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New Halogenated Succinylfluorescein Dyes. Synthesis and Evaluation as Singlet Oxygen Photosensitizers of their Free and Polymer-Bound Forms

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ABSTRACT

*Four halogenated xanthene dyes, viz. 2,4,5,7-tetrabromo-, 4,5-di-iodo-, 2,4,5-tri-iodo-, and 2,4,5,7-tetraiodo-succinylfluorescein have been synthesized, and the tri-iodo and the tetraiodo derivatives have been bound through ester union to a soluble, linear copoly(styrene–*p*-vinylbenzyl chloride). The quantum yields of singlet oxygen generation of free and polymer-bound dyes, relative to Rose Bengal, have been determined in dimethylformamide solution. In the case of the free iodinated dyes, the quantum yield increases with the number of iodines, ranging from 0·30 to 0·44. The two polymeric tri-iodo and tetraiodo dyes present values of 0·33 and 0·34 respectively, and their photostabilities are much higher than those of the corresponding free dyes.*

INTRODUCTION

The synthesis of efficient singlet oxygen photosensitizers based on dyes covalently bound to a polymeric matrix is a current matter of interest in our laboratories. Rose Bengal or Eosine Y has been incorporated, through ester union, to a linear copoly(styrene–*p*-vinylbenzyl chloride) (Scheme 1).[1] The resulting photosensitizers are soluble in organic media and, compared with the free dyes, have an improved photostability and a similar efficiency for

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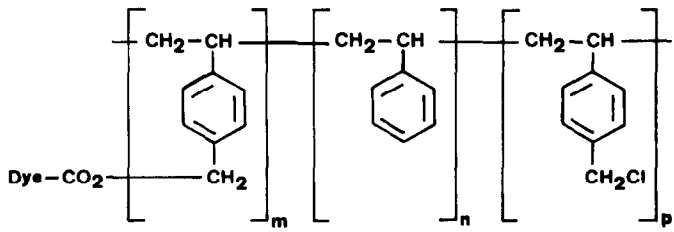


Scheme 1. Structure of linear polymer photosensitizers with a xanthene dye bound through ester union.

singlet oxygen generation. Polymers with percentages of *p*-chloromethylstyrene units ranging from 7 to 54% were used, 12 to 9%, respectively, of these units being occupied with dye molecules. Products with higher dye contents were also obtained, but they lost one of their more interesting properties, i.e. their solubility in organic solvents.

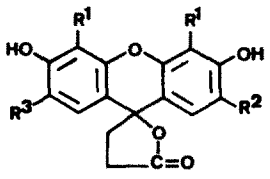
The esterification proceeds with low yield in our experimental conditions. Thus, the said 9% was reached working with a dye excess of about 200% with respect to the CH_2Cl groups in the polymer. From a steric point of view, the anchorage of a dye molecule must have a limited influence on the entrance of another one, because the average space between two neighbouring CH_2Cl groups in this initial polymer matrix is about 30 units of polymerized styrene. More likely, the low reaction yield is a consequence of the steric conformation of the polymer chain in solution, hindering access of the dye to the reactive centers.

A possible way of increasing the yield could be the reaction with xanthene dyes substituted by an aliphatic chain at the 9-position in the xanthene chromophore instead of the 2'-benzoate ring. The smaller volume and the higher mobility of such a group would facilitate access of the dye to the reactive CH_2Cl groups. Moreover, the more flexible bond of the chromophore to the polymer frame would have a positive influence in transfer of its electronic excitation energy.

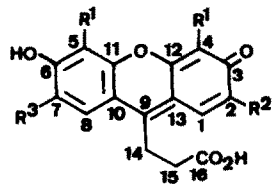
Some xanthene dyes of this structure have been described in the literature. Our attention was mainly focused on 3-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-propanoic acid, also called succinylfluorescein, SF, because it can be obtained by condensation between succinic anhydride and resorcinol, and because it has the xanthenic group of the fluorescein molecule. The fluorescent compound SF was obtained for the first time more than a century ago,[2] although the literature on its chemistry is scarce.[3,4] Aspects relative to its absorption of light,[5,6] its luminescence in the presence of oxygen and bases,[7] and its bactericidal properties[8] have been studied. The SF molecule can adopt different structures in solid form or in solution.[9,10]

The halogenated derivatives of SF 2,4,5,7-tetrabromosuccinylfluorescein

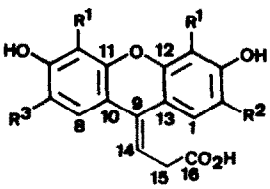
(4BrSF) and 2,4,5,7-tetraiodosuccinylfluorescein (4ISF) are the analogues of Eosine Y and Erythrosine Y (disodium salts of 2,4,5,7-tetrabromo- and 2,4,5,7-tetraiodo-fluorescein, respectively) possessing an aliphatic chain in the 9-position. In the present work we have synthesized both SF derivatives, as well as the 4,5-di-iodo and the 2,4,5-tri-iodo derivatives (2ISF and 3ISF, respectively). All of them have been evaluated as singlet oxygen photosensitizers in dimethylformamide (DMF) as solvent. The two photosensitizers with higher quantum yield of singlet oxygen generation, 3ISF and 4ISF, have been covalently bound to a linear copoly(styrene–*p*-vinylbenzyl chloride), and the resulting polymeric sensitizers PS-3ISF and



