DISSOLVED OXYGEN (DO)

The Azide Modification of the Winkler Method

1. Collection the sample in glass-stoppered BOD bottle of 250-300 mL capacity. Write down the volume of the bottle.
2. Remove the glass stopper from the sample bottle, using a measuring pipet, add 1 ml if manganous sulfate solution followed by 1 ml alkali-iodide-azide reagent. Place the tip of the pipet below the surface of the water so as to allow the heavy solution to flow in without contact with the air
3. Stopper carefully to exclude air bubbles and mix by inverting the bottle a few times
4. Allow the resulting precipitate to settle at least to one half the bottle volume to leave clear sup mate above the manganese hydroxide floc.
5. Remove the stopper again, and with measuring pipet, add 1ml conc. Sulphuric acid
6. Re stopper carefully to prevent air from entering the bottle Mix by inverting several times until the precipitate completely dissolves and the brown or yellow color is distributed uniformly.
7. Titrate with 0.025 N sodium thiosulfate solutions a volume corresponding to 200 ml original sample after correction for sample loss by displacement with reagents. Thus for a total of 2 ml of reagents (1 ml each of MnSO4 and alkali-iodide- azide reagents) in a 300-ml, titrate 200x300 = 201 ml

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1. Gradually add small portions of the sodium thiosulfate titrant while constantly swirling the liquid in the flask, until the sample changes to a pale yellow or straw color
2. Add a few drops of starch indicator solution and continue the titration to the first disappearance of the blue color.
3. Calculation

 mg/L DO=A x N x8000

 Ml of sample

 Where:

A = ml sodium thiosulfate

N= Normality of sodium thiosulfate

Note 1) if the end points is over run, add a measured volume of treated sample and titrate carefully to the proper end point. Correct for the amount of sample added.

2) Disregard subsequent re colorations.

**5210 B. 5-Day BOD Test**

1. General Discussion

*a. Principle:* The method consists of filling with sample, to overflowing, an airtight bottle of

the specified size and incubating it at the specified temperature for 5 d. Dissolved oxygen is

measured initially and after incubation, and the BOD is computed from the difference between

initial and final DO. Because the initial DO is determined shortly after the dilution is made, all

oxygen uptake occurring after this measurement is included in the BOD measurement.

*b. Sampling and storage:* Samples for BOD analysis may degrade significantly during

storage between collection and analysis, resulting in low BOD values. Minimize reduction of

BOD by analyzing sample promptly or by cooling it to near-freezing temperature during storage.

However, even at low temperature, keep holding time to a minimum. Warm chilled samples to

20 ± 3°C before analysis.

1) Grab samples—If analysis is begun within 2 h of collection, cold storage is unnecessary.

If analysis is not started within 2 h of sample collection, keep sample at or below 4°C from the

time of collection. Begin analysis within 6 h of collection; when this is not possible because the

sampling site is distant from the laboratory, store at or below 4°C and report length and

temperature of storage with the results. In no case start analysis more than 24 h after grab sample

collection. When samples are to be used for regulatory purposes make every effort to deliver

samples for analysis within 6 h of collection.

2) Composite samples—Keep samples at or below 4°C during compositing. Limit

compositing period to 24 h. Use the same criteria as for storage of grab samples, starting the

measurement of holding time from end of compositing period. State storage time and conditions

as part of the results.

2. Apparatus

*a. Incubation bottles*: Use glass bottles having 60 mL or greater capacity (300-mL bottles having a ground-glass stopper and a flared mouth are preferred). Clean bottles with a detergent,

rinse thoroughly, and drain before use. As a precaution against drawing air into the dilution

bottle during incubation, use a water seal. Obtain satisfactory water seals by inverting bottles in a

water bath or by adding water to the flared mouth of special BOD bottles. Place a paper or

plastic cup or foil cap over flared mouth of bottle to reduce evaporation of the water seal during

incubation.

*b. Air incubator or water bath,* thermostatically controlled at 20 ±1°C. Exclude all light to

prevent possibility of photosynthetic production of DO.

3. Reagents

Prepare reagents in advance but discard if there is any sign of precipitation or biological

growth in the stock bottles. Commercial equivalents of these reagents are acceptable and

different stock concentrations may be used if doses are adjusted proportionally.

*a. Phosphate buffer solution:* Dissolve 8.5 g KH2PO4, 21.75 g K2HPO4, 33.4 g

Na2HPO4⋅7H2O, and 1.7 g NH4Cl in about 500 mL distilled water and dilute to 1 L. The pH

should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g KH2PO4 or 54.3 g

K2HPO4 in about 700 mL distilled water. Adjust pH to 7.2 with 30% NaOH and dilute to 1 L.

*b. Magnesium sulfate solution:* Dissolve 22.5 g MgSO4⋅7H2O in distilled water and dilute to 1 L.

*c. Calcium chloride solution:* Dissolve 27.5 g CaCl2 in distilled water and dilute to 1 L.

*d. Ferric chloride solution:* Dissolve 0.25 g FeCl3⋅6H2O in distilled water and dilute to 1 L.

*e. Acid and alkali solutions,* 1*N*, for neutralization of caustic or acidic waste samples.

1) Acid—Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute

to 1 L.

2) Alkali—Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.

*f. Sodium sulfite solution:* Dissolve 1.575 g Na2SO3 in 1000 mL distilled water. This

solution is not stable; prepare daily.

*g. Nitrification inhibitor,* 2-chloro-6-(trichloromethyl) pyridine.\*#(98)

*h. Glucose-glutamic acid solution:* Dry reagent-grade glucose and reagent-grade glutamic

acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and

dilute to 1 L. Prepare fresh immediately before use.

*i. Ammonium chloride solution:* Dissolve 1.15 g NH4Cl in about 500 mL distilled water,

adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

*j. Dilution water:* Use demineralized, distilled, tap, or natural water for making sample

dilutions.

4. Procedure

*a. Preparation of dilution water:* Place desired volume of water (¶ 3 *j*) in a suitable bottle

and add 1 mL each of phosphate buffer, MgSO4, CaCl2, and FeCl3 solutions/L of water. Seed

dilution water, if desired, as described in ¶ 4*d*. Test dilution water as described in ¶ 4*h* so that

water of assured quality always is on hand.

Before use bring dilution water temperature to 20 ± 3°C. Saturate with DO by shaking in a

partially filled bottle or by aerating with organic-free filtered air. Alternatively, store in

cotton-plugged bottles long enough for water to become saturated with DO. Protect water quality

by using clean glassware, tubing, and bottles.

*b. Dilution water storage:* Source water (¶ 3 *j*) may be stored before use as long as the

prepared dilution water meets quality control criteria in the dilution water blank (¶ 4*h*). Such

storage may improve the quality of some source waters but may allow biological growth to cause

deterioration in others. Preferably do not store prepared dilution water for more than 24 h after

adding nutrients, minerals, and buffer unless dilution water blanks consistently meet quality

control limits. Discard stored source water if dilution water blank shows more than 0.2 mg/L DO

depletion in 5 d.

*c. Glucose-glutamic acid check:* Because the BOD test is a bioassay its results can be

influenced greatly by the presence of toxicants or by use of a poor seeding material. Distilled

waters frequently are contaminated with copper; some sewage seeds are relatively inactive. Low

results always are obtained with such seeds and waters. Periodically check dilution water quality,

seed effectiveness, and analytical technique by making BOD measurements on a mixture of 150

mg glucose/L and 150 mg glutamic acid/L as a ‘‘standard’’ check solution. Glucose has an

exceptionally high and variable oxidation rate but when it is used with glutamic acid, the

oxidation rate is stabilized and is similar to that obtained with many municipal wastes.

Alternatively, if a particular wastewater contains an identifiable major constituent that

contributes to the BOD, use this compound in place of the glucose-glutamic acid.

Determine the 5-d 20°C BOD of a 2% dilution of the glucose-glutamic acid standard check

solution using the techniques outlined in ¶s 4*d*-*j*. Adjust concentrations of commercial mixtures

to give 3 mg/L glucose and 3 mg/L glutamic acid in each GGA test bottle. Evaluate data as

described in ¶ 6, Precision and Bias.

*d. Seeding:*

1) Seed source—It is necessary to have present a population of microorganisms capable of

oxidizing the biodegradable organic matter in the sample. Domestic wastewater, unchlorinated

or otherwise-undisinfected effluents from biological waste treatment plants, and surface waters

receiving wastewater discharges contain satisfactory microbial populations. Some samples do

not contain a sufficient microbial population (for example, some untreated industrial wastes,

disinfected wastes, high-temperature wastes, or wastes with extreme pH values). For such wastes

seed the dilution water or sample by adding a population of microorganisms. The preferred seed

is effluent or mixed liquor from a biological treatment system processing the waste. Where such

seed is not available, use supernatant from domestic wastewater after settling at room temperature for at least 1 h but no longer than 36 h. When effluent or mixed liquor from a

biological treatment process is used, inhibition of nitrification is recommended.

Some samples may contain materials not degraded at normal rates by the microorganisms in

settled domestic wastewater. Seed such samples with an adapted microbial population obtained

from the undisinfected effluent or mixed liquor of a biological process treating the waste. In the

absence of such a facility, obtain seed from the receiving water below (preferably 3 to 8 km) the

point of discharge. When such seed sources also are not available, develop an adapted seed in the

laboratory by continuously aerating a sample of settled domestic wastewater and adding small

daily increments of waste. Optionally use a soil suspension or activated sludge, or a commercial

seed preparation to obtain the initial microbial population. Determine the existence of a

satisfactory population by testing the performance of the seed in BOD tests on the sample. BOD

values that increase with time of adaptation to a steady high value indicate successful seed

adaptation.

2) Seed control—Determine BOD of the seeding material as for any other sample. This is

the *seed control*. From the value of the seed control and a knowledge of the seeding material

dilution (in the dilution water) determine seed DO uptake. Ideally, make dilutions of seed such

that the largest quantity results in at least 50% DO depletion. A plot of DO depletion, in

milligrams per liter, versus milliters of seed for all bottles having a 2-mg/L depletion and a

1.0-mg/L minimum residual DO should present a straight line for which the slope indicates DO

depletion per milliliter of seed. The DO-axis intercept is oxygen depletion caused by the dilution

water and should be less than 0.1 mg/L (¶ 4*h*). Alternatively, divide DO depletion by volume of

seed in milliliters for each seed control bottle having a 2-mg/L depletion and a 1.0-mg/L residual

DO. Average the results for all bottles meeting minimum depletion and residual DO criteria. The

DO uptake attributable to the seed added to each bottle should be between 0.6 and 1.0 mg/L, but

the amount of seed added should be adjusted from this range to that required to provide

glucose-glutamic acid check results in the range of 198 ± 30.5 mg/L. To determine DO uptake

for a test bottle, subtract DO uptake attributable to the seed from total DO uptake (see ¶ 5).

Techniques for adding seeding material to dilution water are described for two sample

dilution methods (¶ 4 *f*).

*e. Sample pretreatment:* Check pH of all samples before testing unless previous experience

indicates that pH is within the acceptable range.

1) Samples containing caustic alkalinity (pH >8.5) or acidity (pH <6.0)—Neutralize samples

to pH 6.5 to 7.5 with a solution of sulfuric acid (H2SO4) or sodium hydroxide (NaOH) of such

strength that the quantity of reagent does not dilute the sample by more than 0.5%. The pH of

dilution water should not be affected by the lowest sample dilution. Always seed samples that

have been pH-adjusted.

2) Samples containing residual chlorine compounds—If possible, avoid samples containing

residual chlorine by sampling ahead of chlorination processes. If the sample has been chlorinated

but no detectable chlorine residual is present, seed the dilution water. If residual chlorine is

present, dechlorinate sample and seed the dilution water (¶ 4 *f*). Do not test chlorinated/dechlorinated samples without seeding the dilution water. In some samples chlorine

will dissipate within 1 to 2 h of standing in the light. This often occurs during sample transport

and handling. For samples in which chlorine residual does not dissipate in a reasonably short

time, destroy chlorine residual by adding Na2SO3 solution. Determine required volume of

Na2SO3 solution on a 100- to 1000-mL portion of neutralized sample by adding 10 mL of 1 + 1

acetic acid or 1 + 50 H2SO4, 10 mL potassium iodide (KI) solution (10 g/100 mL) per 1000 mL

portion, and titrating with Na2SO3 solution to the starch-iodine end point for residual. Add to

neutralized sample the relative volume of Na2SO3 solution determined by the above test, mix,

and after 10 to 20 min check sample for residual chlorine. (NOTE: Excess Na2SO3 exerts an

oxygen demand and reacts slowly with certain organic chloramine compounds that may be

present in chlorinated samples.)

3) Samples containing other toxic substances—Certain industrial wastes, for example,

plating wastes, contain toxic metals. Such samples often require special study and treatment.

4) Samples supersaturated with DO—Samples containing more than 9 mg DO/L at 20°C

may be encountered in cold waters or in water where photosynthesis occurs. To prevent loss of

oxygen during incubation of such samples, reduce DO to saturation at 20°C by bringing sample

to about 20°C in partially filled bottle while agitating by vigorous shaking or by aerating with

clean, filtered compressed air.

5) Sample temperature adjustment—Bring samples to 20 ± 1°C before making dilutions.

6) Nitrification inhibition—If nitrification inhibition is desired add 3 mg

2-chloro-6-(trichloro methyl) pyridine (TCMP) to each 300-mL bottle before capping or add

sufficient amounts to the dilution water to make a final concentration of 10 mg/L. (NOTE: Pure

TCMP may dissolve slowly and can float on top of the sample. Some commercial formulations

dissolve more readily but are not 100% TCMP; adjust dosage accordingly.) Samples that may

require nitrification inhibition include, but are not limited to, biologically treated effluents,

samples seeded with biologically treated effluents, and river waters. Note the use of nitrogen

inhibition in reporting results.

*f. Dilution technique:* Make several dilutions of sample that will result in a residual DO of at

least 1 mg/L and a DO uptake of at least 2 mg/L after a 5-d incubation. Five dilutions are

recommended unless experience with a particular sample shows that use of a smaller number of

dilutions produces at least two bottles giving acceptable minimum DO depletion and residual

limits. A more rapid analysis, such as COD, may be correlated approximately with BOD and

serve as a guide in selecting dilutions. In the absence of prior knowledge, use the following

dilutions: 0.0 to 1.0% for strong industrial wastes, 1 to 5% for raw and settled wastewater, 5 to

25% for biologically treated effluent, and 25 to 100% for polluted river waters.

Prepare dilutions either in graduated cylinders or volumetric glassware, and then transfer to

BOD bottles or prepare directly in BOD bottles. Either dilution method can be combined with

any DO measurement technique. The number of bottles to be prepared for each dilution depends on the DO technique and the number of replicates desired.

When using graduated cylinders or volumetric flasks to prepare dilutions, and when seeding

is necessary, add seed either directly to dilution water or to individual cylinders or flasks before

dilution. Seeding of individual cylinders or flasks avoids a declining ratio of seed to sample as

increasing dilutions are made. When dilutions are prepared directly in BOD bottles and when

seeding is necessary, add seed directly to dilution water or directly to the BOD bottles. When a

bottle contains more than 67% of the sample after dilution, nutrients may be limited in the

diluted sample and subsequently reduce biological activity. In such samples, add the nutrient,

mineral, and buffer solutions (¶ 3*a* through *e*) directly to individual BOD bottles at a rate of 1

mL/L (0.33 mL/300-mL bottle) or use commercially prepared solutions designed to dose the

appropriate bottle size.

1) Dilutions prepared in graduated cylinders or volumetric flasks—If the azide modification

of the titrimetric iodometric method (Section 4500-O.C) is used, carefully siphon dilution water,

seeded if necessary, into a 1- to 2-L-capacity flask or cylinder. Fill half full without entraining

air. Add desired quantity of carefully mixed sample and dilute to appropriate level with dilution

water. Mix well with a plunger-type mixing rod; avoid entraining air. Siphon mixed dilution into

two BOD bottles. Determine initial DO on one of these bottles. Stopper the second bottle tightly,

water-seal, and incubate for 5 d at 20°C. If the membrane electrode method is used for DO

measurement, siphon dilution mixture into one BOD bottle. Determine initial DO on this bottle

and replace any displaced contents with sample dilution to fill the bottle. Stopper tightly,

water-seal, and incubate for 5 d at 20°C.

