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ORIGINAL PAPER

Photophysical Studies on the Interaction of Formamide and Alkyl Substituted Amides with Photoinduced Electron Transfer (PET) Based Acridinedione Dyes in Water

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Abstract Interaction of photoinduced electron transfer (PET) based acridinedione dye (ADR 1) with amides like formamide, acetamide and dimethylformamide (DMF) were investigated by fluorescence spectral techniques. A fluorescence enhancement accompanied with a blue shift in the emission maximum was observed on the addition of amides to ADR 1 dye, which possess C_6H_4(p-OCH_3) in the 9th position of the basic acridinedione ring. The extent of fluorescence enhancement and the blue shift in the emission maximum of ADR 1 dye is of the order of DMF > acetamide > formamide. DMF, which is more hydrophobic and less polar, results in a higher extent of fluorescence enhancement and a larger shift in the emission maximum towards the blue region. On the addition of amides, the ADR 1 dye prefers to orient towards a more hydrophobic phase surrounded by more number of amide molecules. The fluorescence enhancement of ADR 1 dye is attributed to the suppression of PET process occurring through space. The influence of the hydrophobic nature and the polarity of the amides on the excited state properties of acridinedione dyes are elucidated by steady-state and time resolved fluorescence measurements.

Keywords Acridinedione dyes · Amides · Photoinduced electron transfer · Fluorescence enhancement · Time resolved fluorescence

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Introduction

Acridinedione dyes [1] have a structural similarity with purine derivatives, NADH (Nicotinamide adenine dinucleotide) which makes the dye significantly important in the field of biomedical research. The fluorescence emission maximum, quantum yield and lifetime of the acridinedione dyes varies drastically based on the nature of functional groups present in the 9th and 10th positions [2–4]. Consequently, due to the large variation in the photophyical properties exhibited by acridindione dyes, these dyes have been employed as an ideal host molecule. Owing to its bichromophoric property, acridinedione dyes act as an ideal fluorescent probe for hydrophobic nanocavities [5], hydrogen-bonding assemblies [6, 7], proteins [8] and act as fluorescent sensors [9–11].

Acridinedione dyes have been classified into PET and non-PET based on the substitution in the 9^{th} position of the basic acridinedione ring as shown in Fig. 1. The dyes containing an electron donating moieties (−OCH_3) and –N(CH_3)$_2$ in the para position of C9 carbon of acridinedione dyes are classified as PET dyes.

PET process is the most commonly exploited mechanism for the design of fluorosensors and the resulting fluorescence enhancement on the addition of guest or metal ions is due to the suppression of the PET process [12–14]. Among the PET based acridinedione dyes, the dyes containing (p-OCH_3) in the 9th position exhibits a PET behaviour through space in all solvents [6–8]. A large variation in the fluorescence quantum yield, fluorescence emission and lifetime of PET based acridinedione dye is observed compared to that of the non-PET based dyes. Further, fluorescence spectral techniques has been employed as a tool in elucidating the nature of interaction and the most probable location of ADR 1 dye with various guest

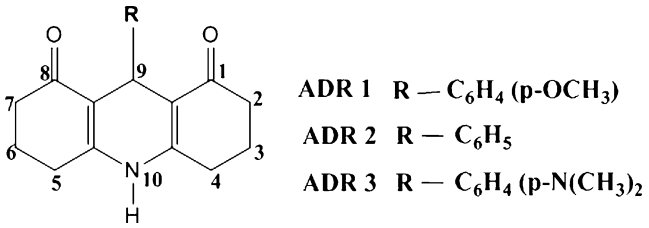


Fig. 1 Structure of PET and non-PET based acridinedione dyes. PET dyes- ADR 1 and ADR 3, non-PET dye- ADR 2

molecules like cyclodextrins urea, bovine serum albumin (BSA) and guanidine hydrochloride (GuHCl) in aqueous solutions [5–8]. It has been well documented that the fluorescence spectral studies (Fluorescence emission maximum and lifetime) of ADR 1 dye in the presence of cyclodextrins or urea or guanidine hydrochloride (GuHCl) or bovine serum albumin (BSA) in aqueous solution is found to be entirely different with that of each other, which clearly reveals that the photophyical properties of ADR 1 dye in different hydrogen-bonding self assemblies are found to be entirely different [6–8]. The present study is largely focused to probe the nature of interaction and the excited state spectral properties of PET and non-PET based acridinedione dyes with hydrogen-bonded self assemblies of water soluble aliphatic amides like formamide, acetamide and DMF.

