LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Limnol. Oceanogr.: Methods 7, 2009, 442–448

Improved cyanolysis protocol for detection of zero-valent sulfur in natural aquatic systems

*Alexey Kamyshny, Jr.**
Department of Biogeochemistry, Max Planck Institute for Marine Microbiology, Celsuisstrasse 1, D-28359 Bremen, Germany

Abstract

We propose a novel protocol for detection of reactive zero-valent sulfur (ZVS) in natural aquatic samples including seawater. Reaction with hot potassium cyanide at slightly acidic conditions recovers ZVS from colloidal fraction of particulate elemental sulfur, polysulfides (S_n^{2-}), and their protonated forms. Preconcentration by partial evaporation of the sample and separation of thiocyanate anions by high-performance liquid chromatography on the C30 reverse phase column modified with poly(ethylene glycol) followed by spectrophotometric detection at 220 nm wavelength allows us to detect reactive ZVS with detection limit of 3 nmol L^{-1} for fresh water samples and 6 nmol L^{-1} for seawater samples. Storage at 4°C for 6 weeks does not change the concentration of thiocyanate in the sample by more than 10%.

Introduction

This work is dedicated to development of the method, which allows quantitative detection of low nanomolar concentrations of colloidal and solubilized elemental sulfur in natural aquatic systems including seawater.

Zero-valent sulfur (ZVS) exists in natural aquatic systems as dissolved sulfur (Boulegue 1978), dispersed solid form (crystalline or amorphous), in a variety of colloidal forms (Steudel et al. 1987; Van Gemerden and Mas 1995; Prange et al. 2002; Dahl and Prange 2006), and solubilized in the presence of sulfide as inorganic polysulfides (S_n^{2-}). Solubility of elemental solid S_8 in pure water is low (19 ± 6 nmol L^{-1} S_8) (Boulegue 1978).

Polysulfides and elemental sulfur are produced by biological and abiotic hydrogen sulfide oxidation in various natural systems (Zopfi et al. 2004 and references therein; Kamyshny et al. 2008). Sulfur-oxidizing bacteria can produce and store sulfur intra- or extra-cellularly (Van Gemerden and Mas 1995). Inorganic polysulfides readily react with organic and inorganic compounds abundant in sediments (Luther 1991; Amrani et al. 2007).

Despite the importance of ZVS compounds, there is no chromatographic technique, which allows detection of ZVS compounds in aqueous systems at low nanomolar concentrations. Electrochemical methods have been applied to detect elemental sulfur. For instance, Luther et al. (1991) were able to measure elemental sulfur with detection limit of 5 nmol L^{-1} by using cathodic stripping square wave voltammetry. Similar detection limit for this technique was reported by Wang et al. (1998). Electrochemical methods are a powerful tool for examining reduced sulfur chemistry in natural waters, but they are not always unambiguous at low ZVS to S(II) ratios.

In this article, we propose a method based on the reaction of hot acidic solution of potassium cyanide with ZVS compounds, followed by chromatographic determination of thiocyanate.

Hydrogen cyanide reacts fast with inorganic polysulfides (S_n^{2-}) and their protonated forms, polythionates ($S_nO_6^{2-}$) with $n > 3$ and colloidal sulfur, but not with the solid (rhombic) sulfur (Karchmer 1970; Szekers 1974; Luthy and Bruce 1979; Koh 1990; Kamyshny in press).

$$S_n^{2-} + (n-1)\,HCN \rightarrow (n-1)\,SCN^- + HS^- + (n-2)\,H^+ \quad (1)$$

$$S_nO_6^{2-} + (n-3)\,HCN \rightarrow (n-3)\,SCN^- + S_3O_6^{2-} + (n-3)\,H^+ \quad (2)$$

$$S(0) + HCN \rightarrow SCN^- + H^+ \quad (3)$$

Thiosulfate and trithionate reactions with cyanide are catalyzed by Cu(II) and La(III) salts, respectively (Koh 1990).

$$S_2O_3^{2-} + HCN \rightarrow SCN^- + SO_3^{2-} + H^+ \quad (4)$$

$$S_3O_6^{2-} + HCN + H_2O \rightarrow SO_3^{2-} + SO_4^{2-} + 3H^+ + SCN^- \quad (5)$$

Two procedures for thiocyanate detection after cyanolysis of

*Corresponding author: E-mail: akamyshn@mpi-bremen.de
Present address: Department of Geology, University of Maryland, College Park, Maryland, 20742, USA

Acknowledgments

This project was supported by the Max-Planck-Society. Author gratefully acknowledges support from the MPG Minerva Program. T. Ferdelman, O. Lev, N. Loboda, and A. Kamyshny Sr. are thanked for extensive comments on the text.

natural sample have been employed: (1) the spectrophotometric detection of SCN^- complex with ferric iron and (2) the ion chromatographic detection of SCN^-. Kamyshny et al. (2008) used ion chromatography to detect thiocyanate after cyanolysis of water from deep aquifer wells. Detection limit for thiocyanate was c.a. 2 µmol L^{-1}. The reaction of ferric iron with thiocyanate (Eq. 6) resulting in a red-colored complex is easily performed but useful only for clear nonsaline water samples.

