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The Influence of Reverse Energy Transport on Emission Anisotropy in Two-Component Viscous Solutions

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The effect of nonradiative reverse energy transport (NRET) in two donor–acceptor systems was studied experimentally. It was found that the NRET occurring in system I; rhodamine 6G (donor) and rhodamine B (acceptor), considerably lowers the emission anisotropy at medium and high concentrations. These results qualitatively confirm the predictions of the approximate theoretical approach of L. Kułak and C. Bojarski (see the preceding paper). In system II; rhodamine 6G (donor) and Nile Blue (acceptor), for which the NRET process does not occur, a good agreement with no-back-transport theory was obtained.

KEY WORDS: Reverse energy transport; emission anisotropy; absorption; fluorescence.

INTRODUCTION

Nonradiative excitation energy transport in disordered systems has been the subject of many papers [1–6]. These works dealt with the direct energy transport from donors to acceptors in one and many steps. Recently significant progress in the theory of energy transport has been made by taking into account not only the direct ($D^* + A \rightarrow D + A^*$) but also the reverse energy transport ($A^* + D \rightarrow A + D^*$) [7–11].

Reverse energy transport is very important, since it takes place in many natural and artificial systems. It can occur especially in the case of different chlorophyll forms [12] and ionic forms of the same dye [13,14]. It should also be taken into account for inhomogeneously broadened systems in which energy transport is present [15,16].

The precondition for energy transfer from A* to D is a partial overlap between acceptor fluorescence and donor absorption bands. The value of such an overlap integral is determined by the energy separation between singlet levels of the D and A molecules [17]. Experimental investigations of the nonradiative reverse energy transport (NRET) process are in the initial stage [18,24]. The only results reported so far concern quantum yield concentration changes in two-component donor–acceptor systems. This is understandable in view of the novelty of the problem and the difficulties in the preparation of appropriate experimental objects. In this paper we report an experimental investigation of the NRET effect on concentration changes in emission anisotropy in two-component viscous solutions.

THEORETICAL BASIS

The coherent theoretical description of the NRET influence on the emission anisotropy has not as yet been elaborated. However, an approximate result for the emission anisotropy can be obtained under a simplifying assumption that the excitation energy transferred from acceptors to monomers affects only the total fluorescence yield η_D and not the fluorescence quantum yield η_i of monomers D_i initially excited by light absorption [11]:

$$r/r_0 = \eta_i/\eta_D = [1 - \alpha' f(\gamma')] (1 - B') \qquad (1)$$

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where

$$B' = \frac{(1 - \alpha')f(\gamma')}{1 - \alpha' f(\gamma')} \frac{(1 - \bar{\alpha}')f(\bar{\gamma}')}{1 - \bar{\alpha}' f(\bar{\gamma}')} \quad (2)$$

$$\gamma' = \gamma_D/2^{1/2} + \gamma_A; \qquad \alpha' = \gamma_D/(2^{1/2}\gamma') \quad (3)$$

$$\gamma' = \gamma_A/2^{1/2} + \bar{\gamma}_D; \qquad \bar{\alpha}' = \bar{\gamma}_D/(2^{1/2}\bar{\gamma}') \quad (4)$$

$$\gamma_D = \frac{\pi^{1/2}}{2} \frac{C_D}{C_{ODD}}; \qquad \gamma_A = \frac{\pi^{1/2}}{2} \frac{C_A}{C_{ODA}} \quad (5)$$

$$\bar{\gamma}_D = \frac{\pi^{1/2}}{2} \frac{C_D}{C_{OAD}}; \qquad \bar{\gamma}_A = \frac{\pi^{1/2}}{2} \frac{C_A}{C_{OAA}} \quad (6)$$

$$f(x) = \pi^{1/2} \times \exp(x^2)[1 - \mathrm{erf}(x)] \quad (7)$$

where C_D and C_A denote the donor and acceptor concentrations, respectively, and C_{ODD},C_{ODA},C_{OAD}, and C_{OAA} are critical concentrations for nonradiative energy transfer from D* to D, D* to A, A* to D, and A* to A, respectively. For $B' = 0$, the well-known expression [19,20] describing energy transport from donors to acceptors can be obtained:

$$r/r_0 = 1 - \alpha' f(\gamma') \quad (8)$$

Numerical analysis of expression (1) as well as optimum choice of physicochemical conditions shows that the influence of NRET on emission anisotropy is expected to be the most important for donor concentrations:

$$10^{-3}\, M/ < C_D < 3{\cdot}10^{-3}\, M \quad (9)$$

and comparable acceptor concentrations. Such a choice enables avoidance of ground-state dimer formation as well as secondary effects which might complicate considerably the interpretation of experimental results.

