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ARTICLE

A colorimetric and ratiometric fluorescent probe for rapid and sensitive detection of sulfite in sugar

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A colorimetric and ratiometric fluorescent probe was developed for the detection of sulfite in sugar. The probe underwent a Michael addition reaction with sulfite, which resulted in significant changes in fluorescence ratios. The probe displayed fast response (within 30 s) and high selectivity towards sulfite over other anions and bio-thiols. The fluorescence intensity ratios were linearly related to the concentrations of SO_3^{2-} ranging from 0 to 15 μM , and the limit of detection was determined to be as low as 12 nM. The probe was employed to determine the concentration of sulfite in sugar with good recoveries.

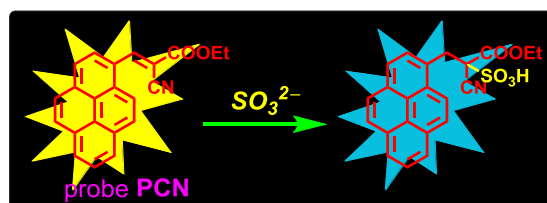
Introduction

In the food and beverage industry, sulfite (SO_3^{2-}) is widely used as preservatives and additives to extend the shelf-life of food and beverage.¹ However, epidemiological studies demonstrate that sulfite is correlated to the cardiovascular diseases such as ischemic heart diseases, myocardial ischemia, spontaneous hypertension and hypoxic pulmonary hypertension.²⁻⁵ Besides, excess amount of sulfite can cause asthma and allergic reactions in some individuals.^{6, 7} Owing to these harmful effects for human, the threshold levels of SO_3^{2-} in food and beverage have been strictly controlled in many countries. To guarantee food safety, it is necessary to monitor the levels of sulfite in food and beverage.

There are some conventional methods for the detection of sulfite, such as titrimetry,⁸ chromatography,⁹ electrochemistry,^{10,11} capillary electrophoresis¹² and flow injection analysis.¹³ Unfortunately, these methods are time-consuming and require complicated pre-treatments, which limits their application. Fluorescent probe is a promising technique due to its non-invasiveness, high sensitivity, high temporal and spatial resolution.¹⁴⁻¹⁸ Up to date, a number of fluorescence probes for sulfite have been developed based on nucleophilic reaction with aldehyde,¹⁹ the selective deprotection of levulinate,^{20,21} and the coordination to metal ions.^{22,23} However, these probes have some disadvantages, such as long respond time (5 min—10 h), unsatisfactory limit of detection and interference from biothiols, proteases or esterases, which limit their application. Besides, most of these probes respond to sulfite with changes only in fluorescent intensity, which suffers from errors

associated with probe concentration, environment effects and instrumental factors.²⁴ In contrast, ratiometric fluorescence probes can utilize the ratios of two emissions at different wavelengths as the detecting signal,²⁵ which are less impressionable to the above mentioned factors and provides more accurate analysis. Therefore, it is highly desired to develop some novel fluorescent probes with colorimetric and ratiometric responses towards sulfite. Recently, some ratiometric fluorescent probes for sulfite have been developed based on Michael addition.²⁶⁻³³ However, there is still plenty room for improvement in terms of selectivity and sensitivity.

Herein, we reported a novel colorimetric and ratiometric fluorescent probe for sulfite, as shown in Scheme 1. The probe would undergo Michael addition reaction with SO_3^{2-} , and a noticeable fluorescence change from yellow to cyan could be observed. The probe shown high selective response towards SO_3^{2-} over other anions and bio-thiols in PBS buffer (pH = 7.4, 10 mM). Furthermore, the probe was used to detect SO_3^{2-} in sugar.



Scheme 1 The proposed sensing mechanism of the probe PCN for SO_3^{2-} .

Results and discussion

Spectral response of the probe PCN toward SO_3^{2-}

The UV-vis absorption spectrum of probe PCN (10 μM) was measured in DMSO/PBS solution (v/v = 1/1, pH 7.40), which was the optimized test condition (Fig. S1). As shown in Fig. 1a. PCN displayed a broad absorption band centred at 430 nm

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with yellow colour (Fig. 1a inset). Upon addition of increasing amount of SO_3^{2-} (0–30 μM), the maximum absorption peak at 430 nm gradually decreased and a new absorption band at 345 nm appeared with a well-defined isosbestic point at 358 nm. When the concentration of SO_3^{2-} reached to 30 μM , these changes in the absorption spectra were found to reach a plateau. Meanwhile, the colour of the solution changed from yellow to colourless (Fig. 1a inset), which suggested that the probe **PCN** could serve as a colorimetric probe for SO_3^{2-} . The absorbance ratios (A_{345}/A_{430}) was linearly ($R^2 = 0.9907$) related to the concentrations of SO_3^{2-} ranging from 0 to 15 μM (shown in Fig. 2a), which indicated that **PCN** could be used to quantitatively detect SO_3^{2-} .

