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A Probe with Aggregation Induced Emission Characteristics for Screening of Iodide

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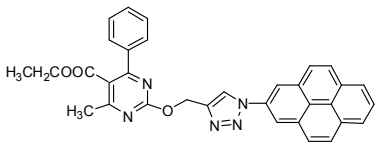
The dilution controlled aggregation enhanced emission of spherically aggregated form of a triazole based probe dies down upon detecting iodide over other inorganic anions. The sensing is realised as a dynamic quenching mechanisn dominated event. Highly selective for iodide, the probe finds application in the detection of iodide in human urine.

Owing to the fundamental roles of inorganic anions in a wide range of biological, chemical and environmental processes, considerable research has been focused recently on the detection of these anions. Among these anions, iodide being an essential micronutrient for thyroid gland functioning and neurological activity, is of particular interest.[1] According to World Health Organization (WHO) estimates, both iodide deficiency and excessive intake can lead to thyroid disorders (hypothyroidism and hyperthyroidism, respectively) and has thus become a matter of concern for public health in many countries.[2] Monitoring of thyroid hormone triiodothyronine (T_3) in biological fluids is a diagnostic tool for thyroidism. T_3 is produced by the action of deiodinases on thyroxine (T_4). Hypothyroidism is usually combated by intake of iodine, which is usually added to salt, it is also present in additives, water sources, medications, and dietary supplements. Also, the use of elemental iodine (oxidized form of iodide) in the synthesis of drugs, dyes and various chemicals[3] and subsequent residual discharge in the environment has caused serious physiological disorders and environmental pollution. Therefore, the rapid, sensitive and selective detection of iodide in food, pharmaceutical products and biological samples nowadays is a challenging task. Although a variety of methods such as, gas chromatography[4], electrostatic ion chromatography[5], indirect atomic absorption spectrometry[6], capillary electrophoresis[7] etc., have been successfully implemented for the detection of iodide, the complexity associated with the detection in real samples such as urine and milk, frequently leads to false positive results from these methods. Operationally simple methods based on photo-physical phenomenon, such as fluorescence emission are receiving considerable attention for developing new sensing methods and are able to be used with the requisite sensitivity. Among the new emerging fluorescent mechanisms, aggregation induced emission enhancement (AIEE) phenomenon has attracted a significant attention.[8] The fluorescent emission of organic fluorophores is often quenched in the aggregated form via such mechanisms as aggregation caused quenching (ACQ).[9] This phenomenon has limited the use of many organic fluorophores in organic light-emitting diodes (OLEDs) and sensing materials i.e. chemo sensors and biosensors etc. On the other hand, some organic molecules that are weakly fluorescent in solution exhibit enhanced fluorescence upon aggregation and the phenomenon was first reported and named as AIEE by Tang et.al.[10] The influence of polarity and viscosity are some of the key factors for tuning the aggregation of AIEE type molecules.[11] In the recent past, inspired by some literature reports on the influence of controlling the ratio of water in solution of aggregates on AIEE,[12] we reported[13] solvent controlled aggregation induced emission enhancement of a triazole linked pyrimidine-pyrene conjugate **6** that modulated emission intensity as well as naked eye fluorescence emission controlled by the degree of aggregation, as depicted in Fig. 1.

In an attempt to explore the behavior of **6** at different stages of aggregation towards a variety of inorganic anions, it was interesting to note the sensitivity of aggregated form at 50% HEPES buffer (HB) fraction to the THF solution of **6**, towards iodide. In the present investigation, we are reporting the quantified results of the sensing behavior of **6** towards iodide ion, in line with the importance of iodide ion. The conjugate **6** is highly soluble in THF but is less emissive.



