THERMOCHROMISM AND PHOTOCHROMISM OF ARYL-SUBSTITUTED ACYCLIC AZINES V: QUENCHING OF THE PHOTOCHEMICAL *E–Z* ISOMERIZATION VIA FÖRSTER ENERGY TRANSFER

K. APPENROTH†, M. REICHENBÄCHER and R. PAETZOLD

Sektion Chemie der Friedrich-Schiller-Universität Jena, DDR-6900 Jena (G.D.R.)

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Summary

The *E–Z* photoisomerization of benzophenone-9-anthraldehyde azine is quenched by dyes of the appropriate singlet energy. The quenching occurs predominantly via Förster energy transfer. The development of a procedure that allows a distinction to be made between direct and sensitized fluorescence for the case of a non-fluorescing donor (azine) and overlapping donor and acceptor spectra is reported.

1. Introduction

Our investigations of 2,3-diazabutadienes (azines) with various substitutions have shown that photochromism is a general property of this class of compound [1, 2]. On irradiation into the longest wavelength absorption band reversible *E–Z* isomerization about the C=N bond takes place, and for benzophenone-9-anthraldehyde azine (BPhAA) only two isomers are possible (Fig. 1). In both cases the S_1 state is photoreactive [3]. When appropriate dyes are used concentration-dependent quenching of the photoisomerization is observed in ethanolic solutions. A possible quenching mechanism is the Förster energy transfer between the azine (donor) and the dye (acceptor).

As azines do not show any fluorescence at room temperature [4] quenching of the azine photoisomerization by the dye would be an example of a singlet–singlet energy transfer between a non-fluorescing donor and an acceptor which obeys the Förster mechanism. At present only a few examples of this behaviour are known [5]. The reason for this paucity of data may be the difficulty in distinguishing between sensitized and unsensitized acceptor fluorescence when the donor and acceptor spectra

†Present address: Sektion Biologie der Friedrich-Schiller-Universität Jena, DDR-6900 Jena (G.D.R.)

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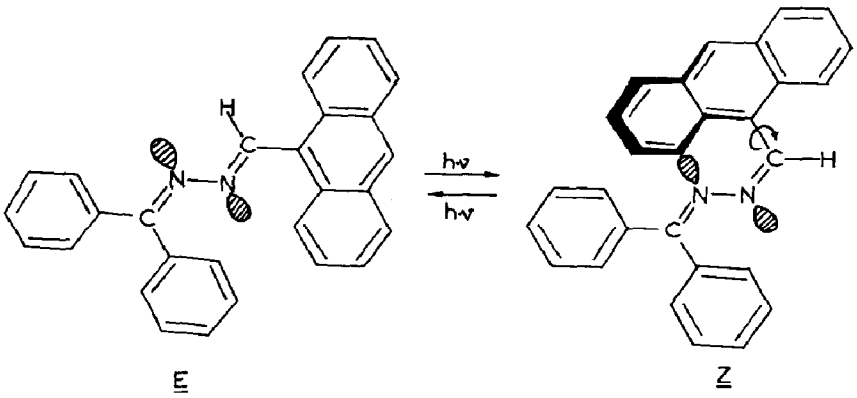

Fig. 1. The *E–Z* isomerization of BPhAA.

overlap. Therefore it is necessary to develop a procedure which allows sensitized fluorescence to be detected for non-fluorescing donors and overlapping donor and acceptor spectra.

2. Experimental details

2.1. Materials

The *E* isomer of BPhAA (*E*-BPhAA) was prepared in the absence of actinic light by standard procedures [6]. The dyes (quenchers) used were purchased from Merck, Schuchardt and Fluka; they were used without further purification. The solvents used were purified by standard methods [7].

2.2. Spectra

The spectra of BPhAA and the dyes were recorded in ethanol using a Cary model 17 spectrometer. The extinction coefficients ϵ' for *E*-BPhAA and *Z*-BPhAA at the irradiation wavelength are 9700 M^{-1} cm^{-1} and 50 M^{-1} cm^{-1} respectively. ϵ' was determined experimentally for erythrosine B (1850 M^{-1} cm^{-1}) and rhodamine B (1400 M^{-1} cm^{-1}). The fluorescence spectra were obtained using a FICA 55 spectrofluorometer (ARL, France). Neither BPhAA isomer showed any fluorescence [4].

