

Numerical Simulations of Thione $S_2 \rightarrow S_0$ Fluorescence Decay: An Aid for Modeling Fast Quenching Kinetics

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Numerical simulations designed to illuminate the opportunities for and limitations on recovering the parameters in the Smoluchowski–Collins–Kimball (SCK) model of time-dependent fluorescence quenching from the data of time-correlated single-photon counting experiments have been carried out. Two “donors” with lifetimes of 880 and 410 ps, corresponding to the unquenched S_2 lifetimes of 2,2,3,3-tetramethylindanethione (TMIT) and 9,9-dimethylanthrathione (DMAT) in inert perfluoroalkane solvents at room temperature, were used for purposes of illustration. The failure of the single-exponential decay model due to the transient effect can be detected at lower quencher concentrations for longer-lived fluorophores. While good values of the diffusion coefficient, effective encounter distance, and scaling parameters can be recovered at sufficiently high quencher concentrations, the simulations show that values of the specific rate constant, κ , can be expected to vary by up to two orders of magnitude when it is recovered from typical time-correlated single-photon counting data even if the data can be reliably described by the SCK model. The ability to differentiate between SCK kinetics and single-exponential kinetics by using fluorophores of varying unquenched lifetimes has also been explored.

KEY WORDS: Fluorescence quenching; transient effect; Smoluchowski–Collins–Kimball model; numerical simulation.

INTRODUCTION

Fast, diffusion-limited bimolecular processes in fluid media play an important role in many chemical, physical, and biological systems. Experiments and theoretical developments designed to describe the kinetics of these processes over their entire temporal range have therefore been active areas of research for many decades. Recently, experiments employing picosecond laser-excited fast fluorescence quenching coupled with time-correlated single-photon counting (TCSPC) emission methods have provided the temporal resolution and sen-

sitivity needed to reveal the complexities of such processes, including the details of the time dependence of their rate coefficients. The phenomenon leading to the time dependency of diffusion-limited rate coefficients is often referred to as the transient effect.

The transient effect is observed in fluorescence decay profiles at times intermediate between the very short-time [picosecond (ps)] static quenching regime and the long-time [typically nanosecond (ns)] regimes in which monoexponential decay characteristic of the classical Stern–Volmer mechanism applies. It is due to quenching which is characterized by events other than simple dynamic bimolecular encounters between an excited fluorophore and an energy (or electron) acceptor. Ware and Nemzek [1] were the first to observe this effect by direct time-resolved emission measurements, and their seminal work has led to several series of papers by

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Ware, Andre, and co-workers, Lakowicz and co-workers, Fleming, Periasamy, and co-workers, and others (Ref. 1, 2 and references therein). For synopses of earlier work, readers are referred to several comprehensive monographs and reviews (Refs. 3–5 and references therein). The interest in fast fluorescence quenching in these studies is twofold. First, fluorescence quenching provides a means of examining the kinetics of bimolecular processes with excellent sensitivity and temporal resolution when mode-locked ps or fs lasers are employed together with fast MCP-PMT detectors. Second, the data from fast fluorescence quenching experiments allow one to determine the functional form of the time-dependent rate coefficient, from which the values of parameters characterizing the donor–quencher–solvent system can be deduced.

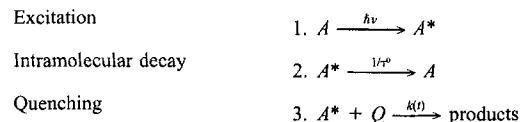
It has been suggested that the thioketones, which exhibit fast S_2 – S_0 radiative decay, may constitute a useful set of compounds for studies of the transient effect [6–11], and we have carried out extensive experimental quenching studies and some initial numerical simulations using one of them, xanthione, whose unquenched S_2 lifetime is 175 ps in inert perfluoroalkane solvents [8–11]. The numerical simulations [6,7] were designed to test the feasibility of distinguishing between the Smoluchowski–Collins–Kimball (SCK) and other models of fluorescence quenching kinetics when using xanthione as a donor and to show how the excited-state lifetimes of the chosen donor affect the ability to extract meaningful values of parameters of interest when the SCK model is valid.

