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Preparation of a renewable sulfide-selective flow through an optical sensor based on immobilization of methylene blue on an agarose membrane

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The characterization of an optical sensor membrane is described for the determination of sulfide ions based on the immobilization of methylene blue on an agarose membrane. Methylene blue is covalently bonded to a transparent epoxy activated agarose film. This optode has a linear range of $6.8 \times 10^{-6} - 7 \times 10^{-3}$ mol L^{-1} at 620 nm. The response time of the optode is about 3 min depending on the concentration of sulfide ions. The sensor can readily be regenerated with water, and the color is fully reversible. The sensor was successfully used to the determination of sulfide in water. The sulfide optical sensor was mounted in a flow cell and successfully applied for on-line sulfide monitoring. No evidence of leaching of the dye or any signal drift was observed.

Key words: optical sensor, agarose membrane, methylene blue, sulfide, epoxy activation

INTRODUCTION

Agarose is a nontoxic gel-type hydrophilic support, chemically inert and microbiologically resistant material that has been widely used as a support in affinity chromatography [1], column preconcentration of metal ions [2, 3] and an optical humidity sensor [4]. Hashemi et al. used transparent agarose membranes as supports for fabrication of a covalently immobilized optical sensor to determinate pH of solutions and Cu^{2+} [5–8]. It was shown that agarose membranes could be easily manufactured, simply activated and functionalized with indicators.

Determination of sulfite is necessary for a variety of studies including groundwater monitoring, food analysis, assessment of biogeochemical processes, in hydrothermal vent fluids, and aquatic sediment pore waters in environmental and industrial samples.

Besides its high toxicity for most organisms, hydrogen sulfide plays a pivotal role in biogeochemical processes at oxicanoxic interfaces, such as in the formation of heavy metal precipitates and oxidation by phototrophic or chemolithotrophic bacteria, respectively [9, 10].

Sulfide analysis is well represented in most branches of the analytical sciences. The titration of sulfide with iodine [11], spectrophotometry [12–13], electrochemical techniques [14–16], gas chromatography [17], and HPLC [18] are among the methods employed for sulfide determination.

During the past decade, the development of optical chemical sensors for the determination of cations and anions has become a rapidly expanding area of analytical chemistry because it offers certain advantages over electrochemical sensors [19–21]. The optical sensors possess the advantages of simple preparation, reasonable selectivity, improved sensitivity and no need for separate reference devices.

In recent years, a number of optodes with different optical principles have been reported for the determination of sulfide. The development of optical sulfide sensors can provide an accessible and rapid method for routine environmental measurements [13, 22].

The aim of this work is to introduce an optical sensor for sulfide determination based on its redox reaction with methylene blue. For this purpose, the dye is immobilized on a transparent agarose membrane and used as a selective membrane sensor for the spectrophotometric determination of sulfide in environmental water samples.

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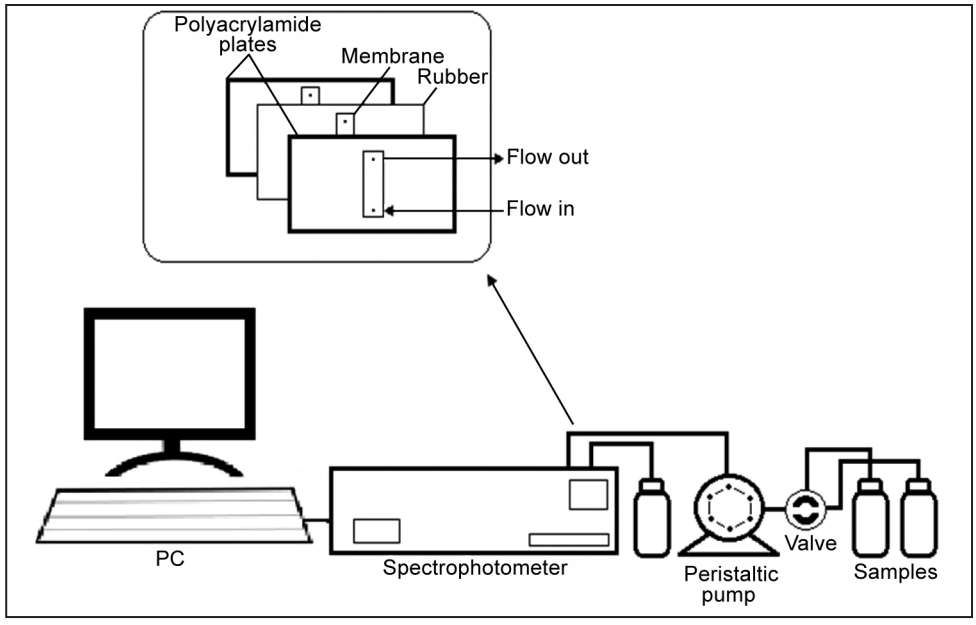

