

THE EFFECT OF TRANSIENT QUENCHING ON THE EXCIMER KINETICS OF 2,5-DIPHENYLOXAZOLE

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Summary

Excimer formation in 2,5-diphenyloxazole (PPO) in ethanol is shown not to obey the expected biexponential kinetics because of the presence of time-dependent quenching. Between 25 and 50 °C the decay of monomer fluorescence can be described by a model based on the linear superposition of excimer reverse dissociation and transient quenching to give values for the diffusion coefficient and the interaction radius. However, at lower temperatures the model provides an incomplete description and the indications are that this is caused by the emergence of a heterogeneous distribution of PPO molecules.

1. Introduction

The phenomenon of intermolecular excimer formation in aromatic molecules has received considerable attention since it was first observed in pyrene by Förster and Kasper [1]. In particular, wide-ranging examples have proved the validity of the Birks kinetic scheme which describes the quenching in terms of simple exponential functions [2]. However, for a diffusion-controlled process such as this, a more general treatment of the quenching would be expected to allow for a transient component such as that predicted by Smoluchowski's treatment of molecular diffusion [3]. This occurs because excited molecules in close proximity to ground state molecules undergo a more rapid rate of quenching than that predicted by Stern-Volmer kinetics. The transient effect is usually assumed to be negligible for molecules of long fluorescence lifetimes in low viscosity solvents since these conditions facilitate mixing of the ground and excited state species such that steady state behaviour is dominant. Transient quenching of fluorescence by molecules containing heavy atoms has been widely studied (see, for example, ref. 4), but there is little experimental evidence for its influence on excimer kinetics owing to self quenching. It has been reported for pyrene excimers

in the viscous medium of biological membranes [5], in which pyrene and its derivatives are now widely used as probes. However, in this case the determination of diffusion coefficients is simplified by there being negligible reverse dissociation of the pyrene excimer to form excited monomer. Deviations from the Birks-type kinetics have been widely reported for intramolecular excimer formation in numerous polymer systems where reverse dissociation can occur (for a recent review see ref. 6), but in these systems the added complications of energy migration and isolated monomer sites have to be considered. Although seemingly complex kinetically, it is perhaps surprising that more attention has not been given to transient behaviour in intermolecular excimer formation for the more general case in which excimer reverse dissociation is present.

Berlman [7] was the first to report excimer formation in 2,5-diphenyl-oxazole (PPO), but most subsequent work [8 - 10] has considered only spectral and quantum yield data. Yguerabide and Burton [11] used pulsed X-ray excitation to observe a bi-exponential decay in excimer-forming solutions of PPO but this early work did not have the benefit of the higher specificity and time resolution of present day fluorometers to the extent that reverse dissociation of the PPO excimer was at that time still an open question. Time resolution is particularly important when studying PPO, which has a fluorescence lifetime of the order of 1 ns as compared with several hundreds of nanoseconds for pyrene. The present applications of PPO as a scintillator, laser dye and fluorescence lifetime standard also warrant a closer look at its photophysical properties.

We have thus set out to investigate the applicability of the accepted excimer kinetic scheme for PPO and thereby determine if transient effects are important and if so how they might be described. The results show that they are indeed far from negligible in low viscosity solvents and that they can be used to determine the interaction radius and diffusion coefficient of PPO at temperatures down to 25 °C, even in the presence of excimer reverse dissociation. However, at lower temperatures, the simple continuum model, wherein solute molecules are considered to be randomly distributed throughout a continuous solvent medium, looks to be inappropriate. This behaviour is of fundamental importance, not only in a fluorescence context, but also with regard to the broader aspect of understanding the collisional dynamics of diffusing reactants in solution.

2. Theory

The Birks kinetic scheme [2] for excimer formation is as follows:



This gives fluorescence response functions of the monomer M and excimer D of the form

$$i_M(t) = B_1 \exp\left(-\frac{t}{\tau_1}\right) + B_2 \exp\left(-\frac{t}{\tau_2}\right) \quad (2)$$

and

$$i_D(t) = B_3 \left\{ \exp\left(-\frac{t}{\tau_1}\right) - \exp\left(-\frac{t}{\tau_2}\right) \right\} \quad (3)$$

where τ_1 and τ_2 are time-independent decay parameters describing the behaviour of the excimer and the monomer respectively in terms of scheme (1).

The exponential containing τ_1 in eqn. (2) is needed to describe the excimer reverse dissociating to give excited monomer with a rate parameter k_{MD} .

Smoluchowski's solution of Fick's second law of diffusion leads to the following expression for the time dependence of the rate of quenching of monomer [2]:

$$k_{DM} = \frac{4\pi NDpR}{1000} \left\{ 1 + \frac{pR}{(\pi Dt)^{1/2}} \right\} \quad (4)$$

where D is the sum of the diffusion coefficients of the ground state and excited molecules, R is the sum of their interaction radii, *i.e.* the encounter distance between the centres of the molecules, N is Avogadro's number and p is the reaction probability per collision. At $t > p^2 R^2 / \pi D$ the time dependence of eqn. (4) becomes negligible and the kinetics given by eqns. (2) and (3) apply.