EXPERIMENTAL

Two systems were investigated:

(1) rhodamine 6G (Rh6G; donor) and rhodamine B (RhB; acceptor) with NRET present; and
(2) Rh6G (donor) and Nile Blue chloride (NB; acceptor) with NRET absent.

Both objects were dissolved in anhydrous glycerol. Rh6G and RhB were additionally purified by multiple crystallization from ethyl alcohol and evaporation in vacuum. Several series of solutions with fixed donor concentrations and different acceptor concentrations were prepared. Some of the data for the systems investigated are listed in Table I.

The cuvette thickness d was small enough to meet the relation

$$2.3\, \epsilon_D^{max}\, C_D\, d < 0.1 \quad (10)$$

where ϵ_D^{max} is the maximum value of the donor extinction coefficient. Under this condition, the influence of secondary effects on emission anisotropy may be neglected [21].

Fluorescence spectra were measured upon frontal excitation and observation of the sample and corrected for the spectral sensitivity of the photomultiplier.

For absorption measurements, a Specord M-40 spectrophotometer was employed.

Emission anisotropy was measured with a single-photon counting apparatus designed and constructed by Dr. A. Kubicki [22,23] (Kubicki method) in our laboratory. A schematic diagram of the system is shown in Fig. 1. An XBO 250 (OSRAM) xenon lamp is the excitation light source. The Glan prism G_e polarizes the excitation light in the vertical plane. The light enters the measuring cells mounted on a rotary table, alternately irradiating the solvent and the sample. The Glan prism G_o (with a polarization plane at an angle of 45° to that of the G_e prism) on the movable table is inserted in the beam of light in order to determine the point $r = 0$, thus constituting a standard for the zero-emission anisotropy, relative to which the results of the measurement of the fluorescence light components are calculated. The Wollaston prism, W, splits the filtered fluorescence light into two components, with polarization planes parallel and perpendicular to the excitation light polarization plane (G_e). Fast photon counting Thorn EMI 9883 QB photomultipliers supplied with a voltage of $-2000\ V_{DC}$, and generating pulses with an amplitude of -0.5 V, were used as detectors. The amplitude-discriminator systems amplify and standardize (in TTL) pulses from the photomultipliers, lowering their frequency so that the counting of pulses can be accomplished with ordinary 10-MHz TTL counters. Such a lowering of the pulse frequencies from photomultipliers should result in an improvement in the linearity of the method by eliminating the recording of two-photon events. The simultaneous measurement of both components enables the effect of the light source intensity fluctuations upon the results to be eliminated. In order to obtain correct results (in particular, for low intensities of the observed light), dark photons and photons originating form scattering and imperfections of the optical elements used are taken into account. The correct determination of the zero point, $r = 0$, for the system is attained by always measuring the value of

Table I. Data Characterizing Both Systems in Glycerol at 293 K

System	Subsystem	Solvent	C_D (M)	r_0 (480 nm)	Viscosity (Pa·s)	n	λ_{ex} (nm)	λ_{obs} (nm)
I	Ia		10^{-3}					
	Ib		$2\cdot10^{-3}$				480	520
	Ic		$3\cdot10^{-3}$					
		Glycerol		0.3702	1.5	1.465		
II	IIa		10^{-3}					
	IIb		$2\cdot10^{-3}$				480	520
	IIc		$3\cdot10^{-3}$					

the ratio of the component intensities, $\rho_0 = I_{\perp 0}/I_{\parallel 0}$, for the emission anisotropy $r = 0$ (G_0 inserted in the beam of light). The correct values of $\rho = I_\perp/I_\parallel$ are obtained upon their calculation relative to ρ_0. The formal idea of the measurement can be given by the following equations:

$$r = \frac{I_\parallel - I_\perp}{I_\parallel - 2I_\perp} = \frac{1 - x}{1 + 2x} \tag{11}$$

$$I_\perp = \frac{N^f_{\perp r} - N^d_{\perp r}}{N^f_{\perp 0} - N^d_{\perp 0}}, \qquad I_\parallel = \frac{N^f_{\parallel r} - N^d_{\parallel r}}{N^f_{\parallel 0} - N^d_{\parallel 0}} \tag{12}$$

