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Spectrofluorimetric determination of trace nitrite with a novel fluorescent probe

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ABSTRACT

A simple, sensitive and selective fluorimetric determination for trace nitrites was developed using an unsymmetrical rhodamine with a high fluorescence quantum yield and large Stokes shift. The method is based on the reaction of the unsymmetrical rhodamine with nitrite in an acidic medium to form a nitroso product, which has much lower fluorescence because the electron-withdrawing effect of the nitroso group influences the system of p-π conjugation between N atom and benzene ring. Under optimum conditions, the fluorescence quenching intensity is linear over a nitrite concentration range of 1.0×10^{-8} to 3.5×10^{-7} M. The detection limit is 2.0×10^{-10} M (S/N = 3). The method was applied to the determination of nitrite in tap water and lake water with satisfactory results. The mechanism for the reaction is also reported.

1. Introduction

Nitrite is not only a very important nitrosating agent used to form nitroso compounds, but also an excellent indicator of the extent of pollution in environmental samples. Furthermore, nitrite salts, which are used as food preservatives, exist widely in the environment, and they can cause various types of noxious effect, such as microsomal enzyme inhibition, and a decrease in the efficiency of a nutritive diet [1,2]. Therefore, the determination of nitrite in water, food and biological and environmental matrices is of vital significance, and in fact a sensitive and accurate method for the determination of nitrite ions is urgent.

Many analytical methods for nitrite have been reported previously, including electrometry [3–5], chromatography [6,7], capillary electrophoresis [8,9], spectrophotometry [10–12] and spectrofluorimetry [13–20]. However, not all are suitable for routine ultra-trace determinations. Spectrophotometric methods [21] suffer from poor sensitivity and interferences from other anions. Chromatographic methods [22] are often time-consuming. Capillary electrophoresis [23] is complicated and uses high-cost instrumentation. Spectrofluorimetry is the most widely employed method for detecting nitrites due to its simplicity, sensitivity, excellent limits of detection and low-cost. In spectrofluorimetric methods, nitrite ions are either chemically reactive or serve as a catalyst for a chemical reaction, the reaction products then have an effect on the fluorescence properties (development, inhibition, enhancement) in either a direct or indirect manner [24]. Rhodamine has been widely used in biochemistry and molecular biology because of its good stability, high extinction coefficient and large fluorescence quantum yield [25]. In this paper, two unsymmetrical rhodamines with high fluorescence quantum yields and large Stokes shifts were synthesized (Fig. 1). They were then tested as fluorescent probes for the determination of nitrite. 6-(2-Carboxyphenyl)-9-(dimethylamino)-3,4-dihydro-2H-chromeno[3,2-g]quinolin-1-ium (**1**) was chosen to detect trace nitrite because of higher fluorescence quantum yield.

2. Experimental

2.1. Apparatus

A RF-5000 spectrofluorimeter (Shimadzu) and Cary Eclipse spectrofluorimeter (Varian) equipped with a 1 cm × 1 cm quartz cell were used for the fluorescence measurements. UV–vis absorption spectra were measured on a computer-controlled Shimadzu UV-2450 spectrometer. ^{1}H NMR was performed on a INOVA 500, BRUKER AV400 and BRUKER AV300 spectrometer using tetramethylsilane as the internal reference.

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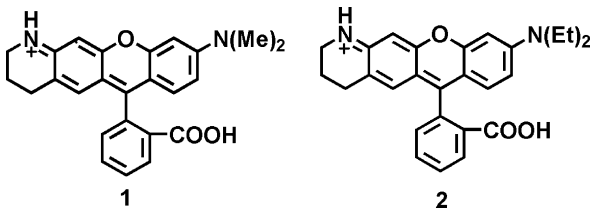

Fig. 1. Molecular structures of compounds **1** and **2**.

2.2. Reagents

All of chemicals were of analytical grade and used without further purification. All solutions were prepared with doubly distilled water.

