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Solvent Effect on Excited-State Hydrogen Transfer and Luminescent Properties of Anthranilic Acid in Solution and in Polymer Matrix

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ABSTRACT

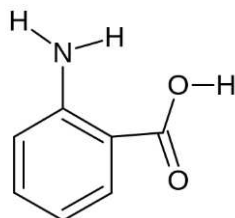
Luminescent properties of anthranilic acid (AA) are studied in solution and in polymer matrix using polar and nonpolar solvents. The fluorescence of AA is found to depend on the environment, which favours improved molecular orientation. In dilute solution, emission arises from the neutral molecule and exhibits a smaller Stokes shift from the long-wavelength absorption band. The fluorescence from concentrated solution shows a large, Stokes-shifted, intense emission band, and the estimated values of Stokes shift are found to be solvent dependent. The Stokes-shifted fluorescence band in polar ethanol and water is attributed to the rotamer formed by intramolecular hydrogen bonding and to a water-bridge structure of AA, respectively. The water cluster of AA formed by solvation in aqueous solution was examined through the fluorescence properties of the molecule. The strongly Stokes-shifted emission band in concentrated carbon tetrachloride solution of AA is assigned to an intermolecular hydrogen-bonded dimer. Concentration-dependent fluorescence studies indicated increased emission intensity of AA without any accompanying spectral change. Polymer films doped with AA were prepared from its solutions in the solvents used in the present study. The behaviour of AA in the polymer matrix was examined by recording the fluorescence spectra of AA-doped polymer films. Polystyrene films doped with AA exhibited dual fluorescence: the band at 410 nm, corresponding to the rotamer formed by intramolecular hydrogen bonding, decreased with increasing wt% of AA, while the band at 437 nm increased. The emission band corresponding to the water-bridge structure of AA was not observed in the AA-doped PVA films. The AA-doped polymer films prepared in the present study by the solvent-evaporation technique are of potential use in electroluminescent light-emitting devices.

Keywords: Anthranilic acid; Excited-state hydrogen transfer; Solvatochromism; Fluorescence; Polymer-doped films; Stokes shift

1. INTRODUCTION

The excited-state intramolecular hydrogen transfer (ESIPT) reaction has been investigated in substituted aromatic acids such as anthranilic acid (AA) [1, 2], salicylic acid (SA) [3], N-phenyl anthranilic acid [4], and others, in different solvents. The observation of a strongly Stokes-shifted visible fluorescence band has been interpreted as arising from an exothermic

intramolecular proton-transfer process in the S_1 (π - π^*) excited state. Weller assumed that the excited-state product is a zwitterion, which led to the concept of intramolecular proton transfer. However, it has recently been shown that in such a process a hydrogen atom, rather than a proton, is moving, since the change in electric dipole moment is not significant [5]. Such processes are therefore currently referred to as excited-state hydrogen-transfer processes and are known to exhibit isomerisation [6], tautomerisation [7], and photochromism [8], among other phenomena. The structural formula of anthranilic acid is shown in Fig. 1.



2-aminobenzoic acid

Fig. 1. Structural formula of anthranilic acid (2-amino benzoic acid).

Anthranilic acid is a fluorescent label used in fluorescence resonance energy transfer studies and enzymatic cleavage assays; it is 2-amino benzoic acid, with the structural formula shown above. Solution studies show that the fluorescence behaviour of AA differs in polar and nonpolar environments, and its properties also depend on the amino group to which it is bound. AA is intensively used as an extrinsic fluorescence probe for peptides [9]. AA dispersed in a rigid matrix is expected to exhibit interesting photophysical properties arising from photo-ion [10], phototautomer [11], hydrogen-dislocation [12], or hydrogen-bonding [13] behaviour, as in other bifunctional acids. Self-organised AA molecules in a rigid matrix are expected to come closer together than even in concentrated solution, favouring intermolecular hydrogen bonding and dimer formation [4]. Polymers are known to provide a good rigid matrix; AA dissolved in a polymer solution in a suitable solvent can be deposited by spin-coating and solvent-evaporation to obtain doped films. Such polymer films are important in the fabrication of light-emitting materials [14], photochromic films [15], and scintillator systems [16, 17]. The fluorescence and absorption spectroscopy of AA-doped polymer films help to explore their utility in device applications [18–20]. This paper reports studies on the absorption and fluorescence properties of AA in different polar and nonpolar solvents and of AA-doped polymer films prepared from these solutions, comparing the film and solution fluorescence to understand the photophysical behaviour of AA molecules.

