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Direct ultraviolet spectrophotometric detection of sulfide in natural waters

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**DIRECT ULTRAVIOLET SPECTROPHOTOMETRIC DETECTION OF SULFIDE IN
NATURAL WATERS**

A Thesis

Presented to

**The Faculty of Moss Landing Marine Laboratory,
San Jose State University**

In Partial Fulfillment

of the Requirements for the Degree

Master in Marine Science

by

Elizabeth Guenther

December 1999

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ABSTRACT

DIRECT ULTRAVIOLET SPECTROPHOTOMETRIC DETECTION OF SULFIDE IN NATURAL WATERS

by Elizabeth A. Guenther

Sulfide is a chemical of great geochemical and biological importance in natural waters. In this paper a new technique is described for the accurate and rapid determination of sulfide in natural waters using direct ultraviolet detection of the HS^- ion. Absorbance data was collected with an ultraviolet spectrophotometer between the wavelength range of 200-400 nm and the concentration of sulfide was determined by least squares regression analyses. Ultraviolet and methylene blue methods for the measurement of dissolved sulfide in natural waters were compared over a wide range of sample types and matrices including freshwater from groundwater wells in Seaside, California, hydrothermal vent fluids from Juan de Fuca Ridge, and porewaters from Elkhorn Slough National Estuarine Research Reserve and Monterey Bay. Good correlation between measurements by both techniques in each environment is observed. Advantages of this method are that it is direct, there is no sample pretreatment required in most cases, it requires no reagents and it is capable of *in situ* or shipboard use. Sulfide concentrations can be determined in complicated media with a detection limit (DL) of 0.8 μM . The detection of sulfide in sediment porewaters (DL 5 μM) that have high organic loading and produce background absorbances of ~ 0.5 A in a 1-cm cuvette provide a stringent test of the UV method.

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PREFACE

In this thesis, a new method to measure sulfide in natural waters is presented. It begins with a background on the sulfur cycle (Chapter 1) and an introduction to the importance of measuring sulfide with an overview of methods that have been used to measure sulfide in natural systems in the past and present (Chapter 2). A detailed description of the ultraviolet method follows in Chapter 2 with emphasis on the UV model. Background to spectral analysis is also included. The results of the application of the Ultraviolet method for groundwater, hydrothermal fluid and sediment porewater samples are presented next. The ultraviolet method is useful for detecting not only sulfide, but also a wide variety of other inorganic compounds that absorb in the UV. While sulfide is the main topic for this thesis other works are in progress for the measurement of nitrate and nitrite and DOC by the UV method and will be the topics of future publications. Chapter 2 of this thesis will be submitted to the journal, *Analytical Chemistry*, and is written in the format that reflects the requirements of the journal.

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CHAPTER 1
INTRODUCTION

BACKGROUND

Sulfide is a chemical of major geochemical and biological importance. In general sulfide is produced by two distinct processes in natural waters. Under anoxic conditions bacteria generate dissolved hydrogen sulfide by the reduction of sulfate. Geochemical processes in hydrothermal systems also produce hydrogen sulfide. Hydrogen sulfide is a constituent of stagnant basins (Black Sea, Cariaco Trench and some Norwegian fjords), sediment porewaters and hydrothermal vent fluids and cold seeps. Figure 1 shows the location of active ridge venting and the numbers show some sites where hydrothermal fluids have been collected and analyzed in the world's oceans.¹ The oxidative decomposition of organic matter in reducing environments exhausts the dissolved oxygen and causes the redox potential to decrease. Such conditions are ideal for sulfate-reducing bacteria both in the water column and in the sediments.² Due to the high concentration of sulfate in seawater (~28 mM), sulfate reduction is the dominating process in marine anoxic habitats, typically accounting for 90-99 % of anaerobic terminal mineralization.³

Although sulfur is a major element in seawater and sulfate behaves as a conservative element, transient forms of sulfur dramatically affect the marine biogeochemistry of many elements. Large changes can occur in the state of metals and non-metals at the interface between anoxic and oxic waters.⁴ This is due to the changes in redox conditions, solubility conditions and bacterial activity. Metal sulfides are highly insoluble, and the production of H₂S can control their sedimentary geochemistry.⁵

Redox processes occurring at or near the sediment-water interface therefore, have important consequences for the geochemical cycling of metals, nutrients, and organic

micropollutants.⁶ In estuaries where the sediments are anoxic due to decomposition of organic matter, certain trace metals (Fe, Mn, Ce and Co) may be more soluble under reducing conditions and will tend to be released to the water. Other metals (Hg, Cd and Zn) form very insoluble metal sulfides and tend to move from the water into the sediment.⁷ The dissolved forms of sulfur (HS^- and S_2^-) may be transformed into metal sulfides such as iron monosulfide (FeS), greigite (Fe_3S_4), and ultimately pyrite (FeS_2). These reduced compounds store the energy fixed by producers in the sediments of salt marshes and estuaries.⁸

On a global scale, hydrothermal plumes are both a source for some elements (Li, Si, Mn, and Fe) and a sink for others (P, Vanadium, As, Pb, and Po).⁹ In high temperature vent systems the rapid precipitation of metal-rich sulfides creates a chimney-like deposit and the channeled flow of hydrothermal fluids generates a visible plume above the chimney mouth resulting in what is termed a black smoker. This activity has proved to be a major source for lithium, rubidium and manganese, and to be a major sink for magnesium and sulfate.¹⁰⁻¹¹

Very high concentrations of hydrogen sulfide may be present in hydrothermal vent fluids. Chemoautotrophic bacteria rely on the availability of sulfide to live and at times also provide sustenance for other animals in anoxic environments through symbiotic relationships. Figure 2 shows a plot of hydrogen sulfide concentration versus magnesium concentration, adapted from Butterfield et al., 1990.¹² Magnesium has been shown to be totally removed from solution during experimental high temperature basalt-seawater interaction,¹² so it effectively serves as a tracer for temperature in Figure 2. The

hydrogen sulfide concentrations in some of these high temperature vent fluids get as high as 8-9 mM. Much of the scatter can be attributed to degassing of the samples prior to measurement.¹²

In anoxic environments, organisms use sulfate as the electron acceptor to oxidize organic matter when oxygen, nitrate, manganese and iron are exhausted.⁶ Many sediments are devoid of free oxygen a few centimeters below the sediment-water interface, such as in estuaries.¹³ Figure 3 shows vertical profiles of hydrogen sulfide, oxygen and pH in through a *Beggiatoa* mat growing on a mud surface.¹⁴ Hydrogen sulfide is not detected until the oxygen concentration is close to 0 μM . The concentration of H_2S goes from 0 to about 600 μM within 3 mm depth.

The reduction of sulfate is shown in Equation (1)¹⁵

where the energy released is equal to 1,588 kcal/mole organic matter.

