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ARTICLE TYPE

Developement of a rhodamine-benzimidazol hybrid derivative as a novel FRET based chemosensor selective for trace level water†

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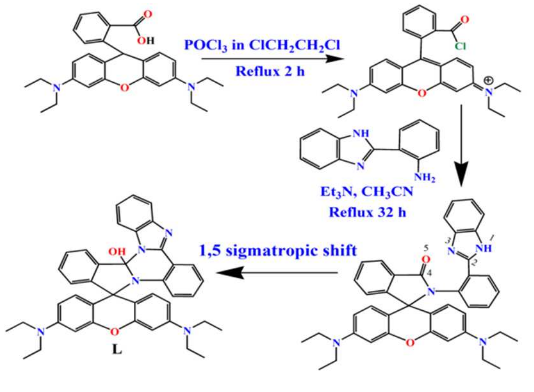
A newly designed rhodamine-benzimidazol hybrid molecule has been developed as a FRET based chemosensor for the selective detection of trace level water in both polar protic and aprotic organic solvents.

During the past few decades, a considerable attention has been paid towards the detection and quantitative analysis of trace water in organic solvents because of its indispensable applications in food, textile, ceramics, electronic, pharmaceutical, petroleum, and environmental monitoring industries.[1] Previously, water content in sample substances was measured upto ≈ 1 ppm level by Karl Fischer coulometric titration method.[2] Although it has been improved through modification, still this method has several disadvantages including long measuring time, the use of toxic and expensive chemical reagents, difficulty in endpoint determination and elimination of interference of other common species. Nowadays, optical water sensors based on fluorescence intensity and naked eye detection are to be particularly attractive on account of their highly sensitive and selective, quick, inexpensive, easy to fabricate, and non-destructive properties as well as their capability of remote and *in situ* monitoring of optical measurements.[3–10] In previous reports several approaches have been taken to facilitate sensitive water detection in many polar aprotic as well as polar protic solvents, including intramolecular charge transfer,[4] proton transfer,[5] photo-induced electron transfer,[6] water-induced decomplexation of dyes with anions,[7] water-induced interpolymer p-stacking aggregation,[8] solvatochromism,[9] etc.[10] However, in most cases the strong polarity and hydrogen bonding from the sensing media play the key role in the determination of water of these sensors and in lowering their LOD, and as a result there is still an increasing demand for designing highly sensitive optical sensors for water in polar protic solvents including a new detection principle for the development of new optical water sensors.

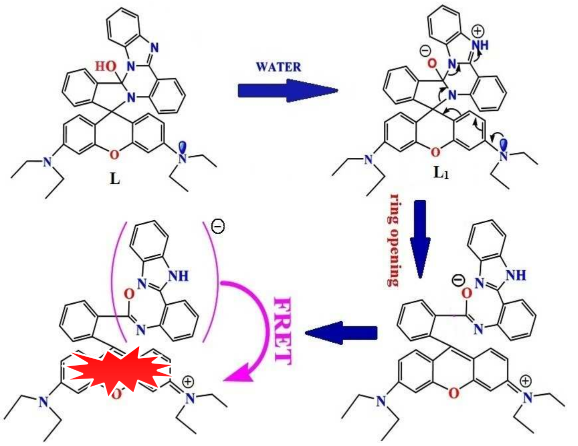
To the best of our knowledge, optical water sensor based on FRET mechanism (not through hydrogen bonding) is a new approach which has not yet been reported. There are a lot of utilization of this novel strategy for water detection to put forward several advantages. The selectivity of this rhodamine-based probe (**L**) towards water is extremely high (Fig. S1) and is independent of the kind of solvents (non-polar, polar, aprotic and protic) as here it responses through FRET which provides a good detection limit.[11] Fluorescence techniques have become powerful tools for sensing different analytes in trace amounts because of its simplicity, high sensitivity and real-time monitoring with a short response time.[12] Among them, FRET, a distance dependent radiationless energy transfer from an excited donor fluorophore to a suitable acceptor fluorophore, is now an important physical phenomenon with considerable interest for the understanding of molecular level interactions in biological systems and with potential applications in optoelectronic and thin film device development due to its sensitivity to distance and intra molecular phenomenon which resist the outer perturbation. It is also known that the distance over which energy can be transferred is dependent on the spectral characteristics of the fluorophores, but is generally in the range of 10–100 Å. Although the efficiency of energy transfer is also dependent over the relative orientation of transition dipoles of both the donor and acceptor and the extent of the spectral overlap between the donor emission and acceptor absorption[11].