$$x = \frac{I_\perp}{I_\parallel} = \left[\frac{N^f_{\perp r} - N^d_{\perp r}}{N^f_{\perp 0} - N^d_{\perp 0}}\right] \Big/ \left[\frac{N^f_{\parallel r} - N^d_{\parallel r}}{N^f_{\parallel 0} - N^d_{\parallel 0}}\right] = \frac{\rho}{\rho_0} \tag{13}$$

where

$$\rho = \frac{N^f_{\perp r} - N^d_{\perp r}}{N^f_{\parallel r} - N^d_{\parallel r}}, \qquad \rho_0 = \frac{N^f_{\perp 0} - N^d_{\perp 0}}{N^f_{\parallel 0} - N^d_{\parallel 0}} \tag{14}$$

$$N^d_{\perp r} = n^d_{\perp r} t_r \qquad N^d_{\parallel r} = n^d_{\parallel r} t_r, \qquad N^d_{\perp 0} = n^d_{\perp 0} t_0, \qquad N^d_{\parallel 0} = n^d_{\parallel 0} t_0 \tag{15}$$

N = the average number of counts in the respective channels
n = the average number of counts per second in the respective channels
t = the time of measurement with the respective super- and subscripts
$\perp, \parallel$ = the components perpendicular and parallel to the excitation light polarization plane
f,d = the measurement for a given sample (fluorescence) and the solvent (dark counts)
o = the measurement for the standard $r = 0$ (G_0 in the light beam investigated)
r = the measurement of anisotropy, r (G_0 outside the light beam investigated)

All quantities (N^f_r, N^d_r, N^f_O, N^d_O, n^d_r, n^d_O) are always measured simultaneously and alternately, and the values taken for calculations are averaged over a great number of measurements (30–200). The employment of stepper motors, an IEC 625 interface system, and a microcomputer enabled the measurement to be fully automated. The measurements reported in this paper were carried out with an accuracy of 0.002.

RESULTS AND DISCUSSION

Figure 2 shows the absorption and fluorescence spectra of R6G, RB, and NB in anhydrous glycerol. In the case of system I, a significant overlap of acceptor fluorescence and donor absorption spectra is shown in Fig. 2a. This overlap enables the NRET process from A* to D. As opposed to system I, the respective spectra of system II are well separated, thus excluding the possibility of NRET (see Fig. 2b).

As discussed in Ref. 24, the presence of dimers for $C_D < 5\cdot10^{-3}$ M can be neglected.

Table II summarizes values of the parameters required for comparison of expressions (1) and (8) with the experimental data. They were obtained from independent measurements, results of which were reported in Refs. 24 and 25. The critical concentrations, as well as the critical distances, were calculated for $\langle\chi^2\rangle = 0.476$, assuming D and A to be statistically distributed fixed dipoles [26,27].

In Figs. 3A–C the experimental results obtained for the emission anisotropy for systems I and II with several donor concentrations are presented. Filled circles correspond to the case where the NRET process is present (system I), and open circles to the case where it does not take place (system II). It can be seen that for each

