UNITED STATES
DEPARTMENT OF THE INTERIOR
GEOLOGICAL SURVEY

SELECTED METHODS OF THE U.S. GEOLOGICAL SURVEY FOR THE ANALYSIS OF WASTEWATERS

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By M. J. Fishman and Eugene Brown

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SELECTED METHODS OF THE U.S. GEOLOGICAL SURVEY FOR THE ANALYSIS OF WASTEWATERS

By M. J. Fishman and Eugene Brown

ABSTRACT

New methods and revisions of selected procedures for the analysis of wastewater in use by laboratories of the U.S. Geological Survey are contained herein. Included are procedures for the determination of total coliform, aluminum, antimony, arsenic, boron, chloride, mercury, molybdenum, tin, vanadium, oil and grease, alpha and beta activity, and radium-226.

INTRODUCTION

Analytical methods approved for use in the laboratories of the U.S. Geological Survey are published in a series of publications entitled "Techniques of Water Resources Investigations of the U.S. Geological Survey" (see selected references). Available publications in this series now include the following:

Methods for collection and analysis of water samples for dissolved minerals and gases,
Determination of minor elements in water by emission spectroscopy,
Methods for analysis of organic substances in water,
Methods for collection and analysis of aquatic biological and microbiological samples,
Methods for analysis of radioactive substances in water and fluvial sediments,
Water temperature - influential factors, field measurement, and data presentation.

Changes in technology continually provide new and improved methods and instrumentation for the study of water-quality characteristics. These developments generally occur at a pace too rapid to permit formal publications to reflect all of the current status of methodology in use. This report makes available recent revisions of procedures contained in several of the above publications, as well as certain additional methods not included therein that are used in Geological Survey laboratories for the analysis of wastewaters. These methods will be included in the next revisions of the appropriate publications on techniques.

The test procedures described in this report are used for measuring both "dissolved" and "total" (dissolved plus suspended) phases of the sample. Each test procedure is identified with a method name that refers to the chief

analytical technique used, and a unique number to differentiate between similar methods for determining the same constituent. The water-quality characteristic and sample phases that can be routinely analyzed using each method are identified by parameter codes also. These numeric codes uniquely identify a water-quality characteristic in terms of its name, units of measurement, and sample phase (dissolved, total, suspended, or bottom material), and are used in processing and analytical data. Parameter codes used by the Geological Survey in its WATSTORE system are identical with those used by the Environmental Protection Agency in its STORET system.

Bacteriological determinations

Coliform bacteria, fecal

The standard procedure for determining fecal coliform concentrations in water is the "Membrane Filter Method" described in *Methods for the Collection and Analysis of Aquatic Biological and Microbiological Samples* (Slack and others, 1973, p. 45-50). Under those conditions where the membrane filter (MF) method cannot be used (for example, for chlorinated effluents or with large concentrations of suspended sediments), the "MPN Procedure" as described in Standard Methods (American Public Health Association and others, 1971, p. 669-672) is used.

Coliform bacteria, total

The standard procedure for determining total coliform concentrations in water is the "Membrane Filter Method" described in *Methods for the Collection and Analysis of Aquatic Biological and Microbiological Samples* (Slack and others, 1973, p. 35-39). Under those conditions where the membrane filter (MF) method cannot be used (for example, with large concentrations of suspended sediment), the MPN method is used.

The "presumptive test" produces results that are directly comparable with the multiple-tube fermentation technique described in Standard Methods (American Public Health Association and others, 1971, p. 662-678). The "Presumptive Field Test" is similar to the "Presumptive Test," except that provision is made for the incubation of samples under field conditions. Both tests are used within the Water Resources Division when circumstances warrant.

Two points should be noted. First, the results obtained from the MPN method are not directly comparable with results obtained with the MF method Second, the precision of the presumptive MPN projecture is poor; for example, when 5 aliquots of each dilution are incubated, the precision of results at the 95 percent confidence limit is 31 to 289 percent of the most probable number of coliform bacteria.

For more precise results, it is preferable to confirm the actual presence of total coliform bacteria determined in the presumptive tests. For positive confirmation, the procedure in *Methods for the Collection and Analysis of Aquatic Biological and Microbiological Samples* (Slack and others, 1973, TWRI, book 5, ch. A4, p. 44-45) starting with step #7.8 (p. 45) is used. Calculations are based upon the number of positive tubes determined in the confirmation test.

Total Coliform Bacteria
(most probable number--MPN) method
Presumptive Test

1. Application

1.1 This method is applicable to all types of fresh and saline waters. It is applicable to waters with large suspended solids content and waters with high counts of noncoliform bacteria.

2. Summary of method

2.1 Decimal dilutions of multiple sample aliquots are inoculated into lauryl tryptose broth. The cultures are incubated at 35°C and examined after 24 and 48 h for evidence of growth and gas production. The most-probable-number (MPN) of coliform organisms in the sample is determined from the distribution of gas-positive cultures among the inoculated tubes.

3. Interferences

3.1 Large concentrations of heavy metals or toxic chemicals may interfere when large volumes of sample are added to small volumes of concentrated tryptose broth.

4. Apparatus

All materials used in bacteriological testing must be free of agents that inhibit bacterial growth.

4.1 Water-sampling bottle

Samples for obtaining water samples under sterile conditions as marketed by General Oceanics, Inc., Hydro Products, Kahl Scientific Instrument Corp., and others. A metallic water sampler, lowered at a speed of 1 m/sec, may be effective for sterile collection of water samples (Kriss and others, 1966). Metallic water-sampling bottles are available from Wildlife Supply Co. (1050 or 1200); Kahl Scientific Instrument Corp. (130WA100); InterOcean Systems, Inc. (206); Foerst Mechanical Specialisities Co. (Improved Water Sampler, Kemmerer-type);

4.2 Durham (fermentation) tubes and serum vials

Serum vials of any type may be used. The size of the vial and durham tube, used for detection of gas production, should be such that the tube is completely filled with medium and at least partly submerged in the vial. The specific choice of fermentation tubes and serum vials depends on the volume of water to be tested and whether the test is to be run in the laboratory or in the field.

The following combinations have been found to be satisfactory for the stated use.

4.2a For testing 50 ml aliquots, use screw cap milk dilution bottles, APHA, Pyrex or Kimax scrum vials; use flint glass culture tubes, 10 x 75 mm, Kimble (73500) or equivalent, as inner, inverted fermentation tubes.

4.2b For laboratory testing of 10 ml or smaller aliquots, use culture tubes, flint glass, 20 x 150 mm, Kimble (73500) or equivalent, and culture tubes, flint glass 10 x 75 mm, Kimble (73500 or equivalent, and test tube caps, 20 mm, Scientific Products (T13990-20) or equivalent.

4.3 Culture-tube rack, galvanized for 20 mm tubes, Thomas-Kolmer or equivalent.

4.4 Incubator with temperature range from 5°C above ambient to 60°C with the capability of maintaining a uniform and constant temperature within 35°C ± 0.5°C. National Appliance (320) or equivalent is satisfactory for laboratory use.

4.5 Sterlizer, stream autoclave, Matheson Scientific (59827), or Market Forge Sterilmatic or equivalent.

4.6 Bottles, milk dilution, APHA, Pyrex or Kimax with screwcaps.

4.7 Pipets, 1.0-ml capacity, presterilized, disposable, glass or plastic with cotton plugs, Millipore (XXX63 001 35) or equivalent, or sterile, disposable, 2.5 ml hypodermic syringes, Becton Dickinson (5610) or equivalent.

4.8 Pipets, 11.0-ml capacity, Corning (7057) or equivalent. Wrap the pipets in kraft paper and sterilize in the autoclave, or place in a pipet box, Matheson Scientific (3593020) or equivalent, and heat in an oven at 170°C for 2 h.

5. Reagents

5.1 Lauryl Tryptose Broth

Difco Bacto Lauryl Tryptose Broth (0241), or BBL Lauryl Sulfate Broth (11338) or equivalent. Prepare according to American Public Health Association and others (1971, p. 651) or according to directions on bottle label.

Place 50 ml of medium containing 71.2 g/l of Difco Bacto Lauryl Tryptose Broth or BBL Lauryl Sulfate Broth in a milk dilution bottle for each 50 ml aliquot of sample to be tested.

Place 10 ml of medium containing 71.2 g/l of Difco Bacto Lauryl Tryptose Broth or BBL Lauryl Sulfate Broth in a 20 x 150 mm culture tube for each 10 ml aliquot of sample to be tested.

Place 10 ml of medium containing 35.6 g/l of Difco Bacto Lauryl Tryptose Broth or BBL Lauryl Sulfate Broth in 20 x 150 ml culture tube for each 1 ml or smaller aliquot of sample to be tested.

In each milk dilution bottle or culture tube, place one 10 x 75 mm durham tube (fig. 1) mouth downward (inverted). Sterilize in upright position at

121°C at 15 psi for 15 min. Air will be expelled from the inverted, inner durham tube during heating; each will fill completely with medium during cooling. Before using, check to see that there are no air bubbles in the inverted durham tubes.

5.2 Buffered dilution water

Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 ml distilled water. Adjust to pH 7.2 with 1 N sodium hydroxide (NaOH). Dilute to 1 litre with distilled water. Sterilize in dilution bottles for 20 min at 121°C at 15 psi. After opening a bottle of stock solution, refrigerate the unused part. Discard contaminated solutions, indicated by slight turbidity of precipitate.

Add 1.2 ml of this stock phosphate buffer solution to 1 litre of distilled water. Dispense in milk dilution bottles in amounts that will provide 99 ml $\pm$ 2.0 after autoclaving at 121°C at 15 psi for 20-30 min. Loosen caps or stoppers prior to sterilizing and tighten when bottles have cooled.

6. Procedure

Samples for bacteriologic examination must be collected in bottles that have been carefully cleaned and autoclaved for 20 min at 121°C at 15 psi. Sterilized milk dilution bottles are ideal sample containers. When the sample is collected, ample airspace must be left in the bottle to facilitate mixing of the sample by shaking. Care must be taken to avoid contamination of the sample and sample bottle at the time of collection and in the period prior to analysis.

To insure maximum correlation of results, the sample sites and methods used for bacteria should correspond as closely as possible to those selected for chemical and plankton sampling. However, sampling for bacteria at depth is complicated by the requirement to avoid contamination of the deeper water layers by bacteria carried from shallower depths on the inner walls of the sampler.

The sample collection method will be determined by the study objectives. In lakes, reservoirs, deep rivers, and estuaries, bacterial abundance may vary transversely, with depth, and with time of day. To collect a surface sample from a stream or lake, open a sterile milk dilution bottle, grasp it near its base, and plunge it, neck downward, below the water surface. Allow the bottle to fill by slowly turning the bottle until the neck points slightly upward. The mouth of the bottle must be directed into the current. If there is no current, as in the case of a lake, a current should be artificially created by pushing the bottle horizontally forward in a direction away from the hand (American Public Health Association and others, 1971, p. 658).

To collect a sample representative of the bacterial concentration at a particular depth, use one of the water-sampling bottles discussed in 4.1 above. For small streams, a point sample at a single transverse position located at the centroid of flow is adequate (Goerlitz and Brown, 1972).

As soon as possible after collection, preferably within 1 h and not more than 6 h, inoculate the decimal dilutions of the sample into the lauryl

tryptose broth serum vials. Samples must be kept cool during the time between collection and inoculation. If inoculation is delayed, ice or refrigerate the sample but do not freeze.

The volumes of decimal dilutions should be such that, after incubation, both positive and negative results are obtained among the range of volumes. The method fails if only positive or only negative results are obtained with all volumes tested.

The following sample volumes are suggested:

1. Unpolluted raw surface water: 0.1- 1.0- and 10.0- and 50.0-ml samples will cover a MPN range of < 1 to ≥ 240 coliform organism per 100 ml.

2. Polluted raw surface water: 0.0001-, 0.001-,0.1- and 1.0-ml will cover a MPN range of 20 to 2.4×10^6 coliform organisms per 100 ml.

6.1 Set up 5 vials of lauryl tryptose broth for each sample volume to be tested.

6.2 If 0.1 ml or more is to be inoculated, transfer the measured samples directly to the serum vials using sterile pipets or presterilized disposable hypodermic syringes.

If the volume of original water sample is less than 0.1 ml, proceed as above after preparing appropriate dilutions by adding the sample to a sterile milk dilution bottle in the following amounts:

Dilution	Volume of sample added to 99 ml dilution bottle	Size of inoculum
1:10	11.0 ml original sample	11.0 ml of 1:10 dilution
1:100	1.0 ml original sample	1.0 ml of 1:100 dilution
1:1,000	1.0 ml of 1:10 dilution	1.0 ml of 1:1,000 dilution
1:10,000	1.0 ml of 1:100 dilution	1.0 ml of 1:10,000 dilution

Note: Use a sterile pipet or hypodermic syringe for each vial. After each transfer between bottles, close and shake the bottle vigorously 25 times. Diluted samples should be inoculated within 20 min after preparation.

6.3 Clearly mark each set of serum vials indicating location, time of collection, sample number, and sample volume. Code each vial for easy identification.

6.4 Place the inoculated vials in the test-tube or other appropriate rack and incubate at $35^{\circ} \pm 1^{\circ}C$ for 24 h $\pm$ 2. Culture vials must be maintained in an upright position.

6.5 Remove vials from incubator and examine. Gas in any amount in the durham tube, oven a pinhead size bubble, constitutes a positive test (fig. 2).

The appearance of an air bubble must not be confused with actual gas production. The broth medium will become cloudy with actual fermentation and small bubbles of gas may appear in the medium outside the durham tube when the serum vial is shaken gently (American Public Health Association and others, 1971, p. 665).

6.6 Autoclave all gas-positive vials for 15 min at 121°C at 15 psi before discarding.

6.7 Replace all gas-negative vials in incubator and incubate for an additional 24 h ± 2 at 35° ± 1°C.

6.8 Remove vials from incubator and examine for gas formation.

Autoclave all remaining vials of lauryl sulfate tryptose broth as in 7.6 before discarding.

7. Calculations

7.1 Record the number of gas-positive vials occurring over all sample volumes tested. When more than three volumes are tested, the results from only three of these are used in computing the MPN. To select the three dilutions for the MPN index, proceed as follows: Take as the first member the smallest sample volume in which all tests are positive (no larger sample volume giving any negative results) and the two next succeeding smaller sample volumes (American Public Health Association and others, 1971 p. 673-674).

In the examples given below, the number in the numerator represents positive tubes; the denominator represents the total number of tubes inoculated:

Example	Decimal Dilutions 1 ml	0.1 ml	0.01 ml	0.001 ml	Combination of positives
a	5/5	5/5	2/5	0/5	5-2-0
b	5/5	4/5	2/5	0/5	5-4-2
c	0/5	1/5	0/5	0/5	0-1-0
d	5/5	3/5	1/5	1/5	5-3-2
e	5/5	3/5	2/5	0/5	

In example c, the first three dilutions should be taken to place the positive results in the middle dilution. When a positive occurs in a dilution higher than the three chosen according to the guideline, as in d, it should be placed in the result for the highest chosen dilution as in e.

A table giving MPN for various combinations of positive and negative results when five 10 ml dilutions, five 1.0 ml, and five 0.1 ml dilutions are tested is shown in table 1. If a series of decimal dilutions other than 10.0-, 1.0- and 0.1-ml is used, record the MPN as the value from the table multiplied by a factor of 10 divided by the volume in which all tests were positive. MPN tables for other combinations of sample volumes and number of tubes at each level of inoculation are given by the American Public Health Association and others (1971, p. 673-676).

8. Report

8.1 The coliform concentration is reported as MPN coliforms per 100 ml. Values less than 10, report whole numbers; 10 or more, report two significant figures.

9. Precision

9.1 Unless large numbers of sample aliquots are used for each decimal dilution, the precision of the MPN procedure is poor. When 5 vials of each decimal dilution are incubated, the precision of results at the 95 percent confidence limit is 31 to 289 percent of the MPN; the precision at the 50 percent confidence limit is 64 to 139 percent of the MPN.

References

American Public Health Association and others, 1971, Standard methods for the examination of water and wastewater (13th ed.): New York, Am. Public Health Assoc., 874 p.

Goerlitz, D. F., and Brown, Eugene, 1972, Methods for the investigation of organic substances in water: U.S. Geol. Survey Techniques Water-Resources Inv., book 5, ch. A3, 40 p.

Kriss, A. E., Lebedeva, M. N., and Tsiban, A. V., 1966, Comparative estimate of a Nansen and microbiological water bottle for sterile collection of water samples from depths of seas and oceans: Deep-Sea Research, v. 13, p. 205-212.

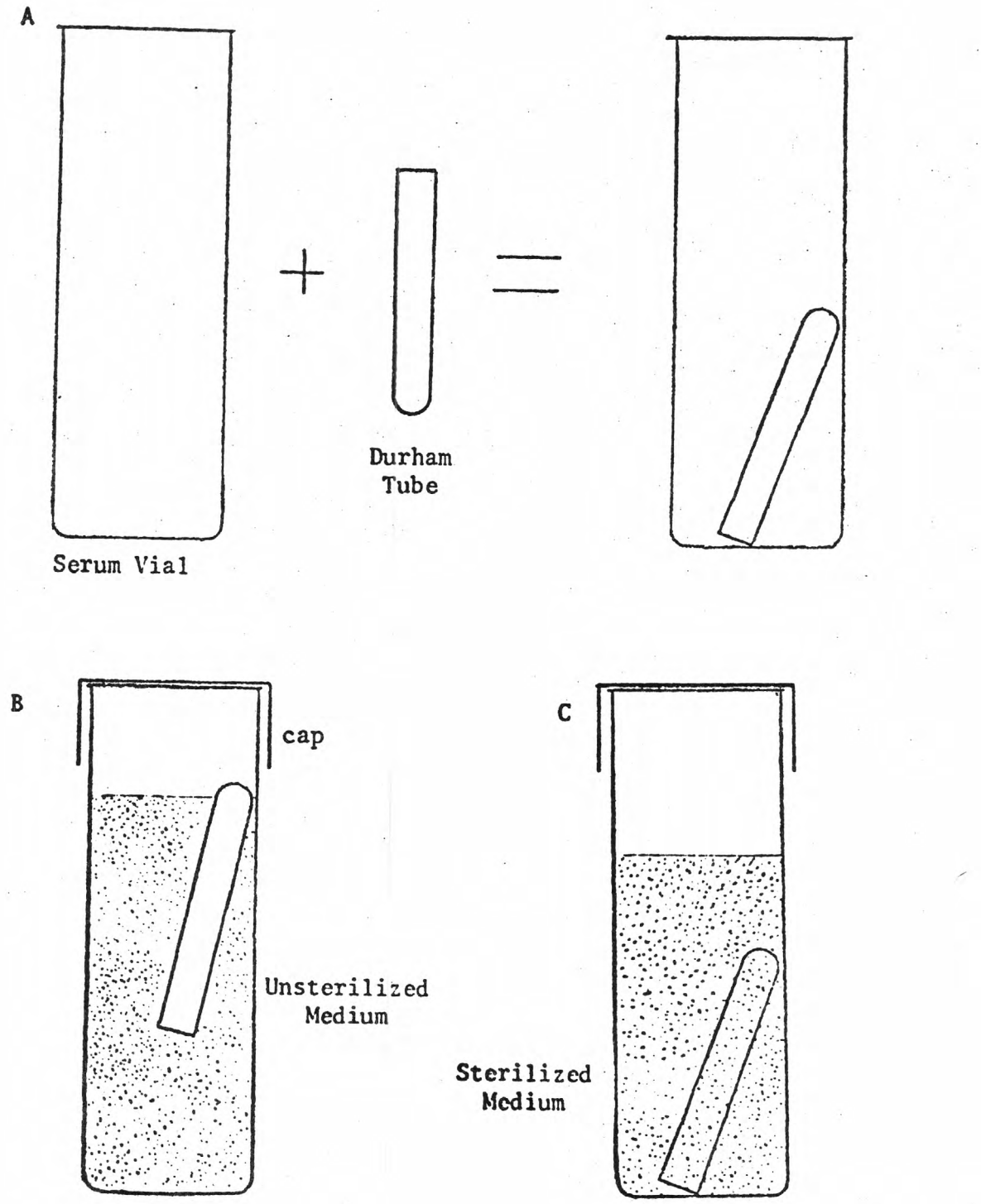

Figure 1.--Preparation of culture tube (step 5.1). A. Invert durham tube inside serum vial. B. Add unsterilized medium and cap. C. Durham tube fills with medium following sterilization.

