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Excited state and free radical properties of rhodamine dyes in aqueous solution: A laser flash photolysis and pulse radiolysis study

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Abstract

The techniques of pulse radiolysis and laser flash photolysis have been used to obtain both novel and improved data on the yields, lifetimes and absorption spectra of the triplet state, radical anion and radical cation of a number of rhodamine dyes. These parameters have also been measured for the widely-studied rhodamine 6G and are compared with previous data obtained using different techniques. In view of the importance of rhodamine dyes in laser action, in photobiology and in other biological processes, the results of this study provide key data on the photophysical and free radical properties of this important class of dyes. © 1997 Elsevier Science S.A.

Keywords: Rhodamines; Laser flash photolysis; Pulse radiolysis; Triplet state; Free radical

1. Introduction

Rhodamine dyes are used, not only for dyeing purposes (e.g. Ref. [1]), but also in lasers [2–4], in the probing of mitochondria [5], in the staining of damaged cells [6], as antitumor agents [7,8] and in photosensitised cell killing [9,10]. For most, if not all, of these processes, an understanding of the role of rhodamine excited states and free radicals in the mechanism of action is of importance. In the case of laser action, for example, the gain coefficient of a rhodamine dye depends on a number of parameters including the absorption cross-sections and population densities of the lowest excited dye singlet and triplet states. Values for the first of these parameters for a number of rhodamine dyes were measured recently by the authors in a picosecond laser flash photolysis study [11]. Although for most laser dyes the quantum yield of intersystem crossing is small, nevertheless, the effectiveness of a dye laser may be impaired if either degradation occurs through the triplet state or if the absorption cross-section of the triplet state is high.

Studies of the properties of the triplet states of rhodamine dyes have relied on either microsecond flash photolysis [12–21] or on steady-state laser techniques [22,23] to produce the triplet state. In the case of flash photolysis, both energy transfer and singlet depletion methods have been applied to derive the absorption spectra and associated molar absorption coefficients of the triplet states of rhodamine 110, rhodamine B, rhodamine 6G, sulforhodamine and rhodamine 575 in a variety of solvents, including water, alcohols, polymer matrices and glassy solvents. Despite this, there is still considerable uncertainty in the triplet state parameters, such as quantum yield, extinction coefficient, lifetimes and absorption spectra. This is not surprising given the anticipated low yield of intersystem crossing (for example, the quantum yield of fluorescence of rhodamine 101 is quoted as unity [24]), pulsed light excitation sources with durations measured in tens of microseconds and sometimes milliseconds, uncertainty of extent of energy transfer, assumptions in the singlet depletion method, limited access to pulsed lasers etc. Thus, for the most widely-studied dye, rhodamine 6G, some triplet absorption spectra were either measured over a restricted wavelength range or have significant spectral contributions from semi- or fully-reduced forms of the dyes. Similarly, only a limited amount of data on molar absorption coefficients of the rhodamine 6G triplet state is available. In ethanol, for example, there is only one set of data giving values at two discrete wavelengths only [15] while in aqueous solutions there are two sets of data comprising a total of five discrete wavelengths in two separate studies [13,19]. Triplet state lifetime and yield data are also sparse and uncertain. In ethanol, triplet lifetimes appear to range from 250 ns to 3400 μs [14,15,25–29]. Triplet state quantum yield values are available for rhodamine 6G [15], rhodamine B [16], rhodamine 110 [16] and rhodamine 123 [30] and range from 2×10^{-3} to unity.

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Rhodamine dye free radicals can be produced as a result of reactions of rhodamine excited states with suitable donors or acceptors. Like xanthene dyes, rhodamines (aminoxanthenes) are expected to produce the corresponding semi-reduced forms more easily than the semi-oxidised forms [31–35]. For rhodamines, this is supported by the observation of fully-reduced forms of the dye following reactions of the triplet state in ethanolic solutions [15]. It has also been proposed that the semi-reduced form of rhodamine 123 is produced on reaction of the dye with porphyrin triplet states so enhancing the photodynamic effect of porphyrins as photosensitisers [36].

The semi-reduced forms of rhodamine dyes can be studied unambiguously using pulse radiolysis techniques in which solvated and hydrated electrons are used to reduce the dye. Thus, the reaction of the solvated electron with rhodamine 6G, rhodamine B, rhodamine 3B and rhodamine 110 has been investigated in ethanolic solution [37,38]. Studies in aqueous solution have, however, been restricted to rhodamine 6G and rhodamine B [38–40]. In the same studies, the reactions of the strong oxidant, ·OH, with the dyes were also investigated. Although it seems likely that the semi-oxidised form of rhodamine dyes was formed in these experiments, it was difficult to make spectral assignments because of the simultaneous formation of free radicals resulting from the addition of ·OH to the dye.

The purposes of the present study are:

1. to produce rhodamine triplet states by energy transfer in nanosecond laser flash photolysis experiments and so determine, their quantum yield, their absorption spectra, molar absorption coefficients and lifetimes. This aspect of the study will also yield completely new data for rhodamine 101, rhodamine 3B and sulforhodamine B.
2. to use the pulse radiolysis technique to produce novel data on the semi-reduced forms of rhodamine 101, rhodamine 3B, sulforhodamine 101 and sulforhodamine B in aqueous solution. Data will also be presented for rhodamine 6G and rhodamine B where significant differences with earlier studies have been observed.
3. to use the pulse radiolysis technique to produce the one-electron oxidised forms of the dyes for rhodamine 6G, rhodamine B, rhodamine 101, sulforhodamine 101 and sulforhodamine B.