The experimental studies of the quenching of xanthione S_2 fluorescence by a large number of quenchers in perfluoroalkane solvents revealed a number of difficulties when interpreting the time-resolved data using the SCK kinetics. The reasons for these difficulties became apparent after doing the numerical simulations, which revealed a strong correlation between some parameters and, also, indicated that one can expect a rather large scatter in the values of some parameters including the intrinsic reaction (interaction) rate and the reaction (interaction) distance. The previous results also indicated that the use of donors with fluorescence lifetimes longer than that of xanthione should help produce more accurate data concerning the kinetics of the bimolecular fluorescence quenching process. In this paper we therefore present more detailed studies of numerical simulations of fluorescence quenching of two “donors” with lifetimes of 880 and 410 ps, corresponding to the unquenched S_2 lifetimes of 2,2,3,3-tetramethylindanethione (TMIT) and 9,9-dimethylanthrathione (DMAT) in inert perfluoroalkane solvents at room temperature. Ear-

lier work leads us to expect that the use of a fluorophore with a longer lifetime will enable us to obtain more accurate values of the parameters in the SCK kinetics model than has been possible previously.

THEORY

The simplest possible kinetic scheme involving a bimolecular fluorescence quenching reaction is



where A and A^* represent the donor in the ground and electronically excited states, respectively, and τ^0 is the lifetime of A^* in the absence of quencher. The bimolecular process, 3, should be understood as any interaction between A^* and Q , either physical or chemical, which leads to the quenching of A^* in circumstances in which the change in concentration of the quencher, $[Q]$, is negligible. For the scheme above, the decay of the donor fluorescence intensity in the presence of a quencher as a function of time may be written as follows:

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

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

The central question concerning us is the functional form of the reaction rate coefficient, $k(t)$, and the temporal fluorescence intensity decay profile, $I(t)$. A number of mechanisms can give rise to a time-dependent quenching rate coefficient, the most familiar of which involves a full or partial diffusion-limiting rate for process 3. According to the Smoluchowski–Collins–Kimball theory the time-dependent reaction rate coefficient is given by [12–15]

$$k(t) = a + b \exp(c^2 t) \operatorname{erfc}(c\sqrt{t}) \quad [2]$$

where

$$a = \frac{4\pi RDN}{1 + \frac{D}{\kappa R}}, \quad b = \frac{4\pi R^2 N}{1 + \frac{D}{\kappa R}}, \quad \text{and}$$

$$c = \left[\frac{\sqrt{D}}{R} \right] \left(1 + \frac{\kappa R}{D} \right), \quad \operatorname{erfc}(x) = \frac{2}{\sqrt{\pi}} \int_x^\infty e^{-z^2} dz$$

Here D represents the sum of the diffusion coefficients of the donor and quencher, R is the sum of the encounter

radii of the donor in the electronic excited state, A^* , and the quencher, respectively, N is Avogadro's constant, and κ represents the specific rate constant [15,16]. By combining Eqs. (1) and (2) one can derive the following expression for $I(t)$ (Refs. 6, 7, and 16 and references therein):

$$I(t) = I(0) \exp\left(\frac{b[Q]}{c^2}\right) \exp\left[-t\left(\frac{1}{\tau^0} + a[Q]\right) - \frac{b[Q]}{c^2} \left(\exp(c^2 t) \operatorname{erfc}(c\sqrt{t}) + \frac{2c\sqrt{t}}{\sqrt{\pi}}\right)\right] \quad [3]$$

In the classical kinetics limit, achieved at long decay times and low quencher concentrations in solvents of low viscosity, Eq. (3) reduces to the standard monoexponential form of the quenched fluorescence decay:

$$I(t) = I(0) \exp[-t/\tau] \quad [4]$$

where τ represents the lifetime of the electronically excited state of the donor in the presence of quencher. One of the objectives of this paper is to provide some insight into the question of when it is possible to observe non-classical kinetics and why sometimes it is not possible.