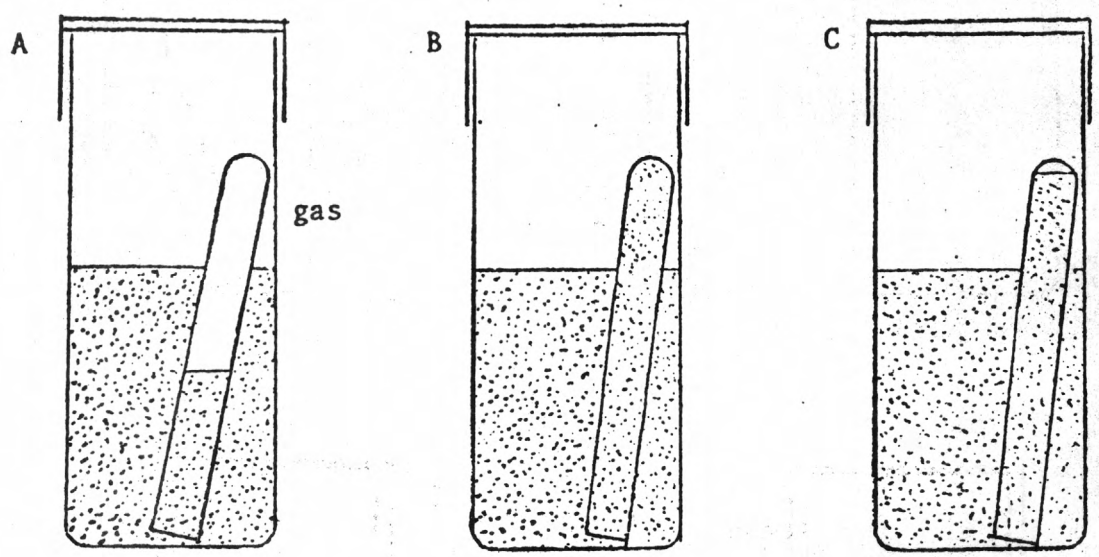

Figure 2.--Examination for gas formation (steps 7.5 and 7.8). A. Positive B. Negative C. Doubtful.

Table 1.--MPN index and 95 percent confidence limits for various combinations of positive and negative results when five 10-ml, five 1-ml, and five 0.1-ml dilutions are used. (American Public Health Association and others, 1971, p. 673).

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence limits		No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence limits	
5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower	Upper	5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower	Upper
0	0	0	<2			4	2	1	26	9	78
0	0	1	2	<0.5	7	4	3	0	27	9	80
0	1	0	2	<0.5	7	4	3	1	33	11	93
0	2	0	4	<0.5	11	4	4	0	34	12	93
1	0	0	2	<0.5	7	5	0	0	23	7	70
1	0	1	4	<0.5	11	5	0	1	31	11	89
1	1	0	4	<0.5	11	5	0	2	43	15	110
1	1	1	6	<0.5	15	5	1	0	33	11	93
1	2	0	6	<0.5	15	5	1	1	46	16	120
						5	1	2	63	21	150
2	0	0	5	<0.5	13						
2	0	1	7	1	17	5	2	0	49	17	130
2	1	0	7	1	17	5	2	1	70	23	170
2	1	1	9	2	21	5	2	2	94	28	220
2	2	0	9	2	21	5	3	0	79	25	190
2	3	0	12	3	28	5	3	1	110	31	250
						5	3	2	140	37	340
3	0	0	8	1	19						
3	0	1	11	2	25	5	3	3	180	44	500
3	1	0	11	2	25	5	4	0	130	35	300
3	1	1	14	4	34	5	4	1	170	43	490
3	2	0	14	4	34	5	4	2	220	57	700
3	2	1	17	5	46	5	4	3	280	90	850
3	3	0	17	5	46	5	4	4	350	120	1,000
4	0	0	13	3	31	5	5	0	240	68	750
4	0	1	17	5	46	5	5	1	350	120	1,000
4	1	0	17	5	46	5	5	2	540	180	1,400
4	1	1	21	7	63	5	5	3	920	300	3,200
4	1	2	26	9	78	5	5	4	1600	640	5,800
4	2	0	22	7	67	5	5	5	>2400		

Total Coliform Bacteria
(most probable number--MPN) method
Presumptive Field Test

1. Application

1.1 This method is applicable to all types of fresh and saline waters. It is applicable to waters with large suspended solids content and waters with high counts of noncoliform bacteria. It is suitable for application at the sample site to eliminate sample transport and storage.

2. Summary of method

2.1 Decimal dilutions of multiple sample aliquots are inoculated into lauryl tryptose broth. The cultures are inculated at 35°C and examined after 24 and 48 h for evidence of growth and gas production. The most-probable-number (MPN) of coliform organisms in the sample is determined from the distribution of gas-positive cultures among the inoculated tubes. The method described herein is similar to the total coliform MPN method (presumptive test), except that provision is made for the incubation of samples under field conditions.

3. Interferences

3.1 Large concentrations of heavy metals or toxic chemicals may interfere when large volumes of sample are added to small volumes of concentrated tryptose broth.

4. Apparatus

All materials used in bacteriological testing must be free of agents that inhibit bacterial growth.

4.1 Water-sampling bottle

Samplers for obtaining water samples under sterile conditions as marketed by General Oceanics, Inc., Hydro Products, Kahl Scientific Instrument Corp., and others. A metallic water sampler, lowered at a speed of 1 m/sec, may be effective for sterile collection of water samples (Kriss and others, 1966). Metallic water-sampling bottles are available from Wildlife Supply Co. (1050 or 1200); Kahl Scientific Instrument Corp. (130WA100); InterOcean Systems, Inc. (206); Foerst Mechanical Specialities Co. (Improved Water Sampler, Kemmerer-type); or equivalent.

4.2 Durham (fermentation) tubes and serum vials

4.2a For testing 50 or 100 ml aliquots, use milk dilution bottles, APHA, Pyrex or Kimax with screwcaps as serum vials; use flint glass culture tubes, 10 x 75 mm, Kimble (73500) or equivalent as fermentation tubes.

4.2b Serum bottles, 10 ml capacity, Wheaton (223739) or equivalent.

4.2c Rubber stoppers, 13 x 20 mm, Wheaton (224183) or equivalent.

4.2d Aluminum seals, one piece, 20 mm Wheaton (224183) or equivalent,

4.2e Fermentation tubes, 25 x 16 mm test tubes, Thomas (9185-R12) or equivalent.

4.2f Crimper, for attaching aluminum seals, Wheaton (224303) or equivalent.

4.2g Decapper, for removing aluminum seals from spent tubes, Wheaton (224183) or equivalent.

4.3 Incubator with temperature range from 5°C above ambient to 60°C. A portable incubator as provided in the Portable Water Laboratory, Millipore (XX63 001 50), or equivalent, which operates on either 110 volts d.c. or 12 volts d.c., is convenient for field use. A larger incubator with a more precise temperature regulation, National Appliance (320) or equivalent, is satisfactory for laboratory use.

4.4 Sterilizer, stream autoclave, Matheson Scientific (59827-20) or Market Forge Sterilmatic, or equivalent.

4.5 Bottles, milk dilution, APHA, Pyrex or Kimax with screwcaps.

4.6 Hypodermic syringes, 2.5 ml, Becton Dickinson (5610) or equivalent.

4.7 Pipets, 1.0-ml capacity, presterilized, disposable, glass or plastic with cotton plugs, Millipore (XX63 001 35) or equivalent.

4.8 Pipets, 11.0-ml capacity, Corning (7057) or equivalent. Wrap the pipets in kraft paper and sterilize in the autoclave, or place in a pipet box, Matheson Scientific (55930-20) or equivalent, and heat in an oven at 170°C for 2 h.

5. Reagents

5.1 Lauryl tryptose broth

Difco Bacto Lauryl Tryptose Broth (0241) or BBL Lauryl Sulfate Broth (11338), or equivalent. Prepare according to American Public Health Association and others (1971, p. 651) or according to directions on bottle label.

Place 50 ml of medium containing 71.2 g/l of Difco Bacto Lauryl Tryptose Broth or BBL Lauryl Sulfate Broth in a milk dilution bottle for each 50 ml aliquot of water to be tested.

Place 9 ml of medium containing 35.6 g/l of Difco Bacto Lauryl Tryptose Broth or BBL Lauryl Sulfate Broth in each 10 ml serum bottle for each 1 ml or smaller aliquot of sample to be tested.

Place one fermentation tube, mouth downward (inverted) in each bottle of broth. Place screwcaps on milk dilution bottles. Loosen caps prior to sterilizing and tighten when bottles have cooled.

Place one fermentation tube, mouth downward (inverted), in each serum bottle. Place rubber stopper in mouth and attach aluminum seal using crimper.

Sterilize in upright position at 121°C at 15 psi for 15 min. Air will be expelled from the inverted, inner durham tube during heating; each will fill completely with medium during cooling. Before using, check to see that there are no bubbles in the inverted durham tubes.

5.2 Buffered dilution water: Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 ml distilled water. Adjust to pH 7.2 with 1 N sodium hydroxide (NaOH). Dilute to 1 litre with distilled water. Sterilize in dilution bottles for 20 min at 121°C at 15 psi. After opening a bottle of stock solution, refrigerate the unused part. Discard contaminated solution, indicated by slight turbidity or precipitate.

Add 1.2 ml of this stock phosphate buffer solution to 1 litre of distilled water. Dispense in milk dilution bottle in amounts that will provide 99 ml ± 2.0 after autoclaving at 121°C at 15 psi for 20 to 30 min. Loosen caps or stoppers prior to sterilizing, and tighten when bottles have cooled.

6. Procedure

Samples for bacteriological examination must be collected in bottles that have been carefully cleaned and autoclaved for 20 min at 121°C at 15 psi. Sterilized milk dilution bottles are ideal sample containers. When the sample is collected, ample airspace must be left in the bottle to facilitate mixing of the sample by shaking. Care must be taken to avoid contamination of the sample and sample bottle at the time of collection and in the period prior to analysis.

To insure maximum correlation of results, the sample sites and methods used for bacteria should correspond as closely as possible to those selected for chemical and plankton sampling. However, sampling for bacteria at depth is complicated by the requirement to avoid contamination of the deeper water layers by bacteria carried from shallower depths on the inner walls of the sampler.

The collection method will be determined by the study objectives. In lakes, reservoirs, deep rivers, estuaries, bacterial abundance may vary transversely, with depth, and with time of day. To collect a surface sample from a stream or lake, open a sterile milk dilution bottle, grasp it near its base, and plunge it, neck downward, below the water surface. Allow the bottle to fill by slowly turning the bottle until the neck points slightly upward. The mouth of the bottle must be directed into the current. If there is no current, as in the case of a lake, a current should be artificially created by pushing the bottle horizontally forward in a direction away from the hand (American Public Health Association and others, 1971, p. 658).

To collect a sample representative of the bacterial concentration at a particular depth, use one of the water-sampling bottles discussed in 4.1 above. For small streams, a point sample at a single transverse position located at the centroid of flow is adequate (Gorelitz and Brown, 1972).

As soon as possible after collection, preferably within 1 h and not more than 6 h, inoculate the decimal dilutions of the sample into the lauryl tryptose broth serum vials. Samples must be kept cool during the time between collection and inoculation. If inoculation is delayed, ice or refrigerate the sample but do not freeze.

The volumes of decimal dilutions should be such that, after incubation, both positive and negative results are obtained among the range of volumes. The method fails if only positive or only negative results are obtained with all volumes tested.

The following sample volumes are suggested:

1. Unpolluted raw surface waters: 0.1, 1.0, 10.0, and 50.0-ml samples will cover a MPN range of < 1 to ≥ 240 coliform organism per 100 ml.

2. Polluted raw surface water: 0.0001-, 0.001-, 0.01-, and 1.0- ml will cover a MPN range of 20 to 2.4×10^6 coliform organisms per 100 ml.

6.1 Set out 5 vials of lauryl tryptose broth for each volume to be tested.

6.2 If the volume to be tested is 0.1 ml or more, transfer the measured samples directly to the serum vials using either sterile pipets, presterilized disposable hypodermic syringes, or other sterile measuring devise such as a graduated cylinder.

If the volume of original water sample is less than 0.1 ml, proceed as above after preparing appropriate dilutions by adding the sample to a sterile milk dilution bottle in the following amounts:

Dilution	Vollume of sample added to 99 ml dilution bottle	Size of inoculum
1:10	11.0 ml original sample	11.0 ml of 1:10 dilution
1:100	1.0 ml original sample	1.0 ml of 1:100 dilution
1:1,000	1.0 ml of 1:10 dilution	1.0 ml of 1:1,000 dilution
1:10,000	1.0 ml of 1:100 dilution	1.0 ml of 1:10,000 dilution

Note: Use a sterile pipet or hypodermic syringe for each vial. After each transfer between bottles, close and shake the bottle vigorously 25 times. Diluted samples should be inoculated within 20 min after preparation.

6.3 When using serum bottles with rubber stoppers, proceed as follows: Remove the inserts from the metal caps and swab the exposed area of the rubber septum with a bit of cotton saturated with 70 percent ethanol or isopropanol.

Carefully invert serum bottles so that the rubber septum is at the bottom. Inoculate the medium with a sterile hypodermic needle by carefully puncturing the septum with the needle and inserting the needle only until the beveled tip is inside the bottle. Discharge the contents of the syringe into the bottle and withdraw the syringe. Agitate the bottle gently to mix the contents.

Carefully return bottle to normal, upright position with stopper at top. Make sure that inverted vial is completely filled with medium and no residual bubbles remain in the vial.

6.4 Clearly mark each set of serum vials indicating location, time of collection, sample number, and sample volume. Code each vial for easy identification.

6.5 Place the inoculated vials in the incubator and incubate at $35^{\circ} \pm 1^{\circ}C$ for 24 h $\pm$ 2. Culture vials must be maintained in an upright position.

6.6 Remove vials from incubator and examine. Gas in any amount in the inverted vial, even a pinhead size bubble, constitutes a positive test. The appearance of an air bubble must not be confused with actual gas production. The broth medium will become cloudy with actual fermentation and small bubbles of gas may appear in the medium outside the durham tube when serum vial is shaken gently (American Public Health Association and others, 1971, p. 665).

6.7 Autoclave all gas-positive vials for 15 min at $121^{\circ}C$ at 15 psi before discarding.

6.8 Replace all gas-negative vials in incubator and incubate for an additional 24 h $\pm$ 2 at $35^{\circ} \pm 1^{\circ}C$.

6.9 Remove vials from incubator and examine for gas formation. Autoclave all vials of lauryl sulfate tryptose broth as in 7.7 before discarding.

7. Calculations

7.1 Record the number of gas-positive vials occurring over all sample volumes tested. When more than three volumes are tested, the results from only three of these are used in computing the MPN. To select the three dilutions for the MPN index, proceed as follows: Take as the first member the smallest sample volume in which all tests are positive (no larger sample volume giving any negative results) and the two next succeeding smaller sample volumes (American Public Health Association and others, 1971, p. 673-674).

In the examples given below, the number in the numerator represents positive tubes; the denominator represents the total number of tubes inoculated:

Example	Decimal Dilutions 1 ml	0.1 ml	0.01 ml	0.001 ml	Combination of positives
a	5/5	5/5	2/5	0/5	5-2-0
b	5/5	4/5	2/5	0/5	5-4-2
c	0/5	1/5	0/5	0/5	0-1-0
d	5/5	3/5	1/5	1/5	5-3-2
e	5/5	3/5	2/5	0/5	

In example c, the first three dilutions should be taken to place the positive results in the middle dilution. When a positive occurs in a dilution higher than the three chosen according to the guideline, as in d, it should be placed in the result for the highest chosen dilution as in e.

A table giving MPN for various combinations of positive and negative results when five 10 ml, five 1.0 ml, and five 0.1 ml dilutions are tested is shown in table 1. If a series of decimal dilutions other than 10.0-, 1.0- and 0.1-ml is used record the MPN as the value from the table multiplied by a factor of 10 divided by the volume in which all tests were positive. MPN tables for other combinations of sample volumes and number of tubes at each level of inoculation are given by the American Public Health Association and others (1971, p. 673-676).

8. Report

8.1 The coliform concentration is reported as MPN coliforms per 100 ml. Values less than 10, report whole numbers; 10 or more, report two significant figures.

9. Precision

9.1 Unless large numbers of sample aliquots are used for each decimal dilution, the precision of the MPN procedure is poor. When 5 vials of each decimal dilution are incubated, the precision of results at the 95 percent confidence limit is 31 to 289 percent of the MPN; the precision at the 50 percent confidence limit is 64 to 139 percent of the MPN.

References

American Public Health Association and others, 1971, Standard methods for the examination of water and wastewater (13th ed.): New York, Am. Public Health Assoc., 874 p.

Goerlitz, D. F., and Brown, Eugene, 1972, Methods for the investigation of organic substances in water: U.S. Geol. Survey Techniques Water-Resources Inv., book 5, ch. A3, 40 p.

Kriss, A. E., Lebedeva, M. N., and Tsiban, A. V., 1966, Comparative estimate of a Nansen and microbiological water bottle for sterile collection of water samples from depths of seas and oceans: Deep-Sea Research, v. 13, p. 205-212.

Table 1.--MPN index and 95 percent confidence limits for various combinations of positive and negative results when five 10-ml, five 1-ml, and five 0.1-ml dilutions are used. (American Public Health Association and others, 1971, p. 673).

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence limits		No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence limits	
5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower	Upper	5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower	Upper
0	0	0	<2			4	2	1	26	9	78
0	0	1	2	<0.5	7	4	3	0	27	9	80
0	1	0	2	<0.5	7	4	3	1	33	11	93
0	2	0	4	<0.5	11	4	4	0	34	12	93
1	0	0	2	<0.5	7	5	0	0	23	7	70
1	0	1	4	<0.5	11	5	0	1	31	11	89
1	1	0	4	<0.5	11	5	0	2	43	15	110
1	1	1	6	<0.5	15	5	1	0	33	11	93
1	2	0	6	<0.5	15	5	1	1	46	16	120
						5	1	2	63	21	150
2	0	0	5	<0.5	13						
2	0	1	7	1	17	5	2	0	49	17	130
2	1	0	7	1	17	5	2	1	70	23	170
2	1	1	9	2	21	5	2	2	94	28	220
2	2	0	9	2	21	5	3	0	79	25	190
2	3	0	12	3	28	5	3	1	110	31	250
						5	3	2	140	37	340
3	0	0	8	1	19						
3	0	1	11	2	25	5	3	3	180	44	500
3	1	0	11	2	25	5	4	0	130	35	300
3	1	1	14	4	34	5	4	1	170	43	490
3	2	0	14	4	34	5	4	2	220	57	700
3	2	1	17	5	46	5	4	3	280	90	850
3	3	0	17	5	46	5	4	4	350	120	1,000
4	0	0	13	3	31	5	5	0	240	68	750
4	0	1	17	5	46	5	5	1	350	120	1,000
4	1	0	17	5	46	5	5	2	540	180	1,400
4	1	1	21	7	63	5	5	3	920	300	3,200
4	1	2	26	9	78	5	5	4	1600	640	5,800
4	2	0	22	7	67	5	5	5	≥ 2400		

Inorganic constituents

Aluminum

Atomic absorption spectrophotometric method--direct (I-1051-75)

Parameters and Codes:

Aluminum, dissolved (µg/1):	01106
Aluminum, total (µg/1):	01105
Aluminum, suspended (µg/1):	01107
Aluminum, total in bottom material (µg/g):	01108

1. Application

1.1 This method may be used to analyze waters, water-suspended sediment mixtures, suspended sediments, and bottom materials containing at least 100 µg of aluminum per litre. Samples containing more than 5,000 µg/1 must be diluted or less scale expansion used. Samples containing less than 100 µg/1, and brines must be analyzed by Method 1052 providing that the interferences discussed in that method are not exceeded.

1.2 Water-suspended sediment mixtures, suspended sediments, and bottom materials may be analyzed by this procedure after preliminary digestion-solubilization by Methods 3485 and 5485, respectively.

2. Summary of method

2.1 Aluminum is determined by atomic absorption spectrophotometry by direct aspiration of the sample into a nitrous oxide-acetylene flame without preconcentration or pretreatment of the sample other than the addition of sodium chloride to control ionization of aluminum and bis (2-ethoxyethyl)ether to enhance the sensitivity (Ramakrishna and others, 1967).

3. Interferences

3.1 Aluminum ionizes slightly in the nitrous oxide-acetylene flame; to control this effect approximately 850 mg of sodium per litre is added to each standard and sample.

3.2 Individual concentrations of sodium (9,000 mg/1), potassium (9,000 mgl), calcium (4,000 mg/1), magnesium (4,000 mg/1), sulfate (9,000 mg/1), chloride (9,000 mg/1), nitrate (9,000 mg/1), and iron (9,000 mg/1) do not interfere. Higher concentration of each constituent were not investigated.

4. Apparatus

4.1 Atomic absorption spectrophotometer

4.2 Refer to the manufacturer's manuals or procedures to optimize output of the instruments for the following parameters.

Aluminum

Grating. . . . . .Ultraviolet

Wavelength . . . . . .309.3 nm

Source (hollow-cathode lamp) .Aluminum

Burner . . . . . .nitrous oxide

Oxidant. . . . . .nitrous oxide

Fuel . . . . . .acetylene

Type of flame. . . . . .fuel rich

5. Reagents

5.1 Aluminum standard solution I, 1.00 ml = 100 µg Al: Dissolve 1.758 g $AlK(SO_4)_2.12H_2O$ in demineralized water, add 1 ml concentrated HNO_3 (sp gr 1.41), dilute to 1,000 ml with demineralized water.

5.2 Aluminum standard working solutions: Prepare a series of standard working solutions containing from 0 to 5,000 µg/l of aluminum by appropriate dilution of aluminum standard solution I. To each standard working solution, add 1.0 ml sodium chloride solution and 1.0 ml bis(2-ethoxyethyl)ether for each 10 ml of standard solution.

5.3 Bix(2-ethoxyethyl)ether: Eastman Kodak Co. Chemical No. 4738, or equivalent.

5.4 Sodium chloride solution, 25.4 g/l: Dissolve 25.4 g NaCl in demineralized water and dilute to 1 litre.

6. Procedure

Samples should be prepared according to instructions in Part III, Section 1, "Sample Preparation".