2. MATERIALS AND METHODS

2.1. Reagents

Anthranilic acid, obtained from S.D. Fine Chemicals, was recrystallised from its saturated solution in ethanol (analytical-reagent grade); its purity was ascertained from its melting point (147°C), electronic spectrum, and similar fluorescence spectra when excited at different excitation wavelengths. The solvents carbon tetrachloride, toluene, ethanol, and water were used after distillation. Polystyrene (PS, Asian Chemicals), polyvinyl pyrrolidone (PVP, Sisco

Research Lab), and polyvinyl alcohol (PVA, Rasayan Lab) were used as polymers for preparing AA-doped polymer films.

2.2. Method

Stock solutions of AA (0.1 wt.%) were prepared in different solvents, and solutions of varying concentration were prepared from these stock solutions. The solutions were deaerated by purging with nitrogen gas prior to fluorescence and absorption measurements, and were further used to prepare AA-doped polymer films. Polymers soluble in the solvents used in the study were selected, and a calculated amount of polymer (2 wt.%) was dissolved in 10 mL of solution and poured into a polypropylene dish kept in an incubator at 40°C [20]. AA was thus doped in polystyrene (PS), polyvinyl alcohol (PVA), and polyvinyl pyrrolidone (PVP) from its solutions in carbon tetrachloride, toluene, water, and ethanol, respectively.

2.3. Film Thickness

The thickness (t) of the films was determined using the relation

$$t = W / (A \cdot d) \quad (1)$$

where W is the weight of the film, A is its area, and d is its density. The thickness of the films was found to be ≈ 0.1 mm [20].

2.4. Optical Measurements

Absorption spectra of the solutions were measured on a UV-Visible spectrophotometer (CHEMITO UV Model-2100). Fluorescence and excitation spectra were recorded on a JASCO (Japan) FP-750 PC spectrofluorometer, using the front-surface attachment provided with the instrument for measurements on the films.

3. RESULTS AND DISCUSSION

The excitation spectra of dilute AA solution (0.00014 wt.%) in toluene, carbon tetrachloride, ethanol, and water are shown in Fig. 2. The excitation spectra in most solvents show sharp bands in the region 300–400 nm and weaker bands in the region 250–300 nm, corresponding to the low-energy π – π^* and high-energy n – π^* transitions, respectively. The π – π^* transition is the most probable in AA because of the greater conjugation and delocalised π -electron system, and the corresponding vibronic bands are more intense. The fluorescence emission of AA is solvent sensitive: the bands are sharp and solvent dependent, and a spectral shift is evident. Unlike salicylic acid and methyl salicylate [21], AA solution does not show dual emission.

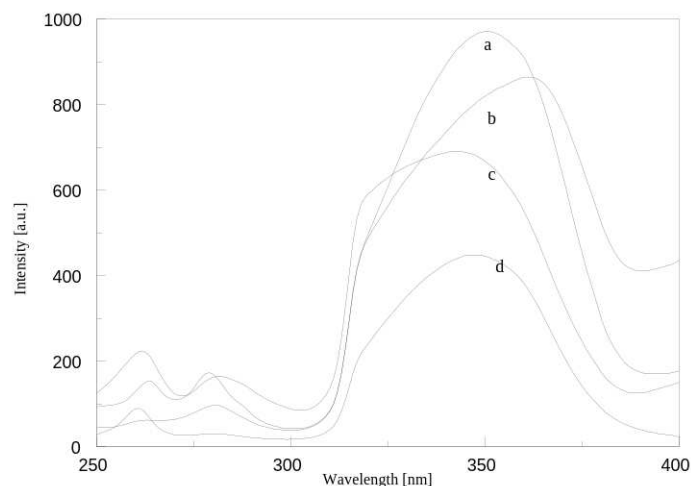


Fig. 2. Excitation spectra of 0.00014 wt.% AA in different solvents: (a) toluene, (b) carbon tetrachloride, (c) ethanol, (d) water.

