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A Tunable Ratiometric pH Sensor Based on Phenanthro[9,10-*d*]imidazole covalently linked with vinylpyridine

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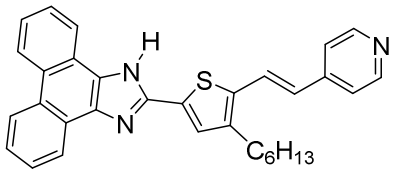
A rational design of novel phenanthro[9,10-*d*]imidazole based pH sensing fluorophore PITP was developed, in which phenanthroimidazole acts as a donor and pyridine acts as an acceptor. Various spectral studies confirm that the protonation occurs at two different nitrogen centres, each of which produces an observable color change depending on the pH of the buffer solution. DFT calculations also support the experimental results. We have shown that PITP can be used as a ratiometric and colorimetric pH sensor in the acidic region.

Design and synthesis of novel fluorophores are of great interest because of their wide range of applications in clinical diagnostics and environmental monitoring.[1-4] Fluorophores can be used as sensors for the detection of biomolecules, cations, anions, biological and ecological pH.[5,6] pH sensors that works under the acidic conditions usually have a single protonation site. Upon protonation on such site there will be a physical or chemical changes occurs either through photoinduced electron transfer (PET) or intramolecular charge transfer (ICT) and in turn used to precisely measure the pH of the medium. pH sensors that deals with single protonation site can only detect a single pH and is widely known.[7-9] To the best of our knowledge, pH sensor molecule having a multiple protonation sites that can sense different pH of the solution is not known till date.

We have designed a molecule in such a way that it can accommodate multiple functional groups having different pKa values, which can alter the ICT depending on the pH of the medium and hence it can sense a different pH under different reaction conditions. For that purpose we chose Phenanthro[9,10-*d*]imidazole in combination with the thiophene tethered pyridine as our target molecule (**PITP**). Under the different pH condition either the imidazolyl nitrogen or the pyridinyl nitrogen get protonated and made significant red shift of more than 115 nm, which is very useful to detect the pH of the analyte under visible region. It is important to note that the **PITP** molecule can act both as ratiometric as well as colorimetric pH sensor under the reaction conditions.

In general, Phenanthro[9,10-*d*]imidazole based molecules act as a metal ion sensors for Hg^{2+} and Fe^{3+} [10-12] to date only very few reports are available for pH sensors that detect within a very narrow pH region.[13,14] In contrast **PITP** can act as a sensor molecule for the region between pH of 2.0 to 7.5. The presence of extended π-conjugation in **PITP** results in large stoke shift, which in turn becomes an essential requirement to detect the wide range of pH of the analyte. Herein, we report the novel synthesis of a phenanthro[9,10-*d*]imidazole based fluorophore **PITP** containing extended π-conjugation in the form of 3-hexyl-thiophene having two protonation sites. We found that it act as an efficient ratiometric and colorimetric pH sensor between a range of pH. The sensing ability of **PITP** was carried out using UV-vis, fluorescence, cyclic voltammetry studies and DFT calculations further support the experimental results. The fluorophore **PITP** was synthesized from 2-bromo-3-hexylthiophene as depicted in scheme 1. Compound **2** was obtained initially by the Vilsmeier-Haack reaction of 2-bromo-3-hexylthiophene and further it was treated with the 9,10-phenoanthrodione in the presence of acetic acid and ammonium acetate at 120° C to get compound **PIT**. The fluorophore **PITP** was obtained by the Heck coupling reaction of vinyl pyridine with **PIT** under the reaction conditions.



PITP

Structure 1

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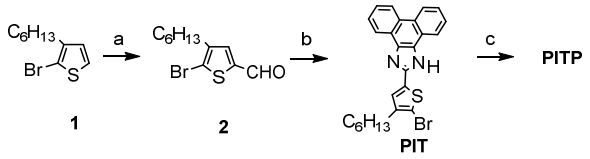
Electronic Supplementary Information (ESI) available: Experimental procedures, spectral data, copies of ^{1}H & ^{13}C NMR and ESI-MS. See DOI: 10.1039/x0xx00000x



Scheme 1 (a) $POCl_3$, DMF, EDC, 80°C, (b) 9,10-phenanthroquinone, NH_4OAc, AcOH, 120°C, (4-vinylpyridine, $Pd(OAc)_2$, DMF, 120°C, TBAB, K_2CO_3.

