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Method for the Determination of Inorganic Polysulfide Distribution in Aquatic Systems

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Inorganic polysulfides have significant technological importance, and their environmental role is gradually being unraveled. But despite their importance, there is still no method for quantification of the individual members of the polysulfide family in nonsynthetic samples. The method is based on fast, single-phase derivatization with methyl trifluoromethanesulfonate followed by one of three modes of sample treatment depending on polysulfide concentration. Under the most aggressive preconcentration treatment involving liquid−liquid extraction, solvent evaporation to dryness, dissolution in *n*-dodecane, and finally HPLC−UV analysis of the dimethylpolysulfane distribution, the minimum detection limits of the individual polysulfides are in the range 15−70 nM. The method was demonstrated for the analysis of synthetic solutions, natural groundwater, polysulfide fortified seawater, and surface water and for time tracing of the distribution of the individual polysulfides during the oxidation of hydrogen sulfide by hydrogen peroxide. The observed speciation was evaluated by comparison with the theoretical distribution of polysulfides at equilibrium with sulfur precipitate showing that the dominant polysulfides' (i.e., tetra- to hexasulfide) concentrations agree well with the predicted distribution (90% of the results fall within less than 30% deviation from the predicted values), whereas up to 3-fold deviation was observed for the less abundant trisulfide and octasulfide species.

Inorganic polysulfides, S_n^{2-}, and their protonated forms are compounds containing sulfur in an oxidation state between elemental sulfur and hydrogen sulfide. Polysulfides are always present in near-neutral and basic aqueous systems containing hydrogen sulfide and sulfur, and as such, they are important intermediates in the oxidation of hydrogen sulfide.[1,2] Polysulfide autoxidation is fast and yields predominantly thiosulfate or sulfur in different proportions depending on the average chain length of the polysulfides.[3] Due to their redox reactivity and high nucleophilicity—and despite their low concentrations in natural systems—they are an important environmental family of precursors for detoxification, cross-linking of natural organic matter, and transition metal ligation and transformation in aquatic systems.[4−11] Their role in the formation of volatile sulfur compounds including dimethyldisulfane, dimethyltrisulfane, and carbonyl sulfide was recently explored.[12,13] However, their reputation goes beyond the environmental arena. They are important for the paper and pulp industry,[14] for photoelectrochemistry, and energy storage[15] as well as for metal finishing and decorative patina.

The polysulfide analysis quest can be divided into three different tasks: (1) determination of the total concentration of inorganic polysulfides; (2) speciation of polysulfides under high pH conditions for evaluation of their thermodynamic constants; (3) determination of the individual polysulfide components in natural or technologically relevant conditions. While the first two issues were addressed and at least partly solved, as detailed below, there is still no satisfactory solution for the third issue, individual quantification of polysulfides, which is the object of this study.

Determination of the Total Concentration of Polysulfides. In general, the useful methods can be classified into titrimetry, electrochemical analysis, spectrophotometry, and transformation of the polysulfides into a measurable chemical entity.

Titrimetry. Iodometric titration of polysulfides has been used to detect the sum of inorganic polysulfides in concentrations of hundredths of micromoles per liter.[16,17]

Electrochemical Methods. Luther's group introduced and developed a range of voltammetric techniques based on mercury and gold amalgam electrodes for the determination of polysulfides and other reduced sulfur species in different natural aquatic

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systems, including porewater, microbial mats, and groundwater.[18−21] Using gold amalgam microelectrodes. the group mapped the concentration profile of total polysulfides in the vicinity of sedimentary oxic−anoxic boundary layers.

Polysulfide Conversion to a Measurable Quantity. Several authors reported successful methods for the stoichiometric conversion of polysulfides into a more stable and therefore more easily quantified chemical species. Acidification followed by elemental sulfur detection was used to determine the total zerovalent sulfur in inorganic polysulfides. The method was applied by Jørgensen et al.[22] for the analysis of Solar Lake water, Sinai, Egypt. A similar method for the extraction of the divalent state sulfur by $ZnCl_2$, leaving quantifiable sulfur precipitate, was applied by Zopfi et al.[23] in Mariager Fjord, Denmark.

Polysulfides' zerovalent sulfur content could be detected in natural samples by cyanolysis,[24,25]

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

though an overestimate of polysulfide sulfur due to partial cyanolysis of colloidal sulfur was noted.

Derivatization of Methyl Iodide and Related Reactrions. Derivatization of inorganic polysulfides with methyl or ethyl iodide was used to quantify total polysulfides in water distribution systems and biofilms.[26] A similar method was used for analysis of subnanomolar polysulfide levels in the oxic layer of Lake Kinneret, Israel.[27] This method gives a large bias, and usually, only the lower dimethylpolysulfanes are found (Me_2S_2, Me_2S_3, Me_2S_4). The concentration of dimethylpolysulfanes obtained by methyl iodide derivatization can exceed actual polysulfide concentration due to the formation of polysulfides during the derivatization.[28] If the derivatization is not concluded within a relatively short time, of the order of 1 s (and the pH remains high enough to facilitate polysulfide formation), the reaction of hydrogen sulfide with sulfur may generate more polysulfides.

Spectrophotometric Techniques. The orange color of polysulfide solutions inspired a number of studies aimed at quantitative determination of polysulfides in the presence of other reduced sulfur species. For relatively pure systems, devoid of optical interferences, polysulfides can be directly quantified by spectrophotometric analysis.[2] Steudel et al.[29] used ion pair chromatography to separate reduced sulfur species and quantify the total polysulfide concentration by a UV detector.

