

Fluorescence Spectroscopic Studies on the Multicomponent System Coumarin 1/Fluorescein/Rhodamine B in Ethanol

Pihong Zhao¹ and Silvio Rodriguez²

Received November 18, 1996; accepted April 1, 1997

The steady-state fluorescence spectra of the three-component system coumarin 1/fluorescein/rhodamine B in 95% ethanol were studied at room temperature. In the appropriate concentration ranges, upon the excitation of coumarin 1 at 377 nm, the ternary dye mixture solution emitted simultaneously with three bands centered at 443, 528, and 575 nm, respectively. The analysis of the data seems to indicate that there is significant energy transfer between these three dyes and that the main contribution corresponds to the Förster nonradiative mechanism. The critical transfer distances between each two of the three components were calculated to be 48.4 Å for the coumarin 1/fluorescein pair, 42.2 Å for the coumarin 1/rhodamine B pair, and 65.5 Å between fluorescein and rhodamine B dyes. The system can potentially be used to obtain three primary outputs at different wavelengths in a dye laser system.

KEY WORDS: Coumarin 1; fluorescein; rhodamine B; fluorescence; non-radiative energy transfer; three-component system.

INTRODUCTION

Studies of nonradiative energy transfer (ET) processes in solution have become an active area of research because of both theoretical and practical interests.⁽¹⁾ Most of the works done in this field are studies of two-component systems⁽¹⁻⁴⁾ containing donors (D) and acceptors (A) of energy in an inert solvent. Since it was found that multicomponent energy transfer systems could be used in dye lasers to give wider ranges of wavelength tunability, there has been an increased interest in these systems. A dye laser system containing three or more components may be used for the improvement of the conversion efficiency and the extension of the wavelength region of lasing. Although studies of three-component energy transfer systems have been re-

ported,^(5,6) because of their complexity, they are mostly qualitative and descriptive in nature. Nevertheless, in order to understand the transfer mechanism, it is necessary to describe the system quantitatively.

The theoretical treatment of excitation energy transfer in multicomponent luminescent solutions appeared in the 1970s. Ketskemény and Kusba^(7,8) derived general equations which describe the shape of the fluorescence spectrum of multicomponent solutions as a function of the emission, absorption, and quantum yield spectrum of each component. Both radiative and nonradiative excitation energy transfer were taken into account. A similar generalization of nonradiative energy transfer referring to the influence of concentration on the luminescent properties of rigid solutions containing multicomponents was carried out by Kusba and Bojarski.⁽⁹⁾ Based on their work, in this paper, we have used their approach to interpret the results of a study of the energy transfer in a coumarin 1, fluorescein, and rhodamine B ternary system in 95% ethanol using steady-state fluorescence spectra.

¹ Chemistry and Materials Science Directorate, Lawrence Livermore National Laboratory, Livermore, California 94550.

² To whom correspondence should be addressed at Chemistry Department, University of the Pacific, Stockton, California 95211.

THEORETICAL BACKGROUND

Electronic energy transfer phenomena between molecules in solution have been recognized for many decades. Förster⁽¹⁰⁾ has given theoretical treatments for nonradiative energy transfer processes through dipole-dipole interaction of donor and acceptor by assuming that the position and orientation of donor and acceptor are fixed during the lifetime of donor emission. The rate constant of the process is given by

$$k_{D^* \rightarrow A} = \frac{1}{\tau_{oD}} \left(\frac{R_{oDA}}{r} \right)^6 \quad (1)$$

where τ_{oD} is the actual lifetime of the excited state of the donor in the absence of acceptor molecules, r is the mutual distance between donor and acceptor, and R_{oDA} , the critical transfer distance, is the separation of the excited donor and acceptor at which the rate of excitation energy transfer is equal to the rate of emission of the excited donor. The critical transfer distance is given by

$$R_{oDA}^6 = \frac{9000 \ln 10 \kappa^2 \phi_{oD}}{128 \pi^5 n^4 N_A} \int_0^\infty F_D(\bar{\nu}) \epsilon_A(\bar{\nu}) \frac{d\bar{\nu}}{\bar{\nu}^4} \quad (2)$$

where $\bar{\nu}$ is the energy in cm^{-1} ; $F_D(\bar{\nu})$ is the spectral distribution of fluorescence, which is normalized on a wavenumber scale; $\epsilon_A(\bar{\nu})$ is the molar absorption coefficient as a function of wavenumber; N_A is Avogadro's number; n is the refractive index of the solvent; ϕ_{oD} is the fluorescence quantum yield of the donor in the absence of acceptor; and κ^2 is an orientation factor. For a random directional distribution the calculated average value of κ^2 is $2/3$, while for the statistical distribution of static dipoles, κ^2 has been experimentally determined⁽¹⁾ to be 0.476. In fact, given that the orientation factor κ^2 is directly proportional to R_{oDA} to the sixth power, the actual value of κ^2 will not significantly affect the calculated critical transfer distances. In this work an orientation factor of $2/3$ was used in the calculations.

Taking into consideration both energy transfer and energy migration (i.e., energy transfer between donor molecules), Bojarski and Domsta⁽¹¹⁾ proposed that the relative quantum yield of the donor in a two-component system is given by

$$\frac{\phi_D}{\phi_{oD}} = \frac{1 - f(\gamma)}{1 - \alpha f(\gamma)} \quad (3)$$

where

$$f(\gamma) = \sqrt{\pi} \gamma \exp(\gamma^2) [1 - \text{erf}(\gamma)] \quad (4)$$

and $\text{erf}(\gamma)$ is the error function:

$$\text{erf}(\gamma) = \frac{2}{\sqrt{\pi}} \int_0^\gamma \exp(-x^2) dx \quad (5)$$

$$\gamma = \gamma_D + \gamma_A = \frac{\sqrt{\pi}}{2} \left(\frac{C_D}{C_{DD}} + \frac{C_A}{C_{DA}} \right) \quad (6)$$

$$\alpha = \frac{\gamma_D}{\gamma_D + \gamma_A} \quad (7)$$

$$C_{DD} = \frac{3}{4\pi N R_{oDD}^3} = \frac{7.35}{R_{oDD}(\text{\AA})^3} \quad (8)$$

$$C_{DA} = \frac{3}{4\pi N R_{oDA}^3} = \frac{7.35}{R_{oDA}(\text{\AA})^3} \quad (9)$$

where C_D and C_A are the concentrations of donor and acceptor, while C_{DD} and C_{DA} are the critical concentrations of donor and acceptor, respectively, all in terms of molarity.

