

## Paper-Based Analytical Device for Rapid Naked-Eye Detection of Sulfide in Water Samples

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**Summary:** This paper describes the development of a low-cost paper-based colorimetric analytical device for the accurate and rapid determination of sulfide. Under optimized conditions, Na<sub>2</sub>S was dropped in the uptake zone I and the probe in the uptake zone II, they converged to the detection zone via capillary force and formed an intense pink resorufin. Sulfide can be quantified based on the average color intensity values of the product "free resorufin". The color intensity is recorded using a camera phone, and quantification was made using Adobe Photoshop. The as-developed analytical device detected sulfide in the range of 5–400 μM (R<sup>2</sup> = 0.982) with the limit of detection (LOD) 1 μM, and was successfully applied in sulfide assay in spiked water samples including tap water and simulated waste water. Colorimetric results from the proposed paper-based colorimetric analytical device were consistent with that from methylene blue (MB) method.

**Keywords:** Sulfide, Paper-based analytical devices, Colorimetry, Resorufin, 4-Chloro-7-nitrobenzofurazan.

### Introduction

Disposable analytical devices are of great concern due to the low-cost, rapid and on-site detection of analytes of interest. Thus, there has been a recent rise in the use of microfluidic paper-based analytical devices (μPADs) as a platform for such analysis. Since they were first reported by the Whitesides' group[1], μPADs is a new alternative device that can be used in food analysis [2-4], environmental assay [5-7], and clinical diagnosis[8-9] owing to their advantages of simplicity, disposability, low cost, portability, low sample volume requirements and so forth.

Hydrogen sulfide (H<sub>2</sub>S), produced widely from various industrial processes, is a colorless, and toxic gas with the unpleasant odor of rotten eggs. Immediate exposure to H<sub>2</sub>S at concentrations exceeding 300 ppm is considered dangerous for human life or health, as is continuous exposure at concentrations exceeding 20 ppm [10]. Just recently, H<sub>2</sub>S, other than nitric oxide and carbon monoxide, is recognized as the third endogenous gaseous signal transmitter and exerts potent effects on neurotransmission mediation, blood pressure reduction, and vascular smooth-muscle relaxation [11-13]. The abnormal level of H<sub>2</sub>S is involved in the symptoms of many diseases such as diabetes, Alzheimer's disease, Down's syndrome, and liver cirrhosis [14-16]. Therefore, an efficient method for

accurate, rapid and sensitive detection of H<sub>2</sub>S levels to guarantee human health is quite important. Because H<sub>2</sub>S is a kind of gaseous molecule, traditional methods for H<sub>2</sub>S detection are mostly based on the detection of dissolved sulfide to indirectly reflect H<sub>2</sub>S levels. Sulfide can exist in aqueous media with different forms such as dissolved H<sub>2</sub>S, bisulphide anion (HS<sup>-</sup>, pK<sub>a1</sub> = 6.88) and sulfide anion (S<sup>2-</sup>, pK<sub>a2</sub> = 14.15), the major sulfide species of H<sub>2</sub>S are depending on pH value of water. Thus, it is of great important to design the simply and efficient sensor for sulfide assay to quantify H<sub>2</sub>S levels in biosystems.

Various analytical techniques existed for the detection of dissolved sulfide including electrochemical methods [17-19], spectrometric methods including fluorometry [23] and colorimetry [20-22, 27, 28, 31-33], gas or ion chromatography and so forth [24-26]. Since the modern society highly demands rapid and accurate analysis with convenient operation and low cost, currently, colorimetric sensors have become an active field, and provide an fascinating analytical platform for industrial and environmental applications owing to the simplicity of the approach, visual signal feedback, facile quantification and no sophisticated instruments. The vast majority of colorimetric methods for sulfide detection are all based on the properties of sulfide

