

Photophysical Studies on the Interaction of N-Acetyl Tryptophanamide (NATA) With Urea Derivatives in Water

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Abstract

Photophysical studies of N-Acetyl Tryptophanamide (NATA) with urea derivatives were carried out in aqueous solution. The urea derivatives were categorised into symmetrical and unsymmetrical urea derivatives. Addition of urea derivatives result in a significant change in the absorbance maximum of NATA. Interestingly, the addition of urea and N, N'-1, 3 dimethylurea (DMU), a symmetrical urea derivative results in a fluorescence enhancement whereas an unsymmetrical urea derivative like N-butyl urea results in a fluorescence quenching. On the contrary, N-methyl urea (MU) exhibits no significant change in the fluorescence intensity, whereas N-ethyl urea (EU) results in a fluorescence enhancement to a considerable extent. The variation in excited state properties of NATA with urea derivatives are correlated to the variation in the excited state dipole moment of the indole moiety microenvironment. Various hydrogen-bonding arrangements exists between NATA and urea derivatives and this is correlated to the shift in the emission maxima towards the red region. NATA exhibits a single exponential decay and the fluorescence lifetime in aqueous solution is 3.0 ns. Addition of urea derivatives results in a significant increase in the fluorescence lifetime irrespective of the nature of the urea derivatives. Butyl Urea(BU), which displays a red shift accompanied with fluorescence quenching of NATA exhibits contrasting properties compared to other urea derivatives is established through fluorescence spectral studies.

Keywords: NATA; Urea derivatives; fluorescence emission, fluorescence enhancement; fluorescence lifetime; hydrogen-bonding.

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INTRODUCTION

Micro heterogeneous systems are broadly classified into molecular aggregates and self-assemblies, which are further divided into several domains, based on their properties in solution and solid state [1]. The term self-assemblies refer to the orientation in the solution phase based on the arrangement of the head group, length of the hydrocarbon group, concentration of the solute and the nature of the interactions between the solute and the solvent molecules [1].

The nature of interaction of sparingly soluble solutes in aqueous solutions is of contemporary interest at both theoretical and practical level, and is of two types: hydrogen-bonding and hydrophobic interactions. Hydrogen-bonding interaction plays an important role in biology allowing the reversible formation of aggregates which are non-covalently linked, and the stability of the multiple hydrogen bonded systems

depends not only on the number of hydrogen bonds but also on the hydrogen-bonding pattern with the solvent molecules [2]. In contrast, hydrophobic interaction is an important factor in many physiochemical processes [1,3-4] and weak non-bonding interactions are important in many biological processes. The hydrophobic interactions are the most important driving force in biological processes [3, 5, 6] and is generally accepted that the hydrophobic interaction is due to an increased ordering or structuring of water molecules in the close vicinity of a non-polar solute [6,7].

Urea and related n-alkyl urea derivatives are classified as organized self-assemblies and are well oriented by strong hydrogen-bonding interactions in both solid and aqueous phase [7]. Large globular proteins like bovine serum albumin (BSA), contain many amino acid moieties are classified as molecular aggregates. The presence of hydrophobic, aromatic and heterocyclic moieties in the biomolecule imparts strong hydrogen-bonding interaction and hydrophobic

interactions in aqueous and in non-aqueous medium [8-10].

The concept of photophysics and photochemistry in micro heterogeneous systems are of utmost importance in the field of chemistry and biology [10-12]. Photophysical studies serve as a vital link in establishing and elucidating the structure and properties of the probe molecules in the micro heterogeneous environment in their ground and excited states [12]. Organised self-assemblies act as an excellent medium for studying the photophysical properties of fluorophores [12-32]. The presence of hydrophilic and hydrophobic group's results in a large variation in the photophysical properties of the probes is a well-known phenomenon [7]. Urea interaction with fluorophores [29,30] has been carried out in both aqueous and non-aqueous solvents, and the influence of urea-solvent hydrogen-bonding properties on the photophysical properties of the probe results in variation in the spectral properties of the fluorescent probe. This provides an excellent approach to study in depth involving the interaction of extrinsic and intrinsic fluorophores in urea and alkyl substituted urea molecular assemblies.

Studies involving the role of hydrogen-bonding interaction and the hydrophobic interaction involving urea on organized micelles [12-22], reversed micelles [22-24], vesicles [13], monolayers [25], polymers [26] and with cyclodextrins [27-32] have been well established. In spite of reports available on the properties of urea and alkyl urea derivatives, there is no concrete evidence about the mechanism and the nature of interaction involving urea and alkyl urea interaction with intrinsic and extrinsic fluorophores. Interestingly, the influence of urea concentration resulting in a large variation in the fluorescence spectral properties of these probes is of utmost importance in the field of spectroscopy. Even though, there are numerous reports based on the interaction of urea with biomolecules and with that of the hydrophobic moieties, the mechanism and the nature of the interaction existing between urea and biomolecules is an area of unanswered domain.