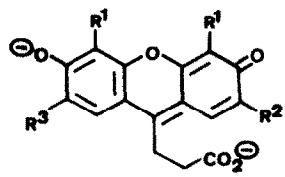
lactone



quinonoid



ethylenic



dianionic

	R^1	R^2	R^3
SF	H	H	H
2ISF	I	H	H
3ISF (cis)	I	I	H
4ISF	I	I	I
4BrSF	Br	Br	Br

PS-4ISF have also been evaluated in the same solvent. In this medium, the acid forms of all these derivatives adopt the corresponding structures with higher absorption in the visible region, i.e. the quinonoid structures with their phenolic group in ionized form.

EXPERIMENTAL

Melting points (mp) were obtained using a Reichert microscope. All SF halogenated derivatives have undefined mp. Microanalysis was performed on a Perkin–Elmer 240C apparatus. Electronic absorption spectra were recorded on Perkin–Elmer models 402 and 554, using as solvents DMF or the mixtures 'MeOH-pH 1 buffer' (0·1 M-HCl/KCl), 1:1 v/v, and 'MeOH-pH 9' buffer (0·033 M-phosphate), 1:1 v/v. Infrared spectra were registered on a Perkin–Elmer 681 apparatus, using the KBr pellet technique. ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian XL-300 spectrometer at 300 MHz and at 75 MHz, respectively, in approximately 0·1 M-solutions in DMSO-d_6,

using tetramethylsilane as internal reference. Mass spectra (MS) were recorded on a VG ZAB spectrometer at 75 eV, with direct injection and heating at 200–250°C; only significant fragments, with *m/z* higher than 100, are described. Thin-layer chromatography (TLC) was performed on silica gel Merck 60 F_{254} in 0·2 mm plates; the usual eluant was the mixture chloroform–ethanol–acetic acid, 75:20:5 by vol. Column chromatography was carried out with silica gel 60 MN, using as eluant the same mixture, but 75:20:1 by vol, and the flash procedure.[11] Traces of silica in the purified solid products were removed by dissolving them in the minimum volume of aqueous 0·1 M-Na_2CO_3; after filtration, the products were recovered by precipitation, adding enough dilute HCl or (for 2ISF) acetic acid. Analytically pure products were obtained after washing with water and drying in vacuum (0·5 torr) at room temperature, in the presence of P_2O_5, for at least a week. High-performance liquid chromatography (HPLC) analysis was run with a Waters M45 pump, a 7125 Rheodyne sample injector (20 μl), a Waters NOVA-PAK C18 (5 μm) reversed-phase column, 0·39 cm × 15 cm, a Pye Unicam 4020 UV detector, reading at 254 nm, and a Philips PM 8252 recorder. Eluants: acetonitrile-pH 2·5 buffer (0·1 M-phosphate), 30:70 v/v (eluant A), or 40:60 v/v (eluant B), working with a flow rate of 1·5 ml min^{-1}. The dye SF was obtained as described elsewhere.[9] Rose Bengal and Erythrosine Y were commercial samples (Fluka).

Synthesis of iodinated derivatives of succinylfluorescein

A solution of 2 g (7 mmol) of succinylfluorescein in 500 ml of pH 9 aqueous buffer (0·2 M-phosphate) was mixed at room temperature with 100 ml of the same buffer, containing 10 g (39 mmol) of iodine and 20 g (120 mmol) of potassium iodide. The reaction was followed by TLC and HPLC. The solution was kept at pH 9 by adding aqueous 30% NaOH. The di-iodo derivative 2ISF was the only detectable compound after 10 min. The tri-iodo derivative 3ISF always appeared in admixture with the tetraiodo product 4ISF. The highest yield of 3ISF (75%) was obtained stopping the iodination reaction when 2ISF was not detected in the medium (3–5 days). After 10 days, the reaction mixture had 85% 4ISF and 15% 3ISF. Longer times or higher temperatures gave rise to an unidentified product difficult to separate by chromatography from 4ISF. The reactions were stopped by the addition of enough 0·1 M-sodium bisulfate solution to eliminate the excess of iodine. At the same time, the iodinated compounds precipitated. After filtration, the products were purified by column chromatography.

4,5-Di-iodosuccinylfluorescein (2ISF)
Brown microcrystals with undefined mp (softening at about 215°C).