2.3. Polarography investigations

The polarographic half-wave potentials of the dyes used were determined with a GWP 673 polarograph (G.D.R.) and a dropping mercury electrode. The dye concentration was 8×10^{-4} M in a 50% ethanolic solution of a 0.1 M phosphate buffer (pH 7.55).

2.4. Quenching of the E–Z photoisomerization

The change d[*E*] in the concentration of the *E* isomer as a result of the $E \rightarrow Z$ photoreaction induced by monochromatic irradiation is given by [8]

$$\frac{d[E]}{dt} = -\phi_{EZ} I_E + \phi_{ZE} I_Z$$

The intensities I_E and I_Z absorbed by isomers E and Z respectively are represented by

$$I_E = I_0 \frac{\epsilon_E'[E]}{A'} (1 - 10^{-A'})$$

$$I_Z = I_0 \frac{\epsilon_Z'[Z]}{A'} (1 - 10^{-A'})$$

where A' is the absorbance, ϵ' is the extinction coefficient and I_0 is the incident light intensity at the irradiation wavelength λ'. The terms $\epsilon_E'[E]/A'$ and $\epsilon_Z'[Z]/A'$ involve only the light absorbed by the E and Z isomers at λ'. Thus both the influence of the dye absorption at λ' and a possible trivial quenching process by the dye are eliminated.

The quantum yields ϕ_{EZ} and ϕ_{ZE} of the photoreactions $E \rightarrow Z$ and $Z \rightarrow E$ respectively are determined by integrating the differential equation given above and performing a graphical or numerical evaluation [8] using a computer program [9]. The quantum yield of the $E \rightarrow Z$ isomerization in ethanol in the absence of the quencher is $\phi_{EZ}^{\circ} = 0.0077$. Since linear extinction difference diagrams are obtained, the photoisomerization also proceeds as a unitary

$$E \underset{h\nu}{\overset{h\nu}{\rightleftharpoons}} Z$$

reaction (*einheitliche Reaktion* [8]) in the presence of the dyes.

The Stern–Volmer quenching constants k_{SV} obtained by plotting $\phi_{EZ}^{\circ}/\phi_{EZ}$ *versus* [Q] are summarized in Table 1. The concentration of BPhAA was 5×10^{-5} M in all experiments and the range of concentration of the quenchers used is given in Table 1. The "dyes" which did not exhibit a quenching effect (*E*-stilbene, anthracene, acridine, fluorenone, methylene blue, night blue and thionine) had concentrations in the range 1.0×10^{-4} - 25×10^{-4} M.

The irradiation wavelength was 436 nm. A high pressure mercury lamp (HBO 200; VEB Narva, Berlin) was used as the light source, and monochromatic radiation was selected using an HgMon 436 filter (VEB Carl Zeiss, Jena).

2.5. Sensitized fluorescence

The dyes erythrosine B and rhodamine B were used to determine the sensitized fluorescence. The fluorescence spectra were recorded using a linear measuring technique: the light from the HBO 200 lamp passed through a 436 nm metal interference filter (VEB Carl Zeiss, Jena), a high intensity monochromator (Bausch and Lomb), the sample and a GDM 1000 monochromator (VEB Carl Zeiss, Jena) and was finally detected using a

TABLE 1

Stern–Volmer constants for the quenching of the $E \rightarrow Z$ photoisomerization of *E*-BPhAA by various dyes in ethanol solutions

Dye	*Concentration* ($\times 10^{-4}$ M)	k_{SV} ($\times 10^{-3}$ M)
Acridine orange	0.2 - 0.6	9.33
Erythrosine B	0.4 - 1.5	4.96
Methyl red	0.6 - 1.1	2.07
Diamond fuchsine	1.0 - 2.8	1.55
Eosin Y	1.0 - 3.0	0.96
Rhodamine B	1.2 - 3.6	0.52
E-thioindigo[a]	1.0 - 2.5	1.03

[a]Non-ionic dye in benzene solvent.

photomultiplier. The irradiation wavelength λ' was 436 nm and the emission wavelength used in the evaluation according to eqns. (11) and (16) below was in the non-absorbing range of donor and acceptor for both dyes ($\lambda_{emission} > 600$ nm). In both cases five emission wavelengths were used in the evaluation.

The concentration of the acceptor (dye) was adjusted to a value such that the overall absorbance A' at the excitation wavelength ($A' = A_{BPhAA}' + A_{dye}'$) was greater than 2.0. The variation in the donor (*E*-BPhAA) concentration ($(1.0 - 4.8) \times 10^{-4}$ M) is limited by its low solubility in ethanol. The use of solvents with a better solubility for BPhAA (*e.g.* toluene) is not possible because the dyes are only slightly soluble in these solvents.

