Thionine as an indicator for use as a hydrogen sulfide optode

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ABSTRACT

The amount of dissolved hydrogen sulfide is an important parameter in many environmental applications. Conventional methods for H_2S detection depend on iodometric titration or spectroscopic measurements. Unfortunatley these methods are not applicable for direct measurements in natural systems. A recently described method for the on-line detection of H_2S is based on quenching of fluorescence of thionine [1,2].The reaction between H_2S and thionine was described as reversible photo-reduction. This reaction was tested in order to design an optical microsensor for the measurement of H_2S in sediments and other biological systems. We immobilised thionine in several matrices and investigated these materials with respect to response time, mechanical stability, the influence of the excitation light and the reversibility. The sensing materials were deposited on the tip of optical fibers. The measuring system for the excitation and detection of the fluorescence consisted of a yellow light emitting diode, a fiber-optic coupler and a photomultiplier. The excitation light was intensity modulated to enable measurements in ambient light. Our results indicate that the thionine based reaction scheme for H_2S detection is not very suitable for use in a H_2S optode due to lack of reversibility, long response times, and the need for regeneration of the sensor chemistry.

Keywords: H_2S, fiber-optic microsensor, microoptode, fluorescence, planar optode

1. INTRODUCTION

In natural systems hydrogen sulfide is an important metabolic product of sulfur reducing bacteria. In aquatic systems sulfide is dissociated through pH dependent equilibria and is mainly found as dissolved H_2S gas in acidic solutions, whereas HS^- and S^{2-} ions become the predominant sulfide species at neutral and alkaline pH values. In marine sediments and biofilms there is a complex vertical structure of different layers of microorganisms which participate in various sulfur transformations. To understand the pathway and microbiology of the sulfur cyle it is necessary to do on-line investigations of e.g. sulfide directly in these layers. The thickness of the layers ranges from few μm up to some mm. Only the use of microsensors with a tip diameter <50 μm can provide a sufficient high spatial resolution for measuring the distribution of various chemical parameters in these systems. The design and the application of different kinds of microsensors are described in the literature[3,4,5]. The most widely used and also well working microsensors in aquatic environments are oxygen and pH sensors. A good microsensor for H_2S is still lacking. The recently described reversible reaction of H_2S with the well known redox fluorescence dye thionine offers a possibility for the design of such microsensors. Here we present a detailed investigation of the thionine based detection scheme for H_2S and an evaluation of various optodes based on immobilized thionine.

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2. EXPERIMENTAL

2.1 REAGENTS AND MATERIALS

The fluorescent dye thionine acetate, sodium dodecylsulfate and the matrix materials tetraethyl orthosilicate, methyltrimethoxysilan, cellulose acetate, cellulose acetate butyrate, and ethyl cellulose were obtained from Aldrich. The silicone E-4 was from Wacker Chemie (Burghausen, Germany) and the silica-gel was from Merck (Darmstadt, Germany). The preparation of thionine dodecylsulfate was done by mixing equimolar amounts of thionine acetate and sodium dodecylsulfate in water. The precipitate was filtered, washed with water and dried in air for 3 days.

2.2 FABRICATION OF THIN FLUORESCENT FILMS

The concentration of the fluorescent dye was in the range of 0.1 mM in all immobilisation matrices. Thin films of cellulose acetate, cellulose acetate butyrate, and ethyl cellulose were prepared by dissolving thionine dodecylsulfate (thiodcs) and the matrix materials separately. After mixing the indicator/polymer solutions were spread out on a glass surface (3 x 6 cm). The thickness of the films was approximately 5 μm. Silicone films were prepared by adsorbing the thiodcs on silica-gel particles, which were mixed with silicone. Sol-gel films were prepared by a previously described acid catalysed procedure[6]. Thionine acetate was used as the fluorescence dye in the sol-gel films. All films showed the intensive blue color of thionine.

