4 Sensors for *In situ* Analysis of Sulfide in Aquatic Systems

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1 INTRODUCTION

Sulfur cycling is of major importance for the biogeochemistry of ecosystems, and here microbial and chemically catalyzed sulfide conversions play a key role [1]. In the environment, sulfide is not only a strong poison to all aerobic organisms through its high affinity to metal containing enzymes [2,3] but is also an important product of anaerobic microbial activity. Under oxic conditions microorganisms are preferentially utilizing oxygen as an electron acceptor, whereas under anoxic conditions other substances such as metal ions (Fe(III), Mn(IV)), nitrate, sulfate or even carbon dioxide are used. In the marine environment sulfate is usually the most available electron acceptor owing to its high

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concentration in seawater (ca 25 mmol L^{-1}) and more than 50% of the carbon mineralization in marine sediments can go via sulfate respiration [4]. This leads to a high H_2S production by microbial sulfate reduction below the oxic/anoxic interface in sediments [5,6], biofilms [7], and in stratified water masses [8,9], where oxygen concentration is low and sulfate availability is high. The sulfide produced reacts with heavy metal ions in the environment and forms precipitates such as FeS, and FeS_2, which in the latter case is preserved in the geological record. A significant amount of sulfide is oxidized via various chemical and microbial pathways involving O_2, NO_3^-, Fe(III) and Mn(IV) [5]. Sulfide oxidation thus binds the sulfur cycle together with the cycling of other key elements in nature [1,5].

In certain areas of industry and waste water treatment, sulfide poses problems. In sewage treatment plants, excessive sulfide production can lead to inhibition of efficient nutrient removal and clogging of activated sludge basins. In sewers, sulfide containing waste water can lead to unpleasant odors and, when oxidized to sulfate, severe corrosion of sewer pipes [10]. Sulfur removal from solids, liquids and gases is of major industrial interest, e.g. in the paper industry [11,12] and the mining and fossil fuel industry [13,14]. Also, corrosion processes due to biofilm formation on immersed surfaces are in part induced by the presence of sulfide-producing bacteria [15,16]. A better understanding of sulfide conversion processes is thus both of general biogeochemical interest and has important socioeconomic implications. This calls for suitable analytical techniques for detecting sulfide in the environment.

In this chapter, we review the current methodology for measuring sulfide in aquatic systems. While we give an overview of currently available analytical techniques, we will focus on direct measurements of sulfide with sensors, that is, devices that can be used directly in natural waters without previous conditioning steps, and that respond specifically and reversibly to sulfide. Special attention is given to sulfide micro-sensors that allow measurements at high spatial (< 0.1 mm) and temporal (t_{90} = 90% of response time < 1–10 s) resolution, with minimal consumption of the analyte, and, therefore, without significant effects on the sulfide equilibria and gradients present in the aquatic enviroment. More general reviews on microsensors and their application in environmental analysis appear elsewhere [17–24] and in other chapters of this book.

2 SULFIDE IN AQUATIC SYSTEMS

Here, we only briefly summarize some important characteristics of sulfide in natural waters. More detailed accounts can be found e.g. in the work of Millero and co-workers [25–27]. In aqueous solution hydrogen sulfide is found to be a weak acid and, neglecting metal complexes and solid phases, the total sulfide concentration, $[S(-II)]_t$, consists of H_2S (dissolved hydrogen sulfide), HS^-

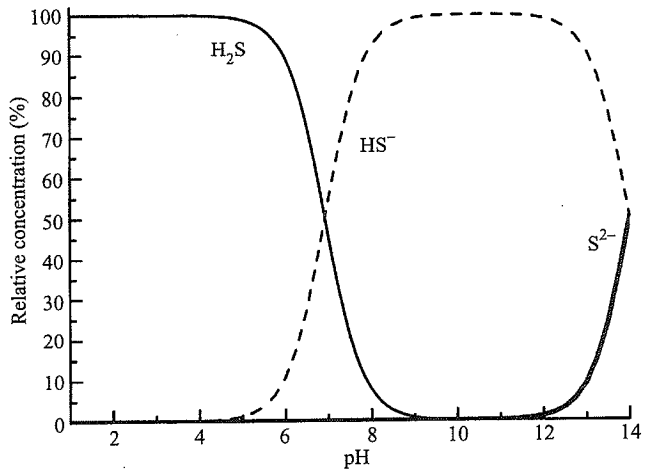

Figure 1. Relative concentrations of the dissociation products of H_2S at different pH (T = 298 °K, $I \leq 1$ mol L^{-1}) calculated from equations (3)–(5). For the calculations we used pK_1 = 6.921 (298 °K) as calculated from equation (6) [28] and $pK_2 \approx 14$ [29]

(hydrogeno sulfide ion), and S^{2-} (sulfide ion). The relation of the actual concentrations of these species is determined by the dissociation constants K_1 and K_2 (equations (1) and (2); Figure 1), and $[S(-II)]_t$ can be calculated from the measured species concentrations, pH, ionic strength and temperature using equations (3)–(5):

$$H_2S \overset{H_2O}{\rightleftharpoons} H_3O^+ + HS^- \qquad K_1 = \frac{[H_3O^+][HS^-]}{[H_2S]} \tag{1}$$

$$HS^- \overset{H_2O}{\rightleftharpoons} H_3O^+ + S^{2-} \qquad K_2 = \frac{[H_3O^+][S^{2-}]}{[HS^-]} \tag{2}$$

$$[H_2S] = \frac{[S(-II)]_t}{1 + \frac{K_1}{[H_3O^+]} + \frac{K_1K_2}{[H_3O^+]^2}} \tag{3}$$

$$[HS^-] = \frac{[S(-II)]_t}{1 + \frac{[H_3O^+]}{K_1} + \frac{K_2}{[H_3O^+]}} \tag{4}$$

$$[S^{2-}] = \frac{[S(-II)]_t}{1 + \frac{[H_3O^+]}{K_2} + \frac{[H_3O^+]^2}{K_1K_2}} \tag{5}$$

The determination of the exact value of the dissociation constants has been a subject of scientific debate. For the first constant, Broderius and Smith[30]

performed a direct photometric determination of H_2S in the gas phase and found the empirical formula, at infinite dilution:

$$pK_1 = 3.122 + \frac{1132}{T} \qquad \text{for } 283\,^\circ\text{K} \leq T \leq 298\,^\circ\text{K} \tag{6}$$

giving a value of $pK_1 = 6.921$ at 298 °K which is in good agreement with the constant found by Barbero *et al.*[31] using the empirical expression:

$$pK_1 = 19.840 + \frac{930.8}{T} - 2.800 \ln T \tag{7a}$$

Other empirical relations for the first dissociation constant as a function of temperature and salinity in natural waters are given by Millero *et al.* [26]:

$$\begin{aligned} pK_1 &= -98.080 + \frac{5765.4}{T} + 15.0455 \ln T \quad \text{at infinite dilution} \\ pK_1^* &= pK_1 - 0.1498\sqrt{S} + 0.0119S \qquad \text{at salinity, } S, \text{ (in ppt)} \end{aligned} \tag{7b}$$

The main problem in the determination of the second dissociation constant is to determine very precisely one of the components HS^- or S^{2-}. Licht and co-workers [32–34] tried to measure sulfide ion using different methods and found a very low value for K_2 of around 10^{-17}. This would mean for most natural conditions (pH 7–9 and 0–10 mmol L^{-1} total sulfide) that the sulfide ion could not be detected by any currently available analytical method except ISE in S(−II) buffered samples.

Licht and co-workers [32–34] used highly concentrated sulfide solutions of 3–6 mol L^{-1}. Physico-chemical parameters in aqueous solutions can, however, only be readily calculated from experimental data when the ionic strength of the solution is located inside the Debye–Hückel region, which describes properties of aqueous solutions up to a maximum ionic strength of about 1 mol L^{-1} (provided empirical corrections are included). Ionic strength outside this region, resulting for instance from sulfide concentrations of 3 mol L^{-1}, leads to a destruction of the water structure and hence to undefined conditions. In addition, there is no way to extrapolate values of Licht and co-workers to infinitely diluted solutions or even to conditions where the ionic strength is inside the Debye–Hückel region.

A more reliable method to get information about the acidity of HS^- was reported by Widmer and Schwarzenbach [29]. They investigated the complex formation of $[HgS_2]^{2-}$ at a mercury electrode and found at an ionic strength of 1 mol L^{-1} that

$$pK_1(20\,^\circ\text{C}) = 6.88 \pm 0.02 \qquad pK_2(20\,^\circ\text{C}) = 14.15 \pm 0.05 \tag{8}$$

The existence of dissociation constants pK_0 and pK_{00} corresponding to the equilibria:

$$H_3S^+ \rightleftharpoons H^+ + H_2S \qquad K_0 = \frac{[H^+][H_2S]}{[H_3S^+]} \tag{9}$$

$$H_4S^{2+} \rightleftharpoons H^+ + H_3S^+ \qquad K_{00} = \frac{[H^+][H_3S^+]}{[H_4S^{2+}]} \tag{10}$$

as proposed by Su *et al.* [35] is not very likely since a decrease of H_2S concentration at lower pH is generally not observed. In addition, the authors used a sulfide ion selective electrode (sulfide ISE) at pH< 5, which is not adequate as these electrodes are responding specifically to the sulfide ion. Even with the amplified technique of Su *et al.*, there is no way to overcome the limitations of the detection principle. Their potential readings must therefore be caused by other phenomena such as chemical destruction of the membrane and formation of potentials at the silver electrode due to the high solubility of Ag_2S in acidic media (see Section 4.2).