Reduced ions such as S^{2-} are used by autotrophic bacteria to convert carbon dioxide (CO_2), water (H_2O) and nitrate (NO_3^-) into essential organic substances necessary for nutrition.¹⁶ This process is referred to as chemosynthesis. In general, the reduction or loss of one sulfate molecule is accompanied by the loss of two and then the regeneration of one molecule of organic carbon.¹⁷ In some coastal sediments approximately half of the oxidation of carbon is due to sulfate reduction.¹⁵ In coastal marine sediments in Denmark sulfate reduction accounted for 53 % of the total mineralization of organic matter.¹⁸

Reduced sulfur compounds such as pyrite are not permanently stored in the sediments of estuaries and salt marshes. Pyrite can undergo biotically mediated oxidation to sulfate and thiosulfate via the oxidizing power of marsh grass roots in salt marsh sediments. Therefore, sulfide oxidation is also an important process.¹⁹ Sulfide oxidizes spontaneously in the presence of oxygen, and the rate of the process is dependent upon sulfide and oxygen concentrations and the pH.³ The oxidation of sulfide results in the formation of several sulfur species, such as thiosulfate, sulfite, elemental sulfur and polysulfide, as well as sulfate.²⁰⁻²¹ Thiosulfate has been shown to be a key intermediate in the sulfur cycle, both as a main product of anoxic sulfide oxidation and as a shunt between oxidative and reductive pathways.²¹

The ability to accurately measure sulfide concentrations in all these environments is extremely important to the understanding of the biological and geochemical processes that occur within them. Methods of analysis, based on colorimetric detection of sulfide derivatives are, however, not well suited to rapid, shipboard or *in situ* analysis. These methods can be time consuming and often involve the use of reagents which may degrade through time.

Over 95 % of the sulfide in seawater at pH 8.0 is in the form of bisulfide (HS^-).⁵ Bisulfide, nitrate, nitrite, bromide, iodide, thiosulfate and organic material, all absorb in the ultraviolet (UV) wavelength range.²²⁻³² Yet, each of these inorganic compounds has a unique absorption spectrum, which when used along with deconvolution methods can provide estimates of the relative concentration of each compound in natural water samples. The UV method does not require the addition of reagents and results can be

generated in minutes compared to hours as with most conventional methods. The UV method can be applied to a wide range of sample types and matrices and is especially important for reactive compounds such as sulfide. Estimates of the half-life of sulfide vary from minutes to more than 24 hours.⁵ Therefore, a quick simple method of detection is desirable. The determination of sulfide by the UV method will be the focus of the remainder of this thesis.

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Figure Captions

Figure 1. Plot of active ridge venting and locations of hydrothermal vent fluids that have been collected and analyzed in the world's oceans (adapted from Humphris et al., 1995).¹

Figure 2. Plot of hydrogen sulfide concentration versus magnesium concentration in hydrothermal vent fluids from a study by Butterfield et al., 1990.¹²

Figure 3. Vertical distribution of H₂S, O₂ and pH through a *Beggiatoa* mat growing on a mud surface. Figure adapted from Jørgensen and Revsbech, 1983.¹⁴

Figure 1.

Figure 2.

Figure 3.

CHAPTER 2

DIRECT ULTRAVIOLET SPECTROPHOTOMETRIC DETECTION OF SULFIDE IN NATURAL WATERS

INTRODUCTION

Hydrogen sulfide is found in many natural waters. It is formed by heterotrophic sulfate reducing bacteria in anoxic waters and as a result of geochemical processes in hydrothermal systems. Although toxic at high concentrations, it is also an energy source for chemosynthetic bacteria where energy released during its oxidation to sulfate drives the phosphorylation of ADP to ATP. Determination of sulfide is important to a variety of studies including groundwater monitoring and assessment of biogeochemical processes in hydrothermal vent fluids and sediment porewaters. Due to its reactivity with O_2 however, sulfide is not detected far from its source, or long after collection, unless it is preserved. Methods capable of rapid measurements in the field are desirable.

A number of methods have been developed to measure sulfide content in natural systems. These include colorimetry with methylene blue,¹⁻² nitroprusside³ and with nitrilotriacetic acid and Fe (III),⁴ coulometric argentometry⁵⁻⁶ and potentiometry (sulfide ion selective electrode).⁷⁻¹⁰ This paper demonstrates the potential of direct ultraviolet spectrophotometric detection of sulfide in natural waters. The advantages of this method include simplicity and speed of data acquisition with the potential to obtain direct chemical measurements at a resolution similar to physical measurements.

Studies have shown that sulfide solutions absorb light directly in the ultraviolet.¹¹⁻
¹² However, many inorganic ions¹³⁻¹⁷ and a broad suite of organic compounds^{11,18-21} exhibit strong absorption below 300 nm. We show that the direct ultraviolet determination of sulfide using peak deconvolution methods yields accurate and precise results in a variety of natural waters. The method involves measuring the ultraviolet

absorbance of the HS^- ion over a range of wavelengths in aqueous samples without the addition of reagents. The concentration of sulfide in a sample can be determined by deconvolution of the HS^- spectra from the complex spectrum of natural fluids using a non-linear least squares fitting approach. Concentrations of sulfide determined directly from UV spectra were compared to measurements made using the conventional methylene blue method.

EXPERIMENTAL SECTION

Reagents

All solutions were prepared from reagent grade chemicals and MQ water (Millipore, Milli-Q water system, $18\ \Omega\ \text{cm}^{-1}$). Primary sulfide standards were prepared by deoxygenating 250 mL of water in a 500-mL glass aspirator bottle with N_2 gas for 1 hour. $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ was rinsed with MQ (in a fume hood) to remove any sodium sulfite and the crystals were wiped dry to remove any excess water before weighing 6g, which were added to the deoxygenated water to produce a 100-mM solution. The glass aspirator bottle was sealed to prevent oxygen contamination. If oxygen is excluded, the solution will be clear and should be stable for approximately 1 to 2 weeks. The sulfide stock solution was standardized by iodometric titration.²

Working standards were prepared daily by drawing portions of the stock primary standard into a syringe and micropipetting them into 100 mL of either MQ water or filtered seawater. Low nitrate ($< 0.5\ \mu\text{M}\ \text{NO}_3^-$) surface seawater was used to prepare the standards for measurements in marine systems. MQ water was used for all other standards. Absorption spectra of working standards were collected within 15 minutes of

the preparation time to avoid changes in sulfide concentration due to oxidation by O₂ in the standard solutions. The surface seawater used for the standards was filtered (≤ 0.45 μm filter) prior to use to exclude sulfide oxidizing bacteria and any particulate material.

The reagents used for the measurement of sulfide by the methylene blue technique were N,N-dimethyl-p-phenylene diamine dihydrochloride ((CH₃)₂N·C₆H₄·NH₂·2HCl, Aldrich), 0.48 g dissolved in 100 mL of 6 M HCl), and ferric chloride (FeCl₃, Eastman Kodak Company, 1.6 g dissolved in 100 mL of 6 M HCl) and they were used as described previously.² Both reagents were stored in brown polyethylene bottles. These reagents should be stable for several months if protected from light and refrigerated.

Sample Collection and Preparation

Well Water Samples

Freshwater samples were collected from groundwater wells in Seaside, Monterey County, California. The samples were collected in brown polyethylene bottles, which were filled completely and capped. Replicate samples were obtained from most of the wells. The bottles were stored in a cooler on ice during transport for a few hours prior to analysis. Standards for these samples were prepared in MQ water. The samples were not filtered or diluted before analysis by the UV method. Well water samples were all within 7.7 to 8.0 pH. Colorimetric methods were also used to determine sulfide and nitrate in these samples.^{1,22}

Hydrothermal Vent Fluid Samples

Vent fluid samples were collected on a research cruise aboard the NOAA *Research Vessel Ronald Brown* above the Juan de Fuca Ridge about 200 miles off the

coast of Oregon and Washington, September 1998. Samples were collected within the caldera of Axial Volcano at a depth of 1500 m using the remotely operated vehicle *ROPOS*. Vent fluid samples were collected from CASM (Canadian-American Seamount) vent field, ASHES (Axial Seamount Hydrothermal Emissions Study) vent field and from hydrothermal vents within the Southeast Rift zone.²³

Samples were drawn from the fluid sampler into plastic syringes. Some samples were filtered at depth upon collection in situ. One of the samples was filtered (0.5 μm Millipore Millex-LCR) in the laboratory to remove visible particulate material. In order to avoid oxidation of the vent sample during filtration, the filter was attached directly to the syringe with which the sample was collected.

