D.Simon et al. International Journal of Recent Research Aspects ISSN: 2349-7688, Special Issue: Conscientious Computing Technologies, April 2018, pp. 664-672

Investigation of Aggregation Induced Emission Enhancement of Anthracene derivatives and Interaction with BSA

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Abstract- **A new three anthracene derivatives of amide (R1), Urea (R2), thiourea (R3) were synthesized and characterized by UV-visible, FT-IR, EI-MS and NMR spectral techniques. The anthracene derivatives are soluble in THF and which aggregates by varying the fraction of water from 0% to 90% (V/V). The emission intensities were increasing by increasing the water fraction. The conjugated organic fluorophore molecules have very high luminescence efficiency in the dilute solution. The significant enhancement in their light emission intensity is called as Aggregated Induced Emission Enhancement (AIEE). The UV-visible absorption spectra have the absorbance at 251-259 nm are decreased with increasing the fraction of H2O and tails are appeared in the range of 450 to 600 nm which indicates that the twisted intra molecular charge transfer (ICT) on their AIEE behavior and the emission spectra of receptors with excitation at 400 nm have a broad emission enhancement with increasing water fraction due to aggregation could be observed because of the free rotation of the phenyl and anthracene rings about the axes of –CH=N- bonds. The AFM spectral studies shows that the size of receptor compounds (R1-R3) WITH 80% H2O are in the range from 100-500 nm which confirms that there is an aggregation of particles in THF and H2O mixture. The SEM analysis of R3 in water fraction (90%) show that the cluster of ordered microsphere to rod shaped nano particles.The interaction between receptor compounds (R1-R3) and BSA has been investigated by using UV-Visible absorption, fluorescence emission spectral techniques. These spectral studies confirms that BSA binds with the receptor compounds through non-covalent types of interactions and fluorescent intensity of receptors compounds enhances gradually by increasing the concentration of BSA. The binding constant values were calculated by the modified Benessi-Hildebrand equation.**

I. INTRODUCTION:

Anthracene derivatives were evidenced as excellent fluorophores and widely used in the developments of fluorescence sensors because of their excellent photoluminescence properties and chemical stabilities. Anthracene fluorescent probes self-assembled to form dimeric structures upon the addition of certain ions to give π–π* excimer fluorescence and also provided the AIE characteristics by tuning the solvent conditions [1]. Aggregation-induced emission (AIE) refers to a photophysical phenomenon shown by a group of luminogenic materials that are non-emissive when they are dissolved in good solvents as molecules but become highly luminescent when they are clustered in poor solvents or solid state as aggregates. In a concentrated solution, the damage (Chemical Quenching) is normally done through the "formation of aggregates" [2] of luminophores [3-8]. These low dimensional organic micro/nanoparticles with well-defined structures (micro/nanoscale hollow, spheres, capsules, tubes, rods, wires and so forth) with the help of π-π stacking, hydrogen bonding, electrostatic interactions and other non-covalent interactions emerges to be relatively elegant as they intend much more flexibility and variability in material design and synthesis [9]. This unique characteristic differentiates them from conventional luminophores and focused on the exploration of their utilities in the development of optical sensors and other important applications. Fluorophores with AIEE characteristics have been successfully utilized in organic light emitting diodes (OLEDs) [10-12]. OLED materials find promising application in next generation flat panel displays and solid-state lighting [13-15]. Recently, a little number of organic π-conjugated molecules was engaged as building blocks to assemble supramolecular one-dimensional (1D) micro/nano- structures which offer several advantages for sensing applications because of their outstanding photoluminescence (PL) efficiency, high reactivity and good processing capability [16−19].

Herein, we use anthracene derivatives to study the influence of H-bonding and steric effects on the coupling behaviour of the chromophores in different phases. For that purpose a set of three compounds was synthesized consisting of a benzene connected to the anthracene through a methylene—amide, urea and thiourea linker. These compounds exhibit remarkable aggregation-induced emission properties, which make them promising candidates as luminescent materials for electro luminescence applications. The anthracene derivative compounds are involved aggregation in THF-Water (v/v) mixture and the results were reported using various spectral techniques like UV-vis, emission, AFM and SEM spectral analysis.

Serum albumin, being the most abundant protein in blood plasma, is responsible for maintaining osmotic blood pressure [20] and for binding and transport of a wide variety of molecules (drugs, metabolites, fatty acids, etc.) [20-22].The binding of different ligands by albumin depends not only on the type of ligand but also in the presence of other ligands in solution (competitive binding) [23] as well as on the conformation of the protein molecules [24].Intrinsic fluorescence, which are caused by aromatic amino acid residues (tryptophan (Trp), tyrosine(Tyr)and phenylalanine(Phe)[25-30].

