lower response because the divalent Mg^{2+} and Ca^{2+} cations are complexing with the free MTB. If depletion has occurred, repack the column with fresh resin.

h. Stock sulfate standard, 1000 mg/L SO_4^{2-}: Dry approximately 2 g sodium sulfate, Na_2SO_4, at 105 °C overnight. Cool in a desiccator. In a 1-L volumetric flask, add 1.479 g of dried sodium sulfate to about 800 mL water. Dissolve by swirling, dilute to the mark with reagent water, and mix by inversion.

i. Standard solutions: Prepare sulfate standards in desired concentration range, using the stock standard (¶ *h* above), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-SO_4^{2-}:2 and follow the method supplied by the manufacturer or laboratory standard operating procedure. Follow quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting adsorbance of standards processed through the manifold versus sulfate concentration.

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-SO_4^{2-}:1 gives results of single-laboratory studies.

b. MDL: A 180-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 5.00-mg/L SO_4^{2-} standard. These gave a mean of 4.80 mg/L SO_4^{2-}, a standard deviation of 0.69 mg/L SO_4^{2-}, and an MDL of 1.8 mg/L SO_4^{2-}.

Reference

1. U.S. Environmental Protection Agency. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11; 1984. Amended June 30, 1986. 49 CFR 43430.

4500-S^{2-}

Sulfide

Approved by Standard Methods Committee, 2000. Editorial revisions, 2021. Joint Task Group: Thomas R. Holm (chair), Robert P. Fisher, Martin S. Frant, Christian Gagnon, Lorne R. Goodwin.

4500-S^{2-} A. Introduction

1. Occurrence and Significance

Sulfide is often present in groundwater and sediment. It is produced by decomposition of organic matter and bacterial reduction of sulfate. It is sometimes found in industrial or municipal wastewater. Hydrogen sulfide escaping into the air from sulfide-containing wastewater causes odor nuisances. The threshold odor concentration of H_2S in clean water is between 0.025 and 0.25 µg/L. Gaseous H_2S is very toxic and has claimed the lives of numerous workers. At levels toxic to humans, it interferes with the olfactory system, giving a false sense of the safe absence of H_2S. It attacks metals directly, and indirectly has caused serious corrosion of concrete sewers because it is oxidized biologically in the presence of oxygen to H_2SO_4 on the pipe wall. Dissolved H_2S is toxic to fish and other aquatic organisms.

Hydrogen sulfide combines with iron and other metals in natural sediments and sludges to form slightly-soluble precipitates. Acid-volatile sulfide (AVS) is an important class of metal sulfides in these anoxic environments. The determination of AVS concentrations has become more prevalent because AVS is considered to be a key binding phase for controlling bioavailability of toxic metals in anoxic sediments.

AVS typically is determined by a purge-and-trap method in which hydrochloric acid is used to volatilize AVS at room temperature. The metals associated with the sulfides can be determined from the supernatant of the purged sample solution by using methods such as those in Part 3000. The hydrogen sulfide produced is trapped in zinc-acetate absorbing solution. AVS concentrations are measured by iodometric titration of the ZnS precipitated in the trap (4500-S^{2-} F). Certain minerals, including iron pyrite, are partially digested by the AVS reagents at elevated temperatures, which may result in a significant overestimation of AVS. Iron pyrite can be partially digested to the extent of less than 10% of the total pyrite present. The addition of stannous chloride ($SnCl_2$) prevents the oxidation of sulfides by any liberated ferric iron.

2. Categories of Sulfides

Four categories of sulfide in water, wastewater, and sediment can be operationally defined:

a. Total sulfide includes dissolved H_2S and HS^- and acid-volatile metallic sulfides present in particulate matter. The pK_{a2} of H_2S is so high that the concentration of S^{2-} is negligible at all pH values. Copper and silver sulfides are so insoluble that they do not respond in ordinary sulfide determinations; they can be ignored for practical purposes.

b. Dissolved sulfide is that remaining after suspended solids have been removed by flocculation and settling. Flocculation and settling are used to separate dissolved and particulate sulfide because sulfide may be oxidized during filtration. Centrifugation also may be used.

c. Acid-volatile sulfide includes amorphous iron monosulfides, including mackinawite (FeS), greigite (Fe_3S_4), and pyrrhotite (FeS), and amorphous monosulfides of other metals. Pyrite, another iron sulfide mineral, is not included in the acid-volatile sulfides.

d. Un-ionized hydrogen sulfide can be calculated from the concentration of dissolved sulfide, the sample pH, and the conditional ionization constant of H_2S.

Figure 4500-S^{2-}:1 shows analytical flow paths for sulfide determinations under various conditions and options. None of the operationally defined sulfide categories includes pyrite or marcasite (FeS_2).

3. Sampling and Storage

Collect water samples with minimum aeration. Either analyze samples immediately after collection or preserve with zinc acetate solution for later analysis. To preserve a sample for a total sulfide determination, put zinc acetate and sodium hydroxide solutions into the sample bottle before filling it with sample. Use 0.2 mL 2 M zinc acetate solution per 100 mL sample. Increase the volume of zinc acetate solution if the sulfide concentration is expected to be greater than 64 mg/L. The final pH should be at least 9. Add more NaOH if necessary. Fill the bottle completely and stopper.

Sample sediments and sludges under nitrogen atmosphere if possible. Store sediment and sludge samples at 4 °C or frozen, and analyze within 2 weeks (1 month for frozen samples) of collection. Do not freeze-dry because acid-volatile sulfide may decompose as a result of oxidation artifacts.

