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ARTICLE TYPE

A reaction based colorimetric as well as fluorescence *'turn on'* probe for the rapid detection of hydrazine†

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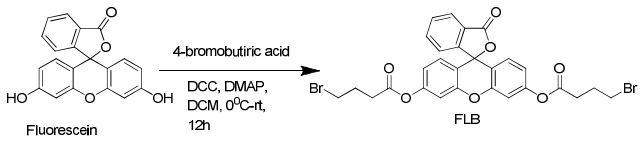
A fluorescein based reactive probe has been designed and synthesized to detect hydrazine selectively over other common analytes. We used here 4-bromobutyrate as a masking unit of fluorescein dye. Hydrazine plays here the role of a de-masking agent to set free the fluorescein dye through a simultaneous substitution-cyclisation-elimination process. This leads to 'turn on' fluorescence with easily discernible color change with a fast response time (< 15 minutes).

In recent decades, the fluorescence spectroscopic method has been used vastly to detect various analytes because of its high sensitivity, specificity, easy to operate; low cost and real-time monitoring.[1] Hydrazine is a well known inorganic compound. It is a strong base with good reducing property[2] and used in many chemical and pharmaceutical industries as corrosion inhibitor, catalyst, pharmaceutical intermediates and textile dyes etc.[3] It is a familiar high-energetic fuel and widely used in rocket-propulsion systems due to its high flammable nature.[4] Despite its usefulness, hydrazine has high toxicity and it was also reported that the exposure of hydrazine cause critical damage to kidney, liver, lungs and the nerve system of human and animal.[5] Thus, the U.S. Environmental Protection Agency (EPA) classified hydrazine as a probable carcinogen and recommended its threshold limit value (TLV) as low as 10 ppb.[6]

Therefore, the construction of small molecule fluorescent probe, which can identify trace amount of hydrazine selectively and delicately, has gained attention in recent years. However, there are only a few hydrazine selective fluorescence chemosensor reported yet.[7] There are many chemodosimeters have been reported based on the deprotection or chemical transformation of a protecting group by the specific deprotecting agent or anlyte.[8] These chemodosimetric systems gained much more attention recently due to their high selectivity. Previously, we have reported a benzothiazole based chemodosimetric system for the selective fluorescence ratiometric detection of hydrazine.[7i] Although, our previous probe showed ratiometric fluorescence change upon reaction with hydrazine, but visible color change, detectable by naked eye, was not observed. The optical/colorimetric sensors have great demand due to their simple and inexpensive detection method i.e. can be easily observable by naked eye without using any equipment. In this regard, we choose here the fluorescein dye as a signalling unit due to its capability of both colorimetric and fluorometric signalling, high quantum yield and water solubility. Considering these advantages of fluorescein dye a large number of sensors have been reported yet.[9]

Based on these considerations, here, we would like to execute a colorimetric and fluoremetric reactive probe enable the selective detection of hydrazine through subsequent substitution-cyclisation-elimination pathway. Probe FLB (FL- Fluorescein and B- Bromobutiric acid) was constructed by connecting 4-bromobutyric acid with fluorescein dye through esterification (Scheme 1). The probe was fully charecterized by ^{1}H NMR, ^{13}C NMR and ESI MS spectroscopy analysis (ESI, Fig. S19□S22).

Scheme 1: Synthetic scheme of the probe (FLB)



As expected, after esterification the dye loss its original fluorescence property and turned to colorless. Addition of hydrazine reserved the original fluorescence and greenish-yellow color of the dye through de-masking of the masking group to free the dye itself via a substantial substitution-cyclisation-elimination sequence.

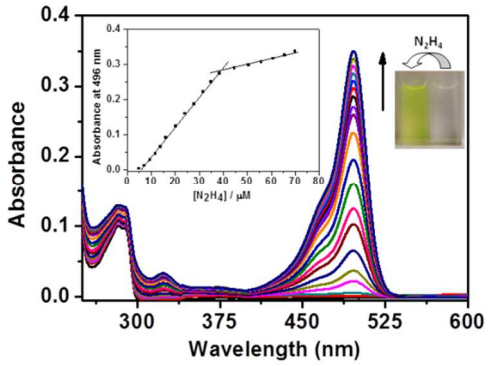


Figure 1: Change of absorption spectra of FLB (20 μM) upon gradual addition of hydrazine (0 to 3 equivalents). Inset: The response curve of absorbance of FLB at 496 nm depending on the hydrazine concentration (left). A photograph of FLB (20 μM), showing visible color change of FLB in absence and presence of 4 equivalents of hydrazine (right).

