

An Interesting Case Where Water Behaves as a Unique Solvent. 4-Aminophthalimide Emission Profile to Monitor Aqueous Environment

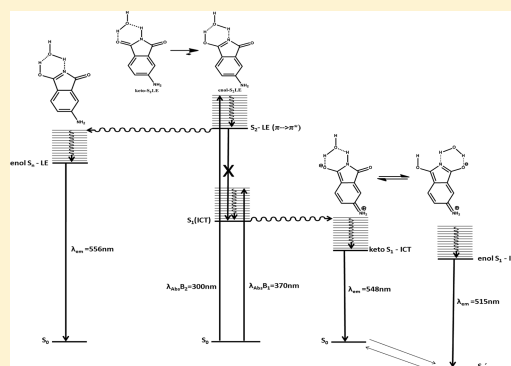
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Supporting Information

ABSTRACT: The behavior of 4-aminophthalimide (4-AP), a common molecular probe utilized in solvation dynamics experiments, was revisited in polar aprotic and protic solvents using absorption, steady-state, and time-resolved fluorescence (TRES) techniques. Also, the deuterium isotope effect was investigated using D₂O as solvent. The absorption spectra of 4-AP consist of two absorption bands with maxima around 300 nm (B2 band) and 370 nm (B1 band) depending on the environment, while the emission feature consists of a single band. In all the solvents investigated (excluding water), the 4-AP photophysics is similar and the emission spectra are independent of the excitation wavelength used. In water the behavior is unique and the emission spectra maximum is different depending on the excitation wavelength used. The emission maximum is 561.7 nm using the excitation wavelength that correspond to the B2 absorption band maximum ($\lambda_{\text{excB2}} = 303.4 \text{ nm}$) but is 545.7 nm when the excitation wavelength that correspond to the B1 absorption maximum ($\lambda_{\text{excB1}} = 370.0 \text{ nm}$) is used. Moreover, while the fluorescence decays of 4-AP in water exhibit no emission wavelength dependence at λ_{excB2} , the situation is quite different when λ_{excB1} is used. In this case, we found a time-dependent emission spectrum that shifts to the blue with time. Our results show that the solvent-mediated proton transfer process displays a fundamental role in the 4-AP emission profile and for the first time a mechanism was proposed that fully explains the 4-AP behavior in every solvent including water. The deuterium isotope effect confirms the assumption because the proton-transfer process is dramatically retarded in this solvent. Consequently, we were able to elucidate not only why in water the emission spectra depend on the excitation wavelength but also why the time-dependent emission spectra shift to the blue with time. Thus, our work reveals the importance that the medium has on the behavior of a widespread dye used as chromophore. This is significant since the use of chromophores without understanding its chemistry can induce artifacts into the interpretation of solvation dynamics in heterogeneous environments, in particular, those provided by aqueous biological systems.



INTRODUCTION

Light absorption by a solute induces a very fast change in electron density, which displaces the solute and its environment from equilibrium with respect to the nuclear coordinates. After that, a system attains equilibrium by nuclear relaxation of the probe and its solvation sphere.^{1–5} This reorganization process takes a few picoseconds in common fluids near room temperature.^{3,6} It is known that the presence in an aromatic ring of an electron donor (amine group for example) conjugated with an electron acceptor moiety (usually carbonyl group) makes that upon electronic excitation of such a molecule significantly changes the electron density distribution.^{1,3,4,7}

Intermolecular hydrogen bonding is an important type of solute–solvent interaction that has been extensively investigated, being particularly significant for polarizable solutes dissolved in protic solvents.^{8,9} Upon photoexcitation, as a result

of differences in charge distribution of the different electronic states, the hydrogen bond formed by solute and solvent molecules in the excited state can undergo reorganization and important changes.⁸ However, knowledge about the process of excited-state hydrogen-bonding dynamics is rather limited because of the extremely short time scale involved.

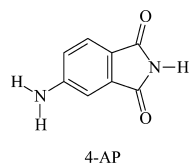
Aminophthalimides have been extensively used as strong fluorescent probes in different environments including nano-materials, organized media, and biological systems.^{4,10–19} This is the case of 4-aminophthalimide (4-AP) (Scheme 1), a molecular probe that exhibits the common emission feature of compounds with an electron-donating moiety (the amine group) conjugated with an electron acceptor moiety (the

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Scheme 1. Molecular Structure of 4-AP



carbonyl of the imido group) in the aromatic ring. Thus, 4-AP is an excellent probe because its fluorescence lifetimes, spectra, and quantum yields are affected greatly by the environment.^{3,7,8,20} It has been reported that the bathochromic shift of the emission spectra of 4-AP in protic solvents is much greater than the one observed in aprotic ones, and the greatest is detected in water. It is believed that as a result of 4-AP excitation there is a redistribution of the charge yielding to an internal charge-transferred state (S_1 -ICT) that has a larger dipole moment (6.5 D) in comparison with the one in the ground state (3.5 D).^{3,21} As a consequence of this charge redistribution, there is a significant change in the energy of the hydrogen bonds that are already present in the ground state.^{3,5} The Stokes shift of this S_1 -ICT emission is very sensitive to the polarity and hydrogen bond donating ability of the medium. Despite the large body of data collected from many studies, there are still many controversies as to the interpretation and origin of 4-AP's photophysical behavior as influenced by the properties of its surrounding media. In summary, the literature gives three main reasons for the strong 4-AP solvatochromism: (i) the increases in the dipole moment value of the excited state relative to its value in the ground state;²¹ (ii) the formation of stronger hydrogen bonds in the S_1 -ICT than in the ground state in protic solvents,^{3,20,22} and (iii) the formation of an emitting enol form of 4-AP as the result of a solvent-mediated proton transfer from the imino or amine groups to the 4-AP carbonyl group.^{10,11} We strongly believe that a composite of the three effects invoked above should be the responsible for the 4-AP photophysics, as we will discuss later.

