

Fluorescence Anisotropies of 4-Dimethylamino- ω -diphenylphosphinyl-*trans*-Styrene in Isotropic Media in the Case of One- and Two-Photon Excitation

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Dedicated to Professor J. R. Lakowicz on the occasion of his 45th birthday

Fluorescence emission anisotropies, r , and mean lifetimes of 4-dimethylamino- ω -diphenylphosphinyl-*trans*-styrene (DDPS) were measured in *n*-butanol at different temperatures from 20 to 80°C for one- and two-photon excitation. When increasing the temperature, the lifetime decreased from 32 to 12 ps, the r values, however, remained constant (0.315 and 0.445 for one- and two-photon excitation, respectively) and were found to be temperature independent. For DDPS in glycerol at –5°C, where rotational diffusion does not occur during the excited state lifetime, the limiting emission anisotropies were $r_0 = 0.382$ and $r_0 = 0.545$ for one- and two-photon excitation, respectively, very little differing from the fundamental emission anisotropies $r_f = 2/5$ and $r_f = 4/7$. The small differences $r_f - r_0$ can be due to vibrations performed after excitation by the DDPS molecule as a result of the *initial shock*.

1. Introduction

Theories of fundamental emission anisotropy (polarization) of photoluminescence of solutions in the case of one-photon excitation were long ago put forward [1–5]. The theory of two-photon excitation, worked out by Pheofilov [6], assumes the existence of intermediate states of excitation. The excitation of the emitting states can be accomplished either by a stepwise absorption of two photons or by the cumulation of the energies of two excited particles by one of them due to a cooperative process [6]. General formulas were also derived by Sato et al. [7] for the emission anisotropy of the S_m – fluorescence emission ($m \geq 2$) of a sample excited by a sequential two-photon excitation process ($S_n \leftarrow S_1 \leftarrow S_0$, $n \geq 2$) with plane-polarized pulsed light. When an ensemble of luminescent molecules (LM) becomes doubly polarization-photo-selected, the fundamental emission anisotropy, r_f , could fall outside the usual limits of $-1/5$ and $+2/5$. The extreme limits for r_f under these conditions were extended to cover the range from $-2/7$ to $4/7$ [6–8].

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Very few experimental papers were devoted to the fundamental emission anisotropy for two-photon excitation with linearly polarized light. In recent papers of Lakowicz et al. [9, 10] the long-lived 1,6-diphenylhexatriene and 2,5-diphenyloxazole molecules (nanoseconds) were examined.

It seemed worthwhile to investigate a molecule displaying of a short lifetime (picoseconds). To this end we have selected the short-lived molecule 4-dimethylamino- ω -diphenylphosphinyl-*trans*-styrene (DDPS), for which the electric dipole moments in the ground- and first excited-states [11], the absorption transition moment directions [12] and the deactivation rate constants of fluorescence and radiationless processes [13] are known. The high fluorescence anisotropy observed in low-viscous *n*-paraffins is due to strong fluorescence quenching resulting in a considerable shortening of the lifetime of this molecule.

The aim of this work is to investigate the fluorescence emission anisotropy of DDPS using one- and two-photon excitation.

2. Fundamental Emission Anisotropy

Fundamental emission anisotropy, i.e. maximum value of the fluorescence anisotropy, r_f , can be ob-

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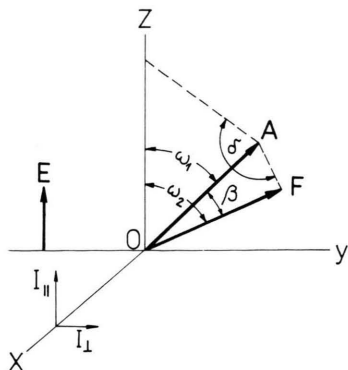


Fig. 1. Geometry of the system. The exciting light E is polarized in the Z direction. The fluorescence intensity is observed along X . Vectors A and F represent the directions of the absorption- and fluorescence transition moments. δ is the angle between the planes formed by the (OZ, A) and (A, F) directions. The position of the angles ω_1 , ω_2 , and β is evident from the figure.

Table 1. Fundamental emission anisotropies, r_f , for different values between angular displacement of the absorption and emission dipoles, β , for three kinds of excitation.