To synthesize the probe **L,** at first rhodamine B acid was converted to its activated acidchloride form by conventional way[13], then the mixture of this produced rhodamine B acid chloride and added *2-amino phenyl benzimidazole* (2-APBZ) was refluxed for 32 h (Scheme 1 and ESI†). The crystallized product (**L**), obtained from dry acetonitrile was characterised using physico-chemical and spectroscopic tools (Figs. S2 to S4). The 1,5 sigmatropic shift[14] is responsible for obtaining the final rearranged cyclic organic moeity (**L**) which is well supported by IR, ^{1}HNMR and ^{13}CNMR data.



Scheme 1 Synthesis of fluorescence FRET sensor **L**

The addition of water to the solution of **L** in dry organic solvents facilitates the formation of fluorescent zwitterionic structure **L$_1$** through the protonation of imidazole nitrogen of the 2-amino phenyl benzimidazole (2-APBZ) unit and deprotonation of alcoholic hydrogen (Figs. S5 and S6). As a result, there was a spirolactone ring opening[15] occured *via* extented conjugation from –NEt$_2$ lone pair to 2-APBZ unit, and as a result, FRET is operated in the molecule (Scheme 2 ESI†) in occurring the red fluorescence. The peak at *ca.* 3449 cm^{-1} attributable to ν_{OH} was observed in the IR spectrum of **L** in dry acetonitrile but it is absent in the spectrum of **L** in 20 % (v/v) water in acetonitrile; whereas, the peak at *ca.* 3158 cm^{-1} attributable to ν_{NH} was obtained in later case but not in former case (Fig. S2). ^{1}HNMR titration was carried using D_2O (Figs.S5 and S6). Here, the signal at *ca.* δ = 12.64 ppm was observed in the spectrum of **L** in dry DMSO-d$_6$ but it was not found when the spectrum of **L** was acquired by adding a few drops of D_2O into DMSO-d$_6$, otherwise some corresponding peaks shifted slightly towrads downfield and some remained unaffected. The peak at arround 73 ppm due to the sp^3 hybridized carbon was observed in the ^{13}CNMR spectrum of **L** in dry DMSO-d$_6$ which also supported the existence of -OH attached to sp^3 carbon instead of any peak corresponding to carbonyl carbon (Fig. S4b).



Scheme 2 Probable mechanistic pathways of sensor **L** for detection of water in organic solvents.

To calculate the detection limit (DL) and quantitation limit (QL)[6,] the calibration curves[11] (Fig. 2) in the lower region were obtained. From the slope (S) of the curve and the standard deviation (σ_{zero}) of seven replicate (of each dry solvent) measurements of the zero level, the DL and QL were estimated using the equation $3\sigma/S$ and $10\sigma/S$, respectively. These values are quite significant compared to the previous reports (*viz*. Table-1).

Fluorescence and absorption spectra of **L** were measured in dry organic solvents namely acetonitrile, methanol, DMSO and THF containing various concentrations of water. A new peak generated at around 575 nm increases in intensity (□ 10 times) with increasing water content in acetonitrile, methanol or DMSO solvent through an isoemissive point at around 547 nm (*viz*. Figs. 1a, 1c and 1e) along with the steady decrease of fluorescence intensity due to benzimidazole unit. In the low water content region below 11 (v/v)%, as shown in Fig. S7, the fluorescence intensities were increased almost linearly with the increase of the water content in the organic solvent. In contrast, the corresponding absorption spectra also exhibited significant changes in absorbance value with a ~20 nm blue shift (Figs. 1b, 1d and 1f), which is a significant support of occurring the FRET[16]. In the absence of water, the rhodamine moiety adopted a closed, non-fluorescent spirolactam form, corresponding to weak spectral overlap between *2-amino phenyl benzimidazole* (2-APBZ) emission and rhodamine absorption. Eventually, FRET was suppressed, and only the blue emission of the donor was observed upon excitation of the 2-APBZ chromophore at 350 nm. Binding to water induced the opening of the fluorescent rhodamine moiety, corresponding to intense absorption in the 2-APBZ emission region. As a result, the spectral overlap was improved, and excitation of the 2-APBZ chromophore resulted in intense strong red emission of rhodamine owing to FRET.