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†Electronic Supplementary Information (ESI) available: [Experimental, Instrumentation, Synthetic scheme for **6**, ^{1}H and ^{13}C NMR spectra of **4** and **6**, Quantum yield calculations, Computational details, Fluorescence data of 6 for pH titration, Interference, Real urine sample analysis, Detection limit calculations]. See DOI: 10.1039/x0xx00000x

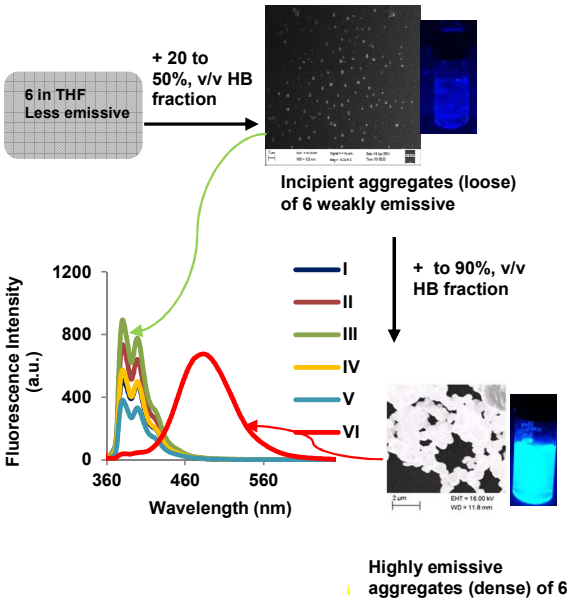

Fig. 1 Schematic representation of the process of aggregation and corresponding emission changes with increasing %age of HB fraction (pH 6.99) in the THF solution of **6**, (i) THF, (ii) 80:20,(iii) 50:50, (iv) 40:60, (v) 30:70 and (vi) 10:90 *v/v* ,THF:HB at $\lambda_{exc.}$= 342 nm.

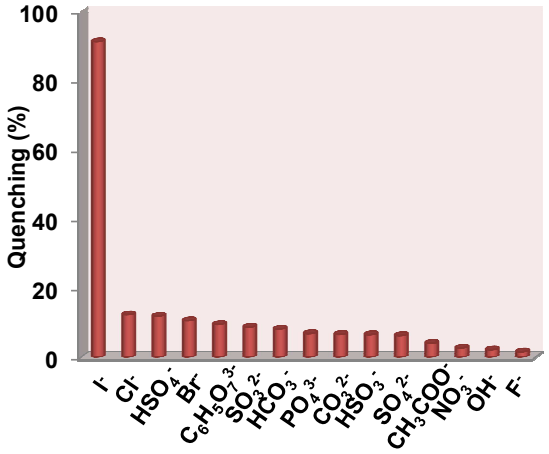

Fig. 2 Percentage fluorescence quenching observed upon addition of (3.71 x 10^{-4} M) aqueous solution of various anions to a solution of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH 6.99) $\lambda_{exc.}$= 342 nm, $\lambda_{em.}$= 380 nm. (For color changes, Fig.S10)

However, upon addition of HB upto certain fraction, it turns significantly emissive owing to aggregation (Fig. 1), and is well in agreement with the literature reports on a variety of organic compounds, which emit strongly in aggregated states.[14]

The compound **6** was readily synthesized[13] through pyridiniumchlorochromate (PCC) promoted dehydrogenation of ethyl-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-phenylpyrimidine-5-carboxylate **1** to ethyl-1,2-dihydro-6-methyl-2-oxo-4-phenylpyrimidine-5-carboxylate **2**, which upon refluxing with $POCl_3$ furnished **3**. Further base catalyzed nucleophilic substitution at C-2 with propargyl alcohol provided **4** which upon copper catalyzed 1,3-dipolar cycloaddition (click reaction) with 2-azidopyrene **5** provided **6**. All compounds exhibited satisfactory spectroscopic as well as micro analytical data (Synthetic scheme, spectroscopic data, ^{1}H and ^{13}C NMR spectra are provided in ESI 4-8).