β (deg)	r_f		
	Excitation		
	one-photon	two-photon	three-photon
0	2/5	4/7	2/3
45	1/10	1/7	1/6
90	-1/5	-2/7	-1/3

served if no depolarizing factors are present. This is the case when the solution is rigid and the concentration of luminescent molecules (LM) so low that no excitation energy migration takes place. The emission anisotropy is defined by [14]

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} = \frac{3}{2} \frac{I_{\parallel}}{I} - \frac{1}{2}, \quad (1)$$

where $I = I_{\parallel} + 2I_{\perp}$ is the total intensity of the emission I_{\parallel} and I_{\perp} are the intensity components parallel and perpendicular to the direction of the electric vector, E , of the excitation light, respectively, and E is parallel to the Z -axis of the reference system (Figure 1). According to Fig. 1, the intensity component I_{\parallel} is time-independent and given by

$$I_{\parallel} = I \langle \cos^2 \omega_2 \rangle, \quad (2)$$

where the angles ω_1 , ω_2 , β and δ satisfy the expression [15]

$$\cos \omega_2 = \cos \omega_1 \cos \beta + \sin \omega_1 \sin \beta \cos \delta. \quad (3)$$

In an isotropic solution, all orientations of the absorption transition dipole, A , and the emission transition dipole, F , determined by angles ω_1 , ω_2 , and δ , are equally probable.

For a given absorption oscillator direction ω_1 , upon squaring (3) and averaging over the azimuthal angle δ ($\langle \cos \delta \rangle = 0$, $\langle \cos^2 \delta \rangle = 1/2$) we obtain the mean value of the emission oscillator [16].

$$\langle \cos^2 \omega_2 \rangle = \left(\frac{3}{2} \cos^2 \omega_1 - \frac{1}{2} \right) \left(\frac{3}{2} \cos^2 \beta - \frac{1}{3} \right) + \frac{1}{3}, \quad (4)$$

where β is constant for a given transition in the LM.

The substitution of the averaged equation (4) into (1) with (2) yields

$$r(\beta, \omega_1) = \left(\frac{3}{2} \langle \cos^2 \omega_1 \rangle - \frac{1}{2} \right) \left(\frac{3}{2} \cos^2 \beta - \frac{1}{2} \right), \quad (5)$$

where

$$\langle \cos^2 \omega_1 \rangle = \frac{\int_0^{\pi/2} \cos^2 \omega_1 f(\omega_1) d\omega_1}{\int_0^{\pi/2} f(\omega_1) d\omega_1} \quad (6)$$

and $f(\omega_1) d\omega_1$ is the direction distribution in the excited state due to the polarized absorption. In general we assume that n photons simultaneously interact with and are absorbed by the LM. We further assume that the n -th photon interacts only with the ensemble of the LMs which were preselected by the first, second and up to $n-1$ -th photons. The orientation distribution in the excited state is in this case given by

$$f(\omega_1) = \cos^{2n} \omega_1 \cdot \sin \omega_1. \quad (7)$$

Using (6) and (7) one finds

$$\langle \cos^2 \omega_1 \rangle = \frac{2n+1}{2n+3}, \quad (8)$$

and (5) becomes

$$r_f(\beta) = \frac{2n}{2n+3} \left(\frac{3}{2} \cos^2 \beta - \frac{1}{2} \right). \quad (9)$$

Thus, for one-photon absorption ($n=1$) the well-known Perrin Equation [2] is obtained while for two-photon absorption ($n=2$), one obtains the equation found in [6, 7, 9, 10].

The fundamental values of r_f for one-, two- and three-photon excitation at different values of β are summarized in Table 1. For $\beta = 54.736^\circ$, $r_f(\beta) = 0$ (cf. (9)) irrespective of the kind of excitation. The pho-

toselection by two- or three-photon absorption can excite a population of the LMs with a better orientation in the excited state than that for one-photon absorption. In accordance with (9), one can expect a 100 per cent orientation of the LMs in the excited state, when $n \rightarrow \infty$, which practically cannot be achieved. A high orientation in the excited state can be attained by exciting a partially oriented system, such as stretched polymer films [16], using the two-photon absorption techniques.

