Dalton Transactions

RSC Publishing

PAPER

View Article Online
View Journal

CHEF induced highly selective and sensitive turn-on fluorogenic and colorimetric sensor for Fe^{3+}†

Cite this: DOI: 10.1039/c3dt51974k

Shyamaprosad Goswami,*[a] Sangita Das,[a] Krishnendu Aich,[a] Deblina Sarkar,[b] Tapan Kumar Mondal,[b] Ching Kheng Quah[c] and Hoong-Kun Fun[c,d]

Received 20th July 2013,
Accepted 12th August 2013

DOI: 10.1039/c3dt51974k

www.rsc.org/dalton

A new fluorescent probe was synthesized from rhodamine-6G and 6-(hydroxymethyl) picolinohydrazide for the sensing of Fe^{3+} in an aqueous environment. The structure of the sensor was confirmed through its single crystal X-ray study. It exhibits a high specificity and sensitivity towards Fe^{3+} over other interfering heavy and transition metal ions (HTM). The turn-on greenish-yellow fluorescence and a colorimetric change from colourless to pink were observed upon addition of Fe^{3+} which evokes almost 116- and 23-fold enhancement in absorbance and emission intensity respectively in an acetonitrile–water (1 : 1, v/v, 25 °C) solution at a neutral pH (pH = 7.2). The Fe^{3+} promoted ring opening of the spirolactam framework to the open chain amide platform of the sensor is responsible for its visible colour change and turn-on fluorescence activity. It also exhibits an excellent performance in the "dip stick" method. Moreover the limit of detection of the probe is in the 10^{-8} M range.

Introduction

Iron, one of the most essential trace elements in biological systems, plays a major role in the function of haemoglobin and various enzymes in the human body and plays crucial roles in cellular metabolism,[1,2] and both its deficiency and excess can induce a variety of diseases,[3] with iron storage and balance being tightly regulated in an organism.[4] Iron being both useful and cytotoxic,[5] its deficiency throughout the developmental phases may lead to permanent loss of motor skills.[6] Accretion of iron in the central nervous system has been involved in a number of diseases such as Parkinson's, Huntington's and Alzheimer's disease, associated with an increased quantity of iron.[5a] Keeping in view the roles played by iron in day-to-day life, the development of techniques for selective determination of iron is in great demand. It is necessary to design simple, highly sensitive and selective chemosensors for Fe^{3+} detection and establish a method for the determination of trace amounts of Fe^{3+} ions. There are many reports of fluorescent receptors to detect heavy and transition metal (HTM) ions, due to the attributes of fluorescence detection, such as simplicity, high sensitivity, low cost and real time monitoring.[7]

In this regard, a rhodamine based probe is highly significant as it has high water solubility and gives a pink colour along with turn-on fluorescence upon binding with metal ions in aqueous medium.[8] In continuation of our research work on the development of '*switching on*' fluorescence sensors[9] for biologically important substrates, herein we report the design, synthesis, and photophysical properties of a new rhodamine 6G-pyridine based probe (RHP). The experimental studies revealed that the probe is highly selective and sensitive towards Fe^{3+} ions in an aqueous environment exhibiting a large CHEF (chelation enhanced fluorescence) effect along with color change detectable by the naked eye.

Experimental

General

Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} plates with a thickness of 0.25 mm. Melting points were determined using a hot-plate melting point

[a]*Department of Chemistry, Bengal Engg. and Science University, Shibpur, Howrah-711 103, India. E-mail: spgoswamical@yahoo.com; Fax: +91 33 2668 2916; Tel: +91 33 2668 2961-3*
[b]*Department of Chemistry, Jadavpur University, Kolkata-700032, India. E-mail: tkmondal@chemistry.jdvu.ac.in*
[c]*X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia*
[d]*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia. E-mail: hfun.c@ksu.edu.sa; Fax: (+966) 146-76220; Tel: (+966) 146-77335*

†Electronic supplementary information (ESI) available: Job plot, association constant determination, detection limit determination, ^{1}H NMR, ^{13}C NMR, ESI MS spectroscopy, fluorescence titration spectra of the receptor with different metal ions, UV-vis titration spectra of the receptor with different metal ions, X-ray data, computational data. CCDC 935238. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3dt51974k

apparatus in an open mouth capillary and are uncorrected. ^{1}H and ^{13}C NMR spectra of RHP were recorded using JEOL 400 MHz and 100 MHz instruments respectively and ^{1}H NMR spectra of compound C were recorded using a Brucker 300 MHz instrument. For NMR spectra, $CDCl_3$ was used as a solvent using TMS as an internal standard. Chemical shifts are expressed in δ units and ^{1}H–^{1}H and ^{1}H–C coupling constants in Hz. IR spectra were recorded on a JASCO FT/IR-460 plus spectrometer using KBr discs. Fluorescence spectra were recorded using a PTI spectrophotometer and UV-vis titration experiments were performed using a JASCO UV-V530 spectrophotometer.