Microptodes were constructed from multimode silica/silica step index fibers with 100 μm core diameter and 140 μm cladding diameter (Radiall, Germany). The fiber was tapered by heating the bare fiber in small flame of a gas burner[5]. Fiber tips from 20 to 40 μm were prepared by cutting the taper at this diameter. The optodes were fabricated by dipping the fiber tip into the described indicator/polymer solutions. After evaporation of the solvent or after the complete reaction of the sol-gel matrix the optodes were ready for use. Dependent on the matrix material used, the process took between 2 and 10 days.

2.3 MEASURING SYSTEMS

Spectroscopical measurements were done in a luminescence spectrometer (Perkin Elmer, LS-50B). Solutions and immobilised indicator films were investigated in a covered standard optical cell (10x10x45 mm).

The setup used for the microoptodes is shown in Figure 1. We used a yellow LED with an emission maximum at 590 nm as excitation light. The light was filtered and coupled via a spherical lens into one branch of an 2 x2 optical fiber coupler (Gould Inc., USA). On the other side of the coupler a fiber-optic switch (DiCon, Fiberoptic Inc.) was inserted between the coupler and the sensor fiber. The switch allowed us to switch off the excitation light for a defined time period. The emission was filtered and detected by a photomultiplier. The LED emission was intensity modulated (f_{mod}= 1 kHz) in order to allow measurements under ambient light conditions. For all fiber connections the standard ST-system was used.

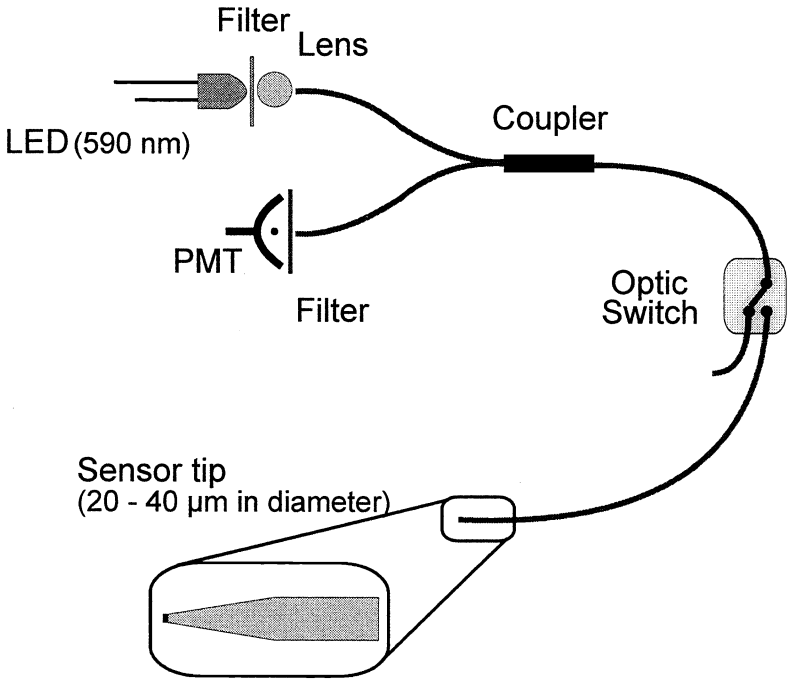


Figure 1: Fiberoptical setup

Hydrogen sulfide solutions were prepared by dissolving sodium sulfide in oxygen free phosphate buffer (pH = 7.5). This system was choosen with regard to the application of the sensor system in seawater systems. Exact defined H_2S concentrations were prepared by the use of an electrochemical H_2S-generator flow cell (AMT Analysentechnik, Rostock, Germany). The generator worked with anoxic 0.02 M sulfuric acid as the carrier solution. A continuously flux of oxygen free acid was reduced in the generator flow cell corresponding to the following equation:

$$H_2SO_4 + 8\,e^- + 8\,H^+ \rightarrow H_2S + 4\,H_2O$$

The H_2S concentration in the solution was regulated by the current in the generator and the flow speed of the pump. Two 3-way-valves allowed the regulated H_2S solutions or analyte free solution in the measuring chamber. Microoptodes were inserted in the measuring chamber, which consisted of a self constructed flow-through-cell of glass. Figure 2 shows the complete measuring setup.