6.1 Add 1.0 ml NaCl solution and 1.0 ml bis(2-ethoxyethyl)ether to 10.0 ml of sample and mix thoroughly.

6.2 While aspirating the blank use auto zero to set instrument digital display to read 0. While aspirating standards use auto concentration to set instrument digital display to read concentrations of standards. Use at least six standards. Calibrate the instrument each time it is set up in operation and check for stability at reasonable intervals.

7. Calculations

7.1 Determine the µg/l aluminum in each sample from the digital display or printer output while aspirating each sample. Dilute samples that exceed the working range and multiply by the proper dilution factors.

7.2 To determine µg/g of aluminum in bottom material samples, first determine the µg/l aluminum in each sample as in 7.1, then:

$$\text{Al in } \mu g/g = \frac{\mu g/l \text{ Al} \times \frac{\text{ml of original digest}}{100}}{\text{wt of sample in grams}}$$

8. Report

8.1 Report aluminum, dissolved (01106), concentrations as follows: Less than 10,000 µg/l, nearest 100 µg/l, 10,000 µg/l and above, two significant figures.

8.2 Report aluminum, total (01105), concentration as follows: See 8.1.

8.3 Report aluminum, suspended (01107), concentration as follows: See 8.1.

8.4 Report aluminum total in bottom material (01108), concentration as follows:

9. Precision

9.1 The precision of this method within the range of approximately 100 to 1300 µg/l may be expressed as follows:

$$S_T = 0.192X + 122.6$$

where

S_T = overall precision, µg/l, and

X = concentration of aluminum, µg/l

References

Ramakrishna, T. V., West, P. W., and Robinson, J. W., 1967, The determination of aluminum and berryllium by atomic absorption spectroscopy: Anal. Chim. Acta, v. 39, p. 81-87.

Atomic absorption spectrophotometric method--chelation-extraction (I-1052-75)

Parameters and Codes:		
	Aluminum, dissolved (µg/1):	01106
	Aluminum, total (µg/1):	01105
	Aluminum, suspended (µg/1):	01107
	Aluminum, total in bottom material (µg/g):	01108

1. Application

1.1 This method may be used to analyze waters and brines containing from 10 to 300 µg of aluminum per litre. Water samples containing more than 300 µg/1 may be either diluted prior to chelation-extraction or analyzed by Method 1051. (NOTE 1)

Note 1: It has been reported that samples up to 1000 µg/1 can be analyzed without dilution.

1.2 Water-suspended sediment mixtures, suspended sediments, and bottom materials may be analyzed by this procedure after preliminary digestion-solubilization by Methods 3485 and 5485, respectively.

1.3 If the iron concentration of the sample exceeds 10 mg/1, determine aluminum by direct atomic absorption (Method 1051).

2. Summary of method

2.1 Aluminum is determined by atomic absorption spectrophotometry following chelation with 8-hydroxyquinoline and extraction with methyl isobutyl ketone (MIBK). The extract is aspirated into a nitrous oxide-acetylene flame of the spectrophotometer.

2.2 Additional information about the principles of the method may be found in Snell and Snell (1959), Fishman (1972).

3. Interferences

3.1 Concentrations of iron greater than 10 mg/1 interfere by suppressing the aluminum absorption.

3.2 Manganese concentrations up to 80 mg/1 do not interfere if the turbidity in the MIBK extract is allowed to settle.

3.3 Magnesium forms an isoluble chelate with 8-hydroxyquinoline at pH 8.0 and tends to coprecipitate aluminum 8-hydroxyquinolate. However, the magnesium 8-hydroxyquinolate forms rather slowly (approximately 4 to 6 min); its interference can be avoided if the aluminum 8-hydroxyquinolate is extracted with MIBK immediately after the sample is buffered to pH 8.

4. Apparatus

4.1 Atomic absorption spectrophotometer.

4.2 Refer to the manufacturers' manuals or procedures to optimize output of the instruments for the following parameters.

Grating	Ultraviolet
Wavelength	309.3 nm
Source (hollow-cathode lamp)	Aluminum
Burner	Nitrous oxide
Oxidant	Nitrous oxide
Fuel	Acetylene
Type of flame	Fuel rich

5. Reagents

5.1 Aluminum standard solution I, 1.00 ml = 100 g Al: Dissolve 1.758 g $AlK(SO_4)_2 \cdot 12H_2O$ in demineralized water, add 1 ml concentrated HNO_3 (sp gr 1.41), and dilute to 1000 ml with demineralized water.

5.2 Aluminum standard solution II, 1.00 ml = 1.00 g Al: Dilute 10.0 ml aluminum standard solution I and 1 ml concentrated HNO_3 to 1000 ml with demineralized water. This standard is used to prepare working standards at the time of analysis.

5.3 Ammonium hydroxide-ammonium acetate buffer solution: Dissolve 200 g $NH_4C_2H_3O_2$ and 70 ml concentrated NH_4OH (sp gr 0.90) in water and dilute to 1 litre with demineralized water.

5.4 8-Hydroxyquinoline solution: Dissolve 20 g 8-hydroxyquinoline in 57 ml glacial acetic acid (sp gr 1.06) and 200 ml demineralized water, and dilute to 1 litre with demineralized water.

5.5 Methyl isobutyl ketone (MIBK).

6. Procedure

Samples should be prepared according to instructions given in Part III, Section 1, "Sample Preparation."

Clean all glassware used in this determination with warm, dilute nitric acid (1 + 9) and rinse with demineralized water immediately before use.

6.1 Pipet a volume of sample containing less than 30 g Al (100 ml maximum) into a 200-ml volumetric flask and adjust the volume to approximately 100 ml.

6.2 Prepare a blank and sufficient standards and adjust the volume of each to approximately 100 ml with demineralized water.

6.3 Add 2 ml 8-hydroxyquinoline solution and mix.

Note 2: For total aluminum add 2.2 ml NH_4OH (concentrated) before proceeding to 6.4.

6.4 Add 10 ml ammonium hydroxide-ammonium acetate buffer solution to one sample and immediately add 10.0 ml MIBK. Shake vigorously for 15 sec. Each sample must be treated individually to avoid interference from magnesium. Each remaining sample, blank, and standard is treated in a like manner.

6.5 Allow the layers to separate and add demineralized water until the ketone layer is completely in the neck of the flask. (NOTE 3)

Note 3: If the layers do not separate, allow to stand overnight.

6.6 While aspirating ketone layer of the blank use auto zero to set instrument digital display to read 0. While aspirating standards use the auto concentration, set instrument digital to read the concentrations of standards. Use at least six standards. Calibrate the instrument each time it is set up in operation and check for stability at reasonable intervals.

7. Calculations

7.1 Determine the µg/l aluminum in each sample run from the digital display or printer output while aspirating each sample. Dilute samples that exceeded the working range, repeat the chelation-extraction, multiply by the proper dilution factors.

7.2 To determine µg/g of aluminum in bottom material samples, first determine the µg/l of aluminum as in 7.1, then

$$\text{Al in } \mu g/g = \frac{\mu g/l \text{ Al} \times \frac{\text{ml of original digest}}{1{,}000}}{\text{wt of sample in grams}}$$

8. Report

8.1 Report aluminum, dissolved (01106), concentrations as follows: Less than 100 µg/l, nearest 10 µg/l; 100 µg/l and above, two significant figures.

8.2 Report aluminum, total (01105), concentrations as follows: See 8.1.

8.3 Report aluminum, suspended (01107), concentrations as follows: See 8.1.

8.4 Report aluminum, total in bottom materials (01108), concentrations as follows:

9. Precision

9.1 Analysis of three test samples, 10 times each, by one operator, resulted in mean values of 10, 57, and 190 µg/l and standard deviations of 1, 3, and 7, respectively.

References

Fishman, M. J., 1972, Determination of aluminum in water: Atomic Absorption Newsletter, v. 11, p. 46-47.

Snell, F. D., and Snell, C. T., 1959, Colorimetric methods of analysis: Princeton, D. Van Nostrand Company, Inc., p. 181-183.

Parameters and Codes:		
	Antimony, dissolved (µg/1):	01095
	Antimony, total (µg/1):	01097
	Antimony, suspended (µg/1):	01096
	Antimony, total in bottom material (µg/g):	01098

Atomic absorption spectrophotometric method (I-1055-75)

1. Application

1.1 This method may be used to analyze waters, water-suspended sediment mixtures, and bottom materials (NOTE 1) containing at least 1 µg antimony per litre. Samples containing more than 15 µg/1 must be diluted.

Note 1: Do not use more than 100 mg of bottom material because severe bumping and loss of antimony will occur.

1.2 Water-suspended sediment mixtures may be analyzed by this procedure after each sample has been thoroughly mixed by vigorous shaking and a suitable aliquot has been rapidly withdrawn from the mixture.

1.3 Bottom materials may be analyzed by this procedure after they have been prepared as directed in Method 0520.

2. Summary of method

2.1 Organic antimony-containing compounds are decomposed by adding sulfuric and nitric acids and repeatedly evaporating the sample to fumes of sulfur trioxide. The antimony so liberated, together with inorganic antimony originally present, is subsequently reacted with potassium iodide and stannous chloride, and finally with sodium borohydride to form stibine. The stibine is removed from solution by aerating and swept by a flow of nitrogen into a hydrogen flame where it is determined by atomic absorption at 217.6 nm (fig. 1).

3. Interferences

3.1 Since the stibine is freed from the original sample matrix, interferences in the flame are minimized.

3.2 Selenium and arsenic, which also form hydrides, do not interfere at concentrations of 100 µg/1. Higher concentrations were not tested.

4. Apparatus

4.1 Atomic absorption spectrophotometer and recorder.

4.2 Refer to the manufacturers' manuals or procedures to optimize output of the instruments for the following parameters:

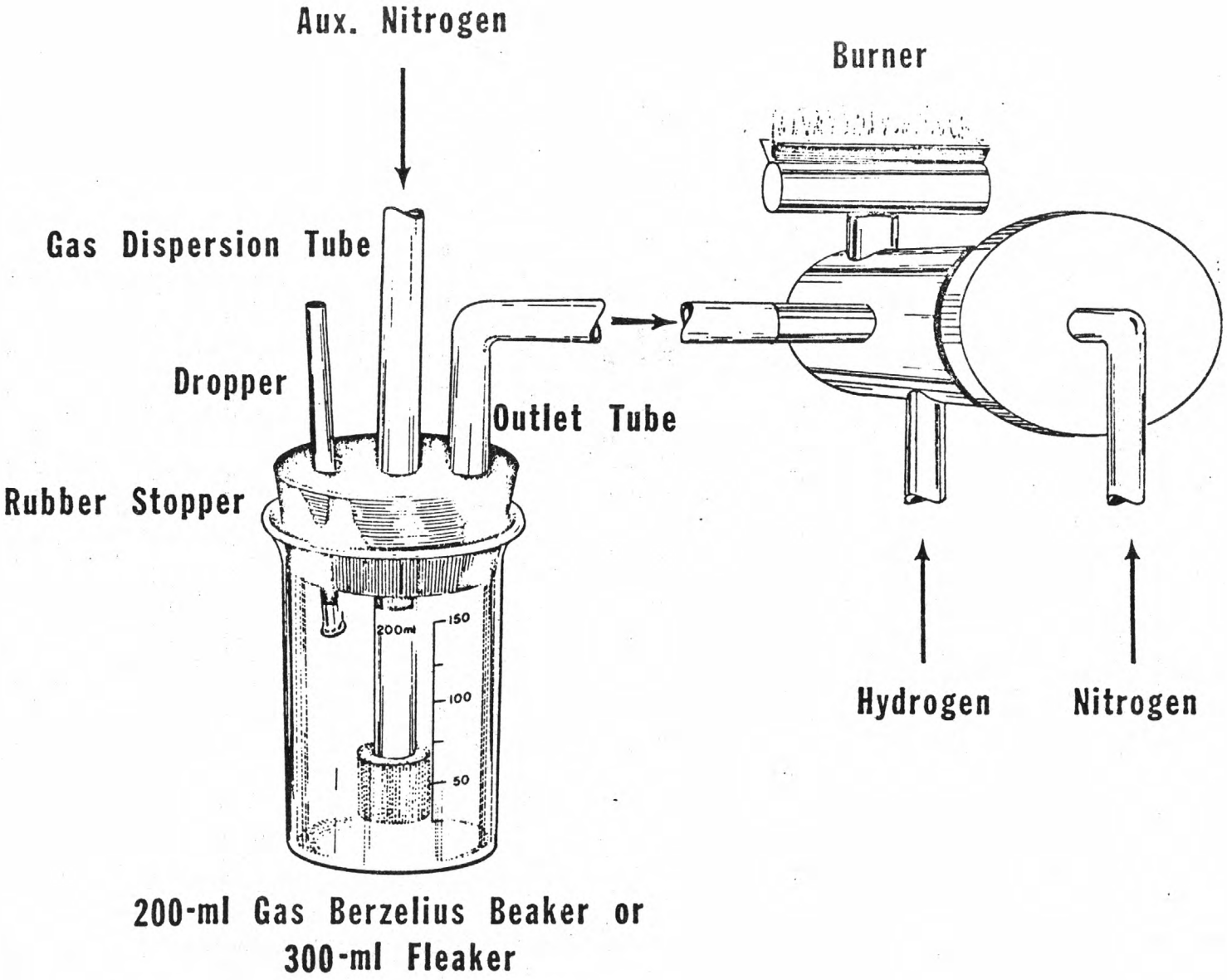

Figure 1.--Stibine vapor analyzer.

Grating. . . . . Ultraviolet

Wavelength . . . . . 217.6 nm

Source (Electrodeless discharge lamp) . . . . . Antimony

Fuel . . . . . Hydrogen

Carrier. . . . . Nitrogen

4.3 Different burners may be used according to manufacturers' instructions.

4.4 Stibine vapor analyzer (fig. 1) consisting of:

4.4.1 Beaker, Berzelius, 200-ml capacity

4.4.2 Gas Dispersion tube, coarse frit (Scientific Glass Apparatus Co. No. JG-8500 has been found satisfactory).

4.4.3 Medicine dropper, 2-ml capacity, minimum, or automatic pipettor, 5-ml capacity.

5. Reagents

5.1 Antimony standard solution I, 1.00 ml = 100 µg Sb: Dissolve 274.3 mg antimony potassium tartrate, $KSbOC_4H_4O_6 \cdot 1/2H_2O$, in demineralized water and dilute to 1,000 ml with demineralized water.

5.2 Antimony standard solution II, 1.00 ml = 10 µg Sb: Dilute 50.0 ml antimony standards solution I to 500.0 ml with demineralized water.

5.3 Antimony standard solution III, 1.0 ml = 0.10 µg Sb: Dilute 5.0 ml antimony standard solution II to 500.0 ml with demineralized water. Prepare fresh before each use.

5.4 Hydrochloric acid, concentrated (sp gr 1.19).

5.5 Nitric acid, concentrated (sp gr 1.41).

5.6 Potassium iodide solution, 15 g/100 ml: Dissolve 15 g KI in 100 ml demineralized water. This solution is stable when stored in an amber bottle.

5.7 Sodium borohydride solution, 4 g/100 ml: Dissolve 4 g $NaBH_4$ pellets in 100 ml demineralized water (Alfa Products No. 14122 has been found satisfactory). Prepare fresh just before each use.

5.8 Stannous chloride solution, 4.6 g per 100 ml concentrated HCl: Dissolve 5 g $SnCl_2 \cdot H_2O$ in 100 ml concentrated HCl (sp gr 1.19). This solution is stable if a few small pieces of mossy tin are added to prevent oxidation.

5.9 Sulfuric acid, 9M: Cautiously, and with constant stirring and cooling, add 250 ml concentrated H_2SO_4 (sp gr 1.84) to 250 ml demineralized water.

6. Procedure

Samples should be collected according to instructions given in "Part I, Sample Collection and Treatment."

6.1 Pipet a volume of sample containing less than 1.5 µg Sb (100 ml max) into a 200-ml Berzelius beaker and dilute to 100 ml with demineralized water.

6.2 Prepare, in 200-ml Berzelius beakers, a blank and sufficient standards containing from 0.0 to 1.5 µg Sb by diluting 0.0 to 15.0 ml portions of antimony standard solution III to 100 ml with demineralized water.

6.3 To each beaker, add 7 ml 9M H_2SO_4 and 5 ml concentrated HNO_3. Add a small boiling chip and carefully evaporate to fumes of SO_3. Maintain an excess of HNO_3 until all organic matter is destroyed. This prevents darkening of the solution and possible reduction and loss of antimony. Cool, add 25 ml demineralized water, and again avaporate to fumes of SO_3 to expel oxides of nitrogen.

6.4 Cool, and adjust the volume of each beaker to approx 100 ml with demineralized water.

6.5 To each beaker, add successively, with thorough mixing after each addition, 8 ml concentrated HCl, 1 ml KI solution, and 0.5 ml $SnCl_2$ solution. Allow about 15 min for reaction.

6.6 Attach one beaker at a time to the rubber stopper containing the gas dispersion tube.

6.7 Fill the medicine dropper with 2 ml $NaBH_4$ solution and insert into hole in rubber stopper.

6.8 Add the $NaBH_4$ solution to the sample solution. After the absorbance has reached a maximum and has returned to the baseline remove the beaker. Rinse the gas dispersion tube in demineralized water before proceeding to the next sample. Treat each succeeding sample, blank, and standard in a like manner.

7. Calculations

7.1 Determine the µg of Sb in each sample aliquot from a plot of scale readings of standards. Exact reproducibility is not obtained, and an analytical curve must be prepared with each set of samples.

7.2 Determine the concentration of Sb in each sample as follows:

$$\text{Sb, in } \mu g/l = \frac{1{,}000}{\text{ml sample}} \times \mu g \text{ Sb in sample.}$$

7.3 Determine the concentration of Sb in µg/g of air-dried sample as follows:

$$\text{Sb in } \mu g/g = \frac{\mu g \text{ Sb per sample}}{\text{sample weight, grams}}$$

8. Report

8.1 Report antimony (Sb), dissolved (01095), concentrations as follows: Less than 100 µg/l, nearest µg/l; 100 µg/l and above, two significant figures.

8.2 Report antimony, total (01097), concentrations as follows: See 8.1.

8.3 Report antimony, suspended (01096), concentrations as follows: See 8.1.

8.4 Report antimony, total in bottom materials (01098), concentrations as follows:

9. Precision

9.1 Analysis of three test samples, 10 times each, by one operator, resulted in mean values of 2.5, 5.3, and 11.6 µg/l and standard deviations of 0.4, 0.3, and 0.3 µg/l, respectively.

Arsenic

Parameters and Codes:		
	Arsenic, dissolved (µg/l):	01000
	Arsenic, total (µg/l):	01002
	Arsenic, suspended (µg/l):	01001
	Arsenic, total in bottom material (µg/g):	01003

Silver diethyldithiocarbamate colorimetric method (I-1060-75)

1. Application

1.1 This method may be used to analyze water samples, water-suspended sediment mixtures and bottom materials containing at least 5 µg arsenic per litre.

1.2 Both inorganic and organic forms of arsenic are determined. To determine only inorganic arsenic, omit the strong-acid digestion, paragraphs 6.3 and 6.4 of the procedure.

1.3 Water-suspended sediment mixtures may be analyzed by this procedure after each sample has been thoroughly mixed by vigorous shaking and a suitable aliquot has been rapidly withdrawn from the mixture.

1.4 Bottom materials may be analyzed by this procedure after they have been prepared as directed in Method 0520.

2. Summary of method

2.1 Organic compounds are decomposed by adding sulfuric and nitric acids and repeatedly evaporating the samples to fumes of sulfur tioxide. The arsenic (V) so liberated, together with inorganic arsenic originally present, is subsequently reduced to arsenic (III) by potassium iodide and stannous chloride, and finally to gaseous arsine by zinc in hydrochloric acid solution. The resultant mixture of gases is passed through a scrubber consisting of boro-silicate glass wool impregnated with lead acetate solution, and into a gas absorber containing silver diethyldithiocarbamate (AgDDC) dissolved in pyridine. Arsine reacts with AgDDC to form a soluble red substance having maximum absorbance at about 535 nm. The absorbance of the solution is measured spectrophotometrically, and arsenic determined by reference to an analytical curve prepared from standards.

2.2 Additional information on the determination is given by Liederman, Bowen, and Milner (1959); Ballinger, Lishka, and Gales (1962); Stratton and Whitehead (1962); and Fresenius and Schneider (1964).

3. Interferences

3.1 Ordinarily, ground- and surface-water samples are relatively free of interferences. Occasional samples may contain hydrogen sulfide; however, commonly encountered quantities are effectively removed by a lead acetate scrubber. Several metals--cobalt, nickel, mercury, silver, platinum, copper, chromium, and molybdenum--interfere with the evolution of arsine. Where such interferences exist, they can be minimized or eliminated by pretreatment of the sample (Liederman and others, 1959). Antimony salts, under the reducing conditions in the generator, form stibine that passes into the absorber and causes high results.

3.2 The blank and standards fade slowly on standing, but not enough to influence results significantly during the first 20 min.

4. Apparatus

4.1 Arsine generator, scrubber, and absorber (fig. 1).

4.2 Spectrophotometer.

4.3 Wavelength. . . . . 535 nm.

5. Reagents

5.1 Arsenic standard solution I, 1.00 ml = 1.00 mg As: Dissolve 1.320 g As_2O_3, dried for 1 hr at 110°C, in 10 ml 10M NaOH and dilute to 1,000 ml with demineralized water. This solution is stable.