Among the aromatic amino acids, an attention is usually paid to Trp, because of its high quantum yield in proteins [29] and sensitivity of its fluorescent properties to the local environment. For instance, when a ligand binds to a site on a protein in the vicinity of Trp, a several times decrease of its fluorescence intensity and/or fluorescence lifetime may occur [29]. Moreover, the wavelength of maximum in the fluorescence spectrum of Trp strongly depends on the polarity of its microenvironment [31, 32]. In the present study, BSA is selected as our protein model because of its long-standing interest in the protein community. We report that the results on the interaction of fluorescent probe of anthracene derivatives with protein like BSA studied by spectroscopic techniques. The absorption titration studies and the fluorescence titration studies was carried out by keeping the concentration of receptors (4 μM) constant while varying the concentration of BSA(2 to 20 μM) was performed.

II. EXPERIMENTAL

Materials and Methods

1-pyrenecarboxaldehyde, Benzohydrazide, 4-phenyl semicarbazide, 4-phenylthiosemicarbazide and Bovine serum albumin (BSA, Fraction V, approximately 99%) were purchased from Sigma-Aldrich Chemical Corp. Solvent tetrahydrofuran (THF), Acetic acid, Acetone were purchased from E-Merck India Ltd. Triply distilled deionized water was used throughout the experiments.

Instrumentation

The UV-vis absorption spectral measurements have been carried out in a quartz cuvette of 1 cm using JASCO 630 UV-vis spectrophotometer. The UV-visible absorption spectra were recorded on a JASCO variant 630 Spectrophotometer. The infra-red spectra of the receptors have been recorded in a JASCO spectrophotometer in solid phase, as KBr pellets and The FTIR spectra were recorded by using Nicolet Si 5 in the range 500- 4000cm-1. The fluorescence emission spectrum were recorded with JASCO FP-8300. The binding constants were calculated using the modified Benesi-Hildebrand equation .Electron spray Ionisation Mass spectrometry (ESI-MS) was recorded in HP Agilent 5973.1H NMR spectra have been acquired on a Bruker 300 MHz NMR spectrometer with CDCl3 solvent. Data is reported as follows: chemical shift in ppm (δ), intergration, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant J (Hz) and assignment. The SEM images were recorded by using JEOL Model: JSM 7000F. The AFM were recorded by using Nanosurf Easyscan 2 AFM.

Preparation of N-(anthracene-1-ylmethylene) benzo hydrazide (R1)

ly added to a solution of anthracene-9-carboxaldehyde in ethanol. The reaction mixture was reflux at 75–80ºC for 3h, yielding the precipitate of **R1** (as mentioned in synthetic route). After evaporating the solvent in vacuum, the residue was filtered and recrystallized with ethanol. The compound **R1** was isolated as yellow solid. Yield = 0.765 g, 94%. FT-IR (KBr): ν_{max} cm^{-1}: 3200 (N-H), 1649 (C=O),1545 (C=N), 1250-1000 (C-C), 900-675(C-H). ^{1}H NMR: (400 MHz, $CDCl_3$): δ 6.99 (s, 1H, J=7.2 Hz, ArH), 7.26 (s, 2H, J=7.2 Hz, ArH), 7.99–8.05 (m, 6H, ArH), 7.51-7.53 (d, 2H, J=8.4 Hz, ArH), 8.53 (s, 1H, -CH=N), 8.67 (s, 1H, ArH), 9.23 (s, 1H, -NH), 9.45 (s, 1H, ArH), 9.63 (s, 1H, ArH). For $C_{22}H_{16}N_2O$ HRMS:MS − ES* (m/z): $[M + H]^+$:Calculated: 324.4, Found: 323.1.