On the other hand, although an effort was made to try to explain the 4-AP emission feature in protic and aprotic solvents, it seems to us that there is important experimental evidence that has not been addressed yet. We have been revisiting the photophysical properties of 4-AP in various solvents and find that there are marked differences between the properties that are observed in organic solvents compared to water. The absorption spectra of 4-AP consist of two absorption bands with maxima around 300 nm (B2 band) and 370 nm (B1 band) depending on the environment, while the emission feature consists of a single band, and it was not possible to find a comprehensive solvatochromic study performed on both absorption bands. Moreover, the origin of the B2 absorption band was not assigned in the literature; thus, it is necessary to assign clearly the origin of these bands. B1 band corresponds to the $S_0 \rightarrow S_1$ (S_1 -ICT) transition that gives the intra-charge-transferred state (ICT) as it was discussed in the literature,^{3,10} and we assign the B2 band to the $S_0 \rightarrow S_2$ (π - π^* S_2 -LE) transition that gives the non-charge-transferred locally excited state (LE) where the dipole moment is much lower than the S_1 -ICT state.²¹

In all the organic solvents studied, the emission spectra (steady and time-resolved) are the same independent of the excitation wavelength used, i.e., B1 or B2, which is the expected behavior for a fluorophore that emits from its lowest excited state (S_1 -ICT).¹ Something really interesting, strange and never

informed happens when the 4-AP photophysics is studied in water where the emission spectra show different emission wavelengths depending on the excitation wavelength used. Moreover, while the fluorescence decays of 4-AP in water exhibit no emission wavelength dependence at λ_{excB2} as it was previously found,¹⁰ the situation is quite different when λ_{excB1} is used. Here, the emission spectra shift to the blue with time; it seems to us that a much more complex and unexplored phenomenon rather than solvation dynamics should be the explanation for 4-AP emission decay in water. Thus, although 4-AP is a very interesting and exciting molecule widely used as chromophore, there are critical aspects of its behavior that are not well understood yet. Therefore, the goal of the present contribution is to unravel the 4-AP photophysics before it will be used as molecular probe in more complex aqueous systems, as we intend to do.

In this work, we want to fully understand the 4-AP emission feature in different organic solvents and in water investigating both molecular probe transitions: $S_0 \rightarrow S_1$ (S_1 -ICT) and $S_0 \rightarrow S_2$ (S_2 -LE). Thus, we performed absorption, steady-state, and time-resolved fluorescence (TRES)¹ techniques on 4-AP dissolved in different organic solvents and water using not only different emission wavelengths but also exciting at different wavelengths. Also, the deuterium isotope effect was investigated including D_2O as solvent. Our results show, for the first time, that the solvent-mediated proton transfer process displays a fundamental role in 4-AP emission profile, and this reaction is unique in water. What is remarkable to highlight is that water is the only solvent that makes complicated and intriguing the 4-AP emission feature, results that show the preponderant role that the excited proton transfer reaction has on the emission profile.

■ MATERIALS AND METHODS

Ultrapure water was obtained from Milli-Q filtered, 18.2 M Ω cm resistivity. The emission of the pure water used (blank) was monitored at the different excitation wavelengths used and no emission bands were detected. All other solvents were of the highest grade available (Aldrich spectral of HPLC grade) and used as received. The molecular probe 4-aminophthalimide (4-AP, Aldrich) was purified by repeated recrystallization from ethanol.³

To dissolve the probe in the different solvents investigated, a 0.01 M solution of 4-AP was prepared in methanol (Sintorgan HPLC quality). The appropriate amount of this solution to obtain a given concentration of the probe in the homogeneous media was transferred into a volumetric flask, and the methanol was evaporated by bubbling dry N_2 ; then, the suitable amount of the different solvents was added to the residue. The dye solutions were transparent and free of solid residues in every solvent used.

The absorption spectra were measured by using Shimadzu 2401 equipment at 25 °C unless otherwise indicated. A Spex fluoromax apparatus was employed for the fluorescent measurements. Corrected fluorescence spectra were obtained using the correction file provided by the manufacturer. The path length used in the absorption and emission experiments was 1 cm. Because the absorption and emission bands are broad, the λ_{max} was measured by taking the midpoint between the two positions of the spectrum where the absorbance is equal to $0.9A_{\text{max}}$. Thus, the uncertainties in λ_{max} are about 0.1 nm.

To apply Kamlet–Taft’s solvatochromic comparison method (KTSCM)²³ to the emission frequency and considering that the maximum frequency of an emission spectrum is not necessarily the maximum of the spectrum on a wavelength scale, a correction was performed.²⁴ Thus, the emission spectrum on a wavelength scale was multiplied by the square of the wavelength and replotted (as $F(\nu) = \lambda^2 F(\lambda)$ vs frequency (ν)) to find the maximum.

Fluorescence decay data were measured with the time-correlated single-photon-counting technique (TCSPC) (Edinburgh Instrument FL-900) with a PicoQuant subnanosecond Pulsed LED PLS 370 (emitting at 378 nm) < 600 ps fwhm. Fluctuations in the pulse and intensity were corrected by making an alternate collection of scattering and sample emission. The quality of the fits was determined by the reduced χ^2 , and for the best fit χ^2 must be around 1.0.²⁵ It must be noted that in any exponential fit (mono or bi) we have tried to perform multi- or monoexponential fitting but the statistics of the decays were not improved or became worse.

Time-resolved emission spectra, TRES, were generated from a set of emission decay times taken at 10 nm intervals spanning the fluorescent spectrum (typically 20 decays). To resolve the convolution with the instrument response on the time-resolved decay data at each emission wavelength, a multiexponential fit was used. The purpose of these fits is simply to represent the decay curves and no physical meaning is ascribed to the derived exponential parameters.¹ Two components were generally required to obtain a satisfactory fit to the data. TRES have been constructed following the procedure described.^{1,26}

RESULTS AND DISCUSSION

Absorption and Emission Studies in Pure Solvents.