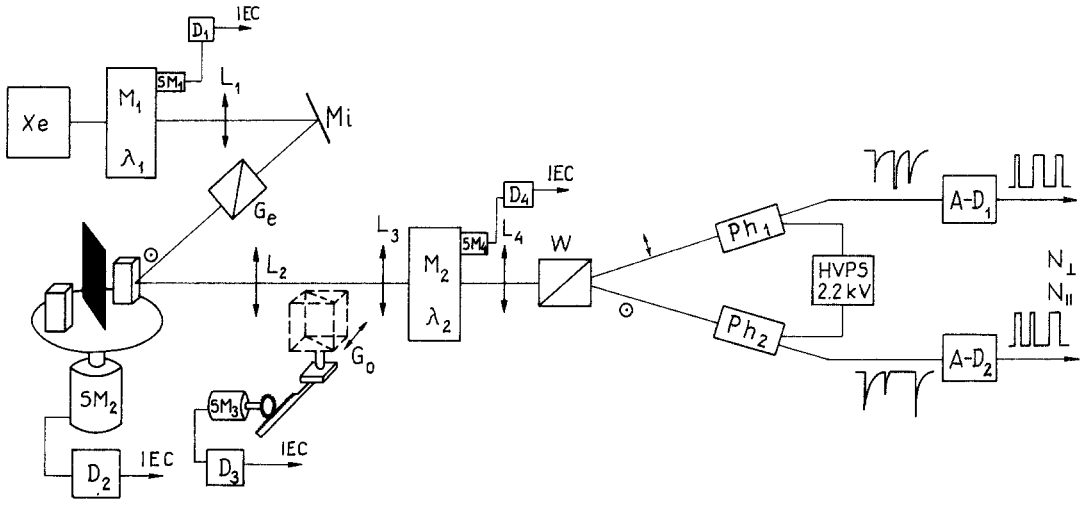

Fig. 1. Schematic diagram of the apparatus: Xe, xenon lamp; M, monochromators SPM-2 (Carl Ziess Jena); L, lenses; Mi, mirror; SM, stepper motors; D, drivers for motors; G, glan prisms; W, Wollaston prism; Ph, Thorn EMI 9883 QB photomultipliers; HVPS, high-voltage power supplier; A-D, amplifier-discriminator system.

subsystem the filled circles lie distinctly lower than the open circles. Also in Fig. 3 these results are compared with those obtained from expressions (1) and (8). In the case of system II, which plays the role of a reference, a good agreement between experiment and expression (8) can be observed over the whole acceptor concentration range. However, for system I, in which the NRET process takes place, the experimental results deviate distinctly from both theoretical curves and are placed between them.

The main reason that the filled circles corresponding to system I are not very well described by curves 1a–1c is the approximate character of expression (1). Within the framework of this approximation, all returns of excitation energy from the set of A* to the set of primarily excited donors D_i are neglected. This assumption causes the emission anisotropy values obtained from (1) to be underrated. It should therefore be emphasized that expression (1) may be treated as the lower limit for the emission anisotropy values in the presence of NRET. On the other hand, expression (8), which corresponds to the no-back-transfer theory, can be considered the upper limit for the NRET effect. The exact NRET theory should therefore lead to the expression for emission anisotropy, the plot of which ought to be placed between lower and upper limits.

When comparing these results to any NRET theory, one should also take into account that acceptors are excited both by nonradiative energy transfer from donors and by direct light absorption. The possibility of radiative transfer from acceptors to donors has been excluded by applying thin enough cuvettes. However, acceptors excited by direct light absorption can also nonradiatively transfer energy to the set of donors. Therefore donors can nonradiatively receive energy from acceptors excited both by nonradiative energy transfer from donors and by direct light absorption. As a result, the effect of NRET is stronger than that predicted by theory, which does not take into account that acceptors can absorb excitation light. The fraction f_r, of acceptors excited by the direct absorption of light can be estimated from the following formula:

$$f_r = X/[X + (1 - X)\eta_{TDA}] \tag{16}$$

where

$$X = \epsilon_A(\lambda_{ex})c_A/[\epsilon_A(\lambda_{ex})c_A + \epsilon_D(\lambda_{ex})c_D] \tag{17}$$

$$\eta_{TDA} = 1 - \eta_D/\eta_O \tag{18}$$

$$\eta_D/\eta_O = [1 - f(\gamma)]/[1 - \alpha f(\gamma)] \tag{19}$$

$\epsilon_A(\lambda_{ex})$ and $\epsilon_D(\lambda_{ex})$ denote decadic molar extinction coefficients for acceptor and donor, respectively, both measured at the excitation wavelength λ_{ex}, η_{TDA} is the nonradiative energy transfer efficiency from D* to A, and η_D/η_O denotes the relative quantum yield of the donor depending on the reduced concentration γ. Function $f(\gamma)$ is defined as in expression (7).

Figure 4 shows the contribution f_r of radiatively excited acceptors to the process of NRET as a function of acceptor concentration. In the same figure the fraction $f_n = 1 - f_r$ of nonradiatively excited acceptors is presented. It can be seen that for the highest acceptor con-