3. Results and discussion

3.1. Investigation of the quenching of the photoreaction

The $E \rightarrow Z$ photoisomerization of *E*-BPhAA can be quenched by adding the following dyes: fluorescein, acridine orange, methyl red, saffranine O, eosin Y, erythrosine B, *E*-thioindigo, phloxine B, rhodamine B, pyronine B and diamond fuchsine. The longest wavelength absorption band of these dyes lies in the range 490 - 555 nm which is close to that of *E*-BPhAA ($\lambda_{max} = 410$ nm).

Seven quenching dyes were selected for the determination of the Stern–Volmer quenching constants k_{SV}. The Stern–Volmer plots were all linear (Fig. 2); the quenching constants k_{SV} are summarized in Table 1. The viscosity of the solvent has only a weak influence (Table 2). Thus the quenching mechanism is not diffusion controlled. Quenching by the anions of ionic dyes [10] can be excluded because neither NaI nor KI in concentrations up to 10^{-3} M has any effect on the $E \rightarrow Z$ photoisomerization. However, the non-ionic dye *E*-thioindigo decreases the isomerization quantum yield markedly.

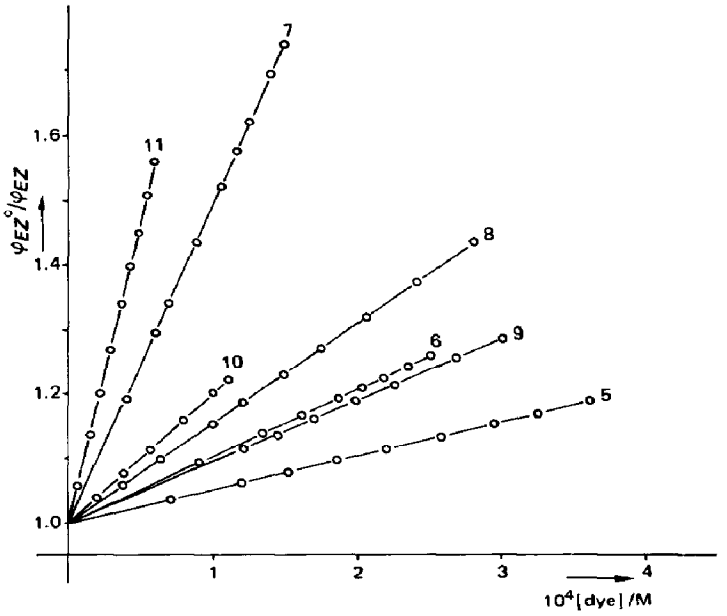

Fig. 2. Experimental plot of $\phi_{EZ}^{\circ}/\phi_{EZ}$ *vs.* the concentration [dye] of the dyes used to quench the $E \rightarrow Z$ photoisomerization: curve 5, rhodamine B; curve 6, *E*-thioindigo; curve 7, erythrosine B; curve 8, diamond fuchsine; curve 9, eosin Y; curve 10, methyl red; curve 11, acridine orange.

TABLE 2

Effect of the viscosity of the solvent on the Stern–Volmer quenching constants k_{SV}

Quencher	k_{SV} ($\times 10^3$ M^{-1}) *in the following solvents*		*Ratio*
	Ethanol	*Ethylene glycol*	
Acridine orange	9.33	7.04	1.33
Rhodamine B	0.52	0.42	1.24

In addition, neither quenching by electron transfer from a reducing agent [11] nor quenching by proton transfer in the excited state [12] is a competitive process because amines (aniline, diphenylamine and diethylamine in concentrations up to 10^{-2} M), phenols (phenol and *p*-nitrophenol in concentrations up to 10^{-2} M) and benzoic acid (in concentrations up to 10^{-3} M) do not influence the quantum yield of the $E \rightarrow Z$ photoisomerization. Since there is no relation between the polarographic half-wave potentials of the dyes and the Stern–Volmer quenching constants (Table 3), electron transfer from the dye to the azine (oxidizing quenching [11]) can also be excluded.

