A Highly Selective and Colorimetric Fluorescent Probe for Hydrazine Detection in Water Samples

Hao WANG,* Xiaoming WU,* Feiyian TAO,** Shaoxiang YANG,*† Hongyu TIAN,* Yongguo LIU,* and Baoguo SUN*

*Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Key Laboratory of Flavor Chemistry, Beijing Technology and Business University, No. 11 Fucheng Road, Haidian District, Beijing 100048, P. R. China
**Research and Development Centre, China Tobacco Sichuan Industrial Co., Ltd., Chengdu 610066, Sichuan, P. R. China

An analytical and practical fluorescent probe (probe 1) was developed to detect hydrazine (N$_2$H$_4$) concentration in real water samples. As different concentrations of N$_2$H$_4$ were added, the color of the probe solution was graded gradually from colorless to pink, which could be observed by the naked eye under UV light at 365 nm. Our research indicates that probe 1 offers a certain practical significance for use as a visible detection agent to detect N$_2$H$_4$ efficiently by distinct color response. Furthermore, our work showed that probe 1 can be successfully applied to detect N$_2$H$_4$ concentrations in real water samples.

Keywords: Fluorescent probe, hydrazine, Michael addition reaction, water

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Introduction

Hydrazine (N$_2$H$_4$) is a strong reducing agent, and plays a major role in chemical, agricultural, pharmaceutical, and military industries. It is used to scavenge oxygen in steam boilers. Due to its high density, N$_2$H$_4$ is widely used in rocket and missile propellants. N$_2$H$_4$ has displayed potentiality in new material fields, including those for fuel cells and chemical blowing agents. However, N$_2$H$_4$ is a water-soluble compound and liable to cause water pollution. As N$_2$H$_4$ can be absorbed by the lungs and skin, it has highly toxic, mutagenic and carcinogenic effects on human health, and can cause serious damage to the lungs, central nervous system, liver and kidneys. So, the acceptable threshold limit value of N$_2$H$_4$ in drinking water is restricted to below 0.02 mg/L in China. The World Health Organization and United States Environmental Protection Agency recommends a threshold limit value of 10 ppb. Although many kinds of fluorescent probes have been applied to detect N$_2$H$_4$, the development of an efficient and visible colorimetric probe to detect N$_2$H$_4$ in water is badly needed. In our previous studies, one probe was developed to detect the level of N$_2$H$_4$ in water. In recent years, several traditional methods have been applied to detect N$_2$H$_4$, including gas chromatography, ultraviolet spectrometry, high-performance liquid chromatography, titration, surface-enhanced Raman spectroscopy and electrochemical methods. However, most of these methods often require time consuming test steps and come with high costs. The fluorescent probe is a sensitive and reliable tool because of its high sensitivity, selectivity, easy operation, real-time detection and convenience of application. In recent years, numerous fluorescent probes for N$_2$H$_4$ detection have been developed based on condensation of aldehyde, deprotection of levulinoyl acetyl or ester groups, transformation from malononitrile to imine and hydrogen bonding. Although many kinds of fluorescent probes have been applied to detect N$_2$H$_4$, the development of an efficient and visible colorimetric probe to detect N$_2$H$_4$ in water is badly needed. In our previous studies, one probe was developed to detect the level of N$_2$H$_4$ in water, but the visible colorimetric changes were not obvious. In order to find a visible colorimetric fluorescent probe, a new probe (E)-3-(6-hydroxynaphthalen-2-yl)-1-(4-nitrophenyl) prop-2-en-1-one (probe 1) was developed based on the Michael reaction in this work, with naphthalene as the fluorophore, and α, β-unsaturated ketone as the Michael acceptor. Probe 1 shows sensitive and visible colorimetric measurements for N$_2$H$_4$ detection by the naked eye under UV light. Especially, the solution of probe 1 showed different colors at different N$_2$H$_4$ concentrations. Probe 1 could be used as a sensor to determine N$_2$H$_4$ levels in water with good recovery.

Experimental

Reagents and chemicals

The chemicals 2-bromo-4'-nitroacetophenone (99%), trimethylamine (99%), triphenylphosphine (99%), and 6-hydroxy-2-naphthaldehyde (98%) were obtained from TCI (Shanghai) Development Co., Ltd. (Shanghai, P. R. China). The analytes sodium sulfate (Na$_2$SO$_4$), sodium chloride (NaCl), magnesium chloride (MgCl$_2$), sodium thiosulfate (Na$_2$SO$_3$), calcium chloride (CaCl$_2$), potassium chloride (KCl), sodium fluoride (NaF), hydrogen peroxide (H$_2$O$_2$), sodium bromide...
(NaBr), potassium iodide (KI), piperazine, triethylamine, L-tryptophan, L-serine, ammonium hydroxide, aniline, ethylenediamine, isonicotinic acid hydrazide, imethyl sulfoxide (DMSO) and tetrahydrofuran (THF) were purchased from Aladdin Industrial Corporation (Shanghai, P. R. China).