Urea: Significance and biological importance

The behaviour of urea is vital in biological and environmental studies because of its involvement as a waste product in our life [15]. Besides, it helps in sustained smooth functioning of the metabolic activities in men and in animals. Further, urea is non-corrosive, non-toxic and commercially available which does not produce any adverse effect to the environment and surroundings. Urea and alkyl urea derivatives are of significant importance due to their excellent hydrogen-bonding properties. Urea is readily soluble in water and possesses characteristic properties on the structure of water. The interest on aqueous solutions of urea and its derivatives are mainly related to the denaturing properties of urea on proteins and on the photophysical

properties of fluorescent probes. Further, urea can act as a host molecule for many hydrocarbon compounds, and this phenomenon is attributed to the extensive hydrogen-bonding network of the urea molecule. Further, urea derivatives are characterized by high solubility in water and alcohols such that it possesses hydrogen bonding abilities; it acts as an electron donor as well as an acceptor with water molecules. Possess contrasting hydrophilic and hydrophobic properties Displaces water molecules near its close vicinity and also acts as a water structure breaker, Formation of dimers, trimers and oligomers in water.

Urea as hydrogen bonded self-assemblies – Structure and properties

The orientation, geometry and the conformation of urea in solid [21, 22, 33] and gaseous states by hydrogen-bonding network has been well established by theoretical studies [23, 24], experimental methods [25-27], and molecular simulation analysis [29,34]. In contrast, the exact geometry of urea in aqueous solution could not be ascertained, even though many reports suggest that urea exists either in a planar or non-planar conformation. When urea molecule is surrounded by large number of water molecules, it brings in further complexity. The complex nature of urea in aqueous solution is not only influenced by the urea-urea, urea-water and water-water hydrogen-bonding interactions, but also depends upon the concentration of urea and the number of water molecules situated in the close proximity of the urea molecule.

Carbonyl and N-H groups in urea act as a hydrogen bond acceptor and donor respectively [30], which provides different types of hydrogen bonding arrangements like urea-urea, urea-water hydrogen bonding interactions [30-32]. The high permittivity and viscosity of urea derivatives in aqueous solutions reveal the existence of strong hydrogen-bonding interactions [35]. Which are very short ranged and short lived. The changes in hydrogen-bonding interactions on increasing or decreasing the concentration of urea derivatives in solution [36] are of significant importance involving both the extrinsic and intrinsic fluorescent probes. The relative stability of urea conformation results from solvent-urea hydrogen-bonding interactions, urea-urea intermolecular hydrogen bonding interactions [37-39] also influence the excited state properties of several micelles.

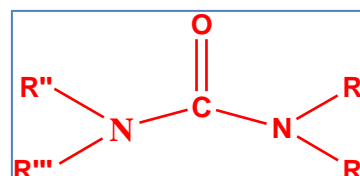
Aqueous solution of urea show exceptional specific properties which results in a large change in the structure of the proteins [28], increase in the solubility of hydrophobic species [37], controls micelle formation and involves in a large variation in photophysical properties of probe molecules. The thermodynamic properties of aqueous urea solution influence the stability of the hydrophobic bond in proteins and peptides have a contrasting effect on the structure of

water. This behaviour is attributed to the ordering or restructuring the position of water molecules in the vicinity of the urea molecule.

Role of alkyl substitution on the hydrogen-bonding properties of urea

Urea and related N-alkyl derivatives were considered as simple model compounds that exhibit strongly opposite features (hydrophilic and hydrophobic) and show the characteristic property of denaturing proteins in aqueous solutions to a varying extent [8]. Intercomponent molecular interaction and the hydrophobic stabilisation of the water structure by urea and alkyl urea derivatives depend on the number of hydrogen atoms and the number of alkyl groups. Alkyl substitution of urea in aqueous solution imparts three major effects [7, 36, 40]; contribution from the methyl group resulting in hydrophobic nature, hindrance of hydrogen-bonding ability of the unsubstituted amino groups and decrease of hydrogen-bonding ability of the carboxyl groups.

Alkyl urea derivatives are peculiar that they have two kinds of functional groups, which perturb water in different ways, generating distinct interactions. Alkylureas, in general prefer the conformation wherein the alkyl group is cis to the carbonyl oxygen. Based on the substitution of the alkyl group, the hydrophobic hydration cospheres of branched alkyl chain contribute relatively little to the hydrophobic interactions, since they are relatively unchanged with respect to increasing concentration. The alkyl urea-protein association or the interaction with hydrophobic moieties in aqueous solution is no longer extra stabilised by hydrogen-bonding [41] alone, but also depends upon the presence of hydrophobic moieties and their symmetry. The structure of urea derivatives is provided in scheme 1.



