**Colorimetric Determination of Sulfide in Turbid Water with a Cost-effective Flow-batch Porous Membrane-based Diffusion Scrubber System**

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A cost-effective flow-batch analysis approach with colorimetric measurement has been developed for sulfide ion determination in turbid water samples without using a conventional pump and valve. Under an acidic condition, sulfide ion was converted to hydrogen sulfide gas and liberated out from other complicated matrices. The porous membrane-based diffusion scrubber was utilized as a gas trapping unit for hydrogen sulfide gas separation/preconcentration. From the correlation of sulfide ion concentration and disappearance of sodium nitroprusside reagent detection by using a homemade LED-photodiode based colorimetry, a linear relationship of sulfide ion concentration and absorbance can be obtained with relative standard deviation (%RSD) less than 5%. The limit of detection was 5.6 μmol L⁻¹. The proposed system was applied for sulfide ion determination in wastewater samples with the recoveries of 91.0 – 105.2%. The proposed system is a robust setup and able to handle turbid water samples without a sample filtering step.

**Keywords** Flow-batch analysis, LED-photodiode-based colorimetry, sulfide determination, porous membrane diffusion scrubber

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**Introduction**

Sulfide ion is one important compound in natural water and environmental systems due to its toxicity to aquatic organisms.¹ Several methods, including spectrophotometry and electrochemistry, have been proposed to determine sulfide ion in various samples.² For the absorption spectrophotometric method, methylene blue chemistry and nitroprusside chemistry have been commonly used for sulfide determination.³ The methylene blue method is based on the oxidative coupling of sulfide ions with N,N-dimethyl-p-phenylenediamine (DMPD) in the presence of Fe(III) in an acidic medium and producing methylene blue dye as reported in the previous articles.³ ⁴ The methylene blue method requires a strongly acidic medium, while the red-violet product of sulfide in the nitroprusside method is formed in an alkaline medium.⁵ ⁶ ⁷

A flow-based analysis system with a gas diffusion unit has been reported for separation of the gas-convertible compounds from the sample matrices.⁸ ⁹ ¹⁰ ¹¹ Flow injection analysis systems coupled with gas diffusion unit (GDU) have been reported for sulfide ion determination by using various detection techniques, such as triangle program coulometric titration,¹² absorption spectrometry via methylene blue method,¹³ ¹⁴ ¹⁵ amperometry,¹⁶ flame photometry¹⁷ and fluorometry via quenching of the fluorescein mercuric acetate (FMA).¹⁸ However, for some turbid samples, a filtering step has been required before introducing the analyte to the flow analysis system.

Sereenonchai et al. adapted a gas diffusion unit in the flow analysis system to be a membraneless vaporization chamber to handle solid samples, such as for calcium carbonate determination in cement.¹⁹ The method is based on the liberation of CO₂ gas in acidic conditions from HCO₃⁻/CO₂ in the cement sample to dissolve into the acceptor solution without using a membrane, and finally, measuring the analyte signal by using a capacitively coupled contactless conductivity detection (C4D) at the outlet of the membraneless vaporization chamber.¹⁹ Timofeeva et al. reported an adapted gas-diffusion chamber with a membrane to connect with a flow injection analysis (GD-FIA) system for ammonium determination in building materials.²⁰ The method is based on the generation of ammonia gas from a solid sample by adding a basic solution. The NH₃ gas was carried by N₂ gas and dissolved into cresol red and thymol blue as an acceptor solution, and finally, the absorbance of the acceptor solution was detected at 580 nm to quantify the NH₄⁺. However, the modified GD-FIA with a conventional pump/valve requires an energy source, which was inconvenient to fabricate as a portable system for on-site monitoring.
The flow-batch analysis approach without using a conventional pump and valve was applied for gas phase chemiluminescence detection for on-site determination as highly sensitive analyzers for determination of arsenic\textsuperscript{21} and dimethyl sulfide\textsuperscript{22} in natural water samples. This design of the flow-batch analysis approach is very interesting in terms of low energy operation and convenience of adaptation for on-site monitoring. Although a highly sensitive signal can be obtained, the systems required an expensive photomultiplier tube. Although the sulfide detection with LED-photodiode based fluorescence based on the quenching of FMA provided a sensitive measurement and simple flow analysis,\textsuperscript{18} the highly toxic reagent of FMA is a drawback. The LED and photodiode colorimeter were adapted for \textit{in-situ} measurement at 535 nm on a gas-permeable porous tube as an optical system for sulfide ion determination in human serum by using nitroprusside chemistry.\textsuperscript{23} In addition, a cost-effective moving liquid drop or mobile drop with a miniature conductivity probe was reported for NH\textsubscript{3} gas measurement converted from NH\textsubscript{4}+ ion without using a conventional pump/valve.\textsuperscript{24} The H\textsubscript{2}SO\textsubscript{4} drop as the gas collection sink was manipulated by a manual stepwise operation. However, the use of liquid drop as a gas collection device could not provide a robust setup for on-site measurement.