The less emissive (Φ=0.175) THF solution of **6** (1 x 10^{-5} M) exhibits enhanced emission intensity with increase in the HB fraction (pH 6.99) to THF, attains (Φ= 0.230) at the addition of 50% of HB (50:50 *v/v*, THF:HB) (Fig. 1, S9). The enhancement in emission intensity of **6** is attributed to the onset of the aggregation (AIEE phenomenon). We propose this state as the incipient (slack) aggregation state,[15] which indeed gets denser with increase in the HB fraction in the THF solution, as is evident from the visual emission (Φ= 0.357 in 10:90 *v/v*, THF:HB) and the SEM images shown in Fig. 1.

Our preliminary investigations revealed that the aggregated form of **6** (in 50:50 *v/v*, THF:HB fraction, pH 6.99) is sensitive to iodide over other inorganic anions. Before quantifying the detection event, pH stability was checked, and was found to be considerably stable in the pH range of 2-13 (Fig. S5). Figure 2 shows the fluorescence response of **6** (1 x 10^{-5} M, in 50:50 *v/v*, THF:HB fraction, pH 6.99) to the addition of various inorganic anions such as F^-, Cl^-, Br^-, I^-, HSO_4^-, SO_3^{2-}, HCO_3^-, PO_4^{3-}, CO_3^{2-}, HSO_3^-, SO_4^{2-}, CH_3COO^-, NO_3^-, OH^-. Comparing the relative fluorescence quenching, a high sensitivity was observed for I^-. Titration of **6** was thus performed with I^- solution (added as sodium iodide) to explore the binding characteristics. Upon incremental addition of the aqueous solution of I^- ion (1 x 10^{-1} M, in H_2O) to the solution of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH 6.99), the fluorescence intensity of the twin emission bands at 380 and 399 nm gradually died down till the addition of 37 eq. (3.71 x 10^{-4} M, in H_2O) of I^-, after which no significant change was observed (Fig. 3). In general, the fluorescence quenching can be either static (through the formation of a complex) or dynamic (due to the random

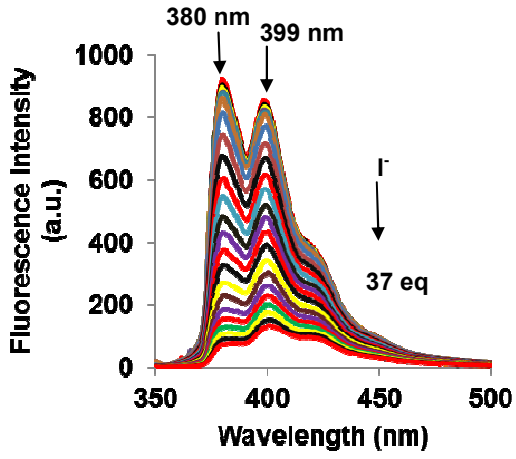

Fig. 3 Changes in emission spectra of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH 6.99 $\lambda_{exc.}$= 342 nm) upon incremental additions of iodide (upto 3.71 x 10^{-4} M), in H_2O, added as NaI).