2) Dilutions prepared directly in BOD bottles—Using a wide-tip volumetric pipet, add the

desired sample volume to individual BOD bottles of known capacity. Add appropriate amounts

of seed material either to the individual BOD bottles or to the dilution water. Fill bottles with

enough dilution water, seeded if necessary, so that insertion of stopper will displace all air,

leaving no bubbles. For dilutions greater than 1:100 make a primary dilution in a graduated

cylinder before making final dilution in the bottle. When using titrimetric iodometric methods

for DO measurement, prepare two bottles at each dilution. Determine initial DO on one bottle.

Stopper second bottle tightly, water-seal, and incubate for 5 d at 20°C. If the membrane electrode

method is used for DO measurement, prepare only one BOD bottle for each dilution. Determine

initial DO on this bottle and replace any displaced contents with dilution water to fill the bottle.

Stopper tightly, water-seal, and incubate for 5 d at 20°C. Rinse DO electrode between

determinations to prevent cross-contamination of samples.

Use the azide modification of the iodometric method (Section 4500-O.C) or the membrane

electrode method (Section 4500-O.G) to determine initial DO on all sample dilutions, dilution

water blanks, and where appropriate, seed controls.

If the membrane electrode method is used, the azide modification of the iodometric method

(Method 4500-O.C) is recommended for calibrating the DO probe.

*g. Determination of initial DO:* If the sample contains materials that react rapidly with DO,

determine initial DO immediately after filling BOD bottle with diluted sample. If rapid initial DO uptake is insignificant, the time period between preparing dilution and measuring initial DO

is not critical but should not exceed 30 min.

*h. Dilution water blank:* Use a dilution water blank as a rough check on quality of unseeded

dilution water and cleanliness of incubation bottles. Together with each batch of samples

incubate a bottle of unseeded dilution water. Determine initial and final DO as in ¶s 4*g* and j.

The DO uptake should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L

Discard all dilution water having a DO uptake greater than 0.2 mg/L and either eliminate source

of contamination or select an alternate dilution water source..

*i. Incubation:* Incubate at 20°C ± 1°C BOD bottles containing desired dilutions, seed

controls, dilution water blanks, and glucose-glutamic acid checks. Water-seal bottles as

described in ¶ 4 *f*.

*j. Determination of final DO:* After 5 d incubation determine DO in sample dilutions,

blanks, and checks as in ¶ 4*g*.

5. Calculation

For each test bottle meeting the 2.0-mg/L minimum DO depletion and the 1.0-mg/L residual

DO, calculate BOD5 as follows:

When dilution water is not seeded:



When dilution water is seeded:



where:

*D*1 = DO of diluted sample immediately after preparation, mg/L,

*D*2 = DO of diluted sample after 5 d incubation at 20°C, mg/L,

*P* = decimal volumetric fraction of sample used,

*B*1 = DO of seed control before incubation, mg/L (¶ 4*d*),

*B*2 = DO of seed control after incubation mg/L (¶ 4*d*), and

*f* = ratio of seed in diluted sample to seed in seed control = (% seed in diluted

sample)/(% seed in seed control).

If seed material is added directly to sample or to seed control bottles:

*f* = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as CBOD5 if nitrification is inhibited.

If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a

DO depletion of at least 2 mg/L and there is no evidence of toxicity at higher sample

concentrations or the existence of an obvious anomaly, average results in the acceptable range.

In these calculations, do not make corrections for DO uptake by the dilution water blank

during incubation. This correction is unnecessary if dilution water meets the blank criteria

stipulated above. If the dilution water does not meet these criteria, proper corrections are

difficult ; do not record results or, as a minimum, mark them as not meeting quality control

criteria.

6. Precision and Bias

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic

acid check prescribed in ¶ 4*c* is intended to be a reference point for evaluation of dilution water

quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L

mixed glucose-glutamic acid solution provided the following results:

Number of months: 14

Number of triplicates: 421

Average monthly recovery: 204 mg/L

Average monthly standard deviation: 10.4 mg/L

In a series of interlaboratory studies,1 each involving 2 to 112 laboratories (and as many

analysts and seed sources), 5-d BOD measurements were made on synthetic water samples

containing a 1:1 mixture of glucose and glutamic acid in the total concentration range of 3.3 to

231 mg/L. The regression equations for mean value, Ä , and standard deviation, *S*, from these

studies were:

Ä = 0.658 (added level, mg/L) + 0.280 mg/L

*S* = 0.100 (added level, mg/L) + 0.547 mg/L

For the 300-mg/L mixed primary standard, the average 5-d BOD would be 198 mg/L with a

standard deviation of 30.5 mg/L. When nitrification inhibitors are used, GGA test results falling

outside the 198 ± 30.5 control limit quite often indicate use of incorrect amounts of seed. Adjust

amount of seed added to the GGA test to achieve results falling within this range.

a. *Control limits:* Because of many factors affecting BOD tests in multilaboratory studies

and the resulting extreme variability in test results, one standard deviation, as determined by

interlaboratory tests, is recommended as a control limit for individual laboratories. Alternatively,

for each laboratory, establish its control limits by performing a minimum of 25 glucose-glutamic

acid checks (¶ 4*c*) over a period of several weeks or months and calculating the mean and

standard deviation. Use the mean ±3 standard deviations as the control limit for future

glucose-glutamic acid checks. Compare calculated control limits to the single-laboratory tests presented above and to interlaboratory results. If control limits are outside the range of 198 ±

30.5, re-evaluate the control limits and investigate source of the problem. If measured BOD for a

glucose-glutamic acid check is outside the accepted control limit range, reject tests made with

that seed and dilution water.

b. *Working range and detection limit:* The working range is equal to the difference between

the maximum initial DO (7 to 9 mg/L) and minimum DO residual of 1 mg/L multiplied by the

dilution factor. A lower detection limit of 2 mg/L is established by the requirement for a

minimum DO depletion of 2 mg/L.

CHEMICAL OXYGEN DEMAND

Open Reflux Method, Titrimetric Method

1. place 5 ml of sample in a 250 or 500 ml refluxing flask
2. Add about 3 glass beads to the reflux flask.
3. Add approximately 1 g mercuric sulfate
4. Very slowly add 5.0 ml sulphuric acid reagent with mixing to dissolve the mercuric sulfate.
5. Cool under the tap while mixing to avoid possible loss of volatile materials.
6. Add 25.0 ml of 0.0417M (0.25 N) potassium dichromate solution
7. Mix until the solution is completely homogeneous.
8. Attach the reflux flask to the condenser and turn on the cooling water
9. Add 75 ml of sulphuric acid reagent through the open end of the condenser Continue swirling and mixing while adding the sulphuric acid reagent
10. Cover the open end of the condenser with a small beaker and reflux for exactly 2 hours.
11. Cool, and wash down the condenser with about 50 ml of distilled water
12. Cool to room temperature under a tap and mix well.
13. Titrate the excess K2Cr2O7 with ferrous ammonium sulfate(FAS) titrant using 2 to 3 drops of Ferroin indicator taking the end point of the titration the first sharp color change from blue-green to reddish brown
14. In the same manner, reflux and titrate a blank containing the reagents and 50 ml of distilled water.
15. Calculation:

 COD as mg O2/L= (A-B) X M X 8,000

 ML of sample

Where A= ml FAS used for the blank

 B= ml FAS used for sample

 C= Molarity of FAS

Note:

1. For samples with a COD of more than 900 mg O2/L, Use a smaller sample

Diluted to 50 ml

1. It is necessary to cover the open end of the condenser to prevent foreign material from entering the refluxing mixture.
2. Neglect the reappearance of the blue-green color after the end point has been reached.
3. For low-COD samples use 0.00417 M (0.025 N) K2Cr2O7 and titrate with 0.025 M (N) FAS. Exercise extreme care with this procedure because even a trace amount of organic matter on the glassware or from the atmosphere may cause gross errors.
4. You can evaluate the technique and quality of reagents by conducting the test on a standard potassium hydrogen phthalate solution.

**AMMONIA NITROGEN**

**Direct Nesslerization Method**

1. Prepare a series of standards by transferring the following amounts of standard

 Ammonium chloride solution to a 50 mL volumetric flask stoppered graduated

 cylinder and diluting to 50 mL with ammonia free distilled water.

|  |  |
| --- | --- |
|  Standard Ammonia Solution, mL |  Ammonia Nitrogen ug /50 mL |
|  0 0.5 1.0 2.0 3.0 4.0 5.0 |  0 5.0 10.0 20.0 30.0 40.0 50.0 |

1. Nesslerize the standards by adding 1.0 mL Nessler’s reagent to each flask with a

 Safety pipet.

1. Stopper and invert several times
2. Read the absorbance 425 nm at least 10 minutes after adding Nessler’s reagent
3. Plot a calibration curve absorbance versus concentration

**Treatment of Samples**

1. Take 100 mL of sample in a 100 mL volumetric flask or graduated cylinder
2. With a measuring pipet add 1 mL zinc surface solution and mix thoroughly
3. Add 0.4 to 0.5 mL 6N sodium hydroxide solution to obtain a PH of 10.5 and mix thoroughly.
4. Let treated sample stand for a few minutes, where upon a heavy flocculent precipitation should fall, leaving a clear and colorless super mate
5. Prepare a filter with a fast filter paper by washing it until it is free of ammonia (Check then filtrate with Nessler’s reagent)
6. Pour estimated 25 mL of the clear liquid through the filter paper. Discard this filtrate.
7. Pour the remaining clear liquid through the same filter and catch the filtrate in a clean 100 mL stoppered graduated cylinder
8. Measure the appropriate volume of the filtrate for the indicated ammonia nitrogen range and transfer it to a 50 mL volumetric flask or graduated cylinder.

|  |  |
| --- | --- |
|  Standard Ammonia Solution, mL |  Ammonia Nitrogen ug/50mL |
| 502510 |  01-1.0 11-2.0 2.1-5.0 |

1. If necessary, dilute to the 50 mL mark with NH3 – fee distilled water.
2. Add 0.05 to 0.1 mL (1 to 2 drops) Rochelle salt solution and mix well.
3. Add mL Nessler’s reagent with a safety pipette.
4. Stopper and mix well.
5. Allow the yellow or brownish color to develop for at least 10 minutes
6. Read the absorbance at 425 nm with a spectrophotometer.
7. Determine the microgram NH3- N from the calibration curve:
8. Calculation:
9. mg/L NH3- = μg NH3-N

 Ml of sample

1. mg/L NH3- = μg NH3-Nx1.22

 Ml of sample

1. mg/L NH4- = μgNH3-Nx1.29

 Ml of sample

*d. Ammonia determination:* Determine ammonia by the titrimetric method (C), the

ammonia-selective electrode methods (D and E), or the phenate methods (F and G).

**4500-NH3 C. Titrimetric Method**

1. General Discussion

The titrimetric method is used only on samples that have been carried through preliminary

distillation (see Section 4500-NH3.B). The following table is useful in selecting sample volume

for the distillation and titration method.

**Ammonia Nitrogen in Sample SampleVolume**

 ***mg/L mL***

5–10 250

10–20 100

20–50 50.0

50–100 25.0

2. Apparatus

*Distillation apparatus:* See Section 4500-NH3.B.2*a* and Section 4500-NH3.B.2*b*.

3. Reagents

Use ammonia-free water in making all reagents and dilutions.

*a. Mixed indicator solution:* Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or

isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol.

Combine solutions. Prepare monthly.

*b. Indicating boric acid solution:* Dissolve 20 g H3BO3 in water, add 10 mL mixed indicator

solution, and dilute to 1 L. Prepare monthly.

*c. Standard sulfuric acid titrant*, 0.02*N:* Prepare and standardize as directed in Alkalinity,

Section 2320B.3*c*. For greatest accuracy, standardize titrant against an amount of Na2CO3 that

has been incorporated in the indicating boric acid solution to reproduce the actual conditions of

sample titration; 1.00 mL = 14 × normality × 1000 μg N. (For 0.02*N*, 1.00 mL = 280 μg N.)

4. Procedure

*a*. Proceed as described in Section 4500-NH3.B using indicating boric acid solution as

absorbent for the distillate.

*b. Sludge or sediment samples:* Rapidly weigh to within ±1% an amount of wet sample,

equivalent to approximately 1 g dry weight, in a weighing bottle or crucible. Wash sample into a

500-mL kjeldahl flask with water and dilute to 250 mL. Proceed as in ¶ 4*a* but add a piece of

paraffin wax to distillation flask and collect only 100 mL distillate.

*c*. Titrate ammonia in distillate with standard 0.02*N* H2SO4 titrant until indicator turns a pale

lavender.

*d. Blank:* Carry a blank through all steps of the procedure and apply the necessary correction

to the results.

5. Calculation

*a. Liquid samples:*



*b. Sludge or sediment samples:*

**

where:

*A* = volume of H2SO4 titrated for sample, mL, and

*B* = volume of H2SO4 titrated for blank, mL.

6. Precision and Bias

Three synthetic samples containing ammonia and other constituents dissolved in distilled

water were distilled and analyzed by titration.

Sample 1 contained 200 μg NH3-N/L, 10 mg Cl−/L, 1.0 mg NO3−-N/L, 1.5 mg organic N/L, 10.0 mg PO43−/L, and 5.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 69.8% and 20%, respectively. Sample 2 contained 800 μg NH3-N/L, 200 mg Cl−/L, 1.0 mg NO3−-N/L, 0.8 mg organic N/L, 5.0 mg PO4 3−/L, and 15.0 mg silica/L. The relative standard deviation and relative error for the 20 participating laboratories were 28.6% and 5%, respectively. Sample 3 contained 1500 μg NH3-N/L, 400 mg Cl−/L, 1.0 mg NO3−-N/L, 0.2 mg organic N/L, 0.5 mg PO4

3−/L, and 30.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 21.6%, and 2.6%, respectively.

**4500-NH3 D. Ammonia-Selective Electrode Method**

1. General Discussion

*a. Principle:* The ammonia-selective electrode uses a hydrophobic gas-permeable membrane

to separate the sample solution from an electrode internal solution of ammonium chloride.

Dissolved ammonia (NH3(aq) and NH4+) is converted to NH3(aq) by raising pH to above 11 with

a strong base. NH3(aq) diffuses through the membrane and changes the internal solution pH that

is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a

chloride ion-selective electrode that serves as the reference electrode. Potentiometric

measurements are made with a pH meter having an expanded millivolt scale or with a specific

ion meter.

*b. Scope and application:* This method is applicable to the measurement of 0.03 to 1400 mg

NH3-N/L in potable and surface waters and domestic and industrial wastes. High concentrations

of dissolved ions affect the measurement, but color and turbidity do not. Sample distillation is

unnecessary. Use standard solutions and samples that have the same temperature and contain

about the same total level of dissolved species. The ammonia-selective electrode responds

slowly below 1 mg NH3-N/L; hence, use longer times of electrode immersion (2 to 3 min) to

obtain stable readings.

*c. Interference:* Amines are a positive interference. This may be enhanced by acidification.

Mercury and silver interfere by complexing with ammonia, unless the NaOH/EDTA solution

(3*c*) is used.

*d. Sample preservation:* Refrigerate at 4°C for samples to be analyzed within 24 h. Preserve

samples high in organic and nitrogenous matter, and any other samples for longer storage, by

lowering pH to 2 or less with conc H2SO4.

2. Apparatus

*a. Electrometer:* A pH meter with expanded millivolt scale capable of 0.1 mV resolution

between −700 mV and +700 mV or a specific ion meter.

*b. Ammonia-selective electrode.*\*#(55)

*c. Magnetic stirrer*, thermally insulated, with TFE-coated stirring bar.

3. Reagents

*a. Ammonia-free water:* See Section 4500-NH3.B.3*a*. Use for making all reagents.

*b. Sodium hydroxide,* 10*N*.

*c. NaOH/EDTA solution,* 10*N*: Dissolve 400 g NaOH in 800 mL water. Add 45.2 g

ethylenediaminetetraacetic acid, tetrasodium salt, tetrahydrate (Na4EDTA⋅4 H2O) and stir to

dissolve. Cool and dilute to 1000 mL.

*d. Stock ammonium chloride solution:* Dissolve 3.819 g anhydrous NH4Cl (dried at 100°C)

in water, and dilute to 1000 mL; 1.00 mL = 1.00 mg N = 1.22 mg NH3.

*e. Standard ammonium chloride solutions:* See ¶ 4*a* below.