The inclusion of the time-dependent k_{DM} given by eqn. (4) modifies the rate equations from which eqns. (2) and (3) are derived to the following:

$$\frac{d[{}^1M^*]}{dt} = k_{MD}[{}^1D^*] - \{k_M + (a + b/t^{1/2})[{}^1M]\}[{}^1M^*] \quad (5)$$

and

$$\frac{d[{}^1D^*]}{dt} = (a + b/t^{1/2})[{}^1M][{}^1M^*] - (k_D + k_{MD})[{}^1D^*] \quad (6)$$

with

$$a = \frac{4\pi NDpR}{1000} \quad (7)$$

and

$$b = \frac{4Np^2R^2(\pi D)^{1/2}}{1000} \quad (8)$$

These coupled differential equations do not lend themselves to a simple analytical solution for $i_M(t)$ and $i_D(t)$ but if $k_{MD} = 0$ they can be solved to give a monomer fluorescence response which is analogous to that used to describe Förster energy transfer [2] and is of the form

$$i_M(t) = B_2 \exp\left(-\frac{t}{\tau_2} - 2b[{}^1M]t^{1/2}\right) \quad (9)$$

where τ_2 is the quenched monomer lifetime for steady state diffusion given by

$$\tau_2 = (k_M + a[{}^1M])^{-1} \quad (10)$$

These expressions have already been shown to be widely applicable to the irreversible deactivation of excited fluorophores undergoing collisions with quencher molecules [4]. However, it is the more general case where $k_{MD} \neq 0$ that concerns us here. As the next step of minimum additional complexity, we have considered the monomer behaviour in terms of a simple linear superposition of terms, *i.e.*

$$i_M(t) = B_1 \exp\left(-\frac{t}{\tau_1}\right) + B_2 \exp\left(-\frac{t}{\tau_2} - 2b[{}^1M]t^{1/2}\right) \quad (11)$$

Although not rigorously derived, this model provides us with an opportunity of describing both the steady state behaviour and the transient behaviour in terms of interpretable exponentials which can be reconvoluted with the excitation pulse to analyse the fluorescence decay. Our findings indicate that it can indeed provide a good description of the kinetics.

3. Experimental details

Fluorescence decay measurements were obtained using a time-correlated single-photon-counting fluorometer with multiplexed dual detection channels which has been described previously [12]. However, the photomultiplier noise performance has recently been improved, without cooling, by an order of magnitude reduction to about 10 Hz with the addition of an Edinburgh Instruments detection system [13]. The late pulsing of this detection system is also negligible in comparison with that reported previously [12] and the overall effect of these improvements has been an enhancement of the instrumental time resolution. The excitation source is an Edinburgh Instruments Model 199F all-metal coaxial flashlamp giving an instrumental response function of about 1.3 ns full width at half-maximum at 50 kHz repetition rate [14]. The excitation wavelength of 310 nm with 5 nm bandwidth was selected with a double monochromator and the emission wavelength of 360 nm selected monomer emission with a bandwidth of 10 nm was chosen. For this sample and these wavelengths no evidence for re-excitation caused

by retrodiffracted light was observed [15]. Reconvolution analysis, which included the rising edge of the decay, was performed on-line using an Edinburgh Instruments model 199M data analyser incorporating a PDP 11/73 microcomputer and non-linear least-squares version 4 programs with a χ^2 goodness-of-fit criterion. As a rule of thumb we have found that if a kinetic model gives $\chi^2 < 1.2$ then decay data with 10^4 counts in the peak will rarely support a more complex kinetic interpretation [16]. In these measurements the fluorescence decay curves were recorded to a higher precision of 4×10^4 counts in the peak and a time calibration of about 90 ps per channel in order to provide a more stringent test of the kinetic model. The typical measurement time for a decay was about 40 min.

The PPO was Aldrich scintillation grade and was used without further purification. The ethanol was BDH spectrosol grade and was found to be free of background fluorescence. The 0.1 M solution was degassed using the freeze-pump-thaw technique and sealed in a 1 cm² quartz cuvette with fluorescence detected through the same face as was used for excitation. The cuvette was tilted, such that excitation light reflected from the front face of the cuvette would pass outside the detection solid angle.

4. Results

Figure 1 shows the measured fluorescence decay curves between 0 and 50 °C. Two decay components attributable to quenched monomer and excimer decay are clearly evident. The extent of reverse dissociation and the rate of excimer decay to give excited monomer are seen to increase as the temperature is raised. However, the χ^2 values obtained by reconvolution analysis of these data, as shown in Table 1, reveal clearly that when fitting over all of the decay the conventional bi-exponential kinetics are inappropriate. This is further illustrated by the weighted residuals in Fig. 2(a), which show that the misfit is large and primarily occurs at shorter times, becoming more pronounced with decreasing temperature. The common shape of the residuals indicates that the same dominant cause of the misfit is involved at all temperatures. Figure 2(b) shows the region and magnitude of the misfit on the decay curve at 25 °C. Scattered excitation light might be expected to have a similar effect on the decay curve but by using a cuvette containing a suspension of Ludox we estimate that the misfit due to scattered light is less than 5% of the misfit observed at 25 °C. Similarly, no improvement in the goodness of fit was obtained by using a polarizing prism at the magic angle of 55° to correct for possible rotational depolarization effects. PPO has only a small spectral overlap of absorption and emission, but we investigated the possibility of self-absorption and radiationless energy transfer by increasing the path length of fluorescence through the solution when exciting it through the diagonal face of a triangular cuvette and detecting the fluorescence at 90° to excitation. Again there was no change in the residuals. In addition, these effects would not be expected to show such a