This work aims to develop a robust, low-energy consuming, and cost-effective device based on a flow-batch analysis approach to couple a porous membrane-based diffusion scrubber without using pump and valve with a homemade LED-photodiode colorimeter for detection. The sulfide ion in turbid wastewater samples was selected as a model analyte to evaluate the proposed device. The analyte would be separated from other sample matrices by the designed gas conversion unit. The proposed system was applied to some turbid water samples or solid samples without the sample pretreatment step.

**Experimental**

**Reagents and chemicals**

All solutions were prepared using analytical reagent grade chemicals and dissolved in deionized water. The stock of 10 mmol L\textsuperscript{-1} standard sulfide solution was prepared daily by dissolving 0.134 g of sodium sulfide hydrate (Na\textsubscript{2}S\textcdot xH\textsubscript{2}O) in 20 mmol L\textsuperscript{-1} of sodium hydroxide solution and adjusting the total volume to the mark of 100-mL of a volumetric flask. This solution was standardized using iodometric titration.\textsuperscript{25} The desired concentrations of standard solutions were freshly prepared by appropriate dilution with the 20 mmol L\textsuperscript{-1} NaOH solution to preserve the S\textsuperscript{2–} ion in solution. The 0.1 mol L\textsuperscript{-1} phosphate buffer solution pH 12 was prepared by dissolving 4.265 g of disodium hydrogen phosphate 2-hydrate in deionized water and diluting to 250 mL. The pH of the solution was adjusted to 12 by adding 1.0 mol L\textsuperscript{-1} of sodium hydroxide solution. The 4.0 mmol L\textsuperscript{-1} sodium nitroprusside as an acceptor solution was prepared by dissolving 0.13 g of sodium nitroprusside in 0.1 mol L\textsuperscript{-1} phosphate buffer pH 12 and adjusting the volume to the mark of 100-mL of a volumetric flask.

Water samples were collected from a drained water canal in the Chiang Mai city area and stored in polyethylene bottles at 4°C in a refrigerator.

**Apparatus and setup**

The analytical system consisted of three parts, as demonstrated in Fig. 1; i) a gas conversion tube, ii) a porous membrane-based diffusion scrubber, and iii) a homemade colorimeter with a data acquisition unit. The gas acceptor solution bottle was hung on the stand with about 30 cm relative height of the solution level and the end of the tubing. The relative height of the solution level can be adjusted for an appropriate solution flow rate. The acceptor solution was flowed through 1.54 mm o.d. PEEK or Teflon tubing, a normally closed 2-way solenoid valve (12 V DC, Nresearch, USA), a porous membrane tube, and a spectrophotometric flow cell. The solenoid valve was manually controlled “ON/OFF” to stop/move the acceptor solution to the spectrophotometric flow cell.