Theoretically, in a solution containing two or more laser dyes, in which the emission bands of one or two dyes located at shorter wavelengths overlap the absorption bands of other dyes located at longer wavelengths, the excitation energy may be transferred successively from the first dye molecule to the last. Therefore, the excitation energy may be efficiently cascaded from shorter to longer wavelengths. In general, in an energy transfer system containing three components, all the possible energy transfer paths can be illustrated⁽⁷⁾ as shown in Fig. 1. In Fig. 1 the thicker arrow represents the incident light. It can excite all three components directly if they absorb more or less at that excitation wavelength. Different circles are used to indicate each of the three components in the system. The open circles denote the first energy donor, the light shaded circles denote the secondary energy donor, and the dark shaded circles stand for the energy acceptor. Lines with arrows indicate the directions of energy transfer. The solid lines indicate the primary energy transfer, while dashed lines indicate the successive transfer of energy. In a three-component system we have to consider both the primary and successive energy transfer processes.

It is assumed that the system contains n types of molecules distributed at random in an inactive medium not taking part in the energy transfer. The components are denoted by numbers from $i = 1$ to $i = n$ in such a manner that the emission spectrum of the j th component does not overlap the absorption of any i th component, if $i < j$. Therefore, the back energy transfer from j th* to i th ($i < j$) does not have to be taken into consideration. Nevertheless, the emission spectrum of the j th component may overlap the absorption spectrum of the same

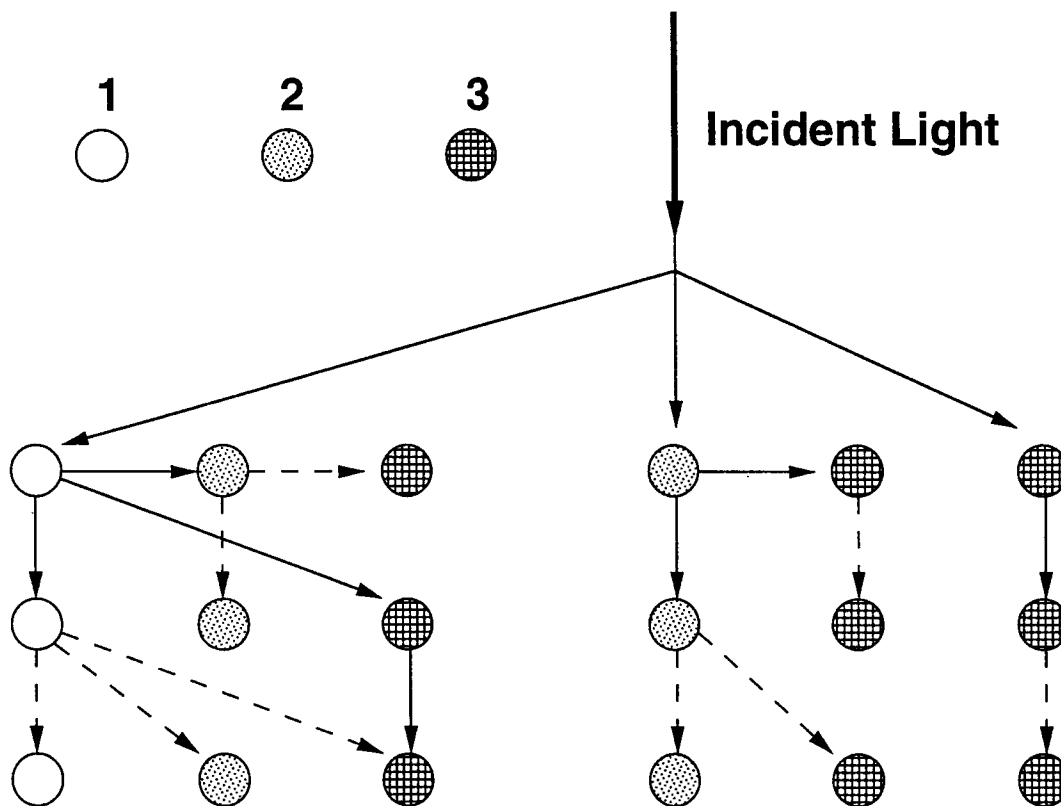


Fig. 1. Energy transfer paths in three-component systems.

component as well as the absorption spectra of components $k > j$. The quantum yield of the i th component ϕ_i can be expressed as⁽⁹⁾

$$\phi_i = \frac{f_{oi} [1 - f(\Gamma_i)]}{1 - a_{oi} (\gamma_{ii}/\gamma_i) f(\gamma_i)} \quad (i = 1, 2, \dots, n) \quad (10)$$

$$\Gamma_i = \sum_{k=i}^n \gamma_{ik} \quad (11)$$

$$\gamma_{ik} = \frac{\sqrt{\pi}}{2} \left(\frac{C_k}{C_{oik}} \right) \quad (12)$$

Analogously, the quantum yield of excitation energy transferred from the i th component to the k th component is

$$\phi_{jk} = \frac{\alpha_{oik} (\gamma_{ik}/\Gamma_i) f(\Gamma_i)}{1 - \alpha_{oi} (\gamma_{ii}/\Gamma_i) f(\Gamma_i)} \quad (k = i \text{ to } n) \quad (13)$$

where ϕ_{oi} is the quantum yield of the i th component in the absence of any j th component $j > i$, $f(\Gamma_i)$ is the error function as defined in Eq. (4), C_k is the concentration of the k th component, and C_{oik} is the critical concentration of the k th component with respect to the i th component.

Also, α_{oik} is the probability that excitation quenching will not occur during the nonradiative energy transfer between the i th and the k th component and is given by

$$\alpha_{oik} = \frac{\int_0^\infty F_i(\lambda) \varepsilon_k(\lambda) \eta_k(\lambda) \lambda^4 d\lambda}{\int_0^\infty F_i(\lambda) \varepsilon_k(\lambda) \lambda^4 d\lambda} \quad (14)$$

where $F_i(\lambda)$ denotes a true quantum fluorescence spectrum, $\varepsilon_k(\lambda)$ is the molar absorption coefficient, and $\eta_k(\lambda)$ is the probability of effective excitation of the k th component molecule as a result of absorption. The value of α_{oik} is usually assumed to be close to unity.

