [20,94,95] illustrate only the effects of using different experimental and data analysis techniques; in Ref. [95] the frequency-domain technique was employed, and in Ref. [94] the method of moments was used for data analysis. Careful inspection reveals similar discrepancies in other systems examined by different techniques—for example indole fluorescence quenched by acrylamide and iodide in water using frequency-domain [95,96] and time-domain [97] techniques.

Non-exponential fluorescence decay in the presence of a quencher has been observed in many other excited donor–quencher–solvent systems. Of note, Beddard et al. [98] used Eqs. (12), (33) and (34) in their analysis of the kinetics of chlorophyll-*a* fluorescence quenching by nitrobenzene in ethanol. Wijnaendts van Resandt [99] studied the kinetics of *N*-acetyltrytophanamide (NATA) fluorescence quenching by potassium iodide in water by time-domain methods and analyzed the data using Eq. (3), i.e., the Smoluchowski model. Wijnaendts van Resandt reports values of $D=1.12\times10^{-5}$ cm^2 s^{-1} and $R=3.4$ Å at 22°C, which are in good agreement with the values $D=1.2\times10^{-5}$ cm^2 s^{-1}, $R=3.8$ Å at 25°C reported later by Periasamy et al. [28], also obtained by time-domain methods but now using Eq. (9) (the SCK model). These results suggest that the data obtained from time-domain quenched fluorescence experiments are reproducible.

6. Application of the SCK model

In a series of papers, Andre et al. [77], Martinho and Winnik [100], Siennicki and Winnik [101], Martinho et al. [102,103], Xu et al. [104], Xu and Winnik [105], Strukelj et al. [106] have used the Smoluchowski–Collins–Kimbal model to describe the kinetics of intramolecular excimer formation. From observations of spectrally resolved fluorescence of the monomer and excimer, they have determined, among other things, the activation energy of excimer formation, the effective distance at which excimer is formed, and the diffusion coefficients in many systems. For instance for methyl 4-(1-pyrene)butyrate in toluene these values at 295 K, are: 10.5 kJ mol^{-1}, 7.6 Å, 15.0×10^{-6} cm^2 s^{-1}, respectively [100]. Using SCK theory, Bohne et al. [107] have determined the lifetimes of the short-lived T_2 states of a number of anthracene derivatives; typical values for 9,10-dichloroanthracene, anthracene and 1-methylanthracene are 23 ps, 11 ps, and 3 ps, respectively. The SCK model has also been used to interpret the results of experiments involving the quenching of short-lived triplet states [107–109].

The SCK model of diffusion-limited fast reactions can be particularly useful in interpreting the results of investigations of the photochemical and photophysical properties of compounds of biological importance [110]. Its application to the electronically excited states of proteins is the subject of Refs. [111,112].

The first reports of non-exponential fluorescence decay of an excited donor in the presence of a quencher confirmed the earlier theoretical predictions and conclusions obtained from determinations of the fluorescence quantum yields, Φ^0/Φ. The ability to record such non-exponential decays became possible as a result of new technological developments [99], new methods of calculation of the instrument response function, $E(t)$ [18,20], and the use of instruments using different methods of detection [98]. A more systematic investigation of the kinetics of fluorescence decay in terms of the SCK theory became possible only near the end of the 1980s thanks to the advent of equipment having substantially improved time resolution, e.g., TCSPC systems employing microchannel plate photomultiplier tubes as detectors [15–17]. This progress is tabulated in Table 1.

Table 1
The characteristics of time-correlated single photon counting equipment used to study SCK kinetics

FWHM	Time/ channel	Year and reference
16700 ps	390 ps	1978 [98]
11400 ps	338 ps	1973 [18], 1975 [20]
2500 ps	200 ps	1992 [123], 1993 [124]
2500 ps	123 ps	1980 [94]
2000 ps	?	1987 [100], 1989 [103], 1991 [104]
400 ps	4.13 ps	1992 [140]
280 ps	42 ps	1988 [24]
150 ps	45 ps	1983 [99]
110 ps	36.2 ps	1988 [116]
90 ps	20.8 ps	1991 [118]
80–90 ps	10.4 ps	1990 [28]
77 ps	10.4 ps	1989 [97]
70 ps	4.6 ps	1990 [39]
65 ps	4.6 ps	1989 [117]
35 ps	4.9 ps	1991 [125]
35 ps	2.75 ps	1993 [141], 1995 [143]

A considerable improvement in the ability to extract valuable information from studies of the kinetics of fluorescence decay has come about with the advent of frequency-domain methods. In 1987, Lakowicz et al. [95] and Joshi et al. [113] studied indole fluorescence quenching by acrylamide and potassium iodide in water as well as 1,2-benzanthracene fluorescence quenching by carbon tetrabromide in propane-1,2-diol by frequency-domain methods, and were the first to analyze the results using the SCK model (Eq. (1) with a reaction rate coefficient $k(t)$ given by Eq. (4)). This produced a much more accurate description of the fluorescence decay than obtained with Eq. (12) [95,113,114]. The values of the parameters determined using SCK theory are in good agreement with those obtained by independent methods, in contrast to the situation when Eq. (12) was used.