Speciation of Polysulfides under Controlled Conditions. A comprehensive discussion of the pros and cons of the currently available procedures for polysulfide speciation under high pH conditions was presented in our recent article[30] and will not be repeated here. Maronny[31] used potentiometric studies to evaluate polysulfide distribution. Cloke[32] followed by Boulegue and Michard[33] developed an approach for polysulfide speciation based on independent determination of polysulfide sulfur and polysulfide sulfide. Teder[34] and Giggenbach[35] used different procedures for the deconvolution of the complex electronic spectra of mixtures of polysulfides. However, the UV information is too dull to provide conclusive identification of the individual spectra of the different polysulfides. This method also cannot be applied to natural samples because of its low sensitivity and due to interference from other, naturally occurring chromophores.

Derivatization by Methyl Trifluoromethanesulfonate (Methyl Triflate). Recently we have reported polysulfide speciation in synthetic solutions.[30] Rapid, single-phase chemical methylation with methyl triflate was used to convert the labile inorganic polysulfides into a stable set of dimethylpolysulfane species that can be preserved for later analysis by HPLC.

$$S_n^{2-} + 2CF_3SO_3CH_3 \rightarrow (CH_3)_2S_n + 2CF_3SO_3^- \quad (2)$$

Since methyl triflate is immiscible in water, the rate-determining step usually involves phase transfer of methyl triflate to the aqueous solution, and the slow transport allows for redistribution of polysulfides prior to completion of the derivatization process. The acid residue of the methylating agent, which is released during the derivatization, decreases the pH and accelerates the rate of polysulfide disintegration. This is by no means restricted to methyl triflate. Other strong capping agents (methyl iodide, methionine, methyl sulfate, phenyl bromide, ethyl iodide, etc.) suffer from the same drawback. To avoid the phase-transfer step and increase the dissolved concentration of the methylating agent, we have carried out the reaction in a methanol−water medium. The method was validated by kinetic tests and by isotope dilution tests, which independently confirmed that the homogeneous derivatization step is faster than polysulfide disproportionation. This allowed us to conclude that the dimethylpolysulfane distribution after derivatization is practically identical to the initial distribution of the inorganic polysulfides.

In our previous research, which was aimed to elucidate the thermodynamic constants governing polysulfide formation, we were able to use pure polysulfide solutions that were prepared at high pH and high polysulfide concentrations, much higher than those relevant to natural aquatic systems. The goal of our current work is to develop a robust analytical method for determination

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of inorganic polysulfide species under environmentally (and technologically) relevant conditions. The theoretical background and formulas of our previous study[30] are equally relevant to the current one and will be used here without explicit detail of their derivation.

MATERIALS AND METHODS

Materials. All reagents, except K_2S_5, were purchased from industrial suppliers. Analytical grade reagents were used unless otherwise stated. Methyl triflate, *n*-pentane, and *n*-dodecane were 99% pure. Methanol was HPLC grade (99.8% pure). K_2S_5 was synthesized and analyzed by the methods described in a previous article and references therein.[13]

Thiosulafte concentration was determined within 6 h of sampling by a Dionex ion chromatograph using IonPac AS14 column, 1 mL/min flow rate of eluent containing 3.5 mM Na_2CO_3 and 1 mM $NaHCO_3$. Method detection limit was 1 μM. Detectable levels of thiosulfate were found only in the natural water sample that was sampled from Zofar 220 well (11μM). The method was inadequate for quantification of the thiosulfate in the hydrogen peroxide oxidation tests.

Natural Samples. The natural water samples were derivatized on-site, immediately after collection. The methylated samples were transferred within 4 h to the laboratory, and the extraction was completed within 24 h of sampling.

Polysulfide Fortified Samples. Polysulfide solutions were prepared by dissolving desirable weights of polysulfides in the natural samples or in 50 mM phosphate buffer set to the specified pH. These solutions were thermostated for at least 12 h to reach equilibrium and filtered through a 0.2-μm filter before derivatization. Buffer and methanol were purged with 99.99% nitrogen for at least 30 min before use. Polysulfides undergo rather fast autoxidation in the presence of oxygen;[3] therefore, all the activities starting from the dissolution of polysulfides in the sample until the addition of the methyl triflate were conducted in a glovebox with less than 0.3% oxygen in the gas phase.

Derivatization and Preconcentration Procedures. Three procedures were applied for sample preparation for HPLC quantification: (1) derivatization without preconcentration; (2) derivatization followed by *n*-dodecane extraction; (3) derivatization, *n*-pentane extraction, complete evaporation of the solvent, and final redissolution of the residue in *n*-dodecane.

Protocol 1: Derivatization and HPLC Analysis without Preconcentration. A set volume of methanol (usually 800 μL) was placed in a 2-mL septum-closed HPLC vial. Two syringes, the first containing the sample (usually 200 μL for high buffer intensity samples) and the second containing methyl triflate, 4 or 6 μL of methyl triflate (depending on pH), were inserted into the septum. The solutions were injected consecutively and immediately into the mixture. The relative volumes of the solutions in the two syringes were varied but the total volume of methanol and sample was kept constant at the 1-mL level. Typically, we added one volume of the sample solution to four volumes of methanol. For the examination of low buffer intensity samples (below ~1.3 mM), e.g., seawater, 800 μL of methanol was first fortified with 100 μL of 50 mM phosphate buffer—preset to the exact pH of the sample—before sample introduction and derivatization, and only then 100 μL of the sample was added. The mixture was fortified with internal standard (solution of 1,2:5,6-dibenzanthracene in 1,4-dioxane), and 20 μL was then injected for HPLC analysis.