4. Procedure

*a. Preparation of standards:* Prepare a series of standard solutions covering the

concentrations of 1000, 100, 10, 1, and 0.1 mg NH3-N/L by making decimal dilutions of stock

NH4Cl solution with water.

*b. Electrometer calibration:* Place 100 mL of each standard solution in a 150-mL beaker.

Immerse electrode in standard of lowest concentration and mix with a magnetic stirrer. Limit

stirring speed to minimize possible loss of ammonia from the solution. Maintain the same

stirring rate and a temperature of about 25°C throughout calibration and testing procedures. Add

a sufficient volume of 10*N* NaOH solution (1 mL usually is sufficient) to raise pH above 11. If

the presence of silver or mercury is possible, use NaOH/EDTA solution in place of NaOH

solution. If it is necessary to add more than 1 mL of either NaOH or NaOH/ EDTA solution, note

volume used, because it is required for subsequent calculations. Keep electrode in solution until

a stable millivolt reading is obtained. Do not add NaOH solution before immersing electrode,

because ammonia may be lost from a basic solution. Repeat procedure with remaining standards,

proceeding from lowest to highest concentration. Wait until the reading has stablized (at least 2

to 3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH3-N/L.

*c. Preparation of standard curve:* Using semilogarithmic graph paper, plot ammonia

concentration in milligrams NH3-N per liter on the log axis vs. potential in millivolts on the

linear axis starting with the lowest concentration at the bottom of the scale. If the electrode is

functioning properly a tenfold change of NH3-N concentration produces a potential change of

about 59 mV.

*d. Calibration of specific ion meter:* Refer to manufacturer’s instructions and proceed as in ¶s

4*a* and b.

*e. Measurement of samples:* Dilute if necessary to bring NH3-N concentration to within

calibration curve range. Place 100 mL sample in 150-mL beaker and follow procedure in ¶ 4*b*

above. Record volume of 10*N* NaOH added. Read NH3-N concentration from standard curve.

5. Calculation



where:

*A* = dilution factor,

*B* = concentration of NH3-N/L, mg/L, from calibration curve,

*C* = volume of 10*N* NaOH added to calibration standards, mL, and *D* = volume of 10*N* NaOH added to sample, mL.

6. Precision and Bias

For the ammonia-selective electrode in a single laboratory using surface water samples at

concentrations of 1.00, 0.77, 0.19, and 0.13 mg NH3-N/L, standard deviations were ±0.038,

±0.017, ±0.007, and ±0.003, respectively. In a single laboratory using surface water samples at

concentrations of 0.10 and 0.13 mg NH3-N/L, recoveries were 96% and 91%, respectively. The

results of an interlaboratory study involving 12 laboratories using the ammonia-selective

electrode on distilled water and effluents are summarized in Table 4500-NH3:I.

**4500-NH3 E. Ammonia-Selective Electrode Method Using Known Addition**

1. General Discussion

*a. Principle:* When a linear relationship exists between concentration and response, known

addition is convenient for measuring occasional samples because no calibration is needed.

Because an accurate measurement requires that the concentration at least double as a result of the

addition, sample concentration must be known within a factor of three. Total concentration of

ammonia can be measured in the absence of complexing agents down to 0.8 mg NH3-N/L or in

the presence of a large excess (50 to 100 times) of complexing agent. Known addition is a

convenient check on the results of direct measurement.

*b.* See Section 4500-NH3.D.1 for further discussion.

2. Apparatus

Use apparatus specified in Section 4500-NH3.D.2.

3. Reagents

Use reagents specified in Section 4500-NH3.D.3.

Add standard ammonium chloride solution approximately 10 times as concentrated as

samples being measured.

4. Procedure

*a.* Dilute 1000 mg/L stock solution to make a standard solution about 10 times as

concentrated as the sample concentrate.

*b.* Add 1 mL 10*N* NaOH to each 100 mL sample and immediately immerse electrode. When

checking a direct measurement, leave electrode in 100 mL of sample solution. Use magnetic

stirring throughout. Measure mV reading and record as *E*1.

*c.* Pipet 10 mL of standard solution into sample. Thoroughly stir and immediately record new

mV reading as *E*2.

5. Calculation

*a.* Δ *E* = *E*1 − *E*2.

*b*. From Table 4500-NH3:II find the concentration ratio, *Q*, corresponding to change in

potential, Δ *E*. To determine original total sample concentration, multiply *Q* by the concentration

of the added standard:

*Co* = *Q Cs*

where:

*Co* = total sample concentration, mg/L,

*Q* = reading from known-addition table, and

*Cs* = concentration of added standard, mg/L.

*c.* To check a direct measurement, compare results of the two methods. If they agree within

±4%, the measurements probably are good. If the known-addition result is much larger than the

direct measurement, the sample may contain complexing agents.

6. Precision and Bias

In 38 water samples analyzed by both the phenate and the known-addition

ammonia-selective electrode method, the electrode method yielded a mean recovery of 102% of

the values obtained by the phenate method when the NH3-N concentrations varied between 0.30

and 0.78 mg/L. In 57 wastewater samples similarly compared, the electrode method yielded a

mean recovery of 108% of the values obtained by the phenate method using distillation when the

NH3-N concentrations varied between 10.2 and 34.7 mg N/L. In 20 instances in which two to

four replicates of these samples were analyzed, the mean standard deviation was 1.32 mg N/L. In

three measurements at a sewer outfall, distillation did not change statistically the value obtained

by the electrode method. In 12 studies using standards in the 2.5- to 30-mg N/L range, average recovery by the phenate method was 97% and by the electrode method 101%.

**4500-NH3 F. Phenate Method**

1. General Discussion

*a. Principle:* An intensely blue compound, indophenol, is formed by the reaction of

ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside.

*b. Interferences:* Complexing magnesium and calcium with citrate eliminates interference

produced by precipitation of these ions at high pH. There is no interference from other trivalent

forms of nitrogen. Remove interfering turbidity by distillation orfiltration. If hydrogen sulfide is

present, remove by acidifying samples to pH 3 with dilute HCl and aerating vigorously until

sulfide odor no longer can be detected.

2. Apparatus

*Spectrophotometer* for use at 640 nm with a light path of 1 cm or greater.

3. Reagents

*a. Phenol solution:* Mix 11.1 mL liquified phenol (≥89%) with 95% v/v ethyl alcohol to a

final volume of 100 mL. Prepare weekly. CAUTION: *Wear gloves and eye protection when*

*handling phenol; use good ventilation to minimize all personnel exposure to this toxic volatile*

*substance.*

*b. Sodium nitroprusside,* 0.5% w/v: Dissolve 0.5 g sodium nitroprusside in 100 mL deionized

water. Store in amber bottle for up to 1 month.

*c. Alkaline citrate:* Dissolve 200 g trisodium citrate and 10 g sodium hydroxide in deionized

water. Dilute to 1000 mL.

*d. Sodium hypochlorite,* commercial solution, about 5%. This solution slowly decomposes

once the seal on the bottle cap is broken. Replace about every 2 months.

*e. Oxidizing solution:* Mix 100 mL alkaline citrate solution with 25 mL sodium hypochlorite.

Prepare fresh daily.

*f. Stock ammonium solution:* See Section 4500-NH3.D.3*d*.

*g. Standard ammonium solution:* Use stock ammonium solution and water to prepare a

calibration curve in a range appropriate for the concentrations of the samples.

4. Procedure

To a 25-mL sample in a 50-mL erlenmeyer flask, add, with thorough mixing after each

addition, 1 mL phenol solution, 1 mL sodium nitroprusside solution, and 2.5 mL oxidizing

solution. Cover samples with plastic wrap or paraffin wrapper film. Let color develop at room

temperature (22 to 27°C) in subdued light for at least 1 h. Color is stable for 24 h. Measure

absorbance at 640 nm. Prepare a blank and at least two other standards by diluting stock ammonia solution into the sample concentration range. Treat standards the same as samples.

5. Calculations

Prepare a standard curve by plotting absorbance readings of standards against ammonia

concentrations of standards. Compute sample concentration by comparing sample absorbance

with the standard curve.

6. Precision and Bias

For the manual phenate method, reagent water solutions of ammonium sulfate were prepared

and analyzed by two analysts in each of three laboratories. Results are summarized in Table

4500-NH3:III.

**4500-NH3 G. Automated Phenate Method**

1. General Discussion

*a. Principle:* Alkaline phenol and hypochlorite react with ammonia to form indophenol blue

that is proportional to the ammonia concentration. The blue color formed is intensified with

sodium nitroprusside.

*b. Interferences:* Seawater contains calcium and magnesium ions in sufficient concentrations

to cause precipitation during analysis. Adding EDTA and sodium potassium tartrate reduces the

problem. Eliminate any marked variation in acidity or alkalinity among samples because

intensity of measured color is pH-dependent. Likewise, insure that pH of wash water and

standard ammonia solutions approximates that of sample. For example, if sample has been

preserved with 0.8 mL conc H2SO4/L, include 0.8 mL conc H2SO4/L in wash water and

standards. Remove interfering turbidity by filtration. Color in the samples that absorbs in the

photometric range used for analysis interferes.

*c. Application:* Ammonia nitrogen can be determined in potable, surface, and saline waters as

well as domestic and industrial wastewaters over a range of 0.02 to 2.0 mg/L when photometric

measurement is made at 630 to 660 nm in a 10- to 50-mm tubular flow cell at rates of up to 60

samples/h. Determine higher concentrations by diluting the sample.

2. Apparatus

*Automated analytical equipment*. An example of the required continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-NH3:1.

3. Reagents

*a. Ammonia-free distilled water:* See Section 4500-NH3.B.3*a*. Use for preparing all reagents

and dilutions.

*b. Sulfuric acid,* H2SO4, 5*N*, air scrubber solution: Carefully add 139 mL conc H2SO4 to

approximately 500 mL water, cool to room temperature, and dilute to 1 L.

*c. Sodium phenate solution:* In a 1-L erlenmeyer flask, dissolve 93 mL liquid (≥89%) phenol

in 500 mL water. In small increments and with agitation, cautiously add 32 g NaOH. Cool flask

under running water and dilute to 1 L. CAUTION: *Minimize exposure of personnel to this*

*compound by wearing gloves and eye protection, and using proper ventilation.*

*d. Sodium hypochlorite solution:* Dilute 250 mL bleach solution containing 5.25% NaOCl to

500 mL with water.

*e. EDTA reagent:* Dissolve 50 g disodium ethylenediamine tetraacetate and approximately

six pellets NaOH in 1 L water. For salt-water samples where EDTA reagent does not prevent

precipitation of cations, use sodium potassium tartrate solution prepared as follows:

*Sodium potassium tartrate solution:* To 900 mL water add 100 g NaKC4H4O6⋅4H2O, two

pellets NaOH, and a few boiling chips, and boil gently for 45 min. Cover, cool, and dilute to 1 L.

Adjust pH to 5.2 ± 0.05 with H2SO4. Let settle overnight in a cool place and filter to remove

precipitate. Add 0.5 mL polyoxyethylene 23 lauryl ether\*#(56) solution and store in stoppered

bottle.

*f. Sodium nitroprusside solution:* Dissolve 0.5 g Na2(NO)Fe(CN)5⋅2H2O in 1 L water.

*g. Ammonia standard solutions:* See Section 4500-NH3.D.3*c* and *d*. Use standard ammonia

solution and water to prepare the calibration curve in the appropriate ammonia concentration

range. To analyze saline waters use substitute ocean water of the following composition to

prepare calibration standards:

**Constituent Concentration**

 ***g/L***

NaCl 24.53

MgCl2 5.20

CaCl2 1.16

KCl 0.70

SrCl2 0.03

Na2SO4 4.09

NaHCO3 0.20

KBr 0.10

H3BO3 0.03

NaF 0.003

Subtract blank background response of substitute seawater from standards before preparing

standard curve.

4. Procedure

*a.* Eliminate marked variation in acidity or alkalinity among samples. Adjust pH of wash

water and standard ammonia solutions to approximately that of sample.

*b*. Set up manifold and complete system as shown in Figure 4500-NH3:1.

*c*. Obtain a stable base line with all reagents, feeding wash water through sample line.

*d*. Typically, use a 60/h, 6:1 cam with a common wash.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold

against NH3-N concentrations in standards. Compute sample NH3-N concentration by

comparing sample response with standard curve.

6. Precision and Bias

For an automated phenate system in a single laboratory using surface water samples at

concentrations of 1.41, 0.77, 0.59, and 0.43 mg NH3-N/L, the standard deviation was ±0.005

mg/L, and at concentrations of 0.16 and 1.44 mg NH3-N/L, recoveries were 107 and 99%,

respectively.

**4500-NH3 H. Flow Injection Analysis (PROPOSED)**

1. General Discussion

*a. Principle:* A water sample containing ammonia or ammonium cation is injected into an

FIA carrier stream to which a complexing buffer, alkaline phenol, and hypochlorite are added.

This reaction, the Berthelot reaction, produces the blue indophenol dye. The blue color is

intensified by the addition of nitroferricyanide. The resulting peak’s absorbance is measured at

630 nm. The peak area is proportional to the concentration of ammonia in the original sample.

Also see Section 4500-NH3.F and Section 4130, Flow Injection Analysis (FIA).

*b. Interferences:* Remove large or fibrous particulates by filtering sample through glass

wool. Guard against contamination from reagents, water, glassware, and the sample preservation

process.

Also see Section 4500-NH3.A. Some interferents are removed by distillation; see Section

4500-NH3.B.

2. Apparatus

*Flow injection analysis equipment* consisting of:

*a. FIA injection valve* with sample loop or equivalent.

*b. Multichannel proportioning pump.*

*c. FIA manifold* (Figure 4500-NH3:2) with tubing heater and flow cell. In Figure

4500-NH3:2, relative flow rates only are shown. Tubing volumes are given as an example only;

they may be scaled down proportionally. Use manifold tubing of an inert material such as

TFE.\*#(57)

*d. Absorbance detector,* 630 nm, 10-nm bandpass.

*e. Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble

formation, degas carrier and buffer with helium. Use He at 140 kPa (20 psi) through a helium

degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing

reagents by weight/weight, use weight/volume.

*a. Buffer:* To a 1-L tared container add 50.0 g disodium ethylenediamine tetraacetate and 5.5

g sodium hydroxide, NaOH. Add 968 mL water. Mix with a magnetic stirrer until dissolved.

*b. Phenolate:* CAUTION: *Wear gloves. Phenol causes severe burns and is rapidly absorbed*

*into the body through the skin.* To a tared 1-L container, add 888 g water. Add 94.2 g 88%

liquefied phenol or 83 g crystalline phenol, C6H5OH. While stirring, slowly add 32 g NaOH.

Cool and invert to mix thoroughly. Do not degas.

*c. Hypochlorite:* To a tared 500-mL container add 250 g 5.25% sodium hypochlorite, NaOCl

bleach solution†#(58) and 250 g water. Stir or shake to mix.

*d. Nitroprusside:* To a tared 1-L container add 3.50 g sodium nitroprusside (sodium

nitroferricyanide), Na2Fe(CN)5NO⋅2H2O, and 1000 g water. Invert to mix.

*e. Stock ammonia standard,* 1000 mg N/L: In a 1-L volumetric flask dissolve 3.819 g

ammonium chloride, NH4Cl, that has been dried for 2 h at 110°C, in about 800 mL water. Dilute

to mark and invert to mix.

*f. Standard ammonia solutions:* Prepare ammonia standards in desired concentration range,

using the stock standard (¶ 3*e*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-NH3:2 and follow method supplied by

manufacturer or laboratory standard operating procedure for this method. Follow quality control

procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting the absorbance of standards processed through the

manifold versus ammonia concentration. The calibration curve is linear.

6. Precision and Bias

*a. Recovery and relative standard deviation:* The results of single-laboratory studies with

various matrices are given in Table 4500-NH3:IV.

*b. MDL:* A 650-μL sample loop was used in the method described above. Using a published

MDL method,1 analysts ran 21 replicates of a 0.020-mg N/L standard. These gave a mean of

0.0204 mg N/L, a standard deviation of 0.0007 mg N/L, and an MDL of 0.002 mg N/L.