The absorption spectra of AA solutions are identical in shape to the excitation spectra. The values of absorption (λ_{ab}), excitation (λ_{ex}), and fluorescence (λ_{em}) maxima, together with the solvent parameters, are summarised in Table 1. Solvent polarity exerts an appreciable effect on the ground-state electronic transition: the absorption wavelength is blue-shifted from low-polarity solvents such as toluene and carbon tetrachloride (~ 340 nm) to the high-polarity solvent water (~ 320 nm), owing to solvation of the solute by the solvent. In contrast, the fluorescence spectrum of AA shows a pronounced red shift with increasing solvent polarity, λ_{em} moving from 396 nm to 420 nm on going from toluene to water. The emission colour changes from violet in toluene to blue in water under 365 nm UV excitation. This solvatochromism is attributed to photo-induced charge transfer in the excited state, associated with stabilisation of the polar emissive excited state by polar solvents [22]. The absorption spectra of AA in different solvent are shown in Fig.3.

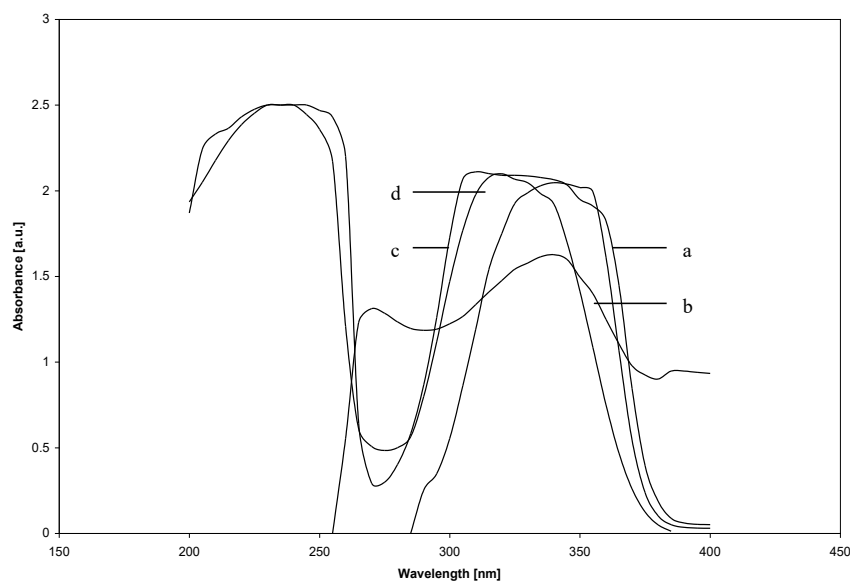


Fig. 3. Absorbance spectra of AA in different solvents: (a) toluene, (b) carbon tetrachloride, (c) ethanol, (d) water.

To better understand the solvent effect on AA fluorescence, the Stokes shift (ν) in different solvents was calculated and correlated with the solvent-polarity parameter, $f(\epsilon, n)$, using

$$\Delta\bar{\nu} = \bar{\nu}_{ab} - \bar{\nu}_{em} = \frac{2\Delta f(\mu_e - \mu_g)^2}{hca^3} + const \quad (2)$$

where ϵ and n are the dielectric constant and refractive index of the solvent, respectively. The values of $f(\epsilon, n)$ and ν are listed in Table 1. The Lippert–Mataga relation was used to describe the solvatochromic behaviour of the fluorophore [23]:

$$\Delta\nu = [2f(\epsilon, n)/hca^3] \cdot (\mu_e - \mu_g)^2 + constant \quad (3)$$

where h is Planck's constant, c is the velocity of light, a is the Onsager radius of the chromophore, and μ_e and μ_g are the dipole moments in the excited and ground states, respectively. Fig. 4 shows the plot of ν versus $f(\epsilon, n)$, the linearity of which confirms the validity of the Lippert–Mataga relation.