Typical absorption and emission spectra of 4-AP in homogeneous media are shown in Figure 1, A and B, respectively. As it can be seen, the absorption spectra consist of two bands that correspond to different transitions: (i) the band around 300 nm assigned to the $S_0 \rightarrow S_2$ ($\pi-\pi^*$ S_2 -LE) transition and that we will call B2 band from now on, and (ii) the band around 370 nm assigned to the $S_0 \rightarrow S_1$ (S_1 -ICT) transition that we will denote as B1 band. On the other hand, the emission spectra consist of a single band that has the same shape and position irrespective of the excitation wavelength used for all solvents (Table 1), excluding water (Table 1, we will come back to this issue later in the work). Also, as reported in the literature, the 4-AP emission intensity is noticeably lower in protic solvents (including water where it is extremely low) than in aprotic solvents.^{5,10,11,22} In addition, in protic solvents the emission energy decreases (red shift of the emission band) markedly in comparison with the band position found in polar aprotic solvents. Thus, as it was previously reported, these facts indicate that the nature of the 4-AP emitting state in protic solvents is distinctly different from that found in aprotic solvents.¹⁰ Nevertheless, we want to call the attention that always it was assumed that 4-AP emits from the S_1 -ICT state in any medium and at any excitation wavelengths used. Our data will show that this assumption does not explain the 4-AP behavior found in water.

The solvent effects are frequently studied by empirical solvent parameters to determine the predominant interactions. One of the most useful approaches for elucidating and quantifying different solute–solvent interactions is the Kamlet–Taft solvatochromic comparison method

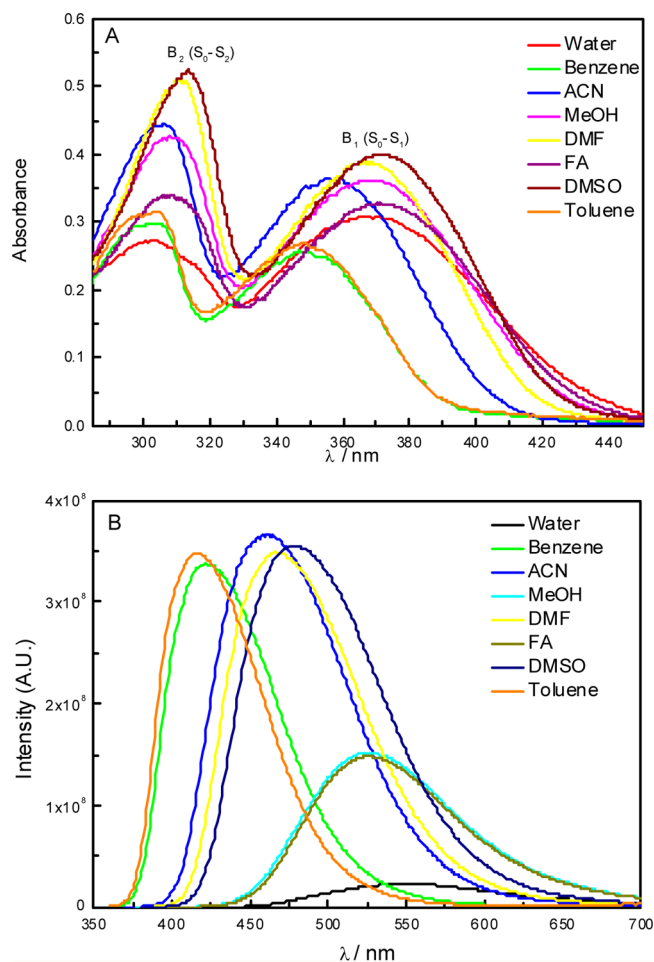


Figure 1. (A) Absorption spectra of 4-AP in different solvents. (B) Emission spectra of 4-AP in different solvents. $\lambda_{\text{exc}} = \text{B1 maximum band}$. $[4\text{-AP}] = 1 \times 10^{-4}$ M.

(KTSCM).²³ According to the KTSCM, absorption and emission band frequencies, ν , can be correlated using eq 1

$$\nu = \nu_0 + s\pi^* + a\alpha + b\beta \quad (1)$$

where π^* is the solvent polarity/polarizability parameter, α is the hydrogen bond donation ability of the solvent, and β is the hydrogen bond acceptance or electron pair donation ability to form a coordinated bond. The coefficients s , a , and b measure the relative sensitivity of ν to the indicated solvent property.^{27–29}

The absorption and emission maxima in all the solvents investigated, as well as the solvent parameters π , α , and β used,^{27–29} are given in Table 1. It must be noted that it was not possible to obtain defined absorption bands in nonpolar or nonpolarizable solvents (low values of π^*) and so they were not included in Table 1. The 4-AP spectra were used to obtain the KTSCM coefficients s , a , and b , given in Table S1 of the Supporting Information. Water was excluded because it strongly deviates from the correlations. The confidence level of the regression is 99.5% (t test). It must be noted that Mukherjee et al.¹⁵ have made the KTSCM correlation using absorption and emission frequencies obtained from the literature. In that work, they only monitored the frequencies that correspond to the B1 absorption band and the emission frequencies that correspond to the emission maxima obtained exciting at λ_{excB1} . Thus, their results are comparable with ours

Table 1. Kamlet–Taft Parameters,^{27–29} Frequencies, and Wavelengths (nm) of the Absorption and Emission Maxima (10³ cm⁻¹) for 4-AP in Different Solvents

