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ORIGINAL ARTICLE

An Acid-Inert Fluorescent Probe for the Detection of Nitrite

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Abstract The fluorescent detection of nitrite ions has been challenging owing to the poor acid-stability of typical fluorescent probes. Herein, an acid-inert rhodamine-based fluorescent probe has been developed for the fast detection of trace amount of nitrite ions with turn-on mode. The detection limit of nitrite ions was determined to be 9.4 nM (S/*N* = 3) and the linear range with high linear correlation was observed between 0.025 to 2.5 μM. A test paper method was developed for rapid visual detection of nitrite ions. Quantitative analysis of two real water samples also confirmed the reliability and sensitivity of the developed fluorescent method.

Keywords Nitrite · Silicon · Rhodamine · Fluorescence enhancement

Introduction

Nitrite (NO_2^-) ion, commonly detected in water and food, has proven to be a great threat to human health [1–4]. Excessive intake of NO_2^- with water or food can lead to a variety of diseases, such as intrauterine growth restriction, spontaneous abortions, infant methemoglobinemia and birth defects in central nervous system [5–7]. Additionally, in acidic environment, NO_2^- play the role as a reaction substrate for the generation of highly carcinogenic N-nitrosamine compounds [8].

Considering the potential toxicity and hazards of NO_2^-, a simple and sensitive detection of NO_2^- level in water and food is highly demanded. Ion chromatography, capillary electrophoresis, colorimetry and electrochemistry are conventionally used for the detection of NO_2^- [3, 9–13]. However, these aforementioned methods are often limited by complicated procedures or low selectivity. In comparison, design of fluorescent probes for NO_2^- has already attracted great attention owing to the high sensitivity and simplicity. So far, several fluorescent probes have been developed for the detection of nitrite [4, 14–21]. These probes are typically based on the diazotization of amine, which requires strong acidic condition. In particular, rhodamine-based fluorescent probes for NO_2^- are especially preferred because of their remarkable chromogenic and fluorogenic responses [14, 15]. Triggered by NO_2^-, diazotization of amine induces the ring-opening of spirolactam in the probe, thereby restoring the absorption and emission. However, most of the developed fluorescent probes are sensitive to H^+, and the rhodamine-based probe is not an exception. In acidic condition, the rhodamine based probes go through a H^+-induced ring-opening process, giving undesired background signals, which leads to the loss of sensitivity and false positive response to NO_2^-. To eliminate these H^+-induced side effects, the detections of NO_2^- are usually restricted to low temperature or extra addition of base [14, 15].

Recently, we have found that the spiroring in Si-rhodamine can tolerate a relatively strong acidic condition, existing as the non-fluorescent spirocyclic form over a wide pH range [22–25]. The stability of the spirolactam suppresses the background signal, thereby providing a promising platform for

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fabricating acid-inert probes with high sensitivity. Herein, we developed a Si-rhodamine-based probe **SiRB-Nitrite** for the detection of NO_2^- (Scheme 1). Under acidic condition with nitrite ions, probe readily undergoes diazotization, resulting in a ring-opening process with remarkable chromogenic and fluorogenic responses. The acid-stability has been further confirmed by comparing the optical properties with its rhodamine analogous **RB-Nitrite** (Scheme 1). Owing to the selectivity and sensitivity, probe **SiRB-Nitrite** has been applied in monitoring NO_2^- level in water.

Materials and Methods

Materials

All reagents and solvents were of the best grade available. Methanol, phosphorus oxychloride, 1,2-dichloroethane, acetonitrile, ethyl acetate, petroleum ether and triethylamine were supplied by Shanghai Chemical Reagent Co. *o*-Diaminobenzene, rhodamine B, sodium nitrite and all salts used in interference experiment were supplied by Adamas-beta. Unless otherwise stated, all commercial reagents were used without additional purification. Solvents were dried according to standard procedures prior to use.

Instrument

All reactions were monitored by thin-layer chromatography (TLC) on gel F254 plates. Flash chromatography was carried out on silica gel (300–400 mesh). NMR spectra were recorded on a Bruker AC-300P spectrometer (300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C) or on a BrukerAC-600P spectrometer(600 MHz for ^{1}H NMR and at 150 MHz for ^{13}C NMR). Spectral data are reported in ppm relative to tetramethylsilane (TMS) as internal standard. High-resolution mass spectra (HRMS) were recorded on an Aglilent Technologies 6538 UHD Accurate-Mass Q-TOF MS spectrometer using ESI. All pH measurements were performed with a pH-3c digital pH-meter with a combined glass-calomel electrode. UV-visible spectra were obtained on a Analytikjena Specord 210 PLUS UV-vis spectrophotometer. Fluorescence spectroscopic studies were performed on a Hitachi F-7000.