For example, in the case of a three-component system, Eq. (10) becomes

$$\phi_1 = \frac{\phi_{o1} [1 - f(\Gamma_1)]}{1 - \alpha_{o11} (\gamma_{11}/\Gamma_1) f(\Gamma_1)} \quad (15)$$

and

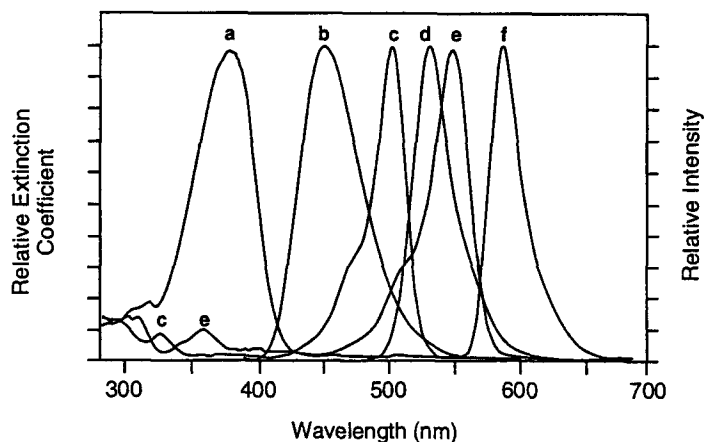


Fig. 2. Absorption and emission spectra of coumarin 1, fluorescein, and rhodamine B in 95% Ethanol with $1 \times 10^{-2} M$ NaOH: (a) absorption spectrum of coumarin 1, $\epsilon_D(377 \text{ nm}) = 2.23 \times 10^4 \text{ mol}^{-1} \cdot \text{cm}^{-1} \cdot \text{L}$; (b) emission spectrum of coumarin 1; (c) absorption spectrum of fluorescein, $\epsilon_A(500 \text{ nm}) = 1.23 \times 10^5 \text{ mol}^{-1} \cdot \text{cm}^{-1} \cdot \text{L}$; (d) emission spectrum of fluorescein; (e) absorption spectrum of rhodamine B, $\epsilon_A(545 \text{ nm}) = 9.84 \times 10^4 \text{ mol}^{-1} \cdot \text{cm}^{-1} \cdot \text{L}$; (f) emission spectrum of rhodamine B.

$$\Gamma_1 = \gamma_{11} + \gamma_{12} + \gamma_{13}$$

$$\gamma_{11} = \frac{\sqrt{\pi}}{2} \left(\frac{C_1}{C_{011}} \right); \quad \gamma_{12} = \frac{\sqrt{\pi}}{2} \left(\frac{C_2}{C_{012}} \right); \quad \gamma_{13} = \frac{\sqrt{\pi}}{2} \left(\frac{C_3}{C_{013}} \right)$$

$$\phi_2 = \frac{\phi_{02} [1 - f(\Gamma_2)]}{1 - \alpha_{022} (\gamma_{22}/\Gamma_2) f(\Gamma_2)} \quad (16)$$

$$\Gamma_2 = \gamma_{22} + \gamma_{23}$$

$$\phi_3 = \frac{\phi_{03} [1 - f(\gamma_{33})]}{1 - \alpha_{033} f(\gamma_{33})} \quad (17)$$

As expected, if the probability $\alpha_{022} = 1$, then Eq. (16) is identical to the Bojarski expression [Eq. (3)] for the donor quantum yield in the two-component system.

EXPERIMENTAL

The dyes coumarin 1, fluorescein, and rhodamine B (Laser Grade, Eastman Kodak), and 95% ethanol (A.R. grade, Aldrich), were used as received. All the solutions including dye stock solutions and dye mixture solutions were prepared daily to avoid aging processes.

The absorption and fluorescence spectra of these dyes were recorded at room temperature, i.e., approximately 25°C. The fluorescence measurements were carried out in a Perkin Elmer LS-5 fluorescence spectrophotometer using a PECLS applications software. The samples were excited at a fixed wavelength, and the emission intensity was recorded as a function of wavelength. Fluorescence spectra were taken from the front face of a 1-mm quartz infrasil cuvette with stopper in order to minimize inner filter effects and solvent evap-

oration. The angles of the incident light and emission light were 40 and 46°, respectively. For unambiguous demonstration of energy transfer between donor and acceptor molecules, the observations of both the quenching of the donor fluorescence and the sensitization of the acceptor fluorescence were recorded at the same time. The fluorescence spectra of 95% ethanol were also taken under the same conditions, but no fluorescence components were found in the solvent. Fluorescence spectra were corrected for the spectral sensitivity of photomultiplier and reabsorption as described elsewhere.⁽¹²⁾ The absorption spectra were recorded using a Perkin Elmer Lambda Array 3840 uv/vis spectrophotometer. When required, more accurate absorption spectra were obtained with a Varian Cary 219 double-beam spectrophotometer with cuvettes of 5.0-cm light path.

RESULTS AND DISCUSSION

The absorption and emission spectra of coumarin 1, fluorescein, and rhodamine B in ethanol with 0.01 M NaOH are shown in Fig. 2. The relatively large spectral overlap between the emission of coumarin 1 and the absorption of fluorescein indicates the possibility of excited state energy transfer from coumarin 1 to fluorescein. Figure 2 also shows the minor overlap of the absorption and fluorescence spectrum of coumarin 1 itself. Thereby, some energy migration is expected to take place simultaneously along with energy transfer. The absorption spectrum of rhodamine B in the wavelength region of 450 to 600 nm has an intense and unsymmetrical band

Table I. The Critical Transfer Distances and Concentrations of the Three Dyes in 95% Ethanol + 0.01 M NaOH

