Concentration Depolarization of the Fluorescence and Phosphorescence of Acridine Orange Cations

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(Received July 15, 1975)

Concentration depolarization of fluorescence and phosphorescence has been studied for Acridine Orange cations in PVA films. Depolarization can be observed with concentrations above 10^{-6} M. For concentrations ranging from 10^{-6} M to 10^{-4} M both depolarization of fluorescence and of phosphorescence obey the Weber equation. The results indicate that singlet-singlet energy transfer is responsible for depolarization of fluorescence, as well as of phosphorescence. Above 10^{-4} M, the effect of the exchange interaction cannot be ignored due to the formation of higher aggregates.

Acridine dyes have a common notable characteristic of readily aggregating. Acridine Orange, a typical acridine dye undergoes many interesting phenomena that remain yet to be studied, although a number of investigations have dealt with the concentration dependence of absorption spectra and the equilibrium between monomers and aggregates.[1,2] Schmidt[3] and Chambers *et al.*[4] have studied the behavior of the triplet state as a function of the dye concentration. It was found that dye molecules aggregate to form a complex in which the molecules are orientated in a fixed direction.

It is the purpose of this paper to demonstrate the favorable energy transfer process in a system of dye molecules embedded in a polymer matrix by means of depolarization of the fluorescence and phosphorescence as a function of the dye concentration.

Experimental

The techniques used to prepare poly vinyl alcohol (PVA) films are similar to those employed by Tanizaki *et al.*[5] PVA solutions were prepared by mixing 20 g of PVA powder with 150 ml of redistilled water, followed by vigorous stirring in a boiling water-bath for 2 h. To this solutions was added 50 ml of a hydrochloric acidified ethanol solution of chromatographically purified Acridine Orange. After careful mixing, the solutions were poured over a glass plate (20× 20 cm) and were spread out to a uniform thickness. The solutions were dried in a dust-free box for a few days at room temperature. The hard films obtained have a typical thickness of about 0.2 mm.

Absorption spectra were measured with a Shimadzu MPS-50L spectrophotometer. A 150 W xenon lamp combined with various interference filters was used to excite the samples. A Nikon G-250 grating monochromator was used for the emission spectra. The exciting light beam falls on the film surface at an angle of 60° and the fluorescence was observed in the direction at right angles with the exciting light beam. For the measurement of polarization, two rotatable film polarizers ("Polaroid" HN-22) were used. A quartz depolarizer was inserted in front of the photomultiplior (HTV-R636). The transmittance of the monochromater for the polarized light was independent of the polarization of the incident light for all wavelengths. To obtain phosphorescence spectra, a cylindrical sector was rotated around the cylindrical Dewar flask in which the films were immersed in liquid nitrogen. Absorption spectra were corrected for the transmittance of the neat PVA film without the dye. The degree of polarization P was corrected for the self-polarization of the apparatus.[6] P was determined by $P=(I_{\parallel}-I_{\perp}f)/(I_{\parallel}+I_{\perp}f)$, where f[7] is a correction factor. $I_{\parallel}$ and $I_{\perp}$ are the observed intensities of vertical and horizontal components of the fluorescence with respect to the vertically polarized exciting light.

Results and Discussion

Figure 1 shows the absorption and fluorescence spectra of Acridine Orange cations in PVA films at 77 K. The room temperature absorption spectra in PVA are essentially identical with those in ethanol solutions. At 77 K, however, there is a remarkable difference between the absorption spectra in ethanol solutions and those in PVA films due to the aggregation of dyes in liquid solutions. The first absorption band with the peak at 495 nm is attributed to the $S_1 \leftarrow S_0$ transition of the dye monomers. The second band around 465 nm appears as a shoulder and becomes more prominent relative to the first band, as the concentration of dye cations increases. It is interesting to note that Acridine Orange cations form dimers in PVA films. In studies by Tanizaki *et al.*,[5] the formation of dimers in PVA films was not observed.