A 2×10^{-5}-M stock solution of compound **1** was prepared by dissolving it in water. A standard nitrite solution (1×10^{-3} M) was prepared by dissolving sodium nitrite in water, which was dried at 100 °C for 2 h. Twenty drops of chloroform (about 0.3 mL) and a pellet of sodium hydroxide (about 5.0 mg) were then added [26]. This standard solution was prepared weekly and kept in a refrigerator. Each day a new working solution was prepared by appropriate dilution of the standard solution. Hydrochloric acid (0.1 M) was prepared from concentrated hydrochloric acid.

2.3. Synthesis of new fluorescence probes

The synthetic route for the new probes is shown in Fig. 2.

2.3.1. 7-Hydroxy-1,2,3,4-tetrahydroquinoline (*3*)

Compound **3** was prepared according to the method of Kulka and Manske [27] in the yield of 25.4%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.85 (q, J = 6.0 Hz, 2H), 2.61 (t, J = 6.0 Hz, 2H), 3.15–3.18 (m, 2H), 4.65 (br, 2H), 5.96 (d, J = 3.0 Hz, 1H), 6.03 (dd, J = 9.0, 3.0 Hz, 1H), 6.64–6.65 (m, 1H).

2.3.2. 2-Carboxyl-4′-dimethylamino-2′-hydroxy benzophenone (*4*)

A solution of 3-dimethylamino phenol (4.11 g, 30.0 mmol) and phthalic anhydride (4.66 g, 31.5 mmol) in toluene (30 mL) was refluxed under N_2 for 3 h, and cooled to 50–60 °C. Then 30 mL of 35% aqueous NaOH (w/w) was added and heated at 90 °C for 6 h. The resulting mixture was poured into H_2O (300 mL), acidified with HCl (10.0 M), and allowed to stand at room temperature for 2 h. The suspension was then filtered. The solid was recrystallized from a mixture of water and methanol, and dried to afford the desired product (5.93 g, 71.3%).

^{1}H NMR (500 MHz, CD_3OD:$CDCl_3$ = 5:1): δ 3.04 (s, 6H), 6.13 (s, 1H), 6.18 (d, J = 7.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 7.36 (d, J = 7.5 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.68 (t, J = 7.5 Hz, 1H), 8.07 (d, J = 7.5 Hz, 1H).

2.3.3. 6-(2-Carboxyphenyl)-9-(dimethylamino)-3,4-dihydro-2H-chromeno[3,2-g]quinolin-1-ium (*1*)

A solution of compound **4** (0.28 g, 1.0 mmol) and compound **3** (0.15 g, 1.0 mmol) in 5 mL methanesulfonic acid was heated under N_2 at 85 °C for 14 h. The cooled mixture was then poured into 20 mL of ice water, neutralized with saturated aqueous Na_2CO_3, and finally filtered. The crude product was dried and purified by column chromatography on silica to afford compound **1** (0.29 g) with a yield of 71.8% (methanol:dichloromethane = 1:20, R_f = 0.1).

^{1}H NMR (300 MHz, CD3OD): δ 1.86–1.96 (m, 2H), 2.67–2.68 (m, 2H), 3.15–3.41 (m, 8H), 6.63 (s, 1H), 6.83–6.93 (m, 3H), 7.12 (d, J = 9.3 Hz, 1H), 7.26 (d, J = 6.9 Hz, 1H), 7.69–7.76 (m, 2H), 8.21 (d, J = 6.6 Hz, 1H).

2.3.4. 2-Carboxyl-4′-diethylamino-2′-hydroxy benzophenone (*5*) and 6-(2-carboxyphenyl)-9-(diethylamino)-3,4-dihydro-2H-chromeno[3,2-g]quinolin-1-ium (*2*)

Compound **5** was prepared similarly to compound **4** in 73.1% yield.

