METHOD # 376.2	Approved for NPDES (Issued 1978)
TITLE:	Sulfide (Colorimetric, Methylene Blue)
ANALYTE:	Sulfide
INSTRUMENTATION:	Spectrophotometer
STORET No.	Total 00745 Dissolved 00746

1.0 Scope and Application

1.1 This method is applicable to the measurement of total and dissolved sulfides in drinking, surface and saline waters, domestic and industrial wastes.
1.2 Acid insoluble sulfides are not measured by this method. Copper sulfide is the only common sulfide in this class.
1.3 The method is suitable for the measurement of sulfide in concentrations up to 20 mg/L.

2.0 Summary of Method

2.1 Sulfide reacts with dimethyl-p-phenylenediamine (p-aminodimethyl aniline) in the presence of ferric chloride to produce methylene blue, a dye which is measured at a wavelength maximum of 625 nm.

3.0 Comments

3.1 Samples must be taken with a minimum of aeration. Sulfide may be volatilized by aeration and any oxygen inadvertently added to the sample may convert the sulfide to an unmeasurable form. Dissolved oxygen should not be present in any water used to dilute standards.
3.2 The analysis must be started immediately.
3.3 Color and turbidity may interfere with observations of color or with photometric readings.

4. Apparatus

4.1 Matched test tubes, approximately 125 mm long and 15 mm O.D.
4.2 Droppers, delivering 20 drops/mL. To obtain uniform drops, hold dropper in vertical position and allow drops to form slowly.
4.3 Photometer, use either 4.3.1 or 4.3.2.
4.3.1 Spectrophotometer, for use at 625 nm with cells of 1 cm and 10 cm light path.
4.3.2 Filter photometer, with filter providing transmittance near 625\nm.

5.0 Reagents

5.1 Amino-sulfuric acid stock solution: Dissolve 27 g N,N-dimethyl-p-phenylenediamine oxalate (p-aminodimethylaniline) in a cold mixture of 50 mL conc. H_2SO_4 and 20 mL distilled water in a 100 mL volumetric flask. Cool and dilute to the mark. If dark discard and purchase fresh reagent. Store in dark glass bottle.

5.2 Amino-sulfuric acid reagent: Dissolve 25 mL amino-sulfuric acid stock solution (5.1) with 975 mL of 1 + 1 H_2SO_4 (5.4). Store in a dark glass bottle. This solution should be clear.

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

5.4 Sulfuric acid solution, H_2SO_4, 1 + 1

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

5.6 Methylene blue solution I: Dissolve 1.0 g of methylene blue in distilled water in a 1 liter volumetric flask and dilute to the mark. Use U.S.P. grade or one certified by the Biological Stain Commission. The dye content reported on the label should be 84% or more. Standardize (5.8) against sulfide solutions of known strength and adjust concentration so that 0.05 mL (1 drop) equals 1.0 mg/L sulfide.

5.7 Methylene blue solution II: Dilute 10.00 mL of adjusted methylene blue solution I (5.6) to 100 mL with distilled water in a volumetric flask.

5.8 Standardization of methylene blue I solution:

5.8.1 Place several grams of clean, washed crystals of sodium sulfide $Na_2S \cdot 9H_2O$ in a small beaker.

5.8.2 Add somewhat less than enough water to cover the crystals.

5.8.3 Stir occasionally for a few minutes. Pour the solution into another vessel. This reacts slowly with oxygen but the change is insignificant over a few hours. Make the solution daily.

5.8.4 To 1 liter of distilled water add 1 drop of solution and mix.

5.8.5 Immediately determine the sulfide concentration by the methylene blue procedure (6) and by the titrimetric iodide procedure (Method 376.1, this manual).

5.8.6 Repeat using more than one drop of sulfide solution or less water until at least five tests have been made in the range of 1 to 8 mg/L sulfide.

5.8.7 Calculate the average percent error of the methylene blue procedure (6) as compared to the titrimetric iodide procedure (Method 376.1).

5.8.8 Adjust by dilution or by adding more dye to methylene blue solution I (5.6).

6.0 Procedure

6.1 Color development

6.1.1 Transfer 7.5 mL of sample to each of two matched test tubes using a special wide tipped pipet or filling to a mark on the test tubes.

6.1.2 To tube A add 0.5 mL amine-sulfuric acid reagent (5.2) and 0.15 mL (3 drops) $FeCl_3$ solution (5.3).

6.1.3 Mix immediately by inverting the tube only once.

6.1.4 To tube B add 0.5 mL 1 + 1 H_2SO_4 (5.4) and 0.15 mL (3 drops) $FeCl_3$ solution (5.3) and mix.

6.1.5 Color will develop in tube A in the presence of sulfide. Color development is usually complete in about 1 minute, but a longer time is often required for the fading of the initial pink color.

6.1.6 Wait 3 to 5 minutes.

6.1.7 Add 1.6 mL $(NH_4)_2HPO_4$ solution (5.5) to each tube.

6.1.8 Wait 3 to 5 minutes and make color comparisons. If zinc acetate was used wait at least 10 minutes before making comparison.

6.2 Color comparison

6.2.1 Visual

6.2.1.1 Add methylene blue solution 1 (5.6) and/or II (5.7) (depending on sulfide concentration and accuracy desired) dropwise to tube B (6.1.4) until the color matches that developed in the first tube.

6.2.1.2 If the concentration exceeds 20 mg/L, repeat 6.2.1.1 using a portion of the sample diluted to one tenth.

6.2.2 Photometric

6.2.2.1 Use a 1 cm cell for 0.1 to 2.0 mg/L . Use a 10 cm cell for up to 20 mg/L.

6.2.2.2 Zero instrument with portion of sample from tube B (6.1.4).

6.2.2.3 Prepare calibration curve from data obtained in methylene blue standardization (5.8), plotting concentration obtained from titrimetric iodide procedure (Method 376.1) versus absorbance. A straight line relationship can be assumed from 0 to 1.0 mg/L.

6.2.2.4 Read the sulfide concentration from the calibration curve.

7.0 Calculations

7.1 Visual comparison: With methylene blue solution 1 (5.6), adjusted so that 0.05 mL (1 drop) = 1.0 mg/L sulfide and a 7.5 mL sample

mg/L sulfide = number drops methylene blue solution I (5.6) + 0.1x [number of drops methylene blue solution II (5.7)].

7.2 Photometric: see 6.2.2.4

8.0 Precision and Accuracy:

8.1 The precision has not been determined. The accuracy is about ± 10%.

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th edition, p. 503, Method 428C (1975).