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Solvent-dependent photophysical properties of aminophenoxazone dyes as optical probes

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Abstract

Photophysical properties of three aminophenoxazone (APO) dyes were investigated in many kinds of pure and mixed solvents to assess the possibility for application as an optical probe. The absorption band underwent a significant red shift which was accompanied by a slight change in the spectral profile when increasing the polarity of the solvent. The wavenumber showed a linear correlation with the solvent polarity/polarizability π^* in aprotic solvents, indicating that the APO dyes are only proton-accepting. The results in dioxane–water deviated downward from this correlation, suggesting that the hydrogen-bonding interaction with water molecules enhances the red shift. The fluorescence spectrum also exhibited a marked red shift and a change in spectral profile when increasing the polarity of the solvent. The relationship between the wavenumber and π^* was nonlinear in aprotic solvents and dioxane–water. A decrease in the rate constant of nonradiative fluorescence decay (k_{nr}) abruptly changed to increase when π^* increased in dioxane–water. This is presumably because two kinds of nonradiative decay process are involved. The alteration of the chemical structure, i.e., the exchange of ethyl to methyl of the substituent at the 7-amino group and the introduction of a methyl group to the 1 position of the phenoxazone ring caused an increase in the Stokes shift and moved the point of the abrupt change in k_{nr} to a larger π^* value. © 1997 Elsevier Science S.A.

Keywords: Optical probe; Absorption; Fluorescence quantum yield; Fluorescence lifetime; Solvatochromism; Hydrogen-bonding interaction; Nonradiative decay process

1. Introduction

Spectroscopic changes observed when dyes are located in various media have been used to evaluate the polarity and viscosity of the microenvironment. Such dyes are obviously most useful as optical probes when small perturbations cause large changes in spectroscopic properties. For this reason, molecules possessing electron donor and acceptor groups attached to the aromatic ring have been examined for application as an optical probe. These structural features will lead to an intramolecular charge transfer upon excitation that will result in a large increase of the dipole moment, and hence will result in a marked solvatochromic effect and a large Stokes shift.

Fluorescence probes such as N-arylaminonaphthalenesulfonates [1,2] and 6-acyl-2-dimethylaminonaphthalenes [3] have since been developed according to the above strategy. However, these molecules are limited by their ultraviolet or near ultraviolet excitation maxima. The long wavelengths of excitation and emission of optical probes will minimize interference from chromophores of target molecules or from other probes, will reduce radiation damage to the target molecules, and so on [4,5]. The realization of these desirable characteristics requires the development and characterization of optical probes with useful excitation and emission responses in this spectral region.

Aminophenoxazone (APO) is a dye family which possesses electron donor (amino group) and electron acceptor (carbonyl group) moieties. Their absorption and fluorescence maxima are expected to exist at sufficiently long wavelengths, which will make the dyes especially promising probes. Some physicochemical characteristics of APO dyes have been reported, e.g., acid–base equilibrium [6], absorption and fluorescence properties [7], fluorescence quantum yield and lifetime [8], nonlinear optical properties [9]. Recently the fluorescence behavior of an APO dye, 1-pentyl-7-dimethylamino-3H-phenoxazin-3-one, has been examined in solutions and some heterogeneous media [5].

In this paper, the solvent effect on the absorption and fluorescence spectra, and fluorescence quantum yield and lifetime was investigated in detail for three APO dyes, whose formulas and abbreviations are shown in Scheme 1. Meas-

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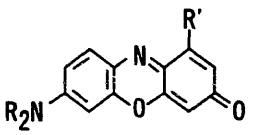

DEPO : R = C_2H_5 R' = H
DMPO : R = CH_3 R' = H
MDMPO : R = CH_3 R' = CH_3

Scheme 1.

urements were conducted in nine aprotic solvents to determine the effect of polarity and were also conducted in a binary mixture of dioxane–water to examine the effect of the hydrogen bonding. Results obtained in dioxane–water are also valuable because optical probes are often used in systems where both hydrophobic organic and aqueous domains exist. In addition, the photophysical properties of the dyes are compared with one another to examine the effect of chemical structure. These results will offer useful information when these dyes are utilized in studies on the microenvironments of various media.