4. Qualitative Tests

A qualitative test for sulfide often is useful. It is advisable in the examination of industrial wastes containing interfering substances that may give a false negative result in the methylene blue method (4500-S^{2-} D).

a. Antimony test: To about 200 mL sample, add 0.5 mL saturated solution of antimony potassium tartrate and 0.5 mL 6 N HCl in excess of phenolphthalein alkalinity.

Yellow antimony sulfide (Sb_2S_3) is discernible at a sulfide concentration of 0.5 mg/L. Comparisons with samples of known sulfide concentration make the technique roughly quantitative. The only known interferences are metallic ions, such as lead, which hold the sulfide so firmly that it does not produce Sb_2S_3, and dithionite, which decomposes in acid solution to produce sulfide.

b. Silver-silver sulfide electrode test: Dilute sample 1:1 with alkaline antioxidant reagent (see 4500-S^{2-} G.3*a*). Measure electrode potential relative to a double-junction reference electrode and estimate the sulfide concentration from an old calibration curve or the example calibration curve in the electrode manual. This gives a reasonable estimate of sulfide concentration if the electrode is in good condition.

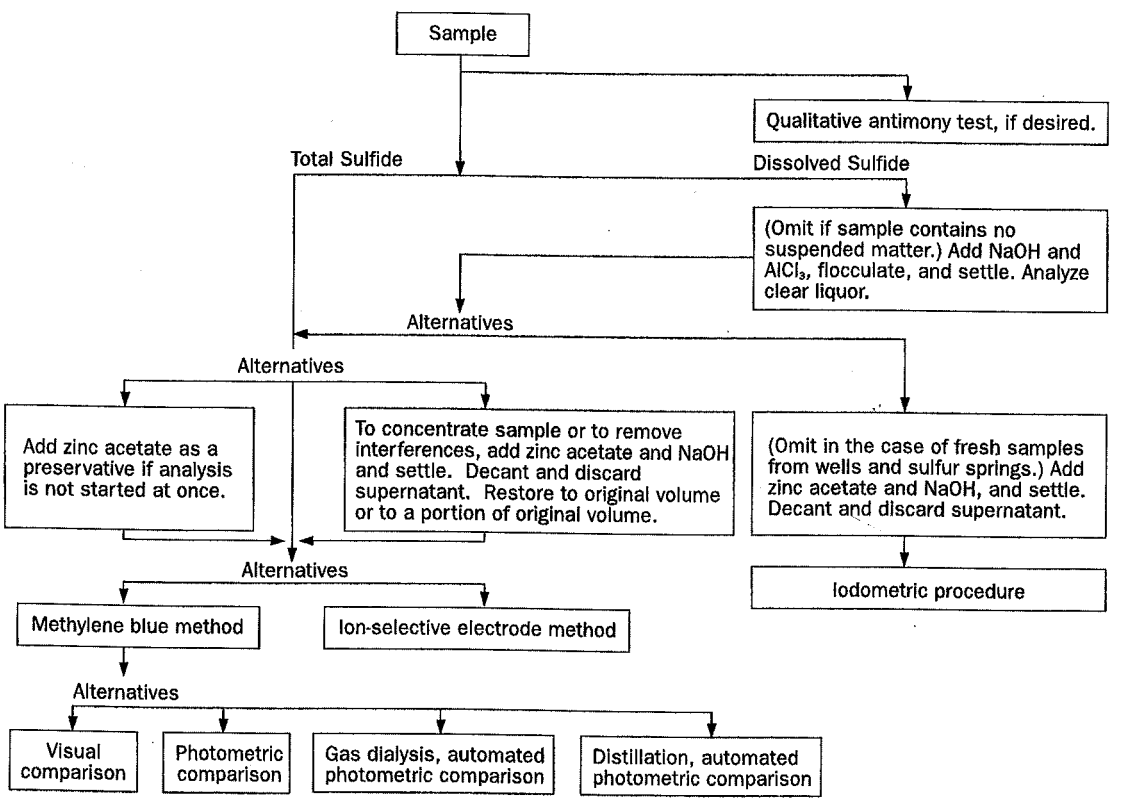

Figure 4500-S^{2-}:1. Analytical flow paths for sulfide determination.

c. Lead acetate paper and silver foil tests: Confirm odors attributed to H_2S with lead acetate paper. On exposure to the vapor of a slightly acidified sample, the paper becomes blackened by the formation of PbS. A strip of silver foil is more sensitive than lead acetate paper. Clean the silver by dipping in a NaCN solution and rinsing. **Caution: NaCN is toxic; handle with care.** Silver is suitable particularly for long-time exposure in the vicinity of possible H_2S sources because black Ag_2S is permanent, whereas PbS slowly oxidizes.

5. Selection of Quantitative Methods

Iodine oxidizes sulfide in acid solution. A titration based on this reaction is an accurate method for determining sulfide at concentrations above 1 mg/L if interferences are absent and if loss of H_2S is avoided. (Note: Many substances can reduce iodine; all of these are potential interferences in procedures using this chemistry.)

The iodometric method (F) is useful for standardizing the methylene blue colorimetric methods (4500-S^{2-} D, E, and I) and is suitable for analyzing samples freshly taken from wells or springs. The method can be used for wastewater and partly oxidized water from sulfur springs if interfering substances are removed first. The automated methylene blue method with distillation (4500-S^{2-} I) is useful for a variety of samples containing more than 1 mg/L S^{2-}.

