



Fluorometric determination of sulfide ions via its inhibitory effect on the oxidation of thiamine by Cu(II) ions

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Abstract

A fluorometric assay is described for sulfide ions determination. It is based on the finding that the oxidation of the non-fluorescent substrate thiamine (TH) by Cu(II) in basic solution to form fluorescent thiochrome is inhibited by sulfide ions. This results in a decrease in fluorescence intensity which is proportional to the concentration of sulfide ions. Under the optimized conditions, the decrease in fluorescence, best measured at excitation/emission wavelengths of 370/440 nm, decreases linearly in the 0.03 to 2.5 μM sulfide ions concentration range. The detection limit is 20 nM. The method shows excellent selectivity over many potentially interfering ions and has been successfully applied to the determination of sulfide ions in spiked tap water, lake water and the synthetic wastewater samples. The method is time-saving and environmentally friendly, and in our perception shows a great potential in water quality inspection and environmental monitoring.

Keywords Fluorimetric assay · Vitamin B1 · Non-fluorescent substrate · Anion detection · Thiochrome · Basic conditions · Water samples

Introduction

Sulfide ions (S^{2-}), a pollutant index for water, are widely distributed in natural and waste water samples [1, 2]. They have been frequently used in many industrial locations, such as tanneries, petroleum refineries, printing, textile, dyes, cosmetic or paper manufacturing plants, where they are either employed as reactant or produced as a by-product of manufacturing or industrial processes [3]. In acidic conditions, S^{2-} are protonated and converted to HS^- or H_2S [4], becoming more hazardous, which will cause serious environmental problems and present a danger to human health. Therefore, developing highly sensitive methods for S^{2-} detection is of great importance. To date, various kinds of analytical

techniques have been utilized for S^{2-} detection, such as titration [5], high performance liquid chromatography [6], capillary electrophoresis [7], electrochemistry [8] and chemiluminescence [9] and fluorimetry [10, 11]. Among these techniques, fluorimetry has attracted significant attention owing to its short detection time, easy operability and high sensitivity [12–14].

Actually, many fluorescent probes have been designed for S^{2-} detection based on their reduction ability, nucleophilicity or binding affinity to metal ions [15–20]. However, it is still challenging for S^{2-} detection. For examples, most of the fluorescent probes are toxic, poorly water-soluble and not utilized in totally aqueous media or they require long response time. Thus, environment-friendly and water-soluble noble metal clusters and carbon quantum dots have been utilized for S^{2-} detection [21, 22]. While the high cost of noble metal salts along with long hydrolysis time limit their further applications. Therefore, establishing time-saving and cost-effective methods is urgently demanded and challenging.

Thiamine (TH), known as vitamin B1, which shows the advantages of low cost, water-solubility and easy accessibility, have been used as non-fluorescent substrate [23]. It can be oxidized by Cu^{2+} to obtain fluorescent thiochrome under basic conditions [24]. However, the applications of Cu^{2+} -TH system for fluorescent detection have been rarely studied.

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In this study, a novel method for S^{2-} detection is proposed based on Cu^{2+} -TH system, where the high binding affinity between Cu^{2+} and S^{2-} suppress the oxidation of TH resulting in the decrease of fluorescence. Based on the above facts, we develop a novel, sensitive and selective method for S^{2-} detection. This method is time-saving without the utilization of fluorescent nanomaterials or probes, which shows high potential in determination of S^{2-} in real samples.

Experimental section

Materials

$Na_2S \cdot 9H_2O$, NaH_2PO_4 , Na_2HPO_4 , $Na_4P_2O_7 \cdot 10H_2O$ and thiamine were supplied by Aladdin Reagent Company (Shanghai, China, www.aladdin-e.com). KNO_3 , $NaNO_3$, $Ca(NO_3)_2 \cdot 4H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$, $Cu(NO_3)_2 \cdot 3H_2O$, $NaOH$ and NaI were obtained from Sigma Aldrich (St. Louis, USA, www.sigmaaldrich.com). NaF , $NaCl$, $NaBr$, Na_2CO_3 , $NaHCO_3$, Na_2SO_4 , Na_2SO_3 , $Na_2S_2O_3 \cdot 5H_2O$, $NaAc$ and $NaNO_3$ were purchased from Damao Chemical Reagent Factory (Tianjin, China, www.dmreagent.com). All reagents were analytical grade and used as received.

Apparatus

Fluorescence measurements were performed on a RF-6000 spectrofluorometer (Shimadzu, Japan, www.shimadzu.com). Both the excitation and emission slits were set as 5.0 nm.