2. Experimental details

2.1. Materials

The following reagent-grade solvents were distilled before use: toluene, benzene, dioxane, ethyl acetate and acetone. The following spectroquality solvents (Katayama) were used as received: acetonitrile, pyridine, dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Water was deionized and distilled. *N,N*-dimethyl-3-nitroaniline (98%; Aldrich) was purified by recrystallization. Rhodamine 6G (laser-grade; Exiton) was used as received. The APO dyes were synthesized by condensation of *N,N*-dialkyl-4-nitrosoaniline and resorcine derivatives in ethanol containing zinc chloride in a similar manner to the procedure described in the literature [10] and were purified by recrystallization from benzene.

2.2. Apparatus and methods

A Shimadzu UV-2200 spectrophotometer was used to record absorption spectra. A Hitachi F-3010 spectrofluorimeter with excitation and emission slits of 3 nm was used to record fluorescence emission and excitation spectra. The emission spectra were measured by excitation at 500 nm and were corrected using *N,N*-dimethyl-3-nitroaniline in benzene-*n*-hexane as a standard. The excitation spectra were obtained after calibration of the excitation system using a Rhodamine B quantum counter. Fluorescence quantum yields were determined relative to a Rhodamine 6G standard ($\phi_f = 0.95$, in ethanol). In order to monitor fluorescence decays, a Horiba NAES-1100 photon-counting apparatus was used together with a Toshiba KL-49 or KL-51 bandpass filter on the excitation side and a monochrometer with a slit width of 14 nm on the emission side. The wavelength of the monochrometer was set at the maximum of the steady-state fluorescence spectrum.

The concentrations of the final dye solutions were of the order of 2×10^{-6} M. The solutions were not degassed.

Solvatochromic scales introduced by Taft and Kamlet [11,12] were used for analyzing absorption and fluorescence properties of the APO dyes. Scales for pure solvents were taken from the literature [13]. Scales for dioxane–water were experimentally determined in the separated work [14].

3. Results and discussion

3.1. Changes in absorption spectrum

Fig. 1 shows the absorption spectra of DMPO in dioxane–water. The absorption band undergoes a significant red shift when increasing the polarity of the solvent. Concomitantly a single broad band in pure dioxane changes to a structured one possessing a short-wavelength shoulder. The change in spectral profile appears in 70 wt% dioxane and becomes prominent in 40 wt% dioxane. The red shift was also observed in a series of aprotic solvents but was accompanied by little change in the spectral shape. DEPO and MDMPO exhibited a similar trend in solvent effect on their absorption spectra. The concentration dependence of the absorption spectrum of DMPO was examined in dioxane–water. A 100-fold increase of the dye concentration caused little change in the spectral shape in 70 and 40 wt% dioxane, indicating that aggregation does not occur in these mixed solvents.

Shifts of absorption spectra of organic dyes can be analyzed in relation to solvatochromic scales introduced by Taft and Kamlet. For a variety of dyes, a plot of the absorption maximum vs. the solvent polarity/polarizability π^* gives a straight line in non-hydrogen-bonding solvents, whereas in hydrogen-bonding solvents, it deviates from the line to the extent of the hydrogen-bond acidity α and the hydrogen-bond basicity β of the solvent [11,12]. Fig. 2 shows the absorption maximum of DEPO, DMPO, and MDMPO in various sol-

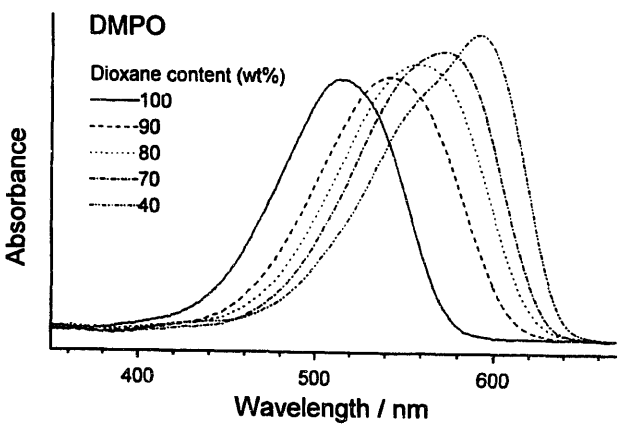

Fig. 1. Absorption spectra for DMPO in dioxane–water.