Protocol 2: Derivatization, Extraction, and HPLC Analysis. A 40-mL sample of methanol was placed in a septum-closed, 100-mL bottle under vigorous magnetic stirring. Two syringes, the first containing 10 mL of polysulfide solution in 50 mM phosphate buffer (or natural aquatic sample) and the second containing 200 or 300 μL methyl triflate (depending on pH) were inserted into the septum. The two solutions were injected consecutively. An 80-mL sample of 375 mM sodium sulfate solution was added, and the solution was extracted by *n*-dodecane. The optimization of the extraction procedure is detailed in the results section yielding an optimal 2 × 1 mL extraction. The extract was fortified by internal standard (solution of 1,2:5,6-dibenzanthracene in 1,4-dioxane), and 20 μL of the mixture was injected for HPLC analysis. For the examination of low buffer intensity samples, 40 mL of methanol was first fortified with 5 mL of 50 mM phosphate buffer—preset to the exact pH of the sample—and then 5 mL of the sample was added followed by the conventional derivatization procedure.

Protocol 3: Derivatization, Extraction, Evaporation, Dissolution, and HPLC Analysis. To decrease the method detection limit and increase its sensitivity, we devised a third, rigorous preconcentration method involving complete drying of the solvent, at the price of a larger bias. A 400-mL sample of methanol was placed in a 1-L open (though maintained in a glovebox) beaker under vigorous magnetic stirring. A 100-mL aliquot of the sample (polysulfide-containing) solution was added as fast as possible and 2 or 3 mL of methyl triflate (depending on pH) was syringe injected immediately afterward into the solution. The reaction mixture was transferred to 2-L separation funnel, 800 mL of 375 mM sodium sulfate solution was added, and the solution was triply extracted by 30 mL of *n*-pentane. The pentane extract was dried over sodium sulfate and transferred to a round-bottom flask. The sodium sulfate salt was washed with 10 mL of pentane, and the solvent was added to the extract. The combined extract was rotor evaporated to dryness under ambient temperature. The dimethylpolysulfane remains were dissolved in 1 mL of *n*-dodecane, and the analysis was carried out as for protocol 2. For the examination of low buffer capacity samples, 400 mL of methanol was first fortified with 50 mL of 50 mM phosphate buffer—preset to the exact pH of the sample—and then 50 mL of the sample was added and derivatization and analysis continued according to the protocol above. Care was taken to minimize the evaporation time and to stop the evaporation immediately after the pentane was removed.

Chromatographic Determination of Dimethylpolysulfanes. The dimethylpolysulfanes were analyzed by reversed-phase liquid chromatography using a mixture of 90% MeOH and 10% water as eluent. A Finnigan MAT HPLC system (equipped with P4000 pump, AS300 autosampler, and UV6000LP detector) with an Alltech reversed-phase C18, 5 μm, 250-mm-length column was used for separation. Concentrations of dimethylpolysulfanes from Me_2S_3 to Me_2S_7 were calculated from calibration curves of the in-house reference materials prepared according to a published protocol.[37] UV detector response to Me_2S_8 was calculated by the algorithm discussed in our previous publication.[30]

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Hydrogen Sulfide Oxidation. To check the applicability of the procedure to dynamic processes involving polysulfide production and consumption, polysulfide formation during the oxidation of sulfide by hydrogen peroxide was studied. A specified volume of hydrogen peroxide was added to a solution of sodium sulfide and monobasic sodium phosphate. After set time intervals, aliquots of the reaction mixture were withdrawn and analyzed by protocol 1. The specified times in the relevant figures represent the time from the mixing of the reactants to the injection of the sample into methanol.

RESULTS AND DISCUSSION

The research comprises three levels of investigation: (1) optimization of the derivatization conditions of inorganic polysulfides in water samples; (2) investigation of various procedures for concentration of the methylated samples in order to increase method sensitivity; (3) examples of the determination of polysulfide concentrations in different matrixes and under dynamic conditions.

Polysulfides undergo rapid disproportionation reactions in aqueous solutions, and therefore, it was impossible to obtain reference materials for the individual polysulfide species. Currently there is also no alternative method that can be used for the validation of the polysulfide distribution by direct comparison. Therefore, method optimization and validation were based on comparison of the total observed polysulfides to the total amount of fortified polysulfides and by a comparison of the observed speciation of polysulfides to the calculated polysulfide distribution. In our previous work,[30] we determined the concentrations of inorganic polysulfides with 2−8 long sulfur atom chains as a function of pH in the presence of sulfur precipitate. Since the goal of work was directly connected to natural water, the comparison was mainly based on comparison of the abundances of the tetra- to octasulfides, the dominant species under the environmentally relevant pH range and at observable polysulfide concentration. At very low polysulfide concentrations or very low and high pH, di- and trisulfides become abundant and then our method is less relevant, partly because it was not validated for disulfides and also because the derivatization of hydrodisulfide (the dominant disulfide form for pH <9.6) proceeds at a slower pace compared to the deprotonated forms.

Optimal Derivatization Conditions. In the first part of the research, we explored the influence of the four dominant derivatization parameters: pH, volume of methyl triflate, ratio of the volumes of the polysulfide sample and methanol, and total concentration of polysulfide species in the sample.