solvents	π^*	β	α	B ₂		B ₁		$\lambda_{\text{exc}} = B_2$		$\lambda_{\text{exc}} = B_1$	
				ν_{abs}	λ_{abs}	ν_{abs}	λ_{abs}	ν_{em}	λ_{emi}	ν_{em}	λ_{emi}
water	1.09	0.18	1.17	32.92	303.4	27.02	370.0	17.82	561.7	18.31	545.7
benzene	0.55	0.10	0.00	33.02	302.3	28.58	350.3	23.64	423.3	23.62	423.5
acetonitrile (ACN)	0.66	0.40	0.19	32.65	306.2	27.97	357.1	21.72	460.2	21.67	461.3
methanol (MeOH)	0.6	0.66	0.91	32.38	308.2	27.06	369.2	18.96	527.4	19.03	526.8
glycerol (GY)	0.62	0.51	1.21	32.16	311.3	26.45	378.4	18.54	539.7	18.58	539.6
dimethylformamide (DMF)	0.88	0.69	0.00	32.14	311.3	27.15	368.9	21.42	467.3	21.38	467.9
formamide (FA)	0.97	0.48	0.71	32.43	308.4	26.90	372.6	18.93	528.2	18.97	527.8
dimethylsulfoxide (DMSO)	1.00	0.76	0.00	31.96	313.4	26.88	372.3	20.93	477.7	20.96	477.5
toluene	0.54	0.11	0.00	32.97	303.7	28.68	349.6	24.02	416.6	23.98	417.0
chloroform (CHCl ₃)	0.58	0.1	0.44	31.33	320.8	28.38	352.5	22.52	444.2	22.58	443.9
<i>n</i> -butanol (<i>n</i> -BtOH)	0.47	0.84	0.84	32.47	308.9	27.00	370.5	19.30	518.5	19.29	519.1
ethanol (EtOH)	0.54	0.75	0.89	32.47	308.9	27.21	367.9	19.30	518.5	19.27	519.0
isopropanol (<i>iso</i> -BtOH)	0.4	0.84	0.79	32.47	308.6	27.11	369.4	19.30	518.6	19.30	518.5
<i>n</i> -pentanol (<i>n</i> -PtOH)	0.4	0.86	0.84	32.47	308.4	27.16	368.2	19.30	518.6	19.26	519.4
<i>n</i> -propanol (<i>n</i> -PrOH)	0.52	0.9	0.84	32.47	308.4	27.15	368.2	19.30	518.5	19.31	518.4
acetone	0.62	0.49	0.08		hidden	27.89	358.3		hidden	21.97	455.6

only for B1 band and the discussion is shown in the Supporting Information.

The data from the 4-AP absorption spectra bands reflect strong solvent interactions of the dye in its ground electronic state. Here, the results are different depending on the electronic transition monitored (Table S1). For the $S_0 \rightarrow S_1$ (S_1 -ICT) transition and in concordance with the literature,¹⁵ the molecule displays bathochromic shift with α , β , and π^* parameters. In this case, the α and β parameters contribute about 17% and 45% while the π^* parameter contributes about 38%. Evidently, for 4-AP the main contribution to its solvathochromism is the hydrogen bond acceptor ability of the solvent (β). On the other hand, for the $S_0 \rightarrow S_2$ (π - π^* S_2 -LE) transition the data also show that the molecule displays bathochromic shift with α , β , and π^* parameters but different susceptibility to them. As it can be seen, the π^* parameter contributes 64% and the molecule seems to be less sensitive to the hydrogen bond abilities of the medium. These results suggest differences in the nature of the excited state populated after the electronic transition, the S_2 -LE (λ_{excB2}) and the S_1 -ICT (λ_{excB1}).

The KTSCM parameters from the emission bands obtained at the two excitation wavelength monitored, λ_{excB2} or λ_{excB1} , show the same parameters values (Table S1). This result shows that in all the solvents studied (except water) the emission comes from the same excited state. From the correlations it is evident that the 4-AP excited state interacts with its environment differently than in its ground state. There is an almost 5-fold increase in the α parameter and almost 2.4-fold increase in the π^* parameter. This implies that 4-AP emission comes from an excited state which is more polar and more hydrogen bond acceptor than the ground state.

Herein, we present our interpretation of the data found in aprotic solvents and in polar protic solvents (except water) in Scheme 2, A and B. In this scheme is shown different processes that have to be taken into account to explain the 4-AP photophysics in different media, including the 4-AP excited states populated after excitation at different wavelengths.

Scheme 2A shows that, in aprotic environments, 4-AP emits always from the same excited state, the keto-ICT state,

independently of the excitation wavelength used as discussed in the literature.⁵

On the other hand, the situation is different in protic solvents as Scheme 2B shows. Exciting 4-AP at λ_{excB2} the π - π^* S_2 -LE state is populated which consists mainly of the 4-AP keto species. After that and in concordance with the literature, the keto species undergoes a solvent-mediated proton transfer reaction to give a mixture of the keto and the enol species.^{7,10} We propose that 4-AP undergoes a fast intramolecular charge transfer reaction only from the keto S_2 -LE state to give the lower energy S_1 -ICT state, from where the molecule emits (the same excited state created if λ_{excB1} is used). The ICT state cannot be created from the enol S_2 -LE state since in this structure the remaining carbonyl group, the one that is not involved in the keto–enol tautomerism, is not conjugated with the amine electron donor group.