In this way, it is anticipated that both new and improved yield, spectroscopic and lifetime data will be available for rhodamine triplet states and free radicals which will contribute significantly to understanding the role of rhodamines in laser action, in the photosensitisation of biological systems and in other applications where their excited states and/or free radicals may be involved.

2. Experimental

'Laser Grade' rhodamine 6G, rhodamine B, rhodamine 101, sulforhodamine 101 and sulforhodamine B were supplied by Coherent Radiant Ltd, Cambridge, UK and Eastman Chemical International, Liverpool, UK. Rhodamine 3B was supplied by A G Electro-Optics Ltd, Tarporley, Cheshire, UK. All dyes were used as supplied. Acridine was used as supplied by the Aldrich Chemical Co Ltd, UK. HPLC grade methanol was supplied by Romil Chemicals Ltd, Loughborough, UK, BDH Chemicals, Poole, UK and by J T Baker-Inc., USA. Absolute ethanol was supplied by James Burroughs (FAD) Ltd, Essex, UK and by Romil Chemicals Ltd, Loughborough, UK.

Triply-distilled water was used to prepare aqueous solutions.

The laser flash photolysis experiments were conducted both at the North East Wales Institute and at the Center for Fast Kinetics Research (CFKR), University of Texas at Austin. In the former set-up, the excitation source was the third harmonic (355 nm) pulse from a J K Lasers System 2000, Q-switched, Nd/YAG laser. The pulse length was about 20 ns. The analysis light source was a pulsed 250W xenon arc lamp (Applied Photophysics Ltd, Model 1410) designed to produce a light pulse of about 1.5 ms duration which is 'flat' to within 5% for 350–400 μs and to within 1% for 100 μs. The analysis beam was focused onto the sample cell using a quartz lens and then transmitted to a monochromator (Applied Photophysics Ltd, Model 7300) and finally to the photomultiplier (RCA IP28A). The signal from the photomultiplier was fed to a Phillips PM 3311 digital oscilloscope. Data processing was performed on a Hewlett Packard HP9153 computer. The quartz sample cell had a pathlength of 1 cm and was filled directly from a reservoir of nitrogen saturated solution, under a small pressure of nitrogen. In the laser experiments performed at CFKR, a Quantel YG580 Q-switched Nd:YAG laser (355 nm, 11 ns pulse) was used. Other details of the apparatus are described elsewhere [42]. Pulse energies were typically adjusted to ensure that < 10% of ground state molecules were converted to excited states. In practice, incident pulse energies were of the order of 1–2 mJ.

The apparatus used in the pulse radiolysis studies has been described in detail elsewhere [43]. It consists of an 8–14 MeV Vickers electron linear accelerator as the source of pulsed radiation. Typical pulse lengths used in this study ranged from 10–50 ns. The optical system consisted of a 500 W xenon lamp, a Kratos GM252 monochromator, a RCA IP28 or Hamamatsu R928 photomultiplier and a Tektronix 7612D digitiser. A 2.5 cm quartz sample cell was used which was automatically filled with a reservoir under a slight pressure of nitrogen with the solution under investigation. For dosimetry, a nitrous oxide saturated aqueous solution of potassium thiocyanate was pulse irradiated. Radiation doses were calculated from the transient absorption of $(SCN)_2\cdot^-$ assuming $G[(CNS)_2\cdot^-] \times \epsilon = 2.23 \times 10^{-4} m^2 J^{-1}$ where G and ϵ represent the radiolytic yield (mol J^{-1}) and molar absorption coefficient of $(CNS)_2\cdot^-$ respectively [48]. The yields of the free radicals, $N_3\cdot$ and $Br_2\cdot^-$ were calculated from the radiation dose assuming radiolytic yields of 0.62

μmol J^{-1} and 0.58 μmol J^{-1} respectively [49]. Similarly, the yield of e^-_{aq}, used to calculate the molar absorption coefficient of the rhodamine radical anions, was taken to be 0.27 μmol J^{-1}.

3. Results and discussion

3.1. Rhodamine triplet state properties

3.1.1. Sensitisation by energy transfer

The acridine triplet state ($E_T = 189$ kJ mol^{-1} [44]) was used to sensitise the formation of rhodamine triplet states. A molar absorption coefficient value for the acridine triplet state of 22 500 dm^3 mol^{-1} cm^{-1} at 440 nm has been reported [45]. In the experiments, described here, the acridine triplet state was produced by excitation at 355 nm in both methanolic and ethanolic solutions. Care was taken to ensure that the laser excitation energy was kept to a minimum so that the decay of the acridine triplet was exponential. Typically, the first-order rate constant was found in our work to be $7.0 \pm 0.5 \times 10^4$ s^{-1}.