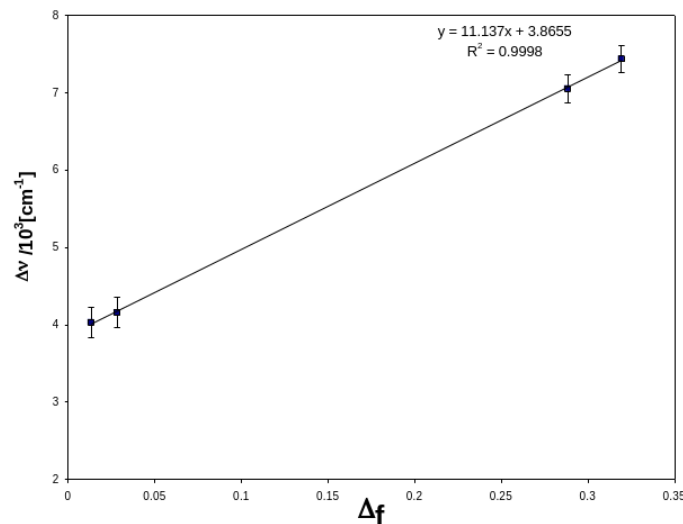


Fig. 4. Plot of Stokes shift (ν) of AA versus solvent-polarity parameter, $f(\epsilon, n)$.

The straight line is a least-squares fit of the form $\nu = a \cdot f(\epsilon, n) + b$, expressed in 10^3 cm^{-1}

$$\nu = a \cdot f(\epsilon, n) + b \quad (4)$$

The linear fit yields $a = 11.137$ and $b = 3.865$, with a squared correlation coefficient of 0.9998. The error bars in Fig. 4 represent the extreme values of ν . The magnitude of the fitted coefficient shows that the hydrogen-bonding properties of the solvents dominate the overall solvent effect. Because only the effect of the solvent-polarity parameter on the Stokes shift is examined in this work, a Student's t-test comparing additional exploratory variables (polarizability, hydrogen-bond donor/acceptor character, etc.) was not undertaken. The linear dependence of ν on $f(\epsilon, n)$, together with the large slope of the plot, indicates that the excited-state species possesses a larger dipole moment than the ground state, consistent with substantial charge redistribution upon excitation [24, 25].

3.1. Effect of Concentration on AA Fluorescence

Concentration-dependent fluorescence studies of AA show more pronounced spectral effects in water and carbon tetrachloride than in toluene and ethanol. The fluorescence spectra of AA in toluene (Fig. 5) show a small Stokes shift of about $4.221 \times 10^3 \text{ cm}^{-1}$ as the AA concentration is increased from 0.0001 to 0.005 wt.%. The emission intensity at λ_{em} increases with concentration owing to increasing intermolecular attractive forces, although the expected hydrogen-bonded-dimer emission is not seen at longer wavelength. The fluorescence spectrum of AA in ethanol (Fig. 6) shows a similar concentration dependence; the emission, at 408–410 nm, is assigned to the intramolecular hydrogen-bonded (IHB) species formed between the amino (donor) and carboxylic (acceptor) groups in the ground state, whose intensity increases with concentration. In water (Fig. 7), the emission wavelength ($\lambda_{\text{em}} = 420 \text{ nm}$) does not change with concentration; the different behaviour in water and ethanol is attributed to different modes of solvation, with the amphoteric AA molecule forming a strong intermolecular hydrogen-bonded species in the excited state.