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The specific reactivity and sensitivity of the probe (FLB) towards hydrazine was investigated through monitor of its ground state and excited state spectral changes upon addition of different cations, anions, and neutral bases [Cd^{2+}, Ag^{+}, Pd^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+}, Fe^{3+}, Co^{2+}, Cr^{3+}, Hg^{2+} (as their chloride salts); F^{-}, NO_3^{-}, OCl^{-}, HSO_4^{-}, HSO_3^{-}, SO_3^{2-}, SO_4^{2-} (as their sodium salts); $NH_2(CH_2)_2NH_2$, NH_3 and NH_2OH].

Only N_2H_4 has been succeeded to perturb the photophysical behaviour of FLB along with a prominent color change from colorless to greenish-yellow was also noticed. Other relevant analytes did not affect the absorption profile, they are almost nonresponsive. FLB (20 μM) in CH_3OH-H_2O (1:1, v/v, pH= 7.1) solution showed an absorbance maxima at 270 nm corresponding to a closed lactone conformation of fluorescein dye and exhibit no absorption features in the region of 450 nm - 550 nm. Upon gradual addition of N_2H_4 to the FLB (20 μM) solution a remarkable increase in the absorbance intensity at 496 nm was observed (Fig. 1). The intensity of the band enhanced regularly with incremental addition of N_2H_4 (0 to 3 equiv). Further addition of hydrazine lead to minor change in absorbance profile of FLB. Accordingly an enhancement (~ 27 folds) in the absorbance intensity was observed, accompanying with a prominent colour change from colourless to greenish-yellow. A linear relationship was observed between the absorbance intensity and concentration of hydrazine added in the range of 5 – 40 μM (Fig. 1, Inset).

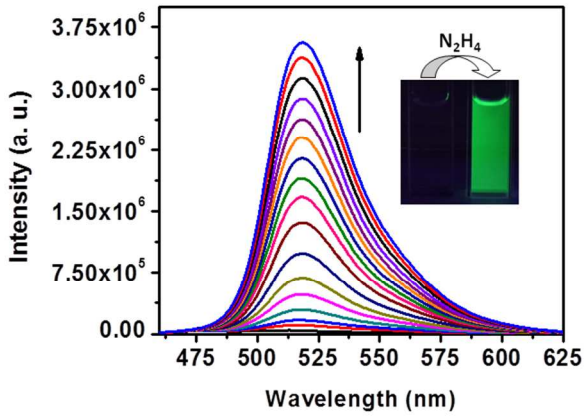

Figure 2: Change of emission spectra of FLB (20 μM) upon gradual addition of hydrazine (0 to 3 equivalents). Inset: Visible emission observed from FLB (20 μM) in absence and presence of 4 equivalents of hydrazine after irradiation under UV light. λ_{ex} = 460 nm.

In the emission spectra, FLB itself showed very weak fluorescence with an emission maxima centred at 516 nm upon excitation at 450 nm in aqueous methanol solution (CH_3OH: H_2O, 1: 1, v/v, 0.1 mM HEPES buffer solution, pH = 7.1). To demonstrate the capability of FLB in the determination of N_2H_4 concentration, the probe FLB (20 μM) was treated with various concentrations of hydrazine (0 - 4 equiv) solution. Upon interaction with hydrazine, intensity of the emission band (516 nm) increases rapidly with increasing concentration of hydrazine (Fig. 2). There was almost 95 times enhancement of fluorescence intensity observed after addition of 4 equiv of hydrazine than the FLB itself. The fluorescence quantum yields (Φ) of FLB (20 μM) itself and after addition of hydrazine (4 equivalents) were calculated using anthracene as reference and these were found to be 0.01 and 0.54 respectively (ESI). Consequently, after addition of hydrazine to the solution of FLB strong green color fluorescence was observed under the UV light (Fig. 2, Inset). This is may be due to the opening of spiro-lactone framework of fluorescein dye, which was formed in situ after the reaction of hydrazine with FLB. An excellent linear correlation between the added N_2H_4 concentrations and the fluorescence intensity was observed in the spectra in the range of 2□39 μM with a good R^2 value of 0.9960 (ESI, Fig. S7).