A typical TCSPC fluorescence quenching experiment consists, in principle, of measuring the instrument response function, $E(t)$, and the observed fluorescence decay profile, $D(t)$, and effecting deconvolution of the two to obtain the fluorescence decay function, $I(t)$. In practice $I(t)$ is often obtained by iterative reconvolution of $E(t,S)$ with trial functions for $I(t)$ until a "best fit" is obtained, i.e.,

$$D(t) = E(t,S) \otimes I(t) \quad [5]$$

where S represents the time-shift parameter which allows for any empirical time shift between $D(t)$ and $E(t)$. The problem, then, is simply to determine the parameters describing the proposed form of the fluorescence decay of the chosen system, $I(t)$, when both $D(t)$ and $E(t)$ are known.

In practice, however, detailed studies of such processes involve some serious limitations from both theoretical and experimental points of view. The most successful method of determining the numerical values of $E(t)$ and then eliminating it from the data-fitting procedure is the reference convolution method, also called the delta function convolution method (DFCM) or the F/F deconvolution method. Detailed descriptions and comparisons of several different methods for solving Eq. [5] can be found in the very detailed papers by van den Zegel *et al.* [17] and by Zuker *et al.* [18]. In the so-called reference method it is assumed that, for a compound subsequently referred to as the reference com-

pound, both the form of the function describing the decay of its fluorescence intensity, $I_r(t)$, and the parameters of this function are known. From measurements of $D_r(t)$ where

$$D_r(t) = E(t,S) \otimes I_r(t) \quad [6]$$

and combining Eqs.[5] and [6], one can then calculate the parameters in the function $I(t)$ since $I_r(t)$, $D_r(t)$, and $D(t)$ are all known.

COMPUTER SIMULATIONS: GENERATION OF TYPICAL FLUORESCENCE DECAY DATA

A full description of the method used for the generation of fluorescence decay data by numerical simulation has been given previously [6,7]. Briefly, the sample and reference fluorescence decay data were generated by convolutions of the same unsmoothed "experimental" instrument response function with a desired SCK function for the sample and with a single-exponential decay function for the reference compound. The data were collected at a temporal resolution of 2.75 ps/channel, and the instrument response functions had a full width at half-maximum (FWHM) of 35 ps. Preexponential factors were adjusted to provide a total of about 40,000 counts in the peak channel. Each pair of functions, $D(t)$ and $D_r(t)$, was generated with different, independently added noise, according to recommended procedures [19].

The values of the parameters τ^{0g} (the unquenched, single-exponential decay time of the fluorophore) were chosen to be 880 and 410 ps, corresponding to the lifetimes of TMIT and DMAT in perfluoro-1,3-dimethylcyclohexane, respectively. A value of τ^g , the single exponential lifetime of the reference compound, of 8 ps was used, corresponding to the lifetime of xanthione in toluene [20]. In both cases, the superscript g indicates that the parameters were used in numerically generated (i.e., not experimentally measured) decay curves.

The parameters describing the fluorescence decay were calculated by the nonlinear least-squares method (Refs. 6 and 7 and references therein). In each case the range of values of each of the calculated or adjustable parameters was determined from the mean plus or minus three standard deviations. In what follows, this range is reported as the maximum and minimum values of each parameter. The criteria for goodness of fit to the generated decay curves were the same as those employed for the evaluation of real experimental data, i.e., the reduced chi-square, Durbin-Watson (DW), and ordinary-runs

Table I. Effect of the Quencher Concentration Used in Calculations on the χ^2 test and on the Minimum (Min) and Maximum (Max) Values of the Lifetime τ and Parameter G_0 Obtained from Fitting a Single-Exponential Function to Generated Values of $D(t)$ for TMIT^a