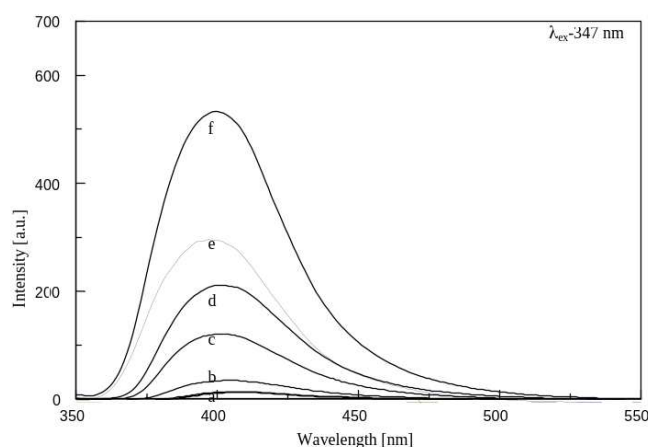


Fig. 5. Fluorescence spectra of AA in toluene at different concentrations: (a) 0.000014, (b) 0.00014, (c) 0.0014, (d) 0.001, (e) 0.01, (f) 0.05 wt.%.

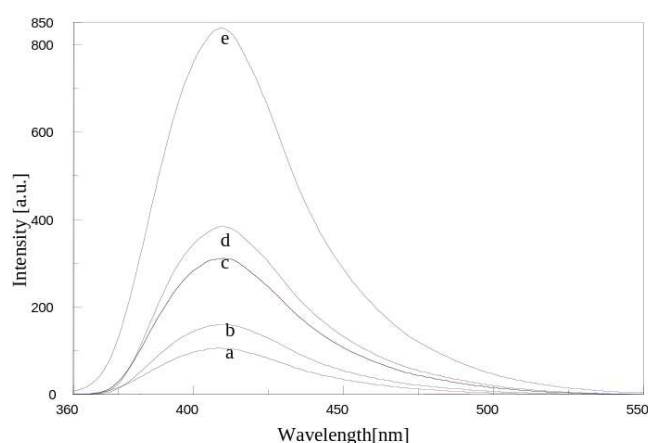


Fig. 6. Fluorescence spectra of AA in ethanol at different concentrations: (a) 0.000014, (b) 0.00014, (c) 0.0014, (d) 0.01, (e) 0.05 wt.

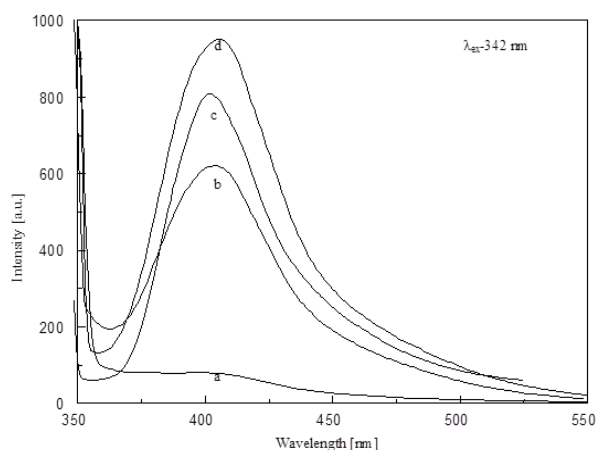


Fig. 7. Fluorescence spectra of AA in water at different concentrations: (a) 0.000014, (b) 0.00014, (c) 0.0014, (d) 0.01, (e) 0.05 wt.%.

The proposed water-bridge structure of AA was further confirmed by fluorescence studies of AA-doped PVA films cast from aqueous solution (Fig. 8). The emission of the doped films peaks at 407 nm, and the 420 nm band, characteristic of solution in water, disappears once AA is dispersed in the rigid PVA matrix; emission intensity increases with the wt.% of AA in the film. Even solid AA shows a single Stokes-shifted emission band at 410 nm, indicating IHB formation between neighbouring AA molecules in the crystalline state. It is therefore concluded that only in aqueous solution does a water molecule form an intermolecular bridge with AA through hydrogen bonding, as shown in Scheme 1.