^{1}H NMR (500 MHz, CD_3OD:$CDCl_3$ = 5:1): δ 1.15 (t, J = 7.0 Hz, 6H), 3.40 (q, J = 7.0 Hz, 4H), 6.09 (d, J = 2.5 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 6.84 (d, J = 9.5 Hz, 1H), 7.35 (dd, J = 7.5, 1.0 Hz, 1H), 7.58 (t, J = 7.5 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H).

Compound **2** was synthesized using the same method as compound **1** with a 74.6% yield.

^{1}H NMR (300 MHz, CD_3OD): δ 1.26 (t, J = 7.2 Hz, 6H), 1.85–1.96 (m, 2H), 2.66–2.67 (m, 2H), 3.23–3.32 (m, 2H), 3.60 (t, J = 7.4 Hz, 4H), 6.65 (s, 1H), 6.82–6.88 (m, 2H), 6.92 (d, J = 9.5 Hz, 1H), 7.08 (d, J = 9.3 Hz, 1H), 7.30 (d, J = 6.9 Hz, 1H), 7.69–7.77 (m, 2H), 8.24 (d, J = 6.6 Hz, 1H).

2.4. Determination of nitrite

A nitrite test solution was prepared by adding 1.0 mL of 2×10^{-5} M compound **1** solution and 2.0 mL of 0.1 M HCl to a 10.0-mL volumetric flask. A certain volume of the standard solution of nitrite was then added and the resulting solution was diluted to 5.0 mL with water. Then the solution was allowed to stand for 30 min at 30 °C, and finally diluted to 10.0 mL with water. The fluorescence intensity was measured at 561 nm with excitation at 538 nm. The slit width was 5 nm for both excitation and emission.

3. Results and discussion

3.1. Spectral properties of new fluorescence probes

The maximum excitation wavelength of compound **1** is at 538 nm ($\varepsilon = 5.40 \times 10^4$ cm^{-1} M^{-1}) and its emission wavelength was at 561 nm. While the maximum excitation wavelength of compound **2** is at 542 nm ($\varepsilon = 5.40 \times 104$ cm^{-1} M^{-1}) with the emission wavelength at 561 nm. Stokes shifts of compounds **1** and **2** are 762.0 and 708.2 nm, respectively. Fluorescent quantum yields of compounds **1** (Φ = 0.79) and **2** (Φ = 0.73) were measured by using rhodamine B (Φ = 0.5 in ethanol) [28] as the standard. Compound **1** has a higher fluorescent quantum yield and larger Stokes shift than compound **2**. The reason is that the diethylamino group of compound **2** has more torsional motion which causes more loss of energy than compound **1**. Therefore, compound **1** was used to detect nitrites.

The excitation and emission spectra of compound **1** are shown in Fig. 3. The fluorescence intensity of compound **1** decreased dramatically when nitrite was added.

3.2. Effect of HCl concentration

Since the reaction proceeds in acidic media, the proper HCl concentration must be selected to ensure compound **1** reacts with the nitrites as completely as possible. Fig. 4 indicates the effect of HCl concentration on the fluorescence quenching intensity (ΔF), where ΔF is the fluorescence intensity difference between the absence and the presence of nitrite. The optimal HCl concentration is 0.05 M.

3.3. Effect of compound *1* concentration

The effect of the concentration of compound **1** on the fluorescent quenching by the formation of the nitroso derivatives was