**NITRITE NITROGEN**

**Diazotization Method**

1. Prepare a series of standards by transferring the following amounts of standard nitrite solution to separate 50-mL volumetric flasks or graduated cylinders.

|  |  |
| --- | --- |
| Standard Nitrite solution mL |  Nitrite Nitrogenμg/50 mL |
|  00.10.20.40.7 1.42.02.5 | 00.050.100.200.350.701.001.25  |

1. Add distilled water to the 50-mL mark, and mix thoroughly.
2. Place the clear colorless sample into a 50-mL volumetric flask or graduated cylinder. If a smaller portion is used, dilute to the 50-mL mark with distilled water ( If the sample contains colloidal suspended solids which can not removed by ordinary filtration, filter through a 0.45 um pore diameter membrane filter)
3. Neutralize if necessary to a PH of about 7.
4. Add 1 mL sulfanilamide solution, mix and allow the reagent to react for 2 to 8 min.
5. Add 1 mL NED di hydrochloride solution and mix immediately.
6. Allow to stand for at least 10 minutes but not more than 2 hours for the reddish purple color to develop.
7. Measure the absorbance of each standard and the sample against a reagent blank in a suitable photometer at 543 nm.
8. Determine the μg of NO2- N in the sample by reference to a standard calibration curve
9. Calculation:
	1. mg/L NO2 – N = μg NO2-N

 Ml of sample

* 1. mg/L NO2 = μg NO2-N x 3.285

 Ml of sample

**NITRATE NITROGEN**

**Phenoldisulfonic Acid Method**

1. Determine the chloride content of the water sample and treat 100 mL with an equivalent amount of silver sulfate solution (1mL for l mg C1) to precipitate the chlorides.
2. Remove the precipitated chloride either by centrifugation or by filtration, coagulating the AgC1 by heat if necessary.
3. If the sample has color of more than 10 unit ( on platinum cobalt scale), decolorize by adding 3 mL aluminum hydroxide suspension to 150 mL sample; stir very thoroughly; allow to stand for a few minutes; then filter, discarding the first portion of the filtrate.
4. Pipette a suitable quantity of the sample or the clarified filtrate into an evaporating dish and neutralize to approximately PH 7.
5. Evaporate to dryness over a hot water bath.
6. Add 2 mL phenoldisulfonic acid reagent and rub the residue thoroughly to insure dissolution of all solids. If needed heat on the water bath a short time to dissolve the entire reside.
7. Dilute with 20 mL of distilled water and add with stirring about 6 to 7 mL of NH4OH or about 5 to 6 mL KOH solution (12N) until maximum yellow color is developed.
8. Remove any resulting flocculent hydroxides by filtration or add the EDTA reagent drop wise with stirring until the turbidity re dissolves
9. Transfer the filtrate of clear solution to a 50-mL volumetric flask or graduated cylinder. Rinse the dish, glass rod and filter paper with distilled water, adding the rinsing to the flask or cylinder until all the colored solution has been transferred.
10. Dilute to the 50- mL mark with distilled water, and mix thoroughly
11. Measure the absorbance at a wave length of 410 nm against a blank prepared from the same volumes of reagents as used for the samples.
12. Construct a calibration curve in the range 0-2 mg/L NO3 – N by adding 0, 0.2, 0.5, 1.0, 3.0, 5.0, and 10 mL of standard nitrate solution to separate evaporating dishes and treating them in the same way as the sample.
13. Determine the μg of NO3- N in the sample by reference to the calibration curve.
14. Calculation:

a) mg/L NO3-N = μg NO3-N

 mL sample

b) mg/L NO3 = μg NO3- N X 4.427

 mL sample

**Note:**

 Nitrite levels in excess of 0.2 mg/L erratically increase the apparent Nitrate concentration as it responds like nitrate. Hence, the nitrite must be converted to nitrate by a suitable oxidizing agent prior to the determination of nitrate.

**Nitrite Conversion**

 To 100 mL of sample add 1 of 1N sulphuric acid and stir, Add drop wise with stirring 0.1N KMn04 solution. Let the treated sample stand for 15 minutes to complete the conversion of nitrite to nitrate. (A faint pink color persists for at least 15 minutes when sufficient KMnO4 is used.) Make the proper deduction at the end of the nitrate determination for the nitrite concentration as determined by the method described in nitrogen nitrite.

 **4500-N NITROGEN\*#(51)**

**4500-N A. Introduction**

In waters and wastewaters the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia, and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas (N2), are biochemically interconvertible and are components of the nitrogen cycle. They are of interest for many reasons.

Organic nitrogen is defined functionally as organically bound nitrogen in the trinegative

oxidation state. It does not include all organic nitrogen compounds. Analytically, organic

nitrogen and ammonia can be determined together and have been referred to as ‘‘kjeldahl

nitrogen,’’ a term that reflects the technique used in their determination. Organic nitrogen

includes such natural materials as proteins and peptides, nucleic acids and urea, and numerous

synthetic organic materials. Typical organic nitrogen concentrations vary from a few hundred

micrograms per liter in some lakes to more than 20 mg/L in raw sewage.

Total oxidized nitrogen is the sum of nitrate and nitrite nitrogen. Nitrate generally occurs in

trace quantities in surface water but may attain high levels in some groundwater. In excessive

amounts, it contributes to the illness known as methemoglobinemia in infants. A limit of 10 mg

nitrate as nitrogen/L has been imposed on drinking water to prevent this disorder. Nitrate is

found only in small amounts in fresh domestic wastewater but in the effluent of nitrifying

biological treatment plants nitrate may be found in concentrations of up to 30 mg nitrate as nitrogen/ L. It is an essential nutrient for many photosynthetic autotrophs and in some cases has

been identified as the growth-limiting nutrient.

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to

nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater

treatment plants, water distribution systems, and natural waters. Nitrite can enter a water supply

system through its use as a corrosion inhibitor in industrial process water. Nitrite is the actual

etiologic agent of methemoglobinemia. Nitrous acid, which is formed from nitrite in acidic

solution, can react with secondary amines (RR′NH) to form nitrosamines (RR′N-NO), many of which are known to be carcinogens. The toxicologic significance of nitrosation reactions in vivo and in the natural environment is the subject of much current concern and research.

Ammonia is present naturally in surface and wastewaters. Its concentration generally is low

in groundwaters because it adsorbs to soil particles and clays and is not leached readily from

soils. It is produced largely by deamination of organic nitrogen-containing compounds and by

hydrolysis of urea. At some water treatment plants ammonia is added to react with chlorine to

form a combined chlorine residual. Ammonia concentrations encountered in water vary from less

than 10 μg ammonia nitrogen/L in some natural surface and groundwaters to more than 30 mg/L in some wastewaters.

In this manual, organic nitrogen is referred to and reported as organic N, nitrate nitrogen as NO3-N, nitrite nitrogen as NO2–-N, and ammonia nitrogen as NH3-N.

Total nitrogen can be determined through oxidative digestion of all digestible nitrogen forms

to nitrate, followed by quantitation of the nitrate. Two procedures, one using a persulfate/UV

digestion (Section 4500-N.B), and the other using persulfate digestion (Section 4500-N.C) are

presented. The procedures give good results for total nitrogen, composed of organic nitrogen

(including some aromatic nitrogen-containing compounds), ammonia, nitrite, and nitrate.

Molecular nitrogen is not determined and recovery of some industrial nitrogen-containing

compounds is low.

Chloride ions do not interfere with persulfate oxidation, but the rate of reduction of nitrate to

nitrite (during subsequent nitrate analysis by cadmium reduction) is significantly decreased by

chlorides. Ammonium and nitrate ions adsorbed on suspended pure clay or silt particles should

give a quantitative yield from persulfate digestion. If suspended matter remains after digestion,

remove it before the reduction step.

If suspended organic matter is dissolved by the persulfate digestion reagent, yields

comparable to those from true solutions are obtained; if it is not dissolved, the results are

unreliable and probably reflect a negative interference. The persulfate method is not effective in

wastes with high organic loadings. Dilute such samples and re-analyze until results from two

dilutions agree.

**4500-N B. In-Line UV/Persulfate Digestion and Oxidation with Flow Injection**

**Analysis (PROPOSED)**

1. General Discussion

*a. Principle:* Nitrogen compounds are digested and oxidized in-line to nitrate by use of

heated alkaline persulfate and ultraviolet radiation. The digested sample is injected onto the

manifold where its nitrate is reduced to nitrite by a cadmium granule column. The nitrite then is

determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion.

The diazonium ion is coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The

resulting pink dye absorbs at 540 nm and is proportional to total nitrogen.

This method recovers nearly all forms of organic and inorganic nitrogen, reduced and

oxidized, including ammonia, nitrate, and nitrite. It differs from the total kjeldahl nitrogen

method described in Section 4500-Norg.D, which does not recover the oxidized forms of

nitrogen. This method recovers nitrogen components of biological origin such as amino acids,

proteins, and peptides as ammonia, but may not recover the nitrogenous compounds of some

industrial wastes such as amines, nitro-compounds, hydrazones, oximes, semicarbazones, and

some refractory tertiary amines.

See Section 4500-N.A for a discussion of the various forms of nitrogen found in waters and

wastewaters, Section 4500-Norg.A and Section 4500-Norg.B for a discussion of total nitrogen

methods, and Section 4130, Flow Injection Analysis (FIA). Also see Section 4500-N.C for a

similar, batch total nitrogen method that uses only persulfate.

*b. Interferences:* Remove large or fibrous particulates by filtering sample though glass wool.

Guard against contamination from reagents, water, glassware, and the sample preservation

process.

2. Apparatus

*Flow injection analysis equipment* consisting of:

*a. FIA injection valve* with sample loop or equivalent.

*b. Multichannel proportioning pump*.

*c. FIA manifold* (Figure 4500-N:1) with tubing heater, in-line ultraviolet digestion fluidics

including a debubbler consisting of a gas-permeable TFE membrane and its holder, and flow

cell. In Figure 4500-N:1, relative flow rates only are shown. Tubing volumes are given as an

example only; they may be scaled down proportionally. Use manifold tubing of an inert material

such as TFE. The block marked ‘‘UV’’ should consist of TFE tubing irradiated by a mercury

discharge ultraviolet lamp emitting radiation at 254 nm.

*d. Absorbance detector,* 540 nm, 10-nm bandpass.

*e. Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and for all solutions. To prevent bubble

formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a

helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

*a. Borate solution,* Na2B4O7⋅10H2O: In a 1-L volumetric flask dissolve 38.0 g

Na2B4O7⋅10H2O and 3.0 g sodium hydroxide, NaOH, in approximately 900 mL water, using a

magnetic stirring bar. Gentle heating will speed dissolution. Adjust to pH 9.0 with NaOH or conc

hydrochloric acid (HCl). Dilute to mark and invert to mix.

*b. Persulfate solution,* K2S2O8: Potassium persulfate solid reagent usually contains nitrogen

contamination. Higher contamination levels result in larger blank peaks.

To a tared 1-L container, add 975 g water and 49 g K2S2O8. Add a magnetic stirring bar,

dissolve persulfate, and dilute to mark. Invert to mix.

*c. Ammonium chloride buffer:* CAUTION: *Fumes. Use a hood.* To a 1-L volumetric flask add

500 mL water, 105 mL conc HCl, and 95 mL conc ammonium hydroxide, NH4OH. Dissolve,

dilute to mark, and invert to mix. Adjust to pH 8.5 with 1*N* HCl or 1*N* NaOH solution.

*d. Sulfanilamide color reagent:* To a tared, dark, 1-L container add 876 g water, 170 g 85%

phosphoric acid, H3PO4, 40.0 g sulfanilamide, and 1.0 g *N*-(1-naphthyl)ethylenediamine

dihydrochloride (NED). Shake to wet solids and stir for 30 min to dissolve. Store in a dark bottle

and discard when solution turns dark pink.

*e. Cadmium column:* See Section 4500-NO3–.I.3*c*, d, and *e*.

*f. Stock nitrate standard,* 1000 mg N/L: In a 1-L volumetric flask dissolve 7.221 g potassium

nitrate, KNO3 (dried at 60°C for 1 h), or 4.93 g sodium nitrite, NaNO2, in about 800 mL water.

Dilute to mark and invert to mix. When refrigerated the standard may be stored for up to 3

months.

*g. Standard solutions:* Prepare nitrate standards in the desired concentration range, using

stock nitrate standards (¶ 3 *f* ), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-N:1 and follow method supplied by

manufacturer, or laboratory standard operating procedure for this method.

Carry both standards and samples through this procedure. If samples have been preserved

with sulfuric acid, preserve standards similarly. Samples may be homogenized. Turbid samples

may be filtered, since digestion effectiveness on nitrogen-containing particles is unknown;

however, organic nitrogen may be lost in the filtration.

5. Calculation

Prepare standard curves by plotting absorbance of standards processed through the manifold

versus nitrogen concentration. The calibration curve is linear.

Verify digestion efficiency by determining urea, glutamic acid, or nicotinic acid standards

(Section 4500-N.C.3*d*) at regular intervals. In the concentration range of the method, the recovery of these compounds should be >95%.

6. Quality Control

See Section 4130B.

7. Precision and Bias

*a. MDL:* Using a 70-μL sample loop and a published MDL method,1 analysts ran 21

replicates of a 0.20-mg N/L standard. These gave a mean of 0.18 mg N/L, a standard deviation of

0.008 mg N/L, and MDL of 0.020 mg N/L.

*b. Precision study:* Ten injections each of a 4.00-mg N/L standard and of a 10.0-mg N/L

standard both gave a relative standard deviation of 0.6%.

*c. Recovery of total nitrogen:* Table 4500-N:I shows recoveries for various nitrogen

compounds determined at 10 mg N/L and 4.0 mg N/L. All compounds were determined in

triplicate.

*d. Ammonia recoveries from wastewater treatment plant effluent with known additions:* To a

sample of wastewater treatment plant effluent, ammonium chloride was added at two

concentrations, 2.50 and 5.00 mg N/L, and analyses were made in triplicate to give mean

recoveries of 96% and 95%, respectively. A sample with no additions also was diluted twofold

in triplicate to give a mean recovery of 99%.

**4500-N C. Persulfate Method**

1. General Discussion

The persulfate method determines total nitrogen by oxidation of all nitrogenous compounds

to nitrate. Should ammonia, nitrate, and nitrite be determined individually, ‘‘organic nitrogen’’

can be obtained by difference.

*a. Principle*: Alkaline oxidation at 100 to 110°C converts organic and inorganic nitrogen to

nitrate. Total nitrogen is determined by analyzing the nitrate in the digestate.

*b. Selection of nitrate measurement method*: Automated or manual cadmium reduction may

be used to determine total nitrogen levels below 2.9 mg N/L. Results summarized in Table

4500-N:II were obtained using automated cadmium reduction.

2. Apparatus

*a. Autoclave, or hotplate and pressure cooker* capable of developing 100 to 110°C for 30 min.

*b. Glass culture tubes:*\*#(52) 30-mL screw-capped (polypropylene linerless caps), 20 mm

OD × 150 mm long. Clean before initial use by autoclaving with digestion reagent.

*c. Apparatus for nitrate determination*: See Section 4500-NO3–.E or Section 4500-NO3–.F.

*d. Automated analytical equipment:* An example of the continuous-flow analytical

instrument consists of components shown in Figure 4500-NO3–:2.

3. Reagents

*a. Ammonia-free and nitrate-free water:* Prepare by ion exchange or distillation methods as

directed in Section 4500-NH3.B.3*a* and Section 4500-NO3–.B.3*a*.

*b. Stock nitrate solution:* Prepare as directed in Section 4500-NO3–.B.3*b*.

*c. Intermediate nitrate solution:* Prepare as directed in Section 4500-NO3–.B.3*c*.

*d. Stock glutamic acid solution:* Dry glutamic acid, C3H5NH2(COOH)2, in an oven at 105°C

for 24 h. Dissolve 1.051 g in water and dilute to 1000 mL; 1.00 mL = 100 μg N. Preserve with 2

mL CHCl3/L.

*e. Intermediate glutamic acid solution:* Dilute 100 mL stock glutamic acid solution to 1000

mL with water; 1.00 mL = 10.0 μg N. Preserve with 2 mL CHCl3/L.gc

*f. Digestion reagent:* Dissolve 20.1 g low nitrogen (<0.001% N) potassium persulfate,

K2S2O8, and 3.0 g NaOH in water and dilute to 1000 mL just before use.

*g. Borate buffer solution:* Dissolve 61.8 g boric acid, H3BO3, and 8.0 g NaOH in water and

dilute to 1000 mL.

*h. Copper sulfate solution:* Dissolve 2.0 g CuSO4⋅5H2O in 90 mL water and dilute to 100

mL.