Fig. 1. Set-up of the measuring flow system

EXPERIMENTAL

Chemicals and reagents

All reagents were of analytical reagent grade. Methylene blue, agarose (medium electroendosmosis), epichlorohydrine and other chemicals were supplied by Merck (Germany). Deionized water was used throughout. A 0.313 M stock solution of sulfide was prepared daily by dissolving 0.7500 g of $Na_2S \cdot 9H_2O$ (Merck) in water and diluting to the mark in a 10 mL volumetric flask. This solution was standardized by iodometric titration [23]. Working solutions were prepared by diluting the standard solutions with phosphate buffer. A phosphate buffer of pH 7.5 was used.

Apparatus

A Jenway (USA) model 3020 pH meter with a combined glass electrode was used for pH adjustment. A Shimadzu (Japan) model 1650 PC double-beam spectrophotometer was used for running absorption spectra. Fig. 1 depicts the set-up of the measuring flow system used. A homemade flow through cell was fabricated from 6 × 4 cm polyacrylamide plates and mounted in place of a sample cell of the spectrophotometer for flow absorption measurements. An agarose membrane sensor and a rubber film, with a 1 × 3 opening, were sandwiched between the two polyacrylamide plates. The plates were held together with four screws. A peristaltic pump (ISMATEC, Japan) was used for pumping solutions through the flow cell, usually with a flow rate of 5 mL min^{-1}. A rotating valve was used for switching between samples.

Preparation of agarose membranes

A 10 mL solution of 4% (w/v) agarose was prepared by dissolution of agarose powder in boiling water. The hot solution was strewed and gently pressed between two 20 × 20 cm glass plates with a 0.2 mm distance, and was let to be cooled to room temperature. The thin and transparent membrane obtained in this way was then cut into pieces and stored in 20% (v/v) ethanol.

Activation of agarose membranes

To about 10 pieces of agarose membranes 3.2 mL of 2 mol L^{-1} sodium hydroxide and 0.78 mL of 6% epichlorohydrine solutions were added in a 25 mL beaker, and the solution was diluted to 10 mL by water. The mixture was heated to 40 °C in a water circulating bath and agitated for 2 h. After cooling, the epoxy-activated membranes were thoroughly washed with water on a glass filter and stored in water at 4 °C. The membranes activated in this way can be stored for a long time before immobilization of dyes.

Preparation of immobilized optical sensor

Some 10 pieces of activated membranes were suction dried and transferred into a beaker containing a 10 mL solution of 5×10^{-4} mol L^{-1} methylene blue in 0.05 mol L^{-1} sodium dihydrogen phosphates.

The pH was then adjusted by dropwise addition of 1 mol L^{-1} sodium hydroxide or hydrochloric acid solution. The mixture was agitated for 24 h in a 40 °C water bath. The resulting blue color membranes were thoroughly washed with water on a glass filter, soaked in water overnight, and washed again with a plenty of water to displace any non-bonded dye. The membranes were ready for use and could be cut in an appropriate size and mounted in the flow cell [5, 8].

Spectroscopic measurements

For calibration curves and response time calculations, absorbance measurements at a fixed wavelength of 620 nm in the flow system were used. Buffered sulfide solutions of the

defined pH were passed over the optical sensors mounted in the flow cell, and absorbance was recorded once it was constant.

RESULTS AND DISCUSSION

Optimization of methylene blue coupling

The effects of different parameters on the preparation of the optical sensor were investigated. It is well known that the thickness of the membrane affects the response time and stability of the membrane [24]. Agarose membranes were prepared with different thicknesses (0.1–0.4 mm). In spite of obtaining faster response to sulfide change when a thin agarose membrane is used, it did not show enough physical stability and was broken to pieces during handling. Thus, a thickness of 0.2 mm was used throughout, as it combines a short response time and a relatively stable film.