collisions between the emitter and the quencher). In case of halogen atoms, the latter mechanism is widely accepted as an external perturbation of the spin-orbital coupling in the π-electron clouds of the emitter (a heavy atom effect).[16] The fundamental parameter for the effectiveness of this perturbation is certainly the atomic number of the perturbing atom and is in the order of I>>Br>Cl>F. Moreover, the Figure 2 also depicts I^- as a better quencher among its group mates. In order to ascertain the quenching mechanism, we further investigated how the fluorescence life-time of the compound **6** varies with added concentration of iodide ions. For static quenching, invariant lifetime with increase in concentration of the quencher is observed, whereas for dynamic, variant response of lifetime versus concentration is observed.[17] Figure 4 shows the fluorescence life-time of **6** at two different concentrations (below and slightly above the saturation point) of iodide. We can see the variant life time from (19.80 ns to 7.8 ns) where decay becomes steeper, consistent with the shorter lifetime, and less linear with increase in I^- concentration. On the basis of above discussion, we propose that the dynamic quenching process has the major contribution to the fluorescence quenching of **6** in the presence of I^- ions. It is important to mention over here that unlike picric acid, which was best detected in 10:90 *v/v*, THF:HB fraction, reported in our earlier investigation,[13] iodide could not be. The main cause for this we propose is the effect of size of the iodide ion. Being larger in size, could comfortably be accepted by the loose aggregates than the dense form of aggregates. Further, the absorption spectrum of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH6.99) exhibits high energy (HE) band at 276 nm and low energy (LE) bands at 326 and 342 nm (Fig. 5). These bands have been assigned as intra-molecular transitions corresponding to the subunits of **6**. The HE (276 nm) band has contributions from HOMO(H)-3, H-4, H-5, H-6 and H-7 to LUMO(L)+1 transitions, with main contribution from H-3 to L+1, whereas the LE bands (326 and 342 nm) get contributions from H, H-1 to L and L+2 transitions, with main contribution from H to L (342 nm) and H to L+2 (326 nm) predicted on the basis of maps of the frontier molecular orbitals (Fig. 6). Upon incremental addition of I^- solution, whole of the spectrum observed a bathochromic shift accompanied by broadening of the bands. This bathochromic shift has been ascribed to the intermolecular charge-transfer between **6** and the iodide ion.[18]

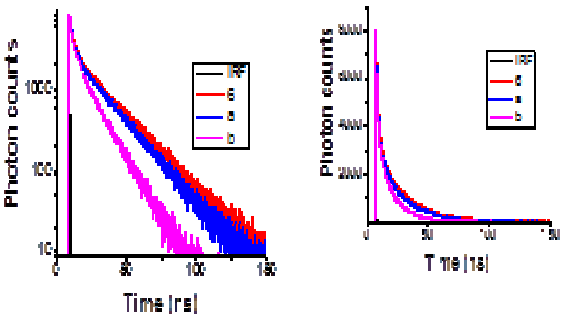

Fig. 4 Life-time decay profile of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH 6.99) before and after addition of iodide (a) 20 eq. , (b) 40 eq. $\lambda_{exc.}$ = 375 nm, IRF = Ludox reference.

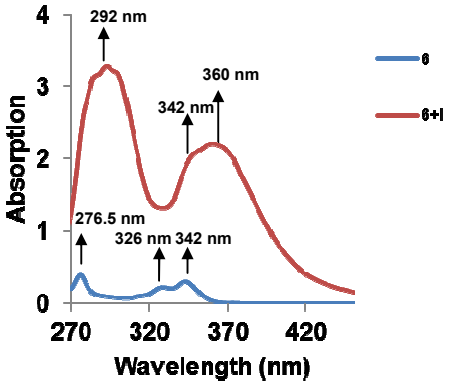

Fig. 5 Change in emission spectra of **6** (1 x 10^{-5} M) in (50:50 *v/v*, THF:HB fraction, pH 6.99) on addition of Iodide (3.71 x 10^{-4} M) in H_2O.

To further enhance the scope of the sensing event, any possible interference by the other inorganic anions including halides was also ruled out by observing the emission spectral behavior of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH 6.99) with I^- (3.71 x 10^{-4} M, in H_2O) at 380 nm, in the presence of other anions (3.71 x 10^{-4} M, in H_2O). The results depicted in Figure S6 exhibit that the compound **6** shows significant resistance to the interference from other anions and thus can be used as a highly selective sensor for iodide. Likewise, Fig. 7 shows a comparison of the changes in the emission spectrum of **6** in response to the addition of Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+} and I^- (Fig. S7a-d). The titrations were performed by gradual addition of respective metal ion solutions (upto 5.00 x 10^{-4} M, in H_2O) or I^- (upto 3.71 x 10^{-4} M, in H_2O) to a solution of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH 6.99). Subsequent to the addition, change in the fluorescence intensity was monitored at 380 nm. Thus, while iodide ion led to 92.06% quenching of the original emission intensity (Fig. 3), Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, respectively caused 5.67%, 6.76%, 12.18% and 21.37% quenching of the original fluorescence intensity (Fig. 7 and S7e).