To account for the effect of concentration on the absorption spectra in PVA films, the monomer-dimer equilibrium of dye molecules is assumed and an ap-

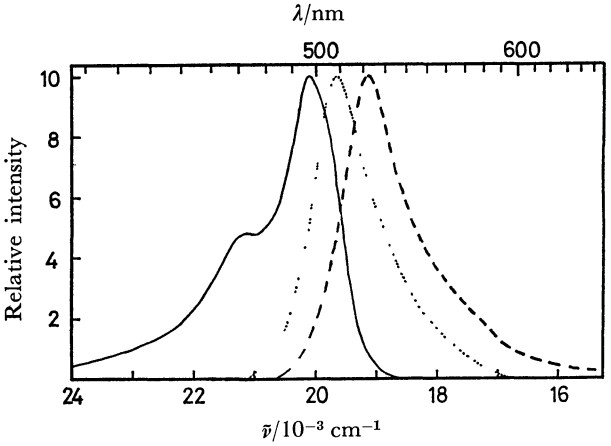

Fig. 1. Absorption and fluorescence spectra of Acridine Orange cations in PVA at 77 K. Absorption; ——: 2.64×10^{-5} M. Fluorescence; ····: 1.32×10^{-6} M. ----: 2.64×10^{-5} M.

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propriate K value, $1.3\times10^4\ M^{-1}$, is obtained for concentrations ranging from 10^{-4} to 10^{-6} M. In the case of ethanol solutions at 10^{-4}—10^{-6} M, a value of $1.4\times10^4\ M^{-1}$ is obtained for the association constant. The equilibrium constant, $1.3\times10^4\ M^{-1}$, can be attributed to that in the film before it hardens and not to that in the hard film. Spectra derived from this dimerization equilibrium display a shoulder at 470 nm in agreement with the results of Zanker.[1] The 495 nm peak and the 470 nm shoulder have been assigned by Sheppard[8] to the 0–0 and 0–1 vibrational transitions, respectively. The association constant in PVA films is independent of the temperature. Therefore, the dimerization equilibrium is the same as that at room temperature.

The fluorescence spectra show good mirror image relationships with the absorption spectra. In all the experiments, no excitation spectra indicate that the samples contain any free base of the dye. The spectra in PVA are rather diffuse, compared with those of ethanol solutions, as well as the absorption spectra. The change of the fluorescence profile with increasing dye concentration is remarkable on the longer wavelength side of the broad emission band, this change appearing to be due to dimer formation. The peak of the broad emission band at 510 nm shifts toward the red by 490 cm^{-1} for a change in the dye concentration from 10^{-6} to 10^{-3} M. The red shift of the fluorescence peak cannot be interpreted in terms of reabsorption, which is negligible in the present optical system.

In ethanol glasses at 77 K, the fluorescence shows three sharp peaks at 510, 535 and 570 nm. In combination with the concentration change of the fluorescence spectra, these bands are assigned as follows. The first is the monomer band and the second that of the dimer. The third band is attributed to the oligomer band because of the appearance of the same peak for the polycrystalline dye films on a glass plate obtained by solvent evaporation from dye cation solutions. Compared with these complex features of solution fluorescence at 77 K, PVA matrix fluorescence spectra appear to be rather simple. The first sharp band is attributed to the monomer band and the second is a weak, but detectable, broad dimer band mixed with that of the oligomer.

In the highest concentration range, the broad intense phosphorescence at 655 nm is observed with a swift decay rate at room temperature. It is expected that the phosphorescence states are established in PVA films because of the slow quenching reaction of the phosphorescence by oxygen. As shown in Fig. 2, phosphorescence spectra in PVA at 77 K give the main peak at 610 nm and the second peak at 655 nm. The lifetime of the phosphorescence is 2.0 s at 10^{-3} M in PVA films at 77 K. If the dimer band is excited at 465 nm, the intensity of the second phosphorescence band is stronger than the first. In addition to the consistency with the excitation spectra, the intensity of the second phosphorescence band becomes stronger as the dye concentration is increased. On the basis of these spectral changes, the second phosphorescence band is attributed to the triplet state