A number of dyes are capable of sensitizing the photochemical $Z \rightarrow E$ isomerization via population of the triplet state [3]. This process also reduces the $E \rightarrow Z$ quantum yield and thus might be misinterpreted as quenching. Such a process can be effective with eosin and erythrosine B

TABLE 3

Comparison of the polarographic half-wave potentials HWP of the dyes with the Stern–Volmer quenching constants k_{SV}

Dye	−HWP (V)	k_{SV} ($\times 10^3$ M^{-1})
Acridine orange	1.067	9.33
Diamond fuchsine	0.773	1.55
Erythrosine B	0.445	4.96
Nile blue	0.413	≈ 0
Eosin Y	0.405	0.96
Methyl red	0.366	2.07
Rhodamine B	0.268	0.52
Methylene blue	0.237	≈ 0

in an air-saturated solution [13]. In the present case, however, the effect is not important because the irradiation wavelength is not in the main absorption region of the dyes, and few of the dyes employed have a sufficiently high intersystem crossing rate.

Hence only the absorption properties of the dyes are important in the quenching process (Fig. 3). Dyes absorbing at significantly longer wavelengths ($\lambda > 600$ nm) than *E*-BPhAA (methylene blue, nile blue, night blue and thionine) and "dyes" absorbing at significantly shorter wavelengths ($\lambda < 400$ nm) (*E*-stilbene, anthracene, acridine and fluorenone) have no influence on the $E \rightarrow Z$ photoisomerization.

Since the main absorption band of all the active quenching substances is close to the longest wavelength absorption band of *E*-BPhAA, a Förster-

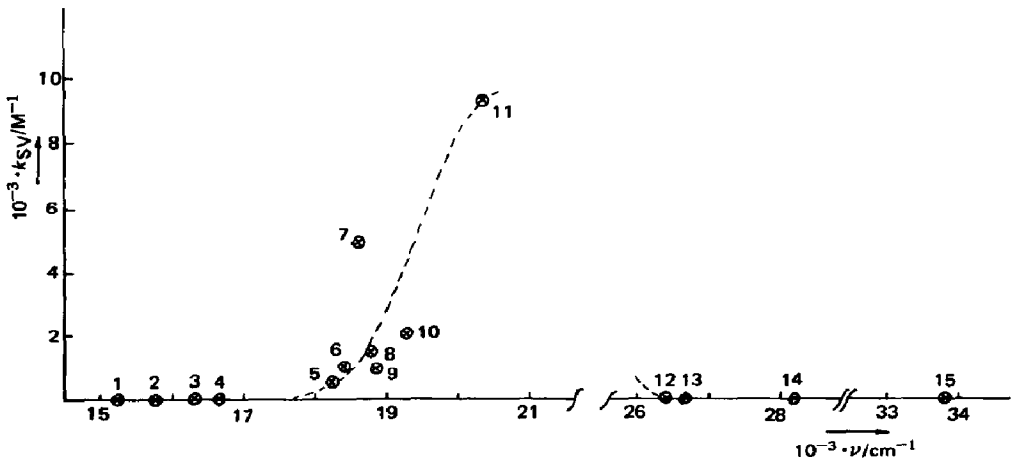

Fig. 3. Plot of the Stern–Volmer quenching constant k_{SV} *vs.* the wavenumber $\tilde{\nu}_{max}$ of the longest absorption band of the dyes used: 1, methylene blue; 2, nile blue; 3, night blue; 4, thionine; 5, rhodamine B; 6, *E*-thioindigo; 7, erythrosine B; 8, diamond fuchsine; 9, eosin Y; 10, methyl red; 11, acridine orange; 12, fluorenone; 13, anthracene; 14, acridine; 15, *E*-stilbene.

type singlet–singlet energy transfer is possible. The relation between the absorption maximum of the acceptor and the Stern–Volmer quenching constants, however, is not a sufficient argument, and therefore the evidence for a Förster energy transfer is not conclusive. Thus a definite decision can be taken only by detecting sensitized fluorescence of the acceptor.

3.2. Sensitized fluorescence

3.2.1. Development of the procedure for detecting sensitized fluorescence

If both the donor D and the acceptor A absorb at the irradiation wavelength λ', the following kinetic scheme applies in the presence of oxygen:

$$D + h\nu \xrightarrow{I_D} {}^1D \tag{1}$$

$$A + h\nu \xrightarrow{I_A} {}^1A \tag{2}$$

$${}^1A \longrightarrow A + h\nu^F \tag{3}$$

$${}^1A \longrightarrow {}^3A \tag{4}$$

$${}^1A \longrightarrow A \tag{5}$$

$${}^1A + A \longrightarrow 2A \tag{6}$$

$${}^1A + D \longrightarrow A + D \tag{7}$$

$${}^1A + O_2 \longrightarrow \ldots \tag{8}$$

$${}^3A + O_2 \longrightarrow \ldots \tag{9}$$

$${}^1D + A \longrightarrow {}^1A + D \tag{10}$$

Analogous equations can also be set up for the donor D.