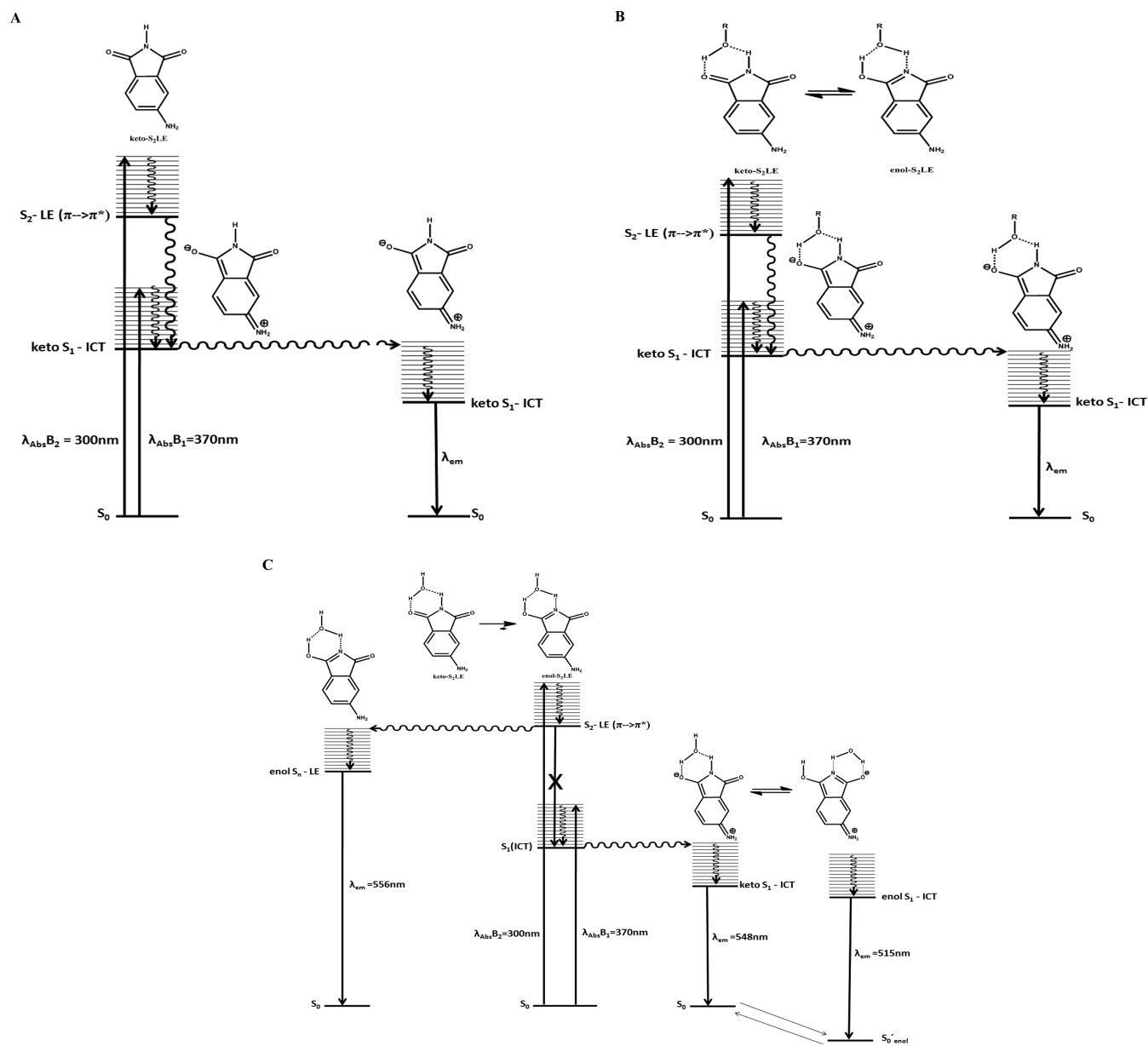
Exciting 4-AP at λ_{excB1} the S_1 -ICT excited state is directly populated and the emission comes from this state. In summary, 4-AP emits from the same excited state, S_1 -ICT, irrespective of the excitation wavelength used. It must be noted that, as it was previously shown, the nonradiative pathway is the favored route for the 4-AP enol species (S_2 -LE) decay.¹⁰

As we have previously mentioned, the 4-AP photophysics behavior in water is completely different from the one observed in all the other solvents investigated. Thus, the next sections will show the results and the explanation for 4-AP photophysics in water.

4-AP in Water. Even though in the literature^{8,10} there are some attempts to explain the odd 4-AP behavior found in water, there are still other pieces of evidence that need to be elucidated. We think that the main problem that causes the confusion in the explanations given in the literature is to assume that the 4-AP emission comes from the same excited state in all protic solvents, including water. However, there is experimental evidence that was not taken into account before and is unique for water. For example, the emission spectra have different wavelength maxima values depending on the excitation wavelength used.

As it can be seen in Figure 2A, the emission maximum wavelength is 561.7 nm at $\lambda_{\text{excB2}} = 303.4$ nm but, it is 545.7 nm at $\lambda_{\text{excB1}} = 370.0$ nm. Also, from Figure 2A it can be observed

Scheme 2. Emission Mechanism and the Solvent-Mediated Proton Transfer for 4-AP in Different Media: (A) Polar Aprotic Solvents at Any Excitation Wavelength; (B) Protic Solvents at Any Excitation Wavelength; and (C) Water at Any Excitation Wavelength



that the emission band is markedly broader and with lower quantum yield at λ_{excB2} than at λ_{excB1} . The tendency is the same at any 4-AP concentration investigated in the 10^{-6} to 10^{-4} M range (not shown).

Moreover, while the emission spectrum maximum is 561.7 nm irrespective of the excitation wavelength used in the red edge of the B2 band (results not shown), the situation is quite different when B1 band is monitored. Figure 2B shows the emission spectra of 4-AP at different excitation wavelengths in the red-edge of the B1 band. As it can be observed, there is a blue shift of around 35 nm of the emission band as the excitation wavelength is changed to lower energy. For example, at $\lambda_{\text{exc}} = 370$ nm the $\lambda_{\text{maxem}} = 546$ nm and, at $\lambda_{\text{exc}} = 420$ nm the $\lambda_{\text{maxem}} = 515.6$ nm. Clearly, the effect is not due to red-edge excitation shift (REES)^{1,30} which reflects slow solvent motion or motional restriction of the chromophores. REES effect

causes that the emission spectrum shifts to longer wavelength when excited at a longer wavelength.^{1,30}

Time-Resolved Emission Spectra (TRES) of 4-AP. In order to gain more information on the 4-AP photophysics in water for the $S_0 \rightarrow S_1$ (S_1 -ICT) transition, we studied the 4-AP behavior in water and other solvents, using time-resolved fluorescence measurements (TRES). TRES is frequently used to study the excited-state dynamics and kinetics of fluorescent molecules in solution.¹

Figure 3A,B shows the fluorescence decays measured at several wavelengths across the emission spectrum of 4-AP in ethanol (profile similar to all the other protic and aprotic solvents used, results not shown) and in water, at $\lambda_{\text{exc}} = 370$ nm (B1 band), which correspond to the $S_0 \rightarrow S_1$ (S_1 -ICT) transition, respectively. Figure 3A shows that the fluorescence decays of 4-AP in ethanol do not exhibit wavelength dependence as it was observed before in protic and aprotic