The methylene blue method (4500-S^{2-} D) is based on the reaction of sulfide, ferric chloride, and dimethyl-*p*-phenylenediamine to produce methylene blue. Ammonium phosphate is added after color development to remove ferric chloride color. The procedure is applicable at sulfide concentrations between 0.1 and 20.0 mg/L. The automated methylene blue method (4500-S^{2-} E) is similar to 4500-S^{2-} D. A gas dialysis technique separates the sulfide from the sample matrix. Gas dialysis eliminates most interferences, including turbidity and color. The addition of the antioxidant ascorbic acid improves sulfide recoveries. The method is applicable at sulfide concentrations between 0.002 and 0.100 mg/L.

Sulfide concentration can be determined using a sulfide ion selective electrode (4500-S^{2-} G) by comparing the potential of the electrode relative to a reference electrode, but careful attention to details of procedures and frequent standardizations are needed to secure good results. The electrode is useful particularly as an endpoint indicator for titrating dissolved sulfide with silver nitrate. The ion-selective electrode method is unaffected by sample color or turbidity and is applicable for concentrations greater than 0.03 mg/L.

6. Preparation of Sulfide Standards

Take care in preparing reliable stock solutions of sulfide for calibration and quality control. Prepare sulfide standards from sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) crystals. These crystals usually have excess water present on the surface, in addition to a layer of contamination from oxidation products of sulfide (polysulfides, polythionates, and sulfate) reacting with atmospheric oxygen. Further, solutions of sulfide are prone to ready oxidation by dissolved and atmospheric oxygen. Use reagent water to prepare sulfide standards and sample dilutions. Degas the water with either argon or nitrogen. Purchase the smallest amount of solid standards possible and keep no longer than 1 year. Preferably handle and store solid sulfide standards and stock solutions in an inert atmosphere glove bag or glove box to reduce contamination due to oxidation.

Preferably remove single crystals of $Na_2S \cdot 9H_2O$ from the reagent bottle with nonmetallic tweezers; quickly rinse in degassed reagent water to remove surface contamination. Blot the crystal dry with a tissue, then rapidly transfer to a tared, stoppered weighing bottle containing 5 to 10 mL degassed reagent water. Repeat the procedure until the desired amount of sodium sulfide is in a weighing bottle. Avoid excess agitation and mixing of the solution with atmospheric oxygen. Quantitatively transfer and dilute the entire contents of the weighing bottle to an appropriate size volumetric flask with degassed reagent water to prepare a known concentration sulfide stock solution (3.75 g $Na_2S \cdot 9H_2O$ diluted to a final volume of 500 mL produces a stock solution in which 1.00 mL = 1.00 mg S^{2-}). Standardize the stock solution using the iodometric method, 4500-S^{2-} F. Alternatively, purchase precertified stock solutions of sulfide. Verify the concentration of the stock solution daily using the iodometric method (4500-S^{2-} F). Store the stock solution with minimum headspace for no more than 1 week.

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4500-S^{2-} B. Separation of Soluble and Insoluble Sulfides

Unless the sample is entirely free from suspended solids (dissolved sulfide equals total sulfide), to measure dissolved sulfide first remove insoluble matter. This can be done by producing an aluminum hydroxide floc that is settled, leaving a clear supernatant for analysis.

1. Apparatus

Glass bottles with stoppers: Use 100 mL if sulfide will be determined by the methylene blue method and 500 to 1000 mL if by the iodometric method.

2. Reagents

a. Sodium hydroxide solution (NaOH), 6 N.

b. Aluminum chloride solution: Because of the hygroscopic and caking tendencies of this chemical, purchase 100-g bottles of $AlCl_3 \cdot 6H_2O$. Dissolve contents of a previously unopened 100-g bottle in 144 mL reagent water.

3. Procedure

a. To a 100-mL glass bottle, add 0.2 mL (nominally 4 drops) 6 N NaOH. Fill the bottle with sample and immediately add 0.2 mL (4 drops) $AlCl_3$ solution. Stopper the bottle with no air under the stopper. Rotate back and forth about a transverse axis vigorously for 1 min or longer to flocculate contents. Vary volumes of these added chemicals to get good clarification without using excessively large amounts and to produce a pH of 6 to 9. If a 500- or 1000-mL bottle is used, add proportionally larger amounts of reagents.

b. Let settle until reasonably clear supernatant can be drawn off. With proper flocculation, this may take 5 to 15 min. Do not wait longer than necessary.

c. Either analyze the supernatant immediately or preserve with 2 N zinc acetate (see 4500-S^{2-} C).

4500-S^{2-} C. Sample Pretreatment to Remove Interfering Substances or to Concentrate the Sulfide

The iodometric method suffers interference from reducing substances that react with iodine, including thiosulfate, sulfite, and various organic compounds, both solid and dissolved.

Strong reducing agents also interfere in the methylene blue method (4500-S^{2-} D) by preventing formation of the blue color. Thiosulfate at concentrations of about 10 mg/L may retard color formation or completely prevent it. Ferrocyanide produces a blue color. Sulfide itself prevents the reaction if its concentration is very high, in the range of several hundred milligrams per liter. To avoid the possibility of false negative results, use the antimony method to obtain a qualitative result in industrial wastes likely to contain sulfide but showing no color by the methylene blue method. Iodide, which is likely to be present in oil-field wastewaters, may diminish color formation if its concentration exceeds 2 mg/L. Many metals (e.g., Hg, Cd, Cu) form insoluble sulfides and give low recoveries.