**Apparatus**

NMR spectra were obtained on a Bruker AV 300 MHz NMR (1H NMR at 300 MHz, 13C NMR at 75 MHz) and TMS was used as an internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. HRMS was carried out on a Bullet Ke Apex IV FTMS. Fluorescence spectra were observed and recorded on a Ri Li F-4600 fluorescence spectrometer. UV-Vis spectra were recorded on a Shimadzu UV-2600 spectrometer.

**Preparation of probe 1**

1. (E)-3-(6-Hydroxynaphthalen-2-yl)-1-(4-nitrophenyl) prop-2-en-1-one. First, 2-bromo-4′-nitroacetophenone (compound 1; 4.88 g, 20 mmol) and triphenylphosphine (compound 2; 5.24 g, 20 mmol) were dissolved in tetrahydrofuran (50 mL). The mixture was heated to reflux for 4 h, and cooled to 25°C. NaOH (2 M, 20 mL), methanol (24 mL) and H2O (28 mL) were added. The mixture was stirred and after keeping at 25°C overnight, compound 4 (yellow solid) was obtained by filtration. Then, compound 4 (1.49 g, 3.48 mmol) and 6-hydroxy-2-naphthaldehyde (compound 5; 0.49 g, 2.9 mmol) were dissolved in tetrahydrofuran (25 mL), refluxed for 14 h, distilled and recrystallized from CHCl3 to obtain probe 1 (orange red solid), yield 38.77% (Scheme 1).

**Preparation of analytes and probe 1**

Probe 1 solution (1 mM) was prepared by DMSO. Analytes Na2SO4, NaCl, MgCl2, Na2SO3, CaCl2, KCl, NaF, H2O2, NaBr, KI, piperazine, triethylamine, L-tryptophan, L-serine, ammonium hydroxide, aniline, and ethylenediamine were dissolved with distilled water prepare 10 mM aqueous solutions. Various concentrations could be obtained by using distilled water to dilute the stock solutions and were used immediately.

**Preparation of sugar and red wine**

Mineral water (bottle, 500 mL) was purchased from Wumei supermarket (Beijing, P. R. China). The tap water was collected from the public water supply system in Beijing. Yellow River water was collected from the Yellow River and Weihai Sea water was collected from Weihai (Weihai, P. R. China). Different concentrations of N2H4 were added, and the 628 nm fluorescence signals of samples were recorded.

**The procedures of N2H4 determination**

The preparation of the test system: 0.02 mL probe solution was dissolved in 0.48 mL DMSO, and added to the ion solution, then the volume way made to 2 mL in a cuvette with phosphate buffer solution (PBS, pH 7.4). The solution was mixed to test the spectrum. Fluorescence spectrophotometer parameters: λex/λem = 399/628 nm, slit width: 10 nm, 20 nm, voltage: 700 V, sensitivity: 2, temperature: 37°C.

**Results and Discussion**

Probe 1 was synthesized through the reaction of 6-hydroxy-2-naphthaldehyde (compound 5) with the Wittig reagent (compound 4) prepared from 2-bromo-1-(4-nitrophenyl) ethanone (compound 1) and triphenylphosphine (compound 2). The Wittig reaction is a classical organic synthetic reaction with mild reaction condition and high selectivity. This synthetic process and purification were simple. 1H NMR, 13C NMR, and HRMS (Figs. S1 – S3, Supporting Information) were used to...
determine the structure of \((E)-3-(6\text{-hydroxynaphthalen-2-yl})-1-(4\text{-nitrophenyl})\) prop-2-en-1-one (probe 1).

The fluorescence response of probe 1 (10 \(\mu\)M) to \(N_2H_4\) was firstly verified in DMSO–H\(_2\)O (v:v = 1:3, pH 7.4; 10 mM PBS buffer) at 37°C. As shown in Fig. 1a, after 200 \(\mu\)M \(N_2H_4\) was added, the fluorescence intensity was detected at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 75 min. The fluorescence intensity at 628 nm continued to increase up to 65 min. The results showed that probe 1 has quite a good response to \(N_2H_4\) in solution of DMSO–H\(_2\)O (v:v = 3:1) and the reaction time was 65 min.