Aqueous sulfide solutions are easily oxidized by oxygen, peroxides (e.g. H_2O_2), halogens, nitric acid, lead dioxide and other oxidants [26,36,37]. Thereby, the final products (see Table 1) as well as the reaction rates are highly determined by the pH. At pH< 6 the reaction rate is very slow, shows maxima at pH 8 and 11 and decreases at pH> 11 [38]. On the overall pH range, heavy metal ions (mainly Fe^{3+}, Fe^{2+} and Ni^{2+}) are increasing the reaction rate [41]. In the case of iron, this is due to the local formation of H_2O_2:

$$Fe^{2+} + O_2 \xrightarrow{H_3O^+} Fe^{3+} + HO_2 \tag{11}$$

$$2HO_2 \rightarrow H_2O_2 + O_2 \tag{12}$$

The sensitivity of sulfide solutions to oxidation plays an important role in the accuracy of analytical determination. Some established analytical procedures require sampling, and sample stabilization, e.g. by the addition of zinc acetate to form zinc sulfide, before the actual analysis is performed. Zinc sulfide is stable for several weeks against oxidation by oxygen [42]. The most important problems involved with sampling are the losses of the analyte by evaporation, adsorption and oxidation prior to the stabilization step. This requires careful handling of samples and specialized procedures for the calibration of all analytical methods, for the determination of sulfide. This is just as important when *in situ*-methods are used and calibrated.

Table 1. Primary oxidation products of sulfide species at different pH

pH range	Main sulfide species	Primary oxidation products	References
acidic	H_2S	S^0, SO_4^{2-}	[38]
neutral	HS^-	$S_2O_3^{2-}, S_nO_6^{2-}, SO_3^{2-}, SO_4^{2-}, S_n^{2-}$	[38,39]
alkaline	HS^- and S^{2-}	$SO_4^{2-}, S_2O_3^{2-}, S^0$	[40]

Calibration of analytical methods for sulfide determination in aqueous samples should involve the following steps:

- A relatively concentrated sulfide solution of about 0.1 mol L^{-1} [S(−II)] is prepared by weighing a certain amount of Na_2S (7–9)H_2O and adding deaerated, deionized water. The determination of the exact sulfide content of this stock solution can be done by iodometric titration [43]. Such concentrated sulfide solutions are stable for months when protected against the impact of oxygen, light and heavy metals, e.g. by storing the solution under argon in brown gas-tight glass flasks. It is not optimal to use flasks with rubber washers or other parts made of silicone, rubber, PVC, Teflon etc. for such long time storage, as these materials are permeable for oxygen and can contain unknown amounts of heavy metals. Furthermore, some rubber materials tend to adsorb sulfide.
- The stock solution is used to make a diluted working solution. The sulfide content can be determined by the methylene blue method [43,44] (see Section 3.1). This diluted working solution is then used to calibrate the analytical method by exactly the same procedure, which is applied on the samples, i.e. stabilization with zinc acetate, dilution steps etc. The working solution cannot be stored and is stable only for some hours.

Another more accurate and easy way to perform calibration of analytical methods for sulfide determination was proposed by Jeroschewski and Schmuhl [45,46]. The method is based on the use of a novel sulfide generator (Figure 2), which performs an electrochemical reduction of HgS in a flow-through apparatus, whereby H_2S is produced in a deaerated, acidic carrier solution. The exact H_2S concentration [μmol L^{-1}] is determined by the flow velocity and the applied current according to the Faraday law:

$$c_{H_2S} = \frac{6 \times 10^4 I}{Fnv} \qquad (13)$$

where I is the applied generator current in μA, F is the Faraday constant in c.mol^{-1}, n is the number of exchanged electrons (2), and v is the flow velocity [mL min^{-1}] of the carrier solution through the generator cell.

The sulfide generator thus performs a coulometrically controlled formation of hydrogen sulfide. The method can only be used in a flow-through configuration, but it is in principle possible to prepare stabilized sulfide solutions with an exactly known content by adding the sulfide-containing carrier solution to a zinc acetate solution. While the sulfide generator is ideal for calibration of e.g. amperometric H_2S sensors, it cannot be used for calibration of sulfide ion-selective electrodes as small amounts of mercury are released, which will interact with the Ag/Ag_2S membrane of such electrodes (see section 4).

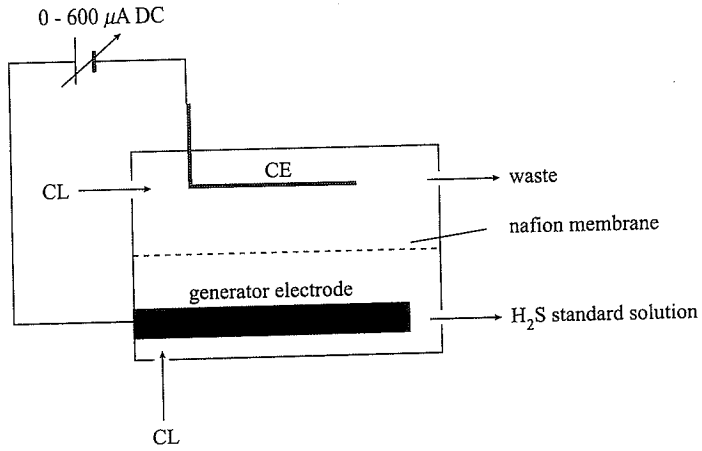

Figure 2. Scheme of a coulometric H_2S generator. CL, carrier solution of 5×10^{-3} mol L^{-1} H_2SO_4; CE, counter electrode; the generator electrode contains HgS; DC, direct current

3 MEASURING TECHNIQUES FOR SULFIDE

A large variety of techniques are available for measuring either total sulfide, sulfide ion or dissolved hydrogen sulfide. The techniques for the determination of $[S(-II)]_t$ usually are *ex situ* methods, i.e. sampling is required. Here the most important techniques are using the methylene blue reaction, other spectrophotometric methods, or chromatographic methods (see Table 2 and 3).

3.1 SPECTROPHOTOMETRIC METHODS

Spectrophotometric methods usually involve conditioning and treatment of the analyte sample with chemical reactions prior to the spectroscopic analysis. There exists a large variety of such analytical methods (Table 2). Only a few authors have proposed direct spectrophotometric determination of H_2S, mostly in the gas phase [76–83]. Although the analytical procedure in this case is rather simple, the direct measurement of H_2S demands a significant amount of (expensive) technical equipment, it suffers from serious interferences, especially from SO_2, and it requires great care to avoid analyte losses through evaporation, adsorption and rapid oxidation. The method is, therefore, seldom used for environmental analysis of sulfide. This is also true for kinetic methods, i.e. sulfide-catalyzed or -inhibited reactions [84–87,103,104] because under most conditions the parameter time cannot be controlled accurately. Furthermore, in natural waters interference with the kinetic reactions, e.g. by metal ions, is a problem.

Table 2. Spectrophotometric methods for sulfide determination in aqueous systems (Fl., Fluorescence; Ph., Photometry; FPh., Flame Photometry; k.Ph., kinetic photometry; MECA, molecular emission cavity analysis; GP-MAS, gas phase molecular absorption spectrometry; LOD, limit of detection)