When both self-quenching ($k_6 = 0$) and cross-quenching ($k_7 = 0$) are neglected, the following equation is obtained [14]:

$$\frac{F_A}{F_A^\circ - F_A} = k_{10}(\tau_D^S)_{O_2}[A] + \frac{\epsilon_A'[A]}{\epsilon_D'[D]}\{1 + k_{10}(\tau_D^S)_{O_2}[A]\} \tag{11}$$

where F_A and F_A° are the fluorescence intensities of the acceptor in the presence and absence respectively of the donor, $(\tau_D^S)_{O_2}$ is the lifetime of ^1D in an oxygen-containing solution, and ϵ_A' and ϵ_D' are the extinction coefficients of A and D respectively at the irradiation wavelength. If eqn. (11) is valid, a straight line with intercept $k_{10}(\tau_D^S)_{O_2}[A]$ should be obtained by plotting $F_A/(F_A^\circ - F_A)$ against $1/[D]$ at constant acceptor concentration. If k_{10} and the lifetime of ^1D are small, the intercept reaches values near zero at low acceptor concentrations. Therefore we have developed a procedure which allows a distinction to be made between direct and sensitized fluorescence.

If it is assumed that there is no cross-quenching ($k_7 = 0$) (see Section 3.2.2), the following equation can be derived from the kinetic scheme given above:

$$\frac{F_A}{F_A^\circ} = \frac{I_A}{I_A^\circ} + \frac{I_D k_{10}(\tau_D^S)_{O_2}[A]}{I_D^\circ\{1 + k_{10}(\tau_D^S)_{O_2}[A] + k_6(\tau_D^S)_{O_2}[D]\}} \quad (12)$$

The absorption intensities of the donor and the acceptor in the presence of the acceptor and the donor respectively are given by the following relations [8]:

$$I_D = \frac{\epsilon_D'[D]I_0}{\epsilon_A'[A] + \epsilon_D'[D]}\{1 - 10^{-(\epsilon_A'[A] + \epsilon_D'[D])d}\} \quad (13)$$

$$I_A = \frac{\epsilon_A'[A]I_0}{\epsilon_A'[A] + \epsilon_D'[D]}\{1 - 10^{-(\epsilon_A'[A] + \epsilon_D'[D])d}\} \quad (14)$$

where I_A° and I_D° are the absorption intensities in the absence of the donor and the acceptor respectively.

If it is assumed that there is no energy transfer ($k_{10} = 0$) and self-quenching is neglected ($k_6 = 0$) [14], we obtain the following equation from eqns. (12) - (14) and the experimental condition $A' > 2.0$ which implies $1 - 10^{-A'} \approx 1$:

$$\frac{F_A^*}{F_A^\circ} = \frac{I_A}{I_A^\circ} = \frac{\epsilon_A'[A]}{\epsilon_A'[A] + \epsilon_D'[D]} \quad (15)$$

where F_A^* is the fluorescence intensity of the acceptor in the absence of energy transfer ($k_{10} = 0$). The ratio F_A^*/F_A° can be calculated from the ratio of the acceptor absorbance to the donor absorbance at λ' (eqn. (15)). This calculated ratio can be related to the ratio F_A/F_A° of the experimental intensities (eqn. (12)) to give the following equation:

$$\frac{F_A}{F_A^*} = \frac{\epsilon_D'}{\epsilon_A'[A]} \frac{k_{10}(\tau_D^S)_{O_2}[A]}{1 + k_{10}(\tau_D^S)_{O_2}[A]}[D] \quad (16)$$

Provided that a singlet–singlet energy transfer is operating, the plot of F_A/F_A^* *versus* [D] is linear with a positive slope. This is direct evidence for enhanced fluorescence of the acceptor (sensitized fluorescence) even under very unfavourable absorption conditions for the donor and acceptor.