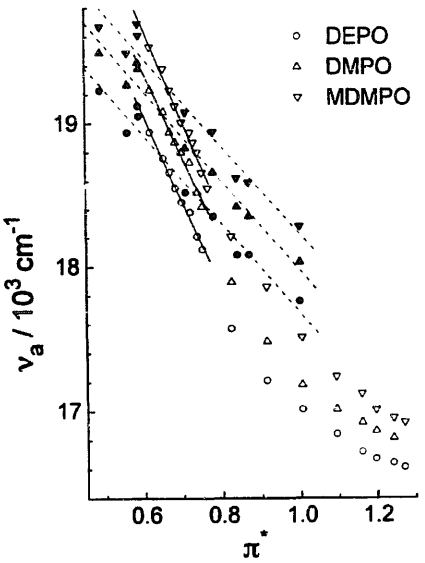

Fig. 2. Absorption maximum in wavenumber for the APO dyes as a function of π^*. Closed symbols are results in aprotic solvents and open symbols, in dioxane–water. The dashed lines correlate maxima for aprotic solvents. The solid lines correlate maxima in dioxane–water in the π^* region smaller than 0.77.

vents as a function of π^*. The dependence of the maximum for these dyes is similar to one another. The results in aprotic solvents show satisfactorily linear correlations. This is because the APO dyes are not proton-donating but only proton-accepting, mainly on the carbonyl group.

On the other hand, the results in dioxane–water deviate from the above straight line. It has been reported that α always increases with π^* in dioxane–water and that this increase is linear in the π^* regions of 0.59 to 0.75 and of 0.82 to 1.1 [14]. Only α should be considered here because the APO dyes are only proton-accepting. The wavenumber decreases linearly with increasing π^* in the π^* region smaller than 0.77. This sensitivity to π^* is higher than that for the aprotic solvents, suggesting that the dye is interacting with water molecules by hydrogen bonding. In addition, presumably the weight fraction of water is larger in the solvation sphere than in the bulk solvent. Preferential solvation by water in nonpolar mixtures of dioxane–water has been reported for 2,6-diphenyl-4-(2,4,6-triphenylpyridinio)phenolate (Reichardt's betaine) [15]. On the other hand, the plot is curved upward in the π^* region larger than 0.82. The sensitivity to π^* in the π^* region larger than 1.0 is lower than that for the aprotic solvents.

3.2. Changes in fluorescence spectrum

Fig. 3 shows the corrected fluorescence spectra of DMPO in various solvents. The fluorescence spectrum undergoes a significant red shift when increasing the polarity of the solvent. The spectrum is structured with a long-wavelength side band in nonpolar solvents such as toluene. The excitation spectrum and fluorescence lifetime of this side band were almost the same as those of the main band. In contrast, the

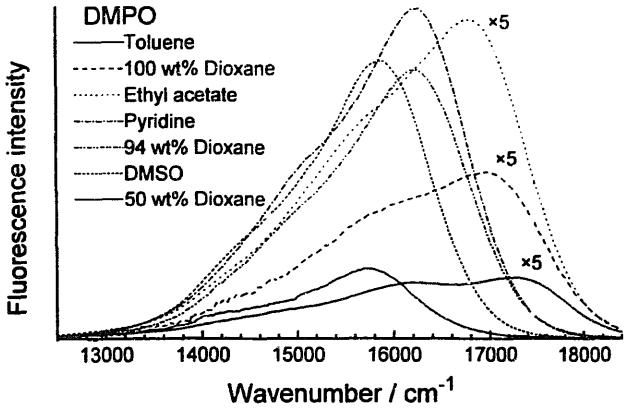

Fig. 3. Corrected fluorescence spectra for DMPO in several solvents.

spectrum is broad in moderately polar solvents such as ethyl acetate to polar solvents such as DMSO. DEPO and MDMPO also exhibited a similar trend in the solvent effect of the fluorescence spectrum.