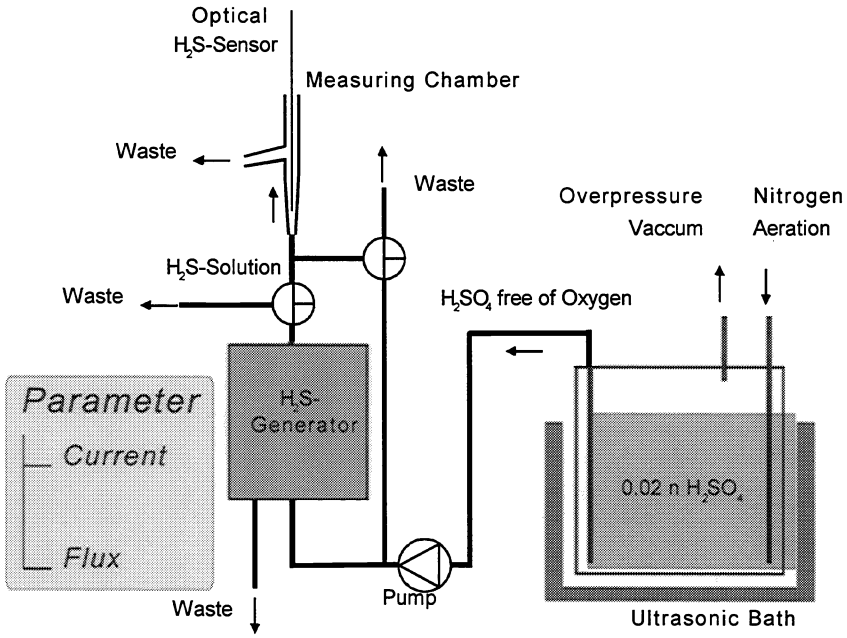


Figure 2: Measuring setup used for microoptode measurements in defined H_2S solutions.

3. RESULTS AND DISCUSSION

3.1 THIONINE IN SOLUTION

Excitation and emmission spectra of thionine acetate disssolved in water are shown in Figure 3.

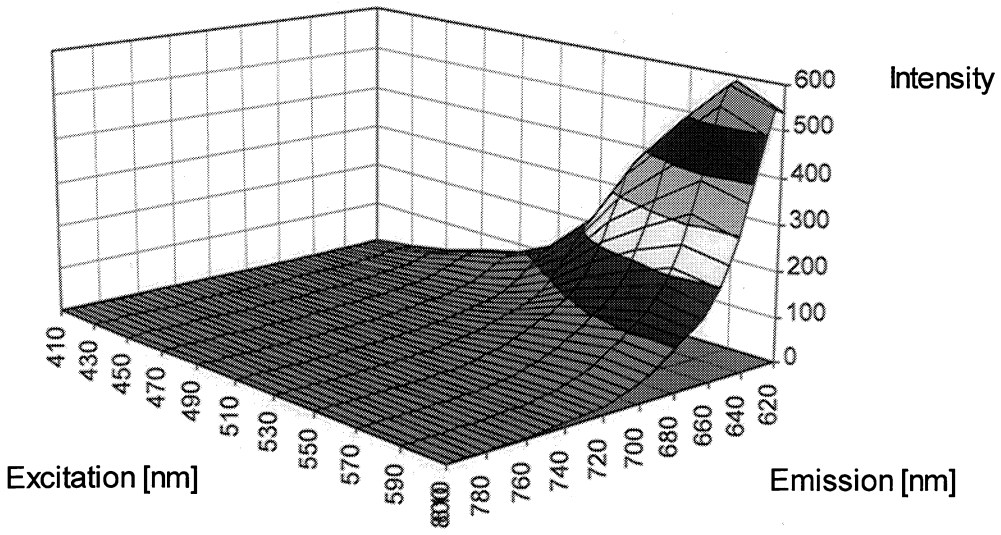


Figure 3: 3D representation of excitation and emission characteristics of thionine acetate in water