Binary system	R_{oDD} (Å)	R_{oDA} (Å)	C_{oDD} (M)	C_{oDA} (M)
Coumarin 1 (D) Fluorescein (A)	21.0	48.4	4.29×10^{-2}	3.49×10^{-3}
Fluorescein (D) Rhodamine B (A)	38.1	65.5	7.19×10^{-3}	1.41×10^{-3}
Coumarin 1 (D) Rhodamine B (A)	21.0	42.2	4.29×10^{-2}	5.27×10^{-3}

with a maximum centered at 552 nm. On the high-energy side of the band there is a shoulder at approximately 510 nm. Spectral overlap between the emission of fluorescein and the absorption of rhodamine B suggests the possibility of nonradiative energy transfer from coumarin 1 to rhodamine B through a molecule of fluorescein. In addition, the overlap between the absorption and the emission spectra of fluorescein and rhodamine B by themselves indicates the likelihood of energy migration for these two dyes. Earlier experimental results demonstrated that the absorption coefficient of these three dyes do not change with increasing concentration up to 10^{-2} M in 95% ethanol. Hence aggregation of any of these dyes may be assumed to be negligible in the range of concentration of this study. Figure 2 also shows that fluorescein and rhodamine B have very low extinction coefficients at the maximum absorption of coumarin 1 (377 nm). In a system in which coumarin 1 is the first donor, fluorescein is the intermediary, and rhodamine B is the acceptor, if coumarin 1 is effectively excited at 377 nm, its fluorescence band overlaps the absorption region of fluorescein, while, in turn, the emission band of fluorescein conveniently extends over the absorption band of rhodamine B. In addition, it must be noted that the emission of coumarin 1 overlaps the absorption of rhodamine B as well. Moreover, the quantum yields of the three dyes are relatively high, being 0.573 for coumarin 1, 0.98 for fluorescein, and 0.95 for rhodamine B. Also, as shown in Fig. 2, there are minimal overlaps between the donor's absorption and the acceptor's emissions; nonradiative and radiative back transfer of the excitation energy from A* to D can be considered negligible. All this information suggests that coumarin 1, fluorescein, and rhodamine B constitute an excellent ternary system of energy transfer. Thus, this is a very promising system in which the excitation energy of coumarin 1 should be transferred to rhodamine B directly as well as through the medium molecules of fluorescein.

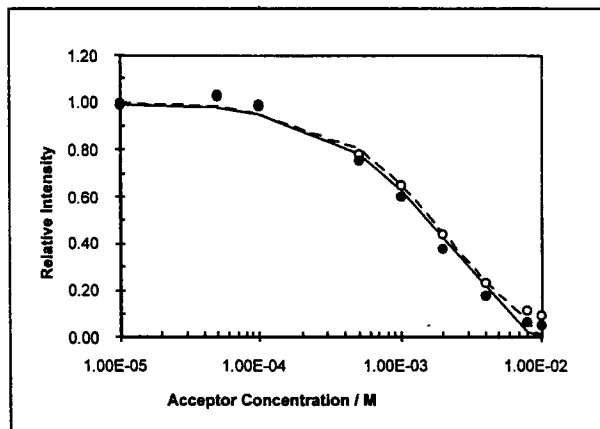


Fig. 3. Fluorescence intensity of coumarin 1 versus concentration of fluorescein in 95% Ethanol with 0.01 M NaOH. Concentration of coumarin 1: 1.00×10^{-2} M. (○○○○○○○○) Corrected experimental points; (●●●●●●●●) uncorrected experimental points; (—) Bojarski's equation; (-----) Förster's equation.

In order to obtain the energy transfer parameters and a better understanding of the three-component system, first, the energy transfers of the three binary systems in 95% ethanol with 0.01 M NaOH have to be examined individually. The related critical transfer distances and concentrations were calculated using Eqs. (2), (8), and (9), respectively. The values are listed in Table I.

As shown in Table I, the coumarin 1/fluorescein and coumarin 1/rhodamine B pairs have moderate critical distances R_{oDA} , while the fluorescein/rhodamine B pair has a longer distance, which reveals a potential for more efficient energy transfer through this pair. On the other hand, coumarin 1 has a R_{oDD} of 21 Å, which is not long enough to result in considerable energy migration, while fluorescein has a relative large R_{oDD} , about 40 Å, which may introduce a significant contribution of energy migration into its binary system.

For the determination of relative quantum yields, the areas under emission curves were used for the values of fluorescence intensities. Because of the overlap between the emission band of coumarin 1 with that of fluorescein, peak analysis software (Peakfit, Jandel Corporation) was used to deconvolute the fluorescence spectra of the mixtures. The ratio of the corrected fluorescence intensities of coumarin 1 in the presence of fluorescein to the intensity of coumarin 1 by itself is equivalent to the corrected relative quantum yields ϕ/ϕ_0 . Figure 3 shows the ϕ/ϕ_0 for coumarin 1 at 1.00×10^{-2} M versus the logarithm of the concentrations of fluorescein.

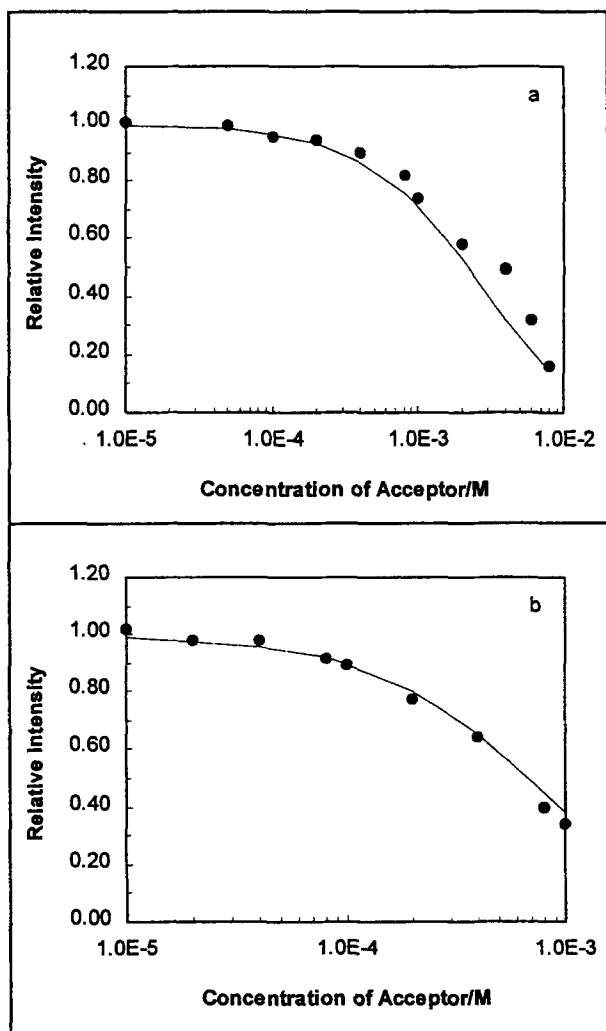


Fig. 4. Donor fluorescence intensity versus concentration of rhodamine B in 95% ethanol with 0.01 M NaOH. (●●●●●●●●) Corrected experimental points; (—) Bojarski's equation. (a) Coumarin 1, 8.0×10^{-3} M; rhodamine B, 1.0×10^{-5} to 8.0×10^{-3} M. (b) Fluorescein, 3.0×10^{-5} M; rhodamine B, 1.0×10^{-5} to 1.0×10^{-3} M.