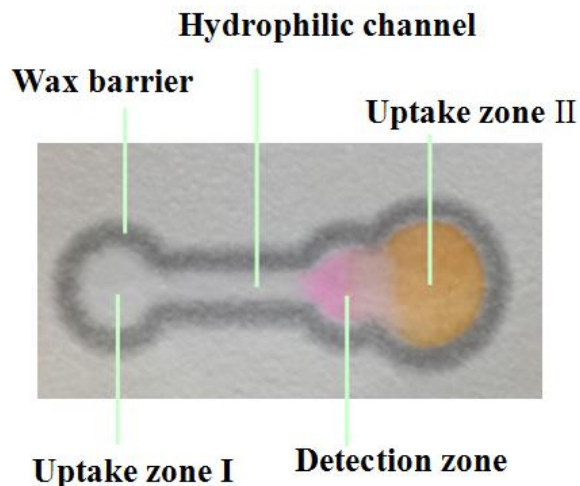
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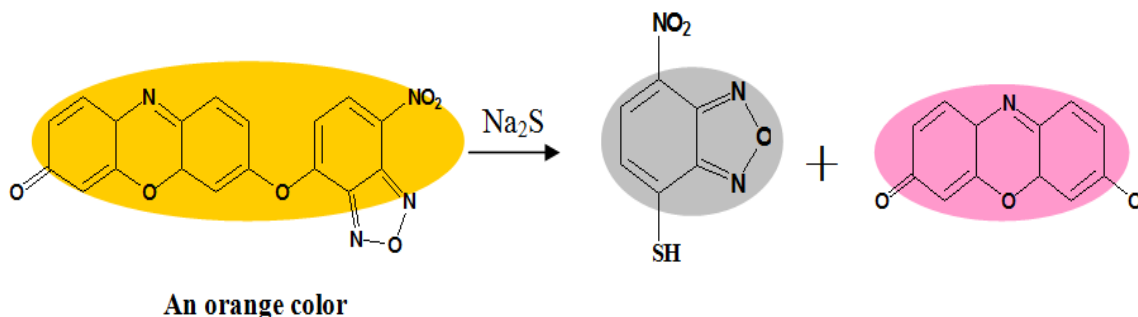
such as the strong reducibility, strong metal affinity to form metal sulfide (CuS, PbS, Ag<sub>2</sub>S and so forth), and strong nucleophilicity. For instance, Yuan *et al.* [27] developed a colorimetric method for sensing sulfide using functionalized Au nanoparticle with the azido-modified surface to recognize the sulfide. The visual method is based on the strong reducibility of sulfides. Cha *et al.* [28] developed a colorimetric method for sensing sulfide based on the formation of a dark brown precipitate of PbS.

Herein, we describe a paper-based sensor for sulfide detection based on the use of the probe. The probe was used here as chromogenic indicators in response to the analyte based on the strong nucleophilicity of sulfides. Hydrophilic paper was employed as analytical substrate for running colorimetric assay, and a wax-printing technique was used to create  $\mu$ PADs. As shown in Scheme-1, the pattern of the  $\mu$ PADs was printed on a single paper and was designed for parallel assays. The principle is displayed in Scheme 1 and 2. In brief, sulfide can be quantified based on the average color intensity values of the product “free resorufin” which come from sulfide-triggered the cleavage reaction of the probe. The probe combines resorufin with 7-nitrobenzofurazan (NBD) and exhibits high stability, a fast color response to sulfide, furthermore, the pink color of the product is more eye-catching and easier to observe than yellow and other colors. Thus, the probe showed higher sensitivity as a naked-eye indicator of sulfide on the as-proposed  $\mu$ PADs. Besides, the fabrication process of  $\mu$ PADs based on wax-printing does not require large microfabrication facilities, which was simpler, less expensive, and faster than other reported methods.

The color intensity is recorded using a common cellphone, and quantification was made using Adobe Photoshop, and the data can be immediately analyzed. Thereby, the combination of the probe and the as-designed  $\mu$ PADs for naked-eye detection of sulfide not only facilitates signal readout recording, but also preserves the simplicity of the technique, demonstrating the potential application in on-site rapid detection and screening.



Scheme-1: The designed  $\mu$ PAD for sulfide detection showing uptake zones, detection zones, and the hydrophilic channel surrounded by the hydrophobic wax barrier. The color of uptake zone II and testing zone were probe RN and the reaction product (probe reacted with sulfide), respectively



Scheme-2: The mechanism of as-proposed colorimetric assay for Na<sub>2</sub>S.