General method of UV-vis and fluorescence titration

UV-vis method. For UV-vis titration we used the solution of the host on the order of 2×10^{-5} M. The solution was prepared in CH_3CN–H_2O (1 : 1, v/v, 25 °C) HEPES buffer. The solution of the guest cations using their perchlorate salts on the order of 2×10^{-4} M was prepared in deionized water using HEPES buffer at pH = 7.2. Different concentrations of the host and increasing concentration of cations were prepared separately. The spectra of these solutions were recorded by means of the UV-vis method.

Fluorescence method. For the fluorescence titration, the solution of the receptor (2 μM) was prepared in CH_3CN–H_2O (1 : 1, v/v, 25 °C) using HEPES buffer. The solutions of the guest cations using their perchlorate salts in the order of 2×10^{-5} M were prepared in deionised water. Here also, various concentrations of the guest and increasing concentration of metal ions were prepared and the spectra were recorded by means of a fluorescence method.

Synthetic method for the preparation of the receptor. Intermediate compounds A and B were prepared according to a literature procedure.[10]

Synthesis of compound C. To a stirred solution of compound B (0.5 g, 2.99 mmol) in methanol (20 ml) excess hydrazine hydrate was added. The reaction mixture was refluxed until the TLC showed complete consumption of the reactant (about 1 h). After completion of the reaction, the solvent was evaporated under reduced pressure and the resulting slurry was poured into ice water. The white precipitate that appeared was filtered and dried under vacuum to afford compound C as a white crystalline solid (0.4 g, 2.39 mmol, yield = 80%). The compound was pure enough to use for the next step without further purification.

M_p = 172 °C.

^{1}H NMR (300 MHz, d^6 DMSO): δ 4.605 (t, J = 6.2 Hz, 4H), 5.433 (t, J = 5.81 Hz, 1H), 7.565 (d, J = 7.8 Hz, 1H), 7.836 (d, J = 7.5 Hz, 1H), 7.949 (t, J = 7.8 Hz, 1H), 9.922 (s, 1H).

TOF MS (ESI, positive): calcd for $C_7H_{10}N_3O_2$ $[M + H]^+$ (m/z): 168.0769; found: 168.0767.

Synthesis of the receptor (RHP). A mixture of compound C (0.5 g, 2.99 mmol) and rhodamine 6G (1.4 g, 2.92 mmol) was taken in 30 ml of methanol and 2 ml of triethylamine was added to it. The resulting solution was stirred for 12 hours under reflux under a nitrogen atmosphere. Upon completion of the reaction, monitored by TLC, the solvent was evaporated by a rota-evaporator and water was added to it and extracted with $CHCl_3$ (3 × 100 ml). The combined organic solvents were dried over anhydrous sodium sulphate and evaporated to get the crude product. The crude product was purified by column chromatography using 3% methanol in chloroform yielding the receptor as a brown coloured solid (0.45 g, yield = 77%).

M_p = 260 °C–262 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 (t, J = 8, 6H), 1.97 (s, 6H), 3.17 (q, J = 14.4, 4H), 4.64 (s, 2H), 6.32 (s, 2H), 6.56 (s, 2H), 7.12 (q, J = 8, 1H), 7.34 (d, J = 7.6, 1H), 7.52 (m, 2H), 7.72 (t, J = 7.8, 1H), 7.90 (d, J = 7.6, 1H), 8.01 (m, 2H), 8.90 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.82, 16.89, 38.58, 64.48, 66.61, 96.75, 105.18, 118.08, 121.86, 123.67, 123.72, 124.28, 129.06, 129.28, 133.36, 137.91, 147.67, 148.18, 151.91, 152.31, 158.50, 161.97, 165.49.

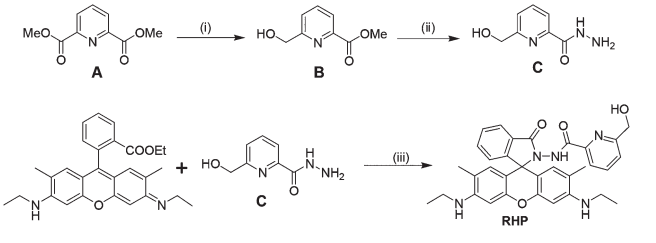
TOF MS (ESI, positive): calcd for $C_{33}H_{33}N_5O_4$ $[M + H]^+$ (m/z): 564.6626; found: 564.3770.

Results and discussion

The synthetic route of RHP is shown in Scheme 1. Compound C was treated with rhodamine 6G to afford the desired receptor.