$$Fe^{3+} + 3SCN^- \rightarrow Fe(SCN)_3 \quad (6)$$

The detection limit of the method is rather high (10 µmol L^{-1}) (Kamyshny in press). Sometimes cyanolysis is performed in organic solvent (carbon disulfide, acetone, and petrol ether) after sediment extraction (Jacobs and Emerson 1982; Troelsen and Jørgensen 1982; Howarth and Jørgensen 1984; Chan and Suzuki 1993; Podgorsek and Imhoff 1999). This method has an important drawback: bacterially produced colloidal sulfur cannot be extracted quantitatively by organic solvents (Janssen et al. 1999). Troelsen and Jørgensen (1982) analyzed ZVS in 1 g sediment pretreated with zinc acetate and reported standard curve with the lowest ZVS amount of c.a. 150 nmol. Jacobs and Emerson (1982) reported detection limit as low as 0.1 µmol L^{-1} for the analysis of ZVS in water column samples, but this sensitivity was achieved by pretreatment of 3 L sample with hydrochloric acid, filtration, and further analysis of filtered sulfur.

In this article, we propose the novel protocol, which increases sensitivity of the cyanolysis method for three orders of magnitude and can be easily applied to both nonsaline and marine water samples. Detection limits are 3 nmol L^{-1} and 6 nmol L^{-1} for Milli-Q and marine water samples, respectively.

Two main improvements of the technique are 1) use of HPLC-UV analysis of SCN^- anion on C-30 reverse phase column derivatized with PEG-20,000 (Rong et al. 2005); and 2) evaporation of the sample after cyanolysis to increase concentration of thiocyanate in the sample.

Materials and procedures

Materials—Milli-Q or North Sea water was used for preparation of all solutions. HPLC grade 99.9% methanol was purchased from Carl Roth GmbH. NaOH (99%) and zinc chloride dehydrate (99.5%) were from Applichem. Boric acid (99.8%), sodium carbonate (99.9%), iron(II) sulfate (99.5%), and sodium thiosulfate (97%) were purchased from Merck. NaCl (99.8%) and HCl 6M solution were purchased from Riedel-de Haën (Sigma-Aldrich). Sodium acetate (99.5%), sodium sulfite (98%), disodium hydrogen phosphate (99%), sodium nitrate (99%), sulfur (99.999%), and PEG-20,000 were purchased from Fluka (Sigma-Aldrich). Acetonitrile (99.9%, HPLC grade), $Na_2S{\cdot}9H_2O$ (98%), KSCN (99%) KCN (98%), sodium sulfate (99%), 2,2'-dithiobis(5-nitropyridine) (96%), and tetrabutylammonium hydrogen sulfate (97%) were purchased from Sigma-Aldrich.

Equipment—A Sykam S1100 pump with Sykam S3200 UV-VIS detector was used for all chromatographic analyses. Nomura Chemical, Japan, Develosil RPAQUEOUS C30 reverse phase column (150 mm × 4.6 mm × 5 µm) was used for high-performance liquid chromatography (HPLC) separation of thiocyanate and polythionates. Zorbax, Knauer, Germany C18 reverse phase column (125 mm × 4 mm × 5 µm) was used for elemental sulfur analysis.

Minisart syringe filters (0.2 µm pore size) were purchased from Sartorius.

Final protocol—Boric acid solution in Milli-Q (1%, 100 mL) is gently boiled in a 250-mL beaker for at least 2 min. A sample (50 mL) is added to boiling boric acid solution followed by 100 µL of 10% KCN solution. Reaction mixture is heated to boiling once more and boiled out gently to the volume of c.a. 10 mL. The beaker is rinsed twice with 1-2 mL of Milli-Q water and rinses are added to the sample. For seawater samples, the volume is adjusted to 14 mL, 200 µL of the sample is injected to HPLC equipped with C30 column, pre-flushed with 5% PEG-20,000 solution for 3 h at 300 µL min^{-1} rate. For detailed description of chromatographic analysis, see Chromatographic methods in Assessment. Freshwater samples, in case the detection limit of less than 6 nmol L^{-1} is required, are transferred to 50 mL beaker and boiled out to the volume of c.a. 4 mL. The beaker rinsed twice with 0.5-1 mL of Milli-Q water and rinses are added to the sample. Sample volume is adjusted to 6 mL. HPLC analysis is as for seawater samples. After cyanolysis, the samples could be stored for at least 4 weeks at room temperature or 6 weeks at 4°C prior to analysis.

Note that during the analysis c.a. 4 mg of HCN released. Due to the toxicity of KCN and HCN, the analysis must be carried in the hood and protective clothes have to be used.