Eliminate interferences due to sulfite, thiosulfate, iodide, and many other soluble substances, but not ferrocyanide, by first precipitating ZnS, removing the supernatant, and replacing it with reagent water. Use the same procedure, even when not needed for removal of interferences, to concentrate sulfide. The automated methylene blue method (4500-S^{2-} E) is relatively free from interferences because gas dialysis separates the sulfide from the sample matrix.

1. Apparatus

Glass bottles with stoppers: See 4500-S^{2-} B.1.

2. Reagents

a. Zinc acetate solution: Dissolve 220 g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ in 870 mL water; this makes 1 L solution.

b. Sodium hydroxide solution (NaOH), 6 N.

3. Procedure

a. Put 0.20 mL (4 drops) zinc acetate solution and 0.10 mL (2 drops) 6 N NaOH into a 100-mL glass bottle, fill with sample, and add 0.10 mL (2 drops) 6 N NaOH solution. Stopper with no headspace and mix by rotating back and forth vigorously about a transverse axis. For the iodometric procedure, use a 500-mL bottle or other convenient size, with proportionally larger volumes of reagents. Vary the volume of reagents added according to the sample so the resulting precipitate is not excessively bulky and settles readily. Add enough NaOH to raise the pH above 9. Let the precipitate settle for 30 min. The treated sample is relatively stable and can be held for several hours. However, if much iron is present, oxidation may be fairly rapid.

b. If the iodometric method is to be used, collect the precipitate on a glass fiber filter and continue at once with the titration according to the procedure of 4500-S^{2-} F. If the methylene blue method (4500-S^{2-} D) is used, let the precipitate settle for 30 min and decant as much supernatant as possible without a loss of precipitate. Refill the bottle with reagent water, shake to resuspend the precipitate, and quickly withdraw a sample. If interfering substances are present in high concentration, settle, decant, and refill a second time. If sulfide concentration is known to be low, add only enough water to bring the volume to one-half or one-fifth of the original volume. Use this technique for analyzing samples of very low sulfide concentrations. After determining the sulfide concentration colorimetrically, multiply the result by the ratio of final to initial volume. No concentration or pretreatment steps to remove interferences are necessary for 4500-S^{2-} E.

4500-S^{2-} D. Methylene Blue Method

1. Apparatus

a. Matched test tubes, approximately 125 mm long and 15 mm o.d.

b. Droppers, delivering 20 drops/mL methylene blue solution. To obtain uniform drops hold the dropper in a vertical position and let drops form slowly.

c. If photometric rather than visual color determination will be used, either:

1) *Spectrophotometer,* for use at a wavelength of 664 nm with cells providing light paths of 1 cm and 1 mm, or other path lengths, or

2) *Filter photometer,* with a filter providing a maximum transmittance near 660 nm.

2. Reagents

a. Amine-sulfuric acid stock solution: Dissolve 27 g *N,N*-dimethyl-*p*-phenylenediamine oxalate in an iced mixture of 50 mL conc H_2SO_4 and 20 mL reagent water. Cool and dilute to 100 mL with reagent water. Use fresh oxalate because an old supply may be oxidized and discolored to a degree that results in interfering colors in the test. Store in a dark glass bottle. When this stock solution is diluted and used in the procedure with a sulfide-free sample, it first will be pink but then should become colorless within 3 min.

b. Amine-sulfuric acid reagent: Dilute 25 mL amine-sulfuric acid stock solution with 975 mL 1 + 1 H_2SO_4. Store in a dark glass bottle.

c. Ferric chloride solution: Dissolve 100 g $FeCl_3 \cdot 6H_2O$ in 40 mL water.

d. Sulfuric acid solution (H_2SO_4), 1 + 1.

e. Diammonium hydrogen phosphate solution: Dissolve 400 g $(NH_4)_2HPO_4$ in 800 mL reagent water.

f. Methylene blue solution I: Use USP grade dye or one certified by the Biological Stain Commission. On the label, report the dye content which must be 84% or more. Dissolve 1.0 g in reagent water and dilute to 1 L. This solution will be approximately the correct strength, but because of variation between different lots of dye, standardize against sulfide solutions of known strength and adjust its concentration so 0.05 mL (1 drop) = 1.0 mg/L sulfide.

Standardization—Prepare 5 known-concentration sulfide standards ranging from 1 to 8 mg/L as described in 4500-S^{2-} A.6, or proceed as follows: Put several grams of clean, washed crystals of $Na_2S \cdot 9H_2O$ into a small beaker. Add somewhat less than enough water to cover crystals. Stir occasionally for a few minutes, then pour the solution into another vessel. This solution reacts slowly with oxygen but the change is insignificant if the analysis is performed within a few hours. Prepare the solution daily. To 1 L reagent water, add 1 drop of Na_2S solution and mix. Immediately determine sulfide concentration by the methylene blue procedure and by the iodometric procedure. Repeat, using more than 1 drop Na_2S solution or smaller volumes of water, until at least 5 tests have been made, with a range of sulfide concentrations between 1 and 8 mg/L. Calculate the average percent error of the methylene blue result as compared to the iodometric result. If the average error is negative (i.e., methylene blue results are lower than iodometric results), dilute the methylene blue solution by the same percentage, so a greater volume will be used in matching colors. If methylene blue results are high, increase the solution strength by adding more dye.

g. Methylene blue solution II: Dilute 10.00 mL of adjusted methylene blue solution I to 100 mL with reagent water.