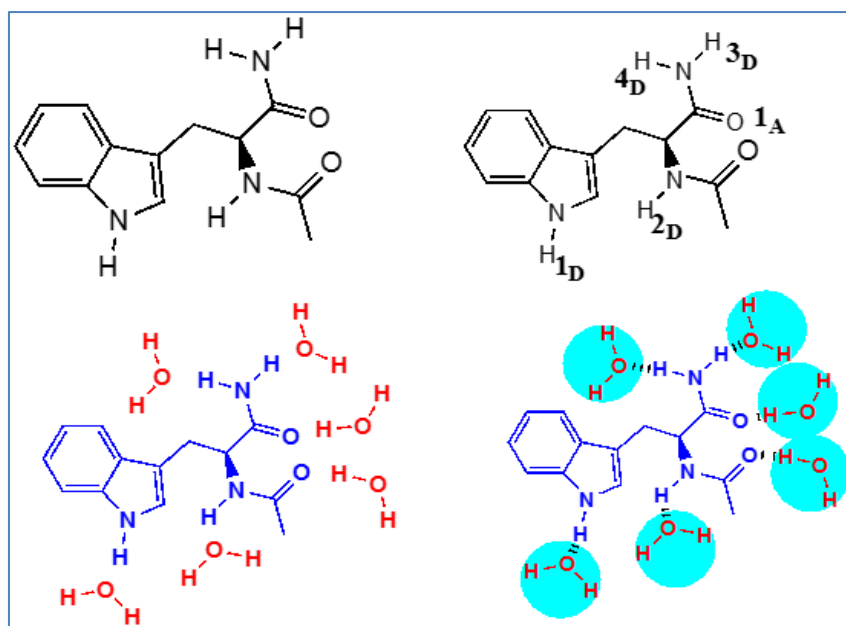
Urea	R	R'	R''	R'''
U	U	H	H	H
MU	CH ₃	H	H	H
EU	C ₂ H ₅	H	H	H
BU	C ₄ H ₉	H	H	H
DMU	CH ₃	H	CH ₃	H
1,1,DEU	H	H	C ₂ H ₅	C ₂ H ₅
TMU	CH ₃	CH ₃	CH ₃	CH ₃

Scheme 1: Structure of urea and alkyl urea derivatives

N-Acetyl-L-tryptophanamide (NATA)

N-Acetyl-L-tryptophanamide (NATA) is an N-terminal and C-terminal blocked analogue of L-tryptophan. L-tryptophan, NATA and NATA-tyr molecules have intrinsic fluorescence which makes these molecules useful in studies pertaining to fluorescence and fluorescence enhancement. The fluorescence of N-acetyl-L-tryptophanamide and proteins was measured and quantified by emission and lifetime studies such that it makes a wonderful probe to study with. The fluorescence of proteins, emitted by aromatic amino acids, is a sensitive indicator of structural changes [1,2]. When a protein is denatured, the fluorescence Spectrum results in a red shift and a decrease in intensity, and hence in a lowering of the $\langle Q \rangle$, as defined by Weber and co-workers [3]. However, it is difficult to primarily attribute the fluorescence change of a protein, which contains various proportions of tyrosine,

phenylalanine, and tryptophan, to environmental changes in the vicinity of the aromatic amino acids. Time-resolved and steady-state fluorescence quenching of NATA by acrylamide and iodide, over a range of viscosities in propylene glycol were carried out in aqueous solution. The quenching of NATA by acrylamide and iodide results in heterogeneity of the intensity decay which increases with the quencher concentration and these are attributed to the complex decays of NATA to transient effects in diffusion and the nature of the fluorophore-quencher interaction. Most of the studies of NATA were concerned with the fluorescence arising from the indole moiety in proteins, but very few studies are available in the literature concerned with fluorescence lifetime studies of NATA with hydrogen-bonding solutes. The Structure of NATA and the Hydrogen-bonding donor and acceptor groups are provided in scheme 2.



NATA and the hydrogen-bonding donor and acceptor groups are provided in scheme 2.

Scheme 2: Structure of NATA and representation of hydrogen-bonding donor and acceptor groups. The scheme represents a theoretical representation of six water molecules around the close vicinity of NATA.

Experimental methods and techniques

Materials

Urea (U) (Molecular biology grade) was obtained from Merck Chemicals, India, Pvt. Ltd. The urea derivatives methylurea (MU), ethylurea (EU), butylurea (BU), 1,3,N,N'-dimethylurea (DMU), 1,1,N,N-dimethylurea (n-DMU), 1,1, N,N-diethylurea (n-DEU), was purchased from Lancaster Chemicals, U.K and Alfa Essar and N-acetyl tryptophanamide (NATA) were purchased from Sigma-Aldrich Ltd. Triple distilled water was used throughout the studies.