Assessment

Polysulfide and colloidal sulfur samples preparation—All samples were prepared under anoxic conditions in the glove-bag ($[O_2] \leq 0.2\%$ in the gas phase) in Milli-Q or North Sea water purged with nitrogen for at least 1 h. Polysulfide solution undersaturated with respect to elemental sulfur was prepared by dissolving sulfur (128 mg) in warm (c.a. 40°C) sodium sulfide solution (704 mg sodium sulfide nonahydrate) in c.a. 50 mL anoxic Milli-Q water. After all sulfur was dissolved (c.a. 2 h), the solution was allowed to cool to room temperature. Next it was acidified with 6M HCl to pH = 9.3 and diluted to 100 mL with anoxic Milli-Q. Concentration of ZVS in this solution (Stock 1) was 40 mmol L^{-1}. Actual concentration of S(II) was measured by the method of Cline (1969) and was found to be 21.5 mmol L^{-1}. Final composition and concentration of the polysulfide solution Stock 1 can be described as 21.5 mmol L^{-1} $Na_2S_{2.86}$. At standard conditions and infinite dilution, the solution of such composition is undersaturated with respect to sulfur at pH > 9.0 (calculated from the set of thermodynamic constants from Kamyshny et al. [2007]).

Stock 1 was prepared once, stored in the glove-box under nitrogen and used on the day of preparation and the next 3 d. Quality of the Stock 1 was monitored each day by three

analyses: determination of total S(II) content by the method of Cline (1969), determination of thiosulfate by derivatization with 2,2′-dithiobis(5-nitropyridine) method (Vairavamurthy and Mopper 1990) and determination of total ZVS by dilution of the sample (75 µL) with 5% zinc acetate solution (1 mL) followed by dilution with methanol to 50 mL. Sulfur in methanol was detected by HPLC.

Thiosulfate is the main contaminant of polysulfide solution prepared from sodium sulfide and sulfur at anoxic basic conditions due to polysulfidic ZVS disproportionation (Giggenbach 1974; Licht and Davis 1997):

$$4S_{dissolved} + 4OH^- \rightleftarrows S_2O_3^{2-} + 2SH^- + H_2O \quad (7)$$

Equilibrium shifts to the right at pH > 9, but the process is slow enough at room temperature to allow storage of polysulfide solutions at anoxic conditions for days even at pH values much higher than pH of Stock 1 (pH = 9.3) (Giggenbach 1974).

No significant difference in S(II) concentration was measured in 4 d: fresh solution contained 21.5 mmol L^{-1} S(II) and in the next 3 d detected concentrations varied in the range 20.7 to 21.8 mmol L^{-1} S(II). Thiosulfate was not detected in fresh solution and after 1-d storage. Thiosulfate concentration after 2 d and 3 d of storage was 9.3 µmol L^{-1} and 32.5 µmol L^{-1}, respectively. The maximum value 32.5 µmol L^{-1} was less than 1/1000 of ZVS concentration (40 mmol L^{-1}). No decrease in ZVS concentration in Stock 1 was detected also by methanol dilution. Detected concentration of ZVS in fresh Stock 1 solution was 37.7 mmol L^{-1} (94% recovery). Concentrations after next 1, 2 and 3 d of storage were in the range 37.6 mmol L^{-1} to 38.5 mmol L^{-1} (94% to 96% recovery).

A final stock of polysulfide ZVS for assessment purposes was prepared by dilution of the primary stock solution to 750 µmol L^{-1} ZVS concentration with Milli-Q water. A final stock of colloidal ZVS was prepared by diluting the primary stock to 750 µmol L^{-1} ZVS concentration with 12 mmol L^{-1} HCl solution in Milli-Q water. The final pH value of the colloidal ZVS was 2.3. At this pH value, more than 99.97% of ZVS was in the form of colloidal sulfur and only 0.027% in the form of polysulfides (calculated from the set of thermodynamic constants from Kamyshny et al. [2007]). Polysulfide ZVS standards and colloidal ZVS standards were used for not more than 4 h after preparation.

Cyanolysis procedure—Due to sensitivity of polysulfide solutions to oxygen and extremely low concentrations of polysulfides in analyzed samples, diluted samples of polysulfides were not prepared. Instead 50 mL Milli-Q or North Sea water were added to reaction mixture together with the volume of polysulfide ZVS standard or colloidal ZVS standard that corresponded to calculated concentration of polysulfidic or colloidal sulfur in 50 mL sample.

Boric acid solution in Milli-Q (1%, 100 mL) was gently boiled in 250 mL beaker for at least 2 min to get rid of oxygen. Milli-Q or North Sea water (50 mL) was added to boiling boric acid solution followed by addition of polysulfide ZVS standard or colloidal ZVS standard. Immediately after addition of ZVS containing sample, 100 µL of 10% KCN solution was added. Reaction mixture was heated to boiling once more and boiled out gently to the volume of c.a. 70% of final sample volume. The beaker was rinsed twice by Milli-Q (c.a. 10% of final volume each rinse) and adjusted to the final volume with Milli-Q water. Samples prepared by using seawater were boiled out to 14–24 mL volume for the set of experiments of recovery dependence on the final volume of the sample. Samples with the lowest final volume (14 mL) were prepared in four replicates. Samples prepared by using Milli-Q water were boiled out to 6–24 mL volume for the set of experiments of recovery dependence on the final volume of the sample. Samples with the lowest final volume (6 mL) were prepared in four replicates. Samples with final volumes of 14–24 mL were boiled out in 250 mL beakers used for cyanolysis. Samples with final volumes of 6–12 mL were boiled to c.a. 15 mL volume in 250 mL beakers and transferred to 50 mL or 25 mL beakers together with two Milli-Q rinses of 250 mL beaker and boiling was continued. Final volumes of all samples used for calibration curve were 14 mL and 6 mL for seawater and Milli-Q water samples, respectively.