3. Procedure

a. Color development: Transfer 7.5 mL sample to each of 2 matched test tubes, using a special wide-tip pipet or filling to marks on test tubes. If the sample has been preserved with zinc acetate, shake vigorously before taking subsample. Add to Tube A 0.5 mL amine-sulfuric acid reagent and 0.15 mL (3 drops) $FeCl_3$ solution. Mix immediately by inverting slowly, only once. (Excessive mixing causes low results by loss of H_2S as a gas before it has had time to react). To Tube B add 0.5 mL 1 + 1 H_2SO_4 and 0.15 mL (3 drops) $FeCl_3$ solution and mix. The presence of S^{2-} is indicated by the appearance of a blue color in Tube A. Color development usually is complete in about 1 min, but a longer time often is required for the fading out of the initial pink color. Wait 3 to 5 min and add 1.6 mL $(NH_4)_2HPO_4$ solution to each tube. Wait 3 to 15 min and make color comparisons. If zinc acetate was used, wait at least 10 min before making a visual color comparison.

b. Color determination:

1) Visual color estimation—Add methylene blue solution I or II, depending on sulfide concentration and desired accuracy, dropwise, to the second tube, until the color matches that developed in first tube. If the concentration exceeds 20 mg/L, repeat the test with a portion of sample diluted 10-fold.

With methylene blue solution I, adjusted so 0.05 mL (1 drop) = 1.0 mg/L S^{2-} when 7.5 mL of sample are used:

$$mg/L\ S^{2-} = no.\ drops\ solution\ I + 0.1\ (no.\ drops\ solution\ II)$$

2) Photometric color measurement—A cell with a light path of 1 cm is suitable for measuring sulfide concentrations from 0.1 to 2.0 mg/L. Use shorter or longer light paths for higher or lower concentrations. This method is suitable for sample concentrations up to 20 mg/L. Zero the instrument with a portion of the treated sample from Tube B. Prepare calibration curves on the basis of colorimetric tests made on Na_2S solutions simultaneously analyzed by the iodometric method, plotting concentration vs. absorbance. A linear relationship between concentration and absorbance can be assumed from 0 to 1.0 mg/L.

Read the sulfide concentration from calibration curve.

4. Precision and Bias

In a study by 2 chemists working in the same laboratory, the standard deviation estimated from 34 sets of duplicate sulfide measurements was 0.04 mg/L for concentrations between 0.2 and 1.5 mg/L. The average recoveries of known additions were 92% for 40 samples containing 0.5 to 1.5 mg/L and 89% for samples containing less than 0.1 mg/L.

5. Quality Control

The quality control practices considered to be an integral part of each method are summarized in Table 4020:1.

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4500-S^{2-} E. Gas Dialysis, Automated Methylene Blue Method

1. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-S^{2-}:2.

The sampler is equipped with a mixer to stir samples before analysis and the gas dialysis membrane, which is maintained at room temperature, separates H_2S from the sample matrix.

2. Reagents

*a. N,N-dimethyl-*p*-phenylenediamine stock solution:* Dissolve 1 g *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride in 500 mL 6 N HCl. Prepare fresh monthly. Store in an amber bottle.

*b. N,N-dimethyl-*p*-phenylenediamine working solution:* Dilute 190 mL *N,N*-dimethyl-*p*-phenylenediamine stock solution to 1 L. Store in an amber bottle. Prepare weekly.

c. Ferric chloride stock solution: Dissolve 13.5 g $FeCl_3 \cdot 6H_2O$ in 500 mL 5 N HCl. Store in an amber bottle. Prepare fresh monthly.

d. Working ferric chloride solution: Dilute 190 mL ferric chloride stock solution to 1 L. Store in an amber bottle. Prepare fresh weekly.

e. Hydrochloric acid (HCl), 6 N.

f. Sodium hydroxide stock solution (NaOH), 1 N.

g. Sodium hydroxide (NaOH), 0.01 N: Dilute 10 mL NaOH stock solution to 1 L.

h. Sulfide stock solution, 1.00 mg S^{2-}/1.00 mL: See 4500-S^{2-} A.6.

i. Sulfide intermediate standard solution: Dilute 10 mL sulfide stock solution to 1 L with water. Prepare fresh daily. Standardize by iodometric titration method, 4500-S^{2-} F. 1 mL $\approx$ 0.01 mg S^{2-}.

j. Sulfide tertiary standard solution: Dilute 50 mL sulfide intermediate solution to 500 mL with 0.01 N NaOH. Prepare fresh daily. Use the standardization value from paragraph *i* above to determine the exact concentration. 1.00 mL $\approx$ 0.001 mg S_2^-.

k. Working sulfide standard solutions: Prepare a suitable series of standards by diluting appropriate volumes of sulfide tertiary standing solutions with 0.01 N NaOH. Prepare fresh daily.

l. Zinc acetate preservative solution: Dissolve 220 g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ in 870 mL water (this makes 1 L solution).

3. Procedure

For unpreserved, freshly collected samples and sulfide working standards, add, in order, 4 drops 2 N zinc acetate, 0.5 mL 6 N NaOH, and 400 mg ascorbic acid per 100 mL. For preserved samples, add 0.5 mL 6 N NaOH and 400 mg ascorbic acid per 100 mL. Shake well.

Let the precipitate settle for at least 30 min. Pour a portion of well-mixed sample or working standard into a sample cup. Set up manifold as shown in Figure 4500-S^{2-}:2 and follow the general procedure described by the manufacturer. Determine absorbance at 660 nm.

4. Calculation

Prepare standard curves by plotting peak heights of standards processed through the manifold against S^{2-} concentration in the standards. Compute S^{2-} sample concentration by comparing the sample response with the standard curve.