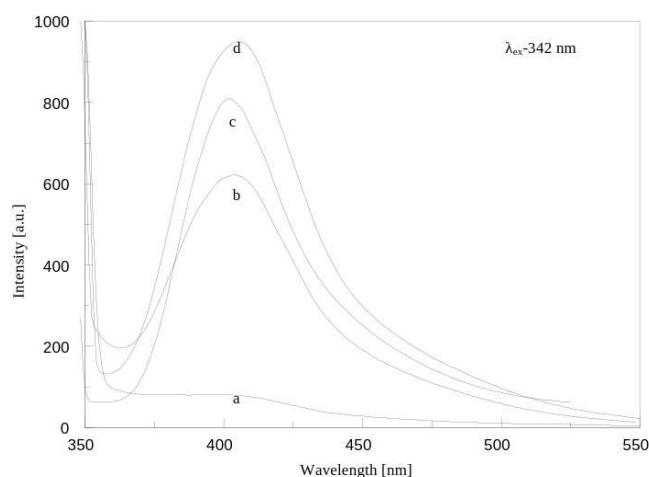


Fig. 8. Fluorescence spectra of AA doped in PVA film (cast from water solution) at different concentrations: (a) 0.000014, (b) 0.00014, (c) 0.0014, (d) 0.01, (e) 0.05 wt.%.

3.2. Fluorescence of AA in Mixed Solvents

The fluorescence spectra of AA in ethanol–water mixtures show that the wavelength of maximum emission shifts gradually to the red as the percentage of water increases (Fig. 9). The 410 nm emission band in 100% (v/v) ethanol undergoes a red shift to 420 nm as the water content increases, ultimately matching the spectrum of AA in pure water. This indicates that intramolecular hydrogen bonding is progressively suppressed and solvation of AA by water, to form the water-bridge structure, increases correspondingly. The intensity of

the 420 nm band also increases with increasing water content, consistent with its assignment to the water-bridge structure; the increase in the density of the water/ethanol mixture appears to enhance solvation of AA, red-shifting λ_{em} .

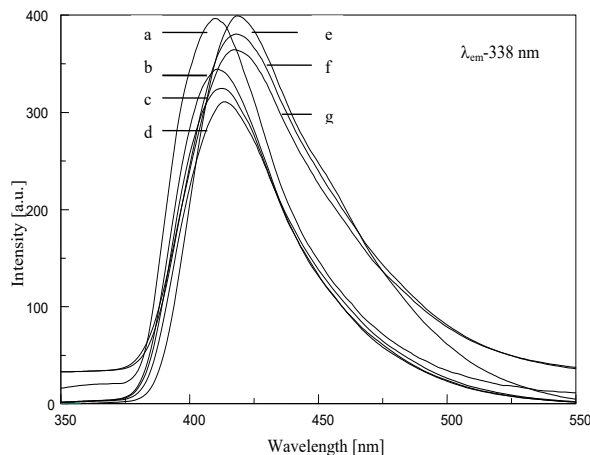


Fig. 9. Normalised fluorescence spectra of AA in water/ethanol mixtures: (a) 0, (b) 10, (c) 20, (d) 40, (e) 80, (f) 90, (g) 100 vol.% water.

3.3 Effect of Acid and Base Addition

Amino-benzoic acid exists as a neutral, uncharged molecule in aqueous solution; the near-identity of the excitation and absorption spectra indicates that solvation does not occur in the ground state, and that solvation of AA by water molecules, and the resulting emission, occurs only in the excited state. In the presence of base (NaOH), deprotonation of the carboxylic acid group in water gives the carboxylate anion of AA in the ground state [26-28]; excitation of this anion at 338 nm gives an emission band at 401 nm (Fig. 10). Because the emission is blue-shifted, formation of a phototautomer via intramolecular hydrogen bonding is unlikely, and the emission is instead assigned to excitation of the anion, as summarised in Scheme 1. Addition of acid (HCl) has no effect on the spectral characteristics of AA, and the emission remains at 419 nm [29].

