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Novel pH tunable fluorescent sensor with dual recognition mode

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ABSTRACT

A novel pH fluorescent chemosensor based on 2-hydroxybenzaldehyde 1-naphthoyl hydrazone (SNH) has been synthesized. This sensor has the ability to respond to both low and high pH values at different wavelengths. In acidic media, the fluorescence of SNH enhanced dramatically at shorter wavelength (λ_{em} = 380 nm, ϕ_f = 0.08) while in basic media the fluorescence at longer wavelength (λ_{em} = 500 nm, ϕ_f = 0.02) increased. At intermediate pH values (5–9), is detected the appearance of a weak emission at 460 nm, likely due to a keto-phototautomer associated to an intramolecular excited-state proton transfer. A complex mechanism tunable in the range of pH 1–11 with at least three fluorescent species at λ_{em} = 380, 460 and 500 nm is proposed.

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1. Introduction

Molecular sensors have been extensively explored in the development of novel functional materials as well as in the detection and measurements of several kinds of analytes. Molecular fluorescent sensors have received an increasingly enormous attention due to their high sensitivity. Fluorescent pH sensors are used in analytical chemistry, bioanalytical chemistry, cellular biology and medicine [1–3].

Most fluorescent pH sensors can be divided into three classes of fluorophores according to the recognition mechanism: photoinduced proton transfer, photoinduced electron transfer or any other mechanism [4].

A variety of molecules have intramolecular hydrogen bonds (H-bonds) that may be photoinduced to undergo proton transfer It is well known that excited-state intramolecular proton transfer (ESIPT) can be observed in a variety of molecules that contain both hydrogen donors (e.g. OH, NH, etc.) and acceptors (>N, >C=O, etc.) in close proximity [5]. An intramolecular hydrogen bond is generally formed in the ground state while in the excited state the proton of the hydroxy or amino group will migrate to the neighboring proton acceptor leading to a phototautomer. [6]. In the general family of 2-(2′-hydroxyphenyl)benzimidazole, -benzoxazole, -benzthiazole and benztriazole the phototautomer gives rise to an emission with large Stokes' shift [7].

Among the pH sensors, most of them are focused on the probes which are sensitive to the physiological pH value of normal body fluids, having in mind biological applications [8]. However, the fluorescent sensors which are sensitive to lower pH (pH < 5) or higher pH (pH > 9), are relatively sparse [9].

Acyl hydrazones can be cation or anion sensors [10]. Bearing this in mind, we devise a new pH chemosensor based on 2-hydroxybenzaldehyde 1-naphthoyl hydrazone, referred to from now on as SNH, reported herein in Scheme 1. This molecule shows off-on fluorescence at lower and higher pH regions with different emission wavelengths.

2. Experimental

2.1. Materials

The syntheses of SNH and its control compound benzaldehyde 1-naphthoyl hydrazone (BNH) were carried out by refluxing the solution of naphthoyl hydrazide in ethanol with corresponding salicylaldehyde or benzaldehyde (1:1 ratio) for 2 h (80% yield) [11]. The crude products were recrystalized from 95% ethanol and characterized by ^{1}H, ^{13}C NMR and ESI-MS data.

2.1.1. SNH

^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 6.921–6.973 (m, 2H), 7.302–7.345 (m, 1H), 7.580–7.645 (m, 4H), 7.800 (d, 1H, J = 7 Hz), 8.016–8.047 (m, 1H), 8.118 (d, 1H, J = 8 Hz), 8.243–8.290 (m, 1H),

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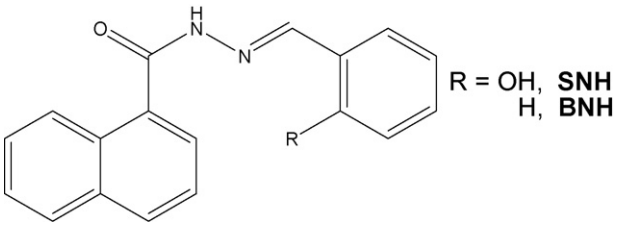


Scheme 1. Structure of SNH and its OH-free control molecule BNH.