In the visible part of the spectrum thionine had a yellow LED compatible excitation maximum at 595 nm. The Stokes-Shift was 25 nm and the emission maximum was thus at 620 nm. Based on these data we did experiments with a 0.025 mM thionine solution (buffer, pH = 7.5). In a nitrogen aerated optical cell oxygen free dye solution was injected. Then H_2S solutions with various contents were added. The cell was closed. A small stirrer inside the cell mixed the solutions continuously. The result of the measurements at 590/620 nm are shown in Figure 4.

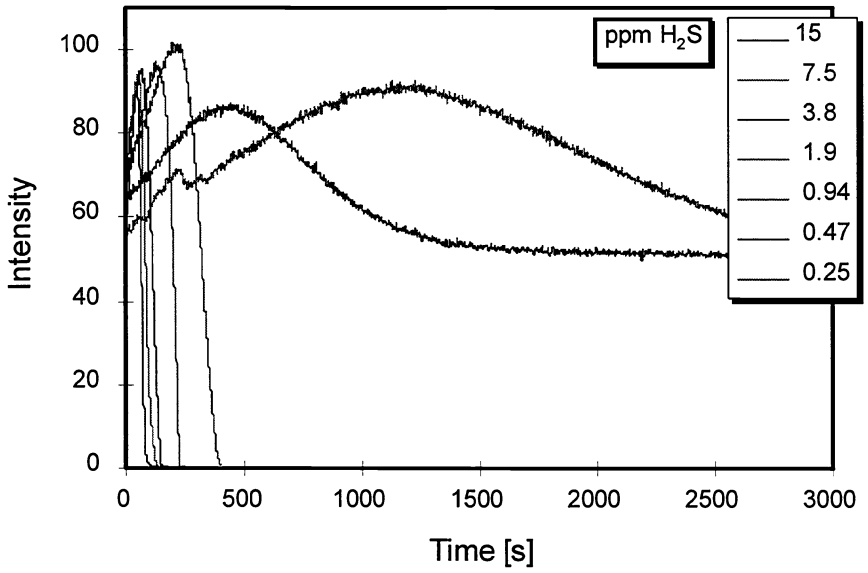


Figure 4: Fluorescence of dissolved thionine in buffered H_2S solutions (pH 7.5) as measured in a fluorometer at 590 nm excitation / 620 nm emission.

All curves showed the development of a maximum in the fluorescence intensity over time. This could be the result of inner filter effects based on a high thionine concentration in the beginning of the reaction. An equilibrium was not reached up to H_2S concentrations of 0.47 ppm. Higher H_2S concentrations reduced the thionine completely to the non-fluorescent form. The observed equilibrium at low H_2S concentrations could be caused by a complete oxidation of the sulfur-(II-) and an excess of thionine. The reaction time was slow. In a well-stirred solution the reaction time ranged from a few minutes up to half an hour for the lowest concentrations.

3.2 Immobilisation matrices

We have tried different matrix materials for the immobilisation of thionine. Table 1 summarizes the qualitative results of our investigations with the described films.

Table 1: Immobilisation matrices (+ = good, o = medium, - = low; more details in text)

Material	Luminescence 590 / 620 nm	Adhesion on a glass surface	Response time
Tetraethyl orthosilicate	+	o	o
Methyltrimethoxysilan Tetraethyl orthosilicate/ 1/1	+	+	-
Cellulose acetate	+	-	+
Cellulose acetate butyrate	-	-	-
Silicone	o	+	-
Ethyl cellulose	+	-	-