Method	Procedure	Dynamic range ($mol\,L^{-1}$)	LOD ($mol\,L^{-1}$)	Interference	References
Fl.	fluorescence quenching of fluorescein mercury acetate (FMA)	$<10^{-5}$	10^{-7}	NO_2^-, SO_3^{2-}, COS, $(CH_3)_2S$, CS_2	[47–49]
Fl.	fluorescence quenching of FMA incorporated in ethylcellulose*	no data	56×10^{-9}	cystein	[50]
Fl.	fluorescence quenching of thionine*				[51, 52]
Fl.	reduction of potassium-1,2-naphtoquinone-4-sulfonate	$(2.5–15) \times 10^{-6}$	No data	$S_2O_3^{2-}$, SO_3^{2-}, NO_2^-	[53]
Ph.	formation of methylene blue	$<1 \times 10^{-3}$†	$(0.3–1) \times 10^{-6}$	I^-, NO_2^-, CS_2, $S_2O_3^{2-}$, CN^-	[28, 43, 44, 54–56]
Ph.	formation of methylene blue (FIA)	$<5 \times 10^{-3}$†	$(0.1–1) \times 10^{-6}$	NH_4^+, CO_3^{2-} (at high conc.), CS_2, $S_2O_3^{2-}$	[51–63]
Ph.	formation of methylene blue (FIA)	$(6–600) \times 10^{-6}$†	$(0.3–20) \times 10^{-6}$		[64–66]
Ph.	addition of HS^- to Brilliant Green	$<62.3 \times 10^{-6}$	0.6×10^{-6}	SO_3^{2-}	[67]
Ph.	indirect determination with formation of SCN^-	$(10–600) \times 10^{-6}$	No data	Br^-, I^-, S(0), $S_nO_4^{2-}$	[68–71]
Ph.	complexation with organic mercury compounds (FIA)	$(0.3–1.1) \times 10^{-3}$	$(10–80) \times 10^{-6}$	oxalate, acetate	[72,73]
Ph.	reaction with cacothelin (FIA)	$(1–1100) \times 10^{-6}$	No data	SO_3^{2-}	[73]
Ph.	exchange with SCN^- and detection with Fe(III)	$(0.3–1.1) \times 10^{-3}$	No data	many other ions	[73]
Ph.	reaction with $[Fe(CN)_5NO]^{2-}$	$<25 \times 10^{-3}$	0.5×10^{-3}	selective	[74]
Ph.	reaction with Fe(III)/nitrilotriacetic acid	$(0.6–3.1) \times 10^{-3}$	No data	selective	[75]
MECA	after evaporation	$<1.4 \times 10^{-3}$	0.6×10^{-6}	all S-compounds	[76]
FPh.	previous separation with IC	$(0.4–1.2) \times 10^{-3}$	No data	selective	[77]
GP-MAS	after evaporation	$<18 \times 10^{-3}$†	$(0.8–22) \times 10^{-6}$	SO_3^{2-}, CN^-, NO_2^-	[78–83]
k.Ph.	reduction of toluidin blue by S^{2-} catalyzed by Se(IV)	$(8.8–53.1) \times 10^{-6}$	1.5×10^{-6}	SO_3^{2-}	[84]
k.Ph.	reaction of methylorange with BrO_3^- catalyzed by S^{2-}	$<156.3 \times 10^{-6}$	5.6×10^{-6}	no data	[85]
k.Ph.	utilizes the iodine–azide reaction	$(0.6–15.6) \times 10^{-6}$	0.3×10^{-6}	Cr(IV), V(V), Ce(IV), $S_2O_3^{2-}$, SO_3^{2-}	[86]
k.Ph.	reaction of Pyronine-G with hypophosphite catalyzed by Pd(II) and inhibited by S^{2-}	$(0.3–6.2) \times 10^{-6}$	0.12×10^{-6}	I^-, Br^-, IO_3^-, SO_3^{2-}, NO_2^-	[87]

* irreversible under anaerobic conditions.
† depending on experimental parameters.

Table 3. Chromatographic methods for sulfide determination in aqueous systems (PID, photoionization detector; FPD, flame photometric detector; SCD, sulfur chemoluminescence detector; amp., amperometric detector; photom., photometric detector; RP-HPLC, reverse phase high pressure liquid chromatography; LOD, limit of detection).

Method	Procedure	Dynamic range (mol L^{-1})	LOD (mol L^{-1})	References
GC/PID	previous enrichment with cryofocussing	$< 2.2 \times 10^{-3}$	12.7×10^{-9}	[88]
HS-GC/MS	cryofocussing of headspace sample (HS)	$< 156 \times 10^{-9}$	29×10^{-9}	[89]
HS-GC/FPD	cryofocussing of headspace sample	$< 0.5 \times 10^{-9}$	0.2×10^{-12}	[90]
GC/SCD	direct aqueous injection	$< 58.8 \times 10^{-6}$	0.25×10^{-6}	[91]
IC/amp.	gas dialysis	$< 2.5 \times 10^{-6}$	0.06×10^{-6}	[92]
IC/amp.	previous enrichment by zinc acetate fixation	$< 1.2 \times 10^{-6}$	0.16×10^{-6}	[93]
IC/amp.	direct injection of dialysed samples (see Chapter 11)	no data	$< 0.31 \times 10^{-6}$*	[94,95]
IC/photom.	post-column reaction of $KBrO_3$+ methylorange catalyzed by sulfide	no data	0.3×10^{-6}	[96]
IC/photom.	post-column reaction with I_2	$< 1.2 \times 10^{-6}$	0.1×10^{-6}	[97]
IC/photom.	post-column reaction with I_2	$< 0.5 \times 10^{-3}$	1.8×10^{-6}	[98]
RP-HPLC	pre-column derivatization to methylene blue	$< 1 \times 10^{-3}$	0.5×10^{-6}	[99]
RP-HPLC	pre-column derivatization to methylene blue and enrichment on silica gel	no data	3.1×10^{-9}	[100]
RP-HPLC	gas dialysis and pre-column derivatization with monobromobimane	$< 10 \times 10^{-6}$	0.04×10^{-6}	[101]
RP-HPLC	pre-column derivatization to 1-methyl-2-thiopyridone	$< 156 \times 10^{-6}$	0.06×10^{-6}	[102]

* depending on column age

The reaction of *N,N*-dimethyl-1,4-phenylenediamine with H_2S, which is catalyzed by Fe(III), via addition of $FeCl_3$, and leads to formation of methylene blue (3,7-*bis*(dimethylamino)-phenothiazin-5-ium chloride), was originally introduced by Fischer in 1883 for the determination of total sulfide in aqueous solution [44]. During the last century, the methylene blue method was modified and adapted to specific analytical problems. The method was applied in various fields of scientific research on sulfide-containing systems, and has become a standard method in analytical chemistry [28,43,54,55]. An important recent improvement was the development of an *in situ* analyzer (Scanner = submersible chemical analyzer) by Sakamoto-Arnold and Johnson [57–59] (see also Chapter 7 in this book), which uses a flow injection analysis (FIA) variation of the methylene blue method. This analyzer has been applied for *in situ* determination of sulfide in hydrothermal vent fields on the deep-sea floor at several kilometers water depth, yielding important and new knowledge about these extreme sulfidic habitats.

Another modification of the original methylene blue method was introduced by Cline [56], and is widely applied in limnological and marine research. Instead of the standardized method, which uses separate solutions of *N,N*-dimethyl-1,4–phenylenediamine and $FeCl_3$, Cline proposed a mixed solution of the two reagents to overcome problems arising with salinity, temperature and pH. We recently compared this approach with the standard method and were not able to realize any advantage (Steuckart *et al.*, unpublished data). Furthermore, the method of Cline shows an important disadvantage if the sample is pH buffered (which is the case in many natural waters). Reliable quantitative results can only be obtained when the pH of the reaction mixture (reagents + sample) is kept very low, as the absorption spectrum of methylene blue depends on its state of protonation (Figure 3A). Also, the sensitivity characteristics of the method vary when mixed reagent solutions are used. Under the same conditions, i.e. same sulfide and reagent concentration and same protonation state of

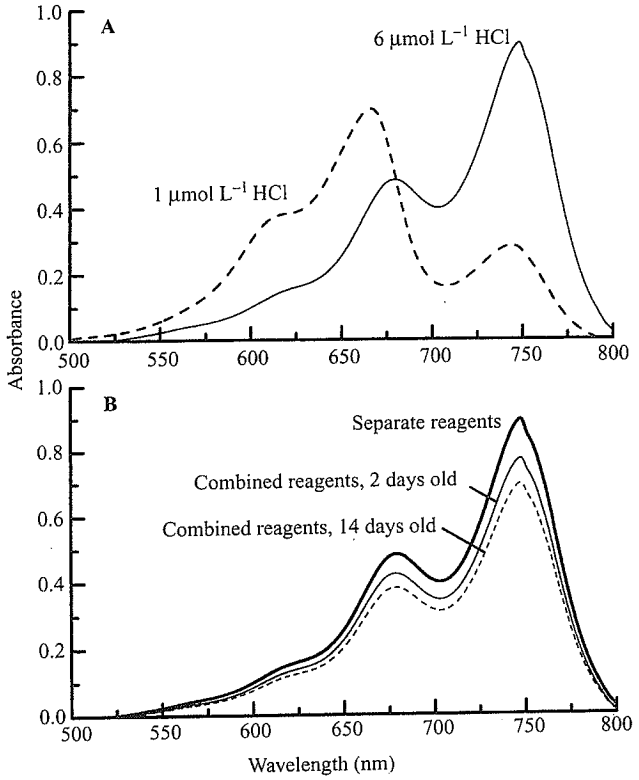

Figure 3. Absorbance spectra of methylene blue: (A) at different pH; (B) obtained with different types of sample/reagent treatment, i.e. with separate reagents, and with 2 d and 14 d old solutions of mixed reagents, respectively (see text for details)

the methylene blue, the mixing of the reagents some hours prior to the analytical procedure leads to a significant drop in sensitivity, which increases with the age of the mixed solution (Figure 3B). The experiments were not performed at the upper border of the dynamic range, i.e. the concentrations of the reagents were always high enough to convert all sulfide to methylene blue, hence resulting in the same theoretical methylene blue concentration. Therefore, the drop in sensitivity can only be explained by a deterioration of the reagent mixture and/or the formation of interfering substances over time. This is consistent with a reaction mechanism proposed by Kubàñ *et al.* [105], where the first step of the methylene blue reaction is the oxidation of the diaminoaniline by Fe(III) leading to a slowly established equilibrium between a cation radical and a quinone diimine, where the latter is not involved in the further reaction with H_2S. In conclusion, we recommend the use of separate reaction solutions when using the methylene blue method.

The formation of ethylene blue instead of methylene blue, by using *p*-diethylaminoaniline as the reactive substrate, has been successfully established for the determination of sulfide in Kraft liquors [64–66]. This method is well suited for sulfide determination even in complex wastewater from paper mills (white, green and black liquors consisting of highly concentrated mixtures of hydroxide and sulfide).