8.551 (s, 1H), 11.245 (s, 1H), 12.242 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 116.346, 118.576, 119.286, 124.864, 125.013, 125.976, 126.381, 127.067, 128.281, 129.416, 129.876, 130.650, 131.372, 132.108, 133.089, 148.159, 157.395, 164.312. ESI-MS: m/z 290.9 (M+H$^+$, MeOH) and 313.0 (M+Na$^+$, MeOH).

2.1.2. BNH

^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 7.198–7.266 (m, 1H), 7.459–7.510 (m, 3H), 7.585–7.635 (m, 3H), 7.759 (d, 3H, J = 7.5 Hz), 7.999–8.048 (m, 1H), 8.102 (d, 1H, J = 8.4 Hz), 8.222 (d, 1H, J = 9 Hz), 8.353 (s, 1H), 12.011 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 124.953, 125.089, 125.513, 126.402, 127.046, 127.115, 128.324, 128.835, 129.942, 130.121, 130.442, 132.851, 133.149, 134.251, 147.716, 164.654. ESI-MS: m/z 275.1 (M+H$^+$, MeOH) and 297.1 (M+Na$^+$, MeOH).

All organic solvents used for spectral study were of spectroscopic grade and used without any further treatment. The water was doubly distilled before used.

2.2. Methods and instruments

The samples were prepared from absolute ethanol stock solution. The aliquots taken were evaporated with a nitrogen stream and then corresponding solvents were added.

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data were acquired in DMSO-d_6 on a Bruker Avance 400 MHz NMR spectrometer using TMS as internal reference.

A Shimadzu UV-1603 spectrophotometer was used to record absorption spectra. Steady-state fluorescence spectra were recorded with a Perkin-Elmer LS-50B spectrofluorimeter, λ_{ex} = 310 and 8 nm slits unless otherwise stated. The instrumental response was corrected by means of a curve provided with the instrument. Fluorescence quantum yields were measured using quinine sulfate as a standard (0.546 in 0.5 M H_2SO_4) [12]. Spectral titration was carried out by introducing sulfuric acid or sodium hydroxide solution into the SNH solution of fixed concentration.

Fluorescence decays were obtained by using the time-correlated single-photon-counting method [13] with a PTI instrument. Excitation was made with a gated flash lamp filled with H_2 and fluorescence decays measurements were performed until a maximum of 10^4 counts. Data analysis was performed by a deconvolution

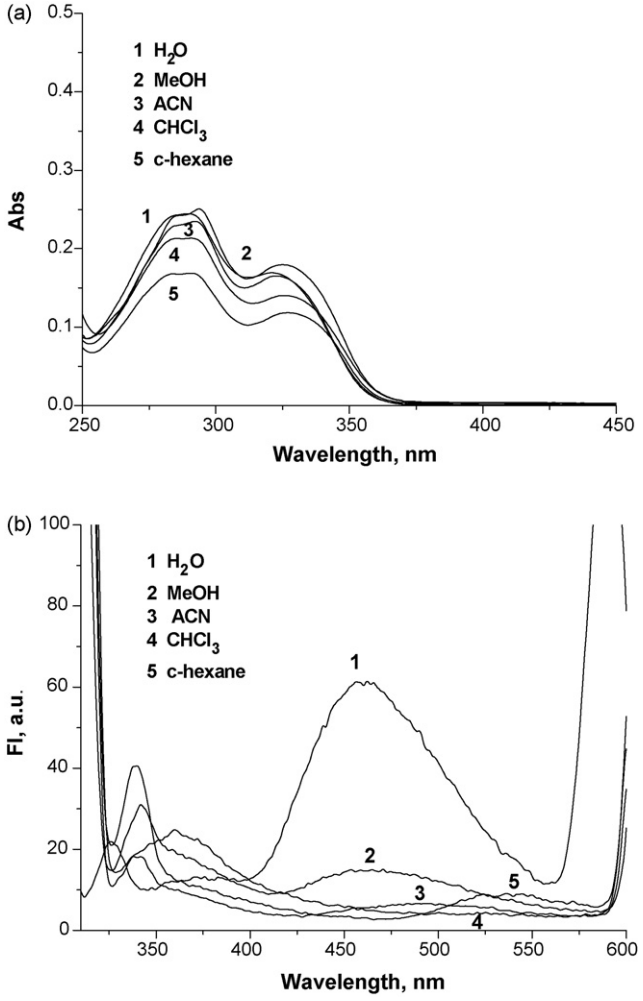


Fig. 1. Absorption (a) and fluorescence emission spectra (b) of SNH in different solvents.