Procedure for S^{2-} detection

The fluorescent analysis for S^{2-} detection was realized as follows. 10 μ L of 0.4 mM $Cu(NO_3)_2$, 10 μ L of various concentrations of freshly prepared of Na_2S , 880 μ L of 0.05 M $NaOH$ and 100 μ L of 1.0 mM TH were added sequentially into a 1.5 mL tube, mixed thoroughly and incubated at 20 $^{\circ}C$ for 15 min. Then, the mixture was transferred for fluorescent measurements. The fluorescence spectra of the mixture were recorded from 390 nm to 600 nm excited at 370 nm. The same detection procedures were used for the selectivity study, except for that S^{2-} was replaced by other ions or the S^{2-} was added simultaneously with other ions.

Detection in water samples

Tap water and lake water samples were collected from our lab and the Jiazi Lake of University of Jinan, respectively. These samples were filtered through a 0.22 μ m membrane and diluted to 10 times and spiked with known concentrations of S^{2-} . The synthetic wastewater samples were prepared according to

previous study [25]. In detail, phenol, $NaCl$, $CaCl_2$, CH_3COONa , Na_2CO_3 , $KSCN$ (each of them is 25 mg) and 7.5 mg of $(NH_4)_2SO_4$ were put into a beaker. Then 50 mL of distilled water were added. Finally, S^{2-} with the finally concentration of 0.1 mM and 0.2 mM were introduced. The same procedures as the standard method was used for S^{2-} detection in spiked water and the synthetic wastewater samples.

Results and discussion

Mechanism of S^{2-} detection

Based on the facts that TH can be oxidized by Cu^{2+} and the solubility product constant of CuS is 6.3×10^{-36} , a possible mechanism of the Cu^{2+} -TH-based system for S^{2-} detection is proposed. Firstly, a remarkable fluorescence signal is found when Cu^{2+} and TH are simultaneously added (a, Fig. 1), due to the oxidation of TH by Cu^{2+} to fluorescent thiochrome [24]. Upon addition of S^{2-} to the Cu^{2+} -TH system, the oxidation process of TH is inhibited with a dramatic decrease of the fluorescence (b, Fig. 1), which may be caused by the binding of Cu^{2+} to S^{2-} due to their extremely low solubility product constant. In sharp contrast, barely any fluorescence signal is observed when Cu^{2+} (c, Fig. 1) or TH (d, Fig. 1) is individually introduced to $NaOH$ solutions, suggesting that both Cu^{2+} and TH show no fluorescence. Based on the above principles, a novel fluorescent method for S^{2-} detection is developed (Scheme 1).

Optimization of detection conditions

To achieve the best performance for S^{2-} detection, several parameters including the concentrations of $NaOH$, Cu^{2+} and

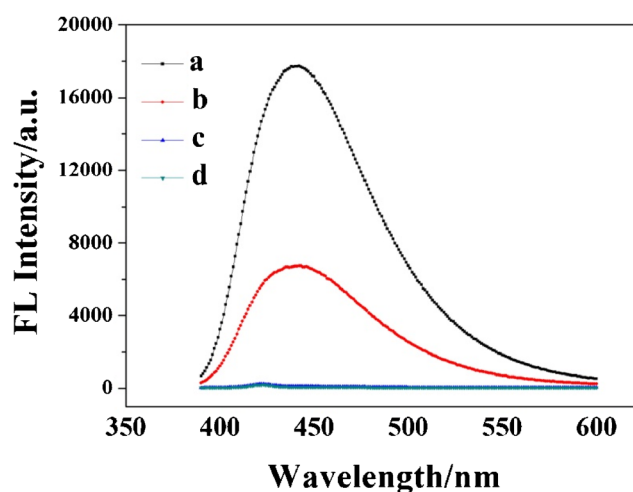
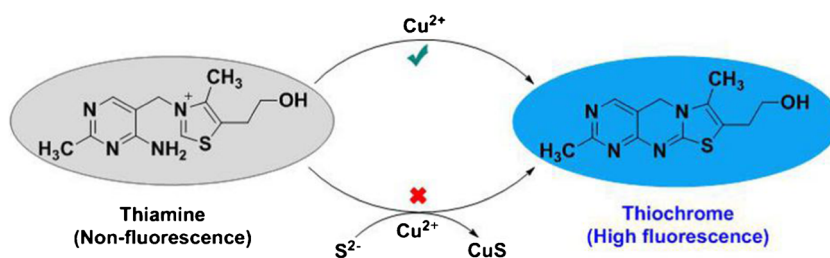


Fig. 1 Fluorescence spectra of Cu^{2+} -TH (a), Cu^{2+} -TH in the presence of 2.0 μ M sulfide ions (b), Cu^{2+} (c) and TH (d). $NaOH$ concentration: 44 mM; Cu^{2+} concentration: 4.0 μ M; TH concentration: 0.1 mM; incubation temperature: 20 $^{\circ}C$; incubation time: 15 min

Scheme 1 Schematic illustration of the Cu^{2+} -TH-based assay for S^{2-} detection



thiamine, incubation temperature and time are studied. We use the decrease of fluorescence intensity, that is, $F_0 - F$ (ΔF , where F_0 and F are the fluorescence intensity of the Cu^{2+} -TH system in the absence and presence of S^{2-} , respectively), to optimize the detection conditions. The optimum conditions should be as follows: (a) the concentration of NaOH is 44.0 mM (Fig. S1), (b) the concentrations of Cu^{2+} and TH are 4.0 μM (Fig. S2) and 0.1 mM (Fig. S3), respectively, (c) the incubation temperature is 20 $^\circ\text{C}$ (Fig. S4) and the time is 15 min (Fig. S5).