cein in 95% ethanol. The theoretical curves calculated from Bojarski's as well as Förster's equations are also presented along with the experimental points in the same figure. The experimentally measured quantum yield ϕ_0 of coumarin 1 used in the calculations was 0.573. The experimental results agreed with the theoretical curves within the limits of experimental errors. It may be seen that differences between the Förster and the Bojarski curves were very small in this system. This is evidence that the energy migration was not a strong competitive process compared with the radiationless energy transfer between donor and acceptor in the system. This is un-

derstandable since the donor-donor critical transfer distance ($R_{oDD} = 21.0 \text{ \AA}$) is much smaller than that of the donor-acceptor ($R_{oDA} = 48.4 \text{ \AA}$). It should be emphasized that the theoretical curves calculated here do not have fitting parameters. Similar experiments were carried out in 95% ethanol solutions having a constant concentrations of coumarin 1 of 5.00×10^{-3} and 5.00×10^{-4} M and varying concentrations of fluorescein. Once again, very good agreements were obtained between the experimental values and the theoretical curves. The details of the study of this two-component system are described elsewhere.⁽¹³⁾

In 1974 Ahmed *et al.*⁽¹⁴⁾ reported laser action in a mixture of coumarin 1-rhodamine B in ethanol. They suggested that, since the two absorption bands overlap, the simultaneous emission from coumarin 1 and rhodamine B is due to the simultaneous excitation of both dyes. However, according to our experiments, energy transfer processes are responsible for the two emission bands in the fluorescence spectra of the mixed solution. Figure 4a represents the corrected relative quantum yields ϕ/ϕ_0 of coumarin 1 at 8.00×10^{-3} M versus the logarithm of the concentrations of rhodamine B in 95% ethanol. The theoretical curve calculated from Bojarski's equation is also shown along with the experimental points in the same figure. The experimental results agreed well with the theoretical curves within the limits of experimental errors.

Energy transfer systems containing fluorescein and rhodamine B have been studied in aqueous solution⁽¹⁵⁾ and in mixed organic solvents.⁽¹⁶⁾ Nonradiative energy transfer and energy migration were verified as the major processes in this binary system in the concentration range of fluorescein from 5×10^{-5} to 2×10^{-2} M. Our experimental results show that when the concentration of donor was fixed at 3×10^{-5} M and the concentration of acceptor varied from 1×10^{-5} to 1×10^{-3} M, the Bojarski theory was valid. The experimental results plotted along with the theoretical curve are presented in Fig. 4b.

In summary, our studies showed that in the appropriate concentration ranges, the donor transfers excitation energy to the acceptor through radiative as well as nonradiative mechanisms in all three of these binary systems. By introducing the corrections for radiative energy transfer and direct absorption of excitation by the acceptor,^(17,18) the experimental data on the relative quantum yields of the donors as a function of the concentration of acceptor in all three binary systems were found to be in excellent agreement with the predictions of theoretical models proposed by Förster⁽¹⁰⁾ and Bojarski and Domsta.⁽¹¹⁾

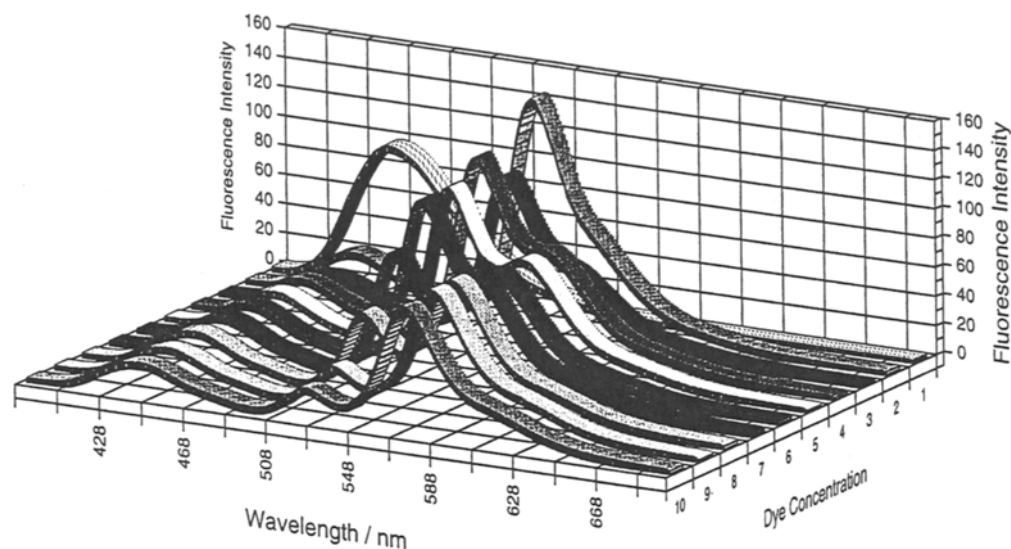


Fig. 5. Emission spectra of solutions containing coumarin 1, fluorescein, and rhodamine B in 95% ethanol with 0.01 *M* NaOH: (1) 2.0×10^{-3} *M* coumarin 1; (2) (1) + 2.0×10^{-3} *M* fluorescein; (3) (2) + 2.0×10^{-5} *M* rhodamine B; (4) (2) + 5.0×10^{-5} *M* rhodamine B; (5) (2) + 8.0×10^{-5} *M* rhodamine B; (6) (2) + 1.0×10^{-4} *M* rhodamine B; (7) (2) + 2.0×10^{-4} *M* rhodamine B; (8) (2) + 4.0×10^{-4} *M* rhodamine B; (9) (2) + 8.0×10^{-4} *M* rhodamine B; (10) (2) + 9.6×10^{-4} *M* rhodamine B.