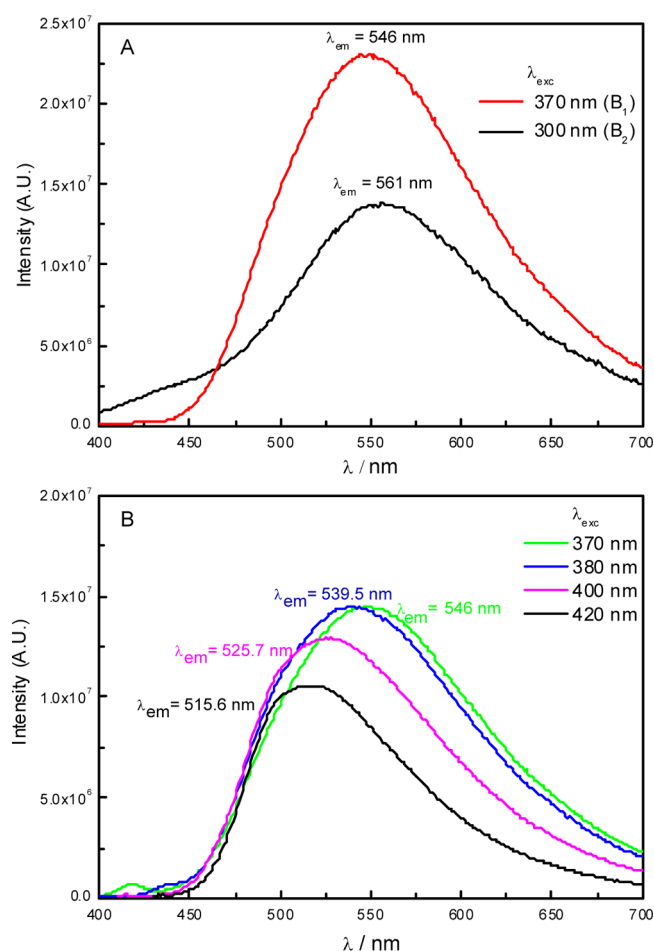


Figure 2. (A) Emission spectra of 4-AP in water at B1 and B2 excitation wavelengths. (B) Emission spectra of 4-AP in water at different excitation wavelengths at the red edge of the B1 band. [4-AP] = 1×10^{-4} M.

solvents and in water at λ_{excB2} .^{5,10,15} However, Figure 3B shows that the fluorescence decays of the molecule exhibit significant wavelength dependence in water at λ_{excB1} .

The time-dependent decays were used to construct the TRES and the results are shown in Figure 4 that clearly show that the TRES is different for ethanol (Figure 4A) and water (Figure 4B). In the former solvent and as expected, there is no dependence with time, indicating that the solvation dynamics is too fast to be detected in our nanosecond setup and that 4-AP emits from the relaxed S_1 -ICT state (see Scheme 2B). On the other hand, in water it was found that on increasing the time the band shapes narrow and the emission spectra shift progressively around 40 nm to shorter wavelengths, results that are absolutely surprising and difficult to understand.

Interestingly, while in every solvent investigated the excitation spectra taken at different emission wavelengths are identical with the absorption spectrum (results not shown), the situation is quite different in water. Figure S1 in the Supporting Information shows the excitation spectra of 4-AP in water at different emission wavelengths that correspond to the 4-AP emission band at $\lambda_{\text{excB1}} = 370.0$ nm. Clearly, the data show two interesting effects: (i) the excitation spectra are different from the absorption spectrum in water (Figure 1A) because in the former the $S_0 \rightarrow S_2$ ($\pi-\pi^*$ S_2 -LE) transition (B2 band) almost disappears and (ii) the excitation spectra shift to higher

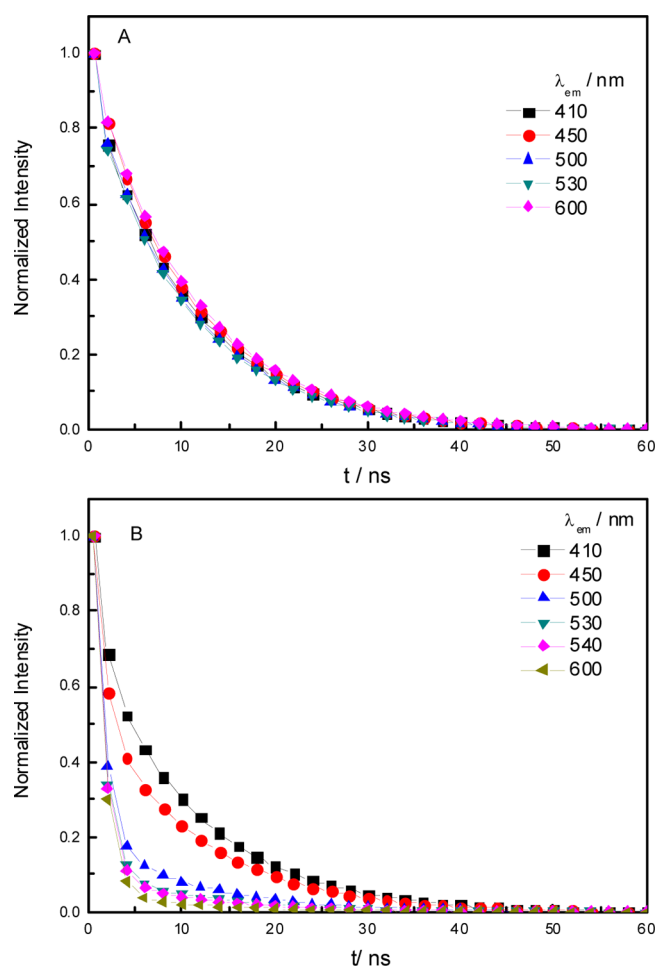


Figure 3. Normalized fluorescence decays of 4-AP in (A) ethanol and (B) water at different emission wavelengths. [4-AP] = 1×10^{-4} M. $\lambda_{\text{exc}} = \text{B1 maximum band}$.

frequencies (hypsochromic shift) as the λ_{em} used to construct the excitation spectra move to longer wavelength values. These data reflect differences in the ground state for the S_1 -ICT $\rightarrow S_0$ transition in water in comparison with all the other solvents and plausibly show that the S_1 state is not populated from the S_2 state.