The homemade colorimeter was fabricated, as illustrated in the circuitry in Fig. S1a (Supporting Information). The green LED (530 nm, Jameco Electronics) was used as a light source, and the internal built-in current-to-voltage converter circuit photodiode (TSL257, Digi-key, USA) was used as a transmitted light detection unit, which was placed in the opposite direction.
of the LED. The positions of both the LED and photodiode were fixed on the metal cuvette sample holder. All components were placed in a black box to prevent stray light, as depicted in the setup in Fig. S1b (Supporting Information). The light source and detector were supplied the current from a standard USB port of a laptop computer or a standard 5 V mobile phone charger. The appropriate applied current to LED was 0.5 to 0.8 mA. A conventional glass absorption spectrophotometric flow cell with 1-cm path length was used in this experiment. The transmittance signal of incident light from the photodiode was recorded as potential (V) and acquired to a computer through the single-channel 12-bit resolution data acquisition unit (Go-link, Vermeer, USA) and the Labquest Logger Pro 3.8.6.2 software. The voltage measuring signal, which correlated with the intensity of transmitted light, was converted to be an absorbance unit.26

The porous membrane-based diffusion scrubber was used as a gas diffusion unit. The 30-cm length of porous polypropylene membrane tube with 0.5 mm i.d. was inserted in a 4-mm i.d. glass tube jacket as annular format as depicted in Fig. 1 and Fig. S6 (Supporting Information). The ends of the porous membrane tube were connected to the 1/16” Teflon tubing for the acceptor solution, and the ends of the glass jacket were connected to the inlet and outlet of H2S gas. The gas conversion tube as a closed system was adapted from a 15-mL disposable conical bottom centrifuge tube, to generate hydrogen sulfide gas from sulfide ion in an acid condition. For the sulfide ion determination in the turbid sample, the disposable conical bottom centrifuge tube was screwed into the designed acrylic cap, as depicted in Fig. S4 (Supporting Information). This design allowed the user to change the disposable conical bottom centrifuge tube to fill/remove the turbid wastewater sample. The cap was drilled to insert the peek tubes (1/16” o.d.) for the nitrogen gas inlet, the hydrogen sulfide outlet to the gas diffusion scrubber, and the acid/sample solution introduction via a disposable plastic syringe. The flow rate of nitrogen gas from the gas conversion tube, and the acid/sample solution introduction were controlled by using a mass flow controller (0 – 2.0 L min –1, Model MQ0002B/C, Azbil, Japan) or pressure regulator incorporated with a needle valve.

Experimental procedure

Firstly, the solenoid valve was switched “ON” to let the sodium nitroprusside (NP) solution move to fill in a porous membrane tube. After the solenoid valve was switched “OFF” to stop the NP solution, an aliquot of 3.0 mL of standard sulfide solution was introduced into the gas conversion tube via a 5-mL disposable syringe. Then, the 3.0 mL of 0.5 mol L –1 phosphoric acid was introduced via another syringe. The mass flow controller (MFC) was opened to let 100 mL min –1 of nitrogen gas blow into the gas conversion tube for 1 min to promote the mixing of the solution and carry the hydrogen sulfide gas to the porous membrane tube. The hydrogen sulfide would diffuse to the porous membrane tube and be captured into the nitroprusside acceptor solution to form a red-violet product. After 1 min of the accumulation step, the solenoid valve was switched “ON” to let the solution containing the colored product move to a spectrophotometric flow cell for detection. After the absorption measurement step, the humidified nitrogen gas, which was blown through deionized water, was purged to the diffusion scrubber for 3 min to remove the rest of H2S gas and avoid carry over contamination in the system.

Results and Discussion

A homemade LED-photodiode based colorimetric detector was utilized in the flow-batch analysis system for sulfide ion determination via nitroprusside chemistry. The analytical system consisted of three parts; i) gas conversion tube to change the sulfide ion to hydrogen sulfide gas, ii) diffusion scrubber for collecting hydrogen sulfide gas into an acceptor solution, and iii) a homemade colorimeter for detection of the resulting coloured product. In this experiment, the sodium nitroprusside in alkaline medium containing 0.1 mol L –1 phosphate buffer pH 12 was selected as an acceptor solution to trap the hydrogen sulfide gas since only one reagent in a single line would be suitable for the simple flow-batch device. The UV-Vis spectra of nitroprusside in buffer solution pH 12 with/without purging of hydrogen sulfide gas is depicted in Fig. S2 (Supporting Information). The maximum absorption wavelength was 571 nm. The green LED, which provides the maximum wavelength at 530 nm, was selected as a light source for H2S gas detection. The temporal profiles of the output transmittance signal as the voltage from the photodiode are depicted in Fig. 2. The absorbance was calculated from the transmittance data after the usual logarithm transformation.