(Analysis. Calculated for $C_{16}H_{10}I_2O_5$: C, 35·8; H, 1·9. Found: C, 35·9; H, 2·1%.) 2ISF could also be purified through its hydrochloride, formed by dissolving in hot aqueous 4% HCl. The salt which separated on cooling was filtered, washed with water and dissolved in the minimum volume of aqueous 30% NaOH. The purified 2ISF was precipitated by adding acetic acid. Visible spectra, λ_{max} (log ε): in DMF, 498 sh (4·32), 536 nm (4·81); in MeOH-pH 1 buffer, 1:1 v/v, 455 nm (4·19); in MeOH-pH 2·5 buffer, 1:1 v/v, 445 sh (3·81), 475 (3·96), 510 sh nm (3·86); in MeOH-pH 9 buffer, 1:1 v/v, 484 sh (4·32), 514 nm (4·73). IR (KBr) ν_{max}: 3000b, 1720m, 1625m, 1590s, 1485vs, 1450vs, 1380vs, 1300vs, 1165s, 1130s, 820m cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6) (recent solutions showed a 1:3 mixture of quinonoid and ethylenic forms, respectively): δ-quinonoid form, 2·55 (m, H-15), 3·47 (m, H-14, overlapped), 6·85 (m, H-2 and H-7, overlapped), 7·96 ppm (broad, H-1 and H-8); δ-ethylenic form, 3·40 (d, J = 7·2 Hz, 2H, H-15), 5·85 (t, J = 7·2 Hz, 1H, H-14), 6·84 (d, J = 8·6 Hz, 1H, H-2), 6·87 (d, J = 8·5 Hz, 1H, H-7), 7·42 (d, J = 8·6Hz, 1H, H-1), 7·44 ppm (d, J = 8·5 Hz, 1H, H-8). ^{13}C-NMR (DMSO-d_6): δ-ethylenic form, 34·6 (C-15), 74·4 (C-4), 75·0 (C-5), 110·3 (C-2), 111·5 (C-7), 113·8 (C-10), 114·6 (C-14), 171·1 (C-13), 124·4 (C-1), 126·2 (C-9), 128·0 (C-8), 151·5 (C-12), 153·2 (C-11), 157·9 (C-3), 158·3 (C-6), 172·9 ppm (C-16). MS *m/z* (%): 536 (M^+, 0·1), 490 (0·2), 480 (0·2), 364 (0·1), 254 (I_2, 100), 127 (I, 34). HPLC: K' 5·86 (eluant A), 1·86 (eluant B).

2,4,5-Tri-iodosuccinylfluorescein (3ISF)

Reddish-brown microcrystals of undefined mp (decomposition between 200 and 250°C). (Analysis. Calculated for $C_{16}H_9I_3O_5$: C, 29·0; H, 1·4. Found: C, 29·4; H, 1·6%.) Visible spectra, λ_{max} (log ε): in DMF, 504sh (4·41), 539 nm (4·94); in MeOH-pH 1 buffer, 1:1 v/v, 450sh (4·09), 478 (4·19), 510sh nm (4·09); in MeOH-pH 9 buffer, 1:1 v/v, 488sh (4·34), 522 nm (4·78). IR (KBr), ν_{max}: 3000b, 1720m, 1625m, 1579m, 1515vs, 1490vs, 1460vs, 1315vs, 1260s, 1150s, 820m cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6); δ quinonoid form (practically the only form in recent solutions), 2·58 (t, J = 7·5 Hz, 2H, H-15), 3·48 (m, 2H, H-14), 7·09 (d, J = 9·0 Hz, 1H, H-7), 8·00 (d, J = 9·0 Hz, 1H, H-8), 8·71 ppm (s, 1H, H-1); δ-ethylenic forms (*cis* and *trans*, 2:5), 3·43 (two overlapped d, J = 7·1 Hz, H-15 *cis* + H-15 *trans*), 5·89 (t, J = 7·1 Hz, H-14 *cis*), 5·95 (t, J = 7·1 Hz, H-14 *trans*), 6·81 (m, H-7 *cis* + H-7 *trans*), 7·46 (d, J = 8·3 Hz, H-8 *trans*), 7·48 (d, J = 8·7 Hz, H-8 *cis*), 7·95 (s, H-1 *cis*), 7·96 ppm (s, H-1 *trans*), assignments deduced from NOE experiments irradiating each H-14. ^{13}C-NMR (DMSO-d_6): δ-quinonoid form, 22·6 (C-14), 34·4 (C-15), 74·5 (C-5), 76·3 (C-4), 103·3 (C-2), 113·2 (C-7), 113·8 (C-13), 117·9 (C-10), 128·5 (C-8), 137·4 (C-1), 151·7 (C-9), 154·4 (C-11), 158·6 (C-12), 164·1 (C-3), 172·8 (C-6), 173·8 ppm (C-16). MS *m/z* (%): 662 (M^+, 0·1), 616 (0·1), 591 (0·2), 535 (0·1), 490

(0·1), 464 (0·2), 337 (0·2), 254 (I_2, 100), 128 (HI, 29), 127 (I, 35). HPLC: K' 27·6 (eluant A), 6·0 (eluant B).