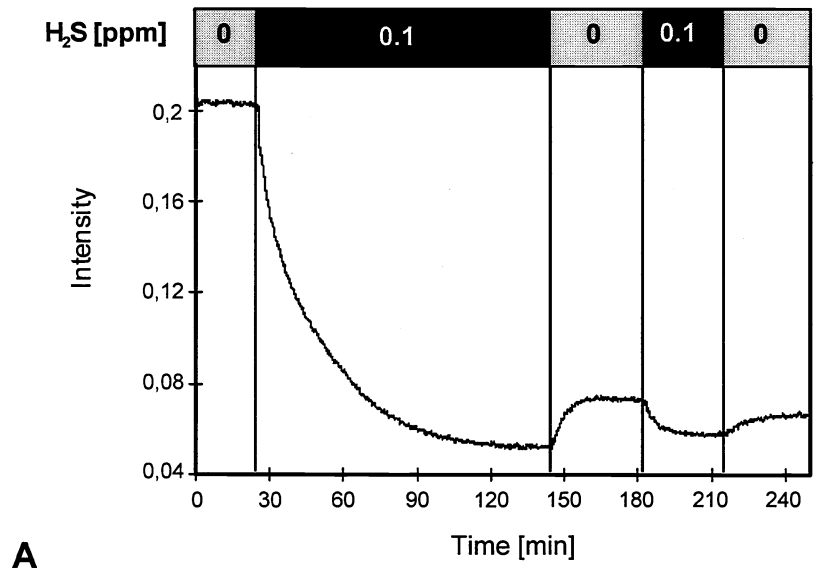
The fluorescence properties of the immobilised dye depended on its solubility in the matrix material after evaporation of the solvent. The silicates and the cellulose products without the butyrate and the system silicon/silica-gel showed a relative high visible fluorescence of the material. Our investigations with regard to the mechanical stability of the optodes was done with films on a glass surface in water. The hydrophilic membranes swell up and lost the adhesion. The same films were used for response time experiments. The planar sensors were placed in a solution which contained 10 ppm H_2S at pH 7. A slow reaction (measured as the decrease of blue color) was obtained. It took 2 hours in case of the cellulose acetate matrix up to a neglible decrease in 2 days in the case of cellulose acetate butyrate. Based on the results of this experiments, cellulose acetate and sol-gel (pure tetraethyl orthosilicate) were used for more detailed investigations.

3.3 CHARACTERISTIC OF THE OPTODES

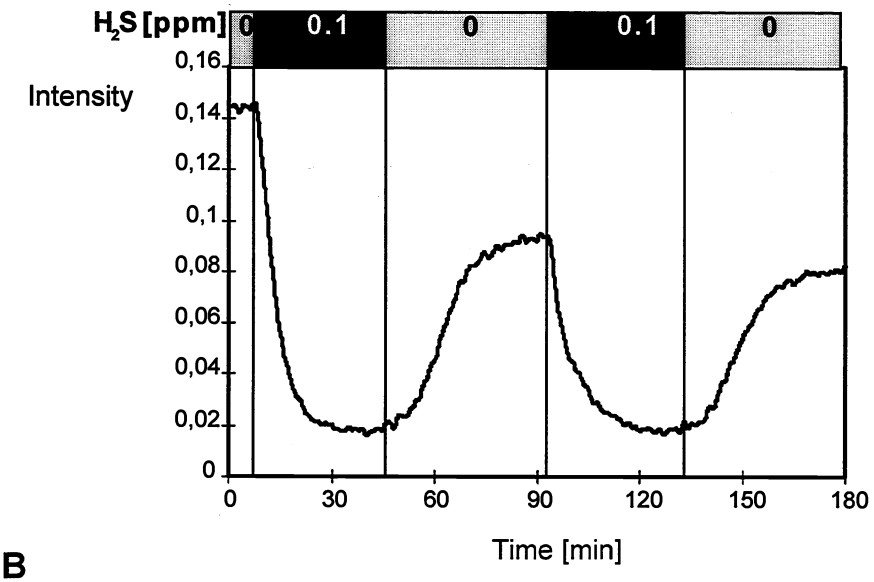
We tested different optodes at pH 7.5. The optodes were dipped alternatively in a solution which contained 0.1 ppm H_2S and an analyte free solution. In Figure 5 the results are shown.