Investigation of the experimental parameters

Hydrogen sulfide in gas conversion tube. The process of S2– ion conversion to H2S is depicted in Fig. 1. The H2S gas concentration depends on the pH of the solution and mole of S2–. At pH < 4, an acidic medium provides a condition for complete H2S(aq) species generation in the gas conversion tube. In this experiment, the various concentrations of phosphoric acid in the range of 0.02 – 4.0 mol L –1 were selected to test the gas conversion process and the effects on the absorbance signal, as shown in the results in Fig. 3a. The 0.5 mol L –1 H3PO4, 3.0 mL was enough to acidify and change the pH of 20 mmol L –1 NaOH in a gas conversion tube. The volume of sulfide solution was investigated in the range of 1 - 5 mL of the 100 µmol L–1 sulfides with a
fixed volume of 3.0 mL acid in the gas conversion tube on the absorbance, as shown in the results in Fig. 3b. Since the size of the gas conversion tube in this experiment was 15 mL, the appropriate volume of the total solution should be less than 6 mL to avoid bubbles from some turbid samples moving to the porous membrane tube. However, a larger volume of analyte introduced would give the larger amount of H$_2$S(aq) in the gas conversion tube and hence would increase the sensitivity of sulfide detection, and less space above the liquid level allows for the possibility of some bubbles in a liquid sample moving to the diffusion scrubber.

Gas collection efficiency in simplified diffusion scrubber. From the previous report, the optimum pH of an acceptor solution was determined to be 12. In this experiment, the sodium nitroprusside concentration was studied in the range of 0.5 - 10 mmol L$^{-1}$ in pH 12. The 4 mmol L$^{-1}$ of sodium nitroprusside concentration was an optimized concentration for the maximum absorbance signal, as shown in the results in Fig. 4a, and this nitroprusside concentration was selected for use in the next experiment.

Use of membrane-based diffusion scrubber (DS) as an online gas collection and analysis has been reported. The gas diffused through the membrane and was trapped by an acceptor solution present inside the membrane tube. The gas collection efficiency depends on the mass transfer of gas molecules diffused to the walls of a cylindrical shape porous tube, which was first presented by Gormley and Kennedy and expressed by the Eq. (1).

\[
f = 1 - A \exp \left( \frac{-B \pi DL}{F} \right)
\]

Where $f$ is the collection efficiency, $A$ and $B$ are dimensionless constants, $D$ is the diffusion coefficient (m$^2$ s$^{-1}$), $L$ is membrane length (m), and $F$ is the gas flow rate (m$^3$ s$^{-1}$). For H$_2$S gas, Eq. (1) was tested, fitted by the experiment, and as a result, the collection efficiency can be expressed in Eq. (2).

\[
f = 1 - 0.819 \exp \left( \frac{3.657 \pi DL}{F} \right)
\]

Where $\varepsilon$ is sink efficiency, which was determined experimentally...
(based on the best fit to Eq. (2)) to be 0.3 for H₂S when using 0.1 M NaOH.²⁷ The Eq. (2) suggested that the gas collection efficiency increases when using a longer membrane tube and lower gas flow rate.

The nitrogen gas was used not only for promoting the mixing of sulfide solution and phosphoric acid in the gas conversion tube but also for carrying the H₂S gas to reach the porous membrane. In this experiment, the effect of the nitrogen gas flow rate was studied in the range of 60 – 500 mL min⁻¹ based on 100 μmol L⁻¹ standard sulfide solution, as shown in the results in Fig. 4b. Although the absorbance would increase with the increasing of nitrogen gas flow rate due to the higher mixing efficiency of acid and sulfide ion, the absorbance signal when using higher nitrogen gas flow rate would slightly decrease due to the shorter residence time of the hydrogen sulfide gas in the diffusion scrubber, as explained in Eq. (2). The longer accumulation time would permit the higher collection efficiency of liberated hydrogen sulfide into the acceptor solution. However, the colored product is not stable, and as a result, the longer accumulation time resulted in the gradual disappearance of the colored product. The effect of accumulation time in the range of 30 – 600 s was investigated, as shown in the results in Fig. 4c. Although the use of 2 min accumulation time provides such as SO₂ from sulfite and CO₂ from carbonate ion, might interfere with the proposed chemistry in this method. Various volatile compounds which were generated in an acid condition, (mAbs unit) = 6.86