An alternative to the classical methylene blue method for environmental sulfide analysis was proposed by Koh *et al.* [68–71] and is based on several variations of the oxidative reaction of sulfide with cyanide to thiocyanide:

$$S^{2-} + I_2 + CN^- \rightarrow SCN^- + 2I^- \qquad (14)$$

Though the methodological parameters (e.g. linear dynamic range, LOD, precision) are similar to those of the methylene blue method, the method is significantly more time consuming and complicated. We conclude that the classical methylene blue method is still the spectrophotometric method of choice for sulfide determination in natural waters.

3.2 CHROMATOGRAPHIC METHODS

Chromatographic methods find many applications in environmental chemistry. They combine powerful separation methods and sensitive detection techniques. The main disadvantage of chromatography is the need for sampling prior to analysis, and *in situ* measurements using chromatographic methods are very complicated to realize. In addition, sample treatment (pre-column or post-column) as well as the separation procedure itself change the chemical and/or biological state of the sample. Therefore, information about the actual situation with respect to sulfide speciation, pH, redox equilibria etc. cannot easily be obtained. In spite of this, gas chromatography (GC), ion chromatography (IC), and high performance liquid chromatography (HPLC) are used for the separation and determination of sulfide in waters (Table 3). Gas chromatography requires the removal of the analyte (H_2S) from the aqueous matrix prior to the analysis followed by enrichment procedures, such as cryofocusing of the headspace [89,90]. Tang and Heaton [91] have shown that it is possible to inject aqueous samples into the gas chromatograph. The detectors normally used for the gas chromatographic determination of H_2S are the photo-ionization detector (PID), the flame photometric detector (FPD), and the sulfur chemoluminescence detector (SCD), which is extremely selective towards sulfur compounds.

Ion chromatographic methods can be performed directly with filtered aqueous samples but problems such as (i) enrichment due to the reaction of sulfide with heavy metal contaminations of the reagents, or (ii) a strong adsorption of sulfide onto the analytical column can be observed [106]. Because of the low dissociation of H_2S, the widespread conductivity detectors cannot be used for sulfide determination. In most cases, ion chromatographic methods are combined with amperometric (Ag versus SCE) [92–95] or photometric (e.g. post-column oxidation with iodine) [97,98] detection principles. More sensitive HPLC methods involve a pre-column derivatization of sulfide mostly to methylene blue and these techniques have detection limits in the nmol L^{-1} range [100–102].

3.3 ELECTROCHEMICAL METHODS

Electrochemistry offers a variety of measuring principles for determining specific sulfur species directly in natural waters (see Table 4). Currently, three types of electrochemical techniques are mostly used for *in situ* environmental analysis of sulfide speciation: (i) the potentiometric sulfide ion-selective electrode, (ii) the amperometric H_2S sensor, and (iii) methods based on voltammetry with either amalgamated gold or Hg-coated iridium microelectrodes. These methods will be discussed in more detail in the following section.

4 SENSORS FOR MEASURING SULFIDE IN AQUATIC SYSTEMS

The quantitative determination of chemical variables in the environment requires analytical methods that are minimally invasive in terms of disturbance of local chemical or redox equilibria and in terms of mechanical disturbance. Electrochemical sensors are suitable analytical tools for this purpose because no sampling and/or sample treatment is required. Such sensors can be constructed with geometric parameters of the sensor tip in the micrometer range, which minimizes mechanical disturbance and, in the case of electrochemical methods, offers many other advantages compared with macroelectrodes. For instance, amperometric and voltammetric methods are characterized by a consumption

Table 4. Electrochemical methods for sulfide determination in aqueous systems (RDE, rotating disc electrode; SMDE, static mercury drop electrode; ISE, ion-selective electrode; LOD, limit of detection)

Method	Procedure	Dynamic range (μmol L^{-1})	LOD (μmol L^{-1})	References
Polarography	cathodic stripping polarography	no data	0.09	[107]
Polarography	differential pulse polarography	< 1500	0.1	[108–110]
Voltammetry (Ag-RDE)	cathodic stripping voltammetry	0.01–10	0.01	[111,112]
Voltammetry (SMDE)	alternating current voltammetry	0.01–900	no data	[113]
Potentiometry	S^{2-}–ISE(Ag/Ag_2S)	$< 10^6$	$< 10^{-17}$*	[114–117]
Potentiometry	S^{2-}–ISE(Ag/Ag_2S/pH)	$< 10^6$	$\leqslant$10; $[S(-II)]_t$	[118–126]
Potentiometry	S^{2-}–ISE (Ag/Ag_2S in FIA with/without gas dialysis)	15–1500 $[S(-II)]_t$	1 – 6	[127–130]
Potentiometry	S^{2-}–ISE (air-gap variation of Ag/Ag_2S)	> 10; $[S(-II)]_t$	0.01; $[S(-II)]_t$	[131]
Potentiometry	CN^-–ISE (Ag/AgCN with acidic evaporation)	> 12; $[S(-II)]_t$	0.3; $[S(-II)]_t$	[132]
Potentiometry	S^{2-}/Cl^-–ISE (Ag/Agx (S,Cl) with FIA)	no data	< 10; $[S(-II)]_t$	[133]
Potentiometry	potentiometric titration with Pb(II) with Pb-ISE	no data	no data	[134]
Potentiometry	S^{2-}–ISE (Co(II)-phtalocyanines and -porphyrines as electrocatalysts)†	depends on pH	depends on pH	[135–137]
Potentiometry	HS^-–ISE (carrier system)	0.2–20; $[S(-II)]_t$	0.06; $[S(-II)]_t$	[138]
Potentiometry	HS^-–ISE (carbon paste electrode)	< 1000; HS^-	no data	[139]
Amperometry	porous Au electrode with pneumato-amperometry	3 – 3300	no data	[140]
Amperometry	H_2S specific biosensor with immobilized *Thiobacillus thiooxidans*	20–400	no data	[141]
Amperometry	H_2S specific sensor with redox mediator	3–90	~ 1	[142–146]
Amperometry	H_2S specific sensor with redox mediator (FIA)	1–750	~ 1	[147,148]
Amperometry	H_2S microsensor	1–750	< 1	[6,149,150]

* Free concentration in buffered medium; value stated by manufacturer (see Section 4.2 for details).
† blocked by I^-, SCN^- and CN^-.

of the analyte through reduction or oxidation. The analytical parameter is the corresponding current which is time dependent in the case of macroelectrodes and is described by the Cottrell equation [151] :

$$i_l = \frac{nFA\sqrt{D}c^*}{\sqrt{\pi t}} \qquad (15)$$

where, i_l is the limiting current, t is the time, n is the number of exchanged electrons, F is the Faraday constant, A is the electrode surface area, D is the analyte diffusion coefficient, and c^* is the bulk concentration of the analyte.

In the case of microelectrodes the limiting current is time independent [151] :

$$i_l = 4r_0\pi nFDc^* \qquad (16)$$

where i_l is the limiting current, r_0 is the electrode radius, and the other parameters have the same meaning as in equation (15). This important measuring characteristic makes microelectrodes very suitable for the analysis of dynamic processes and solute distribution at high spatial and temporal resolution. In addition, since the radius of the electrode, r, is very small, the limiting current, i_l, is also small and analysis in highly resistive solutions is possible since the $i.R$ drop is small. The formation of spherical, hemispherical and cylindrical diffusion layers enhances the mass transport of the analyte to the microelectrode leading to short response times and low dependence of the sensor signal on stirring rate of the sensor tip environment. A more detailed discussion of macro-versus microscale electrochemical sensors can be found in Chapters 8 and 9 of this book.

The different measuring characteristics of macro- and microelectrodes are illustrated in Figure 4. Cyclic voltammetry of a reversible redox system (here: hexacyanoferrate(III)/(II) in alkaline solution) at macroelectrodes shows a typical asymetrical peak of the I/E curve due to a time-dependent diffusion layer thickness, whereas at a microelectrode spherical diffusion leads to a time-independent flux of analyte at the electrode surface, and thus a characteristic S-shape of the I/E curve.

Most sensor principles available for the determination of sulfide species in the environment are based on potentiometry (section 4.2), voltammetry (section 4.3), and amperometry (section 4.4). The available sensors respond either specifically to the sulfide ion or to H_2S, or to the whole of labile S(−II) species. Some attempts to develop HS^- specific sensors have been reported [138,139]. However, a reliable analytical method for HS^- is still to be developed. Also, reversible optical sensors for the determination of S(−II) species have not yet been developed [50–52,152].