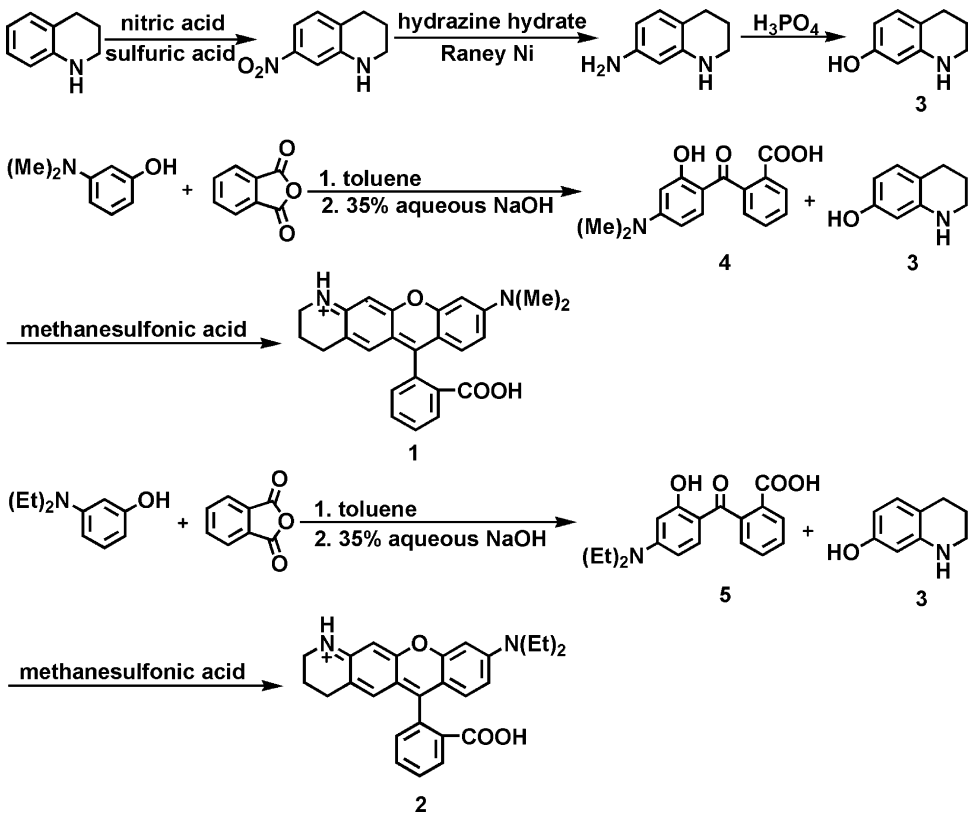

Fig. 2. Synthetic scheme for fluorescent probes **1** and **2**.

studied and the results are shown in Fig. 5. The maximum fluorescent quenching was obtained at 2×10^{-6} M of compound **1**. This concentration was then selected for further studies.

3.4. Effects of reaction time and temperature

The effects of reaction time and temperature on the reaction of compound **1** with nitrite are shown in Fig. 6. Longer times are needed to reach maximum fluorescence quenching at low temperatures (20 °C) than at high temperatures (30–40 °C). The optimal reaction temperature to achieve maximum fluorescent quenching is 30 °C. The optimal reaction time is 30 min. The products were stable for at least 5 h after their formation as judged by UV–vis absorption spectroscopy.

3.5. Linearity, sensitivity and precision

Under the optimized conditions, fluorescence spectra of compound **1** in the presence of nitrites were shown in Fig. 7 in the concentration range of 1.0×10^{-8} to 3.5×10^{-7} M. The fluorescence quenching intensity was linear over nitrite concentration. Based on it, a linear calibration curve can be constructed in this concentration range. The concentration of nitrite can be calculated from the linear

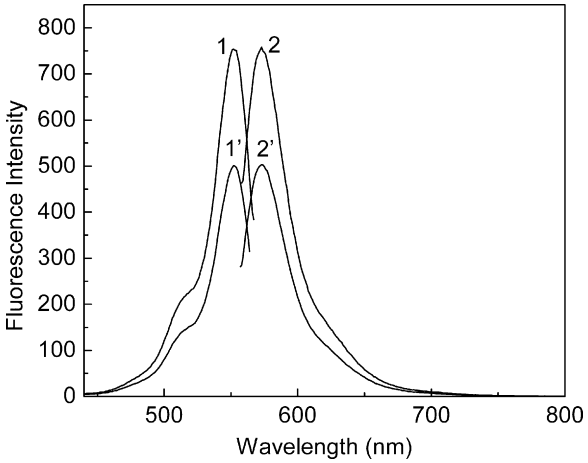

Fig. 3. Fluorescence spectra of compound **1** and compound **1** in the presence of nitrite. $C_{\text{compound 1}} = 2 \times 10^{-6}$ M; $C_{\text{HCl}} = 0.05$ M; nitrite: 1×10^{-7} M; reaction time, 30 min; reaction temperature, 30 °C. 1, 2, excitation and emission spectra of compound **1**, respectively; 1′, 2′, excitation and emission spectra of compound **1** in the presence of nitrite, respectively.