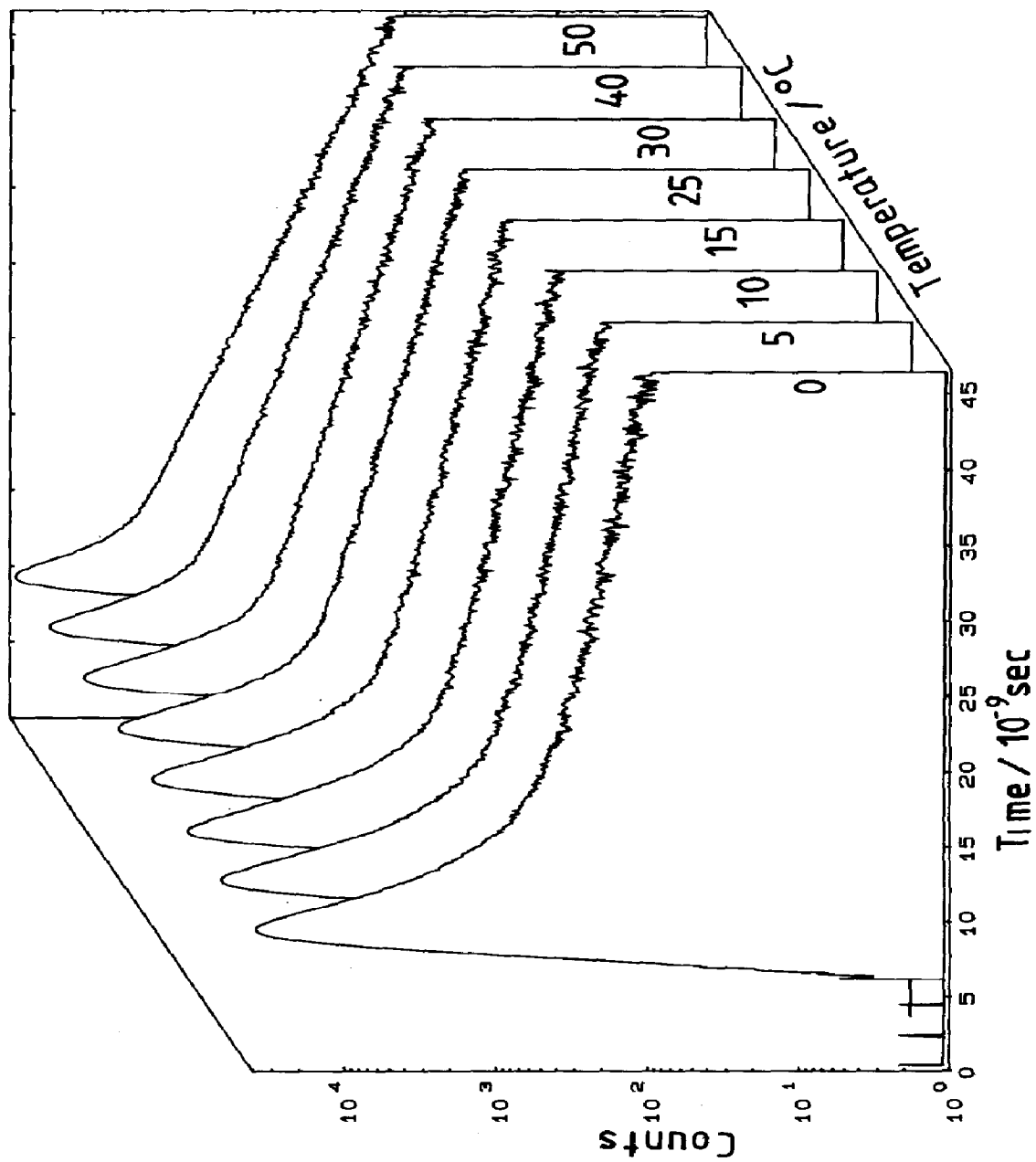


Fig. 1. Fluorescence decay of 0.1 M PPO in ethanol at 360 nm emission vs. temperature.

TABLE 1

χ^2 values for the reconvolution analysis of all of the decay of 0.1 M PPO in ethanol using two exponentials (eqn. (2)), three exponentials and one exponential plus a transient quenching term (eqn. (11)). (The χ^2 values, when reconvoluting and fitting to just the tail of the decay with the two exponential model, are also shown.)