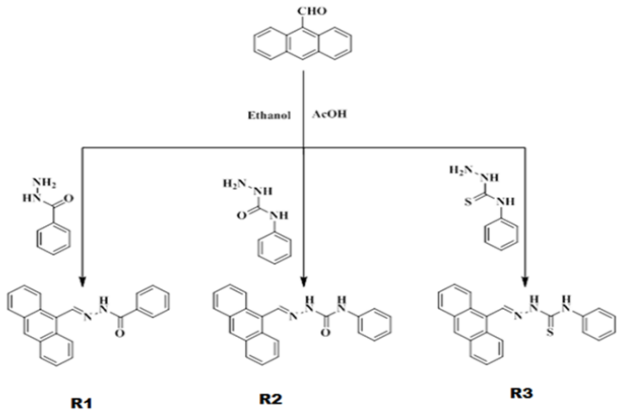
Preparation of N-(antracene-1-ylmethylene) phenyl semicarbazide (R2)

A hot ethanolic solution of 4-phenylsemicarbazide was slowly added to a solution of anthracene-9-carboxaldehyde in ethanol. The reaction mixture was reflux at 75–80ºC for 3h, yielding the precipitate of **R2** (as mentioned in synthetic route). After evaporating the solvent in vacuum, the residue was filtered and recrystallized with ethanol was characterized by various spectral techniques like ^{1}H NMR, FT-IR, Mass, UV–vis absorbance and fluorescence emission spectroscopic techniques. **R2**: The compound **2** was isolated as yellow solid.

Yield = 0.785 g, 93%. FT-IR (KBr): ν_{max} cm^{-1}: 3390 (N-H), 2885 (C=C-H), 1690(C=O), 1596,1533 (C=N), 1250-1000 (C-C), 900-675 (C-H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06 – 7.09 (t, 1H, J=7.2 Hz, ArH), 7.26 – 7.34 (m, 6H, J=7.2 Hz, ArH), 7.53–7.60 (m, 3H, ArH), 8.06 - 8.08 (d, 2H, J=8.4 Hz, ArH), 8.20 (s, 1H, ArH), 8.44 - 8.46(d, 2H, ArH), 8.54 (s, 1H, ArH), 8.90 (s, 1H, -CH=N), 9.13 (s, 1H, -NH), 10.31 (s, 1H, -NH). For $C_{22}H_{17}N_3O$ HRMS:MS − ES* (m/z): $[M + H]^+$: Calculated: 339.4, Found: 338.2.

Preparation of N-(anthracene-1-yl methylene) phenyl thiosemicarbazide (R3)

A hot ethanolic solution of 4-phenyl semithio carbazide was slowly added to a solution of anthracene-9-carboxaldehyde in ethanol. The reaction mixture was reflux at 75–80ºC for 3h, yielding the precipitate of **R3** (as mentioned in synthetic route). After evaporating the solvent in vacuum, the residue was filtered and recrystallized with ethanol was characterized by various spectral techniques like ^{1}H NMR, FT-IR, Mass, UV–vis absorbance and fluorescence emission spectroscopic techniques. **R3**: The compound **3** was isolated as yellow solid. Yield = 0.805 g, 91%. FT-IR (KBr): ν_{max} cm^{-1}: 3445,3328 (N-H), 3142 (=C-H), 1440 (C=S), 1540 (C=N), 1250-1000 (C-H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 – 7.25 (t, 1H, J=7.2 Hz, ArH), 7.37 – 7.40 (t, 1H, J=7.2 Hz, ArH), 7.51 – 7.60 (m, 6H, J=7.2 Hz, ArH), 7.67–7.69 (d, 3H, ArH), 7.88 – 7.90 (d, 2H, J=8.4 Hz, ArH), 8.05 – 8.07 (d, 2H, J=8.4 Hz, ArH), 8.20 (s, 1H, ArH), 8.45 - 8.47 (d, 2H, ArH), 8.55 (s, 1H, ArH), 8.61 (s, 1H, ArH), 8.87 - 8.89 (d, 2H, ArH), 9.06 (s, 1H, -CH=N), 9.30 (s, 1H, -NH), 10.08 (s, 1H, -NH). For $C_{22}H_{17}N_3S$,HRMS:MS − ES* (m/z): $[M + H]^+$: Calculated : 339.4, Found: 338.2.



Scheme: 1 Synthetic Route to Anthracene derivatives

RESULT AND DISCUSSION

synthesis

The synthesis of R1-R3 compounds were depicted in scheme 1 , and the preparation information is provided in Experimental Section: that is, reaction of 9-anthracene carboxaldehyde(0.50 g, 2.42 mmol), with corresponding amine (2.67mmol) dissolved in ethanol solution. A few drops of acetic acid were added and refluxed for 3 hours. The reaction mixture was filtered hot, washed with hot ethanol dried under vacuum to obtain a desired product and used without further purification. The structure of the compound was analyzed by1H-NMR,ESI-MS,FT-IR and UV-visible spectroscopy data.