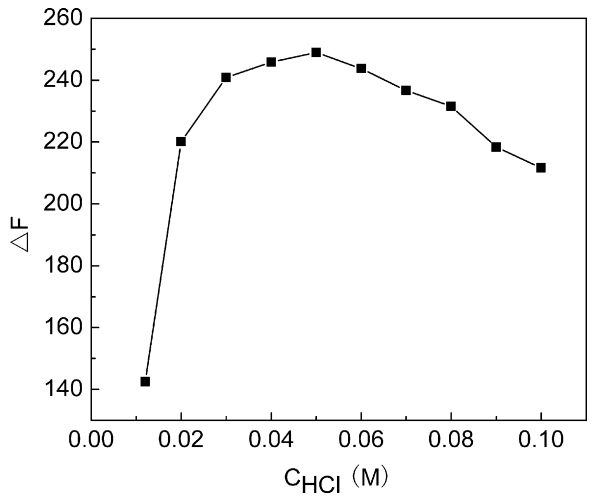

Fig. 4. Effect of HCl concentration. $C_{\text{compound 1}} = 2 \times 10^{-6}$ M; nitrite: 1×10^{-7} M; reaction time, 30 min; reaction temperature, 30 °C.

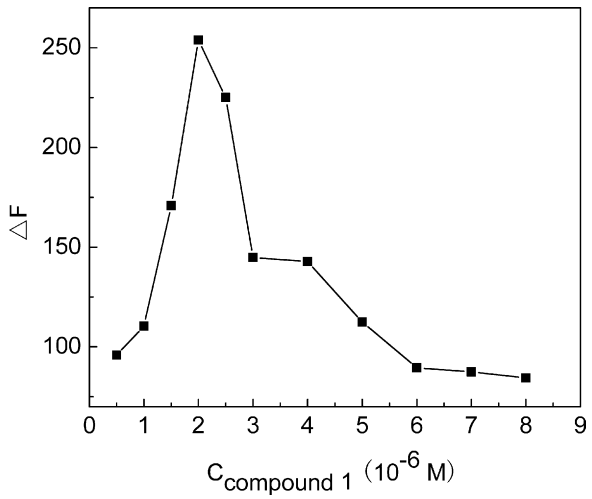

Fig. 5. Effect of compound **1** concentration. C_{HCl} = 0.05 M; nitrite: 1×10^{-7} M; reaction time, 30 min; reaction temperature, 30 °C.

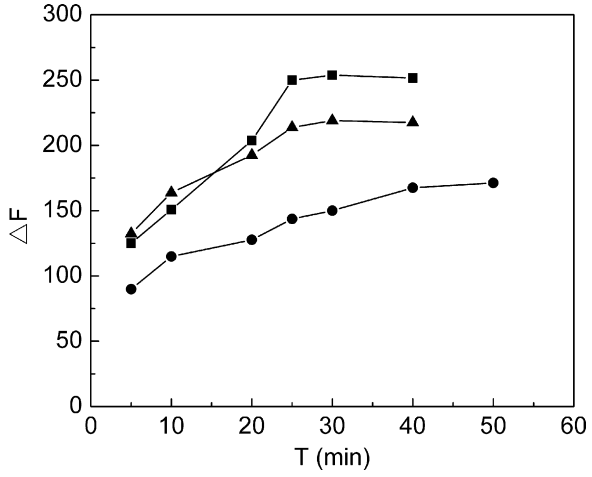

Fig. 6. Effect of time and temperature. $C_{compound\ 1} = 2 \times 10^{-6}$ M; C_{HCl} = 0.05 M; nitrite: 1×10^{-7} M. 20 °C (●), 30 °C (■), 40 °C (▲).