The objective of preparation of such kinds of CHEF induced rhodamine 6G based probes is to get a beautiful spectrum both in emission and in absorbance, which was due to the change in the photophysical properties upon binding with cations. Now the cation binding properties of RHP were observed by employing aqueous solutions of different cations as their perchlorate salts (Fig. 1). The receptor itself does not show any colour in CH_3CN–H_2O (1 : 1, v/v, 25 °C, pH = 7.2), which proves that the structure predominantly exists in its expected spirolactam framework.

In order to perform a UV-vis absorption experiment, solutions of different cations were added separately to the prepared solution of RHP. Only Fe^{3+} was found to perturb the electronic behaviour of RHP, which showed an enhancement of absorption, and no other metals of interest showed any kind of significant effect on the absorption spectrum of RHP (20 μM, in CH_3CN–H_2O, 1 : 1, v/v, pH = 7.2). As shown in Fig. 2, upon addition of Fe^{3+} to the solution of RHP, a new peak arose at



Scheme 1 Synthesis of the receptor (RHP). Reagents and conditions: (i) $NaBH_4$, CH_3OH–$CHCl_3$ (7 : 3, v/v), rt, 2 h. (ii) Hydrazine hydrate, CH_3OH, reflux, 1 h. (iii) CH_3OH, Et_3N, reflux, 12 h.

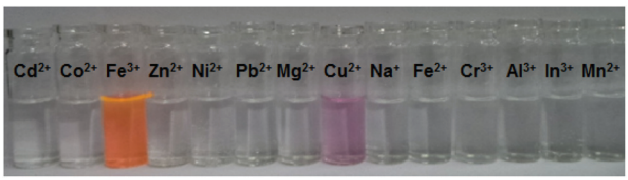


Fig. 1 A photograph of RHP (20 μM), showing visible color changes in the presence of Fe^{3+} and other stated cations (2 equivalents each).

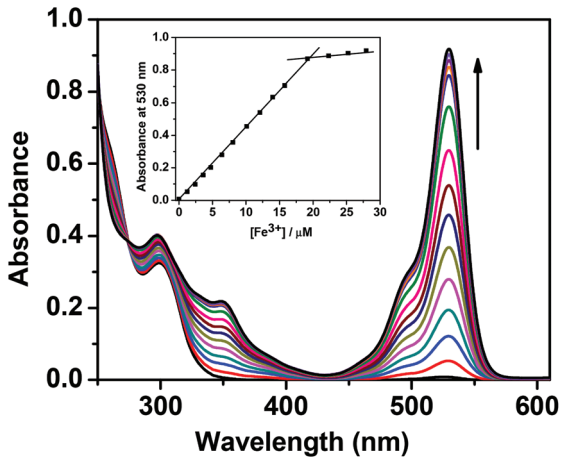


Fig. 2 Changes in absorption spectra of RHP (20 μM) upon gradual addition of Fe^{3+} (0 to 1.5 equivalents). Inset: the linear response curve of the absorbance of RHP at 530 nm depending on Fe^{3+} concentration.

530 nm and became intense with increasing concentration of Fe^{3+}. On addition of 2 equivalents of Fe^{3+} the absorbance intensity of RHP at 530 nm increased by almost 116 times (from 0.007899 to 0.918944 at 530 nm). During the addition of Fe^{3+} the absorption intensity exhibits a beautiful linear curve of the fitness relationship with Fe^{3+} concentration (0 to 19.6 μM) with a good R^2 value of 0.9994. The visual colour change (pink red) was observed during the addition of Fe^{3+} due to complexation of RHP with Fe^{3+} which was accompanied by the opening of the spirolactam ring of RHP. The detection limit of the sensor for Fe^{3+} was determined from the absorption spectral change, upon addition of Fe^{3+}, to be 5.55×10^{-7} M, using the equation $DL = K \times Sb_1/S$, where $K = 3$, Sb_1 is the standard deviation of the blank solution and S is the slope of the calibration curve[11] (ESI†).