2,4,5,7-Tetraiodosuccinylfluorescein (4ISF)
Red microcrystals of mp higher than 300°C. (Analysis. Calculated for $C_{16}H_8I_4O_5$: C, 24·4; H, 1·0; I, 64·45. Found: C, 24·9; H, 1·4; I, 64·1%.) Visible spectra, λ_{max} (log ε): in DMF, 510sh (4·44), 546 nm (5·04); in MeOH-pH 1 buffer, 1:1 v/v, 425sh (3·96), 4·91 (4·13), 516sh nm (4·03); in MeOH-pH 9 buffer, 1:1 v/v, 492sh (4·46), 531 nm (4·97). IR (KBr) ν_{max}: 3000b, 1725s, 1615m, 1585m, 1560vs, 1505vs, 1450s, 1385m, 1340s, 1300s, 1185s, 765w cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ-quinonoid form (the only form in recent solutions), 2·58 (t, J = 7·6 Hz, 2H, H-15), 3·44 (t, J = 7·6 Hz, 2H, H-14), 8·60 ppm (s, 2H, H-1 and H-8); δ-ethylenic form, 3·60 (d, J = 7·1 Hz, 2H, H-15), 6·02 (t, J = 7·1 Hz, 1H, H-14), 7·95 (s, 1H, H-1), 7·96 ppm (s, 1H, H-8). ^{13}C-NMR (DMSO-d_6): δ-quinonoid form, 22·3 (C-14), 34·2 (C-15), 76·2 (C-4 and C-5), 94·0 (C-2 and C-7), 116·6 (C-10 and C-13), 136·8 (C-1 and C-8), 150·0 (C-9), 156·3 (C-11 and C-12), 168·0 (C-3 and C-6), 172·7 ppm (C-16). MS m/z (%): 788 (M^+, <0·1), 730 (0·2), 716 (0·2), 661 (0·1), 616 (0·1), 254 (I_2, 95), 128 (HI, 100). HPLC: K' 12·8 (eluant A), 2·8 (eluant B).

Synthesis of 2,4,5,7-tetrabromosuccinylfluorescein (4BrSF)

A described procedure was followed,[3] with some modifications. A solution of 1 g (3·5 mmol) of succinylfluorescein in 30 ml of pH 9 aqueous 0·2 M phosphate buffer was mixed with a solution of 2·3 g (14·5 mmol) of bromine in 5 ml of the same buffer, at room temperature and with stirring. After 5 min, excess of 0·1 M-sodium bisulfite was added, followed by enough 4% aqueous HCl to precipitate all the reaction product. The solid was filtered, washed with water and purified by column chromatography. Yield of purified compound: 90%. Similar brominations carried out in 100 ml 4% aqueous HCl gave rise in 5 min to quantitative conversion (HPLC analysis) to 4BrSF.

The purified product was obtained as red microcrystals, mp 240–245°C (decomp.) [lit.[3] 250°C (decomp.)]. (Analysis. Calculated for $C_{16}H_8Br_4O_5$: C, 32·0; H, 1·3; Br, 53·3. Found: C, 31·9; H, 1·7; Br, 53·6%). Visible spectra, λ_{max} (log ε): in DMF, 504sh (4·39), 538 nm (4·98); in MeOH-pH 1 buffer, 1:1 v/v, 446sh (4·02), 478 (4·12), 511sh nm (4·04); in MeOH-pH 9 buffer, 1:1 v/v, 490sh (4·47), 522 nm (4·90). IR (KBr) ν_{max}: 3000b, 1750vs, 1715s, 1625m, 1570vs, 1510vs, 1460vs, 1350s, 1300s, 1170s, 1040s, 765w cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ-quinonoid form (the only form in fresh solutions), 2·58 (t, J = 7·5 Hz, 2H, H-15), 3·48 (t, J = 7·5 Hz, 2H, H-14), 8·41 ppm (s, 2H, H-1 and H-8); δ-ethylenic form, 3·47 (d, J = 7·1 Hz, 2H,

H-15), 6·12 (t, $J = 7·1$ Hz, 1H, H-14), 7·81 (s, 1H, H-1), 7·85 ppm (s, 1H, H-8). ^{13}C-NMR (DMSO-d_6): δ-quinonoid form, 22·4 (C-14), 34·3 (C-15), 99·5 (C-4 and C-5), 114·3 (C-2 and C-7), 117·6 (C-10 and C-13), 129·4 (C-1 and C-8), 151·9 (C-11 and C-12), 152·0 (C-9), 164·7 (C-3 and C-6), 172·7 (C-16) ppm; δ-ethylenic form, 34·5 (C-15), 100·8 (C-4), 101·1 (C-5), 106·2 (C-2), 107·6 (C-7), 115·5 (C-13), 118·7 (C-14), 118·9 (C-10), 124·0 (C-9), 125·9 (C-1), 129·4 (C-8), 147·5 (C-11), 149·1 (C-12), 151·6 (C-3), 152·0 (C-6), 172·5 (C-16) ppm. MS *m/z* (%) (M^+ not observed): 555 (0·1), 529 (5), 450 (3), 371 (2), 80 (HBr, 100). HPLC: K' 3·7 (eluant A), 1·3 (eluant B).

