STATE OF MAINE
DEPARTMENT OF
ENVIRONMENTAL PROTECTION

JANET L. MILLS
GOVERNOR

MELANIE LOYZIM
COMMISSIONER

September 24, 2021

Ms. Jennifer Robinson
Compliance Officer
Cooke Aquaculture USA Inc.
P.O. Box 263, Estes Head Road
Eastport, ME. 04631
e-mail: jennifer.robinson@cookeaqua.com

RE: Maine Pollutant Discharge Elimination System (MEPDES) Permit
Maine Waste Discharge License (WDL)
Final General Permit/WDL Coverage Approval Modification

Dear Jennifer:

Enclosed please find a copy of your **final** General MEPDES permit and Maine WDL **modification** which was approved by the Department of Environmental Protection. Please read this permit modification and its attached conditions carefully. Compliance with this permit will protect water quality.

Any interested person aggrieved by a Department determination made pursuant to applicable regulations, may appeal the decision following the procedures described in the attached DEP FACT SHEET entitled "*Appealing a Commissioner's Licensing Decision*."

If you have any questions regarding the matter, please feel free to call me at 207-287-7693.

Your Department compliance inspector copied below is also a resource that can assist you with compliance. Please do not hesitate to contact them with any questions.

Thank you for your efforts to protect and improve the waters of the great state of Maine!

Sincerely,

Gregg Wood
Division of Water Quality Management
Bureau of Water Quality

Enc.

cc: Clarissa Trasko, MDEP/EMRO
Michael Loughlin MDEP/EMRO
Kayleigh Burda, MDEP/EMRO
Pamela Parker, MDEP/CMRO
Laura Crossley, MDEP/CMRO
Lori Mitchell, MDEP/CMRO
Marcy Nelson, MDMR
Ellen Weitzler, USEPA
Nathan Chien, USEPA
Richard Carvalho, USEPA
Alex Rosenberg, USEPA
Maine IFW Environmental Review
Maine Dept. DMR Environmental Review
David Bean, NOAA
Wende Mahaney, USFWS
Anna Harris, USFWS

DEP INFORMATION SHEET

Appealing a Department Licensing Decision

Dated: November 2018 **Contact: (207) 287-2452**

SUMMARY

There are two methods available to an aggrieved person seeking to appeal a licensing decision made by the Department of Environmental Protection's (DEP) Commissioner: (1) an administrative process before the Board of Environmental Protection (Board); or (2) a judicial process before Maine's Superior Court. An aggrieved person seeking review of a licensing decision over which the Board had original jurisdiction may seek judicial review in Maine's Superior Court.

A judicial appeal of final action by the Commissioner or the Board regarding an application for an expedited wind energy development (35-A M.R.S. § 3451(4)) or a general permit for an offshore wind energy demonstration project (38 M.R.S. § 480-HH(1)) or a general permit for a tidal energy demonstration project (38 M.R.S. § 636-A) must be taken to the Supreme Judicial Court sitting as the Law Court.

This information sheet, in conjunction with a review of the statutory and regulatory provisions referred to herein, can help a person to understand his or her rights and obligations in filing an administrative or judicial appeal.

I. ADMINISTRATIVE APPEALS TO THE BOARD

LEGAL REFERENCES

The laws concerning the DEP's *Organization and Powers*, 38 M.R.S. §§ 341-D(4) & 346; the *Maine Administrative Procedure Act*, 5 M.R.S. § 11001; and the DEP's *Rules Concerning the Processing of Applications and Other Administrative Matters* ("Chapter 2"), 06-096 C.M.R. ch. 2.

DEADLINE TO SUBMIT AN APPEAL TO THE BOARD

The Board must receive a written appeal within 30 days of the date on which the Commissioner's decision was filed with the Board. Appeals filed more than 30 calendar days after the date on which the Commissioner's decision was filed with the Board will be dismissed unless notice of the Commissioner's license decision was required to be given to the person filing an appeal (appellant) and the notice was not given as required.

HOW TO SUBMIT AN APPEAL TO THE BOARD

Signed original appeal documents must be sent to: Chair, Board of Environmental Protection, 17 State House Station, Augusta, ME 04333-0017. An appeal may be submitted by fax or e-mail if it contains a scanned original signature. It is recommended that a faxed or e-mailed appeal be followed by the submittal of mailed original paper documents. The complete appeal, including any attachments, must be received at DEP's offices in Augusta on or before 5:00 PM on the due date; materials received after 5:00 pm are not considered received until the following day. The risk of material not being received in a timely manner is on the sender, regardless of the method used. The appellant must also send a copy of the appeal documents to the Commissioner of the DEP; the applicant (if the appellant is not the applicant in the license proceeding at issue); and if a hearing was held on the application, any intervenor in that hearing process. All of the information listed in the next section of this information sheet must be submitted at the time the appeal is filed.

INFORMATION APPEAL PAPERWORK MUST CONTAIN

Appeal materials must contain the following information at the time the appeal is submitted:

1. *Aggrieved Status*. The appeal must explain how the appellant has standing to maintain an appeal. This requires an explanation of how the appellant may suffer a particularized injury as a result of the Commissioner's decision.
2. *The findings, conclusions, or conditions objected to or believed to be in error.* The appeal must identify the specific findings of fact, conclusions regarding compliance with the law, license conditions, or other aspects of the written license decision or of the license review process that the appellant objects to or believes to be in error.
3. *The basis of the objections or challenge.* For the objections identified in Item #2, the appeal must state why the appellant believes that the license decision is incorrect and should be modified or reversed. If possible, the appeal should cite specific evidence in the record or specific licensing requirements that the appellant believes were not properly considered or fully addressed.
4. *The remedy sought.* This can range from reversal of the Commissioner's decision on the license or permit to changes in specific permit conditions.
5. *All the matters to be contested.* The Board will limit its consideration to those matters specifically raised in the written notice of appeal.
6. *Request for hearing.* If the appellant wishes the Board to hold a public hearing on the appeal, a request for public hearing must be filed as part of the notice of appeal, and must include an offer of proof in accordance with Chapter 2. The Board will hear the arguments in favor of and in opposition to a hearing on the appeal and the presentations on the merits of an appeal at a regularly scheduled meeting. If the Board decides to hold a public hearing on an appeal, that hearing will then be scheduled for a later date.
7. *New or additional evidence to be offered.* If an appellant wants to provide evidence not previously provided to DEP staff during the DEP's review of the application, the request and the proposed evidence must be submitted with the appeal. The Board may allow new or additional evidence, referred to as supplemental evidence, to be considered in an appeal only under very limited circumstances. The proposed evidence must be relevant and material, and (a) the person seeking to add information to the record must show due diligence in bringing the evidence to the DEP's attention at the earliest possible time in the licensing process; or (b) the evidence itself must be newly discovered and therefore unable to have been presented earlier in the process. Specific requirements for supplemental evidence are found in Chapter 2 § 24.

OTHER CONSIDERATIONS IN APPEALING A DECISION TO THE BOARD

1. *Be familiar with all relevant material in the DEP record.* A license application file is public information, subject to any applicable statutory exceptions, and is made easily accessible by the DEP. Upon request, the DEP will make application materials available during normal working hours, provide space to review the file, and provide an opportunity for photocopying materials. There is a charge for copies or copying services.
2. *Be familiar with the regulations and laws under which the application was processed, and the procedural rules governing your appeal.* DEP staff will provide this information on request and answer general questions regarding the appeal process.
3. *The filing of an appeal does not operate as a stay to any decision.* If a license has been granted and it has been appealed, the license normally remains in effect pending the processing of the appeal. Unless a stay of the decision is requested and granted, a license holder may proceed with a project pending the outcome of an appeal, but the license holder runs the risk of the decision being reversed or modified as a result of the appeal.

WHAT TO EXPECT ONCE YOU FILE A TIMELY APPEAL WITH THE BOARD

The Board will formally acknowledge receipt of an appeal, and will provide the name of the DEP project manager assigned to the specific appeal. The notice of appeal, any materials accepted by the Board Chair as supplementary evidence, any materials submitted in response to the appeal, and relevant excerpts from the DEP's application review file will be sent to Board members with a recommended decision from DEP staff. The appellant, the license holder if different from the appellant, and any interested persons are notified in advance of the date set for Board consideration of an appeal or request for public hearing. The appellant and the license holder will have an opportunity to address the Board at the Board meeting. With or without holding a public hearing, the Board may affirm, amend, or reverse a Commissioner decision or remand the matter to the Commissioner for further proceedings. The Board will notify the appellant, the license holder, and interested persons of its decision.