Fig. 4 shows the fluorescence maximum of the three APO dyes in various solvents as a function of π^*. The π^* dependence of the maximum for these dyes is similar to one another. The relationship between the wavenumber and π^* is nonlinear in the aprotic solvents. Although the correlation is not very good, the sensitivity to π^* is high in the small π^* region but is much lower in the large π^* region. The relationship between the wavenumber and π^* is also nonlinear in dioxane–water. In the π^* region smaller than 0.77, the plot consists of two straight lines. The maximum has a high sensitivity to π^* in the π^* region of 0.59 to 0.68 but a lower sensitivity in the π^* region of 0.68 to 0.77. Because the APO dyes are considered only hydrogen-bond acceptors, the two kinds of sensitivity may originate from two different emitting states.

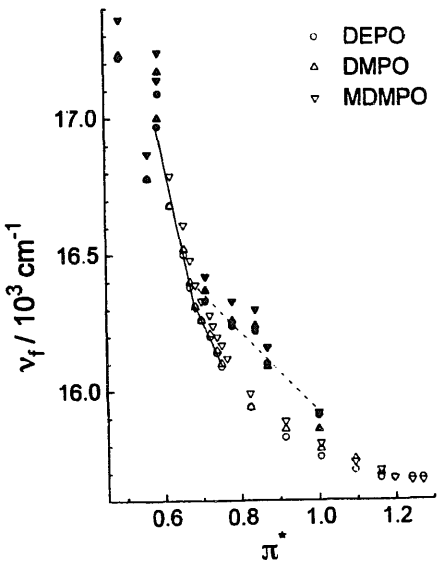

Fig. 4. Fluorescence maximum in wavenumber for the APO dyes as a function of π^*. The closed symbols are results in aprotic solvents and the open symbols, in dioxane–water. The dashed line correlates maxima for DEPO in aprotic solvents in the large π^* region. The solid lines correlate maxima for DEPO in dioxane–water in the π^* regions smaller than 0.68 and of 0.68 to 0.77.

On the other hand, the plot is curved upward in the π^* region larger than 0.82. The sensitivity to π^* in this large π^* region is lower than the two sensitivities in the small π^* region of dioxane–water and is also lower than the two sensitivities in the aprotic solvents.

The spectral shifts of the absorption and fluorescence can be analyzed by means of Lippert-Mataga equation [16,17] to determine the dipole moment difference between APO dyes in the ground and excited states. The Stokes shift $\Delta\nu = (\nu_e - \nu_g)$ in wavenumber should be linearly correlated with a solvent parameter Δf defined as

$$\Delta f = \frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}$$

The slope of the straight line is given by

$$s = \frac{2(\mu_e - \mu_g)^2}{hca^3}$$

where μ_g and μ_e are ground- and excited-state dipole moments, respectively, and a is the Onsager radius.

Fig. 5 shows $\Delta\nu$ *vs.* Δf for the APO dyes in aprotic solvents. The data points are significantly scattered for all dyes. This is probably because the two emitting states exist in

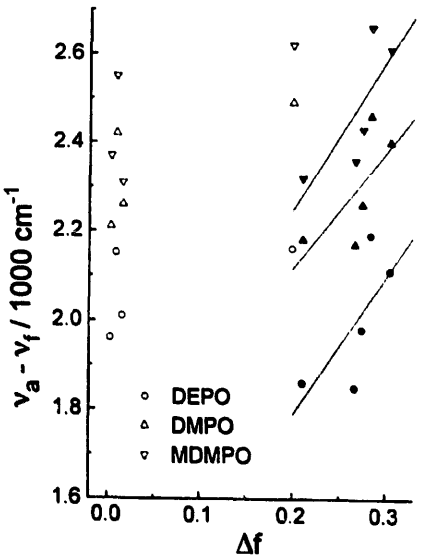

Fig. 5. Stokes shift $\Delta\nu = (\nu_e - \nu_g)$ for the APO dyes as a function of Δf in aprotic solvents. The open symbols correspond solvents with small π^* values and the closed symbols solvents with large π^* values. The straight lines correlate $\Delta\nu$ *with* Δf for results in the latter solvents.