The probe **PCN** itself exhibited an emission band centred at 535 nm in DMSO/PBS solution ($v/v = 1/1$, pH 7.40), as shown in Fig. 1c. Upon treatment of the probe with increasing amount of SO_3^{2-} (0–30 μM), the maximum fluorescence band at 535 nm remarkably decreased and concomitantly a new emission band appeared at 398 nm, providing a ratiometric manner for the detection of SO_3^{2-} . The newly formed emission band centred at 398 nm was increased progressively until the concentration of SO_3^{2-} reached to 30 μM . By plotting the fluorescence intensity ratios (I_{398}/I_{535}) versus the concentrations of SO_3^{2-} , a good linear relationship was obtained with the concentration ranging from 0 to 15 μM , as shown in Fig. 2b. The limit of detection was calculated to be 12 nM based on signal to noise ratio ($S/N = 3$), which was much lower than the threshold levels of Na_2SO_3 in food ($< 30 \text{ mg kg}^{-1}$). To demonstrate practical application of the probe, we tentatively made a paper test strip for the detection of SO_3^{2-} . Neutral filter papers were dipped into **PCN** solution (0.10 mM) for 10 min, and then was dried at ambient temperature. The

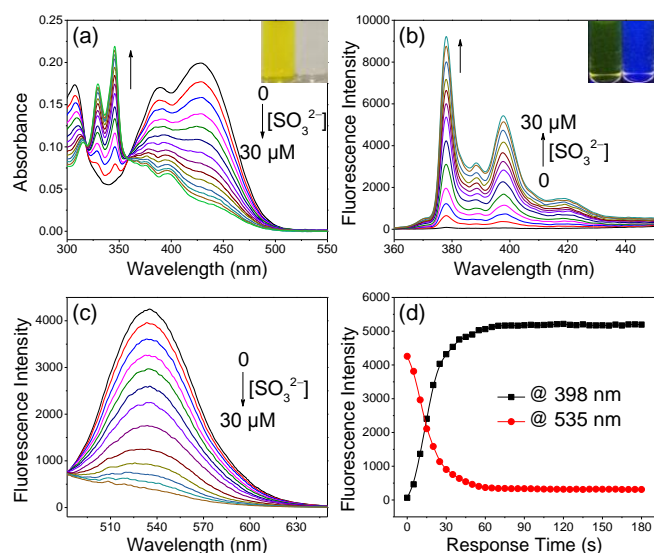


Fig. 1 (a) UV-vis absorption spectra changes and (b-c) fluorescence spectra changes of **PCN** (10 μM) upon addition of increasing amount of SO_3^{2-} (0–30 μM) in DMSO/PBS solution ($v/v = 1/1$, pH 7.40). For (b) $\lambda_{\text{ex}} = 340 \text{ nm}$, slits: 1.0/1.0 nm; for (c) $\lambda_{\text{ex}} = 440 \text{ nm}$, slits: 2.5/5.0 nm. Each spectrum was recorded after 3 min. Inset: (a) The colour and (b) Fluorescence images of **PCN** in the absence and presence of SO_3^{2-} . (d) Time-resolved fluorescence responses of **PCN** (10 μM) to SO_3^{2-} (30 μM) in DMSO/PBS solution ($v/v = 1/1$, pH 7.4). $\lambda_{\text{ex}}/\lambda_{\text{em}} = 340/398 \text{ nm}$, slits: 1.0/1.0 nm (■); $\lambda_{\text{ex}}/\lambda_{\text{em}} = 440/535 \text{ nm}$, slits: 2.5/5.0 nm (●).