In 1988, a series of papers devoted to the kinetics of fluorescence quenching were published [28,115–118], involving the following systems: *n*-acetyltryptophanamide, indole, cresyl violet, zinc tetraphenylporyphyrin, and rhodamine-B donors with potassium hexacyanoferrate (III), potassium hexacyanoferrate (II), ferric chloride, potassium bromate, sodium azide, potassium rhodanate, 1,4-benzoquinone, 1,4-tetrachlorobenzoquinone, potassium iodide and acrylamide as quenchers, all in aqueous solution. Analysis of the fluorescence quenching data was carried out assuming a time-dependent reaction rate coefficient. On the basis of the experimental and numerically generated data describing the donor fluorescence decay in the presence of a quencher, it was showed that the data from TCSPC systems can be successfully analyzed in terms of SCK theory. The results of their studies led to the following conclusions: (i) the kinetics of indole fluorescence quenching by acrylamide in water is well-described by the Smoluchowski model [28]; (ii) the kinetics of indole and cresyl violet fluorescence quenching by potassium iodide in water are well-described by the SCK function [28,116]; (iii) the long-time, type A approximation provides a good description of the kinetics of cresyl violet and indole fluorescence quenching by potassium iodide, while the long-time, type B approximation and the Smoluchowski model give unsatisfactory descriptions [97]; (iv) the kinetics of *n*-acetyltryptophanamide fluorescence quenching with potassium iodide and acrylamide as quenchers can not be satisfactorily described by either the Smoluchowski model or the SCK function [28]; (v) the kinetics of fluorescence decay of none of the systems involving rhodamine-B or cresyl violet as donors and a large number of different quenchers could be described by the long-time, type B approximation [116].

In these works, Joshi et al. [28], Das and Periasamy [115], and Periasamy et al. [116] demonstrated for the first time the significant effect of making an arbitrary choice of the value of the channel shift parameter, *S*, on the values of other parameters calculated in the work-up of TCSPC data. They pay particular attention to appropriate ways of estimating this parameter; however, the method they propose has limited application as it can be used only when the expected values of the other parameters are known.

Dutt and Periasamy [118], also applying the SCK model, determined the changes in the effective distance at which an electron can be transferred from the excited donor to the quencher. They studied the quenching of the fluorescence of zinc tetraphenylporphyrin (ZnTPP) by 1,4-benzoquinone (BQ), and 1,4-tetrachlorobenzoquinone (chloranil, CA) in toluene and acetonitrile. On the basis of their measurements and calculations performed assuming the SCK function (Eq. (5)) and Smoluchowski function (Eq. (3)), they have shown that the distance at which fluorescence can be quenched is by 3 to 5 Å greater for ZnTPP-CA than for ZnTPP-BQ, in both solvents. For example, the reported values of the intrinsic reaction rate constant in acetonitrile are $k_r = (6.6–96) \times 10^{10}$ dm^3 mol^{-1} s^{-1} for ZnTPP-BQ and $k_r = (11–188) \times 10^{10}$ dm^3 mol^{-1} s^{-1} for ZnTPP-CA.

7. Some problems with the SCK model

7.1. Quenching by electron transfer

Eads et al. [39,117] have questioned the earlier successes of the SCK theory in describing the kinetics of fast quenched fluorescence decay in their studies of rhodamine-B fluorescence quenching by potassium ferrocyanide ($K_4Fe(CN)_6$). In these experiments they used both the fluorescence upconversion and the TCSPC detection methods, and provided a comparison of the results obtained by the two measurement techniques.