Analytical performance of the assay

Under the optimized conditions, the analytical performance of this method for S^{2-} detection is evaluated by introduction of various amounts of S^{2-} into the Cu^{2+} -TH system. With the increasing of S^{2-} concentration, the fluorescence intensity at 440 nm decrease gradually (Fig. 2a), while the ΔF increases systematically and reaches a plateau when the S^{2-} concentration is up to 6.0 μM (Fig. 2b). The ΔF displays a good linear relationship versus S^{2-} concentration ranging from 0.03 μM to 2.5 μM . The regression equation is $\Delta F = 5521.34c$ (μM) + 175.88 ($R^2 = 0.997$). The detection limitation is down to 0.02 μM at a signal-to-noise of 3, which is far below the maximum level of S^{2-} (15.0 μM) in drinking water estimated by the World Health Organization. Compared with previously published methods for S^{2-} detection, the proposed method shows comparable or even better sensitivity and detection

linear range (Table S1). The repeatability of this method is demonstrated by six repeated measurements of 2.0 μM of S^{2-} and the relative standard deviation (RSD) of 0.85% is obtained. This result indicates this method is reliable. Moreover, the Cu^{2+} -TH-based method is simple without the need of preparation fluorescent nanomaterials or probes and environment-friendly by using water-soluble TH as non-fluorescent substrate instead of organic and toxic fluorescent probes.

Selectivity

In order to verify the specificity of this method for S^{2-} detection, the fluorescence responses of Cu^{2+} -TH system towards other anions including F^- , Cl^- , Br^- , I^- , SO_4^{2-} , SO_3^{2-} , $\text{S}_2\text{O}_3^{2-}$, H_2PO_4^- , HPO_4^{2-} , $\text{P}_2\text{O}_7^{4-}$, AcO^- , NO_3^- , CO_3^{2-} , HCO_3^- , K^+ , Na^+ , Ca^{2+} , Zn^{2+} and Fe^{3+} both in the absence and presence of S^{2-} are tested and the results are shown in Fig. 3. No obvious changes of the fluorescence intensity are found when these ions are individually added into the system without the introduction of S^{2-} , while the fluorescence intensity decreases dramatically after the addition of S^{2-} . In addition, the effects of these ions on the fluorescence responses are negligible when they are in coexistence with S^{2-} . The above results demonstrate the high selectivity of this method for S^{2-} detection. However, if the concentrations of these ions exceed their tolerable concentrations, it may cause an effect on S^{2-} detection.

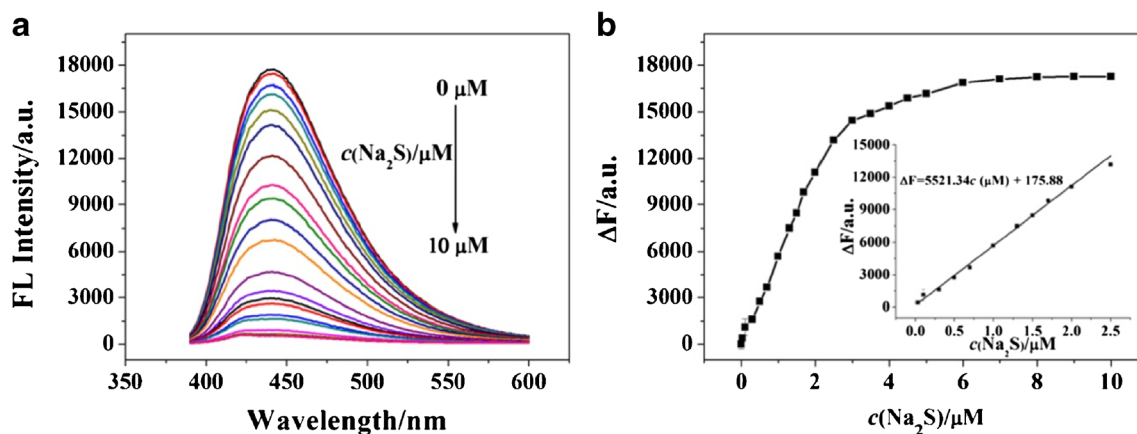


Fig. 2 **a** Fluorescence emission spectra of Cu^{2+} -TH system upon the addition of various concentrations of S^{2-} . From top to down, the concentration of S^{2-} are 0, 0.03, 0.1, 0.3, 0.5, 0.7, 1.0, 1.3, 1.5, 1.7, 2.0,

