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Influence of Diffusion on Excitation Energy Transfer in Solutions by Gigahertz Harmonic Content Frequency-Domain Fluorometry

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Abstract

We report the effects of diffusion on fluorescence resonance energy transfer, as observed by using the frequency-domain technique. Energy transfer between indole (donor) and dansylamide (acceptor) was studied in low and high viscosity solutions. At the current level of resolution, the donor fluorescence decays were satisfactorily analyzed in terms of the theoretical function including translational diffusion proposed by Gosele et al. (*Chem. Phys. Lett.* **1975**, *24*, 519). The Forster equation, which does not consider diffusion, was found to significantly overestimate the critical transfer distance (R_0) in fluid solutions and to provide an inadequate representation of the data. These results demonstrate the ability to determine mutual diffusion coefficients from the frequency response of the donor emission.

Registry No.

Indole, 120-72-9; dansylamide, 1431-39-6

Introduction

The phenomenon of nonradiative resonance electronic energy transfer (RET) has been intensively studied theoretically and experimentally for several decades. Most of these investigations were performed by using steady-state measurements of fluorescence intensity or anisotropy in homogeneous systems. Such measurements have limited information due to averaging of the time-dependent processes, which are characteristic of diffusion-dependent energy transfer. Additionally, steady-state measurements require careful corrections for inner-filter effects, which are substantial at the high acceptor concentrations necessary for intermolecular energy transfer. Time-resolve fluorescence spectroscopy provide direct observation of the time-dependent decay, which are modified by the time-dependent rates of energy transfer.

Recently, several experimental studies of diffusion-dependent energy transfer were reported that used time-domain measurements.^{1–3} However, to our best knowledge, frequency-domain fluorometry has not yet been used for time-dependent energy-transfer measurements, except intramolecular energy transfer.^{4–7} Also, these studies^{4–7} did not consider the effect of diffusion on the energy-transfer process. High resolution of frequency-

domain fluorometry has already been used in recovering distance distributions in macromolecules^{5–8} and transient effects in collisional quenching of fluorescence.^{9–11}

To study diffusion-dependent energy transfer, we chose indole as a donor (D) and dansylamide as an acceptor (A). This D–A system is characterized by Forster distance $R_0 \approx 25 \text{ \AA}$ and has been used in energy transfer studies of macromolecules.^{8,12} Furthermore, we chose to initially study intermolecular energy transfer in homogeneous solutions because of the availability of a theoretical foundation and analytical expression for this case.^{13,14} The measurements were performed in two solvents, in propylene glycol (high viscosity) and in methanol (low viscosity). The concentrations of acceptor were chosen to give approximately 50% quenching of the donor fluorescence.

Theory

There exist several theories describing intensity decay of a donor in the presence of energy transfer in homogeneous solution without^{15–17} and with diffusion,^{13–14,18} referring to only a few. We chose, in our opinion, the most appropriate and commonly used Forster theory,¹⁵ developed latter by Gosele et al.¹⁴ for the case when diffusion occurs.

Energy-Transfer Kinetics without Diffusion (Forster).

Assume the decay of the donor $[I_D(t)]$, in the absence of energy transfer, is a single exponential

$$I_D(t) = I_D^0 \exp(t/\tau_D^0) \quad (1)$$

where τ_D^0 is the decay time of the donor in the absence of acceptor. In the presence of a random distributed acceptor, the donor fluorescence decays nonexponentially according to the relation

$$I_D(t) = I_D^0 \exp \left\{ -\frac{t}{\tau_D^0} - 2\gamma \left(\frac{t}{\tau_D^0} \right)^{1/2} \right\} \quad (2)$$

where $\gamma = C_A/C_A^0$, C_A is molar concentration of the acceptor, and C_A^0 is the molar critical concentration, given by

$$C_A^0 = \frac{3000}{2\pi^{3/2}NR_0^3} \quad (3)$$

N is Avogadro's number and R_0 is the critical transfer distance given by Forster

$$R_0^6 = \frac{9000(\ln 10)k^2\phi_D^0}{128\pi^5Nn^4} \int_0^\infty F_D(\lambda)E_A(\lambda)\lambda^4 d\lambda \quad (4)$$

