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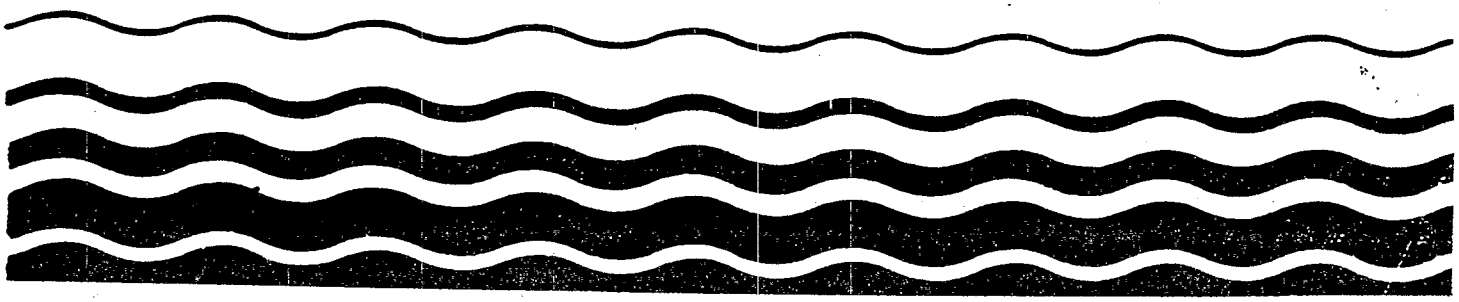
WATER

Draft Analytical Method for Determination of Acid Volatile Sulfide in Sediment



DETERMINATION OF ACID VOLATILE SULFIDE AND SELECTED SIMULTANEOUSLY EXTRACTABLE METALS IN SEDIMENT

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1. SCOPE AND APPLICATION

1.1 This method describes procedures for the determination of acid volatile sulfide (AVS) and for selected metals that are solubilized during the acidification step (simultaneously extracted metal, SEM). As a precipitant of toxic heavy metals, sulfide is important in controlling the bioavailability of metals in anoxic sediments (1). Research has established that the relative amounts of SEM and AVS are important in the prediction of potential metal bioavailability; if the molar ratio of SEM for bivalent metals to AVS exceeds one, the toxic heavy metals in that sample are potentially bioavailable. This method uses the same conditions for release of both sulfide and metal from the sediment and thus provides a useful means of assessing the amount of metal associated with sulfide.

2. SUMMARY OF METHOD

2.1 The AVS in the sample is first converted to hydrogen sulfide (H_2S) by acidification with hydrochloric acid at room temperature. The H_2S is then purged from the sample and trapped in aqueous solution. The amount of sulfide that has been trapped is then determined. The SEM are selected metals liberated from the sediment during the acidification. These are determined after filtration of the sample.

2.2 Two types of apparatus for sample purging and trapping of H_2S are described. One uses a series of Erlenmeyer flasks while the other uses flasks and traps with ground glass stoppers. The former is less costly. The latter is less prone to leakage that causes low recovery of AVS. The latter is recommended when higher degrees of precision are desired and for samples containing low levels of AVS.

2.3 Three means of quantifying the H_2S released by acidifying the sample are provided. The colorimetric method is generally preferred. In the gravimetric procedure, the H_2S is trapped in silver nitrate. The silver sulfide that is formed is determined by weighing (1, 2). This procedure can be used for samples with moderate or high AVS concentrations. Below 10 µmoles AVS/gram dry sediment, accuracy may be affected by incomplete recovery of precipitate and by weighing errors. In the colorimetric method, the H_2S is trapped in sodium hydroxide. The sulfide reacts with N-N-dimethy-p-phenylenediamine to form methylene blue that is measured (3). This procedure is capable of determining AVS concentrations as low as 0.01 µmoles/gram dry weight of sediment. By appropriate sample dilution, the maximum concentration of

AVS which can be determined is at leat 1000 µmoles/gram dry sediment. In an alternative procedure the H_2S is trapped in an antioxidant buffer before using an ion-selective electrode (4, 5).

2.4 After release of the H_2S, the acidified sediment sample is membrane filtered before determination of the SEM by atomic absorption or inductive coupled plasma spectrometic methods (6, 7).

3. DEFINITIONS

3.1 ACID VOLATILE SULFIDE (AVS) - AVS is operationally defined as sulfides that form hydrogen sulfide under the conditions of this test. This includes amorphous, moderately crystalline monosulfides, and other sulfides (8).