Analyzing the results reported in Ref. [117] obtained with TCSPC, the authors showed that the best fit to the experimental donor fluorescence decay in the presence of a quencher is obtained with Green's function, as proposed in Ref. [37]. The values of the Green's function parameters determined from measurements with the two methods employed are almost independent of quencher concentration. However, the sets of values obtained are very different for the two methods. To illustrate the difference, Eads et al. used the following equation from dispersion kinetics [119–121]:

$$I(t)=I(0)\exp(-t/\tau)^{\alpha_d} \qquad (36)$$

Values of α_d obtained by the TCSPC and the upconversion methods were $\alpha_d = 0.90$ and $\alpha_d = 0.55$, respectively. Due to various technical limitations, the fluorescence decay could be observed in the range 0–4000 ps in the former method, but only from 0–200 ps in the latter. These authors conclude that the DSCK theory is not satisfactory for reaction times $t <$ 200 ps.

In Ref. [39], Eads et al. also studied rhodamine-B fluorescence quenching by potassium ferrocyanide, and analyzed the results using; (i) the short and intermediate time model, (ii) the Smoluchowski model, (iii) the Debye SCK approximation, and (iv) the SCK function with an empirical added term describing static quenching. In this case the fluorescence decay of the donor in the presence of a quencher was described by the following equation:

$$I(t)=I(0)\exp\left[-v[\mathrm{Q}](1-\mathrm{e}^{-t/\tau_{\mathrm{p}}})-\frac{t}{\tau^{0}}-[\mathrm{Q}]\int_{0}^{t}k(t')\mathrm{d}t'\right] \tag{37}$$

where $k(t)$ is the reaction rate coefficient defined in Eq. (4), τ_{p} is the lifetime of an $[\mathrm{A}^{*}\cdots\mathrm{Q}]$ contact pair, $v=4/3\pi(\sigma^{3}-R^{3})$ is the volume surrounding a given donor in which the fluorescence quenching reaction takes place, and σ is the effective distance at which the fluorescence quenching reaction takes place.

The rates of electron transfer reactions can also be described using the Wilemski–Fixman form [122] of the theory of nonadiabatic electron transfer. In this case the reaction rate coefficient was found numerically, using the diffusion equation:

$$\frac{\partial p(r,t)}{\partial t}=D\nabla^{2}p(r,t)-k(r)p(r,t) \tag{38}$$

where $p(r,t)$ is the radial distribution function. The reaction rate coefficient, $k(r)$, is defined as follows:

$$k(r)=k(0)\exp[-\beta(r-R)] \tag{39}$$

where $k(0)$ is the 'inner' rate of non-adiabatic electron transfer where $r=R$, (here R is the encounter distance), and β is a constant determining the distance dependence of the electron transfer rate. The reaction rate coefficients $k(t)$ and $k(r)$ are related via Eq. (40):

$$k(t)=\int k(r)p(r,t)\mathrm{d}v \tag{40}$$

Eads et al. [39,117] give numerous examples showing that none of the models provide a uniform or quantitatively correct description of all the available experimental data, and conclude that none of them are completely adequate. They suggest that these inadequacies can be attributed to oversimplifications within the models; too many important physical factors are neglected, e.g., a dependence of the inner reaction rate constant on the geometry of the excited donor–quencher–solvent system, and the dynamics of the interactions between donor and quencher molecules within the solvent 'cage'.

Scully et al. [123,124] recently considered the quenching of fluorescence of 5,10,15,20-tetrakis-(4-sulphonatophenyl)-porphine ($\mathrm{H_2TPPS^{-4}}$) by methylviologen ($\mathrm{MV^{2+}}$) in water (cf. Fig. 3). Using Eq. (24) these authors determined that the distance at which the reaction between the excited donor and quencher takes place is 14 Å and the intrinsic reaction rate constant is 2.7×10^{10} dm^3 mol^{-1} s^{-1}. The value calculated for the intrinsic rate constant for this system is characteristic of the so-called normal Marcus region. The authors emphasize the role of the ionic strength in modifying the kinetics of fast reactions between ions in solution and use this argument to explain the negative conclusions obtained by Eads et al. [39,117].