Table 1
Slope of the regression line m and correlation coefficient r of the Lippert-Mataga plot and derived dipole moment difference between the ground and excited states $\mu_e - \mu_g$ for the APO dyes

	Compound		
	DEPO	DMPO	MDMPO
m	3.07	2.63	3.37
r	0.71	0.71	0.78
$\mu_e - \mu_g$/D	4.4	4.1	4.6

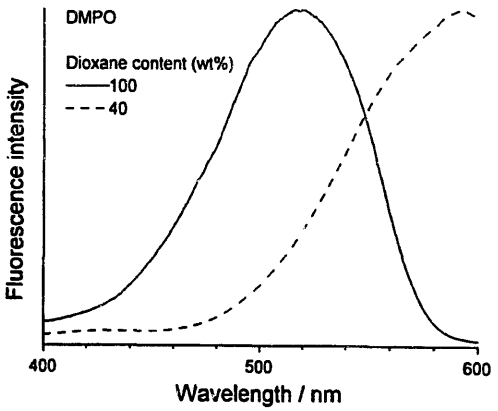

Fig. 6. Corrected excitation spectra for DMPO in pure dioxane and 40 wt% dioxane. Emission wavelength, 620 nm.

aprotic solvents, as suggested above. The determination of properties of a single excited state becomes possible when removing the data points for solvents with the small π^* values. Such separation of solvents into two groups in the Lippert-Mataga plot has been attempted for 9,9-bianthracenes [18,19]. As shown in Fig. 5, although this results in a rather poor correlation, it is still possible to determine the difference $\mu_e - \mu_g$. The calculated values for the dyes as well as the slopes of the regression lines and the correlation coefficients are tabulated in Table 1. In this calculation, the radius a is estimated to be about 4 Å.

Fig. 6 shows excitation spectra of DMPO in pure dioxane and 40 wt% dioxane. The spectrum is in good agreement with the absorption spectrum in both solvents. Similar results were obtained for DEPO and MDMPO.

3.3. Fluorescence quantum yield, lifetime and decay rate constants

Fig. 7 shows the fluorescence quantum yield of the APO dyes in various solvents as a function of π^*. The quantum

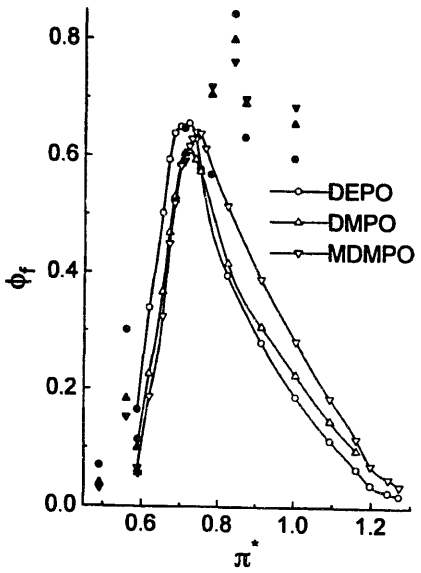

Fig. 7. Fluorescence quantum yield for the APO dyes as a function of π^*. The closed symbols are results in aprotic solvents and the open symbols in dioxane–water.

yield is very strongly dependent on π^*. In aprotic solvents, the quantum yield is low in the small π^* region but is relatively high in the large π^* region. In dioxane–water, the quantum yield rapidly increases with π^* in the small π^* region but decreases in the large π^* region.

The fluorescence decay for the APO dyes were measured in various solvents. The decay curves were well analyzed by single exponential kinetics with chi-square values ranging from 0.87 to 1.61. Fig. 8 shows the fluorescence lifetime as a function of π^*. The dependence on π^* is very strong and similar to that for the fluorescence quantum yield.

Changes in the fluorescence quantum yield and lifetime can in principle be the result of changes in either radiative (k_r) or nonradiative rate constants (k_{nr}). These values are readily calculated using the following relations.

$$k_r = \phi_f / \tau_f \text{ and } k_{nr} = (1 - \phi_f) / \tau_f$$

Fig. 9 shows k_r and k_{nr} of MDMPO in various solvents as a function of π^*. Since the solvatochromic scales due to Taft and Kamlet have been also applicable to rate constants of a number of reactions [13], the π^* dependence of k_r and k_{nr} may provide information about properties of the exited states of the APO dyes.