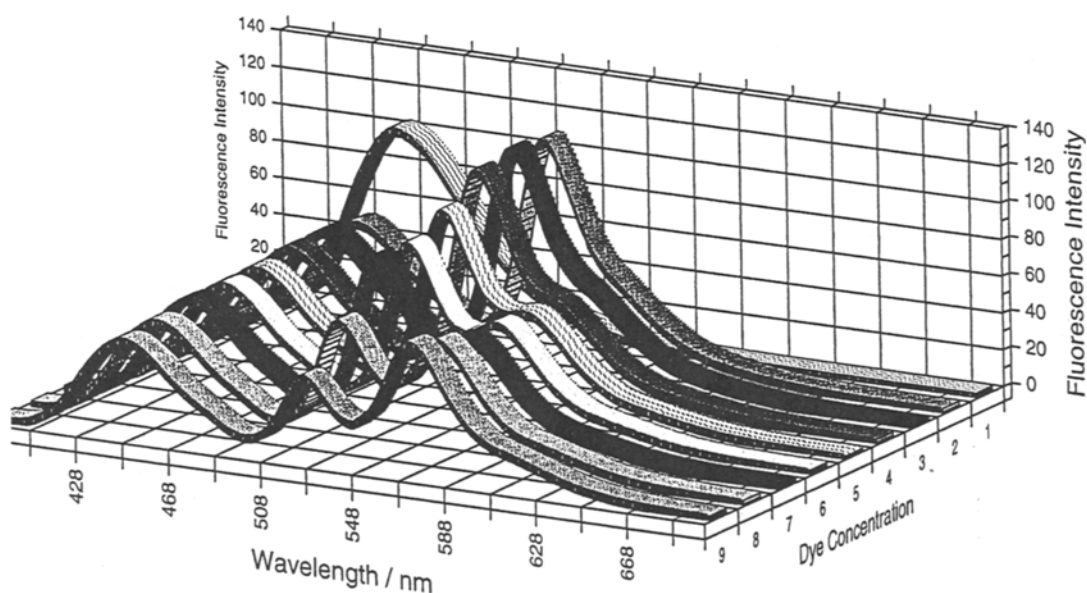


Fig. 6. Emission spectra of solutions containing coumarin 1, fluorescein, and rhodamine B in 95% ethanol with 0.01 *M* NaOH: (1) 2.0×10^{-3} *M* coumarin 1; (2) (1) + 8.0×10^{-4} *M* fluorescein; (3) (2) + 1.0×10^{-5} *M* rhodamine B; (4) (2) + 2.0×10^{-5} *M* rhodamine B; (5) (2) + 5.0×10^{-5} *M* rhodamine B; (6) (2) + 8.0×10^{-5} *M* rhodamine B; (7) (2) + 1.0×10^{-4} *M* rhodamine B; (8) (2) + 2.0×10^{-4} *M* rhodamine B; (9) (2) + 3.8×10^{-4} *M* rhodamine B.

For the three-component system, experiments were carried out by taking the fluorescence spectra of solutions containing fixed concentrations of coumarin 1 and fluorescein and increasing concentrations of rhodamine

B in 95% ethanol with 0.01 *N* NaOH. The results were compared with the fluorescence spectrum of a coumarin 1 solution of the same concentration. The observed emission spectra for the mixture solutions and the so-

Table II. The Shifting of the Emission Maxima (nm) of Coumarin 1, Fluorescein, and Rhodamine B in 95% Ethanol with 0.01 *M* NaOH (Coumarin 1, 2.0×10^{-3} *M*; Fluorescein, 2.0×10^{-3} *M*)

Rhodamine B conc. (<i>M</i>)	Maximum of coumarin 1 (nm)	Maximum of fluorescein (nm)	Maximum of rhodamine B (nm)
0.0	433.3	—	—
0.0	428.3	518.7	—
2.0×10^{-5}	429.2	520.1	553.1
5.0×10^{-5}	429.0	519.7	554.9
8.0×10^{-5}	429.0	519.3	556.1
1.0×10^{-4}	429.0	519.0	556.5
2.0×10^{-4}	429.2	518.1	557.8
4.0×10^{-4}	429.4	516.5	559.5
8.0×10^{-4}	429.8	514.3	561.3
9.6×10^{-4}	430.3	518.6	561.0

lution of coumarin 1 are given in Figs. 5 and 6. It can be seen that the introduction of fluorescein into a coumarin 1 solution dramatically reduced the emission intensity of coumarin 1. Subsequently, with the addition of rhodamine B, both bands corresponding to the emission of coumarin 1 and fluorescein decreased simultaneously. Depending on the concentration of rhodamine B, the emission of coumarin 1 decreased slightly while that of fluorescein decreased considerably. In Fig. 5, the greater than expected decrease in the emission of coumarin 1 and fluorescein for curve 3 is probably due to experimental error. On the other hand, in the solutions containing a constant concentration of coumarin 1 (2.0×10^{-3} *M*) and of fluorescein (2.0×10^{-3} *M*), the fluorescence intensity of rhodamine B increased with concentration until it reached 8.0×10^{-4} *M* (see Fig. 5). At even higher concentrations of rhodamine B, the intensity of its emission decreased because of a change in mechanism in which the concentrational quenching becomes important. These observations demonstrate that the excitation energy is transferred from the excited state of coumarin 1 to the ground state of rhodamine B through an intermediary molecule of fluorescein. Since the three emission bands overlap each other, the spectra were deconvoluted with the peak analysis software Peakfit and the positions of maximum intensity and the areas of each emission band were then determined. The emission maxima of the three bands are listed in Tables II and III.

Both Table II and Table III reveal that the maximum of the rhodamine B emission band shows a red shift with increasing its concentration. This phenomenon suggests a self-reabsorption of the rhodamine B emission on the high-energy side. Moreover, the fluorescein emis-

Table III. The Shifting of the Emission Maxima (nm) of Coumarin 1, Fluorescein, and Rhodamine B in 95% Ethanol with 0.01 *M* NaOH (Coumarin 1, 2.0×10^{-3} *M*; Fluorescein, 8.0×10^{-4} *M*)