Some vent samples had a pH that ranged from 4 to 5. The pH was determined by using pH indicator strips (EM-Reagents). Ammonium hydroxide (0.44 M) was added to these samples to raise the pH to approximately 8.0. The ammonium hydroxide did not interfere with the detection of sulfide in the samples. Gas-rich vent samples were diluted with deoxygenated seawater to prevent degassing. This also provided a natural buffer to the samples so that no more than 60 μL of ammonium hydroxide was added to a volume of 5 ml of the diluted sample. These samples were then analyzed by direct UV spectrometry. Sulfide was measured at sea in each sample by the methylene blue method.

Sediment Porewater Samples

Sediment samples were collected from Elkhorn Slough National Estuarine Research Reserve, Moss Landing, California and from the continental shelf at 98 m (Lat. 36° 44' N., Long. 121° 56' W.) and 85 m depth (Lat. 36° 42' N., Long. 121° 55' W.) in

Monterey Bay. The Elkhorn Slough samples were collected by pushing 10-cm diameter polycarbonate core tubes into a shallow mud flat (Lat. 36° 48' 50" N., Long. 121° 47' 20") at low tide. Approximately 30-cm of sediment was collected. Surface mud was dark brown in the upper 1-5 cm and black below that depth with the very bottom 5-10 cm of sediment appearing gray-black in color. The tube was topped off with surface seawater from the sampling area. Large rubber stoppers were used to seal both ends of the core tube during transport and storage. To ensure production of sulfide, the Elkhorn Slough samples were left in the core tube at room temperature for two weeks prior to porewater extraction.

The Monterey Bay samples were collected aboard the *Research Vessel Point Sur* with a multicorer on March 13, 1998 and with a box corer on April 12, 1999. The core collected on March 13, 1998 was left in the core tube for two weeks in the lab to allow sulfide to develop within the core prior to porewater extraction. The mud from the three cores collected on April 12, 1999 were left in the core tubes for either 1 day, 7 days or 14 days prior to porewater extraction. Initially, the sediment in the core tubes was orange-brown throughout the core. After 1-2 weeks small black spots began to appear randomly throughout the core due to the reduced environment and the reduction of sulfate to form sulfide.

The cores were sectioned in an inflatable glove bag, which was filled with N₂ gas. Centrifuge tubes were filled with mud from each sediment section within the glove chamber. The sealed tubes were centrifuged for approximately 30 minutes at 2500 rpm to separate the porewater. The supernatant solution was filtered with a 0.45 µm filter

under N₂ gas and was analyzed immediately by both the ultraviolet and the methylene blue methods. Some of the Elkhorn Slough samples were diluted with filtered surface seawater from Monterey Bay. These samples were diluted in order to decrease the baseline absorbance due to background signals such as high concentrations of dissolved organic material in these samples and to decrease the absorbance signal due to high concentrations of sulfide in some of the samples.

Analysis of pH Effect

The effect of pH on the absorbance of sulfide standards was determined by measuring the absorbance of pH adjusted sulfide solutions. The pH of the sulfide standards prepared in surface seawater from Monterey Bay, California was adjusted by the addition of 1N HCl for solutions in the pH range of 3 to 7. NaOH was added to the standards for a pH of 9. The pH of the solutions was determined potentiometrically after the addition of the primary sulfide standard and the acid or base.

Apparatus

The pH of the samples was measured with a Corning model 130 pH meter. A Hewlett-Packard HP 8452A diode array spectrophotometer with 2 nm resolution was used to collect absorbance data from 200 to 400 nm. Absorbance of well water and porewater samples was measured with a 1-cm square (Fisherbrand) quartz. Vent samples were measured in a 1-cm Hellma flow-through cuvette with Suprasil I windows. Four solenoid pumps (Lee LPLX0502100AA) were used to propel MQ water, the seawater blank, sulfide standard and sample through the flow-through cuvette (Figure 1). The Lee pumps were controlled by a Metrabyte analog/digital interface and the spectrophotometer

was controlled by HPIB (IEEE 488) interface bus. The commands were written in a Quick Basic 4.5 computer program. Sample and standards were pumped at a rate of approximately 1.6 mL/minute. Standards were analyzed after every 15 samples. Each absorbance spectrum was saved along with the computer-generated estimate of the sulfide concentration in the sample. A Perkin-Elmer Elan 6000 ICP-MS was used to determine iodine in Monterey Bay porewater solutions. Samples were diluted up to 250 fold with 1% HNO₃ before analysis by ICP-MS.

RESULTS AND DISCUSSION

Effect of pH

The absorbance of a 115 μ M sulfide solution versus pH is shown in Figure 2. The percent of sulfide present as HS⁻ in each sample (dotted line), which was calculated with a pK₁ value of 6.60 for seawater,²⁴ is also shown. These results indicate that the bisulfide ion is responsible for the UV absorption of hydrogen sulfide solutions. The optimal pH range for ultraviolet sulfide analysis is 8.0 to 9.0 where HS⁻ constitutes more than 95% of the total sulfide. Above pH 9, the sample absorbance may be complicated due to possible absorbance of polysulfides.²⁴

The UV Model

Many inorganic compounds absorb light at wavelengths less than 300 nm. The absorbance spectra for many of these compounds are shown in Figures 3a and 3b. Bromide, nitrate, and nitrite have a peak absorbance at approximately 204, 202 and 210 nm respectively.¹¹ Bisulfide has a peak absorbance at 230 nm, and is well resolved from the spectra of the other ions. The peak in the spectrum of HS⁻ occurs at a wavelength

similar to that of Γ^- (peak absorbance 226 nm),¹¹ but the HS^- peak is somewhat broader (Figure 3a). The absorbance spectrum of bisulfide and some of the products of sulfide oxidation are shown in Figure 3b. Sulfite and bisulfite have insignificant absorbance spectra compared to the spectra of thiosulfate and bisulfide. Thiosulfate has a peak absorbance at 216 nm.¹¹ Thiosulfate may be a significant shunt in the sulfur cycle, and it has been shown to constitute approximately 68 to 78 percent of the immediate HS^- oxidation products.²⁵ In addition, organic compounds will absorb light in the UV. Studies have shown that the spectrum of natural dissolved organic matter can generally be modeled as an exponential function.¹⁹⁻²⁰ We show below, that each of these compounds can be quantified in a complex mixture.