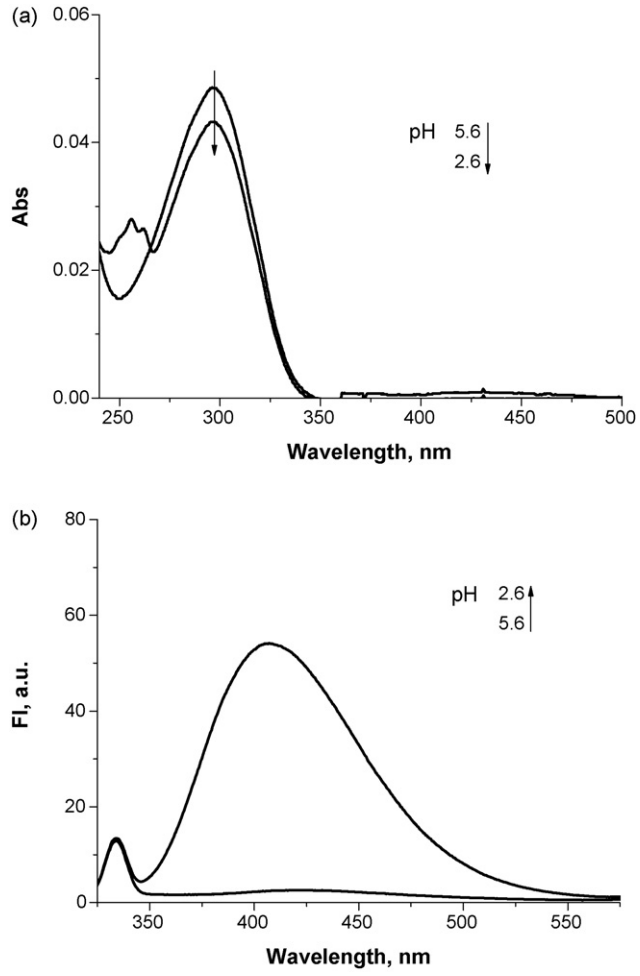
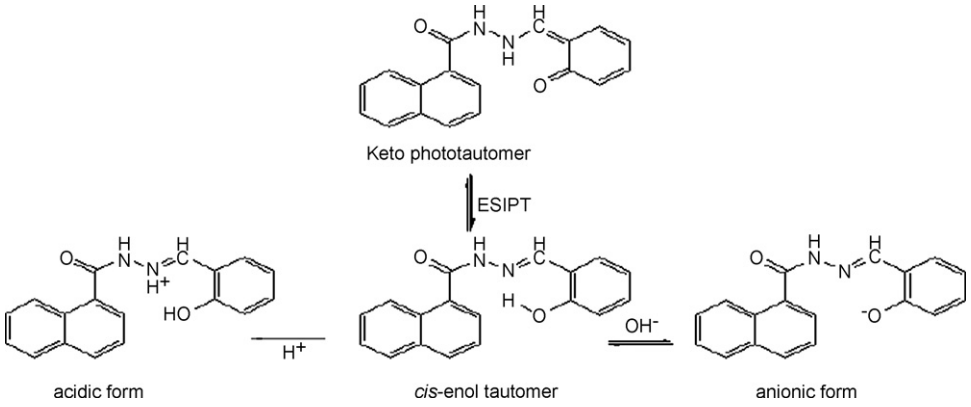


Fig. 2. Absorption (a) and fluorescence emission spectra (b) of BNH at pH 5.6 and 2.6.



Scheme 2.

method using a non-linear least-squares fitting program based on a Marquardt algorithm. The goodness of the fit was evaluated by statistical parameters such as reduced χ^2 and Durbin–Watson (DW) and graphical methods such as the autocorrelation function and weighted residuals.

Quartz cells with 1 cm path length were used.