Using deaerated alcoholic solutions of acridine (typically 1.0×10^{-4} mol dm^{-3}), which also contained the rhodamine dye under investigation at concentrations in the range 1.0–5.0×10^{-5} mol dm^{-3}, it was found that the acridine triplet decayed more rapidly. Fig. 1 shows a typical plot of the acridine triplet state decay constant against rhodamine 6G concentration in ethanolic solution. Similar plots were obtained for rhodamine B, rhodamine 101, rhodamine 3B, sulforhodamine 101 and sulforhodamine B. Analogous experiments were also performed in methanol. In these experiments, the rhodamine dye will also absorb some excitation light. Typically, the proportion of light absorbed varied from 13% to 27%. However, it is unlikely that the direct excitation of rhodamine would lead to observable transient excited states on the timescales studied, particularly since the quantum yield for intersystem crossing is low (vide infra). Thus, the kinetic data for the decay of acridine triplet state are unlikely to contain any contribution from other transient species. The bimolecular rate constants, k_q, for the reaction:

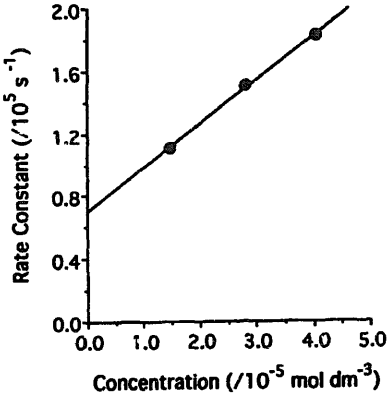

Fig. 1. The effect of rhodamine 6G concentration on the observed rate constant (s^{-1}) for the decay of the acridine triplet state in ethanol. Wavelength of observation = 450 nm; solutions were N_2 saturated.

Table 1
Values of the bimolecular rate constant (dm^3 mol^{-1} s^{-1}) for the reaction of the acridine triplet with rhodamine dyes in alcoholic solution

Dye	k_q (10^9) in ethanol	k_q (10^9) in methanol
Rhodamine 6G	2.8	3.3
Rhodamine B	2.4	3.7
Rhodamine 101	4.6	7.3
Rhodamine B	2.4	4.1
Sulforhodamine 101	2.9	4.3
Sulforhodamine B	3.1	3.8

The error limits are estimated as ± 10%.

$$Ac^{3}* + Rh \xrightarrow{k_q} Rh^{3}* + Ac \quad (1)$$

(where Ac = acridine and Rh = rhodamine) were, therefore, calculated from the slopes of plots such as that shown in Fig. 1 and are summarised in Table 1. The values are close to those expected for diffusion-controlled reactions suggesting that the energy of the acridine triplet state is at least 30 kJ mol^{-1} greater than that of the rhodamine triplet state.

3.1.2. Determination of molar absorption coefficients

These were determined also from the acridine energy transfer experiments on the assumption that reaction 1 represents the only fate of acridine triplets when reacting with rhodamine dyes. Other possibilities include, for example, the production of the semi-oxidised and semi-reduced forms of the rhodamine dyes. However, such species have either different spectral or kinetic properties or both (see below) and are excluded, therefore, as alternative products in the reaction of acridine triplet states and rhodamine dyes. Experimental conditions were selected, therefore, to maximise the energy transfer process (reaction 1) compared to the intrinsic decay of the acridine triplet state. Typically, this required the use of dye concentrations of the order of 4×10^{-5} mol dm^{-3} where about 60% of the acridine triplet states would react to produce rhodamine triplet states. The actual proportions were calculated using data from plots such as Fig. 1. Under such conditions, the acridine triplet state was found to decay by more than 95% within about 20 μs to produce a residual relatively stable absorption assigned to the rhodamine triplet state. These observations represent the most direct and unambiguous evidence, to date, on the production of rhodamine triplet states. The transient absorption assigned to the triplet states of the rhodamine dyes showed maxima between 410 and 440 nm. To determine the molar absorption coefficients at these and other wavelengths, it was necessary to calculate the proportion of acridine triplet states which react with rhodamine dyes to produce rhodamine triplet states.

This proportion, F, was taken to be;

$$F = k_q[Rh]/(k_q[Rh] + k_o) \quad (2)$$

where k_o is the first order rate constant representing the decay of acridine triplet states in the absence of rhodamine dye and was obtained from the intercepts of plots such as that in Fig. 1.

Table 2
Extinction coefficients of rhodamine triplet states in ethanol and in methanol [a]

Dye	λ max (nm)	∈ (dm^3 mol^{-1} cm^{-1})	
		This Study	Previous Studies
Ethanol			
Rhodamine 6G	410	2.3×10^4	1.8×10^4 (Ref. [15])
Rhodamine B	420	4.2×10^4	1.21×10^4 (Ref. [16])
Rhodamine 101	440	8.6×10^3	
Rhodamine 3B	420	7.0×10^4	
Sulforhodamine 101	430	1.5×10^4	
Sulforhodamine B	420	5.4×10^4	
Methanol			
Rhodamine B	420	2.4×10^4	
Rhodamine 101	430	1.0×10^4	
Rhodamine 3B	425	7.2×10^4	

[a] The error limits are estimated as ±45% (see text).

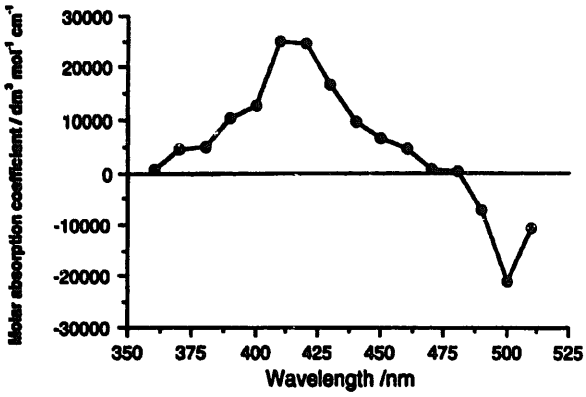

Fig. 2. Difference absorption spectrum of the rhodamine 6G triplet state in ethanol.