The detection limit of the probe for hydrazine was determined from the emission spectral change, upon addition of hydrazine to be 3.881×10^{-8} M, using the equation[10] DL = K × Sb_1/S, where K = 3, Sb_1 is the standard deviation of the blank solution and S is the slope of the calibration curve (ESI, Fig. S7). These results demonstrated that the chemodosimeter (FLB) could detect N_2H_4 quantitatively by the fluorescence spectroscopy method.

To investigate the selective reactivity of the probe (FLB) with hydrazine, absorption and emission titration experiment of the probe in the presence of other analytes were also performed. As shown in Fig. 3, the probe displays a selective response towards hydrazine, whereas the other analytes showed insignificant effect on both the absorption and emission spectra of the FLB.

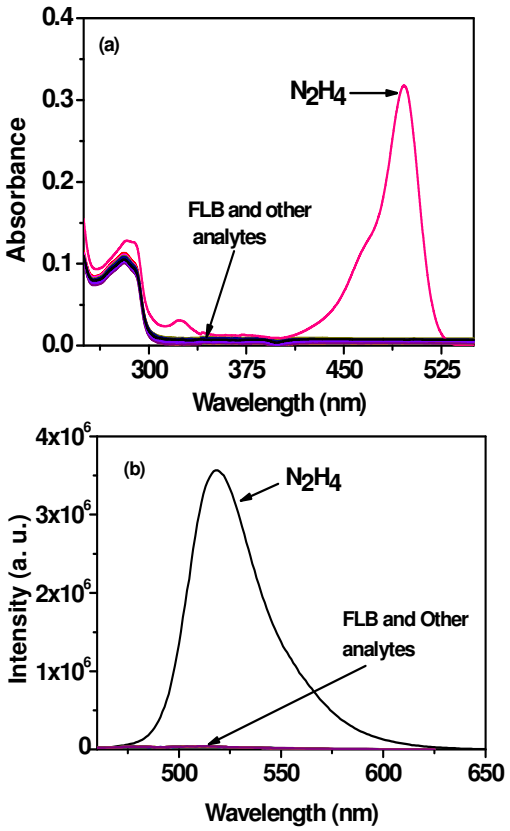

Figure 3: A comparative view of absorption (a) and emission (b) intensities of FLB (20 μM) upon addition of different analytes (4 equivalents).

In order to investigate the effect of acid and base toward the probe, the change of fluorescence intensity of FLB (20 μM) was measured at different pH values. For this purpose, we have used

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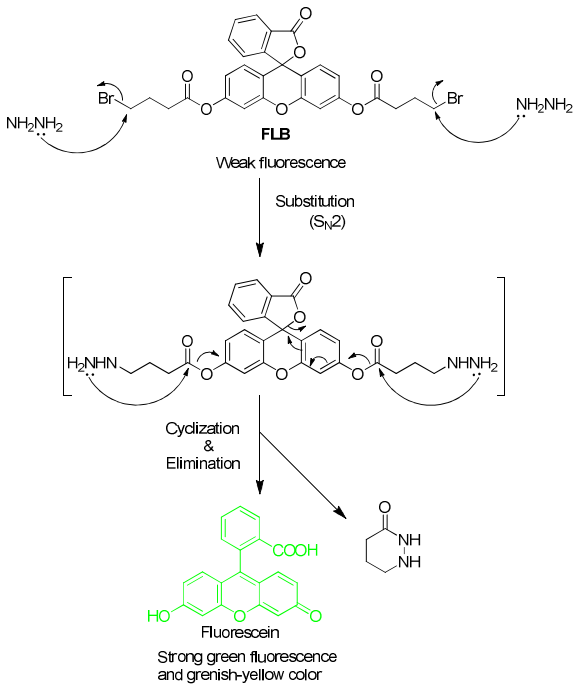
FLB in a mixture of CH_3OH:H_2O (1:1, v/v, 25^0C) solution. From the pH titration experiment it was clear that the chemodosimeter (FLB) showed insignificant change in the pH range of 2 □ 8, indicate that FLB is stable in this pH range and its response toward hydrazine was also almost invariable in this pH range (ESI, Fig. S8). From this experiment it was clear that the detection of N_2H_4 by this probe (FLB) was not at all hampered in this pH range. Thus we employed the near neutral pH (pH = 7.1) for the detection of hydrazine.