Amides are classified as hydrogen-bonded self assemblies, which are also well oriented by strong hydrogen-bonding interactions in water through its hydrogen-bonding donor and acceptor sites. The amides (except acetamide, which is a solid) represent an important class of organic solvents due to their large variation in their viscosity, dielectric constant and hydrogen-bonding acceptor and donor numbers. The polarity of amide largely depends on its alkyl substitution. The structure of DMF-water hydrogen-bonding arrangement is entirely different from that of formamide-water structure and this is presumably attributed to the decrease in the number of hydrogen-bonding sites in DMF than in formamide. Further, the polarity of the amides and the different hydrogen-bonding arrangement of amides (cyclic, sheet, ring, combination of open and closed structures) [15–17] and can influence the excited state properties of fluorescent probes.

Experimental Methods

The amides, formamide, acetamide and DMF were purchased from qualigens India Ltd, and water used in the present investigation was triply distilled. The acridinedione dyes were prepared by following the procedure reported in the literature [18]. The dye stock solution was freshly prepared and the concentration of the dye (1.5×10^{-5} M) was fixed such that the absorbance at the wavelength of excitation (378 nm) was less than 0.2.

Absorption spectra were recorded in Agilent 8453 diode-array spectrophotometer. Fluorescence measurements were carried out in Perkin-Elmer MPF-44B fluorescence spectrophotometer interfaced with PC through Rishcom-100 multimeter. Time resolved fluorescence decays were obtained by the time-correlated-single-photon-counting (TCSPC) method [6]. A diode-pumped Millenia V CW laser (Spectra Physics) was used to pump the Ti-Sapphire rod in a Tsunami picosecond-mode locked Laser system (Spectra Physics). The 750 nm (85 MHz) beam from the Ti-Sapphire laser was passed through a pulse picker (Spectra Physics, GWU 23PS) to generate 4 MHz pulses. The second harmonic output was generated by a flexible harmonic generator (Spectra Physics, GWU 23PS). A vertically polarized 377 nm laser was used to excite the sample. The fluorescence emission of the acridinedione dyes was monitored at the magic angle (54.7°). This was counted by a MCP-PMT apparatus (Hamamatsu R3809U) after alligned through the monochromator and was processed through a constant fraction discriminator (CFD) a time-to-amplitude converter (TAC) and a multichannel analyzer (MC). The instrument response function for this system is around 52 ps. The fluorescence decay was obtained and was further analyzed by using IBH Jobin Yvon (UK) software (DAS-6).

Results and Discussion

Absorption Spectral Studies

The absorption spectrum of ADR 1 and ADR 2 dyes exhibit a maximum around 370–380 nm in water. This peak is assigned to the intramolecular charge transfer(ICT) from the ring nitrogen to the carbonyl oxygen in the acridinedione ring moiety [19, 20]. No significant change in the ICT absorption maximum of ADR 1 dye was observed on the addition of formamide as shown in Fig. 2**.** Similarly, the addition of formamide does not influence the ICT absorption maximum of ADR 2 dye. The absorption spectral studies of ADR 2 dye interaction with formamide is provided in the supporting information (Figure S1).

Absorption spectral studies of formamide to ADR 1 and ADR 2 dyes clearly illustrates that the addition of formamide does not influence the ICT absorption maximum of both PET and non-PET based ADR dyes. Interaction of acetamide and DMF with acridinedione dyes also results no significant change in the ICT absorption maximum similar to that of formamide.

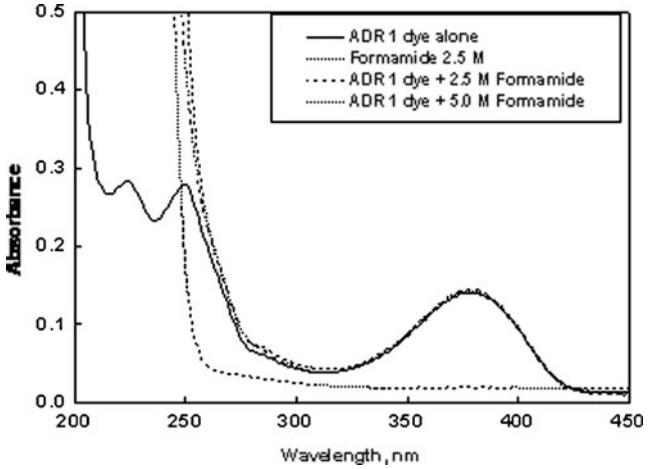


Fig. 2 Absorption spectra of ADR 1 dye in the absence and presence of formamide in water. 1) ADR 1 dye alone(1.5×10^{-5} M), 2) formamide 2.5 M, 3) ADR 1 dye + formamide 2.5 M, 4) ADR 1 dye + formamide 5.0 M