## Experimental

### Chemicals and solutions

Resorufin sodium salt, 4-Chloro-7-nitro-1, 2, 3-benzoxadiazole/ 4-Chloro-7-nitrobenzofurazan (NBD-Cl), dopamine, lactate, glucose, sodium ascorbate, uric acid, 5-hydroxytryptamine, and homocysteine were all purchased from Sigma-aldrich. Cysteine, glutathione and other amino acids, sodium sulfide non-anhydrate ( $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ ), N, N-Dimethyl-p-phenylenediamine monohydrochloride (NDPM), the deuterium generation trifluoroacetic acid ( $\text{CF}_3\text{COOD}$ ) were obtained from Aladdin company. Whatman grade 1 chromatography paper was obtained from Cole-Parmer Whatman (UK). Other chemicals were of at least analytical grade and used as received. Phosphate-buffered saline (20 mM PBS, pH 7.4) was prepared containing 27.2mM NaCl, 0.54mM KCl, 17.4 mM  $\text{Na}_2\text{HPO}_4$ , and 2.8 mM  $\text{KH}_2\text{PO}_4$  and the solution pH was adjusted to 7.4. Aqueous solutions of sulfide, cysteine, GSH, and homocysteine were freshly prepared with 20 mM PBS. A stock solution (1 mM) of probe RN was prepared by dissolving an appropriate amount of the probe in methyl sulfoxide (DMSO) / PBS (v/v, 1:9, pH 7.4). Ultrapure water (over 18 M $\Omega$  cm) from a Milli-Q reference system (Millipore) was used throughout.

### Synthesis of the probe

The probe is synthesized based on the principle as shown in Fig. S1. In detail, a suspension of resorufin sodium salt (70mg, 0.3mmol) in absolute acetonitrile (7 mL),  $\text{K}_2\text{CO}_3$  (15mg, 0.45mmol) was added, followed by stirring at 40 °C for 10 min under an Ar atmosphere. Then, a solution of NBD-Cl (70, 0.45mmol) in  $\text{CH}_3\text{CN}$  (3mL) was added dropwise. The resulting mixture was stirred at 40 °C for 5 h and then diluted with  $\text{CH}_2\text{Cl}_2$  (50 mL). The organic layer was separated, washed three times with water (50 mL  $\times$  3) and brine (50 mL  $\times$  3), and then dried over  $\text{Na}_2\text{SO}_4$ . The solvent was removed by evaporation, and the residue was subjected to silica gel chromatography, eluted with  $\text{CH}_2\text{Cl}_2$ /  $\text{CH}_3\text{OH}$  (v/v, 100:1), affording the probe as an orange solid (0.14 g, 32%).  $^1\text{H}$ NMR (600 MHz, 298K,  $\text{CF}_3\text{COOD}$ ):  $\delta$  7.37 (d, J = 8.1 Hz, 1H), 7.32 – 7.15 (m, 1H), 7.03 (d, J = 9.6 Hz, 1H), 6.49 (dd, J = 9.2, 2.1 Hz, 1H), 162.98, 160.70, 150.75, 145.88, 143.61, 142.39, 136.02, 134.15, 133.78, 132.19, 127.36, 120.58, 115.71, 113.84, 111.96, 110.08, 104.82, 101.74. HR-MS: calcd. For probe RN ( $\text{C}_{18}\text{H}_9\text{N}_3\text{O}_5\text{S}$ ) 399.2730 [M] $^+$ , found: 399.0336 [M] $^+$

### Apparatus

$^1\text{H}$ NMR and  $^{13}\text{C}$ NMR spectra were measured

on a Bruker DMX-400 spectrometer in  $\text{CF}_3\text{COOD}$ . Electrospray ionization mass spectra were recorded in negative mode with a Shimadzu liquid chromatography–mass spectrometry 2010A instrument (Kyoto, Japan). Absorption spectra were recorded in 1 cm quartz cells with a TU-1900 spectrophotometer (Beijing, China).

### Design and fabrication of $\mu\text{PADs}$

The design of  $\mu\text{PAD}$  described here were generated using Inkscape-0.91 software (Scheme-1). The resulting design was printed on a single paper by using the wax printing method. The fabrication process includes two steps: 1) the designed pattern was printed on Whatman grade 1 chromatography paper by using Artesian Solid Ink on a Xerox Phase 8400 wax printer. 2) The wax printed filter paper was then placed in an oven for 100 s at 175°C to allow the wax to melt and penetrate the chromatography paper. The printed area became hydrophobic leaving the unprinted area hydrophilic, creating the hydrophobic barriers to guide fluid movement. The diameters of the uptake zone I, uptake zones II and detection zone were 4, 6, and 4 mm, respectively. The length and width of the hydrophilic channel was 7 mm and 2 mm, respectively.