The interference and selectivity are the two very vital parameter of the feat of a probe. The interference of other analytes toward the detection of hydrazine was investigated by the competition experiment (ESI, Fig. S12). The UV-vis absorbance and emission intensity of FLB was measured after treatment of 2.0 equivalents of N_2H_4 in presence of other analytes (3.0 equivalents). The results showed that detection of N_2H_4 in presence of other relevant analytes were not hampered, that is, the interference for the detection of the N_2H_4 was not observed. The selectivity of the probe towards hydrazine is well executed in this section. So that FLB could be used as a selective and sensitive colorimetric and fluorogenic sensor for N_2H_4.

Scheme 2: Possible mechanism of sensing hydrazine by FLB



The visible color change (colorless to yellow) and strong green fluorescence after addition of hydrazine, explain the opening of spirolactone framework of fluorescein moiety, which is actually a N_2H_4-induced reaction to set free the fluorescein dye. The reaction of FLB with N_2H_4 was illustrated by involving two steps. As shown in Scheme 2, first, nucleophilic substitution of hydrazine occur at the bromo group of FLB to generate a free hydrazide group and then in second step a nucleophilic addition at the carbonyl group from the primary amine group take place with intramolecular cyclization to release the fluorescein moiety, which turned into its ring open form and responsible for the color change as well as fluorescence 'on'.

The charge surface diagram of FLB is in favour of the proposed reaction sequence (ESI, Fig. S14). From the diagram it was clear that the adjacent C-atoms of bromo groups are positively charged (may be because of the –I effect of –Br group). So, the nucleophilic attack of hydrazine possibly takes place at these centres first. The Gibbs free energy change has been found to be -20 Kcal/mol of this reaction which is a responsible factor for the conversion of FLB to fluorescein on addition of N_2H_4.

The transformation of FLB to fluorescein after reaction with hydrazine was evidenced by ^{1}H NMR, ESI MS, UV-vis and emission spectroscopic studies. The reaction product of FLB and N_2H_4 was subjected to mass-spectral analysis and it was found to be (m/z 332.0657) corresponding to fluorescein (ESI, Fig. S23). The ^{1}H NMR spectrum of FLB after addition of 5 equivalents of hydrazine looks similar to that of fluorescein in presence of same amount of hydrazine (ESI, Fig. S24). The comparison of UV-vis and fluorescence spectra of FLB and fluorescein after addition of 5 equivalents of hydrazine were also support the fact (ESI, Fig S10-S11).

In chemodosimetric systems reaction time i.e. the response time is an important factor. Thus we have investigated the required reaction time of FLB with hydrazine in 50% methanolic aqueous solution. We have record the absorption and emission spectral changes of FLB after addition of 5 equivalents of hydrazine in different time interval (ESI, Fig. S1 & S3). With increasing time the absorbance at 495 nm and fluorescence intensity at 516 nm increases repeatedly up to about 12 minutes and then reached to plateau (ESI, Fig. S2 & S4). From these results, it was concluded that the probe is suitable for the rapid detection of hydrazine.

In order to understand the relationship between the structural changes of FLB to fluorescein on addition of N_2H_4 and their electronic spectra, density functional theory (DFT) and time dependent density functional theory (TDDFT) calculations with the B3LYP/6-31+G method basis set using the Gaussian 03W, Revision-D.01[11] program were carried out and visualised using Gauss view program. The optimized geometry and the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of FLB and fluorescein were shown in the figure 4.