Concentration [mol dm ⁻³]	τ (ps)		G_0		χ^2
	Min	Max	Min	Max	
0.088	515.8	519.0	0.0061	0.0066	2.32
0.13	429.7	431.9	0.0063	0.0071	3.63
0.25	285.2	287.1	0.0069	0.0079	8.15

^aSixty randomly generated values of $D(t)$ were used. In the calculations of $D(t)$, the parameter $\tau^{0s} = 880$ ps corresponding to TMIT was used. The values of the remaining parameters were as follows: $\tau_r^s = 8.0$ ps, $R^s = 8.0$ Å, $\kappa^s = 0.1 \times 10^4$ cm s⁻¹, $D^s = 1.0 \times 10^{-5}$ cm² s⁻¹, $S^s = 0.0$ ps, and $D_{\max}(t) = 40,000$.

Table II. Effect of the Quencher Concentration Used in Calculations on the χ^2 Test and on the Minimum (Min) and Maximum (Max) Values of the Lifetime τ and Parameter G_0 Obtained from Fitting a Single-Exponential Function to Generated Values of $D(t)$ for DMAT^a

Concentration [mol dm ⁻³]	τ (ps)		G_0		χ^2
	Min	Max	Min	Max	
0.088	305.3	307.3	0.0065	0.0073	1.85
0.13	271.3	273.1	0.0066	0.0076	2.66
0.25	203.1	204.5	0.0068	0.0076	5.07
0.49	128.4	129.6	0.0074	0.0082	7.16

^aSixty randomly generated values of $D(t)$ were used. In the calculations of $D(t)$, the parameter $\tau^{0s} = 410$ ps corresponding to DMAT was used. The values of the remaining parameters were as follows: $\tau_r^s = 8.0$ ps, $R^s = 8.0$ Å, $\kappa^s = 0.1 \times 10^4$ cm s⁻¹, $D^s = 1.0 \times 10^{-5}$ cm² s⁻¹, $S^s = 0.0$ ps, and $D_{\max}(t) = 40,000$.

(OR) tests, together with the distributions of weighted residuals and autocorrelation functions.

RESULTS AND DISCUSSION

In previous preliminary work [9–11], xanthione, with an unquenched S₂ excited-state lifetime of 175 ps in perfluoroalkane solvents at room temperature, was used as a potential model fluorophore. However, this chromophore proved to be less suitable for modeling purposes than thiones with somewhat longer lifetimes. Therefore simulations were performed in the present work based on the properties of two aromatic thioketones, TMIT and DMAT, with substantially longer fluorescence lifetimes in perfluoroalkane solvents.

First, in order to examine the possibility of differentiating between an “experimental” fluorescence decay

exhibiting the transient effect (described by the SCK function) and a fitted curve described by a single-exponential function, calculations were performed in which $D(t)$ was generated using the SCK function, and the fitted curve was calculated using a single-exponential function. The “best fit” between these two curves was then found using the least-squares method which generated values of the best single-exponential lifetime, τ , and the adjustable scaling parameter, $G_0 = A_r(\max)/A_s(\max)$, where $A(\max)$ is the maximum amplitude of the function (in units of photon counts in one channel of the multichannel analyzer), and the subscripts r and s refer to the reference and sample, respectively. The results of these calculations at several revealing quencher concentrations are presented in Table I for TMIT and Table II for DMAT. The SCK parameters used in generating $D(t)$ are denoted by a superscript “g” and their values are given in the footnotes to the tables. These tables present the range of the “best” single-exponential quenched fluorescence lifetimes, $\tau \pm 3\sigma$, and the range of the adjustable scaling parameters, $G_0 \pm 3\sigma$. The data for TMIT are also presented visually in Fig. 1. The results for DMAT (not shown) are similar.