Instrumental Techniques

Absorption spectrophotometer

Absorption spectrum was recorded using an Agilent 8453 diode array spectrophotometer.

Fluorescence spectrophotometer

Steady state fluorescence measurements were carried out in MPF-44B fluorescence spectrophotometer interfaced with PC through RISHCOM-100 multimeter. For the steady state measurements the excitation and emission slit width were kept at 4 and 2 nm respectively. All the structures were generated in Chem Draw Ultra 8.0 software and the 3D structures were incorporated for the most possible orientation of urea derivatives with NATA molecule.

Measurement of Fluorescence Decays

The fluorescence decay measurements of NATA with amides were carried out using the Time Correlated Single Photon Counting technique (TCSPC) with micro channel plate photomultiplier tube (MCP-PMT) as detector and 290 nm LED as the excitation source. The fluorescence of NATA with urea derivatives was monitored at magic angle (54.7°). This was counted by a MCP-PMT apparatus (Hamamatsu R3809U) after being passed through the monochromatic and was preceded 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 ~50 ps. The obtained fluorescence decays were analyzed by using IBH software (DAS-6) which is based on reconvolution technique using iterative nonlinear least squares method.

RESULTS AND DISCUSSION

Absorption spectral studies

The absorption spectra of NATA in water exhibits absorption maximum around 280 nm and a hump around 290 nm as shown in figure 1. Addition of urea to NATA results no significant change at the longest wavelength absorption maximum which signifies that the longest wavelength absorption maxima is unperturbed on the addition of urea. Urea alone exhibits a strong absorbance less than 240 nm and has been well documented in the literature. The ground state absorption spectral studies of NATA with urea reveal that no significant information regarding the nature of interaction could be ascertained. Due to the close spacing of the vibrational and rotational energy levels, the exact nature of bonding could not be quantified.

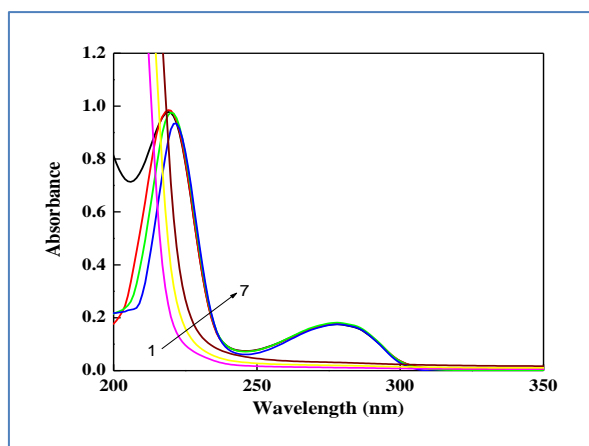


Fig-1: Absorption spectrum of NATA with Urea in water. 1. Urea 0.6 M, 2. Urea 1.2 M, 3. Urea 2.4 M, 4. NATA alone, 5. NATA + Urea 0.6 M, 6. NATA + Urea 1.2 M, 7. NATA + Urea 2.4 M

Introduction of symmetrical urea derivatives like DMU resulted in an increase in absorbance at the longest wavelength absorption maximum of NATA. Introduction of two methyl moieties in the urea molecular framework results in a variation in the absorbance at the longest wavelength absorption maximum. This is found to be entirely different from that of urea, wherein no significant change in the absorption spectrum results. In order to obtain a clear understanding on the photophysical process of NATA with urea derivatives, emission spectral studies were carried out.

Emission spectral studies

On exciting at 290 nm, NATA exhibit a broad emission around 350 ± 2 nm. Addition of urea results in a fluorescence enhancement with slight shift in the emission maximum towards the red region. The emission spectra of NATA with urea are shown in figure 2. The red shift in the emission maxima is presumably attributed to direct hydrogen-bonding of the carbonyl oxygen of urea with the hydrogen atom (N-H) of indole moiety. Similarly, addition of urea results in an increase in the solvent polarity such that an increase in the dipole moment presumably attributes to the shift in the emission towards the red region.

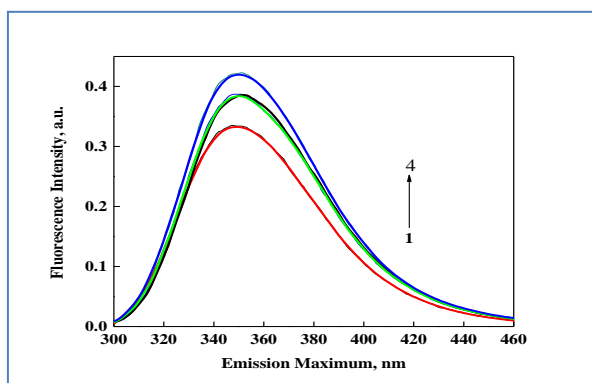


Fig-2: Emission spectra of NATA with Urea in water. λ_{exc} 290 nm. 1. NATA alone, 2. NATA + Urea 0.6 M, 3. NATA + Urea 1.2 M, 4. NATA + Urea 2.4 M

Interestingly, addition of DMU also results in a fluorescence enhancement, but no apparent shift in the emission maxima neither towards the blue nor red region results. However, the extent of fluorescence enhancement in NATA was found to be more in the case of DMU than in urea. Since an alkyl substitution in the urea molecules frame work results in a large variation in the emissive nature of NATA, we extended our studies to unsymmetrical urea derivatives also. The emission spectra of NATA-DMU are provided in figure 3.