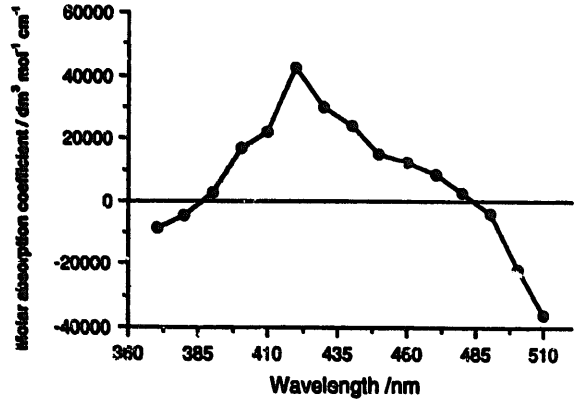

Fig. 3. Difference absorption spectrum of the rhodamine B triplet state in ethanol.

Hence, values of F could be used to calculate the absorbances of the rhodamine triplet state assuming that all acridine triplets, which were quenched, produced an equivalent number of rhodamine triplets. In this instance the concentration of rhodamine triplet states at the end of reaction 1 can be calculated from

$F \cdot A(Ac^T) / \in (Ac^T)$.

where A (Ac^T) represents the observed initial absorbance due to the acridine triplet state, and $\in (Ac^T)$ represents the molar absorption coefficient of the acridine triplet state (taken in this study to be 22,500 dm^3 mol^{-1} cm^{-1} at 440 mm in ethanol [45]). It is then a simple matter to calculate $\in (Rh^T)$ at each wavelength where $\in (Rh^T)$ represents the molar absorption coefficient of the rhodamine triplet state. Such calculations were made for all the rhodamine dyes studied. Table 2 shows the data so obtained.

The main factors which determine the error limits of the molar absorption coefficient measurements are the errors limits of the molar absorption coefficient of the acridine triplet state and the experimentally determined absorbances of the acridine and rhodamine triplet states. These three error limits are assumed to be ±15% [44], ±10% and ±15% respectively making a combined error limit of about ±45%.

The difference absorption spectra representing the difference in molar absorption coefficients between the rhodamine triplet and singlet (ground states) at each of the wavelengths studied are shown in Figs. 2–7 for measurements made in ethanol. The spectra obtained in methanol for rhodamine B, rhodamine 101 and rhodamine 3B are not shown here since they were found to be similar in shape to those obtained in ethanol.

As stated in Section 1, there are relatively few published values of molar absorption coefficients of rhodamine dye

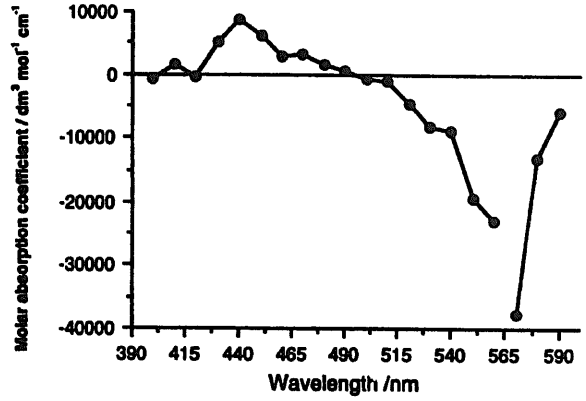

Fig. 4. Difference absorption spectrum of the rhodamine 101 triplet state in ethanol.

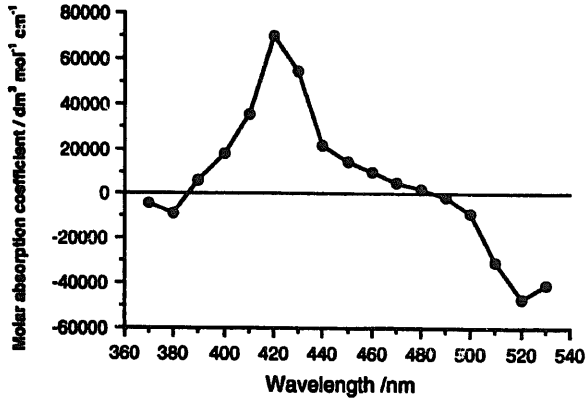

Fig. 5. Difference absorption spectrum of the rhodamine 3B triplet state in ethanol.

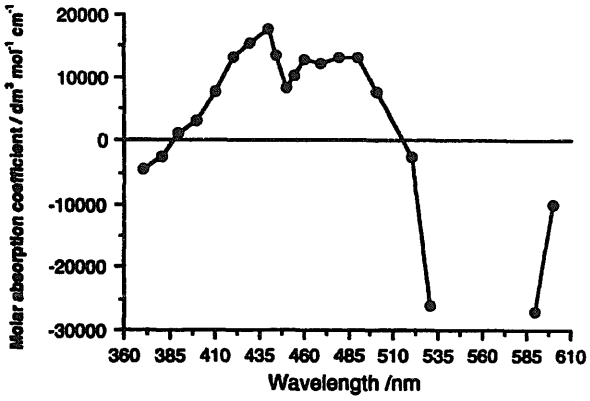

Fig. 6. Difference absorption spectrum of the sulforhodamine 101 triplet state in ethanol.