In case of **L** the fluorescence quantum yield has been calculated in HPLC grade organic solvents and in presence of water and from this measurement it is clear that the fluorescence quantum yield increases from 0.77 to 10.21 (14 times, for acetonitrile, at λ= 575 nm); from 0.77 to 9.32 (12 times, for methanol, at λ= 575 nm) and from 0.77 to 8.28 (10 times, for DMSO ,at λ= 575 nm) upon addition of 30 % (v/v) water. For this reason the probe (**L**) exhibit fluorescence and visual colour changes with increasing water content (Fig. S8).

Table 1 Comparison of the detection limit of this FRET based probe (**L**) with the previuosly reported water sensors.

Detection limit (DL)	Quantitation limit (QL)	Reference
0.038 v/v % (MeCN)	—	4a
0.02 v/v% (THF)	—	4b
0.006 v/v % (MeCN)	—	4c
0.002 v/v% (MeCN)	—	4d
0.0035 v/v% (THF)	—	5a
0.1 wt% (MeCN)	0.4 wt% (MeCN)	6a
0.1 v/v% (EtOH) 0.02 v/v% (MeCN) 0.008 v/v% (DMF)	0.3 v/v% (EtOH) 0.07 v/v% (MeCN) 0.03 v/v% (DMF)	8
0.0026 v/v% (MeOH) 0.0032 v/v% (MeCN) 0.0044 v/v% (DMSO) 0.0033 v/v% (THF)	0.0085 v/v% (MeOH) 0.0108 v/v% (MeCN) 0.0147 v/v% (DMSO) 0.0105 v/v% (THF)	This work

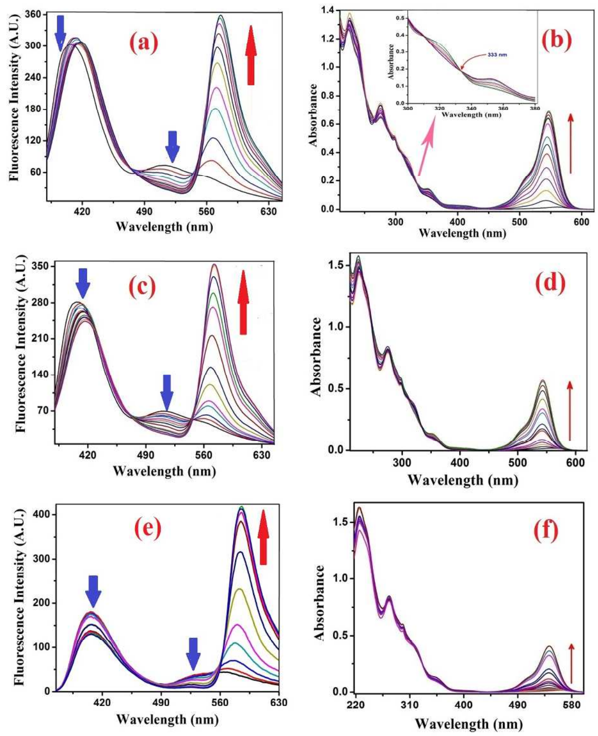


Fig. 1 (a) Fluorescence (λ_{ex} = 350 nm) and (b) absorption spectra of **L** (c = 2.0×10^{-5} M) in acetonitrile containing water (1.43-20 %v/v). (c) Fluorescence (λ_{ex} = 350 nm) and (d) absorption spectra of **L** (c = 2.0×10^{-5} M) in methanol containing water (1.43-25.7 %v/v). (e) Fluorescence (λ_{ex} = 350 nm) and (f) absorption spectra of **L** (c = 2.0×10^{-5} M) in DMSO containing water (1.43-28.6 %v/v).