Aggregation Induced Emission Enhancement of R1-R3 UV-visible absorption spectral study

When a non-emissive molecule is induced to emit by aggregate formation, the molecule is referred to as an "AIE active" molecule. To explore whether receptors (R1-R3) is AIE active or not, the absorption spectra receptors (R1-R3) of were investigated to observe the absorption behaviours of receptors when different fractions of water were added to the THF solutions of the compounds which are given in the Fig. 1.The UV−vis absorption spectrum of receptors (R1-R3) (4 μM) was recorded in the presence of different percentages of water. The receptor compounds shows that the broad band shows the broad absorption peak in the range of 387−400 nm. Upon addition of different fraction of water to receptor compounds, which aggregates due to interaction with water as evident by single characteristic peak have not seen. Generally, blue shift in absorption results from H-type aggregation and red shift in absorption is due to J-type aggregation. However, in the present case blue shift absorption was observed upon increasing the percentage of water, which clearly discloses that H-type aggregates are formed (i.e., head-head interaction).

The absorbance of the entire spectrum increased swiftly showing an absorption tail extending well into the long wavelength region, which was caused by the light scattering or Mie effect of the nanoaggregate suspensions in the solvent mixtures. The results implies that the receptor molecules have aggregated into nanoparticles in the aqueous mixture containing ~80% of water for both R1 and R2, but 90% was observed in R3.The leveling-off tail in the visible region of the absorption spectrum suggests the formation of nanoscopic aggregates of receptormolecules which is due to the Mie effect of the nanoparticles .

The receptor (R1-R3) which exhibited two absorption peaks region at 251-259 nm and 387- 400 nm in pure THF solution. With an increase in water fraction (fw) from 0% to 70%, these two absorption peaks basically remained at the same positions, and a new less intense absorption peak at about 215-223 nm appeared at fw = 20% of water. When fw was further increased to higher values (≥70%) but the absorption peak is suddenly decreased at fw = 80 %. At the same time, leveled-off tails appeared in the visible region in the range of 450-600

nm which can be ascribed to light-scattering effects and indicate the formation of aggregates. In the solvent mixtures with water contents of ≤70%, no leveled-off spectral tails in the long-wavelength region were recorded by the UV-Visible spectra measurements, which confirming that the molecules were dissolved as isolated species in the solvent mixtures. The UV-visible absorption spectrum of R1 and R2 in THF/water mixtures shows outstanding changes at fw≥80%.

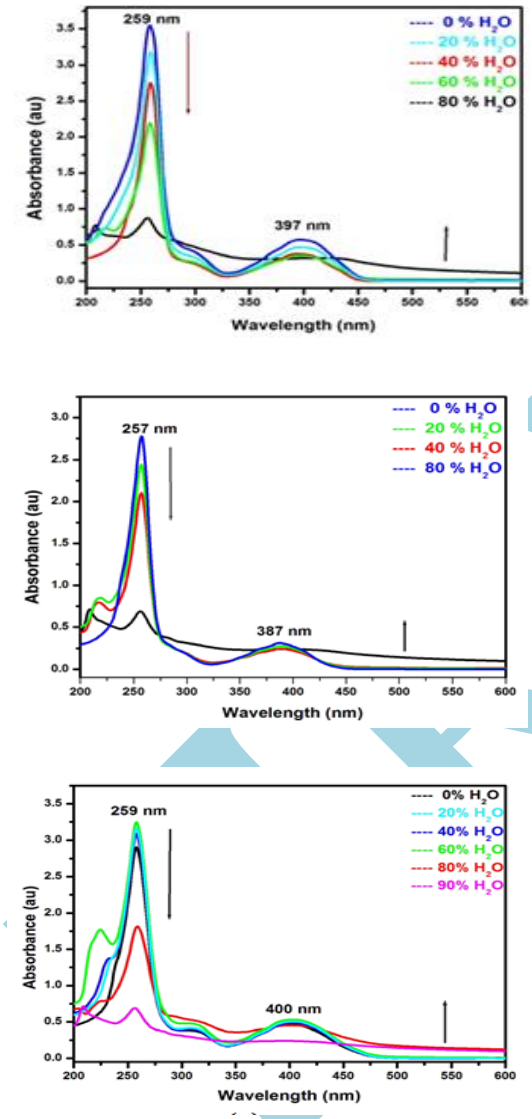
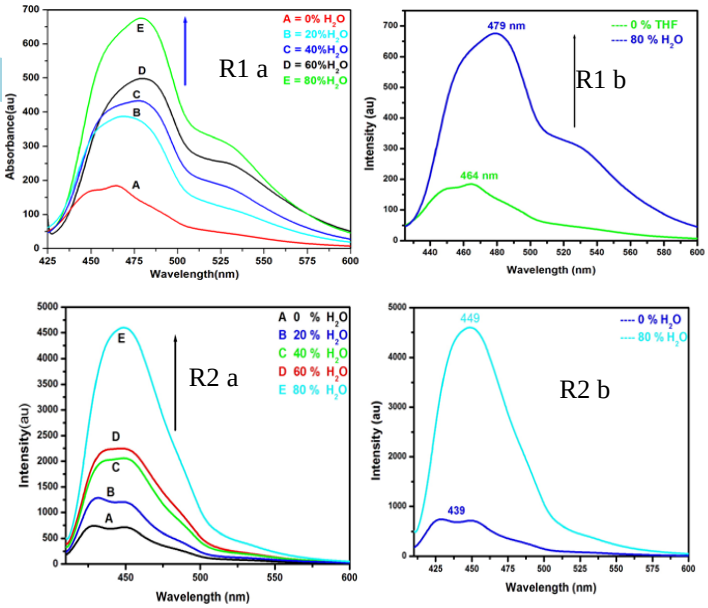