3. Experimental

One- and two-photon experiments were performed using a 10-GHz frequency-domain fluorometer described in detail in [17]. Single-photon excitation was accomplished with the frequency-doubled output of a cavity-dumped pyridine 2 dye laser at 365 nm. For two photon excitation the fundamental output of 730 nm was used, which was focused within the sample using a 5 cm focal length lens. Identical single-photon results were obtained with and without the lens. For the two-photon experiments we used $1.0 \text{ cm} \times 0.5 \text{ cm}$ cuvettes, with the long axis aligned with the incident light and with the focal point positioned about 0.5 cm from the surface facing the incident light. The position of the cuvette was adjusted so that the excitation laser beam crossed the solution near the observation window. For the one-photon experiments we used $0.5 \text{ cm} \times 0.5 \text{ cm}$ cuvettes with excitation and emission near a corner positioned at the center of a $1.0 \text{ cm} \times 1.0 \text{ cm}$ cuvette holder. Emission spectra were obtained using a monochromator with a bandwidth of 10 nm.

The lifetimes and emission anisotropies were measured without the monochromator using Corning 4-96 and 3-75 glass filters. The signal from the solvent alone was lower than 0.3% for both one- and two-photon excitation.

The concentration of DDPS¹ was 10^{-4} M in all the solutions, except for the emission spectra measurements where it was $2 \cdot 10^{-4} \text{ M}$. DDPS was from Dr. D. Gloyna (Humboldt-Universität zu Berlin). All solvents were HPLC or spectroscopic grade.

Figure 2 shows the dependence of the observed emission intensity on the average incident power. The

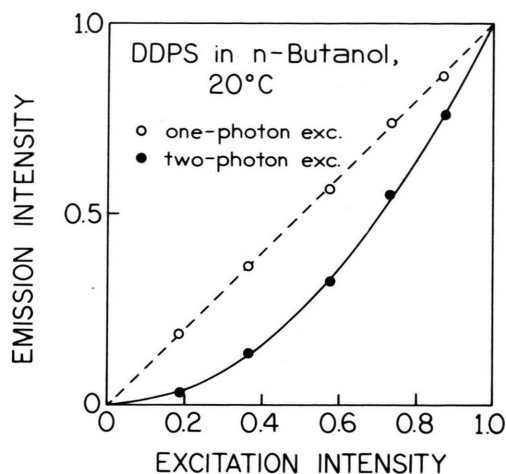


Fig. 2. The dependence of the emission intensity of DDPS in n-butanol at 20°C on the excitation intensity for one- and two-photon excitation.

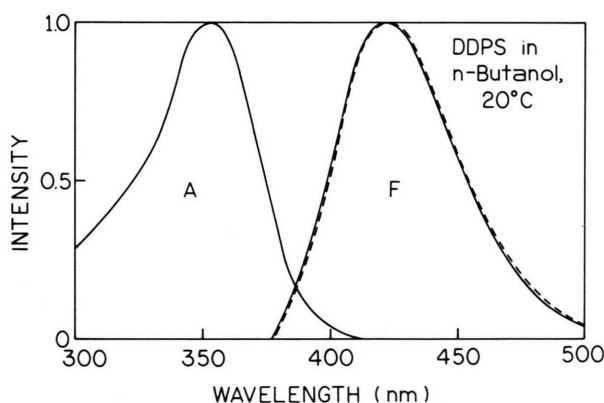


Fig. 3. Normalized absorption and fluorescence of DDPS in n-butanol at 20°C with one (—), $\lambda_{\text{exc}} = 365 \text{ nm}$, and two-photon (---), $\lambda_{\text{exc}} = 730 \text{ nm}$, excitation.

linear and quadratic dependences observed for 365 and 730 nm excitation, respectively, demonstrate for DDPS in n-butanol at 20°C that the long wavelength-induced emission signal is due to a biphotonic process.

4. Results and Discussion

The absorption and fluorescence spectra of DDPS in n-butanol at 20°C are shown in Figure 3. The same fluorescence spectra were obtained using one-photon (solid line) or two-photon (dashed line) excitation. Table 2 summarizes the values of the phase- (τ_p) and modulation- (τ_m) lifetimes measured for DDPS in

¹ In our previous papers [11–13] this molecule was denoted as 2a.