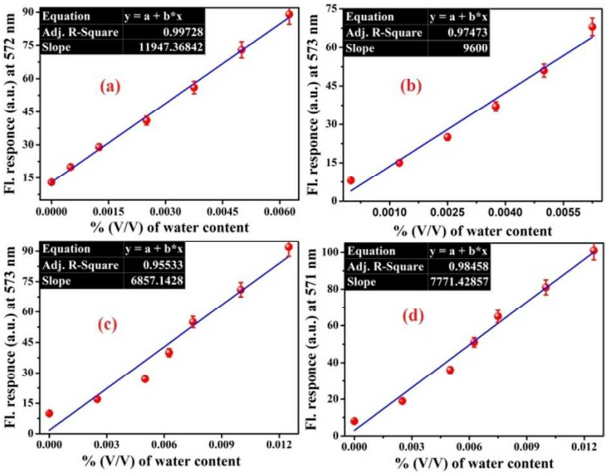


Fig. 2 calibration curve in lower concentration range, with error bars for calculating the DL and QL of **L** as a function of water content in (a) acetonitrile, (b) methanol, (c) DMSO and (d) THF

To strengthen the fact of FRET process, fluorescence life time experiment was performed. In this study, the probe (**L**) was taken in dry DMSO (10 μM) and excited at donor excitation position at 350 nm (from UV-Vis peak of 2-APBZ at 345 nm)[17]. Fig. 3 showed a decrese trend in life time at donor emission position (420 nm) with increase of water content in the dry DMSO from 4.05 ns to 3.51ns, which is in good agreement with the FRET process. A significancant spectral overlap was also observed between 2-APBZ and rhodamine-B moeity (Fig. 4). Again,. Förster distance between donor and acceptor was also calculated (*ca.* 52 A°; ESI†) and this was well supported for occuring FRET.

In summary, a new rhodamine-benzimidazol hybrid molecule has been established as a dual channel chemosensor for the detection of trace level water in both protic and aprotic organic solvents through FRET pathway. This probe is highly selective and sensitive towards water because water have maximum dielectric constant value among all other polar protic solvents. For this reason only water is able to create a dipole in the molecule through zwitterion formation (*viz.* Scheme 2), and this dipolar charge is highly responsible for ring opening (through extended conjugation) in favour of FRET process.

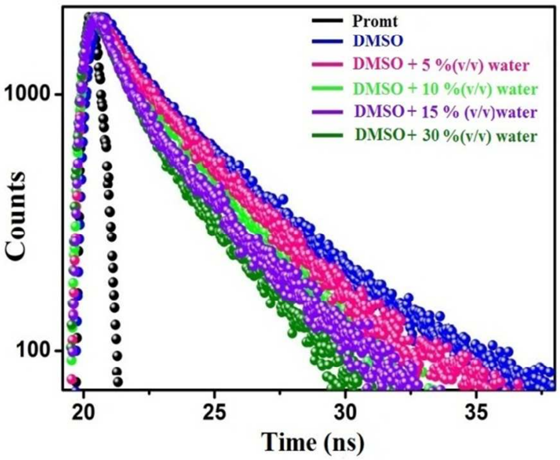


Fig. 3 Fluorescence life time decay profiles of **L** at 420 nm (donor emission position) with increasing water content

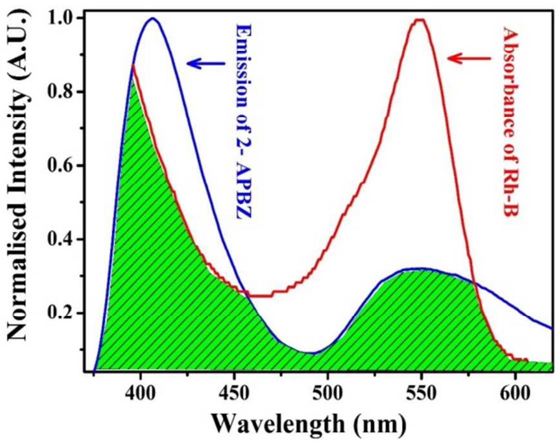


Fig. 4 Overlap spectra of donor emission and acceptor absorbance of **L** (10 μM) in dry acetonitrile.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental section, Figs. S1-S8 Table S1, other electronic format See DOI: 10.1039/b000000x/

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Graphical Abstract

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