Fig. 1 UV- visible absorption spectra of R1-R3 in THF with various fractions of H2O

Fluorescence emission spectral study

The three receptors compound are emissive in nature and on excitation at 400 nm shows a broad emission band at 450-480 nm. The receptor 1 and 2 displays a similar broad emission band at 465 and 466 nm respectively on excitation at 400 nm. The receptor 3 shows a sharp emission band at 479 nm on similar excitation in THF solution. Upon addition of water to the THF solution of receptor compounds, increased with red shift due to strong perturbation of excited receptors with other neighbouring receptor[33] but the emission intensity has drastic changes occurred when fw> 70% of H2O. Successive addition of water enhances the dramatic emission intensity of R1 and R2 at 80% with a red shift (15 nm and 10 nm respectively) from 464 to 479 nm with intensity difference 6 times and for R2 from 439 to 449 nm with intensity difference 5 times was increased but for R3 reaching maximum at 90% of water gets red shifted (11 nm) from 429 nm to 440 nm with intensity difference

7 times has increased. This spectral change indicates that there is a J-aggregation and the emission spectra profile was given as below Fig.2. When the fraction of water content was increased from 0 to 70 vol % no obvious emission enhancement could be observed because of the free rotation of the phenyl and anthracene rings about the axes of the –C=N-N bonds and the resultant non-radiative decay process but at the 80 % H2O (fw = 80%), the fluorescence intensity increased dramatically which is shown in fig. 3.7-3.9 for R1 and R2 and a maximum emission intensity was reached when at fw = 90%, for R3. This should be attributed to the RIR process in the aggregate state, in the fixed the molecular conformation, blocked the non-radiative path, and boosted the fluorescence intensity.The origin of such AIE phenomenon from RIR of pyrene derivatives and tetraphenylethenenaphthyrisine could be accounted to those reported in previous literature[34],[35].



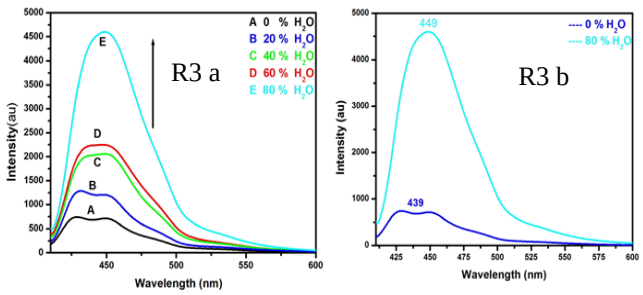


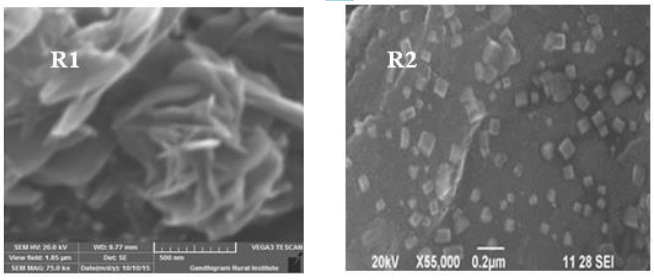
Fig. 2 (a) Emission spectrum of R1-R3 in THF (4 µM) with increasing % of H2O (b) Overlay spectra of 0% H2O (1) and 80% H2O (2)

SEM analysis study

The Aggregation induced emission (AIE) phenomena indicates that anthracene derivatives should adopt twisted conformations that prevent intermolecular π−π stacking and without large planar cores in these molecules, dipole−dipole interactions can also be utilized to achieve ordered self-assemblies, as is commonly observed in the Schiff's base substituted conjugated molecules [36]. Thus, the formation of nanoaggregates is confirmed by scanning electron microscopy (SEM) analysis. Fig.3 shows that the R1 is microstones structure whereas R2 is flower-shaped structure were observed with the diameter of few hundreds of nanometer. But in the SEM spectra of R3 for f_w= 90 % H_2O have rod like structures.