The absorption and emission spectral studies of **PITP** were carried out in a series of protic and aprotic solvents and we infer that the spectral behaviours are very similar in all solvents studied. A strong absorption and emission band is present around 400 and 500 nm respectively, which occurs due to the charge transfer from the phenanthro[9,10-*d*]imidazole donor to the pyridine acceptor within the **PITP** fluorophore. Optical response of **PITP** at different pH was studied in 0.2 M phosphate buffer (containing 60% methanol as a co-solvent). As the pH of the solution is decreased from 7.51 to 4.12, the absorbance around 400 nm decreases with a simultaneous appearance of a new peak at around 450 nm with an isobestic point at 420 nm as shown in Figure 1a. As the pH of the solution decreased further from 4, the newly formed absorption peak around 450 nm is blue shifted (420 nm) along with an isobestic point at 450 nm. The absorbance around 400 nm for **PITP** is due to the neutral form and the new peaks around 450 nm and 420 nm with decrease in pH are due to the successive protonation at pyridine nitrogen (acceptor) and imidazole nitrogen (donor) centers. It can be anticipated that protonation at the donor or acceptor moiety will change the photophysical properties of fluorophore because it affects the efficiency of intramolecular charge transfer within **PITP**. The first protonation at pyridine nitrogen increases the electron accepting character of the acceptor group. This increase in charge transfer results in the red shift of the absorption spectrum. The second protonation at imidazole nitrogen decreases the electron donating character of the donor group results in the blue shift[15,16] of the absorption spectrum (Figure 1b). Similar studies were carried out using **PIT**(**PITP** without the pyridine moiety), upon decreasing the pH of the solution from 7.10 to 1.88 leads to red shift in the absorption spectrum from 355 nm to 361 nm, due to protonation at only the imidazole nitrogen and this shift happened only at pH below 4.0 (Figure S-1). This confirms the second protonation occurs in **PITP** below pH 4.0 is at imidazole nitrogen (donor moiety).

As similar to the absorption spectra, the emission spectra of **PITP** is red shifted by 116 nm with a clear isoemissive point at 620 nm on decreasing the pH from 7.51 to 4.12 (Figure 2). As the pH is decreased, the intensity of the new emission peak around 646 nm enhances with simultaneous decrease in the intensity of the 528 nm peak on exciting at the isosbestic point as shown in Figure 2. The emission around 500 nm for **PITP** is due to the neutral form. The appearance of the new red shifted emission is caused by the protonation at the pyridine moiety, which results in the increasing the electron withdrawing ability of acceptor moiety.

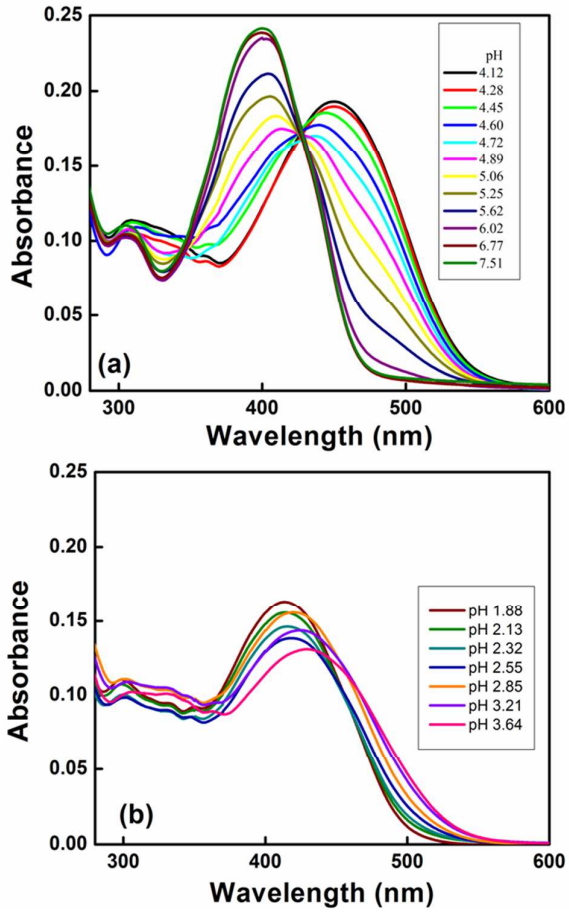


Figure 1 UV-visible spectrum of PITP (a) changes in absorbance of PITP (10 µM) in 0.2 M phosphate buffer (containing 60% methanol as co-solvent) at pH 7.51 to 4.12 (b) changes in absorbance of PITP (10 µM) in 0.2 M KCl-HCl buffer (containing 60% methanol as co-solvent) at pH 3.64 to 1.88.