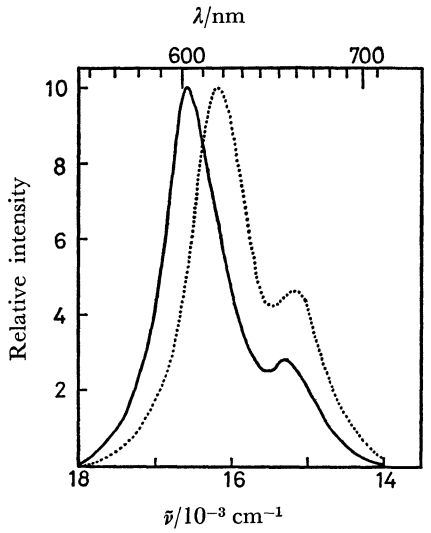

Fig. 2. Phosphorescence spectra of Acridine Orange cations in PVA at 77 K.
——: 7.92×10^{-5} M. ····: 6.12×10^{-4} M.

of the dimer.[4]

However, the energy gap between the triplet states of the monomer and dimer appears to be much larger than the generally accepted values for dimers. The peaks of phosphorescence spectra show a red shift of 600 cm^{-1}, which is larger than that for fluorescence, when the dye concentration increases above 6×10^{-4} M. The energy gap may be interpreted on the basis of the lowering of the triplet state by the formation of complicated aggregates rather than simple dimers.

The second phosphorescence band is shifted makedly toward the red, and is accompanied by an extension of the excitation spectra into the red at concentrations greater than 6×10^{-4} M. Consistent with the present observation, Schmidt[3] and Yamaoka[9] *et al.* have reported the appearance of a broad absorption band with a peak around 570 nm at a high dye concentration above 6×10^{-4} M.

Polarization measurements were made for their fluorescence, phosphorescence and excitation spectra

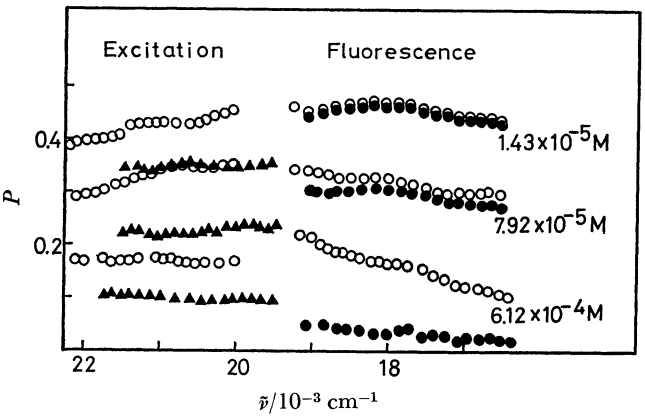

Fig. 3. Polarization degrees of fluorescence and excitation spectra. Fluorescence; ○: excited at 495 nm, ●: excited at 465 nm. Excitation; ○: observed at 525 nm, ▲: observed at 655 nm.

in PVA films. In Fig. 3, the variation of the degree of polarization of excitation as a function of the fluorescence observed at 525 nm is shown. At medium concentrations of 10^{-4}—10^{-6} M, there is a slight, but detectable, difference between the degree of polarization of the monomer at 495 nm and that of the dimer band at 465 nm. The excitation of the dimer band results in a higher depolarization than that of the monomer band. In Fig. 3, fluorescence also exhibits similar differences in the degree of polarization of the monomer, dimer and oligomer bands. Thus, it seems probable that each electronic transition exhibits a certain degree of polarization, but that the separation of each fluorescence band is poor due to the broad emission bands in polymer films. At low concentrations, below 10^{-6} M, it is difficult to obtain a degree of polarization with good reproducibility. At these concentrations, however, the degree of polarization remains constant independent of the fluorescence spectral range. At high concentrations, above 4×10^{-4} M, the structure of the fluorescence spectra disappears in parallel with the fluorescence depolarization.

The fluorescence depolarization indicates energy transfer in PVA films which inhibit molecular diffusion. The plots of $1/P$ *versus* concentration are linear regardless of the exciting or observed wavelengths over the concentration range of 10^{-4}—10^{-6} M, where P is the degree of polarization. Fig. 4 serves to illustrate the linear relation for the case of the monomer-band excitation and observation. For concentrations above 10^{-4} M, $1/P$ at a given concentration is remarkably dependent of the excitation wavelength and the fluorescence wavelength.