Chromatographic methods—Thiocyanate was detected according to the method of Rong et al. (2005). Aqueous solution of PEG-20,000 (5%) was pumped through HPLC column (Nomura Chemical, Japan, Develosil RPAQUEOUS C30 reverse phase column, 150 mm × 4.6 mm × 5 µm) at the rate of 300 µL min^{-1} for 3 h. Mobile phase (300 mmol L^{-1} Na_2SO_4 and 50 mmol L^{-1} NaCl) was pumped through column for at least 2 h for equilibration. This procedure was made every week or more often if peak shape or retention time of thiocyanate changed. A Sykam pump (S1100) and UV-VIS Detector (Sykam S3200) were used. Injection volume was 200 µL. Retention time for thiocyanate was 5.0–5.1 min.

Polythionates (n = 4–6), which also react with cyanide and have to be analyzed in non-cyanolized sample, can be analyzed by exactly the same chromatographic technique. Retention times are 2.3, 3.2, and 9.9 min for tetra-, penta-, and hexathionates, respectively.

Derivatization with 2,2'-dithiobis(5-nitropyridine) (Vairavamurthy and Mopper 1990) to detect thiosulfate concentrations was performed with the sample diluted 20 times with anoxic Milli-Q. HPLC analysis was performed immediately after derivatization. Supelco Discovery C18 reverse phase column (250 mm × 4.6 mm × 5 µm) was used. Dionex GP50 Gradient Pump was used for pumping the mobile phase. Dionex UVD340S Diode Array Detector was used for HPLC analysis at 230 nm wave length. Gradient program described in the original paper (Vairavamurthy and Mopper 1990) was changed for constant flow (1 mL min^{-1}) of the mixture of 50% acetonitrile and 50% aqueous solution containing 50 mmol L^{-1} sodium acetate and 7.5 mmol L^{-1} tetrabutylammonium hydrogen sulfate in Milli-Q water (adjusted to pH 3.5 with 6 mol L^{-1} HC1). This mobile phase allows rapid chromatographic separation of sulfite and thiosulfate derivatives.

Injection volume was 100 µL and retention times were 5.1 min and 5.7 min for sulfite and thiosulfate derivatives, respectively. Detection limits were 400 nmol L^{-1} for both sulfite and thiosulfate.

Elemental sulfur was detected by injection of 100 µL of methanol solution by using Zorbax C18 reverse phase column and pure methanol as a mobile phase at a flow rate 1 mL min^{-1}. A Sykam pump (S1100) and UV-VIS Detector (Sykam S3200) were used for HPLC analysis at 230 nm wavelength. Retention time for sulfur was 4.0 min and the detection limit was 300 nmol L^{-1}.

Calibration curves—The dependence of ZVS recovery based on the final volume of the sample after evaporation as well as importance of precipitation of borates after cooling of the sample was studied on cyanolized solution of polysulfides undersaturated with respect to sulfur (Fig. 1). Concentration of ZVS in these samples was 1.5 µmol L^{-1}. Evaporation of the sample to the volume of less than 20 mL resulted in precipitation. Recoveries and standard deviations for all data points presented in Fig. 1. Recoveries and standard deviations for four replicates for the samples evaporated to the lowest volumes (6 mL for fresh water samples and 14 mL for seawater samples) are summarized in Table 1.

Calibration curves for reactive ZVS were made in the concentration interval from 3 nmol L^{-1} to 1.2–1.5 µmol L^{-1} for polysulfides and colloidal sulfur samples in Milli-Q water (Fig. 2). Final sample volume after evaporation was 6 mL.

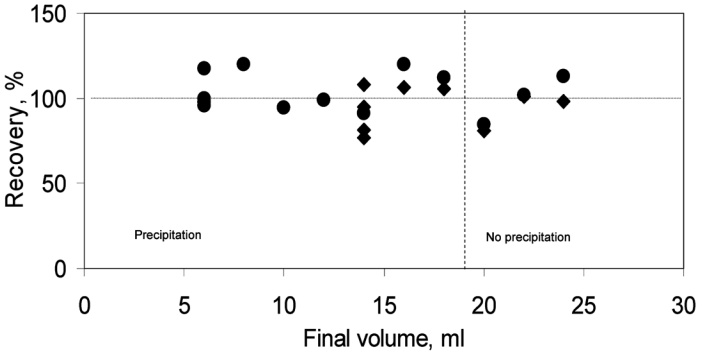

Fig. 1. Recoveries of ZVS as a function of sample final volume after evaporation. Horizontal dashed line show 100% recovery. Vertical dashed line borders between samples where no precipitation was observed to the samples where precipitation was observed. Solid circles: samples in Milli-Q water as a medium; solid diamonds: samples in North Sea water as a medium.