Rhodamine B conc. (<i>M</i>)	Maximum of coumarin 1 (nm)	Maximum of fluorescein (nm)	Maximum of rhodamine B (nm)
0.0	433.4	—	—
0.0	430.8	516.4	—
1.0×10^{-5}	430.9	517.5	554.6
2.0×10^{-5}	430.9	517.3	555.4
5.0×10^{-5}	430.9	516.9	556.3
8.0×10^{-5}	431.0	516.6	556.7
1.0×10^{-4}	431.0	516.6	557.0
2.0×10^{-4}	431.2	515.5	558.4
3.8×10^{-4}	431.3	514.4	559.8

sion band started with a red shift in the maximum, and then it was followed by a blue shift with increasing concentrations of rhodamine B. The initial red shift of the fluorescein band may be due to the addition of the emission band of rhodamine B, which has a considerable overlap on the low-energy side of the former. The following blue shift comes from the absorption of fluorescein emission on the lower-energy side by rhodamine B. This is a typical phenomenon observed in cases of radiative energy transfer from a donor to an acceptor. As expected, the introduction of fluorescein to coumarin 1 solution caused a blue shift on the maximum emission of the latter. Furthermore, an unusual recovery from this blue shift in the maximum of coumarin 1 emission band was observed with increasing concentrations of rhodamine B as well. It may be explained as the result of a competition between the radiative transfer from coumarin 1 to fluorescein and the nonradiative transfer from coumarin 1 to rhodamine B. It seems that the introduction of the third component in this system reduces the probability of radiative energy transfer between the first two components. Nevertheless, radiative energy transfer still takes place simultaneously with nonradiative energy transfer in this multicomponent system, and its effect has to be taken into account.

Conte and Martinho⁽¹⁸⁾ proposed a method for the correction of radiative energy transfer and direct absorption of excitation by the acceptor in a two component energy transfer system. We have modified their equations for the correction of donor's quantum yields in a three component system. By taking in consideration the direct absorption and radiative energy transfer to the second and third components, the fluorescence intensity of the first component is given by

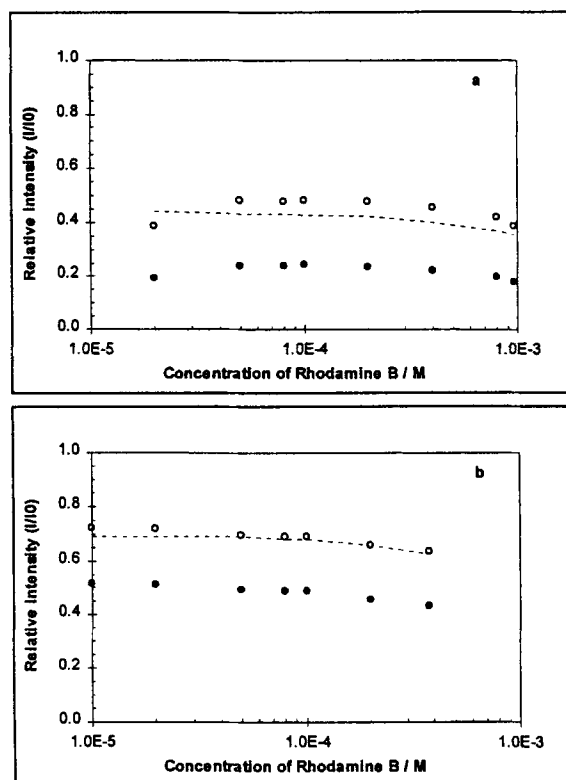


Fig. 7. Relative intensity of coumarin 1 in the presence of fluorescein versus the concentration of rhodamine B in 95% ethanol with 0.01 *M* NaOH. (○○○○○○○○) Corrected experimental points; (●●●●●●●●) uncorrected experimental points; (-----) Bojarski's equation. (a) Coumarin 1, 2.0×10^{-3} *M*; fluorescein, 2.0×10^{-3} *M*; rhodamine B, 0 to 1.0×10^{-3} *M*. (b) Coumarin 1, 2.0×10^{-3} *M*; fluorescein, 8.0×10^{-4} *M*; rhodamine B, 0 to 4.0×10^{-4} *M*.

$$I_{1c} = I_{1\text{exp}} \frac{b + b'}{b} \quad (18)$$

$$b = \varepsilon_1(\lambda_{\text{exc}}) (\sin\beta) C_1 \quad (19)$$

$$b' = [\varepsilon_2(\lambda_{\text{exc}}) \sin\beta + \varepsilon_2(\lambda_{\text{em}}^1) \sin\alpha] C_2 + [\varepsilon_3(\lambda_{\text{exc}}) \sin\beta + \varepsilon_3(\lambda_{\text{em}}^1) \sin\alpha] C_3 \quad (20)$$

where $I_{1\text{exp}}$ is the emission intensity of the first donor obtained experimentally, and I_{1c} is the intensity after corrections. The molar absorptivities of the three components are given by $\varepsilon_1(\lambda_{\text{exc}})$, $\varepsilon_2(\lambda_{\text{exc}})$, and $\varepsilon_3(\lambda_{\text{exc}})$, respectively at the exciting wavelength λ_{exc} . The molar absorptivities of the second and third components at the first component's emission wavelength, λ_{em}^1 , are $\varepsilon_2(\lambda_{\text{em}}^1)$ and $\varepsilon_3(\lambda_{\text{em}}^1)$. The angles of incident light and emission light with the surface of the solution are α and β , respectively. As mentioned before, in our experiments α and β were 40 and 46°, respectively.

For the fluorescence intensity of the second component I_{2c} , we must take into consideration the direct absorption of excitation light, and emission of the first component, by the second component, and the absorption of excitation light, and emission of the first and second components, by the third component. The resulting relations are

$$I_{2c} = I_{2\text{exp}} \frac{c + c'}{c} \quad (21)$$

$$c = [\varepsilon_2(\lambda_{\text{exc}}) \sin\beta + \varepsilon_2(\lambda_{\text{em}}^1)] C_2 \quad (22)$$

$$c' = [\varepsilon_2(\lambda_{\text{exc}}) \sin\beta + \varepsilon_3(\lambda_{\text{em}}^1) + \varepsilon_3(\lambda_{\text{em}}^2) \sin\alpha] C_3 \quad (23)$$

where the denotation of all the symbols is equivalent to those above.

The fluorescence intensities of coumarin 1 in three-component mixture solutions were corrected using Eqs. (18) to (20). The corrected relative quantum yields ϕ/ϕ_0 of coumarin 1 at a concentration of 2.00×10^{-3} *M*, in the presence of 2.00×10^{-3} *M* fluorescein, were plotted versus the logarithm of the concentrations of rhodamine B in 95% ethanol in Fig. 7a. The theoretical curve calculated from Eq. (15) is also shown along with the experimental points in the same figure. A value of 0.812 as the probability α_{011} , as defined by Eq. (14), was used in these calculations. Figure 7b represents the results of similar experiments and data treatment with the same concentration of coumarin 1, 2.00×10^{-3} *M*, but a lower concentration of fluorescein (8.00×10^{-4} *M*). As expected, the relative intensities of coumarin 1 in Fig. 7b were higher than those in Fig. 7a.