Emission Spectral Studies

The emission spectrum of ADR 1 and ADR 2 dyes exhibit maximum around 437 nm. The quantum yield of ADR 1 dye is 0.10±0.02 compared to that of ADR 2 dye (0.90± 0.02) and has been well established [6]. The relatively low quantum yield of ADR 1 dye is attributed to the presence of electron donating methoxy group in the para position of the phenyl ring. Addition of formamide to ADR 1 dye results in a fluorescence enhancement accompanied with a blue shift in the emission maximum as shown in Fig. 3. Addition of acetamide and DMF to ADR 1 dye also results in a fluorescence enhancement accompanied with a larger blue shifted emission compared to that of formamide. The emission spectrum of ADR 1 dye on the addition of acetamide is provided in supporting information (Figure S2).Interaction of ADR 1 dye with amides illustrates that the extent of shift in the emission maximum and fluorescence enhancement is found to be in the order of formamide < acetamide < DMF as shown in Figs. 4 and 5 respectively, which is in the order of hydrophobicity of amides.

Of the amides, formamide is more polar compared to that of water and the dielectric constant of formamide is 1.5 fold greater than that of water. If the shift in the emission maximum of ADR 1 dye had been due to change in the polarity of the medium, a red shift in the emission maximum of ADR 1 dye would have been resulted on the addition of formamide. On the contrary, a shift in the emission maximum towards the blue region was observed, which illustrates that the shift in the emission maximum is not influenced by the polarity of amides. Further, the emission spectra of ADR 1 dye in neat formamide or DMF also resulted in a blue shift in the emission maximum, when compared with that of water.

The extent of fluorescence enhancement of ADR 1 dye on the addition of formamide and DMF is 2.4 and 7.0 fold respectively as shown in Fig. 5. It is well known that the fluorescence intensity of probes is influenced by the viscosity of the solutes, and if the fluorescence enhancement of ADR 1 dye is due to the viscosity of amides, a larger extent of enhancement would have been resulted for formamide (3.302 $mPas^{-1}$) rather than DMF (0.796 $mPas^{-1}$). From the plot of extent of fluorescence enhancement Vs the concentration of amides [M], it is

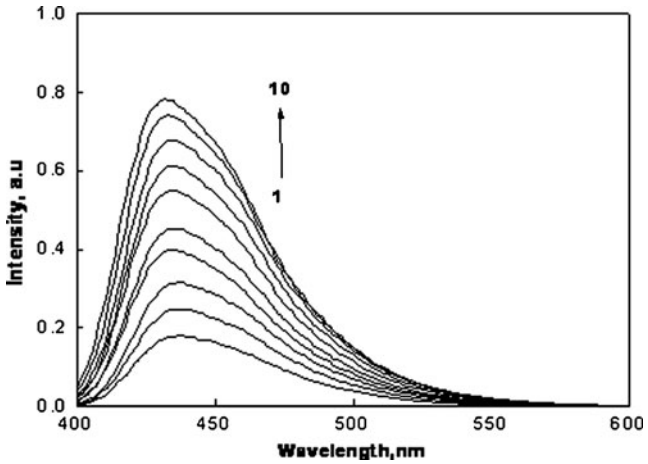


Fig. 3 Fluorescence spectra of ADR 1 dye in the absence and presence of formamide in water λ_{ex}378 nm. 1) ADR 1 dye alone (1.5×10^{-5} M), 2) ADR 1 dye + formamide 2.5 M, 3) ADR 1 dye + formamide 5.0 M, 4) ADR 1 dye + formamide 7.5 M, 5) ADR 1 dye + formamide 10.0 M, 6) ADR 1 dye + formamide 12.5 M, 7) ADR 1 dye + formamide 15.0 M, 8) ADR 1 dye + formamide 17.5 M, 9) ADR 1 dye + formamide 20.0 M, 10) ADR 1 dye + formamide 22.5 M