### Colorimetric assay of sulfide on the $\mu\text{PADs}$

The measurements were conducted as follows: First, the standard solution of  $\text{Na}_2\text{S}$  was prepared by dissolving the compound in PBS. After fabrication of the  $\mu\text{PADs}$ , 6.2  $\mu\text{L}$  of  $\text{Na}_2\text{S}$  standard/sample solution with different concentration was dropped in the uptake zone I and then 6.20  $\mu\text{L}$  of 200  $\mu\text{M}$  probe was dropped in the uptake zone II. They converged into the detection zone via capillary forces, causing the formation of the pink colored compound. After observing color, the  $\mu\text{PAD}$  was allowed to dry for 8 min. For quantitative analysis, the photograph of the results on the paper-based sensor was recorded by using an iPhone 7 camera under a fixed daylight bulb under optimized brightness to guarantee the same brightness. Under optimized conditions, the color intensity has the dependent on the concentration of sulfide. The capture distance will not affect the results because the photograph is scanned using the CS scanning application software (IntSig Information Co., Ltd) that is installed on the iPhone 7. Quantitative image processing was made using Adobe Photoshop (version 7.0). The eyedropper tool in this software was used to quantify the color intensity. The greenish color derivatives are complementary colors (i.e. negative images) of pink-reddish colors, while the blue color derivatives are the complementary colors of orange-brownish. The reaction of sulfide with the probe formed a pink color

onto the detection zone of  $\mu$ PADs. Therefore, inverse green “mean” color intensity was used to quantify the concentration of sulfide provided higher sensitivity than the other colors of the red-green-blue (RGB) color mode in Adobe Photoshop.

#### Sulfide signaling in water samples

Simulated wastewater has a composition of 2.39 mM  $\text{Na}^+$ , 0.281mM  $\text{K}^+$ , 0.288 mM  $\text{Mg}^{2+}$ , 0.250 mM  $\text{Ca}^{2+}$ , 16 $\mu\text{M}$  F, 0.987 mM  $\text{Cl}^-$ , 0.105 mM  $\text{PO}_4^{3-}$ , 0.208 mM  $\text{SO}_4^{2-}$ , 0.484 mM  $\text{NO}_3^-$ , 1.23 mM  $\text{HCO}_3^-$ . 20 mM PBS in tap water and simulated wastewater was prepared. Stock solutions of  $\text{Na}_2\text{S}$  were prepared in tap water and simulated wastewater. The procedure of sulfide detection is the same as colorimetric assay of sulfide standard solution on paper-based devices. The changes in the color intensity for tap water and simulated wastewater were measured as a function of  $\text{Na}_2\text{S}$  concentrations.

### Results and Discussion

#### Preliminary experiment with the probe for colorimetric detection of Sulfide

In this work, the feasibility of the probe strategy on paper-based sensor for sulfide detection was investigated. First, the probe was synthesized by etherification of the 7-hydroxy group of resorufin with 4-Chloro-7-nitro-1,2,3-benzoxadiazole (NBD-Cl). The absorption profile of the probe before and after reacting with sulfide is shown in Fig. 1. The probe itself exhibits a very weak absorption in the long-wavelength region. In the presence of sulfide, their reaction solution produces a strong absorption at about 575 nm with the distinct color shift from nearly colorless to pink (see the inset of Fig 1B). The reason for the absorption enhancement of the probe is attributed to the fact that sulfide-triggered cleavage reaction causes the release of free resorufin[30] (in Scheme 2). The absorption peak at 575 nm is the characteristic absorption peak of free resorufin. These sharp absorption responses and color changes are rather desirable for naked-eye sulfide detection on the  $\mu$ PADs with high sensitivity.

Fig. 1A shows a typical trace of absorption response of the probe solution and the relative intensity had the dependence on the concentrations of sulfide, and the calibration curve is  $A_{585} = 0.00197C_{\text{Sulfide}} + 0.01351$  ( $R^2 = 0.9936$ ) within a linear range of 5–150  $\mu\text{M}$ , the detection limit reached to 0.1 $\mu\text{M}$ .