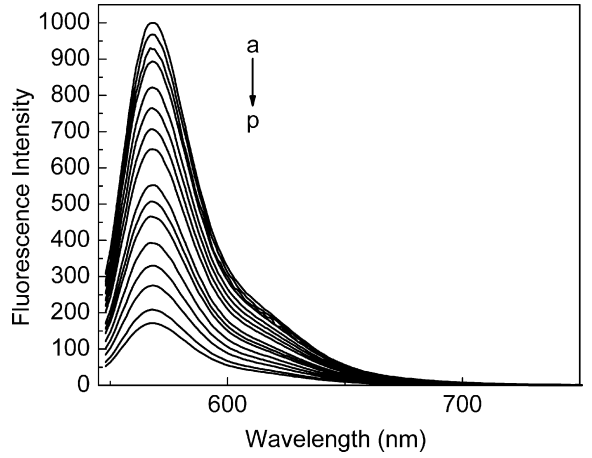

Fig. 7. The fluorescence spectra of the system of compound **1** and nitrite. $C_{compound\ 1} = 2 \times 10^{-6}$ M; C_{HCl} = 0.05 M; nitrite (×10⁻⁶ M); reaction time, 30 min; reaction temperature, 30 °C: (a) 0; (b) 0.01; (c) 0.025; (d) 0.04; (e) 0.07; (f) 0.095; (g) 0.12; (h) 0.14; (i) 0.18; (j) 0.20; (k) 0.22; (l) 0.25; (m) 0.275; (n) 0.3; (o) 0.325; (p) 0.35.

Table 1
Tolerance limits of different ions in the determination of 1.0×10^{-7} M nitrite.

Foreign ion	Tolerance limit (molar ratio)
Mg^{2+}, Hg^{2+}, Ca^{2+}, K^+, Na^+, Mn^{2+}, Zn^{2+}, NH^{4+}, Ba^{2+}, NO^{3-}, CH_3COO^-, $SO_4{}^{2-}$, F^-, $CO_3{}^-$	100,000
$C_2O_4{}^{2-}$, Cl^-, Ce^{3+}, Al^{3+}, Fe^{3+}, Cu^{2+}, Br^-, Fe^{2+}, $PO_4{}^{3-}$	50,000
Tartrate, citric	5,000

Table 2
Analytical results of nitrite in samples with **compound** 1.

Sample	Nitrite added (μM)	Found (μM)	R.S.D. (%)	Recovery (%)
Tap water	0.00	0.0786	2.06	
	0.10	0.1551	1.82	97.3
Lake water	0.00	0.0850	2.18	
	0.10	0.1725	1.36	102.9

regression equation: $\Delta F = 2396.8c + 11.08$ ($r = 0.9995$), where ΔF is the fluorescence quenching intensity and c is the concentration of nitrite (μM). The limit of detection (LOD) is evaluated using $3\sigma/s$, where σ is the standard deviation of the blank signals and s is the slope of the linear calibration plot. The LOD for the determination of nitrite was thus calculated to be 2.0×10^{-10} M. The relative standard deviation ($n = 10$) is 0.69% and 0.53% at 5.0×10^{-8} and 1.0×10^{-7} M nitrite, respectively. The repeatability of the method was confirmed by analysis of a standard nitrite solution over a period of 7 days ($n = 5$). The relative standard deviation was found to be 2.36% at 1.0×10^{-7} M.

In comparison with previously reported spectrofluorimetric methods in detecting trace nitrite, such as 5-aminofluorescein [29], 4-hydroxycoumarin [17], acetaminophen [30], safranine [31], rhodamine 110 [15], TMDABODIPY [32] and TMDCDABODIPY [13], the current method is more sensitive and simple.