Table 1. Recoveries and standard deviations for all data points presented in Fig. 1

Sample medium	Recoveries, % (all data points)	Recoveries, % (four replicates)
Milli-Q water	104 ± 12	103 ± 10
North Sea water	95 ± 12	90 ± 14

Calibration curves for samples in seawater were prepared in the interval from 6 nmol L^{-1} to 6 µmol L^{-1} (Fig. 2). Final sample volume after evaporation was 14 mL. The 1:1 ratio between calculated and measured thiocyanate concentrations was observed at concentrations higher than 100 nmol L^{-1} of reactive ZVS in initial sample. For the samples with lower concentrations of reactive ZVS, the recovery was higher than 100%.

This deviation from the linearity can be explained by the presence of thiocyanate in the samples prior to ZVS addition. This thiocyanate can have the following origins: Milli-Q water or reagents (potassium cyanide and/or boric acid) for fresh water samples and North Sea water or reagents for seawater samples. To quantify these inputs, we analyzed the following: concentration of thiocyanate in Milli-Q, in Milli-Q evaporated from 50 mL to 6 mL volume, cyanolyzed sample with zero concentration of ZVS in Milli-Q (final volume 6 mL), North Sea water, North Sea water evaporated from 50 mL to 14 mL, and cyanolyzed sample with zero concentration of ZVS in North Sea water (final volume 14 mL). Results of those analyses are given in Table 2.

From this data the following sources of thiocyanate in the blank samples may be calculated: (1) Input from reagents (potassium cyanide and/or boric acid): 5.9–9.4 nmol L^{-1}, (2) input from Milli-Q: less than 3 nmol L^{-1}, and (3) input from North Sea water: 13 nmol L^{-1}. KCN is the most probable source of thiocyanate in blank experiments. Content of thiocyanate in KCN calculated from the amount of added KCN (10 mg) and final amount of thiocyanate in 50 mL of blank samples (17–27 ng) is 1.7–2.7 mg kg^{-1}.

This value is much lower than stated in the specification of the most pure KCN available from Sigma-Aldrich (catalogue number 60178), which was used in this study (≤ 100 mg/kg). If the reagent used contains 100 mg kg^{-1} of thiocyanate, it will

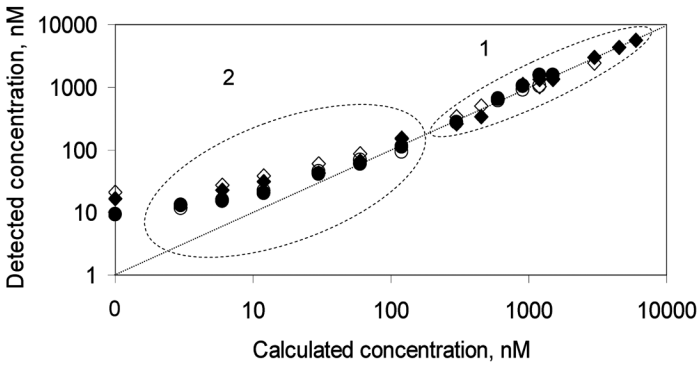

Fig. 2. Concentrations of detected ZVS as a function of initial concentration of polysulfidic or colloidal ZVS. Solid circles: polysulfide samples in Milli-Q water as medium; open circles: colloidal sulfur samples in Milli-Q water as medium; solid diamonds: polysulfide samples in sea water as medium; open diamonds: colloidal sulfur samples in sea water as medium. Oval 1: Range of concentrations, where 1:1 relation between calculated and detected concentrations of ZVS was observed. Oval 2: Range of concentrations, where deviation from 1:1 relation between calculated and detected concentrations of ZVS was observed.

Table 2. Results of cyanolysis analyses of blank samples

Sample	Detected concentration, nmol L^{-1}	Calculated concentration in the initial sample, nmol L^{-1}
Milli-Q water	n.d.	n.d.
Milli-Q water evaporated from 50 mL to 6 mL	n.d.	n.d.
Cyanolysis of Milli-Q	78	9.4
North Sea water	n.d.	n.d.
North Sea water evaporated from 50 mL to 14 mL	46	12.9
Cyanolysis of North Sea water	67	18.8

result in 345 nmol L^{-1} concentration of thiocyanate for 50 mL blank sample, and detection limit will increase to c.a. 100 nmol L^{-1}. Blank experiments with each new batch of cyanide have to be executed before the reagent is used.

Application of HCN gas may solve the background problem, but will significantly complicate an analysis, especially at the field conditions, due to drastic safety precautions that should be taken during work with gaseous hydrogen cyanide.

Recoveries for calibration curve were recalculated taking into account thiocyanate content of the blank samples (Fig. 3). Recoveries and standard deviations for all data points presented in Fig. 3 and separately for low concentration region (< 150 nmol L^{-1}) and for high concentration region (> 150 nmol L^{-1}) are presented in Table 3.