Absorption and Fluorescence Analysis

Absorption spectra were obtained with 1.0-cm glass cells. Fluorescence emission spectra were obtained with a Xenon lamp and 1.0-cm quartz cells. The fluorescence intensity was measured at 685 nm for probes **SiRB-Nitrite**. The slit width was 5.0 nm for both excitation and emission of probe. The photomultiplier voltage was 600 V. Phosphate buffer solution at different pH was respectively added into DMSO (1%, *v*/v) solutions containing probe **SiRB-Nitrite** (250 μM).

Preparation of Colorimetric Test Paper

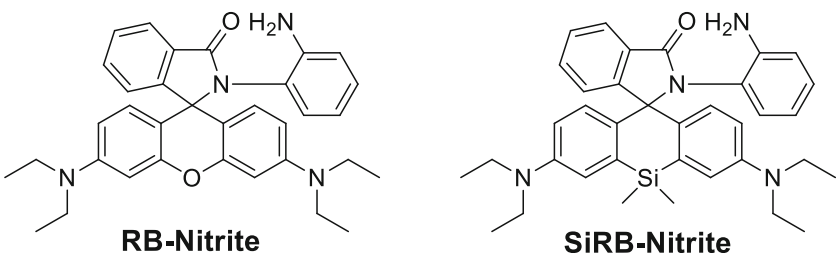
Colorimetric test paper was prepared by immersing strips of white filter paper (10 mm × 30 mm) into a methanol solution of **SiRB-Nitrite** (250 μM) for 30 min and then dried gently. The colorimetric test paper was stored in a vacuum desiccator. Before the usage, the colorimetric test paper was exposed to hydrogen chloride gas for 10 s. Then the paper was dipped into the aqueous solution of nitrite ions for 1 s and dried in air. Blue color appears in the dried colorimetric test paper.

Real Sample Pre-Treatment and Analysis

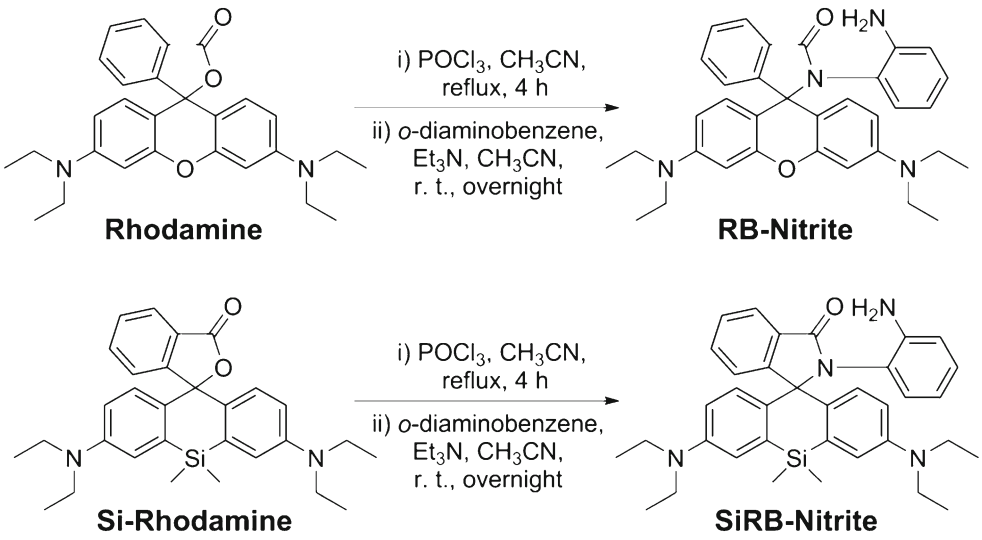
Water samples were taken from the tape water collected from lab and pond water from the university in Shanghai city. River water and tap water were analyzed after filtration and acidification (pH was adjusted to 2.0), which were spiked with NO_2^- at different concentration for the measurement of recovery.