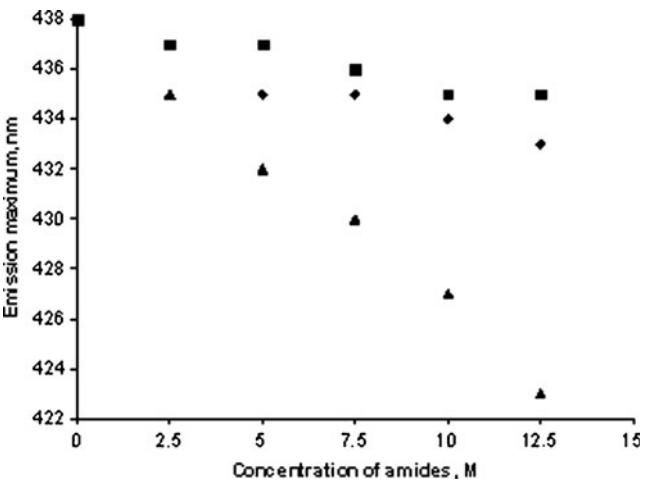


Fig. 4 Shift in the emission maximum of ADR 1 dye as a function of amides in water. formamide (■), acetamide (♦) and DMF (▲)

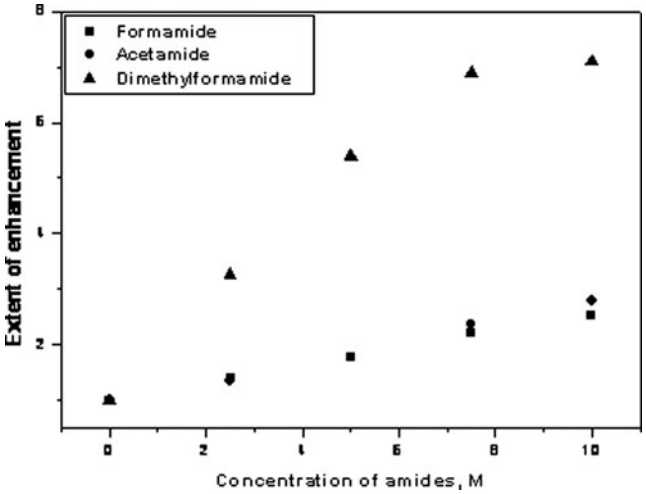


Fig. 5 Extent of fluorescence enhancement of ADR 1 dye on the addition of formamide (■), acetamide (♦) and DMF (▲)

evident that the increase in the fluorescence intensity of ADR 1 dye is not attributed to the viscosity of the amides.

Based on the extent of fluorescence enhancement of ADR 1 dye on the addition of amides, we envisage that the increase in the fluorescence intensity is either attributed to the increase in the hydrophobicity of the amides or due to the gradual decrease in the number of hydrogen-bonding donor groups in amides (DMF > acetamide > formamide). The possibility of hydrogen-bonding interaction between the dye and amides is ruled out based on the following experimental studies.