The fluorescent intensity of probe 1 (10 \(\mu\)M) and probe 1–\(N_2H_4\) (\(N_2H_4\), 500 \(\mu\)M) in different pH was also examined (Fig. 1b). The data suggest that the fluorescent intensity of probe 1 did not change in a wide pH range from 3.0 to 8.0. But probe 1 showed instability from 8.0 to 10.0. As \(N_2H_4\) was added, the fluorescent intensity showed a small increase from pH 3.0 to 6.0 and increased substantially from 6.0 to 8.0, then decrease quickly from 8.0 to 10.0. So, the probe was extremely unstable under pH 8.0 to 10.0. The fluorescence intensity difference between probe 1 and probe 1–\(N_2H_4\) appeared largest in the pH range 7.4 – 8.0. All results indicated that the pH of 7.4 is the optimal condition for further research.

The fluorescence responses of probe 1 (10 \(\mu\)M) and probe 1 with different concentrations of \(N_2H_4\) were evaluated (at pH 7.4, 37°C). As shown in Fig. 2a, free probe 1 has weak fluorescence intensity, but the fluorescence intensity at 628 nm (excitation at 399 nm) increased with the addition of \(N_2H_4\). The large Stokes shift (229 nm) may derive from the ESIPT process. The quantum yield of probe 1 was calculated to be 0.0005 and probe 1–\(N_2H_4\) (1000 \(\mu\)M) was 0.0194, the quantum yield increased 38.8 times (Experiment Section in Supporting Information). As shown in Fig. 2b, upon incremental additions of \(N_2H_4\) (50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 \(\mu\)M), the color of the probe solution was graded gradually from colorless to pink, which could be observed by the naked eye under UV light at 365 nm. This result indicated that the probe 1 can be developed as a sensor for \(N_2H_4\) detection.

At the same time, the fluorescence titrations of probe 1 under a range of \(N_2H_4\) concentrations were measured. As shown in Fig. 3a, as the amount of \(N_2H_4\) increased from 0 to 1 mM, the fluorescence intensity at 628 nm increased. The fluorescence intensity changed tremendously as the amount of \(N_2H_4\) increased from 0.45 to 0.5 mM (Fig. 3b). In addition, the fluorescence intensity at 628 nm exhibited an excellent linear function with the concentrations of \(N_2H_4\) from 0 to 0.45 mM with a correlation coefficient of \(R^2 = 0.9961\) (Fig. 3c) and 0.45 to 0.70 mM with a correlation coefficient of \(R^2 = 0.9980\) (Fig. 3d). The detection limit (LOD) of probe 1 for \(N_2H_4\) was 19 nM, based on \(C_{\text{in}} = 3SD/B\), as defined by IUPAC. These results suggest that probe 1 was highly sensitive to \(N_2H_4\) and useful for quantitative detection of \(N_2H_4\) in the two intervals.

Selectivity and competition are important indexes for a fluorescent probe. To verify the selectivity of probe 1 for \(N_2H_4\), a series of competitive species including \(Na^+\), \(Mg^{2+}\), \(K^+\), \(Ca^{2+}\), \(F^-\), \(Cl^-\), \(Br^-\), \(I^-\), \(SO_3^{2-}\), \(SO_4^{2-}\), \(H_2O_2\), piperazine, triethylamine, L-tryptophan, L-serine, ammonium hydroxide, aniline, ethylenediamine and isonicotinic acid hydrazide were investigated. As shown in Fig. 4, the competitive analytes did not caused any obvious fluorescence increase. At the same time,
competition experiments were conducted by adding N₂H₄ to the solutions containing the above competitors. The fluorescent response of the probe shows that the fluorescence intensity did not change toward N₂H₄ and N₂H₄ + competitor. It clearly indicated that these competitors, especially ammonium hydroxide, aniline, ethylenediamine and isonicotinic acid hydrazide, did not interfere with N₂H₄ detection. This result suggests that probe \(1\) has high selectivity for N₂H₄ and could be used for N₂H₄ detection in a complex environment.

The sensing mechanism may be attributed to the Michael addition reaction of N₂H₄ with the \(\alpha, \beta\)-unsaturated ketone. To evidence the reaction mechanism of probe \(1\) with N₂H₄, the reaction product probe \(1\)-N₂H₄ was verified by 1H NMR (Fig. S4, Supporting Information). After N₂H₄ was added into the solution of probe \(1\), the 1H peaks at 3.00, 3.55 ppm were assigned to the C–CH₂–C=N group and peaks at 5.08 ppm were assigned to the Naphth–CH–N group. The result is implemented by the addition-cyclization of the activated \(\alpha, \beta\)-unsaturated ketone triggered by N₂H₄ and give the formation of compound probe-N₂H₄. The formation of probe \(1\)-N₂H₄ was further confirmed via HRMS (Fig. S5, Supporting Information), where