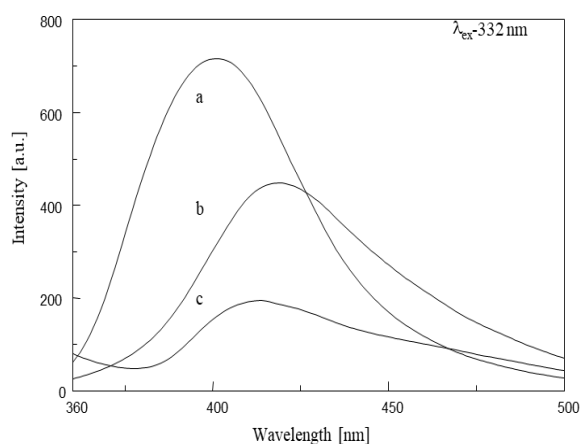


Fig. 10. Fluorescence spectra of AA in the presence of (a) base (NaOH), (b) acid (HCl), and (c) solid AA.

3.4 Fluorescence of AA in Carbon Tetrachloride Solution

The fluorescence spectra of AA in carbon tetrachloride change appreciably with concentration (Fig. 11). At lower concentration, the solution spectrum peaks at 400 nm, with intensity increasing with concentration; above 0.0014 wt.%, the 400 nm band is suppressed and a new band at 436 nm appears, whose intensity grows with AA concentration. This indicates that AA exists in two distinct excited-state forms in carbon tetrachloride: the strongly Stokes-shifted 436 nm band is assigned to the intermolecular hydrogen-bonded dimer, and the 400 nm band to the neutral molecule. Excited-state dimer formation is further confirmed by the fluorescence of AA-doped PS films cast from the same carbon tetrachloride solution (Fig. 12): films with lower AA content show a single band whose intensity increases with concentration, while films above 0.01 wt.% AA show dual emission. Intermolecular dimer formation is favoured at the higher local concentration of solute within the film, and the resulting band is red-shifted [4]; the 436 nm band is therefore assigned to the excited-state dimer of AA. The excited-state behaviour of AA in the different solvents is summarised in Scheme 1.

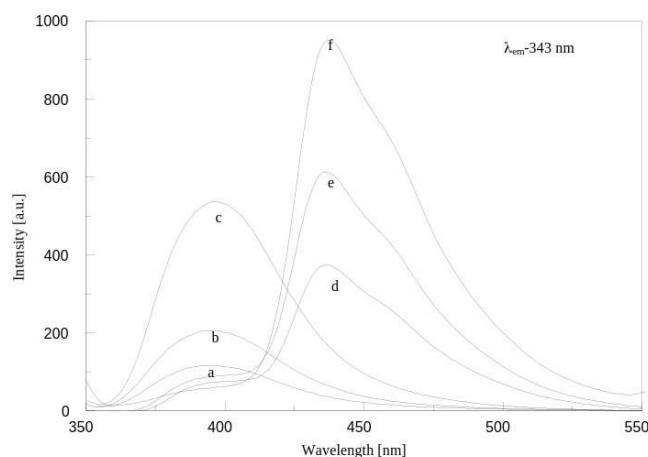


Fig. 11. Fluorescence spectra of AA in carbon tetrachloride solution at different concentrations: (a) 0.000014, (b) 0.00014, (c) 0.0014, (d) 0.001, (e) 0.01, (f) 0.05 wt.%.