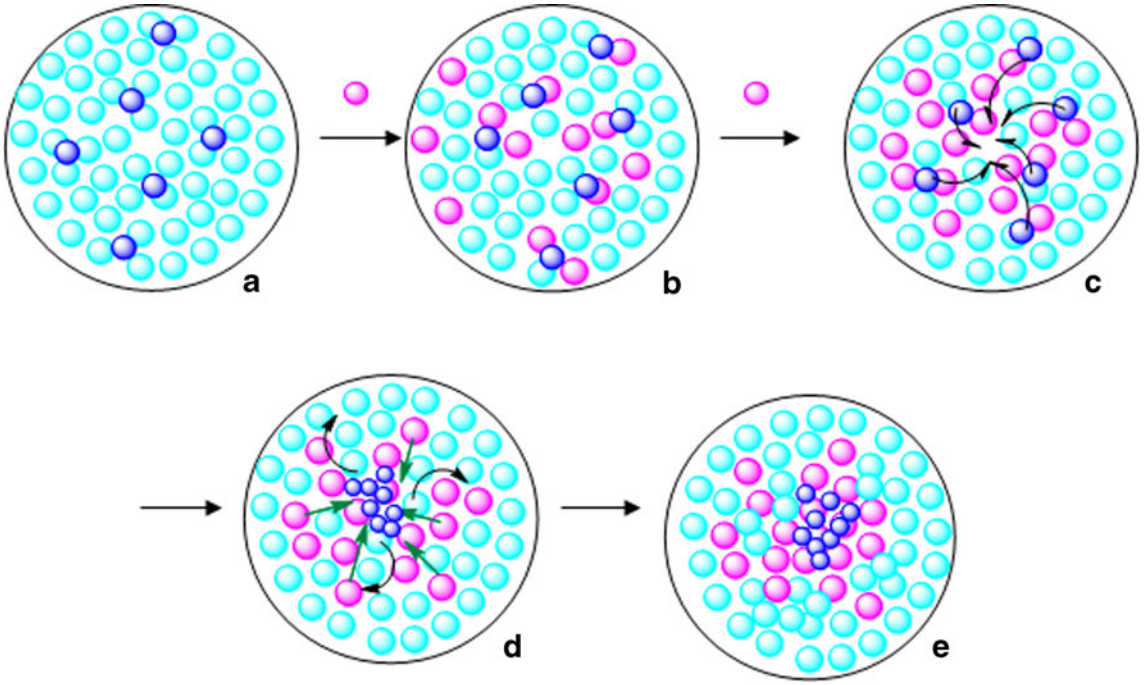
Acridinedione dyes possessing a N-H moiety in the 10^{th} position exhibit a red shifted emission when the N-H hydrogen is hydrogen-bonded. In the present study, no significant shift in the absorption maximum towards the red region is observed, which clearly indicates the absence of a hydrogen-bonding interaction of C = O oxygen of amides with the N-H moiety (10th position) of ADR 1 or ADR 2 dye. It has been well established that the molecular modelling studies portray that the C = O oxygen and the N-H hydrogen of amides form a strong hydrogen-bonding interaction with water molecules. Amides predominantly form a 1:1 (double hydrogen-bonding cyclic structures) rather than 1:2 and 1:3 (Mixture of ring, chain, sheet like structures) complex with water molecules, and behaves as a hydrogen-bonding donor as well as an acceptor. Based on the molecular modelling studies of amides in aqueous solution and photophysical studies, (absorption and emission spectral studies) it is evident that the formation of hydrogen-bonding interaction of the C = O oxygen or the N-H of amide with the N-H hydrogen or C = O oxygen of acridinedione dyes (1st or 5th position) is highly improbable. An uneven distribution of hydrogen-bonding interaction between the amide-amide, amide-water and water-water molecules exist in the aqueous phase at any concentration of amides such that the dye is neither hydrogen-bonded with the amide nor with water. Further, quantum mechanical studies of ADR 1 dye in the presence of water or methanol molecule clearly reveals that the oxygen atom in water is not hydrogen-bonded with the N-H hydrogen (10^{th} position) of acridinedione dye and the solvent molecule is situated near the carbonyl oxygen [9].

We elucidate that the increase in the fluorescence intensity of ADR 1 dye is attributed to the hydrophobic nature of amides only. The dye molecule prefers to orient towards the hydrophobic environment in aqueous solution as shown in scheme 1. The hydrophobic core of amides increases with the increase in the alkyl substitution of amides. The hydrophobic core created by DMF is larger compared to that of acetamide and formamide, such that a larger proportion of hydrophobic domain is created in the case of DMF than in formamide and this is in accordance with the extent of fluorescence enhancement as shown in Fig. 5. Further, the most probable mode of interaction existing between the dye and amides in aqueous solution is hydrophobic interaction. A similar behaviour was also observed in the interaction of urea derivatives with ADR 1 dye in aqueous solution. Tetramethylurea (TMU), which is more hydrophobic than urea, dimethylurea (DMU), methylurea (MU) and ethylurea (EU) resulted in a large extent of fluorescence enhancement [6].

Addition of formamide, acetamide and DMF to ADR 2 dye results no significant change in the fluorescence intensity, which indicates that a fluorescence enhancement is observed in acridinedione dyes, which posses -OCH_3 moiety in the para position of the phenyl ring in the 9th position of the basic acridinedione ring. The concentration and the hydrophobicity of amides influences the suppression of PET process and results in an increase in the fluorescence intensity.