The sensor signal was constant under H_2S free conditions. The endpoint of the thionine reduction took in every experiment more than 30 min. The endpoint signal measured in the H_2S solution was the same in every experiment and corresponded to the non-fluorescent signal of the colorless reduced thionine. The sensor response was not reversible. The back reaction in H_2S free solution didn't show the same fluorescence intensity level as compared to the beginning and there was a strong loss of signal. So we concluded that there must be leaching and/or bleaching of the reduced non-fluorescent thionine form. The optode based on cellulose acetate showed a more stable signal than sol-gel based sensors.

In order to investigate bleaching effects we switched off the excitation light for defined time intervals. In Figure 6 measurements in H_2S solution and analyte free solution is shown. The optode had cellulose acetate as the immobilisation matrix.



A



B

Figure 5: Time recording of signal from an optode based on sol-gel (A) and on cellulose acetate (B)

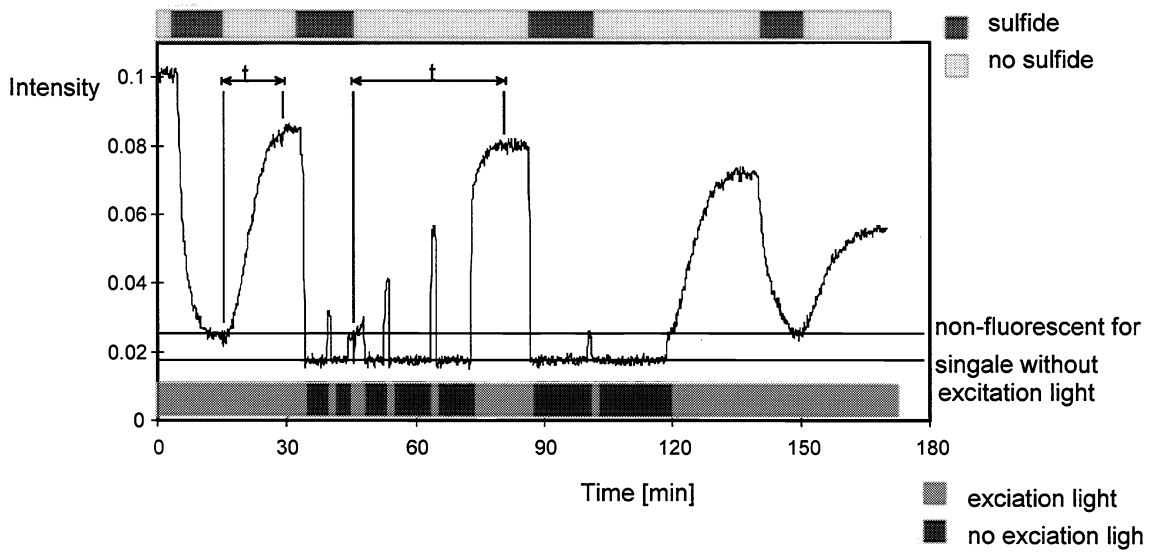


Figure 6: Influence of the exictation light on the reaction of thionine towards H_2S

In general we got the same results as in Figure 5. We obtained a catalysing influence of the excitation light on the system. Especially the oxidation of the non-fluorescent form was influenced by light. The indicated time intervalls in Figure 6 show such a typical caracteristic. The excitation light was necessary for the reaction. Without the light we had a strong increase in the response time. A prevention of bleaching effects was not clearly obtained.

For the calibration measurement of sensors the cellulose actetate optode was choosen. Figure 7 shows a typical time course of these measurements at different H_2S concentrations.