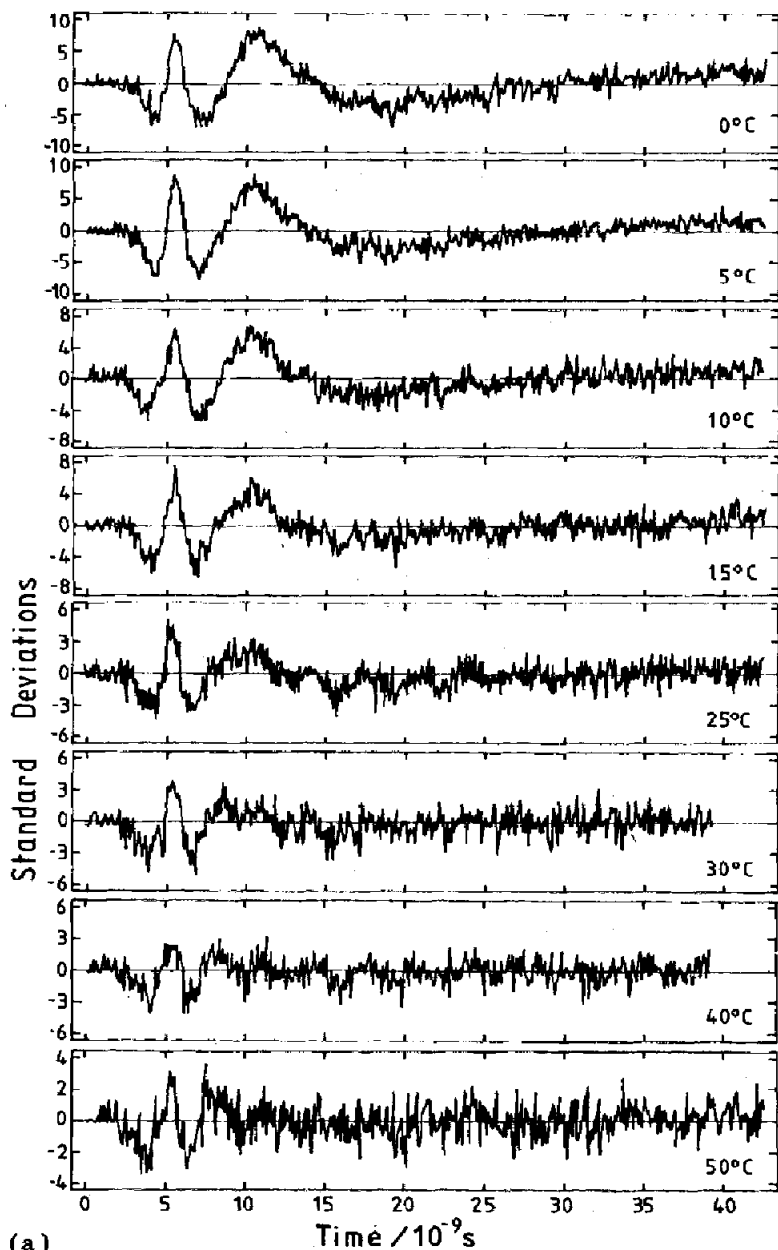
T (°C)	2 Exp	2 Exp (tail)	3 Exp	1 Exp + $t^{1/2}$
0	9.0	3.7	1.3	2.4
5	7.9	2.8	1.3	1.6
10	5.6	2.1	1.3	1.3
15	3.8	1.7	1.4	1.4
25	1.9	1.1	1.1	1.0
30	1.8	1.1	1.1	1.1
40	1.5	1.1	1.1	1.2
50	1.3	1.0	1.0	1.1

marked temperature dependence. Figure 3 shows that a model describing transient quenching and excimer reverse dissociation (eqn. (11)) gives a good fit to the decay at $T \geq 25$ °C. The results of fitting with the three-exponential and transient quenching models are shown in Table 2. It is interesting that Table 1 shows how a three-component model gives as good a fit as that afforded by a superposition of transient quenching and excimer reverse dissociation at higher temperatures. Itagaki *et al.* [17] have made a similar observation for two decay components in the intramolecular excimers of dicarbazolylpropane and poly(1-vinylnaphthalene) when excimer reverse dissociation is neglected. However, the presence of three decay components usually implies that three different excited state species are present, which, although possible in polymeric systems owing to the presence of isolated monomer sites, is harder to rationalize in the simple low molecular weight system considered here. In addition, the intermolecular origin of the departure from bi-exponential kinetics is implied by its strong concentration dependence and the fluorescence in dilute solution being monoexponential. Clearly, what is happening is that at the level of time resolution we have available the third exponential component is just parameterizing the transient-quenching behaviour. However, as our later discussion indicates, the better fits offered by the three-component model at the lower temperatures may have a more physically meaningful interpretation.

Throughout these measurements we have chosen not to investigate the transient effects which should exist in the decay kinetics in the spectral region of excimer emission. This is because at longer wavelengths the spectral overlap of the monomer and excimer emission bands in PPO makes such studies difficult. In fact, because of this a rise time is not observed below 550 nm for 0.1 M PPO.