Time-Resolved Fluorescence Studies

The fluorescence lifetime of the acridinedione dyes exhibit a single exponential behaviour in water. ADR 1 dye exhibits a fluorescence lifetime of 500±10 ps in water [6]. The fluorescence lifetime of ADR 1 dye in solvents like toluene, DMF acetonitrile and methanol is of the order of 300–600 ps, which clearly reveals that the irrespective of the nature of the solvent the PET process occurs through space between the donor and acceptor moiety. The relatively shorter lifetime component of ADR 1 dye in aqueous solution is attributed to PET process through space, and has been well documented [5–8]. The fluorescence lifetime of ADR 2 dye in water exhibits fluorescence lifetime of 8.3±0.1 ns in water. The fluorescence lifetime decay of ADR 1 and ADR 2 dyes in water is provided in the supporting information (Figure S3).



Scheme 1 **a** Uniform hydrogen-bonding interaction between water molecules confined throughout the phase. **b** Addition of amides results in the uneven distribution of amide-amide, amide-water and water-water hydrogen-bonding interactions. **c,d** On increasing the concentration of amides, the position of water molecules are altered such that a more hydrophobic environment is created as the dye molecule tend to orient near the close vicinity of an amide molecule rather with a water molecule. **e** Dye prefers to reside in the hydrophobic core of amide rather with a water molecule

ADR 1 Dye -Formamide Interaction in Water

On the addition of formamide to ADR 1 dye, the fluorescence lifetime decay exhibits a single exponential behavior. The fluorescence lifetime of ADR 1 dye increases to 1.2 ns (2.4 fold enhancement) on the addition of formamide (10.0 M). The fluorescence decay of ADR 1 dye with formamide in water shown in Fig. 6. The single exponential lifetime of ADR 1 dye accompanied with an increase in the fluorescence lifetime on the addition of formamide clearly reveals that there exists no free dye component. The enhancement in the fluorescence lifetime

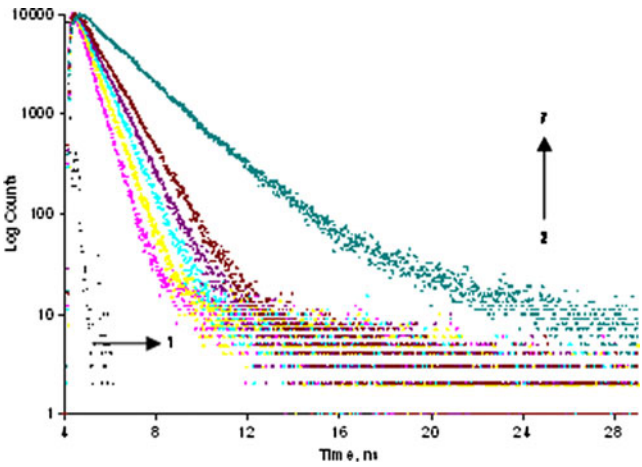


Fig. 6 Fluorescence decay of ADR 1 dye as a function of formamide in water. λ_{ex}378 nm. 1) Laser profile, 2) ADR 1 dye, 3) ADR 1 dye + formamide 2.5 M, 4) ADR 1 dye + formamide 5.0 M, 5)ADR 1 dye + formamide 7.5 M, 6) ADR 1 dye + formamide 10.0 M, 7)ADR 1 dye + formamide 22.5 M

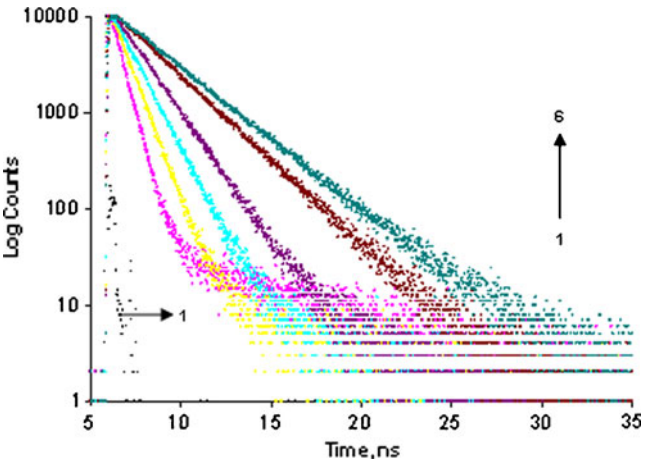


Fig. 7 Fluorescence decay and residuals of ADR 1 dye-DMF decay in water. λ_{ex}378 nm. 1) Laser Profile, 2) ADR 1 dye 3) ADR 1 dye + DMF 2.5 M, 4) ADR 1 dye + DMF 5.0 M, 5) ADR 1 dye + DMF 7.5 M, 6) ADR 1 dye + DMF 10.0 M, 7) ADR 1 dye + DMF 12.5 M