II. JUDICIAL APPEALS

Maine law generally allows aggrieved persons to appeal final Commissioner or Board licensing decisions to Maine's Superior Court (see 38 M.R.S. § 346(1); 06-096 C.M.R. ch. 2; 5 M.R.S. § 11001; and M.R. Civ. P. 80C). A party's appeal must be filed with the Superior Court within 30 days of receipt of notice of the Board's or the Commissioner's decision. For any other person, an appeal must be filed within 40 days of the date the decision was rendered. An appeal to court of a license decision regarding an expedited wind energy development, a general permit for an offshore wind energy demonstration project, or a general permit for a tidal energy demonstration project may only be taken directly to the Maine Supreme Judicial Court. See 38 M.R.S. § 346(4).

Maine's Administrative Procedure Act, DEP statutes governing a particular matter, and the Maine Rules of Civil Procedure must be consulted for the substantive and procedural details applicable to judicial appeals.

ADDITIONAL INFORMATION

If you have questions or need additional information on the appeal process, for administrative appeals contact the Board's Executive Analyst at (207) 287-2452, or for judicial appeals contact the court clerk's office in which your appeal will be filed.

Note: The DEP provides this INFORMATION SHEET for general guidance only; it is not intended for use as a legal reference. Maine law governs an appellant's rights.

STATE OF MAINE
DEPARTMENT OF ENVIRONMENTAL PROTECTION
17 STATE HOUSE STATION AUGUSTA, MAINE 04333-0017

DEPARTMENT ORDER

IN THE MATTER OF

COOKE AQUACULTURE USA INC.) GENERAL PERMIT #MEG130000
NET PEN AQUACULTURE)
GENERAL PERMIT COVERAGE APPROVAL) **MODIFICATION**

NET PEN SITES

W009116-6H-B-M	MEG130029	SAND COVE (EASTW SCN)	BEALS
W009117-6H-B-M	MEG130030	SPECTACLE ISLAND ((EASTW SI)	BEALS
W008228-6H-D-M	MEG130017	CUTLER WEST (MACH CW2)	CUTLER
W009112-6H-B-M	MEG130025	CROSS ISLAND (MACH CI2)	CUTLER
W009114-6H-B-M	MEG130027	CROSS ISLAND NORTH (MACH CIN)	CUTLER
W009040-6H-C-M	MEG130018	DEEP COVE (COB DC)	EASTPORT
W009115-6H-B-M	MEG130028	BROAD COVE (COB BC)	EASTPORT
W009065-6H-C-M	MEG130023	BLACK ISLAND SOUTH (SWAN BIS)	FRENCHBORO
W009113-6H-B-M	MEG130026	BLACK ISLAND (SWAN BI)	FRENCHBORO
W009147-6H-B-M	MEG130032	CALF ISLAND (EASTW CALF)	JONESPORT
W009042-6H-C-M	MEG130020	SOUTH BAY (COB SB)	LUBEC
W008165-6H-E-M	MEG130001	STARBOARD ISLAND (MACH II)	MACHIASPORT
W009066-6H-C-M	MEG130024	SCRAG ISLAND (SWAN HS)	SWANS ISLAND

In compliance with the applicable provisions of *Pollution Control*, 38 M.R.S. §§ 411 – 424-B, *Water Classification Program*, 38 M.R.S. §§ 464 – 470, *Federal Water Pollution Control Act*, Title 33 U.S.C. § 1251, applicable rules of the Maine Department of Environmental Protection (Department) and in consideration of supportive data, agency review comments and other related materials on file, the Department has considered the modification of the Notice of Intent documents submitted by COOKE AQUACULTURE USA INC. (Cooke) for thirteen (13) net pen sites covered under *General Permit – Net Pen Aquaculture*, #MEG130000, issued by the Department on April 10, 2014, and FINDS THE FOLLOWING FACTS.

APPLICATION SUMMARY AND AUTHORIZED DISCHARGES

In April 2014, May 2014 and April 2016, the Department received, as complete for processing, Notice of Intent forms from Cooke for coverage under *General Permit – Net Pen Aquaculture*, #MEG130000 for the thirteen sites listed above. The *General Permit – Net Pen Aquaculture* issued by the Department on April 10, 2014 expired on April 10, 2019 but has been administratively carried forward by the Department until a new *General Permit – Net Pen Aquaculture* is issued. See *Maine Administrative Procedure Act*, 5 M.R.S.A. § 10002, *Rules Concerning the Processing of Applications and Other Administrative Matters*, 06-096 CMR 2(21)(A) (last amended August 25, 2013), and *General Permits for Certain Wastewater Discharges*, 06-096 CMR 529(3)(c) (last amended June 27, 2007).

APPLICATION SUMMARY AND AUTHORIZED DISCHARGES (cont'd)

In the NOI modifications submitted on June 24, 2021, Cooke is requesting the Department formally sanction the use of SM 4500-S^{2-}E in the 18th edition of Standard Methods and as noted in SM 4500-S^{2-}F in the 21st edition of Standard Methods along with the modifications contained in a document entitled, *Maine Department of Environmental Protection Marine Sediment Sampling Guidance* dated July 25, 2017 for the sampling and analysis for sulfides as required by Special Condition I(3) of the General Permit.

CONCLUSIONS

1. The discharge, either by itself or in combination with other discharges, will not lower the quality of any classified body of water below such classification.

2. The discharge, either by itself or in combination with other discharges, will not lower the quality of any unclassified body of water below the classification which the Department expects to adopt in accordance with state law.

3. The provisions of the State's antidegradation policy, 38 M.R.S. Section 464(4)(F), will be met, in that:

   (a) Existing in-stream water uses and the level of water quality necessary to protect and maintain those existing uses will be maintained and protected;

   (b) Where high quality waters of the State constitute an outstanding natural resource, that water quality will be maintained and protected;

   (c) Where the standards of classification of the receiving water body are met or not met, the discharge will not cause or contribute to the failure of the water body to meet the standards of classification;

   (d) Where the actual quality of any classified receiving water body exceeds the minimum standards of the next highest classification, that higher water quality will be maintained and protected; and

   (e) Where a discharge will result in lowering the existing quality of any water body, the Department has made the finding, following opportunity for public participation, that this action is necessary to achieve important economic or social benefits to the State.

ACTION

Based on the findings and conclusions as stated above, the Department APPROVES Cooke Aquaculture USA Inc. request to utilize SM 4500-S^{2-}F(2000) in the 21st edition of Standard Methods along with the modifications contained in a document entitled, *Maine Department of Environmental Protection Marine Sediment Sampling Protocols, Cooke Aquaculture, Effective Starting 2021,* dated August, 2021, (Attachment A of this modification) for the sampling and analysis for sulfides as required by Special Condition I of the General Permit for all thirteen net pen facilities cited on page one of this modification.

Authorization to discharge under this approval becomes effective upon the date of signature below and expires on the effective date of the next *General Permit – Net Pen Aquaculture* renewal.

DONE AND DATED AT AUGUSTA, MAINE THIS 24 DAY OF September, 2021.

DEPARTMENT OF ENVIRONMENTAL PROTECTION

BY:_____
for Melanie Loyzim, Commissioner

PLEASE NOTE ATTACHED SHEET FOR GUIDANCE ON APPEAL PROCEDURES

Application received: June 24, 2021

Application accepted: June 24, 2021

FILED

SEPT 24, 2021

State of Maine
Board of Environmental Protection

Date filed with Board of Environmental Protection

This Order prepared by Gregg Wood, BUREAU OF LAND & WATER QUALITY

4500-S^{2-} SULFIDE*

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 threshhold 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 (Section 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.

---

* Approved by Standard Methods Committee, 2000.

Joint Task Group: (4500-S^{2-}.A, H, I, J)—Thomas R. Holm (chair), Robert P. Fisher, Martin S. Frant, Christian Gagnon, Lorne R. Goodwin; 20th Edition (4500-S^{2-}.I)—Scott Stieg (chair), Bradford R. Fisher, Owen B. Mathre, Theresa M. Wright.