The effect of pH on the coupling of methylene blue to the agarose membranes was tested by variation of synthesis pH, keeping other parameters unchanged. The amount of the coupling was evaluated by absorbance measurements at 620 nm. The results (Fig. 2) show that the maximum coupling has taken place in pH range of 9–11. Therefore, the pH level 10 was chosen for coupling of methylene blue to the agarose membranes.

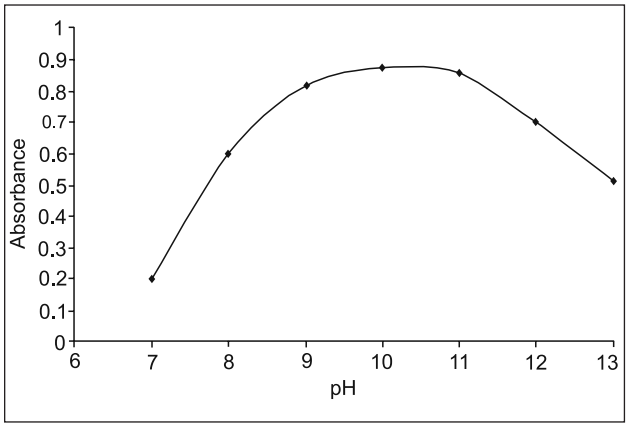

Fig. 2. Absorbance of the immobilized agarose membrane at 620 nm as a function of the synthesis pH. Methylene blue concentration was 5×10^{-4} mol L^{-1}

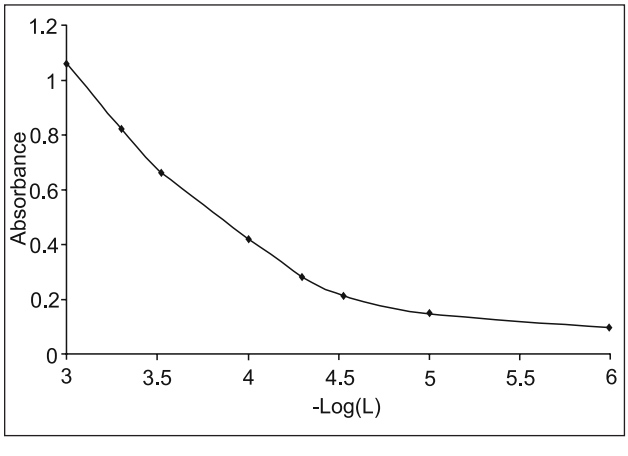

Fig. 3. Absorbance of the immobilized agarose membrane at 620 nm as a function of methylene blue concentration during synthesis

The effect of the solution methylene blue concentration on the dye immobilization on the membrane was also studied. A continuous increase in the membrane absorbance was monitored by increasing the methylene blue concentration from 1×10^{-2} to 1×10^{-6} mol L^{-1}. As it is obvious (Fig. 3), increasing the dye concentration in solution decreases the membrane transmittance significantly. Hence, a concentration of 5×10^{-4} mol L^{-1} with a maximum absorbance of about 0.8 may be considered as optimum.

Optical characteristic of the sensor

The major requirements for an ideal optode membrane are fast response time, high response sensitivity, selectivity, long lifetime and high reproducibility. In this study, all of these parameters were investigated for the proposed sulfide optode membrane.

Fig. 4*a* shows the absorption spectra of free and immobilized methylene blue as a result of change in concentration of the buffered sulfide solutions (pH 7.5) passing through the flow cell.

The absorbance maximum of the immobilized methylene blue is located at 620 nm, and that of the free dye is