Synthesis of polymeric dyes

A solution of 320 mg of a linear copolymer of styrene and 5% *p*-vinylbenzyl chloride,[1] 70 mg (0·09 mmol) of 4ISF (60% molar on CH_2Cl groups in the polymer), and 50 mg of K_2CO_3 in 10 ml of DMF, was heated at 60°C in the dark for 24 h while stirring. After this time, the mixture was cooled and the excess of K_2CO_3 separated by filtration. Traces of unreacted 4ISF and other low-molecular-weight compounds were also separated from the modified polymer by ultrafiltration. The concentrated solution, about 5 ml, was continuously washed and ultrafiltered with dimethylacetamide, until the washings were colourless. The polymeric dye PS-4ISF was dried (10^{-4} torr, 30°C), dissolved in 5 ml of chloroform and precipitated by adding this solution to 70 ml of ethanol–water, 1:1 v/v. After filtration, the separated solid was dried in vacuum to constant weight.

The polymeric dye resulting from the reaction between the same copolymer and 3ISF, PS-3ISF, was obtained in a similar way.

Determination of quantum yields of singlet oxygen production

Solutions in DMF of each dye (10^{-5} M, or, in the case of the two polymer-bound dyes, with a concentration producing the same absorbance at the visible maximum as the corresponding free dye) and diphenylisobenzofuran, DPIBF (10^{-5} M) were placed in 1-cm path-length cuvettes and irradiated in a 'merry-go-round' device that allows the simultaneous irradiation of eight solutions. Two of them were always the actinometric solution, i.e., a methanolic solution of commercial Rose Bengal and DPIBF, both 10^{-5} M. The samples were irradiated with a high-pressure xenon lamp XBO of 500 W (power supply Oriel 6242), situated 13 cm from the samples and in a tubular, horizontal, water refrigerated housing, APR-ALH 220, provided with an elliptical rubidium-coated mirror, an IR filter APR-ALH 1 cooled with running tap-water, and a SFK 11 Schott filter with maximum transmission at 545 nm (window between 516 and 564 nm). With this system, the incident

light intensity was in the order of 10^{-8} einstein s^{-1}, which is about five times higher than the intensity obtained with the same lamp in a conventional system and under similar conditions. Absorbances A_i of the solutions were measured using a Perkin–Elmer 554 spectrophotometer at seven wavelengths equally spaced along the window of the filter. The transmission coefficients of the filter at these wavelengths, θ_i, were previously determined. The variation of the DPIBF concentration with the irradiation time was deduced from the absorbance change at 414 nm (its visible maximum in DMF), taking $\varepsilon = 25\,550$ litre mol^{-1} cm^{-1} at this wavelength.

Calculations were based on the general expression (1), relating the change in the concentration C of the singlet oxygen acceptor with the irradiation time t, in the presence of a singlet oxygen producer,[12] assuming unit efficiency for the energy transfer process triplet dye–triplet oxygen:

$$-\frac{dC}{dt} = I_a \cdot \Phi^1O_2 \cdot \frac{C}{C+\beta} \tag{1}$$

where I_a is the absorbed light intensity (einstein s^{-1} $litre^{-1}$), Φ^1O_2 is the quantum yield of singlet oxygen generation, and β is the ratio between the rate constants of the non-radiative unimolecular decay process and the acceptor oxidation. Under our experimental conditions, I_a can be expressed in terms of the ratio between the incident light intensity I_0 and the volume of the solution, v:

$$I_a = \frac{I_0 \cdot F}{v}; \qquad F = \sum_{i=1}^{n} \frac{\theta_i}{n}(1 - 10^{-A_i}) \tag{2}$$

Parameter F takes into account the absorbances A_i of the irradiated samples (assuming that the dyes are the only absorbents) at all the wavelengths of the incident light, as well as the corresponding transmission coefficients θ_i of the filter employed. In practice, it is enough to measure A_i and θ_i in a limited number of positions, seven in our case. Substituting expressions (2) in (1), integrating and making $y = C/C_0$ (C_0 is the initial acceptor concentration) gives:

$$C_0(1-y) - \beta \ln y = \frac{I_0 \cdot F}{v} \cdot \Phi^1O_2 \cdot t \tag{3}$$

For a given acceptor–solvent pair, i.e. if β is known, Φ^1O_2 can be deduced from the slope m of the plot of the first term of eqn (3) versus the irradiation time. When a dye of known Φ^1O_2 is used as reference, keeping the operating

conditions constant, the unknown Φ^1O_2 value of the other sensitizer can be obtained from the expression:

$$\Phi^1O_2(\text{sens}) = \Phi^1O_2(\text{ref})\frac{m(\text{sens}).F(\text{ref})}{m(\text{ref}).F(\text{sens})} \quad (4)$$

Equation (3) can be employed likewise to determine I_0 if a solution of a sensitizer with known Φ^1O_2 and an acceptor with known β is used as actinometer.