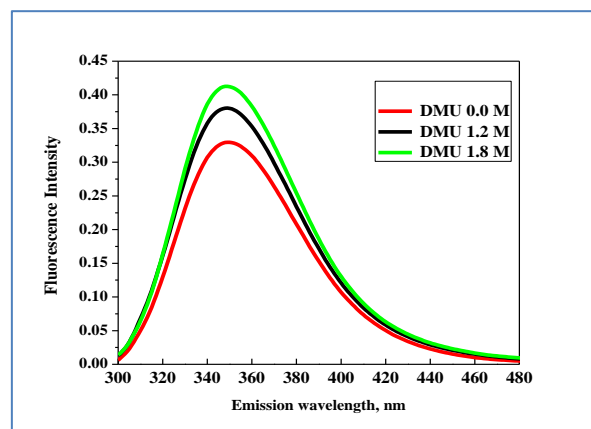


Fig-3: Emission spectra of NATA with DMU in water. λ_{exc} 290 nm

The following unsymmetrical urea derivatives like MU, EU and BU were taken in the present investigation. Addition of MU results in no significant change on the emission maximum of NATA (figure 4). Addition of EU which is an isomer of DMU results in an increase in the fluorescence enhancement similar to that of urea. On the contrary, addition of BU results in a fluorescence quenching accompanied with a larger shift towards the red region. The emission spectroscopy of NATA with EU and BU are shown in figures 5 and 6 respectively. The variation in the fluorescence intensity and shift in the emission maxima of NATA in the presence of unsymmetrical urea derivatives reveals that the extent of methyl substitution, symmetry of the urea molecular framework influences the excited state properties of NATA.

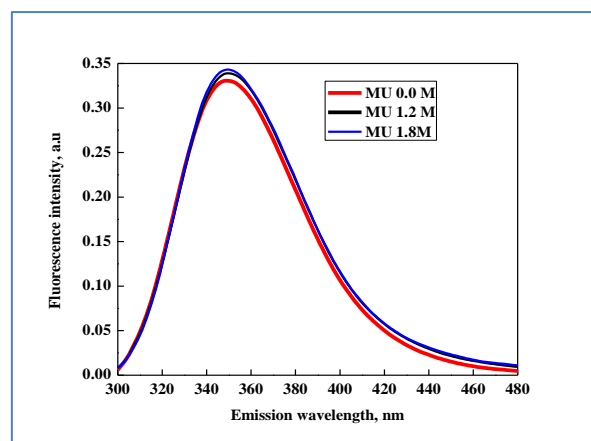
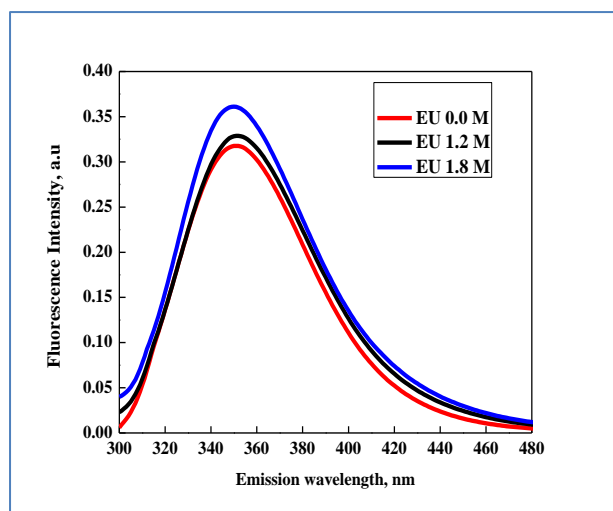
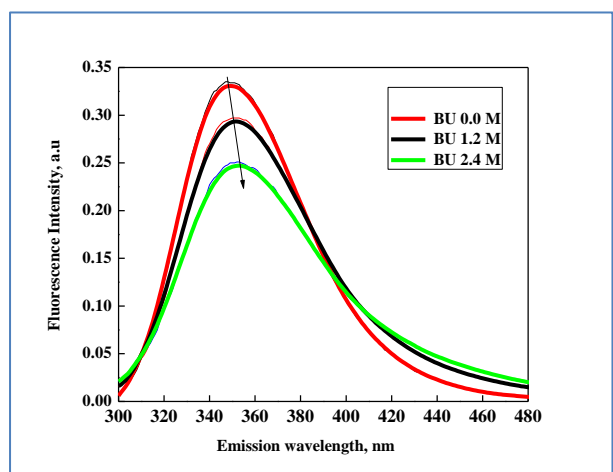