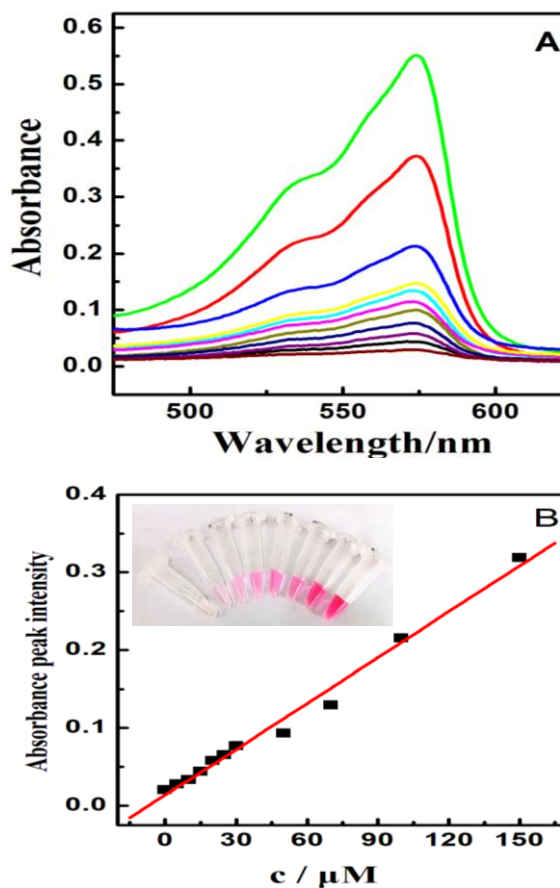


Fig. 1: (A) Absorption spectra of the probe (10  $\mu\text{M}$ ) upon the addition of different concentrations of  $\text{Na}_2\text{S}$  (from the bottom to top: 0, 5, 10, 15, 20, 25, 30, 50, 70, 100, 150  $\mu\text{M}$ ) in DMSO / PBS (v/v, 1:99, pH 7.4). (B) plot of f absorption intensity against  $C_{\text{Sulfide}}$  ( $\mu\text{M}$ ). The color changes of the probe (10  $\mu\text{M}$ ) before and after the reaction with  $\text{Na}_2\text{S}$  (from left to right: 0, 5, 10, 30, 50, 70, 100, 150  $\mu\text{M}$ ) are shown in the inset.

Though the above solution based on the probe can provide an accurate result for sulfide detection by the absorption spectrum, it can only be implemented in a laboratory set-up. To apply colorimetric method of sulfide detection for rapid and on-site analytical applications, a paper-based sensor was applied for running assay. According to the need and requirement of the application, the pattern of the  $\mu$ PADs can be designed for parallel assays and was printed on a single paper as displayed in scheme 1. In detail, after  $\text{Na}_2\text{S}$  solution was dropped into the Uptake zone I, the probe solution was immediately spotted into the Uptake zone II. They flowed and converged to the detection zone rapidly and showed a clear pink color (Scheme 1) via

capillary force. In the absence of  $\text{Na}_2\text{S}$ , the color at the detection zone was pale yellow, attributed to the dilution of the probe. However, the  $\mu\text{PADs}$  developed a pink color instantaneously after applying  $\text{Na}_2\text{S}$  solution (Scheme 1), which can be monitored by naked eyes after 20 s. In view of the low visibility of colors on the  $\mu\text{PADs}$ , colorimetric sensing in paper-based devices generally must use a higher indicator concentration than in solution phase. Herein, 200  $\mu\text{M}$  probe solution was selected on the  $\mu\text{PADs}$ , while 10  $\mu\text{M}$  probe solution selected in solution phase. As seen, pink is more eye-catching and easier to observe than yellow and other colors. Thus, the probe showed higher sensitivity as a naked-eye indicator of sulfide on the as-proposed  $\mu\text{PADs}$ . Thereby, the combination of the probe and the as-designed  $\mu\text{PADs}$  for naked-eye detection of sulfide not only facilitates signal readout recording, but also preserves the simplicity of the technique. The convenience is retained and batch fabrication process does not require large microfabrication facilities, demonstrating the potential application in on-site rapid detection and screening.