Precision and accuracy—Standard deviation is in the range of 7%–18% for individual sets of data (Tables I and III). Precision does not depend on the final volume of sample after evaporation and on the precipitation of borates: 10%–14% for four replicates with lowest final volume versus 12% for all points (Table 1). Precision also does not depend on the sea salts content in the sample: 7%–18% of for Milli-Q samples and 10%–18 % for seawater samples (Tables I and III). Precision does not depend on the form of reactive ZVS (colloidal versus polysulfidic): standard deviations are 7%–18% and 10%–16%, respectively (Tables I and III). For the samples with low reactive ZVS concentrations (< 150 nmol L^{-1} ZVS), which are more affected by the presence of thiocyanate in reagent cyanide, the precision is somewhat lower (standard deviation 16%–18%) than for the samples with higher ZVS concentrations (standard deviation 7%–15 %).

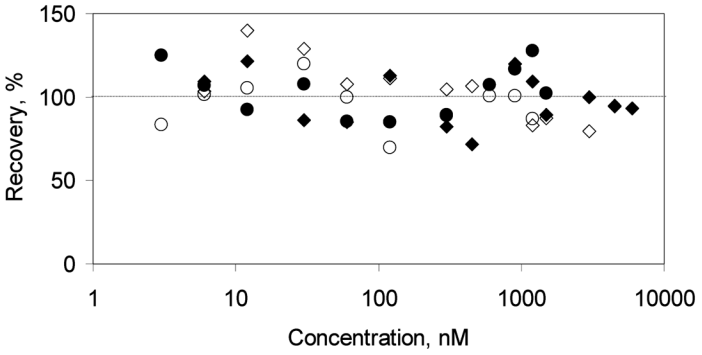

Fig. 3. Recoveries of ZVS as a function of initial concentration of polysulfidic or colloidal ZVS after taking into account SCN^- content of blank samples. Solid circles: polysulfide samples in Milli-Q water as medium; open circles: colloidal sulfur samples in Milli-Q water as medium; solid diamonds: polysulfide samples in sea water as medium, open diamonds: colloidal sulfur samples in seawater as medium.

Accuracy of analyses is high: all average values of recoveries for individual analyses sets (Tables I and III) do not differ from 100% by more than 10% except for the value (18%) for low concentrations of colloidal sulfur concentrations in seawater data set (Table 3). Results of analyses of all samples sets differ from calculated 100% recovery by less than 1.2 standard deviations.

We recommend using the value of 15% standard deviation for analysis of samples that (1) have concentration of reactive ZVS > 150 µmol L^{-1}; and (2) have less than 10% thiocyanate input from thiocyanate content of cyanide reagent. For the samples, which have lower ZVS content or higher thiocyanate content in cyanide, standard deviation of the method is 18%.

Quantitative conversion of tetrathionate to thiocyanate by reaction 2 was shown in Kamyshny et al. (in press). Recovery of tetrathionate by cyanolysis with $Fe(SCN)_3$ spectrophotometric detection was reported to be 101 ± 5 %.

Samples storage—To determine ideal conditions and maximum times for sample storage, samples after cyanolysis were stored at room temperature (22 ± 2°C), at 4°C and at –20°C.

Table 3. Recoveries and standard deviations for the data presented in Fig. 3

Sample description	Recoveries, % (all data points)	Recoveries, % (< 150 nmol L^{-1} ZVS)	Recoveries, % (> 150 nmol L^{-1} ZVS)
Polysulfide solution in Milli-Q	104 ± 15	100 ± 16	109 ± 14
Polysulfide solution in North Sea water	98 ± 15	103 ± 16	95 ± 15
Colloidal sulfur in Milli-Q	96 ± 14	97 ± 18	94 ± 7
North Sea water, colloidal sulfur	103 ± 18	118 ± 16	93 ± 10

Two samples (prepared in Milli-Q water and North Sea water) were stored at each temperature. All samples for these experiments were selected with the initial ZVS concentrations (recalculated for 50 mL sample) in the range of 60 nmol L^{-1} to 900 nmol L^{-1}. The samples were heated to c.a. 60°C, vigorously shaken, and centrifuged before aliquot was taken for analysis. Results of storage experiments are presented in Fig. 4. Detected peak areas decreased below 90% of initial peak area in 54 d for all the samples (both prepared in Milli-Q and North Sea water). The best results were obtained for the samples stored at 4°C. Both Milli-Q and seawater samples showed less than 10% decrease of the peak area in 6 weeks. Samples can be stored also at room temperature with less than 10% peak area loss, but only for 4 weeks. Freezing of the samples and storage at –20°C does not improve the storage stability. For example, for seawater sample stored at –20°C results in decrease of relative peak area to 88% already after 4 weeks.

Interference—To study possible interference with compounds that can be present in natural aquatic systems, polysulfide solution with concentration of ZVS 5 µmol L^{-1} was cyanolized in Milli-Q water and in the presence of following reagents: 50 mmol L^{-1} and 50 µmol L^{-1} of Na_2HPO_4, 50 mmol L^{-1} and 50 µmol L^{-1} of Na_2CO_3, 50 mmol L^{-1} and 50 µmol L^{-1} of $NaNO_3$, 50 mmol L^{-1} and 50 µmol L^{-1} of $FeSO_4$, and 50 mmol L^{-1} of synthetic goethite (FeOOH). All experiments were performed three times.