Fig-4: Emission spectra of NATA with MU in water. λ_{exc} 290 nm

Fig-5: Emission spectra of NATA with EU in water. $\lambda_{\text{exc}} 290 \text{ nm}$ Fig-6: Emission spectra of NATA with BU in water. $\lambda_{\text{exc}} 290 \text{ nm}$

Even though a change in the one of the alkyl substitution (R group) of urea molecular framework, we observed a drastic variation on the fluorescence spectral properties of NATA. This behaviour elucidates that the role of urea derivatives on the fluorescence spectral behaviour of NATA is found to be entirely different. A similar behaviour was also observed in the case interaction of bovine serum albumin (BSA) with urea derivatives in water [40]. This phenomenon is probably ascribed to the following factors such as increase in the hydrophobicity in the urea molecular framework, decrease in the number of hydrogen-bonding donor moieties and extent of hydrogen-bonding network of urea derivatives with water as well with NATA molecule. The variation is shown in the extent of fluorescence enhancement/quenching of NATA with urea derivatives (Figure 7).

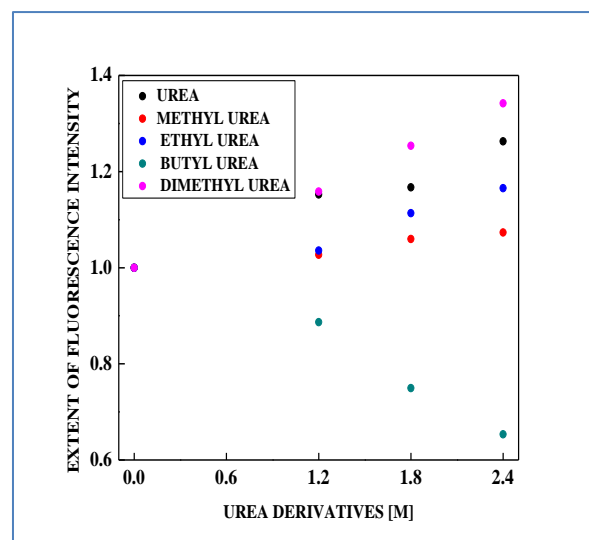


Fig-7: Extent of fluorescence enhancement/quenching of NATA by urea derivatives

Mechanism of fluorescence enhancement

The mechanism of fluorescence enhancement involving a host-guest interaction in aqueous solution or in buffered solutions is mostly attributed to two different mechanisms [28, 36–40]. One mechanism is attributed either to the change in the microenvironment or change in the pH or viscosity of the medium. Another mechanism signifies that the fluorescence enhancement is due to the changes in the bulk viscosity and the polarity around the probe molecule. The gradual addition of solute molecules (guest) resulting in a decrease or increase in the polarity of the medium around the vicinity of the probe (host) has been reported [32]. This mechanism signifies that the increase in the fluorescence intensity accompanied with a blue shifted or red shifted emission is due to the change in the microenvironment around the fluorophore resulting in the stabilization or destabilization of the charge transfer (CT) state. Further, some mechanisms signify that an intermolecular energy transfer from the guest molecule to the probe results in a fluorescence enhancement [36–40]. Apart from these mechanisms, the fluorescence enhancement is also correlated to the binding of the probe-solute resulting in the formation of a stable complex in the excited state.

In our present study, urea, DMU and EU results in a fluorescent enhancement which reveals that the excited state nature of NATA are stabilised and the quantum yield increases significantly. Conversely, BU alone exhibits a fluorescence quenching phenomenon which was found to be dynamic in nature. Urea derivatives exhibit contrasting hydrogen bonding properties in aqueous solution and the role of urea in water molecule-hydrogen bonding with urea derivatives varies considerably.

Time resolved fluorescence lifetime studies

The fluorescence lifetime decay of NATA with symmetrical urea derivatives (U, DMU and TMU) are shown in figures 8-10 respectively and that of unsymmetrical urea derivatives (MU, EU and BU) are provided in figures 11-13 respectively.