As expected, none of the numerically generated data are well modeled by a single-exponential fluorescence decay function, and the fits are worse at higher quencher concentrations. One of the major conclusions of this work is evident from comparisons of these quenching data for TMIT, DMAT, and those previously published for XT [6,7]. Note the quencher concentrations at which the value of χ^2 exceeds some arbitrary value, e.g., $\chi^2 > 2$ at <0.088 mol·dm⁻³ for TMIT, >0.088 mol·dm⁻³ for DMAT, and >0.25 mol·dm⁻³ for XT. Thus, the failure of the single-exponential decay model due to the transient effect can be detected at lower quencher concentrations for *longer-lived* fluorophores, contrary to intuition.

Note in Fig. 1 that, as is the case with real experimental data, we attempt to fit the numerically generated “experimental” and the modeled data over the entire time range. The fits are the worst near the peak of the “experimental” curve, as reflected in the distribution of weighted residuals. Thus, omitting the first part of the decay in any analysis can produce a single-exponential lifetime with an acceptable associated value of χ^2 , but at the expense of ignoring information on the transient effect contained in the early part of the decay and with some negative consequences on the final analysis [11].

The single-exponential decay fits are informative only in the sense that they can signal the presence or absence of the transient effect. However, it is not possible to relate the values of τ and G_0 obtained in single-

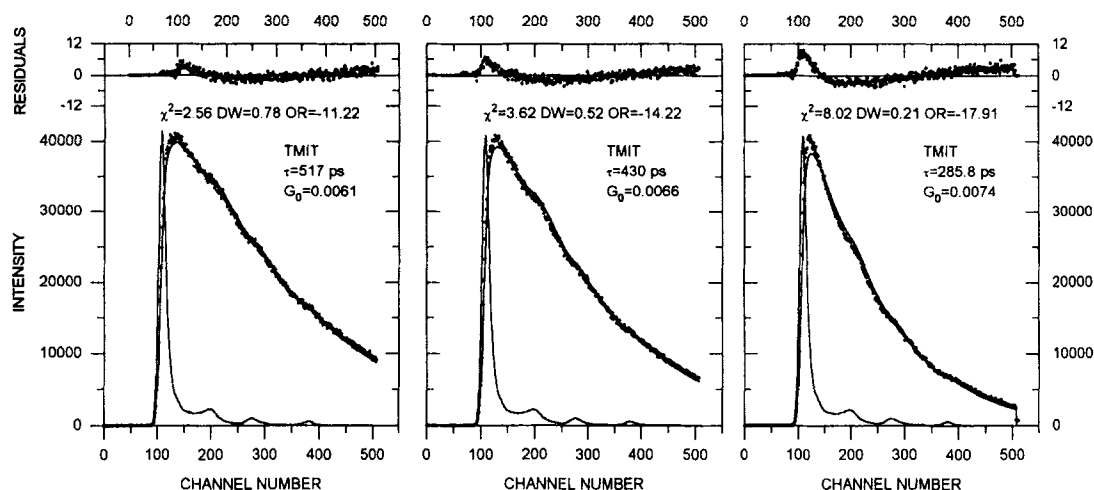


Fig. 1 Typical fitting results: the simulated curve, for TMIT with three concentrations of quencher, was generated using the SCK function, while the fitted curve is the best fit to a single exponential. The instrument response function (dashed lines), simulated plots (dots), and fitted curves (solid lines) are shown. Plots of weighted residuals are given at the top of the graphs. The respective fitted parameter values are shown, 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 at lower concentrations of quencher.