3. Results and discussion

The absorption spectra of SNH in different solvents are shown in Fig. 1a. They are almost independent on solvent polarity, suggesting that the ground-state structure of SNH must be very similar in all solvents. For example, SNH exhibits two typical π–π^* absorption bands centered at λ_{em} = 288 nm ($\varepsilon = 2.08 \times 10^4\ M^{-1}\ cm^{-1}$) and 322 nm ($\varepsilon = 1.42 \times 10^4\ M^{-1}\ cm^{-1}$), respectively, in water. In the

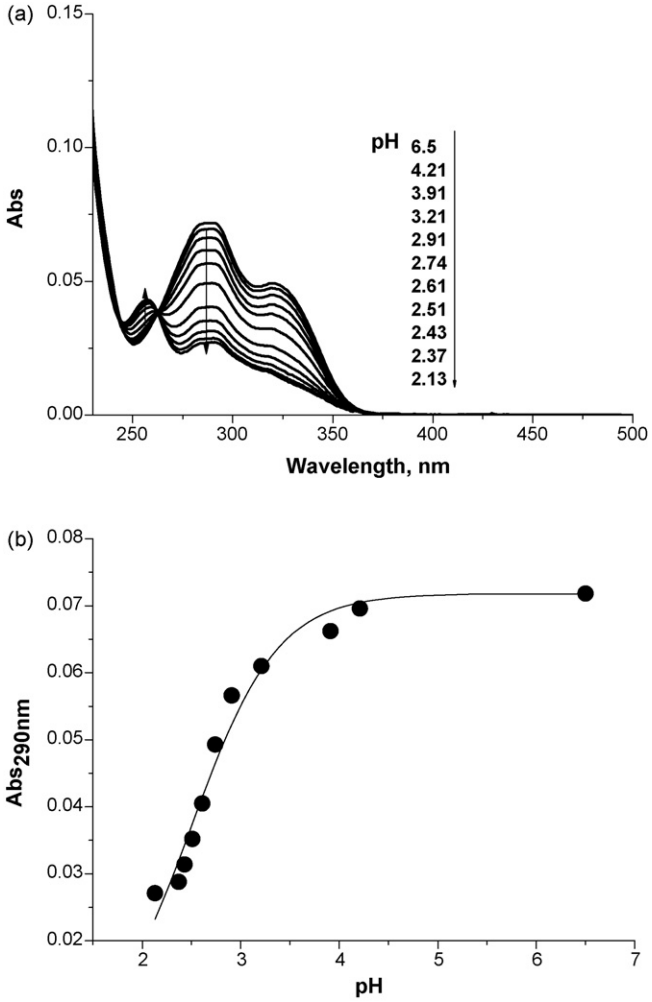


Fig. 3. (a) Absorption spectra of SNH ($c = 3.3 \times 10^{-6}$ mol/L) at different pH values in aqueous solution; (b) pH-dependence of the absorbance of SNH at 290 nm at various pH values.

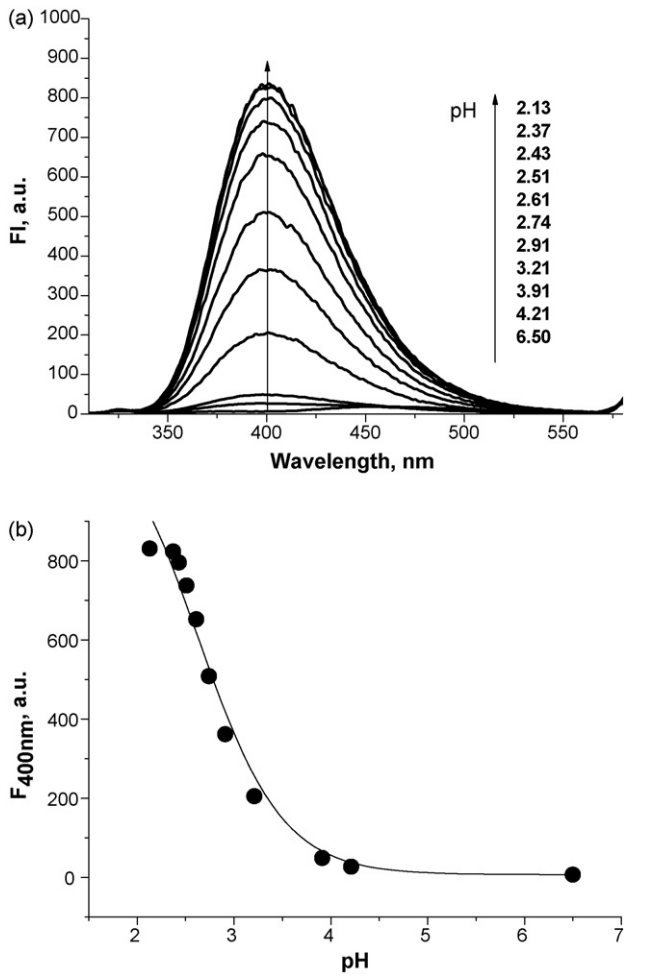


Fig. 4. (a) Fluorescence emission spectra of SNH ($c = 3.3 \times 10^{-6}$ mol/L) at different pH values in aqueous solution; (b) pH-dependence of fluorescence intensity of SNH at λ_{em} = 400 nm at various pH values.