In our case we have used as β value for DPIBF in DMF the one obtained in MeOH, $7{\cdot}3 \times 10^{-5}$.[13] Similarly, the Φ^1O_2 value employed for commercial Rose Bengal in DMF was the one described in MeOH, 0·76.[14] Previous parallel experiments proved that both values are not significantly influenced by this change of solvent.

Photobleaching

Solutions in DMF of Rose Bengal, 3ISF, 4ISF, all 10^{-5} M, and of PS-3ISF and PS-4ISF, both with the same maximum absorbance as the corresponding free dye, were irradiated in the experimental device described above, but without the 545 nm filter. For each dye, the bleaching process was followed by measuring the absorbance decay at its wavelength of maximum absorption, the position of which underwent a small red shift (as much as 1–2 nm) during the experiments.

RESULTS AND DISCUSSION

Structures of the free dyes in solution

The neutral forms of xanthene dyes adopt in solution different structures depending on the medium. In polar solvents, the highly colored, quinonoid structures with their phenolic group in ionized form are present.[15,16] In acidified solvents this ionization is precluded and the possible tautomeric forms can then be studied. The equilibrium constants between lactonic (colorless) and neutral quinonoid (colored) structures in solutions of Fluorescein and Eosine Y in several solvents have been so deduced from visible spectral data.[17] In alcoholic solvents, the proportion of lactonic form in Fluorescein decreases with the increase in the capacity of the solvent to form H-bonds, while the opposite occurs in the case of Eosine Y.[17] When aqueous alkaline solutions of Fluorescein are acidified with HCl or acetic acid, the quinonoid or the zwitterionic form precipitates, respectively.[18] In aprotic solvents (dioxane, DMSO, chloroform, etc.) the corresponding

lactonic forms of Fluorescein,[18,19] Eosine Y[19] and Rose Bengal[20,21] are the only detected structures.

In the case of the SF molecule, our previous work has shown that in 0·1 M-DMSO solutions both the lactonic and the quinonoid structures are initially present, in *ca.* 5:1 mixture. With time, both forms disappear, producing the ethylenic form.[9] In similar solutions of the halogenated SF derivatives, the lactonic forms have not been detected in their ^{1}H-NMR spectra. Instead, the corresponding quinonoid and ethylenic forms are present, the proportions of the former decreasing with time from the preparation of each solution. As expected, two *cis–trans* isomeric ethylene forms were observed for the asymmetric molecule 3ISF.

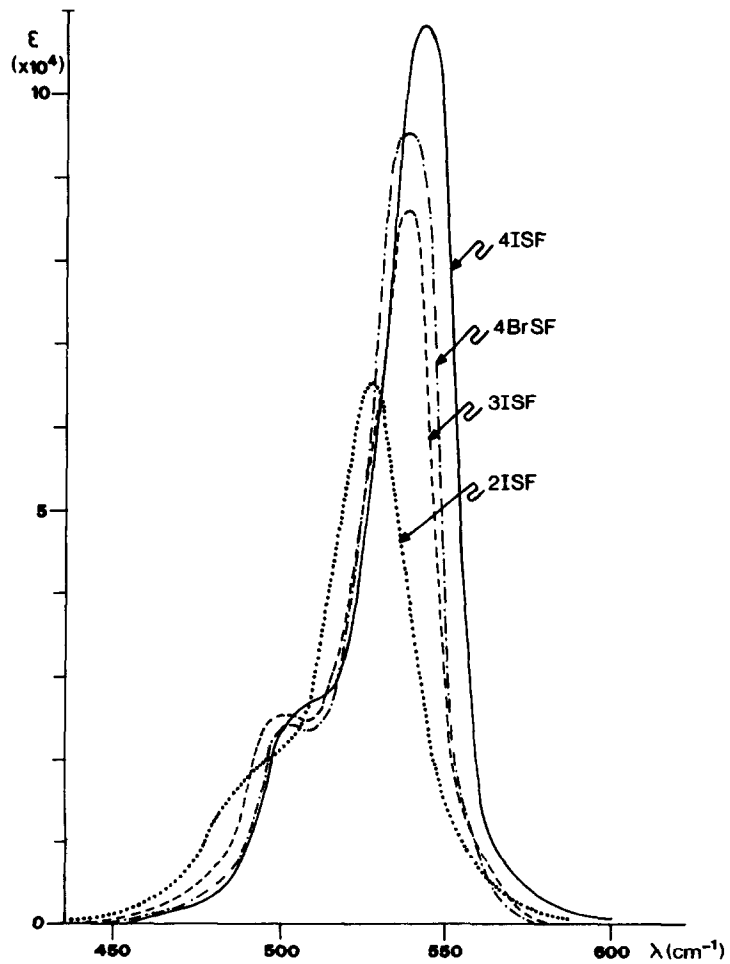


Fig. 1. Visible spectra of halogenated derivatives of succinylfluorescein in *ca.* 10^{-5} M solutions in DMF.