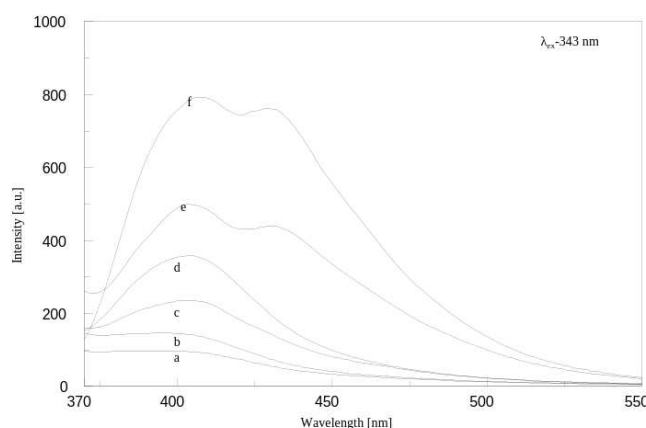
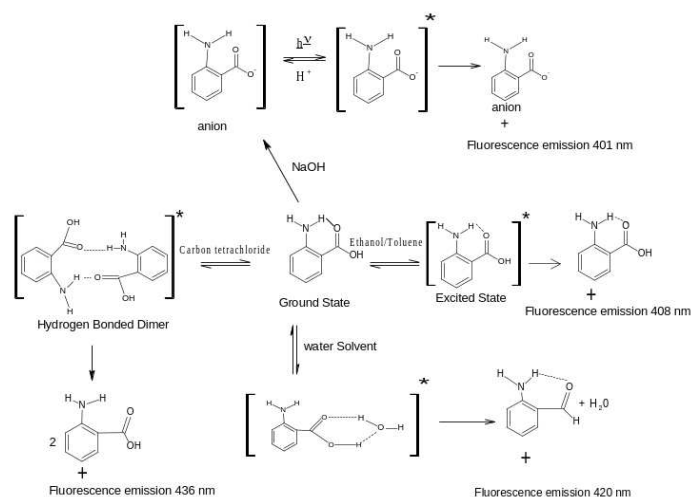


Fig. 12. Fluorescence spectra of AA-doped PS films cast from carbon tetrachloride solution at different concentrations: (a) 0.000014, (b) 0.00014, (c) 0.0014, (d) 0.001, (e) 0.01, (f) 0.05 wt.%.



Scheme 1. Proposed excited-state species of anthranilic acid in the different solvent environments studied.

Table 1. Photophysical properties of 0.00014 wt.% AA in different solvents.

Solvent	$f(\epsilon, n)$	λ_{ab} (nm)	λ_{em} (nm)	γ (cm^{-1})
Toluene	0.01302	340, 347	397	4.221×10^3
Carbon tetrachloride	0.0281	340, 348	396	4.159×10^3
Ethanol	0.2882	318, 364	410	7.056×10^3
Water	0.3193	319, 357	420	7.440×10^3

$f(\epsilon, n)$: solvent-polarity parameter; λ_{ab} : absorption maximum; λ_{em} : emission maximum; γ : Stokes shift.

4. CONCLUSION

Formation of an intramolecular hydrogen-bonded rotamer of AA in the excited state is evident from the fluorescence spectra recorded in toluene and ethanol, whereas dimer formation is favoured in carbon tetrachloride at higher concentration; dilute AA solution in carbon tetrachloride does not show the Stokes-shifted dimer emission. Analysis of the solvent effect on the Stokes shift shows that the hydrogen-bonding properties of the solvent dominate, and are most pronounced in the aqueous environment. The presence of water in ethanol solution shifts the emission towards the red, owing to formation of the water-bridge structure. The blue shift and intensity increase observed in the fluorescence of AA on going from aqueous to nonpolar solution are significant for the use of AA as a fluorescence probe of the local environment of protein molecules.

The fluorescence of the AA-doped films is spectrally similar to that of the corresponding solutions. Casting AA in a polymer film from the solvent environments studied yields AA-doped polymer films that emit towards the red side of the visible spectrum, with high luminescence intensity. The film-preparation technique developed in this work is therefore suitable for application in electroluminescent light-emitting devices.

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Data Availability

The data generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of Interest statement

The authors declare that, they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

“Ethics and Consent to Participate declarations: not applicable.”

“Consent to Publish declaration: not applicable.”

“We confirm that there is no conflict of interest among any of the authors. In accordance with submission guidelines the policies of Journal.”

Credit authorship contribution statement

Madhavi Jadhav: Methodology, Investigation, Supervision, Conceptualization, Methodology, Writing-original draft, Writing & editing. **Uday Lad:** Visualization, Writing & editing. **Nidhi Sharma:** Formal analysis. **Dipali Hodade:** Writing & editing. **Vaishnavi Hubale:** Writing & editing.

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