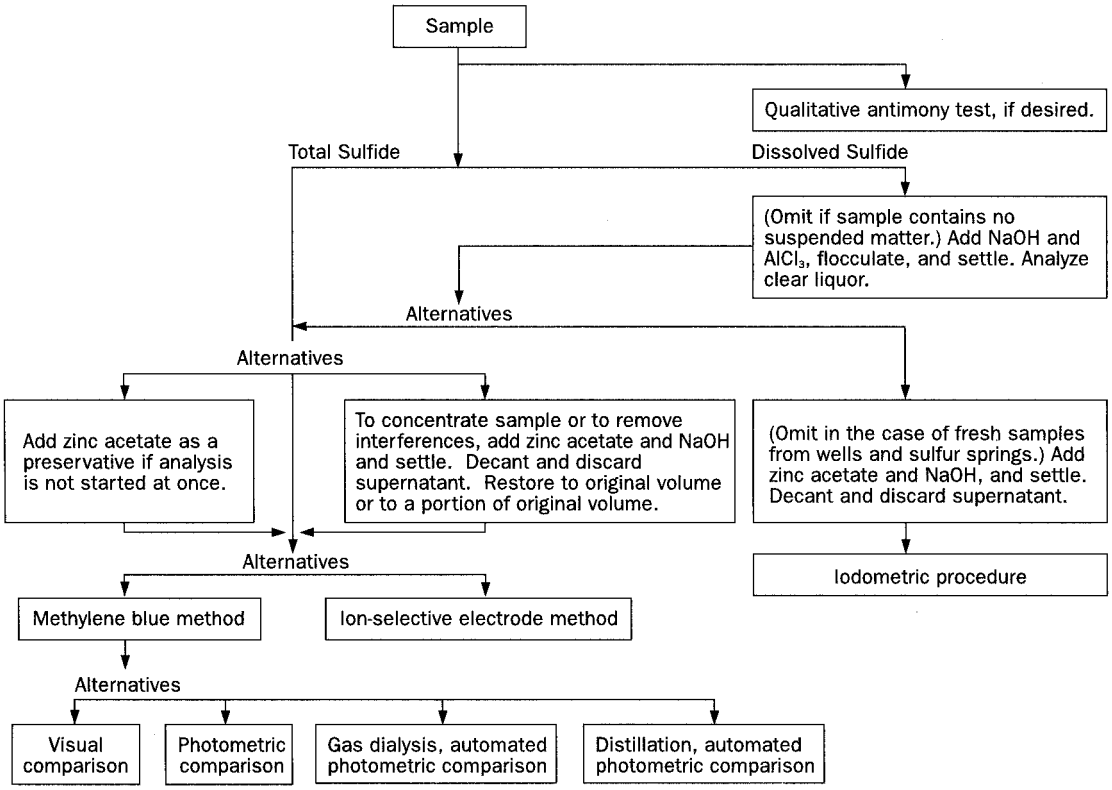


Figure 4500-S^{2-}:1. Analytical flow paths for sulfide determination.

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 sample bottle before filling it with sample. Use 0.2 mL 2*M* zinc acetate solution per 100 mL sample. Increase 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 bottle completely and stopper.

Sample sediments and sludges under nitrogen atmosphere if possible. Store 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 (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 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.

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 formation of PbS. A strip of silver foil is more sensitive than lead acetate paper. Clean the silver by dipping in NaCN solution and rinse. 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. The iodometric method (F) is useful for standardizing the methylene blue colorimetric methods (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 (I) is useful for a variety of samples containing more than 1 mg S^{2-}/L.

The methylene blue method (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 (E) is similar to Method 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.

Potentiometric methods utilizing a silver/sulfide ion-selective electrode (G) may be suitable. The sulfide concentration can be estimated from 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 end-point indicator for titration of 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.

† Many substances can reduce iodine; all of these are potential interferences in procedures using this chemistry.

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 (polysulfides, polythionates, and sulfate) of sulfide 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 reagent bottle with nonmetallic tweezers; quickly rinse in degassed reagent water to remove surface contamination. Blot crystal dry with a tissue, then rapidly transfer to a tared, stoppered weighing bottle containing 5 to 10 mL degassed reagent water. Repeat procedure until desired amount of sodium sulfide is in weighing bottle. Avoid excess agitation and mixing of the solution with atmospheric oxygen. Quantitatively transfer and dilute entire contents of 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 will give a stock solution of which 1.00 mL = 1.00 mg S^{2-}). Standardize stock solution using the iodometric method, Section 4500-S^{2-}. F. Alternatively, purchase precertified stock solutions of sulfide. Verify concentration of stock solution daily using the iodometric method (F). Store stock solution with minimum headspace for no more than 1 week.

7. Bibliography

CRUSE, H. & R.D. POMEROY. 1969. Hydrogen sulfide odor threshold. *J. Amer. Water Works Assoc.* 61:677.

KARCHMER, J.H., ed. 1970. The Analytical Chemistry of Sulfur and Its Compounds. Wiley-Interscience, New York, N.Y.

NICKLESS, G., ed. 1970. Inorganic Sulphur Chemistry. Elsevier Publ., Amsterdam, The Netherlands.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1974. Process Design Manual for Sulfide Control in Sanitary Sewerage Systems. Publ. 625/1-74-005.

BAGARINAO, T. 1992. Sulfide as an environmental factor and toxicant: Tolerance and adaptations in aquatic organisms. *Aquat. Toxicol.* 24:21.

BUTLER, I.B., M.A.A. SCHOONEN & D.T. RICKARD. 1994. Removal of dissolved oxygen from water: a comparison of four common techniques. *Talanta* 41:211.

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 distilled water.

3. Procedure

a. To a 100-mL glass bottle add 0.2 mL (nominally 4 drops) 6*N* NaOH. Fill bottle with sample and immediately add 0.2 mL (4 drops) $AlCl_3$ solution. Stopper bottle with no air under 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 Section 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 (D) by preventing formation of the blue color. Thiosulfate at concentrations 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 distilled water. Use the same procedure, even when not needed for removal of interferences, to concentrate sulfide. The automated methylene blue method (E) is relatively free from interferences because gas dialysis separates the sulfide from the sample matrix.

1. Apparatus

Glass bottles with stoppers: See Section 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 air bubbles under stopper 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 volume of reagents added according to sample so that the resulting precipitate is not excessively bulky and settles readily. Add enough NaOH to raise the pH above 9. Let 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 precipitate on a glass fiber filter and continue at once with titration according to the procedure of Method F. If the methylene blue method (D) is used, let precipitate settle for 30 min and decant as much supernatant as possible without loss of precipitate. Refill bottle with distilled water, shake to resuspend 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 volume to one-half or one-fifth of 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 Method E.

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

1. Apparatus

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

b. Droppers, delivering 20 drops/mL methylene blue solution. To obtain uniform drops hold 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 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 distilled water. Cool and dilute to 100 mL with distilled 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 distilled water.

f. Methylene blue solution I: Use USP grade dye or one certified by the Biological Stain Commission. The dye content should be reported on the label and should be 84% or more. Dissolve 1.0 g in distilled water and make up 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 that 0.05 mL (1 drop) = 1.0 mg sulfide/L.

Standardization—Prepare five 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 solution into another vessel. This solution reacts slowly with oxygen but the change is insignificant if analysis is performed within a few hours. Prepare solution daily. To 1 L distilled 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 five tests have been made, with a range of sulfide concentrations between 1 and 8 mg/L. Calculate average percent error of the methylene blue result as compared to the iodometric result. If the average error is negative, that is, methylene blue results are lower than iodometric results, dilute methylene blue solution by the same percentage, so that a greater volume will be used in matching colors. If methylene blue results are high, increase 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 two matched test tubes, using a special wide-tip pipet or filling to marks on test tubes. If 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-} will be indicated by the appearance of blue color in Tube A. Color development usually is complete in about 1 min, but a longer time often is required for 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 color matches that developed in first tube. If the concentration exceeds 20 mg/L, repeat test with a portion of sample diluted tenfold.

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

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

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 instrument with a portion of treated sample from Tube B. Prepare calibration curves on 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 sulfide concentration from calibration curve.

* Eastman catalog No. 5672 has been found satisfactory for this purpose.

4. Precision and Bias

In a study by two 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. Bibliography

POMEROY, R.D. 1936. The determination of sulfides in sewage. *Sewage Works J.* 8:572.

NUSBAUM, I. 1965. Determining sulfides in water and waste water. *Water Sewage Works* 112:113.

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

1. Apparatus

a. 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.