Some possible interferences
The chemical reaction of sulfide and nitroprusside provides good selectivity for sulfide ion determination.²⁸ However, some volatile compounds which were generated in an acid condition, such as SO₂ from sulfite and CO₂ from carbonate ion, might interfere with the proposed chemistry in this method. Various concentrations of sulfite and carbonate were added into 100 μmol L⁻¹ sulfide standard solution. The absorbance values of the solutions with/without interfering ions were observed and compared as % relative absorbance. When using ±5% relative absorbance values as a definition of interference conditions, it was found that the sulfite and carbonate concentrations up to 100 μmol L⁻¹ did not interfere as depicted in the results of % relative absorbance with/without interfering ions in Table S1 (Supporting Information). In addition, the 0.5 and 1.0 mmol L⁻¹ of some metal ions (As³⁺, Mn²⁺, Cu²⁺, Zn²⁺, Fe³⁺, Fe²⁻) were added to observe the change of absorbance signal. With 0.5 M H₃PO₄, it was found that the solution with additions of Fe²⁻, Fe³⁺, Cu²⁺, and Mn²⁺ can not provide the signal, while Zn²⁺ and As³⁺ partially released from the solution can provide the absorbance signal. These behaviors of metal sulfide correspond with those as reported previously.²⁹

Application of the system for determination of sulfide in water samples
By using the optimum condition, a calibration curve with linear relationship of the absorbance and sulfide ion concentration can be obtained in the range of 5.6 - 89.9 μmol L⁻¹ sulfide with the equation of Y (mAbs unit) = 6.86X (μmol L⁻¹ S²⁻) – 20.27, r² = 0.9848. Moreover, peak area or peak width also exhibits a linear relationship with the sulfide ion concentration as depicted in Fig. S3 (Supporting Information). However, the absorbance is more convenient to perform a standard calibration graph and provide higher slope. When using 3.0 mL of water sample volume, the limit of detection based on the lowest concentration in the standard calibration graph was 5.6 μmol L⁻¹. The percent recoveries, tested by spiking standard sulfide solution into the canal water samples, were 91.0 – 105, as shown in the results in Table 1. The repeatability of the peak height and peak area were found to be 3.5 and 50.1 μmol L⁻¹.

Table 1 The recovery of sulfide ion determination in canal water samples by using the proposed method

| Sample | Concentration of sulfide/μmol L⁻¹ | Recovery, % |
|--------|----------------------------------|-------------|
| Canal 1 | 0.0 | ND³ | 95 |
|        | 60.0 | 56.8 ± 1.0 | 105 |
|        | 70.0 | 73.7 ± 3.9 | 105 |
| Canal 2 | 0.0 | ND³ | 91 |
|        | 60.0 | 54.6 ± 2.8 | 93 |
|        | 70.0 | 65.1 ± 1.5 | 93 |
| Canal 3 | 0.0 | ND³ | 94 |
|        | 60.0 | 56.2 ± 1.3 | 93 |
|        | 70.0 | 64.8 ± 2.5 | 93 |
| Canal 4 | 0.0 | ND³ | 99 |
|        | 60.0 | 59.1 ± 1.4 | 100 |
|        | 70.0 | 69.8 ± 1.0 | 105 |
| Canal 5 | 0.0 | ND³ | 98 |
|        | 60.0 | 58.6 ± 1.0 | 105 |
|        | 70.0 | 73.3 ± 1.9 | 105 |
| Canal 6 | 0.0 | ND³ | 101 |
|        | 60.0 | 60.4 ± 0.9 | 100 |
|        | 70.0 | 69.4 ± 1.3 | 100 |

a. Average ± standard deviation with triplicated results.
b. ND = No detection.