*i. Ammonium chloride solution:* Dissolve 10.0 g NH4Cl in 1 L water. Adjust to pH 8.5 by

adding three or four NaOH pellets as necessary or NaOH solution before bringing to volume.

This reagent is stable for 2 weeks when refrigerated.

*j. Color reagent:* Combine 1500 mL water, 200.0 mL conc phosphoric acid, H3PO4, 20.0 g

sulfanilamide, and 1.0 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Dilute to 2000 mL.

Add 2.0 mL polyoxyethylene 23 lauryl ether.†#(53) Store at 4°C in the dark. Prepare fresh

reagent every 6 weeks. Alternatively, prepare proportionally smaller volumes to minimize waste.

4. Procedure

*a. Calibration curve:* Prepare NO3– calibration standards in the range 0 to 2.9 mg NO3–-N/L

by diluting to 100 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00 . . . 29.0 mL. Treat standards in the same manner as samples.

*b. Digestion check standard:* Prepare glutamic acid digestion check standard of 2.9 mg N/L

by diluting, to 100 mL, a 29.0-mL volume of intermediate glutamic acid solution. Treat digestion

check standard in the same manner as samples.

*c. Digestion:* Samples preserved with acid cannot be analyzed by this method. To a culture

tube, add 10.0 mL sample or standard or a portion diluted to 10.0 mL. Add 5.0 mL digestion

reagent. Cap tightly. Mix by inverting twice. Heat for 30 min in an autoclave or pressure cooker

at 100 to 110°C. Slowly cool to room temperature. Add 1.0 mL borate buffer solution. Mix by

inverting at least twice.

*d. Blank:* Carry a reagent blank through all steps of the procedure and apply necessary

corrections to the results.

*e. Nitrate measurement:* Determine nitrate by cadmium reduction. Set up manifold as shown

in Figure 4500-NO3–:2, but use reagents specified in Section 4500-Norg.C.3.

5. Calculation

Prepare the standard curve by plotting the absorbances or peak heights of the nitrate

calibration standards carried through the digestion procedure against their nitrogen

concentrations. Compute organic N sample concentration by comparing sample absorbance or

peak height with the standard curve.

6. Precision and Bias

See Table 4500-N:II.

**4500-NH3 NITROGEN (AMMONIA)\*#(54)**

**4500-NH3 A. Introduction**

1. Selection of Method

The two major factors that influence selection of the method to determine ammonia are

concentration and presence of interferences. In general, direct manual determination of low

concentrations of ammonia is confined to drinking waters, clean surface or groundwater, and

good-quality nitrified wastewater effluent. In other instances, and where interferences are present

or greater precision is necessary, a preliminary distillation step (B) is required.

A titrimetric method (C), an ammonia-selective electrode method (D), an ammonia-selective

electrode method using known addition (E), a phenate method (F), and two automated versions

of the phenate method (G and H) are presented. Methods D, E, F, G, and H may be used either

with or without sample distillation. The data presented in Table 4500-NH3:I and Table

4500-NH3:III should be helpful in selecting the appropriate method of analysis.

Nesslerization has been dropped as a standard method, although it has been considered a

classic water quality measurement for more than a century. The use of mercury in this test

warrants its deletion because of the disposal problems.

The distillation and titration procedure is used especially for NH3-N concentrations greater

than 5 mg/L. Use boric acid as the absorbent following distillation if the distillate is to be

titrated.

The ammonia-selective electrode method is applicable over the range from 0.03 to 1400 mg

NH3-N/L.

The manual phenate method is applicable to both fresh water and seawater and is linear to

0.6 mg NH3-N/L. Distill into sulfuric acid (H2SO4) absorbent for the phentate method when

interferences are present.

The automated phenate method is applicable over the range of 0.02 to 2.0 mg NH3-N/L.

2. Interferences

Glycine, urea, glutamic acid, cyanates, and acetamide hydrolyze very slowly in solution on

standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5.

Hydrolysis amounts to about 7% at this pH for urea and about 5% for cyanates. Volatile alkaline

compounds such as hydrazine and amines will influence titrimetric results. Residual chlorine

reacts with ammonia; remove by sample pretreatment. If a sample is likely to contain residual

chlorine, immediately upon collection, treat with dechlorinating agent as in Section

4500-NH3.B.3*d*.

3. Storage of Samples

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 h unacidified, or preserve samples by acidifying to pH <2 and storing at 4°C. If acid preservation

is used, neutralize samples with NaOH or KOH immediately before making the determination.

CAUTION: Although acidification is suitable for certain types of samples, it produces

interferences when exchangeable ammonium is present in unfiltered solids.

**4500-NH3 B. Preliminary Distillation Step**

1. General Discussion

The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and

organic nitrogen compounds. It is distilled into a solution of boric acid when titration is to be

used or into H2SO4 when the phenate method is used. The ammonia in the distillate can be

determined either colorimetrically by the phenate method or titrimetrically with standard H2SO4

and a mixed indicator or a pH meter. The choice between the colorimetric and the acidimetric

methods depends on the concentration of ammonia. Ammonia in the distillate also can be

determined by the ammonia-selective electrode method, using 0.04*N* H2SO4 to trap the

ammonia.

2. Apparatus

*a. Distillation apparatus:* Arrange a borosilicate glass flask of 800- to 2000-mL capacity

attached to a vertical condenser so that the outlet tip may be submerged below the surface of the

receiving acid solution. Use an all-borosilicate-glass apparatus or one with condensing units

constructed of block tin or aluminum tubes.

*b. pH meter.*

3. Reagents

*a. Ammonia-free water:* Prepare by ion-exchange or distillation methods:

1) Ion exchange—Prepare ammonia-free water by passing distilled water through an

ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly

basic anion-exchange resin. Select resins that will remove organic compounds that interfere with

the ammonia determination. Some anion-exchange resins tend to release ammonia. If this occurs,

prepare ammonia-free water with a strongly acidic cation-exchange resin. Regenerate the column high blank value.

2) Distillation—Eliminate traces of ammonia in distilled water by adding 0.1 mL conc

H2SO4 to 1 L distilled water and redistilling. Alternatively, treat distilled water with sufficient

bromine or chlorine water to produce a free halogen residual of 2 to 5 mg/ L and redistill after

standing at least 1 h. Discard the first 100 mL distillate. Check redistilled water for the

possibility of a high blank.

It is very difficult to store ammonia-free water in the laboratory without contamination from

gaseous ammonia. However, if storage is necessary, store in a tightly stoppered glass container

to which is added about 10 g ion-exchange resin (preferably a strongly acidic cation-exchange

resin)/L ammonia-free water. For use, let resin settle and decant ammonia-free water. If a high

blank value is produced, replace the resin or prepare fresh ammonia-free water.

Use ammonia-free distilled water for preparing all reagents, rinsing, and sample dilution.

*b. Borate buffer solution:* Add 88 mL 0.1*N* NaOH solution to 500 mL approximately 0.025*M*

sodium tetraborate (Na2B4O7) solution (9.5 g Na2B4O7⋅10 H2O/L) and dilute to 1 L.

*c. Sodium hydroxide,* 6*N*.

*d. Dechlorinating reagent:* Dissolve 3.5 g sodium thiosulfate (Na2S2O3⋅5H2O) in water and

dilute to 1 L. Prepare fresh weekly. Use 1 mL reagent to remove 1 mg/L residual chlorine in

500-mL sample.

*e. Neutralization agent.*

1) *Sodium hydroxide,* NaOH, 1*N*.

2) *Sulfuric acid*, H2SO4, 1*N*.

*f. Absorbent solution, plain boric acid:* Dissolve 20 g H3BO3 in water and dilute to 1 L.

*g. Indicating boric acid solution:* See Section 4500-NH3.C.3*a* and *b*.

*h. Sulfuric acid,* 0.04*N:* Dilute 1.0 mL conc H2SO4 to 1 L.

4. Procedure

*a. Preparation of equipment:* Add 500 mL water and 20 mL borate buffer, adjust pH to 9.5

with 6*N* NaOH solution, and add to a distillation flask. Add a few glass beads or boiling chips

and use this mixture to steam out the distillation apparatus until distillate shows no traces of

ammonia.

*b. Sample preparation:* Use 500 mL dechlorinated sample or a known portion diluted to 500

mL with water. When NH3-N concentration is less than 100 μg/L, use a sample volume of 1000

mL. Remove residual chlorine by adding, at the time of collection, dechlorinating agent

equivalent to the chlorine residual. If necessary, neutralize to approximately pH 7 with dilute

acid or base, using a pH meter.

Add 25 mL borate buffer solution and adjust to pH 9.5 with 6*N* NaOH using a pH meter.

*c. Distillation:* To minimize contamination, leave distillation apparatus assembled after

steaming out and until just before starting sample distillation. Disconnect steaming-out flask and

immediately transfer sample flask to distillation apparatus. Distill at a rate of 6 to 10 mL/min

with the tip of the delivery tube below the surface of acid receiving solution. Collect distillate in

a 500-mL erlenmeyer flask containing 50 mL indicating boric acid solution for titrimetric

method. Distill ammonia into 50 mL 0.04*N* H2SO4 for the ammonia-selective electrode method

and for the phenate method. Collect at least 200 mL distillate. Lower distillation receiver so that

the end of the delivery tube is free of contact with the liquid and continue distillation during the

last minute or two to cleanse condenser and delivery tube. Dilute to 500 mL with water.

When the phenate method is used for determining NH3-N, neutralize distillate with 1*N*

NaOH solution.

*d. Ammonia determination:* Determine ammonia by the titrimetric method (C), the

ammonia-selective electrode methods (D and E), or the phenate methods (F and G).

**4500-NH3 C. Titrimetric Method**

1. General Discussion

The titrimetric method is used only on samples that have been carried through preliminary

distillation (see Section 4500-NH3.B). The following table is useful in selecting sample volume

for the distillation and titration method.

**Ammonia Nitrogen in Sample SampleVolume**

 ***mg/L mL***

5–10 250

10–20 100

20–50 50.0

50–100 25.0

2. Apparatus

*Distillation apparatus:* See Section 4500-NH3.B.2*a* and Section 4500-NH3.B.2*b*.

3. Reagents

Use ammonia-free water in making all reagents and dilutions.

*a. Mixed indicator solution:* Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or

isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol.

Combine solutions. Prepare monthly.

*b. Indicating boric acid solution:* Dissolve 20 g H3BO3 in water, add 10 mL mixed indicator

solution, and dilute to 1 L. Prepare monthly.

*c. Standard sulfuric acid titrant*, 0.02*N:* Prepare and standardize as directed in Alkalinity,

Section 2320B.3*c*. For greatest accuracy, standardize titrant against an amount of Na2CO3 that

has been incorporated in the indicating boric acid solution to reproduce the actual conditions of

sample titration; 1.00 mL = 14 × normality × 1000 μg N. (For 0.02*N*, 1.00 mL = 280 μg N.)

4. Procedure

*a*. Proceed as described in Section 4500-NH3.B using indicating boric acid solution as

absorbent for the distillate.

*b. Sludge or sediment samples:* Rapidly weigh to within ±1% an amount of wet sample,

equivalent to approximately 1 g dry weight, in a weighing bottle or crucible. Wash sample into a

500-mL kjeldahl flask with water and dilute to 250 mL. Proceed as in ¶ 4*a* but add a piece of

paraffin wax to distillation flask and collect only 100 mL distillate.

*c*. Titrate ammonia in distillate with standard 0.02*N* H2SO4 titrant until indicator turns a pale

lavender.

*d. Blank:* Carry a blank through all steps of the procedure and apply the necessary correction

to the results.

5. Calculation

*a. Liquid samples:*



*b. Sludge or sediment samples:*

**

where:

*A* = volume of H2SO4 titrated for sample, mL, and

*B* = volume of H2SO4 titrated for blank, mL.

6. Precision and Bias

Three synthetic samples containing ammonia and other constituents dissolved in distilled

water were distilled and analyzed by titration.

Sample 1 contained 200 μg NH3-N/L, 10 mg Cl−/L, 1.0 mg NO3−-N/L, 1.5 mg organic N/L, 10.0 mg PO43−/L, and 5.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 69.8% and 20%, respectively. Sample 2 contained 800 μg NH3-N/L, 200 mg Cl−/L, 1.0 mg NO3−-N/L, 0.8 mg organic N/L, 5.0 mg PO4 3−/L, and 15.0 mg silica/L. The relative standard deviation and relative error for the 20 participating laboratories were 28.6% and 5%, respectively. Sample 3 contained 1500 μg NH3-N/L, 400 mg Cl−/L, 1.0 mg NO3−-N/L, 0.2 mg organic N/L, 0.5 mg PO4

3−/L, and 30.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 21.6%, and 2.6%, respectively.

**4500-NH3 D. Ammonia-Selective Electrode Method**

1. General Discussion

*a. Principle:* The ammonia-selective electrode uses a hydrophobic gas-permeable membrane

to separate the sample solution from an electrode internal solution of ammonium chloride.

Dissolved ammonia (NH3(aq) and NH4+) is converted to NH3(aq) by raising pH to above 11 with

a strong base. NH3(aq) diffuses through the membrane and changes the internal solution pH that

is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a

chloride ion-selective electrode that serves as the reference electrode. Potentiometric

measurements are made with a pH meter having an expanded millivolt scale or with a specific

ion meter.

*b. Scope and application:* This method is applicable to the measurement of 0.03 to 1400 mg

NH3-N/L in potable and surface waters and domestic and industrial wastes. High concentrations

of dissolved ions affect the measurement, but color and turbidity do not. Sample distillation is

unnecessary. Use standard solutions and samples that have the same temperature and contain

about the same total level of dissolved species. The ammonia-selective electrode responds

slowly below 1 mg NH3-N/L; hence, use longer times of electrode immersion (2 to 3 min) to

obtain stable readings.

*c. Interference:* Amines are a positive interference. This may be enhanced by acidification.

Mercury and silver interfere by complexing with ammonia, unless the NaOH/EDTA solution

(3*c*) is used.

*d. Sample preservation:* Refrigerate at 4°C for samples to be analyzed within 24 h. Preserve

samples high in organic and nitrogenous matter, and any other samples for longer storage, by

lowering pH to 2 or less with conc H2SO4.

2. Apparatus

*a. Electrometer:* A pH meter with expanded millivolt scale capable of 0.1 mV resolution

between −700 mV and +700 mV or a specific ion meter.

*b. Ammonia-selective electrode.*\*#(55)

*c. Magnetic stirrer*, thermally insulated, with TFE-coated stirring bar.

3. Reagents

*a. Ammonia-free water:* See Section 4500-NH3.B.3*a*. Use for making all reagents.

*b. Sodium hydroxide,* 10*N*.

*c. NaOH/EDTA solution,* 10*N*: Dissolve 400 g NaOH in 800 mL water. Add 45.2 g

ethylenediaminetetraacetic acid, tetrasodium salt, tetrahydrate (Na4EDTA⋅4 H2O) and stir to

dissolve. Cool and dilute to 1000 mL.

*d. Stock ammonium chloride solution:* Dissolve 3.819 g anhydrous NH4Cl (dried at 100°C)

in water, and dilute to 1000 mL; 1.00 mL = 1.00 mg N = 1.22 mg NH3.

*e. Standard ammonium chloride solutions:* See ¶ 4*a* below.

4. Procedure

*a. Preparation of standards:* Prepare a series of standard solutions covering the

concentrations of 1000, 100, 10, 1, and 0.1 mg NH3-N/L by making decimal dilutions of stock

NH4Cl solution with water.

*b. Electrometer calibration:* Place 100 mL of each standard solution in a 150-mL beaker.

Immerse electrode in standard of lowest concentration and mix with a magnetic stirrer. Limit

stirring speed to minimize possible loss of ammonia from the solution. Maintain the same

stirring rate and a temperature of about 25°C throughout calibration and testing procedures. Add

a sufficient volume of 10*N* NaOH solution (1 mL usually is sufficient) to raise pH above 11. If

the presence of silver or mercury is possible, use NaOH/EDTA solution in place of NaOH

solution. If it is necessary to add more than 1 mL of either NaOH or NaOH/ EDTA solution, note

volume used, because it is required for subsequent calculations. Keep electrode in solution until

a stable millivolt reading is obtained. Do not add NaOH solution before immersing electrode,

because ammonia may be lost from a basic solution. Repeat procedure with remaining standards,

proceeding from lowest to highest concentration. Wait until the reading has stablized (at least 2

to 3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH3-N/L.