3.6. Effects of foreign ions

The effects of foreign ions (cations and anions) on the determination of 1.0×10^{-7} M nitrite were also studied, and the results are summarized in Table 1. The tolerance limit is defined as the concentration of foreign ion causing at least a 4% relative error in the fluorescence of the sample. Most of ions examined do not interfere with the determination of nitrite.

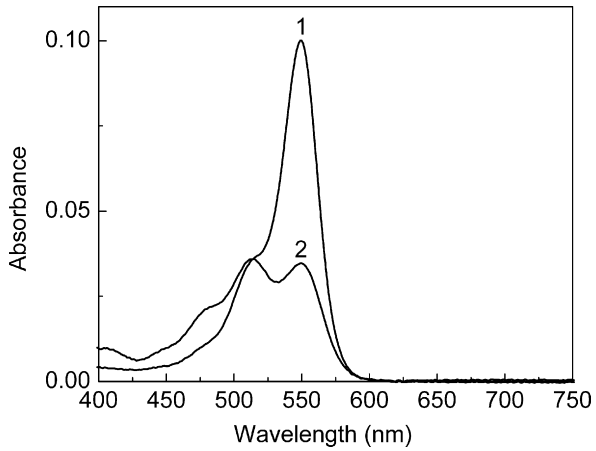

Fig. 8. Absorbance spectra of compound **1** and compound **1** in the presence of nitrite. Ccompound **1** = 2×10^{-6} M; nitrite: 2×10^{-8} M; reaction time, 30 min; reaction temperature, 30 °C. 1, absorbance spectrum of compound **1**; 2, absorbance spectrum of compound **1** in the presence of nitrite.

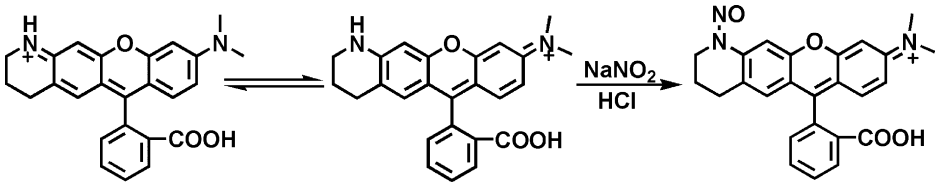

Fig. 9. Reaction of compound **1** with nitrite.

3.7. Sample analysis

The method was applied to the determination of nitrite in tap water and lake water according to the method described in the literature [15]. A 2.0-mL sample of tap water was transferred to a 10.0 mL test-tube and assayed directly. Lake water was analyzed after filtration and dilution by 10 times, of which 0.4 mL was analyzed in a 10.0 mL test-tube. The results are given in Table 2 and show this method is accurate.

3.8. The mechanism of the reaction

Fig. 8 shows the change of the absorption spectra of compound **1** and compound **1** upon nitrite was added. It is clear that a new absorption peak located in 514 nm appeared, with a blue shift of 35 nm in comparison with the original absorption peak. This shows a new substance was formed due to the reaction between nitrite and compound **1** under the experimental conditions. The possible reaction between compound **1** and nitrite is shown in Fig. 9. When compound **1** reacts with nitrite in an acidic medium, the secondary amine group at the 6-position of compound **1** reacts with nitrite to form a nitroso group. This causes lower fluorescence because of the electron-withdrawing effect of the nitroso group. The determination of nitrite is based on this specific reaction.

4. Conclusions

A new fluorescence probe, unsymmetrical rhodamine, was synthesized and used in the spectrofluorimetric determination of trace nitrite in tap water and lake water with satisfactory results. The current method has the advantages of simplicity, good reproducibility, high sensitivity and selectivity for the determination of trace nitrite. The mechanism for the fluorescence quenching involves the formation of an electron-withdrawing nitroso group.

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