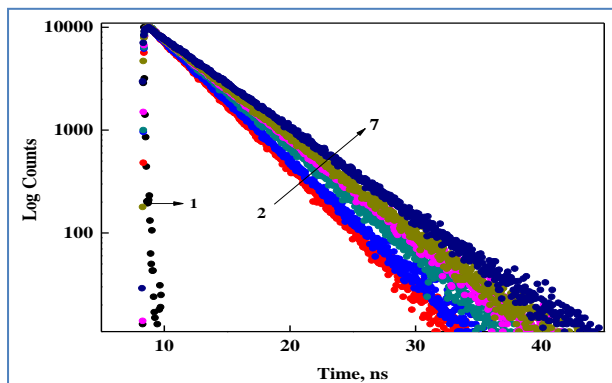


Fig-8: Fluorescence lifetime decay of NATA with Urea in water. λ_{ex} 290 nm LED. 1) LED profile, 2) NATA + U 0.0 M, 3) NATA + U 1.0 M, 4) NATA + U 2.0 M, 5) NATA + U 4.0 M, 6) NATA + U 6.0 M, 7) NATA + U 8.0 M

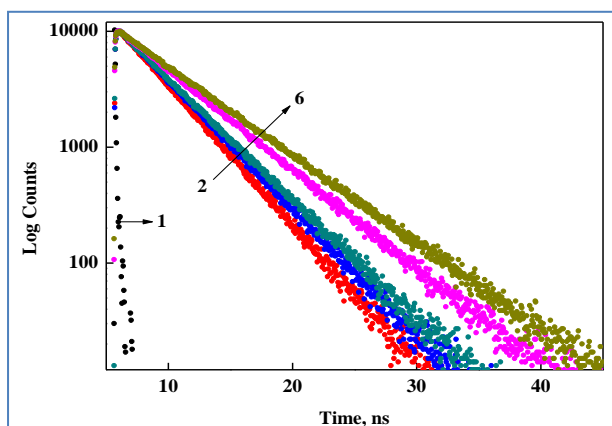


Fig-9: Fluorescence lifetime decay of NATA with DMU in water. λ_{ex} 290 nm LED. 1) LED profile, 2) NATA + DMU 0.0 M, 3) NATA + DMU 0.6 M, 4) NATA + DMU 1.2 M, 5) NATA + DMU 2.4 M, 6) NATA + DMU 4.8 M.

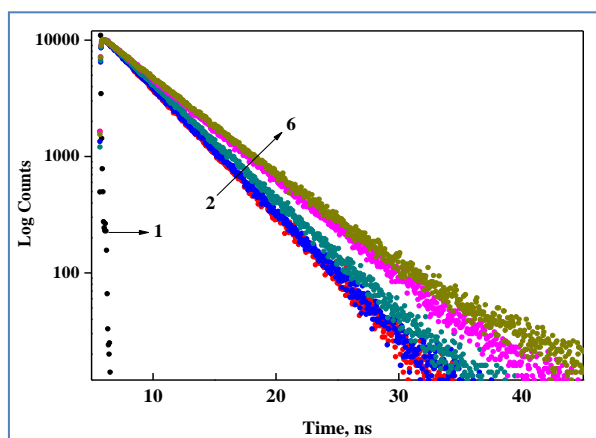


Fig-10: Fluorescence lifetime decay of NATA with TMU in water. λ_{ex} 290 nm LED. 1) LED profile, 2) NATA + TMU 0.0 M, 3) NATA + TMU 0.6 M, 4) NATA + TMU 1.2 M, 5) NATA + TMU 2.4 M, 6) NATA + TMU 4.8 M

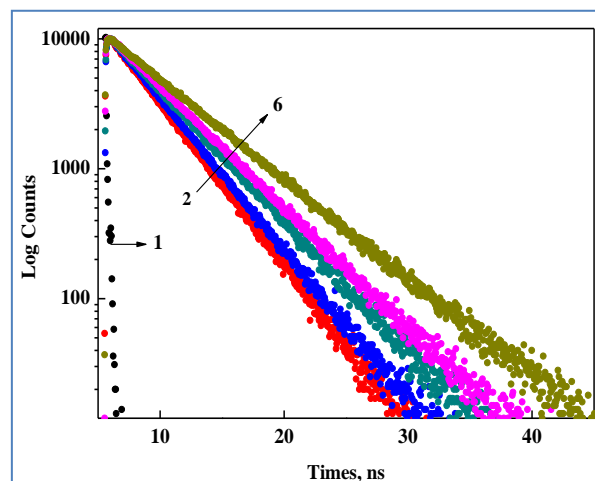


Fig-11: Fluorescence lifetime decay of NATA with MU in water. λ_{ex} 290 nm LED. 1) LED profile, 2) NATA + MU 0.0 M, 3) NATA + MU 0.6 M, 4) NATA + MU 1.2 M, 5) NATA + MU 2.4 M, 6) NATA + MU 4.8 M