pH and Volume of Methyl Triflate. In our previous research on polysulfide speciation, we used 100 μL of methyl triflate per 1 mL of methanol−polysulfide solution mixture at pH >8.0. At this pH, a small bias appeared and becomes more pronounced at lower pH values. Exploring the validity of the derivatization results (by comparison to the calculated distribution), it became apparent that the derivatization procedure complies with the thermodynamic predictions only at intermediate volumes of methyl triflate, which is dependent on the pH of the sample. The analytical option of pH correction that is often used for environmental analysis is unacceptable for polysulfide speciation since their distribution is pH sensitive. Figure 1A,B depicts a comprehensive mapping of the acceptable methyl triflate dose as a function of the sample pH. In Figure 1A,B, nonbiased points (closed circles) mark conditions where the detected concentration of each one of the four dominant polysulfide species (S_4^{2-}, S_5^{2-}, S_6^{2-}, S_7^{2-}) differed by less than 25% from its calculated value. The average bias of the individual polysulfides and the level of inaccuracy in the determination of the total polysulfide concentration were of course much lower. The second level of accuracy—denoted by closed triangles— is characterized by a deviation of a single polysulfide species by less than 50% while all other three polysulfides deviated by less than 25% of the calculated concentrations. The third and lowest level of accuracy is denoted by open triangles. Figure 1b depicts an enlarged section of the low, environmentally relevant pH range. Figure 1c depicts the empirically permissible range of methyl triflate dose, which spans the area confined by the empirical lines: $V_{MeOTf} = 1.6 \times 10^{-5}e^{1.78pH}$ and $V_{MeOTf} = 0.91pH - 1.37$, where V_{MeOTf} denotes the volume in microliters of methyl triflate/mL of derivatized mixture. Panels A and B of Figure 1 demonstrate that for every pH there is a range of permissible methyl triflate doses. When the added methyl triflate is too low, the detected concentrations of lower polysulfides (S_3^{2-}, S_4^{2-}) are biased upward, and the detected concentrations of higher polysulfides (S_6^{2-}, S_7^{2-}, S_8^{2-}) and the sum of polysulfides are biased downward. When excess of methyl triflate is added, both lower and higher polysulfides (S_3^{2-}, S_4^{2-}, S_7^{2-}, S_8^{2-}) were biased upward and their detected concentration could reach up to 30 times the calculated ones.

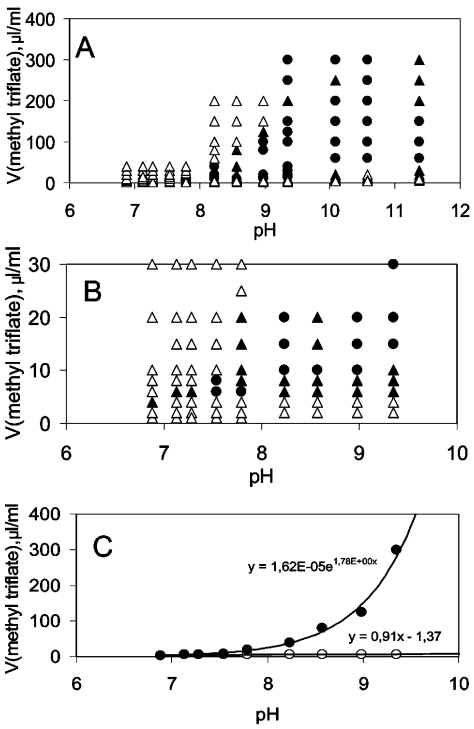

Figure 1. (A) Volume of methyl triflate required for derivatization to give nonbiased results, as a function of pH. (Nonbiased results, closed circle; slightly biased results, closed triangle; severely biased results, open triangle.) (B) Enlargement of the (most important) low pH/low methyl triflate volume part of (A). (C) Linear presentation of boundary between slightly biased and severely biased results. Criteria for "slight" and "severe" biased are reported in the text.

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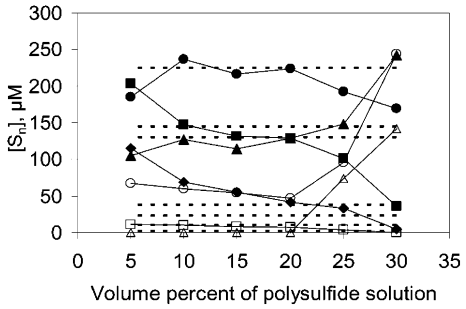

Figure 2. Percent of recovery of different dimethylpolysulfane species depicted as a function of volume percent of polysulfide solution in derivatized mixture. S_3^{2-}, open circle; S_4^{2-}, closed triangle; S_5^{2-}, closed circle; S_6^{2-}, closed rectangle; S_7^{2-}, closed diamond; S_8^{2-}, open rectangle. The open triangles represent the unobserved S_2^{2-} species. Dotted lines present calculated concentrations of polysulfide species: S_5^{2-}, S_6^{2-}, S_4^{2-}, S_7^{2-}, S_3^{2-}, S_8^{2-}, S_2^{2-} (from top).

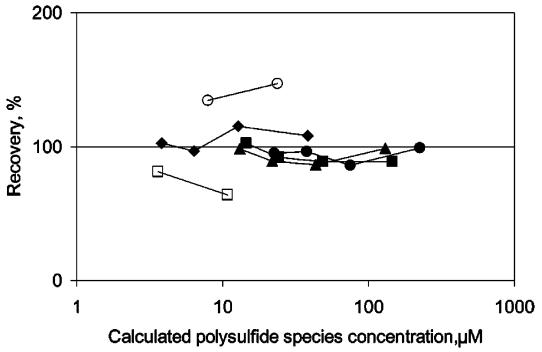

Figure 3. Percent recovery of different polysulfide species depicted as a function of their concentration in the derivatization without preconcentration. S_3^{2-}, open circle; S_4^{2-}, closed triangle; S_5^{2-}, closed circle; S_6^{2-}, closed rectangle; S_7^{2-}, closed diamond; S_8^{2-}, open rectangle.

Table 1. Detection Limits for Different Polysulfide Species

polysulfide	detection limit, μM (protocol 1, no extraction)	detection limit, nM (protocol 2, with extraction)	detection limit, nM (protocol 3, with extraction and evaporation)
S_3^{2-}	10	400	
S_4^{2-}	7	300	70
S_5^{2-}	6	250	30
S_6^{2-}	5	200	30
S_7^{2-}	4	150	15
S_8^{2-}	3	110	

For the high methyl triflate range, methyl triflate hydrolysis lowers the pH and disintegrates the polysulfides. A low methyl triflate dose is insufficient for complete derivatization of the polysulfides. Practically, over a wide pH range, from 7.2 to 9.5, a methyl triflate dose of 6 μL/1 mL of sample−methanol mixture could be used, and for pH >8, 15 μL of methyl triflate/1 mL of sample−methanol mixture could be used.

Sample-to-Methanol Ratio. We were targeting the highest possible sample content in the mixture, to increase the sensitivity of the method. All the solutions in this set of experiments were set to pH 7.77, and the concentration of dissolved K_2S_5 precursor was 6 mM. Figure 2 demonstrates that under our experimental conditions the optimal value, which gave accurate fit to the calculated values (dotted horizontal lines) by mixing 20% sample with 80% methanol though a range of 10−25% sample content, gave acceptable recoveries (corresponding to range marked by full symbols in Figure 1). Throughout the mixing range, the level of trisulfide was biased, though this bias is acceptable considering the fact that the absolute level of trisulfide is less than 6% of the polysulfide pool.

For the 10 and 25% polysulfide sample content, the recovery of heptasulfide was also somewhat in error. The upper possible limit of water in single-phase water−methanol−methyl triflate solution containing 0.6% (v) methyl triflate is ∼30% (v). At higher water content, methyl triflate became insoluble and formed a second phase. However, even when the water content is lower than, but close to, 30%, its dissolution rate is slowed giving a biased polysulfide distribution due to the nonhomogeneity of the solution in the pertinent few seconds of the derivatization. When the level of methanol is too high, the sample buffering becomes insufficient yielding again a biased distribution.

Concentration of Dissolved Polysulfide Precursor (K_2S_5). Experiments with decreased concentration of dissolved polysulfide precursor had two main goals: (1) to check whether at low concentrations of polysulfides the derivatization results were biased and (2) to find the method detection limit for each of the polysulfide species. All solutions in this set of experiments had pH 7.77, and the concentrations of the dissolved polysulfide precursor were 0.6, 1, 2, and 6 mM (Figure 3). In this range of concentrations, no biasing of the S_4^{2-}, S_5^{2-}, S_6^{2-}, and S_7^{2-} species was detected. The recovery of the S_4^{2-}−S_7^{2-} species was 96 ± 8%. The observed concentrations of S_3^{2-} (open circles in Figure 3) were higher than the calculated ones, and those of S_8^{2-} (open squares) were lower than the thermodynamic prediction.

MDL and Precision. Minimum detection limits (MDL) for different polysulfide species are summarized in Table 1, column 2. MDL was set based on a signal that is 200% of the background noise. We have conducted repeatability studies in order to describe the method's precision and its standard deviation. For five samples within pH range 8−9, we have conducted five repeated derivatization and dimethylpolysulfane quantification steps according to protocol 1. The mean relative standard deviations of S_3^{2-}−S_8^{2-} levels were 32, 14, 7, 9, 21, and 24%, respectively.

Optimization of the Derivatization−Extraction Protocol (Protocol 2). Solvent Choice. The main goal of this part of the research was to devise a method for extractive preconcentration of the dimethylpolysulfanes prior to the HPLC analysis. Prerequisites of the chosen solvent included the following: (1) low solubility in methanol−water mixture; (2) lack of interference with the dimethylpolysulfane HPLC chromatogram; (3) good dissolution of all dimethylpolysulfanes. The best solvents that comply with all three prerequisites are hydrocarbons with chain length of 10 or more carbon atoms. *n*-Dodecane was chosen as the extraction solvent in this part of the work. The next section details the optimization of the extractant volumes and number of extractions, the ionic strength, and then the minimal detection limits are determined.

Extraction Volumes. To find the best extraction conditions, derivatization was carried out with solutions containing 400 μM dissolved polysulfide precursor (K_2S_5) at pH 7.75 according to protocol 2. The derivatization mixture was further mixed with 80 mL of 375 mM Na_2SO_4 solution for salting out of dimethylpoly-

Table 2. Recovery of Dimethylpolysulfanes by *n*-Dodecane with and without Salting-Out[a]

DMPS	recovery, % (with 80 mL of 0.375M Na_2SO_4)	recovery, % (with 80 mL of distilled water added)
Me_2S_3	100	90
Me_2S_4	94	77
Me_2S_5	97	88
Me_2S_6	98	96
Me_2S_7	>99	>96
Me_2S_8	>99	>96

[a] Test conditions: 400 μM K_2S_5 sample; pH 7.75; 300 μL of methyl triflate; 40 mL of methanol; 10 mL of polysulfide solution. The postderivatization mixture was further mixed with 80 mL of 375 mM Na_2SO_4 or with 80 mL of distilled water followed by 2 × 1 mL extraction with *n*-dodecane.

sulfanes, and the extraction was carried out four times with different volumes of *n*-dodecane (0.5, 1, 2, 4 mL). The extracts were not mixed, and the level of dimethylpolysulfanes was determined in each of the extracts.

Results of these experiments are summarized in Table S-I (Supporting Information). The extraction tests showed that higher dimethylpolysulfanes are extracted better. In all experiments, the dimethylpolysulfanes were undetectable in the fourth extract. It was concluded that extraction with 2 × 1 mL is sufficient. By this method the recoveries were 94, 97, 98, >99, and >99% for of Me_2S_3 to Me_2S_8, respectively. When the concentration of polysulfides in the initial sample is high enough, a third extraction could be beneficial. After 3 × 1 mL extractions, the recovery was more than 98% for Me_2S_4 and more than 99% for all other species.

Ionic Strength. To determine whether sodium sulfate salting out is required, we repeated the recovery test for 2 × 1 mL extraction with *n*-dodecane without added salt. The results of this experiment are summarized in Table 2. Addition of sodium sulfate solution improved the recovery. For example, the recovery of dimethyltetrasulfane was increased from 77 to 94% by addition of 375 mM Na_2SO_4. Higher concentrations of the sulfate salt could not be used due to salt precipitate in the water−methanol solution.

Calibration and Minimal Detection Limit. This set of tests was conducted in order to extend downward the detection range provided by protocol 1. Figure 3 shows indirectly, by the near 100% recoveries of the individual polysulfides, a linear calibration curve in the range 600 μM−6 mM for the dissolved polysulfide precursor (K_2S_5). Protocol II was used to examine the extension of this range down to 5 μM. The 250, 100, 50, 25, 10, and 5 μM samples of K_2S_5 at pH 7.75 were processed according to protocol 2. Results of these experiments are summarized in Figure 4. The detection limits of the different polysulfide species are summarized in Table 1, column 3. Only for S_3^{2-}, the recoveries were consistently larger than 100%. The recoveries of S_4^{2-}−S_7^{2-} were 101 ± 17%. For 5 μM K_2S_5, all polysulfide species concentrations were below the detection limit.

Optimization of the Derivatization−Extraction−Preconcentration Protocol (Protocol 3). We tried to increase method sensitivity by modification of protocol 2, increasing 10 times both sample and methanol volumes and concentrating the resulting dimethylpolysulfanes by evaporation of the *n*-dodecane. The 100-

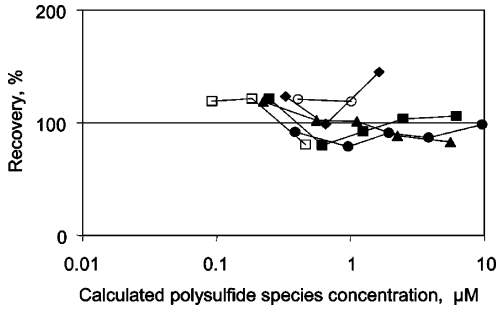

Figure 4. Percent recovery of different polysulfide species depicted as a function of volume percent of polysulfide solution in derivatized mixture (derivatization with preconcentration by extraction with *n*-dodecane). S_3^{2-}, open circle; S_4^{2-}, closed triangle; S_5^{2-}, closed circle; S_6^{2-}, closed rectangle; S_7^{2-}, closed diamond; S_8^{2-}, open rectangle.

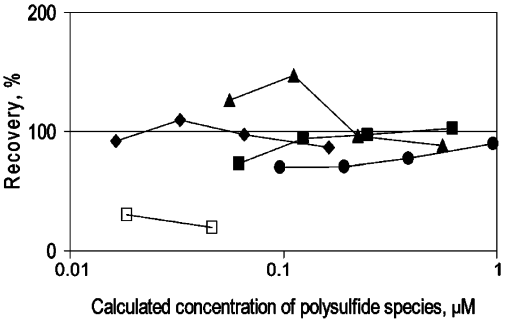

Figure 5. Percent recovery of various polysulfide species depicted as a function of volume percent of polysulfide solution in derivatized mixture after derivatization with preconcentration of dimethylpolysulfanes by pentane extraction and solvent evaporation. S_4^{2-}, closed triangle; S_5^{2-}, closed circle; S_6^{2-}, closed rectangle; S_7^{2-}, open diamond; S_8^{2-}, open rectangle.

mL polysulfide solutions were mixed with 400 mL of methanol and derivatized by 3 mL of methyl triflate. The 800 mL of 375 mM sodium sulfate was added, and the solution was extracted by 3 × 30 mL of *n*-pentane. Dimethylpolysulfane recoveries by *n*-pentane were larger than 95%. Then 1 mL of *n*-dodecane was added immediately to dissolve the dimethylpolysulfanes.

Calibration and Minimal Detection Limit by Protocol 3. K_2S_5 solutions in the range 500 nM−25 μM at pH 7.75 were chosen to complement the detection range of 10 μM−6 mM achieved by protocols 1 and 2. The results of these experiments are summarized in Figure 5. In all these tests, S_3^{2-} was not detected due to the larger noise level and interfering matrix peaks, and the S_8^{2-} concentration was severely biased downward in all tests. The recoveries of all the other polysulfides were within 25% of the calculated values except for the recovery of S_4^{2-} at 70 nM level. Detection limits for different polysulfide species are summarized in Table 1, column 4.

EXAMPLES

To exemplify the applicability of the new method for polysulfide speciation, we selected a set of different water samples from different sources. Unlike the synthetic water samples, the real samples had much lower buffer capacity, and a suitable adjustment had to be carried out according to the protocols of the Materials and Methods section. Each test was carried out with and without the addition of phosphate buffer in order to elucidate its impact.

Table 3. Polysulfide Distribution in Sulfide-Rich Groundwater before and after Additional Spiking with K_2S_5

analysis protocol	total S^{2-}, mM	detected, μM $[S_4^{2-}]$	$[S_5^{2-}]$	$[S_6^{2-}]$	$[S_7^{2-}]$	sum $[S_4^{2-}] - [S_7^{2-}]$
2	0.81[b]	1.17 (80)	1.44 (69)	0.39 (30)	0.10[c] (29)	3.09 (60)
2	1.50	2.52 (93)	3.66 (94)	2.01 (84)	0.76 (123)	8.96 (93)
3	0.81[b]	0.94 (64)	0.86 (41)	0.53 (41)	0.42 (124)	2.75 (53)
3	1.50	1.36 (61)	1.49 (47)	1.67 (85)	0.52 (103)	5.04 (52)

[a] Values in parentheses are percent recovery. [b] No spiking. [c] Lower than reported detection limit.

Our first example involves surface water samples that are artificially fortified with different levels of polysulfides. We also carried out polysulfide determination in hydrogen sulfide-rich groundwater and similar analyses of seawater samples artificially laden with polysulfides. Additionally, we demonstrate the usefulness of the method by tracing the distribution of polysulfides during the oxidation of hydrogen sulfide by hydrogen peroxide.

Surface Water Samples. In this test case, we examined protocol 1 for the examination of surface water samples that were artificially laden with potassium pentasulfide. We carried out three different tests: Test solution 1. Water of Lake Kinneret (better known as the Sea of Galilee, located at the north of Israel (pH 8.75)) was stripped with nitrogen and autoclaved (during which the pH was somewhat increased to 8.87) and then fortified with K_2S_5 to give 0.25−5 mM solutions. Polysulfide determination was then carried out according to protocol 1. Test solution 2. Polysulfide solutions, as for item 1, were prepared, but in this case, derivatization was carried out following the protocol 1 variant for low buffer intensity solutions (i.e., addition of buffered water set to the same pH as the sample). Test 3. Blank analysis with buffered distilled water by protocol 1. The relevant distribution tables are presented in the Supporting Information (Tables S-2, S-3, S-4).

The observed recoveries of the synthetic sample and the surface water determined by modified protocol 1 were very close to each other and to the theoretical calculations. The average recovery of the different polysulfides from the surface water by the modified protocol 1 was 96% with 12% relative standard deviation, very close to the observed recoveries of polysulfides from the synthetic sample (94% with 10% relative standard deviation). Straightforward application of protocol 1 without added buffer gave only 52% recovery with 34% relative standard deviation. The deviation was much less pronounced for high concentrations of polysulfides, since the addition of higher levels of buffered polysulfide solution contributed to pH stabilization (Figure S-1). The buffer capacity of the unbuffered surface water was insufficient to maintain high pH throughout the derivatization process, and methyl triflate hydrolysis (eq 3) decreased the pH and broke down the polysulfides before complete derivatization could take place.

$$CF_3SO_3CH_3 + H_2O = CF_3SO_3^- + H^+ + CH_3OH \quad (3)$$

The individual recoveries of the polysulfides were also encouraging giving 79, 86, and 94% recoveries for the dominant tetra- to hexasulfides, though we encountered some interference in the determination of S_7^{2-}, whose observed concentration was 140% of the calculated level, and the average relative standard deviation of this species was also very high (53%).

Naturally Occurring and Polysulfide Fortified Groundwater. Hydrogen sulfide-rich water was collected from Zofar-220, a groundwater well located in southern Israel. The well water contained 810 μM sulfide, 11 μM thiosulfate, pH 6.93, T 38 °C. Thermodynamically, under such conditions, the total tetra- to heptapolysulfides concentration assuming sulfur saturation should be 5.19 μM. Though these conditions are marginal (corresponding to closed circles in Figure 1) and do not allow accurate speciation, it was important to include a natural polysulfide-laden source in our examples. An additional source for deviation from the calculated values is the high temperature of the groundwater while our thermodynamic constants pertain to 25 °C.

Table 3 shows that the total recovery of the naturally occurring sample is only 60% of the calculated concentrations based on the assumption that the water well is supersaturated with sulfur. However, after K_2S_5 spiking (with 690 μM sulfide as potassium pentasulfide reaching a total of 1.5 mM total sulfide), the total recoveries ranged between 84 (for pentasulfide) and 123% (for heptasulfide) corresponding to 93% average recovery. Therefore, we believe that the reason for the "lower" observed recovery in the natural unfortified sample does not represent experimental bias, but rather it simply reflects the distribution of polysulfides in undersaturated conditions. We have repeated the same tests using protocol 3, and these results are also depicted in Table 3. The observed recoveries for the natural groundwater by protocol 3 were rather low (53%), but they were increased to 52% after the addition of 690 μM sulfide as K_2S_5. A possible explanation for the observed low yield is the presence of organic compounds or traces of metal salts that contributed to the destruction of the organic polysulfanes by the harsh conditions associated with total solvent drying and subsequent dissolution (before the HPLC quantification).

Seawater. We have carried out protocol 1 testing (for low buffer capacity samples) for seawater that was artificially laden with potassium pentasulfide. The seawater samples were deaerated with nitrogen before K_2S_5 was added. We have tested four polysulfide levels corresponding to 0.1, 0.25, 0.5, and 3.0 mM total divalent sulfur. The mean recoveries were 113, 81, 82, and 90% with relative standard deviation levels of 22, 8, 14, and 31% for tetra- to heptasulfide, respectively. The relevant distribution tables are presented in the Supporting Information (Table S-5). The determination of the polysulfides in the seawater was very similar to the surface water analyses. The only reason that the seawater

tests were included here is the fact that upon the addition of the seawater to the methanol immediate precipitation took place resulting in a turbid solution. This apparently had no influence on the observed recoveries.

Comparison of the Observed Distribution of Polysulfides with the Thermodynamic Predictions. Considering the four test case examples (groundwater, Kinneret water, simulated Kinneret water, seawater), it can be concluded that the pentasulfide and hexasulfide are "on the average" biased down by 12.7 and 13% compared to the thermodynamic predictions. Although this bias is rather low, it still exceeds two standard deviations of the respective observations (5.3 and 5.4%, respectively). For all other species, the deviation of the average distribution from the thermodynamic predictions can be attributed to method (im)precision. Some of the observed bias for the pentasulfide and hexasufide species may be attributed to the low recovery of the lower dimethylpolysulfanes (97 and 98% of penta- and hexasulfanes). But there may be another, simpler explanation. The bases for the validative comparison are calculations based on previously reported thermodynamic values[30] rather than comparison with a true value (since the latter is unavailable). However, the thermodynamic predictions have a considerable degree of uncertainty (about 32, 26, 31, and 51% for tetra- to heptasulfide, respectively). A deviation of some 13% falls within the permissible thermodynamic data range.

Sulfide Oxidation by Hydrogen Peroxide. The examples that were obtained thus far represented static concentration with either supersaturated or undersaturated conditions. It was interesting to obtain a dynamic shift from undersaturated to oversaturated conditions and to follow the change in polysulfide distribution that was induced by such a shift. As a test case, we have studied the oxidation of hydrogen sulfide by hydrogen peroxide. We followed the polysulfide evolution after introduction of 10 mM Na_2S and 20 mM H_2O_2 to 12.6 mM NaH_2PO_4. Figure 6A–C depicts the evolution of the total concentration of polysulfides, the distribution of the polysulfides, and the pH change during the oxidation process, respectively. The main products of the oxidation process are sulfur and to a lesser extent sulfate with intermediate formation of polysulfides (eqs 4–6).[2]

$$(n)\mathrm{HS}^- + (n-1)\mathrm{H_2O_2} + (n-2)\mathrm{H}^+ \rightarrow \mathrm{S_n}^{2-} + (2n-2)\mathrm{H_2O}, \quad n = 2-8 \quad (4)$$

$$\mathrm{HS}^- + \mathrm{H_2O_2} + \mathrm{H}^+ \rightarrow \mathrm{S}^0 + 2\mathrm{H_2O} \quad (5)$$

$$\mathrm{HS}^- + 4\mathrm{H_2O_2} \rightarrow \mathrm{SO_4}^{2-} + 4\mathrm{H_2O} + \mathrm{H}^+ \quad (6)$$

Figure 6C shows a slight increase of the pH at the beginning of the reaction and afterward restabilization at somewhat lower pH. Such a shift is to be expected for an oxidation process involving dominant formation of polysulfides and elemental sulfur at its initial stages of the reaction (eqs 4 and 5) and formation of sulfate at longer reaction times (eq 6). The total polysulfide yield indeed supports such a mechanism showing that total polysulfide increases at the beginning of the reaction followed by a decrease at longer reaction times. The new technique for the elucidation of the distribution of polysulfides allowed us to obtain better insight into this process. It is clear that at the very beginning of the reaction period the dominant species is trisulfide and gradually the longer polysulfides become more dominant. This change of polysulfide distribution reflects a transition from undersaturated conditions (or at least a nonequilibrated state) to the establishment of quasi-equilibrium conditions with sulfur precipitate (though hydrogen sulfide level continuously changes).

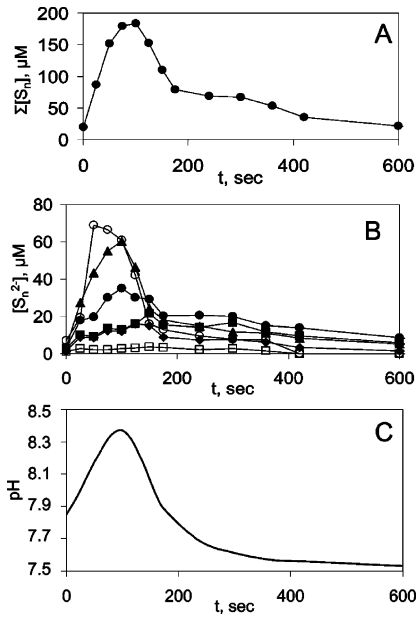

Figure 6. (A) Total concentration of polysulfide species as a function of time in the reaction of 10.0 mM Na_2S and 20.0 mM H_2O_2 (derivatization without preconcentration). (B) Concentration of individual polysulfide species as a function of time in the reaction of 10.0 mM Na_2S and 20.0 mM H_2O_2 in the derivatization without preconcentration. S_3^{2-}, open circle; S_4^{2-}, closed triangle; S_5^{2-}, closed circle; S_6^{2-}, closed rectangle; S_7^{2-}, closed diamond; S_8^{2-}, open rectangle. (C) pH as a function of time during the reaction of 10.0 mM Na_2S and 20.0 mM H_2O_2.

CONCLUSIONS

We provide a way to solve the long-standing question of sulfur speciation in aquatic systems. This new analytical tool provides for the first time a means to study polysulfide speciation in complex sedimentary, pore waters, and sulfate-reducing bacteria environments. Specifically, the new method provides, for the first time, a means to distinguish between systems that are equilibrated with elementary sulfur and those where sulfur level is below saturation or unavailable for sulfur exchange. Polysulfides are reactive species with tremendous impact on aquatic systems, but until now, researchers had to regard the reactivity of the polysulfides as a lumped, united entity. The new tool provides a means to establish the individual distribution of polysulfides and thus to individualize the reactivity of each species.

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SUPPORTING INFORMATION AVAILABLE

Five tables and 1 figure. Table S-1 describes the extraction efficiency of the individual polysulfides vs the number of extractions. Tables S-2−S-4 detail the analysis of fortified polysulfides in surface water samples, showing the importance of buffer addition, and provide a comparison to synthetic sample of the same pH. Table S-5 provides details of the analysis of fortified seawater. Figure S-1 shows the recovery of different polysulfides as a function of added K_2S_5 with and without buffer addition. This material is available free of charge via the Internet at http://pubs.acs.org.

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