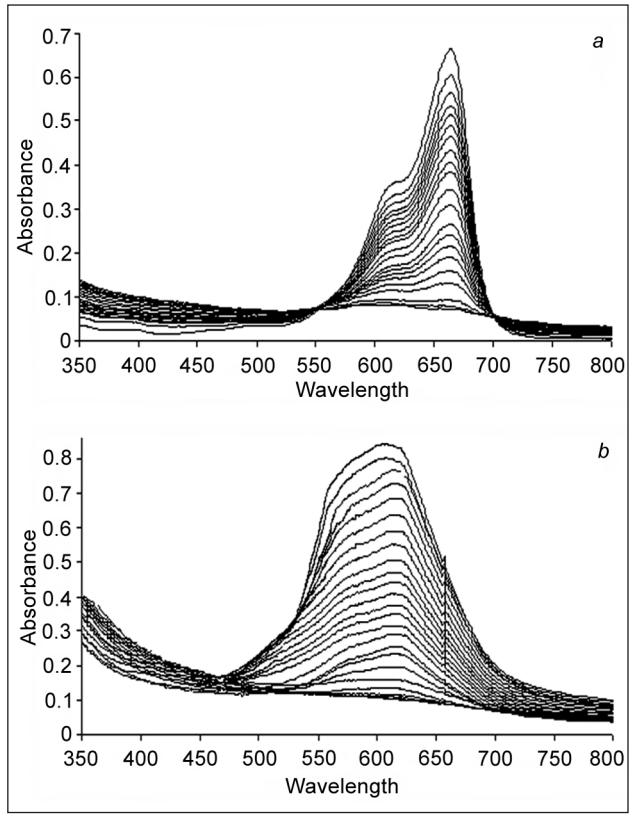

Fig. 4. (*a*) Absorbance spectra of dissolved methylene blue at different sulfide concentration and (*b*) Absorbance spectra of immobilized methylene blue at different sulfide concentration

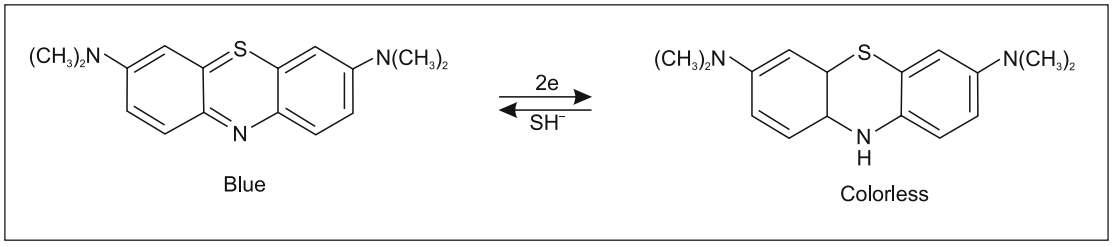

Fig. 5. Reduction reaction of methylene blue by sulfide ion

located at 664 nm. It is necessary to note that the absorption spectrum of the immobilized indicator is blue shifted in comparison to those of their soluble form (620 instead of 664 nm).

As can be seen from Fig. 4*b*, the decrease in the absorption band at 620 nm increases in the membrane as the sulfide concentration rises. The wavelength of 620 nm was selected for further studies because of higher selectivity and sensitivity at this wavelength.

The reduction reaction of methylene blue by sulfide ion has already been reported in the literature, as shown in Fig. 5 [25–28]. It was found that methylene blue undergoes a reduction reaction with sulfide ion to form a colorless product. However, the rate of this reaction is slow, but it sharply increased by the addition of trace amounts of a catalyst. In this study, we examined the catalytic effect of Se (IV), present in the sample solution at a 1.26×10^{-5} mol L^{-1} level, on the absorbance-time behavior of the proposed membrane system in a solution containing 1.5×10^{-4} mol L^{-1} of sulfide at pH 7.5. The resulting absorbance-time plots in the absence and presence of Se (IV) are shown in Fig. 6. As it is obvious, in the presence of Se (IV), the equilibrium absorbance is reached at about 3 min, while it cannot be attained in the absence of the catalyst, even after 8 min. Thus, the results clearly revealed that the operational mechanism of the proposed optical sensor is a catalytic reduction reaction.

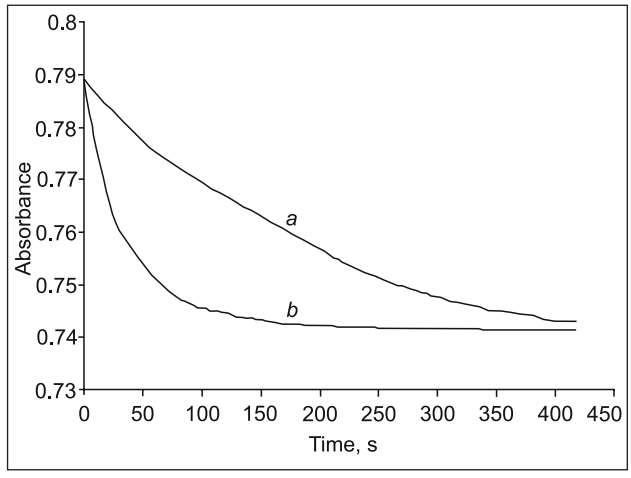

Fig. 6. Time-dependent responses of the optical sensor to a 1.5×10^{-4} mol L^{-1} sulfide solution in the absence (A) and presence (B) of 1.26×10^{-5} mol L^{-1} of Se(IV)