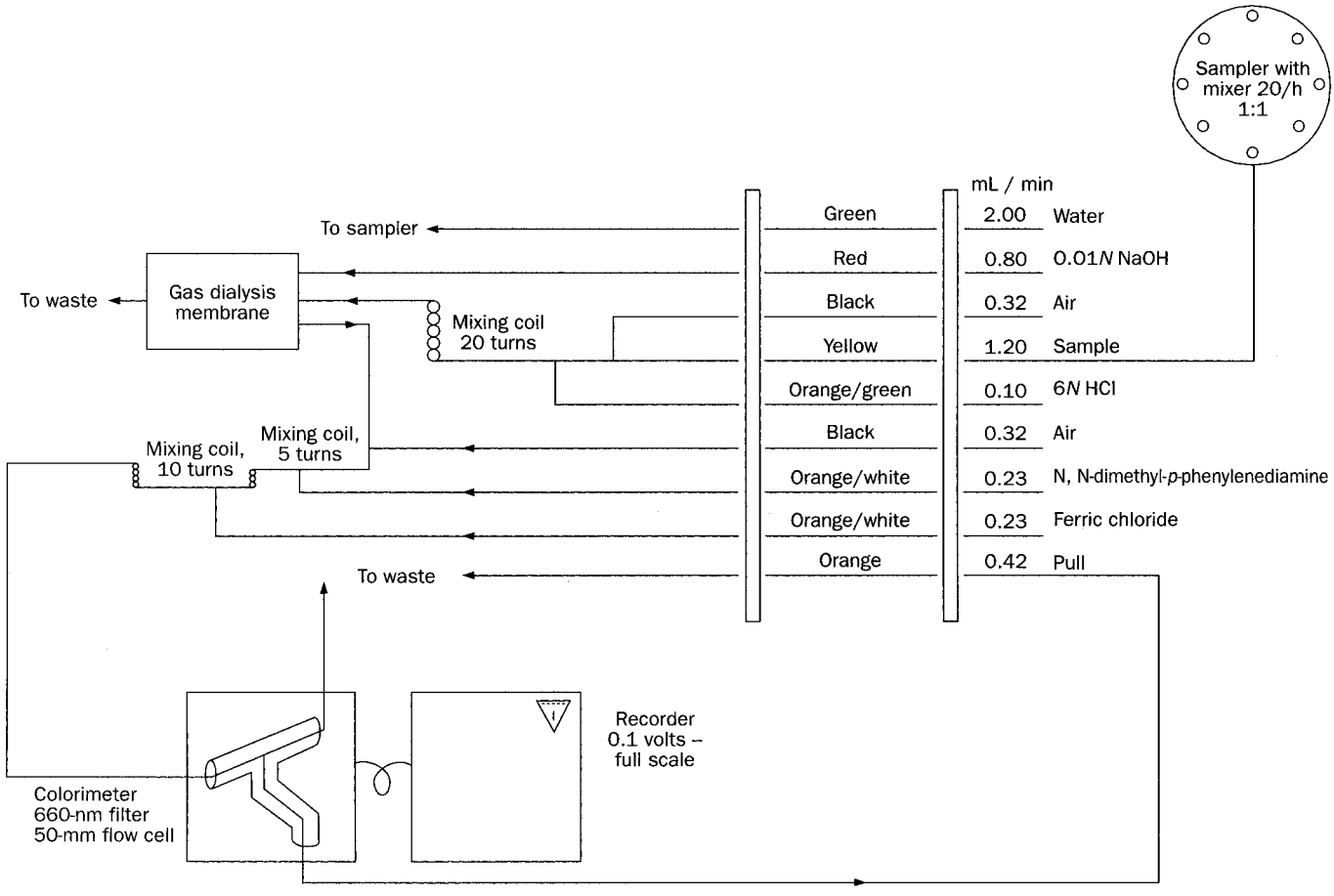


Figure 4500-S^{2-}:2. Sulfide manifold.

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 ≈ 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 standardization value from ¶ 2*i* to determine exact concentration. 1.00 mL ≈ 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/100 mL. For preserved samples, add 0.5 mL 6*N* NaOH and 400 mg ascorbic acid/100 mL. Shake well.

Let 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 sample response with 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 two environmental samples with added S^{2-}, recoveries were 104.2% and 97.6%.

6. Bibliography

FRANCOM, D., L.R. GOODWIN & F.P. DIEKEN. 1989. Determination of low level sulfides in environmental waters by automated gas dialysis/methylene blue colorimetry. *Anal. Lett.* 22:2587.

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 distilled water, if necessary, to bring volume to about 20 mL. Add 2 mL 6*N* HCl. Pipet 200 mL sample into flask, discharging sample under solution surface. If iodine color disappears, add more iodine until color remains. Back-titrate with $Na_2S_2O_3$ solution, adding a few drops of starch solution as end point is approached, and continuing until blue color disappears.

b. If sulfide was precipitated with zinc and ZnS filtered out, return filter with precipitate to original bottle and add about 100 mL water. Add iodine solution and HCl and titrate as in ¶ 2*a* above.

3. Calculation

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

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

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 end point 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.

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

1. General Discussion

a. Principle: The potential of a silver/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 ($1 \times 10^{-6}M$) and 100 mg/L can be measured without preconcentration. For lower concentrations, preconcentration is necessary.

b. Interferences: Humic substances may interfere with Ag/S-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 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).

2. Apparatus

*a. Silver/sulfide 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 suitable cell from a 150-mL beaker and a sheet of rigid plastic (PVC or acrylic) with holes drilled to allow 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 will range from colorless to yellow. Store in a tightly capped brown glass bottle. Discard when 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 S^{2-}/mL 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:III, 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 initial potential. Titrate with 0.1*M* $Pb(ClO_4)_2$. Let electrode potential stabilize and record 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.

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

where:

V_o = 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:

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

where:

C = sulfide concentration, mg/L,
V_{eq} = equivalence volume, mL,
$[Pb]$ = concentration of Pb in titrant, mg/L, and
V_o = 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 Section 4500-S^{2-}.E). CAUTION: *Store in a fume hood.*

d. Sulfide standards: Prepare sulfide standards daily by 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-}:I 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

* Orion 941600 or equivalent.
† Orion 948201 or equivalent.
‡ EG&G Princeton Applied Research K0066, K0060, G0028, or equivalent.

TABLE 4500-S^{2-}:I. 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

sample collection and sulfide determination. If the total sulfide concentration is greater than 0.03 mg/L ($1 \times 10^{-6}M$) and the time delay is only a few minutes, sulfide can be determined directly. Otherwise, precipitate ZnS and filter as described in Section 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 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., less than 0.03 mg/L.) Rinse electrodes, blot dry with a tissue, and read potential of the next highest standard. For a meter that can be calibrated directly in concentration, follow manufacturer's directions. For other meters, plot potential 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 potential when the rate of change is less than 0.3 mV/min. Read 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 are 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 filter with ZnS precipitate in a 150-mL beaker containing a stir bar. Wash sample bottle with 50 mL AAR and 20 mL DRW and pour the washings into the beaker. Stir to dissolve precipitate. Remove 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 mark with DRW. Pour into the electrochemical cell and place the electrodes in the solution. Measure potential as in ¶ 4*c* above. Calculate sulfide concentration (¶ 4*c*).

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

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

where:

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

f. Sulfide determination by titration: Use the same procedure as for standardizing the sulfide stock solution (¶ 3*c*). The minumum 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]

6. References

1. GRAN, G. 1952. Determination of the equivalence point in potentiometric titrations. Part II. *Analyst* 77:661.
2. BAUMANN, E. 1974. Determination of parts per billion sulfide in water with the sulfide-selective electrode. *Anal. Chem.* 46:1345.
3. RATZLAFF, K.L. 1979. Optimizing precision in standard addition measurement. *Anal. Chem.* 51:232.

7. Bibliography

ORION RESEARCH, INC. 1980. Instruction Manual for Silver-Sulfide Electrode.

VIVIT, D.V., J.W. BALL & E.A. JENNE. 1984. Specific-ion electrode determinations of sulfide preconcentrated from San Francisco Bay waters. *Environ. Geol. Water Sci.* 6:79.

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

Hydrogen sulfide (H_2S) and bisulfide ion (HS^-), 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^-:

$$K_{FW} = \frac{\{H^+\}\,[HS^-]}{[H_2S]}$$

The square brackets indicate concentrations and the braces indicate activity. The value of pK_{FW} for H_2S is approximately 7.0 ± 0.3 for the ionic strengths and temperatures likely to be encountered in water-quality monitoring. For seawater, it is convenient to use a stoichiometric equilibrium constant (K_{SW}), which relates the concentrations of H^+, HS^-, and H_2S:

$$K_{SW} = \frac{[H^+]\,[HS^-]}{[H_2S]}$$

The mass-action equations can be rearranged to give:

$$pH - pK = \log\frac{[HS^-]}{[H_2S]}$$

In this equation, pK can be either pK_{FW} or pK_{SW}. The fraction of un-ionized H_2S can either be read from Figure 4500-S^{2-}:3 or calculated with the following equation:

$$\alpha_{H_2S} = \frac{[H_2S]}{S_T} = \frac{1}{10^{pH - pK} + 1}$$

where:

S_T = total dissolved sulfide concentration.

1. Calculation for Fresh Water ($I \leq 0.01M$)

Calculate ionic strength I as in Table 2330:I. Read value of pK_{FW} from Table 4500-S^{2-}:II.