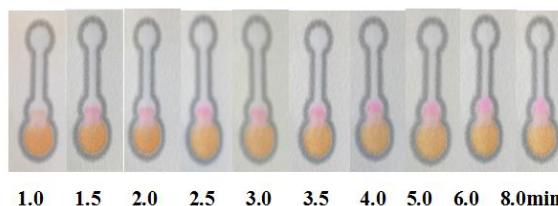
#### Optimization of experimental conditions

In order to obtain the best performance on the as-developed  $\mu\text{PADs}$ , the experimental conditions containing spotted sample volume, pH of buffer solutions, the concentration of the probe, and the color development time on sensitivity was optimized next.

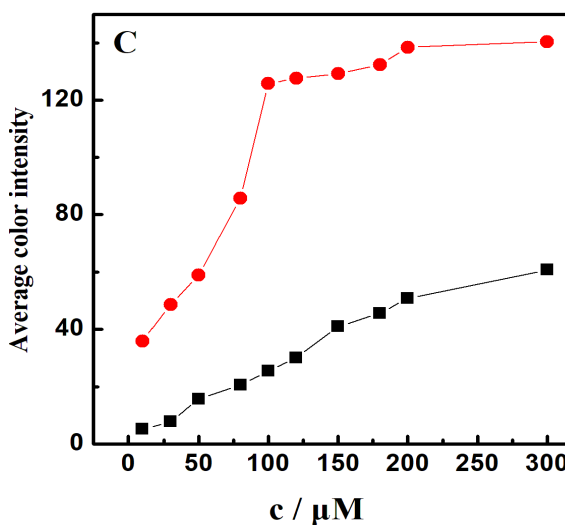
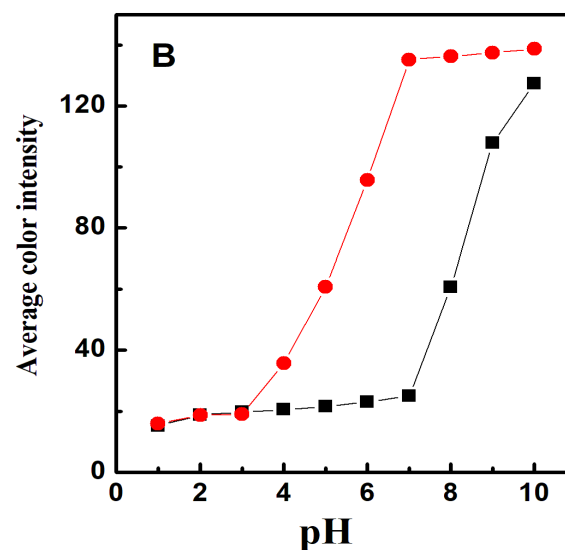
A limited sample volume may cause the color to develop insufficiently, while excessive addition of the solution may result in the reagent to diffuse out of the test zone. Therefore, the spotted sample volume was optimized. The color intensity in volumes ranging between 4.5 to 7.0  $\mu\text{L}$  was measured and the color development time was fixed at 8 min. Investigation showed that the spotted sample volume significantly affected the color intensity and the highest response occurred in 6.2  $\mu\text{L}$ . Hence, the volume of 6.2  $\mu\text{L}$  for sulfide and the probe were chosen for further use.

The pH value is a key factor affecting the major sulfide species ( $\text{H}_2\text{S}$ ,  $\text{HS}^-$ , and  $\text{S}^{2-}$ ) in solution, the stability of the probe and the reaction between sulfide and the probe. In evaluation the effect of pH on the color intensity on the  $\mu\text{PADs}$ , the color development time, the spotted sample volume, and the concentration of the probe were fixed at 8 min, 6.2  $\mu\text{L}$ , and 200  $\mu\text{M}$ , respectively. As seen from Fig.2B, the sulfide sensing became more efficient with pH elevation. At pH 7, the addition of sulfide into the probe solution caused a maximum response after subtracting the background signal. When  $\text{pH} > 8.5$ , the recognition unit of the probe for sulfide sensing is its ether bond, which is easy to

break down into resorufin in basic media resulting in a great background signal. Therefore, taking account of the stability and sensitivity of the probe, and the later application in the physiological conditions and environment monitoring, pH 7.4 was finally selected as the optimal value for subsequent analysis experiments. At pH 7.4,  $\text{HS}^-$  as the major sulfide species exists in the solution.