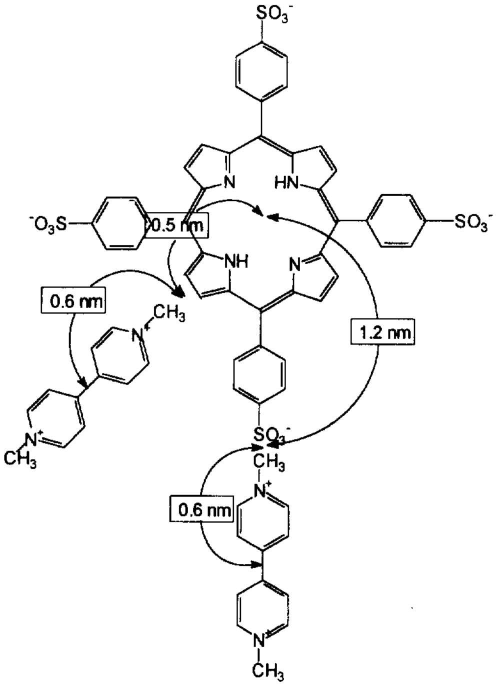


Fig. 3. An analysis of the bimolecular fluorescence quenching reactions between 5,10,15,20-tetrakis-(4-sulfonatophenyl)-porphine and methylviologen in aqueous solution used to determine the reaction distance and the orientation of reacting molecules. After Ref. [123].

Nishikawa et al. [125], Matsuda et al. [126], and Kakitani et al. [127] have used various donor–acceptor pairs with $-\Delta G^{0}$ in the range from 0.37 eV up to 2.21 eV to study the mechanism of photoinduced intermolecular charge separation in acetonitrile solutions using the time-dependent fluorescence quenching method. As electron donors these authors used 9,10-diphenylanthracene, anthracene and 9-methylanthracene, and the acceptors were fumaronitrile, maleic anhydride, 1,4-dicyanobenzene, phthalic anhydride, tetracyanoethylene, pyromellitic dianhydride and tetrachlorophthalic anhydride. The function they used to described the fluorescence decay is of the same form as the SCK function, Eq. (5). The values of the internal reaction rate constants they determined are all larger than those of diffusion-controlled processes, and fall within the range from 2.3×10^{10} dm^3 mol^{-1} s^{-1} to 57.0×10^{10} dm^3 mol^{-1} s^{-1}. Although these electron transfer reactions are very fast, their dependence on $-\Delta G^{0}$ is rather flat. However, the values of the rate constants obtained are scattered, perhaps a reflection of the influence of the character of individual donor–acceptor pairs rather than the effect of $-\Delta G^{0}$.

Murata et al. and Tachiya et al. [128–130] have shown how the Marcus theory can be incorporated into the SCK theory when fluorescence quenching occurs by an electron transfer process. As donors they used 9-cyanoanthracene and 9,10-dicyanoanthracene, as quenchers *p*-anisidine, *N*,*N*-dimethylaniline and aniline in ethylene glycol at 25°C. According to the model used by Murata et al. [128,129], the fluorescence decay function, $P(t)$, is given by the following equation (see also Ref. [145]):

$$P(t)=\exp\left(-\frac{t}{\tau^0}-4\pi[\mathrm{Q}]\int_R^\infty[1-U(r,t)]r^2\mathrm{d}r\right) \tag{41}$$

where R is the sum of the molecular radii of the donor and acceptor, $U(r,t)$ is the survival probability of an $\mathrm{A}^*\cdots\mathrm{Q}$ or $\mathrm{A}\cdots\mathrm{Q}^*$ pair, initially (at $t=0$) separated by distance r. In order to calculate $P(t)$ it is necessary to determine $U(r,t)$ from the diffusion equation, which is written in the form:

$$\frac{\partial U(r,t)}{\partial t}=D\nabla^2U(r,t)-k(r)U(r,t) \tag{42}$$

where D is sum of the diffusion coefficients of the donor and acceptor. Here $k(r)$ is the first-order quenching coefficient, which according to Marcus theory can be expressed as follows:

$$k(r)=\frac{2\pi}{\hbar}J_0^2\exp[-\beta(r-r_0)]\frac{1}{\sqrt{4\pi\lambda k_\mathrm{B}T}}\times\exp\left[-\frac{(\Delta G+\lambda)^2}{4\lambda k_\mathrm{B}T}\right] \tag{43}$$

where J_0 is the magnitude of the electronic coupling energy between the reactant and product states at $r=r_0$ and β is its attenuation coefficient. The reorganization energy λ also depends on r:

$$\lambda=\frac{\mathrm{e}^2}{2}\left(\frac{1}{\varepsilon_\mathrm{op}}-\frac{1}{\varepsilon_\mathrm{s}}\right)\left(\frac{1}{a}+\frac{1}{b}-\frac{2}{r}\right) \tag{44}$$

Here ε_op and ε_s are the optical and static dielectric constants respectively, and a and b are the molecular radii of A and Q. These authors conclude that the modified Marcus equation gives much better results than the SCK theory. However, they also point out that although their time-correlated single-photon counting system had a FWHM ≈ 60 ps, measurements with greater time resolution would be necessary to study these electron transfer reactions in more detail.