![Fluorescence intensity change of probe 1 (10 μM) on addition of various species (300 μM for each. 1, blank; 2, Na⁺; 3, Mg²⁺; 4, K⁺; 5, Ca²⁺; 6, F⁻; 7, Cl⁻; 8, Br⁻; 9, I⁻; 10, SO₃²⁻; 11, SO₄²⁻; 12, H₂O₂; 13, piperazine; 14, triethylamine; 15, l-tryptophan; 16, l-serine; 17, ammonium hydroxide; 18, aniline; 19, ethylenediamine; 20, isonicotinic acid hydrazide. 300 μM for N₂H₄). The test was repeated 3 times.](image)

### Table 1 Determination of the N₂H₄ concentrations in real water samples

| Sample            | N₂H₄ level found/μmol | Added/μmol | Found/μmol | Recovery, % | s (μmol) |
|-------------------|-----------------------|------------|------------|-------------|---------|
| Weihai Sea        | 0.20                  | 200        | 197.8      | 98.90       | 0.003   |
| water             | 0.00                  | 300        | 302.0      | 100.67      | 0.001   |
| Yellow River      | 0.00                  | 200        | 200.2      | 100.10      | 0.002   |
| water             | 0.00                  | 300        | 300.2      | 100.07      | 0.004   |
| Tap water         | 0.00                  | 200        | 199.9      | 99.95       | 0.001   |
| Mineral water     | 0.00                  | 300        | 300.1      | 100.03      | 0.002   |

The sensing mechanism may be attributed to the Michael addition reaction of N₂H₄ with the \(\alpha, \beta\)-unsaturated ketone. To evidence the reaction mechanism of probe \(1\) with N₂H₄, the reaction product probe \(1\)-N₂H₄ was verified by ¹H NMR (Fig. S4, Supporting Information). After N₂H₄ was added into the solution of probe \(1\), the ¹H peaks at 3.00, 3.55 ppm were assigned to the C–CH₂–C=N group and peaks at 5.08 ppm were assigned to the Naphth-CH-N group. The result is implemented by the addition-cyclization of the activated \(\alpha, \beta\)-unsaturated ketone triggered by N₂H₄ and give the formation of compound probe-N₂H₄. The formation of probe \(1\)-N₂H₄ was further confirmed via HRMS (Fig. S5, Supporting Information), where
To prove that probe 1 can be accurate for detecting N2H4, the concentrations (50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 μM) were added. The fluorescence intensity of all these samples were investigated at 628 nm. As shown in Table 1, 2, 20 and 0.20 μM N2H4 were obtained from Weihai Sea and Yellow River water samples, and zero μM N2H4 from tap water and mineral water samples. Probe 1 thus can be detected N2H4 concentration in complex water samples, and the recovery ranged from 98.90 - 100.67% for all real water samples. Our studies showed that probe 1 has good practicability to detect N2H4 levels in real water samples.

To prove that probe 1 can be accurate for detecting N2H4, the concentration of N2H4 in real water samples were used the hygienic standard for hydrazine in water sources (Table S1, Supporting Information). The excellent agreement between the two methods showed the reliability of probe 1. All of the results showed that our proposed probe is feasible and practical for the determination of N2H4 in real water samples.

Many N2H4 fluorescent probes have been designed and used for biological imaging and N2H4 detection in water samples, but visible colorimetric fluorescent probes are rare. In this work, probe 1 exhibited different color changes to different concentrations (50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 μM) of N2H4. The solution of the probe showed different color changes to different concentrations of N2H4 graded gradually from colorless to pink, which could be observed by the naked eye under UV light at 365 nm. Our research indicated that probe 1 has a certain practical significance as a sensor for N2H4 detection in real water samples. Furthermore, probe 1 was successfully used to detect N2H4 concentrations in real water samples.

Conclusions

In summary, a sensitive and visible colorimetric fluorescent probe was developed to detect N2H4 concentration in real water samples. The function of probe 1 relies on the Michael reaction of N2H4 with the α, β-unsaturated ketone, this was verified by 1H NMR, HRMS and 13C NMR studies. When different concentrations of N2H4 were added, the color of the probe solution was graded gradually from colorless to pink, which could be observed by the naked eye under UV light at 365 nm. It indicates that probe 1 has a certain practical significance as a sensor for N2H4 detection in real water samples. Furthermore, our work showed that probe 1 could be successfully applied to detect N2H4 concentrations in real water samples.

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Supporting Information

1H NMR, 13C NMR and HRMS spectra of probe 1; 1H NMR and HRMS spectra of probe 1-N2H4; the detection of water samples by hygienic standard for hydrazine in water sources method. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/

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