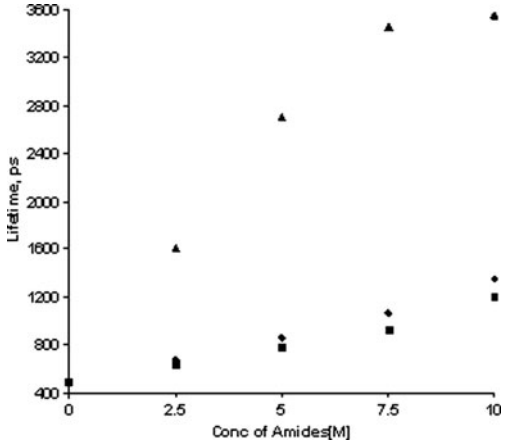


Fig. 8 Fluorescence lifetime of ADR 1 dye on the addition of formamide (■), acetamide (♦) and DMF (▲)

of ADR 1 dye signifies that the dye, which is hydrophobic, prefers to orient near the close vicinity of formamide (hydrophobic) rather in the hydrophilic domain containing large number of water molecules. The increase in the fluorescence lifetime of ADR 1 dye on the addition of formamide further confirm that no free dye exists in solution (500±10 ps). A similar trend was also observed in the interaction of ADR 1 dye with urea derivatives in aqueous solution [6], wherein the fluorescence lifetime of ADR 1 dye in the presence of urea (2.4 M) was around 700 ps.

Interestingly, studies pertaining to the interaction of ADR 1 dye with BSA in water exhibits three lifetime components of 0.5, 2.5 and 7.2 ns of which is correlated to the free dye in the aqueous phase, hydrophilic and hydrophobic phase respectively.

ADR 1 Dye—Alkyl Amides Interaction in Water

An enhancement in the fluorescence lifetime of the dye was also observed on the addition of acetamide and DMF to ADR 1 dye. The fluorescence decay of ADR 1 dye with DMF in water is provided in Fig. 7. The fluorescence lifetime of ADR 1 dye on the addition of acetamide (10.0 M) and DMF (10.0 M) increases to 1.52 (3 fold) and 3.55 ns (7.0 fold enhancement) respectively as provided in Fig. 8. An increase in the fluorescence lifetime of ADR 1 dye is observed on the addition of acetamide and DMF, and the extent of fluorescence enhancement of the dye is of the order DMU > acetamide > formamide.

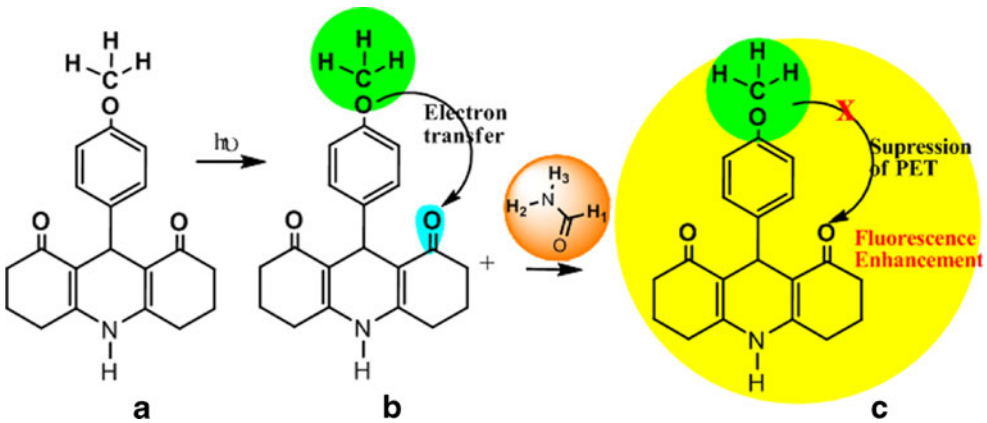
The fluorescence lifetime of 3.5 ns (on the addition of DMF) is attributed to the dye located in the close proximity of DMF rather near the water molecules. DMF, which contains two methyl moieties, impart a hydrophobic environment compared to formamide in the solvent phase, and results in a large hydrophobic environment which is more favourable for the dye to reside as shown in scheme 1, such that the water molecules are gradually displaced from the hydrophobic domain. It has been well documented that ADR 1 dye prefers to orient towards a hydrophobic environment, rather to a hydrophilic phase, as observed in the interaction of ADR 1 dye with β-cyclodextrin [5], urea derivatives [6], guanidine GuHCl [7] and BSA [8].