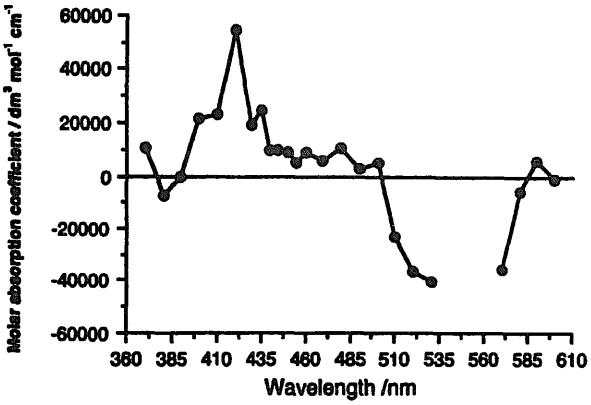

Fig. 7. Difference absorption spectrum of the sulforhodamine B triplet state in ethanol.

triplet states. The values reported here are obtained from energy transfer experiments using nanosecond laser pulses. They do not depend, therefore, on the direct excitation of the rhodamine dyes where extreme experimental conditions are necessary to produce detectable amounts of triplet states, given the very low quantum yield of intersystem crossing (<0.001) reported below. The sensitisation method used here is also not affected by the lifetime of the rhodamine triplet state, as in earlier experiments, when there was limited access to pulsed lasers and hence lamp pulses of the order of ms duration were in common use [15]. It is, however, interesting to note that, despite these reservations, the published molar absorption coefficient value in ethanol at 411 nm for rhodamine 6 G of 18 000 $dm^3\ mol^{-1}\ cm^{-1}$ [15] is reasonably close to that reported here. In contrast, the published value for rhodamine B in ethanol at 419 nm of 12 100 $dm^3\ mol^{-1}\ cm^{-1}$ [16] is much smaller than that reported here. It appears that the interpretation of the spectral data obtained in both sets of earlier experiments was affected, amongst other considerations, by spectral contributions from relatively long-lived photo products [15,16,44]. For two dyes (rhodamine 101 and rhodamine 3B), measurements made in methanol showed no effect of solvent on the molar absorption coefficient for the triplet state. In the case of rhodamine B the different value found in methanol lies within the combined error limit of measurement and hence no attempts have been made here to account for the difference.

3.1.3. Triplet state lifetimes

The lifetimes of the triplet states of rhodamine dyes were also investigated using acridine as sensitiser. Using relatively high rhodamine concentrations, (8×10^{-5} mol dm^{-3} to 1.9×10^{-4} mol dm^{-3}), more than 95% of the acridine triplet states reacted with the rhodamines within 5–10 μs following the laser pulse. The decay of the acridine triplet state obeyed first-order kinetics within this range of rhodamine dye concentrations. The subsequent decay of the triplet state could thus be followed on longer timescales. Analysis of such decay traces for all the rhodamine dyes showed that the decay obeyed second-order kinetics, with no detectable first-order components. The only mechanism which could account for this is the self-reaction of triplet states, presumably to form ground state rhodamine molecules. $k/\in$ values ($cm\ s^{-1}$) are given in Table 3 together with k values calculated using molar absorption coefficients measured in the energy transfer experiments in this study. It can be seen from the Table that the triplet–triplet reaction is very efficient, occurring at rates approaching those of diffusion-controlled reactions. This data represents an improvement on previous data where no information on the kinetic order of the decay was available and where 'lifetimes' were found to range from 250 ns to 3.4 ms [15,25–29]. Such a wide range of lifetime values may indicate that there are varying proportions of first and second-order components in the observed decays of the rhodamine triplet states. In this study, much (>90%) of the initially-formed rhodamine triplet state had disappeared within about 180 μs by second-order processes. On the assumption that earlier studies also produced at least micromolar concentrations of rhodamine triplet state which would be expected to decay initially by second-order kinetics, such decay rates are too rapid to allow meaningful quantitative analysis and interpretation of earlier conventional flash photolysis experiments in which both direct and sensitised experiments were used to produce rhodamine triplet states. The relatively rapid decay observed in this study of the rhodamine triplet states compared to the longer lifetimes of the cation and anion radical forms of the dyes (see later) provides further support to the assignment to the triplet state.

Table 3
Values of $k/\in$ ($cm\ s^{-1}$) and k ($dm^3\ mol^{-1}\ s^{-1}$) for the decay of rhodamine triplet states in methanol [a]

	$k/\in$	k
Rhodamine 6G	8.6×10^5	1.4×10^{10}
Rhodamine B	1.7×10^6	1.5×10^{10}
Rhodamine 101	1.6×10^6	1.1×10^{10}
Rhodamine 3B	1.2×10^6	1.0×10^{10}
Sulforhodamine 101	1.2×10^6	7.2×10^9
Sulforhodamine B	2.0×10^6	1.4×10^{10}

[a] The error limits are estimated as ±10%.

3.1.4. Intersystem crossing yields

Direct excitation at 355 nm of the rhodamine dyes in ethanol and methanol by a 20 μs laser pulse produced no detectable transient absorbance (<0.005) in the region 410–430 nm up to 5 mJ incident energy. At this laser energy, optically-matched solutions of deaerated ethanolic solutions of acridine were also subjected to laser pulses and the transient absorbance of the acridine triplet state measured at 445 nm. Using this absorbance value, together with the known intersystem crossing (ISC) yield of acridine and the molar absorption coefficients in Table 2, the maximum values for the ISC yields of the rhodamine dyes was calculated to be between 0.01 and 0.02 for the dyes studied.