5.2 Arsenic standard solution II, 1.00 ml = 10.0 µg As: Dilute 5.00 ml arsenic standard solution I to 500.0 ml with demineralized water. This solution is stable.

5.3 Arsenic standard solution III, 1.00 ml = 1.00 µg As: Dilute 10.0 ml arsenic standard solution II to 100.0 ml with demineralized water. Prepare fresh before each use.

5.4 Hydrochloric acid, concentrated (sp gr 1.19): Use analytical-grade acid with arsenic content not greater than 1×10^{-6} percent.

5.5 Lead acetate solution, 8.6 g/100 ml: Dissolve 10 g $(CH_3COO)_2Pb \cdot 3H_2O$ in 100 ml demineralized water. Keep tightly stoppered.

5.6 Nitric acid, concentrated (sp gr 1.41): Use analytical-grade acid with arsenic content not greater than 5×10^{-7} percent.

5.7 Potassium iodide solution, 15 g/100 ml: Dissolve 15 g KI in 100 ml demineralized water. This solution is stable when stored in an amber bottle.

5.8 Silver diethyldithiocarbamate (AgDDC) solution, 0.5 g/100 ml: Dissolve 1 g $(C_2H_5)_2NCSSAg$ in 200 ml pyridine. This solution is stable when stored in an amber bottle.

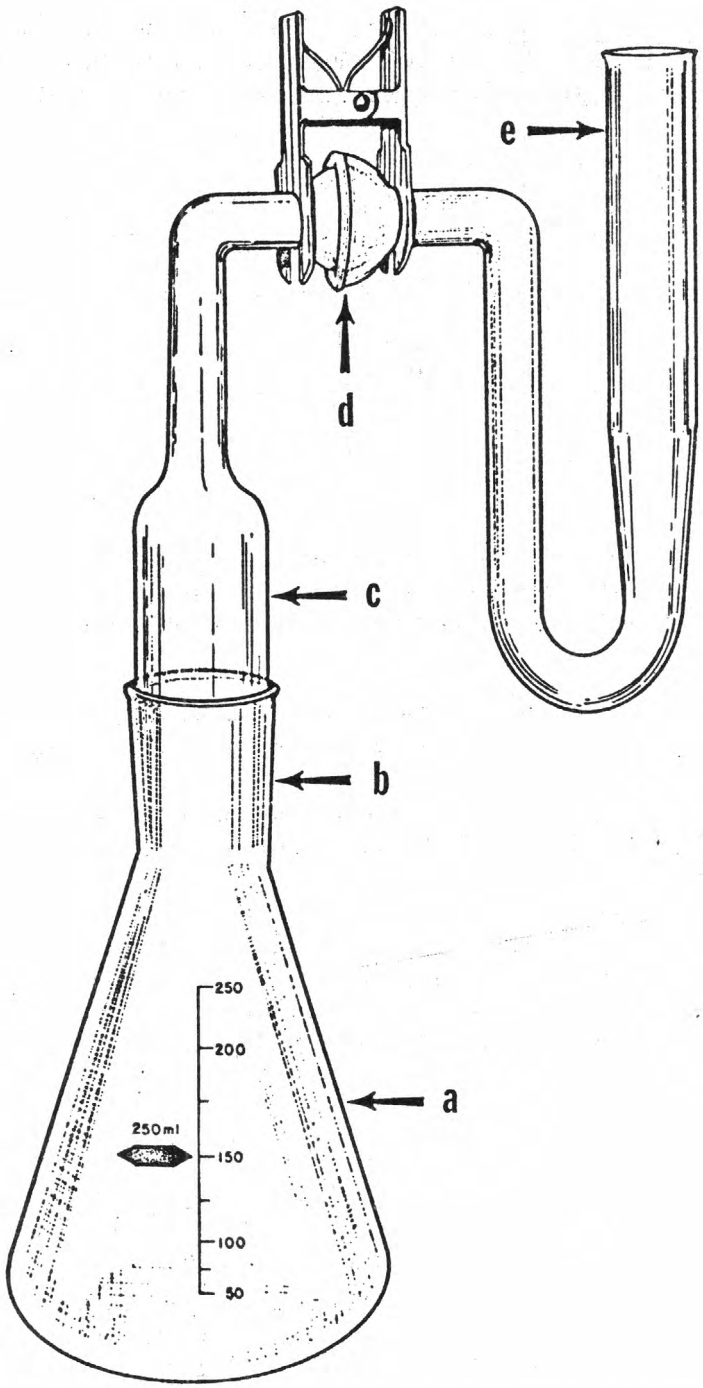

Figure 1.--Arsine generator, scrubber, and absorber. a, Generator, 250-ml erlenmeyer flask (Fisher Scientific Co. No. 10-101B, or equivalent); b, Standard taper neck, ₸24/40; c, Scrubber (Fisher Scientific Co. No. 1-405-5, or equivalent), containing borosilicate glass wool impregnated with lead acetate; d, Ground glass ball-and-socket joint; e, Absorber (Fisher Scientific Co. No. 1-405-10, or equivalent), contains silver diethyldithiocarbamate solution.

5.9 Stannous chloride solution, 33.6 g/100 ml concentrated HCl: Dissolve 40 g arsenic-free $SnCl_2 \cdot 2H_2O$ in 100 ml concentrated HCl. This solution is stable if a few small pieces of mossy tin are added to the bottle to prevent oxidation.

5.10 Sulfuric acid, 9M: Cautiously, and with constant stirring and cooling, add 250 ml concentrated H_2SO_4 (sp gr 1.84) to 250 ml demineralized water.

5.11 Zinc: Granular zinc, about 20 mesh, with arsenic content not greater than 1×10^{-6} percent.

6. Procedure

Samples should be collected according to instructions given in "Part 1, Sample Collection and Treatment."

Clean all glassware used in this determination with warm, dilute HNO_3 (1 + 4) and rinse with demineralized water immediately before each use. The absorbers must also be rinsed with acetone and air dried or briefly oven dried.

6.1 Pipet a volume of sample containing less than 20 µg As (100 ml maximum) into the flask of an arsine generator and dilute to 100 ml.

6.2 Prepare a blank and sufficient standards, and adjust the volume of each to approximately 100 ml with demineralized water.

6.3 To each flask, add 7 ml 9M H_2SO_4 and 5 ml concentrated HNO_3. Add a small boiling chip and carefully evaporate to fumes of SO_3. Cool, add 25 ml demineralized water and again evaporate to fumes of SO_3 to expel oxides of nitrogen. Maintain an excess of HNO_3 until all organic matter is destroyed. This prevents darkening of the solution and possible reduction and loss of arsenic.

6.4 Cool, and adjust the volume to approximately 100 ml. (NOTE 1).

Note 1: If only inorganic arsenic is to be determined, omit steps 6.3 and 6.4.

6.5 To each flask add successively, with thorough mixing after each addition, 10 ml concentrated HCl, 4 ml KI solution, and 1 ml $SnCl_2$ solution. Allow about 15 min for reduction of the arsenic to the tervalent state.

6.6 Place in each scrubber a plug of borosilicate glass wool that has been impregnated with lead acetate solution. Assemble the generator, scrubber, and absorber, making certain that all parts fit and are correctly adjusted. Add 3.00 ml silver diethyldithiocarbamate-pyridine solution to each absorber. Add glass beads to the absorbers until the liquid just covers them.

6.7 Disconnect each generator, add 6 g granular zinc, and reconnect immediately.

6.8 Allow 30 min for complete evolution of the arsine. Warm the generator flasks for a few minutes to make sure that all the arsine is released, and then pour the solutions from the absorbers directly into the spectrophotometer cells. Determine the absorbances of the standards and samples against the blank without unnecessary delay, as the color developed is not permanent.

7. Calculations

7.1 Determine the µg of As in the sample from a plot of absorbances of standards.

7.2 As in µg/l = µg As in sample $X \frac{1,000}{\text{ml sample}}$.

8. Report

8.1 Report arsenic (As), dissolved (01000) concentrations as follows: Less than 10 µg/l, nearest µg/l; 10 µg/l and above, two significant figures.

8.2 Report arsenic (As), total (01002), concentrations as follows: See 8.1.

8.3 Report arsenic (As), suspended (01001), concentrations as follows: See 8.1.

8.4 Report arsenic (As), total in bottom material (01003), concentrations as follows: Less than 100 µg/g; to the nearest µg/g; 100 µg/g and above, two significant figures.

9. Precision

9.1 The precision of this method within its designated range may be expressed as follows:

$$S_T = 0.214X + 3.92$$

where

S_T = overall precision, µg/l, and

X = concentration of arsenic, µg/l.

References

Ballinger, D. G., Lishka, R. J., and Gales, M. E., 1962, Application of the silver diethyldithiocarbamate method to the determination of arsenic: Am. Water Works Assoc. Jour., v. 54, p. 1424-1428.

Fresenius, W., and Schneider, W., 1964, Determination of slight amounts of arsenic with silver diethyldithiocarbamate in water analysis: Z. Anal. Chem., v. 203, p. 417-422.

Liederman, D., Bowen, J. E., and Milner, O. I., 1959, Determination of arsenic in petroleum stocks and catalysts by evolution of arsine: Anal. Chemistry, v. 31, p. 2052.

Stratton, G., and Whitehead, H. C., 1962, Colorimetric determination of arsenic in water with silver diethyldithiocarbamate: Am. Water Works Assoc. Jour., v. 54, p. 86.

Parameters and Codes:		
	Arsenic, dissolved (µg/l):	01000
	Arsenic, total (µg/l):	01002
	Arsenic, suspended (µg/l):	01001
	Arsenic, total in bottom material (µg/g):	01003

Atomic absorption spectrophotometric method (I-1062-75)

1. Application

1.1 This method may be used to analyze waters, water-suspended sediment mixtures, and bottom materials (NOTE 1) containing at least 1 µg arsenic per litre. Samples containing more than 10 µg/l must be diluted. Samples containing more than 100 µg/l should be analyzed by the colorimetric method (1060).

Note 1; Do not use more than 100 mg of bottom material because severe bumping and loss of arsenic may occur.

1.2 Water-suspended sediment mixtures may be analyzed by this procedure after each sample has been thoroughly mixed by vigorous shaking and a suitable aliquot has been rapidly withdrawn from the mixture.

1.3 Bottom materials may be analyzed by this procedure after they have been prepared as directed in Method 0520. Both inorganic and organic forms of arsenic are determined. To determine only inorganic arsenic, omit the strong-acid digestion, paragraphs 6.3 and 6.4 of the procedure.

2. Summary of method

2.1 Organic arsenic-containing compounds are decomposed by adding sulfuric and nitric acids and repeatedly evaporating the sample to fumes of sulfur trioxide. The arsenic (V) so liberated, together with inorganic arsenic originally present is subsequently reduced to arsenic (III) by potassium iodide and stannous chloride, and finally to gaseous arsine by zinc in hydrochloric acid solution. The arsine is removed from solution by aeration and swept by a flow of nitrogen into a hydrogen flame where it is determined by atomic absorption at 193.7 nm (fig. 1).

3. Interferences

3.1 Since the arsine is freed from the original sample matrix, interferences in the flame are minimized.

4. Apparatus

4.1 Atomic absorption spectrophotometer and recorder.

4.2 Refer to the manufacturers' manuals or procedures to optimize output of the instruments for the following parameters:

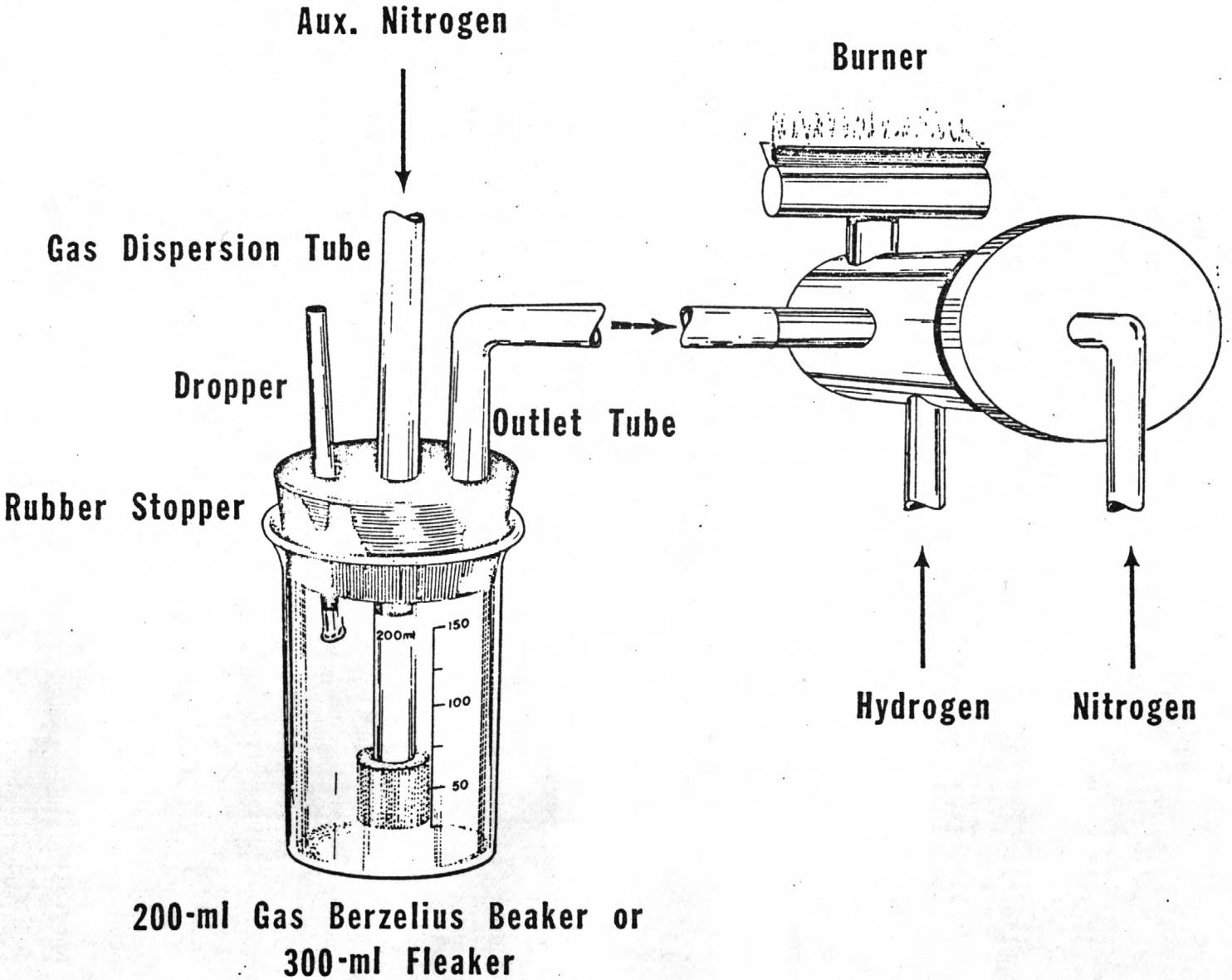

Figure 1.—Arsine vapor analyzer.

Grating. . . . . Ultraviolet

Wavelength counter 193.7 (1937A)

Source (Electrodeless discharge lamp). . . . . Arsenic

Fuel . . . . . Hydrogen

Carrier . . . . . Nitrogen

4.3 Different burners may be used according to manufacturers' instructions.

4.4 Beaker, Berzelius, tall form without pouring spout, 200-ml capacity or fleaker, 300-ml capacity.

4.5 Gas dispersion tube, coarse frit (Scientific Glass Apparatus Co. No. JG-8500 has been found to be satisfactory).

4.6 Medicine dropper, 2-ml capacity, minimum or automatic pipettor, 5-ml capacity.

5. Reagents

5.1 Arsenic standard solution I, 1.00 ml = 1.00 mg As: Dissolve 1.320 g As_2O_3, dried for 1 hr at 110°C, in 10 ml 10M NaOH and dilute to 1,000 ml with demineralized water. This solution is stable. However, it should probably be prepared fresh every six months.

5.2 Arsenic standard solution II, 1.00 ml = 10.0 µg As: Dilute 5.00 ml arsenic standard solution I to 500.0 ml with demineralized water. Discard after three months.

5.3 Arsenic standard solution III, 1.00 ml = 0.10 µg As: Dilute 5.00 ml arsenic standard solution II to 500.0 ml with demineralized water. Prepare fresh before each use.

Note 2: Arsenic standard solution IV, 1.00 ml = 0.040 µg As can also be prepared in order to facilitate preparation of standards above 1.0 µg As: Quantitatively add 100.0 ml arsenic standard solution III to 250 ml volumetric flask that contains 10 ml of 3 percent anhydrous $CaCl_2$ and dilute to 250 ml. Prepare fresh before each use.

5.4 Hydrochloric acid, concentrated (sp gr 1.19): Use analytical-grade acid with arsenic content not greater than 1×10^{-6} percent.

5.5 Nitric acid, concentrated (sp gr 1.41): Use analytical-grade acid with arsenic content not greater than 5×10^{-7} percent.

5.6 Potassium iodide solution, 15 g/100 ml: Dissolve 15 g KI in 100 ml demineralized water. This solution is stable when stored in an amber bottle.

5.7 Stannous chloride solution, 33.6 g/100 ml concentrated HCl: Dissolve 40 g arsenic-free $SnCl_2.2H_2O$ in 100 ml concentrated HCl. This solution is stable if a few small pieces of mossy tin are added to the bottle to prevent oxidation.

5.8 Sulfuric acid 9M: Cautiously, and with constant stirring and cooling, add 250 ml concentrated H_2SO_4 (sp gr 1.84) to 250 ml demineralized water.

5.9 Zinc metal (dust) suspension: Add 10 g zinc dust to 20 ml demineralized water. Fisher Scientific Co. Reagent-grade Zinc metal (dust) No. Z-5 has been found to be satisfactory.

5.10 Calcium chloride, 3 percent solution: Dissolve 30 g anhydrous calcium chloride in sufficient demineralized water and dilute to 1 litre.

6. Procedure

Samples should be collected according to instructions given in "Part I, Sample Collection and Treatment."

Clean all glassware used in this determination with dilute HCl (1 + 4) and rinse with demineralized water immediately before each use.

6.1 Pipet a volume of sample containing less than 1.0 µg As (50 ml max) into a 200-ml Berzelius beaker and dilute to 50 ml.

Note 3: If fleakers are used, maximum volume can be 100 ml. Reagent volumes should then be doubled.

6.2 Prepare, in 200-ml Berzelius beakers or 300-ml fleakers, a blank and sufficient standards containing from 0.0 to 1.0 µg As by diluting 0.0- to 10.0-ml portions of arsenic standard solution III. Dilute each to approximately 50 ml.

Note 4: Standards containing from 0.0 to 1.6 µg As can be prepared by diluting 0.0- to 40.0-ml portions of arsenic standard solution IV.

6.3 To each beaker, or glass beads add 7 ml 9M H_2SO_4 and 5 ml concentrated HNO_3. Add a small boiling chip or glass beads and carefully evaporate to fumes of SO_3. Maintain an excess of HNO_3 until all organic matter is destroyed. This prevents darkening of the solution and possible reduction and loss of arsenic. Cool, add 25 ml demineralized water, and again evaporate to fumes of SO_3 to expel oxides of nitrogen.

6.4 Cool, and adjust each beaker to approximately 50 ml with demineralized water (NOTE 5).

Note 5: If only inorganic arsenic is to be determined, omit steps 6.3 and 6.4.

6.5 To each beaker, add successively, with thorough mixing after each addition, 8 ml concentrated HCl, 4 ml KI solution, and 1.0 ml $SnCl_2$ solution. Allow about 15 min for reduction of the arsenic to the tervalent state.

6.6 Attach one beaker at a time to the rubber stopper containing the gas dispersion tube.

6.7 Fill the medicine dropper with 2 ml zinc dust suspension (NOTE 6) and insert into the hole in the rubber stopper. Alternatively, the zinc dust suspension may be delivered from an automic pipettor.

Note 6: The zinc dust must be kept in suspension by continuous stirring. A magnetic stirrer is satisfactory.

6.8 Add the zinc dust suspension to the sample solution. After the absorbance has reached a maximum and has returned to the baseline remove the beaker. Rinse the gas dispersion tube first in nitric acid (1 + 4), and then in distilled water before proceeding to next sample. Test each succeeding sample, blank, and standard in a like manner.

7. Calculations

7.1 Determine the µg of As in the sample from a plot of absorbances of standards.

7.2 As in µg/l = µg As in sample X $\frac{1{,}000}{\text{ml sample}}$.

7.3 Determine the concentration of As in µg/g of air-dried bottom material sample as follows:

$$\text{As in } \mu g/g = \frac{\mu g \text{ As per sample}}{\text{sample weight, grams}}$$

8. Report

8.1 Report arsenic (As), dissolved (01000), concentrations as follows: Less than 10 µg/l, nearest µg/l; 10 µg/l and above, two significant figures.

8.2 Report arsenic, total (01002), concentrations as follows: See 8.1.

8.3 Report arsenic, suspended (01001), concentrations as follows: See 8.1.

8.4 Report arsenic, total in bottom materials (01003), as follows:

9. Precision

9.1 Analysis of four test samples, 11 times each, by two operators, resulted in mean values of 3.6, 9.1, 18.9, and 32.9 g/l and standard deviations of 0.8, 12., 2.2, and 2.8, respectively.