*c. Preparation of standard curve:* Using semilogarithmic graph paper, plot ammonia

concentration in milligrams NH3-N per liter on the log axis vs. potential in millivolts on the

linear axis starting with the lowest concentration at the bottom of the scale. If the electrode is

functioning properly a tenfold change of NH3-N concentration produces a potential change of

about 59 mV.

*d. Calibration of specific ion meter:* Refer to manufacturer’s instructions and proceed as in ¶s

4*a* and b.

*e. Measurement of samples:* Dilute if necessary to bring NH3-N concentration to within

calibration curve range. Place 100 mL sample in 150-mL beaker and follow procedure in ¶ 4*b*

above. Record volume of 10*N* NaOH added. Read NH3-N concentration from standard curve.

5. Calculation



where:

*A* = dilution factor,

*B* = concentration of NH3-N/L, mg/L, from calibration curve,

*C* = volume of 10*N* NaOH added to calibration standards, mL, and *D* = volume of 10*N* NaOH added to sample, mL.

6. Precision and Bias

For the ammonia-selective electrode in a single laboratory using surface water samples at

concentrations of 1.00, 0.77, 0.19, and 0.13 mg NH3-N/L, standard deviations were ±0.038,

±0.017, ±0.007, and ±0.003, respectively. In a single laboratory using surface water samples at

concentrations of 0.10 and 0.13 mg NH3-N/L, recoveries were 96% and 91%, respectively. The

results of an interlaboratory study involving 12 laboratories using the ammonia-selective

electrode on distilled water and effluents are summarized in Table 4500-NH3:I.

**PHOSPHATE**

**Stannous Chloride Method**

A) Determination of Orthophosphate

1. Prepare the following series of phosphate standards by measuring the indicated volume of standard phosphate solution into separate 100 mL volumetric flasks

 (Or graduated cylinders).

|  |  |
| --- | --- |
| Standard Phosphate Solution. mL |  Phosphate (PO43) μg/100 mL |
|  0 123456 | 0 51015202530 |

1. To the sample, add 0.05 ml 1 drop) of phenolphthalein indicator solution. If the sample turns pink, add strong acid solution drop wise until the color is discharged
2. With a measuring pipette, add 4 mL acid- molybdate solution to each of the standards and sample
3. Mix thoroughly by inverting each flask four to six times.
4. With medicine dropper, add 0.5 mL (10 drops) of stannous chloride solution to each of the standards and sample.
5. Stopper and mix by inverting each flask four to six times
6. After 10 minutes, but before 12 minutes, measure the color photo metrically at 690 nm using distilled water as blank.
7. Construct a calibration curve using the standards and determine the amount of phosphate in μg present in the sample.
8. Calculation

**Calculation**

 a) mg/L PO43 = μg phosphate

 Ml of sample

 b) mg/L P =μg PO43-X 0.32614

 Ml of sample

C) mg/L P2O5 = μg PO43 x 1.4946

 Ml of sample

B) Determination of Total Phosphate

1. Take a 50 mL sample in a 250 mL Erlenmeyer flask and dilute to 100 mL with distilled water
2. Add 1 drop ( 0.05 mL) of phenolphthalein indicator solution
3. If a pink color develops, add strong acid solution one drop at a time until the pink color disappears. Then add 1 mL extra of the acid solution.
4. Boil the acid- treated sample gently for 90 minutes, adding distilled water from time to time to keep the volume between 25 and 50 mL .
5. Cool to room temperature.
6. Stirring the sample constantly; add sodium hydroxide solution until a faint pink color reappears.
7. Transfer sample to a 100 mL volumetric flask or graduated cylinder
8. Rinse the flask, glass beads, and stirring rod with distilled water and add the wash to the flask/cylinder and dilute to the 100 mL mark with distilled water.
9. Complete the determination as described for orthophosphate starting with step 3.
10. Calculate the total phosphate using the formulae given for orthophosphate.

**CHLORIDE**

**Argentometric Method suitable for relatively clear water 0.15-10 mg/l of Cl-**

1. Measure the appropriate sample volume for the indicated chloride range using the

 Following table and transfer to a 250 ml Erlenmeyer flask or porcelain casserole.

|  |  |
| --- | --- |
| Sample volume mL. | Alkalinity range mg/Las CaCO3 |
|  100 50 25 10 |  1-50 51-100 101-200 201-500 |

2. Bring the total volume to 100 mL with distilled water if the sample size is less than

 100 mL

3. Prepare a color comparison blank by placing distilled water in a similar flask and the

 Volume must be equal to that of the sample

4. Add 1 mL potassium dichromate indicator solution to the blank and the sample; and

 Mix

5. To the color comparison blank carefully add from a burette drop by drop silver nitrate

 titrant until the yellow color changes to a brownish tinge.

6. Record the mL silver nitrate titrant consumed.

7. If the sample turns yellow, gradually add silver nitrate titrate from a burette. Shake the

 Flask continuously and continue adding the titrant until the sample turns the same

 Orange- red color as in the color comparison blank.

8. Record mL silver nitrate titrant consumed.

9. Calculation:

 mg Cl/L = ( A-B) X N X 35,450

 Ml of sample

Where

 A= mL titration for sample

 B= mL titration for blank, and

 N= normality of silver nitrate

 Mg NaCl/L = (mg Cl/L) x 1.65

Note:

1. Directly titrate sample in the PH range 7 to 10. Adjust sample PH to 7 to 10

 with H2SO4 or NaOH if not in this range.

1. For highly colored samples clarification with aluminum hydroxide suspension is necessary
2. If sulfide, sulfite thiosulphate is present, add 1 ml hydrogen peroxide and stir for 1 minute.
3. **4500-Cl– D. Potentiometric Method : suitable for colored or turbid water (wastewater)**
4. 1. General Discussion
5. *a. Principle:* Chloride is determined by potentiometric titration with silver nitrate solution
6. with a glass and silver-silver chloride electrode system. During titration an electronic voltmeter
7. is used to detect the change in potential between the two electrodes. The end point of the titration
8. is that instrument reading at which the greatest change in voltage has occurred for a small and
9. constant increment of silver nitrate added.
10. *b. Interference:* Iodide and bromide also are titrated as chloride. Ferricyanide causes high
11. results and must be removed. Chromate and dichromate interfere and should be reduced to the
12. chromic state or removed. Ferric iron interferes if present in an amount substantially higher than
13. the amount of chloride. Chromic ion, ferrous ion, and phosphate do not interfere.
14. Grossly contaminated samples usually require pretreatment. Where contamination is minor, some contaminants can be destroyed simply by adding nitric acid.
15. 2. Apparatus
16. *a. Glass and silver-silver chloride electrodes:* Prepare in the laboratory or purchase a silver
17. electrode coated with AgCl for use with specified instruments. Instructions on use and care of
18. electrodes are supplied by the manufacturer.
19. *b. Electronic voltmeter,* to measure potential difference between electrodes: A pH meter may
20. be converted to this use by substituting the appropriate electrode.
21. *c. Mechanical stirrer,* with plastic-coated or glass impeller.
22. 3. Reagents
23. *a. Standard sodium chloride solution,* 0.0141*M* (0.0141*N*): See Section 4500-Cl–.B.3*c*.
24. *b. Nitric acid,* HNO3, conc.
25. *c. Standard silver nitrate titrant,* 0.0141*M* (0.0141*N*): See Section 4500-Cl–.B.3*b*.
26. *d. Pretreatment reagents:*
27. 1) *Sulfuric acid,* H2SO4, 1 + 1.
28. 2) *Hydrogen peroxide,* H2O2, 30%.
29. 3) *Sodium hydroxide,* NaOH, 1*N*.
30. 4. Procedure
31. *a. Standardization:* The various instruments that can be used in this determination differ in
32. operating details; follow the manufacturer’s instructions. Make necessary mechanical
33. adjustments. Then, after allowing sufficient time for warmup (10 min), balance internal electrical
34. components to give an instrument setting of 0 mV or, if a pH meter is used, a pH reading of 7.0.
35. 1) Place 10.0 mL standard NaCl solution in a 250-mL beaker, dilute to about 100 mL, and
36. add 2.0 mL conc HNO3. Immerse stirrer and electrodes.
37. 2) Set instrument to desired range of millivolts or pH units. Start stirrer.
38. 3) Add standard AgNO3 titrant, recording scale reading after each addition. At the start, large
39. increments of AgNO3 may be added; then, as the end point is approached, add smaller and equal
40. increments (0.1 or 0.2 mL) at longer intervals, so that the exact end point can be determined.
41. Determine volume of AgNO3 used at the point at which there is the greatest change in
42. instrument reading per unit addition of AgNO3.
43. 4) Plot a differential titration curve if the exact end point cannot be determined by inspecting
44. the data. Plot change in instrument reading for equal increments of AgNO3 against volume of
45. AgNO3 added, using average of buret readings before and after each addition. The procedure is
46. illustrated in Figure 4500-Cl–:1. *b. Sample analysis:*
47. 1) Pipet 100.0 mL sample, or a portion containing not more than 10 mg Cl–, into a 250-mL
48. beaker. In the absence of interfering substances, proceed with ¶ 3) below.
49. 2) In the presence of organic compounds, sulfite, or other interferences (such as large
50. amounts of ferric iron, cyanide, or sulfide) acidify sample with H2SO4, using litmus paper. Boil
51. for 5 min to remove volatile compounds. Add more H2SO4, if necessary, to keep solution acidic.
52. Add 3 mL H2O2 and boil for 15 min, adding chloride-free distilled water to keep the volume
53. above 50 mL. Dilute to 100 mL, add NaOH solution dropwise until alkaline to litmus, then 10
54. drops in excess. Boil for 5 min, filter into a 250-mL beaker, and wash precipitate and paper
55. several times with hot distilled water.
56. 3) Add conc HNO3 dropwise until acidic to litmus paper, then 2.0 mL in excess. Cool and
57. dilute to 100 mL if necessary. Immerse stirrer and electrodes and start stirrer. Make any
58. necessary adjustments according to the manufacturer’s instructions and set selector switch to
59. appropriate setting for measuring the difference of potential between electrodes.
60. 4) Complete determination by titrating according to ¶ 4*a*4). If an end-point reading has been
61. established from previous determinations for similar samples and conditions, use this
62. predetermined end point. For the most accurate work, make a blank titration by carrying
63. chloride-free distilled water through the procedure.
64. 5. Calculation
65. 
66. where:
67. *A* = mL AgNO3,
68. *B* = mL blank, and
69. *N* = normality of titrant.
70. 6. Precision and Bias
71. In the absence of interfering substances, the precision and bias are estimated to be about
72. 0.12 mg for 5 mg Cl–, or 2.5% of the amount present. When pretreatment is required to remove
73. interfering substances, the precision and bias are reduced to about 0.25 mg for 5 mg Cl–, or 5%
74. of amount present.

SULFATE

Gravimetric Method with Ignition of Residual

1. Adjust the volume of clarified sample to contain approximately 50 mg of sulfate in a 250 ml volume. Lower concentrations of sulphates may be tolerated if it is impractical to concentrated the sample to the optimum level, but in such cases limit the total volume to 150 ml
2. Adjust the pH with concentrated HCl to pH 4.5-5.0 using a pH meter or the orange color of methyl red indicator. Then, add an additional 1 to 2 ml HCІ.
3. Heat the solution to boiling and while stirring gently, add warm barium chlorides solution slowly until precipitation appears to be complete. Then add about 2 ml in excess
4. Digest the precipitate at 80-90 0C for not less than 2 hours.
5. Filter and wash the precipitate with small portion of warm distilled water until the washings are free of chloride as indicated by testing with AgNO3-HNO3 reagent. Be sure that all of the precipitate is transferred to the paper.
6. Place the filter paper and precipitate in ignited and weighed crucible and dry in the oven
7. Ignite at 8000C for 1 hour, cool in a desiccators and weigh.
8. Calculation:

 mg/L SO24 =mg BaSO4 x 411.6

 Ml sample

 If the silica concentration is above 25 mg/l, it must be removed by the following method prior to sulfate determination.

Removal of silica

1. Evaporate the sample nearly to dryness in an evaporating dish on a steam bath.
2. Add 1ml HCl, tilt the dish, and rotate it until the residue is wetted. Evaporate to dryness and complete the drying at 1800C.
3. If organic matter is present, char over the flame of a burner
4. Moisten the residue with 2 ml distilled water and 1 ml HCl. evapor ate to dryness on a steam bath
5. Add 2 ml HCl, take up the soluble residue in hot water, and filter.
6. Wash the insoluble silica with several small portions of hot distilled water
7. Combine the filtrate and washings and proceed starting with step 1 of the sulfate analysis procedure.

Note

1. If the total cat ion concentration in the sample is 250 mg/l or above, or if the total heavy mental ion concentration in the sample is 10 mg/L or more, pass the sample portion intended for sulfate precipitation through a cat ion-removing exchange column.
2. During the ignition process, do not allow the filter paper to flame
3. It is advisable to use ash less filter paper pulp during filtration as filter aid to reduce the tendency of the precipitate to creep.

**4500-SO4 2– B. (Reserved)**

**4500-SO4 2– C. Gravimetric Method with Ignition of Residue**

1. General Discussion

*a. Principle:* Sulfate is precipitated in a hydrochloric acid (HCl) solution as barium sulfate

(BaSO4) by the addition of barium chloride (BaCl2).

The precipitation is carried out near the boiling temperature, and after a period of digestion

the precipitate is filtered, washed with water until free of Cl–, ignited or dried, and weighed as

BaSO4.

*b. Interference:* The gravimetric determination of SO4 2– is subject to many errors, both

positive and negative. In potable waters where the mineral concentration is low, these may be of

minor importance.

1) Interferences leading to high results—Suspended matter, silica, BaCl2 precipitant, NO3–,

SO3 2– and occluded mother liquor in the precipitate are the principal factors in positive errors.

Suspended matter may be present in both the sample and the precipitating solution; soluble

silicate may be rendered insoluble and SO3 2– may be oxidized to SO4 2– during analysis. Barium

nitrate [Ba(NO3)2], BaCl2, and water are occluded to some extent with the BaSO4 although

water is driven off if the temperature of ignition is sufficiently high.

2) Interferences leading to low results—Alkali metal sulfates frequently yield low results.

This is true especially of alkali hydrogen sulfates. Occlusion of alkali sulfate with BaSO4 causes

substitution of an element of lower atomic weight than barium in the precipitate. Hydrogen

sulfates of alkali metals act similarly and, in addition, decompose on being heated. Heavy

metals, such as chromium and iron, cause low results by interfering with the complete

precipitation of SO4 2– and by formation of heavy metal sulfates. BaSO4 has small but significant

solubility, which is increased in the presence of acid. Although an acid medium is necessary to

prevent precipitation of barium carbonate and phosphate, it is important to limit its concentration

to minimize the solution effect.

2. Apparatus

*a. Steam bath.*

*b. Drying oven,* equipped with thermostatic control.

*c. Muffle furnace,* with temperature indicator.

*d. Desiccator.*

*e. Analytical balance,* capable of weighing to 0.1 mg.

*f. Filter:* Use one of the following:

1) *Filter paper,* acid-washed, ashless hard-finish, sufficiently retentive for fine precipitates.

2) *Membrane filter,* with a pore size of about 0.45 μm.

*g. Filtering apparatus,* appropriate to the type of filter selected. (Coat membrane filter holder

with silicone fluid to prevent precipitate from adhering.)

3. Reagents

*a. Methyl red indicator solution:* Dissolve 100 mg methyl red sodium salt in distilled water

and dilute to 100 mL.

*b. Hydrochloric acid,* HCl, 1 + 1.

*c. Barium chloride solution:* Dissolve 100 g BaCl2⋅2H2O in 1 L distilled water. Filter

through a membrane filter or hard- finish filter paper before use; 1 mL is capable of precipitating

approximately 40 mg SO4 2–.

*d. Silver nitrate-nitric acid reagent:* Dissolve 8.5 g AgNO3 and 0.5 mL conc HNO3 in 500

mL distilled water.

*e. Silicone fluid.\**#(90)

4. Procedure

*a. Removal of silica:* If the silica concentration exceeds 25 mg/L, evaporate sample nearly to

dryness in a platinum dish on a steam bath. Add 1 mL HCl, tilt, and rotate dish until the acid

comes in complete contact with the residue. Continue evaporation to dryness. Complete drying

in an oven at 180°C and if organic matter is present, char over flame of a burner. Moisten residue

with 2 mL distilled water and 1 mL HCl, and evaporate to dryness on a steam bath. Add 2 mL

HCl, take up soluble residue in hot water, and filter. Wash insoluble silica with several small

portions of hot distilled water. Combine filtrate and washings. Discard residue.