Table III. Effect of Quencher Concentration on the Calculated Minimum (Min) and Maximum (Max) Values of the Parameters of the SCK Model Obtained from Fitting an SCK Function to 60 Randomly Generated Values of $D(t)$ for TMIT^a

Concentration [mol dm ⁻³]	R (Å)		D (10 ⁻⁵ cm ² s ⁻¹)		κ (10 ⁴ cm s ⁻¹)		G_0	
	Min	Max	Min	Max	Min	Max	Min	Max
0.088	6.0	13.8	0.4	1.3	0.03	3.2	0.0064	0.0073
0.13	6.6	12.4	0.5	1.2	0.02	1.2	0.0068	0.0075
0.25	6.6	8.7	0.9	1.2	0.06	0.6	0.0081	0.0095
0.49	7.0	8.8	0.9	1.1	0.06	0.24	0.0089	0.0099

^aIn the calculations of $D(t)$, the parameter $\tau^{0*} = 880$ ps corresponding to TMIT was used. The values of the remaining parameters were as follows: $\tau_r^* = 8.0$ ps, $R^* = 8.0$ Å, $\kappa^* = 0.1 \times 10^4$ cm s⁻¹, $D^* = 1.0 \times 10^{-5}$ cm² s⁻¹, $S^* = 0.0$ ps, and $D_{\max}(t) = 40,000$ (cu = 2.75 ps).

exponential fits to the parameters of the SCK kinetics model. To investigate fluorescence quenching within the framework of the SCK theory, it is necessary to determine the values of R , D and κ , because these parameters describe the rate coefficient of the reaction between the excited donor and the quencher, $k(t)$. However, time-resolved fluorescence quenching experiments provide, instead, values of τ^0 , τ_r , the time-base shift parameter S , and the scaling parameter G_0 . We have shown previously [6,7] that some of these parameters are strongly correlated when standard methods are used to extract them from the raw data obtained in time-correlated single-photon counting experiments. In particular, a near-1:1

correlation exists between the values of S and G_0 , and there is also a strong correlation between τ^0 and D . These correlations, and other technical limitations outlined below, make it very difficult to extract physically meaningful parameters of the SCK function from experimental data, as we have found in our previous experimental studies of fluorescence quenching [9–11].

The difficulties of extracting SCK kinetic parameters from data obtained using single-photon counting techniques have been reported previously (Refs. 6 and 7 and references therein). These difficulties are based not only on the above parameter correlations, but also on the fact that both laser and flash lamp excitation sources exhibit jitter in their temporal pulse shapes and arrival times. An additional general problem arises when the instrument response function is measured with a scattering solution so that $E(t)$ is measured at a wavelength different from that of $D(t)$, and often at a different count rate as well. Microchannel plate photomultiplier tubes are less susceptible to the dependence of $E(t)$ on wavelength [21]. However, it is still necessary to examine this dependence if $E(t)$ is obtained by light-scattering methods.

In Table III for TMIT and Table IV for DMAT we present the results of simulations in which the SCK model has been tested on 60 independently and randomly generated sets of $D(t)$ data. The data for TMIT are shown in Fig. 2. These simulations are identical to those reported above except that the SCK function rather than a single-exponential function has been used to

Table IV. Effect of Quencher Concentration on the Calculated Minimum (Min) and Maximum (Max) Values of the Parameters of the SCK Model Obtained from Fitting an SCK Function to 60 Randomly Generated Values of $D(t)$ for DMAT^a

Concentration [mol dm ⁻³]	R Å		D^s (10 ⁻⁵ cm ² s ⁻¹)		κ (10 ⁴ cm s ⁻¹)		G_0	
	Min	Max	Min	Max	Min	Max	Min	Max
0.088	5.9	14.8	0.4	1.4	0.01	3.6	0.0069	0.0080
0.13	6.2	13.7	0.5	1.3	0.02	1.9	0.0072	0.0084
0.25	6.5	9.8	0.8	1.2	0.04	0.65	0.0080	0.0093
0.49	7.0	9.3	0.8	1.2	0.05	0.24	0.0091	0.0106

^aIn the calculations of $D(t)$, the parameter $\tau^{0s} = 410$ ps corresponding to DMAT was used. The values of the remaining parameters were as follows: $\tau_r^s = 8.0$ ps, $R^s = 8.0$ Å, $\kappa^s = 0.1 \times 10^4$ cm s⁻¹, $D^s = 1.0 \times 10^{-5}$ cm² s⁻¹, $S^s = 0.0$ ps, and $D_{\max}(t) = 40,000$ (cu = 2.75 ps).

model the data. Again, the values of the recovered parameters, R , D^s , κ , and G_0 , are presented as maximum and minimum values, i.e., the mean $\pm 3\sigma$. Inspection of the results shows that it is possible to observe a decrease in the uncertainties of R , D^s , and κ at higher quencher concentrations. A similar decrease is also observed when donors with shorter lifetimes are employed. These results are consistent with those reported previously for XT [6,7].

