J Fluoresc (2010) 20:993–1002
DOI 10.1007/s10895-010-0646-9

ORIGINAL PAPER

Photophysical Studies on the Interaction of Acridinedione Dyes with Universal Protein Denaturant: Guanidine Hydrochloride

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Received: 15 November 2008 / Accepted: 18 March 2010 / Published online: 6 April 2010

Abstract Photophysical studies of photoinduced electron transfer (PET) and non-PET based acridinedione dyes with guanidine hydrochloride (GuHCl) were carried out in water and methanol. Addition of GuHCl to photoinduced electron transfer (PET) based acridinedione dye (ADR 1) results in a fluorescence enhancement, whereas a non-PET based dye (ADR 2) shows no significant change in the fluorescence intensity and lifetime. Addition of GuHCl to ADR 1 dye in methanol results in single exponential decay behaviour, on the contrary a biexponential decay pattern was observed on the addition of GuHCl in water. Absorption and emission spectral studies of ADR 1 dye interaction with GuHCl reveals that the dye molecule is not in the protonated form in aqueous GuHCl solution, and the dye is confined to two distinguishable microenvironment in the aqueous phase. A large variation in the microenvironment around the dye molecule is created on the addition of GuHCl and this was ascertained by time-resolved area normalized emission spectroscopy (TRANES) and time-resolved emission spectroscopy (TRES). The dye molecule prefers to reside in the hydrophobic microenvironment, rather in the hydrophilic aqueous phase is well emphasized by time-resolved fluorescence lifetime studies. The mechanism of fluorescence enhancement of ADR 1 dye by GuHCl is attributed to the suppression of the PET process occuring through space.

Electronic supplementary material The online version of this article (doi:10.1007/s10895-010-0646-9) contains supplementary material, which is available to authorized users.

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Keywords Acridinedione dyes · Guanidine hydrochloride · Photoinduced electron transfer · Fluorescence enhancement · Hydrogen-bonding

Introduction

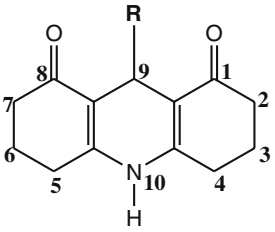
GuHCl, the most widely used protein denaturant [1, 2], plays an important role in the denaturation process of proteins. The chemical and biological aspects of protein folding largely revolve around the hydrogen-bonding properties of GuHCl with the solvent molecules. Aqueous GuHCl solution is the most efficient protein denaturant than urea and micelles which is attributed to very high solubility in water. GuHCl forms molecular cages of extended hydrogen-bonded network with water molecules, clusters and self assemblies [3] in hydrogen-bonding solvents. Very high concentration of GuHCl (6.0 M) exhibit large viscosity and density, which is of significant importance in protein folding [4, 5]. GuHCl possesses a very high ionic strength in water and acts as a strong electrolyte [6]. The presence of positive charge on the imino nitrogen in GuHCl results in an unusual structure than urea. Apart from the solvent mediated hydrogen-bonding interactions, the electrostatic interactions play a major role in the folding of proteins.

GuHCl and urea possess strong hydrogen-bonding properties with solvents and have a tendency to alter the structure of the water molecules, and the biological studies pertaining to GuHCl [7–11] is largely devoted to the concept of folding and refolding of proteins, isolation and inhibition of RNA, disruption of the hydrophobic interactions and solubility of hydrophobic residues in aqueous solutions. The presence of an imine and aminoacetal functional group in GuHCl result in a large variation in

the hydrogen-bonding properties (number of hydrogen-bonding acceptor and donor groups) compared to that of urea in aqueous solutions. The variation in the photophysical properties of an external probe with strong chaotropic agent like GuHCl is an area of unexplored domain and the literature regarding the photophysical properties of an extrinsic fluorescent probe with GuHCl is found to be less compared to that of urea interaction with fluorescent probes in aqueous solution [12–19].

Urea forms hydrogen-bonding self assemblies in water and acts as a hydrogen-bonding donor or acceptor, whereas GuHCl acts as a hydrogen-bond donor only. It has been established that GuHCl is surrounded by 12 to 13 water molecules and six water molecules are involved in a direct hydrogen-bonding interaction with GuHCl [5, 20]. The interaction of water molecules through oxygen with any of the –N-H moiety of GuHCl ion gives almost the same interaction energy. It is known that six water molecules are accommodated in each of the primary hydration sphere as well as in the secondary hydration sphere [5, 20]. An increase in the concentration of GuHCl results in the partial displacement of the water molecules from the secondary hydration sphere resulting in a GuHCl-water-GuHCl hydrogen-bonding network through out the medium. This results in a large variation in the orientation of the hydrophobic and hydrophilic clusters in aqueous phase in the close vicinity of GuHCl. A significant change in the microenvironment of the aqueous phase and a large variation in the hydrogen-bonding properties are promoted on the addition of GuHCl, which is very similar to that of urea.