Equations (21)–(23) were used to correct the observed emission intensities of fluorescein, at concentrations of 2.00×10^{-3} and 8.00×10^{-4} *M*, in the mixture solutions containing these three dyes. Figure 8 shows the plots of the corrected relative intensities ϕ/ϕ_0 of fluorescein in these solutions versus the logarithm of the concentrations of rhodamine B in 95% ethanol. The theoretical curves plotted in the same figure were calculated via Eq. (16) with a value of 0.623 for α_{022} . Once again, the consistency between the theoretical curves and the corrected experimental data is apparent.

CONCLUSIONS

This work is the first attempt to apply theoretical relations to the calculation of fluorescence relative intensities of donors at various concentrations of acceptors

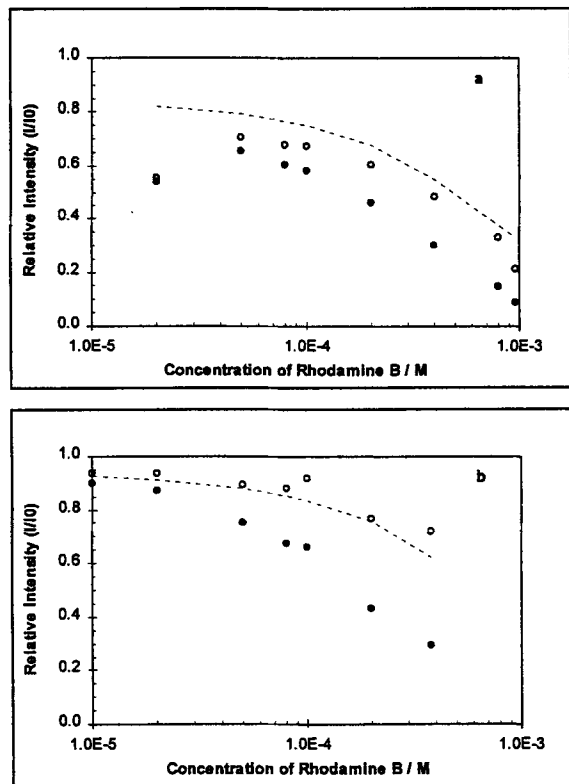


Fig. 8. Relative intensity of fluorescein in the presence of coumarin 1 versus the concentration of rhodamine B in 95% ethanol with 0.01 M NaOH. (○○○○○○○○) Corrected experimental points; (●●●●●●●●) uncorrected experimental points; (-----) Bojarski's equation. (a) Coumarin 1, $2.0 \times 10^{-3} M$; fluorescein, $2.0 \times 10^{-3} M$; rhodamine B, 0 to $1.0 \times 10^{-3} M$. (b) Coumarin 1, $2.0 \times 10^{-3} M$; fluorescein, $8.0 \times 10^{-4} M$; rhodamine B, 0 to $4.0 \times 10^{-4} M$.

in a three component system. The fact that the computed theoretical curves coincide satisfactorily with the experimental results demonstrates that the model and its correction factors represent the data very well. The results seem to indicate that the nonradiative energy transfer, which is accompanied by radiative energy transfer, dominates this three-component energy transfer system. The influence of the second component significantly affects

the energy transfer from component 1 to 3, so that the efficiency of the transfer from 1 to 3 is increased considerably. By regulating the concentrations of these three components, simultaneous emission bands of the three dyes with comparable intensities can be obtained. For example, when the concentrations of the three dyes were coumarin 1 at $2.0 \times 10^{-3} M$, fluorescein at $8.0 \times 10^{-4} M$, and rhodamine B at $2.0 \times 10^{-4} M$ (see Fig. 6), the system gave comparable intensities at three wavelengths of maxima, covering the spectral range from 450 to 600 nm. Since there is efficient energy transfer among these three components, they are good candidates to constitute an energy transfer dye laser system with wide tunable wavelength range.

REFERENCES

1. C. Bojarski and K. Sienicki (1989) *Photochem. Photophys.*, CRC Press, Boca Raton, FL, Vol. 1, p. 1.
2. M. A. Griep and C. S. McHenry (1992) *J. Biol. Chem.* **267**, 3025.
3. M. Miki, S. I. O'Donoghue, Dos, and C. D. Remedios (1992) *J. Muscle Res. Cell Motil.* **13**, 132.
4. M. Debreczeny, K. Sauer, J. H. Zhou, and D. Bryant (1993) *J. Phys. Chem.* **97**, 9852.
5. W. Wang, J. Xu, L. Li, B. Yu, and Z. Guan (1988) *J. Luminesc.* **40/41**, 684.
6. Y. Saito, A. Nomura, and T. Kano (1988) *Appl. Phys. Lett.* **53**, 1903.
7. I. Ketskeménty and J. Kusba (1974) *Acta Phys. Chem. Szeged* **20**, 239.
8. I. Ketskeménty and J. Kusba (1977) *Acta Phys. Chem. Szeged* **23**, 375.
9. J. Kusba and C. Bojarski (1979) *J. Luminescence* **20**, 73.
10. Th. Förster (1959) *Disc. Faraday Soc.* **27**, 7.
11. C. Bojarski and J. Domsta (1971) *Acta Phys. Acad. Sci. Hung.* **30**, 145.
12. J. M. G. Martinho and J. C. Conte (1982) *J. Chem. Soc., Faraday Trans. 2* **78**, 957.
13. P. Zhao (1994) Ph.D. dissertation.
14. S. A. Ahmed, J. S. Gergely, and D. Infante (1974) *J. Chem. Phys.* **61**, 1584.
15. R. P. S. Kushwaha and D. D. Pant (1985) *Indian J. Pure Appl. Phys.* **23**, 442.
16. C. Bojarski, R. Bujko, and P. Bojarski (1991) *Acta Phys. Polonica A* **79**, 471.
17. J. C. Conte and J. M. G. Martinho (1981) *J. Luminesc.* **22**, 273.
18. J. C. Conte and J. M. G. Martinho (1981) *J. Luminesc.* **22**, 285.