(a)



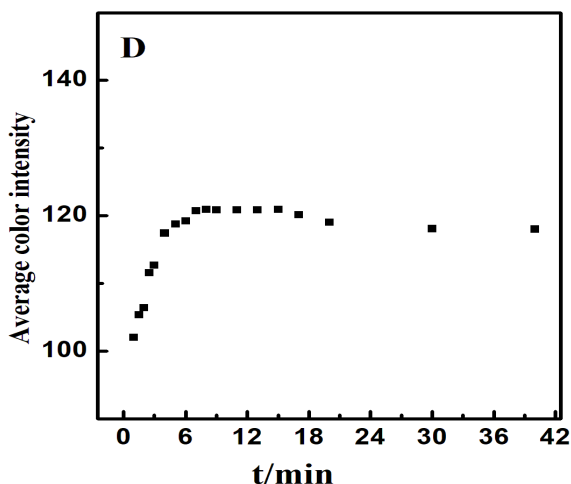


Fig. 2: (A) photographs of  $\mu$ PADs at different reaction times. (B) Effects of pH and (C) the concentration of probe RN on the color intensity. Red line and black line is the color intensity from the probe RN in the absence, and the presence of and 100  $\mu$ M  $\text{Na}_2\text{S}$ . (D) Effects of the reaction time on the color intensity.

The concentration of the probe is crucial for sulfide detection, its effect was examined in the range of 10–300  $\mu$ M. In evaluation the effect of the concentration of the probe on the color intensity, the color development time, the spotted sample volume, and pH were fixed at 8 min, 6.2  $\mu$ L, and 7.4, respectively. The change in the color intensity values of the probe in the absence and the presence of sulfide was determined. As seen in Fig. 2C, the sulfide recognition became more efficient with increasing the concentration of the probe. However, when the concentration of the probe exceed 200  $\mu$ M, the deep yellow color of the probe constructs the great background color and interferes by naked-eye monitoring the pink color from the reaction product resorufin. Therefore, the optimized concentration of the probe was 200  $\mu$ M.

The effect of the color development time on sensitivity was examined next. Dry time affect the development of color. As seen in Fig.2A, The photographs were consecutively recorded every 0.5 min at room temperature immediately after the addition of  $\text{Na}_2\text{S}$  and the probe to the uptake zones. The color intensity was found to plateau at 8 min in Fig.2B. Increasing drying time (exceed 20 min) will cause the reduction of the color signal, which can be attributed to the color indicator concentrated and enriched as the solvent evaporated and the paper

dried. Therefore, the optimized color development time was 8 min.

#### Analytical performance

The performance of  $\mu$ PADs was evaluated for quantitative measurement of  $\text{Na}_2\text{S}$ . Under the optimized conditions, the average color intensity values at the detection zone was proportional to the concentration of  $\text{Na}_2\text{S}$  within a range of 5 to 400  $\mu$ M along with a striking shift of color from faint yellow to pink (Fig 3A). The calibration curve was  $Y=41.198+0.6193 C_S (\mu\text{M})$  ( $R^2 = 0.982$ ) in the range from 5 to 200  $\mu$ M. The average color intensity of one image was calculated by parallel measuring the “mean” value of G channel in different regions of “detection zone” five times by Photoshop. Based on of the  $3\sigma/s$  rule ( $\sigma$  and  $s$  are the standard deviation of the blank signal and the slope of the linear calibration graph, respectively), the detection limit of the assay was estimated to be 1  $\mu$ M sulfide.