Herein it is necessary to give an explanation for the unusual behavior of 4-AP in water. As there are marked differences in the molecular probe photophysics depending on the excited state populated (S_2 or S_1), we will explain both electronic transitions separately. Scheme 2C shows the process for the $S_0 \rightarrow S_2$ ($\pi-\pi^*$ S_2 -LE) and for the $S_0 \rightarrow S_1$ (S_1 -ICT) transitions in water. We want to denote that in the scheme only one water molecule is included for clarity even though it is well-known that probably more than one water molecule can assist in the proton transfer reaction.^{5,8,10} In this way, the probable cyclic hydrogen-bonded structure formed maintains linear hydrogen bonds.^{31,32}

At λ_{excB2} , the $\pi-\pi^*$ S_2 -LE state is populated (Scheme 2C) and, after that, the solvent-mediated proton transfer reaction gives the keto and the enol species.^{7,10} We propose that the main difference found with the other protic solvents (Scheme 2B) is that water seems to be the only solvent where the 4-AP enol species predominates in the tautomeric equilibrium. As it was suggested in the literature, the enol species probably is stabilized by hydrogen bond interaction with water. Moreover,

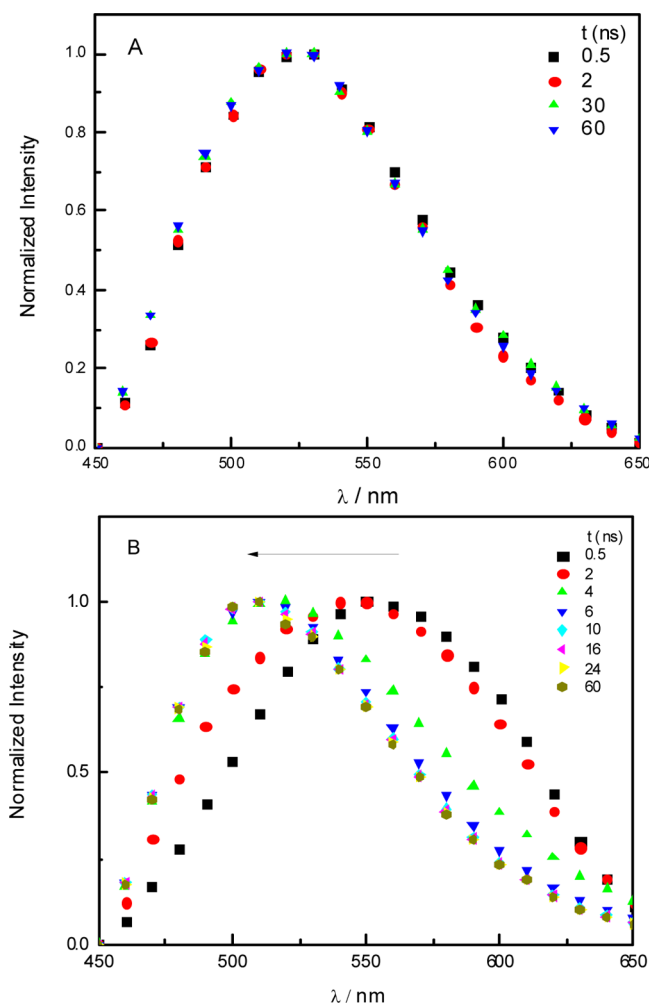


Figure 4. Normalized TRES of 4-AP (A) in ethanol and (B) in water. $\lambda_{\text{exc}} = \text{B1}$ (370 nm). $[4\text{-AP}] = 1 \times 10^{-4}$ M.

it was shown^{5,8,10} that more than one water molecule is involved, making the proton transfer in water an ultrafast process.^{10,11} That is, the relaxed S_n -LE state from where the emission comes consists mainly of the enol proton-transferred species.³³ It must be recalled that the fast intramolecular charge transfer reaction that yields the lower energy S_1 -ICT state does not happen to the enol S_n -LE state since the free carbonyl group of this species is not conjugated with the amine electron donor group, as it was discussed before. Thus, the 4-AP emitting excited state in water is not the keto S_1 -ICT state proposed for the emission in protic solvents (Scheme 2B). It seems that the S_1 -ICT state cannot be populated from the S_2 LE state. As a matter of fact, the broad emission band shown in Figure 2A at λ_{excB2} may indicate that there could be some emission from the keto S_1 -ICT state. It is very likely that, as it was suggested by Das et al.,¹⁰ the marked decrease of the emission quantum yield of 4-AP at λ_{excB2} found in water may be due to the reduction of the enol S_n LE \rightarrow S_0 energy gap due to the excited-state stabilization by water molecules. Thus, there is an enhancement of the nonradiative rates over the radiative pathway. Also, as 4-AP emits only from the enol S_n -LE state, the emission spectra do not show excitation wavelength dependence monitoring the B2 band.

On the other hand, when λ_{excB1} is used the keto S_1 -ICT state is populated directly. We propose that water is the only solvent

that can help that the proton transfer reaction occurs to the charge-transferred species and the keto S_1 -ICT species undergoes the keto – enol tautomerism (Scheme 2C). Under this scenario, the possible 4-AP emitting species are the keto S_1 -ICT and the enol S_1 -ICT species. The answer to the question “why are the 4-AP emission spectra different when λ_{excB2} or λ_{excB1} is used” is that the 4-AP emission states are not the same.

Moreover, Scheme 2C also explains the blue shift of the emission wavelength with time (Figure 4B). We propose that the emission from the enol S_1 -ICT species should be to a S_0' ground state which is different from the S_0 ground state for the keto S_1 -ICT emission. That is, S_0' ground state has a different electronic configuration than the S_0 and the S_0' state is more stabilized by water with the consequent increases of the enol S_1 ICT \rightarrow S_0' energy gap in comparison with the keto S_1 ICT \rightarrow S_0 energy gap.