Interaction of ADR 2 Dye with Amides

No significant change in the fluorescence lifetime of the dye was observed on the addition of formamide to ADR 2 dye. A similar pattern was observed on the addition of acetamide and DMF to ADR 2 dye in water, which clearly illustrates that an enhancement in the fluorescence lifetime of the dye is observed in acridinedione dyes containing a donor moiety in the para position of the phenyl ring.

Mechanism of Fluorescence Enhancement

The fluorescence of probe molecules in general undergoes a significant change in the excited state. The addition of a



Scheme 2 **a** PET dye containing an electron donor group. **b** Electron transfer through space between the donor and acceptor moieties on the absorption of light. **c** Addition of amides promotes the suppression of PET process

guest molecule to a fluorescent probe is accompanied with a change in the emission maxima or fluorescence quenching or enhancement. An excited state process like charge transfer, energy transfer or electron transfer results in an increase or decrease in the fluorescence intensity.

It is well known that the fluorescence enhancement of a PET based acridinedione dyes in the presence of guest molecule (hydrogen-bonded self-assemblies or inclusion complexes, hydrophobic cavities and micelles) clearly indicates the most probable location of the dye [5–8]. Further, acridinedione dyes exhibit a shift in the emission maximum with an increase or decrease in the polarity and this has been well established in the interaction of ADR 1 dyes with different solvents [19, 20].

All the amides are miscible in water at all proportions [21] and it has been well established that the density of formamide and DMF are quite similar to that of water. If the bulk viscosity factor of amides in aqueous solution had been responsible for the fluorescence enhancement of ADR 1 dyes, the addition of amides, irrespective of the nature of hydrophobic moieties present in the amides would have resulted in a similar trend of fluorescence enhancement. On the contrary, we observed a difference in the pattern of increase in the fluorescence lifetime of ADR 1 dye on the addition of amides. From the above observation we postulate the fluorescence enhancement is not due to change in the bulk viscosity of the medium or polarity of the amides, and the mechanism of fluorescence enhancement is presumably attributed to the suppression of the PET process through space between the $–OCH_3$ moiety and the acceptor moiety of the acridinedione ring as shown in Scheme 2. This phenomenon is influenced by the hydrophobicity of amides, wherein the most hydrophobic amides results in a larger extent of suppression of the PET process, resulting in a fluorescence enhancement. A similar behaviour was also observed in the interaction of acridinedione dyes with urea derivatives, where in tetra methyl urea (TMU) which is more hydrophobic than urea (U) and diemthylurea (DMU) results in a larger extent of fluorescence enhancement of ADR 1 dye in aqueous solution.

Urea derivatives have a structural similarity (−N-H and -C = O groups) with amides also exhibit a fluorescence enhancement with ADR 1 dye. No apparent shift in the emission maximum of ADR 1 dye was observed in the interaction of urea derivatives in both water and methanol. Interestingly, a significant shift in the emission maximum of ADR 1 dye towards the blue region was only observed on the addition of formamide, acetamide and DMF. The excited state process of ADR 1 dye are not governed by the polarity of the amides and the amides does not act as a binary mixture or as a co-solvent is emphasized. The hydrophobic nature of the amides predominates over the hydrogen-bonding interaction of solute-solvent interaction and the photophyical properties of ADR 1 dye is largely governed by the change in the environment created by the amides rather by solvent mediated hydrogen-bonding properties.

Conclusion

The suppression of PET process by amides results in an increase in the fluorescence intensity and lifetime of acridinedione dyes containing a $–OCH_3$ moiety in the para position of the phenyl ring situated in the 9th position. The increase in the fluorescence intensity of ADR 1 dye is governed by hydrophobicty of the amides and the polarity of the amides does not play a vital role in the extent of fluorescence enhancement and shift in the emission maximum of ADR 1 dyes. The amides which represents a class of protein denaturants like urea and guanidine hydrochloride (GuHCl) also behaves as an ideal host molecule in studying the photophysical properties of PET based acridinedione dyes in water is emphasized.

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