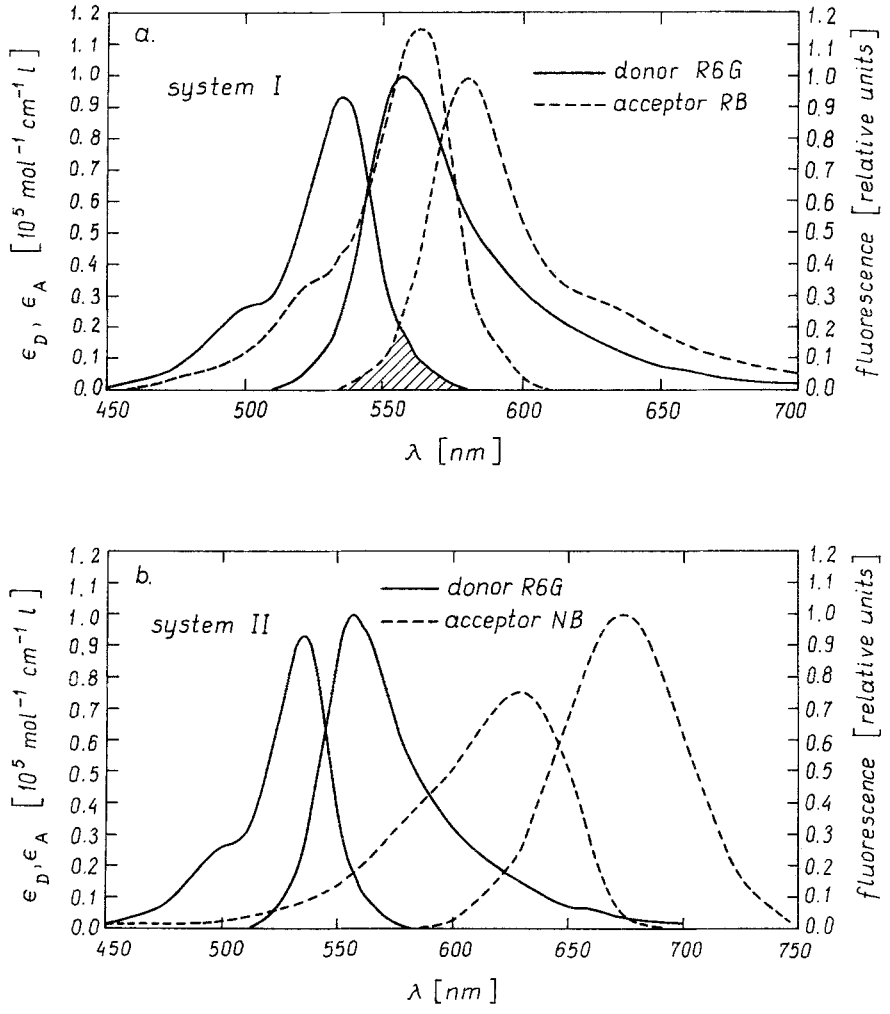

Fig. 2. Absorption and emission spectra of rhodamine 6G, rhodamine B, and Nile Blue chloride in glycerol. (a) System I; (b) system II.

Table II. Values of Energy Transfer for the Systems Investigated

System	R_{ODD} (Å)	R_{ODA} (Å)	R_{OAA} (Å)	R_{OAD} (Å)	$C_{ODD}\cdot10^{-3}$ (*M*)	$C_{ODA}\cdot10^{-3}$ (*M*)	$C_{OAA}\cdot10^{-3}$ (*M*)	$C_{OAD}\cdot10^{-3}$ (*M*)
I[a]	48.92	62.55	51.83	35.48	3.387	1.620	2.848	8.879
II[b]	47.6	46.32	—	0.00	3.675	3.988	—	∞

[a] From Ref. 24.
[b] From Ref. 25.

centrations, the contributions of both fractions are comparable. The conclusion therefore can be drawn that, due to direct light absorption by acceptors, the process of NRET is markedly stronger and can influence the emission anisotropy values, especially at moderate and high concentrations.

We are convinced that an independent estimation of the influence of this effect on emission anisotropy in the presence of NRET can be achieved based on the Monte Carlo simulation method. Further theoretical investigations taking into account the transfer from A* to primarily excited donors also seem important and advisable.