Using the SCK function when fitting “experimental” fluorescence quenching data also yields a value of the specific rate constant, κ . Some of the problems connected with obtaining reliable values of this constant have been discussed previously [6,7]. The further results of the numerical experiments presented here show that κ can be expected to vary by up to two orders of magnitude when it is recovered from typical time-correlated single-photon counting data *even if the data can be reliably described by the SCK model*. Unfortunately the values of κ obtained from SCK fits are difficult to compare with external data, and it is possible that the real values are larger than those obtained from these fitting procedures but that larger rate constants cannot be obtained because the process to which κ pertains does not limit the overall rate of quenching. We conclude that reliable estimates of this parameter are hard to obtain from SCK modeling of TCSPC data and that ascribing physical significance to its magnitude can be of dubious value. The results presented here do, however, help define when reliable data can be obtained from existing fitting procedures.

The above discussion can be made more general and can reveal other interesting aspects of the analysis if one expresses the FWHM of $E(t)$ and the values of the fluorescence lifetimes in channel units (cu), i.e., time

divided by the time per channel. In the work reported above, the channel width is 2.75 ps per channel, so the FWHM of $E(t)$ is 13 cu and the lifetimes of excited thione donors we have used here and elsewhere are 320 cu (TMIT), 220 cu (DMAT), 149 cu, 76 cu, 63 cu (XT), and 37 cu 23 cu (cf. Table V). Table V presents the results of simulations which, on the surface, appear to test the effect of varying the donor fluorescence lifetime on the ability to extract meaningful parameters from a given model. In these simulations the same set of SCK quenching and fitting parameters is employed, the unquenched donor fluorescence lifetime is varied in the simulation, the channel width is maintained at 2.75 ps per channel, and the range of “best” lifetimes recoverable from an attempted fit of the data to a single-exponential decay function is reported. (The quenching parameters are typical of a diffusion-limited process.) Note that the best fit to a single-exponential function is apparently obtained for the shortest donor lifetime, even though the simulation is obtained from an SCK function, and that the failure of a single-exponential function to describe the data is most evident when the donor has the longest lifetime. The effect is due *not* to donor lifetime but, rather, to the limitations of the data analysis method when the nonexponential data (indicating SCK kinetics) are confined to a limited number of data points (channels).

Using this normative means of expressing times, it also becomes relatively easy to determine if previous workers *could have observed* SCK kinetics using the apparatus available to them at the time. For example, Nemzek and Ware were the first to obtain good results using the SCK model to describe the quenching of 1,2-benzanthracene fluorescence (which has an unquenched lifetime of 38.5 ns [1]) by carbon tetrabromide in the high-viscosity solvent 1,2-propanediol. These workers used an apparatus with FWHM = 11.4 ns, which, on the basis of our calculations, suggests that they should have been able to detect SCK kinetics using compounds with fluorescence lifetimes longer than 32 ns.