In summary, Scheme 2C explains the experimental evidence found when λ_{excB1} is used: (i) The dependence on the emission spectra with the excitation wavelength discussed in Figure 2A,B is because there are two different emission species for the 4-AP ICT states. The more stable one, the enol ICT, emits to a different and more stabilized ground state (S_0') than the keto ICT species (S_0). (ii) The reason that the TRES spectra shift to the blue with time (Figure 4B) is because at shorter time the emission arises predominantly from the keto S_1 -ICT species while at longer times the emission comes from the more relaxed enol S_1 -ICT species. (iii) The fact that the excitation spectra for the $S_0 \rightarrow S_1$ (S_1 -ICT) transition practically does not show the B2 band and is not equal to the absorption spectra is explained because the S_1 -ICT state cannot be populated from the enol S_2 -LE species.

It seems that the water-mediated proton transfer reaction in the 4-AP excited state is the key for the explanation of the unique 4-AP photophysics behavior in this solvent. Thus, and in order to shed further light on this process, we have investigated the deuterium isotope effect using D_2O as solvent. It is known the dramatic effect that the deuterium isotope has on ultrafast processes like proton transfer reaction.¹⁰ Das et al.¹⁰ have reported a marked increase in the lifetime and quantum yield of 4-AP in neat D_2O compared with water and they have explained the isotope effect considering that the acidic imide proton is replaced with deuterium in D_2O solvent. It is known that the $S_1 \rightarrow S_0$ nonradiative internal conversion process for 4-AP strongly depends on the N–H stretching mode. On deuteration of the imide proton the frequency of the N–D stretch decreases, causing considerable retardation in the nonradiative $S_1 \rightarrow S_0$ transition. Thus, the enhancement of the emission quantum yield and the lifetime of 4-AP in D_2O was ascribed to the slower $S_1 \rightarrow S_0$ nonradiative process in this solvent.^{10,34} Moreover, the tautomeric equilibrium is also altered replacing hydrogen by deuterium since the proton transfer reaction dramatically slows down.^{5,10} That is, after replacing H by D in the imide group, the excited-state tautomerism is precluded. Similar results were found using the 4-AP *N*-alkyl derivative where it was shown that the tautomerism is absent at least in the excited-state lifetime.^{3,35}

Figure S2A,B in the Supporting Information shows the 4-AP absorption and emission spectra in D_2O (at λ_{excB1} and λ_{excB2}), respectively. As it can be observed, the absorption spectrum consists of two bands that have the same wavelength maxima than in water. Thus, they correspond to the same electronic transitions discussed above. On the other hand, Figure S2B shows that the emission spectra do not depend on the

excitation wavelength used, results that differ markedly from water. Figure S2C shows the 4-AP excitation spectra in D₂O at different emission wavelengths and, as it can be seen, it is identical to the absorption spectra and both transitions are detected. Figure S2D shows the TRES spectra in D₂O and no time dependence was observed. Thus, the behavior of 4-AP in D₂O is similar to the one found in all the other protic solvents (Scheme 2B). In this way, the replacement of the N–H for N–D after 4-AP deuteration considerably inhibits the solvent-assisted proton transfer reaction from the keto S₂-LE species to give the enol S₂-LE species. Therefore, the keto S₁-ICT state can be populated from the keto S₂-LE state as in all protic solvents investigated (Scheme 2B). When the S₁-ICT state is populated from the ground state (λ_{excB1}), D₂O cannot assist in the proton transfer reaction and the 4-AP ICT state does not undergo the tautomerism. The emission comes only from the keto S₁-ICT state as it happens with the other protic solvents (Scheme 2B).

CONCLUSIONS

The unique behavior of 4-AP in water inspired us to perform a detailed investigation of 4-AP in different polar aprotic and protic solvents including water. The strong dependence of the emission band with the excitation wavelength found in water highlighted in the present work did not receive adequate attention in earlier works. The 4-AP absorption spectrum consists of two bands that correspond to different electronic transitions: the band around 300 nm is the S₀ → S₂ (π - π^* S₂-LE) transition (B2 band) and the band around 370 nm is the S₀ → S₁ (S₁-ICT) transition (B1 band). Moreover, in all the organic solvents studied, the emission spectra (steady and time resolved) are the same independent of the excitation wavelength used, i.e., B1 or B2, which is the expected behavior for a fluorophore emitting from its lowest excited state (S₁-ICT). In water the emission spectra have different maxima wavelengths depending on the excitation wavelength used. Also, while the fluorescence decays of 4-AP in water exhibit no emission wavelength dependence at λ_{excB2} , the situation is quite different when λ_{excB1} is used. Now, we found a time-dependent emission spectrum with our nanosecond equipment resolution and, the emission spectra shift to the blue with time. We want to call the attention that we do not say that 4-AP, an organic compound with a small S₁–S₂ energy gap, does not obey Kasha's rule. Rather than that, we explain the 4-AP photo-physics in all the solvents studied considering the solvent proton transfer reaction upon excitation that undergoes the 4-AP LE state. When λ_{excB2} is used, water is the only solvent that produces the enol S₂-LE as the majority species. The enol S₂-LE species cannot yield the S₁-ICT state and the emission comes from the relaxed enol excited state. On the other hand, when λ_{excB1} is used the S₁-ICT state is populated and the emission arises from the keto and enol S₁-ICT states species. The latter species goes to a very well stabilized ground state with the consequent increases in the electronic transition energy gap.

Thus, we show the importance of understanding the chemistry of 4-AP in homogeneous media before using it in more complex environment.

ASSOCIATED CONTENT

Supporting Information

Table S1 with the correlations of 4-AP absorption maxima and emission frequency (10^{-3} cm^{-1}) according to eq 1, Figure S1

with excitation spectra of 4-AP in water at different emission wavelengths, and Figure S2 with (A) absorption spectra of 4-AP in D₂O; (B) emission spectra of 4-AP in D₂O at different excitation wavelength; (C) excitation spectra of 4-AP in D₂O at different emission wavelength; (D) normalized TRES of 4-AP in D₂O. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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DEDICATION

§Dedicated to the memory of Prof. Dr. Elsa Abuin who died on April 18, 2012.

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