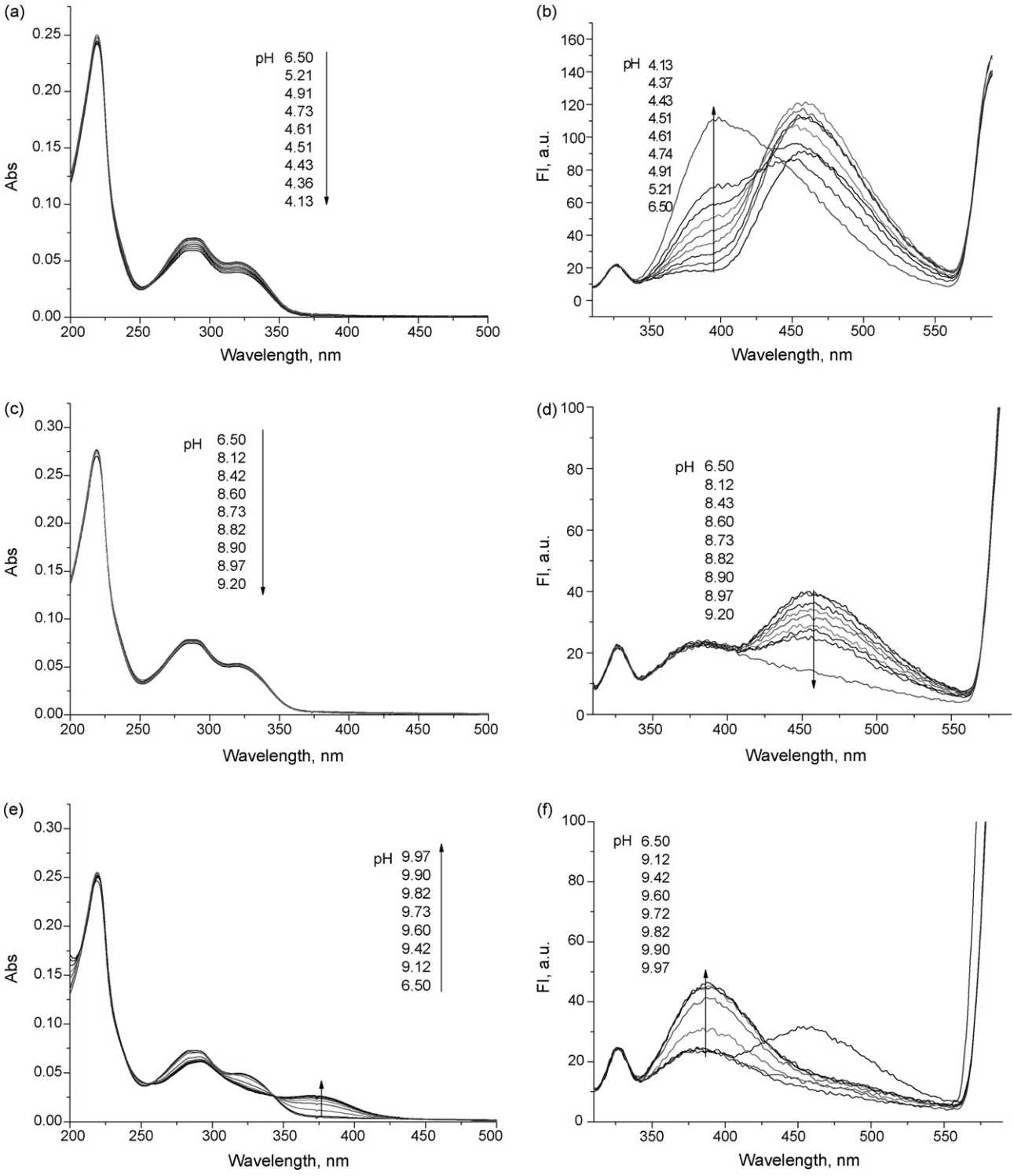


Fig. 5. Absorption (a, c and e) and fluorescence emission spectra (b, d and f) of SNH ($c = 4.2 \times 10^{-5}$ mol /L) at different pH values in ACN–H_2O (2:1, v/v) solution.