The selectivity and interference are the two very important parameters to evaluate the performance of a receptor. Now the sensitivity and selectivity of RHP towards Fe^{3+} were examined by employing different HTM (Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, K^+, Mg^{2+}, Na^+, Mn^{2+}, Ni^{+2}, Al^{3+}, Pb^{2+}, In^{3+} and Zn^{2+}) ions in the RHP solution. RHP shows an extraordinary selective enhancement of absorbance towards Fe^{3+} in a CH_3CN–H_2O (1 : 1) solution at 530 nm, whereas no noticeable change was observed upon addition of other competing metal ions except Cu^{2+}. Upon addition of 2.0 equivalents of Cu^{2+}, the absorbance of the sensor at 530 nm was increased by 7-fold, whereas, it increases by 116-fold in the presence of Fe^{3+}, which is almost 17 times greater than that of Cu^{2+}. This phenomenon indicates that the sensor can be employed conveniently for Fe^{3+} detection by simple visual inspection. So the selectivity of RHP towards Fe^{3+} is thus well proven. A comparative view of absorbance of RHP at 530 nm upon addition of different co-existing metal ions (2 equivalents) is shown in Fig. 3. In order to quantify the stoichiometry of the complex of RHP and Fe^{3+}, a Job's plot analysis (method of continuous variation) was carried out in which the change in absorbance at 530 nm was plotted against molar fractions of RHP and Fe^{3+} under the conditions of an invariant total concentration. The maximum point appears at a mole fraction of 0.5, which corresponds to a 1 : 1 complex formation of RHP and Fe^{3+} (ESI†). The association constant for the metal ion complexation process of the receptor with Fe^{3+} was estimated to be 1.04×10^4 M from a Benesi–Hildebrand plot[12] using data obtained from the UV-vis titration (ESI†). The stoichiometry of such complexation fits nicely with a 1 : 1 (host–guest) binding model.

The fluorogenic response of RHP was investigated by monitoring the fluorescence behaviour upon addition of several metal ions such as Fe^{3+}, Na^+, K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Al^{3+}, Co^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, In^{3+}, Hg^{2+}, Pb^{2+} and Cr^{3+} in CH_3CN–H_2O (1 : 1, v/v, pH = 7.2) as their perchlorate salts. The free receptor (2 μM) exhibits a very weak emission band at 550 nm upon excitation at 505 nm. With the addition of Fe^{3+}, there arises a remarkable enhancement of fluorescence with an emission band at 550 nm, which is accompanied by the opening of the spirolactam ring of the receptor upon chelation with Fe^{3+} (Scheme 2). Notably, the addition of other co-existing metal ions, even in excess amount, caused insignificant changes in the emission intensity of the receptor.

Thus the competitive free cations would have an insignificant influence on RHP and the sensor could be used potentially for fluorogenic detection of Fe^{3+} without any interference. The fluorescence intensity of RHP maintained a good linear relationship with $[Fe^{3+}]$/μM with a good R^2 value of 0.998. On continued addition of Fe^{3+} (0–1.2 equiv.) in the RHP solution, a large enhancement in intensity was observed at 550 nm (23-fold). So this sensor can be specifically used for

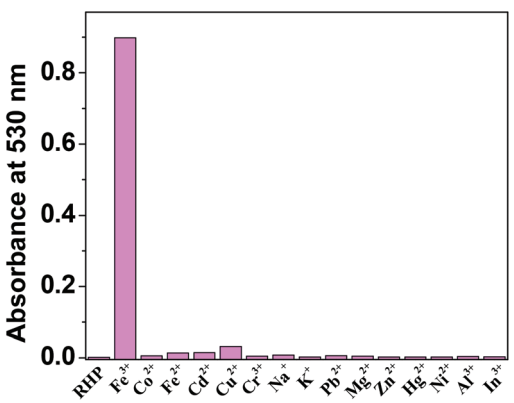
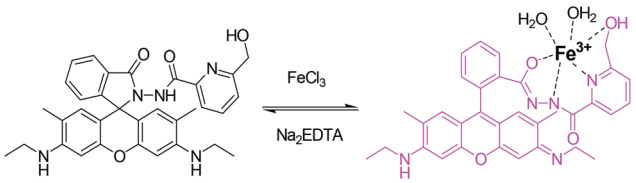


Fig. 3 A comparative view of the absorbance of RHP at 530 nm upon addition of different metal ions (2 equivalents).



Scheme 2 Probable complexation mode of RHP with Fe^{3+}.

the detection of Fe^{3+} fluorometrically. The observed fluorescence spectral changes of the sensor with increasing amounts of Fe^{3+} are shown in Fig. 4.

The selectivity of RHP for Fe^{3+} over other metal ions is shown in Fig. 5. From the fluorescence titration experiment, the detection limit of the sensors was also evaluated and it was found to be 3.6×10^{-8} M. Among all other common metal ions only Fe^{3+} resulted in a pronounced fluorescence enhancement and the association constant (K_a) was determined by the fluorescence titration method for the sensor (RHP) with Fe^{3+} and it was found to be 5.9×10^5 M^{-1} by the Benesi–Hildebrand equation.

The interference of other competing metal ions was investigated by the competition experiment which was conducted with 2.0 equivalents of Fe^{3+} ions in the presence of other metal ions in the same concentration, which is demonstrated in the following Fig. 6. It was observed that the detection of Fe^{3+} in the presence of other metal ions was not hampered; that is, the interference for the detection of Fe^{3+} was not hampered. So RHP could be used as a selective and sensitive colorimetric as well as a fluorometric sensor for Fe^{3+} ions.