The absorbance of a sample is given by the sum of the component absorbances:

$$A_\lambda = \epsilon_{\text{HS},\lambda} * [\text{HS}^-] + \sum_j (\epsilon_{j,\lambda} * [j]) + \exp(a + b*\lambda) + \text{error} \quad (1)$$

where (ϵ) is the molar absorptivity of the subscripted species at wavelength, λ . The sum over components (j) represent all possible combinations of inorganic ions other than HS^- that may be present in the sample. The exponential intercept (a) and the slope (b) represent the background absorption due to organic constituents in a sample.¹⁹⁻²⁰ The error term represents all possible errors including those due to the spectrophotometer. The error produced by removing and replacing the cuvette can be minimized by the use of a flow cell.

Equation (1) can, in principle, be used to determine HS^- , NO_3^- , Br^- , Γ^- and other components in a single sample. However, two factors may complicate the application of Equation (1). Concentrations of HS^- in excess of 200 μM produce absorbances larger

than 1.5 A with a 1-cm cuvette. The low light transmission through these samples increases errors due to stray light and detector dark current. Response is not linear to concentration under these conditions. Secondly, the background absorbance in samples with high dissolved organic carbon (DOC) concentration can deviate significantly from the exponential relationship in Equation (1). An adaptive process must be used in the application of equation (1) to mitigate these factors.

Spectral Analyses and Wavelength Range Determination

The Kalman filter is an adaptive process that has been used to identify the number of components present in an overlapped ultraviolet-visible spectrum and to determine the concentration of the components.²⁶⁻²⁷ A simple Kalman filter is no different from a recursive least-squares fitting approach.²⁷ The Kalman filter technique involves processing the data points in the spectrum one wavelength at a time with an error check to assess how each new data point fits the spectral model.

We have used a modified Kalman filter approach in our work. The concentrations of each compound in the spectral model (Equation 1) are calculated using a multiple non-linear least squares regression over an initial wavelength range. The flow chart in Figure 4 shows the step by step process that is used to determine the wavelength range used in the analysis. The spectra are generally processed from an initial wavelength $\lambda_i = 250$ nm to 300 nm (the end wavelength, λ_e). The estimates for each model parameter (concentration for each component, slope, and intercept) obtained from the non-linear least squares regression analyses of Equation (1) from λ_i to 300 nm, are then used to predict the absorbance at the next lower wavelength in the observed spectrum ($\hat{A}_{\lambda_{i-2}}$ nm).

If the absolute value of the residual absorbance, |(observed absorbance: $A_{\lambda_{i-2} \text{ nm}}$ – predicted absorbance: $\hat{A}_{\lambda_{i-2} \text{ nm}}|$ at this next wavelength ($\lambda_{i-2} \text{ nm}$), which is termed the innovations sequence (I.S.),²⁷ is below a predetermined limit, then that observed absorbance at λ_i will be included in the least squares regression to update all parameters. We continue to decrement through the observed values by successive 2 nm until the predicted innovations sequence at ($\lambda_{i-2} \text{ nm}$) exceeds the maximum allowed or until the observed absorbance of the sample exceeds 1.0 A. The model parameters at this point are used as the final concentration estimates. This same process of choosing the best starting wavelength for the analysis can be used to determine the best ending wavelength for the analysis if negative concentrations are estimated or if absorbance peaks due to dissolved organic material are present at $\lambda > 260 \text{ nm}$.

In a classical Kalman filter, data points are weighted by the innovations sequence and estimates of model parameters can be updated by incrementing sums of squares. Due to the non-linear nature of Equation (1), we use an unweighted non-linear regression and stop the fit when an arbitrary limit for the innovations sequence is exceeded. Systat v. 8.0.1 was used to perform the non-linear least squares regression.

The limit for the innovations sequence depended on the magnitude of the background spectra, which controls the variability of the data about the regression line. This limit is set equal to five times the typical standard error of the regression analysis (SER) for each class of sample analyzed. A value of 0.01 A was used for well water and low sulfide concentration hydrothermal vent fluid samples, which have a relatively low ($< 0.08 \text{ A}$) background absorbances. This corresponds to a concentration of about 1.3

$\mu\text{M HS}^-$ ($\epsilon_{\text{HS}^-} = 7700 \text{ L mol}^{-1} \text{ cm}^{-1}$) near the absorbance maximum of HS^- . The limit for the innovations sequence for high sulfide concentration vent samples was set to 0.015 A. The limit for the innovations sequence for sediment porewaters from Elkhorn Slough was set to 0.03 to 0.05 A based on the actual SER for each sample. This corresponds to concentrations of HS^- equal to 3.9 to 6.5 μM . The 0.05 A corresponds to those samples in Elkhorn Slough with concentrations of DOC greater than 950 μM . The Monterey Bay sediment porewater sample innovations sequence limit was set to 0.01 A.

Only those wavelengths at which the absorbance was less than 1.0 A were used in the analyses due to the non-linear response of the spectrophotometer when absorbance is greater than 1.0 A. Curve fits in seawater initially included components for HS^- and DOC only for the sediment porewaters. HS^- , NO_3^- , Br^- and DOC were included in the curve fit for low sulfide vent fluid samples. HS^- and DOC were included in the curve fit for the high sulfide vent samples. For freshwater, HS^- , NO_3^- and DOC were considered.

If the predicted concentration of a compound of interest was negative, modifications to the model in Equation (1) were necessary. The pattern of the calculated residuals was used to determine what components were missing. The background absorbance due to DOC is fit with the exponential function using absorbance data from 300 nm to the lower wavelength limit for all sample types except sediment porewaters from Elkhorn Slough. These samples required an adjustment to the upper wavelength limit due to high levels of DOC, which is discussed below.

Salt Effect

The array of molar absorptivities, ($\epsilon_{j,\lambda}$), for each compound (j) was determined by calculating the slope of an absorbance versus concentration curve at each wavelength for a series of standards of the compound of interest. To test the effect of salt on the molar absorptivity for sulfide, standards were prepared in varying concentrations of NaCl, as well as seawater (Figure 5). There is a weak effect due to NaCl. Seawater has a larger effect (~5.0 % decrease) on the molar absorptivity (possibly due to the increased number of ion pairs present), relative to MQ and salt. In theory, artificial seawater could be used to prepare standards as long as all of the necessary components are included to produce the same effect as found in natural seawater.

Sulfide and Nitrate in Well Water

Samples of well water represent the simplest natural samples that we examined. These samples contain small amounts of halide ions. The absorbance of samples was less than or equal to 1.0 A at wavelengths greater than 218 nm for all samples. Equation (1) can be applied directly to the well water samples.

Figure 6 shows the absorption spectra of a sample (see Appendix 1) and the component spectra that were determined by a multiple regression fit of Equation (1) using a wavelength range from 220-300 nm. The innovations sequence is less than 0.01 A at all starting wavelengths λ_i greater than 208 nm (see Appendix 2). Constant predictions for both sulfide and nitrate were obtained within the range of starting wavelengths from 210 nm to 222 nm (Figure 7a). The absorbance of all well water

samples was less than 1.0 A at wavelengths greater than 218 nm. Therefore, 220 nm was chosen as the starting wavelength for all of these samples.

Accurate results for both nitrate and sulfide concentrations were obtained in all of the well water samples and the standard errors of the regression analyses were all low (Table 1). The detection limit ($3 \times \text{Standard Deviation}$) for repeated analysis of sulfide in well water was $0.6 \mu\text{M}$. Despite the accurate concentrations, the absorbance residuals obtained across the spectrum after fitting equation (1) are distinctly non-random (Figure 7b). The residual peak at 228 nm may correspond to Γ in this sample at a concentration of approximately $0.33 \mu\text{M}$. It produces the slight shift in the observed versus the predicted absorbance for the well water sample (Figure 6). Dissolved organic carbon probably accounts for the second residual peak at 260 nm. However, the interference due to the presence of these compounds in the samples is small.

Sulfide, Nitrate and Bromide in Hydrothermal Vent Fluids

The spectra of vent fluid samples were more complex than those of well water. The high bromide concentration in seawater ($\sim 850 \mu\text{M}$) and high sulfide concentrations in the samples resulted in absorbances above 1.0. The vent fluid samples were divided into two groups, those containing low sulfide concentrations ($A < 1.0$ at $\lambda = 230 \text{ nm}$) and those with high sulfide concentrations ($A > 1.0$ at $\lambda = 230 \text{ nm}$). Equation (1) was applied directly to samples with a low sulfide concentration. This allowed bromide, nitrate and DOC to be determined as well as HS^- . The high sulfide samples, on the other hand, were modeled with sulfide and DOC as the only constituents.

Figure 8a shows the absorbance spectra of a low sulfide vent sample and the component spectra that were determined by multiple regression of the sample spectrum from 214 to 300 nm. The calculated concentrations for bromide, nitrate and sulfide remain relatively independent of the lower wavelength value that is used in the regression (Figure 8b) and the innovations sequence is small ($< 0.01 A$) between 214 nm and 220 nm.

The absorbance spectrum for a high sulfide vent sample and the component spectra that were determined by non-linear regression fit of Equation (1) from 246 to 300 nm are shown in Figure 9a. The innovations sequence for the high sulfide vent sample is low ($< 0.015 A$) until wavelengths are reached where $A > 1.0$ (Figure 9b). The rapid increase in the innovations sequence may reflect photometric error at high absorbances. The high sulfide concentrations prevent the maximum absorbances of HS⁻ from being used to determine concentrations. However, only the shoulder of the absorbance due to the component is necessary for accurate estimations (Figure 9a). The lower wavelength limit (λ_i) for each high sulfide sample was set to the wavelength where the absorbance was just less than 1.0 A.

Sulfide concentrations in vent fluids predicted by both the ultraviolet method and by methylene blue analysis are compared in Figure 10. There is very good agreement across the concentration range. The detection limit for sulfide in the vent fluids was determined by replicate analysis of a low sulfide sample. The standard deviation (S.D.) of the measurements for sulfide was 0.26 μM , which gives a detection limit ($3 \times S.D.$) of 0.8 μM .

The calculated absorbance residuals (observed absorbance – predicted absorbance) for both spectra of low and high sulfide vent samples are shown in Figure 11. The calculated residuals are not random. The residuals show the possible absorbance of dissolved organic material at 260 to 280 nm. The peak at 218 nm may be due to a product of sulfide oxidation such as thiosulfate (Figure 3b).^{25,28} These signals do not bias the determination of sulfide.

Sulfide in Sediment Porewater

Determination of sulfide in sediment porewater samples presents a stringent test of the ultraviolet method for several reasons. Some of the samples had high baselines (> 0.5 A) after filtration. We believe that this is due to the high concentration of dissolved organic carbon in these samples. Additional inorganic interferences such as Γ are also present at significant concentrations.

Elkhorn Slough Samples

High background absorbances and the presence of a peak near 260 nm (Figure 12) characterize many of these samples. This background absorbance is likely due to concentrations of dissolved organic carbon greater than 950 μM , estimated from the DOC concentration to absorbance at 280 nm relationship reported by Krom and Sholkovitz.²¹ The peak at 260 nm in porewater will bias the exponential baseline to higher absorbance values in a least squares curve fit. This creates an underestimate of HS^- concentrations and negative estimates of HS^- if concentrations are near zero.

One potential approach to solve this problem would be the use of hybrid linear analysis (HLA)²⁹ to model the dissolved organic spectrum. An assessment of the general

applicability of HLA would probably require a larger data set than we have. We therefore used an alternative approach to avoid the upward bias of the baseline by the 260 nm peak. The upper wavelength limit of the regression was reduced to 278 nm, which limits the background spectra primarily to the flat region beyond the peak due to DOC. The wavelength limit of the regression was not reduced for those samples where a peak at 260 nm was not apparent (height less than 0.05 A). These samples produced a low innovations sequence when the spectra of the samples were fit over the wavelength range 234 through 300 nm (Innovations sequence 1 = I.S.₁ in Figure 13). Some of the samples with high baseline absorbances were diluted with surface seawater (Table 2) in order to decrease the effect of the high concentrations of DOC in the samples.

Accurate predictions for sulfide concentrations were obtained by the ultraviolet method at all concentration ranges (Table 2). The slope of the regression line fit to the two sets of data is not significantly different from 1.0 (Figure 14). The largest standard error of the regression (square root of the sum of the squared residuals of the regression divided by the degrees of freedom) calculated for these samples was on the order of 0.016 A. The samples with the highest error tended to be samples that had high baselines. A detection limit ($3 \times \text{S.D.}$) of 5.1 $\mu\text{M S}$ was obtained for the Elkhorn Slough samples.

Monterey Bay Samples

The spectra of the Monterey Bay samples had lower baselines than did the Elkhorn Slough samples. The observed absorbance spectrum of a sample collected from 85-m depth in Monterey Bay on April 12, 1999 is shown in Figure 15. A large peak with a maximum at 226 nm is present. This peak does not appear to be HS^- for several

reasons. The peak decreased with depth in the cores. Sulfide would show the opposite behavior, increasing with depth. The peak is also not discernible in porewater samples that were taken from the cores after sitting at room temperature in stoppered core tubes for 7 to 14 days in the lab, which should promote sulfide formation. Finally, the innovations sequence and standard error of the regression was large for these samples at all starting wavelengths, indicating a model with HS^- did not fit the spectra well.

The peak present in the Monterey Bay samples appeared to be caused by large iodide concentrations. Iodide has a peak absorbance at 226 nm¹¹ (Figure 3a). Iodide concentrations would decrease with depth, unlike sulfide.³⁰ Fitting the observed absorbance spectra with HS^- and I^- components results in considerably smaller standard error of the regression than are observed with just fitting HS^- for the core from March 1998 (Table 3).

The spectra of the Monterey Bay porewater samples are consistent with iodide concentrations up to 50 μM (Figure 16). The presence of dissolved iodine at these concentrations was confirmed by ICP-MS analysis of the pore fluids for the core which was extracted analyzed one day after collection (Figure 16). The total iodine concentration measured by ICP-MS in the core top sample exceeds the iodide concentrations determined by UV spectrophotometry by 33 %. This suggests that 1/3 of the iodine is present as iodate or organic iodine compounds which were not detected by the UV method. The cores from April 1999 have concentrations of sulfide less than 1 μM measured by both the UV and Methylene Blue methods.

The wavelength range from 234 to 278 nm was used to analyze Monterey Bay samples. At 234 nm the innovations sequence is less than the limit (0.01 A) for the sample shown and the predicted sulfide concentration and iodide concentration are relatively constant at this starting wavelength (Figure 17a). Figure 17b shows the innovations sequence determined for different ending wavelengths for a Monterey Bay sample. The innovations sequence is below the limit of 0.01 A at all ending wavelengths (Figure 17b). The end wavelength for the Monterey Bay samples however was set to 278 nm. The lowest standard error for the regressions was obtained when 278 nm was used instead of 300 nm (Table 3). It may be that as with some of the Elkhorn Slough samples, the absorbance due to dissolved organic material can affect the regression analysis when all wavelengths out to 300 nm are used. The predicted sulfide concentrations for the Monterey Bay samples are more negative when ending wavelengths out to 300 nm are used as compared to using just the wavelengths out to 278 nm. The detection limit for sulfide in sediment pore fluids is approximately 5 $\mu\text{M S}$. All of these Monterey Bay samples are below the detection limit for sulfide and the negative concentration estimates are within the bounds of this limit around zero concentration (Table 3).

It is possible to determine an approximate estimate of DOC concentration in these cores from the UV absorbance. A comparison of the measured concentration of DOC by high temperature catalytic oxidation³¹ and the regression calculated absorbance residual peak height at 272 nm for these samples is shown in Figure 18b for the Monterey Bay samples from April 1999. The regression accounts for 66 % of the variability between measured DOC and the residual height calculated by a least squares regression fit of

equation (1) to the observed spectra of the samples from April 1999 from 234–300 nm. The calculated absorbance residual peak height (Figure 18a) for samples can give an approximate indication of the amount of dissolved organic carbon in the sample.

DISCUSSION

The UV method detected nitrate and sulfide concentrations in well water samples with low errors (Table 1). The calculated absorbance residuals for the regression analyses for these samples gave an indication that small amounts of halide ions, specifically iodide ($0.33 \mu\text{M}$), and organic matter may also be present in the samples (Figure 7b). While both of these constituents may be present, they do not appear to bias the detection of nitrate and sulfide in the samples. Accurate estimates for both nitrate and sulfide concentrations were obtained in all of the freshwater samples.

The detection limit for sulfide in hydrothermal vent fluids is $0.8 \mu\text{M}$ by the UV method. Accurate results were obtained for all of the samples and measurements of the concentration could be made in seconds or minutes with the UV method rather than hours as with the methylene blue method. More samples could be run in a shorter amount of time even though the addition of ammonium hydroxide and dilutions with deoxygenated seawater were required. The data in Figure 10 show a close 1:1 relationship and the difference between the methylene blue and UV methods of measurements is small ($r^2 = 0.98$). The calculated absorbance residuals for the vent fluids (Figure 11) also show that dissolved organic material as well as products of sulfide oxidation (thiosulfate) may be present in the samples. The estimated concentration of thiosulfate in the sample in Figure

11a is $1.11\ \mu\text{M}$. These other species did not interfere with the detection of sulfide in the samples however.

The sediment porewaters were more complicated than the well water and the hydrothermal vent fluid samples. High baseline absorbance caused by DOC concentrations greater than $950\ \mu\text{M}$ caused negative estimates of sulfide concentration in some of the samples from Elkhorn Slough when a peak at 260 nm was present. A simple adjustment to the upper wavelength limit was applied to all samples that had a peak height greater than 0.05 A at 260 nm. The maximum error in the detection of sulfide in these samples was on the order of 0.016 A. A detection limit of $5\ \mu\text{M S}$ was calculated for these samples.

The model used for the Monterey Bay samples also required some adjustments. Iodide was present in the samples up to a concentration of $50\ \mu\text{M}$ by the UV method (Figure 16). Iodide has a peak absorbance at 226 nm (Figure 3a) and overlaps the spectrum of sulfide considerably. Some of the Monterey Bay samples also had high dissolved organic material present (Figure 18b). Despite the presence of these other components, reasonable values were obtained for the concentration of sulfide and iodide in the samples collected in March 1998 from Monterey Bay (Table 3). Two thirds of the iodine present in the Monterey Bay samples from April 1999 was detected as iodide by the UV method (Figure 16). This suggests that 1/3 of the iodine in the samples is present as iodate or organic iodine compounds which were not detected by the UV method. The regression of the data in Figure 18b for the Monterey Bay samples from April 1999

accounts for 66% of the variability between DOC measured by high temperature catalytic oxidation³¹ and DOC estimated by the UV method.

Sulfide, iodide, bromide and nitrate levels in a variety of complex natural waters could be monitored with ease and confidence by the ultraviolet method. The sulfide, iodide, bromide and nitrate concentrations could be monitored much quicker and more efficiently than with conventional methods. The accuracy and simplicity of the method suggests that an *in-situ* flow through monitoring system that uses the ultraviolet method would be ideal for measuring sulfide concentrations in hydrothermal vent systems. High resolution mapping of the chemical characteristics of hydrothermal plumes in near real time have provided insight into the variability and distribution of vent plumes.³² It may be possible that the addition of ammonium hydroxide would not be needed if the measurements were made *in-situ* under natural pressure conditions. Degassing of the samples would not be an issue with in situ measurements and it may be possible to determine the amount of sulfide in the sample given the amount present at the specific pH of the sample that is emitted from the vent. A pH measurement would be necessary in conjunction with the in situ sulfide measurement. The use of an *in situ* sensor would enable the acquisition of chemical data at sampling densities approaching those of physical measurements. Bromide concentrations in vent fluids could be obtained either directly in low sulfide samples or by removal of the sulfide from high sulfide vent samples by degassing prior to measuring the absorbance of the sample.

The ultraviolet method has proven applicable for monitoring sulfide in some of the most complex natural systems. The predictions for sulfide by the UV method are

satisfactory and the errors ($< \pm 2.0 \mu\text{M S}$, 1 SD) are within acceptable limits of the study. Nitrate, bromide and iodide concentrations can also be measured by the ultraviolet method. An indication of the amount of dissolved organic carbon in the samples could be inferred as well by examining the absorbance residuals produced by the least squares non-linear regression of the absorbance spectrum of a sample.

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Table 1. Sulfide concentrations determined by both UV and methylene blue in groundwater wells. Nitrate concentrations determined by both colorimetric and UV analyses are also shown. Colorimetric nitrate measurements courtesy of Sara Tanner, Trace Metal Lab, MLM. SE is the standard error of the regression analysis. Where SE equals the square root of the sum of the squared absorbance residuals (SSR) of the regression divided by the degrees of freedom (DF), where $DF = 37$.

Well Sample	UV $\mu\text{M HS}^-$	MB $\mu\text{M HS}^-$	UV $\mu\text{M NO}_3^-$	Colorimetric $\mu\text{M NO}_3^-$	SE $\text{sqrt}(\text{SSR}/\text{DF})$
1	-0.23	0.29	291.7	224.0	0.0009
2	1.66	1.26	16.2	10.8	0.0011
3a	0.16	0.39	39.8	39.4	0.0001
3b	0.14	0.14	39.7	31.9	0.0001
3c	0.20	0.12	39.6	30.9	0.0003
3d	0.15	0.14	39.4	30.4	0.0003
4a	0.36	0.12	146.8	116.2	0.0003
4b	0.30	0.17	146.8	115.0	0.0004
5a	0.26	0.26	333.8	280.8	0.0015
5b	0.20	0.29	333.7	267.4	0.0015

Table 2. Predicted HS⁻ concentration for both UV and MB methods for Elkhorn Slough Estuarine Reserve Porewater Samples. * Indicates dilution of sample with low nitrate filtered surface seawater. (`) Indicates DF = 20 for λ_{a} = 278 and DF = 31 for λ_{a} = 300, where DF is the degrees of freedom for each regression analysis. The standard error (SE) for each regression is shown where SE is equal to the square root of the sum of the squared absorbance residuals (SSR) divided by DF for each regression analysis.

Sample #	UV ($\mu\text{M S}$)	MB ($\mu\text{M S}$)	SE $\text{sqrt}(\text{SSR}/\text{DF})$
1	1.4	1.0	0.016
2	21.4	20.1	0.014
3	0.6	0.8	0.001
4	4.0	1.4	0.006
5	8.4	5.0	0.013
6	26.5	28.2	0.009
7	3.6	4.5	0.015
7*	5.5	5.0	0.004
8*	1199.5	1110.8	0.002
9*	1309.0	1473.1	0.001
10*	422.5	477.8	0.001
11*	83.7	69.2	0.002

Table 3 : Monterey Bay Porewater Samples. Collected March 1998. Analyzed two weeks after collection. SE is the standard error of the regression analysis. SE is equal to the square root of the sum of the squared residuals (SSR) divided by the degrees of freedom (DF) for the regression.

Sample	<u>HS⁻</u>		SE sqrt(SSR/DF)
	UV [μM HS]	MB [μM HS]	
1	16.6	0.6	0.007
2	9.9	1.7	0.004
3	15.8	2.3	0.006
4	4.2	0.7	0.002

Analyzed for HS⁻ only from 234 to 278 nm. DF = 20

Sample	<u>HS⁻</u>		<u>I⁻</u>	SE sqrt(SSR/DF)
	UV [μM HS]	MB [μM HS]	UV [μM I ⁻]	
1	-3.0	0.6	11.5	0.0004
2	-2.0	1.7	7.0	0.0005
3	-2.1	2.3	10.7	0.0005
4	-1.3	0.7	3.3	0.0003

Analyzed for both HS⁻ and I⁻ from 234 to 278 nm. DF = 19

Figure Captions

Figure 1. Diagram of ultraviolet flow through spectrophotometer with four Lee pumps. A Metrabyte DAS 8 analog/digital interface controls the pumps. A HPIB (IEEE 488 parallel) interface bus controls the spectrophotometer. The commands are written in QuickBasic 4.5. A 1-cm square (Fisherbrand) quartz cuvette was used for well water and porewater samples for batch mode collection and a 1-cm Hellma flow-through cuvette with Suprasil I windows was used for vent samples.

Figure 2. Plot of pH versus absorbance of 115 $\mu\text{M HS}^-$, % HS^- was calculated using a pK_1 value of 6.60 for seawater.²⁴

Figure 3. (a) Absorbance spectra for inorganic compounds present in seawater. (b) Absorbance spectra for products of sulfide oxidation.

Figure 4. Flow chart showing the order of the operations that are used to determine the best wavelength range in the analysis. Spectra are generally processed over an initial range of $\lambda_i = 250 \text{ nm}$ to $\lambda_e = 300 \text{ nm}$. A non-linear least squares regression is performed over this wavelength range for the UV model (Equation 1). All steps are followed in the flow chart until the response is STOP or if an adjustment to the model is necessary.

Figure 5. Salt effect on the molar absorptivity for sulfide. Open circles = NaCl solutions, closed circles = seawater.

Figure 6. Observed and predicted absorbance spectra for well water sample #2 (Table 1). The component spectra for nitrate and sulfide are also shown. Data from a non-linear least squares regression from 220-300 nm.

Figure 7. (a) I.S. = Innovations sequence for a well water sample. Sulfide and nitrate concentrations predicted by regression analysis using wavelengths from λ_i to 300 nm. The innovations sequence exceeds the limit of 0.01 A at $\lambda_i = 208$ nm. The arrow shows the I.S. limit. Final estimates of concentrations were computed at $\lambda_i = 220$ nm. (b) Absorbance residuals calculated by least squares non-linear regression for well water sample #2 (Table 1) from 220-300 nm.

Figure 8. (a) Observed and predicted absorbance spectra for a low sulfide vent sample. The component spectra for sulfide and nitrate (inset) and bromide are also shown. Data from non-linear least squares regression analysis from 214-300 nm. (b) I.S. = Innovations sequence. The arrow at 0.01 A shows the limit for the I.S. Sulfide, nitrate and salinity concentrations predicted for each non-linear regression from wavelength λ_i to 300 nm are also shown.

Figure 9. (a) Observed and predicted absorbance spectra for a high sulfide vent sample. The component spectrum for sulfide is also shown. Data from a least squares non-linear regression from 246-300 nm. (b) I.S. = Innovations sequence. The arrow at 0.015 A shows the limit for the I.S. The predicted sulfide concentrations at λ_i -300 nm are also shown.

Figure 10. Sulfide concentration measured by the methylene blue method versus the ultraviolet method for hydrothermal vent fluid samples. The dashed line in the main panel is a least squares fit to all the data. The inset shows the low sulfide values and a 1:1 line.

Figure 11. (a) Calculated absorbance residuals for the regression analysis for a low sulfide vent sample over the wavelength range 214 to 300nm. (b) Calculated absorbance residuals for the regression analysis of a high sulfide vent sample over the wavelength range 246 to 300 nm.

Figure 12. Absorbance spectra for two samples from Elkhorn Slough. A peak near 260 nm in the 20.8 μ M sample (2) causes a significant deviation from the exponential background curve used to model DOC spectra.

Figure 13. (a) I.S. = Innovations sequence at λ_i for two Elkhorn Slough samples. The arrows (1 and 2) show the corresponding I.S. limit for sample (1), limit is 0.03 A, and sample (2), limit is 0.05 A, from Figure 12. Sulfide concentrations predicted for the wavelength ranges λ_i to 300 nm are also shown.

Figure 14. Sulfide concentration measured by the methylene blue method versus the Ultraviolet method for Elkhorn Slough sediment porewater samples. The inset shows the low sulfide values detected in the samples, and a 1:1 line is also shown.

Figure 15. Absorbance spectrum from a sediment porewater sample from a sediment core collected at 85 m depth in Monterey Bay, April 1999. Component spectra for iodide and the baseline obtained with a least squares non-linear regression from 234-300 nm are also shown.

Figure 16. Iodide concentration versus depth for a core collected in Monterey Bay, April 1999, which was extracted and analyzed the day after collection. Open symbols show iodide measurements by the UV method and closed symbols show total iodine

measurements by ICP-MS. This core had $< 1 \mu\text{M HS}^-$ by the methylene blue and UV methods.

Figure 17. (a) I.S. = Innovations sequence at λ_i for the March 1998 Monterey Bay sediment porewater sample. The arrow shows the limit for the I.S. at 0.01 A. Sulfide and iodide concentrations predicted at λ_i -300 nm are also shown. (b) I.S. = Innovations sequence for λ_e . The arrow shows the limit for the I.S. at 0.01 A. Sulfide and iodide concentrations predicted at $240-\lambda_e$ nm are also shown.

Figure 18. (a) Absorbance residuals calculated by least squares non-linear regression fit of equation (1) for a Monterey Bay sample from April 1999, for the wavelength range 240-300 nm. (b) Plot of DOC versus the calculated absorbance residual peak height at 272 nm for all Core #1 samples from Monterey Bay, April 1999. Peak height equals the length of the vertical dotted line called ResH.

Figure 1.

Figure 2.

Figure 3.

Figure 4 .

Figure 5.

Figure 6.

Wavelength (nm)

Figure 7.

Figure 8.

Figure 9.

Figure 10.

Figure 11

Figure 12.

Figure 13.

Figure 14.

Figure 15.

Figure 16.

Figure 17.

Figure 18.

APPENDIX 1

Table of observed absorbance (AU), molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$) for HS^- and molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$) for NO_3^- at each wavelength from 200 to 300 nm in 2 nm increments for groundwater well sample #2 from Table 1.

λ	A_λ	$E_{\text{HS at } \lambda}$	$E_{\text{NO}_3 \text{ at } \lambda}$	λ	A_λ	$E_{\text{HS at } \lambda}$	$E_{\text{NO}_3 \text{ at } \lambda}$
200	0.4674	0.0048	0.0076	256	0.0779	0.0008	0.0000
202	0.3602	0.0042	0.0076	258	0.0770	0.0006	0.0000
204	0.2947	0.0036	0.0075	260	0.0762	0.0004	0.0000
206	0.2531	0.0033	0.0073	262	0.0755	0.0003	0.0000
208	0.2248	0.0031	0.0070	264	0.0746	0.0002	0.0000
210	0.2043	0.0032	0.0065	266	0.0739	0.0001	0.0000
212	0.1884	0.0034	0.0059	268	0.0731	0.0001	0.0000
214	0.1756	0.0038	0.0052	270	0.0724	0.0001	0.0000
216	0.1645	0.0043	0.0045	272	0.0717	4.27E-05	0.0000
218	0.1545	0.0049	0.0037	274	0.0709	3.23E-05	0.0000
220	0.1457	0.0055	0.0030	276	0.0702	2.38E-05	0.0000
222	0.1377	0.0062	0.0024	278	0.0695	2E-05	0.0000
224	0.1303	0.0068	0.0018	280	0.0687	1.8E-05	0.0000
226	0.1237	0.0072	0.0014	282	0.068	1.5E-05	0.0000
228	0.1178	0.0076	0.0010	284	0.0674	1.2E-05	0.0000
230	0.1124	0.0077	0.0007	286	0.0667	1.2E-05	0.0000
232	0.1074	0.0077	0.0005	288	0.066	1.2E-05	0.0000
234	0.1026	0.0074	0.0003	290	0.0652	1.1E-05	0.0000
236	0.0983	0.0070	0.0002	292	0.0646	9E-06	0.0000
238	0.0944	0.0064	0.0001	294	0.0639	9.4E-06	0.0000
240	0.0911	0.0056	0.0001	296	0.063	8.8E-06	0.0000
242	0.0883	0.0048	0.0001	298	0.06232	8.4E-06	0.0000
244	0.0859	0.0040	3.19E-05	300	0.0616	7.2E-06	0.0000
246	0.0841	0.0033	1.80E-05				
248	0.0825	0.0026	6.83E-06				
250	0.0811	0.0020	1.63E-07				
252	0.0798	0.0015	0.0000				
254	0.0789	0.0011	0.0000				

APPENDIX 2

Companion table for Figure 7a showing the equations and the parameters used to determine the innovations sequence and estimated concentrations for groundwater well sample #2 from Table 1.

λ_i	nth parameter Estimate				Non-linear fit of Equation (1) from λ_i to 300 nm gives the following parameters				Predicted $A_{\lambda i}$	I.S. for λ_i	SER
	$A_{\lambda i}$	$E_{HS \text{ at } \lambda i}$	$E_{NO_3 \text{ at } \lambda i}$	parameter Estimate	[HS ⁻]	[NO ₃ ⁻]	b	a			
208	0.2248	0.0031	0.0070	15	1.78	15.41	-0.004	-1.434	0.207	0.017	0.0017
210	0.2043	0.0032	0.0065	14	1.84	15.05	-0.004	-1.430	0.197	0.007	0.0014
212	0.1884	0.0034	0.0059	13	1.84	14.98	-0.004	-1.430	0.187	0.001	0.0013
214	0.1756	0.0038	0.0052	12	1.82	15.15	-0.004	-1.428	0.178	0.002	0.0013
216	0.1645	0.0043	0.0045	11	1.76	15.53	-0.004	-1.422	0.168	0.004	0.0013
218	0.1545	0.0049	0.0037	10	1.66	16.18	-0.005	-1.409	0.159	0.005	0.0013
220	0.1457	0.0055	0.0030	9	1.53	17.08	-0.005	-1.391	0.151	0.005	0.0010
222	0.1377	0.0062	0.0024	8	1.39	18.31	-0.005	-1.369	0.142	0.005	0.0010
224	0.1303	0.0068	0.0018	7	1.23	20.04	-0.005	-1.343	0.136	0.006	0.0009
226	0.1237	0.0072	0.0014	6	1.06	22.48	-0.005	-1.315	0.129	0.006	0.0007
228	0.1178	0.0076	0.0010	5	0.88	25.71	-0.005	-1.288	0.121	0.004	0.0006
230	0.1124	0.0077	0.0007	4	0.69	29.93	-0.005	-1.263	0.115	0.003	0.0005
232	0.1074	0.0077	0.0005	3	0.53	34.90	-0.005	-1.242	0.110	0.002	0.0004
234	0.1026	0.0074	0.0003	2	0.36	41.40	-0.005	-1.222	0.104	0.002	0.0003
236	0.0983	0.0070	0.0002	1	0.20	49.03	-0.005	-1.207	0.100	0.001	0.0003
238	0.0944	0.0056	0.0002								0.0002

λ_i is the initial wavelength used in the regression analysis. $A_{\lambda i}$ is the observed absorbance at λ_i . The molar absorptivities for HS⁻ and NO₃⁻ are also given for λ_i . Parameter estimates are shown for HS⁻, NO₃⁻, the slope (b) and the intercept (a) for non-

linear regression analysis of Equation (1) from λ_i to 300 nm (the end wavelength) for groundwater sample #2 (Table 1). For this sample the analysis is started at $\lambda_i = 238$ nm where $n = 1$. The corresponding parameter estimates for $n = 1$ from the non-linear regression of Equation (1) for the wavelength range 238 to 300 nm are shown in the next four columns. The predicted absorbance (A_{λ_i} or for $n+1$) at the next lower wavelength λ_{i-2} or where $n = n+1$ ($A_{236 \text{ nm}}$) is determined by the following equation when $n = 1$:

$$A_{(n+1, 236 \text{ nm})} = E_{\text{HS at } (n+1)} * [\text{HS}]_n + E_{\text{NO}_3 \text{ at } (n+1)} * [\text{NO}_3]_n + \exp(b_n * \lambda_{n+1} + a_n).$$

Then the innovations sequence (I.S.) is determined by taking the absolute difference between the observed absorbance at $n = n+1$ (236 nm) and the predicted absorbance at $n = n+1$ (236 nm). The innovations sequence limit is calculated by taking three times the standard error of the regression analysis and in the case for well water it is equal to 0.01 A. The determination of the innovations sequence (I.S.) is continued for all possible initial wavelengths (λ_i) until the limit is exceeded. SER is the standard error for each regression analysis from λ_i to 300 nm. The SER should not be greater than the error due to the spectrophotometer which is approximately 0.001A. In this case, 220 to 300 nm was the wavelength range chosen for the groundwater sample analyses.