Table 2. Phase, τ_p , and modulation, τ_m , lifetimes of DDPS in n-butanol at different temperatures.

t (°C)	Frequency (MHz)	Phase (degree)	τ_p (ps)	Modu- lation	τ_m (ps)
20	4087.2	39.6	32	0.775	32
25	4254.2	39.1	30	0.780	30
30	4975.3	40.1	27	0.768	26
35	5400.3	38.9	24	0.777	24
40	5908.8	39.7	23	0.771	22
45	6493.3	39.2	20	0.780	19
50	7677.3	38.8	17	0.789	16
60	8466.6	37.1	14	0.802	14
70	9438.2	37.9	13	0.800	13
80	9438.2	35.3	12	0.822	12

Excitation wavelength: $\lambda_{\text{exc}} = 365$ nm, observation wavelength: 390–600 nm.

Table 3. Steady-state anisotropy of the DDPS fluorescence in n-butanol at different temperatures for one- and two-photon excitation.

t (°C)	η^a (10^{-3} Pa · s)	Emission anisotropy		$\frac{r_2}{r_1}$
		r_1 one-photon excitation $\lambda_{\text{exc}} = 365$ nm	r_2 two-photon excitation $\lambda_{\text{exc}} = 730$ nm	
20	2.948	0.311	0.438	1.408
25		0.312	0.443	1.420
30	2.300	0.308	0.441	1.432
35		0.312	0.444	1.423
40	1.782	0.309	0.442	1.430
45		0.311	0.447	1.437
50	1.411	0.313	0.446	1.425
60		0.309	0.445	1.440
70	0.930	0.318	0.449	1.412
80		0.318	0.452	1.421

^a From Handbook of Chemistry and Physics, CRC, Boca Raton, FL, 1967.

n-butanol at different temperatures from 20 to 80 °C. With growing temperature the lifetime decreased from 32 to 12 ps. In general we found $\tau_p = \tau_m = \tau$, indicating the homogeneous intensity decays. For two-photon excitation the τ values are the same as for one-photon excitation. Short lifetimes τ result from a strong fluorescence quenching due to radiationless *trans-cis*-deactivation. This effect strongly depends on the temperature, since with growing temperature the solvent viscosity decreased.

However, the fluorescence emission anisotropy for one- and two-photon excitation of DDPS in n-butanol remains constant and is temperature-independent (Fig. 4, Table 3). The high values of the emission anisotropies of DDPS in n-butanol, 0.315 and 0.445

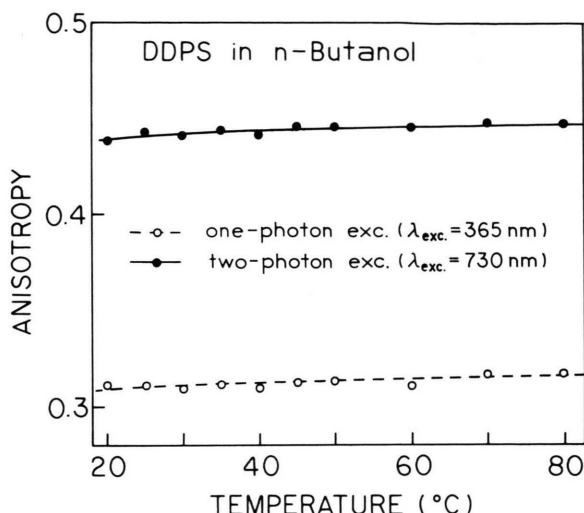


Fig. 4. Steady-state fluorescence anisotropy of DDPS in n-butanol vs. temperature, measured with one- and two-photon excitation.

for one- and two-photon excitation, respectively, are due to a small contribution of the rotational motions to depolarization because the lifetime of the LM is very short. The ratio given in Table 3 is close to the theoretical (see Table 1) value $r_{f2}/r_{f1} = \frac{4/7}{2/5} = \frac{10}{7} = 1.4286$. This means that the absorption transition dipole moments for one- and two-photon excitation are parallel.

The steady state limiting emission anisotropy of DDPS was also examined in glycerol at -5 °C, where the rotational diffusion does not occur during the lifetime of the state. For $\lambda_{\text{exc}} > 355$ nm the limiting emission anisotropy, r_0 , for one-photon excitation is 0.382, being independent of the wavelength. The observed limiting, value of r_0 for two-photon excitation is 0.545, being constant in the whole interval of the excitation wavelengths (Figure 5). The experimental ratio of $r_{02}/r_{01} = 0.545/0.382 = 1.4267$ is in a good agreement with the theoretical ratio $r_{f2}/r_{f1} = 1.4286$. The horizontal arrows in Fig. 5 indicate the theoretical maximum values of the fundamental emission anisotropy, r_f , for collinear absorption and emission transition dipole moments, which are $2/5$ and $4/7$ for one- and two-photon excitation, respectively. Below the exciting wavelength 355 nm, the experimental value of r_{02}/r_{01} is greater than $r_{f2}/r_{f1} = 1.4286$, which should not take place. This can be due to the fact that for $\lambda_{\text{exc}} < 355$ nm the long-wave absorption band (1L_a) partly overlaps the short-wave band (1L_b). The mea-