where k^2 is the orientation factor, ϕ_D^0 the quantum yield of the donor in the absence of acceptor, n the refractive index, $F_D(\lambda)$ the emission spectrum of the donor with the area normalized to unity, $E_A(\lambda)$ the absorption spectrum of the acceptor in units of $\text{M}^{-1} \text{ cm}^{-1}$,

and λ the wavelength in nm. If the molecular orientations are random due to Brownian rotation, then $k^2 = 2/3$. In our analysis using the Forster equation (2), R_0 will be the floating parameter. The influence of diffusion can be monitored in changes in the apparent value of R_0 .

Energy-Transfer Kinetics with Diffusion (Gosele et al.)

Yokota and Tanimoto¹³ used the Pade approximate method to evaluate an expression for Forster transfer in a fluid media. Gosele et al.¹⁴ pointed out that the equation of Yokota and Tanimoto overestimates the transfer rate in the longer time region and proposed an improved formulation

$$I_D(t) = I_D^0 \exp \left\{ -\frac{t}{\tau_D} - 2B\gamma \left(\frac{t}{\tau_D} \right)^{1/2} \right\} \quad (5)$$

The parameter B is given by

$$B = \left(\frac{1 + 5.47 + 4x^2}{1 + 3.34x} \right)^{3/4} \quad (6)$$

Where

$$x = D\beta^{-1/3}t^{2/3} \quad \beta = R_0^6/\tau_D^0 \quad (7)$$

In our fitting analysis, D and/or R_0 will be floating parameters.

Frequency-Domain Theory and Analysis.

We obtained the time-resolved information from the frequency response of the emission to amplitude-modulated light, which is characterized by the frequency (ω)-dependent values of the phase shift (ϕ_ω) and the extent of demodulation (m_ω). The parameters describing the decay law are compared with the calculated (c) values ($\phi_{c\omega}$ and $m_{c\omega}$). For any decay law these values are given by

$$\phi_{c\omega} = \arctan(N_{c\omega}/D_{c\omega}) \quad (8)$$

$$m_{c\omega} = (N_{c\omega}^2 + D_{c\omega}^2)^{1/2} \quad (9)$$

Where

$$N_{c\omega} = \int_0^\infty I(t)\sin(\omega t) dt / \int_0^\infty I(t) dt \quad (10)$$

$$D_{c\omega} = \int_0^\infty I(t)\cos(\omega t) dt / \int_0^\infty I(t) dt \quad (11)$$

and ω is the modulation frequency in radian/s. The goodness-of-fit is characterized by

$$\chi_R^2 = \frac{1}{\nu} \sum_{\omega} \left(\frac{\phi_{\omega} - \phi_{c\omega}}{\delta\phi} \right)^2 + \frac{1}{\nu} \sum_{\omega} \left(\frac{m_{\omega} - m_{c\omega}}{\delta m} \right)^2 \quad (12)$$

where ν is the number of degrees of freedom and $\delta\phi$ and δm are the experimental uncertainties in ϕ_{ω} and m_{ω} . We used values of 0.2° and 0.005 , respectively, which were found to be appropriate for our instrument and measurement techniques. The parameters were determined by the method of non-linear least squares.^{19,20}

For the $t^{1/2}$ -dependent decays (eqs 2 and 5) we used numerical integration to evaluate the sine and cosine transforms (eqs 10 and 11). In particular, we used an adaptive Newton–Cotes nine-point integration,²¹ with care to match the time range of integral to that of the intensity decay. It is important to use small time increments in the early portion of the decay because of the initial rapid decay due to the transient terms.

Materials and Method

Indole and dansylamide (Aldrich) were purified by HPLC just before the experiment. Values of R_0 were calculated by using eq. 4. The quantum yields were obtained relative to tryptophan in water at 20°C , using a value of 0.13 ,²² with appropriate corrections for the refractive index of water and the solvents (propylene glycol, methanol) and for the optical densities of the solutions. The quantum yields of indole at 20°C were found to be 0.34 and 0.32 in propylene glycol and oxygen-free methanol, respectively. The R_0 values are 24.3 \AA in propylene glycol and 26.1 \AA in methanol. The concentration of indole (donor) was about 10^{-4} M . The absorption spectra of the indole-dansylamide mixtures were the sum of that found for the individual chromophores, suggesting that the donor and acceptors were not interacting under our experimental conditions.