Atomic force microscopy study

The Atomic Force microscopy is used to study phenomena such as abrasion, adhesion, cleaning, corrosion, etching, friction, lubrication, plating and polishing and by using AFM one cannot only image the surface in atomic resolution but also measure the force of nano newton scale. AFM images of the receptor compounds (R1-R3) are f_w= 80 % H_2O for R1 & R2 and f_w= 90 % H_2O for R3 shown in Fig 4. The topography of the surface is identical to the anthracene appended receptor and its subsequent closing does not change significantly the surface topography. From the AFM spectroscopy the size of the receptor compounds (R1-R3) are in the range from 100 to 500 nm which confirms that there is an agreegation of particles in the THF/H_2O mixture.



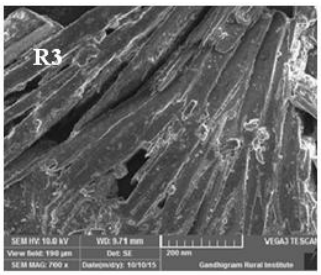


Fig.3 SEM images of Receptor 1 (80 % H2O) Receptor 2(80 % H2O)Receptor 3(90 % H2O)

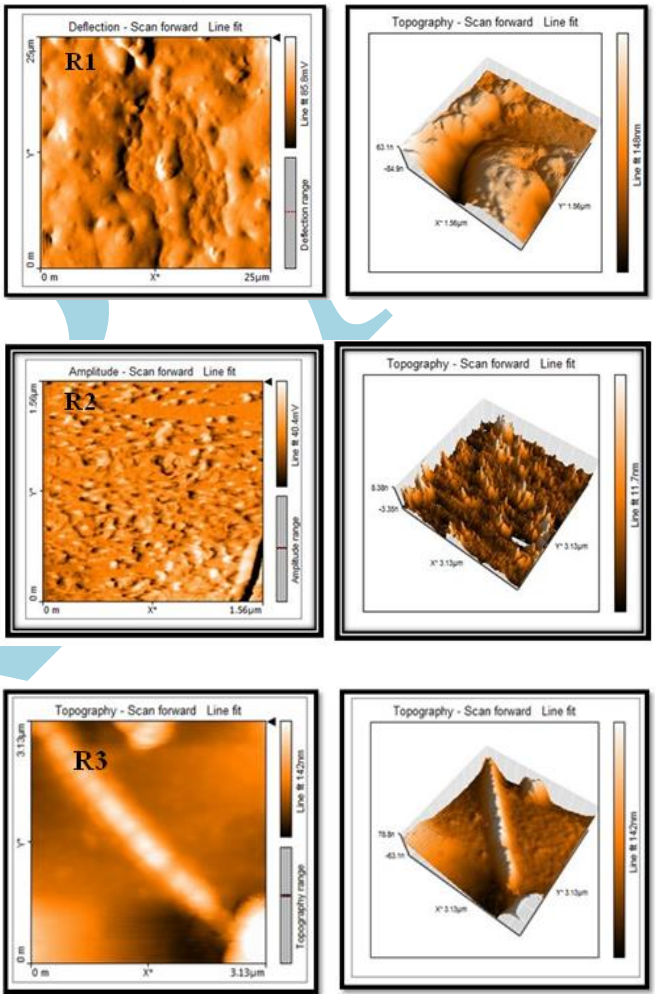


Fig.4 AFM images of Receptor 1(80 % H2O), Receptor 2(80 % H2O),Receptor 1(90 % H2O)

Interaction of receptors with BSA

In the present study, BSA is selected as our protein model because of its long-standing interest in the protein community. Results on the interaction of fluorescent probe of anthracene derivatives with protein like BSA studied by spectroscopic techniques. The absorption titration studies and the fluorescence titration studies and was carried out by keeping the concentration of receptors(R1-R3) with 4µM, constant while varying the concentration of BSA(2 to 20 µM)in phosphate buffer (pH = 7.4) was performed. In order to learn

about the strength of the binding nature of receptors (R1-R3) compounds with BSA, absorption spectral titrations were carried out in the THF medium. BSA has no absorption at 300 – 400 nm, addition of BSA leads to a substantial increase in the absorbance at 300 – 400 nm wavelengths and these spectral changes have been used for the calculation of binding constant (K) of the receptor (1-3) [37-40].