*b. Precipitation of barium sulfate:* Adjust volume of clarified sample to contain

approximately 50 mg SO4 2– in a 250-mL volume. Lower concentrations of SO4 2– may be

tolerated if it is impracticable to concentrate sample to the optimum level, but in such cases limit

total volume to 150 mL. Adjust pH with HCl to pH 4.5 to 5.0, using a pH meter or the orange

color of methyl red indicator. Add 1 to 2 mL HCl. Heat to boiling and, while stirring gently, slowly add warm BaCl2 solution until precipitation appears to be complete; then add about 2 mL

in excess. If amount of precipitate is small, add a total of 5 mL BaCl2 solution. Digest precipitate

at 80 to 90°C, preferably overnight but for not less than 2 h.

*c. Filtration and weighing:* Mix a small amount of ashless filter paper pulp with the BaSO4,

quantitatively transfer to a filter, and filter at room temperature. The pulp aids filtration and

reduces the tendency of the precipitate to creep. Wash precipitate with small portions of warm

distilled water until washings are free of Cl– as indicated by testing with AgNO3-HNO3 reagent.

Place filter and precipitate in a weighed platinum crucible and ignite at 800°C for 1 h. Do not let

filter paper flame. Cool in desiccator and weigh.

1. Calculation



6. Precision and Bias

A synthetic sample containing 259 mg SO4 2–/L, 108 mg Ca2+/L, 82 mg Mg2+/L, 3.1 mg

K+/L, 19.9 mg Na+/L, 241 mg Cl–/L, 0.250 mg NO2 –-N/L, 1.1 mg NO3–-N/L, and 42.5 mg total

alkalinity/L (contributed by NaHCO3) was analyzed in 32 laboratories by the gravimetric

method, with a relative standard deviation of 4.7% and a relative error of 1.9%.

Total solids dried at 103-1050c

Gravimetric Method

1. Clean a porcelain evaporating dish and place it in an oven at 103-1050C or 1 hour, or if the fixed and volatile solids determinations is also to be made, ignite at 550+500C in a muffle furnace for 1 hour
2. Place the dish in desiccators and weigh as soon as it has cooled to balance temperature
3. Thoroughly mix the sample and transfer a sample volume that will yield a residue between 2.5 mg and 200 mg to pre weighed dish. (Care must be taken to keep the solids in suspension while measuring)
4. Place the dish on a steam bath and evaporate the sample to dryness.
5. Dry the dish and residue in an oven maintained at 103-1050c for І hour.
6. Place the dish in desiccators and weigh as soon as it has cooled to balance temperature.
7. Repeat cycle of drying cooling desiccating and weighing until a constant weight is obtained, or until weight loss is less than 4% of previous weight or 0.5 mg.
8. Calculation

mg total solids/l = (A-B) x 1000

 ML sample

Where: A= weight of dried residue + dish, mg, and

 B= weight of dish, mg

Total dissolved Solids Dried at 180 0C

1. Filter measured volume of well-mixed sample through glass-fiber-filter, wash with three successive 10-mL volumes of distilled water, allowing complete drainage between washings, and continue suction for about 3 minutes after filtration is complete.
2. Transfer filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath if filtrate volume exceeds dish capacity successive portions to the same dish after evaporation
3. Dry for at least 1 hours in an oven at 103-1050C, cool in a desiccators to balance temperature, and weigh.
4. Calculation

 mg total dissolved solids/L = (A-B)x1000

 ML sample

Where:

 A= Weight of dried residue= dish, mg and

 B= Weight of dish, mg

Note: 1) prepare glass-fiber filter disk as in total suspended solids determination

1. Use glass-fiber filter disks without organic binder. What man grade 934 AH, Gelman type A/E; Millipore type AP40; or equivalent. Available in diameters of 2.2 cm to 4.7 cm.

TOTAL SUSPENDED SOLIDS (dried at 103- 1050C)

Centrifugation Method

1. Clean an empty centrifuge tube thoroughly and dry at 103-1050c in an oven
2. Cool in a desiccators and weigh (A gram)
3. Place 10 mL of thoroughly mixed sample in the centrifuge tube by means of a pipet
4. Centrifuge for 10 minutes at 2000 rpm.
5. Pour off the supernatant and add distilled water. Stir the tube and centrifuge again for 10 minutes at 2000 rpm.
6. Pour of the water and dry for 1 hour at 103-1050C
7. Cool in a desiccators and weight (B .gram)
8. Calculation:

mg/L suspended solids= (B-A) X І,000,000

 mL of sample

Sludge volume Index (SVI)

Calculate the SVI by the following formula

Sludge Volume Index (mL/g) = Settled Sludge Volume (ML/L) x 1000

 Suspended solids (mg/L)

TOTAL SUSPENDED SOLIDS

Dried at 103-105‑0c

Gravimetric Method

Preparation of glass-fiber disk

1. Insert disk with wrinkled side up in filtration apparatus
2. Apply vacuum and wash disk with three successive 20-mL portions of distilled water continue suction to remove all traces of water, amd discard washing
3. Remove filter from filtration apparatus along with the Gooch crucible, and dry in an oven at 103 to 1050c for 1 hour. If volatile solids are to be measured, ignite at 550 +500C for 15 minutes in a muffle furnace.
4. Cool in desiccators to balance temperature and weighing until a constant weight is obtained or until weight loss is less than 0.5 mg between successive weightings.

SAMPLE ANALYSES

І) Assemble filtering apparatus and filter and begin suction. Wet filter

 With a small volume of distilled water to seat it.

2). Filter a measured volume of well mixed sample through the glass fiber filter.

3). Wash with three successive 10-mL volumes of distilled water, allowing complete drainage between washings and continue suction for about 3 minutes after filtration is complete.

4) Remove the crucible and filter combination from the crucible adapter if a Gooch crucible is used.

5) Dry for at least І hour at 103 to 1050c in an oven,

Cool in a desiccators to balance temperature, and weigh.

1. Calculation

mg suspended solids/L = (A-B)x1000

 ML sample

 Where:

 A= Weight of filter + dried residue, mg

 B= Weight of filter, mg

Chromium

Diphenyl Carbazide Method

Preparation of Calibration Curve

* 1. Pipet measured volumes of standard chromium solution (5μg/mL) ranging from 2.0 to 20.0 mL to give standards for 10 to 100 μg Cr, into 250-mL beakers or conical flasks.
	2. Proceed with subsequent treatment of standards as if they were samples.
	3. Develop color as for sample, transfer a suitable portion of each colored solution to a 1-cm absorption cell, and measure the absorbance at 540 nm.
	4. As reference, use distilled water, correct absorbance readings or standards by subtracting the absorbance of a reagent blank carried through the method.
	5. Construct a calibration curve by plotting corrected absorbance values against micrograms chromium in 102 mL final volume

Oxidation of Trivalent Chromium to Hexa valent Chromium

І). pipette a portion of sample containing 10 to 100 μg Cr into a 125-mL conical flask.

2). Using methyl orange as indicator add conc. Ammonium hydroxide until the solution is just basic to methyl orange, then adds 1+1 H2SO4 drop wise until it is acidic plus 1 mL in excess.

3). Adjust volumes to about 40 mL add a few glass beads, and heat to boiling.

4) Add 2 drops potassium permanganate solution to give a dark red color. If fading occurs add KMnO4 drop wise to maintain excess of about 2 drops.

5). Boil for 2 minutes longer. Add 1 mL sodium azide and continue boiling gently. If the red color does not fade completely after boiling for approximately 30 seconds add another 1 mL sodium aside solution.

6) Continue boiling for 1 minute after the color has faded completely. Cool and add 0.25 mL (5 drops) ortho phosphoric acid

**Color Development**

1). with 0.2N sulphuric acid and using a pH meter, adjust the solution pH to

 1.0+ 0.3.

2). Transfer the solution to a 100-mL Volumetric flask or stopered graduated cylinder, diluted to 100 mL, and mix thoroughly.

3). Add 2 ml diphenyl carbazide solution, mix and let stand 5 to 10 minutes for full color development.

4) Transfer and appropriate portion to 1-cm absorption cell and measure its absorbance at 540 nm. Use distilled water as reference.

5). Correct absorbance reading or sample by subtracting the absorbance of the blank carried through the method.

6) From the corrected absorbance, determine micrograms Cr present by reference to the calibration curve.

7) Calculation

 mg Cr/L =μg Cr

 mL sample

Note: 1) If the sample contains organic matter, it must be subjected to acid digestion to destroy the organic matter.

2.If the solution is turbid after dilution to 100 mL take an absorbance reading before adding carbonize reagent and correct the absorbance reading of the final colored solution by subtracting the absorbance measured previous.

**3500-Cr CHROMIUM\*#(101)**

**3500-Cr A. Introduction**

1. Occurrence and Significance

Chromium (Cr) is the first element in Group VIB in the periodic table; it has an atomic

number of 24, an atomic weight of 51.99, and valences of 1 through 6. The average abundance of

Cr in the earth’s crust is 122 ppm; in soils Cr ranges from 11 to 22 ppm; in streams it averages

about 1 μg/L, and in groundwaters it is generally 100 μg/L. Chromium is found chiefly in

chrome-iron ore (FeO⋅Cr2O3). Chromium is used in alloys, in electroplating, and in pigments.

Chromate compounds frequently are added to cooling water for corrosion control.

In natural waters trivalent chromium exists as Cr3+, Cr(OH)2+, Cr(OH)2+, and Cr(OH)4–; in

the hexavalent form chromium exists as CrO42– and as Cr2O7 2–. Cr3+ would be expected to form strong complexes with amines, and would be adsorbed by clay minerals. Chromium may exist in water supplies in both the hexavalent and the trivalent state although the trivalent form rarely occurs in potable water.

Chromium is considered nonessential for plants, but an essential trace element for animals.

Hexavalent compounds have been shown to be carcinogenic by inhalation and are corrosive to

tissue. The chromium guidelines for natural water are linked to the hardness or alkalinity of the

water (i.e., the softer the water, the lower the permitted level for chromium). The United Nations Food and Agriculture Organization recommended maximum level for irrigation waters is 100

μg/L. The U.S. EPA primary drinking water standard MCL is 0.1 mg/L for total chromium.

2. Selection of Method

The colorimetric method (B) is useful for the determination of hexavalent chromium in a

natural or treated water in the range from 100 to 1000 μg/L. This range can be extended by

appropriate sample dilution or concentration and/or use of longer cell paths. The ion

chromatographic method with photometric detection (C) is suitable for determining dissolved

hexavalent chromium in drinking water, groundwater, and industrial wastewater effluents. The

electrothermal atomic absorption spectrometric method (Section 3113B) is suitable for

determining low levels of total chromium (< 50 μg/L) in water and wastewater, and the flame

atomic absorption spectrometric methods (Section 3111B and Section 3111C) and the

inductively coupled plasma methods (Section 3120 and Section 3125) are appropriate for

measuring concentrations up to milligram-per-liter levels.

3. Sample Handling

If only the dissolved metal content is desired, filter sample through a 0.45-μm membrane

filter at time of collection, and after filtration acidify filtrate with conc nitric acid (HNO3) to pH

<2. If only dissolved hexavalent chromium is desired, adjust pH of filtrate to 8 or above with 1*N*

sodium hydroxide solution and refrigerate. If the total chromium content is desired, acidify

unfiltered sample at time of collection with conc HNO3 to pH <2. If total hexavalent chromium

is desired, adjust the pH of unfiltered sample to 8 or above with 1*N* sodium hydroxide and

refrigerate.

**3500-Cr B. Colorimetric Method**

1. General Discussion

*a. Principle:* This procedure measures only hexavalent chromium (Cr 6+). Therefore, to

determine total chromium convert all the chromium to the hexavalent state by oxidation with

potassium permanganate. NOTE: The oxidation process may not provide total conversion of all

chromium species to Cr 6+.1-3 For total chromium determination, acid-digest the sample (see

Section 3030) and follow with a suitable instrumental analysis technique. The hexavalent

chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solution. A

red-violet colored complex of unknown composition is produced. The reaction is very sensitive,

the molar absorptivity based on chromium being about 40 000 L g–1 cm–1 at 540 nm. To

determine total chromium, digest the sample with a sulfuric-nitric acid mixture and then oxidize

with potassium permanganate before reacting with the diphenylcarbazide.

*b. Interferences:* The reaction with diphenylcarbazide is nearly specific for chromium.

Hexavalent molybdenum and mercury salts will react to form color with the reagent but the intensities are much lower than that for chromium at the specified pH. Concentrations as high as

200 mg Mo or Hg/L can be tolerated. Vanadium interferes strongly but concentrations up to 10

times that of chromium will not cause trouble. Potential interference from permanganate is

eliminated by prior reduction with azide. Iron in concentrations greater than 1 mg/L may

produce a yellow color but the ferric ion (Fe3+) color is not strong and no difficulty is

encountered normally if the absorbance is measured photometrically at the appropriate

wavelength. Interfering amounts of molybdenum, vanadium, iron, and copper can be removed by

extraction of the cupferrates of these metals into chloroform (CHCl3). A procedure for this

extraction is provided but do not use it unless necessary, because residual cupferron and CHCl3

in the aqueous solution complicate the later oxidation. Therefore, follow the extraction by

additional treatment with acid fuming to decompose these compounds.

2. Apparatus

*a. Colorimetric equipment:* One of the following is required:

1) *Spectrophotometer,* for use at 540 nm, with a light path of 1 cm or longer.

2) *Filter photometer,* providing a light path of 1 cm or longer and equipped with a greenish

yellow filter having maximum transmittance near 540 nm.

*b. Separatory funnels,* 125-mL, Squibb form, with glass or TFE stopcock and stopper.

*c. Acid-washed glassware:* New and unscratched glassware will minimize chromium

adsorption on glass surfaces during the oxidation procedure. Do not use glassware previously

treated with chromic acid. Thoroughly clean other used glassware and new glassware with nitric

or hydrochloric acid to remove chromium traces.

3. Reagents

Use reagent water (see Section 1080) for reagent preparation and analytical procedure.

*a. Stock chromium solution:* Dissolve 141.4 mg K2Cr2O7 in water and dilute to 100 mL;

1.00 mL = 500 μg Cr.

*b. Standard chromium solution:* Dilute 1.00 mL stock chromium solution to 100 mL; 1.00

mL = 5.00 μg Cr.

*c. Nitric acid,* HNO3, conc.

*d. Sulfuric acid,* H2SO4, conc, 18*N*, and 6*N*.

*e. Sulfuric acid,* H2SO4, 0.2*N*: Dilute 17 mL 6*N* H2SO4 to 500 mL with water.

*f. Phosphoric acid,* H3PO4, conc.

*g. Methyl orange indicator solution.*

*h. Hydrogen peroxide,* H2O2, 30%.

*i. Ammonium hydroxide,* NH4OH, conc.

*j. Potassium permanganate solution:* Dissolve 4 g KMnO4 in 100 mL water.

*k. Sodium azide solution:* Dissolve 0.5 g NaN3 in 100 mL water.

*l. Diphenylcarbazide solution:* Dissolve 250 mg 1,5-diphenylcarbazide

(1,5-diphenylcarbohydrazide) in 50 mL acetone. Store in a brown bottle. Discard when solution

becomes discolored.

*m. Chloroform,* CHCl3: Avoid or redistill material that comes in containers with metal or

metal-lined caps.

*n. Cupferron solution:* Dissolve 5 g cupferron, C6H5N(NO)ONH4, in 95 mL water.

*o. Sodium hydroxide,*1*N:* Dissolve 40 g NaOH in 1 L water. Store in plastic bottle.

4. Procedure

*a. Preparation of calibration curve:* To compensate for possible slight losses of chromium

during digestion or other analytical operations, treat standards by the same procedure as the

sample. Accordingly, pipet measured volumes of standard chromium solution (5 μg/mL) ranging

from 2.00 to 20.0 mL, to give standards for 10 to 100 μg Cr, into 250-mL beakers or conical

flasks. Depending on pretreatment used in ¶ b below, proceed with subsequent treatment of

standards as if they were samples, also carrying out cupferron treatment of standards if this is

required for samples.

Develop color as for samples, transfer a suitable portion of each colored solution to a 1-cm

absorption cell, and measure absorbance at 540 nm, using reagent water as reference. Correct

absorbance readings of standards by subtracting absorbance of a reagent blank carried through

the method.