3.2 SIMULTANEOUSLY EXTRACTED METALS (SEM) - SEM are operationally defined as metals, commonly cadmium, copper, lead, mercury, nickel and zinc, that form less soluble sulfides than do iron or manganese, and which are at least partially soluble under the conditions of this test.

3.3 METHOD DETECTION LIMIT (MDL) - The minimum concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The MDL is determined from the analysis of a sample that contains the analyte within a given matrix.

3.4 LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water or reagents that is treated exactly as a sample including exposure to all glassware, equipment, and reagents that are used with samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

3.5 STOCK STANDARD SOLUTION - A concentrated solution of the analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

3.6 CALIBRATION STANDARDS - Solutions prepared from the stock standard solution that is used to calibrate the method response with respect to analyte concentration.

3.7 LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water or reagents to which a known quantity of the method analyte is added in the laboratory. The LFB is analyzed exactly like a sample. Its purpose is to determine whether the method is within accepted control limits.

3.8 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) - An environmental sample to which a known quantity of the method analyte is added in the laboratory. The LFM is

analyzed exactly line a sample. Its purpose is to determine whether the sample matrix contributes bias to the analytical results.

4. INTERFERENCES

4.1 Contact with oxygen must be avoided in all stages from sampling to analysis. Consequently, the samples and standards should be protected from air from the time of sampling through the analytical procedure. This can be achieved by deaerating and maintaining the samples under nitrogen or argon at all times.

5. SAFETY

5.1 The toxicity or carcinogenicity of reagents used in this method have not been fully established. Each chemical and environmental sample should be regarded as a potential health hazard and exposure should be minimized. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should be available to all personnel involved in the chemical analysis.

5.2 Hydrogen sulfide is a highly poisonous, gaseous compound having a characteristic odor of rotten eggs. It is detectable in air by humans at a concentration of approximately 0.002 ppm. Handling of acid samples should be performed in a hood or well ventilated area. If a high concentration of hydrogen sulfide is detected in the air by the laboratory staff, sample handling procedures must be corrected. According to Sax (9) an air concentration of 10 ppm of H_2S is permitted for an 8 hour shift for 40 hours per week.

5.3 If samples originate from a highly contaminated area, appropriate sample handling procedures to minimize worker exposure must be followed.

6. APPARATUS AND EQUIPMENT

6.1 Glassware

6.1.1 AVS evolution and H_2S trapping - Glassware in Section 6.1.1.1 is recommended. Glassware in Section 6.1.1.2 may be used, but will not provide as high precision or accuracy for samples.

6.1.1.1 For highest precision and low AVS levels - For each analytical train 500 mL gas washing bottles or oxygen trap, one 250 mL round bottom flask with a septum (Ace Glass 6934 or equivalent), 100 or

250 mL impingers with non-fritted outlets. The round bottom flask contains the sediment and acid is introduced to it by a syringe inserted through the septum. The flasks are connected by tubing. Because sulfide may react with tubing and other surfaces, minimum lengths of tubing should be used as sleeves to connect the glass tubing. The analyst should pay particular attention to the recovery of sulfide from standards in evaluating the apparatus. In all cases the inlets are below the liquid level and the outlets are above the liquid levels. The apparatus is assembled as shown in Figure 1 and more than one analytical train can be connected to a single cylinder of nitrogen or argon if flow controllers are installed in the line. Different amounts of glassware are required for each of the three means of sulfide determination.

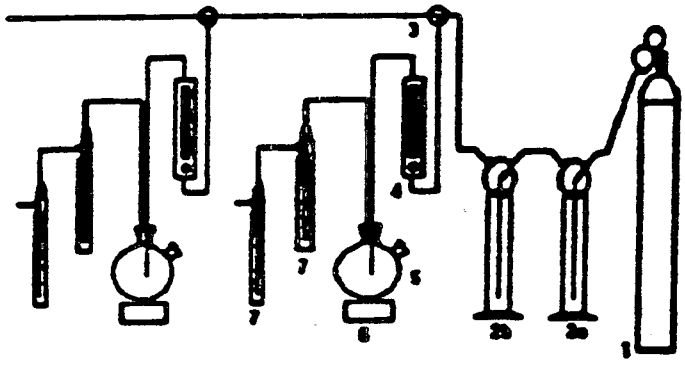

Figure 1. Apparatus for AVS determination: 1. N_2 or Ar cylinder; 2. Gas washing bottles: (a) oxygen scrubbing solution or an oxygen trap may be used in replacement of this gas washing bottle, (b) deionized water; 3. Three-way stopcock; 4. Flow controller; 5. Reaction flask; 6. Magnetic stirrer; 7. Impingers with non-fritted outlets.

6.1.1.2 For routine analysis - Erlenmeyer flasks, 250 mL, are substituted for the gas washing bottle, the round bottom flask and the impingers. The flask size should be consistent with sample size and reagent volumes. A thistle tube fitted with a stopcock or a separatory funnel is provided to introduce acid to the flask containing the sediment sample. This flask is fitted with a three hole stopper. One hole is for the thistle tube or separatory funnel and the other two are for the gas inlet and

outlet. The other flasks are fitted with two hole stoppers; one hole is for the gas inlet and the other is for the gas outlet. The gas inlets are below the liquid level and the gas outlets are above the liquid level. The flasks are connected by tubing. Because sulfide may react with tubing, stoppers and other surfaces, minimum lengths of tubing should be used as sleeves to connect the glass tubing. The analyst should pay particular attention to the recovery of sulfide from standards in evaluating the apparatus.

6.1.2 Evaporating dishes, porcelain, 100 mL.

6.1.3 Assorted calibrated pipettes and volumetric flasks.

6.2 Drying oven - Capable of maintaining a constant temperature in the range of 103-105°C.

6.3 Analytical balance - capable of weighing to 0.0001 g.

6.4 Magnetic stirrer, thermally insulated, and Teflon-coated stirring bar.

6.5 Gravimetric method

6.5.1 Filtering flask.

6.5.2 Filter holder for 47 mm filter.

6.6 Colorimetric method

6.6.1 Spectrophotometer - Capable of measuring absorbance at 670 nm.

6.6.2 Spectrophotometer cells.

6.7 Ion-selective electrode method

6.7.1 Electrometer, pH meter or ion-selective meter - Compatible with the use of ion-selective electrodes.

6.7.2 Sulfide selective electrode.

6.7.3 Double-junction reference electrode.

6.8 Atomic absorption or inductive couple plasma spectrophometer for the determination of SEM.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 All water and reagents used in this method must be free of dissolved oxygen and sulfide. Freshly prepare and use deaerated, deionized water by removing dissolved

oxygen from the deionized water by vigorously bubbling with oxygen-free nitrogen or argon for approximately one hour. Deaerate reagents immediately before use by deaerating with oxygen-free nitrogen or argon.

7.2 Sodium sulfide standard - Required for quality assurance and calibration.

7.2.1 Sulfide stock standard solution, approximately 0.05M or 50 µmoles/mL.

7.2.1.1 Weigh about 12 gram of $Na_2S \cdot 9H_2O$ and dissolve it in 1,000 mL of deionized water. Store in a brown bottle. To prevent air oxidation, the sulfide solution should be maintained under oxygen-free nitrogen or argon.

7.2.1.2 Standardize against thiosulfate solution.

7.2.1.2.1 Pipette 10.00 mL of 0.025N standard iodine solution (Section 7.2.2) into each of two 125-mL Erlenmeyer flasks.

7.2.1.2.2 Pipette 2.00 mL of sulfide stock standard solution into one flask. Pipette 2.00 mL of deionized water, as a laboratory reagent blank, into the other flask.

7.2.1.2.3 Add 5.00 mL of 6M HCl into each flask, swirl slightly, then cover and place in the dark for 5 minutes.

7.2.1.2.4 Titrate each with 0.025N thiosulfate (Section 7.2.3) until the yellow iodine color fades to a pale straw. Just before all the iodine has been titrated, add starch indicator (Section 7.2.4) dropwise to form a pale blue color. Continue the titration with the thiosulfate. The end point is reached when the blue color first disappears.

7.2.1.2.5 Calculate the sulfide concentration as follows:

$$\text{Sulfide } (\mu\text{mol/mL}) = \frac{(T_{blank} - T_{sample}) \times N_{S_2O_3^{2-}}}{V_{sample}} \times \frac{1 \text{ mole } S^{2-}}{2 \text{ equiv } S^{2-}} \times \frac{1000 \text{ µmoles}}{1 \text{ mmole}}$$

where T = volume of titrant used for the blank and sample (mL)

N = concentration of $S_2O_3^{2-}$ titrant

V = volume of sample used (mL), 2.00 mL recommended

7.2.2 Standard iodine solution, 0.025N - Dissolve 20 to 25 gram potassium iodide, KI, in a small volume of deionized water, add 3.2 gram iodine, and dilute to 1,000 mL. Standardize against 0.025N sodium thiosulfate (Section 7.2.3)

7.2.3 Standard sodium thiosulfate solution, 0.025N. May be purchased commercially or prepared in the laboratory. Standardize against potassium bi-iodate.

7.2.3.1 Weigh approximately 6.2 g of sodium thiosulfate, $Na_2S_2O_3 \cdot 5\ H_2O$, into a 500 mL beaker. Add 0.1 g sodium carbonate, Na_2CO_3, and dissolve in 400 mL deionized water. Pour into a 1.0 L volumetric flask and dilute to volume with deionized water.

7.2.3.2 Standardization against potassium bi-iodate, $KH(IO_3)_2$.

7.2.3.2.1 Prepare 0.00208M potassium bi-iodate by dissolving 0.8123 g $KH(IO_3)_2$, previously dried 2 hr at 103-105°C, in distilled water. Pour into a 1.0 L volumetric flask and dilute to volume with deionized water.

7.2.3.2.2 Dissolve approximately 2 g KI, free from iodate, in an erlenmeyer flask with 100 to 150 mL deionized water. Add 1 mL of 6N H_2SO_4 or a few drops of concentrated H_2SO_4 and 20.00 mL of standard bi-iodate solution. Dilute to 200 mL and titrate the liberated iodine with the thiosulfate solution until the yellow color fades to a pale straw color. Then add a couple drops of starch indicator to form a pale blue color and continue the titration with the thiosulfate until the blue color first dasappears..

7.2.3.2.3 20.00 mL of 0.00208M $KH(IO_3)_2$ requires exactly 20.00 mL of 0.025N sodium thiosulfate. For an calculation of the thiosulfate concentration use the following equation:

$$N\left(S_2O_3^{2-}\right) = \frac{g\ KH(IO_3)_2}{mL\ S_2O_3^{2-}} \times \frac{1\ mole\ KH(IO_3)_2}{389.9\ g\ KH(IO_3)_2} \times \frac{12\ equiv\ KH(IO_3)_2}{1\ mole\ KH(IO_3)_2} \times \frac{1000\ mL}{1\ L}$$

7.2.4 Starch indicator - Dissolve 1.0 gram soluble starch in 100 mL boiling deionized water.

7.2.5 Sulfide working standards - Prepare sulfide working standards using the sulfide stock standard solution in Section 7.2.1. The concentrations of the following standards will depend on the exact concentration of the sulfide stock standard determined in Section 7.2.1.2.5. Correct concentrations of the the standards in the following part of this section and the amount of sulfide in standards used in the colorimetric method in Section 12.2.5 by multiplying by a factor of the concentration determined in Section 7.2.1.2.5 divided by 50 µmoles/mL.

7.2.5.1 Prepare sulfide working standard A by diluting 1.00 mL of sulfide stock standard to 100.0 mL. This solution contains 0.5 µmole sulfide/mL, if the concentration of the sulfide stock standard is exactly 0.05M.

7.2.5.2 Prepare sulfide working standard B by diluting 10.00 mL of sulfide stock standard to 100.0 mL. This solution contains 5.0 µmole sulfide/mL, if the concentration of the sulfide stock standard is exactly 0.05M.

7.3 AVS evolution

7.3.1 Hydrochloric acid 6M - Dilute 500 mL of concentrated hydrochloric acid (sp. gr. 1.19) to 1L with deionized water. Dearation of this solution as described in Sections 7.1 and 11.4 is most important.

7.3.2 Nitrogen or argon gas, oxygen free, with regulator and flow controller. An oxygen gas scrubber may be required and is available commercially or deoxygenating solutions may be placed in the first flask or gas washing bottle in the analytical train.

7.3.3 Plastic hypodermic syringe, 30 mL, and needle.

7.4 Gravimetric method

7.4.1 Potassium acid phthalate, 0.05M - Dissolve 10.2 g of potassium acid phthalate, $KHC_8H_4O_4$, in deionized water and dilute to 1L.

7.4.2 Silver nitrate, 0.1M - Dissolve 17 g of silver nitrate, $AgNO_3$, in deionized water and dilute to 1L. Store in a dark bottle.

7.4.3 Glass fiber filters, 1.2 micron - Rinse with deionized water, then predry filters at 103-105°C.

7.5 Colorimetric method

7.5.1 Sodium hydroxide solution, 1M - Dissolve 40 g sodium hydroxide in 1000 mL deionized water.

7.5.2 Sodium hydroxide solution, 0.5M - Dissolve 20 g sodium hydroxide in 1000 mL deionized water.

7.5.3 Mixed diamine reagent, MDR

7.5.3.1 Component A - Add 660 mL concentrated sulfuric acid to 340 mL of deionized water. After the solution cools, dissolve 2.25 g N-N-dimethy-p-phenylenediamine oxalate in it.

7.5.3.2 Component B - Dissolve 5.4 g ferric chloride hexahydrate ($FeCl_3 \cdot 6 H_2O$) in 100 mL concentrated hydrochloric acid and dilute to 200 mL with deionized water.

7.5.3.3 Mixed diamine reagent, MDR - Mix components A and B.

7.5.4 Sulfuric acid solution, 1.0M - Dilute 56 mL concentrated sulfuric acid (H_2SO_4) to 1 L with deionized water.

7.6 Ion-selective electrode method

7.6.1 Sodium hydroxide solution - Dissolve 80 g of sodium hydroxide in 700 mL of deionized water with caution. Cool to room temperature.

7.6.2 Sulfide anti-oxidant buffer (SAOB) - To the sodium hydroxide solution in Section 7.6.1 add and dissolve 74.45 g of disodium ethylenediaminetetraacetic acid and 35.23 g of ascorbic acid. Dilute to 1L with deionized water.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Sulfide ion is unstable in the presence of oxygen. Protect sediment samples from exposure to oxygen during sample collection and storage.

8.2 During storage sulfide can be formed or lost due to biological activity and sulfide can be lost by volatilization or oxidation. Metal speciation can change as a result of changes in sulfide concentration and as a result of other changes in the sample.

8.3 Samples should be collected in wide mouth jars with a minimum of air space above the sediment. If possible, the headspace should be filled with oxygen free nitrogen or argon. The jar lids must have Teflon or polyethylene liners.

8.4 Samples should be cooled to 4°C as soon as possible after collection. Samples maintained at 4°C have been found to have no significant loss of AVS for storage periods up to 2 weeks (3). Holding time for samples should not exceed 14 days.

9. CALIBRATION AND STANDARDIZATION

9.1 Calibrate the photometer with a minimum of four standards and a blank that cover the expected range of the samples. Prepare a calibration graph relating absorbance to the µmoles of sulfide taken.

9.2 Calibrate the sulfide electrode system with a minimum of three standards that cover the expected range of the samples. Standards must be made up in SAOB diluted 1+1 with deionized water. Follow the manufacturer's instructions for use of the electrode.

9.3 Overall sulfide recovery is determined by analysis of a known amount of sodium sulfide standard added to deionized water from which the sulfide is liberated in the analysis train (LFB). Recoveries of 95% ± 10% are expected.

10. QUALITY CONTROL

10.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirement of this program consists of an initial demonstration of laboratory capability, and the analysis of laboratory reagent blanks, fortified blanks and fortified samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data thus generated.

10.2 INITIAL DEMONSTRATION OF PERFORMANCE

10.2.1 The initial demonstration of performance is used to characterize instrument performance, method detection limits, and linear calibration ranges.

10.2.2 Method detection limit (MDL) - The method detection limit should be established for the analyte, using deionized water (blank) fortified at a concentration two to five times the estimated detection limit (10). To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = t \times s$$

where, t = students' t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.14 for seven replicates), and

s = standard deviation of the replicate analyses.

Method detection limits should be determined every six months or whenever a significant change in background or instrument response is expected.

10.2.3 Linear calibration ranges - The upper limit of the linear calibration range should be established by determining the signal responses from a minimum of four different concentration standards covering the expected range, one of which is close to the upper limit. The linear calibration range that may be used for the analysis of samples should be judged by the analyst from resulting data. Linear calibration ranges should be determined every six months or whenever a significant change in instrument response may be expected.

10.3 ASSESSING LABORATORY PERFORMANCE - REAGENT AND FORTIFIED BLANKS

10.3.1 Laboratory reagent blank (LRB) - The laboratory must analyze at least one laboratory reagent blank (Section 3.4) with each set of samples. Reagent blank data are used to assess contamination from the laboratory environment and reagents. If an analyte value in the reagent blank exceeds its determined MDL, then laboratory or reagent contamination should be suspected. Any determined source of contamination should be corrected and the samples reanalyzed.

10.3.2 Laboratory fortified blank (LFB) - The laboratory must analyze at least one laboratory fortified blank (Section 3.7) with each set of 20 samples. Calculate accuracy as percent recovery. If the recovery of the analyte falls outside the control limits (Section 10.3.3), the analyte is judged to be out of control, and the source of the problem should be identified and resolved before continuing analyses.

10.3.3 Until sufficient data become available from within their own laboratory (usually a minimum of twenty to thirty analyses), the laboratory should assess laboratory performance against recovery limits of 85-105%. When sufficient internal performance data becomes available, develop control limits from the mean recovery (x) and the standard deviation (s) of the mean recovery. These data are used to establish upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = x + 3s$$

$$\text{LOWER CONTROL LIMIT} = x - 3s$$

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent twenty to thirty data points.

10.4 ASSESSING ANALYTE RECOVERY - LABORATORY FORTIFIED SAMPLE MATRIX

10.4.1 The laboratory must fortify a minimum of 10% of the routine samples or one fortified sample per set of 20 samples, whichever is greater. At least one sample from each source should be fortified. Ideally, the concentration should at least double the background concentration. Over time, samples from all routine sample sources should be fortified.

10.4.2 Calculate the percent recovery for the analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the control limits established in Section 10.3.3 for the analyses of LFBs. Spike recovery calculations are not required if the spike concentration is less than 10%

of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{(C_s - C_b)}{S} \times 100$$

where

R = percent recovery,

C_s = fortified sample concentration,

C_b = sample background concentration, and

S = concentration equivalent of the fortified sample.

10.4.3 If the recovery of the analyte in the fortified sample falls outside the designated range, and the laboratory performance on the LFB for the analyte is shown to be in control (Section 10.3) the recovery problem encountered with the fortified sample is judged to be matrix related, not system related.

11. GENERATION OF H_2S

11.1 Assemble glassware according to the detection method to be used. The setup in Figure 1 should be followed as a general guide. In all cases a flask or gas washing bottle containing a deoxygenating solution may be placed in the sample train between the nitrogen or argon tank and the first flask. Glassware is specified in Section 6.1.1. It is recommended that nitrogen or argon be controlled by a flow controller, but an equivalent flow rate may be regulated by a clamp and bubble rate determined. In all cases the glassware will minimally consist of a H_2S generating flask and a series of traps.

11.1.1 Gravimetric method - The first flask contains the sediment sample or standard. The second flask contains 175-200 mL of potassium hydrogen phthalate reagent 7.4.1 as an HCl trap. The third and fourth flasks contain 175-200 mL of silver nitrate reagent 7.4.2. If glassware specified in Section 6.1.1.1 is used, the second flask is a gas washing bottle and the third and fourth flasks are impingers.

11.1.2 Colorimetric method - The first flask contains the sediment sample or standard. The second and third flask contain an absorbant of 80 mL 0.5M NaOH reagent 7.5.2. If glassware specified in Section 6.1.1.1 is used, the second and third flasks are impingers.

11.1.3 Ion-selective electrode method - The first flask contains the sediment sample or standard. The second and third flask contain an absorbant of 50 mL SAOB reagent 7.6.2 and 30 mL deionized water. If glassware specified in Section 6.1.1.1 is used, the second and third flasks are impingers.

11.2 One hundred milliliters (100 mL) of deionized water and a magnetic stirring bar are added to the flask that will contain the sediment. The total volume of deionized water plus water contained in the wet sediment sample should not exceed 120 mL to minimize differences in acid concentration among samples. For the computation of the volume of water contained in the wet sediment, see Section 13.3. The traps are filled and deaerated by bubbling nitrogen or argon for 10 minutes at a flowrate of 100 cm^3/min. Reduce flow to 40 cm^3/min.

11.3 Weigh approximately 10 g of wet sediment on an analytical balance. Record weight to the nearest milligram. If AVS concentration is high, a smaller amount of sediment may be required; use of sediment samples smaller than 1-2 grams is not recommended due to sulfide oxidation and sample heterogeneity. Use of large sediment samples is not recommended because significant amounts of acid may be neutralized. Place sediment in the standard taper round bottom flask or the Erlenmeyer flask fitted with the thistle tube or separatory funnel. Parafilm has been found to be free of sulfide (4). Weigh samples on 2 × 2 inch pieces of parafilm and introduce the parafilm and sample to the flask. Rinsing the sample into the flask is not recommended. Purge the sample for 10 minutes with nitrogen or argon at a flowrate of 40 cm^3/min. Stop the flow of gas.

11.4 Using a 30 mL syringe, inject 20 mL of 6M HCl, which has been bubbled with nitrogen or argon gas for 30 minutes, into the reactor through the septum. If the apparatus described in Section 6.1.1.1 is used, add the HCl from the thistle tube or the separatory funnel. Bubble nitrogen or argon through the sample for 1 hour at a flowrate of 20 cm^3/min and magnetically stir the sample at the same time.

11.5 Analyze sulfide contained in sulfide trap by the appropriate analytical procedure in Section 12.

12. ANALYSIS OF SULFIDE

12.1 Gravimetric method

12.1.1 Insure that the final trap, the second silver nitrate trap, contains no precipitate.

12.1.2 Filter the silver sulfide contained in the first sulfide trap through a preweighed 1.2 micron filter. Rinse filter with deionized water. Dry at 103-105°C and weigh.

12.1.3 Calculate the amount of silver sulfide as the difference between the weight of silver sulfide and the filter and the weight of the predried filter.

12.1.4 Calculate the amount of sulfide in the sample:

$$\text{Sulfide in wet sediment (}\mu\text{moles)} = \frac{\text{g } Ag_2S}{247.8} \times 10^6$$

12.2 Colorimetric method

12.2.1 If the AVS concentration is low so that the sulfide contained in the tube trap is less than 15 µmoles, add 10 mL of the mixed diamine reagent (MDR) directly to the NaOH solution in each trap tube to develop the color. Transfer this solution to a 100 mL volumetric flask and dilute to the mark with deionized water. If the sulfide contained in the NaOH in the tube trap exceeds 18 µmoles, transfer the NaOH in each tube trap to a 100 mL volumetric flask. Rinse the trap with deaerated 0.5M NaOH and dilute to volume with NaOH. An appropriate volume aliquot of this solution is used for the analysis. In this case, the aliquot is transferred to a 100 mL volumetric flask, sufficient 0.5M NaOH is added so that the total volume is 80 mL, 10 mL MDR is added, and the solution is diluted to 100 mL with deionized water. Use of sediment samples smaller than 1-2 grams is not recommended due to sulfide oxidation and sample heterogeneity.

12.2.2 After 30 minutes, but before two hours have elapsed, measure the absorbance of light at 670 nm using a half-inch diameter or 1 cm rectangular spectrophotometer cell.

12.2.3 If the absorbance of the sample is greater than 0.6, dilute 10-fold with 1.0M H_2SO_4 and compare to the high range calibration curve.

12.2.4 Normally, the sulfide concentration in second trap tube is close to the blank value in this procedure and is not significant in calculating the concentration of sulfide. If a significant color is developed, the flow rate and amount of sulfide in the standard or sediment should be checked

12.2.5 Preparation of calibration curve - The indicated amounts of sulfide are based on a 0.05M concentration of the sulfide stock standard solution. The procedure indicated in Section 7.2.5 should be used to calculate the exact amount of sulfide in each of the standards.

12.2.5.1 Low range calibration curve - 0.0 - 2.5 µmoles S^{2-} (0.0 - 80 µg S^{2-})

Add 80 mL 0.5 N sodium hydroxide to each of a series of 100 mL of flasks and add 0.00, 1.00, 2.00, 3.00, 4.00, and 5.00 mL of sulfide working standard A to these flasks. These samples contain 0.00,

0.50, 1.00, 1.50, 2.00, and 2.50 μmoles S^{2-}, respectively. Add 10.0 mL of MDR to each and dilute to 100.00 mL with deionized water. After 30 minutes, measure the absorbance at 670 nm.

12.2.5.2 High range calibration curve - 0.0 - 20.0 μmoles S^{2-}

(0.0 - 640 μg S^{2-})

Add 80 mL 0.5M sodium hydroxide in 100 mL flasks and add 0.00, 1.00, 2.00, 3.00 and 4.00 mL of sulfide working standard B to these flasks. These samples contain 0.0, 5.00, 10.00, 15.00, and 20.00 μmoles S^{2-}, respectively. Add 10.0 mL of MDR and dilute to 100.00 mL with deionized water. After 30 minutes, dilute the solution 10-fold with 1.0M H_2SO_4, and measure the absorbance at 670 nm.

12.2.6 Calculate the amount of sulfide (μmoles) in the sample from the calibration curve. If the total volume of NaOH in the trap was not used in the analysis, account for the portion tested.

12.3 Ion-selective electrode method

12.3.1 Calibrate the sulfide electrode and meter according to manufacturer's recommendations, using sulfide standards prepared in SAOB reagent 7.6.2 diluted 1:1 with deionized water.

12.3.2 Transfer the contents of each sulfide trap into a 100-mL volumetric flask. Rinse the trap with deionized water, adding the rinses to the volumetric flask. Dilute to volume with deionized water.

12.3.3 Pour contents of volumetric flask into a 150-mL beaker, add a stirring bar and place on stirrer. Begin stirring with minimum agitation to avoid entrainment of air into the solution and minimize oxidation of the sample during the measurement.

12.3.4 Rinse sulfide and reference electrodes into waste container and blot dry with absorbent tissue. Immerse electrodes in sample solution.

12.3.5 Allow electrode response to stabilize (8-10 minutes), then take measurement of sulfide concentration. Depending on the meter used, the reading may be directly in concentration units if the meter is in the concentration mode and a 2-point calibration has been performed. If the readings are in millivolts, convert millivolts to concentration using the calibration curve obtained from standard solutions.

12.3.6 Calculate the amount of sulfide (μmoles) in the sample.

13. CALCULATION OF AVS CONCENTRATION IN SEDIMENTS

13.1 The sediment dry weight/wet weight ratio (R) must be determined separately. Acid volatile sulfide can be oxidized or altered to non-volatile forms during drying.

13.2 Transfer an aliquot of the sediment to a tared 100-mL tared evaporating dish. Weigh the dish plus the wet sediment. Calculate the wet weight of the sample. Dry the sediment at 103-105°C and weigh. Calculate the dry weight of sediment.

13.3 Determine the ratio of dry weight to wet weight for the sediment sample:

$$R = \frac{W_d}{W_w}$$

where R = ratio of dry weight to wet weight,

W_d = dry weight of sediment sample (g), and

W_w = wet weight of sediment sample (g).

Also, the weight of water, W_{water}, taken in a sample for AVS analysis can be calculated. If the weight of the wet sediment sample taken for the AVS analysis is W_{s+w}, the weight of water contained in the sediment sample would be

$$W_{water} = W_{s+w} - (R \times W_{s+w})$$

The volume of water in the sample equals the weight of water, assuming the density is near unity.

13.4 Compute the sulfide concentration per gram dry weight of sediment:

$$\text{AVS } (\mu\text{moles / g}) = \frac{S}{R \times W_w}$$

where S = the amount of AVS in sediment (µmoles) from Section 12.1.4,

12.2.6, or 12.3.6, as appropriate,

R = ratio of dry weight to wet weight from Section 13.3, and

W_w = wet weight of sediment (g) taken for AVS analysis.

13.5 The QC data obtained during the analysis provides an indication of the quality of the sample data and should be provided with the sample results.

14. DETERMINATION OF SIMULTANEOUSLY EXTRACTED METALS (SEM)

14.1 After the generation of sulfide has been completed, filter the sediment suspension remaining in the H_2S generation flask (Section 11.4) through a 0.2 µ membrane filter

resistant to attack by acid. The filtering apparatus should be soaked in 0.1M HNO_3, then rinsed with deionized water prior to use.

14.2 Transfer the filtrate to a 250-mL volumetric flask. Rinse the filtering flask with distilled water, adding the rinses to the volumetric flask. Dilute to volume with deionized water. The volumetric flasks should be soaked in 0.1M HNO_3, then rinsed with deionized water prior to use. Samples should be analyzed within 2 weeks.

14.3 Determine the concentrations of sulfide binding metals of interest and those which, on a molar basis, are present at more than 1 percent of the AVS concentration. Do not include iron and manganese whose sulfides are less stable than are the sulfides of many trace metals. Metals which may typically be included in SEM are cadmium, copper, lead, mercury, nickel and zinc. In addition, antimony, bismuth and chromium, among others, form insoluble sulfides. If significant concentrations of these or other metals forming insoluble sulfides are present, their concentrations should be taken into account in the computation of SEM. However, if these or other metals which are not divalent are present in significant concentrations, the computation in Section 14.5 must be modified to account for the stoichiometry. Metal concentrations may be determined by by atomic absorption, inductive coupled plasma spectrometic, or another approved method (6, 7). Calibration may be by the method of standard additions or by a calibration curve. If a calibration curve is used, matrix match standards to samples by including 20 mL of 6M HCl per 100 mL for each of the calibration standards. Convert µg/L concentration values to µmoles/L. Multiply the µmoles/L by the solution volume to obtain the µmoles of metal.

14.4 Report the concentrations of each of the metals in the sediment on a µmole per gram dry sediment (µmole/g) basis.

14.5 Calculate the ratio of SEM to AVS:

$$\frac{SEM}{AVS} = \frac{\sum[metal]}{AVS}$$

where SEM is the sum of the concentrations of metals, Σ [metal], for the metals (e.g., cadmium, copper, lead, mercury, nickel and zinc) in Section 14.4 and

AVS is the acid volatile sulfide concentration determined in Section 13.4.

Both SEM and AVS are expressed on a µmole per gram dry sediment (µmol/g) basis. Because metals present in the pore will be included in the analysis, the ratio could be less than that if correction were made for this contribution. This will lead to a conservative estimation of potential bioavailability (1).

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