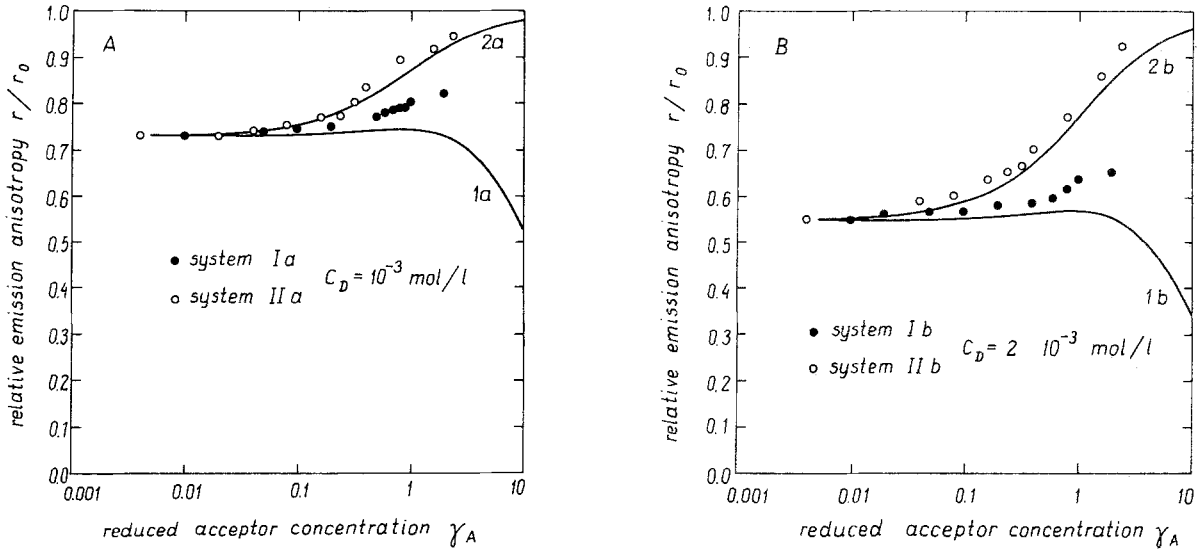

Fig. 3. Concentration dependences of emission anisotropy r/r_0 for systems I (R6G + RB) and II (R6G + NB). (•, ○) Experimental points for systems I and II, respectively; theoretical curves 1a–1c (NRET present) and 2a–2c (NRET absent) are calculated from expressions (1) and (8).

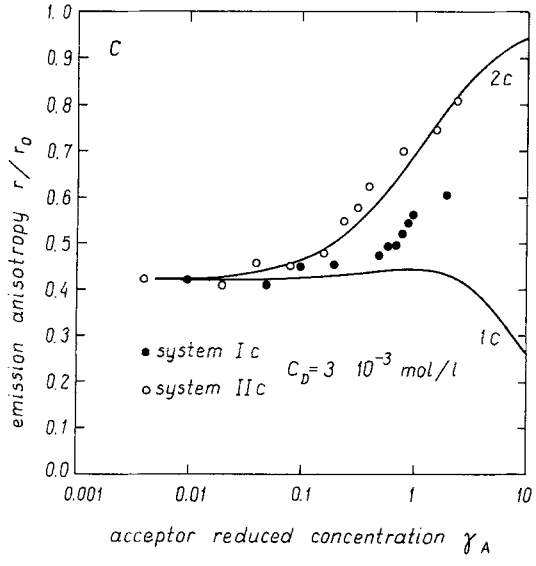

Fig. 3. Continued.

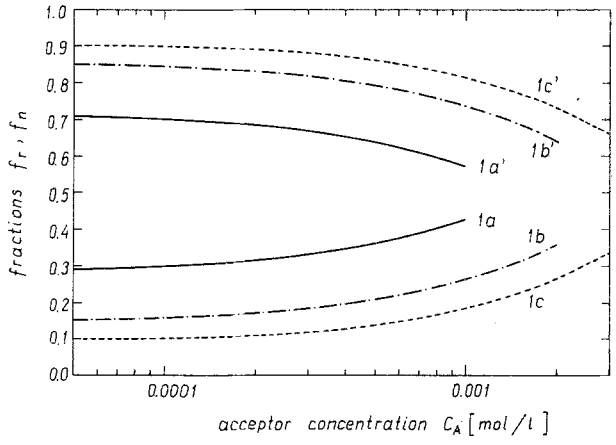

Fig. 4. Relative fractions f_n and f_r of acceptors excited due to nonradiative energy transfer from donors and due to direct light absorption by acceptors, respectively. Curves 1a, 1b, and 1c correspond to f_r fractions, and curves 1a′, 1b′, and 1c′ to the f_n fractions. Curves 1a and 1a′; 1b and 1b′, and 1c and 1c′ correspond to the donor concentrations 10^{-3}, $2{\cdot}10^{-3}$; and $3{\cdot}10^{-3}$ M, respectively.

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