A fluorescence method of detecting sulfur dioxide derivatives

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Abstract. A fluorescence spectrometry method is proposed to detect SO₃²⁻. The sensing mechanism is realized by utilizing the nucleophilic addition reaction between SO₃²⁻ and α, β-unsaturated compounds, exhibiting remarkable blue-shift in the maximum emission wavelength (from 650 nm to 555 nm). Preliminary experiments are conducted and results show that the method exhibits high selectivity and sensitivity toward sulfite over other common anions. The fluorescence intensity ratio at 555 nm and 650 nm (I₅₅₅/I₆₅₀) has a good linearly with sulfite concentration ranging from 0.5 to 15 μM with a detection limit of 0.28 μM. These results provide the theoretical fundamentals for sulfite detection in practical water samples.

1. Introduction

Sulfur dioxide (SO₂) has a stimulant odor and is one of the main contaminants in the atmosphere. Its deleterious effect on the human and environment, together with corrosion action to buildings have attracted more and more attention. A large amount of SO₂ is released by burning raw mineral in industrial activities, resulting in serious environmental problems, for example the acid rains. When SO₂ is dissolved in water, it forms a pH-dependent equilibrium mixture with sulfite (SO₃²⁻) and bisulfite ions (HSO₃⁻). If the pH becomes neutral, HSO₃⁻ usually converts to SO₃²⁻ [1]. There are several conventional analytical methods for the detection of sulfite [2-6], but they are unsuited for routine analysis because of troublesome sample pretreatment, time-consuming and sophisticated device [7]. Therefore, it is necessary to develop a sensitive method for rapid detection of sulfite.

\[
\text{CH}_2=\text{CHCN} + \text{Na}_2\text{SO}_3 + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{SO}_3\text{CH}_2\text{CN}
\]

Figure 1. Reaction equation between α,β-unsaturated compounds and sodium sulfite.

Nucleophilic addition reaction between SO₃²⁻ and α, β-unsaturated compounds (e.g. acrylonitrile)
in aqueous solution has been reported in 1952. The reaction equation is shown in figure 1 [8]. The above mechanism provides a potential approach to design a novel fluorescent probe for detecting $\text{SO}_3^{2-}$. Due to high sensitivity and operation simplicity, fluorescent probes have received more and more attentions in the detection of sulfites [9,10]. In this context, 2-(2-(9-(2-carboxyphenyl)-6-(diethylylamino)-3-oxo-3H-xanthen-2-yl)vinyl)-3-methylbenzothiazol-3-ium (probe 1) possessing the $\alpha,\beta$-unsaturated double bond for sulfite has been developed based on a rhodol-benzothiazole platform. The sensing mechanism is realized by utilizing the nucleophilic addition reaction between $\text{SO}_3^{2-}$ and probe 1, which is schematically illustrated in figure 2. The emission spectra afford remarkable blue shifts from 650 nm to 555 nm, accompanied by the fluorescent color changing from red to orange-yellow. Therefore, a fluorescence spectrometry method to detect $\text{SO}_3^{2-}$ can be established.

2. Experimental

2.1. Reagents
All reagents and solvents were of analytical grade and were used with no further purifications. The water used was double-distilled water. The inorganic salts were $\text{Na}_2\text{SO}_3$, $\text{Na}_2\text{SiO}_3$, $\text{Na}_2\text{SO}_4$, $\text{Na}_2\text{CO}_3$, $\text{NaOAc}$, $\text{NaNO}_3$, $\text{NaNO}_2$, $\text{NaSCN}$, $\text{NaF}$, $\text{NaCl}$, $\text{NaBr}$, KI, NaH$_2$S, NaClO and NaCN from Sinopharm Chemical Reagent Co., LTD. 2-Methylbenzothiazole, iodomethane and methanesulfonic acid were provided by Shanghai Aladdin Bio-Chem Technology Co., LTD.

2.2. Instrumentation
The fluorescence spectra were recorded on a Shimadzu RF-5301 fluorospectrophotometer with a 10 mm quartz cuvette.

2.3. Synthesis of probe 1
The 2-(6-(diethylylamino)-2-formyl-3-oxo-3H-xanthen-9-yl)benzoic acid and 2,3-Dimethylbenzothiazole were synthesized separately according to the reported procedure [11,12], then dissolved together in methanesulfonic acid and stirred at 90 °C for 30 min. Probe 1 was finally obtained by purifying the reaction solution.