The fluorescence intensity and decay measurements were performed by using front-face geometry. Emission spectra were corrected for the inner filter effect. Frequency-domain measurements were performed by using the instrument described previously.²³ The modulated excitation is provided by the harmonic content of a train of 7-ps pulses from a cavity-dumped rhodamine 6G dye laser, which was frequency doubled to 290 nm . The detector is a microchannel plate photomultiplier (Hamamatsu R1564U). The emission was observed through a 340-nm interference Filter, using magic angle polarizer conditions. For all analyses the uncertainties in the phase ($\delta\phi$) and the modulation (δm) measurements were taken 0.2° and 0.005 , respectively. For the frequency-domain measurements the optical densities of the reference and sample cuvette were matched to equalize the optical path lengths.

Results

Emission spectra of indole in methanol at 20°C in the absence and presence of dansylamide are shown in Figure 1. Fluorescence spectra of indole and dansylamide are well separated. A 340-nm interference filter was used to selectively observe only the indole fluorescence.

Concentrations of dansylamide (acceptor) of 0.012 and 0.005 M in propylene glycol and methanol, respectively, resulted in about 50% quenching of indole fluorescence in each solvent. In the pure solvents without acceptor, indole displays dominantly a single-exponential fluorescence intensity decays with lifetimes 4.23 and 4.09 ns, in propylene glycol and methanol, respectively.

Frequency-domain data for the intensity decay of indole fluorescence in propylene glycol in the presence of 0.012 M dansylamide are shown in Figure 2. In the presence of randomly distributed acceptors, the decay became heterogeneous, compared to that found in the absence of acceptors (not shown), due to a range of D-to-A distances and a range of transfer rates. A single-exponential fit (···) is unacceptable, resulting in $\chi_R^2 = 92.8$. The Forster model (- - -) provides a 22-fold improvement in χ_R^2 and the Gosele et al. model (- - -) gives additional 4-fold lower χ_R^2

Frequency-domain data for the intensity decay of indole in methanol at 20 °C in the presence of 0.005 M dansylamide are shown in Figure 3. The single-exponential fit and the fit to the Forster equation (2) without diffusion, are unacceptable, resulting in $\chi_R^2 = 30.7$ and 95.5, respectively. In contrast, the data points are well matched by fit to the Gosele et al. model, resulting in $\chi_R^2 = 1.8$. The recovered parameters for energy transfer are presented in Table I. It should be noted that the effect of diffusion on energy transfer is to compress the donor-frequency response along the frequency axis (Figure 3), as compared to energy transfer without diffusion. That is, the frequency range from 10 to 80° of phase, or 90 to 10% modulation, is decreased by diffusion.

It is interesting to note that the Forester equation (2) provides a reasonable fit ($\chi_R^2 = 4.1$) in the high viscosity solvent but cannot account for the low viscosity data ($\chi_R^2 = 95.8$, Table I). This is because the effect of diffusion on the form of the intensity decay is to make it appear to be more like a single exponential, i.e., more compressed along the frequency axis in Figures 2 and 3. Consequently, eq 2 cannot account for the data, even though the apparent (recovered) value of R_0 is increased to account for the enhanced extent of energy transfer due to translational diffusion.

The diffusion coefficients recovered from least-squares analysis with the Gosele model (eq 5) were 1.03×10^{-6} and 26.4×10^{-6} cm²/s, in propylene glycol and methanol, respectively. These are reasonable values for molecules like indole and dansylamide at these viscosities, 45 and 0.6 cP for propylene glycol and methanol, respectively. It is of interest to determine the uncertainty in the diffusion coefficients recovered from the data. This was accomplished by questioning the range of diffusion coefficients that are statistically consistent with the data. To provide the worse case analysis, we allowed R_0 to be a variable, even though R_0 is in fact known from the spectral data.