Parameter and codes: Boron, dissolved (mg/l): 01020
Boron, total (mg/l): 01022
Boron, suspended (mg/l): 01021

Dianthrimide colorimetric method (I-1110-75)

1. Application

1.1 This method may be used to analyze waters and water-suspended sediment mixtures containing between 20 and 1,000 mg/l. Samples containing more than 1,000 mg/l must be diluted.

1.2 Water-suspended sediment mixtures may be analyzed by this method after preliminary digestion-solubilization by Method 3485.

1.3 This method is not suitable for waters containing high concentrations of oxidizing or reducing materials and dissolved organic matter. However, it is not affected by buffering solutions or high concentrations of total salts.

2. Summary of method

2.1 Boron when heated with 1, 1'-dianthrimide in concentrated sulfuric acid gives a colored complex (Ellis and others, 1949; Rainwater, 1959). The color change ranges from greenish yellow to blue. The reaction producing the blue color depends on the nature of the vessil in which the reaction occurs, the temperature and duration of heating, and the concentration of reagent and of boron. Maximum color development is achieved after the reaction has proceeded for 3 h at 90°C.

3. Interferences

3.1 Traces of moisture will precipitate the reagent and interfere in the determination; therefore, precautionary measures given in the procedure must be followed explicitly. Nitrate and bicarbonate interfere with color development, and must be removed by volatilization as nitric acid and carbon dioxide in the presence of sulfuric acid. Organic matter in high concentrations chars and causes a discoloration of the complex, but this interference is easily recognized; small quantities of organic material cause no trouble. Some success in removal of the organic-material interference has been obtained by heating the sample in the presence of hydrogen peroxide for 1 to 2 h, but it is essential that all nascent oxygen be volatilized before the dianthrimide is added to the sample. When peroxide digestion is used, the final color complex should be compared with standard boron solutions similarly treated. Oxidizing and reducing constituents also interfere. Do not use glassware cleaned with chromic-sulfuric acid.

3.2 Some boric acid is probably volatilized during evaporation of the sample in the presence of sulfuric acid. Prolonged heating or temperatures higher than that recommended volatilizes an excessive amount of boron and decreases the sensitivity of the test. The loss of boron is proportional to the boron content of the sample or standard, hence such loss in no way affects the

linearity of the color development if the heating is uniform. Nonlinearity of the concentration-versus-absorbance curve can result from weak reagents. The standards in step 6.2 of the procedure act as a check on linearity of the reaction and suitability of the working reagent.

4. Apparatus

4.1 Oven, 90°C: Uniformity of temperature throughout the oven is imperative.

4.2 Spectrophotometer

4.3 Refer to manufacturers' manuals or procedures for optimizing instrumental parameters.

Wavelength. . . . . 620 nm.

Note 1: Using a Beckman Model B unit with 23-mm cell, personnel in Atlanta have obtained the following data:

Boron (mg)	Absorbance
1.0	0.26
2.0	.52
5.0	1.30

Using a Coleman Model 55 unit with 10-mm flow-through cell, personnel in Salt Lake City have obtained the following data:

Boron (mg)	Absorbance
2.0	0.100
5.0	.250
10.0	.500
20.0	.950

5. Reagents

5.1 Boron standard solution I, 1.00 ml = 100 µg B: Dissolve 10 g $Na_2B_4O_7 \cdot 10\ H_2O$ in 50 ml demineralized water at 50° to 60°C. Recrystallize by placing in refrigerator for several hours. Dry by removing the water with suction and washing with alcohol followed by ether. Do not dry in oven. Dissolve 0.8820 g in demineralized water and dilute to 1,000 ml. Store in plastic bottle.

5.2 Boron standard solution II, 1.00 ml = 1.0 µg B: Dilute 10.0 ml boron standard solution I to 1,000 ml with demineralized water. Store in plastic bottle.

5.3 1,1'-dianthrimide (or 1,1'-iminodianthraquinone) solution I, 200 mg/50 ml concentrated H_2SO_4. Dissolve 200 mg 1,1'-dianthrimide in 50 ml concentrated H_2SO_4 (sp gr 1.84). The reagent is stable for a long period if the container is sealed and refrigerated. Store in teflon bottle.

5.4 1,1'-dianthrimide solution II, (1 + 19): Dilute 1 volume of 1,1'-dianthrimide solution I with 19 volumes of concentrated H_2SO_4 (sp gr 1.84). The reagent is stable for a long period if the container is sealed and refrigerated. Store in teflon bottle.

5.5 Sulfuric acid, concentrated (sp gr 1.84).

6. Procedure

Samples should be collected according to instructions given in "Part I, Sample Collection and Treatment."

6.1 Pipet a volume of sample containing less than 5.0 µg B (5.00 ml maximum) into the absorption cell, and adjust volume to 5.0 ml.

6.2 Prepare a blank of demineralized water and sufficient standards, and adjust volumes to 5.0 ml.

6.3 Cautiously add 1.0 ml concentrated H_2SO_4 and mix by swirling the contents of the cell. Use of a test tube vibrating mixer is helpful.

6.4 Evaporate overnight in an oven at 90°C. At the end of the evaporation, the solution volume should be between 1.0 and 0.5 ml.

6.5 Add 5.0 ml 1,1'-dianthrimide solution II and mix well by swirling.

6.6 Heat in oven for 3 h at 90°C.

6.7 Immediately after cooling, and with caution, add 10.0 ml concentrated H_2SO_4. Mix thoroughly but carefully with a stirring rod or test tube mixer. The contents must not be spattered on the upper walls of the cell.

6.8 Remove all traces of acid, reagent, and fingerprints from the exterior surface of the cell, and determine the absorbance of the sample and standards against the blank.

7. Calculations

7.1 Determine the µg B in the sample from a plot of absorbances of standards.

7.2 $$\text{B in } \mu g/l = \frac{1{,}000}{\text{ml sample}} \times \mu g \text{ B in sample.}$$

8. Report

8.1 Report boron (B), dissolved (01020) concentrations as follows: Less than 100 µg/l, nearest 10 µg/l; 100 µg/l and above two significant figures.

8.2 Report boron, total (01022), concentrations as follows: See 8.1.

8.3 Report boron, suspended (01021), concentrations as follows: See 8.1.

9. Precision

9.1 Analysis of two test samples by six laboratories using this method resulted in mean values of 101 and 293 µg/l and standard deviations of 17 and 54 µg/l, respectively.

References

Ellis, G. H., Zook, E. G., and Baudisch, Oskar, 1949, Colorimetric determination of boron using 1,1'-dianthrimide: Anal. Chemistry, v. 21, p. 1345.

Automated ferric thiocyanate colorimetric method (I-2187-75)

Parameter and code: Chloride, dissolved (mg/l as Cl): 00940

1. Application

1.1 This method may be used to determine concentrations of chloride in surface, domestic, and industrial waters in the range from 0.0 to 100 mg/l. Chloride in brines must be analyzed by Method 1185-74 or 1186-74.

2. Summary of method

2.1 This method is based upon the displacement of thiocyanate from mercuric thiocyanate by chloride, and the subsequent reaction of the liberated thiocyanate ion with ferric ion to form the intensely colored complex $[Fe(SCN)]^{+2}$. The absorbance of this complex is then measured colorimetrically (O'Brien, 1962; Zall and others, 1956).

$$Hg(SCN)_2 + 2Cl^{-1} \rightarrow HgCl_2 + 2SCN^-,$$

$$(SCN^- + Fe^{+3} \rightarrow [Fe(SCN)]^{+2}.$$

3. Interferences

3.1 Bromide, iodide, cyanide, thiosulfate, and nitrite interfere.

4. Apparatus

4.1 Technicon AutoAnalyzer consisting of a sampler, proportioning pump, cartridge manifold, colorimeter, voltage stabilizer, recorder, and printer.

With this equipment the following operating conditions have been found satisfactory for the ranges from 0.0 to 100 mg/l.

Absorption cell. . . . . 15 mm

Wavelength . . . . . .480 nm

Cam. . . . . .60(6/1) or 40(4/1)

5. Reagents

5.1 Chloride standard solution, 1.00 ml = 0.50 mg Cl^{-1}: Dissolve 0.8242 g primary standard NaCl crystals, dried at 180°C for 1 hr, in demineralized water and dilute to 1,000 ml.

5.2 Chloride working standards: Prepare a blank and 500 ml each of a series of chloride working standards by appropriate quantitative dilution of the chloride standard solution, as follows:

Chloride standard solution (ml)	Chloride concentration (mg/l)
0.0	0.0
5.0	5.0
10.0	10.0
20.0	20.0
30.0	30.0
50.0	50.0
60.0	60.0
80.0	80.0
100.0	100

5.3 Ferric nitrate stock solution, 121 g/l: Dissolve 202 g $Fe(NO_3)_3 \cdot 9H_2O$ in approximately 500 ml demineralized water. Add 31.5 ml concentrated HNO_3 (sp gr 1.41) and dilute to 1 litre. Filter and store in an amber-colored container.

5.4 Mercuric thiocyanate stock solution, 4.17 g/l of methanol: Dissolve 4.17 g $Hg(SCN)_2$ in 500 ml methanol, dilute to 1 litre with methanol, and filter.

5.5 Chloride color reagent: Add 150 ml of ferric nitrate stock solution and 150 ml of mercuric thiocyanate stock solution to demineralized water and dilute to 1 litre. Add 1 ml of Bry-35 per litre. Use amber bottle for storage.

6. Procedure

Samples should be prepared according to instructions given in Part III, Sec. 1, "Sample Preparation and Treatment."

6.1 Set up maniford (fig. 1).

6.2 Allow colorimeter and recorder to warm up for at least 30 min. Adjust baseline to read zero scale divisions on the recorder with all reagents, but with demineralized water in the sample line.

6.3 Beginning with the highest standard, place a complete set of standards in the first positions of the first sample tray followed by a blank and standard reference samples. Place individual standards of differing concentrations in every eighth position of the remainder of this and subsequent sample trays. Fill remainder of each sample tray with unknown samples. At end of last tray place standard reference samples.

Note 1: The sample cups should remain sealed in their packages until just prior to use to avoid contamination. Handle cups carefully to avoid contamination from perspiration on hands.

6.4 Begin analysis. When the peak from the highest standard appears on on the recorder, adjust the STD CAL Control until the flat portion of the peak reads full scale.

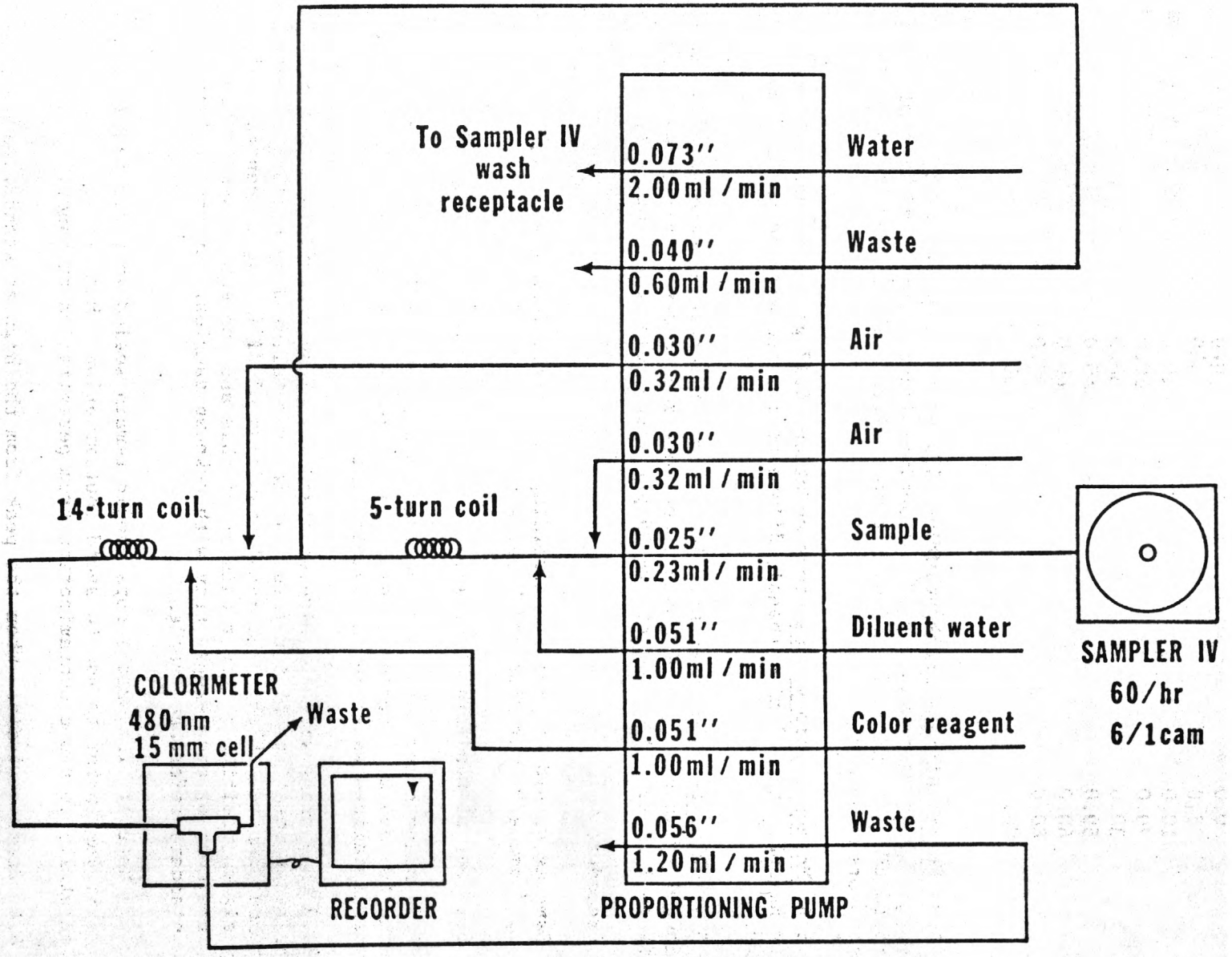

Figure 1.--Chloride manifold.

7. Calculations

7.1 Prepare analytical curve by plotting the height of each standard peak versus its respective chloride concentration.

7.2 Compute the Cl^{-1} concentration of each sample by comparing its peak-height to the analytical curve. Any baseline drift that may occur must be taken into account when computing the height of a sample or standard peak.

8. Report

8.1 Report chloride (Cl^{-1}), dissolved, concentrations as follows: Less than 10 mg/l, nearest tenth; 10 mg/l and above, two significant figures.

9. Precision

9.1 Analysis of two test samples by a single laboratory on 17 and 14 samples resulted in mean values of 26.8 and 72.9 mg/l and standard deviations of 0.6 and 0.7 mg/l, respectively.

References (include all methods)

American Society for Testing and Materials, 1973, Annual Book of ASTM Standards, Part 23: Philadelphia, Am. Soc. Testing Materials, p. 277.

Bergman, J. G., and Sanik, John, Jr., 1957, Determination of trace amounts of chlorine in naptha: Anal. Chemistry, v. 29, p. 241-243.

Clarke, F. E., 1950, Determination of chloride in water: Anal. Chemistry, v. 22, p. 553, 1458.

Collins, W. D., 1928, Notes on practical water analysis: U.S. Geol. Survey Water-Supply Paper 596-II, p. 235-266.

Dubsky, J. V., and Trtilek, J., 1933, Microvolumetric analysis using diphenylearbazide and diphenylearbazone as indicators (mercurimetry): Mikrochemie, v. 12, p. 315.

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Mercury

Atomic absorption spectrophotometric method--flameless (I-1462-75)

Parameters and Codes:		
	Mercury, dissolved (µg/1):	71890
	Mercury, total (µg/1):	71900
	Mercury, suspended (µg/1):	71895
	Mercury, total in bottom material (µg/g):	71921

1. Application

1.1 This method may be used to analyze waters and water-suspended sediment mixtures containing at least 0.1 µg mercury per litre and bottom materials containing less than 1.0 µg mercury in the weighed samples (5 g maximum). Samples containing mercury concentrations greater than 10 µg/1 must be reduced by dilution. Industrial and sewage effluents may be analyzed, as well as samples of fresh and saline water.

1.2 Water-suspended sediment mixtures may be analyzed by this procedure after each sample has been thoroughly mixed by vigorous shaking and a suitable aliquot has been rapidly withdrawn from the mixture.

1.3 Bottom materials may be analyzed by this procedure after they have been prepared as directed in Method 0520.

2. Summary of method

2.1 The flameless atomic absorption procedure is based on the absorption of radiation at 253.7 nm by mercury vapor. Organic mercury compounds, if present, are decomposed by hot (95°C) digestion with potassium permanganate and potassium persulfate in acid solution. The mercuric ions are then reduced to the elemental state with stannous chloride, and the mercury vapor subsequently removed from solution by aeration and passed through a cell positioned in the light path of an atomic absorption spectrophotometer. The percent absorption is plotted on a recorder and mercury determined by reference to an analytical curve prepared from standards. Either a mercury-vapor discharge lamp or a hollow-cathode lamp may be used as a light source.

2.2 This method is based on a procedure described by Hatch and Ott (1968) and is similar in substance to the flameless atomic absorption method in "Methods for Chemical Analysis of Water and Wastes," published by the Water Quality Office of the Environmental Protection Agency (1971).

3. Interferences

3.1 Some samples may contain volatile organic compounds which absorb radiation at 253.7 nm and which may be swept from the solution along with the mercury vapor. These constitute a positive interference and the possibility of their presence must not be overlooked.

4. Apparatus

4.1 Manual technique.

4.1.1 Absorption cell (fig. 1).

Mount and aline the absorption cell in the light path of the spectrophotometer. Position a 60-watt lamp over the cell (10 to 15 cm) to prevent condensation of water vapor. Attach a sufficient length of tubing to the outlet of the cell and vent to a hood. Connect the inlet of the cell to the aerator with a minimum length of plastic tubing. Attach a water aspirator to the outlet of the stopcock (NOTE 1). Position and adjust the cell, both vertically and horizontally, to achieve maximum transmittance.

Note 1: The stopcock must remain closed during analysis and opened only briefly, between samples, to remove residual mercury vapor from the absorption cell.

4.1.2 Aerator

4.1.3 Atomic absorption spectrophotometer and recorder.

4.1.4 Refer to the manufacturer's manuals or procedures to optimize output of the instruments for the following parameters:

Grating . . . . . .Ultraviolet.

Wavelength counter. . . . . .253.7 (2537A).

Source. . . . . .Mercury-vapor discharge lamp.

4.1.5 BOD bottle, 300-ml capacity.

4.2 Automated technique .

4.2.1 Absorption cell, 100 mm long, 10 mm diameter with quartz windows.

4.2.2 Atomic absorption spectrophotometer and recorder

4.2.3 Refer to the manufacturer's manuals or procedures to optimize output of the instruments for the following parameters:

Grating . . . . . .Ultraviolet

Wavelength counter. 253.7 (2537A)

Source. . . . . .Hollowcathode lamp

4.2.4 Technicon autoanalyzer system: Consisting of sampler with stirrer, manifold (fig. 2), proportioning pump, and high temperature heating bath with two distillation coils in series

4.2.5 Vapor-liquid separator (fig. 3).

5. Reagents

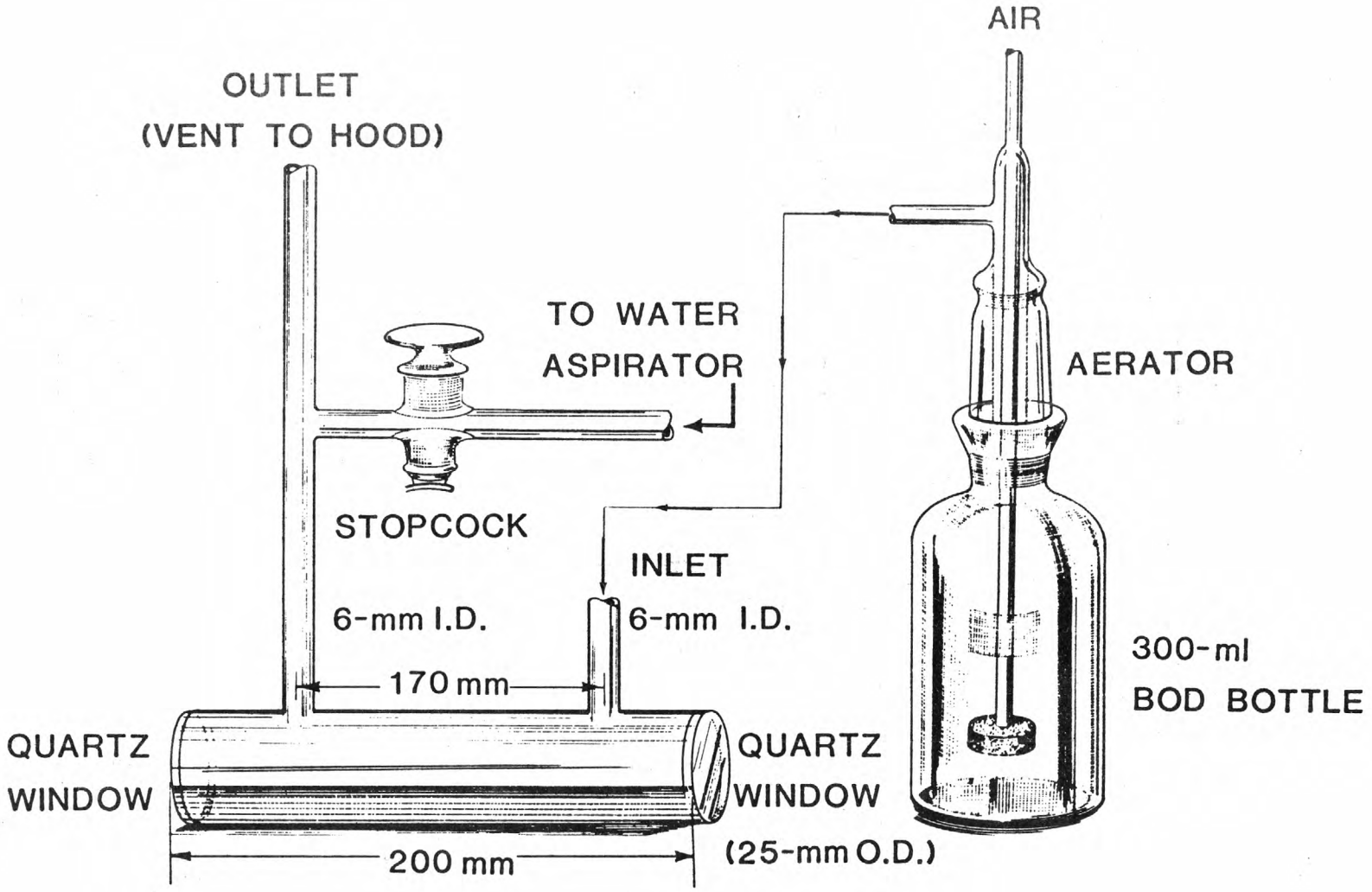

Figure 1.--Mercury absorption cell.