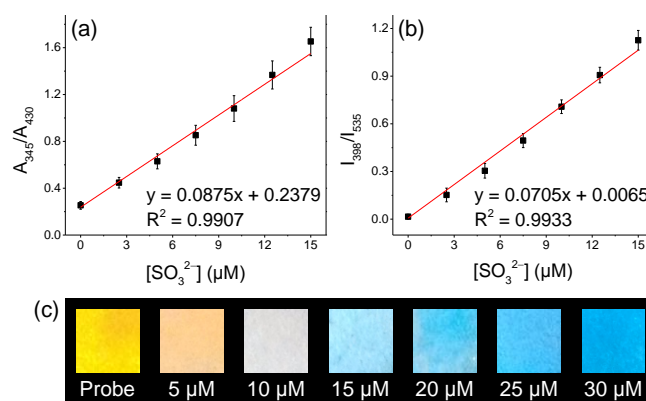


Fig. 2 (a) Linear relationships between (a) UV-vis absorbance ratios and (b) fluorescence ratios of **PCN** (10 μM) versus concentrations of SO_3^{2-} in DMSO/PBS solution ($v/v = 1/1$, pH 7.40). For (b) $\lambda_{\text{ex}}/\lambda_{\text{em}} = 340/398 \text{ nm}$, slits: 1.0/1.0 nm; $\lambda'_{\text{ex}}/\lambda'_{\text{em}} = 440/535 \text{ nm}$, slits: 2.5/5.0 nm. Each spectrum was recorded after 3 min. Error bars are $\pm \text{SD}$, $n = 3$. (c) The fluorescence images of **PCN** test paper with different concentrations of SO_3^{2-} .

test paper display yellow emission under UV light (365 nm). When various concentrations of SO_3^{2-} were dropped onto the test paper, it displayed different colour from yellow to cyan, as shown in Fig 2c.

Time-course and pH-dependent fluorescence responses of **PCN** towards SO_3^{2-}

The time-course fluorescence responses of **PCN** (10 μM) towards SO_3^{2-} (30 μM) were measured, and the fluorescence intensity at 398 nm and 535 nm was plotted as a function of time (s) for data analysis, as shown in Fig. 1d. Upon the addition of 30 μM SO_3^{2-} , the fluorescence intensity of **PCN** promptly increased and reached to a plateau within 30 s, suggesting that **PCN** could serve as a “fast response” fluorescent probe for SO_3^{2-} .

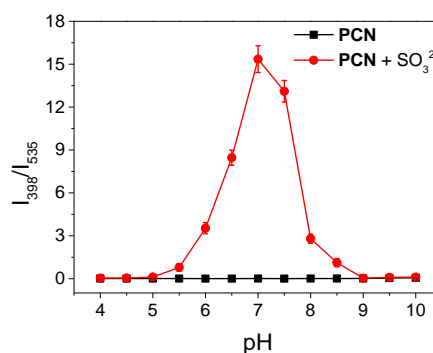


Fig. 3 The pH-dependent ratiometric fluorescence changes of **PCN** (10 μM) in the absence (■) or presence (●) of SO_3^{2-} (30 μM) in DMSO/PBS solution ($v/v = 1/1$). $\lambda_{\text{ex}}/\lambda_{\text{em}} = 340/398 \text{ nm}$, slits: 1.0/1.0 nm; $\lambda'_{\text{ex}}/\lambda'_{\text{em}} = 440/535 \text{ nm}$, slits: 2.5/5.0 nm. Each spectrum was recorded after 3 min. Error bars are $\pm \text{SD}$, $n = 3$.

The pH-dependent fluorescence responses of **PCN** toward SO_3^{2-} were also investigated, as shown in Fig. 3. The fluorescence intensity ratios of **PCN** were invariable from pH 4.0 to 9.0, suggesting that this probe was stable at a wide range of pH values. Upon the addition of 30 μM SO_3^{2-} , the fluorescence intensity of **PCN** increased drastically at pH values

ranging from 5.0 to 8.5. Therefore, **PCN** can be used to detect SO_3^{2-} and HSO_3^- at a wide range of pH values (Fig. S2).

The selectivity of **PCN** towards SO_3^{2-}

To evaluate the selectivity of probe for SO_3^{2-} , various species were examined including some representative anions and small-molecule thiols. As shown in Fig. 4, the probe showed negligible responses to some representative anions (F^- , Cl^- , Br^- , I^- , NO_2^- , NO_3^- , AcO^- , HCO_3^- , CO_3^{2-} , H_2PO_4^- , HPO_4^{2-} , SO_4^{2-} , and $\text{S}_2\text{O}_3^{2-}$). By contrast, noticeable colour change and fluorescence change were detected in the presence of 30 μM SO_3^{2-} , which could be observed by naked eyes (shown in Fig. 4 inset a and b). More importantly, some bio-thiols including cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), did not induce any colour changes and fluorescence changes. These results suggest that the probe has excellent selectivity towards SO_3^{2-} over other anions and biological species. Therefore, this probe has potential application for the detection of SO_3^{2-} in food and beverage.