The uncertainties in the diffusion coefficients can be judged by examining the χ_R^2 surfaces (Figure 4, —). These surfaces were calculated by holding the D fixed at the values indicated on the x axis and allowing R_0 to vary so as to minimize χ_R^2 . These surfaces indicate that the diffusion coefficients are well determined from the data, even in the case of low diffusion (10^{-6} cm²/s) and assuming R_0 is unknown. In propylene glycol, diffusion coefficients from

0.60 to $1.3 \times 10^{-6} \text{ cm}^2/\text{s}$ are consistent with the data. The inability of changes in R_0 to compensate for changes in the diffusion coefficient is probably because the shape of the frequency response, and not just its location along the frequency axis, is affected by changes in the diffusion coefficient.

In the case of methanol, the χ_R^2 surface is steeper, and D values can range only from 23.4 to $27.2 \times 10^{-6} \text{ cm}^2/\text{s}$. This relatively greater certainty of D is because of the increased contributions of diffusion to the transfer process in this low viscosity solvent. Of course, the Forster distance R_0 is known from the optical properties of the chromophores. If R_0 is held constant, then the values of D are determined with still greater certainty (Figure 4, ...)

It should be noted that it is possible to interpret the donor decay data in terms of distance distributions and that the effect of diffusion is to displace the apparent distribution toward the donor.²⁴ However, this model does not provide a correct molecular description of the system, and the recovered distributions are only apparent distributions.

Discussion

In the presence of randomly distributed acceptors, the fluorescence decay of the donor becomes heterogeneous due to a range of D-to-A distances and rates of energy transfer. This is seen from the attempt to fit the data in Figure 2 to the single-exponential model. In propylene glycol, where the mean displacement of molecules during the fluorescence lifetime ($\Delta\bar{x}$) is not significant, the difference between fits to Forster or Gosele et al. models is small. In contrast, in methanol (Figure 3) this difference becomes larger and the fit to the Forster model not acceptable. The Forster model provides a poor fit ($\chi_R^2 = 95.8$) and significantly overestimates critical transfer distance ($R_0 = 37.1 \text{ \AA}$). The heterogeneity of the donor decay (given by χ_R^2 obtained for single-exponential fit) in methanol is lower than that in propylene glycol (Table I) but in both solvents single-exponential fits are not acceptable.

In a fluid solutions the mean diffusion distance during the lifetime of the donor is given by

$$\Delta\bar{x} = (2D\bar{\tau}_D)^{1/2} \quad D = kT/6\pi\eta r \quad (13)$$

Diffusion coefficients may be estimated by the Stokes-Einstein relationship (right), where k is the Boltzmann constant, η the viscosity, and r the molecular radius. In the D–A system, D is the sum of the diffusion coefficients of the donor and acceptor ($D = D_A + D_D$). Calculated $\Delta\bar{x}$ values for the indole–dansylamide system (using $r_D = r_A = 4 \text{ \AA}$) are approximately 2 and 20 \AA in propylene glycol and methanol, respectively. These displacements are much smaller than the R_0 value in propylene glycol and comparable to R_0 in MeOH solutions. The present study has shown that even in solutions in which the diffusion lengths are considerably smaller than the critical transfer distance, frequency-domain measurements provide data for a precise analysis of the energy-transfer kinetics in solution (see Figure 4). The estimated values of D (Table I) are reasonable. It should also be noted that the R_0 values recovered from the Gosele et al. model are in surprisingly good agreement with that estimated from eq 4.

The capability of measuring diffusion coefficients from the energy-transfer data can have widespread applications in chemistry and biophysics. In homogeneous solvents, the data may allow comparison of the data with various models for diffusion. In macromolecules and macromolecular assemblies, the data may allow determination of the dynamics of ions around polyelectrolytes, rate of lipid transport within membranes, and comparison with the molecular dynamics between sites on a protein molecule.