ground state, SNH should exist in a conformational equilibrium between the *cis*- and *trans*-enols, as reported in similar compounds [6]. The peak at $\lambda_{em} = 288$ nm is assigned to the *trans*-enol while that at $\lambda_{em} = 322$ nm should correspond to the *cis*-enol [6]. The control compound BNH which lacks the hydroxyl group only shows in the absorption spectra one band at $\lambda_{em} = 296$ nm in the same conditions Fig. 2a). This observation supported the assumption that SNH coexists as *cis*- and *trans*-enols in the ground state.

As shown in Fig. 1b, SNH showed very weak dual fluorescence ($\phi < 0.001$) in various solvents. The band at higher energy, at $\lambda_{em} = 380$ nm in water, was assigned to the emission from *trans*-enol tautomer. The large Stokes' shifted emission band at $\lambda_{em} = 460$ nm in water can presumably be attributed to the keto-tautomer formed in excited state from *cis*-enol tautomer (Scheme 2) [7]. These assignments are supported by the emission spectra of BNH which shows only one band at $\lambda_{em} = 407$ nm

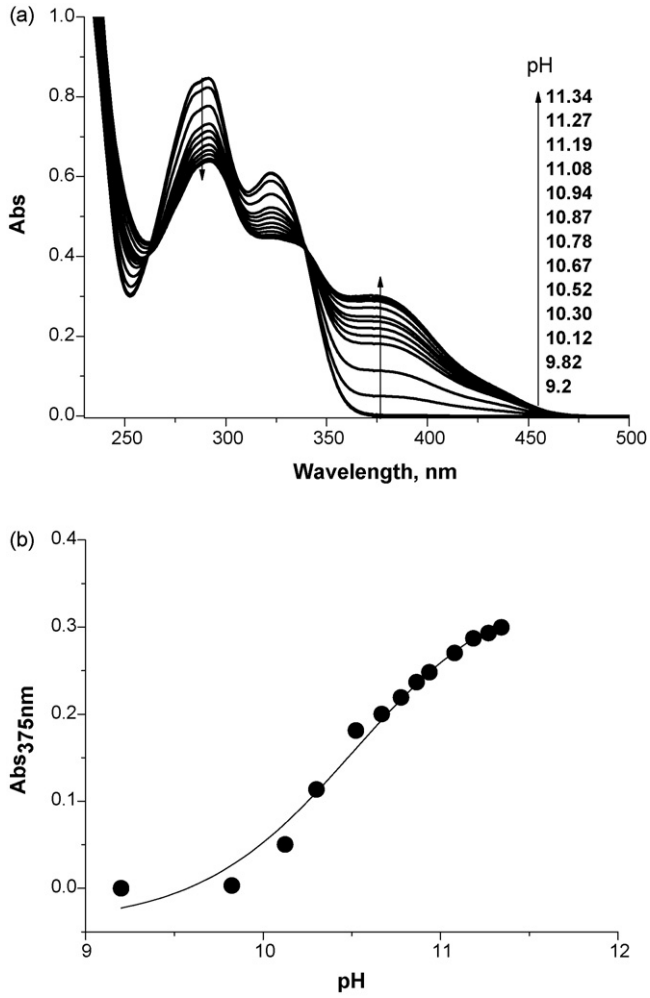


Fig. 6. (a) Absorption spectra of SNH ($c = 4.2 \times 10^{-5}$ mol/L) at different pH values in ACN–H_2O (2:1, v/v) solution; (b) pH-dependence of the absorbance of SNH at 375 nm at various pH values.