Synthesis of Probes SiRB-Nitrite and RB-Nitrite

SiRB-Nitrite To a solution of compound **SiRB** (726 mg, 1.5 mmol) in dry 1,2-dichloroethane (10 mL) at room temperature, phosphorus oxychloride (690 mg, 4.5 mmol) was added dropwise over a period of 5 min. After being refluxed for 4 h, the reaction mixture was cooled and concentrated under vacuum to give a blue solid. This blue solid was further dissolved in dry acetonitrile (10.0 mL). The solution was slowly added to a solution of *o*-diaminobenzene (810 mg, 7.5 mmol) in dry



Scheme 1 Chemical structure of probe **SiRB-Nitrite** and its analogous **RB-Nitrite**



Scheme 2 Synthesis of probe **SiRB-Nitrite** and its analogous **RB-Nitrite**

acetonitrile (10 mL) containing triethylamine (5.0 mL). After stirring at room temperature overnight, the mixture was concentrated in vacuo and the crude product was purified by column chromatography on silica gel (ethyl acetate: petroleum ether = 1: 4) to give **SiRB-Nitrite** as a white solid (458 mg, 53% yield). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.03 (dd, J = 5.6, 2.9 Hz, 1H), 7.54 (m, 2H), 7.17 (d, J = 8.1 Hz, 1H), 6.92–6.86 (m, 1H), 6.51–6.58 (m,7H), 6.32 (t, J = 10.0 Hz, 1H), 5.80 (d, J = 7.7 Hz, 1H), 3.35 (d, J = 1.0 Hz, 8H), 1.16 (t, J = 7.0 Hz, 12H), 0.42 (s, 3H), −0.32 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.34, 154.95, 146.17, 145.50, 137.26, 132.69, 132.30,130.29, 128.87, 127.91, 124.24, 123.53, 122.02, 118.05, 116.76, 114.88, 114.01, 75.04, 53.42, 44.23, 12.45, −0.57, −1.70.

RB-Nitrite To a solution of compound rhodamine B (664 mg, 1.5 mmol) in dry 1,2-dichloroethane (10 mL) at room temperature, phosphorus oxychloride (690 mg, 4.5 mmol) was added dropwise over a period of 5 min. After being refluxed for 4 h, the reaction mixture was cooled and concentrated under vacuum to give a red solid. This blue solid was further dissolved in dry acetonitrile (10.0 mL). The solution was slowly added to a solution of *o*-diaminobenzene (810 mg, 7.5 mmol) in dry acetonitrile (10 mL) containing triethylamine (5.0 mL). After stirring at room temperature overnight, the mixture was concentrated in vacuo and the crude product was purified by column chromatography on silica gel (ethyl acetate: petroleum ether = 1: 4) to give **SiRB-Nitrite** as a light red solid (447 mg, 56% yield).^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (d, J = 6.9 Hz, 1H), 7.63–7.54 (m, 2H), 7.27 (s, 1H), 6.98 (t, J = 7.6 Hz, 1H), 6.69 (d, J = 8.5 Hz, 2H), 6.58 (d, J = 7.9 Hz, 1H), 6.53–6.18 (m, 5H), 6.12 (d, J = 7.8 Hz, 1H), 3.36 (d, J = 6.7 Hz, 8H), 1.18 (t, J = 6.7 Hz, 12H).^{13}C NMR (75 MHz, $CDCl_3$) δ 166.34, 153.84, 152.22, 148.73, 144.42, 132.60, 131.85, 128.63,128.71, 128.34, 124.21, 123.40, 122.02, 118.14, 116.93, 107.97, 97.96, 77.18,67.92, 44.42, 12.38.

Results and Discussion

Probe **SiRB-Nitrite** and its rhodamine analogous **RB-Nitrite** were synthesized according to reported procedures (Scheme 2) [25, 26]. Since the diazotization process requires

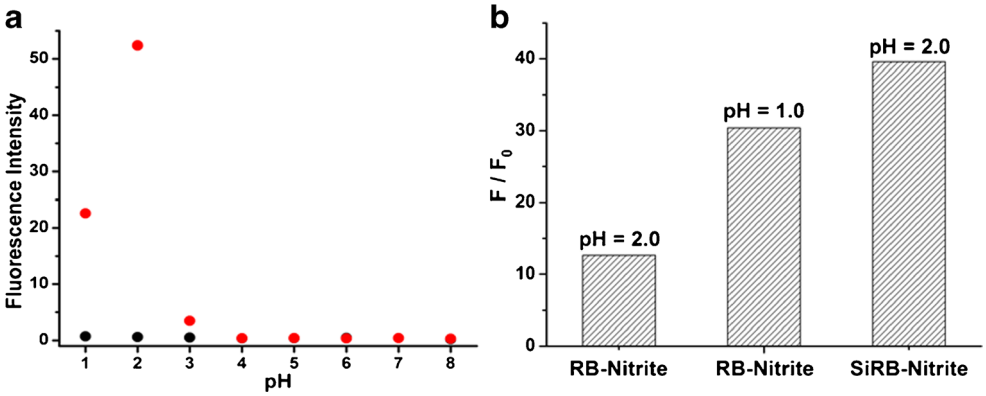


Fig. 1 **a** Effect of pH on the probe **SiRB-Nitrite** (2.5μM, *black circle*) and its fluorescent responses to 1.0 equiv. of NO_2^- (*red circle*). **b** The relative fluorescence intensity changes of probes **RB-Nitrite** (2.5μM) and **SiRB-Nitrite** (2.5μM) in the absence (F_0) or presence (F) of 1.0 equiv. of NO_2^- at pH = 2.0 and pH = 1.0, respectively

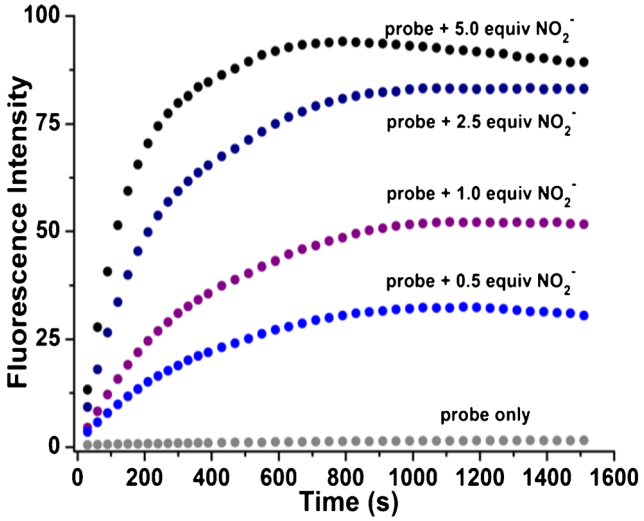


Fig. 2 Time-dependent fluorescence intensity changes of probe **SiRB-Nitrite** (2.5μM) upon addition of various concentrations of NO_2^- at pH = 2.0

acidic condition, the effect of pH on the probe **SiRB-Nitrite** and its fluorescent response to NO_2^- were evaluated in the pH range from 1.0 to 8.0. As illustrated in Fig. 1a, **SiRB-Nitrite** displays constant low background fluorescence within a pH range from 8.0 to 1.0, demonstrating that the probe can tolerate a relatively broad range of pH, even under strong acidic condition (pH = 1.0). In sharp contrast, the reference probe **RB-Nitrite** shows pH-dependent fluorescence enhancement under acidic conditions (Fig. S1and S2, Supporting Information), suggesting that the spirolactam in probe **RB-Nitrite** is sensitive to H^+, resulting in relatively high background fluorescence. The fluorescent response of probe **SiRB-Nitrite** to NO_2^- was carefully examined at a series of pH values. Upon the addition of 1.0 equiv. of NO_2^-, probe **SiRB-Nitrite** presented remarkable fluorescence enhancement with the increasing acidity of solution, and exhibited the best response performance to NO_2^- at pH = 2.0. The fluorescence enhancement of the probe started to decrease with further increasing of acidity.

The reference probe **RB-Nitrite** shared similar responses to 1.0 equiv. of NO_2^- at pH = 2.0 (Fig. S1, Supporting Information). However, the fluorescence enhancements in probe **RB-Nitrite** (pH = 2.0 and pH = 1.0) are both lower than that in probe **SiRB-Nitrite** (pH = 2.0) due to the high background fluorescence in acidic environment (Fig. 1b). Comparing with probe **RB-Nitrite**, it is more feasible to use probe **SiRB-Nitrite** to sensitively monitor NO_2^- without extra procedures.

At room temperature, probe **SiRB-Nitrite** can respond to NO_2^- in relatively short time. As shown in Fig. 2, after adding a certain amount of NO_2^- at pH = 2.0, the fluorescence emission at 685 nm remarkably increases within the first 10 min and reaches a plateau in about 15 min.

Under the optimal conditions, the absorption and fluorescence responses of probe **SiRB-Nitrite** with the titration of NO_2^- were examined. Free probe **SiRB-Nitrite** formed a colorless and non-fluorescent solution, indicating the predominant existence of the spirocyclic form. With the addition of

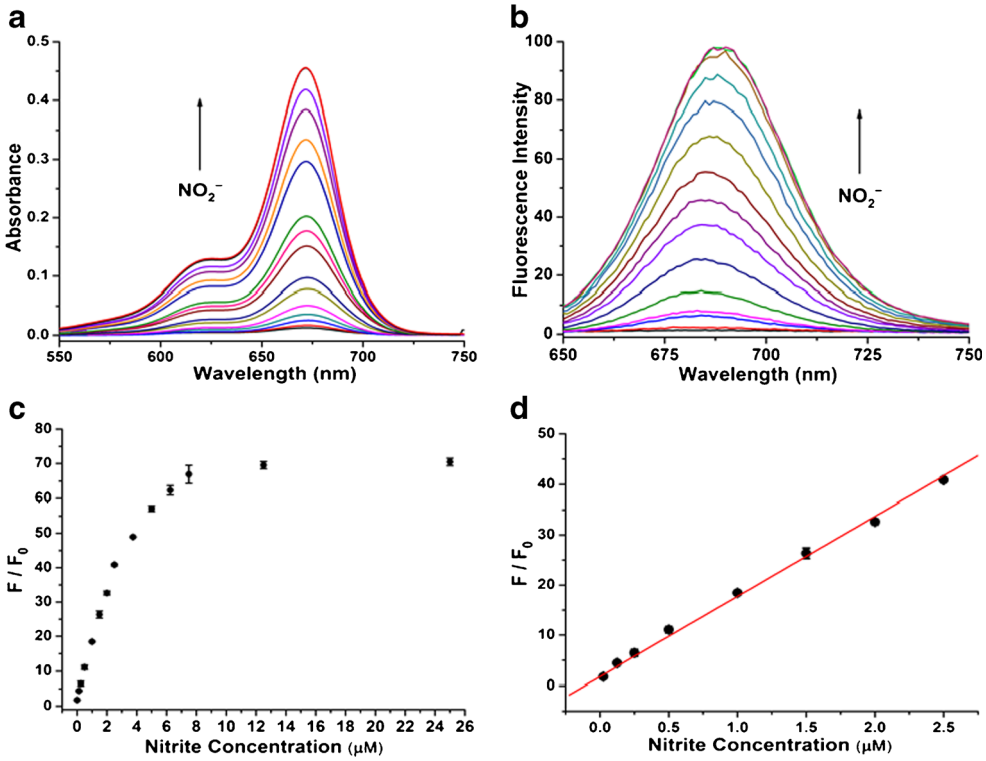


Fig. 3 **a** Absorption change of probe **SiRB-Nitrite** (2.5μM) with the increase of NO_2^- (0.0–25.0μM). **b** Emission change of probe **SiRB-Nitrite** (2.5 μM) with the increase of NO_2^- (0.0–25.0μM). **c** The relative fluorescence intensity (F/F_0) changes with the increase of NO_2^- (0.0–25.0μM). **d** Calibration curve for NO_2^- detection (0.025–2.5 μM). The excitation and emission wavelengths are 630 and 685 nm. Error bars indicate standard deviation for three replicates

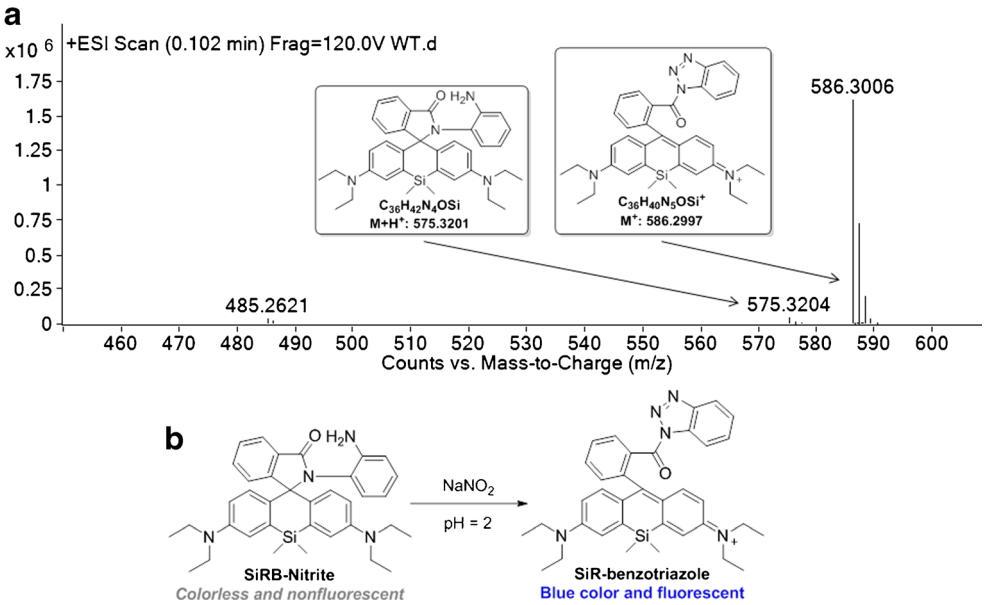


Fig. 4 **a** HRMS analysis of the reaction between probe **SiRB-Nitrite** and nitrite at pH = 2.0. **b** The proposed reaction mechanism of probe **SiRB-Nitrite** with NO_2^-

NO_2^- solution, dramatic color change (colorless to sky blue, Fig. S3, Supporting Information) was observed in the reaction mixture, companied with the change in both absorption and fluorescence emission. In absorption spectra, a sharp peak centered at 675 nm increased rapidly and another gently peak centered at 625 nm gradually increased (Fig. 3a). Concomitantly, a gradual enhancement in fluorescence intensity (λ_{em} = 685 nm) was noted with the increase of NO_2^- (Fig. 3b). The fluorescence enhancement slowed down and reached saturation with 5 equiv. of NO_2^- (Fig. 3c).

A good linear correlation (R^2 = 0.996) between the relative intensity of fluorescence enhancement and NO_2^- concentration from 0.025 to 2.5 μM was observed (Fig. 3d). The detection limit of probe **SiRB-Nitrite** to NO_2^- was determined to be 9.4 nM (S/*N* = 3), which is more sensitive than the maximum allowable level of contamination of nitrite ions in drinking water (1 ppm, 21.7 μM) defined by Environmental Protection Agency [27].

The reaction mechanism of **SiRB-Nitrite** with NO_2^- was revealed by high-resolution Mass Spectroscopy (HRMS) analysis. In the reaction mixture at pH = 2.0, a unique peak at m/z = 586.3006 was clearly found (Fig. 4a). The peak is likely belongs to the highly fluorescent compound **SiR-benzotriazole** (calc. 586.2997), which is the ring-opening product of the probe **SiRB-Nitrite** triggered by NO_2^- in acidic solution (Fig. 4b). We also confirmed the high chemical stability of compound **SiR-benzotriazole** under acidic condition, ensuring the reliability of the analytical application.

Many common cations, including Na^+, K^+, Ca^{2+}, Cu^{2+}, Fe^{3+}, Mg^{2+} and Zn^{2+}, and some common anions in water, such as AcO^-, NO_3^-, I^-, F^-, Cl^-, Br^-, CO_3^{2-} and $C_2O_4^{2-}$ show negligible interfere in the fluorescent emission even at 100-fold higher concentrations of these ions when compared to NO_2^-. In addition, the enhancement in fluorescence intensity resulting from the addition of NO_2^- was not influenced by the

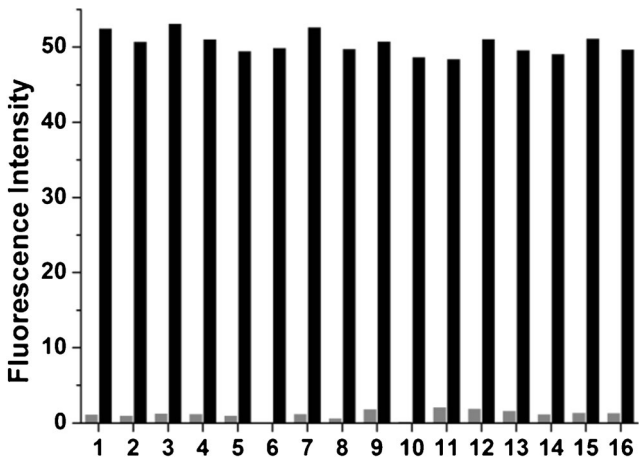


Fig. 5 Fluorescence responses of probe **SiRB-Nitrite** (2.5 μM) to various ions. Gray bars represent the fluorescence response of **SiRB-Nitrite** to the excess ion (250 μM) of interest. Black bars represent the subsequent addition of 2.5 μM NO_2^- to the solution. (1) probe; (2) Na^+; (3) K^+; (4) Ca^{2+}; (5) Cu^{2+}; (6) Fe^{3+}; (7) Mg^{2+}; (8) Zn^{2+}; (9) AcO^-; (10) NO_3^-; (11) I^-; (12) F^-; (13) Cl^-; (14) Br^-; (15) CO_3^{2-}; (16) $C_2O_4^{2-}$

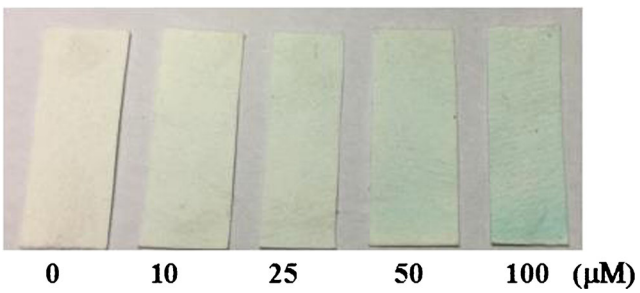


Fig. 6 Photographs of the test paper in the presence of NO_2^-

Table 1 Determination of nitrite ions in water with the proposed method

Samples	NO_2^- added (μM)	NO_2^- found[c] (μM)	Recovery (%)
Tap water[a]	0	-	
	0.30	0.31 ± 0.01	103.3
	0.60	0.62 ± 0.03	103.3
Pond water[b]	0	0.21 ± 0.02	
	0.30	0.52 ± 0.01	102.0
	0.60	0.79 ± 0.01	97.5

[a] Tap water was sampled from our laboratory

[b] Pond water was sampled from the lake in our university

[c] Average of three determinations

presence of other ions (Fig. 5). These results revealed the high selectivity of **SiRB-Nitrite** and thus is suitable for the detection of NO_2^-in complicated real samples.

The fluorescent probe **SiRB-Nitrite** can also work as a turn-on colorimetric probe: visual blue color was observed with the addition of nitrite ions into the solution of the probe (Fig. S3, Supporting Information). Herein, a turn-on colorimetric test paper was prepared for the qualitative analysis of NO_2^-. With the increasing of nitrite ions from 0 to 100 μM, the color of the test paper has vividly changed from pure white to sky blue (Fig. 6). The darkness of the color correlated to the concentration of NO_2^-. With the current concentration of probe, the visible detection limit of NO_2^- is in the range of 10 μM. This simple and convenient method can be useful for the fast on-site detection of NO_2^-.

The unique properties of the probe **SiRB-Nitrite**, including rapid response, high selectivity and good sensitivity for NO_2^-, especially acid-inertance, bring it qualify for complicated practical application. Two water samples, tape water collected from the lab and pond water from the university, were analyzed using this method. The concentration of NO_2^- in tape water was not determined and the amount of NO_2^- in pond water was quantitatively analyzed. Different known amount of NO_2^- solution was directly spiked into the two real samples, and the analysis results were summarized in Table 1. The average recoveries of NO_2^- were in the range of 97.5–103.3% for all the spiked samples with low relative standard deviation (1.0–3.0%).

Conclusion

In conclusion, we have developed an acid-inert fluorescent probe **SiRB-Nitrite** for simple, fast, highly sensitive and selective detection of NO_2^- (Table S1). A test paper is prepared for qualitative detection of NO_2^-. Quantitative analysis of two real water samples also confirmed the reliability of the developed fluorescent method.

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