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A reaction based turn-on type fluorogenic and chromogenic probe for the detection of trace amount of nitrite in water

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ABSTRACT

A turn-on fluorescent probe for the detection of nitrite ion in water is developed based on diazotization reaction of the amino group of the probe in an acidic solution (pH 1). The probe responds selectively to nitrite ion over various other anions with a turn-on type fluorogenic change from colorless to orange by the formation of rhodamine B via an analyte triggered fragmentation process. The fluorescence titration is complete within 1 h with 1 equivalent of nitrite ion. The probe is highly efficient, cost-effective and shows a detection limit of 4.6 ppb.

1. Introduction

An uncontrolled increase in the concentration of nitrite in groundwater, rivers and lakes is a growing threat to public health and the environment [1]. Nitrite is used as a fertilizing agent and preservative of perishable foods. It is also known to play a key physiological role in signaling, blood flow regulation and hypoxic nitric oxide homeostasis [2]. Monitoring of nitrite level in drinking water and food stuffs is of great importance as it reacts with dietary components in the stomach to produce carcinogenic nitrosamines [3]. Another potential danger with the nitrite ions is its ability to convert oxyhemoglobin into methemoglobin when present in the bloodstream and thereby interfering with oxygen transport in the blood [4]. Epidemiologic studies have revealed that elevated concentration of nitrate and nitrite ions in drinking water leads to a number of medical issues like spontaneous abortions, premature birth, intrauterine growth restriction and birth defects of the central nervous system [5–9]. The permitted level of nitrite content in water is just 1 ppm as recommended by the US Environmental Protection Agency (EPA) [10,11]. Therefore, sensitive and selective methods are required for the determination of nitrite.

A number of techniques have been developed for the detection of nitrite ions, based on organic chromophores [12–14], electrochemical detection [15], ion chromatography [16,17] and others [18,19]. Many of these approaches use sophisticated instruments and are expensive and time consuming. In addition, some of these are not adequately sensitive or selective for the determination of trace amount of nitrite ions. Therefore, they cannot be used for real-time and on-site measurement of nitrite. In recent time, a few sensitive colorimetric nitrite sensors using plasmon resonance based gold nanorods [20] and gold nanoparticle probes [21], and a reaction-based probe [22] have been reported. However, probes based on fluorometry [23–26] attract special attention owing to their favorable features such as operational simplicity and cost-effectiveness, in addition to the high sensitivity and selectivity. In particular, reaction-based fluorometric probes, called as chemodosimeters or chemoreactants, which involve target analyte induced fast and irreversible chemical reactions coupled with instant signal transduction, are more efficient in terms of sensitivity and selectivity than chemical probes based on non-covalent interactions in most cases [27]. Application of fluorophore based chemodosimeters in the detection of nitrite ion is rare [23,24]. The reported probes follow tedious procedure and suffer from poor detection limit. We report herein a rhodamine based "turn-on" type fluorogenic chemodosimeter, **1**, which detects trace amount of nitrite ions in water as low as 4.6 ppb. In addition, the naked eye detection level of this probe is well below the range of the EPA recommended MCL for nitrite.

2. Experimental

2.1. Apparatus

NMR spectra were recorded on Bruker AV300 NMR spectrometer. Mass spectra were obtained from Waters Q-TOF micro

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mass spectrometer (ESI^+) and Agilent 6400B LC–MS (ESI^+). Fluorescence spectra were taken on a JASCO FP-6300 spectrofuorometer, the slit width was 2.5 nm for both excitation and emission. Absorption spectra were recorded on a JASCO V570 UV/Vis/NIR spectrophotometer.

2.2. Reagents

Rhodamine B hydrochloride was purchased from Sigma Aldrich and used as received. All other chemicals were obtained from different commercial suppliers and were used without further purification.

2.3. General procedure

Chloroform was distilled from P_2O_5 and acetonitrile was distilled from CaH_2 before use. The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm silica gel plates (60F-254) using UV light (254 or 365 nm) or naked eye for visualization. Probe **1** was dissolved in 95% aqueous ethanol to make $2.0x10^{-3}$ M stock solution, which was diluted to required concentration for measurement. A $1.0x10^{-3}$ M stock solution of $NaNO_2$ was prepared in deionized water (MilliQ, 18 Ω) and other standard solutions of $NaNO_2$ were prepared by further dilution. For study of the effect of different anions and the competitive study of nitrite with other anions, stock solutions (5 mL, 1 mM) were prepared by dissolving $Pb(NO_3)_2$, K_2CO_3, CH_3COONa, NaI, Na_2SO_4, $(Bu)_4N^+Br^-$ and $Na_2S_2O_3$ in deionized water (MilliQ, 18 Ω). All solutions are subjected to filtration through 0.22 μm syringe filter in order to avoid any interference by any particulate matter in fluorescence measurement.

2.4. Synthesis of probe *1*

To a solution of rhodamine B, HCl (100 mg, 0.21 mmol) in dry chloroform (4.0 mL) at room temperature and phosphorus oxychloride (0.058 mL 0.63 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 get rhodamine B acid chloride. This acid chloride was dissolved in dry acetonitrile (2.0 mL) and added dropwise to a solution of o-phenylenediamine (103 mg, 0.95 mmol) in dry acetonitrile (0.6 mL) containing triethylamine (0.8 mL). After stirring for 10 h at room temperature, the mixture was concentrated under vacuum and the crude product was purified by column chromatography (ethyl acetate–petroleum ether 60–80, 20:80) to give compound **1** as white solid in 78% yield; mp 178–179 °C.

^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.14 (*t*, J=7.2 Hz, 12H); 3.25–3.39 (*d*, J=7.2 Hz, 8H); 3.41 (bs, 2H), 6.09 (dd, J_1=1.0 Hz, J_2=7.5 Hz, 1H); 6.26 (bs, 2H); 6.30 (*d*, J=10 Hz, 2H); 6.41 (dt, J_1=1.0 Hz, J_2=7.5 Hz, 1H); 6.55 (dd, J_1=1.0 Hz, J_2=7.5 Hz, 1H); 6.64 (d, J=10 Hz, 2H); 6.95 (dt, J_1=1.0 Hz, J_2=7.5 Hz, 1H); 7.23–7.26 (m, 1H); 7.53–7.57 (m, 2H); 8.01–8.04 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 12.6; 44.4; 68.1; 98.0; 106.9; 108.0; 117.0; 118.2; 122.2; 123.5; 124.3; 128.4; 128.7; 128.8; 128.9; 132.0; 132.7; 144.5; 148.9; 152.4; 154.0; 166.5. ESI–MS (*m*/*z*): 533.4 ($[M+H]^+$), 555.4 ($[M+Na]^+$); calculated 532.2.

3. Result and discussions

3.1. Synthesis and spectral characterization of probe *1*

The colorless and nonfluorescent probe **1** was first synthesized in a "one-step" synthetic procedure as described by Zheng et al. [28] with minor modification from rhodamine B in high yield. The free acid group of rhodamine B HCl was converted to acid chloride by refluxing with phosphorus oxychloride and the resulting crude mixture in acetonitrile was treated with o-phenylenediamine to produce probe **1** in 78% yield. The compound was characterized by ^1H NMR, ^{13}C NMR and ESI–MS, which is in complete agreement with the reported value.

3.2. Study on pH sensitivity and nitrite sensing condition of probe *1*

The function of probe **1** as nitrite detector is based on the diazotization of the amino group of the probe. As the diazotization process requires strong acidic condition, a study on pH dependency of probe **1** was carried out to ensure its stability at low pH. The fluorescence intensity of various solutions of probe **1** within a pH range of 1–9 was measured after 1 h of the addition of the probe at 0–5 °C. Each of the solutions exhibited little or no fluorescence revealing that the spirocyclic form is stable within a wide range of pH at the prevailing condition of diazotization reaction (Fig. 1). Next, the fluorescence intensity of solutions containing probe **1** at pH 1 was measured against time both at low temperature (0–5 °C) and room temperature. To our delight, it was observed that the probe **1** can sustain strong acidic condition (pH 1) at low temperature for several hours with negligible change in fluorescence intensity with respect to a neutral aqueous solution of the probe (Fig. 2) and without developing any visible color in the solution. However, the same acidic solution of probe **1** at room temperature gradually showed increase in fluorescence intensity at λ_{max} 598 nm and development of purple color. The saturation point of fluorescence intensity was reached after exposure of probe **1** at pH 1 for 24 h. From this study we presume that spirocyclic lactam form of probe **1** remains in equilibrium with the ring-opened amide form of probe **1** under acidic condition (Scheme 1) [24]. The presence of ring-opened amide form is negligible even at low pH (viz. pH 1) when the probe solution is kept at 0–5 °C but gradually increases in number at room temperature.

The most suitable condition of diazotization reaction was determined by measuring fluorescence intensity of various solutions at different pH containing 1 equivalent of nitrite ions. Thus, 1 equivalent of $NaNO_2$ was added to each of the solutions within a pH range of 1–9 at 0–5 °C and their fluorescence intensity was recorded after 1 h. This study revealed that the most suitable pH for the sensing reaction is 1 (Fig. 1). The reaction rate gets slower with the decrease in acid strength up to pH 4. As expected, probe **1** does not work at near to neutral or basic pH. Low temperature, a requirement of conventional diazotization reaction, was maintained for all fluorometric studies to avoid any side reaction.

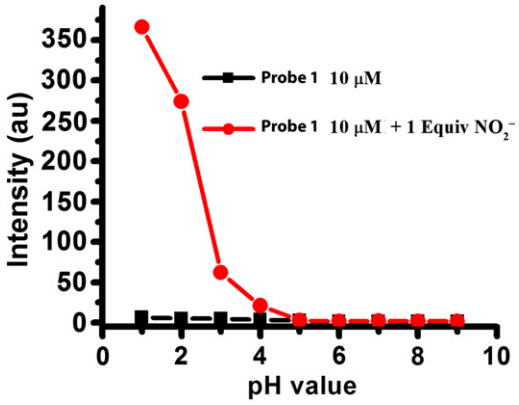

Fig. 1. Fluorescence response of probe **1** (10 μM) after 60 min with and without NO_2^- (10 μM, 1 equiv) in different pH buffer (pH 1–9) at 0–5 °C (excitation at 525 nm).