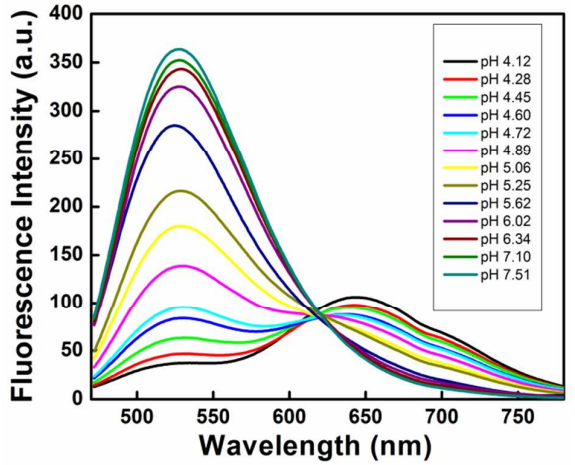


Figure 2 Fluorescence spectra of PITP(10 µM) in of 0.2 Mphosphate

and KCl-HCl buffer solution (containing 60% methanol as a co-solvent) at pH 7.51 to 4.12, excited at 450 nm.

The larger red shift makes this molecule suitable for ratiometric pH sensing in acidic region. Upon decreasing the pH from 7.51 to 4.12 the emission intensity ratio (I_{528} / I_{646}) changes from 8.16 to 0.33 and the pKa (5.47) value was calculated from emission ratio as a function of pH (Figure S-4a).

In addition to absorption and emission spectral changes, the color of the **PITP** solution is changed from colorless to orange-red upon decreasing the pH from 7.5 to 4.0, then changes from orange-red to pale yellow upon decreasing the pH from 3.5 to 2.0 and the color changes are visible to naked eye (Figure 3a). Figure 3b shows the emission color changes of **PITP** solution at three different pH values (the photo was taken under illumination of a UV lamp). The color and spectral changes are reversible, and reverse back the reaction by increasing the pH using aqueous ammonia. Hence, **PITP** can be used as a pH sensor within the three different pH's such as 7.2/6.2/5.2; 4.2; and 3.2/2.2 region.

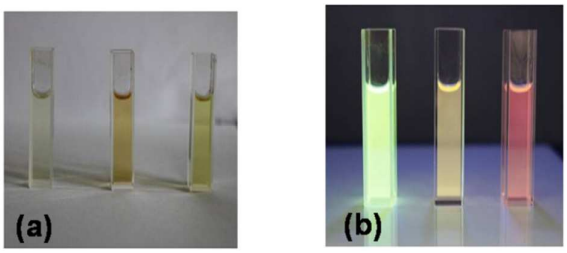


Figure 3 Change in color of PITP at pH 7.5, 4.2 and 2.0 respectively (a) under visible light and (b) under UV light (365 nm).

To establish the optical response of sensor **PITP** observed at different pH, we carried out density functional theory (DFT) calculations using DFT-B3LYP-6-31G level under Gaussian 03 package.[17]DFT study shows that the charge transfer state originating from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) excitation. The HOMO is localized on the phenanthro[9,10-*d*]imidazole(donor), and the LUMO is localized on the thiophene and the vinyl pyridine moiety(acceptor).

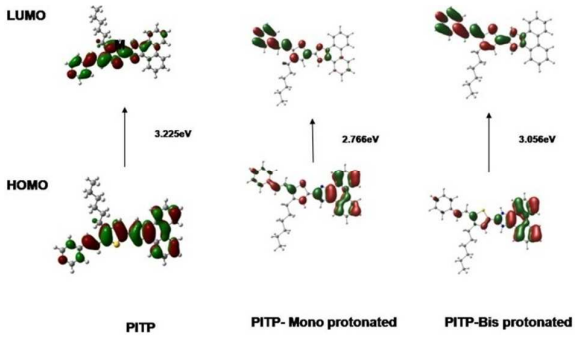


Figure 4 Molecular orbital plots showing HOMO - LUMO energy gaps of PITP, mono and bis-protonated forms of PITP.