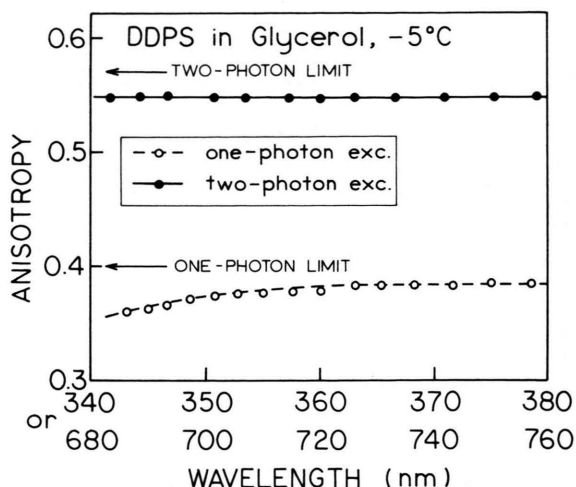


Fig. 5. Steady-state fluorescence anisotropy spectrum of DDPS in glycerol at -5°C . The arrows indicate maximal values of r_f , $2/5$ for one-photon and $4/7$ for two-photon excitation.

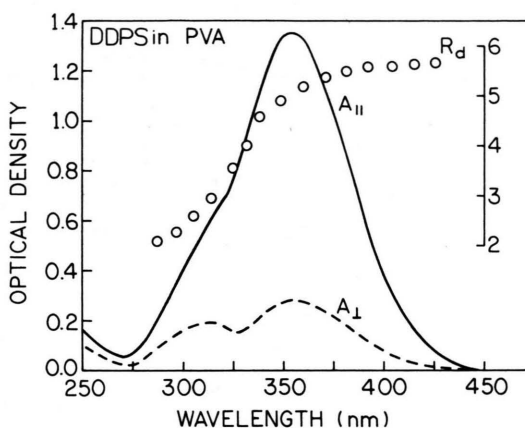


Fig. 6. Optical density components (absorbances) A_{\parallel} and A_{\perp} and the dichroic ratio $R_d = A_{\parallel}/A_{\perp}$ (A_{\parallel} and A_{\perp} are the components of the absorbance $A = \epsilon \cdot C \cdot l$, where ϵ is the molar absorption coefficient in litres per mol \cdot cm, C the concentration in mol/l and l the length in cm) as functions of the wavelength for DDPS in a stretched PVA film.

measurements of the absorbance components A_{\parallel} and A_{\perp} (parallel and perpendicular to the stretching direction (the Z-axis) of a polymer film) and the dichroic ratio $R_d = A_{\parallel}/A_{\perp}$ show that above $\lambda_{\text{exc}} = 355$ nm the value of R_d remains constant (Figure 6). In [12] we have shown that the transition moment of the more intensive 1L_a -band is directed along the long molecular axis, whereas that of the short wave 1L_b -band of DDPS lies at an angle $\varphi = 38^{\circ}$ relative to the 1L_a -band transition moment. The small difference between the fundamental and the limiting anisotropies, $r_f - r_0$, measured with the steady-state technique, can be due to vibrations performed by an LM after the excitation as a result of the *initial shock* [18, 19]. For complex molecules, the deformation vibrations spoil the molecular configuration and the link between the transition dipole moments and the molecules loses its rigidity. The observations of the time dependence of the emis-

sion anisotropy, $r(t)$, for all-*trans*-1,6-diphenylhexa-1,3,5-triene at time $t = 0$, yielded by extrapolation the maximum value $r_f = 2/5$ for one-photon excitation [20]. Later, Fleming et al. [21–24] reported that the theoretical value $r_f = 2/5$ at a zero time was observed for some dyes.

Concluding, it seems worthwhile mentioning that the lack of the temperature dependence of the emission anisotropy of DDPS in *n*-butanol makes this LM a good standard of the emission anisotropy for one- and two-photon excitation.

Acknowledgements

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