The following reagents were found to decrease a recovery of the analysis by more than 20%: 50 mmol L^{-1} of Na_2HPO_4, 50 mmol L^{-1} of Na_2CO_3, and 50 mmol L^{-1} of $FeSO_4$. A drastic recovery decrease was found in the experiments with 50 mmol L^{-1} $FeSO_4$ (6% ± 1% recovery). Suspension of 50 mmol L^{-1} of goethite increases recovery to 131% ± 6 % due to partial oxidation of S(II) moiety of polysulfide to ZVS by Fe(III) prior to completion of cyanolysis. Phosphate, carbonate, nitrate, and iron(II) in concentrations of 50 µmol L^{-1} do not affect recovery of the analysis.

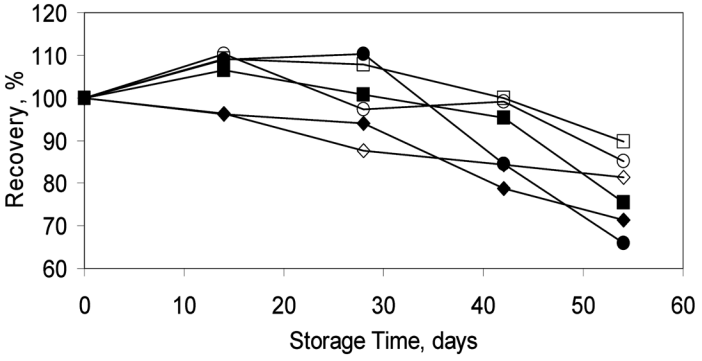

Fig. 4. Relative areas of thiocyanate peaks after storage at three different temperatures. Closed circles: samples in Milli-Q water stored at room temperature; closed rectangles: samples in Milli-Q water stored at 4°C; closed diamonds: samples in Milli-Q water stored at –20°C; opened circles: samples in seawater stored at room temperature; opened rectangles: samples in seawater stored at 4°C; opened diamonds: samples in seawater stored at –20°C.

Discussion

In this work, a method for detection of cyanide-reactive ZVS concentrations in pure water (3 nmol L^{-1} ZVS detection limit) and seawater (6 nmol L^{-1} ZVS detection limit) has been developed. It provides the major improvement of the method performance compared with cyanolysis with ion-chromatography finishing (detection limit 2 µmol L^{-1}, as reported by Kamyshny et al. [2008]), photometric finishing (detection limit 10 µmol L^{-1}, as reported by Kamyshny et al. [in press]), and cyanolysis in organic solvents (150 µmol kg^{-1} sediment, as reported by Troelsen and Jørgensen [1982] and 100 nmol L^{-1}, as reported by Jacobs and Emerson [1982]).

Concentrations of ZVS that can be detected by the developed method are lower than solubility of elemental sulfur in water (c.a. 150 nmol L^{-1} ZVS) (Boulegue 1978). The absence of dependence of ZVS recovery on ZVS concentration at [S(0)] < 150 nmol L^{-1} (Fig. 3) shows that soluble sulfur quantitatively reacts with hydrogen cyanide under experimental conditions, described in this work. Application of this method allows an assay of dissolved elemental sulfur in aquatic samples with high sulfur undersaturation with respect to rhombic sulfur. This method can also help to detect solubility of elemental sulfur in water at different temperatures and salinities and to find if levels of dissolved sulfur at specific conditions can maintain bacterial population using sulfur as an energy source.

Comments and recommendations

One limiting factor for application of this method to the systems with low cyanide reactive ZVS concentrations is a content of thiocyanate in cyanide reagent. Blank experiments must be performed for each new batch of potassium cyanide to evaluate thiocyanate content in the treated sample.

The most time-consuming step of the procedure is evaporation of water to low volumes, which improves the detection limit. If concentrations of ZVS compounds detectable by this method are high enough, the reaction mixture can be boiled for c.a. 10 min only to eliminate HCN from the solution.

References

Amrani, A., J. W. Turner, Q. Ma, Y. Tang, and P.G. Hatcher. 2007. Formation of sulfur and nitrogen cross-linked macromolecules under aqueous conditions. Geochim. Cosmochim. Acta 71:4141-4160.

Boulegue, J. 1978. Solubility of elemental sulfur in water at 298K. Phosphor. Sulfur 5:127-128.

Chan, C. V., and I. Suzuki. 1993. Quantitative extraction and determination of elemental sulfur and stoichiometric oxidation of sulfide to elemental sulfur by Thiobacillus thiooxidans. Can. J. Microbiol. 39:1166-1168.

Cline, J. D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14:454-458.

Dahl, C., and A. Prange. 2006. Bacterial sulfur globules:

Occurence, structure and metabolism. *In* J. M. Shevely [ed.], Inclusions in prokaryotes. Springer Verlag.

Giggenbach, W. F. 1974. Kinetics of the polysulfide-thiosulfate disproportionation up to 240°. Inorgan. Chem. 13:1730-1733.