The limiting polarization P_0, the degree of polarization in the absence of energy transfer is obtained by extrapolation to zero concentration. Weber[10] derived an equation describing the intermolecular energy transfer as revealed by fluorescence depolarization measurements as follows:

$$\frac{1}{P}-\frac{1}{3}=\left(\frac{1}{P_0}-\frac{1}{3}\right)\left(1+\frac{4\pi NR^6\times 10^{-3}}{15(2a)^3}C\right) \qquad (1)$$

in which N is Avogadro's number, C is the concentration in M, R is the critical distance for two oscillators and a is the effective molecular radius. Assuming a value for $2a$ of 10 Å and utilizing the slope of the straight line in Fig. 4, R was calculated under several conditions and is listed in Table 1.

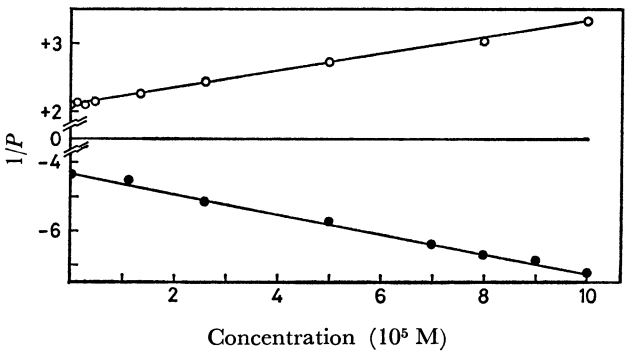

Fig. 4. $1/P$ plots *versus* concentration for fluorescence and phosphorescence spectra. ○; Fluorescence, excited at 495 nm, observed at 525 nm. ●; Phosphorescence, excited at 495 nm, observed at 610 nm.

TABLE 1. CRITICAL DISTANCES OBTAINED FROM FLUORESCENCE DEPOLARIZATION ($10^{-6}-10^{-4}$ M)

Excitation spectra		
Fluorescence	Excitation	
	at 495 nm	465 nm
535 nm	47.5	50.4
570 nm	49.5	52.0
Fluorescence spectra		
Exciting wavelength	Fluorescence	
	at 510—535 nm	570 nm
465	48.3	48.9
500	47.7	48.3

Furthermore, the degree of polarization of the phosphorescence is measured as a function of the concentration at 77 K for two peaks located at 610 and 655 nm. Room temperature phosphorescence is too weak to measure the degree of polarization accurately. The phosphorescence spectra are negatively polarized in contrast with the fluorescence spectra. The degree of polarization around 610 nm is higher than that around 655 nm. The concentration depolarization of phosphorescence is measured for two excitations at 465 and 495 nm while monitoring the phosphorescence peaks. The 465-nm excitation gives a higher depolarization than that at 495 nm.

The results give the relation between the phosphorescence depolarization and the dye concentration as in the case of fluorescence depolarization. A linear relationship between $1/P$ and the concentration is obtained in the concentration range of 10^{-4}—10^{-6} M. Selecting the phosphorescence observed at 610 nm and excited at 495 nm, a linear relation is shown in Fig. 4, for the degree of phosphorescence polarization. Using the slope of Fig. 4, the value of the critical distance R=47.3 Å is obtained from Eq. 1 for the phosphorescence depolarization on the assumption that it is possible to extend the previously mentioned treatment to the case of phosphorescence. This result indicates that the phosphorescence depolarization is also governed by singlet-singlet energy transfer. Above 10^{-4} M, somewhat more complicated features, such as triplet-triplet energy transfer, should be taken into consideration to account for the concentration depolarization of phosphorescence.

The concentration depolarization of phosphorescence has been studied by Chaudhuri and El-Sayed[11] and Iwao *et al.*[12] using the aromatic compounds such as naphthalene and its halogenated or deuterated derivatives. Concerning the primary process of phosphorescence depolarization, the present observations are in agreement with the conclusion obtained by Iwao *et al.*

One of the present authors (T. K.) is greatly indebted to Professor Y. Tanizaki for helpful discussions and suggestions.

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