3.2.2. Results and discussion

Erythrosine B and rhodamine B were used as the acceptors in the detection of sensitized fluorescence by the donor *E*-BPhAA. The absorption spectra of *E*-BPhAA and (*E*–*Z*)-BPhAA are shown in Fig. 4 together with the absorption and fluorescence spectra of erythrosine B and rhodamine B. Figure 5 shows the results predicted by eqn. (11). As expected, the intercepts are near zero, and therefore the rate constant k_{10} for energy transfer cannot be estimated.

The plot of F_A/F_A^* *versus* [D] according to eqn. (16) yields a non-linear relation with a positive slope in the case of erythrosine B, and this is direct evidence for the sensitized fluorescence of erythrosine B on irradiation into the longest wavelength absorption band of *E*-BPhAA (Fig. 6). Thus, a Förster-type energy transfer mechanism is operating.

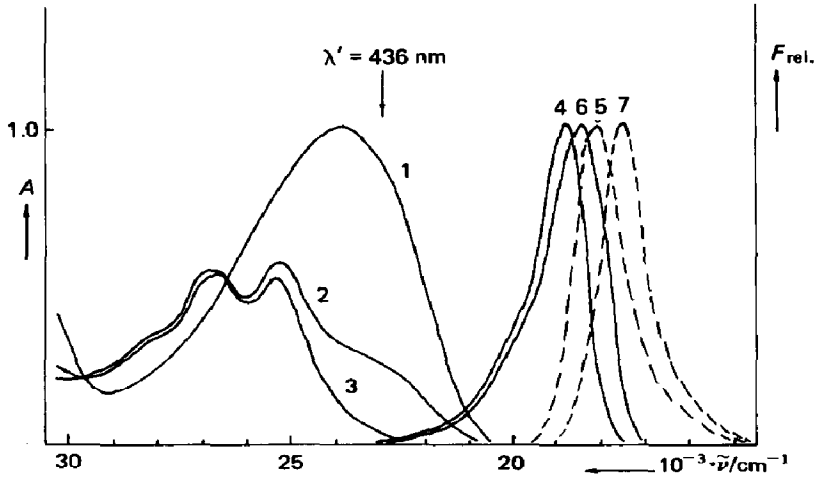

Fig. 4. Absorption spectra of *E*-BPhAA (7.6×10^{-5} M) (curve 1), (*E*–*Z*)-BPhAA (photostationary state) (curve 2), *Z*-BPhAA (7.6×10^{-5} M) (curve 3), erythrosine B (1.0×10^{-5} M) (curve 4) and rhodamine B (1.8×10^{-5} M) (curve 6), and fluorescence spectra of erythrosine B ($A' = 0.048$; $\lambda_{exc} = 534$ nm) (curve 5) and rhodamine B ($A' = 0.043$; $\lambda_{exc} = 544$ nm) (curve 7) in ethanol.