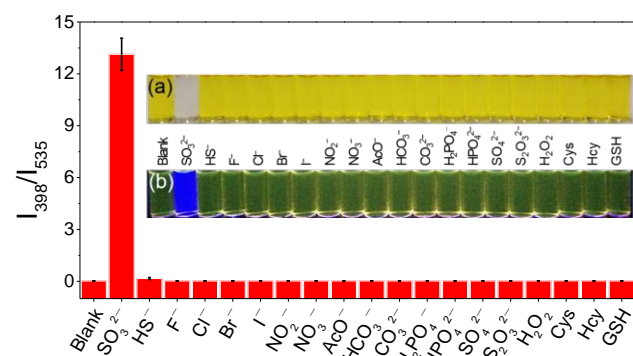


Fig. 4 Fluorescence intensity ratios (I_{398}/I_{535}) of **PCN** (10 μM) upon addition of various anions (5 mM), and reactive sulphur (500 μM HS^- , 5 mM Cys, Hcy and GSH, 30 μM SO_3^{2-}) in DMSO/PBS solution ($v/v = 1/1$, pH 7.40). $\lambda_{\text{ex}}/\lambda_{\text{em}} = 340/398$ nm, slits: 1.0/1.0 nm; $\lambda'_{\text{ex}}/\lambda'_{\text{em}} = 440/535$ nm, slits': 2.5/5.0 nm. Each spectrum was recorded after 3 min. Error bars are \pm SD, $n = 3$. Inset: photographs of **PCN** solutions (30 μM) in the presence of different analytes, taken under the visible light (a) and UV light (b).

The sensing mechanism

^1H NMR titration experiments were performed to investigate the sensing mechanism. As shown in Fig. 5, the proton signal at 9.37 ppm was ascribed to the vinyl group of the probe. Upon addition of 3.0 equiv. of SO_3^{2-} , the proton signal at 9.37 ppm shifted to 5.08 ppm, implying that the vinyl group of **PCN** was interrupted by SO_3^{2-} . Moreover, the formation of a **PCN**- Na_2SO_3 adduct was confirmed by high-resolution mass spectroscopy (Fig. S7), where a dominant peak at an m/z value of 406.0745 (calcd 406.0749) was corresponding to [**PCN** + HSO_3^-]. These results confirmed that SO_3^{2-} added to the vinyl group of **PCN** following Michael addition reaction (shown in Scheme 1).

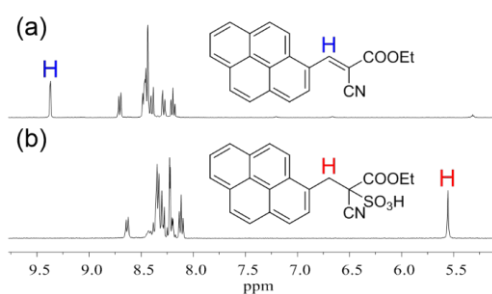


Fig. 5 Partial ^1H NMR spectra of (a) **PCN** in $\text{DMSO}-d_6$ and (b) **PCN** + Na_2SO_3 in $\text{DMSO}-d_6-D_2\text{O}$.

Detection of SO_3^{2-} in real samples by the probe

Finally, we used the probe **PCN** to detect SO_3^{2-} in real samples. Granulated sugar and crystal sugar were purchased from a supermarket, and were used directly in the experiment. To prepare stock solution, 1.0 g of sugar was dissolved in twice-distilled water and diluted to 10 mL. Aliquots of the sugar solution were added to the **PCN** solution (10 μM) in PBS solution ($\text{DMSO}/\text{PBS} = 1/1$, pH 7.4), and the emission intensities at 535 and 398 nm were recorded (Fig. S3). Table 1 shows that the probe **PCN** can determine the concentration of SO_3^{2-} in sugar with good recovery. The amount of Na_2SO_3 in granulated sugar and crystal sugar were determined to be 18.5 and 15.3 mg/kg, respectively.

Table 1 Determination of sulfite in real samples with the probe **PCN**

Samples	sulfite content/ (μM)	Added/ (μM)	Found/ (μM)	Recovery/%
Granulated sugar	2.21	2.0	4.16	97.5
Crystal sugar	1.83	4.0	6.41	105.0
Crystal sugar	1.83	2.0	3.89	103.0
Crystal sugar	1.83	4.0	5.97	103.5

Experimental

Materials and Instrument

Unless otherwise stated, all chemicals and solvents were of analytical grade and used without further purifications. Pyrene-2-carbaldehyde and ethyl cyanoacetate were purchased from Aladdin. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as the internal standard. The chemical shift was recorded in ppm and the following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectra were measured by a HP-1100 LC-MS spectrometer. UV-vis spectra were recorded on a Hitachi UV 3310 spectrometer. Fluorescence spectra were recorded on a Hitachi FL-4700 fluorometer. Fluorescent images were acquired on a Nikon A1 confocal laser-scanning microscope with a 100 objective lens. The solvents used for UV-vis and fluorescence measurements were of HPLC grade.