3.2. One electron oxidation and reduction of rhodamine dyes

3.2.1. One-electron oxidation

Pulse radiolysis techniques were used to produce the powerful one-electron oxidants, $N_3\cdot$ ($E^\circ = 1.34$ V [46]) and $Br_2\cdot^-$ ($E^\circ = 1.65$ V [47]) by irradiating nitrous oxide saturated solutions of either 10^{-2} mol dm^{-3} sodium azide or 10^{-2} mol dm^{-3} potassium bromide. Under these conditions, $N_3\cdot$ and $Br_2\cdot^-$ are produced as follows:

$$H_2O \rightarrow e_{aq}^-, H\cdot, \cdot OH \quad (4)$$

$$e_{aq}^- + N_2O \rightarrow \cdot OH + OH^- \quad (5)$$

$$\cdot OH + N_3^- \rightarrow N_3\cdot \quad (6)$$

$$\text{or } \cdot OH + Br^- \rightarrow Br + OH^- \quad (7a)$$

$$\text{and } Br\cdot + Br \rightarrow Br_2\cdot^- \quad (7b)$$

Under such conditions, $N_3\cdot$ and $Br_2\cdot^-$ are produced in yields of 0.62 and 0.58 μmol J^{-1} respectively within 100 ns following the pulse. In the presence of rhodamine concentrations of the order, of 2×10^{-5} mol dm^{-3}, therefore, both $Br_2\cdot^-$ and $N_3\cdot$ would be expected to react over much longer timescales. By following either the decay of $Br_2\cdot^-$ at 360 nm or the build-up of the transient oxidation product in the case of the azide radical reaction, it was observed that both $N_3\cdot$ and $Br_2\cdot^-$ reacted rapidly with the rhodamine dyes. The transient absorption spectra so produced were found to be identical in the case of rhodamine 6G in both shape and intensity for both $N_3\cdot$ and $Br_2\cdot^-$ reactions. This indicated strongly that only electron transfer was occurring, as proposed for many reactions of these free radicals (reviewed in [50]), and it was therefore decided to use $N_3\cdot$ as the sole oxidant for the remainder of the dyes. For all the dyes studied, the bimolecular rate constant for reactions of $N_3\cdot$ with the dyes was found to be $5.0\pm0.5\times10^9$ dm^3 mol^{-1} s^{-1}. In a typical experiment using $N_3\cdot$ as oxidant and using approximately 2×10^{-5} mol dm^{-3} rhodamine solutions, the oxidation was observed to be complete within 100 μs following the pulse and certainly an order of magnitude faster than the decay of $N_3\cdot$ in the absence of dye. Figs. 8–12 show the transient absorption spectra of the products of the reaction of $N_3\cdot$ with a number of rhodamine dyes. In each figure, the most intense spectrum shown represents the maximum

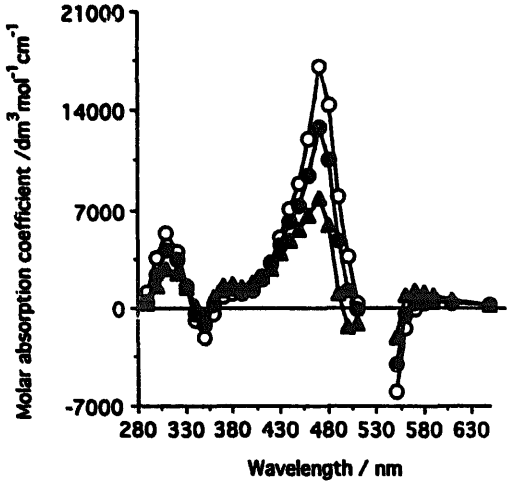

Fig. 8. Transient absorption spectra observed on pulse radiolysis of nitrous oxide saturated aqueous solutions of 10^{-2} mol dm^{-3} sodium azide containing 2.2×10^{-5} mol dm^{-3} rhodamine 6G at pH 7 (○, 86 μs; ●, 274 μs; ▲, 878 μs).

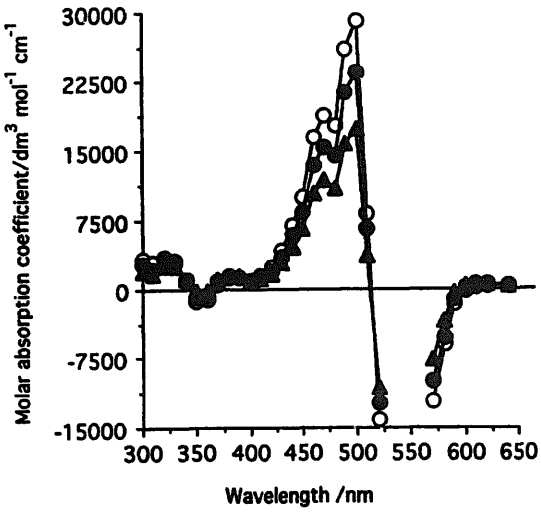

Fig. 9. Transient absorption spectra observed on pulse radiolysis of nitrous oxide saturated aqueous solutions of 10^{-2} mol dm^{-3} sodium azide containing 2.2×10^{-5} mol dm^{-3} rhodamine B at pH7 (○, 66 μs; ●, 312 μs; ▲, 824 μs).

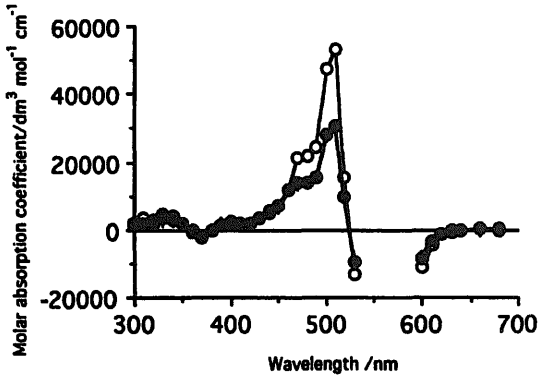

Fig. 10. Transient absorption spectra observed on pulse radiolysis of nitrous oxide saturated aqueous solutions of 10^{-2} mol dm^{-3} sodium azide containing 2.2×10^{-5} mol dm^{-3} rhodamine 101 at pH7 (○, 50 μs; ●, 770 μs.

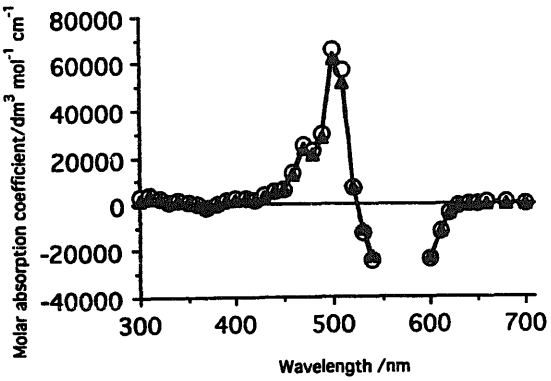

Fig. 11. Transient absorption spectra observed on pulse radiolysis of nitrous oxide saturated aqueous solutions of 10^{-2} mol dm^{-3} sodium azide containing 2.2×10^{-5} mol dm^{-3} sulforhodamine 101 at pH7 (○, 74 μs; ▲, 864 μs).

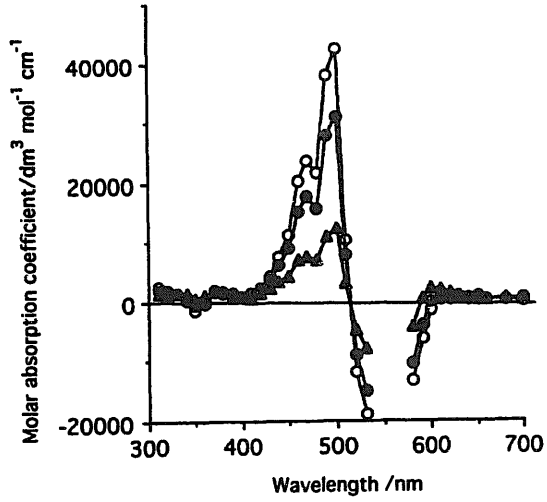

Fig. 12. Transient absorption spectra observed on pulse radiolysis of nitrous oxide saturated aqueous solutions of 10^{-2} mol dm^{-3} sodium azide containing 2.2×10^{-5} mol dm^{-3} sulforhodamine B at pH7 (○, 52 μs; ●, 264 μs; ▲, 1.72 ms).

amount of rhodamine free radical formed at the end of the $N_3\cdot$ reaction. Molar absorption coefficients were calculated assuming a total yield of $N_3\cdot$ of 0.59×10^{-6} mol J^{-1}. All these spectra represent the difference in molar absorption coefficient between the free radical product and the ground state. Hence all show a 'depletion' around 550 nm. In addition, an apparent maximum is seen in the range 470–510 nm for all the dyes studied which is clearly characteristic of the rhodamine free radical species. This species is designated a cation radical as shown in the equation:

$$N_3\cdot + Rh \rightarrow Rh^+\cdot + N_3^- \quad (9)$$

In fact, the actual charges on $Rh^+\cdot$ will vary according to the charges on the parent, Rh. Thus, both rhodamine 6G and rhodamine B have a single positive charge in the parent molecule, rhodamine 101 and sulforhodamine 101 and sulforhodamine B are neutral overall.

From these cation radical spectra, it is clear that cation radicals are not produced in the energy transfer experiments with acridine described earlier (see above) since there are distinct differences between these cation radical spectra and those assigned to the triplet state. Although, this possibility was always regarded as unlikely, in view of the known tendency of xanthene dyes to produce radical anions [37], it was considered important to confirm this view experimentally.

When corrected for the loss of the rhodamine dye ground state absorption (data not shown), the absolute extinction coefficients of the cation radicals confirm the presence of a distinct shoulder at the λ_{max} values seen in Figs. 8–12 i.e. at 470 nm (rhodamine 6G), at 500 nm (rhodamine B), at 500 nm (sulforhodamine B), at 500 nm (sulforhodamine 1001) and at 500 nm (rhodamine 101). In the ground state absorption spectra of these rhodamine dyes, these λ_{max} values appear to correspond with an observable but indistinct shoulder. It would appear therefore that the electronic transition probability giving rise to this band is considerably enhanced in the cation radical states.

The lifetimes of the cation radicals are relatively long. For rhodamine 6G, for example, the decay process lasts well in excess of 20 ms. For sulforhodamine 101, the cation radical showed no decay at all over this timescale. In the structurally-related dye, the rhodamine 101 cation radical optical absorption decayed to about 50% of the original absorption over 270 μs and was found to be stable thereafter up to at least 20 ms. Since the structures of rhodamine 101 and sulforhodamine 101 are so similar, it seems likely that the stable forms of their cation radicals are also similar. It is not clear, however, what type of process the rhodamine 101 cation radical undergoes to achieve a structure similar to that of the sulforhodamine cation radical.

3.2.2. *One-electron reduction*

The reaction of the hydrated electron e_{aq}^-, with rhodamine dyes was studied by pulse-irradiating nitrogen saturated aqueous solutions of the dye containing *t*-butanol (0.1 mol dm^{-3}). Under these conditions, the ·OH radical reacts with *t*-butanol to form relatively unreactive free radicals. The hydrated electron was thus found to react at diffusion-controlled rates with all the rhodamine dyes (see Table 4). The spectra of the transient species formed at the end of the e_{aq}^- reactions (measured at 20 μs) after the pulse) are shown in Figs. 13 and 14.

Table 4
Bimolecular rate constants for the reaction of the hydrated electron with rhodamine dyes [a]

	k (10^{10} (dm^3 mol^{-1} s^{-1})
Rhodamine 3B	1.2
Rhodamine B	2.4
Sulforhodamine B	2.3
Rhodamine 6G	2.3
Sulforhodamine 101	1.2
Rhodamine 101	1.9

[a] The error limits are estimated as ± 10%.

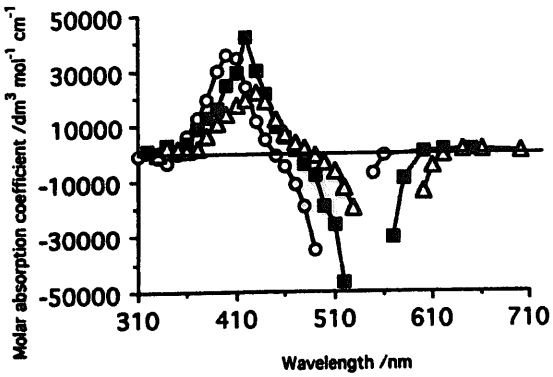

Fig. 13. Transient absorption spectra observed on pulse radiolysis of nitrogen saturated aqueous solutions (pH 7) of rhodamine dye (2×10^{-5}) mol dm^{-3}) containing 0.1 mol dm^{-3} *t*-butanol (○, rhodamine 6G; ■, rhodamine B; △, rhodamine 101 (all 20 μs measured after the pulse).

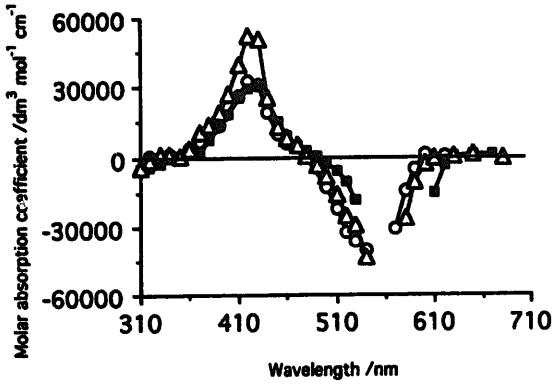

Fig. 14. Transient absorption spectra oserved on pulse radiolysis of nitrogen saturated aqueous solutions (pH 7) of rhodamine dye (2×10^{-5} mol dm^{-3}) containing 0.1 mol dm^{-3} *t*-butanol (○, rhodamine 3B; ■, sulforhodamine 101; △, sulforhodamine B (all measured 20 μs after the pulse).

The rate constants for the reactions of the hydrated electron with rhodamines obtained in this study represent new data for sulforhodamine B, sulforhodamine 101 and rhodamine 101. The values obtained for rhodamine 3B, rhodamine B and rhodamine 6G are the same, within experimental error, as those measured earlier in neutral solutions [38–40]. The lifetimes of the rhodamine radical anions are relatively long undergoing about 25% decay over 15 ms [40–42]. In ethanolic solution, it was found, however, that the radical anions of rhodamine 3B, rhodamine 6G and rhodamine B have even longer lifetimes showing usually 5% or less decay over 15 ms. Only the spectra of the radical anions of rhodamine 6G and rhodamine B have been measured previously in aqueous solution. Although similarly-shaped difference absorption spectra were obtained for these two dyes in the current study, the molar absorption coefficient measured for rhodamine B at 420 nm in this work (4.2×10^4 dm^3 mol^{-1} cm^{-1}) was much greater than that found previously (1.2×10^4 dm^3 mol^{-1} cm^{-1}) [38]. The novel data presented here for the other four rhodamine dyes, as with rhodamine 6G and rhodamine B, show that the radical anion spectra all have characteristic, relatively-intense maxima in the region 410–430 nm. Comparison of the radical anion spectra (Figs. 13 and 14) with the triplet spectra measured in ethanol (Figs. 2–7) show that there are similarities, particularly in the position of the absorption maximum. However, there are sufficient differences in both the intensity and shape of the bands to show that they are not identical. Clearly, therefore, the electronic transitions involved in both the triplet–triplet and radical anion absorptions are very similar.

4. Conclusion

Both novel and improved data on the yield, absorption spectra and lifetimes of the triplet states, radical cations and radical anions have been presented here for a number of rhodamine dyes. Such data is seen as essential to understanding the role of these dyes when used in laser dyes, in the photosensitisation of biological systems and in other applications where their excited states and/or free radicals may be involved.

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