4.1 OPTICAL AND BIOSENSORS FOR SULFIDE

Choi *et al.* [50] proposed an optical sensor based on the fluorescence quenching of fluoresceine by mercury(II) acetate (FMA), which is reacting with sulfide. The measuring principle is reversible only in the presence of oxygen, which is used to regenerate the sulfide sensitivity of the sensor after analytical measurement. A similar behavior was found for the fluorescent dye thionine, which is also irreversibly quenched by sulfide in the absence of oxygen [51,52]. Evidently this imposes severe problems for the practical use of these sensor principles as S(−II) is unstable in the presence of oxygen. Therefore, the currently available

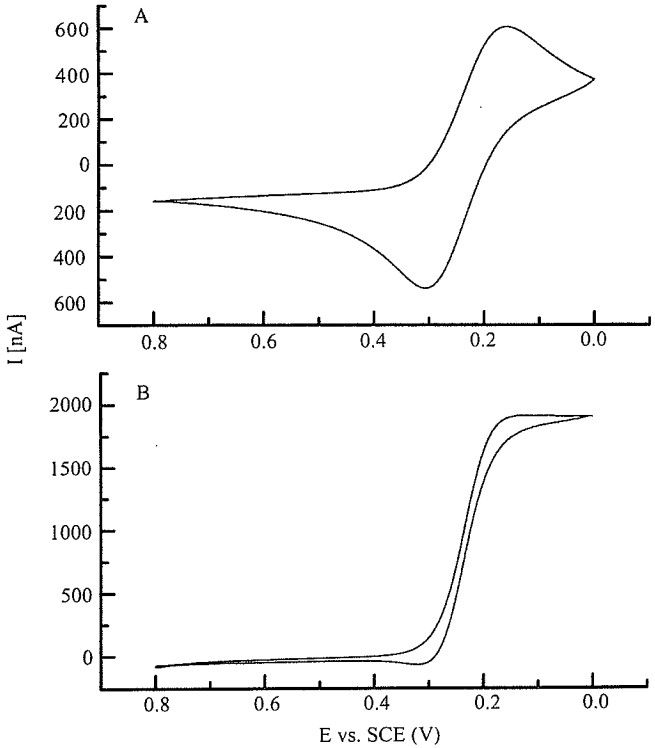

Figure 4. Cyclic voltammogram of 0.05 mol L^{-1} $K_3Fe(CN)_6$ in 0.5 mol L^{-1} carbonate buffer solution measured at a Pt disc electrode. (A) Electrode diameter = 200 μm; scan rate = 0.05 V s^{-1}. (B) Electrode diameter = 10 μm; scan rate 0.1 V s^{-1}

optical sensors do not allow for the continuous determination of sulfide under anaerobic conditions in natural waters. Cardoso *et al.* [152] presented a reversible sensor principle using FMA for the determination of atmospheric hydrogen sulfide, which cannot, however, be used in natural waters.

An interesting possibility for future sensor developments was proposed by Kurosawa *et al.* [141]. Their H_2S specific sensor is based on the oxidation of sulfide by *Thiobacillus thiooxidans* (a colourless sulfur bacterium) with oxygen, which is monitored by a Clark-type oxygen sensor as an internal transducer in the biosensor. More details on various biosensors are presented in Chapter 6 in this book.

4.2 POTENTIOMETRIC SULFIDE SENSORS

Potentiometric methods are based on the measurement of equilibrium potentials without electrochemical consumption of the analyte and are, therefore,

well suited for *in situ* determination. The most important tools used in potentiometry are ion-selective electrodes (ISE) consisting of a solid state or (semi-) liquid membrane and a suitable transducer [153]. The sensor membrane, which has to be electrically conductive and practically insoluble in the medium to be investigated, is the sensitive part of such electrodes. Although this type of electrode is referred to as ion selective, the analytical practice shows interferences by many other species. Problems arise when species are present that may complex one of the components of the membrane or incorporate into the solid membrane (e.g. Cl^-, Br^-, I^- in the case of Ag/Ag_2S membranes) [154]. A more general discussion of potentiometric techniques and ion-selective microsensors can be found in Chapter 5 of this book.

The Ag/Ag_2S electrode, which was originally used as a reference electrode, was first introduced as a potentiometric sulfide ISE by Berner in 1963 [114]. In 1983, Revsbech *et al.* [155] introduced an Ag/Ag_2S microelectrode for use in environmental analysis. More robust needle-type Ag/Ag_2S sensors have also been developed [117].

Silver sulfide is a semiconductor with a high ionic conductance similar to its electrical conductance, i.e. $\sim 5.4 \times 10^{-4}$ S cm^{-1}, with Ag^+ as the mobile ion in an S^{2-} network [156]. The detection principle is based on the formation of equilibrium potentials in the electrochemical chain $Ag/Ag_2S/S^{2-}$ described by the Nernst equation:

$$E = E^0 - \frac{RT}{2F} \ln a(S^{2-}) \tag{17}$$

where $a(S^{2-})$ is the activity of the ion S^{2-}. This implies a strong influence of temperature and ionic strength mainly due to a change in the activity coefficient of the sulfide ion. With the help of equation (5) the calibration curve for sulfide ion (E versus $\ln(a(S^{2-}))$ obtained with the ISE at a given pH and ionic strength can be converted to a calibration curve for total sulfide (E versus $\ln [S(-II)]_t$, which has a theoretical slope of −29 mV $decade^{-1}$ at 298 K. As the potentiometric Ag/Ag_2S electrode responds only to the sulfide ion, and not to HS^- or H_2S, the E versus $\ln[S(-II)]_t$ calibration graph exhibits a parallel shift by 29 mV pH^{-1} at pH 7–13, while at pH < 7 the shift amounts to 59 mV pH^{-1}. The slope of the E versus $\ln[S^{2-}]$ calibration curve is theoretically independent of pH. The theoretical limit of detection can be calculated from the solubility product of Ag_2S[157]:

$$[Ag^+]^2[S^{2-}] = 10^{-51} \tag{18}$$

giving a value of $[S^{2-}] = 6.3 \times 10^{-18}$ mol L^{-1}, which corresponds to 2 pmol L^{-1} of $[S(-II)]_t$ at pH 8.5). In practice, the limit of detection is far higher (about 0.1 μmol L^{-1} of $[S(-II)]_t$) owing to the formation of mixed potentials, silver complexes, elemental silver and other factors leading to the so-called super-Nernstian behavior (see below).

There are several practical problems with the use of Ag/Ag_2S electrodes in complex environmental samples. Dissolved silver ions, heavy metals incorporated (as metal sulfides) in the membrane, halides (as silver halides), and pseudohalides (cyanide) can build new electrochemical chains of the general form $Ag/(Ag, Me)_x(S, Hal)/(Ag^+, Me^{n+}, S^{2-}, Hal^-)$ and hence lead to unpredictable mixed potentials and irreproducible quantitative results [154,158].

In particular, the presence of Hg(II) in solution leads to an irreversible destruction of the Ag_2S membrane by formation of HgS precipitates at the membrane surface. Because of the lower solubility product of HgS as compared with Ag_2S and the unpredictable amount of the HgS formed, uncontrolled mixed potentials occur, which can result in large analytical errors. For that reason, Dobcnik *et al.* [116] proposed to coat the Ag_2S membrane with mercury sulfide in order to improve the sensitivity, the reproducibility and the response time. We tested this modification but were not able to obtain reliable quantitative results because of the non-linearity of the calibration graph [150]. This is consistent with the investigations of De Marco *et al.* [154]. Yu *et al.* [159] presented a modified sulfide microelectrode which was pretreated with $HgCl_2$ solution similarly to the procedure proposed by Dobcnik *et al.* [116]. The calibration of their microelectrode, as well as that of a commercial sulfide ISE used in their study, showed increasing slopes of the calibration curves with decreasing pH up to about 50 mV $(pS^{2-})^{-1}$ at pH 7.2. The authors interpret this behavior as a response of the sulfide ISE to HS^- and S^{2-}, which is in contrast to the well-known theory for the Ag/Ag_2S electrode.

Another problem with the potentiometric sulfide ISE is the so-called super-Nernstian behavior. At low sulfide concentrations ($< 1\ \mu mol\ L^{-1}$ $[S(-II)]_t$ at pH 12.7), reducing conditions and high pH (e.g. by use of so-called sulfide antioxidant buffer, SAOB, with commercial macroelectrodes [115]) the slope of the E versus $\ln[S(-II)]_t$ calibration graph can show a significant deviation from -29 mV decade^{-1} [119,160,161]. This is due to the reduction of Ag_2S under these conditions, which leads to the formation of elemental silver at the electrode surface [156,158].

Frevert and coworkers developed a so-called pH_2S sensor based on the Ag/Ag_2S electrode in conjunction with a pH glass electrode, thus avoiding a liquid junction reference [118–122,125]. Although, it was stated that the influence of ionic strength was reduced by 50% and that the sensor signal was pH independent at $pH < 6$, the basis of Frevert's sensor remains the Ag/Ag_2S electrode including all the above-mentioned problems and disadvantages.

In conclusion, Ag/Ag_2S-based sulfide sensors can be used for environmental analysis under near neutral to alkaline conditions in the water column as well as in sediments and biofilms with detection limits of ca. 1 $\mu mol\ L^{-1}$ for $[S(-II)]_t$. The construction of well-functioning Ag/Ag_2S-based sulfide sensors and calibration of such sensors is, however, complicated by the above-mentioned factors and this puts a limitation on the practical use of such sensors for *in situ* analysis. Nevertheless, such sensors have been used for laboratory [7,155,162] as well as *in situ* applications [117,163,164] in various aquatic systems (see section 5).