(A)

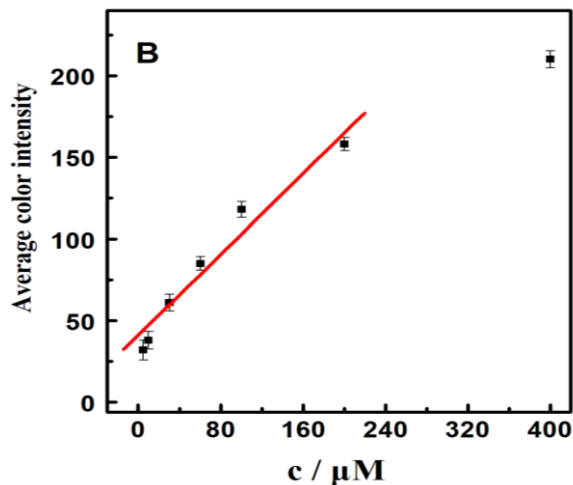


Fig. 3: Paper-based colorimetric sensor for the quantitative analysis of sulfide with different concentration. (A)The photographs and (B) calibration plot of color intensity and the concentrations of sulfide obtained by camera of iPhone 7 (error bar represented the standard deviation at  $n=3$ ).

Table-1: Analytical performance for the detection of sulfide in water samples.

Sample	Added ( $\mu\text{M}$ )	Suggested method	Recovery (%)	RSD%	MB method	Recovery (%)	RSD%
tap water	0	Non-detected			Non-detected		
	3.00	2.95	98.3	0.17	2.98	99.3	0.19
	10.0	10.7	107.0	0.18	10.8	110	0.16
	50.0	51.4	102.8	0.17	51.3	102.6	0.21
Simulated waste-water	100	105	105.0	0.14	107	107.0	0.19
	0	Non-detected			Non-detected		
	10.0	10.8	108	0.17	11.0	110	0.17
waste-water	50.0	50.6	101.2	0.15	50.4	100.8	0.21

The repeatability of the detection strategy based on paper-based analytical device was evaluated for 5 identical  $\mu\text{PADs}$  following the same operation steps. The relative standard deviation (RSD) of inter-day ( $n=15$ ) and intra-day ( $n=15$ ) was 3.2 % and 1.5 %, respectively, showing excellent repeatability of the method.

#### Interference study

To study interferences, we systematically studied the selectivity of the probe for sulfide sensing over various cations ( $\text{K}^+$  and  $\text{Na}^+$ , 500  $\mu\text{M}$ ;  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , 100  $\mu\text{M}$ ;  $\text{Zn}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$ , 50  $\mu\text{M}$ ) and anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{CH}_3\text{CO}_2^-$ ,  $\text{CO}_3^{2-}$ , 200  $\mu\text{M}$ ;  $\text{SO}_3^{2-}$ ,  $\text{S}_2\text{O}_3^{2-}$ , 100  $\mu\text{M}$ ;  $\text{F}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ , 50  $\mu\text{M}$ ). To do this, each kind of the species was assessed under the same conditions just as sulfide. The results demonstrated there was no significant response (under  $\pm 5\%$  deviation) to either one of the interferent tested, suggesting that the proposed  $\mu\text{PADs}$  has a high selectivity for sulfide detection, allowing potential applications in complex samples.

#### Application to water samples

The developed paper-based analytical device was evaluated for detecting sulfide in water samples including tap water and simulated wastewater. To confirm the reliability of data, tap water or simulated wastewater was spiked with the standard solution of sulfide containing different concentrations of  $\text{Na}_2\text{S}$  and was detected using the developed  $\mu\text{PADs}$ . The recovery results are illustrated in Table-1 and are calculated from the calibration plot, the recoveries of sulfide were found in the range of 98.3–107% for tap water and 101.2–108% for simulated wastewater, suggesting that this method is reliable. Furthermore, the results based on the probe were in good agreement with the results based on methylene blue (MB) method perfectly; demonstrating that the developed  $\mu\text{PADs}$  based on the probe is applicable for sulfide detection in water samples.

#### Conclusions

This work describes a paper-based colorimetric analytical device for sulfide detection

based on the use of the probe. A wax-printing technique was used to create the  $\mu\text{PADs}$ . Under optimized conditions,  $\text{Na}_2\text{S}$  was dropped in the uptake zone I and the probe in the uptake zone II, they converged to the detection zone via capillary force and formed an intense pink coloured resorufin. Results can either be observed by naked eyes or analyzed by software. This disposable  $\mu\text{PADs}$  has been used successfully for sulfide assay in spiked tap and simulated wastewater. The simple design, cheap fabrication, and ease of use of  $\mu\text{PADs}$ , make it suitable for use in field measurements of sulfide in resource-limited settings.

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#### Conflict of interest

All the authors have no conflict of interest.

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