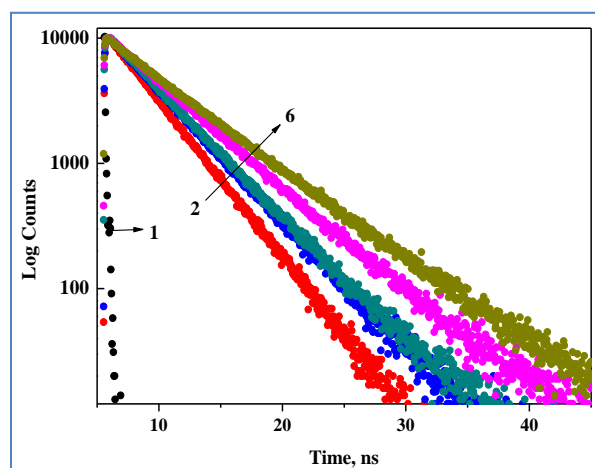


Fig-12: Fluorescence lifetime decay of NATA with EU in water. λ_{ex} 290 nm LED. 1) LED profile, 2) NATA + EU 0.0 M, 3) NATA + EU 0.6 M, 4) NATA + EU 1.2 M, 5) NATA + EU 2.4 M, 6) NATA + EU 4.8 M

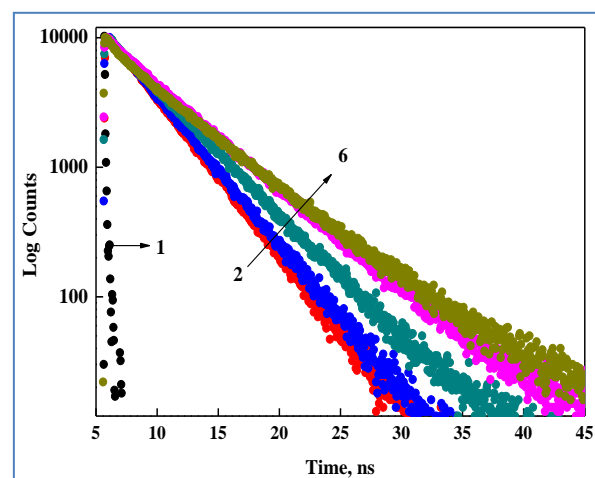


Fig-13: Fluorescence lifetime decay of NATA with BU in water. λ_{ex} 290 nm LED. 1) LED profile, 2) NATA + BU 0.0 M, 3) NATA + BU 0.4 M, 4) NATA + BU 0.8 M, 5) NATA + BU 1.2 M, 6) NATA + BU 1.5 M

The fluorescence lifetime decays of NATA with urea derivatives were monitored at 350 nm using 290 nm LED. Unlike steady state fluorescence measurements, addition of urea derivatives, irrespective of the methyl group substitution, results in an increase in the fluorescence lifetime of NATA. A single lifetime component was only observed even at the highest concentration of urea derivatives (except BU). The fluorescence lifetime of NATA in aqueous solution is around 3.1 ± 0.1 ns in water. The single exponential decay reveals that the excited state nature of NATA is largely stabilised by the presence of urea molecules through a uniform hydrogen-bonding interaction such that a homogeneous microenvironment is created by the urea derivatives. Further, the stabilisation is presumably attributed to hydrogen-bonding interaction and well assisted by the hydrophobic influences of urea derivatives due to the methyl substitution in the urea molecular framework.

Interestingly, the addition BU to NATA results in biexponential decay at a higher concentration (> 1.2 M). This signifies the presence of two distinguishable microenvironments existing in aqueous solution which is attributed to variation in the hydrogen-bonding properties of BU with water and NATA. The presence of two lifetime components with varying amplitude of NATA in the presence of BU confirms the existence of hydrogen-bonding and hydrophobic influences. Even though these combined interactions could be encountered in all the urea derivatives, it was found to be more prominent in the case of BU. BU although less hydrophobic than TMU, results in the creation of a more than one microenvironment in the aqueous phase such that these phases differ in the nature of interaction and this is reflected in the biexponential behaviour of NATA with BU (Figure 13).

CONCLUSION

NATA interaction with urea derivatives by various techniques like UV-Visible, fluorescence emission and Time Correlated Single Photon Counting (TCSPC) technique were employed as a tool in establishing the variation in the photophysical properties of NATA with non-fluorophoric hydrogen-bonding solutes in water. These techniques provide link in establishing the binding nature as well as preferred mode of interaction of NATA with urea derivatives which are found to be predominantly hydrogen-bonding, although hydrophobic interactions coexist. The fluorescence enhancement reveals that the excited state properties of NATA are influenced by the concentration of the urea derivatives which disrupts the water structure and also the microenvironment around the close vicinity of NATA in aqueous solution. Presence of several hydrogen-bonding environments coexists in aqueous medium and this is attributed to the presence of urea derivatives. The fluorescence lifetime of NATA is governed by the concentration of urea

derivatives and this is significant when the ratio of NATA: urea derivatives are of the order of above 1: 1000. Several molecules of urea derivatives stabilise NATA through hydrogen-bonding interaction and impart hydrophobic influences in aqueous medium.

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