Lakowicz et al. [131,132] and Zelent et al. [133] have examined the quenching of *p*-bis-2-(5-phenyloxazolyl)]-benzene (POPOP) by carbon tetrabromide, anthracene by *N*,*N*-diethylaniline, and *N*-acetyl-L-tryptophanamide by acrylamide. Their time-resolved collisional quenching data could not be satisfactorily described by the SCK model, and they therefore introduced a distance-dependent rate of quenching [134], in contradiction to the SCK theory where a finite (specific) rate constant for quenching, κ, is assumed. The differences between these two models of collisional fluorescence quenching are shown diagrammatically in Fig. 4. For the SCK model $k(r)=0$ when $r>a$, and the rate of quenching is described by the interaction of the donor and quencher at the interaction distance. In the distance-dependent rate of quenching model (DDQ) the rate of quenching is dependent on the distance between the donor and quencher and is given by the expression,

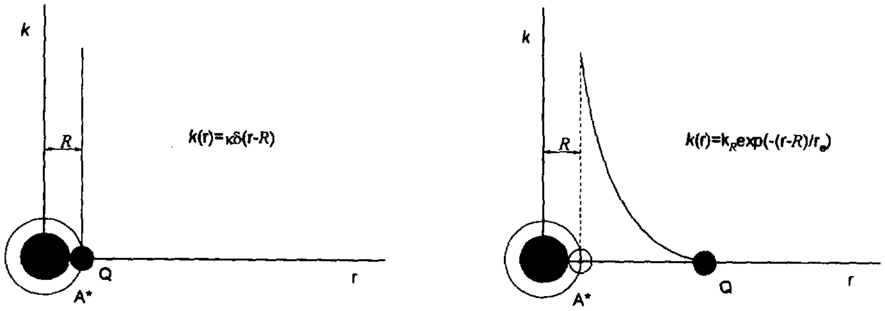
$$k(r)=k_\mathrm{a}\exp[-(r-R)/r_\mathrm{e}] \tag{45}$$

where k_R represents the value of $k(r)$ at the encounter distance $r=R$. Here, R is the distance of closest approach, and r_e is the parameter, characteristic of the interaction, that defines the decrease of $k(r)$ with distance.

Lakowicz et al. and Zelent et al. used the global analysis method in treating their data and noted that the simultaneous analysis of frequency-domain and steady-state data helped significantly in improving the molecular parameters which they recovered. In the future, this kind of analysis is likely to provide more insight into fast reactions studied by fluorescence quenching.

Recently, Shannon and Eads [135] examined the quenching of 7-aminocoumarin fluorescence by aniline and *N*,*N*-dimethylaniline in methanol. They showed that the Smoluchowski model does not describe the fluorescence quenching data in either system, and that the SCK model was acceptable only for the 7-aminocoumarin/aniline system. Their analysis showed that the electron transfer quenching process exhibited a strong donor–acceptor distance dependence, and that it could be treated successfully using the Wilemski–Fixman approximation [122], Eqs. (38)–(40). Within this framework, they interpreted electron transfer in the 7-aminocoumarin/aniline system as a purely nonadiabatic process, whereas *N*,*N*-dimethylaniline showed both adiabatic and nonadiabatic characteristics.

Yabe et al. [136] have also shown recently that even the Smoluchowski model can be used to describe the quenching



ig. 4. Schematic comparison of the Smoluchowski–Collins–Kimball model (on the left) with the distance dependent quenching model (on the right). After ef. [134].

of coumarin-151 with *N*,*N*-dimethylaniline at low concentrations. However, at higher concentrations neither the Smoluchowski model nor the Wilemski–Fixman approaches were adequate. To solve this problem and reproduce the experimental data, these authors used an empirical Lennard–Jones potential between the donor and quencher to establish the spatial distribution of the quencher near the donor.

7.2. *Quenching by neutrals*

If one is to study fast bimolecular reactions using some form of the Smoluchowski–Collins–Kimball theory, it is essential to determine the values of at least three parameters, R, D, and κ, because these parameters enter the equation describing the rate coefficient of the reaction, $k(t)$. However, experimentally one determines the values of other parameters. In the case of fluorescence decay measurements these are τ^0, S, G_0 and τ_r, where S is the time-shift parameter which is often used in the analysis of experimental fluorescence data to correct primarily for the wavelength dependence of the instrument response function [5–7,15,99], and G_0 is a scaling factor. The experimentally measured fluorescence decay, $D(t)$, is the convolution of the instrument response function, $E(t)$, and the fluorescence decay function, $I(t)$, i.e.,

$$D(t)=E(\lambda_{ex},\lambda_{em},t)\otimes I(t) \tag{46}$$

The problem appears to be simply one of determining the function $I(t)$ when both $D(t)$ and $E(t)$ are known. In practice, however, detailed studies of fast quenching processes have serious limitations from both theoretical and experimental points of view [5–7,125,126].