Construct a calibration curve by plotting corrected absorbance values against micrograms

chromium in 102 mL final volume.

*b. Treatment of sample:* If sample has been filtered and/or only hexavalent chromium is

desired, start analysis within 24 h of collection and proceed to ¶ 4*e*. NOTE: Recent evidence4

suggests that preserved samples can be held for 30 d without substantial changes to Cr 6+

concentrations. If total dissolved chromium is desired and there are interfering amounts of

molybdenum, vanadium, copper, or iron present, proceed to ¶ 4*c*. If interferences are not present,

proceed to ¶ 4*d*.

If sample is unfiltered and total chromium is desired, digest with HNO3 and H2SO4 as in

Section 3030G. If interferences are present, proceed to ¶ 4*c*, ¶ 4*d*, and ¶ 4*e*. If there are no

interferences, proceed to ¶ 4*d* and ¶ 4*e*.

*c. Removal of molybdenum, vanadium, iron, and copper with cupferron:* Pipet a portion of

sample containing 10 to 100 μg Cr into a 125-mL separatory funnel. Dilute to about 40 mL with

water and chill in an ice bath. Add 5 mL ice-cold cupferron solution, shake well, and let stand in

ice bath for 1 min. Extract in separatory funnel with three successive 5-mL portions of CHCl3; shake each portion thoroughly with aqueous solution, let layers separate, and withdraw and

discard CHCl3 extract. Transfer extracted aqueous solution to a 125-mL conical flask. Wash

separatory funnel with a small amount of water and add wash water to flask. Boil for about 5 min

to volatilize CHCl3 and cool. Add 5 mL HNO3 and 3 mL H2SO4. Boil samples to the appearance

of SO3 fumes. Cool slightly, carefully add 5 mL HNO3, and again boil to fumes to complete

decomposition of organic matter. Cool, wash sides of flask, and boil once more to SO3 fumes,

assuming elimination of all HNO3. Cool and add 25 mL water.

*d. Oxidation of trivalent chromium:* Pipet a portion of digested sample with or without

interferences removed, and containing 10 to 100 μg Cr, into a 125-mL conical flask. Add several

drops of methyl orange indicator, then add conc NH4OH until solution just begins to turn yellow.

Add 1 + 1 H2SO4 dropwise until it is acidic, plus 1 mL (20 drops) in excess. Adjust volume to

about 40 mL, add two or more acid-washed glass beads, and heat to boiling. Add 2 drops

KMnO4 solution to give a dark red color. If fading occurs, add KMnO4 dropwise to maintain an

excess of about 2 drops. Boil for 2 min longer. Add 1 mL NaN3 solution and continue boiling

gently. If red color does not fade completely after boiling for approximately 30 s, add another 1

mL NaN3 solution. Continue boiling for 1 min after color has faded completely, then cool.

*e. Color development and measurement:* Add 0.25 mL (5 drops) H3PO4. Use 0.2*N* H2SO4

and a pH meter to adjust solution to pH 1.0 ± 0.3. NOTE: Recent work5 identifies the optimum

pH range for color development to be 1.6 to 2.2; the matter of optimum pH range is currently

being considered by *Standard Methods*. Transfer solution to a 100-mL volumetric flask, dilute to

100 mL, and mix. Add 2.0 mL diphenylcarbazide solution, mix, and let stand 5 to 10 min for full

color development. Transfer an appropriate portion to a 1-cm absorption cell and measure its

absorbance at 540 nm, using reagent water as reference. Correct absorbance reading of sample

by subtracting absorbance of a blank carried through the method (see also note below). From the

corrected absorbance, determine micrograms chromium present by reference to the calibration

curve.

NOTE: If the solution is turbid after dilution to 100 mL in ¶ e above, take an absorbance

reading before adding carbazide reagent and correct absorbance reading of final colored solution

by subtracting the absorbance measured previously.

5. Calculation

For digested samples:



where:

*A* = mL original sample, and

*B* = mL portion from 100 mL digested sample.

For undigested samples:



6. Precision and Bias

Collaborative test data from 16 laboratories were obtained on reagent water, tap water, 10%

NaCl solution, treated water from synthetic organic industrial waste, EPA extraction leachate,

process water, lake water, and effluent from a steel pickle liquor treatment plant.6 The test data

yielded the following relationships:

Reagent water:

*St* = 0.037*x* + 0.006

*So* = 0.022*x* + 0.004

Drinking or wastewater:

*S*t = 0.067*x* + 0.004

*S*o = 0.037*x* + 0.002

Leachate:

*S*t = 0.032*x* + 0.007

*S*o = 0.017*x* + 0.004

where:

*St* = overall precision,

*So* = single-operator precision, and

*x* = chromium concentration, mg/L.

**3030 D. Digestion for Metals**

To reduce interference by organic matter and to convert metals associated with particulates

to a form (usually the free metal) that can be determined by atomic absorption spectrometry or

inductively-coupled plasma spectroscopy, use one of the digestion techniques presented below.

Use the least rigorous digestion method required to provide acceptable and consistent recovery

compatible with the analytical method and the metal being analyzed.1-3

1. Selection of Acid

Nitric acid will digest most samples adequately (Section 3030E). Nitrate is an acceptable

matrix for both flame and electrothermal atomic absorption and the preferred matrix for

ICP-MS.4 Some samples may require addition of perchloric, hydrochloric, hydrofluoric, or

sulfuric acid for complete digestion. These acids may interfere in the analysis of some metals

and all provide a poorer matrix for both electrothermal and ICP-MS analysis. Confirm metal

recovery for each digestion and analytical procedure used. Use Table 3030:I as a guide in

determining which acids (in addition to HNO3) to use for complete digestion. As a general rule,

HNO3 alone is adequate for clean samples or easily oxidized materials; HNO3-H2SO4 or

HNO3-HCl digestion is adequate for readily oxidizable organic matter; HNO3-HClO4 or

HNO3-HClO4-HF digestion is necessary for difficult-to-oxidize organic matter or minerals

containing silicates. Although dry ashing is not generally recommended because of the loss of

many volatile elements, it may be helpful if large amounts of organic matter are present.

2. Digestion Procedures

Dilute samples with Ag concentrations greater than 1 mg/L to contain less than 1 mg Ag/L

for flame atomic absorption methods and 25 μg/L or less for electrothermal analysis.2,5,6 To address problems with silver halide solubility in HNO3, digest using method 3030F.3*b*.

Report digestion technique used.

Acid digestion techniques (Section 3030E through I) generally yield comparable precision

and bias for most sample types that are totally digested by the technique. Because acids used in

digestion will add metals to the samples and blanks, minimize the volume of acids used.

Because the acid digestion techniques (Section 3030E and F) normally are not total

digestions, the microwave digestion procedure (Section 3030K) may be used as an alternative.

The microwave method is a closed-vessel procedure and thus is expected to provide improved

precision when compared with hot-plate techniques. Microwave digestion is recommended for

samples being analyzed by ICP-MS. The microwave digestion method is recommended for the

analysis of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Tl,

V, and Zn. Microwave digestion may be acceptable for additional analytes provided its

performance for those elements is validated.

Suggested sample volumes are indicated below for flame atomic absorption spectrometry.

Lesser volumes, to a minimum of 5 mL, are appropriate for graphite furnace, ICP, and ICP-MS.

Do not subsample volumes less than 5 mL, especially when particulates are present. Instead

dilute samples with elevated analyte concentrations after digestion. If the recommended volume

exceeds digestion vessel capacity, add sample as evaporation proceeds. For samples containing

particulates, wide-bore pipets maybe useful for volume measurement and transfer.

When samples are concentrated during digestion (e.g., >100mL sample used) determine

metal recovery for each matrix digested, to verify method validity. Using larger samples will

require additional acid, which also would increase the concentration of impurities.

 **Estimated Metal Concentration**

 ***mg/L***

 **Sample Volume\***

 ***mL***

<0.1 1000

0.1–10 100

10–100+ 10

\*For flame atomic absorption spectrometry.

Report results as follows:



where:

*A* = concentration of metal in digested solution, mg/L,

*B* = final volume of digested solution, mL, and

*C* = sample size, mL.

Prepare solid samples or liquid sludges with high solids contents on a weight basis. Mix

sample and transfer a suitable amount (typically 1 g of a sludge with 15% total solids) directly

into a preweighed digestion vessel. Reweigh and calculate weight of sample. Proceed with one

of the digestion techniques presented below. However, as these digestion methods are

predominantly for dissolved and extractable metals in aqueous samples, other approaches may be

more appropriate for solid samples. For complete mineralization of solid samples, consult

methods available elsewhere.1,4,6,7 Report results on wet- or dry-weight basis as follows:



where:

*A* = concentration of metal in digested solution, mg/L,

*B* = final volume of digested solution, mL, and

*D* = total solids, % (see Section 2540G).

Always prepare acid blanks for each type of digestion performed. Although it is always best

to eliminate all relevant sources of contamination, a reagent blank prepared with the same acids

and subjected to the same digestion procedure as the sample can correct for impurities present in

acids and reagent water. However, blank correction is not recommended for any other sources of

contamination such as impurities adsorbed on glassware.

**3030 E. Nitric Acid Digestion**

Because of the wide variation in concentration levels detected by various instrumental

techniques and the need to deal adequately with sources of contamination at trace levels, this

method presents one approach for high-level analytes (>0.1 mg/L) and another for trace levels

(≤0.1 mg/L).

1. Digestion for Flame Atomic Absorption and High-Level Concentrations

*a*. *Apparatus:*

1) *Hot plate.*

2) *Conical (erlenmeyer) flasks,* 125-mL, or *Griffin beakers,* 150-mL, acid-washed and rinsed

with water.

3) *Volumetric flasks,* 100-mL.

4) *Watch glasses,* ribbed and unribbed.

*b. Reagent:*

Nitric acid, HNO3, conc, analytical or trace-metals grade.

*c. Procedure:* Transfer a measured volume (100 mL recommended) of well-mixed,

acid-preserved sample appropriate for the expected metals concentrations to a flask or beaker

(see Section 3030D for sample volume). In a hood, add 5 mL conc HNO3. If a beaker is used,

cover with a ribbed watch glass to minimize contamination. Boiling chips, glass beads, or

Hengar granules may be added to aid boiling and minimize spatter when high concentration

levels (>10 mg/L) are being determined. Bring to a slow boil and evaporate on a hot plate to the

lowest volume possible (about 10 to 20 mL) before precipitation occurs. Continue heating and

adding conc HNO3 as necessary until digestion is complete as shown by a light-colored, clear

solution. Do not let sample dry during digestion.

Wash down flask or beaker walls and watch glass cover (if used) with metal-free water and then filter if necessary (see Section 3030B). Transfer filtrate to a 100-mL volumetric flask with

two 5-mL portions of water, adding these rinsings to the volumetric flask. Cool, dilute to mark,

and mix thoroughly. Take portions of this solution for required metal determinations.

2. Digestion for Trace-Level (≤0.1 mg/L) Concentrations for ICP and ICP-MS1

*a. Apparatus:*

1) *Block heater,* dry, with temperature control.

2) *Polypropylene tubes*\*#(68), graduated, round-bottom tubes with caps, 17 × 100 mm,

acid-washed and rinsed with metal-free water. Preferably use tubes that simultaneously match

the analysis instrument autosampler and the block digester. A fit with the centrifuge is secondary

but also desirable.

3) *Pipetters,* assorted sizes or adjustable.

4) *Pipet tips*.

5) *Centrifuge.*

*b. Reagent:*

Nitric acid, HNO3, conc, double distilled.†#(69)

*c. Procedure:* Soak new polypropylene tubes and caps overnight or for several days in 2*N*

HNO3. Triple rinse with metal-free water, and preferably dry in poly rackets or baskets in a

low-temperature oven overnight. Store cleaned tubes in plastic bags before use. Pipet tips also

may need to be cleaned; evaluate before use.

Pipet 10 mL well-mixed, acid-preserved sample into a precleaned, labeled tube with a

macropipet. With a minimum volume change (<0.5 mL), add appropriate amount of analyte for

matrix fortified samples. With a pipet, add 0.5 mL conc HNO3 (or 1.0 mL 1 + 1 HNO3) to all

samples, blanks, standards, and quality control samples.

Place tubes in block heater in a hood and adjust temperature to 105°C. Drape caps over each

tube to allow escape of acid vapors while preventing contamination. NOTE: Do not screw on caps

at this time. Digest samples for a minimum of 2 h. Do not let samples boil. Add more conc nitric

acid as necessary until digestion is complete by observation of a clear solution.

Remove tubes from heat and cool. Dilute back to original 10 mL volume with metal-free

water. Adjust over-volume samples to next convenient gradation for calculations and note

volume. (Apply concentration correction from Section 3030D.) If tubes contain particulates,

centrifuge and decant clear portion into another precleaned tube. Tighten screw caps and store at

4°C until ready for analysis.

**3030 F. Nitric Acid-Hydrochloric Acid Digestion**

1. Apparatus

See Section 3030E.1*a*. The following also may be needed:

*Steam bath.*

2. Reagents

*a. Nitric acid,* HNO3, conc, analytical grade or better (see Section 3030E).

*b. Hydrochloric acid,* HCl, 1 + 1.

*c. Nitric acid*, HNO3, 1 + 1.

3. Procedure

*a. Total HNO*3/HCl: Transfer a measured volume of well-mixed, acid-preserved sample

appropriate for the expected metals concentrations to a flask or beaker (see Section 3030D for

sample volume). In a hood add 3 mL conc HNO3 and cover with a ribbed watch glass. Place

flask or beaker on a hot plate and cautiously evaporate to less than 5 mL, making certain that

sample does not boil and that no area of the bottom of the container is allowed to go dry. Cool.

Rinse down walls of beaker and watch glass with a minimum of metal-free water and add 5 mL

conc HNO3. Cover container with a nonribbed watch glass and return to hot plate. Increase

temperature of hot plate so that a gentle reflux action occurs. Continue heating, adding additional

acid as necessary, until digestion is complete (generally indicated when the digestate is light in

color or does not change in appearance with continued refluxing). Cool. Add 10 mL 1 + 1 HCl

and 15 mL water per 100 mL anticipated final volume. Heat for an additional 15 min to dissolve

any precipitate or residue. Cool, wash down beaker walls and watch glass with water, filter to

remove insoluble material that could clog the nebulizer (see Section 3030B), and transfer filtrate

to a 100-mL volumetric flask with rinsings. Alternatively centrifuge or let settle overnight.

Adjust to volume and mix thoroughly.

*b. Recoverable HNO*3/HCl: For this less rigorous digestion procedure, transfer a measured

volume of well-mixed, acid-preserved sample to a flask or beaker. Add 2 mL 1 + 1 HNO3 and 10

mL 1 + 1 HCl and cover with a ribbed watch glass. Heat on a steam bath or hot plate until

volume has been reduced to near 25 mL, making certain sample does not boil. Cool and filter to

remove insoluble material or alternatively centrifuge or let settle overnight. Quantitatively

transfer sample to volumetric flask, adjust volume to 100 mL, and mix.

For trace-level digestion, use precautionary measures similar to those detailed in Section 3030E.

**3030 G. Nitric Acid-Sulfuric Acid Digestion**

1. Apparatus

See Section 3030E.1*a*.

2. Reagents

*a. Nitric acid,* HNO3, conc. (See Section 3030E for acid grades.)

*b. Sulfuric acid,* H2SO4, conc.

3. Procedure

Transfer a measured volume of well-mixed, acid-preserved sample appropriate for the

expected metals concentrations to a flask or beaker (see Section 3030D for sample volume). Add

5 mL conc HNO3 and cover with a ribbed watch glass. Bring to slow boil on hot plate and

evaporate to 15 to 20 mL. Add 5 mL conc HNO3 and 10 mL conc H2SO4, cooling flask or

beaker between additions. Evaporate on a hot plate until dense white fumes of SO3 just appear.

If solution does not clear, add 10 mL conc HNO3 and repeat evaporation to fumes of SO3. Heat

to remove all HNO3 before continuing treatment. All HNO3 will be removed when the solution

is clear and no brownish fumes are evident. Do not let sample dry during digestion.

Cool and dilute to about 50 mL with water. Heat to almost boiling to dissolve slowly soluble

salts. Filter if necessary, then complete procedure as directed in Section 3030E.1*c* beginning

with, ‘‘Transfer filtrate . . .’’