4.3 VOLTAMMETRIC SULFIDE SENSORS

Voltammetry is an electroanalytical method, which enables multi-species analysis by measuring oxidation or reduction currents of chemical species as function of the potential imposed to the electrode. The potential at which the electrode reaction occurs is primarily determined by the redox potential of the electron transfer, but it is additionally influenced by pH (when H_3O^+ is involved in the redox reaction), complexation of the test species and its diffusion properties. A more detailed account of voltammetric techniques in environmental analysis in water and sediment is given by Buffle and Tercier–Waeber in Chapter 9 of this book. In the following, we only address some aspects relating to voltammetric determination of sulfide.

Various voltammetric techniques have been used with Hg electrodes to measure S(−II) in natural waters [110]. Recently, Brendel and Luther [165] used differential pulse polarography at an amalgamated gold electrode (tip diameter $\sim 100\ \mu m$) for the direct measurement of S(−II) in sediment cores. First applications of this technique for the combined measurement of concentration gradients of sulfide, oxygen, I^-, Fe(II), and Mn(II) in porewater of sediments have been reported [166,167]. Also, data from *in situ* profiling of sulfide and other chemical variables in sediments have been obtained by Reimers and Luther (cited in ref. [23]). These studies demonstrate that new information on the complex porewater chemistry of aquatic sediments can be obtained. However, voltammetry with bare electrodes in complex media can be problematic. In the following, we list a few concerns on the limitations of voltammetric techniques with respect to sulfide analysis and give some suggestions for improvement. Nevertheless, besides a need for technical optimizations the approach of performing *in situ* voltammetry with microelectrodes seems very promising and we regard the technique as having a large potential for the quantification of various redox species in natural systems, especially for fine scale analysis of iron and manganese species.

Electrodes with a diameter of $\sim 100\ \mu m$ or larger are not microelectrodes (see also Chapter 9) and, therefore, the well-known advantages of microelectrodes such as measurement in low conductivity freshwaters and independence of stirring rate will not be fully realized. Such relatively large electrodes exhibit currents in the nA range, related to a large local analyte consumption that may disturb local gradients and equilibria around the sensor tip. Microsensors in μm size range have a much smaller consumption of analyte (typical measuring currents in the pA range) and are the only ones to which spherical diffusion occurs. The advantageous measuring characteristics of microelectrodes are

obtained only when the dimensions of the sensor tip, where the reactions take place, are smaller than the thickness of the diffusion layer at the electrode surface, leading to a spherical or hemispherical diffusion field. [150]

One of the main advantages of using mercury as an electrode is its high overpotential for the reduction of water to form hydrogen. When it is coated onto substrates of high solubility such as Au or Ag, however, this advantage is decreased. Glassy carbon electrodes do not suffer from this problem, but the deposition of mercury is not very reproducible on this substrate [168,169]. Buffle and coworkers [170,171] have shown that iridium is by far the best substrate for mercury because of its low solubility in mercury ($< 10^{-6}$ wt%) and its good wettability by Hg.

Another aspect to consider is the measurement of sulfide by oxidation at metal electrodes with pulse techniques. Shimizu *et al.* [172] wrote in 1981: "It may be dangerous to extend the frequently employed pulse techniques to an electrochemical system forming a deposit or film at a solid electrode. A variation of the thickness of the deposited film with time may cause a change in double-layer capacity, and thus capacitive currents may still remain at the current sampling time". In addition, in all cases double peaks are observed at high sulfide concentrations (>10–100 μmol L^{-1}) when Hg electrodes were used with DPP [108,109]. Canterford attributed this phenomenon to the formation of dense HgS films. Davison and Gabbutt [173] observed similar problems with DPP when sulfide concentrations in natural waters exceeded 2 μmol L^{-1}, but at lower concentrations good linear calibration curves were obtained. Normal pulse polarography, in conditions which prevent surface accumulation, can be recommended for higher concentrations, while the reliable determination of sulfide at a very low concentration level is possible by the use of cathodic stripping techniques [107,111,112] or a.c. voltammetry [113] provided the accumulation time is short enough so that a multilayer film is never formed. The important point is that at high concentration the electrode must always be placed at $E < E_{pic}$ (to avoid oxidation of Hg) except during the analysis, where a fast positive scan must be used followed immediately by a return of the electrode potential to $E < E_{pic}$ (The reaction is: $S(-II) + Hg \rightarrow HgS + 2e^-$). At low sulfide concentration, the electrode can be put at $E > E_p$ for a given time to accumulate HgS, but not too long in order to avoid a multilayer film formation, and then a negative scan is used (the reaction is: $HgS + 2e^- \rightarrow Hg + S(-II)$) (see Chapter 9, section 5 for more details).

Electrochemical measurements at an unprotected electrode under complex environmental conditions are problematic because of fouling of the electrode (see Chapter 9) by natural waters, colloidal and particulate forms of organic and inorganic matter that can be reduced, oxidized or simply adsorbed onto the electrode surface. This often interferes with the electrode reaction of interest and drastically perturbs the voltammetric peaks, which can result in large analytical errors, hard to quantify. This is especially true in sediments exhibiting

complex porewater composition and high concentrations and gradients of both inorganic and organic compounds. In some cases electrochemical conditioning between scans can help alleviate such interferences but reproducibility is rarely good and the presence of memory effects should be carefully checked.

The problems associated with *in situ* voltammetry with bare electrodes may also partly apply to bare potentiometric electrodes. Thus, for *in situ* applications a protection of the electrode surface by a membrane, permeable to the test analyte, is usually necessary (a detailed discussion of voltammetry on bare and membrane-covered electrodes is given in Chapter 9).

4.4 AMPEROMETRIC HYDROGEN SULFIDE SENSORS

Most electrochemical gas sensors have a gas permeable membrane, through which the analyte can diffuse into an inner electrolyte compartment, where the electrochemical reactions take place under well-defined conditions. Consequently, gas sensors often exhibit much better measuring characteristics in terms of stability and, especially, selectivity as compared with potentiometric and voltammetric sensors. Gas sensors are well suited for *in situ* analysis and especially Clark-type oxygen microelectrodes have proven to be excellent tools for environmental analysis (see Chapter 1) [174, 175].

An amperometric detection principle for the determination of dissolved hydrogen sulfide in aquatic systems was developed by Jeroschewski and coworkers. [143–148] and several macrosensors for H_2S were realized. In collaboration with our group, a new H_2S microsensor based on this amperometric measuring principle was developed [6,149,150]. As the sensor principle is relatively new in comparison with potentiometric and voltammetric sulfide sensing, we describe the new H_2S microsensor in some more detail below.

The sensor design is based on the Clark principle, i.e. the sensor consists of electrodes in an electrolyte filling an inner compartment, which itself is separated from the analyte solution by a gas permeable membrane (silicone). All electrodes (working electrode, guard electrode, and counter-electrode; Figure 5A) are made of platinum and are placed in a glass casing made of a Pasteur pipette, that is tapered to a tip diameter of a few micrometers and sealed with a thin silicone membrane. The working and guard electrode have a tip diameter of a few micrometers and are prepared by electrochemical etching in concentrated KCN solution. For the sake of mechanical stabilization, electrical insulation towards the guard electrode and minimization of the active electrode surface, the working electrode is coated with a highly resistive glass except for the very tip of a few micrometers. The analyte, H_2S, diffuses through the gas permeable membrane of the casing and is electrochemically determined inside the sensor.

The direct oxidation of sulfide to elemental sulfur at platinum electrodes leads to an inactivation of the electrode surface [176,177]. Hence, the Clark

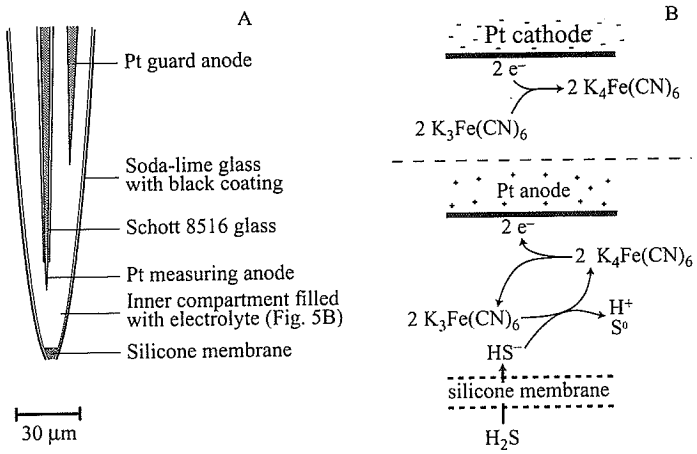

Figure 5. Schematic drawing of an H_2S microsensor tip (A), and the measuring principle of the microsensor (B) (redrawn from Kühl *et al.* [6] by permission of Inter-Research). Note that only the working and the guard electrodes are shown in (A). The Pt counter-electrode is situated further away from the sensor tip up in the bulk part of the electrolyte-filled outer casing. The electrolyte is shielded from photodegradation by painting the outer casing with a black paint. Any optically dense paint which shows a good adhesion to glass can be used. We have good experience with black enamel paint containing xylene as solvent

principle was improved by using a redox mediator (hexacyanoferrate(III)), which oxidizes sulfide to sulfur before it reaches the charged Pt surface. This reaction forms hexacyanoferrate(II) that diffuses to the Pt microanode, where it is reoxidized to hexacyanoferrate(III) (Figure 5B). A more detailed reaction scheme is as follows [149]:
Anode reactions:

$$H_2S + 2[Fe(CN)_6]^{3-} \rightleftharpoons 2[Fe(CN)_6]^{4-} + S^0 + 2H^+ \quad \text{(chemical step)} \quad (19)$$

$$2[Fe(CN)_6]^{4-} \rightleftharpoons 2[Fe(CN)_6]^{3-} + 2e^- \quad \text{(electrochemical step)} \quad (20)$$