There are many ways to treat the data from fast fluorescence quenching experiments. However, even when the best of data treatment methods is used (e.g., the non-linear least squares method [4–6]), difficulties are still experienced when calculations are performed on experimental time-resolved fluorescence decay data for purposes of recovering the values of parameters in the SCK function, Eq. (6). These difficulties have their origins in limitations of both the numerical methods and experimental conditions employed. The experimental difficulties in studying the kinetics of fluorescence quenching of compounds with short fluorescence lifetimes are largely associated with instabilities of the working apparatus, measurement uncertainties, and systematic errors. These factors all cause a deformation (relative shift) of the observed fluorescence decay signals of the sample and the reference compound, and result in propagated errors in the calculated parameters.

Previous theoretical work [1,8,9,42] has suggested that investigations of the kinetics of quenching involving compounds with very short fluorescence lifetimes would be a promising avenue of approach to obtaining more definitive experimental information for modeling studies. The aromatic thioketones are such donors; they have S_2 state lifetimes in perfluoro-1,3-dimethylocyclohexane at room temperature varying over a wide range from 857 ps for 2,2,3,3-tetramethylindanethione to 53 ps for thioxanthione [137–140]. For xanthione, with a lifetime of 178 ps, and 1,3,4,5,6,8-xanthione-d_6, with a lifetime of 600 ps, the kinetics of quenching can be described satisfactorily by the SCK theory [139,141–143]. The parameters such as the sum of donor and quencher radii and the sum of their diffusion coefficients, obtained from this theory, are in rather good agreement with the corresponding values determined by other methods. Unfortunately, a rather large uncertainty in the determination of the proper rate constant κ [139,141–145] prevents obtaining an unambiguous answer to the question of whether the large values of κ are characteristic of the systems studied or instead are a consequence of the model employed. Also the mechanism of quenching of the S_2 states of the thioketones by hydrocarbons is not yet clear.

In order to understand how these experimental difficulties can affect the ability to recover meaningful values of parameters, we have used numerical simulations to control the magnitudes of each source of error, and have used the results of these simulations to test the feasibility of distinguishing among the various models of fluorescence quenching kinetics [144,145]. We have also used simulations to understand the possibilities for advances in our understanding of fast kinetic processes by doing experiments using short-lived fluorophores. Briefly, fluorescence decay data for the sample and the reference compounds were generated by convolution of an SCK function and a single experimental decay function, respectively, with the same unsmoothed instrument response function. The data were collected at 2.75 ps/channel resolution. Two real instrument response functions were used, one with the full width at half maximum (FWHM) equal to 35 ps and another with FWHM = 400 ps. Pre-exponential factors were adjusted to obtain about 40000 as the maximum number of counts in the peak channel. Each generated pair $D(t)$ and $D_r(t)$ had different and independent additions of noise, following recommended procedures [4].

These simulations were limited to a series of hypothetical fluorophores with emission lifetimes shorter than 1 ns, corresponding to the measured unquenched lifetimes of seven thioketones in perfluoro-1,3-dimethylcyclohexane solvent at room temperature [137–141]. The results show that it is possible to determine the parameters of the SCK model from the measured decay curves using the non-linear least-squares fitting technique if sufficiently short excitation pulses and a detector with adequate time resolution are employed. These simulations have also revealed that some parameters are strongly correlated, which explains the difficulties often encountered in fitting decay curves using the SCK theory (Figs. 5 and 6). In particular, these simulations have revealed how the donor lifetime, the channel shift parameter (S), the FWHM of the instrument response function and other parameters used in these modeling calculations can affect the ability to extract the true values of the desired optimized parameters; R, D, κ, S, τ^0, and G_0. The uncertainties in these parameters have also been estimated from this series of simulations, and the results provide experimentalists with a guide