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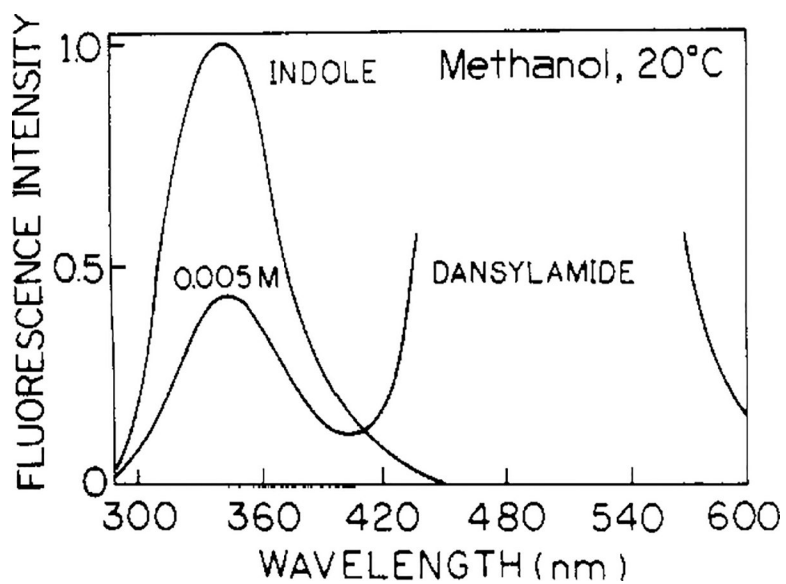


Figure 1. Emission spectra of the donor indole in methanol without and with 0.005 M dansylamide, the acceptor.

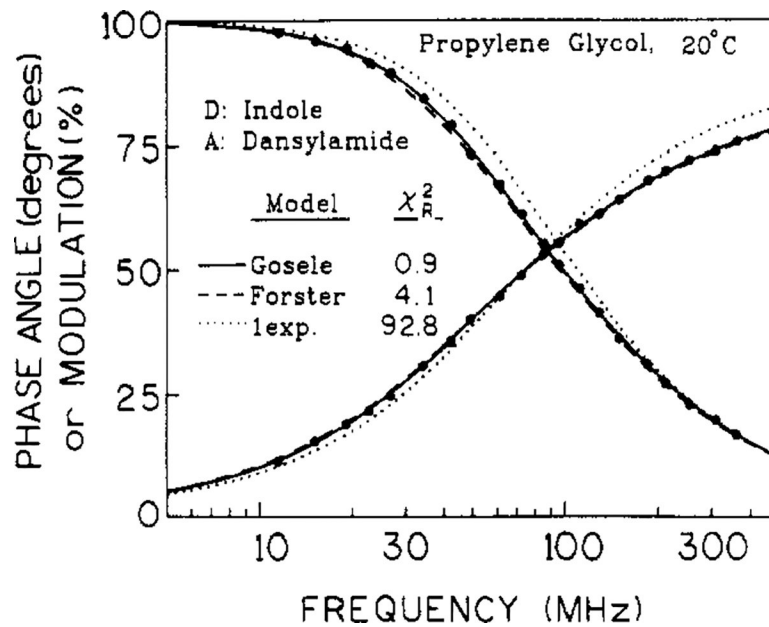


Figure 2. Phase and modulation data for indole fluorescence decay in propylene glycol at 20 °C in the presence of 0.012 M dansylamide. The solid, dashed, and dotted lines are the best fits to the Gosele et al. (eqs 5 and 6), Forster (eq 2), and single-exponential (eq 1) models, respectively.