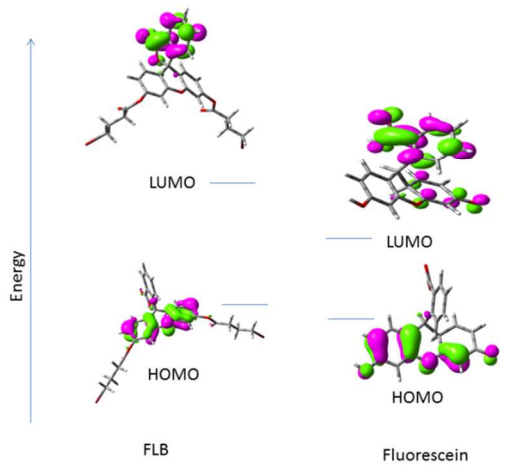


Figure 4: HOMO and LUMO of FLB and the reaction product of FLB + N_2H_4 (fluorescein) obtained from optimized geometry

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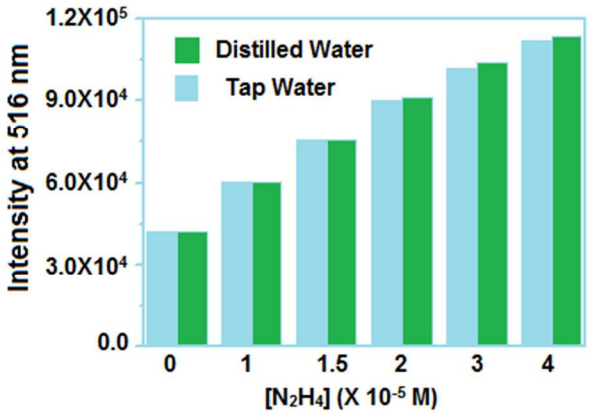
From gas phase TDDFT calculations, a transition at 264.5 nm was calculated which is due to HOMO to LUMO transition of FLB molecule and this is close to the experimentally observed absorption of 270 nm (ESI, Fig. S15). In the case of fluorescein the HOMO to LUMO absorption at 475.1 nm compliments the greenish yellow colour of the solution (ESI, Fig. S16).

When solvent correction was incorporated by CPCM model[12] and 1:1 mixture of water and methanol were chosen as solvent the calculated absorption spectra are observed at 268.3 nm and 481.2 nm for FLB and fluorescein respectively which are due to HOMO to LUMO transition (ESI, Fig. S17- S18).

Here we also explored the practical applications of FLB toward the detection of hydrazine, because of its carcinogenic property and huge use in a variety of industrial processes. The chemodosimertic attacking of hydrazine towards the probe give us an opportunity for the detection of hydrazine in aqueous samples. Both tap water and distilled water have been analysed to examine the "tap-water application". An aliquot of hydrazine was added to both of the tap and distilled water and the recovery of hydrazine by FLB was examined through fluorescence study. Now the healing of hydrazine by FLB was investigated from these two water samples and the examination of these two solutions explored that hydrazine in both solutions established well up to 40 μM concentrations (Fig. 5).

Figure 5: Fluorescence detection of hydrazine in distilled water and tap water by FLB. [FLB] = 10 μM, [Hydrazine] = from 0 to 40 μM, in a



mixture of CH_3OH and HEPES buffer solution (pH = 7.1) (1: 1, v/v). λ_{ex} = 450 nm.

In summary, we present here the design and synthesis of a reactive probe using fluorescein dye, for the selective detection of hydrazine. The probe (FLB) showed significant enhancement in both emission and absorption spectra upon interaction with hydrazine due to the opening of spirolactone framework of fluorescein dye. The reaction of hydrazine with the probe (FLB) to free the fluorescein dye goes through the substantial substitution-cyclization-elimination sequence. The sensing mechanism was supported by ^{1}H NMR, ESI MS and TDDFT studies.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Experimental procedure, synthesis of the probe, NMR, MS, computational data etc.]. See DOI: 10.1039/b000000x/

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