There are a number of sensors which can detect iron only in the solution phase, which would restrict their sensitivity under some special circumstances, for example sensing occurs only through hydration. So in order to investigate a practical application of this sensor we performed an experiment called the "dip-stick" method. It is a very simple but very important

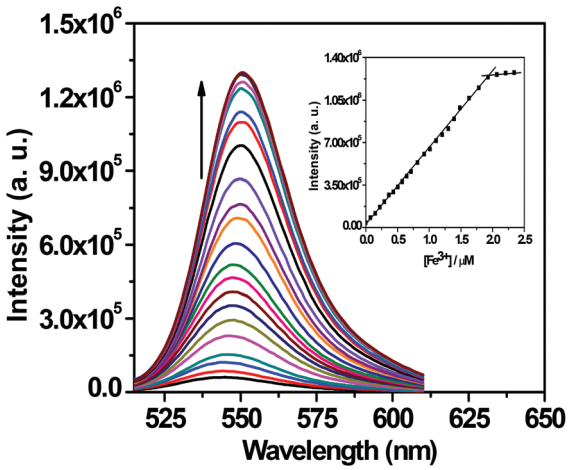


Fig. 4 Changes in emission spectra of RHP (2 μM) upon gradual addition of Fe^{3+}. Inset: the linear response curve of emission intensity of RHP at 550 nm depending on the concentration of Fe^{3+}.

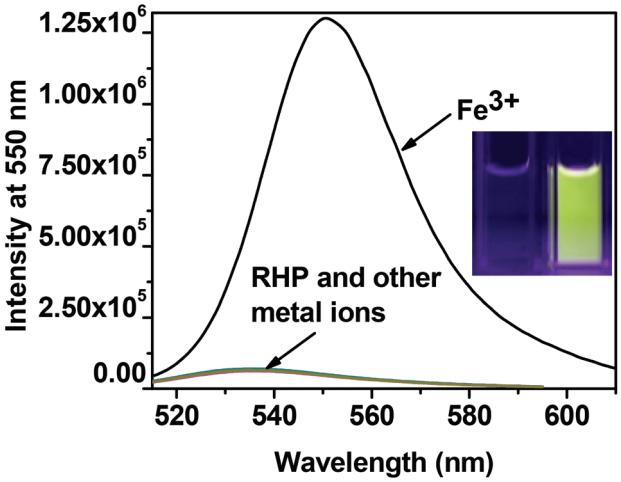


Fig. 5 A comparative view of emission intensities of RHP (2 μM) at 550 nm upon addition of different metal ions (2 equivalents). Inset: a photograph showing the fluorescence changes of RHP in the absence and presence of Fe^{3+} (2 equivalents) after irradiation with UV light.

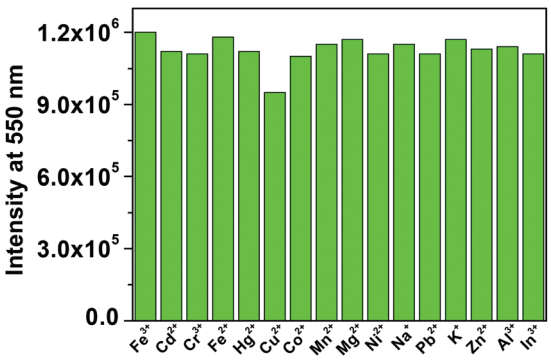


Fig. 6 Changes in emission intensity of RHP (2 μM) at 550 nm upon addition of 2 equivalents of Fe^{3+} along with 2 equivalents of various metal ions. λ_{ex} = 505 nm.

experiment because it gives instant qualitative information without resorting to the instrumental analysis. In order to perform this experiment we prepared TLC plates which were further immersed into the solution of RHP (2×10^{-4} M) in acetonitrile, and then evaporating the solvent to dryness. We immersed the TLC plate in a Fe^{3+} (2×10^{-3} M) solution and then exposed it to air to evaporate the solvent. The color of the TLC plates changes from colorless to pink. This experiment evokes a real time monitoring without using any instrumental help. Just by naked-eye detection and the use of TLC plates we can easily investigate a qualitative instant detection of Fe^{3+} (Fig. 7).

Reversibility of RHP

As is well known, reversibility is an important phenomenon to obtain an excellent chemical sensor. Thus, the chemical reversibility behavior of RHP was studied to examine the reusability of the receptor. Now to demonstrate this fact the absorption and emission titration experiments were conducted using the

Fig. 7 Color changes of RHP on test paper in the absence and presence of Fe^{3+} under ambient light.