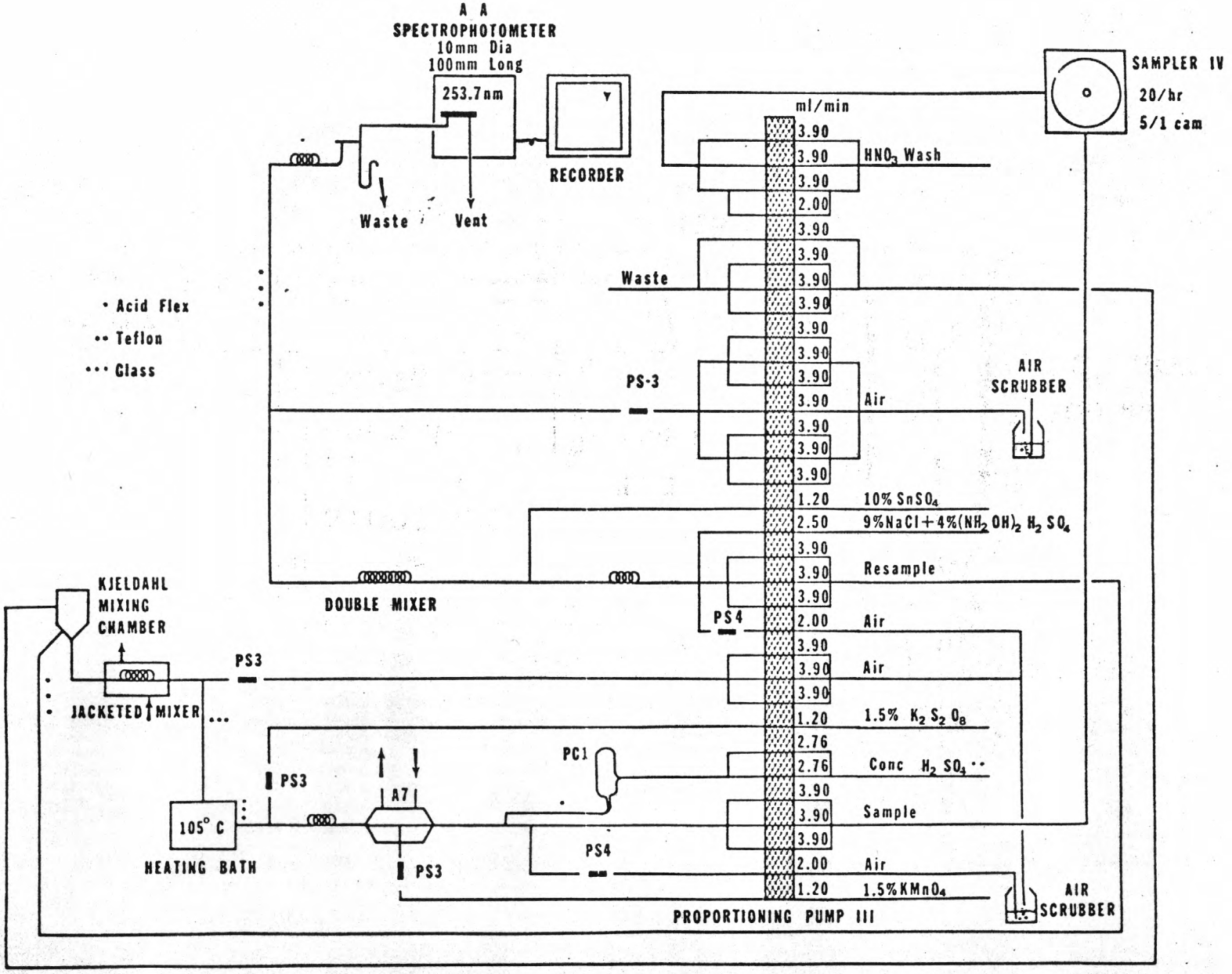

Figure 2.--Mercury manifold.

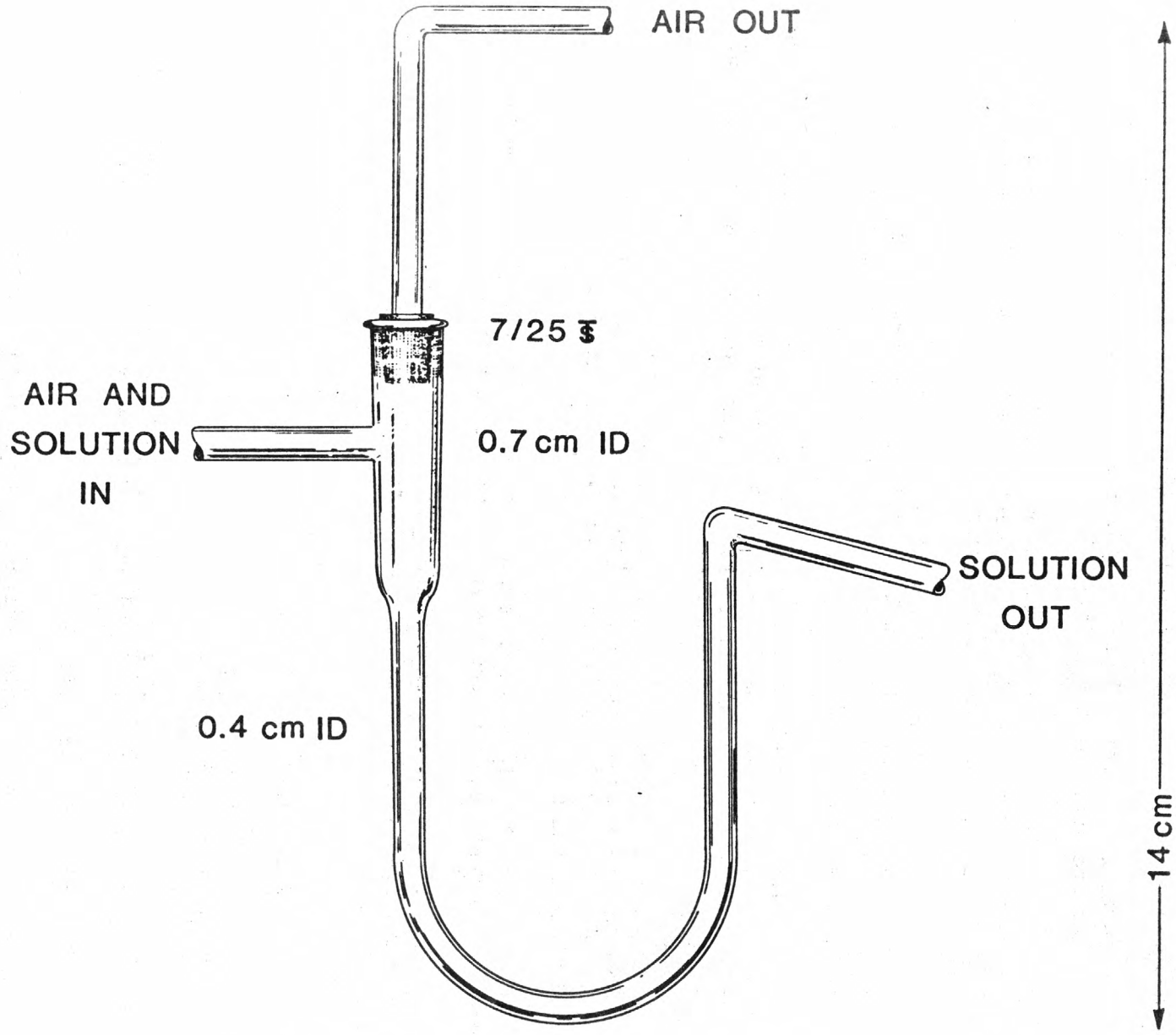

Figure 3.--Vapor-liquid separator

5.1 Manual technique

5.1.1 Hydroxylamine hydrochloride-sodium chloride solution: Dissolve 10 g $NH_2OH \cdot HCl$ and 12 g NaCl in demineralized water and dilute to 100 ml. Prepare fresh daily. Alternatively 12 g hydroxylamine sulfate may be used instead of the hydroxylamine hydrochloride.

Note 2: Larger volumes of this reagent can be prepared if they are kept refrigerated.

5.1.2 Mercury standard solution I, 1.00 ml = 100 µg Hg: Dissolve 0.1712 g $Hg(NO_3)_2 \cdot H_2O$ in demineralized water. Add 1.5 ml concentrated HNO_3 and dilute to 1,000 ml with demineralized water.

5.1.3 Mercury standard solution II, 1.00 ml = 1.00 µg Hg: Dilute 5.00 ml mercury standard solution I and 1.5 ml concentrated HNO_3 to 500.0 ml with demineralized water. This and the following mercury standard solutions must be prepared fresh daily.

5.1.4 Mercury standard solution III, 1.00 ml = 0.050 µg Hg: Dilute 10.0 mercury standard solution II and 1.5 ml concentrated HNO_3 to 200.0 ml with demineralized water. Use this solution to prepare working standards at the time of analysis.

Note 3: Mercury standard solution III, 1.00 - 0.01 µg Hg: has also been found useful. Dilute 5.0 ml mercury standard solution II and 4.0 ml concentrated HNO_3, to 500.0 ml with demineralized water.

5.1.5 Nitric acid, concentrated (sp gr 1.41), with low mercury content: Du Pont reagent-grade acid has been found to be satisfactory.

5.1.6 Potassium permanganate solution, 50 g/l: Dissolve 5 g $KMnO_4$ in demineralized water and dilute to 100 ml. Prepare fresh weekly. Store in glass bottle.

5.1.7 Potassium persulfate solution, 50 g/l: Dissolve 5 g $K_2S_2O_8$ in demineralized water and dilute to 100 ml.

5.1.8 Stannous chloride solution, 74 g/l: Add 22 g $SnCl_2 \cdot 2H_2O$ to 250 ml 0.25M H_2SO_4. Prepare fresh daily.

5.1.9 Sulfuric acid, 0.25M: Cautiously add 14 ml concentrated H_2SO_4 (sp gr 1.84) to demineralized water and dilute to 1 litre.

5.2 Automated technique

5.2.1 Hydroxylamine sulfate-sodium chloride solution: Dissolve 90 g of sodium chloride and 90 g of hydroxylamine sulfate in distilled water to 1 litre. Hydroxylamine hydrochloride may be used in place of hydroxylamine sulfate.

5.2.2 Mercury standard solution I, 1.00 ml = 100 µg Hg: Dissolve 0.1712 g $Hg(NO_3)_2 \cdot H_2O$ in demineralized water. Add 1.5 ml concentrated HNO_3 and dilute to 1,000 ml with demineralized water.

5.2.3 Mercury standard solution II, 1.00 ml = 1.00 µg Hg: Dilute 5.00 ml mercury standard solution I and 1.5 ml concentrated HNO_3 to 500.0 ml with demineralized water. This and the following mercury standard solutions must be prepared fresh daily.

5.2.4 Mercury standard solution III, 1.00 ml = 0.050 µg Hg: Dilute 10.0 ml mercury standard solution II and 1.5 ml concentrated HNO_3 to 200.0 ml with demineralized water. Use this solution to prepare working standards at the time of analysis.

5.2.5 Nitric acid, concentrated (sp gr 1.41), with low mercury content: Du Pont reagent-grade acid has been found to be satisfactory.

5.2.6 Nitric acid, 0.5 percent wash solution. Dilute 5 ml of concentrated nitric acid to 1 litre with distilled water.

5.2.7 Potassium permanganate: 1.5 percent solution, w/v. Dissolve 15 g of potassium permanganate in 1 litre of distilled water.

5.2.8 Potassium permanganate, 0.1N: Dissolve 3.16 g of potassium permanganate in distilled water and dilute to 1 litre.

5.2.9 Potassium persulfate: 5 percent solution, w/v. Dissolve 15 g of potassium persulfate in 1 litre of distilled water.

5.2.10 Stannous sulfate: Add 50 g stannous sulfate to 500 ml of 2N sulfuric acid. This mixture is a suspension and should be stirred continuously during use. Stannous chloride may be used in place of stannous sulfate.

5.2.11 Sulfuric acid, 2N: Dilute 56 ml of conc. sulfuric acid (sp. gr 1.84) to 1 litre with distilled water.

5.2.12 Sulfuric acid, 10 percent: Dilute 100 ml conc. sulfuric acid (sp. gr 1.84) to 1 litre with distilled water.

6. Procedure

6.1 Manual technique

Samples should be collected according to instructions given in "Part I, Sample Collection and Treatment."

Immediately before each use, clean all glassware used in this determination by rinsing, first with warm, dilute HNO_3 (1 + 4), and then with demineralized water.

Mercury

6.1.1 Pipet a volume of sample containing less than 1.0 µg Hg (100 ml maximum) into a 300-ml capacity BOD bottle and adjust the volume to approximately 100 ml.

6.1.2 Prepare a blank of demineralized water and sufficient standards, and adjust the volume of each to approximately 100 ml with demineralized water.

6.1.3 Add 5 ml concentrated H_2SO_4 and 2.5 ml concentrated HNO_3, mixing after each addition.

6.1.4 Add 2 ml $KMnO_4$ solution and shake. Add additional small portions of $KMnO_4$ solution, if necessary, until the purple color persists for at least 15 min.

Note 4: For water-suspended sediment mixtures and bed materials, add 15 ml $KMnO_4$ solution and additional portions if necessary.

6.1.5 Add 2 ml $K_2S_2O_8$ solution, mix, and heat for 2 hours in a boiling-water bath or for 3 hours in an oven at 95°C (NOTES 5 and 6).

Note 5: For water-suspended sediment mixtures and bed materials, add 8 ml $K_2S_2O_8$.

Note 6: BOD bottles may break when placed in boiling water therefore, place the bottles into a cool water bath and then raise the temperature in the bath. The samples must remain in the bath for 2 hours after boiling begins.

Note 7: As an alternative to a boiling-water bath, may be heated for three hours in an oven set at 90°C.

6.1.6 Remove from water bath, cool, and add $NH_2OH \cdot HCl$-NaCl solution in 2-ml increments to reduce the excess permanganate, as evidenced by the disappearance of the permanganate color.

6.1.7 Add 5 ml $SnCl_2$ solution to <u>one</u> sample and immediately attach the bottle to the aerator (NOTE 8). Record the percent absorption. After maximum absorption has been recorded, remove the BOD bottle and open the stopcock to the vacuum. Momentarily pinch off the vent tube in order to remove residual mercury vapor from the absorption cell. Treat each succeeding sample, blank, and standard in a like manner.

Note 8: Use the atomic absorption compressed-air supply or a peristaitic pump to aerate the sample. Adjust the rate of air flow to approximately 2 litres per minute.

6.2 Automated technique

Samples should be collected according to instructions given in "Part I, Sample Collection and Treatment."

Immediately before each use, clean all glassware used in this determination by rinsing, first with warm, dilute HNO_3 (1 + 4), and then with demineralized water.

6.2.1 Set up manifold (fig. 2 and 3).

6.2.2 Prepare a blank of demineralized water and sufficient standards.

6.2.3 Feed all reagents through the system using acid wash solution in the sample line. Allow heating bath to warm to 105°C.

6.2.4 Place a complete set of standards and samples in the sample tray.

6.2.5 Begin analysis.

7. Calculations

7.1 Convert percent absorption readings to corresponding absorbances, and determine the µg of Hg in the sample aliquot from a plot of absorbances of standards. Exact reproducibility is not obtained, and an analytical curve must be prepared with each set of samples.

7.2 Compute the concentration of Hg in the sample as follows:

$$\text{Hg in } \mu g/l = \mu g \text{ Hg } X \frac{1{,}000}{\text{ml sample aliquot}}$$

7.3 Determine the concentration of Hg in µg/g of air-dried bottom material sample as follows:

$$\text{Hg in } \mu g/g = \frac{\mu g \text{ Hg per sample}}{\text{sample weight, grams}}$$

8. Report

8.1 Report mercury (Hg), dissolved (71890), concentrations as follows: Less than 10 µg/l and greater than 0.5 µg/l, nearest 0.1 µg/l: 10 µg/l and above, two significant figures.

8.2 Report mercury, total (71900), concentrations as follows: See 8.1.

8.3 Report mercury, suspended (71895), concentrations as follows: See 8.1.

8.4 Report mercury, total in bottom materials (71921), concentrations as follows: Less than 1.00 µg/g, nearest 0.01 µg/g; 1.00 µg/ and above, two significant figures.

9. Precision

9.1 The precision of this method within its designated range may be expressed as follows:

$$S_T = 0.144X + 0.127$$

where:

S_T = overall precision, µg/1, and

X = concentration of Hg, µg/1.

References

Hatch, W. R., and Ott, W. L., 1968, Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry: Anal. Chemistry, v. 40, p. 2085-2087.

(U.S.) Environmental Protection Agency, 1971, Methods for chemical analysis of water and wastes: Washington, U.S. Govt. Printing Office, p. 121-130.

Molybdenum

Atomic absorption spectrophotometric method--chelation-extraction (I-1490-75)

Parameters and codes: Molybdenum, dissolved (µg/1): 01060
Molybdenum, total (µg/1): 01062
Molybdenum, suspended (µg/1): 01061
Molybdenum, total in bottom material (µg/g): 01063

1. Application

1.1 This method may be used to analyze waters containing from 1 to 25 µg of molybdenum per litre. (NOTE 1).

Note 1: It has been reported that samples up to 100 µg/1 can be analyzed without dilution.

1.2 Water-suspended sediment mixtures, suspended sediments, and bottom materials may be analyzed by this procedure after preliminary digestion-solubilization by Methods 3485 and 5485, respectively, if the iron does not exceed 50 mg/1.

2. Summary of method

2.1 Molybdenum is determined by atomic absorption spectrophotometry following chelation with 8-hydroxyquinoline and extraction with methyl isobutyl ketone (MIBK). The extract is aspirated into a nitrous oxide-acetylene flame of the spectrophotometer (Chau and Lum-Shue-Chan, 1969).

3. Interferences

3.1 The method is free from interference from most elements commonly found in fresh water. Vanadium (V) and iron (III) enhance the absorption while chromium (VI) and tungsten (VI) suppress it. With the addition of ascorbic acid up to 50 mg/1 of iron(III), 1 mg/1 of vanadium(V), and 10 mg/1 of chromium (VI) or tungsten(VI) can be tolerated (Chau and Lum-Shue-Chan, 1969).

4. Apparatus

4.1 Atomic absorption spectrophotometer.

4.2 Refer to the manufacturers' manuals or procedures to optimize output of the instruments for the following parameters.

Grating. . . . . .Ultraviolet
Wavelength counter . . . . . .313.3 nm
Source (Hollow-cathode lamp) .Molybdenum
Oxidant. . . . . .Nitrous oxide
Fuel . . . . . .Acetylene
Type of flame. . . . . .Fuel rich

4.3 Different burners may be used according to manufacturers' instructions.

5. Reagents

5.1 Ascorbic acid solution, 1 g/100 ml: Dissolve 1 g ascorbic acid in 100 ml demineralized water.

5.2 Bromphenol blue indicator solution, 0.1 g/100 ml: Dissolve 0.1 g bromphenol blue in 100 ml 50-percent ethanol.

5.3 Hydrochloric acid, 0.3M: Mix 25 ml concentrated HCl (sp gr 1.19) with demineralized water and dilute to 1 litre.

5.4 8-Hydroxyquinoline-methyl isobutyl ketone solution, 1 g/100 ml: Dissolve 1 g 8-hydroxyquinoline in 100 ml methyl isobutyl ketone. Prepare fresh daily.