The fluorescence decay of a dilute solution of PPO in ethanol gives a good fit to a single-exponential decay time of 1.61 ns [12] which is inde-

pendent of temperature over this range. It is thus interesting to note that below 25 °C the transient-quenching model finds a quenched monomer lifetime longer than this and the χ^2 progressively increases with the misfit shown by the residuals in Fig. 3(a). It is evident that at this point our model based on a simple linear superposition using Smoluchowski's continuum treatment has become inappropriate and the kinetics are even more complicated.



(a)

Fig. 2 (continued).

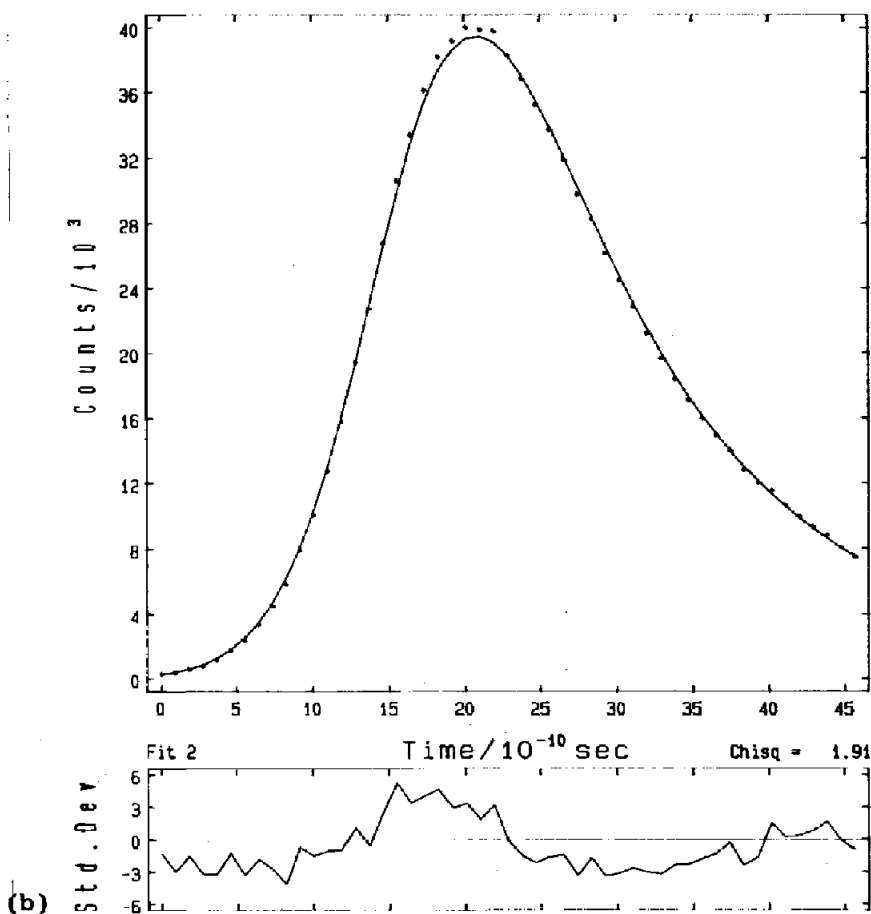


Fig. 2. Bi-exponential reconvolution analysis of the fluorescence decay of 0.1 M PPO in ethanol at 360 nm emission. (a) Weighted residuals vs. temperature. (b) Fit to the decay curve at 25 °C.

TABLE 2

Best-fit decay parameters for 0.1 M PPO in ethanol using a three-exponential model and a model based on the superposition of one exponential and a transient quenching term (eqn. (11))

<i>3 Exp</i>				<i>1 Exp + t^{1/2}</i>		
	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	τ_1 (ns)	τ_2 (ns)	b ($\times 10^5 \text{ M}^{-1} \text{ s}^{-1/2}$)
0	19.9	2.1	0.69	17.6	2.9	2.63
5	19.0	1.71	0.63	18.1	2.77	2.55
10	17.4	1.36	0.55	17.3	2.16	2.28
15	16.4	1.23	0.53	16.3	1.73	2.04
25	14.1	0.92	0.32	14.2	1.24	1.44
30	12.7	0.84	0.18	12.8	1.12	1.32
40	10.6	0.75	0.04	10.6	0.95	1.12
50	8.5	0.71	0.03	8.6	0.93	1.31

5. Discussion

It is convenient to discuss the excimer kinetics in terms of two distinct temperature ranges according to whether or not eqn. (11) is applicable, *i.e.* above and below 25 °C.