Sample calculation: Total sulfide concentration, 1.5 mg S^{2-}/L; pH, 6.87; temperature, 10°C; ionic strength, 0.04. From Table 4500-S^{2-}:II, $pK_{FW} = 7.11$.

$$pH - pK_{FW} = -0.24$$

$$10^{pH - pK_{FW}} = 10^{-0.24} = 0.575$$

$$\alpha_{H_2S} = \frac{1}{1 + 0.575} = 0.63$$

$$0.63 \times 1.5 = 0.95$$

The concentration of un-ionized H_2S is 0.95 mg S^{2-}/L.

2. Calculation for Seawater, Estuarine Water, and Brackish Water

This procedure is the same as that for fresh water. A potential source of error is the determination of the hydrogen ion concentration. If the pH electrode is calibrated using NIST buffers as in Section 4500-H^+, then a correction factor[2] must be determined. Add acid (HNO_3, HCl, or $HClO_4$, not H_2SO_4) to artificial seawater diluted to the salinity of interest and at the temperature of interest to give an acid concentration of 0.001*N*. (Prepare artificial seawater as in Table 8010:III, substituting NaCl for Na_2SO_4 on an equimolar basis and omitting NaF, $SrCl_2 \cdot 6H_2O$, H_3BO_3, KBr, $Na_2SiO_3 \cdot 9H_2O$, Na_4EDTA, and $NaHCO_3$.) Measure the pH. The difference between the negative logarithm of the known acid concentration and the measured pH is the correction factor. For example, if the acid concentration is 0.001*N* and the measured pH is 3.15, the correction factor is 0.15. Subtract 0.15 from measured pH values to get p^{c}H, the negative logarithm of the hydrogen ion *concentration.* (The pH in fresh water corresponds to the negative logarithm of the hydrogen ion *activity.*) Alternatively, calibrate the pH electrode with Tris* buffer in artificial seawater diluted to the salinity of interest and at the temperature of interest.[3] Read pK_{SW} from Table 4500-S^{2-}:III. Calculate the fraction of un-ionized H_2S as for fresh water.

Sample calculation: Total sulfide concentration, 1.5 mg S^{2-}/L; temperature 10°C; pH, 7.15; salinity 25‰. From Table 4500-S^{2-}: III, $pK_{SW} = 6.87$.

$$pH - pK_{SW} = 0.28$$

$$10^{pH - pK_{SW}} = 10^{0.28} = 1.91$$

$$\alpha_{H_2S} = \frac{1}{1 + 1.91} = 0.34$$

$$0.34 \times 1.5 = 0.51$$

The concentration of un-ionized H_2S is 0.51 mg S^{2-}/L.

3. References

1. Millero, F. J. 1986. The thermodynamics and kinetics of the hydrogen sulfide system in natural waters. *Mar. Chem.* 18:121.
2. Sigel, H., A. D. Zuberbuhler & O. Yamauchi. 1991. Comments on potentiometric pH titrations and the relationship between pH-meter reading and hydrogen ion concentration. *Anal. Chim. Acta.* 255:63.
3. Millero, F. J. 1986. The pH of estuarine waters. *Limnol. Oceanogr.* 31:839.

* Trishydroxymethylaminomethane.

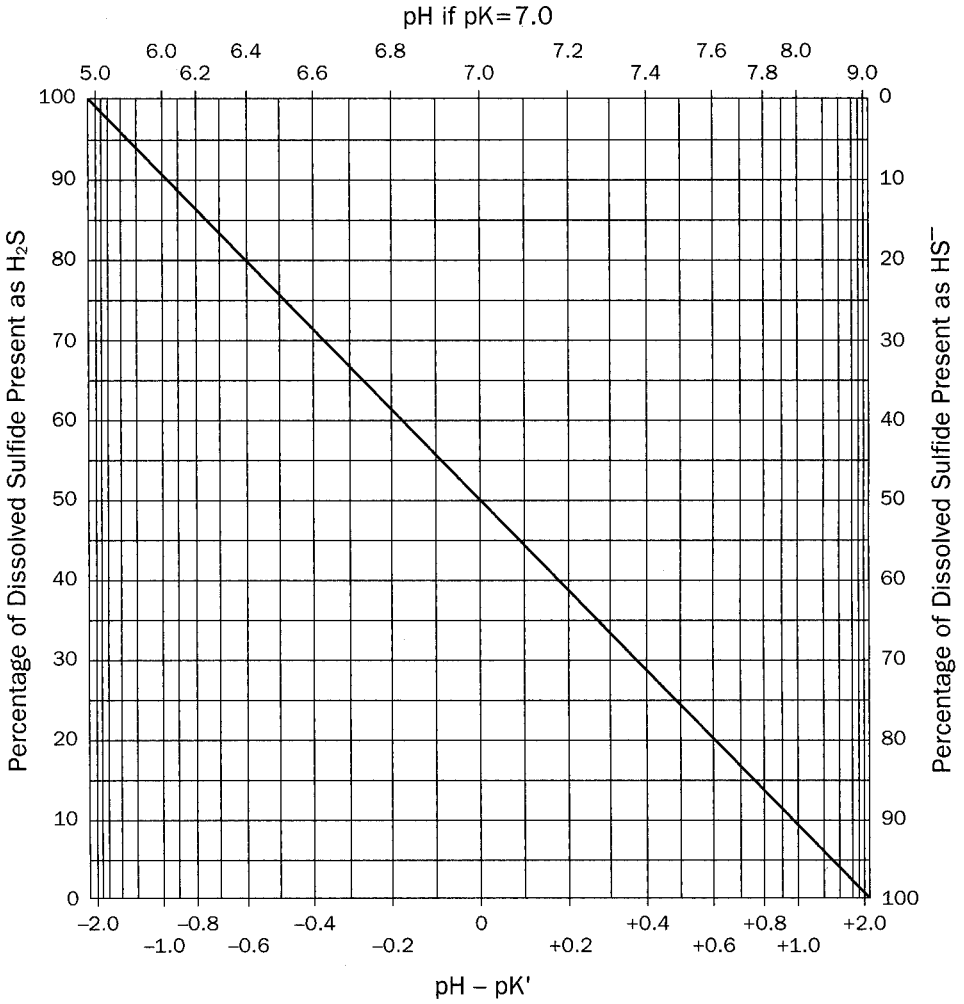


Figure 4500-S^{2-}:3. Proportion of H_2S and HS^- in dissolved sulfide.

TABLE 4500-S^{2-}:II. CONDITIONAL FIRST DISSOCIATION CONSTANT OF HYDROGEN SULFIDE, FRESH WATER*

Temperature	pK_{FW} at Given Ionic Strength						
°C	0.00 mol/L	0.005 mol/L	0.01 mol/L	0.02 mol/L	0.03 mol/L	0.05 mol/L	0.10 mol/L
0	7.36	7.33	7.32	7.30	7.29	7.27	7.24
5	7.28	7.25	7.23	7.22	7.21	7.19	7.16
10	7.20	7.16	7.15	7.13	7.12	7.10	7.07
15	7.12	7.09	7.08	7.06	7.05	7.03	7.00
20	7.05	7.02	7.00	6.99	6.97	6.96	6.92
25	6.98	6.95	6.94	6.92	6.91	6.89	6.86
30	6.92	6.89	6.87	6.86	6.84	6.83	6.79

* Values calculated according to Millero[1].

TABLE 4500-S^{2-}: III. CONDITIONAL FIRST DISSOCIATION CONSTANT OF HYDROGEN SULFIDE, SEAWATER*

Temperature	pK'_{SW} at Given Salinity						
°C	5‰	10‰	15‰	20‰	25‰	30‰	35‰
0	7.17	7.12	7.09	7.07	7.06	7.06	7.06
5	7.08	7.02	6.99	6.97	6.96	6.96	6.96
10	6.99	6.93	6.90	6.88	6.87	6.86	6.86
15	6.91	6.85	6.82	6.80	6.78	6.78	6.77
20	6.83	6.77	6.74	6.72	6.70	6.69	6.69
25	6.76	6.70	6.66	6.64	6.63	6.62	6.61
30	6.70	6.63	6.60	6.57	6.56	6.55	6.54

* Values calculated according to Millero[1].

4. Bibliography

D'YACHKOVA, I. B. & I. L. KHODAKOVSKIY. 1968. Thermodynamic equilibria in the systems S-H_2O, Se-H_2O and Te-H_2O in the 25–300°C temperature range and their geochemical interpretations. *Geochem. Internat.* 1108.

ARCHER, D. G. & P. WANG. 1990. The dielectric constant of water and Debye-Huckel limiting law slopes. *J. Phys. Chem. Ref. Data* 12: 817.