Howarth, R. W., and B. B. Jorgensen. 1984. Formation of ^{35}S-labelled elemental sulfur and pyrite in coastal marine sediments (Limfjorden and Kysing Fjord, Denmark) during short-time $^{35}SO_4^{2-}$ reduction measurements. Geochim. Cosmochim. Acta 48:1807-1818.

Jacobs, L., and S. Emerson. 1982. Trace metal solubility in anoxic fjords. Earth Planet. Sci. Lett. 60:237-252.

Janssen, A. J. H., G. Lettinga, and A. de Keizer. 1999. Removal of hydrogen sulphide from wastewater and waste gases by biological conversion to elemental sulphur—Colloidal and interfacial aspects of biologically produced sulphur particles. Colloids and Surfaces A: Physicochemical and Engineering Aspects 151:389-397.

Kamyshny Jr., A., J. Gun, D. Rizkov, T. Voitsekovski, and O. Lev. 2007. Equilibrium distribution of polysulfide ions in aqueous solutions at different temperatures by rapid single phase derivatization. Environ. Sci. Technol. 41:6633-6644.

———, M. Zilberbrand, I. Ekeltchik, T. Voitsekovski, J. Gun, and O. Lev. 2008. Speciation of polysulfides and zero-valent sulfur in sulfide-rich water wells in southern and central Israel. Aquat. Geochem. 14:171-192.

———, C. G. Borkenstein, and T. G. Ferdelman. In press**.** Protocol for quantitative detection of elemental sulfur and polysulfide zero-valent sulfur distribution in natural aquatic samples. Geostand. Geoanal. Res.

Karchmer, J. K. 1970. Analytical chemistry of sulfur and its compounds. Wiley.

Koh, T. 1990. Analytical chemistry of polythionates and thiosulfate. Anal. Sci. 6:3-14.

Licht, S., and J. Davis. 1997. Disproportionation of aqueous sulfur to sulfide: Kinetics of polysulfide decomposition. J. Phys. Chem. B 101:2540-2545.

Luther III, G. W., A. E. Giblin, and R. Varsolona. 1985. Polarographic analysis of sulfur species in marine porewaters. Limnol. Oceanogr. 30:727-736.

———. 1991. Pyrite synthesis via polysulfide compounds. Geochim. Cosmochim. Acta 55:2839-2849.

———, T. M. Church, and D. Powell. 1991. Sulfur speciation and sulfide oxidation in the water column of the Black Sea. Deep-Sea Res. 38, Suppl. 2:S1121-S1137.

Luthy, R. G., and S. G. Bruce Jr. 1979. Kinetics of reaction of cyanide and reduced sulfur species in aqueous solutions. Environ. Sci. Technol. 13:1481-1487.

Podgorsek, L., and J. F. Imhoff. 1999. Tetrathionate production by sulfur oxidizing bacteria and the role of tetrathionate in the sulfur cycle of Baltic Sea sediments. Aquat. Microb. Ecol. 17:255-265.

Prange, A., R. Chauvistré, H. Modrow, J. Hormes, H. G. Trüper, and C. Dahl. 2002. Quantitative speciation of sulfur in bacterial sulfur globules: X-ray absorption spectroscopy reveals at least three different speciations of sulfur. Microbiology 148:267-276.

Rong, L., L. W. Lim, and T. Takeuchi. 2005. Determination of iodide and thiocyanate in seawater by liquid chromatography with poly(ethylene glycol) stationary phase. Chromatographia 61:371-374.

Steudel, R., G. Holdt, and T. Göbel. 1987. Chromatographic separation of higher polythionates $S_nO_6^{2-}$ (n=3-22) and their detection in cultures of Thiobacillus ferrooxidans; molecular composition of bacterial sulfur secretions. Angew. Chem. Int. Ed. Engl. 26:151-153.

Szekers, L. 1974. Analytical chemistry of sulfur acids. Talanta 21:1-44.

Troelsen, H., and B. B. Jorgensen. 1982. Seasonal dynamics of elemental sulfur in two coastal sediments. Estuar. Coast. Shelf Sci. 15:255-266.

Vairavamurthy, A., and K. Mopper. 1990. Determination of sulfite and thiosulfate in aqueous samples including anoxic seawater by liquid chromatography after derivatization with 2,2'-Dithiobis(5-nitropyridine). Environ. Sci. Technol. 24:333-337.

Van Gemerden, H., and J. Mas. 1995. Ecology of phototrophic sulfur bacteria. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer [eds.], Anoxygenic photosynthetic bacteria. Kluwer Academic Publishers.

Wang, F., A. Tessier, and J. Buffle. 1998. Voltammetric determination of elemental sulfur in pore waters. Limnol. Oceanogr. 43:1353-1361.

Zopfi, J., T. G. Ferdelman, and H. Fossing. 2004. Distribution and fate of sulfur intermediates—sulfite, tetrathionate, thiosulfate, and elemental sulfur—in marine sediments. Geological Society of America, Special Paper 379:97-116.

Submitted 9 May 2008
Revised 16 February 2009
Accepted 7 May 2009