Cathode reaction:

$$2[Fe(CN)_6]^{3-} + 2e^- \rightleftharpoons 2[Fe(CN)_6]^{4-} \quad \text{(electrochemical step)} \quad (21)$$

Brutto reaction:

$$H_2S + 2[Fe(CN)_6]^{3-} \rightleftharpoons 2[Fe(CN)_6]^{4-} + S^0 + 2H^+ \quad (22)$$

A potential difference, ΔE_{pol} (Figure 6), between +80 and +150 mV is imposed between the working or guard electrode and the counter-electrode. The guard electrode serves to shield the measuring electrode from reduced components,

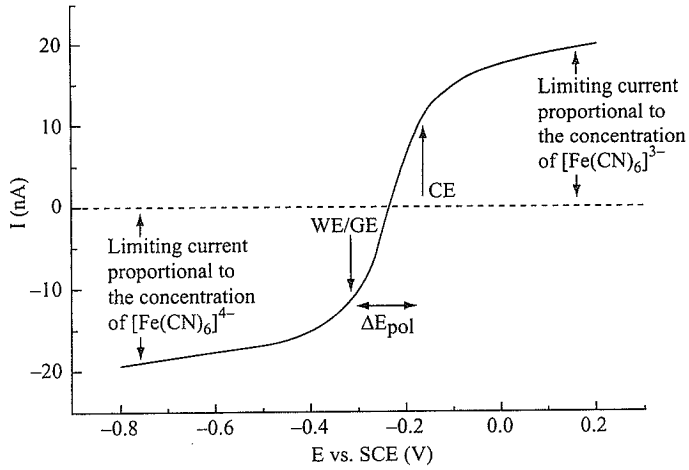

Figure 6. A single-sweep voltammogram of 0.05 mol L^{-1} hexacyanoferrate(II)/(III) in 0.5 mol L^{-1} carbonate buffer measured with a Pt disc electrode (diameter = 5 µm) versus SCE at a scan rate of 0.5 V s^{-1}. The potentials imposed on each individual electrode in a H_2S microsensor are indicated on the figure. WE, working electrode; GE, guard electrode; CE, counter-electrode; ΔE_{pol} = polarization voltage imposed on the sensor

e.g. hexacyanoferrate(II) produced at the counter-electrode, that diffuse towards the sensor tip. The guard electrode thus helps to keep a constant high ratio of hexacyanoferrate(III) to hexacyanoferrate(II) in the microsensor tip compartment, which is a prerequisite for a low zero-current and good signal stability.

The individual potentials of the electrodes are determined by the relative concentrations of hexacyanoferrate(II) and hexacyanoferrate(III) in the inner compartment. This is very important for the lifetime of the sensor. Figure 6 illustrates the potentials of the electrodes with respect to each other. When the sensor is filled with electrolyte, the sensor electrodes are at a potential of ~ 0.27 V versus SCE, i.e. close to the standard potential of the redox system hexacyanoferrate (II)/(III). When the sensor is polarized, the potential of the counter-electrode, CE, is shifted by ΔE_{pol} versus WE/GE towards more reducing potentials and a current can flow between counter-electrode and the measuring and guard electrode, respectively. Over time, equation (22) leads to a slow change of the ratio of hexacyanoferrate(II) to hexacyanoferrate(III), which results in a slow vertical shift of the *i–E* curve in Figure 6, from top to bottom. This results in a slow drift towards more negative potentials of the working, guard and counter-electrodes, respectively. When the working and guard electrode potential gets close to the jump of the *i–E* curve (Figure 6), small changes in the potential, e.g. by electrical noise, lead to high changes of the current, resulting in a lower signal to noise ratio. This can be overcome by a stepwise increase of the polarization voltage. When the concentration of

hexacyanoferrate(II) is so high that the potential of the counter-electrode is shifted to very negative potentials, the sensor lifetime ends and the inner solution must be changed. The latter is, however, seldom practicable with H_2S microsensors. This is only one of several parameters determining the lifetime of the microsensor, which is typically 2–8 weeks. More details of sensor characteristics are discussed elsewhere [6,149,150].

The H_2S microsensor exhibits a linear response from $\sim 0.1\ \mu mol\ L^{-1}$ to >1–2 mmol L^{-1} H_2S, with a sensitivity of 0.2–3 pA $(\mu mol\ L^{-1})^{-1}$. The detection limit is ~0.1–1 $\mu mol\ L^{-1}$ H_2S, depending on the actual sensitivity of the sensor and the accuracy of the ampere meter, which is used in connection with the H_2S microsensor. We recommend the use of picoampere meters with a resolution of 0.1–1 pA, with the possibility for polarizing both measuring and guard electrode. Such meters are commercially available (e.g. from Unisense Aps, Denmark).

The small consumption of H_2S by the microsensor in the test water minimizes the corresponding concentration gradients and, therefore, the effect of external convection at the sensor tip. The sensor signal in stagnant water exhibits a decrease by <1–2% of the value measured in vigorously stirred water. In addition, there is no disturbance of sulfide equilibria at the microsensor tip. Therefore, the sensor signal, for a given total sulfide concentration, at various pH, can be predicted from the protonation equilibiria of the sulfide system (equation (3)). The only siginificant interfering agent is SO_2, which is not important in most natural waters. As the electrolyte of the H_2S microsensor is photodegraded, we recommend shielding the inner electrolyte-filled compartment from bright light by painting the outer sensor casing with an optically dense paint with a good adhesion to glass, e.g. a black enamel paint containing xylene as the solvent.

5 SULFIDE AND H_2S MICROSENSOR APPLICATIONS IN AQUATIC SYSTEMS

Most studies of sulfide in the water column are still based on spectrophotometric methods (see Section 3.1) and relatively few measuring devices equipped with sulfide sensors have been used for profiling the pelagic environment. As a representative example, Eckert *et al.* [125] described a measuring device equipped with a potentiometric Ag/Ag_2S electrode, that was used to monitor the vertical sulfide distribution in the hypolimnion of a stratified lake.

Water sampling and subsequent sulfide analysis or monitoring with large sulfide sensors can in principle provide sufficient resolution for applications in the water column. The consumption of H_2S may, however, be high and stirring effects may therefore be important. Furthermore, with water sampling the risk of degassing or oxidation by O_2 during sampling is very high. *In situ*

measurements are therefore important. It is even more so in the porewaters of sediments and biofilms, where sulfide cycling is of major importance, steep concentration profiles of sulfide and other redox species prevail and must be determined [5–7]. Nevertheless, porewater extraction from defined sediment strata followed by spectrophotometric (Section 3.1) or chromatographic (Section 3.2) analysis are still the most widely used methods for sulfide analysis of sediments in biogeochemistry, despite the inherent limitations in spatial and temporal resolution, and the necessity for sample destruction by this method.

During the last decade a slowly increasing number of studies have used microsensors for measuring sulfide species in benthic systems. Besides the advantages of microsensors over macrosensors in terms of e.g. signal stability, response time and low stirring sensitivity, they are also ideal tools for monitoring steep sulfide gradients owing to their negligible analyte consumption and small mechanical disturbance of the sediment or biofilm matrix. Hence relatively fast and repetitive measurements in practically undisturbed environmental samples become possible, from which detailed information about sulfide producing and consuming processes can be obtained. Below, we list some examples of such sulfide microsensor applications in natural biofilms and sediments, both in the laboratory and *in situ* (Figures 7 and 8)

In spite of the many practical problems with Ag/Ag_2S microsensors discussed in section 4.2, they have been used successfully in many different systems ranging from wastewater biofilms [7,159], sediments and cultures of microorganisms [117,162,179] to microbial mats growing under extreme environmental conditions in hot springs [180] and hypersaline lakes [155,181]. Ag/Ag_2S microsensors were also the first sulfide microsensors to be deployed on benthic lander intruments for autonomous *in situ* profiling of sediments and microbial mats near hydrothermal vents in the deep sea [23,164].

Voltammetric S(−II) microsensors (section 4.3) have been applied in several studies of marine sediment, both in the laboratory and *in situ* [23,166,167]. Although promising, these sensors still need improvement and further development (see Chapter 9). The most recently developed amperometric H_2S microsensor (section 4.4) has already found several laboratory applications in waste water, freshwater and marine biofilms and sediments [6,178,182,183], and the H_2S microsensor now seems to be used more frequently than the Ag_2S microsensor. First *in situ* applications were recently performed in coastal sediments and near shallow water hydrothermal vents [184,185]. The H_2S microsensor seems a favorable alternative to the Ag/Ag_2S microsensor in most applications where pH < 8.5, whereas for studies of more alkaline waters and sediments the Ag/Ag_2S microsensor is still the best choice. Above all, with the H_2S microsensor it is now possible to study sulfide distribution and dynamics at fine scale in acid environments [6]. The fast response time of this sensor has also allowed the first reliable estimates of anoxygenic photosynthesis from measurements of sulfide dynamics around experimental light–dark shift events [186].