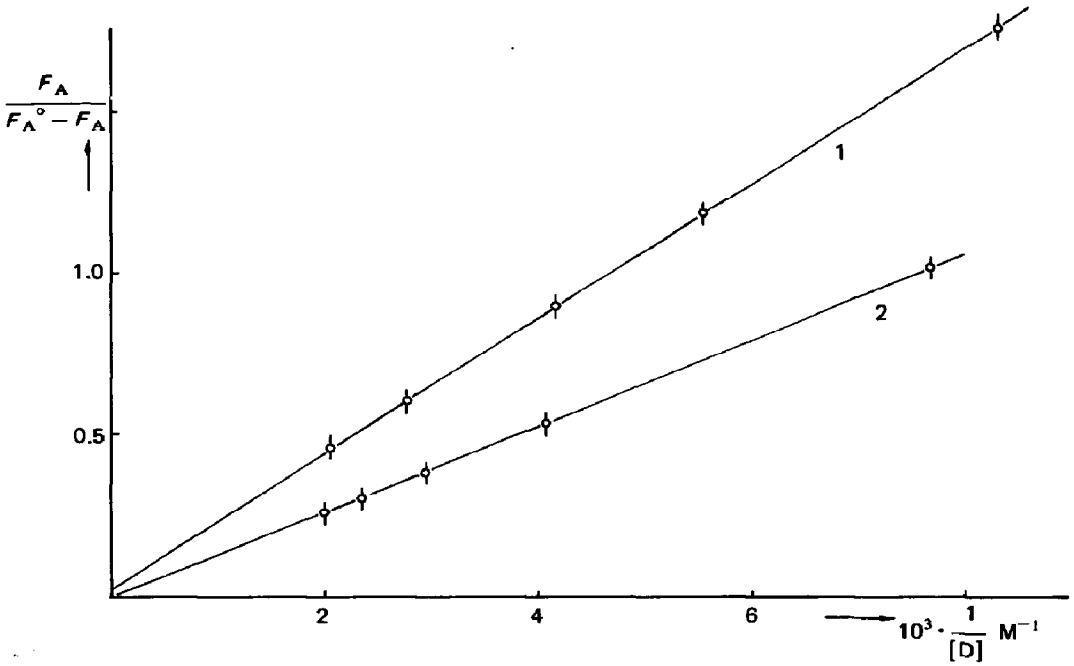

Fig. 5. Plot of $F_A/(F_A^\circ - F_A)$ *vs.* 1/[D] according to eqn. (11): D is *E*-BPhAA; A is erythrosine B (curve 1) or rhodamine B (curve 2).

Sensitized fluorescence cannot be detected when rhodamine B is the acceptor (the plot of F_A/F_A^* *versus* [D] in Fig. 6 does not have a positive slope). Obviously, the fact that the quenching constant is a factor of 10 less than that obtained using erythrosine B (see Table 1) explains why sensitized fluorescence cannot be detected under the experimental conditions.

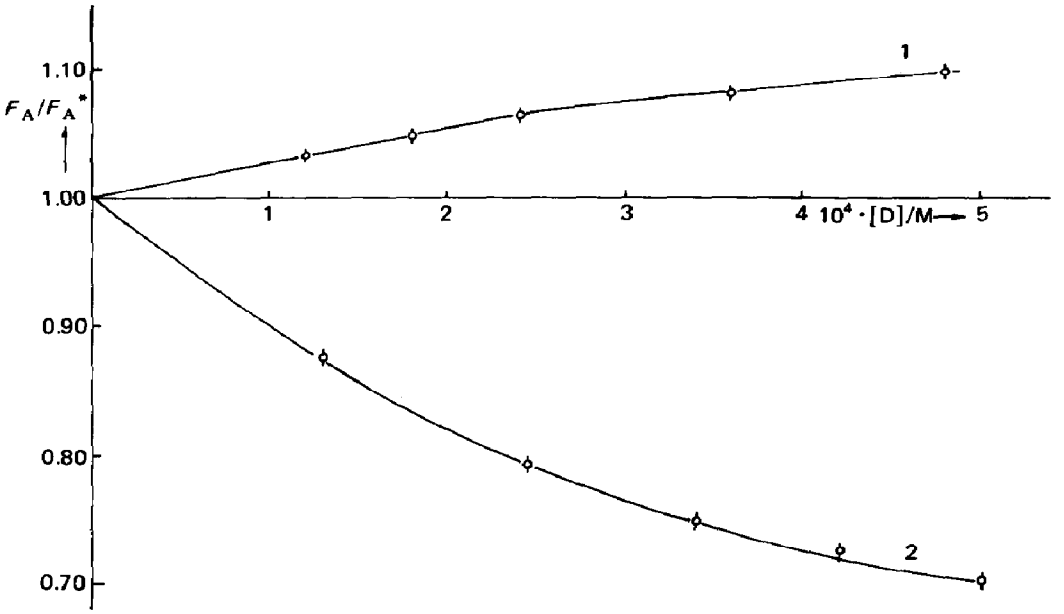

Fig. 6. Plot of F_A/F_A^* *vs.* [D] according to eqn. (16): D is *E*-BPhAA; A is erythrosine B (curve 1) or rhodamine B (curve 2).

The deviation of the ratio F_A/F_A^* from the predicted value of unity with increasing rhodamine B concentration and the non-linearity of the relation F_A/F_A^* *versus* [D] for erythrosine B show that the energy transfer may be complicated by further effects. Thus static quenching must be taken into account, since the spectra of *E*-BPhAA in the presence of the dyes erythrosine B and rhodamine B do not behave in a strictly additive way. However, the experimental results for both the quenching of the $E \rightarrow Z$ photoisomerization and the sensitized fluorescence cannot be explained solely on the basis of static quenching, because quenching of $E \rightarrow Z$ photoisomerization is also observed with diamond fuchsine and *E*-thioindigo which obey the Lambert–Beer law in the presence of the azine. Furthermore, eqn. (16) does not include the effects of cross-quenching (k_7). If this process operates, the ratio F_A/F_A^* will decrease with increasing donor concentration.

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