2.4. Preparation for spectra measurement
The stock solution of probe 1 (1.0 mM) was prepared in 0.5 mL EtOH, and then diluted to 25 mL volumetric flask with twice-distilled water. Solutions of inorganic salt (10 mM) were prepared from $\text{Na}_2\text{SO}_3$, $\text{Na}_2\text{SiO}_3$, $\text{Na}_2\text{SO}_4$, $\text{Na}_2\text{CO}_3$, $\text{NaOAc}$, $\text{NaNO}_3$, $\text{NaNO}_2$, $\text{NaSCN}$, $\text{NaF}$, $\text{NaCl}$, $\text{NaBr}$, KI, NaClO and NaCN. Test solution in DMSO-phosphate buffer (pH 7.4, 20mM, 3:7, v/v) included probe 1 (5 μM) and appropriate analytes. Then resulting solution was kept at 25°C for 15 min, and the fluorescence spectra were measured at wavelengths 530/590 nm and 555/650 nm for excitation and emission, respectively.

3. Results and discussions

3.1. Selectivity experiment
The selectivity of probe 1 for sulfite over other analytes was determined in DMSO-phosphate buffer (pH 7.4, 20mM, 3:7, v/v). In figure 3, the emission spectra at 650 nm decreased dramatically and a new blue-shifted fluorescence peak at 555 nm appeared after addition of sulfite, accompanied by an obvious emission color change from red to orange-yellow. However, other anions afford no obvious changes in fluorescence spectra since their nucleophilic ability is too low to reacting with probe 1 at neutral condition. The experiment results indicate that the probe 1 exhibits a high selectivity toward sulfite over other anions.
3.2. Sensing of sulfite

The sensing behavior toward sulfite was studied by fluorescence titration experiment in DMSO-phosphate buffer (pH 7.4, 20 mM, 3:7, v/v). As shown in figure 4, upon excitation at 590 nm, the free probe displays the strong emission peak around 650 nm. However, upon addition of sulfite (0–60 μM), the peak at 650 nm decreases gradually, along with a new emission peak at 555 nm increases progressively. A blue-shift of 95 nm is observed in the emission spectra, indicating that probe 1 contributes to the dual ratiometric sensing of sulfite.

Figure 3. Fluorescence response of probe 1 (5 μM) towards various analytes.

Figure 4. Fluorescence spectra of probe 1 (5 μM) upon addition of amounts of Na₂SO₃ (0–60 μM), with each spectrum recorded after 15 min.

Figure 5. Linear relationship between I₅₅₅/I₆₅₀ of probe 1 (5 μM) and sulfite concentration. The inset shows the changes of I₅₅₅/I₆₅₀ with the concentration of Na₂SO₃ (0–60 μM).

Figure 5 illustrates the fluorescence intensity ratio I₅₅₅/I₆₅₀ varying with sulfite concentration. In the 12 equiv sulfite treatment, the I₅₅₅/I₆₅₀ increases from 0.034 to 23.25, as a result, a ca. 672-fold enhancement is achieved over the free probe. The I₅₅₅/I₆₅₀ value is proportional to sulfite concentration ranging from 0.5 to 15 μM with a detection limit of 0.28 μM, indicating that probe 1 can detect sulfite quantitatively.
3.3. Response time

The time-dependent fluorescence responses of probe 1 treated with sulfite was obtained in DMSO-phosphate buffer (pH 7.4, 20 mM, 3:7, v/v) (figure 6). Upon addition of sulfite (10 equiv) to the solution of probe 1, I_{555}/I_{650} of the sensing system increases significantly and reaches a plateau after 15 min, whereas I_{555}/I_{650} of the free probe (5 µM) remains almost unchanged at same conditions. Therefore, an assay time of 15 min is acceptable in real-time detection of sulfite.

![Figure 6](image)

**Figure 6.** The time-course fluorescence responses of probe 1 (5 µM) in absence and presence of SO$_3^{2-}$ (10 equiv).

4. Conclusions

In this paper, a method was established based on nucleophilic addition reaction between SO$_3^{2-}$ and probe 1. A new type of colorimetric and ratiometric fluorescent probe 1 for selectively detecting sulfite has been designed. The I$_{555}$/I$_{650}$ increases linearly with sulfite concentration ranging from 0.5 to 15 µM with a detection limit of 0.28 µM. Preliminary experiments were conducted and results show that the method exhibits high selectivity and sensitivity toward sulfite over other common anions. Subsequent researches are under way in our laboratory, and this method is expected to be used in online and real-time monitoring of sulfite in practical water samples.

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