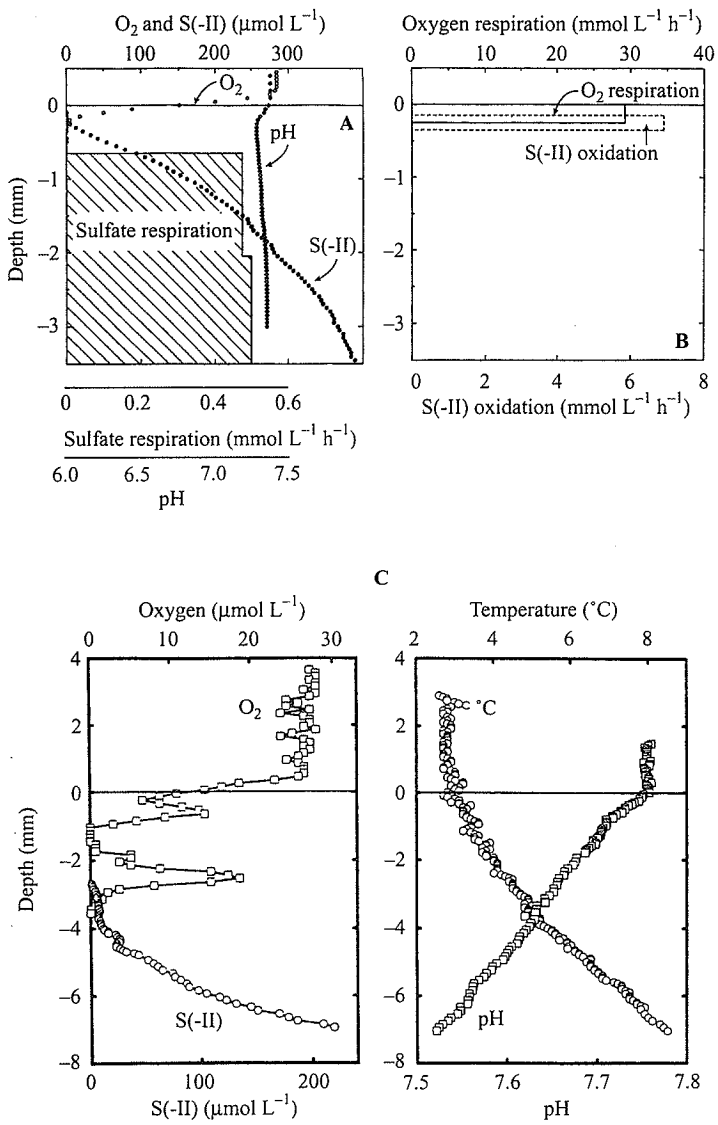

Figure 7. Examples of laboratory (A,B) and *in situ* (C) applications of Ag/Ag_2S microelectrodes. (A,B) Measurements of oxygen and sulfide in a biofilm and modeled reaction rates for sulfide production and consumption (redrawn from Kühl and Jørgenson [7] by permission of American Society for Microbiology). (C) Measurements of sulfide, oxygen, pH and temperature in a microbial mat subject to advective porewater transport; data were obtained *in situ* at a hydrothermal vent at 2 km water depth with a small measuring module controlled by the deep-sea research submersible Alvin. Reprinted with permission from *Nature* [164]. Copyright (1992) Macmillan Magazines Limited

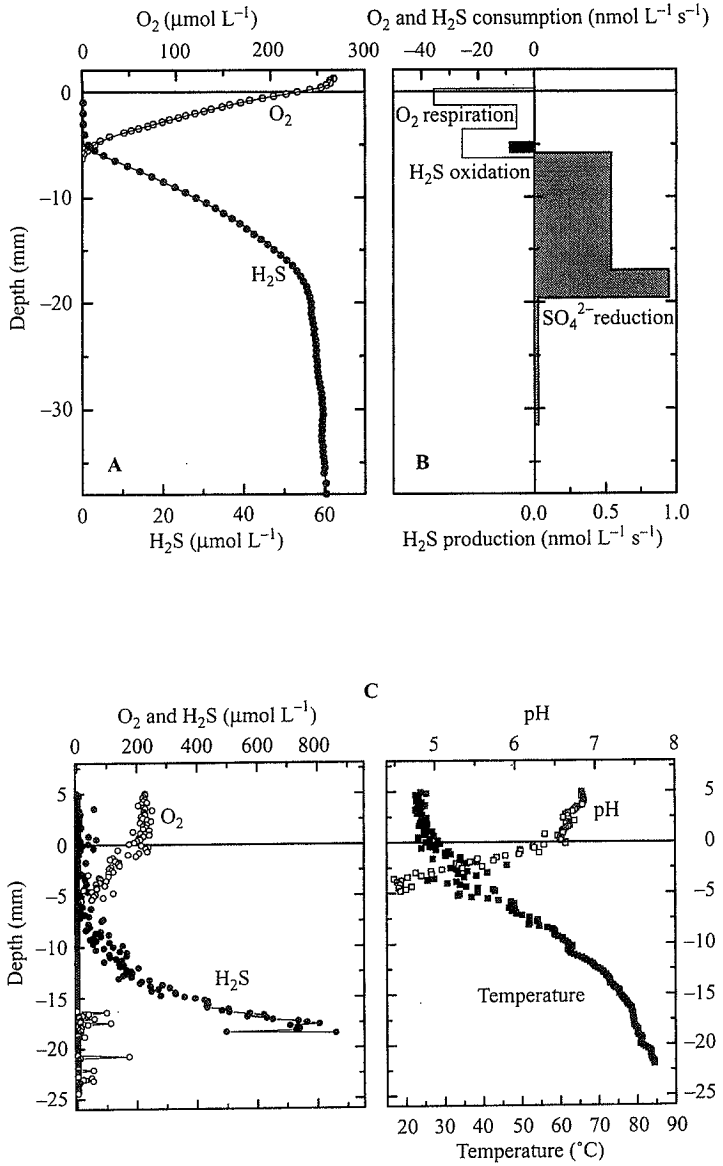

Figure 8. Examples of laboratory (A,B) and *in situ* (C) applications of H_2S microsensors. (A) Oxygen and sulfide measurements in acid (pH < 5) lake sediment. (B) Oxygen and sulfide turnover rates modeled from the microprofiles. (C) *In situ* measurements of oxygen, sulfide, pH and temperature in a sediment near a shallow-water (7 m) hydrothermal vent (Milos, Greece). Redrawn from Kühl *et al.* [6] by permission of Inter-Research, and from ref. [178]

6 SUMMARY AND DIRECTIONS FOR FUTURE RESEARCH

Several sensor techniques are available for direct sulfide analysis in the aquatic environment. Especially microsensors are well-suited for this purpose owing to their minimal consumption of the analyte, fast response times and their ability to measure with minimal invasion the distribution and dynamics of sulfide at high spatial and temporal resolution. Every analytical technique, however, has its advantages and drawbacks, which should be carefully considered for each application. Therefore, we see a need for a detailed experimental comparison of currently available sulfide microsensor techniques. Preferentially, such a comparison should involve measurements not only in defined sulfide solutions but also in defined gradients of sulfide and pH. The latter, would give a much better idea about how the various microsensors affect the analyte gradient itself, and, therefore, how applicable the various techniques are for fine scale measurements in environmental analysis.

Another complicating factor when working with *in situ* sulfide analysis is the instability of dilute sulfide solutions, which requires careful and strictly anaerobic calibration procedures under environmental conditions. For this, more detailed studies of sensor performance as a function of environmental variables such as temperature, salinity, and hydrostatic pressure are required. Besides the use of sulfide standards in traditional dosimetric calibration procedures, new devices may also simplify calibration of sulfide sensors [45].

At the moment, no reliable optical sulfide sensors are available for environmental analysis [20]. However, with the rapid development of optical sensor technology in recent years [19,187,188] optical sulfide sensors will probably become available. Also, advanced gel sampling techniques are becoming available for fine scale sulfide measurements [189] (see also Chapter 11). Last but not least, sulfide measurement with voltammetric microsensors seems a promising direction for future research, provided suitable sensors can be designed to limit interference, when measuring in complex natural systems (Chapter 9). Biosensors for sulfide species have already been developed [141], and with the recent development of new measuring and construction principles for microbiosensors by Revsbech and coworkers (Chapter 6), much better sulfide biosensors can now be realized.

Even if suitable and well-characterized sensors exist, it is a major undertaking to transfer the technology from the laboratory to the field in order to perform *in situ* environmental analysis. An adaptation of the sensors for special measuring platforms needs to be realized and first sucessful deployments of such measuring systems for *in situ* measurements of sulfide have been reported in the literature [92,144,156,159].

In conclusion, with the present array of sulfide sensors, detailed studies of the sulfur cycle can be performed in waters, biofilms and sediments. It must be emphasized, however, that the most suitable techniques have to be carefully

chosen and optimized for each application. The largest gap in our ability to characterize the sulfide equilibria in nature is presently the lack of suitable sensors for measuring HS^-, which is the most abundant sulfide species in most natural aquatic environments.

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