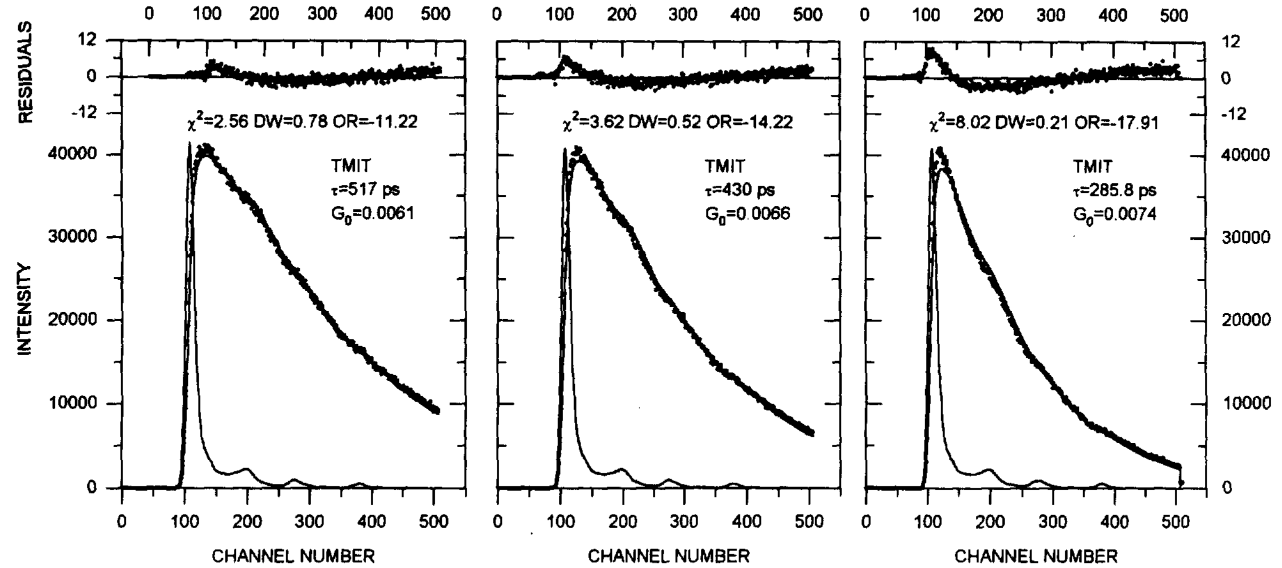


Fig. 5. Typical fitting results: the simulated curve, for TMIT with three different concentrations of quencher, was generated using the SCK function, while the fitted curve is the best fit to a single-exponential. The instrument response function (dashed lines), simulated data (dots) and fitted curves (solid lines) are shown. Plots of weighted residuals are given at the top. The respective fitted parameter values are shown in the Figure, along with the statistical test values. Both the statistical test values and the plots of weighted residuals indicate a poor fit. Note that the quality of fit improves for the lower concentration of quencher. After Ref. [146].