Fe^{3+} complex of RHP (RHP–Fe^{3+}) with Na_2EDTA (ethylenediaminetetraacetic acid disodium salt). From these experiments it was revealed that the pink colour of RHP–Fe^{3+} disappeared with increasing concentration of Na_2EDTA and the emission intensity of the RHP–Fe^{3+} complex also gradually decreased (Fig. 8 and 9). It indicates the decomplexation of RHP–Fe^{3+} as Na_2EDTA strips away Fe^{3+} from the binding zone.

Effect of pH on the optical behavior of RHP

The rhodamine–pyridine containing fluorescent probes are known to be sensitive to a change in pH in the acidic medium.

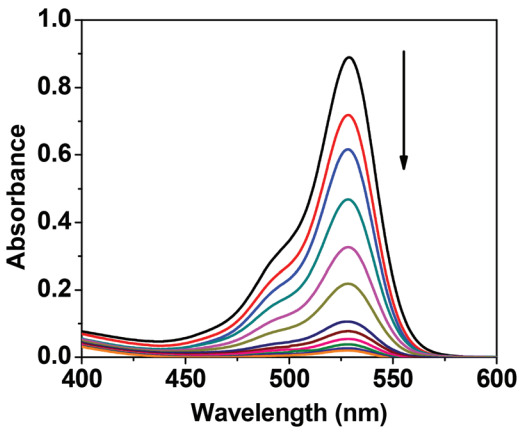


Fig. 8 Absorption titration spectra of RHP-Fe^{3+} (20 μM) upon increasing the concentration of the Na_2EDTA solution (0 to 10 equivalents).

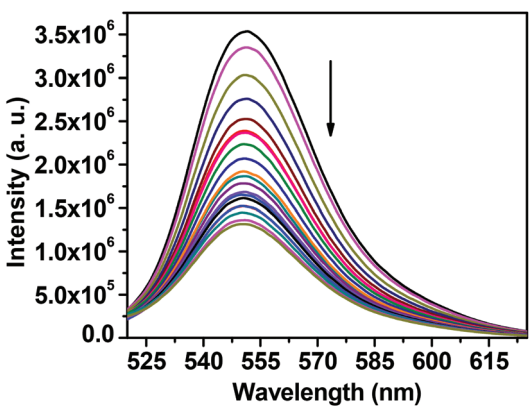


Fig. 9 Fluorescence titration spectra of RHP-Fe^{3+} (20 μM) upon increasing the concentration of Na_2EDTA (0 to 10 equivalents) λ_{ex} = 505 nm.

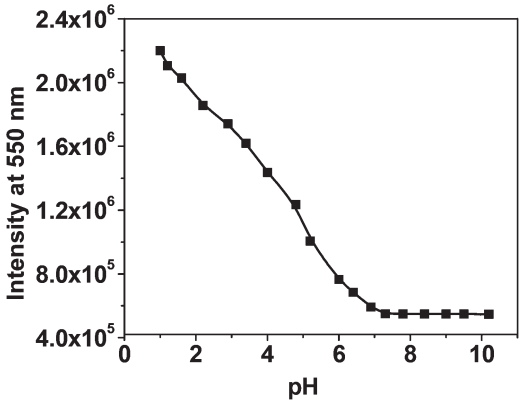


Fig. 10 Fluorescence response of RHP at 550 nm (20 μM, λ_{ex} = 505 nm) as a function of pH in CH_3CN–H_2O (1 : 1, v/v); the pH is adjusted by using aqueous solutions of 1 M HCl or 1 M NaOH.

To observe that phenomenon, a stock solution of RHP (20 μM) was prepared in acetonitrile–water (1 : 1, v/v) and the pH, 1–5 and 5–10.5, was maintained by the addition of a calculated amount of 1 N HCl and 1 N NaOH solutions respectively, and the emission spectra were monitored. The pH–emission plot (Fig. 10) showed insignificant changes in emission intensity of RHP in the pH range 7–10.5, which indicates that the ring predominantly exists in its spirolactum framework in this pH range. But in strong acidic medium (pH > 6) there arose an enhancement in emission intensity, which suggests the protonation induced ring opening of the spiro cyclic framework along with a color change detectable by the naked eye (pink-red). Thus RHP can be used as a sensor for Fe^{3+} in near neutral pH (pH = 7.2).

Crystallographic study

Single crystals of the sensor (RHP) suitable for X-ray studies were obtained by dissolving a powder of the pure compound in $CHCl_3$–CH_3CN (1 : 9, v/v) and slow evaporation of the solution free from vibrations.

In the compound RHP (Fig. 11), the hydroxyl group is disordered over two positions, leading to positional disorder of the two methylene H atoms, with occupancies of 0.556(16) and 0.444(16). The xanthene ring system is almost planar (maximum deviation = 0.0336(13) Å at atom O1) and forms dihedral angles of 89.39(5)° and 83.68(6)° with the essentially planar isoindoline ring system (maximum deviation = 0.0587(13) Å at atom N3) and pyridine ring, respectively, indicating that they are almost perpendicular to each other. The dihedral angle between the isoindoline ring system and pyridine ring is 71.44(7)°.