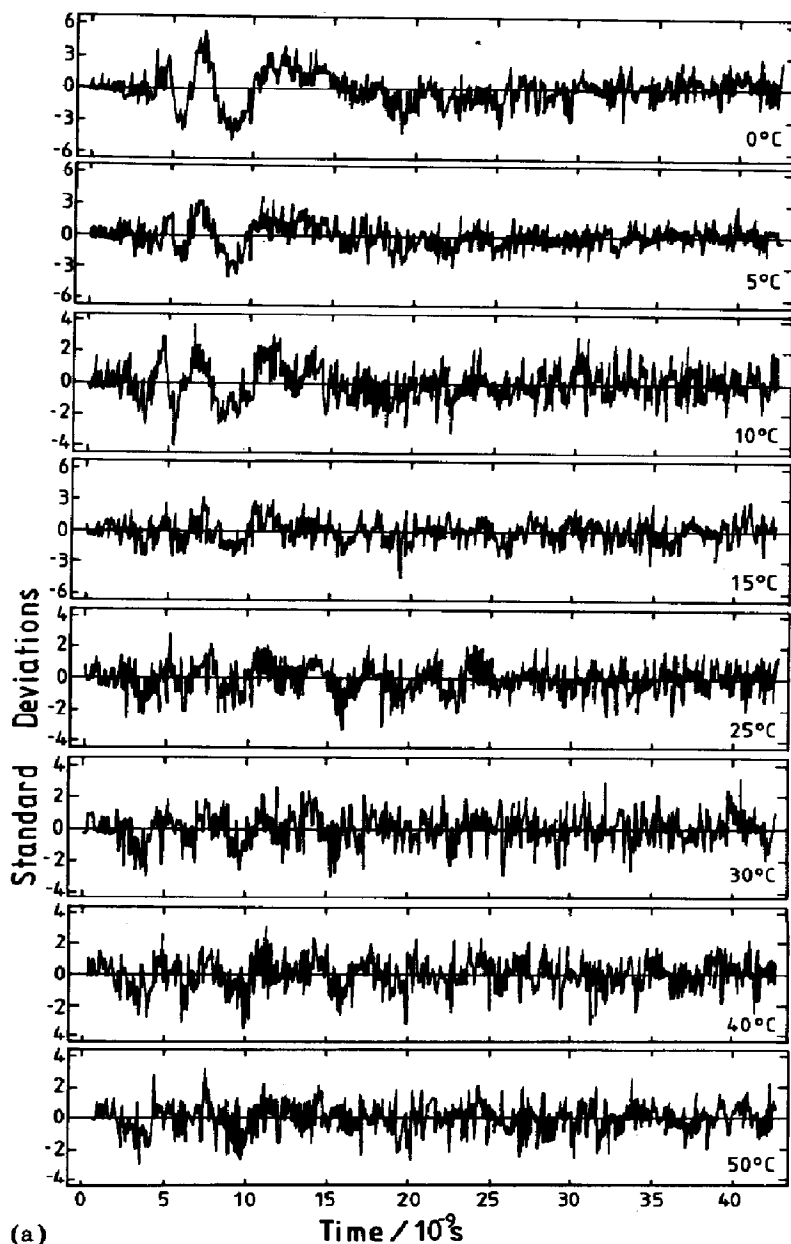


Fig. 3 (continued)

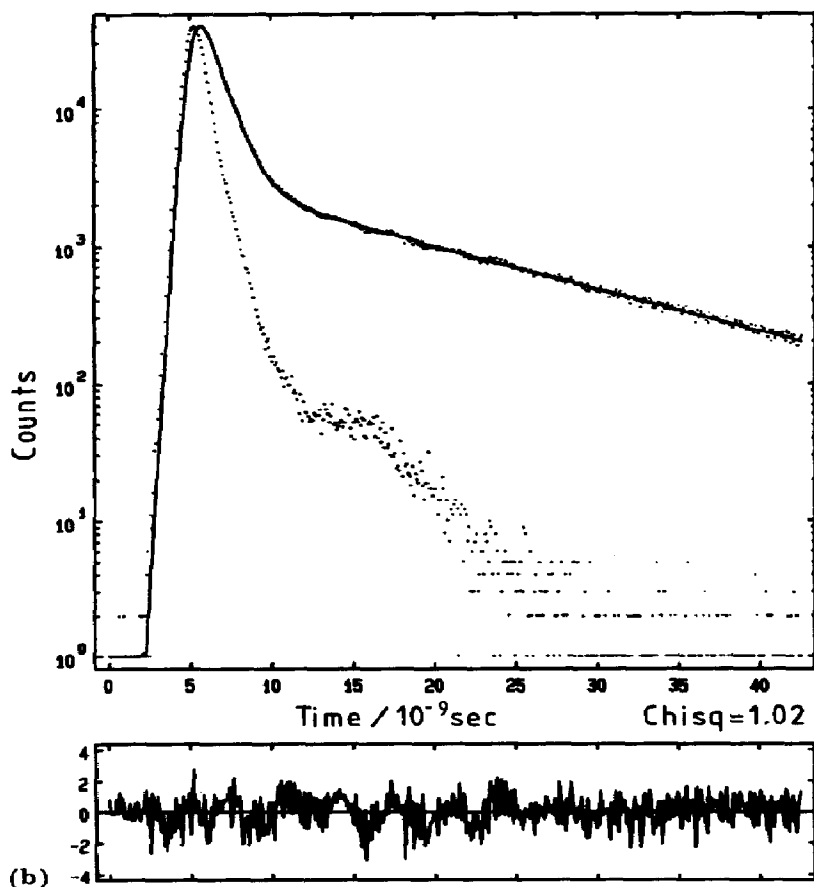


Fig. 3. Reconvolution analysis using a linear superposition of transient quenching and excimer reverse dissociation for the fluorescence decay of 0.1 M PPO in ethanol at 360 nm emission. (a) Weighted residuals vs. temperature. (b) Excitation pulse and fit to the decay at 25 °C.