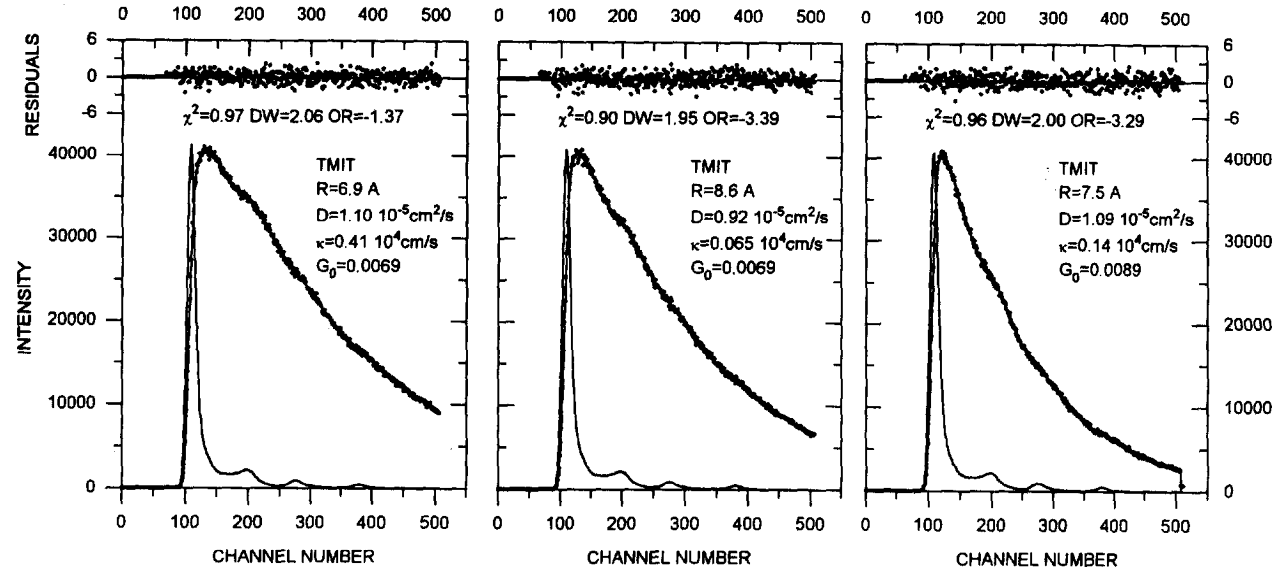


Fig. 6. Typical fitting results for TMIT with three different concentrations of quencher shown in the Figure: both the simulated and the fitted curves are described by the SCK function. The information shown is the same as in Fig. 5. Both the statistical test values and the plots of weighted residuals indicate a good fit. After Ref. [146].

for ensuring that the parameters they obtain from fitting fluorescence decays are accurate. With such simulations in hand, experimentalists should be able to choose optimal conditions for their experiments.

On the basis of the calculations presented in Refs. [139,144,145], it is also easy to understand why Nemzek and Ware were the first to obtain good results using the SCK model for fluorescence quenching. Ware and Nemzek used 1,2-benzanthracene (which has a lifetime of 38.5 ns [18,20]) as a fluorophore, 1,2-propanediol as a high viscosity solvent, and a TCSPC apparatus with a FWHM = 11.4 ns. On the basis of simulations performed for these conditions, Ware and Nemzek should have been able to observe non-exponential SCK kinetics using compounds with fluorescence lifetimes longer than 32 ns, as is the case for 1,2-benzanthracene.

As a result of the above studies we are now able to make recommendations for a set of 'best' experimental conditions and data analysis procedures. First, it is desirable to have data taken simultaneously from several types of experimental measurements (steady state, time-resolved time domain, time-resolved frequency domain) on the same donor–quencher–solvent system. Second, it is desirable in the first instance to design the experiment to test the model, and to choose as simple a system as possible for this purpose: electrically neutral donor and quencher; quenching by intermolecular energy (not electron) transfer with no photochemical complications; minimal association between donor and quencher in both the ground and excited states (weakly interacting species at low concentrations); quencher, donor and solvent independently well-characterized. In later experiments, it would actually be desirable to find systems in which offer more than one experimental observable, for example fast exciplex or quencher fluorescence—the rise and decay of which could be followed in addition to the donor's decay. Excitation light intensity must also be sufficiently low to avoid multiphoton excitation or ground state depletion of the absorber. The temperature dependence or the kinetics should be examined.

In recent papers [143–145], we strongly recommended the use of reference methods to minimize or eliminate errors which are introduced in treatments of TCSPC data by variations in the instrument response function, $E(t)$, with wavelength and count rate. Unfortunately, in almost every study of SCK kinetics to date the temporal instrument response function has been obtained from light scattering measurements, a procedure which can introduce both random and systematic errors into $E(t)$. In our experiments we now use the reference method to minimize the problems associated with variability in the instrument response function with emission wavelength and count rate, and in our calculations we use the non-linear least-squares fitting technique exclusively. In the order to acquire the most reliable results, we also recommend using the sequence of measurements previously described in Refs. [141–143]. Use of the sequence donor plus quencher, followed by standard fluorophore, followed by reference fluorophore helps eliminate instabilities in TCSPC equipment over medium to long data acquisition times. Using this procedure has helped us avoid, in part, the difficulties associated with attempting to determine too many parameters from a single convolution of a complex non-exponential fluorescence decay function [141,143] with a measured instrument response function.

There are still many unresolved problems associated with the acquisition and analysis of fast fluorescence quenching data. The theories presently in use describe the kinetics of fluorescence quenching in solutions in which continuity of the medium is assumed. This assumption necessarily neglects the submicroscopic structure of solvents and phenomena related to this structure. Thus, one of the most important remaining questions in this field involves assessing when the macroscopic description of the medium ceases to yield satisfactory results. In addition, there are still many problems inherent in the methods available to experimentalists [4–6,15]. In this respect, it would be very useful if researchers in the field were to exchange both experimental data and data analysis programs as a means of converging on standard, accepted methodologies. Although much more is to be learned, it is clear that our understanding of the kinetics of fast bimolecular processes has greatly improved in the 25 years since the first observation of non-exponential fluorescence quenching.

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