In the crystal, molecules are linked in an inversion dimer by a pair of intermolecular C–H···O hydrogen bonds (ESI, Table 2†), generating an $R_2^2(16)$ ring motif (Fig. 12). The dimers are further connected by O–H···O and C–H···O hydrogen bonds into a three-dimensional network.

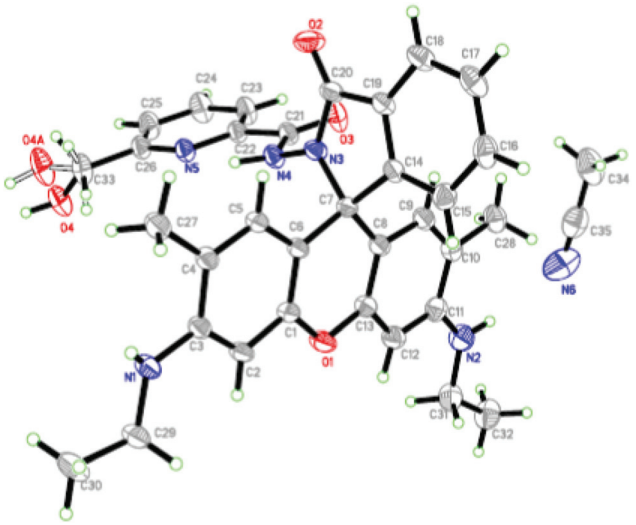


Fig. 11 The structure of RHP, showing displacement ellipsoids drawn at the 30% probability level. Hydrogen atoms are drawn as circles with small radii. Open bonds show the minor disorder component.

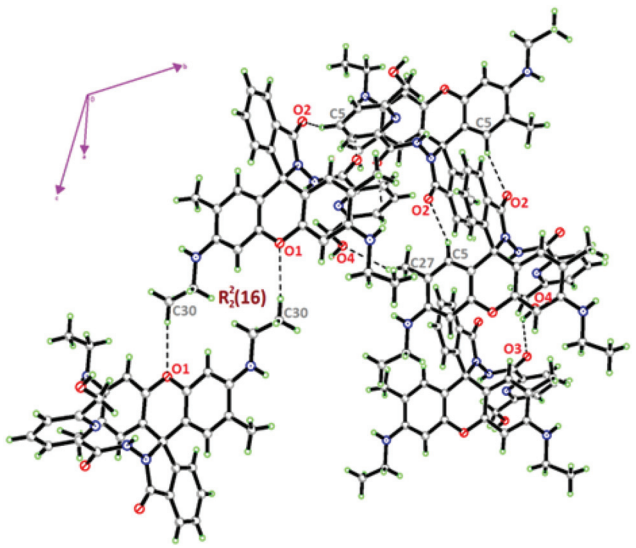


Fig. 12 Part of the crystal packing of RHP showing the hydrogen-bonding network.

Computational study (DFT)

The optimized structures of RHP and its octahedral Fe^{3+} complex obtained by the DFT/B3LYP method are shown in Fig. 13 and 14 respectively. The calculated bond parameters of RHP are well reproduced by the X-ray data. The TDDFT calculation on the optimized geometry of RHP has been performed to understand the electronic transitions in the compound. Some selected vertical electronic excitations of RHP are shown in Table 3 (ESI†). Contour plots of some selected molecular orbitals of RHP are shown in Fig. S12.† The transitions at 375 and 317 nm corresponding to HOMO → LUMO and HOMO → LUMO + 1 transitions are very weak in nature ($f \sim 0.004$). One moderately intense transition at 288 nm corresponds to

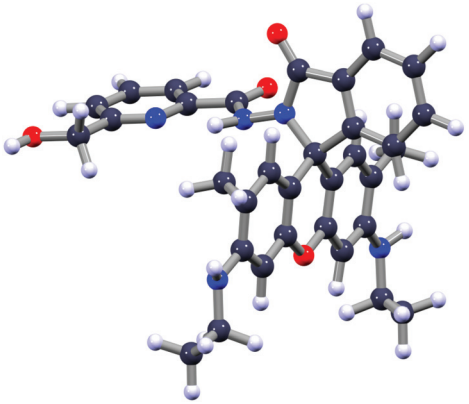


Fig. 13 Optimized structure of RHP by the DFT/B3LYP/6-31G(d) method.