Upon mono- and bis- protonation, the electronic distributions in HOMO is similar to **PITP** (only on phenanthro[9,10-*d*]-imidazole). The electronic distributions in LUMO of mono-protonated form located only on pyridine while in bis-protonated form it is located on pyridine as well as imidazole ring; that results in blue shift of the CT state (Figure 4). The energy difference between HOMO and LUMO of PITP, PITP-H^+ and PITP-$2H^+$ forms is 3.23 eV, 2.77 eV and 3.06 eV respectively. These results are in concordant with our experimental results.

In addition to UV-visible studies, cyclic voltammetric analysis of **PITP** has also been studied at pH 2.0 and 4.2 (Figure S-2). The result is as follows: E_{ox}= 0.961 V, E_{red}= -0.066 V, as the ΔE is > 1000 mV, it is irreversible redox couple. There is a shift in oxidation peak from 0.50 to 0.64 when we change the pH from 2 to 4.2 and the corresponding ΔE values are 0.27 V and 0.59 V. We infer from these values that there is a double protonation occurs ($2e^-$transfer) at pH 2.0 while single protonation ($1e^-$ transfer) occurs at pH 4.2.[18,19]

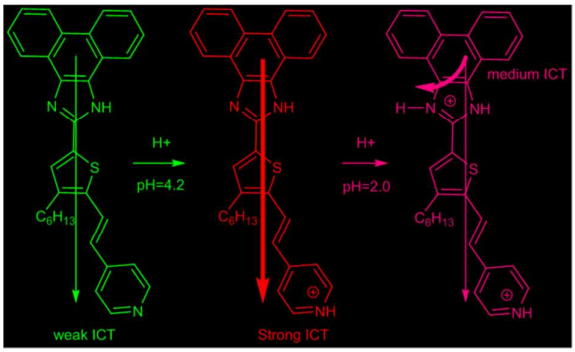


Figure 5 A schematic representation of single and double protonation of PITP at pyridine and imidazole nitrogen centres respectively.

To confirm the protonation site, we recorded the ^{1}H NMR spectra of **PITP** with and without passing HCl vapour (Figure 6b). After which we observe that the protons adjacent to pyridine nitrogen (i.e. H_a and H_b) are shifted from δ 8.20–8.30 to 8.60–8.70 ppm and δ 8.04–8.10 to 8.40–8.50 ppm respectively. Also the imidazole –NH proton at 13.9 ppm is disappeared.This is due to the possible bis-protonation of the pyridine as well as the phenanthro[9,10-*d*]imidazole N atoms, which in turn leads to a fast exchange of protons between the imidazole nitrogens (Figure 6a). The H_{e-h}protons are also shifted downfield to obtain a well-resolved ^{1}H NMR spectrum of **PITP**.

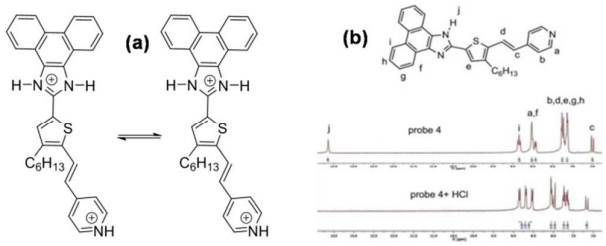


Figure 6(a) Schematic representation of tautomerisation of protonated imidazole, (b) ^{1}H NMR of PITP with and without passing HCl vapour.

In order to show that the **PITP** can act as a pH sensor, we did the following experiment. First the **PITP** flurophore was dissolved in methanol and few drops of it was placed over the Whatman paper and dried in air for few minutes. It was then dipped in different acidic buffer solution (Figure 7). As illustrated in the Figure 7, the change in colour is in accordance with the spectral results suggesting that, the designed fluorophore could act as a pH sensors with in the neutral to acidic region.

Figure 7 Change in color of PITP absorbed on paper upon adding buffer solutions (containing 60% methanol) at pH- 7.5, 4.2, 2.0 (left to right).

In summary we have successfully synthesized a novel phenanthro[9,10-*d*]imidazole based fluorophore having an extended π-conjugation. We observed multiple protonation at two different nitrogen centres of **PITP** by conducting UV-vis, fluorescence, CV and ^{1}H NMR studies. DFT studies of **PITP** supports our experimental results. In conclusion we demonstrated the utility of **PITP** as a ratiometric and calorimentric pH sensor in acidic region.

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