5.1. $T \geq 25$ °C

The low χ^2 values, obtained by fitting to only the tail of the decay with a bi-exponential model, as shown in Table 1, illustrate that the departure from such kinetics is due only to the behaviour at short times. The increasing misfit with decreasing temperature, when fitting over all the decay with bi-exponential kinetics, is what would be expected as the transient quenching increases with decreasing diffusion coefficient. We can thus use eqn. (11) to analyse the transient behaviour in terms of the molecular interaction radius R_m and the diffusion coefficient D_m of either the ground or the excited state species assuming they have identical collisional properties, *i.e.* $R_m = R/2$, $D_m = D/2$ and $p = 1$. The interaction radii shown in Table 3 seem reasonable in comparison with those found from quenching studies with other fluorophores in the absence of reverse dissociation [2, 4, 19]. This indicates that the reaction probability p may indeed be

TABLE 3

Interaction radius R_m , measured molecular diffusion coefficient D_m and Stokes-Einstein diffusion coefficient D_{SE} for excimer formation in 0.1 M PPO in ethanol as a function of viscosity [18] and temperature

T (°C)	η (cP)	R_m (Å)	D_m ($\times 10^{-6}$ cm ² s ⁻¹)	D_{SE} ($\times 10^{-6}$ cm ² s ⁻¹)
25	1.10	8.4	0.7	3.3
30	1.00	6.9	1.3	3.7
40	0.83	5.3	2.7	4.6
50	0.70	5.8	2.6	5.6

close to unity. From the reproducibility of measurement we estimate an error in R_m of about ± 1 Å and in D_m of about $\pm 2 \times 10^{-7}$ cm² s⁻¹.

The values determined for the diffusion coefficient D_m look to be on the low side in comparison with both the diffusion coefficient D_{SE} determined from the Stokes-Einstein equation (assuming a Stokes radius of 6 Å) and available data for quenching studies with similar molecules, which typically report diffusion coefficients of about 10^{-5} cm² s⁻¹ in solvents with a viscosity of about 1 cP [2, 4, 19]. In addition, Table 3 shows that the departure from Stokes-Einstein theory, given by the ratio D_{SE}/D_m , is generally greater as the temperature is reduced. The origin of these low diffusion coefficients has a number of possible explanations which can be traced to the assumptions involved in the formulation of continuum diffusion theory. These are the following: there is no binding of PPO molecules to the solvent; the distribution of PPO molecules is random; there is no repulsion or attraction between diffusing species, *i.e.* there is no correlation between molecular movement; the rate of quenching is diffusion controlled; the solvent is continuous.

Although we have observed transient quenching in the formation of PPO excimers in cyclohexane (*i.e.* a solvent of similar viscosity) it is much less noticeable, implying the presence of a specific solvent effect in ethanol. Difference analysis of steady state spectra (to be published) have revealed evidence of hydrogen bonding of PPO to ethanol. This would have the effect of reducing the diffusion coefficient to an extent which depends on temperature.

Yguerabide and Burton [11] originally introduced the idea of solute domains for PPO in cyclohexane in order to account for the rate of concentration quenching, as determined by spectral measurements, being more than twice that found from the steady state decay time component. However, a greater rate of quenching than that predicted by Stern-Volmer kinetics at higher quencher concentration is precisely how transient quenching would be expected to manifest itself in spectral measurements. Nevertheless, our observations at $T < 25$ °C (see below) do produce some evidence for there not being a completely random distribution of PPO molecules. Computer simulation studies of molecular motion in a two-dimensional fluid predict

that clustering of molecules can occur and that the diffusion coefficient, rather than increasing within a cluster, decreases by about 40% [20]. This type of behaviour may also contribute to the low diffusion coefficients we have determined. The question of correlation between molecular motion takes on an extra dimension in the case of excimers as the very formation of the excimer necessitates an attraction potential over a range which in this case is likely to be comparable with the diffusion length of PPO in its excited state (about 5 Å at 25 °C). On its own this might be expected to increase the diffusion coefficient but the fact that the opposite is observed implies that most PPO molecules are not excited in a location and orientation initially suitable for excimer formation, *i.e.* the excimer formation is diffusion controlled.

5.2. $T < 25\text{ °C}$

The increasing χ^2 values and common shape of the residuals for the bi-exponential fit to all of the decay as shown in Fig. 2 is again consistent with the dominance of transient quenching as the temperature is lowered below 25 °C. However, the high χ^2 values obtained with a bi-exponential fit to just the tail of the decay at lower temperatures, as shown in Table 1, indicate that the departure from such kinetics is not only due to transient quenching. It is not possible to analyse the transient behaviour because of the anomalous τ_2 values and high χ^2 values obtained using eqn. (11). The question arises as to whether eqn. (11) is inappropriate because it is an inexact solution which neglects non-linear terms. Bearing in mind that the exponent describing the transient-quenching behaviour is in itself a general solution if $k_{MD} = 0$, we would expect the misfit to be worse as the intensity and the rate of reverse dissociation increase, *i.e.* at higher temperatures. In fact the opposite is observed and this leads us to examine once again the assumptions of the continuum diffusion theory upon which eqn. (11) is based.