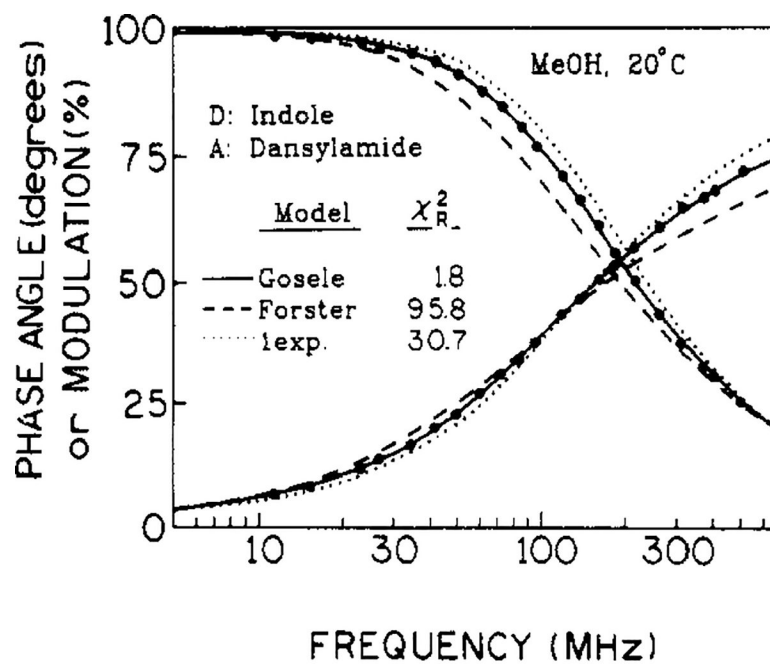


Figure 3. Phase and modulation data for indole fluorescence decay in methanol at 20 °C in the presence of 0.005 M dansylamide. The solid, dashed, and dotted lines are the best fits to the Gosele et al. (eqs 5 and 6), Forster (eq 2), and single-exponential (eq 1) models, respectively.

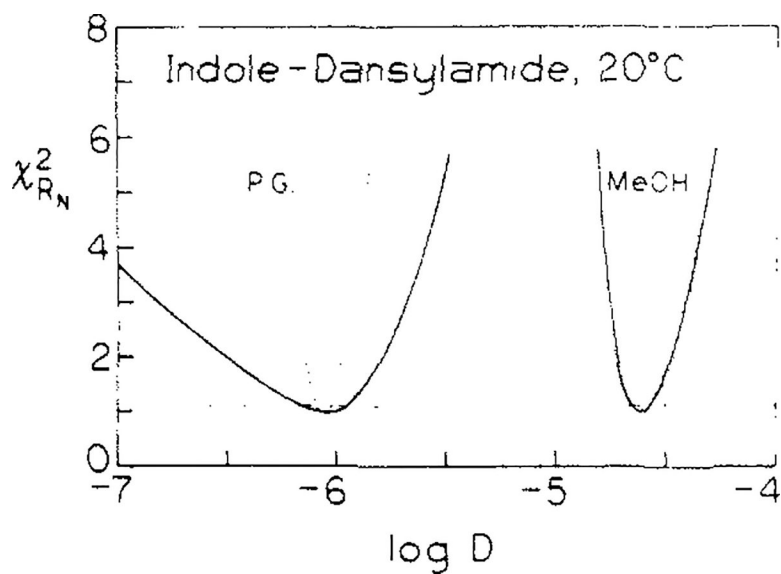


Figure 4. Dependence of χ_R^2 on the diffusion coefficients recovered from Gosele et al. model. The dashed line indicate the highest values of χ_R^2 consistent with random noise in 67% of repetitive measurements. The dotted lines show χ_R^2 dependence on the diffusion coefficients if R_0 are kept constant in analysis.

TABLE I:

Indole Decay Times and D-to-A Diffusion Coefficients in Propylene Glycol and Methanol at 20 °C

solvent	[A]	eq no.	τ_D , ns	R_0 , Å	$10^6 D$, cm ² /s	χ_R^2	
propylene glycol	0	1	4.23			1.4	
		12 mM	1	2.57			92.8
	12 mM	2	$\langle 4.23 \rangle$	24.9			4.1
		2	$\langle 4.23 \rangle$	$\langle 24.3 \rangle^a$			10.6
		5	$\langle 4.23 \rangle$	23.9	1.03		0.9
		5	$\langle 4.23 \rangle$	$\langle 24.3 \rangle$	0.62		1.3
methanol	0	1	4.09			2.4	
		5 mM	1	2.03			30.7
	5 mM	2	$\langle 4.09 \rangle$	37.1			95.8
		2	$\langle 4.09 \rangle$	$\langle 26.1 \rangle$			1152.1
		5	$\langle 4.09 \rangle$	27.8	26.4		1.8
		5	$\langle 4.09 \rangle$	$\langle 26.1 \rangle$	34.0		3.1

^a $\langle \rangle$ indicates that the parameter was held fixed during the analysis.