STUMM, W. & J. J. MORGAN. 1991. Aquatic Chemistry, 3rd ed. John Wiley & Sons, New York, N.Y.

4500-S^{2-} I. Distillation, Methylene Blue Flow Injection Analysis Method

1. General Discussion

a. Principle: Water and wastewater samples are distilled into a sodium hydroxide trapping solution and the distillate is analyzed. Hydrogen sulfide (H_2S) reacts in acid media and in the presence of ferric chloride with two molecules of *N*, *N*-dimethyl-*p*-phenylenediamine to form methylene blue. The resulting color is read at 660 nm.

b. Sample preservation: Because H_2S oxidizes rapidly, analyze samples and standards without delay. To preserve samples, add 4 drops 2*M* zinc acetate to 100 mL sample and adjust pH to >9 with 6*M* NaOH, then cool to 4°C. Samples are distilled into a trapping solution resulting in 0.25*M* NaOH matrix.

Also see Sections 4500-S^{2-}.A, B, and E, and Section 4130, Flow Injection Analysis (FIA).

c. Interferences: This method measures total sulfide, which is defined as the acid-soluble sulfide fraction of a sample. Total sulfide includes both acid-soluble sulfides such as H_2S, and acid-soluble metal sulfides present in suspended matter. This method does not measure acid-insoluble sulfides such as CuS.

Most nonvolatile interferences are eliminated by distillation. Strong reducing agents inhibit color formation at concentrations of several hundred milligrams per liter. Iodide interferes at concentrations greater than 2 mg I/L.

Also see Section 4500-S^{2-}.A and B.

2. Apparatus

a. Distillation apparatus consisting of a glass or polypropylene micro-distillation device* capable of distilling 6 mL or more of sample into a 0.25*M* NaOH final concentration trapping solution.

b. Flow injection analysis equipment consisting of:

1) *FIA injection valve* with sample loop or equivalent.

2) *Multichannel proportioning pump.*

3) *FIA manifold* (Figure 4500-S^{2-}:4) with cation exchange column and flow cell. Relative flow rates only are shown in Figure 4500-S^{2-}:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

4) *Absorbance detector,* 660 nm, 10-nm bandpass.

5) *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min.

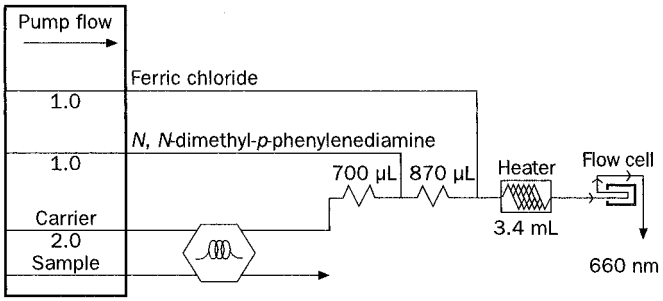


Figure 4500-S^{2-}:4. FIA sulfide manifold.

* Lachat Instruments MICRO DIST or equivalent.

a. Sodium hydroxide diluent, NaOH, 0.25*M:* In a 2-L volumetric flask, dissolve 20 g NaOH in approximately 1800 mL water. Dilute to mark and mix with a magnetic stirrer until dissolved. Store in a plastic container.

b. Hydrochloric acid, HCl, 3*M:* To a tared 1-L container, add 752 g water and then slowly add 295 g conc HCl. Invert to mix.

c. Hydrochloric acid, HCl, 0.20*M:* To a tared 1-L container, add 983.5 g water. Then add 19.7 g conc HCl. Invert to mix.

d. N,N-*dimethyl*-p-*phenylenediamine:* In a 1-L volumetric flask dissolve 1.0 g *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride, $(CH_3)_2NC_6H_4NH_2 \cdot 2HCl$, in about 800 mL 3*M* HCl (¶ 3*b*). Dilute to mark and invert to mix. If solution appears dark, it is likely that the *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride is decomposed; discard, and use fresh reagent.

e. Ferric chloride: In a 500-mL volumetric flask dissolve 6.65 g ferric chloride hexahydrate, $FeCl_3 \cdot 6H_2O$, in about 450 mL 0.20*M* HCl (¶ 3*c*). Dilute to mark with water and invert to mix.

f. Stock sulfide standard, 100 mg S^{2-}/L: In a 1-L volumetric flask dissolve 0.750 g sodium sulfide nonahydrate, $Na_2S \cdot 9H_2O$, in approximately 900 mL NaOH diluent (¶ 3*a*). Dilute to mark and invert to mix. Standardize as in 4500-S^{2-}.F; also see 4500-S^{2-}.A.6.

g. Standard solutions: Prepare sulfide standards in desired concentration range, using stock standard (¶ 3*f*), and diluting with NaOH diluent (¶ 3*a*).

h. Sulfuric acid distillation releasing solution, H_2SO_4, 9*M:* To a tared 500-mL container, add 150.0 g water, then add slowly while swirling, in increments of 40 g, 276 g conc H_2SO_4. CAUTION: *Solution will become very hot.* Allow to cool before using.

4. Procedure

a. Distillation: This procedure is designed for the determination of sulfides in aqueous solutions, solid waste materials, or effluents. To preserve and remove sulfide from interfering substances, distill samples immediately after collection.

Follow manufacturer's instructions for use of distillation apparatus. Add sufficient 9*M* H_2SO_4 (¶ 3*h*) to sample to dissolve ZnS (s), digest total sulfides, and release the sulfide as hydrogen sulfide gas. Immediately place sample on-line with the receiving vessel or collector tube and distill hydrogen sulfide and water in the sample into a 0.25*M* trapping solution.

b. Flow injection analysis: Set up a manifold equivalent to that in Figure 4500-S^{2-}:4 and follow 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 absorbance of standards processed through the manifold versus sulfide concentration.

6. Precision and Bias (MDL)

a. Method detection level (MDL): A 200-μL sample loop was used in the method described above. Using a published method[1], analysts ran 21 replicates of 0.02-mg S^{2-}/L standard. These gave a mean of 0.024 mg S^{2-}/L, a standard deviation of 0.0021 mg S^{2-}/L, and MDL of 0.006 mg S^{2-}/L.

b. Precision: Ten injections of a distilled 0.8 mg S^{2-}/L standard gave a mean of 0.82 mg S^{2-}/L, a standard deviation of 0.0054 mg S^{2-}/L, and percent relative standard deviation of 0.66.

7. Reference

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

4500-S^{2-} J. Acid-Volatile Sulfide

1. Apparatus

See Figure 4500-S^{2-}:5.

a. Reaction vessel, 250-mL, 3-neck flask, standard taper.

b. Gas traps, 125-mL gas scrubbers.

c. Dropping funnel, standard taper to fit reaction vessel.

d. Purge-gas tube, standard taper to fit reaction vessel.

e. Tubing, TFE or polypropylene, 0.635 cm (0.25-in.) OD, to connect reaction vessel to gas traps.

f. Syringe, plastic, 5 mL, lower end cut off to inject sediment into reaction vessel.

g. Compressed gas, cylinder of high-purity nitrogen, regulator, needle valve, rotameter or flow meter (optional).

2. Reagents

a. Hydrochloric acid solution, 9*N:* Add 186 mL conc HCl to about 50 mL distilled or deionized water. Dilute to 250 mL.

b. Stannous chloride solution, 0.53*M :* Dissolve 50 g $SnCl_2$ in 250 mL 9*N* HCl solution.

c. Sodium hydroxide solution, NaOH, 2*N.*

d. Zinc acetate solution, 1*M*, 22%: Dissolve 220 g $Zn(CH_3COO)_2 \cdot 2H_2O$ in 1 L distilled or deionized water.

e. Alkaline zinc solution: Add 150 mL 22% zinc acetate solution to 850 mL 2*N* NaOH solution.

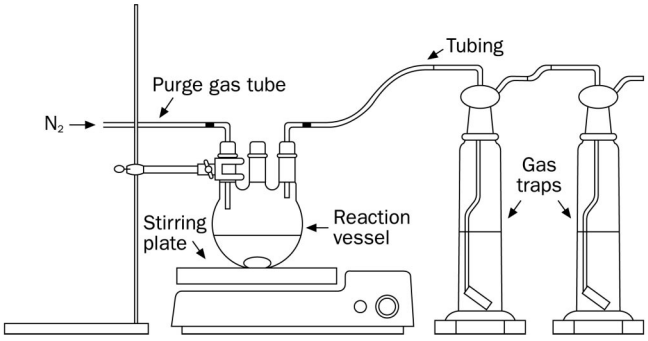


Figure 4500-S^{2-}:5. Apparatus for acid-volatile sulfide analysis.

f. Reagents for the iodometric titration method: See Section 4500-S^{2-} .F.