5. Precision and Bias

In a single laboratory, samples with S^{2-} concentrations of 0.012, 0.015, 0.034, and 0.085 mg/L had standard deviations of 0.001, 0.001, 0.001, and 0.001 mg/L, respectively, with coefficients of variation of 8.3%, 6.3%, 2.9%, and 1.2%, respectively. In 2 environmental samples with added S^{2-}, recoveries were 104.2% and 97.6%.

6. Quality Control

The quality control practices considered to be an integral part of each method are summarized in Table 4020:1.

Bibliography

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4500-S^{2-} F. Iodometric Method

1. Reagents

a. Hydrochloric acid (HCl), 6 N.

b. Standard iodine solution, 0.0250 N: Dissolve 20 to 25 g KI in a little water and add 3.2 g iodine. After iodine has dissolved, dilute to 1000 mL and standardize against 0.0250 N $Na_2S_2O_3$, using starch solution as indicator.

c. Standard sodium thiosulfate solution, 0.0250 N: See Section 4500-O C.2*e*.

d. Starch solution: See Section 4500-O C.2*d*.

2. Procedure

a. Measure from a buret into a 500-mL flask an amount of iodine solution estimated to be an excess over the amount of sulfide present. Add reagent water, if necessary, to bring volume to about 20 mL. Add 2 mL 6 N HCl. Pipet 200 mL sample into the flask, discharging the sample under the solution's surface. If the iodine color disappears, add more iodine until the color remains. Back-titrate with $Na_2S_2O_3$ solution, adding a few drops of starch solution as the endpoint is approached, and continuing until blue color disappears.

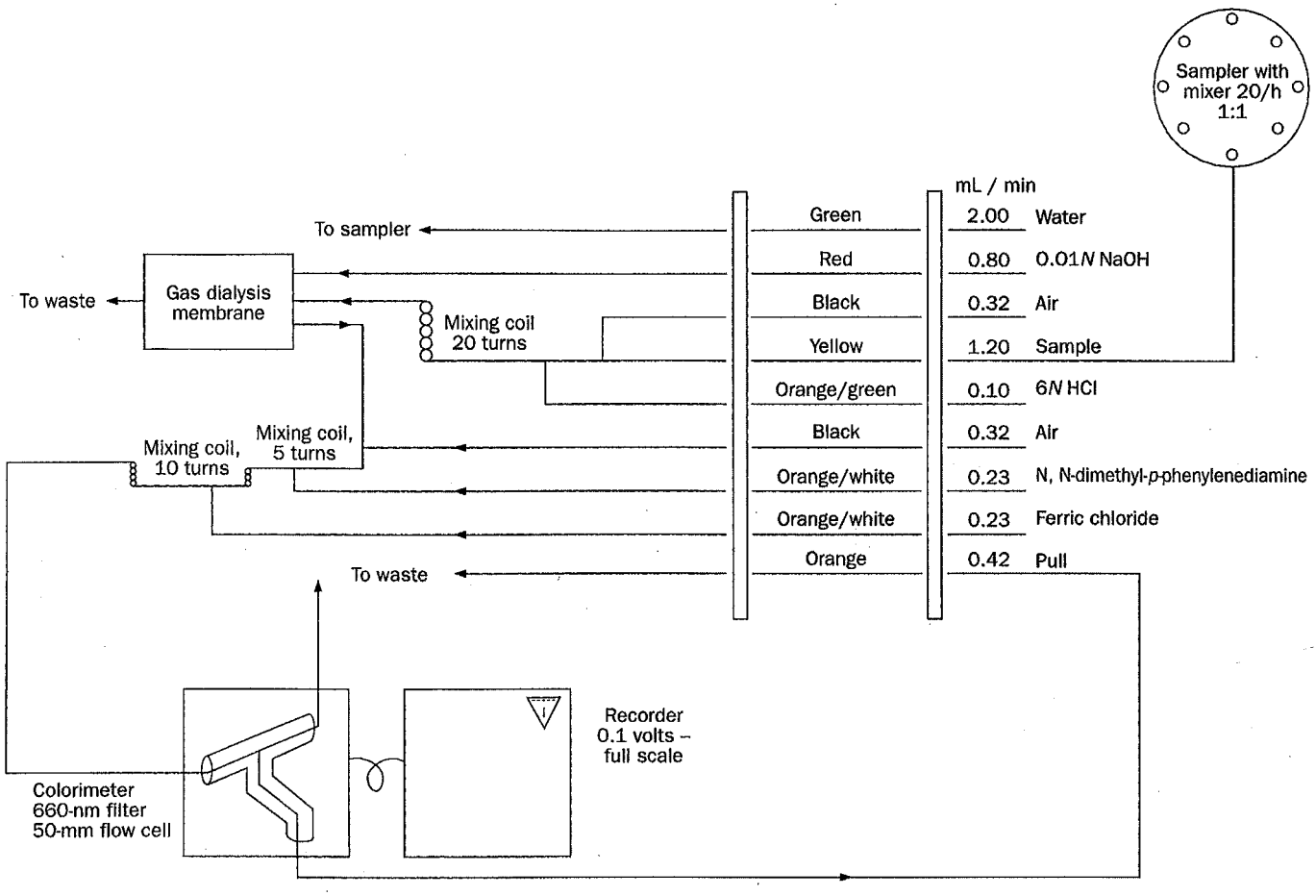

Figure 4500-S^{2-}:2. Sulfide manifold.

b. If sulfide was precipitated with zinc and ZnS filtered out, return the filter with precipitate to the original bottle and add about 100 mL water. Add the iodine solution and HCl and titrate as in paragraph *a* above.