Berlman concluded on the basis of absorption measurements that ground state aggregation did not occur in PPO [7]. We have also found that at both -25 and 50 °C the time-resolved fluorescence spectra of the rising edge of the fluorescence decay are very similar to that for a dilute solution of PPO, again indicating that ground state dimerization is negligible in comparison with excimer formation [21]. At 0.1 M concentration the mean PPO molecular separation is about 25 Å, its molecular length is about 12 Å and our measurements indicate a steady state diffusion length in the excited state of about 5 Å at 25 °C. Hence, there might be expected to exist a significant fraction of pre-formed molecular orientations (as distinct from bound ground state dimers) for which excimer formation is favoured. However, even if ground state dimers were present, their decay kinetics would be indistinguishable from excimers at an emission wavelength of 360 nm and hence would not account for the anomalous τ_2 values observed.

As already mentioned, the presence of a number of decay components in solution usually implies the existence of an equal number of excited

state species. Hence, the significantly better fits at lower temperatures provided by three exponentials as compared with those for the two exponentials of the transient-quenching model might suggest the emergence of a third excited state species in addition to the monomer and excimer. The poorer χ^2 values at lower temperatures, when fitting to just the tail of the decay with a bi-exponential model, would be consistent with this. When reconvoluting eqn. (11) it is difficult to predict how the best-fit parameters might be influenced by an additional decay parameter. We have observed such anomalously high τ_2 values when applying eqn. (11) to homopolymers of vinyl PPO [22] where a distribution of intramolecular excimer decay times occurs owing to there being a distribution of excimer conformations. However, for the low molecular weight species considered here, our findings so far are that the decay parameters are independent of emission wavelength and the time-resolved spectra are independent of delay time after the direct monomer emission has decayed to zero. This implies that the PPO intermolecular excimer has only one conformation, which cannot, in a homogeneous environment, provide more than one decay component.

At concentrations as high as 0.1 M the formation of microcrystals becomes increasingly possible as the temperature is lowered and these might also show their presence as an additional excited state species. Measurements on PPO crystals gave a decay time of about 2.3 ns at 360 nm, which is close to the 2.1 ns component obtained with a three-component fit at 0 °C. However, such a component seems hard to reconcile, if it were due to microcrystals, with the time-resolved spectral evidence [21] which at short delay times showed a fluorescence spectrum very similar to that in dilute solution even though the crystal fluorescence spectrum is markedly different. In addition, the solutions always looked optically transparent, even at 0 °C, with low scattered light levels observed on both visual inspection and measurement in the fluorometer. We thus conclude that microcrystallization is not significant and that only two excited state species, monomer and excimer, are present.

An alternative explanation by which two excited state species could account for the anomalously high τ_2 values obtained with eqn. (11), and the better fits obtained with a three-component model at lower temperatures, is that we are dealing with a heterogeneous distribution of PPO molecules. In this interpretation a dynamic equilibrium exists between more isolated PPO molecules and clusters which are formed by van der Waals forces and in which PPO molecules diffuse as the clusters themselves diffuse through the solvent. Within the clusters the diffusion coefficient and hence the quenching rate would differ from that of more isolated molecules [20] such that the two excited state species of monomer and excimer would each have at least two and more likely a distribution of decay times. The fluorescence spectra of the monomer and excimer would still remain unchanged but the effect on the reconvolution analysis would be similar to that which we have observed in vinyl PPO homopolymers where the presence of a distribution of decay times is established [22].

As a general comment it seems that the deviations from the predictions of simple continuum diffusion theory, observed above and below 25 °C, may well be related, with their effect becoming more pronounced as the temperature is lowered. Indeed, the presence of more than two decay components could in itself contribute to the low diffusion coefficients determined with eqn. (11).

Further work is under way to investigate the extent of transient self-quenching in other scintillator systems and to determine the kinetics of PPO excimer formation in anisotropic media.

6. Conclusions

The conventional time-independent excimer kinetics have been shown to be inappropriate for a fluorophore with a fast fluorescence lifetime in a low viscosity solvent. The transient behaviour can be described for the general case of excimer reverse dissociation taking place and meaningful parameters can be obtained. This might open up opportunities for measuring diffusion coefficients using a wider range of excimeric fluorescence probes which include those undergoing reverse dissociation. It should be noted though that recently Blackwell *et al.* [23] have questioned the validity of the diffusion-controlled self-quenching of pyrene in lipid bilayers and hence its use as a probe of such media. They have suggested that it exists in anisotropic media in equilibrium between two forms, a monomer and an aggregate. Our findings for the behaviour of PPO molecules in the comparatively simple medium of an isotropic and low viscosity solvent suggest that, even when the presence of diffusion control is established, care should be exercised when using excimeric fluorescence probes because of the limitations of the continuum model upon which the description of their quenching dynamics is based.

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