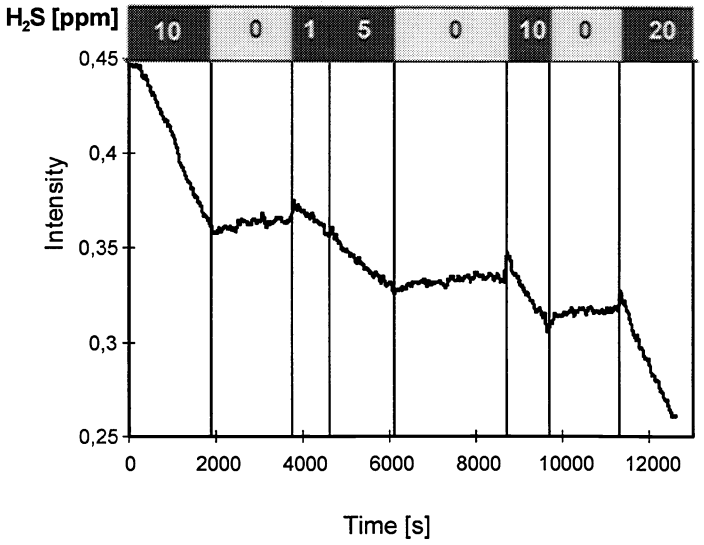


Figure 7: Time course of a cellulose acetate optode at different H_2S concentrations generated by the H_2S generator

Also in these measurements we found an increase of the signal and no regeneration. Dependent on the switch processes at the 3-way-valves we got intensity peaks. These were no real measuring effects. We couldn't find an equilibrium. A time dependent correlation of the H_2S concentration and the decrease of the fluorescence intensity was obtained. In Figure 8 a plot of the signal change per time as a function of the H_2S concentration is shown.

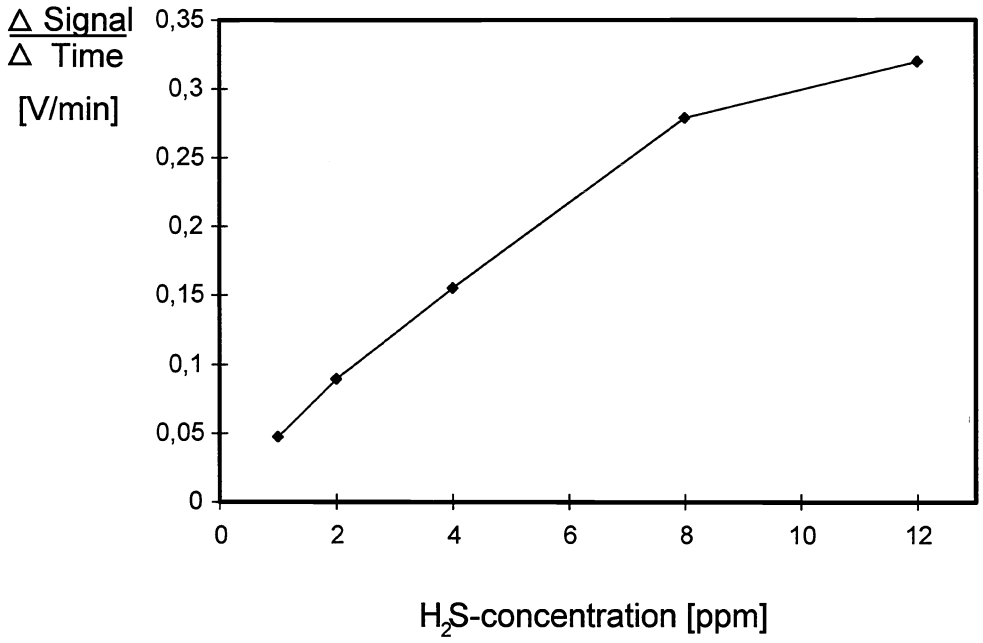


Figure 8: Rate of signal change versus H_2S concentration

After the change to H_2S free solutions we didn't get a significant increase of the fluorescence signal. We investigated this in a separate experiment (Figure 9). The thionine at a cellulose acetate optode was reduced by H_2S in an oxygen free solution (buffer, pH 7.5). After exchange of the medium to a H_2S and oxygen free solution (aerated with nitrogen gas) we got no increase of the signal. The fluorescence increased only after aeration with air. There was no oxidation of the thionine in the abscence of oxygen.