Spectral studies

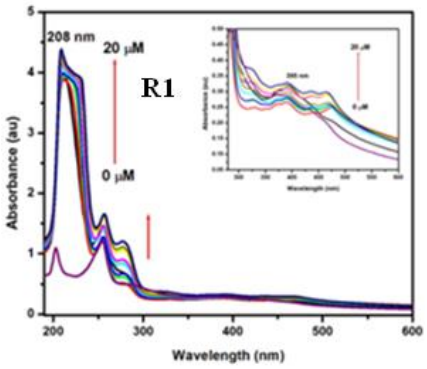
The UV-vis absorption spectral studies which indicated that a gradual increase in absorption at the peak maxima corresponding to the free probe with a simultaneous growth of new red-shifted peaks due to the bound probe. Such bathochromic shifts and hyper chromism has been noted for the binding of several anthracene-derivatives with BSA [41, 42].The spectral shifts clearly imply the change in the probe environment, as the probe is transferred from the aqueous phase to the protein interior. Generally, ground-state complex formation will frequently result in perturbation of the absorption spectra of the fluorophore especially if the interaction occurs by hydrogen bonding [43]. The binding nature of receptor (R1-R3) compounds with BSA, the UV-vis absorption spectral titrations were shown in Fig.5.

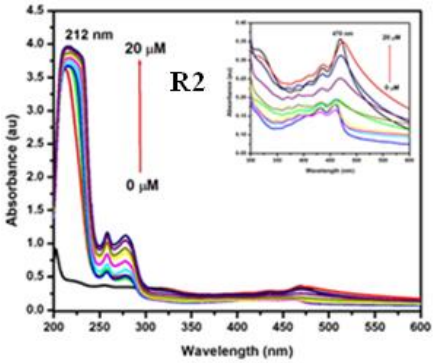
Fluorescence titration experiment is carried out to confirm that the aggregation of receptors is associated with BSA. The receptors (R1-R3) in THF are nearly less luminescent. However, by the addition of BSA can turn on the emissions of receptors with anthracene chromophore at 400 nm showed a broad, reasonably intense monomer emission at 455 nm which was attributed to the π–π*emitting state. Therefore, these receptors could be used as a light-up bioprobe for quantitative detection of BSA. The fluorescence emission spectrum of BSA is shown in Fig.6.On the addition of various concentrations of receptor (R1-R3) compounds to the fixed concentration of BSA, there is a luminescence enhancement as well as a large red shift. This shows the stabilization of the emissive state due to strong binding of the receptor compounds with BSA. The binding constants were calculated using the following modified Benesi-Hildebrand Eqn.

$$\frac{I_0}{I - I_0} = \frac{b}{(a-b)}\left(\frac{1}{K_b[BSA] + 1}\right)$$

The fluorescence emission spectral study confirms that the fluorescent intensity of receptors compounds enhances gradually by increasing the concentration of BSA. Receptors bind to the BSA via non-covalent interactions, such as electrostatic attraction and hydrophobic effect (particularly for the protein with hydrophobic pockets in its native folding structure) to form aggregate. The radiationless transition which activates its fluorescence process and this suppresses the intramolecular rotations of receptors (R1-R3). The investigation indicated that the emission enhancements of the luminophores depended on the competition between the restriction of intramolecular rotation and aggregation with the former causing emission enhancement because of the proximal binding of the chromophores in the protein. The energy transfer was achieved at micromolar concentrations of extremely inefficient in fluid solutions, unless the donors and acceptors are assembled by covalent or non-covalent approaches. The above observations provides new insights in the design of donor and acceptor chromophores, which absorb or emit at desired wavelengths but show moderate to high affinities for serum albumins or other engineered or natural proteins. Progress made in this area may facilitate the construction of a variety of other subunits with specific properties for more efficient solar energy capture, conversion and storage.

The double reciprocal plot(Fig.7) using the equation and Benesi-Hildebrand plot using the equation were confirmed that the binding of receptors with BSA and the binding constant value can be calculated using the equation and given in the table 5.1 .which shows that the binding value of receptor (R2) with BSA has higher than other two receptors than other which close agreement with both UV-vis absorbance and fluorescence emission titration studies. The binding constants (Kb) values for the UV-vis absorption spectral studies and fluorescence emission spectral studies are tabulates in Table 1. From this we concluded that the both values are very close agreement with them.