The effect of pH of the solution on the membrane response

The response characteristic of the prepared membrane sensor was highly dependent on pH. The effect of pH on the reaction between sulfide with methylene blue was studied in the pH range of 5–10 by changing the phosphate buffer, while keeping the sulfide concentration constant at 1.5×10^{-4} M level. As can be seen in Fig. 7, a pH of 7.5 produced the highest signal change while, at lower and higher pH, the absorbance decreased significantly. The observed decrease in the absorbance is most possibly due to the decreased equilibrium concentration of HS^- species at pH other than the optimum value of 7.5. Thus, a phosphate buffer of pH 7.5 was selected for further studies.

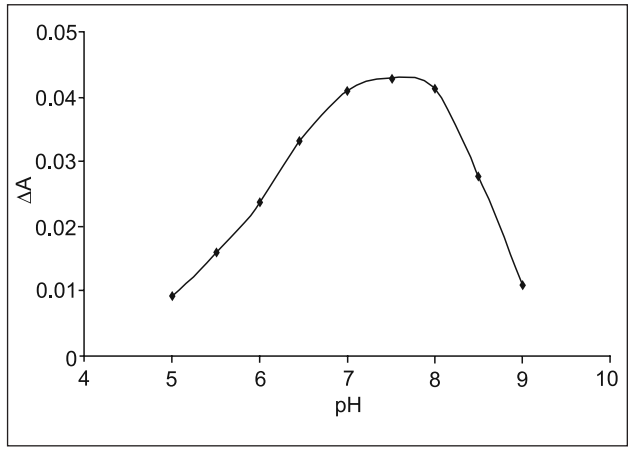

Fig. 7. Effect of pH of test solution of 1.5×10^{-4} mol L^{-1} sulfide on the response of membrane optode

Analytical performance

An important analytical feature of any sensor is its response time. The response time of the sulfide sensor was calculated from absorption profiles at 620 nm. As can be seen in Fig. 8, the absorbance reaches 95% of the steady state signal in about 3 min. The signal levels off after the equilibrium and no signal drift is observed under the experimental conditions.

In spite of the fact that the reaction between methylene blue and sulfide is a redox reaction [25–28], it is found that the color of the membrane after the response to the sulfide ion is reversible and regenerates when the deionized water is

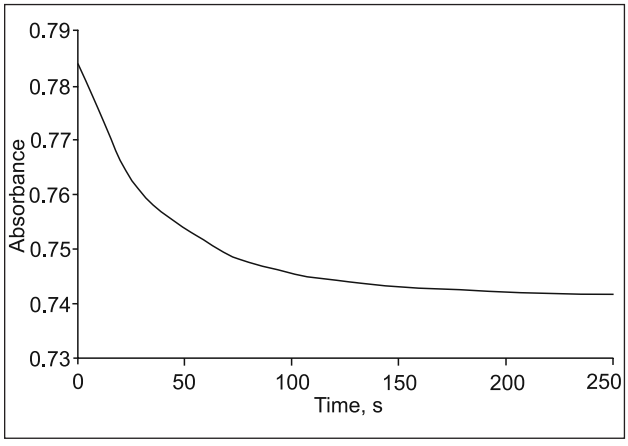

Fig. 8. The absorbance-time plots for the optode membrane in the presence of 1.5×10^{-4} mol L^{-1} sulfide

passed through the flow cell. Water can reverse the response for sulfide about 6 min.

Under the optimum conditions, a calibration graph for sulfide was constructed by plotting absorbance change values at 3 min after the solution to be in contact with a sensing phase as a function of the analyte concentration. The absorbance measurements were expressed as the absorbance difference, which is defined as the difference between the absorbance of the membrane with immobilized methylene blue alone and the absorbance of the membrane in the presence of sulfide ions. The calibration graphs were linear in the range of 6.8×10^{-6}–7×10^{-3} mol L^{-1} for sulfide. The results are shown in Table 1. The detection limit which was estimated as the concentration of analyte producing an analytical signal equal to three times the standard deviation of the blank signal was found to be 0.3×10^{-6}.