5.5 Methyl isobutyl ketone (MIBK).

5.6 Molybdenum standard solution I, 1.0 ml = 100 µg Mo: Dissolve 0.1500 g reagent-grade MoO_3 in 10 ml 0.1M NaOH (warm if necessary). Make just acid with 0.1M HCl and dilute to 1,000 ml with demineralized water.

5.7 Molybdenum standard solution II, 1.00 ml = 1.0 µg Mo: Dilute 10.0 ml molybdenum standard solution I to 1,000 ml with demineralized water.

5.8 Molybdenum standard solution III, 1.0 ml = 0.1 µg Mo: Immediately before use, dilute 10.0 ml molybdenum standard solution II to 100.0 ml with demineralized water. This standard is used to prepare working standards at the time of analysis.

5.9 Sodium hydroxide solution, 2.5M: Dissolve 100 g NaOH in demineralized water and dilute to 1 litre.

6. Procedure

Samples should be prepared according to instructions given in Part III, Section 1, "Sample Preparation".

Clean all glassware used in this determination with warm, dilute HNO_3 (1 + 9), and rinse with demineralized water immediately before use.

6.1 Pipet a volume of sample containing less than 2.5 µg Mo (100 ml maximum) into a 200-ml volumetric flask, and adjust the volume to approximately 100 ml.

6.2 Prepare a demineralizedwater blank with 1.5 ml HNO_3/1 demineralized water and sufficient standards, and adjust the volume of each to approximately 100 ml with demineralized water.

6.3 Add 5 ml ascorbic acid solution and mix.

6.4 Add 2 drops bromphenol blue indicator solution and mix.

6.5 Adjust the pH by addition of 2.5M NaOH until a blue color persists. Add 0.3M HCl by drops until the blue color just disappears; then add 2.0 ml 0,3M HCl in excess. The pH at this point should be 2.3.

6.6 Add 5.0 ml 8-hydroxyquinoline-MIBK solution and shake vigorously for 15 min.

6.7 Allow the layers to separate; then add demineralized water until the ketone layer is completely in the neck of the flask.

6.8 While aspirating the ketone layer of the blank use auto zero to set instrument digital display to read 0. While aspirating standards use the auto concentration to set instrument digital display to read the concentrations of the standards. Use at least six standards. Calibrate the instrument each time it is set up in operation and check for stability at reasonable intervals.

7. Calculations

7.1 Determine the µg/l Mo in each sample from the digital display or printer output. Dilute samples that exceed the working range, repeat the chelation-extraction, and multiply by the proper dilution factors.

7.2 To determine µg/g molybdenum in bottom material samples, first determine the µg/l of molybdenum as in 7.1, then:

$$\text{Mo in } \mu g/g = \frac{\mu g/l \text{ Mo } X \frac{\text{ml of original digest}}{1{,}000}}{\text{wt of sample in grams}}$$

8. Report

8.1 Report molybdenum, dissolved (01060), concentrations as follows: Less than 100 µg/l, nearest µg/l; 100 µg/l and above, two significant figures.

8.2 Report molybdenum, total (01062), concentrations as in 8.1.

8.3. Report molybdenum, suspended (01061), concentrations as in

8.4 Report molybdenum, total in bottom material (01063), concentrations as follows:

9. Precision

The precision of this method within its designated range may be expressed as follows:

$$S_T = 0.072X + 0.447$$

where:

S_T = overall precision, µg/l, and

X = concentration of molybdenum, µg/l

References

American Society for Testing and Materials, 1975, Annual Book of ASTM Standards, part 31: Philadelphia, Am. Soc. Testing Materials, p. 350.

Chau, Y. K., and Lum-Shue-Chan, K., 1969, Atomic absorption determination of microgram quantities of molybdenum in lake waters: Anal. Chim. Acta, v. 48, p. 205-212.

Atomic absorption spectrophotometric method - direct (I-1850-75)

Parameters and codes:		
	Tin, dissolved (µg/l):	01100
	Tin, total (µg/l):	01102
	Tin, suspended (µg/l):	01101
	Tin, total in bottom material (µg/g):	none assigned

1. Application

1.1 This method may be used to analyze waters, water suspended sediment mixtures, suspended sediments, and bottom materials containing at least 500 µg of tin per litre. (NOTE 1)

Note 1: It has been reported that up to 10,000 µg/1 can be analyzed by this method.

1.2 Water-suspended sediment mixtures, suspended sediments, and bottom materials may be analyzed by this procedure after preliminary digestion - solubilization by Methods 3458 and 5485, respectively.

2. Summary of Method

2.1 Tin is determined by atomic absorption spectrophotometry by direct aspiration of the sample into an air acetylene flame without preconcentration or pretreatment of the sample.

3. Interferences

4. Apparatus

4.1 Atomic absorption spectrophotometer

4.2 Refer to the manufacturer's manuals or procedures to optimize output of the instruments for the following parameters.

Grating	Ultraviolet
Wavelength	224.6 nm
Source(hollow-cathode lamp) (or Electrodless Discharge Lamp)	tin
Oxidant	air
Fuel	acetylene
Type of flame	reducing

4.3 Different burners may be used according to manufacturers' instructions.

5. Reagents

5.1 Tin Standard Solution I, 1.00 ml = 1,000 µg Sn: Dissolve 1.000 g of tin metal in 100 ml of concentrated HCl and dilute to 1,000 ml with demineralized water.

5.2 Tin standard working solutions: Prepare a series of standard solutions containing from 0 to 10,000 µg of tin per litre by appropriate dilution of tin Standard Solution I. Standards should contain 10 ml of concentrated HCl for every 100 ml of standard. Standards should be prepared fresh daily.

6. Procedure

6.1 Samples should be prepared according to instructions given in Part III, Section 1, "Sample Preparation".

6.2 While aspirating the blank, set the chart recorder on 0 (NOTE 2). Use at least six standards. Calibrate the instrument each time it is set in operation and check for stability at reasonable intervals.

Note 2: Although a digital readout may be used, the high level of instrument noise makes using a chart recorder preferable.

7. Calculations

7.1 Determine the µg/l tin in each sample from a plot of absorbances of standards. Exact reproducibility is not obtained, and a working curve must be prepared with each set of samples.

7.2 To determine the µg/g of tin in bottom material samples, first determine the µg/l tin in each sample as in 7.1 then:

$$\text{Sn in } \mu g/g = \frac{\mu g/l \text{ Sn} \times \frac{\text{ml of original digest}}{1{,}000}}{\text{wt. of sample in grams}}$$

8. Report

8.1 Report tin, dissolved (01100), concentrations as follows: Less than 1,000 µg/l, nearest 100 µg/l; 1,000 µg/l and above, two significant figures.

8.2 Report tin, total (01102) concentrations as follows: See 8.1.

8.3 Report tin, suspended (01101) concentrations as follows: See 8.1.

8.4 Report tin, total in bottom material, concentration as follows:

9. Precision

9.1 No precision data are available for this method.

Parameter and Code: Vanadium, dissolved (µg/1): 01085

Catalytic oxidation colorimetric method (1880-75)

1. Application

1.1 This method may be used to analyze most waters containing from 0.1 to 5.0 µg of vanadium per litre provided that the interferences identified in section 3 are not exceeded. By reducing the reaction time, concentrations up to 100 µg/1 may be determined.

2. Summary of method

2.1 The oxidation of gallic acid by acid-persulfate is catalyzed by the presence of small amounts of vanadium (Jarabin and Szarvas, 1961). Depending on the amount of vanadium present, the reaction produces a yellow-to-red color, the absorbance of which is measured spectrophotometrically at 415 nm. Under given conditions of reactant concentration, temperature, and reaction time, the extent of oxidation of gallic acid is proportional to the concentration of vanadium present (Fishman and Skougstad, 1964).

3. Interferences

3.1 Several substances interfere, including chloride above 100 mg/1, and bromide and iodide at lower concentrations. Their interference is eliminated or minimized by the addition of mercuric nitrate solution. In the presence of mercuric nitrate, concentrations of 100 mg/1 Cl^{-1}, 0.25 mg/1 Br^{-1}, and 0.25 mg/1 I^{-1} can be tolerated. The following ions interfere when the indicated concentrations are exceeded: silver, 2 mg/1; cobalt, 1 mg/1; nickel, 3 mg/1; copper, 0.05 mg/1; chromium 1 mg/1; and ferrous iron, 0.3 mg/1.

3.2 Nitric acid causes erratic and uncertain results.

4. Apparatus

4.1 Water bath, regulated to $25^{o} \pm 0.5^{o}C$.

4.2 Spectrophotometer,

4.3 Refer to manufacturers' manuals or procedures for optimizing instrumental parameters:

Wavelength. . . . . 415 nm.

5. Reagents

5.1 Ammonium persulfate solution, 100 g/1: Dissolve 100 g $(NH_4)_2S_2O_8$ in demineralized water and dilute to 1 litre.

5.2 Ammonium persulfate-phosphoric acid reagent: Heat 25 ml ammonium persulfate solution to a gentle boil, remove from heat, and add an equal volume of concentrated H_3PO_4 (sp gr 1.69). Let stand for approximately 24 hr before using. Discard after 2 days.

5.3 Gallic acid solution, 2 g/100 ml: Dissolve 1 g gallic acid in 50 ml hot demineralized water and filter through Whatman No. 42 filter paper. Prepare fresh daily.

5.4 Mercuric nitrate solution, 332 mg/l: Dissolve 350 mg $Hg(NO_3)_2 \cdot H_2O$ in demineralized water and dilute to 1 litre.

5.5 Vanadium standard solution I, 1.0 ml = 100 µg V: Dissolve 0.2309 g ammonium vetavanadate (NH_4VO_3), in demineralized water and dilute to 1,000 ml.

5.6 Vanadium standard solution II, 1.00 ml = 1.0 µg V: Dilute 10.0 ml vanadium standard solution I to 1,000 ml with demineralized water.

5.7 Vanadium standard solution III, 1.0 ml = 0.01 µg V: Immediately before use, dilute 10.0 ml vanadium standard solution II to 1,000 ml with demineralized water. This standard is used to prepare working standards at time of analysis.

6. Procedure

Samples should be collected according to instructions given in "Part 1, Sample Collection and Treatment."

6.1 Pipet a volume of sample containing less than 0.08 µg V (10.0 ml maximum) into a 23-mm absorbance cell, and adjust the volume to 10.0 ml.

6.2 Prepare a blank and sufficient standards containing from 0.00 to 0.08 µg V in 23-mm absorbance cells, and adjust the volumes to 10.0 ml with demineralized water (standards must be run with each set of samples).

6.3 Add 1.0 ml mercuric nitrate solution to each sample, standard, and blank and place all cells in a water bath (25°C). Allow 30 to 45 min for samples to reach temperature equilibrium.

6.4 Add 1.0 ml ammonium persulfate-phosphoric acid reagent (temperature equilibrated). Mix and return to water bath.

6.5 Add 1.0 ml gallic acid (temperature equilibrated). Mix thoroughly and return to water bath (NOTE 1).

> Note 1: Since time and temperature are critical factors, the absorbance of each sample must be measured exactly 60 min after the gallic acid is added. When analyzing several samples, this is best accomplished by starting a stopwatch with the addition of gallic acid to the first sample and adding the gallic acid to subsequent samples at exactly 30-s intervals.

6.6 After about 58 min remove from the water bath; and, at exactly 60 min, measure the absorbance at 415 nm, using distilled water as a reference (NOTE 2).

Note 2: All samples may be removed from the water bath 1 or 2 min before the 60-min period is up. The samples are then prepared for measurement, and the absorbance of each sample is measured exactly 60 min after the addition of the gallic acid (30-sec intervals between each measurement).

7. Calculations

7.1 Determine the µg of V in the sample from a plot of absorbances of standards.

7.2 $V \text{ in } \mu g/l = \frac{1{,}000}{\text{ml sample}} \times \mu g \text{ V in sample.}$

8. Report

8.1 Report vanadium (V), dissolved (01085), concentrations as follows: Less than 10 µg/1, one decimal; 10 µg/1 and above, two significant figures.

9. Precision

9.1 The precision of this method within its designated range may be expressed as follows:

$$S_T = 0.069X + 0.422$$

where:

S_T = overall precision, µg/1, and

X = concentration of vanadium, µg/1.

References

American Society for Testing and Materials, 1975, Annual Book of ASTM Standards, part 31: Philadelphia, Am. Soc, Testing Materials, p. 441.

Fishman, M. J., and Skougstad, M. W., 1964, Catalytic determination of vanadium in water: Anal. Chemistry, v. 36, p. 1643.

Jarabin, Z., and Szarvas, p., 1961, Detection of small amounts of vanadium by catalytic reaction with the addition of gallic acid: Acta Univ. Debrecen, v. 7, p. 131-135; Chem. Abs., v. 57, 9192c, 1962.

Organic constituents

Organic matter, extractable (oil and grease)

Organic matter, extractable (O-1555-75)

Parameters and Codes: Organic matter, total extractable (mg/l): 00550
Organic matter, extractable, bottom material (mg/kg): 00553

Trichlorotrifluoroethane extraction--gravimetric method

1. Application

1.1 This method may be used to analyze samples of fresh waters, wastes, samples of water-suspended sediment mixtures, and bottom materials.

1.2 Bottom materials may be analyzed by this procedure after they have been prepared as directed in Method 0520.

2. Summary of method

2.1 A water sample is extracted twice with trichlorotrifluoroethane solvent and the solvent-extract mixture evaporated at 20°C to leave a nonvolatile residue whose weight represents an estimate of the extractable organic matter in the sample.

2.2 The procedure approximates the determination of oils and grease in water, and is similar in substance to Method D 2778-70, in the American Society for Testing and Materials, "1973 Annual Book of Standards, Part 23, Water; Atmospheric Analysis," and to Method 137, Oil and Grease in "Standard Methods for the Examination of Water and Wastewater," 13th Edition (1971).

3. Interferences

3.1 Organic solvents vary considerably in their ability to dissolve oily substances and other organic matter. Any method used to obtain an estimation of the amount of extractable organic matter must, of necessity, be highly empirical. Close attention to all operations of the analytical procedure are required to achieve results that may be compared with confidence.

4. Apparatus

4.1 Dish, aluminum-foil, 110-mm diam., 100-ml capacity.

4.2 Filter paper, Whatman No. 40, or equivalent.

4.3 Graduated cylinder, 1000-ml capacity, glass.

4.4 Separatory funnel, glass stoppered, TFE-fluorocarbon stop-cock, 1000-ml capacity. (NOTE 1).

Note 1: For the determination of extractable organic matter in bottom material, use separatory funnel of 250-ml capacity.

5. Reagents

All reagents must be of analytical reagent quality. Use distilled or distilled-and-demineralized water to prepare all solutions.

5.1 Sodium sulfate (Na_2SO_4), anhydrous, granular.

5.2 Sulfuric acid, concentrated (sp gr = 1.84).

5.3 Sulfuric acid (1 + 1): Slowly and cautiously, with constant stirring and cooling, add 100 ml concentrated H_2SO_4 to 100 ml demineralized water.

5.4 Trichlorotrifluoroethane solvent: 1,1,2-trichlorotrifluoroethane, b. p. 47.6°C.

6. Procedure

6.1 Water-suspended sediment mixtures.

6.1.1 Collect approximately 900 ml of sample in a 1-litre glass bottle. To facilitate collection of sample place a mark on the bottle at the 900-ml level.

6.1.2 Transfer the entire contents of the glass bottle to a 1000 ml graduated cylinder. Record the volume.

6.1.3 Transfer the contents of the graduated cylinder to a 1000-ml separatory funnel, and add 5 ml sulfuric acid (1 + 1). Shake to mix thoroughly.

6.1.4 Rinse the glass bottle with 25 ml trichlorotrifluoroethane solvent and transfer the solvent to the graduated cylinder. Rinse the graduate and transfer the solvent to the separatory funnel. Shake vigorously for 2 min, stopping to vent the pressure as necessary.

6.1.5 Allow the layers to separate and then draw off the solvent and filter it through a small amount of anhydrous Na_2SO_4 placed on a small filter paper in a funnel. Collect the filtrate in a tared aluminum-foil dish.

6.1.6 Repeat steps 6.3 and 6.4, filtering the solvent through the same funnel and adding the filtrate to that already collected in the aluminum-foil dish.

6.1.7 Wash the filter paper with three 5-ml portions of solvent, collecting all washings in the aluminum-foil dish.

6.1.8 Evaporate the solvent collected in the dish at room temperature (20°C) in a well-ventilated fume hood.

6.1.9 Weigh the residue remaining in the dish after evaporation of the solvent.

6.1.10 Evaporate an equivalent volume of solvent (65 ml) in a similar manner from a tared aluminum-foil dish and subtract the residual weight (blank) from the sample-extract residual weight. The weight of the blank so obtained should not exceed 4.0 mg.

6.2 Procedure for bottom materials

6.2.1 Weigh, to the nearest milligram, approximately 1 g of sample. Use sample material prepared as directed in "Sample preparation (0520)." Alternatively, a wet sample may be used.

6.2.2 Quantitatively transfer the weighed sample to a 250-ml capacity separatory funnel. Add approximately 100 ml demineralized water and shake to mix.

6.2.3 Add 5 ml of sulfuric acid (1 + 1). Shake to mix thoroughly.

6.2.4 Add 25 ml trichlorotrifluoroethane and shake vigorously for 2 min, stopping to vent the pressure as necessary.

6.2.5 Allow the layers to separate and then draw off the solvent and filter it through a small amount of anhydrous Na_2SO_4 placed on a small filter paper in a funnel. Collect the filtrate in a tared aluminum-foil dish.

6.2.6 Repeat steps 6.1.5 and 6.1.6, filtering the solvent through the same funnel and adding the filtrate to that already collected in the aluminum-foil dish.

6.2.7 Proceed as directed in paragraph 6.1.7.

7. Calculations

7.1 Determine the milligrams per litre extractable organic matter in the sample as follows:

$$\text{Organic matter, extractable, mg/l} = \frac{R_x - R_b}{\text{ml sample}} \times 1000$$

where

R_x = weight of extracted residue, milligrams, and

R_b = weight of solvent residue (blank), milligrams.

7.2 Determine the mg/kg extractable organic matter in the air-dried sample as follows:

$$\text{Organic matter, extractable, mg/kg} = \frac{R_x - R_b}{\text{sample weight, grams}} \times 1000$$

where

R_x = weight of extracted residue, milligrams, and

R_b = weight of solvent residue (blank), milligrams.

Note 2: If wet bottom material sample is used in preference to air-dried sample, a factor correcting for moisture content must be applied to above equation.

8. Report

8.1 Report organic matter, total extractable (00550), concentrations as follows: Less than 10 µg/l, nearest µg/l; 10 µg/l and above, two significant figures.

8.2 Report organic matter, extractable, air-dried bottom material (00553), concentrations as follows: Less than 10,000 mg/kg, nearest 1,000 mg/kg; 10,000 mg/kg and above, two significant figures.

9. Precision

9.1 Precision data cannot be given for this determination because of the variable nature of the materials being extracted.

References

American Public Health Association and others, 1971, Standard Methods for the examination of water and wastewater (13th ed): New York, Am. Public Health Assoc., Inc., p. 254-256.

American Society for Testing and Materials, 1973, Water; atmospheric analysis, pt 23 of 1973 Book of standards: Philadelphia, Am. Soc. Testing Materials, p. 457-461.

Radiochemical determinations

Gross alpha and beta radioactivity, dissolved and suspended: (R-120-75)

Parameters and codes:

Gross alpha, dissolved, as U natural, (µg/l):	80030
Gross alpha, dissolved, as U natural (µg/l), counting error:	none assigned
Gross alpha, suspended, as U natural, (µg/l):	80040
Gross alpha, suspended, as U natural, (µg/l), counting error:	none assigned
Gross alpha, total, as U natural, (µg/l), sum of parameters 80030 and 80040:	none assigned
Gross alpha, total, as U natural, (µg/l), counting error:	none assigned
Gross beta, dissolved, as cesium 137, (pCi/l):	03515
Gross beta, dissolved, as cesium 137, (pCi/l), counting error:	none assigned
Gross beta, dissolved as strontium/yttrium-90, (pCi/l):	80050
Gross beta, dissolved, as strontium/yttrium-90, (pCi/l), counting error:	none assigned
Gross beta, suspended, as cesium 137, (pCi/l):	03516
Gross beta, suspended, as cesium 137, (pCi/l), counting error:	none assigned
Gross beta, suspended, as strontium/yttrium-90, (pCi/l):	80060
Gross beta, suspended, as strontium/yttrium-90, (pCi/l), counting error:	none assigned
Gross beta, total, as cesium 137, (pCi/l) sum of parameters 03515 and 03516:	none assigned
Gross beta, total, as cesium 137, (pCi/l), counting error:	none assigned
Gross beta, total, as strontium/yttrium-90, (pCi/l), sum of parameters 80050 and 80060:	none assigned
Gross beta, total, as strontium/yttrium-90, (pCi/l), counting error:	none assigned
Gross alpha, suspended, specific activity as U natural, (µg/g):	01518
Gross beta, suspended, specific activity as cesium-137, (pCi/g):	03518
Gross beta, suspended, specific activity as strontium/yttrium-90, (pCi/g):	03517

1. Application

1.1 The method is applicable to any natural water sample, but because of restrictions on the weight of residue which can be accommodated, the sensitivity decreases with increasing concentrations of dissolved solids.