In methanol–aqueous buffers, 1:1 v/v, the SF molecule adopts structures which depend on the buffer pH.[10] Solutions of the halogenated derivatives (except 2ISF) in the methanol-pH 1 buffer show visible spectra with the typical shape of quinonoid neutral structures, i.e. a maximum flanked by two shoulders. In the same medium, 2ISF shows mainly the xanthylium salt form, with a maximum at 455 nm, a pH 2·5 buffer being necessary to obtain a quinonoid type spectrum. For all the halogenated derivatives, the presence of colorless structures, together with quinonoid ones in these acid media cannot be ruled out, since the molar extinction coefficients ε found for the maximum in their visible spectra are somewhat lower than expected. For example, 4BrSF in MeOH-pH 1 buffer, 1:1 v/v, shows $\varepsilon = 13\,200$ at 478 nm (visible maximum), while the methyl ester of Eosine Y, a fixed quinonoid form, in dioxane-pH 0·5 buffer, 1:1 v/v (it suffers fast hydrolysis and bleaching in MeOH-pH 1 buffer, 1:1 v/v) has $\varepsilon = 22\,400$ at 481 nm (maximum).[22] It seems that the 2′-benzoic acid ring in the 9-position in the latter dye cannot be the cause of all this difference.

In the MeOH-pH 9 buffer, 1:1 v/v, all the halogenated SF derivatives show strong absorptions in their visible spectra as a consequence of the ionization of the phenol group in their corresponding quinonoid forms.

In 10^{-5} M solutions in DMF, the halogenated compounds also have the same ionized forms. Their visible spectra in this solvent (Fig. 1) do not change with time (no formation of colourless ethylenic forms) and have similar shapes to the corresponding ones obtained in MeOH-pH 9 buffer, but with the maxima shifted to longer wavelengths and with higher absorptions, as often observed in the visible spectra of dyes when a polar solvent is replaced by a less polar one and with less capacity to form H-bonds. Neutral Eosine Y and Rose Bengal are also completely ionized in DMF solutions of the same concentration. In all cases, the presence of traces of aqueous alkali did not produce absorbance increments. An important consequence of this ionization capacity is that the ionized forms of linear polymer-bound halogenated SF dyes can also be studied in DMF solutions, without the necessity of adding bases.

Structures of soluble polymeric dyes

The two dyes with higher quantum yields of singlet oxygen production (see below), i.e. 3ISF and 4ISF, were covalently bound to a linear polymeric matrix through selective reaction between the carboxylic group of each dye and the chloromethyl groups of the polymer. Such ester unions do not change the xanthene chromophores, so that the corresponding highly colored ionized quinonoid species can be formed. Using a molar ratio dye/initial CH_2Cl groups in the polymer equal to 0·60, only 22% of the

CH_2Cl groups reacted or, in other words, about 1·1% of the aromatic rings in the polymer chains have a dye molecule. This value was determined spectroscopically in DMF solution, using as references the spectra of the free dyes in the same solvent. Polymers with slightly higher dye contents, but insoluble in DMF, were obtained when the initial free dye concentration in the reaction mixture was increased from 0·01 M to 0·1 M (almost saturation).

It is worthwhile noting that, as expected, the amount of each halogenated dye bound to this linear polymer is about twice the amount of Rose Bengal which could be bound to a similar polymer, using the same experimental conditions and without making it insoluble.[1]

Singlet oxygen generation

The four free halogenated dyes and the two polymer-bound dyes have been evaluated, under comparative conditions (see Experimental) as singlet oxygen generators in DMF, using DPIBF as acceptor and commercial Rose Bengal as reference. The results obtained when eqn (3) is applied to the experimental data are shown in Fig. 2. The corresponding quantum yields Φ^1O_2 deduced are given in Table 1. For free iodinated dyes, Φ^1O_2 increases as the number of iodines in the SF molecule increases, as a consequence of