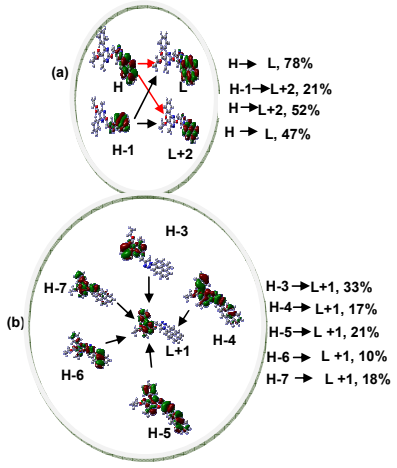

Fig. 6 Frontier molecular orbitals of **6** contributing to (a) LE (b) HE bands.

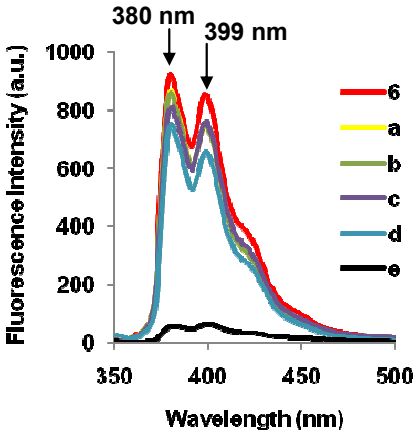

Fig. 7 Changes in emission spectra of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH 6.99 $\lambda_{exc.}$= 342 nm) upon additions of (5.00 x 10^{-4} M) in H_2O, (a-d) perchlorate salt solutions of cations (a) Cr^{3+}, (b) Cu^{2+}, (c) Fe^{2+}, (d) Fe^{3+} and (e) I^- added as NaI.

We further, checked if the presence of these metal ions would cause interference in the detection of iodide by **6**. Thus, the emission (380 nm) behaviour of **6** (1 x 10^{-5} M, 50:50 *v/v*, THF:HB fraction, pH 6.99) upon addition of I^- (3.71 x 10^{-4} M, in H_2O) (Fig. S7f) was noted. It was found that the presence of these metal ions do not cause any significant interference in the detection of iodide.

Further, the applicability of the compound **6** to detect iodide in the real urine sample (pH 6.43) was also evaluated (Fig. S8). For this purpose, change in emission intensity of the blank urine sample (entry 1, Table 1) was noted upon the addition of varying concentration of iodide (entries 2 and 3, Table 1). Based upon the changes in the emission intensity, concentration of iodide in the urine sample as well as recovery (%) was determined (Fig. S8). Detection limit of 49.3 μg (Fig. S9) was calculated, which demonstrates the potential application of **6** in the detection of iodide in biological real samples.

Conclusions

To summarize, a sensing probe for the selective sensing of iodide is reported, that operates predominantly in the dynamic quenching mode of the aggregation induced emission. The efficacy of the probe in determining the urinary iodide concentration is demonstrated.

Table 1 Determination[(a)] of concentration as well as % recovery of Iodide in urine sample.

S. No.	$[I]^-$ (μM)	Total $[I]^-$ (μM)	Emission Intensity (a.u.)[(c)]	$[I]^-$ (μM)[(d)]	Recovery (%)
1.	0[(b)]	0	916.71	1.34	-
2.	2	3.34	913.57	2.91	87.12
3.	4	5.34	908.85	5.27	98.68

[(a)]Average of three independent experiments, [(b)]Blank urine sample, [(c)]380 nm, [(d)]determined (Fig. S8).

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