CONCLUSIONS

Numerical simulations designed to illuminate the opportunities for and limitations on recovering the parameters in the Smoluchowski–Collins–Kimball model of time-dependent fluorescence quenching from the data of time-correlated single-photon counting experiments have been carried out. The failure of the single-exponential decay model due to the transient effect can be detected at lower quencher concentrations for

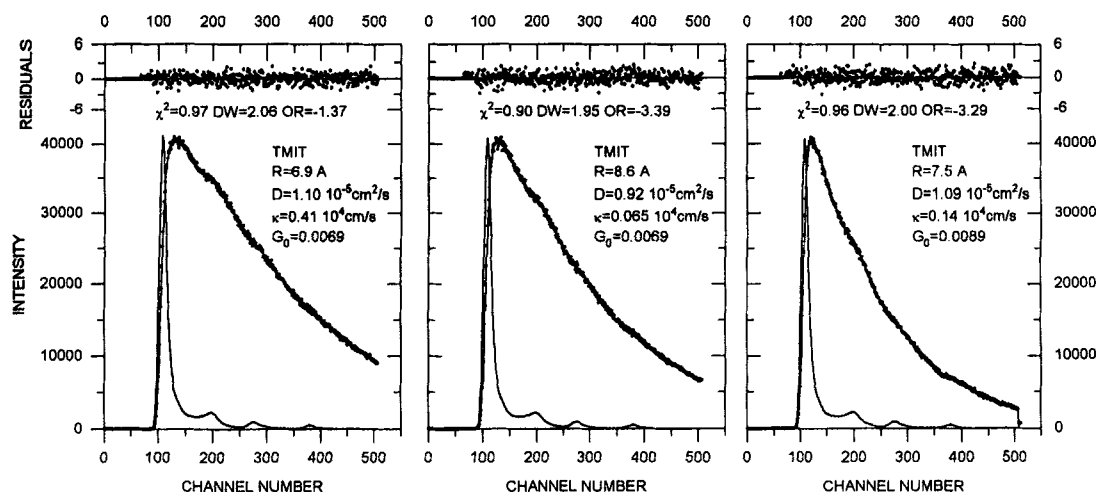


Fig. 2. Typical fitting results for TMIT with three concentrations of quencher shown is the Both the simulated and the fitted curves are described by the SCK function. The information shown in the same as in Fig. 1. Both the statistical test values and the plots of weighted residuals indicate a good fit.

Table V. Effect of Donor Fluorescence Lifetime Used in Calculations of the χ^2 Test and Minimum (Min) and Maximum (Max) Values of the Lifetime τ and Parameter G_0 Obtained from Fitting a Single-Exponential Function to Generated Values of $D(t)$, $[Q]^s = 0.25 \text{ mol dm}^{-3a}$

τ^{0s} (cu)	τ (cu)		G_0		χ^2
	Min	Max	Min	Max	
320	104	105	0.0067	0.0070	7.73
219	89	90	0.0069	0.0073	6.55
149	74	75	0.0068	0.0076	4.91
76	48	49	0.0077	0.0083	2.61
64	43	44	0.0088	0.0094	2.27
37	28	29	0.0092	0.0100	1.39
23	19	19	0.0115	0.0126	1.17

^aIn the calculations of $D(t)$, the value of the parameters were as follows: $\tau_s^s = 8.0 \text{ ps}$, $R^s = 8.0 \text{ \AA}$, $\kappa^s = 0.1 \times 10^4 \text{ cm s}^{-1}$, $D^s = 1.0 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, $S^s = 0.0 \text{ ps}$, and $D_{\text{max}}(t) = 40,000 \text{ (cu} = 2.75 \text{ ps)}$.

longer-lived fluorophores. While consistent values of the diffusion coefficient, effective encounter distance, and scaling parameters of the SCK model can be recovered at sufficiently high quencher concentrations, the simulations show that values of the specific rate constant, κ , can be expected to vary by up to two orders of magnitude when it is recovered from typical time-correlated single-photon counting data even if the data can be reliably described by the SCK model. Simulations designed to test the ability to differentiate between SCK kinetics and single-exponential kinetics by using fluorophores of varying unquenched lifetimes reveals that a single-exponential model appears to fit SCK data better

when short-lived fluorophores are employed. The effect is due not to donor lifetime but, rather, to the limitations of the data analysis method when the nonexponential data (indicating SCK kinetics) are confined to a limited number of data points (channels).

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