In aprotic solvents, the correlation of k_r with π^* is poor in the small π^* region but is good enough in the large π^* region to draw a straight line. In dioxane–water, the dependence of k_r on π^* is linear in the π^* region of 0.65 to 0.77. Both correlation lines are ascending whereas the slope is higher in dioxane–water, suggesting that the hydrogen bonding interaction of water enhances the increase of k_r. Moreover, in dioxane–water, the results significantly deviate from the straight line and become descending below and above the π^* region of 0.65 to 0.77. The k_{nr} value also strongly depends on π^*. It is high in the small π^* region but is much lower in the large π^* region in aprotic solvents. In dioxane–water, k_{nr}

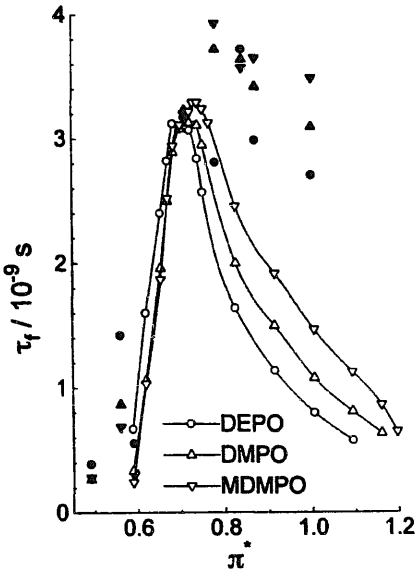

Fig. 8. Fluorescence lifetime for the APO dyes as a function of π^*. See the caption for Fig. 5.

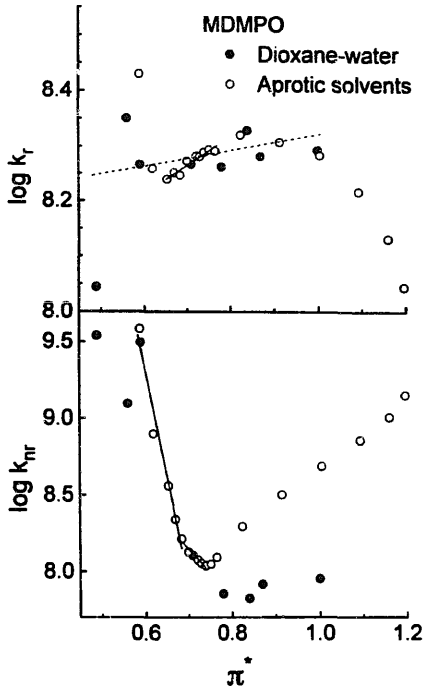

Fig. 9. k_r and k_{nr} for MDMPO as a function of π^*. The closed symbols are results in aprotic solvents and the open symbols in dioxane–water. The dashed line correlates k_r in aprotic solvents. The solid lines correlate k_r in dioxane–water in the π^* region of 0.65 to 0.77 and correlate k_{nr} in dioxane–water in the π^* regions smaller than 0.68 and of 0.68 to 0.74.

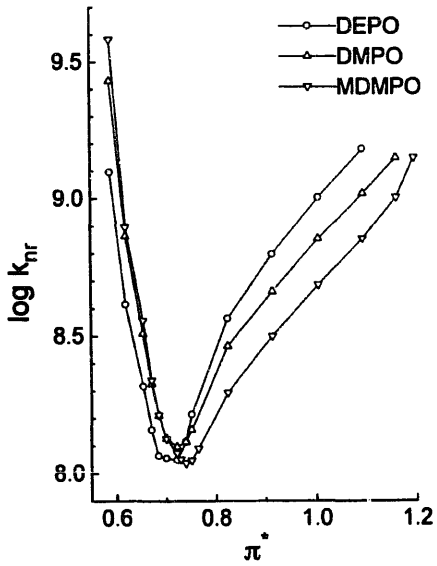

Fig. 10. k_{nr} for the APO dyes in dioxane–water as a function of π^*.

decreases with increasing π^* in the small π^* region but increases in the large π^* region. It is worth noting that the results form a straight line in the π^* region smaller than 0.68. In addition, another linear portion of the π^* dependence of k_{nr} is apparent above this region. Similar behaviors were observed for DEPO and DMPO.