Responses of probe toward various analytes

Stock solution of the probe (1 mM) was prepared in HPLC grade ethanol. Stock solutions of analytes (2.5–5 mM) were

prepared in twice-distilled water. For optical measurements, probe was diluted to 10 μM in DMSO/PBS solution ($v/v = 1/1$, pH 7.4), and 3.0 mL of the resulting solution was placed in a quartz cell of 1 cm optical path length each time. The UV-vis or fluorescent spectra titrations were recorded upon addition of analytes. All spectroscopic experiments were carried out at room temperature.

Preparation and characterization of probe

1-Pyrenecarboxaldehyde (230 mg, 1 mmol) and ethyl 2-cyanoacetate (125 mg, 1.1 mmol) were dissolved in 5 mL of dry ethanol, then piperidine (0.1 mL) and acetic acid (0.1 mL) were added. The reaction mixture was heated at 80 $^{\circ}\text{C}$ under nitrogen atmosphere. After the reaction was completed (monitored by TLC), the reaction mixture was cooled, and the product precipitated from the solution. Then, it was filtrated, and the solid was collected. The crude product was recrystallized with ethanol to give the final product as light yellow needles (286 mg, 86%). ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 9.37 (s, 1H, =CH₂), 8.70 (d, $J = 8.0$ Hz, 1H, Ar-H), 8.48 – 8.43 (m, 5H, Ar-H), 8.39 (d, $J = 8.8$ Hz, 1H, Ar-H), 8.28 (d, $J = 8.8$ Hz, 1H, Ar-H), 8.19 (t, $J = 7.6$ Hz, 1H, Ar-H), 4.43 (q, $J = 7.0$ Hz, 2H, -CH₂-), 1.39 (t, $J = 7.0$ Hz, 3H, -CH₃). ^{13}C NMR (100 MHz, CDCl₃) δ (ppm) 162.91, 152.02, 134.76, 131.44, 131.07, 130.39, 130.23, 129.78, 127.34, 127.06, 126.87, 126.65, 126.25, 125.05, 124.67, 124.60, 124.20, 121.83, 116.13, 104.08, 62.79, 14.28. HR-MS (ESI): calculated for [C₂₂H₁₅NO₂ + H]⁺ 326.1181; Found 326.1138.

Conclusions

In summary, a colorimetric and ratiometric fluorescent probe for SO₃²⁻ was developed based on Michael addition reaction. The probe showed high selectivity and sensitivity toward SO₃²⁻ with fast response and a low limit of detection (12 nM). The fluorescence intensity ratios were linearly related to the concentrations of SO₃²⁻ from 0 to 15 μM , which indicated that PCN could be used to quantitatively detect SO₃²⁻. A simple test paper was developed for rapid monitoring of SO₃²⁻, which displayed different colour in the presence of various amounts of sulfite. Moreover, the probe has been successfully used to determine the levels of SO₃²⁻ in sugar samples. Therefore, this probe has great potential application for food safety and quality control.

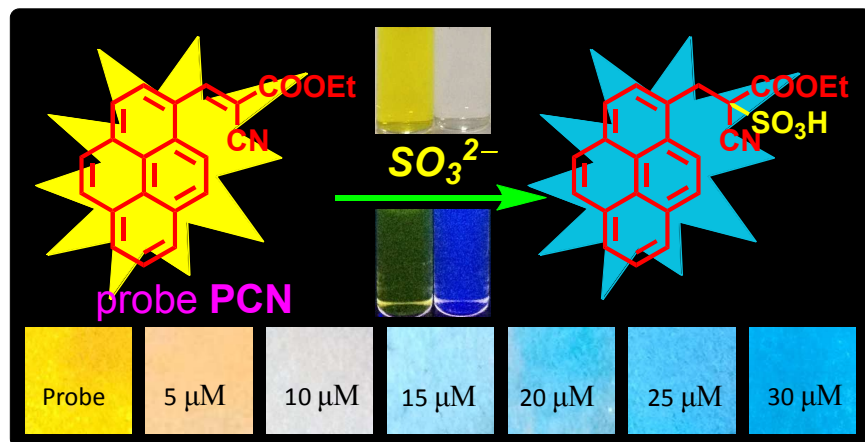
Acknowledgements

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