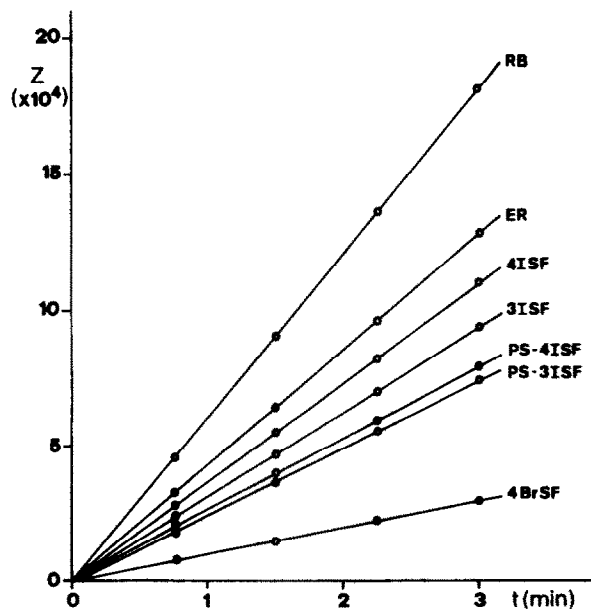


Fig. 2. Plot of the first term of eqn (3) (Z) vs. irradiation time for free and polymer-bound halogenated succinylfluorescein dyes. Results with Rose Bengal (RB) and Erythrosine Y (ER) are included as references.

the known 'heavy atom' effect on the rate of intersystem crossing. The relatively high Φ^1O_2 value obtained for 4ISF, 0·44, is however lower than the one simultaneously determined for Erythrosine Y, 0·54. This difference shows the contribution of the aromatic ring in the 9-position in the latter dye to the production of $^1O_2$. 4BrSF has a lower Φ^1O_2 value than 4ISF. The same result occurs when the corresponding halogenated Fluorescein derivatives Eosine Y (0·32 in methanol[22] or ethanol[23] and Erythrosine Y are compared.

The polymeric dyes PS-3ISF and PS-4ISF have Φ^1O_2 values somewhat lower than those of the respective free dyes. This is the expected result if we take into account the effect of the high local chromophore concentration within the volume encompassed by the macromolecule.[24] Work is in progress to provide a better insight into this behavior. These polymeric dyes can however be qualified as 'good' sensitizers because their Φ^1O_2 values are close to those of the well-established Eosine Y.

TABLE 1
Experimental Quantum Yields of Singlet Oxygen Production of Free and Polymer-Bound Halogenated Derivatives of Succinylfluorescein, in DMF Solution[a]

Sensitizer	Φ^1O_2[b]
2ISF	0·30
3ISF	0·41
4ISF	0·44
4BrSF	0·13
PS-3ISF	0·33
PS-4ISF	0·34

[a] Reference: Φ^1O_2 of Rose Bengal, 0·76 (Ref. 14); Φ^1O_2 found for Erythrosine Y, 0.54.
[b] Average of at least two experiments; errors less than 0·01.

Photostability

No bleaching was observed in any of the dyes after determining their Φ^1O_2 values (6 min irradiation), or on irradiating them for 8 h under the same conditions. Moreover, at the end of the experiments the polymeric dyes were isolated and re-used four times without detecting any variation in their dye contents, molecular weights, visible spectra or Φ^1O_2 values.

Thus in order to check their photostability, DMF solutions of both the

free and the polymeric dyes were studied under more drastic irradiation conditions, i.e. using all the visible and UV output of the lamp to determine Φ^1O_2 values. The results are shown in Fig. 3, where the relative absorption decay of each dye is plotted versus time.

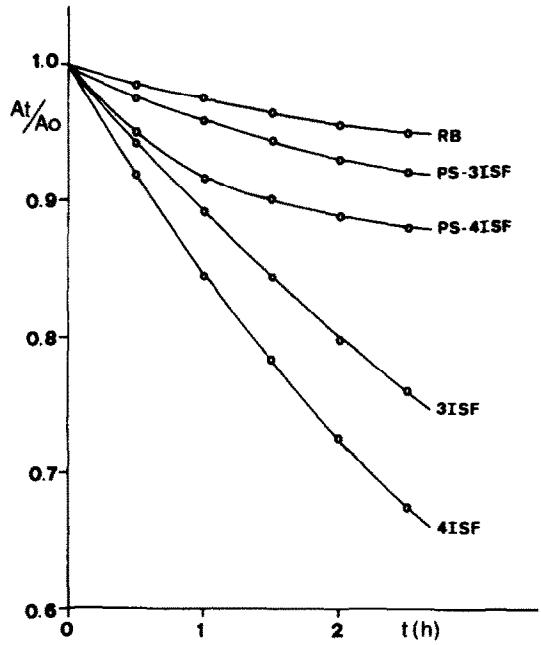


Fig. 3. Comparative photobleaching of the free and polymer-bound halogenated succinylfluorescein dyes. Reference: Rose Bengal (RB).

It is evident that the polymer-bound dyes have higher photostability with respect to the free dyes with the same number of iodine atoms, and the higher the number of iodine substituents, the lower is the photostability.

Apparently, the photobleaching is not related to the loss of iodine atoms. TLC and HPLC analysis of the irradiated 4ISF solution showed absence of the deiodinated derivatives 3ISF and 2ISF in the complex mixture of unidentified compounds produced.

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