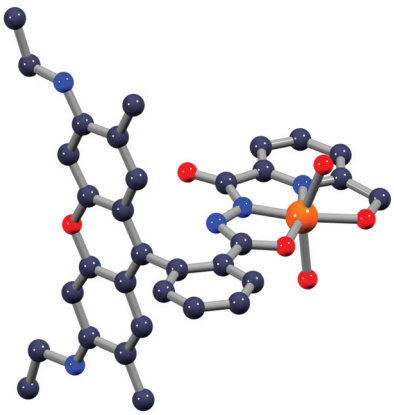


Fig. 14 Optimized structure of RHP-Fe^{3+} (H atoms are omitted for clarity) by the DFT/B3LYP/LANL2DZ/6-31G(d) method.

HOMO − 1 → LUMO + 3. The transitions with high oscillator strength (f) values found at 274 and 254 nm correspond to HOMO → LUMO + 4 and HOMO − 2 → LUMO + 3 transitions respectively.

Conclusions

In summary we report here the synthesis, characterization and cation binding properties of a new rhodamine based probe (RHP) which exhibits an enhanced response both in absorption and fluorescence intensities towards Fe^{3+} in aqueous media at almost neutral pH. Water solubility, reversibility and high sensitivity (both colorimetric and fluorogenic) make the probe suitable for use as an excellent chemosensor for Fe^{3+}.

Acknowledgements

Authors thank the CSIR and DST, Govt. of India for financial support. S.D., K.A. and D.S. acknowledge the CSIR for providing them with fellowships. The authors extend their appreciation to the Deanship of Scientific Research at King Saud

University for funding the work through the research group project no. RGP VPP-207. The authors also thank Universiti Sains Malaysia (USM) for the APEX DE2012 grant (no. 1002/PFIZIK/910323).

Notes and references

1 M. Ghaedi, A. Shokrollahi, R. Mehrnoosh, O. Hossaini and M. Soylak, *Cent. Eur. J. Chem.*, 2008, **6**, 488.
2 S. Bae and J. Tae, *Tetrahedron Lett.*, 2007, **48**, 5389.
3 D. Touati, *Arch. Biochem. Biophys.*, 2000, **373**, 1.
4 (*a*) G. Cairo and A. Pietrangelo, *Biochem. J.*, 2000, **352**, 24; (*b*) E. Beutler, V. Felitti, T. Gelbart and N. Ho, *Drug Metab. Dispos.*, 2001, **29**, 495.
5 (*a*) J. R. Burdo and J. R. Connor, *Biometals*, 2003, **16**, 63; (*b*) J. R. Connor, S. L. Menzies, S. M. St. Martin and E. J. Mufson, *J. Neurosci. Res.*, 1990, **27**, 595; (*c*) R. R. Crichton, S. Wilmet, R. Legssyer and R. J. Ward, *J. Inorg. Biochem.*, 2002, **91**, 9; (*d*) R. S. Eisenstein, *Annu. Rev. Nutr.*, 2000, **20**, 627.
6 (*a*) B. T. Felt and B. Lozoff, *J. Nutr.*, 1996, **126**, 693; (*b*) C. J. Earley, J. R. Connor, J. L. Beard, E. A. Malecki, D. K. Epstein and R. P. Allen, *Neurology*, 2000, **54**, 1698.
7 D. Prabhakaran, M. Yuehong, H. Nanjo and H. Matsunaga, *Anal. Chem.*, 2007, **79**, 4056.
8 (*a*) X. Chen, T. Pradhan, F. Wang, J. S. Kimand and J. Yoon, *Chem. Rev.*, 2012, **112**, 1910; (*b*) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465.
9 (*a*) S. Goswami, D. Sen, N. K. Das, H.-K. Fun and C. K. Quah, *Chem. Commun.*, 2011, **47**, 9101; (*b*) S. Goswami, K. Aich, S. Das, A. K. Das, A. Manna and S. Halder, *Analyst*, 2013, **138**, 1903; (*c*) S. Goswami, K. Aich, A. K. Das, A. Manna and S. Das, *RSC Adv.*, 2013, **3**, 2412; (*d*) S. Goswami, K. Aich and D. Sen, *Chem. Lett.*, 2012, 863.
10 S. Goswami, D. Sen and N. K. Das, *Tetrahedron Lett.*, 2010, **51**, 6707.
11 (*a*) M. Shortreed, R. Kopelman, M. Kuhn and B. Hoyland, *Anal. Chem.*, 1996, **68**, 1414; (*b*) W. Lin, L. Yuan, Z. Cao, Y. Feng and L. Long, *Chem.–Eur. J.*, 2009, **15**, 5096.
12 (*a*) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703; (*b*) D. C. Carter and J. X. Ho, *Adv. Protein Chem.*, 1994, **45**, 153; (*c*) A. Mallick and N. Chattopadhyay, *Photochem. Photobiol.*, 2005, **81**, 419; (*d*) I. Ravikumar, B. N. Ahamed and P. Ghosh, *Tetrahedron*, 2007, **63**, 12940.