Selectivity

Methylene blue is known to have good selectivity for sulfide over many cationic and anionic species. In a phosphate buffer, the membrane optical sensor for sulfide was tested in the presence of different alkali, alkaline earth, transition, and heavy metal ions. It was found that, except those cations that can form insoluble precipitates with the sulfide ion under the experimental conditions used (including Zn^{+2}, Cd^{+2}, Pb^{+2}, Ca^{+2}, Hg^{+2}, Ce^{+3}, Cr^{+3}, and La^{+3}), which can interfere in sulfide ion determination, other cations used have no significant interfering effect on the sulfide ion determination by the proposed optical sensor.

The selectivity of the method for the sulfide ion over other anionic species was determined by adding different amounts of potentially interfering anionic species to a sample solution containing 1.5×10^{-4} mol L^{-1} sulfide at a phosphate buffered pH of 7.5. The tolerance limit was taken as the concentration causing an error of +5% in the determination of sulfide. The results obtained are summarized in Table 2. As can be seen, the anions examined show no significant interfering effects, even at high concentrations, on the sulfide determination by the proposed optical sensor.

Lifetime and stability

The lifetime of the membrane was determined by passing sulfide solution (pH 7.5) through the flow cell including the film. The signal was recorded at the wavelength of 620 nm over a period of about 10 h. No significant loss of the indicator occurs during this time. When the membrane was exposed to light, no drift in signal occurred, and the sensing phase was stable over the experiment with no leaching of the indicator. However, prepared membranes were kept under water when not in use to prevent them from drying out. Additionally, the

Table 1. **Characteristics of calibration graphs for the determination of sulfide**

Slope (mol L^{-1})	Intercept	Correlation coefficient	Linear range (mol L^{-1})	Limit of detection (mol L^{-1})
89.7	0.0353	0.997	6.8×10^{-6}–7.1×10^{-3}	0.3×10^{-6}

Table 2. **Tolerance ratio of different anions on the determination of 1×10^{-5} M sulfide at a phosphate buffered pH of 7.5**

Interfering ion	Tolerance ratio
F^-, Cl^-, Br^-, I^-, ClO_3^-, ClO_4^-, AsO_4^{3-}, $S_2O_3^{2-}$, tartarate, NO_2^-, NO_3^-	100
$H_2PO_4^-$, HPO_4^{-2}, SCN^-, SO_3^-, CH_3COO^-	50

Table 3. **Determination of sulfite and sulfide in water sample**

Sample	Sulfide (mol L^{-1})	
	Added	Found [a]
Tap water	–	N.D. [b]
	1.5×10^{-5}	$(1.4 \pm 0.04) \times 10^{-5}$
	6.2×10^{-5}	$(6.1 \pm 0.02) \times 10^{-5}$
	1.5×10^{-4}	$(1.6 \pm 0.03) \times 10^{-5}$
	3.1×10^{-4}	$(3.2 \pm 0.06) \times 10^{-5}$

[a] Mean ± standard deviation for three determinations

[b] Not detected

effect of dryness on the agarose-based sensor properties was tested by dry storing of the membrane for 3 months. After 30 min soaking of the membrane in deionized water, the optical properties of the sensor were compared to its spectra before dryness and no significant change was observed.

Application

To evaluate the analytical applicability of the proposed method, a sensor was applied for the determination of sulfide in the water sample. The water sample was found to be free from sulfide, so samples were prepared by adding known amounts of sulfide to samples. The results are given in Table 3. The results show that this method is suitable for determination of sulfide concentrations in such samples.

CONCLUSIONS

The chemical immobilization of methylen blue on epoxy activated agarose membranes results in a sensitive sulfide optical sensor with short response time, good reproducibility, long-term stability and no evidence of dye leakage. The sensor may be easily regenerated by water and have the possibility of multiple usages for environmental monitoring of sulfide.

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DAUGKARTINIO JAUTRAUS SULFIDUI PRATEKAMOJO OPTINIO JUTIKLIO PARUOŠIMAS NAUDOJANT METILENO MĖLIO IMOBILIZACIJĄ AGAROZĖS MEMBRANOJE

S a n t r a u k a

Aprašyta sulfido nustatymui skirto optinio jutiklio membrana, paruošta imobilizuojant metileno mėlį agarozės membranoje. Sukurtasis optodas pasižymi tiesine optinės sugerties ties 620 nm priklausomybe $6.8 \times 10^{-6} - 7 \times 10^{-3}$ M ribose. Jutiklis gali būti lengvai regeneruotas vandeniu, jo spalvos kitimas yra visiškai grįžtamas.