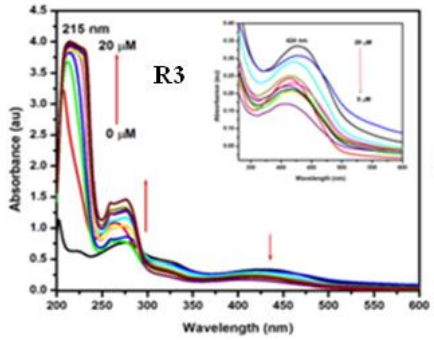


Fig.5 UV-vis absorption spectral changes during the titration of Receptor 3 with BSA Inset: spectral changes at 250-300 nm

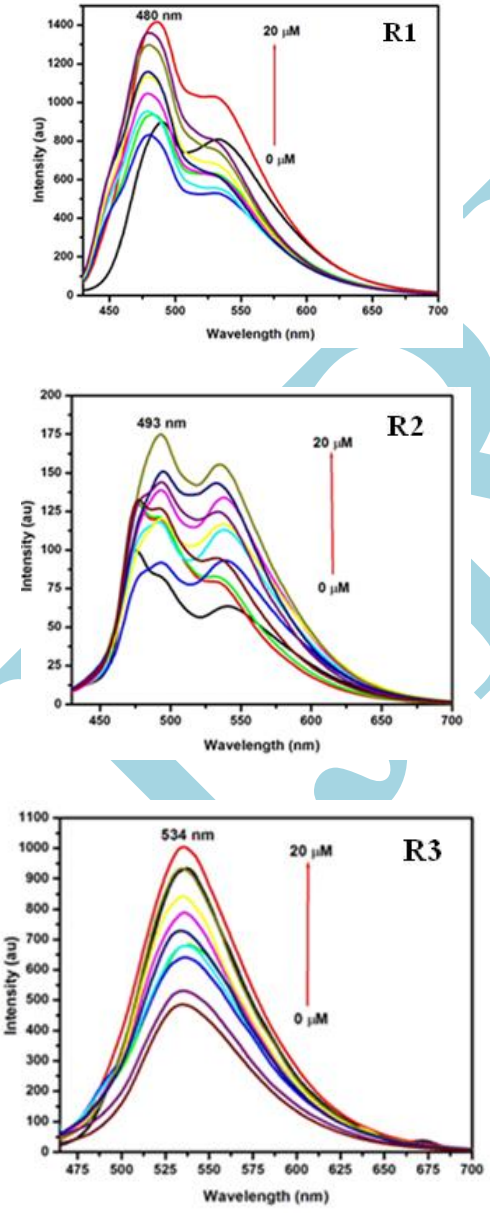


Fig. 6 Fluorescence emission spectral changes during the titration of R1-R3 with BSA

Table.1Binding constants (Kb) values for the interaction of Receptors with BSA

Receptors	K_b , M^{-1}	
	Absorption	Emission
1	1.80 X 10^5	1.70 X 10^5
2	5.42 X 10^7	3.88 X 10^7
3	6.04 X 10^6	2.60 X 10^6

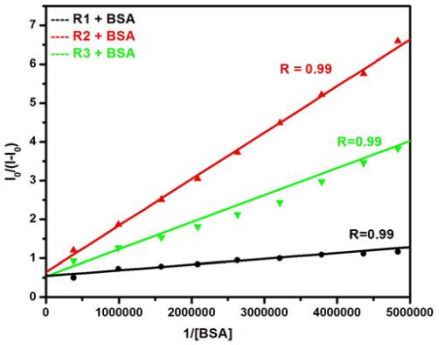


Fig.7 OverlayBenessi-Hildebrand plot of Receptors (1-3) with BSA

CONCLUSION

In this work, we have prepared anthracene derivatives, they are weakly emissive nature in solution state, but it exhibits strong emission in aggregated molecules due to the addition of water i.e, poor solubility of the compound, showing aggregation induced emission enhacement (AIEE) properties. This emission is occur due to the presence of restriction of intramolecular rotation (RIR). The AIEE property of these receptors are explained by UV-Vis absorption, Emission, SEM,AFM spectral studies. The interaction of fluorescent probe of anthracene derivatives with protein like BSA studied by spectroscopic techniques.The double reciprocal plot using the equation and Benesi-Hildebrand plot using the equation were confirmed that the binding of receptors with BSA and the binding constant value can be calculated using the Benasi-Hildebrand equation.

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