c. Sample concentration a/μmol L⁻¹ of some metal ions (As³⁺, Mn²⁺, Cu²⁺, Zn²⁺, Fe³⁺, Fe²⁻) were added to observe the change of absorbance signal. With 0.5 M H₃PO₄, it was found that the solution with additions of Fe²⁻, Fe³⁺, Cu²⁺, and Mn²⁺ can not provide the signal, while Zn²⁺ and As³⁺ partially released from the solution can provide the absorbance signal. These behaviors of metal sulfide correspond with those as reported previously.²⁹

Table 2 Comparison of sulfide ion determination in wastewater samples by using the proposed method and methylene blue method

| Sample | Sulfide concentration/μmol L⁻¹ |
|--------|--------------------------------|
|        | Proposed method | Methylene blue method |
| Wastewater 1 | 73.7 ± 3.9 | 68.3 ± 0.2 |
| Wastewater 2 | 65.1 ± 1.5 | 64.3 ± 0.6 |
| Wastewater 3 | 64.8 ± 2.5 | 69.9 ± 0.1 |
| Wastewater 4 | 69.8 ± 1.0 | 71.7 ± 0.0 |
| Wastewater 5 | 73.2 ± 1.9 | 70.4 ± 0.1 |
| Wastewater 6 | 69.4 ± 1.3 | 65.7 ± 0.2 |
| Reservoir water | 25.4 ± 1.6 | 26.2 ± 0.0 |
| Hot spring water 1 | 48.6 ± 0.2 | 47.9 ± 0.1 |
| Hot spring water 2 | 52.6 ± 3.5 | 50.1 ± 0.1 |

a. Average ± standard deviation of triplicated results.

Benefits of the proposed system
The homemade LED-photodiode based colorimetric detector
provides the benefits of low power consumption from 5 V DC of a USB port, inexpensive fabrication cost, and compactness. The Li-ion power bank could be adapted for future application of the device in on-site monitoring. In order to decrease the cost, a needle valve with a pressure regulator was tested to replace the expensive mass flow controller for controlling the flow rate of nitrogen gas, which was calibrated by using a bubble flow meter. This cheaper part was tested for sulfide ion measurement. The linear relationship of the calibration graph can be obtained with similar performance. Since the colorimetric measurement can be performed after the gas trapping process, this format of measurement is available for further sensitivity enhancement by using the long-path liquid core waveguide cell. The design of the gas conversion tube is applicable and convenient for other gas convertible ions, such as NH$_3$/NH$_4^+$ and HCO$_3^-$/CO$_2$, in solid samples.

Conclusions

A robust, low-energy consumption, and cost-effective manual flow-batch analysis system was successfully developed with a porous membrane based-diffusion scrubber for use in the colorimetric determination of sulfide in turbid water samples without using a conventional pump and valve. This device would be a useful tool for water resource monitoring and management. The proposed method could be an alternative method for the determination of other gas/vapor-convertible compounds, such as NH$_3$/NH$_4^+$ and HCO$_3^-$/CO$_2$, in various sample formats without using a conventional volumetric measuring tool and sample filtering steps. For further development, the proposed system requires fabrication with a more compact setup for on-site wastewater monitoring. The wireless data acquisition unit to acquire a detection signal to a mobile phone, and the programmable control system for the solenoid valve, could be integrated by using a low-cost microcontroller (such as Arduino, https://www.arduino.cc/) to increase the degree of automation.

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Supporting Information

Supporting information includes a circuitry of LED-photodiode based colorimetry (Fig. S1), absorption spectra of nitroprusside in pH 12 buffer solution (Fig. S2), the relationship of sulfide concentration vs. peak width of baseline signal, and peak area of the transmittance signal (Fig. S3), sketch of the acrylic vial cap of the gas conversion tube (Fig. S4), picture of the gas conversion tube (Fig. S5), sketch of the porous membrane based diffusion scrubber (Fig. S6), relative absorbance of spiking ions (Table S1), and comparison of the analytical performance of the proposed method (Table S2). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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