Fig. 10 shows k_{nr} of the three APO dyes in dioxane–water as a function of π^*. Although the decrease in k_{nr} suddenly changes to a rapid increase, the minimum of k_{nr} shifts to a larger π^* value in the order of DEPO ≤ DMPO < MDMPO.

The π^* value at the minimum are 0.71, 0.71, and 0.74 for DEPO, DMPO and MDMPO, respectively.

The sudden change in k_{nr} may come from change in the nonradiative decay process. It seems that there are two types of nonradiative decay process in dioxane–water, one whose rate decreases with increasing π^* of the solvent and the other whose rate increases with π^*. The k_{nr} value was high in the small π^* region but was much lower in the large π^* region in aprotic solvents. This dependence is similar to that in the small π^* region of dioxane–water. It is suggested that, in these solvents, the fluorescence is quenched only by long–range dipolar interactions with solvent molecules. It has been reported that a change of increase to decrease in the fluorescence quantum yield occurred when the water content increases in dioxane–water solutions of *N*-arylaminonaphthalenesulfonates [20]. It has been described that the main process of nonradiative decay from the excited singlet state is the intersystem crossing to triplet in nonpolar mixtures for these dyes. It has been reported that the fluorescence lifetime of acridine in aqueous alcohol increases with water content because the energy barrier for the intersystem crossing increases with solvent polarity [21]. Thus, the polarity dependence of k_{nr} in the small π^* region for the APO dyes could be observed for a few other compounds and is presumably attributed to that of the intersystem crossing. On the other hand, k_{nr} increased with π^* in the large π^* region of dioxane–water. It has been proposed that electron transfer from the charge transfer (CT) excited state to solvent occurs as the most likely nonradiative process in aqueous media for N-arylaminonaphthalenesulfonates [22] but little or no ionization occurs in nonaqueous polar solvents [23]. It seems that short-range hydrogen bonding interactions with solvent molecules are the most likely contributors to the nonradiative decay process in the large π^* region.

3.4. *Effect of chemical structure*

The absorption maximum shifted to the blue in a variety of solvents in the order of DEPO < DMPO < MDMPO (Fig. 2). Such a blue shift by chemical structure was also seen for the fluorescence maximum but was very small (Fig. 4). As a result, the Stokes shift increased in the above order. This is probably because the Frank-Condon (FC) and emitting excited states increase in potential energy in this order but the increase for the latter state is relatively small. The large π^* dependence of the absorption maximum indicates that the FC state is, though incomplete, CT in character. Decreasing the number of carbon atoms of alkyl substituents, i.e. the exchange of ethyl to methyl, at the 7-amino group is considered to inhibit the charge separation and unstabilize the excited states possessing the CT character. The introduction of an alkyl group to the 1 position may have the same effect. Therefore, it is expected that such structural changes raise the potential energy of the FC state. On the other hand, the extent of the solvent relaxation which converts the FC state to the emitting state probably becomes marked in the order of DEPO < DMPO < MDMPO. As a result, the potential energy of the emitting state does not increase very much by the structural change.

The effect of chemical structure was seen on the π^* dependence of k_{nr} (Fig. 10). The point of sudden change in k_{nr} shifted to a larger π^* value in the order of DEPO ≤ DMPO < MDMPO. This indicates that the solvent-induced nonradiative decay becomes dominant over the intersystem crossing in the more polar media when the chemical structure changes as above. This is presumably because the structural change inhibits charge separation as well as hydrogen bonding interactions with solvent molecules.

4. Conclusions

APO dyes used here exhibited interesting features in the photophysical properties. Especially, the absorption and fluorescence were significantly shifted to the red when the dyes were dissolved in a more polar solvent. This property makes APO dyes useful as optical probes of the microenvironments of various media, as proposed recently [5]. The effect of structure modifications of APO dyes were examined and the importance of alkyl substituents at the amino group and on the phenoxazone backbone was pointed out. This observation has implications for the design of more efficient probes.

The APO dyes absorb and emit at long wavelengths. For example, the absorption and fluorescence of DMPO are at 513–595 nm and at 580–637 nm, respectively, in solvents used here. These probes are of greater advantage than conventional ones possessing absorption and emission in the ultraviolet or near ultraviolet region. The large Stokes shifts and appreciable quantum yields of the APO dyes will also improve sensitivity in detection of the fluorescence.

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