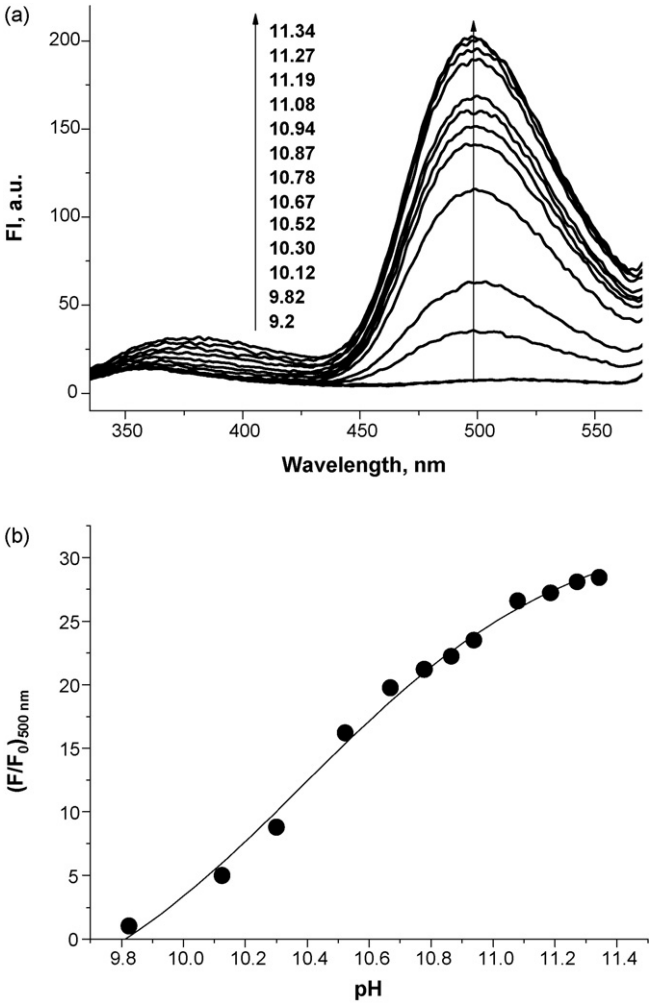


Fig. 7. (a) Fluorescence emission spectra of SNH ($c = 4.2 \times 10^{-5}$ mol/L) at different pH values in ACN–H_2O (2:1, v/v) solution; (b) pH-dependence of the fluorescence intensity ratio F/F_0 at $\lambda_{em} = 500$ nm of SNH at several pH values.

(Fig. 2b). The absence of the lower energy emission band in the fluorescence spectrum of BNH, indicates that this molecule can serve as a non proton-transfer model. Salicylaldehyde benzoylhydrazones, a family of compounds very similar to SNH, showed also dual fluorescence, but only the higher energy emission was detected when the hydroxyl group was derived into methoxyl where no keto-phototautomer can be formed (unpublished results). The longer wavelength emission of SNH undergoes a hypsochromic shift from $\lambda_{em} = 530$ nm in hexane to $\lambda_{em} = 460$ nm in water with increasing solvent polarity, indicating that the dipole moment of excited state is smaller than that of the ground state. This observation is consistent with various other ESIPT systems [14].

There are two functional groups in SNH, namely the basic imine and the acidic phenol moieties, which are sensitive to environmental pH. It can be expected that in excited state, the proton of the phenol group in SNH might transfer to the imine moiety and form a phototautomer which will emit a large Stokes' shifted fluorescence because the species formed is stable in the excited state but not in the ground state. Therefore, if the proton transfer process from the phenol group to the imine moiety is efficiently disrupted in basic media due to the formation of phenolate, the red-shifted tautomer emission will disappear giving rise to the phenolate fluorescence.

The effect of pH on the SNH absorption and fluorescence spectra was investigated altering the solution pH value Lowering the pH of pure water from 5.6 to 2, the absorbance of the original bands at $\lambda_{em} = 288$ and 320 nm decreased while a new band at $\lambda_{em} = 258$ nm is observed. A clear isosbestic point at $\lambda_{em} = 262$ nm is detected (Fig. 3a) which indicates that two species coexist in equilibrium. The changes observed should correspond to the acid form of SNH where the conjugation between both moieties, imine and phenol, is lost. From the titration curve, the pK_a obtained for the imine group in the ground state is 2.5, as shown in Fig. 3b. In these conditions, it was found that the fluorescence of SNH at $\lambda_{em} = 380$ nm enhanced dramatically while that at 460 nm decreases with the pH value of the solution, Fig. 4a. The intensity at $\lambda_{em} = 380$ nm enhanced 120-fold at pH 2.0 as compared to that at pH 6.5. The quantum yield of SNH was measured as 0.08 at pH 2.6, almost 80 times higher than that of SNH at pH 6.5. A single exponential decay with a lifetime of 4.94 ns was found at this pH, exciting at $\lambda_{em} = 302$ nm and collecting the emission at $\lambda_{em} = 400$ nm. Typical titration curves are depicted in Fig. 4b. It was estimated that the pK_a of imine moiety is 2.6 according to equation:

$$\mathrm{pH} = \mathrm{p}K_a + \frac{I - I_A}{I_B - I}$$

where I is the fluorescence intensity at a given wavelength, I_A and I_B are the fluorescence intensities measured at the same wavelength when the indicator is only in the acidic or only in the basic form, respectively [4].

In neutral or basic solution, the photo induced electron transfer (PET) process [15] from the lone pair electron on the imine N atom to the fluorophore (naphthalene) in excited state quenches the fluorescence at λ_{em} = 380 nm. When the lone electron pair of imine N is blocked by the proton, there is a fluorescence enhancement. The decreased fluorescence at λ_{em} = 460 nm can be interpreted as follows: the keto-photoautomer corresponding to the emission at 460 nm cannot be formed because the proton of phenol moiety in excited state cannot migrate to the neighbor N atom of the imine group which is protonated in acidic conditions.

Since the fluorescence quantum yields are very low at higher pHs, it was necessary to increase the concentration of SNH in order to follow the spectral changes. In this pH range it was necessary to use ACN–H_2O (2:1, v/v) binary solvent mixture. The absorption spectra of SNH was almost invariant in the pH range 5–9. In the same pH region, the fluorescence intensity at λ_{em} = 380 nm hardly changed while that at λ_{em} = 460 nm increased in acidic media and decreased in basic media, as shown in Fig. 5a–d. As the pH values are increased from 9 to 10, the absorbance at λ_{em} = 288 and 320 nm decreased concomitantly with a new band increase at λ_{em} = 375 nm due to the phenolate formation. There are two clear isosbestic points at λ_{em} = 264 and 338 nm, respectively. Meanwhile, the fluorescence intensity at λ_{em} = 460 nm decreases concomitantly with the increasing fluorescence intensity at λ_{em} = 380 nm and a shoulder at λ_{em} = 500 nm (Fig. 5e and f). The emission at λ_{em} = 500 nm can be assigned to the phenolate anion formed in basic media (Scheme 2).

It should be noted that the fluorescence of phenolate anion at λ_{em} = 500 nm in neat water solution is too weak to determine the pK_a by fluorescence titration. The fluorescence decays are rather complex and clearly show biexponential kinetics both in the blue and in the green region (unpublished results). Some fluorescence chemosensors which include the phenol group show enhanced fluorescence upon addition of anion in organic solvent due to the formation of phenolate, such as in acetonitrile (indeed, we have observed enhanced fluorescence of SNH in acetonitrile upon addition of fluoride) [10c,16]. Therefore, ACN–H_2O (2:1, v/v) binary solvent mixture was chosen to investigate the effect of hydroxide on the fluorescence of SNH. Similarly to the absorption spectra in the neat water solution, as pH values increased from 9 to 11, a new band increase at λ_{em} = 375 nm was observed with decreasing the absorbance at λ_{em} = 288 and 320 nm (Fig. 6a). From the titration curve, the pK_a of phenol group was estimated as 10.4 in ACN–H_2O (2:1, v/v) mixtures (Fig. 6b). As expected, the emission of phenolate at λ_{em} = 500 nm can be observed obviously with increasing the concentration of hydroxide to the solution of SNH in ACN–H_2O (2:1, v/v) mixtures, as shown in Fig. 7a. The intensity at λ_{em} = 500 nm enhanced 30-fold (ϕ_f = 0.02) and the pK_a of phenol group was estimated as 10.3, Fig. 7b.

4. Conclusions

A new absorption and fluorescence pH sensor based on 2-hydroxybenzaldehyde 1-naphthoyl hydrazone have been prepared and investigated. Due to two functional groups in the molecule, this sensor has the ability to respond to both low and high pH values at different wavelengths. The mechanism of the probe's fluorescence response to pH is complex due to the influence of protonation (imine group)/deprotonation (phenol group).

Acknowledgements

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