3. Procedure

Add 15 mL stannous chloride/hydrochloric acid solution to digestion vessel and 100 mL alkaline zinc solution to each trap. Adjust nitrogen flow rate to approximately 40 to 60 mL/min and flush system for 10 min. Add approximately 6 g fresh wet sediment to digestion vessel using a 5-mL syringe with its distal ends removed. Close outlets with clamps and gently stir the suspension. Allow H_2S generation to take place at room temperature (25±3°C) for 3 h while stirring and with the nitrogen flowing. Combine the solutions from the two traps and titrate as described in Section 4500-S^{2-}.F.

4. Precision and Bias

For marine sediment samples (n=6), an average of 97% of the added ferrous monosulfide was recovered by the diffusion method. Reproducibility of this measurement performed on four samples with concentrations of around 40 to 150 μmols/g dry weight (1.3 to 4.8 mg S/g) was better than 5%.

5. Bibliography

Chanton, J.P. & C.S. Martens. 1985. The effects of heat and stannous chloride on the active distillation of acid volatile sulfide from pyrite-rich marine sediment samples. *Biogeochemistry* 1:375.

Morse, J.W. & J.C. Cornwell. 1987. Analysis and distribution of iron sulfide minerals in recent anoxic marine sediments. *Mar. Chem.* 22:55.

Hsieh, Y.P. & C.H. Yang. 1989. Diffusion methods for the determination of reduced inorganic sulfur species in sediments. *Limnol. Oceanogr.* 34:1126.

Allen, H.E., G.-M. Fu & B.-L. Deng. 1993. Analysis of acid-volatile sulfide (AVS) and simultaneously extracted metals (SEM) for the estimation of potential toxicity in aquatic sediments. *Environ. Toxicol. Chem.* 12:1441.

Lasorsa, B. & A. Casas. 1996. A comparison of sample handling and analytical methods for determination of acid volatile sulfides in sediment. *Mar. Chem.* 52:211.

Leonard, E.N., A.M. Cotter & G.T. Ankley. 1996. Modified diffusion method for analysis of acid volatile sulfides and simultaneously extracted metals in freshwater sediment. *Environ. Toxicol. Chem.* 15:1479.

ATTACHMENT A

Maine Department of Environmental Protection Marine Sediment Sampling Protocols, Cooke Aquaculture, Effective Starting 2021

A. Choosing a Sampling Location

1. Cooke Aquaculture marine aquaculture pen site sample locations have been pre-selected and are identified in the Notice of Intent (NOI), or subsequent notifications, for each facility. Orientation and location of sample must be consistent with Appendix B and C of the General Permit.

2. Sampling locations are to be located using a GPS (Global Positioning System Device) with an accuracy of three (3) meters or less. The sample location's latitude and longitude shall be reported to the Department using degrees, minutes, seconds and tenths of a second. The coordinates should be found using the North American Datum of 1983 (NAD83) as reference.

B. Sample Acceptability for Core and Grab Samples

1. Sediment sample collection, handling, preservation, storage, and analysis must be conducted in accordance with United States Environmental Protection Agency (US EPA) approved methods, where available. The following clarifications and modifications are approved by the Department.

2. Sampling must consist of three replicates at each sample location (triplicate sampling). In this method, each individual triplicate sample is referred to as a replicate.

3. The sediment surface must be relatively level, with minimal sample disturbance or washing.

4. Overlying Water:

    a. Overlying water must be present until the sample is processed to ensure minimal leakage. Processing begins when the sample is removed from

the sample device and placed into another container. If a sample is touching the top of the sample container then there is not adequate overlying water.

b. The overlying water must not be excessively turbid, indicating minimal sample disturbance.

5. There must be minimal visual evidence of sediment loss from the sampling device.

6. If there is a failure to obtain a valid replicate after three consecutive sample attempts with an appropriate sampling device:

   a. The permittee must document the following:

      i. type of sampler used.

      ii. reason for sample limitation (i.e. sediment grain size or sediment depth).

      iii. if any sample was collected.

      iv. photograph any collected material that was deemed invalid.

   b. When all above items have been documented, then it is acceptable to report unable to sample (UTS) for that replicate. Any claim of UTS in a report to the Department must include a narrative describing the sampling impediments and efforts to collect a representative sample as close to the designated compliance sampling location as possible. The narrative must include information described in B.6.a above.

   c. The Department reserves the right to require sampling at alternative location(s) if a sample cannot be collected at a designated sampling location.

C. Sampling Method Type

1. When choosing a sampling method, Cooke must review the amount of sample required for adequate lab analysis and select a sampling method that will satisfy that requirement.

2. Sulfide core or grab samples are considered aqueous samples, not sediment samples. Analysis being conducted for permit compliance demonstration must be conducted on pore water extracted from the samples.

3. Core sampling:

    a. For sulfide sampling a minimum depth of penetration of five (5) cm should be attempted but if a valid sample can be collected with less penetration, then that is acceptable to the Department. When the depth of penetration is less than 5 cm for a sample location then that should be documented in the report.

    b. Replicate core samples for benthic infauna must be four inches, or larger, in diameter and be inserted to the point of resistance or 15 centimeters, whichever is less.

    c. The permittee must report the depth of each core sample.

    d. Core sample equipment must allow for sampling the undisturbed sediment surface. Careful removal of the surface water by siphon, pipet, or core extrusion equipment is recommended for obtaining access to the sediment surface.

    e. For sulfides, once the surface water is removed, the top two (2) centimeters of sample must be collected and deposited into an appropriate container as soon as possible.

    f. Core samples are invalid if:

        i. The sampler was inserted at an angle or tilted upon retrieval.

        ii. If there are less than five centimeters of sample in the core and 2 centimeters of sample cannot be collected for analysis (for sulfide).

iii. If there is sign of wash out/loss of sediment from the core.

iv. The surface water is allowed to drain through the core sample.

g. Invalid or used core remains should not be disposed over the sample site. while sampling activity is still occurring in that location.

4. Grab Sampling:

a. The Department allows use of grab samplers that are capable of sample collection in various sediment types. The Department has approved Van-Veen, Peterson, Ponar and the Ted-Young modified Van-Veen devices. Other sampling apparatus must be approved by the Department prior to use. Requests to use alternative sampling apparatus must be submitted to the Department at least 30 days before the sampling event.

b. If a grab sampler is used, the Department requires the sampler have top opening doors (preferably the doors will have a gasket to prevent water from entering the grab once it has closed) which allow for access, visual examination, and protection of the sample from washout while retrieving the sampler.

c. Acceptable and unacceptable grab samples are illustrated in Attachment #1.

d. Once a valid grab sample is obtained, subsamples are to be taken directly from the grab while the sample is still confined in the grab sampler. Careful removal of the surface water is required (use of siphoning or pipetting is recommended) for obtaining access to the sediment surface. For sulfides, once the surface water is removed, the top two (2) cm of the sample must be collected and deposited into an appropriate container as soon as possible.

e. Precautions should be taken to ensure successive replicates are obtained from substrate that has not been affected by recent sampling. Typically, successive replicates should be taken at least one boat length apart but

ultimately field judgment should be used to ensure that replicates are not taken from disturbed substrate.

D. Field sample processing:

1. The analytical laboratory should be consulted on necessary sample volume prior to sampling. Enough sediment must be collected for the lab to conduct the required analysis.

2. Sample Containers:

    a. Samples must be stored in US EPA approved sample containers per US Code of Federal Regulations (40 CFR Part 136.3) Table II - Required Containers, Preservation, Techniques, and Holding Times.

    b. All containers must be new or pre-cleaned/sterilized from the destination lab or commercial entity.

    c. All containers that are to hold sample for sulfide analysis shall be factory sealed and unopened prior to the sampling event.

    d. Containers for sulfide samples should be filled completely. If sample containers intended for sulfide analysis are not filled completely, the existing headspace must be purged with inert gas prior to capping.

    e. All sample containers should be properly labeled with required laboratory information using a waterproof marker or printed waterproof label prior to sampling.

    f. The replicates must be placed in individual containers for transport to the laboratory, and shall not be composited.

3. Hold Time:

a. The sulfide sample is considered an aqueous sample and hold time begins at the time the sample is collected by the sampler.