2. Summary of method

2.1 The method is an extension of the procedure published by Barker and Robinson (1963) for gross beta radioactivity.

2.2 A representative aliquot but not more than 1 litre of the sample, including suspended solids, is filtered through a tared 0.45 micrometre membrane filter. The filter and retained solids are dried at room temperature and then at 105°C, cooled, and reweighed to determine the weight of non-filtrable residue per litre.

2.3 A filtered volume of the sample containing no more than 150 mg of dissolved solids is evaporated to dryness in a Teflon evaporating dish. The residue is transferred to a tared, 2-inch concentric-ring, stainless-steel planchet, dried in a desiccator, weighed, and counted on a low-background alpha-beta counter. The observed activity is compared with the activity of natural uranium and strontium-90-ytttrium-90 and cesium-137 calibration standards, and results are reported relative to these reference isotopes. Thus, the measured sample activity is reported in terms of the amount of natural uranium and equilibrium strontium-90-ytrrium-90 and cesium-137 activity which would give the same alpha and beta count rates respectively for the same weight of residue. Since reference standards are used, the reported gross activities are only approximations of the true alpha and beta activities of the sample.

2.4 The accuracy of these approximations depends on a number of variables related to the energy distributions of the alpha and beta particles and the similarity of the residues used in preparation of the calibration curves to the actual sample residue. The method must be regarded as a relatively crude, but rapid, semiquantitative measure of gross sample activity.

3. Interferences

3.1 Within its intended purpose, the method is free of interferences, although the accuracy varies considerably with the nature of the alpha and beta emitters, chemical composition of the sample, and uniformity of planchet preparation.

4. Apparatus

4.1 Evaporating dishes, Teflon, 100 ml.

4.2 Hotplate or steam table.

4.3 Infrared drying lamps.

4.4 Low-bakcground counting equipment: Proportional counters capable of measuring both alpha and beta activity are desirable (for example, Beckman Instrument Co. Wide-beta or Low-beta II, or equivalent.

4.5 Membrane filters, 47-mm diameter, 0.45 micrometre pore size, cellulose nitrate or acetate type.

4.6 Planchets, stainless steel, 2-inch diameter, concentric-ring type.

4.7 Specific conductance meter.

4.8 Vacuum desiccator.

4.9 Vacuum filtration apparatus, for 47-mm membrane filters.

5. Reagents. (Naa)

5.1 Calibration solution A: Dissolve 0.284 g $MgSO_4 \cdot 7H_2O$, 0.070 g NaCl, 0.026 g $CaSO_4 \cdot 2H_2O$, 0.109 g $NaHCO_3$, and 0.245 g $CaCO_3$ in distilled water, bubbling CO_2 gas through the solution if necessary to obtain clear solution. Dilute to 2.00 litres.

5.2 Calibration solution B: Dissolve 1.350 g $MgSO_4 \cdot 7H_2O$, 3.510 g NaCl, 1.550 g $CaSO_4 \cdot 2H_2O$, 0.508 g $MgCl_2 \cdot 6H_2O$, and 0.300 g $CaCO_3$ in distilled water, using CO_2 bubbling as necessary to dissolve. Dilute to 2.00 litres.

Note: Composition of the calibration solutions should approximate that of the samples to be analyzed. Solutions A and B were selected to approximate the composition average of 12 major rivers in the United States.

5.3 Cesium-137 standard solution, approximately 500 pCi/ml and acidified to approximately 1N with hydrochloric acid.

5.4 Hydrofluoric acid, 49 percent.

5.5 Strontium-90-yttrium-90 standard solution, approximately 500 pCi/ml combined activity and acidified to approximately 1N with hydrochloric acid.

5.6 Uranium standard solution, 1.00 ml = 100 µg U: Dissolve 0.1773 g of $UO_2(C_2H_3O_2)_2 \cdot 2H_2O$ in approximately 500 ml distilled water. Add 15 ml conc HNO_3 and dilute to 1,000 ml in a volumetric flask. Store in a Teflon bottle.

6. Procedure

6.1 Preparation of beta calibration curves.

6.1.1 Add the following amounts of calibration solutions A and B to 100 ml teflon evaporating dishes:

Dish No.	Ml A	Ml B	Approx. residue wt., mg
1	27.0		10
2		5.7	20
3	81.0		30
4		11.4	40
5	135		50
6		17.1	60
7	189		70
8		22.9	80
9	242		90
10		28.3	100
11	297		110
12		34.2	120
13	351		130
14		40.0	140
15	405		150

To each dish and to four additional 100-ml teflon evaporating dishes (Nos. 16, 17, 18, and 19) add 1.00 ml of strontium/yttrium-90 (Sr/Y-90) standard solution. To dishes 18 and 19 also add 5 drops concentrated NH_4OH.

Procedure is also followed for preparation with cesium-137 standard solution.

6.1.2 Evaporate all the solutions to dryness on a low-temperature hotplate. When 18 and 19 are dry, increase heat to approximately 250°C to volatilize NH_4Cl.

6.1.3 Perform steps 6.6 through 6.11 of the procedure which follows.

6.1.4 Plot the beta efficiency (net counts per minute per picocurie) against the residue weight to obtain the beta calibration curve.

6.1.5 Prepare a beta calibration curve for cesium-137 in the same manner following steps 6.1.1 through 6.1.4 using 1.00 ml of cesium-137 standard solution.

6.2 Preparation of alpha calibration curve.

The alpha calibration curve is obtained in exactly the same manner as the beta curve (step 6.1) except that 100 µg of uranium (1 ml of uranium standard solution) is substituted for the $Sr^{90}Y^{90}$ beta standard. It is important that the uranium standard be in secular equilibrium with respect to uranium-234. The uranium isotopes ratio may be determined by the method described elsewhere in this manual.

Radiochemical determinations

6.3 Sample analysis: Measure the specific conductance of each water sample using a meter. Multiply the specific conductance in μmhos by 0.65 to obtain an approximate value for the total dissolved-solids content of the sample in milligrams per litre. Determine the volume of sample which will contain approximately 100 mg of dissolved solids:

$$\text{Sample volume, } V(1) = \frac{100 \text{ mg}}{\text{mg/1}}$$

The actual sample residue should weigh between 50 and 130 mg.

6.4 Gross radioactivity of the suspended solids is determined as follows: Vigorously agitate the sample bottle containing the total amount of sample collected and quickly pour off 1 litre of sample with suspended solids into a graduated cylinder. Allow sediment to settle and then using suction, filter through a tared membrane filter. When only 100 mls of sample remains to be filtered, swirl to suspend solids and add suspension to filter funnel. Any remaining traces of sediment may be washed out of the graduate using small amounts of filtered sample.

6.5 Carefully rinse solids with a small amount of distilled water and maintain as uniform a deposit as possible. Remove filter, allow to air dry and weigh. If the weight of solids is in excess of 150 mg refilter a fresh aliquot of appropriate volume to obtain less than 150 mg, weigh, dry at 105°C and count for alpha and beta radioactivity.

Although not totally comparable, the same absorption or counting efficiency factors for the respective counting instruments are used as for dissolved solids and the calculations are made in the same way. The weight of suspended solids per litre of sample is also reported (USGS parameter code 00530) and may be used to calculate the radioactivity associated with the total weight of suspended solids per litre of original sample. Specific activity of the solids in picocurie per grams of suspended solids for the equivalent strontium/yttrium-90 or cesium-137 may also be calculated.

6.6 Gross radioactivity of the dissolved solids is determined as follows: Using a tared Teflon dish, evaporate the required volume of water to dryness on a hotplate or steam bath. Remove Teflon dishes from hotplate as soon as they reach dryness to prevent warping of dishes due to excess heat.

6.7 Determine approximate weight of residue by weighing dish plus residue and subtracting tare weight. If the weight of residue falls outside the 50 to 130 mg range, start a new evaporation using a larger or smaller sample volume as required.

6.8 Quantitatively transfer the residues in the evaporating dishes to tared planchets using rubber policemen and a minimum amount of distilled water to effect the transfers. Confine the solution and residue to the three inner concentric rings of the planchet during the transfer.

6.9 Dry the planchets under infrared heat lamps. Police down the evaporating dishes with small additional amounts of distilled water and transfer to planchets.

6.10 Repeat step 6.6. If a residue remains in the evaporating dish after three washes with distilled water, add a small amount of hydrofluoric acid and use for a final policing.

6.11 Disperse the final liquid slurry in the planchets as uniformly as possible, using a stirring rod to disrupt large aggregates. Again evaporate to dryness.

6.12 Place the planchets in a vacuum desiccator and evacuate to complete the drying; weigh the planchets and determine the weight of residue.

6.13 Count the planchets in a low-background alpha-beta counter. Obtain three 50-min counts on each sample.

7. Calculations

Determine the concentration of alpha and beta radioactivity in each planchet using the appropriate counting efficiency factors and the following equations:

$$\text{Gross } \alpha = \frac{S\alpha \ 1000}{F\alpha \quad V} = \mu g/l \text{ as U natural}$$

$$\text{Gross } \beta = \frac{S\beta \ F\beta \ 1000}{V} = \text{pCi/l as } Sr^{90}/Y^{90} \text{ or as } Cs^{137}$$

where

$S\alpha$ = net alpha count rate of sample in counts per minute (cpm)
$S\beta$ = net beta count rate of sample in cpm
$F\alpha$ = alpha factor in spm/μg of natural U
$F\beta$ = beta factor in picocuries per count per minute
V = sample volume in millilitres.

Counting error

Determine σ_N and σ_N , the respective standard deviations of the net alpha or beta counting rates from the equation:

$$\sigma_N = \sqrt{\frac{R_B + R_s}{t_B \quad t_s}}$$

where

R_B = alpha or beta background counting

t_B = number of minutes that background was counted

R_s = sample alpha or beta gross counting rate in spm

t_s = number of minutes that sample was counted.

then, calculate the equivalent alpha or beta activity (±) per litre due to the counting error at the 2 σ (95 percent) confidence level by the following:

$$E_\alpha(\pm) = \frac{2\ \sigma_{N\alpha}\ 1000}{F\alpha V}$$

or

$$E_\beta(\pm) = \frac{2\ \sigma_{N\beta}\ F\beta\ 1000}{V}$$

where

$E_\alpha(\pm)$ = alpha counting error

$E_\beta(\pm)$ = beta counting error.

8. Report

Report values of less than 1 pCi/l or 1 µg/l to one significant figure.

9. Precision

Results for a particular sample are usually reproducible to about ± 20 percent at the 95 percent confidence level.

Radium-226, dissolved (R-141-75)

Parameter and code: Radium-226, dissolved, (pCi/1): 09511

1. Application

1.1 The method is applicable to any water sample.

2. Summary of method

2.1 The method is based on the isolation of radon-222 produced by radium-226 and measurement of the alpha activity of the radon and its short-lived daughters. The method is specific for radium-226 in contrast to the precipitation method of Barker and Johnson (1964). The procedure substitutes a complexing agent to redissolve precipitated barium sulfate, in the emanation method of Rushing (1964). Formerly a complex procedure for resolution involved a strong acid, ashing and evaporation. Radon is measured in a modification of the alpha scintillation cell of Lucas (1957).

2.2 Dissolved radium in filtered water is collected by coprecipitation with barium sulfate. The precipitate is centrifuged and then dissolved in alkaline diethylene triamine penta acetic acid (DPTA). The solution is transferred to a radon bubbler and any radon present is removed by purging with helium gas. Fresh radon is then allowed to grow in. After several days the ingrown radon is purged into an alpha scintillation cell, short-lived daughters are allowed to grow in and the alpha count rate is then determined. The radium-226 concentration in the original water sample is calculated from the radon determination on the basis of the rate of radon production with time.

3. Interferences

3.1 The method is normally specific for radium-226. Radium-223 and radium-224 produce radon-219 and radon-220, respectively. Neither of these interfere directly, but the 10.6 hour lead-212 from radon-220 has alpha emitting daughters which could interfere. Wait for two or three days before counting eliminates the interference. The alpha emitting daughters of radon-219 have no effect if sufficient waiting time is allowed for complete decay of the 36-min lead-211.

4. Apparatus

4.1 Alpha counting apparatus: Scaler and high voltage power supply, preamp and amplifier with discriminator.

4.2 Beaker: 1,500 ml.

4.3 Gas delivery system: For helium gas.

4.4 Mixer: Wiggle plate or ultrasonic type.

4.5 Radon de-emanation train and bubbler (fig. 1).

4.6 Radon scintillation cell and housing (fig. 2).

5. Reagents

5.1 Barium carrier solution: 50 mg barium/ml; dissolve 75.81 g barium chloride ($BaCl_2$) in distilled water and dilute to 1,000 ml.

5.2 Defoaming emulsion: Dow Corning Anti Foam H-10 emulsion, or equivalent; dilute to approximately 4 to 5 percent solution with distilled water before using.

5.3 DTPA-TEA solution: Dissolve 10 g of sodium hydroxide pellets in a beaker containing 60 ml of distilled water and stir in cold water bath until dissolved. Add 20 g of purified diethylene triamine penta acetic acid (DPTA) and continue stirring until dissolved. Add 17 ml of 50 percent triethanolamine, mix and dilute to 100 ml. Store in Teflon bottle.

5.4 Radium, standard solution I, 1 ml = 50.0 pCi: This solution is prepared from National Bureau of Standards encapsulated radium standard No. 4955 which contains 0.100×10^{-6} Ci of radium-226 in 5 ml of 5 percent HNO_3. Rubber gloves should be worn in preparing a standard solution by the following recommended procedure.

5.4.1 Place the vial containing the radium standard in a clean, heavy-wall, small-neck bottle or flask of 250- to 500-ml capacity. Add 50 ml of 3N HCl and stopper securely with a polyethylene stopper.

5.4.2 Place the bottle (or flask) in a durable plastic sack and, holding the stopper firmly in place, shake vigorously to break the vial.

5.4.3 Decant the solution into a 2-litre volumetric flask.

5.4.4 Rinse the bottle with 50 ml of 3N HCl and decant into the 2-litre flask.

5.4.5 Add another 50 ml of 3N HCl and wash thoroughly using the ultrasonic cleaner. Decant into the 2-litre flask.

5.4.6 Rinse with 50 ml of 3N HCl. Decant into the 2-litre flask.

5.4.7 Repeat steps 5.4.4 and 5.4.5 alternately, three more times each.

5.4.8 Dilute the solution in the 2-litre flask to 2 litres with distilled water and mix thoroughly.

The final concentrations of radium and hydrogen ion in the stock solutions are: $(Ra^{+2}) = 50$ pCi/ml and $(H^{+}) = 0.75$ mole/l.

5.5 Radium, standard solution II, 1 ml = 1.000 pCi: Dilute 10.00 ml radium standard solution I and 10 ml of concentrated HCl to 500 ml with distilled water.

5.6 Sulfuric acid with solution: Add 5 ml of concentrated H_2SO_4 and 3 5 drops of Triton X-100 to 4 litres of distilled water.

5.7 Sulfuric acid, concentrated.

6. Procedure

6.1 Precipitation of radium in barium sulfate.

6.1.1 Add 5 ml concentrated hydrochloric acid to 1,000 ml of filtered water sample contained in a 1,500 ml beaker.

6.1.2 Add 1 ml of 50 mg/ml barium carrier to the sample and stir.

6.1.3 Cautiously add 20 ml of concentrated sulfuric acid to each sample with constant stirring. (Use of a 500 ml dispensing flask fitted with a 20 ml delivery head facilitates the acid addition). Stir well after the acid addition. Allow barium sulfate precipitate to settle overnight.

6.1.4 Carefully remove the supernate by decantation or suction and quantitatively transfer the balance of the supernate and precipitate to a 40 ml centrifuge tube using a rubber policeman and small quantities of dilute sulfuric acid-Triton-X-100 wash solution.

6.1.5 Centrifuge as necessary, decant and discard supernate.

6.1.6 Add approximately 10 ml of distilled water and 1.5 ml of DTPA reagent to the precipitate in the centrifuge tube. Disperse the precipitate in each tube by using a wiggle plate mixer or an ultrasonic unit. Place tubes in a wire rack and immerse rack and tubes to a depth of approximately 1 inch in a boiling water bath.

6.1.7 Complete dissolution should occur within a few minutes if the barium sulfate "pellet" was adequately dispersed. Occasionally, total volume in the centrifuge tubes may decrease by 4-5 ml as a result of prolonged heating and the precipitate may not dissolve. Addition of distilled water to bring the total volume to approximately 20 ml 9 max plus additional redispersion and heating will usually result in rapid dissolution of even difficult soluble precipitates. After the precipitate has dissolved, cool the tubes.

6.2 De-emanations

6.2.1 Using a funnel with a fine tip, transfer the cooled solution to a clean bubbler. Wash the centrifuge tube several times with distilled water and add the washings and sufficient additional water to the bubbler to leave approximately 2 cm of air space at the top. Add 1-3 drops of 4 percent silicone defoaming emulsion to the solution in the bubbler to minimize frothing during purging.

6.2.2 Attach stopcock and "O" ring to bubbler using clamp, leaving outlet stopcock on bubbler assembly in open position. Attach helium line (3-5 psi) to inlet side of bubbler. Slowly open stopcock on inlet until a stream of fine bubbles rises from the porous disk. Maintain a steady flow of bubbles through the sample for approximately 20 min to completely purge all ingrown radon from the solution. Close inlet stopcock and allow pressure under porous disc to equalize momentarily. Close outlet and record the day, hour and minute. This is zero time for the growth of radon that will be removed in the second de-emanation and counted.

6.2.3 Allow from 2 to 20 days ingrowth time for radon-222 (samples suspected of high radium-226 values require shorter periods of ingrowth), depending upon the radium-226 concentration in the original sample, volume of sample used, et cetera.

6.2.4 The second de-emanation is made by setting up the bubbler as in 6.2.2 except that the outlet stopcock is closed initially. Attach bubbler to drying tube with "O" ring and clamp. Evacuate purging assembly, including scintillation cell, with vacuum pump for approximately 1.5 to 2 min. Close stopcock at vacuum pump, turn pump off and momentarily crack vacuum pump connection. Open stopcock in helium line above bubbler inlet stopcock and momentarily crack "O" ring connection to purge trapped air from line and bubbler inlet connection. Clamp and allow system to stand for approximately two minutes. If system leaks, manometer meniscus will flatten or manometer will begin to fall. If meniscus remains stable proceed as follows:

6.2.5 Carefully open bubbler outlet stopcock until manometer begins to fall (check porous disk for fine bubbles). Allow vacuum to equilibrate slowly, otherwise there is excessive risk of drawing liquid sample into drying tube). Bubbling will slow appreciably in a few seconds. Slowly open outlet stopcock completely. Then continue with purging by slowly opening bubbler inlet stopcock, checking porous disk carefully for rising bubbles (flow rate must be closely controlled again at this point, to prevent sudden surge of liquid into drying tube). Allow pressure to build up slowly, controlling manometer fall rate to complete purging in 15-20 min. Close cell stopcock approximately 4 mm before manometer indicates atmospheric pressure to guard against pressure leak from cell during counting.

6.2.6 Close purging assembly stopcocks from cell to helium inlet line in sequence as rapidly as possible. Record time. Remove bubbler from assembly quickly and crack outlet stopcock momentarily to release helium pressure.

6.2.7 Place cell in light-tight counting chamber. Allow to age three of four hours before counting. Count overnight (1,000 min) for the average water sample.

6.2.8 Dates, times, counts, and all other pertinent sample information should be recorded on data and calculation sheets.

6.3 Calibration of equipment.

6.3.1 Put 25 ml of radium standard II into a 1,500 ml beaker and dilute to approximately 1,000 ml. Prepare a distilled water blank in a second beaker.

6.3.2 Carry out the analytical procedure as above for the standard and blank for each scintillation cell and counting instrument combination. Count the standard to total of 10,000 counts and count the blank overnight. Blanks are repeated whenever a new batch of reagents is used or whenever a change is made in the system. Instrument backgrounds, cell backgrounds, and cell constants (counting efficiency) varies with each cell-instrument combination. For the ultimate in accuracy each combination must be checked.

7. Calculations

Calculate the radium-226 concentration from the following equation (all values to 3 significant figures):

$$\text{Ra-226 (pCi/l)} = \frac{I_{sample}\ 1000\ f_c}{I_{std}\ V\ f_g\ f_d}$$

where

I_{sample} = counting rate of sample (counts per minute) corrected for background using instrument and scintillation cell combination,

I_{std} = counts per minute for one picocurie of radium std (corrected for background, fraction of equilibrium radon attained, decay before counting, decay during counting) for scintillation cell and instrument combination used,

V = volume of sample in millilitre.

f_g = fraction of the equilibrium amount of radon that had grown in at the time of the second de-emanation. The value of f_g is obtained:

$$f_g = 1 = e^{-\lambda t_1}$$

where

λ = decay constant of radon-222 (1.259×10^{-4} min^{-1}),

t_1 = time elapsed between first and second de-emanations (in minutes),

f_d = fraction of radon remaining after radioactive decay from the end of the second de-emanation to the start of counting. The value of f_d may be calculated from the expression:

$$f_d = e^{-1.259 \times 10^{-4} t_2}$$

where

t_1 = time elapsed between end of second de-emanation to start of the count (in minutes).

f_c = correction factor for decay that takes place during counting.

8. Report

Report concentrations less than 0.10 pCi per litre to one significant figure and values above 0.10 pCi/l to two significant figures.

9. Precision

On the basis of limited data the precision at the 0.10 pCi/l level is estimated at $\pm$ 20 percent. The precision above 0.10 pCi/l is estimated at $\pm$ 10 percent.

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