2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 μM . **b** The ΔF at 440 nm as a function of S^{2-} concentration. Inset shows the linear plot between ΔF and S^{2-} concentration

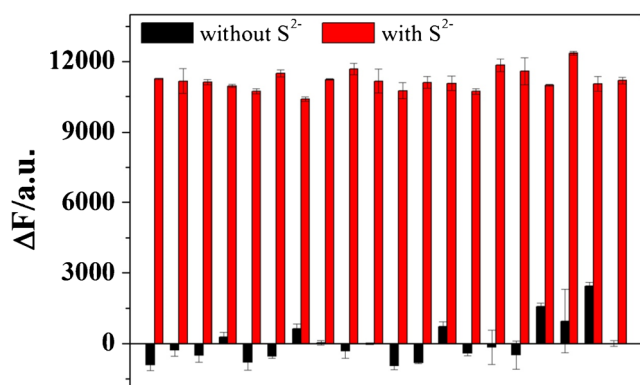


Fig. 3 Fluorescence responses of Cu^{2+} -TH system after the addition of S^{2-} and other ions separately or introduction of S^{2-} and other ions at the same time. From left to right, they are F^- , Cl^- , Br^- , I^- , SO_4^{2-} , SO_3^{2-} , $\text{S}_2\text{O}_3^{2-}$, H_2PO_4^- , HPO_4^{2-} , $\text{P}_2\text{O}_7^{4-}$, AcO^- , NO_3^- , CO_3^{2-} , HCO_3^- , K^+ , Na^+ , Ca^{2+} , Zn^{2+} , Fe^{3+} and the blank. For the absence of S^{2-} , the concentrations of F^- , Cl^- , Br^- , SO_4^{2-} , AcO^- , NO_3^- and CO_3^{2-} are 2.0 mM, I^- , SO_3^{2-} , $\text{S}_2\text{O}_3^{2-}$, H_2PO_4^- , HPO_4^{2-} , $\text{P}_2\text{O}_7^{4-}$, HCO_3^- , K^+ , Na^+ and Ca^{2+} are 0.2 mM, Zn^{2+} is 0.02 mM, Fe^{3+} and S^{2-} are 2.0 μM . For the presence of S^{2-} , the concentrations of F^- and Cl^- are 2.0 mM, Br^- , SO_4^{2-} , AcO^- , CO_3^{2-} , HCO_3^- , K^+ , Na^+ and Ca^{2+} are 0.2 mM, I^- , HPO_4^{2-} , $\text{P}_2\text{O}_7^{4-}$, H_2PO_4^- , NO_3^- and Zn^{2+} are 0.02 mM, SO_3^{2-} , $\text{S}_2\text{O}_3^{2-}$, Fe^{3+} , and S^{2-} are 2.0 μM . Error bar demonstrates the standard deviations of three independent measurements

Analysis of S^{2-} in real samples

To demonstrate the potential application of this method in real samples, the proposed method is used to determine S^{2-} in tap water, lake water and the synthetic wastewater samples. S^{2-} are not detected in either tap water or lake water sample. Then the samples spiked with known concentrations of S^{2-} are analyzed and the results are shown in Table 1. Moreover, this method is used for the analysis of S^{2-} in synthetic wastewater samples and the results are shown in Table S2. Satisfactory recoveries of S^{2-} detection in water samples and acceptable RSD indicate that the Cu^{2+} -TH-based method is highly feasible for S^{2-} determination in real samples.

Conclusions

In summary, a simple, cost effective and environment-friendly method for S^{2-} detection is developed based on Cu^{2+} -TH system. The strong interaction between Cu^{2+} and S^{2-} through

Table 1 The results for S^{2-} detection in tap water and lake water samples

Sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Tap water	1.0	1.01 ± 0.01	101.00	0.99
	2.0	2.00 ± 0.02	100.00	1.00
Lake water	1.0	1.04 ± 0.01	104.00	0.96
	2.0	2.11 ± 0.04	105.50	1.90

CuS formation inhibits the oxidation reaction of TH by Cu^{2+} resulting in fluorescence decrease, which can be used for highly sensitive and selective for S^{2-} detection. Moreover, this method has been successfully applied to detect S^{2-} in real samples with satisfactory results. To the best of our knowledge, this is the first time that Cu^{2+} -TH system has been employed for S^{2-} detection and it may pave the way for the wide applications of Cu^{2+} -TH system in trace analysis.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

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