4. Preservation:

   a. All samples will be preserved by cooling to a temperature of 0º to ≤6ºC immediately after collection. The cooler must include a temperature blank that is checked and documented upon arrival at the laboratory.

   b. Rounding down to 6 °C may not be used to meet the ≤6°C requirement.

   c. After centrifuging is complete, sulfide pore water samples must be removed from the centrifuge tube to a clean vial with preservative. Sulfide samples must be preserved with zinc acetate and sodium hydroxide to a pH > 9. This preservation must be completed on the pore water within 15 minutes of centrifuging. This approval is conditioned on the centrifuging process (pore water extraction) taking place on the day the sample is collected. Sampling events must take this time constraint into consideration.

   d. After preservation with zinc acetate and sodium hydroxide is complete, sulfide pore water samples must be immediately preserved by cooling to a temperature 0º-≤6°C until they can be received by the analyzing laboratory. When the samples are transported, they must be maintained in a cooler at a temperature of 0º-≤6°C. The cooler must include a temperature blank that is checked and documented upon arrival at the laboratory.

   e. For benthic infauna samples, organisms must be fixed in a 10% buffered formalin solution and stained with a 1% Rose Bengal staining solution. After one day or more in the formalin solution the formalin must be replaced with 70% ethanol to preserve the sample.

5. For replicates that are to be analyzed for sulfide, gloves (latex or nitrile) or forceps should be used to remove all non-sedimentary material before placing replicates in containers. This should be done as gently as possible, to keep the

sediment undisturbed.

a. Non-sedimentary material includes large shell fragments, fish, wood waste, and rock (rocks of the sizes that are uncharacteristic of the remainder of the sample). All material removed from the replicate prior to analysis must be documented to include a description of the type, general size and general abundance of material removed from the replicate.

6. Acceptable Field Samples:

   a. Multiple discrete core samples cannot be combined to obtain enough sample for analysis (even if core samples are taken from the same or a similar location). Multiple aliquots of sediment can be removed from a single core sample to obtain enough sample for analysis. Once enough sample is obtained for analysis, core samples must be centrifuged to produce pore water replicates. A replicate must consist of the pore water from a single core sample. The pore water from multiple discrete core samples cannot be combined to produce a single replicate. If centrifuging a single core sample produces enough pore water to fill multiple 13 mL tubes, then all of the pore water from these tubes should be combined to form one replicate prior to analysis.

   b. It is not acceptable to subsample twice for the same parameter from one discrete replicate unless the second sample is for a field duplicate to demonstrate quality assurance of the sampling method.

   c. There should not be multiple subsamples from a discrete grab sample for any physical or chemical parameters when the replicate is for benthic infauna analysis.

   d. There shall be no homogenization of sediment replicates in the field.

7. When sample manipulation for sulfide or benthic infauna is to be conducted on the samples prior to analysis, then methods of manipulation are as follows:

a. Centrifuging. The Department requires centrifuging to obtain pore water from a sediment replicate. The recommended method for centrifuging for isolation of pore water is to:

   i. Hold and centrifuge the replicates at 8,000 - 10,000 x g for thirty (30) minutes at 0-6°C.

   ii. If transfer of sediment replicates is necessary, purge the centrifuge containers with an inert gas prior to replicate transfer, and fill centrifuge tubes so there is no headspace or replace headspace with nitrogen gas.

   iii. The replicate should be homogenized before splitting into centrifuge containers. This should be done in a manner that limits the exposure of the replicate to the atmosphere as much as possible. The Department strongly recommends conducting any homogenization process of the samples within a nitrogen filled glove bag.

b. Filtering:
   i. The Department does not approve the use of filtration for obtaining pore water.

c. Screening:
   i. For Benthic Infauna Collection, samples must be sieved through a 1.0-millimeter mesh sieve. The size of the screen must be reported.

E. Field Sample Documentation

1. For each field replicate, report the following minimum field information:

   a. Project title.

   b. Time and date of collection.

   c. Replicate Identification.

d. Sampler names.

e. Site identification.

f. GPS Location:

i. Coordinates.

ii. Accuracy of the measurement (e.g. within three (3) meters).

iii. Type (e.g. mapping grade, recreational grade, and etcetera), brand, and model of device used.

g. Data source - who collected the data (e.g. DEP, ACME Engineering).

h. Water depth and tidal stage in relation to mean low water. Water depth at sampling locations should be recorded along with the GPS location.

i. Sampler type.

j. Sampler penetration depth.

k. Estimate of the quantity of the sediment in the sampler. This can be addressed in the narrative of the report by indicating the volume of the core sampler attachment that contains sample under normal operating conditions. When a sample does not fill the sampler attachment then that should be indicated in the narrative with an estimation of the volume contained in the sampler attachment.

2. Description of Sediment:

a. Color.

b. Presence of visible biota:

i. *Beggiatoa*-like mats (white, bacteria-like surficial growth on the sediment).

ii. Opportunistic-type polychaete complexes (OPC).

c. Presence, type, and volume of debris.

d. Presence, depth, and thickness of the redox potential discontinuity (RPD) layer (a visual indication (meter not required) of where the sediment turns black). Photo documentation of this layer should be included in the report.

e. Odor of the sediment rated as mild, moderate or strong.

f. A photograph of the replicate.

3. Each field replicate shall be noted on a Chain of Custody (CoC) form that is documented and verified from sample date to date of receipt at the analytical laboratory. The CoC information shall contain a minimum of the following:

a. Name of sampler.

b. Replicate name (identification, date and time of collection).

c. Volume of replicate material.

d. Tests to be conducted on the replicates.

e. Preservative added, if any.

f. Number of replicates.

g. Date, time, and temperature of sample upon receipt at any sample manipulation lab (if one is used). The CoCs and lab reports must include information required to ensure preservation requirements are met.

h. Sample pH confirmation. Each sample being analyzed for sulfide shall be documented to have a pH $\geq$ 9 before it is processed. The pH may be verified by placing a drop of the preserved pore water on a pH test strip.

i. Date, time, and temperature of sample upon receipt at analysis lab.

j. Name of person who took custody of the sample at the analysis lab.

F. Reporting Results

1. Results must be reported in accordance with all the conditions of the General Permit – Net Pen Aquaculture (April 10, 2014). Particular attention should be paid to Special Condition I, Special Condition Q and Appendix A of the General Permit.

2. Results must be compiled into a report that contains the following information:

   a. Copies of the chains of custody (CoC) for all samples.

   b. All laboratory data, including the lab QA/QC data, calibration logs, and laboratory reports showing analytical methods.

   c. Specifics on sample collection procedures, including sample containers, to ensure compliance with the permit.

   d. Sample collection/ analysis time and dates, analysts, analytical results, quality control.

   e. Documentation and explanation for UTS.

   f. Results of sample analysis, including:

      i. Results for the 3 field replicates collected at the four 35 meter locations must be provided. It must include the individual results and the mean of all the results across the site.

      ii. Results for the 3 field replicates collected at the four 5 meter locations must be provided. It must include the individual results and the mean of all the results across the site.

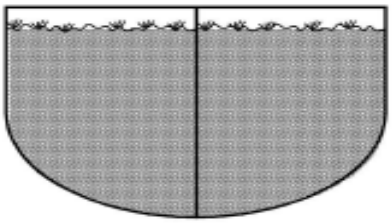
iii. Any non-detect results must be report. Non-detected results must be used in all average calculations required by the Permit. When calculating an average using a non-detect, ½ of the PQL (practical quantitation limit) should be used in place of the non-detect value.

g. The site schematic depicting the actual pen layout must be included. It must include the sample locations with latitude and longitude to the nearest 10^{th} using a GPS with an accuracy of less than three (3) meters.

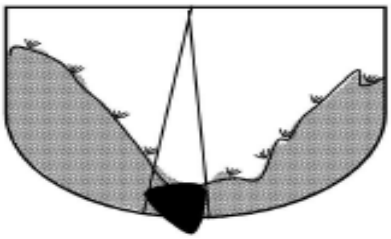
h. Prevailing current direction in relation to true north, tidal height at the time of sampling to the nearest ½ meter above or below mean low water and depth of water at each sample location.

Attachment #1

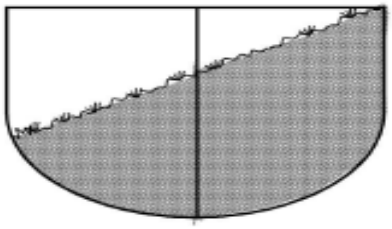
A visual of what valid grab samples should look like.



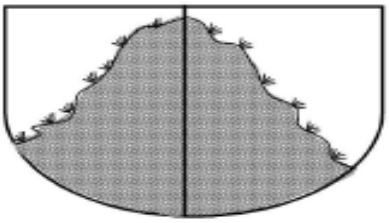
Acceptable if Minimum Penetration Requirement Met and Overlying Water is Present



Unacceptable (Washed, Rock Caught in Jaws)



Unacceptable (Canted with Partial Sample)



Unacceptable (Washed)