3. Calculation

One milliliter 0.0250 N iodine solution reacts with 0.4 mg S^{2-}:

$$\text{mg/L } S^{2-} = \frac{[(A \times B) - (C \times D)] \times 16\,000}{\text{mL sample}}$$

where:

A = mL iodine solution,
B = normality of iodine solution,
C = mL $Na_2S_2O_3$ solution, and
D = normality of $Na_2S_2O_3$ solution.

4. Precision

The precision of the endpoint varies with the sample. In clean waters it should be determinable within 1 drop, which is equivalent to 0.1 mg/L in a 200-mL sample.

5. Quality Control

The quality control practices considered to be an integral part of each method are summarized in Table 4020:1.

4500-S^{2-} G. Ion-Selective Electrode Method

1. General Discussion

a. Principle: The potential of a sulfide ion-selective electrode (ISE) is related to the sulfide ion activity. An alkaline antioxidant reagent (AAR) is added to samples and standards to inhibit oxidation of sulfide by oxygen and to provide a constant ionic strength and pH. Use of the AAR allows calibration in terms of total dissolved sulfide concentration. All samples and standards must be at the same temperature. Sulfide concentrations between 0.032 mg/L (1×10^{-6} M) and 100 mg/L can be measured without preconcentration. For lower concentrations, preconcentration is necessary.

b. Interferences: Humic substances may interfere with sulfide ISE measurements. For highly colored water (high concentration of humic substances), use the method of standard additions to check results. Sulfide is oxidized by dissolved oxygen. Sulfide oxidation may cause potential readings to drift in the direction of decreasing concentration (i.e., to more positive values). Flush the surface of samples and standards with nitrogen to minimize contact with atmospheric oxygen for low-level measurements. Temperature changes may cause potentials to drift either upward or downward. Therefore, let standards and samples come to the same temperature. If samples cannot be analyzed immediately, preserve dissolved sulfide by precipitating with zinc acetate (4500-S^{2-} C).

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 4020:1.

2. Apparatus

a. Sulfide ion selective electrode.
b. Double-junction reference electrode.
c. Electrode polishing strips.
d. pH meter with millivolt scale, capable of 0.1-mV resolution. Meters that can be calibrated in concentration and that perform standard-additions calculations are available.
e. Electrochemical cell: Make a suitable cell from a 150-mL beaker and a sheet of rigid plastic (PVC or acrylic) with holes drilled to allow for insertion of the electrodes and a tube for flushing the headspace with nitrogen. Alternatively, purchase a polarographic cell with gas transfer tube.
f. Gas dispersion tube: Use to deaerate water for preparing reagents and standards.
g. Magnetic stirrer and stirring bar: Use a piece of styrofoam or cardboard to insulate the cell from the magnetic stirrer.

3. Reagents

a. Alkaline antioxidant reagent (AAR): To approximately 600 mL deaerated reagent water (DRW) in a 1-L volumetric flask, add 80 g NaOH, 35 g ascorbic acid, and 67 g Na_2H_2EDTA. Swirl to dissolve and dilute to 1 L. The color of freshly prepared AAR ranges from colorless to yellow. Store in a tightly capped brown glass bottle. Discard when the solution becomes brown.
b. Lead perchlorate, 0.1 M: Dissolve 4.60 g $Pb(ClO_4)_2 \cdot 3H_2O$ in 100 mL reagent water. Standardize by titrating with Na_2H_2EDTA. Alternatively, use commercially available 0.1 M $Pb(ClO_4)_2$ solutions.
c. Sulfide stock solution, 130 mg/L: See 4500-S^{2-} A.6, and dilute 13.0 mL of 1.00 mg/mL S^{2-} stock to 100.0 mL with AAR. Alternatively, add 500 mL AAR and 1 g $Na_2S \cdot 9H_2O$ to a 1-L volumetric flask; dissolve. Dilute to 1 L with DRW. Use deaerated artificial seawater (DASW), Table 8010:3, or 0.7 M NaCl if sulfide concentrations are to be determined in seawater. Standardize stock solution by titrating with 0.1 M $Pb(ClO_4)_2$. Pipet 50 mL sulfide stock solution into the electrochemical cell. (Use 10 mL with a small-volume polarographic cell.) Insert Ag/S electrode and reference electrode and read the initial potential. Titrate with 0.1 M $Pb(ClO_4)_2$. Let the electrode potential stabilize and record the potential after each addition. Locate equivalence point as in Section 4500-Cl^- D.4*a*. Alternatively, linearize the titration curve.[1] Calculate the function F_1 for points before the equivalence point.

$$F_1 = (V_0 + V)10^{\frac{E}{m}}$$

where:

V_0 = volume of stock solution (mL),
V = titrant volume (mL),
E = potential (mV), and
m = slope of calibration curve (mV/log unit).

Plot F_1 as a function of titrant volume. Extrapolate to find the intersection with the x-axis; that is, the equivalence point. Calculate sulfide concentration in the stock solution from:

$$C = \frac{V_{eq}[Pb]}{V_0}$$

where:

C = sulfide concentration (mg/L),
V_{eq} = equivalence volume (mL),
$[Pb]$ = concentration of Pb in titrant (mg/L), and
V_0 = volume of stock solution (mL).