Photophysical studies serve as a vital link in establishing and elucidating the structure and properties of the probe molecules in the microheterogeneous environment in their ground and excited states [21, 22]. Most of the biophysical and biochemical studies of the microheterogeneous systems involve the changes in the microscopic level and are widely applicable to elucidate the nature of interaction between the probe and the host molecule. The present study is largely focused on the interaction of GuHCl with an acridinedione dyes in water and methanol. Acridinedione dyes have structural similarity with purine derivatives, NADH (Nicotineamide adenine dinucleotide), which makes the dye significantly important in the field of biomedical research. The presence of tricyclic structure in acridinedione dyes is capable of protecting the enamine moiety [23, 24]. Further, the reduced form of NADH plays a vital role as the electron source and the reduction of oxygen in the respiratory chain [25]. Acridinedione is a bifunctional molecule and acts as an electron donor or acceptor, and undergoes various interesting reactions in the excited state. The nature of the substituents in the 9th and 10th positions of the basic acridinedione dyes result in a large variation in their photophysical and photochemical properties. The photophysical properties in microheterogeneous systems like polymer matrices [26], cyclodextrin nanocavities [27, 28], molecular self assemblies(urea) [29], and with globular proteins [30], has been elucidated by fluorescence spectral techniques. The following resorcinol based acridinedione dyes ADR 1 (PET) and ADR 2 (non-PET) are used in the present investigation is given below.



ADR 1 R — C_6H_4 (p-OCH3)

ADR 2 R — C_6H_5

Experimental methods

Guanidine hydrochloride (GuHCl) and methanol (HPLC grade) were purchased from SRL chemicals India Ltd and acridinedione dyes were prepared by following the procedure reported in the literature [31–33]. 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 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 [29]. 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°). Photon was counted by a MCP-PMT (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 (MCA). 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). TRANES and TRES plots were constructed as reported elsewhere [34–39].

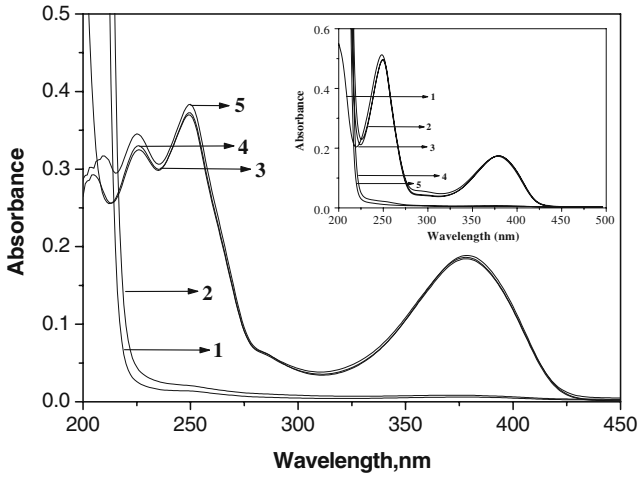


Fig. 1 Absorption spectra of ADR 1 dye with GuHCl in water. *1*) GuHCl 0.6 M, *2*) GuHCl 1.2 M, *3*) Dye alone, *4*) Dye + GuHCl 0.6 M, *5*) Dye + GuHCl 1.2 M. Inset: Absorption spectra of ADR 1 dye- GuHCl in methanol. *1*) Dye alone *2*) Dye + GuHCl 0.6 M, *3*) Dye + GuHCl 0.6 M, *4*) GuHCl 0.6 M, *5*) GuHCl 1.2 M

Results and discussion

Absorption spectral studies

The absorption spectrum of ADR 1 dye shows a maximum at 377 nm and 370 nm in water and methanol respectively [29]. This longest wavelength absorption maximum has been assigned to the intramolecular charge transfer (ICT) from the nitrogen to the carbonyl oxygen [33]. There is no change in the absorbance at the dye absorption maximum of ADR 1 dye and no significant shift in the ICT absorption maximum towards the red or blue region is observed in water and methanol on the addition of GuHCl as shown in Fig. 1. A similar behavior was also observed in ADR 2 dye on the addition of GuHCl. The absorption spectrum of ADR 2 dye as a function of GuHCl is shown in the inset of Fig. 1.The absorption spectra of ADR 1 dye with GuHCl in water (Fig. 1) clearly signifies that the dye is not in the protonated form as ADR 1 H^+, and this was further confirmed by pH variation studies of ADR 1 dye with GuHCl in water. pH measurement of ADR 1 dye in the absence and presence of GuHCl in water were recorded in triple distilled water and no drastic change in the pH of ADR 1 dye was observed on the addition of 1.2 M, 2.4 M, 4.8 M and 6.0 M of GuHCl. This clearly reveals that the dye is not protonated nor deprotonated form.

Steady-state emission spectral studies

On excitation at the ICT absorption maximum of ADR 1 dye results in a fluorescence emission maximum at 436 nm and 430 nm in water and methanol respectively. An enhancement in fluorescence intensity of ADR1 dye is observed on the addition of GuHCl, and the position of emission maxima of ADR 1 dye remains the same even after the addition of high concentration of GuHCl. It has been well established that ADR 1 dye exhibits a shift in the emission maxima when protonated or deprotonated [40] or hydrogen-bonded with the N-H hydrogen [41]. A fluorescence enhancement of ADR 1 dye clearly reveals that the dye is not protonated form (A fluorescence quenching would have been resulted if the dye is in the protonated form [40]). The emission spectra of ADR 1 dye as a function of GuHCl in water is shown in Fig. 2. A gradual shift in the emission maximum towards the red region (429–434 nm) was observed on the addition of GuHCl to ADR 1 dye in methanol, whereas no significant shift in the emission maximum of ADR 1 dye was observed on the addition of GuHCl in water. The emission spectra of ADR 1 dye as a function of GuHCl in methanol is provided in the inset of Fig. 2.

In water a well oriented water-GuHCl hydrogen-bonding arrangement is confined throughout the phase such that the oxygen atom in water molecule does not form any hydrogen-bonding interaction with the N-H hydrogen of ADR 1 dye as shown in Scheme 1. Further the N-H moiety in the 10th position of ADR 1 dye is not involved in a hydrogen-bonding interaction with GuHCl molecule.

PET and fluorescence quantum yield

Acridinedione dyes have been classified into PET and non-PET based on the substitution in the 9th position of the basic acridinedione ring as shown. Among the PET dyes, the dye containing $-OCH_3$ moiety in the phenyl ring

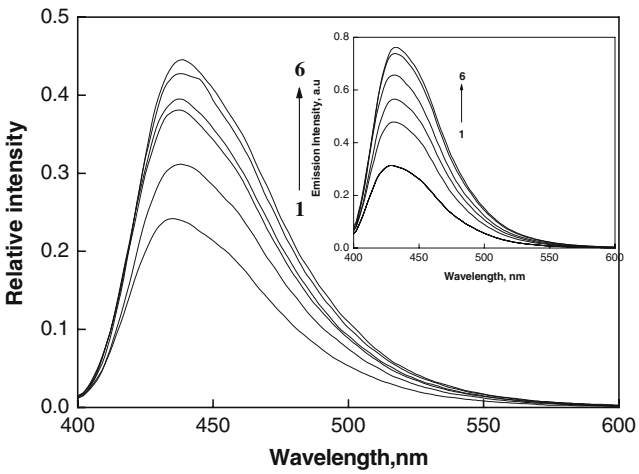
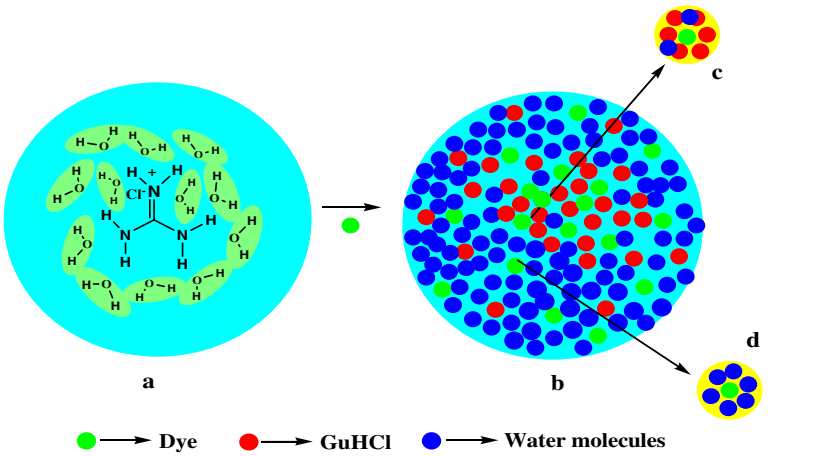


Fig. 2 Emission spectra of ADR 1 dye as function of [GuHCl] in water. 0.0 M, *2*) 0.8 M, *3*) 1.6 M, *4*) 3.2 M, *5*) 4.8 M *6*) 6.4 M. Inset: Emission spectra of ADR 1 dye as function of [GuHCl] in methanol. *1*)0.0 M, *2*) 0.5 M, *3*) 1.0 M, *4*) 2.0 M, *5*) 3.0 M *6*) 4.0 M



Scheme 1 **a** One GuHCl molecule surrounded by 12 water malocules in the solution phase. **b** The probable location of the dye moiety in aqueous GuHCl solution. **c** Hydrophobic microenvironment; The dye molecule is surrounded by more number of GuHVl molecules and less number of water molecules. **d** Hydrophillic microenvironment; The dye molecule is surrounded by water molecules only

a: One GuHCl molecule surrounded by 12 water moelcules in the solution phase.

b: The probable location of the dye moiety in aqueous GuHCl solution.

c: Hydrophobic microenvironment; The dye molecule is surrounded by more number of GuHCl moelcules and less number of water molecules.

d: Hydrophillic microenvironment; The dye molecule is surrounded by water molecules only.

attached in the 9th position exhibits a PET behavior through space in water, methanol and other non-polar solvents. The electron transfer process between the donor moiety to the acceptor moiety results in the decrease of LE state emission and the quantum yield. The experimentally determined fluorescence quantum yield is found to be 0.12 and 0.90 for ADR 1 and ADR 2 dyes in methanol respectively [29] (The quantum yield of PET and non PET based acridinedione dyes are provided in Table 1 of supporting information). It is evident that the quantum yield of ADR 1 dye is very less compared to that of ADR 2 dye, which is attributed to the photoinduced intramolecular electron transfer from the electron deficient excited state of the acridinedione fluorophore in ADR 1 dye [29]. The presence of donor moiety in the para position of the acridinedione structure of ADR 1 dye has resulted in the low fluorescence quantum yield in comparison with that of ADR 2, ADR 3 and ADR 4 dyes.

Time-resolved fluorescence lifetime studies

The fluorescence lifetime of the acridinedione dyes [33] exhibit a single exponential behaviour in aqueous solution and methanol. ADR 1 dye shows the fluorescence lifetime of 500 ± 10 and 400 ± 10 ps in water and methanol respectively [29], and ADR 2 dye exhibits fluorescence lifetime of 8.3 ± 0.1 ns in water and methanol. The relatively shorter lifetime of PET dyes in aqueous solution and methanol is attributed to PET process through space, and has been well documented [29]

a) ADR 1 dye—GuHCl interaction in water.

On the addition of GuHCl to ADR 1 dye, the fluorescence lifetime decay exhibits a biexponential behavior. The fluorescence decay of ADR 1 dye with GuHCl in water is shown in Fig. 3. The biexponential lifetime of ADR 1 dye reveals that the dye molecule is located in two

Table 1 Fluorescence lifetime decay analysis of ADR 1 dye—GuHCl in water

GuHCl (M)	Exp	τ_1(ns)	τ_2(ns)	B_1	B_2	$\chi 2$
0	Single	0.49 ± 0.02	–	100	–	1.18
	Bi	0.50 ± 0.02	5.62	98.50	1.50	1.05
0.8	Bi	0.56 ± 0.02	6.38	95.50	4.50	1.15
1.6	Bi	0.61 ± 0.02	7.48	93.50	6.50	1.09
3.2	Bi	0.69 ± 0.02	7.66	92.50	7.50	1.10
4.8	Bi	0.72 ± 0.03	7.91	91.00	9.00	1.23
6.4	Bi	0.78 ± 0.02	7.91	85.00	15.50	1.20
8.0	Bi	0.81 ± 0.03	7.72	85.00	15.00	1.13

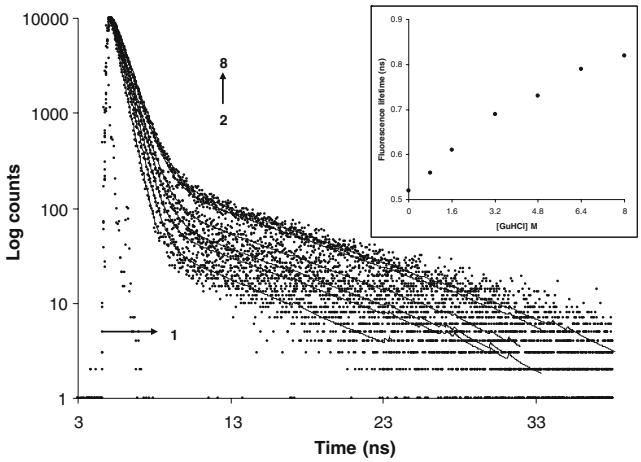


Fig. 3 Fluorescence decay of ADR 1 dye as function of [GuHCl] in water. λ_{ex}378 nm and λ_{em}437 nm. *1*) Laser profile, *2*) 0.0 M, *3*) 0.8 M, *4*) 1.6 M, *5*) 2.4 M, *6*) 3.2 M, *7*) 4.8 M *8*) 6.4 M. Inset: Fluorescence lifetime of ADR 1 dye on the addition of GuHCl

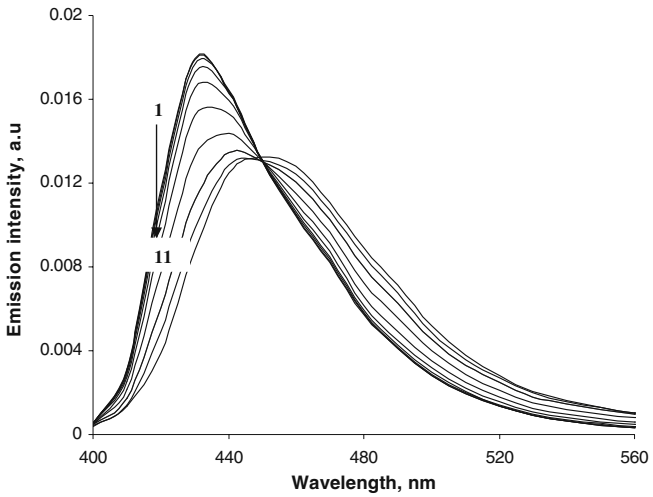


Fig. 4 Time-resolved area normalization emission spectra (TRANES) of ADR 1-dye with GuHCl (8.0 M) in water. Nos 1-11 represent 0 ns, 0.5 ns, 1.03 ns, 1.51 ns, 2.01 ns, 2.50 ns, 3.03 ns, 4.01 ns, 4.99 ns, 6.02 ns, 6.49 ns, 7.02 ns, 7.55 ns, 8.03 ns, 8.5 ns and 9.01 ns. λ_{ex}378 nm and λ_{em}400–600 nm at 10 nm intervals

distinguishable microenvironment in the aqueous medium. In the present study the ADR 1 dye exist in two different microenvironment (Dye predominantly surrounded by water molecule and dye predominantly surrounded by GuHCl) illustrates that the biexponential decay of ADR 1 dye—GuHCl in water is attributed to the location of the dye molecule in a heterogeneous environment. From the fluorescence lifetime decay analysis, we observed an enhancement in the fluorescence lifetime of the dye from 500 ps to 820 ps, accompanied with the evolution of a long lifetime component (8 ns). The fluorescence lifetime of 500 ps is attributed to the location of the ADR 1 dye surrounded predominantly by water molecules only, and the 8.0 ns component is attributed to the dye located in the hydrophobic phase surrounded by less number of water molecules [24]. ADR 1 dye, which is hydrophobic in nature, prefers to orient towards the hydrophobic environment in water rather towards the hydrophilic phase [27–29]. The existence of dye in both hydrophilic and hydrophobic microenvironment is established from time-resolved fluorescence studies as shown in Table 1. The presence of GuHCl results in hydrogen-bonded self assemblies with water molecules and the addition of dye results in the reorientation and displacement of the water molecules in the close vicinity of GuHCl, such that a more hydrophobic microenvironment is created as shown in Scheme 1.

The location of dye in two distinguishable microenvironment was further confirmed by constructing a time resolved area normalization emission spectra (TRANES) and time resolved emission spectra of (TRES) of ADR 1 dye with GuHCl (8.0 M) in water. A clear isoemissive point in the TRANES spectra of ADR 1 dye with GuHCl is observed in the present study and the presence of a single isoemissive point in TRANES analysis of fluorescence is an unambiguous indication of the presence of two emissive species in the system [34, 35]. The TRANES spectra of ADR 1 dye with GuHCl is shown in Fig. 4. TRES, plotted as emission intensity vs wavelength, was constructed and a gradual red shift in the emission maximum of ADR 1 dye was observed as shown in Fig. 5. TRES plot of ADR 1 dye with GuHCl in water clearly illustrates that there exists two different microenvironment in solution and the dye molecule is located in two distinguishable microenvironment.

Addition of GuHCl to ADR 2 dye, results no significant change in the fluorescence lifetime of the dye. Interaction

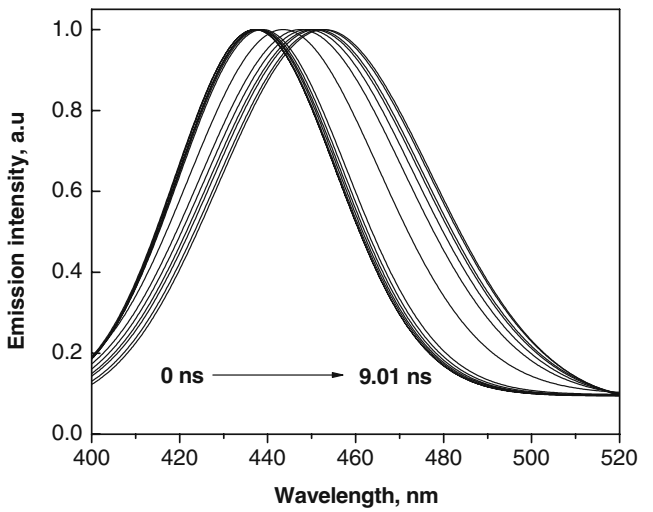


Fig. 5 Time-resolved emission spectra (TRES) of ADR 1-dye with GuHCl (8.0 M) in water at 0 ns, 0.5 ns, 1.03 ns, 1.51 ns, 2.01 ns, 2.50 ns, 3.03 ns, 4.01 ns, 4.99 ns, 6.02 ns, 6.49 ns, 7.02 ns, 7.55 ns, 8.03 ns, 8.5 ns and 9.01 ns. λ_{ex} −370 nm and λ_{em}400–600 nm

of ADR 2 dye (non-PET based dye) with urea derivatives (protein denaturants) in water also results no significant change in the fluorescence lifetime as observed in the present investigation.

There are reports which portray that GuHCl produces large volume changes in aqueous solution in the presence of hydrophobic species [42] and the interaction is primarily by hydrogen bonding or hydrophobic interactions rather than by electrostatic interaction [43]. At high concentrations, GuHCl affect the properties of water determining the hydrophobic interactions, resulting in a large variation in the hydrogen-bonding properties of the solute and the solvent which enhances the solubility of the hydrophobic residues [44]. Increase in the concentration of GuHCl results in the creation of hydrophobic microenvironment and the dye molecules preferably orient towards the hydrophobic phase resulting in the formation of a new lifetime.

b) ADR 1 dye—GuHCl interaction in methanol

The fluorescence lifetime of the dye increases from 390 ps to 1.23 ns at the highest concentration of GuHCl (4.8 M). Interestingly, the addition of GuHCl to ADR 1 dye resulted in a single exponential decay pattern. Single exponential decay characteristics of ADR 1 dye as a function of GuHCl reveals that there exists an uniform and homogeneous microenvironment confined throughout the phase. The fluorescence lifetime decay of ADR 1 dye as a function of GuHCl is shown in Fig. 6 and the fluorescence lifetime is provided in the inset of Fig. 6.

Interestingly, the extent of enhancement is found to be higher in methanol compared in water as shown on Fig. 7. The variation in the fluorescence lifetime of ADR 1 dye on the addition of GuHCl in methanol and water clearly

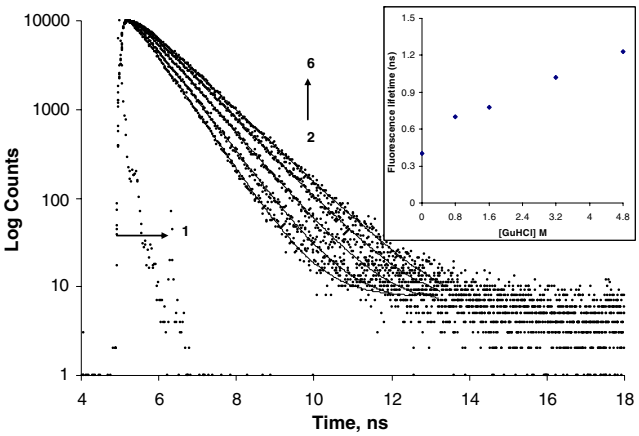


Fig. 6 Fluorescence decay of ADR 1 dye as function of [GuHCl] in methanol. λ_{ex} −370 nm and λ_{em}431 nm. *1*) Laser profile, *2*) 0.0 M, *3*) 0.8 M, *4*) 1.6 M, *5*) 3.2 M, *6*) 4.8 M. Inset: Fluorescence lifetime of ADR 1 dye on the addition of GuHCl

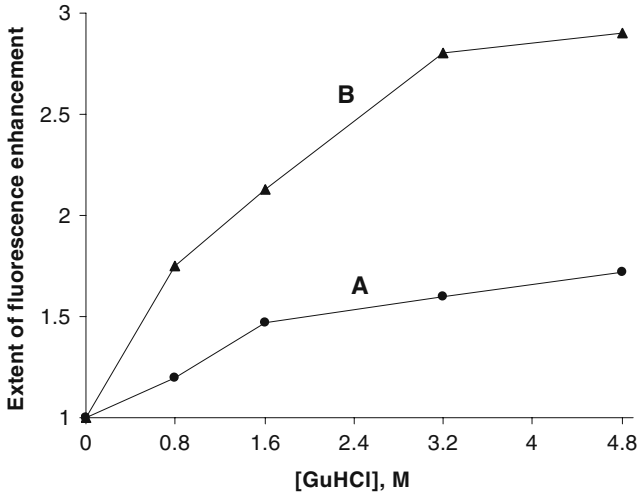


Fig. 7 Extent of fluorescence enhancement of ADR 1 dye as function of [GuHCl]. *A*- Water, *B*- Methanol

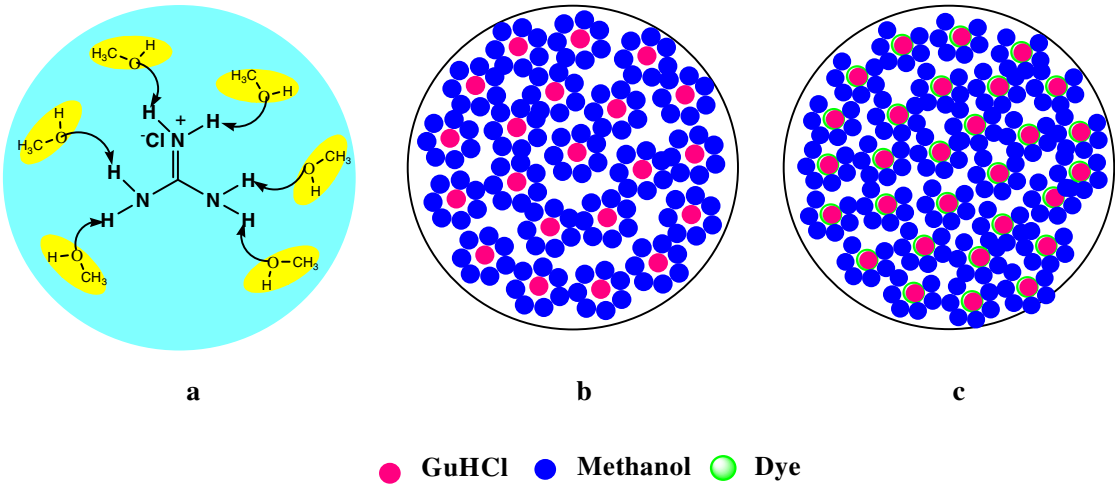
illustrates that the solvent mediated hydrogen-bonding properties and the hydrogen-bonding arrangement of GuHCl with the solvent molecules influences the excited state property of the probe.

The hydrogen-bonding interaction of GuHCl with methanol is through the oxygen atom in methanol with the −N-H hydrogen of GuHCl. Approximately six to seven solvent molecules are involved in a direct hydrogen-bonding interaction with one GuHCl molecule and it forms self assembled clusters through out the medium. A uniform hydrogen-bonding pattern is confined throughout the phase which results in an organized self assembled clusters wherein the microenvironment is found to be the same.

The dye prefers to orient towards the GuHCl-methanol hydrogen-bonding clusters and does not prefer to orient in the solvent phase. This type of hydrogen-bonding pattern is not observed in the case of water; thereby a variation in the microenvironment is resulted such that the dye is located in two different phases. The interaction between the dye and GuHCl is hydrophobic in nature, and this is more pronounced in water. A hydrogen-bonding interaction exists between the nitrogen of GuHCl with N-H hydrogen of ADR 1 dye and presumably resulted in a red shifted emission, when the medium is methanol. In the presence of GuHCl (aqueous medium), the ADR 1 dye molecule prefers to reside in the hydrophobic domain rather in the aqueous phase, whereas in methanol it prefers to reside inside the hydrophobic domain of the cluster and not in the solvent phase (Scheme 2).

Mechanism of fluorescence enhancement

Fluorescence enhancement of a probe in the presence of guest molecule (self-assemblies, biomolecules, micelles,



a) One GuHCl hydrogen-bonded to 6 methanol molecules.

b) GuHCl-methanol hydrogen-bonding self assembled spheres, resulting in a homogenous microenvironment.

c) Location of the dye in the interior of the hydration cospheres.

Scheme 2 **a** One GuHCl hydrogen-bonded to six methanol molecules. **b** GuHCl-methanol hydrogen-bonding self assembled spheres, resulting in a homogenous microenvironment. **c** Location of the dye in the interior of the hydration cospheres

and polymer matrices) is in general attributed to the changes in the polarity and bulk viscosity of the medium [21, 22]. The addition of guest molecule results in a change in the microenvironment surrounding the fluorescent probe. An excited state process like charge transfer, energy transfer or electron transfer also results in a fluorescence enhancement. Apart from these factors the probe can undergo photodissociation accompanied with large change in the pH, resulting in the formation of charged species (protonation or deprotonation).

In the present investigation, the dye does not undergo any dissociation and no drastic change in the pH is observed on the addition of GuHCl and this change hardly influences the photophysical properties of ADR 1 dye. Further, it has been well documented that the photophysical properties of acridinedione dyes are influenced by the viscosity of the medium [26], and REES spectral studies is used as a tool in monitoring the emission spectra in highly viscous medium. On exciting at the red edge of the absorption band of ADR 1 dye as a function of GuHCl (4.8 M) in water, no significant shift in the emission maxima towards the red region is observed. This clearly illustrates that the fluorescence enhancement of ADR 1 dye is not influenced by the viscosity of the solute nor the solvating medium. It is well known that aqueous solution of GuHCl is highly viscous than urea, and carrying out REES studies on urea derivatives (Hydrogen-bonded self assemblies with water molecules) with ADR 1 dye in aqueous solution also results no significant shift in the position of the emission maxima towards the red region. This clearly illustrates that with increase in the viscosity of protein denaturants (GuHCl or urea) hardly influences the excited state properties of acridinedione dye. This is further confirmed by taking a non-PET dye, where in the emission spectra on the addition of GuHCl to ADR 2 dye exhibit no enhancement in fluorescence intensity in water and methanol. Interaction of urea derivatives, cyclodextrins and BSA to ADR 2 dye results no significant change in the fluorescence intensity [27–30].

The most possible mechanism of fluorescence enhancement is attributed to the suppression of the PET process through space and is this was ascertained and confirmed based on the quantum mechanical (QM) calculations. QM studies were carried out to quantify the distance between the donor (Oxygen in O-CH_3) and the acceptor (Carbonyl oxygen) moieties in ADR 1 dye.

Quantum chemical analysis

The molecular and electronic structures of ADR 1 dye were investigated by hybrid Hartree-Fock −DFT computations using Becke's three parameter hybrid-exchange functional and gradient corrected nonlocal correction functional of Lee, Yang and Parr [45]. We have examined the para and the meta conformers of ADR 1 dye by complete structural optimization at B3LYP/6-31+G* level using the G03 software [46]. The role of aqueous and methanol media to mechanism of

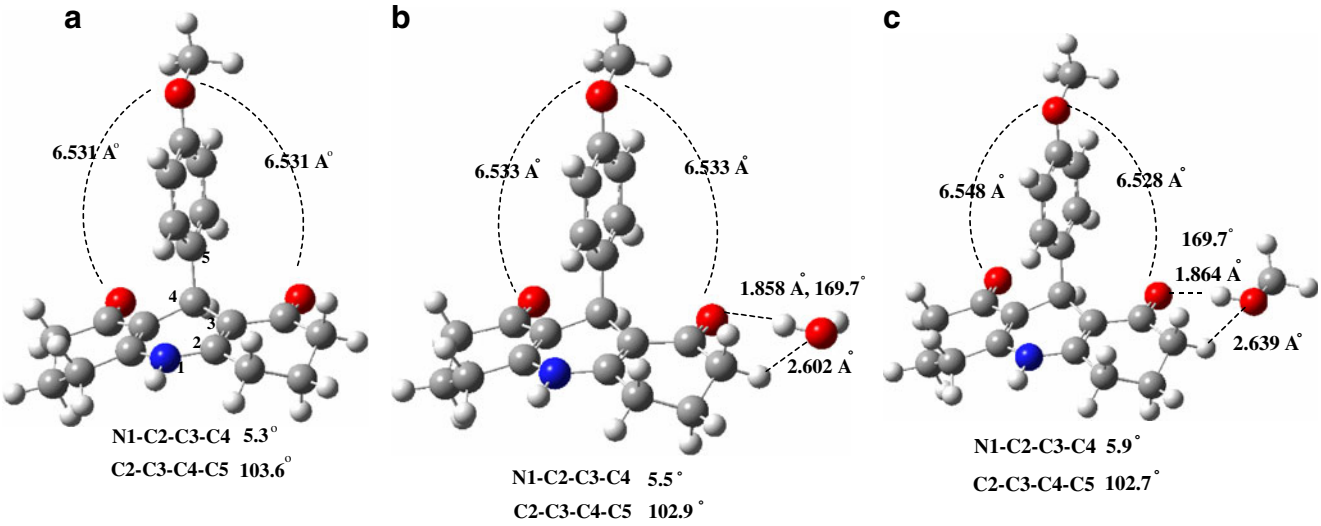


Fig. 8 **a** Optimized structure of ADR 1 dye in gas phase. **b** Optimized structure of ADR 1 dye in the presence of one water molecule. **c** Optimized structure of ADR 1 dye in the presence of one methanol molecule

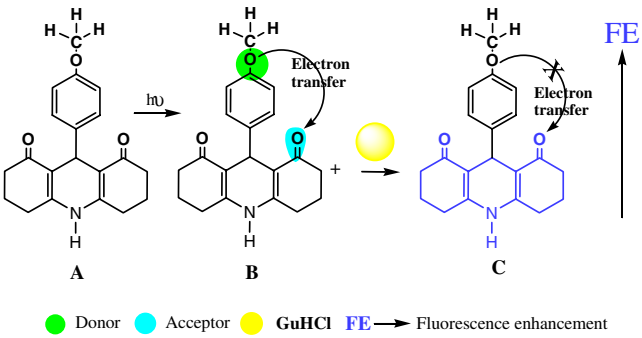
fluorescence enhancement in ADR 1 dye is examined by studying the 1:1 complex of the dye with water as well as methanol. (Vibrational frequency calculations were performed to confirm the optimised structures correspond to genuine minima in the potential energy surface).

Figure 8 shows the optimized structure along with selected structural parameters. The distance between the donor (Oxygen in O-CH_3) and the acceptor (Carbonyl oxygen) moieties in ADR 1 dye is about 6.53 Å which indicates that there is no intramolecular hydrogen-bonding interactions. Although we have studied the structures of the dye with different orientations of the solvent molecule, the optimization study reveals that the conformers shown in Fig. 8b and c possess the lowest energy for the ADR 1 dye….water and ADR 1 dye….methanol complexes. These structures are stabilized by a strong hydrogen-bonding interaction between the hydrogen atom of water/methanol molecule and the carbonyl oxygen acceptor of the ADR 1 dye as shown in Fig. 8b and c. The hydrogen-bond length is about 1.86 Å and the hydrogen-bond angle is 169°. Further there is a weak hydrogen-bonding interaction between the oxygen atom of water/methanol and one of the methylene hydrogen's located ortho to the carbonyl group. Thus it is seen that the solvent molecule is oriented such that it forms a 6-membered ring with the ADR 1 dye through hydrogen-bonding interactions. As a result the distance between the donor and the acceptor moieties in ADR 1 dye is not changed to any significant extent in the solvent water and methanol.

From the above studies it is evident that the fluorescence enhancement of ADR 1 dye in water on the addition of the protein denaturant (GuHCl) is attributed to the suppression of the PET process between the donor moiety (−OCH_3) and the acceptor moiety (C = O), as given in Scheme 3.

Conclusion

The suppression of PET process by GuHCl results in an increase in the fluorescence intensity and lifetime of acridinedione dyes containing a −OCH_3 moiety. The variation in the hydrogen-bonding interaction between GuHCl with water with that of methanol influences the extent of fluorescence enhancement in acridiendione dyes is emphasized. The dye molecule resides in two distinguishable microenvironment in aqueous GuHCl solution, whereas it is confined to the close proximity of GuHCl-methanol clusters. PET suppression of ADR 1 dye in water and



A- PET dye containing an electron donating group

B- Electron transfer through space between the donor and acceptor moiety on absorption of light.

C- Addition of GuHCl results in supression of the PET process, resulting in a fluorescence enhancemnet (FE).

Scheme 3 **a** PET dye containing an electron donating group. **b** electron transfer through space between the donor and acceptor moiety on absorption of light. **c** addition of GuHCl results in suppression of the PET process, resulting in a fluorescence enhancement (FE)

methanol by GuHCl visualizes that the excited state properties of the dye are largely influenced by an increase in the concentration of GuHCl. The hydrogen-bonding interaction of solute-solvent predominates over the solute-dye interaction is emphasized in the present study. The protein denaturant GuHCl behaves as an ideal host molecule in studying the photophysical properties of PET and non-PET based acridinedione dyes is illustrated.

Acknowledgements Financial support by DST-IRHPA and UGC-INNOVATIVE Programme is acknowledged. R.K thanks the UGC for providing the financial assistance.

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