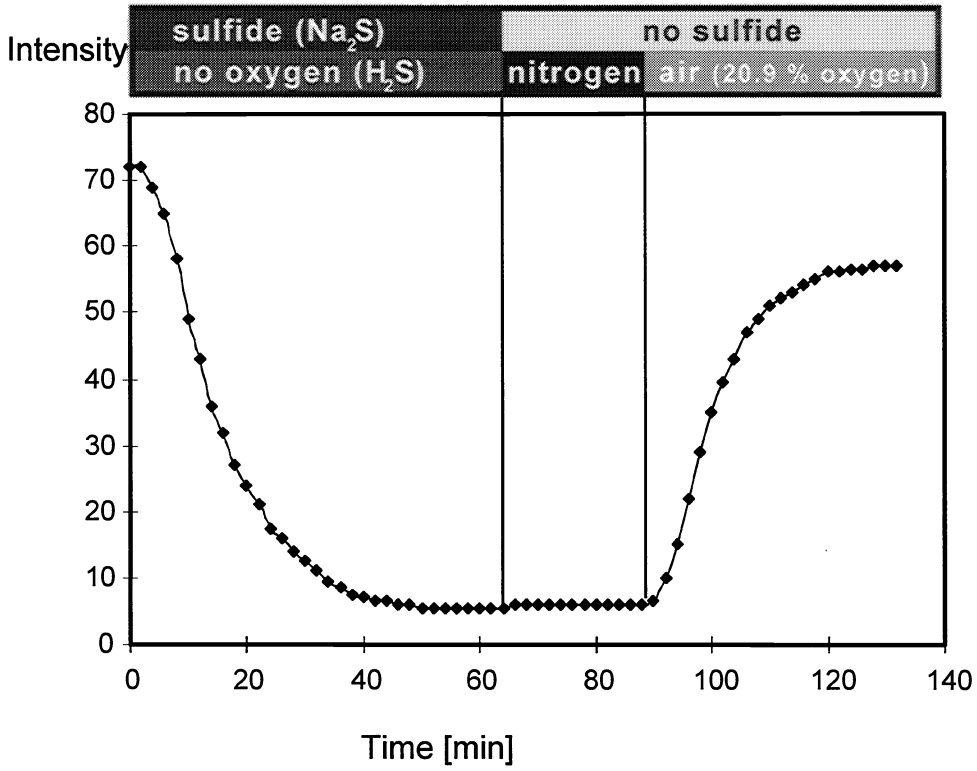


Figure 9: Influence of oxygen on the reversibility of the sensor system

4. SUMMARY AND CONCLUSIONS

We investigated the system thionine and H_2S in different ways. There was a slow reaction between these substances in solution. Only at low H_2S concentrations in the optical cell we obtained an equilibrium. The excess of thionine in comparision to the sulfur-(II-) could be the reason for this equilibrium. With respect to the mechanical stability and the response time we got the best results with cellulose acetate as the immobilisation matrix. The optodes showed a strong decrease of the signal in H_2S solutions based on leaching and/or bleaching. An equilibrium was not observed. The response time could be reduced by a lower concentration of thionine in the sensor matrix. But in this case the fluorescence signal was too low. The described reversible quenching mechanism was not obtained. In our investigations the system showed the following equations:

$$\text{Thionine(fluorescent)} + H_2S \rightarrow \text{Thionine(non-fluorescent)} + \text{Produkt(1)}$$

$$\text{Thionine(non-fluorescent)} + \text{Oxygen} \xrightarrow{h\cdot\upsilon} \text{Thionine(fluorescent)} + \text{Produkt(2)}$$

In our research area only limited applications are possible with the described sensor. The response times and the sensor characteristic do not allow for profile measurements in dynamic biological systems. The experimental conditions with regard to an application in marine systems didn't show the

reversible queching mechanism described in the litterature, and a suitable sensor chemistry which allows the development of fiber-optic based H_2S optodes is thus still lacking. An application of the presented reaction scheme in a flow-injection-analysis system, where a partial regeneration of the thionine system is possible, seems, however, to be practicable.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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