Store stock solution in a tightly capped bottle for 1 week or less. The stock solution also can be standardized iodometrically (see 4500-S^{2-} F). **Caution: Store in a fume hood.**
d. Sulfide standards: Prepare sulfide standards daily by the serial dilution of stock. Add AAR and $Zn(C_2H_3O_2)_2$ solutions to 100-mL volumetric flasks. Add sulfide solutions and dilute to volume with DRW (or DASW). Refer to Table 4500-S^{2-}:1 for volumes. Prepare at least one standard with a concentration less than the lowest sample concentration.

4. Procedure

Check electrode performance and calibrate daily. Check electrode potential in a sulfide standard every 2 h. The procedure depends on the sulfide concentration and the time between sample collection and sulfide determination. If the total sulfide concentration is greater than 0.03 mg/L (1×10^{-6} M) and the time delay is only a few minutes, sulfide can be determined directly. Otherwise, precipitate ZnS and filter as described in 4500-S^{2-} C.
a. Check electrode performance: Pipet 50 mL AAR, 50 mL DWR, and 1 mL sulfide stock solution into the measurement cell. Place Ag/S and reference electrodes in the solution and read potential. Add 10 mL stock solution and read potential. The change in potential should be -28 ± 2 mV. If it is not, follow the troubleshooting procedure in the electrode manual.
b. Calibration: Place electrodes in the most dilute standard but use calibration standards that bracket the sulfide concentrations in the samples. Record the potential when the rate of change is less than 0.3 mV/min. [This may take up to 30 min for very low sulfide concentrations (i.e., <0.03 mg/L.)] Rinse electrodes, blot dry with a tissue, and read the potential of the next highest standard. For a meter that can be calibrated directly in concentration, follow the manufacturer's directions. For other meters, plot the potential

Table 4500-S^{2-}:1. Dilution of Sulfide Stock Solution for Preparation of Standards (100 mL Total Volume)

Dilution	Alkaline Antioxidant Reagent (mL)	Sulfide Solution	Sulfide Solution (mL)	1 M Zinc Acetate (mL)
1:10	45	Stock	10	0.15
1:100	50	Stock	1	0.15
1:1 000	45	1:100	10	0.14
1:10 000	50	1:100	1	0.15

as a function of the logarithm (base 10) of the sulfide concentration. For potentials in the linear range, calculate the slope and intercept of the linear portion of the calibration plot.

c. Sulfide determination by comparison with calibration curve, no ZnS precipitation: Add 40 mL AAR, 0.15 mL (3 drops) zinc acetate, and 50 mL sample to a 100-mL volumetric flask. Dilute to 100-mL with AAR. Pour into the electrochemical cell and insert the electrodes. Record the potential when the rate of change is less than 0.3 mV/min. Read the sulfide concentration from the calibration curve. Alternatively, for potentials in the linear range, calculate the sulfide concentration from:

$$S_{Tot} = 10^{\frac{E-b}{m}}$$

where:

E = electrode potential and
b and m = the intercept and slope of the calibration curve.

For a meter that can be calibrated directly in concentration, follow the manufacturer's directions.

d. Sulfide determination by comparison with calibration curve, with ZnS precipitation: Place the filter with the ZnS precipitate in a 150-mL beaker containing a stir bar. Wash the sample bottle with 50 mL AAR and 20 mL DRW and pour the washings into the beaker. Stir to dissolve precipitate. Remove the filter with forceps while rinsing it into the beaker with a minimum amount of DRW. Quantitatively transfer to a 100-mL volumetric flask and dilute to the mark with DRW. Pour into the electrochemical cell and place the electrodes in the solution. Measure potential as in paragraph *c* above. Calculate the sulfide concentration (¶ *c* above).

e. Sulfide determination by standard addition with or without ZnS precipitation: Measure the Ag/S-ISE electrode potential as in paragraphs *c* or *d* above. Add sulfide stock solution and measure the potential again. Calculate the sulfide concentration as follows:

$$C_o = \frac{fC_s}{(1+f)10^{\frac{E_s-E_o}{m}} - 1}$$

where:

C_0, C_s = sulfide concentrations in sample and known addition,
f = ratio of known-addition volume to sample volume,
E_0, E_s = potentials measured for sample and known addition, and
m = slope of calibration curve (approximately 28 mV/log S^{2-}).

f. Sulfide determination by titration: Use the same procedure as for standardizing the sulfide stock solution (4500-S^{2-} G.3*c*). The minimum sulfide concentration for determination by titration is 0.3 mg/L (10^{-5} M).

5. Precision

For sulfide determination by comparison with the calibration curve, the relative standard deviation varies with the sulfide concentration. RSD values of 23% for 0.0091 mg/L and 5% for 0.182 mg/L have been reported[2] [0.0091 µg/L was below the range for which the potential varied linearly with the logarithm of the sulfide concentration (i.e., the Nernstian range)]. For sulfide determination by standard addition, the precision is greatest if the amount of sulfide added is as large as possible while staying within the linear range.[3]

References

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4500-S^{2-} H. Calculation of Un-ionized Hydrogen Sulfide

Hydrogen sulfide and bisulfide ion, which together constitute dissolved sulfide, are in equilibrium with hydrogen ions:

$$H_2S \rightleftharpoons H^+ + HS^-$$

The fraction of sulfide present as un-ionized H_2S can be estimated with an error of less than 40% from Figure 4500-S^{2-}:3. If more accuracy is needed, use the methods given below. For both fresh water and seawater, it is convenient to define "conditional" dissociation constants, which are valid for the temperature and ionic strength of the water of interest. In the following mass-action equation for fresh water, K'_{FW} is a mixed equilibrium constant that relates the hydrogen ion activity (calculated from the pH) and the concentrations of H_2S and HS^-: